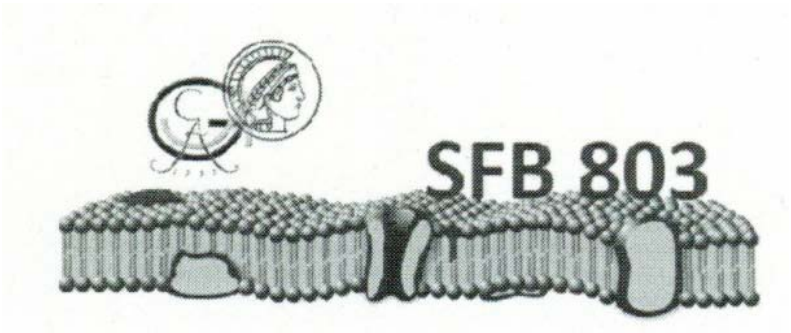
SFB 803 Symposium

Functionality controlled by organization in and between membranes



29.09. – 30.09.2011

University of Göttingen

Max-Planck-Institute for Biophysical Chemistry, Göttingen

funded by the DFG

Program

Thursday, September 29th 2011

09:30 – 09:40	**Claudia Steinem** Welcome
09:40 – 10:20	**Thomas Söllner (University of Heidelberg)** Calcium-dependent membrane fusion of giant unilamellar vesicles requires the combined function of synaptotagmin 1 and complexin
10:20 – 10:40	**Ulf Diederichsen** Artificial peptide and peptide/peptide nucleic acid model systems for SNARE mediated membrane fusion
10:40 – 11:00	**Claudia Steinem** Fusion of vesicles with pore-spanning membranes: Influence of SNARE-derived transmembrane helices and off-membrane molecular recognition
11:00 – 11:30	**Coffee Break**
11:30 – 12:10	**Dieter Langosch (Technical University, Munich)** The backbone dynamics of transmembrane helices
12:10- 12:30	**Guiseppe Sicoli** Distances and orientations between rigid nitroxide spin-labels in peptides by high-field pulsed electron-electron double resonance
12:30 – 12:50	**Axel Munk** Statistical challenges in ion channel recordings and NMR spectroscopy
13:00 – 14:00	**Lunch**
14:00 – 14:40	**Martin Hof (Heyrovsky Institute of Physical Chemistry, Prague)** Hydration and mobility in membranes and enzymes characterized by fluorescence techniques
14:40 – 15:00	**Kerstin Weiß** Quantifying the diffusion of membrane proteins and peptides
15:00 – 15:20	**Daniel Werz** Defined glycosphingolipids to investigate domain formation
15:20 – 15:40	**Kai Tittmann** Structural basis for interphase electron transfer in the peripheral membrane enzyme pyruvate oxidase from *E. coli*
15:40 – 17:00	**Poster Session**
17:00 – 17:40	**Giovanna Fragneto (Institute Laue-Langevin, Grenoble)** Neutrons and model membranes
17:40 – 18:00	**Tim Salditt** Structures and interactions in membrane fusion by x-ray diffraction: from lipid model systems to synaptic vesicles
18:00 – 18:20	**Adam Lange/Christian Griesinger** Solid-state NMR as a tool in structural biology: VDAC1 and the type three secretion system needle
19:00	**Conference Dinner**

Friday, September 30th 2011

08:30 – 09:10	**John Fricks (Pennsylvania State University, PA, USA)** Multiple scales in molecular motor models
09:10 – 09:30	**Christoph Schmidt** Mechanosensing by primary cilia in kidney epithelial cells and characterization of PC1/PC2 channels in supported lipid model membranes
09:30 – 09:50	**Christian Ducho** Structural insights into protein-ligand-membrane interactions for the bacterial membrane protein MraY: synthesis and application of chemical probes
09:50 – 11:10	**Coffee Break / Poster Session**
11:10 – 11:50	**Christoph Zaba (University of Natural Resources and Life Sciences, Vienna)** Proteopolymersomes: in vitro production of a membrane protein in polymersome membranes
11:50 – 12:10	**Bert de Groot** The dynamics of protein membrane interactions
12:10 – 12:30	**Markus Zweckstetter** The human voltage-dependent anion channel: Structure, dynamics and membrane interactions
12:30 – 12:50	**Marcus Müller** Coarse-grained simulation of collective phenomena in membranes
13:00 – 14:00	**Lunch**
14:00 – 14:40	**Carsten Schultz (EMBL, Heidelberg)** Tools for imaging and manipulating membrane biology
14:40 – 15:00	**Jomo Walla** Synaptotagmin-1 activity is modulated by cis- and trans-membrane interactions
15:00 – 15:20	**Gregory Bubnis** Forcing membranes to bend and fuse
15:20 – 15:30	**Claudia Steinem** Concluding remarks
15:30	**Departure**

Speakers Abstracts

Calcium-Dependent Membrane Fusion of Giant Unilamellar Vesicles Requires the Combined Function of Synaptotagmin 1 and Complexin

Jörg Malsam, Daniel Parisotto, Tanmay Bharat[1], Andrea Scheutzow, Jean Michel Krause, John Briggs[1] & Thomas H. Söllner

Heidelberg University Biochemistry Center
[1] EMBL Heidelberg

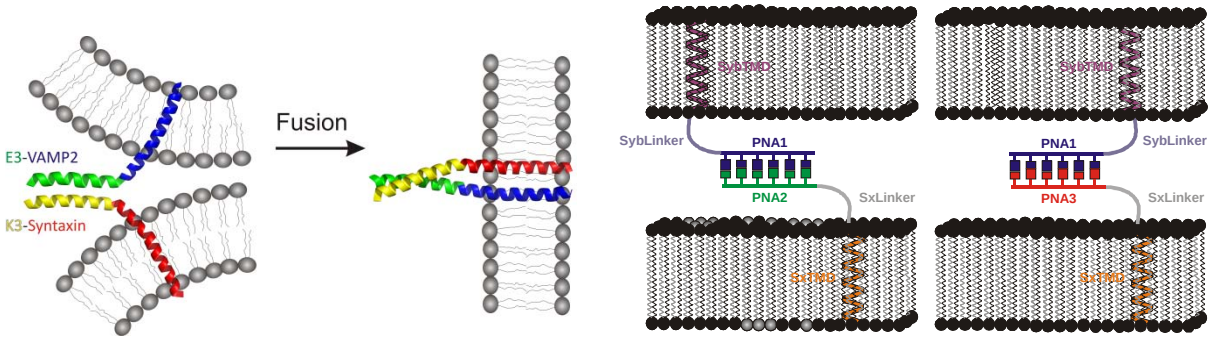
Regulated exocytosis of neurotransmitters requires the tight coupling of the minimal membrane fusion machinery (SNARE proteins) to a signal (calcium) which triggers transmitter release within less than a millisecond. Therefore, a calcium-sensing machinery (synaptotagmin and complexin) controls the final steps of SNARE complex assembly and synchronizes membrane fusion. *In vivo* and *in vitro* experiments have established that synaptotagmin 1 functions as the main calcium sensor. Complexin knock-out studies in *Drosophila* indicate that complexin controls the rate of spontaneous fusion events. To test if this set of proteins is sufficient to confer calcium regulation and to reveal the underlying mechanisms, we analyzed the function of complexin and synaptotagmin 1 in a reconstituted fusion assay, containing giant unilamellar vesicles. The results demonstrate that synaptotagmin 1 stimulates the overall fusion reaction in the absence of calcium, whereas complexin clamps with its accessory/inhibitory alpha-helix this calciu1m-independent stimulation. Cryo-EM studies show that complexin arrests membrane fusion at a vesicle docking stage. Addition of physiological calcium levels triggers fast and synchronous fusion and complexin potentiates this reaction.

Artificial peptide and peptide/peptide nucleic acid model systems for SNARE mediated membrane fusion

Antonina Lygina, Karsten Meyenberg, Annika Groschner, Geert van den Bogaart,[#] Reinhard Jahn,[#] Ulf Diederichsen

Institute of Organic and Biomolecular Chemistry, Tammannstr. 2, 37077 Göttingen
[#]Max-Planck-Institute for Biophysical Chemistry, Am Faßberg 11, 37077 Göttingen

Specific aggregation of transmembrane domains (TMDs) mediated by recognition units outside the lipid bilayer is well-known in membrane fusion processes like the SNARE protein-mediated fusion.[1] In order to mechanistically evaluate the fusion process and especially the role of the TMDs, the linker regions, and the recognition units, hybrid biooligomers were synthesized containing the TMDs and peptides for recognition in a coiled-coil motif or SNARE derived TMDs in a hybrid oligomer with peptide nucleic acids.[2,3] The artificial constructs allow the variation of recognition motifs, combinations of TMDs, relative orientation of recognizing oligomers, modifications at the *C*-terminal end of the helix, and variations of the linker region. Lipid mixing and content mixing fusion essays were applied to evaluate the potential of the artificial oligomers for fusing vesicle membranes. Thereby, the influence of recognition units, TMDs, terminal modification and linker region on the fusion process was detected.



1. Jahn, R., Scheller, R. H. (2006) SNAREs – engines for membrane fusion. *Nat. Rev. Mol. Cell Biol.* 7, 631.
2. Lygina, A. S., Meyenberg, K., Jahn, R., Diederichsen, U. (2011) Transmembrane Domain Peptide/Peptide Nucleic Acid Hybrid as a Model of a SNARE Protein in Vesicle Fusion. *Angew. Chem. Int. Ed.*, DOI: 10.1002/anie.201101951.
3. Meyenberg, K., Lygina, A. S., van den Bogaart, G., Jahn, R., Diederichsen, U. (2011) SNARE derived peptide mimic inducing membrane fusion. *Chem. Commun.*, DOI: 10.1039/C1CC12879E.

Fusion of vesicles with pore-spanning membranes: Influence of SNARE-derived transmembrane helices and off-membrane molecular recognition

Ines Höfer, Henrik Neubacher, Antonina Lygina, Karsten Meyenberg, Gesa Pähler,[#] Andreas Janshoff,[#] Ulf Diederichsen, Claudia Steinem

Institute of Organic and Biomolecular Chemistry, Tammannstr. 2, 37077 Göttingen
[#]Institute of Physical Chemistry, Tammannstr. 6, 37077 Göttingen

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. Even though experiments based on artificial membrane systems have significantly contributed to our current knowledge on membrane fusion processes, there are still a number of drawbacks associated with these assays. In this project, we aim to overcome some of these disadvantages by developing and applying a new fusion assay based on pore-spanning membranes. Pore-spanning membranes are achieved by spreading of giant unilamellar vesicles on a functionalized highly ordered porous silicon substrate. We were able to demonstrate that these pore-spanning membranes are well suited to monitor different stages of the fusion process by time-elapsed fluorescence microscopy. Single fusion events can be observed upon the addition of Texas Red DHPE doped unilamellar vesicles with Oregon Green DHPE doped pore-spanning membranes in a time resolved manner. Lipid mixing during the fusion process is followed by the occurring Förster resonance energy transfer.
With the established setup, the influence of the insertion of SNARE mimetics in pore-spanning membranes and unilamellar vesicles, respectively on the fusion efficacy has been investigated. The insertion of synthetic minimal SNARE proteins as well as PNA-sequences coupled to the native SNARE-transmembrane domain resulted in very efficient fusion. In contrast, if only the off-membrane SNARE motif is coupled to a lipid anchor, no lipid mixing is observed. From our results we conclude that a destabilization of the vesicles by SNARE-transmembrane helices is pivotal to cause lipid mixing with pore-spanning membranes.

Höfer, I., Steinem, C. (2011) A membrane fusion assay based on pore-spanning membranes. *Soft Matter* 7, 1644-1647.

The backbone dynamics of transmembrane helices

Dieter Langosch

TUM, Freising

The backbones of transmembrane helices are dynamic in a sense that they unfold locally and transiently on nanosecond timescales. We determined the backbone dynamics of natural and designed transmembrane helices by deuterium/hydrogen-exchange experiments and Molecular Dynamics simulations. Our findings suggest that amino acids that are known to be helix-destabilizing enhance dynamics. In one example, we studied the implications of this effect for the mechanism of membrane fusion. We will present data indicating that enhanced TM-helix dynamics supports their fusogenicity. In another example, we investigated the backbone dynamics of the transmembrane helix of the transmembrane domain of the amyloid precursor protein (APP-TMD). Here, we asked whether backbone dynamics of the alpha-helical APP-TMD is influencing its processing.The results show that the N-terminal part of the transmembrane domain of the APP exhibits higher backbone dynamics than the C-terminal part which is probably due to the helix-destabilising glycines occuring in the double GxxxG motif at the N-terminus. Moreover, we compared APP-TMD dynamics with those of other TMDs. These findings raise the question to which extent the helix backbone dynamics distinguishes the APP-TMD from non-substrate TMDs.

Distances and orientations between rigid nitroxide spin-labels in peptides by high-field pulsed electron-electron double resonance

[1]Giuseppe Sicoli, [1]Igor Tkach, [1]Soraya Pornsuwan, [2]Sven Stoller, [2]Tatiana Baranova, [2]Ulf Diederichsen and [1,2]Marina Bennati

[1]Max-Planck Research Group Electron Paramagnetic Resonance, Max-Planck Institute for Biophysical Chemistry Am Faßberg 11, Göttingen, Germany and [2]Institute for Organic and Biomolecular Chemistry, Tammannstr. 2, 37077 Göttingen

Recently pulsed electron-electron double resonance (PELDOR) spectroscopy at high magnetic fields has been successfully employed not only for distance measurement but also for determination of the mutual orientation between protein radicals [1, 2]. To obtain structural information in biological macromolecules by EPR, in particular to study the organization of peptides in membranes, the nitroxide spin-label is a widely used paramagnetic center that can be inserted at the desired position in the peptide or in the lipid chain of a membrane. However, the determination of orientations between two nitroxide labels is generally aggravated by the flexibility of the nitroxide side-chain. We have been developing an experimental protocol to determine orientations between nitroxide spin labels in peptides by means of high field EPR spectroscopy. The protocol comprehends new hardware for orientation selection during distance measurements [3], the incorporation of new rigid nitroxide labels for peptides [4] and finally the experiment and analysis of a spin labeled peptide. The data will be discussed in view of the possibility to extend the method to study peptides domains into the trans-membrane regions.

1. Denysenkov V. P., Biglino D., Lubitz W., Prisner T. F., Bennati M., *Angew. Chem. Int. Ed.*, 47, 1224-1227 (**2008**)
2. Sicoli G., Argirević T., Stubbe J., Tkach I., Bennati M., *Appl. Magn. Reson.*, 37, 539-548 (**2010**)
3. Tkach I., Sicoli G., Hoebartner C., Bennati M., *J. Magn. Reson.*, 209, 341-346 (**2011**)
4. S. Stoller, G. Sicoli, T. Baranova, M. Bennati, U. Diederichsen, *Angew. Chem. Int. Ed*. in press.

Statistical Challenges in Ion Channel Recordings and NMR Spectroscopy

Axel Munk, Thomas Hotz, and Axel Munk

Institute for Mathematical Stochastics, Georg-August-Universität Göttingen

In this talk we will illustrate the interplay of mathematical statistics and chemical resp. biophysical research by means of two examples. First, we show how multi-scale analyses of ion channel recordings allow to reconstruct the channel's behaviour under minimal assumptions in an objective, data-driven manner. Second, we will comment on optimal sampling designs for indirect times in NMR correlation spectroscopy which help to improve the parameter estimates, which is of importance especially for large molecules such as membrane proteins.

Hydration and mobility in membranes and enzymes characterized by fluorescence techniques

Martin Hof

J. Heyrovský Institute of Physical Chemistry, Academy of Sciences of the Czech Republic, Dolejškova 3, CZ-18223 Prague 8, Hof@jh-inst.cas.cz

Fluorescence is certainly one of the most widely used spectroscopic techniques in biological orientated sciences. One reason for that is that fluorescence can be used in all kind of model systems and in living cells. Using a fluorescent reporter molecule one can gain -depending on the specific techniques chosen- information on location, dynamics and polarity of the labeled system of interest. Our lab significantly contributed to the development of novel fluorescence techniques including the **fluorescence solvent relaxation technique**[1-3], **z-scan Fluorescence Correlation Spectroscopy**[4,5], Fluorescence Lifetime Correlation Spectroscopy[6], and Dynamic Saturation Optical Microscopy[7].

In a considerable part of this lecture the fluorescence solvent relaxation (SR) technique will be introduced. SR refers to the dynamic process of solvent reorganization in response to an abrupt change in charge distribution of a fluorescent dye via electronic excitation. While in pure water an average (integral) SR time of about 0.3 ps has been determined, the SR processes in **biomembranes** is occurring mainly on the nanosecond timescale[2]. When applied to membranes monitoring this SR process by recording time dependent fluorescence shifts yields defined information of **hydration and mobility** at different depth within the bilayer[3]. In this contribution we will give an introduction to that technique and discuss two recent applications, namely the influence of **„Hofmeister" ions**[8] and **lipid oxidation** [9,10] on the physical chemical properties of phospholipid bilayers.

While the application of the SR technique in membrane sciences has been for the last almost 15 years mainly pursued by our group, a much higher number of publication have appeared within the last ten years presenting SR studies in enzymes. Those studies will be summarized and a recent work dealing with hydration and mobility at the active site of enzymes will be highlighted[11]. Moreover, the role of those parameters in the **enantioselectivity** of certain designed **dehalogenase enzymes** will be discussed[12].

References:

(1) Hof, M. *Solvent Relaxation in Biomembranes. Applied Fluorescence in Chemistry, Biology and Medicine.* (1998), ed. Rettig, W.; Strehmel, B.; Schrader, S.; Seifert, H., Berlin: Springer, 439-456, ISBN 3-540-64451-2

(2) Sýkora, J.; Kapusta, P.; Fidler,V.; Hof, M. *On What Time-Scale Does Solvent Relaxation in Phospholipid Bilayers Happen?* (2002), Langmuir, 18, 571-574

(3) Jurkiewicz, P.; Cwiklik, L.; Jungwirth, P.; Hof, M. *Lipid hydration and mobility: An interplay between solvent relaxation experiments and molecular dynamic simulations.* Biochimie doi:10.1016/j.biochi.2011.06.027

(4) Benda, A.; Beneš, M.; Mareček, V.; Lhotský, A.; Hermens, W.Th.; Hof, M. *How to Determine Diffusion Coefficients in Planar Phospholipid Systems by Confocal Fluorescence Correlation Spectroscopy.* (2003), Langmuir 19, 4120-4126.

(5) Macháň, R.; Hof, M. *Lipid diffusion in planar membranes investigated by fluorescence correlation spectroscopy.* Biochimica Et Biophysica Acta-Biomembranes 2010*, 1798*, 1377-1391;

(6) Humpolickova, J.; Benda, A.; Sykora, J.; Machan, R.; Kral, T.; Gasinska, B.; Enderlein, J.; Hof, M. *Equilibrium dynamics of spermine-induced plasmid DNA condensation revealed by fluorescence lifetime correlation spectroscopy* Biophysical Journal 2008, *94*, L17-19;
(7) Humpolíčková, J.; Benda, A.; Macháň, R.; Enderlein, J.; Hof, M. *Dynamic saturation optical microscopy: employing dark-state formation kinetics for resolution enhancement.* Physical Chemistry Chemical Physics 2010, *12*, 12457-12465;
(8) Vácha, R.; Jurkiewicz, P.; Petrov, M.; Berkowitz, M.L.; Böckmann, R.A.; Barucha-Kraszewska, J.; Hof M.; Jungwirth, P. *Mechanism of Interaction of Monovalent Ions with Phosphatidylcholine Lipid Membranes.* Journal of Physical Chemistry B 2010, *114*, 9504-9509;
(9) Beranova, L.; Cwiklik, L.; Jurkiewicz, P.; Hof, M.; Jungwirth, P. *Oxidation Changes Physical Properties of Phospholipid Bilayers: Fluorescence Spectroscopy and Molecular Simulations* Langmuir 2010, *26*, 6140-6144
(10) Volinsky, R.; Cwiklik, L.; Jurkiewicz, P.; Hof, M.; Jungwirth, P.; Kinnunen, P.K.J. *Oxidised Phosphatidylcholones reconstitute phospholipid "scramblase" activity in liposomes.* Biophysical Journal, in press;
(11) Jesenska,A.; Sýkora, J.; Olžyńska, A.; Brezovský, J.; Zdráhal, Z.; Damborský, J.; Hof, M. *Nanosecond Time-Dependent Stokes Shift at the Tunnel Mouth of Haloalkane Dehalogenases.* Journal of the American Chemical Society 2009, 131, 494 −501
(12) Jan Sykora, Jan Brezovsky, Tana Koudelakova, Maryna Lahoda, Andrea Fortova, Tatsiana Chernavets, Radka Chaloupkova, Veronika Stepankova, Zbynek Prokop, Ivana Kuta Smatanova, Martin Hof, Jiri Damborsky. *Dynamics and Hydration: Two Factors to be Considered in the Rational Design of Enzyme Catalysts*, submitted

Quantifying the Diffusion of Membrane Proteins and Peptides

Jörg Enderlein and Kerstin Weiß

3. Physikalisches Institut, Georg-August-Universität Göttingen

The Saffman-Delbrück (SD) model[1], which is often used to describe protein diffusion in lipid bilayers, has been both challenged[2,3] and supported[4] by recent publications. The model assumes the proteins to be cylinders that diffuse through an infinite two-dimensional bilayer and predicts a logarithmic dependence of the protein's diffusion coefficient D_{SD} on its hydrodynamic radius R.

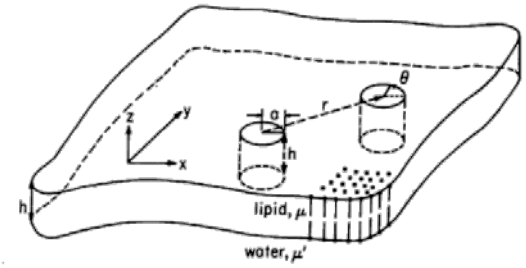


Fig. 1: Illustration of the assumptions of the Saffman-Delbrück model. Proteins are modeled as cylinders that diffuse through an infinite two-dimensional lipid bilayer.

Our project aims to precisely measure translational and rotational diffusion coefficients of proteins in lipid bilayers by means of dual-focus fluorescence correlation spectroscopy (2f-FCS). For our investigations we use Black Lipid Membranes (BLMs) as model systems.
Proteins and peptides of different size have been reconstructed into BLMs and their diffusion coefficient has been determined. Our preliminary results indicate that the diffusion of proteins much larger than the lipid can be described with the SD model. The diffusion of proteins with a size similar to the lipid, however, seems to be better characterized by a Stokes-Einstein-like model, where the protein's diffusion coefficient is inversely proportional to its hydrodynamic radius.
In the future, different environmental conditions will be employed to investigate their effect on protein diffusion within the lipid bilayer. Moreover, we are aiming to derive a size / structure – diffusion relationship for rotational diffusion of proteins in a membrane.

References:

[1] Saffman, P.G., Delbrück, M., *Proc. Nat. Acad. Sci. USA,* **1975**, *72*, 3111 – 3113.
[2] Gambin, Y., Lopez-Esparza, R., Reffay, M., Sierecki, E., Gov, N.S., Genest, M., Hodges, R.S., Urbach, W., *PNAS*, **2005**, *103*, 2098 – 2102.
[3] Kriegsmann, J., Gregor, I., von der Hocht, I., Klare, J., Engelhard, M., Enderlein, J., Fitter, J., *ChemBioChem*, **2009**, *10*, 1823 – 1829.
[4] Ramadurai, S., Holt, A., Krasnikov, V., van den Bogaart, G., Killian, J.A., Poolman, B., *J. Am. Chem. Soc.*, **2009**,*131*, 12650 – 12656.

Defined glycosphingolipids to investigate domain formation

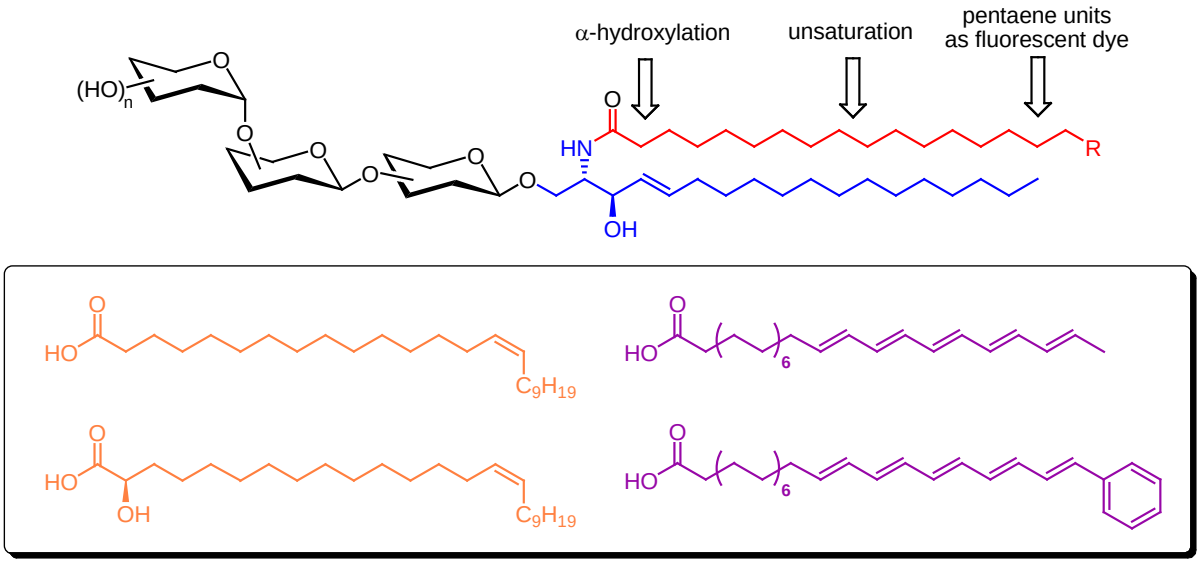
Annika Ries, Lukas Patalag and Daniel B. Werz

Institute of Organic and Biomolecular Chemistry, Georg-August-University Göttingen

Commonly, glycerophospholipids are the major components of eukaryotic cell membranes whereas glycosphingolipids occur to a minor extent. 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.

Many investigations use extracted glycosphingolipids being mixtures of a variety of different lipids. Besides the carbohydrate head group they might differ in the type of the fatty acid (saturated, unsaturated, α-hydroxylated or unsaturated and α-hydroxylated). Therefore, synthetic efforts to access lipids in pure form are of utmost importance. In order to visualize glycolipids several pentaene fatty acids have been prepared as well. In terms of sterics the latter resemble their native counterparts to major extent [2,3].

Syntheses and first biophysical investigations using the synthetic materials will be discussed.



[1] K. Simons, E. Ikonen, *Nature* **1997**, *387*, 569-572.
[2] L. Kuerschner, C. S. Ejsing, K. Ekroos, A. Shevchenko, K. I. Anderson, C. Thiele, *Nature Methods* **2005**, *2*, 39-45.
[3] A. Ries, L. Patalag, D. B. Werz, *unpublished results*.

Structural basis for interphase electron transfer in the peripheral membrane enzyme pyruvate oxidase from *E. coli*

Astrid Sitte, Danilo Meyer and Kai Tittmann

Göttinger Zentrum für Molekulare Biowissenschaften, Georg-August-Universität Göttingen, Justus-von-Liebig-Weg 11, 37077 Göttingen

Pyruvate oxidase from *E. coli* is a peripheral membrane enzyme and catalyzes the oxidative decarboxylation of the cytosolic metabolite pyruvate and concomitant transfer of two reducing equivalents to the membrane-dissolved electron carrier ubiquinone 8. Our previous studies provided molecular insights into a “redox-gated” membrane binding mechanism, according to which substrate induced reduction of the flavin cofactor leads to a structural rearrangement of the membrane binding C-terminal domain triggering recruitment of the protein from the cytosol to the cell membrane. In the cytosolic, oxidized state, the auto-inhibitory C-terminal membrane anchor adopts a half-barrel/helix fold that occludes the active site. Upon reduction, the C-terminus is expelled and becomes structurally flexible thereby freeing the active site. Formation of a C-terminal amphipathic helix is then observed upon contact of the reduced protein with the membrane or phospholipids.

Two key questions were addressed in our recent studies on pyruvate oxidase. First, how does pyruvate oxidase suppress an off-pathway reoxidation of the two-electron reduced flavin cofactor with molecular oxygen to give hydrogen peroxide as product, a reaction that efficiently takes place in most flavo-oxidases? Obviously, in pyruvate oxidase, the latter reaction would uncouple pyruvate oxidation from the respiratory chain. Second, we aimed at elucidating how electrons are shuttled from the reduced enzyme to the final acceptor ubiquinone. Several mechanisms can be envisaged including long range transfer from the active site into the membrane or cytosol-membrane interphase as well as direct transfer at the active site, which would require transient interphase transit of the ubiquinone.

Novel crystallographic and functional analysis infer an active site tyrosine residue as being a gatekeeper that sterically blocks access of dioxygen to the flavin C4a oxidase site. Variants with substitutions of the tyrosine exhibit enhanced “short circuit” oxidase activities. Newly determined crystal structures of pyruvate oxidase with ubiquinone strongly suggest that the redox reaction between the enzyme-bound, reduced flavin and membrane dissolved ubiquinone takes place at the enzyme active site. This finding necessitates transient transfer of ubiquinone from the membrane phase into the active site over a distance of ~10 Å. Possible reasons for the apparent interphase transit of ubiquinone will be discussed.

Neutrons and Model Membranes

Giovanna Fragneto[1]

[1] *Institut Laue-Langevin, 6 rue Jules Horowitz, BP 156 F-38042 Grenoble*¶

Biological systems near interfaces represent one of the most dynamic and expanding fields in science and technology and have been at the centre of recent major scientific and technological advances [1]. Biological interfaces include the surfaces of cells and organelles within cells where many biological mechanisms happen, as well as artificial mimics of biological surfaces.

Current research in membrane protein biophysics highlights the emerging role of lipids in shaping membrane protein function. Cells and organisms have developed sophisticated mechanisms for controlling the lipid composition and many diseases are related to the failure of these homeostatic regulatory mechanisms. One of the recent advances in the field is the discovery of the existence of coexisting micro-domains within a single membrane, important for regulating some signaling pathways. Many important properties of these domains remain poorly characterized.

The characterization and analysis of bio-interfaces represent a challenge. Performing measurements on these few nanometer thick, soft, visco-elastic and dynamic systems is close to the limits of the available tools and methods. *Neutron and x-ray reflectometry* are rapidly developing techniques for these studies and are attracting an increasing number of biologists and biophysicists at large facilities. As the deuteration of proteins is becoming an active field of research, the use of fully deuterated or partially deuterated proteins has opened up new possibilities in the study of lipid protein interactions or protein structures at lipid surfaces.

Examples of recent reflectivity studies on the structure and fluctuations of model membranes and their interaction with proteins will be presented as well as advances in the structural determination of membranes with complex lipid composition (including cholesterol, gangliosides, ceramides) and a recent ILL project for the extraction and purification of natural deuterated lipids.

[1] G. Fragneto, Eur. Phys. J. E., 30 (2) (2009) 239.

Structures and interactions in membrane fusion by x-ray diffraction: from lipid model systems to synaptic vesicles

Tim Salditt, Sebastian Aeffner, Simon Castorph, Sajal Ghosh, Andre Beerlink,

Institut für Röntgenphysik Matthew Holt,
Reinhard Jahn. MPI biophysikalische Chemie

Despite many biochemical and numerical studies, the structural pathways and intermediates of membrane fusion and the associated energetic barriers remain rather elusive due to the tremendous experimental challenge of probing non-crystallographic structures in aqueous media at the nanoscale. We have used advanced interface senstive synchrotron x-ray diffraction to study the formation of membrane fusion intermediates in a large number of multi-component lipid model systems, yielding a rather universal fusion stalk structure [1]. At the same time, the osmotic pressure necessary to induce stalk formation depends strongly on the lipid mixture. Based on the data, we derive a simple fusion 'predictor', based on the measured hydration repulsion.

Beyond simple lipid model systems, we have extended the x-ray structure analysis to the level of native synaptic vesicles (SV) in solution [2] and in the presence of lipid bilayers and monolayers [3].
We show that the width and density of the SV protein layers can be resolved. Furthermore, binging SVs into contact with model bilayers and monolayers, that structural reorganization of the lipid systems can be monitored. The collective monolayer response to SV injection in the subphase is probed at controlled levels of Ca. Finally, we discuss new methods to resolve membrane contours and structural details locally by x-ray imaging [6].

[1] S. Aeffner, T. Reusch, B. Weinhausen, and T. Salditt Membrane fusion intermediates and the effect of cholesterol: An in-house X-ray scattering study Eur. Phys. J. E 30, 205–214 (2009); S. Aeffner et al., in preparation.

[2] Simon Castorph, Dietmar Riedel, Lise Arleth, Michael Sztucki, Reinhard Jahn, 7Matthew Holt, Tim Salditt Structure Parameters of Synaptic Vesicles Quantified by Small-Angle X-Ray Scattering Biophys.J. 98 1200, (2010).

[3] S.K. Ghosh, S. Castorph, O. Konovalov, R. Jahn, M. Holt and T. Salditt In vitro study of interaction of synaptic vesicles with lipid membranes New J. Phys. 12, 105004 (2010); S.K. Ghosh et al. submitted to Biophys.J.

Solid-State NMR as a Tool in Structural Biology: VDAC and the TTSS Needle

Adam Lange

Max Planck Institute for Biophysical Chemistry, Göttingen, Germany

In the last decade, solid-state NMR has emerged as a powerful technique in structural biology. For instance, ssNMR opens new ways to study membrane proteins in their natural lipid environment, or insoluble disease-associated protein aggregates. Moreover, functional filamentous assemblies such as the needle of the Type Three Secretion System (TTSS) – composed of multiple copies of a single small protein – can be readily studied. In terms of membrane proteins our main interest lies on the voltage-dependent anion channel (VDAC) that is located in the mitochondrial outer membrane and constitutes the major pathway for the transport of ADP and ATP. Recently we have determined the native conformation of the N-terminal part of human VDAC1 in liposomes by ssNMR [Schneider et al. Angewandte Chemie 2010]. This region has so far been difficult to observe with solution NMR. Here, we report the measurement of dipolar order parameters for residues in the N-terminus as well as in other parts of the molecule. Our data show that the N-terminus is rather rigid and indicate that it plays an important stabilizing role for the □-barrel protein.

Furthermore, we have recently started to structurally characterize the needle of the *S. typhimurium* TTSS [Poyraz, Schmidt, Seidel et al. NSMB 2010]. Based on an optimized *in vitro* needle preparation we can now obtain ssNMR spectra of much higher quality. Progress in resonance assignment of the needle protein PrgI and detection of distance restraints will be reported. In this regard, we are applying a novel approach for the study of intermolecular interfaces based on equimolar mixtures of [1-^{13}C]- and [2-^{13}C]-glucose labeled proteins [Loquet et al. JACS 2010, JACS 2011].

Multiple Scales in Molecular Motor Models

Will Hancock, Peter Kramer, Scott McKinley, and John Fricks

Dept of Bioengineering
Pennsylvania State University

Dept of Mathematical Sciences
Rensselaer Polytechnic Institute

Dept of Mathematics
University of Florida

Dept of Statistics
Pennsylvania State University

Molecular motors, such as kinesin and dynein, carry cargo through a cell along a microtubule network. The heads of these motors step along a microtubule and are on the order of nanometers, while the cargo size and the distance traveled can be on the order of hundreds of nanometers. Two examples of mathematical models of motors that bridge these spatial scales will be discussed.

In the first example, a stochastic model for variable-length stepping of kinesins engineered with extended neck linkers will be developed. This requires consideration of the separation in microtubule binding sites between the heads of the motor at the beginning of a step. This separation is a stationary process and can be included in the calculation of standard experimental quantities though a semi-Markov model. A corresponding matrix computational framework will be discussed for conducting computer experiments that is more computationally efficient than large-scale Monte Carlo simulation; this efficiency greatly eases sensitivity analysis, an important feature when there is considerable uncertainty in the physical parameters of the system.

The second example involves multiple motors attached to a common cargo. The behavior of intracellular cargo should depend strongly on the number of motors that attach the cargo to microtubules. However, convincing evidence for the influence of multiple motors on transport has proved elusive. In fact, some authors now claim that cargo interact with microtubules *in vivo* almost exclusively through one motor at a time. In order to better understand how both single and multiple motors can influence the motion of a cargo, a mathematical model for microtubule-motor-cargo dynamics is developed that emphasizes the spatial configuration and the resultant distribution of forces generated on and by the cargo. In the analysis, the comparison in performance of multiple motors versus a single motor is dependent on the applied external force. At small force regimes, multiple motors are found to move cargo more slowly than a single motor, while at high force regimes (such as near stall force) superlinear improvement in transport performance is observed. Although this phenomenon has been observed in stochastic simulation, the presented results depend on rigorous asymptotic methods that lead to the specification of explicit conditions under which different transport phenomena arise. Theoretical predictions are confirmed by experimental data from multiple sources.

Mechanosensing by primary cilia in kidney epithelial cells and characterization of PC1/PC2 channels in supported lipid model membranes

Christoph Schmidt

III. Physikalisches Institut, Georg-August-Universität, Friedrich-Hund-Platz 1, 37077 Göttingen

We are pursuing a two-pronged approach to understand the mechanosensory capabilities of kidney epithelial cells. These cells use primary cilia, which are universally found in almost all cells, to sense fluid flow in kidney ducts. The primary signal is believed to be generated by TRP channels (PC1/PC2) inserted in the membrane of the cilia, which are activated by the deformation of the cilia in extracellular fluid flow. We have set up specialized instrumentation and have employed microfabrication to construct chambers in which we can probe the channels in model lipid bilayers supported by porous substrates with built-in electrodes for electrical recording. We have completed construction and test experiments. We have also managed to produce channel protein.
In the second approach, we have established MDCK cell cultures and have constructed instrumentation with which we can mechanically stimulate primary cilia on MDCK cells with an optical trap. In first tests we have probed the mechanical properties of the cilia and their anchoring in the cell surface.

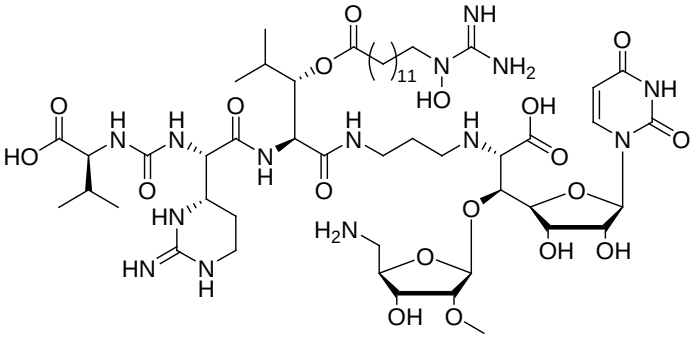
Structural insights into protein-ligand-membrane interactions for the bacterial membrane protein MraY: synthesis and application of chemical probes

Anatol P. Spork, Oliver Ries, Martin Büschleb, Stephanie Wohnig, and Christian Ducho

Georg-August-University Göttingen, Department of Chemistry, Institute of Organic and Biomolecular Chemistry, Tammannstr. 2, D-37 077 Göttingen

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. Results from synthetic studies on muraymycins and muraymycin analogues will be presented, particularly with respect to their unusual nucleoside core structure[4-6] and to the lipid structure bearing the *N*-hydroxy-guanidine moiety.[7]

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. [7] O. Ries, A. Ochmann, C. Ducho, *Synthesis* **2011**, 2357-2368.

Proteopolymersomes: in vitro production of a membrane protein in polymersome membranes

Christoph Zaba

University of Natural Resources and Life Sciences, Vienna

Polymersomes are stable self-assembled architectures which mimic cell membranes. For characterization, membrane proteins can be incorporated into such bio-mimetic membranes by reconstitution methods, leading to so-called proteopolymersomes. In this work, we demonstrate the direct incorporation of a membrane protein into polymersome membranes by a cell free expression system. Firstly, we demonstrate pore formation in the pre-formed polymersome membrane using α-hemolysin. Secondly, we use claudin-2, a protein involved in cell-cell interactions, to demonstrate the in vitro expression of a membrane protein into these polymersomes. Surface plasmon resonance (Biacore) binding studies with the claudin-2 proteopolymersomes and claudin-2 specific antibodies are performed to show the presence of the in vitro expressed protein in polymersome membranes.

The dynamics of protein membrane interactions

Rodolfo Briones, and Bert L. de Groot

Computational biomolecular dynamics group, Max Planck Institute for biophysical Chemistry, Göttingen, Germany

Membrane proteins rarely function as independent structural units but rather require a specific membrane environment to be functional. Which are the interactions between membrane proteins and the surrounding lipids that lead to this interdependence? Molecular dynamics simulations of the mitochondrial voltage gated anion channel VDAC1 and the water channel AQP0 will be presented that provide detailed information on both the membrane structure around the protein as well as global dynamics underlying conductance and selectivity. Key interactions between protein and lipids are identified that define lipid ordering around AQP0. For VDAC1, a global conformational transition towards elliptical structures has been characterized, of which the ion conductance and selectivity are commensurate with channel gating observed by electrophysiology. Finally, using the recently developed functional mode analysis, a collective conformational gating transition was identified for the yeast aquaporin AQY1, that allows the transmission of a pressure signal from the membrane to the protein water pore, that is not in contact with the membrane directly. Thereby, a possible mechanism for mechanosensitive gating has been identified.

Coarse-grained simulation of collective phenomena in membranes

Giovanni Marelli [(a)], Yuliya Smirnova [(a)], Marc Fuhrmans [(a)], Jelger Risselada [(b)], and Marcus Müller [(a)]

[(a)] Institut for Theoretical Physics, Georg-August University, 37077 Göttingen, Germany
[(b)] Theoretical Molecular Biophysics Group, Max-Planck-Institute for Biophysical Chemistry, 37077 Göttingen, Germany

Using coarse-grained models for lipid membranes and inclusions, mimicking peptides, we study the formation of pores and stalks and their interaction. The coarse-grained models and simulation techniques allow us to calculate the free-energy of stalks and analyze its dependence on the lipid architecture. Additionally, the membrane deformation and the pressure profile around peptides or in the vicinity of pores is investigated. The relevance of these structures to collective phenomena like fusion or spreading of bilayer membranes on substrates is discussed.

The human voltage-dependent anion channel: Structure, dynamics and membrane interactions

Markus Zweckstetter

Max Planck Institute for biophysical Chemistry, Göttingen, Germany

The voltage-dependent anion channel (VDAC), located in the outer mitochondrial membrane, acts as a gatekeeper for the entry and exit of mitochondrial metabolites. Here we reveal functional dynamics of isoform one of VDAC (VDAC1) by a combination of solution NMR spectroscopy, Gaussian network model analysis, and molecular dynamics simulation. Micro- to millisecond 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. Molecular dynamics simulations reveal that a charge on the membrane-facing glutamic acid 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 micro- to millisecond dynamics in solution. Because E73 is necessary for hexokinase-I-induced VDAC channel closure and inhibition of apoptosis, our results imply that micro- to millisecond dynamics in the N-terminal part of the barrel are essential for VDAC interaction and gating.

Tools for imaging and manipulating membrane biology

Carsten Schultz

European Molecular Biology Laboratory, Cell Biology and Biophysics Unit, Meyerhofstr. 1, 69117 Heidelberg, Germany. schultz@embl.de

The Schultz group develops tools for imaging and for modulating cellular enzyme activities in the context of intracellular signal transduction networks. Currently we are focusing on lipid-mediated signal transduction. By using total synthesis, we generated a set of membrane-permeant, bioactivatable (caged) lipid derivatives including diacylglycerol and phosphoinositide derivatives. These were used to study lipid diffusion and metabolism in living cells. We further applied phosphoinositide derivatives to investigate endocytosis and gained new insight in the function of these lipids in growth factor receptor signaling. Membrane-permeant photoactivatable phosphoinositide derivatives modulated endocytotic and membrane trafficking events with spatial and temporal control.
If time permits, the development of lipidated FRET reporters able to specifically monitor enzyme activity on membranes will be presented. These reporters may be used to very early monitor the onset of lung emphysema formation in COPD patients in the future.

Synaptotagmin-1 activity is modulated by cis- and trans- membrane interactions

Peter Jomo Walla

In neurotransmission synaptotagmin-1 tethers membranes via SNAREs and lipids and promotes quick SNARE-mediated fusion upon Ca^{2+}-triggering. However, recent fusion experiments indirectly suggested that this *trans* membrane tethering competes with *cis* interactions, in which synaptotagmin interacts with its own membrane and which potentially plays an important regulative role. Here we show with a membrane tethering assay which Ca^{2+}-, lipid-, and SNARE-binding structures of membrane-bound, full-length synaptotagmin are responsible for the competing interactions.

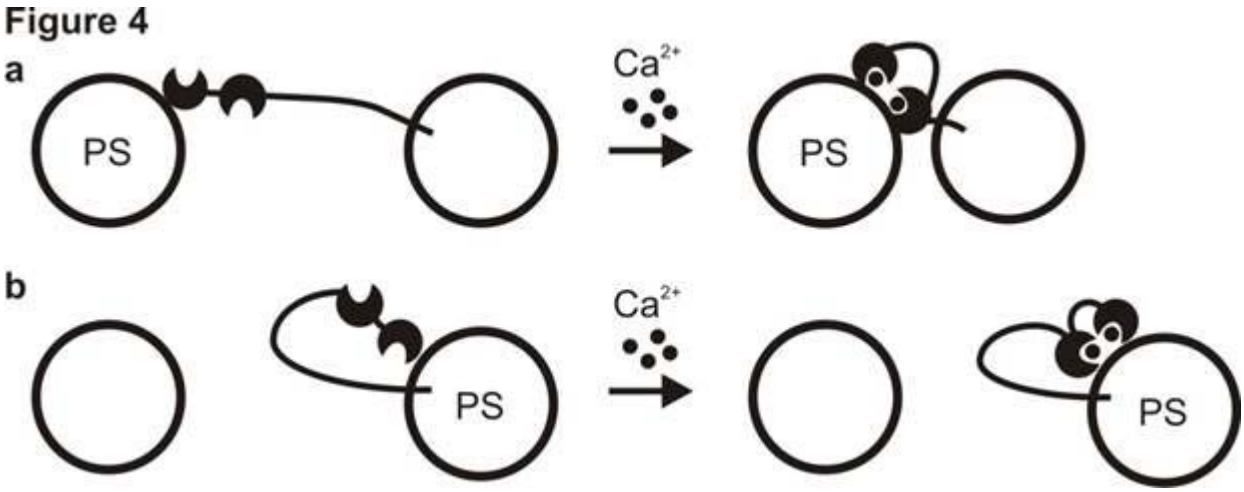


Figure. Membrane tethering by synaptotagmin-1. (**a**) Scheme of membrane tethering by *trans* interactions with anionic phosphatidylserine (PS). (**b**) Back-binding by *cis* interactions dominates over *trans* tethering, regardless of Ca^{2+}, PIP2 and/or SNAREs.

A. Cypionka , A. Stein , J. M. Hernandez , H. Hippchen , R. Jahn , P. J. Walla "Discrimination between docking and fusion of liposomes reconstituted with neuronal SNARE-proteins using FCS", PNAS, 106, 44, 18575-18580 (**2009**).

Forcing Membranes to Bend and Fuse

Gregory Bubnis, H. Jelger Risselada, Helmut Grubmüller

Max Planck Institute for Biophysical Chemistry
Department of Theoretical and Computational Biophysics

The energetics, kinetics, and atomistic details of protein regulated membrane processes such as tubulation and fusion are of great current interest. Molecular Dynamics (MD) simulations, however, provide limited insight to these processes due to diffusive, fluid lipid dynamics. To overcome this limitation, we are developing a scheme to prevent diffusion without altering the dynamics. For trajectory analysis, we can compute collective membrane motions (using e.g. PCA) and rigorously describe conformational transitions. Furthermore, during MD simulations we can enforce conformational constraints and compute free energies via umbrella sampling. Using this approach we have computed the free energy of a stalk-hemifusion-pore pathway for 20nm DOPC vesicles and found a 52 kBT barrier. In additon, we have also determined free energies and bending moduli of highly curved (~10nm^-1) membranes.

Posters

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Aeffner, S.	Stalk structures in lipid bilayer fusion studied by x-ray diffraction
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Briones, R.	Effect of gp41 HIV-1 peptides in model membranes by molecular dynamics simulations
Eggeling, C. **Honigmann, A.** **Müller, V.**	STED microscopy reveals nanoscopic details of membrane dynamics
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Gattin, Z.	Voltage-dependent anion channels under the ssNMR loupe
de Ghellinck, A.	Studies on reconstituted native lipid bilayer by neutron reflectometry
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Weiß,K.	Quantifying the diffusion of membrane proteins and peptides

Stalk structures in lipid bilayer fusion studied by x-ray diffraction

Sebastian Aeffner and Tim Salditt

Institut für Röntgenphysik, Georg-August-Universität Göttingen, Germany

Biological membrane fusion is a ubiquitous process in life. By now, it is well accepted and confirmed by simulations that it involves the formation of lipidic non-bilayer intermediates [1,2]. The first connection between two lipid bilayers about to merge is the so-called stalk. Due to its length scales of few nanometers, the only method providing sufficient resolution to study stalks experimentally is x-ray diffraction on the rhombohedral phase of phospholipids where stalks are arranged on a crystal lattice [3].
We use this technique to study stalks in different lipids and lipid mixtures with unprecedented resolution and propose a strategy to quantify the curvatures of lipid monolayers based solely on experimental data. In addition, we evaluate the powerful hydration forces at close membrane separation opposing stalk formation.
Our results indicate that the structure of stalks in different lipid systems is highly conserved, with subtle yet systematic changes upon variation of acyl chain length or cholesterol content. Addition of phosphatidylethanolamine (PE), in contrast, does not lead to observable changes. Within the framework of the continuum theory of membrane bending, we find that the energy contribution due to Gaussian curvature dominates the mean curvature term. In total, the free energy barrier for stalk formation is determined by hydration energy, which is considerably reduced by addition of cholesterol or PE.

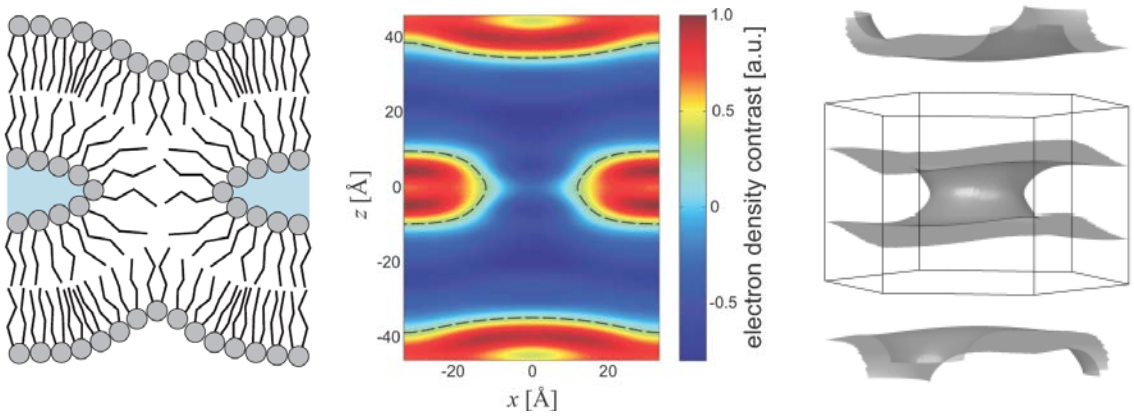


Figure 1: Sketch of a stalk (*left*), electron density map indicating a stalk in the rhombohedral phospholipid phase (DOPC/DOPE 1:1) (*center*) and corresponding electron density isosurface in *3d* used for evaluation of monolayer curvatures (*right*).

[1] L. Chernomordik, M.M. Kozlov, *Nature Struct. Mol. Biol*. **15** (2008) 675-683.
[2] S.J. Marrink, A.H. DeVries, D.P. Thieleman, *Biochim. Biophys. Acta* **1788** (2009) 149–168.
[3] L. Yang, H.W. Huang, Science **297** (2002) 1877-1879.

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

Theresa Kaufeld, Christopher Battle, Christoph F. Schmidt

Drittes Physikalisches Institut, Georg-August-Universität Göttingen

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 TRPP2 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 and optimized, which combines an epi-fluorescence microscope with an optical trap and also offers the possibility to perform electrophysiological experiments.

We have fabricated porous substrates suitable for simultaneous electrical recording and fluorescence microscopy to study ion channels. Lipid bilayer and substrate characteristics such as resistance and capacitance have been determined.

References:

Nauli et al. “Polycystins 1 and 2 mediate mechanosensation in the primary cilium of kidney cells” (2003) *Nature Genet*. **33**, 129–37.

Kartik Venkatachalam, Craig Montell “TRP Channels“ *Annu. Rev. Biochem.* (2007) **76**, 387-417.

S.J. Wilk, L. Petrossian, M. Goryll, T.J. Thornton, S.M. Goodnick, J.M. Tang, R.S. Eisenberg “Integrated electrodes on a silicon based ion channel measurement platform” (2007) *Biosensors and Bioelectronics* **23**,183-190.

Slavoj Kresák, Tibor Hianikb and Renate L. C. Naumann “Giga-seal solvent-free bilayer lipid membranes: from single nanopores to nanopore arrays” (2009) *Soft Matter* **5**, 4021-4032.

Effect of *gp41* HIV-1 peptides in Model Membranes by Molecular Dynamics Simulations

Rodolfo Briones[1], Anna Neumann[1,2] and Bert de Groot[1]

1. Max Planck Institute for Biophysical Chemistry, Göttingen, Germany. 2. Gdansk University of Technology, Gdansk, Poland.

gp41/32 are proteins from HIV-1/2 that help viruses to get the inside of the T4 white cells. From these proteins (~300 aas) the first 12-23 aas are known as the Fusion Peptide (FP)[1]. This peptides penetrates and destabilize membranes in a similar way than amiloyd peptides (Alzheimer) and some antimicrobial peptides (alamethicin, mellitin). In this work we studied by molecular dynamics the effect of the first 12 aas of gp41 (HIV-1 BRU strain) on DMPC membrane patches using the Gromos96-43a1 force field. Peptide(s) in α-helical (1ERF) and extended conformations were placed in water and pre-inserted in the membrane as starting configurations. In the μs timescale of the simulations, we observe alpha to extended interconversion of these peptides in the membrane. We also observe local and global changes in the lipid properties that could be related with the peptide's capability of destabilize biological membranes.

1. Charloteaux et al. J. Mol. Biol. (2006) 3559, 597-609.

STED microscopy reveals nanoscopic details of membrane dynamics

Veronika Mueller, Alf Honigmann, Stefan W. Hell and Christian Eggeling

Max Planck Institute for Biophysical Chemistry, Department of Nanobiophotonics, Am Fassberg 11, 37077 Göttingen, Germany

Cholesterol-assisted lipid interactions such as the integration into lipid nanodomains are considered to play a functional part in a whole range of membrane-associated processes, but their direct and non-invasive observation in living cells is impeded by the resolution limit of >200nm of a conventional far-field optical microscope. We report the detection of single diffusing lipid molecules in nanosized areas in the plasma membrane of living cells using the superior spatial resolution of stimulated emission depletion (STED) far-field nanoscopy. By combining a (tunable) resolution of down to 30 nm with tools such as fluorescence correlation spectroscopy (FCS), we obtain new details of molecular membrane dynamics. Sphingolipids or other proteins are transiently (~ 10 ms) trapped on the nanoscale in often cholesterol-mediated molecular complexes [1]. Distinct differences show up between different lipids and molecules. The novel observations may shed new light on the role of lipid-protein interactions for membrane bioactivity.

[1] Eggeling et al., Nature 2009: 457, 1159-1163.

AFM and laser trapping Measurements of Lipid Bilayer Mechanics

Iwan A.T. Schaap and Frederic EGHIAIAN

III Physikalisches Institut, 37077 Göttingen

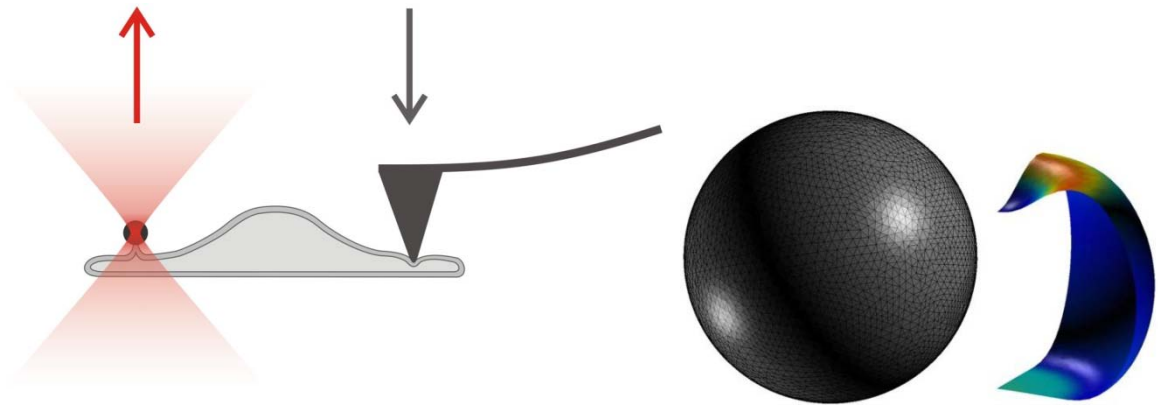
We perform force spectroscopy measurements on viruses and cells in order to understand the mechanical role of the lipid bilayer in their development and persistence. Our systems are:

1) Liposomes: AFM nano-indentation experiments and finite element analysis of small unilamellar vesicles allow us quantifying the elasticity and rupture limits of lipid bilayers with biological compositions, and the temperature-dependency of their behaviour.

2) Myelin cells: We use a vertical laser trap to measure the membrane tension via tether extraction experiments. We investigate how cell differentiation alters the mechanics of the membrane during the formation of myelin sheets from cell precursors.

3) Enveloped viruses: We investigate the effects of viral spike proteins (which are membrane-embedded) and the matrix protein on the rigidity and curvature of the envelope of influenza virus. Our findings may have implications in viral budding and persistence.

4) Fluorescent rotors: We are developing the application of a rotor with a viscosity dependent fluorescent life-time to measure the viscosity and bending rigidity of lipid bilayers using optical microscopy.



Vesicles at Surfaces – Adsorption, Rupture and Spreading

Marcus M*ü*ller, and Marc Fuhrmans

Universität Göttingen,Institut für Theoretische Physik

Upon adsorption onto sufficiently attractive surfaces, otherwise stable lipid vesicles can deform, rupture, fuse with neighbouring vesicles and spread on the surface to form continuous bilayers. While this process is used in a number of technical applications like, e.g., the creation of supported bilayers, the exact pathway taken, as well as the interplay between the different stages, is not understood very well. We use dissociative particle dynamics (DPD) simulations to study systems of one or two lipid vesicles subjected to a 9-3 Lennard-Jones-potential representing the attractive surface. To cover the relatively large system sizes and time scales necessary to observe these collective phenomena and still generate sufficient statistics, we employ a solvent-free coarse-grained model.

Voltage-dependent anion channels under the ssNMR loupe

Adam Lange, and Zrinka Gattin

Max Planck Institute for Biophysical Chemistry, Göttingen, Germany

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 1976[1]. To date, multiple VDAC isoforms (VDAC1, VDAC2, and VDAC3) have been identified in a variety of organisms, including yeast, plants, mouse, and humans. The structural information about VDAC1 (mouse and human) is known only from 2008[2-4] which revealed a novel 19-stranded β-barrel architecture with an N-terminal α-helix.

The importance of the N-terminal part of VDAC1 was already demonstrated with N-terminal truncation mutants where it was found that the truncation mutants exhibit lower conductance than the full-length channel[5]. This result was taken as an indication that the N-terminal helix may not lie inside the pore, but forms part of the barrel wall.

In our group we have determined the native conformation of the N-terminal part of human VDAC1 in liposomes by solid state NMR[6], which was difficult to be observed by solution-state NMR[2-4]. We found out that the stabilizing role of the N-terminus on the β-barrel depends crucially on a hydrophobic contact involving leucine-10 from the N-terminus and valine-143 from β-strand 9. Further investigations of the order parameter underlined the rigid nature of the N-terminal helix.

Another point mutation, the mutation of the glutamate-73 in β-strand 4 to either glutamine or valine, was shown to have a different, more stabilizing effect on the β-barrel[7].

In our present study we continue to investigate from the solid state NMR view, the influence of two point mutations L10N and E73V, separately as well as one double mutation, on the β-barrel (de)stabilization.

1. Schein, S. J.; Colombini, M.; Finkelstein, A. J Membr Biol 1976, **30**, 99–120.

2. Ujwal, R.; Cascio, D.; Colletier, J.-P.; Faham, S.; Zhang, J.; Toro, L.; Ping, P.; Abramson, J. PNAS 2008, **105**, 17742–17747.

3. Hiller, S.; Garces, R. G.; Malia, T. J.; Orekhov, V. Y.; Colombini, M.; Wagner, G. Science 2008, **321**, 1206-1210.

4. Bayrhuber, M.; Meins, T.; Habeck, M.; Becker, S.; Giller, K.; Villinger, S.; Vonrhein, C.; Griesinger, C.; Zweckstetter, M.; Zeth, K. PNAS 2008, **105**, 15370-15375.

5. De Pinto, V.; Reina, S.; Guarino, F.; Messina, A. J. Bioenerg. Biomembr. 2008, **40**, 139-147.

6. Schneider, R.; Etzkorn, M.; Giller, K.; Daebel, V.; Eisfeld, J.; Zweckstetter, M.; Griesinger, C.; Becker, S.; Lange, A. Angew. Chem. Int. Ed. 2010, **49**, 1882-1885

7. Villinger, S.; Briones, R.; Giller, K.; Zachariae, U.; Lange, A.; de Groot, B. L.; Griesinger, C.; Becker, S.; Zweckstetter, M. PNAS 2010, **107**, 22546-22551

Studies on reconstituted native lipid bilayer by neutron reflectometry

Giovanna Fragneto [1], Michele Sferrazza [2], Hanna Wacklin [3] and Alexis de Ghellinck[1,2]

[1] Institut Laue Langevin, Grenoble, France
[2] Université Libre de Bruxelles, Brussels, Belgium
[3] European Spallation Source, Lund, Sweden

Neutron reflectometry is a powerful technique which allows getting information along the normal of an interface. It is particularly adapted for the study of lipid bilayers since neutrons do not destruct soft bio-material, the wavelength of cold neutrons correspond to the Ångström-nanometer scale and allow isotopical labeling via selective deuteration. Indeed, neutron scattering in matter is dependent of the nuclei and not of electron density as X-ray. The difference of scattering length between hydrogen and deuterium being especially high, it is possible to highlight some parts of the membrane by substituting hydrogen by deuterium without affecting the physic of the system.

However, researchers studying lipid bilayer by means of neutron scattering use generally model membrane composed from one to three synthetic lipids, while biological membrane are constituted of several different molecules such as glycerolipids, sphingolipids, sterols and membrane proteins. This is partly because it is very hard to get enough amount of various deuterated biomaterial. In this study, we plan to investigate the structure of reconstituted native lipid bilayer, either hydrogenated or deuterated, by neutron reflectometry. To do so, yeast cells are grown in hydrogenated and deuterated media. Lipids are then extracted and deposited on a silicon substrate to be analysed by neutron reflection. Moreover, we will use amphotericin B as a pilot. Amphotericin B is a famous antibiotic used against fungal infections. It is thought its activity results from the interaction with ergosterol, the main lipid present in fungal membrane. However, it seems that at higher concentration, amphotericin B could react with phospholipid as well, suggesting there are more than one way of interaction. We will investigate the reaction of the membrane with amphotericin B by reconstituting different lipid bilayers which contain or not ergosterol. This will be a first attempt to determine how the complexity affects the function of the membrane.

The fusion of vesicles with pore-spanning membranes mediated by peptide nucleic acid recognition motifs

Ines Höfer, Antonina S. Lygina, Ulf Diederichsen and Claudia Steinem

Georg-August-University Göttingen, Institute of Organic and Biomolecular Chemistry, Tammannstr. 2, 37077 Göttingen, Germany

In an effort to gain new insights into protein-mediated fusion processes we established a new vesicle/planar membrane fusion assay. With our setup we want to overcome the drawbacks of other membrane model systems such as black lipid membranes or solid supported membranes.
The fusion of large unilamellar vesicles (600 nm in diameter) with pore-spanning membranes allows for the investigation of single fusion events by means of fluorescence microscopy. Vesicles are doped with Texas Red DHPE, while solvent-free pore-spanning membranes are prepared by spreading giant unilamellar vesicles doped with Oregon Green DHPE. Lipid mixing can be verified by the occurrence of Förster Resonance Energy Transfer (FRET).
Fusion was mediated by hybridization of complementary peptide nucleic acid (PNA)-single strands.[1] The PNA recognition motif was coupled to the native SNARE-transmembrane/linker domain which was incorporated into the lipid bilayer. The insertion of PNA-sequences resulted in very efficient fusion. The PNA sequences, identical in both membranes or complementary in vesicle and pore-spanning membrane, barely influenced the fusion efficiency. Destabilization of the membrane by the transmembrane anchor appears to be in this case the key to cause fusion whereas the molecular recognition plays a minor role.

1. Lygina, A. S.; Meyenberg, K.; Jahn, R.; Diederichsen, U., Transmembrane Domain Peptide/Peptide Nucleic Acid Hybrid as a Model of a SNARE Protein in Vesicle Fusion. *Angew Chem Int Ed Engl*, DOI: 10.1002/anie.201101951.

Automatic Jump Detection in Ion Channel Recordings

Thomas Hotz[1], Hannes Sieling[1], Ingo Mey[2], Ole Schütte[2], Claudia Steinem[2], Axel Munk[1]

[1]Institute for Mathematical Stochastics, Georgia Augusta University of Goettingen
[2]Institute of Organic and Biomolecular Chemistry, Georgia Augusta University of Goettingen

Ion channel recordings can ideally be thought of as piecewise-constant signals plus white noise; in practice, they are however only observed after a low-pass filter has been applied. We report on a new method to reconstruct the block signal: based on a multi-scale test statistic, we compute the signal with the minimal number of jumps such that the hypothesis of the residuals being noise cannot be rejected. We demonstrate the usefulness of this approach on simulated and real data. Furthermore, a custom-built software for ion channel recordings will be shown.

Robust Designs for Choosing Indirect Times in NMR Spectroscopy

Thomas Hotz[1], Fabian Telschow[1], Saskia Villinger[2], Sheng Qi Xiang[2], Markus Zweckstetter[2], Christian Griesinger[2], Axel Munk[1]

[1]Institute for Mathematical Stochastics, Georgia Augusta University of Goettingen
[2]NMR based Structural Biology, Max Planck Institute for Biophysical Chemistry

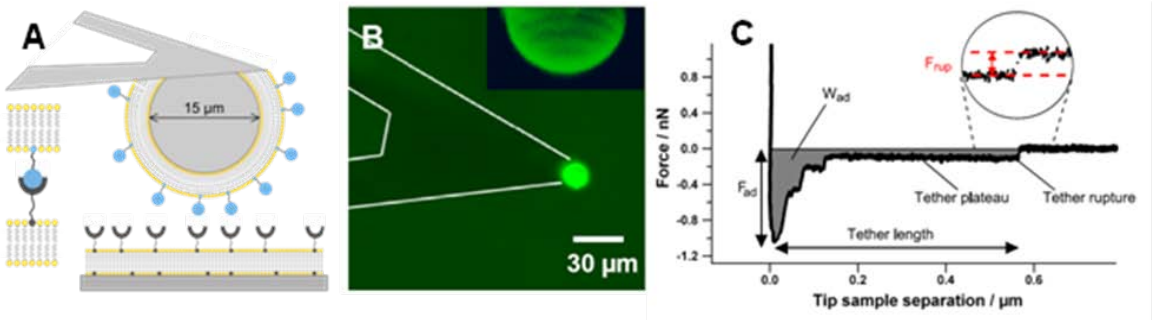
In two dimensional Nuclear magnetic resonance (NMR) spectroscopy, e.g. NOESY (NOE spectroscopy), each indirect time domain corresponds to measuring one free induction decay. One therefore wants to choose the indirect times, i.e. the design points, in an optimal way to obtain the best parameter estimates for the signal components' amplitudes and phases with as few measurements as possible. Assuming the spectrum's frequencies and decay parameters to be known, the data are well described by a linear model in the unknown (complex) amplitudes. One could now determine the optimal design by minimising the estimators' variance. We discuss the drawbacks of such designs and show how to compute the worst-case error if one includes an unknown bias that models systematic deviations, e.g. because the decay is not perfectly known. Using a tailor-made reproducing kernel Hilbert space over the positive half-line for the biases, we show how optimal designs can be computed.

Force spectroscopy of membrane-membrane interactions

G. Pähler*, M. Oelkers*, E. Sunnick[*], D. Yuksel[**], C. Brand*, E. Kriemen[***], D. Werz[***], K. Kumar[**], B. Geil[*], A. Janshoff[*], and B. Lorenz[*]**

[*] Institute of Physical Chemistry, Goettingen University
[**] Chemistry Department, Tufts University, Boston, MA
*** Institute of Organic and Biomolecular Chemistry, Goettingen University

We present here a new technique to measure interactions between lipid membranes in order to mimic cellular processes involving membrane-membrane contact. By extracting interaction forces and surface adhesion energy from our measurements, we are able to comment on the impact of membrane functionalization in the context of cell-cell contact formation. The following setup was used in our experiments:



A: Experimental setup, B: Fluorescently labeled membrane on colloidal probe, C: Force-distance curve producing deflection of cantilever upon interaction between membranes

A colloidal probe cantilever was coated with a ligand-functionalized lipid membrane (A, B) and brought into contact with a second membrane displaying the corresponding receptor molecule. Subsequently, the membranes were pulled apart and the strength of interaction between the membranes was measured (C). The advantage of our experimental approach is its versatility. We are able to vary receptor concentration, fluidity of the lipid membranes, buffer conditions, membrane contact time as well as force rates acting on the bonds. Consequently, we can access the number and off-rates of bonds established during membrane contact. We can also estimate the strength of single bonds. In a first setup, we used a homophilic sulphated disaccharide interaction to mimic the glycocalyx of marine sponges. In a second system, we aimed to mimic the docking within a eukaryotic membrane fusion process by coupling peptides to lipid bilayers that work as minimal fusion machines in a heterophilic coiled-coil interaction.

Minimalistic model of lipid membranes and hydrophobic inclusions: the effect of oil in the stalk.

Giovanni Marelli, Jelger Risselada, Yuliya Smirnova and Marcus Müller

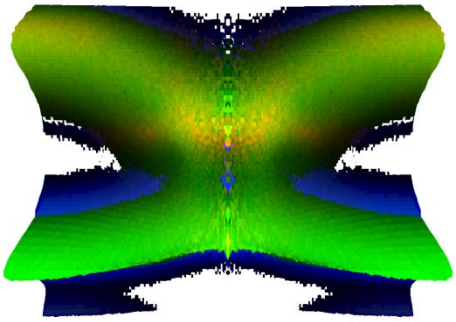
Institut für theoretische Physik, Georg August Universität Göttingen

A stalk between two opposed membranes modifies the conformation of the local lipids and changes the mechanical properties of the membrane in its surroundings.

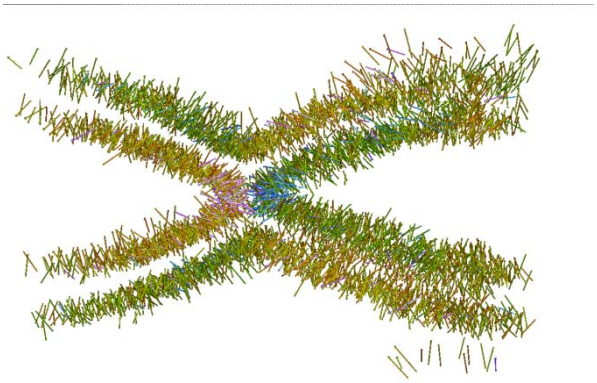
There are different topologies and shapes of stalks depending on the lipid composition of the membranes. For instance between two membranes in the lamellar phase (PC) the stalk is metastable and has a circular shape, between two membranes in the inverted hexagonal phase (PE) the stalk is stable and elongates spanning all the length of the simulation box.

In our minimal coarse-grained model of the lipids, which only accounts for the amphiphilicity of the molecules, changing the length of the hydrophilic heads we can describe both an elongated stalk (PE membranes) or a circular one (PC membranes) and calculate averaged profiles exploiting the symmetry of the system, radial and normal (r,z) for circular stalks and linear and normal (x,z) for elongated ones, respectively.

For the different stalks we calculate the thickness, density and tension profile and we investigate how the presence of small chains (either hydrophilic or amphihpilic) can relax the tension at the ends of the stalk and change its shape.



Side view of a stalk between two opposed PE membranes. The chains are represented as vectors and the pink/light blue colors represent the vectors between 30 and 60 degrees.



Radial density profile around a circular stalk. The upper bilayer is enriched in small hydrophobic chains. In green is represented the density of hydrophobic beads, in blue the hydrophilic and in red the small oil chains which fill the upper end of the stalk which is more depleted in lipids.

Investigation of Synaptotagmin 1 Using Microscale Thermophoresis

Karsten Meyenberg[a], Geert van den Bogaart[b], Reinhard Jahn[b] and Ulf Diederichsen*[a]

[a] Institut für Organische und Biomolekulare Chemie, Georg-August-Universität Göttingen, Tammannstr. 2, 37077 Göttingen

[b] Max-Planck-Institut für biophysikalische Chemie, Am Faßberg 11, 37077 Göttingen

Synaptotagmin 1 is a synaptic vesicle protein mainly serving as a calcium sensor and thereby triggering rapid neurotransmitter release. It is composed of a single transmembrane domain followed by a large cytoplasmic domain consisting of a 61-residue unstructured linker and tandem C2-type domains, called C2A and C2B. These domains bind two and three calcium ions, respectively (affinity from 60 µM – 1 mM) and interact with anionic lipids and SNARE proteins.[1]

Microscale Thermophoresis (MST) was introduced as a new technique to determine binding constants of biomolecules.[2] We employed MST to measure the intrinsic calcium binding affinities of the calcium binding synaptotagmin C2AB domain and found it to be in a very good agreement with Isothermal Titration Calorimetry (ITC) experiments.[3] The experiments were performed with and without a fluorescent label using a NT-647 fluorophor and the proteins intrinsic tryptophan fluorescence, respectively. As a negative control experiment for both, no binding to magnesium ions was observed.[4] We were able to show that MST allows following the binding of ions to proteins by measuring the change in thermophoresis of the comparably much larger protein.

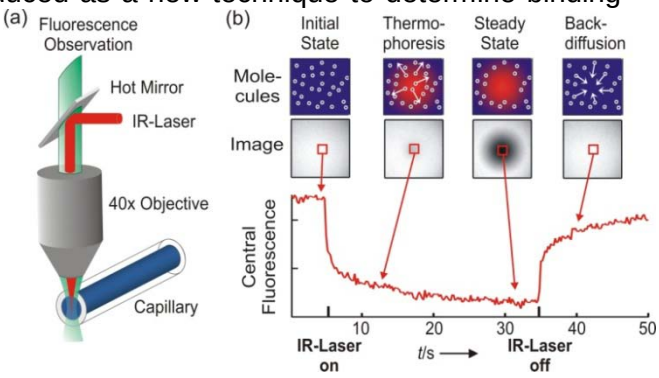


Figure 1 a) Experimental setup for a MST measurement. b) Typical signal response from MST experiments.[2]

Furthermore, we developed a new assay to distinguish between free floating and so-called docked vesicles based on MST experiments.[4] Therefore, lipid vesicles with different thermophoretic behaviors were employed to dissect docked and undocked vesicles. A clear change in the MST signal was observed when synaptotagmin 1 bearing vesicles were added to vesicles without protein indicating the tethering of vesicles. This effect turned out to be strong for wild-type synaptotagmin 1 and weak for modified synaptotagmin and vesicles without posphatidylinositol 4,5-bisphosphate, respectively.

In summary, Microscale Thermophoresis is a well-suited method for revealing binding events to proteins, both for small ions and large vesicles. In further MST experiments we will focus on the determination of active binding sites on vesicle surfaces.

[1] E. R. Chapman, Annu. Rev. Biochem. 2008, 77, 615-641.
[2] P. Baaske, C. J. Wienken, P. Reineck, S. Duhr and D. Braun, Angew. Chem. Int. Ed. 2010, 49, 1-5.
[3] A. Radhakrishnan, A. Stein, R. Jahn and D. Fasshauer, J.Biol.Chem. 2009, 284, 25749–25760.
[4] K. Meyenberg, G. van den Bogaart, Applikation Note Nanotemper Technologies GmbH, München.
[5] G. van den Bogaart, S. Thutupalli, J. H. Risselada, K. Meyenberg, M. Holt, D. Riedel, U. Diederichsen, S. Herminghaus, H. Grubmüller, R. Jahn, Nat. Struct. Mol. Biol. 2011, 18, 805-812.

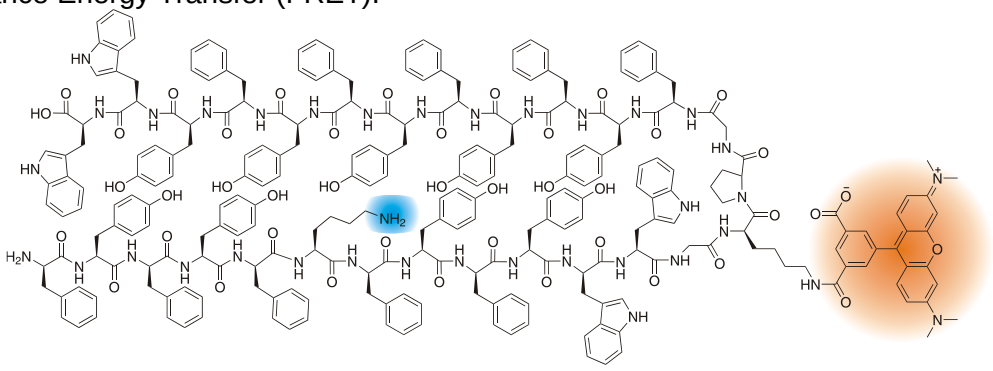
Synthesis of Artificial Membrane Peptides: Recognition and Assembly within the Membrane

Stefan Müllar[a], Ulrike Rost[a], Anna V. Lübben[a], Ulf Diederichsen[a]

[a]*Georg-August-Univerität Göttingen, Institut für Organische und Biomolekulare Chemie, Tammannstraße 2, 37077 Göttingen*

email: smuella@gwdg.de

Recognition between membrane anchored protein species is crucial for a variety of cellular processes like transport, signal transduction, posttranslational modification and membrane insertion of nascent proteins. While some membrane proteins function as monomers others need to assemble into oligomeric structures to carry out their biological function.[1] Based on the structural requirements of a recently reported homodimeric peptide pore motif for membrane insertion, novel D,L-alternating double helical hairpins were designed in structural analogy to the natural antibiotic gramicidin A.[2] As reported previously, a dimerization of these hairpin peptide/PNA conjugates could be observed and proven to be triggerable by the applied temperature.[3] Our research focuses on the induced aggregation of artificial transmembrane domains (TMDs) within lipid bilayer complexes mediated by recognition of nucleobase pairing or electrostatic interaction of charged amino acids. Therefore, nucleobases or lysine/glutamic acid residues, respectively, were introduced in the center of the homodimer/hairpin-TMD. Fluorophores were indroduced applying an orthogonal protecting group strategy. The functionalized TMDs were reconstituted in large unilamellar vesicles (LUVs). The in-membrane pore formation by adopting $\beta^{5.6}$-double helices will be investigated using CD spectroscopy. Further investigation will be performed by using the fluorescence probes to determine the dynamic aggregation process via Fluorescence Resonance Energy Transfer (FRET).



[1] Z. W. Hall, *Trends Cell. Biol.* **1992**, *2*, 66-68.

[2] a) E. Alexopoulos, A. Küsel, G. M. Sheldrick, U. Diederichsen, I. Usón, *Acta Cryst.* **2004**, *D*60, 1971-1980. b) A. Küsel, Z. Khattari, P. E. Schneggeburger, A. Banerjee, T. Salditt, U. Diederichsen, *ChemPhysChem* **2007**, *8*, 2336-2343.

[3] P. E. Schneggenburger, S. Müllar, B. Worbs, C. Steinem, U. Diederichsen, *J. Am. Chem. Soc*., **2010**, *132*, 8020-8028.

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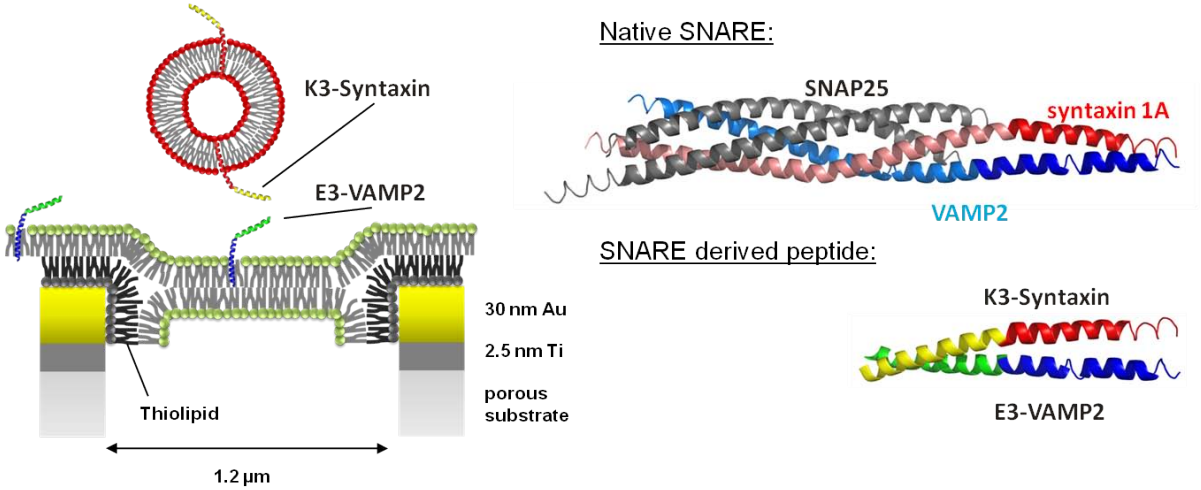
Single membrane fusion events mediated by SNARE-derived peptides observed on pore-suspending membranes

Henrik Neubacher, Lando Schwenen, Ines Höfer, Karsten Meyenberg, Ulf Diederichsen and Claudia Steinem

Georg-August-Universität Göttingen, Institute of Organic and Biomolecular Chemistry, Tammannstr. 2, 37077 Göttingen, Germany

We developed a new versatile single-vesicle fusion assay to study the molecular mechanisms of membrane fusion, based on pore-suspending membranes. This system offers advantages over other model membrane systems, such as black lipid membranes (BLMs) or solid supported membranes (SSMs). Specifically, it provides a solvent-free environment with aqueous compartments that are accessible on both sides of the membrane. With this assay, we were able to observe single fusion events of large unilamellar vesicles (LUVs) with pore-suspending membranes using confocal laser scanning fluorescence microscopy. Membranes were prepared by spreading of giant unilamellar vesicles (GUVs) on highly ordered porous silicon substrates. GUVs were doped with the fluorescent dye Oregon Green DHPE, LUVs with Texas Red DHPE. Membrane fusion could be observed through Förster resonance energy transfer that occurs if the two lipid dyes come in close proximity as a result of lipid mixing. Membrane fusion was mediated using SNARE-derived artificial peptides, consisting of a coiled-coil-forming three-heptad repeat segment that is linked to a SNARE-transmembrane domain anchoring it to the lipid membrane.

Currently, a setup is being developed to simultaneously monitor the lipid mixing and content release that occurs upon fusion. One aim is to entrap the content of fused vesicles in pores that are closed at the bottom. This content release is detected by fluorescence microscopy. To achieve this, we use nanoporous anodic alumina substrates. Their material properties combine closed pores and optical transparency.



Reconstitution of large dense-core vesicle (LDCV) fusion in vitro: electrostatic effect of ATP is required for Ca2+-dependent fusion

Yongsoo Park, Javier Matias Hernandez, Geert van den Bogaart, Saheeb Ahmed, Dietmar Riedel, and Reinhard Jahn

Department of Neurobiology, Max-Planck-Institute for Biophysical Chemistry, Am Fassberg 11, D-37077 Göttingen, Germany

SNARE (soluble N-ethylmaleimide-sensitive factor attachment protein receptor) proteins are the core machinery for vesicle fusion. However, reconstitution of large dense-core vesicle (LDCV) fusion in a SNARE-dependent manner has never been proposed. Reconstitution system using purified native vesicles provides the advantage to investigate the direct molecular mechanisms by which vesicle fusion is mediated. Here we show the complete reconstitution of LDCV fusion in vitro; i) LDCV fusion is SNARE-dependent, ii) Ca2+ increases LDCV fusion, and iii) PI(4,5)P2 (phosphatidylinositol-4,5-bisphosphate) is essential for Ca2+-induced LDCV fusion. Surprisingly, the electrostatic effect of ATP is required for Ca2+-induced vesicle fusion by reversing the restriction of synaptobrevin and synaptotagmin-1. This report provides the first evidence that Ca2+-triggered vesicle fusion process is dependent on ATP not by the enzymatic activity, but by the electrostatic effect.

Synthesis of modified gramicidin A derivatives (gA) for analysis of membrane-peptide-interactions

Tatjana Polupanow[1], **Peter Pohl**[2], **Guillem Portella**[3], **Ole Schütte**[1], **Bert L. de Groot**[4], **Claudia Steinem**[1] **and Ulf Diederichsen**[1]

[1] Institut für Organische und Biomolekulare Chemie, Georg-August-Universität Göttingen, Tammannstr. 2,
D-37077 Göttingen, Germany
[2] Institut für Biophysik, Altenbergerstr. 69, A-4040 Linz, Austria
[3] Institute for Research in Biomedicine, Baldiri Reixac 10-12, E-08028 Barcelona, Spain
[4] Max-Planck-Institut für Biophysikalische Chemie, Am Faßberg 11, D-37077 Göttingen, Germany

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 $\beta^{6.3}$ - helical dimer and a double-helical $\beta^{5.6}$ - structure.[2] These structures demonstrate single-file transfer through channels. However, recent molecular dynamics simulations clearly identified lipid head groups as deterrents of the water flux due to their ability to block the channel entrance (Figure 1 A).[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 ion and water permeability. In order to counteract and to regulate the stability and function gA-derivatives with covalently attached fatty acids were synthesized. Water flux analyses demonstrate an increase of water permeation of acylgramicidin in comparison to the native gA (Figure 1 B). Single channel measurements show channel characteristics of acylgramicidin (Figure 1 C, upper and zero level correspond to the open and closed state of the channel). Circular dichroism experiments of acylgramicidin show the $\beta^{6.3}$- helical conformation, the preferred conformation upon incorporation in lipid bilayers of gramicidin. Furthermore, BODIPY-labeled gA-derivatives were synthesized for FRAP measurements to reveal additional structural and functional information of acylgramicidin within a lipid bilayer.

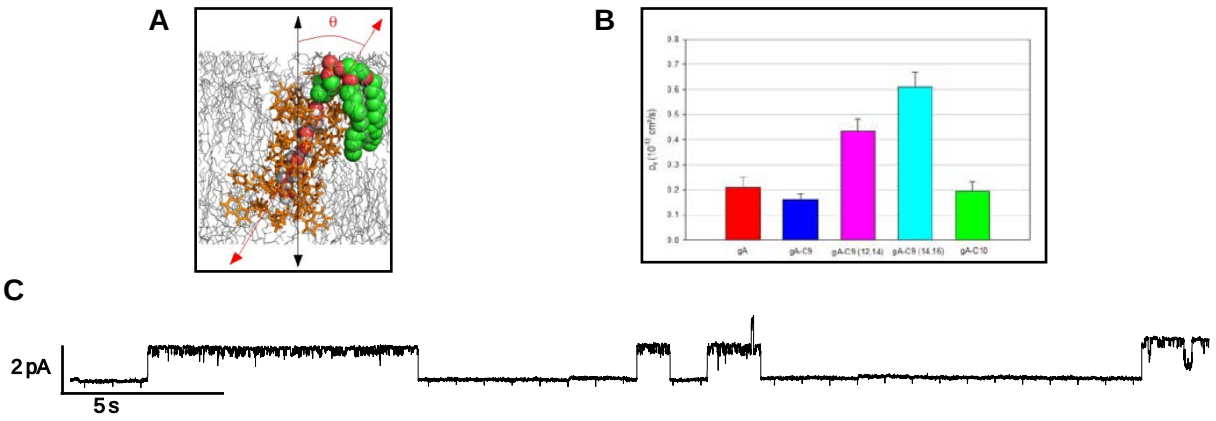


Figure 1. **A** MD - simulations. Tilt of the channel Θ: 14.3 ± 0.1°. Head groups of lipid molecules disturb ion and water permeability. **B** Water flux and **C** single channel measurements on gA-derivatives with covalently attached fatty acids.

References

1. B. A. Wallace, *Annu. Rev. Biophys. Biophys. Chem.* **1990**, *19*, 127-157.
2. B. L. de Groot, D. P. Tieleman, P. Pohl, H. Grubmüller, *Biophys. J.* **2002**, *82*, 2934-2942.
3. G. Portella, *Determinants of water and ion permeation through nanopores studied by Molecular Dynamics simulations*, Dissertation, Göttingen **2008**.

Glycosphingolipids to Investigate Domain Formation

Lukas J. Patalag, Waldemar Preis, Claudia Steinem, Daniel B. Werz, Annika Ries and Alexander Orth

Institute of Organic and Biomolecular Chemistry, Georg August University of Göttingen,

Tammannstr. 2, D-37077 Göttingen, Germany, E-mail: aorth@gwdg.de, aholken@gwdg.de

Glycosphingolipids can be found in all eukaryotic cell membranes, but mainly in the plasma membrane. They are known to form clusters, so called membrane domains, which can move within the fluid lipid 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 study the influence of different structural motifs on domain formation in artificial lipid bi-layers and their consequences for protein binding.

Another interesting focus is the investigation of new fluorescent tags which are closer to the native structure of glycosphingolipids. Up to now fluorescence spectroscopy in order to study domain formation needs bulky fluorescent tags like NBD and BODIPY, which change the chemical and physical properties of glycosphingolipids dramatically and makes the observation of lipid rafts quite difficult. Conjugated polyene lipids show a strong similarity to natural lipids and were already used as tags by *Kueschner et al.*[2] for studying membrane phase partitioning. Such fatty acid precursors would be a good possibility to install a fluorophore in the glycosphingolipid backbone.

Our attempt is to build up different sets of glycosphingolipids, where either the carbohydrate part or the fatty acid part differs. Different chain lengths, the grade of saturation and α-hydroxylation of the fatty acids are considered. As D-*erythro*-(2*S*,3*R*)-sphingosine[3] is the main sphingoid base in mammalian tissue, no structural change in this building block is envisaged. After successful synthesis these glycosphingo-lipids will be studied in artificial lipid bilayer and monolayer systems using biophysical methods like the Langmuir trough as well as fluorescence and atomic force microscopy.

References

1. A. Wedeking, G. van Echten-Deckert, *Curr. Org. Chem.* **2007**, *11*, 579-589.
2. L. Kuerschner, C. S. Ejsing, K. Ekroos, A. Shevchenko, K. I. Anderson, C. Thiele, *Nat. Methods* **2005**, *2*, 39-45.
3. P. M. Koskinen, A. M. P. Koskinen, *Synthesis* **1998**, *8*, 1075-1091.

Synthesis of *O*-acylated 3-hydroxyleucine derivatives for the preparation of muraymycin nucleoside lipopeptide antibiotics

Anne Ochmann[1], Markus Granitzka[2], Christian Ducho[1] and Oliver Ries[1]*

Georg-August-University Göttingen, Department of Chemistry, [1]Institute of Organic and Biomolecular Chemistry, [2]Institute of Inorganic Chemistry, Tammannstr. 2-4, 37077 Göttingen, *ories@gwdg.de

In 2002, *McDonald et al.* discovered nineteen structurally related naturally occurring nucleoside lipopeptide 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 (*e.g.* muraymycin A1 **1**, Figure 1). Structural diversity is achieved through the absence or presence of an aminoribose unit attached to the uronic acid moiety and a differentially *O*-lipidated (2*S*,3*S*)-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 **2** as starting material which can be stereoselectively converted into key intermediate **3** and then finally into *O*-acylated 3-hydroxyleucine derivatives **4** containing different lipid moieties.

*Figure 1. Structure of muraymycin A1 **1** and synthesis of O-acylated 3-hydroxyleucine derivatives*

Furthermore, the synthesis of *N*-alkyl-*N*-hydroxyguanidines for the preparation of the lipid side chain of the biologically most potent muraymycin A1 **1** is currently investigated [2]. Previously reported syntheses were carried out in aqueous media and are therefore not applicable for the derivatisation of lipophilic compounds [3-5]. Thus it is important to establish an efficient synthesis which can be performed in organic solvents. Recent results from these investigations will also be presented.

References

[1] McDonald *et al.*, *J. Am. Chem. Soc.* **2002**, *124*, 10260-10261.
[2] Ries, O., Ochmann, A., Ducho, C., *Synthesis* **2011**, 2357-2368.
[3] Heesing, A., Peppmöller, R., *Z. Naturforsch.* **1967**, *22*, 820-824.
[4] Maehr, H., Leach, M., *J. Org. Chem.* **1974**, *39*, 1166-1168.
[5] Widmer, J., Keller-Schierlein, W., *Helv. Chim. Acta* **1974**, *57*, 657-664.

Simulating the backbone dynamics of transmembrane helices

Christina Scharnagl [1,2] , and Dieter Langosch [2]

Technische Universität München
[1] Fakultät für Physik, [2] Wissenschaftzentrum Weihenstephan

In an effort to systematically study the relation between backbone flexibility and amino acid sequence of transmembrane domains (TMDs) we analyze the conformational dynamics of alpha-helical model peptides by molecular dynamic (MD) simulations. Our peptides represent either the TMDs of fusion proteins or the TMD of the amyloid precursor protein (APP-TMD). Their backbone dynamics has been characterized experimentally by deuterium/hydrogen-exchange (DHX) and the functional relevance of dynamics has been investigated.

The simulation results show that local deficiencies of side-chain packing, e.g. due to restricted side-chain mobility within the context of an alpha-helix, enhance backbone dynamics in a sequence-specific way.

Local DHX rates are calculated from the occupancies of intra-helical hydrogen-bonds und describe the DHX-kinetics in good agreement with the experiment. This validates the simulations which reveal different site-specific dynamical features.

Furthermore, we compare the peptide dynamics in isotropic solvent mimicking an apolar helix-stabilizing surrounding (80% TFE v/v, used for the DHX experiments) and an explicit membrane environment (DOPC:DOPE:DOPS 3:1:1). The impact of the membrane on helix dynamics will be discussed.

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

Astrid Sitte[1], Piotr Neumann[2], Annett Weidner[3], Danilo Meyer[1], Kai Tittmann[1]

[1] Albrecht-von-Haller-Institute, Georg-August University Göttingen
[2] Institute for Microbiology and Genetics, Georg-August University Göttingen
[3] Institute of Biochemistry and Biotechnology, Martin-Luther University Halle-Wittenberg

Pyruvate oxidase from *E. coli* (*Ec*POX) is a thiamine diphosphate- and flavin-dependent enzyme that catalyses the oxidative decarboxylation of pyruvate into acetate and CO_2. The two reducing equivalents of this redox reaction are initially transferred to the flavin cofactor, leading to the exposure of the membrane anchor (alpha-peptide). Subsequently, membrane binding triggers enzyme activation (by increasing the catalytic efficiency) followed by electron transfer to ubiquinone-8 (Q_8), a mobile electron carrier at the membrane. The addition of amphiphiles, such as SDS, and limited proteolytic digestion can mimic the activation process. A central research goal of our 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 Q_8 shall be analyzed.
To study the contribution of electrostatic and hydrophobic interactions on the activation process, an *Ec*POX variant with three alanin-substituted residues (D328A, D348A, Q537A) was generated. In wt these residues predominantly clamp the membrane anchor via salt bridges and hydrogen bonds to the protein surface, whereas in the triple mutant these interactions are impaired. The triple variant shows similar steady state kinetics and proteolytic activation parameters like wt, but has higher activity when activated by SDS. Preliminary analysis of X-ray structures revealed no significant changes of the variant compared to wt. These results implicate a minor role of electrostatic interactions for the stabilization of the alpha-peptide in the enzyme-bound state and thus a mainly hydrophobic driven binding.

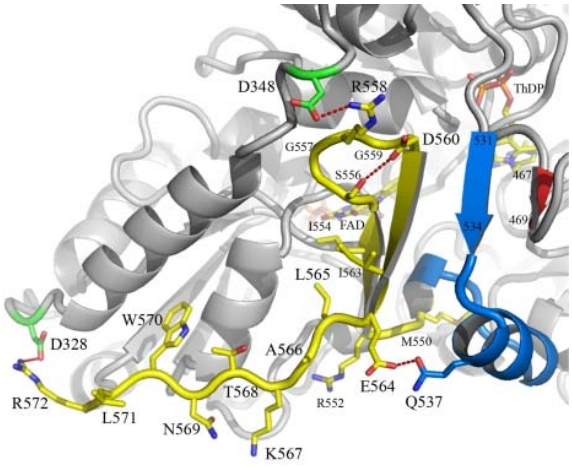


Fig. 1: Structure of non-activated *Ec*POX wt showing key electrostatic interactions of the membrane anchor (yellow) with the protein surface.

Additionally, we want to analyze the electron transfer from the flavin cofactor to Q_8, which is dissolved within the membrane bilayer. Both a direct electron transfer from the FAD to Q_8 at the active site, or indirect transfer via the protein backbone into the membrane seem possible. Recent X-ray structures of *Ec*POX in complex with Q_0 (a water-soluble Q_8 analog) suggest a direct electron transfer mechanism.

How viral peptides generate membrane curvature?

Giovanni Marelli, Marc Fuhrmans and Marcus Müller, and Yuliya Smirnova

Institute for Theoretical Physics, Soft Matter and Biophysics
Friedrich-Hund-Platz 1
D-37077 Göttingen

Membrane deformations are involved in many biological processes. One of such processes of key importance is membrane fusion [1]. Before the proper fusion process starts, two membranes approach each other to a short distances on the order of 1 nm. The strong hydration repulsion force dominates the membrane-membrane interaction at these distances, and it is proportional to the area of the membrane. In order to minimize the contact area, the membrane can bend and form a dimple. The concomitant bending energy can be provided by peptides. A class of interfacial amphipathic peptides, to which some viral peptides belong, is studied by molecular simulation. We study an interaction of such peptides with the membrane by means of coarse grained simulations using two different approaches [2,3], which allow us to explore different time and length scales. In order to understand the underlying mechanisms of membrane bending by peptides, we evaluate such quantities as the free energy of bending, pressure profiles, curvature and thickness of the membrane.

[1] L.V. Chernomordik, M.M. Kozlov. Nat. Struct. Mol. Biol. 15, 675 (2008)
[2] M. Hömberg, M. Müller. J. Chem. Phys. 132, 155104 (2010)
[3] Y.G. Smirnova, S.J. Marrink, R. Lipowsky, V. Knecht. J. Am. Chem. Soc. 192, 6710 (2010)

Synthesis of nucleosyl amino acids and muraymycin analogues for the investigation of the bacterial membrane protein MraY

Christian Ducho*, Stefan Koppermann, Daniel Wiegmann and Anatol P. Spork

Georg-August-University Göttingen, Department of Chemistry, Institute of Organic and Biomolecular Chemistry, Tammannstr. 2, 37 077 Göttingen, Germany. *Correspondence to: cducho@gwdg.de

Due to the emerging resistances of bacteria towards established antibiotics, there is an urgent need for the development 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. Currently, there is no commercially available antibiotic addressing MraY as a target, but natural products (nucleoside antibiotics) are known to efficiently inhibit this membrane protein. Muraymycins (*e.g.*, muraymycin A1 **1** and A5 **2**, Fig.)[1] display a high potential for detailed structure-activity relationship (SAR) studies as there are known MraY-inhibiting truncated analogues with reported remarkable activities against *Staphylococcus aureus*. These 5'-*epi*-analogues such as **3** and **4** (Fig.) exhibit a much higher antibiotic activity compared to the corresponding compounds bearing the natural product-like (5'*S*)-configuration (not displayed).[2]
Based on this result and due to the similar biological potencies of aminoribosylated antibiotic **1** and non-aminoribosylated derivative **2**,[1] we designed 5'-deoxy analogues of **3** and **4** with

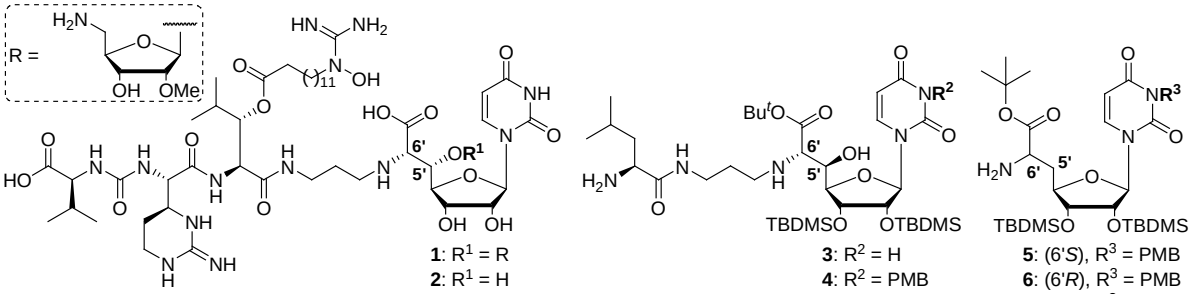


*Fig. Muraymycin A1 **1** and A5 **2** as representatives of the naturally occurring muraymycin antibiotics. Truncated 5'-epi-muraymycin analogues **3** and **4** with reported biological activity. Novel nucleosyl amino acids **5**-**8** for the synthesis of unprecedented 5'-deoxy muraymycin analogues.*

both the naturally occurring (6'*S*)- and the epimeric (6'*R*)-configuration. We developed a concise and highly stereoselective strategy for the synthesis of the nucleosyl amino acids **5**-**8** (Fig.), which was accomplished by asymmetric hydrogenation employing chiral rhodium(I) catalysts.[3] In combination with other synthetically derived nucleosyl amino acids (not displayed),[4,5] a set of building blocks for the preparation of muraymycin analogues is now available. The evaluation of their antibacterial potency 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.

[1] L. A. McDonald, L. R. Barbieri, G. T. Carter, E. Lenoy, J. Lotvin, P. J. Petersen, M. M. Siegel, G. Singh, R. T. Williamson, *J. Am. Chem. Soc.* **2002**, *124*, 10260-10261.
[2] A. Yamashita, E. Norton, P. J. Petersen, B. A. Rasmussen, G. Singh, Y. Yang, T. S. Mansour, D. M. Ho, *Bioorg. Med. Chem. Lett.* ***2003****, 13, 3345-3350.*
[3] A. P. Spork, S. Koppermann, B. Dittrich, R. Herbst-Irmer, C. Ducho, *Tetrahedron: Asymmetry* **2010**, *21*, 763-766.
[4] A. P. Spork, C. Ducho, *Org. Biomol. Chem.* **2010**, *8*, 2323-2326.
[5] A. P. Spork, S. Koppermann, C. Ducho, *Synlett* **2009**, 2503-2507.

Functional dynamics in the voltage-dependent anion channel

Saskia Villinger[1], Rodolfo Briones[2], Karin Giller[1], Adam Lange[1], Bert de Groot[2], Christian Griesinger[1], Stefan Becker[1] and Markus Zweckstetter[1]

[1] Dept. of NMR-based Structural Biology, Max Planck Institute for Biophysical Chemistry, Am Fassberg 11, 37077 Göttingen, Germany
[2] Dept. of Theoretical and Computational Biophysics, Max Planck Institute for Biophysical Chemistry, Am Fassberg 11, 37077 Göttingen, Germany

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 analysis (4). Micro- to millisecond 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. MD simulation reveals that a charge on the membrane-facing glutamic acid 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 micro- to millisecond 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 (5), our results imply that micro- to millisecond dynamics in the N-terminal part of the barrel are essential for VDAC interaction and gating.

References

1. Lemasters JJ, Holmuhamedov E (2006) Voltage-dependent anion channel (VDAC) as mitochondrial governator--thinking outside the box. *Biochim Biophys Acta* 1762:181-190.
2. Shoshan-Barmatz V *et al.* (2010) VDAC, a multi-functional mitochondrial protein regulating cell life and death. *Mol Aspects Med* 31:227-285.
3. Bayrhuber M *et al.* (2008) Structure of the human voltage-dependent anion channel. *Proc Natl Acad Sci U S A* 105:15370-15375.
4. Villinger S *et al.* (2010) Functional dynamics in the voltage-dependent anion channel. *Proc Natl Acad Sci U S A* 107:22546-22551.
5. Zaid H, Abu-Hamad S, Israelson A, Nathan I, Shoshan-Barmatz V (2005) The voltage-dependent anion channel-1 modulates apoptotic cell death. *Cell Death Differ* 12:751-760.

Electrophysiological Investigation of different hVDAC1 Ion-Channels

Conrad Weichbrodt, Claudia Steinem*

Georg-August-Universität Göttingen, IOBC, Tammannstr. 2, D-37077 Göttingen
email: cweichb@gwdg.de

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 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 the electrophysiological properties under different conditions are investigated. Furthermore, the properties of the wild type and a mutant of hVDAC1 are compared. The hVDAC1 protein forms a 19-stranded β-barrel with a N-terminal helix lying inside this pore. As this helix is believed to alter the protein characteristics, the mutant contains small modifications in this part.

For the determination of electrophysiological properties, human VDAC1, expressed in *E. coli*, purified and finally refolded in the detergent LDAO, is reconstituted in giant unilamellar lipid vesicles (GUVs) with sizes of about 1-100 µm in diameter. These GUVs are spread over an aperture in an borosilicate chip in order to obtain protein-doped lipid membranes. Use of a Port-a-Patch® device (Nanion Technologies, Munich, Germany) allows for high success rates in membrane-formation as well as their direct utilization for successive research.[2]

Both hVDAC1 isoforms primarily tend to be in a fully open state with conductances around 4 nS in 1 M KCl. As potentials exceeding 30 mV are applied, the proteins gate into 'closed' states with decreased conductances in the range of 1-2 nS more frequently with increasing potential. The mutant, carrying three additional amino acids (Arg-Gly-Ser) and thus introducing an additional positive charge, reveals a slightly higher tendency to stay in a subconductive state. This state is characterized by a significantly higher noise and short openings to the fully conducting state. This behaviour led to the idea that the RGS-mutant might represent a lower conductive, if not the 'closed' state of the hVDAC1 protein.

However, the electrophysiological properties of both variants have to be studied under different conditions to further evaluate this assumption.

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[1] V. Shoshan-Barmatz et al., *Current Phamaceutical Design,* **2006**, *12*, 2249-2270;

[2] N. Fertig et al., *Assay and Drug Development Technologies*, **2003**, *1*(5), 665-673.

Quantifying the Diffusion of Membrane Proteins and Peptides

Jörg Enderlein, Ingo Gregor, Qui Van and Kerstin Weiß

3. Physikalisches Institut, Georg-August-Universität Göttingen

Protein diffusion in lipid bilayers is often described by the Saffman-Delbrück model[1], which has been both challenged[2,3] and supported[4] by recent publications. The model assumes the proteins to be cylinders that diffuse through an infinite two-dimensional bilayer and predicts a logarithmic dependence of the protein's diffusion coefficient D_{SD} on its hydrodynamic radius R.

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

Here, k_B denotes Boltzmann's constant, T is the Temperature, μ and μ' are the viscosities of the membrane and the surrounding medium, respectively, and γ denotes Euler's constant.

The current project aims to precisely measure translational and rotational diffusion coefficients of proteins in lipid bilayers by means of dual-focus fluorescence correlation spectroscopy (2f-FCS). As model system, Black Lipid Membranes (BLMs) are investigated. BLMs are generated using a novel device, the Ionovation Bilayer Explorer, which monitors bilayer formation and stability via capacitance measurements. Moreover, both sides of the bilayer can be perfused individually and the setup allows access of the bilayer with a high numerical aperture objective.

A wide variety of protein sizes and environmental conditions will be employed to investigate the dependence of lateral diffusion on protein shape, membrane curvature, ionic strength and lipid-protein-interactions. Furthermore, the project aims to find a size / structure – diffusion relationship for rotational diffusion of proteins in a membrane. The results will allow for a critical re-evaluation and potential extension of the Saffman-Delbrück model.

References:

[1] Saffman, P.G., Delbrück, M., *Proc. Nat. Acad. Sci. USA,* **1975**, *72*, 3111 – 3113.
[2] Gambin, Y., Lopez-Esparza, R., Reffay, M., Sierecki, E., Gov, N.S., Genest, M., Hodges, R.S., Urbach, W., *PNAS*, **2005**, *103*, 2098 – 2102.
[3] Kriegsmann, J., Gregor, I., von der Hocht, I., Klare, J., Engelhard, M., Enderlein, J., Fitter, J., *ChemBioChem*, **2009**, *10*, 1823 – 1829.
[4] Ramadurai, S., Holt, A., Krasnikov, V., van den Bogaart, G., Killian, J.A., Poolman, B., *J. Am. Chem. Soc.*, **2009**,*131*, 12650 – 12656.

Participants

Aeffner, Sebastian
Institut für Röntgenphysik, Universität Göttingen, Friedrich-Hund-Platz 1, 37077 Göttingen
Phone: +49-551-39-9389, Fax: +49-551-39-9430, e-mail: saeffne@gwdg.de

Battle, Christopher
III. Physikalisches Institut, Universität Göttingen, Friedrich-Hund-Platz 1, 37077 Göttingen
Phone: +49-551-39-13829, Fax: +49-551-39-7720, e-mail: cbattle@physik3.gwdg.de

Bennati, Marina Prof. Dr.
Max-Planck-Institut für biophysikalische Chemie, Am Faßberg 11, 37077 Göttingen
Phone: +49-551-201-1911, Fax: +49-551-201-1467, e-mail: marina.bennati@mpibpc.mpg.de

Briones, Rodolfo Dr.
Max-Planck-Institut für biophysikalische Chemie, Am Faßberg 11, 37077 Göttingen
Phone: +49-551-201-2309, Fax: +49-551-201-2302, e-mail: rbrione@gwdg.de

Bubnis, Gregory Dr.
Max-Planck-Institut für biophysikalische Chemie, Am Faßberg 11, 37077 Göttingen
Phone: +49-551-201-2309, Fax: +49-551-201-2325, e-mail: gbubnis@gwdg.de

Diederichsen, Ulf Prof. Dr.
Institut für Organische und Biomolekulare Chemie, Universität Göttingen, Tammannstr. 2, 37077 Göttingen
Phone: +49-551-39-3221, Fax: +49-551-39-22944, e-mail: udieder@gwdg.de

Ducho, Christian Jun.Prof. Dr.
Institut für Organische und Biomolekulare Chemie, Universität Göttingen, Tammannstr. 2, 37077 Göttingen
Phone: +49-551-39-3285, Fax: +49-551-39-9960, e-mail: cducho@gwdg.de

Eggeling, Christian Dr.
Max-Planck-Institut für biophysikalische Chemie, Am Faßberg 11, 37077 Göttingen
Phone: +49-551-201-2553, Fax: +49-551-201-2513, e-mail: ceggeli@nmr.mpibpc.mpg.de

Eghiaian, Frédéric Dr.
III. Physikalisches Institut, Universität Göttingen, Friedrich-Hund-Platz 1, 37077 Göttingen
Phone: +49-551-39-7725, Fax: +49-551-39-7720, e-mail: feghiai@physik3.gwdg.de

Enderlein, Jörg Prof. Dr.
III. Physikalisches Institut, Universität Göttingen, Friedrich-Hund-Platz 1, 37077 Göttingen
Phone: +49-551-39-18933, Fax: +49-551-39-7740, e-mail: enderlein@physik3.gwdg.de

Fragneto, Giovanna Dr.
Institut Laue-Langevin, 6,rue Jules Horowitz, F-38042 Grenoble Cedex 9, France
Phone: +33-476-2070652, Fax: +33-476-207120, e-mail: fragneto@ill.fr

Fricks, John Dr.
Dept. of Statistics, 32 Thomas Bldg., Penn State University, University Park PA 16802-2111
Phone: +1-814-865-3532, e-mail: fricks@stat.psu.edu

Fuhrmans, Marc Dr.
Institut für theoret. Physik, Universität Göttingen, Friedrich-Hund-Platz 1, 37077 Göttingen
Phone: +49-551-39-91211, Fax: +49-551-39-9566, e-mail: fuhrmans@theorie.physik.uni-goettingen.de

Gattin, Zrinka Dr.
Max-Planck-Institut für biophysikalische Chemie, Am Faßberg 11, 37077 Göttingen
Phone: +49-551-201-2215, Fax: +49-551-201-2202, e-mail: ziga@nmr.mpibpc.mpg.de

de Ghellinck, Alexis
Institut Laue-Langevin, 6,rue Jules Horowitz, F-38042 Grenoble Cedex 9, France
Phone: +33-476-2071-52, e-mail: deghellinck@ill.fr

Griesinger, Christian Prof. Dr.
Max-Planck-Institut für biophysikalische Chemie, Am Faßberg 11, 37077 Göttingen
Phone: +49-551-201-2200, Fax: +49-551-201-2202, e-mail: cigr@nmr.mpibpc.mpg.de

de Groot, Bert Prof. Dr.
Max-Planck-Institut für biophysikalische Chemie, Am Faßberg 11, 37077 Göttingen
Phone: +49-551-201-2308, Fax: +49-551-201-2302, e-mail: bgroot@gwdg.de

Grubmüller, Helmut Prof. Dr.
Max-Planck-Institut für biophysikalische Chemie, Am Faßberg 11, 37077 Göttingen
Phone: +49-551-201-2300, Fax: +49-551-201-2302, e-mail: hgrubmu@gwdg.de

Höfer, Ines
Institut für Organische und Biomolekulare Chemie, Universität Göttingen, Tammannstr. 2, 37077 Göttingen
Phone: +49-551-39-3224, Fax: +49-551-39-3228, e-mail: ihoefer@gwdg.de

Hof, Martin Prof. Dr. DSc
J. Heyrovsky Institute of Physical Chemistry, Academy of Sciences of the Czech Republic, Dolejskova 3, Cz-18223 Prague 8
Phone: +420-266-053-264, Fax: +420-266-582-677, e-mail: martin.hof@jh-inst.cas.cz

Honigmann, Alf Dr.
Max-Planck-Institut für biophysikalische Chemie, Am Faßberg 11, 37077 Göttingen
Phone: +49-551-201-2511, Fax: +49-551-201-2505, e-mail: ahonigm@gwdg.de

Hotz, Thomas Dr.
Institut für mathem. Stochastik, Universität Göttingen, Goldschmidstr. 7, 37077 Göttingen
Phone: +49-551-39-13517, Fax: +49-551-39-13505, e-mail: hotz@math.uni-goettingen.de

Jahn, Reinhard Prof. Dr.
Max-Planck-Institut für biophysikalische Chemie, Am Faßberg 11, 37077 Göttingen
Phone: +49-551-201-1635, Fax: +49-551-201-1639, e-mail: rjahn@gwdg.de

Kaufeld, Theresa
III. Physikalisches Institut, Universität Göttingen, Friedrich-Hund-Platz 1, 37077 Göttingen
Phone: +49-551-39-7712, Fax: +49-551-39-7720, e-mail: kaufeld@physik3.gwdg.de

Koszinowski, Konrad Dr.
Department Chemie, University of Munich, Butenandtstr. 5-13, 81377 München
Phone: +49-89-2180-77772, Fax: +49-89-2180-9977658, e-mail: konrad.koszinowski@cup.uni-muenchen.de

Lange, Adam Dr.
Max-Planck-Institut für biophysikalische Chemie, Am Faßberg 11, 37077 Göttingen
Phone: +49-551-201-2214, Fax: +49-551-201-2202, e-mail: adla@nmr@mpibpc.mpg.de

Langosch, Dieter Prof. Dr.
Lehrstuhl für Chemie der Biopolymere, TU München, Weihenstephaner Berg 3, 85354 Freising
Phone: +49-8161-71-3500, Fax: +49- 8161-71-4404, e-mail: tlangosch@tum.de

Lorenz, Bärbel
Institut für Physikalische Chemie, Universität Göttingen, Tammannstr. 6, 37077 Göttingen
Phone: +49-551-39-22390, e-mail: blorenz@gwdg.de

Marelli, Giovanni
Institut für theoret. Physik, Universität Göttingen, Friedrich-Hund-Platz 1, 37077 Göttingen
Phone: +49-551-39-91211, Fax: +49-551-39-9631, e-mail: Marelli@theorie.physik.uni-goettingen.de

Meyenberg, Karsten
Institut für Organische und Biomolekulare Chemie, Universität Göttingen, Tammannstr. 2, 37077 Göttingen
Phone: +49-551-39-3235, Fax: +49-551-39-22944, e-mail: kmeyenb@gwdg.de

Meyer, Danilo Dr.
Albert-von-Haller-Institut, Universität Göttingen, Justus-von-Liebig-Weg 11, 37077 Göttingen
Phone: +49-551-39-14000, e-mail: dmeyer2@gwdg.de

Müllar, Stefan
Institut für Organische und Biomolekulare Chemie, Universität Göttingen, Tammannstr. 2, 37077 Göttingen
Phone: +49-551-39-3243, Fax: +49-551-39-22944, e-mail: smuella@gwdg.de

Müller, Marcus Prof. Dr.
Institut für theoret. Physik, Universität Göttingen, Friedrich-Hund-Platz 1, 37077 Göttingen
Phone: +49-551-39-13888, Fax: +49-551-39-9631, e-mail: mmueller@theorie.physik.uni-goettingen.de

Müller, Veronika
Max-Planck-Institut für biophysikalische Chemie, Am Faßberg 11, 37077 Göttingen
Phone: +49-551-201-2519, Fax: +49-551-201-2505, e-mail: vmuelle@gwdg.de

Munk, Axel Prof. Dr.
Institut für mathem. Stochastik, Universität Göttingen, Goldschmidstr. 7, 37077 Göttingen
Phone: +49-551-39-172111, Fax: +49-551-39-13505, e-mail: munk@math.uni-goettingen.de

Neubacher, Henrik
Institut für Organische und Biomolekulare Chemie, Universität Göttingen, Tammannstr. 2, 37077 Göttingen
Phone: +49-551-39-3249, Fax: +49-551-39-3228, e-mail: hneubac@gwdg.de

Park, Yongsoo
Max-Planck-Institut für biophysikalische Chemie, Am Faßberg 11, 37077 Göttingen
Phone: +49-551-201-1624, Fax: +49-551-201-1499, e-mail: ypark@gwdg.de

Polupanow, Tatjana
Institut für Organische und Biomolekulare Chemie, Universität Göttingen, Tammannstr. 2, 37077 Göttingen
Phone: +49-551-39-3793, Fax: +49-551-39-22944, e-mail: tpolupa@gwdg.de

Ries, Annika
Institut für Organische und Biomolekulare Chemie, Universität Göttingen, Tammannstr. 2, 37077 Göttingen
Phone: +49-551-39-3230, e-mail: aholken@gwdg.de

Ries, Oliver
Institut für Organische und Biomolekulare Chemie, Universität Göttingen, Tammannstr. 2, 37077 Göttingen
Phone: +49-551-39-3204, e-mail: ories@gwdg.de

Risselada, Jelger Dr.
Max-Planck-Institut für biophysikalische Chemie, Am Faßberg 11, 37077 Göttingen
Phone: +49-551-201-2325, e-mail: hrissel@gwdg.de

Salditt, Tim Prof. Dr.
Institut für Röntgenphysik, Universität Göttingen, Friedrich-Hund-Platz 1, 37077 Göttingen
Phone: +49-551-39-9427, Fax: +49-551-39-9430, e-mail: tsalditt@gwdg.de

Scharnagl, Christina Dr.
Fakultät für Physik, TU München, Maximus-von-Imhof-Forum 4, 85354 Freising
Phone: +49-8161-71-3557,e-mail: chrstina.scharnagl@tum.de

Schmidt, Christopher Prof. Dr.
III. Physikalisches Institut, Universität Göttingen, Friedrich-Hund-Platz 1, 37077 Göttingen
Phone: +49-551-39-7740, Fax: +49-551-39-7740, e-mail: c.f.schmidt@physik3.gwdg.de

Schultz, Carsten Dr.
EMBL Heidelberg, Meyerhofstr. 1, 69117 Heidelberg
Phone: +49-6221-387-0, e-mail: schultz@embl.de

Schwenen, Lando
Institut für Organische und Biomolekulare Chemie, Universität Göttingen, Tammannstr. 2, 37077 Göttingen
Phone: +49-551-39-3212, Fax: +49-551-39-3228, e-mail: lschwenen@gwdg.de

Sicoli, Giuseppe
Max-Planck-Institut für biophysikalische Chemie, Am Faßberg 11, 37077 Göttingen
Phone: +49-551-201-1379, Fax: +49-551-201-1383, e-mail: gsicoli@gwdg.de

Sitte, Astrid
Albert-von-Haller-Institut, Universität Göttingen, Justus-von-Liebig-Weg 11, 37077 Göttingen
Phone: +49-551-39-14000, e-mail: asitte@gwdg.de

Smirnova, Yulya Dr.
Institut für theoret. Physik, Universität Göttingen, Friedrich-Hund-Platz 1, 37077 Göttingen
Phone: +49-551-39-91211, Fax: +49-551-39-9631, e-mail: ysmirno@gwdg.de

Söllner, Thomas Prof. Dr.
Biochemie-Zentrum, Universität Heidelberg, Im Neuenheimer Feld 328, 69120 Heidelberg,
Phone: +49-6221-54-5342, Fax: +49-6221-64-5341, e-mail: thomas.soellner@bzh.uni-heidelberg.de

Spork, Anatol
Institut für Organische und Biomolekulare Chemie, Universität Göttingen, Tammannstr. 2, 37077 Göttingen
Phone: +49-551-39-3204, e-mail: aspork@gwdg.de

Steinem, Claudia Prof. Dr.
Institut für Organische und Biomolekulare Chemie, Universität Göttingen, Tammannstr. 2, 37077 Göttingen
Phone: +49-551-39-3294, Fax: +49-551-39-3228, e-mail: csteine@gwdg.de

Telschow, Fabian
Institut für mathem. Stochastik, Universität Göttingen, Goldschmidstr. 7, 37077 Göttingen
Phone: +49-551-39-13515, Fax: +49-551-39-13505, e-mail: f.telschow@stud.uni-goettingen.de

Tittmann, Kai Prof. Dr.
Albert-von-Haller-Institut, Universität Göttingen, Justus-von-Liebig-Weg 11, 37077 Göttingen
Phone: +49-551-39-14430, Fax: +49-551-39-5749, e-mail: ktittma@gwdg.de

Van, Qui Dr.
III. Physikalisches Institut, Universität Göttingen, Friedrich-Hund-Platz 1, 37077 Göttingen
Phone: +49-551-39-7727, Fax: +49-551-39-7720, e-mail: q.van@physik3.gwdg.de

Villinger, Saskia
Max-Planck-Institut für biophysikalische Chemie, Am Faßberg 11, 37077 Göttingen
Phone: +49-551-201-2219, Fax: +49-551-201-2202, e-mail: savi@nmr.mpibpc.mpg.de

Walla, Peter Jomo Prof. Dr.
Max-Planck-Institut für biophysikalische Chemie, Am Faßberg 11, 37077 Göttingen
Phone: +49-51-201-1087, Fax: +49-551-201-1501, e-mail: pwalla@gwdg.de

Wasilczuk, Katarzyna
Institut für mathem. Stochastik, Universität Göttingen, Goldschmidstr. 7, 37077 Göttingen
Phone: +49-551-39-172111, Fax: +49-551-39-13505, e-mail: k.wasilczuk2@stud.uni-goettingen.de

Weichbrodt, Conrad
Institut für Organische und Biomolekulare Chemie, Universität Göttingen, Tammannstr. 2, 37077 Göttingen
Phone: +49-551-39-3209, Fax: +49-551-39-3228, e-mail: cweichbr@gwdg.de

Weiß, Kerstin
III. Physikalisches Institut, Universität Göttingen, Friedrich-Hund-Platz 1, 37077 Göttingen
Phone: +49-551-39-7745, Fax: +49-551-39-7720, e-mail: kweiss@physik3.gwdg.de

Werz, Daniel B. Dr.
Institut für Organische und Biomolekulare Chemie, Universität Göttingen, Tammannstr. 2, 37077 Göttingen
Phone: +49-551-39-3251, Fax: +49-551-39-9476, e-mail: dwerz@gwdg.de

Zaba, Christoph
Institut für Nanobiotechnologie, Muthgasse 11/II, A-1190 Wien, Austria
Phone: +43-1-47654-2247, e-mail: christoph.zaba@boku.ac.at

Zweckstetter, Markus Dr.
Max-Planck-Institut für biophysikalische Chemie, Am Faßberg 11, 37077 Göttingen
Phone: +49-551-201-2220, Fax: +49-551-201-2202, e-mail: zwecks@gwdg.de