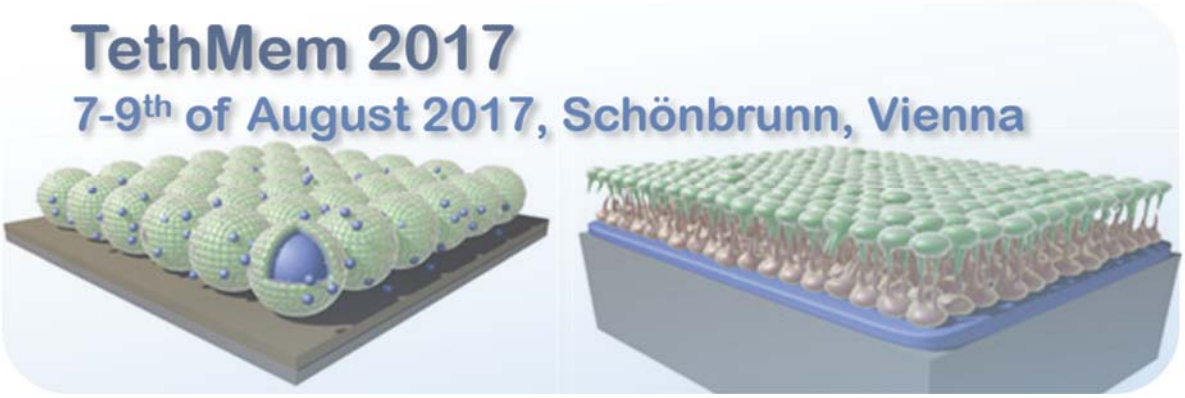

Poster abstracts

Single-molecule immunoassay in the nanogap-junction with a fluid lipid bilayer

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Single-molecule observation techniques are potentially useful for the diagnostic applications due to sensitivity and high information content. However, it is generally challenging to detect specific biological molecules at the single-molecular sensitivity in the presence of diverse coexisting molecules. We recently developed a novel biosensing platform for single-molecule observation by combining a model biological membrane and a nanometric gap structure (nanogap-junction)[1]. A micropatterned membrane composed of polymeric and fluid bilayers was generated on a glass substrate. By bonding the polymeric bilayer and an elastomer sheet (PDMS: polydimethylsiloxan) via an adhesion layer having a finite thickness (e.g. silica nanoparticles), a nanogap-junction was formed between the fluid bilayer and PDMS. Molecules that could specifically interact with the fluid bilayer were selectively transported into the nanogap-junction and detected. For applying the nanogap-junction to single molecule diagnostics, we detected a non-labeled biomarker, prostate specific antigen (PSA), by the sandwich immunoassay in the nanogap-junction. Single PSA molecules were observed even by the simple epi-illumination, showing higher signal-to-noise ratio than the conventional total internal reflection fluorescence microscopy (TIRF). The results demonstrate the potential of nanogap-junction for single molecule-based diagnostic applications.

1. Ando, K. *et al.*, Nanometric Gap Structure with a Fluid Lipid Bilayer for the Selective Transport and Detection of Biological Molecules, *Langmuir,* **32**, 7958 (2016)

Ladderane Phospholipids Slow Transmembrane Diffusion of Protons as a Result of Dense Lipid Packing

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Anaerobic ammonium oxidizing (anammox) bacteria convert ammonium and nitrite to nitrogen gas, which forms a critical part of the nitrogen cycle. Anammox bacteria carry out their metabolism in a membrane-bound organelle called the anammoxosome, which is comprised of the unique ladderane lipids. Ladderane lipids, named for the ladder-like structure of fused cyclobutane rings in the fatty acid tails, have not been found anywhere else in nature, suggesting that they play a critical role in the anammox metabolism. As anammox bacteria have not been grown in axenic culture and grow extremely slowly in enrichment cultures, researchers have not been able to isolate sufficient quantities of pure ladderane lipids to determine the biophysical properties of membranes composed of ladderane lipids. Without knowledge of the physical properties of ladderane lipids or genetic tools for studying the lipids *in vivo*, their biological function remains unknown. The Burns lab at Stanford has recently developed efficient synthetic routes to naturally occurring ladderane phospholipids, as well as unnatural analogs [1]. Using these synthetic molecules, we show that ladderane lipids have physical properties that are distinct from conventional straight-chain lipids. In particular, ladderane lipids form more dense membranes with smaller diffusion coefficients and monolayers with denser lipid packing. By varying the identities of the fatty acid tails, we establish structure-function relationships for the different ladderane structures. These physical properties result in membranes with much slower rates of transbilayer diffusion of protons, which are pumped across the anammoxasome membrane and used to produce ATP. These results suggest that ladderane lipids in the anammoxosome of anammox bacteria may prevent the dissipation of the proton gradient during the slow anommox metabolism. This role for ladderane lipids in the anammoxasome may partially explain why nature evolved such unique lipids for anammox bacteria. These studies also highlight the potential for synthetic chemistry to provide insight into challenging problems in membrane biology. Collaboration with Noah Burns' group at Stanford.

[1] “Chemical synthesis and self-assembly of ladderane phospholipids,” J. A. M. Mercer, C. M. Cohen, S. R. Shuken, A. M. Wagner, M. W. Smith, F. R. Moss III, M. D. Smith, R. Vahala, A. Gonzalez-Martinez, S. G. Boxer, N. Z. Burns, *Journal of the American Chemical Society*, 138, 15845–15848 (2016).

IMPACT OF MEMBRANE-ASSOCIATION ON SEMISYNTHETIC LIPIDATED PRION PROTEIN VARIANTS

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Prion diseases are based on the conversion of cellular (PrP^{C}) into misfolded, aggregated scrapie prion protein (PrP^{Sc}) on the outer leaflet of the cell membrane.[1,2] Membrane attachment via a C-terminal glycosylphosphatidylinositol (GPI) anchor seems to be crucial for prion toxicity.[3] In order to investigate the mechanisms of PrP conversion and toxicity, conformational changes, localization and orientation of PrP on the membrane and during cellular processing shall be determined by using semisynthetic, fluorescently labeled PrP variants. In a semisynthetic approach based on expressed protein ligation (EPL) recombinant full length (FL, aa23-231), N-terminally truncated (T, aa90-231), central region deleted (ΔCR, aa23-104/126-231) PrP thioesters linked to GPI-mimicking fluorescently labeled and non-labeled peptides are produced.[4-6] These PrP variants are analyzed using quartz crystal microbalance with dissipation monitoring (QCM-D) on lipid bilayers and imaging spectroscopy in live cells.

References:

1. Morillas, M.; Swietnicki, W.; Gambetti, P.; Surewicz, W.K. J. Biol. Chem. 1999, 274(52), 36859–36865.

2. Baron, G.S.; Caughey, B. J. Biol. Chem. 2003, 278(17), 14883–14892.

3. Priola, S.A.; McNally, K.L. Prion 2009, 3, 134–138.

4. Olschewski, D.; Seidel, R.; Miesbauer, M.; Rambold, A.S.; Oesterhelt, D.; Winklhofer, K.F.; Tatzelt, J.; Engelhard, M.; Becker, C.F.W. Chem. Biol. 2007, 14, 994–1006.

5. Becker, C.F.W.; Liu, X.; Olschewski, D.; Castelli, R.; Seidel, R.; Seeberger, P.H. Angew. Chem. Int. Ed 2008, 47, 8215–8219.

6. Chu, N.K.; Becker, C.F.W. FEBS Lett. 2013, 587, 430–435.

Controlling the distance between a patterned model membrane and the substrate with polymer brushes

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Substrate-supported planar bilayer (SPB) is being developed as versatile models of the biological membrane. We previously developed a micropatterned membrane consisting of polymeric lipid bilayer and fluid bilayer on a substrate, where the polymeric bilayer worked as a mechanically-stable framework of embedded fluid bilayer and membrane proteins. However, reconstituted membrane proteins tend to become immobile due to the proximity to substrate. Here, we report on the formation of hydrophilic polymer brush from the polymeric bilayer to control the distance between the substrate and the patterned membrane. Polymer brushes of 2-methacryloyloxyethyl phosphorylcholine (MPC) were formed by the activators regenerated by electron transfer-atom transfer radical polymerization (ARGET-ATRP) in aqueous solution in the presence of fluid bilayer. After the brush formation, polymeric bilayers were lifted by the polymer brush, whereas the distance between fluid bilayer and substrate remained small. We are currently studying the structural changes upon the brush formation in order to improve the control over the membrane-substrate separation. A hybrid membrane of patterned lipid bilayer and hydrophilic polymer brush should offer opportunities to incorporate and study membrane proteins in a quasi-native state.

Membrane binding and aggregation of neuronal acidic protein of 22kDa (NAP-22) studied with a patterned model membrane

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Neuronal acidic protein of 22kDa (NAP-22; also called CAP-23 or BASP1) is a neuron-enriched membrane protein. As an intrinsically disordered protein, NAP-22 interacts with several proteins and lipids, whose comprehensive function is, however, still unknown. NAP-22 associates with lipid bilayer with a myristoyl chain, and accumulates in the detergent resistant membrane (DRM), suggesting its affinity to lipid rafts. However, its binding and aggregation behaviors on the cell membranes are not fully understood. We quantitatively studied the binding and aggregation by using a patterned model membrane having defined compositions and geometries. We found that acidic lipids such as phosphatidylserine (PS) promoted membrane binding and aggregation. Presence of acidic lipids also affected the affinity of NAP-22 to lipid rafts. On the other hand, enhanced NAP-22 adsorption and aggregation was found on DRM in the patterned bilayers, but not on its lipid extracts, implying that protein components in DRM may be involved in its interactions with NAP-22. The systematic study using a model membrane gives insight into the nature of NAP-22 binding and aggregation, and should help to elucidate its roles in neurons.

Fabricating a nanometric gap junction by attaching a patterned lipid bilayer with PDMS via polymeric materials

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We are developing a single molecule observation technique of membrane proteins based on a model biological membrane and a nanometric gap structure (nanogap-junction). The nanogap-junction is formed from a patterned membrane composed of polymeric and fluid bilayers and a polydimethylsiloxane (PDMS) sheet, which are attached with an adhesion layer having a finite thickness, such as lipid vesicles and silica nanoparticles. The adhesion layer controls the thickness of nanogap-junction. For observing membrane proteins in the nanogap-junction, it is important to fabricate the nanogap-junction by using biocompatible materials that are resistant to nonspecific adsorption. To this end, we are developing a polymer-based adhesion layer and a technique to passivate the surface of PDMS. As the adhesion layer, we coated silica nanoparticles with hydrophilic polymer brushes of 2-methacryloyloxyethyl phosphorylcholine (MPC) and 2-aminoethyl methacrylate (AMA). For passivating the PDMS surface, we applied an amphiphilic random copolymer composed of MPC, AMA, and 3-trimethoxysilyl-propyl methacrylate (TSM). We discuss on the obtained results and suitability of these materials for realizing biocompatible nanogap-junction that are amenable to the single molecule observation of membrane proteins.

Degradation of drug delivery vehicles

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The degradation of supported lipid bilayers (SLBs) and supported lipid bilayers with grafted poly-(ethylene glycol) polymers (PEG-SLBS) with Phospholipase A2 (PLA2) was investigated using Quartz Crystal Microbalance with Dissipation Monitoring (QCM-D), an interfacial acoustic sensing method. Here planar SLBs and PEG-SLBs were used as a model system for spherical liposomes and PEG-liposomes, containing phospholipids, used in drug delivery. PEG-liposomes are considered as more stable and safer in the bloodstream of patients through reduced protein adsorption. Most drug delivery system degradation studies focus on the effect of opsonization of delivery vehicles through antibodies and parts of the complement system in serum. However, secreted and immobilized lipases and phospholipases can be found in nearly every part of the body. Phospholipids are the substrate for phospholipases and therefore this enzyme class could be considered as problematic for drug delivery with liposomes. As expected, unprotected SLBs are degraded by PLA2. Interestingly this study shows that PEG protected SLBs, even at high PEG density, do not offer a sustainable protection against PLA2 degradation. In fact, an increased initial rate of mass loss through degradation is observed for SLBs with dense and thick PEG shells.

Interaction-dependent structure of biomimetic soft interfaces : new methods for neutron reflectometry studies

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The study of the structure of biological interfaces and the molecular rearrangements as a response to the interaction with the surrounding environment encounters significant methodological restrictions. This is reasoned by the very limited number of techniques that probe buried interfaces with high resolution and in a perturbation-free manner. Neutron reflectometry is able to meet these fundamental requirements, and it also allows to highlight individual parts of the molecular assemblies at the interface by isotopic labelling.

In our group, we exploit the capability of this technique to study various biomimetic interfaces, from polymer-decorated lipid monolayers to liquid-supported bilayers, under controlled interaction conditions. In particular, we are at the cutting edge of the method by developing new approaches to correlate structure and interfacial forces of interacting membranes at any scenario from macroscopic separation to full adhesion. Such achievement is of crucial importance for the understanding of the physical mechanism of many biological processes based on recognition, fusion and transfer at the interface of biomembranes.

Development of model biological membranes for the functional reconstitution of membrane proteins

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Substrate-supported planar lipid bilayers (SPBs) are developed as a platform for sensitive analyses of membrane lipids and proteins. However, reconstituting transmembrane proteins into SPBs poses significant technological challenges. In the present work, we are developing model biological membranes that are suitable for the reconstitution of transmembrane proteins. A micropatterned SPB composed of polymeric and fluid bilayers was employed and its two- and three-dimensional structures were extended by the following approaches. First, we controlled the distance between the membrane and the substrate by forming hydrophilic polymer brushes of poly-2-methacryloyloxyethyl phosphorylcholine on the polymeric bilayer. Bacteriorhodopsin could be reconstituted into the embedded fluid bilayers with a reduced number of immobile molecules. Second, we physically separated the locations for the reconstitution of membrane proteins and their observation. We deposited a liquid-crystalline lipid source in the patterned bilayer, and observed that fluid bilayers spontaneously spread into the patterned polymeric bilayers. The lipid source would be used as the location of protein reconstitution, so that only successfully reconstituted proteins that can diffuse into the patterned membrane are analyzed. These methodologies should enhance the incorporation and functional analyses of transmembrane proteins in a model membrane.

Roles of lipid rafts in phototransduction studied with a micropatterned model membrane

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Phototransduction is believed to be regulated by lipid rafts. However, the regulation mechanism is still poorly understood. It is important to evaluate the affinity of membrane proteins to lipid rafts (raftophilicity) for revealing the regulation mechanism. We developed a methodology for evaluating raftophilicity by using a supported planer lipid bilayer (SPB) having patterned liquid-ordered (Lo) (raft model) and liquid-disordered (Ld) (non-raft model) bilayer domains[1]. In the present study, we quantitatively evaluated the raftophilicities of the major membrane proteins in the phototransduction of bullfrog retina (rhodopsin (Rh), G-protein transducin (Gt), phosphodiesterase (PDE6), rhodopsin kinase (GRK1), and S-modulin (S-mod)) (Fig.1).

The raftophilicity of Rh was heighted upon crosslinking by antiboby or associating with Gt by photoactivation. The raftophilicity of the α-subunit of Gt (Gαt) was lower than that of αβγ-trimer. PDE6, which is activated by Gαt has very low raftophilicity. Furthermore, GRK1, which phosphorylates Rh, had a low raftophilicity. These raftophilicities are generally consistent with observations in the disk membrane in retina. We discuss the implications of the obtained results in connection with the observations in the disk membrane and kinetic model simulations.

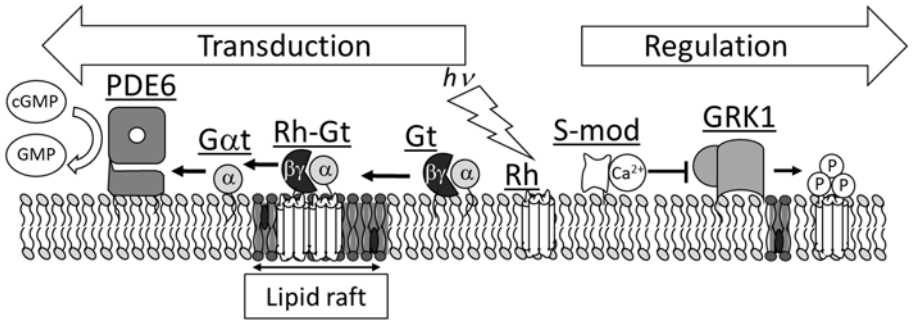

Fig.1. Scheme of the phototransduction: Rh relays the photo stimulation to PDE6 via Gt. GRK1 regulates the phototransduction by phosphorylating photo-activated Rh, whereas S-mod inhibits

the GRK1 activation depending on the Ca^{2+} concentration.

1. Tanimoto, Y., Okada, K., Hayashi, F., Morigaki, K. *Biophys. J.* **109,** 2307–2316 (2015).