created an constitutively open-channel even at pH 9. Using Fab-assisted crystallization methods we have "trapped" the structure of this mutant in a partially open state. Although the activation gate appears to be in the early stages of opening, full opening appears to be inhibited by lattice forces.

886-Plat Structure of Acid-sensing Ion Channel

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Acid-sensing ion channels (ASICs) are ligand-gated ion channels activated by the simplest ligand protons. These voltage-independent sodium channels belong to the epithelial sodium channel/degenerin family of ion channels and are implicated in perception of pain, ischemic stroke, mechanosensation, learning and memory. Here we report the crystal structure of a deletion mutant of ASIC1 from chicken at 1.9 Å resolution and low pH state. The receptor is a homotrimer with a chalice-like shape and is composed of short intracellular amino and carboxyl termini, 2 transmembrane helices per subunit, glycosylated extracellular domains rich in disulfide bonds, and bound chloride ions. The extracellular domain contains grooves enriched in acidic residues and carboxyl-carboxylate pairs within 3 Å, suggesting that at least one carboxyl group bears a proton. Electrophysiological studies on aspartate-to-asparagine mutants confirm that these carboxyl-carboxylate pairs are involved in proton sensing. A disulfide-rich 'thumb' domain lies between the acidic residues and the transmembrane pore, coupling the binding of protons to the opening of the ion channel and demonstrating that proton activation involves long-range allosteric conformational changes.

Platform R: Self Assembled Session: Membrane Dynamics and Biological Function

887-Plat Membrane-active Peptides and Drugs: Kinetic and Equilibrium Experiments

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One of the difficulties in membrane research concerns how to reconcile the results of equilibrium experiments with that of corresponding kinetic experiments. Take the example of membrane-active antimicrobial peptides. One can study the peptide-lipid interactions by examining the physical properties of the peptide-lipid mixtures. With a high degree of precision and reproducibility, we have detected and measured the effect of membrane thinning by peptides, peptide orientation change with concentration, pore formation in fluid membranes, and, more recently, reconstructed the electron density image of a pore from X-ray anomalous diffraction. But pore formation in cell membranes caused by water-soluble peptides occurs as a kinetic process. Typical kinetic experiments are performed with a vesicle suspension, for example measuring the content leakage when peptides are introduced into the suspension. How does one compare the kinetic experiments with the effects seen in peptide-lipid mixtures? In this talk, I will present a solution by the experiment of individual giant unilamellar vesicles (GUVs). We will see that the responses of individual GUVs depend on the poorly understood physics of defect, therefore they are not individually reproducible, contrary to the equilibrium experiments. Nevertheless the responses of individual GUVs reveal information not obtainable by suspension experiments, and they are consistent with the equilibrium results. We will also see this new method can distinguish different membrane-active effects between an antimicrobial peptide and an amphiphilic drug.

888-Plat Influence of Small Drugs on Permeablility and Fluctuation Lifetimes of Membranes

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It has long been known that lipid membranes become more permeable in their chain-melting regime. Unfortunately, examples that document such phenomena are rare and the data quality is often poor. In this work, we monitored membrane vesicle permeation by fluorescence correlation spectroscopy (FCS) and showed that the permeation of fluorescence dyes through such membranes follows the heat capacity profiles of the lipid system. This is due to the known coupling of the heat capacity with the elastic constants that determine the magnitude of the work necessary to create a transient pore. Since anesthetic molecules are able to change the lipid melting profiles they should influence their permeability in a predictable manner. Here, we confirmed these predictions using our FCS permeation experiment. Addition of octanol to lipid membranes is shown to have a significant effect on the permeability, depending on the state of the membranes. Further, when using black lipid membranes (BLM) one finds that permeation events for ions through the membranes appear in quantized steps that resemble those found for ion channel proteins. Both, current intensities and open lifetimes are quite similar to those found for such proteins.

Here we show that the open lifetimes are related to the overall permeability. Using pressure-perturbation calorimetry we found that relaxation processes are proportional to the heat capacity profile. Within the melting transition relaxation processes are slow. The application of anesthetics (and other drugs) has a predictable influence on the relaxation times. Since the pore formation is a consequence of density fluctuations, the relaxation time scales are closely coupled to pore open times.

As a result, we find a self-consistent coupling of membrane permeability and the open lifetimes of lipid pores, and a coherent and predictable effect of anesthetic and other drugs.

889-Plat Additive-induced Domain Formation In Membranes

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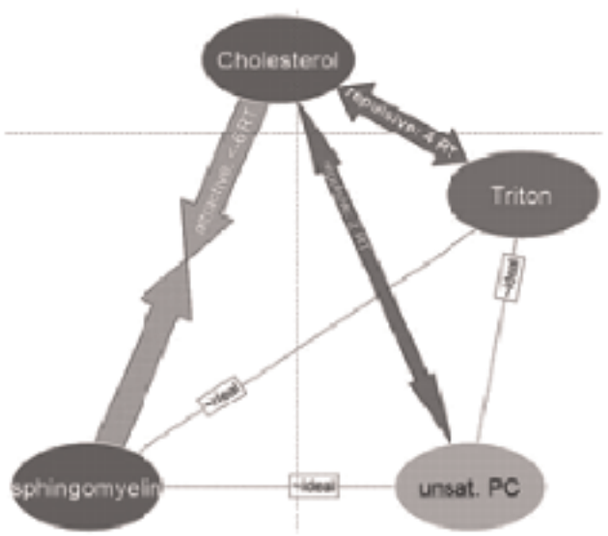
The finding that the detergent Triton (TX100) can stimulate the formation of ordered domains which are, at higher Triton concentration, isolated as detergent-resistant membrane (DRM) fraction has challenged the assumption that DRMs represent in vivo domains (rafts) isolated from cell membranes. Recent studies have not only confirmed these findings but provided a detailed understanding of the interactions of detergents with membranes being capable of forming liquid ordered domains. Detergent-induced domain formation was predicted to require a significant non-ideal, unfavorable interaction of the detergent with at least one component favoring ordered domains. We have indeed observed such a repulsive interaction between TX100 and cholesterol at 37degC.

In the absence of TX100, the non-ideal effects may be too weak to overcompensate the entropy of mixing and induce domains (for a given composition and temperature). Addition of TX100 shifts the balance between mixing and demixing by introducing energetically unfavorable encounters of TX100 and cholesterol in the fluid phase which can be eliminated by separating the two components from each other into different domains. The effect is not limited to TX100. The results provide insight into potential mechanisms of domain regulation in vivo and the interpretation of DRMs.



890-Plat Visualizing Association Of Signaling Proteins In Lipid Microdomains

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Two-photon fluorescence microscopy on giant unilamellar lipid vesicles and tapping-mode atomic force microscopy (AFM) are applied to follow the insertion of fluorescently labeled and dually lipidated N-Ras proteins with different anchor systems into heterogeneous lipid bilayer systems. Ras proteins are involved in the regulation of cell differentiation, mitosis, growth control, and cell cycle regulation, acting as molecular switches shuttling between active GTP-bound and inactive GDP-bound states. The lipid bilayers consist of canonical raft mixtures, which - depending on the concentration of the constituents - separate into liquid-disordered (ld), liquid-ordered (lo) or solid-ordered (so) phases. By combining both techniques, we were able to detect partitioning of N-Ras in lipid domains of canonical raft mixtures at length scales from the micrometer to the nm range. The results provide direct evidence that partitioning of N-Ras occurs preferentially into liquid-disordered lipid domains, which is also reflected in a faster kinetics of incorporation into the fluid lipid bilayers. The phase sequence of preferential binding of N-Ras to mixed microdomain lipid vesicles is ld $>$ lo $\gg$ so for all anchor systems studied. Intriguingly, we detect, using the better spatial resolution of AFM, also a large proportion of the lipidated protein located at the ld/lo phase boundary, thus leading to a favorable decrease in line tension that is associated with the rim of the demixed phases. Such an interfacial adsorption effect may serve as an alternative vehicle for association processes of signaling proteins in membranes.

891-Plat Structure and Dynamics of the Acyl Chain of Lipid Modified Membrane Proteins Studied by 2H Solid-State NMR and MD Simulation

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Lipid modification is a common motif for membrane binding of proteins involved in signal transduction. Here, we have studied structure and dynamics of the lipid chains of human N-Ras and GCAP-2 proteins. N-Ras carries a farnesyl and a palmitoyl chain at the C-terminus, while GCAP-2 is myristoylated at the N-terminus. Experimental ^{2}H NMR studies were conducted for lipid modified Ras peptides in host membranes of varying hydrophobic thickness (DLPC, DMPC, POPC, DPPC/Chol (10:6)).

Atomically resolved S_{CD} order parameters and R_{1Z} relaxation rates were measured. Order parameters of the lipid modifications depended strongly on the membrane environment indicating different packing of the lipid modifications. In addition, plots of R_{1Z} versus the squared S_{CD} allowed investigation of the flexibility of the Ras hydrocarbon chains in the different environments. Furthermore, orientation dependent relaxation rates of the peptide in DMPC were determined showing a very weak dependence on the orientation of the sample in the magnetic field. This indicates large amplitude motions of the Ras hydrocarbon chains. These experimental rates were fitted using an analytical model considering slow order fluctuations and collective motions. Thus, peptide dynamics in the membrane could be modeled by an anisotropic diffusion tensor with principal values of $D_{\parallel} = 2.1 \times 10^9\ s^{-1}$ and $D_{\square} = 4.5 \times 10^5\ s^{-1}$. Similarly, the orientation dependence of the relaxation rates was well reproduced by a 100 ns MD simulation. The 16:0 chain of Ras is

depicted as highly mobile undergoing large amplitude motions. In contrast, the 14:0 chain of GCAP-2 is well incorporated into its DMPC or POPC/POPE/POPS/cholesterol host matrix. It shows a moderate mobility within the lipid environment, comparable to the acyl chains of the host membrane lipids.

892-Plat The Impact of Collective Molecular Dynamics on Physiological and Biological Functionalities of Artificial and Biological Membranes

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The spectrum of fluctuations in biomimetic and biological membranes covers a large range of time and length scales, ranging from the long wavelength undulation and bending modes of the bilayer with typical relaxation times of nanoseconds and lateral length scales of several hundred lipid molecules, down to the short-wavelength, picosecond density fluctuations involving neighboring lipid molecules. New developments and improvements in neutron scattering instruments, sample preparation and environments and, eventually, the more and more powerful neutron sources open up the possibility to study collective excitations, e.g. phonons, in artificial and biological membranes. The goal of this project is to seek relationships between collective dynamics on various length scales on the one hand, and macroscopic phenomena such as transmembrane transport on the other hand.

The combination of various inelastic neutron scattering techniques enlarges the window of accessible momentum and energy transfers and allows one to study structure and dynamics on length scales ranging from the nearest-neighbor distances of lipid molecules to length scales of more than 100 nm, covering time scales from about 0.1 ps to almost 1 μs (1–6s). By using multiple instruments, from spin-echo to triple-axis spectrometers, we have successfully probed these fluctuations over the desired range of length and time scales [1]. Dynamical light scattering extends this range to even slower motions.

We present first results how bilayer permeability, elasticity and inter protein excitations can be determined from the dynamical experiments in the different regimes of length and time scales.

References

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893-Plat Protein Folding In Membranes: Insights from Neutron Diffraction Studies of a Membrane Beta-Sheet Oligomer

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Studies of the assembly of the hexapeptide Acetyl-Trp-Leu5 (AcWL5) into β-sheets in membranes have provided insights into membrane protein folding. Yet, the exact structure of the oligomer in the lipid bilayer is unknown. Here we use neutron diffraction to study the disposition of the peptides in bilayers. We find that pairs of adjacent deuterium labeled leucines have no well-defined peak or dip in the transmembrane distribution profiles, indicative of heterogeneity in the depth of membrane insertion. At the same time, the monomeric homolog AcWL4 exhibits a homogeneous, well-defined, interfacial location in neutron diffraction experiments. Thus, while the bilayer location of monomeric AcWL4 is determined by hydrophobicity matching, or complimentarity within the bilayer, the AcWL5 molecules in the oligomer are positioned at different depths within the bilayer because they assemble into a staggered transmembrane β-sheet. The AcWL5 assembly is dominated by protein-protein interactions rather than hydrophobic complimentarity. These results have implications for the structure and folding of proteins in their native membrane environment and highlight the importance of the interplay between hydrophobic complimentarity and protein-protein interactions in determining the structure of membrane proteins.

894-Plat Rhodopsin Adjusts Helical Content To Match Membrane Hydrophobic Thickness

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It is well known that the rhodopsin-dependent steps of the visual process are exquisitely sensitive to membrane composition. In particular, bilayer hydrophobic thickness was identified as key factor modulating the MII/MI equilibrium after photoactivation. Such dependence was explained by the hydrophobic matching theory which predicts that lipid bilayer thickness adjusts to the hydrophobic length of transmembrane helices. Here, we examined whether dramatic changes in bilayer thickness from altering hydrocarbon chain length in the series 14:0(d27)-14:1-PC, 16:0(d31)-16:1-PC, 18:0(d35)-18:1-PC, 20:0(d39)-20:1-PC result in adjustment of hydrocarbon chain length to rhodopsin. Experiments were conducted on dark-adapted rhodopsin, as well as on the photointermediates M-I, M-II, M-III and opsin. By ^{2}H NMR on the sn-1 chain perdeuterated lipids we probed for changes of bilayer thickness. The response of rhodopsin to membrane hydrocarbon chain lengths was followed by circular dichroism, spectrophotometry, tryptophan fluorescence, and calorimetry. We observed a significant increase in helical content with increasing bilayer thickness, corresponding to an extension of transmembrane helices by 1–2 turns. In contrast changes of lipid order to rhodopsin incorporation were mostly absent. Photoactivation did not alter lipid order or helical content. However, the MII/MI equilibrium, the kinetics of formation of photointermediates, as well as the temperature of thermal denaturation of rhodopsin responded strongly to changes of bilayer thickness. The data suggest that rhodopsin adjusts to changes of membrane hydrophobic thickness by increasing the length of transmembrane helices rather than inducing changes of membrane hydrophobic thickness. We propose that changes of helical content are linked to stability of photointermediates.