binding energy. Together, the simulations and experimental results suggest that PI-PLC binding to PC-rich membranes is mediated by aromatic-rich surface-accessible regions of the protein that engage in a myriad of transient pi-cation interactions with choline headgroups. This PC-binding mechanism may be utilized by other peripheral membrane proteins.

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Hydrophobic Matching Between Phospholipid Bilayers and Helical Peptides Affects the Memrbane Affinity of Sterols

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The lateral organization of cell membranes is important for several cellular functions including signaling and membrane trafficking. In addition pathogen uptake and influenza virus budding is associated with sphingolipid and cholesterol enriched lateral membrane domains. The lateral structure of membranes is in part controlled by lipid self-organization, but it is becoming increasingly clear that also membrane proteins can play an active role in the maintenance of the lateral structure. Cholesterol is thought to have an important role in lateral organization of eukaryotic cell membranes. As cholesterol also has been implicated to take part in the sorting of cellular transmembrane proteins it is a good starting point to determine how transmembrane proteins influence the lateral sorting of cholesterol in phospholipid bilayers. Insight into this can be obtained by studying how cholesterol interacts with bilayer membranes of different composition in the presence of different transmembrane peptides, mimicking the transmembrane helices of proteins. By measuring the equilibrium partitioning of the fluorescent cholesterol analogue cholestatrienol (CTL) between large unilamellar vesicles and methyl-beta-cyclodextrin the effect of hydrophobic matching on the affinity of sterols for phospholipid bilayers was determined. The results showed that increasing positive mismatch led to higher affinity of the sterol for the bilayers. This suggests that hydrophobic matching could affect the lateral organization of cholesterol in cell membranes and have an important role especially in membrane trafficking.

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A Flim-FRET Study of the Raft-Associated Proteins in Erythrocytes Expressing the Miltenberger Blood Group Antigen Subtype III (Mi.III) Phenotype

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Lipid rafts are dynamic signaling hubs packed with ordered lipids on the cell membrane. Lipid rafts in erythrocytes can be classified into two types: flotillin-associated rafts and stomatin-associated rafts. We previously found by biochemical means that these two types of rafts coalesce on the erythrocytes expressing the Mi.III blood type. Mi.III is one of the most important blood groups in the field of transfusion medicine in Southeast Asia. Mi.III encompasses a characteristic hybrid structure of glycophorin A and glycophorin B, termed Gp.Mur, and is part of the band 3 macrocomplexes on the erythrocyte surface. Band 3 is known to be partially associated with erythrocyte rafts. The objective of this study was to examine if Gp.Mur was associated with rafts on Mi.III+ cells. We assessed the protein-protein interaction between Gp.Mur and band 3 by measurements of FRET using fluorescent lifetime imaging microscopy (FLIM). In these experiments, band 3 and GFP-Gp.Mur fusion constructs were co-transfected into HEK-293 cells, and band 3 was labeled with Alexa Fluor-568 prior to FLIM-FRET measurements. Here, GFP served as the donor and Alexa Fluor-568 as the acceptor fluorophore if FRET occurred. We found that the fluorescence lifetime of the donor was significantly shorter at some surface edges in the cells coexpressing Gp.Mur-GFP and band 3; this phenomenon however was not observed in the cells expressing Gp.Mur-GFP only. We thus conclude that Gp.Mur and band 3 interact within 10 nm on particular regions of the plasma membrane, some of which are likely lipid rafts. Because Mi.III+ erythrocytes are superior in CO_2 metabolism, pH homeostasis and cell membrane resilience, as compared to the non-Mi.III cells, their unique raft organization is expected to have functional implications.

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Membrane Interaction of α-Synuclein in Different Aggregation States

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Aggregated α-synuclein in Lewy bodies and Lewy neurites are hallmarks of Parkinson's disease (PD). Recent observations that aggregated α-synuclein is propagated to healthy neurons grafted into brains of PD patients prompted our study. We asked whether any, and if so which, molecular form(s) of α-synuclein can pass over model phospholipid bilayers. Confocal fluorescence microscopy was used to study the binding of Alexa488 labeled α-synuclein to giant unilamellar vesicles (GUVs) and to monitor if the α-synuclein is transported over the phospholipid bilayers. The GUVs were composed of mixtures of DOPC and DOPS or DOPC and cardiolipin at different molar ratios to vary the membrane charge. All lipids chosen for this study are common in human membranes. Cardiolipin is primarily found in the mitochondrion, i.e. an organelle that is implicated in PD pathogenesis.
We studied membrane binding and transport for monomeric, fibrillar as well as on-pathway α-synuclein samples. To enable preparation of samples representing defined time points during the aggregation lag period, conditions were identified that resulted in reproducible aggregation kinetics at moderate shaking and at quiescent conditions. We observed association to lipid membranes for on-pathway species and equilibrium aggregates, but not for monomers. Particularly strong association was found between α-synuclein and GUVs that contained cardiolipin or DOPS. By contrast, α-synuclein did not associate with GUVs containing DOPC only. No transport of α-synuclein over the GUV bilayer was observed under any of the conditions studied. Labeled α-synuclein was never observed inside the GUVs although clearly visible in the surrounding buffer. This suggests that the transport of α-synuclein over membranes, which has been observed in several previously published cell culture experiments, requires additional molecular components and/or an active transport mechanism.

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Influence of the Membrane Composition of Retinal Photoreceptors on the Reversible Binding of a Neuronal Calcium Sensor Protein (NCS), Recoverin : A Solid-State NMR and FTIR Study

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NCS proteins consist of 4 domains called EF-Hand, which are 2 α-helices joined by a loop with a highly conserved sequence. Binding of calcium ions by this loop induces important conformational changes in the protein. Most of the NCS also have a N-terminal sequence recognized by N-myristoyl transferase which is responsible for the acylation of the proteins. At low calcium concentration, the myristoyl group is sequestered into a hydrophobic cavity. The binding of 1 to 4 Ca^{2+} leads to the extrusion of the myristoyl (known as the calcium myristoyl switch) and the exposure of many hydrophobic residues allowing the protein to go from a cytosolic form to a membrane bound form. This property has an important biological function in the visual phototransduction cascade. In fact, the absorption of a photon by the visual pigment rhodopsin leads to an important decrease of calcium level in photoreceptors and recoverin, one of the NCS family, plays a key role in the recovery phase of visual excitation by inhibiting rhodopsin kinase at high calcium level. An interesting fact is that the membrane composition of the photoreceptor rod outer segments is known to be very different from that of other membranes, with more than 60% of the lipids being polyinsaturated. We have determined in the present study how the membrane composition affects the reversible membrane binding of recoverin. More specifically, ^{31}P solid-state NMR and ^{2}H solid-state NMR have been used to get information respectively about the lipid polar head groups and the recoverin myristoyl group. FTIR was also used to study the structure of recoverin (amide I' band) and the lipid acyl chains (CH_2 symmetric stretch band).

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Identifying Motifs for Phosphatidylcholine Activation of Bacterial Phosphatidylinositol-Specific Phospholipase C Enzymes

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Phosphatidylinositol-specific phospholipase Cs (PI-PLCs) secreted by pathogenic bacteria are often virulence factors. For one well-studied example, *Bacillus thuringiensis* PI-PLC (B*t*PI-PLC), the presence of the non-substrate lipid phosphatidylcholine (PC) in membranes enhances both membrane binding and enzymatic activity towards phosphatidylinositol (PI). A strip of four surface-exposed Tyr residues near the rim of the α/β-barrel are involved in this enhancement, and it has been proposed that this strip contributes to a specific PC binding site. The related PI-PLC from *Staphylococcus aureus* (S*a*PI-PLC), has similar kinetic characteristics to B*t*PI-PLC, but has only two Tyr residues in the same region. While S*a*PI-PLC can also be specifically activated towards PI cleavage by incorporation of PC into assay systems, S*a*PI-PLC membrane binding as a function of PC content is considerably weaker than that of B*t*PI-PLC. Mutagenesis of the two S*a*PI-PLC Tyr residues (Y253S/Y255S) reduces interfacial activity of S*a*PI-PLC. Adding the two 'missing' Tyr residues (N254Y/H258Y) did not further enhance S*a*PI-PLC specific activity but it dramatically enhanced binding of the protein to PC-rich