(GUV's) are made from phospholipids using electroswelling. To mimic the mushroom morphologies of dendritic spines, a micromanipulator is used to pull membrane tubes from the GUV lipid bilayer.
Diffusion is measured by attaching quantum dots to lipids or receptors in the GUV membrane using biotin-streptavidin bonds. Particle tracking is then used to determine diffusion in the GUV membrane, and escape times from GUV to tube.
Results show a strong dependence of GUV and tube size on escape times, confirming the idea that connection strengths of mushroom shaped dendritic spines are much stabler than the strengths of stubby shaped dendritic spines.

1524-Pos Board B254
Minimal Viral Potassium Channels for Studying Protein/Lipid Interaction
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The channel proteins from Chlorella viruses are miniature versions of K^+ channels. They are the structural equivalents of the pore module of complex K^+ channels from eukaryotes and they include the selectivity filter, the cavity and gates. The channel KcvNTS is with only 82 amino acids particularly small and molecular dynamics simulations suggest that the channel is quasi fully embedded in a di-myristoylphosphatidylcholin (C14) lipid bilayer [1]. This close interaction between the channel protein and its lipid environment offers the possibility to examine lipid/protein interaction for a channel pore in a systematic manner. For this purpose we reconstituted the purified channel protein in lipid bilayers with different chain lengths, phospholipid head groups and in the absence and presence of detergents or cholesterol. The analysis of single channel activity shows that the membrane composition affects channel gating but not the unitary conductance. Most interesting is a voltage dependency of the channel, which is introduced by the presence of cholesterol, detergent or bilayers with long chain length.
Reference
1. Braun, C.J., et al., Viral potassium channels as a robust model system for studies of membrane-protein interaction. Biochim Biophys Acta, 2013.

1525-Pos Board B255
Oligomeric States and Cooperative Gating in Clusters of Mechanosensitive Membrane Proteins
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The mechanosensitive channel of large conductance (MscL) provides a paradigm for mechanosensation and mechanotransduction. It also provides a model system for how bilayer material properties regulate membrane protein function. The basic phenomenology of MscL gating as a function of membrane tension, and the dependence of MscL gating on bilayer material properties, can be understood by considering the difference in membrane deformation energy between open and closed states of MscL. However, even basic issues such as the physiologically relevant oligomeric states of MscL remain a matter of intense debate. Is MscL a tetramer or a pentamer under physiological conditions, or do both structures occur in cell membranes? Moreover, MscL proteins have recently been observed to form clusters, and the gating behavior of MscL in clusters was found to be different from the gating behavior of single MscL. What are the physical mechanisms behind the clustering of MscL, and what is the biological significance of MscL clusters? The complexity of the observed MscL structures and the large size of MscL clusters have so far prohibited a theoretical analysis of the relation between the oligomeric states of MscL and the properties of MscL clusters. Here we develop a mathematical approach capable of predicting the coupling between MscL shape and function. Combining this approach with the theory of regular lattices of polygons, we study the interplay between the oligomeric state of MscL, membrane-mediated interactions between MscL proteins, the structure of MscL clusters, and cooperative gating of MscL. We predict that the architecture of MscL clusters may provide a signature of MscL shape, with distinct molecular structures of MscL yielding distinct spatial and orientational organization patterns. Our work establishes a bridge between the oligomeric state of MscL and the self-assembly of MscL into clusters.

1526-Pos Board B256
Mechanisms Underlying the Uncoupling of Binding and Gating in the Nicotinic Receptor and its Prokaryotic Homologs
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In PC membranes lacking activating cholesterol and anionic lipids, the nicotinic acetylcholine receptor (nAChR) adopts an uncoupled conformation. The uncoupled nAChR retains agonist-binding, but does not usually undergo conformational transitions to the open or desensitized state. The fact that the uncoupled nAChR does not desensitize in most membranes, despite binding agonist, requires that the activation energy between uncoupled and coupled (resting, open, and desensitized) conformations is insurmountably high. Reconstitution of the nAChR into membranes with relatively thick hydrophobic cores, however, leads to slow (minutes to hours) ligand-induced conformational transition to ultimately the desensitized state. The large energy barrier(s) between uncoupled and coupled conformations is(are) consistent with the current model of uncoupling, which proposes that the transmembrane α-helix, M4, from each subunit becomes solvated by lipid, thus weakening interactions between M4 and the Cys-loop. Weakened interactions may lead to an altered Cys-loop conformation that does not interact effectively with the transmembrane domain to elicit agonist-induced channel gating. In this presentation, we will discuss current experiments aimed at elucidating the mechanisms underlying lipid-dependent uncoupling. In particular, we will focus on structural and mutagenesis data obtained using the model prokaryotic pLGICs, ELIC and GLIC. The data highlight the importance of M4 in pLGIC gating and in dictating the lipid sensitivities of the different prokaryotic pLGICs.

1527-Pos Board B257
P-Glycoprotein: Purification, Incorporation and Activity in Nanodiscs
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P-glycoprotein (P-gp), a member of the ATP Binding Cassette (ABC) superfamily, is a drug transporter that effluxes a broad spectrum of therapeutic agents. P-gp is expressed in many tissues important in drug disposition including the intestine, liver, kidneys, and blood-brain-barrier. P-gp acts to decrease drug absorption following oral administration, facilitate elimination from the body, and decrease drug exposure in tissues such as the brain. This 170 kDa protein consists of two similar halves, each composed of 6 helical transmembrane regions and an ATP-binding domain. Genetic variations in the ABCB1 gene that encodes P-gp lead to alterations in P-gp expression and activity, which can affect multidrug resistance and drug disposition. The goals of this study are to biochemically and biophysically characterize P-gp to understand how ABCB1 genetic polymorphisms alter activity. The first step in our study is to obtain functional protein incorporated into our model membrane system, the nanodisc. A nanodiscs is a discoidal system whose composition (lipid, cholesterol) can be custom tailored and is held in shape by two belt proteins, which also govern the size of the disc. This poster describes our path to producing active P-gp in nanodiscs using E. coli total lipids and two sizes of belt protein. The next step is to conduct studies to determine how ABCB1 genetic polymorphisms can lead to differences in transport activity and structural dynamics. Understanding these differences, as related to polymorphisms, can potentially lead to a method to reverse multidrug resistance.

1528-Pos Board B258
The Effect of Detergent on the Oligomerization of a 7-Transmembrane Protein
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Membrane proteins serve a broad range of functions necessary for life; in fact, they are coded for by 30% of genes in the human genome. Unfortunately, the