transport across reconstituted lipid bilayers and compare ionic flow through gramicidin A channels under applied DC and AC signals. For these measurements, we adapted a Planar Lipid Bilayer workstation with a frequency mudulator in order to handle applied AC signals and focused our studies to lithium transport to correlate with previous measurements of lithium effects on lipid interactions. Studying the behaviour of lipid bilayers under AC voltages will allow for further insight on how the cell's plasma membrane reacts to suddent variations in voltages.

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Regulation of Ion Channel Function by the Host Lipid Bilayer Examined by a Stopped-Flow Spectrofluorimetric Assay

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To examine the function of ligand-gated ion channels in a defined membrane environment we developed a robust sequential-push fluorescence-based stopped-flow assay. The method is based on earlier studies (Moore and Raftery, PNAS, 1980, 77, p. 4509) (Karpen et al, Anal. Biochem., 1983, 135, p. 83) (Ingólfsson and Andersen, 2010, Assay Drug Dev. Technol., 8, p. 427), in which channel activity is determined using a channel-permeable quencher (e.g., thallium, Tl^+) of a water-soluble fluorophore (ANTS) encapsulated in large unilamellar vesicles in which the channel of interest has been reconstituted. To validate the method, we explored the activation of wild type (WT) as well as a non-inactivating (E71A) mutant KcsA channels, by extravesicular protons (H^+). For either channel type, the day-to-day variability in the reconstitution yield (as judged from the time course of fluorescence quenching) is less than 10%. WT and E71A KcsA activation curves are indistinguishable, and the activation curve for E71A KcsA is similar to that obtained previously using single-channel electrophysiology (Thompson et al., 2008, PNAS, 105(19):6900). We then investigated the regulation of KcsA activation by changes in lipid bilayer composition. We found that increasing the acyl chain length (from C18:1 to C22:1 in di-acyl-PC), but not the mole fraction of POPG (above 0.25) in the bilayer-forming phospholipid mixture, alters KcsA proton gating. The bilayer thickness-dependent shift in the activation curve indicates an apparent decrease in H^+ affinity and cooperativity. The method's reproducibility, control over bilayer environment and time resolution makes it a powerful assay for exploring ligand-activation and inactivation of ion channels, and how these processes vary with changes in the channels' lipid bilayer environment.

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A New Supported Membrane System for Studying the Lipid Effects on a Kv Channel

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Recent studies have demonstrated that annular lipids may directly change the conformation of a voltage-gated ion channel (lipid-dependent gating). We found it was challenging to insert membrane proteins into a bilayer that mimics a eukaryotic cell membrane, especially the membranes that contain sphingomyelin and cholesterol. A new system is needed to overcome this technical barrier. In this study we developed a stable unilamellar vesicle system that is supported by unidirectionally inserted membrane proteins, and offers the capability of controlling lipid composition with relative ease. A voltage-gated potassium channel, KvAP, was used as a model system, and was selectively anchored onto the surface of micron-sized beads. Our data suggest that with a high surface density of channel molecules, it is feasible to introduce various lipids and form a continuous unilamellar membrane around the beads. The unilamellar nature of the bilayers was demonstrated by cryo-electron microscopic observations. The functionality of the channels in the membrane was examined by electrical recordings of their voltage-gated activities. Our results showed cholesterol, even at a low concentration, exerts strong effects on the open probability of the KvAP channel. We anticipate that this novel membrane system will provide a new technique to study how lipids influence the function of membrane proteins.

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The Role of Trp in Arg-Rich Paddle Domain-Lipid Interaction

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Paddle domains in voltage-gated K^+ channels exert their function to mediate voltage gating by altering their positions depending on membrane potential. Two Arg-rich paddle domains, KvAPp from the K_v channel from *Aeropyrum pernix* and HsapBKp from the BK channel from *Homo sapiens*, have been shown to be able to induce a thinning of a bilayer which may be the key of gating mechanism. However, differences between the two paddle domains were also observed, e.g., calcein leakage in large unilamellar vesicles induced by HsapBKp is more pronounced and HsapBKp can probably undergo structural transitions indicated by other spectroscopic results. The presence of a Trp in HsapBKp but not in KvAPp may be the reason for the different direct membrane effects. Therefore, in this study we have investigated the role of the Trp in the human BK channel. We have used a range of spectroscopic techniques to elucidate the role of the Trp residue by studying HsapBKp and a variant where Trp was replaced by Ala, and KvAPp and the variant where Ala was replaced by Trp. As a comparison, we also investigated a model transmembrane peptide, KALP21, and a variant of this peptide where an Ala residue in the middle of the hydrophobic region was substituted by Trp. The results show that a Trp in the middle of the sequence alters the structure, and changes the way that the motifs interact with lipids. A Trp promotes disruption of fast-tumbling small bicelles as well as magnetically aligned bicelles. The presence of a Trp residue explains the differences by which the Arg-rich motifs from the K_v and BK interact with the membrane.

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Solubilization, Purification and Characterization of the Potassium Channel Kcsa in its Native Lipid Environment: The Power of Native Nanodiscs

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The common use of detergents in solubilizing integral membrane proteins to allow for their purification and biophysical study involves the complete disruption of the lipid bilayer, which can result in a decrease of protein stability and function. We report here the detergent-free solubilization and affinity purification of the tetrameric potassium channel KcsA in native nanodiscs stabilized by a styrene-maleic acid (SMA) copolymer. The polymer self-inserts into the membrane and stabilizes and isolates discoidal proteolipid particles, thus conserving the native lipid environment of KcsA. Analysis of the lipids isolated from native nanodiscs by thin layer chromatography and mass spectrometry revealed an enrichment of the anionic lipids cardiolipin and phosphatidylglycerol in close proximity to the channel. Using an SDS-PAGE assay as well as circular dichroism and fluorescence spectroscopy, we found the thermal stability of the KcsA tetramer to be higher in native nanodiscs as compared to detergent micelles. Together, these findings highlight the potential of the use of native nanodiscs as a general tool in the study of membrane proteins.

Key words:
lipid-protein interactions, styrene-maleic acid copolymer, nanodisc, membrane-protein solubilization, KcsA.

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GUV Based Mimicry of Dendritic Spine Morphology Permits to Test Hypotheses on Ltp and Learning

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Memory and learning are believed to be regulated by the strength of the connections in the synapses of the 100 billion neurons in the human brain. In the synapse the signal is transmitted between the presynaptic axon and the postsynaptic dendrite by neurotransmitters. The postsynaptic site is usually a protrusion on the dendrite called dendritic spine. Receptors in the membrane of the dendritic spine define the strength of a synapse. Thus the strength and stability of synapses are stored in the dendritic spine and are thought to be dependent on spine morphology (Yuste & Bonnhoeffer, 2001). Therefore the mushroom shape of mature dendritic spines is predicted to function as a receptor trap, locally increasing receptor density.
We developed a mimetic system to investigate dendritic spine morphology and its effects on receptor confinement and diffusion. Giant unilamellar vesicles