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## Subgroup: Membrane Biophysics

**1-Subg**
**Flavonoids Regulate Eag1 Channels**
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[1]University of Washington, Seattle, WA, USA, [2]deCODE Biostructures Inc, Bainbridge Island, WA, USA.
Ether-a-go-go (Eag) family channels, which include hErg1 channels, are voltage-gated K+ channels that are important in cardiac and neural function. From the amino acid sequence of Eag family channels, we identified two probable ligand-binding sites based on their similarities with well-characterized ligand-binding domains. The first putative ligand-binding domain is in the carboxy-terminal region and shares sequence similarity with the cyclic nucleotide-binding domain (CNBD) of proteins modulated by cyclic nucleotides. The second is a Per-Arnt-Sim (PAS) domain in the amino-terminal region. As no ligands have been reported for Eag family channels, we categorize these channels as orphan receptors. We reasoned that a chemical screen of metabolites will lead us to physiologically relevant channel-regulators. Using a novel, high through-put screen of the ''Fragments of Life'' chemical library of metabolites and metabolite-like compounds (deCODE Biostructures, Bainbridge Island WA) and inside-out patch-clamp recording, we have identified the flavonoid quercetin, and various structurally-related flavonoids, as regulators of Eag1 channels. Flavonoids are secondary plant metabolites found in all vascular plants that share a three-ring structure. Flavonoids have existed in nature for more than a billion years, and thus have interacted with evolving organisms for eons. Identified flavonoids slow channel deactivation and shift the conductance/voltage relationship toward more negative potentials in a concentration-dependent manner. For example, 10 uM quercetin shifted the Vhalf of the conductance/voltage relation an average of −13 mV, and slowed the tau of deactivation from 3 to 9 ms (in 5 separate experiments). Flavonoids retained their activity on Eag1 constructs lacking the PAS domain, the entire amino-terminus, or the post-CNBD of distal-carboxy terminal region. Further, identified flavonoids bind to the the isolated CNBD in a fluorescence-based binding assay. Thus, we predict that flavonoids activate Eag1 channels by binding to the CNBD of the carboxy-terminal region.

**2-Subg**
**The KvLm Potassium Channel in Asymmetric Bilayer**
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Bilayer lipid composition and charge asymmetry markedly alter the inactivation phenotype of a voltage-gated $K^+$ channel[1]. Here we investigate the effects of lipid head-group size and charge asymmetry on the permeation and gating of a sensor-less pore module from a voltage-gated potassium-selective ion channel (KvLm-PM) by using Droplet Interface Bilayers (DIBs). In DIBs a lipid bilayer is formed by contacting two aqueous droplets submerged in the hydrocarbon medium[2]. When each droplet contains a different lipid an asymmetric bilayer forms at the droplet interface. Five lipids of equal carbon chain length that differ in their head-group size and charge were selected to supplement DPhPC bilayers and form both symmetric and asymmetric bilayers. We found that a negative charge in either leaflet favors channel opening. Head-group charge also affects the unitary conductance of KvLm-PM. For example when both leaflets are negative, the conductance is increased.
[1]Schmidt D, MacKinnon R. Voltage-dependent K+ channel gating and voltage sensor toxin sensitivity depend on the mechanical state of the lipid membrane. Proc. Nat. Acad. Sci. USA 2008 DEC 9; 105(49): 19276-19281.
[2]Hagan Bayley *et al*. Droplet interface bilayers. Mol. BioSyst., 2008, 4, 1191-1208.

## Subgroup: Membrane Structure & Assembly

**3-Subg**
**Membrane-Active Peptides Derived From HIV-1 GP41: Could They Become Useful Therapeutic Tools?**
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Fusion between cell and viral membranes constitutes a key step of the human immunodeficiency virus (HIV) infectious cycle. To this end, the fusogenic gp41 transmembrane Env subunit makes use of a collapsible ectodomain structure (the hairpin or six-helix bundle) that opens and closes and two membrane-transferring regions, the fusion peptide (FP) and the membrane-proximal external region (MPER), which ensure coupling of hairpin closure to apposition and fusion of cell and viral membranes. The isolation of natural products, short peptides and neutralizing IgG-s, that interact with FP and MPER, respectively, and block the viral infection, suggests that these conserved regions might represent alternative targets for clinical intervention. FP and MPER-derived peptides have been shown to be membrane-active. Here, it is discussed the potential use of these specimens in the development of HIV fusion inhibitors and immunogens.

**4-Subg**
**Mechanisms of the Interactions Between Antimicrobial Peptides and Model Membranes: A Thermodynamic Hypothesis**
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The relation between the sequences of antimicrobial peptides, their mechanism, and specificity remains poorly understood. The specificity of these polypeptides varies, but there are no striking differences in their sequences. The generally accepted view that binding affinity of cationic peptides toward anionic bacterial membranes determines functional specificity is supported by experiments in model membranes, but beyond that little is clear. On the basis of the mechanisms of several different antimicrobial, cytolytic, and cell-penetrating peptides, including melittin, magainin, cecropin, delta-lysin, mastoparans, transportans, and some of their variants, we have proposed the hypothesis that it is the thermodynamics of peptide insertion into the membrane, from a surface-bound state, that determines their mechanism. We have begun to test this hypothesis by designing variants of the original set of peptides examined. The first results, which include the thermodynamics and kinetics of interaction of these peptides with phospholipid vesicles, are discussed here and compared to those obtained for the original set.
This work was supported by NIH grant GM072507.

**5-Subg**
**Mixing and Matching Detergents For Membrane Protein NMR Structure Determination**
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One major obstacle to membrane protein structure determination is the selection of a detergent micelle that mimics the native lipid bilayer. Currently, detergents are selected by exhaustive screening because the effects of protein-detergent interactions on protein structure are poorly understood. We have investigated the structure and dynamics of integral membrane proteins in different detergents by nuclear magnetic resonance (NMR) and electron paramagnetic resonance (EPR) spectroscopy and by small-angle X-ray scattering (SAXS). Initial results suggest that matching the micelle dimensions to the protein's hydrophobic surface avoids exchange processes that reduce the completeness of the NMR observations. We have extended these studies to additional membrane proteins with different properties to obtain a more complete understanding of the physical characteristics required of micelles to stabilize membrane protein folds. These findings provide a basis for the rational selection of micelles that may advance membrane protein structure determination by NMR.

**6-Subg**
**What Drives Membrane Protein Folding?**
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Understanding the forces that drive protein folding is essential for many of the central quests of structural biology including structure prediction, design and deciphering evolution. Nevertheless, we still have a rudimentary understanding of membrane protein folding energetics. I will describe tools we have developed for studying the factors that stabilize membrane proteins. Application of these methods suggests that packing forces, rather than hydrogen bonding, dominate membrane protein stabilization. Given the importance of dispersion forces, it is possible that membrane proteins might utilize packing more effectively than soluble proteins. We find that membrane proteins are not necessarily more efficiently packed, but manage to bury more surface area than soluble proteins. The results have implications for membrane protein evolution and their susceptibility to disease-causing mutations.