revealed association of *hERG1a*, *hERG1b*, and *SCN5A* mRNAs and their corresponding proteins hERG1a, hERG1b and $Na_V1.5$, indicative of a co-translational complex. Furthermore, the transcripts can be co-regulated in iPSC-CMs as indicated by a coordinate decrease of *SCN5A*, *hERG1a* and *hERG1b* transcript levels upon *hERG1b*-specific silencing. Whole-cell patch clamp revealed a corresponding reduction in the magnitude of the potassium I_{Kr} and sodium I_{Na} currents. The co-translational association and co-regulation of transcripts may represent a general mechanism by which cardiac cells coordinate expression and thus activity of different ion channel types. This mechanism may shed new light on cardiac rhythm maintenance and arrhythmogenic disorders, as well as other ion channel-related diseases.

1867-Plat

A Hyperpolarization-Activated Proton Channel in Zebrafish Sperm

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Most fish reproduce by external fertilization. Gametes are released into the aquatic habitat and the motile sperm need to find the egg. Ion channels in the plasma membrane of sperm are important players in the fertilization process. We have identified in sperm of the zebrafish *Danio rerio* a hyperpolarization-activated ion channel highly similar to HCN channels found in heart and brain. Strikingly, the channel is highly selective for protons that are carried into the cell during hyperpolarization. The selectivity of the channel is about as high as that of the Hv1 proton channel. We are currently investigating the molecular determinants of this proton selectivity by targeted mutagenesis and electrophysiological characterization.
The change in selectivity from Na+/K+ as in HCN channels to H+ as in the zebrafish channel investigated here is probably an evolutionary adaptation to enable fertilization in freshwater, where ion concentrations, especially that of Na^+, are particularly low.

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Insights on Gating Functions of Cytosolic Domains of Connexin26 Hemichannels Revealed by a Human Pathogenic Mutation (N14K)

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Connexin 26 (Cx26) is a hexameric, transmembrane protein. At the cell membrane, it can be found forming hemichannels or gap junction channels (GJCs) with opposing cells. The N-terminal domain (NT) of these channels is folded into the pore, playing an important role in permeability and gating. A group of mutations within the NT that produce aberrant hemichannels with increased basal activity is responsible for Keratitis-Ichthyosis-Deafness (KID) syndrome. In this study we focus on N14K, an NT KID mutant. Structural data reveals an interaction between the NT and cytoplasmic loop (CT) of the channel, where position 14 is located. Here, we explored how the N14K mutant affects the interactions between these two regions and consequently, promotes gain in function. Assessing macroscopic and single-channel recordings, we observed that the N14K mutant shows an increase in the energy barrier for the transition between open and closed states, shifting calcium sensitivity, voltage sensitivity, and deactivation time constants. Correlation analysis of Cx26 WT molecular dynamics (MD) simulations identified several sites of NT-CL interaction: These include interactions between N14 and residues H100 and Y97, and between K15 and E101. Interestingly, the same analysis performed on the Cx26 N14K MD simulations showed that the insertion of a Lys at position 14 completely disrupted the NT-CL interactions. To test this, we used double mutant cycle analysis, which showed that the NT-CL interaction does occur in the WT channel and is disrupted in the N14K mutant. Our data suggest that disruption of NT-CL interaction facilitates hemichannel opening and stabilizes the open state. In addition, it provides a mechanistic understanding of how mutations at position 14 cause human disease.

Platform: Membrane Structure

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Lipid Organization in Simulations of Cell Membranes

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Cell membranes contain multiple lipid and protein components, that are organized in the membrane plane. The membrane lateral organization is currently viewed as dynamic nano-scale clusters/assemblies of lipids and proteins, but the nature of these clusters remains elusive. They are described by a number of theories, the most common being the "raft hypothesis". According to these theories, the clusters/rafts could represent domains of an Lo-like phase, or composition (critical) fluctuations, or a micro-emulsion. The clusters lie below the spatio-temporal resolution of the most experimental techniques, but are supported by a large number of indirect studies. These studies have shown that cell membrane extracts separate into Lo and Ld phases at lower (room) temperatures, but the phase state at higher (physiological) temperatures is to be uncovered.
Here we investigate the temperature-dependent phase behaviour of a model membrane with a realistic lipid composition. The model is based on an idealized plasma membrane, with an asymmetric distribution of components between the leaflets [JACS, 2014, 136, 14554]. We use molecular dynamics simulations with the coarse-grained Martini force field. We investigate the changes in the membrane lateral structure in a wide temperature interval of 260-330 K, and observe dynamic nano-scale lipid clusters. We characterize the properties of these clusters, including their local composition and structure, sizes and lifetimes, coupling between the leaflets, and lipid flip-flop.

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Cholesterol-Induced Membrane Organization Promotes Influenza Virus Binding

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The influenza virus attaches to a cell surface by binding sialic-acid containing receptors with the viral ligand hemagglutinin. Stable viral binding requires more than one viral ligand- host receptor interaction to simultaneously occur. Thus, binding can be modulated by the spatial distribution of target receptors in the host membrane. We show that membrane sterols critically control viral attachment and propose that sterols alter the nanoscale clustering of viral receptors to facilitate binding. Using single virus fluorescence microscopy, we demonstrated that viral binding is dependent on the cholesterol content of the target membrane. Fluorescently labeled viral particles preferentially bound synthetic membranes supplemented with increasing amounts of cholesterol (0-40 mol%). Other sterols exhibited a similar effect on binding, independent of their ability to support liquid-liquid phase separation. To develop a molecular explanation for cholesterol's effect, we ran a series of course grained molecular dynamics simulations of lipid bilayers containing the viral receptor, disialoganglioside GD1a. While simulated GD1a molecules self-associated independent of cholesterol, the dissociation rate between pairs of GD1a molecules was a function of bilayer cholesterol concentration. Additionally, cholesterol increased the order parameter of simulated GD1a lipid tails. We suggest that, by preordering the viral receptor in its monomeric state, cholesterol lowers the entropic penalty of receptor association. This in turn promotes the formation of GD1a multimers and increases the influenza virus binding avidity of the lipid bilayer. Our findings assign a critical role to the host cellular membrane in viral infectivity and reveal sterol-dependent membrane organization not associated with phase separation.

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Nanoscale Membrane Curvature Generated by Cholera Toxin Subunit B: The Effects of Lipid Cross-Linking and Lipid Phase

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With polarized localization microscopy (PLM), we have resolved the inherent membrane bending capability of cholera toxin subunit B (CTxB) in supported lipid bilayers. PLM is single-molecule localization microscopy technique capable of revealing membrane orientation with super-resolution. Membrane buds of <50 nm radius were observed to grow into >200 nm radius and extended tubules with dependence on the membrane tension, CTxB concentration, and the number of GM1 bound per CTxB. However, the membrane bending induced by CTxB was apparently independent of the lipid phase characteristics of the GM1 or the surrounding lipids. The CTxB was (12 ± 4)x more concentrated on the positive curvature top and (26 ± 11)x more concentrated on the negative Gaussian curvature neck of the nanoscale membrane buds compared to the surrounding planar supported lipid bilayer. Whereas CTxB is frequently used as a marker for liquid-ordered lipid phases, the coupling between CTxB and membrane bending provides an alternate