face significant free energy barriers, but with very different underlying mechanisms: the energetics of charged species involving strong interactions with water molecules and lipid head groups throughout the bilayer while the neutral species experiences simple dehydration. We found that local membrane deformations preferentially stabilize the charged form leading to a significant pKa shift only at the bilayer center. However, we conclude that the energetics for arginine movement in membranes is governed almost exclusively by the protonated form. In contrast, analog molecules experience much greater shifts due to exaggerated variations in energetics across the bilayer, emphasizing the important role played by the host protein. The large free energy barrier experienced by arginine suggests that these side chains would not be exposed to the lipid hydrocarbon region of a bilayer, with implications for models of voltage gated ion channel activity.

## 2102-Pos Effects of Cholesterol and Phosphoethanolamine on Thermodynamics for Self-Association of an Inert Transmembrane

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**Board B217**

Physico-chemical properties of bilayers, such as hydrophobic thickness, membrane rigidity, and lateral pressure should affect self-association of transmembrane helices, which is a crucial thermodynamic step for membrane protein folding, by changing the balance between helix-helix, helix-lipid, and lipid-lipid interactions. We have measured the complete set of thermodynamic parameters ($\Delta G$, $\Delta H$, $\Delta S$, and $\Delta C_p$) at 5–55°C for antiparallel dimer formation of the inert transmembrane helix X-$(AALALAA)_3$-Y (X = NBD and Y = $NH_2$ (**I**) or X = Ac and Y = DABMI(**II**)) by fluorescence resonance energy transfer from **I** to **II** in PC vesicles (Biochemistry **2006**, *45*, 3370). Here, we examine the effects of cholesterol and PE, which modulate the rigidity and lateral pressure of bilayers, respectively. It was confirmed that the peptide assumes a transmembrane helical structure in POPC bilayers containing cholesterol (30 mol%) or POPE (50 mol%), by polarized ATR-FTIR spectroscopy. Stronger dimerization was observed in POPC/cholesterol (7/3) vesicles ($\Delta G = -20.9$ kJ mol$^{-1}$, $\Delta H = -65.3$ kJ mol$^{-1}$, $-T\Delta S = 44.4$ kJ mol$^{-1}$, and $\Delta C_p = 1.3$ kJ K$^{-1}$mol$^{-1}$) than in POPC vesicles ($\Delta G = -12.8$ kJ mol$^{-1}$, $\Delta H = -27.9$ kJ mol$^{-1}$ and $-T\Delta S = 15.1$ kJ mol$^{-1}$) at 35°C. The effect of POPE on thermodynamics for the formation of the antiparallel dimer will also be reported.

## 2103-Pos Properties of WALP23 in Mixtures of Oriented Lipids Investigated by Solid-State $^2$H and $^{31}$P NMR Spectroscopy

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**Board B218**

Biological membranes represent complex mixtures of lipids of different types with varying hydrocarbon chain lengths. In this project the behavior of model WALP23 peptides (Killian *et al*. 1996. *Biochemistry 35*:1037; Strandberg *et al.* 2004. *Biophys J 86*:3709) in lipid mixtures was investigated by using $^{31}$P as a probe for the lipid head groups and $^2$H-alanine as a probe for the peptide. The bilayer normal was aligned either parallel (β=0°) or perpendicular (β=90°) to the applied magnetic field. WALP23 (acetyl-GWW$(LA)_8$LWWA-ethanolamide) has a hydrophobic length approximately corresponding to the hydrophobic portion of DOPC (di-C18:1Δ9c) bilayers. In shorter DLPC (di-C12:0), the peptide "kinks" at position Leu$^{12}$ (Daily *et al. Biophys J* in press), while in longer DEuPC (di-C22:1Δ13c) it induces an isotropic lipid phase. Therefore, it was of interest to study WALP23 behavior in lipid mixtures composed of differing proportions of DLPC, DOPC and DEuPC. It was found that the bilayer structure is generally preserved in lipid mixtures, as shown by $^{31}$P NMR, although some isotropic phase may be induced depending upon the peptide/lipid ratio and the DEuPC/(total lipid) ratio. WALP23 incorporates into the lipid bilayers, judging by the two-fold reductions of quadrupolar splitting magnitudes at β=90° compared to β=0°. The influence of bilayer lipid composition upon the geometry and average orientation of WALP23 will be examined in detail.

### Intracellular Channels

## 2104-Pos Wetting, Ionic Conductance, and Structural Stability of the Phospholamban Pentamer

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**Board B219**

Phospholamban (PLN) is a 6-kDa membrane protein that forms a stable pentamer in a lipid bilayer membrane. As a monomer, PLN directly inhibits the Calcium ATPase, thereby regulating cardiac muscle contraction. Earlier reports have suggested that a PLN oligomer can also regulate calcium flux across the sarcoplasmic reticulum membrane by acting as a calcium ion channel. The recent NMR structure of a PLN pentamer revealed a 7.2-A-diameter, entirely hydrophobic pore. In this study, we used molecular dynamics to investigate the stability of the NMR model and determine whether the PLN pentamer can transport ions. A microscopic model of the PLN pentamer was constructed by merging the NMR structure with a patch of a solvated lipid bilayer membrane and physiological solution. To explore the structural dynamics of the pentamer, the system was equilibrated for over 30 ns. Within less than one nanosecond of the onset of equilibration, a chain of water that was initially placed in the pentamer's pore disappeared. After several nanoseconds, the pentamer underwent structural rearrangements resulting in partial closure of the pore at the cytoplasmic side.

The average pore radius decreased from 3.6 to 2.4 angstroms. Without water in the pore, the PLN pentamer is highly unlikely to exhibit significant ion conductance. To quantitatively demonstrate this conjecture, steered molecular dynamics was employed to induce permeation of chloride and calcium ions through the pore. During each passage, the forced ions were observed to partially lose their hydration shells. A chain of water formed transiently in the pore after each ion passage. The results of our study strongly suggest that a PLN pentamer, in the conformation resolved by NMR, does not form an ion channel.

**Ryanodine Receptors**

## 2105-Pos Generation of the GFP-tagged Type 1 Ryanodine Receptor Mutants by Transposon-based Random Insertion Approach

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**Board B220**

Ryanodine receptor (RyR) is a family of intracellular $Ca^{2+}$ release channels in the sarcoplasmic reticulum in skeletal muscle and plays an important role in excitation-contraction coupling. A relatively small C-terminal region (500–1,000 residues) of the RyR constitutes an ion channel and the other N-terminal region forms a large cytoplasmic assembly which serves as the regulatory domain. The structure-function relationship of RyR, especially in the cytoplasmic assembly, however, remained still unclear because of its large size. A tansposon-based random insertion approach is a powerful tool to study the structure-function relationship of large proteins. In this study, we applied this method to investigate the domain structure and function of the type 1 RyR (RyR1) by inserting GFP. The Tn5 transposon encompassing the kanamycin-resistant gene (*Kan*$^r$) which was flanked by two unique restriction enzyme sites (*Asc*I and *Pac*I) was randomly inserted into full-length RyR1 cDNA via in vitro transposon reaction using the EZ::TN transposase. Clones with an in-frame insertion of transposon were selected by kanamycin resistance, followed by DNA sequencing. Then, *Kan*$^r$ was replaced by genes encoding GFP variants by *Asc*I / *Pac*I digestion. The resultant clones were transfected into Flp-In T-REx HEK293 cells to screen GFP fluorescence. The GFP-positive clones were tested by caffeine-induced $Ca^{2+}$ release and some of these clones showed a reduced caffeine sensitivity. We are currently investigating [$^3$H] ryanodine binding and single channel activity of the GFP-tagged RyR1 mutants which was stably expressed in HEK293 cells. The transposon-based random insertion approach should be a powerful tool to study structure-function relationships of the RyR channels.

## 2106-Pos Interaction Of The K3614-N3643 Calmodulin-binding Domain With The C4114-N4142 Region Of The Ryanodine Receptor (RyR1) Is Involved In The Mechanism Of Ca2+/agonist-induced Channel Activation

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**Board B221**

We tested the hypothesis that the interaction of the $K^{3614}$-$N^{3643}$ calmodulin (CaM) binding domain (CaMBD) with its neighboring domain (e.g. CaM-like domain) serves as an intrinsic regulator of RyR1 by performing the following experiments (a and b). (a) We raised antibodies against synthetic peptides corresponding to the CaMBD and the $C^{4114}$-$N^{4142}$ region, which is a part of the CaM-like domain. Both antibodies produced significant inhibition of [$^3$H] ryanodine binding activity, and the antibody concentration required for inhibition increased with an increase of [$Ca^{2+}$] from 0.1 to 1.0 μM. These data suggest that the binding of antibody to either side of the interacting domains interfered with $Ca^{2+}$-dependent formation of a 'channel activating link' between the two regions. (b) In order to monitor the state of inter-domain interaction and its changes during the $Ca^{2+}$/agonist-mediated channel activation, we employed the Stern-Volmer fluorescence quenching analysis of the accessibility of the fluorescent probe MCA, which was incorporated to the partner domain of CaMBD, to a large size fluorescence quencher, BSA-QSY. The quencher accessibility of the attached probe (namely, the size of the gap between the interacting domains) decreased with an increase of [$Ca^{2+}$] from 0.03 μM to 2.0 μM. The $Ca^{2+}$-dependent decrease in the quencher accessibility was more pronounced in the presence of 4-CmC, and was reversed by 1 mM $Mg^{2+}$ (well-known inhibitor of $Ca^{2+}$/agonist-induced channel activation). These results suggest that the $K^{3614}$-$N^{3643}$ and $C^{4114}$-$N^{4142}$ regions of RyR1 interact with each other in a $Ca^{2+}$/agonist-dependent manner, which serves as a mechanism of $Ca^{2+}$/agonist-dependent activation of the RyR1 $Ca^{2+}$ channel.

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## 2107-Pos Transient Relief Of Mg2+ Inhibition Of RyR1 Ca2+ Channel Is A Part Of The Mechanism Of 'Domain Switch'-mediated Channel Activation

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**Board B222**

Relatively high concentrations of $Mg^{2+}$ (~1 mM), normally present in the myoplasm, inhibit $Ca^{2+}$/pharmacological agonist (PA)-dependent channel activation, but the voltage sensor-mediated physi-