a comparative study of the structural properties of IL2 in β1AR, β2AR and 5HT2AR, as well as the P5.37A mutants. We found that IL2 in both β1AR and β2AR folds back to the original conformations even after unfolding at 1210K. IL2 in 5HT2AR folded from an initial non-helical conformation into a helical one with similar fold and orientation as in β1AR. In the P3.57A mutants of IL2 in 5HT2AR and β1AR, the helical structure melted. Together, our calculations indicate the existence of at least two major conformational families for IL2 (in agreement with crystal structures), and the role of a conserved Pro in the interconversion between them.

2215-Pos Board B185

An Atomic-level Model for the Periplasmic Open State of Lactose Permease

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Membrane transport proteins play significant roles in human physiology, drug transport, bacterial resistance to antibiotics, and diseases. Lactose permease of *E. coli* (LacY) transports various disaccharides and is a member of the major facilitator superfamily of proteins that exists in a broad range of organisms from archaea to the human central nervous system. Since only the atomic-level structure for the cytoplasmic open state of LacY has been determined, it is our objective to obtain a structure (or set of structures) of LacY open to the periplasm by utilizing a two-step hybrid approach of molecular simulations. In the first step, self-guided Langevin dynamics (SGLD) with an implicit membrane but explicit water is used to enhance conformational sampling. SGLD was found to significantly enhance protein motions compared to identical implicit membrane molecular dynamics (MD) simulations. Significant periplasmic conformational changes are only observed in simulations with Glu^{269} protonated and a disaccharide in the binding site, which is based on several simulations with different initial structures. LacY helix-helix distances obtained from double electron-electron resonance (DEER) experiments (Smirnova et al., *PNAS*, 2007) are used to select protein conformations consistent with a periplasmic open state. In the final step, explicit membrane MD simulations with screened structures from the implicit membrane simulations converged to periplasmic open structures. This hybrid implicit/explicit bilayer approach results in LacY structures that transition from a periplasmic closed state (pore radius, R_p, of ~1Å) to one fully open the periplasm (R_p= 3Å). The helices on the outside of the protein are the first to fan out (H-III/IV then H-VIII) before there is a concerted motion of the periplasmic half. This two-step simulation approach in conjunction with experiments may be successful in predicting conformational changes of other membrane proteins.

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Stochastic Switching Into Hydrolytically Active Conformations In A Homodimeric ABC Exporter

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ATP binding cassette (ABC) transporters are a large family of membrane proteins with high clinical relevance in, for example, bacterial multidrug resistance, tumor resistance, cystic fibrosis, or insulin secretion. Bacterial ABC exporters are homodimers in which each identical half contributes a transmembrane domain (TMD) and a nucleotide binding domain (NBD). Many mammalian ABC transporters, instead, consist of asymmetric halves. ABC transporters are thought to hydrolyze MgATP only at one of their two nucleotide binding sites at a time. In homodimeric ABC exporters, the process of switching one of the binding sites into a hydrolytic conformation ought to be stochastic. Recent evidence suggests that the asymmetry in the binding sites of various mammalian exporters induces a directional preference in their nucleotide hydrolysis that may improve the choreography of complex transport processes. Currently, it is poorly understood how exactly the switching of only one binding site into a hydrolytically favorable conformation occurs. Furthermore, it is mostly unknown how this conformational change is reflected at the NBD-TMD interface. In this study, we apply molecular dynamics simulations to probe the switching of the MgATP-bound bacterial multidrug exporter Sav1866 into pre-hydrolytic states. The simulations are performed of the full-length structure embedded in a phospholipid bilayer. Our simulations show that the switching in Sav1866 is of stochastic nature. We identify specific changes at the binding sites that characterize a pre-hydrolytic conformation, and show that the switching event causes pronounced changes in NBD-TMD interactions. We also extend our findings to asymmetric transporters and suggest mechanisms of directionality in the nucleotide handling of some mammalian ABC transporters.

2217-Pos Board B187

Understanding the conformational changes in Ca-APTase using Coarse-grained and All-atom simulations with Dynamic Importance Sampling

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The sarcoplasmic reticulum (SR) ATPase (SERCA) actively transports calcium ions across the membrane. A number of sequential steps are involved in the catalytic cycle, starting with the binding of two Ca2+ ions to the ground state (E2) to form a phosphorylated intermediate (ADP.E1P.2Ca2+). ADP dissociation is followed by the isomerization of E1P.2Ca2+ to E2P.2Ca2+ and dissociation of Ca2+, finally hydrolytic cleavage of Pi from E2P. Twenty five mutations have been identified on the Actuator (A), Phosphorylation (P) and Nucleotide binding (N) domains that have significant impact on the structure and function of Ca-ATPase (Toyoshima et al, Biochemistry (2005), 44, 8090-8100). While a lot has been studied about the relative positions of domains and the structural changes involved in the catalytic cycle, the actual kinetics and conformational transitions are yet to be explored. The main focus of this research is to study the impact of these mutations on the kinetics of reactions involving conformational changes in the catalytic cycle of SERCA. Since this required generating many sets of transitions between intermediate states, we have implemented the coarse-grained protein and lipid model (Marrink et al, JCTC(2007) 4(5), 819-834) in CHARMM. Coarse-grained models have been used to address the problem of time scales inaccessible to the all atom approach. We use Dynamic Importance Sampling (DIMS) to generate transitions between the intermediate states. Transitions are generated between each mutated open and closed conformational state in both coarse-grained and all atom model in CHARMM. A comparison of both sheds light on the kinetics and the nature of transitions involving structural changes during the opening and closing of the pump.

2218-Pos Board B188

Mechanisms and Energetics of Protein/Peptide Interactions in Biological Membranes

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Understanding the delicate balance of forces governing helix/β-hairpin interactions in transmembrane proteins is central to understanding membrane structure and function. These membrane constituent interactions play an essential role in determining the structure and function of membrane proteins, and protein interactions in membranes, and thus form the basis for many vital processes, including transmembrane signaling, transport of ions and small molecules, energy transduction, and cell-cell recognition. ''Why does a single transmembrane helix or β-hairpin have specific orientations in membranes?'' ''What are the roles of hydrogen bonds, close packing, and helix-lipid or β-hairpin-lipid interactions in helix or β-hairpin associations in membranes?'' ''How do these interactions change the membrane structures?'' ''How do transmembrane domains transmit signals across membranes?'' These are fundamentally important biophysical questions that can be addressed by understanding the delicate balance of forces governing helix/β-hairpin interactions in membranes. Recently, we has published novel methods and their applications that begin to address the complicated energetics and molecular mechanisms of these interactions at the atomic level by calculating the potentials of mean force (PMFs) along reaction coordinates relevant to helix/β-hairpin motions in membranes, and dissecting the total PMF into the contributions arising from physically important microscopic forces [1-5]. In this work, I will summarize our research accomplishment so far, and present recent research activities to elucidate the influence of helix tilting on ion channel gating and the molecular basis of transmembrane signaling.

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2219-Pos Board B189

Protein Modification Analysis of GM2 Activator Protein Mutants by High Performance nano-LC ESI FT-ICR Mass Spectrometry

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GM2AP is an 18 kDa protein that is involved in the catabolism of the ganglioside GM2. GM2AP extracts GM2 from intralysosomal vesicles and orients the

oligosaccharide head group for hydrolytic cleavage by the hydrolase, HexA. Mutations in GM2AP or HexA lead to an accumulation of GM2 in the lysosomes, causing lysosomal storage diseases such as Tay Sachs or the AB variant of Sandhoff's disease. Our lab is utilizing site directed spin-labeling (SDSL) with electron paramagnetic resonance (EPR) spectroscopy to probe the conformational changes and the membrane bound orientation of GM2AP. This protein contains eight naturally occurring cysteine (CYS) residues involved in four disulfide bonds. With site directed mutagenesis, a ninth CYS residue is introduced as the reporter site for spin labeling . A series of 10 single CYS mutants have been generated. To validate the EPR results, the mass spectrometry protocol described here was developed to characterize spin-labeled GM2AP samples. For mass spectrometry measurements, either biotin-linked maleimide (BM) or 4-maleimide tempo (4MT) were used to modify and trap available CYS residues in a thioesterbond. The remaining eight native CYS residues, which are disulfide bonded, are then reduced and modified with iodoacetamide. Samples were analyzed by high performance nano-liquid chromatography electrospray ionizaton Fourier-transform ion cyclotron resonance mass spectrometry (FT-ICR MS) equipped with a 14.5 T magnet. FT-ICR spectra of enzymatically digested BM-labeled or 4MT-labeled GM2AP were utilized to determine to which CYS residue is modified with the maleimide moeity. Those sites labeled with acetamide are inferred to have been disulfide bonded. The fragment that contains the maleimide moeity tells us, which CYS residue (and how many CYS residues) is accessible for reaction with the spin label for EPR studies.

2220-Pos Board B190

Purification And Reconstitution Of The Connexin43 Carboxyl Terminus Attached To The 4Th Transmembrane Domain In Detergent Micelles

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In recent years, reports have identified that many eukaryotic proteins contain disordered regions spanning greater than 30 consecutive residues in length. In particular, a number of these intrinsically disordered regions occur in the cytoplasmic segments of plasma membrane proteins. These intrinsically disordered regions play important roles in cell signaling events, as they are sites for protein-protein interactions and phosphorylation. Unfortunately, in many crystallographic studies of membrane proteins, these domains are removed because they hinder the crystallization process. Therefore, a purification procedure was developed to enable the biophysical and structural characterization of these intrinsically disordered regions while still associated with the lipid environment. The carboxyl-terminal domain from the gap junction protein connexin43 attached to the 4th transmembrane domain (TM4-Cx43CT) was used as a model system (residues G178-I382). The purification was optimized for structural analysis by nuclear magnetic resonance (NMR) because this method is well suited for small membrane proteins and proteins that lack a well-structured three-dimensional fold. The TM4-Cx43CT was purified to homogeneity with a yield of ~6 mg per liter from C41(DE3) bacterial cells, was reconstituted in the anionic detergent 1-palmitoyl-2-hydroxy-sn-glycero-3-[phospho-RAC-(1-glycerol)], and circular dichroism and NMR were used to demonstrate that the TM4-Cx43CT was properly folded into a functional conformation by its ability to form a-helical structure and associate with a known binding partner, the c-Src SH3 domain, respectively.

2221-Pos Board B191

A Refinement Protocol to Define the Structure, Topology and Depth of Insertion of Membrane Proteins using Hybrid Solution/Solid-state NMR Restraints

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To fully describe the folding space and ultimately the biological function of membrane proteins (MPs), it is necessary to determine their interactions with lipid membranes. This intrinsic property of MPs referring to as structural topology is not possible to resolve using x-ray crystallography or solution NMR. Here, we present a hybrid solution and solid-state NMR approach for the simultaneous determination of the structure, topology, and depth of insertion of MPs. Distance and angle restraints obtained from solution NMR of MPs solubilized in detergent micelles are combined with backbone orientational restraints (15N chemical shift anisotropy and 15N-1H dipolar couplings) derived from solid-state NMR in orientated lipid bilayers (PISEMA) into a hybrid objective function. In addition, a supplementary energy term, Ez (insertion depth potential), is used to ensure the correct positioning of helical MPs domains with respect to a virtual membrane. The hybrid objective function is optimized using a two-stage simulated annealing protocol and is implemented into XPLOR-NIH software for general use. To validate the hybrid method, the effects of chemical shift tensor orientations, principal tensor values, and dipolar constant magnitudes on the structural ensemble are determined.

The hybrid method is applied to monomeric and pentameric PLN (phospholamban), a integral MP that regulates sarco(endo)plasmic reticulum Ca-ATPase (SERCA) function in cardiac muscle. The hybrid conformational ensemble defines the structure, topology and depth of insertion of PLN in lipid bilayer simultaneously. This ensemble is compared with solution NMR structures in DPC micelle obtained using conventional solution NMR data (NOEs, J-couplings) and residual dipolar coupling as orientational restraints.

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2222-Pos Board B192

Solution and Solid-State NMR Analysis of Phosphorylated and Pseudo-Phosphorylated Phospholamban

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Phospholamban (PLN) is a 52 residue transmembrane protein that regulates the Ca2+ ATPase (SERCA) of muscle cells. At low μM Ca2+ concentration, PLN binds to SERCA and decreases the rate of uptake of Ca2+ into the sarcoplasmic reticulum (SR) thereby promoting muscle relaxation. This inhibitory activity is reversed by phosphorylation of PLN at Serine-16 or Threonine-17 in response to ß-adrenergic stimulation. Phosphorylation of PLN is associated with increased cardiac contractility of muscle cells due to the larger Ca2+ load into the SR.

PLN has become important as a therapeutic target in fighting heart failure, a complex disease associated with impaired cardiac contractility. It has been shown that delivering of phospho-mimiking PLN (Ser-16 → Glu substitution) to failing mice cardiomyocytes significantly improves contractility.

Although functional effects of PLN phosphorylation have been extensively studied, the mechanistic details of how phosphorylated and pseudo-phosphorylated (S16E) PLN interacts with SERCA to reverse inhibition are still unclear. In here we present data on the structural characterization of Ser-16 phosphorylated and S16E monomeric and pentameric phospholamban (AFA-PLN and WT-PLN) in the presence and absence of SERCA as probed by solution and solid-state NMR spectroscopy in detergent micelles and oriented lipid bilayers. For solid-state NMR, SERCA and PLN were reconstituted in planar lipid bilayers and uniaxially aligned on glass plates. Residue-specific information as well as topology of PLN monomer and pentamer was determined by PISEMA experiments.

2223-Pos Board B193

Hybrid Solution and Solid-State NMR Analysis of SERCA/Phospholamban Interactions in lipid membranes: From Structural Dynamics to Function

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Phospholamban (PLN) is a sarcoplasmic reticulum (SR) integral membrane protein that regulates calcium translocation in cardiac muscle. Upon interaction with SERCA (the SR calcium ATPase). PLN decreases the rate of calcium uptake, reducing the apparent affinity of the enzyme for Ca^{2+} ions. This process is reversed by adrenergic stimulation of protein kinase A, which phosphorylates PLN at Ser16, re-starting the muscle contraction cycle. Here, we present the hybrid solution and solid-state NMR structural analysis of PLN in its pentameric (storage), monomeric (active), and SERCA-bound forms in lipid membranes. This knowledge about the structural dynamics PLN under these different stages is used to steer the extent of PLN control on SERCA activity. These findings lay the groundwork for the rational design of PLN loss-of-function mutants to be used in gene therapy.

2224-Pos Board B194

Topology of Phospholamban when Bound to Ca2 ± ATPase by Solid-State NMR

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Calcium cycling in muscle cells drives the relaxation and contraction of both skeletal and heart tissue. The sarcoplasmic reticulum (SR) Ca^{2+}-ATPase is central in the relaxation of the heart, accounting for ~70% of calcium sequestration. Phospholamban (PLN) is a small integral membrane protein regulator of Ca^{2+}-ATPase. Its inhibition of the enzyme is shown in calcium dependence ATPase activity curves, resulting in decreased ATPase affinity for Ca^{2+}. While there have been several successful attempts to gain structural knowledge of the complex between PLN and Ca^{2+}-ATPase, no high-resolution structure exists.