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.

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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.

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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.

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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