Membrane Protein Structure II

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Rhodopsin in Bovine Rod Outer Segment Disk Membranes Exhibits Two Thermal Transitions

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The photoreceptor, rhodopsin is a GPCR in rod outer segment disk membranes. Activation by light converts the dark-adapted form, rhodopsin is to the bleached form, opsin. Differential scanning calorimetry (DSC) studies showed that rhodopsin and opsin each exhibit an irreversible scan rate dependent endothermic transition (T_m) at approximately 72°C and 55°C respectively. We report here a scan rate dependent exothermic transition. Solubilization was used to examine the contribution of the bilayer. Freeze-thaw cycles were used to examine rhodopsin aggregation in these transitions. Disk membranes were sequentially solubilized in octylglucoside (OG) until rhodopsin was completely delipidated. DSC experiments were performed using a MicroCal VP-DSC microcalorimeter. Samples were scanned at 15, 30, 60 and 90°/hr. Because the protein transitions are irreversible, a second scan was used to determine the baseline. As the OG partitioned into the bilayer the endothermic T_m and E_{act} (activation energy of denaturation) rapidly decreased. Both then remained constant following rhodopsin solubilization. At low detergent concentration the exothermic T_m increased rapidly then remained constant after solubilization. Unlike the endothermic E_{act}, the degree of solubilization had little effect on the exothermic transition E_{act}. Freeze-thaw cycles generated disks in which rhodopsin was aggregated. Increasing aggregation resulted in a decrease in the T_m of the exothermic transition, but had no effect on the endothermic T_m of either rhodopsin or opsin. These results are consistent with earlier cross-linking studies suggesting the exothermic transition results from aggregation. These results indicate that the rhodopsin endothermic transition is due to weakening of the tertiary structure interactions accompanied by changes in the packing of the trans-membrane helices as well as changes in protein-lipid interactions. CD data indicate that rhodopsin secondary structure is almost unchanged.

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NMR Structure of Water-Solubilized Transmembrane Domain of Nicotinic Acetylcholine Receptor

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No high-resolution experimental structure of the transmembrane (TM) domains of the nicotinic acetylcholine receptor (nAChR) is currently available. We have addressed some critical issues related to structure determination by strategically mutating 22 hydrophobic, putatively lipid-facing residues to hydrophilic ones, so that the resulting TM domains have improved water solubility while maintaining the native fold. Two different designs based on the nAChR α1 subunit were attempted. The first (TM123-4) consisted of the four TM domains in their natural sequential arrangement, with an artificial linker between TM3 and TM4. The second (TM4-123) has TM4 moved before TM1. TM123-4 failed to be expressed by E. coli, suggesting possible cell toxicity. In contrast, TM4-123 was expressed with high yield and produced stable samples for structure characterization by high-resolution NMR. Monomeric TM4-123 structure was resolved to a backbone RMSD of 1.2 Å. The overall structure of TM4-123 is similar to that of the cryo-EM model, except that the very short TM2-TM3 loop (VPLI) found in the cryo-EM structure was replaced by a significantly longer loop (LIPSTSSA) and shifted by 5 residues towards the TM2 domain. More interestingly, 'TSSA' in this loop exists in two conformations: a rigid helix and a flexible coil, and these two conformations are in slow exchange on the NMR timescale. Given that the interaction between the TM2-TM3 loop and the extracellular domain likely mediates channel gating, the two conformations in this important loop might represent the critical transitions required for the channel to open. The high-resolution structure of water-solubilized nAChR TM domains allows us to better understand the structural basis of channel function (Funded by NIH R37GM049202, R01GM056257, and P01GM055876).

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The Active Conformation Of Opsin (ops*): Role In Signal Transduction And Regeneration

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Functional modules are specific subsets of the proteome, which fulfil an autonomous function in living cells. Functional modules in retinal photoreceptors include those devoted to phototransduction and regeneration [1]. In signal transduction, rhodopsin is kept inactive in the dark by a set of structural elements and residues conserved in the E(D)RY and NPxxY(x)5,6F regions of the receptor protein. On light activation, the same residues are crucial determinants of the Meta II state [2], with the hallmark of a motion of the sixth of the seven transmembrane helices [3]. The same elements stabilize the ligand free active form of the opsin apoprotein, Ops* [4], and build up new interactions of Ops* with the G-protein α-subunit C-terminal interaction site [5]. The Ops* state can thus act as the key element in both functional modules of the rod cell. In signal transduction, the Ops* conformation is present in the highly active Meta II photoproduct. In rhodopsin regeneration, Ops* facilitates the uptake of 11-*cis*-retinal into the binding site [6] and causes the visual system to behave as if it is experiencing an ''equivalent background'' [7].

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2213-Pos Board B183

Agonist and Inverse agonist Induced Conformation Changes in G-protein Coupled Receptors

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Conformational flexibility in G-protein coupled receptors and the effect of ligand binding on this flexibility is important for an understanding of functional specificity of ligands. Ligands of varied efficacies such as agonists, partial agonists, inverse agonists, and antagonists have different effects on the receptor conformation depending on its binding affinity and efficacy. 3D structural information of these ensemble of conformations stabilized by various ligands would be very critical to understand the molecular basis of ligand efficacy. To this end, we have developed a computational method, Ligand Induced Transmembrane Conformational changes (LITiCon), based on simultaneous optimization of the ligand induced movement of the seven transmembrane helices, followed by molecular dynamics simulations in explicit lipid and water for predicting the conformational changes that occur on ligand binding. We have studied the conformational changes resulting from the activation of bovine rhodopsin starting from the crystal structure of the inactive (dark) state. The predicted active state of rhodopsin satisfies all of the 30 known experimental distance constraints and also correlates well with the G-transducin peptide bound opsin crystal structure. Details of these results will be presented. Results for agonist, partial agonist and inverse agonist bound to β2-adrenergic receptor and β1-adrenergic receptor will also be presented. We find that the agonist induced larger conformational changes in the transmembrane region as compared to the inverse agonist. Our calculations for the chemokine receptor CCR3 shows that the small molecule antagonist UCB35625 binds in a different location in two similar receptors CCR1 and CCR3 whereas the small molecule agonist CH0076989 binds only to CCR3 and not to CCR1.

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Probing the Dynamic Structure and Function of Intracellular Loop 2 in Structurally Cognate GPCRs

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The increasingly frequent identification of functional attributes of loops connecting TMS in transmembrane proteins such as GPCRs, prompted us to address the structures of loops in the context of specific mechanistic hypotheses about their role in protein-protein interaction underlying signaling processes. Experimentally and computationally-derived evidence suggest that these functional properties of the loops are directly dependent on their specialized structure and positioning relative to the rest of the protein. The intracellular loop 2 (IL2) of class A GPCRs is such an example. It is known to be involved both in G-protein-coupling and in regulating β-arrestin binding and β-arrestin-mediated internalization. Structural information about IL2 reveals differences in conformation even between nearly identical short loops in different GPCRs (e.g., IL2 has different secondary structure in β1AR and β2AR: helical v.s. non-helical), as well as roles for key residues (e.g., replacing of the well conserved Pro3.57 by Ala in β2AR and 5HT2CR decreases β-arrestin binding and internalization (Marion et al, JBC 2006)). We have used a recently described *ab initio* method for loop structure prediction (Mehler et al, Protein 2006) for

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.

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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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