assemblies which alter the permeability of lipid membrane for ions and small molecules. These so called channel or pore forming proteins can either have one, two or three TM spanning domains. Albeit structural information is available for some of them, neither the number of monomers forming the functional pore or the proper orientation of most of the monomeric units within the assembly is available.
Computational methods are used to model the functional pore architecture of some of the proteins encoded by HIV-1, SARS-Co and Polio virus. Based on these findings novel pore motives need to be discussed and verified by experiments.

1735-Pos Board B579
Predicting Strained Regions for Folding and Protein-Protein Interaction in Bacterial Outer Membranes
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Beta-barrel membrane proteins are found in the outer membrane of Gram-negative bacteria, mitochondria, and chloroplasts. Exposed on the surface of bacterial cells, many beta-barrel proteins are initial sites of attachment by bacteriophages and bacterial toxins. Little information is known on the stability of local regions of these membrane proteins, and how they interact with each other. We have developed a model to quantify energy level of transmembrane strands in beta-barrel membrane proteins. We showed that our predictions on PagP are consistent with recent experimental NMR studies of protein dynamics [1] and mutant studies of folding stability [2]. We further found that the strands located in the interfaces of protein-protein interactions are considerably less stable by our model We have further developed a method to predict interface of protein-protein interaction of beta-barrel membrane proteins. In a data set of 25 beta-barrel membrane protein structures with less than 32% pairwise sequence identity, we can predict whether the protein will form a monomeric or multimeric structure with 91% accuracy and identify the protein-protein interaction interface with an accuracy of 86% in leave one out tests using sequence information only. We also report results of predicting genome-wide protein-protein interaction in bacterial outer membranes.
[1] Evanics F., Hwang P.M., Cheng Y., Kay L.E. and Prosser R.S. (2006). Topology of Outer-Membrane Enzyme: Measuring Oxygen and Water Contacts in Solution NMR Studies of PagP. *J. Am. Chem. Soc.,* **128,** 8256-8264.
[2] Huysmans G.H.M., Radford S.E., Brockwell D.J. and Baldwin S.A. (2007). The N-terminal Helix is a Post-assembly Clamp in the Bacterial Outer Membrane Protein PagP. *J. Mol. Biol.,* **373**, 529-540.

1736-Pos Board B580
Molecular Dynamics Simulation of Transmembrane Helix Dissociation
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In a typical single-molecule force spectroscopy (SMFS) experiment with a transmembrane protein (TM), a protein surface loop or tail is grabbed by the atomic force microscope cantilever and helices unwind as the TM is pulled in a direction perpendicular to the plane of the membrane. This process is unrelated to the insertion of helical segments from the translocon into the bilayer in a direction parallel to the plane of the membrane. We now use molecular dynamics simulations (MD) to consider a SMFS experiment in which TMs are pulled apart in the plane of the lipid bilayer, so that the helix-helix interactions can be studied. Parallel-versus perpendicular-pulling energy. We calculated the work of separating the helices of the glycophorin A dimer in a sodium dodecyl sulfate (SDS) micelle. Pulling perpendicular to the membrane plane requires about four times the work as parallel pulling to separate the helices. Point pulling. Using MD, we pulled Ala 82 of glycophorin A in a direction parallel to the membrane plane. For 150 ps, the two helices separated smoothly, essentially as rigid rods. However, both Ile 76 side chains remain in van der Waals contact, locking the dimer together. Further pulling unfolds the backbone around Ala 82, until the Ile 76 groups separate after 600 ps. Similar results were observed for a two-helix fragment of bacteriorhodopsin in an SDS micelle. Pulling on Ile 52, the helices separate smoothly for 150 ps. However, during this motion, Leu 13 and Leu 61 remain locked in van der Waals contact. We conclude that SMFS of helical membrane proteins pulled in the direction of the membrane plane would provide valuable information about the interactions that stabilize integral membrane proteins. Experimental implementation will require development of tethering groups compatible with bicelles.

1737-Pos Board B581
The Interactions Of Secondary Structural Elements In The Achitecture Of Membrane Proteins
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Membrane proteins exist in an anisotropic environment which strongly influences their architecture, stability and folding. Previous studies have mainly focused on the transmembrane domains of alpha helical membrane proteins. However it is becoming increasingly apparent that their architecture is richer than a simple bundle of transmembrane alpha-helices, notably due to helix deformations and other membrane embedded structures. Here, we make use of available databases and tools to investigate the weight and role of structural heterogeneity in the supra molecular organization of membrane proteins. Using a non redundant subset of alpha helical membrane proteins, we have annotated and analysed the statistics of several types of structural element such as incomplete helices, intramembrane loop, helical extensions of helical transmembrane domains, extracellular loops and helices lying parallel to the membrane surface. The different structural elements and their composition were studied in relation to their immediate membrane environment. Calculation of hydrophobicity using different scales show that different structural elements appear to have affinities coherent with their position in the membrane. The considerable information content found in the amino-acid compositions of the different elements, suggests that the annotation scheme used might be useful for structural prediction. In a second step we have investigated the energetics of interaction between the structural elements. We show that while the folding in integral membrane polypeptides seems to be dominated by the interactions between transmembrane helices the same is not true of the assembly of complex membrane proteins. In most multisubunit membrane proteins the interactions between multiple domains are important in driving the assembly. Notably interactions at the surface of the membrane and between soluble domains are often very important.

1738-Pos Board B582
A Novel Pain Management Paradigm: Conformational Studies of the Mu-Opioid Receptor
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The mu opioid receptor (MOR) is a Class A G protein-coupled receptor. Naloxone, a MOR antagonist, has been shown experimentally to have partial agonist properties in a transmembrane helix 4 (TMH4) S196L/A MOR and full agonist activity in a triple mutant that combines the TMH4 mutation (S196L) with two mutations in TMH7, T327A and C330S (Claude-Geppert et. al, J. Pharmacol. Exp. Ther. 2005). We hypothesized that the loss of a serine in TMH4, the loss of a threonine in TMH7 and addition of a serine in TMH7 will modify the wild-type (WT) conformation of both helices and that this change may result in naloxone preferring the MOR activated state. The hydrogen bonding capacity of Ser/Thr residues in α-helices can be satisfied by an intrahelical hydrogen bond interaction, in either the *g-* or *g+* conformation, between the O-γ atom and the *i-3* or *i-4* carbonyl oxygen . Ser/Thr residues in the *g-* conformation can induce a bend in an α-helix (Ballesteros et. al, Biophysical J. 2000), as well as changes in wobble angle and face shift (Zhang et al. Mol. Pharmacol. 2005). Using the Monte Carlo/simulated annealing technique, Conformational Memories, we explored the effect of Ser/Thr in *g-* on the conformation of WT MOR and the mutant TMH4 and TMH7s (Whitnell et. al, J. Comput. Chem. 2007). For TMH4, the (wobble angle, face shift) was found to be (-36.4°, 22.1°) for WT vs. (5.7°, 4.6°) for S196L , causing the top of TMH4 to move away from the TMH bundle. For TMH7, a significant difference was found in the average wobble angles of WT(52.52° ± 21.72°) vs. mutant TMH7 (120.55° ± 23.43°), causing the top of TMH7 to move into the bundle. [Support: NIH DA023905 and DA021358]

1739-Pos Board B583
The Kv1.2 Paddle Chimera Channel in a Lipid Bilayer
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The Kv1.2 paddle chimera structure (Long et al. 2007, Nature 450: 376) is the first voltage-dependent ion channel structure that reveals all residues at high resolution. In addition, fragments of lipid acyl chains and headgroups are also revealed. We present results of an atomistic molecular dynamics simulation of the Kv1.2 paddle-chimera tetramer in a POPC bilayer in excess water. The initial configuration includes the crystallographic waters and lipid fragments (reconstructed as POPC molecules) observed in the crystal structure (PDB ID 2R9R). Similar to previous simulation studies of Kv1.2 (Treptow and Tarek 2006, Biophys. J. 90:L64; Jogini and Roux 2007, Biophys. J. 93:3070), we find the basic side chains of the S4 helix in a polar environment formed by water molecules, lipid headgroups, and acidic side chains. As suggested by the crystal structure, the H-bond network that solvates each voltage sensor domain is interrupted by F233, a side chain highly conserved among Kv channels. The lipid fragments observed in the crystal structure are fully consistent with the lipid bilayer configuration and present a distinctive dynamics that contrasts with the bulk-like behavior of the lipid molecules far from the protein.

These results suggest that these lipid fragments should be considered structural lipids, and would imply an important role for the lipid-protein interactions in the channel stability and crystallization. Research supported by NIGMS and NSF.

1740-Pos Board B584

Dimer Conformation Of Amyloid Precursor Protein Fragments In Membrane

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The aggregation/oligomerization of Aβ peptides has been known as an essential element of the pathogenesis of Alzheimer's disease (AD). Aβ peptide is a product of amyloid precursor protein (APP) cleaved by β-secretase at the extracellular region and γ-secretase at the transmembrane region. Therefore, to know the structure of APP in membrane is essential for understanding the initial stage of AD.

Recent experimental studies showed that APP in membrane exists in a homodimeric form. Because APP contains three Gly-xxx-Gly (GxxxG) motifs, the dimer conformation is likely stabilized by Cα-H…O type hydrogen bonds between two segments. A mutant, in which two Gly in the second GxxxG are replaced by Leu, exists also in a dimeric form, but the cleavage by γ-secretase is prohibited.

To obtain structural models of APP dimers, we performed replica-exchange molecular dynamics (REMD) simulations of two APP fragments (D23-K55) of wild type and the mutant in implicit membrane. Starting from random configurations, stable dimeric forms were predicted for wild-type APP. Major conformations have Cα-H…O hydrogen bonds between different segments. Two different dimeric forms were found in the mutant. In one form, two APP segments were crossed and strongly packed at the fifth Gly. In another form, which is major conformation, two segments were aligned in parallel with hydrophobic residues facing each other (Leu-zipper type). Thus, the driving forces to promote the dimerization with APP in membrane differ between WT and the mutant. These structural models can explain the difference in the conformational stability of APP dimer in membrane and the cleavage of APP by γ-secretase.

1741-Pos Board B585

Curvature Dynamics of α-Synuclein Familial Parkinson's Disease Mutants: Molecular Dynamics Simulations of the Micelle- and Membrane-Bound Forms

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α-synuclein remains a protein of interest due to its propensity to form fibrillar aggregates in neurodegenerative disease and its putative function in synaptic vesicle regulation. We have performed a series of all-atom molecular dynamics simulations of wild-type α-synuclein and the three Parkinson's disease familial mutants, A30P, A53T, and E46K, bound to an SDS detergent micelle. Our analysis explains how α-synuclein adapts to, and alters, highly curved membrane surfaces through helical bending. We find that α-synuclein binding induces significant deformation in the micelle, flattening the structure and decreasing its surface area. Similar effects on biological membranes would relieve curvature stress, ameliorating the propensity to fuse, and would perhaps explain α-synuclein's role in stabilizing synaptic vesicles. Consistent with the experimentally determined behavior of A30P, the proline dramatically destabilizes the secondary structure, inducing reversible unfolding up- and down-stream of the substitution. The E46K mutation provides an additional electrostatic interaction between the protein and micelle, offering an explanation for the mutants increased affinity. In the case of the A53T mutant, recent NMR data suggested that an enthalpic interaction might be responsible for a slight rigidification of the helical structure. We show that the equilibrium structure of A53T contains a very tight hydrogen-bond between the threonine's hydroxyl and the backbone carbonyl of Val49. We speculate as to the potential effects of these dynamic and structural changes, on α-synuclein's role in neural signaling. In order to assess the applicability of these results to biological membranes, we have performed simulations of the wild-type and mutants on a DOPS bilayer. These simulations reproduce the major conclusions of the micelle-bound simulations, suggesting that the detergent micelle is an adequate model system.

1742-Pos Board B586

Computational Study of Transmembrane Domain of Cytokine Receptor Family

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Cytokine receptor family represents an important group of proteins which play an essential role in transducing signals from extracellular via the transmembrane domain to the intracellular compartment of the cell. Understanding these transmembrane signaling at the molecular level is one of the most important, challenging biophysical problems. Transmembrane signaling is thought to be transmitted through the membrane receptors which undergo a variety of conformational changes upon ligand binding, ions and cofactors. In our study, we have modeled the transmembrane domains of four major cytokine receptors: human growth hormone receptor, human prolactin receptor, human erythropoietin receptor, and human thrombopoetin receptor. We have built the models using only sequence information and have applied replica exchange molecular dynamics simulations to sample the conformational space of the TM helices. We will present possible conformational changes of each transmembrane domain based on the clustering of the sampled conformations and the representative structures from each cluster. Furthermore, we will also present the molecular dynamics simulation studies of these models in explicit lipid environments to characterize the stability, orientation, and helix-lipid interactions of the models in lipid bilayers.

1743-Pos Board B587

A Microsecond Time Scale Molecular Dynamics Simulation of B2AR in a Membrane

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We present the results of a 1 microsecond all-atom molecular dynamics simulation of a B2AR monomer embedded in a lipid bilayer. The initial model was derived from the crystal structure of the carazolol-bound B2AR-T4 lysozyme fusion protein (Rosenbaum et al, Science 2007 318:1266-73), where the T4-lysozyme and carazolol ligand were removed. Singular value decomposition and CA-RMSD analyses show a remarkably stable structure with three large-scale conformational substates. The majority of motions associated with these states occur at the ends of the transmembrane helices. The protein core, and in particular, the key structural/functional regions around the highly conserved proline residues of TM5, TM6, and TM7 remain very stable. Water rapidly infiltrates the protein core, forming pockets that are persistently hydrated, including the ligand binding pocket. Remarkably, the distorted Pro-kink in TM6 is stabilized by individual water molecules with very long residence times; the simulation is able to perfectly mimic this feature that is observed in the crystal structures of bovine and squid rhodopsin and in B1AR and B2AR, and is probably common of other class A GPCRs.

Genome Packaging & Manipulation II

1744-Pos Board B588

A Combined Approach for Structure Determination of a Human Rad51 Protein Filament: from Computer Modeling to Site-Specific Linear Dichroism

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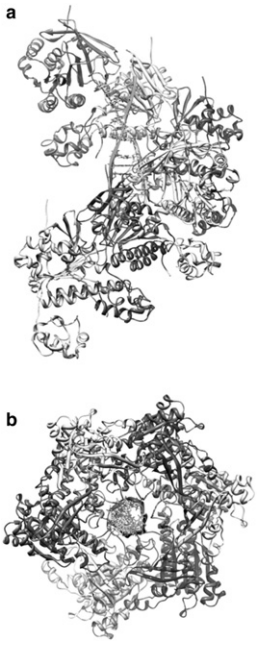

The human Rad51 protein plays a crucial role in homologous recombination and DNA repair. The details of the recombination process, which is essential to all cells and has been evolutionarily conserved, are still to be revealed. Structural information on Rad51-DNA complexes in solution can contribute to mechanistic insight. We here combine molecular modeling of the filamentous structure of human Rad51 protein with experimental data for angular orientations of aromatic residues of a Rad51-DNA filament in solution obtained by Site-Specific Linear Dichroism (SSLD), a spectroscopic technique in combination with protein engineering. The resulting structural model is in fair agreement with a filament structure previously deduced from electron microscopy. We show that the filament has ability to house a DNA molecule and that putative DNA binding loops are strategically positioned for interactions with DNA.