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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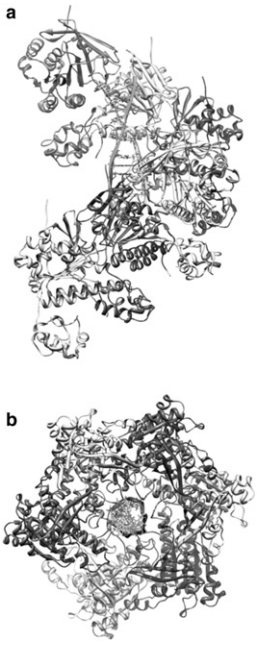

The human Rad51 protein plays a crucial role in homologous recombination and DNA repair. The details of the recombination process, which is essential to all cells and has been evolutionarily conserved, are still to be revealed. Structural information on Rad51-DNA complexes in solution can contribute 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.