2516-Pos
Do Cation-PI Interactions Occur in Lipid Bilayers Between Phosphatidylcholine Headgroups and Interfacially Localized Tryptophans?
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Lipids can modulate membrane protein activity in many different ways. To understand the basic priciples governing this complex lattice of interactions between lipids and membrane proteins, simple model peptides have been created. Among those are the families of WALP and KALP peptides, which consist of a poly-(leucine-alanine) stretch flanked by tryptophans and lysines, respectively.
Here we focused on studying how electrostatic interactions between these flanking residues and the lipid polar head groups can affect the behavior of the peptides and the lipids. We used 2H NMR on Ala-d4 labeled peptides to map changes in the orientation of the peptides in phosphatidylcholine bilayers in the absence and presence of the anionic lipid phosphatidylglycerol and we used 14N NMR to monitor changes in structure and dynamics of the phosphocholine head groups. Surprisingly, we found that WALP peptides, which are uncharged, are more sensitive to incorporation of negatively charged lipids, than their positively charged equivalents, the KALP peptides. As a possible explanation we raised the hypothesis that WALP peptides are sensitive to the concentration of phosphatidylcholine lipids in the membrane, due to favorable cation-pi interactions between the tryptophans and the choline moieties of the lipids. This hypothesis was supported by results from high resolution solid state NMR experiments, designed to monitor Trp-choline interactions. The existence of such a favorable interaction may shed new light on understanding the behavior of membrane proteins, in particular since in such proteins Trp frequently occurs as flanking residue at the lipid/water interface.

2517-Pos
Single Tryptophan Mutants of Galleria Mellonella Apolipophorin III: Binding Interaction to Lipopolysaccharides
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Apolipoproteins have been shown to interact with lipopolysaccharides, thereby providing protection against sepsis. To gain insight in the binding interaction, apolipophorin III (apoLp-III) from the Greater wax moth, Galleria mellonella, was used as a model. The protein bears a unique tyrosine residue which has been used to monitor LPS binding interaction. To improve the binding analysis, single tryptophan mutants were engineered. The introduction of a tryptophan in apoLp-III provides a stronger fluorescence signal, allowing for less protein and decreasing light scatter problems when determining the binding interaction with the three components of LPS (Lipid A, core and O-antigen polysaccharides). Five single-tryptophan mutants (F20W, L80W, L119W, I138W and F145W) were designed and produced in a bacterial expression system. The secondary structure of the mutant proteins was similar to that of the wild-type protein. The protein stability, measured as the resistance to Gdn-HCl induced denaturation, was slightly decreased. This indicates that the overall α-helical structure was not affected by the introduction of tryptophan. Upon LPS binding, the tryptophan fluorescence emission increased for I138W and F145W-apoLp-III, decreased for F20W and L119W-apoLp-III, and no significant difference was observed for L80W-apoLp-III. Thus the tryptophan residues relocate into distinct environments, indicating that the apoLp-III helices bind to different parts of LPS.

2518-Pos
Simulations of Surfactant and Lipid Assemblies with Generalized Born Implicit Solvent Models
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In recent years, all-atom and coarse-grained models have been developed for theoretical studies of surfactant and lipid assemblies. Here we describe the development of hybrid models incorporating atomic-level representation of surfactant molecules and continuum description for solvent. Specifically, we show that atomistic simulations of neutral, anionic and cationic surfactants can be performed using the generalized Born implicit solvent model by careful parameterization of atomic input radii. We applied the new models to constant pH molecular dynamics simulations of acid-base titration of fatty acid solubilized in dodecyl triehyleneglycol either (DE3), dodecylsulfaphate (SDS) and dodecyl tetramethylammonium (DTA) micelles. We show that simulation results are able to reproduce the experimental data and offer atomically detailed explanation for the abnormal titration behavior of fatty acid in cationic micelles. The combined atomistic solute and continuum solvent models significantly reduce computational and represent an attractive alternative approach for theoretical studies of interfacial phenomena involving surfactant and lipid assemblies.

2519-Pos
Voronoi Analysis of Lipid Surface Area in Protein-Membrane Systems
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All-atom molecular dynamics (MD) simulations are powerful tools to study dynamics and functions of membrane proteins at the atomic level. In these simulations, NPAT ensemble, where the cell area is fixed, has been widely used to reproduce the experimental value of lipid surface area around proteins. However, the surface area for each lipid molecule can be under- or overestimated due to the deformation of membranes. It may cause artificial conformational deviations of membrane proteins during log MD simulations. To overcome this problem, we proposed a novel algorithm to calculate the lipid surface area in protein-membrane systems using Voronoi tessellation. We analyzed 100-ns MD trajectory data of the SecY channel of *Thermus thermophilus* (ttSecYE) and *Methanococcus jannaschii* (mjSecYEβ), Fab-ttSecYE complex, and sarcoplasmic reticulum (SR) Ca^{2+}-pump in the NPAT ensemble. We found that in the simulations of Fab-ttSecYE and Ca^{2+}-pump the averaged surface area for 'bulk' lipids, which are located more than 18 Å away from the proteins, agreed with the experimental values, while slightly larger surface areas were obtained in the simulations of ttSecYE and mjSecYEβ. In order to reproduce more reliable membrane environment, we performed a short NPT simulation until the lipid surface areas only for 'bulk' lipids were converged to the experimental values, and then the cell area was fixed with the NPAT ensemble as a production dynamics. By this procedure, a more valid MD trajectory data was obtained, where the membrane thickness and order parameter of bulk lipid molecules were also consistent with the experimental values.

2520-Pos
Potential of Mean Force Between Ionizable Amino Acid Side Chains in Lipid Bilayer
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Potentials of mean force (PMF) between ionizable amino acid side chains (Arg, Lys, His, Glu/Asp) in different protonation states in palmitoyl oleoyl phosphatidylcholine lipid bilayer were obtained from all-atom explicit solvent molecular dynamics simulations and the adaptive biasing force approach available with NAMD.[1,2] Side chains (SC) were constrained in different orientations: collinear, stacked and T-shaped and placed into the bilayer interface. The most structured PMFs were observed for unlike-charged ions or pairs with neutral SCs in collinear orientation. Contact pairs (CP) occurred at a distance of 2.6-3.1 Å with the strongest interaction of −9.6 kcal/mol between Arg^+ and Glu^- ions. Like-charged SCs in this orientation displayed less stable contact minima at greater distances or solvent separated minima. All pairs in stacking approach showed similar, well-structured PMF profiles with CPs at ~3.8 Å. The strongest interaction between like-charged pairs was observed for stacked arginines. Like-charged pairs constrained in T-shaped geometry mostly displayed slightly stable solvent separated minima. A relationship between water and phosphate coordination numbers, contact pair minima and free energy barriers was found. There is also dependence of PMF shapes on H-bonding between amino acids. Generally, interactions between ionizable SCs are more attractive and the PMFs are more structured in a lipid bilayer than in water.[3]
[1] Darve, E.; Pohorille, A. *J. Chem. Phys*. **2001**, *115*, 9169.
[2] Darve, E.; Wilson, M.; Pohorille, A. *Mol. Simul*. **2002**, *28*, 113.
[3] Masunov, A.; Lazaridis, T.*J. Am. Chem. Soc.* **2003** *125*, 1722.

2521-Pos
Water Under the BAR
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The generation of high-curvature membranes by Bin/amphiphysin/Rvs (BAR) domain containing proteins is a topic of intense current interest. Details regarding the mechanism of curvature generation are debated, with some emphasizing for the importance of electrostatic attraction between the protein and the membrane, and others pointing to the insertion of amphipathic N-terminal helices. Here, we present evidence from molecular simulations of single amphiphysin N-BAR domains that, even when tightly bound to highly curved membranes, a considerable amount of water is found between the protein in the membrane.

Furthermore, this water results in significant electrostatic screening, an important consideration when building theoretical and computational models of membrane remodeling. We suggest that this electrostatic screening is at least partly responsible for our observations that multiple, oligomerized N-BAR domains largely fail to bend flat membranes. Our results support the insertion of hydrophobic moieties as the major driving force of membrane remodeling by N-BAR domains.

2522-Pos

Mesoscopic Simulations of Membrane Protein Trafficking and Signal Transduction Across Membranes

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Palmitoylation is a frequent posttranslational modification that triggers the membrane association of soluble proteins. Besides those peripheral membrane proteins (PMPs) also many transmembrane proteins are subject to lipid modifications, hence indicating that these membrane anchors may also regulate the trafficking of transmembrane proteins. Using coarse-grained membrane simulations we find that palmitoylation indeed significantly alters the tilting of transmembrane proteins with respect to the bilayer normal. Cluster formation and partitioning behavior due to hydrophobic mismatching with the surrounding lipid bilayer is also altered, therefore allowing for ample possibilities to regulate the trafficking of transmembrane proteins via palmitoylation. Using the same simulation approach, we also have studied the trafficking of peripheral membrane proteins (PMPs). In particular, we have observed a cross-leaflet oligomerization of PMPs due to membrane mediated attraction. The strength of this effect is determined by the radii and membrane anchor lengths of the involved PMPs. Since both of these might be altered, for example by ligand binding, the observed cross-leaflet oligomerization may be the fundamental process by which PMPs can trigger an intracellular signalling cascade without the need for accessory transmembrane factors.

2523-Pos

Positioning of Proteins in Membranes of Variable Lipid Composition

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A novel anisotropic solvent model of the lipid bilayer has been developed and applied for calculating energetically optimal translational and rotational positions of proteins in different types of biological membranes. The spatial positions are refined for the entire set of ~900 distinct protein structures currently in the OPM (Orientations of Proteins in Membranes) database (http://opm.phar.umich.edu). The bilayer is represented as a fluid anisotropic solvent described by profiles of dielectric constant, solvatochromic dipolarity/polarizability parameter, and hydrogen bonding acidity and basicity parameters that change gradually along the bilayer normal, including the lipid head group region. The profiles of several artificial phospholipid bilayers have been calculated based on the published distributions of their molecular segments determined by neutron and X-ray scattering. The profiles were also simulated for biological membranes based on their lipid composition including eukaryotic plasma membrane and bacterial inner and outer membranes. Transfer energy of the protein includes a solvent accessible surface area-dependent contribution (first solvation shell energy) and a long-range electrostatic component for group dipole moments and ionized groups, as well as ionization energy. Application of this model to transmembrane and peripheral proteins from the OPM resulted in a more precise and reliable calculation of their spatial positions and membrane binding affinities. Membrane-binding regions of numerous peripheral proteins have been identified during Protein Data Bank screening. The analysis of membrane association for peripheral proteins from the Structural Genomics projects helps to assign their biological functions, as illustrated for proteins from calycin and SpoIIAA superfamiles.

2524-Pos

Modeling Lipid-Mediated Transmembrane Protein Aggregation

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Many transmembrane proteins play crucial roles in cell signaling, and lipid-mediated association of these proteins may well have a role to play in these pathways in addition to protein-specific interactions. We seek to gain insight into the large-scale aggregation effects induced by lipid-mediated hydrophobic driving forces previously revealed in coarse-grained molecular simulations [de Meyer, Venturoli, and Smit. Biophys J. (95) 2008]. The molecular coarse-grained model of transmembrane peptides and lipid bilayers focused on the impact of hydrophobicity and of simple molecular structures on the association between small numbers of peptides. We build on this previous work by developing a computationally feasible model of protein-protein interaction which captures the driving forces relevant for aggregation of small numbers of peptides as well as the highly non-additive effect of the surrounding lipid as the peptides further aggregate. Such a model is better able to capture large-scale aggregation and organization of proteins via the lipid bilayer and to explore the consequences of the driving forces of aggregation at the experimentally relevant time scales and length scales.

2525-Pos

Modeling the Membrane Role in Ca^{2+}-ATPase Catalytic Cycle

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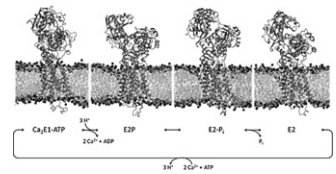
A deep understanding of the function of membrane proteins requires that we understand the direct and indirect effects of the lipid environment. Deformations of the bilayer to accommodate the protein induce energy penalties and potentially change the free energy between conformational states and thereby change the distribution of protein conformations. The lipid bilayer thus plays a regulatory role for the function of a membrane protein.

Structures of the Ca^{2+}-ATPase from sarcoplasmic reticulum, SERCA, have been determined by X-ray crystallography in several different functional states. These structures have provided a unique opportunity to study how the protein interacts with the membrane throughout the functional cycle by all-atom molecular dynamics (MD) simulations.

MD simulations have been performed with four different structures of SERCA representing a Ca^{2+}- and ATP-bound state (Ca_2E1-ATP); a state with the luminal Ca^{2+}-exit path open and the protein phosphorylated (E2P); and two dephosphorylated occluded states with bound protons, one with inorganic phosphor still bound (E2-Pi) and one without (E2). Our results show how the POPC-membrane and the protein in different functional states undergo mutual adaption (see figure) and how the hydrophobic mismatch and protein area profile change during the functional cycle.



2526-Pos

Monte-Carlo Simulations of Peptide-Membrane Interactions: Web-Server

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Short peptides interact with biological membranes in many ways. For example, antimicrobial peptides destabilize bacterial cell membrane, while fusion peptides of viral proteins promote membrane fusion. Short peptides may mimic the interaction of integral membrane proteins with the membrane and thus are a convenient model system to study the folding and insertion of membrane proteins into the hydrophobic environment of the membrane. Along with various experimental techniques, computational methods are also used in research of peptides-membranes interactions. We have previously developed a Monte Carlo (MC) simulations model for the investigation of linear α-helical peptides with membranes. This model was tested on an assortment of peptides, such as Magainin2, penetratine, M2δ peptide (a transmembrane segment from the acetylcholine receptor δ-subunit), melittin and NK-2 and its derivatives. The results of the simulations correlated very well with empiric data. Moreover, these computations were used to guide further experimental efforts. Encouraged by these studies, we are establishing a web-server to allow external users to perform simulations of their peptides of interest in membrane and water environments. The server will provide a possibility to choose the amino acid sequence of the peptide, the ratio of zwitterionic-to-acidic lipids and width of the bilayer, and the ionic strength. The results will include the free energy of membrane-association of the peptide, its helical content upon membrane interaction as well as its predicted location in the membrane.

Membrane Structure II

2527-Pos

Probing the Membrane Deformations Induced by Binding of Membrane Proteins: Alpha-Synuclein and CRAC

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