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2335-Pos Board B305

Kinetics Of Peptide (pHLIP) Insertion And Folding In A Lipid Bilayer Membrane

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The early stages of folding a membrane protein have been conceptualized in terms of the formation of independently stable transmembrane helices, followed by their association to form a bundle, and then followed by further insertions, rearrangements, and binding events. A part of this notion is the formation of transmembrane helices, which is catalyzed by the translocon for hydrophobic sequences, but which can also occur spontaneously for moderately polar sequences. We study spontaneous insertion and folding across a lipid bilayer of moderately polar membrane peptide pHLIP - pH Low Insertion Peptide. pHLIP has three major states: (I) soluble in water or (II) bound to the surface of a lipid bilayer as an unstructured monomer, and (III) inserted across the bilayer as a monomeric α-helix. The existence of three distinct equilibrium states makes it possible to separate the process of peptide attachment to a lipid bilayer from the process of peptide insertion/folding. The transitions between states could be easily monitored by the changes of tryptophan fluorescence and circular dichroism signals. We performed steady-state and stopped-flow fluorescence and CD measurements to reveal the molecular mechanism of pHLIP insertion and folding within a POPC lipid bilayer and to calculate the activation energy of formation of transmembrane helix. Global mode analysis allowed us to monitor changes of entire tryptophan fluorescence spectrum during the transition from the state II to the state III.

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Membrane Remodeling By N-bar Domains At All Scales: Theory And Simulation Of The Ensemble Effect

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We address the concentration and composition dependent remodeling of cell membranes by N-BAR domains through a combination of large scale atomistic molecular dynamics simulations and mesoscopic simulation. The atomistic simulations approach the problem from the short length- and timescale end, studying the relationship between curvature induction, N-BAR oligomerization, and membrane composition on timescales up to 100 nsec and lengthscales up to 50 nm. The results of the atomistic simulations systematically motivate the mesoscopic field theory, which does a remarkable job of predicting experimental morphologies observed at 500 nm lengthscales for a range of conditions.

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Structural And Conformational Analysis Of A Peptide-Detergent Complex By Molecular Dynamics Simulations

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It is now well known that membrane proteins have a great quantitative and qualitative importance. Their stabilization in detergent micelles, which mimic their natural environment, is an essential step in their structural and functional study. The detergent choice is largely based on empirical approaches and the nature of the complex they form is barely understood. To discover the nature of such complexes, we have realized molecular dynamics simulations of a system composed of the transmembrane alpha helix of the Glycophorin A (GpA) and di-hexanoyl-phosphatidyl-choline. A study of the interactions between the different elements of the system, and their dynamics allowed us to discover the structuration of such complex in a bilayer and the behaviors related to the faces of the peptide. The "GxxxG" dimerization motif of GpA interacts barely with the detergents, which allow them to maximize their cohesion. The study of the peptide's faces also revealed that the topology of the peptide would be a determinant factor in the structuration of the complex. Understanding the functioning such systems is a step toward the rationalization of the phenomena in place in the stabilization of membrane proteins and their interaction modulation by the environment.

2338-Pos Board B308

Detergent Localization In Model Proteo-bicelles

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Several methods for crystallization of membrane proteins for structure determination have been published, including those which use discoidal membranes called bicelles. The bicelle-based method has proven to be a stable platform resulting in well-diffracting crystals of G-protein coupled receptors, and other proteins including bacteriorhodopsin. In typical 'empty' bicelles - those devoid of protein - long chain phospholipids make up the core of the disk, and micelle-forming detergents "cap" the disk by forming the rim. Short chain phospholipid and cholate analog detergents (e.g. DiC$_6$PC, DHPC and CHAPSO) are included in this "capping" category having been shown to associate preferentially with the bicelle rim. In proteo-bicelles, formed by the mixture of protein-detergent complexes with preformed bicelles, a second type of detergent containing a sugar headgroup (e.g. octylglucoside and maltoside) is introduced. This second type of detergent has proven to be effective in membrane protein purification and stabilization. In this study, we use small angle neutron and x-ray scattering to explore the structure and phase-behavior changes induced by sugar-headgroup type detergents on bicelles and their influence on bicelle-based membrane protein crystallization. Preliminary results suggest that these sugar headgroup amphiphiles partition more heavily into the core of bicelles than their short chain phospholipid and cholate counterparts. An understanding of the roles of these amphiphiles in modifying the meso-structures which eventually lead to crystallization is a critical next step in furthering our understanding of the membrane protein crystallization process in these systems.

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Penetration of Aromatic Residues into Membrane Bilayers: A New Approach

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Penetration of Aromatic Residues into Membrane Bilayers: A New Approach
Lipid bilayers are characterized by a unique molecular motional regime that makes it possible to apply both solid-state NMR and solution-state NMR methods together for structural studies. The combination of magic angle spinning (MAS) with the high-resolution ^{1}H NOESY NMR experiment is an established method for measuring through-space ^{1}H¼ ^{1}H dipolar couplings in biological membranes, and has been applied extensively in the past to biological membranes to determine the location of bound drugs and peptides. The segmental motion of the lipid acyl chains along with the overall rotational diffusion of the lipids provides sufficient motion to average the ^{1}H dipolar interaction to within the range where MAS can be effective. One drawback of the approach is the relatively long NOESY mixing times needed for relaxation processes to generate significant crosspeak intensity. In order to drive magnetization transfer more rapidly, we introduce the use of solid-state radiofrequency driven dipolar recoupling (RFDR) pulses during the mixing time. We compare the established ^{1}H MAS NOESY experiment with a new ^{1}H MAS RFDR experiment on dimyristoylphosphocholine, a bilayer forming lipid, and show that the ^{1}H MAS RFDR experiment provides considerably faster magnetization exchange than the ^{1}H MAS NOESY experiment. We apply the method to model compounds containing basic and aromatic amino acids bound to membrane bilayers to illustrate the ability to locate the position of aromatic groups that have penetrated to below the level of the lipid headgroups.

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Characterization Of Phosphoinositide Monolayers By Infrared Spectroscopy And Epifluorescence Microscopy At The Air/water Interface

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Phosphoinositides have been shown to mediate a large variety of important physiological processes by affecting the activity and the localization of membrane associated proteins. Phosphoinositide properties are largely determined by the characteristics of their headgroup, which at physiological pH is highly charged but is also capable of hydrogen bond formation. For phosphoinositide mediated signaling events to occur, it requires the local enrichment of phosphoinositides, which depend on the interchange between attractive and repulsive forces. Factors expected to affect mutual phosphoinositide interaction are pH, cations, or positively charged proteins. We have characterized the structural properties of dipalmitoyl phosphatidylinositol mono-, bis- and trisphosphate monolayer films at the air/water interface by infrared reflection-absorption spectroscopy (IRRAS) as well as by direct visualization of domain formation of each phosphoinositide derivative by epifluorescence microscopy in the presence of low and high monovalent salt concentrations. It has been observed that on pure water subphases the surface pressure/area (Π/A) isotherms for all phosphoinositide derivatives were characteristic for a condensed monolayer whereas a monolayer expansion was found for medium (10 mM) and high salt concentrations (150 mM). IRRAS measurements showed that this