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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.

2336-Pos Board B306
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.

2337-Pos Board B307
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_6PC, 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.

2339-Pos Board B309
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 $^1H/_4$ ^{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.

2340-Pos Board B310
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

was associated with a larger molecular tilt angle and a reduced acyl chain order. Using epifluorescence microscopy, domain formation has been observed for each phosphoinositide derivative as the monolayer goes from a liquid phase to the liquid condensed phase. In addition, membrane organization can be affected by the binding and interaction of various proteins or peptides to the lipids. Surface pressure/time experiments along with epifluorescence microscopy will investigate these interactions for each phosphoinositide monolayer highlighting the distinct properties of phosphoinositide/protein interactions.

2341-Pos Board B311

Orientation of Single-Span Transmembrane Peptides Investigated by Independent Solid-State NMR Methods: GALA and PISEMA

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Model peptides of the ''WALP'' family have been shown by deuterium solid-state NMR to adopt small average tilt angles in model membranes; however, molecular dynamics simulations have consistently predicted larger tilt angles. It has been argued that peptide molecular motion could potentially compromise the observed tilt angles deduced from solid-state NMR. It was therefore of interest to employ an independent technique to address the discrepancy between experimental and theoretical methods.

Here we report the analysis of the average orientation of single-span transmembrane peptides acetyl-G<u>X</u>ALW(LA)$_6$LWLA<u>X</u>A-[ethanol]amide (<u>X</u>WALP23, where <u>X</u> = K or G) in mechanically aligned lipid bilayers using two distinct solid-state NMR methods. GALA (Geometric Analysis of Labeled Alanines) employs the ^{2}H signals of labeled alanine side chains, while the PISEMA (Polarization Inversion Spin Exchange at the Magic Angle) technique is based upon the ^{15}N-^{1}H signals from the peptide backbone. Due to the different angles between the helical axis and the C_α-C_β or N-H bond vectors, these methods are expected to provide different sensitivities toward the molecular motion.

GALA analysis of <u>X</u>WALP23 orientation in DLPC, DMPC and DOPC revealed that both peptides are tilted with respect to the membrane normal, with the magnitude of tilt dependent on membrane thickness (8-17° for KWALP23 and 6-13° for GWALP23). For comparison, PISEMA experiments were performed in DLPC, where the tilt is highest. The results from the two independent NMR methods are similar, with the tilt difference not exceeding 3 degrees (Vostrikov, V. *et al.* 2008. *J Am Chem Soc*, 38:12584). Although molecular dynamics simulations have not yet been performed for <u>X</u>WALP23 peptides, such calculations would be of interest for comparison with the experimental results from ^{2}H NMR and ^{15}N NMR, and with existing simulations for WALP19 and WALP23.

2342-Pos Board B312

Conformation of the Transmembrane Domain of the Anthrax Toxin Receptor

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The human receptor for anthrax toxin is a single span membrane protein of 368 amino acids that binds to the antigens of *Bacillus anthraces*, the bacterium that causes anthrax. The transmembrane (TM) domain of the receptor spans residues 319-343 and has the sequence GSILA^{5}IA^{7}LLILFLLLA^{16}LA^{18}LLWWFWA. Through the use of solid phase peptide synthesis, we have incorporated deuterated alanines into the native domain (except with Gly instead of Ser) and a related TM domain with Trp anchors on both ends of the peptide, GWWLA^{5}IA^{7}LLILFLLLA^{16}LA^{18}LLWWFWA. To enable a more complete analysis of the backbone geometry and possible helix tilt in lipid bilayer membranes, we also introduce deuterated Ala instead of Leu^9, Leu^{11}, or Leu^{14} in selected samples. For the native sequence in DMPC, the Ala methyl quadrupolar splittings are 12.9, 23.4, 13.1 and 23.9 kHz for alanines 5, 7, 16 and 18, respectively (β=0 sample orientation), compared to 13.5, 7.7, 18.9, and 0.7 for the modified ''double anchored'' sequence. Once the data sets for the Leu→Ala substitutions are complete, we will seek to define the geometry and orientation of the domains that are anchored on one or both ends using the 'GALA' method for DMPC and DOPC. We also seek to compare the bilayer-incorporated domain structures with results obtained at 1/100 (peptide/detergent) in sodium dodecyl sulfate (SDS) micelles. Based upon solution NMR of the TM domains in micelles of deuterated SDS, there appear to be multiple conformations of both the single- and double-anchored domains. With appropriate assignments of the solution NMR resonances (in progress), we expect to be able to define one or more major or minor conformations in SDS.

2343-Pos Board B313

Studying Membrane Proteins Using Covalent Assemblies Of Well-defined Model Peptides

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Membrane protein research is an important, but problem-riddled field. Since structure and function of these hydrophobic proteins is highly influenced by the surrounding lipids, often simple model systems are used in which the general principles of protein-lipid interactions can be studied systematically. Examples of such model systems are the WALP peptides (Ac-GW2(LA)8LW2A-NH2) in vesicles of varying lipid composition. The α-helical WALP peptides mimic the features of natural membrane proteins and their behaviour in lipid bilayers has been well characterized. However, most proteins have multiple membrane spanning segments. To mimic such systems and to study the effect of oligomerization and/or cross sectional diameter of the protein on peptide-lipid interactions we decided to construct covalent assemblies of WALP-peptides. Two strategies were followed.

First, cysteine-containing WALP analogs, that can be oxidized to form covalent dimers, were synthesized. Dimerization was monitored by SDS-PAGE and HPLC. An interesting additional feature of the analogs is the possibility to investigate whether there is a preferred helix interaction site, by introducing cysteines at different positions along the helix axis.

As a second approach we used a cyclic peptide-based scaffold to which WALP monomers were attached by the so-called click-reaction. With this method a covalent tetramer was synthesized, as shown by SDS-PAGE, gel permeation chromatography and mass spectrometry. The design is flexible. It was adapted to facilitate characterization and can be modified to meet specific requirements, like antiparallel versus parallel peptide arrangements or the incorporation of suitable labels.

The properties of the dimers and tetramers are now being tested in a membrane environment and their interactions with lipids are being compared to those of monomers (e.g. efficiency of lipid flip-flop and lipid chain order). Results of these studies will be shown.

2344-Pos Board B314

Biophysical Studies Of The Membrane Interactions Of A Transthyretin Fragment TTR(10-20)

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TTR(10-20) is a peptide derived from transthyretin, a 127 amino acid amyloid protein. Previous studies on TTR(10-20) have shown that this peptide could form protofibrils and fibrils *in vitro*. These are very rich β-sheet structures, insoluble at physiological pH. *In vivo*, protofibrils and fibrils deposit on tissues and lead to degenerescence. The goal of the present study is to characterize peptide structure and membrane interactions using different spectroscopic techniques.

The secondary structure of TTR(10-20) was determined by Fourier transform infrared (FTIR) spectroscopy. More specifically, the information about the peptide secondary structure is given by the amide I band, which was monitored as a function of temperature and lipid composition of the bilayer. On most spectra, there is an aborption band around 1620 cm^{-1}, corresponding to an intermolecular β-sheet structure such as protofibrils and fibrils. The area of this band increases with increasing temperature while the band corresponding to disordered structures decreases. The nature of the lipid head group also appears to have an impact on the aggregation of TTR(10-20).

The lipid bilayer is also affected by the presence and the proportion of peptide. Solid-state ^{31}P and ^{2}H nuclear magnetic resonance spectroscopy were used in the present study to determine the location of the peptide in the bilayer. More specifically, ^{31}P NMR is used to investigate the effect of TTR(10-20) on the lipid head group while ^{2}H NMR is used to investigate the effect of the peptide on the lipid acyl chains.

2345-Pos Board B315

Molecular Dynamics Simulations Of Alpha Synuclein In The Presence Of Sds Micelles

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Alpha synuclein (αS) is the principle protein in the Lewy body plaques that are found in the brains of patients suffering from Parkinson's disease. Oligomerization of αS is believed to be the initial step in the mechanism by which the disease causes neuronal death. Sodium dodecyl sulfate (SDS) is known to enhance the rate at which αS aggregates. Molecular dynamics simulations