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.

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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