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

Vitaly V. Vostrikov[1], Christopher V. Grant[2], Stanley J. Opella[2], Roger E. Koeppe II[1].
[1]University of Arkansas, Fayetteville, AR, USA, [2]University of California, San Diego, La Jolla, CA, USA.

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

Christopher L. Mazzanti, Denise V. Greathouse, James F. Hinton, Jennifer D. Lemmons, Roger E. Koeppe II.
University of Arkansas, Fayetteville, AR, USA.

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

Tania Rutters-Meijneke, Dirk T.S. Rijkers, Rob M.J. Liskamp, J. Antoinette Killian.
Utrecht University, Utrecht, Netherlands.

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)

Myriam Laneville, Michéle Auger.
CREFSIP, CERMA, Department of Chemistry, Université Laval, Quebec, QC, Canada.

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

James C. Patterson[1], Hongyi Yang[1], Desmond R. Parker[2].
[1]University of Alabama at Birmingham, Birmingham, AL, USA, [2]Talladega College, Talladega, AL, USA.

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

were performed on αS in the absence and presence of SDS micelles to probe which specific protein-protein and protein-lipid interactions are involved as SDS denatures αS determining the structure and dynamics. In the absence of SDS, individual αS molecules rapidly fold to form globular structures in which the acidic C-terminus interacts strongly with the more basic N-terminus. Simulating these structures in the presence of SDS micelles shows an initial electrostatic interaction between the protein and lipid, but the tertiary structure remains compact over short time scales, instead of forming the extended structures obtained by others via NMR measurements. However, as the simulations progress, the electrostatic interactions between the protein and lipid become less favorable because the sulfate groups of the micelles compete with the acidic residues of the C-terminus. Meanwhile the interaction between hydrophobic residues and the lipid acyl chains increases with time. These results suggest that monomeric and soluble αS requires the presence of lipids to overcome the strong attraction between the N- and C-terminus prior to aggregation, or that soluble multimeric forms of αS are the primary agents of aggregation.

2346-Pos Board B316

A Molecular Dynamics Study on the Binding and Interaction of the Amyloid-Beta (1-42) Peptide with Phospholipid Bilayers

Charles H. Davis, Max L. Berkowitz.
University of North Carolina-Chapel Hill, Chapel Hill, NC, USA.

The Amyloid-Beta (A-Beta) peptide is a key aggregate species in Alzheimer's disease. While aspects of the A-Beta peptide aggregation pathway have previously been elucidated, the initial conversion of monomer peptides into an oligomer during aggregation is not clearly understood. One potential mediator of these early stages of aggregation is interactions of A-Beta with cell membranes, particularly anionic cell membranes. We use unconstrained and umbrella sampling molecular dynamics simulations to investigate interactions between the 42-amino acid A-Beta peptide and model bilayers consisting of zwitterionic dipalmitoylphophatidylcholine (DPPC) lipids and anionic dioleoylphosphatidylserine (DOPS) lipids. From this work, we determine that A-Beta binds to the surface of DPPC and DOPS bilayers over the small length scales used in simulations. Our results also support the hypothesis that the charge on the bilayer surface and on the peptide affects both the free energy of peptide-membrane binding and the distribution of the peptide on the bilayer surface. Finally, no significant secondary structure change is observed in the peptide during the timescales used in these simulations. This result may indicate all-atom simulation times are too short to observe secondary structure changes in this system or that structure change during the oligomerization process requires peptide-peptide interactions. Our work demonstrates that interactions between the A-Beta peptide and lipid bilayers promote a peptide distribution on the bilayer surface that is prone to peptide-peptide interactions, which can influence the propensity of A-Beta to aggregate into higher order structures.

2347-Pos Board B317

Use of Transmembrane Peptides to Investigate Arginine Interactions with Lipid Bilayers

Vitaly V. Vostrikov, Denise V. Greathouse, **Roger E. Koeppe**.
University of Arkansas, Fayetteville, AR, USA.

With a pK $>$ 12, the guanidinium side chain of arginine (Arg; R) is positively charged over a very wide pH range. There has been much recent discussion concerning the energetics of Arg inserting into a lipid bilayer, or Arg crossing a lipid bilayer. The topic holds significant intrinsic intellectual interest and also is important for understanding the gating mechanism of voltage-dependent transmembrane channels. We address this problem by direct experimental observation using a designed transmembrane peptide that has interfacial tryptophan (Trp; W) anchors. Within membrane-spanning, alpha-helical GWALP23, acetyl-GGALW^{5}LALALAL^{12}ALALALW^{19}LAGA-amide (Vostrikov, *et al.* 2008. *J Am Chem Soc* **130**, 12584), we have substituted either R^{12} or R^{14} near the putative helix midpoint. Models suggest that the W^{5} and W^{19} side chains project from essentially the same side of the GWALP23 alpha-helix, with R^{14} projecting from the *opposite* face and R^{12} from the *same* face as the W^{5} and W^{19} anchors. The R^{12} side chain in effect is situated *between* the W anchors. Based upon solid-state NMR spectra from oriented lipid/peptide samples, specifically the deuterium quadrupolar splittings from several ^{2}H-labeled alanines in each Arg-containing peptide, the properties of these sequence isomers depend heavily upon the location of the Arg. We find that GWALP23-R14 adopts a transmembrane orientation with a tilt of about 17° in DOPC (compared to a tilt of about 6° for GWALP23 itself). By contrast, GWALP23-R12 seems to assume several different orientations with respect to a hydrated bilayer of DOPC; one or more of these orientations may represent surface-bound peptide.

2348-Pos Board B318

Influence of Proline upon the Folding and Geometry of the WALP19 Transmembrane Peptide

Rachel E. Thomas, Vitaly V. Vostrikov, Roger E. Koeppe.
University of Arkansas, Fayetteville, AR, USA.

The orientations, geometries and lipid interactions of designed, specifically anchored, transmembrane (TM) peptides have attracted significant experimental and theoretical interest. Because Pro will introduce a known discontinuity into an alpha-helix, we have sought to measure the extent of helix kinking caused by a single proline within an isolated TM helical domain, and to address the question: To what extent do the individual N-terminal and C-terminal segments adjust their tilts with respect to the bilayer normal in response to the proline? For this purpose, we synthesized WALP19-P10, acetyl-GWWLALALAP^{10}ALALALWWA-ethanolamide, and included pairs of deuterated alanines by using 60-100% fmoc-d_4-Ala at selected sequence positions. Remarkably, solid-state ^{2}H NMR spectra from oriented, hydrated samples (1/40, peptide/lipid; using DOPC, DMPC or DLPC) reveal signals from many of the Ala Cα deuterons as well as the Ala Cβ methyls; whereas signals from backbone Cα deuterons had not been observed for WALP19 without Pro. For example the magnitudes of the ^{2}H quadrupolar splittings are 70 and 10.7 kHz for the Ala^{11} Cα-D and side-chain methyl groups, respectively. We are considering possible reasons for the apparent ''unmasking'' of the backbone resonances in the presence of the proline. At the same time, the observed backbone resonances provide valuable additional data points for evaluating the segmental tilt angles of the N- and C-terminal segments. In order to make available still more data points for the Geometric Analysis of Labeled Alanines (GALA), we also are substituting selected leucines with d_4-Ala. Together the results suggest that the central proline influences not only the geometry but also the dynamics of WALP19.

2349-Pos Board B319

Comparison of Mechanical and Magnetic Alignment of WALP-like Peptides for Solid-State NMR

Nicholas J. Gleason, Vitaly V. Vostrikov, Roger E. Koeppe II.
University of Arkansas, Fayetteville, AR, USA.

Oriented lipid/protein and lipid/peptide samples for solid-state NMR spectroscopy can be prepared by using either hydrated lipid bilayers that are mechanically aligned on glass plates or mixed lipid bicelles (''bilayered micelles'') that are magnetically aligned in solution. The bicelles consist of a combination of long- and short-chain lipids that form lipid bilayer discs in which a planar portion is formed by the longer lipids while the sides are capped by the shorter lipids. In this study we compare the solid-state ^{2}H and ^{31}P NMR spectra from bilayer and bicelle samples containing deuterated peptides. Example peptides having deuterated alanines include WALP19 (a-GWW(LA)$_6$LWWA-e), WALP23 (a-GWW(LA)$_8$LWWA-e) and GWALP23 (a-GGALW(LA)$_6$LWLAGA-e) in which ''a'' is acetyl and ''e'' is ethanolamide. Using bicelles having two orientations, as well as mechanically aligned bilayers, we compare the measured ^{2}H quadrupolar splittings and the deduced average peptide tilt. Additional variables include the lipid composition, peptide-to-lipid ratio and temperature.

2350-Pos Board B320

Half-Anchored WALP Peptides: Effect Of Anchor Position On Peptide Orientation

Johanna M. Froyd-Rankenberg, Denise V. Greathouse, Roger E. Koeppe II.
University of Arkansas, Fayetteville, AR, USA.

Peptides of the ''WALP'' family, acetyl-GWW(LA)$_n$LWWA-[ethanol]amide, provide an opportune model for investigating protein/lipid interactions. Because the motional behavior of the N- and C-terminal tryptophan (W) residues is different (van der Wel [2007] *Biochemistry*, 46:7514), it is of interest to investigate how the positions of the anchoring tryptophans will influence the average peptide orientation. To address this question, we synthesized ''half-anchored'' WALP peptides having only one pair of anchoring tryptophans at either the amino or carboxy terminus. These peptides are acetyl-GGWW(LA)$_8$-ethanolamide and acetyl-(AL)$_8$WWG-ethanolamide, which we designate as ''N-anchored'' and ''C-anchored'', respectively. The hydrophobic lengths of these peptides are similar to that of WALP23, but unlike WALP23 they are anchored to the lipid bilayer membrane on only one side. We find that the half-anchored WALP peptides incorporate into lipid bilayers and assume defined orientations. Unlike shorter half-anchored analogs that contain only three or four Leu-Ala pairs, these longer peptides with eight Leu-Ala pairs show no signs of aggregation and therefore allow further investigation of the peptide/lipid interactions. Circular dichroism spectra indicate that the N-anchored