to consequently deliver its contents at a rate appropriate for maximum therapeutic benefit. It should also possess a large drug loading capacity and retain its contents over the course of treatment. While liposomal systems have experienced success with extending circulation, content retention and controlled release remain problematic. The vesosome - a large lipid bilayer enclosing many smaller liposomes - is the most suitable candidate for addressing these issues. The external lipid bilayer offers a second barrier of protection for interior components and also serves as the anchor for active targeting components. Furthermore, internal compartmentalization permits customization of separate environments for multiple therapeutics and release triggers, highlighting the vesosome's potential as a single site, single dose, multiple component drug treatment.
To assess the viability of the vesosome as a drug carrier, its in vivo lifetime and biodistribution was examined in live animals. Our work examines how these properties are affected by lipid composition and the addition of other functional components, including ones for controlled release and active targeting.

2330-Pos Board B300
Hyperglycemia Promotes Membrane Cholesterol Crystalline Domain Formation Through Lipid Peroxidation: Inhibition with Atorvastatin Metabolite
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Insulin resistance and poor glycemic control contribute to atherogenesis through the chemical and structural modification of cell membrane lipids. The direct contribution of glucose to these membrane alterations, however, is not well understood. In this study, small angle x-ray diffraction and spectrophotometry were used to examine the autoxidative effects of glucose on lipid oxidation and structural organization in model membranes comprised of dilinoleoylphosphatidylcholine (DLPC) and cholesterol. Membranes were prepared at cholesterol-to-phospholipid (C/P) mole ratios ranging from 0.2 to 0.8 in order to model physiologic and hyperlipidemic conditions. Changes in membrane lipid organization and unit cell periodicity (*d*-space) were correlated with lipid hydroperoxide (LOOH) concentration measured at 24 hr intervals. The effects of glucose on lipid peroxidation were more pronounced at elevated levels of membrane cholesterol, with LOOH levels 20% higher at 0.8 C/P than at 0.2 C/P. At 0.6 C/P, glucose treatment resulted in a concentration-dependent increase in LOOH formation as compared to control. These changes corresponded to a reduction in membrane bilayer width (51 Å to 49 Å) and the progressive formation of highly-ordered cholesterol crystalline domains (*d*-space value of 34 Å). Treatment with atorvastatin hydroxy metabolite, a statin with scavenger antioxidant properties, inhibited the membrane-altering effects of hyperglycemia in a dose-dependent manner, even at elevated cholesterol levels. These data demonstrate that glucose directly stimulates lipid peroxidation and subsequent changes in membrane structure, including the formation of immiscible cholesterol crystalline domains. Insights from this study may also serve as a model for better understanding the membrane structural changes associated with diabetes and related complications.

2331-Pos Board B301
Characterization of a New Biomimetic Multilayer System for Biomembrane Interaction Studies
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Layer-by-layer (LbL) deposition methods were shown to be especially suitable for polyelectrolyte multilayers (PEM) as stable and functional supports for various bio-mimetic systems. Here, results from an investigation of the interaction between chitosan/heparin PEM films and small unilamellar lipid vesicles (SUV) are presented. The membranes were composed of a mixture of zwitterionic POPC and its cationic counterpart E-POPC thus having a positive surface charge density. Surface Plasmon Resonance (SPR) was applied to continuously monitor the self-assembly process of physisorption of subsequent PE layers and to report the deposition efficiency and dynamics. A terminating lipid bilayer was successfully deposited on top of the PEM films, both with chitosan and heparin as uppermost PE layer. The lipid layer could be totally removed by detergent application without damage to its PEM cushion. The PE film itself was studied by atomic force microscopy (AFM) in its dry and also in its fully hydrated state. The integrity and homogeneity of the terminal lipid bilayer on its PEM cushions was also visualized with the AFM technique. Currently, neutron reflectivity is being applied to further investigate of the multi-layer structure of the composite film and its hydration. Experiments with confocal microscopy and applying SFS (sum frequency spectroscopy) are under preparation.

Interfacial Protein-Lipid Interactions I

2332-Pos Board B302
Interaction of Tea Catechin (−)-Epigallocatechin Gallate with Lipid Bilayers
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A major component of green tea extracts, catechin (-)-Epigallocatechin gallate (EGCg) has been reported to be biological active and interacting with membranes. A recent paper reported drastic effects of EGCg on giant unilamellar vesicles (GUVs). In particular, EGCg above 30 μM caused GUVs to burst. Here we investigated the effect of EGCg on single GUVs at lower concentrations, believing that its molecular mechanism would be more clearly revealed. We used the micropipette aspiration method, by which the changes of surface area and volume of a GUV could be measured as a result of interaction with EGCg. We also used X-ray diffraction to measure the membrane thinning effect by EGCg. To understand the property of EGCg, we compared its effect with other membrane-active molecules, including pore-forming peptide magainin, the turmeri (curry) extract curcumin, and detergent Triton X100. We found the effect of EGCg somewhat unique. Although EGCg readily binds to lipid bilayers, its membrane area expansion effect is one order of magnitude smaller than curcumin. EGCg also solubilizes lipid molecules from lipid bilayer without forming pores, but its effect is different from Triton X100.

2333-Pos Board B303
How Do Electrostatic Interactions Affect The Behavior Of Transmembrane Peptides?
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It has been shown that changes in physical properties of the membrane, such as surface charge or fluidity, affect the activity of embedded proteins. This is likely related to the presence of polar and/or aromatic residues that are often observed in the interfacial regions of those proteins. There mutation often results in modification of their activity. This raises the question: how do those polar and/or aromatic residues affect the orientation and dynamic behavior of transmembrane segments of proteins, thus affecting protein activity?
Here we try to understand how electrostatic interactions affect transmembrane segments by use of simplified model systems consisting of KALP and WALP peptides. These peptides are composed of alternating alanine and leucine stretches flanked with lysines or tryptophans residues respectively. The peptides are embedded in vesicles containing Zwitterionic (DMPC), negatively charged (DMPG, DMPS, DMPA), or positively charged (DMTAP) lipids. The samples are then analyzed with 2H or 14N and 31P wide line solid state NMR methods.
The results show that lipid composition affects transmembrane peptides in different ways depending on whether they are flanked with lysines or tryptophans. The results highlight the different properties of salt-bridge interactions and cation-pi interactions, and their possible implications in membrane protein activity.

2334-Pos Board B304
Orientation Of A Transmembrane Peptide Under Positive Mismatch By Computer Simulations
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This work deals with the orientation of a transmembrane model peptide (WALP23) under positive mismatch, assessed by atomistic molecular dynamics simulations. Emphasis was given to link our results to deuterium solid state NMR data of the same system under the same mismatch conditions. So far, small tilt angles were extracted from the experimental quadrupolar splittings using a geometric analysis, called the GALA method. The backcalculation of these NMR quadrupolar splittings from our simulations showed a good fit with experimental data only if several hundred of nanoseconds trajectories were considered. Some coarse-grained simulations allowed us to reach the NMR time scale (a few microseconds) and led to the same observation. For both types of simulation we found that some averaging effects may affect the interpretation of NMR data, and thus larger tilt angles than previously estimated are likely to occur.

Reference:
Özdirekcan et al. On the orientation of a designed transmembrane peptide: towards the right tilt angle? J. Am. Chem. Soc. (2007), 129, 15174 -15181.

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