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.