dynamics may be important for understanding the diffusion water transport in the top layer of skin, stratum corneum. Here, we investigate the heterogeneity of model skin membranes on the nanoscale using site-directed FRET approach. Using Bodipy donors and acceptors (Bodipy-FL and Bodipy-TR, respectively) covalently attached to ceramide, cholesterol, and fatty acid, it is possible to investigate the compositional heterogeneity of model skin lipid membranes using FRET efficiency measurements for various combinations of donors and acceptors. Using this approach, it is possible to estimate the co-localization of different lipid species on the length scale less than ~10 nm (~2 Förster radii for Bodipy probes). Measurements of FRET efficiency as a function of acceptor-to-donor molar ratio show strong co-localization between ceramides, fatty acids, and cholesterol. We show that the position of Bodipy group in cholesterol fluorescence analogs affects the partitioning of the probe within lipid bilayers. We also show that site-directed FRET can be used for characterizing interactions between the biomembranes and detergents.

1801-Pos Board B711
Raft Recruitment Processes and Oligomerization State of Integrons Studied in Polymer-Tethered Single and Double Bilayer Systems
Amanda P. Siegel, Ann Kimble-Hill, Rainer Jordan, Christoph A. Naumann.
Specific lipid environments are increasingly recognized as a crucial factor affecting membrane protein function in plasma membranes. Unfortunately, this topic has remained elusive, due to the challenging characterization of small and transient plasma membrane heterogeneities. To overcome this impasse, we present an experimental model membrane platform based on polymer-supported single and double bilayers containing stable raft-mimicking domains. The flexible platform lets us probe the effect of native lipids in domain-specific protein sequestration and protein oligomerization state. Here we show significant ligand-induced changes in integrin sequestration. Remarkably, preliminary results indicate that integrins do not change their oligomerization state on the addition of lipids in lipid environments with varying concentrations of cholesterol. These results strongly suggest that ligands induce changes to integrin conformation and/or dynamics without inducing changes in integrin oligomerization state, and in fact these ligand-induced conformational changes impact protein-lipid interactions.

1802-Pos Board B712
A biosynthetic Membrane-Anchor/Protein System Based on a Genetically Encoded "Aldehyde Tag"
Chaojie Zhen, Ian P. McCabe, David Rabuka, Rebecca A. Bader, Martin B. Forstner.
The covalent binding of an aldehyde side-chain containing protein to a lipid with an aminooxy-modified head group opens a versatile avenue to bio-functionalize lipid membranes without compromising function and dynamic properties of both the protein and the lipid membrane. It was recently found that the site-specific insertion of a 6 amino acid consensus sequence into a protein is sufficient to target it for post-translational modification by formylyglycine-generating enzyme (FGE). FGE will enzymatically turn the cysteine in the consensus motif into a formylglycine, thus leading to the site-specific introduction of an aldehyde side chain for further chemical modification. We have engineered the consensus sequence to the C-terminus of an Enhanced Green Fluorescence Protein (EGFP) which was co-expressed with FGE in E. Coli. Lipids were chemically modified to bear a reactive aminooxy group and then conjugated with the aldehyde tagged EGFP. The resulting EGFP-lipid constructs were successfully incorporated into solid supported lipid bilayer as verified by fluorescence microscopy. Membrane integrity as well as protein and lipid motilities were investigated using both fluorescence recovery after photo-bleaching and fluorescence correlation spectroscopy. In order to determine integration efficiency, the surface concentration of EGFP-lipid constructs was monitored as a function of their solution concentration and incubation time. This site specific lipidation strategy promises to allow for the use of a variety of possible lipid anchors as well as to provide unprecedented freedom in the choice of the lipidation site on the protein.

1803-Pos Board B713
Incorporation of Membrane Proteins and Electrodes into a Suspended Lipid Bilayer Platform
Laura D. Hughes, Steven G. Boxer.
Membrane proteins are notoriously difficult to study. While supported lipid bilayers offer stability and allow the application of surface measurement techniques, integral membrane proteins are often not fully functional when close to a solid surface. We have developed a membrane interferometer which allows free standing membranes suspended above an atomically flat silicon surface to be studied by fluorescence interference measurements (Prasad V. Ganesan and Steven G. Boxer, PNAS; 2009,106, 5627-5632). In this platform, a lipid bilayer is suspended across a micron-sized well, allowing the use of Variable Incidence Angle Fluorescence Interference Contrast Microscopy (VIA-FLIC; Caroline A. Frankin, Prasad V. Ganesan, and Steven G. Boxer, Biophys J., 2005, 89, 2759-2769). The interferometry measurements in VIA-FLIC can be used to determine the height of fluorescent dyes relative to the mirror with an axial resolution of a few nanometers. Moreover, by incorporating electrodes on both sides of the suspended bilayer, we wish to perform concurrent conductance and optical measurements that will allow us to study the function and conformation of single membrane proteins at the same time. Progress towards incorporating ion channels into the interferometer and making electrophysiology measurements will be reported.

1804-Pos Board B714
The Residence Time and Processivity Study of the Ras/Sos Interaction
Ras is a membrane-bound small GTPase protein that plays a central role in the signal transduction pathways that control cell proliferation, differentiation, and apoptosis. Its deregulation is a hallmark of many cancers and developmental defects. Son of Sevenless (SOS) is a guanine nucleotide exchange factor (GEF) enzyme that activates Ras by catalyzing the conversion of Ras from the GDP- to the GTP-bound state. SOS has two binding sites for Ras, a catalytic site and an allosteric site, which can both be occupied simultaneously by membrane-bound Ras. Previous studies have shown that binding to the allosteric site by Ras-GTP will localize SOS to the membrane and therefore stimulate the nucleotide exchange activity of the catalytic site (positive-feedback), raising the question of whether SOS is processive, capable of remaining surface bound while catalyzing the nucleotide exchange of multiple Ras. In this study we employ fluorescence microscopy on Ras functionalized supported lipid bilayers to demonstrate that the catalytic core of SOS (SOScat) is processive. In the absence of GTP, SOScat remains surface bound via Ras in a non-processive state for ~hours. Addition of GTP triggers processive turnover of multiple Ras by surface bound SOScat. Using single molecule TIRF microscopy, the result indicates that most of the initial surface bound SOScat rapidly desorbs when GTP is added, and that most of the Ras turnover is catalyzed by a small but processive fraction of the initial SOScat population.

1805-Pos Board B715
Elucidation of Carbohydrate-Phospholipid Interactions - a Quantum Chemical Study
Ramakrishnan Parthasarathi, Jianhui Tian, S. Gnanakaran.
Many pathogens induced potential causative immune responses are determined by the interaction of a virulence factor containing carbohydrates with host membranes. Here, we seek a basic understanding of the nature of interactions between carbohydrate and lipid to dissect their role in molecular recognition. A hybrid quantum mechanics/quantum mechanics (QM/QM) scheme is described here, to explore the structural basis and energetics of carbohydrate-phospholipid interactions. This method is used to study two different phospholipids (POPC and DOPC) and mannose interactions using density functional theory (DFT). Carbohydrate-lipid interactions are probed with respect to competing interactions to water. The results clearly reveal the intrinsic nature of interactions between the carbohydrate and phospholipid system. The significance of the OH - O, CH · · O and CH - · π interactions in the stabilization of the intermolecular complexes can be observed from the results. The calculated average interaction energies for the various carbohydrate-water-lipid complexes show both mannose and water preferably interact with POPC over DOPC. The interplay between conventional and nonconventional hydrogen bonding and non-polar interactions is crucial in the recognition and further stabilization of carbohydrate-phospholipids complexes. This first hybrid QM/QM method on carbohydrate-lipid interaction demonstrates that mannose interactions with phospholipids could result in alterations in charge distributions and conformations. Finally, we have compared these QM energies with Molecular Mechanics (MM) based energies for the same interactions to aid in the refinement of the all-atom lipid-carbohydrate force field.

1806-Pos Board B716
Understanding the Phase Changes of Coarse-Grained Model Bilayers Through Computational Calorimetry
Jocelyn M. Rodgers, Jesper Sorensen, Frederick J.-M. de Meyer, Birgit Schiott, Berend Smit.
In this study, we assess the thermodynamic behavior of a variety of coarse-grained lipid bilayer models across the temperature range which experimentally produces the gel phase, the ripple phase, and the liquid crystalline phase. Computational model systems including both lipids and water are often validated by...