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 Integrins 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 into which transmembrane proteins are incorporated ($\alpha v\beta 3$ and $\alpha 5\beta 1$ integrins). This flexible platform lets us probe the effect of native ligands in domain-specific protein sequestration and protein oligomerization state. Here we show significant ligand-induced changes in integrin sequestering. Remarkably, preliminary results indicate that integrins do not change their oligomerization state on the addition of ligands 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 formylglycine-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 Ajo-Franklin, 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

Hsiung-Lin Tu, Lars Iversen, Wan-Chen Lin, Jeffrey Iwig, Jodi Gureasko, John Kuriyan, Jay Groves.

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 pathogen 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 & 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 phospholipids system. The significance of the $OH\cdots O$, $CH\cdots O$ and $CH\cdots\pi$ 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

comparison to structural or thermodynamic data at ambient conditions or by comparison to the variation of structural data as a function of temperature. Here we compare these models on new grounds by using a computational version of differential scanning calorimetry, an experimental technique widely employed to observe phase transitions in model bilayer systems. The goal of such an endeavor is to gain insight into the driving forces behind phase changes in single component model lipid systems. The strong interest in the phase behavior of multi-component bilayers as simple models of cell membranes requires that we also more deeply understand the phase behavior of the pure lipid bilayer computational models first. This study yields progress in understand the driving forces of each model and the trade-offs in choosing various coarse-grained models.

1807-Pos Board B717

The Lateral Stress Profiles of Lipid Bilayers Compared with Spontaneous Lipid Curvature Using Computer Simulations

Alex J. Sodt, Richard W. Pastor.

For transmembrane protein folding, it is hypothesized that the lateral stress profile of the lipid bilayer is an important factor for comparing protein conformations, such as the open and closed states of a channel. For example, according to this theory, the positive pressure at the bilayer interior and negative pressure at the bilayer surface stabilize protein conformations with an hourglass shape. However, the lipid-dependent lateral stress profile of a lipid bilayer is not directly measurable by experiment, but rather is inferred, e.g., from the spontaneous curvature of its constituent lipids, a geometric parameter that may be measured by x-ray scattering of the hexagonal phase of lipid/water systems. The lateral stress profile of model bilayers may be measured by computer simulations, in this work using the latest all-atom lipid CHARMM forcefield. By simulating both the fluid bilayer and hexagonal phases of lipid systems with varied spontaneous curvature, we seek to bridge the gap between experimentally measured spontaneous curvature of lipids and their characteristic bilayer lateral stress profile.

1808-Pos Board B718

Molecular Dynamics Simulation Studies of Cardiolipin Bilayers

Kevin C. Song, Richard M. Venable, Wonpil Im, Richard W. Pastor.

Molecular dynamics (MD) simulations of tetramyristoyl cardiolipin (TMCL) and tetraoleoyl cardiolipin (TOCL) were carried out with the newly developed CHARMM lipid force field (FF), C36, and with head group charges q= −1 and −2. The surface areas per lipid, A_L, for q=-1 are 126 ± 0.1 Å^2 for TOCL and 111 ± .1 Å^2 for TMCL. These are 1.8 times than those of the diacyl equivalents: 63 Å^2 for dimyristoylphosphatidylcholine (DMPC) at 328K, and 69 Å^2 for dioleoylphosphatidylcholine (DOPC) at 310K. Area compressibility, K_a, of TOCL equals 340 ± 40 dyn/cm, approximately 50% higher than experimentally obtained for DOPC (and most diacyl lipids); an experimental value for K_a for cardiolipins is not presently available. The areas and compressibilities for TOCL from the present simulation differ substantially from those obtained by Dahlberg and Maliniak using the FF of Berger et al. under the same conditions (A_L= 99 Å^2; K_a = 1100 dyn/cm). The origin of the differences appears to be in the ion binding to the surfaces of the cardiolipin bilayer. Under FF of Berger et al., ions bind closer to the carbonyl group in the lipid chain region whereas C36 CHARMM FF results ion binding closer to the negatively charged phosphate groups of the head group. Deuterium order parameter measurements are underway to determine which FF yields areas more representative of the fluid state of cardiolipin bilayers.

1809-Pos Board B719

Effect of Extrinsic Constraints on Lipid Bilayers

Sameer Varma, Michael Teng, H. Larry Scott.

The structure, dynamics and organization of cell membranes are not passive consequences of lipid equilibration in a two-dimensional milieu. Instead, a rapidly advancing body of work now suggests that these physical properties are actively and spatiotemporally regulated by a variety of intrinsic and extrinsic constraints. Extrinsic constraints, or interfacial templating, involve dynamics and fluctuations in the underlying cytoskeleton and cytoskeleton-binding membrane proteins, which actively re-compartmentalize the membrane fluid, modulating molecular diffusion and organization in membrane bilayers. In addition, modulations in the polar environment surrounding the membrane, including pH, ionic strength and composition, also influence membrane equilibration and dynamics. Precisely how these constraints influence membrane properties remains incompletely understood. Here we introduce and employ three different types of model membrane configurations that resemble physical constraints offered by the cytoskeleton. By analyzing statistics from microsecond-long atomistic molecular dynamics simulations, we develop detailed correlations between the properties of the lipid bilayers and the interfacial constraints. Our main finding is that despite the presence of nanometer-thick water layers buffering the interaction between the cytoskeletal constraints and the lipid bilayers, the cytoskeletal constraints induce a striking asymmetry between the properties of the bilayer leaflets.

1810-Pos Board B720

Molecular Modeling of Domain Formation upon Protein Adsorption in Lipid Bilayers

Doris M. Grillo, Igal Szleifer, Monica Olvera de la Cruz.

The mechanisms that govern domain formation in phospholipid bilayers are unclear. Understanding the underlying principles of domain formation in model lipid bilayers will provide with insights on how protein adsorption leads to domain formation and also will lead to the effective design of novel biotechnology applications that can take advantage of the structure to function relationship in biology. In this work, the thermodynamics and structural properties of domain formation upon protein adsorption in model lipid membranes are studied through the application of a three dimensional molecular theory that includes a complete description of the relevant interactions of the entire phospholipid molecules. This theoretical approach takes into account the electrostatic interactions of the hydrophilic phospholipid headgroups as well as the attractive packing interactions of the phospholipid acyl tails. The proposed theory considers in an explicit manner the molecular conformations, size, shape and charge density of each molecule within a mean-field level approximation for the intermolecular interactions. The results show how domain formation in lipid bilayers depends on several biologically relevant environments such as different salt concentrations, solution pH and phospholipid composition of the bilayer, i.e. , the chemical structure and number density of the different phospholipid molecules present in the bilayer. The molecular theory provides with the tools for understanding the fundamental principles of phospholipid domain formation by giving insights on how the membrane responds to changes in its chemical environment. Moreover, since the theory includes an explicit description of the phospholipid headgroups, the coupling between the physical states of the two leaflets of the bilayer as the result of protein adsorption onto one side of the bilayer is also described.

1811-Pos Board B721

External Electric Field in the Atomistic Simulation of Membrane Systems

Anatoly Dryga, Arieh Warshel.

The relationship between the membrane voltage and the gating of voltage activated ion channels has been a problem of great current interest. Although the macroscopic representation of external potential is well-known, incorporation of external voltage in a consistent molecular model is not trivial. In the current work, the effect of external electric field as well as the effect of ionic strength is introduced into the Coarse-Grain molecular simulation approach. This simulation technique allows us to calculate effect of transmembrane potential in an atomistic simulation. The resulting model is validated with known results(Debye-Huckel, Gouy-Chapman, and membrane in electrolyte solution).Preliminary results for K channel and its gating process are discussed.

Membrane Active Peptides I

1812-Pos Board B722

The Molecular Basis for Antimicrobial Activity of Pore-Forming Cyclic Peptides

Gemma Moiset, Anna D. Cirac, Jacek T. Mika, Armagan Koçer, Durba Sengupta, Siewert-Jan Marrink, Bert Poolman.

The mechanism of action of antimicrobial peptides is still not understood in molecular detail. Here, we present a molecular-dynamics and biophysical study of a cyclic antimicrobial peptide, c(KKLKKWKKLQ), and its inactive linear analogue. We establish that, relative to the linear peptide, the cyclic one binds stronger to negatively-charged membranes, folds at the interface and subsequently penetrates deeper into the bilayer. In the simulations, the cyclic peptide caused large perturbations in the bilayer and cooperatively opened a disordered toroidal pore, 1-2 nm in diameter. Electrophysiology measurements confirm discrete poration events of size 1.5-2 nm by the cyclic peptide. By employing dual-color fluorescence burst analysis, we show that both peptides are able to fuse/aggregate liposomes but only the cyclic peptide is able to form pores with a size of ~2 nm. Fluorescence Resonance Energy Transfer (FRET) proved the fusiogenic activity of the cyclic versus the aggregation of vesicles caused by the linear peptide. The results provide detailed insight on the molecular basis of the activity of cyclic antimicrobial peptides.