the proximal leaflet and lack of appropriate protein insertions but also to understand the bilayer-nanoparticle interactions. Here, we demonstrate the formation of multiphase lipid bilayers on nanoporous silica xerogels and compare it with mica supported bilayers. It was observed that the lipid bilayer follows the surface contours by AFM (Atomic Force Microscopy). This was also confirmed by the quantitative fluorescence analysis. The lateral diffusion coefficient of the lipids on silica xerogel was found to be lower than on mica by both FRAP (Fluorescence Recovery After Photobleaching) and FCS (Fluorescence Correlation Spectroscopy) experiments. The basic reason for this reduction was the bilayer following the surface contours. The domains on silica xerogel were observed to be symmetric and larger than the domains on mica. This reflects the possible effect of the support on the phase behavior of the lipid mixture. Ternary mixtures containing cholesterol were also prepared and the substrate effect on phase behavior was investigated.

1869-Plat

Understanding The Nucleation and Growth of Lipid-Lipid Phase Separation at Nanometer-Length-Scale: A Small Angle Neutron Scattering Study

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Although lipid-lipid phase separation has been studied extensively in model lipid membranes, most of the available information is limited to micron-size domains. Lack of information on the nanometer-length-scale is a big hurdle in understanding the fundamental basis for lipid domain nucleation, in the first place, and growth. How do small unstable domains grow? What is the effect of temperature and membrane curvature? To answer these questions, this work presents Small Angle Neutron Scattering (SANS) studies on small Unilemellar Vesicles (ULVs) (diameter varying from 30nm to 400nm) made of 1:1 and 3:7 ratios of deuterated DPPC (dDPPC) and hydrogenated DLPC respectively. Small vesicles with varying sizes not only provide a means to control the curvature, but also limit the amount of available lipids for domain growth. Experiments were performed in contrast matched conditions, such that the scattering length density of the solvent and that of the homogeneous lipid vesicle (above the melting transition temperature, Tm, of the mixture) were exactly equal. As the sample temperature was lowered below Tm, the lipids started to phase separate and an excess scattering characteristic of lipid segregation into nano-size domains was observed. Notable trends were observed in the scattering curves as the temperature was lowered below the Tm of the mixtures. Interestingly, these results show that the phase separation behavior varies significantly between the small and large size vesicles. Insight in to the basic mechanism for the formation of lipid-lipid phase separation as a function of temperature and curvature will be discussed based on the analysis of the scattering intensities and Differential Scanning Calorimetry experiments.

1870-Plat

Cholesterol Displacement By Ceramide In Sphingomyelin-containing Liquid-ordered Domains, And Generation Of Gel Regions In Giant Lipidic Vesicles

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Fluorescence confocal microscopy and differential scanning calorimetry are used in combination to study the phase behaviour of bilayers composed of PC:PE:SM:Chol equimolecular mixtures, in the presence or absence of 10 mol% egg ceramide. In the absence of ceramide, separate liquid-ordered and liquid-disordered domains are observed in giant unilamellar vesicles. In the presence of ceramide, gel-like domains appear within the liquid-ordered regions. The melting properties of these gel-like domains resemble those of SM:ceramide binary mixtures, suggesting Chol displacement by ceramide from SM:Chol-rich liquid-ordered regions. Thus three kinds of domains coexist within a single vesicle in the presence of ceramide: gel, liquid-ordered, and liquid-disordered. In contrast, when 10 mol% egg diacylglycerol is added instead of ceramide, homogeneous vesicles, consisting only of liquid-disordered bilayers, are observed.

1871-Plat

How Membrane Curvature Can Sort Proteins

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While it is a well known fact that membrane proteins interact via electrostatic, hydrophobic and van der Waals forces, only recently attention was drawn to a new kind of fundamental interaction that is mediated by the membrane itself. Proteins that locally impose a membrane curvature create a deformation that is probed by other, similar proteins. Since it is very difficult to quantitatively measure and discriminate the different types of interactions in the case of proteins, we study a model system which allows the exclusive observation of membrane mediated interactions alone. Building on our earlier experiments on fully phase separated vesicles (Semrau et al., PRL, 2008) we study biomimetic vesicles with bulging liquid ordered domains. These domains deform the surrounding membrane and create a repulsive force between them. This leads to a wealth of measurable effects: we observe hindered domain fusion, a preferred domain size and, most importantly, an intriguing sorting mechanism. Domains of different sizes spontaneously demix and form regions of equally sized domains. Quantitative measurements of the repulsive force allow us to build a model for the sorting that is independently confirmed in Monte Carlo simulations. The observed sorting provides a new mechanism for protein sorting in the endoplasmic reticulum and for membrane mediated protein aggregation.

1872-Plat

Lateral Stress and Spontaneous Curvature in Mixed Membranes

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A key aspect of membrane mechanics is the balance of attractive and repulsive forces involving lipid head groups and acyl chains [1]. In particular, demethylation of PC lipids and addition of polyunsaturated chains [2] have been shown to alter substantially the activity of membrane receptors [3, 4] and of ion channels. How to best describe membrane forces? An appealing theoretical description is the lateral stress profile, which is easily calculated from computer simulations. However the lateral stress profile is not yet accessible experimentally. Experimental observables (which can be related to the lateral pressure profile if desired) are (i) the *spontaneous curvature* values as obtained from X-ray scattering, and (ii) the lateral *mean-torque profile* obtained from solid-state ^{2}H NMR [1]. We report and analyze experimental data on PE/PC and saturated-polyunsaturated lipid mixtures to show how measured shifts in the chain mean-torque profiles correspond to shifts in the balance of forces between headgroups and acyl chains. We first determine changes in the mean-torque profile caused by variations in lipid composition. We then determine a geometric parameter called the projected segmental area, directly related to the spontaneous curvature of individual monolayers. Last, we show that lateral compression energies are sufficiently large to provide a thermodynamic driving force for protein conformational changes. Our approach reveals that a universal chain packing profile exists for saturated acyl chains, and that the measurable shift in the force balance gives rise to the observable membrane mechanics. This approach provides a new framework for relating lipid composition to membrane function.

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Platform AB: Molecular Dynamics

1873-Plat

Automated Event Detection and Activity Monitoring in Long Time-Scale Molecular Dynamics

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Molecular dynamics trajectories of biological systems contain many events of great scientific interest, including conformational transitions, folding processes, and translocations of ligands and reaction products. In proteins these events often correspond to high-level tertiary or quaternary structure rearrangements, which alter the contacts between amino acid residues. Due to advances in computer architecture and software, molecular dynamics trajectories representing such structure-changing events have become easy to generate, but their length complicates scientific interpretation. The goal of this work is to simplify an important part of the analysis workflow (and to complement traditional visual inspection) by automating the mining of long trajectories. We present

several new algorithms and implementation techniques that enable the detection of significant structure-changing events in a molecular dynamics trajectory. These algorithms include a coarse graining of side chain contacts, a contact metric based on higher-order generalizations of the Delaunay tetrahedralization, and median filters for detecting significant shifts in the ensemble mean of the resulting time series. We have also developed numerical techniques for suppressing trivial re-crossing events and a new kernel-based estimator of the contact alteration activity. These methods will be disseminated in a newly developed package, ''TimeScapes,'' which is compatible with molecular dynamics trajectories generated from any of a variety of popular simulation programs. Tests on microsecond time scale simulations suggest that the implementation is efficient and requires very little parameterization. The analysis provides a detailed listing of broken and formed contacts, and reliably detects allosteric and folding transitions, as well as stable intermediates, in the protein dynamics.

1874-Plat
Searching For the Hinge of E3 Ubiquitin Ligase Machinery with MD Simulations
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In Cullin-RING E3 ubiquitin ligases, substrate binding proteins, such as VHL-box, SOCS-box or the F-box proteins, recruit substrates for ubiquitination, accurately positioning and orienting the substrates for ubiquitin transfer. Yet, how the E3 machinery precisely positions the substrate is unclear. We performed molecular dynamics simulations for seven substrate binding proteins: Skp2, Fbw7, β-TrCP1, Cdc4, pVHL, SOCS2, and SOCS4, in the unbound form and bound to Skp1 or Elongin C. All seven proteins have two domains: one binds to the substrate; the other to E3 ligase modules Skp1/Elongin C. In all seven cases, the flexible inter-domain linker serves as a hinge rotating the substrate binding domain, optimally and accurately positioning it for ubiquitin transfer. A conserved proline is noticed in the linker of all seven proteins. The prolines pucker substantially and the pucker is associated with the backbone rotation toward the E2/ubiquitin. We further observed that the linker flexibility could be regulated allosterically by binding events associated with either domain. Thus searching for the allosteric sites to regulate the flexibility could provide a new strategy for drug discovery targeting the ubiquitin system. This project has been funded in whole or in part with Federal funds from the National Cancer Institute, National Institutes of Health, under contract number NO1-CO-12400.

1875-Plat
Identification Of Two Distinct Inactive Conformations Of The Beta-2 Adrenergic Receptor Reconciles Structural And Biochemical Observations
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Understanding the mechanisms of signaling proteins such as G-protein-coupled receptors (GPCRs) requires definition of their conformational states and the pathways connecting those states. The recent crystal structures of the beta-2 and beta-1 adrenergic receptors in a presumably inactive state constituted a major advance toward this goal, but also raised new questions. Although earlier biochemical observations had suggested that the beta adrenergic receptors possessed a set of contacts between helices 3 and 6, known as the ionic lock, which was believed to form a molecular switch for receptor activation, the crystal structures lacked these contacts. The unexpectedly broken ionic lock has provoked a great deal of speculation, raising questions about whether the structures accurately represent the inactive receptor state and whether the ionic lock plays a role in activation of these and other GPCRs. To address these questions, we performed microsecond-timescale molecular dynamics simulations of the beta-2 adrenergic receptor in multiple wild-type and mutant forms. Our observations of the behavior of the ionic lock, along with the formation of several novel structural elements in the extramembrane loops during our simulations, paint a more complete picture of the inactive state of the beta adrenergic receptors, reconciling the crystal structures with biochemical studies.

1876-Plat
Atomic Level Description of GPCR Activation Revealed by Microsecond Time Scale Molecular Dynamics
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Previously, we had reported the results of microsecond molecular dynamics simulations for the interaction of 2-arachidonoylglycerol (2AG), the endogenous ligand of the Class A cannabinoid CB2 receptor, with the CB2 receptor in an explicit POPC lipid bilayer[1]. These results show the initial stages of agonist binding to and activation of the CB2 receptor. Analysis of these trajectories reveals that upon the binding of 2AG, which occurs via lipid between transmembrane helix 6 (TMH6) and TMH7, the intracellular portions of TMH3 and 6 separate with a concurrent breaking of an intracellular salt bridge. The latter event has been probed by an Essential Dynamics analysis of the trajectory during the binding event. This analysis indicates that a single eigenvector captures the motion of the breaking of this salt bridge and the opening of the intracellular surface of the receptor, events that are believed to be associated with activation. These results will be presented and discussed, particularly in light of recent experimental results of spin label measurements by Altenbach et al. [2] and the crystal structures of opsin [3,4] which both show an intracellular separation of between 6-7 Å between the ends of TMH3/TMH6 of rhodopsin upon light activation.
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1877-Plat
Potential of Mean Force Calculations of Ion Permeation in Gramicidin A Channel
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The potential of mean force (PMF) for K^+ ion permeation through the gramicidin A (gA) channel were calculated from the molecular dynamics (MD) simulations with four different force fields (FF): CHARMM27, CHARMM27 with dihedral-based correction map (CHARMM27+CMAP), CHARMM27 with a improved FF parameters for tryptophan indole ring (CHARMM27+Trp), and CHARMM27 with the CMAP and the improved FF parameters for Trp (CHARMM27+CMAP+Trp). When comparing the PMFs obtained with these four different FF, we find that both CHARMM27 and CHARMM27+Trp predict free energy profiles that are in semi-quantitative agreement with measurements of the conductance and dissociation constant. The combination CHARMM27+Trp gives the best agreement. However, the CHARMM27+CMAP yields a larger barrier in the PMF and CHARMM27+CMAP+Trp generates a deeper binding potential well. These calculations illustrate the sensitivity of the PMF controlling ion permeation to subtle changes in the FF. We also compute a 2-ion PMF for a doubly occupied gA channel. The effect of the number of water molecules in the channel on the effective ion-ion interactions is also studied. Elucidating the properties of the doubly occupied channel is important because experiments are often carried out at fairly high concentration where double ion occupancy is predominant.

1878-Plat
A Solvent-Free Coarse-Grained Model for Quantitative POPC Bilayer Simulations
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We presented an implicit solvent CG model in a bottom-up scheme for simulations of 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) bilayer membranes. The usage of implicit solvent enables membrane simulations on large length- and time-scale at modest computational expense. Despite an improved computational efficiency, the model preserves chemical specificity and quantitative accuracy in comparison with top-down solvent-free CG bilayer models. In the CG model, each of the CG sites was associated with the center-of-mass of a specific group of atoms in the all-atom representation of POPC. The bonded and non-bonded interaction parameters together with the effective cohesive interactions mimicking the hydrophobic effect were systematically derived by matching radial distribution functions, density and pressure profiles of the bilayer, and self-assembly of lipids in all-atom simulations of POPC phospholipids. The CG model is especially useful for studies of large-scale phenomena in membranes which require a detailed description of chemical specificity, e.g. membrane patches interacting with movable and transformable membrane proteins/peptides.

1879-Plat
Interaction of Fullerene with Model Cell Membranes: a Computer Simulation Study
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