Our results indicate that LPA-membrane interactions depend on initial surface pressure, phospholipid headgroup and degree of acyl chain saturation, presence and amount of cholesterol, aqueous media conditions, and aggregation state of LPA. These data suggest that, in addition to its function as a ligand for specific GPCR, LPA interacts directly with the target membrane, constituting a role for this phospholipid as a physical regulatory molecule for LPA cellular signaling pathways.

3590-Pos

Molecular Hydration Investigated using Extended Membrane Surfaces

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In biological cells and in solutions, biomolecules are in constant competition for water. The availability of water is critical to a wide array of processes such as protein folding, molecular recognition, and cell signaling. To understand these mechanisms, we distinguish between the water molecules residing in the vicinity of biological macromolecules and the most distant water molecules in the bath. What happens on the surface of macromolecules? How to investigate the role and the properties of interfacial water? The problem is that the spatial extent of hydration layers is very small, on the order of nanometers or less. How to produce an experimentally measurable signal? Our approach to investigate the many remaining mysteries of molecular hydration is by using the naturally extended water surfaces within multilamellar lipid structures. We show how addition of salts, sugars, and most buffers make the interlamellar (D) spacing of synthetic phospholipids membranes to increase. In contrast to this behavior, low concentrations of highly hygroscopic molecules such as PEG (polyethylene glycol) and DMSO (dimethyl sulfide) are found to decrease the D-spacing. A very interesting case is that of the small molecular weight PEG 400 with unusual effects on the interfacial hydration, in such a way that at some concentration threshold the PEG molecules overcome a mixing barrier and become included in the forbidding interlamellar water space. This behavior is due to competitions between the strong exclusions forces from extended hydration surfaces and entropy. We are currently investigating how biological relevant molecules such as ''Factor V'' (BSA Bovine Serum Albumin) are modifying cellular osmoregulation and its effects on membranes stress. Knowing how molecules interact at the lipid-water interface could prove beneficial in the drug design of anesthetics, cryoprotectants of mammalian cells, and in general, of molecular stressor affecting biological cells.

Signaling & Membrane Transformations

3591-Pos

Determination of Threshold Forces for Tether Formation in Vesicles

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Membrane tether experiments, which involve applying point forces to membranes to form thin tubes of the membrane, provide a powerful method to drastically alter membrane curvature. To study the formation of membrane tethers, we have designed and built a magnetic force transducer (MFT) using microfabrication techniques. MFTs have traditionally suffered from an inability to precisely calibrate the force. Quantitative determination of the tether formation force using theoretical models of membrane mechanics, may be done very accurately if the applied force is known with sub-piconewton accuracy. Our initial results indicate our ability to control the amplitude of the force up to 10 pN within +/- 0.2 pN over a constant length of 50 microns. We have used this device to determine tether formation forces from POPC giant unilamellar vesicles and find that formation forces range from 3 to 10 pN. When tethers are repeatedly pulled from the same vesicle, the formation force is constant, suggesting that individual GUVs may have differences in their mechanical properties.
A particular advantage of our device is that a wide range of dynamic force profiles can be applied via a computer-controlled interface, enabling studies of responses of membranes to dynamic force application at kilohertz frequencies.

3592-Pos

Quantifying Pathogen Recognition of the Cell Membrane: Simple Sugars Simulate the Functions of Complex Glycans

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The first step leading to the breaching of the cell membrane and infection of healthy cells is often the multivalent recognition and adhesion of glycan binding proteins (GBPs) on pathogens and glycans on host cell membranes. Such multivalent interaction depends critically on the mobility and density of signaling molecules on the membrane surface. While glycan microarrays have been used in exploring multivalent interactions, the lack of mobility and the difficulty in controlling surface density both limit their quantitative applications. Here we apply a fluidic glycan microarray, with glycan density varying for orders of magnitude, to profile cell surface interaction using a model system, the adhesion of Escherichia coli (E. coli) to mannose. We show the quantitative determination of monovalent and multivalent adhesion channels; the latter can be inhibited by nanopartices presenting a high density of mannosyl groups. These results reveal a new E. coli adhesion mechanism: the switching in the FimH adhesion protein avidity from monovalent to multivalent as the density of mobile mannosyl groups increases; such avidity switching enhances binding affinity and triggers multiple fimbriae anchoring. Affinity enhancement towards FimH has only been observed before for oligo-mannose due to the turn on of secondary interactions outside the mannose binding pocket. We suggest that the new mechanism revealed by the fluidic microarray is of general significance to cell surface interactions: the dynamic clustering of simple sugar groups (homogeneous or heterogeneous) on the fluidic membrane surface may simulate the functions of complex glycan molecules.

3593-Pos

Dynamical Basis of the Enhancement of the Enzymatic Activity of Factor VIIa by Tissue Factor

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Activation of coagulation factors is regulated by their binding to and complex formation on the surface of anionic membrane resulting in an increase of the enzymatic activity by several orders of magnitude. Complex formation of tissue factor (TF) and factor VIIa (FVIIa) on anionic membranes constitutes one of the key steps in the coagulation cascade. The mechanisms for the enhancement of the enzymatic activity of FVIIa by TF is not fully understood, primarily due to the lack of atomic models for the membrane-bound form of the TF:FVIIa complex.
We report a first membrane-bound model of the TF:FVIIa complex resulting from large-scale molecular dynamics simulations. The dynamics of FVIIa and soluble TF (sTF) was investigated in solution and on the membrane, both in their isolated and complex forms. Our model of membrane-bound GLA domain was used to construct the membrane-bond models of the TF:FVIIa complex and monomers. All-atom simulations were performed for tens of nanoseconds to investigate the protein dynamics after establishing optimal protein-protein/protein-lipid interactions.
The results reveal that sTF restricts the motion of FVIIa, thus optimally orienting its catalytic triad for the interaction with its substrate factor X (FX). Several direct interactions between the membrane lipids and the side chains of sTF, including the regions of K159-K166 and D180-N184 that form the exosite of the substrate FX, are observed. Interestingly, sTF sustains two distinct orientations against the membrane and different conformations of the K159-K166 loop, in the isolated and complex forms. These findings suggest that TF orients itself on the membrane surface through the interactions both to the membrane and FVIIa, independent of its trans-membrane anchoring helix and that the exosite on TF is available for FX binding only after TF:FVIIa complex is formed.

3594-Pos

Regulation of Phospholipase C Beta - Rac1 Cytoskeletal Pathways by Gamma Synuclein

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The breast cancer specific gene protein 1 also known as γ-synuclein is undetectable in normal or benign breast lesions, but it is highly expressed in infiltrating breast cancer. The precise role of γ-synuclein in malignancies is not well known. We investigated whether γ-synuclein might affect activity of phospholipase Cβ2 (PLCβ2). PLCβ2 is also absent in normal breast tissue, but it is highly expressed in breast tumors where it is correlated with the progression and migration of the tumor. Expression of PLCβ2 is highly correlated with expression of g-synuclein. We found that γ-synuclein binds PLCβ2 in vitro with high affinity, Kd = 23 +/- 3 nM. PLCβ2 is activated by heterotrimeric G protein and by members of Rho family of GTPases (in particular Rac1), which are

critical regulators of cytoskeletal remodeling, cell adhesion and motility. γ-synuclein alters binding between Rac1 and PLCβ2. This observations and activity measurements suggests that γ-synuclein mediates cell motility and invasiveness through Rac1-PLCβ2 pathway.

3595-Pos

PTEN and Ci-VSP Show Similar Phosphoinositide Binding Preferences

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The *Ciona intestinalis* voltage sensor containing phosphatase (Ci-VSP) is a voltage dependent phosphatidylinositol phosphatase with two main domains, the voltage sensing domain (VSD) and the phosphatase domain (PD). Ci-VSP's PD bears homology with the phosphatase domain of the tumor suppressor protein PTEN (phosphatase and tensin homolog deleted chromosome 10). Recently, it has been proposed that the linker between the VSD and PD Ci-VSP constitute a Phosphoinositide Binding Motif (PBM). The PBM of Ci-VSP shares high homology with PTEN's N-terminal, which, in turn, is known to bind $PI(4,5)P_2$ leading to an allosteric activation of PTEN. Similarly to the PBM of PTEN, we have found that a peptide representing the Ci-VSP's PBM ($Ci\text{-}VSP_{240\text{-}271}$) binds $PI(4,5)P_2$ significantly more strongly than other phosphatidyinositol bisphosphates. A Ci-VSP chimera created by replacing Arg 257 and Lys 258 with the amino acids found at the corresponding positions in the PTEN sequence, Gln 16 and Glu17, lead to a peptide that showed significantly reduced binding to $PI(4,5)P_2$. While $Ci\text{-}VSP_{240\text{-}271}$ exhibited a mixture of random and a-helical secondary structural elements, it was found that the chimeric Ci-VSP showed an increased b-sheet content. Molecular dynamics simulations were performed using the package NAMD and showed that the peptide does not form a helical structure and its charged residues interact in a pairwise fashion with $PI(4,5)P_2$. Those pairs are form by R245 and R246, K252 and R253, and R254 and R257. Experimentally, the binding of the PBM has a great influence on the rate of return of the sensing charges. However, in a deletion mutant lacking the PD we observed that the PBM alone is not enough to limit the rate of return of the sensing currents. These observations strongly suggest that the binding of the PBM might be stabilized by the PD.

3596-Pos

Membrane Phosphatidylserine and Plasma Ca2+ Levels Switch Factor Xa from an Inactive Dimer to an Active Monomer

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The main role of factor Xa(fXa) in the coagulation cascade is association with factor Va to catalyze the proteolytic activation of prothrombin to thrombin. Phosphatidylserine (PS) triggers both this fXa-fVa association and formation of inactive fXa dimers both in solution and on a membrane. We show here that binding of fXa to PS-containing membranes promotes proteolytic activity at low Ca2+ but inhibits it at high Ca2+ concentration, with the transition of FXa from active to inactive form on PS-containing membranes being a sigmoidal function of Ca2+ concentration. We modeled this membrane regulation of fXa activity to obtain kcat/KMdimer = 0 M-1s-1 and Kd,surfacedimer = (40 ± 25)●10-15 mol/(dm)2 at 4 mM Ca2+. This surface dimerization constant corresponds to a solution-phase Kddimer = 1 nM at 10 μM lipid concentration, nearly the same as observed (20 nM) for short-chain PS-triggered formation of fXa dimers in solution. fXa was activated by membrane binding below 1.1 mM Ca2+ but inactivated above this Ca2+ concentration. This resulted because the dimerization constant increased with decreasing Ca2+ concentrations (calculated at at 3.08, 1.54, and 1.16 mM Ca2+, respectively). Just below the normal range of free plasma Ca2+ concentration (1 - 1.3 mM), addition of PS-containing membranes promotes factor Xa activity, while at physiological [Ca2+], fXa is inhibited by dimerization, which depends critically on [Ca2+]. Supported by USPHS grant HL072827.

3597-Pos

Quantitative Analysis of Binding Affinities of PI(4,5)P2 Sensor Domains in Living Cells by Using the Voltage-Controlled PI(4,5)P2-5'-Phosphatase, Ci-VSP

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Dynamic changes in the phosphoinositide (PI) concentration in the cell membrane play an important role in the regulation of many cellular processes. Of particular interest is the PI phosphatidylinositol 4,5-bisphosphate ($PI(4,5)P_2$), a genuine signaling molecule that, e.g., controls the function of some ion channels.

Plasma membrane PI concentration in living cells is often monitored with genetically encoded fluorescence labeled PI-binding probes such as the $PI(4,5)P_2$ specific $PLC\delta_1$-PH-GFP and tubby-GFP probes. Knowledge of their $PI(4,5)P_2$ affinities is essential in selecting the most suitable probes and for appropriate interpretation of experimental results. These affinities of $PI(4,5)P_2$ probes apparently differ and can be modified by mutagenesis; however, a quantitative analysis of affinity in vivo is lacking.

To address this issue we employed the voltage dependent $PI(4,5)P_2$-5′-phosphatase Ci-VSP which alters plasma membrane $[PI(4,5)P_2]$ as a function of membrane voltage in a graded and reversible manner (Halaszovich et al., 2009, JBC 284:2106-13). We co-expressed Ci-VSP with various GFP-tagged $PI(4,5)P_2$ probes and used total internal reflection microscopy (TIRF-M) to measure membrane association of the probe at different membrane voltages. Generally, the TIRF signal decreased upon depolarization, indicating the translocation of the probe from the cell membrane into the cytosol in response to the decrease in $[PI(4,5)P_2]$. Voltage-dependent changes of TIRF signals allowed construction of fluorescence-voltage relations for each probe that can be used as surrogate $PI(4,5)P_2$ binding curves. Comparison of these curves defined the rank order of $PI(4,5)P_2$ affinities of the various probes.

We conclude that Ci-VSP is a valuable tool for analyzing $PI(4,5)P_2$ affinities in living cells, which we demonstrated by determining the affinities of fluorescence labeled $PI(4,5)P_2$-probes.

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3598-Pos

Optical Control of Cell Death: Translation of a Temporal Process to a Spatial Display

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The ability to arrest a dynamic physiological process and display the progression as a sequence of events has proved successful for the imaging of transport through transmembrane channels under cryogenic conditions. If cellular processes that advance in response to insult could be similarly arrested and displayed, it would open the door to investigation of biophysical and biochemical questions that are currently difficult to address. We have developed such a spatial cellular array, which might be utilized to study a broad spectrum of stress-induced physiological processes. As an illustration, we show that the process of optically induced cellular apoptosis can be translated from the temporal progression to a spatial array. Following graded doses of short-wavelength ultraviolet radiation, cells presented to a surface undergo progressively more advanced stages of the apoptotic cascade, depending upon their position in the array, as measured by caspase-8 and caspase-3 activation. This broadly applicable tool, exemplified here by the display of optically induced apoptosis, could facilitate the study of a wide range of optically and oxidatively stiumulated processes.

3599-Pos

Patch Clamped Giant Unilamellar Vesicles Containing Reconstituted Ion Channels

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There is increasing interest in how the chemical and physical properties of lipid membranes affect the function of mammalian ion channels. As evidenced by recent work on KvAP and Kv1.2, as well as studies of the physical nature of patches themselves, these effects are both intriguing and difficult to study. For the TRP family of ion channels these questions are especially important as membrane lipids, such as phosphoinositide (4,5) bisphosphate (PIP2), play an active role in regulating their functional properties. Although much work has been done to address the mechanism by which lipids regulate TRP channels in intact cells and excised patches, the physical interactions that govern channel regulation are still unknown. We report here on our efforts to establish high resolution control over membrane chemical composition and physical properties. We find that giant unilammelar vesicles can be synthesized with desired lipid compositions and subsequently studied using patch-clamp techniques. Reconstitution of functional TRP channels into these synthetic lipid membranes would provide a well-controlled experimental paradigm for studying the function and mechanism of channel-lipid interactions.