These expansions could be destabilized by adding a PI3K inhibitor, or by depleting the membrane cholesterol suggesting that the formation of these branches is due to membrane lipid domain formation around nlg-1 clusters. To confirm the role nlg-1 clustering, we have resorted to polarization fluorescence lifetime imaging microscopy to spatially resolve the nlg-1 oligomerization state at different location through out these expansions. We have exploited the photophysical properties of cerulean, a fluorescent protein, to assess the interneuroligin distances and decipher nlg-1 molecular interactions. These measurements confirmed that neuroligin tight clustering was involved in the formation of membrane cholesterol rich domains enabling the recruitment of PI3K, which in turns promotes the growth and the maintenance of these expansions.

3584-Pos

Don't Fence Me in: Evidence for a 'fence' that Impedes the Diffusion of PIP_2 Into and Out of Nascent Phagosomes in Macrophages

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To account for the many roles that phosphatidylinositol 4,5-bisphosphate (PIP_2) plays (e.g., in phagocytosis, exocytosis, activation of ion channels) a number of investigators have suggested there are separate pools of PIP_2 in the plasma membrane. Recent experiments show that the free concentration of PIP_2 is indeed enhanced in nascent phagosomes, syntaxin clusters, and the furrows of dividing cells. Kinases that produce PIP_2 (PIPkins) are also concentrated in these regions. But how is the PIP_2 produced by these PIPkins prevented from diffusing rapidly away? *First*, proteins could act as 'fences/corrals'around the perimeter of these regions. *Second*, some factor (e.g., a protein that acted as a PIP_2 buffer) could decrease significantly the diffusion coefficient, D, of PIP_2 within these regions. We used FCS and FRAP to investigate these two possibilities in the nascent phagosomes of J774 macrophages injected with fluorescent PIP_2. FCS measurements show PIP_2 diffuses with similar fast diffusion coefficients in the nascent phagosomes and in the bulk (unengaged) plasma membrane: $D = 0.6 \pm 0.3$ $\mu m^2/s$ and 0.8 ± 0.2 $\mu m^2/s$, respectively. FRAP measurements show the fluorescence from PIP_2 recovers slowly (>100 s) after photobleaching the entire nascent phagosome but recovers rapidly (<10 s) in a comparable area of the plasma membrane outside the cup. These results support the first hypothesis: a 'fence'impedes the diffusion of PIP_2 into and out of nascent phagosomes. The nature of the PIP_2 fence remains an enigma: although actin filaments are concentrated at the perimeter of the forming phagosomes, electrostatic and Brownian Dynamics calculations suggest individual negatively charged actin filaments near the membrane do not significantly impede the diffusion of PIP_2 into or out of the cup.

3585-Pos

Hard to Fence You in: Computational Approaches to Explore the Hypothesis that Actin Filaments Impede PIP2 Diffusion in Membranes

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Experiments described by Golebiewska et al. at this meeting suggest the existence of a 'fence'that impedes the diffusion of phosphatidylinositol 4,5-bisphosphate (PIP2) into and out of nascent phagosomes in macrophages. Although the nature of the fence remains an enigma, actin filaments are plausible components. They are highly negatively charged (as is PIP2), are swept away from the central region and are concentrated at the perimeter of the forming phagosome. To explore the actin fence hypothesis, we have used (1) Poisson-Boltzmann continuum electrostatics and a grid-based repulsive potential to describe a fence model made of a single layer of actin filaments, and (2) Brownian dynamics to describe the diffusion of PIP2 molecules modeled as single spheres. The simulations with actin filaments positioned parallel to the membrane indicate that a single filament without attached proteins does not significantly impede the diffusion of PIP2. A helical stripe of basic residues on the acidic actin filament provides a hole in the putative fence through which PIP2 can diffuse, no matter how close the filament is positioned to the membrane. Results from simulations of PIP2 diffusion out of corrals formed of multiple layers of actin filaments, and mazes of non-electrostatic barriers will also be presented.

3586-Pos

Regions of Correlated Fluctuations in Membrane Lipid Concentrations as a Consequence of Charged Cytoplasmic Lipids

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The cytoplasmic leaflet of the mammalian plasma membrane is characterized by charged lipids such as phosphatidylserine and phosphatidylinositol and its phosphorylated derivatives PIP and PIP2. Their concentrations vary from about ten to less than one percent. The charges are balanced by counter ions in the cytosol. As a consequence there are electric dipole moments in the cytoplasmic leaflet. The extra-cellular leaflet has essentially no charged lipids. In contrast to the electric dipole moments of the lipid head groups in both leaves which, due to their opposite orientation, essentially cancel one another at large distances, the dipole moments of the charged lipids in the cytoplasmic leaf interact via a long-ranged force. As a consequence, the fluctuations in density of these dipole moments are characterized by a non-zero length which depends upon the dipole density and temperature. Because the tails of these lipids couple them to the lipids of the extra-cellular leaflet, composition fluctuations in the outer leaflet will also display characteristic sizes. Thus the presence of charged lipids in the cytoplasmic leaflet results in coupled fluctuations in both leaves of a characteristic size. Such coupled regions could be important in the signaling processes which are associated with the charged lipids.

3587-Pos

Probing Spatial Organization in Cell Membrane at the Immunological Synapse

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The large-scale spatial arrangement of cell surface molecules has been gradually realized to regulate specific cellular outcomes in many cellular processes. This phenomenon is particularly striking in the antigen recognition by T cells. Signaling through discrete T cell receptors (TCRs) in the context of immunological synapse, involves the orchestrated movement and reorganization of TCRs on multi length scales. Microcluster movement is believed to be associated with centripetal actin flow, but the underlying physical mechanism remains unclear. By using the hybrid live T cells-supported membrane system, our study to probe the membrane spatial organization of T cells at their immunological synapse and its connection with TCRs movement will be discussed.

3588-Pos

The Effects of High Voltages on the Morphology of a Dppc Lipid Bilayer

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Biological membranes are often subject to large voltages compared to their small thickness, especially true in nerves where fluxes of ions and corresponding voltage changes are thought to be the main mechanism behind the nerve signal. Yet the effects that these voltages have on the phospholipids that makes up the membrane are largely unknown. Lipids of biological membranes are often charged or swittterionic, high electrical fields should be expected to have a large effect on their organization and thermodynamical properties. Fluorescence microscopy is utilized to image the effects of high voltage fields on the domain structure of a model system consisting of a Langmuir-Blodgett monolayer of phospholipids.

3589-Pos

Lysophosphatidic Acid Interactions with Model Membranes: a Novel Cell Signaling Regulatory Mechanism?

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Lysophosphatidic acid (LPA), the structurally simplest of the glycerophospholipids, is a potent second messenger whose functional diversity makes it a compelling target in lipid research. LPA, the effects of which include cell motility and proliferation, platelet activation, fertility and development, and neuropathic pain, is believed to act through a family of G protein-coupled receptors (GPCR). Since some members of this family of proteins are localized in ordered lipid domains (membrane rafts), a role for LPA in altering and re-ordering membranes as part of regulation of the signaling pathway cannot be discounted.
As part of a series of efforts to obtain biophysical information about the effects of LPA on membranes, we have employed the Langmuir monolayer technique and isothermal titration calorimetry (ITC) to measure the kinetics and thermodynamics of LPA intercalation into lipid films and bilayers of various compositions representing different physical phases known to exist in biomembranes.