containing phosphatidylcholine (PC), an uncharged lipid; phosphatidylglycerol (PG), a charged lipid; and cholesterol. These phase diagrams have several interesting features. Miscibility in membranes containing charged lipids occurs over similar ranges of temperatures and lipid compositions as in membranes containing only uncharged lipids. The coexisting liquid phases differ primarily in their phospholipid content such that one phase has a high concentration of charged lipid. Adding salt to the system causes an increase in transition temperatures at some membrane compositions, consistent with electrostatic screening, whereas the transition temperatures at other compositions fall.

2550-Pos

Activity and Ordering of Mixed Phosphatidylethanolamine/Dihydrocholesterol Monolayers

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Cholesterol is thought to be important for the structure and assembly of lipid rafts, and its interaction with other membrane lipids has been a topic of great interest. This study focuses on the interactions between 1,2-dimyristoyl-*sn*-glycero-3-phosphoethanolamine (DMPE) and dihydrocholesterol (Dchol) in Langmuir monolayers using fluorescence microscopy (FM), beta-cyclodextrin (CD) desorption assays, and grazing incidence x-ray diffraction (GIXD). Similar to our previous results for 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC)/Dchol monolayers [Biophys. J. 2007, 93, 2038-2047], FM and CD assays show 2 regimes for the DMPE/Dchol system. Short-ranged lateral ordering was observed using GIXD that was also consistent with our recent work on sphingomyelin (SM)/Dchol monolayers [Phys. Rev. Lett. 2009, 103, 028103]. We investigate how the smaller headgroup of DMPE affects the surface morphology, Dchol chemical activity, and lateral structure compared to monolayers of Dchol with DMPC or SM.

2551-Pos

Group III Secretory Phospholipase A_2 Enhances Alpha-Secretase-Dependent Amyloid Precursor Protein Processing Through Alterations in Membrane Fluidity

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Phospholipases A_2 (PLA_2s) are responsible for maintenance of phospholipids homeostasis in cell membrane and implicated in neurodegenerative disease including Alzheimer's disease (AD). Among many types of secretory PLA_2s, type III secretory PLA_2 ($sPLA_2$-III) is expressed in neuronal cells and contributes to cell differentiation and survival. Yet the role of $sPLA_2$-III in AD has not been explored. We studied the effects of $sPLA_2$-III and its hydrolyzed products, including arachidonic acid (AA), palmitic acid (PA) and lysophosphatidylcholine (LPC), on cell membrane fluidity in relations to amyloid precursor protein (APP) processing, which is an important cellular process in AD to produce either neuroprotective α-secretase-cleaved soluble APP ($sAPP_\alpha$) or neurotoxic amyloid-β peptide (Aβ). Differentiated human neuroblastoma (SH-SY5Y cells) treated with $sPLA_2$-III and AA, not PA and LPC, was found to increase $sAPP_\alpha$ secretion and these changes were accompanied by increased membrane fluidity and accumulation of APP at the cell surface. All the treatments altered neither total APP expression nor expression of α-secretases, including ADAM 9, 10, and 17. Taken together, our results support the hypothesis that $sPLA_2$-III enhances $sAPP_\alpha$ secretion through its action to increase membrane fluidity and recruitment of APP at the cell surface. This study provides insights into potential therapeutic approaches for AD treatment.

2552-Pos

Nonlinear Effect of Sucrose on Lamellar-Hexagonal Phase Transition Kinetics

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The topological nature of the lamellar-hexagonal phase transition in lipids makes it a useful tool in the study of pore formation. This phase transition in lipid-water systems is sensitive to the addition of various solutes such as sucrose. In our study of lipid SOPE (1-stearoyl-2-oleoyl-*sn*-glycero-3-phosphoethanolamine), we find that equilibrium lamellar-hexagonal phase transition temperature decreases linearly with sucrose concentration. However, we find that the phase transition kinetics vary in a strikingly nonlinear fashion. The speed of the transition greatly slows with even small concentrations of sucrose and then plateaus as the concentration increases.

2553-Pos

Structure of a DOTAP Lipid Bilayer: A Concerted Neutron Scattering and Molecular Dynamics Study

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Non-phospholipid, cationic 1,2-dioleoyl-3-trimethylammonium propane (DOTAP) lipid based membranes fail to support the function of a voltage-dependent K+ channel due to the lack of a phosphate group (Schmidt et al., Nature 444, 775-779, 2006). However, the specific effects of the presence or absence of phosphate groups in the channel membrane environment on the voltage-sensing mechanism remain unknown. Before addressing the question of why DOTAP is not a suitable membrane environment for Kv channels, a detailed structural characterization of the pure DOTAP lipid bilayer system is required. Here, we employ molecular dynamics simulations in combination with neutron scattering experiments for a detailed atomistic study of a DOTAP lipid bilayer. All-atom molecular dynamics simulations of DOTAP bilayer at 9.4 waters/lipid were performed at constant pressure and temperature. One-dimensional structural data obtained from the neutron scattering experiments is used to validate the molecular dynamics simulations, which in turn provide the structural details at the atomistic level. We also compare the properties such as alkyl chain order parameter, area per lipid, and headgroup hydration, packing and orientation for a DOTAP lipid bilayer to zwitterionic phospholipid bilayers and propose the underlying physical-chemical reasons for the differences observed.

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2554-Pos

The Effect of Fatty Acids with Different Unsaturations on Membrane Fluidity and Alpha-Secretase-Dependent Amyloid Precursor Protein Processing

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Fatty acids are important dietary ingredients, which are implicated in neurodegenerative disease including Alzheimer's disease (AD). Yet their roles are not fully understood. We investigated the effects of fatty acids with different unsaturations (number of double bonds) including stearic acid (SA, 18:0), oleic acid (OA, 18:1), linoleic acid (LA, 18:2), α-linolenic acid (ALA, 18:3), arachidonic acid (AA, 20:4), eicosapentaenoic acid (EPA, 20:5), docosahexaenoic acid (DHA, 22:6) on cell membrane fluidity in relations to amyloid precursor protein (APP) processing, which is an important cellular process in AD to produce either neuroprotective α-secretase-cleaved soluble APP ($sAPP_\alpha$) or neurotoxic amyloid-β peptide (Aβ). Differentiated human neuroblastoma (SH-SY5Y cells) treated with AA, EPA and DHA, not SA, OA, LA and ALA, increased $sAPP_\alpha$ secretion, which was accompanied by increased membrane fluidity. Our results showed that the fatty acids with four or more double bonds, including AA, EPA and DHA, promoted $sAPP_\alpha$ secretion through increasing membrane fluidity. This study provides the potential dietary strategies for the prevention of AD.

2555-Pos

PLA_2 Type IIA Increases Platelet Plasma Membrane Rigidity During Cold Induced Activation

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There is a wide discussion regarding the origin and function of lipid domains in human cells. An example in which lipid domains may play a functional role is the plasma membrane of human platelets. Platelets are susceptible to chilling, activating when the temperature falls below 20 °C. This limitation represents a crucial issue in terms of storage of platelets. It has been previously shown that cold-induced platelet activation is correlated with the formation of macroscopic lipid domains during cooling. This is a result of the fact that platelets present low cholesterol content (15 mol%), which results in the presence of a cooperative lipid melting transition centered at 15°C. This transition is responsible for the formation of the macroscopic lipid domains during cooling. Human secretory phopholipase A_2 type IIA ($sPLA_2$-IIA) catalyses the hydrolysis of the sn-2 ester bond in glycerolipids to produce fatty acids and lysolipids. Recently we have shown that its activity is triggered by the local enrichment of anionic lipids in fluid domains during phase coexistence. Since human platelets

expose their anionic lipid content during chilling, we propose that PLA2-IIA is triggered during this process, and that the activity of the enzyme on the fluid domains induces an overall increase in rigidity in the platelet plasma membrane due to an increase in the proportion of ordered lipids. We present a combined FTIR and Laurdan fluorescence spectroscopy study on the thermotropic phase behavior of human platelets, and show that during cold-induced activation membrane rigidity increases. We present results on how the activity of sPLA2-IIA is regulated by the thermotropic phase behavior of the platelet plasma membrane after platelets have been artificially activated through sonication. We show that PLA2-IIA activity increases the rigidity of these activated platelet plasma membranes.

2556-Pos

Neutron Scattering and MD Simulation Study of DOPC and DOPC/cholesterol Bilayers

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Cholesterol represents about 35% of the lipid content in the plasma membrane. Relevant cellular processes that have been associated with cholesterol-enriched microdomains include, among others, signaling, trafficking, cytoskeleton organization, apoptosis, and cell adhesion. In this work we studied the effects of cholesterol on model bilayers of DOPC using a combination of molecular dynamics simulations and neutron scattering experiments. Taking advantage of the higher scattering length of deuterium relative to hydrogen, the location of labeled components can be determined by difference-structure analysis of the scattering length density profiles. By labeling the terminal methyl groups of DOPC and a cholesterol ring, we determined their respective mass distributions across the membrane in DOPC and DOPC/cholesterol bilayers. The neutron data reveal the terminal methyl groups in a pure DOPC bilayer visit the head group-water interface as observed by the prominent wings in the mass distribution. A narrower terminal methyl group distribution of DOPC in the presence of cholesterol suggests an enhancement of the order of the lipid acyl chains. Cholesterol heads, on the other hand, point toward the aqueous interface, as expected. Order parameters S_{CD} calculated from the MD simulations confirm that DOPC is more ordered in presence of cholesterol. The structural analysis based on MD simulation in concert with neutron diffraction experiments provides valuable data for advancing understanding of role of cholesterol in altering transbilayer communication in cholesterol-enriched microdomains found in biological membranes.

Membrane Receptors & Signal Transduction II

2557-Pos

Distribution and Dynamics of RBL IgE Receptors (FcεRI) Quantitatively Observed on Planar Ligand-Presenting Surfaces

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There is considerable interest in the signaling mechanisms of immunoreceptors, especially when triggered with membrane-bound ligands. When T cells, B cells, or mast cells bind to monovalent ligands on fluid supported lipid bilayers, receptor clustering, signaling, and receptor redistribution into immunological synapses follow. We have quantitatively studied the kinetics of redistribution of IgE receptors (FcεRI) on RBL-2H3 mast cell surfaces. To separate the kinetics of receptor redistribution from cell spreading, the initial cell-substrate contact time was precisely defined (± 50 ms) by micropipette cell manipulation. Using total internal reflection fluorescence, the distribution and dynamics of receptor clusters were imaged. We find strong quantitative evidence that initial receptor aggregation occurs at cellular protrusions, which are visible bright spots/regions on cells contacting ligand-free surfaces. The initial size of these regions is independent of the substrate and the presence or absence of ligand, and they were found to be randomly distributed over the interfacial contact area. Using a finite-element diffusion model, we found that the initial rate of accumulation of receptors at the protrusions is consistent with diffusion-limited trapping with $D \sim 10^{-1}$ $\mu m^2/s$. At longer timescales, individual clusters on ligand-bearing membranes were observed to move with both a diffusive and a directed component of motion; clusters eventually coalesced near the center of the contact region (~ 1 minute). The dynamics of the early cluster motion is similar to the dynamics of membrane fluctuations of cells on ligand-free fluid membranes. Thus, the same cellular machinery may be responsible for both processes.

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2558-Pos

Multi-Color Quantum Dot Tracking to Correlate FcεR1 Aggregate Size with Mobility

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The high affinity IgE receptor, FcεRI, is the primary immune receptor expressed on mast cells and basophils. These receptors bind circulating IgE with high affinity and are activated when multivalent allergen crosslinks IgE-bound receptors, initiating a complex signaling cascade that leads to the release of inflammatory mediators by degranulation. It is also well documented that FcεRI crosslinking leads to receptor immobilization. However, the exact mechanism of crosslink-induced activation is not completely understood.
We have used single quantum dot (QD) tracking to characterize the mobility of QD-IgE-FcεRI in the resting and activated state. We have previously shown that crosslink-induced immobilization is depended on the actin cytoskeleton as well as the antigen dose. Furthermore, we have determined that small, mobile clusters are signaling competent and that immobilization is the trigger for internalization rather than signal initiation. In these experiments, a combination of QD-IgE and dark IgE were crosslinked with multivalent antigen; therefore, the cluster size could not be directly correlated with mobility. We have now developed an experimental strategy and algorithmic approach to differentiate between monomers, dimers, trimers, and tetramers. Using a four-color beam splitter, we can simultaneously track FcεRI complexes labeled with four spectrally distinct QDs (525, 585, 655, and 705) at rates up to 30 frames/s. From these data, we use QD localization and channel overlay accuracies to identify monomers, dimers, trimers, and tetramers and determine the diffusion coefficient for each type of aggregate. We demonstrate this approach with both simulated and experimental data.

2559-Pos

Fluorogen Activating Peptides for Single Molecule Localization-Based Superresolution and Single Particle Tracking of FcεRI Subunits

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Fluorogen activating peptides (FAPs) are genetically expressible tags that increase the fluorescence excitation cross-section of dye binding partners by up to four orders of magnitude. The binding of FAPs with corresponding fluorogens is characterized by nanomolar affinity, corresponding to bound lifetimes of up to ten seconds. FAPs exhibit resistance to photobleaching, with single peptides repeatedly binding and activating new dye molecules. The resulting long observation lifetimes for single peptides make the FAP system a convenient, expressible probe for single particle tracking on live cells. The intermittency due to the equilibrium of dye binding and unbinding can be used for localization-based superresolution.
We apply this technology to study the localization and dynamics of the high affinity IgE receptor, FcεRI, the primary multi-subunit receptor on mast cells and basophils. Circulating, allergen-specific IgE molecules bind to FcεRI receptors via the α-subunit. Receptor crosslinking by cognate multivalent allergen initiates the immune response by activation of the immunoreceptor tyrosine-based activation motifs on the β- and γ-subunits. We have previously characterized the dynamics of the FcεRI by tracking of quantum dot (QD)-labeled IgE bound to FcεRIα. To compare the behavior of the α- and γ-subunits, we have generated an FAP-tagged FcεRI γ-subunit that is expressed on the cell surface of rat basophilic leukemia cells and binds an exogenous fluorogen based on the malachite green dye. We use this probe to determine dynamics of the γ-subunit during resting and activated states and to generate superresolution images of FcεRIγ distribution. We use QD-IgE, in conjunction with the FAP-FcεRIγ, for two-color single particle tracking to observe the relative spatial and temporal dynamics of the α- and γ-subunits.

2560-Pos

Receptor Cluster Size Affects Signaling in Breast Epithelial Cancer Cells

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Our purpose is to study a potentially novel mechanical regulatory mechanism in the EphA2 signaling pathway. EphA2 is a receptor tyrosine kinase that is known to be up-regulated in 40% of human breast cancers and plays an active role in metastasis. Activation of EphA2 occurs after binding to its ligand, presented on an opposing cell membrane. Monomer binding is followed by