1425-Pos

Measurement of the Duration and Critical Exponent of Concentration Fluctuations in Lipid Bilayers Near the Critical Point

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Membranes containing a wide variety of ternary mixtures of high chain-melting temperature lipids, low chain-melting temperature lipids, and cholesterol undergo lateral phase separation into coexisting liquid phases at a miscibility transition. Large composition fluctuations appear in lipid membranes prepared near miscibility critical points. We have measured the effective dynamic critical exponent relating the decay time τ_0 of membrane composition fluctuations to the wavenumber k through= $\tau \sim k^{-z_{eff}}(\xi)$, where the correlation length ξ characterizes the size of the largest fluctuations. We find that z_{eff} increases from roughly 2 at $\xi \rightarrow 0$ to $z_{eff} = 2.31 \pm 0.03$ at $\xi = 16\mu m$. Changes in lipid composition are known to affect membrane protein activity. We find that submicron membrane fluctuations corresponding to a wavenumber of $(50nm)^{-1}$ persist for at least 0.8 ± 0.3 ms, on the order of times required for changes in protein configuration (e.g. 1ms). Therefore, similar and longer-lived fluctuations in cell membranes can potentially alter protein function.'' To our knowledge, we present the first measurement of in a 2-dimensional system of conserved order parameter, whether with or without conserved momentum.

1426-Pos

Interaction of a Novel Iron Chelator with Model Membranes

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A novel fluorescent hexadentate iron chelator developed in our laboratory has been shown to inhibit the growth of *Mycobacterium avium* inside macrophages. Apart from its high affinity to iron, the compound seems to possess a key molecular structure to cross biological membranes, thus reaching the targets to deprive bacteria from iron.

To get insight on the partition and location of this new compound, fluorescence spectroscopic studies are being performed in large unilamellar liposomes.

To be able to separate liposome surface effects from lipophilicity, we measured the fluorescence anisotropy of two fluidity probes (DPH and TMA-DPH) in DMPC and DMPG liposomes prepared with the iron chelator. The results indicate that the primary interaction is near the lipid headgroup, with a partial molecular immersion in the outer leaflet, favouring negatively charged lipids. Our results suggest the importance of the first membrane penetration through the outer headgroups in the effectiveness of bacteriostatic agents with intracellular activity.

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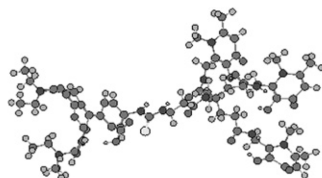

1427-Pos

Unraveling the Mechanism of Membrane Binding by Annexin 5

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Protein-membrane interactions are a vital mechanism of propagating signals both across the membrane and between cells. To control the magnitude and specificity of this type of cell signaling at the membrane, clustering of similar lipids and proteins has been observed in the cell via the formation of lipid microdomains. To address the thermodynamic basis of this type of signal propagation, we investigated how lipid microdomains form in response to annexin a5 binding to model membranes using Isothermal Titration Calorimetry (ITC). Annexins are known to bind to negatively charged (e.g., phosphatidylserine [PS]) membranes in a Ca2+ dependent manner that lead to the formation of PS-enriched microdomains. Based on Differential Scanning Calorimetry (DSC) results, we suggest that that annexin functions to order lipid acyl chains upon binding and that the ordering of phospholipids can lead to the formation of these microdomains. Using ITC, we have analyzed the membrane binding affinity of annexin for both gel and fluid state mixtures. Binding analysis of these isotherms show that annexin binds fluid state mixtures with a significantly lower Kd than gel state lipids, which would be consistent with the hypothesis that the ordered nature of gel state lipids reduces the binding affinity of annexin for that lipid. In addition, because the binding is entropically dominated but exhibits greater affinity for fluid compared to gel state lipids, we suggest that annexin binding is driven by the release of water molecules as fluid lipids have more water of hydration. Interestingly, the enthalpy associated with the binding process for both gel and fluid state lipid mixtures is weak, which is indicative of a similar binding mechanism for the mixtures, albeit that binding of lipid is exothermic for fluid state and endothermic for gel state.

1428-Pos

Drug Release from Liposomes can be Modulated by the Extent of Cholesterol Superlattice in the Lipid Membrane

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Liposomes have been used as drug carriers for targeted delivery. Much attention has been paid to the stealth properties of the liposome in order to avoid the immune system and have a prolonged circulation time. An aspect in the liposome design as a drug delivery system that has been relegated is the passive drug leakage from liposomes. In this work we investigated how lateral distribution of lipids in membranes can affect the overall leakage of an entrapped drug. For this study we used the antivascular drug Combretastatin A4 disodium-phosphate (CA4P) that has entered clinical trials for the treatment of a variety of cancers and is naturally fluorescent. CA4P was encapsulated in liposomes composed of 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC)/cholesterol at a quenching concentration (30 mM). Cholesterol content was varied in steps of 0.4 mol% in a range of concentrations covering the theoretically predicted critical mole fractions (Cr, e.g., 20.0, 22.2, 25.0, 33.3, 40.0 and 50.0 mol%) for maximal sterol superlattice formation. The non-encapsulated CA4P was removed by size exclusion column chromatography. The leakage was followed in real time by exciting CA4P at 328nm and reading fluorescence at 400nm. The results obtained show that at Cr the leakage of CA4P is faster than at non-critical mole fractions. Although cholesterol superlattice domains have tighter lipid packing, the defects that are produced in the interfaces between regular and irregular domains enhance the overall membrane permeability. Therefore the extent of cholesterol superlattice can be used to modulate the release of encapsulated drugs. Ongoing work is aimed to observe how this modulation will affect CA4P treatment using endothelial and mammary cancer cell lines. (supported by DOD breast cancer program)

1429-Pos

Measuring Passive Transport Using Confocal Microscopy of Giant Lipid Vesicles

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The ability of a molecule to pass through the plasma membrane without the aid of any active cellular mechanisms is central to that molecule's pharmaceutical characteristics. Existing techniques for measuring this passive transport capacity are hampered by the presence of an unstirred layer (USL) which dominates transport considerations across the bilayer. We are developing assays based on confocal microscopy of giant unilamellar vesicles (GUVs) that allow for the detailed investigation of passive transport processes and mechanisms. At the size of GUVs (generally less than 100 μm), the effect of the USL on membrane transport processes is minimized, giving more accurate values of membrane permeability.

We have constructed several series of drug-like fluorescent molecules by covalently modifying the dye 4-nitrobenzo-2-oxa-1, 3-diazole (NBD). A series of molecules of increasing hydrophilicity was constructed from polyethylene glycol (PEG) having 4, 8, and 12 repeating units. Alkane chains with 3, 5, and 7 carbons were used as the hydrophobic representatives. Transport of both series of modified NBD molecules was observed by tracking NBD fluorescence as the molecules passed through the GUV membrane. Weak acyclic acid with 2, 4, and 6 carbons were also examined, using a pH-sensitive dye to track their transport.

An analytical theoretic passive transport model was devised, original data was regressed to the model, and permeability was calculated for each dataset. Finite element modeling (FEM) was used to simulate the experiments. The simulation supported the experimental results well.

1430-Pos

Buffer Properties Revealed with Model Lipid Membranes

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Numerous kinds of buffers including MOPS, MES and HEPES are used to control the pH of biological samples. We have investigated how these particular buffers alter the charge state of lipid membranes. As measured by x-ray scattering, polar but neutral lipids such as phoshatidylcholine (PC) form multilamellar vesicles (MLVs) in buffer solutions. Previous work has shown that the highly uniform equilibrium spacing between lipid membranes is easily influenced by the presence of weakly binding charges such as bromide ions in monovalent salt solutions [1]. In general, the MLVs shrink or expand (swell)

depending on how the balance of attractive and repulsive force is shifted by the nature of the aqueous solution. We performed small angle x-ray scattering measurements to reveal how the buffers modify lipid interactions. Buffers loosely associate with the lipid membrane and alter their surface charge causing the MLVs to swell. Interestingly, as opposed to monovalent salts which charge up the PC membranes negatively, MOPS charges PC membranes positively. We have used small angle x-ray scattering to measure the modification of membrane forces and we have measured the diffusion of lipid aggregates in electric fields to determine the charging effect of the buffers on PC membranes. By measuring how buffers modify the electrical state of lipid membranes we can better understand how buffers behave at the interface of biological membranes.

[1] H. I. Petrache, T. Zemb, L. Belloni, and V. A. Parsegian. *Proc. Natl. Acad. Sci.*, 2006, 103:7982-7987.

1431-Pos

Molecules Pushing Molecules: Dynamic Consequences of Crowding

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Membrane pores, such as alpha-hemolysin, sieve molecules to provide passage. Large polymers are excluded while monomers and small polymers can pass. At high concentrations, flexible polymers lose their size that exists under dilute conditions. Rather, flexible polymers look more like strings with regions of limited coherence. This transition is clear from the shift in osmotic pressure vs. polymer concentration: van't Hoff regime in the dilute limit but des Cloizeaux regime at higher concentrations. Under crowded conditions, a polymer previously unable to enter the alpha-hemolysin pore suddenly enters when the apparent limiting size is in the region of limited coherence. Mixtures of small and large polyethylene glycols show exclusion in this range where the larger species exert stress that drives the smaller polymers into and across pores at concentrations far larger than those in the bathing solution. This coupling of polymer activities and consequent conferred mobility creates a new form of crowding-driven transport.

1432-Pos

Effect pf PAH Concentration on Sops Liposomes

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We have prepared SOPS liposomes using the hydration technique. Optical microscopy experiments show that the size and shape of the liposomes do not change when they are swell with a glucose/sucrose solution. To the SOPS liposome system we add the polyelectrolyte PAH (Poly-Allylamine Hydrochloride), producing a drastic change in the liposome structure. We have studied the influence of PAH on the liposome shape and size distribution by means of Differential interference contrast microscopy (DIC). The results show that PAH interacts with the SOPS liposomes forming PAH-SOPS complexes.

1433-Pos

Localized Photothermal Heating of Temperature Sensitive Liposomes

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A drug delivery system consisting of a temperature sensitive liposome coupled to hollow gold nanoshells allows precise spatial and temporal control of drug release. A small fraction of lysolipid in a primarily dipalmitoylphosphatidylcholine (DPPC) liposome lowers the membrane transition temperature to that obtainable by mild hyperthermia, while simultaneously enhancing the membrane permeability at the transition temperature. Hollow gold nanoshells coupled to the liposomes heat the membrane when irradiated by a continuous wave near-infrared laser. The heat generated by the nanoshells can be tuned to control local membrane temperature, and hence the membrane permeability and rate of drug release. This system could be used to deliver anticancer drugs directly to a tumor site. Additionally, the ability to correlate drug release with membrane temperature allows us to empirically determine the local heat generated by the hollow gold nanoshells upon laser irradiation.

1434-Pos

Phase Diagram of a 3-Component Lipid Mixture Containing a Polyunsaturated Phosphatidylcholine

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Polyunsaturated acyl chains have special influences on the mixing and phase behaviors of lipid mixtures. Their high degree of unsaturation affects the physical properties of biomembranes in ways that are still not fully understood, and their high concentration in some membranes makes them key players in membrane structure. We are investigating the 3-component phase diagram for the biologically relevant mixture of brain-SM/ 18:0-22:6 PC/ cholesterol. Fluorescence microscopy imaging of giant unilamellar vesicles (GUVs) was employed for phase boundary visualization and phase identification of the 3-component mixture. Fluorescent lipid probes having complementary partitioning behavior are used in FRET measurements to enable more quantitative analysis. Of particular interest is the region of Lo + Lα phase coexistence, which shows macroscopic phase separation.

1435-Pos

Investing Early Signaling Events in IgE-FcεRI Activation Using SEM

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Antigen-mediated cross-linking of immunoglobulin E (IgE) bound to its high affinity receptor FcεRI on mast cells initiates a transmembrane signaling cascade that results in cell activation and exocytotic release of chemical mediators involved in allergic response. Plasma membrane lipids and proteins redistribute as part of this transmembrane signaling process. To understand the functional role of these redistributions, resolution of their size, composition and structure on the nanometer scale is required. We utilize high resolution scanning electron microscopy (SEM) to directly visualize sub-micron membrane domains in intact cell membranes. In our experiments, the distribution of gold-labeled proteins and lipids is analyzed at the surface of intact fixed cells using backscattered electron detection. In parallel, we also observe membrane topography using secondary electron detection. We use a pair-correlation function analysis to quantify protein distributions and parameterized domain size. We have mapped the distribution of a variety of proteins, both related and non-related to the IgE signaling pathway. Using this experimental and quantitative method, we observe dramatic changes in the nano-scale membrane distribution of IgE due to stimulation with multivalent ligands. In resting cells, IgE receptors are clustered into small domains less than 30nm. After stimulation, receptors redistribute into large domains that are correlated at long length-scales and subsequently reduce in size at long stimulation times. We also observe cross-linking-dependent rearrangement of several inner leaflet-associated proteins implicated in early signaling events. In contrast, outer leaflet GPI-anchored proteins are not affected. We have also quantified the co-redistribution of IgE with other membrane proteins after stimulation using cross-correlation functions. These findings provide valuable insights into the mechanisms that drive the selective nanoscopic reorganization of plasma membrane proteins during immune cell signaling.

1436-Pos

Fluorescence Measurements in Fruit Fly (Drosophila Melanogaster)

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Drosophila melanogaster is a widely used animal model in developmental biology. In *Drosophila*, the wealth of genetic tools allows expression of any given marker or construct in specific cells or tissues within the organism. This is especially advantageous since particular cells can be studied in their natural 3D organization, avoiding possible artefacts and deviations from the physiologically relevant situation that may be introduced in cell cultures. For the study of molecular dynamics within cells and cell membranes on a single molecule level, we performed fluorescence correlation spectroscopy (FCS) within the *Drosophila* embryonic nervous system. Using a GAL4 driver expressed in a small subset of neurons, we expressed fluorescently tagged fusion membrane proteins, CD8 and flotillins-2, in two identified motor neurons per hemisegment of the embryonic central nervous system (CNS). We obtained autocorrelation curves for membrane and cytoplasmic probes which show diffusion times that correspond to their respective subcellular locations. By additionally expressing (non-tagged) proteins which influence lipid metabolism, example ceramidase, we are able to follow changes in the molecular dynamics of membrane proteins. With this approach, we are studying the biophysical properties of the cellular membrane *in vivo* and *in situ* and will extend this in the future to different genetic backgrounds. The study shows that *in vivo* analyses provide us greater insights into the role of membrane dynamics in the context of development, differentiation and pathogenesis of diverse diseases.

1437-Pos

The Influences of Electric Fields on Lipid Membranes

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