

endocytosis and exocytosis, and protein trafficking. Cholesterol-rich lipid domains have been hypothesized to exist in a liquid-ordered phase and play an important role in cellular functions. Here, we test the hypothesis that cholesterol diffuses as a complex with other lipids in a bilayer. In addition, we examine the role of lipid variation and proteins on the biophysical properties of biomembranes using comparative studies of giant unilamellar vesicles (GUVs) and plasma membrane vesicles (GPMVs) isolated from Hs578Bst live cells. The fluorescence dynamics assay used here includes two-photon fluorescence lifetime imaging, fluorescence resonance energy transfer, and diffusion (both rotational and translational). Different fluorescent lipid analogs are used in these studies to probe both the hydrophobic core and the head group region. Our comparative studies on GUVs and GPMVs serve as a platform to test our understanding of lipid-lipid and lipid-protein interactions in these biomembrane models.

2819-Plat

Two-dimensional Calorimetry: Imaging Thermodynamics and Kinetics of Phase Transitions of Biological Membranes

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Differential scanning calorimetry (DSC) is a relatively rapid and informative biophysical method for studying thermotropic phase behavior of biological membranes. More recently a pressure perturbation calorimetry has been introduced. The latter method is capable of characterizing membrane thermal volume expansion coefficient and kinetics associated with the phase transition. Notably, pressure perturbation calorimetry requires both pressure jump accessory and, most importantly, fast and sophisticated temperature control system to ensure constant sample temperature upon the pressure jump. Here we describe a calorimetry procedure and associated method of data analysis to characterize sample thermal properties as a two-dimensional (2D) object (temperature-time thermal image) whereas data obtained by conventional calorimetry are essentially one-dimensional. In brief, the method utilizes mathematical formalism of the Radon transform (back-projecting algorithm) to separate temperature and time dimensions from a series of thermal flux measurements obtained by a conventional DSC calorimeter at different scanning rates. By this manner static and time-dependent (i.e., relaxation) thermodynamic parameters of an object become resolved and displayed as a single 2D temperature-time thermal image. There are two main advantages of our 2D-calorimetry method: 1) 2D temperature-time thermal image separates and characterizes equilibrium and non-equilibrium thermal properties of a sample; 2) the method improves signal-to-noise ratio for conventional DSC measurements of equilibrium heat capacity as a function of temperature. We demonstrate these advantages of the 2D calorimetry method on examples of imaging thermodynamics and heat relaxation properties of lipid bilayers composed from single and mixed phospholipids with and without cholesterol. We also show that confining lipid bilayers inside nanopores of ca. 175 nm in diameter results in heterogeneous heat transfer kinetics while conventional equilibrium calorimetry curves remain unperturbed. Supported by the DOE Contract DE-FG02-02ER15354.

2820-Plat

Raft recruitment of Membrane Proteins by Native Ligands and GPI-Anchored Proteins: A Model Membrane Study

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The role of cholesterol/sphingolipid-enriched lipid heterogeneities, also known as lipid rafts, in membrane protein function represents one of the fascinating topics in cell biophysics and cell biology. Progress in this research area has been slow, due to the small size and transient character of such heterogeneities in plasma membranes of resting cells. To overcome these experimental complications, here we describe two-types of protein-induced membrane protein recruitment processes to/from raft-like domains using a model membrane platform based on a polymer-tethered phospholipid bilayer. The domain-specific quantification of reconstituted membrane proteins in the bilayer is achieved using a combined setup of epifluorescence microscopy and confocal fluorescence correlation spectroscopy. In the first case, we present experimental data concerning the raft recruitment of membrane proteins due to the binding to their native ligands [receptor/ligand pairs investigated: FcγRIII/IgA; urokinase plasminogen activator receptor (uPAR)/ urokinase plasminogen activator (uPA); $\alpha_v\beta_3$ integrin/vitronectin; and $\alpha_5\beta_1$ integrin/fibronectin]. Interestingly, the ligand binding process causes substantial translocations among GPI-anchored proteins (FcγRIII and uPAR) and membrane-spanning receptors ($\alpha_v\beta_3$ and $\alpha_5\beta_1$) to/from raft-like domains. Remarkably, under the experimental conditions chosen, no ligand-induced receptor oligomerization can be observed. In

the second case, we explore the impact of uPAR-integrin complex formation on the affinity of $\alpha_v\beta_3$ and $\alpha_5\beta_1$ for lipid rafts. The uPAR/integrin complexation is determined for different ligand states using single molecule fluorescence microscopy. Importantly, our results confirm that such complexes enhance the affinity of integrins for raft domains, thus suggesting a mechanism of GPI protein-induced recruitment of transmembrane proteins to raft domains. Finally, the impact of monolayer and bilayer-spanning raft-mimicking domains on protein recruitment processes is discussed.

2821-Plat

Electromechanical Forces and Flexoelectricity in Plasma Membranes

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Flexoelectricity is a phenomenon analogous to piezoelectricity which describes the evolution of electric fields in two dimensional liquid crystals in response to curvature changes (and, conversely, changes in curvature due to applied electric fields). It provides a mechanism for cellular electromechanical transduction in flexoelectric membranes, a process which is important to the physiology of outer hair cells of the inner ear and touch sensitive nerve cells.

We have developed a simple electrostatic model for a bilayer containing anionic phospholipids. Charged ions surrounding the bilayer are distributed according to the Poisson-Boltzmann equation. The correct way to treat the adsorption of positive ions into the bilayer (the "Stern layer" of Gouy-Chapman-Stern theory) is not clear. Different regimes are investigated, including a Langmuir Isotherm and a fixed chemical potential approach. It is found that the presence/absence of a flexoelectric effect is critically dependent upon how this adsorption is handled.

Additionally, recent experimental work conducted on cell membrane tethers which are extended in an optical trap setup provides direct estimates for the tensile forces in these tethers as a function of applied voltages. We describe a model which gives quantitative agreement with these measurements.

2822-Plat

Characterizing Changes In The Structure And Orientation Of Supported Model Membranes Upon Binding Of Cholera Toxin B

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Cholera is caused by a protein toxin secreted by the bacterium *Vibrio cholerae*. This toxin is a member of the AB₅ superfamily of toxins composed of an active (A) and a binding (B) pentameric unit. The five binding subunits of the pentamer bind specifically to the GM₁, which is present in the outer leaflet of the host's plasma membrane; however, the mechanism for the translocation of the toxin is not well understood. Model membranes consisting of DMPC+cholesterol+GM₁ were supported on gold electrode surfaces. The changes in the structure and orientation of the model membrane upon binding of the cholera toxin B unit were explored using differential capacitance, chronocoulometry and polarization modulation infrared reflection absorption spectroscopy (PM-IRRAS). Changes in the structure and orientation of the toxin protein with respect to a changing electric field were similarly investigated. The IR data suggests that the binding of the toxin to the membrane causes a decrease in the number of *gauche* conformers, which is caused by the constriction of the acyl chains due to interactions between the toxin and the GM₁ glycolipids. The bound toxin induces some minor defects in the membrane; however, these defects are not significant enough to cause measurable changes in the average orientation of the membrane lipids. The major change in the bilayer upon binding of the cholera B unit was a remarkable decrease in the relative hydration of the membrane. This decrease in hydration is mostly like due to the separation of the bilayer from the aqueous electrolyte by the presence of the bound protein layer on the membrane surface. This work is part of ongoing study to understand the mechanism for translocation of the cholera toxin across the plasma membrane.

2823-Plat

COPI Coat Assembly Occurs on Liquid Disordered Domains and the Associated Membrane Deformations are Limited by Membrane Tension

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Cytoplasmic coat proteins are required for cargo selection and budding of tubulovesicular transport intermediates that shuttle between intracellular

compartments. To better understand the physical parameters governing coat assembly and coat-induced membrane deformation, we have reconstituted the Arf1-dependent assembly of the COPI coat on giant unilamellar vesicles using fluorescently labelled Arf1 and coatomer. Membrane recruitment of Arf1-GTP occurs exclusively on disordered lipid domains and does not induce optically visible membrane deformation. In the presence of Arf1-GTP, coatomer self-assembles into weakly curved coats on membranes under high tension, while it induces extensive membrane deformation at low membrane tension. These deformations appear to have a composition different from the parental membrane since they are protected from phase transition. These findings suggest that the COPI coat is adapted to liquid disordered membrane domains where it could promote lipid sorting and that its mechanical effects can be tuned by membrane tension.

2824-Plat

The Origin of Antimicrobial Resistance and Fluidity Dependent Membrane Structural Transformation by Antimicrobial Peptide Protegrin-1

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In order to kill bacteria, antimicrobial peptides (AMPs) need to intercalate into bacterial membranes, diffuse laterally, and forms pore. Membrane fluidity is thus a critical aspect in AMP-mediated killing. We studied the proposition in this work by systematically examining the effect of membrane fluidity on the disruption of lipid bilayer by AMP protegrin-1 (PG-1). In a fluid supported bilayer patch, PG-1 induces edge instability, then pore-like surface defects at low concentration, and finally wormlike micelles at higher concentrations. We show that the lipid in fluid phase is more susceptible to the formation of pore and wormlike micelle. The progress of destabilizing gel phase to structural transformations occurred along with crack formation and disordering of the gel phase into fluid phase. In addition, we found that, even with the same phase, longer chain length contributed to the PG-1 resistance. Lastly, we provide clear evidence that altering the fluidity of the bilayer can give rise to AMP resistance. These results agree with other studies in which membrane lysis by antimicrobial peptide occurs preferentially at temperatures above the liquid crystal-gel phase transition of the lipid bilayers. Our works provide possible explanation to one of the physical mechanisms of AMP resistance developed by bacteria in the nature.

Platform AW: Ryanodine Receptors

2825-Plat

A Mutation Associated with Catecholaminergic Polymorphic Ventricular Tachycardia in the Cardiac Ryanodine Receptor (RyR2-V2475F) Yields a Highly Arrhythmogenic Channel

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Mutations in *RyR2*, the gene encoding for the cardiac ryanodine receptor (RyR2), are associated with Catecholaminergic Polymorphic Ventricular Tachycardia (CPVT), an arrhythmogenic syndrome characterized by the development of adrenergically-mediated ventricular tachycardia in individuals with an apparently normal heart. CPVT-associated mutations are proposed to result in hyperactive RyR2 channels that "leak" Ca^{2+} excessively during diastole, thus creating a favorable substrate for the development of tachyarrhythmias; however, the vast majority of *RyR2* mutations (~70) fall within three domains of the RyR2 protein that control several aspects of channel function, including Ca^{2+} regulation, phosphorylation, e-c coupling, and FKBP12.6 interaction, among others, thus, a single molecular mechanism of arrhythmogenesis appears unlikely. We expressed CPVT-associated RyR2 mutations, R176Q, R414C, V2475F and G4671R in HEK293 cells and performed [³H]ryanodine binding and single channel experiments to determine the Ca^{2+} dependence of wild type (WT) and CPVT-associated RyR2 mutants. Surprisingly, R176Q, R414C and G4671R yielded no apparent phenotype and displayed Ca^{2+} -activation profiles identical to WT, with threshold for activation at ~pCa7 and maximal activation at ~pCa5. Conversely, V2475F departed from the above mutants and displayed a significantly higher activity at pCa7 (diastolic [Ca^{2+}]), suggesting a more severe phenotype for this mutation. Insertion

of the V2475F mutation in the murine *RyR2* gene yielded mice with moderate cardiac hypertrophy. Furthermore, β -adrenergic stimulation of isolated hearts with isoproterenol induced tachyarrhythmias and fibrillation to a significantly higher extent than in WT mice. Thus, the V2475F mutation yields a highly arrhythmogenic RyR2 channel that induces some structural remodeling. These findings demonstrate that there is heterogeneity of channel dysfunction among CPVT mutants, and that the severity of the dysfunction probably stems from the location of the mutation.

2826-Plat

Mutant Ryanodine Receptor-dependent Calcium Leak, RyR2 Open Probability, Calcium Sparks And Cardiac Arrhythmogenesis

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Ca^{2+} leak from the sarcoplasmic reticulum (SR) represents a major mechanism underlying arrhythmic disease in the heart. Nevertheless the links between cardiac ryanodine receptor (RyR2) single channel behavior, Ca^{2+} sparks, and Ca^{2+} waves remains surprisingly enigmatic. Here we investigate the relationship between a known missense mutation in RyR2 (R2474S) and Ca^{2+} sparks. R2474S has been shown to cause catecholaminergic polymorphic ventricular tachycardia (CPVT) in humans and knockin mice under conditions of stress and catecholamine exposure. Heterozygous CPVT-mutant RyR2-R2474S/WT (RS/WT) or wild-type (WT) channels were isolated from the hearts of 1) resting, control mice or 2) mice which underwent exercise stress testing ('stressed'). Using the lipid bilayer method (cytosolic [Ca^{2+}]_{cis} = 150 nM to approximate diastolic concentrations), RyR2-RS/WT from 'stressed' mice showed a significant gain-of-function defect as evidenced by increased open probability ($P_o = 0.134 \pm 0.008$, n=7) versus WT (0.018 ± 0.008 , n=7; $P < 0.05$). When isolated cardiac myocytes were pre-treated with isoproterenol (1 μM) to mimic catecholaminergic stress and following 1 Hz field pacing, fluo-4-AM loaded RS/WT cells showed a significantly increased spark rate ($341.8 \pm 115.7\%$ vs. $108.1 \pm 44.6\%$) when compared to WT cells. These findings suggest that changes in RyR2 P_o , Ca^{2+} sparks and arrhythmogenesis are linked mechanistically. How the ensemble of findings are interrelated dynamically, however, is model-dependent and this modeling will be presented. These findings suggest that our approach to the investigation of Ca^{2+} dependent arrhythmogenesis broadens understanding of molecular cardiac defects in disease and lays the foundation for the development and testing of novel therapeutic agents.

2827-Plat

Reperfusion after Ischemia Causes Cytosolic Calcium Overload Due to Rapid Calcium Release from the Sarcoplasmic Reticulum

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After a brief ischemic insult, a sustained contractile dysfunction occurs manifested as a sluggish recovery of pump function (myocardial stunning). Substantial evidence supports that myocardial dysfunction is triggered by Ca^{2+} overload during reperfusion (R). Previous results from different laboratories including our own, describe a cascade of events triggered by R that involves the activation of Na^+/H^+ and $\text{Na}^+/\text{Ca}^{2+}$ (NCX) exchangers, with enhanced Ca^{2+} influx. Whether this Ca^{2+} influx directly produces the increase in cytosolic Ca^{2+} or this increase occurs as a consequence of sarcoplasmic reticulum (SR) Ca^{2+} release triggered in turn by the Ca^{2+} influx, is not known. To address this issue, we performed 12 min of global no-flow ischemia followed by R in the isovolumic Langendorff perfused mouse heart positioned on a Pulsed Local Field Fluorescence microscope and loaded with fluorescent dyes (Rhod-2 or Mag-Fluo-4 to assess cytosolic or SR Ca^{2+} , respectively). The results indicated an initial increase in diastolic Ca^{2+} during early R that gradually returned to pre-ischemic levels. This increase was associated with a decrease in SR Ca^{2+} content that recovered within 10 min, as a mirror image of the diastolic Ca^{2+} profile. Additional experiments in which caffeine pulses (20 mM) were applied, confirmed that SR Ca^{2+} content was greatly diminished at the onset of R and gradually recovered within 10 min of R. The present findings indicate that the increase in diastolic Ca^{2+} that occurs upon R is due to a SR Ca^{2+} release and not just because of the Ca^{2+} entry through the reverse NCX mode, as has been previously thought.