153-Plat
Analysis of Membrane Domains by Freeze-Fracture Replica Labeling EM
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In comparison to proteins, relatively little is known about how lipids distribute in the membrane, mainly because microscopic methods for proteins are often not appropriate for lipids. We have been working on an EM method using freeze-fracture replica labeling combined with quick-freezing (Fujita et al, Nat Protoc 5, 661-669, 2010). This method physically fixes membrane molecules thus avoiding the problem of chemical 'unfixability' of lipids and has been applied successfully to examine lipids such as gangliosides GM1 and GM3, and phosphoinositides PtdIns(4,5)P2 and PtdIns3P.
In the present study, we developed a method for PtdIns(3,5)P2, which is critical for the endosome/lysosome functionality. A fluorescent biosensor for PtdIns(3,5)P2 was reported (Li et al, PNAS 110, 21165-21170, 2013), but detailed distribution of PtdIns(3,5)P2 within individual membranes is yet to be determined. To specifically label PtdIns(3,5)P2, we utilized recombinant GST-ATG18 as a probe and blocked its binding to PtdIns3P by co-presence of excess recombinant p40phox PX domain. The labeling specificity was verified by control experiments, including the use of Saccharomyces cerevisiae lacking FAB1 kinase activity.
By use of the methodology, we found that PtdIns(3,5)P2 is concentrated in a membrane domain of yeast vacuole in the process of hyperosmosis-induced fragmentation. PtdIns3P showed much less (or no) concentration than PtdIns(3,5)P2, whereas transmembrane proteins including Vph1p are largely excluded from the domain. The PtdIns(3,5)P2-rich membrane domain usually forms in close contact with another vacuole or the nuclear membrane, and indentations that eventually lead to vacuolar fission form in the domain. In HeLa cells, PtdIns(3,5)P2 was labeled in vesicles with or without tubular extentions, which morphology suggested them to be endosomes. The result corroborated that PtdIns(3,5)P2 could exist in limited membrane domains of endosome/lysosome compartments.

154-Plat
Imaging Sub-Diffraction Membrane Curvature Dynamics during Clathrin Mediated Endocytosis
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Proteins can generate and recognize curved membranes that in turn, modulate protein clustering, diffusion and biochemical reactions. The biophysical mechanisms of this dynamic relationship influence numerous biologically important events including receptor signal transduction, endocytosis, exocytosis and viral assembly. New approaches are needed to measure the dynamics of membrane bending in conjunction with protein recruitment and assembly. Here, I will discuss our work using back focal plane (BFP) spinning total internal reflection fluorescence (360-TIRF) microscopy to create uniform evanescent fields for quantitative imaging of molecular recruitment and assembly. We have paired these methods with BFP positioning for polarized-TIRF microscopy of membrane-oriented lipophilic fluorophores to image the sub-resolution membrane curvature. Using these methods, we have imaged the membrane bending dynamics during assembly of clathrin and dynamin at single endocytic sites in living cells. Our data demonstrate that this method is capable of imaging sub-resolution endocytic events and within the context of their surrounding topography. Together, these methods are enabling new insights into the interplay between assembly, reaction dynamics and membrane topography in living cells.

155-Plat
Long acyl Chain Sphingolipids Govern Visible Microdomains and Cholesterol in Both Model and Plasma Membranes
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Lateral membrane microdomains, or lipid rafts, are considered fundamental to a wide range of biological processes. The association of cholesterol and sphingolipids promotes transient, nanoscopic protein-protein interactions that enhance membrane signal transduction. In giant unilamellar vesicles (GUVs) or giant plasma membrane vesicles (GPMVs), these microdomains are readily resolvable by optical microscopy. However, similar visible microdomains have not been observed in living cells, which has been a long-standing mystery. A unique feature of the native plasma membrane is the high degree of transbilayer lipid asymmetry and, particularly, with long acyl chain sphingolipids exclusively in the outer leaflet. The impact of asymmetry on optically resolvable microdomains is not known, nor is the role of long acyl chain sphingomyelin (SM). Here, we show that GUVs fail to form visible microdomains if long acyl chain SM is exclusively in the outer leaflet but, if long acyl chain SM is placed in both leaflets, GUVs readily produce visible microdomains. With a novel methodology capable of analyzing cholesterol in the individual leaflets, we obtained evidence that long acyl chain SM, not short acyl chain SM, drives cholesterol into the inner-leaflet of large unilamellar vesicles. This depletes cholesterol from the outer leaflet, thereby providing a mechanism for abolishing visible microdomains. We finally confirm that, in native plasma membrane - human erythrocytes, the majority of cholesterol is indeed in the inner leaflet. We conclude that native plasma membrane cannot form visible microdomains, primarily due to lack of cholesterol in the outer leaflet as the consequence of exofacial long acyl SM. Our findings therefore resolve the long-standing puzzle and uncover a novel fundamental organization principle for the plasma membrane.

156-Plat
Using Lipopolysaccharides to Create 3-D Multicomponent Biomimetic Membranes on Solid Supports
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Cell membranes are complex, multi component systems that contain dynamic elements such as lipid rafts and protein rich domains. Lipid Bilayer Assemblies (LBAs) can be used as model membrane systems for investigating interactions and responses to different stimuli but the level of complexity that can be studied is limited. Previous studies have shown the ability to create these LBAs on glass microspheres as well as flat substrates. These microspheres introduce a new level of complexity by creating mobile samples that can be analyzed using new techniques and also provide a curved surface more similar to biological cells. In previous studies we have demonstrated that Lipopolysaccharides (LPS) from gram-negative bacteria can be used to pattern LBAs in a simple, iterative manner. In this study, we have applied this LPS-modification approach to introduce new elements into LBAs on glass microspheres. We have found that LPS can be used to form static holes throughout the membrane while keeping the membrane intact. These holes can be backfilled with proteins such as BSA, and lipids, allowing membrane repair and or introduction of new components into the system. This technique gives rise to a multicomponent system that can be manipulated while still maintaining lipid fluidity in three dimensions. These new findings are being used to better understand LPS-membrane interactions, lead to new approaches for potential biosensor design, and provide a new platform for investigating cell membrane interactions. Future directions include using these coated microspheres as an LPS biosensor that can be analyzed using flow cytometry, introducing additional components into the system, such as receptors, gel phase lipids, and complex proteins to investigate membrane interactions and binding events and exploring as a template for the generation of janus-type particles.

157-Plat
GPI-Anchored Proteins do not Reside in Ordered Domains in the Live Cell Plasma Membrane
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The organization and dynamics of proteins and lipids in the plasma membrane, and their role in membrane functionality, have been subject of a long-lasting debate. Specifically, it is unclear to what extent membrane proteins are affected by their immediate lipid and protein environment and vice versa. Studies on model membranes and plasma membrane spheres indicated characteristic preferences of proteins for ordered or disordered lipid phases, however, whether such phases do indeed exist in live cells is still not known. Here, we used a micropatterning approach combined with super-resolution microscopy to quantify the influence of a glycosylphosphatidylinositol-anchored protein (GPI-AP) - a typical marker of ordered lipid phases - on its molecular environment directly in the live cell plasma membrane. Intriguingly, local enrichment and immobilization of a GPI-anchored mGFP - even at highest densities - did not nucleate the formation of ordered membrane regions. At a molecular scale, immobilized mGFP-GPIs essentially behaved as inert obstacles that reduced the mobility of other membrane constituents according to their molecular size and shape. Overall we conclude that GPI-APs do not significantly influence their membrane environment over distances beyond their actual physical size. Further, our

results imply that the outer leaflet of the plasma membrane is in a homogenously mixed single phase state under physiological conditions.

Symposium: Emergent Properties and Collective Behaviors of Complex Systems

158-Symp
Scaling Laws Governing Growth and Division of Single Bacterial Cells
Aaron Dinner.
Chemistry, The University of Chicago, Chicago, IL, USA.
Are there simple quantitative relationships that govern the behaviors of whole organisms? If so, what form do they take? To address these questions, we study growth and division of Caulobacter crescentus, a paradigm for polar cell development and cell cycle control. Using a unique combination of technologies that yields unprecedented statistical precision, we find that their mean sizes grow exponentially in time and that their size distributions collapse to a single curve when rescaled by their means. An analogous result holds for the division-time distributions. We account for these observations with a minimal stochastic model that is based on an autocatalytic cycle. It predicts the scalings, as well as specific functional forms for the universal curves. Our experimental and theoretical analysis reveals a simple physical principle governing these core biological processes: a single timescale governs noisy bacterial growth and division despite the complexity of underlying molecular mechanisms.

159-Symp
Driving with the Brakes on: An Incoherent Transcriptional Circuit Patterns the Drosophila Embryo
Angela DePace.
Systems Biology, Harvard University, Boston, MA, USA.
We used modeling and quantitative experiments to uncover a case where a bifunctional transcription factor regulates the same expression pattern in opposite ways. I will discuss the functional implications of this type of transcriptional circuit, and how our results highlight the need for new methods to determine how promoters integrate information from multiple enhancers.

160-Symp
Temporal Frequency of Directional Sensing and Collective Migration in Dictyostelium
Satoshi Sawai.
Graduate School of Arts and Sciences, University of Tokyo, Tokyo, Japan.
In the social amoebae Dictyostelium discoideum, stimulation by chemoattractant cAMP invokes a transient rise in cytosolic cAMP that is secreted and excite other cells in the neighborhood. The adaptive cAMP response is the key property that supports the synchronized oscillations and waves of extracellular cAMP that direct cell aggregation. Here, we report on simultaneous live-cell observations of propagating cAMP waves, cell motion and the intracellular signals involved in the leading edge formation. We show that, for early aggregation stage, there is a good correspondence between the waves indirectly observed by means of light-scattering and those observed by FRET cAMP indicators. During the later cell streaming stage, there is a clear transition in the oscillation frequency from about 6 to 3 minutes. The timing of this shift in periodicity coincides with the onset of cell streaming - a distinct mode of cell migration of elongated cells that are aligned and appear tightly connected from the back of a cell to the front of another. Observations of Ras activation, phosphatidylinositol (3,4,5)-trisphosphate (Pip3), F-actin and cytosolic cAMP revealed that, during the 6-min cAMP oscillations, Ras, Pip3 and F-actin are synchronized in phase with cAMP in a biphasic manner. The secondary peak corresponds to lateral pseudopod formation observed during the resting phase of the cAMP oscillations. In contrast, after the transition to 3-min oscillations, lateral pseudopod formation disappears and cell migration becomes ballistic and highly efficient. The present results suggest that these modes of cell migration are closed associated with the underlying kinetics of the directional sensing and cell polarity.

161-Symp
The Emergence of Heart Failure as a Consequence of Myocardial Metabolic Dysfunction
Daniel A. Beard.
Molecular and Integrative Physiology, University of Michigan, Ann Arbor, MI, USA.
It is widely appreciated that heart failure is associated with alteration in metabolic/energetic state of the myocardium. However, it remains unknown what exactly is the nature these alterations, and if/how these changes might impact the ability of the heart to generate pressure and pump the blood through the circulation. Like many questions associated with the etiology of complex disease, this is a chicken-or-the-egg question: To what extent does mechanical/structural remodeling precede and cause the metabolic changes observed to occur with mechanical failure of the organ? To what extent can mechanical failure be explained by diminished metabolic function? We developed a multi-scale multi-physics simulation of the cardiovascular system to probe the hypothesis that since variety of cellular processes in the heart depend critically on ATP, alterations in ATP hydrolysis potential could result in mechanical dysfunction at the level of the cardiac myocyte resulting in impaired contractile performance of the heart and, ultimately, heart failure. Since heart failure is a whole-body disease, with clinical presentation influenced by cardiac, pulmonary, renal, and neurohumoral function this investigation requires an integrated simulation spanning molecular/metabolic function to whole-body cardiovascular dynamics. Accounting for myocardial metabolism (normal versus changes associated with heart failure), electrophysiology and calcium handling, cross-bridge kinetics and mechanics, and neurohumoral signaling in the context of a whole-body model of cardiovascular dynamics, simulations reveal the extent to which the key clinical features emerge as consequences of metabolic dysfunction at the cellular level. These theoretical predictions are validated based on pharmacological interventions targeting key metabolic pathways in the heart.

Symposium: Protein Evolution and Allosteric Networks

162-Symp
Understanding Enzyme Molecular Evolution toward Thermal Adaptation using Multistate Computational Protein Design
Corey J. Wilson.
School of Engineering and Applied Science, Yale University, New Haven, CT, USA.
A careful balance between structural-stability and flexibility (allostery) are hallmarks of enzymatic function and temperature can affect both properties. Canonical (fixed-backbone) enzyme design strategies currently do not consider the role of these properties. Here we describe the rational design of 100 temperature-adapted adenylate kinase (AdK) enzymes via a novel multistate design strategy that takes into consideration the impact of conformational changes to the backbone structure and stability, based on boundaries set by molecular evolution. In turn, each AdK protein variant was expressed and experimentally characterized for thermostability and temperature-adapted function. Comparison of the experimental temperature of maximum activity to the melting-temperature across all 100 variants reveals a strong correlation between these two parameters. In turn, experimental stability data was used to produce accurate predictions of thermostability via multistage nodes, providing the requisite complement for de novo temperature-adapted enzyme design. To the best of our knowledge the rational design of temperature adaptive enzyme function has not been realized, prior to this work. In principle, this level of design-based analysis can be applied to any protein, paving the way toward identifying and understanding the hallmarks of enzymatic allostery as it relates to the thermodynamic and structural limits of function.

163-Symp
Allosteric Networks in Thrombin
Elizabeth Komives.
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Serine proteases are found ubiquitously in both eukaryotes and prokaryotes however the dynamic motions of this largest peptidase family remains unknown. All serine proteases have a double β-barrel core surrounded by connecting loops and helices, but compared to the prototypical serine protease, chymotrypsin, thrombin has more extended loops that are thought to impart greater specificity. We analyzed apo-thrombin and active site-bound (PPACK-thrombin) using a combination of MD, accelerated MD and NMR. Community analysis of the MD ensembles identified groups of residues linked through correlated motions and physical contacts. AMD simulations, calibrated on measured residual dipolar couplings, revealed correlated loop motions connecting the active site with distal allosteric regulator binding sites. The backbone dynamics profile (from ps to ms motions) of thrombin was determined in both states using R_1, R_2, ^{15}N-$\{^1H\}$NOEs, TROSY Hahn-Echo, and relaxation dispersion experiments. The apo thrombin backbone is highly dynamic and shows motions across multiple time scales. The various surface loops move on different time scales. The substrate-binding loops showed intermediate time