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to the cell cycle. To test this hypothesis, we isolated GPMVs from populations of cells synchronized using a double Thymidine block that arrests cells at the border between G1 and S phases. After Thymidine is removed from the culture media, cells proceed synchronously through one cell cycle and GPMVs are isolated at time-points corresponding to S, G2, M, and G1 phases. We find that critical temperatures are significantly elevated in the cell cycle phases that immediately preceding cell division (G2 and M) compared to the remainder of the cell cycle phases (G1 and S), consistent with our observations of higher overall transition temperatures in more rapidly dividing cells. We speculate that membrane heterogeneity arising from this critical point may play a role in cytokinesis or other processes vital for cell division. If this hypothesis is correct, it may suggest that biochemical perturbations that lower critical temperatures in GPMVs can be used to inhibit cellular proliferation, possibly providing a novel treatment strategy for some cancers.

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Cytoskeletal Pinning Prevents Large-Scale Phase Separation in Model Membranes

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During the last decades, artificial lipid bilayers have become an important tool in studies of properties of the plasma membrane of cells. One important feature of cell membranes, which has been difficult to recapitulate in the artificial bilayer systems, is the membrane-associated cytoskeleton. The cytoskeleton is believed to affect diffusion of lipid and protein molecules in the plasma membrane, and is considered to be one of the reasons for the sub-resolution size of membrane domains by preventing large-scale phase separation. Mimicking the eukaryotic actin-based cytoskeleton in vitro is inefficient and complicated, owing to the number of components involved and the nature of membrane binding of the actin-network complex. Here we describe a minimal cytoskeletal network formed by the prokaryotic tubulin homologue, FtsZ. FtsZ has been modified to interact with the membrane through a membrane targeting sequence (MTS) from MinD, another prokaryotic protein. FtsZ-MTS efficiently forms a highly interconnected network on the membrane with a concentration-dependent characteristic mesh size, much similar to the eukaryotic network underlying the plasma membrane. Using giant unilamellar vesicles formed from a quaternary lipid mixture, we demonstrate that, on the one hand, the artificial membrane-associated cytoskeleton suppresses large-scale phase separation below the phase transition temperature, and, on the other hand, preserves phase separation above transition temperature. Our experimental observations support the ideas put forward in our previous simulation study [1]: In particular, the picket-fence effect on phase separation explains why micrometer-scale membrane domains are observed in isolated, cytoskeleton-free giant plasma membrane vesicles, but not in intact cell membranes. The experimentally observed suppression of large-scale phase separation much below the transition temperatures also serves as an argument in favor of the cryoprotective role of the cytoskeleton.

[1] J. Ehrig, E. P. Petrov, P. Schwille, Biophys. J. 100 (2011) 80.

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Extracellular, Membrane and Intracellular Proteins that Alter Integrin Cell Membrane Diffusion and Clustering

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Integrins are ubiquitous membrane proteins that are involved in cell adhesion and signaling across the cell membrane. We are elucidating the role of other membrane proteins, extracellular ligand, and intracellular proteins in altering integrin clustering and diffusion using several fluorescence microscopy techniques. Our work provides vital information on the molecular mechanism of integrin function through altered dynamics and membrane organization. Clustering is measured using fluorescence resonance energy transfer (FRET) and stimulated emission depletion imaging (STED). In order to noninvasively measure membrane structure and dynamics, we have developed a novel STED instrument for fluorescence lifetime imaging with 40-nm spatial resolution, which is below the diffraction limit of light. Diffusion is measured using fluorescence recovery after photobleaching (FRAP) and single particle tracking (SPT). Select membrane proteins that we study include focal adhesion kinase and epidermal growth factor receptor, while select cytoplasmic proteins that we study include paxillin, vinculin, actin and focal adhesion kinase. Among our interesting findings, we have determined that other membrane proteins do not significantly alter the percentage of mobile integrins, but significantly constrain integrin diffusion; the role of cytoplasmic proteins in altering integrin clustering depends on the concentration of extracellular ligand available for binding; and increasing integrins' ligand affinity reduces the population of mobile integrins and also reduces the diffusion coefficient of integrins that remain mobile. We hypothesize that altered partitioning into membrane nanodomains is the main mechanism for altered integrin clustering and diffusion when the concentration of select cytoplasmic, membrane or extracellular protein is altered; and we are further testing this hypothesis using STED lifetime imaging.

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Ligand Receptor Binding Rate Kinetics via K-Space Image Correlation Spectroscopy: An in Silico Study

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To achieve a fundamental understanding of intra-cellular signalling pathways, it will be necessary to measure the rates of reaction between chemically reacting and interacting macromolecules. The magnitude of binding rates plays a very important molecular regulatory role in a range of systems including the binding of antigens to T-cell receptors, which can lead to differing immune-responses, as well as rates of hydrolysis of GTPs with Rac or Rho proteins, which lead to temporal changes in actin filament lengths and cell motility. Here, we develop an image correlation technique which may be used to study such systems in conjunction with fluorescence microscopy. We simulate receptors diffusing in 2D with diffusion coefficients varying from 0 to 10 pixels^2/frame. Receptors are only visible as point emitters when a fluorescent ligand is bound to the receptor; ligand binding kinetics are created by turning receptors 'on' or 'off' with average life-times varying from 3 to 20 frames drawn from a negative exponential distribution. Photobleaching is similarly incorporated by permanently turning diffusing receptors 'off' after average times varying from 20 to 400 frames. The image time-series is generated by convolving a Gaussian function of fixed radius with the point emitter distribution to create 128x128 pixel images with background noise added to give a signal to noise ratio of 3. We use k-space image correlation spectroscopy (kICS) and develop a two-state kinetic binding model with freely diffusing receptors on a 2D membrane to capture the membrane binding kinetics of the image time-series. We show that kICS can accurately recover on/off binding rates at the 95% confidence level for over 75% of simulations. Typically, percentage errors are less than 30% for weak photobleaching and within 60% for strong photobleaching effects.

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Single Molecule Tracking of Annexin V in Cushioned DMPC Assemblies

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Using single molecule imaging, this study describes the phase behavior and mobility of individual transmembrane (TM) proteins and compares those results with the bulk phase behavior of the biomimetic membrane in which they have been incorporated. To accomplish this a TM protein, Annexin V, was incorporated into a cushioned planar supported 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC) / L-α-lysophosphatidyl-serine (Brain-PS) biomimetic assembly and its mobility between 30 and 16 C was measured. Fluorescence microscopy and Fluorescence Recovery After Photobleaching (FRAP) were used to verify the structural integrity and the phase behavior (median melting temperature, TC = 22 C) of the lipid assembly. The spatial confinement of individual Annexin V molecules was measured in three distinct phase regions: (1) a homogenous liquid crystalline phase region ($L\alpha$) in which Annexin V was unconfined ($\geq$ 25 C), (2) a two-phase region ($L\alpha$ + gel-phase-$P\beta'$) in which Annexin V displayed intermediate confinement (24 - 20 C), and (3) a gel-phase region ($P\beta'$) with included nanoscopic domains that are enriched with PS and surround a single Annexin V TM protein (19 - 16 C); the mobility of Annexin V in these domains is highly confined. At early time lags, Annexin V moves with apparent Brownian-like behavior at all temperatures but the diffusion coefficients have very different magnitudes and temperature dependence. A possible mechanism for nanoscopic domain formation will be discussed.

Protein-Nucleic Acid Interactions I

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A SAXS Study of the CRISPR Associated Gene (Cas) Csn2 in the Presence and Absence of Calcium Ions

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Small angle X-ray scattering (SAXS) was used to study the solution structures of the *E. Faecalis* Csn2 protein, a *cas* gene required for DNA spacer acquisition