1067-Plat
Single Molecule Kinetics of ENTH Binding to Lipid Membranes
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The Epsin N terminal homology (ENTH) domain is a peripheral membrane protein that transiently associates with membranes by binding the signaling lipid phosphatidylinositol 4,5-bisphosphate (PIP2). This protein exemplifies general principals of spatio-temporal control in lipid-protein and protein-protein interactions. For example, ENTH assists clathrin mediated budding by intercalating into the lipid head group's region thus stabilizing non-spherical membrane shapes. We capture the different stages in tubulation of liposomes induced by ENTH binding using cryo transmission electron microscopy.
To gain further insight into the interactions of ENTH with model membranes of well controlled compositions we image single ENTH binding events via total internal reflection fluorescence microscopy. Frame rates in excess of 75 exposures per second and subsequent single fluorophore tracking provides insight into details of ENTH binding kinetics at different PIP2 concentrations. The resulting distributions of residence times and step-sizes of the ENTH diffusion on the membrane show significant deviations from simple kinetics and one component diffusion processes.

1068-Plat
Probing Molecular Interactions in Biological Membranes by Solid-State NMR
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The interaction between integral membrane proteins and the lipid bilayer is vital in the regulation of many biological processes. Here we report preliminary solid-state NMR studies on two systems where interactions at the lipid/protein interface play an important role in the regulation and trafficking of integral membrane proteins.
The anaesthetic octanol has been shown to interact with several sites on the nicotinic acetylcholine receptor (nAChR) one of which is in the lipid/protein interface. In preliminary studies we are investigating how octanol interacts with the lipid bilayer. Analysis of the ^{1}H-^{13}C dipolar couplings present in 2D dipolar/chemical shift spectra we have been able to measure changes in bilayer fluidity at pharmacologically relevant concentrations. We have been able to correlate these results with 2D-^{1}H-NOE-MAS spectra enabling us to locate the position of the octanol within the lipid bilayer. As these methods function with isotopes at natural abundance levels, we are currently applying them to native nAChR membranes to see if changes in the order parameter profile observed in model lipid bilayers are reproduced in native receptor membranes.
The interaction of proteins with lateral domains (rafts) in lipid bilayers has been proposed to play an important role in the trafficking of proteins within cells. We are utilizing exchange spectroscopy to study the partitioning of Fukutin, a protein linked to the onset of muscular dystrophy, into lipid rafts. Exchange measurements have enabled us to observe the restricted lateral diffusion of phospholipids in the presence of lateral domains formed in vesicles formed from ternary lipid mixtures. The affects of these lateral domains on the structure and oligomeric state of the Fukutin is also being probed.

1069-Plat
Single Molecule Fluorescence Studies of Membrane Targeting Proteins: Lateral Diffusion in Supported Bilayers Reveals Additional Lipid Binding Sites
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Protein-membrane docking via conserved membrane-targeting domains is essential for many cell signaling processes. Recently developed, in vitro applications of single-molecule total internal reflection fluorescence microscopy (TIRFM) can reveal features of the membrane-bound state that may remain hidden during bulk measurements. In particular, lateral diffusion on supported lipid bilayers can provide information on properties of membrane-docked states. Here, we measure lateral diffusion of multiple membrane-targeting pleckstrin homology (PH) and C2 domains and full-length proteins to determine the relationship between membrane binding and lateral diffusion. We find that increasing the number of peripherally bound lipid molecules slows diffusion, as expected based on both classical diffusion and established theory for membrane proteins. Furthermore, we use lateral diffusion measurements in supported bilayers of defined composition to identify and characterize a novel, secondary binding site specific for phosphatidylserine in the membrane-docked state of a representative PH domain. Overall, this study shows that in vitro single-molecule TIRFM provides a new window into the molecular mechanisms of membrane docking reactions.

1070-Plat
Ceramide And The FasR In Plasma Membrane Spheres
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We have induced plasma membrane spheres formation in human cells in culture expressing a FasR-eGFP construct in order to study the effect of the membrane lipids organization in the receptor lateral distribution, diffusion and aggregation state. The induction of domain coalescence with Cholera Toxin subunit B produced micron-sized domains into which the receptor preferentially partitioned. We have used fluorescence correlation spectroscopy to assess the effects of domain coalescence, FasL binding, an agonistic antibody and sphingomyelinase treatment on the dynamics of lipid and receptor molecules in the membrane.

Platform R: Fluorescence Spectroscopy

1071-Plat
Live Cell Fluorescence Fluctuation Analysis Of Cdc42 Polarity Maintenance In Live Yeast
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Biological processes such as development, cell motility, and immune response depend on the maintenance of robust cell polarization. Cdc42, a conserved Rho-GTPase and master regulator of cell polarization is recycled by two complementary mechanisms: actin-mediated transport/endocytosis and cytosolic recycling through the Rho-GDP dissociation inhibitor (GDI) Rdi1p. However, the molecular interactions controlling these recycling mechanisms are not well understood. Here, fluorescence correlation spectroscopy (FCS), and cross correlation spectroscopy (FCCS), along with fluorescence recovery after photobleaching (FRAP) are implemented to study recycling of Cdc42 at the polarized site and dynamics and interactions of cytosolic Cdc42p in living yeast cells. FRAP of GFP-Cdc42p at the polar tip upon Cdc42 mutation, Rdi1 deletion, or LatA treatment to inhibit actin transport reveal that multiple recycling mechanisms work in concert to maintain polarization of Cdc42p. Using FCCS, we observe strong cytosolic interaction of Rdi1p-mCherry and GFP-Cdc42p. While *in vitro* experiments have shown that Rdi1p forms a stable complex with Cdc42p-GDP, we observe little *in vivo* interaction of Rdi1p with Cdc42 mutants locked in GDP or GTP forms, suggesting the GTPase cycle of Cdc42p is necessary for the ability of Rdi1p to bind Cdc42p *in vivo*. Relative abundance of 'fast' and 'slow' diffusing populations of Cdc42 depends upon the ability of Rdi1p to bind Cdc42 and the GTPase cycle of Cdc42p. In addition, the role of Rdi1p phosphorylation in the regulation of Rdi1p-mediated Cdc42 recycling, and a mathematical model of the spatial relationship of the multiple pathways for Cdc42p polarity maintenance relative to the polar cap will be presented.

1072-Plat
Fluorescence Fluctuation Spectroscopy for Probing the Ionic Atmosphere of Nucleic Acids
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Two-beam fluorescence cross-correlation spectroscopy coupled with continuous flow capillary electrophoresis (2bFCCS-CFCE) was used to study the relationship between diffusion and effective charge of a fluorescently-labeled 40-base polythymine single-stranded DNA (ssDNA) as a function of divalent magnesium ion concentration. Cross-correlation analysis of the fluorescence monitored from two spatially offset microscopic detection volumes revealed the diffusion and electrophoretic migration of ssDNA at a range of magnesium concentrations and electric field strengths. The effective charge of the ssDNA could then be determined using simple calculations. It was found that as the magnesium concentration in the buffer solution increased, the diffusion of the ssDNA also increased, while the effective charge of the ssDNA decreased. This was believed to be caused by increased association of the magnesium counterions with the negatively charged backbone of the ssDNA, which partially neutralized the negatively charged functional groups and allowed the ssDNA to adopt a more compact structure. To our knowledge, this is the first demonstration of the measurement of effective charge of ssDNA in solution relative to magnesium ion concentration.