the dyes; (3) Appropriate description for the spatial distribution of the fluorophore by fast accessible volume (AV) simulations [2] to determine the dye positions relative to the biomolecule; (4) Search for possible structures via a FRET positioning system using a spring-network algorithm. Possible structures are generated either by a model-based approach with rigid body docking or model free by selecting suitable models from a huge structure library; (5) Docking is repeated many times to find all possible arrangements and assure the completeness of generated structural ensemble; (6) The obtained models are ranked according to their violation of FRET constraints and steric clashes. Then they are assigned to clusters of related structural organization in order to judge the uniqueness of structural models; (7) The precision (RMSD) of the structure models is determined using a bootstrapping procedure. We demonstrate the accuracy of high-precision (hp) FRET in two experiments - determination of the DNA position in HIV-1 reverse transcriptase primer/template complexes and arrangement of a primer/template DNA bound by HIV-1 reverse transcriptase and analysis of the internal structural heterogeneity of human guanylate binding protein 1 (hGBP1). These studies show that hpFRET studies are valuable tools to complement the structure information obtained by classical methods.


Platform: Interfacial Protein-Lipid Interactions I

1190-Plat Visualization of Supported Lipid Bilayer Remodelling by s-mgm1 using Correlated Confocal Fluorescence and Atomic Force Microscopy

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In yeast, the GTPase s-mgm1 is responsible for the fusion of inner mitochondrial membranes, a process essential for maintenance of normal mitochondrial morphology and function. Direct, real-time visualization of the effects of s-mgm1 upon mitochondrial mimic membranes is particularly relevant to elucidating the mechanism by which it acts. Here, we utilize both confocal microscopy and AFM to demonstrate that s-mgm1 spontaneously induces GTP-independent pinching and tubulation of lipids in the gel phase. Subsequent addition of GTP causes further remodelling of the membrane. Similar experiments using ATR-FTIR suggest that the membrane induces increased order in protein conformation. Our data is consistent with a model by which s-mgm1 promotes fusion of opposing membranes by pinching and tubulation.

1191-Plat Controlled Protein Confinement in Phase-Separated Membranes Tethered onto Micro-Patterned Supports

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Phase-separation of lipid membranes into liquid-disordered (l_d) and liquid-ordered (l_o) phases has been recognized as a key principle for the functional organization of the plasma membrane. In classic model systems such as GUVs, the spatial organization of phase separated membranes is a stochastic, time-dependent process, which depends on the lipid composition and often leads to a complete coalescence of the lipid phases. We have here established an approach for a spatial control of lipid phase separation in tethered polymer-supported membranes (PSM). On a dense poly(ethylene glycol) polymer brush functionalized with hydrophobic tethers, contiguous, highly fluid PSM were obtained by means of fusion of SUVs. Free diffusion of lipids and reconstituted transmembrane proteins in these PSM was confirmed by FRAP, FCS and single molecule tracking. Strikingly, phase separation of ternary lipid mixtures (DOPC/SM/cholesterol) in PSM into l_d and l_o phases was dependent on the properties of the anchoring group. We exploited these features for assembly of l_d domains into defined structures using micropatterned tethers. Within isolated micropatterns, l_d and l_o phases self-assembled into stable, reproducible membrane architectures. By binary micro-patternning of different tethering groups into complementary areas, ternary lipids mixtures separated into l_d and l_o phases controlled by the geometry of the underlying tethers. Transmembrane proteins reconstituted in these phase-separated PSM strictly partitioned into the l_o phase. Hence, the l_d phase could be used for confining transmembrane proteins into microscopic and submicroscopic domains. The permeability of these barriers for lipids and proteins and thus their exchange between adjacent l_d compartments can be globally and locally controlled by the temperature. These features have been exploited for probing interactions and diffusion of a transmembrane receptor in the context of l_d and l_o phases. 1) Roder, F.; et al. Anal Chem 2011, 83, 6792-6799.

1192-Plat Lipid-Protein Interactions in Nanodiscs: How to Enhance Stability

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Lipid-protein interactions can function as “co-factors” that affect the properties / function of transmembrane proteins. Herein, the interaction between anionic dimyristoylphosphatidylglycerol (DMPG) and zwitterionic dimyristoylphosphatidylcholine (DMPC) with the amphipatic membrane scaffold protein (MSP), were studied. Two 25 kDa MSP wrap around the circumference of discoidal bilayer in a belt-like manner to form a nanodisc [1,2]. The membrane-like structure of nanodiscs has been used for reconstitution of membrane proteins in a native-like environment. Differential scanning fluorometry was employed to characterize lipid-protein interactions in these particles by evaluating changes in MSP denaturation temperature and lipid gel-liquid phase transition as a function of nanodisc lipid composition and ionic strength. Small-angle X-ray scattering and size-exclusion chromatography were used to determine the overall structure of the nanodisc. We suggest the nanodisc lipid is divided into a lipid rim that interacts with the internal face of the MSP helical segments and a central region that is composed of the closely located nanodisc lipids maintaining a more bulk-like lipid behavior. This finding is important for reconstitution of membrane proteins since the presence of a ‘lipid rim’ serves to prevent contact between the membrane protein and the MSP. Furthermore, the presence of two distinct lipid environments reduces the available area for reconstituted membrane proteins in the nanodisc. We also show that the negatively charged DMPG has a high preference for the rim due to its negatively charged headgroup. Finally, we conclude that DMPG stabilizes the nanodisc in a twofold manner: i) DMPG ‘freezes’ the MSP conformation preventing flexibility / dissociation that may lead to aggregation. ii) DMPG also contributes to prevention of aggregation due to electrostatic repulsion between the negatively charged lipids on neighboring nanodiscs.


1193-Plat The HSP70 Interaction with Phosphatidyl Serine on Membranes is the Initial Step its Release into the Extracellular Medium

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The HSP70 Interaction with Phosphatidyl Serine on Membranes is the Initial Step its Release into the Extracellular Medium. These extracellular Hsp appear to play signaling role in the activation of the systemic response to stress. The question that arises is how hsp that do not display any consensus secretory signal or hydrophobic domains are inserted into membranes and secreted into the extracellular medium. We have previously shown that Hsp70, the major inducible hsp, was released embedded into the membrane of export or extracellular vesicles (ECV). The possible mechanism for Hsp70 insertion into membranes was investigated. We found that Hsp70 displayed a high specificity for phosphatidyl serine (PS) on the membrane, even if this lipid is combined with larger amounts of other phospholipids. The interaction of Hsp70 with PS was demonstrated by insertion into liposomes, changes in tryptophan fluorescence after exposure to artificial lipid membranes, and fluorophore leakage from liposomes. In addition, we showed extracellular Hsp70 bound to cells displaying PS on the surface, but not to surface PS negative cells. We propose that insertion of Hsp70 into membranes is a spontaneous process requiring the presence of PS. Therefore, we suggest that the insertion of Hsp70 in cellular membranes is the initial step in the export of this signaling molecule into the extracellular environment.