reduced viral fusion and infectivity. Furthermore, neutralizing anti-gp41 antibodies disrupt the MPER hinge function by perturbing MPER hinge orientation, and/or extracting part of the MPER from the membrane. The interaction can be a stepwise rearrangement through an apparent scoop-like movement of the antibodies' long and unique CDRH3 segments. Mutations of the CDRH3 segments reduced the ability of the antibodies to extract MPER residues from the membrane, without affecting peptide binding in solution. In addition, MPER-membrane interaction and antibody binding are modulated by lipid composition and cholesterol content. These findings have revealed important features of gp41-antibody interaction at the viral membrane interface.

189-Plat

Small Angle Neutron and X-Ray Scattering Reveal Conformational Differences in Detergents Affecting Rhodopsin Activation

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Understanding G-protein-coupled receptor (GPCR) activation plays a crucial role in the development of novel improved molecular drugs. During photoactivation, the retinal chromophore of the visual GPCR rhodopsin isomerizes from the 11-cis conformation to the all-trans conformation, yielding an equilibrium between inactive Meta-I and active Meta-II states [1]. The principal goals of this work are to address whether the activation of rhodopsin leads to a single state or a conformational ensemble, and how the protein organizational structure changes with the detergent environment. We used small-angle neutron scattering (SANS) and small-angle X-ray scattering (SAXS) to answer the above questions. Both SANS and SAXS are powerful techniques to study the macromolecular structures in solution within the length scale from angstroms to several nanometers. In our experiments, rhodopsin is solubilized in CHAPS detergent, which favors the inactive Meta-I state. By contrast, dodecylmaltoside (DDM) detergent stabilizes the active Meta-II state [2]. Notably SANS with contrast-variation enables the separate study of the protein structure within the detergent assembly [3], and suggests a looser structure of rhodopsin in DDM versus CHAPS micelles. Such results are consistent with the SAXS data fitted by either a core-shell ellipsoid or core-shell cylindrical model, describing a monolayer of detergent molecules surrounding the rhodopsin molecule. Moreover, the SAXS experiments with different rhodopsin to detergent ratios delineate the role of the detergent in stabilization of the protein in solution. Our combined approach of SANS and SAXS studies reveals the protein structural changes associated with GPCR activation in the case of visual rhodopsin.

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190-Plat

Determining the Free Energy of Membrane Protein Dimerization in Lipid Bilayers

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Experimental determination of the free energy of membrane protein oligomerization in lipid bilayers is difficult. There are few membrane protein systems that allow for this equilibrium measurement, and for strong complexes, the amount of dilution necessary to observe dissociation often obscures experimental detection. We have devised a new model system for measuring equilibrium dimerization in membranes, using the homodimeric CLC-ec1 Cl^-/H^+ antiporter. A mutated version of CLC-ec1 that bears a single tryptophan substitution on the dimerization interface (CLC-W) shifts the protein to the monomeric state in detergent micelles. We quantitatively labelled CLC-W with Cy3 or Cy5 fluorophore and reconstituted the protein into 2:1 POPE/POPG lipids. Then, we measured macroscopic Förster resonance energy transfer (FRET) in large membranes to assess CLC-W dimerization. Mixing CLC-W-Cy3 and CLC-W-Cy5 in detergent before reconstitution, or fusing CLC-W-Cy3 liposomes with CLC-W-Cy5 liposomes yields a FRET signal indicative of dimer formation and equilibrium exchange. We also confirmed that the protein was folded, by measuring Cl- transport function. We then investigated dissociation of CLC-W by "traditional" dilution in membranes. Since wild-type CLC-ec1 does not undergo dimer exchange, we used CLC-ec1-Cy3 mixed with CLC-ec1-Cy5 to determine the "all-monomer" background signal, and co-labelled CLC-ec1-Cy3/Cy5 to determine the "all-dimer" FRET signal, at various Cy3:Cy5 labeling ratios and protein:lipid densities. With this, we observe that CLC-W begins to dissociate at a density of 1 protein per 300,000 lipids. At densities below 1 protein per 650,000 lipids, macroscopic FRET is obscured by background scattering and so we turn to single-molecule fluorescence microscopy to measure CLC-W stoichiometry by fluorophore photo-bleaching. These studies demonstrate that we have pushed the observable limit of this reaction, expanding our ability to measure the free energy of membrane protein assembly in lipid bilayers.

191-Plat

A FRET Analysis of the FtsB-FtsL Transmembrane Domain Interactions of the E.coli Divisome Suggests a Higher Order Oligomeric Complex with a 1:1 Stoichiomtery

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FtsB and FtsL are two essential integral membrane proteins of the bacterial division complex or 'divisome', both characterized by a single transmembrane helix and a juxta-membrane coiled coil domain. The two domains are important for the association of FtsB and FtsL, a key event for their recruitment to the divisome that in turn enables recruitment of the late divisomal components and subsequent completion of the division process. We have previously established that the transmembrane domain of FtsB self-associates in Escherichia coli membranes using a biological assay in vivo (Biochemistry 2013, vol. 52 pp. 2574-85). We hypothesized that the FtsB dimer forms a core for the lateral association of FtsL, leading to the the assembly of a higher-order oligomeric FtsB-FtsL complex. Here we present a biophysical analysis performed in vitro that further supports this hypothesis. Using FRET, we have measured the association of fluorophore-labeled transmembrane domains of FtsB and FtsL in both detergent and lipid. Our findings demonstrate that these helices form a very stable higher-order oligomeric complex with a 1:1 FtsB:FtsL stoichiometry in isolation. The data also suggest that the transmembrane component is likely to be a major contributor to the stability of the FtsB-FtsL complex. We also present a design strategy to further elucidate the oligomeric state of this complex using single molecule fluorescence microscopy.

192-Plat

Membrane-Induced Dimerization of Coagulation Factor VIII

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Coagulation Factor VIII (FVIII) is a blood plasma protein expressed as five domains denoted as A1, A2, A3, C1 and C2 that exist as a non-covalently bound heterodimer of a light (A3, C1, C2) and heavy (A1, A2) chain with a combined molecular mass of ~170 kDa. FVIII is activated by thrombin to FVIIIa, which is the cofactor to the serine protease Factor IXa (FIXa) within the membrane-bound Tenase complex that assembles on the activated platelets' surface during the propagation phase of coagulation. FVIIIa is a heterotrimer, due to an additional cleavage between the A1 and A2 domains.

Although the function of FVIII has been well characterized, little is known about its membrane-bound structure and mechanism of the Tenase complex assembly. To fill this knowledge gap, we have engineered negatively charged lipid nanotubes (LNT) that resemble the activated platelet surface and on which FVIII and FVIIIa can be helically organized. The helically organized FVIII-LNT and FVIIIa-LNT were further subjected to structural analysis by Cryo-electron microscopy (Cryo-EM) to elucidate the structural basis of FVIII activation and function. To achieve this, we carried out helical reconstruction with the iterative real space helical reconstruction algorithm (IHRSR). Our results show that membrane-bound FVIII forms dimers that associate tightly through heavy chain - heavy chain interactions. These interactions involve the A1 and A2 domains surface that don't overlap with the A2-A3 interface containing the FVIIIa binding sites to FIXa. We therefore propose that membrane-induced dimerization of FVIII is required to stabilize its membrane-bound conformation and facilitate its interaction with FIXa. The resulting model of a binary Tenase complex would therefore consist of two molecules of FIXa and two molecules of FVIIIa, which fits its function in coagulation: increasing of more than a 100,000 fold the FIXa proteolytic activity.