TonB-binding region of these transporters, called the Ton box, undergoes dramatic structural changes from an immobile to a mobile configuration upon substrate binding. However, this change is not observed in the crystal structures of BtuB. When SDSL is performed with protecting osmolytes such as polyethylene glycols (PEGs), which are contained in the buffers used in crystallization, this order-disorder transition of the Ton box is abolished in BtuB. Here we find that the substrate-dependent Ton box transition in FecA is also modulated by PEGs, and we show that the five residues N-terminal to the Ton box of BtuB are in conformational exchange between folded and unfolded states. Upon addition of PEGs, this equilibrium is shifted towards the folded state. Larger molecular weight PEGs produce a larger apparent shift in the conformational free energy, consistent with the finding that PEGs are excluded from protein surfaces as a result of steric interactions. The observation of conformational exchange in the N-terminus provides an explanation for differences seen between the *in surfo* and the *in meso* crystal structures of BtuB and suggests that each of these structures represents one conformational substate among a family of substates that are normally sampled by the protein. This work illustrates how SDSL and osmolytes may be used to characterize and quantitate conformational equilibria in membrane proteins.

260-Pos

Proximity of the EGF Receptor Kinase Domain To the Plasma Membrane

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Understanding the activation mechanism of the epidermal growth factor receptor (EGFR) is of fundamental importance in anticancer drug development. Crystal structures establish the two kinase domains in the receptor dimer bind to and activate each other by an allosteric mechanism. However, information about the conformation of the juxtamembrane (JM) region and the proximity of kinase domains to the membrane in live cells is lacking. Electrostatic potential calculations suggest that both the positively charged N-terminal JM region and a positive face of the kinase domain could bind to the negatively charged inner leaflet of the plasma membrane. The objective of this report is to investigate the proximity of the kinase domain of EGFR to the plasma membrane. We truncated the EGFR after the kinase domain and genetically tagged it with the monomeric yellow fluorescent protein (mYFP). We measured FRET between a plasma membrane target cyan fluorescent protein (PMT-CFP) and the truncated EGFR- mYFP (tEGFR-mYFP). The observed FRET is consistent with the hypothesis that the kinase domain is located close to the membrane in the absence of ligand. Addition of epidermal growth factor (EGF) produced a decrease in FRET, which indicates the kinase domain may dissociate from the membrane during activation.

261-Pos

Distinct Topologies For the HIV-1 Transmembrane Glycoprotein gp41 C-Terminal Tail on Cellular and Viral Lipid Membranes

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The HIV-1 envelope (Env) transmembrane protein, gp41, is typically considered a type I membrane protein with an extracellular N-terminus, a single membrane-spanning domain, and a C-terminus forming a ~150 residue intracytoplasmic tail. However, published studies indicate an alternative or dynamic topology for portions of the C-terminal tail (CTT) that results in exposure of CTT segments on the membrane surface. To distinguish between these alternative models, we evaluated the accessibility of a reference CTT sequence, the ''Kennedy epitope'' (KE), in viral and cellular membranes to map CTT topology relative to the lipid bilayer. KE accessibility in cell-associated Env was defined by reactivity of native or VSV-G epitope-tagged KE to specific monoclonal antibodies (MAbs), measured by FACS of intact cells. In parallel, KE exposure in virion-associated Env was characterized by MAb binding to intact virions as measured in immunoprecipitation and surface plasmon resonance (SPR) spectroscopy assays. FACS analyses of live cell-associated Env demonstrated significant reactivity of the KE with MAbs to native or VSV-G epitope-tagged KE. No reactivity was observed in cells expressing Env with the VSV-G epitope substituted into a cytoplasmic domain. In contrast to cellular Env, KE in the context of virions failed to react with MAbs directed to the native KE sequence, as measured both by immunoprecipitation and SPR assays. However, MAbs specific for a membrane-proximal sequence bound virions in both assays. Together, the results of these accessibility assays indicate that the KE sequences of gp41 are accessible to antibody binding in cell surface-expressed, but not virion-associated, Env. These observations suggest that the CTT may assume distinct topologies (reflected in KE exposure) that depend on the membrane environment (viral/cellular) and that parts of the CTT may be (transiently) exposed on the membrane surface.

262-Pos

Membrane Topology of Hepatitis C Virus Protein NS4B

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Hepatitis C virus (HCV) protein NS4B is a poorly characterized highly hydrophobic integral membrane protein which is associated with membranes of the ER or an ER-derived modified compartment. NS4B induces the formation of intracellular membrane changes that are visible by electron microscopy, the so called membranous web. This membranous web has been postulated to be the HCV RNA replication complex. Since morphogenesis and budding has been suggested to take place in ER modified membranes, a function of NS4B might be to induce a specific ER membrane alteration that serves as a scaffold for the formation of the HCV replication complex. If that were true, NS4B might have a critical role in the HCV cycle. It has been predicted that NS4B possess four / five transmembrane (TM) domains. Two helical elements have been predicted in the C-terminal part of the protein, pointing out to a yet unknown common function of the C-terminal globular part. Additionally, RNA binding properties through a nucleotide-binding motif have recently been reported, as well as a new membrane association segment in the N-terminal portion of the protein. Many questions remain unanswered about the NS4B topology but due to the highly hydrophobic nature of the protein, a detailed structure determination using experimental techniques will not be obtained in the near future. In order to define the membrane topology of NS4B we have analyzed the spatial distribution of the spatial hydrophobicity of thirty-one sequences of protein NS4B pertaining to different strains representing the seven major genotypes of HCV. Our study shows that NS4B has at least five and possibly six TM domains.

263-Pos

Evolutionary Conservation of Phospholipid-Binding Sites in Membrane Proteins

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Membrane proteins evolved to function in a diverse phospholipid environment. Experimental evidence suggests that some phospholipid molecules are recruited by membrane proteins as co-factors or ligands that tightly bind to specific sites on the protein surface and play important functional roles. In this work we have assessed the evolutionary conservation of phospholipid-binding sites in several alpha-helical and beta-barrel membrane proteins. We first identified the membrane protein surface residues and residues that are in close contact with co-crystallized phospholipids in the x-ray structures of adrenergic receptor, photosynthetic reaction center, Kcsa potassium channel, formate dehydrogenase and ferric hydroxamate uptake receptor using methods of computational geometry such as Delaunay triangulation and alpha shape. We next collected orthologous cDNA sequences for every protein and used posterior probability analysis of evolutionary selection pressure measured as ω-ratio with the aid of PAML package to identify phospholipid-facing residues under strong purifying selection pressure. We show that protein residues interacting with co-crystallized phospholipids are collectively more conserved than the rest of the phospholipid-facing residues with statistically significant p-values in the range 10e-7 - 10e-3. Additionally, we found that every phospolipid-binding site on the membrane protein surface contains from 3 to 5 residues that experience strong purifying selection pressure similar to the functionally important buried residues.

264-Pos

Transmembrane Protein Association in a Biomimetic Medium

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A plethora of membrane proteins studies have been performed on micellar systems containing detergent solubilised proteins. A major drawback of these systems is their rather poor mimicry of biological membranes. Therefore, development of new biologically relevant membrane systems is justified.
We illustrate an original approach combining fluorescence recovery after fringe pattern photobleaching (FRAPP) with the use of an optically isotropic, extremely fluid phase of bilayers (the sponge phase). In this biologically relevant system, well suited for spectroscopic studies, the spacing between two adjacent bilayers and the thickness of the bilayers can be easily and very precisely tuned. So when the membranes are sufficiently separated, the only possible interactions occur between proteins embedded in the same bilayer, whereas when the membranes come together, interactions between proteins embedded in adjacent membranes may also occur.