Membrane-Proximal External Region of the Env glycoprotein can be enhanced via single-site modification of membrane-interacting Ab areas with synthetic aromatic compounds. Potency enhancement in cell-entry inhibition and standard neutralization assays correlated with an increase in affinity for the native antigen in virions and did not compromise neutralization breadth. Thus, we have established an optimization procedure with the potential of improving functionality of Abs that bind immunotherapeutic targets at membrane surfaces.

1183-Pos

Water for Sterol: an Unusual Mechanism of Sterol Egress from a StARkin Domain

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Previously we identified a new family of endoplasmic reticulum membrane proteins that possess sterol-binding StARkin domains. These Lam/GramD1 proteins are implicated in intracellular sterol homeostasis, a function that requires them to be able to bind sterols. Here we show how these proteins exchange sterol molecules with membranes. An aperture at one end of the StARkin domain enables sterol to enter/exit the binding pocket. Strikingly, the wall of the pocket is fractured along its length, exposing bound sterol to solvent. We considered whether hydration of the pocket could mediate sterol entry/exit. Large-scale atomistic molecular dynamics simulations reveal that sterol egress involves widening of the fracture, penetration of water into the cavity and consequent destabilization of the bound sterol. The simulations also identify polar residues along the fracture that are important for sterol release. Their replacement with alanine affects the ability of the StARkin domain to bind sterol, catalyze intervesicular sterol exchange and alleviate the nystatin-sensitivity of *lam2Δ* yeast cells. These data suggest an unprecedented, water-controlled mechanism of sterol acquisition and discharge from a StARkin domain

1184-Pos

Sigma-1 Receptor Remodels Endoplasmic Reticulum Membrane

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Sigma-1 Receptor (S1R) is a single-transmembrane intracellular receptor, primarily residing in the endoplasmic reticulum (ER) membrane. Pharmacological activation of the receptor has been shown to be neuroprotective in a number of brain disorders including Alzheimer's, Huntington's diseases, Amyotrophic lateral sclerosis. It has been proposed that S1R functions as an auxiliary regulatory subunit for various ion channels and receptors, modulating their activity and gating properties. However, the molecular mechanism underlying S1R function in the cell remains unknown. In our study we explored S1R-lipid interactions using a combination of biophysical and biochemical approaches. First, we confirmed that S1R localizes at the specific ER compartment - mitochondria-associated membranes (MAM). Then, we showed that wild-type S1R binds to major raft components such as cholesterol and sphingomyelins. We identified protein motifs responsible for S1R-cholesterol interactions. Intact cholesterol-binding sequence was essential for proper S1R targeting to the MAM regions. Finally, we purified and then reconstructed recombinant S1R in artificial lipid bilayers (supported lipid bilayers and giant unilamellar vesicles). Bilayers of different lipid composition were used to study S1R protein-lipid interactions. We discovered that in the presence of cholesterol S1R forms S1R-enriched microdomains, a function that is modulated by sigma-ligands. We propose that on the molecular level S1R may function as a cholesterol-recruiting protein of the ER. This can explain how S1R can modulate and control numerous physiological pathways and provides a new perspective on ER compartmentalization.

1185-Pos

Modulation of Insulin Receptor Kinase Activity by Lipid Environment

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Membrane receptors involved in signal transduction play a crucial role in cellular communication with its environment. They are embedded in the plasma membrane, which contains a high level of SM and cholesterol. Ordered lipid domains (lipid rafts) can be formed by mixtures of sphingomyelins (SM) and cholesterol, while disordered lipid domains are rich in unsaturated lipids, such as phosphatidylcholines (PC), which are also abundant in the plasma membrane. Previous studies have reported that receptor tyrosine kinases, such as insulin receptor (IR), have differential distribution in lipid rafts and varying activation when cellular cholesterol levels are altered. Therefore, we are studying the effect of lipid properties on IR activity. We previously found that after removal of cholesterol IR activity is fully restored when cellular cholesterol is replaced by raft-supporting sterols, partially restored by an intermediate raft-supporting sterol, and not restored with raft-disrupting sterols. This change in kinase activity could be due to IR association with lipid domains, due to a change in membrane thickness caused by sterols, or due to direct interactions between IR and sterols that involve the surface of sterols important for forming ordered domains. We checked the effect of membrane thickness on purified IR activity (as measured by autophosphorylation) with a series of maltoside detergents with varying alkyl chain lengths, and found some increase in kinase activity with an increase in alkyl chain lengths. Preliminary studies using our method for exchanging plasma membrane outer leaflet phospholipid found little, if any, change of IR autophosphorylation upon substitution of endogenous lipid with SM (which should form rafts) or unsaturated PCs (which should not form rafts). To see if membrane width is important, these experiments will be repeated with shorter acyl chain PC.

1186-Pos

Improved Solubility of Membrane Proteins with zSMA Polymers

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Styrene-maleic acid (SMA) copolymers have been used for solubilization and reconstitution of membrane proteins (MPs) into nanodiscs. These polymer-encased nanodiscs (SMALPs; SMA lipid particles) are a promising platform for studies of MPs in a near-physiologic environment. SMALPs can also be produced starting with synthetic membranes of well-controlled compositions for studies of the regulatory role of lipids on MPs. One drawback of the SMA copolymers is their limited buffer compatibility and flexibility for various applications. We previously demonstrated that in contrast to SMA, our zwitterionic styrene-maleic acid-derivative copolymers (zSMAs) do not aggregate at low pH or in the presence of polyvalent cations, and can be used to solubilize MPs and produce nanodiscs of controlled sizes. Here we present data on solubilization and stabilization capability of different zSMA copolymers. We also produce zSMAPLs by reconstitution of MPs in an active state from specifically formulated proteoliposomes. We expect that the chemical versatility of this new group of copolymers will open new opportunities for applications built on the reconstituted MPs, or involved with drug targeting and delivery. This work was supported in part by NSF grants DMR-1623241 and CBET-1623240.

1187-Pos

Characterizing the Translocation of Charged Peptide Loops across Lipid Bilayers with Molecular Dynamics Simulations

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The hydrophobic core of the cell membrane is considered largely impermeable to charged molecules because of the large free energy barrier and corresponding long timescale (~hours) for charge translocation. Contradicting this view, a variety of water-soluble peptides are known to translocate charged groups across the cell membrane on a surprisingly rapid timescale, on the order of few seconds at least for some peptides. In this work, we study the interconversion of a peptide with charged flanking loops between a surface-adsorbed and membrane-embedded state, which requires the translocation (or ''flipping'') of charged loops across the bilayer. We utilize all-atom Temperature Accelerated Molecular Dynamics (TAMD) simulations to predict the likelihood of loop flipping without predefining a specific translocation pathway. We show that membrane-exposed charged residues accelerate the flipping of charged peptide loops by stabilizing intramembrane water defects. We further demonstrate that this computational approach can identify multiple flipping pathways without specifying them *a priori*. The position of charged residues in the transmembrane helix also affects the flipping pathways and the apparent flipping free energy barrier. These detailed molecular-level insights of peptide translocation pathways may be valuable for designing small cationic peptides for applications in gene and drug delivery. Moreover, the methodology and findings discussed here can generalize to more complex behaviors, such as the large-scale conformational rearrangements of integral membrane proteins.

1188-Pos

Membrane Curvature Effects on Rhodopsin Activation Investigated by Time-Resolved Electronic Spectroscopy

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