Title

Conformational diversity and contraction of the denatured state ensemble of a helical membrane protein

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lumen of the transmembrane β-barrel, must rearrange to form a pore and permit passage of the substrate, which is initially bound at the TBDT's extracellular face. Vitamin B12 transport in *E. coli* is facilitated by the TBDT BtuB. After B12 binds to BtuB, TonB binds to the periplasmic face of BtuB and couples it to the proton motive force of the inner membrane. However, the role of TonB and the rearrangement of the plug domain remain enigmatic with some studies historically focusing on a force-mediated unfolding of the plug domain through TonB, while others recently proposed a force-independent model where TonB breaks a salt bridge (the ''Ionic Lock''). Here we report hydrogen deuterium exchange (HDX) measurements of BtuB embedded in *E. coli* OMs that provide thermodynamic information of the plug domain at the residue level. We find that a region surrounding the Ionic Lock is destabilized 10^2-10^3-fold upon B12 binding, despite being located over 20 Å from the binding site. A further comparison of HDX data on the B12-bound binary and the B12+TonB-bound ternary complexes indicates that the binding of B12 alone is sufficient to unfold the Ionic Lock region, while only minor changes are induced by the addition of a soluble TonB construct. Some of these additional changes occur in the third substrate binding loop, supporting a recently proposed site for pore formation, although pore formation itself does not obviously occur. Our results provide insight into the allosterically-activated intermediate state that can be created by B12 binding, which is likely the first major step on BtuB's transport pathway.

2267-Pos

Building the autophagosome: the interplay between WIPI2 and ATG16L1 at the endoplasmic reticulum

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Autophagy is a conserved catabolic pathway that recycles nutrients for sustaining cellular metabolism. The autophagosome, a double-membrane structure that engulfs its cargo and delivers it to the lysosome for degradation, is the hallmark of autophagy. Autophagosomes evolve from precursor structures initiated at specific subcellular membrane compartments, including the endoplasmic reticulum (ER). Phosphatidylinositol 3-phosphate (PI3P) signaling is responsible for recruiting the autophagy proteins WIPI2 and ATG16L1 at the autophagosome initiation site. The current autophagy model identifies WIPI2 as the PI3P-sensor able that recruits ATG16L1 to promote the growth and closure of the autophagosome. In this study, we determined the mechanism by which the ER acts as a platform for the initiation of autophagosome formation. Using genome editing, we tagged WIPI2 and ATG16L1 with HaloTag for fluorescent labeling. To determine the molecular mechanisms underlying ATG16L1 and WIPI2 localization at the ER, we employed single-molecule live-cell imaging in combination with machine learning superresolution image reconstruction. Surprisingly, ATG16L1 is recruited to the ER independently of WIPI2. Pharmacological inhibition of PI3P signaling reduced both ATG16L1 and WIPI2 residence time on the ER. On the other hand, inhibition of PI-kinases increased lateral mobility of WIPI2 and ATG16L1, without compromising ER tethering. Single-molecule binding assays performed on supported-lipid bilayers confirmed that cell-extracted ATG16L1 has a higher affinity for PI3P, independently of WIPI2. Our results suggest the existence of ER-bound reservoirs of WIPI2 and ATG16L1 that re-localize to initiation sites upon autophagy triggering. By direct observation - both in live cells and model lipid membranes - our approach delivers a high-resolution molecular roadmap to monitor autophagy initiation at the lipid membrane interface.

Posters: Membrane Protein Folding

2268-Pos

Conformational diversity and contraction of the denatured state ensemble of a helical membrane protein

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Defining the denatured state ensemble (DSE) of both soluble and membrane proteins is essential to understanding protein folding, thermodynamics, chaperone action, degradation, translocation, and cell signaling. While many studies have focused on water-soluble proteins, the DSE of membrane proteins is less well characterized. We reconstituted the DSE of a helical-bundle membrane protein GlpG in native-like *E. coli* lipid bilayers using our steric trapping method, which couples spontaneous denaturation of doubly biotinylated GlpG to the binding of two bulky streptavidin molecules. Using limited proteolysis and mass spectrometry, we mapped the flexible regions in the DSE. Using our paramagnetic biotin derivative and double electron-electron resonance spectroscopy, we determined the dimensions of the DSE. Finally, we employed our *Upside* model for MD simulations to generate DSEs including the collapsed and fully expanded DSEs in the bilayer. We find that the DSE is highly dynamic involving the topology changes of transmembrane segments and their unfolding. The DSE is expanded relative to the native state, but only at 55-90% of the fully expanded condition. The degree of expansion depends on the local protein packing and lipid composition. Our result emphasizes that the contraction of the DSE and whether transmembrane helices remain in the bilayer is a function of the relative strength of the protein-protein, protein-lipid and lipid-lipid interactions, which can be influenced by both the chemical and physical properties of the lipid bilayer in combination with those of the transmembrane helices.

2269-Pos

Dissecting the impact of interfacial tryptophans on CLC-ec1 dimerization in membranes

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Experimentally, the CLC-ec1 chloride/proton antiporter forms stable dimers in 2:1 POPE/POPG bilayers with a dissociation constant of 2 subunits per 100 million lipids. Dimerization involves association of two large non-polar interfaces that are driven together, by the energetic gain that results from avoiding a thinned-membrane defect exclusive to the monomeric state. Previous experiments demonstrated that substitutions of tryptophans on the dimerization interface (I201W/I422W) shift the equilibrium to the monomeric state without impacting fold or function. The reason why tryptophans elicit this striking effect has been unclear. Do the tryptophans cause a steric repulsion in the dimer, i.e. acting as warts, or do they form preferential interactions with the membrane in the monomer, i.e. acting as hooks? To investigate this, we carried out all-atom molecular dynamics simulations of wild-type (WT) and I201W/I422W models of CLC-ec1 in POPC lipid bilayers. Monomer and dimer models were simulated for an accumulated time of 30 microseconds, and the trajectories were analyzed to compare changes in membrane structure, non-bonded interactions and lipid dynamics. In the monomeric state, the tryptophans lead to a more pronounced membrane deformation compared to WT. While the I201W/I422W dimer remained associated throughout the simulation, the subunits exhibited increased fluctuations compared to WT. Analysis of the net van der Waals interactions for dimer and monomer suggests that while I201W/I422W stabilizes the dimer due to more favorable protein-protein interactions, greater energetic gains stabilize the monomeric state as a result of improved protein-lipid interactions, supporting the hooks mechanism. Altogether, these studies provide a basis for further analysis of the energetics and mechanism of CLC-ec1 dimerization in membranes.

2270-Pos

Van't Hoff analysis of CLC-ec1 dimerization in *Escherichia coli* lipid membranes

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What are the thermodynamic forces that drive hydrophobic membrane proteins to form stable complexes with other hydrophobic proteins in the greasy lipid solvent? In order to investigate this question, we must have a way of dissecting the changes in thermodynamic properties, i.e. enthalpy and entropy, associated with membrane protein reactions. The CLC-ec1 chloride/proton antiporter forms a homodimer that participates in an association equilibrium with a