We present studies of interactions between proteins of the efflux pump of Pseudomonas aeruginosa: MexB (the rotor of the pump connecting the interior of the bacterium and the periplasm), OprM (a pipe connecting the periplasm to the outside of the bacterium) and perplasmic MexA. The mode of interaction, the size of protein complexes and their stoichiometry were determined. In particular we show that MexA and OprM interact only if they are incorporated in opposite bilayers. The population of this complex reaches its maximum when the bilayers are separated by a distance of about 200 A, which is the thickness of the Pseudomonas aeruginosa periplasm. The stoichiometry of the above complex will be presented as well as results describing Mexa-MexB and MexB-OprM associations.
We will thus demonstrate the versatility of our system, which is well suited to study the associations of membrane proteins in a biologically relevant environment.

265-Pos
Studying Membrane Protein Thermodynamics Using a Steric Trap
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An understanding of the molecular forces that specify a protein's structure is essential for many of the central quests of structural biology. While there has been a larger effort to understand soluble protein folding, we know very little about membrane protein folding energetics. We are developing a novel method to study the forces that stabilize membrane proteins in lipid bilayers, which we termed the 'Steric Trap method.' The steric trap method couples protein unfolding to a measurable binding event by exploiting steric repulsion and the high affinity of the streptavidin/biotin interaction. To do this, we introduce two biotin tags on a target protein that are close in space and employ monovalent streptavidin (mSA) as our steric trap. A single mSA can bind without steric hindrance to the folded protein, but a second mSA can only bind when the protein unfolds due to steric overlap. Thus, the binding affinity of the second streptavidin provides a measure of unfolding free energy because binding is coupled to unfolding. We have developed this method on the water-soluble protein, dihydrofolate reductase (DHFR). When two biotin-labeling sites were rationally designed to be close to one another in space and near the enzyme active site of DHFR, enzymatic activity was reversibly abolished upon incubation with a molar excess of monovalent streptavidin. Incubation with a stabilizing ligand shifted streptavidin binding curves, confirming that the steric trapping can quantitatively detect changes in protein stability. Results on our application of the steric trap method to membrane proteins will be presented. The steric trap method could be a powerful tool for measuring protein association affinities, studying unfolding energetics and investigating membrane protein unfolded states in the context of membrane environments.

266-Pos
Measuring the Thermodynamic Stability of Strong Protein-Protein Interactions in Lipid Bilayers Using a Steric Trap
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Protein-protein interactions within cell membranes play crucial roles in the assembly of membrane proteins and cell signaling. Although thermodynamic analysis of binding affinity is essential for understanding the stability, specificity, and function of membrane proteins, these measurements can be difficult to make for high affinity interactions in lipid bilayers. To address this problem, we have developed a steric trap method, which couples the dissociation of a membrane protein complex to another measurable binding event. The method postulates that a concomitant binding of two bulky monovalent streptavidins (mSA) to a doubly biotinylated protein complex occur only when the protein is dissociated due to the steric hindrance. This leads to an attenuated binding affinity of the second mSA, which is directly correlated to the stability of a target interaction. We tested the method using a glycophorinA transmembrane domain fusion to staphylococcal nuclease (SNGpA), which forms a stable dimer in various lipid environments. Equilibrium binding of mSA to the enzymatically biotinylated SNGpA exhibited two distinctive phases, which corresponds to the tight first mSA binding and the weaker second binding in decyl maltoside (DM) micelles and palmitoyloleoyl phophatidylcholine (POPC) bilayers. The stability of GpA dimer extracted from the second binding event at different micellar concentrations yielded the dissociation constants (K_d) of 10^{-8}~10^{-7} M, which agree well with the previous results. The stability of GpA dimer is enhanced in POPC bilayers by ~4 orders of magnitude at comparable mole fractions. The difference free energies between wild-type and destabilized mutants in both systems correlate with the equilibrium sedimentation data measured in C_8E_5 micelles. Our results suggest that the steric trap method provides a powerful tool to study the strong protein-protein interactions in lipid bilayers.

267-Pos
Monitoring and Optimizing Detergent Concentration For Membrane Protein Crystallization While Following Protein Homogeneity
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Detergent concentration is critical to growing quality membrane protein crystals. Since the optimal detergent concentration lies just below the detergent phase boundary, the starting detergent concentration must be minimized for initial crystallization trials. However, the majority of Pure, Homogenous and Stable targets contain excessive levels of detergent micelles upon concentration using molecular weight cut-off filters. Size Exclusion Chromatography (SEC) and a Tetra Detector Array (refractometer, viscometer, light scattering, and UV detectors; TDA) are now being successfully utilized to monitor and optimize detergent concentration while assaying PDC homogeneity during purification and concentration for crystallization. In doing so, the oligomeric state, size, shape and the detergent:protein ratio of the Protein Detergent Complex (PDC) is measured.
Five different membrane proteins using 3 different detergents (OG, DDM, and FC14) and 4 different methods will be presented where detergent was successfully minimized while maintaining PDC homogeneity. Methods utilized where ultra filtration (centrifugal and high-pressure molecular weight cut-off filters) plus SEC and dialysis, changing detergent isomer, Ni-NTA and ion exchange chromatography.
Detergent micelle SEC retention volume, dn/dc, Rh, IV, mass and behavior on different molecular cut-off filters and formats are all being measured using TDA. As expected, there is a direct correlation of measured excess micelle concentration to crystal phase separation and diffraction quality. Unexpectedly, free micelles in the presence of PDCs tend to be highly retained on cut-off filters which would freely pass a pure detergent micelle system; therefore, when measuring whether a micelle is retained or passed through by specific molecular cut-off filters and formats, it must be measured using a PDC system and not just buffered detergent controls.

268-Pos
A Semi-Quantitative Analysis of Detergent Exchange For Integral Membrane Proteins
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Biophysical and biochemical studies of membrane proteins often require the protein to be analyzed in a detergent different from the one used for purification. Detergent exchanges are often achieved through size exclusion chromatography. Despite the widespread usage of this approach, it is not clear how one can determine the exchange efficiency, and how two different detergents interact during the chromatography process. Here we seek to semi-quantitatively analyze the process of detergent exchange using thin layer chromatography. We choose a bacterial potassium channel KcsA as our model protein, and studied the exchange of this protein in various non-ionic and zwitterionic detergents.

269-Pos
Biochemical Definition of 'harsh' Vs. 'mild' Detergents For Membrane Protein Solubilization
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Selection of a solubilizing detergent for membrane proteins is typically based on its ability to maintain the native structure and/or function of the molecule of interest. Descriptors of detergents as 'harsh' or 'mild' in terms of their propensity to denature membrane protein structures may act as a qualitative guide to this process, but the basis of the variable effects of detergents on native membrane protein folds is not yet fully explained. Previous work by our group suggested that the ability of the 'harsh' detergent sodium dodecylsulfate (SDS) to denature a series of wild-type and mutant model helical membrane-soluble 'hairpin' (helix-loop-helix) proteins depends on their level of detergent binding, as manifested by significant variability in their electrophoretic mobilities on SDS-PAGE, and in circular dichroism (CD) spectra and hydrodynamic radii [Rath, Nadeau et al., *PNAS USA* 106, 1760-65 (2009)]. Here we have extended this work to the characterization of a corresponding library of hairpin proteins in sodium perfluorooctanoate (SPFO), a surfactant considered to be non-denaturing. The 'hairpin' library has been developed from helix-loop-helix constructs from transmembrane segments 3 and 4 from the cystic fibrosis transmembrane conductance regulator (CFTR). We find that SPFO-solubilized 'hairpins' exhibit significantly less variability vs. their SDS-solubilized counterparts in their electrophoretic mobilities, CD spectra, and hydrodynamic radii. In addition, SPFO favours more stable helical structure while binding hairpins