of the transmembrane helix dimer Glycophorin A, the simplest prototype for membrane protein folding. We find that although the native state is a local minimum in the force field used, it is only metastable. After a simple force field modification to improved dimer stability, we have studied the assembly rate and mechanism using transition-path sampling. We find that the overall rate of dimer assembly is comparable to that for similar transmembrane helix dimers, and that assembly proceeds first by formation of a non-native dimer, followed by a transition to the fully native state with formation of the characteristic GXXXG packing motif.

1234-Pos Board B143

Molecular Mechanism of Polypeptide Insertion into Bilayer and Exit

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Using the pH-triggered insertion of the pHLIP (pH Low Insertion Peptide) peptide to enable kinetic analysis, previously we showed that insertion occurs in several steps, with a rapid interfacial helix formation followed by a much slower insertion pathway to give a transmembrane helix. The reverse process of unfolding and peptide exit from the bilayer core, which can be induced by a rapid rise of the pH from acidic to basic, proceeds through different intermediate states. To gain insights into molecule mechanism of membrane-associated folding and unfolding we designed and investigated three single-Trp variants, were tryptophan fluorophores were placed at the positions 6 (W6-pHLIP), 17 (W17-pHLIP) and 30 (W30-pHLIP) of 32-residues pHLIP. Kinetics of membrane insertion and exit of various parts of the polypeptide were investigated in great details. The obtained results allowed us to complete molecular model of pHLIP propagation into bilayer and exit triggered by pH changes.

1235-Pos Board B144

Mechanisms by Which Lipids Shape the Reaction Coordinate of GlpG Protease

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Rhomboid proteases are membrane-embedded enzymes whose catalytic activity depends on the composition of the lipid membrane that surrounds them. This makes rhomboids an excellent model system to dissect mechanisms by which lipids shape the reaction mechanisms of membrane proteins. Extensive all-atom molecular dynamics simulations of the *Escherichia coli* rhomboid, GlpG, indicate that the orientation of GlpG and lipid interactions at the substrate docking site, which are essential for substrate binding, depend on the lipid membrane composition. Different protein conformations can associate with different orientations in the membrane, suggesting that protein orientation is a dynamic parameter that changes during the reaction coordinate. The computations provide a comprehensive view of the molecular picture of GlpG/lipid interactions in different lipid membrane environments. This research was supported in part by funding from the Excellence Initiative of the German Federal and State Governments provided via the Freie Universität Berlin, and by an allocation of computing time from HLRN, the North-German Supercomputing Alliance (bec00076).

1236-Pos Board B145

A Minimal Helical Hairpin Motif Recapitulates Misfolding and Pharmacological Rescue of Cystic Fibrosis Transmembrane Conductance Regulator (CFTR)

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A major challenge in the development of cystic fibrosis (CF) disease mechanism-based therapies is the incomplete understanding of the molecular basis of CFTR misfolding and its drug rescue by pharmacological correctors. Here we use a helical hairpin construct—essentially the simplest *in vitro* model of membrane-protein tertiary contacts—containing transmembrane (TM) helices 3 and 4 of CFTR TM domain 1 and its corresponding CF-phenotypic mutant (V232D in TM4) to recapitulate the misfolding and drug-rescue effects observed for full-length CFTR *in vivo*. Employing a single-molecule Förster resonance energy transfer spectroscopy approach to study hairpin conformations in phosphatidylcholine lipid bilayers, we find that the WT hairpin is well-folded, while the V232D mutant assumes an open hairpin conformation in bilayer thicknesses mimicking the endoplasmic reticulum. Titration of V232D hairpin with the CFTR corrector Lumacaftor (VX-809) reverses hairpin opening to restore a compact state as in the WT. The observed membrane escape of TM4 in the V232D hairpin recapitulates cell-based mutational analyses of full-length CFTR, which suggest that a loss of local nonpolar interactions propagates among several TM domains to produce misfolding of the V232D mutant CFTR. Thus, our findings provide *in vivo*/*in vitro* correlates and molecular-level insights into the structural effects of a disease-causing CFTR mutation and its pharmacological rescue. We anticipate our approach to be a promising tool for studying misfolding and drug rescue of other CFTR mutants and for facilitating the development of mechanism-based therapies of other misfolding-prone helical membrane proteins.

1237-Pos Board B146

Functional Stabilization of Purified Human CFTR by NBD1 Mutations and by Phosphatidylserine

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Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) is a chloride channel of the ABC transporter superfamily. It contains two transmembrane domains that form the ion pore, and two nucleotide binding domains (NBD1 and NBD2) that bind and hydrolyze ATP to gate the channel, regulated by phosphorylation. This complex and nonrobust integral membrane protein is a key target for cystic fibrosis drug development. Here we used a systematic and sequential approach to compare the effect of single and multiple site NBD1 mutations on stability of both the NBD1 domain alone, and on purified full length human CFTR. We demonstrate that mutations that stabilize NBD1 structure, quantified by calorimetry, also improve the structural stability of purified, full-length CFTR in a predictable manner. Moreover, in either the NBD1 domain or in full length CFTR, the effect of single mutations is additive when combined. Combinations of up to six mutations S492P/A534P/I539T/M470V/S495P/R555K yielded a super stabilized CFTR variant (6SS-CFTR) with highly improved structural stability, exhibiting a thermal unfolding >20 ^{o}C higher than wild type CFTR. NBD1 stabilizing mutations also improved biogenesis and cell surface expression in a mammalian expression system. These stabilized proteins all displayed robust ATPase activity, and better retained functional stability after heat treatment. Replica exchange molecular dynamics simulations performed on the most stable CFTR construct suggested better packing of the mutated side chain. Further stabilization of purified CFTR could be achieved specifically with addition of phosphatidylserine, complementing the stabilizing effects of introduced mutations. A practical outcome of these studies is the observation that structurally stabilized CFTR has improved solution properties and maintains enzymatic function, allowing for the purification of implicitly properly folded protein in quantities sufficient for future biophysical and structural studies.

Posters: Transcription

1238-Pos Board B147

Roles of Upstream Promoter DNA in Bacterial Transcription Initiation

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Far-upstream promoter DNA plays multiple roles in bacterial transcription initiation. α-CTD of RNA polymerase (RNAP) and transcription factors regulate initiation rate by binding to this upstream region (1). Even without factor binding, upstream truncations between −100 and −40 greatly reduce the rate of open complex formation at λP_R (2) and lacUV5 (3) promoters. Both closed and open promoter complexes (CC, OC) exhibit strong FRET between cyanine dyes at −100 (upstream) and +14 (downstream) positions of λP_R DNA (4), demonstrating bending and wrapping of upstream DNA around RNAP. These results confirm and extend what was known from real-time footprinting of CC and OC (5) and AFM on OC (6). Here we use stopped-flow fluorescence FRET and PIFE assays to determine the kinetics of bending and wrapping the initial