A signal feature of an ion channel is a conduction pathway that is continuous from the cytoplasm to the extracellular space, through which ions move by electrodiffusion, driven solely by an electrochemical potential gradient. We constructed homology models of CFTR using the prokaryotic ABC transporter, Sav1866, as a template; and employed molecular dynamics (MD) simulations to explore the local conformational landscape. These models make predictions for the structure and properties of the anion-conduction path that can be tested experimentally. Predictions for "pore-lining" side chains can be compared with the results of cysteine scanning. The shape and geometry of the pore can be discerned by constructing a space-filling model (casting) of the pore interior that can be used to predict the cut-off point for the reactivity of substituted cysteines toward channel-permeant and channel-impermeant, thiol-directed probes. Predictions for the electrostatic potential in and around the pore can be compared to expectations for a pathway that selects for anions over cations. Finally, the model can be used to predict possible "binding sites" for pore-blocking molecules such as GlyH-101. Supported by NIH, The Cystic Fibrosis Foundation, The Wellcome Trust and the BBRC.

1964-Symp
Linking the Catalytic Cycle of the Nucleotide Binding Domains to Channel Gating in CFTR
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The chloride channel CFTR is an ATP Binding Cassette (ABC) protein, a member of a family of active transport proteins. Following a mechanism conserved among all ABC proteins, binding of ATP to CFTR's two cytosolic nucleotide binding domains (NBDs) induces formation of a stable intramolecular NBD1/NBD2 dimer with two ATPs occluded at the interface, and ATP hydrolysis disrupts this dimer. While in homologous active transporters dynamic formation/dissociation of the NBD dimer is coupled to flipping between inward- and outward-facing TMD conformations, in CFTR these events are coupled to opening/closure of the chloride permeation pathway, allowing real-time detection of these conformational events: using single-channel patch-clamp recordings we seek to understand the precise *timing* and *direction* of molecular motions associated with each gating step. A limitation for reconstructing *timing* is that conformational transitions that are not associated with pore opening/closure go undetected in our recordings. However, information on these steps is hidden in the distributions of open (burst) and closed (interburst) dwell times. Our studies on the distributions of burst durations identified two kinetically distinct open states (pre- and posthydrolytic), and provided estimates for their life times. The peaked shape of the distribution violates microscopic reversibility and suggests nonequilibrium gating for wild-type CFTR, with pore closure strictly coupled to ATP hydrolysis. This coupling is partially or fully disrupted in various catalytic site mutants. Burst distributions obtained in the presence of ATP analogs, or for NBD1 mutants, suggest a crosstalk between the two ATP binding sites. The *direction* of motions can be studied by detecting gating-associated changes in energetic coupling between select position pairs. Using thermodynamic mutant cycles we have identified a conserved pair of residues within NBD2 which appears to form a molecular switch that signals ATP binding.

1965-Symp
Mutations that Disrupt Formation of Functional CFTR
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Cystic fibrosis (CF) is caused by a loss-of-function of the cystic fibrosis transmembrane conductance regulator (CFTR) channel. Many mutations that cause CF, including the most common disease allele ΔF508, interfere with CFTR function because the mutant protein does not efficiently fold into the native channel structure. Significantly, when these mutant proteins are induced to fold, in experimental systems, some CFTR function is recovered, suggesting an avenue for therapeutic development. Exploiting this opportunity requires detailed knowledge of the basic processes of CFTR folding and the steps altered by the disease-causing mutations. Models of CFTR place the critical F508 residue on the surface of one of the two nucleotide binding domains (NBD1) at a predicted interface with the intracellular loop (ICL4) in the second of two transmembrane domains (TMD2). A variety of biophysical, biochemical, and cell biological studies demonstrate that CFTR folds in a hierarchical manner, with folding of the domains occurring first, during translation and, later, the partially folded domains associating to form the final, functional CFTR structure. Consistent with the location of F508 in the structural models, its deletion interferes with both the folding of NBD1 and with subsequent steps of domain-domain association. The detailed energetics and kinetics of these processes provide insight into the fundamental mechanisms by which integral membrane proteins achieve their native structures and reveal obstacles to and suggest strategies for improving the folding efficiency of the mutant CFTR protein. Supported by NIH-NIDDK, NIH-NIDCR, Reata Pharmaceuticals, CFF.

1966-Symp
Emergent Properties of Proteostasis in CFTR Folding and Misfolding
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The cell exploits both the dynamic and emergent properties of the protein homeostasis or proteostasis program to generate and maintain proteomic profiles in the cytosol, and to support protein trafficking through the exocytic and endocytic pathways in diverse cell, tissue and organismal environments. It is now apparent that folding diseases such as cystic fibrosis (CF) are a consequence of mutations that compromise the kinetics and/or stability of protein folding pathways mediated by the proteostasis network (PN). Mutations challenge the synthesis and maintenance of the cystic fibrosis transmembrane conductance regulator (CFTR) that functions as a chloride channel at the cell surface. Mutations triggering CF compromise tissue (intestine-pancreas-lung) function during development and in response to aging. An important goal is to understand the composition, protein-protein interactions and signaling mechanisms utilized by the PN to promote wild-type CFTR function, how they are altered in CF and how they can be targeted by small molecule pharmacologic chaperones/proteostasis regulators to restore function. The inherent capacitance and evolvability of the proteostasis program to deal with folding stress highlights the potential of using the emergent properties of the PN to significantly impact the problem of protein misfolding in inherited disease.

Platform AR: Membrane Physical Chemistry

1967-Plat
Thermodynamic Equivalence of Hydration and Osmotic Stress in Membrane Deformation
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Membrane deformation and tension potentially affect the conformational energetics of membrane proteins such as rhodopsin though non-specific lipid-protein interactions [1]. The question arises how membrane deformation can alter these protein-lipid interactions and thus affect membrane protein function. Through usage of osmolytes and dehydration we observe deformation in DMPC-d_{54} membranes via solid-state ^{2}H NMR [2]. Measured order parameters allow deformations to be accessed at the molecular level. Stresses from dehydration and osmotic pressure are thermodynamically equivalent because the change in chemical potential when transferring water from the interlamellar space to the bulk water phase corresponds to an induced pressure. Due to equivalence of the two stresses, we directly relate membrane hydration to an applied osmotic pressure via the order parameters. A unified theoretical framework predicts an equation of state for the membrane system that depends inversely on the number waters per lipid as confirmed by experimental data [2]. We extend this thermodynamic framework via a mean-torque model [3] to analyze the compressibilities of the lipid and water components. Increases in osmotic pressure and dehydration reduce the compressibility of the water space so that greater work is required to remove water from the membranes at low hydration. The cross-sectional area per lipid decreases and thickness increases non-linearly with an increase of osmotic pressure and dehydration. Changes in membrane thickness can result in hydrophobic mismatch which directly affects protein-lipid interactions and thereby affect membrane protein function. Our findings demonstrate the ability to change membrane structure in a controlled manner for the investigation of pressure and hydration sensitivity of membrane proteins.
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