ATP flux. Therefore, we determined the conduction state of the channel with regard to the ATP permeation. To understand ATP permeation through VDAC, we solved the structure of murine VDAC1 (mVDAC1) in the presence of ATP revealing a low-occupancy binding site. Guided by these coordinates, we initiated hundreds of molecular dynamics (MD) simulations to construct a Markov State Model (MSM) of ATP permeation using the software (Beauchamp et al. JCTC 2011). These simulations show a high ATP flux generated from multiple pathways through the channel, consistent with our structural data and previously reported physiological permeation rates.

751-Pos  Board B506
Genomics-Aided Structural Modeling of an Antiparallel Homodimeric Fluoride Channel
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Fluoride Channel (FLCs) are small membrane proteins widespread in bacteria, single-celled eukaryotes, and plants. Only recently characterized, FLCs act as fluoride-specific ion channels, forming antiparallel dimers of four-helix transmembrane bundles. Little else is known about this protein family. To gain insight into the structure and function of the FLCs, we use direct-coupling analysis (DCA) and ab initio molecular modeling to generate all-atom models of the E. coli Fluc homodimer EC2. DCA uses multiple sequence alignments to infer the interdependencies between residue positions in protein families and is robust at predicting protein contacts from sequence alone. Taking into account simple geometric considerations and strong experimental evidence for an antiparallel homodimer, we are able to parse the inter- and intra-monomeric contacts predicted by DCA. These contacts are used to bias a conformational search performed by a custom Rosetta fold-and-dock protocol. Final refined models are further relaxed with all-atom molecular dynamics simulations in an explicit membrane environment. Possible mechanisms for fluoride selectivity and permeation are discussed in light of the model. This study demonstrates the utility of DCA in the ab initio modeling of oligomers, suggests a novel sequence-based approach to identify dual-topology proteins, and provides a strong foundation for more directed experimental characterizations of the Fluc family proteins.

752-Pos  Board B507
Mouse CFTR Exhibits Multiple Characteristic Differences from Human CFTR
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Currently available murine CF models have failed to replicate CF-like spontaneous obstructive lung disease until challenged with bacteria or LPS. It has been reported that the delF508-mCFTR mutant does not exhibit mistrafficking behavior typical of the human CFTR, and that mCFTR single channel conductance is distinctly different from hCFTR. We have investigated and compared the differences between mCFTR and hCFTR will provide a tool in identifying and previously reported physiological permeation rates.

754-Pos  Board B509
Interaction of the Isolated Nucleotide Binding Domains of CFTR Channels
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The cystic fibrosis transmembrane conductance regulator (CFTR) is a chloride channel that belongs to the ATP binding cassette (ABC) superfamily. Defective function of CFTR is responsible for cystic fibrosis, a lethal genetic disease manifesting as defective chloride transport across the epithelial surfaces of various tissues. The structure of CFTR comprises two transmembrane domains (TMDs) that form the channel pore, two intracellular nucleotide-binding domains (NBDs) and a unique regulatory domain. The opening of CFTR channels is coupled to ATP binding to the NBDs and subsequent NBD dimerization. ATP hydrolysis leads to dimer separation and channel closure. In the past few years tremendous progress has been made in the characterization of CFTR gating, but the conformational changes behind the gating transitions observed in these functional studies can only be inferred based mostly on the crystal structures available from a few ABC transporters. Dynamic structural information governing the mechanisms behind CFTR function at the molecular level is still lacking. Recent biochemical breakthroughs in purifying CFTR now make it possible to address some of the outstanding questions in the CFTR field.

Our goal is to investigate the NBD dimer formation and separation (dimerization dynamics) using the state-of-the-art single-molecule Förster resonance energy transfer (smFRET) technique for WT and mutant CFTR channels. Here we show our progress so far in this work, namely the characterization of the purified NBDs: the apparent binding affinity, hydrolysis competence and FRET data demonstrating the association of two isolated domains in the presence of ATP. The purpose of this study is to address fundamental questions about the molecular mechanisms behind the function of CFTR channels, and when completed, will no doubt enhance our understanding of the relationship between structure, dynamics and function.

755-Pos  Board B510
Non-Equilibrium Gating of CFTR Revealed by Nitrate as Charge Carriers
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CFTR, a member of the ATP-binding cassette protein superfamily, is a phosphorylation-activated but ATP-gated chloride channel. Like other anion channels, CFTR’s pore is permeable to a wide variety of anions, including bromide, nitrate, iodide and bicarbonate. From the shift of reversal potentials under bi-ionic conditions for chloride, bromide and nitrate, we obtained a permeability sequence: NO3-> Br-> Cl-, a result consistent with previous reports, but the macroscopic conductance sequence, NO3-> Br-> Cl-, contradicts previously published Cl-> NO3-> Br-. Nonetheless, single-channel studies reveal a conductance sequence of Cl-> NO3-> Br-, suggesting bromide and nitrate may affect CFTR gating. By analyzing single channel kinetics, we found that NO3-> I- indeed increases the open probability (0.71 ± 0.01 versus 0.51 ± 0.02 with Cl-) by increasing the opening rate and prolonging the open time. Interestingly, when examining recordings from patches containing one single channel in nitrate-based bath, we observed two distinct open-channel conductance levels (the smaller O1 state and the larger O2 state), a phenomenon similar to the effect of the R352C mutation on CFTR. Furthermore, statistical analysis of the pattern of gating transitions also reveals a prevalent