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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'Flucs' are small membrane proteins widespread in bacteria, single-celled eukaryotes, and plants. Only recently characterized, Flucs 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 Flucs, 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 large 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 protein family.

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 as seen in delF508-hCFTR, and that mCFTR single channel behavior is distinctly different from hCFTR. We have investigated and compared the channel behavior of mCFTR and hCFTR expressed in Xenopus oocytes using electrophysiology combined with mutagenesis. The results are: (1) GlyH-101 blocked hCFTR with Kd 4.9 μM while GlyH-101 blocked mCFTR with Kd 32 μM at VM=-60 mV. GlyH-101 failed to block R334A and R334C in both hCFTR and mCFTR. (2) GlyH-101 activated mCFTR but not hCFTR in a concentration-dependent manner with Kd 103 μM. GlyH-101 only exhibited the activation effect on R334A-mCFTR. (3) 10 μM VX-770 transiently increased hCFTR ~30%, while activated mCFTR in a time-dependent manner to > 2 fold. (4) Glibenclamide (Glyb) only inhibited hCFTR (Kd 22 μM at Vm = −120 mV) but also inhibited mCFTR (Kd 18 μM at Vm = −120 mV) and activated mCFTR (Kd 5 μM at Vm = 100 mV). Similar phenomena were seen in both whole cell (TEVC) and inside-out macropatch configuration. (5) Whereas human CFTR opens to the full open state (f) over 98% of the open time, mCFTR occupies the f state 60% of the open time, with the rest spend in the subconductance 1 (s1) or subconductance 2 (s2) at Vm= −100mV. Conclusion: (1) mCFTR channel behavior differs from hCFTR in multiple respects. (2) The differences between mCFTR and hCFTR will provide a tool in identifying the binding sites and mechanism of VX-770 and GlyH-101. (NIH R01-DK 056481).

753-Pos Board B508

Probing Structure and Conformational Changes in the Extracellular Loops of CFTR

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CFTR is a member of the ABC transporter family, and as such bears closer homology to transporters than to other channels. However, it is unknown whether, like transporters, the transmembrane domains of CFTR undergo an inward-to-outward facing transition upon NBD dimerization. To test this, we made cysteine substitutions in the extracellular loops (ECL) 1 and 4, which appose each other in inward-facing but not outward-facing structures of ABC transporters. We found that the macroscopic current of D110C/K892C-CFTR channels (but not corresponding single mutants) was increased in the presence of DTT. Multichannel patch recordings revealed that DTT increased NP_O of double cysteine mutant channels, indicating that they were locked into a closed state by a spontaneous disulfide bond prior to DTT. To further understand the kinetics of the interaction, we applied Cd^{2+} trapping and found that D110C alone is transiently bound by Cd^{2+}, causing a rapidly reversible 33 +/- 4.6% inhibition of current. By contrast, Cd^{2+} inhibited D110C/K892C-CFTR irreversibly and to a greater extent (72.5 +/-6.8%) under identical cumulative exposure. Results were consistent in 2 mM, 20 uM and 200 nM Cd^{2+}. K892C was reversibly modified to a small extent (1.5 +/- 0.9%) and E115C (near the end of ECL1) was not functionally modified by Cd^{2+}, but was labeled by MTS-TAMRA, indicating accessibility. The data suggest: 1) ECL1 and ECL4 come into close proximity in a closed state of CFTR; 2) K892 is located away from the conductance pathway of open CFTR; and 3) ECL1 is oriented such that D110 is closer to the pore than E115. Experiments are currently investigating the single channel effect of Cd^{2+} on ECL mutants and state-dependent kinetics of Cd^{2+} coordination by D110C/K892C and other ECL pairs. (NIH R01-DK 056481).

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 manifested in defective chloride transport across the epithelial cells in 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 biding affinity, hydrolysis competence and FRET data demonstrating the association of the 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- 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

transition of C→O1→O2→ C (46.3%) over C→O2→O1→ C (< 5%), a telltale sign for a violation of detailed balance and hence demanding an input of free energy to drive the gating transition in a preferred direction. In addition, a considerable fraction of openings contain more than one O1→O2 transition (38%), supporting the idea that more than one ATP molecule is hydrolyzed within an opening burst. Overall our studies indicate that nitrate, as a charge carrier, can be a new tool to probe CFTR's non-equilibrium gating cycle.

756-Pos Board B511

Chloride Transport Inhibition Causes Calcium-Dependent Arrhythmic Activity in Isoproterenol-Treated Rabbit Cardiomyocytes

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During ß-adrenergic stimulation, chloride outward current mediated by CFTR has been proposed to aid repolarization and shorten action potential duration. With sustained stimulation and absent a Cl- extrusion mechanism, CFTR-mediated outward current may result in intracellular Cl- accumulation and collapse of the Cl- electrochemical gradient, leading to arrhythmias. Recently, we identified robust expression of an electroneutral K-Cl cotransporter (KCC) in vertebrate cardiomyocytes and have proposed that it plays a crucial role in Cl- homeostasis by countering channel-mediated Cl- accumulation during ß-adrenergic stimulation. We tested the hypothesis that both CFTR and KCC activity are critical during ß-adrenergic stimulation in paced (1Hz) acutely isolated adult rabbit cardiomyocytes. Application of novel inhibitors of either CFTR (10μM CFTR Inh-172) or KCC (2μM 11k) did not appreciably alter the regular Ca transients during steady state pacing in rabbit cardiomyocytes. Addition of 300nM isoproterenol increased Ca transient amplitude and accelerated [Ca]i decline (as expected). However, in this state, the application of either CFTR or KCC inhibitor induced prominent aftercontractions, indicative of cellular Ca overload and arrhythmogenic activity. We hypothesized that these two inhibitors elicit arrhythmic activity via distinct mechanisms: CFTR inhibition may acutely prolong action potentials directly contributing to Ca loading (independent of altered [Cl-]i), whereas KCC inhibition might allow CFTR current to dissipate the [Cl-]o/[Cl-]i gradient and thus indirectly reduce CFTR-mediated outward current (by reduced driving force). We are currently testing this working hypothesis using a novel ratiometric fluorescent protein-based Cl- sensor.

757-Pos Board B512

Anion Permeation through Excitatory Amino Acid Transporters

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Glutamatergic synaptic transmission critically depends on excitatory amino acid transporters (EAATs) that remove released neurotransmitters from the synaptic cleft and thereby ensure low extracellular glutamate concentrations in the central nervous system. EAATs are thermodynamically coupled glutamate/Na^+/H^+/K^+ transporters and anion-selective channels. EAAT anion channels control neuronal excitability and synaptic communication, and their physiological importance is further corroborated by the recently identified association of altered EAAT anion conduction with neurological disorders. The five mammalian EAATs differ in their effectiveness as glutamate transporters and anion channels. However, pore properties of the known isoforms such as anion selectivity and unitary current amplitudes appear to be closely similar. Although important structural information on secondary-active glutamate transport has been resolved in recent years, the molecular mechanisms underlying anion permeation are still unknown. We here performed molecular dynamics (MD) simulations of the prokaryotic EAAT homologue GltPh to elucidate how these transporters conduct anions. Our results are validated by fluorescence quenching experiments on single-tryptophane mutants of GltPh and patch-clamp recordings of mammalian EAATs. Whereas outward- and inward-facing conformations of GltPh were found to be non-conductive in MD simulations, a voltage-dependent lateral movement of the mobile glutamate transport domain from an intermediate conformation led to the opening of an anion-selective conduction pathway. Amino acid substitutions of homologous pore-forming residues have similar effects on experimental EAAT2/EAAT4 and simulated GltPh single-channel conductances and anion/cation selectivities. Thus, the here identified anion conduction pathway appears to be conserved within the whole glutamate transporter family. Our results highlight how the glutamate transporter family accommodates an anion channel together with a transporter in one single protein.

758-Pos Board B513

Investigating the Structure-Function Relationship of the Phosphate-Selective Channel OprP

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The outer membrane porin OprP of Pseudomonas aeruginosa is a highly phosphate-selective channel. It is induced under the condition of phosphate starvation and facilitates the high-affinity uptake of phosphate ions across the outer membrane of bacteria [1]. An investigation of the structure-function relationship of OprP is required to understand the anion and phosphate selectivity of this porin in particular and to expand the present understanding of ion selectivity of different channels in general. To this end, we investigated the wild-type OprP and several important mutants of OprP to decode the phosphate selectivity of the channel [2, 3]. Mutants helped to probe the individual contribution of important residues toward the selectivity of OprP. Both electrophysiological bilayer measurements and free-energy molecular dynamics (MD) simulations were carried out to monitor the change in ion selectivity and phosphate binding affinity of various mutants compared to wild-type OprP. Results obtained from MD simulations were in qualitative agreement with experiments and complemented experimental observations by providing atomistic details regarding function and dynamics of OprP. Molecular details learned from such studies could be exploited to engineer the channel for various applications [4, 5].

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759-Pos Board B514

Description of the Structural Determinants of the hPepT1-Ligand Interactions

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Membrane transporters are responsible of the transport of solutes into the cell and play a key role in pharmacokinetics of prescription drugs. hPepT1 belongs to the Solute Carrier 15 gene family (SLC15) and transports peptides and peptidomimetic drugs (e.g., β-lactam antibiotics) across the cell membrane. Mutations in hPepT1 are associated with various disease (e.g., pancreatic cancer) and differential drug response among individuals.The study presented here describes the interactions of this transporter with its ligands using computational methods.We have first built by homology modeling the distinct conformations involved in the secondary active transport mechanism using prokaryotic transporters templates. The models have then been used for the docking of known ligands and for developing rules for their binding and transport specificities.

760-Pos Board B515

Dynamics of Ca^{2+}-Dependent Regulation of the Cardiac Na^+/Ca^{2+} Exchanger

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The Na^+/Ca^{2+} exchanger (NCX) is an important ion transport mechanism for the movement of Ca^{2+} into and out of cardiac myocytes. Allosteric regulation of NCX has been intensively studied in excised giant patches under steady-state conditions and also in whole cell systems where only Ca^{2+} dynamics was examined. However, it has been difficult to distinguish between the role(s) of Ca^{2+} as an allosteric regulator and Ca^{2+} as a transported ion. Additionally, there are parallel complex regulatory elements that control spatially resolved $[Ca^{2+}]_i$ within cardiac myocytes. In this study, we compared the dynamic changes of I_{NCX} and $[Ca^{2+}]_i$ in non-transfected HEK293T cells, in cells expressing canine wild-type NCX, and in cells expressing the constitutively