with thirty-five deubiquitylase enzymes, we identified Usp8 as the specific DUB involved in deubiquitylation of the endocytosed Kc a3.1. This result was confirmed in vivo, by measuring membrane Kc a3.1 ubiquitination and degradation rate in the presence of the wild type or catalytically inactive mutant of Usp8. Thus, overexpression of wild type Usp8 accelerates channel deubiquitylation, while the mutant Usp8 strongly enhanced accumulation of ubiquitinated Kc a3.1. Interestingly, in both cases the rate of channel degradation was delayed. In conclusion, we demonstrate that poly-ubiquitylation mediates the targeting of membrane Kc a3.1 to the lysosomes and also that Usp8 regulates the rate of Kc a3.1 degradation by deubiquitylating Kc a3.1 before delivery to lysosomes.

Anion Channels

1437-Pos Board B347
Binding of GlyH-101 in the Pore of the CFTR Chloride Channel
GlyH-101 is a small molecule (MW: 493) that carries a single negative charge (pKa: 5.5) under physiological conditions (~ pH 7.4) and blocks the cystic fibrosis transmembrane conductance regulator (CFTR) chloride channel by entering from the extracellular side and binding to a site or sites within the pore (Muanprasat et al., J. Gen. Physiol. 124: 125-137). However, the precise binding sites for this molecule have yet to be identified. We used virtual ligand docking software, “Glide” (Schrodinger Inc.) to identify potential GlyH-101 binding sites within molecular models of CFTR derived by means of molecular dynamics simulation from a homology model based on Sav 1866 (Alexander et al., Biochemistry 48: 10078-10088). These sites were evaluated by determining the extent of occlusion of reactive cysteines by the blocker. The results suggest that the binding of GlyH-101 near a narrow portion of the pore could reduce the reactivity of T338C with [Au(CN)2]- and MTSES- determining the extent of occlusion of reactive cysteines by the blocker.

1438-Pos Board B348
A hidden Intermediate State for Priming ATP-Hydrolysis During Open Conformation in CFTR Channels
Yoshiro Sohma, Ying-Chu Yu, Masato Yasui, Tzyh-Chang Hwang.
Cystic Fibrosis Transmembrane conductance Regulator (CFTR) anion channel, a member of ABC transporter superfamily, gates following ATP-dependent conformational changes of the nucleotide binding domains (NBDs). Reflecting the hundreds of milliseconds duration of the channel open state corresponding to the dimerization of two NBDs, macroscopic WT-CFTR currents usually showed a fast, single exponential relaxation upon removal of cytoplasmic ATP. Mutations of tyrosine1219, a residue critical for ATP binding in second NBD (NBD2), induced a significant slow phase in the current relaxation, suggesting that weakening ATP binding affinity at NBD2 increases the probability of the stable open state. The slow phase was effectively diminished by a higher affinity ATP analogue. These data suggest that a stable binding of ATP to NBD2 is required for normal CFTR gating cycle and the instability of ATP binding frequently halts the gating cycle in the open state presumably through a failure of ATP hydrolysis at NBD2. In addition, two distinct components of the current relaxation suggests the existence of a hidden intermediate state of CFTR chloride channel which success or failure of ATP-hydrolysis is destined during open conformation.

1439-Pos Board B349
Energetic Coupling at the Nucleotide-Binding Domain/Transmembrane Domain Interface of CFTR
Daniella R. Mualem, Sabine Kobayter, Arun Kelay, László Csanády, Paola Vergani.
CFTR belongs to the superfamily of ABC transporters, which generally couple vectorial transport of diverse compounds across membranes to hydrolitic cy- cles at conserved nucleotide-binding domains (NBDs). CFTR, alone, functions as an ion channel. As in other ABC proteins, ATP binds on the surface of the two NBDs, which then can form a head-to-tail NBD dimer, with two composite catalytic sites at the interface (site 1 and site 2). ATP hydrolysis at site 2 triggers dimer dissociation. The conformational signals generated by NBD dimer formation and dissociation are transmitted to the transmembrane domains (TMDs) where they result in opening and closing, respectively, of the ion-permeation pathway. To investigate the coupling mechanism linking the NBDs to the channel gates, we selected target sites for mutagenesis by analysing multiple sequence alignments. Two pairs of sites gave high correlation scores and mapped close (on homology models) at the domain-swapped interface linking active composite site 2 to intracellular loop 4: E543-E1046 and P499-T1057. The formalism of double mutant cycles was applied to test for interaction between coevolving side chains. Single mutations E543A and E1046A altered opening rate and burst duration, but the changes were additive, suggesting there is no change in energetic coupling between these target sites during hydrolytic gating. However, for mutations at P499 and T1057, changes in opening rates in hydrolytic background and open probability in non-hydrolytic (K1250R) background were less than additive, consistent with P499 and T1057 moving closer during channel opening (forming a contact in the transition state for opening which is maintained in the open state). The results are consistent with homology modelling studies suggesting that channel opening may be preceded by a sliding movement of the NBDs coupled to a twisting of the TMDs (Cell Mol Life Sci 66:3469-3486).

1440-Pos Board B350
Electrostatic Basis of Anion Over Cation Selectivity in the CFTR Chloride Channel
Oliver Beekstein, Yohei Norimatsu, Christopher Alexander, David C. Dawson, Mark S. Sansom.
The cystic fibrosis transmembrane conductance regulator (CFTR) is an ATP-gated, anion-selective channel that is the product of the gene mutated in the inherited disease, cystic fibrosis. Chloride conductance via CFTR is a key component of the salt and water secretory system that maintains mucous hydration in the airway lumen. CFTR belongs to the large family of ABC transporter proteins, but it is the only member known to function as an ion channel. The channel is highly selective for anions over cations, but the structural basis for the observed charge selectivity is unknown. Individual point mutations generally do not seem to strongly affect charge selectivity, suggesting the possibility of a mechanism that is not highly dependent on local structural specialization such as that seen in the K-channel selectivity filter. We used a recent structural model of CFTR, based on the crystal structure of the Sav1866 ABC transporter and experimentally validated by cysteine scanning [1], to begin a computational investigation of the structural basis of the selectivity of the CFTR channel for anions over cations. Poisson-Boltzmann calculations suggest that an excess of basic residues in the transmembrane domain creates a large, anion-stabilizing region that includes the channel pore and extends towards the extracellular vestibule. The results indicate that electrostatic interactions affecting a large volume of the channel are a major contributor to anion over cation selectivity of the CFTR channel, similar to the situation observed in OpnP [2].


1441-Pos Board B351
Studies on Correlated Pairs at the NBD-NBD and NBD-TMD Interfaces in CFTR
Andras Szollosi, Daniella R. Mualem, Paola Vergani, László Csanády.
CFTR is an ATP-binding cassette ( ABC)-protein, which contains two Nucleo- tide Binding Domains (NBDs) and two Transmembrane Domains (TMDs), but is unique among these proteins in that ATP binding, formation of a tight NBD-dimer (sandwiching two ATPs at two composite sites) and hydrolysis of one nucleotide drives opening/closure of a transmembrane chloride channel pore. We investigated interactions between residues at the NBD1-NBD2 interface of the catalytically inactive site 1, and the NBD-TMD boundary. Target pairs suggested by coevolution analysis were tested experimentally using mutant cycles. At the NBD1-NBD2 interface we studied pairs T460 (NBD1 Walker A)-H1348 (NBD2 signature sequence), and T460-H1375 (NBD2 D-loop). Mutation T460S decreased burst duration (t_b) and open probability (P_o), mutation H1348A markedly prolonged t_b while slightly reducing P_o. But mutant cycles built on t_b, maximal opening rates, as well as P_o and closing rates in non-hydrolytic (K1250R) background have so far not revealed significant changes in coupling between either positions 460-1348 or positions 460-1375 during various steps of the gating cycle. Hence we conclude that site 1 does...