have been observed, leading to the view that the antipore cycle only involves local conformational changes near the chloride binding sites. This view has been challenged recently (Elvington et al., 2009), in which a residue up to 25 Å away from the chloride binding sites was shown to undergo substrate-induced, antiport-dependent conformational changes. To gain further understanding of this conformational change, we are using specific 13C-methyl labeling to facilitate the study of the CIC-ecl structure and dynamics by NMR. Use of a monomeric CIC-ecl (Robertson et al., 2010) greatly improves spectral quality of this 50-kD (per subunit) membrane protein. Heteronuclear single quantum correlation (HSQC) spectra reveal substrate-dependent spectral changes that may correspond to functionally relevant conformational change. Resonance assignment is in progress.

2654-Pos Board B424
Lysine 210 Regulates Voltage-Dependent Gating of the Anion-Proton Exchanger CIC-5
Matthias Grieschat, Christoph Fahlke, Alexi K. Aleksov. Institute for Neurophysiology, Hannover Medical School, Hannover, Germany.

The CIC-type Cl-/H+ exchanger CIC-5 displays complex voltage-dependent gating that regulates the number of active transporters and is modified by the concentration of internal and external transport substrates. So far, the molecular basis of CIC-exchanger gating is insufficiently understood. One residue, a conserved amino acid facing the anion permeation pathway at the external side of the protein, is known to be a crucial determinant of this process. Adjacent to this so-called gating glutamate (E211), there is a positive amino acid (K210) that is also highly conserved among the CIC family. Studies of the corresponding residue in CIC-1 (Fahlke et al. (1997) Nature 390, 529-532) demonstrated the importance of this residue for anion selectivity. Because of the well-established connection between anion permeation and voltage-dependent gating in CIC proteins, this residue might also shape the voltage dependence of CIC-5. To test this hypothesis, we introduced the point mutation K210C in the non-conducting E268Q CIC-5. Because the gating process, observed at positive voltages is associated with prominent gating currents, we assessed the effects of K210C by measuring the voltage dependence of the nonlinear capacitances of CIC-5. The charge neutralization K210C shifted the voltage dependence of the bell shaped capacitance curve of CIC-5 by ~25 mV to the left, which indicates that this residue is part of the voltage sensing machinery of the transporter. Surprisingly, the modification of K210C with the thiol-reactive reagent MTSET that restores the positive charge at this position induced additional 40 mV towards hyperpolarized potentials. Therefore, not only the positive charge of the lysine K210 but also the size and the pk of its side chain contribute to shaping the voltage dependence of CIC-5.

2655-Pos Board B425
Rate Constants for Anion Transport by Steroid-Based Synthetic Anion Transporters
Hongyu Li, Germinal Magro, Luke W. Judd, Peter R. Brotherhood, David N. Sheppard, Anthony P. Davis. University of Bristol, Bristol, United Kingdom.

Cholapods are steroid-based synthetic anion transporters that bind anions with high affinity and promote their efflux from liposomes. To better understand the mechanism of action of cholapods, we evaluated the rate constants for anion transport by the cholapods AS09, LJ09 and TL145 using the method developed by Lauger (Science. 1972; 178:24-30) to study valinomycin-mediated cation translocation. Membranes were formed by painting a mixture of POPE/Cholesterol (7/3) plus cholapod at a ratio of 250:1 and bathed in symmetric anion-containing solutions (10 - 200 mM). Voltage steps from 100 mV to +200 mV lasting 5 seconds were applied to membranes. For Cl- transport, all cholapods had similar rate constants to those for Cl- transport with the exception of TL145, in which a significantly smaller value of KAS was observed. This result might explain the poor performance of TL145 in flux assays used to observe Cl-/NO3- exchange. In conclusion, our studies demonstrate that movement of the anion-cholapod complex across the lipid membrane is the rate-limiting step for cholapod-mediated anion transport. This knowledge will guide the development of more efficacious synthetic anion transporters. [Supported by the EPSRC].

2656-Pos Board B426
NMR Structure of a Double-Transmembrane Fragment from NHE1
Brian L. Lee, Claudia Alves, Larry Fliegel, Brian D. Sykes. University of Alberta, Edmonton, AB, Canada.

The Na+/H+ exchanger isoform 1 (NHE1) is an integral membrane protein important in the regulation of intracellular pH in the heart. NHE1 contains an N-terminal transmembrane domain that exchanges one intracellular H+ for one extracellular Na+ and a C-terminal cytoplasmic tail involved in regulation. Its membrane domain is predicted to contain 12-14 transmembrane helices, however no other detailed structural information is currently available. Our labs have previously used solution-state NMR spectroscopy to determine structures of individual transmembrane helices of NHE1 in either detergent micelles or organic solvents and have found them to contain non-helical regions which correspond to functionally important regions in the protein as determined by mutagenesis studies. To gain additional insights into the structure and function of NHE1 we are investigating the structure of a construct such as a double-transmembrane helix fragment of NHE1 containing TM 6-7 (amino acids 226-274). We have been able to produce unlabelled and 15N labelled peptide using a maltose binding protein fusion construct. We found the peptide has favourable spectral properties for solution-state NMR structure determination in detergent micelles. We will characterize the structure of the peptide in detergent micelles using solution-state NMR and in magnetically aligned bicelles using solid-state NMR experiments.

2657-Pos Board B427
Structure and Function of a Bacterial Homolog of the SLC10 Family Bile Acid Transporters
Xiaoming Zhou1, Elena J. Levin1, Matthias Quick1, Yaping Pan1, Hua Huang2, Ruchika Sharma1, James Love3, Ming Zhou1. 1Columbia University, New York, NY, USA, 2New York Structural Biology Center, New York, NY, USA.

The SLC10 family bile acid transporters (BATs) in mammals play critical roles in driving enterohepatic circulation and maintaining bile salt and cholesterol homeostasis by transporting bile acids into cells. For example, the uptake of bile acids from the portal blood into the liver is mediated primarily by the Na+/taurocholate cotransporting polypeptide (NTCP; SLC10A1), whereas in the ileum the apical sodium-dependent bile acid transporter (ASBT; SLC10A2) takes up bile acids from the lumen into the epithelial cells. To understand better the transporting mechanism of BATs, we have crystallized and solved the structure of a bacterial homolog of BAT to a resolution of 3.0 Å. The bacterial BAT shares 54% sequence similarity with NTCP and 60% similarity with human ASBT, and many of the NTCP and ASBT residues known to be essential for function are conserved in the bacterial BAT. The overall structural fold of the bacterial BAT unexpectedly resembles that of the Na+/K+ exchanger isoform 1 (NHE1), although the bacterial BAT is likely a Na+-dependent acid symporter and the two do not have significant sequence similarity. In combination with functional assays to monitor binding and transport of bile acids by the bacterial BAT, the structure suggests possible mechanisms for substrate selectivity and translocation.

2658-Pos Board B428
Unraveling the Structural Organization of Potassium Uptake System KtrAB
Ricardo Pires, João Morais-Cabral. IBMC, Porto, Portugal.

Potassium ion flux across cellular membranes is mediated by a variety of protein channels and transporters. While ion channels provide pores through which select ions move down their electrical or chemical gradients at very high flux rates, ion transporters, on the other hand, show saturation for their substrates and may drive ions up their electrochemical gradient. Together, ion channels and transporters are critical components in the maintenance of cellular homeostasis, including osmolarity and pH as well as in electrical impulse propagation in higher organisms. We have previously demonstrated the RCK-domain octameric ring conformation for KtrAB potassium transporter. We focused on further characterizing this structural organization, obtaining high resolution atomic details by X-ray crystallography. We have therefore optimized overproduction and purification of full-length cytoplasmic (KtrA) and membrane (KtrB) components as well as the complex assembly. Ultimately, structural details will help understand conduction and regulation of potassium ion transport in KtrAB.