2648-Pos Board B418
Building a Transporter: Toward the Chemical Semisynthesis of Integral Membrane Protein Transporters
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Recent years have seen remarkable progress in the elucidation of crystal structures of a number of transporters. Notable examples include 1) GltPh, an archebacterial orthologue of the aspartate/glutamate and neutral amino acid family 2) LeuT, a eubacterial orthologue of the glycine/GABA and biogenic amine family, and 3) LacY, an E. coli permease of the major facilitator superfamily. Information gleaned from these structures raises a number of questions concerning the mechanism of substrate recognition, ion selectivity and the coupling of ionic gradients to substrate uptake. Addressing these questions requires the ability to precisely modify the proteins. Chemical synthesis is a very powerful method for protein modification as it enables the incorporation of a wide range of unnatural amino acids and protein backbone modifications. For the chemical synthesis of these transporters, we are developing a synthetic approach in which regions of interest, such as the ion binding sites or the substrate binding pocket, are obtained by peptide synthesis while the rest of the protein is obtained by recombinant means. We are utilizing GltPh as a model transporter for the development of our approach and progress towards the synthesis of the GltPh transporter will be presented.

2649-Pos Board B419
Reconstitution of an Arginine-Agmatine Antiporter in an Oriented System
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The arginine-dependent extreme acid resistance system plays a critical role for enteric bacteria to survive the harsh gastric environment. At the center of this multi-protein system is an arginine-agmatine antiporter called AdiC. To maintain cytoplasmic pH, AdiC imports arginine and exports its decarboxylated product agmatine, resulting in a net extrusion of one proton in each turnover. An obstacle to quantifying AdiC's transport mechanism is the random orientation of AdiC protein in reconstituted liposomes. To overcome this problem, we introduced a mutation, S26C, near the substrate-binding site. This mutant exhibits similar substrate recognition and pH-dependent activity as wild-type protein, but loses function completely upon reaction with MTS reagents. The membrane-impermeant MTSES can then be used as a cleanly sided inhibitor to ''silence'' those S26C-AdiC proteins whose extracellular portion projects from the external side of the liposome. Alternatively, the membrane-permeant MTSEA and membrane-impermeant reducing reagent, TCEP, can be used together to inhibit proteins in the opposite orientation. With this approach, we demonstrate that the activity of AdiC increases to a plateau at pH 4 as the extracellular side is acidified. Interestingly, the optimal pH for the cytoplasmic side is 5.5 and further acidification strongly inhibits AdiC's function. These findings recapitulate the gastric environment where the extracellular and cytoplasmic pH of bacteria are 2-4 and 5-6, respectively. Steady-state kinetic analysis found that for both sides of AdiC, the Michaelis-Menten constants of arginine and agmatine are in the same range. Competition experiments show that argininamide, which mimics the carboxylate-protonated form of arginine, suppresses arginine transport more effectively from the cytoplasmic side. This oriented system allows more precise analysis of AdiC-mediated substrate transport than has been previously available and permits comparison to the situation experienced by the bacterial membrane under acid stress.

2650-Pos Board B420
Characterization of a Novel CLC Homolog from Citrobacter Koseri
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CLC chloride-transport proteins are expressed ubiquitously and are vital to several physiological processes. This family is distinctive in that some members are chloride ion channels while others are chloride/proton antiporters. To better understand the mechanistic similarities and differences between CLC proteins, we have characterized a novel bacterial homolog from Citrobacter koseri called CLC-b. CLC-b is 24% identical and 42% similar in amino acid sequence to CLC-ec1, but lacks several amino acids near the chloride binding sites that are conserved in most CLCs. Despite lacking these regions, we found that CLC-b does transport chloride ions. CLC-b contains an isoleucine at the position equivalent to the intracellular proton transfer glutamate (E203 in CLC-ec1). Since all known CLC ion channels contain a hydrophobic residue at this position whereas all antiporters contain a protonatable residue at this position, we had hypothesized that CLC-b would be an ion channel. To our surprise, we found that CLC-b is a chloride/proton antiporter. Hence, a protonatable residue at the intracellular glutamate position is not necessary for proton transfer. We are currently working to test the ion selectivity and the effects of introducing a glutamate at this position.

2651-Pos Board B421
Characterization of a Novel CLC-ec1 Inhibitor
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CLC chloride channels and transporters are vital to many physiological processes. Defects in these proteins can lead to diseases that affect muscle, cardiovascular system, kidneys, and bones. Historically, high affinity inhibitors have played crucial roles in the characterization of ion transporter structure and function and in the treatment of disease. However, there is a dearth of known CLC inhibitors. To search for improved inhibitors, we synthesized derivatives of DIDS (4,4'-diisothio-cyanostilbene-2,2'-disulfonic acid), a small molecule that inhibits several CLC proteins with low affinity and specificity. We found that linking DIDS and octanoic acid generated a CLC-ec1 inhibitor (OADS) that inhibits CLC-ec1 with low micromolar affinity. We are characterizing the mechanism of inhibition using flux assays, electrophysiology, isothermal titration calorimetry, photoaffinity labeling, and mutagenesis. Given that OADS and OADS derivatives that inhibit ClC-ec1 are rather hydrophobic, we also explored their potential to alter lipid bilayer properties using a gramicidin based fluorescence assay.

2652-Pos Board B422
Structural Investigations of ClC-ec1, a Large Integral Membrane Protein, using Solution-State NMR and Nanodisc Technology
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CLC antiporters mediate Cl^-/H^+ exchange across cell membranes in organisms ranging from bacteria to mammals. This exchange is accomplished through elegant coupling of protein conformational changes to ion binding, unbinding and translocation events. To understand this mechanism, molecular details of the different conformations and the dynamics of their interchange must be known. While X-ray crystallographic structures have provided essential molecular pictures, the details of protein conformational change have remained elusive. To address this issue, we are using solution-state NMR to probe conformational change in ClC-ec1, a well-characterized CLC homolog of known structure. Selective ^{13}C labeling of lysine residues and the N-terminus is achieved by post-translational reductive methylation. ^1H-^{13}C HSQC spectra of these samples reveal reversible, substrate-dependent spectral changes that may reflect protein conformational changes (studies in progress). A chronic concern with the use of detergent-solubilized protein is the possible effects of the non-native detergent environment. To address this concern we are also implementing nanodisc technology. Nanodiscs present a promising alternative for studying membrane proteins such as CLCs, offering both a native bilayer environment and a size small enough for solution-state NMR studies. Each disc consists of two amphipathic helices wrapped around a bilayer patch of lipids surrounding the membrane-embedded protein. The protocol for the incorporation of the monomeric, ^{13}C-methylated mutant form of ClC-ec1 was optimized and the samples tested for homogeneity and monodispersity. The ^1H-^{13}C HSQC spectra of methylated, monomeric ClC-ec1 in nanodiscs are being investigated, with preliminary results indicating substrate-dependent spectral changes in this system. While further experiments will be needed to determine if these spectral changes represent functionally-relevant conformational changes, these studies demonstrate the potential for using solution-state NMR and nanodisc technology to study ClC-ec1 structure in a native lipid environment.

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Monitoring Substrate-Driven Conformational Changes of ClC-ec1 by [Methyl-13C] Methionine NMR
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ClC-ec1, a bacterial Cl^-/H^+ antiporter of the CLC family, has been crystallized under many conditions. Yet, only one major conformation of the protein has