1084-Plat
Transport Properties of a Prokaryotic CLC Transporter Assayed by Solid-Supported Membrane Electrophysiology
Juan J. Garcia-Celma, Adrian Szydelko, Raimund Dutzler.
University of Zurich, Zurich, Switzerland.
EcClC (or ClC-Ec1), a prokaryotic member of the ClC family of channels and transporters of known structure, acts as a coupled chloride/proton antiporter. To investigate its electrogenic activity, proteoliposomes containing reconstituted EcClC were adsorbed on a solid-supported membrane (SSM) electrode. The application of concentration jumps of different anions results in robust transient currents with a selectivity sequence that agrees with previously reported measurements on planar lipid bilayers. The transient currents increase at acidic pH with an apparent pKa of 4.6.
The pH dependences of three mutations that are known to impair proton translocation while preserving chloride transport (mutations E148A, Y445A, and E203Q) have also been investigated. In agreement with previous results, E148A shows weak pH dependence. The transport impaired Y445A and the uncoupled E203Q present a shift in the apparent pKa towards less acidic values. Mutation E203Q, in addition, presents residual electrogenic activity at neutral pH values. Taken together, our results support the idea that chloride transport in the wild-type varies with pH mainly in response to titration of the external glutamate (E148).

1085-Plat
On the Mechanism of Gating Charge Movement of the Chloride/Proton Antiporter CLC-5
Giovanni Zifarelli, Silvia De Stefano, Ilaria Zanardi, Michael Pusch.
CNR - Biophysics, Genova, Italy.
Structural and functional studies identified two critical residues for the transport mechanism of CLC transporters; the so-called gating glutamate (E211 in ClC-5) that controls the access of the anions to the extracellular space and is critical for anion/proton coupling and the proton glutamate (E268 in ClC-5), likely the intracellular entry/exit point for protons. However, the mechanism of voltage-sensitivity of CLC transporters is still poorly understood.
Interestingly, it has been recently reported that the E268A mutant of the endosomal Cl-/H+ antiporter ClC-5, beside inhibiting steady-state transports, exhibits transient currents upon voltage steps to large positive voltages. These transient currents may offer the possibility to glean information on the molecular details of transport coupling and voltage-sensitivity. Here we studied the dependence of the transient currents on the extracellular and intracellular pH and Cl- concentration. We conclude that the transient currents represent the movement of an intrinsic gating charge followed by the voltage dependent binding of extracellular Cl-ions. In addition, we find that the gating glutamate mutation E211D abolishes stationary transport but displays transient currents which are shifted by ~ 150 mV compared to the proton glutamate mutation, identifying E211 as a major component of the voltage sensing mechanism of ClC-5.

1086-Plat
***Bath Salts*: A Synthetic Cathinone Whose Two Major Components Act Similar to Methamphetamine and Cocaine on the Human Dopamine Transporter**
Krasnodara N. Cameron, Renata Kolanos, Ernesto Solis, Rakesh H. Vekariya, Richard A. Glennon, **Louis J. De Felice**.
Virginia Commonwealth University, Richmond, VA, USA.
β-Keto-amphetamine analogs (synthetic cathinones) represent a new and rapidly growing class of abused substances. Members include cathinone (*khat*) and the mixture of mephedrone and MDPV (*bath salts),* which is increasingly popular in the United States. Similar to amphetamine and methamphetamine, cathinone and methcathinone work primarily at the dopamine transporter (DAT) as dopamine (DA) releasing agents and CNS stimulants. Theoretically, hundreds of synthetic cathinones are structurally possible, and more than a dozen analogs have been designated as illegal. With few exceptions, however, little is known about the pharmacology or mechanism of these new and powerful drugs. Furthermore, most cathinone analogs are unavailable in pure form for scientific investigation. We are synthesizing racemic mixtures and optical isomers of synthetic cathinones, some of which are already on the clandestine market, to investigate their pharmacology and mechanism of action. Electrophysiological studies of *bath salts* on hDAT-expressing frog oocytes show that one component, mephedrone, has an electrical signature similar to methamphetamine, while another component, MDPV, has the electrical signature of cocaine. In particular, 10 μM mephedrone elicits an inward current at −60 mV that persists long after the drug is removed externally, similar to the *molecular stent* mechanism described for S(+)Amphetamine (Rodriguez-Menchaca et al., British J Pharmocology, 2011). MDPV on the other hand elicits an outward current under similar conditions, indicative of a blocking agent similar to cocaine. We have verified MDPV block of hDAT in ^{3}H-DA uptake experiments. Our results indicate that *bath salts* contain a DA releasing agent and a DA reuptake inhibitor. The two drugs have different kinetics and rather than cancel each other they would exacerbate the effect of either drug applied alone.

1087-Plat
Direct Observation of Conformational Exchange in the Small Multidrug Resistance Transporter EmrE
Emma Morrison[1], Greg Dekoster[1], Supratik Dutta[1], Michael Clarkson[2], Reza Vafabakhsh[3], Dorothee Kern[2], Taekjip Ha[3], **Katherine Henzler-Wildman**[1].
[1]Washington University, St. Louis, MO, USA, [2]Brandeis University, Waltham, MA, USA, [3]University of Illinois, Urbana, IL, USA.
Small multidrug resistance (SMR) transporters provide an ideal system to study the minimal requirements for active transport across a membrane. EmrE is an E. coli SMR transporter that exports a broad class of polyaromatic cation substrates, thus conferring resistance to drug compounds matching this chemical description. As a secondary active antiporter, EmrE drives the uphill export of each substrate molecule by coupling it to the downhill import of 2 protons across the inner membrane. EmrE is proposed to function via a single-site alternating access model. In this well-established model, transporters are inherently dynamic proteins, converting between inward- and outward-facing conformations in order to move substrate molecules across a membrane barrier. There is general agreement that the minimal functional unit is an EmrE homodimer, but a great deal of controversy remains regarding its structure, topology, and detailed mechanism. We have used a combination of NMR and FRET experiments to directly follow the kinetics and structural changes occurring during individual steps in the transport cycle. Our results reveal that EmrE forms an antiparallel homodimer and exchanges between inward- and outward-facing states at a rate of 5 s-1 when bound to the substrate tetraphenylphosphonium. Furthermore, the inward- and outward-facing states are identical except that they have opposite orientation. These findings reconcile the controversial asymmetric EmrE crystal structure with the functional symmetry of residues in the active site and have important implications for the energetics of proton-driven coupled antiport.

1088-Plat
Mechanistic Investigations into the Multi-Drug Resistance Transporter, EmrE
Emma A. Morrison, Gregory T. DeKoster, Yongjia Liu, Katherine A. Henzler-Wildman.
Washington University in St. Louis, Saint Louis, MO, USA.
EmrE, an *E. coli* small multidrug resistance transporter, exports a broad range of toxic polyaromatic cations, thus imparting resistance to drug compounds of this type. According to the proposed single-site alternating access model of antiport, EmrE converts between inward- and outward-facing structures during the transport cycle, using the import of two protons across the inner membrane to drive the export of one substrate molecule. Interconversion between the inward- and outward-facing conformations must occur only in the substrate-bound state in order to achieve coupled antiport. Our research focuses on this coupling between substrate binding and conformational exchange.
Conformational exchange between inward- and outward-facing states under substrate-bound conditions is the key step to efflux. We have directly monitored conformational exchange in tetraphenylphosphonium+-bound EmrE using ZZ-exchange NMR spectroscopy. This gives a rate constant of about 5 sec^{-1} for TPP^+-bound EmrE converting from an inward- to outward-facing conformation or vice versa. Stopped-flow kinetics were employed to measure the ligand on/off rates. The inward- and outward-facing states are identical in this system. This facilitates analysis of all the microscopic steps in the transport cycle, and has significant implications for the energetics of the transport process.
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1089-Plat
Mechanism and Regulation of Urea Permeation in a Mammalian Urea Channel
Elena J. Levin[1], Yu Cao[1], Giray Enkavi[2], Matthias Quick[1], Yaping Pan[1], Emad Tajkhorshid[2], Ming Zhou[1].
[1]Columbia University, New York, NY, USA, [2]University of Illinois at Urbana-Champaign, Urbana, IL, USA.
To maintain constant fluid volume and osmolarity in the face of infrequent access to water, terrestrial animals accumulate high concentrations of urea in the kidney interstitium to allow the reabsorption of water. This mechanism is dependent on the facilitated diffusion of urea through members of a family of