transitions of $C_{\text{O1}} \rightarrow O_{2} \rightarrow C (46.3\%)$ over $C_{\text{O2}} \rightarrow O_{1} \rightarrow C (< 5\%)$, 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 $O_{1} \rightarrow O_{2}$ transition (38%), suggesting 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.

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Chloride Transport Inhibition Causes Calcium-Dependent Arrhythmic Activity in Isoproterenol-Treated Rabbit Cardiomyocytes
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During β-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 β-adrenergic stimulation. We tested the hypothesis that both CFTR and KCC activity are critical during β-adrenergic stimulation in paced (1Hz) acutely isolated adult rabbit cardiomyocytes. Application of novel inhibitors of either CFTR (100μM CFTR inhibitor 172) or KCC (2μM N11k) did not appreciably alter the regular Ca transients during steady state pacing in rabbit cardiomyocytes. Addition of 100μM isoproterenol increased [Ca2+]o transient amplitude and accelerated [Ca2+]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-]), whereas KCC inhibition might allow CFTR current to dissipate the [Cl-/Cl+]o 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.

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Anion Permeation through Excitatory Amino Acid Transporters
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Glutamatergic synaptic transmission critically depends on excitatory amino acid transporters (EAATs) that release neurotransmitters from the synaptic cleft and thereby ensure low extracellular glutamate concentrations. EAATs are thermodynamically coupled glutamate/Na+/H+/K+ transporters and anion-selective channels. EAAT 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 GlpTph to elucidate how these transporters conduct anions. Our results are validated by fluorescence quenching experiments on single-tryptophan mutants of GlpTph and patch-clamp recordings of mammalian EAATs. Whereas outward- and inward-facing conformations of GlpTph 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 GlpTph 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.

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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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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 Ca2+-Dependent Regulation of the Cardiac Na+/Ca2+ Exchanger
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The Na+/Ca2+ exchanger (NCX) is an important ion transport mechanism for the movement of Ca2+ 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 Ca2+ dynamics was examined. However, it has been difficult to distinguish between the role(s) of Ca2+ as an allosteric regulator and Ca2+ as a transported ion. Additionally, there are parallel complex regulatory elements that control spatially resolved [Ca2+]i within cardiac myocytes. In this study, we compared the dynamic changes of iNCX and [Ca2+]i in non-transfected HEK293T cells, in cells expressing canine wild-type NCX, and in cells expressing the constitutively