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their effects on hDAT inhibition by GBR12909, a DAT specific inhibitor were tested. Interestingly, all tested ions reduced GBR12909 potency by ~50% in a similar fashion. Among them, isethionate seemed to alter GBR12909 inhibition the most, increasing the IC_{50} value 3-fold and the *h* value 2-fold. Since isethionate contains a chemical moiety observed in some atypical DAT inhibitors, it might directly compete with GBR12909 binding. Based on the experimental data and the crystal structure of leucine transporter with ligands, putative organic ion binding sites on hDAT were speculated.

1840-Pos Board B570

A Quantitative Model of Amphetamine Action on the Serotonin Transporter

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Background and purpose: Amphetamines bind to the plasmalemmal transporters for the monoamines dopamine (DAT), norepinephrine (NET) and serotonin (SERT); influx of amphetamine leads to efflux of substrates. Various models have been put forth to account for this amphetamine-induced reverse transport in mechanistic terms. A most notable example is the molecular stent hypothesis, which posits a special amphetamine-induced conformation that is not foreseen in alternate access models of transport. The current study was designed to evaluate the explanatory power of these models and the molecular stent hypothesis.
Experimental approach: Xenopus laevis oocytes and HEK293 cells expressing human (h)SERT were voltage clamped and exposed to serotonin (5-HT), p-chloroamphetamine (pCA) or methylenedioxyamphetamine (MDMA).
Key results: In contrast to currents induced by 5-HT, pCA-triggered currents through SERT decayed slowly (i.e., with a half-life of 20 s at 3 micromolar) in Xenopus laevis oocytes once the agonist was removed (consistent with the molecular stent hypothesis). However, when SERT was expressed in HEK293 cells, currents induced by 3 micromolar or 100 micromolar pCA decayed 10 or 100 times faster, respectively, after pCA removal.
Conclusions and implications: This discrepancy in decay rates is inconsistent with the molecular stent hypothesis. In contrast, a multi-state version of the alternate access model accounts for all the observations and reproduces the kinetic parameters extracted from the electrophysiological recordings. A crucial feature that explains the action of amphetamines is their lipophilic nature, which allows for rapid diffusion through the membrane.

1841-Pos Board B571

Real Time Imaging of SGLT1 Location and Activity in Mammalian Cell Lines

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Real time imaging of SGLT1 location and activity in mammalian cell lines.
In polarized epithelial cells with apical and basal membranes SGLT translocation to the apical surface is a complex and poorly understood process. We have engineered SGLT1 constructs linked to YFP or CFP to investigate transporter insertion into plasma membrane of COS, CHO and HEK cells. Activity was assessed in parallel, using the patch clamp technique, a FRET based glucose sensor and radioactive uptake. Insertion is both variable and highly regulated. In COS cells SGLT1 is constitutively inserted into the plasma membrane and active. In HEK cells SGLT1 is mainly in the endoplasmic reticulum, and its activity varies from cell to cell. In CHO cells SGLT1 is degraded, but localizes to mitochondria when co-expressed with EGFR (not endogenously present in WT CHO cells), or after incubation with the proteasome inhibitor III (MG-262, 1 μM). Incubation of HEK and CHO cells with the cholesterol inhibitor MβCD (15 mM) increases both insertion and activity, suggesting that SGLT1 internalization is lipid raft mediated. To test whether lipid raft mediated internalization involves caveolin, experiments were carried out in HEK cells co-expressing SGLT1 with caveolin WT, or the caveolin dominant negative P132L. Our data suggest that P132L may increase insertion of SGLT1 into the plasma membrane. Activity will have to be assessed to confirm this preliminary data. Finally, the lack of effect of the dominant negative dynamin (K44A) rules out a clathrin-dependent process and further supports lipid raft involvement. We propose that SGLT1 translocation to the apical surface involves a lipid raft-dependent route with stopover at the basal membrane. Accordingly, a decrease in membrane cholesterol content would cause accumulation of SGLT1 in the basal membrane and decrease insertion into the apical membrane.

1842-Pos Board B572

Insight into the Mechanism of Water Permeation through the Sodium-Galactose Transporter vSGLT from Long Molecular Dynamics Simulations

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Sodium-glucose transporters (SGLTs) mediate sugar transport as well as water flow across the cell membrane. Using a series of long molecular dynamics simulations generated on the special-purpose Anton supercomputer totaling nearly 16 microseconds in aggregate, we investigated the mechanism of water permeation through the inward-facing state of the bacterial sugar transporter vSGLT from *Vibrio parahaemolyticus*. Our simulations reveal conformational changes in the extracellular gate that create a transient water channel through the transporter. We developed a novel channel detection algorithm to characterize water pathways through the protein and identify key residues that control water flow. The simulations contain multiple, independent instances in which the galactose, initially bound to the transporter, spontaneously exits to solution. By comparing water flow through vSGLT when the sugar is bound, exiting and free in solution, we show that water flow, while modulated by galactose occupancy, is not coupled to substrate release.

1843-Pos Board B573

Energetics of Urea Permeation through Sodium-Dependent Galactose Cotransporter vSGLT

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In human kidneys, the glomeruli filter about 54 g of urea from the blood each day and approximately 12-14 g of which is reabsorbed in the proximal tubule that is believed to be devoid of urea channels or uniporters. Experimental evidence suggests that human sodium-dependent glucose cotransporters (hSGLTs) may be involved in urea uptake. Our Molecular dynamics (MD) simulations with the inward-facing structure of vSGLT (PDB ID: 3DH4) resulted in permeation of a single urea molecule in presence of a bound galactose. Separately, long-timescale MD simulations (1.5 μs) performed on the Anton supercomputer in presence of urea did not result in any urea permeation event. During these simulations, sugar was observed to escape the binging pocket indicating that the presence of galctose in the binding pocket may facilitate urea escape to the periplasm. Here we present our results on the study of energetics of urea transport along the previously observed pathway. Umbrella sampling simulations are performed to calculate the potential of mean force (PMF) of urea exit. Separate sets of umbrella sampling simulations are performed in presence and absence of a bound galactose to investigate our hypothesis that the bound galactose facilitates urea permeation. Two different force field parameters for urea are used to inspect the validity of the previously observed urea permeation event.

1844-Pos Board B574

Sodium-Galactose Transporter: The First Steps of the Transport Mechanism Investigated by Molecular Dynamics

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Sodium-Galactose Transporter (SGLT) is a secondary active symporter able to accumulate sugars like glucose/galactose into cells using the electrochemical gradient of Na^+ across the membrane. This transport is believed to occur via an alternating-access mechanism in which the protein, switching from an outward to an inward-conformation, guarantees a correct uptake of sugar molecules important in intestinal absorption and renal reabsorption. The protein belongs to the five-helix inverted repeat (5HIR) superfamily of sodium-dependent cotransporters where, despite the low sequence identity, it has been observed a common structural core of 10 transmembrane helices.
In 2008 Faham et al solved the crystal structure of SGLT of the Vibrio parahaemolyticus bacterium (vSGLT), where the Na^+ ion was not detected. The protein was so classified as representing an 'ion-releasing' state (Li et al, Biophys J, 2009). Moreover, the precise mechanism of the binding/unbinding of Na^+ and galactose from the inward-facing conformation and the gating role

of Y263 were not clear. In this study, using classical molecular dynamics (MD) simulations and bias-exchange metadynamics, we identified a candidate ion-retaining state of the transporter (Bisha et al, J Chem Theor Comput, 2013). Furthermore, we found that the interplay between the two ligands is based on a weakly coupled mechanism in which the role of Y263 seems to be not relevant for the exiting of the galactose towards the cytoplasm.

1845-Pos Board B575

Coupling of Ion Binding and Conformational Equilibrium in Na^+-Driven Secondary Active Transporters

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In secondary active transporters, the electrochemical potential of ions across the membrane is used to fuel the ‘‘uphill’’ translocation of the substrate via the alternating access mechanism. The mechanism of this crucial coupling, however, remains unclear, despite significant recent experimental and computational studies. Mhp1, Na^+/Benzyl-hydantoin transporter, has become a key model for the secondary active transporters sharing the LeuT-fold topology. In the present study, we employed molecular dynamics (MD) simulations to study the impact of Na^+-binding on the structure and dynamics of Mhp1 in multiple functional states and on the transition between them. Microsecond-long equilibrium MD simulations suggest that Na^+ binding stabilizes the substrate-binding conformation in the outward-facing (OF) state, thereby conferring high affinity for substrate binding. Furthermore, the results of a special-protocol time-dependent biased simulation and subsequent free energy calculation for state transition, illustrate that Na^+ binding can increase the free energy barrier along the OF-IF transition. All the results suggest that cation binding reshapes the free-energy landscape of the ion/protein complex, thereby shifting the conformational preference toward a specific OF structure, which is favorable for substrate-binding. The increased substrate affinity provided by Na^+ binding will facilitate capturing the substrate from its low-concentration environment by the transporter. The results, therefore, provide a deeper and more comprehensive understanding for the ion-coupling mechanism of secondary active transporters.

1846-Pos Board B576

Mechanism of Sodium/Proton Antiport in NhaA

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The transmembrane protein NhaA from Escherichia coli is a prototypical sodium/proton antiporter. It enables the bacterium to grow under high salt conditions while homologous proteins in eukaryotes are involved in pH and cell volume regulation. A number of acidic and basic residues have been shown to be essential for the transport of one sodium ion for two protons but the mechanistic details of their involvement have not been fully determined. Furthermore, the conformational changes involved in the transport mechanism were not known. We present an unpublished crystal structure of NhaA in the inward facing conformation and of the homolog NapA in the outward facing conformation [1]. Using modelling and computer simulations we show how NhaA can function according to the alternating access model, resulting in large relative domain motions that are incompatible with previous structural models for transport in NhaA. Our structure of NhaA contains a salt bridge between the two conserved residues Asp163 and Lys300. With the help of molecular dynamics simulations we critically examine competing models for the molecular mechanism of the stoichiometric transport of two protons for one sodium ion, including one in which Lys300 maintains an active role in proton transport.

[1] Lee et al, Nature 501 (2013), 573.

1847-Pos Board B577

Functional Evaluation of NHE6 Mutation Associated with Syndromic Autism and Tau Deposition

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Mutations in the endosomal Na^+/H^+ exchanger NHE6 (*SLC9A6*) are associated with autism co-morbid with epilepsy and severe X-linked intellectual disability. In this work, using evolutionary conservation analysis, we built a model-structure of NHE6 based on the crystal structures of bacterial NhaA and NapA and used it to predict functional consequences of NHE6 mutations associated with autism and tau deposition. Based on this analysis we located the patient mutation p.Trp338_Thr340del in NHE6, corresponding to TM helix VII in NhaA. We showed that NHE6 transports protons out of the endosomal lumen to regulate trafficking of the amyloid precursor protein and processing to Abeta. We also studied the effect of normal and mutant NHE6 on tau aggregation and toxicity using a cell culture model of inducible tau expression. Taken together, these studies will advance our understanding of the mechanistic link between NHE6 and the trafficking and processing of endosomal cargo in neurons and glia and will provide insight into the molecular pathophysiology of autism and related disorders.

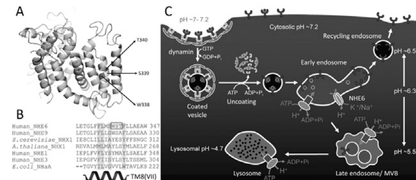

Figure: Model of NHE6 (**A**) depicting amino acid residues (**B**) deleted in syndromic autism. **C:** NHE6 regulates endosomal pH in vesicle trafficking.

1848-Pos Board B578

The Aspartate Transporter in Motion - Combining Steered Molecular Dynamics with Lanthanide Resonance Energy Transfer based Distance Measurements

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Excitatory amino acid transporters (EAAT’s) or glutamate transporters belong to the SLC1 family of the neurotransmitter transporters and mediate the re-uptake of glutamate from the synaptic cleft. Crystal structures of GltPh, an archeal homologue of the mammalian glutamate transporter, have been solved in several states, providing a starting point for understanding the conformational changes that accompany substrate transport. In this study we aim to integrate molecular dynamics simulations and Lanthanide Resonance Energy Transfer (LRET) based distance measurements to study the molecular motions that accompany substrate transport. Steered Molecular Dynamics (SMD) simulations were used to obtain insights into the transition path that lead to internalization of substrate from the extracellular milieu. Our simulations revealed the existence of an intermediate state along the transition path from the outward-occluded to the inward-occluded conformation. Our simulations highlighted the existence of gatekeeper interactions at the transition from the intermediate state to the inward-facing state. Based on the dynamics observed from our simulations, cysteine mutants were designed to observe the conformational changes in vitro. Site directed mutagenesis was used to insert genetically encoded lanthanide binding tags (LBT) and also cysteines which act as fluorophore docking sites to perform LRET based distance measurements, thus generated LBT mutants were expressed and purified. The wild type and mutant proteins were expressed and purified using affinity column chromatography, donor decay signals were recorded for LBT insertion mutants to confirm the insertion of tags. Furthermore radioligand binding assays were performed with the mutants and they were found to be functional. The distance measurements made with LRET were compatible with the distances observed in the crystal structure.

1849-Pos Board B579

Transition Metal FRET to Study Conformational Changes in Glutamate Transporter

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Crystal structures of the bacterial glutamate transporter homologue GltPh developed show a series of different states of the transporter: an outward state with HP2 open, an outward state with HP2 closed, an inward occluded state and a recently reported intermediate conformation between outward and inward state. These crystal structures suggest that glutamate transporters undergo a series of conformational changes during substrate binding and transport. Previously we have used classical FRET methods to measure these conformational changes in human glutamate transporters EAAT3 during the glutamate transport cycle. But due to the long R_0 value of most FRET pairs, their large sizes and long flexible linkers, classical FRET methods are not always well-suited for mapping intramolecular movements in proteins. Here we used a new transition metal ion FRET method, which enables us to measure distance change within a very short range to better study the conformation change during the transport cycle. By labeling the FRET donor