the L-type calcium channel, and we demonstrate one variant's decreased affinity to the target site. In comparison, sequence variants affecting critical residues of the junctophilin-binding site in L-type calcium channels are also linked to cardiac arrhythmias. The structural and functional insights from our studies provide evidence of junctophilin's role in important physiological processes.

Platform: Membrane Pumps, Transporters, and Exchangers

123-Plat
Visualizing the binding mode of the antidepressant vilazodone on the serotonin transporter by cryo-EM

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The serotonin transporter (SERT) belongs to the family of neurotransmitter sodium symporters (NSS) and is responsible for serotonin recycling and signal termination in the synaptic cleft of neurons. SERT is a therapeutical target for psychiatric disorders such as major depression. The standard medical treatment involves administration of selective serotonin reuptake inhibitors (SSRIs). All investigated SSRIs function as competitive inhibitors to serotonin. However, SERT also possesses an allosteric binding site and allosteric inhibitors may produce a novel therapeutic profile. Vilazodone is a novel SSRI. Its binding mode to SERT has not been fully explored. Recent work (Zhang et al. 2020) showed that the vilazodone binding site is located in the substrate binding (S1) site but protruding into the allosteric (S2) site. Here, we report that vilazodone is a non-competitive inhibitor of serotonin uptake and impedes dissociation of [^{3}H]imipramine, suggesting an allosteric mechanism of action. We solved the SERT structure in complex with imipramine and vilazodone by cryo-EM. It showed allosteric binding of vilazodone in a unique pose. The structural data is further substantiated with radioligand binding experiments, mutagenesis mechanistic data and MD simulations, showing that vilazodone's binding pocket is located at the S2 extracellular vestibule. Our findings highlight the versatility of the S2 site for drug targeting and demystify the unique and high affinity binding pose of vilazodone.

124-Plat
Voltage dependence of monoamine transporter function is determined by handling of intracellular potassium

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The transporters for dopamine (DAT), norepinephrine (NET), and serotonin (SERT) tap the transmembrane Na+ gradient to fuel their concentrative power. It is, however, conceivable that these carrier proteins may also utilize other energy sources such as membrane voltage and/or the transmembrane K+ gradient. In order to delineate these forces, we investigated the role of intracellular cations and voltage on substrate transport through DAT, NET, and SERT. To this end, we simultaneously recorded substrate-induced currents and uptake of the fluorescent substrate APP+ (4-(4-dimethylamino)phenyl-1-methylpyridinium) into single HEK293 cells expressing either DAT, NET or SERT under voltage control. These measurements were conducted in the whole-cell patch-clamp configuration, which allowed for control of the intra- and extracellular ion composition via the electrode and bath solution, respectively. We show that DAT and NET-mediated substrate uptake is voltage-dependent but SERT-mediated uptake is voltage-independent. This difference between SERT and DAT/NET arises due to differential handling of intracellular K+. In DAT and NET, intracellular K+ binding was transient in nature that precluded K+ antiport. Antiport of K+, on the other hand, led to voltage-independent and transmembrane K+ gradient powered substrate uptake by SERT. Thus, there seems to be a trade-off; these closely related transporters can either maintain constant uptake or harvest membrane potential for concentrative power, which can be attributed to subtle differences in the kinetics of co-substrate ion binding.

125-Plat
Tonic inhibition of the chloride/proton antiporter CLC-7 by pi(3,5)p2 is crucial for lysosomal pH maintenance

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The acidic luminal pH of lysosomes, maintained within a narrow range, is essential for proper degrative function of the organelle and is generated by the action of a V-type H^+ ATPase, but other pathways for ion movement are required to dissipate the voltage generated by this process. ClC-7, a Cl^-/H^+ antiporter responsible for lysosomal Cl^- permeability, is a candidate to contribute to the acidification process as part of this ''counterion pathway''. The signaling lipid PI(3,5)P2 modulates lysosomal dynamics, including regulating lysosomal ion channels, raising the possibility that it could contribute to lysosomal pH regulation. Here we demonstrate that depleting PI(3,5)P2 by inhibiting the PIKfyve kinase causes lysosomal hyperacidification, primarily via an effect on ClC-7. We further show that PI(3,5)P2 directly inhibits ClC-7 transport and that this inhibition is eliminated in a disease-causing gain-of-function ClC-7 mutation. These observations suggest an intimate role for ClC-7 in lysosomal pH regulation.

126-Plat
Accessing intracellular membrane transport proteins: a novel electrophysiological approach for lysosomal and mitochondrial channels and transporters

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Lysosomal, mitochondrial and other internal membranes are moving into focus of transport protein research. De-orphanization of intracellular transport proteins is ongoing and pharmacological interest is rising. For example, the lysosomal leak channel TMEM175 is investigated in the context of neurodegenerative diseases, the amino acid transporter SLC15A4 is linked to inflammatory processes and mitochondrial pumps are relevant for drug safety.

There are several approaches to study the function of these proteins, but the intracellular location is preventing the application of many established methods. Frequently plasma membrane mutants are generated to enable the application of conventional techniques like patch clamp electrophysiology or HTS fluorescence approaches. However, investigating these proteins in their physiological environment, the intracellular membrane, for example using patch clamp for direct recordings from lysosomes or mitoplasts, is very challenging, requires extensive training and provides a very low throughput.

We explored the application of SSM-based electrophysiology to study electrogenic intracellular transporters and channels in the original membrane. Capacitive sensors were functionalized to enable the adsorption of lysosomal/mitochondrial membrane samples. Activity of a specific transporter or channel was triggered by fast perfusion with a substrate and the resulting current was recorded. We optimized the purification of the membranes and the sensor formation. Activity of lysosomal expressed TMEM175, mitochondrial proton pumping complexes, ANT and other transport proteins was measured. We investigated signal specificity, reproducibility and success rate. Substrate affinity, transport kinetics and inhibition assays were performed.

We conclude that this novel approach is a robust and straightforward method, providing a high information content with an unusually high success rate and throughput for a direct electrophysiological intracellular assay. This technique can become a valuable addition to the existing portfolio of functional assays for intracellular transport proteins.

127-Plat
Intermediates, state transitions, and ligand coupling in the dynamic mechanism of the hEAAT3 glutamate transporter

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The most abundant excitatory neurotransmitter in the brain, L-Glu, is involved in a variety of neuronal signaling processes. This explains the keen interest in the excitatory amino acid transporter (EAAT) family and its role in the regulation of intra- and extra-cellular Glu concentrations. The human EAAT3 protein (hEAAT3) operates by an elevator mechanism to transfer the substrate from an

outward-facing state (OFS) to an inward-facing state (IFS) from which the Glu can be released into the cell. To investigate the transition mechanism between the end states from molecular dynamics (MD) simulations we have generated > 2 milliseconds of trajectory data by following an elaborate adaptive sampling procedure. Constructed free energy space model for the OFS to IFS transition was used to evaluate a Markov State Model for the process. It revealed three stable intermediates (I1, I2, I3) between the OFS and IFS states. The highest energy barrier was registered for the last stage (I3 to IFS). The structure of the hEAAT3 in I3 revealed a transmembrane channel with ~30% opening probability, which we show to be able to pass chloride anions. This suggests a similarity between I3 and the chloride conducting state described recently for the bacterial analog GltPh. To establish the mechanistic ''triggers'' for the state-to-state transition in the substrate transfer from OFS to IFS we have applied N-Body Information Theory (NbIT). One of the identified allosteric pathway connects the gates at the extracellular end of the hEAAT3 to the substrate binding site and the protonation of E374 residue in OFS state. The detailed dynamic changes at the interface between scaffold and transport domains of the transporter suggest a set of mutations predicted to stabilize the I3 intermediate.

128-Plat
Role of a conserved ion-binding site tyrosine in ion selectivity of the Na/K pump
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The Na^+/K^+ pump hydrolyses ATP to export three Na^+ and import two K^+ across the plasmalemma. Crystal structures identified three ion-binding sites within the pump's α-subunit. Site-I and site-II bind either Na^+ or K^+, while site-III exclusively binds Na^+. A conserved site-III tyrosine (*Xenopus*-α1 Y780) has been proposed to stabilize Na^+ through H^+-bonding and a cation-π interaction. We studied the effect of mutating Y780 on apparent ion affinities ($1/K_{0.5}$) utilizing electrophysiological and biochemical techniques, after heterologous expression in *Xenopus* oocytes and COS-1 cells. Changes in $K_{0.5}$ for Na^+_o were evaluated indirectly by the ouabain-sensitive transient-charge movement elicited by voltage pulses in the absence of K^+_o when the pump transits between Na^+-bound and Na^+-free states. The center ($V_{1/2}$) of the Boltzmann distribution describing this voltage-dependent process changes 20-25 mV for every twofold change in apparent Na^+ affinity (a leftward or rightward shift corresponds to reduced or increased affinity, respectively). $V_{1/2}$ was −51 mV (wild-type), −180 mV (Y780A), −137 mV (Y780F) and −33 mV (Y780Q). We measured Na^+_i affinity from the Na^+_i-dependent phosphorylation reaction in COS-1 cell membrane preparations. The $K_{0.5,Na+i}$ was 0.5 mM (wild-type), 24 mM (Y780A), 8.1 mM (Y780F) and 0.25 mM (Y780Q). Thus, Na^+ affinity was equally affected on both sides of the membrane, reduced by mutations disrupting H-bond, and increased when H-bond was maintained. The contribution of a cation-π interaction to ion binding was evaluated by introducing fluorinated tyrosine and phenylalanine derivatives (with reduced cation-π interaction energies) by non-sense suppression. Fluorination did not alter Na^+ affinity. The interaction with K^+ was also studied. All Y780 substitutions increased $K_{0.5}$ for K^+, indicating that the structure of an intricate H-bond network is also essential for binding and selectivity of site-I and site-II.
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129-Plat
Spectroscopy studies of the human mitochondrial ABCB10 transporter
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ABCB10 is an inner mitochondrial membrane ATP-binding cassette (ABC) transporter essential for heme synthesis and protection against oxidative stress. Like other ABC exporters, ABCB10 is expected to work through an alternating-access mechanism, where binding and/or hydrolysis of ATP by the nucleotide binding domains (NBDs) switches the accessibility of the substrate binding pocket from the inner side (mitochondrial matrix) to the outer side (intermembrane space), leading to translocation of substrate. However, most of this information comes from snap shots of X-ray models of a large variety of ABC transporters in detergent micelles, often with non-physiological ATP-analogs bound, inactivated by mutations or drugs, and at low temperature. Under such conditions, these models typically do not show conformational changes induced by substrate binding. To overcome some of these limitations, we use Luminescence Resonance Energy Transfer (LRET) to investigate conformational changes during the active ATP hydrolysis cycle of the purified human ABCB10 reconstituted in a lipid bilayer. LRET provides 1) distance measurements between LRET probes strategically positioned in the protein and 2) dynamic changes in distribution of molecules adopting different conformations. Our data show conformational changes induced by ATP binding, which are highly dependent on the temperature, demonstrating the importance of performing structural studies at physiological temperature. Additionally, since we recently identified that biliverdin (an antioxidant heme degradation product) is a substrate for ABCB10, we have studied the conformational changes that the transporter undergoes during its basal and substrate-activated ATPase cycle. ABC transporters present a basal ATPase activity that is increased in the presence of substrate, although the molecular bases of such activation are still unclear. Contrarily to X-ray crystallography studies, our spectroscopy data show a clear effect of substrate binding to the transporter, further stressing the importance of studying these transporters in near-physiological conditions.

130-Plat
Structures of multidrug resistance protein MRP4 reveal basis of substrate specificity
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ATP-binding cassette (ABC) transporters are a ubiquitous family of transmembrane proteins which hydrolyze ATP to facilitate the movement of substrates across cell membranes. Despite common architectural features, individual eukaryotic ABC transporters have evolved to transport a broad spectrum of substrates. The multidrug resistance protein MRP4, a member of the MRP sub-family of transporters, has been linked to the efflux of diverse endogenous and exogenous substrates, including prostaglandins, cyclic nucleotides, conjugated steroids, and other chemical scaffolds. To date, the functional characterization of MRP4's transport properties has been limited to in vivo cell-based assays, providing little clarity on the structural basis of MRP4's substrate specificity or how this specificity diverges from other closely related MRPs. In this work, we present the first in vitro characterization of MRP4, identifying multiple substrates that strongly stimulate ATPase activity while ruling out others. Using cryoEM, we determined the structures of MRP4 in nucleotide-free, substrate-bound, and ATP-bound states. These structures, along with comparisons to the closely related MRP1, provide insight into the determinants of substrate binding, inform on disease-relevant mutants, and map out the coupled cycle of MRP4 substrate transport and ATP-hydrolysis.

Symposium: Imaging and Modeling of the Brain

131-Symp
Modeling brain morphogenesis by integrating growth behavior on cell and tissue scales
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The cerebral cortical surface of the mature human brain is folded, and the pattern of gyri (ridges of the folds) and sulci (valleys of the folds) relates to behavioral attributes, such as the risk for neuropsychiatric disease. Axons, which populate white matter tissue adjacent to the cortex and mediate long-range neural connections in the brain, are known to grow in response to applied mechanical force. This connection between macroscopic forces and cellular development indicates that brain morphogenesis relates to its function at maturity. One proposed approach for modeling the process of cortical folding is to view the developing brain as viscoelastic material in which inelastic deformation (tissue growth) is induced by sustained elastic deformation (instantaneous tissue response to applied force). Experimental assessment of this viewpoint requires knowledge of region-dependent tissue growth rates, material properties of brain tissue components throughout the relevant developmental period, and the ability to express the cellular responses to mechanical forces induced by tissue growth. Here we describe an experimental model for measuring these parameters under conditions that produce normal and abnormal folding patterns. The ferret brain folds over the 30-day period from postnatal day (P)8 to P38. We present growth data from high-resolution magnetic resonance imaging (MRI) of 10 normally-sighted ferrets (5 males) evaluated at 6-day intervals from P8 to