In Shaker K^+ channels, lanthanide binding tags (LBT) were encoded in 4 consecutive positions on the top of the S4 segment and in 4 consecutive positions on the top of the S3 segment. To constrain the LBT position a truncated S3-S4 linker Shaker construct was used.
Tagged channels were expressed in *Xenopus laevis* oocytes and LRET-based distance measurements were conducted between Tb^{3+} ions bound to the LBT and Bodipy-Fl attached to the pore-blocker Agitoxin-2. Distance measurements for each of the tagged Shaker constructs were repeated with 3 toxins labeled at positions D20C, Q13C and N5C, respectively. Distances were determined in the three main conformational states of the channel: closed, open and open-inactivated.
Voltage-dependent K^+ channels are comprised of 4 subunits, symmetrically arranged around a central pore. In our measurements each of the subunits carried a LBT. With the toxin bound to the channel pore, energy is transferred from the 4 donors on the channel to the 1 acceptor element on the toxin. Due to this geometry the lifetime of the sensitized emission decay is composed of 4 exponential components corresponding to 4 donor-acceptor distances (Posson, Selvin 2008). We could determine all 4 distances by fitting a geometrical model to the decay and also determine the positions of the bound Tb^{3+} ion in the LBT in x, y and z. The resultant coordinates are used to refine the models based on the crystal structure of $K_V1.2$ for the closed, open and open-inactivated states. The most important finding of this study is that the position of the voltage sensor changes, not only when going from the closed to the open state, but also when going from the open to the open-inactivated state.
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1958-Plat
Probing The Length Of The Gating Pore In K-channels By Mutations Along The Spiral Arginine Thread of S4
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Voltage-gated ion channels sense voltage by moving arginine residues located in the S4 segment across the membrane electric field. According to the helical screw model these arginines, which gates the channel, move through a defined molecular gating pore. Tombola et al., 2007 were able to show a leak current (omega current) when the first arginine R1 was substituted with a smaller amino acid. For Nav1.2 channels, Sokolov et al., 2005 reported that the leak current only appears when the two outermost arginines are replaced by glutamine. In the present study, we probe the length of the gating pore and ask for the minimum number of amino acids which should occupy the gating pore in order to block it. To check that, the short Alanine 359 which lies next to R1S (362) was replaced by arginine. We expected that A359R will mimic the function of R1 and block or at least diminish the omega current. Approximately 80% of the omega current was blocked compared to the classical R1S construct. The mutation of the second arginine R2 to serine (**R1**,R2S,**R3**) also shows a little omega current. In both of these mutations ,(**A359R**, R1S,**R2**) (**R1**,R2S,**R3**), two long amino acids are separated by one short amino acid. However, the construct with the double mutation (**R1**, R2S, R3S, **R4**) produced a large omega current. These findings suggest that the length of the narrow part of the gating pore is just about two inter-arginine distances.

1959-Plat
Structural Basis For The Coupling Between Activation And Inactivation Gating In Potassium Channels
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We have known the structure for the closed-state of a potassium channel pore domain (PD) for more than a decade. However, major progress in understanding the molecular basis for activation and inactivation gating in K-channels had to wait until high-resolution structural information of the channel in the open state became available. Recently, we solved the structure KcsA in it fully open conformation, as well four others partial openings, which richly illustrated the channel activation-inactivation pathway. Analysis of these open structures suggested that residue F103 in TM2 interacts with the c-terminal end of the pore helix, compressing the pitch of its first helical turn. As a consequence, the distance between E71-D80 side chains is shortened, strengthening the carboxyl-carboxylate interaction that leads to a non-conductive conformation of the selectivity filter. Perturbation mutagenesis at position 103, affected gating kinetics as predicted from our structural analysis: small side chain substitutions F103A and F103C severely impaired inactivation kinetics, suggesting an allosteric coupling between the inner helical bundle and the selectivity filter. Free energy calculations show strong open state interaction-energies between F103 and surrounding residues. Similar interactions were probed in the Shaker K-channel by mutating highly conserved I470, equivalent to F103, to a smaller side chain. In the mutant I470A, inactivation was abrogated, suggesting that a similar mechanism underlies inactivation coupling in eukaryotic potassium channels. A crystallography study of these mutants in the open KcsA will be reported.

1960-Plat
Mechanism Of Increased Bk Channel Activation From A Channel Mutation That Causes Epilepsy
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Concerted depolarization and calcium rise during action potentials activate large-conductance calcium- and voltage- activated (BK) potassium channels, whose robust potassium currents increase the rate of action potential repolarization. Gain-of-function BK channels, both in mouse knockout of the inhibitory β4 subunit, and in humans with (αD434G) mutation have been linked to epilepsy. Here, we investigate mechanisms underlying the gain of function effects of the equivalent mouse mutation (αD370G), its modulation by the β4 subunit and potential consequences of the mutation on BK currents during action potentials. Kinetic analysis in the context of the Horrigan-Aldrich allosteric gating model revealed that changes in intrinsic and calcium-dependent gating largely account for the gain-of-function effects. D370G causes a greater than 2-fold increase in intrinsic gating equilibrium constant (1.65e-6 versus 6.6e-7) and an approximately 2-fold decrease in calcium dissociation constants (closed channel: 5.2 versus 11.3 μM, open channel: 0.54 versus 0.92 μM). Contrary to a previous report, co-expression of β4 produced similar changes in G-V relationships and gating kinetics for wildtype and mutant channels, suggesting that αD370G channels can be inhibited by β4. In physiological recording solutions, we established calcium dependence of BK current recruitment during action potential-shaped stimuli. D370G reduces K1/2 for both α (6.3 versus 13.7 μM) and α/β4 (15.0 versus 24.8 μM) channels. Although increased recruitment of BK currents by the mutation for both channel types are highly calcium dependent, greater effects were observed for the α/β4 BK channels. These results suggest that the D370G enhancement of intrinsic gating and apparent calcium affinity allow a greater contribution of BK current in sharpening of action potentials both in the presence and absence of the inhibitory β4 subunit.

Platform AL: Membrane Transporters & Exchangers

1961-Plat
Conformational Coupling of the Nucleotide-Binding and the Transmembrane Domains in ABC Transporters
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With the recent discovery of several crystal structures of complete ABC transporters, an alternating access model for substrate transport has been hypothesized, in which the transporter is open to the cytoplasm in the *resting* state and only accessible extracellularly in its ATP-bound, *intermediate* state. To test the hypothesized transport mechanism, we use molecular dynamics simulations to investigate the conformational changes and detailed interactions between structural components of ABC transporters in a membrane environment. Starting from the crystal structure of an intact maltose transporter which is trapped in the intermediate state, 50 ns or longer simulations are performed on the complete transporter, as well as on the transmembrane domains (TMDs) in the presence or absence of other components, and the conformational coupling of different domains is analyzed. We find that in the presence of nucleotide binding domains (NBDs) and the absence of nucleotides, the TMDs tend to open the cytoplasmic end, consistent with the prevailing transport mechanism. However, the cytoplasmic opening is not observed when the NBDs are absent, suggesting that the cytoplasmic-open state is dictated by the separation of the NBDs, and not as a result of the natural tendency of the TMDs to stay open. Furthermore, the results show that the opening of NBDs is propagated to TMDs through the mechanical engagement of the two helices at the EAA loop of the TMDs, which requires the formation of a 3-helix bundle together with the helix next to the Q-loop at the NBD helical subdomain. In the absence of NBDs the two coupling helices are completely decoupled from the rest of the TMDs, undergoing large fluctuations relative to the rigid TMD structures and show no conformational correlation to the other two EAA helices.

1962-Plat
Simulating Efflux Pumps: The Extrusion Mechanism of Substrates
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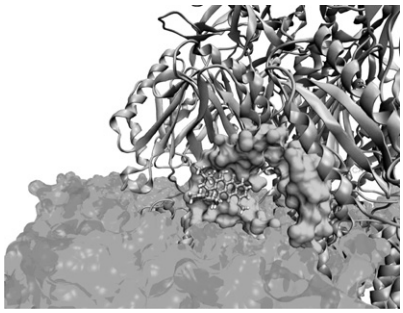
Bacteria use multidrug efflux pumps to extrude toxic substrates through their cell membranes. The RND transporters of the AcrAB-TolC (E.Coli) and MexAB-OprM (P.Aeruginosa) systems are able to export structurally and chemically different substrates, being responsible of multidrug resistance. While the energy conversion takes place in the transmembrane domain of AcrB and MexB, the energy is transducted towards the periplasmic part and used there to initiate what is believed to be a three-cyclic peristaltic pumping. Using different computational methods like adaptive bias force (ABF) and targeted molecular dynamics (TMD), we have investigated the mechanism of substrate uptake and pumping. With ABF we have studied the passage of antibiotics from the periplasm and protein-lipid interface into the inner pore of the pump, while TMD has been used to assess the effect of conformational changes on the extrusion of drugs (located into one of the proposed binding pockets). Finally, analysis of water distribution in the transmembrane region represents an important step to identify features of the energy transduction process. Comparison between the active pumps AcrB and MexB (which show different resistance patterns despite their homology) provide insights into the microscopic details of their functioning.



1963-Plat

Phenylalanine 508 Forms Intra-domain Contact Crucial To CFTR Folding And Dynamics

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Cystic fibrosis (CF) is the most prevalent genetically inherited lethal disease in the United States, with an especially high incidence rate among people of European descent and affects at least one in every 2500-4000 newborns. In 90% of CF patients, the disease is caused by the deletion of phenylalanine-508 (Phe508) from the first nucleotide-binding domain (NBD1) of the cystic fibrosis transmembrane conductance regulator (CFTR), a member of the ATP-binding cassette (ABC) transporter family. Prior experimental studies suggest that the Phe508 deletion cause the aberrant folding of NBD1 and the misassembly of various CFTR domains. To gain a fuller understanding of the role of Phe508 in channel function and of the impact of its deletion, we constructed a 3D structural model of CFTR using the high-resolution structure of another ABC transporter, Sav1866. The CFTR model predicts, and we validated experimentally, that Phe508 mediates an interaction between NBD1 and the fourth cytoplasmic loop in the second membrane-spanning domain (MSD2). This interface explains the observed sensitivity of CFTR assembly to many disease-associated mutations in CL4 as well as in NBD1. We also confirmed other intra-domain contacts between the cytoplasmic and membrane-spanning domains that were predicted by the CFTR model. Furthermore, our electrophysiological measurements and molecular dynamics simulations show that these interfaces regulate channel gating and are highly dynamic. Aside from advancing understanding of CFTR structure and function, this study identifies the region of CFTR that should be targeted in treating cystic fibrosis.

1964-Plat

Substrate-Dependent Conformational Changes of a Glutamate Transporter Homologue

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Glutamate transporters tightly control the extracellular concentration of glutamate in the brain, ensuring excitatory neurotransmission and cell integrity. These transporters utilize the energy of pre-existing ionic gradients to pump the transmitter against steep concentration gradients. Active transport is achieved by alternately exposing the ion and substrate binding sites to the extra- and the intracellular milieu and the catalytic cycle includes at least three conformational states: outward-facing, occluded and inward-facing. The crystal structures of a prokaryotic glutamate transporter homologue, GltPh, in complex with an inhibitor and a substrate showed the transporter in the outward-facing and occluded states, respectively, revealing how substrate and ions reach their binding sites from the extracellular medium. However, the conformational changes mediating the release of ligands into the cytoplasm are unknown. To gain insights into this process, we investigated ligand-dependent conformational transitions of the detergent-solubilized purified GltPh. Cysteine cross-linking experiments show that in the absence of ligands, helical hairpin 2 (HP2), which serves as an extracellular gate of the transporter, approaches TM2 bringing together residues that are more than 25 Å apart in the GltPh structures. The direction of HP2 movement is opposite of the observed in the outward-facing state, suggesting that the gate remains closed in the novel state of GltPh. We further probed the substrate-dependent solvent accessibility changes of key regions of the transporter by determining the kinetics of the fluorescence increase upon the reaction of fluorescein maleimide with single cysteine mutants. These experiments reveal a striking pattern: cytoplasmic residues become more solvent exposed and extracellular residues become less solvent-exposed upon substrate dissociation. Our results suggest that the ligand-free state is an inward-facing state of the catalytic cycle and demonstrate that the transporter can be conformationally constrained in this state by cysteine cross-linking.

1965-Plat

A Role for Topologically-Inverted Structural Repeats in Secondary Active Transport by Membrane Proteins of the LeuT Fold

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Structures of secondary active transporters often reveal the presence of large structural repeats. In the case of LeuT from the Na^+-dependent neurotransmitter symporter (NSS) family, the two repeats, each composed of five transmembrane helices, have inverted topology with respect to the plane of the membrane. An asymmetry between these two repeats in the extracellular-facing conformation of LeuT is responsible for the formation of the extracellular pathway. Structural modeling was performed in order to exchange the conformations of the two repeats. This resulted in a ~25° net rotation of a four-helix bundle around an axis in the plane of the membrane, which remarkably, created a conformation of LeuT in an intracellular-facing state. That is, the substrate binding site became exposed on the cytoplasmic side, and closed on the extracellular side. The model therefore proposes a cytoplasmic pathway for proteins with the LeuT fold. We show that accessibility of residues in this cytoplasmic pathway is consistent with a substantial number of biochemical accessibility measurements on single-cysteine mutants at the cytoplasmic face of the homologous mammalian serotonin transporter (SERT). In addition, the inward-facing structure of the related sugar transporter vSGLT shows significant similarities (Faham *et al*, Science 2008). Comparison of the two states of LeuT suggests an alternating-access mechanism in which a bundle of four transmembrane helices 'rocks' within the structure of the remaining helices. More generally, these results suggest that topologically-inverted repeats in other secondary active transporters may provide useful clues in understanding their mechanisms.

1966-Plat

Detecting Transport-related Conformational Changes In The Glutamate Transporter Homologue, Glt_{Ph}.

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Glutamate transporters (EAATs) are essential in clearing glutamate from the synapse, allowing precise control over excitatory synaptic function. Much has been learned in recent years about how these proteins function, however little is known about how the structural basis of this function; how do protein conformational changes lead to transport activity? A homolog of these proteins, Glt_{Ph} from *Pyrococcus horikoshii*, is functionally similar to the mammalian proteins; co-transporting substrate and sodium ions whilst having an uncoupled chloride conductance. Importantly, however it has been crystallized and its structure determined, making it an excellent model for understanding how structure and function are related in the EAATs. In order for transport to proceed, conformational changes must occur that allow alternating access of the substrate binding site to both the internal and external solutions. Using fluorescein maleimide labeling and protease accessibility studies on single cysteine mutants of Glt_{Ph} we have identified regions of the protein involved in this process. Changes in accessibility are seen in the presence of both aspartate and TBOA, a non-transported competitive inhibitor. Surprisingly, our accessibility studies implicate heretofore unexpected areas of the Glt_{Ph} protein in the conformational changes associated with substrate and inhibitor binding. We are also further investigating functional aspects of Glt_{Ph} transport using radioligand flux assays.

1967-Plat

Crystal Structure Of Chloride Transporter From A Cyanobaterium

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The CLC family of chloride transporting proteins is constituted of chloride channels and chloride-proton antiporters. Of the transporter subclass CLC_ec1 from E.coli has been characterized extensively structurally and functionally,