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
Tamer M. Gamal El-Din, Dominik Grögler, Hansjakob Heldstab, Claudia Lehmann, Nikolaus G. Greeff.
University of Zurich, Zurich, Switzerland.
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
Luis G. Cuello, Vishwanath Jogini, D. Marien Cortes, Albert C. Pan, Dominique G. Gagnon, Julio F. Cordero-Morales, Sudha Chakrapani, Benoit Roux, Eduardo Perozo.
University of Chicago, Chicago, IL, USA.
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
Bin Wang[1], Brad S. Rothberg[2], Robert Brenner[1].
[1]UT Health Science Center San Antonio, San Antonio, TX, USA, [2]Temple University School of Medicine, Department of Biochemistry, Philadelphia, PA, USA.
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
Po-Chao Wen, Emad Tajkhorshid.
University of Illinois at Urbana-Champaign, Urbana, IL, USA.
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
Robert Schulz[1], Attilio V. Vargiu[2], Francesca Collu[2], Matteo Ceccarelli[2], Ulrich Kleinekathöfer[1], **Paolo Ruggerone**[2].
[1]Jacobs University Bremen, Bremen, Germany, [2]Universita' di Cagliari, Monserrato (CA), Italy.