they have no sequence homology to apoA-I, the peptides bind to lipids in a manner similar to that of apoA-I (i.e. antiparallel double-belt on the edge of the lipid disc). Molecular dynamics simulations of the lipid-bound peptide mimetic 4F were performed for 30 ns in explicit water using CHARMM22/27 parameters. The peptides were arranged in a stacked and staggered conformation to determine if there was any difference in the stability of the belt structure of the peptides. In the initial model, 16 straight alpha-helical chains of 4F were placed around two leaflets of 108 dimyristoylphosphatidylcholines (DMPC). In both simulations all peptides remain in contact with the lipid. The staggered model gives a more circular shape while the stacked model distorts into an oval shape. The staggered model also has a lower conformational energy than the stacked model, indicating that peptide-lipid complexes in which the peptides are staggered may be the more stable form. Salt bridge analysis shows there are three additional salt bridge interactions formed that are not present in the stacked conformation. These interactions may be a contributing factor for the more stable form of the staggered conformation.

Platform AK: Voltage-gated K Channels - Gating: Gating Motions & Modulations

1953-Plat

The KCNE1 Subunit Modifies S2-S4 Interactions in the KCNQ1 Subunit of the I_{Ks} Channel Complex

Dick Wu, Kelli Delaloye, Jianmin Cui.
Washington University, St. Louis, MO, USA.

The I_{Ks} channel is formed by the coexpression of KCNE1 with KCNQ1. KCNE1 modifies KCNQ1 to bring about the characteristic I_{Ks} current that is essential for terminating ventricular action potentials. The objective of this study is to examine how KCNE1 modifies channel activation by altering the interactions between S2 and S4 in the voltage sensing domain (VSD) of KCNQ1. S2 and S4 contain a series of negatively (E1, E2) and positively (R1, R2, R4) charged residues conserved across all Kv channels, which are essential for voltage-dependent activation. We tested the accessibility of E1C by MTS reagents and found that E1C can be modified by $MTSES^-$ only when KCNE1 is present, suggesting that KCNE1 changes the packing of E1C. Likewise, E2 interactions are also altered by KCNE1. E2Q generates constitutively open channels with apparent partial inactivation, a phenotype distinctly different from WT KCNQ1. However, coexpressing E2Q with KCNE1 produces channels that are nearly identical to WT I_{Ks} in activation and deactivation, as if the drastic perturbations caused by E2Q in KCNQ1 were inconsequential to the function of I_{Ks}. Consistent with this view, in KCNQ1 a secondary mutation R2E can rescue the non-functional E2R. However, this double mutant remains non-functional in the presence of KCNE1. Therefore, E2 and R2 interact in KCNQ1 but not when the channel is coexpressed with KCNE1. Taken together, our data indicate that the association of KCNE1 either directly or allosterically changes the environment around E1 and breaks the interaction between E2 in S2 and R2 in S4. These findings offer new insight into the impact of KCNE1 on the structure of the VSD in KCNQ1, revealing a novel mechanism by which KCNE1 may modulate voltage-dependent activation in KCNQ1.

1954-Plat

Wild-Type KCNQ1 Modulates the Gating of the LQT1 Mutation R231C

Daniel C. Bartos[1], Jennifer L. Smith[1], Jennifer A. Kilby[2], Craig T. January[2], Brian P. Delisle[1].
[1]University of Kentucky, Lexington, KY, USA, [2]University of Wisconsin, Madison, WI, USA.

KCNQ1 mutations are linked to type 1 Long QT Syndrome (LQT1). *KCNQ1* encodes the voltage-gated K^+ channel α-subunit, Kv7.1, and LQT1 mutations typically reduce Kv7.1 current ($I_{Kv7.1}$). The missense mutation, R231C-Kv7.1, is associated with LQT1 and sinus bradycardia (Lupoglazoff et al., JACC 2004), but functional studies suggest that this mutation yields a constitutively activated large $I_{Kv7.1}$ (Rocheleau et al., JGP 2007). To better understand the molecular phenotype of R231C-Kv7.1, we transfected HEK293 cells with cDNA encoding the auxiliary K^+ channel subunit MinK1 and WT-Kv7.1, R231C-Kv7.1, or WT- and R231C-Kv7.1 (since LQT1 follows a dominant inheritance pattern). We measured $I_{Kv7.1}$ by prepulsing cells from -80 to 90mV in 10-mV increments for 5s, followed by a test-pulse to -50mV. $I_{Kv7.1}$ measured from cells expressing R231C-Kv7.1 was maximally activated at all potentials, and, compared to WT $I_{Kv7.1}$, increased the maximal peak tail $I_{Kv7.1}$ by ~350% (n=6-8 cells, $p<0.05$). In contrast, cells expressing WT- and R231C-Kv7.1 reduced the maximal peak tail $I_{Kv7.1}$ by ~50% compared to WT $I_{Kv7.1}$ (n=4-6 cells per group, $p<0.05$). We plotted the peak tail $I_{Kv7.1}$ measured during the test-pulse, as a function of the prepulse for cells expressing WT-Kv7.1 or WT- and R231C-Kv7.1, and described the data with the Boltzmann equation to calculate the midpoint potential ($V_{1/2}$) and slope factor (k) for peak tail $I_{Kv7.1}$ activation. Cells expressing WT-Kv7.1 had a $V_{1/2}$ of 19 ± 2mV and a k of 15 ± 2mV/e-fold change (n=6), and cells expressing WT- and R231C-Kv7.1 had a $V_{1/2}$ of -16 ± 5mV (n=5, $p<0.05$) and a k of 21 ± 2mV/e-fold change, ($p<0.05$). Cells expressing WT- and R231C-Kv7.1 also had a constitutively activated $I_{Kv7.1}$ that was ~22% of the maximal peak $I_{Kv7.1}$. These data demonstrate that WT-Kv7.1 dramatically alters the R231C-Kv7.1 phenotype and emphasize the importance of co-expressing WT-Kv7.1 and LQT1 mutations.

1955-Plat

Gated Motions and Interactions Between the Intra-Cellular Domains of the I_{KS} Channel Subunits

Yoni Haitin[1], Reuven Wiener[1], Dana Shaham[1], Enbal Ben-Tal[1], Asher Peretz[1], Liora Shamgar[1], Olaf Pongs[2], Joel Hirsch[1], Bernard Attali[1].
[1]Tel Aviv University, Tel Aviv, Israel, [2]Universitaet Hamburg, Hamburg, Germany.

Kv7.1 α-subunit assembles with the KCNE1 auxiliary subunit to form the cardiac I_{KS} K^+ channel. Mutations in these subunit genes produce the long QT syndrome, a life-threatening ventricular arrhythmia. Here we studied the static interactions and the voltage-dependent molecular rearrangements of the intra-cellular domains of the I_{KS} channel complex. The I_{KS} subunits were tagged with ECFP and/or EYFP and expressed in *Xenopus* oocytes. Simultaneous spectral analysis of the fluorescence resonance energy transfer (FRET) were combined with TEVC recordings of K^+ currents. In the channel closed state, a strong constitutive FRET signal between the C-termini of Kv7.1 and KCNE1 was observed. This static FRET signal was increased by 2-fold with a C-terminal truncation of Kv7.1 (Δ622-676). In addition, a marked FRET signal was observed between C-terminally CFP/YFP labeled Kv7.1 subunits, and between the N- and C-termini of double tagged α-subunits. Upon channel opening at +30 mV, concomitantly with I_{KS} K^+ currents recording, a voltage-dependent FRET elevation was detected between the C-termini of Kv7.1 and KCNE1 and between the N- and C-termini of the doubly-tagged Kv7.1. Notably, both K^+ currents and dynamic FRET changes were abolished by coexpressing the KCNE1 LQT5 mutant D76N along with Kv7.1. Direct interactions between the C-termini of Kv7.1 and KCNE1 were further explored by the use of purified recombinant peptides in a series of *in-vitro* pull-down experiments. These experiments indicated that the KCNE1 C-terminus physically interacts with the coiled-coil helix-C of the tetramerization domain. Thus, we suggest that the tetramerization domain of Kv7.1 possesses an additional function as an intra-cellular docking site for KCNE1. Moreover, we demonstrate that channel gating is propagated to the C-termini of both subunits, and accompanied by a spatial rearrangement of the channel complex.

1956-Plat

Using Voltage Clamp Fluorometry to Track Voltage Sensor Movement in a Mammalian Kv1.2 Channel in the Presence of the Kvbeta1.2 Subunit

Christian J. Peters, Moninder Vaid, Andrew Horne, David Fedida, Eric A. Accili.
University of British Columbia, Vancouver, BC, Canada.

The N-termini of Kv1 α-subunits bind co-translationally with cytosolic Kvβ-subunits, with 1:1 symmetry. Kvβ-subunits of three distinct families have been found in neural and cardiac tissue, and members of the Kvβ1 family confer fast inactivation and slowed deactivation when co-assembled with Kv1 α-subunits. These effects may be due to a blocking action by the Kvβ1 N-terminus. Kvβ1 subunits also cause an apparent hyperpolarizing shift in the activation curve of Kv1 channels, which may be a consequence of block by the Kvβ1 N-terminus, due to premature saturation of deactivating tail currents, or alternately may be due to an allosteric interaction between Kvβ1 and Kv1 α-subunits, modifying voltage sensor movement. Here, we use voltage clamp fluorometry to directly track the movement of the Kv1.2 voltage sensor in the absence or presence of the Kvβ1.2 subunit, or an N-terminally-truncated Kvβ1.2 subunit which does not produce fast inactivation. While Kvβ1.2 led to a spike-and-decay current waveform and a hyperpolarized shift in ionic current activation, the voltage dependence of ON gating charge movements were unaffected. Kvβ1.2 also slowed Kv1.2 fluorescence and current deactivation, implying that the return of the voltage sensor to its pre-activation position followed the closing of the activation gate. These findings suggest that the hyperpolarizing shift in channel activation is a consequence of pore block by the Kvβ1.2 N-terminus, and not an allosteric effect on the Kv1.2 voltage sensor, and that block prevents both closure of the activation gate and the return of the S4 helix upon repolarization.

1957-Plat

LRET Measurements In The Three Major Conformations Of The Shaker K Channel

Walter Sandtner, Jerome Lacroix, Janice Robertson, Ludivine Frezza, Clark Hyde, Francisco Bezanilla, Ana M. Correa.
University of Chicago, Chicago, IL, USA.

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.
Support: AHA07257632(WS), NIHGM30376(FB), NIHGM68044(AMC).

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.