did not alter the structure of the selectivity filter but did eliminate ion binding specifically to the S2 site. We introduced an equivalent ester substitution at the 2' position in the selectivity filter of the voltage-gated K^+ channel KvAP and found that this substitution dramatically slowed inactivation, similar to the effect observed in the KcsA channel. Our results suggest that ion occupancy at the S2 site is necessary for C-type inactivation in K^+ channels.

1183-Plat
Regulation of ion Permeation in the Selectivity Filter of Potassium Channels
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Ion efflux through potassium channels is regulated by a main gate controlled by ligands and/or the transmembrane voltage. The opening of the main gate let K ions flow down their gradient, diffusing through the selectivity filter of the channel, which can also contribute to the regulation of the ionic current by changing its conformation. Functional and structural data have brought evidence of interaction between the main gate and the selectivity filter of potassium channels. It is understood that opening the main gate favors the inactivated state of the filter, whereas closing the main gate stabilize the conducting state of the filter.
We have performed molecular dynamics simulations and potential of mean force calculations showing that the selectivity filter of the KcsA channel in its canonical conducting state, as captured by crystallography, does not allow the diffusion of ions at a rate compatible with experimental data. However, our calculations reveal that the opening of the main gate favors a transient conformational state of the selectivity filter that allows transport rate near the ion diffusion limit. The selectivity filter is thus expected to go from a pre-conducting state with high ion binding affinity to a conducting state with lower ion binding affinity, eventually transiting to the inactivated state. The detailed balance of forces involved in the fine regulation of ion permeation is well described by the latest generation of classical force-fields, which can reveal structural features of ion channels with an unprecedented level of precision.

1184-Plat
Initial Steps of Inactivation at the K^+ Channel Selectivity Filter
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K^+ efflux through K^+ channels can be controlled by C-type inactivation, which is thought to arise from a conformational change near the channel's selectivity filter. Inactivation is modulated by ion binding near the selectivity filter. However, the molecular forces that initiate inactivation remain unclear. We probe these driving forces using single-channel electrophysiology and molecular simulation of MthK, a prototypical K^+ channel. We observed that either Mg^{2+} or Ca^{2+} can reduce unitary current through MthK channels. However, Ca^{2+}, but not Mg^{2+}, can enhance entry to the inactivated state. Molecular simulations illustrate that in the MthK pore, Ca^{2+} ions can partially dehydrate, enabling selective accessibility of Ca^{2+} to a site at the entry to the selectivity filter. Upon reaching the site, Ca^{2+} can drive redistribution of K^+ within the selectivity filter, facilitating a conformational change within the filter and subsequent inactivation. These results support an ionic mechanism that precedes changes in channel conformation, to initiate inactivation.

1185-Plat
Mapping Conformational States in VDAC1 Channel: Complexity of Gating Landscape
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The Voltage-Dependent Anion Channel (VDAC) controls the fluxes of small ions and key respiratory substrates across the mitochondrial outer membrane (MOM). Like many other voltage-gated channels it displays a characteristic ability to switch or 'gate' between the so-called 'open', high conducting and 'closed', low conducting states. The gating conformations exhibit switch in ion selectivity from anionic to cationic for open and close states, respectively. Although a high-resolution structures for VDAC channels are available, the molecular mechanism of VDAC voltage-gating is still under debate. The very location of the voltage sensor and residues contributing to the observed gating charge and ion selectivity are yet to be established. The combination of replica-exchange MD simulations and all-atom MD simulations were used in this work to map possible conformational states of the VDAC1. Over 2 microseconds of atomistic simulations were used for a construction of Markov-State network of conformational transition. The permeation properties of the cluster representatives were assessed with non-equilibrium MD simulations under applied voltage as well as with Grand-Canonical Monte-Carlo/Brownian Dynamics Simulations. We found that up to 5 well-defined conformational states (3 open and 2 close states) exist in good agreement with the experimental data on VDAC gating. Therefore gating dynamics of VDAC1 channel can not be reduced to a simple two-state model. The computations of the reversal potential show that one of the closed states is lacking any selectivity, while the second closed state is weakly cation selective. All of the open-states display well-pronounced anion selectivity. These structural models suggest that collective transition of an entire beta-barrel domain represents a gating event in the conformational cycle. The computed gating charge is in good accord with experimental measurements.

Platform: Member Organized Session - Mechanics at the Cell Surface

1186-Plat
Antigen-Specific TCR-Pmhc Catch Triggers T-Cell Signaling by Rapidly Accumulating Successive Bond Lifetimes Prolonged by Optimal Force
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T-cell receptor (TCR) binding to the peptide-major histocompatibility complex (pMHC) initiates adaptive immune responses. However, the mechanism by which binding triggers intracellular signaling remains unclear. Mechanical force has been suggested to facilitate T-cell signaling; but the biophysical properties of TCR-pMHC interaction under force and their impact on T-cell signaling and antigen discrimination have not been characterized. Here, using an ultrasensitive mechanical assay with simultaneous Ca^{2+} imaging, we show that 10-pN force prolongs TCR-pMHC bond lifetimes for agonists (*catch*) but shortens those for antagonists (*slip*), thereby amplifying antigen discrimination. Both the magnitude and duration of force are important for calcium triggering as maximal Ca^{2+} was induced by 10-pN force applied via pMHC and via anti-TCR. This force produced the longest lifetime for the agonist pMHC catch bond but the longest lifetime for the antibody slip bond was produced by 0-pN force, which did not trigger Ca^{2+}. Single-cell analysis revealed that high Ca^{2+} requires early and rapid accumulation of bond lifetimes and low Ca^{2+} corresponds to high number of short TCR engagements prior to long lifetime accumulation. Our data support a model where mechanical force on the TCR induces signaling intermediates depending on the magnitude, duration, frequency, and timing of force, such that agonists form long-lived catch bonds at high two-dimensional (2D) association rates to activate the T cell digitally, whereas antagonists forms short-lived slip bonds that fail to activate.

1187-Plat
How Cellular Geometry Regulates Traction Stresses in Adherent Cells
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Cells generate mechanical stresses via the action of myosin motors on the actin cytoskeleton. While the molecular origin of force generation is well understood, we currently lack an understanding of the regulation of force transmission at cellular length scales. Here we experimentally decouple the effects of substrate stiffness, focal adhesion density and cell morphology to show that the total amount of work a cell does against the substrate to which it is adhered is regulated by the cell spread area alone. For a given spread area, we find that local curvature along the cell edge regulates the distribution and magnitude of traction stresses to maintain a constant strain energy. Finally, we present a physical model of the adherent cell as a contractile gel under a uniform boundary tension and mechanically coupled to an elastic substrate that quantitatively captures the spatial distribution and magnitude of traction stresses. With this model, we can accurately predict the spatial distribution and magnitude of traction stresses based entirely on cell morphology.

1188-Plat
To Adhere or not to Adhere: Regulation of Self-Contact Elimination by Membrane Fusion
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Mutual, homophilic cell-cell adhesion between epithelial cells is required for proper maintenance of epithelial barrier function. While opposing membranes