**178-Plat**
**High Temperature Sensitivity is Intrinsic to Voltage-Gated Potassium Channels**
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Temperature-sensitive transient receptor potential (TRP) ion channels are members of the large tetrameric cation channels superfamily but are considered to be uniquely sensitive to heat, which has been presumed to be due to the existence of an unidentified temperature-sensing domain. We find that the homologous voltage-gated potassium (Kv) channels also exhibit high temperature sensitivity comparable to that of TRPV1, which is detectable under specific conditions when the voltage sensor is functionally decoupled from the activation gate through either intrinsic mechanisms or mutations. Interestingly, mutations could tune Shaker channel to be either heat-activated or heat-deactivated. Therefore, high temperature sensitivity is intrinsic to both TRP and Kv channels. Our findings suggest important physiological roles of heat-induced variation in Kv channel activities. Mechanistically our findings indicate that temperature-sensing TRP channels may not contain a specialized heat-sensor domain; instead, non-obligatory allosteric gating permits the intrinsic heat sensitivity to drive channel activation, allowing temperature-sensitive TRP channels to function as polymodal nociceptors.

**179-Plat**
**Permeation and Dynamics of an Open-Activated TRPV1 Channel**
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Transient receptor potential (TRP) ion channels compose a large and diverse protein family, found in yeast and widespread in the animal kingdom. TRP channels work as sensors for a wide range of cellular and environmental signals. Understanding how these channels respond to physical and chemical stimuli has been hindered by the limited structural information available until now. The three-dimensional structure of the vanilloid receptor 1 (TRPV1) was recently determined by single particle electron cryo-microscopy, offering for the first time the opportunity to explore ionic conduction in TRP channels at atomic detail. In this study, we present molecular dynamics simulations of the open-activated pore-domain of TRPV1 in the presence of three cationic species: $Na^+$, $Ca2^+$ and $K^+$. The dynamics of these ions while interacting with the channel pore allowed us to rationalize their permeation mechanism in terms of a pathway involving three binding sites at the intracellular cavity, and the extracellular and intracellular entrance of the selectivity filter. Furthermore, conformational analysis of the pore in the presence of these ions reveals specific ion-mediated structural changes in the selectivity filter, which influences the permeability properties of the TRPV1 channel.

**180-Plat**
**The L596-W733 Bond between S4-S5 Linker and TRP Domain Maintains Basal Activity and Enables Inactivation of TRPV4**
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Unlike other cation channels, all TRP (Transient Receptor Potential) channel subunits have a TRP-domain helix immediately trailing S6 that bears the gate. The role(s) of TRP-domain helix is unclear. Recent cryo-EM TRPV1 structures revealed that this helix forms a bond with the beginning of the S4-S5 linker. By homology modeling, we identified the corresponding L596-W733 bond in TRPV4 (Vanilloid type 4). L596P, likely a gain-of-function (GOF) mutation, causes bone-developmental blockage of the spondylometaphyseal dysplasia Kozlowski type (SMDK) in human. Our previous screen also isolated W733R as a GOF that suppresses grows of yeast expressing TRPV4. Here we show, when expressed in Xenopus oocytes, TRPV4 with L596P or W733R mutation displays normal depolarization-induced activation and outward rectification. However, as expected from their biological GOF phenotypes, these mutant channels indeed have higher basal open probabilities and limited responses to the strong agonist GSK1016790A. In addition, W733R current also fails to inactivate after activation during depolarization. Systematic substitutions of W733 with amino acids of different properties produce similar electrophysiological defects. The results can be consistently interpreted in the context of the homology model of TRPV4 that we have developed. Our results indicate that the TRP domain stabilizes various functional conformations by bonding to other structures, especially to the S4-S5 linker.

**181-Plat**
**Comparative Sequence Analysis Suggests a Unified Gating Mechanism for TRP Channels**
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The transient receptor potential (TRP) channel superfamily plays a central role in the transduction of diverse stimuli in eukaryotes. Their transmembrane regions assemble in tetramer, similarly to voltage-gated potassium (Kv) channels. Given the degree of structural similarity between the two superfamilies, an intriguing question concerns the sequence determinants of such highly divergent activation mechanisms. To provide insight into this question and to investigate the fascinating hypothesis of a conserved allosteric activation mechanism shared amongst TRP channels, we have performed comparative sequence analysis on large, comprehensive ensembles of TRP and Kv channel sequences. We observe sequence features throughout the TRP channel TM core that are not shared with Kv channels. When interpreted in light of the recently resolved TRPV1 structures, our results suggest a novel, unified model of TRP channel gating.

**182-Plat**
**Effects of Inactivation of TRPM7 Kinase Activity on its Channel Activity in Mice**
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Transient receptor potential (TRP) family channels are involved in sensory pathways and are activated by various environmental stimuli. Among the members of this family, TRPM7 is a unique fusion of an ion channel and a C-terminus kinase domain that is ubiquitously expressed. TRPM7 is a key membrane protein governing cellular $Mg2^+$ homeostasis in mammals since its channel pore is permeable to $Mg2^+$ ions and can act as a $Mg2^+$ influx pathway. Moreover, TRPM7 channel activity is inhibited by intracellular $Mg2^+$. Mechanistic links between its kinase activity and channel function have remained uncertain, partly due to embryonic lethality of TRPM7 gene deletion in mice. In this study, we generated kinase inactive knock-in mutant mice by mutagenesis of a key lysine residue involved in $Mg2^+$-ATP binding. K1646R mutant mice were normal in development and general locomotor activity. In peritoneal macrophages isolated from adult animals the basal activity of TRPM7 channels prior to cytoplasmic $Mg2^+$ depletion was significantly potentiated, while maximal current densities measured after $Mg2^+$ depletion were unchanged in the absence of detectable kinase function. The inhibition of TRPM7 channel currents by 300 uM intracellular $Mg2^+$ or spermine was similar in WT and K1646R macrophages. Serum total $Ca2^+$ and $Mg2^+$ levels were not significantly altered in kinase-dead mutant mice either. Our findings suggest that 1) abolishing TRPM7 kinase activity does not impair its channel activity, but rather, potentiates basal current magnitudes; 2) kinase activity is not essential for regulation of mammalian $Mg2^+$ homeostasis.

**183-Plat**
**Temperature and Voltage Coupling to TRPM8 Channel Opening**
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Expressed in somatosensory neurons of the dorsal root and trigeminal ganglion, the transient receptor potential melastatin 8 (TRPM8) channel is a Ca2+-permeable cation channel activated by cold, voltage, PIP2 and menthol. Although TRPM8 channel gating has been characterized at the single channel and macroscopic current levels, there is currently no consensus regarding the extent to which temperature and voltage sensors couple to the conduction gate. In the present study we extended the range of voltages at which the TRPM8-induced ionic currents were measured and made careful measurements of the maximum open probability the channel can attain at different temperatures by means of fluctuation analysis. The first direct measurements of TRPM8 channel temperature-driven conformational rearrangements provided here suggest that temperature alone is able to open the channel and that the opening reaction is voltage-independent. Voltage is a partial activator of TRPM8 channels, since absolute open probability values measured with fully activated voltage sensors are less than 1 and they decrease as temperature rises. By unveiling the fast temperature-dependent deactivation process, we show