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Structural Insights into the Mechanisms and Pharmacology of K_{2P} Potassium Channels

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Abstract

Leak currents, defined as voltage and time independent flows of ions across cell membranes, are central to cellular electrical excitability control. The K_{2P} (*KCNK*) potassium channel class comprises an ion channel family that produces potassium leak currents that oppose excitation and stabilize the resting membrane potential in cells in the brain, cardiovascular system, immune system, and sensory organs. Due to their widespread tissue distribution, K_{2P} s contribute to many physiological and pathophysiological processes including anesthesia, pain, arrhythmias, ischemia, hypertension, migraine, intraocular pressure regulation, and lung injury responses. Structural studies of six homomeric K_{2P} s have established the basic architecture of this channel family, revealed key moving parts involved in K_{2P} function, uncovered the importance of asymmetric pinching and dilation motions in the K_{2P} selectivity filter (SF) C-type gate, and defined two K_{2P} structural classes based on the absence or presence of an intracellular gate. Further, a series of structures characterizing K_{2P} :modulator interactions have revealed a striking polysite pharmacology housed within a relatively modestly sized (~70 kDa) channel. Binding sites for

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A.M.N., P.E.D., and D.L.M. wrote and edited the manuscript and figures. D.L.M. supervised and coordinated the effort.

CRedit authorship contribution statement

Andrew M. Natale: Conceptualization, Visualization, Writing - original draft, Writing - review & editing. **Parker E. Deal:** Conceptualization, Visualization, Writing - original draft, Writing - review & editing. **Daniel L. Minor:** Conceptualization, Visualization, Writing - original draft, Writing - review & editing, Project administration, Funding acquisition.

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small molecules or lipids that control channel function are found at every layer of the channel structure, starting from its extracellular side through the portion that interacts with the membrane bilayer inner leaflet. This framework provides the basis for understanding how gating cues sensed by different channel parts control function and how small molecules and lipids modulate K_{2P} activity. Such knowledge should catalyze development of new K_{2P} modulators to probe function and treat a wide range of disorders.

Keywords

K_{2P} channel; selectivity filter C-type gate; M2 and M4 helix transmembrane motions; polysite pharmacology

Introduction

The term ‘leak’ tends to have negative connotations, is often used as a pejorative, especially in politics, and is something best avoided by those worried about roofs, buckets, or other important barriers or containers. To the contrary, leak is not a bad thing for cells, especially those involved in producing the bioelectricity that runs our hearts, brains, muscles, and sensory organs. Indeed, background or ‘leak’ potassium currents, defined broadly as those lacking voltage or time dependency, have been recognized for >70 years as essential actors in affecting the electrical excitability of cells.^{1–3} Ion channel proteins responsible for this activity first became apparent ~25 years ago with the identification of the founding members of what is now known as the K_{2P} (KCNK) family of potassium channels: $K_{2P1.1}$ (TWIK-1),⁴ $K_{2P2.1}$ (TREK-1),⁵ $K_{2P3.1}$ (TASK-1),⁶ and $K_{2P4.1}$ (TRAAK)⁷ (Figure 1(a)).^{8,9} The subsequent study of K_{2P} s has revealed that these channels are much ‘more than just leak’.¹⁰ K_{2P} s are highly regulated by diverse factors including both chemical and physical gating cues^{9,11,12} and although their pharmacology remains underdeveloped, they present promising targets for new therapeutics aimed at pain, migraine, ischemia, depression, glaucoma, and lung injury. Structural studies over the past ~8 years have begun to reveal the core architecture responsible for K_{2P} function, as well as a rich array of sites that can be targeted by small molecule modulators. Here, we focus on these key molecular advances and highlight some of the exciting new directions that are on the horizon for this very positive form of leak.

The K_{2P} family of channels belongs to the larger voltage-gated ion channel (VGIC) superfamily.¹³ There are 15 human K_{2P} subunits^{8,9} comprising six subfamilies that are regulated by various stimuli (Figure 1(a)). The name ‘ K_{2P} ’ derives from their unique subunit architecture. Each K_{2P} subunit comprises two pore-forming domains, PD1 and PD2. Both of these domains contain two transmembrane helices (M1-M2 and M3-M4) that are bridged by a pore helix (P1 and P2) and selectivity filter (SF1 and SF2) (Figure 1(b)). K_{2P} subunits dimerize to create a channel having an intrinsically heterotetrameric pore due to the fact that PD1 and PD2 are not identical. Unlike voltage-gated or inward rectifier channels from the VGIC superfamily, K_{2P} s conduct ions over the entire physiological voltage range, in line with their role as ‘leak’ channels. This leak current is not invariant and can be tuned by a remarkable array of chemical and physical gating cues that include pressure,

temperature, extracellular and intracellular pH, lipids, phosphorylation, and agents such as volatile anesthetics and antidepressants.^{9,14}

Studies of K_{2P} function have uncovered that members of this family are important for diverse classes of physiological responses and pathological conditions such as action potential propagation,^{15,16} anesthetic responses,^{17,18} microglial surveillance,¹⁹ sleep duration,²⁰ pain,^{21–23} arrhythmia,²⁴ ischemia,^{17,25,26} cardiac fibrosis,²⁷ depression,²⁸ migraine,²⁹ intraocular pressure regulation,³⁰ pulmonary hypertension,³¹ and lung injury.³² Due to their unusual topology, diverse gating stimuli, and poor pharmacology, K_{2P}s have remained the most poorly understood potassium channel class.^{8,9,33–35} Nevertheless, the structures of six homomeric K_{2P}s are now known (Figure 1 (a))^{36–41} and reveal a conserved overall architecture that defines the K_{2P} family (Figure 1(c) and (d)).

In addition to the need to answer basic questions about how K_{2P}s function and are regulated, the lack of good pharmacological tools to manipulate K_{2P}s remains a challenge for understanding and controlling their physiological functions.^{42,43} The demand to address this deficit has not gone unnoticed and a number of recent discoveries of a variety of K_{2P} modulators are beginning to define new pharmacological tools directed at this channel family.^{23,37–38,44–49} Further, in spite of their modest size (~70 kDa), the first studies of K_{2P} structural pharmacology have revealed a strikingly lush structural landscape for functional control by various types of modulators.^{37,40,50,51} This unexpectedly rich polysite pharmacology provides the means to dissect mechanisms of K_{2P} function and is the starting point for new ventures to elaborate K_{2P} pharmacology.

K_{2P} structure: Similar to other potassium channels, but different

Common to other potassium channels, each of the K_{2P} pore domains carries the potassium channel selectivity filter signature sequence (TxTTxGYGD),⁵² encompassing the strands that directly coordinate the permeant ions and the C-terminal end of the pore helix (Figure 2(a)). There are several notable deviations from this conserved sequence. In PD1, the first Thr of this sequence is always a hydrophobic residue and the final Asp is replaced with Asn, His, Tyr, or Met. In PD2, the 'Y' of the 'GYG' is either Phe or Leu. Despite these sequence differences, which may impact function, structures of various K_{2P} members show that the fundamental architecture of what is essentially the active site of the channel is not different from other potassium channels (Figure 2(b)).^{36–41}

Most of the K_{2P} structure is within the membrane (Figure 1(c)). PD1 and PD2 assemble to form the single pore in which M2 and M4 line the central cavity and M1 and M3 face the bilayer. The two transmembrane pairs, M1/M2 and M3/M4 interact closely with each other to form the supporting structure of the P1 and P2 pore helices and their attendant selectivity filters, respectively. The four SF elements are arranged along the channel central axis where they coordinate the permeant ions (Figure 2(b)). Because of their dimeric structure, the K_{2P} pore is intrinsically heterotetrameric having two PD1s and two PD2s arranged around the central axis (Figure 2(c)). The linker connecting the SF to the inner helix of its pore domain has different lengths in PD1 versus PD2 (Figure 2(a) and (d)). The SF1-M2 linker has six residues, similar to most other potassium channels, whereas the SF2-M4 linker is more than twice that length having between 12–14 residues (Figure 2(a) and (d)). The one exception is

K_{2p}18.1 (TRESK) in which the SF2-M4 linker has the canonical six residue length. These differences have important consequences for the role of the SF in channel gating and point to the SF2-M4 loop as a unique functional feature of K_{2ps}.⁵³

K_{2ps} have a distinctive extracellular structure termed the Cap domain that is formed by a pair of helices from each subunit.^{36,39} One of these helices is contiguous with the M1 transmembrane helix (Figure 1(c)). The Cap has a largely conserved disulfide bond at its peak^{54,55} and forms an archway over the mouth of the channel pore that creates a bifurcated pathway, termed the 'Extracellular ion pathway' (EIP),^{36,39} through which potassium ions exit the channel under physiological conditions (Figure 1(c)).

Structural studies have uncovered one other unusual aspect of the K_{2p} pore domain architecture. In K_{2p}2.1 (TREK-1),^{37,51,53} K_{2p}3.1 (TASK-1),³⁸ K_{2p}4.1 (TRAAK),^{56–58} K_{2p}5.1 (TASK-2),⁴¹ and K_{2p}10.1 (TREK-2),⁴⁰ the M1 segment is domain swapped between the subunits, an arrangement enabled by the interposition of the Cap domain helices between M1 and P1. The result is that the PD1 structural module is comprised of M1 from one subunit and M2, P1, and the selectivity filter of the other (Figure 2(c)). Domain swapping, which involves the formation of intertwined protein assemblies by exchange of identical structural elements, is observed in many classes of soluble and transmembrane proteins,^{59–62} although its functional relevance is often not clear.^{59,61,63} The only K_{2p} structure in which this arrangement has not been reported is K_{2p}1.1 (TWIK-1)³⁶. Further, such pore domain intertwining has not been seen in other VGIC superfamily members. The predominance of domain-swapped pores in the K_{2p} family raises questions about how such interlocked structures form during K_{2p} biogenesis, how easily they are taken apart, and whether such interlinking affects K_{2p} stability or function. Moreover, some K_{2p} subunits can assemble as heterodimers.^{29,64–71} Although it is not known whether heterodimeric K_{2ps} also have this domain swap, the impact of associating M1 of one subunit with the M2/P1 elements of the pore domain from the heterodimer partner could result in functional differences between domain-swapped *versus* non-domain swapped versions if both are able to form. Biogenesis and folding of most channels, including K_{2ps}, is largely a black box. Unravelling how these domain swapped structures form, and their consequences for function and regulation is a key question for future studies.

K_{2p} channel moving parts

In order to respond to signals, a protein must have some moving parts. The structures of K_{2ps} determined to date have revealed three moving elements that are tied to function. Two of these involve the two pore-lining helices, M2 and M4.^{40–41,56–58} The third encompasses the structure that forms and supports the selectivity filter C-type gate.^{37,40,41,53}

Unlike most members of the VGIC superfamily, K_{2p} gating is primarily controlled at the selectivity filter (SF) C-type gate.^{53,72–77} Structures from the TWIK³⁶ and TREK subfamilies^{37,40,51,53,56–58} showed channels that lack an intracellular barrier and that have an unhindered path from the cytoplasm to the SF (Figure 3(a)). These observations are consistent with functional data indicating that these channels,^{72–75,78} as well as other K_{2ps}, such as K_{2p}18.1 (TRESK),⁷⁸ lack an intracellular gate. Prior functional studies suggested that some K_{2ps} may have an intracellular gate^{79–80} and two recent structures of

representatives from the TASK³⁸ and TALK⁴¹ subfamilies have provided the first structural examples of how such a gate forms. In K_{2p}3.1 (TASK-1) a pair of short helical segments (~two turns) at the distal end of M4 kink to produce a barrier at the intracellular mouth of the channel termed the ‘X-gate’³⁸ (Figure 3(b)). By contrast, the intracellular barrier of the pH 6.5 K_{2p}5.1 (TASK-2) structure shows a pinching between the two M4 helices that appears mediated by a pair of lysine sidechains⁴¹ (Figure 3(b)). Thus, it appears based on the current available data that the K_{2ps} will break into two structural classes, those without an inner gate, such as the TREK subfamily and K_{2p}1.1 (TWIK-1), and those that have some type of intracellular barrier like K_{2p}3.1 (TASK-1)³⁸ and K_{2p}5.1 (TASK2).⁴¹ Understanding these class distinctions and whether there are additional variations to this classification will be important for delineating gating mechanisms and for developing subtype selective pharmacologies that can exploit these differences. Defining how the other K_{2p} homodimers fit into these classes, and uncovering whether there are further variations on these themes, especially among heterodimeric K_{2ps}, is an important line of investigation for the near future.

The ‘up’ and ‘down’ movements of M4

The most obvious moving part of K_{2ps} is the M4 helix. The position of this helix varies when compared across structures from different subtypes (Figure 3(a)) and in multiple structures of K_{2p}4.1 (TRAAK)^{56–58} and K_{2p}10.1 (TREK-2).⁴⁰ The extremes of these M4 positions have been termed ‘up’ and ‘down’ (Figures 3(a) and 4(a) and (b)). In the ‘down’ conformation M4 is approximately straight, crosses the membrane at a ~45° angle and makes no contacts with the M2 helix of the opposite subunit. This conformation leaves a 5–10Å gap that faces the lipid bilayer. ‘Down’ structures have shown that hydrophobic entities (either detergents or lipids),^{36,56,58} as well as the inhibitor norfluoxetine (Figure 4(b)) can occupy a site formed by this gap that is just under the P2 helix, termed the ‘Fenestration site’. It has been proposed that lipid tails may reach through this gap to block ion permeation⁵⁸; however, functional tests have not supported this hypothesis.^{74,81,82} In the ‘up’, conformation, M4 bends at its midpoint near a conserved glycine (G260 in human K_{2p}4.1 (TRAAK)) towards the bilayer core by approximately 25°. This conformational change allows the C-terminal half of M4 to pack against M2, closes the fenestration (Figure 4(a) and (b)), and also changes the shape of the channel near the intracellular end of M4 that is thought to be the principal site of lipid modulation by phosphatidylinositol 4,5-bisphosphate (PIP₂) in the TREK subfamily.^{37,57} Intriguingly, functional and molecular simulation studies have suggested that the hydrophobicity of particular M2 and M4 residues near the fenestration may affect function in some K_{2ps} by enabling transient dewetting of the central pore through a process termed ‘hydrophobic gating’.^{83–85} However, it remains unclear how this process might be regulated by the state of the M4 helix.

These up/down M4 motions are reminiscent of similar motions of the pore-lining helices of fourfold symmetric channels from the VGIC superfamily, but whereas in VGICs a similar motion towards an ‘up-like’ conformation of four pore-lining helices leads to an opening of the intracellular gate, this is not so for K_{2ps}. In both the ‘up’ and ‘down’ conformations some K_{2ps} have an unobstructed path for ions to traverse from the cytoplasm to the selectivity filter (Figure 3(a)). Consequently, assigning functional correlates to the

‘up’ and ‘down’ conformations has not been straightforward. Initial reports from studies of the mechanosensitive TREK subfamily had contradictory interpretations with one suggesting that the ‘up’ state⁵⁸ represented the active state – reasoning that the expanded cross sectional area of the ‘up’ state would be favored by in-plane membrane tension, while another observed that the ‘down’ state resulted from gain-of-function (GOF) mutations.⁵⁷ It has been noted that the mechanosensitive TREK K_{2P} subfamily shows relatively small differences in cross sectional area between ‘up’ and ‘down’ states compared to analogous gating changes in other mechanosensitive channels (ex. $A < 5 \text{ nm}^2$ in K_{2P}10.1 (TREK-2) *versus* 20 nm^2 in MscL and 120 nm^2 in Piezo1)¹². Nevertheless, molecular dynamics studies of K_{2P}10.1 (TREK-2) have shown that lipid bilayer stretching favors the ‘up’ state, likely as a result of complex changes in the internal pressure of the membrane and protein-lipid packing alterations, in addition to protein cross-sectional area changes.^{82,86–87} To add to the complexity, functional experiments have shown that activation is not a simple dichotomy of an active ‘up’ state and inactive ‘down’ state, but in fact, both the ‘up’ and the ‘down’ states are active and are sensitive to additional regulation.⁸¹

Based on the established centrality of the SF gate and the observation that activators reduce the mobility of the C-type gate,^{37,53} it has been proposed that M4 movements are coupled to the dynamics of the selectivity filter C-type gate, providing a rationalization for how both extreme positions could result in active channels.^{37,53,87–88} This structural explanation for resolving the apparent paradox with an ‘everybody wins’ mechanism is attractive, even though the details of the energetic coupling between M4 and the SF gate remain incompletely understood. Intriguingly, a recent report describes a K_{2P}10.1 (TREK-2) high activity mode that retains sensitivity to norfluoxetine, and thus, must be in a ‘down’ conformation.⁸⁹ This result supports the idea that the ‘down’ state can be a high activity state as inferred from the studies of gain-of-function mutants⁵⁷ and underscores that there remains much to be learned about how M4 motions in K_{2P}s influence activity.

Both K_{2P}3.1 (TASK-1)³⁸ and K_{2P}5.1 (TASK-2)⁴¹ structures that show a closure at the intracellular mouth of the channel have a ‘down’ M4 position that includes additional structural rearrangements that block the pore (Figure 3(b)). It is clear from the physical barriers made by the X-gate and the intracellular pH regulated gate that some part of these elements must move to let both ions and blocker molecules pass, an idea supported by functional studies of K_{2P}3.1 (TASK-1),^{38,79} K_{2P}5.1 (TASK2),⁹⁰ and the drosophila K_{2P} KCNK0.⁸⁰ In the case of K_{2P}5.1 (TASK-2)⁴¹, the motions required to open the inner gate are modest, largely involving changes in the sidechains. The distal end of the X-gate has a region termed the ‘latch’ that interacts extensively with M1 and M2, and it has been proposed that disruption of these interactions would allow M4 to move towards and ‘up’ conformation as the gate opens.³⁸ Interestingly, mutations in and around the latch structure in TASK subfamily members are linked to pulmonary hypertension and a developmental disorder known as Birk-Barel syndrome,⁹¹ further highlighting the importance of this element in channel function.⁹² Understanding the extent of such motions, as well as the stability of these inner gate structures is a key next step that will enrich our understanding of how M4 moves, how changes in its position affect K_{2P} function, how its motions can be exploited to gain control of specific K_{2P}s, and the extent to which such M4 motions are present in other K_{2P} family members.

Buckling motions of M2

The TREK subfamily represents a group of polymodal ion channels that are affected by both physical and chemical gating cues.¹² Structural studies of two K_{2P}4.1 (TRAAK) GOF mutants that impact responses to temperature and pressure activation^{72–73} showed that the M4 ‘down’ state resulted in a second, functionally relevant conformational change.⁵⁷ The ‘down’ motion of M4 separates this helix from M2 of the neighboring subunit, but creates a new interface with M2 from its own subunit. These changes deform M2 in a way that leads to its buckling at a conserved glycine GXG motif found in the TREK subfamily⁵⁷ (Figure 4(c) and (d)). Interestingly, the deformation includes an apparent backbone hydrogen bond shift from $i, i + 4$ (Leu161-Gly165) to $i, i + 3$ (Ala162, Gly165) in human K_{2P}4.1 (TRAAK) (PDB: 4RUE)⁵⁷ (Figure 4(d)), a low-energy rearrangement that has been associated with functionally relevant helix deformations in other membrane proteins.⁹³ Mutation at this GVG motif glycine impacts function and supports the idea that M2 buckling is important for TREK subfamily activation. It is interesting that this glycine is also conserved in the TASK and TALK subfamilies (Figure 4(c)) and coincides with the center of a $\sim 5^\circ$ bend in M2. A better understanding of the potential roles of motions in M2 as well as other parts of the K_{2P} transmembrane structure may uncover additional factors that influence K_{2P} dynamics and function.

Pinching and dilation of the K_{2P} the selectivity filter ‘C-type’ gate

Numerous studies have established the primacy of the selectivity filter (C-type) gate for K_{2P} function.^{72–74,76,77,90,94–96} This form of gating occurs in various potassium channel classes and displays a hallmark sensitivity to external potassium due to its dependency on interactions between the permeant ions and selectivity filter.^{73,74,76,97–101} The interdependency on channel:permeant ion interactions for function can be thought of as a type of ‘substrate assisted catalysis’ wherein interactions with the permeant ion are essential for maintaining the integrity of the selectivity filter active conformation. Although structural studies of exemplar homotetrameric potassium channels have uncovered various types of selectivity filter rearrangements attributed to C-type gating,^{100,102–108} whether the essence of potassium channel C-type gating involves pinching,^{100,102–105} dilation¹⁰¹, or more subtle selectivity filter changes^{106–108} has remained unresolved. Furthermore, even though structures of different K_{2P}s brought to light changes in M2 and M4 conformations that impact activity^{36,37,39,40,56–58}, these studies did not reveal selectivity filter conformational changes that could explain how K_{2P} C-type gating occurs (Figure 2(b)). Notably, all were determined under conditions of high concentrations (150–200 mM) of permeant ions. Thus, the extent to which K_{2P} C-type gating mechanisms are similar to those described for homotetrameric channels^{100,102–107} and whether the innate heterodimeric K_{2P} selectivity filter architecture conferred unique properties to their C-type gates had remained unclear.

Recent crystal structures of K_{2P}2.1 (TREK-1) determined under seven different potassium concentrations spanning 0–200 mM, alone and complexed with a small molecule C-type gate activator, ML335, together with molecular dynamics simulations demonstrated that K_{2P} selectivity filters use both pinching and dilation mechanisms to control the C-type gate.⁵³ These structures show that under low potassium concentrations (<50 mM [K⁺])

both selectivity filter elements that interact with the permeant ions, SF1 and SF2, undergo potassium-dependent structural rearrangements that are accompanied by the loss of the potassium ions at the S1 and S2 sites in the outer portion of the selectivity filter (Figure 5(a)). These conformational changes pinch SF1 at the extracellular mouth of the pore and dilate the exterior portion of SF2 and unfold the linker that connects SF2 to M4 (SF2-M4 loop) (Figure 5(a)). Hence, K_{2P} s use both types of mechanisms that have been proposed to control potassium channel selectivity filter C-type gates – pinching and dilation. Binding of the small molecule activator ML335 to a site behind the selectivity filter, the K_{2P} modulator pocket, completely suppresses these conformational changes and the loss of potassium ions at low potassium concentrations (Figure 5(b) and (c)).⁵³ These studies also identified an important functional role for the uniquely long SF2-M4 loop found in the K_{2P} family. This channel element is stabilized by a conserved hydrogen bond network, the ‘M3 glutamate network’, centered on Glu234 from the M3 helix, unfolds when SF2 dilates, and connects the C-type gate with gating cues sensed by other parts of the channel. Disruption of the M3 glutamate network by design in $K_{2P}2.1$ (TREK-1) and $K_{2P}3.1$ (TASK-1)⁵³ or by a $K_{2P}3.1$ (TASK-1) mutation associated with pulmonary hypertension⁹² impairs function and establishes the key role of this network and the SF2-M4 loop in diverse K_{2P} s even for those, such as $K_{2P}3.1$ (TASK-1), that have intracellular gates. Together, these findings show that asymmetric order–disorder transitions enabled by the K_{2P} heterodimeric architecture are at the heart of K_{2P} gating mechanisms.

Several other structural studies have also hinted at asymmetric motions in the K_{2P} SF gate. In the $K_{2P}10.1$ (TREK-2) ‘M4-down’ structures SF1, but not SF2, adopts a small inward pinch relative to its position in the ‘M4-up’ structure.⁴⁰ A similar motion has been observed in SF1 of the pH 6.5 structure of $K_{2P}5.1$ (TASK-2) relative to the pH 8.5 structure.⁴¹ In both cases, these changes distort the coordination environment of ions at the S0 and S1 positions of the selectivity filter and no ions were observed in those sites. These selectivity filter changes are not as extreme as those seen in $K_{2P}2.1$ (TREK-1)⁵³ and the degree to which they diminish ion permeation is not yet clear. Nevertheless, such local, asymmetric structural distortions of the selectivity filter further highlight the intrinsically heterodimeric character of the K_{2P} architecture.

Taken together, these findings support a model in which under basal conditions the K_{2P} C-type gate transits between an inactive state in which the K_{2P} selectivity filter and its supporting architecture are dynamic and has a low potassium ion occupancy^{53,77} to a rigidified, conductive state in which most potassium binding sites are occupied (Figure 5(d)).³⁷ Ion permeation requires limiting filter mobility which can be achieved by ligand binding to the K_{2P} modulator pocket or by conformational changes transmitted through the M4 helix.³⁷ These structural changes shift the conformational equilibrium of the channel to the active state, enable permeant ions to organize and stabilize the K_{2P} conductive state^{37,50,77} and may also be induced by the action of modulators that act on other sites in the channel.^{50,109} Developing a better understanding of the types of SF changes that can control K_{2P} function, how conformational changes in transmembrane elements affect the SF gate, and how diversification of intrinsically asymmetric K_{2P} architecture through heterodimer formation augments C-type gate function remain key unsolved issues.

The polysite pharmacology of K₂P_s

Although K₂P_s have been largely viewed as pharmacological orphans, this perspective is changing rapidly due to both structural studies and the ongoing identification and characterization of new K₂P modulators^{42,43,110} (Tables 1 and 2). Structural studies of K₂P_s complexed with different types of modulators show a remarkably rich structural landscape for functional control, especially given the modest size of these channels (~70 kDa). Binding sites for modulators have been found at every layer of the protein, starting from its extracellular side through the portion that interacts with the membrane bilayer inner leaflet (Figure 6). This polysite pharmacology comprises five structurally defined binding sites for small molecules or lipids arranged from the outside to the inside: the Keystone inhibitor site,⁵¹ the K₂P modulator pocket,³⁷ the Vestibule site,³⁸ the Fenestration site,^{40,50} and the Modulatory lipid site.³⁷ Each offers a distinct structural environment and mechanism for controlling K₂P function. As K₂P modulators have been the subject of many recent reviews,^{42,43,110,111} we focus here on the current state of knowledge of the K₂P structural pharmacology and try to provide context for some of the growing number of K₂P modulators whose sites of action remain to be structurally defined.

The Keystone inhibitor site – ‘Finger in the dam’ polynuclear ruthenium amines

The trinuclear oxo-bridged ruthenium amine polycation ruthenium red (RuR) (Figure 6, Table 1)¹¹² has many biological applications,¹¹³ including a ~50 year legacy as an inhibitor of diverse ion channels. RuR inhibits two TREK subfamily K₂P_s, K₂P4.1 (TRAAK)^{68,114} and K₂P10.1 (TREK2),¹¹⁴ as well as K₂P9.1 (TASK-3).^{115–117} Functional studies established that a negatively charged residue at the K₂P Cap domain base comprises a key RuR sensitivity determinant in natively RuR sensitive channels.^{115–117} Further, mutational studies showed that placing a negatively charged residue at the Cap base is sufficient for rendering a non-RuR sensitive K₂P responsive to RuR inhibition.^{51,114}

Structural studies of an RuR-sensitive K₂P2.1 (TREK-1) mutant, I110D,⁵¹ have recently revealed the mechanism by which this inhibition occurs (Figure 6). RuR inhibits K₂P_s using a 1:1 stoichiometry matching prior functional studies^{68,114} that places one ruthenium amine moiety directly over the channel pore at the site of the S0 ion, while the remainder of the RuR molecule occupies one of the two branches of the EIP (Figure 6). The negatively charged residues that form the RuR-sensitivity determinant at the Cap domain base constitute the ‘Keystone inhibitor site’ and directly coordinate RuR through a multipronged interaction to several RuR elements (Figure 6). Further structural and functional investigation demonstrated that the dinuclear ruthenium amine, Ru360 (Table 1),¹¹⁸ an inhibitor of the mitochondrial calcium uniporter^{119–121} not previously known to affect potassium channels, also binds to the Keystone inhibitor site in a similar way to RuR, although with lower affinity, likely due to its decreased positive charge.⁵¹ Replacing the SF1 Asn147 at the SF external mouth with an acidic residue in conjunction with the I110D mutation yielded designer RuR super-responders having IC₅₀s in the low nanomolar range.⁵¹ Because of the shared K₂P pore architecture, combination of this protein engineering strategy with efforts to make novel polyruthenium amine derivatives¹²² could provide new means for exploring the function of any K₂P subtype. Together, these studies define a ‘finger in the dam’ mechanism in which polynuclear ruthenium amines binding to the Keystone

inhibitor site creates an electrostatic and physical barrier that prevents the flow of potassium ions through the selectivity filter. The ability of these relatively simple compounds to reach through the EIP to stop function raises the possibility that other classes of molecules could work using the same mechanism. In particular, biologics, such as nanobodies or peptide toxins, may be particularly suited to exploiting this unique feature of the EIP.

The K_{2P} modulator pocket – A cryptic small molecule binding site for K_{2P} control

Structures of $K_{2P2.1}$ (TREK-1) complexed with two novel activators, ML335 (N-[(2,4-dichlorophenyl)methyl]-4-(methanesulfonamido) benzamide) and ML402 (N-[2-(4-chloro-2-methylphenoxy)ethyl] thiophene-2-carboxamide), revealed the presence of an L-shaped pocket behind the selectivity filter in the P1-M4 interface, the K_{2P} modulator pocket (Figure 6, Table 2). This small molecule binding site resides in an intersubunit interface involved in C-type gating^{72,73} and is unrelated to any previously known small molecule binding pocket in the VGIC superfamily.³⁷ Both compounds bind in similar ways, act as molecular wedges that stabilize the P1-M4 interface, and directly activate the channel selectivity filter C-type gate.^{37,53} In the unliganded structure, P1-M4 interface interactions occlude the K_{2P} modulator pocket. Small movements of a few residues are required for the compounds to bind, making this a cryptic site that relies on conformational change, similar to cryptic sites described for soluble proteins.¹²³ The intersubunit interface that these two compounds stabilize is also the site of GOF mutations^{57,72,73} and highlights the general importance of intersubunit interfaces for channel control.

ML335 and ML402 show a strong subtype selectivity, activating $K_{2P2.1}$ (TREK-1) and $K_{2P10.1}$ (TREK-2) but not $K_{2P4.1}$ (TRAAK).³⁷ Selectivity originates from a single lysine residue on the N-terminal end of M4 that makes a cation- π interaction with the upper ring of the activators (Figure 6). The equivalent $K_{2P4.1}$ (TRAAK) residue is a glutamine and exchanging $K \rightarrow Q$ in $K_{2P2.1}$ (TREK1) and $Q \rightarrow K$ in $K_{2P4.1}$ (TRAAK) at this position is sufficient for rendering the former insensitive to ML335 and ML402 activation and the later sensitive to activation by both compounds.³⁷ The importance of a single amino acid difference in an otherwise conserved small molecule binding pocket underscores the potential for exploiting local differences and structural knowledge to develop subtype-selective K_{2P} modulators.

There are no known natural ligands for the K_{2P} modulator pocket, but given that P1-M4 interface is central for integrating gating cues that arise in other parts of the protein, particularly the C-terminal tail,^{37,53,72,73} it seems likely that endogenous compounds such as lipids, metabolites, signaling molecules, or regulatory proteins may target this site. Defining its roles in other K_{2P} subfamilies and identifying natural effectors for this site remain important directions for future study.

The Fenestration site – A dual-action binding site

The M4 transmembrane helix is a key moving part of K_{2Ps} and in the TREK subfamily this element conveys gating cues from temperature,^{57,72,73,81} pressure,^{73,81,82} and phosphorylation⁷² to the C-type gate. The M4 ‘down’ state creates an opening just below the P2 pore helix that faces the center of the membrane bilayer,^{36,39,57,58} the ‘Fenestration

site'. Structural studies of K_{2P}10.1 (TREK-2) have demonstrated that the K_{2P} inhibitors fluoxetine and norfluoxetine bind here⁴⁰ (Figure 6, Table 1). Remarkably, crystal structures of a K_{2P}10.1 (TREK2) complex with a brominated version of an activator, BL-1249, although not defining density for the entire compound, strongly indicate that this molecule, and perhaps other activators, also bind to the Fenestration site created by the 'down' M4 position.⁵⁰ Hence, the Fenestration site has the curious property of being a dual-action site where ligand binding can either stimulate or suppress channel activity.

How can the binding of a small molecule to the same site yield opposite functional outcomes of inhibition and activation? Clearly, the answer cannot be in stabilization of the M4 'down' state over the 'up' state, as the binding of both inhibitors and activators to the Fenestration site requires an M4 'down' conformation.^{40,50} Given the location of the Fenestration site just below the selectivity filter, one plausible mode of action would be for Fenestration site binders to affect the C-type gate conformational dynamics.⁸⁹ Additionally, Fenestration site activators, such as BL-1249, may stabilize the filter by using a negatively charged moiety to recruit potassium ions to the aqueous cavity and thereby alter ion site occupancies in the selectivity filter.⁵⁰ Notably, the Fenestration site is found throughout the VGIC superfamily and serves as the site of action for multiple types of activators of different classes of potassium channels.⁵⁰ Understanding the relationship between Fenestration site occupation and its effects on the selectivity filter C-type gate, how permeant ion recruitment activates the SF gate, and the properties of activators and inhibitors that can inhabit this site is an important challenge for the future development of K_{2P} modulators. Further, unlike the K_{2P} modulator pocket which is composed of elements of the same subunit chain, the Fenestration site comprises elements from both chains of the K_{2P} dimer (Figures 4(c) and 6). Hence, it has potential as a site for the discovery or design of compounds that exploit these differences to achieve selective modulation of homodimeric *versus* heterodimeric K_{2P}s.

The Vestibule site – X-gate traps

Potassium channels can be blocked by compounds such as quaternary amines that enter the central cavity and block ion flow through the filter.^{124–126} K_{2P}s share this property (Table 1) and it has been exploited to probe various features of the K_{2P} inner pore.^{50,74} Recently, structures of K_{2P}3.1 (TASK-1) complexed with two different inhibitors, BAY10000493 ((4-([2-(4-bromophenyl)imidazo[1,2-a]pyridin-3-yl)methyl]piperazin-1-yl)(2-fluorophenyl)methanone) and BAY2341237 (4-([2-(4-chlorophenyl)imidazo[1,2-a]pyridin-3-yl)methyl]piperazin-1-yl)[6-(trifluoromethoxy)pyridin-2-yl]methanone) have revealed a novel mode in which these compounds are trapped in the intracellular vestibule by the closure of the cytoplasmic X-gate.³⁸ Notably, neither compound interacts directly with the X-gate, but rather makes intimate contacts to the upper part of the vestibule (Figure 6). Nevertheless, the stability of the X-gate can affect how readily these compounds can be washed out of the channel.³⁸ Both compounds also make many contacts to residues from each subunit, raising the possibility that it may be possible to identify derivatives that can distinguish homomeric from heteromeric channels. Understanding the interplay between compounds that bind to the vestibule site and the motions of the X-gate is an interesting new direction that will likely aid in the development of other compounds that can affect K_{2P}s at the vestibule site.

The Modulatory lipid site – PIP₂ and the C-terminal tail

The signaling lipid PIP₂ is an important modulatory lipid for TREK subfamily channels.^{127–129} The likely PIP₂ site of action has been located in a series of K_{2p}2.1 (TREK-1) structures.^{37,53} These show the presence of a phospholipid that copurified with the channel and that was bound to a groove formed by the M1/M2/M4 interface (Figure 6). The phosphoinositol headgroup contacts an electropositive patch on the C-terminal tail comprising five residues implicated in PIP₂ modulation (Arg297, Lys301, Lys302, Lys304, and R311)^{127,128} (Figure 6). This region also contains two other regulatory sites, the Glu306 intracellular proton sensor¹³⁰ and Ser300 inhibitory phosphorylation site.¹³¹ Notably, the PIP₂-interacting residues are in a part of the M4 helix that is most affected by movements of between the ‘up’ and ‘down’ positions. Hence, it seems likely regulation of these channels by the modulatory lipid, intracellular pH sensor, and phosphorylation site are all tightly intertwined with M4 motions.¹³² Further study is needed to unravel questions about these interactions such as probing whether other activatory lipids such as phosphatidyl serine and phosphatidic acid^{127,133} compete with PIP₂ at this site, whether this site can be targeted by small molecules, and to define how changes in this lower part of the channel impact the dynamics and function of the C-type gate. Further, the effects of PIP₂ on K_{2p}2.1 (TREK-1) are complex, involving activation and inhibition.^{128,134} Determining if the Modulatory lipid site has a role in both actions, whether there are additional lipid binding sites, and whether other activating lipids that bind TREK subfamily members^{134,135} (Table 2) affect this site or other parts of the channel is an important direction for future studies.

Members of the TASK and TALK subfamilies are also known to bind and be modulated by specific lipids^{129,136} and recent structures have begun to hint at the molecular details of these interactions. Structures of K_{2p}3.1 (TASK-1) revealed a potential binding site for cholesterol in a site contacting M1, M2, M4, and key elements of the X-gate that is roughly spatially coincident with K_{2p}2.1 (TREK-1) PIP₂ site.³⁸ Mutations at this site and depletion of cholesterol from bilayers in functional experiments modify K_{2p}3.1 (TASK-1) activity, indicating a role for cholesterol or its analogues in stabilizing X-gate conformations. K_{2p}5.1 (TASK-2) is known to be activated by a variety of phosphatidylinositol lipids that interact with its C-terminal tail.¹³⁶ The K_{2p}5.1 (TASK-2) structures show that a polybasic region suspected to be involved in these interactions exists at least in part as a membrane parallel helical extension of M4, similar to the electropositive PIP₂ binding patch on M4 in K_{2p}2.1 (TREK-1).⁴¹ While most of the molecular details of how lipids regulate K_{2ps} remain to be elucidated, it is intriguing that many key determinants for lipid modulation are at the distal end of the movable M4 helix, suggesting that they exploit its motion to convey gating commands to the C-type gate.⁷² Given that most of the K_{2p} mass is solvated by the lipid bilayer, emerging approaches such as direct measure of K_{2p}:lipid interactions by mass spectrometry¹³⁵ and the ability to image K_{2ps} in lipid environments⁴¹ will have a key role in uncovering the variations on the use of the Modulatory lipid site and existence of other specific lipid binding sites housed with the K_{2p} architecture.

General anesthetics – A site for gases in the pore

Various type of general anesthetics comprise one of the best studied K_{2p} modulator classes.⁴³ Volatile anesthetics such as halothane and isoflurane activate K_{2p}2.1 (TREK-1),

K₂P9.1 (TREK-2), K₂P3.1 (TASK-1), K₂P9.1 (TASK-3) and K₂P18.1 (TRESK) (Table 2).^{137,138} Although no structural information is yet available about where such molecules bind, a number of studies indicate that they have a specific binding site within the K₂P inner cavity^{139–143}. In particular, the recent use of an unbiased photoaffinity labeling approach utilizing the isoflurane derivative azi-isoflurane combined with mass spectrometry has identified a key glycine in M2 of K₂P2.1 (TREK-1) that sits at the junction where M2, M3, and M4 converge¹⁴³ (Figure 4(c), Table 2). This site is a hotspot for residues involved in responses to stretch, thermal responses, and the activator BL-1249.^{40,57,58,109} These findings set the stage for further studies of how anesthetics influence K₂P function that will be important for resolving the ongoing debates about the relative contributions of direct binding modes versus proposed mechanisms of indirect modulation,¹⁴⁴ defining whether other activators of the TREK and TRESK families, such as chloroform, nitrous oxide, and xenon (Table 2) target the same region, and defining why some anesthetics such as halothane activate K₂P2.1 (TREK-1)¹³⁷ but inhibit K₂P13.1 (THIK-1) and K₂P12.1 (THIK-2).¹⁴⁵

K₂P modulators - structural matchmaking

The desire to turn K₂Ps into a channel class with good pharmacology has produced a growing list of activators and inhibitors (Tables 1 and 2).^{42,43,110,111} The mechanisms of action and binding sites for these modulators remain to be defined by direct methods such as X-ray crystallography, cryo-electron microscopy, or chemical crosslinking. Here, we recount some of these exciting molecules, particularly those where docking or mutagenesis studies have provided suggestions for potential binding sites and place them in the context of the defined structural pharmacology sites mentioned above.

Vestibule site modulators – An investigative TASK to be undertaken

Many potassium channels are blocked by compounds that act within the central cavity^{124–126,146} and functional studies have long indicated that K₂Ps share this proclivity for block from the intracellular side^{74,142,147} (Table 1). Structural definition of the Vestibule site delimited by the X-gate in K₂P3.1 (TASK-1)³⁸ together with prior studies identifying the TASK subfamily central cavity as a hotspot for the action of a varied antagonists¹¹¹ highlight the potential for modulators to act in the K₂P inner cavity. Mutational and computational studies with antagonists A1899,¹⁴⁷ A293,¹⁴⁷ PK-THPP,¹⁴⁸ doxapram,^{142,149} ML365¹⁵⁰ and DR16.1¹⁵¹ indicate they share key interactions with hydrophobic residues directly below the selectivity filter and inhibit channel activity by directly blocking the channel pore (Table 1).^{142,147,152,153} While small differences between K₂P3.1 (TASK-1) and K₂P9.1 (TASK-3) lead to differences in the potency and selectivity of these blockers, the chemical structures of the inhibitors share a similar topology, incorporating aromatic rings, hydrogen bond acceptors and hydrophobic groups.¹¹¹ Aristolochic acid (AristA), a compound identified in traditional medicinal plants, was found to activate K₂P2.1 (TREK-1) and K₂P10.1 (TREK-2) while having no effect on K₂P4.1 (TRAAK) and causing inhibition of K₂P18.1 (TRESK) (Table 2).¹⁵⁴ Mutations at residues F145 and F352 in the presumptive K₂P18.1 (TRESK) inner pore ablated the blocking effect of AristA,¹⁵⁴ a result that suggests that AristA binds in the inner cavity. It would seem that exploiting differences between K₂Ps that have an inner gate and those that do not might be a productive direction for developing subfamily specific compounds that act at the Vestibule site. Further, whether

there are variations on the theme of the Vestibule site and whether multiple, distinct binding pockets exist within the K_{2P} inner cavity remains to be discovered.

Fenestration site – Or very close

Nonsteroidal anti-inflammatory fenamates such as flufenamic acid (FFA) activate members of the TREK subfamily^{155–157} and $K_{2P}18.1$ (TRESK).¹⁵⁸ Based on the behavior of the related compound BL-1249^{50,109} the Fenestration site seems like a strong candidate for their site of action (Table 2). It is thought that the negatively charged tetrazole moiety of BL-1249 creates a binding site for potassium ions in the central cavity and thereby stabilizes the selectivity filter C-type gate.⁵⁰ Similar structural motifs can be found in FFA as well as other suspected Fenestration site activators ML67–33^{44,50} and DCPIB⁵⁰ (Table 2), raising the possibility that these structurally diverse compounds activate K_{2P} s using this shared mechanism. Based on a combination of mutagenesis and molecular dynamics simulations, the local anesthetic bupivacaine has also been proposed to bind at the Fenestration site¹⁵⁹ (Table 1). Other local anesthetics, such as lidocaine and ropivacaine, by virtue of their structural similarity to bupivacaine, seem likely to bind at the Fenestration site to inhibit these channels. The effect of local anesthetics may be more complicated, as in addition to direct action, they have been proposed to indirectly antagonize $K_{2P}2.1$ (TREK-1) through inhibition of phospholipase D2 (PLD2).¹⁶⁰ A highly selective $K_{2P}9.1$ (TASK-3) agonist, CHET3 (Table 2), has also been predicted to bind underneath the selectivity filter between the M2 and M4 helices in an analogous position to BL-1249.⁴⁹ The $K_{2P}2.1$ (TREK-1) inhibitor, TKIM (Table 1) may also bind in the inner cavity.¹⁶¹ Defining the extent to which these and other modulators bind at the Fenestration site, the Vestibule site, or some variation of both, and whether conformational changes in the position of M4 are linked to action at such sites should provide new perspectives on the richness and druggability of what is clearly a hotspot of K_{2P} modulation by small molecules.

K_{2P} modulator pocket – Will it open for other modulators?.—No natural or synthetic activators of the K_{2P} modulator pocket besides ML335 and ML402 are yet known. However, two small molecule screens have identified TREK subfamily activators that may act on the K_{2P} modulator pocket or its surrounding elements. Mutagenesis and computational modelling suggest that C3001a acts at the K_{2P} modulator pocket, although perhaps in a different manner than ML335 and ML402 (Table 2).¹⁶² T2A3 and the bioactive lipid 11-deoxy prostaglandin F2a were identified as highly specific $K_{2P}10.1$ (TREK-2) activators through a high-throughput thallium flux screen (Table 2).⁴⁵ Functional studies of $K_{2P}2.1$ (TREK-1)/ $K_{2P}10.1$ (TREK-2) chimeras identified a short amino acid sequence in the SF2-M4 loop that governs the $K_{2P}10.1$ (TREK-2) selectivity of both activators.⁴⁵ The SF2-M4 loop is a key element of the K_{2P} modulator pocket, is one of the few divergent portions of these two TREK subfamily channels (Figure 2(A)), and has an important role in C-type gating.⁵³ Interestingly, the chemical structure of T2A3 is strikingly similar to ML335 and ML402. The main difference is the presence of an additional benzoate moiety, which is attached to part of the shared scaffold known as the ‘upper ring’.³⁷ Hence, it seems likely that T2A3 would bind $K_{2P}10.1$ (TREK-2) in a pose similar to ML335 and ML402 in which the T2A3 benzoate would protrude from the K_{2P} modulator pocket and enable T2A3 to interact with the SF2-M4 loop in a way that capitalizes on the net charge difference

between the K_{2P}10.1 (TREK-2) and K_{2P}2.1 (TREK-1) SF2-M4 loops (+1 versus -2 net charge at pH 7.0, respectively).¹⁶³ These structural features could explain the selective activation of K_{2P}10.1 (TREK-2) by T2A3 and its ability to inhibit K_{2P}2.1 (TREK-1) at high concentrations. Further investigation into the pharmacological basis of how K_{2P} modulator pocket binders can exploit differences among the SF2-M4 loops, which is one of the most diverse elements of this binding site (Figure 2(a)), could offer routes to more potent and selective K_{2P} modulators.

How many more modulatory sites are packed into the K_{2P} structure?—The five structurally defined modulator sites within the compact K_{2P} 70 kDa frame provide a surprisingly rich framework for modulators to influence K_{2P} function. Intriguingly, there are suggestions that the count of sites where external agents can influence K_{2P} function will not stop at five. For example, the small molecule TKDC, an inhibitor of the TREK subfamily (Table 1), has been proposed to act on a site on the external part of the Cap.¹⁶⁴ 2-APB, an activator of K_{2P}2.1 (TREK-1) and K_{2P}10.1 (TREK-2) has been proposed to have a binding site within the channel C-terminal domain based on truncation studies¹⁶⁵ (Table 2). Pranlukast, a leukotriene receptor antagonist, is a relatively selective K_{2P}10.1 (TREK-2) activator (Table 2)¹⁶⁶ that based on mutational studies does not act at either the K_{2P} modulator pocket or the BL-1249 site, leaving open the possibility that other sites that mediate activation are present in the K_{2P} framework. While the proposed modes of action of these compounds as well as the many other inhibitors and activators (Tables 1 and 2) require structural validation, there is good reason to believe that the number of sites comprising of the polysite pharmacology of K_{2P}s will increase.

Perspectives.—The diversity of moving parts that affect K_{2P} function and the many modulator binding sites housed within the compact K_{2P} architecture underscores the structural complexity that undergirds channels known by the deceptively simple moniker of 'leak potassium channels'. Understanding how the various K_{2P} moving parts work together to control function and respond to natural gating cues remains a crucial challenge. Without question, structure determination efforts will continue to deepen our understanding of how gating cues impact the SF gate and inform the extent to which other K_{2P}s can be classified into those having an inner gate and those without such a feature. Promising lines of inquiry include obtaining structures of the remaining uncharacterized K_{2P}s homodimers, structures of K_{2P}s in lipid environments, additional structures of K_{2P}:modulator complexes, especially for those whose binding sites are not yet clear (Tables 1 and 2), and structures of K_{2P} heterodimers. Given the diverse structural sites where modulator binding can influence function, sorting out the synergies among the defined modulator binding sites and whether multiple modulators can work in concert to influence function is also a key next step. The question of the role of domain-swapping in function remains unsolved. Given how tightly intertwined the subunits in the domain-swapped configuration are, understanding how this feat of architectural gymnastics comes about during channel biogenesis and assembly and the extent to which such intertwining can be undone during the life cycle of a K_{2P} channel remains an intriguing and unaddressed direction.

The importance of K_{2P} heterodimer formation is becoming ever more appreciated,⁷¹ and a key open question is how heterodimer formation affects the structure and dynamics of K_{2P} relative to the homomeric isoforms. As the Keystone inhibitor site, the Fenestration site, and the Vestibule site have contributions from each subunit, variations in the residues that comprise these sites has the potential to provide another layer of diversification in function and perhaps pharmacology between homodimer and heterodimer K_{2P}s. Further, in cells K_{2P}s are not lone actors but are part of larger assemblies of signaling and scaffolding proteins.¹⁶⁷ The involvement of K_{2P}s in multiprotein assemblies is still understudied and steps to understand how the formation of K_{2P}-centered protein complexes affect function, structure, and pharmacology is a key direction for addressing the diverse roles of this channel family in physiologically relevant settings. Together, these recent advances in K_{2P} structure and structural pharmacology, the ever growing appreciation of the importance of K_{2P}s in physiological and pathophysiological function,^{9,43,168–170} and the key K_{2P} frontier questions outlined here show that these ‘leak channels’ are far more fascinating and important than their much maligned name might suggest.

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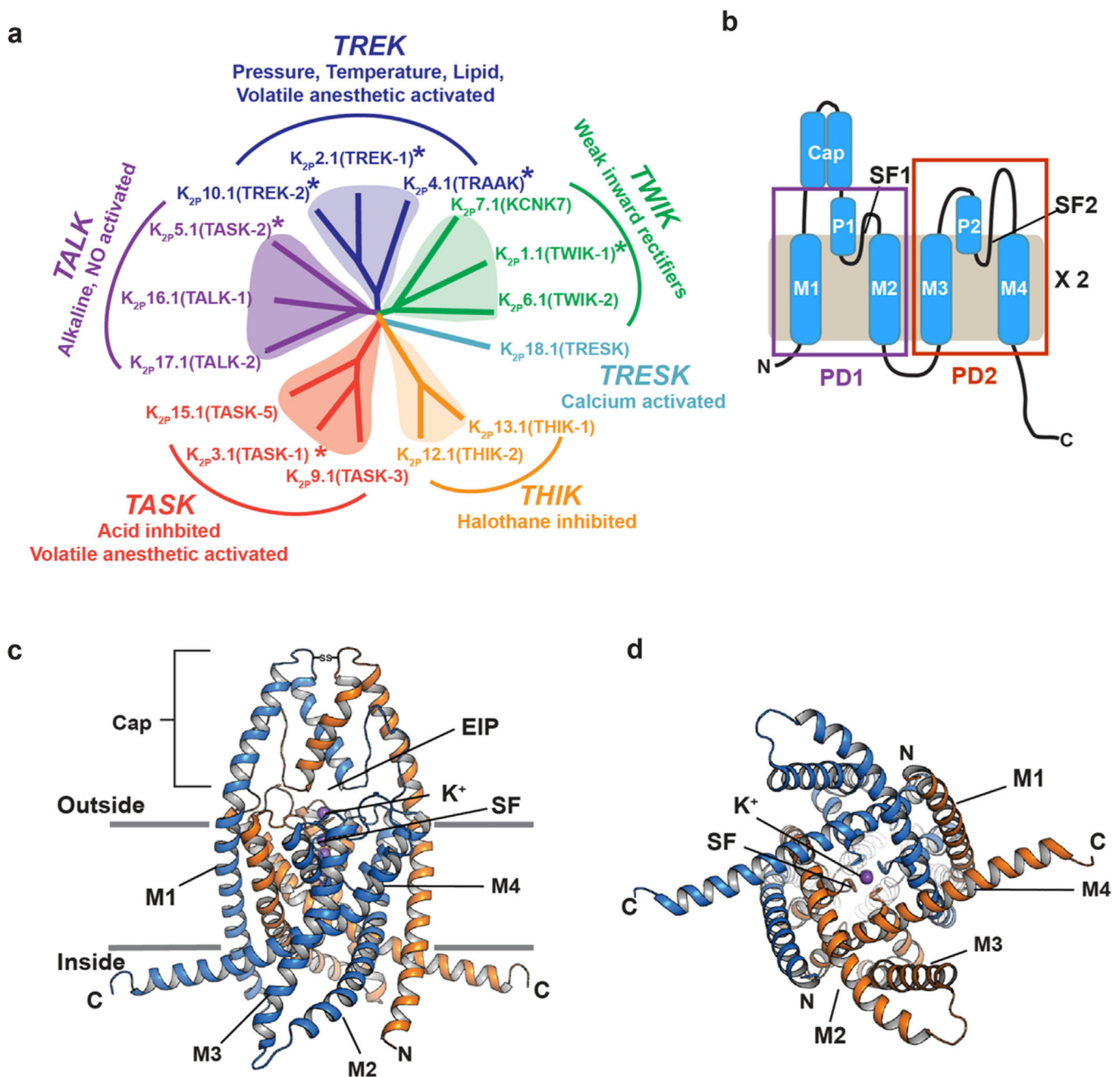
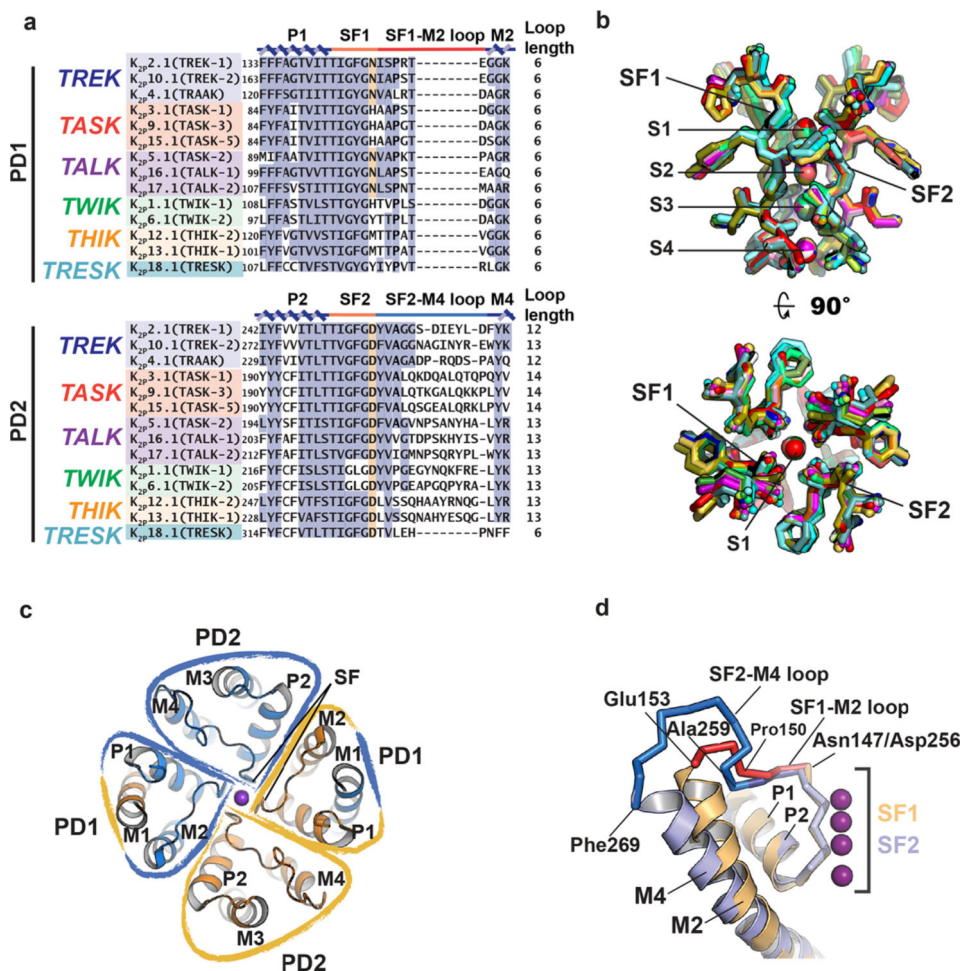


Figure 1. K_{2p} channel family relationships and structure. (a) K_{2p} family dendrogram. Subfamilies and key characteristics are indicated. Asterisks indicate structurally characterized K_{2ps} . (b) K_{2p} subunit diagram. Pore domains 1 and 2 (PD1 and PD2), transmembrane helices (M1–M4), pore helices (P1 and P2), selectivity filters (SF1 and SF2), and Cap domain are indicated. (c and d) Cartoon diagram of the $K_{2p}2.1$ (TREK-1) structure (PDB:6CQ6).³⁷ Chains are colored marine and orange. Potassium ions are purple. Grey lines indicate membrane. c, side view, (d) cytoplasmic view. Channel elements are labeled as in ‘b’. ‘EIP’ denotes the extracellular ion pathway.

**Figure 2.**

K_{2p} pore region structure. (a) Sequence alignment of selectivity filter and loop regions of PD1 and PD2 for the indicated channels. P1, M2, P2, and M4 helices (blue), SF1 and SF2 (orange), SF1-M2 loop (red), and SF2-M4 loop (light blue) are indicated. Terminal residue of the selectivity filter is highlighted. (b) Superposition of the selectivity filters and permeant ions for: K_{2p}1.1 (TWIK-1) (3UKM)³⁶ (red), K_{2p}2.1 (TREK-1) (6CQ6)³⁷ (smudge), K_{2p}2.1 (TREK1):ML335 (6CQ8)³⁷ (deep salmon), K_{2p}2.1 (TREK-1):ML402 (6CQ9)³⁷, K_{2p}3.1 (TASK-1) (6RV2) (orange)³⁸, K_{2p}3.1 (TASK-1):BAY1000493 (6RV3) (yellow orange)³⁸, K_{2p}3.1 (TASK-1):BAY2341237(6RV4) (olive)³⁸, K_{2p}4.1 (TRAAK) (3UM7)³⁹ (aquamarine), (4I9W)⁵⁶ (limon), (4WFE) (forest green)⁵⁸, (4WFF) (white)⁵⁸, (4WFG) (grey)⁵⁸, (4WFH) (black)⁵⁸, K_{2p}4.1 (TRAAK) G124I (4RUE) (blue)⁵⁷, K_{2p}4.1 (TRAAK) W262S (4RUF) (lime green)⁵⁷, K_{2p}5.1 (TASK-2) pH 6.5 (6WLV) (deep olive)⁴¹, K_{2p}5.1 (TASK-2) pH 8.5 (6WM0) (light teal)⁴¹, K_{2p}10.1 (TREK-2) (4BW5)⁴⁰ (pink), (4XDJ)⁴⁰ (magenta), (4XDK)⁴⁰ (purple). SF1, SF2 and ion binding positions, S1-S4, are indicated. Ions are shown as spheres and colored according to the parent structure. (c) K_{2p} pore domains (PD1 and PD2) highlighting the inherent heterotetrameric nature of the pore and the domain-swapped positions of M1. (d) Superposition of K_{2p}2.1 (TREK-1) (PDB:6CQ6)³⁷ P1-SF1-M2 (orange) and P2-SF2-M4 (slate). SF1-M2 loop (red) and SF2-M4 loop (blue) and portions having a shared conformation (dark blue) are indicated.

Residue labels indicate the SF1-M2 and SF2-M4 loop ends and structural divergence point (Pro150/Ala259). GenBank sequences in 'a' are: K_{2p}2.1 (TREK-1), AAD47569.1; K_{2p}10.1 (TREK-2), AAL95705.1; K_{2p}4.1 (TRAAK), AAF64062.1; K_{2p}3.1 (TASK-1), AAC51777.1; K_{2p}9.1 (TASK-3), AAF63708.1; K_{2p}15.1 (TASK-5), AAG33127.1; K_{2p}5.1 (TASK-2), AAC79458.1; K_{2p}16.1 (TALK-1), AAK49532.1; K_{2p}17.1 (TALK-2), AAK49533.1; K_{2p}1.1 (TWIK-1), AAB01688.1, K_{2p}6.1 (TWIK-2), AAD22980.1; K_{2p}12.1 (THIK-2), AAG32313.1; K_{2p}13.1 (THIK-1), AAG32314.1; K_{2p}18.1 (TRESK), BAC78527.1.

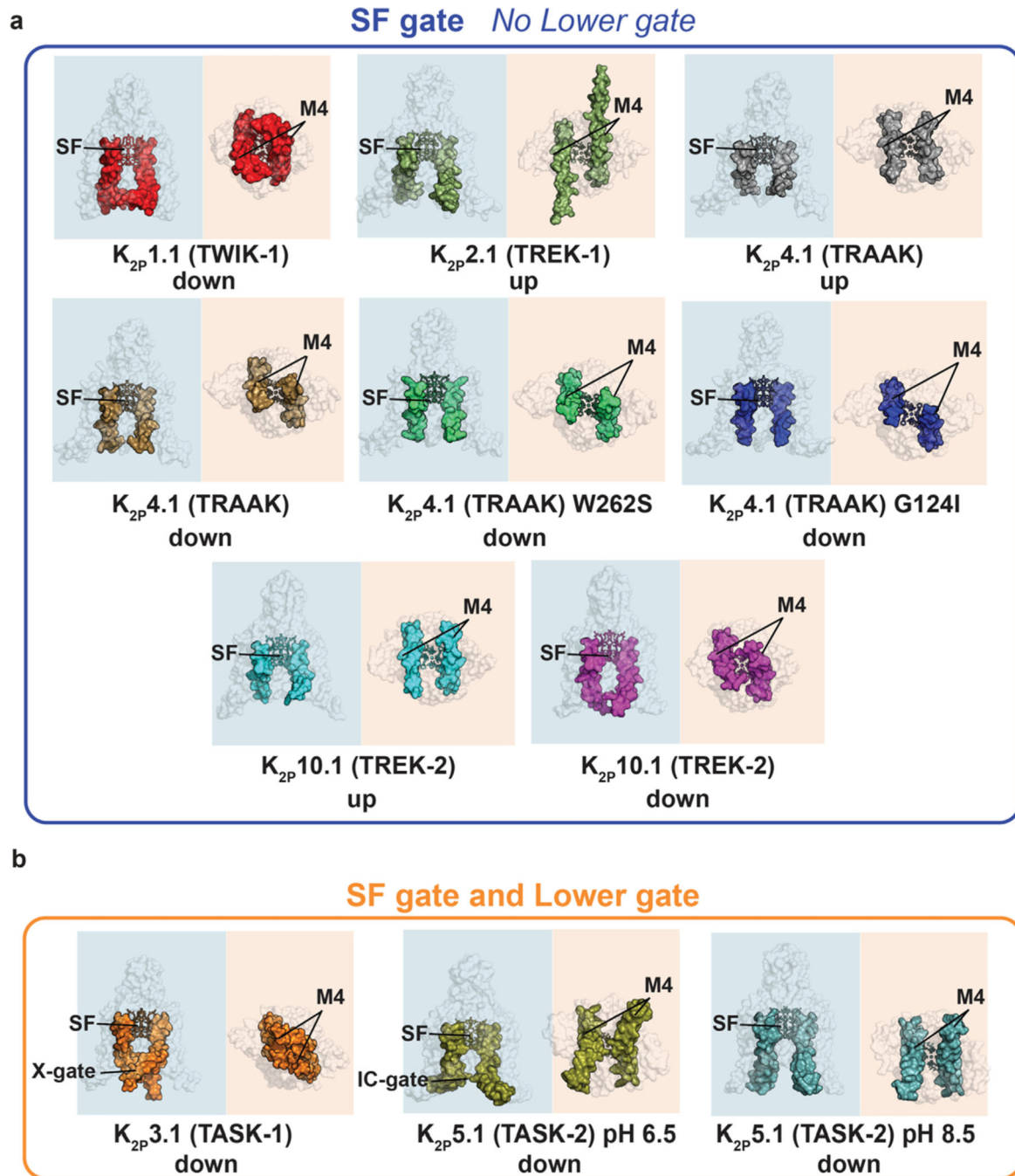


Figure 3.

K_{2P} structure comparison highlighting the M4 position and presence or absence of a lower gate. (a) K_{2P} s having only the SF gate: $K_{2P1.1}$ (TWIK-1) (PDB:3UKM),³⁶ $K_{2P2.1}$ (TREK-1) (PDB:6CQ6),³⁷ $K_{2P4.1}$ (TRAAK) (PDB:4WFG),⁵⁸ $K_{2P4.1}$ (TRAAK) (PDB:4WFF),⁵⁸ $K_{2P4.1}$ W262S (PDB:4RUF),⁵⁷ $K_{2P4.1}$ (TRAAK) G124I (PDB:4RUE),⁵⁷ $K_{2P10.1}$ (TREK-2) (PDB:4BW5),⁴⁰ $K_{2P10.1}$ (TREK-2) (PDB:4XDJ).⁴⁰ (b) K_{2P} s having both an SF gate and a Lower gate $K_{2P3.1}$ (TASK-1) (PDB:6RV2)³⁸ and $K_{2P5.1}$ (TASK-2) pH 8.5 (PDB:6WM0).⁴¹ SF, M4, X-gate, Intracellular gate (IC gate) are indicated. ‘Up’ and

'down' indicate M4 position. Shading indicates view from the side (blue) and intracellular face (orange).

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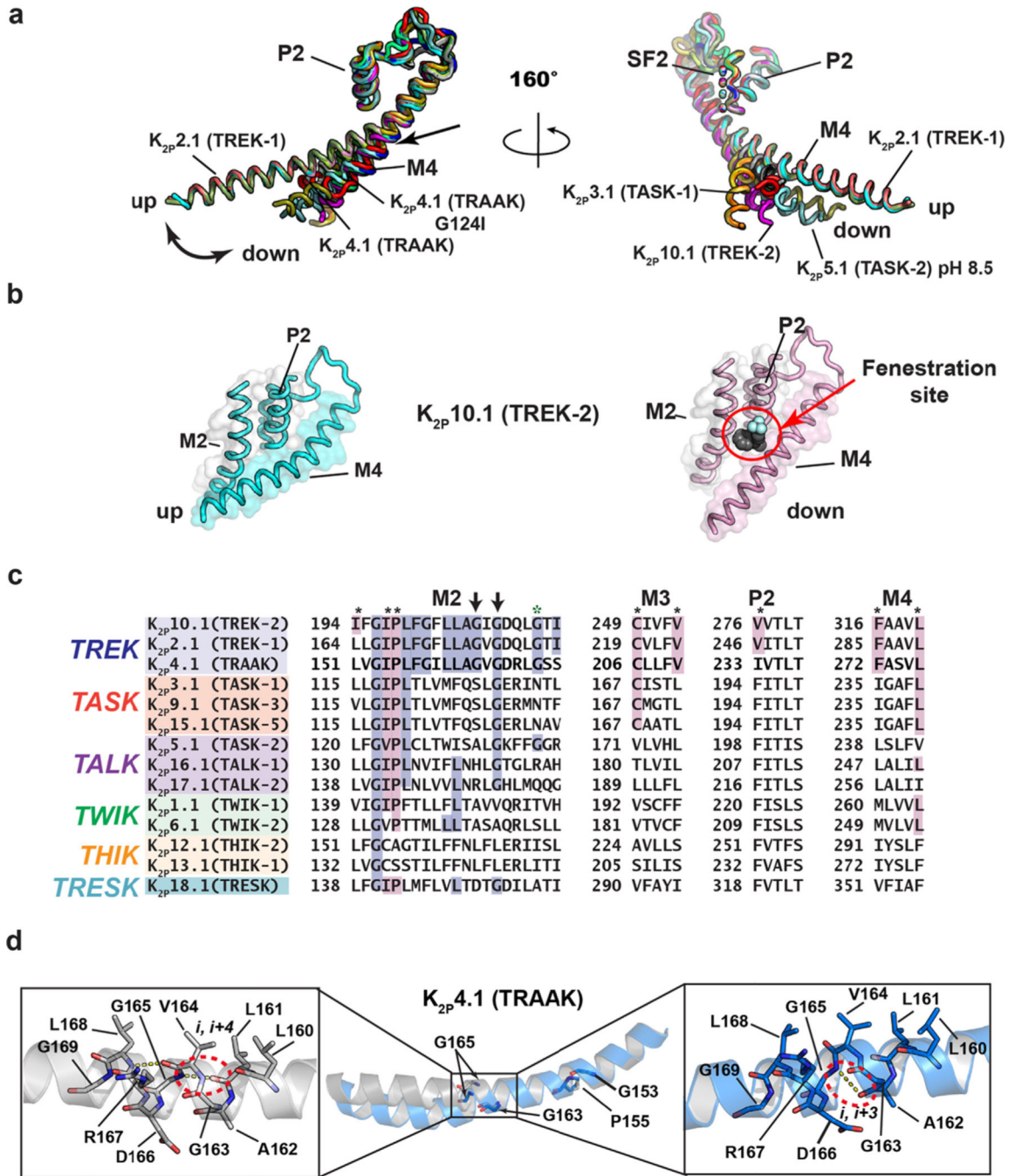


Figure 4.

K_{2P} transmembrane moving parts. (a) Superposition of the P2-SF2-M4 portion for: K_{2P}1.1 (TWIK-1) (3UKM)³⁶ (red), K_{2P}2.1 (TREK-1) (6CQ6)³⁷ (smudge), K_{2P}2.1 (TREK-1):ML335 (6CQ8)³⁷ (deep salmon), K_{2P}2.1 (TREK-1):ML402 (6CQ9)³⁷ (yellow orange),³⁸ K_{2P}3.1 (TASK-1) (6RV2) (orange),³⁸ K_{2P}3.1 (TASK-1):BAY1000493 (6RV3) (yellow orange),³⁸ K_{2P}3.1 (TASK-1):BAY2341237(6RV4) (olive),³⁸ K_{2P}4.1 (TRAAK) (3UM7)³⁹ (aquamarine), (4I9W)⁵⁶ (limon), (4WFE) (forest green),⁵⁸ (4WFF) (white),⁵⁸ (4WFG) (grey),⁵⁸ (4WFH) (black),⁵⁸ K_{2P}4.1 (TRAAK) G124I (4RUE) (blue),⁵⁷ K_{2P}4.1 (TRAAK)

W262S (4RUF) (lime green),⁵⁷ K_{2P}5.1 (TASK-2) pH 6.5 (6WLV) (deep olive),⁴¹ K_{2P}5.1 (TASK-2) pH 8.5 (6WM0) (light teal),⁴¹ K_{2P}10.1 (TREK-2) (4BW5)⁴⁰ (pink), (4XDJ)⁴⁰ (magenta), (4XDK)⁴⁰ (purple). Select structures are indicated. Arrow indicates point of M4 bend. (b) View of the ‘up’ (left) and ‘down’ (right) M4 conformations in K_{2P}10.1 (TREK-2). Fenestration site is occupied by norfluoxetine (space filling) and is indicated by the red circle. (c) Sequence comparisons of the M2 helix and elements that frame the Fenestration site. Residues that interact with the Fenestration site ligand are indicated by the black asterisks and are highlighted in magenta (cf. Figure 6). TREK subfamily ‘GVG’ sequence is indicated by the arrows. Green asterisk indicates the position of azo-isoflurane labeling.¹⁴³ (d) Buckling at the conserved TREK family ‘GVG’ sequence in K_{2P}4.1 (TRAAK). Dashed red oval highlights the site of the $i, i + 4$ to $i, i + 3$ hydrogen bond shift. Residue numbers are from human K_{2P}4.1 (TRAAK) PDB:4RUE.⁵⁷ Genbank sequences in ‘c’ are the same as those in Figure 2(a).

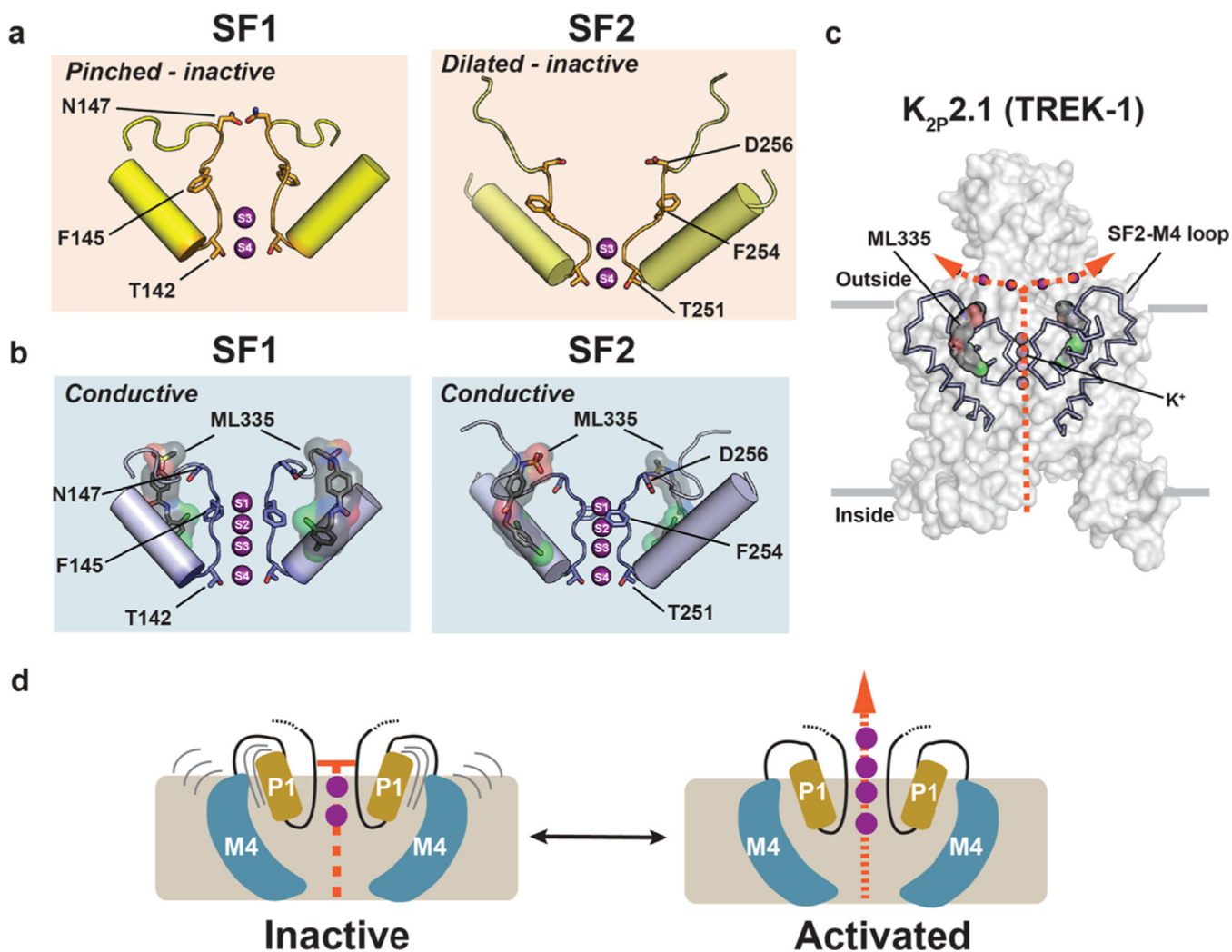


Figure 5. Structural changes in the K_{2p} selectivity filter C-type gate. (a) SF1 and SF2 structures in low [K⁺] showing the pinched and dilated conformations. (b) SF1 and SF2 structures in low [K⁺] showing the conductive conformation with ML335 (surface and sticks). (c) Structure of K_{2p}2.1 (TREK-1) (solid) highlighting SF2 and the surrounding structure (wireframe), ML335 (black, space filling), and potassium ions (purple spheres). Orange arrows show the flow of K⁺ through the channel. Grey bars indicate the membrane. (d) Model for K_{2p} gating at the K_{2p} C-type gate. Grey lines indicate mobile elements. Only P1 and M4 are depicted for simplicity. Activation involves rigidification of the SF gate elements and increased ion flow. K⁺ ions are shown as purple spheres. ML335 is shown in space filling. In 'a' and 'b' ion positions and select SF residues are indicated.

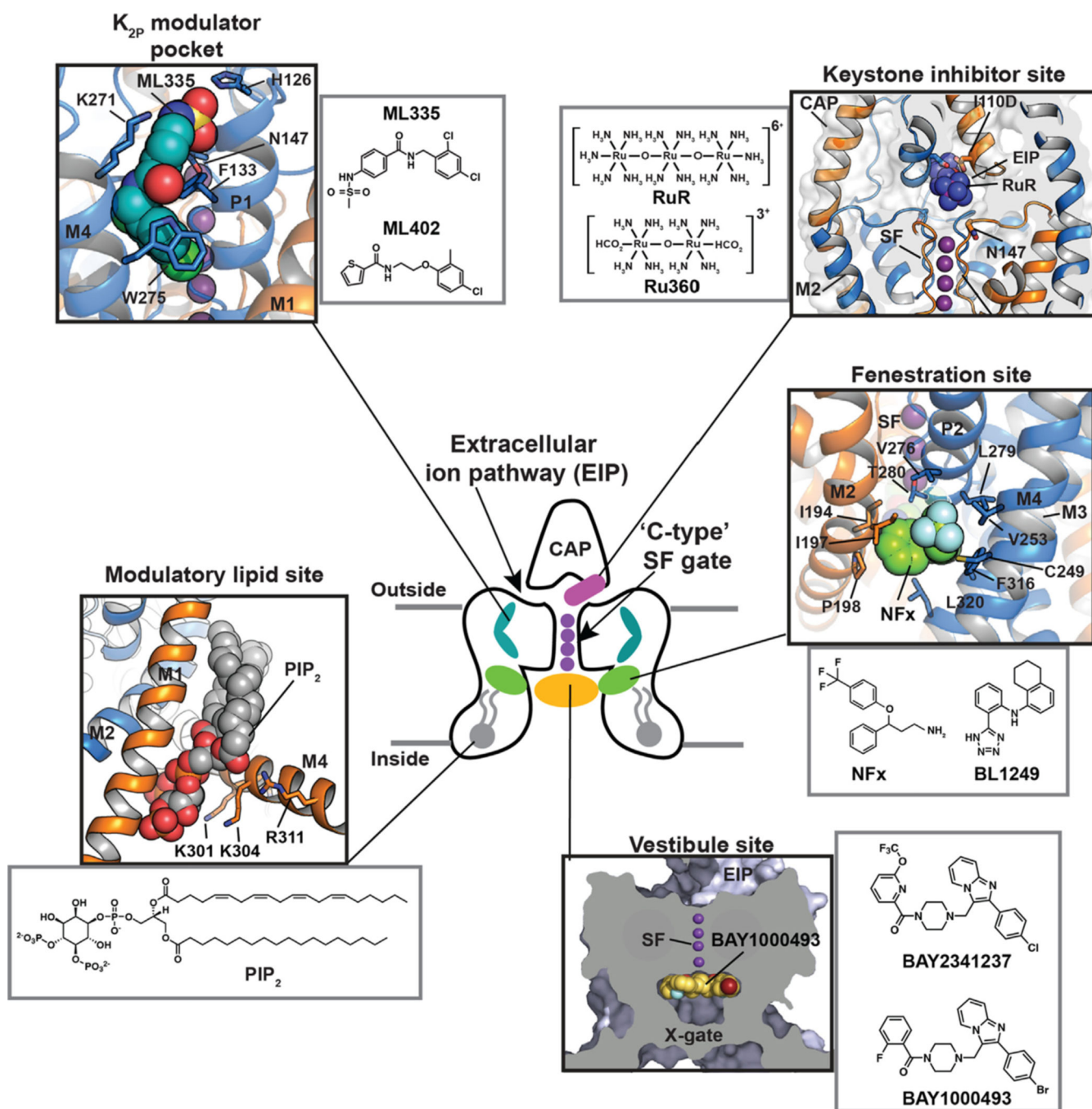


Figure 6. Polysite model of K_{2P} structural pharmacology. Central cartoon shows the locations of structurally defined K_{2P} small molecule binding sites including the Keystone inhibitor site (magenta) (PDB: 6V3I),⁵¹ K_{2P} modulator pocket (cyan) (PDB:6CQ8),³⁷ Fenestration site (green) (PDB:4XDK),⁴⁰ Modulatory lipid site (grey) (PDB:6W8C),⁵³ and Vestibule site (orange) (PDB: 6RV3).³⁸ Cap, Extracellular ion pathway (EIP), and 'C-type' SF gate are indicated. Potassium ions are shown (purple). Grey lines denote the lipid bilayer. Black

boxes show the details of the individual sites. Grey boxes show modulator chemical structures. X-gate is not shown in the central cartoon.

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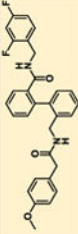
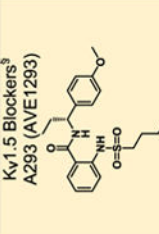
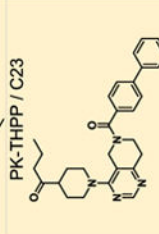
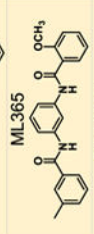
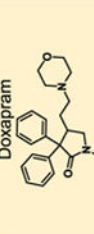
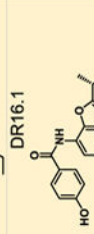
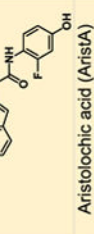
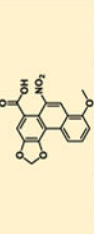
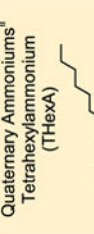
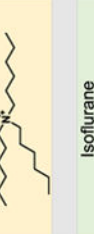
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Table 1

K₂P inhibitors 171–192,194

Chemical Structure	Inhibition	No Inhibition*	IC ₅₀ (μM)	Reference
	K _{2p} 10.1 (TREK-2) K _{2p} 9.1 (TASK-3) K _{2p} 2.1 (TREK-1) I110D K _{2p} 4.1 (TRAAK)	Keystone inhibitor site K _{2p} 2.1 (TREK-1) K _{2p} 3.1 (TASK-1) K _{2p} 5.1 (TASK-2) K _{2p} 13.1 (THIK-1) K _{2p} 18.1 (TRESK)	0.23 ± 0.06 K _{2p} 10.1 (TREK-2) ^a 0.114 ± 0.021 K _{2p} 9.1 (TASK-3) ^a 1.7 ± 0.1 K _{2p} 4.1 (TRAAK) ^a 0.287 ± 0.054 K _{2p} 2.1 (TREK-1) I110D ^a	[51, 114-116]
	K _{2p} 10.1 (TREK-2) K _{2p} 9.1 (TASK-3) K _{2p} 2.1 (TREK-1) I110D	K _{2p} 2.1 (TREK-1) K _{2p} 3.1 (TASK-1)	2.8 ± 1.2 K _{2p} 10.1 (TREK-2) ^a 15.6 ± 2.7 K _{2p} 9.1 (TASK-3) ^a 11.3 ± 1.8 K _{2p} 2.1 (TREK-1) I110D ^a	[51]
Fenestration Site				
	K _{2p} 2.1 (TREK-1) K _{2p} 10.1 (TREK-2) K _{2p} 4.1 (TRAAK) (weak)		19 ± 2 K _{2p} 2.1 (TREK-1) ^b 37.9 ± 7.7 K _{2p} 2.1 (TREK-1) ^b 28.7 ± 7.6 K _{2p} 10.1 (TREK-2) ^b	[171, 172]
	K _{2p} 2.1 (TREK-1) K _{2p} 10.1 (TREK-2) K _{2p} 5.1 (TASK-2) K _{2p} 4.1 (TRAAK) (weak)		9 K _{2p} 2.1 (TREK-1) ^b 2.7 K _{2p} 10.1 (TREK-2) ^a 4.9 ± 0.5 K _{2p} 10.1 (TREK-2) ^b 17 ± 5 K _{2p} 5.1 (TASK-2) ^b	[81, 89, 171, 173]
	K _{2p} 3.1 (TASK-1) K _{2p} 5.1 (TASK-2) K _{2p} 9.1 (TASK-3) K _{2p} 18.1 (TRESK) K _{2p} 4.1 (TRAAK) K _{2p} 2.1 (TREK-1) K _{2p} 10.1 (TREK-2) K _{2p} 3.1 (TASK-1) K _{2p} 2.1 (TREK-1)		41 ± 10 K _{2p} 3.1 (TASK-1) ^a ≈20 K _{2p} 5.1 (TASK-2) ^a	[159, 174, 175]
	K _{2p} 18.1 (TRESK) K _{2p} 3.1 (TASK-1) K _{2p} 9.1 (TASK-3) (weak)	Activates K _{2p} 2.1 (TREK-1) K _{2p} 10.1 (TREK-2) K _{2p} 4.1 (TRAAK)	0.14 ± 1.44 K _{2p} 18.1 (TRESK) ^a 0.95 ± 1.61 K _{2p} 3.1 (TASK-1) ^a 50.72 ± 2.74 K _{2p} 9.1 (TASK-3) ^a	[176]
Vestibule Site				
	K _{2p} 3.1 (TASK-1) K _{2p} 9.1 (TASK-3)	K _{2p} 18.1 (TRESK) K _{2p} 6.1 (TWIK-2)	0.0095 K _{2p} 3.1 (TASK-1) ^a 0.0151 K _{2p} 9.1 (TASK-3) ^a	[38]
	K _{2p} 3.1 (TASK-1) K _{2p} 9.1 (TASK-3)	K _{2p} 18.1 (TRESK) K _{2p} 6.1 (TWIK-2)	0.0076 K _{2p} 3.1 (TASK-1) ^a 0.0023 K _{2p} 9.1 (TASK-3) ^a	[38]
	K _{2p} 3.1 (TASK-1) K _{2p} 9.1 (TASK-3) K _{2p} 18.1 (TRESK) (weak) K _{2p} 13.1 (THIK-1) (weak) K _{2p} 17.1 (TALK-2) (weak)		0.0351 ± .0038 K _{2p} 3.1 (TASK-1) ^a 0.318 ± .030 K _{2p} 9.1 (TASK-3) ^a 0.9 ± 0.1 K _{2p} 18.1 (TRESK) ^a 2.2 ± 0.2 K _{2p} 13.1 (THIK-1) ^a 8.1 ± .2.3 K _{2p} 17.1 (TALK-2) ^a	[147, 152]

	K _{2P} 10.1 (TREK-2) (weak) K _{2P} 5.1 (TASK-2) (weak) K _{2P} 2.1 (TREK-1) (weak) K _{2P} 4.1 (TRAAK) (weak)	8.4 ± 1.1 K _{2P} 10.1 (TREK-2) ^a 12.0 ± 2.2 K _{2P} 5.1 (TASK-2) ^a 23.8 ± 1.1 K _{2P} 2.1 (TREK-1) ^a >20 K _{2P} 4.1 (TRAAK) ^a	[152, 177]
	K _{2P} 3.1 (TASK-1) K _{2P} 9.1 (TASK-3) K _{2P} 5.1 (TASK-2) (weak) K _{2P} 2.1 (TREK-1) (weak) K _{2P} 17.1 (TALK-2) (weak)	0.222 ± 0.038 K _{2P} 3.1 (TASK-1) ^a 0.95 ± 0.1 K _{2P} 9.1 (TASK-3) ^a 8.1 ± 1.9 K _{2P} 5.1 (TASK-2) ^a 9.8 ± 1.7 K _{2P} 2.1 (TREK-1) ^a 18.1 ± 0.9 K _{2P} 17.1 (TALK-2) ^a	[148]
	K _{2P} 3.1 (TASK-1) K _{2P} 9.1 (TASK-3) K _{2P} 2.1 (TREK-1) (weak)	0.30 ± 0.02 K _{2P} 3.1 (TASK-1) ^b 0.035 ± 0.005 K _{2P} 9.1 (TASK-3) ^b >10 K _{2P} 2.1 (TREK-1) ^b	[150]
	K _{2P} 3.1 (TASK-1) K _{2P} 9.1 (TASK-3) (weak)	≈0.004 K _{2P} 3.1 (TASK-1) ^d ≈3.2 K _{2P} 9.1 (TASK-3) ^d	[149]
	K _{2P} 3.1 (TASK-1) K _{2P} 9.1 (TASK-3)	≈0.410 K _{2P} 3.1 (TASK-1) ^a ≈37 K _{2P} 9.1 (TASK-3) ^a ≈ 9 K _{2P} 3.1 (TASK-1) / K _{2P} 9.1 (TASK-3) heterodimer ^a	[178]
	K _{2P} 3.1 (TASK-1) K _{2P} 9.1 (TASK-3)	14.2 ± 3.4 K _{2P} 9.1 (TASK-3) ^b 21.2 ± 5.5 K _{2P} 3.1 (TASK-1) ^b	[154]
	K _{2P} 18.1 (TRESK) Activates K _{2P} 2.1 (TREK-1) K _{2P} 10.1 (TREK-2)	13 ± 2 K _{2P} 18.1 (TRESK) ^c	[74]
	K _{2P} 9.1 (TASK-3) K _{2P} 18.1 (TRESK) K _{2P} 2.1 (TREK-1)	≈0.3 K _{2P} 9.1 (TASK-3) ≈0.5 K _{2P} 18.1 (TRESK) ≈1 K _{2P} 2.1 (TREK-1)	[137, 138, 179]
	Anesthetic Site K _{2P} 4.1 (TRAAK) K _{2P} 16.1 (TALK-1) Activates K _{2P} 2.1 (TREK-1) K _{2P} 10.1 (TREK-2) K _{2P} 9.1 (TASK-3) K _{2P} 18.1 (TRESK) K _{2P} 4.1 (TRAAK) Activates K _{2P} 2.1 (TREK-1) K _{2P} 10.1 (TREK-2) K _{2P} 3.1 (TASK-1) K _{2P} 9.1 (TASK-3)	≈160 K _{2P} 18.1 (TRESK) ^c	[137, 138, 179]
	K _{2P} 13.1 (THIK-1) K _{2P} 12.1 (THIK-2) K _{2P} 16.1 (TALK-1) K _{2P} 17.1 (TALK-2)	≈300 K _{2P} 18.1 (TRESK) ^c	[137, 138, 179-181]

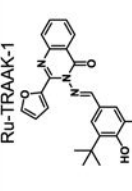
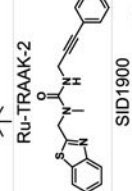
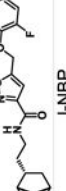
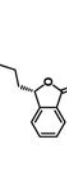
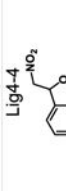
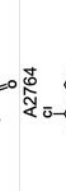
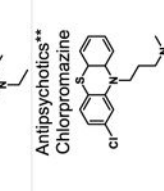
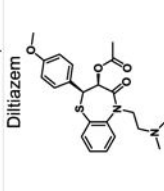
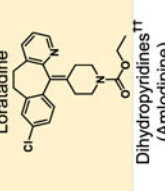
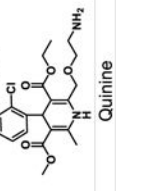

Diethylether	K _{SP} 3.1 (TASK-1)	K _{SP} 18.1 (TRESK) K _{SP} 4.1 (TRAAK) Activates	[137]
Chloroform 	K _{SP} 6.1 (TWIK-2) K _{SP} 16.1 (TALK-1) K _{SP} 17.1 (TALK-2)	K _{SP} 2.1 (TREK-1) K _{SP} 4.1 (TRAAK) K _{SP} 3.1 (TASK-1) K _{SP} 13.1 (THIK-1) Activates	[137]
Other Ligands			
TKDC 	K _{SP} 2.1 (TREK-1) K _{SP} 10.1 (TREK-2) K _{SP} 4.1 (TRAAK) (weak)	4.9 ± 0.6 K _{SP} 2.1 (TREK-1) ^c 5.2 ± 1.9 K _{SP} 10.1 (TREK-2) ^c 65.9 ± 7.0 K _{SP} 4.1 (TRAAK) ^c	[164]
TKN1 	K _{SP} 2.1 (TREK-1) K _{SP} 10.1 (TREK-2) K _{SP} 4.1 (TRAAK)	6.5 ± 1.7 K _{SP} 2.1 (TREK-1) ^c 4.4 ± 1.0 K _{SP} 10.1 (TREK-2) ^c 15.7 ± 3.1 K _{SP} 4.1 (TRAAK) ^c	[164]
TKN2 	K _{SP} 2.1 (TREK-1) K _{SP} 10.1 (TREK-2) K _{SP} 4.1 (TRAAK)	3.8 ± 0.6 K _{SP} 2.1 (TREK-1) ^c 1.7 ± 0.8 K _{SP} 10.1 (TREK-2) ^c 10.3 ± 1.5 K _{SP} 4.1 (TRAAK) ^c	[164]
TKIM 	K _{SP} 2.1 (TREK-1) K _{SP} 10.1 (TREK-2) K _{SP} 4.1 (TRAAK) (weak)	2.96 ± 0.66 K _{SP} 2.1 (TREK-1) ^c 0.84 ± 0.10 K _{SP} 10.1 (TREK-2) ^c	[161]
Spadin Ala-Pro-Leu-Pro-Arg-Trp-Ser-Gly-Pro-Ile-Gly-Val-Ser-Trp-Gly-Leu-Arg PE 22-28	K _{SP} 2.1 (TREK-1)	0.04 K _{SP} 2.1 (TREK-1) ^o	[182, 183]
Gly-Val-Ser-Trp-Gly-Leu-Arg T21 T21 Compounds 	K _{SP} 2.1 (TREK-1) K _{SP} 10.1 (TREK-2)	0.00012 K _{SP} 2.1 (TREK-1) ^o	[184]
ML45 	K _{SP} 2.1 (TREK-1) K _{SP} 10.1 (TREK-2) K _{SP} 4.1 (TRAAK)	≈2.81 K _{SP} 2.1 (TREK-1) ^o ≈3.65 K _{SP} 10.1 (TREK-2) ^o	[45]
BTP3/Py-3 	K _{SP} 2.1 (TREK-1) K _{SP} 10.1 (TREK-2) K _{SP} 5.1 (TASK-2)	≈21 K _{SP} 2.1 (TREK-1) ^o	[44]
NPBA 	K _{SP} 2.1 (TREK-1) K _{SP} 10.1 (TREK-2) K _{SP} 5.1 (TASK-2)	0.89 ± 0.27 K _{SP} 2.1 (TREK-1) ^o 1.40 ± 1.12 K _{SP} 10.1 (TREK-2) ^o 2.42 ± 0.39 K _{SP} 5.1 (TASK-2) ^o	[185]
ML308 	K _{SP} 3.1 (TASK-1) K _{SP} 18.1 (TRESK) Activates K _{SP} 9.1 (TASK-3)	7.5 K _{SP} 9.1 (TASK-1) ^o	[48]
	K _{SP} 9.1 (TASK-3) K _{SP} 3.1 (TASK-1) (weak)	0.413 K _{SP} 9.1 (TASK-3) ^o 3.2 K _{SP} 3.1 (TASK-1) ^o	[186]

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 Ru-TRAAK-1	K _{sp} 4.1 (TRAAK) K _{sp} 6.1 (TWIK-2) K _{sp} 3.1 (TASK-1) K _{sp} 18.1 (TRESK) (weak)	K _{sp} 13.1 (THIK-1) K _{sp} 16.1 (TALK-1)	0.385 ± 0.078 K _{sp} 4.1 (TRAAK) ^f	[46]
 Ru-TRAAK-2	K _{sp} 4.1 (TRAAK) K _{sp} 9.1 (TASK-3) (weak) K _{sp} 18.1 (TRESK) (weak)	K _{sp} 6.1 (TWIK-2) K _{sp} 13.1 (THIK-1) K _{sp} 16.1 (TALK-1)	0.719 ± 0.079 K _{sp} 4.1 (TRAAK) ^f	[46]
 SID1900	K _{sp} 2.1 (TREK-1)		29.72 ± 2.4 K _{sp} 2.1 (TREK-1) ^g	[187]
 I-NBP	K _{sp} 2.1 (TREK-1)		0.06 ± 0.03 K _{sp} 2.1 (TREK-1) ^c	[188]
 Lig4-4	K _{sp} 2.1 (TREK-1)		2.06 K _{sp} 2.1 (TREK-1) ^c	[189]
 A2764	K _{sp} 18.1 (TRESK) K _{sp} 2.1 (TREK-1) (weak) K _{sp} 10.1 (TREK-2) (weak) K _{sp} 3.1 (TASK-1) (weak) K _{sp} 5.1 (TASK-2) (weak) K _{sp} 16.1 (TALK-1) (weak)	K _{sp} 4.1 (TRAAK) K _{sp} 9.1 (TASK-3) K _{sp} 13.1 (THIK-1)	11.8 K _{sp} 18.1 (TRESK) ^a	[190]
Antipsychotics** Chlorpromazine 	K _{sp} 2.1 (TREK-1) K _{sp} 10.1 (TREK-2)		2.7 ± 0.3 K _{sp} 2.1 (TREK-1) ^e	[172, 191]
 Diltiazem	K _{sp} 2.1 (TREK-1) K _{sp} 10.1 (TREK-2)		≈ 180 K _{sp} 2.1 (TREK-1) ^e ≈ 330 K _{sp} 10.1 (TREK-2) ^e	[155]
 Loratadine	K _{sp} 18.1 (TRESK)		0.49 ± 0.23 K _{sp} 18.1 (TRESK) ^p 63.4 ± 39.6 K _{sp} 9.1 (TASK-3) ^d	[192]
 Dihydropyridines** (Amlodipine)	K _{sp} 2.1 (TREK-1)		0.43 K _{sp} 2.1 (TREK-1) ^g	[193]
 Quinine	K _{sp} 1.1 (TWIK-1)	K _{sp} 6.1 (TWIK-2)	≈ 60 K _{sp} 1.1 (TWIK-1)	[4, 194]

	K _{2p} 2.1 (TREK-1)	≈41 K _{2p} 1.1 (TWIK-1)	[193, 195]
Anandamide	K _{2p} 2.1 (TREK-1) ^{††} K _{2p} 3.1 (TASK-1)	K _{2p} 1.1 (TWIK-1) K _{2p} 6.1 (TWIK-2) K _{2p} 4.1 (TAAK) K _{2p} 2.1 (TREK-1) ^{††} K _{2p} 10.1 (TREK-2) K _{2p} 5.1 (TASK-2) K _{2p} 9.1 (TASK-3)	5.1 K _{2p} 2.1 (TREK-1) ^{††} 0.7 K _{2p} 3.1 (TASK-1) ^c

Green indicates structurally validated inhibitors. Yellow indicates inhibitors supported by mutagenesis or mutagenesis combined with simulation studies. Cells used to measure activity are indicated as follows:

- ^aOocytes,
- ^bHEK cells,
- ^cCHO Cells,
- ^dThallium flux assay,
- ^eCOS-7 cells,
- ^ftsA201 cells, AZF cells,
- ^hhippocampal pyramidal neurons,
- ^lLysosome flux assay.
- ^{*} Only K₂Ps tested against the inhibitor are listed.
- [†] Other SSRIs such as citalopram and paroxetine inhibit K₂Ps and likely bind to the fenestration site.
- ^{††} Other structurally similar local anesthetics such as lidocaine, ropivacaine, mepivacaine and etidocaine likely bind K₂Ps similarly to bupivacaine.
- [§] Many Kv1.5 blockers have been identified as being more potent antagonists for TASK channels. In addition to A1899 and A293, ICAGEN-4, MSD-D and A1899 derivatives have all been shown to potentially inhibit TASK channels.
- ^{||} K₂Ps are traditionally insensitive to quaternary ammoniums applied extracellularly, however are sensitive to intracellular application. Quaternary ammoniums with longer alkyl chains inhibit K₂Ps more strongly than those with short alkyl chains.
- ^{**} Both typical antipsychotics and atypical antipsychotics (fluphenazine, haloperidol, loxapine, clozapine, etc.) have been shown to modulate K₂P channels.
- ^{†††} Other dihydropyridines such as nifedipine and mifedipine antagonize TREK channels.

¹⁹⁵ however this was contested by Liu *et al.* 193
K2P2.1 (TREK-1) was first reported to be insensitive to 3 μ M anandamide,¹⁹⁵ however this was contested by Liu *et al.* 193

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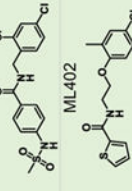
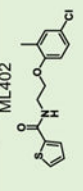
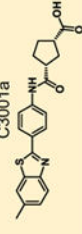
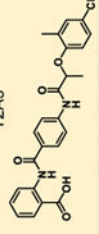
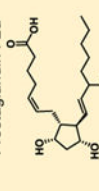
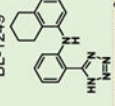
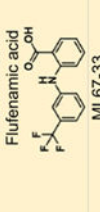
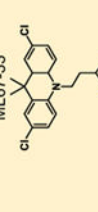
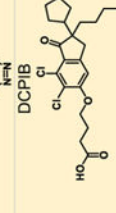
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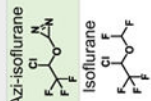

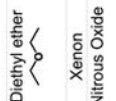
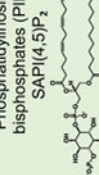
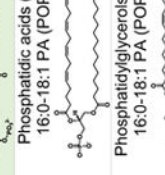
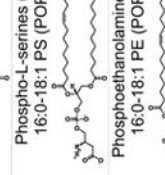
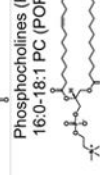
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Table 2

K₂P activators^{176,179,181,192,196–202}

Chemical Structure	Activation	No Activation*	EC ₅₀ (μM)	Reference
	K _{Sp} 2.1 (TREK-1) K _{Sp} 10.1 (TREK-2)	K _{Sp} 4.1 (TRAAK)	14.3 ± 2.7 K _{Sp} 2.1 (TREK-1) ^a 5.2 ± 0.8 K _{Sp} 2.1 (TREK-1) ^b 5.2 ± 0.5 K _{Sp} 10.1 (TREK-2) ^b	[37]
	K _{Sp} 2.1 (TREK-1) K _{Sp} 10.1 (TREK-2)	K _{Sp} 4.1 (TRAAK)	13.7 ± 7.0 K _{Sp} 2.1 (TREK-1) ^a 5.9 ± 1.6 K _{Sp} 10.1 (TREK-2) ^a 5.9 ± 1.6 K _{Sp} 10.1 (TREK-2) ^b	[37]
	K _{Sp} 2.1 (TREK-1) K _{Sp} 10.1 (TREK-2) K _{Sp} 4.1 (TRAAK) (weak)	K _{Sp} 3.1 (TASK-1) K _{Sp} 9.1 (TASK-3) K _{Sp} 18.1 (TRESK) K _{Sp} 13.1 (THIK-1)	12.81 K _{Sp} 2.1 (TREK-1) ^b 11.31 K _{Sp} 10.1 (TREK-2) ^b 15.29 K _{Sp} 4.1 (TRAAK) ^b	[162]
	K _{Sp} 10.1 (TREK-2)	K _{Sp} 2.1 (TREK-1) K _{Sp} 9.1 (TASK-3) K _{Sp} 16.1 (TALK-1)	≈ 10 K _{Sp} 10.1 (TREK-2) ^d	[45]
	K _{Sp} 10.1 (TREK-2)	inhibits K _{Sp} 2.1 (TREK-1)	≈ 0.294 K _{Sp} 10.1 (TREK-2) ^d	[45]
Fenestration Site				
	K _{Sp} 2.1 (TREK-1) K _{Sp} 10.1 (TREK-2) K _{Sp} 4.1 (TRAAK) (weak)	K _{Sp} 3.1 (TASK-1) K _{Sp} 5.1 (TASK-2) K _{Sp} 9.1 (TASK-3) K _{Sp} 13.1 (THIK-1) K _{Sp} 1.1 (TWIK-1) K _{Sp} 18.1 (TRESK)	5.5 ± 1.2 K _{Sp} 2.1 (TREK-1) ^a 8.0 ± 0.8 K _{Sp} 10.1 (TREK-2) ^a 48 ± 10 K _{Sp} 4.1 (TRAAK) ^a	[50, 109]
	K _{Sp} 2.1 (TREK-1) K _{Sp} 10.1 (TREK-2) K _{Sp} 4.1 (TRAAK) (weak) K _{Sp} 9.1 (TASK-3) (weak) K _{Sp} 5.1 (TASK-2) (weak)		≈ 100 K _{Sp} 2.1 (TREK-1) ≈ 100 K _{Sp} 10.1 (TREK-2) > 100 K _{Sp} 4.1 (TRAAK)	[154, 157]
	K _{Sp} 2.1 (TREK-1) K _{Sp} 10.1 (TREK-2) K _{Sp} 4.1 (TRAAK)	K _{Sp} 3.1 (TASK-1) K _{Sp} 5.1 (TASK-2) K _{Sp} 9.1 (TASK-3) K _{Sp} 18.1 (TRESK)	36.3 ± 1.0 K _{Sp} 2.1 (TREK-1) ^a 9.7 ± 1.2 K _{Sp} 2.1 (TREK-1) ^b 30.2 ± 1.4 K _{Sp} 10.1 (TREK-2) ^a 27.3 ± 1.2 K _{Sp} 4.1 (TRAAK) ^a	[44]
	K _{Sp} 2.1 (TREK-1) K _{Sp} 10.1 (TREK-2) K _{Sp} 4.1 (TRAAK)	inhibits K _{Sp} 18.1 (TRESK) K _{Sp} 3.1 (TASK-1) K _{Sp} 9.1 (TASK-3)	I/I ₀ = 4.69 ± 1.37 K _{Sp} 2.1 (TREK-1) [†] I/I ₀ = 8.45 ± 2.37 K _{Sp} 4.1 (TRAAK) [‡]	[176, 196]

	Anesthetic Site		
	K _{SP} 2.1 (TREK-1)	K _{SP} 4.1 (TAAAK)	735 ± 192 K _{SP} 2.1 (TREK-1) [143]
	K _{SP} 2.1 (TREK-1) K _{SP} 10.1 (TREK-2) K _{SP} 3.1 (TASK-1) K _{SP} 5.1 (TASK-2) K _{SP} 9.1 (TASK-3) K _{SP} 18.1 (TRESK)	K _{SP} 4.1 (TAAAK) inhibits K _{SP} 13.1 (THIK-1) K _{SP} 12.1 (THIK-2) K _{SP} 16.1 (TALK-1) K _{SP} 17.1 (TALK-1) K _{SP} 6.1 (TWIK-2)	[137, 138, 179-181, 197]
	K _{SP} 2.1 (TREK-1) K _{SP} 10.1 (TREK-2) K _{SP} 18.1 (TRESK) K _{SP} 3.1 (TASK-2)	K _{SP} 4.1 (TAAAK) K _{SP} 3.1 (TASK-1) K _{SP} 13.1 (THIK-1) inhibits K _{SP} 6.1 (TWIK-2) K _{SP} 17.1 (TALK-1) K _{SP} 17.1 (TALK-2)	[137, 197, 198]
	K _{SP} 2.1 (TREK-1) K _{SP} 10.1 (TREK-2) K _{SP} 18.1 (TRESK) K _{SP} 3.1 (TASK-2)	K _{SP} 4.1 (TAAAK) K _{SP} 3.1 (TASK-1) K _{SP} 13.1 (THIK-1) inhibits K _{SP} 6.1 (TWIK-2) K _{SP} 17.1 (TALK-1) K _{SP} 17.1 (TALK-2)	[137, 197, 198]
	K _{SP} 2.1 (TREK-1)	K _{SP} 4.1 (TAAAK) inhibits K _{SP} 3.1 (TASK-1) K _{SP} 9.1 (TASK-3)	[137]
	K _{SP} 2.1 (TREK-1) K _{SP} 18.1 (TRESK) K _{SP} 2.1 (TREK-1)	K _{SP} 9.1 (TASK-3)	[197, 198] [197, 198]
	K _{SP} 2.1 (TREK-1)	Modulatory lipid site	[197, 198]
	K _{SP} 2.1 (TREK-1) K _{SP} 4.1 (TAAAK)	K _{SP} 2.1 (TREK-1) ^o 0.87 ± 0.11 K _{SP} 2.1 (TREK-1) ^h 3.4 ± 0.3 K _{SP} 4.1 (TAAAK)	[37, 127, 128, 134, 135]
	K _{SP} 2.1 (TREK-1) K _{SP} 4.1 (TAAAK)	15.7 ± 3.0 K _{SP} 2.1 (TREK-1) ^h 1.3 ± 0.1 K _{SP} 4.1 (TAAAK)	[134, 135]
	K _{SP} 2.1 (TREK-1) K _{SP} 4.1 (TAAAK)	176.9 ± 30.3 K _{SP} 2.1 (TREK-1) ^h 11.1 ± 0.4 K _{SP} 4.1 (TAAAK)	[134, 135]
	K _{SP} 2.1 (TREK-1) K _{SP} 4.1 (TAAAK)	168.4 ± 29.1 K _{SP} 2.1 (TREK-1) ^h 22.3 ± 1.4 K _{SP} 4.1 (TAAAK)	[134, 135]
	K _{SP} 4.1 (TAAAK)	5.3 ± 0.2 K _{SP} 4.1 (TAAAK)	[135]
	K _{SP} 4.1 (TAAAK)	11.1 ± 0.1 K _{SP} 4.1 (TAAAK)	[135]

Other Activators				
	K _{2P} 2.1 (TREK-1) K _{2P} 10.1 (TREK-2) K _{2P} 4.1 (TRAAK)	K _{2P} 3.1 (TASK-1) K _{2P} 9.1 (TASK-3) K _{2P} 18.1 (TRESK)	486 ± 135 K _{2P} 2.1 (TREK-1) ^a 1220 ± 390 K _{2P} 10.1 (TREK-2) ^b	[199, 200]
	K _{2P} 9.1 (TASK-3)	K _{2P} 2.1 (TREK-1) K _{2P} 10.1 (TRAAK)	1.4 ± 0.2 K _{2P} 9.1 (TASK-3) ^b 2.5 ± 0.2 TASK-1/3 heterodimer ^b	[49]
	K _{2P} 9.1 (TASK-3)	K _{2P} 2.1 (TREK-1) K _{2P} 10.1 (THIK-1) K _{2P} 2.1 (TREK-1) K _{2P} 3.1 (TASK-1) K _{2P} 18.1 (TRESK) K _{2P} 4.1 (TRAAK) K _{2P} 5.1 (TASK-2) K _{2P} 18.1 (TRESK)	6.7 K _{2P} 9.1 (TASK-3) ^c	[48]
	K _{2P} 2.1 (TREK-1) K _{2P} 10.1 (TREK-2)	K _{2P} 9.1 (TASK-3) K _{2P} 10.1 (TASK-3) K _{2P} 2.1 (TREK-1) K _{2P} 5.1 (TASK-2) K _{2P} 13.1 (THIK-1) K _{2P} 4.1 (TRAAK)	0.76 ± 0.1 K _{2P} 2.1 (TREK-1) ^d 0.89 ± 0.3 K _{2P} 2.1 (TREK-1) ^b	[201]
	K _{2P} 4.1 (TRAAK) K _{2P} 2.1 (TREK-1) K _{2P} 10.1 (TREK-2)	inhibits K _{2P} 18.1 (TRESK) K _{2P} 3.1 (TASK-1) K _{2P} 9.1 (TASK-3)	≈2 K _{2P} 10.1 (TREK-2) ^d ≈3 K _{2P} 2.1 (TREK-1) ^d ≈3 K _{2P} 18.1 (TRESK) ^d >10 K _{2P} 9.1 (TASK-3) ^a	[166]
	K _{2P} 2.1 (TREK-1) (weak) K _{2P} 10.1 (TREK-2) (weak)	inhibits K _{2P} 18.1 (TRESK)	1.2 ± 0.1 K _{2P} 4.1 (TRAAK) ^a 6.9 ± 1.2 K _{2P} 2.1 (TREK-1) ^a 3.8 ± 0.4 K _{2P} 10.1 (TREK-2) ^a	[7, 11, 77]
	K _{2P} 9.1 (TASK-3)	K _{2P} 3.1 (TASK-1) K _{2P} 5.1 (TASK-2) K _{2P} 1.1 (TWIK-1) K _{2P} 10.1 (TREK-2) K _{2P} 18.1 (TRESK) K _{2P} 13.1 (THIK-1)	≈1 K _{2P} 9.1 (TASK-3) ^d	[202]
	K _{2P} 2.1 (TREK-1) K _{2P} 10.1 (TREK-2) (weak)	K _{2P} 4.1 (TRAAK) K _{2P} 9.1 (TASK-3)	0.06 ± 0.01 K _{2P} 18.1 (TRESK) ^d	[23, 47]
	K _{2P} 2.1 (TREK-1) K _{2P} 10.1 (TREK-2) (weak)	K _{2P} 4.1 (TRAAK) K _{2P} 9.1 (TASK-3)	0.24 ± 0.02 K _{2P} 18.1 (TRESK) ^d	[47]
	K _{2P} 18.1 (TRESK)	K _{2P} 9.1 (TASK-1)	0.65 ± 0.10 K _{2P} 18.1 (TRESK) ^d	[192]
	K _{2P} 18.1 (TRESK)	K _{2P} 9.1 (TASK-1)	0.65 ± 0.10 K _{2P} 18.1 (TRESK) ^d	[192]

Green indicates structurally validated activators Yellow indicates activators supported by mutagenesis or mutagenesis combined with simulation studies. Cells used to measure activity are indicated as follows

^a Oocytes,

^b HEK cells,

^c CHO Cells,

^d Thallium flux assay,

^e COS cells,

^f tsA201 cells,

^g AZF cells,

^h Fluorescent binding assay,

ⁱ K_d values from native mass spectrometry with K2p4.1a (TRAAK) isoform.

* Only K2Ps tested against the activator are listed (other K2Ps may not have been tested).

† Other closely related fenamates like mefenamic acid and niflumic acid possess similar activity and selectivity to flufenamic acid.

‡ I/I_0 values at 10 IM DCPiB. EC₅₀ values were not determined as E_{max} was not reached, even at very high DCPiB concentrations (100 IM).

§ While the EC₅₀s for Pramlukast against the TREK family are nearly identical, the fold-activation observed for K2P10.1 (TREK-2) was much higher than for K2P2.1 (TREK-1) or K2P4.1 (TRAAK).