Physical mechanism for gating and mechanosensitivity of the human TRAAK K⁺ channel

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Activation of mechanosensitive ion channels by physical force underlies many physiological processes including the sensation of touch, hearing and pain1–5. TRAAK (also known as KCNK4) ion channels are neuronally expressed members of the two-pore domain K⁺ (K2P) channel family and are mechanosensitive6. They are involved in controlling mechanical and temperature nociception in mice7. Mechanosensitivity of TRAAK is mediated directly through the lipid bilayer—it is a membrane-tension-gated channel8. However, the molecular mechanism of TRAAK channel gating and mechanosensitivity is unknown. Here we present crystal structures of TRAAK in conductive and non-conductive conformations defined by the presence of permeant ions along the conduction pathway. In the non-conductive state, a lipid acyl chain accesses the channel cavity through a 5 Å-wide lateral opening in the membrane inner leaflet and physically blocks ion passage. In the conductive state, rotation of a transmembrane helix (TM4) about a central hinge seals the intramembrane opening, preventing lipid block of the cavity and permitting ion entry. Additional rotation of a membrane interacting TM2–TM3 segment, unique to mechanosensitive K2Ps, against TM4 may further stabilize the conductive conformation. Comparison of the structures reveals a biophysical explanation for TRAAK mechanosensitivity—an expansion in cross-sectional area up to 2.7 nm² in the conductive state is expected to create a membrane-tension-dependent energy difference between conformations that promotes force activation. Our results show how tension of the lipid bilayer can be harnessed to control gating and mechanosensitivity of a eukaryotic ion channel.

How physical force gates mechanosensitive ion channels in animals is unknown1–5. Mechanosensitivity in the bacterial MscL and MscS ion channels is proposed to result from a membrane-tension-dependent energy difference between open and closed conformations due in part to cross-sectional area expansion with opening6–11. The eukaryotic K⁺ channel TRAAK, like MscL and MscS, is gated open by membrane tension12. TRAAK is a member of the K2P K⁺ channel family. The architecture of K2Ps is unique among metazoan K⁺ channels because two of the four subunits that form canonical K⁺ channels are fused into a single protein chain, thus, K2Ps are dimers12–14. Constraints imposed by this architecture preclude direct extension of the canonical mechanism of gating four-fold-symmetric K⁺ channels involving symmetric dilation of a helical bundle at the intracellular channel surface15,16. Crystal structures of TRAAK12,13 revealed intramembrane openings to the lipid bilayer between subunits and a role for lipids in gating the channel has been hypothesized14.

Figure 1a shows the effect of stretching the membrane on TRAAK channel activity. Pressure application to inside-out patches induces a rapid and reversible increase of K⁺-selective current. The construct used for these studies removed the disordered carboxy terminus from TRAAK because it improved the resolution of X-ray crystal structures by ~0.25 Å without affecting function. We determined the structure of TRAAK purified in decyl maltoside detergent and 150 mM K⁺ in complex with Fab from mouse monoclonal antibodies at 2.5 Å resolution (Fig. 1b, Extended Data Table 1).

We find in this structure that TRAAK is asymmetric because transmembrane helix 4 (TM4) from each subunit adopts a different conformation (Fig. 1b–e). TM4 from subunit A (TM4A) is ‘up’—it is kinked approximately halfway through the membrane around the hinge glycine conserved in K⁺ channels (G268) and packs hydrophobic residues on its extracellular-facing side against the cytoplasmic-facing side of TM2B (Fig. 1c). This creates a continuous protein surface facing the membrane that seals the channel cavity from lipids (the ~3 Å-wide gap between L276 and I151 is smaller than an acyl chain). On the other side of the channel, TM4B is ‘down’—it traverses the membrane without kinking at the hinge glycine (Fig. 1d). This creates a ~5 Å-wide intramembrane opening between TRAAK subunits that exposes the channel cavity to the inner leaflet of the lipid bilayer.

An elongated tube of electron density consistent with a ten-carbon acyl chain extends from this intramembrane opening to the central cavity underneath the selectivity filter (Fig. 1e). We cannot distinguish crystallographically whether this acyl chain is from a co-purified lipid or a detergent molecule because of the lack of strong density features outside of the channel. This suggests that TRAAK does not tightly bind the head group of the molecule occupying the cavity. Electrophysiological evidence presented below, the presence of acyl chains in analogous positions in the structure of the related K2P TWIK1 (ref. 14), the hydrophobic nature of the site (Extended Data Fig. 1) and geometric considerations (a typical acyl chain from an inner leaflet lipid can reach the site) lead us to believe that a lipid acyl chain can occupy the cavity in a membrane.

We wondered whether the presence of this acyl chain would preclude ion binding in the cavity of TRAAK. We used the anomalous dispersion of X-rays by the permeant ion Tl⁺ as a sensitive measure of ion occupancy to test this idea. A structure in which 150 mM Tl⁺ replaced K⁺ was determined at 3.0 Å resolution. The overall structure in Tl⁺ is essentially identical to the structure in K⁺ (Extended Data Table 2). The cavity is similarly exposed to the membrane on the side of TM4B and acyl chain density is observed, although the intracellular half of TM4B is apparently disordered. Anomalous density maps show five Tl⁺ ions along the conduction pathway: one above the extracellular mouth of the pore and four in the selectivity filter (Fig. 1f). No Tl⁺ is observed in the cavity site that is occupied by lipid.

We screened for crystallization conditions to capture TRAAK with both intramembrane openings sealed and identified chemical additives that promoted this conformation (Fig. 1b, g–j). The best diffracting K⁺- and Tl⁺-bound crystals contained 4% (vol/vol) polypropylene glycol or 52 mM trichloroethanol (a known TRAAK activator17) and structures were determined to 2.5 Å and 3.0 Å resolution, respectively (Extended Data Table 1). Compared to the previous structures, TM4A remains in the same up conformation (Fig. 1g). The conformation of TM4B changes by bending around the hinge G268 to pack against TM2A and seal the intramembrane opening (Fig. 1h). With both intramembrane openings sealed, elongated acyl chain density is no longer observed in the cavity of these structures. In its place, there is approximately spherical density (Fig. 1i) and the Tl⁺-containing structure has a strong (5.1σ) additional density that is not seen in K⁺-bound crystals (Fig. 1j).
anomalous peak in the cavity (Fig. 1j). Although we do not observe density for the additives that promote this conformational change, trichloroethanol functionally activates TRAAK channels in patches 17.

The structures show that when TM4 is down, a lipid acyl chain can access the channel cavity through intramembrane openings and compete with ion binding. When TM4 is up, the intramembrane opening is sealed and an ion occupies the cavity site. These observations lead us to the following hypothesis: the TM4-down, lipid-blocked structure is a non-conductive TRAAK conformation, whereas the TM4-up structure is a conductive TRAAK conformation.

This hypothesis predicts that a lipid less capable of entering through the intramembrane opening would render channels more conductive. To test this, we reconstituted purified TRAAK in phosphatidylcholine with either unbranched (PC) or branched diphytanoyl (DPhPC) acyl chains and recorded from proteoliposome patches (Extended Data Fig. 2). We observed higher levels of current in DPhPC compared to PC. This could result from either more active channels or more efficient reconstitution (that is, more channels) in DPhPC. When the patch is inflated, the mechanically activatable component is not proportionally increased as would be expected if there were simply a larger number of channels. Thus, the baseline activity of TRAAK is higher in DPhPC, consistent with the notion that the diphytanoyl chain is less able to enter the cavity and block ion conduction.

A second prediction from the model is if TM4 is trapped in the up conformation, the channel should be more conductive. To test this, we exploited the large conformational change in TM4 to design a double cysteine mutant TRAAK(I159C, R284C) that could form disulphide bridges only in the predicted conductive conformation (Fig. 2a, b). The

Figure 1 | Structures of TRAAK in non-conductive and conductive conformations. a, Current response to pressure applied to an inside-out patch from a TRAAK-expressing cell held at 0 mV in a tenfold [K+] gradient (E_{K_+} = -59 mV). Recordings are vertically offset with red lines at the zero current level. Inset, current–voltage relationship from the same patch before and during pressure application. b, Stereo view of an overlay of conductive (blue) and non-conductive (red) conformations of TRAAK in K^+ (grey). The central cavity (with ligands removed) is marked with an asterisk. c–f, Non-conductive TRAAK structures. c, d, Membrane view of the cytoplasmic half of TM4A (c) and TM4B (d) with a cavity-bound acyl chain (cyan). Panel d is rotated 180° about the conduction axis from c. e, Cytoplasmic view clipped to the cavity plane with F_o–F_c positive omit density at 2.5σ (green) around the acyl chain (red). f, Anomalous density (grey) at 3σ (extracellular ion) or 5σ (selectivity filter ions) around Tl^+ (green) in the conduction pathway of non-conductive TRAAK with a cavity-bound acyl chain (cyan). g–j, Conductive TRAAK structures in the same views as c–f. The cavity-bound ion is shown in F_o–F_c positive omit density (i, green) and anomalous density (j, grey) at 3σ.
TRAARK(I159C, R284C) mutant was rapidly activated upon exposure to oxidizing solution (greater than tenfold within 1 min) and activation continued over several minutes to 23.8 ± 4.7-fold (Fig. 2c, e, Extended Data Fig. 3). Wild-type channels in contrast showed only a small effect (1.67 ± 0.19-fold activation) (Fig. 2d, e, Extended Data Fig. 3). Single cysteine mutants or three other double cysteine mutants close in space to TRAARK(I159C, R284C) showed no effect relative to wild-type (Fig. 2e). Therefore, the activation of TRAARK(I159C, R284C) in oxidizing solution depends on the three-dimensional orientation of the cysteine pair. These results suggest that the formation of a disulphide bridge between TM4 and TM2 stabilizes a conductive conformation of TRAARK.

As in the case of TRAARK reconstituted in DPhPC, the mechanically activatable component of oxidized TRAARK(I159C, R284C) is a smaller fraction of the baseline current compared to reduced TRAARK(I159C, R284C) or wild-type TRAARK (Fig. 2f, Extended Data Fig. 3). In principle, if all channels were locked open with a disulphide bridge, we should observe no further opening with mechanical perturbation. However, TRAARK open probability is low in all contexts studied (Extended Data Fig. 4). Together, the increased baseline activity and relative reduction of mechanosensitivity in these experiments are consistent with the proposed TM4-mediated gating of TRAARK.

We asked whether comparison of the structures could provide additional insight into the physical basis of TRAARK mechanosensitivity. Figure 3a shows the spectrum of conformational changes observed in TRAARK structures (Extended Data Table 3). The cytoplasmic half of TM4 is observed in one non-conductive (TM4-down, red) and two conductive (TM4-up) conformations. From the non-conductive conformation, rearrangement of the intracellular half of TM4 by a ~15° bend (around G268) and a ~15° right-handed rotation about the helical axis gives the orange TM4-up conformation. Alternatively, a ~25° bend and a ~10° rotation gives the blue, green, or yellow TM4-up conformations. The transition from a non-conductive to conductive state can occur by movement of TM4 alone (compare the red and green structures) or with additional conformational changes (Fig. 3a). In the blue and orange structures, the linked inner extension of TM2–TM3 rotates ~10–15° towards TM4. This can only occur if TM4 is up because a rotated TM2–TM3 would sterically clash with TM4 in a down conformation (Extended Data Fig. 5). By creating a steric barrier to the non-conductive conformation of TM4, rotation of TM2–TM3 may stabilize the conductive conformation. In the blue, orange, and yellow structures, the selectivity filter to TM4 linker rearranges and the extracellular end of TM4 is laterally displaced from the conduction axis compared to the red structure. This displacement is observed only in TM4-up structures, although unlike with the TM2–TM3 rotation, there is no apparent structural constraint that necessitates the changes be coupled.

The effect of membrane tension on TRAARK activity can be observed in a simple experiment. A cell expressing TRAARK recorded in whole-cell mode gives ~1,000 pA of current (Extended Data Fig. 4a, b). An outside-out patch excised from this cell runs up over several minutes to give ~250 pA and applying pressure to the patch gives ~1,000 pA of current (Extended Data Fig. 4c, d). It is surprising to observe that a patch containing a small fraction of the whole cell membrane (~1%) gives currents similar in magnitude to the whole cell. A logical explanation is that membrane tension varies in these recordings. The whole-cell has near zero tension, the excised patch has low to intermediate tension (~1–5 mN m⁻¹), and applying pressure to the patch gives ~12 mN m⁻¹ tension. From these experiments we can estimate that TRAARK has very low basal activity near zero tension (~1% of maximum activity) and is activated over a broad tension range from ~0.5–4 mN m⁻¹ to ~12 mN m⁻¹.

We want to understand whether the structural differences between conductive and non-conductive states can explain the mechanosensitiv-
cavity between subunits (bound by the inner halves of TM2, TM3 and TM4) (Figs 3 and 4). In the conductive channel this cavity becomes sealed by the elevation of TM4 and rotation of TM2–TM3 to create a flatter membrane-facing surface. The non-conductive channel is more wedge-shaped, the conductive channel more cylindrical, and these different shapes have energetic consequences 20. A cylinder matches the average flat plane of a membrane better than a wedge. A wedge forces the membrane to curve where it meets the surface of the channel and may stabilize the conductive conformation. Area expansion and reduced mid-plane bending of the membrane (grey bars) result in a membrane-tension-dependent energy difference between conformations that explains the mechanosensitivity of TRAAK.

Conformational changes upon channel opening increase the channel cross-sectional area versus membrane depth calculated between each conductive conformation and the non-conductive conformation (positive values indicate area expansion upon channel opening, colours as in a). Depth is on the same scale as a with grey indicating bilayer boundaries. c, Cytoplasmic view of non-conductive and conductive conformations clipped at −9 Å depth. Intramembrane openings (asterisks) or TM4 positions are indicated.

Our data support a model for gating and mechanosensitivity of TRAAK shown in Fig. 4. The conformational change in the two TM4s that gates TRAAK is similar to the conformational change in four inner helices that gates canonical K+ channels 15,16. However, instead of opening a protein seal to the cytoplasm, TM4 movement in TRAAK seals intramembrane openings to prevent lipid from blocking ion conduction. It is possible that other channels might use this unprecedented gating mechanism.

The closely related K2Ps TREK1 and TREK2 share the key functional and structural features of TRAAK 8,12,21. Predictions of area expansion and energy differences from electrophysiological studies of TREK1 agree closely with the values calculated for TRAAK here. Increasing the interaction of a cytoplasmic sensor region immediately following TM4 with the membrane is proposed to activate TREK channels 24–28, but how this sensor is physically coupled to a channel gate is unknown. Extension of the model presented here suggests a simple explanation: increasing the proximity of the intracellular end of TM4 to the membrane in TREK channels stabilizes a conductive TM4-up conformation.

Comparison of TRAAK and TWIK1 suggests an explanation for why among K2Ps only TRAAK subfamily channels are mechanosensitive 8. TWIK1 was observed in a TM4-down conformation with intramembrane openings filled with acyl chains 24. Pore dehydration of TWIK1 has been proposed to stabilize the non-conductive state and introducing polar residues in the cavity increases channel activity, consistent with a hydrophobic barrier to conduction 29. TRAAK has a wider cavity than TWIK1, which expands in the conductive conformation (Extended Data Fig. 1). Two structural differences appear to restrict the TM4s of TWIK1 from adopting a membrane-sealing conformation. First, an amphipathic helix after TM4 in TWIK1 anchors the cytoplasmic end of the helix to the membrane. Second, TM4 in TWIK1 interacts extensively with the cytoplasmic half of TM2–TM3. In TRAAK, the amphipathic nature of TM2–TM3 results in its lateral extension in the membrane away from TM4, enabling free rotation of the inner half of TM4. If TM4 is prevented from rotating to an up conformation in TWIK1, gating would not produce the area expansion and shape changes we propose underlie the mechanosensitivity of TRAAK.

Online Content Methods, along with any additional Extended Data display items and Source Data, are available in the online version of the paper; references unique to these sections appear only in the online paper.

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Protein expression and purification. Cloning of a codon-optimized variant of the Homo sapiens TRAAK gene (UniProt Q07982-2) and heterologous expression in Pichia pastoris was previously described. The construct used in this study is C-terminally truncated by an additional ten amino acids compared to our previous reports and similarly incorporates two mutations to remove N-linked glycosylation sites (N104Q/N108Q) and is expressed as a C-terminal PreScission protease-cleavable EGFP–10X His fusion protein. TRAAK1–290(N104Q,N108Q)–SNS–LEVLFQ/GP–EGFP–H10 is referred to as TRAAK in the text for simplicity.

Frozen Pichia cells expressing TRAAK were disrupted by milling (Retsch model MM301) 5 times for 3 min at 25 Hz. All subsequent purification steps were carried out at 4°C. For crystallization in K⁺, cell powder was added to lysis buffer (50 mM Tris pH 8.0, 150 mM KCl, 6 mM DM) with 10 mM, 30 mM, and 300 mM imidazole pH 8.0 EDTA pH 8.0 (1 mM final) and PreScission inhibitor (1:50 WT:WT) were added to the elution before incubation with gentle rocking overnight. Cleaved protein was concentrated (50 kDa MWCO) and stirred gently for 3 h. Resin was collected on a column and serially washed and eluted in buffer (50 mM Tris pH 8.8, 150 mM KCl, 6 mM DM) with 10 mM, 30 mM, and 300 mM imidazole pH 8.0 EDTA pH 8.0 (1 mM final) and PreScission inhibitor (1:50 WT:WT) were added to the elution before incubation with gentle rocking overnight. Cleaved protein was concentrated (50 kDa MWCO) and applied to a Superdex 200 column (GE Healthcare) equilibrated in SEC buffer (20 mM Tris pH 8.0, 150 mM KCl, 1 mM EDTA, 4 mM n-dodecyl-β-D-maltoside (DM)).

Fab was prepared from monoclonal antibody 13E9 against TRAAK as described and buffer exchanged on a HitTrap desalting column (GE Healthcare) into 20 mM Tris 150 mM KCl pH 8.0 for crystallization in KCl or 20 mM Tris 150 mM KNO3 pH 8.0 for crystallization in TINO3. TRAAK–Fab complexes were prepared by incubating purified channel concentrated (50 kDa MWCO) to ~10 mg/mL with purified Fab concentrated (10 kDa MWCO) to ~30 mg/mL at a 1:2.5 molar ratio in SEC buffer for 10 min at 4°C. The TRAAK–Fab complex was separated from excess free Fab on a Superdex 200 column (GE Healthcare) equilibrated in SEC buffer. TRAAK–Fab complexes were concentrated (10 kDa MWCO) to 30 mg/mL for crystallization.

For crystallization in Tl⁺, TRAAK–Fab complexes were purified identically, except that 150 mM KNO3 replaced the 150 mM KCl in all buffers except for the final SEC buffer in which 150 mM TINO3 replaced the 150 mM KCl.

Crystallization, data collection, and structure determination. Crystals were grown in drops of 0.25–0.35 μL protein added to an equal volume of reservoir, in hanging drops over a 100 μL reservoir, in centrifugation at 35,000g for 45 min. Cobalt resin (Clontech) was added to the supernatant (1 mL resin per 5 g cell pellet) and stirred gently for 3 h. Resin was collected on a column and serially washed and eluted in buffer (50 mM Tris pH 8.8, 150 mM KCl, 6 mM DM) with 10 mM, 30 mM, and 300 mM imidazole pH 8.0, 1 mM leupeptin, 10 μg/mL soy trypsin inhibitor, 1 mM benzamidine, and 1 mM phenylmethylsulfonyl fluoride added immediately before use) at a ratio of 1 g cell pellet per 4 mL buffer. Membranes were extracted for 3 h with stirring followed by centrifugation at 35,000g for 45 min. Crystals were collected at the energy giving a maximum imaginary scattering correction of weak data at high resolution (to CC1/2 < 0.5) and recording for 10 s without data collection. A cryoloop was mounted over the crystals and withdrawn into a cryoprotecting solution (20% (vol/vol) PEG 400, 0.1 M HEPES pH 7.2 (adjusted with NaOH) and bath solution was 5 mM HEPES, 150 mM NaCl, 3 mM MgCl₂, 5 mM EGTA, pH 7.2 (adjusted with KOH) and bath solution was 10 mM HEPES, 15 mM CaCl₂, 3 mM MgCl₂, 1 mM CaCl₂, pH 7.3 (adjusted with NaOH). For experiments in Fig. 2 and Extended Data Fig. 3, the reducing bath solution additionally contained 10 mM DTT added from a freshly thawed 1 M stock of DTT in water. Reducing solution was used within one hour of preparation. For recordings from proteoliposomes, pipette solution was 5 mM Hepes, 180 mM NaCl, 20 mM KCl, pH 7.2 (adjusted with NaOH) and bath solution was 5 mM Hepes, 150 mM KCl, 3 mM MgCl₂, pH 7.2 (adjusted with KOH). Perfusion was accomplished with a nitrogen pressurized micro-perfusion system (VC-3-8, ALA Scientific). All recordings were performed in a tenfold concentration gradient of K⁺ to KCl, for cell recordings, pipette solution was 5 mM Hepes, 150 mM KCl, 3 mM MgCl₂, 5 mM EGTA, pH 7.2 (adjusted with KOH) and bath solution was 10 mM HEPES, 15 mM CaCl₂, 3 mM MgCl₂, 1 mM CaCl₂, pH 7.3 (adjusted with NaOH). For experiments in Fig. 2 and Extended Data Fig. 3, the reducing bath solution additionally contained 10 mM DTT added from a freshly thawed 1 M stock of DTT in water. Reducing solution was used within one hour of preparation. For recordings from proteoliposomes, pipette solution was 5 mM Hepes, 180 mM NaCl, 20 mM KCl, pH 7.2 (adjusted with NaOH) and bath solution was 5 mM Hepes, 150 mM KCl, 3 mM MgCl₂, pH 7.2 (adjusted with KOH). Perfusion was accomplished with a nitrogen pressurized micro-perfusion system (VC-3, ALA Scientific).

The positions of essentially all C atoms in the protein ribbon were determined automatically using Phenix. Positions of side-chain atoms and close proton pairs were determined manually using the program Arapaima. All structures were analyzed using the program MolProbity. Structures were compared after alignment of pore helices and selectivity filters. Structures were superimposed with the program Coot followed by manual adjustment and addition of close atomic interactions. Molecular graphics were generated with PyMol (Version 1.7.2 Schrödinger, LLC) or VMD (Visual Molecular Dynamics).
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Extended Data Figure 1 | The central cavity in conductive and non-conductive TRAAK conformations. a, b, View from the membrane plane of the TRAAK central cavity in the non-conductive (a) and conductive (b) conformations. The exposed surface of hydrophobic amino acids are colored white, arginine and lysine are blue, glutamate and aspartate are red, and polar residues are green. The positions of K⁺ ions in the filter are outlined and residue T277 in TM4 is indicated with an asterisk. c, Diameter of the ion conduction pathway as a function of distance through the membrane for non-conductive TRAAK (red), conductive TRAAK (blue) and TWIK1 (grey, PDB: 3UKM). The green box indicates the position of the selectivity filter and dashed grey lines are the approximate boundaries of the lipid membrane. The ~10 Å diameter constriction formed partially by T277 is indicated with an asterisk. The pore diameter is larger in TRAAK than in TWIK1 and expands below T277 in the conductive conformation.
Extended Data Figure 2 | Reconstituted TRAAK activity in different lipids.

a, Current recorded from TRAAK proteoliposome patches as a function of holding voltage (mean ± s.e.m., n = 9 patches each from three separate experiments). Current through TRAAK reconstituted in phosphatidylcholine lipids with branched acyl chains (1,2-diphytanoyl-sn-glycero-3-phosphocholine, DPhPC) was significantly higher than in non-branched acyl chains (egg L-α-phosphatidylcholine, PC) at each voltage measured (5.0-fold higher at 0 mV, \( P < 0.0001 \), Student’s \( t \)-test).

b, c, Representative recording of pressure (lower trace) activation of TRAAK current (upper trace) in PC (b) or DPhPC (c) lipids.

d, Quantification of pressure activation of TRAAK in PC and DPhPC (mean fold pressure activation at 0 mV ± s.e.m., n = 9 patches each from three separate experiments, \( ***P < 0.0001 \), Student’s \( t \)-test).
Extended Data Figure 3 | Representative electrophysiological recordings from wild-type TRAAK and TRAAK(I159C, R284C). In these experiments and those in Fig. 2, inside-out patches from cells expressing wild-type or mutant TRAAK channels were excised and perfused with reducing bath solution (with 10 mM DTT). After stabilization of the patch (TRAAK channels exhibit a gradual run-up of current following excision to an equilibrium value, for example, Extended Data Fig. 4), the perfusion solution was switched to oxidizing bath solution (no DTT). a, b, Representative voltage family from a TRAAK(I159C, R284C) patch during perfusion of reducing (a) and oxidizing (b) solution. The voltage family protocol is illustrated. c, d, Same as a, b, but from a wild-type TRAAK patch. e, f, Current response (upper) to pressure application (lower) at 0 mV from the same TRAAK(I159C, R284C) patch during perfusion of reducing (e) or oxidizing (f) bath solution. g, h, Same as e, f, but from a wild-type TRAAK patch. Scale is shown between each pair of recordings in reducing and oxidizing bath solutions.
Extended Data Figure 4 | Basal activity and tension activation of TRAAK.

a, Whole cell current from a TRAAK-expressing cell during a voltage step protocol in a tenfold gradient of [K+] (Em = −59 mV, holding voltage = −80 mV, ΔV = 10 mV, indicated steps shown). Red dashed line indicates zero current level. b, Current–voltage relationship from experiment in a. c, Currents (upper traces) recorded from an outside-out patch excised from the same cell as in a, b. The voltage protocol in a was used with an additional pressure step (lower trace) during each voltage step. d, Current–voltage relationship from data in b (mean current 5 min after patch excision before pressure (red) and peak current during pressure step (grey)) and a recording immediately after pulling the patch (red dashes). The excised patch contains <1% of the whole cell membrane area, but gives ~25% of the whole cell current before and similar current during a pressure step. This is explained by very low basal activity of TRAAK with near-zero membrane tension (whole cell) and channel activation by increasing membrane tension over a broad range (intermediate tension in an excised patch to high tension in a pressurized patch).
Extended Data Figure 5 | Detailed view of TM2–TM3 rotation in TRAAK. Stereo view from the cytoplasm of an overlay of non-conductive (red) and conductive TM2–TM3 rotated (blue) conformations. Amino acids that sterically prevent TM2–TM3 rotation when TM4 is down are shown as sticks. TM2–TM3 rotates about hinges at positions G169 and G205. This rotation can only occur if TM4 is up because amino acids L172, F201 and G205 on TM2–TM3 shift (0.75–2.1 Å) to a position that would sterically clash with amino acids Y271 and V275 on TM4 in a down conformation. Translation of Y271 and V275 3.1–4.1 Å in TM4 up conformations creates space for the TM2–TM3 rotation.
Extended Data Table 1 | Data collection and refinement statistics

| Data collection |
|-----------------|
| **Conductive K⁺ bound** | **Conductive Tl⁺ bound** | **Nonconductive K⁺ bound** | **Nonconductive Tl⁺ bound** |
| Space group | P2₁ | P2₁ | P2₁ | P2₁ |
| a, b, c (Å) | 80.6, 138.5, 96.8 | 80.8, 138.8, 96.3 | 80.7, 138.9, 96.5 | 81.0, 138.8, 96.6 |
| α=γ, β (°) | 90, 95.1 | 90, 94.6 | 90, 95.1 | 90, 94.6 |
| Wavelength (Å) | 1.0332 | 0.9781 | 1.0332 | 0.9781 |
| Resolution (Å) | 50-2.5 (2.5-2.5)* | 50-3.0 (3.0-3.0) | 50-2.5 (2.5-2.5) | 50-3.0 (3.0-3.0) |
| R_p (%) | 6.3 (>100) | 9.7 (>100) | 9.3 (>100) | 15.3 (>100) |
| R_w (%) | 4.3 (97.6) | 5.8 (98.6) | 4.0 (94.2) | 7.0 (97.9) |
| CC1/2 (%) | (35.1) | (58.6) | (44.5) | (44.2) |
| l/σl | 22.8 (0.9) | 14.7 (0.9) | 34.8 (1.0) | 11.3 (0.6) |
| Completeness (%) | 99.8 (99.8) | 99.6 (99.7) | 99.9 (100) | 100 (100) |
| Redundancy | 3.8 (3.7) | 5.6 (5.7) | 10.1 (10.3) | 10.5 (10.7) |

| Refinement |
|------------|
| Resolution (Å) | 48.2 - 2.5 | 48.0 - 3.0 | 48.1 - 2.5 | 48.2 - 3.0 |
| No. reflections† | 65770 | 40099 | 66997 | 39746 |
| R_work / R_free | 19.9 / 23.4 | 20.2 / 23.6 | 21.3 / 24.5 | 20.3 / 23.9 |
| No. atoms | | | | |
| Total | 10698 | 10599 | 10585 | 10506 |
| K⁺ / Tl⁺ | 6 | 8 | 5 | 7 |
| Decane | 0 | 0 | 10 | 10 |
| B-factors | | | | |
| All atoms | 90 | 109 | 98 | 112 |
| K⁺ / Tl⁺ | 89 | 94 | 137 | 170 |
| Decane | n/a | n/a | 101 | 131 |
| R.m.s deviations | | | | |
| Bond lengths (Å) | 0.008 | 0.006 | 0.008 | 0.006 |
| Bond angles (°) | 1.058 | 0.898 | 1.002 | 0.908 |
| Ramachandran | | | | |
| favored (%) | 96.69 | 97.37 | 97.14 | 96.51 |
| allowed (%) | 3.16 | 2.48 | 2.71 | 3.26 |
| disallowed (%) | 0.15 | 0.15 | 0.15 | 0.23 |

Data from 1, 1, 3 and 2 crystals were merged for the conductive K⁺, conductive Tl⁺, non-conductive K⁺ and non-conductive Tl⁺ structures, respectively.

* Values for the highest resolution shell are shown in parenthesis.
† 5% of these reflections were used to calculate R_free.
Extended Data Table 2 | Pairwise root mean square deviation (Å) between conformationally static regions between TRAAK structures

|                        | Conductive K⁺ bound PDB: 4WFE | Nonconductive K⁺ bound PDB: 4WFH | Conductive Ti⁺ bound PDB: 4WFG |
|------------------------|-------------------------------|---------------------------------|-------------------------------|
| Nonconductive K⁺ bound | 0.217                         | -                               | -                             |
| PDB: 4WFH              |                               |                                 |                               |
| Conductive Ti⁺ bound   | 0.186                         | 0.227                           | -                             |
| PDB: 4WFG              |                               |                                 |                               |
| Nonconductive Ti⁺ bound| 0.221                         | 0.137                           | 0.193                         |
| PDB: 4WFH              |                               |                                 |                               |

Conformationally static regions of the TRAAK channel in these structures include the entire A chain and residues 1–155 of chain B.
Extended Data Table 3 | Summary of TRAAK channel conformations in crystal structures

| State as a homodimer | PDB ID | Chain ID | TM4 inner half conformation | TM2-TM3 conformation | TM4 outer half conformation |
|----------------------|--------|----------|-----------------------------|----------------------|-----------------------------|
| Nonconductive        | 4I9W   | B        | Down                        | Out                  | Up                          |
|                      | 4WFF   | B        | Down                        | Out                  | Up                          |
|                      | 4WFH   | B        | Down                        | Out                  | Up                          |
|                      | 3UM7   | B        | Down                        | Out                  | Up                          |
|                      | 3UM7   | A        | Down                        | Out                  | Up                          |
| Conductive           | 4WFE   | A        | Up                          | In                   | Displaced                   |
|                      | 4WFF   | A        | Up                          | In                   | Displaced                   |
|                      | 4WFG   | A        | Up                          | In                   | Displaced                   |
|                      | 4WFH   | A        | Up                          | In                   | Displaced                   |
|                      | 4I9W   | A        | Up                          | In                   | Displaced                   |
|                      | 4WFE   | B        | Up                          | Out                  | Displaced                   |
|                      | 4WFG   | B        | Up                          | Out                  | Displaced                   |

Structures shown in Fig. 3 are highlighted with the colour used in the figure.