



Supporting Online Material for

Crystal Structure of the Human K2P TRAAK, a Lipid- and Mechano-Sensitive K⁺ Ion Channel

Stephen G. Brohawn¹, Josefina del Marmol¹, Roderick MacKinnon¹

¹Laboratory of Molecular Neurobiology and Biophysics, The Rockefeller University, Howard Hughes Medical Institute, 1230 York Avenue, New York, New York 10065, USA.

correspondence to: Roderick.MacKinnon@rockefeller.edu

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Materials and Methods

Cloning, expression, and purification. A gene corresponding to *H. sapiens* TRAAK (GI:13124080) amino acids 1-419 was codon-optimized for eukaryotic expression, synthesized (Genewiz, Inc.), amplified by PCR, and ligated into the EcoR1/Xho1 restriction sites of a modified pPICZ-B vector (Invitrogen). The resulting protein is linked at the C-terminus to EGFP and a 10xHis tag via a short linker (SNS) followed by a PreScission protease cleavage site (LEVLFG/GP). Purified full-length protein did not crystallize and was N-glycosylated (data not shown). The construct was modified by PCR to truncate the C-terminal 119 residues and mutate two predicted N-linked glycosylation sites (N104Q, N108Q) for crystallization. Human TRAAK_{1-300(N104Q,N108Q)}-SNS-LEVLFG/GP-EGFP-H10 is referred to as TRAAK in the text for clarity.

Vector was linearized with Pme1 and transformed into *P. pastoris* strain SMD1163 by electroporation. Transformants were selected by plating on YPDS plates with 1mg/mL zeocin. Expression levels of individual clones were compared by FSEC screening of small-scale culture inductions (43). Large-scale expression was performed in a fermentor. Overnight cultures of cells grown in YPD with 1mg/mL zeocin were added to 3L minimal media to an OD₆₀₀ ~1 and grown overnight at 29°C with glycerol feeding and pH maintained at 5.0 by addition of NH₄OH. Cells were then starved to deplete glycerol, temperature was reduced to 27°C, and induction was initiated with slow addition of methanol. Expression continued for ~48-60 hours. Cells were pelleted, frozen in liquid nitrogen, and stored at -80°C.

Cells were disrupted by milling (Retsch model MM301) 5 times for 3 minutes at 25 Hz. All subsequent purification steps were carried out at 4° C. Cell powder was added to lysis buffer (50 mM Tris pH 8.0, 150 mM KCl, 60 mM dodecyl- β -D-maltoside (DDM, Affymetrix), 0.1 mg/mL DNase 1, 0.1 μ g/ml pepstatin, 1 μ g/ml leupeptin, 1 μ g/ml aprotinin, 0.1 mg/ml soy trypsin inhibitor, 1 mM benzamidine, and 0.1 mg/ml AEBSF, with 1 mM phenylmethanesulfonyl fluoride added immediately before use) at a ratio of 1g cell pellet/4mL lysis buffer. Membranes were extracted for 3 hours with gentle stirring followed by centrifugation at 35000xg for 45 minutes. Cobalt resin (Clontech) was added to the supernatant (1mL resin / 5g cell pellet) and stirred gently for 3 hours. Resin was collected on a column and serially washed and eluted in IMAC buffer (50 mM Tris pH 8.0, 150 mM KCl, 6 mM DDM) with 10 mM, 30 mM, and 300 mM imidazole pH 8.0. EDTA (1mM final) and PreScission protease (~1:50 wt:wt) were added to the elution before incubation with gentle rocking overnight. Cleaved protein was concentrated 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, 1 mM DDM). For TI⁺ bound crystals, protein was prepared identically except for substitution of KNO₃ for KCl in lysis and IMAC buffers and TINO₃ for KCl in SEC buffer.

Crystallization and structure determination. Pure protein was concentrated (50kDa MWCO, Millipore) to ~ 10 mg/mL for crystallization. 0.4 μ L protein was added to 0.9 μ L reservoir (21-24% PEG400) in hanging drops. The largest crystals appeared within 1 week and grew to full size cuboids with two approximately equal length faces (~ 0.15 x 0.15 x 0.4 mm) in 3-6 weeks at 4° C. Crystals were cryoprotected by addition of 1 μ L SEC buffer with 30% PEG400 to drops and immediately harvested and frozen in

liquid nitrogen. CH_3Hg^+ -derivatized crystals were prepared by adding a trace amount of solid CH_3HgCl to drops with crystals and incubating over reservoir for 4-12 hours before harvesting. Crystal mother liquor and cryoprotection solution was supplemented with 1 mM n-dodecylphosphocholine (Fos-choline-12, Affymetrix) for native and CH_3Hg^+ -derivative crystals as it was found to improve x-ray diffraction.

Data were collected for native and Tl^+ crystals at APS beamline 23-IDD and for CH_3Hg^+ -derivatized crystals at APS beamline 23-IDB and processed with HKL2000 (44). Data were anisotropic and native data were elliptically truncated and scaled (45) to $3.8 \times 3.3 \times 3.8 \text{ \AA}$ prior to anisotropic scaling with Phaser (46) and sharpening by application of a negative isotropic B factor of -74 to the data.

Seven Tl^+ sites were located by Shelx (47) and refined with Sharp (48) from the Tl^+ derivative data in a multiple-wavelength with anomalous dispersion (MAD) experiment. Positions and phases were further refined in Sharp using isomorphous and anomalous differences in a MAD plus native experimental configuration. Density modification resulted in continuous and interpretable electron density for the majority of the channel (fig. S5). There are two TRAAK protomers forming one channel in the asymmetric unit. Utilization of an early stage helical model during initial rounds of density modification in Sharp to guide solvent envelope estimation improved definition of fine features and weakly defined loop regions in the experimental map. For register information, cysteines in native crystals were derivatized with CH_3Hg^+ . Five Hg sites were consistently found with PhaserEP (49) in multiple datasets from derivatized crystals using partially refined models of TRAAK as starting phase information. Use of PhaserEP to search log-likelihood gradient maps was found to be more sensitive than searching

model-phased anomalous difference Fourier maps for the weaker Hg sites. The Hg positions correspond to 5 of the 8 cysteines in the asymmetric unit: C146 and C206 from each protomer and C218 in protomer B. One cysteine from each protomer is disulfide bonded at the top of the helical cap and so is not expected to react with CH_3Hg^+ . C218 in protomer A is either not observed crystallographically as a Hg^+ adduct due to disorder or is not chemically modified.

The channel was modeled by iterative manual building in Coot (50) and refinement in Refmac (51). A late stage model was improved by refinement in CNS with simulated annealing and a deformable elastic network using the starting model as a reference structure (52). Refinement was aided by incorporation of experimental phase and two-fold local NCS restraints and converged to an $R_{\text{free}}=32.3\%$ with good geometry (Table S1). Strict two-fold NCS restraints were not used, as there exist small but significant differences in the relative orientations of some regions of the channel including the outer helices and helical cap. Two loops and residues at each protomer terminus were not modeled due to lack of interpretable electron density. The final model consists of TRAAK residues 25-106, 112-187, and 190-290 in protomer A, residues 27-104, 112-179, and 193-290 in protomer B, and five K^+ ions.

We note that while protein mediated crystal contacts between the helical cap and the pore domain 1-2 linking region are observed along the **b** axis in the crystal, both **a** and **c** axes lack well defined protein mediated contacts (Fig. S6). While consistent with the severe anisotropy of the data (strong **b** direction, weak **a** and **c** directions), poor packing can be indicative of incorrect space group determination as a result of apparent pseudosymmetry. Molecular replacement in each of the other seven primitive

orthorhombic enantiomorphs followed by refinement of the top solutions failed to produce a convincing solution with observable packing in all lattice directions. Attempts to find solutions in alternatively processed data (in primitive monoclinic, centered monoclinic, centered orthorhombic, and primitive tetragonal lattices) also failed. There are 24 residues at the N terminus and 19 (10 from TRAAK and 9 remaining from the linker and protease cleavage site) at the C terminus of each protomer that are unmodeled due to poor electron density. Either of these regions if even partly extended would be of sufficient length to bridge the $\sim 20\text{\AA}$ gap between channel layers in the crystal. Alternatively, detergent molecules/micelles or other solvent molecules may contribute to lattice formation. Regardless, we are confident that our ability to refine the structure to good statistics indicates a reliable model.

Electrophysiological recordings from CHO cells. Full length TRAAK and the truncated and mutated TRAAK construct used for crystallization were cloned into a pCEH vector for mammalian cell expression. CHO cells (ATCC) were maintained in DMEM-F12 (Gibco) containing 10% FBS. Cells were plated onto poly-D-lysine-coated glass coverslips (BD BioCoat) ~ 24 hrs before transfection with Lipofectamine2000 (Invitrogen) following manufacturers protocol. After 48-72 hrs, coverslips were transferred to the recording chamber. Immediately before recording, media was replaced by bath solution. All recordings were performed at room temperature. Recordings were obtained with an Axopatch 200B amplifier (Molecular Devices) using standard whole-cell patch-clamp and excised outside-out patch techniques. Recordings were filtered at 1 kHz with sampling at 10 kHz. Pipettes of 1.5-2 M Ω resistance (for whole cell recordings) and 2-3 M Ω resistance (for outside-out patch recordings) were pulled from borosilicate

glass and fire polished. Currents were recorded during voltage steps from -100 to 40 mV in 10mV increments from a holding potential of -80 mV. Voltage ramps were obtained by holding at -100mV and increasing to 40mV in 800 msec. For arachidonic acid (AA) activation experiments, cells were continuously perfused with either bath solution or bath solution containing 100 μ M AA. Currents were recorded from the same cell before and ~1 min after perfusion of AA. For pressure activation experiments, positive pressure was applied to patches through a syringe connected to the pipette. Pipette solution was 150 mM KCl, 3 mM MgCl₂, 5 mM EGTA, 10 mM Hepes (pH 7.2). Bath solution was 3 mM MgCl₂, 1 mM CaCl₂, 10 mM Hepes (pH 7.3) and 15 mM KCl and 135 mM NaCl for activation experiments and either 5 mM KCl and 145 mM NaCl, 15 mM KCl and 135 mM NaCl, 70 mM KCl and 80 mM NaCl or 150 mM KCl for voltage ramp experiments.

Reconstitution in lipid vesicles. Purification of TRAAK was carried out identically except SEC buffer was 20 mM Hepes pH 8.0, 150 mM KCl, 1 mM EDTA, 1 mM DDM. Protein was concentrated to 1 mg/mL and added to 10mg/mL 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphoethanolamine: 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phospho-(1'-*rac*-glycerol) (POPE:POPG) (3:1) lipid vesicles in dialysis buffer (20mM Hepes pH 7.4, 150 mM KCl, 1 mM EDTA) with 10 mM DDM at a protein to lipid ratio of 1:100 (w/w). The mixture was rocked overnight before dialyzing in 50 kDa MWCO tubing for 1 week against 10 4L changes of dialysis buffer with Bio-Beads (Bio-Rad) added to the final three changes. Vesicles were frozen in liquid nitrogen and aliquots stored at -80°C until required.

Flux assay. Frozen vesicles were thawed and briefly sonicated prior to the assay. 10 μ L of vesicles were added to 190 μ L of flux assay buffer (20 mM Hepes pH 7.4, 150

mM NaCl, 1 mM EDTA, 2 μ M 9-amino-6-chloro-2-methoxyacridine (ACMA)).

Fluorescence was recorded every 10 seconds (excitation $\lambda=410$ nm, emission $\lambda=490$ nm).

After 30 seconds of baseline fluorescence was monitored, K⁺ flux was initiated by

addition of 1 μ M *m*-chlorophenyl hydrazone (CCCP) to collapse the electrical potential.

The chemical gradient was terminated by addition of the K⁺ ionophore valinomycin to

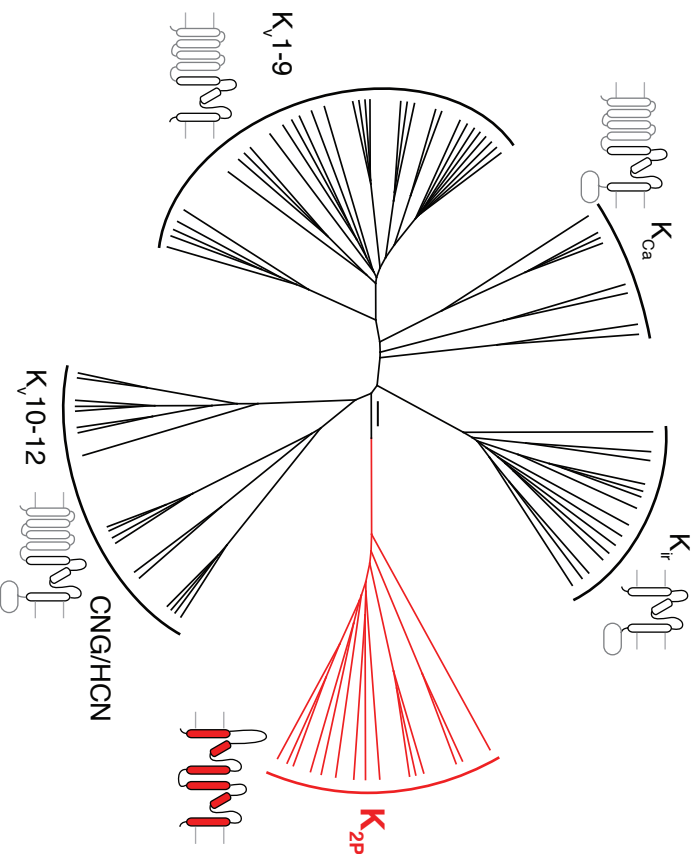
0.02 μ M and fluorescence was monitored until equilibrium reached.

Lipid bilayer recordings. Vesicles from the same reconstitution used for the flux assay were thawed and briefly sonicated prior to use. Bilayer experiments were performed essentially as previously described (53). Planar lipid bilayers of 3:1 (w:w) 1,2-diphytanoyl-*sn*-glycero-3-phosphocholine:1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphate (DPhPc:POPA) were painted over a 300 μ m polystyrene hole separating two chambers. Vesicles were added to the *cis* chamber filled with 4 mL 10 mM Hepes pH 7.5, 150 mM KCl while the *trans* side contained 3 mL 10 mM Hepes pH 7.5, 15 mM NaCl. Once vesicles were fused with the bilayer NaCl was made 150 mM on the *trans* side. Measurements were made with the voltage-clamp method in whole-cell mode using an Axopatch 200B amplifier, a DigiData 1440A analogue-to-digital converter, and Clampex software (Axon instruments). Analogue data were filtered at 1 kHz and sampled at 10 kHz.

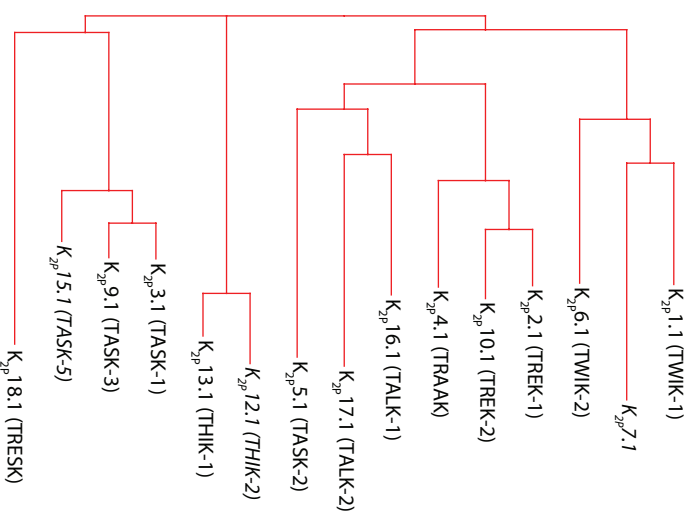
Software. Crystallographic programs from the CCP4 suite were used throughout structure determination (54). Structure figures were generated with Pymol (55).

Alignments were made with MAFFT (56) and visualized with JalView (57).

A



B



C

	TRAAK (K2P_4.1)	TREK1 (K2P_2.1)	TREK2 (K2P_10.1)	TWIK1 (K2P_1.1)	TWIK2 (K2P_6.1)	TWIK3 (K2P_7.1)	TALK1 (K2P_16.1)	TALK2 (K2P_17.1)	TASK2 (K2P_5.1)	THIK2 (K2P_12.1)	THIK1 (K2P_13.1)	TASK1 (K2P_3.1)	TASK3 (K2P_9.1)	TASK5 (K2P_15.1)
TREK1 (K2P_2.1)	39.2													
TREK2 (K2P_10.1)	41.0	59.0												
TWIK1 (K2P_1.1)	28.0	29.7	29.6											
TWIK2 (K2P_6.1)	33.0	29.3	28.1	44.3										
TWIK3 (K2P_7.1)	28.2	25.4	28.4	39.3	33.2									
TALK1 (K2P_16.1)	33.2	30.1	30.2	28.1	30.4	28.5								
TALK2 (K2P_17.1)	35.0	30.5	27.5	24.3	31.0	25.1	35.2							
TASK2 (K2P_5.1)	28.0	27.3	25.9	27.0	28.4	23.5	30.9	31.4						
THIK2 (K2P_12.1)	24.9	25.7	23.5	25.9	27.0	25.8	26.3	23.1	24.9					
THIK1 (K2P_13.1)	25.5	23.9	23.3	25.8	24.0	22.3	24.7	24.1	23.4	63.8				
TASK1 (K2P_3.1)	27.1	26.3	24.5	29.6	30.2	26.1	29.8	28.3	26.5	24.2	28.2			
TASK3 (K2P_9.1)	23.7	26.2	26.2	27.2	27.9	22.6	28.0	24.4	24.6	21.4	24.1	58.9		
TASK5 (K2P_15.1)	27.8	29.4	27.0	28.5	29.7	27.8	29.2	27.8	29.5	27.4	27.1	51.4	55.1	
TRESK (K2P_18.1)	21.9	19.7	20.3	22.1	20.2	20.1	24.9	20.9	20.5	21.1	21.0	23.1	24.4	22.5

Fig. S1.

Evolutionary relationships and unique structural architecture of K2P channels. (A)

Unrooted phylogenetic tree of the K⁺ ion channel superfamily. The tree was calculated from a sequence alignment of the 88 human K⁺ channel superfamily pore domains (58). K2P channels form a clade distinct from other K⁺ channels (the voltage-gated (K_v1-9), Ca²⁺-activated (K_{Ca}), inward-rectifying (K_{ir}), and cyclic nucleotide-gated (CNG/HCN and K_v10-12 channels)). The architecture of each family is illustrated as a cartoon from N- (left) to C-terminus (right). Note that some K_{Ca} channels contain an S₀ helix placing the N-terminus on the extracellular side. Cylinders represent helices drawn with respect to the membrane (gray lines) with extracellular solution above. A K⁺ channel pore domain (black outlines) consists of two membrane spanning helices (the outer and inner helices) flanking a membrane reentrant pore helix and selectivity filter. K2P channels (red) have two concatenated pore domains per protomer, while other channels have one.

Accessory domains in other channels are drawn in light gray. **(B)** K2P phylogenetic tree.

The tree was calculated from a sequence alignment of the 15 human channel pore domain 1 sequences. The K2P channels can be divided into six subfamilies based on sequence similarity: TRAAK/TREK (TRAAK, TREK-1, and TREK-2), TWIK (TWIK-1 and TWIK-2), TALK (TALK-2 and TASK-2), THIK (THIK-1 and THIK-2), TASK (TASK-1, TASK-3, and TASK-5) and TRESK. Channels for which functional expression has not been demonstrated are italicized in **(B)**. **(C)** Pairwise percentage of identical residues between human K2P channels. Sequence conservation between subfamilies of K2P channels (~20-30%) is comparable to that between channels from other K⁺ channel clades (e.g. between K_v and K_{ir} channels).

PD1 outer helix

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TAAK 1 M-----TTAPQEPPARPLQAGSGAGPAPGRAMRSTLLA LALVLL YLVSGALVFRAL E 54
TREK-1 M-----AAPDLLPKSA-----AQNSKPRLSFSTKPTVLSRVESDIT I NVMKWKT VSTIFLVVLL YL I GATVFKALE 69
TREK-2 1 MFFLYTDFFLSLVAVPAAAPVCQPKSATNGOPPAPAPTPTPRLS ISSRATVVA RMEGT SQGGLQTVMKWKTVVAI FVVVVV YLVTGGLVFRAL E 94
TWIK-1 M-----LQSLAGSSCVRLVERHRSAWCFGL VLGYLL YLVFGAVVFSV E 45
TWIK-2 1 M-----RRGALLAGALAAAYAA YLVLLGALLVVARLE 29
TWIK-3 1 M-----GGLRPWSRYGL VVAHLLALGLGAVVFAQLE 32
TALK-1 1 M-----PSAGLCSWGGRVLP LLAAYCV YLLLGATIFQLLE 36
TALK-2 1 M-----YRPRARAPEGRVRCVAVPTVL LLAYLAYLALGTGVFVLE 44
TASK-2 1 M-----VDRGPL TSAIIFYLAIGAIFVELE 27
THIK-2 1 M-----SSRSRPPPPRRSRRRLPRPSCCCCRSHLNEDTGRFVL AALIGL YLVAGATVFSAL E 61
THIK-1 1 M-----AGRGFSWGP-----GHLNEDNARFLL AALIVL YLLGGAAVFSAL E 42
TASK-1 1 M-----KRQNVRTLAI IVCTFT YLLVGA VFDAL E 30
TASK-3 1 M-----KRQNVRTLAI IVCTFT YLLVGA VFDAL E 30
TASK-5 1 M-----RRPSVRAAG VLCTLC YLLVGA VFDAL E 30
TRESK 1 M-----EVSGHPQARRCCPEALGKLPFG--LCFLCLVTV ALVGA VFSAL E 45

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Helical cap helix 1 Helical cap helix 2 PD1 pore helix PD1 filter

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TAAK 55 QPH EQQAQRELGEVREKFLRAH PCVSDQE GLLIK--EVAD LGG GADPETNSTSNSS--HSA WDLGSAFF SGT I I T T I GYGNVALRT DAGR 143
TREK-1 70 QPH EISQRTTIVIQKQTFISQH SCVNSTE DELIQ--QIVAI NAGI I PLGNTSNQIS--H WDLGSSFF F AGT V I T T I GFGNI SPRT EGGK 156
TREK-2 95 QPF ESSQKNTIALEKAEFLRDH VCSPQEL ETLIQ--HALD DNA GVSPIGNSSNNS--H WDLGSAFF F AGT V I T T I GYGNV APST EGGK 181
TWIK-1 46 LPYEDLLRQELRKLKRRFLEEH ECLSEQQ EQFLG--RVLE SNY GVSVLSNAGSNWN--WDFTSALF F AST V L S T T I GYHTV PLSDGK 131
TWIK-2 30 GPH EARLRAELET LRAQLQRS PCVAAPA DAFVE--RVLA GRL GRVVLANASGSANADPA WDFASALF F AST I I T T I GYGYT T PLT DAGK 120
TWIK-3 33 GPACRLQAE LRAELAAFQAEHRA CLPPGA EELLG--TALATQAHGVSTLGNSEGR T--WDLPSALL F ASIL T T T GYGHM PLSPGK 119
TALK-1 37 RQA EQASRDQQLKRLRFL ENY T CLDQWAMEQFVQ--VIME WVK GVNPKGNSTNPSN--WDFGSSFF F AGT V V T T I GYGNL APST EAGQ 122
TALK-2 45 GRAAQDSSRSFQRDKWELLQNF T CLDRPA DSLIR--DVVQ YKN GASLLSNTTSMGR--WELVGSF F SVST I T T I GYGNL SPNT MAAR 130
TASK-2 28 EPHWKEAKKNYYTQKLHLLKEF PCLGQEG DKILE--VVSD AGQVVAITGNQTFNN--WNWPNAMI F AT V I T T I GYGNV PKT PAGR 112
THIK-2 62 SPG AEARARWGATLRNFSAAH--GVAEPE RAFLR--HYEA LAA GVRADALRPR--WDFPGA F Y VGT V V S T I GFGMTT PAT VGGK 143
THIK-1 43 LAH RQAKQRWEERLANFSRGH--NLSRDEL RGF LR--HYEE A TRAGI RVDNVRPR--WDFTFGA F Y VGT V V S T I GFGMTT PAT VGGK 124
TASK-1 31 SEP ELIERQLELRQQLRARY--NLSQGGYEELER--VVLRLKPHKAGVQ--WRFAGS F Y A I T V I T T I GYGHAA P T DGGK 107
TASK-3 31 SDH MREEEKLKAEERIIRKGY--NISSEYRQLEL--VILQSEPHRAGVQ--WKFAGS F Y A I T V I T T I GYGHAA P T DGGK 107
TASK-5 31 SEA SGRQRLLVQKRGALRRKF--GFAEDYRELER--LALQ EPHRAGRQ--WKFPGS F Y A I T V I T T I GYGHAA P T DGGK 107
TRESK 46 DGQVLVAADGGEFEK--FLEEL--CRILNCSETVVEDRKQDLQGLKQVQPWFNRTH--WSFLSSLF F CCT V F S I V GYGYI YPVT RL GK 130

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PD1 inner helix

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TAAK 144 LFCIFAYLVGIP LFGILLAGV CDRLGSSLRHGI GHIEAI-----182
TREK-1 157 IFCI IYAL LGIP LFGFLLAGV CDQLGTIFGKGI AKVEDT-----195
TREK-2 182 IFCI IYAL LGIP LFGFLLAGV CDQLGTIFGKSI ARVEKV-----220
TWIK-1 132 AFCI IYSVIGIP FTLLFLTAVVQRI TVHVTRR--PVLYF-----168
TWIK-2 121 AF SIAFALLGV PTTMLLLTASAQRLLSLLTHV--PLSWL-----157
TWIK-3 120 AF CMVYALGL PASLALVATLRHCLLPVLSR--PRAWV-----155
TALK-1 123 VFCVYAL LGIP LNVIFLNHL CTGLRAHLA--AIERW-----157
TALK-2 131 LFCIFAYLVGIP LNLVVLNRL CHLMQGGVNHWASRLGGT-----169
TASK-2 113 LFCVYVYVGV PLCLTWISAL CKFFGGRAK--RLGQF-----147
THIK-2 144 ALIAYVGF CAGTILFFNFLFLERI ISLLAFIMRACRER-----182
THIK-1 125 IFLIYVGV CCSSTILFFNFLFLERLIT I IAYIMKSCHQR-----163
TASK-1 108 VFCMFYAL LGIP LTLVMFQSL CERINTLVRYL LHRAKKG-----146
TASK-3 108 VFCMFYAVL GIP LTLVMFQSL CERMNTFVRYL LKR I KCC-----146
TASK-5 108 VFCMFYAL LGIP LTLVTFQSL CERLNAVVRRL LLAAKKC-----146
TRESK 131 YL CMLYAL FGIP LMLVLTDT CDILATILSTS YNRFRKFPFFTRPLL SKWCKPSL FKKKPPDKPADEAVPQII I SAEELPGPKLGT CPSRPPSCSM 225

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PD2 outer helix

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TAAK 183 -FLKW-----HVPPPELVRLSAML FLLIGCLLFV-----LPTPFV CYM-----E-DWSKLE 227
TREK-1 196 -FIKW-----NVSQTKIRIIST IIFILFGCVLFV-----ALPAII KHI-----E-GWSALD 240
TREK-2 221 -FRKK-----QVSQTKIRIVST IFLIFLAGCIVFV-----TIPAVI KYI-----E-GWTALE 265
TWIK-1 169 -HIRW-----GFSKQVVAI VHAVLLGFTVVSCEF--FIPAAV SVL-----E-DDWNFLE 214
TWIK-2 158 -SMRW-----GWDPRAACWHLVALLGVVTVCF--LVPAVI AHL-----E-EAWSFLD 203
TWIK-3 156 -AVHW-----QLSPARAALLQAVLGLLVASSFV--LLPALV WGL-----E-QGDCSLG 201
TALK-1 158 -EDR-----PRRSQVLQVLGLALFLTGLTVLIL--IFPPMV SHV-----E-GWSFSE 201
TALK-2 170 -LTKR-----WQDPDKARWLAGSGALLSGLLFL--LLPPLL SHM-----E-GWSYTE 210
TASK-2 148 -LTKR-----GVSLRKAQICTVI FIVWGLVHL--VIPPV MVV-----E-GWNYIE 192
THIK-2 183 -QLRRSGLLPATFRRGALS EADSLAGWKPSVYVHLL I LGLFAVLLS--CCASAMYSV-----E-GWDYVD 245
THIK-1 164 -QLRRRGGALPQESLKDAGQCEVDSL AGWKPSVYVHLL I LCTASILIS--CCASAMYTP I-----E-GWSYFD 226
TASK-1 147 -L-----GMRRADVSMMNMT IGFSSICTL--CIGAAA SHY-----E-HWTFQ 188
TASK-3 147 -L-----GMRNTDVSMMNMT VGF FSCMGTL--CIGAAA SOC-----E-EWSFFH 188
TASK-5 147 -L-----GLRWT CVSTENLVVAGLLACAATL--ALGAVA SHF-----E-GWTFH 188
TRESK 226 ELFERSHALEKQNTL-----QLPPQAMERSNSCP ELVLRGLSYSI ISNLDEVGQVERLDI PLPI IALIV AYI SCAAA I LPFW TQLDFEN 312

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PD2 pore helix PD2 filter PD2 inner helix

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TAAK 228 AIYFVIVLT TIVGFGDYVAGA-----DP-RQDSPA YQPLVWF--W L L G I AYFASVLT T I GNWL-----RVVSRRT R 291
TREK-1 241 AIYFVVI TLT IGF GDYVAGG-----S-DIEYDFY KPVVWF--W LV G I AYFAAVLSMI GDWL-----RVI SKKT K 304
TREK-2 266 SIYFVVLT TIVGFGDFVAGG-----NA-GI NYREW KPLVWF--W LV G I AYFAAVLSMI GDWL-----RVL SKKT K 330
TWIK-1 215 SFYFCFIS ST IGLGDYVPEE-----GY-NQKFREL YKIGITC--Y L L G I AMLVLETFC ELHELK KFRKMF VYKK 284
TWIK-2 204 AFYFCFIS ST IGLGDYVPEE-----AP-GQP YRAL YKVLTV--Y L F G I VAMVLVLTQFRHVSDDLHGLTEL I LPP 273
TWIK-3 202 AVYFCFSS ST IGL EDLLPGR-----GR-SLHPV IYHLGQLALLGYL L G I LAMLLAVETFS EL PQVRAMGKFFRPSG 273
TALK-1 202 GFYFAF I T LST IGF GDYVGHPLNF I T P S G L L P S Q E P F Q T P H G K P E S Q Q I P G S F Q K V S S M N V W P L S G M H S P G L A F P L-----PDCN I PDQ 286
TALK-2 211 GFYFAF I T LST IGF GDYVIGM-----NP-SQRYPLW KNMVSL--W L F G M A W L A L I K L I L S Q L E T P G R V C S C H H 279
TASK-2 193 GLVYF I T I ST IGF GDYVAGV-----NP-SANYHAL YRFVEL--W Y L G A W L S L F V N W K V S M F--VEVHKA I KRRR 261
THIK-2 246 SLVYFCFV FST IGF GDVLSQ-----HA-AYRNQGL YRLGNFL--F I L L G V C C I Y S L F N V S I L I K Q L N W M L R K L S C 315
THIK-1 227 SLVYFCFVAFST IGF GDVLSQ-----NA-HYESOGL YRFANFV--F L M G V C C I Y S L F N V S I L I K Q L N W M L R K M D S 296
TASK-1 189 AY YCF I T L T IGF GDYVALQ-----KDQALQTQPQ VAFSFM--Y L T G I T V I G A F L N L V L R F-----MTMNEDE 254
TASK-3 189 AY YCF I T L T IGF GDYVALQ-----TKGALQKPL YVAFSFM--Y L V G I T V I G A F L N L V L R F-----LTVNSDE 254
TASK-5 189 AY YCF I T L T IGF GDYVALQ-----SGEALQRKLP YVAFSFL--Y I L L G I T V I G A F L N L V L R F-----LVSADWP 254
TRESK 313 AFYFCFVLT T IGF GDTVLEH-----PNFLLFSI--Y I V G M E I V F I A F K L V Q N R L I D I Y K N V M L F F A K 375

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TRAAK 292 AEMGGLTAQAASWTGTVTARVTQ-----RAGPAAPPEKEQPLLPPPPCPAQPLGRPRSPSP 349
TREK-1 305 EEVGEFRAHAAEWANVTAEFKET-----RRRLSVEIYDKFQRATSI----KRKLSAELAGNHN 359
TREK-2 331 EEVGEIKAHAAEWKANVTAEFRET-----RRRLSVEIHDKLQRAATIRSMERRRLGLDQRAHSL 389
TWIK-1 285 DKDE-----DQVHI-----EHDQLSFSSI TDQAAGMKEDQKQN 318
TWIK-2 274 PCPASFNAD-----DRVDILGPQPESHQQLSASSHTDYASIPR----- 313
TWIK-3 274 PVTAE-----DQGGIL-----GQDELALSTLPPAAPASGQAPAC- 307
TALK-1 287 ERFRLPHGAW-----KFWPLPLPSNSKWAPMWLGSQAQV----- 322
TALK-2 280 SSKEDFKSQSW-----RQGPDPREPESHSPQQGCYPEGPMGI----- 316
TASK-2 262 RRKESFESSPHSRKALQVKGSTASKDVNIFSLSKKEETYNDL I KQI GKKAMKTSGGGETGP 356
THIK-2 316 RCCARCCPAPGAPLA-----RRNAITPGSRLRRRLAALGADPAARDSDAEGRRLS 365
THIK-1 297 GCCPQC--QRGLLRS-----RRNVVMPGSRVNRRCNISIETDGVA-ESD TDGRRLS 343
TASK-1 255 KRDAEHRALLT-----RNGQAGGGGGGSAHTTDTASSTAAAGGGGRNVY 300
TASK-3 255 RRDAEERASLA-----GNRNS-----MVIHIPEEPRPSRP-----RYK 287
TASK-5 255 ERAARPP-----SPRPPGAPESRGLWLP RRPARSVGSA----- 287
TRESK 376 GKFYHLVKK----- 384

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TRAAK 350 EKAQPPSPPTASALDYPSENLA FIDESSDTQSERG-----CPLPRAPRGRRRNP NPKPVPRGPGR 411
TREK-1 360 QELTPCRRRLSVNHLT SERD-----VLPPLLKTESIYLNGLTPHCAGEEIAVIENIK 411
TREK-2 390 DMLSPEKRSVFAALDTGR----FKASSQESI NNRPNLRLKGPEQL NKHGQGA SEDNI I NKF 480
TWIK-1 319 EPFVATQSSACVDGPANH-----GSTSRLTKRKNKDLKKTLPEDVQKIYKTFRNY 336
TWIK-2 -----
TWIK-3 -----
TALK-1 -----
TALK-2 317 QHLEPSAHAAGCGKDS-----SDEETS KSSLEDNLAGEESPQQ 332
TASK-2 357 EVSQT LRSKGHVSRSPDEEAVARAPEDSSPAPEVFMNQLDR I SEECEP WDAQDYHPL I FQDAS 451
THIK-2 366 GELI SMRDLTASNKVS LALLQKQLSETANGYPR-----SVCVNT RQNGFSGGVGALGI MNNRL 430
THIK-1 344 GEMI SMKDLLAANKASLAI LKQLSEMANGCPH-----QTSTLARDNEFSGGVGAFI MNNRL 408
TASK-1 301 AEVLHFQSMC SCLWYKSREKLQYSI PMI I PRDLSTSDT CVEQSHSPGGGGGRYSDT PSRRCL 394
TASK-3 288 ADVPD LQSVCSCTCYRSQD---YGGRSVAPQNSFSAKLAPHYFHSI SYKIEE I SPSTLKNSLFP 374
TASK-5 288 SVFCHVHKLERC---ARDNLGFSPPS-----SPGVVRGGQAPRPGARWKS I - 330
TRESK -----

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TRAAK 412 PRDKGVPV----- 419
TREK-1 -----
TREK-2 481 LDEEKKEEET EKMCNSDNSSTAMLDCI QQHAEL ENGM I PTDTKDREPENNSLLED RN 538
TWIK-1 -----
TWIK-2 -----
TWIK-3 -----
TALK-1 -----
TALK-2 -----
TASK-2 452 GAEAKAPLNMGEFPSSSESTFTSTES ELSVPYEQLMNEYNKANS PKGT----- 499
THIK-2 -----
THIK-1 -----
TASK-1 -----
TASK-3 -----
TASK-5 -----
TRESK -----

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Fig. S2.

Multiple sequence alignment of human K2P channels. Alignment of the 15 human K2P channels is colored by conservation in a ramp from white (not conserved) to dark blue (highly conserved). Secondary structure of TRAAK is indicated above the sequences and labeled with PD1 and PD2 signifying pore domain 1 and 2, respectively. Large gaps in the alignment are shown as dashed black lines, residues not observed in the crystal structure as dashed gray lines, loops and non-helical secondary structure as solid gray lines, K⁺ selectivity filters as green lines, and helices as cartoons. Helices in pore domain 1 are colored blue and helices in pore domain 2 are colored orange. In the helical cap, hydrophobic core-forming residues are marked with green boxes and the disulfide bonded C78 is marked with a yellow box above the sequence. In the amphipathic segment of the pore domain 1 inner helix, hydrophobic residues highlighted in Fig. 5 are marked with green boxes and basic residues highlighted in Fig. 5 are marked with red boxes above the sequence. The hinge glycine (G153) and kink proline (P155) in the pore domain 1 inner helix are also marked with a green box above the sequence. Secondary structure is drawn until the last residue present in the crystal construct of the TRAAK channel (Q300).

Fig. S3.

Multiple sequence alignment of TRAAK/TREK K2P channels. Alignment of four TRAAK, four TREK-1, and four TREK-2 channels is colored by conservation in a ramp from white (not conserved) to dark blue (highly conserved). Secondary structure of TRAAK is indicated above the sequences and labeled with PD1 and PD2 signifying pore domain 1 and 2, respectively. Large gaps in the alignment are shown as dashed black lines, residues not observed in the crystal structure as dashed gray lines, loops and non-helical secondary structure as solid gray lines, K⁺ selectivity filters as green lines, and helices as cartoons. Helices in pore domain 1 are colored blue and helices in pore domain 2 are colored orange. In the helical cap, hydrophobic core-forming residues are marked with green boxes and the disulfide bonded C78 is marked with a yellow box above the sequence. In the amphipathic segment of the pore domain 1 inner helix, hydrophobic residues highlighted in Fig. 5 are marked with green boxes and basic residues highlighted in Fig. 5 are marked with red boxes above the sequence. The hinge glycine (G153) and kink proline (P155) in the pore domain 1 inner helix are also marked with a green box above the sequence. Secondary structure is drawn until the last residue present in the crystal construct of the TRAAK channel (Q300). Abbreviations used are: Hs, *Homo sapiens*, Rn, *Rattus norvegicus*, Bt, *Bos taurus*, Tn, *Tetraodon nigroviridis*, Gg, *Gallus gallus*, Dr, *Danio rerio*, Xl, *Xenopus laevis*.

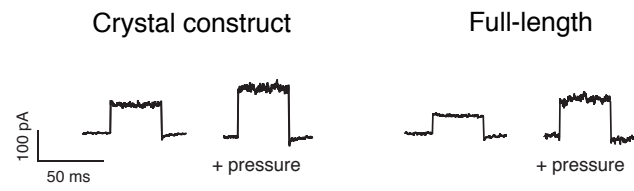
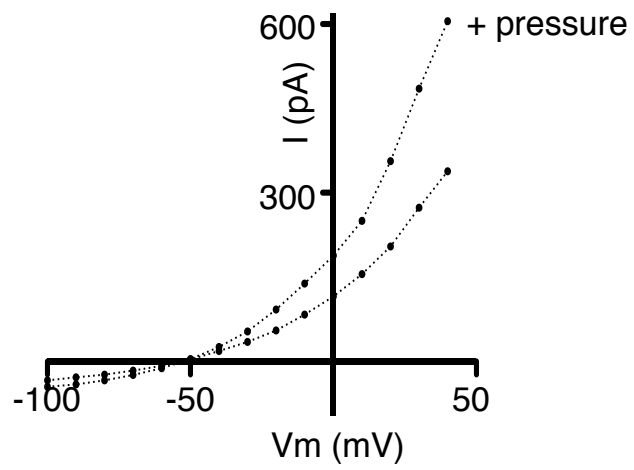
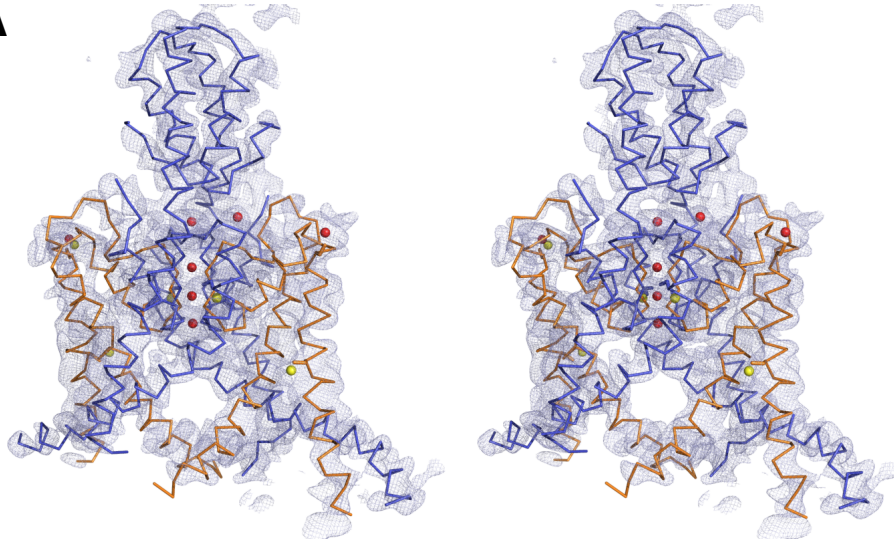
A**B**

Fig. S4.

Pressure activation of TRAAK. (A) Pressure activation of the crystal construct and full-length TRAAK channels. A representative current recording during a voltage pulse from -80 to -10 mV was made before and during the application of positive pressure. (B) Current-voltage relationship is plotted from outside-out patch recordings of the crystal construct of the TRAAK channel during voltage pulses from -100 to 40 mV from a holding potential of -80 mV before and during the application of positive pressure through the patch pipette (+ pressure).

A



B

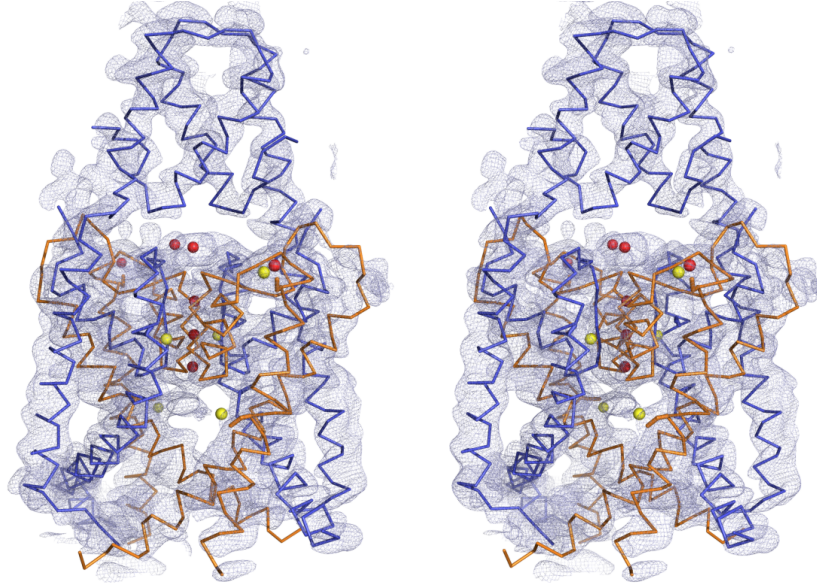


Fig. S5.

The TRAAK structure solution. (A) Stereo view of TRAAK similar to the view in Fig. 1C. The view in (B) is rotated $\sim 70^\circ$ counterclockwise. Electron density (light blue mesh) calculated from experimental phases and used for initial model building is shown around the final TRAAK model in wire representation with pore domain 1 colored blue and pore domain 2 colored orange. Phases were calculated with Sharp (48) from a multiple-wavelength isomorphous replacement with anomalous scattering experiment using two-wavelength data from a Tl^+ -containing derivative and K^+ -containing native data. A solvent fraction of 0.75 was used for density modification within Sharp, the map is calculated from 31-3.3 Å, and is contoured at 1.5σ . Seven Tl^+ sites determined with Sharp are shown as red spheres. Five Hg sites determined from CH_3Hg^+ -derivatized crystals with Phaser EP (49) using a preliminary TRAAK model as starting phase information are shown as yellow spheres. Cysteine residues in the final TRAAK model proximal to the Hg^+ positions are shown as sticks.

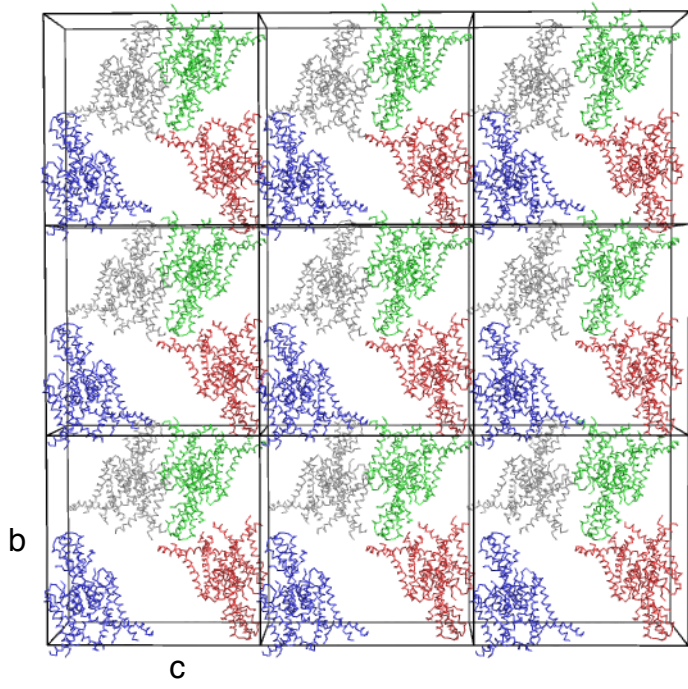
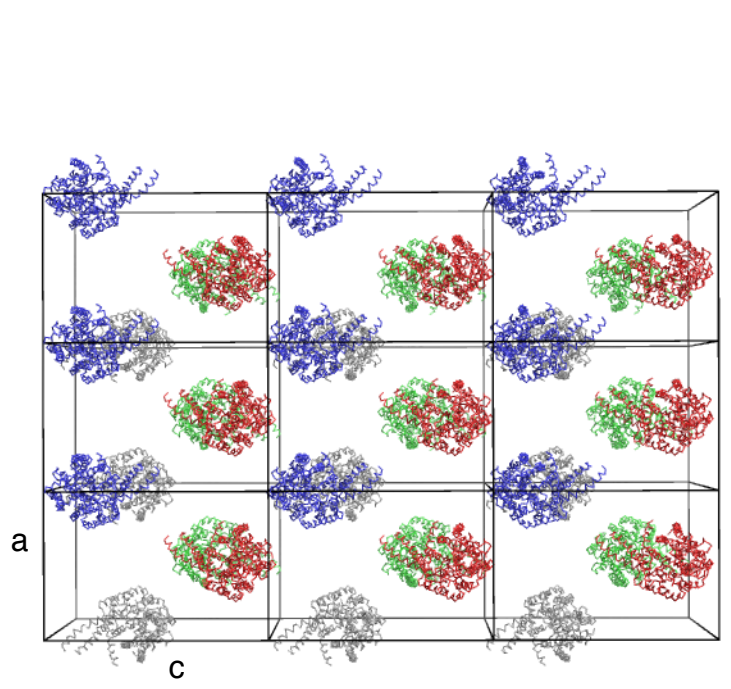
A**B**

Fig. S6.

TRAAK crystal packing. (A,B) Two views of the TRAAK crystal lattice: along the **a** axis in (A) and along the **b** axis in (B). Unit cells are drawn as boxes and TRAAK molecules in each unit cell are shown in different color ribbons. Crystals diffracted anisotropically with a strong (3.3 Å) **b** direction and weak (3.8 Å) **a,c** directions.

Consistently, well-defined packing interactions exist along the **b** axis, with the helical cap from one channel forming crystal contacts with the cytoplasmic side of the neighboring channel. The **a** and **c** directions, however, lack clear protein-mediated contacts.

Presumably micelle- or detergent- mediated contacts and/or poorly discernable protein contacts propagate the lattice in these directions.

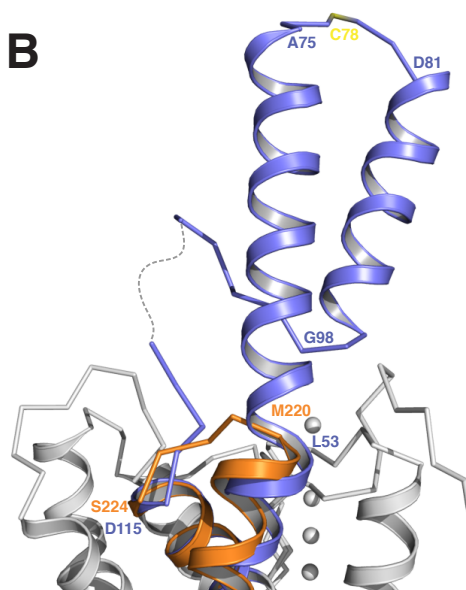
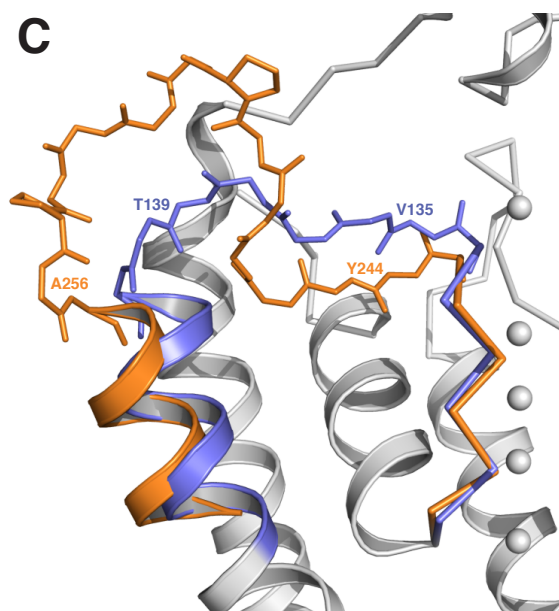
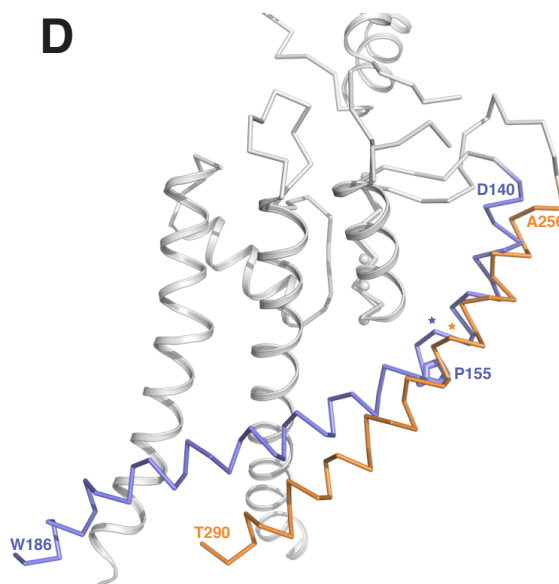
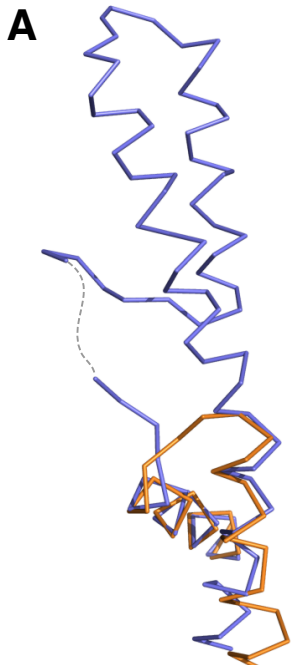
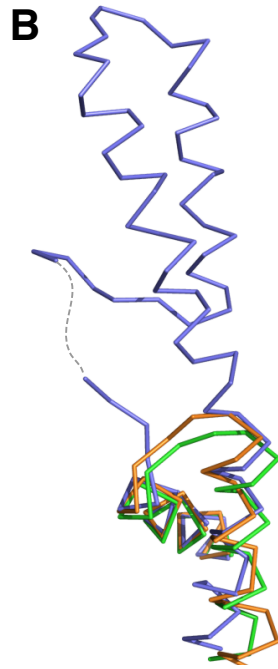
A**B****C****D**

Fig. S7.

Structural asymmetry in TRAAK. (A) Overall structural differences between pore domains in TRAAK. Pore domain 2 (orange) is shown in the same view as in Fig. 1C with pore domain 1 (blue) superimposed. (B-D) Detailed views of structural differences between pore domain 1 and pore domain 2. (B) Difference between the outer helix-pore helix connections. Residues 107-111 lack interpretable electron density and are drawn as a dashed gray line. (C) Difference between the selectivity filter-inner helix connections. (D) Difference between the inner helices. Stars indicate the position of the hinge glycine in each pore domain inner helix. The first and last residue in each region and residues referred to in the text are labeled in (B-D).



TRAAK pore domain 1
TRAAK pore domain 2



TRAAK pore domain 1
TRAAK pore domain 2
MthK (3LDC)



TRAAK pore domain 1
TRAAK pore domain 2
KcsA (1K4C)

Fig. S8.

Comparison of outer helix-pore helix connections in TRAAK, MthK, and KcsA.

(A,B,C) Views of the outer helix-pore helix connection similar to that in Fig. S7B.

TRAAK is shown as wires with pore domain 1 blue and pore domain 2 orange in (A). In

(B), the analogous region from MthK (green) (25) is superimposed. In (C), the analogous region (the turret, yellow) from KcsA (27) is superimposed.

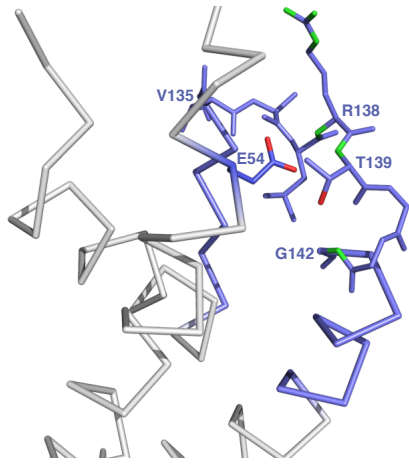
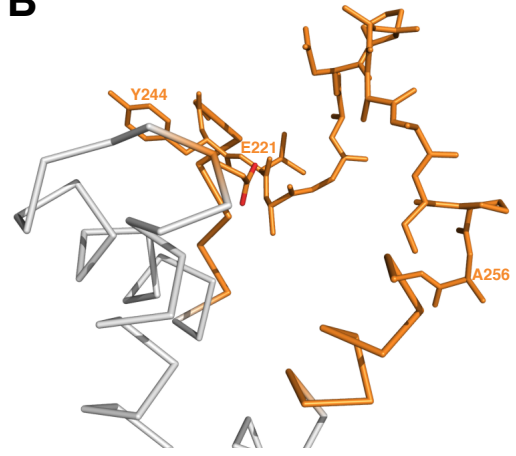
A**B**

Fig. S9.

Detailed view of the selectivity filter-outer helix connection difference between

TRAAK pore domain 1 and pore domain 2. Views of pore domain 1 (**A**) and pore

domain 2 (**B**) are rotated 180° with respect to (Fig. S7C). The region is shown as sticks

and ribbons with pore domain 1 blue and pore domain 2 orange. Surrounding protein is

shown as wire. In pore domain 1 (**A**), oxygen atoms (red) and amides (green) forming the

conserved K⁺ channel outer helix-inner helix interaction network are displayed. T139 is

positioned to hydrogen bond with the backbone amide of G142 at the extracellular end of

the inner helix. The backbone amides of R138 and T139 are in turn positioned to interact

with the side chain of the conserved E54 from the extracellular end of the outer helix.

This set of interactions is conserved in all known K⁺ channel structures except for the

eukaryotic inward rectifiers (28) where a disulfide bond between cysteines in analogous

positions to T139 and E54 tethers the inner helix to the channel core. In (**B**), E221 is

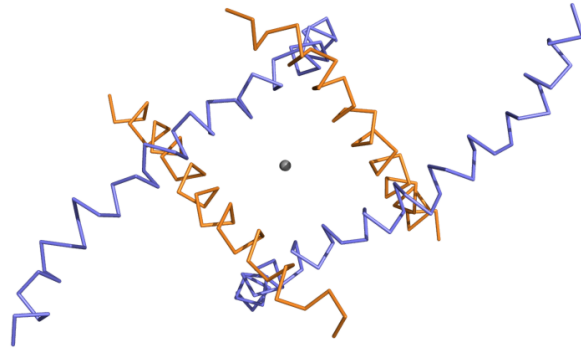
shown, but it does not form a similar interaction network with residues at the

extracellular end of the inner helix in pore domain 2 due to the extended linker and lateral

displacement of the outer helix from the channel core. E221 is in an analogous position to

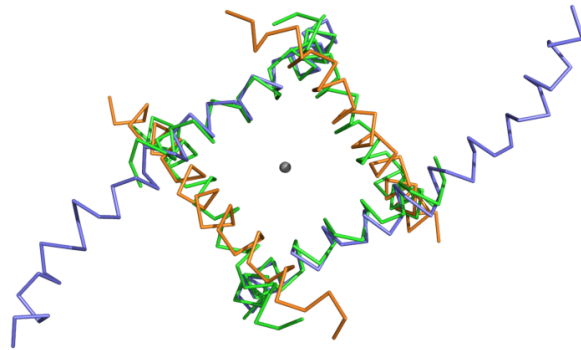
E54 from pore domain 1, but is not conserved in pore domain 2 of K2P channels.

A



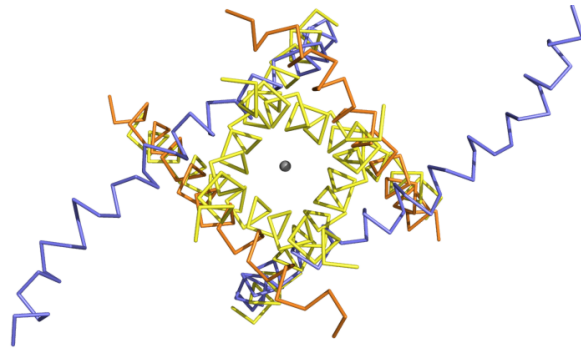
TRAAK pore domain 1
TRAAK pore domain 2

B



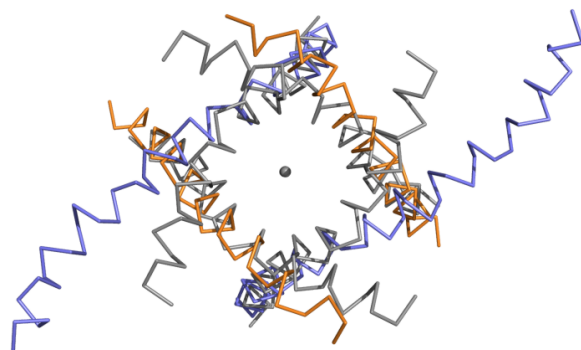
TRAAK pore domain 1
TRAAK pore domain 2
MthK (3LDC)

C



TRAAK pore domain 1
TRAAK pore domain 2
KcsA (1K4C)

D



TRAAK pore domain 1
TRAAK pore domain 2
Kv1.2-2.1 (2R9R)

Fig. S10.

Comparison of the inner helix arrangement in TRAAK, MthK, KcsA, and Kv1.2-

2.1. (A) Inner helices of TRAAK in wire representation viewed from the cytoplasmic side. Pore domain 1 is colored blue and pore domain 2 is colored orange with K⁺ ions shown as gray spheres. In **(B)**, the inner helices from MthK (green, open conformation) (25) are superimposed. In **(C)**, the inner helices from KcsA (yellow, closed conformation) (27) are superimposed. In **(D)**, the inner helices from Kv1.2-2.1 (gray, open conformation) (30) are superimposed.

Table S1. Crystallographic data collection and model refinement statistics.

Data collection				
Data set	Native ^a	Tl ⁺ peak	Tl ⁺ inflection	CH ₃ Hg ⁺ peak
Space group	p2 ₁ 2 ₁ 2 ₁	p2 ₁ 2 ₁ 2 ₁	p2 ₁ 2 ₁ 2 ₁	p2 ₁ 2 ₁ 2 ₁
Lattice constants (Å)	a=87.9 b=130.9 c=132.8 α=β=γ=90°	a=87.5 b=130.7 c=132.1 α=β=γ=90°	a=87.5 b=130.7 c=132.1 α=β=γ=90°	a=87.2 b=128.6 c=135.5 α=β=γ=90°
Beamline	APS 23-IDD	APS 23-IDD	APS 23-IDD	APS 23-IDB
Wavelength (Å)	0.97833	0.97833	0.97914	1.00604
Resolution (Å)	40.0 - 3.3 (3.4 - 3.3) ^b	40.0 - 4.2 (4.3 - 4.2)	40.0 - 4.2 (4.3 - 4.2)	40.0 - 5.0 (5.1 - 5.0)
Unique reflections	17761	11492	11619	6940
I/σI	25.6 (1.4)	15.6 (1.3)	14.2 (1.1)	34.0 (1.2)
Redundancy	3.7 (4.5)	3.6 (3.7)	3.6 (3.7)	7.9 (8.1)
Completeness (%)	73.4 (4.9)	98.2 (99.3)	98.4 (99.3)	99.8 (100)
R _{sym} ^c	0.056 (0.948)	0.089 (0.943)	0.067 (>1.0)	0.059 (>1.0)
Refinement				
Resolution (Å)	31.2 - 3.3			
Number of reflections	16792 (880) ^d			
R _{work} (%)	31.7			
R _{free} (%)	32.3			
Protein atoms, K ⁺ ions	3740, 5			
Mean B value ^e	167.7			
Ramachandran plot (%) ^f	93.1 / 6.9 / 0			
R.M.S.D. bond lengths (Å) ^g	0.008			
R.M.S.D. bond angles (°)	1.172			

^a Native data were anisotropically truncated to 3.8 x 3.3 x 3.8 Å prior to scaling.

^b Numbers in parentheses represent values for the highest resolution shell.

^c $R_{sym} = \sum I_i - \langle I \rangle / \sum I_i$, where $\langle I \rangle$ is the average intensity of symmetry related reflections.

^d Number of reflections in free set.

^e An additional isotropic B value of -74 was applied to the scaled data.

^f The three values represent the percentage of residues in the most favored, additionally allowed, and disallowed regions, respectively.

^g Root mean-squared deviation from ideal values.