The openings may provide a way for hydrophobic K⁺ channel inhibitors, such as THA, to reach the channel via the membrane and suggest that screening of lipophilic compounds may be an effective strategy for ion channel drug discovery. The openings create a physical connection between the membrane and the pore and thus may be a means of coupling properties of the membrane such as lipid composition or mechanical tension with K₂P channel function.

Subunit interface. K₂P1 has an unusual transmembrane molecular surface that may have important functional consequences. The two P domains within each K₂P1 subunit form a continuous molecular surface throughout the transmembrane region (Fig. 5A). However, the surface formed at the interface between the two subunits is markedly different from the intramolecular surface and from the transmembrane surfaces of tetrameric K⁺ channel structures determined to date (Fig. 5B). In K₂P1, openings between the subunits expose the central cavity to the hydrophobic core of the lipid membrane. The two openings, one on each side of the dimer, are sealed off at the top at the level of the selectivity filter and at the bottom by the C helix and consequently span much of what would correspond to the inner leaflet of the membrane. They are located between the M2 helix of one subunit and the M4 helix of the other (Figs. 1A and 5B). The separation between these helices arises from M4 being more perpendicular to the membrane than M2 and from a bend in M2 that occurs at Pro267. Pro267 is conserved in most K₂P channels, suggesting that analogous openings may be present in other K₂P channels.

Electron density maps revealed tubes of density within the openings just below the selectivity filter (Fig. 5, B and C). The density is consistent with two alkyl chains of about 11 carbons each. The alkyl chains could arise from detergent used in purification or from bound lipids that copurified with K₂P1. Two lines of evidence support the binding of alkyl chains within the openings. First, the recently determined crystal structure of a prokaryotic voltage-dependent Na⁺ channel contains analogous openings (termed fenestrations) (44). The fenestrations were filled with electron density that could be attributed to lipid alkyl chains, suggesting that this may be a property common to other channels. Second, lipids are known to affect TREK-1 channel function, and residues that play a role in lipid sensing correspond to positions along the C helix of K₂P1, which is located directly below the openings (9–12, 45).

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

Stephen G. Brohawn, Josefina del Mármo, Roderick MacKinnon* TRAAK channels, members of the two–pore domain K⁺ (potassium ion) channel family K₂P, are expressed almost exclusively in the nervous system and control the resting membrane potential. Their gating is sensitive to polyunsaturated fatty acids, mechanical deformation of the membrane, and temperature changes. Physiologically, these channels appear to control the noxious input threshold for temperature and pressure sensitivity in dorsal root ganglia neurons. We present the crystal structure of human TRAAK at a resolution of 3.8 angstroms. The channel comprises two protomers, each containing two distinct pore domains, which create a two-fold symmetric K⁺ channel. The extracellular surface features a helical cap, 35 angstroms tall, that creates a bifurcated pore entryway and accounts for the insensitivity of two–pore domain K⁺ channels to inhibitory toxins. Two diagonally opposed gate-forming inner helices form membrane-interacting structures that may underlie this channel’s sensitivity to chemical and mechanical properties of the cell membrane.

Cellular electrical signaling relies on a resting potential difference that originates in the membrane’s permeability to K⁺ (1). Termed “potassium leakage” by Hodgkin and Huxley (2), cellular leak currents are approximated by the behavior of constitutively open
K⁺-selective pores but deviate in detail, suggesting more complex underlying channel behavior. Leak K⁺ currents are now recognized as resulting from the activity of two–pore domain K⁺ ion channels (K2P channels) (3). Discovered as the final member of the K⁺ channel family (4–6), K2P channels have remained less well characterized than other K⁺ channels. Perhaps the prospect of studying simple “leakage” has somewhat delayed progress.

The view of K2P channels as passive K⁺ leaks has recently changed. K2P channels control the resting potential in many cells, and their regulation can directly tune cellular excitability. K2P channels have among the most varied sets of ion channel modulators, including mechanical force, temperature, lipid interaction, voltage, pH, post-translational modification, and accessory protein interaction (3, 7, 8). Pharmacologically, K2P channels are targets of antidepressants, inhalational anesthetics, neuroprotective agents, and respiratory stimulants (9–12). Their deregulation underlies human pathophysologies including Birk-Barel syndrome and familial migraine with aura (13, 14).

K2P channels constitute one of five clades within the K⁺ channel superfamily (fig. S1, A and B). Although their sequences are diverse (figs. S1C and S2), K2P channels have two shared characteristics. First, K2P channels contain two concatenated pore domains on a single protein chain protomer (fig. S1A); each pore domain contains an outer and inner membrane-spanning helix flanking a membrane-reentrant segment that forms a pore helix and K⁺ selectivity filter. K⁺ channels of known structure have one pore domain per protomer and form four-fold symmetric tetramers. Second, K2P channels contain a segment of ~60 amino acids on the extracellular side of the first pore domain. This segment has often been referred to as a “loop,” and its role in channel function has been unknown.

The best-studied subfamily of K2P channels includes TRAAK (TWIK-related arachidonic acid–stimulated K⁺ channel), TREK-1, and TREK-2 (fig. S3) (15–17). Unlike other K2P channels, they are mechanosensitive and modulated by polyunsaturated fatty acids and temperature (18–21). Physiologically, TRAAK and TREK-1 appear to regulate the noxious input threshold for temperature and pressure sensitivity in mouse dorsal root ganglia (22). These channels are highly sensitive to the structure and chemical composition of the lipid bilayer, but a mechanistic

![Fig. 1. Analysis of TRAAK channel activity.](image)

(A) Macroscopic currents from Chinese hamster ovary (CHO) cells expressing the crystal construct of TRAAK. Current was measured in whole-cell mode during a voltage ramp from −100 mV to 40 mV from a holding potential of −80 mV in 800 ms. Curves recorded from cells in 5, 70, and 150 mM external [K⁺] are displayed. The inset is a plot of reversal potential determined from voltage ramps recorded from cells in 5, 15, 70, and 150 mM external [K⁺]. (B) Arachidonic acid activation of the crystal construct of the TRAAK channel. (C) Arachidonic acid activation of the full-length channel. Current-voltage relationship is plotted from whole-cell recordings during voltage pulses from −100 to 40 mV from a holding potential of −80 mV before and after perfusion of arachidonic acid (+AA). (D) K⁺ flux assay in which K⁺ efflux drives the carbonyl cyanide m-chlorophenyl hydradize (CCCP)–mediated uptake of protons, which are detected by fluorophore 9-amino-6-chloro-2-methoxyacridine (ACMA). Vesicles were loaded with 150 mM K⁺, 0 mM Na⁺, and assayed in buffer with 0 mM K⁺, 150 mM Na⁺. Relative fluorescence change recorded from TRAAK-reconstituted (red) and empty (black) lipid vesicles is shown. Addition of the K⁺ ionophore valinomycin results in flux from crystal construct TRAAK-reconstituted and empty vesicles. (E) Current recorded from TRAAK reconstituted into planar lipid bilayers under bi-ionic conditions. Current was recorded during voltage pulses from −120 to 120 mV from a holding potential of 0 mV with internal 150 mM K⁺, 0 mM Na⁺ and external 0 mM K⁺, 150 mM Na⁺ by electrophysiological convention. Zero current level is indicated by a dashed red line. (F) Current-voltage relationship plotted from data in (E).
rationale for these biophysical properties has not been possible in the absence of structural information.

**Crystal structure of TRAAK.** Human TRAAK was recombinantly expressed and purified from *Pichia pastoris*. Diffracting crystals were obtained by mutating two consensus N-linked glycosylation sites (Asn<sup>104</sup> → Gln, Asn<sup>108</sup> → Gln), and the predicted intrinsically unstructured C-terminal region was truncated (to Gln<sup>300</sup>). Four complementary approaches were taken to assess the functional integrity of this modified construct. First, macroscopic currents were examined in cells expressing TRAAK (Fig. 1A). Channels displayed approximate time and voltage independence of activation. Current-voltage relationships did not indicate strong rectification. Expressed channels were K<sup>+</sup>-selective, as demonstrated by the change in reversal potential as a function of external K<sup>+</sup> concentration ([K<sup>+</sup>] (Fig. 1A, inset). Second, channel activation by arachidonic acid and mechanical force was examined. TRAAK channels were robustly activated above basal levels after perfusion of arachidonic acid (Fig. 1B). The extent of activation above basal levels by arachidonic acid was comparable to that measured from full-length TRAAK channels (Fig. 1C). Application of positive pressure through the pipette to outside-out patches also activated TRAAK channels (fig. S4). [Future quantitative assessments of the gating response to mechanical perturbation will require simultaneous measurements of pressure and membrane curvature to ascertain tension as the independent variable (23)].

Third, purified TRAAK was reconstituted into lipid vesicles and examined in a K<sup>+</sup> flux assay. A decrease in fluorescence indicative of K<sup>+</sup> flux was observed from TRAAK-reconstituted, but not empty, vesicles loaded with high [K<sup>+</sup>]<sub>i</sub> into buffer without K<sup>+</sup> (Fig. 1D). Fourth, recordings were made with planar lipid bilayers into which purified TRAAK channels were reconstituted (Fig. 1E). The bilayer recordings show voltage-independent channels exhibiting exquisite selectivity for K<sup>+</sup> over Na<sup>+</sup> (Fig. 1F). Taken together, these data show that the channel we have expressed, purified, and crystallized exhibits the fundamental biophysical properties of previously described TRAAK channels (24).

Crystals of TRAAK grown in 150 mM KCl diffracted to 3.8 Å. Initial attempts at molecular replacement using known K<sup>+</sup> channel structures were unsuccessful. The structure was thus solved with phases determined from a multiple-wavelength isomorphous replacement with anomalous scattering experiment using a crystal grown in the presence of TlNO<sub>3</sub> substituted for KCl. Additional register information was obtained from anomalous scattering data collected from crystals derivatized with CH<sub>3</sub>Hg<sup>+</sup> to covalently label cysteine residues. The experimentally phased maps were of sufficient quality to model the majority of the structure (fig. S5), and the final TRAAK

![Fig. 2. Structure of TRAAK. (A) Left: Ribbon representation of a single TRAAK protomer viewed from the membrane plane with the extracellular solution above. Approximate positions of the lipid bilayer boundaries are indicated as gray bars. Pore domain 1 is colored blue, pore domain 2 is colored orange, and potassium ions are shown as green spheres. Right: Illustration of TRAAK protomer organization. Approximate boundaries of structural features are indicated in the illustration and labeled in the structure: N and C terminus, outer helix (OH), helical cap, pore helix (PH), selectivity filter (F), and inner helix (IH). (B) A view of the TRAAK channel from the cytoplasmic solution. The protomer shown in (A) is rotated 90° both into the page and clockwise, and the second protomer is half-transparent. (C) Stereo view of TRAAK viewed from the membrane plane with the protomer shown in (A) rotated 90°. The disulfide bond bridging the apex of the helical cap is shown in stick representation, with the cysteine sulfur colored yellow. Note that part of the cytoplasmic extension of protomer B (residues 180 to 187) is not present in the final TRAAK model because of weak electron density features. Here, it is modeled from a superposition of the well-defined region in protomer A for visual clarity. Loops not modeled in the structure because of a lack of interpretable electron density are drawn as dashed gray lines.
model was refined to $R_{work}/R_{free} = 31.7/32.3\%$ with good geometry (table S1).

TRAAK crystallized in space group $p2_12_12_1$ with two protomers, each containing two non-identical pore domains—pore domain 1 and pore domain 2—in the asymmetric unit, forming a single $K^+$ channel with a molecular two-fold axis (along the $K^+$ conduction pathway) noncoincident with crystal symmetry axes (fig. S6). Viewed from the bilayer plane, the transmembrane region of the channel spans $\sim 35$ Å in height and is formed by the outer helix, pore helix, $K^+$ selectivity filter, and inner helix from the four pore domains (Fig. 2A). Extensions of the inner helices and the linker between pore domains 1 and 2 protrude $\sim 10$ Å further into the cytoplasmic side. On the extracellular side of the transmembrane region is a structural unit we term the helical cap, extending $\sim 35$ Å above the membrane. The helical cap is not observed in other ion channels of known structure. When viewed from the cytoplasmic side of the membrane, the channel is rhomboid-shaped; the inner helices of pore domain 1 are separated by $\sim 75$ Å across a long axis, whereas the inner helices of pore domain 2 are separated by $\sim 50$ Å across a short axis (Fig. 2B).

**Structural asymmetry in TRAAK.** Structural differences between pore domains 1 and 2 in each protomer of TRAAK result in deviation from the four-fold symmetry of known $K^+$ channel structures and generate an approximately two-fold symmetric channel instead (Fig. 2C). Differences between TRAAK pore domains 1 and 2 are most evident in three regions: the outer helix–pore helix connection, the filter–inner helix connection, and the inner helix (fig. S7A).

The difference between the outer helix–pore helix connections in pore domains 1 and 2 is shown in fig. S7B. In pore domain 1, the $\sim 60$–amino acid connection forms a two-helix hairpin extending $\sim 35$ Å into the extracellular solution. The helices from each protomer pack together to form the helical cap and are covalently linked by a disulfide bond between inter-helix turns at the cap apex. A conserved glycine allows a tight turn at the bottom of the second helix leading to an extended loop containing the N-linked glycosylation sites and connecting to the pore helix. The outer helix–pore helix connection in pore domain 2 forms a short four-residue linker structurally similar to the corresponding “turret”

---

**Fig. 3.** Convergent symmetry of the TRAAK pore helices and selectivity filter. Stereo view, from the membrane plane with extracellular solution above, shows a comparison between the TRAAK and KcsA (27) pore helices and selectivity filters. Pore helices and selectivity filter chains closest to the viewer are removed. Pore helices are shown as wires and selectivity filters as backbone sticks. TRAAK pore domain 1 is blue, TRAAK pore domain 2 is orange, and KcsA is gray, with backbone carbonyl and threonine hydroxyl oxygen atoms from selectivity filter residues shown in red. $K^+$ positions in TRAAK (green spheres) occupy canonical positions S0 to S4 from the extracellular to the intracellular side.

**Fig. 4.** The TRAAK helical cap. (A) Stereo view of the helical cap viewed from the membrane plane with the extracellular solution above. TRAAK is shown as gray ribbons, with the helical cap blue within a semitransparent surface representation. A Ti$^+$ anomalous-difference Fourier map (red mesh) calculated from 30 to 4.2 Å and contoured at 6σ is shown around Ti$^+$ ions (red spheres). (B) The hydrophobic core of the helical cap. The helical cap is shown as a blue ribbon with hydrophobic residues (L65, F72, L73, P77, L84, L87, I88, V91, A92, A94) as green sticks. The C78 disulfide bond is shown as blue sticks with yellow sulfur atoms. Abbreviations: A, Ala; C, Cys; F, Phe; I, Ile; L, Leu; P, Pro; V, Val. (C) Cartoon depiction of the bifurcated ion pathway created by the TRAAK helical cap. View is analogous to (A). TRAAK (black) in a membrane (gray) coordinates four ions (red circles) in the selectivity filter and one ion directly above in site S0. A sixth ion is present in one of the two possible extracellular access/egress pathways (red arrows) that extend into the page and toward the viewer. The TRAAK helical cap blocks ion access from above and laterally in the image plane.
regions in the prokaryotic K⁺ channel MthK (25) and the nonselective cation channel NuK (26) (fig. S8).

The difference between the selectivity filter—inner helix connections in pore domains 1 and 2 is shown in fig. S7C. In pore domain 1, this connection is short and forms an interaction network conserved in most K⁺ channel structures tethering the extracellular end of the outer helix and inner helix (fig. S9), whereas in pore domain 2, this connection is longer and forms an extended bridge to the inner helix. The interaction network observed in pore domain 1 is absent because of the extended linker and lateral displacement of the pore domain 2 inner helix from the channel core.

The difference between the inner helices in pore domains 1 and 2 is shown in fig. S7D. The pore domain 1 inner helix is kinked halfway through the bilayer and projects toward the cytoplasmic side at an angle ~25° more acute to the membrane plane than the pore domain 2 inner helix. Pro155, two residues C-terminal to the hinge glycine conserved in all K⁺ channels, permits this helix distortion because of its lack of a hydrogen bond donor at the backbone amide position. Pro155 is conserved in K2P channel pore domain 1 inner helices [except in THIK-1,2 (fig. S2)] and not observed in pore domain 2 or in other ion channels. The pore domain 1 inner helix is also longer by three helical turns that lead to the cytoplasmic connection between pore domains 1 and 2.

Convergent four-fold symmetry at the selectivity filter. The pore helix and selectivity filter are the most conserved regions of K⁺ channels. The majority of K⁺ channel selectivity filters share the canonical sequence Thr-x-Gly-Tyr-Gly-Asp-x (where x denotes a hydrophobic amino acid). In TRAAK, the selectivity filter is made from the sequences Thr-Val-Gly-Tyr-Gly-Asn-Tyr (pore domain 1) and Thr-Ile-Gly-Phe-Gly-Asp-Val (pore domain 2). Comparison of TRAAK to the prototypical K⁺ channel KcsA (27) demonstrates that the overall two-fold symmetric channel converges to an essentially four-fold symmetric pore helix and selectivity filter (Fig. 3). Only minor deviations due to conservative side chain substitutions between the two TRAAK pore domains were observed. The atomic positions of K⁺ coordinating oxygens from selectivity filter residues in TRAAK align well with those from KcsA (root mean square deviation = 0.6 Å). We modeled four K⁺ ions in positions S1 to S4 and a fifth ion in the extracellular site S0 on the basis of observed electron density from native crystals grown in 150 mM KCl, Tl⁺ positions in derivative crystals determined from anomalous scattering data, and chemical knowledge of the ion conduction pathway. The convergence to nearly perfect four-fold symmetry at the pore helix and selectivity filter in the two-fold symmetric TRAAK channel implies strong evolutionary pressures maintaining the integrity of nature’s solution to highly selective K⁺ conduction through an ion channel.

The TRAAK helical cap structure and implications for K⁺ access and pharmacology. The helical cap is unprecedented in ion channel structures and is likely a conserved feature of K2P channels. The interface between protomers is large (~850 Å²) and forms a complementary hydrophobic core (Fig. 4, A and B). The region is conserved in length among K2P channels, with residues that form the hydrophobic core more conserved than those that are solvent-exposed (fig. S2). The interprotomer disulfide-bonded cysteine is conserved in most K2P channels (fig. S2) and was correctly predicted to covalently link TWIK protomers (28). The TRAAK helical cap is modestly askew of the molecular two-fold axis along the ion conduction pathway. This is presumably the result of crystal packing between the helical cap from one channel and a neighboring channel in the crystal (fig. S6). As a result, residues 55 to 60 from one protomer are extended, whereas the rest of the cap displays approximate two-fold symmetry.

What might be the functional ramifications of the helical cap? The rigid helical bundle of the cap is positioned above the mouth of the channel pore tethered to pore domain 1 outer helices, bridging opposite sides of the channel. The surface of the helical cap is far enough above the pore entrance to allow unhindered K⁺ access from two sides. However, the cap blocks K⁺ access from above and from the two tethered sides, creating a bifurcated extracellular pathway for ions to the pore (fig. 4, A and C). Consistently, we observed a Tl⁺ ion along one branch of the split pathway to the pore in crystals grown in the presence of TlNO₃ in place of KCl (Fig. 4, A and C).

K2P channels are not responsive to known K⁺ channel blockers including pore-blocking toxins from the extracellular side (6, 16). The TRAAK structure provides a simple explanation for these observations: The presence of the helical cap above the pore would sterically prevent toxin access to the channel mouth. A similar steric occlusion mechanism was proposed for Kir2.2 on the basis of its atypically tall turrets surrounding the pore entrance (29). By fully preventing access to the mouth of the channel from above and two

Fig. 5. TRAAK channel inner helices and gating implications. (A) View from the membrane, with extracellular solution above, of the amphipathic segment of the pore domain 1 inner helix (as in Fig. 1C). TRAAK is shown in wire representation with inner helices from pore domain 1 as ribbons and key residues as sticks within a semitransparent gray surface. Pore domain 1 is colored blue, pore domain 2 is colored orange, and K⁺ ions are shown as green spheres. The hinge glycine (Gly151), kink proline (Pro155), and hydrophobic face of the amphipathic segment (Leu168, Leu172, Ile176, Ile179, Ile182, Phe183, Trp186) are colored green. Basic residues conserved in TRAAK (Arg167, Arg173, His174, His178, Lys185) on the solution accessible face of the amphipathic helix are colored blue. (B) A view rotated 90° with respect to (A). The lipid bilayer accessible surface of the lateral opening into the TRAAK channel central cavity closest to the viewer is additionally colored yellow.
sides, the helical cap in K2P channels more effectively restricts molecular access to the pore entryway.

Implications of TRAAK structure for channel gating. The crystal structure of TRAAK has an open inner helical gate (Fig. 2C). The central cavity under the selectivity filter is wide and forms a vestibule continuous with the cytoplasmic solution, presenting no steric hindrance to ion flux. The pore domain 1 inner helices are wide open, with the smallest constriction measuring ~12 Å, similar to the most open configuration observed in Mthk (Fig. S10) (25). The pore domain 2 inner helices are slightly less open, with the smallest constriction measuring ~10 Å, but this is still more open than observed in open-state Kv structures (Fig. S10) (30).

It is interesting to speculate on the potential for inner helix gating in TRAAK. Recent studies have suggested that the closely related K2P TREK-1 has an inner gate that is constitutively open (31, 32). Three features of the TRAAK pore domain 1 inner helix may be relevant if TRAAK has a similarly constitutively open inner gate: the presence of Pro and His points toward the bilayer lipid layer opposite a series of basic residues (Arg, Lys, His) pointed toward the membrane-cyttoplasm interface. An additional basic residue (Lys) farther along the helix is also directed toward the same plane. These five residues are conserved in basic character among TRAAK channels (fig. S3). The amphipathic helix is thus positioned to interact with both the hydrophobic tails and acidic head groups of membrane lipids. This structure extended the pore domain 1 inner helix, which extends like a tendril out into the lipid membrane inner leaflet, may be related to this channel's ability to respond to both mechanical and chemical properties of the cell membrane.

Another consequence of the inner helix structure in TRAAK is illustrated in Fig. 5B. The relative orientation of inner helices creates an extended lateral opening to the central cavity from the membrane. Between the two protomers, a gap ~5 Å wide extends through the bilayer from the bottom of the selectivity filter to the ends of the pore domain 2 inner helix and pore domain 1 outer helix on the cytoplasmic side. Although we observed electron density from the central cavity to the lateral openings in TRAAK, we were not able to confidently assign and model a ligand at this resolution. Lateral openings have been observed in other ion channels, including the K+ channel KcsA (27) and the voltage-gated sodium channel (42), but the size and shape of the openings in TRAAK are striking. This bilayer-facing opening results in a large protein surface accessible to the membrane and is a potential site for the interaction of lipids and other hydrophobic molecules with the TRAAK channel.

References and Notes

Acknowledgments: We thank M. Becker, R. Sanishvili, and staff at beamline 23-ID/B at the Advanced Photon Source (APS), F. Murphy, K. Rajasekharan, and staff at beamline 24-IDC at APS, and H. Robinson and staff at beamline X29 at the National Synchrotron Light Source for assistance at the synchrotrons, and members of the MacKinnon laboratory for helpful discussions. S.G.B. is a postdoctoral fellow of the Helen Hay Whitney Foundation, J.d.M. is a Howard Hughes Medical Institute International Student Research Fellow, and R.M. is an investigator in the Howard Hughes Medical Institute. The authors declare no conflict of interest. The x-ray crystallographic coordinates and structure factors are available in the Protein Data Bank, entry 3UM7. S.G.B. purified and crystallized TRAAK; collected, processed, and refined crystallographic data; and performed the flux assay and analyzed data. J.M. performed and analyzed data from electrophysiology experiments in CHO cells. R.M. performed and analyzed data from bilayer experiments. S.G.B and R.M. wrote the paper.
Supporting Online Material for

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

Stephen G. Brohawn, Josefina del Mármol, Roderick MacKinnon*

*To whom correspondence should be addressed. E-mail: mackinn@mail.rockefeller.edu

Published 27 January 2012, Science 335, 436 (2012)
DOI: 10.1126/science.1213808

This PDF file includes:
Materials and Methods
Figs. S1 to S10
Table S1
References
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 (LEVLFQ/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<sub>1-300(N104Q,N108Q)</sub>-SNS-LEVLFQ/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 OD<sub>600</sub> ~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 µg/ml pepstatin, 1 µg/ml leupeptin, 1 µg/ml aprotinin, 0.1 mg/ml soy trypsin inhibitor, 1 mM benzamidine, and 0.1 mg/ml AEBSF, with 1 mM phenylmethysulfonyl 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 Tl⁺ bound crystals, protein was prepared identically except for substitution of KNO₃ for KCl in lysis and IMAC buffers and TlNO₃ for KCl in SEC buffer.

**Crystallization and structure determination.** Pure protein was concentrated (50kDa MWCO, Millipore) to ~ 10 mg/mL for crystallization. 0.4 µL protein was added to 0.9 µL 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 µL SEC buffer with 30% PEG400 to drops and immediately harvested and frozen in
liquid nitrogen. \( \text{CH}_3\text{Hg}^+ \)-derivatived crystals were prepared by adding a trace amount of solid \( \text{CH}_3\text{HgCl} \) 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 \( \text{CH}_3\text{Hg}^+ \)-derivative crystals as it was found to improve x-ray diffraction.

Data were collected for native and \( \text{Tl}^+ \) crystals at APS beamline 23-IDD and for \( \text{CH}_3\text{Hg}^+ \)-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 x 3.3 x 3.8 Å prior to anisotropic scaling with Phaser (46) and sharpening by application of a negative isotropic B factor of -74 to the data.

Seven \( \text{Tl}^+ \) sites were located by Shelx (47) and refined with Sharp (48) from the \( \text{Tl}^+ \) 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 \( \text{CH}_3\text{Hg}^+ \). 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$_3$Hg$^+$. 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 ~20Å 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 MΩ resistance (for whole cell recordings) and 2-3 MΩ 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 µM 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 μL of vesicles were added to 190 μL of flux assay buffer (20 mM Hepes pH 7.4, 150
mM NaCl, 1 mM EDTA, 2 μM 9-amino-6-chloro-2-methoxyacridine (ACMA)).

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

After 30 seconds of baseline fluorescence was monitored, K⁺ flux was initiated by addition of 1 μM m-chlorophenyl hydrazone (CCCP) to collapse the electrical potential. The chemical gradient was terminated by addition of the K⁺ ionophore valinomycin to 0.02 μM 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 μM 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).
**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 (Kᵥ1-9), Ca²⁺-activated (KᵥCa), inward-rectifying (Kᵢᵦ), and cyclic nucleotide-gated (CNG/HCN and Kᵥ10-12 channels)). The architecture of each family is illustrated as a cartoon from N- (left) to C-terminus (right). Note that some KᵥCa 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 (TRAACK, 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 Kᵢᵦ and KᵥCa channels).
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*. 
**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).
**Fig. S5.**  
The **TRAAK structure solution.** (A) Stereo view of TRAAK similar to the view in Fig. 1C. The view in (B) is rotated ~70° 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 CH₃Hg⁺-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.
**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.
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).
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.
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

TRAAS pore domain 1
TRAAS pore domain 2

B

TRAAS pore domain 1
TRAAS pore domain 2
MithK (3LDC)

C

TRAAS pore domain 1
TRAAS pore domain 2
KcsA (1K4O)

D

TRAAS pore domain 1
TRAAS 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.

<table>
<thead>
<tr>
<th>Data collection</th>
<th>Native*</th>
<th>T- peak</th>
<th>Tr-infection</th>
<th>CH-Hg* peak</th>
</tr>
</thead>
<tbody>
<tr>
<td>Data set</td>
<td>p2₁/2₁</td>
<td>p2₁/2₁</td>
<td>p2₁/2₁</td>
<td>p2₁/2₁</td>
</tr>
<tr>
<td>Space group</td>
<td>p2₁/2₁</td>
<td>p2₁/2₁</td>
<td>p2₁/2₁</td>
<td>p2₁/2₁</td>
</tr>
<tr>
<td>Lattice constants (Å)</td>
<td>a=87.9 b=130.9 c=132.8</td>
<td>a=87.5 b=130.7 c=132.1</td>
<td>a=87.5 b=130.7 c=132.1</td>
<td>a=87.2 b=128.6 c=135.5</td>
</tr>
<tr>
<td>α=β=γ=90°</td>
<td>α=β=γ=90°</td>
<td>α=β=γ=90°</td>
<td>α=β=γ=90°</td>
<td>α=β=γ=90°</td>
</tr>
<tr>
<td>Beamline</td>
<td>APS 23-IDD</td>
<td>APS 23-IDD</td>
<td>APS 23-IDD</td>
<td>APS 23-IDD</td>
</tr>
<tr>
<td>Wavelength (Å)</td>
<td>0.97833</td>
<td>0.97833</td>
<td>0.97914</td>
<td>1.00004</td>
</tr>
<tr>
<td>Resolution (Å)</td>
<td>40.0 - 33.3 (3.4 - 3.3)</td>
<td>40.0 - 4.2 (4.3 - 4.2)</td>
<td>40.0 - 4.2 (4.3 - 4.2)</td>
<td>40.0 - 5.0 (5.1 - 5.0)</td>
</tr>
<tr>
<td>Unique reflections</td>
<td>17761</td>
<td>11492</td>
<td>11619</td>
<td>6940</td>
</tr>
<tr>
<td>I/σ(I)</td>
<td>25.6 (1.4)</td>
<td>15.6 (1.3)</td>
<td>14.2 (1.1)</td>
<td>34.0 (1.2)</td>
</tr>
<tr>
<td>Redundancy (%)</td>
<td>3.7 (4.5)</td>
<td>3.6 (3.7)</td>
<td>3.6 (3.7)</td>
<td>7.9 (8.1)</td>
</tr>
<tr>
<td>Completeness (%)</td>
<td>73.4 (4.9)</td>
<td>98.2 (99.3)</td>
<td>98.4 (99.3)</td>
<td>99.8 (100)</td>
</tr>
<tr>
<td>Rmerge (%)</td>
<td>0.056 (0.948)</td>
<td>0.089 (0.943)</td>
<td>0.067 (&gt;1.0)</td>
<td>0.059 (&gt;1.0)</td>
</tr>
</tbody>
</table>

Refinement

| Resolution (Å) | 31.2 - 3.3 |
| Number of reflections | 16792 (880)² |
| Rmerge (%) | 31.7 |
| Rmerge (%) | 32.3 |
| Protein atoms, K+ ions | 3740, 5 |
| Mean B value* | 167.7 |
| Ramachandran plot (%) | 93.1 / 6.9 / 0 |
| R.M.S.D. bond lengths (Å)* | 0.008 |
| R.M.S.D. bond angles (*) | 1.172 |

* Native data were anisotropically truncated to 3.8 x 3.3 x 3.8 Å prior to scaling.
² Numbers in parentheses represent values for the highest resolution shell.
Rmerge = Σ | I(I) − <I> | / Σ I(I), where <I> is the average intensity of symmetry related reflections.
Rmerge (%) = Σ | I(I) − <I>| / Σ I(I).
Rmerge (%) = Σ | I(I) − <I>| / Σ I(I).
Number of reflections in free set.
¶ An additional isotropic B value of 74 was applied to the scaled data.
1 The three values represent the percentage of residues in the most favored, additionally allowed, and disallowed regions, respectively.
* Root mean-squared deviation from ideal values.
References


55. Schrodinger, LLC. (2010).

