The lysosomal potassium channel TMEM175 adopts a novel tetrameric architecture

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TMEM175 is a lysosomal K\(^+\) channel that is important for maintaining the membrane potential and pH stability in lysosomes\(^1\). It contains two homologous copies of a six-transmembrane-helix (6-TM) domain, which has no sequence homology to the canonical tetrameric K\(^+\) channels and lacks the TVGYG selectivity filter motif found in these channels\(^2\)–\(^4\). The prokaryotic TMEM175 channel, which is present in a subset of bacteria and archaea, contains only a single 6-TM domain and functions as a tetramer. Here, we present the crystal structure of a prokaryotic TMEM175 channel from Chamaesiphon minutus, CmTMEM175, the architecture of which represents a completely different fold from that of canonical K\(^+\) channels. All six transmembrane helices of CmTMEM175 are tightly packed within each subunit without undergoing domain swapping. The highly conserved TM1 helix acts as the pore-lining inner helix, creating an hourglass-shaped ion permeation pathway in the channel tetramer. Three layers of hydrophobic residues on the carboxy-terminal half of the TM1 helices form a bottleneck along the ion conduction pathway and serve as the selectivity filter of the channel. Mutagenesis analysis suggests that the first layer of the highly conserved isoleucine residues in the filter is primarily responsible for channel selectivity. Thus, the structure of CmTMEM175 represents a novel architecture of a tetrameric cation channel whose ion selectivity mechanism appears to be distinct from that of the classical K\(^+\) channel family.

Acidic lysosomal organelles mediate crucial biological processes such as degradation, catabolite export, and metabolism sensing\(^5\)–\(^7\), and defects in these processes can result in lysosomal storage diseases\(^8\)–\(^10\). The lysosomal membrane is decorated with channels and transporters that regulate the ionic homeostasis and physiological functions of the lysosome\(^1\),\(^1\)\(^1\),\(^1\)\(^2\). Recently, the previously uncharacterized transmembrane protein 175 (TMEM175) was identified as the bona fide endosomal and lysosomal K\(^+\) channel, whose function is important for setting the lysosomal membrane potential and maintaining pH stability\(^3\). TMEM175 deficiency has been shown to play a critical role in the pathogenesis of Parkinson disease\(^1\)\(^3\),\(^1\)\(^4\).

Mammalian TMEM175 contains two homologous 6-TM domains and has been suggested to function as a dimer\(^1\). As TMEM175 lacks the TVGYG motif that is found in canonical 6-TM K\(^+\) channels\(^2\)–\(^4\) and does not show sequence homology to these channels, it would be expected to adopt a distinct structure and K\(^+\) selectivity mechanism from classical K\(^+\) channels. The prokaryotic TMEM175 (bacTMEM175), which is found in some bacteria and archaea, contains a single copy of a 6-TM domain in each subunit (Extended Data Fig. 1). Several bacTMEM175s can be overexpressed and purified as tetramers in solution (Extended Data Fig. 2), supporting the prediction that the eukaryotic TMEM175, with its two 6-TM domains, would function as a dimer. The bacTMEM175 from C. minutus, CmTMEM175, yielded the best-diffracting crystals and is the focus of this study.

We first tested whether CmTMEM175 functions as a K\(^+\)-selective channel. We could not record CmTMEM175 channel current using electrophysiology, so we used a \(^{86}\)Rb flux assay\(^1\)\(^5\) to measure K\(^+\) permeation and selectivity, on the basis of the premise that CmTMEM175 can conduct K\(^+\) as well as Rb\(^+\) (see Methods). In brief, CmTMEM175 was reconstituted into liposomes containing high concentrations of KCl. When the proteoliposomes are transferred to a low-salt reaction buffer containing \(^{86}\)Rb, the K\(^+\) concentration gradient across the lysosomal membrane drives K\(^+\) efflux through CmTMEM175, generating an electrical driving force for the influx of radioactive \(^{86}\)Rb (see Methods). Figure 1a shows the time-dependent accumulation of \(^{86}\)Rb inside K\(^+\)-loaded proteoliposomes, confirming that CmTMEM175 is permeable to K\(^+\) and Rb\(^+\). The same flux assay performed using proteoliposomes loaded with NaCl shows much weaker liposomal \(^{86}\)Rb accumulation (Fig. 1b), indicating that CmTMEM175 conducts K\(^+\) better than Na\(^+\).

To evaluate channel selectivity, we performed a competition assay by measuring \(^{86}\)Rb influx in the presence of various external monovalent cations; conductive ions would compete with \(^{86}\)Rb and decrease its lysosomal accumulation over time. The competition assay indicated that CmTMEM175 is permeable to K\(^+\), Rb\(^+\) and Cs\(^+\), which caused a reduction in \(^{86}\)Rb accumulation in comparison to the control experiment (Fig. 1c). The channel was unable to conduct Na\(^+\) or the large organic cations valinomycin, gramicidin A or large organic cations, which caused a reduction in \(^{86}\)Rb accumulation in comparison to the control experiment (Fig. 1c).

### Figure 1

**\(^{86}\)Rb flux assay of CmTMEM175.** 

- **a**, Time-dependent \(^{86}\)Rb influx into CmTMEM175 proteoliposomes prepared in KCl; control liposomes contained no protein. Data are normalized against the maximum \(^{86}\)Rb influx obtained by adding 1.0 mM of valinomycin to the reaction. 
- **b**, Time-dependent \(^{86}\)Rb flux into CmTMEM175 proteoliposomes prepared in NaCl. Data are normalized against the maximum \(^{86}\)Rb influx obtained by adding 1.0 mM of valinomycin to the reaction.
- **c**, Competition assay of \(^{86}\)Rb influx in the presence of external monovalent cations. Three concentrations of each cation (0.1, 0.5 and 1.0 mM; X denotes the cation) were tested. Data are normalized against the control measurement, in which no external cations were added. All data points are mean ± s.e.m. of 2–4 measurements.

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cation N-methyl-d-glucamine (NMDG\(^+\)), neither of which had any obvious effect on \(^{86}\)Rb influx. These results show that CmTMEM175 is selective for K\(^+\), Rb\(^+\) and Cs\(^+\) over Na\(^+\), consistent with the selectivity of eukaryotic TMEM175.

The structure of CmTMEM175 was determined and refined to 3.3 Å (see Methods and Extended Data Fig. 3). Consistent with the biochemical data, CmTMEM175 adopts a four-fold symmetric, square-shaped tetramer in the crystal with dimensions of about 61 Å on each side and a height of about 60 Å (Fig. 2). Each subunit contains six transmembrane (TM) helices with both N- and C- termini predicted to be on the cytosolic side. TM1–3 are positioned on the inner circle of the channel tetramer and participate in inter-subunit interactions that are central to the assembly of the ion conduction pore. TM4–6 are positioned on the periphery of the channel and do not contribute to any contacts with neighbouring subunits. Not surprisingly, TM1–3 have higher sequence conservation than TM4–6 among TMEM175 homologues. TM1 and TM2 are linked by an extended, strand-like tail and two short helices (H1 and H2). This linker protrudes outwards from the main body of the transmembrane domain and encloses the external open entrance of the ion conduction pore in the channel tetramer. TM3 is bent into two segments (TM3a and TM3b) owing to the presence of a conserved proline (Pro102) (Fig. 2 and Extended Data Fig. 1).

The structure of CmTMEM175 is completely different from that of canonical 6-TM homo-tetrameric K\(^+\) channels\(^{16,17}\) (Extended Data Fig. 4). First, all six transmembrane helices of CmTMEM175 are tightly packed within each subunit without undergoing any domain swapping. Second, the two central pore elements in canonical K\(^+\) channels, the pore helix and the TVGYG selectivity filter, are not present in CmTMEM175. Third, in CmTMEM175, TM1 instead of TM6 forms the pore-lining inner helix at the centre of the channel. Finally, a search of the structure database using DALI\(^{18}\) yielded no similar structures, indicating that the 6-TM architecture of CmTMEM175 represents a previously undescribed fold.

The pore-lining TM1 helix is the most conserved region in the TMEM175 family. Four TM1 helices form a bundle crossing, which, along with their extended C-terminal tails, create a hourglass-shaped ion permeation pathway (Fig. 3a–c). No specifically bound ions were observed along the pathway, even in crystals soaked with heavier monovalent or divalent cations. The bundle crossing juncture contains three layers of highly conserved hydrophobic residues—Ile23, Leu27 and Leu30 from each TM1 helix—that form a 10-Å-long bottleneck with a diameter of about 3 Å at each constriction point. The N-terminal half of TM1 lines the cytosolic entrance of the hourglass pathway with a negatively charged surface potential (Fig. 3d) whereas the extended C-terminal tail of TM1 and the C-terminal half of TM3 (TM3b) enclose the extracellular entrance of the pathway with a slightly negative surface potential.

The N-terminal part of TM1 also contains the \(\alpha_{RxxxFSD}^{18}\) signature sequence motif, which is important for TMEM175 channel function\(^1\). These residues coordinate a network of specific inter- and intra-subunit interactions with several other highly conserved residues on TM2 and TM3, and play a crucial role in the tetrameric assembly of the channel. As shown in Fig. 3e, Arg12 forms a cation–π interaction with His74 from TM2 of a neighbouring subunit; Phe16 forms π–π stacking with Trp70 from TM2 of the neighbouring subunit; Ser17 forms an intra-subunit hydrogen-bonding network with His73 on TM2 and
Asn91 on TM3; and Asp18 forms a hydrogen bond with Trp70 of the same subunit, which participates in π–π stacking with Phe16 of the neighbouring subunit.

With wide open entrances on both sides of the membrane, the only possible region that can control ion flux through CmTMEM175 is the narrow hydrophobic bottleneck. Intuitively, this poses a conundrum given our understanding of what constitutes a K\(^+\)-selective ion conduction pore: how can charged ions move across this narrow tunnel, whose size is equivalent to that of a closed tetrameric cation channel gate\(^{19-21}\)? However, ion permeation through a narrow hydrophobic pathway is not unprecedented. One example is the pentameric bestrophin channel, in which three layers of hydrophobic residues create a 10-Å-long, 3-Å-wide ion pathway resembling that of CmTMEM175\(^{22,23}\) (Extended Data Fig. 5). Notably, the eukaryotic bestrophin channel, whose narrow pathway is formed by three layers of isoleucine, phenylalanine and phenylalanine, is an anion-selective channel, whereas the bacterial bestrophin, which contains isoleucine, provides a plausible explanation\(^{26-29}\). Computationally, nanochannels and the radius-dependent selectivity of these nanochannels might provide a plausible explanation\(^{26-29}\). Computationally, nanochannels with a sub-nanometre pore size (that is, \(r < 5\) Å) have been suggested to achieve higher selectivity for K\(^+\) than for Na\(^+\), because the energetic cost of shedding water from the ion hydration shell or constraining a hydrated ion inside a narrow hydrophobic pore is lower for K\(^+\) than Na\(^+\). With its extremely narrow hydrophobic pathway (3 Å in diameter), ion passage through the CmTMEM175 filter would require a partially dehydrated form with only two coaxial water molecules remaining, one on each side, and is expected to be energetically unfavourable. Two features of the TMEM175 structure may help in overcoming the energy barrier for ion passage. First, the electronegative surface potential on both entrances can attract and stabilize the positively charged ions and effectively increase the local cation concentration. Second, the hourglass architecture of the ion pathway effectively reduces the thickness of the membrane barrier, allowing the membrane potential to drop across the 10 Å filter region and, thereby, substantially enhancing the electrochemical driving force for ion permeation. It is also worth noting that the narrow pathway observed in the CmTMEM175 structure is likely to represent a closed filter with the minimum size, as the side chains of two neighbouring Ile23 residues are only about 3.3 Å apart (Fig. 3), reaching the closest van der Waals distance between two non-covalent carbon atoms. It is unclear how TMEM175 is gated, but we suspect that

We also performed mutagenesis on the first layer of isoleucine residues (Ile23) on CmTMEM175 by replacing them with cysteine, alanine or asparagine, and analysed the selectivity of the mutated channels using the \(^{86}\)Rb flux assay. These mutants were expressed, purified and reconstituted into liposomes loaded with KCl, and their selectivity was measured by a competition assay with external K\(^+\), Na\(^+\) or NMDG\(^+\) (Fig. 6b). Both external K\(^+\) and Na\(^+\), but not NMDG\(^+\), resulted in a concentration-dependent decrease in \(^{86}\)Rb influx into proteoliposomes bearing the CmTMEM175 mutants, indicating that the mutant channels can conduct both Na\(^+\) and K\(^+\). This result confirms the central role of the layer 1 Ile23 residues in defining the K\(^+\) selectivity of CmTMEM175.

With a structure so different from canonical K\(^+\) channels, how does TMEM175 achieve its K\(^+\) selectivity? The narrow hydrophobic filter of CmTMEM175 is reminiscent of hydrophobic nanochannels such as synthetic organic nanopores or single-walled carbon nanotubes, and the radius-dependent selectivity of these nanochannels might provide a plausible explanation\(^{26-29}\). Computationally, nanochannels with a sub-nanometre pore size (that is, \(r < 5\) Å) have been suggested to achieve higher selectivity for K\(^+\) than for Na\(^+\), because the energetic cost of shedding water from the ion hydration shell or constraining a hydrated ion inside a narrow hydrophobic pore is lower for K\(^+\) than Na\(^+\). With its extremely narrow hydrophobic pathway (3 Å in diameter), ion passage through the TMEM175 filter would require a partially dehydrated form with only two coaxial water molecules remaining, one on each side, and is expected to be energetically unfavourable. Two features of the TMEM175 structure may help in overcoming the energy barrier for ion passage. First, the electronegative surface potential on both entrances can attract and stabilize the positively charged ions and effectively increase the local cation concentration. Second, the hourglass architecture of the ion pathway effectively reduces the thickness of the membrane barrier, allowing the membrane potential to drop across the 10 Å filter region and, thereby, substantially enhancing the electrochemical driving force for ion permeation. It is also worth noting that the narrow pathway observed in the CmTMEM175 structure is likely to represent a closed filter with the minimum size, as the side chains of two neighbouring Ile23 residues are only about 3.3 Å apart (Fig. 3), reaching the closest van der Waals distance between two non-covalent carbon atoms. It is unclear how TMEM175 is gated, but we suspect that...
METHODS

Protein expression and purification. Twenty genes of prorakocyte TMEM175 homologues were chemically synthesized (Biomatik), subcloned into PET15b expression vectors and tested for expression in *E. coli*, stabilized in n-decyl-β-D-maltopyranoside (DM) detergent, and purified to homogeneity. TMEM175 from *C. minutus* (CmTMEM175) yielded the best diffracting crystals for structure determination in this study and is used for the description of protein expression and purification. These TMEM175 clones were transformed into *E. coli* BL21 (DE3) cells and were grown to 2YT medium initially at 37 °C to an optical density (OD600) of 0.4, and then at a reduced temperature of 30 °C; overexpression was induced with 0.1 mM isopropyl-β-D-thiogalactoside (IPTG) at an OD600 of 0.6. Cells were harvested 6 h after induction and homogenized with an emulsiflex-C5 homogenizer (Avastin) in buffer containing 50 mM HEPES, pH 7.4, 200 mM KCl, 1 μg/ml leupeptin, 1 μg/ml pepstatin A, 2 μg/ml DNaseI, 1 μg/ml aprotinin, and 1 mM PMSF. Cell lysate was incubated with 1% (w/v) n-dodecyl-β-D-maltopyranoside (DDM; Anatrace) for 3 h at room temperature for protein extraction. The supernatant was collected after a 45 min centrifugation at 48,384g, supplemented with 5 mM imidazole and loaded onto a Talon Co2+ affinity column (Clontech) pre-equilibrated with 20 mM HEPES pH 7.4, 200 mM KCl and 1 mM DDM. The protein was eluted with an equilibration solution containing 300 mM imidazole and digested overnight at 4 °C with thrombin (1 U per 5 g wet cells; Roche) to remove the hexamidastagine tag. The sample after digestion was concentrated using a 100 kDa cut-off Amicon Centrifugal Filter (EMD Millipore) and further purified by gel filtration using a Superdex-200 (10/30 GL) column (GE Healthcare) in 20 mM HEPES, pH 7.4, 200 mM KCl, 0.5 mM decyl maltose neopentyl glycol (DMNG, for cryo-crystallization and X-ray analyses) or 3 M total (for biochemical assays). Peak fractions were collected and concentrated for further experiments. To facilitate model building, more than ten CmTMEM175 mutants with a single-cysteine substitution at various parts of the protein were generated using QuikChange II Site-Directed Mutagenesis Method (Agilent). All mutants were expressed, purified and crystallized in similar conditions to the wild-type protein.

Crystallization. CmTMEM175 was purified in DMNG and concentrated to 6 mg/ml for crystallization. Crystals were grown at 20 °C using the sitting-drop vapour diffusion method. Crystals appeared within 3 days under conditions of 16–22% PEG 1000, 50 mM CaCl2, 50 mM MgCl2 and 100 mM NaAc, pH 4.6, and grew to their full size in 2–4 weeks. Crystals were cryoprotected by adding a cryosolution containing 25% PEG 1000, 10% glycerol, 50 mM CaCl2, 50 mM MgCl2, 0.5 mM DMNG and 100 mM NaAc, pH 4.6 directly into the crystallization drop and flash-frozen in liquid nitrogen. To obtain experimental phasing, crystals were derivatized overnight by adding methyl mercury chloride (CH3HgCl) solubilized in a stabilization solution of 25% PEG 1000, 50 mM CaCl2, 50 mM MgCl2, 0.5 mM DMNG, 100 mM NaAc, pH 4.6 into the crystallization drops to a final concentration of 1 mM.

Two different types of crystal were obtained under the same crystallization conditions: one has a C2 space group and the other is P1. C2 crystals diffracted up to 3.3 Å and were used for structure determination, whereas P1 crystals generally diffracted up to 4.0 Å. We noticed that freshly purified protein tended to be crystallized in the P1 space group, whereas leaving purified protein on ice for three days before crystallization yielded more C2 crystals. The structures of P1 crystals were also determined by molecular replacement method using a wild-type C2 crystal structure as the search model and showed no obvious difference.

Data collection and structure determination. X-ray diffraction data were collected using synchrotron radiation source (Advanced Photon Source 23IDB and 23IDD, Argonne National Laboratory, IL) and were measured with 1.0 or 1.5 mM protein, and were measured in 100–215 mM ammonium sulfate and 0.1 M Tris–HCl, pH 8.0. All crystals belonged to space group C2 with cell dimensions of a = 152.66 Å, b = 108.88 Å, c = 119.31 Å, α = 90°, β = 117.24°, γ = 90°, and contained four subunits per asymmetric unit. To maximize the anomalous signal, the mercury-derivative data were collected for 40 h before the reaction. The native data were derived from 30% amino acid residues on the Ramachandran plot. The pore radius of the ion conduction pathway was determined using the program HOLE27 and the electrostatic potentials were calculated using the program APBS40. All structure figures were prepared with Pymol41.

Protein reconstitution and 86Rb flux assay. All proteins used in the flux assay were purified in DMNG detergent and reconstituted into lipid vesicles composed of 1-palmitoyl-2-oleoyl-phosphatidylethanolamine (POPE, 7.5 mg/ml) and 1-palmitoyl-2-oleoyl-phosphatidylglycerol (POPG, 2.5 mg/ml) at 2 μg protein per mg lipid using the same method as described below with the following modifications: 20 mM DM was used to solubilize the lipid and the detergent–lipid–protein mixture was dialysed against a reconstitution buffer of 10 mM HEPES buffered with 4 mM NMDG, pH 7.4, and 450 mM KCl or NaCl to slowly remove the detergent. The reconstituted liposome samples were kept at −80 °C in 100-μl aliquots.

The 86Rb flux assay was performed as described below. Liposomes were thawed and sonicated in a bath sonicator for 40s before the assay. To remove extra-liposomal salt (NaCl or KCl), 100-μl samples were washed through a pre-spun Sephacry G-50 fine gel filtration column (1.5 ml bed volume in a 5 ml disposable spin column) swollen in 400 mM sorbitol, 10 mM HEPES buffered with 4 mM NMDG, pH 7.4. 160 μl of liposome samples collected after this buffer exchange step was added to 320 μl 86Rb flux buffer (400 mM sorbitol, 10 mM HEPES buffered with 4 mM NMDG, pH 7.4, 30 μM NaCl or KCl, and 5 μM 86RbCl). At desired time points, 60 μl of this reaction mixture was passed through a pre-spun Sephadex G-50 fine gel filtration column as described above to remove extra-liposomal 86Rb and stop the flux. The eluate was mixed with 10 ml scintillation cocktail and its radioactivity was measured in a scintillation counter. For time-dependent measurement of 86Rb influx, the radioactivity of each sample was normalized against the maximum 86Rb influx. The maximum 86Rb influx for K+-loaded liposomes was obtained by adding 1 μg/ml valinomycin to a 60-μl reaction mixture and allowing the influx to proceed for 2 min before sample collection using Sephadex G-50 column. 10 μg/ml gramicidin A was used to obtain the maximum 86Rb influx for Na+-loaded liposomes. For competition assay, CmTMEM175-containing liposomes were loaded with KCl and flux was allowed to proceed for 30 min before the radioactivity levels in liposomes were measured. The tested ions (Na+, K+, Rb+ and Cs+ and NMDG+) were added directly to the flux buffer, and the final concentrations of 0.1, 0.5, and 1.0 or 5.0 mM after mixing with liposomes, and samples of reaction mixture were collected 30 min after the reaction. The radioactivity of each sample in the competition assay was normalized to the reaction mixture without competition ions.

Chemical cross-linking. Cross-linking experiment was performed using the amine-reactive cross-linking reagents disuccinimidyl suberate (DSS) and diisuccinimidyl glutarate (DSSG, ProteoChem) on bacTMEM175 channels from *Chryseobacterium sp.* and *Streptomyces collinus* following the manufacturer’s suggested protocol. Both reagents were prepared as 50 mM stock in anhydrous dimethylsulfoxide (DMSO). The protein samples used for the cross-linking reaction were dialysed overnight in 0.1 M sorbitol and 10 mM dimethylsulfoxide (DMSO). The protein samples used for the cross-linking reaction were dialysed overnight in 0.1 M sorbitol and 10 mM dimethylsulfoxide (DMSO). The cross-linking reactions had a concentration of about 1 mg/ml and were purified in amine-free dimethylsulfoxide (DMSO). The protein samples used for the cross-linking reactions had a concentration of about 1 mg/ml and were purified in amine-free dimethylsulfoxide (DMSO). The radioactivity of each sample in the competition assay was normalized to the reaction mixture without competition ions.

Electrophysiology. Instead of using whole lysosome patch as previously described, we were able to express human TMEM175 in HEK293 cells and measure plasma membrane channel activity using whole-cell patch clamping. Human TMEM175 and its mutants were cloned into pC6GFP-EM Plasmid 21–125 μl of plasmid-containing C-terminal GFP-tagged wild-type or mutant human TMEM175 was transfected into HEK293 cells grown in a six-well tissue culture dish using Lipofectamine 2000 (Life Technology). 24–48 h after transfection, cells were dissociated by trypsin treatment, kept in complete serum-containing medium and re-plated on 35-mm tissue culture dishes in a tissue culture incubator until the heavy atom positions were determined in SHELXD32 and refined in AutoShar33. The initial phases were improved by solvent flattening with SOLOMON34 and four-fold non-crystallographic symmetry averaging with RESOLVE35, generating an electron density map of sufficient quality for model building. The model was manually built in COOT16 and refined with Phenix32. To facilitate accurate model building, more than ten mutants containing single cysteine substitution at various parts of the protein were generated, crystallized and derivatized with CH3HgCl. The heavy atom sites from these mutants and wild-type crystals provided unique registry for 11 residues throughout the protein, allowing us to accurately model the structure of CmTMEM175. The final model was refined to 3.3 Å with Rwork = 25.9% and Rfree = 28.2% and contained residues 7–144 and 154–202 from the protein, covering 93% of the protein sequence. Geometry analysis of the final model with Procheck36 gave statistics of 97.5% and 2.5% for the most favoured and additional allowed regions, respectively, on the Ramachandran plot. The pore radius of the ion conduction pathway was analysed using the program HOLE27 and the electrostatic potentials were calculated using the program APBS40. All structure figures were prepared with Pymol41.
recording. In most recordings, patch clamp in the whole-cell configuration was used to measure TMEM175 current in HEK293 cells. Patch clamp in the inside-out configuration was used to measure the Zn$^{2+}$ and 4-AP block of TMEM175 from the intracellular side. The standard extracellular solution contained (in mM): 145 sodium methanesulfonate (Na-MS), 5 NaCl, 1 MgCl$_2$, 1 CaCl$_2$, 10 HEPES buffered with Tris, pH 7.4. The intracellular solution contained (in mM): 150 Cs-MS, 5 MgCl$_2$, 10 EGTA, 10 HEPES buffered with Tris, pH 7.4. The patch pipettes were pulled from borosilicate glass (Harvard Apparatus) and heat polished to a resistance of 3–5 MΩ. Data were acquired using an AxoPatch 200B amplifier (Molecular Devices) and a low-pass analogue filter set to 1 kHz. The current signal was sampled at a rate of 20 kHz using a Digidata 1322A digitizer (Molecular Devices) and further analysed with pClamp 9 software (Molecular Devices). After the patch pipette attached the cell membrane, the gigaseal (5–10 GΩ) was formed by gentle suction. The whole-cell configuration was formed by short zap or suction to rupture the patch. The holding potential was set to 0 mV. All $I$–$V$ curves of TMEM175 and its mutants were obtained using voltage pulses ramping from −100 to +100 mV over 800 ms. To measure the ion selectivity, the extracellular solution (bath) was changed to (in mM): 150 XMS ($X$ = Li, Na, K, or NMDG), 1 MgCl$_2$, 1 CaCl$_2$, 10 HEPES buffered with Tris, pH 7.4. All data points are mean ± s.e.m. ($n$ ≥ 5).

The ion permeability ratios were calculated with the following equation: $P_{\text{Cs}}/P_{\text{X}} = [\text{X}]_{\text{out}}/([\text{Cs}]_{\text{out}} \exp(E_{\text{rev}}/FRT))$ where $X$ represents Li, Na, K, or Rb, $E_{\text{rev}}$ is the reversal potential, $F$ is Faraday’s constant, $R$ is the gas constant, and $T$ is the absolute temperature.

**Data availability.** The atomic coordinates and structure factors have been deposited in the Protein Data Bank under accession number 5VRE.

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Extended Data Figure 1 | Sequence comparison between prokaryotic and eukaryotic TMEM175 channels. a, Topologies of prokaryotic (left) and eukaryotic (right) TMEM175 channel subunits. b, Sequence alignment of prokaryotic TMEM175 proteins and the first 6-TM domains of eukaryotic TMEM175 proteins. c, Sequence alignment of prokaryotic TMEM175 proteins and the second 6-TM domains of eukaryotic TMEM175 proteins. Secondary structure assignments are based on the CmTMEM175 structure. Asterisks mark the three hydrophobic filter residues. Blue triangles mark the RxxxFSD motif and the residues participating in the inter- and intra-subunit interactions with the motif.
Extended Data Figure 2 | Biochemical analysis of bacterial TMEM175 channels. a, Gel filtration profiles of three purified bacTMEM175 channels on Superdex-200 (10/30 GL column) in 20 mM HEPES, pH 7.4, 200 mM KCl and 3 mM DM. All purified bacTMEM175 channels eluted as a monodispersed peak at a position much larger than a monomer, indicating oligomerization. Arrows indicate the elution peaks of three standard proteins (29, 66 and 200 KDa) on the same column. b, Cross-linking reaction of purified bacTMEM175 channels from Streptomyces collinus (left gel) and Chryseobacterium sp. (right gel) with the cross-linking reagents DSS and DSG. Samples were analysed by SDS–PAGE and detected by coomassie blue staining. The purified proteins migrate as multiple bands on SDS–PAGE corresponding to the sizes of monomer to tetramer. The cross-linking reaction promotes the formation of a cross-linked tetramer, demonstrating that these bacterial channels form tetramers in solution. For gel source data, see Supplementary Fig. 1.
Extended Data Figure 3 | Electron density maps of CmTMEM175. a, $2F_o - F_c$ electron density map of one subunit (contoured at 1.5σ). b, $2F_o - F_c$ electron density map of the filter region (contoured at 1.5σ). The front and back subunits have been removed for clarity. The side chains of the three layers of hydrophobic residues are coloured in magenta.
Extended Data Figure 4 | Structural comparison between K⁺ channels. **a**, CmTMEM175; **b**, Shaker-like K⁺ channel (Kv1.2-2.1 chimaera, PDB code: 2R9R). Each subunit is individually coloured. Both structures are viewed from the extracellular side.
Extended Data Figure 5 | Structural comparison of the ion conduction pathways. Ion conduction pathways are shaded grey. a, CmTMEM175; b, chicken bestrophin-1 (PDB code: 4RDQ); c, bacterial bestrophin from *Klebsiella pneumoniae* (KpBest, PDB code: 4WD8). Insets show zoomed-in views of the narrow filters.
Extended Data Figure 6 | Ion selectivity and pharmacological properties of human TMEM175. a, Partial sequence alignment of TM1 helices from hTMEM175 and CmTMEM175; the three layers of hydrophobic residues are boxed and the RxxxFSD motif is shaded red. b, I–V curve from a control cell. The pipette and bath solutions contained 150 mM Cs\(^+\) and 150 mM Na\(^+\), respectively. c, Extracellular Zn\(^{2+}\) and 4-AP blockade of human TMEM175. Currents were recorded using whole-cell patches with 150 mM extracellular Na\(^+\) (bath) and 150 mM intracellular Cs\(^+\) (pipette). d, Intracellular Zn\(^{2+}\) and 4-AP blockade of human TMEM175. Currents were recorded using inside-out patches with 150 mM intracellular Na\(^+\) (bath) and 150 mM extracellular Cs\(^+\) (pipette). Recordings shown in c and d indicate that human TMEM175 is sensitive to Zn\(^{2+}\) or 4-AP block from both sides. e, I–V curves of wild-type human TMEM175. Currents were recorded using whole-cell patches in bi-ionic conditions. The pipette solution contained 150 mM Cs\(^+\) and the bath solution contained 150 mM X\(^+\) (X = NMDG, Li, Na, K or Rb). f, I–V curves of the I46N/I271N (at layer 1) double mutant of human TMEM175 in bi-ionic conditions. Currents were recorded using the same conditions as e. g, Summary of reversal potentials of hTMEM175 and its mutants and the calculated relative permeability between Cs\(^+\) and K\(^+\) or Na\(^+\); shown are mean ± s.e.m. of ≥5 measurements. h, Summary of reversal potentials of human TMEM175 and I46N/I271N mutant with various monovalent cations in the bath solutions and the calculated relative permeability of these ions in comparison to Cs\(^+\).
Extended Data Table 1 | Data collection and structure refinement statistics

| Dataset                      | Native                        | Hg derivative               |
|------------------------------|-------------------------------|----------------------------|
| **Data collection**          |                               |                            |
| Space group                  | C2                            | C2                         |
| **Cell dimensions**          |                               |                            |
| \(a, b, c\) (Å)              | 152.66, 108.88, 119.33        | 150.80, 107.85, 118.14     |
| \(\alpha, \beta, \gamma\) (°)| 90, 117.24, 90                | 90, 116.72, 90             |
| Wavelength (Å)               | 1.0332                        | 1.0064                     |
| Resolution (Å)               | 50.00-3.30 (3.36-3.30)\textsuperscript{a} | 50.00-3.50 (3.36-3.50)   |
| \(R_{	ext{merge}}\)         | 0.087 (1.596)                 | 0.071 (1.503)              |
| \(CC_{1/2}\)                | (0.581)                       | (0.633)                    |
| \(I/\sigma\)                | 30.5 (1.4)                    | 26.0 (1.0)                 |
| Completeness (%)             | 99.0 (98.4)                   | 95.5 (95.4)                |
| Redundancy                   | 3.6 (3.4)                     | 7.2 (6.0)                  |
| **Refinement**               |                               |                            |
| Resolution (Å)               | \(3.7 \ast 3.6 \ast 3.3\)\textsuperscript{b} |                            |
| No. of reflections           | 21668                         |                            |
| \(R_{	ext{work}}/ R_{	ext{free}}\) | 0.2594/0.2818               |                            |
| No. atoms                    | 5972                          |                            |
| Protein                      |                               |                            |
| B-factors                    |                               |                            |
| Protein                      | 106.59                        |                            |
| R.m.s deviations             |                               |                            |
| Bond lengths (Å)             | 0.011                         |                            |
| Bond angles (°)              | 1.280                         |                            |

\textsuperscript{a}The number in parentheses shows the value in the highest-resolution shell.

\textsuperscript{b}The data were elliptically truncated to \(1/3.7\) Å \(^{-1}\), \(1/3.6\) Å \(^{-1}\) and \(1/3.3\) Å \(^{-1}\) along \(a^{*}\), \(b^{*}\) and \(c^{*}\) directions, respectively.

\textsuperscript{c}\(R_{	ext{free}}\) was calculated with 5\% of reflection data.