Cryo-electron microscopy structure of the Slo2.2 Na\(^{+}\)-activated K\(^{+}\) channel

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Na\(^{+}\)-activated K\(^{+}\) channels are members of the Slo family of large conductance K\(^{+}\) channels that are widely expressed in the brain, where their opening regulates neuronal excitability. These channels fulfill a number of biological roles and have intriguing biophysical properties, including conductance levels that are ten times those of most other K\(^{+}\) channels and gating sensitivity to intracellular Na\(^{+}\). Here we present the structure of a complete Na\(^{+}\)-activated K\(^{+}\) channel, chicken Slo2.2, in the Na\(^{+}\)-free state, determined by cryo-electron microscopy at a nominal resolution of 4.5 Å. The channel is composed of a large cytoplasmic gating ring, in which resides the Na\(^{+}\)-binding site and a transmembrane domain that closely resembles voltage-gated K\(^{+}\) channels. In the structure, the cytoplasmic domain adopts a closed conformation and the ion conduction pore is also closed. The structure reveals features that can explain the unusually high conductance of Slo channels and how contraction of the cytoplasmic gating ring closes the pore.

Potassium channels control the excitability of electrically active cells by regulating the resting membrane potential in response to a variety of stimuli. One such stimulus is an increase in intracellular Na\(^{+}\), which occurs following repeated membrane depolarization. Slo2.2, also known as Slack or KCNT1, is opened by increases in intracellular Na\(^{+}\) (refs 2–8). Mutations in KCNT1, the gene that encodes the Slo2.2 protein, are linked to a variety of intellectual disabilities, including mental retardation, ataxia, and seizures (Ref. 9). Slo2.2 has also been found in other cell types, including nociceptive and sensory neurons, where it is hypothesized to influence pain sensitivity, and in epithelial cells of the thick ascending limb of Henle’s loop, where it is involved in ion reabsorption (Ref. 10).

Slo2.2 is a member of the Slo family of large conductance K\(^{+}\) channels, which are characterized by a transmembrane domain (TMD) containing six or seven transmembrane helices, and a large cytoplasmic domain (CTD) containing two regulator-of-K\(^{+}\)-conductance (RCK) domains. High-resolution structural data do not currently exist for full-length Slo channel, but structures of isolated CTDs of Slo1, a Ca\(^{2+}\)- and voltage-activated channel, were determined in Ca\(^{2+}\)-free (closed) and Ca\(^{2+}\)-bound (open) conformations (Ref. 11). In these structures the CTDs are organized into tetrameric gating rings that expand upon Ca\(^{2+}\) binding (Ref. 12). This expansion appears to be sufficient to open an inner helix gate in the transmembrane channel (Ref. 13). However, solvent accessibility experiments carried out in Slo1 and in Slo2.1 (a channel that shares a high degree of sequence similarity to Slo2.2, also Na\(^{+}\)-activated) have led to a hypothesis that these channels never fully close an inner gate, but instead close at the selectivity filter (Ref. 14–16). Thus, the basic question is where the gate is in Slo channels has remained unanswered.

Cryo-electron microscopy analysis

To determine the structure of a full-length Slo2.2 channel, a construct encoding the entire chicken KCNT1 gene (84% sequence identity to human KCNT1; Extended Data Fig. 1) was heterologously expressed in Spodoptera frugiperda cells. Images of frozen-hydrated preparations of detergent- and lipid-solubilized Slo2.2 tetramers in the absence of Na\(^{+}\) were recorded using a direct electron detector (Extended Data Fig. 2a). Using single-particle analysis, a cryo-electron microscopy (cryo-EM) density map of a Slo2.2 tetramer was calculated at a nominal resolution of 4.5 Å with C\(_{60}\) symmetry imposed (Extended Data Fig. 2d and Extended Data Table 1). The density map contains two domains into which the tetrameric structures of a Slo1 gating ring (Ref. 24) and the voltage-dependent K\(^{+}\) channel (Kv) chimera \(\alpha\)-subunit (Ref. 25) could be manually fitted. During fitting it became apparent that the density corresponding to the periphery of the TMD was of poorer quality than the density corresponding to the gating ring (Extended Data Fig. 3a). To assess the source of disorder in the TMD, 3D maximum-likelihood classification of the particle images was performed, yielding five similar but non-identical subclasses. These subclasses were related by a rotation of the TMD with respect to the gating ring about the fourfold axis (Ref. 1b). In the two most extreme subclasses, the rotation angle is 7°. Consequently, the larger mass of the gating rings resulted in them being well aligned in the reconstruction, whereas the smaller TMD is blurred, especially at the perimeter furthest from the fourfold axis. Separate focused refinements of the TMD and the gating ring using soft masks improved the maps for both domains (Fig. 1a) and provided enough detail to build a model starting with a voltage-dependent K\(^{+}\)-channel structure for the TMD and a Slo1 gating-ring structure for the gating ring (Fig. 1c–e). Extended Data Fig. 6 and Extended Data Table 1). Strong K\(^{+}\) ion density (6.5 \(\sigma\)) is present in the selectivity filter and weaker density (4 \(\sigma\)) is present in the central cavity and in the inner pore closer to the cytoplasm (Fig. 1e and Extended Data Fig. 5a). Prior knowledge of the location of K\(^{+}\) ions in the selectivity filter and central cavity of K\(^{+}\) channels from X-ray crystallographic studies (Ref. 31) supports our assignment of these densities as ions rather than noise along the fourfold axis (Extended Data Fig. 5b).

The gating-ring and TMD models were built and refined independently against phases and amplitudes calculated from the focused refinement maps using reciprocal-space algorithms. The gating ring...
Structure of Slo2.2

The tetrameric Slo2.2 channel is 120 Å in length along an axis perpendicular to the membrane plane (Fig. 2b). More than half of this length corresponds to the gating ring, which protrudes into the cytoplasm. Viewed down the fourfold axis, the TMD is approximately 80 Å on the square edge and the gating ring is 95 Å (Fig. 2b). Each subunit polypeptide chain builds up the Slo2.2 channel in three layers: a TMD layer and two RCK domain layers (RCK1 and RCK2), which form the gating ring (Fig. 2b, c).

The TMD is similar in topology to a Kv channel, to which Slo2.2 is related. The central ion conduction pore is surrounded in the membrane by voltage-sensor-like domains, which we refer to as S1–S4 domains to emphasize their voltage-independent uniqueness (Fig. 2b, c). As in Kv channels, the S1–S4 domains are arranged as appendages that project laterally into the lipid membrane. At a level of greater structural detail, we note two important distinctions between the TMDs of Kv1.2 and Slo2.2.

The first distinction is the manner in which the surface of the S1–S4 domain forms its contacts with the pore. In the Kv channel, the voltage sensor’s interaction with the pore occurs mainly through the extracellular half of S1 and through the S4–S5 linker (Fig. 3). This configuration leaves the charged S4 helix free to move in response to voltage differences across the membrane and exert force on the S4–S5 linker to gate the channel. In Slo2.2, the extracellular half of S1, nearly the entire S4 and a helix from the S1–S2 connecting segment form an extensive contact surface with the pore. The Kv voltage sensor and Slo2.2 S1–S4 domains have essentially the same helical structure, but their different orientations with respect to the pore reconfigure the contact surface (Fig. 3). In Slo2.2, S4 runs antiparallel to and in close contact with S5, as if its function is to help glue the S1–S4 domain to the pore rather than to provide a mobile voltage sensor. This possibility is consistent with the amino acid sequence of Slo2.2, which shows two positive- and two negative-charged amino acids in or near S4 instead of the excess positive charges observed in the S4 of a Kv channel and in the S4 sequence of Slo1 and Slo3 (ref. 33). Furthermore, mutations involving these positive and negative charges in Slo2.2 do not produce large changes in voltage-dependence.

Thus, S1–S4 does not appear to function as a mobile voltage sensor in Slo2.2. A surprising observation, which may be related to the differences in S4, is that we cannot detect cryo-EM density for an S4–S5 helical linker in Slo2.2. Higher-resolution data will be required to tell whether this linker is partially disordered or simply not present as a helix. Its absence in Slo2.2 is conspicuous, and implies that this region serves a different function than in Kv channels.

The second distinction is the manner in which the TMD connects to the gating ring. The Kv channel contains a CTD consisting of a T1 subunit. These are held at a distance of nearly 15 Å away from the membrane plane and consequently ions can gain access to the transmembrane pore through lateral openings between the TMD and the CTD, near the membrane surface. In contrast, in Slo2.2 the TMD and gating ring form a complementary interface near the membrane surface. This is most easily appreciated in a surface representation of Slo2.2 (Fig. 2c): the gap at the perimeter of the interface would be filled in if a full side-chain model (instead of polyalanine) of the S1–S4 domain had been built. The relatively snug fit between the TMD and gating ring in Slo2.2 has important implications for ion conduction and gating.

Figure 1 | Cryo-EM structure of chicken Slo2.2. a, Cryo-EM density map of chicken Slo2.2 following focused refinements of the TMD and the gating ring. The gating-ring map is filtered to 4.2 Å and the TMD map is filtered to 4.5 Å. The S1–S4 domain is green, the pore domain is yellow, the RCK1 domain is blue and the RCK2 domain is red. b, Density map of two of the 3D subclasses (coloured in red and blue) filtered to 7 Å and aligned by their gating rings. The density slice corresponds to the region of the TMD between the dashed lines. c, d, Fragments of the density map corresponding to helix αD in the gating ring (c) and the pore helix in the TMD (d). The refined model is shown in stick representation. Large side chains that were used to register the sequence are labelled. e, Central section of the density map in a through the TMD calculated at 4 σ (red) and 6.5 σ (blue) with densities corresponding to K⁺ ions labelled.
Ion conduction

In contrast to the Kv channel, the continuous pore across the membrane and through the gating ring makes Slo2.2 more like an inward-rectifier K⁺ channel, which also has an extended ion pathway, owing to a tightly engaged CTD³⁶,³⁷ (Fig. 4a). In inward rectifiers, however, the extended pore is narrower and contains binding sites for blocking ions, a property that gives rise to inward rectification³⁶,³⁸. In contrast, in Slo2.2 the gating ring creates a massive funnel that begins nearly 40 Å wide and gradually narrows as it approaches the pore inside the TMD. The inner surface of the funnel is highly electronegative, owing to the presence of many aspartate and glutamate amino acids (Fig. 4c). This electrostatically negative funnel, by functioning as a cation attractor, undoubtedly contributes to the unusually high conductance (nearly 200 pS for Slo2.2: about 20 times that of a canonical Kv channel such as Shaker³⁹) of Slo channels (Extended Data Fig. 7a). Indeed, Slo channels are the highest-conductance K⁺ channels known, and a recent mutagenesis study with Slo1 showed that the gating ring contributes to the high conductance⁴⁰.

The ion conduction pathway leading from the cytoplasm up to the selectivity filter has a single constriction that is formed by the crossing of four transmembrane S6 (inner) helices (Fig. 4b). The pore contracts to a minimum radius at Met333, which points its β-carbon towards the centre of the pore. Cryo-EM density for this side chain of Met333 is not well resolved; however, modelling the six most frequently observed rotamers of Met yields a range of pore diameters—defined by diagonally opposed van der Waals surfaces—ranging from 4 Å to 6 Å (Fig. 4b and Extended Data Fig. 8).

A hydrated K⁺ ion has a diameter closer to 8 Å; therefore, the structure is compatible with a closed conformation, consistent with the Na⁺-free conditions under which this structure was determined. Adding to the physical barrier that the van der Waals constriction at the level of Met333 would present to a permeating K⁺ ion, the local electrostatic environment would raise the barrier further (Fig. 4c). The band of blue colour seen in Fig. 4c (positive electrostatic potential) coincident with the constriction is due to Arg335 and Lys337 on the inner helices and Arg396 on the surface of the gating ring; these positive-charged amino acids impose a long-range cation-repulsive potential. Thus, the inner helix gate appears closed to K⁺ diffusion by virtue of its narrow constriction and positive electrostatic potential at the level of the constriction.

Gating inferences from structure

The Slo2.2 gating ring is similar in overall structure to the gating rings in Slo1 and Slo3 (refs 24, 25, 41). Two non-identical RCK domain pairs then assemble with each other through ‘assembly interfaces’ to form the ring (Fig. 5). The assembly interface is essential to the function of Slo1 because it contains the high-affinity Ca²⁺-binding site known as the ‘Ca²⁺ bowl’²⁶. Instead of a Ca²⁺ bowl, Slo2.2 has an extended α-helix, αQ, which interacts across the assembly interface with an adjacent subunit (Fig. 5). A helix-turn-helix structure (αX’, αY’ and αZ’) is also unique to the assembly interface in Slo2.2. This helical elaboration is provided by an approximately 110-residue insertion in the RCK2 domain that is conserved and

Figure 2 | Architecture of Slo2.2. a, Domain organization of chicken Slo2.2. b, Ribbon diagram of Slo2.2. The S1–S4 domain is green, the pore domain is yellow, the RCK1 domain is blue and the RCK2 domain is red. The approximate width of the membrane is marked by the grey lines. c, Surface depiction of Slo2.2 with front and rear subunits removed for clarity.

Figure 3 | Interactions between pore and S1–S4 domains. Ribbon diagram of the S1–S4 domain of Slo2.2 (left) and the voltage-sensor domain of Kv chimaera (middle). The S1–S4 and voltage-sensor domains are green and the pore domains are yellow. Red spheres represent the residues on S1, S4 and the S1–S2 linker that are close enough to interact with the pore domain. Superposition (right) of the S1–S4 domain of Slo2.2 (green) with the voltage-sensor domain of Kv chimaera (magenta) by aligning S1, S2 and S3.
unique to Slo2 channels (Extended Data Fig. 1). Putative Na\(^+\)-binding-site amino acids, identified through mutagenesis, do not fall within the assembly interface\(^{45}\). If this means that Na\(^+\) in Slo2.2 activates from a different locus than does Ca\(^{2+}\) in Slo1, then we should not be too surprised—the prokaryotic K\(^+\) channel MthK contains a Ca\(^{2+}\)-activated gating ring in which Ca\(^{2+}\) binds near the flexible interface rather than the assembly interface\(^{43}\). Apparently the conformations of RCK-based gating rings can be modulated in a variety of ways.

A possible locus for Na\(^+\) binding was identified in two studies using mutagenesis\(^{42,44}\). Both studies found that Asp812 (or the corresponding residue in Slo2.1, Asp757) reduced Na\(^+\) activation. In one study, a second residue (His817 in Slo2.2) was also found to affect Na\(^+\) activation; however, position 817 is located 15 Å away from Asp812 and thus could not possibly form a single binding site along with Asp812 (ref. 42). Additional studies including a structure of Slo2.2 in the presence of Na\(^+\) are required to better understand Na\(^+\) activation in Slo2.2.

Crystal structures of isolated (from the TMD) Slo1 gating rings in the absence and presence of Ca\(^{2+}\) show that when Ca\(^{2+}\) binds to the Ca\(^{2+}\) bowl the N lobe of RCK1 undergoes a conformational change\(^{24,25}\). This change causes the four N lobes (one from each subunit) on the surface of the gating ring to expand like the petals of an opening flower (Extended Data Fig. 9). This expansion is illustrated by marking a residue (Lys343) on the N lobe that connects to S6 (the inner helix gate) through a linker (Extended Data Fig. 9). The distance between diagonally opposed Lys343 residues is approximately 81 Å and 94 Å in closed and opened Slo1 gating rings, respectively. The equivalent residue in Slo2.2 (Lys351) is 73 Å from its diagonally opposed molecular symmetry mate, and the position of the N lobe is similar to that in the closed Slo1 gating ring. Thus, the Slo2.2 gating ring is closed, as is the inner helical gate to which it is connected. Cryo-EM densities show a weak connection between Lys351 on the N lobe and Lys337 on S6 (Fig. 6b). This connection supports the idea that expansion of the N lobes upon Na\(^+\) binding to the gating ring is mechanically connected to pore opening through the connecting linker. But the structure raises an additional mechanistic possibility. The interface that is formed between complementary-shaped surfaces on the TMD and the gating ring involves the RCK1 N lobes. If the N lobes expand when Na\(^+\) binds, as occurs when Ca\(^{2+}\) binds to the gating ring of Slo1, then the expansion would do more than pull on the linkers to S6; it would also presumably exert forces on the TMD through the protein–protein interface\(^{45}\). Such a mechanism of force transfer across the interface might explain why the S1–S4 domain in Slo2.2 is firmly attached through a large interface with the pore—so that conformational changes within the gating ring can be efficiently transmitted to the pore via the S1–S4 domain.

This study visualizes a Na\(^+\)-activated Slo2.2 K\(^+\) channel in its ligand-free, closed conformation. The structure supports three important concepts concerning the function of this class of K\(^+\) channels: (1) a closed inner helix gate, analogous to that observed\(^{46}\) or deduced\(^{47}\) in other K\(^+\) channels, would prevent K\(^+\) conduction through restrictive van der Waal surfaces and an unfavourable electrostatic environment; (2) expansion of N lobes on the gating ring would open the pore through a polypeptide linker and through the protein–protein interface between the gating ring and the TMD; and (3) a massive electrostatic funnel facing the cytoplasm helps to explain why Slo channels are the highest-conductance K\(^+\) channels.

Figure 5 | Slo2.2 gating ring. Wire diagram of the Slo2.2 gating ring with the boundary of one subunit highlighted by the dashed circle (left). The flexible interface that mediates interactions between the RCK1 and RCK2 domains of each subunit and the assembly interface that mediates interactions between adjacent subunits are shown. The assembly interfaces for Slo2.2 (middle) and Slo1 (right) with one subunit shown as a surface and the second shown as ribbons. The RCK1 domains are blue and the RCK2 domains are red. The Ca\(^{2+}\) ion resolved in the Slo1 Ca\(^{2+}\) bowl is shown as a yellow sphere.

Figure 4 | Slo2.2 ion conduction pathway. a, Surface representation of the Slo2.2 pore and ribbon diagram with front and rear subunits excluded for clarity. The K\(^+\)-accessible surface was determined using HOLLOW.
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Author Contributions R.K.H. performed the experiments. P.Y. provided assistance with protein expression and purification. Z.L. aided with sample preparation and data collection. T.W. provided assistance with protein expression. R.K.H and R.M. designed the experiments and analysed the results. R.K.H. and R.M. prepared the manuscript with input from all co-authors.

Author Information The 3D cryo-EM density maps of Slo2.2 with low-pass filter and amplitude modification have been deposited in the Electron Microscopy Data Bank under accession numbers EMD-3062 (Slo2.2 whole channel), EMD-3063 (Slo2.2 gating ring) and EMD-3064 (Slo2.2 TMD). Atomic coordinates for the atomic model of full-length Slo2.2, Slo2.2 gating ring and Slo2.2 TMD have been deposited in the Protein Data Bank under accession numbers 5A6E, 5A6F and 5A6G, respectively. Reprints and permissions information is available at www.nature.com/reprints. The authors declare no competing financial interests. Readers are welcome to comment on the online version of the paper. Correspondence and requests for materials should be addressed to R.M. (mackinn@rockefeller.edu).
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METHODS

No statistical methods were used to predetermine sample size.

Expression and purification. A synthetic gene fragment encoding residues 1 to 1201 of the Spodoptera frugiperda baculovirus were purchased from Bio Basic Inc. The resulting fragment was cloned into a modified pFastbac vector (Invitrogen) containing green fluorescent protein and a 1D4 antibody recognition sequence (TETSQVAPA) on the C terminus. S29 (Spodoptera frugiperda) cells infected with the baculovirus were cultured at 27 °C for 72 h in supplemented Grace’s insect cell medium (Invitrogen). Cells were washed with ice-cold phosphate-buffered saline and extracted for 3 h at 4 °C with buffer containing 50 mM Hepes pH 7.4, 300 mM KCl and 40 mM dodecyl-β-D-maltopyranoside (DDM) in the presence of a protease inhibitor cocktail (2 μg ml⁻¹ leupeptin, 2 μg ml⁻¹ aprotinin, 2 μg ml⁻¹ pepstatin A, 1 mM benzamidine, 100 μg ml⁻¹ 4-(2-aminoethyl) benzenesulfonyl fluoride hydrochloride and 100 μM phenylmethylsulfonyl fluoride). The insoluble fraction was removed by centrifugation at 35,000g for 45 min at 4 °C and the remaining soluble fraction was incubated with 1D4-affinity resin pre-equilibrated with 20 mM Hepes pH 7.4, 300 mM KCl and 4 mM DDM. The suspension was mixed for 5 h at 4 °C. Beads were collected on a column by gravity and then washed with 10 column volumes of wash buffer (20 mM Hepes pH 7.4, 300 mM KCl, 4 mM DDM and 0.1 mM 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphoethanolamine (POPE):1-palmitoyl-2-oleoyl-sn-glycero-3-phosphoglycerol (POPG) (3:1 w/w)). The protein was digested with PreScission protease (20:1 w/v ratio) on the column overnight at 4 °C to remove the affinity tag and then eluted with two column volumes of wash buffer. Concentrated protein was further purified by size-exclusion chromatography on a Superose 6 (GE Life Sciences) column in 20 mM Hepes pH 7.4, 300 mM KCl, 1.5 mM DDM and 0.05 mg ml⁻¹ POPE:POPG (3:1 w/w). Peak fractions were pooled and concentrated to 3–5 mg ml⁻¹ for cryo-EM analysis.

Electron microscopy sample preparation and imaging. 3.5 μl of purified channel was pipetted onto glow-discharged copper Quantifoil R 1.2/1.3 holey carbon grids (Quantifoil). Grids were blotted for 3.5 s at 85% humidity and then washed in liquid nitrogen-cooled liquid ethane using an FEI Vitrobot Mark IV (FEI). Grids were transferred to an FEI Titan Krios electron microscope operating at an acceleration voltage of 300 keV. Images were recorded in an automated fashion on a Gatan K2 Summit (Gatan) detector set to super-resolution counting mode with a super-resolution pixel size of 4.16 Å from a movie (pixel size of 1.04 Å at the image plane) for a total accumulated dose of approximately 40 electrons per square Ångström on the specimen over 25 subframes (approximately 1.5 electrons per square Ångström per subframe).

Image processing and map calculation. Dose-fractionated super-resolution images were 2 × 2 binned (resulting in a pixel size of 1.04 Å) for whole-frame motion correction with motioncor2. Following motion correction, the sum of the 25–30 single-contrast images was 4 × 4 binned (pixel size of 4.16 Å) for manual particle selection. 40,643 particles were manually selected in Boxer from 2,243 images and then extracted from the motion-corrected images in RELION51. The effects of the contrast transfer function were estimated by ctffind352 and CTER53, 22,154 particles were subjected to iterative stable alignment from 2,243 images and then extracted from the motion-corrected images in RELION51. The 3D classification were combined, yielding a subset of 11,303 images. The angular parameters of these images were determined by FREELIGN using an iterative process in which two refinement cycles were run with the mask followed by two without the mask. This iterative procedure of refining with and without mask application was necessary, owing to the small size of the TMD relative to that of the whole channel. The map of the transmembrane region achieved a final resolution of 5.2 Å by FSC.

Model building. The structure of the closed Slo1 gating ring24 (Protein Data Bank code 3NAF) was docked into the gating-ring map using UCSF Chimera and then manually rebuilt in Coot to fit the density. For the regions lacking homology with Slo1, secondary structure elements were first built into the density map and then connected with manually built loops. Once the backbone was traced, the sequence was registered using a combination of identification of large side chains and sequence homology with Slo1. The structure of the Kv chimera α-subunit30 (PDB code 2R9R) was docked into the TMD map using UCSF Chimera using the selectivity filter and pore helix as a reference. The positions and tilts of S5 and S6 were adjusted by moving the helices as rigid domains to fit the density, and then the loops connecting them were manually rebuilt. The sequence was registered using the presence of large side chains, the proline kink and sequence homology among K⁺ channels. The S1–S4 domain was built as six polyalanine helices in coot (S0, 15 residues; S1, 28 residues; S1–S2, 37 residues; S2, 27 residues; S3, 32 residues; S4, 26 residues). The helices were assigned on the basis of homology with Kv chimera, and roughly agree in length with those predicted by hydrophathy analysis. A model of the full channel was generated by manually docking the independently determined structures for the gating ring and the TMD into the map of the full channel.

Reciprocal space refinement. The masked cryo-EM density maps were transplanted to a new unit cell that extended 10 Å from the model in all directions, and then solvent flattened by creating a mask that extends 3 Å away from the model and setting the density outside of this mask to approximate the solvent content of a protein crystal. Reciprocal-space structure factors were calculated from the map using solvent-flattened phases computed using FFT. The phases were blurred in a resolution-dependent fashion by setting the figure of merit equal to the FSC and subsequently used to calculate Hendrickson–Lattman coefficients. Coordinate and B-factor refinement of the gating-ring and TMD models were performed in Phenix64, using X-ray scattering factors against a maximum likelihood with an experimental phase probability distribution target function, with 5% of the reflections excluded as a free set to monitor overfitting. Secondary structure, geometric, B-factor and Ramachandran restraints were used throughout refinement. Rigid-body refinement of the full channel model was performed in Phenix64 using X-ray scattering factors against a maximum likelihood with an experimental phase probability distribution target function, with 5% of the reflections excluded as a free set to monitor overfitting.

Overfitting of the models during refinement was also evaluated using a method previously described41. Briefly, all atoms of the refined models were randomly displaced by 0.1 Å and then subjected to one round of coordinate and B-factor refinement against phases and amplitudes calculated from one of the independently determined half maps. Following this refinement cycle, the FSC was calculated between the resultant model and both of the half maps, as well as the full map. Structure figures were prepared with UCSF Chimera54, Pymol (Pymol version 1.7.2 Schrödinger, LLC), HOLLOW65 and APBS56,67. All structure calculations were performed using software compiled by SBGrid.75

Reconstitution and electrophysiological recordings from planar lipid bilayers. Purified channels were reconstituted into octyl maltoside (Anatrace)-solubilized 3:1 w/v POPE:POPG lipid vesicles as described80. Detergent was removed by dialysis for 5 d against a detergent-free buffer containing 10 mM Hepes-KOH pH 7.5, 450 mM KCl and 2 mM dithiothreitol at 4 °C, with daily buffer exchanges. After 5 d, all residual detergent was removed by incubating the reconstituted channels with Bio-Beads (Bio-Rad) for 2 h at room temperature. The reconsti-
tuted channels were aliquoted and flash frozen into liquid nitrogen before storage at $-80^\circ$C.

Planar lipid bilayer experiments were performed as described previously.\(^{70,71}\) Lipids of desired compositions were prepared by dissolving argon-dried lipids in decane to a final concentration of 20 mg ml\(^{-1}\). Lipid solutions were painted over a 300-$\mu$m hole in a polystyrene partition that separated the two chambers to form the planar lipid bilayer. Reconstituted channels were pipetted onto the chamber side of the bilayer after thinning of a planar lipid bilayer had been detected via monitoring of electrical capacitance. All recordings were performed using the voltage-clamp method in whole-cell mode. Analogue signals were filtered at 1 kHz using a low-pass Bessel filter on an Axopatch 200B amplifier (Molecular Devices) in whole-cell mode and digitized at 10 kHz using a Digidata 1400A analogue-to-digital converter (Molecular Devices). The pClamp software suite (Molecular Devices) was used to control membrane voltage and record current. All electrophysiological recordings were replicated in at least three independent experiments.

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Extended Data Figure 1 | Sequence alignment of Sło channels. a, Sequence alignment of chicken Sło2.2 with human Sło2.2 and human Sło2.1. b, Predicted position of transmembrane helices in Sło2.2 S1–S4 domain on the basis of hydropathy analysis using Jpred 4 (ref. 72). c, d, Structure-based sequence alignment of chicken Sło2.2 TMD with rat Kv chimera (c) and chicken Sło2.2 gating ring with human Sło1 gating ring (d). Helices are blue and β-strands are red.
Extended Data Figure 2 | Full channel 3D reconstruction of chicken Slo2.2.
a, Representative micrograph of detergent- and lipid-solubilized Slo2.2 in vitreous ice. b, Selected 2D class averages. c, Ab initio model of Slo2.2. d, FSC curve of the full channel reconstruction with the nominal resolution estimated to be 4.5 Å on the basis of the FSC = 0.143 (dashed line) cut-off criterion.
Extended Data Figure 3 | Focused refinement of the gating ring and the TMD. a, 3D density map of the full channel reconstruction, coloured according to local resolution (in ångströms). b, c, 3D density map calculated following focused refinement using a mask to only include the gating ring (b) and the TMD (c), coloured according to local resolution (in ångströms). d, FSC of the full channel reconstruction (estimated resolution of 4.5 Å), the gating-ring-focused refinement reconstruction (4.2 Å) and the TMD-focused refinement reconstruction (5.2 Å).
Extended Data Figure 4 | Validation of the Slo2.2 model. a, Refinement statistics for the Slo2.2 full channel, TMD and gating-ring models. b, c, FSC curves for cross-validation of the refined gating ring (b) and TMD (c) models. The black curves are the refined model compared to the full data set, the red curves are the refined model compared to half map 1 (used during test refinement) and the blue curves are the refined model compared to half map 2 (not used during test refinement).
Extended Data Figure 5 | K\(^+\) ions in Slo2.2.  

a, Central section of the density maps of the two independently calculated half maps (coloured in green and red) with densities corresponding to K\(^+\) ions labelled.

b, Superposition of the Slo2.2 selectivity filter (green) with KcsA (PDB code 1K4C) selectivity filter (yellow). Density peaks resolved in the Slo2.2 selectivity filter at 6.5 \(\sigma\) are shown as blue meshes. K\(^+\) ions resolved in KcsA are shown as grey spheres.
Extended Data Figure 6 | Representative segments of the cryo-EM density map. a–d, Selected regions of the gating-ring density (a, b) and the TMD density (c, d) maps with the refined model.
Extended Data Figure 7 | Single channel conductance of Slo2.2. **a**, Single channel current–voltage relationship (mean ± s.e.m.) for Slo2.2 in planar lipid bilayers. Single channel conductance is about 200 pS. **b**, Representative recordings of Slo2.2 held at −80 mV, −40 mV and 0 mV in planar lipid bilayers. Chamber solution contained 135 mM NaCl and 15 mM KCl, and cup solution contained 150 mM KCl. **c**, Histogram of Slo2.2 currents when held at −80 mV, −40 mV and 0 mV, as labelled.
Extended Data Figure 8 | Inner helix gate. a, Ribbon diagram of the Slo2.2 pore with Met333 side chains modelled as spheres. b, Pore radius plot as a function of distance from the extracellular surface for Slo2.2 with Met333 modelled as each of the six most frequently observed rotamers, as labelled. For distances less than about 40 Å, the curves coincide.
**Extended Data Figure 9 | Slo2.2 gating ring is in a closed conformation.**

Wire diagrams of Slo1 gating ring in the open (top left) and closed (top right) conformations. The mobile RCK1 N lobe is black and the rest of the gating ring is grey. The N-terminal residue of the gating ring, Lys343, is shown as a pink sphere. Wire diagram of the Slo2.2 gating ring (bottom) with the RCK1 N-lobe blue and the rest of the gating ring light blue. The N-terminal residue of the gating ring, Lys351, is shown as a pink sphere.
## Extended Data Table 1 | 3D reconstructions of chicken Slo2.2 by cryo-EM

| Refinement       | Full channel | TMD       | Gating ring |
|------------------|--------------|-----------|-------------|
| Space group      | P4           | P4        | P4          |
| Cell dimensions  |              |           |             |
| a, b, c (Å)      | 163.6, 163.6, 138.3 | 126.3, 126.3, 70.3 | 161.2, 161.2, 96.0 |
| α, β, γ (°)      | 90, 90, 90   | 90, 90, 90 | 90, 90, 90  |
| Resolution (Å)   | 138.4.5      | 90 - 4.5  | 114 - 4.2   |
| No. reflections  | 21,796       | 6,760     | 18,225      |
| R \text{free}/R \text{free} | 29.0/22.5 | 26.2/28.3 | 25.1/27.6   |
| No. protein atoms| 5,991        | 1,440     | 4,561       |
| Wilson b         | 237.0        | 226.8     | 225.6       |
| Mean B-factor    | 608.4        | 278.4     | 297.2       |
| R.m.s. deviations|              |           |             |
| Bond lengths (Å) | 0.010        | 0.004     | 0.011       |
| Bond angles (°)  | 1.28         | 0.71      | 1.38        |
| Ramachandran     |              |           |             |
| Favored (%)      | 89.7         | 94.1      | 88.0        |
| Allowed (%)      | 10.2         | 5.9       | 11.8        |
| Outliers (%)     | 0.1          | 0.0       | 0.2         |
| MolProbity       |              |           |             |
| Clash score*     | 7.75         | 3.5       | 9.0         |
| Rotamer outliers (%) | 2.1   | 1.2       | 2.3         |
| Overall score    | 2.23         | 1.59      | 2.35        |

* steric overlaps >0.4 Å per 1000 atoms