In neurons, the loading of neurotransmitters into synaptic vesicles uses energy from proton-pumping vesicular- or vacuolar-type adenosine triphosphatases (V-ATPases). These membrane protein complexes possess numerous subunit isoforms, which complicates their analysis. We isolated homogeneous vesicular- or vacuolar-type adenosine triphosphatases (V-ATPases). These membrane protein complexes possess numerous subunit isoforms, which complicates their analysis. We isolated homogeneous rat brain V-ATPase through its interaction with SidK, a Legionella pneumophila effector protein. Cryo-electron microscopy allowed the construction of an atomic model, defining the enzyme’s ATP:proton ratio as 3:10 and revealing a homolog of yeast subunit f in the membrane region, which we tentatively identify as RNaseK. The c ring encloses the transmembrane anchors for cleaved ATP6API/Ac45 and ATP6AP2/PRR, the latter of which is the (pro)renin receptor that, in other contexts, is involved in both Wnt signaling and the renin-angiotensin system that regulates blood pressure. This structure shows how ATP6API/Ac45 and ATP6AP2/PRR enable assembly of the enzyme’s catalytic and membrane regions.

vesicular- or vacuolar-type adenosine triphosphatases (V-ATPases) are ATP-hydrolysis-driven proton pumps that are essential for acidification of endosomes, lysosomes, and the trans Golgi network, as well as for acid secretion by osteoclasts, kidney intercalated cells, and some tumor cells (1, 2). ATP hydrolysis in the V-ATPase catalytic V1 region drives rotation of a central rotor subcomplex and leads to proton translocation through the membrane-embedded V0 region. V-ATPase activity is regulated by reversible separation of the V1 and V0 regions, which inhibits ATP hydrolysis in the isolated V1 complex and makes the V0 complex impermeable to protons (3, 4). In neurons, V-ATPase activity energizes synaptic vesicle membranes, allowing transporters to load the vesicles with neurotransmitters (Fig. 1A) (1, 2). Fusion of synaptic vesicles with the presynaptic membrane requires separation of the V1 and V0 regions, but it is not known how these events are coordinated (5). The regulated release of neurotransmitters from synaptic vesicles into the synaptic cleft allows signal propagation from the axon terminal of a presynaptic neuron to the dendrite of a postsynaptic neuron. After the release of neurotransmitters, subsequent endocytosis and regeneration of synaptic vesicles occurs via clathrin-independent and clathrin-mediated routes (6), with the formation of clathrin-coated vesicles temporarily blocking V-ATPase activity (7). In the Saccharomyces cerevisiae enzyme, which is the most thoroughly characterized enzyme to date, the V1 region contains subunits A, c, c′, c″, d, and e as well as ATP6API, also known as Ace5, and ATP6AP2, also known as the (pro)renin receptor (10, 11). ATP6AP2/PRR is involved in several signaling pathways (12), including the renin-angiotensin system for regulating blood pressure and electrolyte balance (10, 11, 13) and Wnt signaling in stem cells and embryo development (14). The precise arrangement of subunits in the mammalian V0 region remains unclear. Further, mammals have multiple isoforms of some subunits in both V1 and V0 that are expressed in a tissue-dependent and cellular-compartment–dependent way. These include two isoforms of subunit B, two of C, two of E, three of G, four of a, two of d, and two of e (15, 16). Mass spectrometry of purified rat-brain synaptic vesicles detected V-ATPase subunits A, B1, B2, C1, D2, E1, F1, GI, G2, a1, a4, c1, ATP6API/Ac45, and ATP6AP2/PRR (17).

To isolate V-ATPase for structural analysis, we developed a purification strategy based on the high-affinity interaction of the enzyme with the Legionella pneumophila effector protein SidK (18, 19). Although procedures capable of obtaining highly purified synaptic vesicles have been described previously (17, 20), these procedures enhance purity at the cost of yield, which complicates or precludes structure determination. Instead, we used a two-step differential centrifugation procedure to collect rat synaptic vesicles and clathrin-coated vesicles along with other cell membranes. Membranes were solubilized with detergent and SidK (residues 1 to 278) fused to a C-terminal 3xFLAG tag was used with M2-agarose to purify V-ATPase. With this approach, a single ~2-g rat brain provided ~200 μg of highly purified V-ATPase (Fig. 1B). Mass spectrometry of tryptic fragments (fig. S1A and tables S1 and S2) identified the bands on the SDS–polyacrylamide gel electrophoresis (SDS-PAGE) gel as V-ATPase subunits A, B2, C1, D1, E1, F, G2, a1, c1, and d1. A low-intensity band on the gel corresponding to subunit G1 was also detected, which, from gel densitometry, appears to constitute 17 ± 3% of subunit G in the preparation (±SD, three independent purifications). Subunit G1 may be a component of some V-ATPase complexes in synaptic vesicles (17). Alternatively, subunit G may be from copurifying lysosomal V-ATPase (21). The glycoprotein ATP6API/Ac45 and the small and hydrophobic proteins ATP6AP2/PRR and subunit e2, which do not stain clearly with Coomassie, were detected after cleavage with trypsin or chymotrypsin (Fig. 1B, fig. S1A, and table S3). The subunit isoforms identified are all consistent with the V-ATPase from synaptic vesicles (17). This homogeneity could be because of the synaptic vesicle V-ATPase being the predominant form of the enzyme in the brain or because of V-ATPases from other cellular compartments in the brain having the same isoform composition as the synaptic vesicle enzyme. RNaseK, a hydrophobic protein recently found to associate with mammalian V-ATPase (22), was also detected after cleavage with trypsin or chymotrypsin (Fig. 1B, fig. S1A, and table S3). Subunit H, which dissociates from bovine brain V-ATPase when treated with oxidizing agents (23), was not detected by mass spectrometry, despite the absence of these agents from the preparation. Subunit H is part of the mammalian V-ATPase (7, 17) and is needed for full enzyme activity (24). Loss of subunit H during purification of mammalian V-ATPase is markedly different from the subunit’s behavior in S. cerevisiae (25). This difference is noteworthy because the protein’s physiological role of mechanically blocking ATP hydrolysis in the isolated V1 region likely requires a strong attachment to the enzyme (26, 27). Although tryptic peptides from subunits B1, C2, a2, a3, and a4 were also detected (table S4), integrated peak intensities for these peptides in extracted ion chromatograms were two to three orders of magnitude lower than for peptides from subunits B2, C1, and a1, which indicates that its abundance is negligible for structural studies.

Native mass spectrometry further demonstrated the homogeneity of the enzyme preparation (Fig. 1C, red, and fig. S1, B and C). Spectra show the V1 region, presumably because of dissociation of the complex during analysis. The V1 region has a native mass of 683369 ± 144 Da, consistent with a subunit composition of A3, B2, D, E1b, F, G2b, and SidK (Fig. 1C, fig. S1B, and table S5). Fragmentation of the V1 region using a higher-energy collisional dissociation (HCD) voltage of 50 to 250 V confirmed the isoform composition of the V1 region as B2, C1, and E1 (fig. S1B). Specifically, spectra for subunit G showed a native mass of 13578 ± 1 Da (Fig. 1C, right), consistent with subunit G2 (13578 Da) but not G1 (13621 Da), both of which are N-terminally acetylated (fig. S1C). Lower abundance peaks were also seen for the V1 region missing subunit C1 (Fig. 1C, green) and...
envelope (during ATP hydrolysis, as seen with the detergent-solubilized V-ATPase disassembling because subunit H is not present or to the preparation yielded three three-dimensional be due to free V1 complexes that remain ac-

Table 5. Differences between calculated and measured masses for each peak (± SD of fit) and the calculated mass depending on subunit composition (table S5). The difference between calculated and measured masses is indicated. m/z, mass/charge ratio. (D) Composite cryo-EM map (left) and atom model (right) of brain V-ATPase in rotational state 1. Scale bar, 25 Å.

Cryo–electron microscopy (cryo-EM) of the preparation yielded three three-dimensional (3D) maps, corresponding to ~120°-rotations of the rotor subcomplex between states (25). These rotational states of the enzyme had overall resolutions of 3.9, 4.0, and 3.9 Å (figs. S3 and S4). Focused refinement of rotational state 1 was able to improve the resolution to 3.8 Å for the membrane region and 3.6 Å for the catalytic region of the complex. Together, these maps allowed the reconstruction of an atomic model for most of the complex, with a few components—including parts of subunits E1 and G2, the soluble N-terminal domain of subunit a1, subunit C1, and several luminal loops in the membrane region—modeled as backbone with truncated side chains (Fig. 1D, table S6, and fig. S5). Similar to the yeast V0 structure (8, 29), no density was apparent for the loop between residues 667 and 712 in subunit a1. Owing to the averaging that occurs during cryo-EM image analysis, the minor population of complexes in the preparation possessing subunit G1 rather than G2 could produce a map that shows a weighted averaging of both isoforms. Alternatively, images of complexes containing subunit G1 may be excluded during 2D- and 3D-image classification if they do not average coherently with the majority population of complexes to produce high-resolution map features. Where the map shows high-resolution features for subunit G, it accommodates subunit G2 better than G1 (fig. S6), which is consistent with most of the V-ATPase complexes containing subunit G2. Interpolating between the three rotational states produced a movie (movie S1) that shows the conformational changes in the enzyme that couple ATP hydrolysis in the V1 region to proton pumping through the V0 region. These changes illustrate the flexibility of the enzyme, particularly in the peripheral stalks, subunit C1, and N-terminal domain of subunit a1.

Previous high-resolution insight into the structure of the eukaryotic V1 region has been limited to cryo-EM of the intact yeast V-ATPase at ~7-Å resolution (19, 25, 29) and a 6.2- to 6.7-Å resolution crystal structure of the autoinhibited

Fig. 1. Overall structure of brain V-ATPase. (A) Cycle of synaptic vesicle loading, docking and priming, fusion, and recycling. (B) SDS-PAGE of rat brain V-ATPase isolated with 3×FLAG SidK1-278 and gel filtration chromatography. (C) Native mass spectrometry (MS) of V1 region (left) and native mass spectrometry of dissociated subunit G (right) at a higher-energy collisional dissociation (HCD) voltage of 250 V. The charge state for one peak per subunit is indicated. The table shows the measured mass for each peak (± SD of fit) and the calculated mass depending on subunit composition (table S5). The difference between calculated and measured masses is indicated. m/z, mass/charge ratio. (D) Composite cryo-EM map (left) and atom model (right) of brain V-ATPase in rotational state 1. Scale bar, 25 Å.
yeast V1p(C) complex (27). These maps only allowed visualization of α helices in the structure. The current structure of the V1 region (Fig. 2A) enables comparison with atomic models of prokaryotic V/A-ATPase catalytic regions (30–32) as well as numerous atomic models of ATP synthase F1 regions (33). ATP synthases possess three catalytic and three noncatalytic nucleotide binding sites, found at the interfaces between subunits α (corresponding to V-ATPase subunit B) and β (corresponding to subunit A) (33). In contrast, and consistent with structures of the bacterial V/A-ATPase (31, 34), the mammalian V1 region lacks noncatalytic nucleotide binding sites and shows only a single-bound nucleotide in one of the catalytic sites (Fig. 2, B and C). This nucleotide could be modeled in the density map as adenosine 5′-diphosphate (ADP), consistent with biochemical analysis of the M. sexta V-ATPase (28). The three pairs of catalytic subunits in F- and V-type ATPases interchange between three different conformations, originally described for the F1p-ATPase as ATP-bound, ADP-bound, and empty (33). In the present structure, each catalytic AB pair similarly adopts one of three conformations (Fig. 2, B and C). Comparison of the conformation of catalytic AB pairs with crystal structures of a prokaryotic V1p/A1 region suggests that the ADP-bound site is in a posthydrolysis state (Fig. 2, B and C) (34) and that the site in an open conformation (clockwise from the ADP-bound site when viewed from V1 toward V0) is in a state with high affinity for ATP (31) (Fig. 2, B and Cii). The conformation of the third pair of AB subunits does not appear to correspond to previous structures, but its nucleotide-binding pocket is occluded (Fig. 2, B and Ciii), suggesting a low affinity for nucleotide (31). Therefore, the enzyme imaged here appears to be poised to bind ATP. Three copies of SidK (27) are bound to the three A subunits (fig. S7). The structural consequences of SidK binding to the mammalian V1 region are not known, but SidK perturbs the conformation of the yeast V1 region only subtly (19). Although lower-resolution structures of the yeast enzyme have suggested that subunit G does not contact the rest of the V1 region (25, 27), the present structure shows that it does participate in linking the EG heterodimer to the catalytic A2B3 complex (31). This connection relies on residues from the N terminus of subunit B2 and C-terminal residues of subunits E1 and G2 (Fig. 2D, asterisks) and includes a structure where β strands from both subunit B2 and E1 form a single β sheet (Fig. 2E, purple arrowhead), also seen in a recent cryo-EM map of a prokaryotic V/A-ATPase (32). Numerous mutations in subunits A, B1, B2, and E1 are linked to disease and can be mapped onto the structure (fig. S8).

The V1 region contains subunits a1, d1, e2, e4, ATP6AP1/Ac45, ATP6AP2/PRR, and the c ring (Fig. 3A). Previous high-resolution structures of S. cerevisiae V1 regions were determined from auto-inhibited complexes separated from their V1 regions (8, 9, 29), whereas the structure presented here is within the context of an assembled V-ATPase. Consequently, the soluble N-terminal domain of subunit a1 is found in its non-inhibitory conformation and does not make contact with subunit d1 (Fig. 3B, green). The membrane-embedded C-terminal domain of subunit a1 includes eight transmembrane α helices (Fig. 3C) and creates the two offset half-channels that allow proton translocation (8). The subunit starts with a v-shaped insertion into the lipid bilayer formed by two short α helices that do not entirely cross the membrane (α1 and α2), followed by four transmembrane α helices (α3 to α6) and two highly tilted transmembrane α helices (α7 and α8) that create the surface that contacts the c ring. This fold closely follows yeast Vph1p and Stv1p (8, 29), with root mean square deviations between Cα atoms of 1 and 0.8 Å, respectively (fig. S9A). The major difference between these structures is the insertion of a structured linker with α helices connecting transmembrane α3 and α4 in subunit a1 (Fig. 3C and fig. S9B). The c ring
contains nine copies of subunit c and one of subunit c′′ (Fig. 3B, pink and purple). As expected from the lack of a homologous gene in mammals, the ring does not contain the subunit c′ that is found in yeast. Conserved Glu residues from protomers of the c ring are each capable of carrying a single proton during proton translocation (Fig. 3B, red spheres). As with the yeast V_0 region, subunit c′′ breaks the pattern of conserved proton-carrying Glu residues on alternating outer α helices of the c ring (Fig. 3B, red arrow) (8). The presence of ten protomers in the c ring sets the ATP: proton ratio at three ATP molecules hydrolyzed for every ten protons translocated. With typical concentrations of nonfermentable carbon sources (41), recent analysis of both the vacuolar and Golgi forms of the S. cerevisiae V-ATPase identified the hypothetical protein YPR170W-B as a transmembrane α-helical hairpin subunit, named subunit f (8, 29). YPR170W-B is highly conserved in fungi, but deletion of the gene did not cause the Vma_8 V-ATPase deficiency phenotype in yeast, where cells can grow on medium buffered to pH 5.5 but not pH 7.5, are sensitive to extracellular calcium and a variety of heavy metals, and cannot grow on medium with typical concentrations of nonfermentable carbon sources (41). The structure of the brain V-ATPase revealed density for a similar transmembrane α-helicain hairpin in the VO region in a position corresponding to the yeast subunit f (Fig. 3, red). Bioinformatic analysis suggests RNAseK, a conserved metazoan protein (42), as a homolog of S. cerevisiae subunit f, sharing 32% sequence identity and 52% sequence similarity (Fig. S1A). Immunoprecipitation of RNAseK previously revealed that it is associated with V-ATPase (22) and mass spectrometry done in this study shows that RNAseK is present in this enzyme preparation (tables S1 to S3). Consistent with a role

Fig. 3. Structure of the V_0 region. (A) Surface representation with cryo-EM density for subunits f, ATP6AP1/Ac45, and ATP6AP2/PRR. Scale bar, 25 Å; NTD, N-terminal domain; CTD, C-terminal domain. (B) Viewed from V_1 with conserved proton-carrying Glu residues as red spheres. The direction of ATP-hydrolysis-driven rotation of the ring is indicated. Red arrow indicates the symmetry-breaking Glu residue of subunit c′. (C) Cartoon representation viewed parallel to the plane of the lipid bilayer. (D) Proton path through the V_0 region. (E) Surface representation of a1CTD viewed parallel to the plane of the lipid bilayer. The gray arrow indicates the expected location of an opening between α7 and α8 leading toward the luminal half-channel. (F) Close-up view of the luminal terminus of the luminal half-channel, showing the interaction of subunits f, e2, a1, and the unidentified density. Scale bar, 10 Å.
in V-ATPase activity, RNAseK is necessary for viral endocytosis and replication (22, 43). The cryo-EM density for subunit f, although lacking the necessary resolution to unambiguously identify the protein as RNAseK, is consistent with the expected size of RNAseK and accommodates most of its bulky side chains (fig. S5B). Together, this evidence tentatively identifies RNAseK as subunit f in mammalian V-ATPases. Whether or not RNAseK functions as a ribonuclease within the V-ATPase is not known, but we note that the in vitro assays that established this protein and its homologs as ribonucleases (42, 44) were performed in the absence of detergents or other membrane mimetics, despite clear transmembrane \( \alpha \) helices in hydropathy analysis of the protein’s amino acid sequence (fig S1B). An additional unidentified protein-like density previously found in both the vacuolar and Golgi forms of the \( S. \ ceravisae \) V-ATPase (9, 29) is also present in the mammalian complex (Fig. 3, C to F, purple). All four of the membrane-embedded components from this part of the enzyme interact at the opening of the luminal proton half-channel, with the linker between \( \alpha \)3 and \( \alpha \)4 of subunit a1 acting as a scaffold for packing the luminal loop connecting the transmembrane \( \alpha \) helices of subunit f, the C-terminal sequence of subunit e2, and part of the unknown component (Fig. 3F). These four components are all elongated relative to their yeast counterparts to accommodate their interaction (figs. S9 to S11).

Unlike in the yeast V-ATPase, three rather than two transmembrane \( \alpha \) helices are located within the lumen of the \( c \) ring (Fig. 4A). The centermost \( \alpha \) helix corresponds to the N-terminal \( \alpha \) helix of subunit c\(^{\prime}\), which is also seen in the yeast V-ATPase (8, 9, 29). The other two transmembrane \( \alpha \) helices are from ATP6AP1/Ac45 and ATP6AP2/PRR (Fig. 4A and fig. S5C). ATP6AP1/Ac45 mutations in humans can lead to immunodeficiency, cognitive impairment, liver dysfunction, and abnormal protein glycosylation (45), whereas mutations in ATP6AP2/PRR can result in neurodegeneration as well as X-linked parkinsonism and epilepsy (12). Both proteins are frequently mutated in granular cell cancers (46). Immature ATP6AP1/Ac45 contains two soluble N-terminal domains on the luminal side of the membrane, separated by a furin cleavage site (47) (Fig. 4B, bottom).

In the map, the soluble region of ATP6AP1/Ac45 (fig. 4D) and ATP6AP2/PRR (D). Arrowheads indicate regions of ATP6AP1/Ac45 and ATP6AP2/PRR that contribute to the binding surface for subunit d1. (E) Subunits c\(^{\prime}\), c\(^{\prime}\)a, and ATP6AP2/PRR interact with the second luminal domain of ATP6AP1/Ac45. Scale bar, 10 Å. (F) Subunits c\(^{\prime}\), ATP6AP1/Ac45, ATP6AP2/PRR, and d1 create a network of interactions that connect the vesicle lumen and the cytoplasm. Scale bar, 25 Å.
Ac45 appears as a single low-resolution domain approximately 30 Å by 20 Å by 30 Å in size (Fig. 4C, light blue), which is consistent with only the second luminal domain being in the complex. Further, although mass spectrometry detected peptides from uncleaved ATP6AP1/Ac45, it suggested that the cleaved form is predominantly present (fig. S12, A and C). The C-terminal transmembrane α helix of ATP6AP1/Ac45 is homologous with that of the yeast V-ATPase subunit Voap1 (45) and is found in an equivalent position inside the c ring (9). This transmembrane α helix stretches approximately halfway across the thickness of the lipid bilayer, terminating as a C-terminal tail that interacts with two of the subunits in the c ring and subunit d1 before entering the cytoplasm (Fig. 4C, light blue). ATP6AP2/PRR is known to associate with mammalian V-ATPase (10, 11) and is essential for biogenesis of active V-ATPase (46). The strong density for its transmembrane α helix in the map suggests that every complex includes the subunit (fig. S5C). Consistent with an emerging role for V-ATPases in cell signaling (49), the presence of ATP6AP2/PRR in the complex has tied V-ATPases to the renin-angiotensin system for regulation of blood pressure and electrolyte balance (10, 11, 12), Wnt signaling (14, and other pathways (12). The gene for ATP6AP2/PRR encodes an N-terminal extracellular or luminal soluble domain and a transmembrane anchor (Fig. 4B, top). The soluble domain increases the angiotensin I-generating activity of renin and can function in both a membrane-bound form and, when released by proteolysis, a soluble form (50, 51). In the structure, the renin-activating domain of ATP6AP2/PRR is missing (Fig. 4D, yellow). The transmembrane anchor consists of a long α helix and a short α-helical turn, connected by an extended linker with N- and C-terminal tails. The location of ATP6AP2/PRR’s transmembrane region, captured within the stable c ring, dictates that, after its incorporation into the c ring, the protein must remain associated with the V-ATPase. Mass spectrometry detected some intact ATP6AP2/PRR in the preparation, but, consistent with the cryo-EM density, the cleaved transmembrane region alone was much more abundant (fig. S12, B and D). In contrast to the renin-angiotensin system, Wnt signaling relies on ATP6AP2/PRR remaining membrane-anchored, with interaction of proteins with the extracellular or luminal part of ATP6AP2/PRR leading to signaling in the cytoplasm (14). The absence of the soluble domain of ATP6AP2/PRR from the structure suggests that ATP6AP2/PRR’s function in Wnt signaling involves either a subpopulation of intact ATP6AP2/PRR molecules that are not associated with V-ATPase or a different population of V-ATPase or V0 complex that contain intact ATP6AP2/PRR.

The structure shows numerous interactions between ATP6AP1/Ac45, ATP6AP2/PRR, subunits d1 and c1, and multiple c-subunits (Fig. 4, E and F). Because these proteins are all part of the rotor subcomplex, the interactions persist during rotation and are also found in rotational states 2 and 3 (fig. S13). In the lumen, the soluble domain of ATP6AP1/Ac45 interacts with the N-terminal tail of cleaved ATP6AP2/PRR, the linker that connects subunit c1 to its N-terminal α helix in the middle of the c ring, and the N terminus of subunit c9 (Fig. 4E). Near the cytoplasmic surface of the V0 region, the C-terminal tail of ATP6AP1/Ac45 and the short C-terminal α helix of ATP6AP2/PRR, including d2 of the 19 residues in its intracellular domain (14), are sandwiched between subunits of the c ring and subunit d1 (Fig. 4, C and D, arrow). V0 complexes assemble in the endoplasmic reticulum (ER), with the incorporation of subunit d allowing ER release (52, 53) and subsequent binding of the V1 region (53). Free subunit d in the cytoplasm (54) does not bind the V1 complexes that are preassembled there (55), which suggests that the conformation of subunit d changes upon incorporation into V0, making it competent to interact with subunit D of V1 (Fig. 1D). Indeed, subunit d adopts a more open conformation when it engages subunit D of the central rotor in the intact V-ATPase and a more closed conformation when V1 is detached and subunits d and D are disconnected (fig. S14). The C-terminal tails of ATP6AP1/Ac45 and ATP6AP2/PRR produce part of the surface onto which subunit d1 assembles (Fig. 4, C, D, and F). This structure explains why mutations in ATP6AP1/Ac45 and ATP6AP2/PRR are associated with related disease phenotypes (12, 45, 46). It also explains why ATP6AP1/Ac45 and ATP6AP2/PRR appear to work together to allow subunit d binding, ER release, and subsequent V1 assembly onto V0 in the mammalian V-ATPase (9, 56). ATP6AP1/Ac45 and ATP6AP2/PRR could also be involved in the reverse process, with conformational changes within them altering the conformation of subunit d1, disrupting its interaction with subunit D, and triggering separation of the V1 and V0 regions.

REFERENCES AND NOTES

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proteomics and native mass spectrometry experiments and C.V.R. supervised the mass spectrometry. J.L.R. and Y.M.A. wrote the manuscript and prepared the figures with input from the other authors. **Competing interests:** The authors declare no competing interests. **Data and materials availability:** Cryo-EM maps are deposited in the Electron Microscopy Data Bank under accession numbers EMD-21317 to 21319 for maps of the intact complex and 21345 to 213453 for maps from focused refinement. Atomic models are deposited in the Protein Data Bank under accession numbers 6VQ6, 6VQ7, and 6VQ8 for composite models and 6VQA, 6VQB, 6VQC, 6VQD, 6VQE, 6VQF, 6VQG, and 6VQH for models built into maps from focused refinement. The SidK-3×FLAG expression vector is available from J.L.R.

**SUPPLEMENTARY MATERIALS**

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Materials and Methods

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Structure of V-ATPase from the mammalian brain
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Snapshots of a rotary pump
Vesicular- or vacuolar-type adenosine triphosphatases (V-ATPases) are ATP-hydrolysis–driven proton pumps. In neurons, V-ATPase activity generates a proton gradient across the membrane of synaptic vesicles so that neurotransmitters can be loaded into the vesicles. Abbas et al. developed a method to purify V-ATPase from rat brain and determined the structure of the entire complex by cryo-electron microscopy. Native mass spectrometry showed that the preparation was homogeneous and complemented structural studies by confirming the subunit composition. Three rotational states were resolved at better than 4-angstrom resolution, providing insight into the conformational changes that couple ATP hydrolysis to proton pumping.
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