Citrate is best known as an intermediate in the tricarboxylic acid cycle of the cell. In addition to this essential role in energy metabolism, the tricarboxylate anion also acts asboth a precursor and a regulator of fatty acid synthesis\(^1\). Thus, the rate of fatty acid synthesis correlates directly with the cytosolic concentration of citrate\(^2,3\). Liver cells import citrate through the sodium-dependent citrate transporter NaCT (encoded by \(SLC13A5\)) and, as a consequence, this protein is a potential target for anti-obesity drugs. Here, to understand the structural basis of its inhibition mechanism, we determined cryo-electron microscopy structures of human NaCT in complexes with citrate or a small-molecule inhibitor. These structures reveal how the inhibitor—which binds to the same site as citrate—arrests the transport cycle of NaCT. The NaCT–inhibitor structure also explains why the compound selectively inhibits NaCT over two homologous human dicarboxylate transporters, and suggests ways to further improve the affinity and selectivity. Finally, the NaCT structures provide a framework for understanding how various mutations abolish the transport activity of NaCT in the brain and thereby cause epilepsy associated with mutations in \(SLC13A5\) in newborns (which is known as \(SLC13A5\)-epilepsy\(^6–8\)).

NaCT—along with the low- and high-affinity dicarboxylate transporters, NaDC1 and NaDC3—is a member of the mammalian solute carrier family 13 (\(SLC13\))\(^19,20\). The three human proteins have amino acid identities between 45% and 55% and display distinct expression patterns—NaCT is expressed only in the liver, brain and testis. Whereas all three proteins catalyse sodium-driven carboxylate uptake, NaCT is the major transporter of citrate\(^11,21\), the uptake of which can be enhanced by lithium\(^12\) (Extended Data Fig. 1a, b).

The central role of NaCT in fatty acid biosynthesis makes it a particularly attractive drug target for the treatment of obesity, diabetes and cardiovascular diseases through the reduction of fat storage\(^22,23\). Several new small molecules have been developed as NaCT inhibitors\(^24\) (Extended Data Fig. 1c, d). In particular, the compound PF-06649298 (PF2) exhibits high affinity and selectivity for NaCT, although its apparent inhibitory concentration (IC\(_{50}\)) varies between cell types\(^25,26\). Treating mice with this compound reduced citrate uptake in the liver. The location of the PF2-binding site in NaCT and the nature of the inhibition mechanism, however, are both controversial. Transport activity and inhibition measurements show that PF2—which contains a dicarboxylate moiety—is both a substrate and an inhibitor, entering the cell and exerting inhibition at the substrate site from the cytosolic side\(^25–27\).
suggesting direct competition with substrate. On the other hand, electrophysiological evidence suggests that PF2 is a state-dependent, allosteric inhibitor\(^2\). The structural basis of the selectivity of PF2 remains similarly uncertain. In contrast to related compounds that inhibit all three human di- and tricarboxylate transporters, PF2 is highly selective towards NaCT\(^23,24\) (Extended Data Fig. 1c, d). To resolve this ambiguity in the selectivity and inhibition mechanism of PF2 requires high-resolution structural information on NaCT.

Currently, there is no structure available for mammalian SLC13 proteins. Information on the substrate and Na\(^+\)-binding sites has been inferred from the structures of the bacterial SLC13 homologue from *Vibrio cholerae*, VcIndy\(^27,28\). VcIndy is a dimeric protein in which each protomer consists of a scaffold domain and a transport domain. The transport domain contains two conserved Ser-Asn-Thr (SNT) signature motifs, which both bind to Na\(^+\) and form major parts of the substrate-binding site. Although homology models of human SLC13 proteins based on the VcIndy structures have been used to guide mutagenesis experiments\(^29,30,33\), the low sequence identity of 26–33\% limits the accuracy of such models.

To understand the molecular mechanism of human NaCT, as well as the inhibition mechanism and selectivity of PF2, we determined its structures in complexes with citrate or PF2.

**Structure of NaCT in complex with citrate**

When overexpressed in HEK293 cells, NaCT was able to mediate Na\(^+\)-driven citrate uptake (Extended Data Fig. 2a). We first determined its substrate-binding affinity and oligomeric state in detergent solution. In agreement with their roles as substrates and co-substrates, citrate, succinate and lithium\(^32,33\) all stabilized the solubilized protein in a thermostability screen\(^34,35\) (Fig. 1a and Extended Data Fig. 2b). Purified NaCT binds to citrate in detergent solution, with a dissociation constant (\(K_d\)) of 148 ± 28 μM (Extended Data Fig. 2c–e). Finally, the purified protein ran as a single, sharp peak with a protein mass of 125 ± 2 kDa (Extended Data Fig. 2f), which is in agreement with the expected dimer mass of 126,124 kDa.

We set out to determine the structure of NaCT in complex with citrate and sodium using cryo-electron microscopy (cryo-EM) (Extended Data Fig. 3), with the addition of lithium to increase the stability of NaCT. The NaCT–citrate particles in ice showed a strong preferred orientation (Extended Data Fig. 4a, d), yielding a reconstruction from this anisotropic dataset with a sphericity\(^36\) of around 0.86. To overcome the preferred orientation problem, we collected images from specimens tilted at 0°, 20° and 40° (Extended Data Figs. 3, 4). This strategy allowed us to obtain a map of the NaCT–citrate complex with a nominal resolution of 3.04 Å, enabling direct model building (Fig. 1b, Extended Data Fig. 5a–e and Extended Data Table 1).

**NaCT dimer structure**

The NaCT–citrate structure represents the inward-facing, substrate- and Li\(^+\)/Na\(^+\)-bound (C\(_i\)-Na\(^+\)-S) state (Extended Data Fig. 1a, b). NaCT forms a homodimer, and each protomer consists of a scaffold domain and a transport domain (Fig. 1c, Extended Data Figure 5f, g), in agreement with an elevator mechanism proposed for the NaCT homologue VcIndy\(^27,28\). The scaffold domain is formed by transmembrane (TM) α-helices TMs 1–4 and 7–9, while the transport domain consists of TMs 5, 6, 10 and 11, as well as the helix hairpins HP\(_{in}\) and HP\(_{out}\) (Fig. 1d). Both TMs5 and TM10 are broken in the middle of the membrane, with the halves of these helices connected by loops L5ab and L10ab, respectively. The N-terminal half of the protein, from TM2 to TM6, is related to the C-terminal half (TM6 to TM11) by an inverted repeat pseudo-symmetry\(^37\) (Fig. 1d). Finally, density for one glycan unit is observed connected to C-terminal half (TM6 to TM11) by an inverted repeat pseudo-symmetry\(^37\) (Fig. 1d). Finally, density for one glycan unit is observed connected to Asn562, the only N-glycosylation site in the protein\(^11\). Although the overall fold of NaCT is similar to that of VcIndy\(^27\) – as both proteins have a backbone root mean square deviation of 1.968 Å—the structural details are different (Extended Data Fig. 6c).

The scaffold domain is stabilized by a number of interactions that enable it to cradle the transport domain through the reaction cycle\(^29,38,39\). These include the π–π interactions, hydrogen bonds and salt bridges (Arg102–Asp398 and Lys107–Glu305) (Extended Data Fig. 6f), in addition to numerous van der Waals interactions. Many of these interactions...
rigidify the two horizontal ‘arm’ helixes, H4c and H9c, that connect the transport domain to the scaffold domain. Notably, Arg108 is central to the interaction of H4c, H6b and TM7 (Extended Data Fig. 6f) and, when it is mutated, the transport activity of NaCT is abolished. Between protomers, the interface of the two scaffold domains has an area of 2,291 Å². Such a large interface area, along with the hydrogen bond at Arg76–Asp85′ and π–π interaction between Trp408 and Trp408′ from the neighbouring protomer, further stabilizes the two scaffold domains together into a rigid framework.

As with the scaffold domain, the transport domain of NaCT is stabilized by a number of internal hydrogen bonds and, particularly, π–π interactions. Presumably, this keeps the domain rigid and compact for rigid-body movement during substrate translocation. Additional rigidity comes from prolines in the sequences and, importantly, from interactions between aromatic side chains. Before a bend in TM6 at Pro259, three aromatic side chains, Phe258, Phe254 and Phe250 on one side of that helix pack against Trp330 and Phe331 of the scaffold domain (Extended Data Fig. 6g). On the back side of TM6, Phe256 interacts with Trp547 from TM11. Similarly, Phe500 and Phe502 from L10ab pack against Trp399 of the scaffold domain. These clusters of aromatics stabilize the protein and help to keep hairpins HPa and HPab and intra-membrane loops S5a and S6ab fixed within the transport domain.

In contrast to the extensive networks of interactions within the scaffold and transport domains, the interface between domains is dominated by small, hydrophobic residues, with only two apparent hydrogen bonds (Asn249–Asp334 and Asn249–Arg333). This results in a relatively smooth interface, and presumably a low energetic barrier to transport domain movement during substrate translocation.

Substrate-binding sites

In our NaCT–citrate map, at equivalent positions of the two Na⁺ ions in Vclndy, weak densities for Na⁺ ions are found (Fig. 2). The presence of cations at these positions agrees with the sodium-dependent binding of NaCT to citrate. The relatively weak density at each site is probably owing to their partial occupancy by weakly scattering Li⁺, which has a higher affinity for the Na⁺ sites. Na₁ is coordinated by the backbone oxygens of Ser136, Trp138 and Gly226 and the side chain of Asn141, within the clamshell-like structure formed by S5ab and the tip of HPa (Fig. 2a). Located at the symmetry-related clamshell on the C-terminal side of the transport domain, Na₂ is similarly surrounded by the carboxyls Thr460, Thr463 and Ala507 and the side chains of Thr460 and Asn465 (Fig. 2b). In agreement with the assignment of these Na⁺ sites, the affinity of NaDC3 for Na⁺ was reduced when residues in the equivalent region were mutated, supporting the role of the second SNT motif in Na⁺ coordination. Compared with Vclndy, the Na⁺ coordination in NaCT is different due to both variations in the amino acid sequence and movement of the polypeptide backbone (Extended Data Fig. 6c, e). Although NaCT cotransports one substrate with four Na⁺ ions, locations for the remaining two sodium-binding sites are not apparent in the map.

In a basic pocket between the Na₁ and Na₂ clamshells in the NaCT–citrate map, a density that appears to be citrate is found (Fig. 2c and Extended Data Fig. 6a–c). We propose that the two SNT motifs in Slc13a5 family members (Ser140-Asn141-Thr142 and Ser464-Asn465-Val466 in NaCT) and their neighbouring residues are chiefly responsible for interacting with two carboxylate moieties. Indeed, mutations in the equivalent residues of the two SNT signatures in both the human NaDC3 and the rabbit NaDC1 proteins led to markedly reduced transport activities. Furthermore, we hypothesize that S5ab has a major role in distinguishing various tri- and dicarboxylates (Extended Data Fig. 6c, e). Mutating the equivalents of Thr227, Gly228 and Pro229 in this loop in human NaDC3 reduced its affinities to dicarboxylates but not to citrate. By contrast, the NaCT(G228A) mutant displayed less than 10% of the wild-type citrate transport activity. A mutation at the equivalent position in NaDC3, A234D, causes leukoencephalopathy with
NaCT(I410V) mutants, the IC\textsubscript{50} increased to 300 \(\mu\text{M}\) and 20 \(\mu\text{M}\), respectively to the wild-type (WT) protein is 5 \(\mu\text{M}\), whereas for the NaCT(G409Q) and NaCT(I410V) mutants, the IC\textsubscript{50} increased to 300 \(\mu\text{M}\) and 20 \(\mu\text{M}\), respectively (\(n = 6\)). Data are mean \(\pm\) s.e.m. c Sequence alignment of the human SLC13 transporters. Positions of the two residues in NaCT that interact with the tert-butyl group of PF2 are indicated. The equivalent of Gly409 in NaCT is asparagine in both NaDC3 and NaDC1. d Schematic drawing showing the proposed inhibition mechanism of PF2. The dicarboxylate moiety of PF2 (circle) binds to the citrate site of NaCT in its inward-facing, \(C_i\)-Na\textsuperscript{+} state and blocks sodium release from the \(Na_1\) and \(Na_2\) sites. At the same time, the modified benzene ring of PF2 (triangle) interacts with the scaffold domain, preventing the transition of the transport domain to an outward-facing conformation. Together, the two types of interactions arrest the transporter in its \(C_i\)-Na\textsuperscript{+} state and inhibit transport.

markedly reduced \(\alpha\)-ketoglutarate uptake\cite{44}. Furthermore, a number of missense mutations in SLC13A5 associated with epilepsy\cite{6–8} occur near the citrate-binding site in NaCT, suggesting that these might interfere with substrate binding (Fig. 2d and Extended Data Fig. 6d).

PF2-binding site
To understand the mechanism and selectivity of NaCT inhibitors, we validated the interaction of PF2 and PF-06761281 (PF4a) with NaCT. Notably, although the protein was thermostabilized by both inhibitors, the effect of the highly selective PF2 was much stronger than the less selective and lower affinity PF4a (Fig. 1a). We subsequently determined a cryo-EM structure of the NaCT in complex with PF2 (Extended Data Fig. 7 and Extended Data Table 1). As with the NaCT–citrate complex, the PF2 complex in ice suffered from severe preferred orientation problems. We therefore used the same tilting data-collection strategy, determining a NaCT–PF2 map with a resolution of 3.12 \(\AA\). The protein structure of the NaCT–PF2 complex is essentially identical to that of the NaCT–citrate complex, with an r.m.s.d for all non-hydrogen protein atoms of 0.624 \(\AA\).

In the NaCT–PF2 map, a PF2 molecule occupies the substrate-binding site of each transporter protomer, with its carboxylate moieties located at the citrate site (Fig. 3a, b and Extended Data Fig. 8c–f). Similar structures of the Na1 and Na2 sites of NaCT in both PF2 and citrate structures are observed (Extended Data Fig. 8g), indicating that sodium is required for PF2 binding. The carboxylate-binding residues generally overlap with the binding site of citrate, coordinated by the side chains of Ser\textsubscript{140}, Asn\textsubscript{141}, Thr\textsubscript{142}, Ser\textsubscript{464}, Asn\textsubscript{465} and Thr\textsubscript{508} (Fig. 3d). The middle hydroxyl group interacts with Thr\textsubscript{227} and the backbone nitrogen of Gly\textsubscript{228} (Extended Data Fig. 8e). At the other end of PF2, the tert-butyl group interacts with a cavity on the cytosolic surface of the scaffold domain, lined by Leu\textsubscript{56} from TM3, and Pro\textsubscript{407}, Gly\textsubscript{409} and Ile\textsubscript{410} from the scaffold limits the movement of the transport domain towards the extracellular space (Fig. 4a and Extended Data Fig. 8f). We propose that these interactions of PF2 with the scaffold domain block the sliding movement of the transport domain that is necessary to return the transporter to the outward-facing conformation. Simultaneously, PF2 binding prevents sodium release and stops the \(C_i\)-Na\textsuperscript{+} state transition (Extended Data Fig. 1a, b). Thus, the reaction cycle of the transporter is arrested by PF2 in the \(C_i\)-Na\textsuperscript{+} state (Fig. 4d), resulting in inhibition.

The NaCT–PF2 structure also explains the selectivity of PF2 for NaCT over the two human dicarboxylate transporters, NaDC1 and NaDC3\cite{23–25}. The PF2-interacting residue Gly409 in NaCT is an asparagine at the equivalent position in both NaDC3 and NaDC1 (Fig. 4e). Similarly, the Ala\textsubscript{57} of NaCT is replaced by Ser\textsubscript{60} in NaDC3. At both positions, these larger side chains in NaDC3 and NaDC1 would sterically clash with the tert-butyl group of PF2, thereby reducing PF2 binding and inhibition. In agreement with this model, neither PF4 nor PF4a—either with a smaller or no moiety at the tert-butyl position—show a strong preference for any of the three SLC13 transporters (Extended Data Fig. 1c, d). Our model predicts that mutating Gly409 in NaCT to an asparagine, as found in NaDC1 and NaDC3, should significantly reduce its inhibition by PF2. However, as the NaCT(G409N) mutant retained less than 10% of the wild-type transport activity\cite{24}, we tested this hypothesis with a glycine-to-glutamine mutant instead. In HEK293 cells transfected with the NaCT(G409Q) mutant, the IC\textsubscript{50} of PF2 was found to increase by 60-fold, from 5 \(\mu\text{M}\) to 300 \(\mu\text{M}\) (Fig. 4b), without compromising transport activity (Extended Data Fig. 8h). This observation supports the notion that the interaction of the tert-butyl group of PF2 with this pocket makes the molecule selective towards NaCT.

**Discussion**

The structure of the NaCT–PF2 complex explains the varying selectivity of the inhibitors for NaCT compared with NaDC1 and NaDC3. The difference in selectivity of PF2 and PF4a must arise from their modified ring structures, as their citrate-mimicking moieties are the same (Extended Data Fig. 1c). The tert-butyl group of PF2 sterically prevents it from binding to NaDC1 or NaDC3, explaining the 200-fold difference in IC\textsubscript{50}. Furthermore, the rugged PF2 binding pocket suggests that further modifications on the PF2 structural scaffold may improve the affinity and selectivity of the inhibitor (Fig. 3c). In particular, the inhibitor molecule can be modified at the tert-butyl position to improve both van der Waals and hydrophilic interactions.
Finally, these structures enable us to understand how mutations in SLC13A5 associated with epilepsy hinder the activity of NaCT. For the more than 40 mutations identified, their cellular localization and biochemical analysis has suggested two pathogenesis types: type-I mutations affect the localization and proteolytic susceptibility of the protein; whereas type-II mutations directly abolish transport activity. Now we can further subdivide each type based on their location and apparent biophysical mechanisms (Fig. 2d and Extended Data Fig. 6d). Within the type-I mutations, type-Ia mutations are nonsense or frameshift mutations that result in incomplete protein molecules (Extended Data Table 2). Type-Ib mutations, although producing full-length proteins, are hypothesized to destabilize the transporter. For example, the change in side chain volume caused by the Y82C substitution may prevent dimer formation and therefore hinder transport. Of the type-II mutations, those close to the citrate- and Na+-binding sites (Ila) are expected to alter substrate or cation affinity. Finally, type-Ib mutations are located at the interface between the scaffold and transport domains and probably obstruct the conformational changes necessary for substrate translocation. Although these mutations all abolish citrate uptake—causing SLC13A5-epilepsy by reducing cellular energy supply and altering neurotransmitter synthesis—their pathogenic mechanisms are distinct and therefore each type will require a unique therapeutic strategy.

Online content
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**Article**

**Methods**

**Overexpression**

Baculoviruses bearing the wild-type SLC13A5 gene with an N-terminal decahistidine–TEV tag were prepared using the Bac-to-Bac system and amplified in the Spodoptera frugiperda SF9 cell line. Protein was expressed by infection of the Trichoplusia ni BTI-Tn-SB1-4 cell line at 27 °C for 72 h. In parallel, the overexpression of the SLC13A5 gene with an N-terminal eGFP tag in HEK293 cells was monitored using confocal microscopy.

**Whole-cell transport assay**

HEK-293 cells (ATCC CRL-1573) were seeded in 24-well plates (PerkinElmer, 1450), with around 120,000 cells per well. The following day, the cells were transfected with NaCT in a pNGFP vector, containing an N-terminal GFP tag. Transfection was accomplished using Lipofectamine LTX reagent (Invitrogen) according to manufacturer’s protocol, with 300 ng DNA and 0.66 μl Lipofectamine per well.

Approximately 48 h after transfection, [3H]citrate uptake experiments were carried out based on previously published studies. Cells were washed with a buffer containing 140 mM NaCl, 2 mM KCl, 1 mM MgCl₂, 1 mM CaCl₂ and 10 mM HEPES, with pH adjusted to 7.4 using 1 M Tris solution. The wash buffer was removed and replaced with the same buffer containing PF2 at the indicated concentration for 15 min. This buffer was then removed and replaced with buffer containing the same concentration of PF2 and 500 μM total citrate, including 20 μM [3H]citrate (PerkinElmer, NEC160) for 30 min. For the transport kinetics assay, cells were washed with citrate-free buffer as above, then incubated in buffer containing the indicated total citrate concentration including 50 μM [3H]citrate for 15 min. After incubation, the [3H]citrate buffer was removed and washed three times with 0.6 ml ice-cold buffer containing 140 mM choline chloride, 2 mM KCl, 1 mM MgCl₂, 1 mM CaCl₂, and 10 mM HEPES, with pH adjusted to 7.4 using 1 M Tris solution. After washing the buffer was completely removed, and 0.5 ml of Ultima Gold XR scintillation cocktail (PerkinElmer, 601311) was added and mixed on a shaker for 5 min. Counts were measured using a Wallac 1450 MicroBeta liquid scintillation counter. All steps were carried out at room temperature.

**Thermostability assay**

A thermostability assay was used to search for compounds that stabilized wild-type NaCT. BTI-Tn-SB1-4 cells were infected with a baculovirus containing the SLC13A5 gene with an N-terminal eGFP tag. Cells were collected as previously described and solubilized in a buffer of 50 mM Tris pH 8.0, 200 mM NaCl, 10% glycerol and 1.3% dodecyl-maltoside (DDM). Solubilized lysates were incubated with 100 μM of various compounds at 37 °C for 20 min, and subsequently injected into a Shodex KW803 analytical SEC column on a Waters HPLC and eluted with buffer containing 0.05% DDM at a rate of 0.5 ml min⁻¹. The mass of the NaCT protein was determined using a Wyatt miniDAWN TREOS 3 angle-static light scattering detector, a Wyatt Optilab rEX refractive index detector and a Waters 2489 UV absorbance detector. The differential refractive index (dn/dc) for DDM, 0.128 ml g⁻¹, was calculated using the refractive index detector. The size of the protein–detergent conjugate was deconvoluted following a published method, in which contributions from co-purifying lipids were not distinguished from those of the detergent.

**Amphipol exchange**

After Ni²⁺-NTA column purification, amphipol (PMAL-C8 (Anatrace)) was added to the DDM-purified protein at a 1:5 protein:amphipol weight ratio. The mixture was incubated at 4 °C overnight with gentle agitation. To remove detergent, Bio-Beads were incubated with sample at 4 °C for 2 h at 4 °C with gentle agitation. The Bio-Beads were then removed by centrifugation at 4,500 rpm. Samples were further purified by SEC in buffer containing 25 mM Tris pH 7.5, 100 mM NaCl, 0.1 mM TCEP and either 10 mM LiCl (for NaCT–citrate) or 10 mM LiCl and 100 μM PF2 for NaCT–PF2.

**Cryo-EM specimen preparation and image processing**

All cryo-EM grids were prepared by applying 3 μl of protein at around 0.75 mg ml⁻¹ to a glow-discharged UltrAuFoil R1.2/1.3 300-mesh grid (Quantifoil) and blotted for 2.5–4 s under 100% humidity at 4 °C before plunging into liquid ethane using a Mark IV Vitrobot (FEI).

Micrographs of the NaCT–citrato complex in amphipol were acquired on a Titan Krios microscope with a K2 direct electron detector and an energy filter slit width of 20 eV. The magnification used was 130,000×, with a super-resolution pixel size of 0.524 Å. Leginon was used to target holes with 0 to 100 nm of ice, measured as described previously, at varying specimen tilt angles of 90°, resulting in 1,151,799 particles from all micrographs. Selected particles were combined for multiple rounds of 2D classification. Initial particle picks with a specimen tilt angle of 50° were also excluded due to increased ice thickness and excess particle movement. Initial particle picks from the 20° micrographs were used as templates for picking using Topaz, yielding 1,151,799 particles from all micrographs. Particle stacks were uploaded to cryoSPARC and data from the different specimen tilts were processed separately using two-dimensional classification. Selected particles were combined for multiple rounds of three-dimensional classification (ab initio model generation and heterogeneous three-dimensional refinement with two or more classes.
and C$_2$ symmetry imposed). Finally, a single class of 563,708 particles was selected for nonuniform refinement after signal subtraction and symmetry expansion to C$_2$. The dynamic mask threshold was systematically screened to minimize masking artefacts during local refinement, resulting in the 3.04 Å map. Directional Fourier shell coefficients were calculated using a published script. Higher order CTF aberration correction, with or without grouping by image shift, did not improve the quality of the map. A map was also calculated in cisTEM following the previously published strategy for processing anisotropic data, confirming the overall resolution and substrate densities.

Micrographs of the NaCT–PF2 complex in amphipol were acquired on a Titan Krios microscope with a K2 direct electron detector. The magnification used was 22,500×, with a super-resolution pixel size of 0.537 Å. Legion was used for automated data collection of 1,778,711 and 1,143 micrographs from 0°, 20° and 40° tilted specimens, respectively. Each micrograph was dose-fractioned over 50 frames, with an accumulated dose of 69 e$^-$/Å$^2$. On-the-fly data quality was monitored by running MotionCor$^2$ and CTFFind$^4$ under control of Appion$^5$. Images were acquired with image shifts up to 8 μm, with hardware beam tilt correction enabled in Legion.

For the NaCT–PF2 dataset, Warp was used for frame alignment and CTF estimation, and micrographs with an overall resolution worse than 5.5 Å were excluded from subsequent steps. Initial particle picks from the 20° micrographs were used as templates for replicating using Topaz, yielding 1,044,228 particles from all micrographs. After multiple rounds of three-dimensional classification (ab initio model generation and heterogeneous three-dimensional refinement with two or more classes with C$_2$ symmetry) a single class of 600,946 particles was selected for nonuniform refinement, resulting in a 3.18 Å map. Imposing C$_2$ symmetry did not change the resolution or the appearance of the map, so C$_2$ symmetry was chosen. Local refinement after systematically screening the dynamic mask threshold resulted in the 3.12 Å final map. Higher order CTF aberration correction, with or without grouping by image shift, did not improve the quality of the map. A map was also calculated in cisTEM following the previously published strategy for processing anisotropic data, confirming the overall resolution and PF2 density.

Within the NaCT–citrate complex map, the density for the bound substrate is significantly weaker than the surrounding protein or the corresponding inhibitor density in the NaCT–PF2 complex map. This is consistent with previous observations that negatively charged moieties give weaker density than neutral or positively charged groups of a cryo-EM map. This is proposed to be due to electron radiation damage or the charge dependence of electron scattering. In either case, the triple-negative charge of the citrate would exacerbate these effects.

Model building and refinement

All maps were sharpened using ‘auto-sharpen map’ in Phenix, built in Coot, and refined in Phenix real space refine. The NaCT–citrate map was sharpened, and the model refined, to 3.04 Å. Owing to weak ligand density in the NaCT–citrate map, the Vcxndy structure (PDB: SULD) was used as a reference when orienting citrate and Na+ within the NaCT-binding site. All figures were prepared in Chimera and PyMol.

Reporting summary

Further information on research design is available in the Nature Research Reporting Summary linked to this paper.

Data availability

Electron microscopy densities and protein models have been deposited in the Electron Microscopy Data Bank and Protein Data Bank for the NaCT–citrate (EMD-22457, 7JSK) and NaCT–PF2 (EMD-22456, 7JSJ) complexes. All other data are available from the corresponding authors upon reasonable request. Source data are provided with this paper.

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Author contributions J.S. expressed and purified the protein. J.S., N.K.K. and J.K.H. conducted biochemical studies. D.B.S. froze grids. D.B.S., B.W. and W.J.R. collected and processed the cryo-EM images. D.B.S. built the atomic models. D.B.S. and D.-N.W. analysed the structures. D.B.S., J.K.H., J.A.M., W.J.R. and D.-N.W. wrote the manuscript. All authors participated in the discussion and manuscript editing. D.-N.W. supervised the research.

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Extended Data Fig. 1 | Kinetic cycle of NaCT and molecular structures of its substrates and inhibitors. a, b, Kinetic cycle (a) and schematic model (b) of the SLC13 transport cycle. C_o, outward-facing conformation; C_i, inward-facing conformation; S, substrate. The number of cotransported Na^+ for different SLC13 transporters varies between 3 and 4, but only two are shown here. All available biochemical evidence indicates that sodium ions bind before and release after the substrate. c, Molecular structures of the NaCT substrate, citrate and various inhibitors. d, IC_{50} of inhibitors for NaCT and the dicarboxylate transporters NaDC1 and NaDC3. Whereas PF2 is highly selective towards NaCT, PF4 and PF4a inhibit all three human di/tricarboxylate transporters.
Extended Data Fig. 2 | Purification and functional characterization of human NaCT. 

a, Michaelis–Menten plot showing the citrate dependence of Na\(^+\)-driven radioactive citrate uptake into HEK293 cells that expressed eGFP–NaCT. HEK293 cells transfected with an eGFP vector were used as a control. All data points include six biological replicates from two independent experiments, with error bars indicating the s.d. Inset, representative confocal image of HEK293 cells transfected with the eGFP–NaCT construct, from four biological replicates. Scale bar, 10 μm.

b, Analytical fluorescence SEC of detergent-solubilized cell lysate of Hi5 cells overexpressing an eGFP–NaCT construct. Peak height represents the protein concentration, whereas the peak sharpness indicates the protein homogeneity. The cell lysate was solubilized in DDM detergent, incubated with various compounds at 37 °C, and loaded onto an analytical SEC column on HPLC.

c, Preparative SEC of NaCT following Ni\(^{2+}\)-NTA affinity purification. d, Representative SDS–PAGE of purified NaCT, from twenty biological replicates.

e, NaCT binding to citrate in detergent solution as measured by tryptophan fluorescence quenching. All points include three biological replicates, with error bars indicating the s.d. The K\(_d\) was found to be 148 ± 28 μM.

f, Molecular mass measurements of DDM-purified NaCT using multi-angle light scattering. The measured mass of 125 ± 2 kDa agrees with the molecular weight of a dimeric NaCT of 126.124 kDa calculated from the protein sequence.
Extended Data Fig. 3 | Characterization of the NaCT–citrate cryo-EM specimens and flow chart of image processing.  

**a**, Violin plot showing the distribution of ice thickness in electron micrographs from specimens tilted at 0°, 20°, 40° and 50°. The plot widths correspond to ice thickness distribution. Theoretically, the ice thickness at 20°, 40° and 50° tilts would increase from 0° by 6%, 30% and 56%, respectively. The actual number of electron micrographs with ultra-thin ice (5–20 nm) decreased significantly with the tilt angle.  

**b**, Violin plot showing the distribution of the average horizontal particle displacements from the first five frames of each electron micrograph. The beam-induced particle displacements increased with the tilt angle.  

**c**, Violin plot showing the distribution of micrograph CTF fit resolution of the micrographs. The image quality markedly deteriorated for those recorded from 50° tilted specimens.  

**d**, Flow chart of image processing of the NaCT–citrate images. Only images collected from specimens tilted at 0°, 20° and 40° were included in the processing and the generation of the finals maps.
Extended Data Fig. 4 | Cryo-EM data collection from 0°, 20° and 40° tilted specimens and image processing of the NaCT–citrate complex.

**a,** Orientation distribution of particles from a NaCT–citrate complex reconstruction using only particles from 0° tilt micrographs. At 0° sample tilt most of the particles are top views (viewed along the membrane normal). Side views (viewed from within the membrane plane) are relatively rare. The number of side views and top views differ by three orders of magnitude, indicating a considerable degree of preferred orientation.

**b, c,** Orientation distribution of particles from a NaCT–citrate complex reconstruction using particles from 40° specimen tilt (b) and all micrographs at 0°, 20° and 40° specimen tilts (c). With tilting, the orientation distribution of particles becomes much more isotropic, alleviating the preferred orientation problem.

**d, e,** The 30 most populous classes from two-dimensional classification of particles from the 0° (d) and 40° (e) tilted specimens. The 0° classes are dominated by top views, with few side and oblique views. By contrast, the 40° micrographs include clear side- and oblique-view classes.
Extended Data Fig. 5 | Structure determination of NaCT–citrate complex.

a, Cryo-EM map FSC curve of the NaCT–citrate complex reconstruction using all micrographs. Arrows indicate the nominal map resolution of 3.04 Å, based on a threshold of FSC = 0.143.

b, Directional FSC curves of the NaCT–citrate complex reconstruction. Each purple trace is an individual FSC calculated from a conical wedge of the overall spherical shell, sampled on a 500-point Fibonacci spherical grid. The global FSC curve (the yellow trace), as calculated by averaging all directional FSC curves, also indicates a resolution of 3.04 Å.

c, Mask used for refinement using cryoSPARC.

d, Local resolution of the map.

e, Example cryo-EM densities showing the quality of the chain tracing of the NaCT–citrate model. All of the key helices that are involved in citrate and sodium ion binding are shown. The density for peripheral helix TM1 is poorly resolved, with the helix loosely attached to the rest of the protein.

f, Model of NaCT dimer. The scaffold domain and the transport domain in each protomer is coloured green and pink, respectively.

g, Model of the NaCT protomer as viewed from the cytosol. C2 symmetry was used for the image reconstruction and model refinement. The two protomers are identical, with a root mean square deviation of 0.002 Å.
Extended Data Fig. 6 | Features of the NaCT–citrate structure. a, Cryo-EM density map around the citrate-binding sites. All of the densities are shown at the same contour levels. The density for citrate is coloured red. b, Electrostatic surface of the citrate-binding site. The sodium ions at Na1 and Na2 were included in the calculations. c, Overlay of the NaCT–citrate and VcIndy–succinate (PDB: 5UL7) structures, along with their respective substrates, shown in green and grey, respectively. d, Locations of the SLC13A5-epilepsy missense mutations within the NaCT structure as viewed from the cytosol. e, Sequence alignments of the first SNT motif (left), L5ab–TM5b (centre) and second SNT motif (right) of SLC13 family proteins and bacterial homologues. The second SNT motif in NaCT has a sequence of Ser-Asn-Val. f, Interaction of Lys107 and Arg108 on H4c with other residues on H6b and TM7. g, Aromatic clusters near TM6.
Extended Data Fig. 7 | Cryo-EM data collection from tilted specimens and reconstruction FSC curve of the NaCT–PF2 complex. a, Orientation distribution of particles from a NaCT–PF2 complex reconstruction using only particles from images of 0° tilt specimens. At 0° sample tilt most of the particles are top views, whereas side views are relatively rare. The number of side views and top views differ by up to three orders of magnitude, indicating a considerable degree of preferred orientation. b, c, Orientation distribution of particles from a NaCT–PF2 complex reconstruction using particles from 40° tilt (b) and all micrographs collected at 0°, 20° and 40° tilts (c). With tilting, the particle views become much more isotropic, alleviating the preferred orientation problem. d, Cryo-EM map FSC curve of the NaCT–PF2 complex reconstruction using all micrographs. e, Cryo-EM map of the NaCT–PF2 complex with a resolution of 3.12 Å. f, Local resolution of the map. g, Example cryo-EM densities showing the quality of the chain tracing of the NaCT–PF2 model. All of the key helices that are involved in PF2 and sodium ion binding are shown.
Extended Data Fig. 8 | Map and structural model of the NaCT–PF2 complex.

a, b, Structure of the NaCT–PF2 complex as viewed from the membrane plane (a) and the cytosol (b). c, d, Cryo-EM density map around the PF2-binding sites. All of the densities are shown at the same contour levels. The density for PF2 is coloured red.

e, f, PF2-binding site as viewed from within the transport domain. g, Packing of the scaffold domain side chains around PF2. The scaffold and transport domains are coloured green and pink, respectively. Residues Leu56, Ala57, Gly409 and Ile410 are shown as spheres.

h, Overlay of the NaCT–citrate and NaCT–PF2 structures in green and blue, respectively. The loops enclosing Na1 and Na2 sodium-binding sites move by around 1 Å, more tightly enclosing both sites in the NaCT–PF2 complex. h, Na+-driven citrate uptake into HEK293 cells transfected with various eGFP-tagged NaCT mutants. Each data point includes three biological replicates, with error bars indicating the s.d. NaCT(G409Q) and NaCT(I410V) mutants retained wild-type level activity and were used to measure inhibition by PF2 in Fig. 4b.
## Extended Data Table 1 | Cryo-EM data collection and structure determination of NaCT

|                  | NaCT-Citrate | NaCT-PF2 |
|------------------|--------------|----------|
| **EMDB ID**      | EMD-22457    | EMD-22456|
| **PDB ID**       | 7JSK         | 7JSJ     |
| Microscope       | Krios-NYU    | Krios-NYSBC |
| Magnification    | 130,000      | 22,500   |
| Voltage (kV)     | 300          | 300      |
| Movies           | 4,240        | 3,632    |
| Electron dose (e-/Å²) | 57.71      | 69.42    |
| Defocus range (µm) | 1.2 – 1.7   | 1.3 – 2.3|
| Collection mode  | Super-resolution | Super-resolution |
| Effective pixel size (Å) | 0.518      | 0.537    |

### Data processing

|                                      | NaCT-Citrate | NaCT-PF2 |
|--------------------------------------|--------------|----------|
| Initial number of particles          | 1,151,799    | 1,044,228|
| Final number of particles            | 563,708      | 600,946  |
| Symmetry imposed                     | C1           | C1       |
| B-factor sharpening (Å²)              | 96.62        | 50.86    |
| Map resolution* (Å)                  | 3.04         | 3.12     |

### Model refinement

|                                      | NaCT-Citrate | NaCT-PF2 |
|--------------------------------------|--------------|----------|
| Non-hydrogen atoms                   | 7372         | 7388     |
| Protein residues                     | 936          | 936      |
| Ligands                              | 8            | 8        |
| Mean B factor                         |              |          |
| Protein (Å²)                         | 65.83        | 26.08    |
| Ligands (Å²)                         | 74.59        | 28.65    |
| RMS deviations                       |              |          |
| Bond lengths (Å)                     | 0.011        | 0.008    |
| Bond angles (°)                      | 1.133        | 1.075    |
| Molprobity score                     | 2.41         | 2.67     |
| Clash score                          | 6.57         | 9.15     |
| Poor rotamers (%)                    | 4.74         | 7.23     |
| Ramachandran plot                    |              |          |
| Favored (%)                          | 90.61        | 90.61    |
| Allowed (%)                          | 9.17         | 9.17     |
| Outliers (%)                         | 0.22         | 0.22     |
| Model resolution (Å)                 | 3.5          | 3.3      |

*Resolution determined using a gold-standard FSC threshold of 0.143.
## Extended Data Table 2 | Classification of SLC13A5-epilepsy mutations

| Type | Mutations | Proposed effect |
|------|-----------|-----------------|
| Ia   | E160*, E171fs, R333*, W341*, I367fs, P407fs, G484fs | Early termination and frame shift |
| Ib   | P68Q, Y82C, G130D, T145K†, G423E†, P487L, L488P, L492P | Folding or dimerization defect |
| IIa  | T142M, G219R†, T227M, P505L | Substrate- and Na⁺-binding defect |
| IIb  | C50R, H106R, L111R†, G417E, S427L, D524H | Conformational change defect |

*Early termination.
†Mutations that can be classified as more than one type.
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  - A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
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  - Only common tests should be described solely by name; describe more complex techniques in the Methods section.
  - A description of all covariates tested
  - A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
  - A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
  - For null hypothesis testing, the test statistic (e.g. F, t, r) with confidence intervals, effect sizes, degrees of freedom and P value noted
    - Give P values as exact values whenever suitable.
  - For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
  - For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
  - Estimates of effect sizes (e.g. Cohen's d, Pearson's r), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

| Data collection | Data analysis |
|-----------------|---------------|
| Leginon (version 3.5) | CryoSparc (version 2.15), Phenix (version 1.18), COOT (version 0.8.9.1), UCSF Chimera (version 1.13.1), PyMOL (version 2.3.0) |

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The cryo-EM density maps and corresponding coordinates have been deposited to the Electron Microscopy Data Bank (EMDB) and the Protein Data Bank (PDB), respectively. The accession numbers are listed as follows: NaCT-Citrate (EMD-22457, 7JSK) and NaCT-PF2 (EMD-22456, 7JSJ). All other data are available from the authors upon reasonable request.
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Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

| Sample size | The transport and inhibition study samples sizes of N=3 and N=6 for each time and concentration point, respectively, were chosen to ensure reproducibility based on prior experimental experience. |
| Data exclusions | No data were excluded from the analysis. |
| Replication | Inhibition assay of each mutant were performed using three technical replicates in each of two separate transfection on separate days (biological replicates). Transport assays were preformed using three biological replicates. All replicates were successful. |
| Randomization | For cryo-EM studies, particles were randomly assigned to half-maps for resolution determination. No randomization was not needed for transport or inhibition assays. |
| Blinding | Blinding was not used for structural or transport experiments. The results are not subjective or prone to being biased by individual investigators, therefore blinding is not necessary. |

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