Crystal structure of the plant dual-affinity nitrate transporter NRT1.1

Ji Sun1, John R. Bankston2, Jian Payandeh1, Thomas R. Hinds1, William N. Zagotta3 & Ning Zheng1,3

Nitrate is a primary nutrient for plant growth, but its levels in soil can fluctuate by several orders of magnitude. Previous studies have identified Arabidopsis NRT1.1 as a dual-affinity nitrate transporter that can take up nitrate over a wide range of concentrations. The mode of action of NRT1.1 is controlled by phosphorylation of a key residue, Thr 101; however, the post-translational modification switches the transporter between two affinity states remains unclear. Here we report the crystal structure of unphosphorylated NRT1.1, which reveals an unexpected homodimer in the inward-facing conformation. In this low-affinity state, the Thr 101 phosphorylation site is embedded in a pocket immediately adjacent to the dimer interface, linking the phosphorylation status of the transporter to its oligomeric state. Using a cell-based fluorescence resonance energy transfer assay, we show that functional NRT1.1 dimerizes in the cell membrane and that the phosphomimetic mutation of Thr 101 converts the protein into a monophasic high-affinity transporter by structurally decoupling the dimer. Together with analyses of the substrate transport tunnel, our results establish a phosphorylation-controlled dimerization switch that allows NRT1.1 to uptake nitrate with two distinct affinity modes.

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By comparing the structures of NRT1.1 and a bacterial peptide transporter from *Streptococcus thermophilus*, PepTSt, we confirm that eukaryotic and prokaryotic members of the NRT1/PTR family of MFS transporters share a similar overall architecture (Extended Data Fig. 4). The plant nitrate transporter, nonetheless, has three unique and conserved structural elements, including a well-structured N-terminal cytoplasmic segment, a disulphide bond-stabilized extracellular loop, and a partially ordered central linker sequence (Fig. 1b). Consistent with the sequence divergence between the NRT1/PTR and NRT2/NNP nitrate transporter families, the structures of NRT1.1 and two bacterial NRT2/NNP family members, NarK (ref. 24) and NarU (ref. 26), share few common features except for the MFS fold (Extended Data Fig. 4).

The residue mutated (P492L) in the *chil*-9 mutant, which lost the transporter but not the sensor function of NRT1.1 (ref. 17), is located at the short TMH10–TMH11 loop (Fig. 1b). Its mutation probably affects the structural coordination of the two helices.

**NRT1.1 dimer in the crystal**

So far, crystal structures of more than ten MFS transporters have been determined in the monomeric form. The DDM-solubilized NRT1.1 protein was also isolated in a monomeric state as determined by size-exclusion chromatography-coupled multi-angle light scattering measurements (Extended Data Fig. 5a). A closer examination of the two NRT1.1 molecules in the asymmetric unit, however, reveals a possible biological dimer.

In the crystal, the two adjacent non-crystallographically related NRT1.1 molecules are juxtaposed in a side-to-side fashion with their N-terminal halves facing and interacting with each other (Figs 1a and 2a, b). The intermolecular packing is predominantly mediated by TMH3 and TMH6, which are located at a peripheral edge of the canonical MFS fold. Although crystal contacts may not always reflect biological interactions, two prominent features of the crystallographic dimer arrangement support its physiological relevance. First, the overall topology of the putative NRT1.1 dimer is perfectly compatible with its transporter function at the membrane (Fig. 2a, b). Second, the interface between the two NRT1.1 molecules is extensive and complementary with a total surface area of ~2,160 Å² (Fig. 2d–f and Extended Data Fig. 6). Overall, the two inward-facing NRT1.1 molecules give rise to a putative ‘in-phase’ dimer assembly, which is about 90 Å wide and 50 Å thick (Fig. 2b). When viewed from the side, the two substrate-transporting tunnels are not in parallel with the central two-fold axis but slant at an ~15° angle in opposite directions (Fig. 2c).

**Functional dimerization of NRT1.1**

To dissect the biological relevance of the NRT1.1 dimer observed in the crystal, we first used a crosslinking experiment to assess the potential of detergent-solubilized NRT1.1 to dimerize in solution. Despite its low efficiency, an amine reactive crosslinker was able to crosslink NRT1.1 in a concentration-dependent manner (Fig. 3a). The crosslinked products migrated on SDS–polyacrylamide gel electrophoresis (PAGE) with a size corresponding to a NRT1.1 dimer, indicating that DDM-solubilized NRT1.1 is capable of forming a transient dimer in a membrane-free environment.

Because solubilization by DDM might interfere with NRT1.1 dimer formation, we next performed fluorescence resonance energy transfer (FRET) spectroscopy experiments with the nitrate transporter expressed in the membrane of *Xenopus* oocytes, which allowed us to examine the oligomeric state of NRT1.1 in the same lipid environment where its dual-affinity transporter activity has been measured. We separately fused the N terminus of NRT1.1 with either the mGerulian variant of cyan fluorescent protein (mCFP) or the mCitrine variant of yellow fluorescent protein (mYFP), which constitute a FRET pair with an $R_0$ of ~50 Å for 50% energy transfer efficiency (Fig. 3b). In the structure...
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Fig. 7b). This result strongly suggests that the plant dual-affinity nitrate transporter 1 (Extended Data Fig. 1). In the structure of the putative NRT1.1 dimer, the N-terminal ends of the two NRT1.1 molecules are about 40 Å away from each other (Extended Data Fig. 7a). Therefore, FRET is expected to occur if NRT1.1 dimerizes in the membrane in the same fashion as seen in the crystal structure. As shown in Fig. 3c, a strong FRET signal measured by a spectrum-based approach was detected between the co-expressed mCFP–NRT1.1 and mYFP–NRT1.1 fusion proteins, but not in the negative control (Extended Data Fig. 7b). This result strongly suggests that the plant dual-affinity nitrate transporter can form a homodimer not only in the crystal but also in the cellular membrane.

Thr 101 phosphorylation as a dimerization switch

The phosphorylation site residue Thr 101 is strictly conserved among plant NRT1.1 orthologues and represents one of the hallmarks of the dual-affinity nitrate transporter (Extended Data Fig. 1). In the NRT1.1 structure, Thr 101 is located at the N-terminal end of TMH3 and is entirely buried in a hydrophobic pocket formed among TMH2, TMH3 and TMH4 (Fig. 3d, e). Notably, this pocket is directly adjacent to the dimer interface with one of its walls demarcated by three hydrophobic interface residues: Leu 96, Leu 100 and Ile 104 (Figs 2d and 3e). Together, these results not only confirm the functional relevance of the NRT1.1 dimer, but also indicate a dimerization-based switching mechanism for the dual-affinity nitrate transporter—unmodified NRT1.1 forms a structurally coupled homodimer and functions as a low-affinity transporter, whereas phosphorylated NRT1.1 undergoes dimer decoupling and adopts a high-affinity state.

Substrate binding site and proton coupling

In both protomers of the refined NRT1.1 dimer structure, an island of strong electron density is present in the middle of the transport tunnel from a to c map calculated before the nitrate was modelled in. THMs are numbered (orange). E476 is a side chain of Thr 101 and the transporter tunnel with the clustered EXXER motif and K164. c Nitrate uptake activities of the H356A mutant relative to wild-type NRT1.1 in the presence of 10 mM or 0.1 mM nitrate, y axis is the percentage of nitrate uptake compared to wild type. All results are the mean ± s.d. of one experiment in quintuplicates or sextuplicates. d, Sequence alignment of eight NRT1 family members from Arabidopsis thaliana in regions surrounding H356 and F511 of NRT1.1.

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![Figure 3](image3.png)

**Figure 3** | NRT1.1 dimerization controlled by Thr 101 phosphorylation. a, Crosslinking of NRT1.1 with increasing concentrations of ethylene glycol bis-succinimidylsuccinate (EGS). b, The design of FRET assay. Dashed lines indicate the 11-residue-long linkers between the fluorescence proteins and the structurally resolved NRT1.1 N terminus. c, FRET measurements of wild-type (WT) and mutant NRT1.1. The mCFP–HCN–mYFP–NRT1.1 pair was used as negative control. Consistent with the loss of FRET signal, the T101A mutant failed to be crosslinked in solution (Extended Data Fig. 5b). d, A close-up view of Thr 101 at the NRT1.1 dimer interface. e, Thr 101-interacting residues with their side chains shown as sticks.

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![Figure 4](image4.png)

**Figure 4** | Substrate binding and energy coupling in NRT1.1. a, Intracellular view of the nitrate-binding pocket. Nitrate is shown as a stick representation together with electron density contoured at 4σ from a F̅ 2ρ map calculated before the nitrate was modelled in. THMs are numbered (orange). E476 is a His 356-interacting residue, the mutation of which abolishes the transporter function of NRT1.1 (Extended Data Fig. 3b). b, Side view of the putative nitrate-binding site and the transporter tunnel with the clustered EXXER motif and K164. c, Nitrate uptake activities of the H356A mutant relative to wild-type NRT1.1 in the presence of 10 mM or 0.1 mM nitrate. y axis is the percentage of nitrate uptake compared to wild type. All results are the mean ± s.d. of one experiment in quintuplicates or sextuplicates. d, Sequence alignment of eight NRT1 family members from Arabidopsis thaliana in regions surrounding H356 and F511 of NRT1.1.
omitted from the cryo-protection buffer, this electron density completely disappeared from both NRT1.1 molecules (Extended Data Fig. 8). The position of the nitrate density is slightly shifted between the two NRT1.1 molecules, which might reflect the mode of substrate release (Extended Data Fig. 8).

Distinct from the nitrate/nitrite binding sites of NarK and NarU, which coordinate the substrate(s) with two opposing conserved Arg residues\(^{24,25}\), the nitrate-binding pocket in NRT1.1 is predominantly formed by hydrophobic residues, including Leu 49, Val 53, Leu 78 and Phe 511 (Fig. 4a, b). His 356 on TMH7 is the only polar residue that is in close contact with nitrate, the precise binding mode of which cannot be resolved due to the resolution limit of the structure. On the basis of its close proximity to the nitrate density and the crystallization condition (pH = 4.5), His 356 probably stabilizes the substrate in the pocket through a charge–charge interaction. Its side-chain conformation, meanwhile, is supported by two nearby residues: Tyr 388 and Glu 476. Although Tyr 388 and two other polar residues, Thr 360 and Thr 48, are also around the substrate, their hydroxyl groups do not seem to be at the optimal hydrogen bond distance (Fig. 4a).

To validate the substrate-binding site, we mutated His 356 and compared the nitrate uptake activities of the wild-type and mutant transporters\(^{41}\). In support of a critical role of His 356 in substrate transport, its mutation to alanine completely abolished the transport activity of NRT1.1 at both high and low nitrate concentration (Fig. 4c). Notably, His 356 is not conserved among plant NRT1.1 orthologues and Arabidopsis NRT1 family members, which harbour either a tyrosine or a hydrophobic amino acid (Leu, Met or Phe) at the equivalent position (Fig. 4d). This key residue, nevertheless, has closely co-evolved with the adjacent residue, Phe 511. Among all NRT1.1 orthologues and paralogues, a combination of a polar and a hydrophobic side chain has been generally maintained between the two residues, indicating that one of them is responsible for specific nitrate binding (Fig. 4d and Extended Data Figs 1 and 2). NRT1.1 is unique among all Arabidopsis NRT1 family members by featuring a histidine at the nitrate-binding pocket. This charged residue provides a plausible explanation for the high-affinity nitrate uptake activity acquired by NRT1.1, which is otherwise a member of LAT5. Furthermore, the replacement of the histidine residue by tyrosine in some of the plant NRT1.1 orthologues raises a question about their dual affinity transporter function (Extended Data Fig. 1).

In the PepTSt\(^3\) structure, a conserved motif, EXXERFXYY, on TMH1 has been identified to have an important role in proton coupling. Part of this motif, EXXER, is also found in all plant NRT1.1 orthologues (Extended Data Fig. 1). Together with the conserved residue Lys 164, this motif presents a cluster of interacting residues under the nitrate-binding pocket and facing towards the transport tunnel (Fig. 4b). Consistent with a key function in the symport cycle, alanine mutation of each of the four residues abrogated the transporter activity of NRT1.1 in the oocyte-based nitrate uptake assay (Extended Data Fig. 3b). Surprisingly, these four residues have been simultaneously evolved into non-charged residues in two Arabidopsis NRT1 family members: AtNRT1.5 and AtNRT1.8 (Extended Data Fig. 2). Their documented pH-dependent nitrate transporter activities necessitate an alternative proton-coupling mechanism.

**Cytoplasmic structural elements**

NRT1.1 has an ~30-amino-acid-long N-terminal cytoplasmic segment, which is highly conserved among its plant orthologues (Extended Data Figs 1 and 2). In the crystal, this sequence adopts a well-ordered loop structure and forms a pronounced cleft between the NRT1.1 dimer (Extended Data Fig. 9). With several strictly conserved residues exposed to the solvent, this cleft presents a putative two-fold symmetric protein–protein interaction site with a potential role in recruiting kinases and phosphatases. Although the central linker sequence is mostly disordered in the crystal, its N-terminal region forms a stable amphipathic \(\alpha\)-helix (Figs 1b and 2a), providing yet another potential protein-docking site.

**Figure 5 | A dimerization switch model.** The non-phosphorylated and structurally coupled NRT1.1 dimer functions as an ‘in-phase’ homodimeric low-affinity nitrate transporter (right). Once phosphorylated, the NRT1.1 dimer is decoupled, and each molecule functions as an independent high-affinity nitrate transporter (left). Different shapes of the putative substrate-binding site at the central transport tunnel reflect its differential nitrate-binding properties.

**Discussion**

The crystal structure of NRT1.1 reveals a biologically relevant dimer, the dynamic coupling and decoupling of which is controlled by the phosphorylation of a single residue near the dimer interface. Because the same post-translational modification switches the mode of action of the dual-affinity transporter, we propose that dimer assembly and disassembly enables NRT1.1 to toggle between the low-affinity and high-affinity states with an overlapping, if not the same, substrate-binding site. In this model (Fig. 5), structural engagement of two protomers at the interface allosterically regulates the affinity of substrate binding at the central transport tunnel. Whether dimer decoupling itself is sufficient to shift NRT1.1 into the high-affinity mode awaits future analysis.

Previous structural studies of several MFS members have established a ‘rocker-switch’ mechanism of substrate transport, in which the transporters cycle through outward-facing, occluded, and inward-facing conformations\(^{22,25}\). The structure of the dimeric unmodified NRT1.1 reveals a buried Thr 101 phosphorylation site in the inward-facing conformation. Thr 101 phosphorylation, therefore, probably occurs when the dimeric transporter adopts either the outward-facing or occluded conformation. It is equally possible that the unmodified dimeric transporter is in equilibrium with the monomeric form, which is susceptible to phosphorylation.

Despite the current structure, questions remain as to how NRT1.1 senses nitrate and transduces the signal. Our structure reveals only one nitrate-binding site within the substrate transport tunnel. However, there might be an additional nitrate-binding site responsible for signalling, which is excluded from the inward-facing conformation. Previous studies have suggested that dephosphorylation of Thr 101 is required for the low-affinity sensor function of NRT1.1 (ref. 17). It is possible that dimerization mediates this signalling function of the transporter in the same manner as found in many common cell-surface receptors. The high-affinity sensor function of NRT1.1, on the other hand, might involve an entirely different mechanism.

Transporter oligomerization and phosphorylation have been implicated in the proper functions of a number of MFS members, such as LacS (ref. 34), GLUT1 (ref. 35), TetL (ref. 36) and hRFC (ref. 37). The crystal structure of the NRT1.1 dimer not only establishes a structural framework for understanding its dual-affinity nitrate transporter and receptor activities, but also reveals how protein oligomerization and post-translational modification can synergistically expand the functional capacity of an MFS transporter.

**METHODS SUMMARY**

Detailed descriptions of the following experimental procedures can be found in the Methods: protein expression and purification; protein crystallization, data collection and structure determination; nitrate transporter assay in oocytes; FRET assay and data analysis; and details for crosslinking experiment.
Online Content Any additional Methods, Extended Data display items and Source Data are available in the online version of the paper; references unique to these sections appear only in the online paper.

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Author Contributions J.S. and N.Z. conceived and J.S. conducted the protein purification and crystallization experiments. J.P. provided experimental suggestions. J.S. and N.Z. determined and analysed the structures. J.S., T.R.H. and N.Z. conceived and J.S. and T.R.H. conducted SEC-LS-RF-UV experiments. J.S., J.R.B., W.N.Z. and N.Z. conceived and J.S. and J.R.B. conducted FRET experiments. J.S. and N.Z. conceived and J.S. and T.R.H. conducted SEC-LS-RI-UV experiments. J.S. and N.Z. wrote the manuscript with inputs from all authors.

Author Information Structural coordinates and structural factors are deposited in the Protein Data Bank under accession number 4OH3. 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 N.Z. (nzeng@uwashington.edu).
METHODS

Protein expression and purification. The full-length *Arabidopsis thaliana* NRT1.1 was cloned into pFastBac vector with a C-terminal 8×His tag. Recombinant baculovirus was generated using the Bac-to-Bac system (Invitrogen). Monolayer High Five insect cells were infected for protein expression. Cells were collected 72 h after infection, washed and lysed with a buffer containing 20 mM Tris-HCl, pH 8.0, 200 mM NaCl, 10 mM NaNO₃ (buffer A). NRT1.1 was subsequently solubilized by DDM (Anatrace) at a final concentration of 1.5% with 1–2 h incubation at 4°C. Solubilized NRT1.1 was separated from the insoluble fraction by high-speed ultracentrifugation and applied to a Ni-NTA gravity column (GE Healthcare). The bound NRT1.1 protein was then washed and eluted in the presence of 0.02% DDM with buffer A supplemented with 20 mM and 200 mM imidazole, respectively. The 8×His tag was cleaved by thrombin overnight and the NRT1.1 protein was further purified by size-exclusion chromatography with a Superdex 200 column (GE Healthcare) in buffer A supplemented with 0.02% DDM. The peak fractions containing NRT1.1 were pooled and the protein was concentrated to 10 mg ml⁻¹ using a 50-kDa MWCO centrifugal device (Ambion).

Crystallization, data collection and structure determination. The NRT1.1 crystals were grown at 4°C by the hanging-drop vapour diffusion method, using 2 μl protein sample mixed with 1 μl reservoir solution containing 100 mM sodium acetate, pH 4.5, 30% PEG3000 and 3% MPD. Crystals of maximal sizes were obtained after 1 month. 7.5% ethylene glycol was added during crystal harvest and data collection as cryo-protectant. The heavy-atom derivative crystals were obtained by soaking in the presence of 1 mM ethyl mercury thiosalicylate (EMTS) for 2 h. All data sets were collected at the BL8.2.1 and BL8.2.2 beamlines at the Advanced Light Source. The single anomalous dispersion (SAD) data set was collected near the mercury absorption edge (μ = 1.008 Å). X-ray diffraction data were integrated and scaled with the HKL2000 package. Improved molecular replacement by Rosetta combined with SAD was used to determine the initial phase using PHENIX with a 3.5 Å mercury derivative data set. Initial structural models were built, refined and rebuilt using COOT and PHENIX. The final model was built and refined with a native data set of 3.25 Å resolution. All the structure model figures in the paper were prepared using PyMol.

Transporter assay. The transporter assays in oocytes were carried out as previously described. Briefly, genes were constructed into pGEMHE vector and kit as in the transporter assay and injected into oocytes. For NRT1.1 mutants, the background signal from the oocytes injected with water was subtracted from the final uptake data, which were subsequently normalized to the wild-type protein. Every data point in the experiments was measured and averaged on more than five oocytes.

FRET assay. The mCerulean variant of cyan fluorescent protein and mCitrine variant of yellow fluorescent protein were individually fused to the N terminus of NRT1.1 with a three-alanine linker. Then CRNAs were prepared using the same vector and kit as in the transporter assay and injected into oocytes. The FRET signal from the surface membrane was measured after incubation for 3 days using the Zeiss LS-710 confocal microscope as previously described. Briefly, emission spectra of mCerulean and mCitrine were collected using laser excitation at 458 and 488 nm, respectively, and an emission window of 3.2 nm. FRET was calculated using a spectrum-based approach to remove contaminations caused by donor emission and direct excitation of the acceptor fluorophore. mCerulean spectra were collected from oocytes expressing mCerulean-tagged NRT1.1 transporters alone and then subtracted from spectra taken from oocytes expressing both mCerulean- and mCitrine-tagged transporters. The resulting extracted mCitrine emission spectrum, F₄₅₈₆ₓ, contains two components: one caused by direct excitation, F₄₅₈₆₄₅₈, and the other by FRET, F₄₅₈₆₅₄₈₅₈. F₄₅₈₆₅₄₈₅₈ was normalized to total mCitrine emission excited directly with 488 nm light (F₄₈₈₅₈). The resulting ratio, termed ratioA, can be expressed as ratio A = F₄₅₈₆₄₅₈/F₄₈₈₅₈ = F₄₅₈₆₄₅₈/F₄₅₈₆₅₄₈₅₈ + F₄₅₈₅₄₈₂₅₄₈. The direct excitation component, F₄₅₈₆₄₅₈/F₄₅₈₆₅₄₈₅₈, termed ratioA₀, was experimentally determined with oocytes expressing mCitrine-tagged transporters only. The ratio between ratioA and ratioA₀ (FR), directly proportional to FRET efficiency, was calculated as follows: FR = ratioA/ratioA₀ = 1 + F₄₅₈₆₅₄₈₅₈/F₄₅₈₆₄₅₈. Images were analysed in ImageJ (National Institute of Health) by drawing regions of interest around the fluorescent membranes and using the measure stack feature. The data were then analysed using programs written in MATLAB (MathWorks).

Crosslinking experiment. Purified NRT1.1 protein was dialysed in PBS buffer, pH 7.4, supplemented with 0.01% DDM for 24 h with one buffer exchange. The protein was then concentrated to 5 mg ml⁻¹, and 2 μl ethylene glycol bis-succinimidylsulfate (EGS) (Pierce) was added in 18 μl protein sample at increasing concentrations (0.1 mM, 0.3 mM, 1 mM, 3 mM and 10 mM). The reaction was carried out at room temperature for 30 min and stopped by a 50 mM Tris-HCl buffer, pH 8.0. One-fifth of the reaction solution was analysed by SDS–PAGE.

SEC-LS-RI-UV measurement. Size-exclusion chromatography coupled with light scattering, refractive index, and ultraviolet absorption (SEC-LS-RI-UV) was done under the SEC-MAL system, which consisted of a P900 HPLC pump (GE), a UV-2077 detector (Jasco), a Tri Star Mini Dawn light scattering instrument (Wyatt), and an Opti Lab T-Rex refractive index instrument (Wyatt). 20 μl of purified and DDM-solubilized NRT1.1 (5 mg ml⁻¹) was injected into a Superdex 200 (10/300GL) gel filtration column and eluted isocratically at 0.5 ml min⁻¹ in a buffer containing 20 mM Tris, 200 mM NaCl, 5 mM NaN₃, 0.02% DDM, pH 8.0. The extinction coefficient of NRT1.1 at 280 nm was calculated from the amino acid sequence (E = 1.268 ml g⁻¹ cm⁻¹). DDM has no absorbance at 280 nm. The specific refractive index of NRT1.1 and DDM was assumed to be 0.186 ml g⁻¹ and 0.147 ml g⁻¹, respectively. Data collection and analysis was performed with Astra 6 software (Wyatt). Total molecular mass and individual masses of the protein and the detergent were determined with Astrasoft software using protein conjugate analysis.

Both peak overlap and peak broadening were corrected with Astra 6 software. The SEC-MAL system was pre-calibrated with BSA.

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Extended Data Figure 1 | Sequence alignment of plant NRT1.1 orthologues. Alignment and secondary structure assignments of NRT1.1 orthologues from Arabidopsis thaliana (At), Brassica napus (Bn), Oryza sativa (Os), Sorghum bicolor (Sb), Populus trichocarpa (Pt), Vitis vinifera (Vv) and Zea mays (Zm). Strictly conserved residues are coloured in blue. Green dots indicate the EXXER motif. Orange empty squares indicate dimer interface residues. Red triangles indicate residues in the substrate-binding pocket. The red dot indicates the energy-coupling residue. Dashed lines represent the disordered region in the crystal structure.
Extended Data Figure 2 | Sequence alignment of Arabidopsis NRT1 family members. Alignment and secondary structure assignments of the NRT1 family members from Arabidopsis thaliana. Strictly conserved residues are coloured in blue. The two residues potentially important for substrate binding are indicated by a red triangle with green stroke. The energy coupling residue is indicated by a red dot. Dashed lines represent the disordered region in the crystal structure.
Extended Data Figure 3 | Nitrate uptake and electron density map of NRT1.1. a, Measurement of nitrate uptake by NRT1.1 was carried out in *Xenopus* oocytes in the presence of increasing concentrations of nitrate. A Q-test was used to identify statistical outliers in data. All data points are mean ± s.d. of one experiment in quintuplicates or sextuplicates. The data were fit with a two site nonlinear binding curve using Prism. b, Relative nitrate uptake activities of NRT1.1 mutants to the wild-type protein measured in the *Xenopus* oocyte-based assay in the presence of 10 mM nitrate. All results are the mean ± s.d. of one experiment in quintuplicates or sextuplicates. c, The overall 2Fo–Fc map of the NRT1.1 dimer contoured at 1.5σ. d, e, Two representative helices and their 2Fo–Fc maps contoured at 1.5σ. f, An island of density from the 2Fo–Fc map contoured at 1.5σ is assigned to the head group of DDM bound between TMH5 and TMH8.
Extended Data Figure 4 | Structural comparison of NRT1.1 and other MSF transporters. 

**a**, Cutaway views of PepTst, GlpT and LacY showing their shared inward conformation as observed for NRT1.1. 

**b**, Overall structural comparison of NRT1.1 and PepTst with their N-terminal and C-terminal domains (NTD and CTD) coloured in pale green and cyan, respectively. Superposition of their NTDs and CTDs are shown separately. 

**c**, Comparisons between NRT1.1 and two bacterial NRT2 family nitrate transporters, NarK and NarU.
Extended Data Figure 5 | Assessment of the oligomerization state of detergent-solubilized NRT1.1. a, SEC-LS-RI-UV analysis of DDM-solubilized NRT1.1. Normalized light scattering signal from the 90° detector, ultraviolet absorption signal, and the refractive index signals are plotted in blue, green and red lines, respectively. The highest peak contains NRT1.1 bound to DDM. The calculated masses of the protein-detergent micelle complex (magenta), DDM micelle (cyan), and the NRT1.1 protein (black) are shown. The complex contains about 196 detergent molecules (molecular mass 510.62 Da) and 1 NRT1.1 molecule (67 kDa). The second peak belongs to the detergent micelle with a mass of 79 kDa or 155 detergent molecules.

b, Crosslinking of the NRT1.1 T101D mutant protein with increasing concentrations of EGS. The protein was purified in the presence of 0.1% digitonin.
Extended Data Figure 6 | Shape complementarity and conservation of the NRT1.1 dimer interface. a, Two representative cross-section views of the NRT1.1 dimer interface that are parallel to the membrane. The top cross-section goes through the plane defined by Ala 110 and Val 229 in the two NRT1.1 protomers. The bottom cross-section goes through Ala 104 and Ala 237. b, Conservation surface mapping of NRT1.1 residues at the dimer interface among NRT1.1 orthologues (left) and among Arabidopsis NRT1 family members (right). A colour ramp (white, pale yellow, bright orange, to deep orange) is used to indicate the degree of conservation of surface residues. The arrow indicates the N-terminal segment.
Extended Data Figure 7 | Spatial relationship between the N terminus of the two NRT1.1 protomers. a, The N termini of the two NRT1.1 protomers in the crystal structure are about 42 Å apart and are shown in two orthogonal orientations. b, Spectral quantification of the FRET. Emission spectra measured from an oocyte expressing wild-type NRT1.1 (top left), NRT1.1(T101D) (top right), NRT1.1(T101A) (bottom left), and wild-type mCitrine–NRT1.1 and mCerulean–HCN2 (bottom right). The spectra are colour coded as follows: cyan, 458 nm excitation of oocytes expressing mCerulean constructs alone; black, 488 nm excitation of oocytes expressing both mCitrine and mCerulean constructs; red, 458 nm excitation of oocytes expressing both mCitrine and mCerulean constructs; green, subtracted spectrum (red minus cyan). The dashed line is the position of the peak of the fluorescence signal after excitation at 458 nm of the mCitrine–NRT1.1 only expressing oocytes (the position of the Azero or no FRET peak).
Extended Data Figure 8 | Putative nitrate-binding site in the two NRT1.1 protomers. a, b, Intracellular view of the substrate binding site in the two copies of NRT1.1 within the dimer. To compare the relative position of the substrate to its surrounding residues, distances (Å) between the nitrogen atom of the modelled nitrate and select amino acid atoms in its vicinity are shown with dashed lines and are indicated. Nitrate is shown in sticks with electron density contoured at 4σ from a Fo-Fc map calculated without the substrate. c, d, Side view of the substrate binding site. e, f, A comparison of the putative substrate density between the NRT1.1 structures determined with a cryo-protectant solution containing 10 mM or 0 mM nitrate. g-i, Electrostatic potential surface of the NRT1.1 substrate pocket. The surface colours are clamped between red (−20 kT e−) and blue (+20 kT e−). Nitrate is shown as spheres. Two global views of the electrostatic potential surface of NRT1.1 are shown for comparison.
Extended Data Figure 9 | The conserved cleft formed by the N-terminal segment of NRT1.1. Overall and close-up views of the N-terminal segments of NRT1.1 within the dimer are shown in surface representation. The cleft-forming residues, which are strictly conserved in the NRT1.1 orthologues, are labelled and shown in stick representation.
Extended Data Table 1 | Data collection, phasing and refinement statistics (SAD)

|                            | Native          | Hg Derivative   |
|-----------------------------|-----------------|-----------------|
| **Data collection**         |                 |                 |
| Space group                 | C2221           | C2221           |
| Cell dimensions             |                 |                 |
| a, b, c (Å)                 | 84.0, 188.5, 262.8 | 84.7, 189.9, 262.8 |
| α, β, γ (°)                 | 90, 90, 90      | 90, 90, 90      |
| Resolution (Å)              | 50.0-3.2 (3.26-3.20)* | 50.0-3.50 (3.56-3.50)* |
| R_{sym} or R_{merge}        | 0.07 (0.91)     | 0.08 (0.65)     |
| lmax                         | 17.92 (2.05)    | 26.96 (1.84)    |
| Completeness (%)            | 99.1 (84.6)     | 98.2 (96.5)     |
| Redundancy                  | 5.9 (4.9)       | 7.0 (5.5)       |
| **Refinement**              |                 |                 |
| Resolution (Å)              | 3.25            |                 |
| No. reflections             | 30437           |                 |
| R_{work} / R_{free}         | 0.236 / 0.305   |                 |
| No. atoms                   | 8060            |                 |
| Protein                     | 8004            |                 |
| Ligandation                 | 56              |                 |
| Water                       |                 |                 |
| B-factors                   | 94.50           |                 |
| Protein                     | 94.50           |                 |
| Ligandation                 | 93.40           |                 |
| Water                       |                 |                 |
| R.m.s deviations           |                 |                 |
| Bond lengths (Å)            | 0.010           |                 |
| Bond angles (°)             | 1.40            |                 |

*Highest-resolution shell is shown in parenthesis.