Supplementary Information for

A structural framework for unidirectional transport by a bacterial ABC exporter

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Figure S1. Screening candidate residues in NaAtm1 for disulfide crosslinking. (A) Partial sequence alignments of NBDs of various ABC transporters. (B) SDS-PAGE of the products of crosslinking with Cu (II)(1,10-phenanthroline)$_3$ under different conditions for the three cysteine variants in this report. RT = room temperature, SS = crosslinked species, SH = uncrosslinked (disulfide reduced) species.
Figure S2. NaA527C inward-facing occluded structures. (A) Location of selenium sites in the four transporters present in the selenomethionine-substituted NaA527C asymmetric unit. The selenium sites identified in Autosol of Phenix (1) are shown in red spheres, the sulfur atoms of methionine residues from the refined model are shown in yellow spheres, and nucleotides are shown in sticks with Mg$^{2+}$ shown in green spheres. (B) Disulfide bridges in the four transporters in the asymmetric unit. The Cα positions corresponding to C527 in the two chains are depicted as grey and yellow spheres for transporters #1-3, and grey and red spheres for transporter #4, separately. (C) Composite omit map showing the electron densities for the bound MgADP in different chains of NaA527C in the asymmetric unit.
Figure S3. Structural alignments of NaA527C in the inward-facing occluded conformations. (A) Alignment of NaA527C inward-facing occluded conformation #1 (yellow) to NaA527C inward-facing occluded conformation #2 (red) with an overall rmsd of 1.7 Å. The relative rotation of the α-helical subdomains in the NBDs between the two states is shown in (B). (C) Alignment of NaA527C inward-facing occluded conformation #1 (yellow) to NaAtm1 inward-facing conformation (PDB ID: 4MRN) (grey) with an overall rmsd of 2.1 Å. (D) Alignment of NaA527C inward-facing occluded conformation #2 (red) to NaAtm1 inward-facing conformation (PDB ID: 4MRN) (grey) with an overall rmsd of 4.4 Å. Nucleotides are shown in sticks with Mg$^{2+}$ shown as green spheres.
Figure S4. Binding of GS-Hg to NaA527C inward-facing occluded structures. Anomalous electron density maps calculated from data collected at the Hg edge, contoured at the 5σ levels (dark blue) for NaA527C crystallized in the presence of GS-Hg. For comparison, the structure of NaAtm1 with GS-Hg bound (PDB ID: 4MRV) is indicated (left) with mercury shown in purple sphere. Nucleotides are shown in sticks with Mg$^{2+}$ shown as spheres.
Figure S5. **NaAtm1 in the occluded conformations.** (A) Disulfide bridge formed by S526C in the NaS526C structure with the Cα positions shown as grey and blue spheres. (B) Composite omit map showing the electron densities for the bound ATP in the dimeric NaS526C structure. (C) T525C residues in the NaT525C occluded structure with the Cα positions shown as grey and purple spheres. (D) Composite omit map showing the electron densities for the bound ATP in the dimeric NaT525C structure. (E) Composite omit map showing the electron density for the bound ATP in the dimeric NaE523Q structure. (F) NaT525C overall structural alignment to NaS526C with an overall rmsd of 0.5 Å. (G) NaE523Q overall structural alignment to NaS526C with an overall rmsd of 0.5 Å. (H) NaE523Q overall structural alignment to NaT525C with an overall rmsd of 0.7 Å. In (F-H), NaS526C is colored in blue, NaT525C in purple, and NaE523Q in pink. Nucleotides are shown in sticks.
Figure S6. Structural alignments of wild type NaAtm1 with MgAMPPNP in the occluded conformation. (A) Composite omit map showing the electron densities for the bound MgAMPPNP in the three copies of NaAtm1 in the asymmetric unit. NaAtm1 occluded structure alignments to (B) NaS526C with an overall rmsd of 1.1 Å, (C) NaT525C with an overall rmsd of 0.9 Å, and (D) NaE523Q with an overall rmsd of 1.1 Å. In (B-D), NaAtm1 occluded structure colored in orange, NaS526C in blue, NaT525C in purple and NaE523Q in pink. Nucleotides are shown in sticks with Mg\(^{2+}\) shown as green spheres.
Figure S7. Single particle cryo-EM structure of NaAtm1 in the closed conformation stabilized with MgADPVO₄. (A) Examples of 2D classes. (B) Fourier shell correlation (FSC) curve showing the resolution estimate for the final reconstruction, generated from the final refinement in cryoSPARC 2 (2). (C) Density fitting for different TM helices and nucleotides (MgADPVO₄). Overall structural alignments to the occluded crystal structures of (D) NaS526C, (E) NaT525C, (F) NaE523Q and (G) NaAtm1 occluded with rmsds of 2.0 Å, 2.1 Å, 2.0 Å and 1.7 Å, respectively. In (D-G), the NaAtm1 closed structure is shown in green, NaS526C in blue, NaT525C in purple, NaE523Q in pink and NaAtm1 occluded structure is shown in orange. Nucleotides are shown as sticks and Mg²⁺ as green spheres.
Figure S8. Single particle cryo-EM structure of NaAtm1 in the wide-open inward-facing conformation. (A) Examples of 2D classes. (B) FSC curve showing the resolution estimate for the final reconstruction, generated from the final refinement in cryoSPARC 2 (2). (C) Density fitting for different TM helices. Overall structural alignments of the wide-open inward-facing conformation (cyan) to (D) NaAtm1 inward-facing conformation (PDB ID: 4MRN) (grey), (E) NaA527C inward-facing occluded state #1 (yellow), and (F) NaA527C inward-facing occluded state #2 (red) crystal structures with rmsds of 5.8 Å, 8.9 Å, and 9.3 Å, separately.
Figure S9. TM6 comparisons for different ABC transporter systems. (A) TM6 (residues 300 to 340) arrangements of representative NaAtm1 structures. (B) TM6 (residues 288 to 335) arrangements of representative PglK structures. (C) TM6 (residues 290 to 333 of chain A and residues 275 to 319 of chain B) arrangements of representative TmrAB structures. (D) TM6 (residues 324 to 370 and residues 968 to 1013) arrangements of representative ABCB1 structures. The corresponding PDB IDs and the conformational states are labeled below the structures.
Table S1. Coupling efficiencies between ATP hydrolysis and substrate translocation for ABC transporters. Coupling efficiencies for different ABC transporter systems (3-17). Coupling efficiencies are either presented in the corresponding reference or calculated based on the reported ATPase and transport activities of the transporter. Coupling efficiency = ATPase activity/transport activity.

| Transporters | ATPase activity (nmol/min/mg) | Transport Activity (nmol/min/mg) | Coupling efficiency | References                  |
|--------------|-------------------------------|---------------------------------|---------------------|------------------------------|
| ABCC3        | 200                           | 1.200                           | 0.17                | Zehnpfennig et al (2009)     |
| MpfFGK       | ~1.5 - 3                      | ~0.5 - 2                        | 1.4 - 17            | Davidson et al (1990)        |
| OpuA         | ~80 - 120                     | ~30 - 70                        | 2                   | Patzlaff et al (2003)        |
| GlnPQ        | 15 (min⁻¹)                    | 8.5 (min⁻¹)                     | 2                   | Lyoklama et al (2018)        |
| Pgp          | ~750 - 1,300                  | ~500                            | 2                   | Eytan et al (1996)           |
| ABCG5/8      | 50                            | 2.2                             |                      | Wang et al (2006)            |
| MpfFGK       | ~1.2 - 8                      | ~0.3 - 2                        | 4 - 10              | Dean et al (1989)            |
| Pgp          | ~110                          | ~6                              | 18                  | Dong et al (1996)            |
| HisP         | 580                           | 19                              | 31                  | Nikaido and Ames (1999)      |
| ABCG2        | ~750                          | ~22                             | 34                  | Manolaridile et al (2018)    |
| TmrAB        | ~1,100                        | ~30                             | 37                  | Hofmann et al (2019)         |
| BltCDF       | ~400                          | ~4                              | 100                 | Borths et al (2005)          |
| NaAtm1       | 150                           | 1.5                             | 100                 | This study                   |
| HmuUV        | ~130                          | ~1.1                            | 120                 | Woo et al (2012)             |
| MpfFGK       | 4,000                         | 1.2                             | 3,300               | Chen et al (2001)            |
| ABCB6        | 610                           | 0.03                            | 20,000              | Chavan et al (2013)          |
Table S2. Raw ATPase activities of NaAtm1 and variants in both proteoliposomes and detergent. The ATPase activities were measured in triplicate at 10 mM MgATP and 2.5 mM GSSG at 37 °C.

| Conditions            | Variants         | NaAtm1 | NaA527C | NaS526C | NaT525C | NaE523Q |
|-----------------------|------------------|--------|---------|---------|---------|---------|
|                       | **In proteoliposomes**                        |        |         |         |         |         |
| + 10mM MgATP          |                  | 51.90  | 1.26    | 55.21   | 13.28   | 1.22    |
|                       |                  | 77.01  | 2.97    | 52.58   | 15.39   | 1.86    |
|                       |                  | 67.60  | 2.93    | 54.32   | 13.43   | 1.00    |
| Average               |                  | 66 ± 13| 2 ± 1   | 54 ± 1  | 14 ± 1  | 1.4 ± 0.4|
| + 10mM MgATP + 2.5mM GSSG |          | 118.68 | 7.55    | 69.50   | 20.88   | -0.06   |
|                       |                  | 179.94 | 5.66    | 70.29   | 21.00   | -0.46   |
|                       |                  | 157.94 | 6.18    | 68.06   | 20.55   | 0.48    |
| Average               |                  | 152 ± 31| 6 ± 1   | 69 ± 1  | 20.8 ± 0.2| 0.0 ± 0.5|
|                       | **In detergent (DDM/C12E8)**                      |        |         |         |         |         |
| + 10mM MgATP          |                  | 122.53 | 11.50   | 103.90  | 57.47   | 5.20    |
|                       |                  | 125.12 | 13.32   | 111.80  | 57.37   | 5.76    |
|                       |                  | 97.76  | 12.30   | 112.40  | 56.94   | 3.30    |
| Average               |                  | 115 ± 15| 12.4 ± 0.9| 111 ± 2 | 57.3 ± 0.3| 5 ± 1    |
| + 10mM MgATP + 2.5mM GSSG |          | 215.77 | 14.16   | 104.30  | 42.67   | 3.25    |
|                       |                  | 200.91 | 15.01   | 103.80  | 42.01   | 4.76    |
|                       |                  | 202.41 | 15.10   | 103.20  | 42.88   | 4.26    |
| Average               |                  | 206 ± 8| 14.8 ± 0.5| 103.8 ± 0.6| 42.5 ± 0.5| 4.1 ± 0.8|
Table S3. Raw transport activities of NaAtm1 and variants. The transport activities for various controls and the different NaAtm1 variants were measured in triplicate at 10 mM MgATP and 2.5 mM GSSG at 37 °C.

| Samples                  | Transport rate (nmol/mg protein) at various time points (min) | Transport rates (nmol/min/mg) |
|--------------------------|-----------------------------------------------------------------|-------------------------------|
|                          | 0                  | 15               | 30               | 45               | 60               | 75               |                  |
| NaAtm1 PLS +MgATP +GSSG | 28.17              | 45.49            | 69.95            | 81.08            | 113.96           | 141.67           | 1.488             |
| NaAtm1 PLS +GSSG        | 28.05              | 49.95            | 64.09            | 94.01            | 125.89           | 139.02           | 1.554             |
| Average                  | 26.57              | 54.53            | 77.96            | 83.45            | 114.33           | 149.99           | 1.528             |
| NaAtm1 PLS +MgATP       | 26.69              | 26.22            | 30.82            | 34.07            | 34.73            | 39.73            | 0.179             |
| NaAtm1 PLS +GSSG        | 26.08              | 28.78            | 37.44            | 36.06            | 38.74            | 32.31            | 0.1137            |
| Average                  | 26.5 ± 0.3         | 29 ± 2           | 35 ± 4           | 35 ± 1           | 35 ± 3           | 37 ± 4           | 0.14 ± 0.04       |
| NaAtm1 PLS +MgATP       | 2.84               | 1.03             | 2.95             | 2.98             | 1.13             | 1.46             | -0.0135           |
| NaAtm1 PLS +GSSG        | 3.39               | 1.59             | 3.58             | 2.29             | 2.17             | 1.83             | -0.01406          |
| Average                  | 3.2 ± 0.3          | 1.7 ± 0.7        | 3.5 ± 0.6        | 2.5 ± 0.5        | 1.7 ± 0.5        | 0 ± 2            | -0.03 ± 0.03      |
| NaAtm1 PLS +MgATP       | 3.30               | 1.80             | 3.34             | 2.64             | 1.48             | 1.76             | -0.01782          |
| NaAtm1 PLS +GSSG        | 3.39               | 1.75             | 3.75             | 2.18             | 1.31             | 1.59             | -0.02279          |
| Average                  | 3.4 ± 0.1          | 1.2 ± 0.9        | 3.7 ± 0.3        | 2.7 ± 0.6        | 1.6 ± 0.4        | 2 ± 1            | -0.01 ± 0.02      |
| Liposomes +GSSG         | 20.25              | 22.67            | 29.57            | 17.30            | 20.47            | 16.88            | -0.06508          |
| NaAs27C PLS +MgATP +GSSG| 23.88              | 19.64            | 31.19            | 30.18            | 26.25            | 37.11            | 0.1618            |
| NaS526C PLS +MgATP +GSSG| 24.36              | 26.97            | 32.05            | 31.50            | 33.88            | 35.79            | 0.1473            |
| Average                  | 23 ± 3             | 24 ± 4           | 32 ± 1           | 31.2 ± 0.9       | 30 ± 4           | 36.1 ± 0.9       | 0.16 ± 0.01       |
| NaS526C PLS +MgATP +GSSG| 22.86              | 42.06            | 53.21            | 66.26            | 77.55            | 79.59            | 0.7679            |
| NaS526C PLS +MgATP +GSSG| 21.26              | 41.68            | 59.75            | 61.69            | 84.84            | 92.92            | 0.9328            |
| Average                  | 22.2 ± 0.9         | 41 ± 2           | 58 ± 4           | 64 ± 2           | 82 ± 4           | 92 ± 12          | 0.9 ± 0.1         |
| NaT525C PLS +MgATP +GSSG| 23.32              | 25.75            | 15.61            | 49.92            | 58.39            | 63.36            | 0.6332            |
| NaT525C PLS +MgATP +GSSG| 20.60              | 26.69            | 40.53            | 51.72            | 52.88            | 37.39            | 0.3309            |
| Average                  | 22 ± 1             | 26.1 ± 0.5       | 32 ± 14          | 48 ± 5           | 57 ± 4           | 53 ± 14          | 0.5 ± 0.2         |
| NaE523Q PLS +MgATP +GSSG| 21.82              | 26.31            | 30.16            | 31.69            | 32.19            | 32.12            | 0.1345            |
| NaE523Q PLS +MgATP +GSSG| 16.01              | 24.62            | 30.91            | 8.99             | 35.00            | 33.71            | 0.1862            |
| Average                  | 19 ± 3             | 24 ± 2           | 28 ± 4           | 24 ± 13          | 34 ± 2           | 33 ± 1           | 0.19 ± 0.06       |
Table S4. Data collection and refinement statistics of \textit{NaA527C}.

|                       | \textit{NaA527C} native | \textit{NaA527C} SeMet |
|-----------------------|--------------------------|------------------------|
| Beamline              | SSRL 12-2                | SSRL 12-2              |
| Wavelength (Å)        | 0.97346                  | 0.97949                |
| Resolution range (Å)  | 39.37 - 3.70 (3.82 - 3.70)| 39.72 - 4.50 (4.66 - 4.50) |
| Space group           | P1                       | P1                     |
| Unit cell (Å, °)      | 129.18 133.61 134.26 110.619 98.282 101.2 | 129.19 133.14 134.27 109.61 98.51 101.87 |
| Total reflections     | 863652 (61595)           | 972530 (99987)         |
| Unique reflections    | 84628 (7646)             | 47277 (4067)           |
| Multiplicity          | 7.1 (7.3)                | 20.6 (21.4)            |
| Completeness (%)      | 97.55 (89.28)            | 99.3 (99.3)            |
| Mean I/σ(I)           | 7.31 (0.74)              | 8.1 (1.4)              |
| Wilson B-factor       | 141.79                   | 163.94                 |
| R-merge               | 0.167 (3.050)            | 0.291 (3.241)          |
| R-meas                | 0.180 (3.283)            | 0.258 (3.320)          |
| R-pim                 | 0.067 (1.206)            | 0.066 (0.712)          |
| CC1/2                 | 0.999 (0.412)            | 0.998 (0.761)          |
| CC*                   | 1.000 (0.764)            |                        |
| Reflections used in refinement | 83473 (7643) |                     |
| Reflections used for R-free | 4185 (346) |                     |
| R-work                | 0.240 (0.356)            |                        |
| R-free                | 0.288 (0.400)            |                        |
| CC(work)              | 0.787 (0.709)            |                        |
| CC(free)              | 0.841 (0.703)            |                        |
| Number of non-hydrogen atoms | 39839                  |                        |
| macromolecules        | 36015                    |                        |
| ligands               | 224                      |                        |
| Protein residues      | 4722                     |                        |
| RMS (bonds) (Å)       | 0.004                    |                        |
| RMS (angles) (*)      | 0.95                     |                        |
| Ramachandran favored (%) | 97.95                   |                        |
| Ramachandran allowed (%) | 1.96                     |                        |
| Ramachandran outliers (%) | 0.09                    |                        |
| Rotamer outliers (%)  | 0.40                     |                        |
| Clashscore            | 6.13                     |                        |
| Average B-factor      | 178.14                   |                        |
| macromolecules        | 178.04                   |                        |
| ligands               | 194.08                   |                        |

*Statistics for the highest-resolution shell are shown in parentheses.
Table S5. Data collection and refinement statistics of NaS526C.

| **NaS526C SeMet** |    |
|-------------------|---|
| **Beamline**      | SSRL 12-2 |
| **Wavelength (Å)**| 0.9738 |
| **Resolution range (Å)** | 39.71 - 3.40 (3.522 - 3.40) |
| **Space group**   | P 21 21 21 |
| **Unit cell (Å, °)** | 95.5 134.58 190.12 90 90 90 |
| **Total reflections** | 916579 (84633) |
| **Unique reflections** | 34343 (3016) |
| **Multiplicity**  | 20.7 (25.1) |
| **Completeness (%)** | 98.64 (89.27) |
| **Mean | Sigmal(θ) | 17.20 (1.19) |
| **Wilson B-factor** | 138.15 |
| **R-merge**       | 0.148 (3.919) |
| **R-meas**        | 0.151 (4.000) |
| **R-pilr**        | 0.029 (0.792) |
| **CC1/2**         | 1.000 (0.588) |
| **CC**            | 1.000 (0.861) |
| **Relections used in refinement** | 33962 (3012) |
| **Relections used for R-free** | 1690 (153) |
| **R-work**        | 0.192 (0.265) |
| **R-free**        | 0.234 (0.377) |
| **CC(work)**      | 0.727 (0.820) |
| **CC(free)**      | 0.865 (0.750) |
| **Number of non-hydrogen atoms** | 8021 |
| **macromolecules** | 8859 |
| **ligands**       | 62 |
| **Protein residues** | 1147 |
| **RMS (bonds) (Å)** | 0.002 |
| **RMS (angles) (°)** | 0.57 |
| **Ramachandran favored (%)** | 98.77 |
| **Ramachandran allowed (%)** | 1.23 |
| **Ramachandran outliers (%)** | 0.00 |
| **Rotamer outliers (%)** | 0.88 |
| **Clashscore**    | 7.07 |
| **Average B-factor** | 196.93 |
| **ligands**       | 197.86 |

*Statistics for the highest-resolution shell are shown in parentheses.*
**Table S6. Data collection and refinement statistics of NaT525C.**

|                                | NaT525C native | NaT525C SeMet |
|--------------------------------|----------------|--------------|
| **Beamline**                   | APS GM/CA 23-IDB | APS GM/CA 23-IDB |
| Wavelength (Å)                 | 1.033202       | 0.972338     |
| Resolution range (Å)           | 39.1 - 3.65 (3.78 - 3.65) | 39.31 - 3.90 (4.21 - 3.90) |
| Space group                    | P 21 21 21     | P 21 21 21   |
| Unit cell (Å, °)               | 94.164 135.415 191.592 90 90 90 | 93.78 136.37 192.48 90 90 90 |
| Total reflections              | 246061 (222521) | 237026 (49769) |
| Unique reflections             | 27912 (2712)   | 23170 (4675)  |
| Multiplicity                   | 8.8 (8.1)      | 10.2 (10.6)  |
| Completeness (%)               | 97.77 (97.54)  | 99.9 (99.9)   |
| Mean I/σI0                     | 14.33 (1.11)   | 12.1 (1.6)    |
| Wilson B-factor                | 172.32         | 180.32       |
| R-merge                        | 0.076 (0.002)  | 0.100 (1.852) |
| R-meas                         | 0.080 (2.146)  | 0.106 (1.945) |
| R-pim                          | 0.027 (0.758)  | 0.033 (0.590) |
| CC1/2                          | 0.999 (0.634)  | 1.000 (0.596) |
| CC                            | 1.000 (0.881)  |              |
| Reflections used in refinement | 27348 (2699)   |              |
| Reflections used for R-free    | 1356 (128)     |              |
| R-work                         | 0.251 (0.430)  |              |
| R-free                         | 0.285 (0.475)  |              |
| CC(work)                       | 0.713 (0.364)  |              |
| CC(free)                       | 0.877 (0.304)  |              |
| Number of non-hydrogen atoms   | 8835           |              |
| Macromolecules                 | 8773           |              |
| Ligands                        | 62             |              |
| Protein residues               | 1135           |              |
| RMS (bonds) (Å)                | 0.003          |              |
| RMS (angles) (*)               | 0.63           |              |
| Ramachandran favored (%)       | 97.70          |              |
| Ramachandran allowed (%)       | 2.30           |              |
| Ramachandran outliers (%)      | 0.00           |              |
| Rotamer outliers (%)           | 0.22           |              |
| Clashscore                     | 7.07           |              |
| Average B-factor               | 214.38         |              |
| Macromolecules                 | 214.56         |              |
| Ligands                        | 189.06         |              |

*Statistics for the highest-resolution shell are shown in parentheses.*
Table S7. Data collection and refinement statistics of NaE523Q.

|                           | NaE523Q SeMet          |
|---------------------------|------------------------|
| Beamline                  | SSRL 12-2              |
| Wavelength (Å)            | 0.97946                |
| Resolution range (Å)      | 38.63 - 3.30 (3.419 - 3.30) |
| Space group               | P 21 21 21             |
| Unit cell (Å, °)          | 89.346 115.354 184.536 90.90 90 |
| Total reflections         | 387647 (39220)         |
| Unique reflections        | 29277 (2661)           |
| Multiplicity              | 13.2 (13.6)            |
| Completeness (%)          | 99.32 (98.72)          |
| Mean | | |
| Wilson B-factor           | 103.58                 |
| R-merge                   | 0.190 (2.680)          |
| R-meas                    | 0.200 (2.784)          |
| R-pim                     | 0.054 (0.748)          |
| CC1/2                     | 1.000 (0.700)          |
| CC*                       | 1.000 (0.908)          |
| Reflections used in refinement | 29179 (2645)   |
| Reflections used for R-free | 1434 (127)         |
| R-work                    | 0.234 (0.330)          |
| R-free                    | 0.300 (0.439)          |
| CC(work)                  | 0.684 (0.848)          |
| CC(free)                  | 0.827 (0.615)          |
| Number of non-hydrogen atoms | 8842                  |
| macromolecules            | 8780                   |
| ligands                   | 62                     |
| Protein residues          | 1136                   |
| RMS (bonds) (Å)           | 0.002                  |
| RMS (angles) (°)          | 0.63                   |
| Ramachandran favored (%)  | 97.52                  |
| Ramachandran allowed (%)  | 2.48                   |
| Ramachandran outliers (%) | 0.00                   |
| Rotamer outliers (%)      | 1.77                   |
| Clashscore                | 8.24                   |
| Average B-factor          | 134.03                 |
| macromolecules            | 133.82                 |
| ligands                   | 163.57                 |

*Statistics for the highest-resolution shell are shown in parentheses.
### Table S8. Data collection and refinement statistics of NaAtm1.

|                       | NaAtm1 native | NaAtm1 SeMet |
|-----------------------|---------------|--------------|
| **Beamline**          | SSRL 12-2     | SSRL 12-2    |
| **Wavelength (Å)**    | 0.97946       | 0.9793       |
| **Resolution range (Å)** | 39.33 - 3.35  | 39.65 - 3.60  |
| **Space group**       | P 21          | P 21         |
| **Unit cell (Å, °)**  | 169.648 92.498 237.691 90 110.34 90 | 170.10 92.21 237.47 90 110.58 90 |
| **Total reflections** | 688114 (60865) | 559063 (30390) |
| **Unique reflections**| 98507 (9041)  | 79094 (4376) |
| **Completeness (%)**  | 97.85 (91.11) | 98.1 (95.8)  |
| **Mean / sigma(I)**   | 9.43 (0.97)   | 7.5 (1.1)    |
| **Wilson B-factor**   | 91.20         | 94.99        |
| **R-merge**           | 0.174 (1.768) | 0.211 (1.942) |
| **R-meas**            | 0.185 (1.927) | 0.228 (2.100) |
| **R-pick**            | 0.071 (0.754) | 0.086 (0.787) |
| **CC1/2**             | 0.999 (0.530) | 0.994 (0.466) |
| **CC**                | 1.000 (0.832) |
| **Reflections used in refinement** | 97914 (9035) |
| **Reflections used for R-free** | 4897 (425)    |
| **R-work**            | 0.254 (0.353) |
| **R-free**            | 0.282 (0.365) |
| **CC(work)**          | 0.740 (0.685) |
| **CC(free)**          | 0.529 (0.606) |
| **Number of non-hydrogen atoms** | 26975 |
| **macromolecules**    | 26783         |
| **ligands**           | 192           |
| **Protein residues**  | 3464          |
| **RMS (bonds) (Å)**   | 0.003         |
| **RMS (angles) (°)**  | 0.60          |
| **Ramachandran favored (%)** | 97.35       |
| **Ramachandran allowed (%)** | 2.59        |
| **Ramachandran outliers (%)** | 0.06        |
| **Rotamer outliers (%)** | 0.58        |
| **Clashscore**        | 8.33          |
| **Average B-factor**  | 120.02        |
| **macromolecules**    | 120.15        |
| **ligands**           | 101.67        |

*Statistics for the highest-resolution shell are shown in parentheses.*
### Table S9. Cryo-EM data collection, refinement and validation statistics

|                         | Closed                      | Inward-facing               |
|-------------------------|-----------------------------|-----------------------------|
| **Data Collection and processing** |                             |                             |
| Microscope              | Titan Krios at S2C2         | Titan Krios at Caltech Cryo-EM facility |
| Camera                  | Gatan K3                    | Gatan K2 Summit             |
| Magnification           | x25,000                     | x165,000                    |
| Voltage (kV)            | 300                         | 300                         |
| Exposure (e Å²)         | 48.6                        | 36                          |
| Pixel size (Å)          | 0.823                       | 0.834                       |
| Defocus Range (μm)      | -1.7 to -2.4                | -1.0 to -3.5                |
| Initial Particle Image (no.) | 3,978,816                | 1,145,444                   |
| Final Particle Image (no.) | 166,278                    | 102,076                     |
| Symmetry Imposed        | C2                          | C2                          |
| Map Resolution (Å)      | 3.03                        | 3.88                        |
| FSC Threshold           | 0.143                       | 0.143                       |
| Map Resolution Range (Å) | 2.8 - 3.8                  | 3.8 - 4.5                   |
| **Refinement**          |                             |                             |
| Initial Model Used      | PDB ID: 6PAR                | PDB ID: 4MRN                |
| Model Resolution (Å)    | 3.0                         | 3.9                         |
| FSC Threshold           | 0.143                       | 0.143                       |
| Map Sharpening B-factor (Å) | -86                       | -121                        |
| Model composition       |                             |                             |
| non-hydrogen atoms      | 9144                        | 9156                        |
| protein residues        | 1198                        | 1178                        |
| ligands                 | ADP; 2; Mg: 2; VO4: 2       |                             |
| Average B-factors (Å)   | 94.1                        | 22.5                        |
| protein                 |                             |                             |
| ligands                 | 66.7                        | -                           |
| R.m.s. deviations       |                             |                             |
| Bond length (Å)         | 0.000                       | 0.007                       |
| Bond angles (°)         | 0.967                       | 0.929                       |
| **Validation**          |                             |                             |
| MolProbity score        | 2.2                         | 1.67                        |
| Clashscore              | 10.2                        | 5.1                         |
| Rotamer outliers        | 4.9                         | 1.7                         |
| Ramachandran plot       |                             |                             |
| Ramachandran favored (%)| 97.3                        | 96.6                        |
| Ramachandran allowed (%)| 2.7                         | 3.4                         |
| Ramachandran outliers (%)| 0                          | 0                           |
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