

Table S1. Data collection and refinement statistics of *NaA527C*

	<i>NaA527C</i> native	<i>NaA527C</i> SeMet
Beamline	SSRL 12-2	SSRL 12-2
Wavelength (Å)	0.97946	0.97949
Resolution range (Å)	39.37 - 3.70 (3.832 - 3.70)	39.72 - 4.50 (4.66 - 4.50)
Space group	P 1	P 1
Unit cell (Å, °)	129.18 133.61 134.26 110.619 98.282 101.2	129.19 133.14 134.27 109.61 98.51 101.67
Total reflections	603652 (61595)	972530 (99987)
Unique reflections	84628 (7646)	47277 (4667)
Multiplicity	7.1 (7.3)	20.6 (21.4)
Completeness (%)	97.55 (89.28)	99.3 (99.3)
Mean I/sigma(I)	7.31 (0.74)	8.1 (1.4)
Wilson B-factor	141.79	163.64
R-merge	0.167 (3.050)	0.291 (3.241)
R-meas	0.180 (3.283)	0.298 (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	36839	
macromolecules	36615	
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 S2. Data collection and refinement statistics of NaS526C**

	<b>NaS526C SeMet</b>
<b>Beamline</b>	SSRL 12-2
<b>Wavelength (Å)</b>	0.97938
<b>Resolution range (Å)</b>	39.71 - 3.40 (3.522 - 3.40)
<b>Space group</b>	P 21 21 21
<b>Unit cell (Å, °)</b>	95.5 134.58 190.12 90 90 90
<b>Total reflections</b>	916579 (84633)
<b>Unique reflections</b>	34343 (3016)
<b>Multiplicity</b>	26.7 (25.1)
<b>Completeness (%)</b>	98.64 (89.27)
<b>Mean I/sigma(I)</b>	17.26 (1.19)
<b>Wilson B-factor</b>	138.15
<b>R-merge</b>	0.148 (3.919)
<b>R-meas</b>	0.151 (4.000)
<b>R-pim</b>	0.029 (0.792)
<b>CC1/2</b>	1.000 (0.588)
<b>CC*</b>	1.000 (0.861)
<b>Reflections used in refinement</b>	33962 (3012)
<b>Reflections used for R-free</b>	1696 (153)
<b>R-work</b>	0.192 (0.295)
<b>R-free</b>	0.234 (0.377)
<b>CC(work)</b>	0.727 (0.820)
<b>CC(free)</b>	0.865 (0.750)
<b>Number of non-hydrogen atoms</b>	8921
<b>macromolecules</b>	8859
<b>ligands</b>	62
<b>Protein residues</b>	1147
<b>RMS (bonds) (Å)</b>	0.002
<b>RMS (angles) (°)</b>	0.57
<b>Ramachandran favored (%)</b>	98.77
<b>Ramachandran allowed (%)</b>	1.23
<b>Ramachandran outliers (%)</b>	0.00
<b>Rotamer outliers (%)</b>	0.88
<b>Clashscore</b>	7.07
<b>Average B-factor</b>	196.93
<b>macromolecules</b>	196.93
<b>ligands</b>	197.86
**Statistics for the highest-resolution shell are shown in parentheses.	

Table S3. Data collection and refinement statistics of NaT525C

	<b>NaT525C native</b>	<b>NaT525C SeMet</b>
<b>Beamline</b>	APS GM/CA 23-IDB	APS GM/CA 23-IDB
<b>Wavelength (Å)</b>	1.033202	0.979338
<b>Resolution range (Å)</b>	39.1 - 3.65 (3.78 - 3.65)	39.31 - 3.90 (4.21 - 3.90)
<b>Space group</b>	P 21 21 21	P 21 21 21
<b>Unit cell (Å, °)</b>	94.164 135.415 191.592 90 90 90	93.78 136.37 192.48 90 90 90
<b>Total reflections</b>	246061 (22321)	237126 (49769)
<b>Unique reflections</b>	27912 (2712)	23170 (4675)
<b>Multiplicity</b>	8.8 (8.1)	10.2 (10.6)
<b>Completeness (%)</b>	97.77 (97.54)	99.9 (99.9)
<b>Mean I/sigma(I)</b>	14.33 (1.11)	12.1 (1.6)
<b>Wilson B-factor</b>	172.32	180.32
<b>R-merge</b>	0.076 (2.002)	0.100 (1.852)
<b>R-meas</b>	0.080 (2.146)	0.106 (1.945)
<b>R-pim</b>	0.027 (0.758)	0.033 (0.590)
<b>CC1/2</b>	0.999 (0.634)	1.000 (0.586)
<b>CC*</b>	1.000 (0.881)	
<b>Reflections used in refinement</b>	27348 (2699)	
<b>Reflections used for R-free</b>	1356 (126)	
<b>R-work</b>	0.251 (0.430)	
<b>R-free</b>	0.285 (0.475)	
<b>CC(work)</b>	0.713 (0.364)	
<b>CC(free)</b>	0.877 (0.304)	
<b>Number of non-hydrogen atoms</b>	8835	
<b>macromolecules</b>	8773	
<b>ligands</b>	62	
<b>Protein residues</b>	1135	
<b>RMS (bonds) (Å)</b>	0.003	
<b>RMS (angles) (°)</b>	0.63	
<b>Ramachandran favored (%)</b>	97.70	
<b>Ramachandran allowed (%)</b>	2.30	
<b>Ramachandran outliers (%)</b>	0.00	
<b>Rotamer outliers (%)</b>	0.22	
<b>Clashscore</b>	7.07	
<b>Average B-factor</b>	214.38	
<b>macromolecules</b>	214.56	
<b>ligands</b>	189.06	

\*\*Statistics for the highest-resolution shell are shown in parentheses.

**Table S4. Data collection and refinement statistics of NaE523Q**

	<b>NaE523Q SeMet</b>
<b>Beamline</b>	SSRL 12-2
<b>Wavelength (Å)</b>	0.97946
<b>Resolution range (Å)</b>	38.63 - 3.30 (3.419 - 3.30)
<b>Space group</b>	P 21 21 21
<b>Unit cell (Å, °)</b>	89.346 115.354 184.536 90 90 90
<b>Total reflections</b>	387847 (39220)
<b>Unique reflections</b>	29277 (2861)
<b>Multiplicity</b>	13.2 (13.6)
<b>Completeness (%)</b>	99.32 (98.72)
<b>Mean I/sigma(I)</b>	11.11 (1.01)
<b>Wilson B-factor</b>	103.58
<b>R-merge</b>	0.190 (2.680)
<b>R-meas</b>	0.200 (2.784)
<b>R-pim</b>	0.054 (0.748)
<b>CC1/2</b>	1.000 (0.700)
<b>CC*</b>	1.000 (0.908)
<b>Reflections used in refinement</b>	29179 (2845)
<b>Reflections used for R-free</b>	1434 (127)
<b>R-work</b>	0.234 (0.330)
<b>R-free</b>	0.300 (0.439)
<b>CC(work)</b>	0.684 (0.848)
<b>CC(free)</b>	0.827 (0.615)
<b>Number of non-hydrogen atoms</b>	8842
<b>macromolecules</b>	8780
<b>ligands</b>	62
<b>Protein residues</b>	1136
<b>RMS (bonds) (Å)</b>	0.002
<b>RMS (angles) (°)</b>	0.63
<b>Ramachandran favored (%)</b>	97.52
<b>Ramachandran allowed (%)</b>	2.48
<b>Ramachandran outliers (%)</b>	0.00
<b>Rotamer outliers (%)</b>	1.77
<b>Clashscore</b>	8.24
<b>Average B-factor</b>	134.03
<b>macromolecules</b>	133.82
<b>ligands</b>	163.57

\*\*Statistics for the highest-resolution shell are shown in parentheses.

Table S5. Data collection and refinement statistics of *NaAtm1*

	<i>NaAtm1</i> native	<i>NaAtm1</i> SeMet
Beamline	SSRL 12-2	SSRL 12-2
Wavelength (Å)	0.97946	0.9793
Resolution range (Å)	39.33 - 3.35 (3.47 - 3.35)	39.85 - 3.60 (3.67 - 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	686114 (60885)	559063 (30390)
Unique reflections	98507 (9041)	79094 (4376)
Multiplicity	7.0 (6.4)	7.1 (6.9)
Completeness (%)	97.85 (91.11)	98.1 (95.8)
Mean I/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	0.189 (1.927)	0.228 (2.100)
R-pim	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 S6a. Raw transport activities for all variants**

Samples	Time (min)					
	0	15	30	45	60	75
<b>NaAtm1 PLS +MgATP +GSSG</b>	28.17	45.49	69.95	81.08	113.06	141.67
	28.05	49.95	64.09	94.01	125.89	139.62
	26.57	54.53	77.96	83.45	114.33	149.99
<b>NaAtm1 PLS +GSSG</b>	26.69	26.22	30.82	34.07	34.73	39.73
	26.08	28.78	37.44	36.06	38.74	32.31
	26.59	30.69	36.27	35.48	31.99	38.69
<b>NaAtm1 PLS +MgATP</b>	2.84	1.03	2.95	2.98	1.13	1.48
	3.39	1.59	3.58	2.29	2.17	1.83
	3.39	2.54	4.10	2.08	1.65	-1.82
<b>NaAtm1 PLS</b>	3.30	1.80	3.34	2.64	1.48	1.76
	3.39	1.76	3.75	2.18	1.31	1.59
	3.39	0.16	4.03	3.30	2.04	3.95
<b>LS +GSSG</b>	20.25	22.67	29.57	17.30	20.47	16.88
	14.00	21.70	25.88	18.91	16.76	16.41
	12.87	20.42	29.74	2.07	19.66	19.29
<b>NaA527C PLS +MgATP +GSSG</b>	23.88	19.64	31.19	30.18	26.25	37.11
	24.36	26.97	32.05	31.50	33.88	35.79
	19.28	26.69	33.64	31.78	29.64	35.41
<b>NaS526C PLS +MgATP +GSSG</b>	22.86	42.06	53.21	66.26	77.55	79.59
	21.26	41.68	59.75	61.69	84.84	92.92
	22.58	37.91	61.09	64.64	82.63	103.12
<b>NaT525C PLS +MgATP +GSSG</b>	23.32	25.75	15.61	49.92	58.39	63.36
	20.60	26.69	40.53	51.72	52.88	37.39
	21.82	25.94	39.12	42.16	59.62	57.18
<b>NaE523Q PLS +MgATP +GSSG</b>	21.82	26.31	30.16	31.69	32.19	32.12
	16.01	24.62	30.91	8.99	35.00	33.71
	17.88	21.80	23.30	32.07	35.00	34.47

**Table S6b. Raw ATPase activities for all variants**

Conditions	Variants				
	NaAtm1	NaA527C	NaS526C	NaT525C	NaE523Q
<b>Proteoliposomes + 10mM MgATP</b>	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
	56.09				
	68.27				
	60.02				
<b>Proteoliposomes + 10mM MgATP + 2.5mM GSSG</b>	118.68	7.55	69.50	20.88	-0.08
	179.94	5.66	70.29	21.00	-0.46
	157.94	6.18	68.06	20.55	0.48
	216.80				
	206.72				
	201.87				
<b>Detergent (DDM/C12E8) + 10mM MgATP</b>	122.53	11.50	108.90	57.47	5.20
	125.12	13.32	111.60	57.37	5.76
	97.76	12.30	112.40	56.94	3.30
	134.40				
	127.08				
	131.24				
<b>Detergent (DDM/C12E8) + 10mM MgATP + 2.5mM GSSG</b>	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
	288.59				
	290.64				
	295.58				