Supporting Information

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SI Methods

Expression and Purification of Art(QN)₂. The artQ and artN genes were cloned from T. tengcongensis genomic DNA into a pACYCDuet vector (Novagen) that can coexpress artQ and artN stimultaneously using separate T7 promoters. An engineered C-terminal 6× His tag was fused to artQ (encoding the transmembrane subunit), which was cloned into multiple cloning sites-2, whereas the artN gene (ATPase subunit) was inserted in multiple cloning sites-1. Overexpression of Art(QN)₂ was induced in E. coli strain C43 by 0.5 mM isopropyl-β-D-thiogalactoside when the cell density reached $OD_{600} = 1.2$. After growth for 4 h at 37 °C, the cells were collected, resuspended in buffer containing 25 mM Tris-HCl pH 8.0 and 200 mM NaCl, and lysed by sonication. Cell debris was removed by centrifugation at $15,422 \times g$ for 15 min. The supernatant was subsequently subjected to ultracentrifugation at $173,021 \times g$ for 1 h. The membrane fraction (pellet) was resuspended in buffer A containing 25 mM Tris HCl pH 8.0, 20 mM imidazole, and 500 mM NaCl. After the addition of n-dodecyl-β-D maltopyranoside (DDM; Anatrace) at a final concentration of 1.5% (wt/vol), the mixture was incubated for 5 h with slow stirring at 4 °C. After another ultracentrifugation step at $173,021 \times g$ for 30 min, the supernatant was collected and loaded onto Ni²⁺-nitrilotriacetate affinity resin (Ni-NTA; Qiagen). The resin was then washed with Buffer A supplemented with 0.02% DDM. Protein was eluted from the affinity resin with Buffer A supplemented with 0.02% DDM and 200 mM imidazole-HCl pH 8.0. Protein-containing fractions were pooled, concentrated, and applied to a gel-filtration resin (Superdex-200 HR 10/30; GE Healthcare), previously equilibrated with 25 mM Tris-HCl pH 8.0, 200 mM NaCl, 5 mM DTT, and 0.2% DM. The peak fractions were collected and concentrated to 8 mg/mL for crystallization trials.

Expression and Purification of Artl. The artI gene was cloned from T. tengcongensis genomic DNA into a pet21b vector (Novagen) with a C-terminal 6× His tag. Overexpression of ArtI was induced in E. coli strain BL21(DE3) by 0.5 mM isopropyl-β-Dthiogalactoside when the cell density reached $OD_{600} = 1.2$. After growth for 10 h at 23 °C, the cells were collected; resuspended in Buffer A containing 25 mM Tris·HCl pH 8.0, 500 mM NaCl, and 20 mM imidazole; and lysed by sonication. After ultracentrifugation at $13,000 \times g$ for 1 h, the supernatant was loaded onto Ni-NTA resin. After a washing step using Buffer A, protein was eluted with Buffer A supplemented with 200 mM imidazole-HCl pH 8.0. Protein-containing fractions were pooled, concentrated, and subjected to gel filtration (Superdex-200; GE Healthcare) in 25 mM Tris pH 8.0, 200 mM NaCl, and 5 mM DTT. The peak fractions were collected and concentrated to 15 mg/mL for crystallization trials.

Denaturation and Renaturation of Artl. For ATPase assays, ArtI was subjected to a denaturation/renaturation procedure to remove prebound substrate (1). In brief, 10 mL of ArtI (1 mg/mL) was denatured by adding guanidinium hydrochloride to a final concentration of 6 M and subsequently dialyzed against 1L of buffer containing 25 mM Tris·HCl pH 8.0, 200 mM NaCl, and 6 M guanidinium hydrochloride for three times at an interval of 12 hours. Renaturation was achieved by dialysis against 1L of buffer containing 25 mM Tris·HCl pH 8.0 and 200 mM NaCl for five times at an interval of 12 hours. Subsequently, the dialysate was ultracentrifuged at 220,000 × g for 15 min to remove aggregated protein and concentrated by a centrifugal device (Amicon;

EMD Millipore). Glycerol and DTT were added to concentrations of 10% (vol/vol) and 10 mM, respectively.

Preparation of Proteoliposomes. Incorporation of Art(QN)₂ variants into liposomes prepared from *G. stearothermophilus* total lipids was carried out as described previously (2). In brief, lipids (20 mg) were dried under a stream of nitrogen, redissolved in 1 mL of 50 mM Mops-KOH pH 7.5, containing 1% n-Octyl-β-D-glucopyranoside (β-OG; Anatrace), and sonicated for 15 min. Subsequently, Art(QN)₂ variants (50 µg) were added to 125 µL of the lipid–detergent mixture, resulting in a final volume of 300 µL. Proteoliposomes were formed by removal of detergent by adsorption to Biobeads (100 mg; BioRad) at 4 °C overnight. After the beads were replaced with a new batch, incubation continued for another 2 h. The mixture was centrifuged for 1 min at 10,000 × *g* to pellet the beads. Then proteoliposomes were recovered by ultracentifugation for 30 min at 220,000 × *g*, resuspended in 50 mM Mops-KOH pH 7.5, and assayed for ATPase activity.

ATPase Assay. ATPase activity was assayed essentially as described previously (2). Reactions were started by adding 3 mM MgCl₂ and 2 mM ATP to preheated (70 °C) proteoliposomes in the presence of 50 mM Mops (pH 7.5), ArtI (35 μ M), L-arginine or L-histidine (100 μ M each), and *ortho*-vanadate (1 mM) (where indicated). Aliquots (25 μ L containing 3 μ g of protein) were taken in 2-min intervals and placed into wells of a microtiter plate containing 25 μ L of a 12% (wt/vol) SDS solution. The amount of liberated phosphate was determined colorimetrically with ammonium molybdate complexes using Na₂HPO₄ as the standard.

ITC Measurements. Calorimetric experiments were conducted at 25 °C with a MicroCal iTC200 instrument (GE Healthcare). The protein sample (ArtI, 22–260 aa) was dialyzed against the buffer containing 20 mM Hepes pH 7.5 and 200 mM NaCl. Arginine and other amino acids were prepared in the same buffer and the pH was checked before use. The data were fitted using the Origin 7 software package (Microcal).

Crystallization, Data Collection, and Structure Determination. Crystals were grown at 18 °C by the hanging-drop vapor diffusion method. Crystals of Art(QN)₂ complex appeared overnight in the well buffer containing 12% (wt/vol) PEG 4000, 0.1 M sodium chloride, 0.1 M sodium citrate tribasic dehydrate pH 5.0, 0.1 M magnesium chloride, and 8 mM 3-[(3-cholamidopropyl)dimethylammonio]-2-hydroxy-1-propanesulfonate (CHAPSO), typically growing to full size in ~3-4 d. Before data collection, crystals were transferred into a solution containing 2.5 mM ATP, 18% (vol/vol) glycerol, 12% (wt/vol) PEG 4000, 0.1 M sodium chloride, 0.1 M codium citrate tribasic dehydrate pH 5.0, 0.1 M magnesium chloride and 8 mM CHAPSO. Crystals of ArtI were grown in the well buffer containing 0.2 M sodium chloride, 0.1 M Bis-Tris pH 6.5, and 25% (wt/vol) PEG 3350.

All of the data were collected at the Shanghai Synchrotron Radiation Facility Beamline BL17U and integrated and scaled using the HKL2000 package (3). Further processing was carried out using programs from the CCP4 suite (4). Data collection statistics are summarized in Table S1. The molecular replacement was performed with PHASER (5) using *E. coli* Methionine ABC Transporter (PDB ID code 3DHW) as a model. The final model rebuilding was performed using Coot (6), and the protein structure was refined with phenix (7) using noncrystallographic symmetry and stereochemistry information as restraints. The structure

of ArtI was solved with molecular replacement using amino acid ABC transporter substrate-binding protein AbpA from

Streptococcus pneumoniae (PDB ID code 4I62) as a model, and structural figures were generated in PyMOL (8).

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Fig. S1. Representative views of the electron density of Artl in complex with Arg and $Art(QN)_2$ in complex with substrates. (*A*) The 2Fo-Fc electron density map, shown in blue mesh, is contoured at 1.5 σ , and the Fo-Fc electron density map, shown in green mesh, is contoured at 5 σ . (*B*) Electron density for the bound Arg. The 2Fo-Fc electron density map, shown in blue mesh, is contoured at 1.5 σ , and the Fo-Fc electron density map, shown in green mesh, is contoured at 3.3 σ . (*C*) Electron density for the bound His. The 2Fo-Fc electron density map, shown in blue mesh, is contoured at 1.5 σ , and the Fo-Fc electron density map, shown in green mesh, is contoured at 3.3 σ .

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I.tengcongensis_Arti	MNKKSLFLAF	AVVFALAFML	SGCGSKENTV	DQ1	KQKGVIVMGI	SADFPPFEFH	KVEGGKDEIV	GFDIDIANAI 73
G. stearothermophilus_ArtJ	RKGLFVAV	VAALLMALAA	CGGKSTETSS	S SGGDGG	ATKKKVVVGT	DAAFAPFEYM	QKGKIV	GFDVDLLDAV 71
S. enterica_HisJ	MKKLA	LSLSLVLAFS	SATAA	FA	AIPQKIRIGT	DPTYAPFESK	N AQGELV	GFDIDLAKEL 59
S. enterica_LAO	MKKTV	LALSLLIGLG	ATAAS	· · · · · YA	ALPQTVRIGT	DTTYAPFSSK	D AKGEFI	GFDIDLGNEM 59
G.caldoxylosilyticus_Artl	KKSLLLF	IASILLIGLL	SACGTGGQKN	G SG	EQKKVLKMGT	SADYAPFEYI	DTAK - GNDII	GFDVDLAKMI 69
B.licheniformis_ArtI	WMLL	VVTACVTFAL	TACGTSS	SS	DDKKTLVMGT	SADYPPFESK	D GDQ I V	GFDVDLANAL 59
E.acidaminophilum_ArtP2	- ILALVVSIS	LVFALTA - CG	KKDGASSD	AGKLEQI	KESGKLVLGT	SADYPPFEFH	KEINGKDEIV	GFDIEIAKAM 73
P.larvae_ArtP	ASVLSLV	LAGSLIAGCG	QKDKNAG	DAASTA	APNGKLVLAT	SADYPPYEFH	KLVDGKDSII	GFDIEIAKEI 70
								
T tenacongensis Artl	AKKIGVKIEL	KDMDEKGI IP	ALOAGRVDMV	AGMTPTAER	KKSVDESDLY	YDSROVVVVK	ND SPISKE	DDI KVKTIAV 151
G stearothermophilus Art.	MKAAGLDYEL	KNIGWDPLFA	SLOSKEVDMG	ISGITITDER	KOSYDESDEY	FEATOVILVK	QG SPVKNA	LDLKGKTIGV 149
S enterica His.	CKRINTOCTE	VENPLDALIP	SLKAKKIDAL	MSSLSITEKR	OOFIAFTDKL	YAADSRLVVA	KNS-DIOPTV	ASLKGKRVGV 138
S enterica LAO	CKRMOVKCTW	VASDEDALIP	SLKAKKIDAI	ISSLSITDKR	QOELAESDKL	YAADSRLIAA	KGS - PVOPTL	ESLKGKHVGV 138
G caldoxylosilyticus Artl	AKELGYELOL	VDMDETGLIP	ALOSGKVDEV	LAGMTPTEKR	KKSVDESDVY	YVARNMIVSK	KG SGIKTV	EDLKGKTVGV 147
B licheniformis Art	AFKTGHELEV	KDMDENGLVT	ALKTNKVDIV	ISGMTPTPKR	KKOVDESNVY	YTAHNMIVTK	KS SGIKSI	DDI KGKTVGV 137
E acidaminophilum ArtP2	AKELGVELEI	KDMKEDGLLA	ALSTGKVDIV	LAGMNPTEDR	KKAVDESOLY	YREVOSVVVR	TESADLIKTL	DDLKGKKVAV 153
Planae ArtP	AKDMGKELEV	KDMRYDALLA	ALOSGTADIV	AGMTPTPER	OKNYDESDLY	YTAOHAVVTL	FENKDKYKNP	EDLKGKKIVI 150
	-				-			
T tongoongonoio Arti	OLOTTOEEAA					KAVIKE		DELNNVENCE 222
G stearethermonbilus Art I	ONATTGOEAA	EKLEGKOP	HIKKEETTVV	AIMELLNCCV	DAVITONAVA	NEVVKN		EDDKNEASEV 222
G. stearothermophilus_Arts	LOCTTOETEC	NEHWARKO I	FIVEVOCODN	LYSDLTACRI	DAAEODEVAA	SECEL KORVO	KDYKECODAV	KDEKLEOVOT 217
S. enterica_HISJ	LOGSTOFAYA	NEHWAPKG-1	DVVAVANODI	I YSDL TACRI	DAALODEVAA	SEGELKOPAG	KEYAEAGPSV	KDEKLFGVGT 217
S. enterica_LAO	OTOSIQEATA	NUNWRING-V	KIESPNRIDE	LIGELOACRE	DAALLEDTVA	SEGFLKQPAG	NCKLECHTM	RURATFODGT 21/
G.caldoxylosilylicus_Arti	QIGSIQEGEA	KELTROVN I	KVENDNDISD	LTEELKACRE	DAALLEDIVA	KUTLKNS	-NGRLEGHTM	PIS-EQEAGS 221
B.lichenilormis_Arti	OKOTTMEAMA	COUMAESE	KVENKNKISU	LIEEIKAGKF	DAATTEUTVA		-NEELVGINL	FREPDERAGS 211
E.acidaminophilum_ArtP2	OKCELOEELA	KR LECAR	- INGLORVID	VVLELKNKKV	DASLIEKDVA		-NPELMLIEI	ELS-PEDIGF 224
P.larvae_ArtP	UKGSTUEETA	KK-TEGAD	- LUELGKIGD	LIQELKNKKA	DASTIERPVA	ANTVKA	-NKGLATIDL	ILQ-AEDAGS 220
-					000			
I.tengcongensis_Arti	AVAVAKGNKS	LLDVVNEVIK	ELKQSGEYDK	LVDKWFKQ	260			
G. stearothermophilus_ArtJ	TGMTFPKNSE	LKAKVDEALK	NVINSGRYTE	TTKKWFGKEP	KLDKLKQ 269			
S. enterica_HisJ	GMGLRKEDNE	LKEALNKAFA	EMRADGIYEK	LAKKYFDFDV	TGG 260			
S. enterica_LAO	GVGLRKDDTE	LKAAFDKALT	ELRODGIYDK	MAKKYFDFNV	TGD 260			
G.caldoxylosilyticus_ArtI	ATAFPKGSK-	LKDEFNKVLQ	EKMKNGEVDK	LIKKWFDQ	258			
B.lichenitormis_ArtI	ATAFKKGSG-	LIEKFNKALE	EMEKSGELDK	LKEKWF	246			
E acidaminophilum ArtP2		VAKTNELLK	STTDOGKIDE	FV	256			

Fig. 52. The residues participating in substrate binding are conserved in Artl homologs from different species. Shown is the sequence alignment of Artl homologs from representative organisms. Conserved residues are highlighted in blue. The key residues involving in substrate recognition are indicated by red triangles. Sequence alignment was carried out using ClustalW (9).

Plarvae ArtP AVAIKKGNKE LVDQVNKTLK RLKDEKKMDQ FIEEATKQ -- ---- 258



Fig. S3. Artl displays different binding affinities for Arg and His. (*A*) ITC curves of Arg titrated into unloaded Artl. Pink trace, 500 μ M arginine titrated into 35 μ M protein; blue trace, 500 μ M arginine titrated into buffer. The K_d value of arginine titrated into buffer was not detectable. The first peak in the thermogram was not used for analysis. (*B*) ITC curves of histidine, glutamate, and threonine titrated into unloaded Artl. Pink trace, 2 mM histidine titrated into buffer; green trace, 1 mM glutamate titrated into 100 μ M protein; purple trace, 1 mM of threonine titrated into 100 μ M protein. K_d values of glutamate and threonine titrated into unloaded Artl, as well as histidine titrated into buffer, were not detectable. The first peak in the thermogram was not used for analysis.



Fig. 54. $Art(QN)_2$ binds to two substrates and two ATPs. (A) Ribbon diagram of TM helices surrounding two substrate binding sites viewed along the membrane bilayer from the extracellular side (left) or from cytoplasmic side (right). ArtQ subunits are colored in cyan and magenta. Two Arg molecules are shown in ball-and-stick models. (B) Overall structure of the ArtN dimer in complex with ATPs. The Walker A motif (residues 34–41) is colored blue, the Walker B motif (residues 157–162) is colored magenta, and the ABC signature motif (residues 137–146) is colored orange, ArtN subunits are colored gray and green, respectively. Two ATP molecules are shown in ball-and-stick models. (C) Representative view of the electron density of ATP-bound Art(QN)₂. The 2Fo-Fc electron density map, shown in blue mesh, is contoured at 1.5 σ , and the Fo-Fc electron density map, shown in green mesh, is contoured at 3 σ .



T.terigeorigenais_Arter	A0D 11 - 220	
G. stearothermophilus_ArtM	YSTQ 218	
S. enterica_HisM	QHVSSK- 235	
S. enterica_HisQ	VKRADL - 228	
D.alkaliphilus_HisM	TRIRD 220	
Y.fragilis_ArtQ	MSTDDRG 218	
P.larvae_HisM	IKRA 217	
PaenibacillusspArtQ	MGQR 217	
C.subterraneus_HisM	AGD R 220	
T.siderophilus_HisM	AGD R 220	

Fig. S5. The residues involved in substrate binding are conserved in ArtQ homologs from different species. Sequence alignment of ArtQ homologs from representative organisms. ArtQ^{P66}, ArtQ^{L67}, ArtQ^{N98}, ArtQ^{Y102}, ArtQ^{E152}, ArtQ^{V155}, ArtQ^{E159}, and ArtQ^{M156} residues involved in interacting with substrate are highly conserved. Those residues are highlighted in blue and indicated by red triangles. Invariant amino acids are highlighted in blue. Sequence alignment was carried out using Clustal W (9). Secondary structural elements of ArtQ are indicated.



Fig. S6. The residues of ArtQ involved in substrate binding are conserved in the HisM/HisQ subunits of the His-, Lys-, Arg-transporter from *Salmonella enterica* serovar Typhimurium (S. Typhimurium). (A) Three-dimensional structure of HisM/HisQ from S. Typhimurium based on the structure of ArtQ. Superposition of ArtQ (cyan), HisM (pink), and HisQ (orange). Representative models of the residues from the substrate-binding site as well as Arg (R) /His (H) are shown in ball-and-stick models. (*B*) Stereoview of the structural alignment between ArtQ (cyan) and HisM (pink). In ArtQ and HisM, residues that mediate substrate binding are shown as cyan and pink sticks, respectively. Invariant and variant residues are indicated in black and magenta, respectively. (C) Stereoview of the structural alignment between ArtQ (cyan) and HisQ, residues substrate binding are shown as cyan and orange sticks, respectively. Invariant and variant residues are indicated in black and magenta, respectively. (C) Stereoview of the structural spectively. Invariant and variant residues are indicated in black and magenta, respectively.





Superposition of ArtN (cycan) and MalK (magenta) in open state.

Fig. 57. The ArtN dimer with two bound ATPs is in "semi-open" state. (A) Stereo views of the structural alignment between the ArtN dimer and the semi-open state of the MalK dimer of the maltose transport system from *E. coli* (PDB ID code 3PV0). The MalK dimer is in orange, and the ArtQ dimmer is in cyan. (*B*) Stereo views of the structural alignment between the ArtN dimer and the open state of the MalK dimer (PDB ID code 3FH6). The MalK dimer is in magenta, and the ArtQ dimer is in cyan.



Fig. S8. No obvious changes are seen in apo-, ATP-, Arg-, His-, and Arg/ATP-bound states of Art(QN)₂. Shown is structural alignment among apo-bound (purple), ATP-bound (blue), Arg-bound (yellow), His-bound (orange), and Arg-/ATP-bound (cyan) states of Art(QN)₂.

Table S1. Data collection and refinement statistics

PNAS PNAS

Type	Apo-bound Art(QN) ₂	Arg-bound Art(QN) ₂	Arg/ATP-bound Art(QN) ₂	ATP-bound Art(QN) ₂	His-bound Art(QN) ₂	Arg-bound Artl
Data collection statistics	(beamline: SSRF-BL17U; det	ector: ADSC Quantum 315r; so	oftware: HKL2000)			
Space group Unit cell	C2221	C2221	C2221	C2221	C2221	P1
a, b, c, Å	96.096, 114.209, 302.019	96.379, 114.779, 300.202	96.765, 114.098, 298.636	96.527, 115.257, 301.546	96.247, 115.264, 301.185	39.026, 41.409, 75.604
α, β, γ, °	90, 90, 90	90, 90, 90	90, 90, 90	90, 90, 90	90, 90, 90	103.73, 102.72, 90.12
Wavelength, Å	0.9793	0.9793	0.9793	0.9793	0.9793	0.9789
Resolution, Å	41.54–2.8 (2.9–2.8)	49.8–2.59 (2.68–2.59)	49.50-2.50 (2.59-2.50)	43.5-3.00 (3.11-3.00)	34.72–2.80 (2.90–2.80)	30.68-1.48 (1.53-1.48)
R _{merge} , %	11.3 (65.0)	9.2 (63.6)	8.9 (82.4)	10.2 (55.4)	9.5 (49.0)	4.1 (12.0)
l/sigma	15.9 (2.14)	34.7 (3.0)	19.2 (1.71)	22.3 (2.2)	22.0 (2.1)	28.0 (10.3)
Completeness, %	95.59 (79.30)	99.8 (100)	99.3 (94.8)	98.7 (91.6)	92.9 (74.4)	95.9 (85.9)
Redundancy	6.7 (5.4)	6.8 (6.2)	5.9 (4.9)	5.5 (4.6)	4.3 (3.6)	2.2 (2.1)
Structure refinement sta	atistics (software: phenix.refi	ne)				
R _{work} /R _{free}	22.8/27.6	22.2/26.5	22.4/26.0	20.6/25.3	22.6/27.1	15.5/18.3
No. of atoms						
AII	7,046	7,086	7,134	7,108	7,068	4,508
Side chains	3,410	3,438	3,426	3,410	3,424	1,845
Main chains	3,636	3,648	3,644	3,636	3,644	1,916
Macromolecule	7,046	7,086	7,070	7,046	7,068	3,761
Other entities	0	65	64	62	0	747
Average B-factor, Å ²						
AII	92.7	73.2	88.1	93.0	86.5	12.8
Side chains	93.6	73.9	88.9	93.3	87.2	12.2
Main chains	91.9	72.5	87.4	92.0	85.9	8.0
Macromolecule	92.7	73.2	88.1	92.6	86.5	10.0
Other entities		73.2	85.5	132.2		27.0
Rmsd						
Bond lengths, Å	0.010	0.01	0.009	0.010	0.009	0.006
Bond angles, $^{\circ}$	1.33	1.26	1.28	1.29	1.24	1.003
Ramachandran						
Favored, %	94.0	96.5	97.0	96.23	96.67	98.52
Outliers, %	0.11	0.22	0.00	0.00	0.00	0.52
PDB ID codes	4YMS	4YMT	4YMU	4YMV	4YMW	4YMX
Values in parentheses f_{calc} is the calculated prot	are for the highest-resolution stein structure factor from the a	hell. $R_{merge} = \Sigma_h \Sigma_i I_{h,l} - I_h / \Sigma_h \Sigma_l I_h$ itomic model (R_{free} was calculat	<i>i</i> , where <i>l_h</i> is the mean intensity ed with 5% of the reflections).	of the <i>i</i> observations of symme	try-related reflections of h . $R =$	$\Sigma F_{obs} - F_{calc} /\Sigma F_{obs},$ where