

Appendix

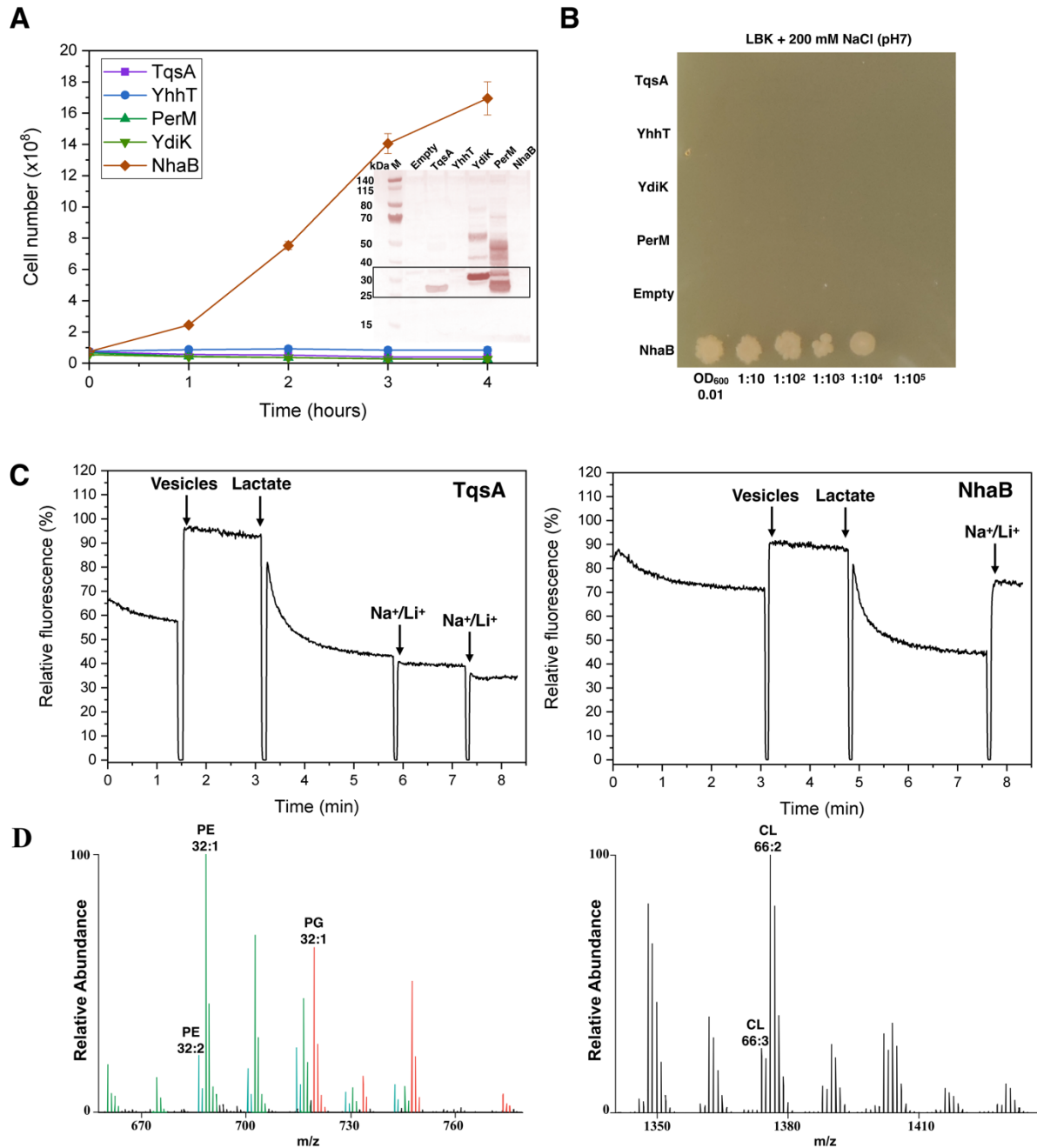
Cryo-EM structures of pentameric autoinducer-2 exporter from *E. coli* reveal its transport mechanism

Radhika Khera¹, Ahmad R Mehdipour^{2,3}, Jani R Bolla^{4,5,6}, Joerg Kahnt⁷, Sonja Welsch⁸, Ulrich Ermler¹, Cornelia Muenke¹, Carol V Robinson^{4,5}, Gerhard Hummer^{2,9}, Hao Xie^{1*}, Hartmut Michel^{1*}

* Address correspondence to Hao Xie, Hao.Xie@biophys.mpg.de or Hartmut Michel, Hartmut.Michel@biophys.mpg.de

Table of Contents:

Appendix Figure S1-	page 2
Appendix Figure S2-	page 4
Appendix Figure S3-	page 5
Appendix Figure S4-	page 6
Appendix Figure S5-	page 8
Appendix Figure S6-	page 10
Appendix Figure S7-	page 11
Appendix Figure S8-	page 12
Appendix Figure S9-	page 13
Appendix Figure S10-	page 14
Appendix Table S1-	page 18



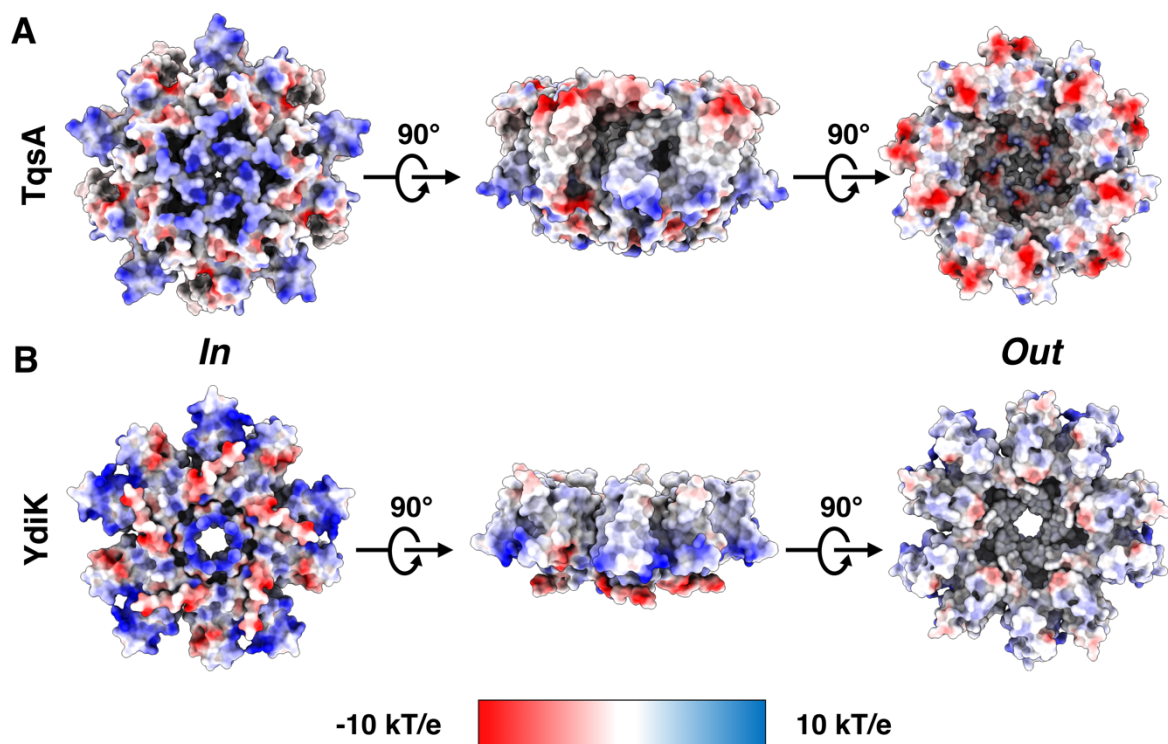
Appendix Figure S1- Sodium proton antiporter assays and lipidomic analysis of purified TqsA.

A Growth complementation sodium proton antiporter assay in LBK medium with 200 mM NaCl. The starting OD₆₀₀ of all the cultures was maintained around 0.06-0.07. Western blot analyses for the expression of the exporters are also included in the plot. *E. coli* NhaB couldn't be detected via Western blot either due to low expression levels or inaccessible Strep-II tag.

B Similar growth complementation sodium proton antiporter assay on LBK agarose medium with 200 mM of NaCl with *E. coli* NhaB as a positive control.

C Acridine orange assay profiles for AI-2 exporter TqsA on the left and the positive control *EcNhaB* on the right.

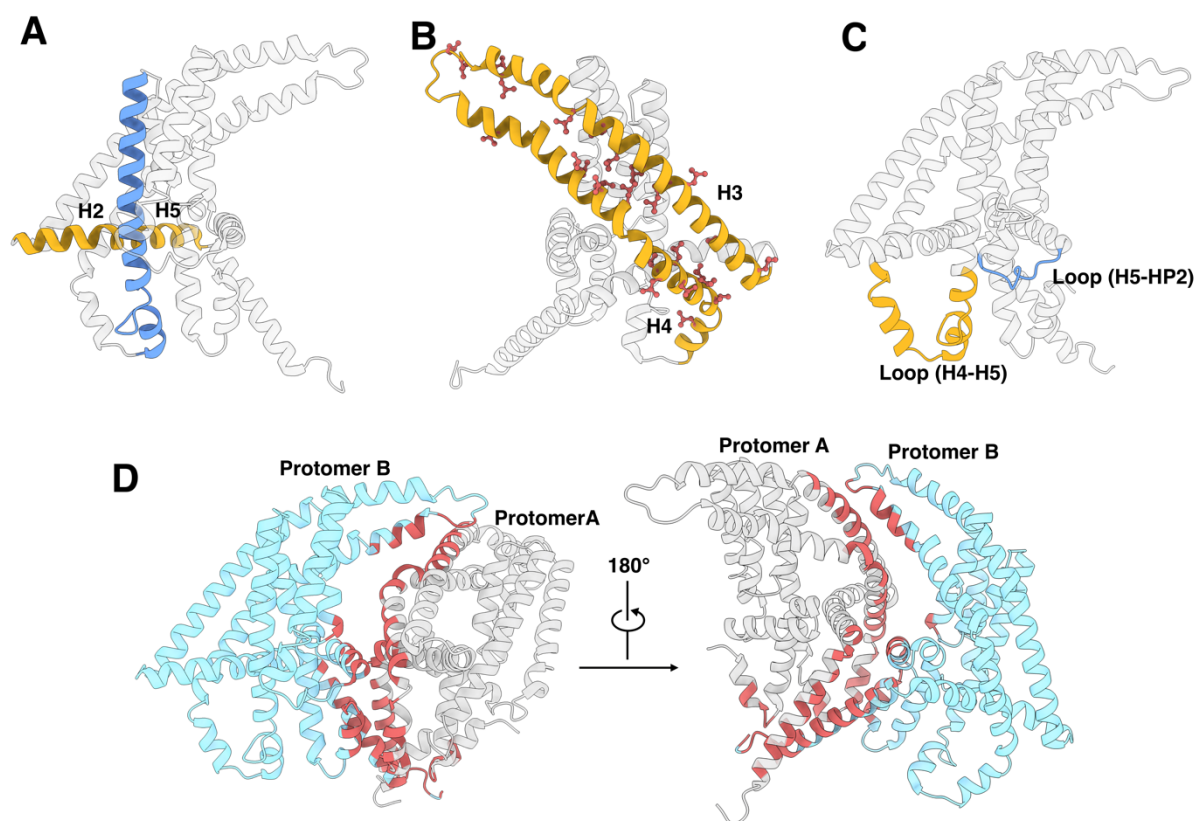
D Lipidomic analysis of GDN-purified TqsA. *E. coli* lipids, phosphatidylethanolamine (PE), Phosphatidylglycerol (PG) and cardiolipin (CL) were observed.



Appendix Figure S2- Electrostatic potential surface maps of TqsA and YdiK.

A, B Electrostatic surface potentials of TqsA (**A**) and YdiK (**B**) calculated by APBS are colored ranging from blue (+10 kT/e) to red (-10 kT/e).

C Close-up view on the charges around the docking site of AI-2 in TqsA.



Appendix Figure S3- Structural features of Al-2 exporter, TqsA.

A Cross shaped architecture formed by helices H2 and H5 at the interface of scaffold and transport domains in TqsA is highlighted. Yellow is used for the scaffold domain (N-half) and blue for the transport domain (C-half).

B “Leucine zipper like motif” covering the tilted antiparallel helix bundle (H3 and H4) of the TqsA monomer is illustrated in this subsection.

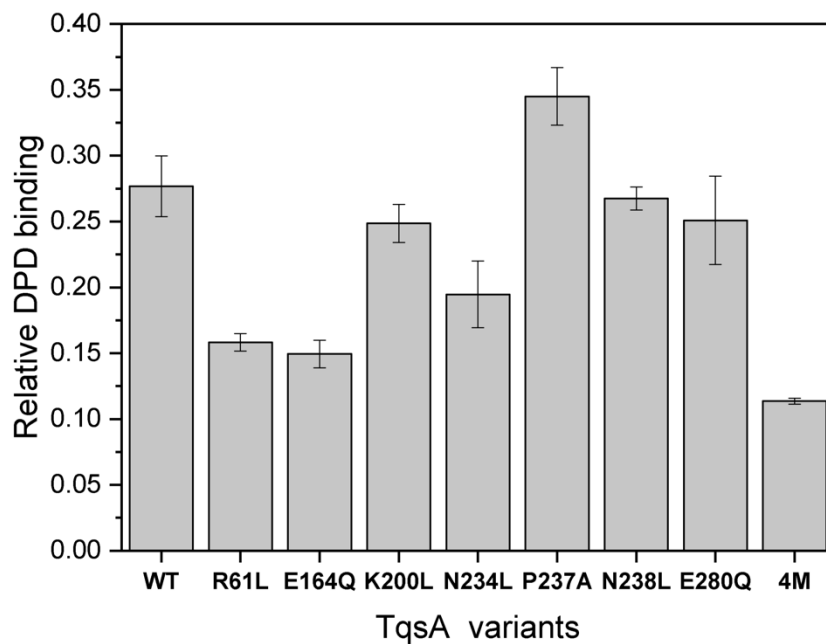
C Loops suspected essential for the elevator motion of the transport domain in TqsA are focused on in the figure.

D The dimer interface within the homo-pentameric complex is highlighted in the figure for TqsA. The interacting residues are shown in red. The major interacting residues are located at the N- and C- terminus of TqsA. Loops connecting helices H3-H4 and H4-H5 also appear essential. The dimer interface and the interacting residues were calculated using PDBePISA. The estimated solvation free energy ΔG for the formation of the dimer interface is ~ 39.7 kcal/mol.

A

Location on TqsA structure	Motif B H5	Motif C HP1	Motif D Loop (H6-HP2)
<i>E. coli</i> (TqsA)	Y L V L K 200	N Y I P N238	G N I L E P R I M G R G L288
<i>H. andaensis</i> (Upf0118)	F I K A Q 217	D F V P I255	R R T V E P K V M G R H I304
	↓ pH response	↓ Na ⁺ /H ⁺ antiporter activity	

B

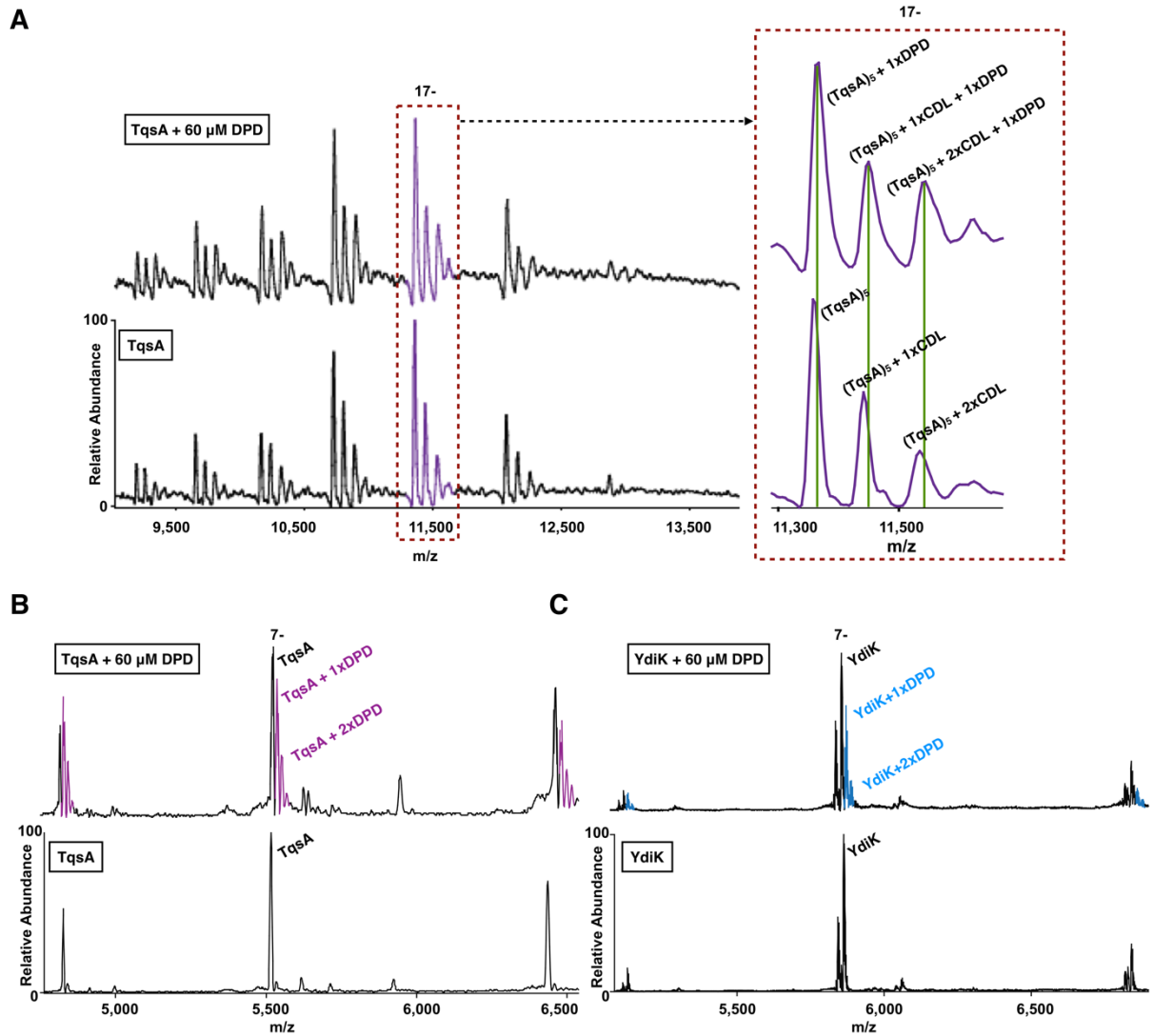


Appendix Figure S4- Upf0118 critical motifs placement and Native-MS on TqsA variants

A A sequence comparison of TqsA and Upf0118 is shown, especially focusing on the motifs harboring functionally critical residues of Upf0118. The residues reported essential for the transporter activity of Upf0118 are highlighted in green and the residue K215 essential for pH response of Upf0118 member is shown in red (Wang *et al*, 2020).

B Relative AI-2 binding study on TqsA variants using Native-MS is shown in the figure. The samples include the wild type represented as WT, single mutants, and a quadruple mutant

with multiple mutations near the proposed binding site shown as 4M which comprises four mutations N234L, P237A, N238L and E280Q.

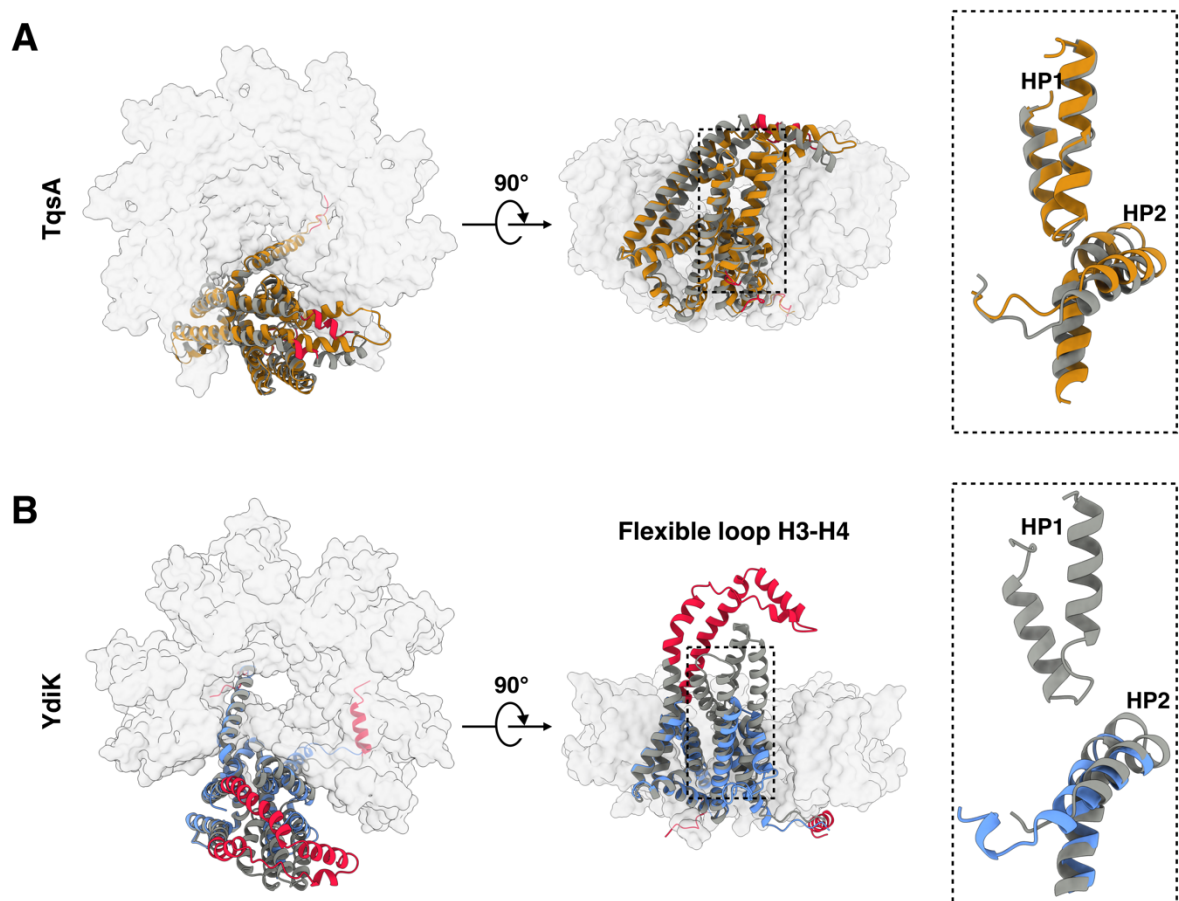


Appendix Figure S5- Interaction analysis of AI-2 exporters with AI-2/DPD using Native-MS.

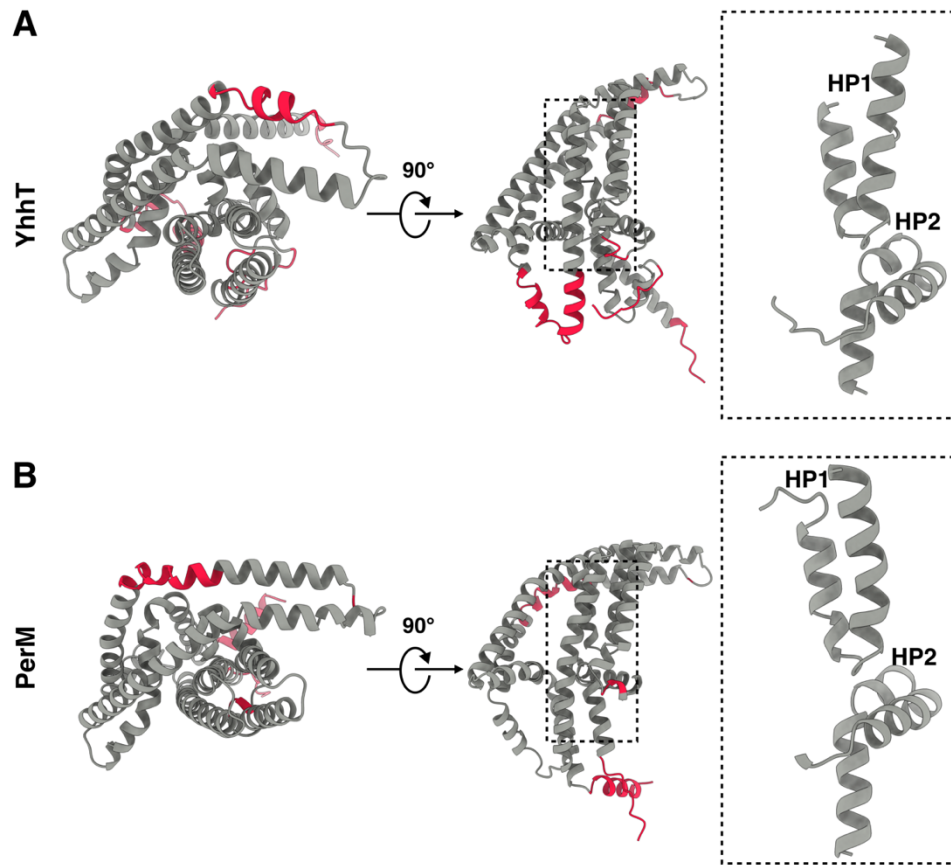
A Two spectra for TqsA are shown in the figure, the upper one after the addition of DPD and the lower one devoid of any substrate. The peaks corresponding to the pentameric TqsA in both spectra are highlighted in purple. For clarity, a zoomed-in view of the 17- charge state is shown on the right as an inset. CDL in the figure represents a cardiolipin molecule. The binding of DPD to pentameric TqsA is evident from the shift in the peak centre.

B The spectra of TqsA in C8E4 detergent with and without the addition of AI-2 show that the protein is majorly in the monomeric form and the additional peaks developed around it in the upper spectrum are highlighted in purple which indicates AI-2 interaction with the protein.

C The spectra of YdiK in C8E4 also showed a similar behavior that was observed in the case of TqsA in b, further confirming that YdiK also binds to AI-2 (highlighted in blue).



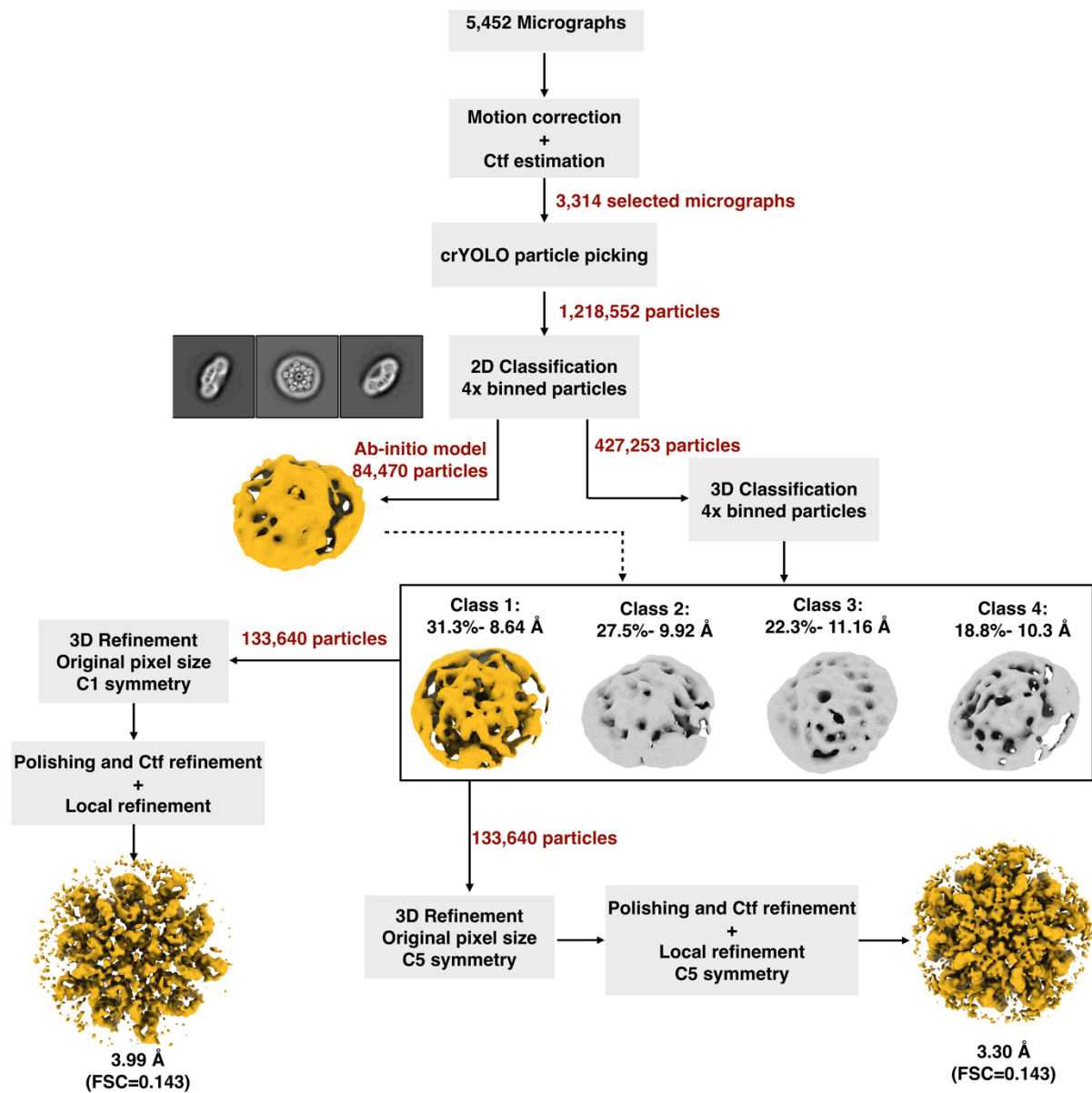
Appendix Figure S6- Comparison between cryo-EM and AlphaFold predicted structures of Al-2 exporters: A, B: Two orthogonal views of superimposition of the cryo-EM and AlphaFold predicted monomer structures of TqsA (**A**) and YdiK (**B**). The monomer structure is shown as a ribbon diagram in the pentameric complex. Cryo-EM structures of TqsA and YdiK are shown in brown and blue respectively. The predicted structures are shown in grey and low confidence areas with a per-residue confidence score lower than 70 are highlighted in red. The magnified view of the superimposed helical hairpins is shown on the right for both proteins.



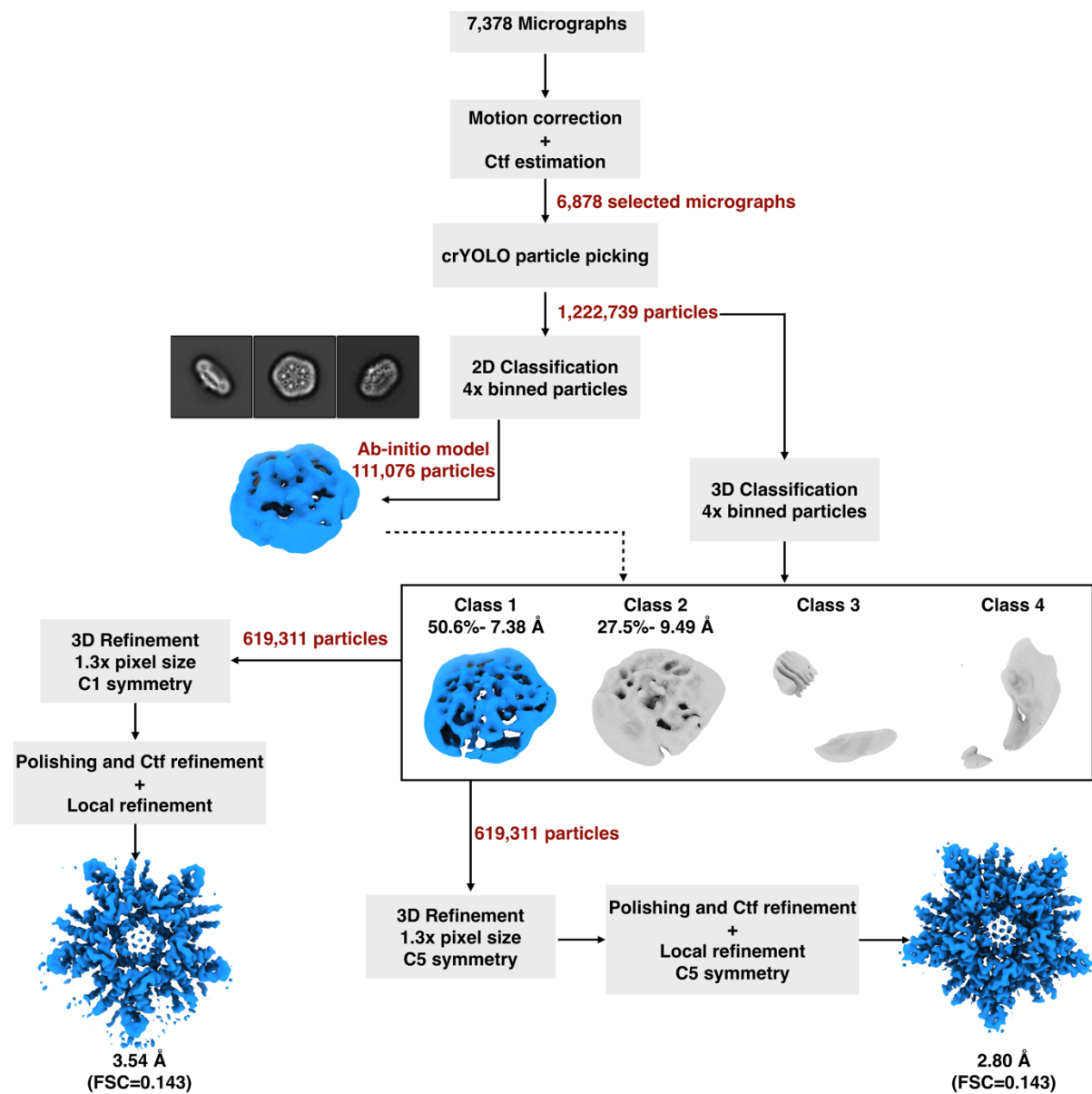
Appendix Figure S7- AlphaFold predicted monomer structures of YhhT and PerM.

Both the predicted structures are shown in grey with two different views, **A:** YhhT and **B:** PerM.

The low confidence areas in the structures are highlighted in red. The helical hairpins for both the predicted models are shown magnified on the right.



Appendix Figure S8- TqsA single particle processing workflow. Detailed single particle cryo-EM workflow adopted for TqsA dataset is described.



Appendix Figure S9- YdiK single particle processing workflow. Detailed single particle cryo-EM workflow adopted for YdiK dataset is shown.

	10	20	30	40	
<i>TqsA_Ec/1-344</i>	-----	-----	-----	MAKPIITLNGLKIVIM	16
<i>YhhT_Ec/1-349</i>	-----	-----	-----	METPQPDKTGMHILLK	16
<i>PerM_Ec/1-353</i>	-----	-----	-----	MLEMLMQWYRRRFSDPEA	18
<i>BARG_01524_Ba/1-658</i>	---MVKKPTPARQTVTKTIRQSETIGRNGDIHVNVEVESFSGASV	42			
<i>YueF_Bs/1-369</i>	-----	-----	-----	MLKSKVHFWTLQILFV	16
<i>F8FLY5_Pm/1-357</i>	-----	-----	-----	MDRFSKNSLLLWMVYMLLA	19
<i>Upf0118_Ha/1-352</i>	-----	-----	-----	MFRLSKRQWILL	13
<i>YtvI_Bs/1-371</i>	-----	-----	-----	MNQSYITIFFRTLFI	16
<i>Yct2_Bp/1-385</i>	MNKVNKINAYTCSSFQLHTIMKISFYVVERGFELAAFFTRRTIWI	46			
<i>Q188A3_Cd/1-363</i>	-----	-----	-----	MNEQDHTSYQNKQRFLLNFCYLVV	24
	50	60	70	80	90
<i>TqsA_Ec/1-344</i>	LGMVLVILCG-----	IRFAAEITVPFILALFIAVILNPLVQHMV	55		
<i>YhhT_Ec/1-349</i>	LASLVVILAG-----	IHAADIIIVQLLLALFFAIVLNPLVTFWI	55		
<i>PerM_Ec/1-353</i>	IALLVILVAG-----	FGIIFFSGLLAPLLVAIVLAYLLEWPTVRLQ	60		
<i>BARG_01524_Ba/1-658</i>	RRQAGFWLGAMAFFVFLFVLVFSVLLPFVAGMALAYFLDPVADRLE	88			
<i>YueF_Bs/1-369</i>	LLIIFVATKVSFVFQPFIVFISTLFFPMLIAGILYFINPVVRL-	61			
<i>F8FLY5_Pm/1-357</i>	LIILFMLQQLRPLFVGIIYQFLREILTPFIAMIISYVLNPVVTLLG	65			
<i>Upf0118_Ha/1-352</i>	LLGILFVVAG-----	YFILPVSVPILIALITALLFNPAVRWMQ	51		
<i>YtvI_Bs/1-371</i>	SMTAGSIAAA-----	YYSFPLTYPFLIALILSSVIHPVVDYLD	54		
<i>Yct2_Bp/1-385</i>	ISILLFLVAA-----	YFILPVSPLVAALLTALILTAVNALQ	84		
<i>Q188A3_Cd/1-363</i>	ILGLLMLLKGK-----	YAFSVLLPFVFAFLIASLLNKPVLVYIS	61		
	100	110	120	130	
<i>TqsA_Ec/1-344</i>	-RWRVPRLAVSILMTIIVMAMVLLLAYLGSALNELTRTLPQYRNS	100			
<i>YhhT_Ec/1-349</i>	-RRGVQRPVAITIVVVVMLIALTALVGVLAASFNEFISMLPKFNKE	100			
<i>PerM_Ec/1-353</i>	-SIGCSRWATSIVLVVFGILLMAFVVLPIAWQQGIYILIRDMPG	105			
<i>BARG_01524_Ba/1-658</i>	-KVGLSRLAASIVILLIFLMILIGLMIVVPILATQLADFI SNLPG	133			
<i>YueF_Bs/1-369</i>	-EKKIPRTL SILLIYLLFI GLLAFISASVGP IITAQVTGLFNNLPD	106			
<i>F8FLY5_Pm/1-357</i>	-ARKVPRTI AVLIIYAVFITSTTVVLMNVI PMFVRQLAELNEHMPD	110			
<i>Upf0118_Ha/1-352</i>	FRFRLNRKMAVTIVFLLFVIMIGLLGTAYAVTRAVTQLVELADNAPS	97			
<i>YtvI_Bs/1-371</i>	KVTGFPRTINVLGVLAFFLLAAFGLVLTILVAEIVTGTAYLAKTLP	100			
<i>Yct2_Bp/1-385</i>	RKTKIKRNVAVMLVFTVFVVFGLSGGYIATKAITQGTQIVENSPQ	130			
<i>Q188A3_Cd/1-363</i>	EKLLIKRGIVATISVFLFFLIAGIIVSVIGTYLIYGIKEIFHFLPT	107			
	140	150	160	170	180
<i>TqsA_Ec/1-344</i>	IMTPLQALEP LLQ-----	RVGIDVSVDQLAHYIDPNAAMTLL	137		
<i>YhhT_Ec/1-349</i>	LTRKLFK LQEMLP-----	FLNLHMSPERMLQRMDS EKVVFT	137		
<i>PerM_Ec/1-353</i>	MLNKLSDFAATLPR-----	RYPALMDAGIIDAMAENMR SRMLTM	144		
<i>BARG_01524_Ba/1-658</i>	YITQLQSLLANRDS EWLK---	KYIGIDSTVIQQNLSSLLQQGAGFL	176		
<i>YueF_Bs/1-369</i>	YIKQIQALT KDLS-----	HSQWFTWMMNQDY-VSISKIEQSL	142		
<i>F8FLY5_Pm/1-357</i>	VAYKAQSVVQNMN-----	ELQFLPDSVRTGINQSLSKLENGI	147		
<i>Upf0118_Ha/1-352</i>	YINQINNVLINWQN-NMN---	SFTQNMPSEFVDKVSVELQNTIDT	139		
<i>YtvI_Bs/1-371</i>	HISTFISYCEKLFTHIQLPYNELTLLFQELETNQQASIVTHIQT	146			
<i>Yct2_Bp/1-385</i>	YISDINRAWLNFR-NLE---	EKYENLPPELVQEINITVTNTLSDL	172		
<i>Q188A3_Cd/1-363</i>	MFEELVIPLETETVI-----	KEVELFSGSFNFSFIELIEANMPVL	146		
	190	200	210	220	
<i>TqsA_Ec/1-344</i>	TNLLTQ----LSNAMS S-----	IFLLLLTVLFMLLE	164		
<i>YhhT_Ec/1-349</i>	TALMTG----LSGAMAS-----	VLLLVMTVVFMLE	164		
<i>PerM_Ec/1-353</i>	GDSVVKIS--LASLVGLLTIAVY-----	LVLP LMVFFLLKD	179		
<i>BARG_01524_Ba/1-658</i>	STLLQS----LWNSGKSLIDIAG-----	LFVVTPVVAFYMLD	210		
<i>YueF_Bs/1-369</i>	TSFLQN----LPQNI TSSLSAVFGVVNTLV IITVPFILFYMLKD	184			
<i>F8FLY5_Pm/1-357</i>	SLAIAN----YINSIGSTINTLF-----	LIFIIPFVAFYILKD	181		
<i>Upf0118_Ha/1-352</i>	TQTLSQKL--QLSNIAAFAAKIP----	EYLI SFLVYLIALFLFMLE	179		
<i>YtvI_Bs/1-371</i>	GDSAAKNAGLLSHILEMIPRFALLPNTAAVLIFSLLATTFMTKD	192			
<i>Yct2_Bp/1-385</i>	RSNISDRN--LIQDITSLISSIP----	GYLVTFLVYLIALFLFMLE	212		
<i>Q188A3_Cd/1-363</i>	MNEMSVFIVLSNIN VSGITDIVSSVPLLFMKTIITIVASIFIAID	192			

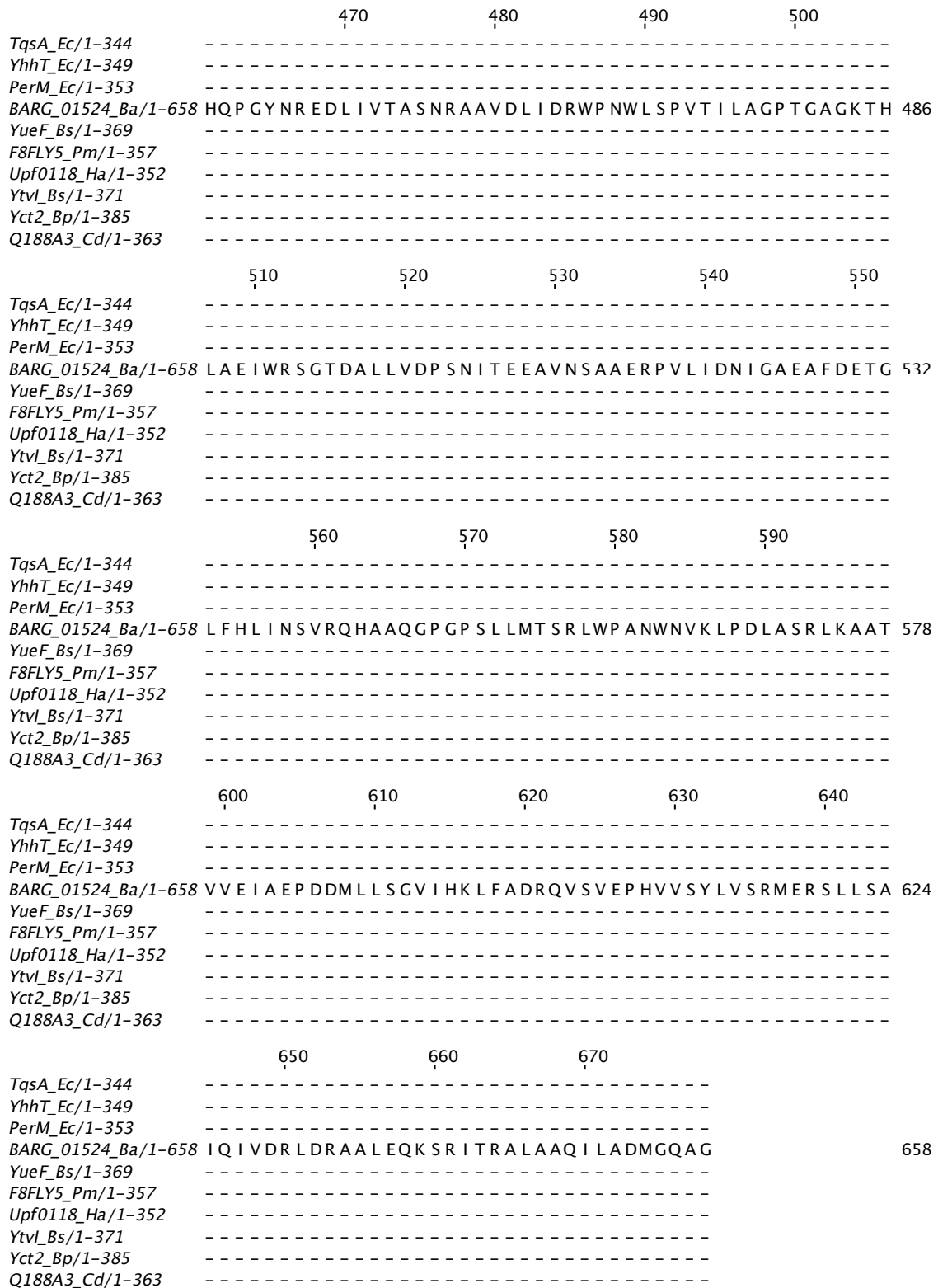
	240	250	260	270	
<i>TqsA_Ec/1-344</i>	V P Q L P G K F Q Q M M A R -- P V E G M A A I Q R A I D S V S H Y L V L K T A I S I I T G	208			
<i>YhhT_Ec/1-349</i>	V R H V P Y K M R F A L N N -- P Q I H I A G L H R A L K G V S H Y L A L K T L L S L W T G	208			
<i>PerM_Ec/1-353</i>	K E Q M L N A V R R V L P R N - R G L A G Q V W K E M N Q Q I T N Y I R G K V L E M I V V G	224			
<i>BARG_01524_Ba/1-658</i>	W D R M V N S I D S W V P R K Q L H T V R R I A R E M N A A V A G F I R G Q G T L C L I L G	256			
<i>YueF_Bs/1-369</i>	G H R F P H L A V K I L P A S Y R T E G L K I F K D L S D T L A A Y F Q G Q L L I C L F V G	230			
<i>F8FLY5_Pm/1-357</i>	F Q L L E K T T L A I V P R N H R K E I V S M L M D I D T A L G N Y I R G Q F T V C M I V G	227			
<i>Upf0118_Ha/1-352</i>	L P R L K D K M H G N F T E S T S E K V K F M N A R L S Y V V F G F L K A Q F L V S I V I F	225			
<i>YtvI_Bs/1-371</i>	W H K L K A M L V L I L P D R V T A N S K A I S S E L K K A M T G F I K A Q A V L V F I T M	238			
<i>Yct2_Bp/1-385</i>	L P R L R E K L Y S Y L S E R T K E K V N F M T S R L S Y V I W G F F K A Q F L V S I I I F	258			
<i>Q188A3_Cd/1-363</i>	Y P T I K R F I V L Q I P K N K S Y L L A E A K A F T I D T I I K C G F S Y L L I F A I T F	238			

	280	290	300	310	320	
<i>TqsA_Ec/1-344</i>	L V A W A M L A A L D V R F A F V W G L L A F A L N Y I P N I G S V L A A I P P I A Q V L V	254				
<i>YhhT_Ec/1-349</i>	V I V W L G L E L M G V Q F A L M W A V L A F L L N Y V P N I G A V I S A V P P M I Q V L L	254				
<i>PerM_Ec/1-353</i>	I A T W L G F L L F G L N Y S L L L A V L V G F S V L I P Y I G A F V V T I P V V G V A L F	270				
<i>BARG_01524_Ba/1-658</i>	T Y Y A I G L T L T G L N F G L L I G F F A G L I S F I P Y I G S F V G L A L A I G V A L V	302				
<i>YueF_Bs/1-369</i>	T A C F I G Y L I A G L P Y A L I L G I V M A I T N I I P Y V G P F L G A A P A V I V G F M	276				
<i>F8FLY5_Pm/1-357</i>	L L A Y I G Y W L I G M P Y A L L A C V V A V F N I I P Y L G P F L G A A P A L I V A A T	273				
<i>Upf0118_Ha/1-352</i>	V V C L I G L F W I T P E V A I V M S L I I W I V D F V P I I G S I V I L G P W A L Y M L I	271				
<i>YtvI_Bs/1-371</i>	V I V F I G L S L L K V E H A A T I A F L I G L V D L L P Y L G A G S V F V P W I L Y L S I	284				
<i>Yct2_Bp/1-385</i>	I V T L I G L L F I A P E V A L L M A F I I W L I D F V P I I G S I V I L A P W A I F Q L I	304				
<i>Q188A3_Cd/1-363</i>	S E L Y V G F V I I N I S Y A G I I A L F I A L L D I L P V L G T G S I L I P W C I I S L F	284				

	330	340	350	360	
<i>TqsA_Ec/1-344</i>	F N G -- F Y E A L L V L A G Y L L I N L V F G N I L E P R I M G R G L G L S T L V V F L S	298			
<i>YhhT_Ec/1-349</i>	F N G -- V Y E C I L V G A L F L V V H M V I G N I L E P R M M G H R L G M S T M V V F L S	298			
<i>PerM_Ec/1-353</i>	Q F G - A G T E F W S C F A V Y L I I Q A L D G N L L V P V L F S E A V N L H P L V I I L S	315			
<i>BARG_01524_Ba/1-658</i>	Q F W P D W I M V C T V A A V F F L G Q F I E G N I L Q P K L V G S S V G L H P V W L M F A	348			
<i>YueF_Bs/1-369</i>	D S -- - P A K A L F A I I V V V I V Q Q L D G N L L S P L V I G K R L N T H P L T I I L L	319			
<i>F8FLY5_Pm/1-357</i>	I S -- - V K M V L F V I L V N T A C Q I M E G N V I S P Q V V G R S L K M H P L F I I F A	316			
<i>Upf0118_Ha/1-352</i>	V G D -- I A M G G Q L A M L A I I L L A I R - R T V E P K V M G R H I G L S P L A T L I A	314			
<i>YtvI_Bs/1-371</i>	T G Q -- - L P Q A I G I G I L Y L V V L I Q R Q L T E P K I L S K S I G I D P L A T L I A	327			
<i>Yct2_Bp/1-385</i>	V G D -- V S T G S K L L I L A A V L L I I R - R T V E P K V M G K H I G L S P L A T L I A	347			
<i>Q188A3_Cd/1-363</i>	L G D -- - Y S M A L G I A L L Y I V I T I I R N I I E P K L V G K H M E L H P V L A L A S	327			

	370	380	390	400	410	
<i>TqsA_Ec/1-344</i>	L I F W G W L L G P V G M L L S V P L T I I V K I A L E Q T A G G Q S I A V L L S D L N K E	344				
<i>YhhT_Ec/1-349</i>	L L I W G W L L G P V G M L L S V P L T S V C K I W M E T T K G G S K L A I L L G P G R P K	344				
<i>PerM_Ec/1-353</i>	V V I F G G L W G F W G V F F A I P L A T L I K A V I H A W P D G Q I A Q E - - - - -	353				
<i>BARG_01524_Ba/1-658</i>	L F A F G S L F G F T G M L V A V P A A A A V G V L V R F A L N S Y L R S P M Y D P A N N R	394				
<i>YueF_Bs/1-369</i>	L I G A G S F G G I L G M I L A V P V Y A V V K A F F L N I V R L I K L R Q R S R L E E N A	365				
<i>F8FLY5_Pm/1-357</i>	L L V G G E I A G I A G L I L A V P F F A V M K V I L Q H V F S Y Y I H R R T P T - - - -	357				
<i>Upf0118_Ha/1-352</i>	M Y I G L Q L I G L M G F I L G P L L V I A F N S A K E A G I I R W N F K L - - - - -	352				
<i>YtvI_Bs/1-371</i>	L F A G F K L F G F L G L I A G P A V L V I I Q A F I T T G A L K E I W S Y I T V Q Q K - -	371				
<i>Yct2_Bp/1-385</i>	M Y L G L M L F G V I G F I I G P L L V I A F T S A K E A G I I K L N F K L - - - - -	385				
<i>Q188A3_Cd/1-363</i>	M L T G L H F F G F I G L F G I P L L I A F L K K L N D K E I I H I L H - - - - -	363				

	420	430	440	450	
<i>TqsA_Ec/1-344</i>	- - - - -				
<i>YhhT_Ec/1-349</i>	S R L P G - - - - -				349
<i>PerM_Ec/1-353</i>	- - - - -				
<i>BARG_01524_Ba/1-658</i>	S N P D A G P L I E A G D N T G R E K W D G A K I R W D L M G A A M H D A P R Q I P L N L E				440
<i>YueF_Bs/1-369</i>	K P A E - - - - -				369
<i>F8FLY5_Pm/1-357</i>	- - - - -				
<i>Upf0118_Ha/1-352</i>	- - - - -				
<i>YtvI_Bs/1-371</i>	- - - - -				
<i>Yct2_Bp/1-385</i>	- - - - -				
<i>Q188A3_Cd/1-363</i>	- - - - -				



Appendix Figure S10- Multiple sequence alignment of the AI-2 exporters. Homologs considered include AI-2 exporters from *E. coli* (TqsA, YhhT and PerM), *Brucella abortus* (Uniprot: A0A7U8JIH7), *Paenibacillus mucilaginosus* (Uniprot: F8FLY5), *Bacillus subtilis*

(YueF, Uniprot: O32095), *H. andaensis* (Upf0118, Uniprot: A0A1W5X0D5), *B. subtilis* (YtvI, Uniprot O50485), *Bacillus pseudofirmus* (YCT2, Uniprot: Q04454) and *Clostridium difficile* (Uniprot: Q188A3).

Appendix Table S1- Primers used in the study

Primers	Sequence (5'-3')
TqsA_fwd	GAGGAATTAACCATGGCAAAGCCGATCATCACGC
TqsA_rev	GTGGCTCCAAGCGCTCTCTTTATTGAGATCGCTTAACAGAACGG
YdiK_fwd	GAGGAATTAACCATGGTAAATGTTCGTCAGCCCAGGG
YdiK_rev	GAGGAATTAACCATGGTAAATGTTCGTCAGCCCAGGG
YhhT_fwd	GAGGAATTAACCATGGAAACCCCTCAACCCGATAAAACG
YhhT_rev	GAGGAATTAACCATGGAAACCCCTCAACCCGATAAAACG
PerM_fwd	GAGGAATTAACCATGCTCGAAATGTTGATGCAATGGTATCG
PerM_rev	GTGGCTCCAAGCGCTTTCTTGCGCGATTTGCCCA
Aq_740_fwd	CAGGAGGAATTAACCATGAACAAACTCTCCCTTTTCGTTTATCTATTTTT
Aq_740_rev	CGGGTGGCTCCAAGCGCTAATACCTGAGTGAACCTTCTCCTGTAAATT