Supplementary Discussion

Structural determination of CusA

Data collection and refinement statistics are summarized in Tables S1 and S2. The resulting experimental electron density maps (Fig. S1) based on MIRAS reveal that the asymmetric unit consists of one protomer. The native crystal structure of CusA has been determined to a resolution of 3.52 Å (Table S1). Currently, 97.9% of the residues (residues 5-504 and 516-1040) are included in our final model. The final structure is refined to R_{work} and R_{free} of 23.7% and 27.9%, respectively. Superimposition of the structure of CusA with the structure of AcrB (pdb code:1IWG)[5] results in a high RMSD of 11.4 Å for 1,003 C^{α} atoms, suggesting highly significant differences between these two transporters.

Reconstitution and stop-flow transport assay

Although all of our experimental results strongly suggest that these five methionine pairs/clusters (Fig. 4) are important for metal transport, we cannot definitively rule out the possibility that these mutations may affect the structure of the pump and in that way impair metal binding. Nonetheless, our collective experiments provide direct evidence that CusA is capable of taking up Ag^{+} from the cytoplasm. It should be noted that no decrease in fluorescence signal was detected in the absence of a pH gradient (i.e. no differential pH) between the inside and outside of the proteoliposomes, or if the intravesicular pH is higher than the extravesicular pH (i.e. reverse pH gradient) (Fig. S13).

Superimposition of the structures of CusA and AcrB

We superimposed a protomer of apo-CusA onto each protomer of the "asymmetric" AcrB (pdb code: 2DHH).[7] Likewise, we also superimposed a protomer of CusA-Cu(I) onto those of the AcrB pump. These superimpositions gave high overall RMSDs, each RMSD exceeding 11 Å. However, when the portion of the apo-CusA protomer that contains only sub-domains PC1, PC2, PN1 and PN2 was superimposed onto those of the "extrusion", "binding" and "access" conformers of AcrB (2DHH),[7] we obtained much smaller RMSDs of 4.7 Å, 5.6 Å and 5.5 Å. Similarly, superimpositions of the periplasmic domain (only containing PC1, PC2, PN1 and PN2) of a protomer of CusA-Cu(I) onto those of the "extrusion", "binding" and "access" conformers of AcrB (2DHH)[7] gave RMSDs of 6.2 Å, 5.0 Å and 5.3 Å, respectively. These superimpositions suggest that the conformation of the apo-CusA protomer may correspond to the "extrusion" state, and that the CusA-Cu(I) protomer structure may represent the "binding" conformation of the pump. If this is the case, CusA may go through a cyclic conformational change, from the "access", through to the "binding" and finally to the "extrusion" conformer, as suggested for the AcrB pump,[7-9] to export copper/silver.

We also superimposed the Cu(I)-binding site of CusA onto the doxorubicin binding site of AcrB[7]. The superimposition suggests that the locations of the bound copper and drug molecule are within 3 Å apart (Fig. S14).

Supplemental Figures

Fig. S1. Stereo view of the experimental electron density map at a resolution of 3.8 Å. (a) The electron density map contoured at 1.2 σ is in blue. Each subunit of CusA consists of 34 methionines. 33 selenium sites are found with a single SeMet crystal. The anomalous maps of the selenium sites contoured at 4 σ are in green. The Cα traces of CusA are in red. (b) Anomalous maps of the 33 selenium sites (contoured at 4 σ). The selenium sites corresponding to the 11 methionines forming the methionine-residue relay network are in green. The rest of the 22 selenium sites are in purple. The Cα traces of CusA are in red. (c) Representative section of the electron density in the second domain of CusA. The electron density (colored blue) is contoured at the 1.2 σ level and superimposed with the final refined model (yellow, carbon; red, oxygen; blue, nitrogen).

Fig. S2. Sequence and topology of CusA and AcrB. Alignment of the amino acid sequences of CusA and AcrB were done using CLUSTAL W. *, identical residues; :, >60% homologous residues. Secondary structural elements are indicated: TM, transmembrane helix; Nα and Nβ, helix and strand, respectively, in the N-terminal half; Cα and Cβ, helix and strand, respectively, in the C-terminal half. The CusC or TolC docking domain is divided into two sub-domains, DN and DC; whereas the pore domain is divided into four sub-domains, PN1, PN2, PC1 and PC2. The sequence and topology of CusA are shown at the top, and those for the AcrB pump are shown at the bottom.

Fig. S3. Experimental electron density of the periplasmic domain of CusA. (a) The electron density map of apo-CusA (3.8 Å-resolution) contoured at 1.2 σ is in blue. The

Cα traces of the periplasmic domain of apo-CusA are in red. (b) The electron density map of CusA-Cu(I) (4.1 Å-resolution) contoured at 1.2 σ is in purple. The Cα traces of the periplasmic domain of CusA-Cu(I) are in green. The bound copper is shown as a brown sphere.

Fig. S4. Ion pairs in the transmembrane domain viewed from the cytoplasmic side. Residues D405 of TM4, E939 of TM10 and K984 of TM11 that form ion pairs, which may play an important role in proton translocation, are in yellow sticks. The six methionines, M391, M403, M410, M486, M501 and M1009, are shown as pink sticks.

Fig. S5. Docking of CusB to CusA. (a) Side view of the docked complex of CusBA. The three CusB protomers are shown in green ribbons. The trimeric CusA is in gray surfaces. Sub-domains PN2 and PC1 of CusA are in red and blue, respectively. Specific interaction is found to occur between Domain 2 of CusB and the groove formed between DN and DC sub-domains of CusA to further stabilize the complex. (b) Top view of the docked complex of CusBA.

Fig. S6. Stereo view of the methionine-residue relay network. (a) The transmembrane domain of a subunit of CusA viewed from the cytoplasmic side. The six methionines that form three pairs in the transmembrane region are in green sticks. (b) The periplasmic domain of a subunit of CusA viewed from the periplasmic side. The five methionines that form a triad and a pair in the periplasmic domain are in green sticks.

Fig. S7. Alignment of amino acid sequences of the HME-RND-type Cu(I) and/or Ag(I) efflux pumps using CLUSTAL W. *, identical residues; :, >60% homologous residues. The alignment suggests that the methionine residues forming the relay network are conserved among these 30 different pumps. Six of these methionines located at the transmembrane region are highlighted with gray bars.

Fig. S8. Channel in the CusA pump. (a) Stereo view of the channel formed by the front protomer of apo-CusA (red) leading through the transmembrane and periplasmic domains is in gray color. This channel was calculated using the position of the sulfur atom of residue M672 as a starting point. The diameter of this channel ranges between 4.4 and 8.9 Å. The 11 methionines forming the relay network are in spheres (green, carbon; red, oxygen; blue, nitrogen; orange, sulfur). The distances between consecutive methionine pairs, from the cytoplasm to the periplasm, are 10.1, 12.1, 17.5 and 14.0 Å, respectively. Two other CusA protomers behind the front protomer are shown as blue wires. (b) Stereo view of the channel formed by the front protomer of Cu(I) bound-CusA (green) leading through the transmembrane and periplasmic domains is in gray color. This channel was calculated using the position of the sulfur atom of residue M672 as a starting point. The diameter of this channel ranges between 5.6 and 9.1 Å. The 11 methionines forming the relay network are in spheres (pink, carbon; red, oxygen; blue, nitrogen; orange, sulfur). The distances between consecutive methionine pairs, from the cytoplasm to the periplasm, are 9.4, 13.0, 20.0 and 14.7 Å, respectively. The bound Cu(I) is completely buried inside the channel. Two other CusA protomers behind the front protomer are shown as blue wires. The funnel formed by sub-domains DN and DC is

indicated with a dotted curve. For clarity, the channels formed by the other two protomers at the back are omitted. The calculations were done using the program CAVER (http://loschmidt.chemi.muni.cz/caver).

Fig. S9. A cartoon of a proteoliposome containing the CusA trimers. The CusA trimers are in green. The intravesicular space is loaded with the fluorescence indicator PGSK (blue star). Ag^+ (red sphere) is then added into the extravesicular medium for metal transport.

Fig. S10. The neighbor dependent opening closing motions. We show the effects of the strong internal motions on the CusA trimer. In the center is a diagram of the periplasmic domain looking downward, towards the inner membrane. We build an elastic network model using the formalism of Atilgan et al.[34] to predict the natural motions of CusA. On each of three sides we show the effect of two of the structure's natural motions. These motions describe coupled opening and closing of adjacent periplasmic metal entry sites. Black wedges are shown in open and closed form, indicating the approximate angle between the edges of PC1 and PC2 in each motion. From these the alternating open/close motion is evident.

Fig. S11. The swinging motion of PC2 coupled to TM8, as computed with the same elastic network model used for Fig. S10. This motion is similar to the difference between the apo and bound crystal forms displayed in Fig. 2. An arrow indicates the motion of

swinging from the red conformation to the green. For clarity PC2 and TM8 are shown in thicker lines than the remainder of the monomer.

Fig. 12. Expression level of the CusA pumps. An immunoblot against CusA of crude extracts from 50 μg dry cells of strain BL21(DE3)Δ*cueO*Δ*cusA* expressing the CusA pumps (M755I, lane 1; M391I, lane 2; M410I, lane 3; M486I, lane 4; wild-type, lane 5; marker, lane 6; M573I, lane 7; M623I, lane 8; M672I, lane 9) is shown.

Fig. S13. Stopped-flow transport assay of reconstituted wild-type CusA with extravesicular Ag^+ ion at different intravesicular and extravesicular pHs. The stopped-flow traces are the cumulative average of four successive recordings (intravesicular pH = 6.6 and extravesicular pH = 7.0, black curve; intravesicular pH = extravesicular pH = 6.6, blue curve; intravesicular pH = extravesicular pH = 7.0, green curve; intravesicular pH = 7.0 and extravesicular pH = 6.6, pink curve; control liposome, red curve).

Fig. S14. Comparison of the CusA and AcrB binding sites. Superimposition of a subunit of Cu(I) bound-CusA (green) onto the "binding" protomer of AcrB (orange) with doxorubicin (pdb code: 2DH6).[7] In this superimposition, the bound copper and doxorubicin are 3 Å apart. The bound Cu(I) and doxorubicin are colored red and blue, respectively.

Table S1. Data collection and refinement statistics of apo-CusA.

	Native CusA	Au(III)	$Ta_6Br_{12}^{2+}$	Se (peak)
Data collection				
Space group	*R*32	*R*32	*R*32	*R*32
Cell dimensions				
a, *b*, *c* (Å)	178.42, 178.42, 285.75	179.31, 179.31, 287.10	178.34, 178.34, 284.23	178.21, 178.21, 285.57
α, β, γ (°)	90, 90, 120	90, 90, 120	90, 90, 120	90, 90, 120
Wavelength (Å)	0.9791	1.0398	1.0089	0.9791
Resolution (Å)	50-3.52 (3.62-3.52)	50-4.31 (4.50-4.31)	50-3.52 (3.66-5.52)	50-3.68 (3.81-3.68)
R_{sym} or R_{merge}	5.7 (43.5)	10.2 (35.9)	6.2 (37.7)	7.5 (46.9)
$I/\sigma I$	32.5 (4.0)	17.3 (5.2)	21.7 (3.4)	19.7 (2.6)
Completeness (%)	94.2 (95.8)	100 (100)	99.9 (100)	100 (100)
Redundancy	6.9 (6.9)	3.2 (2.8)	3.8 (3.9)	3.9 (3.9)
Refinement				
Resolution (Å)	50-3.52			
No. reflections	432,346			
R_{work}/ R_{free}	0.238/0.275			
No. atoms				
Protein	7,884			
Ligand/ion	0			
Water	0			
R.m.s deviations				
Bond lengths (Å)	0.004			
Bond angles (°)	0.874			

*Highest resolution shell is shown in parenthesis.

Table S2. Data collection and refinement statistics of the CusA-Cu(I) and CusA-Ag(I) complexes.

	Cu(I) (peak)	Cu(I)	Ag(I)
Data collection			
Space group	*R*32	*R*32	*R*32
Cell dimensions			
a, *b*, *c* (Å)	178.21, 178.21, 285.57	179.14, 179.14, 286.12	179.99, 179.99, 287.98
α, β, γ (°)	90, 90, 120	90, 90, 120	90, 90, 120
Wavelength (Å)	1.3779	1.0333	1.3779
Resolution (Å)	50-4.10 (4.25-4.10)	50-3.88 (4.03-3.88)	50-4.35 (4.51-4.35)
R_{sym} or R_{merge}	8.9 (42.4)	9.0 (42.6)	9.2 (39.6)
$I/\sigma I$	11.8 (1.8)	10.5 (2.1)	14.9 (2.0)
Completeness (%)	96.6 (98.7)	99.5 (98.8)	99.7 (99.9)
Redundancy	2.5 (2.5)	3.2 (3.1)	3.3 (3.4)
Refinement			
Resolution (Å)		50-3.88	50-4.35
No. reflections		346,057	258,908
$R_{work/}$ R_{free}		0.261/0.296	0.271/0.312
No. atoms			
Protein		7,802	7,782
Ligand/ion		1	1
Water		0	0
R.m.s deviations			
Bond lengths (Å)		0.005	0.006
Bond angles (°)		0.995	1.252

*Highest resolution shell is shown in parenthesis.

Table S3. MICs of copper for different CusA mutants expressed in *E. coli* BL21(DE3)Δ*cueO*Δ*cusA*

Gene in BL21(DE3)Δ*cueO*Δ*cusA*	MIC (mM) of $CuSO_4$
Empty vector	0.50
cusA (wild-type)	2.25
cusA (M573I)	0.50
cusA (M623I)	0.50
cusA (M672I)	0.50
cusA (M391I)	1.25
cusA (M410I)	1.75
cusA (M486I)	1.75
cusA (M755I)	1.75
cusA (D405A)	0.50
cusA (E939A)	0.50
cusA (K984A)	0.50
cusA (E412A)	1.00[a]
cusA (S453A)	1.00[a]
cusA (C353A)	2.25[b]
cusA (C375A)	2.25[b]

[a]Residues E412 and S453, located in the vicinity of the triad formed by D405, E939 and K984, may also involve in the proton-relay network.
[b]The CusA pump contains only two cysteine residues, C353 and C375, which may not involve in copper resistance.

Table S4. MICs of silver for different CusA mutants expressed in *E. coli* BL21(DE3)Δ*cueO*Δ*cusA*

Gene in BL21(DE3)Δ*cueO*Δ*cusA*	MIC (μM) of $AgNO_3$
Empty vector	10.0
cusA (wild-type)	30.0
cusA (M573I)	12.5
cusA (M623I)	12.5
cusA (M672I)	12.5
cusA (M391I)	10.0
cusA (M410I)	17.5
cusA (M486I)	17.5
cusA (M755I)	12.5
cusA (D405A)	12.5
cusA (E939A)	12.5
cusA (K984A)	12.5
cusA (E412A)	12.5[a]
cusA (S453A)	15.0[a]

[a]Residues E412 and S453, located in the vicinity of the triad formed by D405, E939 and K984, may also involve in the proton-relay network.

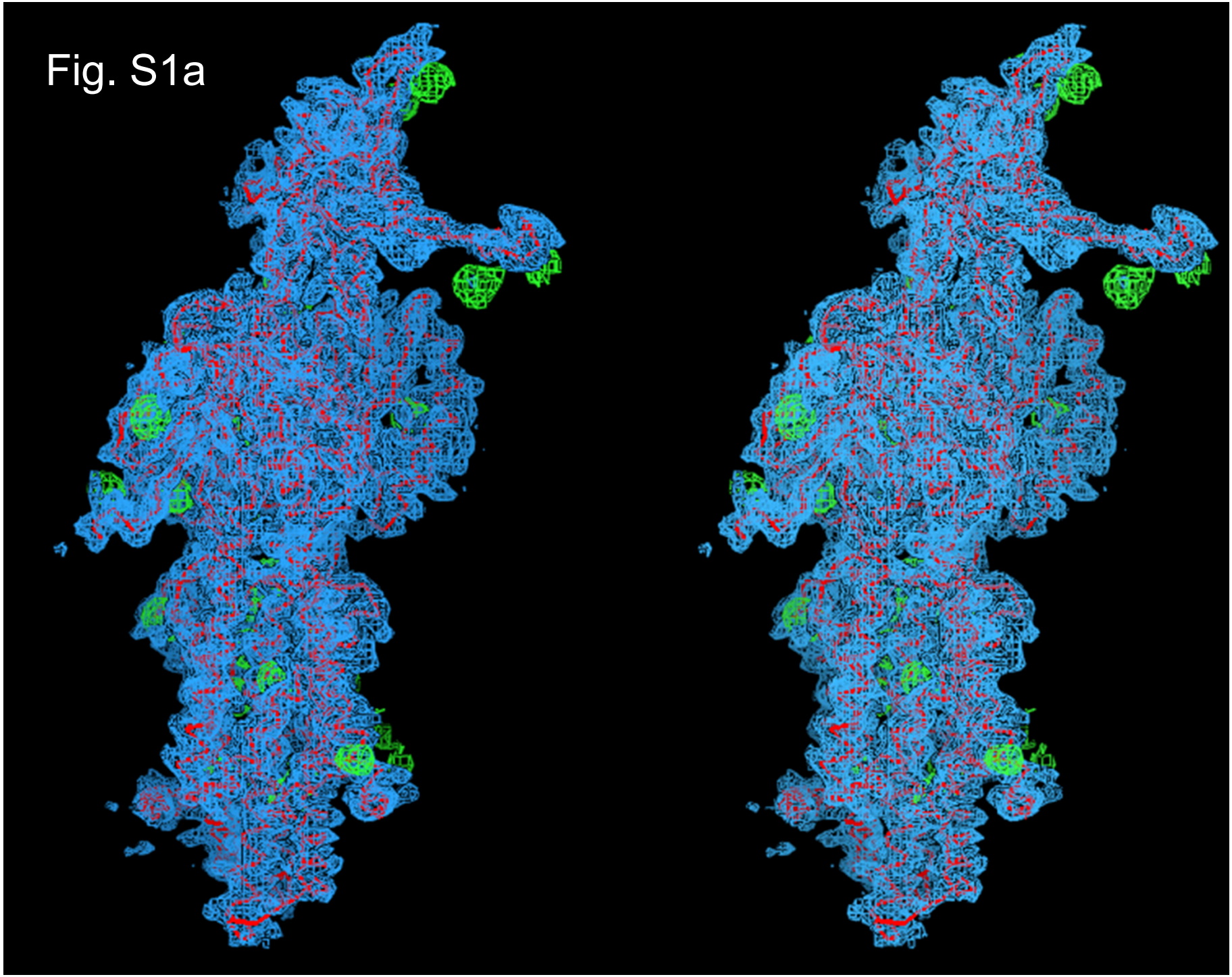


Fig. S1a

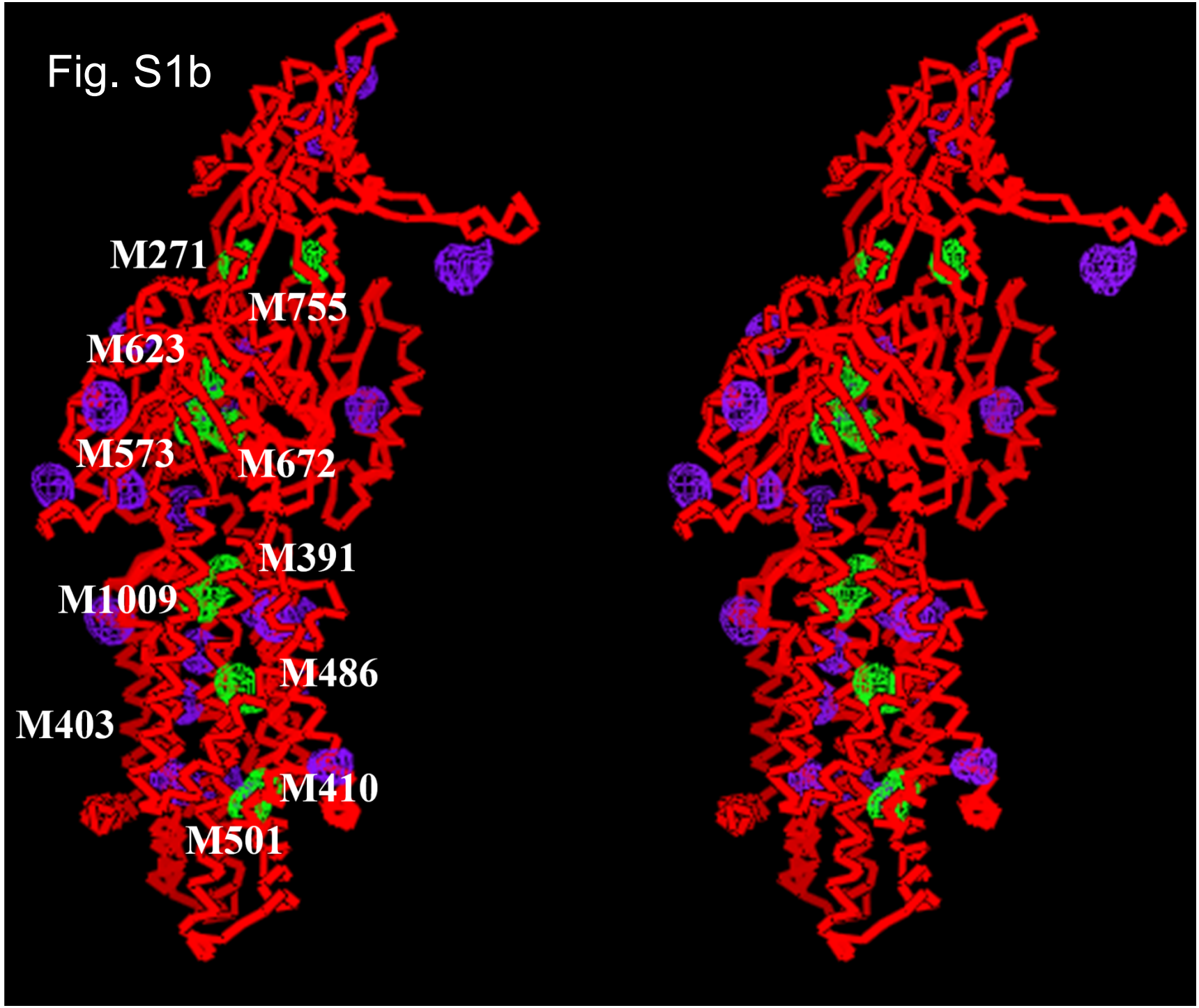


Fig. S1b
M271
M755
M623
M573
M672
M391
M1009
M486
M403
M410
M501

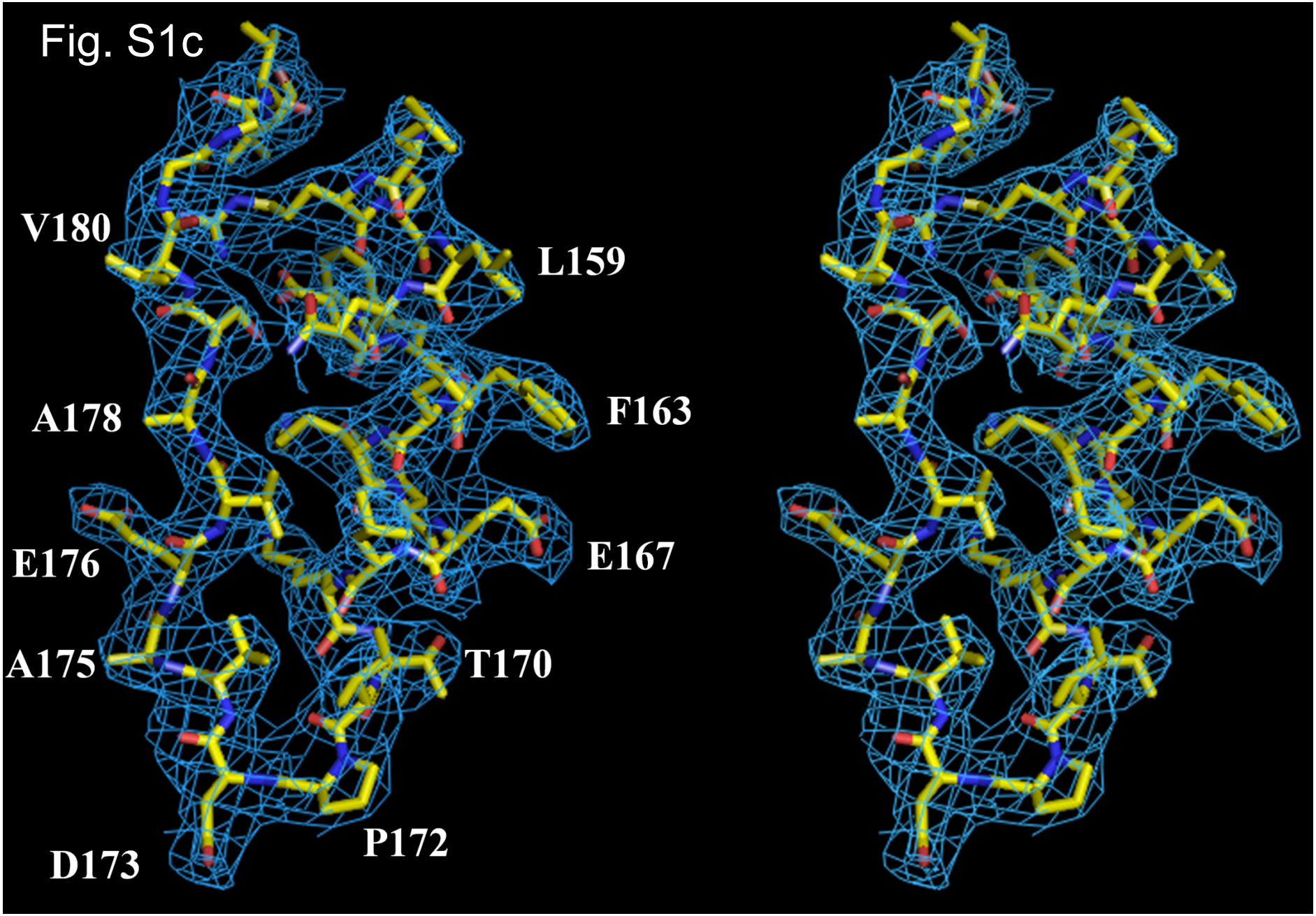


Fig. S1c
V180
L159
A178
F163
E176
E167
A175
T170
P172
D173

Fig. S2

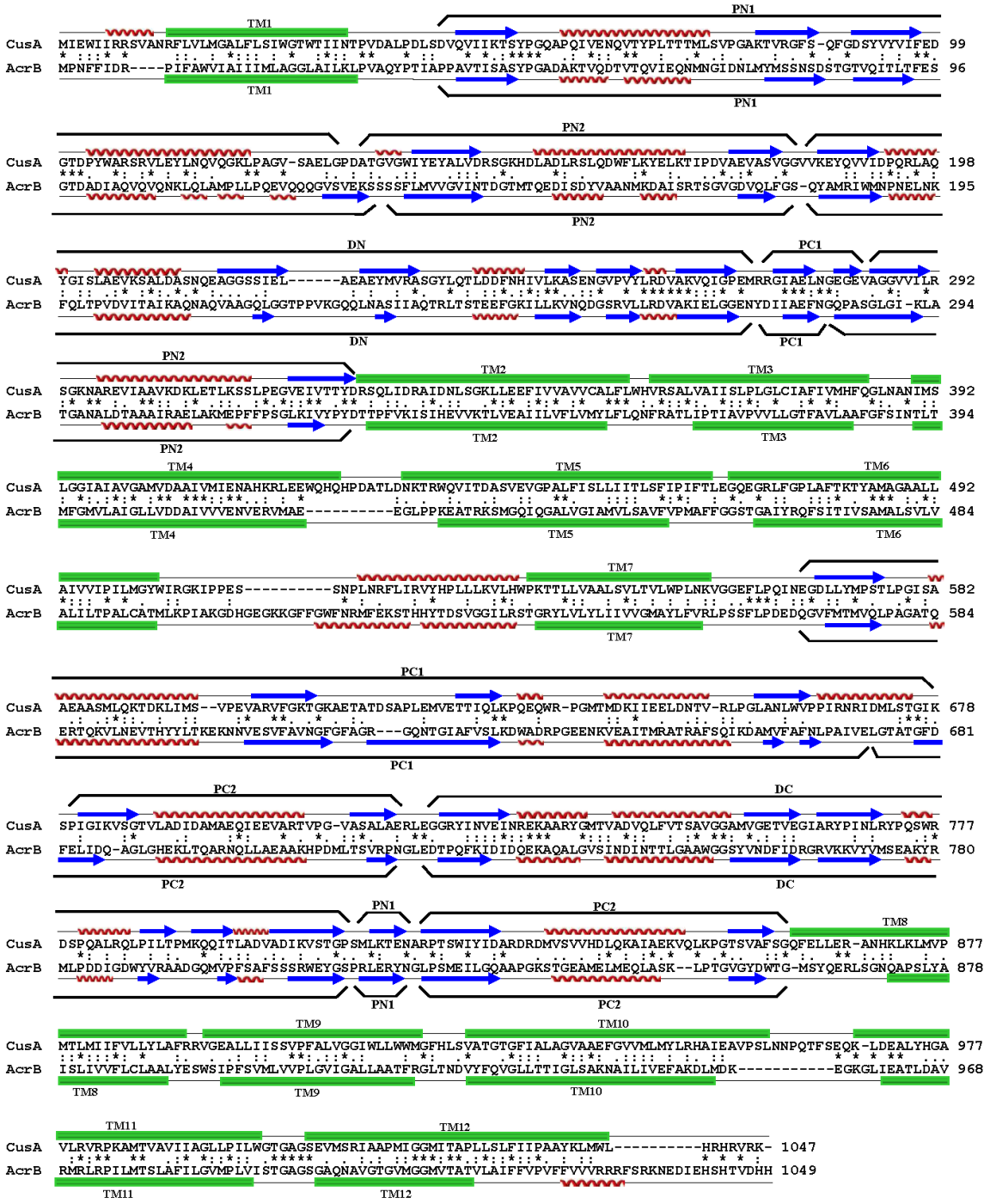

CusA MIEWIIRRSVANRFLVLMGALFLSIWGTWTIINTPVDALPDLSDVQVIIKTSYPGQAPQIVENQVTYPLTTTMLSVPGAKTVRGFS-QFGDSYVYVIFED 99
AcrB MPNFFIDR----PIFAWVIAIIIMLAGGLAILKLPVAQYPTIAPPAVTISASYPGADAKTVQDTVTQVIEQNMNGIDNLMYMSSNSDSTGTVQITLTFES 96
CusA GTDPYWARSRVLEYLNQVQGKLPAGV-SAELGPDATGVGWIYEYALVDRSGKHDLADLRSLQDWFLKYELKTIPDVAEVASVGGVVKEYQVVIDPQRLAQ 198
AcrB GTDADIAQVQVQNKLQLAMPLLPQEVQQQGVSVEKSSSSFLMVVGVINTDGTMTQEDISDYVAANMKDAISRTSGVGDVQLFGS-QYAMRIWMNPNELNK 195
CusA YGISLAEVKSALDASNQEAGGSSIEL------AEAEYMVRASGYLQTLDDFNHIVLKASENGVPVYLRDVAKVQIGPEMRRGIAELNGEGEVAGGVVILR 292
AcrB FQLTPVDVITAIKAQNAQVAAGQLGGTPPVKGQQLNASIIAQTRLTSTEEFGKILLKVNQDGSRVLLRDVAKIELGGENYDIIAEFNGQPASGLGI-KLA 294
CusA SGKNAREVIAAVKDKLETLKSSLPEGVEIVTTYDRSQLIDRAIDNLSGKLLEEFIVVAVVCALFLWHVRSALVAIISLPLGLCIAFIVMHFQGLNANIMS 392
AcrB TGANALDTAAAIRAELAKMEPFFPSGLKIVYPYDTTPFVKISIHEVVKTLVEAIILVFLVMYLFLQNFRATLIPTIAVPVVLLGTFAVLAAFGFSINTLT 394
CusA LGGIAIAVGAMVDAAIVMIENAHKRLEEWQHQHPDATLDNKTRWQVITDASVEVGPALFISLLIITLSFIPIFTLEGQEGRLFGPLAFTKTYAMAGAALL 492
AcrB MFGMVLAIGLLVDDAIVVVENVERVMAE---------EGLPPKEATRKSMGQIQGALVGIAMVLSAVFVPMAFFGGSTGAIYRQFSITIVSAMALSVLV 484
CusA AIVVIPILMGYWIRGKIPPES----------SNPLNRFLIRVYHPLLLKVLHWPKTTLLVAALSVLTVLWPLNKVGGEFLPQINEGDLLYMPSTLPGISA 582
AcrB ALILTPALCATMLKPIAKGDHGEGKKGFFGWFNRMFEKSTHHYTDSVGGILRSTGRYLVLYLIIVVGMAYLFVRLPSSFLPDEDQGVFMTMVQLPAGATQ 584
CusA AEAASMLQKTDKLIMS--VPEVARVFGKTGKAETATDSAPLEMVETTIQLKPQEQWR-PGMTMDKIIEELDNTV-RLPGLANLWVPPIRNRIDMLSTGIK 678
AcrB ERTQKVLNEVTHYYLTKEKNNVESVFAVNGFGFAGR---GQNTGIAFVSLKDWADRPGEENKVEAITMRATRAFSQIKDAMVFAFNLPAIVELGTATGFD 681
CusA SPIGIKVSGTVLADIDAMAEQIEEVARTVPG-VASALAERLEGGRYINVEINREKAARYGMTVADVQLFVTSAVGGAMVGETVEGIARYPINLRYPQSWR 777
AcrB FELIDQ-AGLGHEKLTQARNQLLAEAAKHPDMLTSVRPNGLEDTPQFKIDIDQEKAQALGVSINDINTTLGAAWGGSYVNDFIDRGRVKKVYVMSEAKYR 780
CusA DSPQALRQLPILTPMKQQITLADVADIKVSTGPSMLKTENARPTSWIYIDARDRDMVSVVHDLQKAIAEKVQLKPGTSVAFSGQFELLER-ANHKLKLMVP 877
AcrB MLPDDIGDWYVRAADGQMVPFSAFSSSRWEYGSPRLERYNGLPSMEILGQAAPGKSTGEAMELMEQLASK--LPTGVGYDWTG-MSYQERLSGNQAPSLYA 878
CusA MTLMIIFVLLYLAFRRVGEALLIISSVPFALVGGIWLLWWMGFHLSVATGTGFIALAGVAAEFGVVMLMYLRHAIEAVPSLNNPQTFSEQK-LDEALYHGA 977
AcrB ISLIVVFLCLAALYESWSIPFSVMLVVPLGVIGALLAATFRGLTNDVYFQVGLLTTIGLSAKNAILIVEFAKDLMDK----------EGKGLIEATLDAV 968
CusA VLRVRPKAMTVAVIIAGLLPILWGTGAGSEVMSRIAAPMIGGMITAPLLSLFIIPAAYKLMWL----------HRHRVRK- 1047
AcrB RMRLRPILMTSLAFILGVMPLVISTGAGSGAQNAVGTGVMGGMVTATVLAIFFVPVFFVVVRRRFSRKNEDIEHSHTVDHH 1049
PN1
PN2
DN
PC1
PC2
DC
TM1
TM2
TM3
TM4
TM5
TM6
TM7
TM8
TM9
TM10
TM11
TM12

Fig. S3

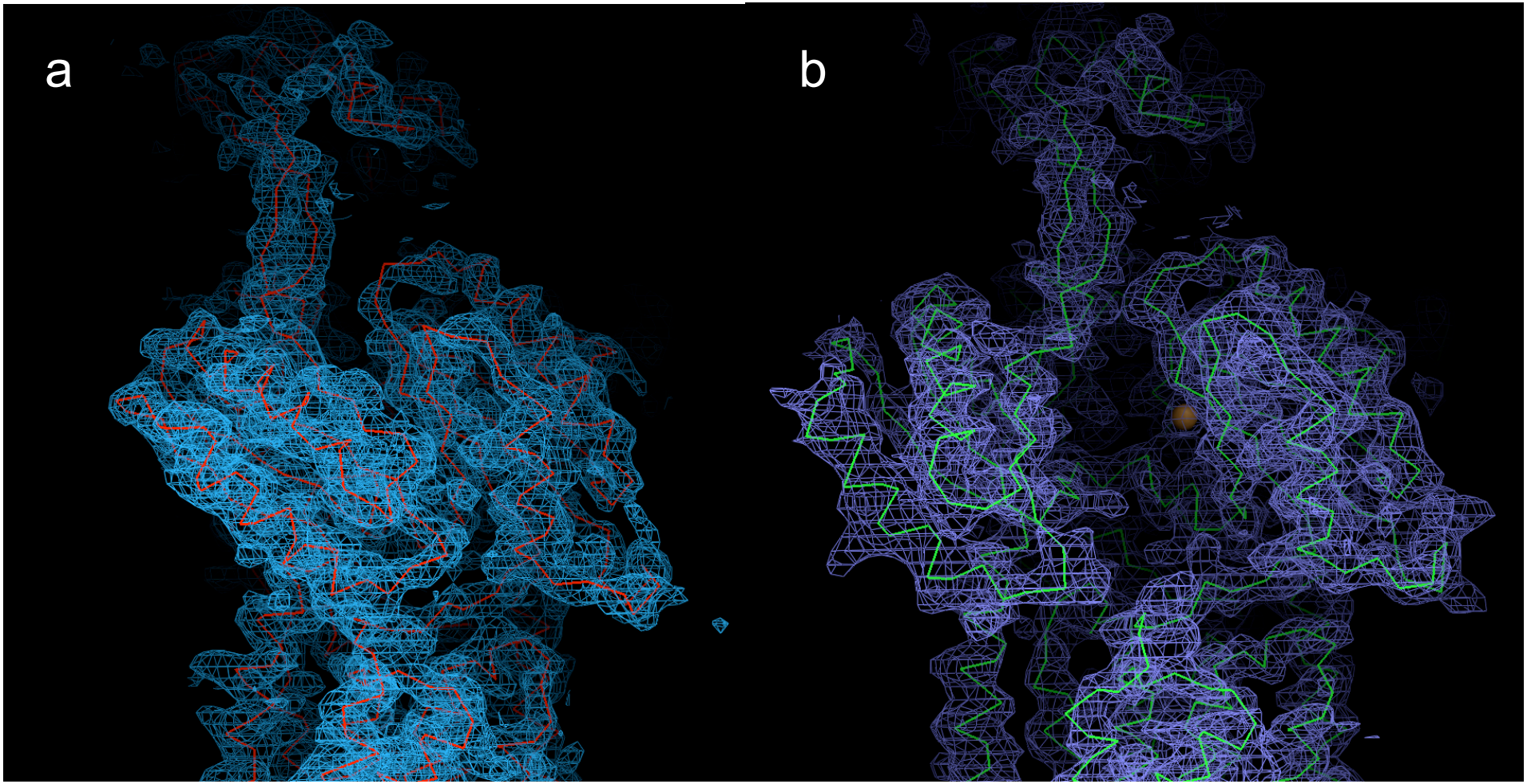


Fig. S4

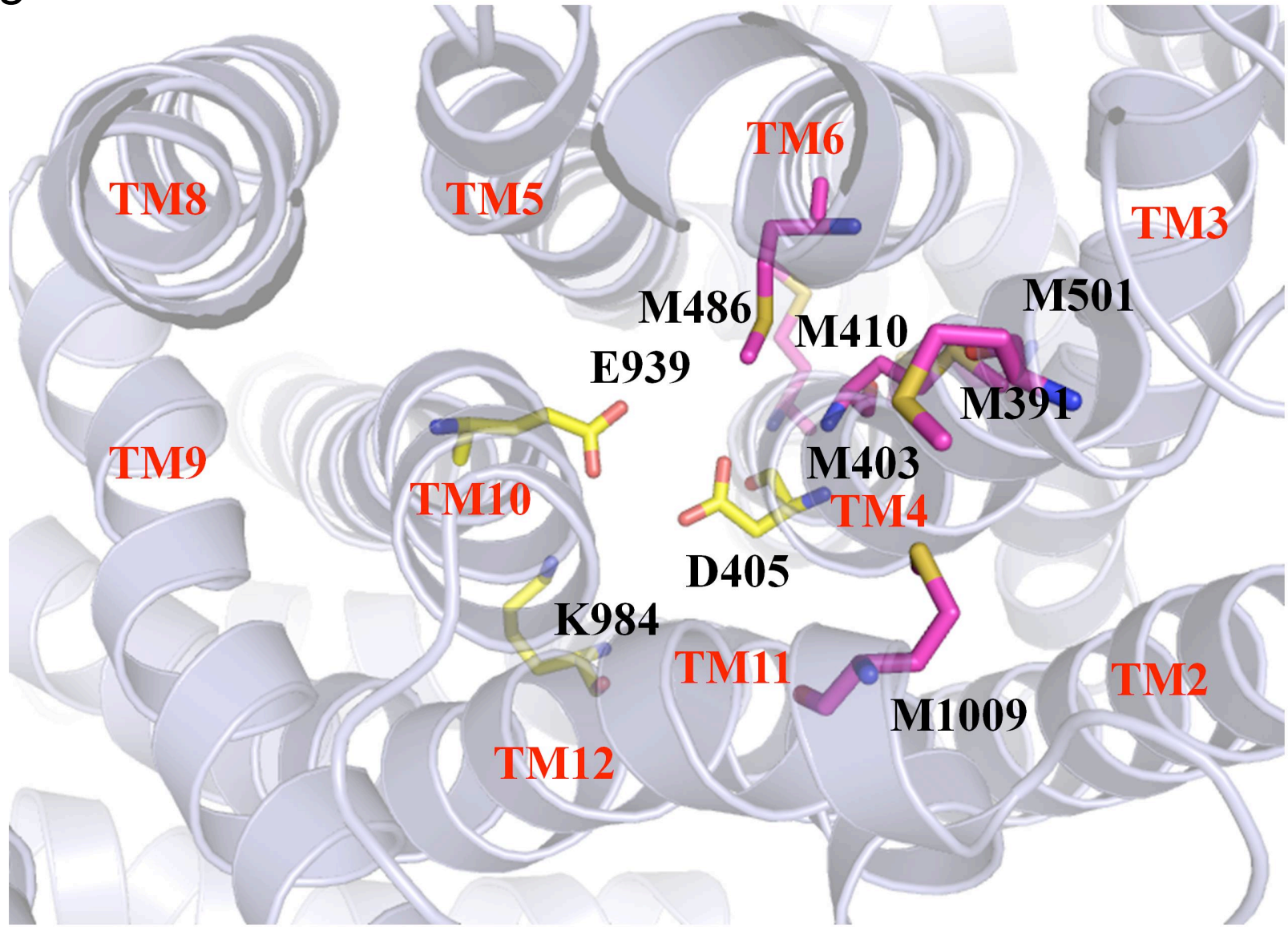

TM6
TM8
TM5
TM3
M486
M501
M410
E939
M391
TM9
M403
TM10
TM4
D405
K984
TM11
TM2
M1009
TM12

Fig. S5

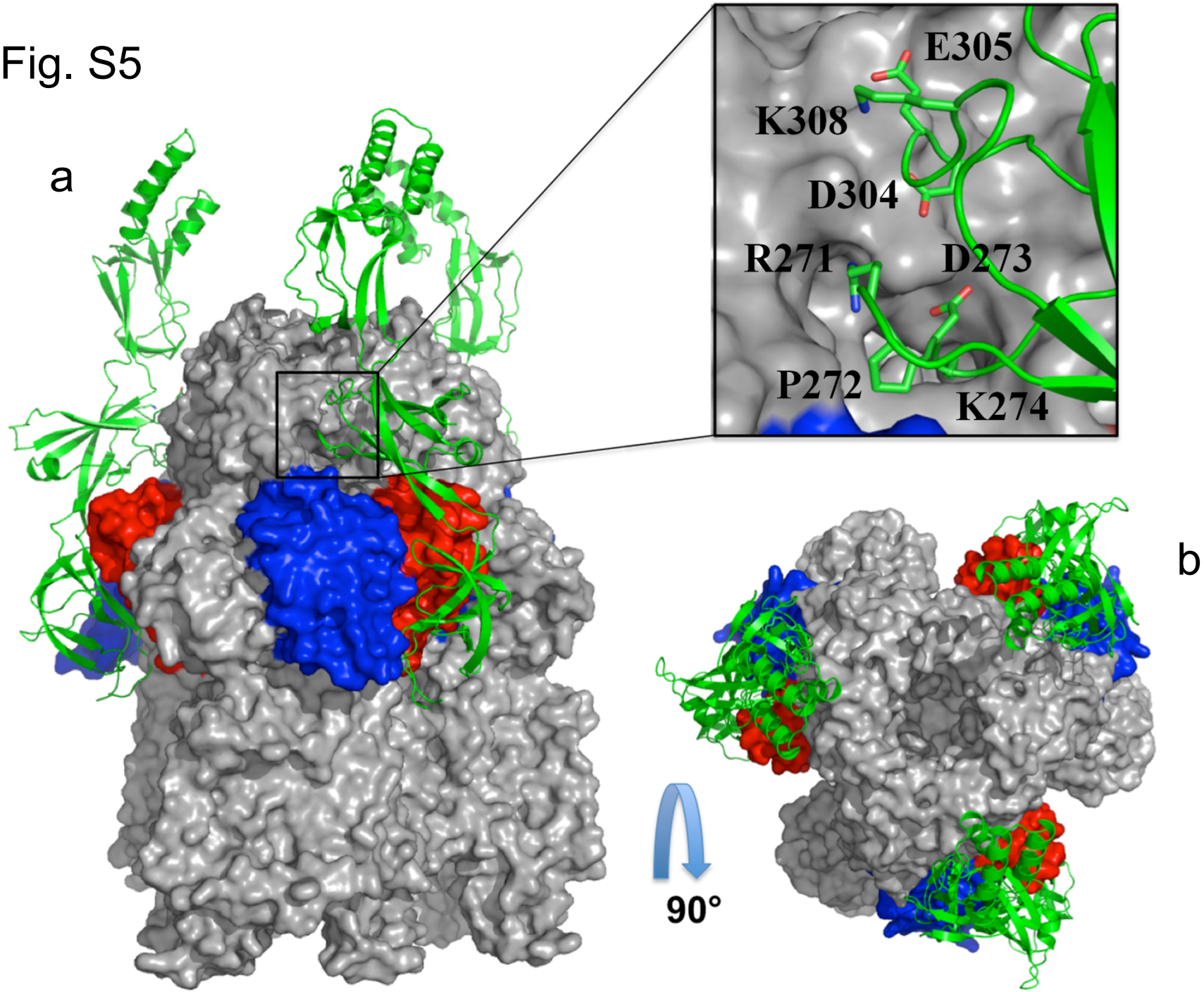


Fig. S6a

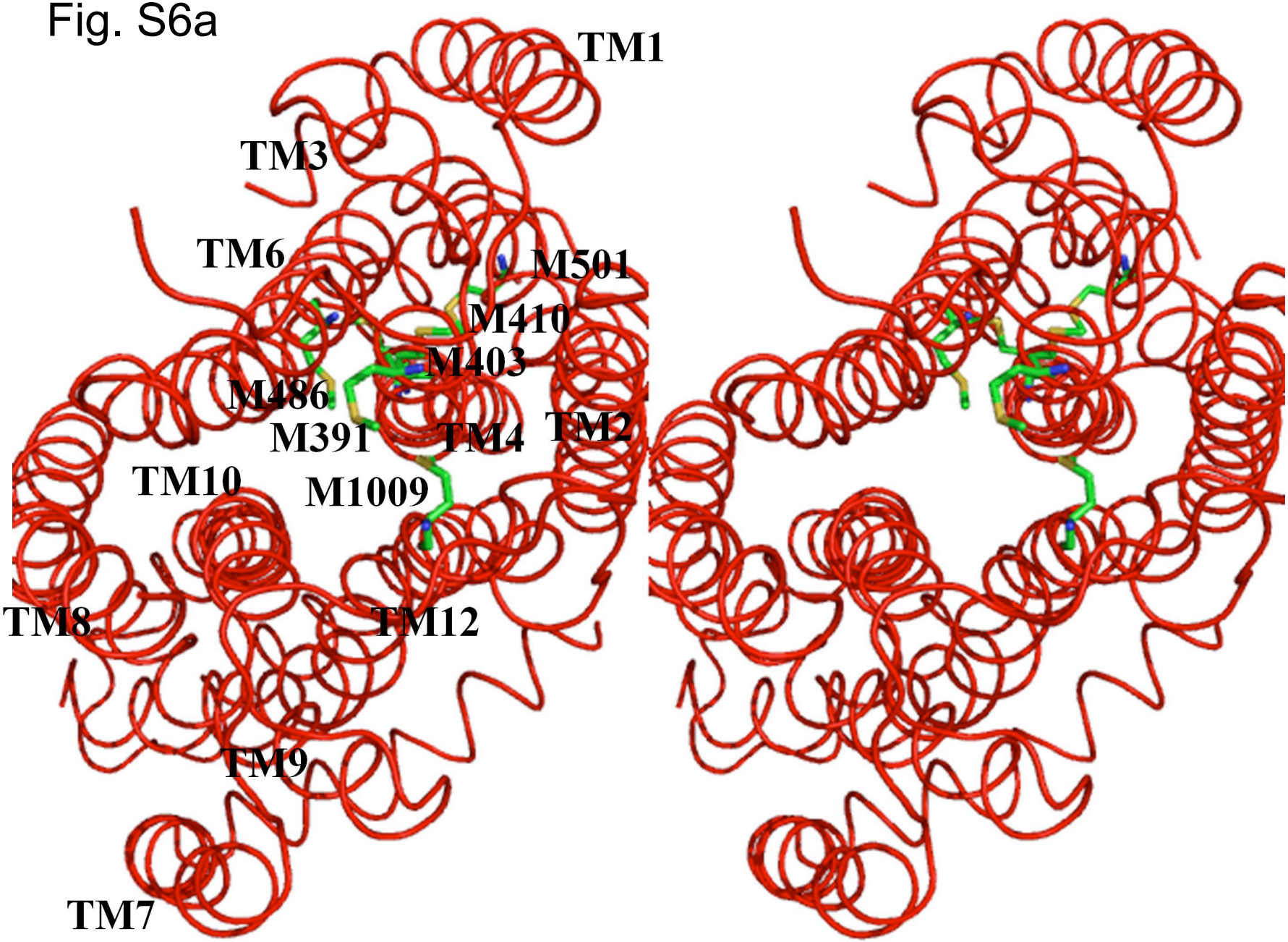

TM1
TM3
TM6
M501
M410
M403
M486
M391
TM4
TM2
TM10
M1009
TM8
TM12
TM9
TM7

Fig. S6b

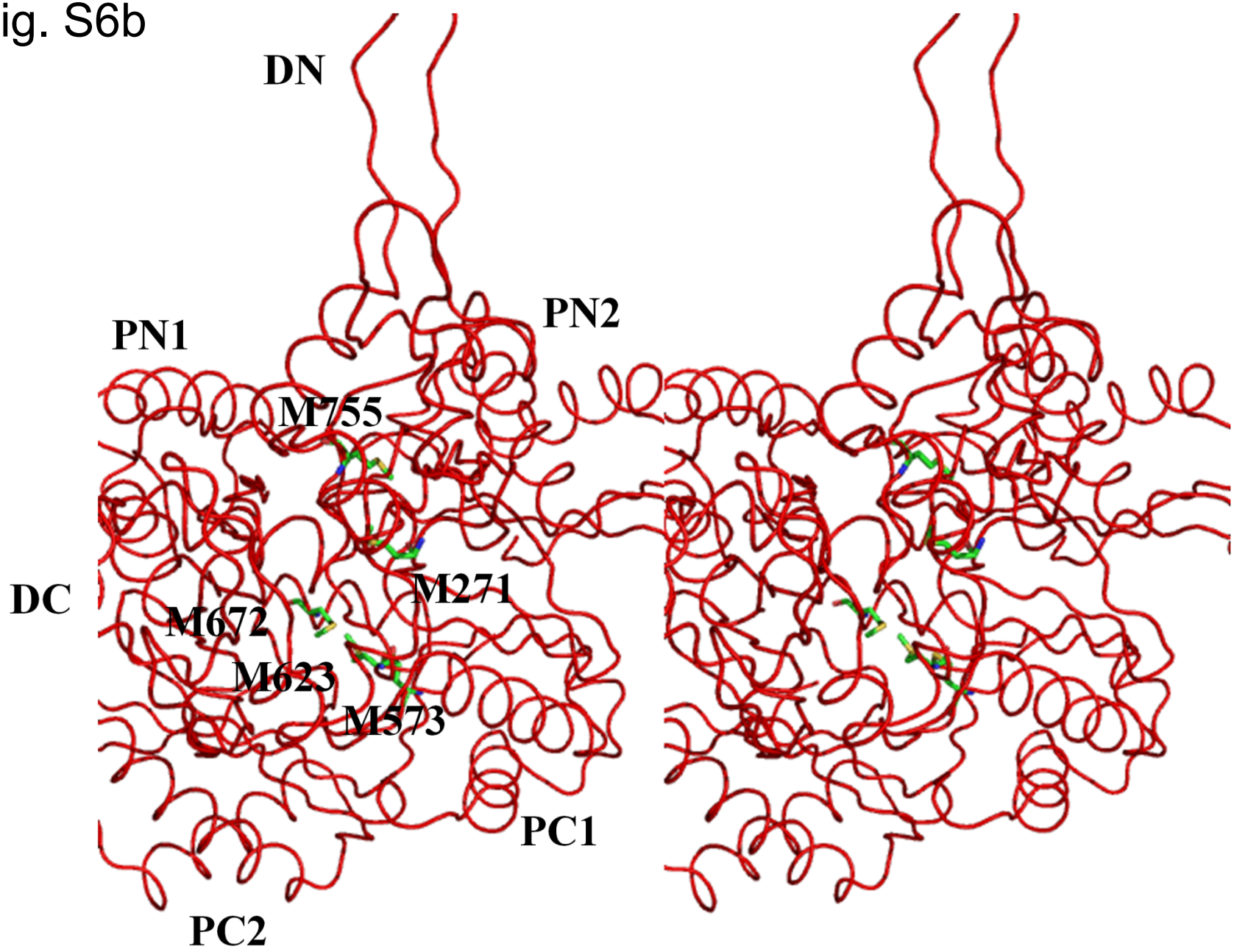
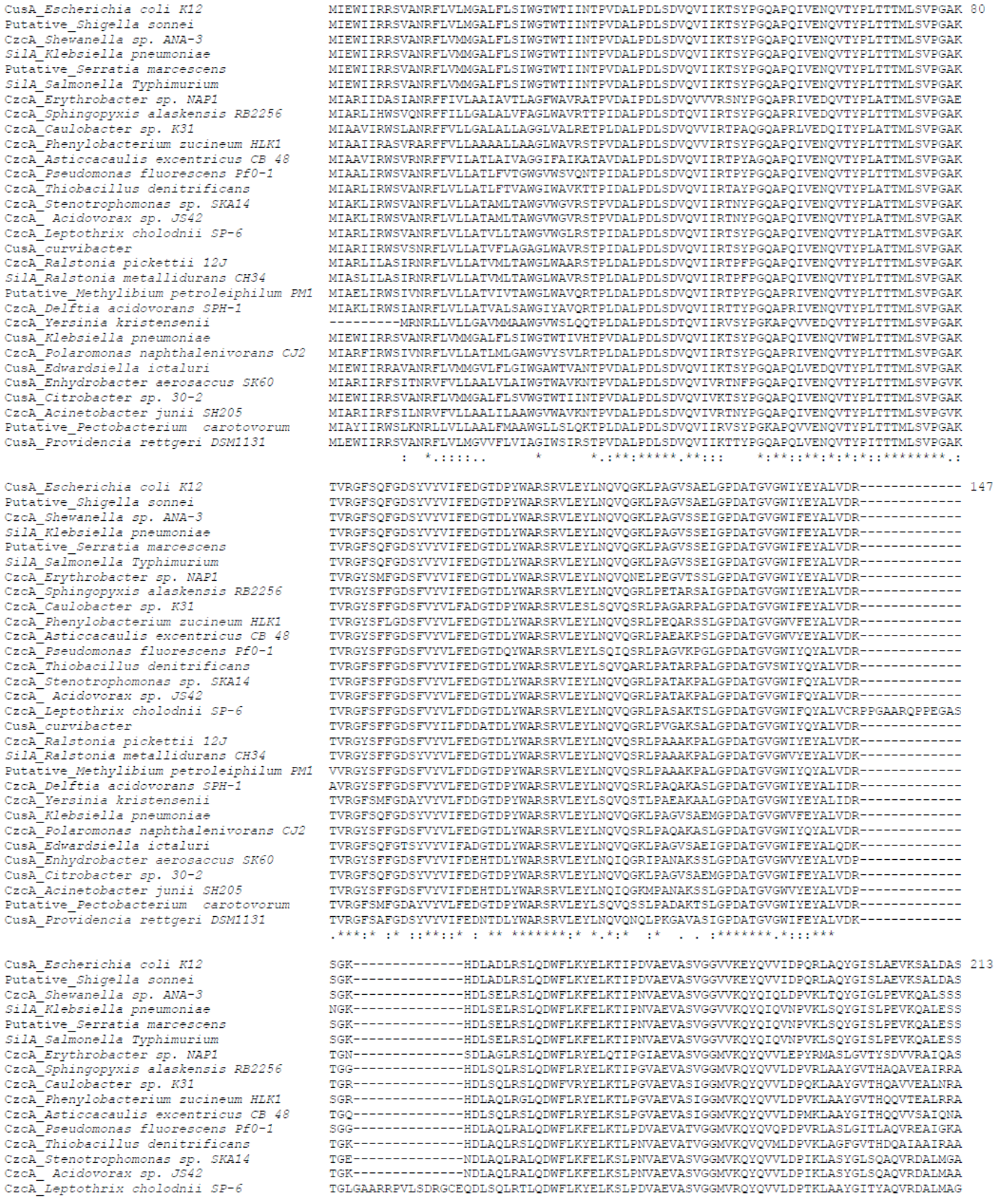


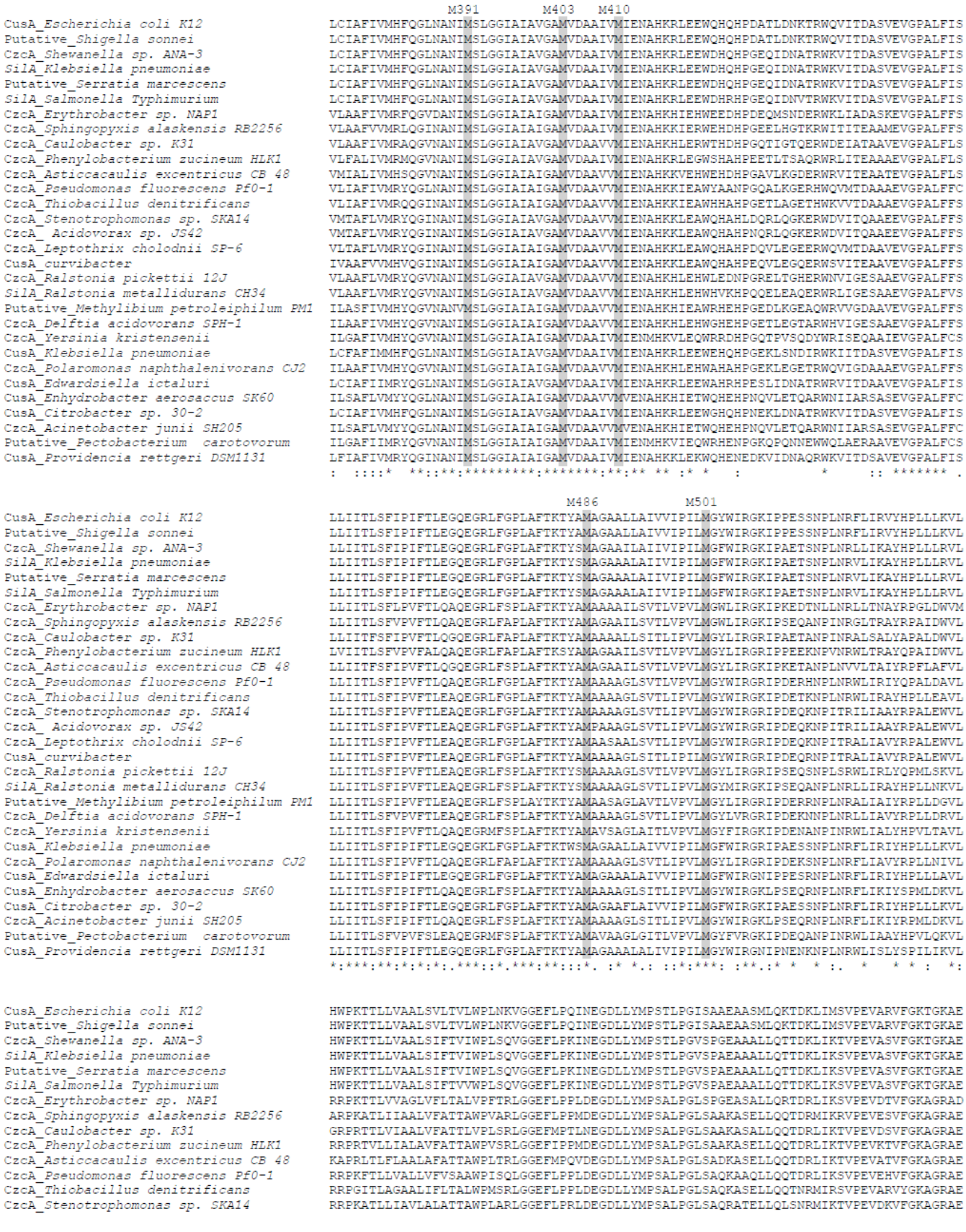
Fig. S7


CusA_Escherichia coli K12
Putative_Shigella sonnei
CzcA_Shewanella sp. ANA-3
SilA_Klebsiella pneumoniae
Putative_Serratia marcescens
SilA_Salmonella Typhimurium
CzcA_Erythrobacter sp. NAP1
CzcA_Sphingopyxis alaskensis RB2256
CzcA_Caulobacter sp. K31
CzcA_Phenylobacterium zucineum HLK1
CzcA_Asticcacaulis excentricus CB 48
CzcA_Pseudomonas fluorescens Pf0-1
CzcA_Thiobacillus denitrificans
CzcA_Stenotrophomonas sp. SKA14
CzcA_ Acidovorax sp. JS42
CzcA_Leptothrix cholodnii SP-6
CusA_curvibacter
CzcA_Ralstonia pickettii 12J
SilA_Ralstonia metallidurans CH34
Putative_Methylibium petroleiphilum PM1
CzcA_Delftia acidovorans SPH-1
CzcA_Yersinia kristensenii
CusA_Klebsiella pneumoniae
CzcA_Polaromonas naphthalenivorans CJ2
CusA_Edwardsiella ictaluri
CusA_Enhydrobacter aerosaccus SK60
CusA_Citrobacter sp. 30-2
CzcA_Acinetobacter junii SH205
Putative_Pectobacterium carotovorum
CusA_Providencia rettgeri DSM1131

```
CusA_curvibacter                          TGG--------------HDLSQLRALQDWFLKFELKTLPNVAEVASVGGMVKQYQVVLDPLKLNGYGITYEQVRQALTNG
CzcA_Ralstonia pickettii 12J              TGR--------------HDLAQLRALQDWFLRFELKALPNVAEVASVGGMVKQFQVVLQPDRLRAFNLTQAKVLAALKGA
SilA_Ralstonia metallidurans CH34         SGQ--------------HDLSQLRALQDWFLRFELKSLPNVAEVASLGGMVKQFQIVLLPDRLRAYNLSQGKVLAALKGA
Putative_Methylibium petroleiphilum PM1   SGT--------------MDAGQLRALQDWFLKYELKTVPNVAEVASVGGMVRQYQVLLDPDKLAAYNVPHGAVIDAIRNA
CzcA_Delftia acidovorans SPH-1            SGK--------------MDISQLRALQDWFLKYELKTVPDVAEVASVGGMVRQYQIVLDPDKLAAYGIPHTKVVEAIQRA
CzcA_Yersinia kristensenii                TGK--------------HSLADLRALQDWTLKFELKTVPNVSEVASVGGMVRQYQVVLDPERMRALNLSHQQIASAIADS
CusA_Klebsiella pneumoniae                SGK--------------HDLAELRSLQDWFLKYELKTIPNVSEVASVGGVVKEYQIVVDPMKLTQYGISLGEVKSALDAS
CzcA_Polaromonas naphthalenivorans CJ2    SGT--------------QDLGQLRALQDWFLKYELKTVPNVAEVASIGGMVRQYQIVLDPQKLAAYAIPHTKVAEAVQKA
CusA_Edwardsiella ictaluri                SGQ--------------HDLAELRSLQDWFLKYELKTIPNVAEVAAVGGVVKQYQIQVDPLKLAQYGISLAEVKRAIGAA
CusA_Enhydrobacter aerosaccus SK60        TGQ--------------HDLAQLRSIQDWFLKYELKTLPNVAEVATIGGMVKQYQVVLDPSKMAALGVTQNNVIEAIQKA
CusA_Citrobacter sp. 30-2                 SGK--------------HDLAELRSLQDWFLKYELKTIPNVAEVASVGGVVKQYQVVIDPMKLTQYGISLADVKSALDTS
CzcA_Acinetobacter junii SH205            TGQ--------------HDLSQLRSIQDWFLKYELKTLPNVAEVATIGGMVKQYQVVLDPSKMAALGVTQNNVIEAIQKA
Putative_Pectobacterium  carotovorum      SGK--------------YSLADLRGFQDWLLKYELKTVPDVAEVASVGGMVKQYQIVVDPERMRTLGITHQQIVSAVKAA
CusA_Providencia rettgeri DSM1131         TGK--------------HNLAELRSLQDWFLKYELKALPNVSEVATVGGVVKSYQILVDPLKLSQFSVTLPEIKQAVEMA
                                          .*               . . ** :*** :::**:::*.::***::**:*:. *:   * ::    :       *:  .

CusA_Escherichia coli K12                 NQEAGGSSIELAEAEYMVRASGYLQTLDDFNHIVLKASENGVPVYLRDVAKVQIGPEMRRGIAELNGEGEVAGGVVILRS 293
Putative_Shigella sonnei                  NQEAGGSSIELAEAEYMVRASGYLQTLDDFNHIVLKARENGVPVYLRDVAKVQIGPEMRRGIAELNGEGEVAGGVVILRS
CzcA_Shewanella sp. ANA-3                 NQEAGGSSVEIAESEYMVRASGYLQTIDDFKNIVLKTGENGVPVYLRDVARIQMGPEMRRGIAELNGQGEVAGGVVILRS
SilA_Klebsiella pneumoniae                NQEAGGSSVEMAEAEYMVRASGYLQSIDDFNNIVLKTGENGVPVYLRDVARVQTGPEMRRGIAELNGQGEVAGGVVILRS
Putative_Serratia marcescens              NQEAGGSSVEMAEAEYMVRASGYLQSIDDFNNIVLKTGENGVPVYLRDVARVQTGPEMRRGIAELNGQGEVAGGVVILRS
SilA_Salmonella Typhimurium               NQEAGGSSVEIAEAEYMVRASGYLQSIDDFNNIVLKTGENGVPVYLRDVARVQTGPEMRRGIAELNGQGEVAGGVVILRS
CzcA_Erythrobacter sp. NAP1               NQEAGGSVVEMGEAEFMVRASGYLTSLNDFRQIPLKSVGNGIPVTLSDVATIQLGPEMRRGIAELNGEGEVAGGIVVLRQ
CzcA_Sphingopyxis alaskensis RB2256       NQEAGGSVLELAEAEYMVRASGYLRSLADFRAIPLRTASGGIPVTLGDVATIQLGPEMRRGIADLNGEGEVAGGIIVLRQ
CzcA_Caulobacter sp. K31                  NQEAGGSVVEMAEAEYMVRASGYLSSLDDFAAVPVKTAAGGVPIALGQVATIQIGPEMRRGVAELNGQGEVTGGVVVLRS
CzcA_Phenylobacterium zucineum HLK1       NQEAGGSVLELGEAEYMVRASGYLKTLEDFEAVPLRTAAGGVPVRLGDVAFVQLGPEMRRGVAELNGEGEVAGGVVILRS
CzcA_Asticcacaulis excentricus CB 48      NGESGGSVLELAEAEYMVRAGGYLKTLDDFRAIPLKTAAGGVPVTLGEVATVQIGPEMRRGVAELNGQGEVAGGVVILRS
CzcA_Pseudomonas fluorescens Pf0-1        NQETGGAVLEMAETEFIVRASGYLKTLDDFRAIPLKLGAGGVPVTLGDVATVQTGPDMRRGITELDGQGETVGGVVILRS
CzcA_Thiobacillus denitrificans           NQETGGAVLELAETELMVRASGYLESVADFRAIPLKL-AGTTPVSLGDVAHIQLGPEMRRGIGELDGEGEAVGGVVVLRS
CzcA_Stenotrophomonas sp. SKA14           NQETGGSVLELSGAEYMVRASGYLKTLDEFREIPLTA-RGGVPVRLGDVATLQIGPEMRRGIAELDGEGEVAGGVVVLRS
CzcA_ Acidovorax sp. JS42                 NQETGGSVLELSGAEYMVRASGYLKTLDEFREIPLTA-RGGVPVRLGDVAMLQIGPEMRRGIAELDGEGEVAGGVVVLRS
CzcA_Leptothrix cholodnii SP-6            NQETGGSVLELAEVEYMVRASGYLRSLDDFRTIPLAA-RGGIPVKLGDVATVQIGPEMRRGIAELDGEGEVAGGVVVLRS
CusA_curvibacter                          NQETGGSVLELAEAEYMVRVNGYLRTLEDFRAIPLSS-RGGTAVRLGDVATVQVGPEMRRGVAELDGQGEVAGGVVLLRS
CzcA_Ralstonia pickettii 12J              NQETGGSVLELGEAEYMVRASGYLKTLDDFRQIPLMTNDAGIAVHLGDVATVQLGPEMRRGIAELNGQGEVAGGVVILRS
SilA_Ralstonia metallidurans CH34         NQETGGSVLELGEAEYMVRATGYLKTLDDFRQVPIVTSDAGIPVRLGDVAVIQLGPEMRRGIAELNGQGEVAGGVIILRS
Putative_Methylibium petroleiphilum PM1   NQEAGGSVLELGEAEYVVRASGLLATLDDFRKIPLSATDAGVSVRLGDVARIQVGPEMRRGIGELDGEGEATGGVIVMRS
CzcA_Delftia acidovorans SPH-1            NQETGGSVLELGEAEYMVRASGYLGSLEDFRKVPLMTTAAGVSVRLGDVARVQIGPEMRRGIGELNGEGEAVGGVIVMRS
CzcA_Yersinia kristensenii                NQEGGGSVLEMGAAEYMVRASGYLKTLDDFRNIVITV-REGIPILLSDVATVRIGPEMRRGVAELNGEGEVAGGIIVMRY
CusA_Klebsiella pneumoniae                NQEAGGSSVELAEAEYMVRASGYLQTLDDFKNIVLKTGDNGVPVYLGDVARVQIGPEMRRGIAELNGEGEVAGGVVILRS
CzcA_Polaromonas naphthalenivorans CJ2    NQEAGGSVLELGEAEYMVRATGYLKGLEDFRAIPLTTTAGGVSVRLGDVATIQLGPEMRRGIGELDGEGEAVGGVIIMRS
CusA_Edwardsiella ictaluri                NQEAGGASLELAEAEYMVRASGYLRTLDDFNHIVLKSGQDGVPITLQDVARLQIGPEMRRGITELNGEGEVAGGVVILRS
CusA_Enhydrobacter aerosaccus SK60        NQETGGSVLEMAETEYMVRASGYLKTLDDFRQIPLRTNSLGVPVSLGDVATIQLGPEMRRGITELNGQGETVGGVVILRA
CusA_Citrobacter sp. 30-2                 NQEAGGSSVEMAEAEYMVRASGYLQTLDDFNNIVLKTGADGVPVYLRDVARVQIGPEMRRGIAELNGEGEVAGGVVILRS
CzcA_Acinetobacter junii SH205            NQETGGSVLEMAETEYMVRASGYLKTLEDFRQIPIKTNSFGIPVTLGDVATIQLGPEMRRGITELNGQGETVGGVVILRA
Putative_Pectobacterium  carotovorum      NQENGGSVLELGEAEYMVRTTGYLKSMQDFNHVVITTRN-GIPVLLQDVATLREGPEMRRGIAELNGEGEVAGGIIVLRY
CusA_Providencia rettgeri DSM1131         NQEAGGSSIEIAEAEYMVRASGYLQSIDDFNKIYLKTPENGVPIYLQDVARIQEGPEMRRGVAELNGEGEVVGGVILLRS
                                          * * **: :*:.  * :**. * *  : :*  : :       .: * :** :: **:****: :*:*:**..**::::*

CusA_Escherichia coli K12                 GKNAREVIAAVKDKLETLKSSLPEGVEIVTTYDRSQLIDRAIDNLSGKLLEEFIVVAVVCALFLWHVRSALVAIISLPLG 373
Putative_Shigella sonnei                  GKNAREVIASVKDKLETLKSSLPEGVEIVTTYDRSQLIDRAIDNLSGKLLEEFIVVAVVCALFLWHVRSALVAIISLPLG
CzcA_Shewanella sp. ANA-3                 GKNAREVITAVRDKLDTLKASLPEGVEIVTTYDRSQLIDRAIDNLSYKLLEEFIVVAVVCALFLWHVRSALVAIISLPLG
SilA_Klebsiella pneumoniae                GKNARDVITAVRDKLETLKASLPEGVEIVTTYDRSQLIDRAIDNLSYKLLEEFIVVAVVCALFLWHVRSALVAIISLPLG
Putative_Serratia marcescens              GKNARDVITAVRDKLETLKASLPEGVEIVTTYDRSQLIDRAIDNLSSKLLEEFIVVAIVCALFLWHVRSALVAIISLPLG
SilA_Salmonella Typhimurium               GKNARDVITAVRDKLETLKASLPEGVEIVTTYDRSQLIDRAIDNLSSKLLEEFFVVAIVCALFLWHVRSALVAIISLPLG
CzcA_Erythrobacter sp. NAP1               GEDARATIAAVEEKLDVLQASLPEGVEIVTTYDRSKLIDASIENLTGKLIAEFIIVAIVCALFLWHVRSALVAIVTLPLG
CzcA_Sphingopyxis alaskensis RB2256       GADARSAIMAVEAKLAELKKGLPKGVEVVTTYDRSQLIDHAVENLTHKLIEEFIVVALVCALFLWHVRSALVAIVTLPLG
CzcA_Caulobacter sp. K31                  GEDARATLKAVQDKLKTLKASLPPGVEIVTTYDRSGLIDRAIDNLKSKLIEEFIVVALVCALFLWHLRSALVAIIALPLG
CzcA_Phenylobacterium zucineum HLK1       GKNARETIQAVRGKLEELKRSLPPGVEVVTTYDRSQLIDRAIANLSQKLLEEFVVVAIVCGLFLWHARSALVAILTLPLG
CzcA_Asticcacaulis excentricus CB 48      GKNAMETISAVKAKLAELKKSLPPGVEVVTTYDRSQLIGRAIGNLREKLIEEFLVVAIVCALFLWHVRSALVAILTLPIG
CzcA_Pseudomonas fluorescens Pf0-1        GKNARETIAAVKAKLDDMKSSLPPGVEIVTTYDRSKLIDRAVENLSHKLLEEFIVVALVCGIFLWHLRSSLVAIISLPVG
CzcA_Thiobacillus denitrificans           GKNARETIAAVKAKLDTLKVSLPAGVEIVPTYDRSQLIDRAIDNLSDKLVEEFIVVALVCALFLWHLRSSLVAIVSLPLG
CzcA_Stenotrophomonas sp. SKA14           GKNAQETIAAVKAKLAELQGSLPPGVEIVTTYDRSALIERAIRNLTTKLGEEFLVVALVCALFLWHLRSALVAIISLPLG
CzcA_ Acidovorax sp. JS42                 GKNAQETIAAVKAKLAELQGSLPPGVEIVTTYDRSALIERAIRNLTTKLGEEFLVVALVCALFLWHLRSALVAIISLPLG
CzcA_Leptothrix cholodnii SP-6            GKNAQSTITAVKAKLAELQKSLPAGVEIVTTYDRSALIERAIENLTHKLIEEFIVVAAVCALFLWHLRSALVAIIALPLG
CusA_curvibacter                          GKNAQETIRAVKTKLAELQKSLPPGVEVVTTYDRSALIARAIDNLSAKLLEEFVVVALVCVLFLGHLRSALVAIISLPLG
CzcA_Ralstonia pickettii 12J              GKNALETIEAVKAKLATLRTSLPEGVEIVPVYDRSGLIKRAVDNLTHKLIEEFIVVAVVCLVFLFHLRSALVAIVSLPLG
SilA_Ralstonia metallidurans CH34         GKNALETINAVKAKLQTLQKSLPDGVEVVTVYDRSALISRAVDNLTTKLLEEFAVVAVVCLLFLFHLRSALVAIISLPLG
Putative_Methylibium petroleiphilum PM1   GKNALETIAAVKTKIASLQASLPKGVEIVPTYDRSSLIERAVENLSHKLIEEFIVVALVCVVFLFHLRSAFVAIVTLPLG
CzcA_Delftia acidovorans SPH-1            GKNALQTIAAVKGKLQGLASSLPRGVEIVPVYDRSGLIERAVENLTHKLIEEFIVVAVVCFLFLFHLRSALVAIISLPLG
CzcA_Yersinia kristensenii                GKNALETINGVKEKLQQIQRSLPAGVEIVPVYDRSHLITQAIDSLSFKLLEEFLVVAVICSLFLFHFRSALVAIITLPLG
CusA_Klebsiella pneumoniae                GKNAREVISAVKAKLASLQSSLPEGVEVVTTYDRSQLIDRAIDNLSHKLLEEFIVVALVCALFLWHVRSALVAIISLPLG
CzcA_Polaromonas naphthalenivorans CJ2    GKNALETIAAVKAKMAQLQASLPQGVEVVTTYDRSGLINRAVDNLTYKLLEEFLVVAVVCFVFLFHLRSALVAIISLPLG
CusA_Edwardsiella ictaluri                GENARQVITAVKAKLAALQGSLPPGVEVVTTYDRSQLIDRAIDNLSTKLIEEFIVVALVCALFLWHVRSALVAIVSLPLG
CusA_Enhydrobacter aerosaccus SK60        GKNARETITAVKAKLAELQQSLPKGVQVVPVYDRSKLIDRAVENLSHKLIEEFIVVALVCVLFLWHLRSAMVAIVSLPLG
CusA_Citrobacter sp. 30-2                 GKNAREVISAVKAKLDTLKSSLPEGVEVVTTYDRSQLIDRAIDNLSYKLLEEFIVVALVCALFLWHLRSALVAIISLPLG
CzcA_Acinetobacter junii SH205            GKNARETITAVKAKLAELQQSLPKGVQVVPVYDRSKLIDRAVENLSHKLIEEFIVVALVCGLFLWHLRSAMVAIVSLPLG
Putative_Pectobacterium  carotovorum      GKNALNTLHAVKARLQEIQKSLPAGVEIVPTYDRSQLIEHAIDTLSFKLLEEFVVVAIICALFLFHFRSALVAIISLPLG
CusA_Providencia rettgeri DSM1131         GENARNVIQDVKQKLEDLSASLPEGIEIITTYDRSILIDNAIDNLSYKLLEEFIVVALVCAVFLWHFRSALVAIISLPLG
                                          * :*  .:  *. ::  :  .** *:::::.**** **  :: .*  **  ** :** :* :** * **::***::**:*
```


M391
M403
M410
M486
M501
CusA_Escherichia coli K12
Putative_Shigella sonnei
CzcA_Shewanella sp. ANA-3
SilA_Klebsiella pneumoniae
Putative_Serratia marcescens
SilA_Salmonella Typhimurium
CzcA_Erythrobacter sp. NAP1
CzcA_Sphingopyxis alaskensis RB2256
CzcA_Caulobacter sp. K31
CzcA_Phenylobacterium zucineum HLK1
CzcA_Asticcacaulis excentricus CB 48
CzcA_Pseudomonas fluorescens Pf0-1
CzcA_Thiobacillus denitrificans
CzcA_Stenotrophomonas sp. SKA14
CzcA_ Acidovorax sp. JS42
CzcA_Leptothrix cholodnii SP-6
CusA_curvibacter
CzcA_Ralstonia pickettii 12J
SilA_Ralstonia metallidurans CH34
Putative_Methylibium petroleiphilum PM1
CzcA_Delftia acidovorans SPH-1
CzcA_Yersinia kristensenii
CusA_Klebsiella pneumoniae
CzcA_Pectobacterium carotovorum
CusA_Providencia rettgeri DSM1131

```
CzcA_ Acidovorax sp. JS42                RRPKATLLIAVLALATTAWPLARLGGEFLPRLDEGDLLYMPSALPGLSAQRATELLQLSNRMIKTVPEVDKVFGKAGRAE
CzcA_Leptothrix cholodnii SP-6           RWPKATLAAALLVLATTAWPLMQLGGEFLPKLDEGDLLYMPSALPGLSAQKAGELLQLTNRMIKTVPEVERVFGKAGRAE
CusA_curvibacter                         LRPKSTLAIAVLAFATTLWPVSQLGGEFLPPLDEGDLLYMPSALPGLSAQKASELLQITNRMIRTVPEVERVFGKAGRAE
CzcA_Ralstonia pickettii 12J             AYPKATVAIAIVLLGATAWPILRTGGEFMPPLDEGDLLYMPSALPGLSAGKAAQLLQQTDRLIKTVPEVASVYGKAGRAD
SilA_Ralstonia metallidurans CH34        RYPKTTVALAGVLLVATAWPIMRTGGEFMPPLDEGDLLYMPSALPGLAAGKASQLLQQTDRLIKTVPEVASVFGKAGRAD
Putative_Methylibium petroleiphilum PM1  AWPKATIAVALLLLASTAWPILKIGGEFMPPLDEGDLLYMPTALPGLSAGKAAELLQQTDRLIKTLPEVQSVYGKAGRAE
CzcA_Delftia acidovorans SPH-1           RAPKLTLVVALVVLATSLWPLRHIGGEFMPRLDEGDLLYMPSALPGLSAGKAAELLQQTDRLIKTVPEVASVYGKAGRAE
CzcA_Yersinia kristensenii               ARPKTTLAIAGMLLLATLYPLSRLGSEFMPALDEGDLLYMPSTLPGISVREASHLLQQTDRLIKTVPEVDTVFGKAGRAE
CusA_Klebsiella pneumoniae               HWPKTTLLIALLSILTVAWPLNRVGGEFLPQINEGDLLYMPSTLPGISAAQAADMLQKTDKLIMTVPEVARVFGKTGKAE
CzcA_Polaromonas naphthalenivorans CJ2   RAPKMTLVVAGIVLATSLWPLQHIGGEFMPRLDEGDLLYMPSALPGLSSGKAGELLQQTDRLIKTVPEVASVYGKAGRAE
CusA_Edwardsiella ictaluri               RWPRMTLLVAAFSIVTVIWPLERLGGEFLPNINEGDLLYMPSTLPGISPAQAAQLLQTTDKLIRQVPEVASVFGKSGKAE
CusA_Enhydrobacter aerosaccus SK60       AHPKTILLGALLIFLISLFPLTRLGGEFLPNMDEGDLLYMPSALPGLSAAKASELLQQTDRMIKTVPEVATVFGKAGRAE
CusA_Citrobacter sp. 30-2                HWPKTTILVAVLSILTVVYPLNKVGGEFLPQINEGDLLYMPSTLPGISAAQAADMLQKTDKLIMTVPEVARVFGKTGKAE
CzcA_Acinetobacter junii SH205           AYPKTTLLVALLIFLVSLYPLTRLGGEFLPNMDEGDLLYMPSALPGLSAAKASELLQQTDRMIKTVPEVATVFGKAGRAE
Putative_Pectobacterium  carotovorum     SYPKTTLLVSGLLLLLTLFPLSRLGSEFMPPLDEGDLLYMPSTLPGISAREAGRLLQQTDRLIKTVPEVESVFGKAGRAE
CusA_Providencia rettgeri DSM1131        AWPKTTLLIGFLSLFTVIWPLKHLGGEFLPAINEGDLLYMPSTLPGVSPAQAAFLLQNTDKLIKTIPEVDTVFGKVGKAE
                                           *   :  .   .     *. : *.**:* ::********::***::  .*  :** ::::*  :***  *:** *:*:

CusA_Escherichia coli K12                TATDSAPLEMVETTIQLKPQEQWRPGMTMDKIIEELDNTVRLPGLANLWVPPIRNRIDMLSTGIKSPIGIKVSGTVLADI 693
Putative_Shigella sonnei                 TATDSAPLEMVETTIQLKPQDQWRPGMTMDKIIEELDNTVRLPGLANLWVPPIRNRIDMLSTGIKSPIGIKVSGTVLADI
CzcA_Shewanella sp. ANA-3                TATDSAPLEMMETTIQLKPEDQWRPGMTIDKIIEELDRTVRLPGLANLWVPPIRNRIDMLSTGIKSPIGIKVSGTVLSDI
SilA_Klebsiella pneumoniae               TATDSAPLEMVETTIQLKPEDQWRPGMTIDKIIDELDRTVRLPGLANLWVPPIRNRIDMLSTGIKSPIGIKVSGTVLSDI
Putative_Serratia marcescens             TATDSAPLEMVETTIQLKPEDQWRPGMTIDKIIEELDRTVRLPGLANLWVPPIRNRIDMLSTGIKSPIGIKVSGTVLSDI
SilA_Salmonella Typhimurium              TATDSAPLEMVETTIQLKPEDQWRPGMTIDKIIDELDRTVRLPGLANLWVPPIRNRIDMLSTGIKSPIGIKVSGTVLSDI
CzcA_Erythrobacter sp. NAP1              TATDPAPLTMFETTIRFKPREEWRPGMTPEKLVEELDRAVQVPGLANVWVPPIRNRIDMLATGIKSPIGVKVSGENLGEI
CzcA_Sphingopyxis alaskensis RB2256      TATDPAPLEMFETTIHFKPRDQWRPGMTPEKLVEELDAKVKVPGLANIWIPPIRNRIDMLATGIKSPIGVKVSGSDLAEL
CzcA_Caulobacter sp. K31                 SATDPAPLEMFETTIRFKPRDQWRPGMTPEKLVEELDRTVAVPGLSNVWVPPIRNRIDMLATGIKSPIGVKVSGADLAQL
CzcA_Phenylobacterium zucineum HLK1      TATDPAPLEMFETTIQFKPRGEWRPGMTPEKLVEELDRAVQVPGLANVWVPPIRNRIDMLATGIKSPIGVKVSGPDLAQL
CzcA_Asticcacaulis excentricus CB 48     SATDPAPMEMFETTIQFKPRDQWRPGMTPDKLVEELDRTVKVPGLANVWVPPIRNRIDMLATGIKSPIGVKVSGSNLAEL
CzcA_Pseudomonas fluorescens Pf0-1       TATDPAPLEMFETTIQFKPHDQWRPGMTPEKLVEELDRVVRVPGLTNIWIPPIRNRIDMLATGIKSPIGVKVAGNDLAQI
CzcA_Thiobacillus denitrificans          TATDPAPLEMFETTIQFKPREQWRAGMTPDELVEELDRAVRVPGLANIWVPPIRNRIDMLATGIKSPVGVKIAGSHLADI
CzcA_Stenotrophomonas sp. SKA14          TATDPAPLEMFETTVKLKPREQWRPGMTPEKLVEELDRAVKIPGLSNIWIPPIRNRIDMLATGIKSPIGVKVTGNDLGVI
CzcA_ Acidovorax sp. JS42                TATDPAPLEMFETTVKLKPREQWRPGMTPEKLVEELDRAVKIPGLSNIWIPPIRNRIDMLATGIKSPIGVKVTGNDLGVI
CzcA_Leptothrix cholodnii SP-6           SATDPAPIEMFETTIQLKPREQWRAGMTPDKLVEELDRAVKVPGLSNLWVPPIRNRIDMLATGIKSPIGVKVTGGDLAAI
CusA_curvibacter                         TATDPAPMEMFETTVQLKPREQWRVGMTPEKLIETLDQAVKVPGLSNIWIPPIRNRIDMLATGVKSLIGVKVNGTDLATI
CzcA_Ralstonia pickettii 12J             SATDPAPIEMFETTIQFKPHDQWRPGMTTDKLVEELDRVVKVPGLSNIWVPPIRNRIDMLATGIKSPVGIKVAGTDLKEI
SilA_Ralstonia metallidurans CH34        SATDPAPIEMFETTIRFKPKDQWRPGMTTDKLVEELDRIVKVPGLSNIWVPPIRNRIDMLATGIKSPVGIKVAGTDLKEI
Putative_Methylibium petroleiphilum PM1  TATDPAPMEMFETTIQFKPKDQWRDGMTSDRLVEELDRIVKVPGLSNIWVPPIRNRIDMLATGIKSPVGVKVAGTDLAEI
CzcA_Delftia acidovorans SPH-1           SATDPAPLEMFETTIQFKPRDQWRAGMTTDKLVEELDRIVKVPGLSNIWVPPIRNRIDMLATGIKSPVGVKVAGTDLATI
CzcA_Yersinia kristensenii               TATDPAPLTMIETTIRFKPKDQWRPGMTMDKLIEELDATVKVPGIANLWVPPIRNRLDMLSTGIKSPVGIKVNGKNVQQI
CusA_Klebsiella pneumoniae               TATDSAPLEMVETTIQLKPQDQWRPGMTMEKIVEELDKTVRLPGLANLWVPPIRNRIDMLSTGIKSPIGIKVSGTNLADI
CzcA_Polaromonas naphthalenivorans CJ2   TATDPAPMEMFETTIQFKPKDQWRAGMTQDQLVEELDRIVQVPGLSNIWVPPIRNRIDMLATGIKSPVGVKVAGTDLQTI
CusA_Edwardsiella ictaluri               TATDSAPLEMIETTIQLKPQEQWRPGMTMDRIIAELDATVRLPGLANLWVPPIRNRIDMLSTGIKSPIGIKVSGTVLADI
CusA_Enhydrobacter aerosaccus SK60       SATDSAPLEMFETTIQFKPRSEWRSGMTPDKLVKELDKAVQVPGLTNIWVPPIRNRIDMLATGVKSPIGIKISANDLQDI
CusA_Citrobacter sp. 30-2                TATDSAPLEMVETTIQLKPQDQWRPGMTMDKIIAELDKTVRLPGLANLWVPPIRNRIDMLSTGIKSPIGIKVSGNVLADI
CzcA_Acinetobacter junii SH205           SATDPAPLEMFETTIQFKPRSEWRSGMTPDKLVKELDKAVQVPGLTNIWVPPIRNRIDMLATGVKSPIGIKISANDLQDI
Putative_Pectobacterium  carotovorum     TATDPAPLTMLESTIRLKPRNQWREGMTMDKLVAELDRTVSLPGIVNVWVPPIRNRLDMLATGIKSPVGIKVNGNNLADI
CusA_Providencia rettgeri DSM1131        TATDSAPMEMIETTIRLKPQAQWREGMTLEKIIDELDETVRLPGVANLWVPPIRNRIDMLSTGVKSPIGIKVSGRDLEEI
                                         :***.**: *.*:*:::**. :** *** :.::  **  * :**: *:*:******:***:**:** :*:*: .  :  :

CusA_Escherichia coli K12                DAMAEQIEEVARTVPGVASALAERLEGGRYINVEINREKAARYGMTVADVQLFVTSAVGGAMVGETVEGIARYPINLRYP 773
Putative_Shigella sonnei                 DAMAEQIEEVARTVPSVASALAERLEGGRYINVEINREKAARYGMTVADVQLFVTSAVGGAMVGETVEGIARYPINLRYP
CzcA_Shewanella sp. ANA-3                DVTAQSIEAVAKTVPGVVSALAERLEGGRYIDVDINREKASRYGMTVGDVQLFVSSAIGGAMVGETVEGVARYPINIRYP
SilA_Klebsiella pneumoniae               DATAQSIEAVAKTVPGVVSVLAERLEGGRYIDIDINREKASRYGMTVGDVQLFVSSAIGGAMVGETVEGVARYPINIRYP
Putative_Serratia marcescens             DATAQSIEAVAKTVPGVVSALAERLEGGRYIDVDINREKASRYGMTVGDVQLFISSAIGGATVGETVEGVARYPINIRYP
SilA_Salmonella Typhimurium              DATAQSIEAVAKTVPGVVSVLAERLEGGRYIDIDINREKASRYGMTVGDVQLFVSSAIGGAMVGETVEGVARYPINIRYP
CzcA_Erythrobacter sp. NAP1              ERVALQVENVAKQVPGVSSALAERLSGGRYVDVDIDRVAAARFGLNIADVQQIVSGAIGGANIGRTVEGLARYPINVRYP
CzcA_Sphingopyxis alaskensis RB2256      DRIAHDVETAAKAVPGVSSALAERLTGGRYVDVEIDRLAAARFGLNIADVQEIVAGAIGGENVGQTVEGLARYPINVRYP
CzcA_Caulobacter sp. K31                 DRVAQQIAQVAKTVPGVSSALAERLAGGRYVDVRIDRAAAGRYGLNIADVQAIVSGAIGGQTVGETVEGLARYPISVRYP
CzcA_Phenylobacterium zucineum HLK1      DRVAAQVEAVARRTPGVSSALAERLTGGRYVDVSIDRSAAARFGLNIADVQEIVSGAVGGETIGETVEGVARYPISVRYP
CzcA_Asticcacaulis excentricus CB 48     DRIAHDVETVAKTVPGVSSALAERLTGGRYVDVDIDRAAAGRYGLNTSDVQSIISGAVGGENVGETVDGLARYPINVRYP
CzcA_Pseudomonas fluorescens Pf0-1       DAATQAVERVAKGVPGVSSALAERLTGGRYIDVDIDRNAAGRYGLNISDVQSIVSGAIGGENIGETIEGLARFPINIRYP
CzcA_Thiobacillus denitrificans          DRIAQDVERVAKTVPGVSSALAERLTGGRYVDVDIDRAAAARYGMNIGDVQSAVANLIGGENIGETVEGLARYPINVRYP
CzcA_Stenotrophomonas sp. SKA14          DRIAAEVEQVAKGIPGVTSSLAERLTGGRYVDVQIDRVAAGRYGLNIADVQAVIAGAVGGENVSETVEGLARFPINLRYA
CzcA_ Acidovorax sp. JS42                DRIAAEVEQVAKGIPGVTSSLAERLTGGRYVDVQIDRVAAGRYGLNIADIQAVIAGAVGGENVSETVEGLARFPINLRYA
CzcA_Leptothrix cholodnii SP-6           DRVAARIEQVAKGVPGVSSALAERLTGGRYVDVQIDRAAASRYGLNIADVQALVAGAIGGENVTETIEGRARFPVNLRYP
CusA_curvibacter                         DRIASQIEQVAKSVPGVSSALAERLTGGRYVDVQIDRVAAGRYGLNINDVQALVSGAIGGENVAETIEGLARFPINLRYP
CzcA_Ralstonia pickettii 12J             DRLTTRIEETVKTVPGVTSALAERLSGGRYIDVDINRQAAGRYGLNIDDVQSIVSSAIGGDNVGEVVDGLARFPINLRYP
SilA_Ralstonia metallidurans CH34        DRLATRIEEAVKTVPGVTSALAERLTGGRYIDVDIDRAAAGRYGLNIEDVQSIVSSAIGGETVGEVVDGLARFPINVRYP
Putative_Methylibium petroleiphilum PM1  DRIASEVEKAVRGVPGVSSALAERLTGGRYVDVNINRDTAARYGMNIADVQSVISTAVGGDNVGETVEGLQRFPINVRYP
CzcA_Delftia acidovorans SPH-1           DRLTGEIERTLKDVPGVSSALAERLTGGRYVDVNIRRDDAARYGLNIADVQSVVSSAVGGENIGETVEGLQRFPINLRYP
CzcA_Yersinia kristensenii               EQVAQKIEQVVRKVPGVTSALSERLAGGRYVDIDIDRKRAARYGVSVKELQSLVETVIGGQNIGETIEGRERYPINLRYP
CusA_Klebsiella pneumoniae               DAIAEQIEGVARTVPGVTSALAERLVGGRYLNIDIQREKAARYGMTVGDVQLFVSSAIGGAMVGETVEGVERYPINIRYP
CzcA_Polaromonas naphthalenivorans CJ2   DKLSSEIERALKDVPGVSSALAERLTGGRYVDVNIKRDAAASFGLNIADVQSVISSAVGGENIGETVEGLQRFPINMRYP
CusA_Edwardsiella ictaluri               DATAQRIEAVARGVPGVVSALAERLEGGRYIDIQIQRQRAARYGMTVADVQLFVSSAIGGAMVGETVEGVARYPIALRYP
CusA_Enhydrobacter aerosaccus SK60       DRVAQQIEQVAKQVPGVSSALAERLTGGRYVDVDINRMQAARYGLNINDVQQIVSSAIGGENIGETVEGLARYPINVRYP
CusA_Citrobacter sp. 30-2                DATAEQIEEVARTVPGVSSALAERLVGGRYLNVDINRERAARYGMTVGAVQLFVTSAVGGAMVGETVEGIARYPINLRYA
CzcA_Acinetobacter junii SH205           DRVAQQIEQVAKQVPGVSSALAERLTGGRYVDVDINRMQAARYGLNINDVQQIVSSAIGGENIGETVEGLARYPINVRYP
Putative_Pectobacterium  carotovorum     ERTAAQIEQVVKQVPGVTSALAERLAGGRYIDIDIDRQRAARYGVSVEELQSVVSTLIGGQNIGETIEGRQRYPINIRYP
CusA_Providencia rettgeri DSM1131        DALAQEIEKVTKSVPGVVSVLAERLVGGRYIDVKIDREKAARFGMSVSDVQVYISMATGGEMIGETVEGIERYPISIRYP
                                         :  :  :  . :  *.* * *:*** ****::: * *  *. :*:.  :*  :    **  : ..::*  *:*: :**.
```

```
CusA_Escherichia coli K12                    QSWRDSPQALRQLPILTPMKQQITLADVADIKVSTGPSMLKTENARPTSWIYIDARDRDMVSVVHDLQKAIAEKVQLKPG 853
Putative_Shigella sonnei                     QSWRDSPQALRQLPILTPMKQQITLADVADVKVSTGPSMLKTENARPTSWIYIDARDRDMVSVVHDLQKAIAEKVQLKPG
CzcA_Shewanella sp. ANA-3                    QDYRNSPQALREMPILTPMKQQITLGDVADIKVVSGPTMLKTENARPASWIYVDARGRDMVSVVNDIKTAISEKVKLRPG
SilA_Klebsiella pneumoniae                   QDYRNSPQALREMPILTPMKQQITLGDVADIKVVSGPTMLKTENARPASWIYVDARGRDMVSVVNDIKTAISEKVKLRPG
Putative_Serratia marcescens                 QDYRNSPQALKQMPILTPMKQQITLGDVADIKVVSGPTMLKTENARPASWIYIDARGRDMVSVVNDIKTAISQKVKLRPG
SilA_Salmonella Typhimurium                  QDYRNSPQALKQMPILTPMKQQITLGDVADINVVSGPTMLKTENARPASWIYVDARGRDMVSVVNDIKTAISEKVKLEPG
CzcA_Erythrobacter sp. NAP1                  REIRDSLAELHALPLLTPSGQQITLGTVAQVSVSDGPPMLKNEQGRLISVVYVDTRGRDLTSVVSDLQAAVSEGVDLPAG
CzcA_Sphingopyxis alaskensis RB2256          REIRDSLENLQALPILTRSGQQITLGTVALLRISDGPPMLKTENGRLSTWVYVDVRGRDLASVVGDIQTAIAGQVKLSPG
CzcA_Caulobacter sp. K31                     REVRGDLEALRALPILTPSLQQITLGTVAKVVVNEGPPMLKSENGRPTTFVYVDVRGRDLSSVVADLQRAVAKDVRPVAG
CzcA_Phenylobacterium zucineum HLK1          RELRDSLDGLRSLPVLTPAGQQITLGAVADIRVADGPPMLKTENARPSTWIYVDVRGRDLNSVVKDLQQAVAKEVKAPPG
CzcA_Asticcacaulis excentricus CB 48         REDRDSLEGLRNLPILTPSGAQITLGTVAKVEIAEGPPMLKTENGRPSTWVYIDVRDRDLASVVGDLQKAVAKDVKLSPG
CzcA_Pseudomonas fluorescens Pf0-1           REWRDSPGALEQLPIYTPSGSQITLGMLAKIKVTDGPPMLKSENARPSGWVYIDVRGRDIASVVADLRRAVNEQVKLQPG
CzcA_Thiobacillus denitrificans              REWRDTPDKLAALPILAPNGSRITLGMVAAVRVSDGPPMLKSENARPSGWVYVDVRGRDLASTVADLQAAVAAGVKLEPG
CzcA_Stenotrophomonas sp. SKA14              REWRDSPQRLAELPIFTPMGQQITLGTVARIAITDGPPMLKSENARPSGWVYVDVRGRDLASVANELRDAIGQQVKLEPG
CzcA_ Acidovorax sp. JS42                    REWRDSPQRLAELPIFTPMGQQITLGTVARIAITDGPPMLKSENARPSGWVYVDVRGRDLASVANELRDAIGQQVKLEPG
CzcA_Leptothrix cholodnii SP-6               REWRDTPQRLAQLPILTPMGQQITLGSVARVAISDGPPMLKSENARPSGWVYVDVRGRDLAAVANDLRTAVTRDVPLEAG
CusA_curvibacter                             REWRDTPEKLAQLPILTPMGQQITLGTVAKVRITDGPPMLKTENARPSNWVYVDVRGRDLASVANDLRQAVSQQVKLEPG
CzcA_Ralstonia pickettii 12J                 RDYRDSVEQLRRLPIVTDRGQQIVLSDVADIRVVQGPPMLRSENARLSGWIYVDIRGRDLRSAVKDMQAAVTKAVPMPAG
SilA_Ralstonia metallidurans CH34            RDDRDSIDALRRLPIVTDSGLQIALADVARITVMQGPPMLRSENARLSGWVYVDIRGRDLRSTVRDMQAAVAKAVPMPAG
Putative_Methylibium petroleiphilum PM1      REVRDSLEKLRKLPFVTPRGARIVLSDVAELRISDGPPMLRSENARLAGFVYVDIRGRDLKSAVIDMQKVVAEQVKLTPG
CzcA_Delftia acidovorans SPH-1               REMRDSLEKLRVLPVLTGSGQRLVLSDVADIRVADGPPMLRSENARLSGWVYVDIRDRDLRSAVQDMQQAVEKQVKLPPG
CzcA_Yersinia kristensenii                   REIRDSLQKLRDLPVVTASGGQVALSELADIKVTEGPPMLKSENARLSDWVYVDLRGRDLKSAVTDMQQAVAEQVKLPEG
CusA_Klebsiella pneumoniae                   QSYRDSPETLRQLPILTPLKQQIVLGDVAEVKVVTGPSMLKTENARPTSWIYIDARDRDMVSVVHDLQQAIGKEVKLKPG
CzcA_Polaromonas naphthalenivorans CJ2       RELRDSMETLRSLPFVTERGQRVVLSDVADIRITDGPPMLRSENARLSGWVYVDIRDRDLRSAVQDMQKAVTEKVKLPPG
CusA_Edwardsiella ictaluri                   QSWRDSPEALRQLPILTPLQQQITLGDVAGVRVVSGPSMLKTENARPASWIYIDARGRDMVSVVEDLKRAIQQQVTLAPG
CusA_Enhydrobacter aerosaccus SK60           REIRDSLEALRNLPIVTESGQQIVLSSVANIQITDGPPMLKSENSRPSGWVYVDVQGRDLASVVQDLKQSIDQKVKRSSA
CusA_Citrobacter sp. 30-2                    QSYRDSPEALRNLPILTPMKQQITLADVADVTVVSGPSMLKTEDARPTSWIYIDARDRDMVSVVNDLKKAIAEKVQLKPG
CzcA_Acinetobacter junii SH205               REIRDSLEALRNLPIVTESGQQIVLSSVANIQITDGPPMLKSENSRPSGWVYVDVQGRDLASVVQDLKQSIDQKVKRSSA
Putative_Pectobacterium  carotovorum         RELRDSLQKLRDLPIVTANGSRVMLAELADIRVSEGPPMLKSENSRLSDWIYVDLRGRDLKSAVEDMQQAVTQQVTLPEG
CusA_Providencia rettgeri DSM1131            QDYRNSVSAMKLLPILTPSKQQIVLSDVADVSVNMGAPMLKTENARPTSWIYIDARDRDMVSVIKDIDTAIKDNIQMRPG
                                             :. *.    :  :*. :    :: *. :* : :  *..**:.*:.*    :*:* :.**: :.  ::   :   :    .

CusA_Escherichia coli K12                    TSVAFSGQFELLERANHKLKLMVPMTLMIIFVLLYLAFRRVGEALLIISSVPFALVGGIWLLWWMGFHLSVATGTGFIAL 933
Putative_Shigella sonnei                     TSVAFSGQFELLERANHKLKLMVPMTLMIIFVLLYLAFRRVGEALLIISSVPFALVGGIWLLWWMGFHLSVATGTGFIAL
CzcA_Shewanella sp. ANA-3                    TSVAFSGQFELLEHANKKLKLMVPMTVMIIFILLYLAFRRVDEALLILMSLPFALVGGIWFLYWQGFHMSVATGTGFIAL
SilA_Klebsiella pneumoniae                   TSVAFSGQFELLEHANKKLKLMVPMTVMIIFILLYLAFRRADEALLILMSLPFALVGGIWFLYWQGFHMSVATGTGFIAL
Putative_Serratia marcescens                 TSVSFSGQFELLEHANKKLKLMVPMTVMIIFILLYLAFRRVDEALLILMSLPFALVGGIWFLYWQGFHMSVATGTGFIAL
SilA_Salmonella Typhimurium                  TSVAFSGQFELLEHANKKLKLMVPMTVMIIFILLYLAFRRVDEALLILMSLPFALVGGIWFLYWQGFHMSVATGTGFIAL
CzcA_Erythrobacter sp. NAP1                  VSISYAGQFEYLTRANERLQVVVPATLGIIFVLLYLIFRRFDEALLVMGTLPFALTGGFWLLYLMGYNQSVATSVGFIAL
CzcA_Sphingopyxis alaskensis RB2256          VSIAYSGQFEYLTRAIDRLKLVIPATLVIIFVLLFLIFGRFDEALLIMATLPFALSGGFWLLYLLGYHQSVATGVGFIAL
CzcA_Caulobacter sp. K31                     VTISYSGQFEYLQRAVERLKIVVPATLLIIFVLLYLTFRRFDEAALIMGALPFALTGGFWALYLLGFNQSVATGVGFIAL
CzcA_Phenylobacterium zucineum HLK1          VSIAYSGQFEYLERAAERLKLVVPVTLAIIFVLLYVTFGRFDEAALIMLTLPFALTGGVWTLYLLGYEQSVATGVGFIAL
CzcA_Asticcacaulis excentricus CB 48         VSIAYSGQFEYLQRAIERLKLVVPATLVIIFLLLYVTFGRFDEAALIMLTLPFALTGGIWTLYLLGFHQSVATGVGFIAL
CzcA_Pseudomonas fluorescens Pf0-1           MSLSYSGQFEFLERANARLKLVVPATLLIIFVLLYLTFARFDEALLIMATLPFALSGGAWFLYLLGYNLSVATGVGFIAL
CzcA_Thiobacillus denitrificans              MSLAYSGQFEFMERANARLKLVVPATLLIIFVLLYLTFSRFDEAVLIMATLPFALTGGVWFLYAMDYQLSVATGVGFIAL
CzcA_Stenotrophomonas sp. SKA14              VSITYSGQFEYMERANARLKVVVPATLLIIFVLLYLTFARVDEAGLIMATLPFALTGGIWFLYLLNYNLSIATGVGFIAL
CzcA_ Acidovorax sp. JS42                    VSITYSGQFEYMERANARLKVVEPATLLIIFVLLYLTFARVDEAGLIMATLPFALTGGIWFLYLLNYNLSIATGVGFIAL
CzcA_Leptothrix cholodnii SP-6               MSVAYSGQFEYLERANARLKVVVPATLLIIFVLLYLTFSRFDEALLIMATLPFALTGGVWFLYLLGYQMSVATGVGFIAL
CusA_curvibacter                             VSITYSGQFEFLERANARLKVVVPATLLIIFVLLYLTFSRLDEAALIMATLPFALTGGVWFLYLMGYHLSVATGIGFIAL
CzcA_Ralstonia pickettii 12J                 YALSWSGQFEYLERASAKLQVVVPFTLLIIFVLLYLVFGRIDEALLIMGTLPLALIGGFWLLYLLGYNLSVAGVVGFIAL
SilA_Ralstonia metallidurans CH34            YALSWSGQFEYLERATAKLKVVVPFTLLIIFVLLYLVFGRLDEAMLIMGTLPLALIGGFWLLYLLRYNLSVAGIVGFIAL
Putative_Methylibium petroleiphilum PM1      YSVSWSGQFEFLERATAKLKVVVPFTLGIIVVLLYLTFRRFDEALLILATLPFALIGGFWLLYLLGYNLSVAGAIGFIAL
CzcA_Delftia acidovorans SPH-1               YSISWSGQFEFLERATAKLKVVVPFTLLIIFVLLYLTFGAFGEALLIMAALPFALVGGIWLLYLLGYNLSIAGAVGFIAL
CzcA_Yersinia kristensenii                   VSLSWSGQFEYLERATAKLKIVLPVTLMIIFVLLYVTFSSVRDAALIMATLPFALIGGVWLLYGLGYNFSVAAAVGFIAL
CusA_Klebsiella pneumoniae                   ISVSYSGQFELLERANQKLKLMVPMTLMIIFVLLYLAFRRVGEALLIITSVPFALVGGIWFLYWMGFHLSVATGTGFIAL
CzcA_Polaromonas naphthalenivorans CJ2       YSISWSGQFEFLERATAKMKIVVPFTLMIIFVLLYLTFKRFDEAVLIMATLPFALVGGIWLLYLLSYNLSVAGAVGFIAL
CusA_Edwardsiella ictaluri                   TSVSFSGQFELLEHANRKLMLMVPMTLVIIFVLLYLAFRRVEEALLILMSLPFALVGGIWFLYWQGFHLSVATGTGFIAL
CusA_Enhydrobacter aerosaccus SK60           MSISYSGQFEFMERANARLKVVIPITLMIIFLLLYLIFRQVQDAVLIMATLPFALIGGIWAMYLSDYHFSVAIAVGFIAL
CusA_Citrobacter sp. 30-2                    TSVAFSGQFELLERASHKLKLMVPMTLMIIFVLLYLAFRRFGEALLIITSVPFALVGGIWFLYWMGFHMSVATGTGFIAL
CzcA_Acinetobacter junii SH205               MSISYSGQFEFMERANARLKVIIPITLMIIFLLLYLIFRQVQDAVLIMATLPFALIGGIWAMYLSGFHFSVAIAVGFIAL
Putative_Pectobacterium  carotovorum         VSLSWSGQFEYLERATEKMKVVVPFTLLIIFVLLYVTFNRIKDALLIMATLPFALIGGVWLLYILGYNLSVAGAVGFIAL
CusA_Providencia rettgeri DSM1131            LSYSFTGQFELLERANQKLMLMVPATIMIIFVLLYLAFRRFSEALLILLSLPFALVGAIWFLYWMNFNLSVATGTGFIAL
                                              : :::**** : :*  :: :: * *: **.:**:: *    :* *:: ::*:** *. * ::   :. *:*   *****

                                                                                                                                M1009
CusA_Escherichia coli K12                    AGVAAEFGVVMLMYLRHAIEAVPSLNN--PQTFSEQKLDEALYHGAVLRVRPKAMTVAVIIAGLLPILWGTGAGSEVMSR1011
Putative_Shigella sonnei                     AGVAAEFGVVMLMYLRHAIEAEPSLNN--PQTFSEQKLDEALYHGAVLRVRPKAMTVAVIIAGLLPILWGTGAGSEVMSR
CzcA_Shewanella sp. ANA-3                    AGVAAEFGVVMLMYLRHAIEAHPKLSR--KETFTPEGLDEALYHGAVLRVRPKAMTVAVIVAGLLPILWGTGAGSEVMSR
SilA_Klebsiella pneumoniae                   AGVAAEFGVVMLMYLRHAIEAHPELSR--KETFTPEGLDEALYHGAVLRVRPKAMTVAVIIAGLLPILWGTGAGSEVMSR
Putative_Serratia marcescens                 AGVAAEFGVVMLMYLRHAIEAHPELSR--KETFTPEGLDEALYHGAVLRVRPKAMTVAVIIAGLLPILWGTGAGSEVMSR
SilA_Salmonella Typhimurium                  AGVAAEFGVVMLMYLRHAIEAHPELSR--KETFTPEGLDEALYHGAVLRVRPKAMTVAVIIAGLLPILWGTGAGSEVMSR
CzcA_Erythrobacter sp. NAP1                  AGVSAEFGVIMLIYLKSALARRSGAL-------DADGVGEAIREGALLRVRPKAMTVAVILAGLFPVLIGTGAGSEVMSR
CzcA_Sphingopyxis alaskensis RB2256          AGVAAEFGVVMLIYLKSTLEDRGPDP-------DMAQVESAVREGALLRVRPKAMTVAVIIAGLLPALVGGGAGSEVMSR
CzcA_Caulobacter sp. K31                     AGVSAEFGVVMLIYLKQALAERQGVL-------SDADLSQAIREGALLRVRPKAMTVAVILAGLMPILIGHGAGSEVMSR
CzcA_Phenylobacterium zucineum HLK1          AGVAAEFGVVMLIYLKHALAERGPRP-------DAADVEAAIREGALLRVRPKAMTVAVILAGLFPILIGHGAGSEVMSR
CzcA_Asticcacaulis excentricus CB 48         AGVSAEFGVIMLIYLKDALKARGPDP-------SPREVETAVSEGALLRVRPKAMTVCVILAGLLPILFGHGAGSEVMSR
CzcA_Pseudomonas fluorescens Pf0-1           AGVSAEFGVIMLLYLKNAWAEREELG-----DHSERGLVAAIREGAVQRVRPKAMTVAVIIAGLLPILLGSGTGSEVMSR
CzcA_Thiobacillus denitrificans              AGVSAEFGVIMLLYLKNAWDAKREEGKTGEADLGEADLLEAIREGAVQRVRPKAMTVAVIVAGLLPILLGSGTGSEVMSR
```

```
CzcA_Stenotrophomonas sp. SKA14          AGVAAEFGVVMLIYLKQALAERCPDR----REPTREELLDAIREGAVLRVRPKAMTVAVILAGLVPIVWSSGTGSEVMSR
CzcA_ Acidovorax sp. JS42                AGVAAEFGVVMLIYLKQALAERCPDG----REPTREELLDAIREGAVLRVRPKAMTVAVILAGLVPIVWSSGTGSEVMSR
CzcA_Leptothrix cholodnii SP-6           AGVAAEFGVVMLLYLKHALQDRLAAG----ASPSAALVDDAIREGAVLRVRPKAMTVAVILAGLVPIVWSSGTGSEVMSR
CusA_curvibacter                         AGVAAEFGVVMLLYLKQALADRESQG----AAPGVAMVLDAIREGAVLRVRPKAMTVAVILAGLFPIVWGSGTGSEVMSR
CzcA_Ralstonia pickettii 12J             AGVAAEFGVIMLLYLKHAWTERQENG-----QTSTQALLEAIQEGAVLRVRPKAMTVAVILAGLLPIMWSDGTGSEVMQR
SilA_Ralstonia metallidurans CH34        AGVAAEFGVIMLLYLKHAWHDRLARG-----EDTLDALLDAIQEGAVLRVRPKAMTVAVILAGLIPIMWSHGTGSEVMQR
Putative_Methylibium petroleiphilum PM1  AGVAAEFGVIMLLYLKQAWEARLARG-----LSSDADLMDAIREGAVLRVRPKAMTVAVIIAGLVPIMFGGGTGSEVMQR
CzcA_Delftia acidovorans SPH-1           AGVSAEFGVIMLLYLKNAWQERADQG-----KTSEADLLDAIREGAVLRVRPKAMTVAVILAGLLPIMWGTGTGSEVMQR
CzcA_Yersinia kristensenii               AGVAAEFGVIMVLYLNQAVKKHQRPG----IAMTASEMSAAIHEGAVLRVRPKAMTVATIMAGLLPIMWGGGTGSEVMQR
CusA_Klebsiella pneumoniae               AGVAAEFGVVMLMYLRHAIEAEPSLKN--PQTFSVDKLDEALYQGAVLRVRPKAMTVAVIIAGLLPILWGTGAGSEVMSR
CzcA_Polaromonas naphthalenivorans CJ2   AGVSAEFGVIMLLYLRHAWD--ERLAQGKTN---AEDLLDAIREGAVLRVRPKAMTVAVILAGLLPIMWGTGTGSEVMQR
CusA_Edwardsiella ictaluri               AGVAAEFGVVMLMYLRQAIE--QDPALSHTGRFTPAALDEALYHGAVLRVRPKAMTVAVIIAGLLPILWGTGAGSEVMSR
CusA_Enhydrobacter aerosaccus SK60       AGVAAEFGVVMLFYLKQAIEHAQQSLSSSTASLTEQQLNEAIHTGAVLRVRPKAMTVAVILAGLIPILLGTGTGSELMSR
CusA_Citrobacter sp. 30-2                AGVAAEFGVVMLMYLRHAIE-AEPALEN-PQTFTADKLDEALYHGAVLRVRPKAMTVAVIIAGLLPILWGTGAGSEVMSR
CzcA_Acinetobacter junii SH205           AGVAAEFGVVMLFYLKQAVEHAQQNLSSSTTSLTEQQLNDAIRTGAVLRVRPKAMTVAVILAGLIPILLGTGTGSELMSR
Putative_Pectobacterium  carotovorum     AGVSAEFGVIMLLYLNHAVEKHR--V--AGQALSRQQLMDAIHEGAVLRVRPKMMTVATIMAGLLPIMWGGGSGSEIMQR
CusA_Providencia rettgeri DSM1131        AGVAAEFGVVMLIYLRHAIEDKEKQQG--ISKLSNQDLDRALYEGAVLRVRPKAMTVAVILAGLLPILLGTGVGSEVMSR
                                         ***:*****:*::**. :                  :  *:  **: ***** ***..*:***.* : . * ***:*.*

CusA_Escherichia coli K12                IAAPMIGGMITAPLLSLFIIPAAYKLMWLHR----HRVRK---------------   1047
Putative_Shigella sonnei                 IAAPMIGGMITAPLLSLFIIPAAYKLMWLHR----HRVRK---------------   1047
CzcA_Shewanella sp. ANA-3                IAAPMIGGMITAPLLSLFIIPAAYKLIWLRR----HKKSVS--------------   1048
SilA_Klebsiella pneumoniae               IAAPMIGGMITAPLLSLFIIPAAYKLIWLRR----HKKSVS--------------   1048
Putative_Serratia marcescens             IAAPMIGGMITAPLLSLFIIPAAYKLIWLRR----HKKSVS--------------   1048
SilA_Salmonella Typhimurium              IAAPMIGGMITAPLLSLFIIPAAYKLIWLRR----HKKSVS--------------   1048
CzcA_Erythrobacter sp. NAP1              IAAPMVGGMITAPLLSMFVIPAAYLLMRRPR---PERPTQPQGEEECVPQPS---   1055
CzcA_Sphingopyxis alaskensis RB2256      IAAPMVGGMLTAPLLSMFVIPAAYLLMRQRK---AKPNPQPD-------QP----   1047
CzcA_Caulobacter sp. K31                 IAAPMIGGMLSAPLLSMFVVPAAYLLIERRR---LSRPRGPT-----IAHG----   1049
CzcA_Phenylobacterium zucineum HLK1      IAAPMIGGMLTAPLLSMFVVPAGYLLLRRRR---PSRTSILK------GDPQ---   1049
CzcA_Asticcacaulis excentricus CB 48     IAAPMIGGMLTAPLLSMLVVPAAYLLIRRRR---RSNPPQSN------SQQ----   1048
CzcA_Pseudomonas fluorescens Pf0-1       IAAPMVGGMVTAPLLSLFVIPAAYRLMRRRR---LPAENKSPQSDDASIQP----   1056
CzcA_Thiobacillus denitrificans          IAAPMVGGMITAPLLSMFVIPAAYYLMRRPR---PQPHAPAHPIHSSGEHP----   1060
CzcA_Stenotrophomonas sp. SKA14          IAAPMLGGMVTAPLLSLFVIPAAYLLMRKPR------------------------   1039
CzcA_ Acidovorax sp. JS42                IAAPMLGGMVTAPLLSLFVIPAAYLLMRKPR------------------------   1039
CzcA_Leptothrix cholodnii SP-6           IAAPMLGGMVTAPLLSLFIVPAVYALLRRRS------------------------   1066
CusA_curvibacter                         IAAPMLGGMITAPLLSLFIIPAAYRMMRVRT---SRRLN----------------   1044
CzcA_Ralstonia pickettii 12J             IAAPMVGGMITAPLLSLFVVPAVYLLLRRRG---SGQSIPQPPSHDKVSR-----   1055
SilA_Ralstonia metallidurans CH34        IAAPMVGGMVTAPLLSMFVVPAVYLLLRRR----LLPSPSTTAKQAELERAV---   1056
Putative_Methylibium petroleiphilum PM1  IAAPMVGGMVTAPLLSMFVVPAIYRLVRRRRRASAGEAAAAVPGKSSIPTPAPSAA   1063
CzcA_Delftia acidovorans SPH-1           IAAPMVGGMVTAPLLSMFVIPAAYLLMRRSR-----ERKASPWMFWRRHRAAA--   1056
CzcA_Yersinia kristensenii               IAAPMIGGMVSAPLLSMLVIPAVYMLLHKKE----SKQQ----------------   1034
CusA_Klebsiella pneumoniae               IAAPMIGGMITAPLLSLFIIPAAYKLMWLSR----HRGKRSE-------------   1049
CzcA_Polaromonas naphthalenivorans CJ2   IAAPMVGGMITAPLLSMFVIPAVYLLMRRPRETQRTSGRFWRRRAVV--------   1055
CusA_Edwardsiella ictaluri               IAAPMIGGMITAPLLSLFIIPAAYKLIWLHRQRRATR----RTDSSA--------   1054
CusA_Enhydrobacter aerosaccus SK60       IALPMVGGMISAPLLSMFVIPAAYQLLIKRKLSKN--------------------   1048
CusA_Citrobacter sp. 30-2                IAAPMIGGMITAPLLSLFIIPAAYKLMWLHRHRGQK-------------------   1047
CzcA_Acinetobacter junii SH205           IALPMVGGMISAPLLSMFVIPAVYQLLIKRKLSTH--------------------   1048
Putative_Pectobacterium  carotovorum     IAAPMIGGMVSAPLLSMLVIPAVYLLLHKGDSGLLKR------------------   1045
CusA_Providencia rettgeri DSM1131        IAAPMIGGMITAPLLSLFIIPAAYKLICQLR----HKK-----------------   1045
                                         ** **:***::*****::::** * ::
```

Fig. S8a

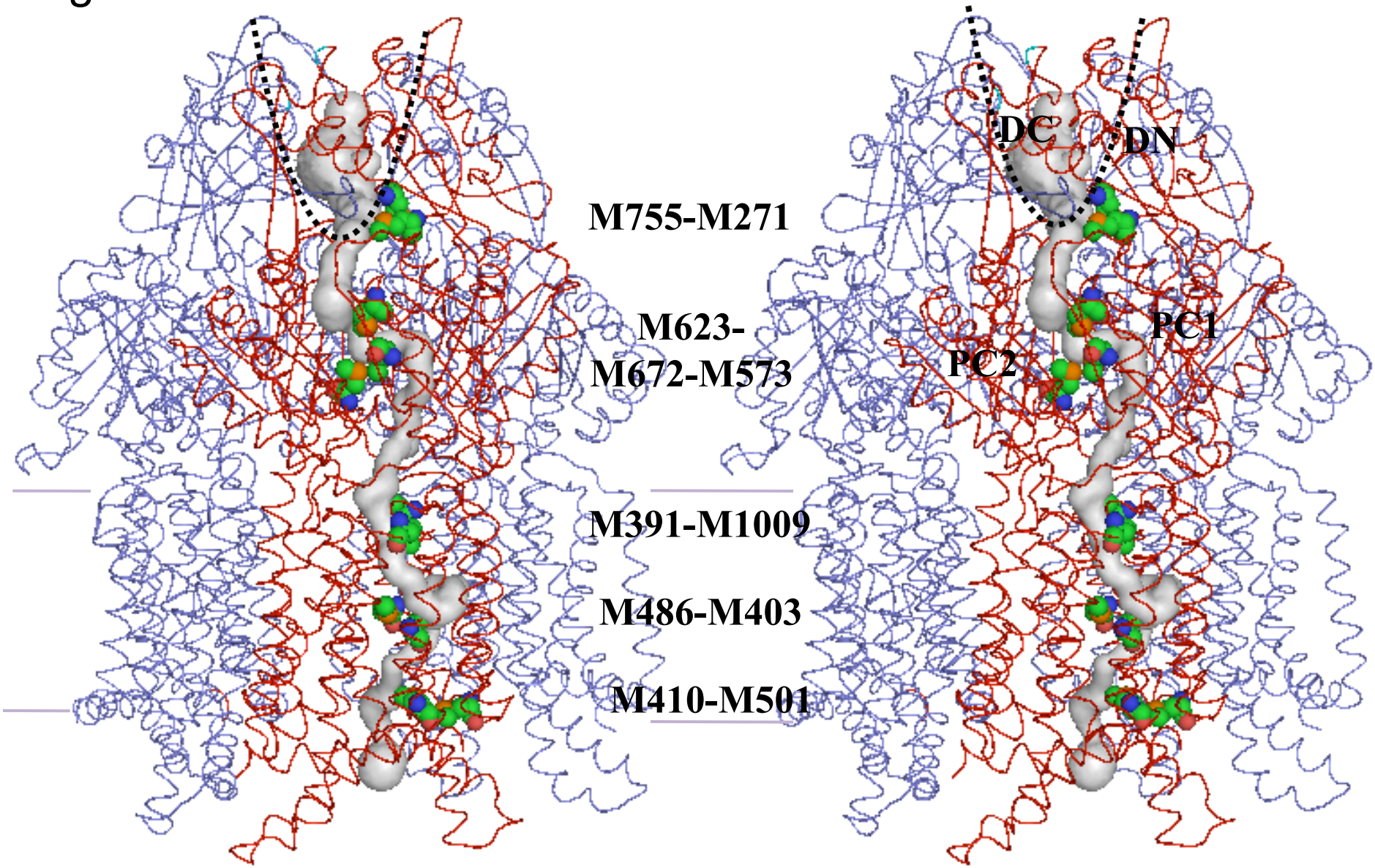

DC
DN
M755-M271
M623-
M672-M573
PC1
PC2
M391-M1009
M486-M403
M410-M501

Fig. S8b

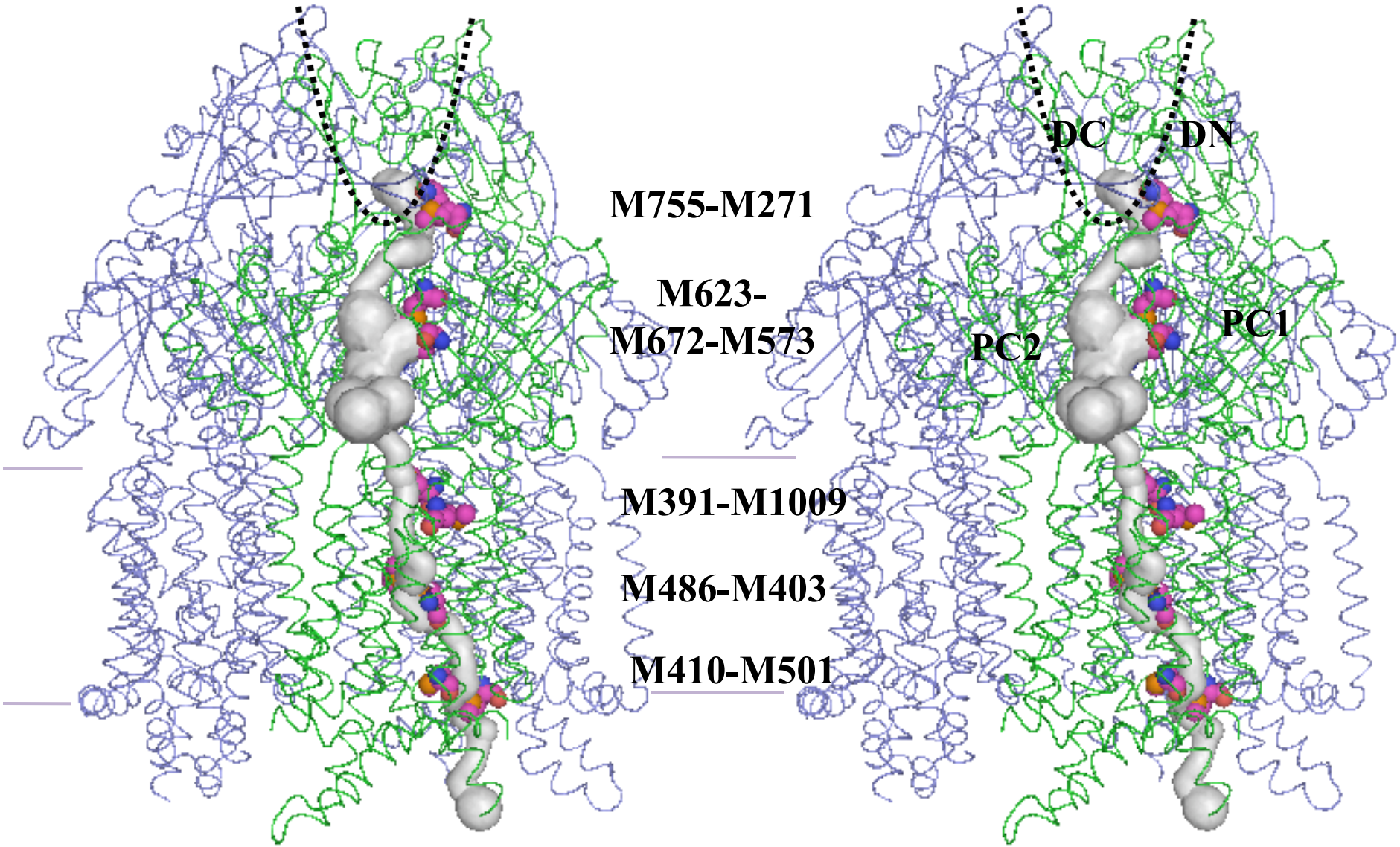

DC
DN
M755-M271
M623-
M672-M573
PC2
PC1
M391-M1009
M486-M403
M410-M501

Fig. S9

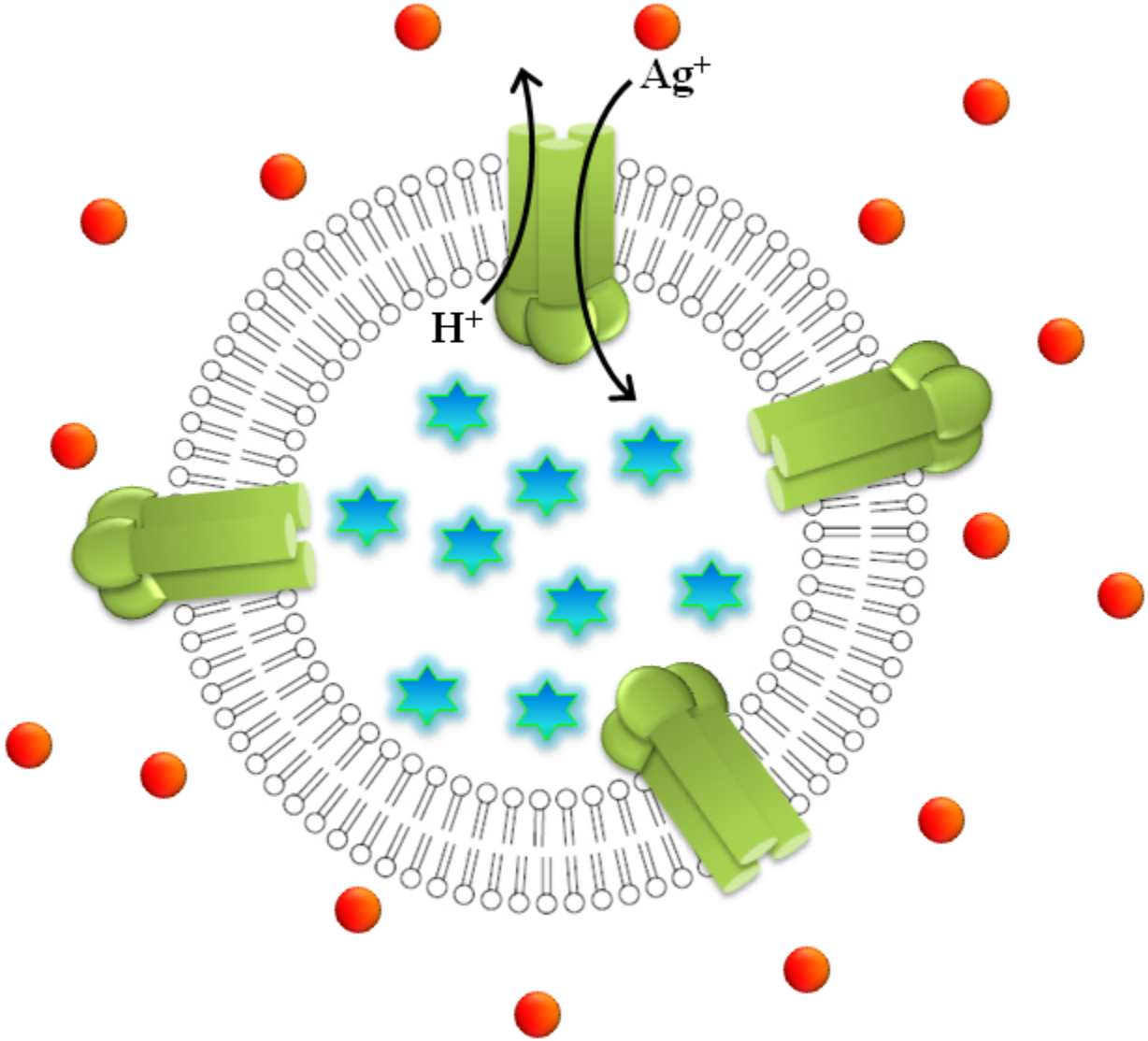


Fig. S10

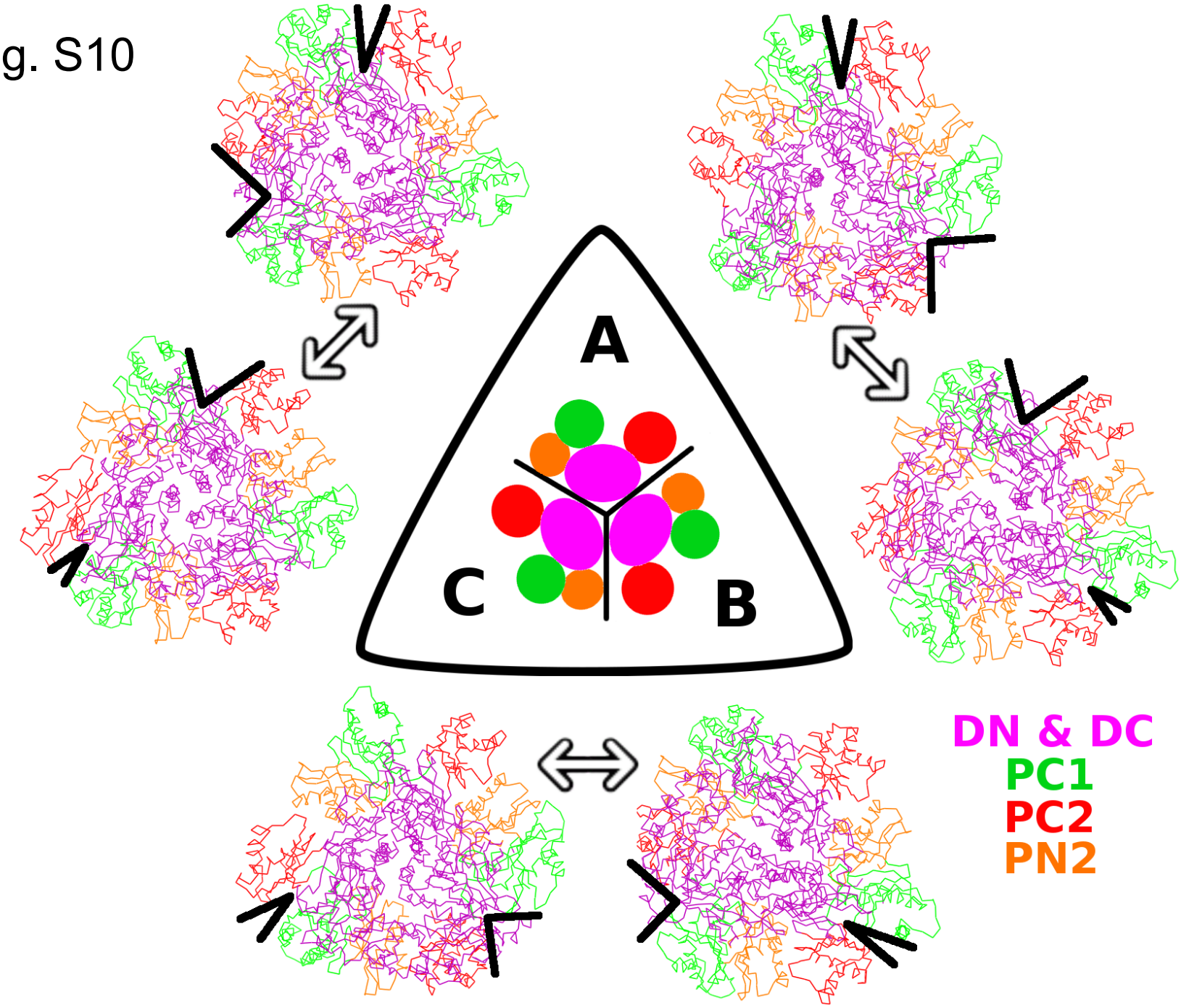


Fig. S11

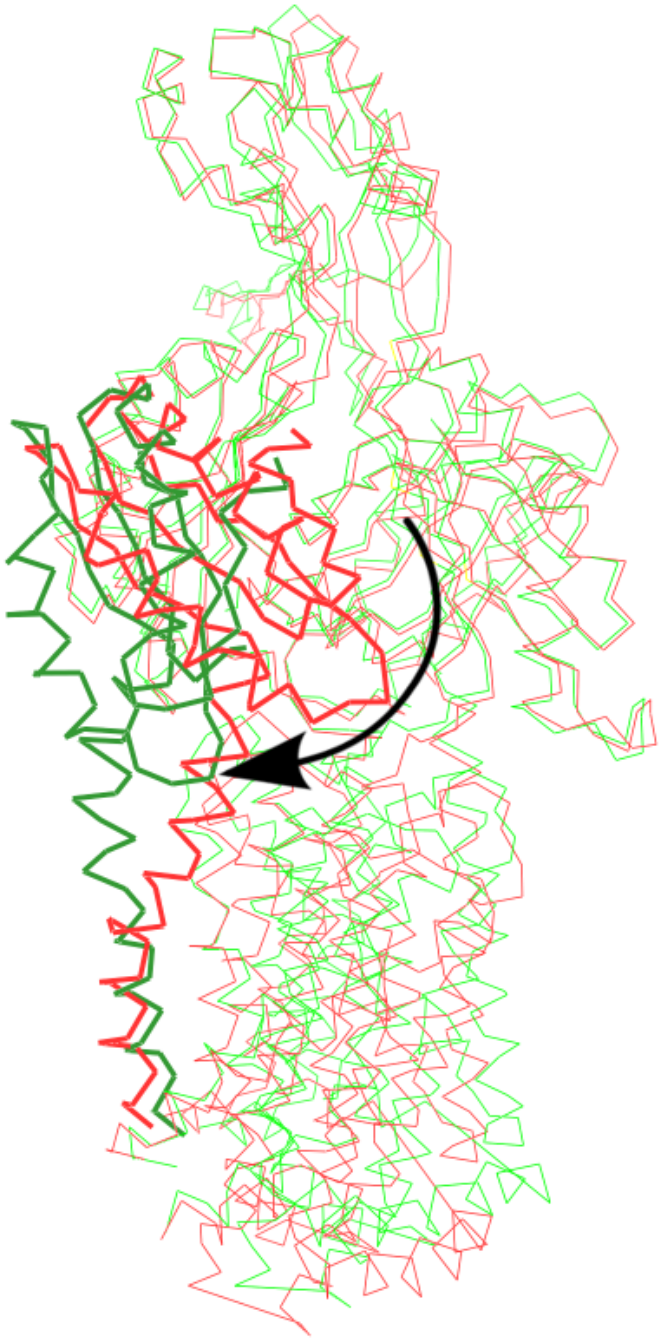


Fig. S12

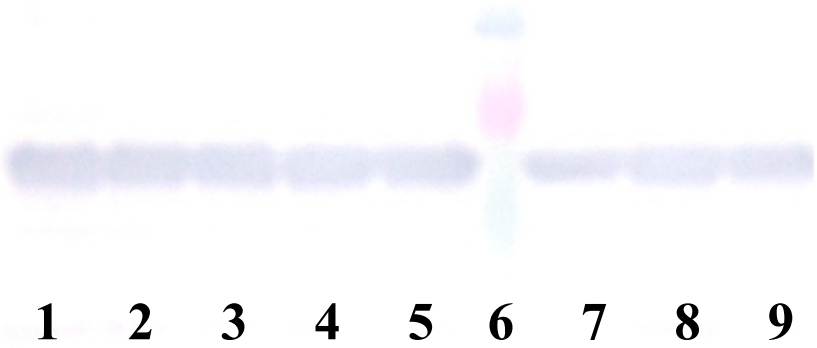


Fig. S13

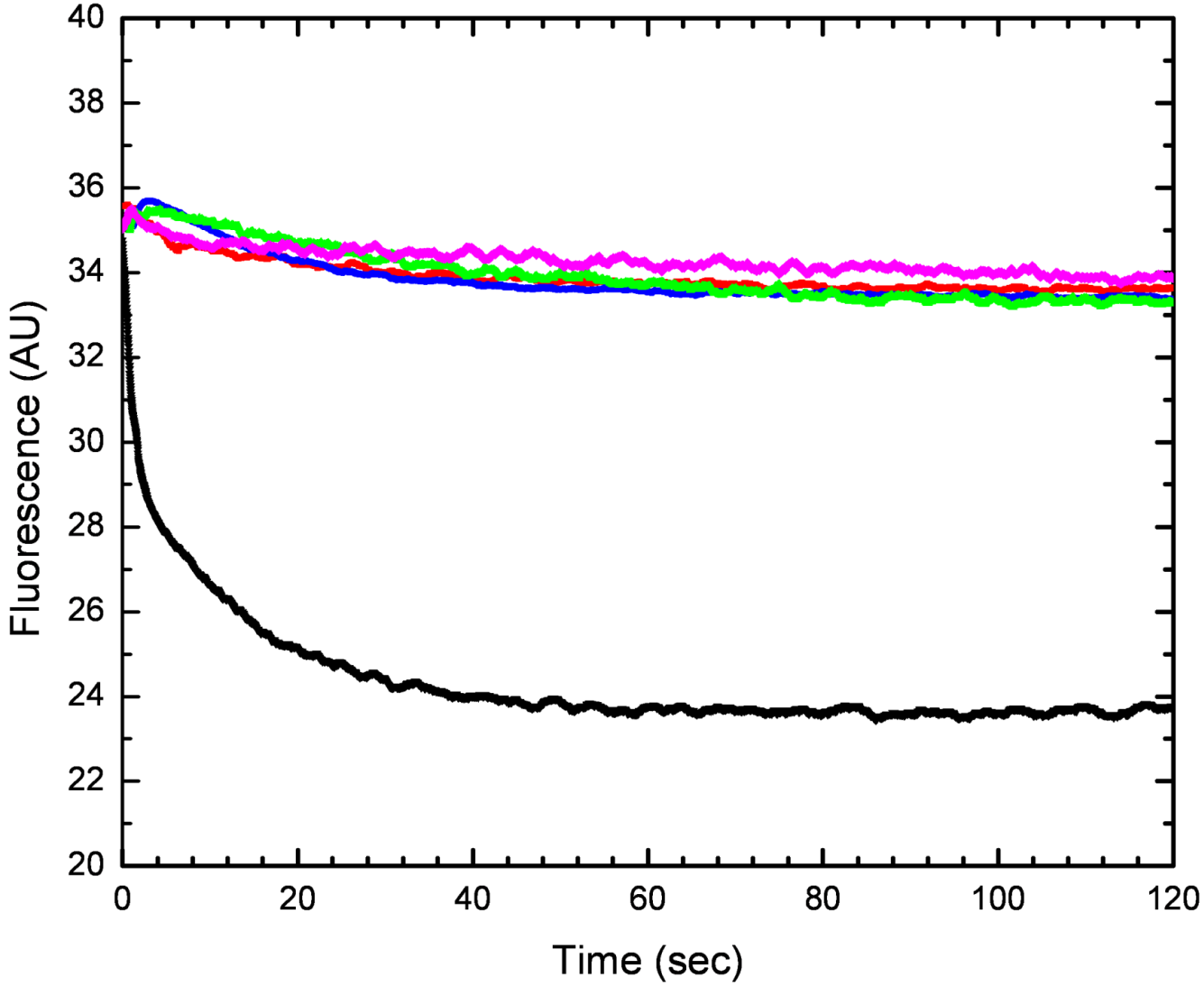
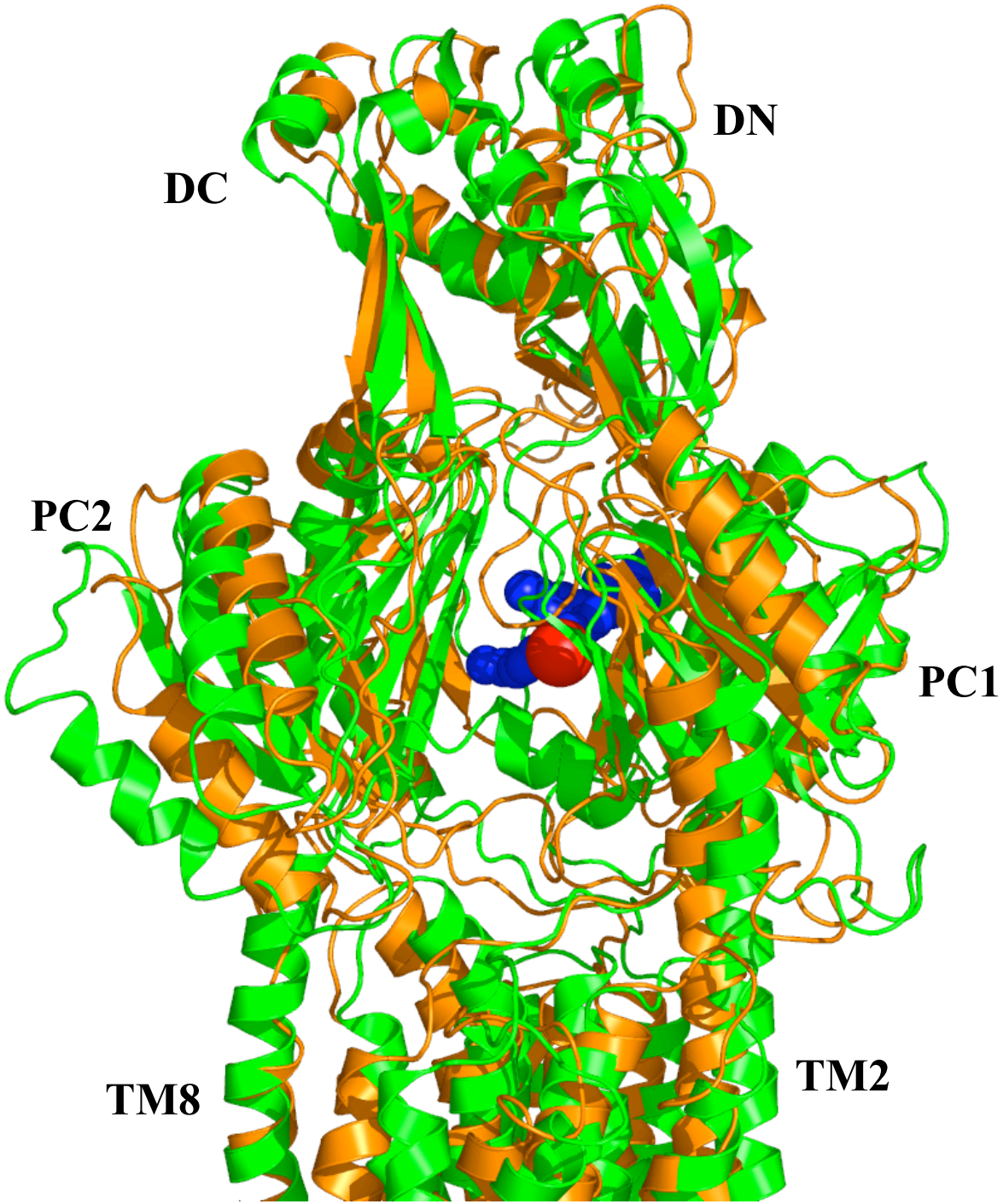

Fluorescence (AU)
40
38
36
34
32
30
28
26
24
22
20
0
20
40
60
80
100
120
Time (sec)

Fig. S14



nature

MANUSCRIPT CHECKLIST

Manuscript number . . . 2009-12-15523C

Author name . . . EDWARD . . . YU . . . . . .

Article type . . Letters . . to . . Nature . . .

The following checklist is intended to aid the publication of your manuscript. Please read through the list carefully and amend your manuscript as appropriate. To avoid delays in the production process, please complete and return this sheet with the final version of your manuscript.

Text

Nature will edit text to conform to house style and for clarity and accessibility. Please check the following points when preparing the final version of your manuscript.

Letters to *Nature*

- ☑ Title no longer than 90 characters (including spaces)
- ☑ First paragraph referenced and no longer than 300 words
- ☑ No subheadings in main text
- ☑ Methods for print no longer than 300 words
- ☑ Online-only Methods provided as part of the main text file?
- ☑ No more than 30 references

Articles

- ☐ Title no longer than 75 characters (including spaces)
- ☐ First paragraph unreferenced and no longer than 150 words
- ☐ No more than 6 main subheadings of up to 40 characters (including spaces)
- ☐ Methods for print no longer than 300 words
- ☐ Online-only Methods provided as part of the main text file?
- ☐ No more than 50 references

All manuscripts (if applicable)

- ☑ Abbreviations and symbols defined on first use
- ☑ Italic font used for variables, genes and non-English languages; bold font for vectors
- ☑ Equations and special characters in Word in 'normal text' or Symbol font (Word Equation Editor avoided where possible)
- ☑ Authored in Compatibility Mode if using Word 2007 (i.e., as a Word 97-2003 document; saved as .doc format, NOT .docx)
- ☑ Brief title provided for each figure, table and box
- ☑ Figure legends no more than 100 words each (300 words each for contributions without methods sections)
- ☑ Author contribution statement included (a statement of responsibility that specifies the contribution of every author; see http://www.nature.com/authors/editorial_policies/authorship.html)
- ☑ Declaration and listing of any competing interests included in manuscript in the Author Information section (http://www.nature.com/nature/authors/policy/competing.html)
- ☑ Database accession numbers included for sequences determined in this paper
- ☑ Structure data in *Nature* standard tables

For TeX users

Word or rtf formats are preferred, unless there is very extensive use of special characters or equations. Such formats enable on-screen subediting and the preparation of a 'pre-proof', when the editing can be checked before formal proofs are prepared. We have found that this minimizes the chance of errors being inadvertently introduced.

- ☐ Word conversion not practicable

Artwork

Contact our subeditors at authors@nature.com for further help and advice on preparing text for publication.

Nature will edit figures to conform to house style and for clear reproduction in print. Standard figure sizes are 89 mm wide (single column) and 183 mm wide (double column); figures can also be a column-and-a-half width where necessary (120–136 mm). Please check the following points when preparing your figures.

Electronic figure formats

- ☑ Preferred file formats for line (or vector) artwork and graphs are unflattened eps, postscript, pdf and Illustrator (ai) files
- ☑ Preferred file formats for photographic images are TIFF and Photoshop (minimum resolution 300 dpi). If you have created images with separate components on different layers, please send us the Photoshop file (.psd) with the layers intact
- ☑ For a full list of acceptable electronic formats, see http://www.nature.com/nature/authors/submissions/final

When preparing and sending figures

- ☑ Text in an editable format and in sans serif font (Helvetica, Arial)
- ☑ Text and lines clearly visible when figure is sized for page: type size 6–8 point; lines preferably 0.5 point (0.18 mm)
- ☑ Colour images in CMYK (not RGB) mode; ensure you are happy with the conversion from RGB to CMYK before submission
- ☑ Suggestions for cover artwork submitted if desired
- ☑ Completed colour costs form submitted with manuscript (http://www.nature.com/nature/authors/submissions/final/colfigs_Jan2009.pdf)
- ☐ For reviews/Insight articles, permission obtained for any republished or redrawn illustrations

See http://www.nature.com/nature/authors/submissions/final/Final_artwork.pdf for detailed guidelines. For further help and advice, e-mail our art editors at art@nature.com.

Nature does not edit online-only material. Please check the following points when preparing Supplementary Information:

Supplementary Information

- ☑ Supplementary Information referred to in main text (print version) of paper
- ☑ Summary diagram/figure included as part of the Supplementary Information (optional)
- ☐ Separate text file ('SI Guide') provided with a brief description (file format and content) of each item
- ☐ We recommend that all Supplementary Information is supplied as a single PDF file, where possible.

See http://www.nature.com/nature/authors/submissions/final for advice on preparing Supplementary Information.

E-mail authors@nature.com if you have additional queries.

nature

Dear Author,

Your paper may be eligible for our advance online publication (AOP) programme. In this programme, your paper will be published online on *Nature*'s website at www.nature.com/nature as soon it is typeset and your proof corrections returned. It will be published in print as soon afterwards as space is available. The two versions of the paper are identical.

Our offer of this service is conditional on your agreement to the following:

- Your text, figures and any supplementary information are **all** included when you send us the final version of your paper for formal acceptance, and are **all in *Nature*-preferred format** (within length and below maximum sizes) as specified in our format instructions at http://www.nature.com/nature/authors.
- The corresponding (or otherwise designated) author undertakes to read and return the proofs **within 48 hours** of their receipt.
- If figures are in colour, the corresponding author signs and returns the the colour-figure payment form*, **before** the paper is formally accepted.
- The corresponding author signs and returns the "author licence" form **with** the returned proof, **not later.**
- **No changes** can be made to the paper once the online version is published.

Please sign and return this letter, preferably scanned and uploaded with the final version of your manuscript, or fax letter to +44 207 843 4596 if this is not possible. Please specify below the author responsible for returning proofs if different from the corresponding author.

I agree to the above terms and conditions for AOP publication of my paper in *Nature:*

Name: EDWARD YU

Manuscript number: 2009-12-15523C

Name and contact details (phone, fax and email) of author responsible for returning proofs **if not the corresponding author:**

*Note, the offer of AOP publication is not related to authors' ability to contribute to costs of reproduction of colour figures.

nature publishing group npg

nature

Corresponding Author Name: EDWARD YU

Address: IOWA STATE UNIVERSITY, A115 Zaffarano, Ames, IA 50011, USA

Manuscript Reference Number: 2009-12-15523C

COLOUR FIGURES IN *NATURE*

Nature is committed to providing authors with the highest quality figures, and we use the latest techniques to achieve this reproduction. We recognise, however, that excessive colour figure and page charges can sometimes place an enormous burden on authors. We therefore charge authors a fee of £735 for the first colour figure and £262.50 per figure for additional colour figures; this covers part, but not all, of our additional costs for four-colour reproduction. (Please note that in EU countries VAT will be added if you are unable to provide us with your VAT details below.)

No author will be denied publication based on his/her inability to meet these charges. If we agree that colour is essential but you are unable to meet the costs, they will be waived if you explain the circumstances to the editor who handled your manuscript before your paper is published. Otherwise you will be billed after publication.

PLEASE COMPLETE AND SIGN THE SECTION BELOW, DELETING WHICHEVER ALTERNATIVE DOES NOT APPLY, AND RETURN A COPY OF THIS SHEET TO *NATURE*.

☑ I agree to pay the sum requested by *Nature* in the event that colour is considered essential.

☐ I am unable to meet the costs of colour for the following reason:

For EU member countries, please tick the box and provide the VAT number if your Institution/University is exempt from VAT:

☐ My Institution/University is a VAT registered paying member of the EU

VAT NUMBER | | | | | | | | | | | | | |

SIGNED: Edward Yu

NAME IN BLOCK LETTERS: EDWARD YU