The topology of the L-arginine exporter ArgO conforms to an N_{in}-C_{out} configuration in *Escherichia coli*: Requirement for the cytoplasmic N-terminal domain, functional helical interactions and an aspartate pair for ArgO function

Amit Pathania[1, 2] Arvind Kumar Gupta[3] Swati Dubey[1, 2] Balasubramanian Gopal[3] and Abhijit A. Sardesai[1#]

[1]Laboratory of Bacterial Genetics, Centre for DNA Fingerprinting and Diagnostics Hyderabad, India, [2]Graduate Studies, Manipal University, India and [3]Molecular Biophysics Unit, Indian Institute of Science, Bangalore, India.

[#]Author for correspondence Abhijit A. Sardesai, abhijit@cdfd.org.in

SUPPLEMENTAL MATERIAL

Table S1. Plasmid list

Plasmid	Description
pHYD3025	A modified version of pTrc99A described in reference 2.
pHYD2805	Derivative of the plasmid pET21b in in which *argO*$_{CHA}$ expression is under the transcriptional control of the P_{T7} promoter, encoding ArgO bearing a hemagglutinin (HA) tag appended to its C-terminus. *argO*$_{CHA}$ is present in the NdeI XhoI sites.

pHYD2835	pHYD3025 in which *argO*$_{CHA}$ expression is under the transcriptional control of the P$_{trc}$ promoter, *argO*$_{CHA}$ is present in the NdeI SalI sites of pHYD3025 and is described in reference 2.
pHYD2835.1	Derivative of the plasmid pHYD2835 encoding ArgO bearing the G20C amino acid substitution.
pHYD2835.2	Derivative of the plasmid pHYD2835 encoding ArgO bearing the Q22R amino acid substitution.
pHYD2835.3	Derivative of the plasmid pHYD2835 encoding ArgO bearing the V118E amino acid substitution.
pHYD2835.4	Derivative of the plasmid pHYD2835 encoding ArgO bearing the D128Y amino acid substitution.
pHYD2835.5	Derivative of the plasmid pHYD2835 encoding ArgO bearing the S156F amino acid substitution.
pHYD2835.6	Derivative of the plasmid pHYD2835 encoding ArgO bearing the F160S amino acid substitution.
pHYD2835.7	Derivative of the plasmid pHYD2835 encoding ArgO bearing the V118E and the I51T amino acid substitutions.
pHYD2835.8	Derivative of the plasmid pHYD2835 encoding ArgO bearing the V118E and the A60P amino acid substitutions.
pHYD2835.9	Derivative of the plasmid pHYD2835 encoding ArgO bearing the V118E and the V132A amino acid substitutions.

pHYD2835.10	Derivative of the plasmid pHYD2835 encoding ArgO bearing the S156F and the I51T amino acid substitutions.
pHYD2835.11	Derivative of the plasmid pHYD2835 encoding ArgO bearing the S156F and the A60P amino acid substitutions.
pHYD2835.12	Derivative of the plasmid pHYD2835 encoding ArgO bearing the S156F and the V132A amino acid substitutions.
pHYD2835.13	Derivative of the plasmid pHYD2835 encoding ArgO bearing the I51T amino acid substitution.
pHYD2835.14	Derivative of the plasmid pHYD2835 encoding ArgO bearing the A60P amino acid substitution.
pHYD2835.15	Derivative of the plasmid pHYD2835 encoding ArgO bearing the V132A amino acid substitution.
pHYD2835.16	Derivative of the plasmid pHYD2835 encoding ArgO bearing the D47A amino acid substitution.
pHYD2835.17	Derivative of the plasmid pHYD2835 encoding ArgO bearing the D47E amino acid substitution.
pHYD2835.18	Derivative of the plasmid pHYD2835 encoding ArgO bearing the D128A amino acid substitution.
pHYD2835.19	Derivative of the plasmid pHYD2835 encoding ArgO bearing the D128E amino acid substitution.
pHYD2835.20	Derivative of the plasmid pHYD2835 encoding ArgO lacking the N-

	terminal 28 amino acids. ArgO expressed from the P_{trc} promoter, the ORF is designated $argO_{N\Delta 28}$.
pHYD2865	*phoA* lacking first 26 codons, present in the SmaI site of pCL1920, *phoA* can be excised by digestion with XhoI
pHYD2868	pHYD3025 in which $mscL_{CHA}$ expression is under the transcriptional control of the P_{trc} promoter, encoding MscL bearing a hemagglutinin (HA) tag appended to its C-terminus. $mscL_{CHA}$ is present in the NdeI SalI sites of pHYD3025.
pHYD2869	pHYD3025 in which $argO_{NHA}$ expression is under the transcriptional control of the P_{trc} promoter, encoding ArgO bearing a hemagglutinin (HA) tag appended to its N-terminus. $argO_{NHA}$ is present in the NdeI SalI sites of pHYD3025.
pHYD2869A	pHYD3025 in which *argO* expression is under the transcriptional control of the P_{trc} promoter, encoding ArgO. *argO* is present in the NdeI SalI sites of pHYD3025.
pHYD2869B	Contains $argO_{NHA}$ present in the NdeI XhoI sites of pOK12.
pHYD2869B8	Derivative of pHYD2869B containing a Sal1 site engineered after the 8th natural codon of *argO*.
pHYD2869B36	Derivative of pHYD2869B containing a Sal1 site engineered after the 36th natural codon of *argO*.
pHYD2869B66	Derivative of pHYD2869B containing a Sal1 site engineered after the 66th

	natural codon of *argO*.
pHYD2869B110	Derivative of pHYD2869B containing a Sal1 site engineered after the 110th natural codon of *argO*.
pHYD2869B146	Derivative of pHYD2869B containing a Sal1 site engineered after the 146th natural codon of *argO*.
pHYD2869B181	Derivative of pHYD2869B containing a Sal1 site engineered after the natural codon 181 of *argO*.
pHYD2869B201	Derivative of pHYD2869B containing a Sal1 site engineered after the natural codon 201 of *argO*.
pHYD2869C8	Derivative of pHYD2869B8 containing *phoA* isolated as an XhoI fragment from pHYD2865 present in its SalI site.
pHYD2869C36	Derivative of pHYD2869B36 containing *phoA* isolated as an XhoI fragment from pHYD2865 present in its SalI site.
pHYD2869C66	Derivative of pHYD2869B66 containing *phoA* isolated as an XhoI fragment from pHYD2865 present in its SalI site.
pHYD2869C110	Derivative of pHYD2869B110 containing *phoA* isolated as an XhoI fragment from pHYD2865 present in its SalI site.
pHYD2869C146	Derivative of pHYD2869B146 containing *phoA* isolated as an XhoI fragment from pHYD2865 present in its SalI site.
pHYD2869C181	Derivative of pHYD2869B181 containing *phoA* isolated as an XhoI

fragment from pHYD2865 present in its SalI site.

pHYD2869C201 Derivative of pHYD2869B201 containing *phoA* isolated as an XhoI fragment from pHYD2865 present in its SalI site.

pHYD2869D8 pHYD3025 in which a translational fusion of the codon 8 of *argO* to codon 27 of *phoA* is placed under the expression control of the P_{trc} promoter. The translational fusion was isolated as an NdeI XhoI fragment from the plasmid pHYD2869C8 and cloned into the NdeI SalI sites of pHYD3025.

pHYD2869D36 pHYD3025 in which a translational fusion of the codon 36 of *argO* to codon 27 of *phoA* is placed under the expression control of the P_{trc} promoter. The translational fusion was isolated as an NdeI XhoI fragment from the plasmid pHYD2869C36 and cloned into the NdeI SalI sites of pHYD3025.

pHYD2869D66 pHYD3025 in which a translational fusion of the codon 66 of *argO* to codon 27 of *phoA* is placed under the expression control of the P_{trc} promoter. The translational fusion was isolated as an NdeI XhoI fragment from the plasmid pHYD2869C66 and cloned into the NdeI SalI sites of pHYD3025.

pHYD2869D110 pHYD3025 in which a translational fusion of the codon 110 of *argO* to codon 27 of *phoA* is placed under the expression control of the P_{trc} promoter. The translational fusion was isolated as an NdeI XhoI fragment from the plasmid pHYD2869C110 and cloned into the NdeI SalI sites of

pHYD3025.

pHYD2869D146 pHYD3025 in which a translational fusion of the codon 146 of *argO* to codon 27 of *phoA* is placed under the expression control of the P_{trc} promoter. The translational fusion was isolated as an NdeI XhoI fragment from the plasmid pHYD2869C146 and cloned into the NdeI SalI sites of pHYD3025.

pHYD2869D181 pHYD3025 in which a translational fusion of the codon 181 of *argO* to codon 27 of *phoA* is placed under the expression control of the P_{trc} promoter. The translational fusion was isolated as an NdeI XhoI fragment from the plasmid pHYD2869C181 and cloned into the NdeI SalI sites of pHYD3025.

pHYD2869D201 pHYD3025 in which a translational fusion of the codon 201 of *argO* to codon 27 of *phoA* is placed under the expression control of the P_{trc} promoter. The translational fusion was isolated as an NdeI XhoI fragment from the plasmid pHYD2869C201 and cloned into the NdeI SalI sites of pHYD3025.

pHYD2869.1 Derivative of the plasmid pHYD2869 encoding ArgO lacking the last 10 amino acids with the corresponding ORF present between the NdeI and SalI sites of pHYD3025 and expressed from the P_{trc} promoter. The *argO* ORF is designated as $argO_{C\Delta 10}$.

pHYD2869.2 Derivative of the plasmid pHYD2869 encoding ArgO lacking the last 40 amino acids with the corresponding ORF present between the NdeI and

	SalI sites of pHYD3025 and expressed from the P_{trc} promoter. The *argO* ORF is designated as $argO_{C\Delta 40}$.
pHYD2870	Derivative of pHYD2869 in which an ORF $argO_{CL}$ encoding a cysteine-less ArgO is placed under the expression control of the P_{trc} promoter. The translated polypeptide bears the C43A, C52A and C189A amino acid substitutions.
pHYD2870.1	Derivative of pHYD2870 encoding ArgO bearing the M15C amino acid substitution.
pHYD2870.2	Derivative of pHYD2870 encoding ArgO bearing the S59C amino acid substitution.
pHYD2870.3	Derivative of pHYD2870 encoding ArgO bearing the T90C amino acid substitution.
pHYD2870.4	Derivative of pHYD2870 encoding ArgO bearing the A117C in pHYD2870 amino acid substitution.
pHYD2870.6	Derivative of pHYD2870 encoding ArgO bearing the L158C amino acid substitution.
pHYD2870.7	Derivative of pHYD2870 encoding ArgO bearing the R175C amino acid substitution.
pHYD2870.8	Derivative of pHYD2870 encoding ArgO bearing the L98C amino acid substitution.

pHYD2870.10	Derivative of pHYD2870 encoding ArgO bearing the L81C in pHYD2870 amino acid substitution.
pHYD2870.13	Derivative of pHYD2870 encoding ArgO bearing the L71C in pHYD2870 amino acid substitution.
pHYD2870.15	Derivative of pHYD2870 encoding ArgO bearing the L165C amino acid substitution.
pHYD2870.17	Derivative of pHYD2870 encoding ArgO bearing the Q7C amino acid substitution.
pHYD2870.19	Derivative of pHYD2870 encoding ArgO bearing the S65C amino acid substitution.
pHYD2870.20	Derivative of pHYD2870 encoding ArgO bearing the W109Camino acid substitution.
pHYD2870.21	Derivative of pHYD2870 encoding ArgO bearing the R146C amino acid substitution.
pHYD2870.22	Derivative of pHYD2870 encoding ArgO bearing the A179C amino acid substitution.
pHYD2870.23	Derivative of pHYD2870 encoding ArgO bearing the D201C amino acid substitution.
pHYD2870.24	Derivative of pHYD2870 encoding ArgO bearing the N28C amino acid substitution.

pHYD2870.25	Derivative of pHYD2870 encoding ArgO bearing the M38C amino acid substitution.
pHYD2870.26	Derivative of pHYD2870 encoding ArgO bearing the A43C amino acid substitution.
pHYD2870.27	Derivative of pHYD2870 encoding ArgO bearing the A52C amino acid substitution.
pHYD2870.29	Derivative of pHYD2870 encoding ArgO bearing the L41C amino acid substitution.
pHYD2870.30	Derivative of pHYD2870 encoding ArgO bearing the V142C amino acid substitution.
pHYD2870.31	Derivative of pHYD2870 encoding ArgO bearing the P144C amino acid substitution.
pHYD2870.32	Derivative of pHYD2870 encoding ArgO bearing the S135C amino acid substitution.

The ancestral plasmids pTrc99A, pHYD3025, PCL1920 and pOK12 are described in references 1, 2, 3 and 4 respectively.

Table S2. PCR primers used in this study (appropriate destination plasmids are italicized)

Primer ID	Sequence of Primer (5'to 3')
JGLARGOHAF	CGGAATATCCCATATGTACCCATACGATGTTCCTGACTATGCGGG CGGCCCATTTTCTTATTACTTT
	pHYD2869
JGLARGOFP	CGGAATATCCCATATGTTTTCTTATTACTTT
	pHYD2869A
JGLARGOR	TCCATCAGACTCGAGCTAACTGAACAAGGCT
	pHYD2869, pHYD2869A
JGLCYS1RP	AGGACCAAATCGCTGATAGCAGCAAGTAAGGCAATCATAATGT
	pHYD2870
JGLCYS2FP	GCTATCAGCGATTTGGTCCTGATTGCTGCCGGGATTTTTGGTGG
	pHYD2870
JGLCYS2RP	TCCCACAACCAGATTGATAATG
	pHYD2870
JGLCYS3FP	CATTATCAATCTGGTTGTGGGAGCTGTTATGTGGTTTATTGCCTT
	pHYD2870
JGLM15CFP	CTTGCACTTGGGGCGGCTTGCATCCTACCGCTCGGTCCA
	pHYD2870.1
JGLM15CRP	TGGACCGAGCGGTAGGATGCAAGCCGCCCCAAGTGCAAG
	pHYD2870.1
JGLS59CFP	GCCGGGATTTTTGGTGGCTGCGCGTTATTGATGCAGTCG
	pHYD2870.2

JGLS59CRP	CGACTGCATCAATAACGCGCAGCCACCAAAAATCCCGGC
	pHYD2870.2
JGLT90CFP	GGTTTTGGCGCTTTTAAATGCGCAATGAGCAGTAATATT
	pHYD2870.3
JGLT90CRP	AATATTACTGCTCATTGCGCATTTAAAAGCGCCAAAACC
	pHYD2870.3
JGLA117CFP	ATTATCGCCACCATGTTGTGCGTGACCTGGCTGAATCCG
	pHYD2870.4
JGLA117CRP	CGGATTCAGCCAGGTCACGCACAACATGGTGGCGATAAT
	pHYD2870.4
JGLL158CFP	ACAATTAGCGCCTCTTTCTGCTGGTTCTTTGGTCTGGCT
	pHYD2870.6
JGLL158CRP	AGCCAGACCAAAGAACCAGCAGAAAGAGGCGCTAATTGT
	pHYD2870.6
JGLR175CFP	TGGCTGGCACCGCGTCTGTGCACGGCAAAAGCACAGCGC
	pHYD2870.7
JGLR175CRP	GCGCTGTGCTTTTGCCGTGCACAGACGCGGTGCCAGCCA
	pHYD2870.7
JGLL98CFP	ATGAGCAGTAATATTGAGTGCGCCAGCGCCGAAGTCATG
	pHYD2870.8
JGLL98CRP	CATGACTTCGGCGCTGGCGCACTCAATATTACTGCTCAT
	pHYD2870.8
JGLL81CFP	GGCGGCGTAGCCTTCTTGTGCTGGTATGGTTTTGGCGCT

	pHYD2870.10
JGLL81CRP	AGCGCCAAAACCATACCAGCACAAGAAGGCTACGCCGCC
	pHYD2870.10
JGLL71CFP	TCGCCGTGGTTGCTGGCGTGCGTCACCTGGGGCGGCGTA
	pHYD2870.13
JGLL71CRP	TACGCCGCCCCAGGTGACGCACGCCAGCAACCAC
	pHYD2870.13
JGL165CFP	TGGTTCTTTGGTCTGGCTTGCCTCGCAGCCTGGCT
	pHYD2870.15
JGL165CRP	AGCCAGGCTGCGAGGCAAGCCAGACCAAAGAACCA
	pHYD2870.15
JGLARGOQ7FP	TTTTCTTATTACTTTTGTGGTCTTGCACTTGGGG
	pHYD2870.17
JGLARGOQ7RP	CCCCAAGTGCAAGACCACAAAAGTAATAAGAAAA
	pHYD2870.17
JGLARGOS65FP	CGCGTTATTGATGCAGTGTCCGTGGTTGCTGGCGCTGG
	pHYD2870.19
JGLARGOS65RP	CCAGCGCCAGCAACCACGGACACTGCATCAATAACGCG
	pHYD2870.19
JGLARGOW109FP	TGAAGCAAGGCAGATGCAAAATTATCGCCACCA
	pHYD2870.20
JGLARGOW109RP	TGGTGGCGATAATTTTGCATCTGCCTTGCTTCA
	pHYD2870.20

JGLARGOR146FP	TTGATGTGGAACCAAAATGCTGGTTTGCACTCGGGAC
	pHYD2870.21
JGLARGOR146RP	GTCCCGAGTGCAAACCAGCATTTTGGTTCCACATCAA
	pHYD2870.21
JGLARGOA179FP	GCACCGCGTCTGCGCACGTGTAAAGCACAGCGCATTATCA
	pHYD2870.22
JGLARGOA179RP	TGATAATGCGCTGTGCTTTACACGTGCGCAGACGCGGTGC
	pHYD2870.22
JGLARGOD201FP	CCTTGCAGCTGGCGAGATGCGGTATTGCTCATGCAC
	pHYD2870.23
JGLARGOD201R	GTGCATGAGCAATACCGCATCTCGCCAGCTGCAAGG
	pHYD2870.23
JGLARGON28FP	CAGGGCATACGTCGTCAGTACCACATTATGATTGCCTTAC
	pHYD2870.24
JGLARGON28RP	GTACTGACGACGTATGCCCTGACACATCACAAAAGCATTT
	pHYD2870.24
JGLARGOM38FP	ATTGCCTTACTTTGTGCTATCAGCGATTTGGTCCTGATTTG
	pHYD2870.25
JGLARGOM38RP	GATAGCACAAAGTAAGGCAATACAAATGTGGTACTGACGACG
	pHYD2870.25
JGLARGOCYS43RP	AGGACCAAATCGCTGATAGCACAAAGTAAGGCAATCATAATGT
	pHYD2870.26

JGLARGOCYS52FP	GCTATCAGCGATTTGGTCCTGATTTGCGCCGGGATTTTTGGTGG
	pHYD2870.27
JGLL41CNWFP	TACCACATTATGATTGCCTGCCTTGCTGCTATCAGCGAT
	pHYD2870.29
JGLL41CNWRP	ATCGCTGATAGCAGCAAGGCAGGCAATCATAATGTGGTA
	pHYD2870.29
JGLS135CFP	ACTTTTGTTGTACTGGGCTGTCTTGGCGGGCAACTTGAT
	pHYD2870.32
JGLS135CRP	ATCAAGTTGCCCGCCAAGACAGCCCAGTACAACAAAAGT
	pHYD2870.32
JGLV142CFP	CTTGGCGGGCAACTTGATTGCGAACCAAAACGCTGGTTT
	pHYD2870.30
JGLV142CRP	AAACCAGCGTTTTGGTTCGCAATCAAGTTGCCCGCCAAG
	pHYD2870.30
JGLP144CFP	GGGCAACTTGATGTGGAATGTAAACGCTGGTTTGCACTC
	pHYD2870.31
JGLP144CRP	GAGTGCAAACCAGCGTTTACATTCCACATCAAGTTGCCC
	pHYD2870.31
JGLARGOG8FP	TTTCTTATTACTTTCAAGGTGTCGACCTTGCACTTGGGGCGG
	pHYD2869A8
JGLARGOG8RP	CCGCCCCAAGTGCAAGGTCGACACCTTGAAAGTAATAAGAAA
	pHYD2869A8
JGLARGOH36FP	CATACGTCGTCAGTACCACGTCGACATTATGATTGCCTTACTTTG

	pHYD2869A36
JGLARGOH36RP	CAAAGTAAGGCAATCATAATGTCGACGTGGTACTGACGACGTATG
	pHYD2869A36
JGLARGOP66FP	TTATTGATGCAGTCGCCGGTCGACTGGTTGCTGGCGCTGGT
	pHYD2869A66
JGLARGOP66RP	ACCAGCGCCAGCAACCAGTCGACCGGCGACTGCATCAATAA
	pHYD2869A66
JGLARGOK110FP	TGAAGCAAGGCAGATGGAAAGTCGACATTATCGCCACCATGTTGG
	pHYD2869a110
JGLARGOK110RP	CCAACATGGTGGCGATAATGTCGACTTTCCATCTGCCTTGCTTCA
	pHYD2869A110
JGLARGOR146FP	TTGATGTGGAACCAAAACGCGTCGACTGGTTTGCACTCGGGACAATT
	pHYD2869A146
JGLARGOR146RP	AATTGTCCCGAGTGCAAACCAGTCGACGCGTTTTGGTTCCACATCAA
	pHYD2869A146
JGLARGOR181FP	CAAAAGCACAGCGCGTCGACATTATCAATCTGGTTGTGGG
	pHYD2869A181
JGLARGOR181RP	CCCACAACCAGATTGATAATGTCGACGCGCTGTGCTTTTG
	pHYD2869A181
JGLARGOD201F	CCTTGCAGCTGGCGAGAGACGTCGACGGTATTGCTCATGCACAAG
	pHYD2869A201
JGLARGOD201RP	CTTGTGCATGAGCAATACCGTCGACGTCTCTCGCCAGCTGCAAGG

	pHYD2869A201
JGLMSCLFP	GAATTCCATATGAGCATTATTAAAGA
	pHYD2868
JGLMSCLHARP	AAGTAGCTCGAGCTACGCATAGTCAGGAACATCGTATGGGTAAG AGCGGTTATTCTGCT
	pHYD2868
JGLPHOAFP	TCCATCAGACTCGAGCCTGTTCTGGAAAACCGGGCT
	pHYD2865
JGLPHOARPSTOP	TCCATCAGACTCGAGCTATTTCAGCCCCAGAGCGGCTTT
	pHYD2865
JGLD47AFP	TTACTTTGTGCTATCAGCGCTTTGGTCCTGATTTGCGCC
	pHYD2835.16
JGLD47ARP	GGCGCAAATCAGGACCAAAGCGCTGATAGCACAAAGTAA
	pHYD2835.16
JGLD47EFP	TTACTTTGTGCTATCAGCGAATTGGTCCTGATTTGCGCC
	pHYD2835.17
JGLD47ERP	GGCGCAAATCAGGACCAATTCGCTGATAGCACAAAGTAA
	pHYD2835.17
JGLD128AFP	AATCCGCATGTTTACCTGGCTACTTTTGTTGTACTGGGC
	pHYD2835.18
JGLD128RP	GCCCAGTACAACAAAAGTAGCCAGGTAAACATGCGGATT
	pHYD2835.18
JGLD128ENEWFP	AATCCGCATGTTTACCTGGAAACTTTTGTTGTACTGGGC
	pHYD2835.19
JGLD128ENEWRP	GCCCAGTACAACAAAAGTTTCCAGGTAAACATGCGGATT

	pHYD2835.19
JGLUPTO200MISS	AAGTAGCTCGAGCTATCTCGCCAGCTGCAAGGCAA
	pHYD2869.1
JGLUPTO171MISS	AAGTAGCTCGAGCTATGCCAGCCAGGCTGCGAGAAG
	pHYD2869.2
JGLUPTO28MISS	GAATTCCATATGCAGGGCATACGTCGTCAGTAC
	pHYD2835.20
JGL51TFP	TGCGCCGGGATTTTTGGTGGCAGCGCGTTATTGATGCAGTC
	pHYD2835.7, pHYD2835.10, pHYD2835.13
JGL51TRP	CCACCAAAAATCCCGGCGCAAGTCAGGACCAAATCGCTGATAG
	pHYD2835.7, pHYD2835.10, pHYD2835.13
JGL60PFP	TTATTGATGCAGTCGCCGTGGTTGCTGGCGCTGGTCACCTG
	pHYD2835.8, pHYD2835.11, pHYD2835.14
JGL60PRP	CCACGGCGACTGCATCAATAACGGGCTGCCACCAAAAATCCC
	pHYD2835.8, pHYD2835.11, pHYD2835.14
JGL132AFP	CTGGGCAGCCTTGGCGGGCAACTTGATGTGGAACCAAAAC
	pHYD2835.9, pHYD2835.12, pHYD2835.15
JGL132ARP	TTGCCCGCCAAGGCTGCCCAGTGCAACAAAAGTATCCAGGTAA
	pHYD2835.9, pHYD2835.12, pHYD2835.15

Table S3. Bacterial strains

Strain	Genotype
GJ4823	MC4100[a] *argO205*::Tn*10*dTet, described in reference 5
GJ9099.1	F$^-$ *araC14 leuB6 secA206 lacY1 proC14 tsx-67* Δ(*ompT- fepC*)*266 entA403 glnX44 trpE38 rfbC1rpsL109 xylA5 mtl-1 thiE1 argO205*::Tn*10*dTet hns_{FL}::Kan[b]

[a]MC4100 was from the laboratory collection and [b]hns_{FL}::Kan encodes the nucleoid protein H-NS bearing a 3x FLAG epitope at its C-terminus.

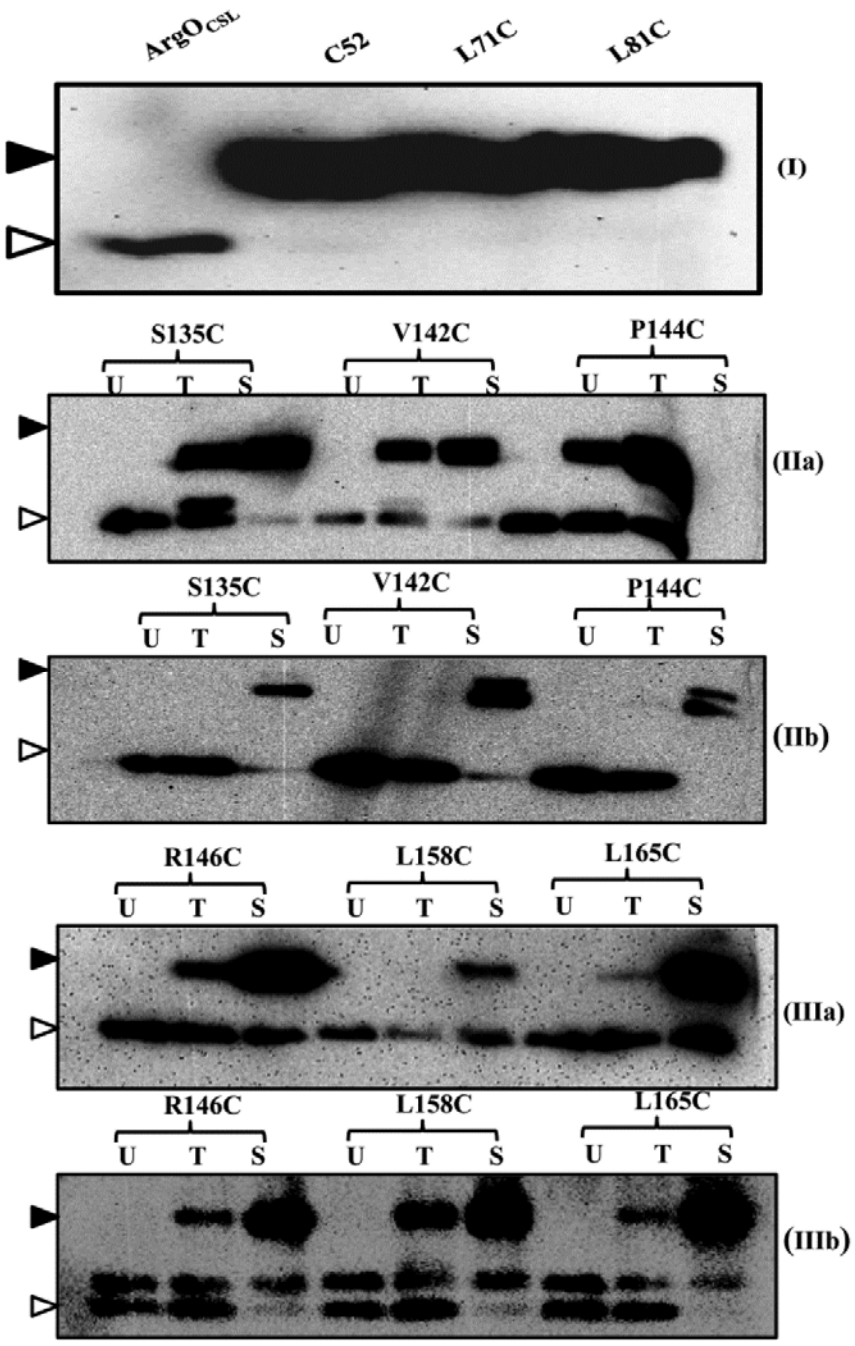

Figure S1. Cys accessibility depicting immunodetection of the ArgO:MAL-PEG adducts. Cultures of GJ9099.1 bearing plasmids encoding the indicated single cysteine substituted ArgO, were processed and the ArgO:MAL-PEG (IIa-IIIa) and the H-NS:MAL-PEG (IIb-IIIb) adducts

were detected with western blotting with anti-HA and anti-FLAG antibodies respectively. U, T and S stand for cell suspensions that were not treated with MAL-PEG, treated with MAL-PEG and treated with MAL-PEG following sonication. The positions of the ArgO:MAL-PEG, H-NS:MAL-PEG adducts and free ArgO and H-NS species are marked with filled and open triangles respectively. Panel I depicts the imnnodetection with anti-HA antibody of ArgO:MAL-PEG adducts obtained after treatment of cultures of GJ9099.1 bearing plasmids encoding the Cys-less ArgO ($ArgO_{CSL}$) and its indicated single cysteine substituted derivatives with MAL-PEG following whole cell protein precipitation (6) and SDS solubilization. All plasmids are derivatives of pHYD2870 that encodes the Cys-less ArgO expressed from the P_{trc} promoter and are described in Table S1.

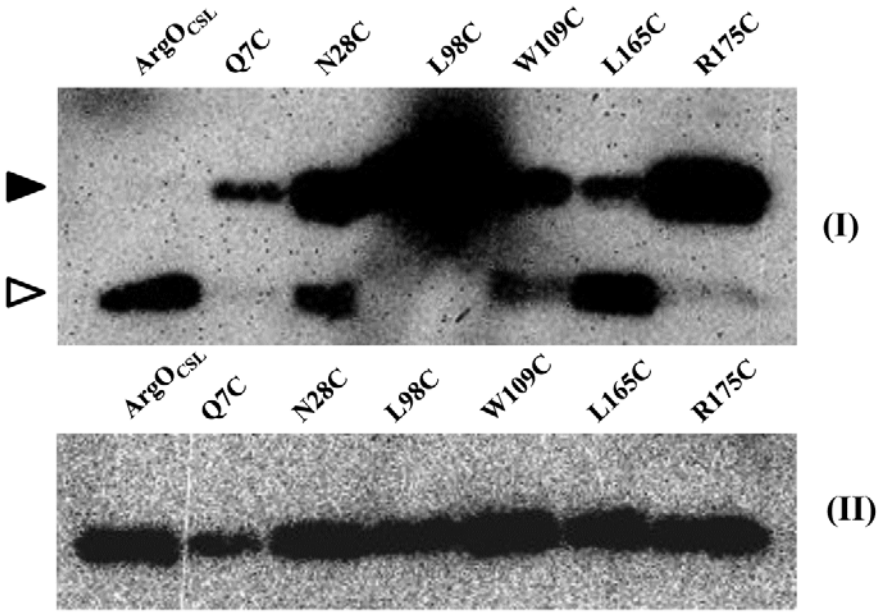

Figure S2. Detection of ArgO:MAL-PEG adducts in crude membrane fraction. Crude membrane fractions isolated from (I) Mid-log phase cultures of GJ9099.1, containing the plasmid expressing the Cys-less ArgO ($ArgO_{CSL}$) and its derivative plasmids bearing the indicated Cys substitutions were A_{600} normalized, sonicated, treated with MAL-PEG and their crude membrane fractions were isolated and solubilized. 20 μg total membrane protein from each fraction was electrophoreded on an SDS-PAGE gel and the ArgO:MAL-PEG adducts were detected following immublotting with anti-HA antibody. (II) Cellular proteins levels of $ArgO_{CSL}$ and its indicated cysteine substituted ArgO proteins. A_{600} normalized sample loading of mid-log phase cultures of transformants of GJ9099.1 containing plasmids encoding the indicated *argO* variants was performed and the levels of the indicated ArgO proteins were detected with immnoblotting with anti-HA antibody. The positions of the ArgO:MAL-PEG adducts and of $ArgO_{CSL}$ are marked with filled and open triangles respectively.

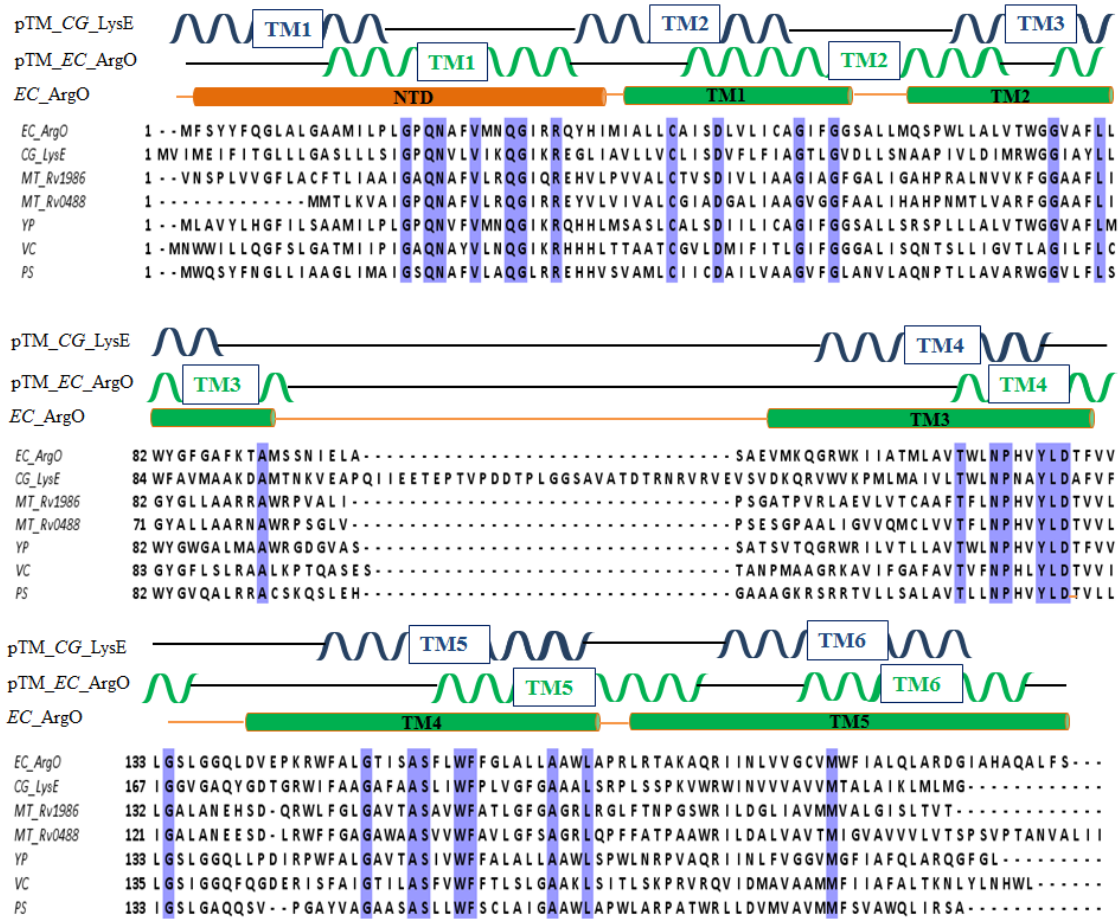

Figure S3. Multiple sequence alignment of ArgO and its orthologs. The sequence alignment was performed using PROMALS3D (7). The alignment (in CLUSTAL format) was edited using Jalview 2.9.0b2 (8) with conserved residues highlighted in blue. The sequence based prediction of secondary structural elements (predicted transmembrane α-helices, referred to here as pTM) for LysE (dark blue) and ArgO (green) are shown above the inferred secondary structure of ArgO. TM prediction was performed using TMpred (9). The experimental data described in this study guided the assignment of the transmembrane helices (green cylinders) and the cytoplasmic N-terminal domain (orange cylinder, NTD) of ArgO. *EC*, *CG*, *MT*, *YP*, *VC*, *PS* stand for *E. coli*, *C. glutamicum*, *M. tuberculosis*, *Y. pestis* and *P. syringae* respectively.

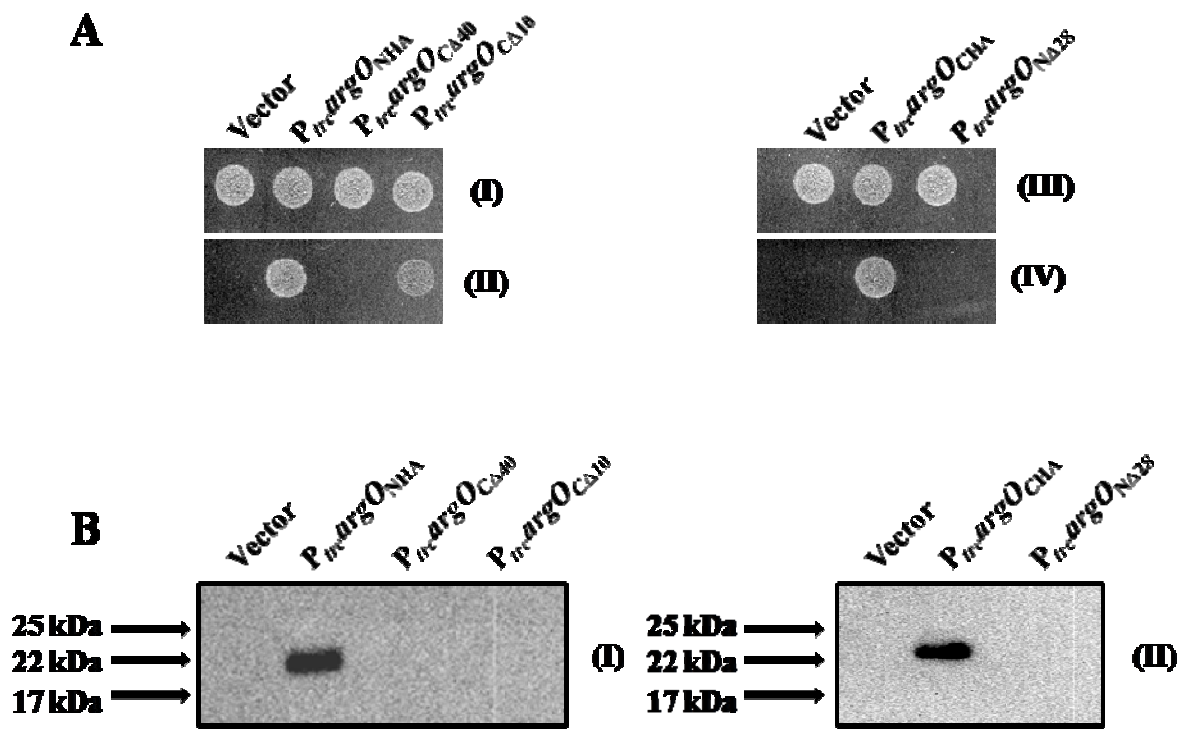

Figure S4. (A) Phenotypes of ArgO bearing deletions of its N and C terminal regions. 5 μl of 10000 fold diluted stationary phase cultures of GJ4823 bearing the vector or the plasmid pHYD2869 ($P_{trc}argO_{NHA}$) expressing ArgO bearing an appended N-terminal HA tag and its derivatives pHYD2869.2 ($P_{trc}argO_{C\Delta40}$) and pHYD2869.1 ($P_{trc}argO_{C\Delta10}$) encoding ArgO lacking the last 40 and the last 10 C-terminal amino acids respectively, were spotted on the surface of an MA agar plate (I) and an MA agar plate containing 2 μg/ml of CAN (II). 5 μl of similarly diluted stationary phase cultures of GJ4823 bearing the vector or the plasmid pHYD2835 ($P_{trc}argO_{CHA}$) expressing ArgO bearing an appended C-terminal HA tag and its derivative pHYD2835.20 ($P_{trc}argO_{N\Delta28}$) encoding ArgO lacking its 28 N-terminal amino acids, were spotted on the surface of an MA agar plate (III) and an MA agar plate containing 2 μg/ml of CAN (IV). (B) Levels of the corresponding ArgO proteins detected by immunoblotting with anti-HA antibody. A_{600}

normalized sample loading was performed and the indicated ArgO encoding plasmids are described in Table S1.

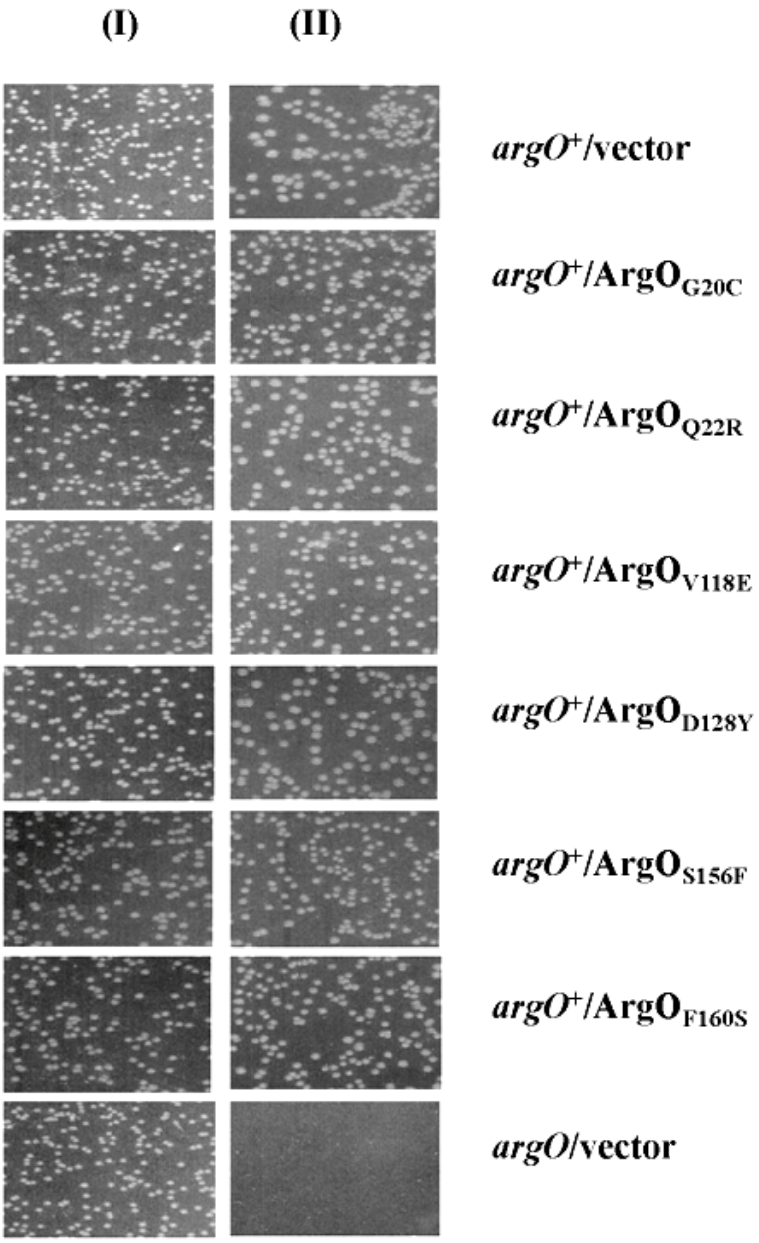

Figure S5. Effect of overexpression of functionally defective ArgO variants from the P_{trc} promoter on chromosomally encoded ArgO function. Dilutions ($3X10^{-6}$) of stationary phase cultures of MC4100 (*argO*$^{+}$) bearing the vector and plasmids encoding ArgO bearing the indicated substitutions and GJ4823 (*argO*, MC4100 *argO205*::Tn*10*dTet), bearing the vector were spread on (I) MA agar plates containing 10 μM IPTG and (II) MA agar plates containing 10 μM

IPTG and 20 μg/ml of CAN. Sections of plates in I and II were photographed after 24 and 36 hours respectively and the plasmid series pHYD2835.1 to pHYD2835.6 (Table S1) encode ArgO with the indicated amino acid substitution.

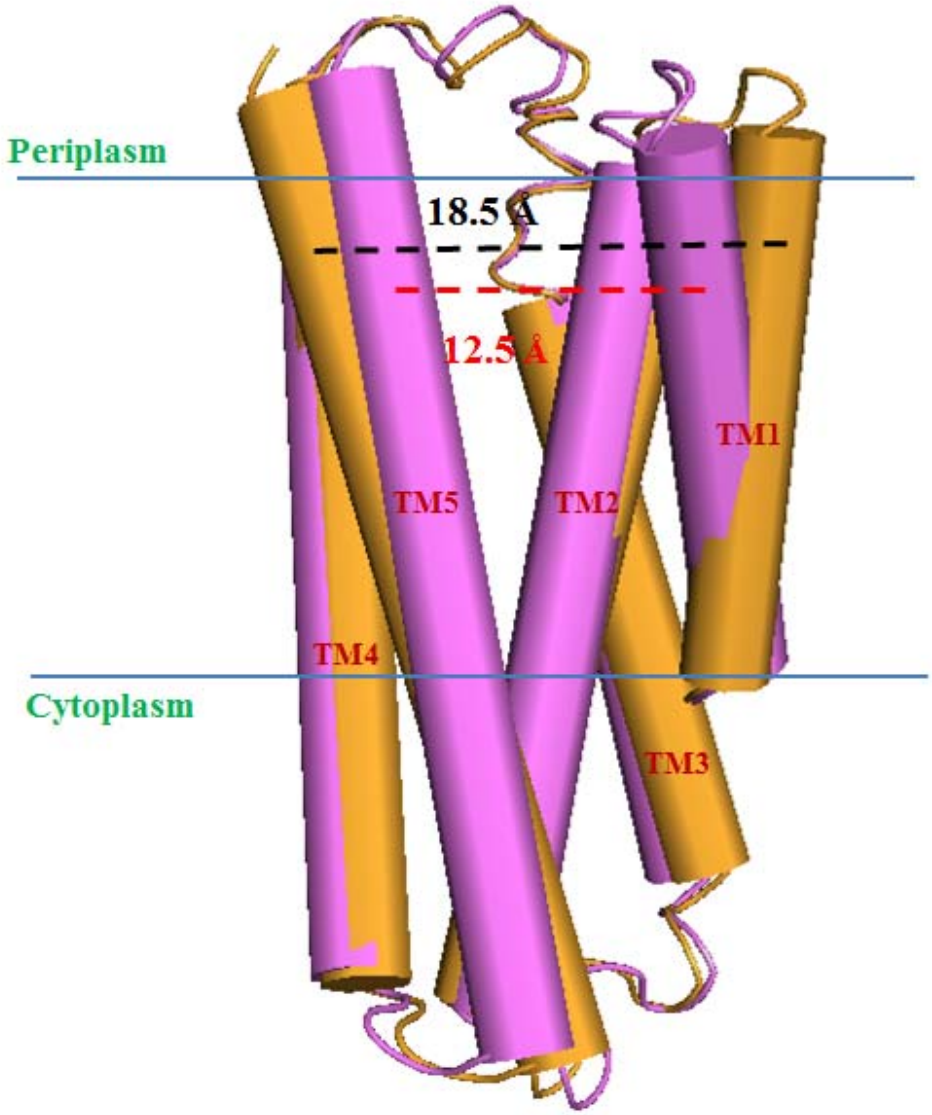

Figure S6. Superposition of a representative conformational variant of ArgO obtained by Normal Modes simulations. Low frequency normal modes revealed breathing motions between the transmembrane helices. A representative conformation of ArgO generated in the course of the normal modes analysis (magenta) was superimposed on ArgO (orange). Low frequency normal modes for the ArgO model were obtained using the online server ElNémo (10).

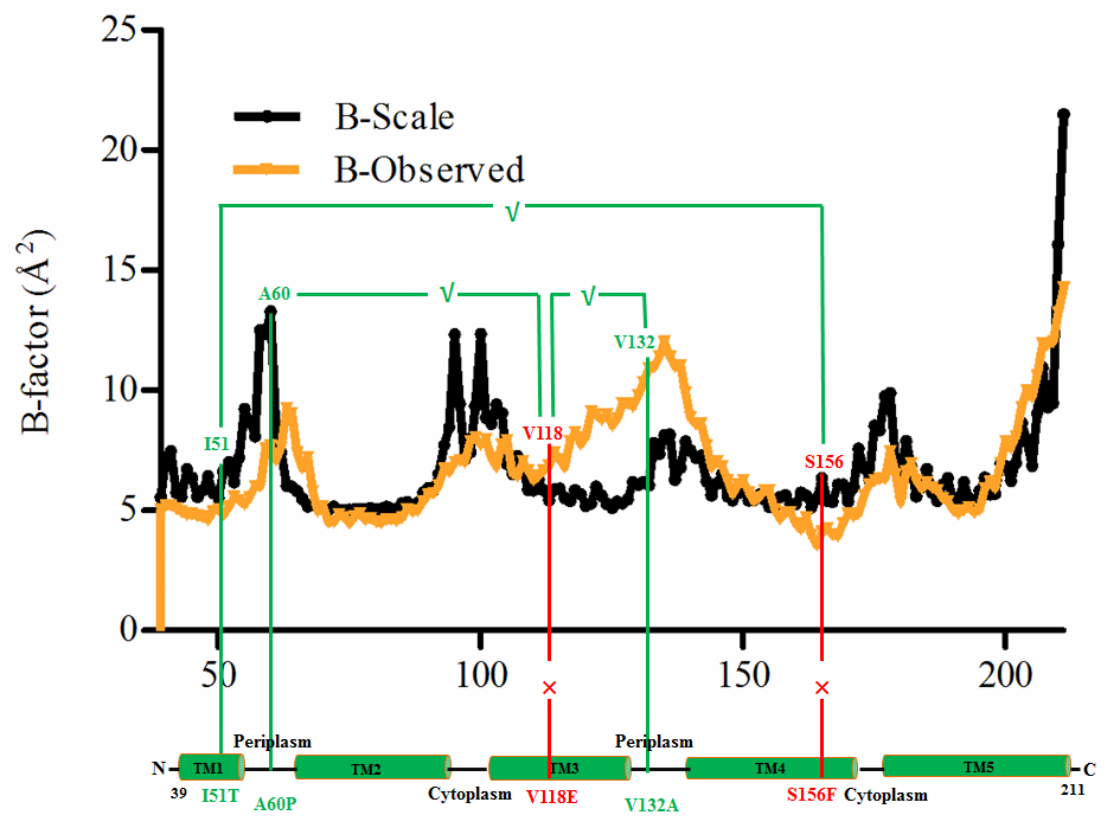

Figure S7. B-factor analysis (dynamic fluctuation) of residues of ArgO. The variation in B-factors for the reference model (black) is compared with that obtained from low frequency normal modes simulations (orange). The location of the indicated amino acids of ArgO is marked to provide an estimate of dynamic fluctuations at these positions. The pairing of amino acid substitutions (green) that compensate for ArgO disabling substitutions (red) is indicated. The compensatory pairs shown are V118E A60P, V118E V132A and S156F I51T.

REFERENCES

1. **Amann E, Ochs B, Abel KJ.** 1988. Tightly regulated *tac* promoter vectors useful for the expression of unfused and fused proteins in *Escherichia coli*. Gene. **69:**301-315.

2. **Pathania A, Sardesai AA.** 2015. Distinct paths for basic amino acid export in *Escherichia coli*: YbjE (LysO) mediates export of L-lysine. J. Bacteriol. **197:**2036-2047.

3. **Lerner CG, Inouye M.** 1990. Low copy number plasmids for regulated low-level expression of cloned genes in *Escherichia coli* with blue/white insert screening capability. Nucleic Acids Res. **18:**4631.

4. **Vieira J, Messing J.**1991. New pUC-derived cloning vectors with different selectable markers and DNA replication origins. Gene. **100:**189-194.

5. **Nandineni MR, Gowrishankar J.** 2004. Evidence for an arginine exporter encoded by *yggA* (*argO*) that is regulated by the LysR-type transcriptional regulator ArgP in *Escherichia coli*. J. Bacteriol. **186:**3539-3546.

6. **Wessel D, Flügge UI.** 1984. A method for the quantitative recovery of protein in dilute solution in the presence of detergents and lipids. Anal Biochem. **138**:141-143.

7. **Pei J1, Kim BH, Grishin NV.** 2008. PROMALS3D: a tool for multiple protein sequence and structure alignments. Nucleic Acids Res. **36:**2295-2300.

8. **Waterhouse AM1, Procter JB, Martin DM, Clamp M, Barton GJ.** 2009. Jalview Version 2--a multiple sequence alignment editor and analysis workbench. Bioinformatics. **25:**1189-1191.

9. **Hofmann K**, **Stoffel W**. 1993. TMbase-a database of membrane spanning proteins segments. BiolChem Hoppe-Seyler. **347**:166.

10. **Suhre K**, **Sanejouand Y-H**. 2004. ElNémo: a normal mode web server for protein movement analysis and the generation of templates for molecular replacement. Nucleic Acids Res. **32**:W610-W614.