

SUPPLEMENTARY INFORMATION

Spatial characteristics of the efflux pump MexB determine inhibitor binding

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Supplementary Materials

Supplementary Figure 1. Alignment of *E. coli* AcrB and *P. aeruginosa* MexB.

Supplementary Figure 2. Growth of *Escherichia coli* cells in the presence of various concentrations of ABI-PP without antibiotics

Supplementary Figure 3. Growth of *Escherichia coli* cells in the presence of 0.016 µg/mL of levofloxacin and various concentrations of ABI-PP

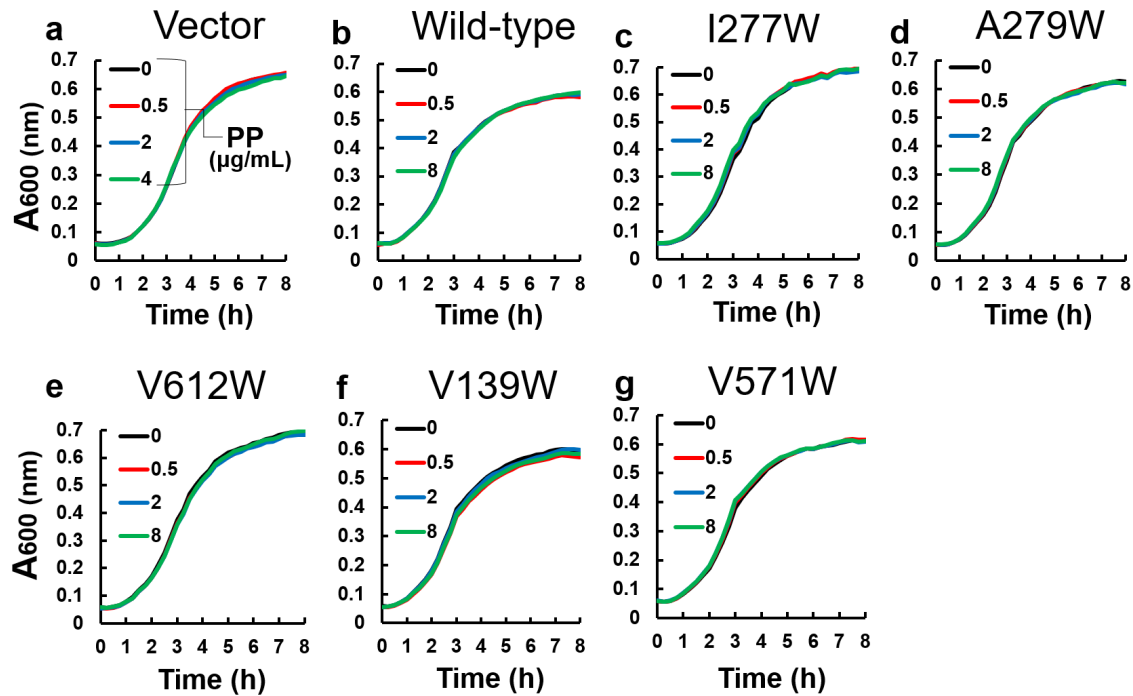
Supplementary Figure 4. Growth of *Escherichia coli* cells in the presence of 0.125 µg/mL of aztreonam and various concentrations of ABI-PP

MexB	1	MSKFFIDRPIFAWVIALVIMLAGGLSILSLPVNQYPAIAPPAIAVQVSYPGASAETVQDT	60
AcrB	1	M FFIDRPIFAWVIA++IMLAGGL+IL LPV QYP IAPPA+ + SYPGA A+TVQDT	60
MexB	61	VVQVIEQQMNGIDNLRYSSESNSDGSMTITVTFEQGTDPDIAQVQVQNKQLQATPLLPQ	120
AcrB	61	V QVIEQ MNGIDNL Y+SS S+S G++ IT+TFE GTD DIAQVQVQNKQLA PLLPQ	120
MexB	121	EVQRQIRVTKAVKNFLMVGVSVDGSMTKEDLSNYIVSNIQDPLSRTKGVGDFQVFGS	180
AcrB	121	+FLMVGVS++TDG+MT+ED+S+Y+ +N++D +SRT GVG D Q+FGS	180
MexB	181	QYSMRIWLDPAKLNYSYQLTPGDVSSAIAQNVQISSGQLGGLPAVKGQQLNATIIGKTRL	240
AcrB	181	QY+MRIW++P +LN +QLTP DV +AI+AQN Q+++GQLGG P VKGQQLNA+II +TRL	240
MexB	241	QTAEQFENILLKVNPDGQVRLKDVADVGLGGQDYSINAQFNGSPASGIAIKLATGANAL	300
AcrB	241	+ E+F ILLKVN DGS+V L+DVA + LGG+++ I A+FNG PASG+ IKLATGANAL	300
MexB	301	DTAKAIRQTIANLEPFMPQGMKVVPYDTPPVVSASIEHVVKTLGEAILLVFLVMYFLQ	360
AcrB	301	DTAAAIRAELAKMEPFFPSGLKIVYPYDTPFVKISIEHVVKTLVEAILLVFLVMYFLQ	360
MexB	361	NFRATLIPTIAVPVLLGTGFLAAFGFSINTLTMFGMVAIGLLVDDAIVVVENVERVM	420
AcrB	361	NFRATLIPTIAVPVLLGTGFLAAFGFSINTLTMFGMVAIGLLVDDAIVVVENVERVM	420
MexB	421	AEEGLSPREARKSMGQIQGALVGIAMVLSAVFLPMAFFGGSTGVYIRQFSITIVSAMAL	480
AcrB	421	AEEGL P+EA RKSMGQIQGALVGIAMVLSAVF+PMAFFGGSTG IYRQFSITIVSAMAL	480
MexB	481	SIVALILTPALCATMLKPIEKGDHGEHKAGFFGWFNRMFLSTTHGYERGVASILKHRAP	540
AcrB	481	SV+VALILTPALCATMLKPI KGDHGE K GFFGWFNRMF +TH Y V IL+	540
MexB	541	YLLIYVVIVAGMIWMFTRIPTAFLPDEDQGVLFQVQTPPGSSAERTQVVVDSMREYLLE	600
AcrB	541	YL++Y++IV GM ++F R+P++FLPDEDQGV VQ P G++ ERTQ V++ + Y L	600
MexB	601	KESSSVSVFTVTGFGFAGRGQSSGMAFIMLKPWEERPGENSVFELAKRAQMHHFSFKD	660
AcrB	601	KE++V SVF V GF FAGRGQ++G+AF+ LK W +RPG EN V + RA F KD	660
MexB	661	AMVFAFAPPVLELGNATGFDLFLQDQAGVGEVLLQARNKFLMLAAQNP-ALQVRPENG	719
AcrB	661	AMVFAF P+++ELG ATGFD L DQAG+GHE L QARN+ L AA++P L VRPENG	720
MexB	720	MSDEPQYKLEIDDEKASALGVSLADINSTVSIAWGSSYVNDFIDRGRVKRYVQGRPDAR	779
AcrB	721	+ D PQ+K++ID EKA ALGVS+ DIN+T+ AWG SYVNDFIDRGRVK+VY+ R	780
MexB	780	MNPDDLSKWWYVRNDKGMVFPNFAFATGKWEYGSPLKERYNGVPAMEILGEPAPGLSSGDA	839
AcrB	781	M PDD+ WYVR G+MVPF+AF++ +WEYGS+LERYNG+P+MEILG+ APG S+G+A	840
MexB	840	MAAVEEIVKQLPKGVGYSWTGLSYEERLSGSQAPALYALSLLVFLCLAALYESWSIPFS	899
AcrB	841	M +E++ +LP GVG Y WTG+SY+ERLSG+QAP+LYA+SL+VFLCLAALYESWSIPFS	900
MexB	900	VMLVVPLGVIGALLATSMRGLSNDVFFQVGLLTTIGLSAKNAILIVEFAKEL-HEQGKGI	958
AcrB	901	VMLVVPLGVIGALLA + RGL+NDV+FQVGLLTTIGLSAKNAILIVEFAK+L ++GKG+	960
MexB	959	VEAAIEACRMRLRPIVMTSLAFILGVVPLAISTGAGSGSQHAIGTGVIGGMVTATVLAI	1017
AcrB	961	+EA +++ RMRLRPI+MTSLAFILGV+PL ISTGAGSG+Q+A+GTGV+GGMVTATVLAI	1019

Supplementary Figure 1. Alignment of *E. coli* AcrB and *P. aeruginosa* MexB.

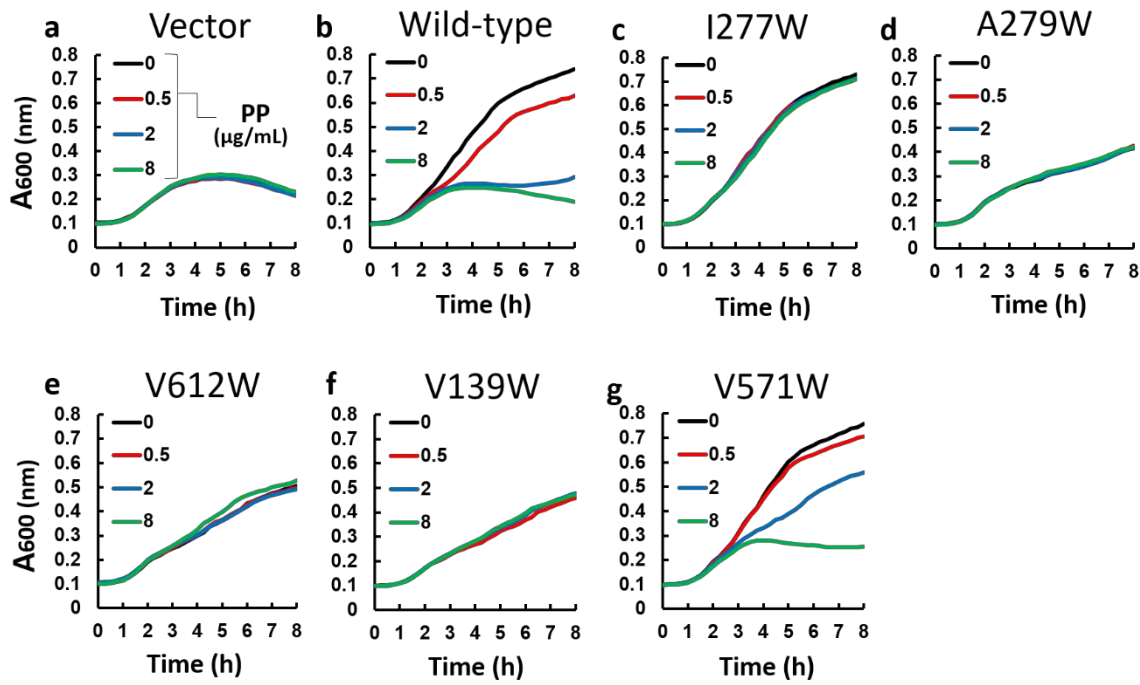
Alignment of *Escherichia coli* AcrB (Ref. NP_414995.1) and *Pseudomonas aeruginosa*

MexB (Ref. NP_249117.1) by BLAST. Identity 71%, positives 84%, Gaps 0%.



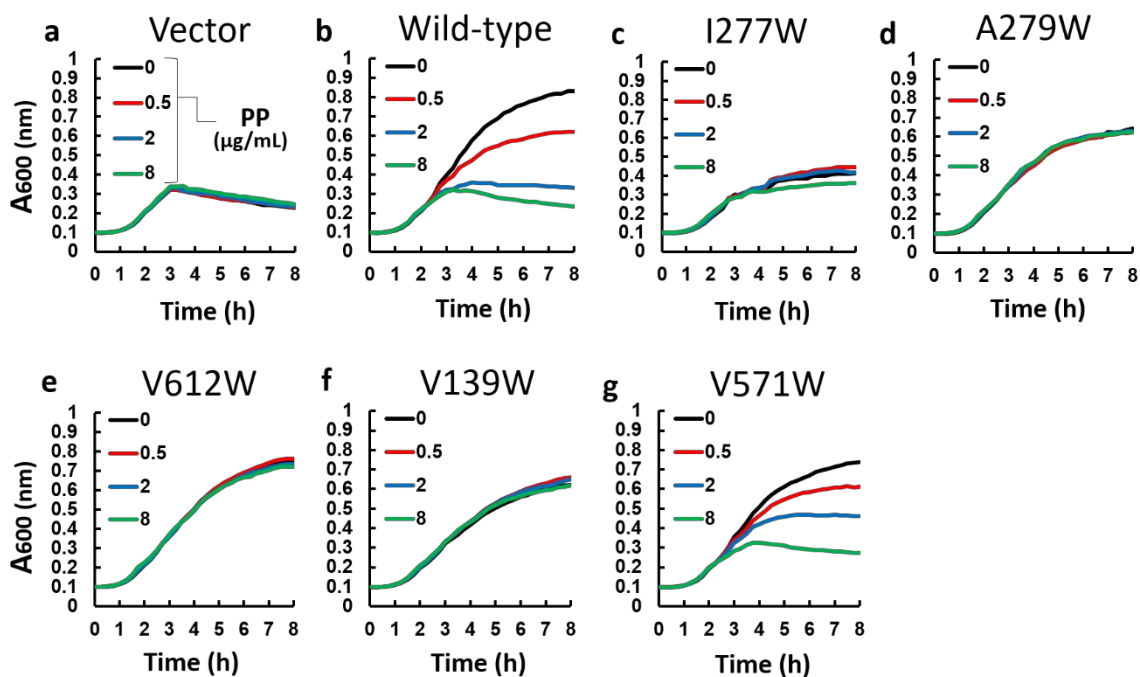
Supplementary Figure 2. Growth of *Escherichia coli* cells in the presence of various concentrations of ABI-PP without antibiotics

a The growth of *E. coli* MG1655 Δ *acrB* Δ *tolC* cell harbouring the plasmid pMMB67HE in the presence of various concentrations of ABI-PP without antibiotics. **b-g** The growth of *E. coli* MG1655 Δ *acrB* Δ *tolC* cells expressing wild-type MexB (**b**), MexB(I277W) (**c**), MexB(A279W) (**d**), MexB(V612W) (**e**), MexB(V139W) (**f**) and MexB(V571W) (**g**) in the presence of various concentrations of ABI-PP without antibiotics. These tests were performed in triplicates. Abbreviations: PP, ABI-PP inhibitor.



Supplementary Figure 3. Growth of *Escherichia coli* cells in the presence of 0.016 µg/mL of levofloxacin and various concentrations of ABI-PP

a The growth of *E. coli* MG1655 Δ *acrB* Δ *tolC* cell harbouring the plasmid pMMB67HE in the presence of 0.016 µg/mL of levofloxacin and various concentrations of ABI-PP. **b-g** The growth of *E. coli* MG1655 Δ *acrB* Δ *tolC* cells expressing wild-type MexB (b), MexB(I277W) (c), MexB(A279W) (d), MexB(V612W) (e), MexB(V139W) (f) and MexB(V571W) (g) in the presence of 0.016 µg/mL of levofloxacin and various concentrations of ABI-PP. These tests were performed in triplicates. Abbreviations: PP, ABI-PP inhibitor.



Supplementary Figure 4. Growth of *Escherichia coli* cells in the presence of 0.125 µg/mL of aztreonam and various concentrations of ABI-PP

a The growth of *E. coli* MG1655 Δ *acrB* Δ *tolC* cell harbouring the plasmid pMMB67HE in the presence of 0.125 µg/mL of aztreonam and various concentrations of ABI-PP. **b-g** The growth of *E. coli* MG1655 Δ *acrB* Δ *tolC* cells expressing wild-type MexB (b), MexB(I277W) (c), MexB(A279W) (d), MexB(V612W) (e), MexB(V139W) (f) and MexB(V571W) (g) in the presence of 0.125 µg/mL of aztreonam and various concentrations of ABI-PP. These tests were performed in triplicates. Abbreviations: PP, ABI-PP inhibitor.