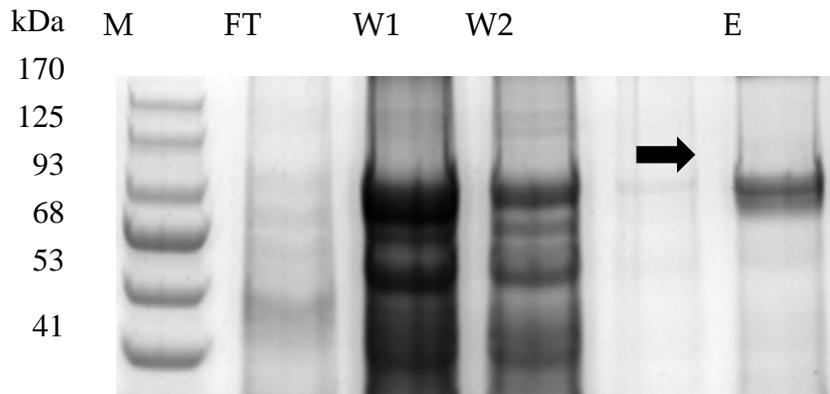


(A)



(B)

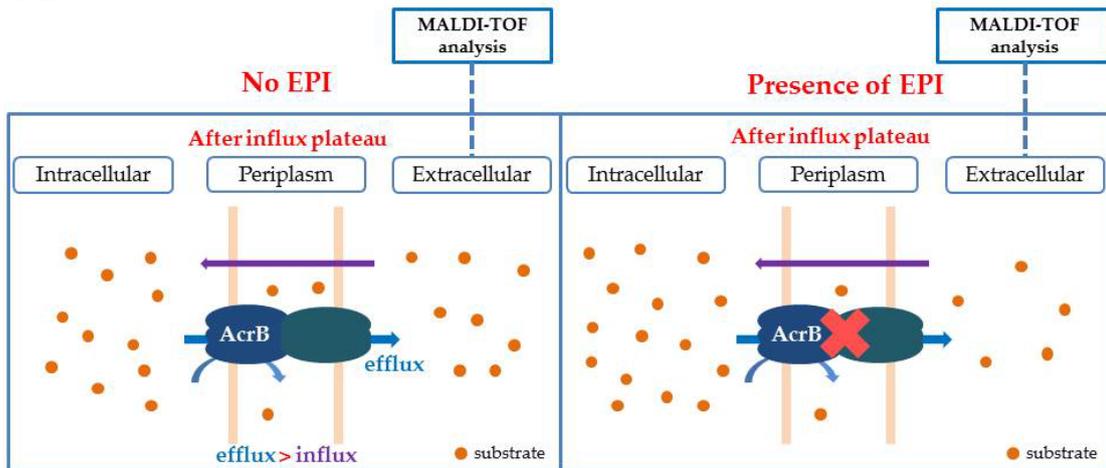
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101 DIAQVQVQNK LQLAMPLLPQ EVQQQGVSVe KSSSSFLMVV GVINTDGTMT
151 QEDISDYVAA NMKDAISRtS GVGdVQLFGS QYAMRIWMNP NELNKFQLTf
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251 LKVNQDGSRV LLRDVAKIEL GGENYDIAE FNGQPASGLG IKLATGANAL
301 DTAAlRAEL AKMEPFfPSG LKIVVPYDTT PFVKISIHEV VKTLVEAILL
351 VFLVMYLFfLQ NFRATLIPTI AVPVLLGTF AVLAAFGFSI NTLTfMGfVL
401 AIGLLVDDAI VVENVERVM AEEGLPPKEA TRKSMGQIQG ALVGIAMVLS
451 AVFVPMAffG GSTGAIYRQF SITIVSAMAL SVLVALILTP ALCATMLKPI
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651 ATRAFSQIKD AMVFAFNLPa IVELGTATGF DFELIDQAGL GHEKLTQARN
701 QLLAEAAKHP DMLTSVRPNg LEDTPQfKID IDQEKAQALG VSINDINTTL
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801 FSAFSSSRWE YGSPRLERYN GLPSMEILGQ AAPGKSTGEA MELMEQLASK
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901 VMLVVPLGVI GALLAATFRG LTNDVYfQVG LLTTIGLSAK NAILIVEfAK
951 DLMDKEGKGL IEATLDAVRM RLRPILMTSL AFILGVMPfLV ISTGAGSGAQ
1001 NAVGTGVMGG MVTATVLAIF FVPVfFVVVR RRFsRKNEDI EHSHTVDHh
```

**Figure 1. Identification of the purified AcrB protein. (A)** SDS-PAGE. M: protein molecular weight marker; FT: flow through; W1: wash by 30 mM imidazole; W2: wash by 50mM imidazole; E: elution by 500 mM imidazole. The black arrow indicated overexpressed AcrB. **(B) AcrB sequence coverage determined by MS.** Taxonomy: *E. coli* K-12. Sequence coverage: 41%. Identified peptides were shown in red.

**Table S1.** The IC<sub>50</sub> of Kam3, and Kam3-AcrB against dyes and drugs.

Drug group and drug	IC <sub>50</sub> (µg/mL)		Relative resistance
	Kam3	Kam3-AcrB	
<b>Macrolide</b>			
Erythromycin	7.812	250	32
Clarithromycin	10.937	87.5	8
<b>Quinolone</b>			
Norfloxacin	0.78	1.56	2
<b>Tetracycline</b>			
Tetracycline	0.098	0.39	4
<b>Rifampicin</b>	0.625	1.25	2
<b>Dyes</b>			
Hoechst 33342	7.812	15.625	2
Ethidium bromide	25	100	4
Nile red	>16	>16	1

(C)



Increased substrates in the extracellular space as monitored by using MALDI-TOF. Fewer substrates in the extracellular space as compared with the no EPI group.

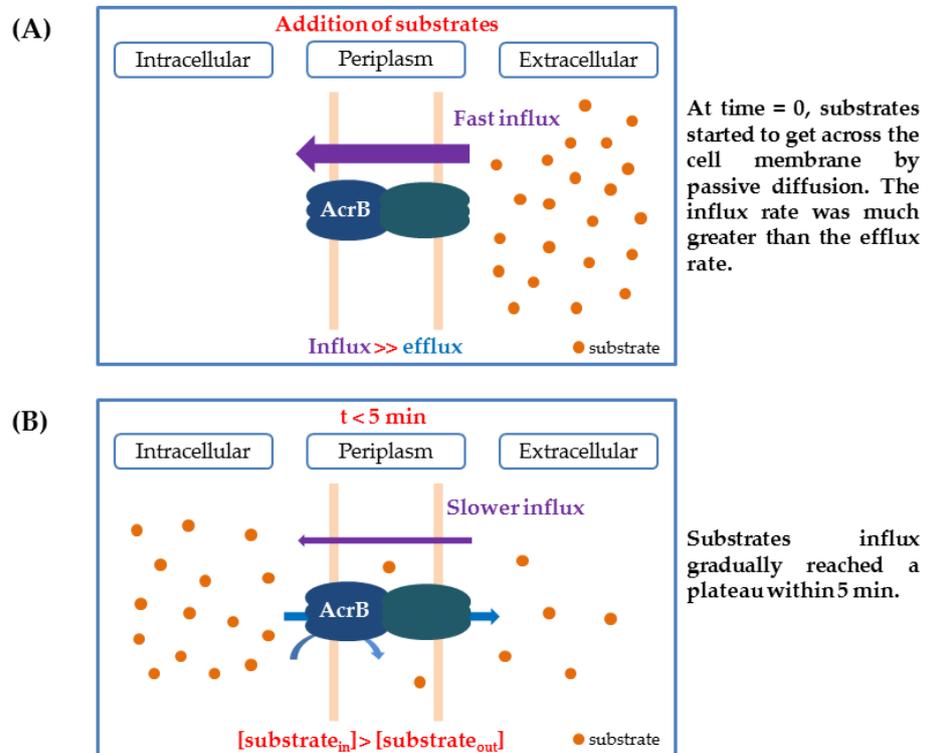
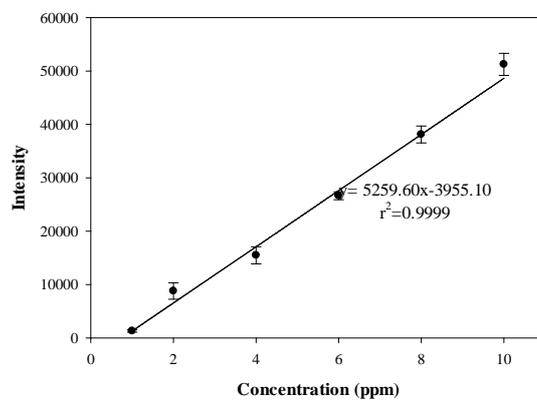
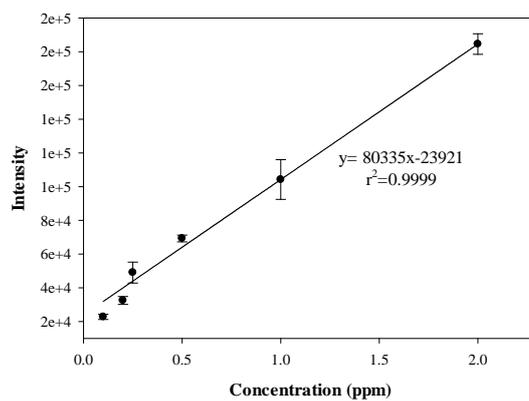


Figure S2. The influx and efflux of the substrates when they are incubated with the *E. coli* cells.

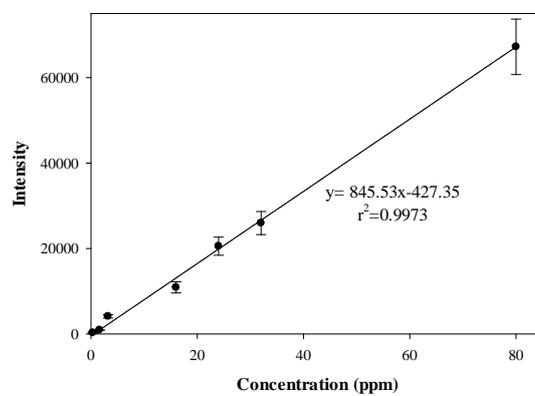
(A)



(B)

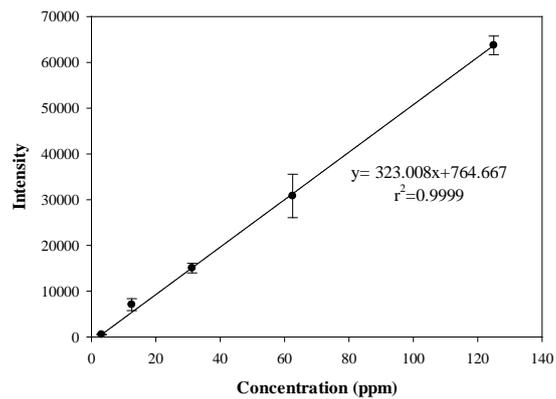


(C)

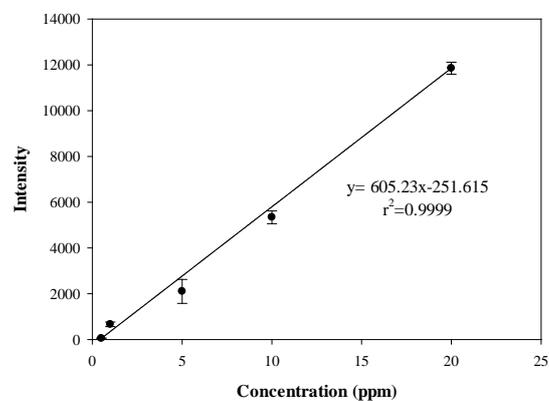


**Figure S3.** The calibration curves of dyes (A) Hoechst 33342 (B) EtBr (C) Nile red. Values were expressed as mean  $\pm$  standard deviation (SD) ( $n = 3$ ).

(A)



(B)



**Figure S4.** The calibration curves of dyes (A) Erythromycin (B) Rifampicin. Values were expressed as mean  $\pm$  standard deviation (SD) ( $n = 3$ ).