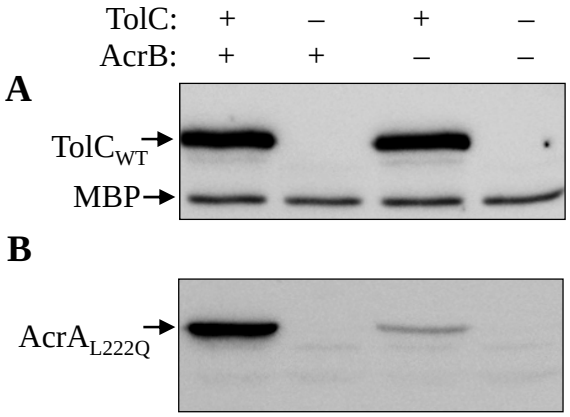

Supplemental Fig. 1. A requirement of the tripartite complex for $AcrA_{L222Q}$ stabilization. $AcrA_{L222Q}$'s stability was determined in the presence (+) or absence (-) of TolC and/or AcrB. Two identical sets of protein samples, obtained from cultures grown overnight at 37^{o}C, were analyzed by SDS-PAGE and electro-blotted onto PVDF membrane. Proteins were detected by Western blot analysis using antibodies against TolC-MBP (A) and $AcrA_{6His}$ (B). All proteins were expressed from genes present at their normal chromosomal locations.

Table S1. Primers used for site-directed mutagenesis[a].

Alterations	Primer sequence (5' to 3')
$TolC_{147}$ GC deletion	Forward: CCAACGTTTTAACGTGGCTGGTAGCGATCACCG
	Reverse: CGGTGATCGCTACCAGCCACGTTAAAACGTTGG-3
$TolC_{150}$ GC insertion	Forward: CCAACGTTTTAACGTGGCTGGTAGCGGCATCACCGACGTGCAGAACG
	Reverse: CGTTCTGCACGTCGGTGATGCCGCTACCAGCCACGTTAAAACGTTGG
$TolC_{A147G}$	Forward: CAACGTTTTAACGTGGGGGGTAGCGGCATCACC
	Reverse: GGTGATGCCGCTACCCCCCACGTTAAAACGTTG
$TolC_{L148S}$	Forward: CCAACGTTTTAACGTGGGCTCCGTAGCGATCACCGACGTGC
	Reverse: GCACGTCGGTGATCGCTACGGAGCCCACGTTAAAACGTTGG
$TolC_{V149S}$	Forward: CGTTTTAACGTGGGCCTGTCAGCGATCACCGACGTGCAG
	Reverse: CTGCACGTCGGTGATCGCTGACAGGCCCACGTTAAAACG
$TolC_{A150G}$	Forward: CGTTTTAACGTGGGCCTGTCAGGGATCACCGACGTGCAGAACGC
	Reverse: GCGTTCTGCACGTCGGTGATCCCTGACAGGCCCACGTTAAAACG
$TolC_{G147A}$	Forward: CCCAACGTTTTAACGTGGcCCTGGTAGCGATCACCG
	Reverse: CGGTGATCGCTACCAGGGCCACGTTAAAACGTTGGG
$TolC_{148AA149}$	Forward: CCAACGTTTTAACGTGGGCgcGGcAGCGATCACCGACGTGCAGAACGC
	Reverse: GCGTTCTGCACGTCGGTGATCGCTgCCgcGCCCACGTTAAAACGTTGG
$TolC_{147AAA149}$	Forward: CCACCCAACGTTTTAACGTGGcCgcGGcAGCGATCACCGACGTGCAGAACGC
	Reverse: GCGTTCTGCACGTCGGTGATCGCTgCCgcGgCCACGTTAAAACGTTGGGTGG
$TolC_{147AG148\rightarrow GL}$	Forward: CCAACGTTTTAACGTGGGGCTGAGCGGCATCACCGACGTGC
	Reverse: GCACGTCGGTGATGCCGCTCAGCCCCACGTTAAAACGTTGG
$TolC_{Q142C}$ (WT)	Forward: CCGTCAATTAGATCAAACCACCtgtCGTTTTAACGTG
	Reverse: CACGTTAAAACGacaGGTGGTTTGATCTAATTGACGG
$TolC_{Q142C}$ (Turn 1 quadruple mutant)	Forward: GCGATCTACCGTCAATTAGATtgtACCACCCAACGTTTTAACGTG
	Reverse: CGCTAGATGGCAGTTAATCTAacaTGGTGGGTTGCAAAATTGCAC
$AcrA_{S83G}$	Forward: CCTATCAGGCGACATACGACgGTGCGAAAGGTGATCTGGCG
	Reverse: CGCCAGATCACCTTTCGCACcGTCGTATGTCGCCTGATAGG

[a]Other primer sequence information is available upon request.

Table S2. Minimum inhibitory concentrations (μg/ml) of carbonyl cyanide *m*-chlorphenylhydrazone (CCCP) against various *Escherichia coli* strains.

Strains	MIC
Wild type	8-16
Δ*acrAB*	4-8
Δ*emrAB*	4-8
Δ*acrAB*, Δ*emrAB*	2-4
Δ*tolC*	1

MIC values were determined by a two-fold dilution method. The inhibitor was mixed with approximately 5 x 10^5 cell/ml. Cultures were incubated for 18 h at 37^{o}C without shaking.

Table S3. Effect of AcrA suppressors on TolC and AcrA levels in TolC turn 1 $_{147}$AGSG$_{150}$ quadruple mutant and TolC wild-type backgrounds.

AcrA protein	TolC turn 1 mutant		TolC wild type	
	TolC levels	AcrA levels	TolC levels	AcrA levels
$AcrA_{WT}$	0.50	1.00	1.00	1.00
$AcrA_{S83G}$	0.48	1.01	1.05	0.93
$AcrA_{T111P}$	0.54	0.64	0.87	0.66
$AcrA_{A135T}$	0.59	1.12	0.97	1.49
$AcrA_{T153P}$	0.49	1.09	0.89	1.40
$AcrA_{L252R}$	0.58	0.94	1.00	1.25

Proteins levels were determined from 2 to 4 independent Western blots, with a margin of error of 10% or less.