

Supplemental Table 1. Primers used in this work.

Name	Sequence (5'-3')
MdfA-F	gactggtggacagcaaatgggtcgcggatccgaattcgagatgcaaaataaattagcttc
MdfA-R	gtggtggtgctcgagtgcgccgcaagcttgcgacggagttacccttcgtgagaatttc
E136D-F	ttcaggaatccttcaagacgcggtttgatcaagatcac
E136D-R	gtgatcttgatacaaaccgctcttcaaggattcctgaa
A137E-F	caggaatccttcaagaggaggttgatcaagatcaccg
A137E-R	cggtgatcttgatacaaaccctcttcaaggattcctg
G50D-F	acaatatcaggcggacattgattgggtcctacttcaatg
G50D-R	cattgaagtaggaacccaatcaatgtccgctgatattgt
E135D-F	attcaggaatccttcgacgaggcggttgatc
E135D-R	gatacaaaccgctcgtcgaaggattcctgaat
S368R-F	tcggtattgaaatcagaaaacatgcctggct
S368R-R	agccaggcatgtttctgattcaataccga
W372R-F	aatcagcaaacatgcccggctgaacgggggc
F	
W372R-R	gccccggtcagccgggcatgttgctgatt
R	
L339R-F	tggcgcgattaacccggttgccagcgata
L339R-R	tatcgctggcaaaccgggtaatcgcacca
G333R-F	ggtctggcgaatgcgagactggtgcgatta
G333R-R	taatcgaccagtctcgattcgccagacc
K346R-F	gccagcgatatgagtagaggtacggtttctgc
K346R-R	gcagaaaccgtaccttactcatatcgctggc
K369R-F	tattgaaatcagcagacatgcctggctg
K369R-R	cagccaggcatgtctgctgattcaata
E136R-F	aattcaggaatccttcaagggcggttgatcaagat
E136R-R	atcttgatacaaaccgcccttcaaggattcctgaatt

Supplemental Table 2. Detailed information of the natural variations of MdfA across *E. coli* isolates.

Variation	Accession				
G50D	WP_1697519	EFA6854405.1			
	78.1				
E135D	HCJ5848385.				
	1				
E136D	MXH51204.1	WP_257160858	EFA7074832		
		.1	.1		
A137E	EEV1120356.				
	1				
S368R	WP_0968513	WP_137536365			
	24.1	.1			
W372R	WP_0973455	EFG3719515.1	QKV15594.1	WP_032300665	
	30.1			.1	
L339R	MCN8686451				
	.1				
G333R	MBF5229523.				
	1				

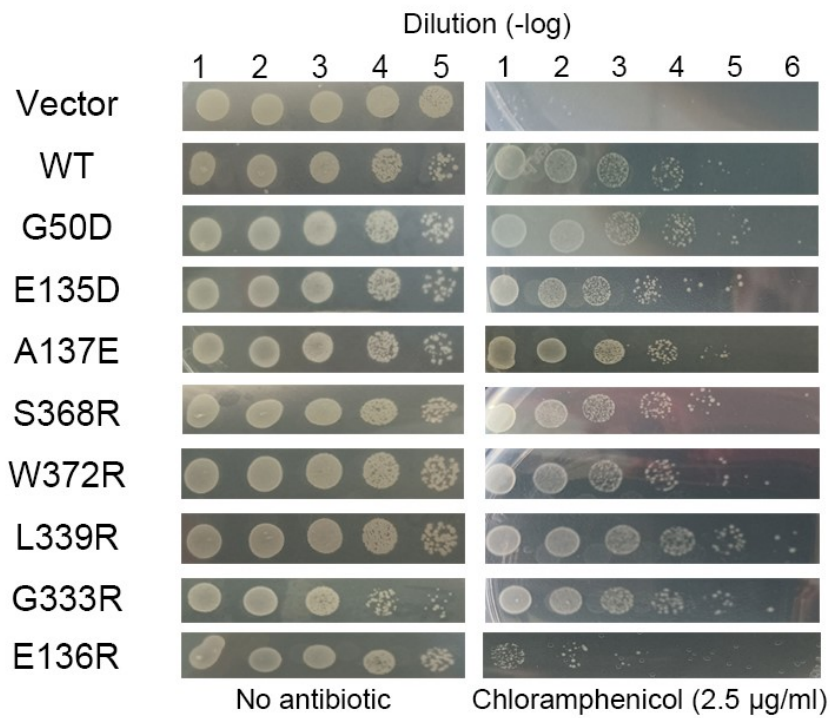


Figure S1. Chloramphenicol resistance levels of the other MdfA variants were not changed. Chloramphenicol resistance assay of the *E. coli* strains. Cultivations of the strains were 10-folds diluted and plated on solid LB medium containing 2.5 µg/ml chloramphenicol.

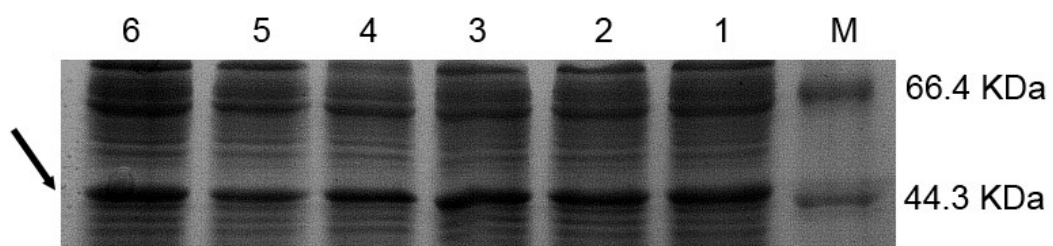


Figure S2. SDS-PAGE analysis of the recombinant *E. coli* strains. SDS-PAGE

samples were harvested at the cultivation of 12 h. M: Protein maker (TAKARA premix marker low molecule weight, 3595Q). Lane 1 to Lane 6: MdfA, MdfA^{E136D}, MdfA^{E136D-K346R}, MdfA^{K346R}, MdfA^{K369R}, MdfA^{W372R}. Arrow indicated the position of MdfA and variants (45 KDa).

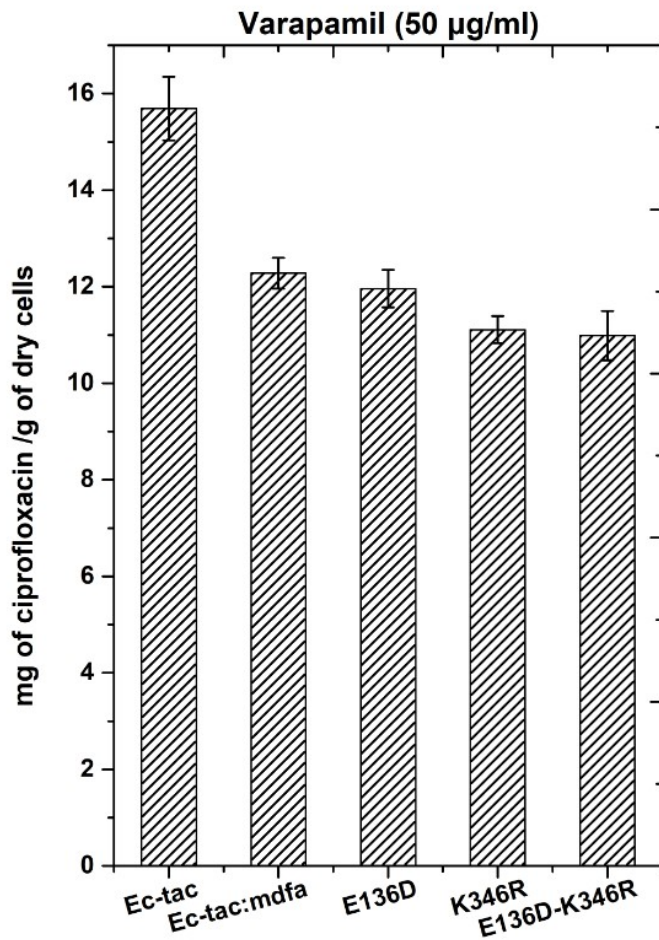


Figure S3. Intracellular ciprofloxacin concentrations were reversed by verapamil.

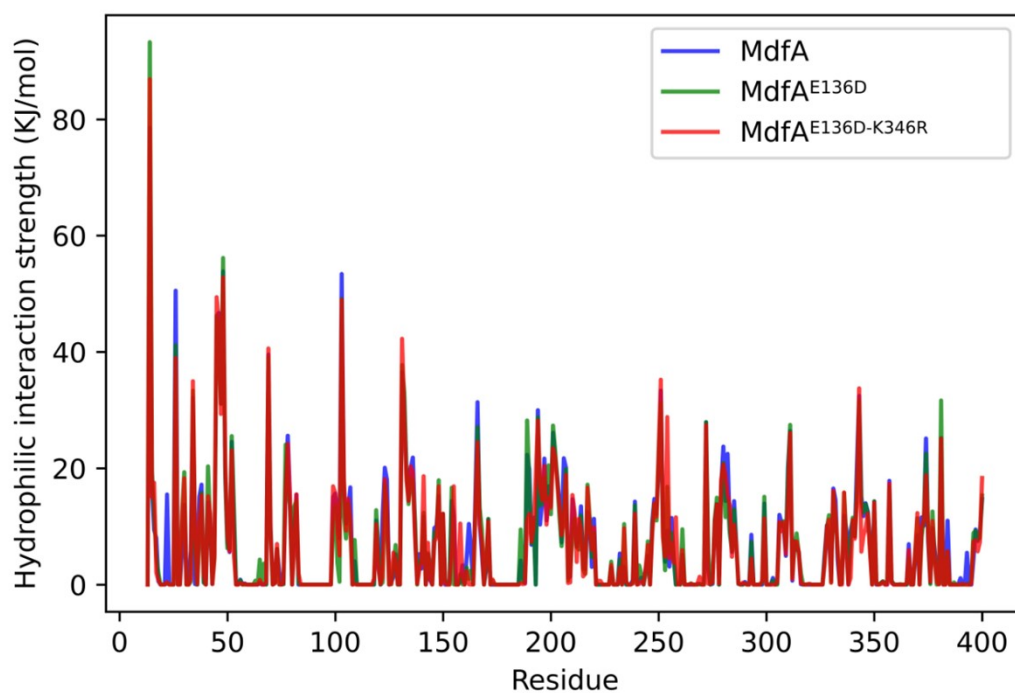


Figure S4. Detailed data of MdfA's hydrophilic interactions with water at the inward-facing conformations. Hydrophilic interactions were calculated with the built-in function of YASARA software suit.

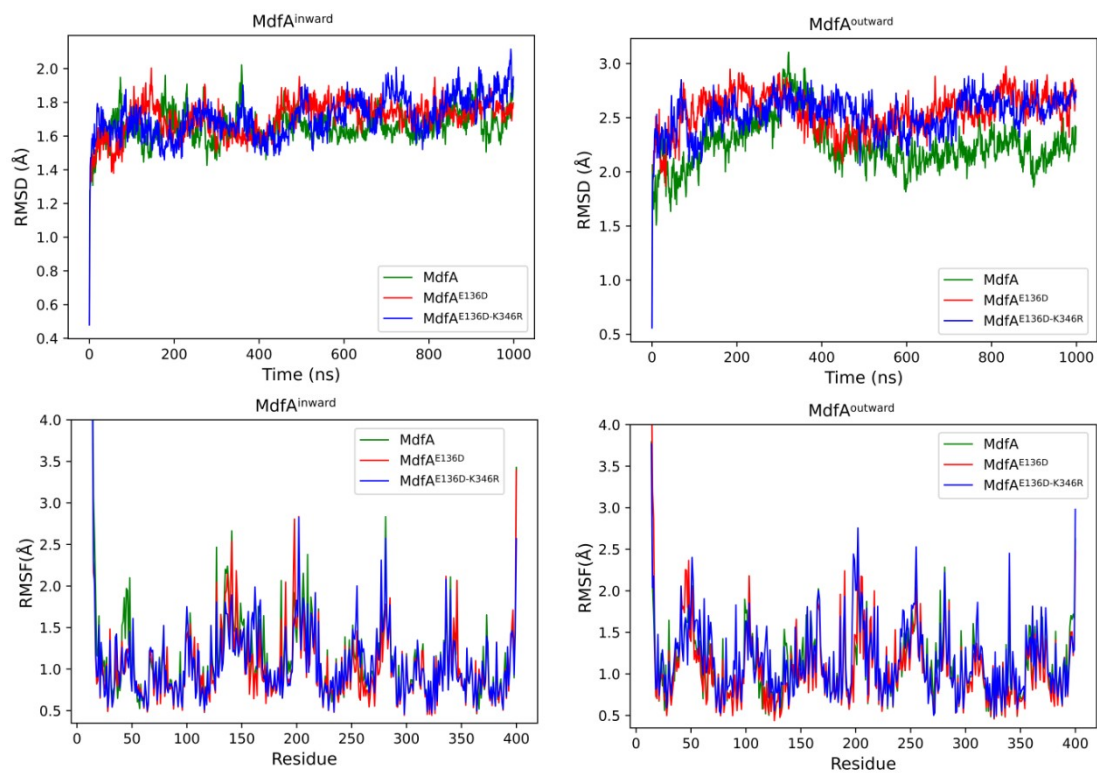


Figure S5. Flexibilities of MdfA's variants were not reduced compared to wild type MdfA. RMSD and RMSF were used to elucidate the total and local flexibilities of the protein, respectively.