Supplementary Information and Data

TABLE 1: Strains and plasmids

Strains/ plasmids	***Description***	***Source***
***E. coli* strains**		
NovaBlue	Source of DNA for PCR amplification of *macAB* and *tolC* genes – *recA1 endA1 gyrA96 thi-1 hsdR17 supE44 relA1 lac* [F' *proAB lacIqZΔM15 Tn10*(Tetr)]	Novagen
C43(DE3)	Expression host for pET vectors - F- *ompT hsdSB* (*rB-mB-*) *gal dcm (DE3)*	**1**
BL21(DE3)	*F-*, omp*T*, hsd*Sβ(rβ-mβ-),* dcm, gal*, (DE3) ton*	Stratagene
KAM3(DE3)	Antibiotic sensitive host strain used for expression of pDuet vectors – *ΔacrB*	**2**
(*ΔtolC*)TG1	Antibiotic sensitive host strain used for expression of pDuet vectors – *ΔtolC*	**3**
(*ΔtolC*)TG1(DE3)	λDE3 lysogenic strain to enable T7 polymerase production for use with pET vectors	This study
Plasmid cloning vectors		
pET21a	Expression of His-tagged proteins in *E. coli*	Novagen
pACYCDuet	Simultaneous expression of two proteins in *E. coli*	Novagen
pGEX6p-3	Expression of GST-tagged proteins in *E. coli*	GE Healthcar
Plasmid constructs		
pET-mtrD	*mtrD* cloned into pET21a(+) using NdeI-mtrD F and XhoI-mtrD R primers	This study
pET-mtrE	*mtrE* cloned into pET21a(+) using NdeI-mtrE F and HindIII-mtrE R primers	This study
pET-Δ34mtrC	*mtrC* mutant, encoding a derivative truncated at position 34, cloned into pET21a(+) using NdeI-Δ34-mtrC F and XhoI-mtrC R primers	This study
pET-mtrC hairpin	*α*-helical hairpin domain of mtrC cloned into pET21a(+) using NdeI-mtrC hairpin F and NdeI-mtrC hairpin R primers	This study
pACYCDuet-mtrD	*mtrD* cloned into MCS1 of pACYCDuet, using BamHI-mtrD F and HindIII-mtrD R primers.	This study
pACYCDuet-mtrC/mtrE	*mtrC* cloned into MCS1 of pACYCDuet using BamHI-mtrC F, HindIII-mtrC R, *mtrE* cloned into MCS2 of pACYCDuet using NdeI-mtrE F, KpnI-mtrE R.	This study
pACYCDuet-mtrC/mtrD/mtrE	*mtrCmtrE* cloned into MCS1 of pACYCDuet using BamHI-mtrC F, EcoRI-mtrC-SD-R, EcoRI-mtrE -ATG F and SalI-mtrE R; *mtrD* cloned into MCS2 of pACYCDuet using NdeI-mtrD F and KpnI-mtrD R.	This study
pGEX6p-3-NT-mtrC-GST	*NT-mtrC* cloned into pGEX6p-3 using MtrC NT For BamH1and MtrC NT Rev Xho1 –GST-tag fusion	This study
pACYCDuet-mtrC-S	*NT-mtrC* cloned into pACYC using MtrC NT For Nde1 and MtrC NT Rev Xho1 – S-tag fusion	This study

1. Miroux B, Walker J. (1996) *J. Mol Biol*. **260**:289-98.

2. Morita Y, Kodama K, Shiota S, Mine T, Kataoka A, Mizushima T, Tsuchiya T. (1998) *Antimicrob Agents Chemother*. **42**:1778-82.
3. Nagakubo S, Nishino K, Hirata T, Yamaguchi A. (2002) *J Bacteriol*. **184**:4161-7.

TABLE 2: Primers

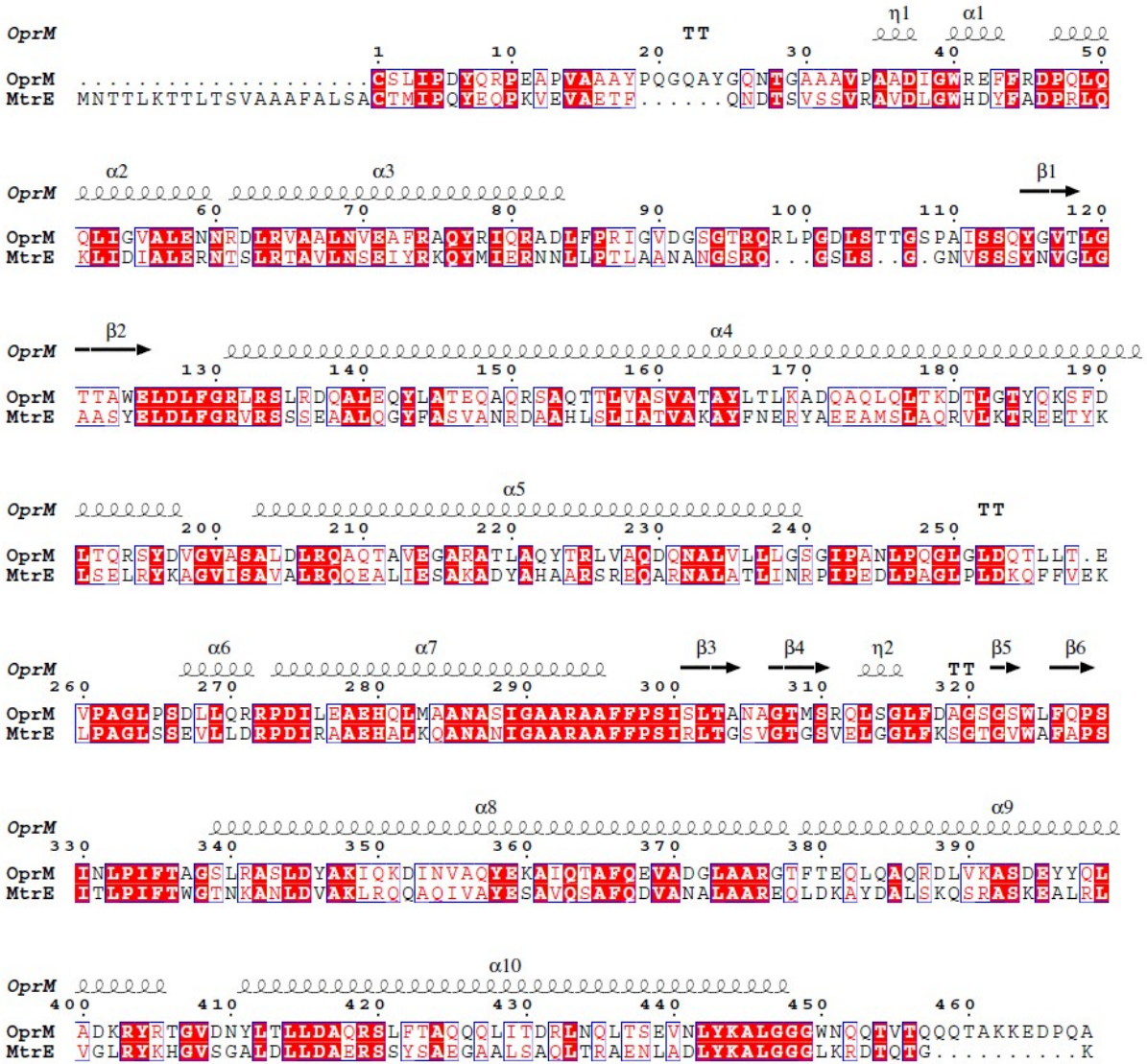
Primers	
NdeI-mtrD F	**CATATG**GCTAAATTCTTTATCGACCGCCCCATTTTCG
XhoI-mtrD R	**CTCGAG**ATATTGTTTATCGTCCGAACCGGTTATACCCG
BamHI-mtrD F	**GGATCC**GGCTAAATTCTTTATCGACCGCCCCATTTTCG
SalI-mtrD R	**GTCGAC**ATATTGTTTATCGTCCGAACCGGTTATACCCG
KpnI-mtrD R	**GGTACC**ATATTGTTTATCGTCCGAACCG
NdeI-mtrC F	**CATATG**GGCTTTTTATGCTTCTAAGGCGATGCGTGCG
XhoI-mtrC R	**CTCGAG**TTTCGCTTCAGAAGCAGGTTTGGCTTCAG
BamHI-mtrC F	**GGATCC**GGCTTTTTATGCTTCTAAGGCGATGCGTGCG
SalI-mtrC R	**GTCGAC**TTTCGCTTCAGAAGCAGGTTTGGCTTCAG
NdeIΔ34-mtrC F	**CATATG**GGCGGGCAGCCTGCGGGTCGG
NdeI-mtrC hairpin F	**CATATG**ATCGACAGTTCCACTTATGAAGC
XhoI-mtrC hairpin R	**CTCGAG**AATGCGCGAACGGTTCAGATTG
NdeI-mtrE F	**CATATG**AATACTACATTGAAAACTACCTTGACCTCTGTTG
HindIII-mtrE R	**AAGCTT**TTTGCCGGTTTGGGTATCCCGTTTCAATCCGC
BamHI-mtrE F	**GGATCC**GAATACTACATTGAAAACTACCTTG
KpnI-mtrE R	**GGTACC**TTTGCCGGTTTGGGTATCCCGTTTCAATCCGC
EcoRI-mtrC SD R	**GAATTC**TAATAATTCCTCTTATTTCGCTTCAGAAGCAGG
EcoRI-mtrE ATG F	**GAATTC**ATGAATACTACATTGAAAACT
NcoI-mtrC hairpin F	**CCATGG**AGATCGACAGTTCCACTTATGAAGG
MtrC NT For BamH1	**GGATCC**GGCGGGCAGCCTGCGGGTCGGGAA
MtrC NT Rev Xho1	**CTCGAG**TTATTTCGCTTCAGAAGCAGGTTTGGCTTCAGATGCCGTC
MtrC NT For Nde1	**CATATG**GGCGGGCAGCCTGCGGGTCGGGAA
MtrC NT Rev Xho1	**CTCGAG**TTTCGCTTCAGAAGCAGGTTTGGCTTCAGATGCCGTC

Notes: Bolded sequences indicate restriction endonuclease sites. Underlined sequences indicate start and stop codons.

TABLE 3: Primers used for mutagenesis of *mtrE*

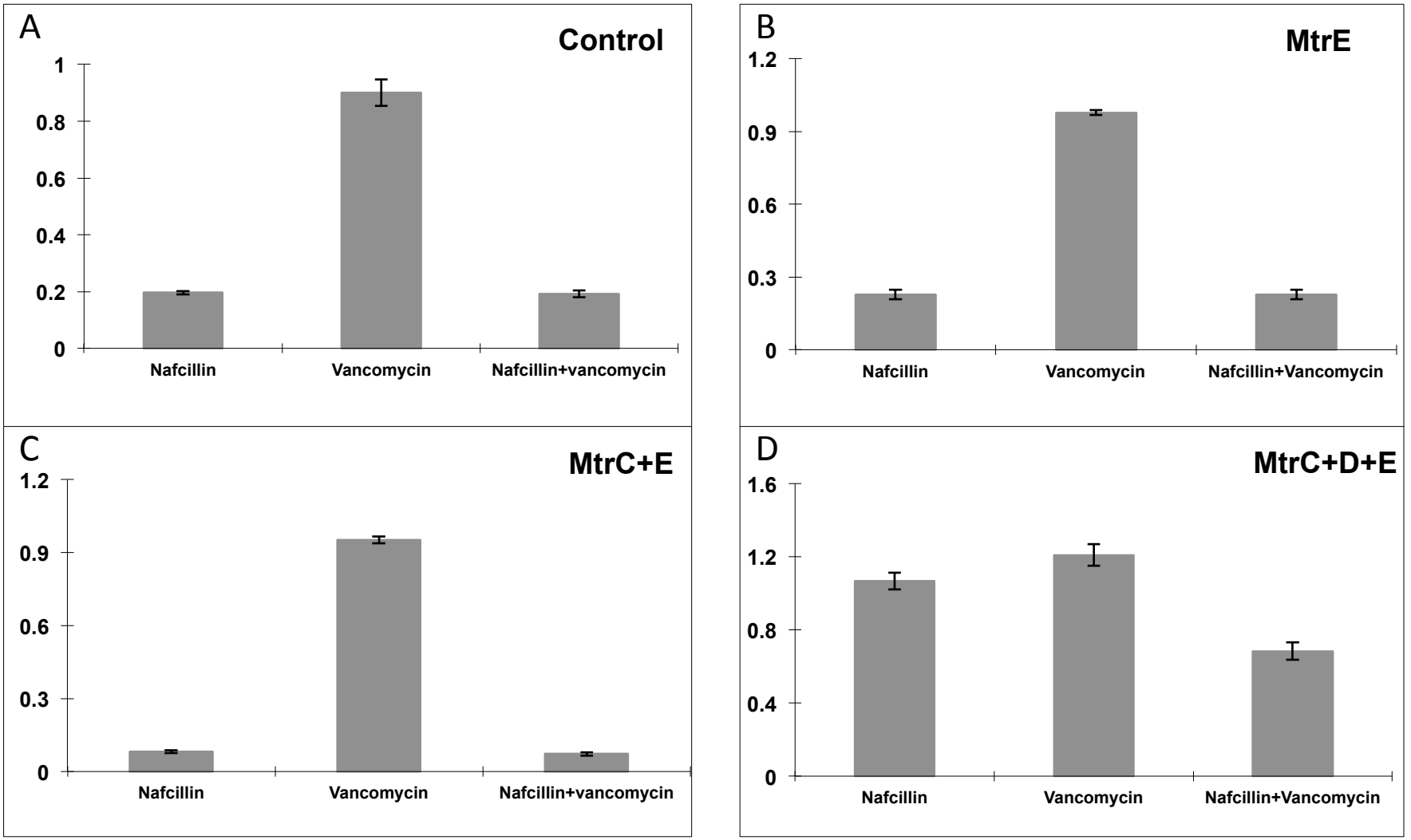
MtrE Derivatives	**Primers**
N198L	For 5′ CGCGCGAGGAAACCTACCTAGCTGTCCGAATTG 3′ Rev 5′ CAATTCGGACAGCTAGGTAGGTTTCCTCGCGCG 3′
R239E	For 5′ CCGCGAACAGGCGGAGAATGCCTTGGCAAC 3′ Rev 5′ GTTGCCAAGGCATTCTCCGCCTGTTCGCGG 3′
K397E	For 5′ CTATGACGCTTTAAGCGAGCAAAGCCGCGCCTC 3′ Rev 5′ GAGGCGCGGCTTTGCTCGCTTAAAGCGTCATAG 3′
Q441E	For 5′ GGCTTTGTCGGCAGAGCTGACCCGCGCCG 3' Rev 5′ CGGCGCGGGTCAGCTCTGCCGACAAAGCC 3'
E434K	For 5′ GCAGCTATTCGGCGAAAGGTGCGGCTTTG 3′ Rev 5′ CAAAGCCGCACCTTTCGCCGAATAGCTGC 3′

Figure 1



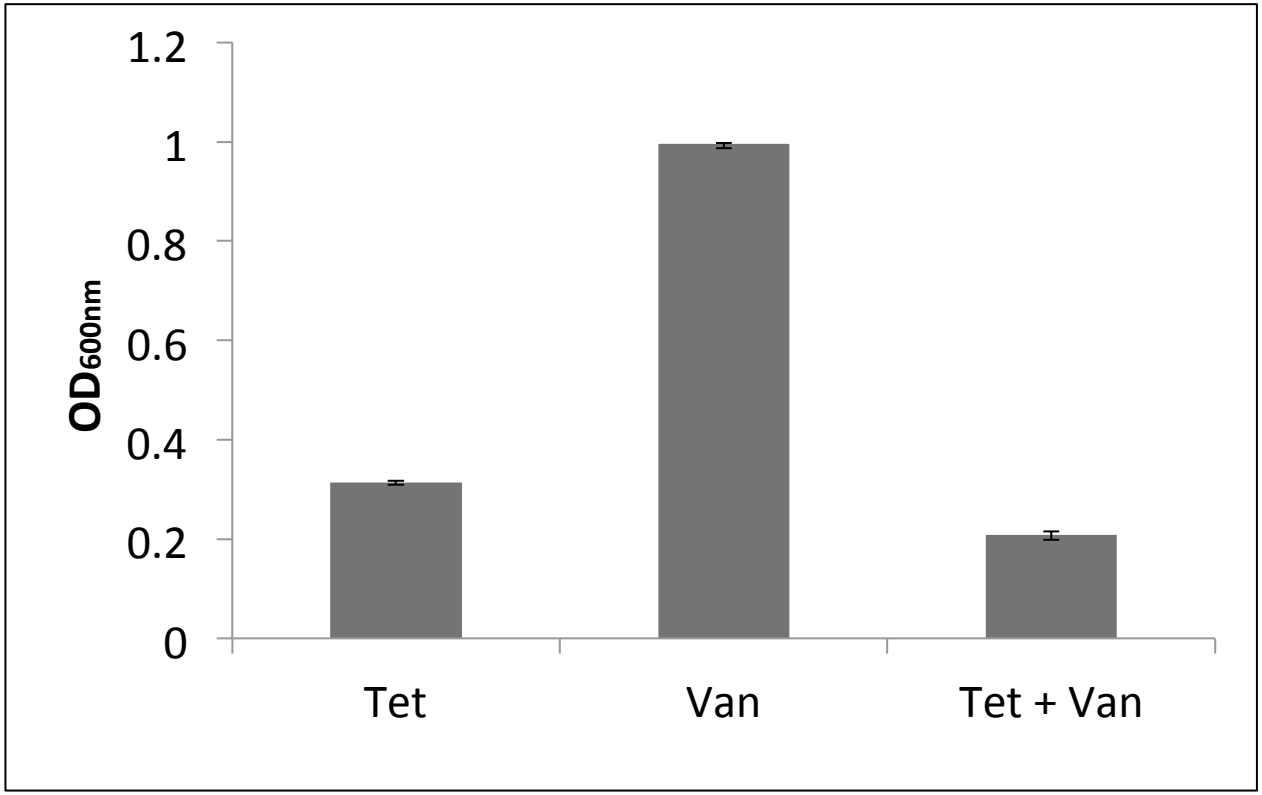
A structural alignment of MtrE and OprM indicating the identical and homologous residues as well as the secondary structure elements in OprM as determined by X-ray crystallography.

Figure 2



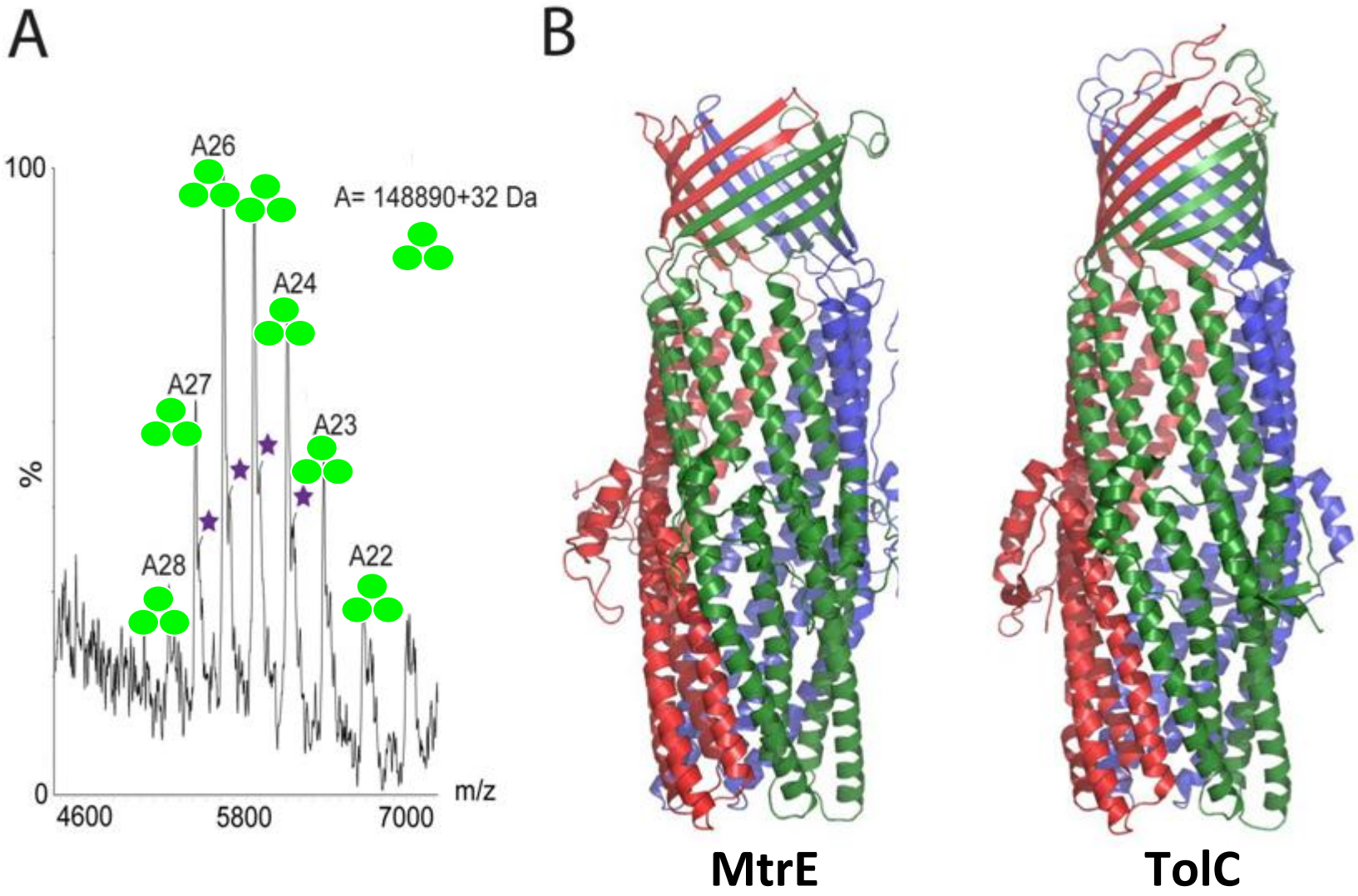
Drug-induced opening of the MtrE channel. A bar chart showing the extent of inhibition of the growth of *E. coli* cells in response to 64 μg/ml nafcillin or/and 150 μg/ml vancomycin, of strain KAM3(DE3), harboring the plasmids **(A)** pACYC, **(B)** pACYC-MtrE, **(C)** pACYC-MtrCE and **(D)** pACYC-MtrCDE, as indicated. For each strain the OD_{600} was determined after growth for 24 hours in the absence and presence of nafcillin and/or vancomycin and the growth inhibition was determined as the ratio of these measurements made in triplicate. In comparison to the control strain transformed with pACYC, the strains expressing MtrCDE, but not MtrE or MtrCE, were resistant to nafcillin and susceptible to vancomycin in the presence, but not the absence, of nafcillin. This data suggests that the the MtrD-nafcillin complex is required to trigger opening of the MtrE channel that enables vancomycin to enter the cells.

Figure 3



The binding of tetracycline to MtrD triggers opening of the MtrE channel. A bar chart showing the extent of inhibition of the growth of *E. coli* cells in response to 3 μg/ml tetracycline or/and 150 μg/ml vancomycin, of strain *ΔtolC* TG1(DE3), harboring the plasmid pACYC-MtrCDE. For each strain the OD_{600} was determined after growth for 24 hours in the absence and presence of tetracycline and/or vancomycin and the growth inhibition was determined as the ratio of these measurements made in triplicate. The cells are clearly insensitive to vancomycin, indicating that the MtrE channel is closed within the MtrCDE assembly. There is a small, but reproducible, further decrease in the growth of cells treated with both tetracycline and vancomycin, compared to those treated solely with tetracycline. This data suggests that the MtrD-tetracycline complex is required to trigger opening of the MtrE channel that enables vancomycin to enter cells.

Figure 4



A molecular model for trimeric MtrE. (A) Mass spectrum of trimeric MtrE. The charge states and measured mass of the trimer is illustrated. The asterik indicates that a small molecule is bound to the protein complex, likely corresponding to a lipid.
(B) Comparison of the homology model generated for MtrE with the crystallographic structure of TolC.