Supplemental Figures

Fig. S1. Sequence and topology of *N.gonorrhoeae* MtrF. Related to Figure 1. Alignment of the amino acid sequences of the AbgT family of transporters was done using CLUSTAL W. *, identical residues; :, >60% homologous residues. Secondary structural elements are indicated: TM, transmembrane helix; α , helix. The sequence and topology of *N. gonorrhoeae* MtrF are shown at the top. Conserved residues involved in lining the channel of the inner core of the protein are highlight with green bars.

Fig. S2. Stereo view of the electron density maps of MtrF at a resolution of 3.95 Å. Related to Figure 1. (a) The electron density maps are contoured at 1.2 σ . The C α traces of the MtrF dimer in the asymmetric unit are included. Anomalous signals of the four Ta₆Br₁₂²⁺ and six W₆(μ -O)₆(μ -Cl)₆Cl₆²⁻ cluster sites (both contoured at 4 σ) found in the asymmetric unit are colored red and white, respectively. (b) Anomalous maps of the 30 selenium sites (contoured at 4 σ). Two protomers forming a dimer of MtrF are found in the asymmetric unit. Each protomer contributes 15 selenium sites corresponding to the 15 methionines (red). The C α traces of the two MtrF monomers are colored green and cyan. (c) Representative section of the electron density at the interface of TM2 and TM6 of MtrF. The electron density (colored slate) is contoured at the 1.2 σ level and superimposed with the final refined model (green, carbon; red, oxygen; blue, nitrogen).

Fig. S3. Representative gel filtration experiment. Related to Figure 1. The experiment demonstrated that MtrF exists as a dimer in solution. The *y* axis values were defined as: $K_{av} = (V_e - V_0)/(V_T - V_0)$, where V_T , V_e , and V_0 are the total column volume, elution

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volume, and void volume of the column, respectively. Standards used were the trimeric *E. coli* CusC channel (M_r 12,400) and monomeric *N. gonorrhoeae* NorM efflux pump (M_r 29,000). The void volume was measured using blue dextran (M_r 2,000,000).

Fig. S4. Surface representation of a cross section of the MtrF protomer. Related to Figure 2. The channel formed within the outer core of the MtrF protomer is colored purple.

Fig. S5. Expression level of the MtrF pumps. Related to Figure 3. An immunoblot against MtrF of crude extracts from 50 μ g dry cells of strain BL21(DE3) $\Delta abgT\Delta pabA$ expressing the MtrF wild-type and mutant (D193A, S417A, W420A, P438A, R446A, D449A, and P457A) pumps are shown.

Fig. S6. Copy numbers of MtrF in the cell. Related to Figure 3. An immunoblot against MtrF of crude extracts from 1.1×10^9 cells of BL21(DE3) $\Delta abgT\Delta pabA/pET15b\Omega mtrF$ (lane 1). 6 ng (lane 2), 12 ng (lane 3), 30 ng (lane 4), 60 ng (lane 5), 120 ng (lane 6) and 300 ng (lane 7) of the purified MtrF protein were used as standards. The program ImageJ (Schneider et al., 2012) suggests that the crude cell extracts contain 21 ng MtrF protein, which should correspond to ~200 copies per cell.

Fig. S7. Representative isothermal titration calorimetry for the binding of sulfanilamide to MtrF. Related to Figure 5. (a) Each peak corresponds to the injection of 10 μ l of 40 μ M monomeric MtrF in buffer containing 20 mM Tris-HCl pH 7.5 and 0.03% DDM into the reaction containing 1.0 mM sulfanilamide in the same buffer. (b) Cumulative heat of reaction is displayed as a function of the injection number. The solid line is the least-square fit to the experimental data, giving a K_D of 1.14 ± 0.01 μ M.

Supplemental References

Schneider, C.A., Rasband, W.S., and Eliceiri, K.W. (2012). NIH Image to ImageJ: 25 years of image analysis. Nature Methods *9*, 671-675.

		— (TM1a) — (TM1b		TM2a
N - M	VCOTDADDCC				
NgMtrF	MSQTDARRSG	RFLRTVEWLGNMLPHPVT	LFIIFIVLLLIASAVGA	YFGLSVPDPRPVGAKGRADDGL-	-IHVVSLLDADGLIKILTHTVKNFTGFA 94
ECADGT AbabaT	MSMSS1PSSSQS	-GKLYGWVERIGNKVPHPFL	LFIYLIIVLMVTTAILS	AFGVSAKNPTDGTP	-VVVKNLLSVEGLHWFLPNVIKNFSGFA 89
NmM+rF	MSOTDTORDG	-BELETVEWLGNMLPHPVTI	LETTETVILLITASAVGA	VEGLSVPDPRPVGAKGRADDGL_	-IVIVSLINADGEIKILTHTVKNETGEA 94
CcAbgT	MSDAAPPVSSPPPRQK	GLLGVVERLGNLLPEPVM	FVWLILGLMVLSAIGQ	ALGWSASITYAGDEAPQFGELENGVL	TYAASSLFSEANLARLFTEMPKTLTSFA 105
SpAbgT		-MRFLNIVERLGNLLPHPIT	L <mark>F</mark> ALFCVAVILISGIAG	YFELTVADPRPVGSHGRSADGL-	-IHVVSLMNAEGLRMIVSNLVTNFTGFT 85
Pm MtrF	MTTTQQQKKGS	KFLHTVEWLGNMLPHPVT	L <mark>F</mark> MIFIVLLLITSALGE	YFGLAVADPRPEGVKGRAADGM-	-IYVVSLLNAEGLSRILTNLVKNFTNFA 95
VcAbgT	MTRREQMSSSASINQNAPKKPL	[TRFLDGVEYLGNLLPHPIT]	LFAIFCVVLLVASGIAG	YFELSVVDPRPEGAKGRAADGM-	-IHVVSLLNADGLELIVTNLVKNFVGFA 108
BhAbgT	-MKPAPHVELKPNQRGVI	FVRFLDIIEKYGNKLPDPIM	LFVIMAVLILICSAIFS	ALGTSAVHPGTGEE	-IEVVNLLNGEGFILILTELVNNFTSFP 94
PdAbgT SalbaT	MECKHOOKCC II	-MRALNVVERAGNKLPDPVT	IFLLLCIIVVILSAVIS	NLGVEEIHPSTKEV	-VKVVNLLEKEQIQSYLGSIVTNFQSFA 77
TdlbaT	MISKRQQKGS	- GEL KOVERIGNKLPDPSVI	TEL TI STIVITUSALAR	TLUASAULASIU	-TCAVSLINI DGI BYLINTATKNETCEA 90
CdAbqT	MSTTTPPHKTAP	-SGFLGKIEQLGNRLPDPFW	IFAFLAIIVAISSWIGS	AIGMTAVNPQDGST	-VEVTNLLTKEGATKMVSEAVNNFVAFP 89
2		:* ** :*.*	:* : : : : : :	_	
		HP1a	НР1Ь		тмзь
					11100
NgMtrF	PLGTVLVSLLGVGIAEKSGLISALMRLLL	TKSPRKLTTFMVVFTGILSN	TASELGYVVLIPLSAVI	FHSLGRHPLAGLAAAFAGVSGGYSAN	LFLGTI <mark>D</mark> PLLAGITQQAAQIIHPDYVVG 214
EcAbgT	PLGAILALVLGAGLAERVGLLPALMVKMAS	SHVNARYASYMVLFIAFFSH:	ISSDAALVIMPPMGALI	FLAVGRHPVAGLLAAIAGVGCGFTAN	LLIVTT <mark>D</mark> VLLSGISTEAAAAFNPQMHVS 209
AbAbgT	PLGVVLVAMLGLGVAEQSGLLSVSLASLV	RRSSGGALVFTVAFA <mark>GVLS</mark> SI	LTVDAGYVVLIPLAGLV	FQLAGRPPIAGIATAFAAVSGGFSAN	LLVGPV <mark>D</mark> ATLAGLSTEAAHIIDPDRTVA 201
NmMtrF	PLGTVLVSLLGVGIAEKSGLISALMRLLL	TKSPRKLTTFMVVFTGILSN	TASELGYVVLIPLSAII	FHSLGRHPLAGLAAAFAGVSGGYSAN	LFLGTIDPLLAGITQQAAQIIHPDYVVG 214
CcAbgT	PLGLVLVVILGAAVAERSGLFSALIRASL	REAPKRILTPLVVIIGMVSH	HASDAAYVVFIPLAGLL	YAAVGRHPLAGIAAGFAAVSGGFAGN	LTPGQFDVVLFGFTQEAARIIDPTWTMN 225
SpAbgT DrmM+rF	PLGTVLVALLGVGIAERSGLLSAAMRALV	IGASKRLVTVTIVFAGIMSN	PAAELGYVVLIPMAAMI	FHSLGRHPLAGLAAAFAGVSGGYSAN	LLLGTVDPLLSGITEAAARMIDPDYSVG 205
Vabam	DI CUVI VANI CVA I AFUSCI I SA MOCI VI	ACA SYDMUTETUTE ACTION	TASELGI VVLIPLAATI	FUEL CRUPT ACT AN A FACUSCOVE AN	
BhAbaT	PLGLVLVVMLGVGVAFABIISGHISAAII(GEVI	NAPRKIIIPTIVIVANIGN	AAADAAMVVLPPTVAMT	FTALGRHPLAGLAAAYASVAGGFSAN	LILSMLDPLVAGFTOTGAOMIDPDYVAN 214
PdAbgT	PLGLVLVTMLGAGVAEKSGFMEVLMKKGIS	KVPOKLVTVAIVFAGMLSH	TAADVGFIILPPLAALV	FLGIGRHPLVGMFAAFAGVAGGFAAN	VMLSTTOVLLAGFTIPAAOMMDPSYOGN 197
SaAbgT	ALGLVLAVMIGIGVAEKTGYFDKLMISVV	NRAPRFLILPTIILIGILGS	TAGDAATIILPPLAAML	FIKIGYHPIAGLTMAYASAVGGFAAN	IVVGMQDALVYSFTEPATRIVSDSIKTN 209
<i>Td</i> AbgT	PLGTVLVAMLGVGVAEWTGLINTSLKKLLS	GVHPRLLTVVVVFAGIMSNV	VASDAGYVVV <mark>IP</mark> LG <mark>A</mark> IV	FANAGRHPMAGLAAAFAGVSGGFSAN	LMLGTI <mark>D</mark> PLLTGITVEALHNAGMDIAID 210
<i>Cd</i> AbgT	PLGVIITVMLGVSVAEHSGFISALVRAMVA	AKVGPKTLTYVVALA <mark>GVTG</mark> S	IASDAVYVI <mark>LIS</mark> LGAMS	FRALGRSPIVGAMVAFAASSAGFNAS	LILNIT <mark>D</mark> VLLSGISTSAAQLVDPEYHVS 209
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		α1			
	— <u>TM4</u>	//////		TM5	
NoMtrE		CDVOCDI COFFEETDUCNI		THDI PYPOT THACUNPTAT CALLA	
ECAbaT	VIDNWYFMASSYVVI.TIVGGI.TDKITEP	LGOWOGNSDEKLOT		ITESORFGLETAGVVFIALSALLA	IMVIPADGILRHPEIGLVAGS 509
AbAbaT	ATGNYWFT TASTFLYTGLYTLTTRTLTEP	LAHANTVADASVDAPOTHS-		RAMKWTGLT-LATLLAGL	ALLV LPNDAPLR HPDTGSVLGS 289
NmMtrF	PEANWFFMVASTFVIALIGYFVTEKIVEP	LGPYQSDLSQEEKDIRHSNI	Е	ITPLEYKGLIWAGVVFVALSALLA	WSIVPADGILRHPETGLVSGS 309
<i>Cc</i> AbgT	PLGNWWYILAIVVVFTPIAWFLTDKVVEP	LGPWGGQADDALKAELAKS	A	VTADEKRGLKFAGLAALAIVALFA	ALSLIPGFTPLID-ETKTGPAQLT 322
<i>Sp</i> AbgT	PEVNWYFMFVSTFV1TFLGAFVTEKIVEP	LGKYQDGDADETVLQS-ME:	S	VSAIEKRGLKWAGLSVLILAIMLA	LLVVPEGAPLRHPDTGLVSGS 299
Pm MtrF	PEANWFFMAASTFVIAFIGYFITEKIVEP(LGPYNSHLSQEELDLQHSNI	E	VSPLERKGLRYAGLVFLILCALLA	WTVVPENGILRDPKTGLVTGS 310
VcAbgT	PEVNWYFMAASTFVIAILGAFVTEKIVEP	LGKYDVSEASDDLSQDKMG	5	LTALEKKALAYAGLAVVVVSALLA	WTIVPADGVLRGEDGLVSGS 322
BhAbg'I'	PAMNYYFLVASCLVLVPVAVWVTTKIVEP	EGTYTGEVEETTG-		VIKEEKKGLRWAGISVVILAVVFL	FLTVPEQALLRDPETGS-LTVS 302
FUADGI	VAMNEVETA A SUULT DETT I VERTIARI	CKADDEL WADDAREWEER		LSELENKGIKIALLSLLVVVVVIV	TTAL DEUSEDNARMOPEIGSILSSNA 209
TdabaT	PTCNWFFMIVSTFILTVVGTFVTFKIVEK	LGTYNGSYKPDNMPIS			IGLEGI.PKI.PGI.AVI.REIDPKTGESSIS 306
CdAbgT	TLANYFFVVASFLVLALIITAVTELFVKN	ARQLVDHDHIDHSELSFRDI	DDHPDLGAKTDEELAEE	IALHSGEIRALTIAGVAFIGMLAVYF	ALLFVPASPFYSE-ESAMSS 320
-	* :: .:. :*				
	TM6		TM7b	TM7c	HP2a HP2b
NgMtrF	PFLK <mark>S-IVVFIFLLFALPGIV<mark>YG</mark>RITRSL</mark>	RGEREVVNAMAESMSTLGLY	LVIIFFAAQFVAFFNWT	NI <mark>G</mark> QYIAVKGAVF <mark>L</mark> KEVGLAGSVLFI	GFILICAFINLMIG <mark>SAS</mark> AQ <mark>W</mark> AVTAPIFV 428
<i>Ec</i> AbgT	PFIKG-IVPLIILFFFVVSLA <mark>YG</mark> IATRTI	RQADLPHLMIEPMKEMAGF	IVMVFPLAQFVAMFNWS	NMGKFIAVGLTDILESSGLSGIPAFV	GLALLSSFLCMFIASG <mark>S</mark> AI <mark>W</mark> SILAPIFV 417
AbAbgT	PFIHG-LVVIVALIAGICGAVYGRVSGQF	RNSGAVITAMEVTMASMAGY	LVLMFFAAQFVAWFNYS	QLGLLLAVKGAAWLGALTVPKVVLLL	LFVVLTALINLMIG <mark>SAS</mark> AKWSILAPVFI 408
NmMtrF	PFLKS-IVVFIFLLFALPGIVYGRVTRSL	RGEQEVVNAMAESMSTLGLY	LVIIFFAAQFVAFFNWI	NIGQYIAVKGATFLKEVGLGGSVLFI	GFILICAFINLMIGSASAQWAVTAPIFV 428
CCADGT Spaba	PFYGA-LIAGFMMLFLAGGVAYGVGVGTVI	(TEGDVVNMMADGVRSVAPY.	LVFAFFAAHFVAMFNWS	RLGPIAAIHGAE'I'LKAMNLPAPLLLV	SVLGFSSVLDLFIGSASARWSALAPVVV 441
DmM+rF	DELKS_IVAFIFILEAIDCTVVCIVTKSI	GERDININAMAEAMSTI CI VI	UTTEEASOEVAFENWT	NICOVIAVEGANEI NEVCI HCCII EM	GETLICATINIMIGSAGAGMANTAFITY 410
VcAbaT	PFLKS-IVAFIFIFFAIPGYVYGRVVGTM	TDRDVINAMAKSMSSMGMY	IVLVFFAAOFVAFFSWI	KFGOVLAVLGADFLKDIGLTGPMLFF	AFILMCGYINLMIGSASAOWAVTAPIFV 429
BhAbaT	PFMTG-TVPTMMVFFLVPALVYGFVAKVF	SSKDVADHLAKSMSNMGTY	TVTAFVAAOMTAFFNWS	OLGPTVATKGANFT.OTTGFTGLPLLL	GETVTAALTNI MVASASAKWATLAPVEV 421
PdAbgT	PLMKG-IVPIITIIFLTPGLVYGKVSKKI	SDKDLVSMMGSSMSDMGGY	IVLAFIASQFINLFNLS	NLGTILSITGAKLLÄESGIPSYGLII	GFILLSGFINLFVG <mark>SAS</mark> AK <mark>W</mark> AILAPIFV 408
SaAbgT	PLING-VGLIILVVFLVPGLVYGILSKEI	NTKDLGKMFGDAVGSMGTF	IVIVFFAAQLLAYLKWS	NLGIIAAVKGAKLLEHQNGIVLIL	GIIVLSAMVNMLIG <mark>SAS</mark> AK <mark>W</mark> GILGPIFV 421
<i>Td</i> AbgT	NFMHGGLLPVILLFLIPGLIYGKKTGKI	ISSHDLVKGMSQAMSSMGGY	LVLSFFAAQFVNYFGKT	NLGTIISVNGANFLKSIGFTGLPLII	SFVIISAFLNLFMG <mark>SAS</mark> AK <mark>W</mark> AIMAPIFV 426
<i>Cd</i> AbgT	PLVKA-VTVPISLMFLGLGVVYGITIKSI	TSLGDIPAFMAKGLTTLIPM	VVLFFMVAQFLAWFQWS	NLGIWTAIKGAELLQRWDLPVYVLFA	AVVLAVALLNLTITSG <mark>S</mark> AQ <mark>W</mark> ALMAPVIV 439
	• • • • • • • • •		• • • • • • •		• • • • • • • • • • • • • • • • • • • •
		—	- TM	<mark>) </mark>	
<i>Ng</i> MtrF	PMLMLAGYAPEVIOAAYRIGDSVTNIITP	MSYFGLIMATVMKYKKDAG	VGTLISMMLPYSAFFLI	AWIALFCIWVFVLGLPVGPGAPTLYP	AP 522
<i>Ec</i> AbgT	PMFMLLGFHPAFAQILFRIADSSVLPLAP	/SPFVPLFLGFLQRYKPDAK	L <mark>G</mark> TYYSLVL <mark>PYP</mark> LIFLV	VWLLMLLAW-YLVGLPIGPGIYPRLS	508
AbAbgT	PMLMLLGIS <mark>P</mark> EASQAAY <mark>R</mark> VGDSSTNIITP	MPYFVLVLGFARRYQPETG	I <mark>G</mark> TLIALML <mark>PYSL</mark> TLLL	GWSVLLGVW-IGFGWPLGP	492
NmMtrF	PMLMLAGYAPEVIQAAYRIGDSVTNIITP	MSYFGLIMATVIKYKKDAG	VGTLISMMLPYSAFFLI	AWIALFCIWVFVLGLPVGPGAPTFYP	AP 522
CcAbgT	PMFMLLGISPEMTTAAYRMGDSFTNLMTP	MSYFPLVLAMTRRWDPSMG	VGSLLALMLPYALAFMV	AGVAMTLAW-VAFDWPLGPAAQVHYT	PPGGLLK 539
SPADGT DmM+	PRIME VGIARETIQAAIRIGUSVTNLVTP	MENECT TRADUCTION	IGTLIATMLPYTLVFFV	GWIAFFFLWVFGFGLFVGPGAATYYT	r 311 NO 522
VCAbaT	PMLMLTGISPELIQAAIRIGUSVTNIITP PMLMLVGYAPEVTOAAVPICOSVTNIITP	MSYFGLIMATVLKYKKDAG	VGTLVSMMLPYSVAFLV IGTLTATMI.PYSVVFMU	AWSAMFTIWVFVLGLPVGPNSPMFYP GWSLLFYVWVFVLGLPVCPCAATVVT	AQ 523 P 534
BhAbam	PMFMIJEYSPAFTOAAVPVCDSTTNDTTD	ILPYFATAL TFAKKVNDETC	IGTEMSSI.I. PVSTAFAU	TWITLETVW-YLLCLDLCDCEVTUTU	T 513
PdAbgT	PMFMLLDFNPALTQIAYRIGDASTNPISP	FPYFPVILAFARRYDKDIG	IGTVISNMIPYSVVFTL	IEIIILLF-MGIGIPLGPGGGISYV	L 500
SaAbgT	PMLILIGFHPAFTQVIYRVGDSITNPITP	MPYLPLLLTYAQKYDKRMK	LGALLSSLMPYSIALSI	VWTVFVIIW-FLLGIPVGPGGPIFVK	512
<i>Td</i> AbgT	PMMVNLGLSPALTQVAYRIGDSSTNLITP	MSYFAMIVVFMKKYDEDSG	LGTLISTMLPYSIAFLL	SWIGLMIIW-YIFGLPLGPGAFIHI-	516
<i>Cd</i> AbgT	PMMMYVGIS P EVTQMLFRIGDSPTNIITP	SPYFALALTFLQRYYKPAG	VGTLVSLALPYSIAMLV	GWFVFFIVW-YALGVPLGPGTPMHFQ	QG 532
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		•	$W_{6}(\mu - O)_{6}(\mu -$	
Data set	Native MtrF	$Ta_6Br_{12}^{2+}$	$Cl)_6Cl_6^{2-}$	Se (peak)
Data Collection				
Wavelength (Å)	0.98	1.25	1.02	0.98
Space group	P65	P65	P65	P65
Resolution (Å)	50 - 3.95	50 - 6.53	50 - 5.70	50 - 4.60
	(4.11 – 3.95)	(6.74 - 6.53)	(5.93 – 5.70)	(4.76 - 4.60)
Cell constants (Å)				
a	120.77	120.57	120.51	116.37
b	120.77	120.57	120.51	116.37
с	233.90	231.33	231.75	224.88
α, β, γ (°)	90, 90, 120	90, 90, 120	90, 90, 120	90, 90, 120
Molecules in ASU	2	2	2	2
Redundancy	4.7 (4.0)	5.7 (5.8)	5.6 (6.5)	4.4 (3.8)
Total reflections	868,348	28,1275	189,1056	1796,647
Unique reflections	16,832	3814	5533	9712
Completeness (%)	98.2 (92.8)	99.9 (99.9)	99.7 (99.0)	99.9 (100)
R_{merge} (%)	5.8 (46.8)	5.3 (49.5)	8.6 (48.7)	9.4 (59.0)
Ι/σ	29.65 (0.88)	45.42 (2.60)	34.14 (1.7)	19.35 (1.95)
Phasing				
Number of sites		4	6	30
Phasing power (acentr	ric/centric)	1.71	/0.99	
Figure of merit		0.	59	
Refinement	MtrF			
Resolution (Å)	50 - 3.95			
R _{work} (%)	29.9			
$R_{\text{free}}(\%)$	33.6			
RMSD bond lengths	2210			
(Å)	0.009			
RMSD bond angles				
(°)	1.52			
Ramachandran plot				
most favoured (%)	89.0			
additional allowed				
(%)	10.0			
generously allowed	1.0			
(70)	1.0			
aisallowed (%)	0.0			

Table S1. Data collection, phasing and structural refinement statistics. Related to Figure 1.

Primer	Sequence
D193A-forward	5'-CTGTTCCTGGGCACCATTGCTCCGCTGCTGGCCGGTATC-3'
D193A-reverse	5'-GATACCGGCCAGCAGCGGAGCAATGGTGCCCAGGAACAG-3'
S417A-forward	5'-GGTAGTGCTGCCGCACAATGGGCAGTGACCGCACCGATCT-3'
S417A-reverse	5'-CCATTGTGCGGCAGCACTACCGATCATCAGGTTAATAAA-3'
W420A-forward	5'-CGCACAAGCGGCAGTGACCGCACCGATCTTCGTTCCG-3'
W420A-reverse	5'-GGTCACTGCCGCTTGTGCGGAAGCACTACCGATCATCAG-3'
P438A-forward	5'-GGCTATGCTGCGGAAGTCATTCAGGCCGCATACCGC-3'
P438A-reverse	5'-GACTTCCGCAGCATAGCCTGCCAGCATCAGCATCGG-3'
R446A-forward	5'-GTCATTCAGGCCGCATACGCCATCGGTGATTCAGTTACC-3'
R446A-reverse	5'-GGTAACTGAATCACCGATGGCGTATGCGGCCTGAATGAC-3'
D449A-forward	5'-GCCGCATACCGCATCGGTGCTTCAGTTACCAATATTATC-3'
D449A-reverse	5'-GATAATATTGGTAACTGAAGCACCGATGCGGTATGCGGC-3'
P457A-forward	5'-ATCACGGCGATGATGTCGTATTTTGGTCTGATTATG-3'
P457A-reverse	5'-CGACATCATCGCCGTGATAATATTGGTAACTGAATC-3'

Table S2. Primers for site-directed mutagenesis. Related to Figure 3.

Table S3. MICs of sulfamethazine, sulfadiazine, sulfathiazole and sulfanilamide for different MtrF variants expressed in *E. coli* BL21(DE3) $\Delta abgT\Delta pabA$. Related to Figure 5.

Gene in BL21(DE3) $\Delta abgT\Delta pabA$	Sulfamethazine (µg/mL)	Sulfadiazine (µg/mL)	Sulfathiazole (µg/mL)	Sulfanilamide (µg/mL)
Empty vector	62.5	31.25	62.5	500
<i>mtrF</i> (wild-type)	2000	>250	>500	4000
<i>mtrF</i> (D193A)	1000	31.25	62.5	2000
mtrF (S417A)	125	31.25	125	2000
mtrF (W420A)	125	31.25	62.5	2000
<i>mtrF</i> (P438A)	62.5	31.25	62.5	1000
<i>mtrF</i> (R446A)	1000	>250	>500	2000
<i>mtrF</i> (D449A)	62.5	31.25	62.5	1000
<i>mtrF</i> (P457A)	62.5	62.5	125	1000

	$K_D(\mu M)$	$\Delta H (\text{kcal} \cdot \text{mol}^{-1})$	ΔS (cal•mol•deg ⁻¹)
Sulfamethazine	0.33 ± 0.02	-580.2 ± 5.9	27.7
Sulfadiazine	12.74 ± 0.62	-1900.0 ± 131.8	16.0
Sulfathiazole	1.52 ± 0.07	-267.3 ± 8.0	25.7
Sulfanilamide	1.14 ± 0.01	-135.2 ± 1.1	26.7
PABA	0.54 ± 0.02	-319.2 ± 8.6	27.6

Table S4. Binding of sulfamethazine, sulfadiazine, sulfathiazole, sulfanilamide and p-aminobenzoic acid by MtrF. Related to Figure 5.

	$K_D(\mu M)$	$\Delta H (\text{kcal} \cdot \text{mol}^{-1})$	ΔS (cal•mol•deg ⁻¹)
Sulfamethazine	10.78 ± 1.17	-233.4 ± 10.7	21.9
Sulfadiazine	105.82 ± 25.6	-41103 ± 2065.0	4.4
Sulfathiazole	50.76 ± 8.9	-713.5 ± 28.4	17.3
Sulfanilamide	6.80 ± 1.5	-63.1 ± 3.0	23.4
PABA	41.15 ± 8.5	-425.3 ± 18.7	18.6

Table S5. Binding of sulfamethazine, sulfadiazine, sulfathiazole, sulfanilamide and p-aminobenzoic acid by the W420A mutant. Related to Figure 5.