Supplementary Information for

A "drug sweeping" state of the TriABC triclosan efflux pump from *Pseudomonas aeruginosa*

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Tables S1 to S2

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Other supplementary materials for this manuscript

None.

Supplementary Information Text

Table S1, *Related to Figure 1A*. Antimicrobial susceptibility of *E. coli* Δ9-Pore[a] cells producing the plasmid-borne AcrAB and TriABC efflux complexes with the separate TriA and TriB (TriA,B) and fused TriAB proteins.

Variants	0% L-Arabinose		0.1% L-Arabinose	
	SDS (μg/mL)	Triclosan (ng/mL)	SDS (μg/mL)	Triclosan (ng/mL)
-	9	4	9	4
AcrAB	>2500	128	312	128
TriA,BC	39-78	16	78	16
TriABC	>2500	64	156-312	64

[a] Addition of arabinose to growing E. coli Δ9-Pore induces the expression of a large pore in the outer membrane that enables permeation of compounds into periplasm (Krishnamoorthy et al., 2016).

Table S2, *Related to Figures 2, 3 and 7*. I-TASSER homology modelling of TriC, TriA, and TriB polypeptide folds. Proteins homologues, found in the PDB, are listed for TriC, TriA, and TriB subunits that are considered to have the same topological fold (as identified by TM-align) and limited to those homologues having TM-score > 0.5 (Xu and Zhang, 2010).

TriC subunit

RANK	PDB HIT	PROTEIN	TM-SCORE[A]	RMSD[B]	IDEN[C]	COV[D]
1*	3ne5	CusA	0.955	1.02	0.209	0.963
2	4k0e	ZneA	0.913	1.73	0.220	0.936
3	3d9b	AcrB	0.904	3.21	0.200	0.976
4	4mt1	MtrD	0.889	3.26	0.204	0.958
5	3jd8	NPC1	0.519	6.11	0.073	0.641

TriA subunit

RANK	*PDB HIT*	*PROTEIN*	*TM-SCORE[a]*	*RMSD[b]*	*IDEN[c]*	*COV[d]*
1*	3lnnA[¶]	ZneB	0.900	0.71	0.215	0.909
2	4dk0A	MacA	0.501	5.04	0.130	0.708

TriB subunit

RANK	*PDB HIT*	*PROTEIN*	*TM-SCORE[a]*	*RMSD[b]*	*IDEN[c]*	*COV[d]*
1*	2v4dM[¶]	MexA	0.859	1.77	0.219	0.909
2	4dk0A	MacA	0.611	4.41	0.191	0.798
3	2f1mC	AcrA	0.568	3.43	0.215	0.658
4	4l8jA	BACEGG	0.559	3.63	0.180	0.664
5	3fppB	MacA	0.543	3.98	0.200	0.681

[a] Ranking of proteins is based on TM-score of the structural alignment between the query structure and known structures in the PDB library (Zhang and Skolnick, 2004).

[b] RMSD is the RMSD between residues that are structurally aligned by TM-align. TM-align is an algorithm for sequence-order independent protein structure comparisons (Zhang and Skolnick, 2005).

[c] IDEN is the percentage sequence identity in the structurally aligned region.

[d] Cov represents the sequence coverage of the alignment by TM-align and is equal to the number of structurally aligned residues divided by length of the query protein.

[¶] Last letter in PDB code identifies the subunit as there may be more than one subunit

* The confidence of each model is quantitatively measured by C-score that is calculated based on the significance of threading template alignments and the convergence parameters of the structure assembly simulations. C-score is typically in the range of [-5, 2], where a C-score of a higher value (> -1.5) signifies a model with a higher confidence and vice-versa (Yang et al., 2015).

- TriC: C-score for 1st ranked model = 1.70 indicating a correct fold.
- TriA: C-score for 1st ranked model = - 0.78 indicating a correct fold.
- TriB: C-score for 1st ranked model = - 0.16 indicating a correct fold.

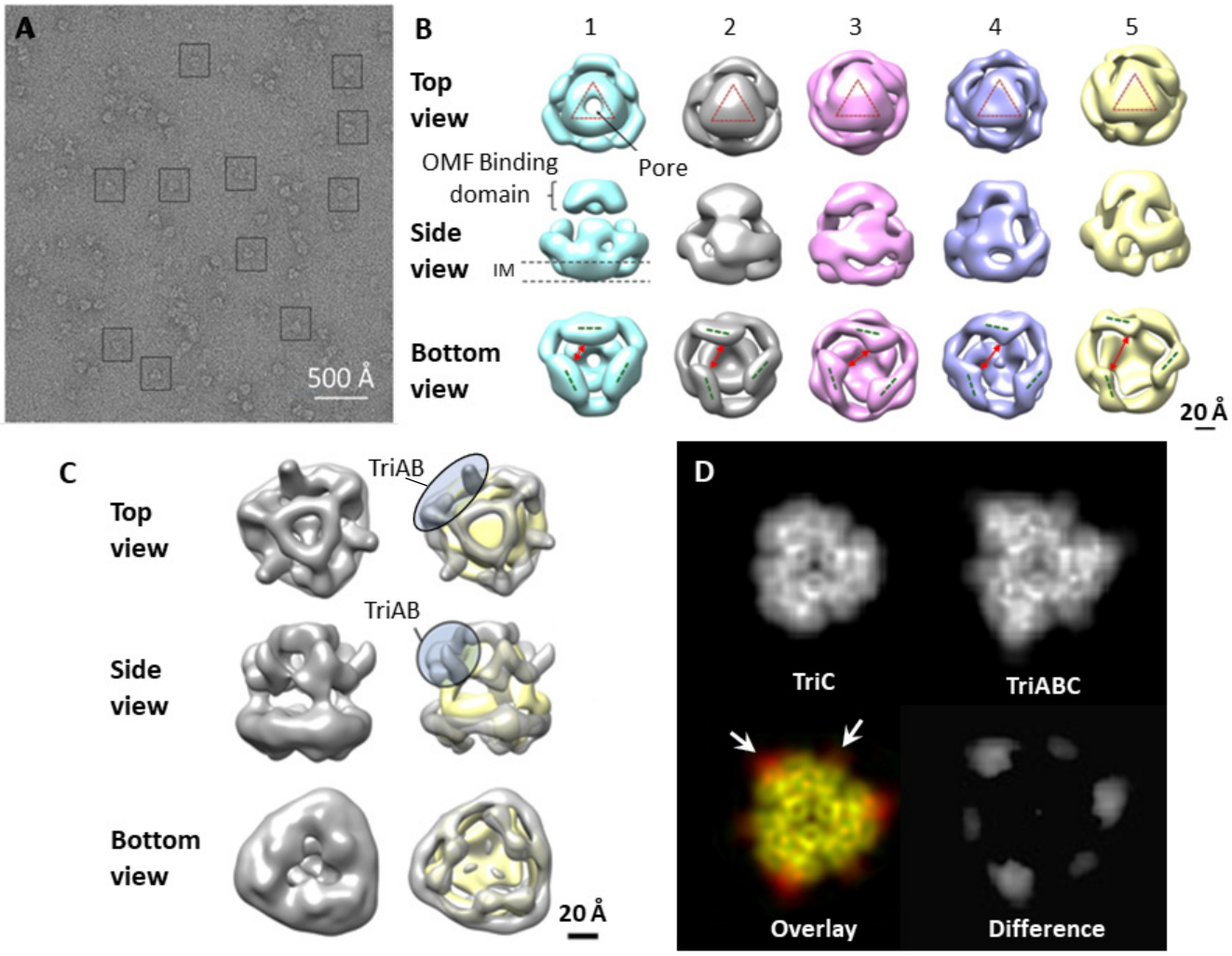

Figure S1, *Related to Figure 2*. Low resolution structure of the purified TriC.

A. Negative stain EM image of purified TriC.

B. Five different negative staining density maps of TriC, C3 averaged and calculated by Maximum-likelihood three-dimensional classification (ML3D), were obtained with 3370, 3500, 4836, 6217 and 2992 particles (volumes 1 to 5 respectively). The isosurfaces are shown after alignment of the OMF binding domain, which has a characteristic triangular shape) at a threshold of 330A^3. All TriC complexes are approximately 110 Å in height and width.

C. Negative staining structure of purified TriABC in grey and its overlay with the TriC structure (average of all classes) in yellow. Putative density for TriAB is shown highlighted.

D. Comparison of top view class averages of TriC and TriABC (cryo-EM data of ~80,000 particles of TriC of exclusively top views). In this overlay, TriABC is colored in red and with TriC

superposed in yellow. The difference density was interpreted as the location of the TriAB MFPs. The TriC top view was aligned to the TriABC top view using SPARX (Hohn et al., 2007). The colored overlay was prepared with ImageJ (Schneider et al., 2012).

The figure was prepared using Chimera (Pettersen et al., 2004).

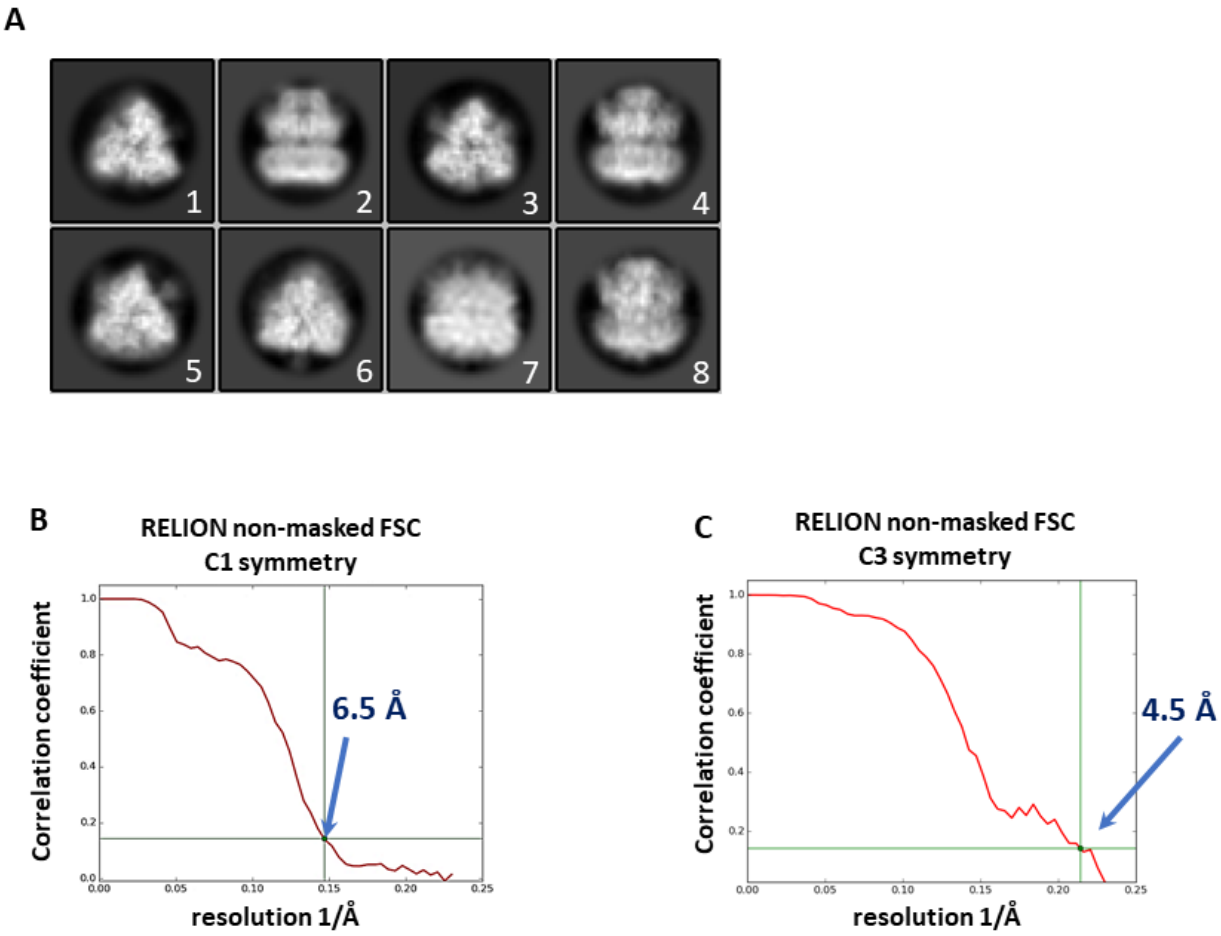

Figure S2, *Related to Figure 2*. 2D classification of TriABC particles and Gold-standard FSC of cryo-EM density maps

A) Cryo-EM reference-free 2D class averages of TriABC in DDM, showing periplasmic domains and detergent corona. Note the weak density features clearly projecting into the periplasm in sub-panels 3, 5 and 7.

B) Gold-standard Fourier shell correlation (FSC) curve of the C1 cryo-EM density map.

C) Gold-standard Fourier shell correlation (FSC) curve of the C3 cryo-EM density map after three-fold symmetry averaging.

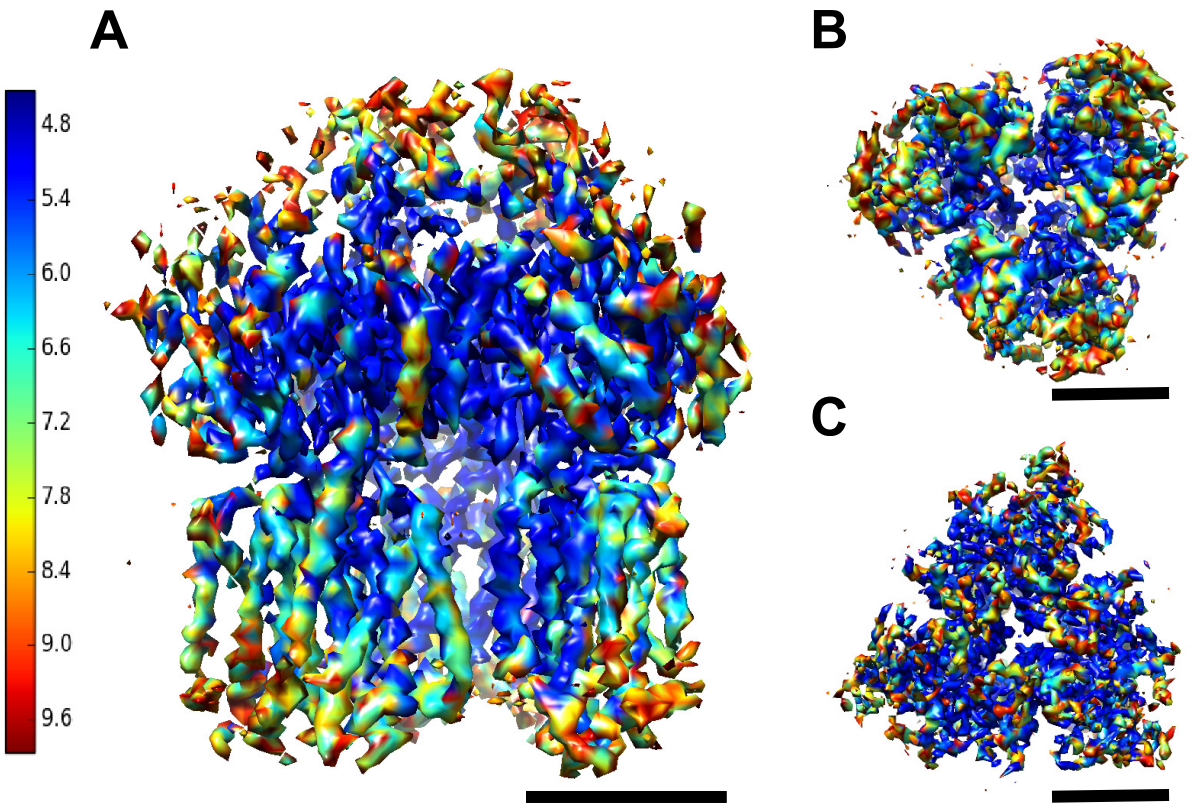

Figure S3, *Related to Figure 2*: Local resolution of the cryo-EM C3 map of TriABC. Local resolution was estimated using Monores (Vilas et al., 2018). A mask was generated in Chimera at a threshold ~ 4.0 σ with smooth 2 pixels borders. Mask and map were used as inputs for the Monores Protocol inside SCIPION (de la Rosa-Trevin et al., 2016). Side (A), top (B) and bottom views (C) are shown. Scale bar, 50Å.

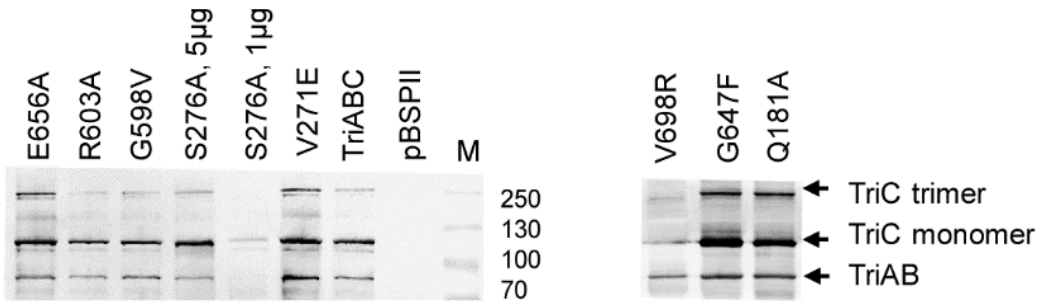

Fig. S4, *Related to Figure 8*. Expression of TriABC and its TriC variants in *E. coli* Δ9-Pore cells. Exponential cells were induced with 1 mM IPTG for five hours, total membrane fractions isolated by ultracentrifugation and analyzed by immunoblotting with anti-TriC polyclonal antibody (Weeks et al., 2015). TriAB is also seen in these immunoblots because of the cross-reactivity of the polyclonal antibodies (Weeks et al., 2015).

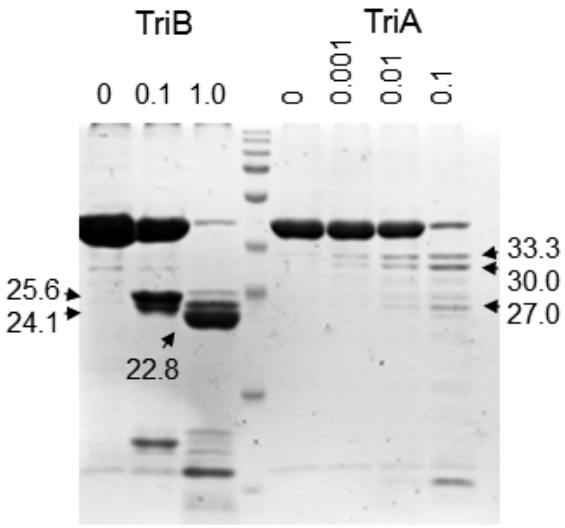

Fig. S5, *Related to Figure 8*. Tryptic digest of the purified TriA and TriB proteins. Proteins were purified by metal affinity chromatography as described (Weeks et al., 2015). Proteins (3-5 μg/mL) were mixed with trypsin (Molecular Biology grade, Sigma) in the concentrations indicated at the top of gel (μg/mL) and incubated at 37°C for 30 min. Proteolytic fragments were separated on 16% SDS-PAGE and stained with Coomassie Brilliant Blue. The same samples after separation by SDS-PAGE were transferred onto PVDF membrane, stained and the indicated bands were cut-out from the membrane and processed for the N-terminal sequencing. The molecular masses in kD of the identified bands are shown.

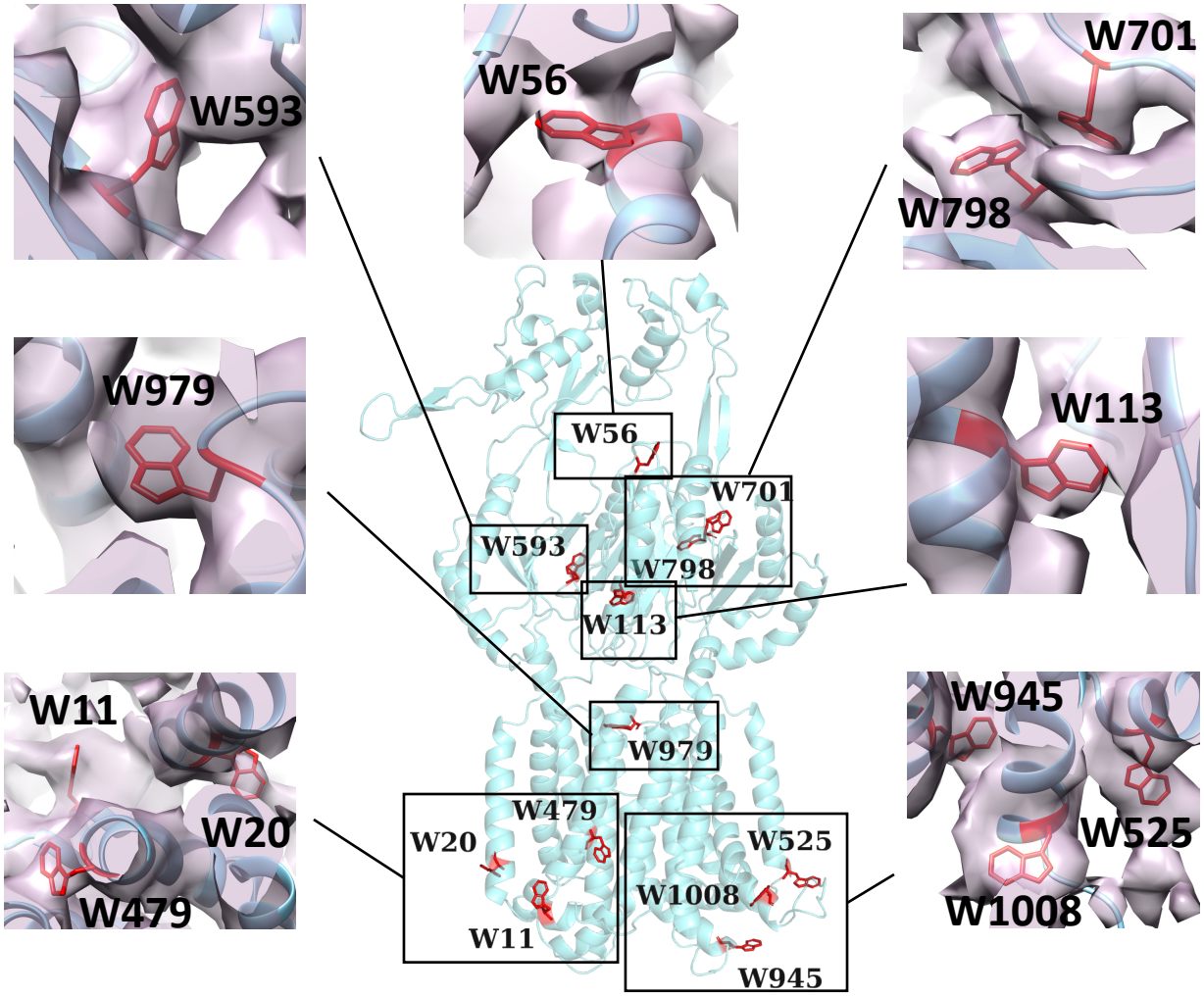

Fig. S6, *Related to Figures 2-3.* Regions of the density map showing Trp residues of the folded TriC polypetide sequence. Bulky side chains of the Trp residues of TriC were unambiguously associated with EM density using a 3σ cutoff (using UCSF Chimera). The cartoon of TriC model is shown in pale blue colors (using Pymol) and side chains of Trp residues are displayed in red. Map densities are shown as light pink isosurfaces. Viewing orientations were slightly changed for display clarity.