Dissection of protonation sites for antibacterial recognition and transport in QacA, a multi-drug efflux transporter

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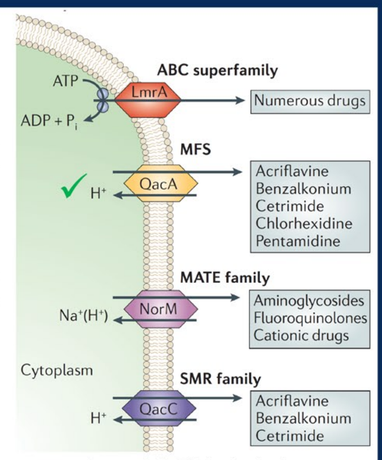
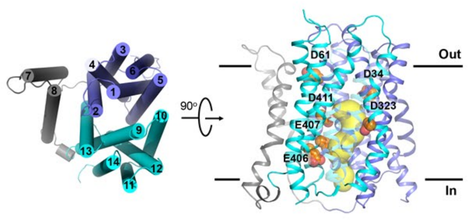
Introduction

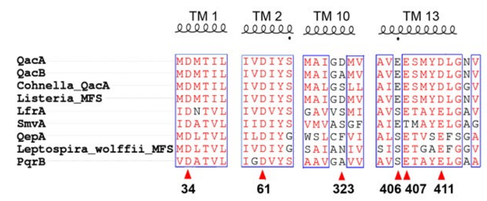
Integral membrane transporters of major facilitator superfamily mediate proton driven multi-drug efflux in pathogenic organisms. The current study aids in understanding QacA, a drug:H^+ antiporter that mediates antibacterial efflux in community and hospital associated strains of methicillin resistant *Staphylococcus aureus* and displays an extensive ability to transport a diverse array of lipophilic cations. H^+-driven transport depends on protonation of acidic residues in the binding pocket which then serve as sites for direct substrate binding or have indirect effects, facilitating transport. We dissect the roles of individual acidic residues in the drug binding vestibule of QacA and identify them as being either essential for substrate recognition and transport or as sites that get protonated and aid in the transport process.

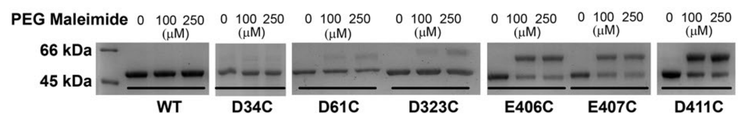


Laura J. V. Piddock *et. al*
Nature Reviews Microbiology (2006)

Homology model of QacA

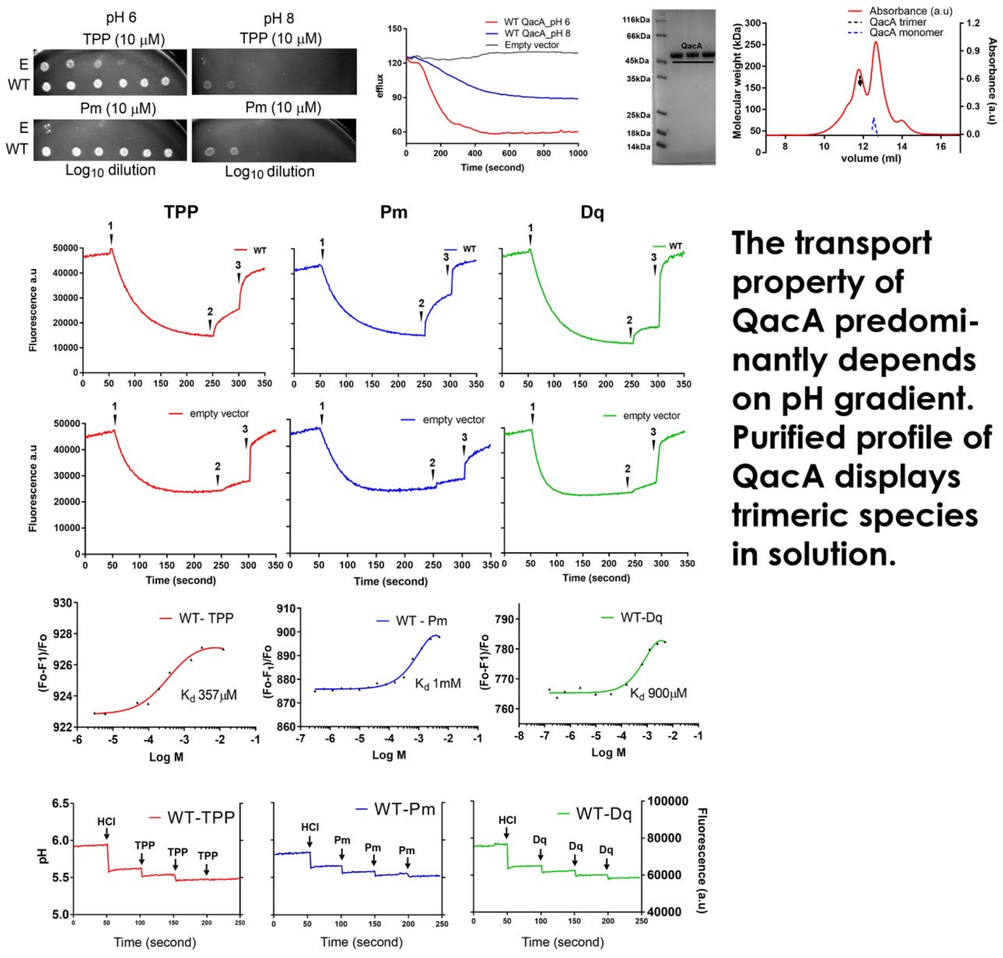






Six acidic residues were identified in the vestibule of QacA. Among these residues, D34, D61, E407 and D411 are highly conserved across different species. PEG-MAL accessibility of D411C, E407C and E406C supports inward-open conformation of the transporter in solution.

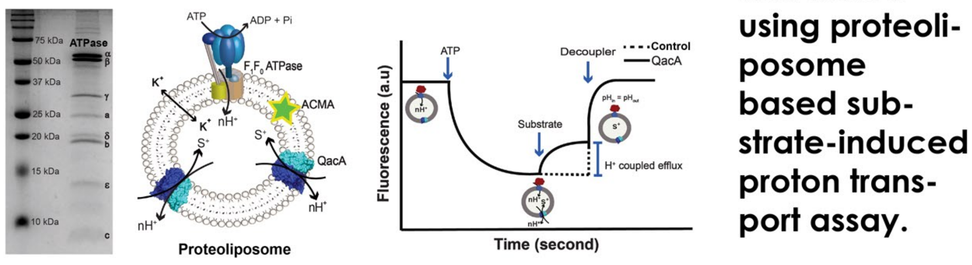
Functional characterisation of WT-QacA



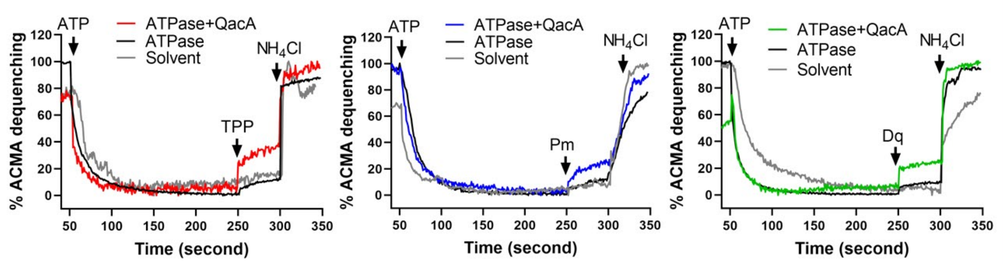
The transport property of QacA predominantly depends on pH gradient. Purified profile of QacA displays trimeric species in solution.

Inside-out vesicle based proton release assay indicates WT-QacA is transport active in membrane environment. Higher μM to mM K_d values were determined by binding assay with the substrates tested. Substrate induced proton release assay displays substrates were recognized by protonatable residues and compete out H^+.

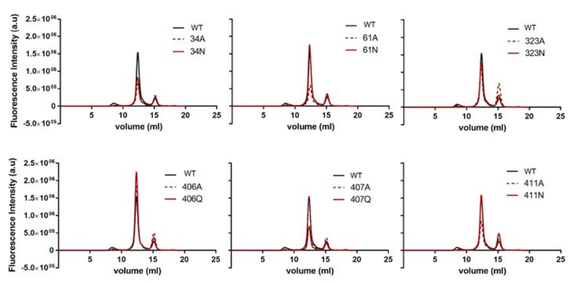
Purified QacA is reconstitutively active

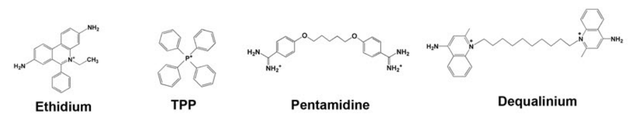


The activity of purified QacA was tested using proteoliposome based substrate-induced proton transport assay.

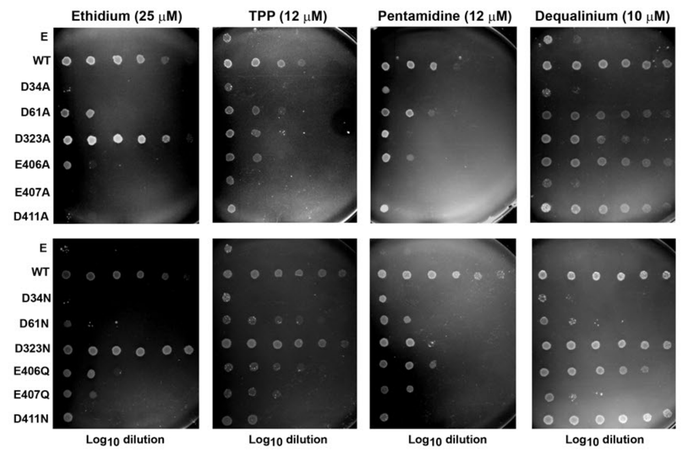


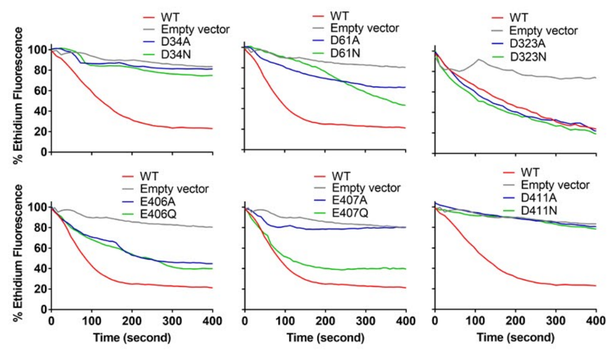
Residues required for transport activity



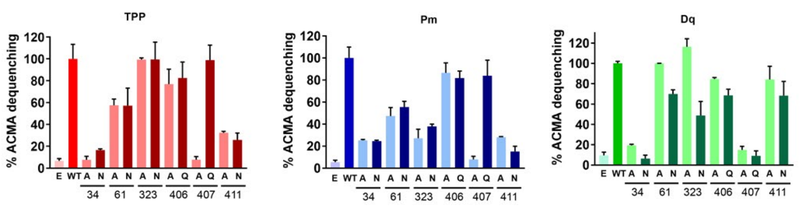


Active site mutants of QacA are well expressed and display homogeneous protein profile. Survival assay indicates D34, D323, E407 and D411 are crucial for efflux of some of the antibacterial compounds tested. Whole cell based ethidium efflux assay displays the crucial role of D34 and D411 in ethidium transport. It was also observed that permanent protonation mimicking substitution at E407 turns the transporter active.



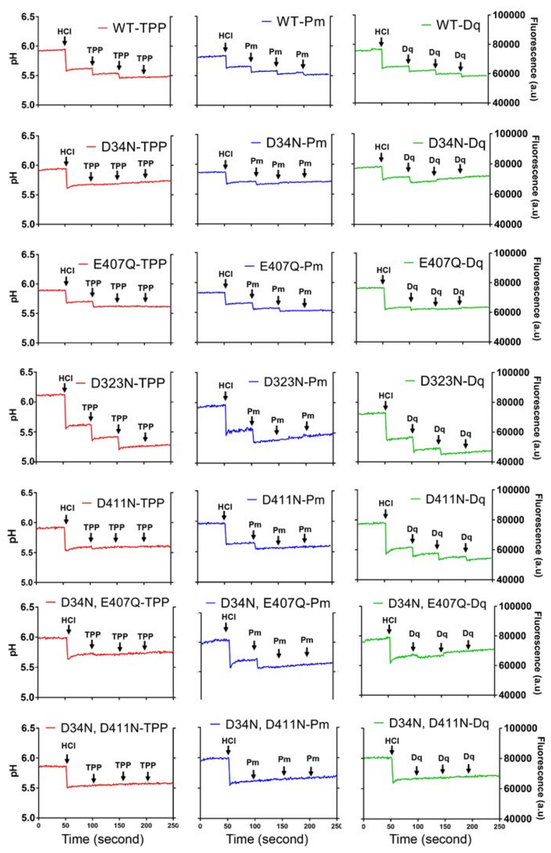


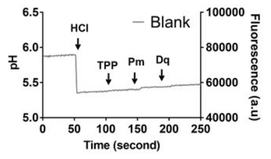
Substrate driven proton release assay using inside-out vesicles indicates D34 and D411 are essential for transport process of QacA. Protonation at E407 is required to facillitate efflux. The requirement of E407 in case of Dq transport instead of D411 displays subtrate level promiscuity. D323 is specifically needed for Pm efflux, but not for all the divalent cations.



Substrate recognition sites in QacA

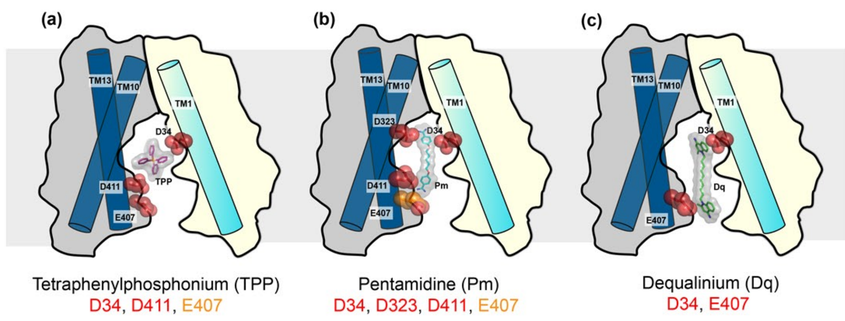
mutants	ligand	K_d (mM)
WT	TPP	0.36 ± 0.07
D34N	TPP	-
D61N	TPP	0.93 ± 0.10
D323N	TPP	0.30 ± 0.05
E406Q	TPP	0.93 ± 0.10
E407Q	TPP	1.65 ± 0.48
D411N	TPP	-
WT	Pm	1 ± 0.17
D34N	Pm	-
D61N	Pm	0.90 ± 0.20
D323N	Pm	-
E406Q	Pm	1 ± 0.13
E407Q	Pm	0.58 ± 0.2
D411N	Pm	-
WT	Dq	0.90 ± 0.21
D34N	Dq	-
D61N	Dq	0.79 ± 0.17
D323N	Dq	0.50 ± 0.10
E406Q	Dq	0.87 ± 0.3
E407Q	Dq	-
D411N	Dq	0.71 ± 0.2





Binding assays and substrate-induced proton release assay displays D34 and D411 are the substrate recognition sites. In case of Dq E407, instead of D411, is the secondary recognition site. D323 is required for Pm binding.

Conclusion



- **A homology model of QacA with 14 TM helices was built and used to test the discrete roles of acidic residues within the vestibule in substrate recognition and H+-coupled antibacterial efflux.**
- **Four out of six residues identified in the binding pocket play essential or conditional roles in the transport of diverse substrates.**
- **Acidic residues in the cytosolic half of TM13 (E407, D411) are important for promiscuous substrate recognition and enhancing the H+:drug stoichiometry during translocation.**

Acknowledgements.
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Majumder et. al., *J. Mol. Biol* (2019)