How pathogens detox: the bacterial antibiotic efflux system protein TolC.

Many bacteria 'detox' by actively pumping toxic molecules, including some antibiotics, across the cell membrane out of the cytoplasm using proteins called efflux pumps. This mechanism is medically important as it can contribute to antibiotic resistance. Gram-negative bacteria, including pathogenic *Salmonella, Escherichia,* and *Shigella* species, possess a further outer membrane across which toxins must be transported to get them out of the cell. To achieve this, these bacteria use a protein called TolC, which acts as a channel spanning both the periplasmic space (the region between the two membranes) and the outer membrane (Figure 1). TolC (PDB 1ek9 view-1) is a tube-shaped assembly, inserted in the outer membrane and open to the environment. It projects into the periplasmic space and contacts the inner membrane.

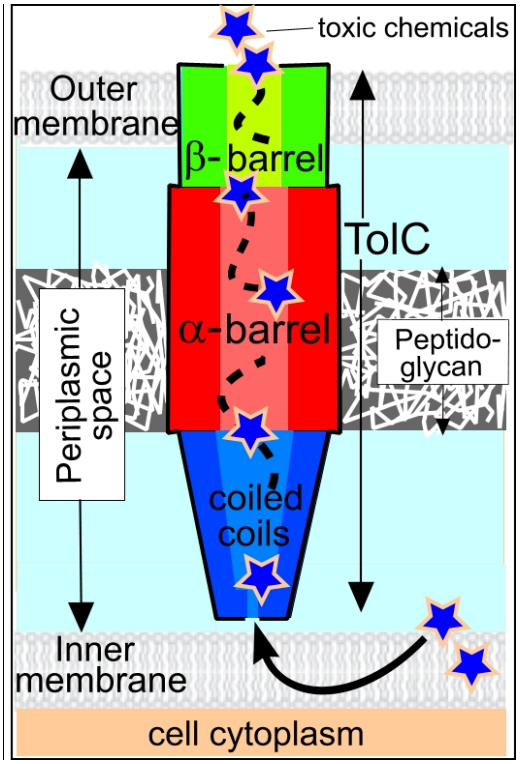

Fig. 1. Figure 1: TolC is a hollow structure that allows toxic chemicals to migrate to the exterior. A curved arrow shows how an associated pump could deliver toxic compounds.

Spot the joins.

TolC is made up of three identical protein subunits, of more than 400 amino acid residues each, that assemble in the outer membrane. (View-1) shows how the subunits pack together to form one continuous tube.
The TolC tube consists of three distinct structural sections (shown schematically in Figure 1 and view-1). At one end of the tube, a β-barrel (green) crosses the outer membrane, the central section is an α-helical barrel (red) and the final section consists of α-helices twisting together like the blades of a camera iris in an arrangement known as a coiled coil (blue). Over most of its length, the tube has an inner diameter of over 20Å, wide enough to allow quite large molecules to flow through, but the coiled-coil section tapers to an inner diameter of less than 5Å at its end (view-2). This narrowing prevents leaking out of molecules that are larger than small ions or water, and molecules from the environment getting in. Although there is evidence that TolC connects directly with one of a number of efflux pumps located in the inner membrane, the available structures in the PDB are of the unconnected 'closed' forms.

Closing in on Crick's coiled-coil concept.

The coiled-coil section reduces the diameter of the channel and so prevents TolC from acting as an open conduit between the periplasm and the environment. The coiled-coil domain shown in view-2 is constructed from six pairs of α-helices, two from each protein chain. Figure 2 shows how side-chains pack between the α-helices in each pair. Such packing is thermodynamically most stable when the residues involved are hydrophobic. Optimal packing of this hydrophobic core favours an overall twist to each pair of α-helices which can be seen clearly in TolC (view-2). Coiled coils, a common packing arrangement in α-helical proteins, were first predicted by Francis Crick (of DNA fame) back in 1953 (ref. 1).

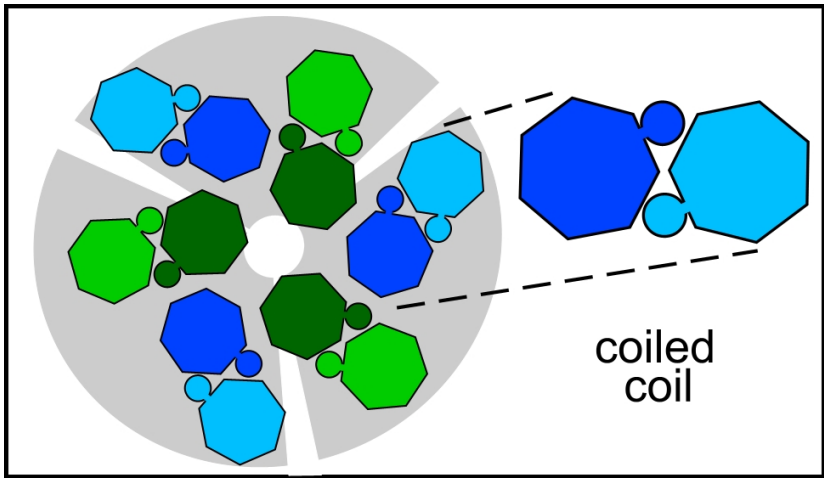

Figure 2: Cross-section of the coiled-coil region. Each subunit contributes two pairs of helices (light and dark blue/green). Side-chains packing shown in inset.

Untwisting a coiled coil to get an α-helical barrel.

The central section of the TolC structure crosses the periplasm and consists of a barrel-like arrangement of 12 α-helices - four from each subunit (view-3). Unlike the coiled-coil section, these long α-helices are arranged with their axes parallel and untwisted. But if a coiled coil is the most stable arrangement, how is the parallel assembly stabilised?
In the coiled-coil section of TolC, the packing of side-chains holds the α-helices together and causes them to twist around each other. In an α-barrel, packing of side chains also holds the helices together but in a different arrangement. Here, the side chains towards the inside of the barrel tend to be small and those on the outside tend to be larger (Figure 3) (ref. 2). This asymmetry straightens the helices so that they stay parallel rather than coil around each other and also favours a cylindrical architecture, maintaining a constant diameter of the barrel (view-3).

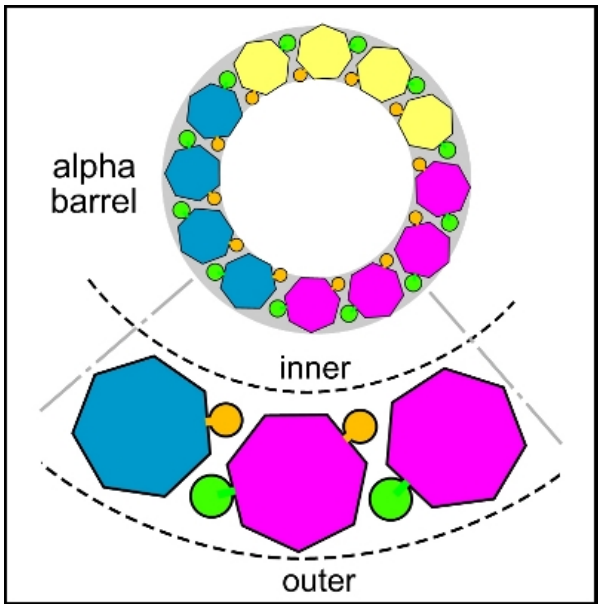

Figure 3: Side-chain packing between helices in the α-barrel. Helices are shown in cross-section as seven-sided shapes. Side chains towards the centre are smaller than those on the outside.

The β-barrel - like a porin, except it's a "pore out".

The β-barrel section of TolC spans the barrier that is the outer membrane. It consists of 12 anti-parallel β-strands, four from each subunit (view-4). Anti-parallel β-strands were described in an earlier Quips. β-barrels are commonly found in proteins that span the bacterial outer membrane. The porin family of outer-membrane proteins uses this motif but the porin β-barrel is formed from only one protein chain, whereas in TolC there are three. Porins only facilitate passage of molecules across the outer membrane whilst TolC transports molecules across both the periplasm and the outer membrane.

Key interactions keep TolC shut.

The TolC coiled-coil section is critical to its function. This section forms the periplasmic entrance at the inner membrane. It is here that TolC connects to an efflux pump, which spans the inner membrane and pumps small molecules into TolC, ready for transport out of the cell.
In the absence of an efflux pump, however, TolC must constrict to prevent the periplasmic contents from leaking out. A key set of side chains at the end of the coiled-coil section interact via hydrogen bonds (H-bonds) to form the constriction where the diameter of the tube is reduced to less than 5Å (view-5). This H-bond network forms only if the coiled coils from all three subunits fit together to give a 'closed' state.
To assess the importance of these H-bonds, specific mutations have been introduced that disrupt the H-bond network. Removing one hydrogen bond (by mutating Arg367 to Ser) relaxes the twist of the coils slightly (PDB entry 2wmz). A larger effect is seen when two hydrogen bonds are removed (by mutating Arg367 to Ser/Glu combined with Tyr362 to Phe) (PDB entry 2vde). These mutations widen the radius of the pore by 1.5Å, in an iris like movement (ref. 3) (view-6). This widening increases the flow of small ions through the

pore, an effect that can be measured for the isolated TolC mutants. The mutated forms remain functionally active and couple with inner-membrane efflux pumps to transport toxic chemicals from the cell (ref. 4).
In order for toxic chemicals to enter TolC, the coiled coil iris needs to open further than observed in these mutated forms and it has been proposed that interaction with the efflux pump causes this opening (ref. 5). However, this has yet to be confirmed by a structure of the complex.

Further exploration.
Your first view of any structure from the PDB is afforded by a set of carefully chosen images, conveying key features of the structure, on its PDBe summary page. The images for the TolC structure 1ek9 are explained in the mini tutorial that accompanies this Quips.

Acknowledgement

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References

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Ref. 2 Calladine CR *et al.* (2001) How to untwist an alpha-helix: structural principles of an alpha-helical barrel. *J. Mol. Biol.* **305** 603-18.

Ref. 3 Pei *et al.* (2011) Structures of sequential open states in a symmetrical opening transition of the TolC exit duct. *Proc Natl. Acad. Sci. U S A.* **108** 2112-7.

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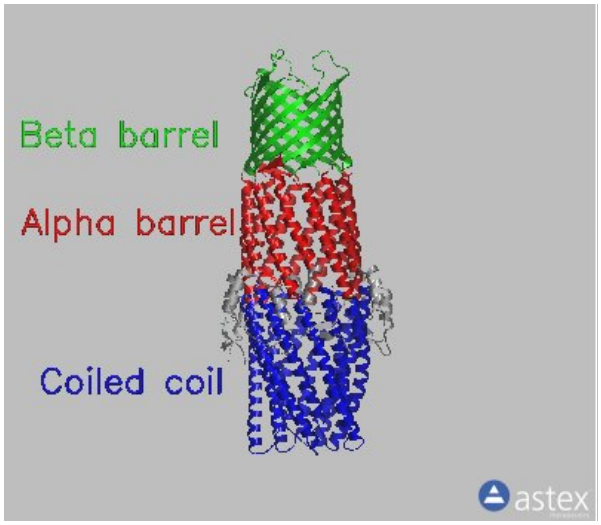
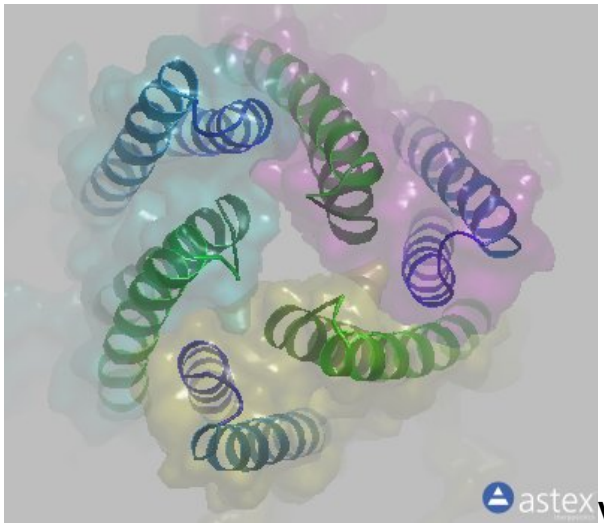
Ref. 5 Koronakis V *et al.* (2001) Crystal structure of the bacterial membrane protein TolC central to multidrug efflux and protein export. *Nature* **405** 914-9.

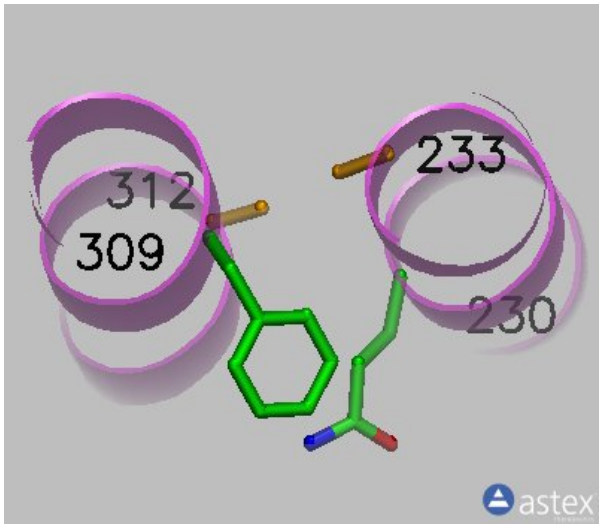
Views



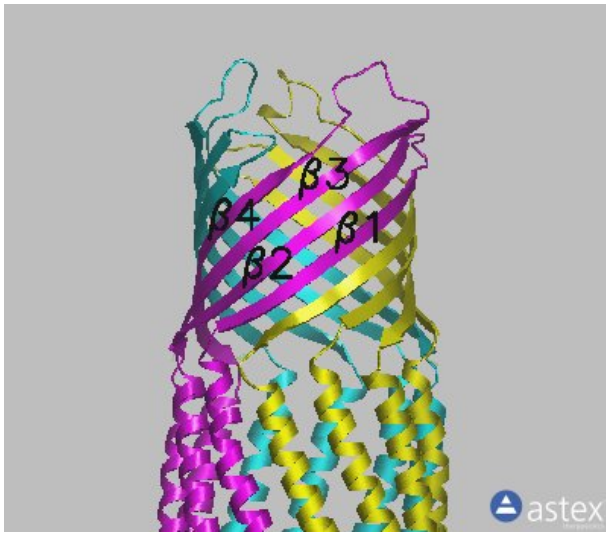
View 1: Trimeric construction of the TolC tube. Initially, each subunit is shown in a different colour (magenta, yellow, cyan). Then, the individual sections of TolC are shown in green, red and blue. The grey sections are not part of these domains.



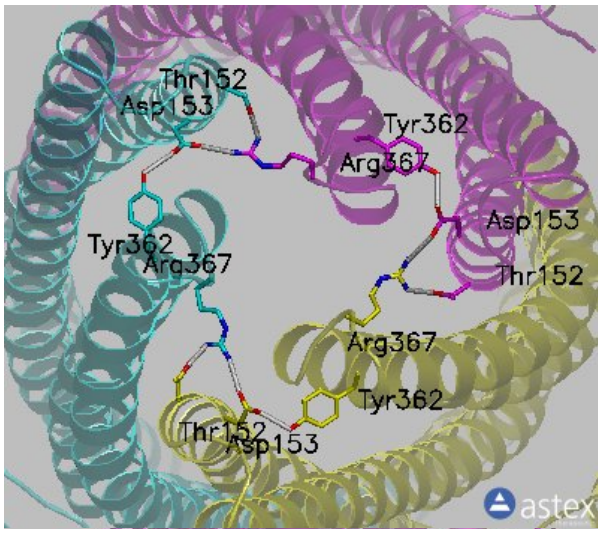
View 2: Coiled coils close off the inner membrane end of TolC. Four helices from each subunit (coloured blue, light blue, green and light green) twist together to reduce the diameter of the tube. Each subunit is shown as a surface (magenta, yellow, cyan).



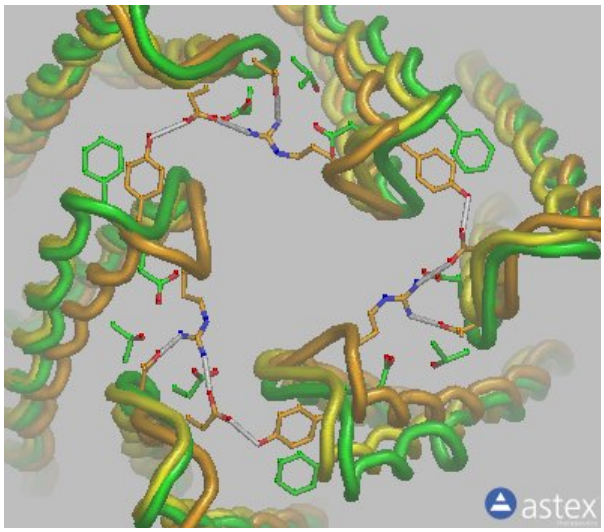
View 3: Alpha-helical barrel section of TolC. The barrel wall is formed from 12 parallel α-helices. Small side chains (orange) are located on the inside of the barrel and larger sidechains (green) are located on the outside.



View 4: TolC β-barrel. Twelve anti-parrallel β-strands, 4 contributed by each of the three subunits (shown in magenta, yellow, cyan) form the β-barrel which is over 20Å in diameter.



View 5: The bottom end of the coiled-coil section. Interacting residues (Arg367, Tyr362, Asp153, and Thr152) are shown as sticks and the hydrogen bonds as white lines.



View 6: Mutant forms of TolC widen the barrel opening at the end of the coiled-coil section. The wild-type structure is shown in orange and superimposed on the structures of the Arg367Ser mutant (yellow) and Arg367Ser, Tyr362Phe double mutant (green).