

SUPPLEMENTARY ONLINE DATA Characterization of a pre-export enzyme–chaperone complex on the twin-arginine transport pathway

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1	MNNNDLFQAS	RRRFLAQLGG	LTVAGMLGPS	LLTPRRATAA	QAATDAVISK	EGILTGSHWG
61	AIRATVKDGR	FVAAKPFELD	KYPSKMIAGL	PDHVHNAARI	RYPMVRVDWL	RKRHLSDTSQ
121	RGDNRFVRVS	WDEALDMFYE	ELERVQKTHG	PSALLTASGW	QSTGMFHNAS	GMLAKAIALH
181	GNSVGTGGDY	STGAAQVILP	RVVGSMEVYE	QQTSWPLVLQ	NSKTIVLWGS	DLLKNQQANW
241	WCPDHDVYEY	YAQLKAKVAA	GEIEVISIDP	VVTSTHEYLG	REHVKHIAVN	PQTDVPLQLA
301	LAHTLYSENL	YDKNFLANYC	VGFEQFLPYL	LGEKDGQPKD	AAWAEKLTGI	DAETIRGLAR
361	QMAANRTQII	AGWCVQRMQH	GEQWAWMIVV	LAAMLGQIGL	PGGGFGFGWH	YNGAGTPGRK
421	GVILSGFSGS	TSIPPVHDNS	DYKGYSSTIP	IARFIDAILE	PGKVINWNGK	SVKLPPLKMC
481	IFAGTNPFHR	HQQINRIIEG	LRKLETVIAI	DNQWTSTCRF	ADIVLPATTQ	FERNDLDQYG
541	NHSNRGIIAM	KQVVPPQFEA	RNDFDIFREL	CRRFNREEAF	TEGLDEMGWL	KRIWQEGVQQ
601	GKGRGVHLPA	FDDFWNNKEY	VEFDHPQMFV	RHQAFREDPD	LEPLGTPSGL	IEIYSKTIAD
661	MNYDDCQGHP	MWFEKIERSH	GGPGSQKYPL	HLQSVHPDFR	LHSQLCESET	LRQQYTVAGK
721	EPVFINPQDA	SARGIRNGDV	VRVFNARGQV	LAGAVVSDRY	APGVARIHEG	AWYDPDKGGE
781	PGALCKYGNP	NVLTIDIGTS	QLAQATSAHT	TLVEIEKYNG	TVEQVTAFNG	PVEMVAQCEY
841	VPASOVKS					

Figure S1 The E. coli TorA precursor primary sequence

The points of natural proteolysis, and deliberate truncation, of the TorA precursor are highlighted. The 39-residue N-terminal twin-arginine signal peptide is highlighted in red. The position of the engineered initiation site for the construct expressing the signal-less TorA protein is indicated by the black arrow. The location of the experimentally determined trypsin-cleavage site for TorA within the TorA–TorD^{*His*} complex is indicated by the red arrow. This is also the point of truncation for the TorA_{ACT} protein. A small fraction of the TorA sample is subject to degradation during the purification procedure. Following IMAC, a small fraction of TorA is found to be degraded from the C-terminus and loses the purple-coloured stretch of polypeptide. Following the subsequent SEC step, a small fraction of TorA within the TorA–TorD^{*His*} complex becomes proteolysed at the N-terminus and further at the C-terminus (blue). This shows that the extremities of TorA are not shielded or protected by the tightly bound TorD^{*His*} protein, are exposed to solvent and are therefore susceptible to proteolysis.

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Figure S2 X-ray structure of S. massilia TorA

The C-terminal Domain IV is shaded in blue and the approximate position of the trypsin-cleavage site determined for *E. coli* TorA in the TorA–TorD^{*His*} and TorA_{Δ SP}–TorD^{*His*} complexes is indicated. The image was created using PyMOL (http://www.pymol.org) with PDB code 1TMO.



Figure S3 Comparison of SAXS scattering curves

Theoretical curves were calculated from *S. massilia* TorD (PDB code 1N1C) monomer (blue line) and dimer (red line) and *E. coli* DmsD (PDB code 3CW0) monomer (black line) using CRYSOL and compared with experimental scattering curves of TorD^{His} (green dots).

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Figure S4 SAXS scattering curve of the TorA-TorD^{His} complex

Fitted curve of TorAD^{His} rigid body modelling conducted using the TorA structure (PDB code 1TMO) (split into two components: Domains I–III and Domain IV) and the DmsD structure (PDB code 3CWO), performed using SASREF. The fitted curve is shown in comparison with the *E. coli* TorAD^{His} scattering curve.

Table S1 TMAO reductase activity in strains producing variants of TorA

Crude cell extracts were prepared from anaerobically grown cells before TMAO-dependent Benzyl Viologen-oxidation assays were performed. Results are means \pm S.D. of three measurements.

Strain and plasmid	Complex produced	TMA0 reductase activity (μ mol of Benzyl Viologen oxidized/min per mg of protein)
GB426 (<i>tor</i> ⁻ , <i>dms</i> ⁻) + pQE80 (control) GB426 + pQE80TorADHis GB426 + pQE80TorAdelSSDHis GB426 + pQE80TorAtruncDhis	– TorA/TorD ^{His} TorA _{∆SP} /TorD ^{His} TorA _{∆CT} /TorD ^{His}	$\begin{array}{c} 0.15 \pm 0.01 \\ 28.8 \pm 4.0 \\ 14.8 \pm 1.6 \\ 0.12 \pm 0.01 \end{array}$

Table S2 Characterization of the TorA and TorD proteins by CD

Proportions of secondary-structural elements were calculated from CD analysis for each of the indicated samples.

Protein	Total helix (%)	Total strand (%)	Turns (%)	Unordered (%)
TorA ^{His} (experimental)	26	25	20	29
TorD ^{His} (experimental)	56	7	17	20
TorA ^{<i>His</i>} and TorD ^{<i>His</i>} combined (theoretical)	31	22	19	27
TorA–TorD ^{His} (experimental)	28	22	22	29
TorA _{△SP} -TorD ^{His} (experimental)	20	29	22	30

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