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SUPPLEMENTARY ONLINE DATA

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

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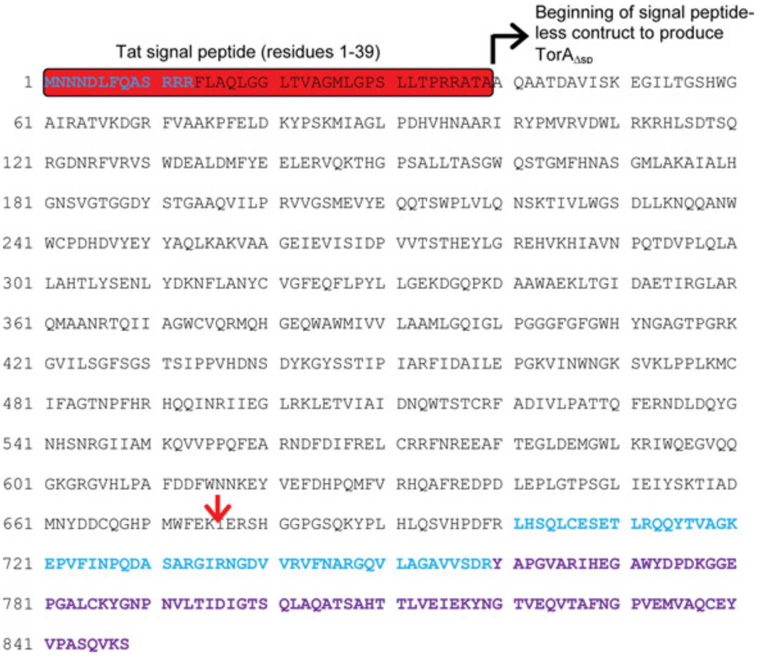

Figure S1 The *E. coli* TorA precursor primary sequence

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$_{\Delta CT}$ 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$_{\Delta SP}$–TorD^{His} complexes is indicated. The image was created using PyMOL (http://www.pymol.org) with PDB code 1TMO.

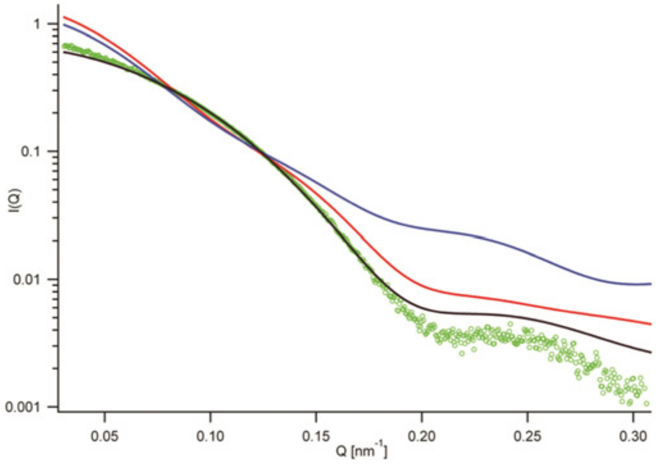

Figure S3 **Comparison of SAXS scattering curves**

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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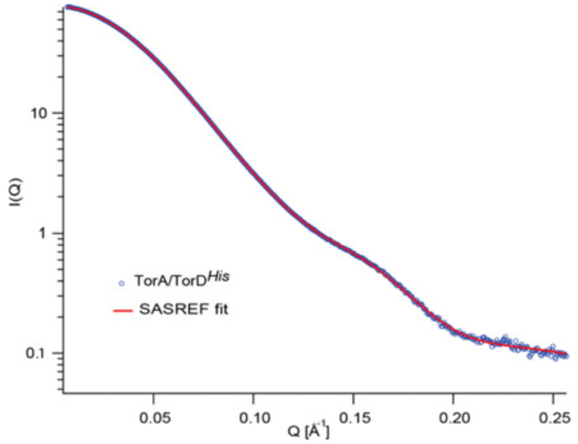

Figure S4 **SAXS scattering curve of the TorA–TorD^{His} complex**

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 3CW0), 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 ± S.D. of three measurements.

Strain and plasmid	Complex produced	TMAO reductase activity (μmol of Benzyl Viologen oxidized/min per mg of protein)
GB426 (*tor*$^-$, *dms*$^-$) + pQE80 (control)	–	0.15 ± 0.01
GB426 + pQE80TorADHis	TorA/TorD^{His}	28.8 ± 4.0
GB426 + pQE80TorAdelSSDHis	TorA$_{\Delta SP}$/TorD^{His}	14.8 ± 1.6
GB426 + pQE80TorAtruncDhis	TorA$_{\Delta CT}$/TorD^{His}	0.12 ± 0.01

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^{His} and TorD^{His} combined (theoretical)	31	22	19	27
TorA–TorD^{His} (experimental)	28	22	22	29
TorA$_{\Delta SP}$–TorD^{His} (experimental)	20	29	22	30