Supplementary Information:

Structural analysis of the interaction between the bacterial cell division proteins FtsQ and FtsB

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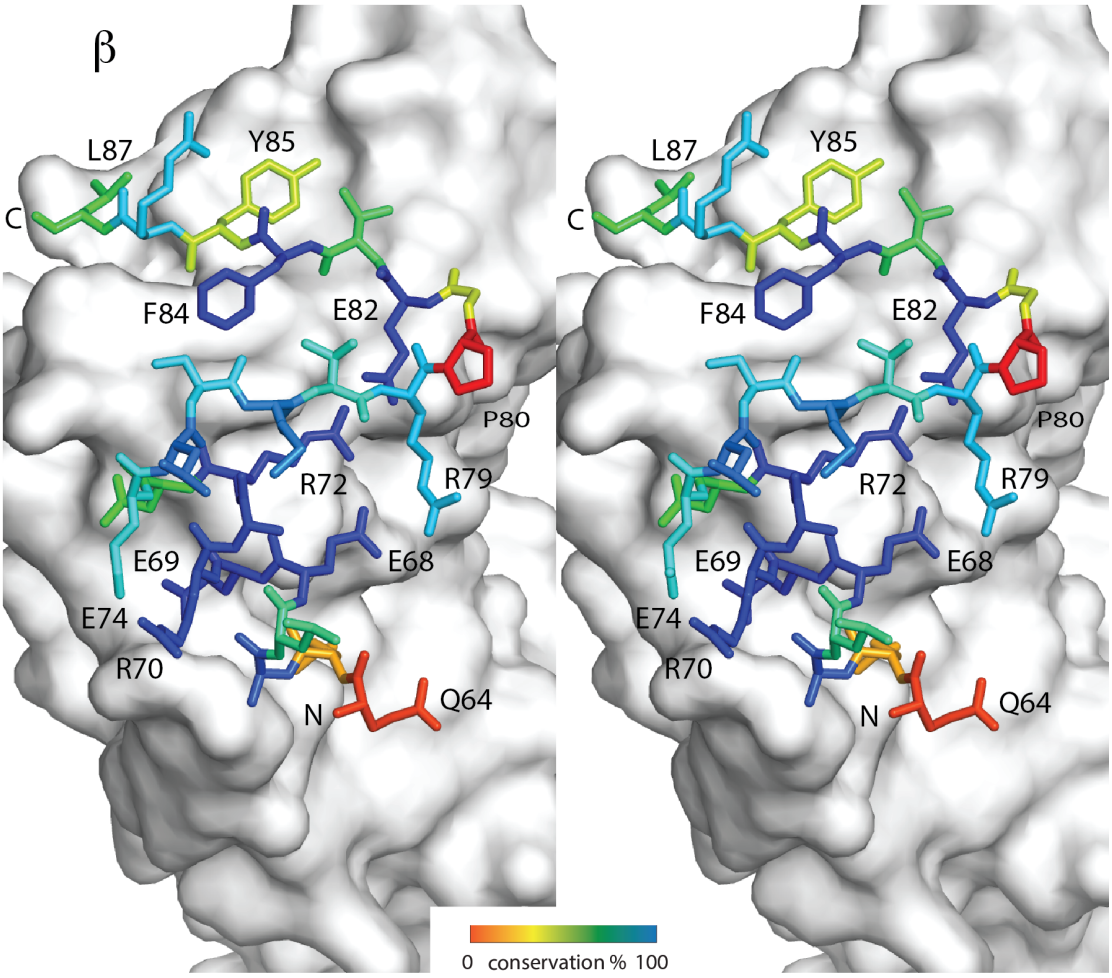
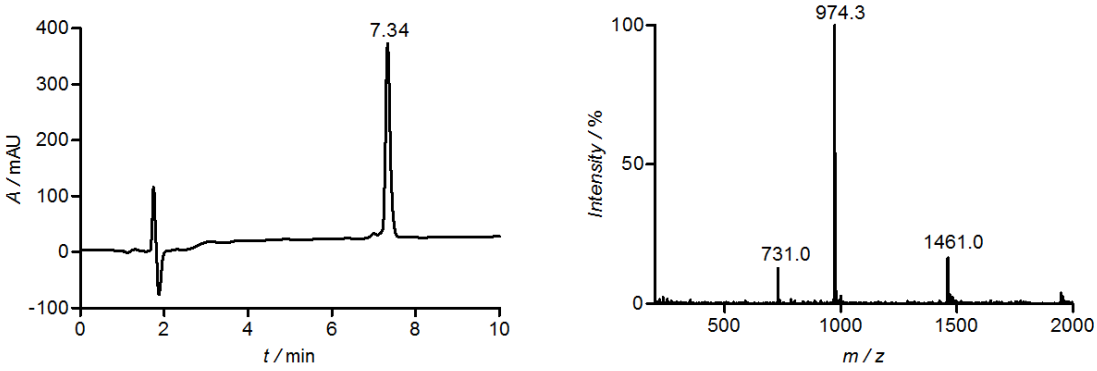
Content:



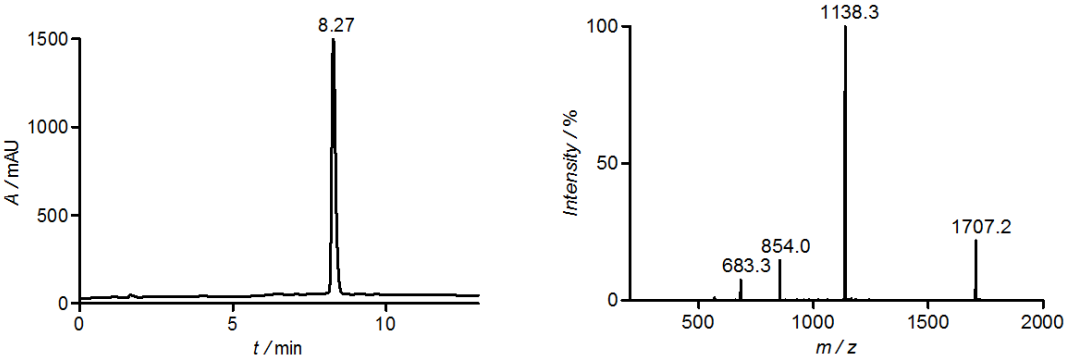
Supplementary Figure S1. Sequence conservation-based colouring of FtsB surface, shown in stereo. Around 100 sequences similar to *E. coli* FtsB (database ID FTSB_ECOLI) were identified using BLAST and sequence conservation was determined from a CLUSTAL OMEGA multiple sequence alignment with CONSURF (http://consurf.tau.ac.il/2016/). FtsB residues 64-87 are shown as a stick model coloured by conservation from blue (most conserved residues in the alignment) to red (least conservation). FtsQ is shown as a grey molecular surface. All residues involved in major interactions with FtsQ are conserved in FtsB, in addition to internal interactions, such as R72 - E82.

N-terminus	formula, sequence	mw [g*mol^{-1}]	calculated m/z	found m/z
Ac	$C_{126}H_{203}N_{39}O_{41,}$ QEALEERARNELS(Nle) TRPGETFYRL-NH_2	2919.5	1460.25 / 973.83 730.63 / 584.7	1461.0 / 974.3 / 731.0
FITC PEG	$C_{151}H_{223}N_{41}O_{48}$ QEALEERARNELS(Nle) TRPGETFYRL-NH_2	3411.6	1706.3 / 1137.87 853.65 / 683.12	1707.2 / 1138.3 854.0 / 638.3

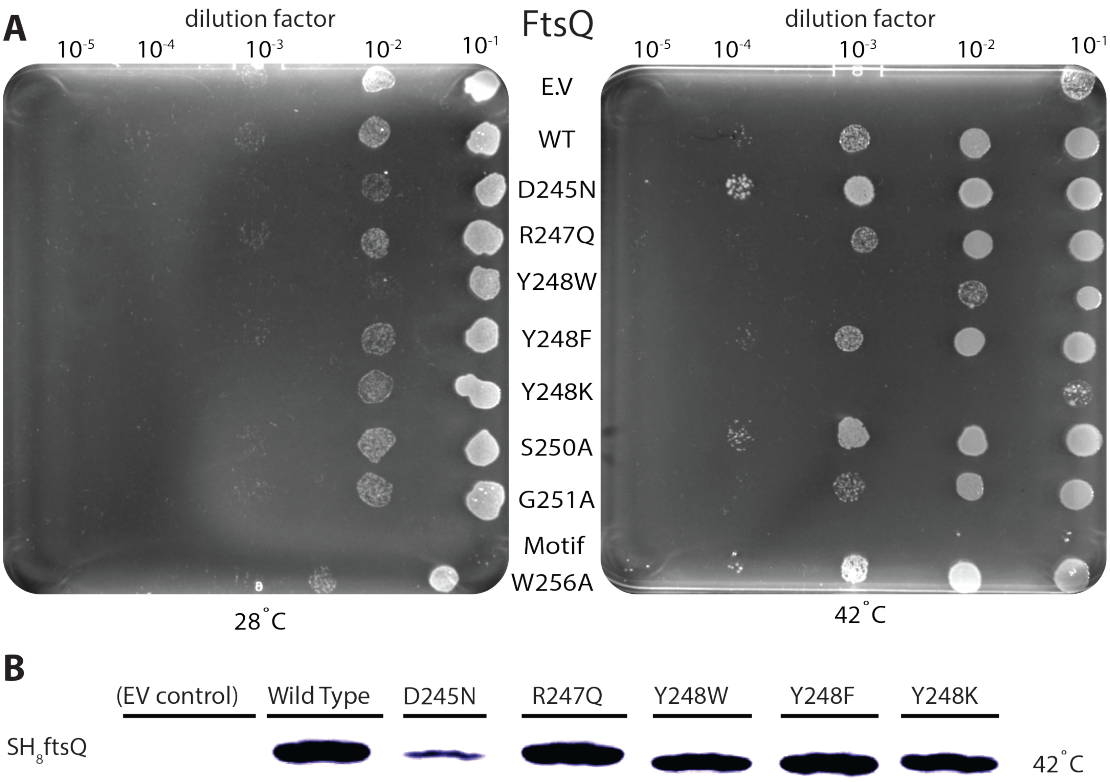
a)



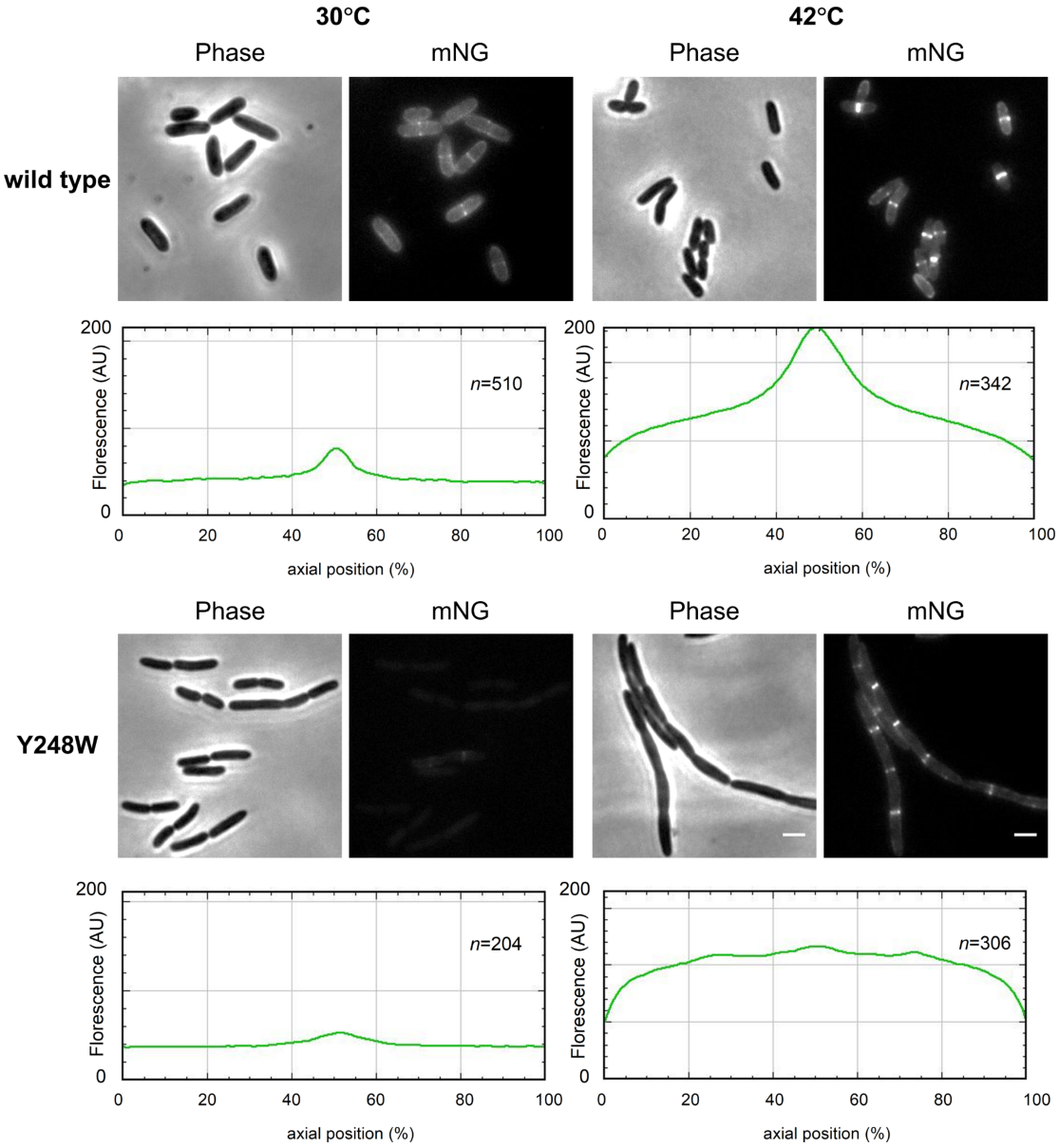
b)



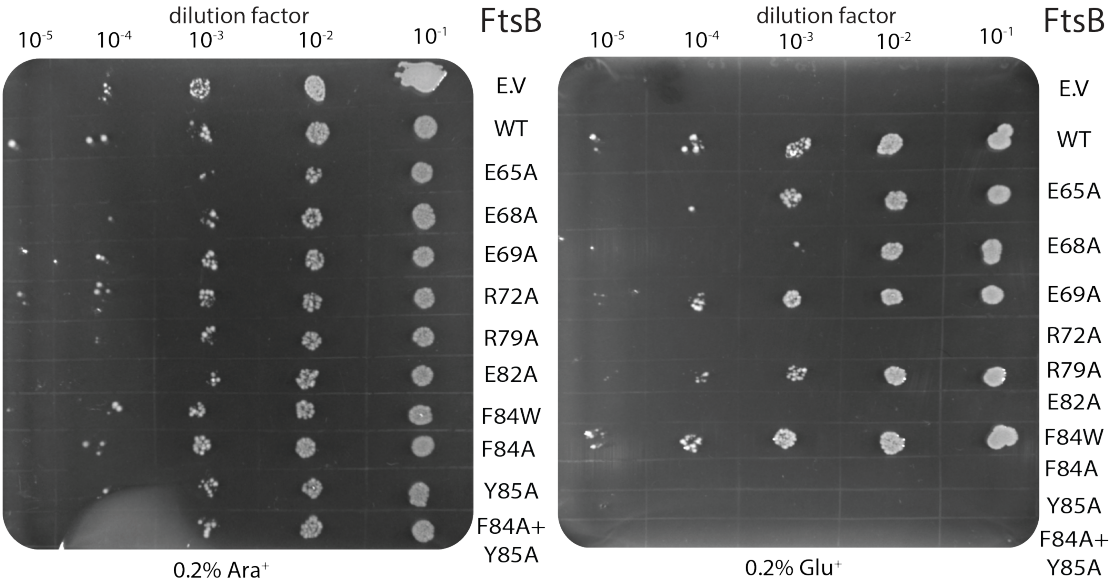
Supplementary Figure S2. Table: peptide characteristics (molecular formula, one-letter code sequence, molecular weight, calculated and detected m/z ratios for multi charged masspec ions ($[M+nH]^{n+}$). Abbreviations: Ac: acetyl; FITC: fluoresceinisothiocyanat fluorophore; PEG: 8-amino-3,6-dioxaoctanoyl linker; Nle: norleucine amino acid replacing methionine 77. **A)** HPLC chromatogram at 210 nm of peptide used for co-crystallisation with FtsQ (Figure 3D), gradient: 20-50 % ACN in 10 min and mass spectra of corresponding peptide. **B)** HPLC chromatogram at 210 nm of FITC PEG peptide for fluorescence polarisation (Figure 3D), gradient: 30-60 % ACN in 10 min, starting at 3 minutes and mass spectra of corresponding peptide.



Supplementary Figure S3. A) Spot assay of FtsQ mutants used in Figure 4A. B) Western blot showing expression levels of FtsQ mutants used in A).

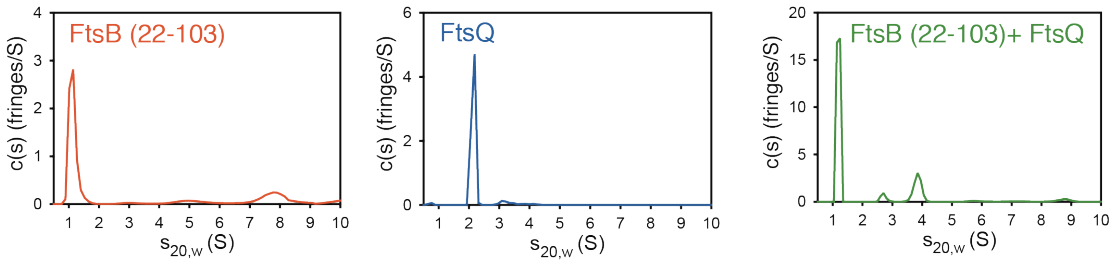


Supplementary Figure S4. Fluorescence microscopy localisation of FtsQ (Y248W) mutant protein in *E. coli* cells. Phase contrast images, fluorescent images and fluorescence profiles per cell (from 0 to 200 AU) plotted against normalised cell length (from 0 to 100 %) of ts FtsQ LMC531 strain expressing wild type FtsQ or FtsQY248W fused to mNeonGreen (mNG) at 30 or 42°C. *n* indicates number of cells. The graphs show the average total fluorescence per cell plotted against the normalised cell length. Filaments have therefore a higher fluorescence per cell. In addition, the expression of the proteins is higher at the non-permissive temperature. Scale bar represents 2 µm.



Supplementary Figure S5. Spot assay showing FtsB mutants used in Figure 4B.

A



B

Protein	SEC-MALS Molar mass (kDa)	AUC Sedimentation coefficient ($S_{20,w}$)	AUC Calculated mass (kDa)
FtsB (22-103) 12.4 kDa	11.4	1.1	11.0
	65.5	3.5	58.6
	119	5.1	104
		8.0	203
FtsQ 26.2 kDa	27.9	2.2	22.9
		3.2	42.3
FtsB+FtsQ 38.6 kDa	21.9	1.2	12.0
		2.71	41
	67.8	3.84	69

Supplementary Figure S6. A) Analytical ultracentrifugation (AUC) of FtsQ, FtsB and in complex. B) Summary table of SEC-MALS (Figure 5A) and AUC data (panel A) describing the properties of FtsQ, FtsB and their complexes.