# FtsA forms actin-like protofilaments

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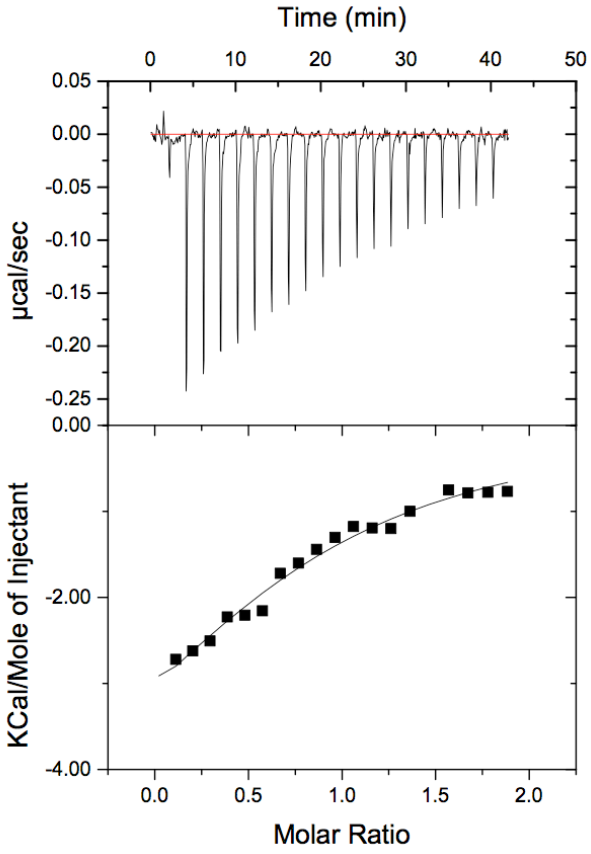
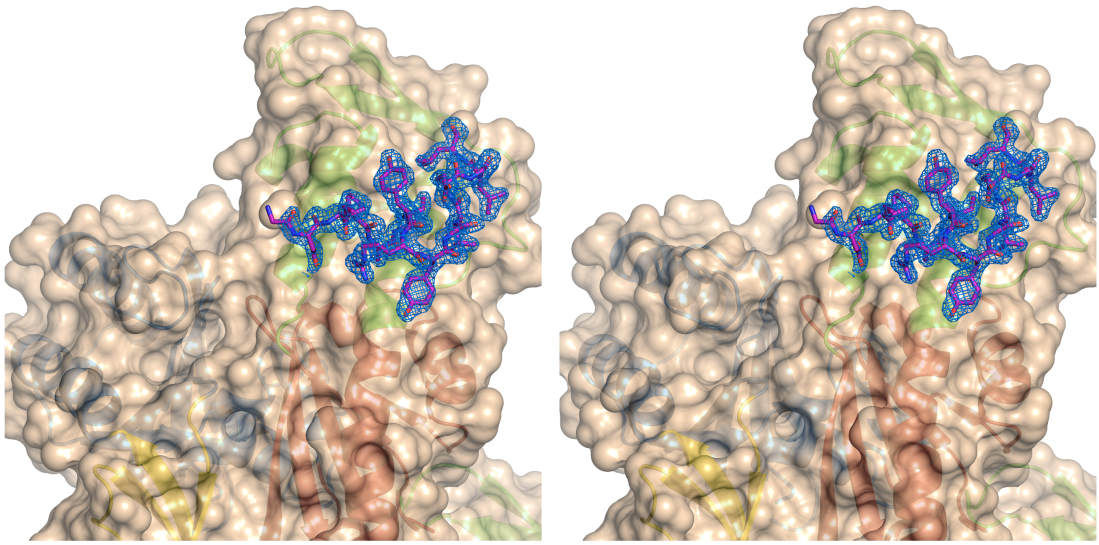
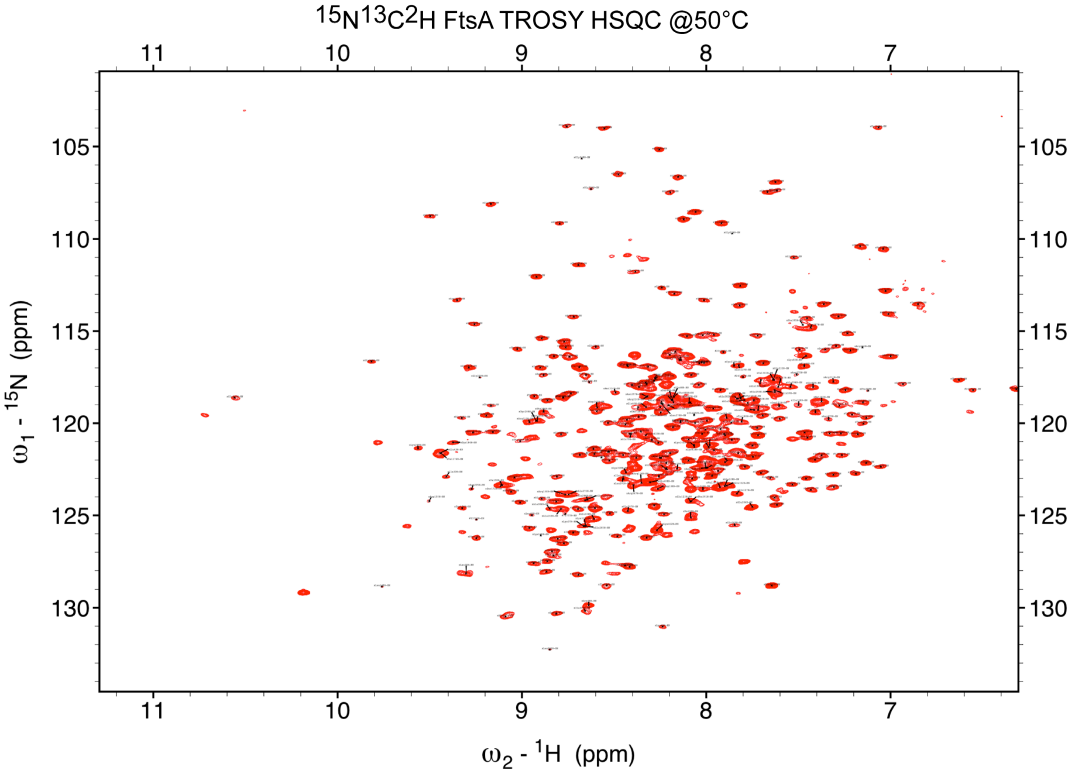
**Supplementary Figures.**



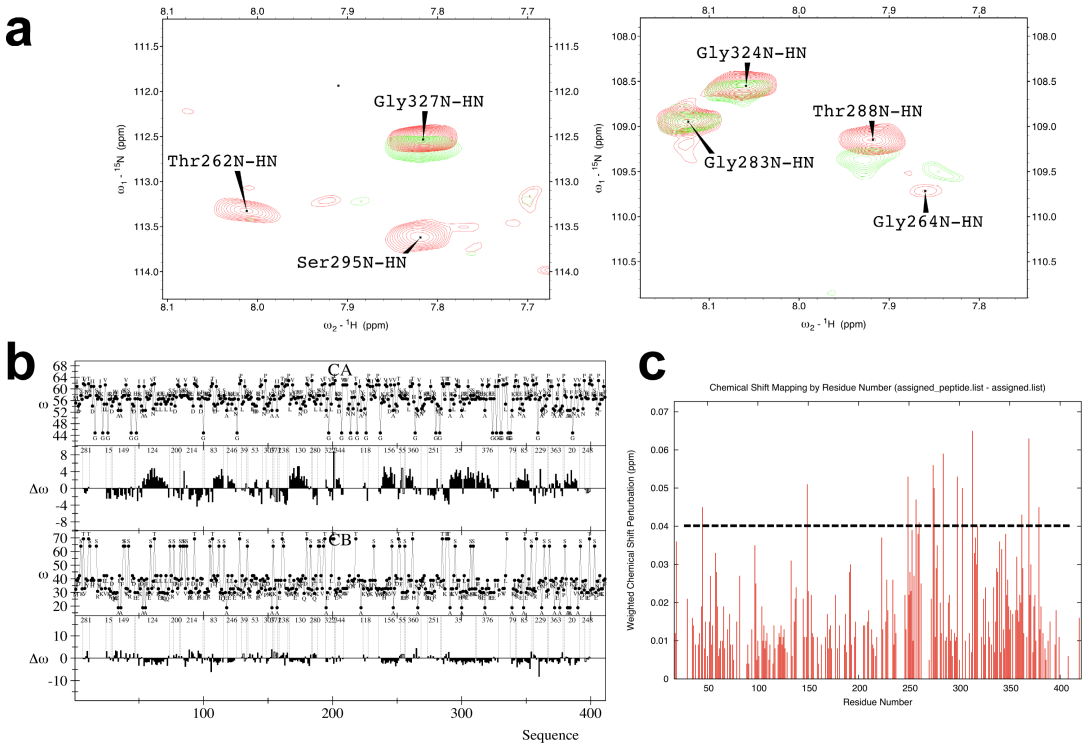

**Supplementary Figure 1**. ITC analysis. Shown is the experiment where 500 µM FtsZ peptide was titrated into 55 µM FtsA.



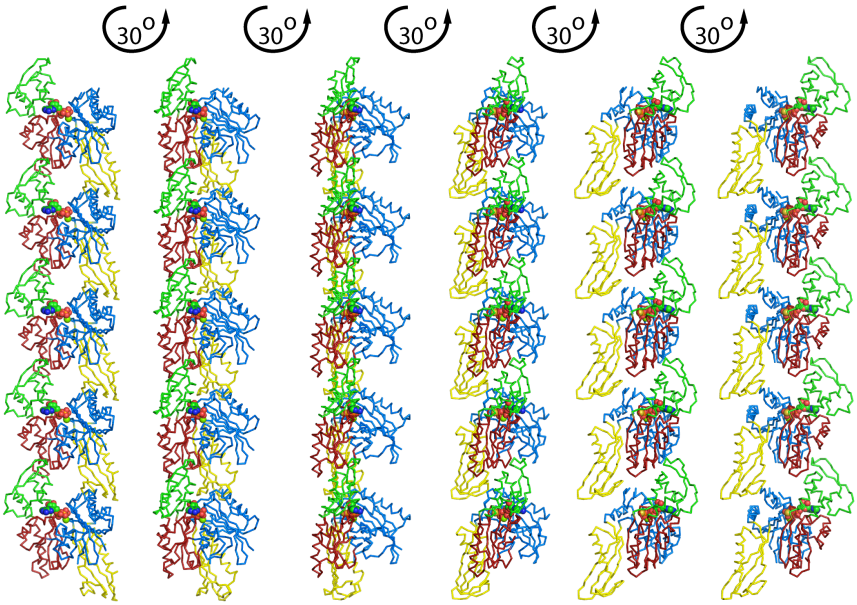
**Supplementary Figure 2**. Stereo view of the FtsA/FtsZ interaction site. For the peptide, the final 2FoFc electron density map at 1.8 Å is shown.



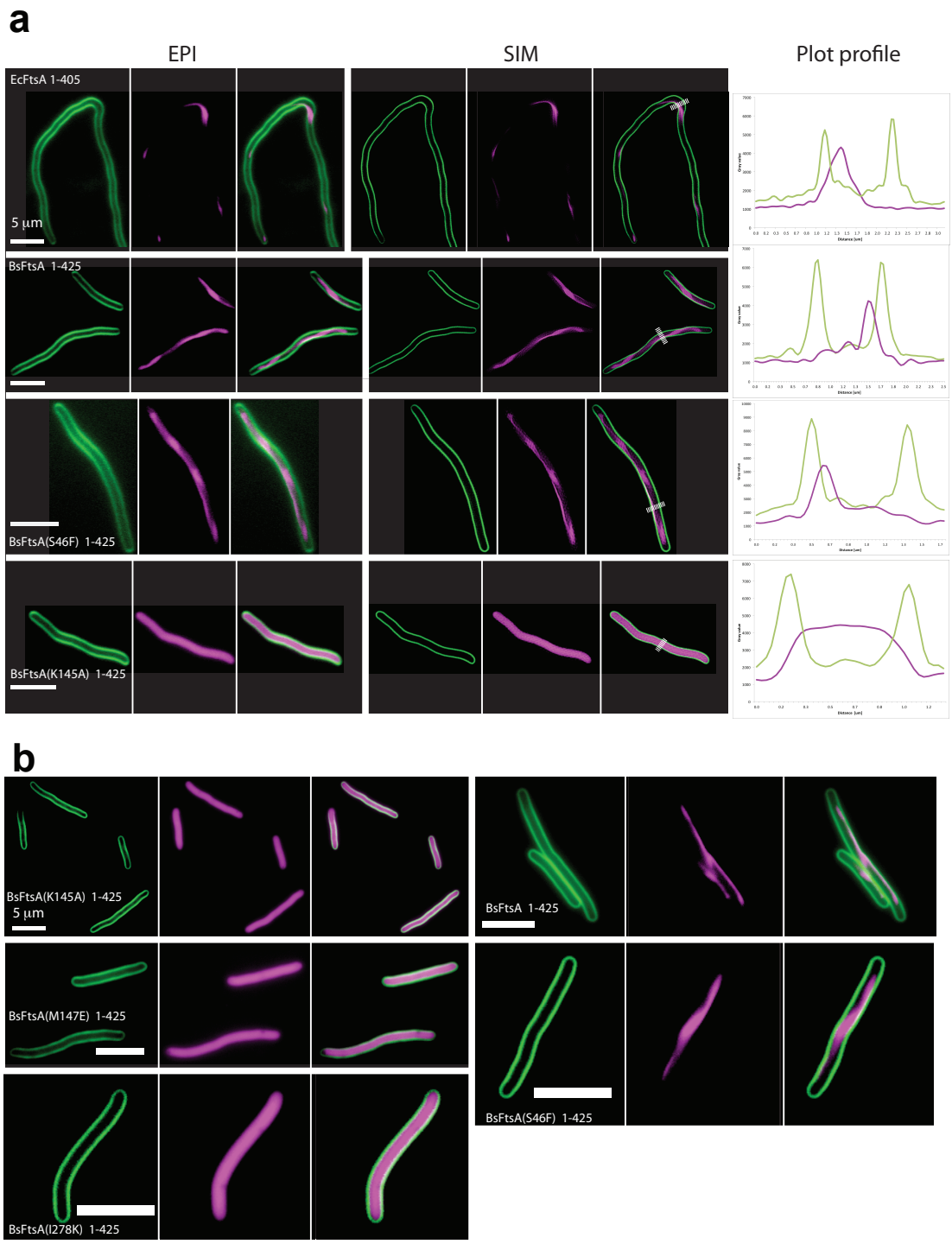

**Supplementary Figure 3.** $^{1}H$,$^{15}N$ TROSY spectrum of a $^{15}N$,$^{13}C$,$^{2}H$ triple labeled sample of TmFtsA (~50 kDa) recorded at 50˚C and 16.4 T .



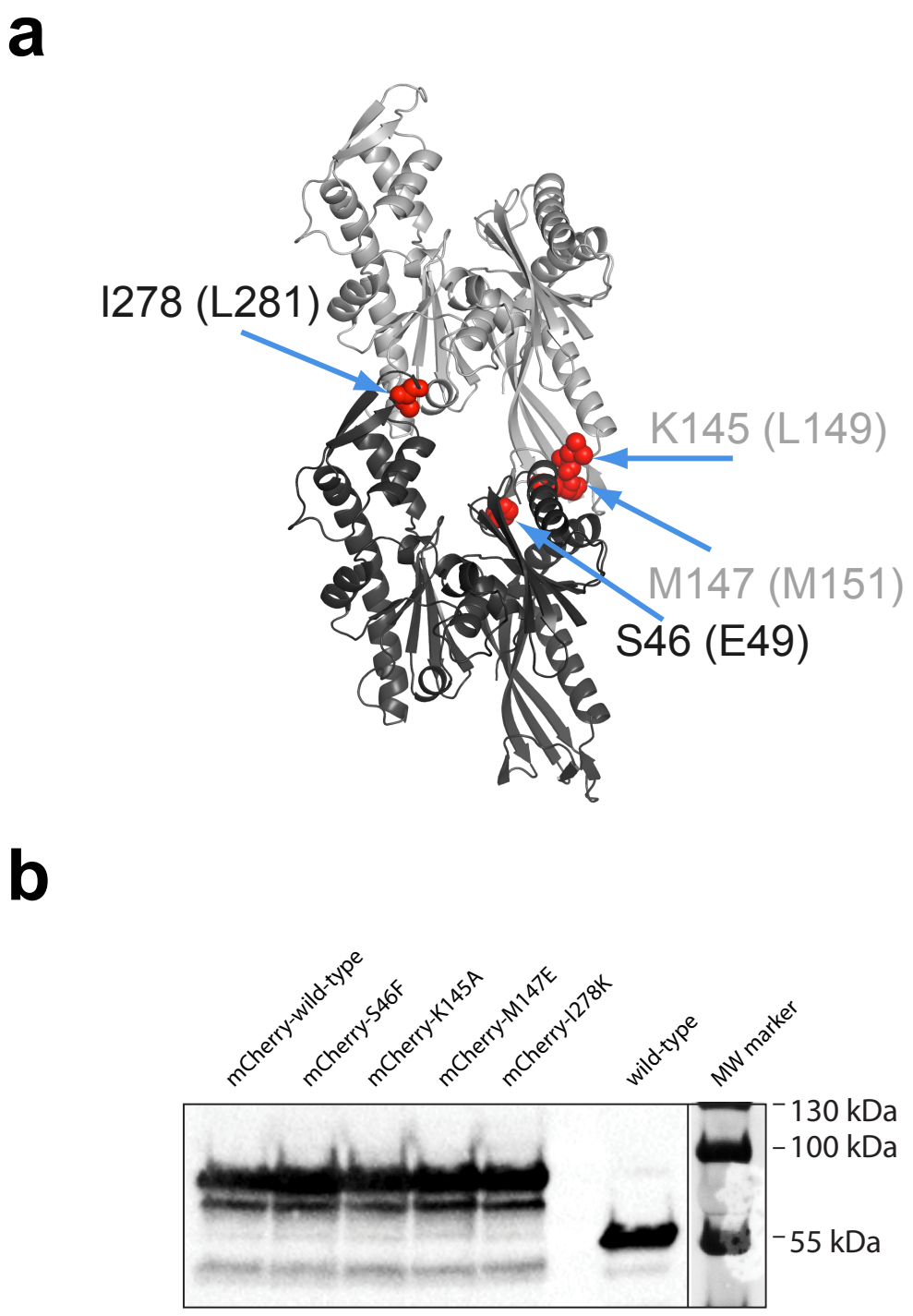

**Supplementary Figure 4**. Solution NMR analysis allowed to independently confirm the FtsZ peptide-binding site on FtsA's surface. a) Examples of exchange broadened (left) and shifted (right) resonances. b) MAPPER program output showing that more than 80 % of FtsA's primary sequence has been assigned. c) A graph representing weighted chemical shift perturbations observed after the addition of the 16 mer FtsZ peptide. Residues shifted more than the threshold (dotted line) and those that disappeared upon peptide binding have been mapped onto the crystal structure (Fig. 2b).



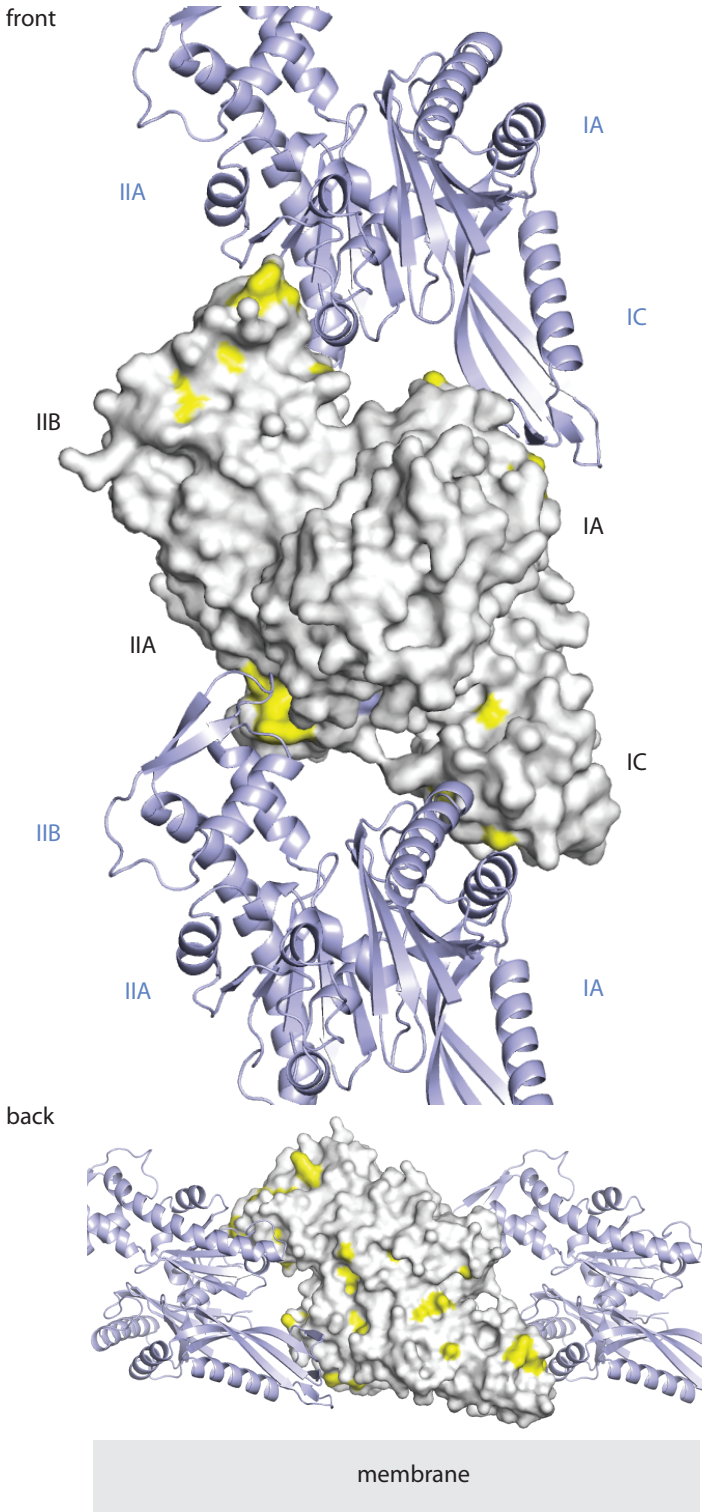

**Supplementary Figure 5.** 180° view of FtsA polymer. Each monomer has an ATPγS molecule bound and every filament is rotated 30° in respect to the previous one.



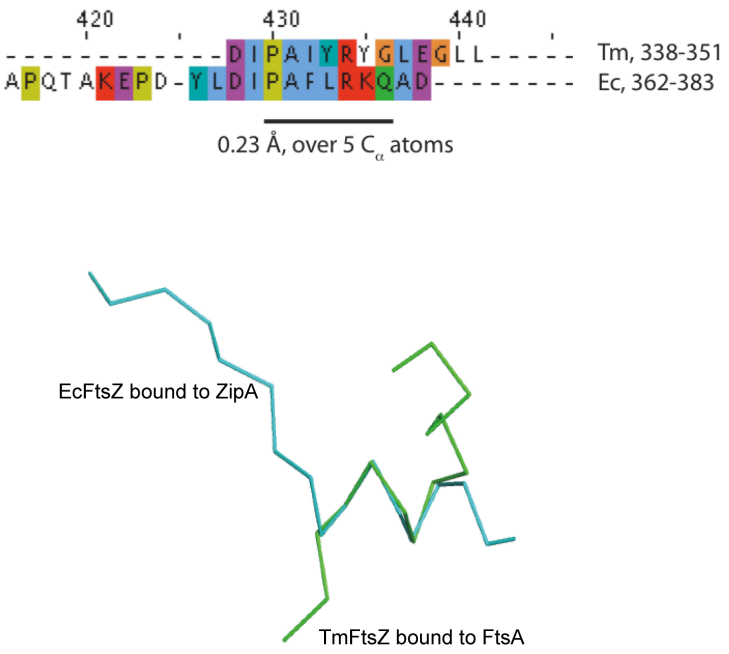

**Supplementary Figure 6.** Structured illumination microscopy (SIM) visualizes FtsA polymers. a) Comparison of images captured with epifluorescence (left) and SIM (middle). Plot profiles along dotted lines (SIM, right) show distributions of mCherry-labeled FtsA in cells. b) Corresponding conventional epi images of cells overexpressing different variants of *B. subtilis* FtsA. Left panels – polymerization-deficient mutants. Right panels – polymerizing FtsA.



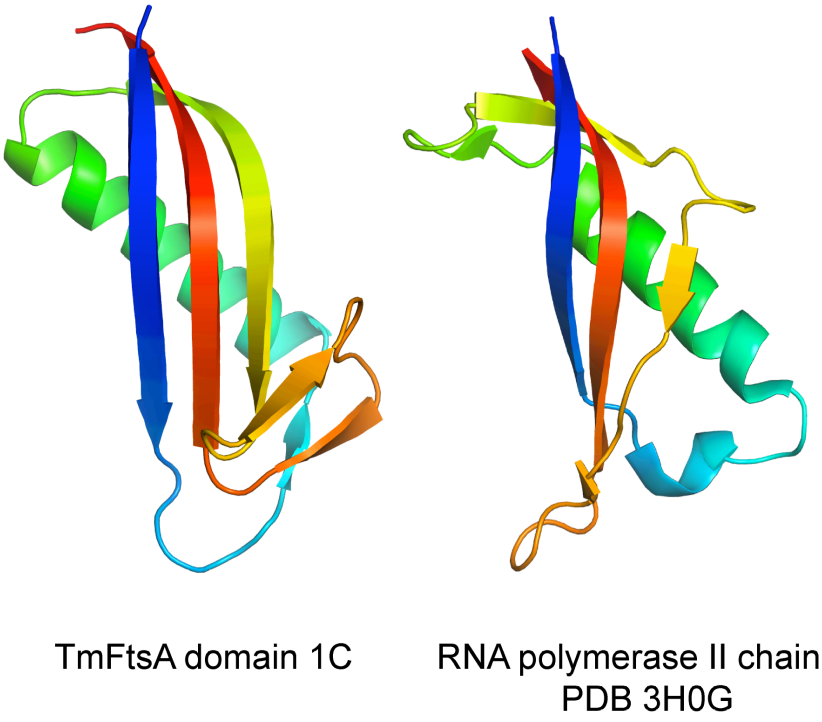

**Supplementary Figure 7.** Analysis of FtsA mutants. a) FtsA dimer with mutations marked that were introduced on the polymerization interface (in red). *B. subtilis* residue numbers followed by *T. maritima* residue numbers (in brackets). b) Western blot analysis shows that all the mutants are stable *in vivo* and are produced at comparable levels to the wild-type FtsA protein




**Supplementary Figure 8.** An independent study by the Lutkenhaus group[1] identified residues in FtsA that allow to bypass ZipA when mutated and affect FtsA's self-interaction in a yeast-two-hybrid assay (yellow). All four major interaction sites light up (top). Bottom: residues on the inside of the canonical actin fold light up as well, indicating possible double filament formation by FtsA.




**Supplementary Figure 9.** Superposition of the TmFtsZ C-terminal peptide (this work) and the corresponding peptide of EcFtsZ when bound to ZipA[2].




**Supplementary Figure 10.** DALI server identified RNA polymerase II chain G as the one having the most similar fold to the 1C domain of FtsA with Z score 6.3 (PDB ID 3H0G).

**Supplementary Table 1.**

Plasmids used in this study.

| plasmid | descritption | tag | resistance | promoter | vector | ref |
|---|---|---|---|---|---|---|
| pSZ1 | TmFtsA (1-419) | 6xHis | Amp | T7 | pET15b | this work |
| pSZ6 | TmFtsA (1-419) | no | Kan | T7 | pET9b | this work |
| pSZ7 | TmFtsZ (1-351) | Intein-chitin | Amp | T7 | pTXB1 | this work |
| pSZ8 | TmFtsA (1-419) | Intein-chitin | Amp | T7 | pTXB1 | this work |
| pSZ19 | ParM-Z | Intein-chitin | Amp | T7 | pTXB1 | this work |
| pSZ41 | BsFtsA (1-440) | no | Kan | T7 | pET9b | this work |
| pSZ47 | BsFtsA (1-425) | no | Kan | T7 | pET9b | this work |
| pMZ114 | EcFtsA (1-405) | no | Kan | T7 | pET9b | this work |
| pSZ49 | EcFtsA (1-405) | mCherry | Amp | T7 | pQW310 | this work |
| pSZ50a | BsFtsA (1-425) | mCherry | Amp | T7 | pQW310 | this work |
| pSZ50b | BsFtsA_K145A (1-425) | mCherry | Amp | T7 | pQW310 | this work |
| pSZ50c | BsFtsA_M147E (1-425) | mCherry | Amp | T7 | pQW310 | this work |
| pSZ50d | BsFtsA_I278K (1-425) | mCherry | Amp | T7 | pQW310 | this work |
| pSZ50e | BsFtsA _S46F (1-425) | mCherry | Amp | T7 | pQW310 | this work |
| pSZ61a | BsFtsA (1-440) | no | Amp/Spc | Pspac | pAPNC213 | this work |
| pSZ61b | BsFtsA_K145A (1-440) | no | Amp/Spc | Pspac | pAPNC213 | this work |
| pSZ61c | BsFtsA_M147E (1-440) | no | Amp/Spc | Pspac | pAPNC213 | this work |
| pSZ61d | BsFtsA_I278K (1-440) | no | Amp/Spc | Pspac | pAPNC213 | this work |
| pSZ61e | BsFtsA _S46F (1-440) | no | Amp/Spc | Pspac | pAPNC213 | this work |
| pSZ61f | BsFtsA_Q87Stop (1-440) | no | Amp/Spc | Pspac | pAPNC213 | this work |

**Supplementary Table 2.**

Strains used in this study.

| strain | genotype | reference |
|---|---|---|
| Escherichia coli C41 (DE3) | BL21(DE3) derivative | Miroux and Walker[3] |
| Bacillus subtilis S62 | ftsA279 trpC2 metE3 rpoB2 | Karmazyn-Campelli[4] |
| Bacillus subtilis S62C | ftsA279 trpC2 metE3 rpoB2 aprE::Pspac-ftsaK145A | this work |
| Bacillus subtilis S62D | ftsA279 trpC2 metE3 rpoB2 aprE::Pspac-ftsaM147E | this work |
| Bacillus subtilis S62E | ftsA279 trpC2 metE3 rpoB2 aprE::Pspac-ftsaI278K | this work |
| Bacillus subtilis S62F | ftsA279 trpC2 metE3 rpoB2 aprE::Pspac-ftsaQ87End | this work |
| Bacillus subtilis S62G | ftsA279 trpC2 metE3 rpoB2 aprE::Pspac-ftsa | this work |
| Bacillus subtilis S62H | ftsA279 trpC2 metE3 rpoB2 aprE::Pspac-ftsaS46F | this work |

**Supplementary Movie 1.** Movie moving through Z-slices through an electron cryotomogram of an E. coli cell over-expressing TmFtsA 1-419. The cell membrane forms many tubes that are interleaved by filaments that are presumed to be made of FtsA. Membrane deformation is caused by FtsA's filamentation combined with its membrane affinity through the C-terminal amphipathic helix. Corresponds to Fig. 4b, top right.

**Supplementary references.**

1. Pichoff, S., Shen, B., Sullivan, B. & Lutkenhaus, J. FtsA mutants impaired for self-interaction bypass ZipA suggesting a model in which FtsA's self-interaction competes with its ability to recruit downstream division proteins. *Mol Microbiol* **83**, 151-167 (2012).

2. Mosyak, L. et al. The bacterial cell-division protein ZipA and its interaction with an FtsZ fragment revealed by X-ray crystallography. *EMBO J* **19**, 3179-3191 (2000).

3. Miroux, B. & Walker, J. E. Over-production of proteins in Escherichia coli: mutant hosts that allow synthesis of some membrane proteins and globular proteins at high levels. *J Mol Biol* **260**, 289-298 (1996).

4. Karmazyn-Campelli, C., Fluss, L., Leighton, T. & Stragier, P. The spoIIN279(ts) mutation affects the FtsA protein of Bacillus subtilis. *Biochimie* **74**, 689-694 (1992).