SUPPLEMENTAL INFORMATION

Self-organization of FtsZ polymers in solution reveals spacer role of the disordered C-terminal tail


MATERIALS AND METHODS

FtsZ proteins

Full-length untagged FtsZ from B. subtilis (BsFtsZ) was overexpressed in E. coli C41(DE3) cells and purified by ammonium sulfate precipitation, ion exchange and hydrophobic chromatography, with ~0.05 guanine nucleotide bound per FtsZ (1). Untagged truncated BsFtsZ(1-318) (BsFtsZ-ΔCt) was obtained from a pHis17 plasmid in which a truncated version of the ftsZ gene (2) (from 1 to 954 bp, plus a TAA stop codon) was cloned into the NdeI/BamHI sites. BsFtsZ-ΔCt was purified similarly to the full length protein, except precipitation with 60% ammonium sulfate. His-tagged versions of full-length Bs-FtsZ and truncated BsFtsZ(1-315) were obtained through the expression from pAB20 and pAT19 plasmids respectively. The pAB20 plasmid (3) has an ftsZ copy from B. subtilis integrated in pET28a vector (Novagen) at Nhel and NotI sites and it was used to generate pAT19 by the insertion of a stop codon at the I316 position. The ftsZ gene constructs were confirmed by complete open reading frame sequencing. The His-tagged proteins were overexpressed in E. coli BL21(DE3) and purified using Ni-affinity chromatography in 50mM Tris-HCl, 50 mM KCl, 10% glycerol, pH 8.0, with an imidazole gradient (50mM to 1M). His-tags were cleaved with thrombin yielding proteins GSHMAS-BsFtsZ (tag-BsFtsZ) and GSHMAS-BsFtsZ(1-315) (tag-BsFtsZ-ΔCt) respectively. The N-terminal residues were confirmed by Edman sequencing purified tag-BsFtsZ-ΔCt with an Applied Biosystems Procise 494 sequencer. Two additional chromatographic steps were used: anion exchange (HiTrapQ HP) in 50 mM Mes-KOH, 5 mM MgCl2, 10% glycerol, pH 6.5, with a KCl gradient (50mM to 1M) and size exclusion (Superdex 75) in 50mM Tris-HCl, 50 mM KCl, 1 mM EDTA, 10% glycerol, pH 7.5. These proteins contained ~0.8 guanine nucleotide bound.
The C-terminal constructs BsFtsZ-ΔC17 (4), BsFtsZ-ΔCTL25, BsFtsZ-ΔCTL50, BsFtsZ-CTLA50, BsFtsZ-CTLA100 and BsFtsZ CTLA249 (5) were expressed from their corresponding plasmids (Table S1), kindly provided by the authors, in C41(DE3) cells and purified as native untagged BsFtsZ, with minor modifications. BsFtsZ-AC17 and BsFtsZ-ΔCTL25 were precipitated with 45% ammonium sulfate, BsFtsZ-ΔCTL50 with 65%, BsFtsZ-CTLA50 with 40%, BsFtsZ-CTLA100 and BsFtsZ-CTLA259 with 30% ammonium sulfate. These proteins contained 0.03 to 0.08 guanine nucleotide bound per FtsZ.

FtsZ from *E. coli* (EcFtsZ) was overproduced in transformed *E. coli* BL21(DE3) cells and purified by Ca\(^{2+}\)-precipitation and anion-exchange, with ~0.8 guanine nucleotide bound (6).

Thermophilic FtsZ from *M. jannaschii* (MjFtsZ) was overexpressed in *E. coli* BL21(DE3) pLyS cells, purified by ammonium sulfate precipitation, ion exchange and hydrophobic chromatography, containing ~0.45 guanine nucleotide bound (7).

**Polymerization conditions**

BsFtsZ assembly experiments were performed in 50 mM Tris-HCl, 50 mM KCl, 1 mM EDTA, 10 mM MgCl\(_2\), pH 7.4 (Tris50 buffer), at 25 °C. Polymerization was started by addition of 0.1 mM GMPCPP or 1 mM GTP and it was monitored by right angle light scattering at 350 nm (0.5 nm band-pass) using a Fluromax-4 spectrofluorometer. Aliquots of the polymer solutions were adsorbed to carbon-coated grids, negatively stained with 2% uranyl acetate and examined with a Jeol 1230 electron microscope operated at 100 kV.

EcFtsZ assembly experiments were performed in 25 mM Pipes/KOH, 250mM KCl, 1 mM EDTA, 10 mM MgCl\(_2\), pH 7.5 (Pipes250 buffer) at 25 °C, or in Tris50 buffer as above for comparison. Assembly was started by nucleotide addition: 0.1 mM GMPCPP, or 1 mM GTP, or a GTP (1mM) regenerating system consisting of 1 U/mL acetate kinase and 7.5 mM acetyl phosphate, or 1 mM GDP (negative control) or 0.1 mM GMPCP (negative control).

MjFtsZ was assembled in 50 mM Mes/KOH, 50 mM KCl, 1 mM EDTA, 10 mM MgCl\(_2\), pH 6.5 (Mes50 buffer) at 55 °C, with 0.2 mM GMPCPP or 4 mM GTP.

FtsZ polymer formation was measured by isothermal pelleting and protein concentration measurement (1). The Cr values of BsFtsZ and tag-BsFtsZ-ΔCt were 6.6 ± 1.0 µM and 1.6 ± 0.2 µM respectively with 2 mM GTP, 2.2 ± 0.4 µM and 1.0 ± 0.1 µM with 0.1 mM GMPCPP. Tag-BsFtsZ(1-382) assembled with Cr values similar to BsFtsZ (5.8 ± 1.4 µM with GTP and 1.5 ± 0.8 µM with GMPCPP).
Biochemical methods

The hydrolysis of GTP (2mM) or GMPCPP (0.1 mM) was measured from the released inorganic phosphate (1). The hydrolysis rate values given are referred to polymerized FtsZ, that is, total FtsZ concentration (10 to 20 \( \mu \)M) minus the Cr.

To determine nucleotide content in FtsZ polymers, nucleotides were extracted from FtsZ polymer pellets by the addition of \( \text{HClO}_4 \) (7) and analyzed employing an anion exchange column (VYDAC 3021C4.6, 10 \( \mu \)m, 4.6 mm x 250 mm) with a gradient of 25 mM NaH2PO4/Na2HPO4, pH 2.8 to 125 mM NaH2PO4/Na2HPO4, pH 2.9, detecting nucleotides by absorbance at 254 nm, with an AKTAPurifier system (GE Healthcare).

Sedimentation velocity experiments with unpolymerized FtsZ were made employing a Beckman XLI analytical ultracentrifuge with the interference optics (8) and analyzed with SEDFIT (9). Sedimentation coefficients of FtsZ ring models were calculated with HYDRO++ (10) using the BsFtsZ monomers sedimentation coefficient, with HYDROPRO (11) from SaFtsZ atomic model coordinates, and with HYDROMIC (12) from the electron microscopy volume of EcFtsZ rings.

SAXS experiments

Time-resolved small angle synchrotron X-ray solution scattering measurements of FtsZ and its polymers were performed at the ALBA BL11-NCD beam line. The camera length was 2.1 m and the X-ray energy 10 keV (\( \lambda = 1.24 \) Å). An ADSC Quantum 210r CCD detector (210 x 210 mm\(^2\); 4096 x 4096 pixel) was employed, with a 6 mm centered beam stop, which provided a useful range of \( q = 0.01 \) to 0.22 Å\(^{-1}\). The scattering vector modulus \( q \) is defined as \( q = 4\pi (\sin \theta) / \lambda \), where \( 2\theta \) is the angle of incident to scattered radiation and \( \lambda \) the X-ray wavelength. The \( q \) values were calibrated using the diffraction maxima of silver behenate. Preliminary SAXS data were acquired at former ESRF Spanish BM16 with a similar q-range. Comparative static measurements of truncated BsFtsZ-\( \Delta \)Ct and full length BsFtsZ were subsequently made at the ESRF BM29-BioSAXS beam line using the capillary sample changer robot and Pilatus 1M detector.

A vertical thermostated sample cell with mica windows and an optical path of 3 mm was employed at ALBA BL11-NCD. The degassed samples (180 \( \mu \)L) were loaded from the bottom with protein electrophoresis pipette tips (BioRad # 223-9915), which were introduced through a channel at the top, avoiding bubble formation. The X-ray beam size was
approximately 600 \(\mu\text{m}\) (horizontal) \(\times\) 138 \(\mu\text{m}\) (vertical). The protein solution was scanned, irradiating during 0.5 s, each 15 s, in a series of 63 non-overlapping evenly spaced positions within a 3mm (horizontal) \(\times\) 7 mm (vertical) area, by automatically controlling the position of the sample stage and the shutter. This procedure avoided radiation damage. The dead time between sample loading and the first measurement was \(~\text{2 min.}\)

The X-ray scattering data were integrated with the FIT2D software (http://www.esrf.eu/computing/scientific/FIT2D/), normalized for the incident intensity, processed with PRIMUS (13) and analyzed with GMOM (14). For each protein sample, the scattering by a carefully matched buffer reference was subtracted from the data. The time-resolved data were subtracted frame by frame, employing a PerL script (E.R.A., unpublished).

The instrumental set-up was tested measuring the scattering profile of delipidated bovine serum albumin (BSA, Sigma; 4.3, 2.2 and 1.1 g/L in Tris50 buffer, which afforded a radius of gyration value \(R_G = 27.0 \pm 1.5 \text{ Å}\) and was superimposable within experimental error to a standard scattering profile of BSA from the Small Angle Scattering Biological Data Bank (SASBDB; entry SASDA32, 25.6 g/L BSA in 50 mM Hepes, 50 mM KCl; \(R_G = 29 \text{ Å}\)). To test the instrument performance with large protein assemblies, we measured the scattering profile of microtubules assembled from tubulin (120 \(\mu\text{M}\)) with docetaxel (130 \(\mu\text{M}\)) in 10 mM sodium phosphate buffer, 6 mM MgCl\(_2\), 1 mM GTP, pH 6.7, at 37 °C. The position of the scattering maxima \((q, \text{Å}^{-1})\) was very close to our reference values (15): \(J_{01}, 0.029\) (ref. 0.031); \(J_{02}, 0.056\) (ref. 0.057); \(J_{03}, 0.086\) (ref. 0.086); \(J_n, 0.118\) (ref. 0.121); \(J_3, 0.159\) (ref. 0.160); \(J_{n-3}, 0.179\) (ref. 0.177).

**X-ray scattering by FtsZ polymer models**

FtsZ polymer models were constructed FilaSitus program (http://situs.biomachina.org/fila/; (16)), employing the filament crystal structure of *Staphylococcus aureus* FtsZ (SaFtsZ; PDB entry 3vo8). Theoretical scattering curves were generated with CRYSOL (https://www.embl-hamburg.de/biosaxs/crysol.html, (17)). Default parameters were used with the following exceptions: maximum order of harmonics 30, order of Fibonacci grid 18, maximum q-value 0.30 and number of points 256 (except ring models that were calculated with 1024 points).

**Model analysis of X-ray scattering by FtsZ single filaments.** For convenience in this detailed description of model analysis, we will focus first on single filaments (Figure 4) and the higher angle scattering and then proceed to multiple filaments (Figure 2B) and lower angle scattering features. We constructed single filaments made of 140 FtsZ monomers with
different curvature angles between consecutive monomers of 0° (straight, 6160 Å long), 0.5, 1, 1.5, 2 and 2.57° (a closed 1960 Å diameter ring) (Fig. S2). The direction of curvature employed is similar to that of SaFtsZ-GTP filaments in molecular dynamics simulations, which leaves the FtsZ C-terminal end on the outside, and the curvature angles are within the range observed (18). The straight model has a sharp peak at \( q = 0.143 \text{ Å}^{-1} \), corresponding to the 44 Å spacing between subunits along the filament. However, this peak could hardly be appreciated in the experimental SAXS profiles, except in BsFtsZ-ΔCt (Figure 2A) and in the more concentrated EcFtsZ polymer samples (Figure 3). The 0.14 Å\(^{-1}\) peak disappears in the curved single filament models, but undulations appear in the higher angle region that have not been experimentally observed. These undulations are out of phase in the different models and tend to smooth out in linear combinations of SAXS profiles calculated for filaments with different degrees of curvature, thus simulating filament flexibility (Fig. S2, dark grey line). As the filaments curve beyond a semi-circle and approach ring closure, a series of periodic ripples evenly spaced each ~0.003 Å\(^{-1}\) appear throughout the model scattering profile. These ripples correspond to the subsidiary maxima of a \( J_0 \)-like Bessel function arising from rings with mean diameter ~2000 Å and their spacing is inversely proportional to the ring diameter (19). Combining rings of close sizes (100, 120 and 140 monomers) hardly smoothed the model ripples (Fig. S2, light grey line); notice that these features are absent from the experimental data (Figures 2 - 3). Mini-rings made of 16 monomers (224 Å diameter) give a sinusoidal pattern with a longer period (~0.03 Å\(^{-1}\)). Reversing the direction of curvature to have the FtsZ C-terminal end on the inside of the mini-rings (as in tubulin protofilaments peeling outwards from disassembling microtubule ends) does not change their scattering profile at our resolution. Combining mini-rings of close sizes (14, 16 and 18 monomers) smoothed the model scattering profile in the middle region but left periodic features in the low and high angle zones (Fig. S2) that have not been experimentally observed.

The effects of varying the filament length were examined with increasing number of monomers at two different curvature angles (Fig. S3). The ~0.14 Å\(^{-1}\) maximum is more marked in models covering circular arches up to 80° and disappears in the longer models, which show undulations and ripples as above. However, the higher angle undulations smooth by combining filaments of close sizes (100, 120 and 140 monomers) with 1° curvature angle between consecutive monomers; similarly combining filaments (40, 60 and 80 monomers) with 3.5° curvature angle cancels the ripples along the profile (Fig. S3, grey lines), and certain individual S-shape models lack ripples (Fig. S3, models \( m \) and \( n \)). Thus, single filament models of variable curvature and length (Figs. S2 and S3), rather than rings of close sizes or
straight filaments, are qualitatively compatible with the smooth higher angle zone of the experimental EcFtsZ and BsFtsZ polymer SAXS.

In order to more closely modeling X-ray scattering by FtsZ polymer solutions it has to be taken into account that these always contain a concentration of unassembled protein, the critical protein concentration (Cr) required for assembly, which depends on the FtsZ species and solution conditions (20, 21). We thus simulated the Cr effects on SAXS with linear combinations of the model filaments scattering curves (Fig. S2 and S3) with a small proportion (5%; exp. value Cr = 0.088 g/L) of the scattering by a similar number of unassembled FtsZ monomers. This procedure softens the 0.14 Å \(^{-1}\) maximum of straight models and the higher angle undulations of curved models, making their corresponding model mixture qualitatively compatible with the EcFtsZ polymers experimental data (see Figure 3); however, this is clearly not the case for ring model mixtures, in which characteristic sinusoidal oscillations remain at low and mid angles after mixing with unassembled FtsZ monomers. The amplitude of these oscillations clearly exceeds experimental noise: for example, the oscillations of ring model mixture scattering intensity at 0.12 Å \(^{-1}\) have relative amplitude (± 26%) five times larger than the normalized standard deviation (5 %) of the scattering by the concentrated EcFtsZ sample (Figure 3). Taking into account that FtsZ filaments were not crystal-straight during molecular dynamics simulations (18) or in cryo-EM (20, 22), we concluded from the model analysis that the smooth higher angle zone of the experimental EcFtsZ polymers SAXS is compatible with single filament FtsZ models of variable curvature and length. Such ensemble of FtsZ filament configurations may include S-shapes, as the model shown in Figure 3, as well as less curved S- and C-shapes, and a proportion of multiple filaments (analyzed below) should not be excluded. However, a polymer population consisting of rings of close sizes (100-120-140 monomers) appears incompatible with the EcFtsZ SAXS data; we cannot rule out annealed filament tangles of this size. Mini-ring models (14-16-18 monomers) are also inconsistent with the data (Figure 3). However, a 20% of FtsZ forming mini-rings and 80% in curved filaments would be qualitatively compatible with the EcFtsZ SAXS data.

**Model analysis of X-ray scattering by FtsZ filament bundles.**

We next constructed multiple filament models, in order to capture the ~0.09 Å \(^{-1}\) and ~0.05 Å \(^{-1}\) maxima of the BsFtsZ experimental profiles (Figure 2A). For simplicity, we first analyze straight filaments and the ~0.09 Å \(^{-1}\) maximum and then proceeded to multiple curved filaments and the ~0.05 Å \(^{-1}\) maximum. Two parallel filaments with a 70 Å center to center
distance reproduce the 0.09 Å\(^{-1}\) maximum (Figure S4, models i and j). Importantly, these model filaments do not touch each other, and touching filaments do not give rise to this maximum (Figure S4, models f, g, h; 48 Å center to center distance). A sharp monomer repeat peak at 0.14 Å\(^{-1}\) appears in straight models (c, d, e, i) that is modulated into a characteristic bulge by shifting one filament with respect to the other by half a monomer length (compare models i and j). Rotating the in-phase filaments by 90° and making them closer (38 Å center to center) slightly softened this peak, whereas performing the same operation with the shifted filament pair gave a different, wider maximum centered around 0.17 Å\(^{-1}\) (not shown). Of note, the 0.15 Å\(^{-1}\) bulge in multiple filament models resembles the 0.14 Å\(^{-1}\) experimental feature of truncated BsFtsZ-ΔCt (Figure 2A), which lacks the 0.09 Å\(^{-1}\) maximum as the touching filaments models (Figure S4, model f). This 0.09 Å\(^{-1}\) maximum becomes prominent in non-touching filament ribbons upon increasing the number of strands (models i, k, l).

Nevertheless, straight models hardly reproduce the 0.05 Å\(^{-1}\) maximum, which corresponds to a 126 Å characteristic distance between filaments. This feature is better captured by triple curved filaments (Figure S5, models a-d). Three concentric filaments, made of 65-60-55 subunits each, radially spaced 70 Å display both an scattering maximum at 0.09 Å\(^{-1}\) and a shoulder at 0.05 Å\(^{-1}\) (model a). But they also have a prominent minimum at 0.15 Å\(^{-1}\) and peak at 0.18 Å\(^{-1}\) that are absent from the data; these unwanted model features can be suppressed by introducing some disorder, which we simulated by displacing one (models b and c) or two of the filaments (model d) by 36 Å perpendicularly to the curvature plane, and readjusting to 70 Å the distance between neighbor filaments. Model d, or a combination of models, resemble better the ~0.05 Å\(^{-1}\) and ~0.09 Å\(^{-1}\) maxima together with the smooth higher angle scattering by BsFtsZ polymers. It is conceivable that bundles of more than three curved, 70 Å -spaced filaments, can also reproduce the SAXS features. However, circularly closed triple ring models (Figure S5, models e-h; mean diameter 1470 Å) or their combination showed the periodic maxima typical of rings, now modulated by inter-ring interference, which are absent from the experimental profiles. Three stacked rings models or an equivalent flat helix show reinforced ring maxima (Figure S5, model i).

Combining FtsZ triple filament models with a 5% of unassembled monomers as before (BsFtsZ experimental Cr = 0.076 g/L), we found that triple curved filaments reproduce the 0.05 and 0.09 Å\(^{-1}\) scattering maxima and smooth higher angle region of the BsFtsZ-GMPCPP polymer solutions. These models are exemplified in Figure 2B by an off-plane triple curved filament, which is made of 65 + 60 + 55 crystallographic FtsZ monomers and has an end-to-
end distance of 1430 Å (note that length should actually be variable). Straight triple filaments resemble less well the BsFtsZ experimental data whereas the triple ring models generate ripples that are absent from the data. The lower angle scattering (q = 0.01 to ~ 0.02 Å⁻¹) by the models is not as steep as in BsFtsZ-GMPCPP polymers, which indicates that the later are still larger scattering objects, as indicated by their turbidity in white light. We suggest that they form by aggregation or annealing of curved filaments bundles similar to those described by the models. We conclude from this model analysis that the experimental BsFtsZ–GMPCPP polymer SAXS can be explained by loose bundles of ≥ 3 curved protofilaments of variable length, laterally spaced 70 Å, and their aggregates. We found straight filament bundles less likely and ordered circularly closed FtsZ rings incompatible with the SAXS profiles of BsFtsZ. In contrast, the distinct scattering by the polymers of tail-less BsFtsZ-ΔCt-GMPCPP can be explained by straight tight bundles of ≥ 2 protofilaments, which is exemplified by a triple filament in Figure 2B.

**Scheme of a FtsZ bundle with C-terminal tails**

The C-terminal tails connecting protofilaments were constructed as follows. The last 15 C-terminal amino acid residues of SaFtsZ were initially predicted with the I-Tasser server (23) to form a helical structure. Subsequently, potential binding sites for this segment in the FtsZ subunit of the contiguous filament were searched using the Frodock 2.0 server (24), and one of the 10 best solutions was selected. The rest of the peptide chain linking to the FtsZ core was completed using the loops generation RCD+ server (25). Several C-tails were then reconfigured for Fig. 6, and the protein electrostatic contact potential displayed with PyMOL.

**Cryo-electron microscopy**

Samples were applied to holey carbon grids (Quantifoil) after glow-discharge and immediately blotted and vitrified using a GATAN or FEI Vitrobot cryo-plunger. Micrographs were taken at x40000 nominal magnification in a Jeol 1230 electron microscope operated at 100 kV and equipped with a Gatan liquid nitrogen specimen holder for cryo-EM. Cryo-EM images were taken from the hole areas, where the ice lacks any supporting film underneath, under low dose conditions and different defocus, with a CMOS Tvips TemCam-F416 camera, at 2.84 Å per pixel. Image-J software was used for filament thickness measurements and diffractogram calculations. Mini-ring images were picked and subsequently aligned and classified using SCIPION package (http://scipion.cnb.csic.es; (26)).
SUPPLEMENTAL REFERENCES


Table S1: FtsZ proteins and expression plasmids employed in this work

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SUPPLEMENTAL FIGURE LEGENDS

Figure S1. Effects of small molecule modulators of FtsZ polymer assembly. Related to main text and Figure 2. The scattering profiles of BsFtsZ (50 μM) assembled with GTP or GMPCPP, plus the polymer stabilizer PC190723 (60 μM) or the inhibitor UCM53 (60 μM) are shown as indicated. In each case, the grey tracing corresponds to a sample with nucleotide and no modulator, whereas the black points are from a parallel sample to which the modulator has been added.

Figure S2. X-ray scattering by FtsZ single filament models with varying curvature. Related to main text and Figure 4. Models were built with head-to-tail associated SaFtsZ monomers (indicated by the enlarged ribbon diagram: see Methods). Model a is a straight protofilament made of 140 monomers with 0° bending angle per monomer, model b with 0.5° angle, model c with 1° angle, model d with 1.5° angle, model e with 2° angle. The dark grey profile below is a linear combination of the scattering profiles of curved models b (30%), c (40%) and d (30%). Models f, g and h are closed rings made of 140 monomers (with 2.57° bending angle), 120 and 100 monomers respectively. The light grey profile is a combination of scattering by ring models f (30%), g (40%) and h (30%). Model i, j, k are small rings made of 14, 16 and 18 monomers (only model j is shown). The light grey dashed line is a combination of the scattering by ring models i (30%), j (40%) and k (30%). Each scattering profile is plotted using the same color as its corresponding model.

Figure S3. X-ray scattering by FtsZ single curved filament models of varying length. Related to main text and Figure 4. Curved models were constructed with 1° and 3.5° bending angles between consecutive monomers. Models a, b, c, d, e, f and g (1° angle) are respectively made of 20, 40, 60, 80, 100, 120 and 140 monomers. Models h, i, j, k and l (3.5° angle) are respectively made of 20, 40, 60, 80 and 100 monomers. S-shape model m is built of 60 + 60 monomers with 3.5° and -3.5° bending angles. Model n is made of four 36 monomer sections with alternating 5° and -5° bending angles. Each calculated scattering profile is displayed in the same color as its model, with continuous (1°) or dash lines (3.5°, 5°). The continuous grey line is a combination of the scattering profile of model e (30%), f (40%) and g (30%). The dash grey line corresponds to a combination of models i (30%), j (40%) and k (30%).

Figure S4. X-ray scattering by FtsZ multiple straight filament models. Related to main text and Figure 2. Model a is a SaFtsZ monomer, models b are a straight crystallographic
heptamer and a curved GTP-bound molecular dynamics heptamer (18). Models e, d, and e are straight single filaments respectively made of 100, 120 and 140 monomers. Model f is made of two touching 140-monomer filaments with a lateral center to center distance of 48 Å; models g and h are corresponding triple and quadruple filaments respectively; model i two non-contacting filaments with a 70 Å distance; model j is similar to model i but one of the filaments has been axially shifted by half a monomer distance. Models k and l are respectively triple and quadruple filaments built as model j.

**Figure S5. X-ray scattering by Fts Z multiple curved filament models.** Related to main text and Figure 2. Model a, shown in orthogonal and end views, consists of three concentric arcs made of 65-60-55 monomers each, radially spaced 70 Å (with curvature angles of 3.138°, 3.437° and 3.800°). In models b and c the central or one side ring has been displaced 36 Å away from the plane defined by the other two rings, and the distance between rings readjusted to 70 Å. In model d the central and one side rings have been similarly displaced from the plane of the other ring. The calculated scattering profiles are shown in the same colors as the models. The scattering profiles at the bottom part of the figure correspond to closed rings of the same dimensions (models e-h). The dashed blue line is the scattering profile of a stack of three 110 FtsZ monomer rings consecutively spaced 7 Å (model i, not shown). A flat helix with corresponding dimensions gave a very similar scattering profile. The grey line is a combination of the scattering profiles of model b (30%), model c (40%) and model d (30%). The light grey line at the bottom is an equivalent combination of closed ring models.

**Figure S6. Electron micrographs of negatively stained BsFts Z and BsFts Z-ΔCt polymers.** Related to Figure 4. 50 μM FtsZ was polymerized in Tris50 buffer with 10 mM MgCl2 and 0.1 mM GMPCPP or 1 mM GTP at 25 °C and polymers formed were observed by EM. Bar, 2000 Å.

**Figure S7. Cryo-EM of BsFts Z, tag-BsFtsZ-ΔCt and tag-BsFtsZ Z polymers with GTP.** Related to Figure 4. 50 μM BsFtsZ (A), tag-BsFtsZ-ΔCt (B) and tag-BsFtsZ (C) were assembled in Tris50 buffer with 10 mM MgCl2 and 1 mM GTP at 25 °C, and polymers formed were visualized by cryo-EM; bar, 1000 Å; enlarged areas (bar, 500 Å) from each picture and their diffractogram are shown and some more examples of BsFtsZ are included to show spacing variability obtained from different areas (D). The distance between filaments in bundles of BsFtsZ (black bars; mean ± s.d.: 66.0 ± 7.5 Å (GTP); 70.5 ± 8.6 Å (GMPCPP)) and tag-BsFtsZ-ΔCt (gray bars; 55.2 ± 11.0 Å (GTP) 57.8 ± 13.2 Å (GMPCPP)) were
measured and the distribution plotted (D, E). Notice that the spread of these distributions may partially result from inherent measurement errors.

**Figure S8. Cryo-EM of disassembling BsFtsZ polymers.** Related to main text. (A) light scattering time courses of 2 g/L BsFtsZ assembly in Tris50 buffer with 10 mM MgCl₂ and 0.1 mM GMPCPP or 1 mM GTP; nucleotides were added at time 0. Samples were taken at different stages during de-polymerization and observed by cryo-EM. BsFtsZ assembled with GMPCPP at 70-50% (B) and 20-5% (C) light scattering. D, BsFtsZ assembled with 0.1 mM GMPCP. E, BsFtsZ assembled with GTP at 50-30% (E) and 20-10% (F) light scattering. G, BsFtsZ assembled with 1 mM GDP. Bar, 1000 Å.

**Figure S9. Cryo-EM of BsFtsZ C-terminal constructs polymers.** Related to main text. BsFtsZ-ΔC17 (A and B), BsFtsZ-ΔCTL25 (C and D), BsFtsZ-ΔCTL50 (E and F) are described in main text. Each BsFtsZ construct (50 μM) was assembled in Tris50 buffer with 10 mM MgCl₂ and 0.1 mM GMPCPP at 25 °C, in the absence (A, C, E) or presence of 20 μM PC190723 (B, D, F), and polymers formed were visualized by cryo-EM. Bar, 1000 Å. The insets in each case are enlarged areas (bar, 500 Å) and their computed diffractograms where the spacing of the main equatorial spots is indicated.

**Figure S10. Cryo-EM of BsFtsZ C-terminal constructs polymers.** Related to main text. BsFtsZ-CTLA50 (A and B), BsFtsZ-CTLA100 (C and D), BsFtsZ-CTLA249 (E and F) are described in main text. Each BsFtsZ construct (50 μM) was assembled in Tris50 buffer with 10 mM MgCl₂ and 0.1 mM GMPCPP at 25 °C, in the absence (A, C, E) or presence of 20 μM PC190723 (B, D, F), and polymers formed were visualized by cryo-EM. Bar, 1000 Å. The insets in each case are enlarged areas (bar, 500 Å) and their computed diffractograms where the spacing of the main equatorial spots is indicated. A linear correlation of the spacing between protofilaments and the C-terminal length (excluding BsFtsZ-CTLA249) in the absence (G) and presence of PC190723 (H) resulted in 0.31 ± 0.02 Å and 0.38 ± 0.05 Å per residue, respectively.

**Figure S11. Electron micrographs of negatively stained EcFtsZ polymers with GMPCPP.** Related to Figure 5. EcFtsZ (4 μM except where indicated) was polymerized in Pipes250 buffer with 10 mM MgCl₂ and 50 μM GMPCPP at 25 °C. The assembly reaction was monitored by light scattering and samples were taken at different stages of de-polymerization due to nucleotide consumption. (A) Initial plateau (100% light scattering). (B)
depolymerizing sample (40% light scattering); bars (A,B), 2000 Å. (C, D) representative large circular structures observed at 40% light scattering; (E) highly curved and mini-ring like structures observed at 5% light scattering in EcFtsZ (50 μM) depolymerizing samples; bars (C,D,E), 1000 Å.

**Figure S12. Mg$^{2+}$-induced self-association of EcFtsZ with GDP and GMPCPP analyzed with sedimentation velocity (AUC).** Related to main text. Sedimentation coefficient distribution $c(s)$ of (A) EcFtsZ in Pipes250 buffer with 1 mM GDP and 10 mM MgCl$_2$, 25 °C, at 1.4 g/L (black line, $s_{20,w} = 2.4, 4.2$ and 5.8 S), 2 g/L (blue line, $s_{20,w} = 3.3, 4.9$ and 6.3 S) and 4 g/L (red line, $s_{20,w} = 3.1, 4.6, 5.8$ and 7.1 S); (B) EcFtsZ with 0.2 mM GMPCP instead of GDP, at 2 g/L (black line, $s_{20,w} = 3.4, 4.8$ and 6.2 S), 3 g/L (blue line, $s_{20,w} = 3.3, 5.0$ and 6.5 S) and 4 g/L (red line, $s_{20,w} = 2.8, 4.1, 5.4$ and 6.8 S). Note that in a self-association system in rapid equilibrium, the velocity of neither the slow nor the fast peaks at a finite protein concentration represents the sedimentation of any particular species; only when the association constant and concentration are large enough, the sedimentation of the fast peak may approach that of the rings (27). First theoretical estimates for the sedimentation coefficient of mini-rings made of 16 FtsZ monomers (Figure 5H) were calculated (Methods). For a ring of 220 Å diameter made of spheres equivalent to a 3.2 S monomer (Figure 1), $s_{20,w}^{0} = 15.3$ S; for a model ring made of monomer core structures (Figure S2, model j), $s_{20,w}^{0} = 13$ S; and from the ring average cryo-EM volume (Figure 5H), $s_{20,w}^{0} = 14$ S.
Figure S1
Figure S2
Figure S3
Figure S4
Figure S5

![Graph showing log I(q) vs q (Å⁻¹) for different samples labeled a-h. The graph includes end view markers for a-d and e-h.](image-url)
Figure S6

BsFtsZ-wt  BsFtsZ-ΔCt

GMPcPP

GTP
Figure S7

(A) BsFtsZ

(B) tag-BsFtsZ-ΔCt

(C) tag-BsFtsZ

(D) BsFtsZ

(E) GTP

(F) GMPCPP
Figure S8

A

Time (min)

GMP CPP

GTP

light scattering (au)

0 10 20 30

B 70-50% scat. (8 min)

C 20-5% scat. (11 min)

D GMPCP

E 50-30% scat. (18 min)

F 20-10% scat. (21 min)

G GDP
Figure S9
Figure S10

(A) BsFtsZ-CTLA50

(B) +PC190723

(C) BsFtsZ-CTLA100

(D) +PC190723

(E) BsFtsZ-CTLA249

(F) +PC190723

(G) Graph showing spacing between protofilaments (Å) vs. C-terminal length (number of residues)

(H) Graph showing spacing between protofilaments (Å) vs. C-terminal length (number of residues)