Supplemental Data

EXPERIMENTAL PROCEDURES

Proteins expression and purification. Untagged, full-length *ftsZ* from *B. subtilis* (ATCC 23857D) cloned into pHis17 (1) was sequenced. The deduced protein sequence was identical to the Uniprot *B. subtilis* FtsZ sequence P17865 (16 June 2009). The construction was transformed into *E. coli* C41(DE3) cells, grown in 2xYT medium (1L) with 100ug/mL ampicillin and expression induced with 1 mM IPTG (at Abs_{600nm} = 0.5, 3h at 37 °C). The cell pellet was resuspended in 20 ml of TKM buffer (50 mM Tris-HCl, 50 mM KCl, 1 mM EDTA, 5 mM $MgCl_2$, 10% glycerol, pH 8.0). Bs-FtsZ was purified as described (1) with modifications. PMSF (200 μg/mL) and leupeptin (2 μg/mL) were added to the cells in TKM buffer. Cells were disrupted by sonication and centrifuged at 100,000 x g for one hour. The protein in the supernatant was precipitated with 40% ammonium sulphate, resuspended in 50 mM Mes–KOH, 5 mM $MgCl_2$, 10% glycerol, pH 6.5, and loaded into a 5 mL anion-exchange chromatography HiTrap Q HP column (GE Healthcare). FtsZ was eluted using a 40 minute long 0-1M KCl gradient at 1ml/min. FtsZ was concentrated and subjected to a hydrophobic chromatography with a 1 ml Phenyl HP column (Amersham) in 50mM KH_2PO_4/K_2HPO_4-KOH, 1mM EDTA, 0.8M $(NH_4)2SO_4$, pH 7.5, eluted by depletion of $(NH_4)_2SO_4$ in a 100-0% gradient for 20 minutes at 1ml/min. Finally the protein was subjected to gel-filtration chromatography (Sephacryl S300 26/60 column, GE Healthcare) in 50 mM Tris–HCl, 50mM KCl, 1 mM EDTA, 10% glycerol, pH 7.5, concentrated to < 0.5 mL (about 1mM Bs-FtsZ) and stored at -80ºC. Bs-FtsZ preparations contained ~0.1 guanine nucleotide bound per FtsZ, determined by perchloric acid extraction (2). The molecular mass of full length Bs-FtsZ was confirmed by MALDI-TOF mass spectrometry. The Bs-FtsZ concentration was determined spectrophotometrically, after subtracting the nucleotide contribution (2), employing an extinction coefficient 2560 M^{-1} cm^{-1} at 280 nm (2 Tyr, 0 Trp residues).

The complete *S. aureus* (Mu50/ATCC 700699) *ftsZ* ORF was amplified with flanking oligos carrying targets for *NdeI* and *BamHI* enzymes, digested, cloned into pET29 and sequenced. The construction was transformed into *E. coli* BL21(DE3)pLys cells, grown in LB medium (1L) with 50ug/mL kanamycin and chloramphenicol and expression induced with 1 mM IPTG (3h at 37 °C). Sa-FtsZ was purified and its molecular mass confirmed as for Bs-FtsZ. It contained ~0.05 guanine nucleotide per FtsZ. The Sa-FtsZ concentration was determined spectrophotometrically, after subtracting the nucleotide contribution (2), employing and extinction coefficient 1960 M^{-1} cm^{-1} at 260 nm (14 Phe, 0 Tyr, 0 Trp residues).

FtsZ from *E. coli* was overproduced in transformed *E. coli* BL21(DE3) cells and purified with the Ca^{2+}-precipitation and anion-exchange method as described (2), yielding preparations with ~0.8 guanine nucleotide per FtsZ. The concentrations of Ec-FtsZ, Bs-FtsZ and Sa-FtsZ stocks were compared by quantifying their Coomassie stained bands in the same SDS-PAGE gels. Purities of the three different FtsZ are shown by Fig. S6.

Tubulin was purified from bovine brain and microtubules assembled in glycerol containing buffer with 1 mM GTP, pH 6.7, 37 ºC, as described (3), employing the scattering and electron microscopy methods described below.

Synthetic compounds. PC190723 (4) was > 99% pure in HPLC analysis. The absorption spectrum of PC in methanol has maxima at 200, 220 and 296 nm and a shoulder at 304 nm. The extinction coefficient at 296 nm is 7300 ± 300 M^{-1} cm^{-1} (7085 ± 200 M^{-1} cm^{-1} in DMSO).

DFMBA and DFHMBA were synthesized following described procedures (5). DFMBA: ^{1}H RMN (300 MHz, acetone-d^{6}): 3.89 (s, 3H); 6.98 (td, J = 9.0, 2.0 Hz, 1H); 7.14-7.22 (m, 2H); 7.42 (s, 1H). mp = 169.5-170.6 ºC. DFMBA was > 99% pure in HPLC-MS analysis. HPLC conditions: MeOH/H_2O-0.1% HCOOH-0.1% NH_4OH, 0-100% for 45 min, 0.5 mL/min, detection at 210/230/254/280 nm for 60 min, t_R: 18.5. *m*/*z* 188 [M+H]$^{+}$.

DFHBA: ^{1}H RMN (300 MHz, acetone-d^{6}): 6.87 (td, J = 8.8, 1.8 Hz, 1H); 6.99-7.07 (m, 1H); 7.26 (s, 1H); 7.48 (s, 1H); 8.85 (s, 1H). mp = 127.0-128.2 ºC. DFHBA was > 99% pure in HPLC-MS analysis. HPLC conditions: MeOH/H_2O-0.1 % HCOOH-0.1% NH_4OH, 0-100% for 45 min, 0.5 mL/min, detection at 210/230/254/280 nm for 60 min, t_R: 12.4. *m*/*z* 172 [M-H]$^{-}$.

CTPM was synthesized as follows. To a solution of 2-(benzyloxymethyl)-6-chlorothiazolo[5,4-*b*]pyridine (5) (1 g, 3.74 mmol) in distilled CH_2Cl_2 (2 mL), a solution of BBr_3 in DCM (19 mL, 1M) was added dropwise. The mixture was stirred at room temperature for 2 h. After the completion of the reaction, $NaHCO_3$ (18 mL, sat. solution) was added at 0 ºC and the mixture extracted with DCM (3x 40 mL). The organic layers were dried (Na_2SO_4) and evaporated. The resultant solid was purified by column chromatography on silica (Hexane/EtOAc 8:2 to EtOAc) to afford the desired compound in 83% yield. ^{1}H RMN (300 MHz, $CDCl_3$): 5.09 (s); 8.21 (d, J = 2.2 Hz, 1H); 8.55 (d, J = 2.2 Hz). ^{13}C RMN (75 MHz, $CDCl_3$): 63.6 (CH_2); 129.8 (CH); 130.3 (C); 146.4 (CH); 146.9 (C), 156.3 (C); 176.2 (C). mp = 194.3-195.5 ºC. CTPM was > 99% pure in HPLC-MS analysis. HPLC conditions: MeOH/H_2O-0.1 % HCOOH-0.1% NH_4OH, 0-100% for 45 min, 0.5 mL/min, detection at 210/230/254/280 nm for 60 min, t_R: 123.0. *m*/*z* 201.0 [M-H]$^{+}$

MBA was from Aldrich.

Monitoring FtsZ assembly with light scattering. FtsZ (10μM in 0.5 mL filtered buffer, to which ligands were added) was placed in a 10 x 2 mm (excitation path) cell in a photon-counting Horiba-Jovin-Yvon FluoroMax-4 spectrofluorometer thermostated at 25ºC. The 350 nm light scattered at 90º (with 0.5 nm excitation and emission band pass), was normalized by the internal reference beam and was recorded in 1 second time frames. The reaction was started by consecutive additions of 10 mM $MgCl_2$ and guanine nucleotide. The arbitrary scattering units employed through this work are 10^{-6} x counts per second (sample) / microAmps (reference).

Electron Microscopy. For negative staining, samples (10-20μl) were adsorbed to carbon-coated copper electron microscopy grids and stained with 2% uranyl acetate. For cryo-electron microscopy (cryo-EM), samples were applied to grids with holey carbon film (Quantifoil) after glow-discharge and immediately blotted and vitrified using the automatic GATAN cryo-plunge. Micrographs were taken at x40000 magnification in a Jeol 1230 electron microscope operated at 100 kV and equipped with a GATAN liquid nitrogen specimen holder for cryo-EM. Cryo-electron micrographs were taken from the hole areas, where the ice lacks any supporting film underneath, under low dose conditions and different defocus. Micrographs were digitized with a Minolta Dimage Scan Multi Pro scanner at 2400 dpi, corresponding to 0.265 nm per pixel. To measure the thickness of the FtsZ filaments, the images were saved as .PNG files and measured with Image-J. Measurements obtained in pixels were multiplied by 0.265 nm per pixel (6). Cryo-EM images were subsequently low-pass filtered to remove the noise present at high frequencies using EMAN.

FtsZ polymers pelleting. Samples (0.1 mL) were prepared in polycarbonate ultracentrifuge tubes in a thermostat at 25ºC. Control samples included one where GTP was substituted with GDP and another lacking $MgCl_2$. Polymerization was initiated by the addition of nucleotide or of 10μM FtsZ and typically allowed to proceed for 15 minutes before centrifugation at 386,000 x g (100,000 rpm) for 20 or 80 minutes at 25ºC in a TLA-100 rotor with an Optima TLX ultracentrifuge (Beckman). To pellet FtsZ bundles with reduced filament sedimentation, samples were centrifuged at 15,000 x g (20,000 rpm). Supernatants were removed and added one equivalent volume of SDS-PAGE sample buffer. Pellets were carefully resuspended in one volume of sample buffer and one volume buffer added. Pellet and supernatant aliquots from each sample were loaded with a 15 minute eletrophoretic shift between them into the same lanes of SDS-12% polyacrylamide gels. Gels were stained with Coomassie blue, scanned and the protein bands quantified using Quantity One software (BioRad).

Screen of solution conditions. The effects of the solution variables on the spontaneous and PC-enhanced assembly of FtsZ (10 μM) were screened with series of small volume sedimentation and electron microscopy tests, complemented with light scattering. Substitution of K by Na reduced polymerization. Bs-FtsZ polymerization was sensitive to the buffer anion. The order of effectiveness was Mes > Hepes > Pipes > Mops ~Bis-Tris (50 mM, pH 6.6). For example, Bs-FtsZ barely assembled in Pipes with GTP (in contrast with Ec-FtsZ), but formed filaments with GMPCPP or bundles upon PC addition.

Binding of PC to FtsZ polymers. Samples (0.2 mL) containing 10 μM FtsZ and different concentrations of PC in Hepes assembly buffer pH 6.8 with 2 mM GTP were centrifuged for 20 min as above. The supernatants were collected and the pellets were resuspended in the same buffer. To measure the concentration of pelleted FtsZ and FtsZ in the supernatant, 5 μl of the samples were separated and applied to a SDS-PAGE gel. Ten micromolar taxol was added as the internal standard to each sample. Both the pellets and the supernatants were then extracted three times with an excess volume of dichloromethane, dried in vacuum, and dissolved in 35 μl of a methanol/water (v/v: 80/20) mixture. PC bound to pelleted polymers and free in the supernatant were determined by HPLC analysis with a C-18 column (Eclipse XDB-C18, 4.6 x 150 mm, 5 μm bead size) eluted with a gradient of 5 mM ammonium acetate to acetonitrile (10% to 90%) in 30 minutes and at a flow rate of 0.5 ml/min.

GTPase. GTP hydrolysis in FtsZ solutions at 25ºC was measured from the released inorganic phosphate employing the malachite green method (7). GTPase units are defined as mol of phosphate per mol protein per minute.

Binding of ^{3}H-GTP to FtsZ. Samples (0.2 mL) containing 1 μM FtsZ and 1 μM ^{3}H-GTP (Amersham; diluted into cold GTP to give about 50000 cpm per sample) were prepared in polycarbonate ultracentrifuge tubes at 25ºC. Control samples contained and excess of GDP (100μM). Samples were centrifuged at 386000 x g for 1.5 hours in a TLA100 rotor at 25 25ºC. Samples were carefully divided into a top and a bottom half, an equivalent volume of buffer (100μl) was added and the free GTP concentration was determined in the protein-depleted top half of tubes, after dilution in 3.8 ml of Beckman ReadySafe solution, employing a LKB Wallac 1219 Rackbeta liquid scintillation counter (PerkinElmer Life Sciences). Bound GTP was determined by difference to the total GTP concentration. In each assay, controls without FtsZ were included, and concentrations were corrected for the small amount of ^{3}H-GTP sedimented in the absence of protein.

REFERENCES

1.Oliva, M.A., Trambaiolo, D., and Löwe, J. (2007) *J. Mol. Biol*. **373**, 1229-1242.

2. Rivas, G., López, A., Mingorance, J., Ferrándiz, M.J., Zorrilla, S., Minton, A.P., Vicente, M,, and Andreu, J.M. (2000) *J. Biol. Chem.* **275**, 11740-11749.

3. Andreu, J.M. (2007) *Meth. Mol. Medicine* **137**, 17-28.

4. Haydon, D.J., Stokes, N.R., Ure, R., Galbraith, G., Bennett, J.M., Brown, D.R., Baker, P.J., Barynin, V.V., Rice, D.W., Sedelnikova, S.E., Heal, J.R., Sheridan, J.M., Aiwale, S.T., Chauhan, P.K., Srivastava, A., Taneja, A., Collins, I., Errington, J., and Czaplewski, L.G. (2008) *Science* **321**, 1673-165.

5. Brown, D.R., Collins, I., Czaplewski, L.G., and Haydon, D.J. (2007) Preparation of substituted benzamides and pyridylamides as antibacterial agents. *PCT Int. Appl*. WO 2007107758 A1 20070927.

6. Huecas, S., Llorca, O., Boskovic, J., Martin-Benito, J., Valpuesta, J.M., and Andreu J.M. (2008) *Biophys. J.* **94**, 1796-1806.

7. Kodama, T., Fukui, K., and Kometani, K. (1986) .*J. Biochem.* **99**, 1465-1472.

8. Abal, M., Souto, A.A., Amat-Guerri, F., Acuña, A.U., Andreu, J.M., and Barasoain, I. (2001) *Cell Motil. Cytoskeleton* **49**, 1-15.

TABLE S1. Bs-FtsZ polymorphic assemblies observed under various solution conditions and with PC190723 at 25 ºC

POLYMER TYPE (spontaneous)	NUCLEOTIDE, CATION	BUFFER
short filament (few)	GTP/GMPCPP	Hepes 250 mM KCl pH 6.8
Filaments	GTP/GMPCPP, Mg^{2+}	Hepes 50 mM KCl pH 6.8 or pH 7.5 Hepes 250 mM KCl pH 6.8
curved filaments	GMPCPP, Mg^{2+}	Hepes 50 mM KCl pH 6.8
coils ~200 nm Ø	GMPCPP, Mg^{2+}	Hepes 50 mM KCl pH 6.8 Mes 50 mM KCl pH 6.8
toroids ~150 nm Ø	GMPCPP/GTP, Mg^{2+}	Hepes 50 mM KCl pH 6.8

		Mes 50 mM KCl pH 6.8
POLYMER TYPE (with PC190723)		
toroids ~300 nm Ø	GTP	Hepes 50 mM KCl pH 6.8
curved filaments	GTP/GMPCPP	Hepes 250 mM KCl pH 6.8
coils ~200 nm Ø	GTP/GMPCPP	Hepes 250 mM KCl pH 6.8
ribbon and bundle	GTP/GMPCPP, Mg^{2+}	Mes 50 mM KCl, Hepes 50 mM KCl pH 6.8, eventually Hepes 250 mM KCl
helical bundle	GTP, Mg^{2+}	Hepes 50 mM KCl pH 7.5

FIGURE LEGEND

Fig. S1. Polymers formed by Bs-FtsZ (10 μM) in Hepes250 assembly buffer with 2 mM GTP (A), 2 mM GTP plus 20 μM PC (B), 2 mM GTP plus 10 μM $MgCl_2$ (C), and 2 mM GTP plus 10 mM $MgCl_2$ and 20 μM PC (D). The bar indicates 200 nm

Fig S2. PC-induced Bs-FtsZ polymers sedimentation in Hepes250 assembly buffer with 10 mM $MgCl_2$ and GMPCPP (0.1 mM, upper panel) or GTP (2 mM, lower panel). The solid circles are with 20 μM PC, void circles without PC, solid triangles with GMPCP or GDP, void triangles without $MgCl_2$. The X-axis intercept values were: GTP, 0.0 ± 0.1 μM; GMPCPP, 0.1 ± 0.4 μM.

Fig. S3. Effects of PC on the binding of ^{3}H-GTP (1 μM) to Bs-FtsZ (1 μM) (circles) and Ec-FtsZ (squares) in Hepes50 assembly buffer with 10 mM $MgCl_2$. The effects of controls with 100 μM GDP added are indicated (triangles).

Fig. S4. A comparison of the effects of PC (20 μM, solid red lines) on the polymerization of FtsZ from susceptible (Bs, Sa) and resistant bacteria (Ec) (dashed lines are without PC). A, Bs-FtsZ. B, Sa-FtsZ. C, Ec-FtsZ. D, Ec-FtsZ with GTP regenerating system (1 unit/mL acetate kinase and 15 mM acetyl phosphate) monitored by light scattering. Conditions: Hepes50 buffer with 10 mM $MgCl_2$ and 1 mM GTP, 10 μM FtsZ, 25 ºC.

Fig. S5. Spindle pole delocalizing effects of taxol on mitotic of A549 human lung carcinoma cells. A and B, no taxol. C and D, 300 nM taxol (24 hour). This figure is only intended to illustrate the analogy, saving systems differences, with the delocalizing effects of PC on the FtsZ ring of *B. subtilis* cells (see Fig. 2B in ref. 4). The bar indicates 10 μm. The microtubules were stained with a monoclonal antibody to alpha-tubulin and a secondary fluoresceinated antibody (green) and DNA with Hoescht 33342. Figure courtesy of I. Barasoain, Centro de Investigaciones Biológicas (see ref. 8 for related results).

Fig. S6. SDS-PAGE of FtsZ preparations. Purities were: Bs-FtsZ 98%, Sa-FtsZ 93% and Ec-FtsZ > 98%, estimated by scanning and quantifying Coomassie blue stained gels. The mobilities of apparent molecular weight markers (KDa) are indicated on the left. Trace contaminants identified by tryptic peptide MALDI-MS/MS fingerprinting in Bs-FtsZ preparations were beta-galactosidase (LacZ) and DnaK from the Ec expression system.

Fig. S1

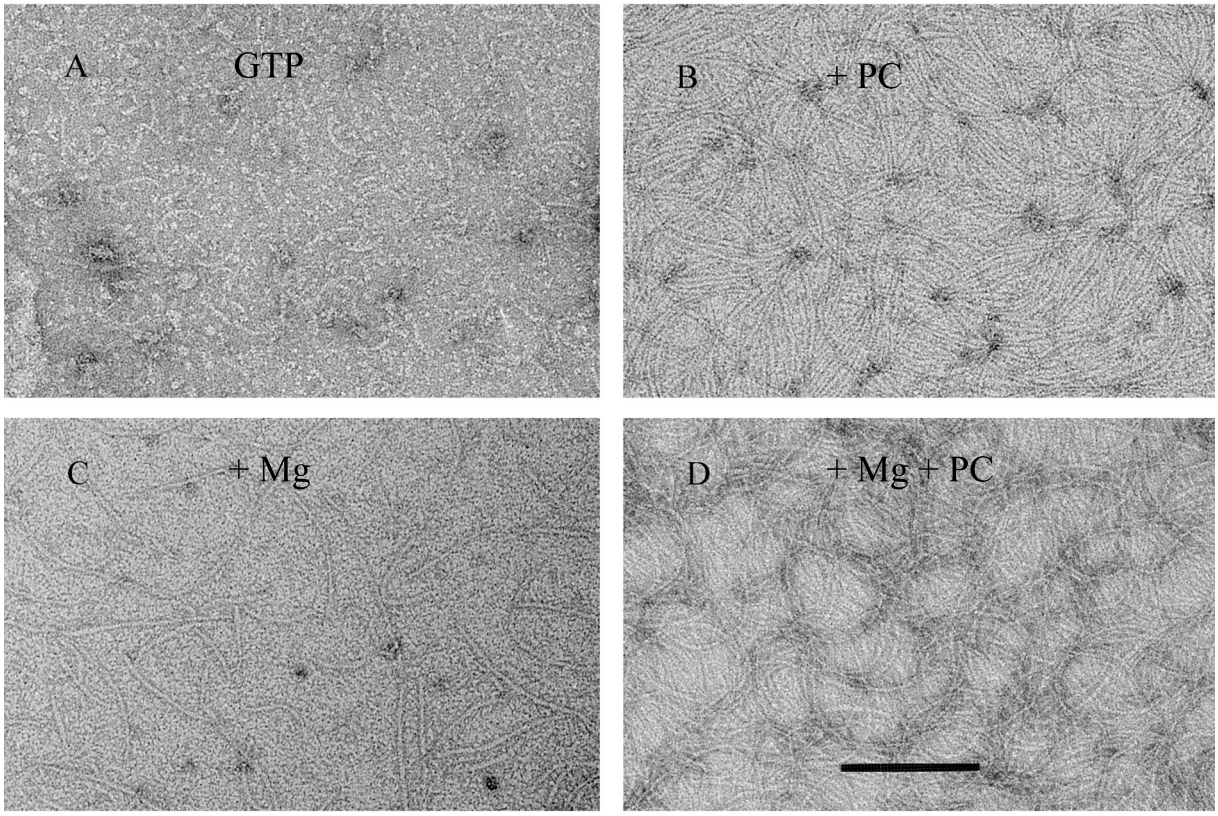
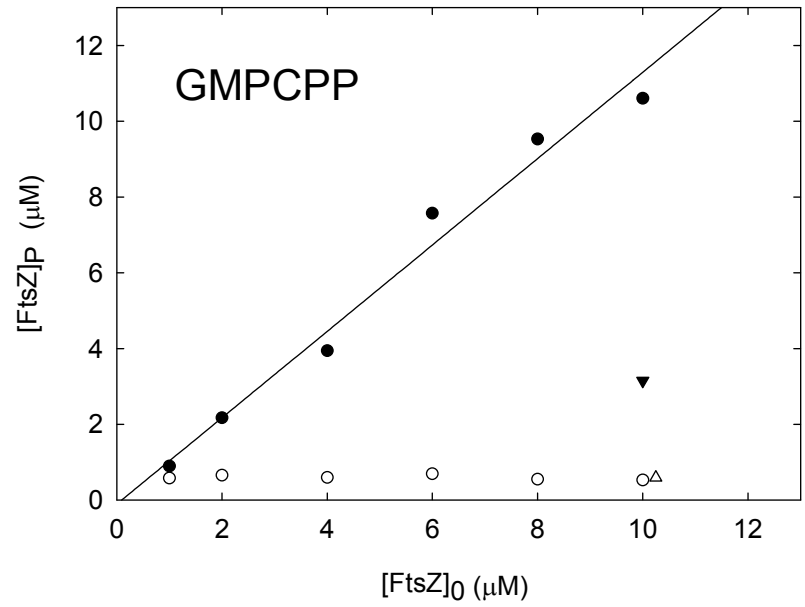


Fig. S2


GMPCPP
[FtsZ]P (μM)
[FtsZ]0 (μM)
0
2
4
6
8
10
12

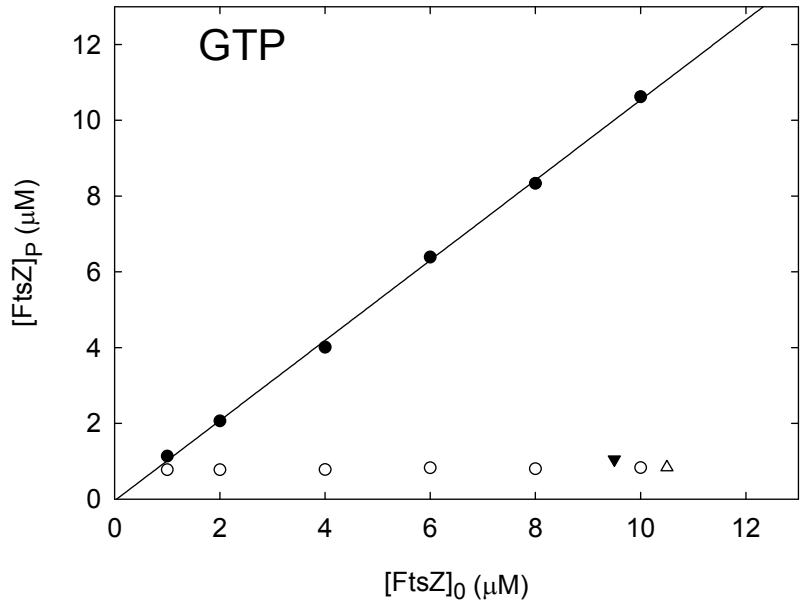

GTP
[FtsZ]P (μM)
[FtsZ]0 (μM)
0
2
4
6
8
10
12

Fig. S3

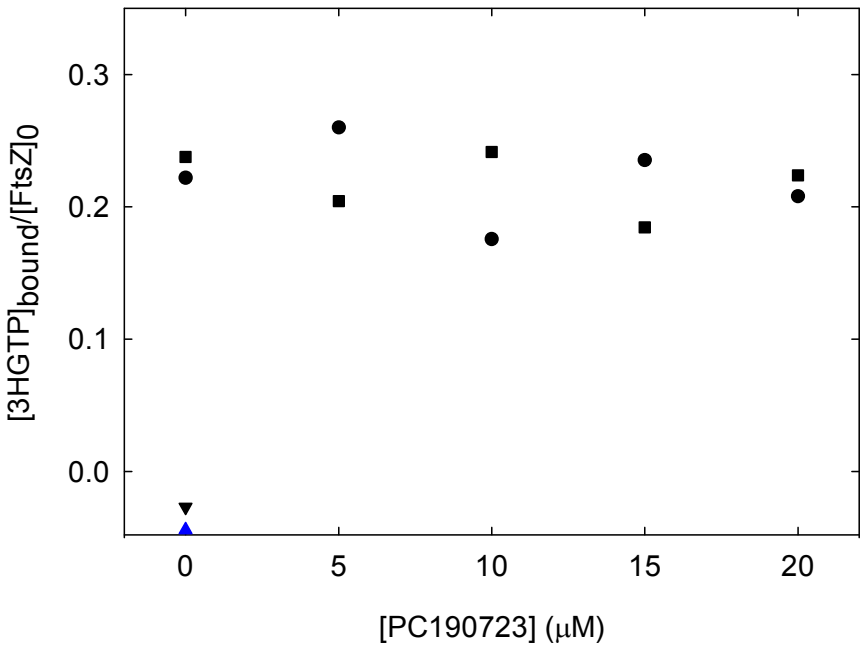


Fig. S4

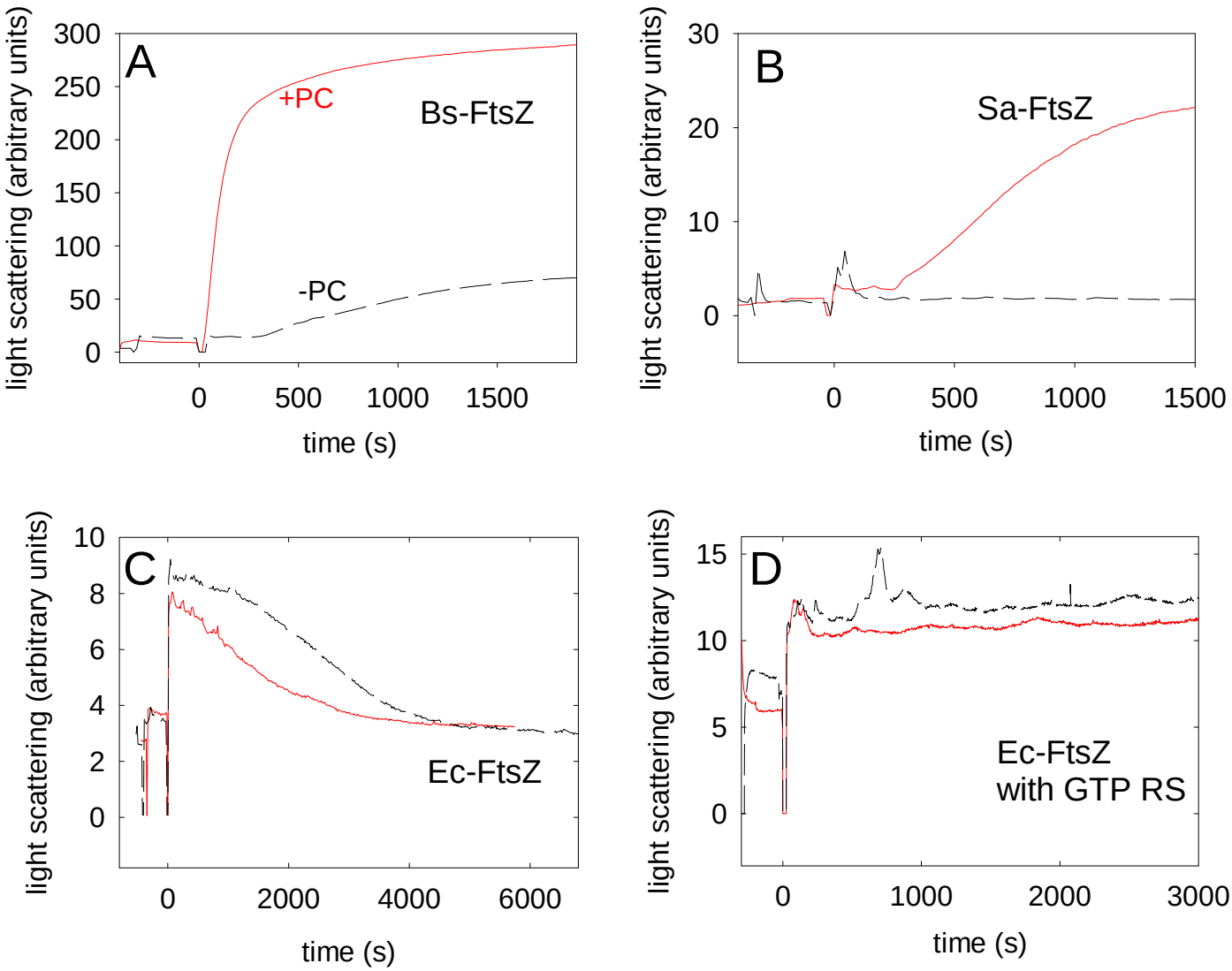
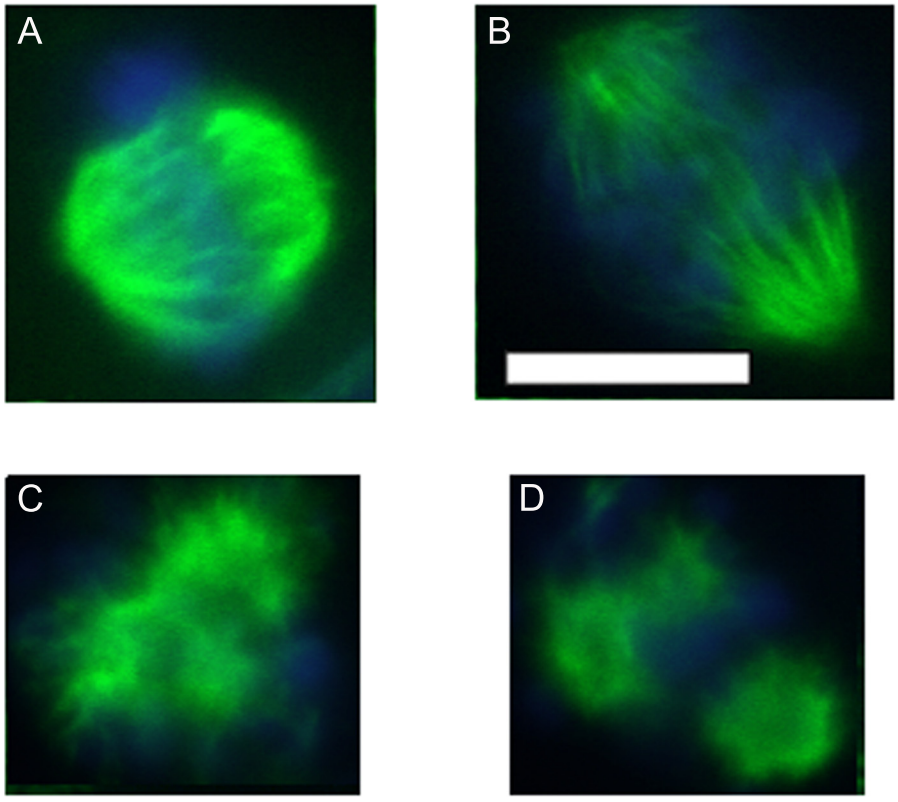
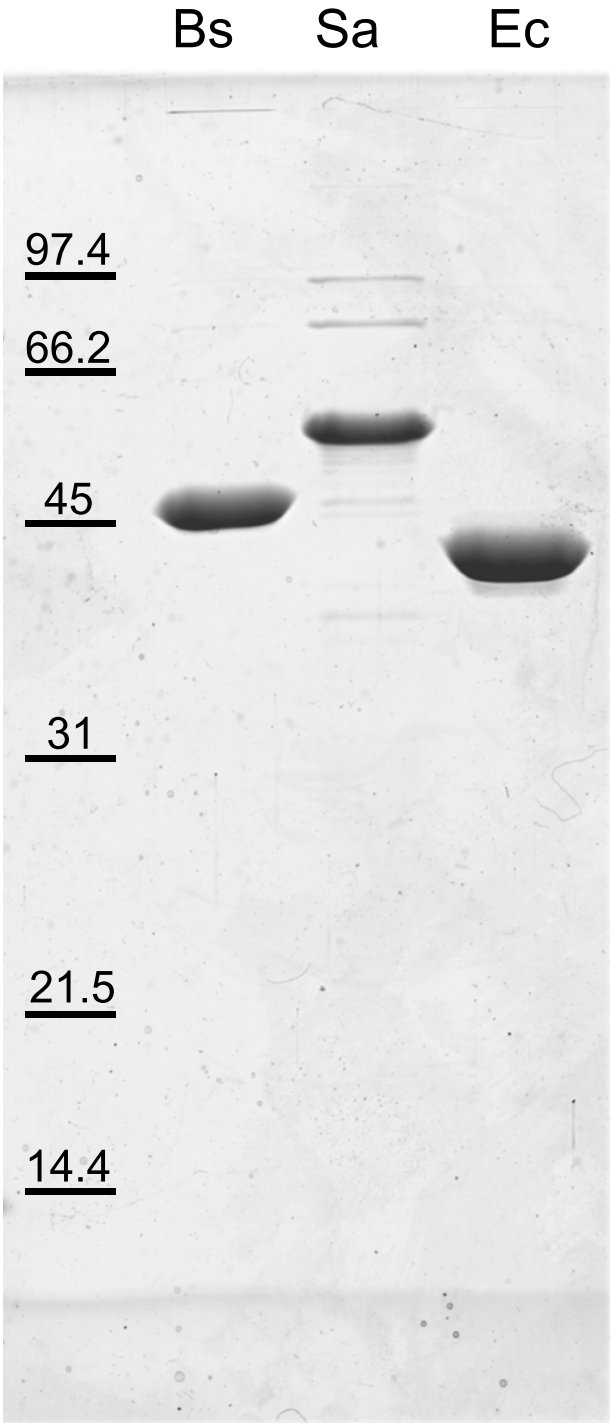

A
+PC
-PC
Bs-FtsZ
light scattering (arbitrary units)
time (s)
B
Sa-FtsZ
light scattering (arbitrary units)
time (s)
C
Ec-FtsZ
light scattering (arbitrary units)
time (s)
D
Ec-FtsZ
with GTP RS
light scattering (arbitrary units)
time (s)

Fig. S5




Bs
Sa
Ec
97.4
66.2
45
31
21.5
14.4