Supporting Information

Chiral vortex dynamics on membranes is an intrinsic property of FtsZ, driven by GTP hydrolysis

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Supplementary methods

Sedimentation velocity. Sedimentation velocity analysis was performed at 0.5 mg/ml of FtsZ-YFP-mts (0.5 mg/ml) equilibrated in 50 mM Tris-HCl, 150 mM KCl, pH 7.5 buffer, in the absence or presence of GTP and $MgCl_2$, as specified. The experiments were carried out at 38,000 rpm and at 20 ºC in an XL-I analytical ultracentrifuge (Beckman-Coulter Inc.) equipped with a UV-VIS detection system, an An-50 Ti rotor and 12 mm double-sector centrepieces. The sedimentation coefficient distributions were calculated by least-squares boundary modelling of sedimentation velocity data using the c(s) method as implemented in the SEDFIT program[1].

GTPase activity. FtsZ GTPase activity was determined using the BIOMOL GREEN reagent for phosphate detection (Enzo). In brief FtsZ at 5 µM concentration is measured every 20 seconds after adding 1 mM GTP for a total of 7 time points. After 15 minutes of incubation with Biomol Green, the samples are measured OD_{620nm}. The data is later fit to a standard curve of 40 µM phosphate.

(1) Schuck P. (2000) Size-distribution analysis of macromolecules by sedimentation velocity ultracentrifugation and lamm equation modeling. Biophys J. 78:1606-1619

Supplementary figures

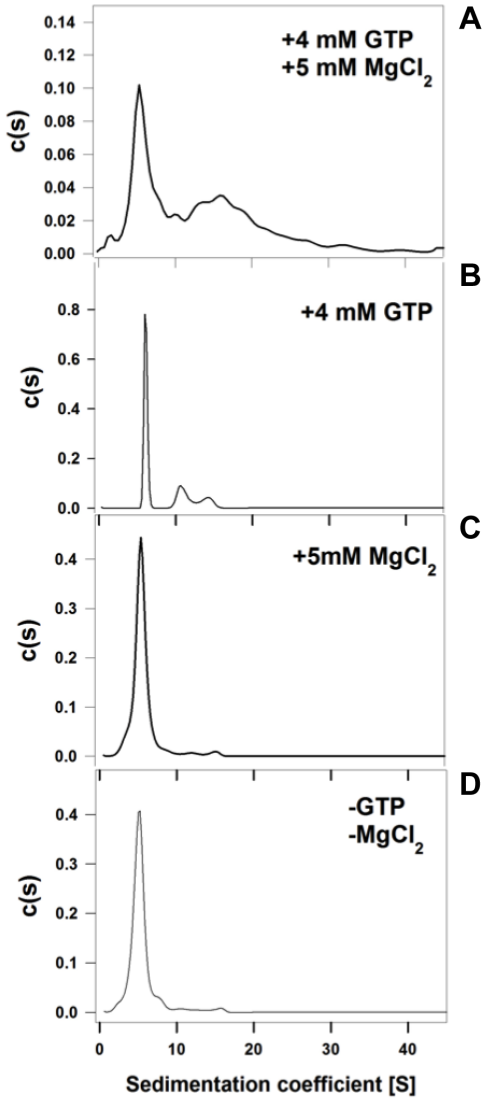
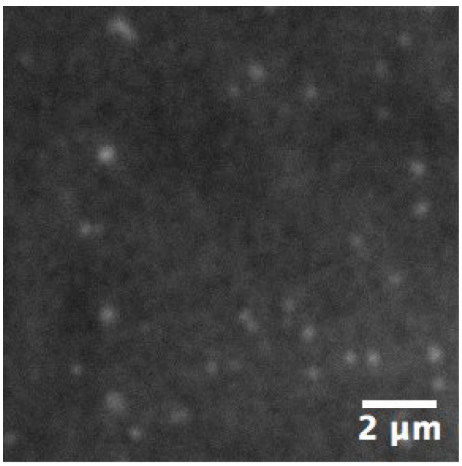


FIGURE S1. Distribution of sedimentation coefficients obtained from sedimentation velocity analysis of FtsZ-YFP-mts with 150 mM of salt. Four different conditions were tested. (A) When GTP and Mg^{2+} are absent, polymers are not observed, and the sedimentation coefficient distribution shows a single main peak, comparable with monomeric protein, at 5.5 S. (B) Similar results are observed when only Mg^{2+} is added. (C) In the presence of only GTP, some oligomers are present. (D) At 4 mM GTP and 5 mM Mg^{2+}, the sedimentation-velocity behaviour changes dramatically when compared with the other conditions. The sample is polydisperse. The distribution profile shows polymers of different sizes.



0 mM GTP

FIGURE S2. No visible structures are observed when GTP is absent. Representative image after adding 0.5 µM FtsZ-YFP-mts in buffer solution with 5 mM Mg^{2+}.

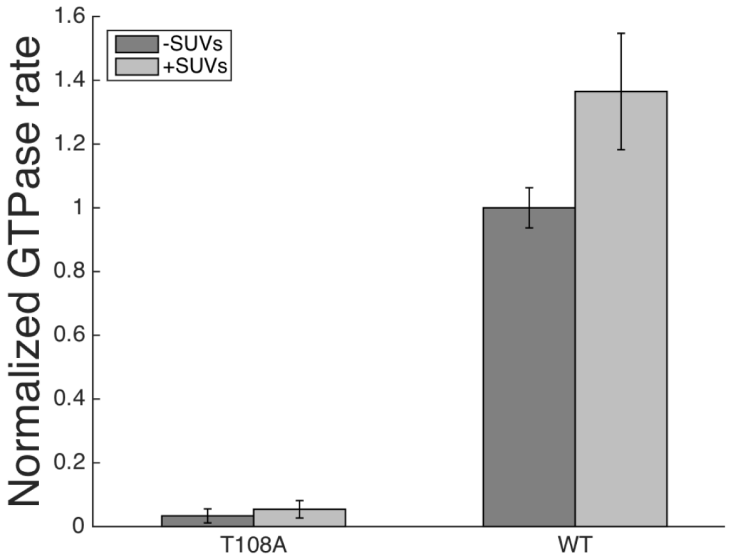


FIGURE S3. GTPase activity of FtsZ-YFP-mts (5 µM) or FtsZ*[T108A]-YFP-mts (5 µM) in the absence or presence of phospholipids (4 mg/ml). The corresponding rates where normalized to the GTP activity of FtsZ-YFP-mts in the absence of phospholipids. We observed that the GTPase activity of FtsZ*[T108A]-YFP-mts was almost zero. GTPase activities were determined using the BIOMOL GRENN assay (Enzo). Error bars correspond to s.d. from three different experiments.

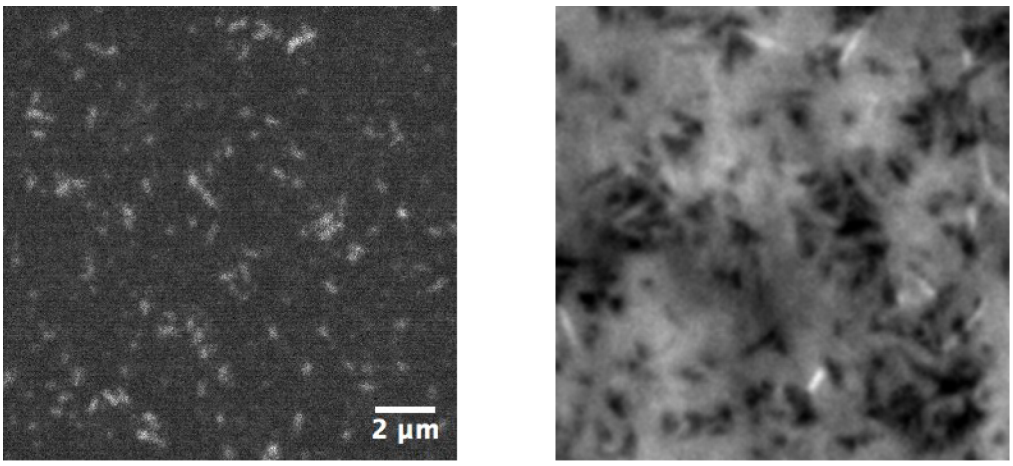


FIGURE S4. Representative images of FtsZ-YFP-mts at low (left panel) and high (right panel) protein concentrations. No polymers could be detected at 0.1 µM. On the contrary, when 1 µM of FtsZ-TFP-mts is added, polymer networks were observed almost instantly at the vicinity of the membrane. Dynamic rings were only noticed at intermediated protein concentrations.

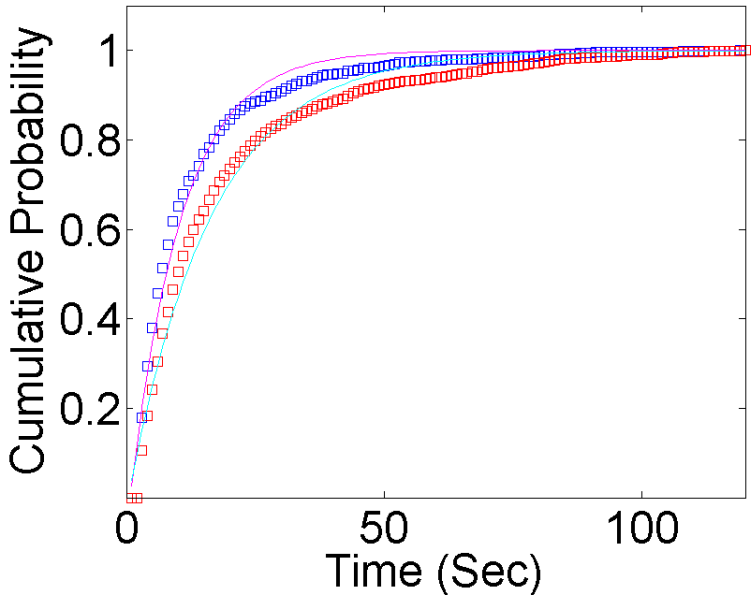


FIGURE S5. To show that photobleaching contribution does not affect the trend observed with the single molecule results from **Figure 5**, we performed the same set of experiments with half laser intensity. Blue squares represent the cumulative probability of ~3000 events at 4 mM whereas red squares refer to the cummulative probability of ~6000 events at 0.04mM. The fit to an single exponential $1 - exp(-t/\tau)$ for both GTP concentrations reveals than τ for low concentration GTP is about 1.5 fold longer than high GTP (~10.9 sec), agreeing with the findings of the main text.