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Structural and Functional Model for Ionic (K⁺/Na⁺) and pH Dependence of GTPase Activity and Polymerization of FtsZ, the Prokaryotic Ortholog of Tubulin.

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SUMMARY

Bacterial cell division occurs through the formation of a protein ring (division ring) at the site of division, FtsZ being its main component in most bacteria. FtsZ is the prokaryotic ortholog of eukaryotic tubulin; they share GTPase activity properties and the ability to polymerize *in vitro*. To study the mechanism of action of FtsZ, we used molecular dynamics (MD) simulations of the behavior of the FtsZ dimer in the presence of GTP-Mg²⁺ and monovalent cations. The presence of a K⁺ ion at the GTP binding site allows the positioning of one water molecule that interacts with catalytic residues Asp235 and Asp238, which are also involved in the coordination sphere of K⁺. This arrangement might favor dimer stability and GTP hydrolysis. Contrary to this, Na⁺ destabilizes the dimer and does not allow the positioning of the catalytic water molecule. Protonation of the GTP gamma-phosphate, simulating low pH, excludes both monovalent cations and the catalytic water molecule from the GTP-binding site and stabilizes the dimer. These MD predictions were contrasted experimentally by analyzing the GTPase and polymerization activities of purified *Methanococcus jannaschii* and *Escherichia coli* FtsZ proteins *in vitro*.

Keywords: FtsZ; molecular dynamics; GTPase; polymerization; monovalent cations; pH dependence.

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Abbreviations used: MD, molecular dynamics; FtsZ_{EC}, *Escherichia coli* FtsZ; FtsZ_{MJ}, *Methanococcus jannaschii* FtsZ.

FtsZ is the central protein of the prokaryotic cell division machinery. It is the most abundant among all the known essential division proteins, the first to localize to the division site, together with FtsA and ZipA^{1; 2}, and the most conserved throughout the bacterial and archaeal domains^{3; 4; 5}. FtsZ localizes to the cell center prior to division and forms a protein ring, the Z-ring, beneath the inner cell membrane⁶. Rings formed by several FtsZ filaments, 5 nm in diameter and spaced an average of 9.3 nm apart, have been observed *in vitro*^{7; 8}. In addition to the central role that it plays in division as an organizer for the rest of the division complexes, it has been suggested that the Z-ring itself has an active motor function during cytokinesis⁹. Force-generating mechanisms have been previously proposed based on the structural or physical properties of FtsZ filaments^{10; 11; 12}, but specific molecular explanation is lacking.

Sequence and structural comparisons of FtsZ to eukaryotic tubulin indicate that the two proteins are homologous^{3; 13}. As tubulin, FtsZ binds GTP and GDP *in vitro*, has GTPase activity and polymerizes in a GTP-dependent manner^{3; 13}. Nucleotide binding is independent of any other factor, but the catalytic and polymerization activities depend on the presence of divalent and monovalent cations^{14; 15; 16}. The physiological divalent cation is Mg⁺⁺, which is bound to the phosphates of the nucleotide and it has been implicated in oligomerization¹⁷.

In the case of FtsZ, the role of the monovalent cation is not known. The binding affinity is very low, in the mM range, and the cation itself has not been detected in crystallographic structures, experimentally obtained in the presence of lithium salts and in the absence of Na⁺ or K⁺. There are some differences between bacterial species with regard to the cation preference, so while FtsZ from *Azotobacter vinelandii* or *Thermotoga maritima* are activated by Na⁺ and K⁺, FtsZ from *E. coli* shows some preference for K⁺ at neutral pH, but may be activated by Na⁺ at low pH (6.0-6.5) or high protein concentration¹⁸. In *M. jannaschii*, FtsZ activity depends exclusively on the presence of K⁺; it is not measurable for any Na⁺ concentration at neutral pH¹⁹. In *E. coli* FtsZ, cation binding is

linked to polymerization, whereby the uptake of one ion is associated with the incorporation of one monomer into the growing polymer¹⁶.

Biochemical studies and macromolecular structures of several proteins bound to K⁺ or Na⁺ at the active sites show a significant role for monovalent cations in the activity of a broad range of enzymes^{20; 21; 22}. It is not known which is the physiological role that the activation of FtsZ by the monovalent cation might have. In spite of the low binding affinity, the intracellular concentration of K⁺ is probably always high enough to saturate FtsZ in growing cells (0.1-0.5 M)¹⁶ and therefore, K⁺ cannot temporally or spatially control FtsZ polymerization. It has rather been proposed that K⁺ provides a weak interaction between monomers that might be important in determining the highly dynamic behavior of FtsZ polymers¹⁶.

In the present work, we studied the role of monovalent cations in FtsZ activity by means of molecular dynamics (MD) and we have corroborated those *in silico* results by measuring GTPase activity and polymerization.

Modeling structural stability of FtsZ dimer in presence of K⁺ or Na⁺

We aim to explain the differential behavior of FtsZ in the presence of Na⁺ or K⁺^{16; 19} and used MD techniques to simulate the effect of these ions on the FtsZ dimer structure. We used the crystallized form of the *Methanococcus jannaschii* FtsZ dimer²³ as a base. We hoped to perform additional simulations with the FtsZ dimer from *E. coli*, but no crystal structure has yet been obtained for this protein and the quality of homology models of the *E. coli* FtsZ dimer is too poor for them to be used in MD. We performed computational simulation using the structure of FtsZ from *M. jannaschii* (FtsZ_{MJ}) and *in vitro* analysis of GTPase and polymerization activities of FtsZ_{MJ} and *E. coli* FtsZ (FtsZ_{EC}).

The crystal structure of FtsZ_{MJ} (PDB entry 1W5A)²³ was subjected to initial equilibration before starting MD simulation using the AMBER suite²⁴. Two different conditions, by either adding K⁺ or Na⁺, were used in a Coulombic potential grid to ensure the electrical neutrality of the system. Figure 1a shows the positioning of the Na⁺ or K⁺ atoms in the solvent boxes around the FtsZ_{MJ} dimer after equilibration. In both cases an atom of the monovalent cation was found in the interface between the two protein monomers (Fig 1a, black arrows), close to the GTP binding site. This is likely a consequence of the negative charge of GTP. Next, a 5 nanoseconds MD simulation was performed, and changes in structure of FtsZ were followed by measuring root mean square deviation (RMSD) values of the whole protein dimer and of each monomer with respect to the initial position in the crystal. To measure changes in dimer arrangement, the distances between selected pairs of aminoacids located at the interface between monomers were continuously annotated. Those pairs of residues correspond to two of the three protein-protein contacts described in the original report of FtsZ_{MJ} dimer structure²³: the Cα-Cα distance between Asp 26 (upper monomer) and Lys 80 (lower monomer), positioned in the area of the H0 helix-contact (blue arrows in Figure 1a); and the Cα-Cα distance between Glu 302 (upper monomer) and Lys 205 (lower monomer), located in the opposite position of the interface between monomers (red arrows in Figure 1a). Continuous measurements of the RMSD and selected distances are shown in Figure 1b. No significant protein movements were observed during the MD trajectory when K⁺ was used as the counterion, indicating that the contacts between domains remained unchanged, with an average global RMSD of the upper monomer of 1.88 ± 0.11 Å in the last 2 ns of the simulation. However, in the presence of Na⁺, RMSD values for the displacement of the upper monomer with respect to the lower one increased to 2.87 ± 0.24 Å on average in the last 2 ns, and the distance between Glu 302 and Lys 205 increased from 4 to 13 Å. This indicates the rupture of the selected contact. No relevant intra-domain movements were observed in either case, nor were changes in the relative position of GTP and Mg⁺⁺ with respect to the contact residues in the lower monomer of FtsZ.

GTP binding site and GTPase activity.

Our results suggested some important changes in the dimer interface between the presence of K⁺ or Na⁺. Figure 2a shows the structure of the catalytic site of the two dimers after 5ns of MD simulation in detail. In the presence of K⁺, the GTP binding site has a water molecule located in a position compatible with the attack distance of the γ -phosphorus of GTP. This water molecule is in the coordination sphere of the K⁺ ion (the distance between the K⁺ and the water oxygen is 3 Å), and at the same time it is in contact with the catalytic residues Asp 235 and Asp 238 of the upper monomer. However, in the presence of Na⁺ (Fig. 2a, right), the smaller coordination sphere of this cation does not allow the positioning of a water molecule in the vicinity (including the original water molecules located in the active site in the crystal structure); its position is occupied by oxygen atoms from the Asp 238 residue and a phosphate group. Here, the absence of the water molecule necessary to hydrolyze the GTP must result in the absence of GTPase activity in the dimer.

Thus, we propose that K⁺ might activate the GTPase activity of FtsZ, but Na⁺ cannot. As both Na⁺ and K⁺ are bound to the same site in the dimer structure, our results also suggest that Na⁺ might be a competitive inhibitor of K⁺ for the GTPase activity of FtsZ.

In addition, although both ions retain the capacity to connect the two protein monomers via the GTP molecule and maintain the dimerized structure, the final positioning of bridging residues is different (Figs 1b and 2a). This affects their polymerization properties.

To test this hypothesis, the GTPase activities of purified FtsZ_{MJ} and FtsZ_{EC} were calculated in the presence of different concentrations of K⁺ and Na⁺. As shown in Figure 2b, Na⁺ is a competitive inhibitor of K⁺ for FtsZ_{MJ}, as predicted by the MD model. In contrast, Na⁺ did not compete with K⁺ in FtsZ_{EC}, most likely due to differences in the affinities for the two cations in this

system¹⁶. It should be noted that the MD simulation was done only for FtsZ_{MJ}, as there are no structures of FtsZ_{EC} available.

Correspondence between GTPase activity at different concentrations of Na⁺ and the capacity of FtsZ to polymerize was analyzed by light-scattering (Fig. 2c). In agreement with the corresponding GTPase activity and MD simulation results discussed above, FtsZ_{MJ} polymerization was almost completely inhibited when the Na⁺ concentration was increased from 0 to 250 mM (Fig. 2c, right) even at a high K⁺ concentration (500 mM). This confirms the role of Na⁺ as a competitive inhibitor of this process. FtsZ_{EC} polymerization at a K⁺ concentration of 150 mM (Fig. 2c, left) was also partially inhibited when Na⁺ concentration was increased from 0 to 100 mM, but in agreement with the results obtained for GTPase activity, polymer formation cannot be completely inhibited by further increases in Na⁺ concentration, even to 1M.

Effect of pH on GTPase activity and polymerization of FtsZ.

Different reports indicate that at pH values lower than 6.8 FtsZ_{EC} GTPase activity decreases, but it retains its capacity to polymerize even in the absence of K⁺^{13; 14; 18; 19}. To study the apparent contradiction between GTPase activity and polymerization capabilities, we have compared results obtained from MD and from biochemical techniques. MD simulations of low pH conditions can be done by protonation of histidine residues and GTP, as pKa values of both molecules are in the same range, around 6.8²⁵. As there are no histidine residues located in the vicinity of the active site of FtsZ, and they are not involved in essential interactions for maintaining FtsZ dimer structure, acidic conditions were established only by protonation of the gamma-phosphate of GTP prior to MD equilibration. The first consequence of GTP protonation was the absence of the positive monovalent cation (both Na⁺ and K⁺) in the dimer interface during equilibration. This was probably due to the lower negative charge compared to unprotonated GTP (Fig. 3).

After equilibration, a 5-nanosecond MD simulation was performed to measure the stability of the dimerized structure. As shown in Figure 3b, global RMSD values did not fluctuate over values higher than 2 Å and the distances measured between selected residues located in the interface did not increase significantly. This indicates that the whole dimer remained stable. In addition, during the simulation, no water molecule was found at a distance compatible for the necessary attack that leads to GTP hydrolysis (Fig. 3a), so GTPase activity was concluded to be inhibited. In summary, the simulated conditions for the FtsZ dimer interface at low pH showed the absence of GTPase activity and the stabilization of the dimer in the protonated FtsZ structure. Then, it is predicted a simultaneous decrease of GTPase activity parallel to a decrease in pH, and the presence of stable polymers at all acidic pHs, independently of the GTPase activity.

To test this hypothesis, the GTPase activities of FtsZ_{MJ} and FtsZ_{EC} were measured *in vitro* over a wide range of pH values, from 4.8 to 8.0. Figure 3c summarizes the results. In agreement with the MD prediction, values of GTPase activity for both molecules significantly decrease when pH changes from 7.0 to 6.0, and become undetectable at pH values lower than 5.5. The putative denaturalization of FtsZ at these pH values was discarded, as it has been reported that FtsZ_{EC} structure denaturalization occurs only at pH values around 3.0²⁶, being the protein completely structured at pH 6.0²⁷. The experiment was also done at pH values of up to 8.0, although these conditions cannot be simulated in the MD environment, as all Arg and Lys residues were already completely protonated at neutral pH in the simulation. The behavior of FtsZ_{MJ} and FtsZ_{EC} was similar at all measured pH values, exhibiting the GTPase activity of FtsZ_{MJ} higher variability at pH values in the range 6.5 - 8.0 when compared to those of FtsZ_{EC}. Finally, to analyze the capacity of FtsZ to polymerize in the absence of GTPase activity at acidic pH values, we performed a series of light-scattering experiments using FtsZ_{EC} and FtsZ_{MJ}. In accordance with the profiles for GTPase activity at low pH values, both proteins showed increasing maximum signal values and maintained them for long periods (up to 10 min. at pH 6.0) when the experiments were performed at lower pH

values (Fig. 4a). To ensure that the same polymers were obtained at different pH values, aliquots of different extracts were analyzed by electron microscopy. The results, shown in Figure 4b (FtsZ_{EC}) and 4c (FtsZ_{MJ}), indicate the presence of polymers at all pH values. At lower pHs there was a trend to form filament bundles, that gave higher light scattering signal, and could be detected by electron microscopy. Filament bundling has been described before, and is related to the formation of stable filaments with low GTPase activities^{13; 27}. This is in agreement with the model predicted by MD, at lower pH values, the dimer interface is more stable, the GTPase activity is lower, and this might favour the filaments bundling.

In conclusion, the analysis of the behavior of FtsZ by MD simulation and by measuring GTPase activity and polymerization revealed an important role for potassium ions in positioning a catalytic water molecule in the active GTPase site and thus maintaining appropriate contact between residues at the dimer interface. Furthermore, it has been shown that GTPase activity depends on the protonation state of the gamma-phosphate of GTP, which offers a molecular explanation for the capacity of FtsZ to form stable polymers at low pH values, in the complete absence of GTPase activity.

Finally, a MD model for the preference of FtsZ_{MJ} for K⁺ over Na⁺ as the monovalent ion and for the inhibitory role of Na⁺ in the active center of the protein has been offered. This model can be extended to other enzymes complexed to monovalent cations^{21; 22}.

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References

1. Rueda, S., Vicente, M. & Mingorance, J. (2003). Concentration and assembly of the division ring proteins FtsZ, FtsA, and ZipA during the *Escherichia coli* cell cycle. *J Bacteriol* **185**, 3344-51.
2. Vicente, M. & Rico, A. I. (2006). The order of the ring: assembly of *Escherichia coli* cell division components. *Mol Microbiol* **61**, 5-8.
3. Erickson, H. P. (2007). Evolution of the cytoskeleton. *Bioessays* **29**, 668-77.
4. López-Viñas, E. & Gómez-Puertas, P. (2004). Sequence and structural alignments of eukaryotic and prokaryotic cytoskeletal proteins. In *Molecules in time and space: Bacterial shape, division and phylogeny* (Vicente, M., Tamames, J., Valencia, A. & Mingorance, J., eds.), pp. 155-172. Kluwer Academic/Plenum Publishers.
5. Mingorance, J., Rico, A. I. & Gómez-Puertas, P. (2004). Bacterial Morphogenes. In *Molecules in time and space: Bacterial shape, division and phylogeny* (Vicente, M., Tamames, J., Valencia, A. & Mingorance, J., eds.), pp. 173-194. Kluwer Academic/Plenum Publishers.
6. Lutkenhaus, J. (2007). Assembly dynamics of the bacterial MinCDE system and spatial regulation of the Z ring. *Annu Rev Biochem* **76**, 539-62.
7. Li, Z., Trimble, M. J., Brun, Y. V. & Jensen, G. J. (2007). The structure of FtsZ filaments in vivo suggests a force-generating role in cell division. *EMBO J* **26**, 4694-708.

8. Mingorance, J., Tadros, M., Vicente, M., Gonzalez, J. M., Rivas, G. & Velez, M. (2005). Visualization of single Escherichia coli FtsZ filament dynamics with atomic force microscopy. *J Biol Chem* **280**, 20909-14.
9. Osawa, M., Anderson, D. E. & Erickson, H. P. (2008). Reconstitution of contractile FtsZ rings in liposomes. *Science* **320**, 792-4.
10. Horger, I., Velasco, E., Mingorance, J., Rivas, G., Tarazona, P. & Velez, M. (2008). Langevin computer simulations of bacterial protein filaments and the force-generating mechanism during cell division. *Phys Rev E Stat Nonlin Soft Matter Phys* **77**, 011902.
11. Horger, I., Velasco, E., Rivas, G., Velez, M. & Tarazona, P. (2008). FtsZ bacterial cytoskeletal polymers on curved surfaces: the importance of lateral interactions. *Biophys J* **94**, L81-3.
12. Surovtsev, I. V., Morgan, J. J. & Lindahl, P. A. (2008). Kinetic modeling of the assembly, dynamic steady state, and contraction of the FtsZ ring in prokaryotic cytokinesis. *PLoS Comput Biol* **4**, e1000102.
13. Mingorance, J., Rueda, S., Gomez-Puertas, P., Valencia, A. & Vicente, M. (2001). Escherichia coli FtsZ polymers contain mostly GTP and have a high nucleotide turnover. *Mol Microbiol* **41**, 83-91.
14. Mukherjee, A. & Lutkenhaus, J. (1999). Analysis of FtsZ assembly by light scattering and determination of the role of divalent metal cations. *J Bacteriol* **181**, 823-32.
15. Sossong, T. M., Jr., Brigham-Burke, M. R., Hensley, P. & Pearce, K. H., Jr. (1999). Self-activation of guanosine triphosphatase activity by oligomerization of the bacterial cell division protein FtsZ. *Biochemistry* **38**, 14843-50.
16. Tadros, M., Gonzalez, J. M., Rivas, G., Vicente, M. & Mingorance, J. (2006). Activation of the Escherichia coli cell division protein FtsZ by a low-affinity interaction with monovalent cations. *FEBS Lett* **580**, 4941-6.

17. Rivas, G., Lopez, A., Mingorance, J., Ferrandiz, M. J., Zorrilla, S., Minton, A. P., Vicente, M. & Andreu, J. M. (2000). Magnesium-induced linear self-association of the FtsZ bacterial cell division protein monomer. The primary steps for FtsZ assembly. *J Biol Chem* **275**, 11740-9.
18. Lu, C., Stricker, J. & Erickson, H. P. (1998). FtsZ from *Escherichia coli*, *Azotobacter vinelandii*, and *Thermotoga maritima*--quantitation, GTP hydrolysis, and assembly. *Cell Motil Cytoskeleton* **40**, 71-86.
19. Oliva, M. A., Huecas, S., Palacios, J. M., Martin-Benito, J., Valpuesta, J. M. & Andreu, J. M. (2003). Assembly of archaeal cell division protein FtsZ and a GTPase-inactive mutant into double-stranded filaments. *J Biol Chem* **278**, 33562-70.
20. Suelter, C. H. (1970). Enzymes activated by monovalent cations. *Science* **168**, 789-95.
21. Page, M. J. & Di Cera, E. (2006). Role of Na⁺ and K⁺ in enzyme function. *Physiol Rev* **86**, 1049-92.
22. Di Cera, E. (2006). A structural perspective on enzymes activated by monovalent cations. *J Biol Chem* **281**, 1305-8.
23. Oliva, M. A., Cordell, S. C. & Lowe, J. (2004). Structural insights into FtsZ protofilament formation. *Nat Struct Mol Biol* **11**, 1243-50.
24. Case, D. A., Cheatham, T. E., 3rd, Darden, T., Gohlke, H., Luo, R., Merz, K. M., Jr., Onufriev, A., Simmerling, C., Wang, B. & Woods, R. J. (2005). The Amber biomolecular simulation programs. *J Comput Chem* **26**, 1668-88.
25. Kaczmarek, P., Szczepanik, W. & Jezowska-Bojczuk, M. (2005). Acid-base, coordination and oxidative properties of systems containing ATP, L-histidine and Ni(II) ions. *Dalton Trans*, 3653-7.
26. Santra, M. K. & Panda, D. (2007). Acid-induced loss of functional properties of bacterial cell division protein FtsZ: evidence for an alternative conformation at acidic pH. *Proteins* **67**, 177-88.

27. Beuria, T. K., Shah, J. H., Santra, M. K., Kumar, V. & Panda, D. (2006). Effects of pH and ionic strength on the assembly and bundling of FtsZ protofilaments: a possible role of electrostatic interactions in the bundling of protofilaments. *Int J Biol Macromol* **40**, 30-9.
28. Diaz, J. F., Kralicek, A., Mingorance, J., Palacios, J. M., Vicente, M. & Andreu, J. M. (2001). Activation of cell division protein FtsZ. Control of switch loop T3 conformation by the nucleotide gamma-phosphate. *J Biol Chem* **276**, 17307-15.
29. Hoenig, M., Lee, R. J. & Ferguson, D. C. (1989). A microtiter plate assay for inorganic phosphate. *J Biochem Biophys Methods* **19**, 249-51.
30. Stoll, V. S. & Blanchard, J. S. (1990). Buffers: principles and practice. *Methods Enzymol* **182**, 24-38.

Figures

Fig. 1. Molecular dynamics analysis of FtsZ dimer stability in the presence of K⁺ or Na⁺. MD simulations were performed using the SANDER module of the AMBER9 package ²⁴. Initial conditions were established using the crystallized structure of *Methanococcus jannaschii* FtsZ dimer (PDB code 1W5A²³) surrounded by a solvent box created by with a minimum distance of 12 Å from the edge and the closest atom of the solute. Na⁺ or K⁺ ions were added to neutralize the whole system. Adaptation of the system to the AMBER force field was performed by 10000 steps of energy minimization using a cutoff of 10.0 Å. During the initial heating phase (200 ps), temperature was raised from 0 to 298° K, restraining the position of Cα atoms in the solute with a force constant of 20 kcal·mol⁻¹. In a second phase, the force constant was reduced stepwise. After equilibration, 5 ns of unrestrained MD was performed in each case. The SHAKE algorithm was applied to constrain bonds involving hydrogen atoms to their equilibrium values. The list of non-bonded pairs was updated every 25 steps, and coordinates were saved every 2-ps. Periodic boundary conditions were applied and electrostatic interactions were represented using the smooth particle mesh Ewald methods with a grid spacing of ~ 1 Å. **(a).** Cartoon representation of the FtsZ dimer showing the positioning of K⁺ (left) or Na⁺ (right) ions placed in a shell around the system using a Coulombic potential in a grid of 1 Å as implemented in the XLEaP module of AMBER9. Black arrows indicate the presence of an atom of K⁺ or Na⁺ in the GTP site of the FtsZ dimer. Blue and red arrows point to the measured contacts between the two monomers (E302-K205 and D26-K80 respectively). **(b).** Left: RMSD values measured for the whole dimer (black), lower monomer (orange) and upper monomer (green) during a 5 ns MD trajectory in the presence of K⁺ (top panel) or Na⁺ (bottom panel) cations. Right: continuous measuring of Cα-Cα distances between residues E302-K205 (blue) and D26-K80 (red) during a 5 ns MD trajectory in the presence of K⁺ (top panel)

or Na⁺ (bottom panel). Distortion of the FtsZ dimer in the presence of Na⁺ is patent in both RMSD and C α -C α measurements (see text).

Fig. 2. Differential GTPase activity and polymerization of FtsZ in the presence of K⁺ or Na⁺. **(a).** Schematic representation of the active site for the FtsZ_{MJ} dimer after a 5 ns MD simulation in the presence of K⁺ (left) and Na⁺ (right). Residues directly involved in GTP hydrolysis from lower (Asp 72) and upper (Asp235, Asp238) FtsZ monomers are depicted. The presence of a water molecule in the appropriate position for catalysis of the GTP molecule, only in the presence of K⁺ (not of Na⁺), is indicated. **(b).** Left: the effect on FtsZ_{MJ} and FtsZ_{EC} GTPase activities of increasing the concentration of Na⁺ in the presence of different concentrations of K⁺. Right: Double reciprocal plot of FtsZ_{MJ} GTPase activity at increasing concentration of K⁺ in the presence of different concentrations of Na⁺. FtsZ_{EC} was purified by the Ca²⁺-induced precipitation method ¹⁷. FtsZ_{MJ} was purified in a Ni²⁺-affinity column ²⁸. Proteins were stored at -80°C and, prior to use, *M. jannaschii* FtsZ was further purified by a heat shock (15 minutes at 65°C) followed by centrifugation, and both FtsZ_{MJ} and FtsZ_{EC} proteins were desalted by exchanging the storage buffer with TM buffer (FtsZ_{MJ}: 50mM Tris, pH 6.5, and 10mM MgCl₂; FtsZ_{EC}: 50mM Tris, pH 7.5, and 10mM MgCl₂) in a 5ml Hi-TrapTM desalting column (Pharmacia Biotech). To test polymerization in the absence of Na⁺, the GTPase activity was detected either by thin-layer chromatography¹³ or by a colorimetric assay ²⁹ using 10μM of FtsZ and GTP (Tris salt; G9002, Sigma) as substrate. Activity values were calculated by measuring the slope of the lineal part of the enzymatic kinetics (eight measurements done in triplicate for each experiment) in presence of GTP. **(c).** Light scattering analysis of the assembly of purified FtsZ_{EC} (left) and FtsZ_{MJ} (right) at different concentrations of Na⁺ in the presence of 150 mM of K⁺ for FtsZ_{EC} or 500 mM of K⁺ for FtsZ_{MJ}. Polymerization was followed by 90° light scattering in a Hitachi F-2500 fluorescence spectrophotometer at excitation and emission wavelengths of 350 nm. The light scattering signal was corrected for background signal obtained

before the addition of GTP or with GTP in the absence of monovalent salts. All the assays were performed at 25°C and pH 7.5 for FtsZ_{EC} and at 55°C and pH 6.5 for FtsZ_{MJ}. Arrow indicates the point at which GTP (0.5 mM, final concentration) was added.

Fig. 3. Effect of pH on FtsZ dimerization and GTPase activity. **(a).** Active site for FtsZ_{MJ} after 5ns MD simulation in the presence of γ -protonated GTP. The location of the protonated group is indicated (arrow). **(b).** Continuous measuring of RMSD (upper panel, color codes as in Fig. 1) and of Ca-Ca distances between residues E302-K205 and D26-K80 (blue and red lines respectively, lower panel) during a 5 ns MD trajectory simulating low pH conditions. **C.** GTPase activity measured for purified FtsZ_{EC} (left) and FtsZ_{MJ} (right) at different pH values. To cover a wide range of pH values and to avoid buffer-specific effects, several buffers, with overlapping pH ranges, were used. Citrate-phosphate buffers, providing the widest range of buffer capacity with constant ionic strength, were done as described by Stoll and Blanchard³⁰.

Fig. 4. Polymerization of FtsZ at low pH. **(a).** Light scattering analysis of the assembly of purified FtsZ_{EC} at 10 μ M (left) and FtsZ_{MJ} at 5 μ M or 10 μ M (right). Polymerization in citrate-phosphate buffers at the indicated pH values was followed by 90° light scattering as indicated in Fig. 2. Arrow indicates the point at which GTP (0.5 mM, final concentration) was added. **(b).** Electron microscopy photographs of FtsZ_{EC} at acidic pH values. Electron microscopy was performed as described previously¹³. FtsZ was incubated in 100 μ l of polymerization buffer. GTP was added to 0.5 mM and after 1 minute the samples were applied to 400-mesh collodion-coated glow-discharged grids, negatively stained with 2% uranyl acetate, and inspected with a Jeol 1200EXII electron microscope. Bar: 0.25 μ m. **(c).** Electron microscopy photographs of FtsZ_{MJ} (lower panel) at acidic pH values. Experimental conditions as in (b).

(a)

(b)

Figure 1

Figure 2

Figure 3

Figure 4