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Dimer dynamics and filament organization of the bacterial cell division protein FtsA

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Abstract

FtsA is a bacterial actin homolog and one of the core proteins involved in cell division. While previous studies have demonstrated the capability of FtsA to polymerize, little is known about its polymerization state *in vivo*, or if polymerization is necessary for FtsA function. Given that one function of FtsA is to tether FtsZ filaments to the membrane, *in vivo* polymerization of FtsA imposes geometric constraints and requires a specific polymer curvature direction. Here we report a series of molecular dynamics simulations probing the structural dynamics of FtsA as a dimer and as a tetrameric single filament. We found that the FtsA polymer exhibits a preferred bending direction that would allow for its placement parallel to FtsZ polymers underneath the cytoplasmic membrane. We also identified key interfacial amino acids that mediate FtsA-FtsA interaction, and propose that some amino acids play more critical roles than others. We performed *in silico* mutagenesis on FtsA and demonstrated that while a moderate mutation at the polymerization interface does not significantly affect polymer properties such as bending direction and association strength, more drastic mutations change both features and could lead to non-functional FtsA.

Keywords

bacterial cytoskeleton; polymer mechanics; actin family; molecular dynamics; cell division

1 Introduction

In bacteria, cell division is carried out by an array of spatially and temporally regulated proteins collectively known as the divisome [1-4]. These proteins assemble at the division site, initiate constriction that pulls the cytoplasmic membrane inward, synthesize new cellular materials, and, during the last stage of division, separate the two daughter cells. How these actions are conducted in succession has remained largely unknown, as most division proteins still have unassigned functions or operate via unknown mechanisms. Achieving a full understanding of the divisome is further hindered by the high degree of protein-protein interactions amongst division proteins, including self-interactions, as revealed through interaction assays and imaging techniques [1,5-10]. Advanced

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computational methods with molecular resolution, in combination with emerging structural data [11-16], offer the potential to reveal the functional mechanisms of essential bacterial division proteins. For example, we recently applied atomistic molecular dynamics simulations to study the most conserved bacterial division protein, FtsZ, and revealed the structural basis of its hydrolysis-induced force generation that potentially drives constriction [17].

Since FtsZ does not interact directly with the cytoplasmic membrane, it needs to bind to other proteins in order to transmit mechanical force to the membrane and the cell wall. Two divisome proteins have been identified to act as such “membrane anchors”: ZipA and FtsA [18]. Although both proteins are essential, FtsA is thought to be more important, as it is the second most conserved bacterial division protein after FtsZ [19,20] (ZipA is only found in *proteobacteria* [21]), and mutations in *ftsA* can bypass the requirement for ZipA in *Escherichia coli* [22,23]. Interestingly, FtsA is a bacterial homolog of actin [20, 24], and, like actin, FtsA from *Streptococcus pneumoniae* and *Thermotoga maritima* polymerizes into filaments *in vitro* [13,25]. In *E. coli* and *Bacillus subtilis* cells, FtsA tagged with fluorescent proteins appears to assemble into filament-like structures [13,26-28]. When *E. coli*, *B. subtilis*, and *T. maritima* FtsA is over-expressed in *E. coli*, linear FtsA filaments can be visualized through electron cryotomography [13]. Together with earlier genetics experiments suggesting FtsA has a tendency for self interaction [29-32], it has become evident that FtsA is capable of polymerization [13]. However, the physiological role of FtsA polymerization during division is still unclear, and specifically it remains to be addressed if FtsA exists in a polymerized or a monomeric state when forming functional complexes with FtsZ (Fig. 1), which itself has been shown to polymerize *in vivo* through cryo-electron microscopy and super-resolution microscopy, forming the so-called Z-ring [33,34].

Since FtsA tethers FtsZ to the membrane, the architecture of the two proteins imposes some geometric constraints on the polymerization of FtsA. First, FtsA interacts with the membrane via a conserved amphipathic helix formed between residues 411 and 419 (*T. maritima* numbering is used unless specified otherwise) at its C-terminus [27,35]. The amphipathic helix is connected to the core structure of FtsA via a linker region (residues 390 to 410) with an unknown structure. Therefore, unless the 21-residue linker is substantially extended, a hypothesized functional FtsA polymer would require residues 390 to be located at the outer edge of the polymer facing the cytoplasmic membrane (Fig. 1a). At the same time, FtsA interacts with FtsZ through its 2B domain [18,28]. Specifically, charged residues 301, 304, and 293 from FtsA were shown to establish electrostatic interactions with FtsZ [13]. Accordingly, in order for an FtsA filament to be sandwiched between the membrane and the FtsZ filament, residues 301 of FtsA would need to be located at the inner edge of the FtsA polymer (Fig. 1a; residues 304 and 293 are in close proximity to 301). Taken together, in order for FtsA to establish proper interactions with both the membrane and FtsZ, functional FtsA polymerization *in vivo* is only possible if the intrinsic curvature direction of the FtsA polymer adopts a “390_{out}-301_{in}” configuration following the terminology for transmembrane α -helices [36]). In contrast, if the intrinsic curvature of the FtsA polymer deviates significantly from the 390_{out}-301_{in} direction, then the feasible physiological organizations of FtsA would involve either only monomeric units (Fig. 1b), or FtsA polymerization in a direction perpendicular to the FtsZ polymer (Fig. 1c).

Here, we report a series of molecular dynamics simulations probing the intrinsic filament curvature of FtsA. We found that a wild-type FtsA single filament, either as a dimer or tetramer, consistently adopted the 390_{out}-301_{in} configuration throughout each simulation, suggesting that FtsA polymerization parallel to FtsZ during physiological division function is feasible. We then identified conserved amino acids involved in FtsA-FtsA interaction, and

noted that while some are critical in polymerization, others play only moderate roles. We propose that mutations of the former group of amino acids abolish FtsA function, while mutations of the latter group can be tolerated. Through *in silico* mutagenesis, we also found that FtsA polymer direction and polymerization strength can be altered by drastic amino acid substitutions. These results provide a structural basis for interpreting recent experimental efforts characterizing phenotypes of single mutations in FtsA, and shed light on the potential large-scale and dynamic structures formed by the complex system of division proteins during bacterial cytokinesis.

2 Results

An FtsA filament prefers the 390_{out}-301_{in} configuration

An unbiased all-atom molecular dynamics simulation was carried out for a *T. maritima* FtsA dimer placed in an explicit solvent environment (Fig. 2a). During the 90-ns simulation, each FtsA monomer remained structurally stable with subtle motions between the monomers. To visualize the effect of monomer-monomer motions on the structure of an FtsA single filament, we constructed models of FtsA filaments by replicating the monomer-monomer interface observed in the molecular dynamics simulation. At the beginning of the simulation, the FtsA dimer crystal structure forms a filament with a curvature direction substantially deviating from the 390_{out}-301_{in} configuration (Fig. 2b). However, within 30 ns, the filament quickly adopted the 390_{out}-301_{in} configuration, and this direction of bending persisted throughout the remainder of the simulation (Fig. 2c). To verify that the monomer-monomer interface in an FtsA dimer is representative of a polymerization interface in a filament, we also conducted simulations with a short FtsA filament composed of four monomers (Fig. S1-S3). In the two simulations performed, the tetrameric FtsA filament adopted the 390_{out}-301_{in} bending direction within 10 ns.

To quantitatively measure the direction of bending in the dimer simulation, we define a deviation angle ϕ as follows. First, the ideal bending axis is defined; rotation of an FtsA monomer around this axis places residues 390 on the outer edge of the curvature, and residues 301 on the inner edge (Fig. 3a). At each time step of the dimer simulation, the actual bending axis was measured (Fig. 3b), and the angle between the actual bending axis and the ideal bending axis is the deviation angle ϕ (Fig. 3c). Smaller magnitudes of ϕ indicate that the FtsA filament bending direction is closer to the 390_{out}-301_{in} configuration. As shown in Fig. 3d, the simulation began with large values of ϕ , but quickly decreased and fluctuated around 0°. To confirm this behavior, we performed a repeat simulation, and observed a similar trend for the time evolution of ϕ (Fig. 3d), as well as a comparable distribution of ϕ values (Fig. 3e), suggesting that the intrinsic curvature direction of FtsA naturally adopts the 390_{out}-301_{in} configuration. Therefore, our results indicate that FtsA polymerization parallel to FtsZ polymers is feasible (Fig. 1a).

Mechanical properties of an FtsA polymer match those of FtsZ

While our data suggest that the intrinsic curvature direction would allow FtsA polymers to be situated parallel to an FtsZ filament, an additional condition for such organization is that the two polymers should exhibit comparable degrees of curvature. To ascertain whether this was the case, we measured the three rotational angles between the FtsA monomers during each of our simulations using a previously reported protocol [17]. Briefly, the coordinate system, $\{\mathbf{d}_1, \mathbf{d}_2, \mathbf{d}_3\}$, is defined for each FtsA monomer at each time step of the trajectory. The unit vector $\mathbf{d}_1$ is approximately aligned with the vector pointing from the center-of-mass position of the bottom monomer to that of the top monomer, such that rotation around $\mathbf{d}_1$, denoted by α , measures the amount of twisting motion between the monomers (Fig. 4a). The unit vector $\mathbf{d}_2$ is the ideal bending axis shown in Fig. 3a, and rotation around $\mathbf{d}_2$,

denoted by α_2 , corresponds to a bending motion (Fig. 4b). We chose to define positive values of α_2 as bending that leads to the 390_{out}-301_{in} configuration, whereas negative values of α_2 correspond to the opposite, 390_{in}-301_{out} configuration. Lastly, $\mathbf{d}_3$ is the vector perpendicular to $\mathbf{d}_1$ and $\mathbf{d}_2$, and rotation around $\mathbf{d}_3$ corresponds to the bending motion orthogonal to bending around $\mathbf{d}_2$ (Fig. 4c).

Throughout both wild-type FtsA simulations, there was little twisting motion between the monomers (Fig. 4a). Bending around $\mathbf{d}_2$, the ideal bending axis, began with small values of α_2 , then increased to above 10° (Fig. 4b). In contrast, α_3 , which measures bending around $\mathbf{d}_3$ orthogonal to the ideal bending axis, approached zero (Fig. 4c). The time courses of these three rotation angles are in accordance with the observation that the FtsA dimer preferred to bend around the ideal bending axis, reflecting its intrinsic 390_{out}-301_{in} configuration (Fig. 3d).

To characterize the mechanical properties of an FtsA polymer, we measured the mean bending angles $\langle \alpha \rangle$ and the bending angle variances σ^2 for both simulations (Fig. 4d). The mean bending angles were 10.8° and 13.6° for the first and second wild-type FtsA simulation, respectively. Given that the length of each FtsA monomer along the polymerization axis is ~5 nm, the radius of curvature of an FtsA polymer was estimated to be 21 and 27 nm. In comparison, independent electron microscopy studies have revealed that, in the presence of GTP, purified FtsZ assembles *in vitro* into either straight or gently curved filaments with a radius of curvature of ~100 nm, and GDP-FtsZ filaments are highly curved with a radius of curvature of ~10 nm [16,37-40]. In our prior molecular dynamics simulations of FtsZ dimers, we similarly found that GTP-FtsZ is less curved (~70 nm) and GDP-FtsZ is more curved (~10 nm) [17]. During cell division, active FtsZ polymers may contain some GTP-FtsZ and some hydrolyzed GDP-FtsZ [41], and these mixed polymers could adopt radii of curvature between 10 and 70 nm.

The mechanical stiffness k of an FtsA polymer is related to the bending angle variance σ^2 via $k = k_B T / \sigma^2$, where k_B is the Boltzmann factor and T is temperature. For an FtsZ dimer, we have measured its bending angle variance σ^2 to be 10 deg² in the GTP-bound state and 14-28 deg² in the GDP-bound state [17], whereas σ^2 measured here for FtsA are 10.4 and 20.1 deg². In sum, mechanical properties of an FtsA polymer such as radius of curvature and stiffness do not differ significantly from those measured for FtsZ experimentally and computationally, indicating that aligning FtsA and FtsZ polymers in parallel does not incur significant energy costs and further supporting the feasibility of functional FtsA polymerization *in vivo*.

Identification of key interfacial residues

From our molecular dynamics simulations of an FtsA dimer, we identified specific amino acids that contribute to stabilizing the monomer-monomer interaction. We define these key amino acids as those that form stable inter-monomer interactions. For simplicity in connecting to experimental data from various bacterial species such as *E. coli* and *B. subtilis*, we only consider highly conserved amino acids. We note that these key amino acids potentially have varying levels of contribution to interface stabilization, as seen in a ranking by the closest distance to their inter-monomer interaction partners (Table 2). Assuming proper polymerization is a prerequisite for FtsA function, we hypothesize that the highly ranked amino acids are essential for FtsA polymerization, and that disrupting these residues would lead to non-functional FtsA. For the lower ranked amino acids, we hypothesize that they still contribute, but perhaps single mutations of these amino acids only moderately weaken FtsA-FtsA interaction and can be tolerated without fully abolishing FtsA function.

The highest ranked amino acid that has been previously subjected to mutagenesis study is M151, corresponding to M147 in *B. subtilis*. The M147E mutation was introduced in *B. subtilis* on a vector in a strain with endogenous temperature-sensitive FtsA. The resulting cells were unable to divide at high temperature [13], suggesting that M147 is essential for the division function of FtsA. In contrast, several single amino acid substitutions in FtsA have been shown to support cell division in *E. coli* [22,23,42], and three of these amino acids are conserved between *E. coli* and *T. maritima* (R357, D140, and G48; corresponding to R357, D138, and G49 in *E. coli*). All three amino acids appear in the list of key interfacial residues, but are ranked lower at positions 8, 11, and 24 (Table 2). It is thus possible that while these amino acids also play an important role in FtsA polymerization, they are not as critical as the higher ranked residues, and can withstand mutations to a certain degree.

Highly conserved amino acids are clustered in FtsA

Mapping the highly conserved amino acids back to the FtsA dimer structure, we noted that these residues are not scattered across the protein, but form two major clusters (Fig. 5). One cluster surrounds the ATP-binding site, which is expected as FtsA belongs to the actin family of proteins with a common ATPase domain [20,43,44]. Another cluster of highly conserved amino acid resides at the monomer-monomer interface, and is formed mostly between domains 1C and 1A (Fig. 5). Notably, domain 1C of FtsA is involved not only in FtsA-FtsA self-interaction and polymerization, but also in the recruitment of downstream cell division proteins such as FtsN and FtsI [5,30,31,45-47]. For FtsN, it was recently demonstrated that a set of conserved, positively charged amino acids in FtsN are essential in establishing proper interaction with the 1C domain of FtsA [46]. Interestingly, we identified two conserved, negatively charged amino acids in domain 1C (D140 and D150; Fig. 5) that potentially serve as the FtsN binding site. Since these two amino acids are also involved in FtsA polymerization (Table 2), FtsA-FtsA self-interaction might be in competition with FtsA-FtsN interaction, and, as previously proposed, temporally regulated polymerization and depolymerization of FtsA that is synchronized with division stage might be essential for its function [23].

Severe mutations at the polymerization interface shift polymer properties

To assess the effect of mutation on FtsA polymer structural dynamics, we performed four *in silico* mutagenesis simulations. We chose to perform the mutations on D140, changing it to three different amino acids (E, A, and R). The choice of D140 was based on several observations. First, D140 was identified to be a key interfacial amino acid, but is ranked in the middle in terms of its potential contribution to interfacial stability (Table 2). Second, a mutation in the corresponding amino acid in *E. coli* (D138E) was shown to preserve FtsA function while slightly weakening FtsA-FtsA self-interaction [23]. However, this mutation is relatively moderate (compared to other functional *E. coli* FtsA mutants such as R286W, R357A, and G49D [22,23]), leading us to hypothesize that more drastic mutations at this site would introduce more severe defects in FtsA function. Third, D140 and D150 potentially serve dual roles as mediators of both FtsA-FtsA interactions and FtsA-FtsN electrostatic interactions (Fig. 5).

We performed simulations with three different mutations with varying degrees of disruption: D140E, D140A, and D140R. Three repeat simulations were carried out for D140R, which is the most drastic mutation changing the negatively charged aspartic acid into a positively charged arginine. We tracked the distributions and cumulative distributions of the deviation angle ϕ throughout all four simulations (Fig. 6). For the minimally disruptive mutation D140E, we found that the time evolution and distributions of ϕ were very similar to the wild-type simulations (Fig. 6a-c and Fig. 3d and e). In contrast, the D140A mutation consistently deviated away from the $390_{\text{out}}-301_{\text{in}}$ configuration and fluctuated around $\phi \sim$

–20° toward the end of the simulation. These features are recapitulated in a comparison of the cumulative distributions of ϕ from the various FtsA dimer simulations (Fig. 6c), which shows a high degree of similarity among the two wild-type simulations and the D140E simulation, and a shift to smaller values for the D140A mutant. The most drastic mutation, D140R, introduced erratic behavior in ϕ , which in one simulation reached above 60°, and in the other dipped below –20° (Fig. 6d). In addition, the three D140R simulations lead to very different distributions of ϕ (Fig. 6e and f), suggesting inherent variability of ϕ in this mutant. Based on these data, we suggest that while moderate mutations such as D140E do not change the FtsA polymer conformation, more aggressive mutations such as D140A and D140R can alter the intrinsic bending direction of the FtsA polymer. Indeed, the D140E mutation in *E. coli* (D138E) was shown to complement an FtsA depletion strain [23], suggesting that D140E FtsA remains functional. If proper polymer configuration is needed to establish correct contact with FtsZ and the membrane, it is possible that D140A and D140R FtsA mutants would lose their function in part due to their inability to anchor FtsZ to the membrane.

For the most drastic interfacial mutant, D140R, we carried out two simulations of a tetrameric D140R FtsA filament, with similar simulation protocols as for the wild-type FtsA tetramer (Materials and Methods and Supplementary Data). Unlike the wild-type FtsA tetramer, which adopted the 390_{out}-301_{in} curvature direction in two independent simulations, the bending direction of a D140R FtsA tetramer was more varied and differed between the two simulations (Figs. S1-S2). These tetramer-based observations are consistent with our D140R FtsA dimer simulations.

In addition, we also measured the relative rotations (α_1 , α_2 , and α_3) between the FtsA monomers in the dimeric mutant simulations (Fig. 7). In comparison with the wild-type results (Fig. 4a-c), the simulation with the benign D140E mutation led to similar values of α_1 , α_2 , and α_3 , namely, small twisting angle α_1 , significant bending around $\mathbf{d}_2$ (the ideal bending axis), and little bending around the orthogonal $\mathbf{d}_3$ (red traces in Fig. 7a). The resemblances of rotational angles extracted from the D140E simulation and the wild-type simulations indicate that this mutation does not change the mechanical properties of the FtsA polymer such as the radius of curvature and stiffness. In contrast, the more drastic mutations, D140A and D140R, introduced different behaviors in α_1 , α_2 , and α_3 , such as consistently non-zero twisting angles and non-zero bending angles around $\mathbf{d}_3$, orthogonal to the ideal bending axis (Fig. 7). These measurements reinforce the observation that while the D140E mutation, shown previously to preserve FtsA function [23], does not affect FtsA polymer mechanics significantly, severe mutations such as D140A and D140R could bring about physiologically relevant alterations in FtsA polymer properties.

Inter-monomer contact is affected by drastic mutations

To evaluate the effect of mutations on FtsA-FtsA self-interaction, we measured the contact surface area between the two FtsA monomers during the seven dimer simulations as a crude metric for interaction strength (Table 3) [48]. We found that the two simulations with wild-type FtsA resulted in comparable inter-monomer contact surface area. The least disruptive mutation, D140E, also led to wild-typelike inter-monomer contact surface area. It should be noted that the corresponding mutation in *E. coli* (D138E) was shown to slightly weaken FtsA-FtsA self-association through a yeast two-hybrid assay [23]; it is possible that our measurement is not sensitive enough to capture this difference. On the other hand, mutation D140A caused an increase in inter-monomer contact surface area, potentially due to the small side chain providing sites suitable for close contact and compact packing [49-53]. Lastly, the three D140R simulations consistently led to lower inter-monomer contact surface area, suggesting a reduced degree of FtsA-FtsA self-interaction. Such partial loss of

monomer-monomer association would lead to increased flexibility at the polymerization contact, and could introduce variability in bending direction, possibly explaining the observed differences in the deviation angle measurements (Fig. 6d-f).

3 Discussion

Using all-atom molecular dynamics simulations, we observed that an FtsA dimer and a tetrameric polymer consistently exhibited a preferred bending direction that would allow for its placement between the cytoplasmic membrane and FtsZ, aligned parallel to an FtsZ polymer (Fig. 1a). This result supports a previously proposed model in which FtsA polymerizes during physiological function, anchoring FtsZ polymers to the membrane and potentially forming a disconnected “A-ring” adjacent to the Z-ring [13].

While our data suggest that functional polymerization of FtsA parallel to the FtsZ polymers is feasible, it should be noted that we cannot rule out alternative FtsA models such as those shown in Fig. 1b and c, or determine whether or not FtsA polymerization is required for its function. In addition, we note that the majority of our simulations were performed on an FtsA dimer. However, it is possible that the FtsA dimer interface might not fully represent the general dynamics of the polymerization interface. For example, in a dimer, the motion of each monomer is only constrained by one neighbor, whereas in a filament most of the monomers are associated with two neighboring units, altering the potential motions at each polymerization interface. Also, when replicating the monomer-monomer interface to construct a model of a filament, small deviations at the interface are multiplied and amplified, whereas the similarities between neighboring interfaces will be dictated by the degree of long-range coupling between non-adjacent subunits. Nonetheless, our observation of preferred polymer bending direction is very consistent for wild-type FtsA across two dimer simulations, and is also supported by two simulations with an FtsA tetramer. Other elements to consider are FtsA-binding proteins that are also essential to cell division, such as FtsZ and FtsN. Complex formation between these division proteins will likely affect FtsA polymer properties. Future simulations incorporating more members of the divisome will further elucidate how the complex process of bacterial cell division is achieved.

In light of recent mutagenesis experiments demonstrating that certain single amino acid substitutions at the FtsA monomer-monomer interface decrease FtsA self-interaction [23], we classified key interfacial amino acids into two groups: those critical to polymerization and hence cannot be altered, and those that can tolerate mutations up to a certain degree depending on their molecular properties. We propose that amino acid M151, corresponding to M147 in *B. subtilis*, belongs to the former group [13], while the amino acids identified in Ref. 23 belong to the latter group. To test our hypothesis, we performed *in silico* mutagenesis on D140, and found that the most benign mutation (D140E) preserved the curvature direction of FtsA and led to very little change in inter-monomer contact surface area, while the more drastic mutations (D140A and D140R) altered both polymer properties. In comparison, the D140E mutation in *E. coli* FtsA (D138E) was previously demonstrated to maintain FtsA function [23], and upcoming studies will illuminate if the more drastic mutations can still support cell division.

Interestingly, the most highly conserved amino acids in FtsA, besides those near the ATP-binding pocket, are clustered near the polymerization interface between domains 1A and 1C. Given the dual roles of domain 1C in establishing FtsA-FtsA polymerization and interactions with other division proteins [5,30,31,45-47], mutations of interfacial amino acids in 1C could affect FtsA function in multiple ways. For example, our results suggest that drastic perturbations in FtsA D140 can lead to too high (D140A) or too low (D140R) FtsA-FtsA affinity (Table 3), altered polymer bending direction that impedes proper

anchoring of FtsZ (Fig. 6), and/or failure to establish electrostatic interactions with FtsN (Fig. 5). For these reasons, loss of FtsA function due to single amino acid substitutions might originate from multiple molecular sources, making it difficult to unambiguously attribute phenotypes of FtsA mutants to specific mechanisms.

To date, more than a dozen essential bacterial division proteins have been identified, as perturbation of these proteins introduces division defects and lethality [1,2]. The essentiality of these proteins is often challenged as studies have provided cases in which mutations of a particular division protein allow for the removal of other otherwise essential division proteins. For example, various mutations in FtsA in *E. coli* have been shown to bypass the need for ZipA [22,23], FtsN [54], and FtsK [55]. In light of the complexity of divisome protein-protein relationships and the apparent redundancy in division proteins, it should be helpful to identify essential molecular interactions and their corresponding functions in cell division. To this end, combining high-resolution structural information obtained through crystallography with computational simulations and modeling can potentially generate the molecular and dynamic information necessary to decipher the mechanisms of the bacterial divisome. Future studies integrating multiple types of division proteins, or membrane-protein interactions, will continue to advance our understanding of how the bacterial division machinery functions.

4 Materials and Methods

Equilibrium molecular dynamics

All simulations were performed using the molecular dynamics package NAMD [56] with the CHARMM27 force field [57,58], including CMAP corrections [59]. In the FtsA dimer simulations, water molecules were described with the TIP3P model [60]. Long-range electrostatic forces were evaluated by means of the particle-mesh Ewald summation approach with a grid spacing of $< 1 \text{ \AA}$. An integration time step of 2 fs was used [61]. Bonded terms and short-range, non-bonded terms were evaluated every time step, and long-range electrostatics every other time step. Constant temperature ($T = 310 \text{ K}$) was maintained using Langevin dynamics [62], with a damping coefficient of 1.0 ps^{-1} . A constant pressure of 1 atm was enforced using the Langevin piston algorithm [63] with a decay period of 200 fs and a time constant of 50 fs. For the FtsA tetramer simulations, an implicit solvent environment was used to reduce computational cost [64], and stability of the polymerization interfaces was maintained using a restraining method [65] before free equilibrium dynamics. Further details are provided in the Supplementary Data (Figs. S1-S3).

Simulated systems

Eleven molecular dynamics simulations were performed as described in Table S1 in the Supplementary Data; seven involved FtsA dimers and four involved FtsA tetramers. For each simulation, the crystallographic structure of *T. maritima* FtsA dimer bound to ATP molecules (PDB code: 4A2A [13]) was used with the corresponding mutation. In the FtsA dimer simulations, water and neutralizing ions were added around FtsA dimer, resulting in final simulation sizes of up to $\sim 200,000$ atoms. Each dimer simulation was performed for $\sim 90 \text{ ns}$. Setup, analysis, and rendering of the simulation systems were performed with the software VMD [66]. For measurements of average values and distributions, only the last 20% of each of the simulation trajectories was used to ensure that the system had reached equilibrium.

Identification of highly conserved amino acids

~ 400 FtsA sequences from different bacterial species were obtained from BLAST and PSI-BLAST [67], and aligned with COBALT [68]. Conservation scores were computed for each

amino acid position using a feature in the statistical coupling analysis software package [69]. Amino acids with conservation scores in the top 15% were designated as highly conserved.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Highlights

- It is unknown if FtsA, a bacterial actin homolog, is polymerized during division.
- We probed FtsA polymer properties with molecular dynamics simulations.
- Functional polymerization of FtsA parallel to FtsZ filaments is feasible.
- • Drastic mutations at the FtsA-FtsA interface change polymer properties.
- • Understanding FtsA polymer dynamics aids in elucidation of its functional mechanism.

Figure 1. Models for the *in vivo* organization of FtsA and FtsZ. (a) FtsA forms polymers sandwiched between the cytoplasmic membrane and an FtsZ polymer; the two polymers are parallel. This organization requires that residues 390 (green circles) are positioned at the outer curvature and residues 301 (blue circles) at the inner curvature of the FtsA polymer (the 390_{out}-301_{in} configuration). (b) Binding of the FtsZ polymer to the membrane by FtsA monomers does not impose such a geometric constraint. (c) Similarly, there is no geometric constraint on FtsA polymers if they are oriented perpendicular to the FtsZ polymer.

Figure 2. Molecular dynamics simulation of an FtsA dimer indicate that filament bending direction allows for the parallel alignment of FtsA and FtsZ polymers

(a) An example simulation system with an FtsA dimer (red and pink) bound to ATP (green). Water box is shown in transparent gray and neutralizing ions are shown as blue spheres. (b) An FtsA filament constructed by replicating the geometry of the monomer-monomer interface. Six FtsA monomers are shown in alternating red and pink for distinction, and the approximate protein surface is shown in transparent gray. Residues 390 and 301 are shown as green and blue spheres, respectively. At $t = 0$, the curvature of the filament deviates from the $390_{\text{out}}-301_{\text{in}}$ direction. (c) Within 30 ns, the filament adopted the $390_{\text{out}}-301_{\text{in}}$ bending direction, which persisted throughout the rest of the 90-ns simulation.

Figure 3. Wild-type FtsA dimers exhibit only small deviations from the ideal bending direction (a-c) The deviation angle ϕ is defined to be the angle between the ideal bending axis (black arrow) and the actual bending axis (orange arrow) tracked during the simulation. (d) Time courses of ϕ for the two repeat simulations with a wild-type FtsA dimer; both exhibited small ϕ values that stabilized around 0° toward the end of the simulations. (e) Normalized distributions of ϕ . Averaged values of ϕ are provided in Table 1.

Figure 4. Characterizing the relative rotations between FtsA monomers

(a) Time courses of θ_1 , measuring the “twisting” motion between the monomers. (b) Time courses of θ_2 , measuring the “bending” motion between the monomers around the ideal bending axis (defined in Fig. 3a). (c) Time courses of θ_3 , measuring the bending motion between the monomers orthogonal to θ_2 . (d) Normalized distributions of the bending angle θ_2 . Raw data are shown as filled circles, and Gaussian fits are shown as solid lines, with the means $\langle \theta_2 \rangle$ and variances σ^2 given in units of degrees and degrees², respectively.

Figure 5. Locations of highly conserved amino acids in FtsA

For clarity, only the top 10% most highly conserved amino acids are shown. Dimerized FtsA monomers are shown in red and pink, ATP molecules in green, and highly conserved amino acids from the two monomers are shown as yellow and blue spheres. The two highly conserved, negatively charged amino acids in domain 1C located near the dimerization interface (D140 and D150) are shown in light blue.

Figure 6. Interface mutants show varying degrees of deviation from the ideal bending direction
Time courses (a,d), distributions (b,e), and cumulative distributions (c,f) of ϕ for the simulations with mutated FtsA. While the least disruptive mutation, D140E, did not alter the bending direction significantly, the other two mutations, D140A and D140R (three repeat simulations), displayed markedly different bending directions. Average values of ϕ are provided in Table 1.

Figure 7. Severe mutations at the FtsA polymerization interface alter the mechanical properties of the polymer

The three rotation angles between the FtsA monomers measured during simulations with dimeric FtsA mutants. Twisting and bending angles are defined in Fig. 4a-c. The least disruptive mutation, D140E, did not substantially change the mechanical properties of the FtsA polymer, reaching comparable values of θ_1 , θ_2 , and θ_3 compared to wild-type FtsA. The more severe mutations, D140A and D140R, led to noticeably different trajectories of θ_1 , θ_2 , and θ_3 .

Table 1**Deviation angle ϕ**

Measured in seven wild-type and mutated FtsA dimer simulations as an approximate metric for deviation from the ideal, $390_{\text{out}}-301_{\text{in}}$ bending direction.

Simulation	Deviation angle ϕ (°)
WT-1	-4±18
WT-2	-5±27
D140E	-2±24
D140A	-17±10
D140R-1	-12±15
D140R-2	40±33
D140R-2	17±25

Table 2**Key interfacial amino acids**

Only highly conserved amino acids are considered and ranked by their closest inter-monomer contacts.

Rank	Residue	Ave. dist. to closest inter-monomer contact (Å)
1	D150	1.57±0.03
2	D52 ^{a,b}	1.74±0.08
3	M151 ^b	1.94±0.16
4	V145	1.99±0.06
5	F176	2.06±0.13
6	G44 ^{a,b}	2.18±0.27
7	I50 ^b	2.27±0.17
8	R357 ^{a,b}	2.41±0.40
9	M172	2.52±0.36
10	D244 ^{a,b}	4.06±0.38
11	D140 ^{a,b}	4.22±0.63
12	G207 ^{a,b}	4.37±0.12
13	G331 ^{a,b}	4.77±0.09
14	P148 ^{a,b}	4.84±0.15
15	V333 ^{a,b}	5.12±0.21
16	S256	5.14±0.19
17	L199 ^{a,b}	5.39±0.27
18	I242	5.64±0.18
19	A153	5.91±0.94
20	Y137 ^a	5.93±0.16
21	V245	6.22±0.16
22	Y361	6.68±0.13
23	N216	6.98±0.52
24	G48 ^{a,b}	7.54±0.28
25	E257 ^{a,b}	7.93±0.22
26	S17	8.26±0.21
27	V241 ^a	8.60±0.47

^a Amino acid is conserved between *T. maritima* and *E. coli*.

^b Amino acid is conserved between *T. maritima* and *B. subtilis*.

Table 3
Monomer-monomer contact surface area

Measured in seven wild-type and mutated FtsA dimer simulations as an approximate metric for interaction strength.

Simulation	Inter-monomer contact surface area ($\text{\AA}^2$)
WT-1	801 $\pm$ 50
WT-2	820 $\pm$ 57
D140E	812 $\pm$ 38
D140A	970 $\pm$ 81
D140R-1	795 $\pm$ 55
D140R-2	759 $\pm$ 35
D140R-3	789 $\pm$ 55