# Efficient models of polymerization applied to FtsZ ring assembly in *Escherichia coli*

Álvaro Ruiz-Martínez[a], Thomas M. Bartol[b], Terrence J. Sejnowski[b,c,d,1], and Daniel M. Tartakovsky[e,1]

[a]Department of Mechanical and Aerospace Engineering, University of California, San Diego, La Jolla, CA 92093; [b]Computational Neurobiology Laboratory, Salk Institute for Biological Studies, La Jolla, CA 92037; [c]Howard Hughes Medical Institute, Salk Institute for Biological Studies, La Jolla, CA 92037; [d]The Division of Biological Studies Sciences, University of California, San Diego, La Jolla, CA 92093; and [e]Department of Energy Resources Engineering, Stanford University, Stanford, CA 94305

Contributed by Terrence J. Sejnowski, March 27, 2018 (sent for review November 27, 2017; reviewed by Ajay Gopinathan and Alexander Lukyanov)

**High protein concentrations complicate modeling of polymer assembly kinetics by introducing structural complexity and a large variety of protein forms. We present a modeling approach that achieves orders of magnitude speed-up by replacing distributions of lengths and widths with their average counterparts and by introducing a hierarchical classification of species and reactions into sets. We have used this model to study FtsZ ring assembly in *Escherichia coli*. The model's prediction of key features of the ring formation, such as time to reach the steady state, total concentration of FtsZ species in the ring, total concentration of monomers, and average dimensions of filaments and bundles, are all in agreement with the experimentally observed values. Besides validating our model against the in vivo observations, this study fills some knowledge gaps by proposing a specific structure of the ring, describing the influence of the total concentration in short and long kinetics processes, determining some characteristic mechanisms in polymer assembly regulation, and providing insights about the role of ZapA proteins, critical components for both positioning and stability of the ring.**

mathematical model | complex kinetics | in vivo concentration | bundling | FtsZ ring assembly

Protein polymerization is central to cell functioning, contributing to cell division, motility, and intracellular transport. In a cell's cytoplasm, interacting monomers form long polymers called filaments, which assemble and disassemble dynamically by elongation and annealing mechanisms. These filaments attach to the cell's membrane and constitute fundamental building elements of the cytoskeleton. In eukaryotic cells, both actin-based microfilaments and tubulin-based microtubules form bundles of different characteristics (1–3). For example, cell migration due to filopodia formation is regulated by the polymerization of long and tight filaments and by their subsequent bundling (1, 4), and F-actin polymerization and bundling are critical processes in the birth, growth, and final form of mushroom-shaped dendritic spines as well as in the guidance and migration of neuronal growth cones (4–7). In prokaryotic cells, such as *Escherichia coli* or *Bacillus subtilis*, FtsZ and MreB proteins (homologues of eukaryotic tubulins and actins) are the most dominant components of their cytoskeletons. Whereas FtsZ is responsible for cell division, MreB controls the cell width. In both eukaryotic and prokaryotic cells, continuous turnover of monomers between the cytosol and the network of polymers regulates the shape and size of filaments and bundles (5, 6, 8–10). Assembly and disassembly of polymers are, therefore, permanent activities even in the steady state.

The importance and ubiquity of polymer assembly provided an impetus for development of its kinetics models, many of which (e.g., refs. 8, 11–15) aim to describe in vivo or in vitro observations of FtsZ assembly. Initial stages of FtsZ polymerization have been adequately captured with the eight-equation model (8, 12). The latter describes only the first seconds of polymerization for different FtsZ strains and buffer conditions, rather than the whole process of FtsZ assembly. The model's failure to handle later times and in vivo FtsZ concentrations stems from its inability to account for hydrolysis effects and transformations of filaments and bundles. Current models of full FtsZ assembly (e.g., refs. 11, 13, and 15) use hundreds or even thousands of rate equations. Table 1 provides a comparison of these models in terms of their complexity, applicability range, and ability to predict the salient features of FtsZ assembly.

We present a modeling framework that is (many) orders of magnitude faster than the existing alternatives (e.g., those included in Table 1); this speed-up is achieved by replacing distributions of lengths and widths with their average counterparts and by introducing a hierarchical classification of species and reactions into sets. As in previous models, monomers, filaments, and bundles are defined as interacting species; a system of coupled ordinary differential equations (ODEs) describes the temporal evolution of the species concentrations. Unlike those models, our approach involves a hierarchical classification of these species such that, for example, bundles are assembled from filaments that, in turn, are built from monomers. The resulting model comprises ODEs describing the dynamics of the concentrations of species classes and the exchange of elemental quantities (e.g., a monomer in filaments or a filament in bundles) between the classes.

While some kinetic models gain in computational efficiency by replacing filaments of different sizes with filaments of an average length (11, 14, 16), they all treat bundles differing by a single filament as distinct species. Hence, their computational cost increases with total protein concentration, $C_{\mathrm{tot}}$. That is because higher concentrations of $C_{\mathrm{tot}}$ result in larger polymers and

## Significance

**Our modeling framework yields accurate and computationally efficient quantitative predictions of complex kinetics of polymerization processes in biological systems. The resulting model consists of 10 differential equations, regardless of the total concentration of proteins. This is in contrast to previous polymerization models, in which the number of equations increases with the total concentrations, reaching into the thousands. Consequently, our model is orders of magnitude faster than its existing alternatives. It can be used to predict polymerization kinetics at high concentrations characteristic of in vivo processes and, especially, their compartmentalized and spatially distributed representations.**

Author contributions: A.R.-M., T.M.B., T.J.S., and D.M.T. designed research; A.R.-M. performed research; and A.R.-M., T.M.B., T.J.S., and D.M.T. wrote the paper.

Reviewers: A.G., University of California, Merced; and A.L., Harvard Medical School.

The authors declare no conflict of interest.



[1]To whom correspondence may be addressed. Email: terry@snl.salk.edu or tartakovsky@stanford.edu.

This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1719391115/-/DCSupplemental.

**Table 1. Comparison of the kinetic in vitro models in terms of their complexity, applicability range, and ability to predict the observed features of FtsZ assembly**

| Model, reference | (8, 12) | M1 in ref. 11 | M2 in ref. 11 | M3 in ref. 11 | (13) | (14) | AFM |
|---|---|---|---|---|---|---|---|
| Number of ODEs | 8 | 500 | 500 | 1,254 | 300 | 17 | 10 |
| Short time | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| Long time | No | Yes | Yes | Yes | Yes | Yes | Yes |
| Low $C_{tot}$ | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| High $C_{tot}$ | No | No | No | No | Yes | Yes | Yes |
| Filament length | No | Dist | Dist | $Ave^+$ | Dist | Ave | Ave |
| Bundle width | No | No | 2 filaments | $Dist^+$ | No | Dist | Ave |
| $C_{cr}^1$ | $Yes^-$ | Yes | Yes | Yes | $Yes^-$ | Yes | Yes |
| $C_{cr}^2$ | No | No | No | No | No | Yes | Yes |

M1, M2, and M3 designate the single-filament, two-filament bundling, and multifilament bundling models introduced in ref. 11, respectively; AFM denotes our Average Feature Model; $C_{tot}$ is the total concentration of FtsZ monomers in all forms; low and high $C_{tot}$ refers to its values of 2 μM and 10 μM, respectively; $C_{cr}^1 = [Z^{na}]_{ss} + [Z]_{ss} \approx 0.7$ μM is the critical concentration at which polymerization begins, and it is computed as the sum of the steady-state concentrations of nonactivated (GDP-bound) and activated (GTP-bound) FtsZ monomers, respectively; $C_{cr}^2 \approx 3.0$ μM is the critical value of concentration $C_{tot}$ at which bundling becomes pronounced. The abbreviations "Ave" and "Dist" denote average and distribution, respectively; the superscripts $^+$ and $^-$ denote the overestimated and underestimated predictions, respectively.

bundles and, consequently, increase the variability of their sizes; the latter enlarges the number of species and ODEs describing their dynamics. At relatively high concentrations, some of the models comprise hundreds or thousands of ODEs (Table 1). In contrast, the number of ODEs in our model (10 or 11, depending on the presence of a membrane) does not change with $C_{tot}$.

We use in vitro and in vivo FtsZ ring assembly in *E. coli* to demonstrate the veracity and computational efficiency of our model. This complex kinetics process involves a plethora of chemical reactions and species; large concentrations accompanying in vivo assembly of the FtsZ ring put this phenomenon out of reach of most current models. Our approach requires an addition of a single ODE to account for the influence of the membrane and FtsA, ZipA, and ZapA proteins. The resulting 11-ODE model accurately predicts key observed features of the ring formation, such as time to reach the steady state, total concentration of FtsZ species in the ring, total concentration of monomers, and average dimensions of filaments and bundles. It also allows one to generate a hypothesis, for example, about the role of ZapA proteins in positioning and stability of the FtsZ ring.

## Average Feature Model of Polymerization

We reduce multiple sizes of polymers to a species called "filament" and "wide bundle" whose average features are tracked in time. The resulting model comprises 10 ODEs. Our model does not provide information about the exact binding sites where species attach or detach. Instead, it estimates variations in concentration of monomers, filaments, and/or bundles. Consequently, we refer to it as an Average Feature Model, or AFM.

The first critical concentration, $C_{cr}^1$, is the minimum concentration of FtsZ proteins in the monomeric form at which polymerization begins, and it establishes two regimes of polymerization. The first regime, $C_{tot} \leq C_{cr}^1$, admits only monomers such that $[Z^{na}] + [Z] \approx C_{tot}$, where $[Z^{na}]$ and $[Z]$ denote concentrations of nonactivated (GDP-bound) and activated (GTP-bound) FtsZ monomers, respectively. The second regime, $C_{tot} > C_{cr}^1$, allows for FtsZ polymerization and bundling, with $C_{cr}^1 = 0.7$ μM (8).

**Short-Time Kinetics.** The first protofilaments obtained by combining the corresponding number of monomers are denoted by $Z_i$ with $i = 2, 3$. Longer polymers (i.e., filaments) are denoted by $F$. The basic structures (monomers, protofilaments, filaments, thin bundles, and wide bundles) and their graphical representations are summarized in *SI Appendix*, Fig. S1. We describe the early-time kinetics of polymerization with the reduced version (14) of the activation–nucleation–elongation model (8); the latter was used in refs. 16–18 to describe the kinetics of actin polymerization. In so doing, we express the kinetics of all of the processes involved in FtsZ assembly, from its nonactivated monomeric form to long bundles of filaments, in terms of fundamental unimolecular and bimolecular reactions. These are summarized in Table 2 and represented graphically in *SI Appendix*, Fig. S1.

The process of activation is described by reaction I in Table 2, with forward and backward reaction rates $k_{ac}^+$ and $k_{ac}^-$, respectively. Activation and deactivation of monomers occurs due to their interactions with GTP and GDP nucleotides, respectively, even though they are not represented explicitly in our model. The process of nucleation is represented by reaction II in Table 2, with forward and backward reaction rates $k_{nu}^+$ and $k_{nu}^-$, respectively. Formation of the nucleus of two monomers (nucleation or dimerization) is a critical stage of initialization of the FtsZ assembly (12); it also determines the rate of assembly of the polymer network. The elongation process is modeled by reactions III–V in Table 2, with forward and backward reaction rates $k_{el}^+$ and $k_{el}^-$, respectively.

**Long-Time Kinetics.** The first bundles of $k$ filaments are denoted by $B_k$ with $k = 2, 3$. Bigger structures of laterally attached filaments are referred to as wide bundles and are denoted by

**Table 2. Eighteen reactions comprising our FtsZ kinetics model**

| Reactions | Chemical equations |
|---|---|
| Reaction I | $Z^{na} \rightleftharpoons Z$ |
| Reaction II | $2Z \rightleftharpoons Z_2$ |
| Reactions III–V | $Z + Z_2 \rightleftharpoons Z_3$, $Z + Z_3 \rightarrow F$, $Z + F_{z-} \rightleftharpoons F_{z+}$ |
| Reaction VI | $2F_{f-} \rightleftharpoons F_{f+}$ |
| Reactions VII–XI | $2F \rightleftharpoons B_2$, $F + B_2 \rightleftharpoons B_3$, $F + B_3 \rightarrow B_w$, $F + B_{w;f-} \rightleftharpoons B_{w;f+}$, $2B_{w;b-} \rightleftharpoons B_{w;b+}$ |
| Reactions XII and XIII | $F_{z+} \rightarrow F_{z-} + Z^{na}$, $F_{z+,f+} \rightarrow 2F_{z-,f-} + Z^{na}$ |
| Reactions XIV–XVI with $i = 2, 3$ | $B_{i;z+,b+} \rightarrow 2B_{i;z-,b-} + Z^{na}$, $B_{i;z+} \rightarrow B_{i;z-} + Z^{na}$, $B_{w;z+} \rightarrow B_{w;z-} + Z^{na}$ |
| Reactions XVII and XVIII with $i = 2, 3$ | $Z + B_{i;z-} \rightarrow B_{i;z+}$, $Z + B_{w;z-} \rightarrow B_{w;z+}$ |

The subscripts $z^-/f^-/b^-$ (and $z^+/f^+/b^+$) designate a monomer/filament/bundle lost (or gained) by a species.

$B_w$. We assume that filaments and bundles have the same length when they connect laterally and that bundles grow laterally into 3D structures. With these simplifications, the process of filament annealing is represented by reaction VI in Table 2, with forward and backward reaction rates $k_{\text{an}}^{+}$ and $k_{\text{an}}^{-}$, respectively. The process of filament bundling is modeled similarly to elongation/annealing of filaments; that is, bundles of up to three filaments are explicitly defined by reactions VII–XI in Table 2, with forward and backward reaction rates $k_{\text{bu}}^{+}$ and $k_{\text{bu}}^{-}$, respectively. The former rate depends on the number of filaments comprising both reactants; the latter rate varies with the number of filaments comprising the reactant and $\bar{L}_{\text{fb}}^{m}$, an average filament length (expressed as the number of monomers in a filament)—that is, $k_{\text{bu}}^{-} = k_{\text{bu}}^{-}(\bar{L}_{\text{fb}}^{m})$.

Hydrolysis of both filaments and bundles contributes to the turnover of monomers between a network of filaments/bundles and the ambient solution.

Dissociation of monomers from filaments after GTP hydrolysis is modeled by irreversible reactions XII and XIII in Table 2, with rates $k_{\text{hy/dis}}^{1}$ and $k_{\text{hy/dis}}^{2}$, respectively. To model dissociation of monomers from bundles after GTP hydrolysis, we supplement the two reactions used in ref. 14 with a third one for wide bundles (reactions XIV–XVI in Table 2). The first of these reactions has rate $k_{\text{hy/dis}}^{2}$, and the remaining two have rate $k_{\text{hy/dis}}^{3}$. Biochemical implications and limitations of our conceptualization of monomer turnover are discussed in ref. 14.

Finally, attachment of monomers to bundles is represented by reactions XVII and XVIII in Table 2, with attachment rate $k_{\text{mb}}$. These reactions account for interactions between activated monomers and the bundles and attachment of the former to the latter.

**Concentration of Species Sets.** A key component of AFM is a classification of the FtsZ species into different sets (Table 3). Exchange of FtsZ structures between these sets is defined in terms of the elementary reactions collated in Table 2. A set containing all FtsZ species, $\mathbb{S}_{\text{z}}$, includes monomers ($m$), filaments ($f$), and bundles ($b$) and is endowed with average filament length ($\bar{L}_{\text{fb}}^{m}$) and bundle width ($\bar{f}_{\text{wb}}$, the number of filaments in a bundle). It comprises a subset of monomers and protofilaments, $\mathbb{S}_{\text{mp}}$, and a subset of filaments and bundles, $\mathbb{S}_{\text{fb}}$, such that $\mathbb{S}_{\text{z}} = \mathbb{S}_{\text{mp}} \cup \mathbb{S}_{\text{fb}}$. The total concentration (in $\mathbb{S}_{\text{z}}$) of FtsZ monomers in all forms, $C_{\text{tot}}$, is the sum $C_{\text{tot}} = C_{\text{mp}}^{m} + C_{\text{fb}}^{m}$ of the concentration (in $\mathbb{S}_{\text{mp}}$) of monomers in the monomer and protofilament forms and the concentration (in $\mathbb{S}_{\text{fb}}$) of monomers in the filament and bundle forms, with both concentrations defined in Table 3. Transfer of monomers from $\mathbb{S}_{\text{mp}}$ to $\mathbb{S}_{\text{fb}}$ is due to a reaction set $\mathbb{R}_{\text{mp}\to\text{fb}}^{m}$ (see Table 3). The number of monomers (in both monomer and protofilament forms) involved in reaction $R_1$ is $m_{\text{r}}^{1} = 4$ for the reactants and $m_{\text{p}}^{1} = 0$ for the reaction product; the same for reaction $R_k$ ($k = 2, 3, 4$) is $m_{\text{r}}^{k} = 1$ and $m_{\text{p}}^{k} = 0$. Likewise, transfer of monomers from $\mathbb{S}_{\text{fb}}$ to $\mathbb{S}_{\text{mp}}$ is due to a reaction set $\mathbb{R}_{\text{fb}\to\text{mp}}^{m}$. For each reaction $R_n$ ($n = 1, \cdots, 6$), $m_{\text{r}}^{n} = 0$ and $m_{\text{p}}^{n} = 1$. Then,

$$\frac{\mathrm{d}C_{\text{fb}}^{m}}{\mathrm{d}t} = \sum_{\substack{k=1 \\ \mathbb{R}_{\text{mp}\to\text{fb}}^{m}}}^{4} \kappa_k (m_{\text{r}}^{k} - m_{\text{p}}^{k}) - \sum_{\substack{n=1 \\ \mathbb{R}_{\text{fb}\to\text{mp}}^{m}}}^{6} \kappa_n (m_{\text{p}}^{n} - m_{\text{r}}^{n}), \qquad [1]$$

where $\kappa_k$ ($k = 1, \cdots, 10$) are the reaction rates for reactions $R_k$ from the reaction sets $\mathbb{R}_{\text{mp}\to\text{fb}}^{m}$ and $\mathbb{R}_{\text{fb}\to\text{mp}}^{m}$. This formulation conserves mass and energy, although the principle of microscopic reversibility, or detailed balance, is violated (see *SI Appendix, section S1*).

The subset $\mathbb{S}_{\text{fb}}$ is, in turn, subdivided into subsets of filaments and thin bundles, $\mathbb{S}_{\text{ftb}}$, and wide bundles, $\mathbb{S}_{\text{wb}}$ (see Table 3). The total concentration (in $\mathbb{S}_{\text{fb}}$) of FtsZ filaments in filament and bundle forms, $C_{\text{fb}}^{f}$, is the sum $C_{\text{fb}}^{f} = C_{\text{ftb}}^{f} + C_{\text{wb}}^{f}$ of the concentration (in $\mathbb{S}_{\text{ftb}}$) of filaments in the filament and thin bundle forms, $C_{\text{ftb}}^{f}$, and the concentration (in $\mathbb{S}_{\text{wb}}$) of filaments in the wide bundle form, $C_{\text{wb}}^{f}$. (Both $C_{\text{ftb}}^{f}$ and $C_{\text{wb}}^{f}$ are defined in Table 3.) Transfer of filaments from $\mathbb{S}_{\text{ftb}}$ to $\mathbb{S}_{\text{wb}}$ is due to a reaction set $\mathbb{R}_{\text{ftb}\to\text{wb}}^{f}$. The number of filaments (in both filament and thin bundle forms) involved in reaction $R_1$ is $f_{\text{r}}^{1} = 4$ for the reactants and $f_{\text{p}}^{1} = 0$ for the reaction product; for reaction $R_2$, these are $f_{\text{r}}^{2} = 1$ and $f_{\text{p}}^{2} = 0$. The transfer from $\mathbb{S}_{\text{wb}}$ to $\mathbb{S}_{\text{ftb}}$ is due to reaction $R_3$; it results in $f_{\text{r}}^{1} = 0$ and $f_{\text{p}}^{1} = 1$. Then,

$$\frac{\mathrm{d}C_{\text{wb}}^{f}}{\mathrm{d}t} = \sum_{\substack{k=1 \\ \mathbb{R}_{\text{ftb}\to\text{wb}}^{f}}}^{2} \kappa_k (f_{\text{r}}^{k} - f_{\text{p}}^{k}) - \sum_{\substack{n=1 \\ \mathbb{R}_{\text{wb}\to\text{ftb}}^{f}}}^{1} \kappa_n (f_{\text{p}}^{n} - f_{\text{r}}^{n}), \qquad [2]$$

where $\kappa_k$ are the reaction rates for reactions $R_k$ from the reaction sets $\mathbb{R}_{\text{ftb}\to\text{wb}}^{f}$ and $\mathbb{R}_{\text{wb}\to\text{ftb}}^{f}$ (see Table 3).

The definitions of $C_{\text{fb}}^{m}$ and $C_{\text{wb}}^{f}$ relate to the average structural features to the concentrations

$$\bar{L}_{\text{fb}}^{m} = \frac{C_{\text{fb}}^{m}}{[F] + 2[B_2] + 3[B_3] + C_{\text{wb}}^{f}}, \qquad \bar{f}_{\text{wb}} = \frac{C_{\text{wb}}^{f}}{[B_w]}. \qquad [3]$$

A smallest filament consists of four monomers (i.e., has the length $\bar{L}_{\text{fb}}^{m} = 4$). The latter is achieved instantaneously once $[F]$ becomes larger than zero. To consider all species, we define an average total length, $\bar{L}_{\text{tot}}^{m}$, which includes the first oligomers ($Z_2$ and $Z_3$):

$$\bar{L}_{\text{tot}}^{m} = \frac{2[Z_2] + 3[Z_3] + C_{\text{fb}}^{m}}{[Z_2] + [Z_3] + C_{\text{fb}}^{m}/\bar{L}_{\text{fb}}^{m}}. \qquad [4]$$

It represents the average length of filaments in all forms (short oligomers, longer filaments, and bundles). Similarly, a smallest wide bundle consists of four filaments (i.e., has the width $\bar{f}_{\text{wb}} = 4$). This value is achieved instantaneously once $[B_w]$ becomes larger than zero. To consider all species, we define an average total number of filaments per bundle, $\bar{f}_{\text{tot}}$, which includes the first

**Table 3. Species, reaction, and concentration of species sets**

| Types of sets | Notation |
|---|---|
| Species sets | $\mathbb{S}_{\text{z}} = \mathbb{S}_{\text{mp}} \cup \mathbb{S}_{\text{fb}} = \{Z^{\text{na}}, Z, Z_2, Z_3, F, B_2, B_3, B_w; m, f, b; \bar{L}_{\text{fb}}^{m}, \bar{f}_{\text{wb}}\}$, $\mathbb{S}_{\text{mp}} = \{Z^{\text{na}}, Z, Z_2, Z_3; m\}$, $\mathbb{S}_{\text{fb}} = \mathbb{S}_{\text{ftb}} \cup \mathbb{S}_{\text{wb}} = \{F, B_2, B_3, B_w; m, f, b; \bar{L}_{\text{fb}}^{m}, \bar{f}_{\text{wb}}\}$, $\mathbb{S}_{\text{ftb}} = \{F, B_2, B_3; m, f; \bar{L}_{\text{fb}}^{m}\}$, $\mathbb{S}_{\text{wb}} = \{B_w; m, f, b; \bar{L}_{\text{fb}}^{m}, \bar{f}_{\text{wb}}\}$ |
| Reaction sets | $\mathbb{R}_{\text{mp}\to\text{fb}}^{m} \equiv \{R_1 : \text{IV};\ R_2 : \text{V}_{\text{FW}};\ R_3 : \text{XVII};\ R_4 : \text{XVIII}\}$, $\mathbb{R}_{\text{fb}\to\text{mp}}^{m} \equiv \{R_1 : \text{V}_{\text{BW}};\ R_2 : \text{XII};\ R_3 : \text{XIII};\ R_4 : \text{XIV};\ R_5 : \text{XV};\ R_6 : \text{XVI}\}$, $\mathbb{R}_{\text{ftb}\to\text{wb}}^{f} \equiv \{R_1 : \text{IX};\ R_2 : \text{X}_{\text{FW}}\}$; $\mathbb{R}_{\text{wb}\to\text{ftb}}^{f} \equiv \{R_1 : \text{X}_{\text{BW}}\}$ |
| Concentrations of species sets | $C_{\text{tot}} = C_{\text{mp}}^{m} + C_{\text{fb}}^{m}$, $C_{\text{mp}}^{m} = [Z^{\text{na}}] + [Z] + 2[Z_2] + 3[Z_3]$, $C_{\text{fb}}^{m} = \bar{L}_{\text{fb}}^{m}\left([F] + 2[B_2] + 3[B_3] + \bar{f}_{\text{wb}}[B_w]\right)$, $C_{\text{fb}}^{f} = C_{\text{ftb}}^{f} + C_{\text{wb}}^{f}$, $C_{\text{ftb}}^{f} = [F] + 2[B_2] + 3[B_3]$, $C_{\text{wb}}^{f} = \bar{f}_{\text{wb}}[B_w]$ |

FW and BW designate forward and backward reactions, respectively.

**Table 4. Timing of FtsZ ring formation for a characteristic range of in vivo FtsZ concentrations, $C_{tot,C;0} = 6 - 18$ μM**

| $C_{tot,C;0}$, μM | $t_{att}$, s | $t_{\bar{L}}$, s | $t_{mon}$, s | $t_{\bar{f}}$, s |
|---|---|---|---|---|
| 6.0 | 19 | 49 | 220 | 244 |
| 12.0 | 10 | 53 | 217 | 240 |
| 18.0 | 8 | 40 | 210 | 233 |

oligomers ($Z_2$ and $Z_3$), longer filaments ($F$), and the first thin bundles ($B_2$ and $B_3$):

$$\bar{f}_{tot} = \frac{2[Z_2] + 3[Z_3] + C_{fb}^m}{2[Z_2] + 3[Z_3] + \bar{L}_{fb}^m([F] + [B_2] + [B_3] + [B_w])}. \quad [5]$$

Average characteristics $\bar{L}_{fb}^m$, $\bar{L}_{tot}^m$, $\bar{f}_{wb}$, and $\bar{f}_{tot}$ play a crucial role in reducing the number of species and, therefore, the number of equations used to describe the protein assembly process. *SI Appendix*, *section S1* contains ODEs corresponding to the reactions involved in the short- and long-term kinetics processes described above.

**Model's Calibration, Validation, and Computational Cost.** The system of 10 ODEs is parametrized and calibrated on the steady-state, low-concentration ($C_{tot} = 0.7 - 3.0$ μM) data from ref. 8 (see *SI Appendix*, *section S2*). The resulting model is validated by comparing its fit-free predictions with the transient, low-concentration data and the steady-state high-concentration ($C_{tot} = 3.0 - 10.0$ μM) data, both from ref. 8 (*SI Appendix*, *section S3*). This comparison demonstrates AFM's ability to accurately predict time evolution of nonactivated and activated monomers, the first critical concentration $C_{cr}^1$ at which polymerization begins, an average size of filaments and bundles, and the second critical concentration $C_{cr}^2$ at which bundles appear.

This predictive power of AFM is achieved at a fraction of the cost of its nearest competitor (14), which, in turn, is orders of magnitude faster than the models consisting of hundreds of ODEs (see Table 1). The computational efficiency of AFM, vis-à-vis ref. 14 and other models of this kind, is magnified when it is used to simulate in vivo polymerization phenomena, which are characterized by high total concentrations. For $C_{tot} = 200$ μM, AFM is about 2 orders of magnitude faster than ref. 14 (see *SI Appendix*, *section S4* for details).

**FtsZ-Ring Assembly in *E. coli*.** We use AFM to describe the Z-ring formation in the middle of an *E. coli* cell, within a torus adjacent to the cell membrane (CM) of volume $V_{CM}$. A typical cell has a volume $V_{CELL} = 1.374$ μm$^3$ and contains $5000 - 15000$ FtsZ molecules, which translates into a range of FtsZ concentrations in the cytosol, $C_{tot,C;0} = 6 - 18$ μM (19–21); at the midcell, close to the membrane, the concentration is one to two orders of magnitude higher than that. Only a few polymerization models can handle such concentrations, and even the most computationally efficient among them (14) (Table 1) would require hundreds of ODEs to handle all bundle sizes. AFM accomplishes the same with 11 ODEs, adding only one equation for the total concentration of FtsZ species (in all forms) attached to the membrane, $C_{tot,CM}^a$.

CM contributes to significant physical, chemical, and structural differences between in vitro and in vivo polymerization. The augmented AFM accounts for some of these differences by incorporating the FtsZ species' attachment to and detachment from a CM. The parameters relevant to this process as well as other parameters describing cell geometry and bundling/dissociation kinetics come from the literature, so that predictions reported below are made without any fitting parameters. (A detailed formulation of the augmented AFM is provided in *SI Appendix*, *section S5*.)

**Timing of FtsZ Ring Formation.** We define four stages of the ring formation in terms of their characteristic times: time it takes the FtsZ protofilaments in the cytoplasm to attach to the mid-CM and occupy all binding sites, $t_{att}$; time to reach a constant average length of all filaments and bundles in the ring, $t_{\bar{L}}$; time to reach a constant concentration of monomers at the midcell region ($C_{m,CM}^d$), $t_{mon}$; and time to reach a constant average number of filaments per bundle in the ring, $t_{\bar{f}}$.

Let $P$ denote an FtsZ ring property and $P^\infty$ its value at $t \to \infty$; $P$ stands for $C_{tot,CM}^a$ at time $t_{att}$, $\bar{L}_{tot}^m$ at time $t_{\bar{L}}$, $C_{m,CM}^d$ at time $t_{mon}$, and $\bar{f}_{tot}$ at time $t_{\bar{f}}$. We compute these times by inverting the condition $|P(t) - P^\infty|/P^\infty < 0.01$. The results, reported in Table 4, reveal that changes in the total concentration $C_{tot,C;0}$ appreciably affect $t_{att}$ (early-time kinetics), while having an almost negligible impact on the other three characteristic times (long-time kinetics). These results identify the timing for three distinct stages of the ring formation (time $t = 0$ corresponds to the moment at which the Ter region is already located at the center of the cell).

***Short-time kinetics.*** Attachment of FtsZ protofilaments to the binding sites at the midcell takes 8 to 19 s. Although there are no data about the first seconds of in vivo polymerization to verify this prediction, the values that our model estimates are quite similar to the turnover half-times of FtsA (12 to 16.3 s) (22) and ZipA mutants [7.81 to 9.01 s, or 0.111 to 0.128 s$^{-1}$) (23), which is the range of FtsZ turnover rate values, since according to ref. 24 both FtsZ and ZipA may undergo similar dynamic exchanges]. It seems reasonable to think that the emergence of the first FtsZ oligomers at the membrane will depend strongly on the time that FtsA and ZipA, both responsible for the attachment of FtsZ to the membrane, remain themselves attach to the membrane.

***Intermediate kinetics.*** Elongation of the species up to their average length takes 40 to 50 s. This is in line with the observations (25, 26) that ring assembly takes $\sim 1$ min. This suggests correspondence between the complete longitudinal elongation of the species and the ring formation.

***Long-time kinetics.*** Continuous exchange of monomers between the ring and the cytosol accompanies the formation of large FtsZ structures, at 3.5 to 4 min. This is consistent with the observed time interval, $\sim 4$ min, between the ring's central positioning and the onset of septation (27).These findings imply that dissociation of monomers after GTP hydrolysis plays a fundamental role in rearrangement of filaments and bundles, while formation of wide bundles contributes to regulation of the dissociation in live cells, just as it does in in vitro studies (14). Finally, the timing predictions obtained with our model and the in vivo version of ref. 14 are compared in *SI Appendix*, *section S4*.

**FtsZ Ring Features.** Our model predicts FtsZ concentrations in the ring to be around 40 times larger than cytosolic concentrations (Table 5). Variations in the total concentration $C_{tot,C;0}$ do not materially affect the average length of the species at steady state, $\bar{L}_{tot}^{m,\infty} = 24$ to 25 monomers. That value corresponds to the experimentally observed characteristic length of 120 to 125 nm (since the monomer's diameter is 5 nm) (8) and falls within

**Table 5. FtsZ ring features for a characteristic range of in vivo FtsZ concentrations, $C_{tot,C;0} = 6 - 18$ μM, at steady state ($t \to \infty$)**

| $C_{tot,C;0}$, μM | $C_{tot,CM}^{a,\infty}$, μM | $\bar{L}_{tot}^{m,\infty}$ | $C_{m,CM}^{d,\infty}$, μM | $\bar{f}_{tot}^{\infty}$ |
|---|---|---|---|---|
| 6.0 | 237 | 24.43 | 1.13 | 9.4 |
| 12.0 | 474 | 24.47 | 1.20 | 16.4 |
| 18.0 | 711 | 24.49 | 1.25 | 22.7 |

**Table 6. Large FtsZ structures for a range of in vivo FtsZ concentrations, $C_{tot,C;0} = 6 - 18$ μM**

| $C_{tot,C;0}$, μM | $\mathcal{R}^{\%}_{wb}$ | $\bar{f}_{clu}$ | $\bar{f}_{CLS}$ | $\mathcal{N}^{CLS}_{clu}$ | $\mathcal{N}_{CLS}$ | $\mathcal{N}_{clu}$ |
|---|---|---|---|---|---|---|
| 6.0 | 85.1 | 6.1 | 19.6 | 3.2 | 2.8 | 9.0 |
| 12.0 | 91.6 | 7.6 | 35.0 | 4.6 | 3.4 | 15.6 |
| 18.0 | 94.0 | 9.5 | 48.5 | 5.1 | 3.7 | 18.9 |

The structures are characterized by the percentage of FtsZ proteins in the wide-bundles form, $\mathcal{R}^{\%}_{wb}$; the average number of filaments per bundle in a cluster, $\bar{f}_{clu}$; average width of CLSs, $\bar{f}_{CLS}$; number of clusters per CLS, $\mathcal{N}^{CLS}_{clu}$; number of CLSs, $\mathcal{N}_{CLS}$; and number of clusters, $\mathcal{N}_{clu}$.

the range, $100 - 200$ nm, found in other in vitro experiments (28–30).

The predicted steady-state concentration of attached monomers, $C^{a,\infty}_{tot,CM} = 0.9$ to 1.25 μM, is insensitive to the total concentration $C_{tot,C;0}$ (Table 5) and falls within the range of values of the first critical concentration in wild-type cells, $C^{1}_{cr,wt}$ (12). We postulate this feature to be representative of polymer assembly regulation, both in in vitro and in in vivo.

The total cytosolic concentration $C_{tot,C;0}$ does affect the average number of filaments per bundle at steady state, $\bar{f}^{\infty}_{tot}$ (Table 5). To investigate the prevalence of this form of FtsZ proteins at steady state, we consider the percentage of FtsZ proteins in the form of wide bundles, $\mathcal{R}^{\%}_{wb} \equiv 100\% \times C^{f,a}_{wb,CM}/([F] + 2[B_2] + 3[B_w] + C^{f,a}_{wb,CM})$. Table 6 reveals that, at steady state, $\mathcal{R}^{\%}_{wb} =$ 85%–94%, depending on $C_{tot,C;0}$; that is, wide bundles are the dominant species.

Once $C^{a}_{tot,CM} = C^{a,max}_{tot,CM}$—that is, all FtsZ protofilaments are attached to the mid-CM—bundles form cross-linked structures (CLSs) along the ring. We refer to these structures as "clusters," which in our model are formed at time $t = t_{att}$ and consist of bundles with an average number of filaments $\bar{f}_{clu} = \bar{f}_{tot}(t_{att})$. The predicted values $\bar{f}_{clu} = 6.1 - 9.5$ (Table 6) imply the average width of FtsZ–ZapA sheets of $12.2 - 19$. This is consistent with the experimentally observed maximal number of FtsZ–ZapA filaments perfectly aligned at the mid-CM, $\bar{f}_{clu,ZapA} < \bar{f}^{max}_{wb,ZapA} = 20$ (see *SI Appendix, section S7* for details). The predicted minimal value, $\bar{f}_{clu,ZapA} = 12.2$, indicates that the two-layered sheets have to occupy at least half of the axial width of the ring. When that occurs, bundle interactions in the tangential direction, which induce bundles to push and lift each other, dominate their axial interactions.

For $t > t_{att}$, $\bar{f}_{clu}$ remains constant as clusters interact only along the ring-forming CLSs. Since wide bundles contain most of the FtsZ at steady state, we define an average CLS width as $\bar{f}_{CLS} = \bar{f}_{wb}(t \to \infty)$. A number of clusters per CLS is $\mathcal{N}^{CLS}_{clu} \equiv \bar{f}_{CLS}/\bar{f}_{clu}$, and a number of CLSs is $\mathcal{N}_{CLS} \equiv [B_w]\, V_{CM} N_A / 10^{21}$, where $N_A$ is Avogadro's number. A number of clusters in the entire ring is $\mathcal{N}_{clu} = \mathcal{N}^{CLS}_{clu}\mathcal{N}_{CLS}$. Our model predicts $\mathcal{N}_{clu} = 9.0 - 18.9$ (Table 6), which conforms to the observed range of $10 - 20$ clusters (31).

To sum up, our model predicts FtsZ rings that are mostly composed of 3 to 4 large structures, which are partially attached to the membrane and comprise 3 to 5 cross-linked clusters each. These clusters contain 6 to 10 filaments made up of 24 monomers each. The entire network also includes ZapA tetramers, which reinforce the lateral bonds of the clusters and the cross-links between clusters, as observed in vitro (32) and in vivo (33). *SI Appendix, section S4* includes a comparison between the ring feature predictions of our model and the in vivo version of ref. 14.

**ZapA Deficiency.** A recent experimental study (33) investigated in vivo polymerization in the absence of ZapA proteins. Our model predicts the resulting FtsZ concentrations to be only 10 times larger than their in vitro counterparts (Table 7). The average length ($L^{m}_{tot}$) and concentration ($C^{d}_{m,CM}$) of detached monomers are insensitive to the total cytosolic concentration $C_{tot,C;0}$. The predicted monomer concentrations $C^{d}_{m,CM}$ fall within the experimentally observed range of critical concentrations, $C^{a,\infty}_{tot,CM} = 0.9 - 1.25$ μM.

The model also predicts the predominant presence of non–cross-linked and dispersed thin clusters along the entire cell, as observed (33). In the absence of ZapA, $t_{att} = 20 - 30$ s is twice as long as that in the case with ZapA, while $t_{\bar{L}} = 40 - 50$ s remains about the same. The times for bundling and dissociation of monomers after GTP hydrolysis, $t_{mon} \sim t_{\bar{f}} = 100 - 120$ s, are half of their counterparts in the presence of ZapA. Since FtsZ structures are more dispersed and interactions happen less frequently, the equilibrium is reached faster.

## Conclusions

We developed a computationally efficient model of protein polymerization, which relies on concentrations and average features of different species. Orders of magnitude speed-up is achieved by replacing distributions of lengths and widths with their average counterparts and by introducing a hierarchical classification of species and reactions into sets. The resulting model consists of 10 or 11 ODEs, regardless of the total concentration of proteins. This is in contrast to previous polymerization models, in which the number of ODEs increases with the total concentrations, reaching into the thousands. Consequently, our model can be used to predict polymerization kinetics at high concentrations characteristic of in vivo processes and, especially, their compartmentalized representations.

We have used this model to study in vitro and in vivo FtsZ ring assembly in *E. coli*, a complex kinetics process with a large number of chemical reactions and species involved. The model's computational performance is not affected by the large concentrations of proteins located at the midcell, near the membrane. The model's predictions of key features of the ring formation, such as time to reach the steady state, total concentration of FtsZ species in the ring, total concentration of monomers, and average dimensions of filaments and bundles, are all in agreement with the experimentally observed values. Besides validating our model against the in vivo observations, this study fills some knowledge gaps by proposing a specific structure of the ring, describing the influence of the total concentration in short and long kinetics processes, determining some characteristic mechanisms in polymer assembly regulation, and providing insights about the role of ZapA proteins, a critical component for both positioning and stability of the ring.

The orders of magnitude computational speed-up provided by our model comes at a cost. An explicit representation of bundle size distribution (14) would improve a description of the ring's structure (e.g., heterogeneity of the bundle network). It would avoid overestimation of robustness at the sides of the ring where proteins like MinC promote debundling and depolymerization (29). The influence of bundling on the dissociation of monomers upon the GTP-hydrolysis process is also related to the size of the

**Table 7. FtsZ species features for a characteristic range of in vivo FtsZ concentrations, $C_{tot,C;0} = 6 - 18$ μM, in the absence of ZapA at steady state**

| $C_{tot,C;0}$, μM | $C^{a,max}_{tot,CM}$, μM | $\bar{L}^{m}_{tot}$ | $C^{d}_{m,CM}$, μM | $\bar{f}_{tot}$ |
|---|---|---|---|---|
| 6.0 | 48 | 35.43 | 0.92 | 1.92 |
| 12.0 | 104 | 35.84 | 0.95 | 3.49 |
| 18.0 | 160 | 35.86 | 1.00 | 4.76 |

bundles, which is captured by the model (14). Nevertheless, our results demonstrate that the models based on average characteristics yield predictions at least as accurate as those computed with their distribution-based model counterparts.

**ACKNOWLEDGMENTS.** This work was supported in part by the Howard Hughes Medical Institute, National Institutes of Health Grants MH079076 and P41-GM103712, the Salk Innovation Award, the Defense Advanced Research Projects Agency EQUiPS program, and National Science Foundation Grant DMS-1620103.

1. Dixon RD, et al. (2008) Palladin is an actin cross-linking protein that uses immunoglobulin-like domains to bind filamentous actin. *J Biol Chem* 283:6222–6231.
2. Erb ML, et al. (2014) A bacteriophage tubulin harnesses dynamic instability to center DNA in infected cells. *eLife* 3:6222–6231.
3. Walczak CE, Shaw SL (2010) A MAP for bundling microtubules. *Cell* 142:433–443.
4. Mattila PK, Lappalainen P (2008) Filopodia: Molecular architecture and cellular functions. *Nat Rev Mol Cell Biol* 9:446–454.
5. Koskinen M, Hotulainen P (2014) Measuring F-actin properties in dendritic spines. *Front Neuroanat* 8:1–14.
6. Medeiros NA, Burnette DT, Forscher P (2006) Myosin II functions in actin-bundle turnover in neuronal growth cones. *Nat Cell Biol* 8:216–226.
7. Penzes P, Rafalovich I (2012) Regulation of the actin cytoskeleton in dendritic spines. *Adv Exp Med Biol* 970:81–95.
8. Chen Y, Erickson HP (2005b) Rapid in vitro assembly dynamics and subunit turnover of FtsZ demonstrated by fluorescence resonance energy transfer. *J Biol Chem* 280:22549–22554.
9. Chen Y, Erickson HP (2009) FtsZ filament dynamics at steady state: Subunit exchange with and without nucleotide hydrolysis. *Biochemistry* 48:6664–6673.
10. Rottner K, Stradal TEB (2011) Actin dynamics and turnover in cell motility. *Curr Opin Cell Biol* 23:569–578.
11. Lan G, Dajkovic A, Wirtz D, Sun SX (2008) Polymerization and bundling kinetics of FtsZ filaments. *Biophys J* 95:4045–4056.
12. Chen Y, Bjornson K, Redick SD, Erickson HP (2005a) A rapid fluorescence assay for FtsZ assembly indicates cooperative assembly with a dimer nucleus. *Biophys J* 88:505–514.
13. Surovtsev IV, Morgan JJ, Lindhal PA (2008) Kinetic modeling of the assembly, dynamic steady state and contraction of the FtsZ ring in prokaryotic cytokinesis. *PLoS Comput Biol* 4: e1000102.
14. Ruiz-Martinez A, Bartol TM, Sejnowski TJ, Tartakovsky DM (2016) Efficient multiscale models of polymer assembly. *Biophys J* 110:185–196.
15. Dow CE, Rodger A, Roper DI, van den Berg HA (2013) A model of membrane contraction predicting initiation and completion of bacterial cell division. *Integr Biol* 5:778–795.
16. Falzone TT, Lenz M, Kovar DR, Gardel ML (2012) Assembly kinetics determine the architecture of $\alpha$-actin in crosslinked F-actin networks. *Nat Commun* 3:266–272.
17. Frieden C, Goddette DW (1983) Polymerization of actin and actin-like systems: Evaluation of the time course of polymerization in relation to the mechanism. *Biochemistry* 22:5836–5843.
18. Sept D, Xu J, Pollard TD, McCammon JA (1999) Annealing accounts for the length of actin filaments formed by spontaneous polymerization. *Biophys J* 77:2911–1919.
19. Hale CA, de Boer AJ (1997) Direct binding of Ftsz to ZipA, an essential component of the septal ring structure that mediates cell division in E. coli. *Cell* 88:175–185.
20. 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* 185:3344–3351.
21. Erickson HP, Anderson DE, Osawa M (2010) FtsZ in bacterial cytokinesis: Cytoskeleton and force generator all in one. *Microbiol Mol Biol Rev* 74:504–528.
22. Geissler B, Shiomi D, Margolin W (2007) The ftsA* gain-of-function allele of Escherichia coli and its effects on the stability and dynamics of the Z ring. *Microbiology* 153:814–825.
23. Anderson DE, Gueiros-Filho FJ, Erickson HP (2004) Assembly dynamics of FtsZ rings in Bacillus subtilis and Escherichia coli and effects of FtsZ-regulating proteins. *J Bacteriol* 186:5775–5781.
24. Stricker J, Maddox P, Salmon ED, Erickson HP (2002) Rapid assembly dynamics of the Escherichia coli FtsZ-ring demonstrated by fluorescence recovery after photobleaching. *Proc Natl Acad Sci USA* 99:3171–3175.
25. Addinall CSG, Cao C, Lutkenhaus J (1997) Temperature shift experiments with an ftsZ84(Ts) strain reveal rapid dynamics of FtsZ localization and indicate that the Z ring is required throughout septation and cannot reoccupy division sites once constriction has initiated. *J Bacteriol* 179:4277–4284.
26. Sun Q, Margolin W (1998) FtsZ dynamics during the division cycle of live Escherichia coli cells. *J Bacteriol* 180:2050–2056.
27. Tsukanov R, et al. (2011) Timing of Z-ring localization in Escherichia coli. *Phys Biol* 8:066003.
28. Romberg L, Simon M, Erickson HP (2001) Polymerization of FtsZ, a bacterial homolog of tubulin: Is assembly cooperative? *J Biol Chem* 18:11743–11753.
29. Dajkovic A, Lan G, Sun SX, Wirtz D, Lutkenhaus J (2008) MinC spatially controls bacterial cytokinesis by antagonizing the scaffolding function of FtsZ. *Curr Biol* 18:235–244.
30. Popp D, Iwasa M, Narita A, Erickson HP, Maéda Y (2009) FtsZ condensates: An in vitro electron microscopy study. *Biopolymers* 91:340–350.
31. Coltharp C, Buss J, Plumer TM, Xiao J (2016) Defining the rate-limiting processes of bacterial cytokinesis. *Proc Natl Acad Sci USA* 113:1044–1053.
32. Dajkovic A, Pichoff S, Lutkenhaus J, Wirtz D (2010) Cross-linking FtsZ polymers into coherent Z rings. *Mol Microbiol* 78:651–668.
33. Buss J, et al. (2013) In vivo organization of the FtsZ-ring by ZapA and ZapB revealed by quantitative super-resolution microscopy. *Mol Microbiol* 89:1099–1120.