Treadmilling and length distributions of active polar filaments

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The cytoskeleton is a network of filamentous proteins, notably, actin filaments and microtubules. These filaments are active as their assembly is driven by the hydrolysis of nucleotides bound to the constituting protomers. In addition, the assembly kinetics differs at the two respective ends, making them active polar filaments. Experimental evidence suggests, that, in vivo, actin filaments and microtubules can grow at one and shrink at the other end at the same rate, a state that is known as treadmilling. In this work, we use a generic discrete two-state model for active polar filaments to analyze the conditions leading to treadmilling. We find that a single filament can self-organize into the treadmilling state for a broad range of monomer concentrations. In this regime the corresponding length distribution has a pronounced maximum at a finite value. We then extend our description to consider specifically the dynamics of actin filaments. We show that actin treadmilling should be observable in vitro in the presence of appropriate depolymerization promoting factors. © 2013 AIP Publishing LLC [http://dx.doi.org/10.1063/1.4825248]

I. INTRODUCTION

The proteins actin and tubulin can both assemble into non-covalently bound linear aggregates known as filamentous actin (F-actin) and microtubules, respectively. They are the main constituents of the cytoskeleton, which drives a number of vital processes in living cells and forms important cellular structures. F-actin and microtubules owe their key roles in life to two features, which distinguish them from most conventional linear polymers. First of all, the two ends of these polymers are structurally different. In addition, actin and tubulin can bind nucleotides: adenosine-diphosphate (ADP) and -triphosphate (ATP) in the case of actin, guanosine-diphosphate (GDP) and -triphosphate (GTP) in the case of tubulin. These two properties characterize F-actin and microtubules as active polar filaments. Here, “active” refers to the fact that through hydrolysis of a nucleoside triphosphate energy can be fed into the filaments on the scale of their respective subunits. It is of paramount importance for many cellular processes and structures, that the rates at which actin or tubulin subunits are added to or removed from F-actin or microtubules, respectively, depend on the end and also on the nucleotide bound.4

The polarity and activity of cytoskeletal filaments are exploited by cells in various ways. For example, polymerization forces are used to generate cell protrusions.5 The two properties can also lead to a state called treadmilling, in which a filament grows at one end at the same rate as it shrinks at the other.6,7 Some experimental evidence for treadmilling was given in vivo8–10 as well as in vitro.11,12 Treadmilling offers possible explanations for the constant size of cell structures such as stereocilia or mitotic spindles/phragmoplasts in presence of filament turnover.13 However, the conditions under which treadmilling occurs remain unclear and whether it plays an important role in vivo is still debated.

The polymerization of actin has been studied since the 1960s. In early studies, the degree of polymerization was investigated by the birefringence of actin solutions.4,14 Advances in microscopy made it possible to observe the growth and shrinkage of filaments directly in vitro.15 The rate constants for the addition and removal of different types of monomers could be determined in this way.16–18 These studies revealed, in particular, that the critical concentrations depend only slightly on the state of the end,15,19 such that the critical concentration is dominated by the state of the monomer. Today, by using microfluidic devices, the dynamics of individual actin filaments can be studied in controlled environmental conditions, giving, for example, insights into the dynamics of phosphate release from actin filaments.15 In the future, microfluidic devices could be designed to observe the length of individual actin filaments under constant environmental conditions.20

Similarly, the assembly of microtubules has been intensively studied.21,22 Notably, it was found that the plus-end can switch between relatively long phases of net growth and net shrinkage.23 Due to the complicated microtubule structure with typically 13 protofilaments, a thorough understanding of microtubule assembly dynamics remains elusive, though.

Extensive work has also been done to study the dynamics of isolated actin filament and microtubule ends from a theoretical point of view.24–29 However, the study of isolated ends provides only limited information about the assembly dynamics. Indeed, as a consequence of nucleoside triphosphate (NTP) hydrolysis and the structural polarity, a gradient in the state of the protomers can be generated along the filament. Earlier studies of the length distribution of active polar filaments have largely ignored the consequences of this gradient,30–35 which can effectively lead to a coupling between the two ends.36 For this statement, we tacitly assumed that NTP hydrolysis on a protomer occurs at a rate
independent of the neighboring protomers’ states. If vectorial NTP hydrolysis is assumed, where hydrolysis is restricted to protomers with a neighboring protomer carrying a nucleoside diphosphate (NDP), the ends remain independent and fluctuations are large. For actin, however, there is now strong experimental support for random ATP hydrolysis\textsuperscript{15, 38, 39} and, for microtubules, theoretical arguments also suggest random GTP hydrolysis.\textsuperscript{40}

Here, we present a theoretical study of the length dynamics of active polar filaments. We focus on the effects of the coupling between the two filament ends through a NTP gradient. Our study reveals a remarkable property of active polar filaments: For a broad range of monomer concentrations, the assembly rates at the two ends can self-adjust to become equal but of different sign and thus lead to treadmilling. This state is thus not restricted to a specific value of the free monomer concentration. Furthermore, in this state, the length distribution is unimodal and length fluctuations can be significantly smaller than the average.

The work is organized as follows. We first introduce a generic two-state lattice model displaying the essential features of active polar filaments. We study the ensuing steady-state length distribution by simulations and analytics. We then investigate the existence of treadmilling for F-actin. To this end we extend our model to include a third monomer state, which accounts for actin bound to ADP-P\textsubscript{i}. Remarkably, we find that while actin filaments naturally grow at one and shrink at the other end, treadmilling with equal rates of opposite sign at both ends can only be expected in presence of a depolymerization-inducing factor acting at the minus-end.

II. A TWO-STATE MODEL FOR TREADMILLING

The essential physics underlying the assembly of active polar filaments can be studied considering two states of the subunits. To this end, we introduce a kinetic two-state model and identify different dynamic regimes, notably including treadmilling, as a function of the external monomer concentrations. We then investigate in detail the dynamics of subunits within the filament as well as the attachment and detachment dynamics at the filament ends. Finally, we will estimate the typical length of a treadmilling filament.

A. Molecular processes and simulation results

We describe a filament as a linear polar lattice with each site representing one filament subunit. Each subunit is in one of two states, corresponding to the degree of phosphorylation of the nucleotide bound to an actin monomer or a tubulin dimer, see Fig. 1. One state will be denoted as the “T”-state representing a subunit bound to a NTP, the other as the “D”-state representing a subunit bound to a NDP. Note, that actin filaments and microtubules are helical structures consisting of several protofilaments. Hence they sever at a different rate than subunits are added or removed at the ends. We will neglect the process of filament severing. Our representation of the filament as a one-dimensional lattice is appropriate if the conditions at the filament ends depend only on the state of the outmost subunit.

Subunits within the lattice switch between the two states. The rate at which a subunit switches form the T-state to the D-state is denoted by $\omega_{de}$ and the rate of the opposite process by $\omega_{re}$. For all processes we assume that the corresponding rates only depend on the state of the affected subunit, but not on that of neighboring subunits. As stated in the Introduction, this notably implies that we are assuming random NTP hydrolysis.

Single subunits can be added to and removed from both ends of the lattice. We will index the lattice sites such that $i = 1$ corresponds to the plus-end and the site $i = L$, with $L$ being the length of the lattice corresponds to the minus-end. Furthermore, we denote the various rates of T-subunit addition and removal at the plus-end by $k_{on}^{T+}$ and $k_{off}^{T+}$, respectively, and at the minus-end by $k_{on}^{T-}$ and $k_{off}^{T-}$, respectively. Analogously, $k_{on}^{D+}$, $k_{off}^{D+}$, $k_{on}^{D-}$, and $k_{off}^{D-}$ denote the corresponding rates for D-subunits. In a dilute solution, the addition rate of subunits is proportional to the corresponding monomer concentration. For example, $k_{on}^{T+} = r_{on}^{T+}c^{T}$ for T-subunits at the plus-end with $c^{T}$ being the concentration of unbound T-subunits and $r_{on}^{T+}$ the corresponding molecular rate. According to our assumption that all rates depend only on the subunit state, the equilibrium constants for subunit addition and removal are independent of the filament end, that is,

\[
\frac{k_{on}^{T+}}{k_{off}^{T+}} = \frac{r_{on}^{T+}}{r_{off}^{T+}} \quad \text{and} \quad \frac{k_{on}^{D+}}{k_{off}^{D+}} = \frac{r_{on}^{D-}}{r_{off}^{D-}}. \tag{1}
\]

We performed stochastic simulations of the above processes in presence of subunit reservoirs, such that the various subunit attachment rates are constant. We consider the limit of a diluted solution such that different filaments are independent of each other. An array of length zero is treated as a filament nucleus to which subunits can be added at both ends. This situation corresponds to a constant number of filaments in the system and we will discuss possible experimental realizations below.

Our simulation algorithm followed the Gillespie scheme\textsuperscript{41} and the first $10^5$ s of simulated time were neglected to suppress transients. The state of the system was sampled at intervals of length $t_{\text{samp}}$. To avoid correlations between two successive samples, we chose a timestep of $t_{\text{samp}} = 5/\omega_{de}$, because $\omega_{de}$ was typically the smallest non-zero rate we used. Results were obtained for $10^6$ samples or more.\textsuperscript{42}
We expect treadmilling to occur if the rates fulfill the following conditions: We assume that T-state subunits are more tightly bound to the filament than D-state subunits, that is \( k^{-}_{off} > k^{T+}_{on} \) and \( k^{D+}_{on} < k^{-}_{off} \). Furthermore, T-state subunits should favor the filamentous state, \( k^{T+}_{on} / k^{-}_{off} = k^{T-}_{off} / k^{T+}_{on} > 1 \), whereas D-state subunits should typically be removed from the lattice, \( k^{D+}_{on} / k^{D-}_{off} = k^{D-}_{off} / k^{D+}_{on} < 1 \). Net polymerization and net depolymerization then occur at different ends if subunits at the plus-end are more likely to be found in the T-state, in contrast to subunits at the minus-end, which are more likely to be in the D-state. Therefore, T-state monomers should be added to the plus-end more rapidly than they switch to the D-state, \( k^{T+}_{on} \gg \omega_{de} \) and they should be added to the minus-end at a slower rate, \( k^{T-}_{off} \lesssim \omega_{de} \).

Figure 2 presents simulation results for two parameter sets fulfilling the constraints discussed in the previous paragraph. The lattices indeed treadmill as can be inferred from the positions of the lattice ends shown in the inset. The length distributions are unimodal in both cases and differ strongly from the exponential distributions observed for equilibrated polymer solutions,4,35 see Fig. 2(a). As anticipated above, the probability for a subunit to be in the T-state along the lattice shows an accumulation of T-states at the plus-end, whereas D-subunits dominate at the minus-end, see Fig. 2(b).

To gain an intuitive understanding of the treadmilling state, we note that the gradient in the probability to find a subunit in the T-state along the lattice implies an effectively length-dependent subunit removal rate at the minus-end. Since the probability to find a D-state subunit at the minus-end increases with the filament length, the same holds for the net depolymerization rate at the minus-end. Now, if the (length-independent) polymerization rate at the plus-end lies in-between the depolymerization rates for lattices of lengths one and infinity, the lattice should attain a stable state, for which the rate of subunit addition at the plus-end equals the subunit removal rate at the minus-end.36 By a slight abuse of language, we can express this mechanism also in terms of critical concentrations (a concept that should in principle be reserved to equilibrium systems): to get treadmilling, the monomer concentrations do not need to be set to specific critical values known a priori. Rather, the system will auto-regulate its length and hence its depolymerization rate such that the (fixed) concentrations present in the systems equal the critical concentration. Correspondingly, the treadmilling state is robust against changes in the monomer concentrations.

Let us emphasize that the parameter sets used for Fig. 2 fulfill the conditions given in Eq. (1), see Table I. The only free energy source for driving treadmilling is thus the positive difference between the chemical potentials of free T-state and of free D-state subunits. It follows, in particular, that cooperative effects during subunit addition or removal are not needed for generating treadmilling or unimodal length distributions.

### B. The effectively length-dependent depolymerization rate

To gain deeper understanding of the steady state length distribution, we will now determine the effectively length-dependent depolymerization rate. The starting point for these considerations is the T-state gradient along the lattice. We denote by \( \Theta_i \) the probability of finding lattice site \( i \geq 1 \) of an

![Figure 2](image-url)

**FIG. 2.** Simulation of the two-state model. (a) Steady-state length distribution for two parameter sets, see Table I. Vertical dashed lines indicate the corresponding typical filament lengths \( L_{typ} \) determined in Sec. II C. (Inset) Corresponding positions of plus- (filled symbols) and minus-ends (empty symbols) as a function of time. (b) Probability \( \Theta_i \) for site \( i \) to be in the T-state in filaments of length \( L \geq i \). Corresponding profiles calculated in Secs. II B 1 and II B 2. Full lines represent the gradient for deterministic growth and dashed lines stochastic growth with constant rate.
array of length $L > i$ in the T-state. After determining this distribution, we will proceed to calculate the probability of finding the site $i = L$ in the T-state.

1. The stability gradient for a constant elongation rate

The probability $P_T(t)$ to find a subunit in the T-state at time $t$ after its incorporation into the filament evolves according to

$$\frac{d}{dt} P_T = -\omega_{de} P_T + \omega_{re} (1 - P_T). \quad (2)$$

This equation is complemented by the initial condition $P_T(t = 0) = \Theta_1$, where $\Theta_1$ will be determined in Sec. II B 2. We thus have

$$P_T(t) = \frac{\omega_{re}}{\omega_{de} + \omega_{re}} + \left(\Theta_1 - \frac{\omega_{re}}{\omega_{de} + \omega_{re}}\right) e^{-(\omega_{de} + \omega_{re})t}. \quad (3)$$

From this time-dependent probability we can infer the probability along the filament if we can relate the position of a subunit in the filament to the time that has passed since it was incorporated. This is simple if the filament grows at a constant velocity $\langle v_a \rangle$ by adding T-state monomers to the plus-end. Neglecting for a moment that the filament is assembled from discrete subunits, the position of a subunit at a time $t$ after its addition to the filament is simply $x = \langle v_a \rangle t$. In the case of a constant elongation velocity, we thus have

$$\Theta_i = P_T((i - 1)/\langle v_a \rangle) \quad (4)$$

and the characteristic length of the exponential gradient is

$$\Lambda = \frac{\langle v_a \rangle}{\omega_{de} + \omega_{re}}, \quad (5)$$

see Eq. (3).

This approximation neglects fluctuations in the polymerization process. If we account for these fluctuations by assuming a Poissonian addition of monomers at the filament’s plus end, the T-state gradient is only weakly affected, see Fig. 2(b). More importantly, it also neglects depolymerization effects at the plus-end, which correspond to “back steps” of a subunit from site $i$ to $i - 1$.

2. Dynamics of the growing end

The next step is to determine the average attachment rate at the plus-end. It is given by the sum of all attachment rates minus all detachment rates, that is,

$$\langle v_a \rangle = k_{on}^{T+} + k_{on}^{D^+} - \Theta_1 k_{off}^{T+} - (1 - \Theta_1) k_{off}^{D^+}. \quad (6)$$

Note, that this equation can also have solutions $\langle v_a \rangle < 0$, which would correspond to shrinkage at the plus-ends as is observed for microtubules after a catastrophe.

To determine the value of $\Theta_1$ let us consider its time evolution. Employing a mean-field ansatz to replace the joint probability of finding monomers 1 and 2 in the T-state by $\Theta_1 \Theta_2$, we write

$$\frac{d}{dt} \Theta_1 = (k_{on}^{T+} + \omega_{de}) (1 - \Theta_1) + k_{off}^{D^+} (1 - \Theta_1) - k_{off}^{T^+} (1 - \Theta_2) \Theta_1 - (k_{on}^{D^+} + \omega_{de}) \Theta_1. \quad (7)$$

The first term describes the addition of a T-subunit to a plus-end in the D-state as well as the transformation of a D-subunit into a T-subunit at the plus-end. The second term accounts for removal of a D-subunit from the plus-end with the new plus end being a T-subunit. The remaining terms account for the corresponding processes that lead to a loss of a T-subunit at the plus end. Using

$$\Theta_2 = (1 - e^{-1/\Lambda}) \omega_{re}/(\omega_{de} + \omega_{re}) + \Theta_1 e^{-1/\Lambda}, \quad (8)$$

see Eq. (3), the steady state value of $\Theta_1$ can be calculated from Eq. (7). The expression for $\Theta_1$ becomes explicit in the limit of fast subunit addition as compared to the rate of state changes, such that $\Lambda \gg 1$ and thus $\Theta_1 \approx \Theta_2$. In Fig. 3(a) we compare the positions of the plus-ends for the simulations presented in Fig. 2 with their position expected from the mean velocity $\langle v_a \rangle$ given by Eqs. (6)–(8).

3. Dynamics of the shrinking end

With the result for the dynamics of the growing plus-end, we can now proceed to calculate the average subunit removal rate at the minus-end. We start our analysis by considering the case when subunits can only be removed from the minus-end but not added to it, $k_{on}^{T-} = k_{on}^{D^-} = 0$. Similar to Eq. (6) for

![FIG. 3. Dynamics of lattice ends for the same parameters used in Fig. 2. (a) The position $x$ of the plus-end as a function of time for a single realization. Curves are shifted for better visibility. Lines represent $x = \langle v_a \rangle t$ with $\langle v_a \rangle$ from Eq. (6) and $\Theta_1$ from Eq. (7) with $\Theta_2 = \Theta_1$ (dashed) or $\Theta_2$ from Eq. (8). (b) The average depolymerization rate as a function of the filament length. Symbols represent the results of stochastic simulations. The dashed line shows $\langle v_d \rangle$ as explained in the text.](image-url)
plus-end growth, the average depolymerization rate $\dot{v}_d$ at the minus-end can be written as

$$\dot{v}_d = k_{\text{off}}^D (1 - T_-(L)) + k_{\text{off}}^T T_-(L),$$

with $T_-(L)$ being the probability to find a T-subunit at the minus end of a lattice of length $L$. Note, that the value of $T_-(L)$ is in general different from the value of $\Theta_L$. This is clearly illustrated by considering the limit of $k_{\text{off}}^D \to \infty$. In that case, $T_-(L) = 1$ as any D-subunit reaching the minus end is instantaneously removed, while at the same time $\Theta_L < 1$. In the stationary state, $T_-$ is given by

$$\left[\omega_{\text{de}} + k_{\text{off}}^T (1 - \Theta_{L-1})\right] T_-(L) - \left[\omega_{\text{re}} + k_{\text{off}}^D \Theta_{L-1}\right] (1 - T_-(L)) = 0.$$

This equation reflects that the probability of having a T-subunit at the minus end can change either by nucleotide exchange in the subunit at the tip or by its detachment. Using Eq. (4) to determine $\Theta_{L-1}$, this equation can be solved for $T_-(L)$:

$$T_-(L) = \frac{\omega_{\text{re}} + k_{\text{off}}^D \Theta_{L-1}}{\omega_{\text{re}} + \omega_{\text{de}} + k_{\text{off}}^D \Theta_{L-1} + k_{\text{off}}^T (1 - \Theta_{L-1})}.$$

Let us now consider the general case, when subunits can also be added to the minus-end. These subunits form a “cap” with a distribution of nucleotide states that does not follow the exponential profile present in the rest of the filament. The cap can be treated as a factor that transiently inhibits subunit removal at the minus end. With probability $\alpha$ the minus end carries a cap, which appears at constant rate $k_{\text{on}}^D + k_{\text{on}}^T$. Knowing the value of $\alpha$, one obtains the average depolymerization rate $\langle \nu_d \rangle$ from Eq. (9) by replacing the depolymerization rates $k_{\text{off}}^D$ and $k_{\text{off}}^T$ in the calculation above by $k_{\text{off}}^D (1 - \alpha)$ and $k_{\text{off}}^T (1 - \alpha)$, respectively. Before describing, how the value of $\alpha$ can be estimated, we present in Fig. 3(b) the average subunit removal rate in steady state as a function of the lattice length obtained from our calculation and the corresponding values extracted from the simulations presented in Fig. 2.

4. Dynamics of the cap at the minus-end

We now estimate the probability $\alpha$ of finding a minus-end cap formed by subunits having been added to the lattice at the minus-end. As in the full model introduced in Sec. II A, subunits in the lattice change states at rates $\omega_{\text{de}}$ and $\omega_{\text{re}}$, they are added to the minus-end at respective rates $k_{\text{on}}^D$ and $k_{\text{on}}^T$ and removed at rates $k_{\text{off}}^D$ and $k_{\text{off}}^T$, see Fig. 4(a), inset. Lattice sites are numbered such that the oldest subunit added at the minus-end is at $i = 0$. Its dynamics is neglected. The dynamics is thus very similar to simple models used for studying the dynamic instability of microtubules, see, for example, Ref. 43. Stochastic simulations analogous to those described in Sec. II A reveal an exponential length distribution $C(L) = (1 - \alpha) \alpha^L$, where $\alpha$ is the probability of finding a cap of a finite length, see Fig. 4(a). An exponential length suggests the existence of effective on- and off-rates, $k_{\text{on}}$ and $k_{\text{off}}$, that are independent of the system’s state, in particular, its length. In that case, $\alpha = k_{\text{on}} / k_{\text{off}}$. The effective on-rate is given by $k_{\text{on}} = k_{\text{on}}^D + k_{\text{on}}^T$, whereas we have for the effective off-rate $k_{\text{off}} = \tau_{-} k_{\text{off}}^D + (1 - \tau_{-}) k_{\text{off}}^T$. Here, $\tau_{-}$ is the (now length-independent) probability that the subunit at the minus-end is in the T-state.

To estimate $\tau_{-}$, we consider the total rates at which the system gains and loses T-state subunits, respectively. In steady state they have to be equal leading to

$$k_{\text{on}}^T + \omega_{\text{de}}(\langle L \rangle - \langle N \rangle) = k_{\text{off}}^T \alpha \tau_{-} + \omega_{\text{de}} \langle N \rangle,$$

where $\langle L \rangle$ is the average lattice size and $\langle N \rangle = \sum_{i=1}^{\infty} C(L) T_{L,i}$ the average number of T-subunits in the system. Numerically, the distribution of T-subunits along a lattice of length $L$ is found to be given by $T_{L,i} = \tau_{-} \beta^{L-i}$, where $0 < \beta < 1$ and $0 < i \leq L$, such that

$$N = \frac{\alpha \tau_{-}}{1 - \alpha \beta}.$$
from the T- to the D-state and vice versa, \( \beta = k_{TD}/k_{DT} \), where
\[
k_{TD} = \alpha \tau_-(\omega_{0e} + k_{on}^{D-} + k_{off}^{T-} \alpha (1 - P_{TT})),
\]
and
\[
k_{DT} = \alpha (1 - \tau_-)(\omega_{0e} + k_{on}^{D-} + k_{off}^{T-} \beta P_{TD}).
\]
In these expressions \( P_{TT} \) and \( P_{TD} \) are the conditional probabilities of subunit \( L - 1 \) to be in the T-state, when subunit \( L \) is in the T- or D-state, respectively. They obey the normalization condition \( P_{TT}(1 - \tau_-) + P_{TD}(1 - \tau_-) = T_{L-1} \). To obtain a closed expression for the conditional probabilities, we write them in the form \( P_{TT} = T_{L-1} \tau_+ \) and \( P_{TD} = T_{L-1} (1 - \tau_-) \). One might be tempted to neglect correlations and to write \( T_{L-1} = \beta \tau_- \). The normalization condition then implies \( \tau_- = 0 \) or 1, which are both bad approximations. A better estimate is obtained by using
\[
k_{off}^{D-} P_{TT} \tau_- + k_{off}^{D-} P_{TD}(1 - \tau_-)
\]
\[
\approx k_{off}^{D-} T_{L-1} \tau_- + k_{off}^{T-} T_{L-1}(1 - \tau_-).
\]
Equations (14) and (15) can thus be expressed in terms of \( \alpha \), \( \beta \), and \( \tau_- \) only, such that we obtain a closed system for determining \( \alpha \). A comparison of our estimate with results from numerical simulations is given in Fig. 4(b).

**C. The typical filament length**

Having calculated the average net polymerization rate at the plus- and the average net depolymerization rate at the minus-end, we can determine the typical lattice length \( L_{typ} \).

The typical lattice length is the most probable length and determined by the condition that lattice growth and shrinkage balance each other
\[
\langle \nu_a \rangle = \langle \nu_d \rangle (L_{typ}).
\]
Since \( \langle \nu_d \rangle \) is a monotonically increasing function of the length \( L \) this equation has a unique solution if \( \langle \nu_d \rangle (L = 0) < \langle \nu_a \rangle < \langle \nu_d \rangle (L = \infty) \). If \( \langle \nu_d \rangle (L = 0) > \langle \nu_d \rangle (L = \infty) \), then filaments grow faster than they shrink and the typical filament length diverges. In the case \( \langle \nu_d \rangle (L = 0) > \langle \nu_d \rangle (L = \infty) \), lattices always disassemble faster than they grow. Since in our model a lattice of length zero grows again, the average length will be greater than zero and the length distribution exponential. Thus, if the depolymerization rate at the minus-end exceed the polymerization rate at the plus-end for all filament lengths, then the typical length is \( L_{typ} = 0 \).

For the simulations presented in Fig. 2(a), the typical filament length obtained from Eq. (17) is indicated by vertical dashed lines. In order to get a more comprehensive picture, we present in Fig. 5 the typical filament length obtained as a function of the concentration \( c^T \) of free T-monomers and of the T-subunit detachment rate \( k_{off}^{T+} \) at the barbed end. Here, we have assumed that the attachment rates \( k_{on}^{T+} \) and \( k_{on}^{T-} \), of T-monomers at the plus and minus ends are proportional to \( c^T \).

This relation holds for dilute solutions. Moreover, the rates obey relations (1). Note, that they imply \( k_{on}^{T+} = K k_{off}^{T+} \) with \( K = const \) if all other parameter values are fixed. For the parameters used in Fig. 5, see Table I, the contribution of D-subunit addition and removal from the plus end is negligible, such that the average growth rate is \( \langle \nu_a \rangle \approx k_{on}^{T+} - k_{off}^{T+} = k_{off}(K - 1) \). As a consequence, \( \langle \nu_a \rangle \) and hence the typical filament length increase with increasing \( k_{off} \) as long as \( K > 1 \), which is the case for the values in Table I. The agreement between simulation and analytic results is satisfactory in light of the approximations made and indicates that our analysis indeed captures the essential features of the two-state model.

**D. Phenomenological characterization of the length distribution**

In Fig. 6(a), we present the length distributions \( P_L \) from Fig. 2(a) on a semi-logarithmic scale. This presentation suggests an exponential tail of the distribution. The full distribution can be fitted with good accuracy to a convolution of an exponential and a Gaussian distribution, see Fig. 6(a). A convolution of a Gaussian with a Poissonian results, for example, if some fast processes are coupled to a slow Poissonian process. Indeed, the central limit theorem implies that the length distribution resulting from the joint action of the fast processes yields a Gaussian distribution, \( P_{fast}(L) = e^{-(L - \bar{L})^2/2\sigma^2}/\sqrt{2\pi\sigma} \) with positive average \( \bar{L} \) and standard deviation \( \sigma \). The slow process keeps its Poissonian character, \( P_{slow}(L) = e^{-L/\bar{L}}/\bar{L}_s \) with average \( \bar{L}_s \). The total length \( L \) results from adding the contributions of all processes, such that
cess. The parameter \( \bar{\nu} \) represents the transition from the T- to the D-state as the relevant slow process.

A. Actin length distributions

III. ACTIN DYNAMICS

FIG. 6. Phenomenological characterization of the length distribution. (a) The length distributions from Fig. 2 in a semi-logarithmic plot. Solid lines: convolution of a Gaussian with a Poissonian distribution, Eq. (19). Fit parameters are \( \sigma = 12 \) (□) and 43 (○), \( \bar{L} = 20 \) (□) and 104 (○), and \( \bar{L}_s = 27 \) (□) and 192 (○). (b) The fit parameter \( \bar{L}_s \) as a function of the typical length gained during the average time for phosphate release \( \langle \nu_b \rangle / \omega_{de} \). Dashed line represents \( \bar{L}_s = 1.64 \langle \nu_b \rangle / \omega_{de} \). Parameters are the same as for (□) in (a) except for \( \omega_{de} \), which was varied.

\[
\mathcal{P}(L) = \int_{-\infty}^{\infty} dL \int_{0}^{\infty} d\bar{L} \mathcal{P}_{\text{fast}}(L) \mathcal{P}_{\text{slow}}(\bar{L}) \delta(L - \bar{L} - L) \\
= \frac{e^{-L/\bar{L}_s}}{\sqrt{2\pi \sigma \bar{L}_s}} \int_{-\infty}^{\infty} d\bar{L} e^{\bar{L}^2 / (2\bar{L}_s^2)} e^{-\left(\bar{L} - \bar{L}_s\right)^2 / 2\sigma^2}.
\]

(18)

For the parameters given in Table I, we can identify the transition from the T- to the D-state as the relevant slow process. The parameter \( \bar{L}_s \) should thus essentially be given by the average length gained by the filament before the newly added monomer has switched from the T- to the D-state, that is, \( L_s \approx \langle \nu_b \rangle / \omega_{de} \). A plot of the values of \( \bar{L}_s \) from fits to the length distribution obtained from simulations yields \( \bar{L}_s = 1.64 \langle \nu_b \rangle / \omega_{de} \), see Fig. 6(b), in line with our reasoning.

18, see Table II. The rates of nucleotide exchange for actin monomers in a filaments were taken from Refs. 44 and 15.

In Fig. 8(a), the effective addition and removal rates at the barbed and pointed ends are shown as a function of the concentration \( c^T \) of free T-monomers. As for the two-state model discussed above, for all values of \( c^T \), the filament grows at the barbed end. Also the pointed end grows on average if \( c^T \) exceeds a certain level (phase IV). Below that value the pointed end shrinks on average. Treadmilling in the strict sense, when monomer removal at the pointed end on average balances exactly growth at the plus end, occurs below a second threshold concentration. Correspondingly, the filament grows indefinitely above that threshold (III and IV). In the treadmilling state, the typical filament length is zero below a third threshold concentration of free T-monomers (I). In phase II, the typical filament length is finite.

For comparison, the velocities of isolated filament ends in a solution of T-subunits obtained by Vavylonis et al.26 are presented in the same plot. For these curves, the bulk of the filament is supposed to consist of ADP-actin only. They indicate that the dynamics of the pointed and the barbed end decouple in regions III and IV, which goes along with the

FIG. 7. A three-state model for actin dynamics. White arrowheads represent T-, gray arrowheads P-, and black arrowheads D-subunits. Within the filament, T-monomers are transformed into the P-state at rate \( \omega_{TP} \) and back at rate \( \omega_{PT} \); monomers in the P-state turn into D-states at rate \( \omega_{PD} \) and back at rate \( \omega_{DP} \). At filament ends, the transition from the P- to the D-state is faster than in the bulk and occurs at rates \( \omega_{TP} \) or \( \omega_{PD} \), respectively.
TABLE II. Parameter values for polymerization and depolymerization of all three types of monomers to barbed and pointed ends as determined by Fujiwara et al., complemented by the rates for hydrolysis and phosphate release from independent measurements. All rate constants are given in units of s⁻¹, all concentrations in μM.

<table>
<thead>
<tr>
<th>Rates</th>
<th>Fig. 8(a)</th>
<th>Fig. 8(b)</th>
<th>Fig. 9</th>
</tr>
</thead>
<tbody>
<tr>
<td>$k_{on}^{\bar{c}T}$</td>
<td>$11.6^a × c^T$</td>
<td>$11.6^a × c^T$</td>
<td>$11.6^a × c^T$</td>
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<tr>
<td>$k_{on}^{cT}$</td>
<td>$13^a × c^T$</td>
<td>$13^a × c^T$</td>
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<tr>
<td>$kP_{on}^{\bar{c}P}$</td>
<td>$3.4^a × c^P$</td>
<td>$3.4^a × c^P$</td>
<td>$3.4^a × c^P$</td>
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<tr>
<td>$kP_{on}^{cP}$</td>
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<td>$0.11^a × c^P$</td>
<td>$0.11^a × c^P$</td>
</tr>
<tr>
<td>$k_{on}^{\bar{c}D}$</td>
<td>$2.9 × c^D$</td>
<td>$2.9 × c^D$</td>
<td>$2.9 × c^D$</td>
</tr>
<tr>
<td>$k_{on}^{cD}$</td>
<td>$0.09^a × c^D$</td>
<td>$0.09^a × c^D$</td>
<td>$0.09^a × c^D$</td>
</tr>
<tr>
<td>$k_c^{\bar{c}T}$</td>
<td>$1.4^a$</td>
<td>$1.4^a$</td>
<td>$1.4^a$</td>
</tr>
<tr>
<td>$k_c^{cT}$</td>
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<td>$0.8^a$</td>
<td>$0.8^a$</td>
</tr>
<tr>
<td>$kD_{off}^{\bar{c}P}$</td>
<td>$0.16^b$</td>
<td>$0.16^b$</td>
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<tr>
<td>$kD_{off}^{cP}$</td>
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<td>$0.02^a$</td>
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<tr>
<td>$kD_{off}^{\bar{c}D}$</td>
<td>$5.8^b$</td>
<td>$5.8^b$</td>
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<tr>
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<tr>
<td>$\omega_{\bar{c}P}$</td>
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<td>$\omega_{cP}$</td>
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<td>$0.0006^b$</td>
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<tr>
<td>$\omega_{DP}$</td>
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<td>0</td>
</tr>
<tr>
<td>$c_D$</td>
<td>0</td>
<td>0</td>
<td>0</td>
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</tbody>
</table>

$^a$Values are taken from Ref. 18.
$^b$Values are taken from Ref. 15.
$^c$Values are taken from Ref. 44.

A hint towards the origin of treadmilling is given by the behavior of isolated pointed and barbed ends as illustrated by the curves form Ref. 26. In regions I and II, the depolymerization at the pointed end always exceeds the net polymerization rate at the barbed end (in fact, throughout region I and in a large part of region II, the barbed end shrinks on average).

In order to explain the non-monotonic dependence of the net assembly rates on the concentration of free T-monomers, let us first note that the net depolymerization rate at an isolated pointed end in regions I and II exceeds the net polymerization rate of an isolated barbed end. As a consequence, the filament length distribution is monotonically decreasing and there is a high probability that the filament consists of a single monomer. In this case, the monomer is “removed” at the combined removal rates of the barbed and pointed end. As the concentration of T-monomers in solution increases, two things happen: First, the average filament length increases and thus the probability of the filament to consist of a single subunit decreases. Second, the probability to find a P-monomer instead of a D-monomer at the filament ends increases. Since P-subunits have a much lower probability to be removed from the pointed end than D- or T-subunits, both effects reduce the rate at which the pointed end shortens. The typical filament length, however, increases monotonically in region I and II and diverges at the critical concentration that marks the transition to phase III.

Finally, it is worth mentioning that the large number of parameters of the three-state model begs the question of how sensitive our results are to variations in these rates. In all cases we examined no other than the four phases discussed above could be detected. When increasing the depolymerization rates at the pointed end, phase II could shrink significantly or the system could transit directly from phase I to III. To see whether treadmilling in the strict sense and consequently length regulation of actin filaments could be observed in vivo, one thus has to take into account the recently observed diverging filament length in these phases. Whereas deviations from the behavior of isolated ends might have been expected at the pointed end, at the barbed end, they might come as a surprise. Indeed, this finding points to a different mechanism underlying treadmilling than discussed for the two-state model above. Another indication for this difference is the fact that, in the three-state model, the probability to find a filament of zero length is finite, whereas it is essentially zero for the two-state model.
accelerated phosphate release rate at filament ends,\(^\text{15}\) which leads to an effective increase in the depolymerization rate.

### B. Accelerated phosphate release at the filament ends

We modified the three-state model presented in Sec. III A to account for the accelerated phosphate release at the filament ends.\(^\text{15}\) In Fig. 8(b), we present the corresponding growth rates of the pointed and the barbed ends as a function of the free monomer concentration. The treadmilling state with a unimodal length distribution vanishes in this case. The reason is that fast phosphate release at the barbed end prevents the formation of a gradient along the filament. Indeed, in the relevant range of free monomer concentrations below 0.06 \(\mu\text{M}\), the typical rate of monomer addition at the barbed end is 0.2 \(s^{-1}\), see Fig. 8(b), whereas, on average, ATP hydrolysis is completed in 3 s and phosphate release in 0.6 s. Consequently, the filament consists almost exclusively of ADP-actin.

To prevent phosphate release at the barbed end and thus to generate a gradient in ATP along a filament necessary for unimodal length distributions, the concentration of free monomers must exceed some threshold value. For such large concentrations and the parameters used above, however, the pointed end does not shrink fast enough to compensate for barbed end growth. As a consequence, conditions need to be changed to increase the filament disassembly rate at the pointed end that allows for fast actin treadmilling.

As shown in Fig. 9(a), for sufficiently large depolymerization rates, the treadmilling state can indeed be unimodal. Phase II, in which filament length regulation is observed, appears for \(k_{\text{off}}^{\text{D}} \gtrsim 1\) and broadens for increasing depolymerization rates. The closer the parameters are to the boundary between region II and region III, the longer the filaments get, preserving their peaked character, see Fig. 9(b). For a D-monomer removal rate of \(k_{\text{off}}^{\text{D}} = 2.5 \text{ s}^{-1}\) and \(c^T = 0.25 \mu\text{M}\), the average length is roughly 400 monomers. This value corresponds to a filament length of 1 \(\mu\text{m}\), which could be measured in experiments.

A possible way to increase the disassembly rate at the pointed end might be to add ADF/Cofilin. ADF1 from \textit{Arabidopsis thaliana} targets filamentous ADP-actin with high specificity\(^\text{45}\) and enhances its removal rate by a factor of 25 as compared to ADF1-free ADP-actin subunits without filament severing.\(^\text{11}\) In addition, treadmilling velocities of \(\approx 2\) subunits/s were measured in presence of ADF/Cofilin.\(^\text{11}\) The increase of the treadmilling velocity compared to the case without ADF/Cofilin might be essentially due to an increase of the concentration of free actin monomers resulting from the ADF/Cofilin-induced accelerated depolymerization. Note, however, that this finding remains controversial.\(^\text{45}\) More recent evidence suggests that ADF/Cofilin rather severs actin filaments.\(^\text{46, 47}\) In that case, too, the filament length distribution might be unimodal.\(^\text{48}\)

### IV. CONCLUSIONS

In this work, we analyzed the dynamics of active polar filaments in presence of a reservoir of monomers. Several different regimes could be identified. For sufficiently large monomer concentrations a filament will show unbounded growth. In the opposite case, the filament length can either be distributed exponentially as is the case for passive filaments or fluctuate around a finite typical value. To describe bounded length distributions it was essential to consider the gradient of the nucleotide phosphorylation state along the filament. The gradient leads to an effectively length-dependent depolymerization rate at the minus-end, which can qualitatively explain the three regimes. Fluctuations, however, play an important role in determining the actual length distribution.

We then considered the specific case of actin to see, whether a unimodal length distribution could be observed in \textit{vitro}. Our description corresponds to a situation, where a single filament is observed under constant environmental conditions.
conditions, notably a fixed concentration of monomers. Such a situation could, for example, be realized in a microfluidic device. Based on published rates for actin, however, there do not seem to exist conditions to observe treadmilling in the strict sense for actin. The reason is essentially that the depolymerization at the pointed end is too small. Only by adding an appropriate depolymerization promoting factor this non-equilibrium state might be reached.

Since the essential property preventing treadmilling of actin filaments is insufficient filament disassembly at the pointed end, we expect this result to hold also if details of the model are modified. For example, we assumed that the rates of ATP-hydrolysis and of phosphate release in the filament bulk do not depend on the state of neighboring actin monomers. Alternatively, ATP-hydrolysis and phosphate release of ADP-Pi might be cooperative within the actin filament. Other works have considered situations in which the rates of hydrolysis and phosphate release depend on the states of the neighboring subunits in the filament lattice. For low cooperativity, Eq. (2) should have nonlinear terms to account for this effect and the exponential gradients that were found for independent monomers should then be sigmoidal. An extreme case of such a scenario is known as vectorial hydrolysis, in which hydrolysis or phosphate release only occur at the boundaries of homogenous regions in the filament. In this case, the filament length is always exponentially distributed.

Maybe somewhat surprisingly, it thus turns out that it might be easier to observe the treadmilling state for microtubules than for actin-filaments. Indeed, there have been reports on treadmilling microtubules in vitro, but some questions remained concerning the true nature of the states observed there. For a theoretical analysis, one would need a better understanding of the microtubule assembly and disassembly process. This is difficult due to the large number of protofilaments in a microtubule and because conformational changes of tubulin after GTP hydrolysis might add a genuine mechanical aspect to the disassembly of microtubules.

As we have seen, the existence of a gradient along an active polar filament is essential for generating treadmilling. Such gradients cannot only be generated by nucleotide hydrolysis. They also form, for example, if some accessory protein binds with constant rate to the filament, because the distance of a monomer from the plus-end in the filament correlates with the time passed since it was incorporated into the filament. It has been shown that for severing proteins, this gradient will lead to a unimodal length distribution. Additional active processes can also reinforce gradients. Notably, molecular motors generate a gradient, when accumulating at an end due to directed motion. If the motors affect the assembly or disassembly rates, interesting effects on the length distribution can be generated.

Concerning the situation in vivo, our findings suggest that for the actin cytoskeleton treadmilling might not play a role in the actin cortex. This is in line with recent measurement of the actin turnover in the cortex suggesting a scenario in which filaments grow on average as long as they are bound to formins or the Arp2/3 complex, but shrink otherwise. In other cellular structures such as filopodia or stereocilia, in contrast, treadmilling with a unimodal length distribution might result from length-dependent growth rates. These could be a consequence of applied forces or result from a spatially varying actin monomer concentration.

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