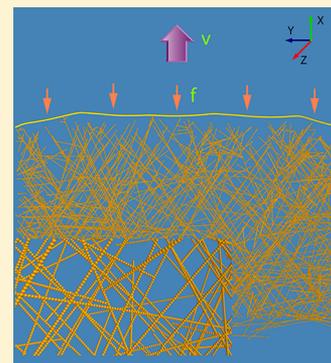


Molecular Transport Modulates the Adaptive Response of Branched Actin Networks to an External Force

Longhua Hu and Garegin A. Papoian*

Department of Chemistry and Biochemistry and Institute for Physical Science and Technology, University of Maryland, 2216 Chemistry Building, College Park, Maryland 20742, United States

ABSTRACT: Actin networks are an integral part of the cytoskeleton of eukaryotic cells and play an essential role in determining cellular shape and movement. Understanding the underlying mechanism of actin network assembly is of fundamental importance. We developed in this work a minimal motility model and performed stochastic simulations to study mechanical regulation of the growth dynamics of lamellipodia-like branched actin networks, characterized by various force–velocity relations. In such networks, the treadmilling process leads to a concentration gradient of G-actin, and thus G-actin transport is essential to effective actin network assembly. We first explore how capping protein modulates force–velocity relations and then discuss how actin transport due to diffusion and facilitated transport such as advective flow tunes the growth dynamics of the branched actin network. Our work demonstrates the important role of molecular transport in determining the adaptive response of the actin network to an external force.



INTRODUCTION

Actin is a globular protein ubiquitous in eukaryotic cells. Actin molecules polymerize into filaments *in vivo*, which are further assembled into networks of distinct structures such as finger-like bundles in filopodia and dendritically branched meshes in lamellipodia. Actin dynamics play important roles in many cellular processes such as cellular motility and endocytosis.^{1,2}

In eukaryotic cells, growth of polymers such as microtubules or actin filaments against a mechanical barrier may generate mechanical work.³ During their movement, cells experience resistive forces from their surrounding extracellular matrix and from other cells that may impede their motion. The crawling movement of the cells is an integrated physical process in which membrane protrusion is often the first important step.⁴ An interesting aspect of the membrane protrusion regulation is the question of how actin dynamics depends on the external force. The corresponding force–velocity relations indicate how easily the actin network can overcome external mechanical obstacles. Numerous prior studies investigated the force–velocity relations for various motility systems.^{5–19,19–28} However, the forms of the force–velocity relations are distinct for different motility models, and no unified explanation is commonly accepted for the multitude of force–velocity relations observed *in vitro* and *in vivo*.

The tethered filament elastic Brownian ratchet model developed by Mogilner and Oster predicts that the velocity decays rapidly with small forces and less rapidly at larger force.⁶ In addition, it is expected that the force–velocity relation is sensitive to the mechanism of nucleation of actin filaments. Carlsson investigated various mechanisms for growth velocities of branched actin networks against obstacles:⁹ in the autocatalytic model, new actin filaments are generated on existing filamentous branches, and the model predicts a

constant velocity regime in the force–velocity relation; whereas in the nucleation model, new branches are generated independently of the existing branches, and the velocity decreases steadily with the external force. The autocatalytic model is supported by an experimental work by Wiesner et al.¹¹ Stochastic simulations by Schaus and Borisy showed that 2-D lamellipodial protrusion velocity depends also on the mechanism of how the mechanical work of protrusion is shared among individual filaments:¹³ flexible filaments can share the mechanical work more evenly, which helps the performance of the lamellipodial protrusion.

Marcy et al. performed direct measurements of the forces generated during actin-based propulsion using micromanipulation force-probe experiments, from which the force–velocity relations were measured and fit with an empirical formula:¹⁰ they found that force–velocity curves were linear for pulling forces and decayed more weakly for pushing forces. Schreiber et al. studied how polymerization kinetics of a branched actin network coupled to excluded volume effect drives cell motility.²⁴ In their computational model, adhesion was also included; this model produces the force–velocity relationship with a plateau in the regime of modest force, consistent with the experiment done by Prass et al.²² Keren et al. investigated the mechanism of shape determination in motile cells and suggested a general form of the force–velocity relation $v = v_0[1 - (f/f_{\max})^w]$.¹⁵ By varying the parameter w , many observed force–velocity relations can be approximated. The force–velocity relation in keratocyte lamellipodial protrusion is

Special Issue: Peter G. Wolynes Festschrift

Received: May 26, 2013

Revised: August 19, 2013

Published: August 20, 2013

concave, for example.¹⁷ Protrusion velocity is constant at small loading forces until ultimately the cell is stalled at higher forces.

Prior studies also differed from each other by postulating different force-generation mechanisms. From Brownian dynamics simulations based on the self-diffusiophoretic mechanism, where an object generates a steady-state concentration gradient that drives the motion of the object, Lee and Liu found that at small loads, the velocity is controlled by the reaction rate, while at high loads the stall pressure is determined by the mechanical properties of the branched actin network.¹⁶ Gerbal et al. described an elastic model to study how propulsive forces on a bacterium of *Listeria* is generated via the addition of actin filaments to its tail.⁷ Brangbour et al. proposed an entropy-based mechanism of force generation.²⁷ In their model, generation of the force is not controlled by the rate of polymerization but due to the restriction of filaments' orientational fluctuations, and velocity first decreases fast with the force, followed by a slow decay. According to this model, the force–velocity relation depends on the density of actin filaments: at a certain force, velocity increases with the density of filaments and eventually saturates.²⁷

In an interesting *in vitro* experiment studying the growth of actin networks,²³ Parekh et al. measured the velocity of the growing actin network for a wide range of forces (up to the stall force of ~ 300 nN), and they showed that the force–velocity relations are history-dependent. To interpret the experimentally observed phenomena of the force–velocity relations showing both convex and concave forms as well as the load-history dependence, Weichsel and Schwarz showed by stochastic simulations and mathematical modeling that changes in the network growth velocity induce a transition between two different orientational patterns of actin filaments: a $\pm 35^\circ$ pattern and a -70° , 0 , and 70° pattern.²⁵ Smith and Liu, however, suggested that the convex and concave forms of the force–velocity relations are regulated by the interplay between the branching and capping processes.²⁸ Tsekouras et al. calculated the filament density and the force–velocity relation for a simple system containing only a small number of parallel filaments with mean-field method and simulations, and they found that there exists two regimes in the force–velocity relation: at low forces, filaments spread out and only one or a few filaments are in contact with the barrier while velocity is close to its maximum; at high forces, filaments accumulate at the barrier.²⁶

Despite extensive studies on the force–velocity relations of various actin networks, the following fundamental issue of actin network growth has not been adequately addressed: in a motility system with limited concentration for molecular components, how does molecular transport affect the force–velocity relation? In this work, we study the force–velocity relation for the lamellipodium-like actin meshwork. Membrane protrusion due to actin dynamics in cells is controlled by complicated mechano-chemical processes. How does the external force affect the underlying reaction-diffusion processes in the cytoplasm? Our objective was to investigate the effect of molecular transport on the force–velocity relation. After a study of how the force–velocity relation is modulated by capping proteins, we then investigate the force–velocity relation by varying the diffusion coefficient to examine the role of molecular transport. Furthermore, we consider the case of facilitated transport of G-actin, which mimics the delivery of G-actin monomers to the membrane leading edge through fluid flow or via molecular motors. We shed light on the underlying

molecular mechanism of actin dynamics in our computational model, obtaining insights into the way molecular transport affects the adaptive response of the growing actin mesh to various external forces of different magnitudes.

MODEL AND METHODS

Our model consists of a reaction–diffusion system coupled with the mechanics of actin network and the plasma membrane. The reaction–diffusion system is simulated with the compartment-based stochastic simulation approach introduced in our previous work.²⁹ The basic feature of the compartment-based stochastic simulation is that the whole simulation region (3D) is partitioned into compartments in which reactions occur, and molecules diffuse between neighboring compartments. The compartments at the base level are coupled to a reservoir in which the concentrations of molecules are fixed. Various average concentrations for the reactive region can be obtained by adjusting the rate of particle exchange between the bulk region and the base compartments of the reactive region. The model motility system contains molecules of actin, capping proteins, and Arp2/3 complex. The model allows detailed, microscopic description of the underlying mechanism controlling the growth of the actin network.

In our previous work,^{29,30} which focuses on the regulation of actin network growth by molecular components such as the concentration of capping proteins, actin filaments are modeled as rigid polymers; this approximation is generally sufficient for the study of mechano-chemical regulation of the actin dynamics in lamellipodium because the typical length of actin filaments in lamellipodia is much smaller than the persistence length of actin filaments. In this work, we study the growth of actin network in which the length of filaments may be comparable to the persistence length ($l \sim l_p$), when actin filaments are considered as semiflexible polymers.

The actin filaments are modeled as polymer chains composed of monomers whose positions are denoted as $\vec{r}_i = (x_i, y_i, z_i)$, and the interactions between neighboring monomers along a chain are modeled with a harmonic potential, similar to the discrete semiflexible harmonic chain model.³¹ For an actin filament with N monomers, there are $N - 1$ bonds $\vec{u}_i = \vec{r}_{i+1} - \vec{r}_i$, $i = 0, 1, \dots, N - 2$. We assume that all torsion angles are equally likely and independent of each other (freely rotating chain model). The total stretching and bending energy of the bonds is

$$E_{r,\text{tot}} = \frac{1}{2}k_r \sum (|\vec{r}_{i+1} - \vec{r}_i| - \delta)^2 + \frac{k_f}{\delta} \sum (1 - \cos \theta_i) \quad (1)$$

where $\delta = 2.7$ nm is the size of a monomer and k_r is the spring constant, which is taken to be $k_r = 4000k_B T/\text{nm}^2$, estimated from the stiffness value of ~ 43 pN/nm for a $1 \mu\text{m}$ filament.^{32,33} The flexural rigidity k_f is related to the persistence length l_p by the relation $k_f = l_p k_B T$,³⁴ and the angle θ_i between two consecutive bonds is related to the bond vectors by the relation $\cos \theta_i = (\vec{u}_i \cdot \vec{u}_{i+1}) / (|\vec{u}_i| |\vec{u}_{i+1}|)$.

Actin monomers cannot occupy the same position because of the excluded volume, which is modeled with the following repulsive potential

$$E_V = E_0 \exp\left(-\frac{r}{r_0}\right) \quad (2)$$

where $E_0 = k_B T$. For computational efficiency, we adopted the linked-list method³⁵ to calculate the pairwise interactions.

The plasma membrane can generally be considered as a 2-D elastic sheet, commonly described with the Monge representation.^{36,34} We write the protrusion position (the x direction) at the membrane surface point (y, z) as $x = x(y, z)$. Because lamellipodia are flat with thickness ~ 200 nm (along the z direction here), we approximate the front membrane configuration as $x = x(y, z) \equiv h(y)$. That is, we ignore the height variation along the z direction on the membrane surface. This allows us to write the Helfrich Hamiltonian for the membrane in a simplified form:

$$E_m = \frac{k_b}{2} \int (\nabla^2 h(y))^2 dy + \frac{\gamma}{2} \int (\nabla h(y))^2 dy$$

where k_b is the effective bending rigidity and γ is the effective surface tension coefficient, and in our simulation $k_b = 100k_B T \cdot \text{nm}$ and $\gamma = 0.5k_B T/\text{nm}$. To reduce the finite size effect of the plasma membrane, we apply periodic boundary conditions (PBCs) for the plasma membrane (along the y direction). This is realized by connecting the two boundary points with an elastic spring

$$E_{\text{pbc}} = \frac{1}{2} k_{\text{pbc}} (h(y=0) - h(y=y_{\text{max}}))^2 \quad (3)$$

where the spring constant $k_{\text{pbc}} = 25k_B T/\text{nm}^2$. We do not explicitly include in our model the ventral membrane and dorsal membrane, which are treated implicitly as hard-wall surfaces, which the filaments cannot cross.

The actin network is mechanically coupled with the plasma membrane. Polymerizing actin filaments deform the membrane and generate membrane protrusion. This in turn affects the rate of actin polymerization, which is described by the widely used Brownian ratchet model:³⁷

$$k_{\text{poly}} = k_{\text{on}}[A] \exp\left(-\frac{f\delta_{\text{eff}}}{k_B T}\right) \quad (4)$$

where f is the load on polymerizing filaments and δ_{eff} is the effective distance. $[A]$ represents the local concentration of G-actin and $k_{\text{on}} = 11.6 \mu\text{M}^{-1} \text{s}^{-1}$ ³⁸ is the polymerization rate constant. When the barbed end of a polymerizing filament is closer to the plasma membrane, the load on it becomes larger, which reduces the rate of polymerization correspondingly. The interaction between actin filaments and the plasma membrane is derived from the steric repulsive potential, modeled with the exponential form

$$\mathcal{H}_{\text{fm}} = E_0 \exp\left(-\frac{l}{\lambda}\right) \quad (5)$$

where l is the distance between the barbed end of a filament and the plasma membrane, and $\lambda = 0.1\delta$ is the characteristic distance.²⁹

The external field term representing the external resistive force acting on the leading-edge membrane is

$$\mathcal{H}_{\text{field}} = \beta \int h(y) dy \quad (6)$$

where β is the strength of the external field and is varied to get different forces as we study the force–velocity relation of the actin network.

During simulations, the positions of the plasma membrane at successive Gillespie steps are recorded, from which the growth

velocity of the actin network can be derived. A snapshot of the simulated motility system is shown in Figure 1. The equilibrium

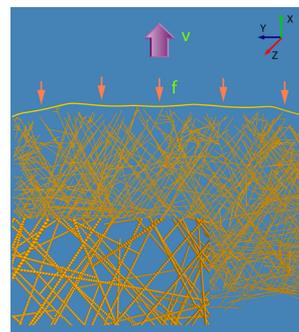


Figure 1. Snapshot of the simulated motility system shows the actin network (orange) and the plasma membrane (gold). The inset depicts in greater detail the 3-D branched actin network.

configurations of the actin network and plasma membrane can be obtained by minimizing the energy of the filament–membrane system. Such method is common in studying the actin networks.^{39,40} There are no motor proteins included in the motility system; motor proteins would generate active contractility and affect the assembly and mechanics of the actin network.^{41–44} The actin network is enclosed by the plasma membrane (either the explicit part or the implicit part, as described above) and in contact with the substrate and the bulk part of model cell (both implicit). The force balance for the system would be satisfied from the energy minimization procedure. The time scales of the relaxation of actin filaments and the plasma membrane need to be considered so we can make proper approximations to find their configurations during the actin polymerization process. The equilibration time for membrane fluctuation is on the order of nanoseconds to microseconds,⁴⁵ and membrane relaxation is much faster than the rate of polymerization with typical G-actin concentration ($\sim 10 \mu\text{M}$).²⁹ The relaxation time of filaments depends on their length.^{33,46} In our simulated motility system, actin filaments are typically very stiff, and hence their mechanical relaxation is much faster compared with the rate of polymerization.

RESULTS AND DISCUSSION

Studies of actin network assembly are often performed *in vitro* with abundant monomeric G-actin in the system, therefore the role of G-actin transport in such actin networks is rarely discussed. In a lamellipodia-like actin network, however, monomeric G-actin diffuses from the rear edge, where filaments depolymerize so monomeric G-actin is recycled, to the membrane leading edge, where G-actin is consumed as a result of the polymerization. Thus such a motility system may display G-actin concentration gradient and G-actin transport may be important.^{47,48} Our stochastic simulation model takes into account the heterogeneity of the concentration profile and allows us to explore the role of G-actin transport in shaping the force–velocity relation of the actin network. The primary aim of this study is to explore the effect of molecular transport on growth dynamics of the actin network. In this section, we discuss the force–velocity relation for the lamellipodia-like branched actin network under various scenarios.

Force–Velocity Relation of the Branched Actin Network. We investigate the force–velocity relation for a minimal motility system that contains actin, capping protein, and Arp2/

3 complex. Different values of the external force are obtained by varying the strength of external field term β in eq 6. We also vary concentrations of molecular components so that we may compare results in different motility conditions. Capping proteins are known to be capable of regulating the density of the leading-edge filaments. Thus, by varying the concentration of capping proteins, we can study the force–velocity relation and see how it depends on the density of the actin system. Of course, this is not the only way to obtain an actin network of different filament densities. Arp2/3 complex, for example, directly mediates branch formation and plays a critical role in determining actin network density, and its concentration can be adjusted as well.

We plot in Figure 2 the force–velocity relation (v – f), which shows that the velocity (v) of the actin network growth

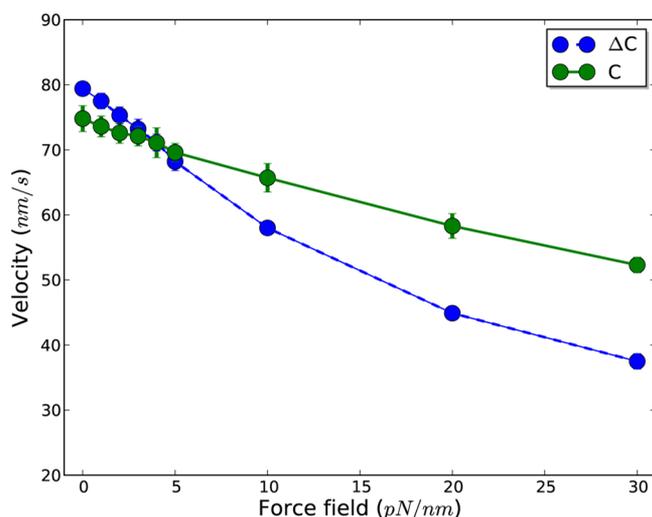


Figure 2. Mechano-chemical regulation of actin network growth by the external force and capping proteins: the external force hinders the motion; this hindrance is modulated by capping proteins, leading to a variation in the rate of the velocity decay with the external force: with capping proteins, the velocity decays slower. C in the Figure denotes the motility system with capping proteins, whereas ΔC denotes the motility system without capping proteins.

decreases as a function of the external force (f). From the slope of the force–velocity relation, we can see that the decrease in velocity is faster in the regime of modest forces than in the regime of high forces. This result is consistent with the tethered filament elastic Brownian ratchet model⁶ and also consistent with the experimental result on the *Listeria* system.⁴⁹ The force–velocity relation exhibiting such form is also a feature of the entropy-based mechanism of force generation.²⁷ The autocatalytic model,⁹ however, predicts a force–velocity relation with a distinct plateau region and finds its support from computer simulations or experimental work such as refs 22 and 24. We point out that the autocatalytic model assumes an unlimited pool of G-actin in the motility system and hence may be rather unrealistic for systems where this condition is not satisfied. Although our model and the autocatalytic model share the similarity in the aspect that filament nucleation depends on the existing filaments in the nucleation zone, in our motility system, molecular components, such as monomeric G-actin and Arp2/3 complex, are limited. In such a case, a plateau regime in the force–velocity relation may not be observed.

Interestingly, the force–velocity relation decays slower for the motility system with capping proteins because in this case the density of the actin network is lower, hence, the growth of the actin network is limited less by the bottleneck of molecular transport. It should be pointed out that capping proteins may enhance the actin-based motility.^{29,30} This phenomenon—higher velocity for the motility system with capping proteins—can be seen in the large-force regime (Figure 2), which corresponds to the regime of high filament density. (See Figure 3.) Capping proteins reduce the filament density, lessen the bottleneck of molecular transport, and thus could facilitate the actin network growth dynamics.

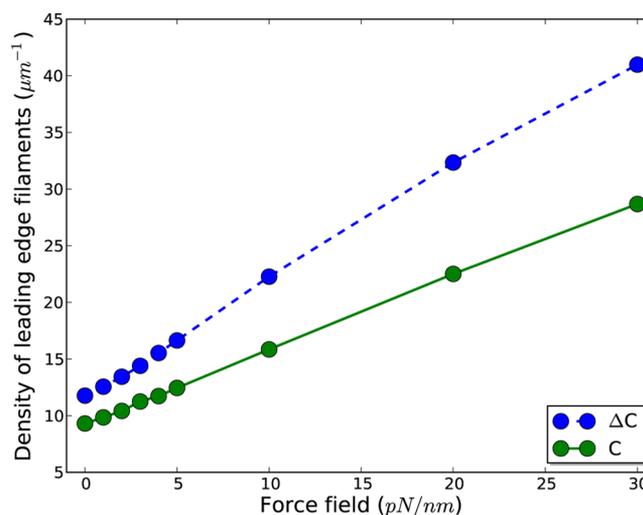


Figure 3. External force leads to increased density of the leading-edge filaments; capping proteins decrease the density of the leading-edge filaments. Furthermore, the increase in the density of the leading-edge filaments due to the external force becomes slower with the addition of capping proteins.

In Figure 3 we show the density of the leading-edge filaments, which increases as the external force becomes larger. Thus, despite the fact that mechanical force is not directly related to filament density—unlike capping protein or Arp2/3 complex—it nevertheless regulates the actin network morphology indirectly. This is consistent with the tethered filament elastic Brownian ratchet model,⁶ and also qualitatively agrees with the AFM experiments.^{23,50,51} Such type of density pattern change has also been shown in a motility system containing a small number of parallel filaments.²⁶

The trend of the density of the leading-edge filaments is inversely correlated with the local concentration of monomeric G-actin for polymerization and the load on polymerizing filaments (Figure 4). We note that as the density of the leading-edge filaments becomes larger there are fewer G-actin monomers available for polymerization. The load on polymerizing filaments also increases as a function of the external force, as expected, albeit there are more filaments to support the membrane. We conclude that when the external force is increased both the chemical aspect (G-actin) and the mechanical aspect (the load on polymerizing filaments) of the motility system become the bottleneck for rapid polymerization. Furthermore, for the motility system without capping proteins, its density of leading-edge filaments grows faster, leading to the faster decay of local G-actin monomers as a function of the external force and, correspondingly, also a

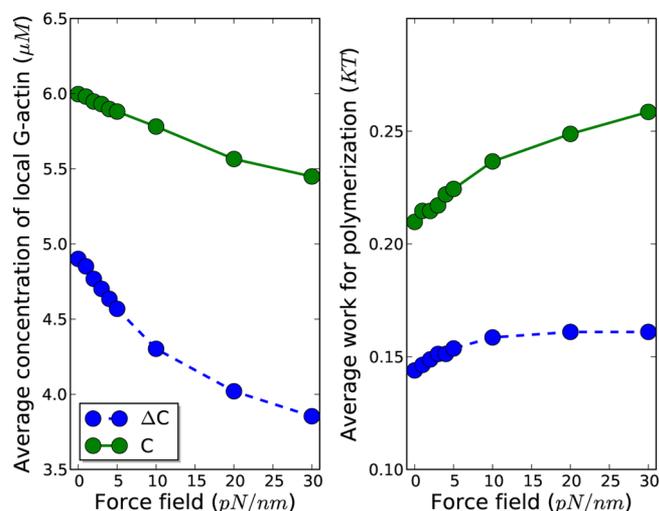


Figure 4. With the external force, the density of the leading-edge filaments increases (see Figure 3), and this leads to less monomeric G-actin available for polymerization. Because capping proteins slow down the density increase due to the external force, the decrease in monomeric G-actin available for polymerization slows down with higher external forces. The work actin filaments have to do to polymerize increases with the external force.

steeper force–velocity relation. Thus, our model highlights the role of molecular transport in regulating the force–velocity relation of the branched actin network.

External Force Enhances Arp2/3 Complex-Mediated Nucleation of Actin Filaments. The density of the leading-edge filaments is correlated with the rate of nucleation of filaments and the protrusion speed.²⁹ From Figure 5, we can

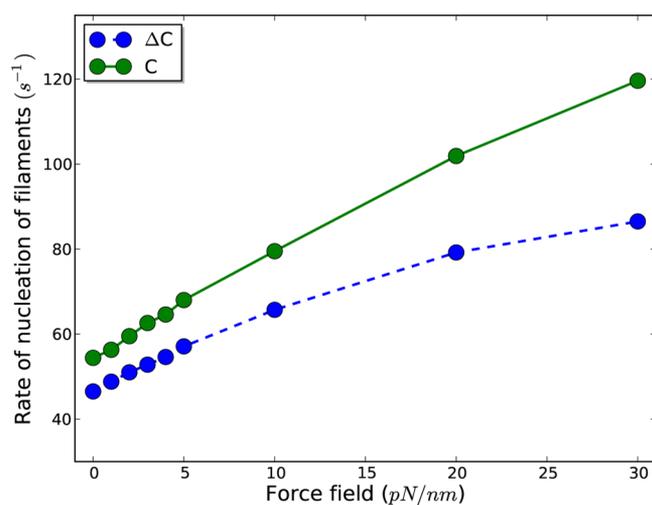


Figure 5. Rate of nucleation of actin filaments is enhanced by the external force.

see that the external force enhances the rate of nucleation, the favorable factor that contributes to the density of leading-edge filaments increasing as a function of the external force. Arp2/3 complex-mediated nucleation requires both Arp2/3 complex and actin molecules as well as preexisting filaments that can provide potential branching sites. We examined each of these factors and found that the underlying mechanism behind the force-enhanced nucleation of filaments is that there are more F-actin monomers located in the nucleation zone (Figure 6). We

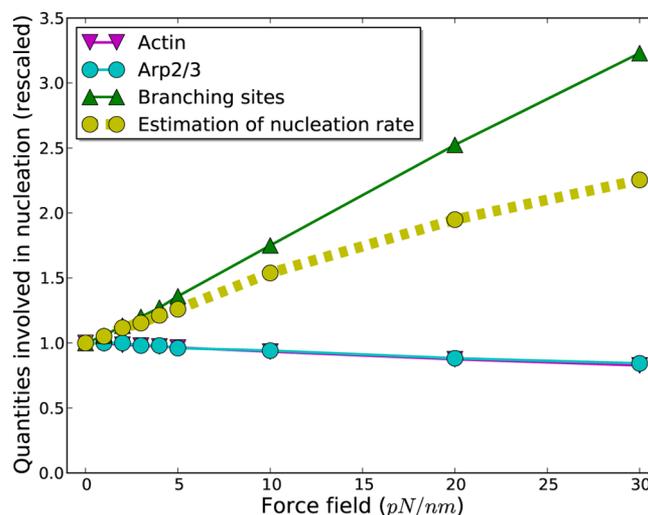


Figure 6. Trends of the various factors (Actin, Arp2/3, and the number of branching sites) involved in the nucleation process indicate that the enhanced nucleation by the external force is correlated with more branching sites located in the nucleation zone: among the three quantities, only the number of branching sites increases as a function of the external force. A “mean-field” estimation of the nucleation rate from the product of the three quantities is also provided to illustrate the enhanced nucleation of actin filaments by the external force.

already know that the external force inhibits the polymerization of actin filaments and slows down actin network growth (Figure 2); as a result, barbed ends of actin filaments tend to stay in the nucleation zone, leading to increased density of the leading-edge filaments. Thus, more F-actin monomers are located in the nucleation zone that could serve as potential branching sites.

The enhancement of Arp2/3-mediated nucleation of actin filaments due to the external force indicates an adaptive response mechanism of the motility system: increasing the external force on plasma membrane leads to a higher load on the filaments that support the plasma membrane, and to counteract the increasing load, more filaments may be nucleated to share the load, alleviating the burden due to higher external forces. This also clearly shows the coupling between the mechanical and chemical aspects of the motility system.

Actin Network Growth Dynamics Is Limited by Slow Diffusion. Intracellular transport is important to many cellular processes.⁵² Actin filaments in lamellipodia undergo a treadmilling process, and the turnover of actin network requires effective transport of monomeric G-actin from the rear of lamellipodia, where disassembly of filaments occurs, to the front of lamellipodia, where filaments polymerize to push the plasma membrane. The concentration of cytoplasmic G-actin is critical to actin network assembly and lamellipodial protrusion.^{53,54} In this section, we investigate the effect of passive diffusion, while the effect of advection/active transport of molecules will be examined in the next section.

In the preceding section, we have shown that the concentration of monomeric G-actin at the leading edge is essential to the growth dynamics of the actin network because the rate of polymerization of individual filaments is proportional to the local concentration of G-actin. The cytoplasm of cells is crowded.^{52,55–57} The cytoskeleton structure has a strong influence on the diffusion of macromolecules,⁵⁸ and the

diffusion coefficient varies depending on the viscosity of the cytoplasm.^{59–61} It is of interest to study how actin network growth dynamics is affected by the transport of molecules. Stuhrmann et al. found that actin network length and growth speed scales with the diffusion coefficient of G-actin with exponents 0.715 and 0.356, respectively.⁶²

We investigate how actin network growth is limited by the diffusion process by varying the diffusion coefficient. Our simulation results show that when the diffusion coefficient is changed from 20 to 10 $\mu\text{m}^2/\text{s}$ (labeled as “fast diffusion” and “slow diffusion”, respectively) the growth of actin network becomes slower, and this effect is more pronounced in the regime of larger forces (Figure 7). The rate of nucleation of

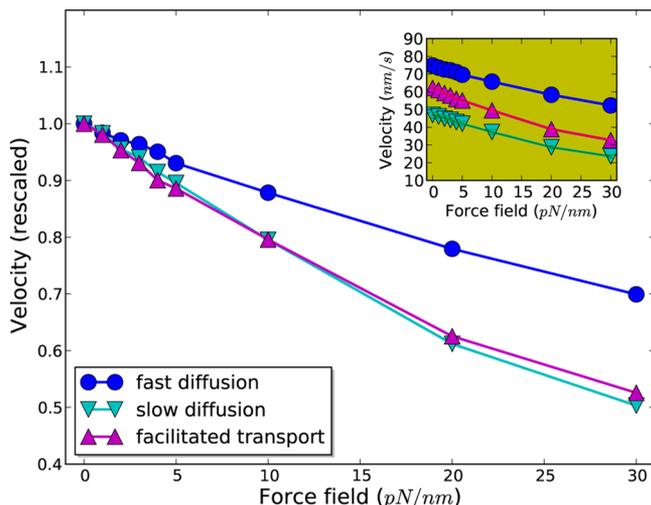


Figure 7. Comparison of the actin network growth dynamics for the cases of slow diffusion (cyan), facilitated transport (magenta), and the control (blue). With the reduced diffusion coefficient, delivery of molecules becomes a bottleneck for efficient actin network growth, and thus the velocity decreases faster as a function of the external force. Larger absolute values of the velocity result from facilitated transport. (See the inset.)

filaments also becomes smaller: the nucleation of actin filaments requires monomeric G-actin; when monomeric G-actin is scarce in the nucleation zone—which is close to the plasma membrane—due to the diffusion bottleneck, there is a drop in the rate of nucleation compared with the fast diffusion case (data not shown).

In summary, when the diffusion coefficient becomes smaller, molecular transport becomes a bottleneck for actin network growth because the diffusion flux is proportional to the diffusion coefficient: $j \propto D$. The effect of slow diffusion on actin network growth is more distinct in the regime of large forces, where due to the high density of the leading-edge filaments that polymerize the supply of G-actin is more limited than that of the regime of small forces.

How Does the Facilitated Transport of G-Actin Affect the Force–Velocity Relation? Diffusion is an ubiquitous way for molecular transport on the microscopic scale. In addition to the passive diffusion—which is due to the thermal agitation—the transport of molecules in cells may also be realized through active process resulting from motor protein activity or advective flow due to intracellular fluid flow. Many studies have emphasized the importance of facilitated transport on actin dynamics. Zicha et al. found that in the cytoplasm the

transport of monomeric G-actin to the protruding membrane leading edge through diffusion only may not be adequate to explain protrusion behavior and that hydrodynamic flow supplying monomeric actin from the region of rapid depolymerization to the site of rapid polymerization may be needed.⁶³ Naoz et al. developed a model of active localization of proteins, and they showed that protein localization by actin treadmilling and molecular motors can exert a strong effect on stereocilia shape and treadmilling rate.⁶⁴ In the nerve growth cone, membrane leading-edge-directed fluid flow was found to increase the concentration of G-actin at the leading edge to various extents and may significantly accelerate the actin treadmilling process.⁶⁵ Actin turnover is required to maintain an enhanced concentration of monomeric actin in the peripheral of neuronal growth cone.⁶⁶ In addition to the convective flow, active transport due to myosin motor proteins contributes to the delivery of G-actin to the membrane leading edge and optimal cell migration.⁶⁷ In neuronal cells, membrane proteins of migrating cells exhibit net forward translocation in the form of biased drift—in addition to the Brownian motion—driven by myosin-II-dependent active transport.⁶⁸ Zhuravlev et al. developed a model for active transport of G-actin by molecular motors in cellular protrusions such as filopodia and investigated how active transport regulates the length of these cellular protrusions.⁶⁹ Hydrodynamic flow and active transport could be coupled because the cytoplasmic flow can result from the motion of actively transported cargo itself.⁷⁰

If the availability of monomeric G-actin is a limiting factor that affects the growth velocity of the actin mesh network, then it is possible to design a motility system using actin transport to control the force–velocity relation. To see how facilitated transport of monomeric G-actin affects the force–velocity relation, we assume in our simulation a biased diffusion of monomeric G-actin toward the membrane leading edge, that is, $D_f > D_b$, where D_f is the coefficient of forward diffusion (from the rear edge to the leading edge) and D_b is the coefficient of backward diffusion. Such a biased diffusion could be caused by the motor proteins actively carrying monomeric G-actin to the leading-edge membrane or through advective hydrodynamic flow. The idea of utilizing the biased diffusion method is not to model the motor proteins or the advection explicitly but is simply to mimic the effect of the facilitated transport of molecules to the membrane leading edge. Note that advective transport of other molecules such as Arp2/3 complex is also possible, but here for simplicity we focus on facilitated transport of G-actin only.

In Figure 8 we show the G-actin concentration profiles for the various cases studied. G-actin concentration decreases toward the membrane leading edge. Compared with the case of slow diffusion, G-actin concentration is enhanced for the cases of fast diffusion and facilitated transport; such enhancement favors fast polymerization. Transport of molecules due to the drift of fluid is small compared with the diffusion.⁷¹ Thus, we consider small biased diffusion—the case where no inverse concentration gradient may occur—and study how biased diffusion affects actin network growth. In Figure 9, we show velocity for a few values of the extent of the diffusion bias, defined as $(D_f - D_b)/D_b$. Our simulation results show that the facilitated transport of G-actin to the membrane leading edge can significantly increase the growth velocity of the actin network—for a 3% difference in diffusion bias the velocity could be $\sim 30\%$ higher—but the overall shape of the force–velocity relation may not be noticeably different (Figure 7).

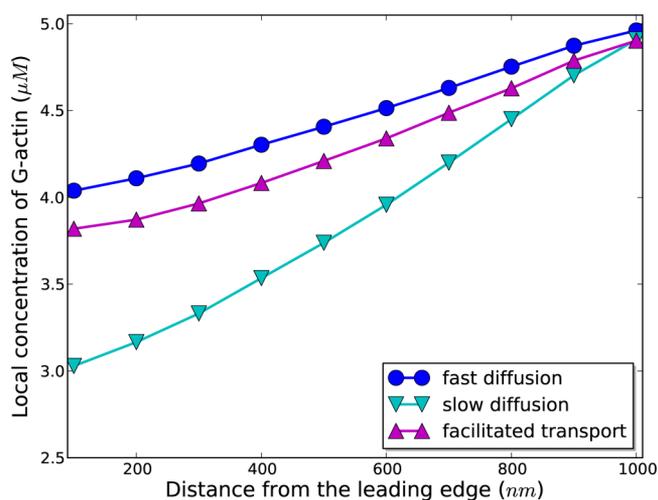


Figure 8. Concentration profiles in the case of fast diffusion (blue), slow diffusion (cyan), and facilitated transport (magenta).

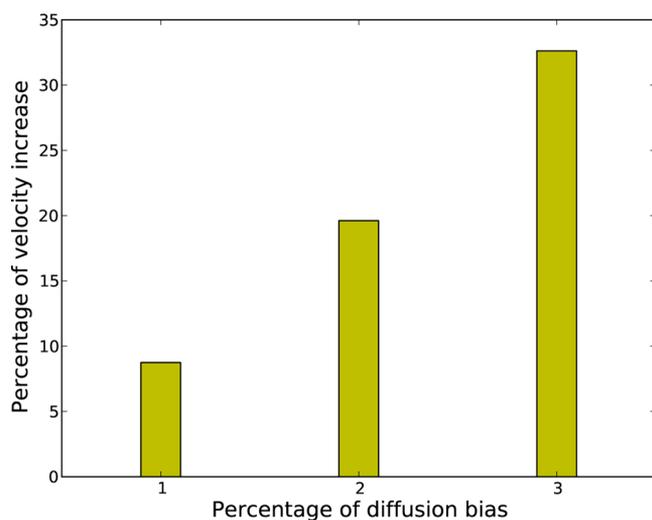


Figure 9. Facilitated transport enhances the actin network growth. The horizontal axis indicates the relative difference between the diffusion coefficients for forward diffusion and backward diffusion.

The rate of nucleation is also enhanced due to the abundance of monomeric G-actin at the leading edge resulting from the facilitated transport.

Prior computational studies have shown that active transport plays an important role in the growth of filopodia-like protrusion.^{69,72} It should be pointed out that in addition to the dimensionality difference between the filopodial (1D) and lamellipodial (3D) actin networks, actin transport is much more complicated in modeling lamellipodia-like branched actin networks because the density of filaments is not constant. This nonconstant density of filaments complicates understanding of the transport of G-actin to the leading edge.

Reorganization of the Actin Network. We have shown that the density of leading-edge filaments increases as a function of the external force. It turns out that external force also affects the length of actin filaments.

We find that there is a correlation between the average length of actin filaments and the external force acting on the mesh network: the average length of actin filaments decreases as the external force increases (Figure 10). Shorter filaments are stiffer and less prone to buckling. Thus, both the density of the

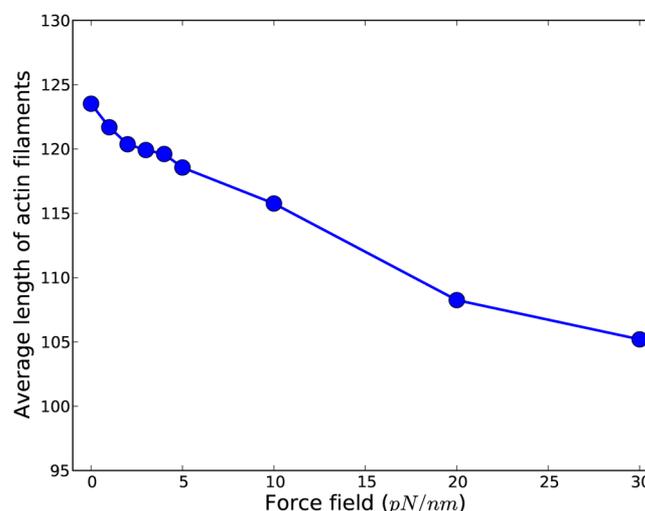


Figure 10. Average length of actin filaments decreases as a function of the external force.

leading-edge filaments and the average length of filaments indicate that when the load on actin network becomes larger the organization of the actin network changes accordingly to counteract the effect of the increasing load.

CONCLUSIONS AND FUTURE WORK

Membrane protrusions due to actin polymerization are controlled by the complicated mechano-chemical processes in which the biochemical factors, such as G-actin and regulatory proteins, and biophysical factors, such as mechanical force, are coupled. Using stochastic simulations, we have investigated how the external resistive force acting on the membrane affects the growth dynamics and organization of the lamellipodia-like dendritically branched actin networks. We have shown that the external force affects the growth velocity of the actin network. The extent of velocity decrease, though, is affected by a number of factors, including the density of leading-edge filaments. Our results demonstrate that in this type of motility system where there exists a treadmilling process, growth dynamics of the actin network may be limited by molecular transport. Facilitated transport such as active transport and advective fluid flow helps deliver G-actin to membrane leading edge and can enhance the growth of the actin network. Our simulation results also demonstrate how the external force enhances the nucleation activity.

The model presented here has its limitations in fully interpreting *in vivo* experiments. Our computational model system contains no myosin motors, and we also assume strong adhesion of the actin network to the substrate and no slippage of the network. Thus, there is no retrograde flow in our model. It should be noted that when the external force is large enough adhesion bonds may be easily broken. Thus the strong adhesion assumption in our model is applicable to a range of modest force. The formation of focal adhesions induces slow actin flow.⁷³ In future modeling, we will introduce some simple model of the substrate to study the retrograde flow effect. For example, constant retrograde flow to mimic the relative motion between the actin network and the substrate may be incorporated into the computational model.^{69,72,74,75} To explicitly model the interaction between the substrate and the actin network, we may use the lattice-spring model.⁷⁶

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: gpapoian@umd.edu. Tel:301-405-8667.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We acknowledge financial support from the National Science Foundation under CAREER award CHE-0846701. Computations were carried out using the Deepthought High Performance Computing Cluster at the University of Maryland.

■ REFERENCES

- (1) Pollard, T. D.; Cooper, J. A. Actin, A Central Player in Cell Shape and Movement. *Science* **2009**, *326*, 1208–1212.
- (2) Bray, D. *Cell Movement: From Molecules to Motility*; Garland Publishing: New York, 2001.
- (3) Hill, T. L.; Kirschner, M. W. *Proc. Natl. Acad. Sci. U.S.A.* **1982**, *79*, 490–494.
- (4) Lauffenburger, D. A.; Horwitz, A. F. Cell Migration: A Physically Integrated Molecular Process. *Cell* **1996**, *84*, 359–369.
- (5) Mogilner, A.; Oster, G. Cell Motility Driven by Actin Polymerization. *Biophys. J.* **1996**, *71*, 3030–3045.
- (6) Mogilner, A.; Oster, G. Force Generation by Actin Polymerization II: The Elastic Ratchet and Tethered Filaments. *Biophys. J.* **2003**, *84*, 1591–1605.
- (7) Gerbal, F.; Chaikin, P.; Rabin, Y.; Prost, J. An Elastic Analysis of *Listeria Monocytogenes* Propulsion. *Biophys. J.* **2000**, *79*, 2259–2275.
- (8) Carlsson, A. E. Growth of Branched Actin Networks against Obstacles. *Biophys. J.* **2001**, *81*, 1907–1923.
- (9) Carlsson, A. E. Growth Velocities of Branched Actin Networks. *Biophys. J.* **2003**, *84*, 2907–2918.
- (10) Marcy, Y.; Prost, J.; Carlier, M.-F.; Sykes, C. Forces Generated during Actin-Based Propulsion: A Direct Measurement by Micro-manipulation. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 5992–5997.
- (11) Wiesner, S.; Helfer, E.; Didry, D.; Ducouer, G.; Lafuma, F.; Carlier, M.-F.; Pantaloni, D. a Biomimetic Motility Assay Provides Insight into the Mechanism of Actin-Based Motility. *J. Cell Biol.* **2003**, *160*, 387–398.
- (12) Mogilner, A. on the Edge: Modeling Protrusion. *Curr. Opin. Cell Biol.* **2006**, *18*, 32–39.
- (13) Schaus, T. E.; Borisy, G. G. Performance of a Population of Independent Filaments in Lamellipodial Protrusion. *Biophys. J.* **2008**, *95*, 1393–1411.
- (14) Burroughs, N. J.; Marenduzzo, D. Nonequilibrium-Driven Motion in Actin Networks: Comet Tails and Moving Beads. *Phys. Rev. Lett.* **2007**, *98*, 238302.
- (15) Keren, K.; Pincus, Z.; Allen, G. M.; Barnhart, E. L.; Marriott, G.; Mogilner, A.; Theriot, J. A. Mechanism of Shape Determination in Motile Cells. *Nature* **2008**, *453*, 475–480.
- (16) Lee, K.; Liu, A. J. Force-Velocity Relation for Actin-Polymerization-Driven Motility from Brownian Dynamics Simulations. *Biophys. J.* **2009**, *97*, 1295–1304.
- (17) Heinemann, F.; Doschke, H.; Radmacher, M. Keratocyte Lamellipodial Protrusion Is Characterized by a Concave Force-Velocity Relation. *Biophys. J.* **2011**, *100*, 1420–1427.
- (18) Zimmermann, J.; Enculescu, M.; Falcke, M. Leading-Edge-Gel Coupling in Lamellipodium Motion. *Phys. Rev. E* **2010**, *82*, 051925.
- (19) Zimmermann, J.; Brunner, C.; Enculescu, M.; Goegler, M.; Ehrlicher, A.; Kas, J.; Falcke, M. Actin Filament Elasticity and Retrograde Flow Shape the Force-Velocity Relation of Motile Cells. *Biophys. J.* **2012**, *102*, 287–295.
- (20) Campas, O.; Mahadevan, L.; Joanny, J.-F. Actin Network Growth under Load. *Biophys. J.* **2012**, *102*, 1049–1058.
- (21) Amin, L.; Ercolini, E.; Shahapure, R.; Migliorini, E.; Torre, V. The Role of Membrane Stiffness and Actin Turnover on the Force Exerted by DRG Lamellipodia. *Biophys. J.* **2012**, *102*, 2451–2460.
- (22) Prass, M.; Jacobson, K.; Mogilner, A.; Radmacher, M. Direct Measurement of the Lamellipodial Protrusive Force in a Migrating Cell. *J. Cell Biol.* **2006**, *174*, 767.
- (23) Parekh, S. H.; Chaudhuri, O.; Theriot, J. A.; Fletcher, D. A. Loading History Determines the Velocity of Actin-Network Growth. *Nat. Cell Biol.* **2005**, *7*, 1219–1223.
- (24) Schreiber, C. H.; Stewart, M.; Duke, T. Simulation of Cell Motility That Reproduces the Force Velocity Relationship. *Proc. Natl. Acad. Sci. U.S.A.* **2010**, *107*, 9141–9146.
- (25) Weichsel, J.; Schwarz, U. S. Two Competing Orientation Patterns Explain Experimentally Observed Anomalies in Growing Actin Networks. *Proc. Natl. Acad. Sci. U.S.A.* **2010**, *107*, 6304–6309.
- (26) Tsekouras, K.; Lacoste, D.; Mallick, K.; Joanny, J.-F. Condensation of Actin Filaments Pushing against a Barrier. *New J. Phys.* **2011**, *13*, 103032.
- (27) Brangbour, C.; du Roure, O.; Helfer, E.; Demoulin, D.; Mazurier, A.; Fermigier, M.; Carlier, M.-F.; Bibette, J.; Baudry, J. Force-Velocity Measurements of a Few Growing Actin Filaments. *PLoS Biol.* **2011**, *9*, e1000613.
- (28) Smith, D. B.; Liu, J. Branching and Capping Determine the Force-Velocity Relationships of Branching Actin Networks. *Phys. Biol.* **2013**, *10*, 016004.
- (29) Hu, L.; Papoian, G. A. Mechano-Chemical Feedbacks Regulate Actin Mesh Growth in Lamellipodial Protrusions. *Biophys. J.* **2010**, *98*, 1375–1384.
- (30) Hu, L.; Papoian, G. A. How Does the Antagonism between Capping and Anti-Capping Proteins Affect Actin Network Dynamics? *J. Phys.: Condens. Matter* **2011**, *23*, 374101.
- (31) Kierfeld, J.; Niamploy, O.; Sa-yakanit, V.; Lipowsky, R. Stretching of Semiflexible Polymers with Elastic Bonds. *Eur. Phys. J. E* **2004**, *14*, 17–34.
- (32) Kojima, H.; Ishijima, A.; Yanagida, T. Direct Measurement of Stiffness of Single Actin Filaments with and without Tropomyosin by in Vitro Nanomanipulation. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 12962–12966.
- (33) Howard, J. *Mechanics of Motor Proteins and the Cytoskeleton*; Sinauer Association, Inc: Sunderland, MA, 2001.
- (34) Boal, D. H. *Mechanics of the Cell*; Cambridge University Press: Cambridge, U.K., 2002.
- (35) Allen, M. P.; Tildesley, D. J. *Computer Simulation of Liquids*; Oxford University Press: New York, 1989.
- (36) Safran, S. A. *Statistical thermodynamics of surfaces, interfaces, and Membranes*; Addison-Wesley: Reading, MA, 1994.
- (37) Peskin, C.; Odell, G.; Oster, G. Cellular Motions and Thermal Fluctuations. *Biophys. J.* **1993**, *65*, 316–324.
- (38) Pollard, T. D. Rate Constants for the Reactions of ATP- and ADP-actin with the ends of actin Filaments. *J. Cell Biol.* **1986**, *103*, 2747–2754.
- (39) Head, D. A.; Levine, A. J.; MacKintosh, F. C. Distinct Regimes of Elastic Response and Deformation Modes of Cross-Linked Cytoskeletal and Semiflexible Polymer Networks. *Phys. Rev. E* **2003**, *68*, 061907.
- (40) Dasanayake, N. L.; Michalski, P. J.; Carlsson, A. E. General Mechanism of Actomyosin Contractility. *Phys. Rev. Lett.* **2011**, *107*, 118101.
- (41) Shen, T.; Wolynes, P. G. Stability and Dynamics of Crystals and Glasses of Motorized Particles. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 8547–8550.
- (42) Shen, T.; Wolynes, P. G. Nonequilibrium Statistical Mechanical Models for Cytoskeletal Assembly: Towards Understanding Tensegrity in Cells. *Phys. Rev. E* **2005**, *72*, 041927.
- (43) Wang, S.; Wolynes, P. G. On the Spontaneous Collective Motion of Active Matter. *Proc. Natl. Acad. Sci. U.S.A.* **2011**, *108*, 15184–15189.
- (44) Wang, S.; Wolynes, P. G. Active Contractility in Actomyosin Networks. *Proc. Natl. Acad. Sci. U.S.A.* **2012**, *109*, 6446–6451.
- (45) Gov, N. S.; Safran, S. A. Red Blood Cell Membrane Fluctuations and Shape Controlled by ATP-Induced Cytoskeletal Defects. *Biophys. J.* **2005**, *88*, 1859–1874.

- (46) Gittes, F.; Mickey, B.; Nettleton, J.; Howard, J. Flexural Rigidity of Microtubules and Actin Filaments Measured from Thermal Fluctuation in Shape. *J. Cell Biol.* **1993**, *120*, 923–934.
- (47) Novak, I. L.; Slepchenko, B. M.; Mogilner, A. Quantitative Analysis of G-Actin Transport in Motile Cells. *Biophys. J.* **2008**, *95*, 1627–1638.
- (48) Smith, M. B.; Kiuchi, T.; Watanabe, N.; Vavylonis, D. Distributed Actin Turnover in the Lamellipodium and FRAP Kinetics. *Biophys. J.* **2013**, *104*, 247–257.
- (49) McGrath, J. L.; Eungdamrong, N. J.; Fisher, C. I.; Peng, F.; Mitchison, T. J.; Kuo, S. C. The Force-Velocity Relationship for the Actin-Based Motility of *Listeria Monocytogenes*. *Curr. Biol.* **2003**, *13*, 329–332.
- (50) Chaudhuri, O.; Parekh, S. H.; Lam, W. A.; Fletcher, D. A. Combined Atomic Force Microscopy and Side-View Optical Imaging for Mechanical Studied of Cells. *Nat. Methods* **2009**, *6*, 383–387.
- (51) Chaudhuri, O.; Fletcher, D. A. In *Protrusive Forces Generated by Dendritic Actin Networks during Cell Crawling*; Carrier, M.-F., Ed.; Springer: New York, 2010.
- (52) Bressloff, P. C.; Newby, J. M. Stochastic Models of Intracellular Transport. *Rev. Mod. Phys.* **2013**, *85*, 135–196.
- (53) Kiuchi, T.; Ohashi, K.; Kurita, S.; Mizuno, K. Cofilin Promotes Stimulus-Induced Lamellipodium Formation by Generating an Abundant Supply of Actin Monomers. *J. Cell Biol.* **2007**, *177*, 465–476.
- (54) Kiuchi, T.; Nagai, T.; Ohashi, K.; Mizuno, K. Measurements of Spatiotemporal Changes in G-Actin Concentration Reveal Its Effect on Stimulus-Induced Actin Assembly and Lamellipodium Extension. *J. Cell Biol.* **2011**, *193*, 365–380.
- (55) Ellis, R. J. Macromolecular Crowding: An Important but Neglected Aspect of the Intracellular Environment. *Curr. Opin. Struct. Biol.* **2001**, *11*, 114–119.
- (56) Ridgway, D.; Broderick, G.; Lopez-Campistrous, A.; Ruaini, M.; Winter, P.; Hamilton, M.; Boulanger, P.; Kovalenko, A.; Ellison, M. J. Coarse-Grained Molecular Simulation of Diffusion and Reaction Kinetics in a Crowded Virtual Cytoplasm. *Biophys. J.* **2008**, *94*, 3748.
- (57) Dlugosz, M.; Trylska, J. Diffusion in Crowded Biological Environments: Applications of Brownian Dynamics. *BMC Biophys.* **2011**, *4*, 3.
- (58) Klann, M. T.; Lapin, A.; Reuss, M. Stochastic Simulation of Signal Transduction: Impact of the Cellular Architecture on Diffusion. *Biophys. J.* **2009**, *96*, 5122–5129.
- (59) Drenckhahn, D.; Pollard, T. D. Elongation of Actin Filaments Is Diffusion-Limited Reaction at the Barbed End and Is Accelerated by Inert Macromolecules. *J. Biol. Chem.* **1986**, *261*, 12754–12758.
- (60) Shaevitz, J. W.; Fletcher, D. A. Load Fluctuations Drive Actin Network Growth. *Proc. Natl. Acad. Sci. U.S.A.* **2007**, *104*, 15688–15692.
- (61) Atilgan, E.; Wirtz, D.; Sun, S. X. Mechanics and Dynamics of Actin-Driven Thin Membrane Protrusion. *Biophys. J.* **2006**, *90*, 65–76.
- (62) Stuhmann, B.; Huber, F.; Kas, J. Robust Organizational Principles of Protrusive Biopolymer Network in Migrating Living Cells. *PLoS One* **2011**, *6*, e14471.
- (63) Zicha, D.; Dobbie, I. M.; Holt, M. R.; Monypenny, J.; Soong, D. Y. H.; Gray, C.; Dunn, G. A. Rapid Actin Transport during Cell Protrusion. *Science* **2003**, *300*, 142–145.
- (64) Naoz, M.; Manor, U.; Sakaguchi, H.; Kachar, B.; Gov, N. S. Protein Localization by Actin Treadmilling and Molecular Motors Regulates Stereocilia Shape and Treadmilling Rate. *Biophys. J.* **2008**, *95*, 5706–5718.
- (65) Craig, E. M.; Goor, D. V.; Forscher, P.; Mogilner, A. Membrane Tension, Myosin Force, And Actin Turnover Maintain Actin Treadmill in the Nerve Growth Cone. *Biophys. J.* **2012**, *102*, 1503–1513.
- (66) van Goor, D.; Hyland, C.; Schaefer, A. W.; Forscher, P. The Role of Actin Turnover in Retrograde Actin Network Flow in Neuronal Growth Cones. *PLoS One* **2012**, *7*, e30959.
- (67) Fan, Y.; Eswarappa, S. M.; Hitomi, M.; Fox, P. L. Myo1c Facilitates G-Actin Transport to the Leading Edge of Migrating Endothelial Cells. *J. Cell Biol.* **2012**, *198*, 47–55.
- (68) Wang, D.; She, L.; Sui, Y.; Yuan, X.; Wen, Y.; Poo, M. Forward Transport of Proteins in the Plasma Membrane of Migrating Cerebellar Granule Cells. *Proc. Natl. Acad. Sci. U.S.A.* **2013**, *109*, E3558–E3567.
- (69) Zhuravlev, P. I.; Lan, Y.; Minakova, M. S.; Papoian, G. A. Theory of Active Transport in Filopodia and Stereocilia. *Proc. Natl. Acad. Sci. U.S.A.* **2012**, *109*, 10849–10854.
- (70) Trong, P. K.; Guck, J.; Goldstein, R. E. Coupling of Active Motion and Advection Shapes Intracellular Cargo Transport. *Phys. Rev. Lett.* **2012**, *109*, 028104.
- (71) Mogilner, A.; Edelstein-Keshet, L. Regulation of Actin Dynamics in Rapidly Moving Cells: A Quantitative Analysis. *Biophys. J.* **2002**, *83*, 1237–1258.
- (72) Zhuravlev, P. I.; Papoian, G. A. Protein Fluxes along the Filopodium As a Framework for Understanding the Growth-Retraction Dynamics: The Interplay between Diffusion and Active Transport. *Cell Adhes. Migr.* **2011**, *5*, 448–456.
- (73) Alexandrova, A. Y.; Arnold, K.; Schaub, S.; Vasiliev, J. M.; Meister, J.-J.; Bershadsky, A. D.; Verkhovskiy, A. B. Comparative Dynamics of Retrograde Actin Flow and Focal Adhesions: Formation of Nascent Adhesions Triggers Transition from Fast to Slow Flow. *PLoS One* **2008**, *3*, e3234.
- (74) Lan, Y.; Papoian, G. A. The stochastic dynamics of filopodial Growth. *Biophys. J.* **2008**, *94*, 3839–3852.
- (75) Zhuravlev, P. I.; Papoian, G. A. Molecular Noise of Capping Protein Binding Induces Macroscopic Instability in Filopodial Dynamics. *Proc. Natl. Acad. Sci. U.S.A.* **2009**, *106*, 11570–11575.
- (76) Paszek, M. J.; Boettiger, D.; Weaver, V. M.; Hammer, D. A. Integrin Clustering Is Driven by Mechanical Resistance from the Glycocalyx and the Substrate. *PLoS Comput Biol.* **2009**, *5*, e1000604.