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A model actin comet tail disassembling by severing

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Abstract

We use a numerical simulation to model an actin comet tail as it grows from the surface of a small object (a bead) and disassembles by severing. We explore the dependence of macroscopic properties such as the local tail radius and tail length on several controllable properties, namely, the bead diameter, the bead velocity, the severing rate per unit length, and the actin gel mesh size. The model predicts an F-actin density with an initial exponential decay followed by an abrupt decay at the edge of the tail, and predicts that the comet tail diameter is constant along the length of the tail. The simulation results are used to fit a formula relating the comet tail length to the control parameters, and it is proposed that this formula offers a means to extract quantitative information on the actin gel mesh size and severing kinetics from simple macroscopic measurements.

1. Introduction

Actin polymerization provides the forces necessary for several types of cell movement and shape changes, including the protrusive forces at the leading edge of many motile cells [1] and the membrane deformation forces required during endocytosis [2]. A wide variety of intracellular pathogens, including bacteria such as *Listeria monocytogenes* [3] and viruses such as the vaccinia virus [4], have developed means to hijack the cell's actin-based machinery for their own movement. *Listeria* achieves this result by expressing a surface protein, ActA, that directly activates the actin nucleation activity of the Arp2/3 complex [5]. An asymmetric distribution of ActA [6] induces the formation of an actin network on one side of the bacterium which pushes the bacterium through the cytoplasm and facilitates infection [7]. The resulting network resembles a "comet tail" that trails behind the moving bacterium. Other intracellular pathogens use similar mechanisms that lead to the formation of an actin comet tail trailing behind the pathogen [8]. Such actin tails are also observed behind endocytic vesicles as they move from the plasma membrane into the cytosol [9].

The actin based motility of *Listeria* can be reconstituted in a variety of cellular extracts [10, 11] and in pure protein solutions [12]. Polystyrene beads [13] and oil droplets [14] coated with Arp2/3 activators are able to form a comet tail and exhibit actin-based motility in a manner similar to intracellular pathogens. These *in vitro* systems allow actin motility to be studied with biochemical, biophysical, and mechanical techniques that would be difficult or impossible to employ *in vivo*, and such studies have greatly enhanced our understanding of actin based motility [15, 16]. They have led to the identification and characterization of many actin binding proteins (ABPs) [17, 18], and have helped elucidate the physical mechanisms of actin-based force production [19, 20].

The actin processes involved in motility can be loosely divided into three stages: a filament nucleation stage, an elongation stage, and a disassembly stage. The first two stages are those most relevant to infection and the biophysical characterization of motility, and most studies have focused on these stages. The disassembly step is equally important for motility; for

large cellular structures, such as lamellipodia, disassembly replenishes the supply of G-actin required for continued polymerization, while for uniformly coated beads disassembly is required for the symmetry breaking event that creates a comet tail from an initially uniform distribution of actin. Several lines of evidence indicate that actin networks disassemble by the cofilin-mediated severing of individual filaments [21, 22, 23, 24]. Here we study the structure of a highly crosslinked actin comet tail as it disassembles via severing. The primary goal of this work is to understand how the macroscopic structure of the actin tail, especially the tail length, is determined by the microscopic network structure and chemical dynamics. Such a relationship will help to interpret experimental measurements on actin comet tails, and may inspire new measurements on the disassembly of comet tails.

Measurements of actin disassembly in the comet tail typically fall into two categories. In the first, actin turnover is measured by measuring the change of fluorescence intensity as a function of time at a fixed location on the tail [25, 7, 26]. Such measurements give a decay time of about 30–60 s that is independent of position along the tail. In the second, disassembly in the tail is monitored by measuring the actin tail length under different conditions. Such measurements were used to identify [17, 18] and characterize [11] proteins responsible for regulating actin turnover. To date, neither type of measurement has allowed quantitative measures of the underlying chemical rates or microscopic structure of the comet tail. In the first, measurements are often made in a single, poorly characterized system, *in vivo* or in a cell extract. In the second, only a qualitative measure of protein activity is needed and only a limited amount of quantitative data is obtained.

Most theoretical studies of actin comet tails have focused on either particle motility [27, 28, 29] or the initial symmetry breaking event which creates a tail from a uniform actin gel [29], with actin disassembly included in an *ad hoc* fashion or completely ignored. When disassembly is included, it is usually treated as a first order decay reaction [30], as would be appropriate for a single depolymerizing filament. Such a treatment may not accurately describe the disassembly of a highly crosslinked network, such as the comet tail, that disassembles by the severing of individual filaments [31].

We recently published a study on the severing-induced disassembly of the highly crosslinked network in model lamellipodium (LP), where we analytically derived and numerically verified scaling relationships between the LP structure and the fundamental microscopic parameters of the system [32]. There it was shown that the length of the network is proportional to the polymerization velocity and inversely proportional to an effective severing rate per link, which was simply related to the other rates in the system. Here we extend the previous study by using the same numerical simulation to model the smaller networks associated with moving bacteria and beads. Now, in addition to the microscopic parameters of the system, the network structure will also depend on the size of the bead. This complication is absent in an LP, where the width of the network is much larger than all other length scales in the system. One consequence of the finite network size is that the length of the actin comet tail is no longer strictly proportional to velocity, as it was for an LP, but has nonlinear corrections that become important for rapidly moving beads.

Using our numerical simulations, we find that the concentration of F-actin in the tail at first decays exponentially as a function of distance from the bead surface, but then abruptly decreases to zero near the edge of the tail. The width of the comet tail is nearly constant over the entire length of the tail, a result which compares favorably to some experimental images. We use the simulation data to determine the functional form of a formula relating the comet tail length to the four control parameters in the system, namely, the bead width, the bead velocity, the severing rate per unit length, and the actin gel mesh size. The formula contains

three parameters which must be fit from the simulation data, and we determine these parameters for the ordered cubic lattice that we use as a coarse grained approximation to the real actin network lattice, as well as some other ordered lattices. The functional form of the relationship holds for all lattices studied to date, which suggests (but does not prove) that the same functional form will hold for a more realistic, dendritically branched actin network. The determination of these constants for a more realistic actin lattice is beyond the scope of this paper, but will be required before this formula can be used to accurately fit experimental data. Here we use the fitted parameters from several ordered lattices to place bounds on the parameters for the actin lattice. We propose that, once these constants are determined for the actual actin network, this formula can be used to obtain information on the mesh size and severing kinetics from simple measurements of actin tail lengths.

2. Simulation Methods and Theory

2.1. Simulation

A detailed description of our stochastic simulation method can be found in Ref. [32]. Briefly, we use a coarse-grained approach where we model the actin comet tail as a simple cubic lattice, where the nodes represent actin crosslinks and the links represent filament sections of length ζ . The nodes in this model represent crosslinks by actin crosslinking proteins, such as filamin and fascin, as well as the crosslinks at Arp2/3 branch points. (See Sec. 4.1.1 for further discussion of this approximation.) At the bead surface (assumed flat) are M^2 nucleation sites arranged in an $M \times M$ square lattice, which fixes the initial tail width to be $W_0 = (M - 1)\zeta$. New filaments grow from the bead surface with a polymerization velocity v . Filament sections can exist in one of N states, where, roughly, the first state corresponds to a section of ATP subunits, the second state corresponds to a section of ADP + P_i subunits, the third state corresponds to a section of ADP subunits, and any other states correspond to the binding of various ABPs. It is assumed that only the N th state can sever. Figure 1 shows a schematic of a small network ($M = 2$) where the filament sections can exist in one of three states.

During each time step, a filament in state i will transition to state $i + 1$ with probability $P_i = r_i \Delta t$, where r_i is a transition rate per link and Δt is the time step. In Fig. 1, state changes occurred at the locations labeled (a), (b), and (c). Filaments in the N th state can sever and be removed from the network with probability $P_s = r_s \Delta t$, where r_s is a severing rate per unit link. It is convenient to write the severing rate as $r_s = \zeta k_s$, where k_s is a severing rate per unit length. In Fig. 1, filaments at (d) and (e) severed during the time step. After each time step, a recursive algorithm identifies all filament sections in the connected cluster, defined as those segments that are connected to the bead surface through other segments. Any filament sections not in the connected cluster are assumed to diffuse away rapidly and are removed from the network. In Fig. 1, the severing event at (e) disconnected all filaments farther from the bead, and all of these filaments are removed from the network. Lastly, filament annealing is modeled by allowing vacant sections adjacent to connected nodes to grow new filaments with probability $P_a = r_a \Delta t$, where r_a is an annealing rate per link. In Fig. 1, an annealing event occurred at (f).

We measure three variables during a simulation, the total F-actin concentration profile, the network width profile, and the average network length. All data collection begins after a network reaches steady state. Then, after each time step, we count the total number of actin filament sections a distance x from the bead surface. The time average of these measurements give the F-actin density, $c(x)$. The network width, $W(x)$, is obtained by taking a cross-section at position x and measuring the distance from the center of the network to the farthest connected node in the direction of each of the four faces, averaging the vertical and horizontal distances, and then taking a time average over all simulation runs. Fig. 2

illustrates this procedure. (Defining $W(x)$ as the width along a network diagonal did not change the results.) After each time step, the distance from each nucleation site to its farthest connected node is calculated, and the spatial and time average of these measurements is used to calculate L .

2.2. Analytic Theory

In Ref. [32] it was shown that an accurate description of the system could be obtained by modeling the filament aging, severing, and annealing processes as simple chemical transitions. In the absence of annealing the dynamical system described by these chemical transitions can be solved exactly, with the result that the total F-actin density is

$$c(x) = \sum_{k=1}^N \frac{\prod_{i \neq k}^N r_i}{\prod_{j \neq k}^N (r_j - r_k)} e^{-r_k x / v}, \quad (1)$$

where x is the distance from the bead surface and the density is normalized so that $c(0) = 1$. With this normalization the average length of the network is simply $L = \int c(x) dx$. (This definition exactly matches the length measured in the simulations for a single filament, and it gives a highly accurate approximation for the connected networks examined here [32].) Using Eq. (1) to evaluate the integral, we find

$$L = v / r_{\text{eff}}, \quad (2)$$

where r_{eff} is an effective severing rate that is related to the harmonic mean of the transition and severing rates,

$$\frac{1}{r_{\text{eff}}} = \sum_{i=1}^N \frac{1}{r_i}. \quad (3)$$

The important implication of Eq. (3) is that a system with many microscopic states can be directly mapped onto a system with one state by using the effective severing rate. For a system with annealing the mapping is not exact but remains a very good approximation. In what follows we focus on systems with a single microscopic state, with the understanding that the above mapping allows us to extend our results to multiple state systems.

The length of an actin comet tail will depend on three length scales: the bead width, W_0 , the mesh size, ξ , and a third length scale, $L_0 = \sqrt{v/k_s}$, which is proportional to the average length of a single filament that disassembles by severing [31]. The quantity L_0 is a natural length scale in any network that disassembles by severing. The probability that a link is severed after a time T is $P_{\text{sev}}(T) = 1 - (1 - r_s \Delta t)^{T/\Delta t}$, which is obtained by noting that the link has had $T/\Delta t$ chances to sever with a probability $r_s \Delta t$. By noting that $T = x/v = n\xi/v$ and taking the limit as $\Delta t \rightarrow 0$, we find that the probability of finding a link severed at position $x = n\xi$ is

$$P_{\text{sev}}(x) = 1 - \exp(-n\xi^2 k_s / v), \quad (4)$$

which shows that the structure of the comet tail depends on v and k_s only through the ratio $L_0^2 = v/k_s$. When $W_0/\xi \gg 1$ the geometry of the tail is similar to the geometry of the LP and we expect $L \propto v/r_s$, or

$$\lim_{W_0 \rightarrow \infty} L \propto L_0^2/\xi, \quad (5)$$

as in Eq. 2. For small beads ($W_0/\xi < 10$) we expect finite size effects to reduce the length of the comet tail. The effect of these corrections can be included by Defining a dimensionless scaling function f such that $L = f(W_0, \xi, L_0)L_0^2/\xi$.

Because f is dimensionless it can only depend on the dimensionless ratios W_0/ξ and ξ/L_0 . The scaling function must increase monotonically with either ratio. To see this, we vary each ratio independently but with the factor L_0^2/ξ fixed, so that changes in L correspond to changes in f . As W_0/ξ increases (with ξ and L_0 fixed) the number of severing events required to disassemble the network also increases. This increases the lifetime of the network and thus increases the network length. The dependence on the second ratio is easiest to understand if we write it as $\xi/L_0 = (\xi/L_0^2) \sqrt{v/k_s}$, which shows that if L_0^2/ξ is fixed, then at fixed k_s we have $\xi/L_0 \propto \sqrt{v}$. Increasing the polymerization velocity at fixed network size (W_0/ξ fixed) must increase the length of the actin comet tail.

The scaling function must asymptotically approach a constant as either ratio becomes large. To see this, it is useful to recall that f is a measure of surface effects, and that in an infinitely wide network, we know $f = \text{const}$. Surface effects become important when the size of the average disconnected cluster approaches the width of the network. Increasing W_0/ξ increases the network size and thus makes surface effects less important, and as $W_0/\xi \rightarrow \infty$ we approach the infinitely wide network limit. To understand the asymptotic behavior of f as a function of the second ratio, it helps to write it as $\xi/L_0 = \sqrt{r_s/(v/\xi)}$, which shows that it is a measure of the ratio of the rate of link destruction, r_s , to the rate of link creation, v/ξ . If the rate of link destruction increases then the size of the average disconnected cluster decreases. When the size of the average disconnected cluster becomes much smaller than the network width, then surface effects becomes unimportant and we recover the infinitely wide network limit. Below, we will use these facts and the simulation results to determine an approximate analytic form for the scaling function.

3. Results

Figure 3(a) shows a snapshot from a simulation with three microscopic states with $W_0 = 250$ nm and $\xi = 50$ nm. Figure 3(b) shows a magnified view of the network near the surface of the bead. Here the network is dense and highly crosslinked with very few vacancies. Figure 3(c) shows a magnified view near the end of the comet tail. In this region large voids have opened up in the network, and the network is less dense with fewer crosslink points. Notice that although the total number of vacancies steadily increases, the average width of the network remains relatively constant until the very edge of the comet.

Figures 4(a)–(b) shows the actin filament density, c , as a function of distance from the bead for a network with $W_0 = 600$ nm and $\xi = 50$ nm. These parameters were picked to approximate the network in a *Listeria* comet tail. Figure 4(a) shows the simulation results for tails both with (black) and without (gray) annealing, along with the analytical solution given by Eq. (1), which is simply a decaying exponential with a decay length of about $3 \mu\text{m}$. The

simulation result without annealing agrees with the analytic result near the bead surface, but at a certain distance from the bead the simulation results abruptly decay to zero while the analytical result continues with a smooth exponential decay. The abrupt decay in the simulation results is due to the cooperative nature of disassembly by severing, where multiple severing events are required to remove sections of the network. When annealing is included the filament concentration decays more slowly and the abrupt decay at the edge of the network is smoothed out.

The fluorescence intensity of a comet tail labeled with fluorescent actin will be directly proportional to the actin filament density, and thus such experiments offer a direct way to measure the spatial profile of the actin filament density. However, the sharp features predicted by our simulation results will be masked in a fluorescence experiment because of the diffraction limited resolution of the microscope and because of background noise. The experimentally measured profile will be a blurred profile that is a convolution of the real profile with a blurring function that is approximately a Gaussian with standard deviation σ . In Fig. 4(b) we show the blurred concentration density, c_b , that would be observed for the distributions in Fig. 4(a) with $\sigma = 1 \mu\text{m}$, which is about 2–4 times the diffraction limit. We chose this value because in Ref. [33] it was found that a simple blurred exponential with $\sigma = 1 \mu\text{m}$, which is shown as the thin solid line in Fig. 4(b), gave an excellent fit to experimentally measured *Listeria* actin comet tail profiles. Fig. 4(b) shows that the abrupt decay in our simulation results is greatly reduced in the blurred concentration, and the differences between the simulation results and the simple exponential decay are less pronounced.

We noticed that our simulations produced comet tails with a nearly constant width along their entire length (see Fig. 3). This behavior was surprising; we expected to see the comet tail taper because nodes at the surface of the comet require fewer severing events than interior nodes to disconnect from the network. To quantify this behavior, we measured the average width of our network as a function of position from the bead surface, $W(x)$. The results are shown in Fig. (5) for the two networks shown in Fig. 4. We see that the width of the network is nearly constant for the entire length of the tail, which is in stark contrast to the exponential decay observed for the actin density. The width is within 10% of the initial width for over 85% of the tail length without annealing and for over 70% of the tail length with annealing.

To compare these results to the behavior of real tails, we obtained published images of experimental comet tails and measured their widths as a function of distance along the tail. The behavior of real tails seems to vary: under some experimental conditions tails appear to have a constant width [20, 34], while under other conditions the tails taper as they decay [10, 11, 13]. We were not able to discern a pattern in the data, although it appears that the constant width tails were predominantly obtained in pure protein solutions, while the tapered tails were obtained in vivo or in cell extracts. The square data points in Fig. 5 represent experimentally measured diameters from a single fluorescently labeled actin comet tail with a constant width. The width profile of this tail is in qualitative agreement with the simulation data; the comet tail diameter is essentially constant over its entire length, decaying abruptly to zero at the very edge of the tail.

The length of an actin comet tail is the easiest variable to measure in both phase contrast and fluorescent microscopy. This macroscopic quantity is directly controlled by the bead velocity, the actin gel mesh size, and the severing/disassembly rate. Measurements of the length therefore offer a wealth of information about the microscopic properties of the system. In Fig. 6(a) we plot the average length as a function of network width for four values of ζ . When the width is large compared to the mesh size ($W_0/\zeta > 20$)

the actin tail length becomes independent of the network width. In this regime the system responds as if the network were infinite in extent and matches the networks used to study lamellipodia in Ref. [32]. When the network width is only slightly larger than the mesh size ($W_0/\xi < 10$) the effects of the finite network size decrease the network length relative to the large width networks.

The cause of the length decrease at small W_0 is easiest to understand by considering the smallest possible network in our model, $W_0/\xi = 1$. Here there are only four nucleation sites and the entire comet tail can be disconnected by just four severing events that occur at the same distance from the bead surface. These catastrophic severing events significantly decrease the length of the comet tail. More generally, the probability of a catastrophic severing event, one that forms a disconnected cluster on the order of the network size, decreases rapidly as the size of the network grows. The asymptotic behavior at large W_0 can be understood as follows: if the network width is much greater than the average size of a disconnected cluster, then the network behaves as if it were infinitely wide, and increasing W_0 further has no effect.

In Fig. 6(b) we plot L as a function of ξ for various bead widths. For large beads the length of the actin network scales inversely with ξ , as expected from our previous work. For smaller beads the log-log plot shows deviations from this relationship at large ξ . The differences between the finite networks and the true 3D limit are not as pronounced in Fig. 6(b) as they were in Fig. 6(a), which shows that the finite size corrections have a weaker dependence on the mesh size than they do on the bead diameter.

As discussed in Sec. 2.2, we hoped to use our simulation data to determine a formula relating the network length to the three controllable parameters: $L_0 = \sqrt{v/k_s}$, ξ , and W_0 . This formula must have the form $L = f(W_0, \xi, L_0) L_0^2 / \xi$, where the finite size corrections are described by the scale factor f . We first scaled all network lengths by L_0^2 / ξ and plotted this quantity versus $(W_0/\xi)(\xi/L_0)^n$ for different values of n . As shown in Fig. 7, the data collapse onto a single curve with $n = 1/3$. The shape of the curve suggested an exponential dependence on the arguments, and trial and error with various exponential fits gave the formula,

$$L = A \left\{ 1 - B \exp \left[-\alpha \sqrt{\left(\frac{W_0}{\xi} \right) \left(\frac{\xi}{L_0} \right)^{1/3}} \right] \right\} \frac{L_0^2}{\xi}, \quad (6)$$

with $A = 1.36$, $B = 1.92$, and $\alpha = 1.66$. This curve is plotted in Fig. 7 and is seen to be in excellent agreement with all of the simulation results. The curves described by Eq. (6) are shown in Fig. 6(a)–(b) with the accompanying simulation data to better illustrate the agreement between the simulation data and this formula. In almost all cases the curves given by Eq. 6 - with A , B , and α fixed to the values listed above - differ by no more than 5% from the simulation results, and by no more than 2% when $W_0 > 3\xi$. The only notable deviations occur at the smallest widths, when $W_0 = \xi$ ($M = 2$), where the curve deviates from the simulation data by 12.5% for $\xi = 100\text{nm}$ and by about 7% for the other mesh sizes. These are the thinnest tails we can model with our course grained approach, and larger deviations in this limiting case are not surprising. The exponential decay in Eq. (6) shows that finite size effects decrease rapidly as either W_0/ξ or ξ/L_0 grows.

4. Discussion

4.1. Model Assumptions

Here we modeled the growing actin comet tail as a perfect cubic lattice that disassembles as links are removed from the network. This is an oversimplification and it is natural to question how the results presented here relate to an actual actin comet tail. A detailed discussion of the model assumptions can be found in Ref. [32], including a justification of the coarse grained approach and the effects of filament diffusion and network disorder. Here we focus on two assumptions of particular relevance to actin comet tails.

4.1.1. Lattice Structure and Network Geometry—The use of a cubic lattice may appear questionable because its six-fold coordination does not match the coordination observed in actin networks, namely, 3-fold coordination at Arp2/3 branch sites and 4-fold coordination at sites of other actin crosslinkers like α -actinin or fascin. In Ref. [32] we investigated several 2D and 3D lattices as models for an LP, including a cubic lattice, diamond lattice, and a body centered cubic (BCC) lattice in 3D. In all cases we found that the actin density profile matched the profile predicted by Eq. (1) up to a point where the local concentration reached the lattice percolation threshold, where the actin density rapidly dropped to zero. For the smaller networks considered here the behavior is identical but the concentration where the simulation density deviates from the analytic prediction is shifted above the percolation threshold. The magnitude of this shift may be different for different lattices. Thus, the values of the fit constants in Eq. (6) may be different for different lattices, but the dependence on the microscopic length scales will be the same. This behavior should persist in a physical network with disorder and crosslinks with varying coordination number [32].

In vitro, actin networks are sometimes grown using Arp2/3 complex without additional crosslinkers, and in this case the crosslinks will correspond to threefold coordinated branch points. This case differs from the regular lattices considered here because of the preferential orientation of barbed ends toward the obstacle. This orientation implies that at a typical crosslink there will be two links pointing toward the obstacle and one away from it. The resulting hierarchical network structure consists of connected dendritic clusters, which are likely strongly entangled. A dendritic cluster differs from the geometries considered here in that severing a single link will break the cluster into two disconnected fragments. However, this effect will be mitigated by the entanglement of the clusters, since the steric constraints preventing filaments from passing through each other will prevent these large fragments from diffusing away rapidly unless many links are severed. Thus we expect the main qualitative features observed here to persist in more realistic models based on Arp2/3-induced branching.

The comet tails considered here are nucleated from a square grid on the bead surface and thus have square cross-sections. Physical comet tails are usually nucleated from cylindrical or ellipsoidal objects and thus will have a more circular cross-section. Using a circular cross-section in our simulations would have a minimal effect on the tail properties. For very small beads the number of filaments is so small that the major changes come from changing filament number and not from the geometry of those filaments. The nearly constant width of simulated networks up to the point where rapid disassembly occurs (see Fig. 5) shows that surface effects are minimal, and thus for large beads the properties of the tail can not depend on the cross-sectional shape.

Likewise, the near constant width of the tail should not depend on the underlying lattice. Nodes at the surface of the comet will have fewer links, regardless of the lattice structure. If the reduced number of links at the surface would lead to tapering for any lattice, then it

should lead to tapering for all lattices. In fact, we did not observe tapered tails for the cubic, diamond, or BCC lattice. Nor was tapering observed with a 2D square lattice. Tapering of comet tails is thus likely not due to structural effects, but rather associated with disassembly mechanisms beyond those considered in our model. One mechanism that might lead to tapering is if severing occurs more rapidly at the surface of the bead, perhaps because cofilin diffuses to the center of the bead more slowly. At this point we have no data to support such an assumption, and in fact the tails are so thin that reduced diffusion is unlikely to play a factor.

One significant difference between the networks considered here and real actin comet tails is that real comet tails are usually wider than the diameter of the bead or bacterium. There are two reasons for this: uncapped growth at the surface and growth on the sides of the bead. If the capping protein (CP) concentration is low enough, then free barbed ends will continue to grow for some time even when they leave the vicinity of the bead. This can lead to dramatic morphological changes at very low CP, as exemplified by the “fishbone” tails shown in Fig. 7 of Ref. [20] at 50nM gelsolin (which functions as a capping protein in this case). Our model does not capture the features of such an extreme case, but under ordinary conditions such extraneous growth is limited and represents only a minor perturbation on the width of the network. Of more consequence is the growth on the side of the bead, which certainly makes the tail wider than the bead. Such an effect is missing in our model because we consider nucleation from a flat surface. To map our model onto a more realistic case, we interpret W_0 as the width of the network before it begins depolymerizing. As such, an appropriate approximation is to take W_0 as the thickness of the network through a plane tangential to the back end of the bead.

4.1.2. Mechanical Effects—The elastic properties of the actin network are important for the symmetry breaking event that leads to bead motility [35, 36, 29], and in some limits the velocity of the bead is controlled by these elastic properties [27, 37, 38, 39, 30]. In our simulation and analytic theory we have neglected mechanical effects. It has been shown that mechanical equilibrium is achieved in the tail approximately one bead radius away from the bead surface [39]. Usually this region constitutes a negligible fraction of the total tail length and can thus be ignored. For smaller actin tails this region might constitute 10 – 20% of the tail. In this case our model can still be applied, but the “bead surface” should instead be taken to be a plane through a section of the actin tail that has already reached mechanical equilibrium but close enough to the tail so there is no significant depolymerization. It is conceivable that a tail may be so short relative to its width that our theory no longer applies, but we have not encountered any such extreme tails in published images, and in fact most tails have lengths much larger than their widths.

4.2. Experimental Implications

The reconstitution of the actin-based motility of bacteria and beads *in vitro* offers a controllable environment that should allow some predictions of this theory to be tested. Direct measurements of the spatial dependence of the F-actin concentration are complicated by background noise and by the optical diffraction limit. These effects will obscure the sharp features that are a hallmark of severing mediated disassembly and will make it difficult to distinguish between models.

The length of the actin comet tail is easier to measure, and measurements of the length can both test the predictions of Eq. 6 and can offer a means to measure two important parameters, the mesh size and the severing rate. Many models should reproduce $L \propto v/(\xi k_s)$ in the large bead limit, and so the interesting prediction of our model is the prefactor in Eq. (6), $f(W_0/\xi, \xi/L_0)$. With the available data we are unable to distinguish if our functional form

is better than a constant prefactor. Many of the data sets are too small to distinguish between the two models [17, 11]. The largest relevant data set, representing about 250 length measurements as a function of velocity, was obtained on *Listeria* moving in cell extract [33]. There is a large variation in the motility of individual *Listeria* in the same medium, which appears to reflect variations in the surface properties of individual bacteria and not any variation in the local environment. Thus, the data is very noisy and we are unable to use it to distinguish between models. A study using a more uniform system such as beads coated with a high density of actin nucleator should minimize the variation between moving particles and allow for cleaner data that can distinguish between models.

Each of the four parameters W_0 , v , k_s , and ζ can be controlled in an experiment, although not necessarily independently. W_0 will be proportional to the bead size. Under most conditions, v will be directly proportional to the free actin monomer concentration, $[G]$. The cofilin concentration, $[C]$, will determine k_s because of cofilin's severing [21, 24] and debranching [40] activity, but the relationship between k_s and $[C]$ is nonlinear [40]. Cofilin is also known to bind G-actin [11], and this interaction will decrease both free cofilin and free G-actin, affecting both the severing rate and the velocity. In general, ζ should be roughly inversely proportional to the actin crosslinker concentration, $[X]$, and to the active Arp2/3 concentration, $[A]$. It can also be controlled by varying the capping protein concentration, $[CP]$, although there might not be a simple relationship between ζ and $[CP]$. For example, Ref. [39] reports a non-monotonic relationship between a capping protein (gelsolin) and the mesh size. As with cofilin, the steady state values of the other protein concentrations can be changed when $[X]$ or $[CP]$ are varied. For example, increasing $[CP]$ will generally inhibit polymerization, which will increase the available free G-actin and increase the polymerization velocity [34]. Conversely, increasing $[A]$ decreases v [34].

Simultaneous measurements of the actin tail length and the bead velocity could be used to make quantitative measurements of the gel mesh size and filament severing rates. One way to do this is to fix W_0 , k_s , and ζ while varying v . This can be achieved by varying $[G]$ while keeping $[C]$, $[X]$, and $[CP]$ fixed. This is conceptually straightforward, but, as mentioned above, protein-protein interactions must be accounted for to ensure the desired steady state levels of these proteins. A plot of L/v vs v could then be fit to the form

$L/v = a_0(1 - b_0 \exp(-c_0 \sqrt{W_0/v^{1/6}}))$. From Eq. (6) we have $a_0 = A/(k_s \zeta)$ and $c_0 = \alpha(k_s/\zeta^4)^{1/12}$, where A and α are the fitted constants in Eq. (6) for a dendritically branched actin network, and thus we obtain the mesh size and the severing rate per unit length as

$$\zeta = \left[\frac{A}{a_0} \left(\frac{\alpha}{c_0} \right)^{12} \right]^{1/5} \quad (7)$$

and

$$k_s = \left[\left(\frac{A}{a_0} \right)^4 \left(\frac{c_0}{\alpha} \right)^{12} \right]^{1/5}. \quad (8)$$

By repeating the above measurements while varying the cofilin and crosslinker concentrations one could determine ζ and k_s as functions of $[C]$ and $[X]$. (Again, protein-protein interactions must be taken into account when calculating the steady state levels of these proteins. Varying $[C]$ will also vary $[G]$, and thus the amount of $[G]$ actin will have to be varied at the same time.) We believe that measurements of this sort have the potential to

teach us much about the severing activity and structure of actin gel systems and have not been fully exploited for quantitative measurements. Models such as the present one can be a crucial aid in this effort.

4.2.1. Bounding the parameters for a dendritically branched actin network—

The method just proposed relies on an accurate determination of the constants A and α . The constants found here for the cubic lattice cannot be used for the actin network because these constants depend on the lattice structure. An accurate determination of A and α requires a detailed simulation of a dendritically branched network and is beyond the scope of this paper. However, we can put bounds on the values of these quantities for the actin network by examining other ordered lattices. The constant A is the proportionality factor giving

$L = AL_0^2/\xi$ as $W_0 \rightarrow \infty$. From Ref. [32] we know that the network density drops exponentially until it reaches c_p , the network percolation threshold, at which point the density drops abruptly to zero. Thus, we can estimate the length by setting $c_p = \exp(-\xi L/L_0^2)$, which gives

$$A_{\text{est}} = \ln(1/c_p). \quad (9)$$

For the cubic lattice this gives $A_{\text{est}} = 1.39$, which is within 3% of the value determined from our simulation. Table 1 shows that results of similar accuracy are obtained for the BCC, diamond, and square lattices. The percolation threshold for an actin lattice, which has a mix of three-fold and four-fold coordination, must be greater than c_p for the diamond lattice. A rigorous upper bound is given by c_p for the honeycomb lattice (3-fold coordinated in 2D), but if the crosslinker concentration is not too low then a significant fraction of the actin network will be 4-fold coordinated, and thus c_p for the square lattice gives a reasonable upper bound. These considerations give $0.69 < A_{\text{actin}} < 0.94$, with A_{actin} likely to be similar to A_{diamond} and thus in the upper part of this range.

We were not able to develop an analytic approximation for α , but from Table 1 we see that α is a decreasing function of coordination number and dimension. Following the same reasoning as above, we bound α_{actin} by the values for the square lattice and the diamond lattice, which gives $1.01 < \alpha_{\text{actin}} < 1.40$, with the actual value probably lying in the upper part of this range.

4.3. Conclusion

We used a numerical simulation of an idealized actin comet tail to determine an approximate analytic formula, Eq. (6), relating the tail length to the width of the bead, the bead velocity, the actin mesh size, and the severing rate per unit length. The formula has three free parameters which are determined by the lattice structure, and we placed bounds on these parameters for the actin lattice. The formula provides a means to extract data on the mesh size and severing rate from simultaneous measurements of the actin tail length and bead velocity. We have proposed an experiment to determine the mesh size and severing rate as functions of various protein concentrations, such as cofilin, capping protein, or various crosslinking proteins. However, in order to derive useful information from the experiment, further modeling is required to obtain the best fit parameters for an actual dendritically branched actin network.

The model predicts an F-actin density with an initial exponential decay but with an abrupt decay at the edge of the tail. This abrupt decay is a hall-mark of severing mediated disassembly, but may be difficult to observe with fluorescence microscopy because such

sharp features are obscured by background fluorescence and the diffraction limited resolution of optical microscopes. Our model produces actin tails with a near constant diameter, in agreement with some experimental tails but in disagreement with others. The factors causing tapering are beyond those included in the present model, and establishing their nature could shed valuable light on the actin disassembly process.

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Figure 1.

Schematic of the growing actin comet tail illustrating the changes that can occur during each time step. The figure on the left shows a section of the network at time t , and the figure on the right shows the same network one step later at time $t + \Delta t$. A new layer of filament segments was added at the bead surface during this time step. The network has three states: state 1 (red), state 2 (blue), and state 3 (green). Segments changed state at the locations marked (a), (b), and (c). Severing events occurred at the locations marked (d) and (e), and annealing events occurred at the location marked (f). See the text for more discussion.

Figure 2.

Calculation of $W(x)$. The three images above represent cross-sections of a 5×5 network at three different positions, x_1 , x_2 , and x_3 . Connected nodes are black, disconnected nodes (empty sites) are white, and the links are omitted for simplicity. To find $W(x)$, we begin at the center of the network and find the farthest connected node in the direction of each of the four faces of the square cross-section, and we sum these distances and divide by 2 to get an average width. For example, at x_1 we have $W(x_1) = (2\xi + 2\xi + 2\xi + 2\xi)/2 = 4\xi$, which is what we expect for the full 5×5 lattice. Similarly, $W(x_2) = 3.5\xi$ and $W(x_3) = 2.5\xi$. The time average of such measurements gives $W(x)$.

Figure 3.

(a) A snapshot of a small actin comet tail ($W_0/\xi = 5$) where the filament segments can exist in one of three states, state 1 (red), state 2 (blue), and state 3 (green). The boxed regions in (a) are magnified in (b) and (c). The x axis measures the distance from the bead surface. Parameters: $v = 0.03 \mu\text{m}$, $\xi = 50 \text{ nm}$, $k_{\text{eff}} = 0.33 \text{ s}^{-1} \mu\text{m}^{-1}$, $r_1 = r_2 = r_3 = 0.05 \text{ s}^{-1}$, and $r_a = 0 \text{ s}^{-1}$.

Figure 4.

The F-actin density profile in the actin comet tail as a function of distance from the edge of the bead. (a) Simulation results without (black) and with (gray) annealing. The thin line is the solution to the simple kinetic theory, Eq. (1). (b) The curves in (a) convolved with a Gaussian kernel with $\sigma = 1 \mu\text{m}$ to simulate the diffraction and background noise in an experimental situation. The sharp features of the actual distribution are lost because of the limits on experimental resolution. Parameters: $N = 1$, $W_0 = 600 \text{ nm}$, $\zeta = 50 \text{ nm}$, $v = 0.03 \mu\text{m}$, $k_s = 0.66 \mu\text{m}^{-1} \text{ s}^{-1}$, $r_a = 0$ (black) or $r_a = 0.14 \text{ s}^{-1}$ (gray).

Figure 5.

The width of an actin comet tail as a function of position from the bead surface. The dots represent simulation results without (black) and with (gray) annealing. The squares are measurements extracted from a fluorescent image of a comet tail in Fig. 7 of Ref. [20] (leftmost comet of the 100 nM gelsolin frame). The left and bottom axes refer to the simulation results, and top and right axes refer to the experimental results. Simulation parameters are as in Fig. 4.

Figure 6.

(a) The length of an actin comet tail as a function of width for fixed ξ . Curves are shown for four different values of ξ : 10 nm (filled circles), 25 nm (open triangles), 50 nm (open squares), and 100 nm (open circles). (b) The length of an actin comet tail as a function of mesh size for fixed radius. Curves are shown for six radii: 0.10 μm (open circles), 0.20 μm (open squares), 0.40 μm (open triangles), 0.60 μm (gray circles), 1.0 μm (gray squares), and 2.0 μm (gray triangles). In both plots $v = 0.03 \mu\text{m/s}$ and $k_s = 0.25 \mu\text{m}^{-1} \text{s}^{-1}$ were fixed. The solid curves are fits to Eq. (6) with the appropriate parameters fixed. The dashed line has a slope of -1 , and illustrates the asymptotic slope approached as $W_0 \rightarrow \infty$.

Figure 7.

The average lengths of many networks scaled by the appropriate values of L_0^2/ξ and plotted as a function of the single parameter $(W_0/\xi)(\xi/L_0)^{1/3}$. All of the data collapse onto a single curve when plotted as a function of this parameter. The solid line is the fit to the data given by Eq. (6). Four of the networks had $v = 0.03 \mu\text{m/s}$ and $k_s = 0.25 \mu\text{m}^{-1} \text{s}^{-1}$ fixed while W_0 was varied for different values of ξ : 100 nm (solid circles), 50 nm (open circles), 25 nm (open squares), and 10 nm (open triangles). Three of the networks had $\xi = 0.05 \mu\text{m}$ and $k_s = 0.20 \mu\text{m}^{-1} \text{s}^{-1}$ fixed as v varied for different values of W_0 : 100 nm (gray squares), 250 nm (gray diamonds), and 400 nm (open diamonds).

Table 1

Values of the fitting constants A , B , and α in Eq. (6) for four periodic lattices, and the estimated value of A from Eq. (9).

Lattice	A	B	α	A_{est}
BCC	1.67	2.18	2.14	1.71
Cubic	1.36	1.92	1.66	1.39
Diamond	0.91	2.86	1.40	0.94
Square	0.73	1.40	1.01	0.69