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The Effects of Filament Aging and Annealing on a Model Lamellipodium Undergoing Disassembly By Severing

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Abstract

We construct a simplified model of a lamellipodium and use numerical simulations to study its properties as it disassembles by filament severing. The growing lamellipodium is modeled as a 2D or 3D periodic lattice of crosslinked actin filaments. At each time step a new layer of actin filaments is added at the membrane, existing filaments are severed stochastically, and disconnected sections of the network are removed. Filament aging is modeled by including several different filament chemical states. Filament annealing is included by allowing existing filaments to grow new filaments. The properties of the model are studied as functions of the number of states and the severing and annealing rates. We find that the network width is proportional to the sum of the average lifetimes of the states, and is well modeled by a simple kinetic theory. The length of the network scales linearly with actin concentration and has a finite width even at high severing protein concentrations. The edge of the growing network becomes sharper as either the number of states or the dimensionality is increased. Annealing increases the average length of the network, and we find that the network length diverges at a critical annealing rate.

1. Introduction

At the leading edge of most motile cells is a thin, sheet-like extension called the lamellipodium (LP). Within this narrow region the cell generates protrusive forces by the polymerization of an actin network. The processes involved in creating and maintaining this highly dynamic structure have been the subject of intense experimental and theoretical studies for decades (for a review see [1]). These processes can be organized into three broad categories: actin filament nucleation and elongation, filament aging (including changes in the chemical states of actin subunits and association with various actin binding proteins), and filament disassembly. The nucleation and elongation step is most directly responsible for cell motility and protrusion, and most theoretical studies have focused on modeling these two effects [2,3].

The disassembly step is equally important for cell motility; without disassembly the pool of monomeric actin (G-actin) would be depleted and protrusion would cease. The disassembly of a polymer network can occur by depolymerization of individual filaments, by filament severing, or by some combination of these two processes. Several lines of evidence indicate that severing controls the rate of depolymerization *in vivo* (see below). In this paper we study the structure of a model LP as it undergoes both aging and disassembly via severing. The main goal is to understand how the size and structure of the LP are determined by the fundamental control parameters of the network: the total number of states, the transition rates between these states, the severing rate, and the annealing rate. To this end, we use a combination of analytic

theory and numerical simulation to derive and verify scaling relationships between the control parameters and the network size and structure.

Network growth at the leading edge proceeds via a dendritic nucleation mechanism [4,5,6,7] which gives rise to a network of short, highly branched filaments right at the leading edge [8]. As filaments move away from the membrane they are acted upon by several actin binding proteins (ABPs), some of which crosslink nearby filaments [8]. Thus, the actin network in the LP is best thought of as an elastic gel, composed of filaments interacting via branch and crosslink points, rather than as a collection of independent filaments.

When an actin subunit is incorporated into the growing actin network it begins an aging process that involves changes in its chemical state and binding partners. An actin subunit can exist in one of three states depending on the state of its associated nucleotide, an ATP state, an ADP- P_i state, or an ADP state [6]. *In vivo*, newly added subunits are almost entirely in the ATP state [6]. Subunits convert to the ADP- P_i state through rapid ATP hydrolysis (with a rate of $\sim 0.3 \text{ s}^{-1}$ [9]), and subsequently convert to the ADP state through P_i release. P_i release is slow *in vitro*, $\sim 0.003 \text{ s}^{-1}$ [10], but *in vivo* the rate is accelerated so that the total aging process from ATP to ADP actin only takes 10 – 30 s [11]. Actin subunits can also bind various ABPs, so that the full state of a subunit is determined by both its chemical state and any associated ABPs.

The natural depolymerization rate of an actin filament is too slow to account for the rate of disassembly observed *in vivo* [12], and suggests that disassembly is a regulated process. *In vivo*, exposed barbed ends are rapidly capped by CP, and most pointed ends are capped by Arp2/3 complex, and both cappers inhibit depolymerization [11]. It is therefore assumed that depolymerization is preceded by a mechanism that exposes or creates filament ends.

Actin depolymerizing factor/cofilin (ADF/cofilin) is a small ABP localized to the LP of motile cells that has been found to sever actin filaments both *in vitro* [13,14] and *in vivo* [15]. The severing activity of ADF/cofilin depends on its interaction with other proteins and on the nucleotide state of the actin filament, and our present understanding is that ADF/cofilin preferentially binds to and severs the ADP portion of an actin filament [13,11]. In this view filament aging is a timing mechanism for disassembly, where severing is inhibited in the newly formed ATP and ADP- P_i sections, and is accelerated in the older ADP sections of a filament [16].

Most theoretical studies have focused on the role of actin assembly in motility, with disassembly included in an ad hoc manner, usually by an extremely rapid depolymerization rate [17] or by treating disassembly as a boundary condition [18,19,20]. Realistic treatments of filament aging and disassembly by depolymerization and severing were included in several models for the *in vitro* length distribution of individual filaments [21,22,23]. For our purposes, the most important result of these models is that filaments with many states can be described by an effective severing rate that is given by the geometric mean of the transition rates between states and the severing rate of the final state.

Extensions of these models were used to study an LP composed of 1D, non-interacting filaments [24], which allowed for the calculation of the total F-actin density profile in the LP. While this model based on independent filaments serves as a useful starting place, it is now apparent that an accurate description of the LP must include the effects of filament branching and crosslinking.

Mogilner and Edelstein-Keshet [25] developed a quasi-1D model of an LP based on rate equations that included the effects of branching and explicitly treated depolymerization throughout the LP, either through a severing mechanism or a debranching mechanism. This model showed that with *in vivo* capping rates, the two disassembly mechanisms are equivalent.

Furthermore, this model predicted that the F-actin density decays exponentially from the leading edge.

Carlsson [26] used a coarse grained model of an LP to study network disassembly in the presence of filament crosslinking. The LP was modeled as a simple cubic lattice in up to three dimensions, and was allowed to disassemble via severing. Annealing was included but filament aging was not (all filament subunits were in the same state). This study revealed the cooperative nature of severing in higher dimensions, which leads to a more abrupt decay of the filament density than that seen in 1D. Annealing led to larger networks and broadened the LP edge. Furthermore, at a critical annealing rate the network failed to decay completely, instead leaving an asymptotic tail whose density depended on the ratio of the annealing and severing rates.

Here we extend the previous study by including the effects of filament aging. The primary goal of this study is to understand how a many state model with filament interactions in higher dimensions differs from the many state, 1D, independent filament theory. Specifically, we ask if there is a single effective severing rate that determines the network length and the sharpness of the F-actin profile, as in the 1D case. We also investigate the effects of annealing, and ask if simple scaling relationships exist between the annealing rate and the network properties of the many state model.

We find that in higher dimensions the effects of multiple actin states are well modeled using a simple kinetic theory. The model can be characterized by a single effective severing rate that is related to the harmonic mean of the transition and severing rates. This prediction differs from the 1D model, where the corresponding effective severing rate is related to the geometric mean of the transition and severing rates [23]. We find that the length of the network increases and the network boundary becomes sharper as the number of states increases. Under the combined effects of aging and annealing, we find that a two parameter characterization consisting of the effective severing rate and the annealing rate provides an approximate description of the network properties. The critical annealing rate - the rate at which the network length diverges - depends only on the effective severing rate and not on the number of states.

This paper is organized as follows. In Sec. 2 we describe the model of the LP and the parameters that characterize the model. In Sec. 3 we analyze a mathematical model based on a set of chemical transitions that is found to describe the properties of the LP. In Sec. 4 we present the results from the simulations and compare those results to the predictions of the analytic model developed in Sec. 3. In Sec. 5 we discuss the validity of the assumptions used in developing the model and the biological implications of our results. In Sec. 6 we present our conclusions.

2. Simulation Methods

We model the lamellipodium as a simple cubic lattice in two or three dimensions, with nodes at the lattice points and links connecting each node to its nearest neighbors. The network grows by adding new links at the leading edge with a constant polymerization velocity, v . We do not attempt to model the polymerization and disassembly of individual actin subunits. Rather, the links represent sections of individual actin filaments of length δ , where δ is the average distance between branching/crosslink points along a filament, and the nodes represent crosslinks or branch points between filaments. It is assumed that the average filament length is much greater than the mesh size. In this regime the time required to disassemble the network is much larger than the time required for individual severing events, and the coarse grained approach used here is a reasonable approximation.

We allow each filament segment to exist in one of several states. The states here are not explicitly defined, but they are intended to correspond to different chemical and mechanical states of the actin filament section. Segments are always added at the membrane (hereafter

referred to as the wall) in the first state, which can be thought of as a section with all ATP subunits and without any associated ABPs. As the link ages it is converted to other states, each of which has an associated transition rate. These transitions are intended to model ATP hydrolysis, P_i release, and the binding of various ABPs. In addition, link states can represent different structural states, such as a filament section that has severed between two crosslink points but which is still attached to the network. We assume that a link can sever completely and be removed from the network only when it converts to the final state.

We include annealing by allowing a vacant section of the network adjacent to any connected node to grow a new filament segment. A new segment may be added between two connected nodes or at a location with one connected node and one removed node. In the latter case, the newly added segment creates a new node in the previously vacant position, and that new node can then act as a nucleation site for additional annealing. The newly added segments are always in the first state, and they then begin to age as the network grows.

Figure 1 shows a schematic of a 2D network and illustrates its time evolution. The image on the left shows the network at step n , and the image on the right shows the same network one time step later, at step $n + 1$. Here it is assumed that filament segments exist in one of three states: red segments are in the first state, blue segments are in the second state, and green segments are in the third state. At each time step a new layer of segments is added at the leading edge. Subsequently, every filament segment in the network, including those newly added at the leading edge, are allowed to change state with some transition probability $P_i = r_i \Delta t$, where r_i is the transition rate for going from state i to state $i + 1$ and Δt is the time step. Then, any filament segments in the final state are allowed to sever with some probability $P_s = r_s \Delta t$, where r_s is the severing rate of a link. In figure 1, segments at positions (a), (b), and (c) have changed from the first to the second state, and the segment at position (d) changed from the second to the third state. Filament segments at positions (e) and (f) were severed during the time step. After severing, a recursive algorithm is used to determine which nodes remain in the connected cluster, defined as the set of nodes with an unbroken path of links back to the leading edge. All nodes not in the connected cluster are removed from the network at this time. In figure 1, the severing event at position (f) left a disconnected cluster in the upper righthand corner of the network, which has been removed. Finally, we model filament annealing and polymerization away from the leading edge by allowing new segments to grow from connected nodes into vacant sections of the network with probability $P_a = r_a \Delta t$, where r_a is an appropriate annealing rate for a section of filament. In figure 1, the annealing event at (g) has reconnected two connected nodes, while the annealing event at (h) has reintroduced a node that was previously removed from the network.

2.1. Lattice Parameters

In [26] the appropriate mesh size for a cubic lattice was estimated to be $\delta \approx 0.04 \mu\text{m}$, which corresponds to about 15 actin subunits. This estimate was obtained as a lower limit by assuming an F-actin concentration of 1 mM in the LP. Increasing the mesh size by a factor of 5 (with the severing rate per unit length held fixed) only changed the size of the network by $\sim 30\%$, and decreasing the mesh size had an even smaller effect on the network properties. We assume that in the multistate model the system properties will continue to be relatively insensitive to the exact value of the mesh size. Therefore, in this study we fix $\delta = 0.04 \mu\text{m}$.

In [26] the polymerization velocity in a lamellipodium was taken to be $v = 0.03 \mu\text{m/s}$, which is an estimate based on speckle velocities measured in [27]. Here we fix the polymerization velocity at the same value. The mesh size and polymerization velocity define the time step, $\Delta t = \delta/v \approx 1.3 \text{ s}$. Although this time step is large compared to the time scales involved in individual subunit dynamics, it is sufficiently small to capture the stochastic nature of the processes considered in the coarse grained model.

We found that 100 parallel filaments were sufficient to capture the true 2D behavior of an LP sheet. A 10-fold increase in the number of filaments in a 2D sheet generally changed the results by less than 1% (data not shown). A 3D system is formed by stacking several 2D sheets, each with 100 parallel filaments. The height of our 3D network was chosen to approximate the thickness of a real lamellipodium. Estimates of LP thickness range from about 100–200 nm [28,29]. Here we assume a thickness of 160 nm, which corresponds to 5 stacked 2D sheets.

2.2. Other Parameters

In addition to the lattice parameters, the system properties will also depend on the number of states, the transition rates between the states, the severing rate of the final state, and the annealing rate. We allowed all of these parameters to vary in a range that gave physically sensible widths of about 1–10 μm . The appropriate number of states is not clear, but is probably in the range of 3 to 6. The lower limit comes from considering ATP hydrolysis and subsequent P_i release, and then assuming an intrinsic severing rate for ADP sections. The upper value assumes that segment removal requires two ADF/cofilin binding events and an initial severing event, with a second severing event actually removing the filament section from the network. In [23] it was assumed that a subunit goes through 5 steps before severing, in the middle of this range. Here, we will consider state numbers in the range of 1 to 10, with the larger values used solely to investigate the mathematical properties of the model.

2.3. Data Collection

Data collection started once a simulation reached a steady state. At each time step we recorded the filament density for each state, the node density, and the LP boundary density. The boundary is defined as the location of the subunit in each 1D filament of a 2D or 3D network that is farthest from the wall, and the boundary density is simply the spatial probability distribution of boundary sections. Simulations were run until the calculated boundary density converged to a smooth curve.

3. Analytical Theory

Here we present simple mathematical models that describe the filament distribution, size, and shape of a LP. We begin with the 1D theory in order to emphasize the profound difference between 1D and higher dimensional networks.

3.1. One Dimensional Theory

3.1.1. Severing without annealing—The filament and boundary distributions for a 1D filament undergoing severing can be obtained analytically [22,23]. Here we will summarize the major results for models with multiple states, which we will use below. Consider a 1D filament with a constant polymerization velocity, v , where segments can exist in one of m states, with the transition between states i and $i + 1$ characterized by a transition rate, r_i . Assume that the m th state can be severed with a severing rate $r_s \equiv r_m$. Then the 1D filament density is given by [23]

$$c_{\text{tot}}(x) = \exp \left(-\frac{r_m}{v} \int_0^x ds \int_0^s du P(u) \right), \quad (1)$$

where $P(u) = S_m(u)/\delta$ is the probability per subunit of severing at position u , and $S_m(u)$ is given by equation (10) below. (Note that, here and below, all filament densities are given as dimensionless quantities normalized so that $c(0) = 1$.) In the limit that all of the rates are slow compared to the polymerization rate, $r_i\delta/v \ll 1$, $\forall i$, equation (1) becomes

$$c_{\text{tot}}(x) \approx \exp \left(-\frac{K_m}{(m+1)!} \left(\frac{x}{\delta} \right)^{m+1} \right), \quad (2)$$

where $K_m = \prod_{i=1}^m (r_i \delta / v)$. For a single state model (2) reduces to a Gaussian, $c_{\text{tot}}(x) = \exp(-r_s x^2 / 2\delta v)$.

In 1D the filament distribution is simply related to the boundary distribution through $b(x) = -\partial c(x) / \partial x$. With the boundary distribution we can directly calculate the average length of the network,

$$L = \int_0^\infty x b(x) dx, \quad (3)$$

and the sharpness of the boundary, which we quantify with a dimensionless “spread parameter,”

$$\eta = \frac{\sqrt{\sigma^2}}{L}, \quad (4)$$

which is the ratio of the standard deviation of the boundary distribution to the average length of the network. Note that there is an inverse relationship between the η and the sharpness of the network: a network with a more abrupt boundary will give a smaller η than a network that decays gradually. In terms of the boundary density and the network length, we have

$$\eta = \left(\int_0^\infty x^2 b(x) dx - L^2 \right)^{1/2} / L. \quad (5)$$

From (2), we find that

$$L_m = \frac{\delta}{m+1} \Gamma \left(\frac{1}{m+1} \right) \left(\frac{(m+1)!}{K_m} \right)^{\frac{1}{m+1}}, \quad (6)$$

and

$$\eta_m = \sqrt{\frac{4^{\frac{1}{m+1}} (m+1) \Gamma(1/(m+1)+1/2)}{\sqrt{\pi} \Gamma(1/(m+1))}} - 1 \quad (7)$$

where $\Gamma(x)$ is the gamma function. Notice that η depends only on the number of states in the system and is independent of the transition and severing rates. Equations (2) and (6) show that a 1D system with many states is equivalent to a 1D system with one state, with an effective severing rate proportional to the geometric mean of the m rates.

3.1.2. Severing with annealing—In 1D new filament sections can only anneal at the pointed end and in this case annealing is equivalent to pointed end polymerization. In a single state model, the effect of annealing is simply to increase the polymerization velocity from v to $v + r_a \delta$. In a multistate model, annealing will effectively increase the polymerization velocity and will create a collection of “younger” filament sections near the boundary of the filament.

However, because a single severing event removes all filament sections farther from the wall, in steady state this collection of younger segments never grows very large. Thus, the primary effect of annealing in a multistate model is to effectively increase the polymerization velocity.

3.2. Higher Dimensional Theory

3.2.1. Severing without annealing—In 1D, a single severing event is sufficient to remove all of the filament sections that are farther away from the wall. This leads to catastrophic severing events in which a filament section severs at or near the leading edge and thereby removes the entire LP. Such catastrophic severing events are absent in higher dimensions. In higher dimensions it takes many severing events to detach a section of the network, making each individual severing event less significant for network disassembly. This allows us to model the system as a simple set of chemical transitions,

$$S_1 \rightarrow S_2 \rightarrow \cdots \rightarrow S_m \rightarrow E, \quad (8)$$

where S_i represents the i th state and E is an “empty” state, which is just a vacant section in the network. In this model we neglect all of the reverse reaction rates. For the transitions between chemical states, irreversibility is rigorously justified [30,10]. More generally, the constant flux from S_1 to E consists of a series of reactions that are all strongly driven forward, and this allows us to ignore any backward reactions.

Assuming a constant polymerization velocity allows us to relate the spatial and temporal coordinates through $x = vt$. Using this to rewrite time derivatives in terms of spatial derivatives, the reaction (8) is described by the system of equations

$$\begin{aligned} v \frac{\partial S_1}{\partial x} &= -r_1 S_1 \\ v \frac{\partial S_2}{\partial x} &= r_1 S_1 - r_2 S_2 \\ &\vdots \\ v \frac{\partial S_m}{\partial x} &= r_{m-1} S_{m-1} - r_m S_m \\ v \frac{\partial E}{\partial x} &= r_m S_m \end{aligned} \quad (9)$$

with the boundary condition $S_1(0) = 1$, $S_i(0) = 0$ for $i \neq 1$, and normalized so that $E(x) = 1 - \sum_i S_i(x)$. The boundary conditions ensure that all newly added filament sections are in the first state. These equations can be solved exactly, with the solutions

$$S_n(x) = \left(\prod_{i=1}^{n-1} r_i \right) \sum_{k=1}^n \frac{\exp(-r_k x/v)}{\prod_{j \neq k}^n (r_j - r_k)}, \quad (10)$$

for $n = 1 \dots m$, and

$$E(x) = 1 - \sum_{k=1}^m \frac{\prod_{i \neq k}^m r_i}{\prod_{j \neq k}^m (r_j - r_k)} e^{-r_k x/v}. \quad (11)$$

We will sometimes be interested in the limit where all of the rates are the same, in which case (11) reduces to

$$E(x) = 1 - e^{-rx/v} \sum_{k=0}^{m-1} \frac{1}{k!} \left(\frac{rx}{v}\right)^k. \quad (12)$$

We now make the assumption that the boundary probability density is related to the density of the empty state E through $b(x) = \partial E(x)/\partial x$. This relationship is exact in 1D and should be a very good approximation in higher dimensions. With this assumption the average length of the multi-state network is given by (3), which can be solved using (11) to give

$$L_m = v \sum_{k=1}^m \frac{1}{r_k}. \quad (13)$$

This equation is easy to interpret. The average lifetime of state i is $\tau_i = 1/r_i$, so equation (13) says that the average length of the network is simply the polymerization velocity times the average time from polymerization to severing. When all of the rates are equal this becomes $L_m = vm/r$, which shows that the network size scales linearly with the number of states. Equation (13) motivates us to define an effective severing rate proportional to the harmonic mean,

$$\frac{1}{r_{\text{eff}}} = \sum_i \frac{1}{r_i}, \quad (14)$$

and predicts that systems with the same effective severing rate will have similar properties regardless of their number of states and the individual rates. This result should be contrasted with the 1D result, where the effective severing rate was instead related to the geometric mean of the rates.

The spread parameter of this model can be calculated using equations (5) and (11), and is given by

$$\eta = r_{\text{eff}} \sqrt{\sum_{k=1}^m \frac{1}{r_k^2}}. \quad (15)$$

Notice that if one of the rates becomes much slower than the others, then that rate dominates r_{eff} and the sum in (15), and in this case η rises to a constant value, independent of the other rates.

3.2.2. Severing with annealing—In higher dimensions with annealing included, we again model the multi-state network with a simple kinetic theory, but now allow the empty state to transition to the first state with an annealing rate r_a . The chemical equations are

and describe a system cycling through several different states. With annealing included, the rate equations become

$$\begin{aligned}
v \frac{\partial S_1}{\partial x} &= r_a f(E) - r_1 S_1 \\
v \frac{\partial S_2}{\partial x} &= r_1 S_1 - r_2 S_2 \\
&\vdots \\
v \frac{\partial S_m}{\partial x} &= r_{m-1} S_{m-1} - r_m S_m \\
v \frac{\partial E}{\partial x} &= r_m S_m - r_a f(E),
\end{aligned} \tag{17}$$

where $f(E)$ is a function to be specified. If the network is completely disassembled, then there are no nodes to serve as nucleation sites for new segments and the annealing term must vanish. Conversely, if there are no empty sites in the lattice, then there are no places for new pieces to anneal and again the annealing term must vanish. These conditions force the function $f(E)$ to satisfy $f(0) = f(1) = 0$. The simplest function that satisfies these conditions is a parabola, $f(E) = E(1 - E)$, and we will use this form to model annealing.

There is no closed form solution to the model above, but the above analysis of equations (9) suggests that the network properties may be controlled by just two parameters, the effective severing rate as defined in (14), and the annealing rate. The system defined by equations (17) always has a fixed point at $E = 1$, $S_i = 0$, $\forall i$. By studying the stability of this fixed point, we have found that this model predicts a transition to an infinite network when

$$\beta r_a > r_{\text{eff}}, \tag{18}$$

where $\beta = |f'(1)|$, and $\beta = 1$ in the model discussed above. Equation (18) predicts that the critical annealing rate depends only on the effective severing rate and not on the number of states in the model.

4. Simulation Results

4.1. Severing without Annealing

Figure 2 shows a snapshot of a simulation of a 2D LP with three states, with the first state in red, the second state in blue, and the third state in green. The simulation methods were described in Sec. 2. The region near the wall, where segments are added, is dominated by segments in the first state. Segments that transition to the third state can sever, and older sections of the network are riddled with holes where nodes have been removed. However, notice that there are still a significant number of segments in the first and second state even at the boundary of the network. It is evident that only some fraction of the network has to convert to the third state before the network can completely disassemble.

Figure 3 shows the filament density, $c(x)$, and the boundary density, $b(x)$, for a system with three states in 1D, 2D, and 3D. In these systems the transition rates and the severing rate are all equal, $r_1 = r_2 = r_s = 0.02 \text{ s}^{-1}$. Figures 3(a–c) show the filament state densities and the total F-actin density for a 1D (a), 2D (b), and 3D (c) network. The points are the results of the numerical simulation, and the curves are the analytic solutions given by equations (1) and (10). In 1D, the analytic and numerical results are in excellent agreement. For the 2D and 3D network, the filament densities near the wall decrease or increase in a simple exponential fashion, in agreement with the predictions of the simple kinetic theory. However, near the edge of the network the cooperative nature of severing becomes apparent and the network decays more rapidly than exponential.

The primary difference between the 2D and 3D network is how long they follow the simple exponentials predicted by (10): the 3D network follows these curves out to a larger distance

before dropping off more rapidly. Our simulation results suggest that the actual filament density begins to decrease more rapidly than the analytic prediction when the total filament density, c_{tot} , is equal to a critical “breakoff” density, c_B . To determine the breakoff density, we write $c_{\text{tot}}(x) = c_a(x)n(x)$, where $c_a(x)$ is the analytic result and $n(x)$ is a step-like function that characterizes the cooperative nature of severing. We determined the breakoff point, x_B , from our simulations by finding the location where $-n''(x)$ attained half its maximum value. From x_B we obtain $c_B = c_{\text{tot}}(x_B)$. Histograms of c_B showed a sharp peak near $c_B = 0.51$ for 2D lattices and $c_B = 0.28$ for 3D lattices (data not shown). These are very close to the bond percolation thresholds for the respective lattices ($1/2$ for the 2D square lattice, 0.2488 for the 3D cubic lattice). Thus, we find that the stochastic results follow the analytic results until the *local* filament density is close to the bond percolation threshold for the lattice, at which point the filament density decreases rapidly to zero and the network falls apart. A theory based on this fact would allow for accurate calculations of network lengths, but since the focus here is in using our coarse grained theory to understand scaling relationships, such calculations will not be presented here. (The discrepancy between c_B and the bond percolation threshold in 3D arises because a stack of five 2D lattices is not large enough to accurately model with an infinite 3D lattice. With ten 2D layers we find $c_B \approx 0.25$, in much better agreement with the bond percolation threshold.)

Figure 3(d) shows the network boundary densities for the 1D, 2D, and 3D network. It is evident that the network size increases significantly as we go from 1D to 2D, and increases further for the 3D network. The boundary distribution, which is a direct measure of the sharpness of the lamellipodium boundary, narrows as the dimensionality is increased. These effects were noted in [26], and are direct manifestations of the cooperative nature of the severing events that lead to disassembly.

In figure 4 we plot L and η as a function of $1/r_s$ for a network with three states, where $r_1 = r_2 = 0.02 \text{ s}^{-1}$ are held fixed and $r_3 = r_s$. Results are shown for a 1D, 2D, and 3D network. The length of the network increases dramatically as the dimensionality is increased from 1 to 2, and increases a little further in 3D. With these parameters, (6) predicts that the 1D network length is given by $L_3 = 0.528 \mu\text{m} (1/r_3)^{1/4}$. This curve is plotted in figure 4(a), and the agreement with the simulation results is seen to be excellent. We do not expect the simple theory for higher dimensions to give exact quantitative results, but (13) predicts that in 2D and 3D we will have $L_3 = A + B/r_s$ for some constants A and B , and indeed the simulations give a linear scaling with $1/r_s$.

Figure 4(b) shows that the spread parameter of a 2D network is about 70% smaller than the spread parameter of a 1D network, and the parameter decreases another 50% for a 3D network. For the 1D network, (7) predicts that η is independent of the rates, and for three states (7) gives $\eta = 0.281$. This value is plotted as a horizontal dashed line in the figure, and we see that this is an excellent approximation to the 1D results. For 2D and 3D networks, we find that η decreases as $1/r_s$ increases, and that for large $1/r_s$, η is nearly constant. The constancy at large $1/r_s$ is consistent with the result of the kinetic theory, equation (15). However, the kinetic theory incorrectly predicts that η increases with increasing $1/r_s$. Furthermore, the kinetic theory gives an asymptotic value for η that is about an order of magnitude larger than that found in the simulations. The analytic solutions of the kinetic theory have long exponential tails (see figure 3) that dramatically increase the spread of the boundary density over that seen in the simulations, and cause the order of magnitude error in the analytic expression for η .

In figure 5 we plot L and η as a function of state number for systems where all of the rates are identical, $r_i = 0.02 \text{ s}^{-1}$, $\forall i$. We show results for up to ten states to demonstrate the trend and clarify the dependence of η on the number of states. Equation (13) predicts that $L \propto m$ for higher dimensions, and in fact this scaling relation holds to high accuracy. Equation (13)

predicts that the slope of these lines should be $v/r_s = 1.5\mu\text{m}$. Remarkably, the slope of the 2D curve agrees with this value to better than 2%. The slope of the 3D curve, $\sim 1.7\mu\text{m}$, is only slightly larger than the predicted value.

Figure 5(b) shows that the sharpness of the LP boundary increases as either the number of states increases or as the dimensionality is increased. When all of the rates are equal, (15) predicts that $\eta \propto 1/\sqrt{m}$. A log-log plot (not shown) reveals that the decay is algebraic but is slightly faster than that predicted by the kinetic theory, $\eta \propto 1/m^{0.72}$.

The strongest prediction of the kinetic theory is that the length of the network only depends on r_{eff} . Thus, the length does not depend in an explicit manner on the number of states in the system, nor is there a different dependence on the severing rate compared to the transition rates. Furthermore, the theory predicts that the size of the network scales as $1/r_{\text{eff}}$. To test these predictions, we simulated many networks with 1 to 4 states and with many combinations of rates, but always keeping r_{eff} fixed. (The combinations of rates were picked to adequately sample the hypersurface defined by (14) with r_{eff} held fixed. In general, the individual rates varied over about two orders of magnitude, from $\sim 0.001\text{ s}^{-1}$ to $\sim 0.1\text{ s}^{-1}$.) The results of these tests are shown in figure 6, where we plot the average length and the spread parameter for all of the combinations tested as a function of $1/r_{\text{eff}}$. In these plots the data from 2D networks is shown as circles and the data from 3D networks is shown as diamonds, and in the insets we have expanded the data sets for $r_{\text{eff}} = 0.01\text{ s}^{-1}$ to show the number of data points used at each value of r_{eff} . Figure 6(a) shows that the lengths of the 2D and 3D networks scale linearly with $1/r_{\text{eff}}$, in agreement with the kinetic theory. For each r_{eff} , the lengths of the 2D networks vary by 20 – 30%, while the lengths for the 3D networks vary by 3 – 7%. These variations arise because of the discrete nature of the simulations, a feature which is not described by the continuum kinetic theory, which predicts a vanishing variation in the lengths. The variation of the 3D network lengths is much smaller than the variation of the 2D network lengths. The better agreement between the kinetic theory and the 3D networks compared to 2D networks can be understood as follows: the kinetic theory is based on the assumption that individual severing events are unimportant to the overall network structure. This assumption is more valid in 3D networks, where the 6-fold coordination makes each individual severing event less important than in the 4-fold coordinated 2D network. Still, the 20 – 30% variation of the lengths of 2D networks is insignificant compared to the two orders of magnitude variation in the individual rates, and overall the results of these tests are in excellent agreement with the predictions of the kinetic theory.

Figure 6(b) shows the spread parameter for the same networks considered in figure 6(a). For a fixed r_{eff} , the spread parameter varies by 70 – 80% for 2D networks, and by 40 – 60% for 3D networks. The kinetic theory gives an expression for η , (15), that does not predict a simple scaling with r_{eff} .

4.2. With Annealing

In figures 7(a–b) we plot the filament state densities and total F-actin density for 2D and 3D networks with three states with annealing included, and compare the results to the same networks without annealing. The black points are the simulation results with annealing, and the dashed lines are the simulation results for an equivalent network without annealing. The severing and transition rates are all equal, $r_1 = r_2 = r_3 = 0.02\text{ s}^{-1}$. The annealing rate is $r_a = 0.005\text{ s}^{-1}$ for the 2D network and $r_a = 0.002\text{ s}^{-1}$ for the 3D network. These annealing rates are slow (in the sense that they are much slower than the critical annealing rate, discussed below), but large enough to easily visualize the qualitative effects of annealing. The solid curves are the solutions to the kinetic theory in equations (17) with $f(E) = E(1 - E)$. For the slow annealing rates considered here the solutions to (17) are in excellent agreement with the simulation results near the wall. At some distance away from the wall, the simulation results drop abruptly to

zero, whereas the solutions to (17) have long exponential tails. This is the same behavior seen in the networks without annealing discussed in Sec. 4.1.

Figure 7(c) shows the boundary density for the 2D and 3D networks both with and without annealing. It is evident that the network size increases when annealing is included, as expected. The sharp edge of the network is broadened when annealing is turned on. This broadening is partly due to the new growth that occurs close to the boundary of the network.

Figure 8 shows the average length for various one state 2D and 3D networks as a function of the ratio of the annealing rate to the severing rate. Here we see that when the length is scaled by L_0 , the length of an equivalent network in the absence of annealing, the data points collapse onto two curves, one for 2D networks and another for 3D networks. The effect of annealing is much stronger in 3D, in the sense that at equivalent annealing rates, the fractional change in the size of a 3D network is much greater than the fractional change of a 2D network. This effect can be understood by considering that more severing events are required to disassemble the 3D network than the 2D network. Thus, vacant sections remain in the network longer and have more opportunity to anneal, effectively increasing the annealing rate for the entire network.

Lastly, notice that the length of the network appears to diverge at some critical annealing rate, $r_{a,crit}$. Simulations show that above the critical annealing rate the network length diverges and the filament density asymptotically approaches some constant value determined by the ratio of the annealing rate to the severing rate. The critical annealing rate in 3D is substantially less than the critical annealing rate in 2D for the reasons discussed in the previous paragraph.

We asked if, in the presence of annealing, the network properties will depend only on r_{eff} , and not on the number of states or any of the individual transition and severing events. In figure 9 we plot the average length and the spread parameter for several 2D and 3D networks as a function of r_a/r_{eff} . The number of states varied from one to four, and for each number of states and each value of r_{eff} we measured data for two different networks with widely different values of the transition and severing rates. (In general the individual rates varied by about an order of magnitude, but no attempt was made to completely sample the hypersurface of rates defined by a fixed r_{eff}). The agreement between the different curves is qualitative but not quantitative; the length and the spread parameter each increase as r_a is increased, and the networks appear to diverge at a critical annealing rate that depends on dimensionality. The variation in the lengths is about 20 – 30% for 2D networks and about 10 – 40% for 3D networks. The variation in spread parameter is 40 – 50% for 2D networks and 10 – 40% for 3D networks. These variations are small enough such that the ratio r_a/r_{eff} serves as a useful characterization of the network.

The kinetic theory does predict that the critical annealing rate should depend only on r_{eff} , and we tested this prediction by examining the behavior of the networks as the critical annealing rate was approached from both above and below. Figure 10 shows upper and lower bounds for the critical annealing rate for 2D and 3D networks with different numbers of states. We see that the ratio $r_{a,crit}/r_{eff}$ is nearly independent of r_{eff} and the number of states, as predicted by the simple kinetic theory.

5. Discussion

We have made a number of assumptions and simplifications in developing this coarse grained model of a LP. Here we discuss the validity of these assumptions and how the properties of the network differ with different assumptions. We also discuss the biological implications of the results of our model.

5.1. Model Assumptions

5.1.1. No disorder at leading edge—The networks studied here are created as perfect crystalline lattices at the wall and so lack any disorder. In any real network there will be a certain amount of disorder induced by the stochastic nature of polymerization, branching, and crosslinking. To study the effects of disorder in the network we randomly removed a certain fraction of the new segments at the wall at each time step, which imparted a controllable amount of disorder to the network. We find that the results of this paper are fairly robust against the presence of disorder for networks both with and without annealing. At a fixed amount of disorder, network length still scales inversely with r_{eff} , and η is nearly constant for small r_s with the other rates fixed (data not shown). For one state at 15% disorder, a plot of L vs. $1/r_{\text{eff}}$ can be fit to a linear curve with $R^2 = 0.9997$ (compare $R^2 = 0.9999$ without disorder). For multiple states, plots of L vs. r_{eff} yield variations in the length similar to those observed in figure 6. There are no obvious qualitative changes in the shape or structure of the network at low to medium disorder (< 15% of links removed).

5.1.2. Cubic Lattice—In this paper we used a simple cubic lattice to model the growing LP, which is a gross simplification to the complicated meshwork found at the leading edge of cells. However, the results of Sec. 4.1 suggest a universal, lattice dependent behavior: the total filament density will follow the analytic theory until the density reaches the bond percolation threshold for the lattice, at which point the cooperative nature of severing reduces the filament density abruptly to zero. To investigate this suggestion, we modeled the LP with a hexagonal lattice and a honeycomb lattice in 2D, and with a body centered cubic (BCC) lattice and a diamond lattice in 3D. The breakoff densities for each lattice were calculated as outlined in Sec. 4.1, and in all cases the breakoff densities agreed with the bond percolation threshold for the lattice (data not shown). Bond percolation thresholds are known for many 2D and 3D lattices, including random lattices and quasi-periodic lattices. If the LP was modeled with a more realistic lattice structure than that considered here, the size of the LP could be calculated with the analytic theory presented here and the appropriate bond percolation threshold. Importantly, the effective severing rate and the scaling relationships derived from the analytic theory are independent of the lattice.

5.1.3. Crosslinks Inhibit Depolymerization—Our model is based on the assumption that the filaments in an LP can be treated as a collection of links separated by crosslink locations (nodes), and that these links can be removed without affecting the state of neighboring filament sections. If link removal consists of a severing event followed by depolymerization, the assumption of independent state changes is equivalent to the assumption that crosslinks inhibit depolymerization. This assumption has a sound theoretical basis: the crosslinked subunits have more binding partners and are thus more tightly bound to the network. This assumption is further supported by recent experimental work on several different actin crosslinkers, which showed that actin crosslinking proteins do indeed inhibit depolymerization [31].

5.1.4. Size of the Time Step—The data presented in this paper were obtained from simulations with a time step of $\Delta t = \delta/v \approx 1.3$ s. As mentioned in section 2.1, this time step is sufficiently small to capture the stochastic nature of our simulations. We have run simulations with the time step reduced by a factor of a hundred and found that the results are relatively insensitive to the size of the time step. For the fastest rates considered here, the network size increases by about 10%, while for most rates the network size increases by less than 2%. These variations do not affect our general conclusions about the scaling of network properties with the microscopic rates.

5.1.5. Mechanism of Annealing—The results of our simulations depend on whether annealing occurs before or after the removal of disconnected pieces during each time step. If

annealing occurs before removal we will occasionally rescue large sections of the network that would have otherwise been removed, and this will prolong the network lifetime. Thus, simulations where we anneal before removing disconnected pieces will give larger networks than those where we remove disconnected pieces first. However, qualitative features of the network, such as the existence of a critical annealing rate, are not affected by this choice.

Ultimately, the correct option depends on the relative rates of annealing and polymerization compared to the diffusion rate of small sections of the network. Here we have assumed that a new section of the network can grow a length $\delta = 0.04 \mu\text{m}$ in $\sim 1.3\text{s}$. The diffusion constant of an actin monomer in solution is $D \approx 30 \mu\text{m}^2/\text{s}$ [28], although in the crowded environment of the cell this may be as low as $D \approx 5 \mu\text{m}^2/\text{s}$ [32]. Here we will use this smaller value, which will give us lower bounds on the diffusion rate of filament sections. The diffusion constant of a filament scales inversely with the length of the filament [33]. The filament sections here contain about 15 subunits, which allows us to estimate the diffusion constant of a filament as $D_{\text{fil}} \approx 0.3 \mu\text{m}^2/\text{s}$. In the time step considered here, the filament section travels a distance

$\sqrt{\langle r^2 \rangle} = 6D_{\text{fil}}\Delta t \approx 1.5 \mu\text{m}$. This distance is almost 40 times the mesh size. Therefore, it is usually the case that a disconnected section of the network will diffuse away much more rapidly than it can be annealed to the network. This justifies the approach used in the text, where we removed disconnected sections before allowing annealing.

5.1.6. State of Annealed Filaments—For simplicity, we have assumed that all annealed filaments are in the first state. Because “annealing” here was meant to include new polymerization away from the leading edge, this assumption seems justified. More generally, we could assume that a new filament segment has probability P_1 of adding in the first state, P_2 of adding in the second state, etc. On average, newly added filaments require fewer state changes before severing, and this leads to smaller networks. In this case there is an additional term $+P_i r_{af}(E)$ in each line of (17), it can be shown that the network length diverges when

$$\beta r_a > \left(\sum_{i=1}^m \sum_{j=1}^i \frac{P_j}{r_i} \right)^{-1} \equiv r'_{\text{eff}}, \quad (19)$$

where r'_{eff} is a probability dependent effective severing rate, and $r'_{\text{eff}} = r_{\text{eff}}$ when $P_1 = 1$. Plots of the network length and spread parameter as a function of r_a/r'_{eff} show variations similar to those seen in figure 9 (data not shown). Thus, we find that with any set of P_i 's our major conclusions still hold, namely, that a two parameter model accurately captures the effects of annealing on network structure, and that there exists a critical annealing rate above which the network length diverges.

5.2. Biological Implications

To apply the model developed here to physical situations, it is necessary to make some assumptions about how the various rates scale with protein concentrations. It is generally accepted that the polymerization velocity is directly proportional to the actin monomer concentration, $v \propto [A]$. In this case, equation (13) predicts that the LP width is directly proportional to the monomer concentration, $L \propto [A]$. Although this result makes intuitive sense, several theories, notably those based on 1D models, have found different scaling relationships. In particular, [23] finds $L \propto [A]^{5/6}$.

It is well established that ADF/cofilin can sever actin filaments [13], accelerate P_i release from an ADP- P_i subunit [13], and debranch Arp2/3 induced branches [34]. In *in vitro* experiments,

the severing activity of ADF/cofilin has a non-monotonic dependence on ADF/cofilin concentration, peaking at low concentrations (~ 10 nM) and vanishing at higher concentrations [35,36]. The P_i release rate and debranching rate increase linearly with ADF/cofilin concentration at low concentrations, and saturate at high concentrations, about $5 \mu\text{M}$ for debranching [34] and about $50 \mu\text{M}$ for P_i release [13]. In our model there is little difference between a debranching event and a severing event (both create an uncapped filament that then depolymerizes to the next crosslink). Thus, for low ADF/cofilin concentrations both the P_i release rate and the severing rate increase linearly with $[\text{ADF/cofilin}]$, and our model predicts that the size of the network scales as $L \propto C_0 + C_1/[\text{ADF/cofilin}]$. This implies that the length becomes nearly independent of ADF/cofilin concentration before the ADF/cofilin activities themselves saturate. In [37], it was found that the lengths of *Listeria* actin comet tails (a model system for an LP) were independent of ADF/cofilin concentration for $[\text{ADF/cofilin}] > 5 \mu\text{M}$, a result that is consistent with the predictions of our theory.

6. Conclusion

In this paper we have studied the combined effects of filament aging and annealing in a coarse grained model of a lamellipodium undergoing disassembly via severing. We have emphasized the dramatic difference between the effects of severing in 1D models compared to models in higher dimensions.

The most important finding of this paper is that, to an excellent approximation, the properties of 2D and 3D networks with many states and different transition and severing rates can be described by a single effective severing rate, proportional to the harmonic mean of the transition and severing rates. By contrast, the 1D theory predicts an effective severing rate given by the geometric mean of the rates. The fact that the networks can be described by an effective severing rate shows that the severing rate is no more important than any of the other transition rates in determining the size of the network, and shows that the network properties do not depend explicitly on the number of states in the model. This is a useful finding because the number of states changes needed before severing occurs in the cell is currently the subject of much investigation and may not be well defined. We found that the size of the network scales inversely with r_{eff} , and that the spread of the boundary decreases algebraically with r_{eff} .

When annealing is included we find that the network properties are still reasonably described by a single effective severing rate. The effects of annealing are more pronounced in 3D compared to 2D, with the same ratio of r_a/r_{eff} giving much larger networks in 3D. We found that the critical annealing rate, the rate at which the network size diverges, does depend only on r_{eff} . This is somewhat surprising, as it is not obvious that the critical annealing rate should depend as strongly on transition rates between middle states as it does on the severing rate of the final state.

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Figure 1.

A schematic of the growing actin network illustrating the changes that can occur during each time step. The figure on the left shows a section of the network at step n , and the figure on the right shows the same network at step $n + 1$. This schematic corresponds to a network with three states, where red segments are in the first state, blue segments are in the second state, and green segments are in the third state. A new row of filament segments are added at the wall at each time step. Here, segments changed state at the locations marked (a), (b), (c), and (d). Severing events occurred at the locations marked (e) and (f), and annealing events occurred at the locations marked (g) and (h). See the text for more discussion.

Figure 2.

A snapshot of a 2D actin network for a system with three states. The wall is located at the bottom of the figure and the network is growing upwards. State 1 is shown in red, state 2 is shown in blue, and state 3 is shown in green. The transition and severing rates are all equal, $r_1 = r_2 = r_s = 0.02 \text{ s}^{-1}$, and there is no annealing, $r_a = 0$.

Figure 3.

The filament and boundary densities for a 1D, 2D and 3D network with three states. (a–c) The filament densities for the individual states and the total amount of F-actin for a 1D (a), 2D (b), and 3D (c) network. The black points are numerical results from the simulation. The solid curves are the analytic solutions given by equation (10) corresponding to state 1 (red), state 2 (green), and state 3 (blue). (d) Simulation results for the boundary density of the 1D, 2D, and 3D networks in (a–c). For all networks the transition and severing rates are the same, $r_1 = r_2 = r_s = 0.02 \text{ s}^{-1}$, and there is no annealing, $r_a = 0$.

Figure 4.

The average length and the spread parameter for networks with three states, plotted versus $1/r_s$ with $r_1 = r_2 = 0.02 \text{ s}^{-1}$ held fixed. (a) The average length of a 1D, 2D, and 3D network. The points are the simulation results. The curve through the 1D data is the analytical solution given by equation (6). The 2D and 3D data points are well fit by linear curves, as predicted by equation (13). (b) The spread parameter for a 1D, 2D, and 3D network. The dashed line in the figure is the analytical prediction of the 1D theory, given by equation (7).

Figure 5.

The average length (a) and the spread parameter (b) for an LP as the number of states is varied in a 1D, 2D, and 3D network. The network length has a linear dependence on the number of states, as predicted by equation (13). Here all of the rates are equal, $r_i = 0.02 \text{ s}^{-1}$.

Figure 6.

The average length (a) and spread parameter (b) for various 1D, 2D, and 3D networks plotted against $1/r_{\text{eff}}$. Data for several networks with different numbers of states are shown at each value of r_{eff} . Each network had a different combination of rates but with the same value of r_{eff} .

Figure 7.

The filament state densities, total F-actin density, and boundary densities for three state 2D and 3D networks with annealing. (a–b) The filament state densities and total F-actin density for a 2D (a) and 3D (b) network. The black points are the simulation results. For comparison, the simulation results for an equivalent network without annealing are shown as dashed lines. The solid curves are the numerical solutions to equation (17), with $f(E) = E(1 - E)$, for filaments in state 1 (red), state 2 (green), state 3 (blue), and the total F-actin density (black). (c) The boundary density for the networks shown in (a) and (b). The boundary densities are shown for the networks with annealing (points) and without annealing (dashed line) for comparison. The

transition and severing rates are equal, $r_1 = r_2 = r_s = 0.02 \text{ s}^{-1}$. The annealing rate are $r_a = 0.005 \text{ s}^{-1}$ for the 2D network and $r_a = 0.002 \text{ s}^{-1}$ for the 3D network.

Figure 8.

The average length of 2D and 3D networks with one state as a function of the ratio of the annealing rate to the severing rate. Circles represent 2D networks and squares represent 3D networks. The different colors correspond to different severing rates: 0.06 s^{-1} (red), 0.04 s^{-1} (blue), 0.02 s^{-1} (green), and 0.01 s^{-1} (black). Although the lengths of these networks vary greatly, the data all collapse onto two curves when scaled by the size of the network without annealing.

Figure 9.

The average length and spread parameter for several 2D and 3D networks as a function of r_a/r_{eff} . The symbols correspond to the network conditions as in figure 6.

Figure 10.

Bounds on the critical annealing rates for several values of r_{eff} and networks with one to four states. 2D networks always have a larger critical annealing rate than 3D networks with the same r_{eff} . The possible ranges for networks with different numbers of states are shown in different colors: red (one state), blue (two states), green (three states), and black (four states). The bars represent the range of possible values for $r_{a,crit}$ obtained by studying very large networks near the critical annealing rate.