Disassembly of actin networks by filament severing

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Abstract. The disassembly of actin networks by filament severing is studied by simulation of a model in which the actin filaments are crosslinked in a periodic network and begin to sever as soon as they are created at the cell membrane. The actin network is defined as the set of all sites which have a connecting path leading to the membrane. An extension of this model including annealing effects, corresponding to restoration of links between nodes, is studied as well. The simulations show that in the presence of only severing, the network density drops abruptly at a critical distance from the network, which is inversely proportional to the severing rate. This result is explained by a mean-field theory based on a layer-by-layer approach in which the average size of an in-layer connected cluster is used to calculate the probability of a given site having a connected path to the membrane, via the next layer in. Inclusion of annealing effects leads to a broadening of the attached portion of the network. At a critical value of the annealing rate, the network density develops a component that does not decay away from the membrane.
1. Introduction

The polymerization of the intracellular protein actin is a key step in most cellular processes involving cell migration. The polymerization exerts protrusive forces which can propel the cell or help it sample its environment. The polymerized actin is often in the form of a dendritic network attached to the cell membrane. The filaments in this network are connected by both branch points and crosslinking proteins that join filament sides at random points. A substantial amount of theoretical effort, as reviewed in [1], has been devoted over the past 15 years to understanding the physical mechanisms by which polymerization of actin generates protrusive force. This analysis has demonstrated several mechanisms by which either active, i.e. coupled to hydrolysis of ATP (adenosine triphosphate), or passive polymerization of individual filaments can convert the chemical energy of polymerization into protrusive forces on the order of piconewtons per filament tip. The combined effects of large numbers of filaments pushing on the cell membranes have also been studied by a variety of models, including both those based on continuum elasticity [2]–[4] and those based on explicit growth of actin networks using stochastic-growth algorithms [5, 6]. These studies have demonstrated the importance of the elasticity of the actin network, and the dependence of the number of pushing filaments on opposing force.

Disassembly of this intracellular actin network is equally crucial for cell migration. Without such disassembly, nearly all of the actin in a cell would polymerize and there would be little free actin left to drive cellular protrusion in response to external stimuli. However, there have been few systematic studies of actin network disassembly comparable to the studies of protrusion. There have been several studies of the effects of severing on bulk solutions of actin [7]–[11]. The filament-length distribution of free filaments in a lamellipod has also been treated, using rate equations based on the population of filaments as a function of length and distance from the leading edge [12]. However, in most continuum treatments of protrusion and cell migration (see, for example, [13, 14]), disassembly has been treated via a simple lifetime term based on first-order kinetics, which causes the density of F-actin to decay exponentially as a function of time or distance from the membrane. Experimental studies based on actin speckle dynamics [15] suggest that at least two time-scales are involved in actin network disassembly. The first is the time that a typical actin subunit remains in the network, which was measured to be about 25 s. The second is the time required for a continuous mass of actin moving in from the leading (outer) edge of the cell to reach the inner edge of the lamellipodium and disassemble, which
I find to be about 200 s from the quoted retrograde flow speed of $1.5 \mu\text{m min}^{-1}$ \cite{15} and a visually estimated lamellipodium width of 5 $\mu\text{m}$. At present, there is no systematic study which relates the F-actin profile as a function of distance from the membrane, or the average filament lifetime, to the both mechanism of disassembly and the structure of the actin network. As a result, some very basic questions about the disassembly of actin networks remain unanswered, such as the nature of the competition between severing and annealing in network disassembly.

In this paper, I present an analysis of the combined effects of severing and annealing on actin network disassembly, based on a very simple geometric model of the actin network in which the filaments are arranged in a square or simple cubic geometry. Such a geometry is highly idealized, but the model is sufficiently rich to allow one to establish the connection between the severing and annealing rates on the one hand, and the lamellipodial actin density profile on the other hand. I treat this model via both simulation and analytic theory. I show that the lamellipodial density profile does not have the simple exponential decay obtained by simple first-order kinetic equations. In the presence of only severing, the density drops rapidly at a critical distance from the leading edge. When annealing is included, the drop is less abrupt but the behavior is still far from an exponential decay. I also find that there is a transition in the lamellipodial shape as a function of the annealing rate. At a critical value of this rate, the lamellipodium density develops a component which does not decay with distance away from the membrane. The organization of rest of the paper is as follows: the methods section describes the model and method of simulation, the results section presents the numerical results and analytic theory and the conclusion section describes the biological consequences.

2. Methods

The actin network is described as a square or cubic lattice of nodes, corresponding to crosslinking or branch points, with nearest neighbors connected by links corresponding to segments of filaments (see figure 1). The lattice grows at a steady growth velocity $v$ away from the membrane, which I take to be the $x$-direction. The lattice constant $\xi$ corresponds to the mesh size of the actin network, defined as the average distance between crosslinks or branch points. The layers in the lattice are denoted by the index $n$. The filament length is not explicitly defined in the model, but we assume that it is much larger than the mesh size. In this case, the time required to disassemble part of the network will be much greater than the time required to sever
a typical filament, and will thus correspond more closely to the time required to sever the length of a filament between the two crosslinks. Severing of filaments corresponds to breaking of one of the links connecting the nodes. It occurs at a rate \( k_{sev} \) per link per unit of time. This definition is used for convenience in the mathematical analysis. Severing rates are often given as rates per unit length per unit time, and such a rate would need to be multiplied by \( \xi \) to obtain the rate that we use in our calculations and simulations. In the simulations, connectivity information for each node is stored in an array that mirrors the topology of the network. The model studied here is closely related to the problem of bond percolation in two or three dimensions. It differs in that the fraction of intact links decreases away from the membrane in the manner described above.

When annealing is absent, severing can be treated in a ‘one-shot’ fashion as follows. After the network has grown long enough to establish a steady state structure in the vicinity of the leading edge, the probability of a given link not being severed is proportional to \( \exp(-k_{sev}A) \), where \( A \) is the age of the link—the time since it was created at the membrane. Thus \( A = x/v \), where \( x \) is the \( x \)-coordinate of the center of the link, and \( x = 0 \) corresponds to the membrane where the filaments are ‘born’. To establish whether or not the link is broken, I evaluate the output of a random number generator on the unit interval. If it is greater than \( \exp(-k_{sev}A) \) the link is severed. Thus severing of different links is assumed to be uncorrelated.

I assume that fragments of the network that are cut off by severing will diffuse away rapidly. Then the parts of the network that remain, and would appear in a bulk fluorescence or speckle microscopy experiment, are those which are connected to the membrane by a path of unsevered links. I will denote these parts of the network as the ‘attached component’. To ascertain which nodes are in the attached component, I begin with a given node at the membrane, and calculate the connected cluster of nodes containing that node. This is done by a recursive C algorithm, which for a given node loops over all of the neighbors connected to that node and calls itself for each neighbor. Each node that is found in this fashion is assigned an integer variable which assigns it to cluster 1. Then this procedure is repeated, starting with the first node along the membrane which does not belong to cluster 1, except that each ‘daughter’ node is now assigned to cluster 2. (I caution the reader that this procedure can lead to deep levels of recursion calls, which may exceed the stack size defined by the C compiler. In this case, the stack size can be increased by using the ‘ulimit -s’ command of Unix. I used a limit of 60 000, measured in kilobytes, in comparison with a typical system limit of 8192.) The attached component then consists of all the clusters which are attached to the membrane. The model assumes that all the filaments at the membrane are attached to it. It is likely that only some of these filaments are attached to the membrane, as assumed, for example, in the ‘tethered–ratchet’ model [16]. This will not affect the results significantly, since the clusters of the connected component typically have a large area of contact with the membrane and thus will in all likelihood contain at least one attachment.

Annealing is included by allowing links that have been severed to heal, with a probability of \( k_{ann} \) per unit time. Only links that are between two nodes of the attached component can be healed. Therefore, the probability of healing at a particular point depends on the history of the other links in the system, and the simple ‘one-shot’ approach does not work. I thus perform a dynamic simulation in which at each time step, the leading edge moves one lattice step of length \( \xi \) in the \(-x\)-direction and severing/annealing events occur with probabilities \( k_{sev}\Delta t \) and \( k_{ann}\Delta t \). These probabilities are much less than unity for the simulation parameters that I have used, justifying the approximation of moving an entire lattice step during each time step. The simulation is run until a steady state of the actin distribution is found.
3. Results

The model is not sufficiently detailed to treat a particular system quantitatively, but for concreteness I choose definite values of the parameters. I use a network velocity $v = 0.03 \, \mu\text{m s}^{-1}$, typical of the upper range of values obtained in [15]. I take a network mesh size $\xi = 0.04 \, \mu\text{m}$, which is a lower limit obtained by using a density of 1 mM for F-actin in the lamellipodium and assuming the filaments to be arranged in a simple cubic array. The mesh size is hard to define precisely, and presumably varies considerably between the cell types. I find that the results are only weakly sensitive to the choice of $\xi$. The values of $k_{\text{sev}}$ and $k_{\text{ann}}$ are treated as variables to explore possible behaviors. Figure 2 shows typical two-dimensional network geometries without (frame a) and with (frame b) annealing. In the absence of annealing,
Figure 3. Scaled actin network density as function of distance \( x \) from leading edge, in severing-only model, with \( k_{\text{sev}} = 0.01 \text{ s}^{-1} \). Solid circles: one-dimensional results. Open circles: two-dimensional results. Squares: three-dimensional results. Solid lines are analytic theory for one, two and three dimensions.

the attached component has a random surface, but the extent of the randomness is quite limited. There are very few internal severed links. In the presence of annealing, the shape of the surface is much rougher, and there are more internal severed links.

The roughness of the surface of the attached component is reflected in the normalized F-actin density \( P(x) \) plotted for the case of no annealing in figure 3 as a function of distance \( x \) from the membrane. This distribution is obtained for each discrete value of \( x \) (a multiple of \( \xi \)) as the fraction of nodes at \( x \) that belong to the attached component. Thus for small \( x \), \( P(x) \) is close to one because almost all of the links are intact near the membrane. The plotted values of \( P(x) \) are obtained by averaging over numerous simulation runs, 1000 for the one-dimensional case and 100 for the two- and three-dimensional cases. In the two-dimensional case, I use a lamellipodium width (parallel to the leading edge) of 100 lattice spacings. In the three-dimensional case, I use a height of 10 lattice spacings. I have performed some calculations with larger values of the height to verify that 10 lattice spacings are sufficient to mimic three-dimensional behavior. The one-dimensional results in the figure correspond to a line of nodes in the \( x \)-direction, and the first break in this line defines the end of the attached component. Thus the contribution from each run \( P(x) \) changes abruptly from one to zero, but the average over many runs has the smooth appearance indicated. For all three cases considered in figure 3, the behavior of \( P(x) \) is different from the simple exponential behavior that one would expect from first-order kinetics. In the one-dimensional case, the dependence, as I shall prove below, is Gaussian (proportional to \( \exp(-\text{const} \times x^2) \)). In the two- and three-dimensional cases, \( P(x) \) is very flat for small \( x \), and then at a crossover value drops rapidly to zero. Visual examination of the two- and three-dimensional cases after scaling of the \( x \)-axis shows that the shapes of the two- and three-dimensional curves are nearly identical. The behavior of \( P(x) \) can be understood via a probabilistic analysis of the network severing statistics. This analysis is exact in one dimension, but requires a mean-field approximation in two and three dimensions.

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3.1. One-dimensional analysis

I define a discrete analog of \( P(x) \), \( P_n \), as the probability that the site (there is only one site in a layer in one dimension) in layer \( n \) away from the membrane is part of the attached component. Then, since severing is uncorrelated by assumption, and the site in layer \( n-1 \) must be in the attached component if the site in layer \( n \) is,

\[
P_n = \exp\left(-\frac{1}{2}k_{\text{sev}}\xi/v\right)P_{n-1}
\]

and

\[
P_n = \prod_{m \leq n} \exp\left[-(m - \frac{1}{2})k_{\text{sev}}\xi/v\right],
\]

where I have used the fact that \( P_0 = 1 \). Then, combining the exponentials on the right-hand side of equation (2), and using the fact that \( \sum_{m \leq n} (m - \frac{1}{2}) = n^2/2 \), I have

\[
P_n = \exp\left(-n^2k_{\text{sev}}\xi/v\right).
\]

As figure 3 shows, the agreement between the simulations and equation (3) is very close.

3.2. Two-dimensional analysis

This analysis follows the same general approach as that of the one-dimensional analysis. But now a site in layer \( n \) can also be connected to the attached component below it by indirect paths, in which one or more lateral steps in layer \( n \) are taken before a step to layer \( n-1 \) is made. To account for this possibility, I define an in-layer cluster size \( S_n \) as the average size of the connected in-layer cluster containing a typical node in the cluster. Figure 4 shows an example with \( S_n = 5 \), where a site at the center of the five-site cluster is indirectly connected to the main cluster. Defining \( p(n) = \exp(-nk_{\text{sev}}\xi/v) \) as the probability that a given link in layer \( n \) has not been severed, one readily shows that if one starts at a certain node in the layer and goes in one direction in the layer, the typical number of nodes included before a broken link is encountered is \( 1/(1 - p(n)) \). An equal number of nodes is encountered going in the other direction. Adding these two contributions, and subtracting unity to avoid doublecounting, I obtain

\[
S_n = \frac{2}{(1 - p(n))} - 1.
\]

Then I assume that the probability of a site in layer \( n \) being in the attached component is \( P_{n-1} \) times the probability that any one of the sites in a cluster of size \( S_n \) is connected to the layer \( n-1 \):

\[
P_n = P_{n-1}\left[1 - (1 - p(n - \frac{1}{2}))^{S_n}\right].
\]
where \( p(n - \frac{1}{2}) \) is defined by obvious analogy with \( p(n) \). Thus
\[
P_n = \prod_{m<n} [1 - (1 - p(m - \frac{1}{2}))^{S_m}] .
\] (6)

Figure 3 shows that this mean-field theory gives reasonably good agreement with the simulation results, underestimating the width by about 10%. The reason that the curve is sharper than in one dimension is that the ratio \( P_m/P_{m-1} \) takes longer to drop appreciably below unity in the two-dimensional case. Even if the severing probability \( 1 - p(m - \frac{1}{2}) \) is appreciable, the exponent \( S_m \) is large enough to make its contribution to \( P_m \) small. For example, if \( p(m - \frac{1}{2}) \) is \( \frac{2}{3} \), \( S_m \) is about 5, and the deviation of \( P_m/P_{m-1} \) from unity is less than 1%. In figure 3, this corresponds to \( x = n\xi = \ln(3/2)v/k_{sev} = (0.4 \times 0.03 \, \mu m \, s^{-1})/0.01 \, s^{-1} = 1.2 \, \mu m \). The difference between the one- and two-dimensional cases may also be understood in terms of the cooperativity in the two-dimensional case. Several bond-breaking events are required to detach a piece of the network, so the detachment probability increases more suddenly as a function of time than in one dimension.

### 3.3. Three-dimensional analysis

Here, there is no exact formula for \( S_n \) (which is now the typical area of a typical cluster in two-dimensional bond percolation [17]). If \( p(n) \) is larger than the bond percolation threshold of 1/2 for a two-dimensional layer, then \( S_n \) is infinite and I assume that \( P_n/P_{n-1} = 1 \). Below the percolation threshold, numerical simulations [18] suggest the asymptotic form
\[
S_n = 0.134[1/2 - p(n)]^{-2.43} .
\] (7)

I have used this form, together with equation (6), to calculate the mean-field approximation to \( P_n \) in three dimensions. As seen in figure 3, the agreement with the numerical results is worse than in the two-dimensional case. The sharpness of the drop as a function of \( x \) is considerably underestimated. This may occur because even if the average two-dimensional cluster size is very large, there are numerous small clusters which will have less chance of being connected to the next layer in, and these are not accounted for in the mean-field theory. These small clusters are also present when \( p(n) \) exceeds the percolation threshold, so that the assumption that \( P_n/P_{n-1} = 1 \) is not completely accurate. In addition, the asymptotic form that I use for \( S_n \) may give a poor description of behavior away from the percolation threshold.

Figure 5(a) shows a corresponding plot with annealing included, in the two-dimensional case. It is seen that the decay of the average density away from the membrane is much smoother, and in fact is quite similar to the Gaussian shape found in the one-dimensional case. The mean-field theory can be extended to treat annealing as follows. We set up the following rate equation for the probability of a given link to be unsevered, where the time evolution is regarded as occurring as the link moves away from the membrane:
\[
\frac{dp}{dt} = -k_{sev} p + k_{ann} [1 - p] .
\] (8)

The form of these two terms is such that only an unbroken link can be severed, and only a severed link can be annealed. Given the boundary condition that \( p(0) = 1 \), the solution to this equation is:
\[
p(t) = [k_{ann} + k_{sev} \exp(-n(k_{sev} + k_{ann})t)]/(k_{sev} + k_{ann}) ,
\]
which when re-expressed in terms of \( n = tv/\xi \), becomes
\[
p(n) = [k_{ann} + k_{sev} \exp(-n(k_{sev} + k_{ann})\xi/v)]/(k_{sev} + k_{ann}) .
\] (9)
Figure 5. Scaled actin network density as function of distance $x$ from leading edge, for severing-and-annealing model in two dimensions. (a) Solid line: simulations for $k_{\text{sev}} = 0.06 \text{ s}^{-1}$ and $k_{\text{ann}} = 0.06 \text{ s}^{-1}$. Dotted line: simulations for $k_{\text{sev}} = 0.06 \text{ s}^{-1}$ and $k_{\text{ann}} = 0$, shown for reference. Dashed line: analytic theory for $k_{\text{sev}} = 0.06 \text{ s}^{-1}$ and $k_{\text{ann}} = 0.06 \text{ s}^{-1}$. (b) $k_{\text{sev}} = 0.06 \text{ s}^{-1}$ and $k_{\text{ann}} = 0.13 \text{ s}^{-1}$ (lower curve) or $k_{\text{ann}} = 0.16 \text{ s}^{-1}$ (upper curve).

This result can be substituted in equations (6) and (7) to calculate $P_n$. The results are shown in figure 5(a). As seen in the figure, the initial part of the curve, which is considerably broader than it would be in the absence of annealing, is obtained reasonably well. However, the large-$x$ tail in the mean-field theory is not seen in the simulation results.

For larger $k_{\text{ann}}$, a transition in the shape of $P(x)$ is found. Above a critical value of $k_{\text{ann}}$, the actin density develops a component that does not decay away from the membrane. For the value $k_{\text{sev}} = 0.06 \text{ s}^{-1}$ used in figure 5, the transition is at about $k_{\text{ann}} = 0.14 \text{ s}^{-1}$. Figure 5(b) shows results for $k_{\text{ann}} = 0.13 \text{ s}^{-1}$ and $k_{\text{ann}} = 0.16 \text{ s}^{-1}$, and the non-decaying component is clearly seen in the latter results. As $k_{\text{ann}} \to \infty$, $P(x)$ goes to unity everywhere. When $k_{\text{ann}}$ increases from below the critical value, $P(x)$ gradually develops a large-$x$ tail, whose slope eventually decreases until it becomes the constant component. This transition is not seen in the mean-field theory.

Figure 6 shows results of a system having a thickness of 0.2 $\mu$m, corresponding to a typical lamellipod, again for $k_{\text{sev}} = 0.06 \text{ s}^{-1}$. Here the transition is at a smaller value than in the two-dimensional case because of the higher connectivity, about $k_{\text{ann}} = 0.062 \text{ s}^{-1}$. The curves showing $P(x)$ just below and above the transition show a behavior similar to that in two dimensions.
For purposes of comparing with potential experiments in which the severing or annealing rates are modified in a cell, for example, by RNAi knockdown, it is of interest to establish how the lamellipodium thickness depends on the severing and annealing rates. Figure 7 shows the average half-thickness of the lamellipodium,

\[ \langle x \rangle = \xi \sum_n n P_n \left/ \sum_n P_n \right. \] (10)

as a function of $1/k_{\text{sev}}$ in the absence of annealing, in the one-, two- and three-dimensional cases. In one dimension, $\langle x \rangle$ is proportional to $1/\sqrt{k_{\text{sev}}}$. This proportionality is obtained straightforwardly from equation (10) if the sum in equation (3) is treated as an integral. This is valid because our value of $\xi$ used in our simulations is much smaller than the characteristic distance over which $P(x)$ varies. This assumption is expected to hold under cellular conditions because the mesh size is on the order of tenths of a micron or less, and the lamellipodium density varies over distances on the order of a micron.

In two and three dimensions, $\langle x \rangle$ is seen to be proportional to $1/k_{\text{sev}}$. This result can be understood as follows. We take the logarithm of both sides of equation (6) and again treat the right-hand side of the resulting equation as an integral. We thus obtain

\[ \ln P_n = \int_0^n [1 - (1 - p(m))^{(2/(1-p(m))-1)}] \, dm. \] (11)

Here, we have replaced $p(m - \frac{1}{2})$ by $p(m)$ for simplicity of calculation, which will not change the results significantly since $\xi$ is small. Recalling that $p(m) = \exp(-mk_{\text{sev}}\xi/v)$, and making the change of variables $u = mk_{\text{sev}}\xi/v$, I obtain

\[ \ln P_n = \frac{v}{k_{\text{sev}}\xi} \int_0^{nk_{\text{sev}}\xi/v} [1 - (1 - \exp(-u))^{(2/(1-\exp(-u))-1)}] \, du, \] (12)
Figure 7. Average half-thickness of lamellipodium as a function of inverse severing rate. (a) Solid circles: one-dimensional geometry. (b) Open circles: two-dimensional geometry. Half-solid circles: three-dimensional geometry.

which, aside from the \( n \)-independent prefactor, is a function only of the product \( nk_{\text{sev}}\xi/v \). Thus writing \( P_n = f\left( nk_{\text{sev}}\xi/v \right)^{v/k_{\text{sev}}\xi} \), and taking equation (10) as an integral, gives

\[
\langle x \rangle = \xi \int_0^\infty m f\left( mk_{\text{sev}}\xi/v \right)^{v/k_{\text{sev}}\xi} \, dm \int_0^\infty f\left( mk_{\text{sev}}\xi/v \right)^{v/k_{\text{sev}}\xi} \, dm.
\]

(13)

Making the change of variables \( w = mk_{\text{sev}}\xi/v \) gives

\[
\langle x \rangle = \frac{v}{k_{\text{sev}}} \int_0^\infty w f\left( w \right)^{v/k_{\text{sev}}\xi} \, dw \int_0^\infty f\left( w \right)^{v/k_{\text{sev}}\xi} \, dw
\]

(14)

As seen above in figure 3, \( P(x) \) (and thus \( P_n \)) in two and three dimensions have a behavior that is reasonably close to that of a step function. If one assumes that they are step functions dropping from unity to zero, \( f(w) \) takes on only the two values zero and one. Then the integral in equation (14) is independent of the exponent containing \( k_{\text{sev}} \), and \( \langle x \rangle \propto 1/k_{\text{sev}} \).

The proportionality of \( \langle x \rangle \) to \( 1/k_{\text{sev}} \) suggests that the width \( 2\langle x \rangle \) of the lamellipodium might be described by a simple criterion based on probability of a bond being unsevered at
the edge of the lamellipodium. One could speculate that the edge of the lamellipodium is defined by a critical probability $p_c$, so that $p(n) = p_c$, where $n = 2 \langle x \rangle / \xi$. For example, in a two-dimensional geometry $p_c$ could be the bond percolation threshold of $\frac{1}{2}$. The value of $p_c$ extracted in this fashion from our two-dimensional results with $\xi = 0.04 \, \mu m$ and $k_{sev} = 0.01 \, s^{-1}$, where $\langle x \rangle = 1.15 \, \mu m$ (cf figure 7), is $\exp(-k_{sev}2\langle x \rangle / v) = \exp(-0.75) = 0.47$. The linearity of $\langle x \rangle$ as a function of $1/k_{sev}$ implies that this value of $p_c$ will describe all other values of $k_{sev}$ well. The assumption of a critical $p_c$ implies that $\langle x \rangle$ should be independent of $\xi$. We have tested this assumption by performing additional calculations for $\xi = 0.2 \, \mu m$. We find that, in general, the values of $\langle x \rangle$ are about 30% larger than those for $\xi = 0.04 \, \mu m$. Given the factor of five change in $\xi$, this 30% change in $\langle x \rangle$ means that the approximation of constant $p_c$ is reasonable, although not quantitatively accurate. The reason that smaller values of $\xi$ lead to smaller values of $\langle x \rangle$ can be understood from equation (6). At a value of $x$ where $p(n) = p_c$, there have been more multiplications by factors less than unity if $\xi$ is smaller, so $P_n$ is also smaller. I do not have a compelling theoretical argument justifying the use of the constant $p_c$ criterion. However, its accuracy may be connected with the smallness of $\xi$ relative to the width of the lamellipodium. I have performed some calculations with values of $\xi$ smaller than 0.04 $\mu m$, and I find that $P(x)$ converges to a well-defined limit. This means that in the limit of small $\xi$, $\langle x \rangle$ is rigorously independent of $\xi$, justifying the $p_c$ criterion.

This result allows us to analyze the effect of the mesh size in a system where the severing rate of a link is proportional to $\xi$, as might be expected in a cell. Again considering the two cases $\xi = 0.04 \, \mu m$ and $\xi = 0.2 \, \mu m$, the latter case would have a value of $k_{sev}$ five times larger than the former. The effect on $2\langle x \rangle$ would then contain two contributions: a factor of five reduction from the five-fold increase in $k_{sev}$, and about a 30% increase from the effect of increased mesh size at a given $k_{sev}$. The net result is about a factor of four reduction in the lamellipodium width. In general, one might expect that an increase in the mesh size at a constant severing rate per unit length will lead to a decrease in the lamellipodium width of a magnitude similar to, but somewhat less than, the increase in the mesh size.

Figure 8 shows the effect of annealing on $\langle x \rangle$, for three values of $k_{sev}$. Here, we consider the lamellipodial geometry, again with height 0.2 $\mu m$. The plots are only taken up to the threshold values of $k_{ann}$. It is seen that the $\langle x \rangle$ plots curve strongly upward before the transition, but do not diverge as the transition is approached. These results allow us to address the two timescales that are seen in speckle microscopy studies of lamellipodial disassembly, discussed above. For concreteness, I define the speckle lifetime $\tau_{speckle}$ as the average lifetime of a speckle generated at the leading edge ($x = 0$). Then $\tau_{speckle} = 1/k_{sev}$, since severing is the only process that eliminates speckles in our model, and annealing only comes into play once severing has occurred. I define the network lifetime $\tau_{net}$ as the average time required for a part of the network to reach the inner edge of the lamellipodium, where it disassembles. I will take this to occur at $2\langle x \rangle$. Thus $\tau_{net} = 2\langle x \rangle / v$. Consider the $k_{sev} = 0.1 \, s^{-1}$ curve, for which $\tau_{speckle} = 10 \, s$. At $k_{ann} = 0$, $\tau_{net} = 2 \times 0.2 \, \mu m/(0.03 \, \mu m \, s^{-1}) = 13 \, s$, so the two times are essentially the same. On the other hand, at $k_{ann} = 0.12 \, s^{-1}$, $\langle x \rangle \simeq 1.5 \, \mu m$, so that $\tau_{net} = 100 \, s$. Thus $\tau_{net}$ is almost an order of magnitude larger than $\tau_{speckle}$, consistent with the speckle microscopy data [19]. This finding emphasizes the need for a treatment of actin disassembly dynamics based on the competition between severing and annealing.
Figure 8. Average half-thickness of lamellipodium with height 0.2 µm as a function of $k_{\text{ann}}$. Solid circles: $k_{\text{sev}} = 0.06$ s$^{-1}$. Open circles $k_{\text{sev}} = 0.08$ s$^{-1}$. Squares: $k_{\text{sev}} = 0.1$ s$^{-1}$.

4. Conclusion

This paper has described a very simple model, which cannot quantitatively describe a real lamellipodium. However, some of the results are general enough that they should hold in biological cells. The cooperativity of disassembly that was seen most clearly in figure 3 is one of these. In general, one expects that it should take more than one severing event to detach part of the lamellipodium. This should be reflected in the behavior of the F-actin density away from the lamellipodium, which should have an accelerated dropoff at a certain distance. The effect will be modulated by several other effects in the cell. For example, we have already seen in figures 5 and 6 that annealing can reduce the sharpness of the drop in $P(x)$, although the behavior is still far from the smooth exponential decay that is found in simple first-order kinetics. The irregularity of the actin network structure will also reduce the cooperative effect. For example, some filaments will be connected to the network at only one point, and detaching part of these filaments requires only one severing event. This part of the disassembly will therefore be non-cooperative. Another important component of disassembly that is not included in our calculations is pointed-end depolymerization. The effects of depolymerization on the F-actin density profile depend on the relative magnitudes of the severing time and the depolymerization time (the time required for an average filament to depolymerize). If the depolymerization time is longer than the severing time, then disassembly is dominated by severing and the present analysis is valid. If, on the other hand, the depolymerization time is shorter than the severing time, depolymerization must still be preceded by severing or debranching, since in the ‘as-grown’ network the pointed ends are capped. In this case, one should think of disassembly as a two-step process in which severing/debranching events occur first. Filaments then disappear quickly after these events. This will lead to an initial downward
slope of the intensity as a function of distance from the leading edge, resulting from single-filament severing/depolymerization events. But the cooperativity effects will still be present since there will be regions where several filaments in close proximity have disappeared, which will allow whole chunks of the network to detach and diffuse away. Finally, tensile stresses induced by myosin II in the cell interior are expected to accelerate severing. The general effect of the stress should be to enhance the cooperativity of disassembly. In regions with a lower density of filaments, the stress will be spread over a smaller number of filaments, leading to a larger stress per filament. This will accelerate the severing of these filaments, causing low-density regions to disassemble faster than in the absence of the stress. Such stresses are also found in the absence of myosin, in actin comet tails assembled on *Listeria* and biomimetic analogs [2]. The present model, with its flat leading edge, cannot treat these systems directly. However, the enhancement of cooperativity by tensile stress should be seen here as well.

I cannot establish the extent to which the cooperative behavior will survive in specific cases. However, in some cases the observed behavior of the F-actin density as a function of distance from the membrane does display features similar to those found here. In figure 9, I show the F-actin fluorescence intensity as a function of distance from the edge of a carcinoma cell that is obtained by scanning the pixel intensities depicted in figure 1(a) of [19]. A shoulder is seen on the right-hand side of the peak, which could be a blurred remnant of a sharper drop resulting from cooperative behavior. Such a shoulder is not seen in an exponential decay, nor in a blurred exponential decay. I note that some previous studies (see, for example, [20]) have found a zone of increased F-actin at the base of the lamellipodium, which is not seen in the present results. The origin of this effect is not well established, but it has been attributed [20] to the formation of arc-like bundles resulting from lateral flow of preformed filaments. Such lateral flow effects are not included in the present model.

The other general feature of the results that I feel will persist in more realistic models is the effect of annealing on the network decay dynamics. Increasing annealing, or reducing severing
should increase the gap between the two actin decay times—the speckle lifetime and network lifetime. These effects should be observable by genetic manipulations that knock down severing proteins such as ADF/cofilin or proteins such as cortactin which stabilize branch points.

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