

Filamin Cross-Linked Semiflexible Networks: Fragility under Strain

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The semiflexible *F*-actin network of the cytoskeleton is cross-linked by a variety of proteins including filamin, which contains Ig domains that unfold under applied tension. We examine a simple filament network model cross-linked by such unfolding linkers that captures the main mechanical features of *F*-actin networks cross-linked by filamin proteins and show that, under sufficient strain, the network spontaneously self-organizes so that an appreciable fraction of the filamin cross-linkers are at the threshold of domain unfolding. We propose and test a mean-field model to account for this effect. We also suggest a qualitative experimental signature of this type of network reorganization under applied strain that may be observable in intracellular microrheology experiments of Crocker *et al.*

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The cytoskeleton of eukaryotic cells is a cross-linked biopolymer network [1–3]. Its principal constituent is a stiff filamentous protein aggregate (*F*-actin) that is cross-linked densely on the scale of its own thermal persistence length. Because of the combination of filament stiffness and dense cross-linking this *semiflexible* polymer gel differs fundamentally from the better understood *flexible* polymer gels that are the products of modern synthetic chemistry.

There has been considerable progress in understanding the complex relationship between the mechanical properties of semiflexible networks and the mechanical properties of their constituent filaments [4–11]. Still, the current understanding of semiflexible networks is based on simplified systems, while the cytoskeleton is a highly heterogeneous chemical system. Cytoskeletal filaments are polydisperse in length and have a greater distribution of mechanical properties than the model systems studied. Furthermore these filaments are cross-linked by a plethora of highly structured proteins that play an active role in generating internal stresses and in sensing externally imposed stress. One class of cross-linking proteins (e.g., filamin) contains numerous repeat “Ig” domains that unfold at a critical pulling force [12,13].

In this Letter we explore one aspect of the chemically complex cytoskeleton and show that, for a sufficiently stretched filamin cross-linked gel, the population of cross-links at a certain tension grows exponentially or faster up to the unfolding force of the domains. Thus at moderate applied stresses the system appears to adjust its mechanical properties so as to achieve a strain state in which a significant fraction of its cross-linkers are poised at the unbinding transition of their internal domains. Molecular motor activity acting on this cross-linker population should yield a broad, continuous distribution of relaxation time scales via mechanically induced subcritical cross-link unfolding. Such a broad relaxation time distri-

bution has been termed soft glassy rheology [14] and has been observed in the cytosol [15] by measurements of low-frequency intracellular strain fluctuations. In our model this fragile state is generated by externally applied stress, but we expect an *in vivo* network built with filamin cross-linkers to evolve into such a state due to the action of internal molecular motors (e.g., myosin—not considered in our model).

We study via numerical simulation a random, statistically homogeneous, two-dimensional, isotropic filament network. These networks are formed in a manner identical to that of Head *et al.* [8]. At filament intersections we add a cross-linker of zero rest length that exerts constraint forces but no constraint torques. Part of a model network constructed by this procedure is shown in Fig. 1. The filament sections between cross-links are modeled as independent linear springs with fixed extension modulus μ . The large

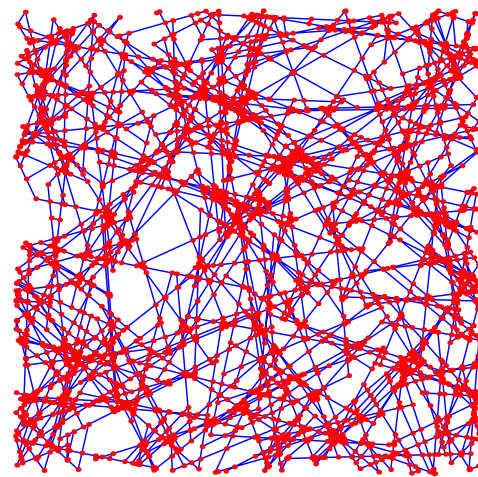


FIG. 1 (color online). One quarter of a model network showing the *F*-actin filaments in blue and the filamin cross-linking agents in red.

deformation, nonlinear behavior of semiflexible networks with freely rotating cross-links has been shown to be dominated by semiflexible filament stretching instead of bending [16]. For this study we neglect filament bending, allowing the filaments to bend without energetic penalty at each cross-link point. We anticipate that our results are essentially independent of network dimensionality since network connectivity, not the dimensionality of the space in which the network is embedded, should control the collective mechanical properties of the system.

At forces below the unfolding force, the force-extension relation of the filamin cross-linkers is that of a wormlike chain [17]. When an Ig domain unfolds the contour length of the filamin increases, adding enough length to relax most of the tension at fixed extension. For simplicity, we model the filamin as a simple spring with spring constant k_f and we take the additional contour length generated during any unfolding event ℓ_f to be a constant. The critical unfolding force of a domain is $k_f \ell_f$. We neglect the rate dependence of this unfolding force [18]. Though the physiological filament cross-linkers have a finite number of unfolding domains (24), we will take our sawtooth force-extension curve to have an infinite number of branches.

Our network has periodic boundary conditions and is sheared by adding a constant horizontal offset to filaments that crossed the top and bottom boundaries. At the beginning of each strain step all nodes are moved affinely, then the node positions are relaxed by a conjugate gradient routine to reach local force equilibrium. Since the cross-linker force-extension curve is a sawtooth, there are many possible equilibrium states of the network. The multiplicity of equilibrium states requires the use of imposed displacements smaller than the sawtooth length ℓ_f so that equilibrium is achieved at the smallest filamin extension. Such a procedure is computationally intractable in light of the need to average our results over many realizations of the network. To reduce this computational overhead we use a two step equilibration procedure that finds a state close to desired one, but allows for strain steps 100 times larger. In the first step we replace the sawtooth force law for the cross-linkers by the following:

$$\mathbf{f} = \begin{cases} k_f \mathbf{x} & |\mathbf{x}| < \ell_f, \\ k_f \ell_f & |\mathbf{x}| \geq \ell_f. \end{cases} \quad (1)$$

The combined network of linear elastic filaments and constant force cross-links is equilibrated. We then reimpose a sawtooth force law for the cross-linkers and equilibrate the network again. As the network relaxes during this final equilibration step, the force on each filamin must be less than $k_f \ell_f$, so the cross-links will stay on the same sawtooth branch. Since the rest of the network was originally equilibrated at the critical pulling force, the sawtooth force law could not have reached force equilibrium on any earlier sawtooth branch assuming all filamin linkers act independently. In practice, collective relaxations of the

network push individual cross-links onto different sawtooth branches in this final step. We found, however, that such coordinated relaxation events had a negligible quantitative effect on the data.

We study networks at a filament density such that there are on average 30 cross-links per filament. At this cross-link density we find the network behavior is independent of total filament length for lengths greater than 0.1 (in simulation units). Accordingly, we used monodisperse filaments of length 0.2. This construction produced an average of 1100 filaments and 16 500 cross-links per network instance. For these values we find negligible system-size effects. The average distance between cross-links is then $\langle \ell_c \rangle = 0.2/30 = 6.7 \times 10^{-3}$. This construction has been shown to produce an exponential distribution of ℓ_c [19]. The length of the filamin domains (the sawtooth length) is set to $\ell_f = 1.3 \times 10^{-3}$, so that $\ell_f / \langle \ell_c \rangle = 0.2$. This ratio is approximately equal to the expected physiological value [20,21] (taking $\langle \ell_c \rangle \sim 0.1 \mu\text{m}$ for dense networks). To fix an energy scale we set the extensional modulus μ of the filaments to unity. The average spring constant for a filament segment can then be determined from the mean distance between cross-links by $k_R = 1/\langle \ell_c \rangle = 150$. The range of cross-linker spring constant values studied here is $10^1 < k_f < 10^4$.

Figure 2 shows the measured equilibrium distributions of cross-link lengths, modulo the sawtooth length ℓ_f , for strained networks having several values of spring constant k_f . The statistical weight for finding a cross-link extension (modulo ℓ_f) is exponentially enhanced towards length ℓ_f for values of $k_f < 10 \times \langle k_R \rangle$ and grows faster than exponentially near length ℓ_f for values $k_f > 10 \times \langle k_R \rangle$. The enhancement of the number of such filamins poised at the unbinding transition (critical cross-linkers) is one of principal results of our work. Apart from the noise floor, the shape of the distribution appears to be strain independent.

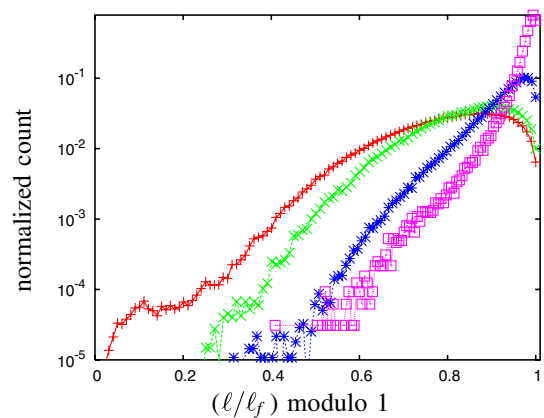


FIG. 2 (color online). Distribution of normalized cross-linker lengths ℓ/ℓ_f modulo 1 in equilibrated networks with, from shallowest to steepest slopes, respectively, $k_f = 100, 200, 600,$ and 2000 .

To understand the growth of the critical cross-linker population, we consider a mean-field model for the behavior of a single cross-link in an effective elastic medium representing the rest of the network. The surrounding effective medium is modeled as a single harmonic spring with spring constant k . Reflecting the network structure, the cross-linker is connected in series with the effective network spring. We set the total strain on the two springs in series (by fixing their total length) so that the cross-link has crossed at least one branch of the sawtooth function. Upon the further application of extensional strain, the two springs in series will both linearly increase their extension until the filamin spring with spring constant k_f reaches its maximum force $k_f \ell_f$ where it is poised at the top of its sawtooth force-extension curve.

Now consider an infinitesimal increase in the total extension that drives the unfolding of one more filamin domain. Before the extension the two springs were in force balance so that $k_f \ell_f = kx$ where x represents the extension of the medium spring. After the infinitesimal extension, the system achieves force balance on the next branch of the saw-tooth filamin force-extension curve so that the extension of the filamin spring is now increased by $\ell_f - d$ while the extension of the medium spring is decreased to $x - (\ell_f - d)$. Force balance requires that d , the distance between the current extension of the filamin spring and the edge of the next sawtooth, is given by $d(k) = k\ell_f/(k + k_f)$. In other words, the combined system once equilibrated with the filamin spring at its maximal force is now equilibrated with that filamin spring on its next sawtooth branch at a smaller force. The strain in the surrounding medium has also decreased due to the extension of one more filamin domain.

To maintain force balance, the filamin spring cannot relax its length more than $\ell_f - d$. Upon further extension the filamin spring will only extend until another domain unbinds. Thus in steady-state the filamin spring will evenly sample all extensions (modulo ℓ_f) between $\ell_f - d(k)$ and ℓ_f . For a given value of the spring constant of the medium we expect that the extensions (modulo ℓ_f) of the filamin cross-linkers x_f to be uniformly distributed between the bounds given above so that this distribution can be written as

$$P(x_f, k) = d(k)^{-1} \Theta(x_f - [\ell_f - d(k)]), \quad (2)$$

where Θ is a step function. Different cross-links in the network, however, will not have the same local environments; the values of k will be sampled from some statistical distribution $K(k)$. Integrating over that distribution we write the probability of finding a given filamin length (modulo ℓ_f) x_f :

$$P(x_f) = \int_{k_f(\ell_f - x_f/x_f)}^{\infty} \frac{k + k_f}{\ell_f k} K(k) dk. \quad (3)$$

The step function fixes the lower limit on the k integral.

We examine the distribution of the local spring constants in the random network and concentrate on the high- k tail of that distribution. One may imagine that the value of the effective spring constant of the medium is controlled by small number of chains of many springs. Each chain of springs represents one path for force propagation through the random network and is made up of a large number of statistically independent springs connected in series. In order to find an extremely large value of the effective spring constant k it must be that for one of the force paths *all* of the constituent spring constants are large, since the compliance of the springs in series will be dominated by any single soft spring. We expect the probability of such a rare event to be Poisson distributed so that, in the high- k tail, the distribution $K(k)$ takes the form

$$K(k) \sim H(k)e^{-k/\bar{k}}, \quad (4)$$

where H is some regular function characterizing the small- k behavior of the distribution ($H(x) \rightarrow \text{const}$ as $x \rightarrow \infty$) and the constant \bar{k} is undetermined. We note that our arguments justifying this exponential form for the distribution are independent of network dimensionality, and thus the distribution should be the same for three-dimensional networks. Combining Eqs. (3) and (4), we find that $P(x_f)$ takes the form

$$P(x_f) \simeq \frac{\bar{k}}{\ell_f} \exp\left(\frac{k_f(x_f - \ell_f)}{\bar{k}x_f}\right) + \frac{k_f}{\ell_f} \Gamma\left(0, \frac{k_f(\ell_f - x_f)}{\bar{k}x_f}\right), \quad (5)$$

where Γ is the incomplete gamma function, as long as $k_f \frac{\ell_f - x_f}{x_f}$ is large enough that $K(k)$ within the integral in Eq. (3) can be replaced by its high- k asymptotic form. Equation (5) shows the sought after exponential peak as $x_f \rightarrow \ell_f$.

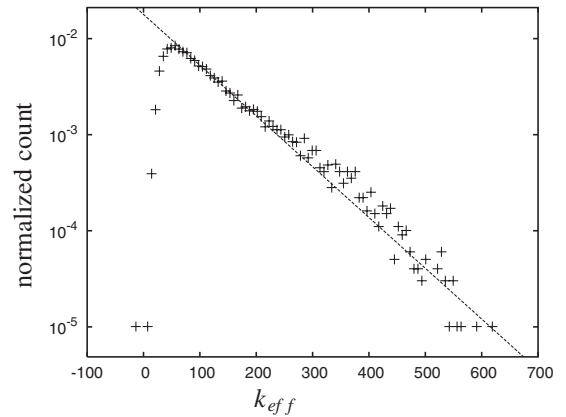


FIG. 3. Distribution of local effective spring constants, sampled on small sets of highly stretched filamin cross-linkers with $k_f = 600$. The solid line is a fit to Eq. (4) with k_f/\bar{k} set to 7.3.

Using Eq. (5) we may determine the ratio k_f/\bar{k} using the slope of the numerically measured distribution $P(x_f)$ shown in Fig. 2. Fitting the data for $k_f = 600$, we find $k_f/\bar{k} = 7.3$. By numerically sampling the local mechanical response in many realizations of the network, we independently verify the principal physical insight in the above discussion: for small values of k_f , $K(k)$ has an exponential tail in the high- k limit. These data are presented in Fig. 3 for $k_f = 600$. The dashed line shows the expected asymptotic (high- k) exponential distribution of the local effective spring constant with $k_f/\bar{k} = 7.3$ as required to fit the $P(x_f)$ using Eq. (5). The quantitative agreement between the measured decay rate of the distribution (crosses) and that calculated from the mean-field theory using the observed filamin length distribution $P(x_f)$ supports the mean-field theory. More generally, we find that even if the local spring constant distribution $K(k)$ strongly deviates from a simple exponential form (as it does at higher values of k_f) the basic relation between $P(x_f)$ and $K(k)$ given by Eq. (3) holds [22].

We have found that the strained filamin cross-linked network develops into a fragile mechanical state in which a large fraction of the cross-linking filamins reach a critical strain where they are poised at the brink of domain unfolding. While this work explores a nonthermal system, we expect that thermal fluctuations will merely unbind the subpopulation of cross-linkers within $k_B T/k_f \ell_f$ of their unbinding length and thereby lead to decreased value of the effective ℓ_f in our model [15]. Fluctuating internal stresses in the system due to molecular motor activity acting on this highly fragile state should produce large strain fluctuations due to the coordinated failure of the many critical cross-linkers. The formation of this critical state under stress may explain the necessity of filamin for the enhancement of low-frequency intracellular strain fluctuations [23]. This behavior should manifest itself once the average stress per filament exceeds the Ig domain unfolding stress of ~ 100 pN [21], which for a dense network of ten filaments per μm^2 of cross section amounts to ~ 1 kPa of applied stress.

We have presented a simple, mean-field theory that successfully explains the development of this fragile state under applied strain. There are a number of extensions of this work that remain to be considered. Foremost among these is the exploration of the effect of filamin-type cross-linkers in semiflexible gels where the filaments each have a finite bending modulus. The development of a complete model that includes the effect of internally generated random stresses due to the action of molecular motors will also be an important step towards the direct calculation of the low-frequency dynamics of this biopolymer gel that determines cellular mechanics.

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