The semiflexible F-actin network of the cytoskeleton is cross-linked by a variety of proteins including filamin, which contain Ig-domains that unfold under applied tension. We examine a simple semiflexible 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 sufficiently high 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 an explanation of this organization based on a mean-field model and 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, 2, 3]. Its principal constituent is a stiff 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, 5, 6, 7, 8, 9, 10, 11]. Since much of this work addresses highly simplified systems, one may ask how well does the current understanding of semiflexible networks elucidate the rheology of the cytoskeleton, which is a highly heterogenous chemical system. Cytoskeletal filaments are polydisperse in length and have a greater distribution of mechanical properties (due to e.g. filament bundling) than the model semiflexible network 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 contain numerous repeat domains, such as titin[12, 13] and filamin[14, 15] that unfold reversibly at a critical pulling force.

In order to begin to address the mechanical effect of this chemical heterogeneity in the cytoskeleton, we investigate networks cross-linked by filamin–like proteins containing multiple unfolding domains. We find that, above a certain strain threshold, the population of cross-links at given tension grows rapidly up to the critical unfolding tension of the domains. Thus, the system appears to self-consistently adjust its mechanical properties so as to reach a highly fragile state in which a large fraction of its cross-linkers are poised at the unbinding transition of their internal domains. The evolution of this high susceptibility state in which the system responds to small stress fluctuations with anomalously large strain fluctuations may contribute to the large nonthermal low-frequency intracellular strain fluctuations as measured by Hoffman et al.[16]. The network may evolve into this high susceptibility state under the action of internal molecular motors (e.g. myosin – not considered in our model) so that small fluctuations in motor protein activity leads to the coordinated unfolding of numerous filamin cross-linkers and the consequent large-scale cytoskeletal rearrangement event.

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.[3]. At filament intersections we add a cross-linker of zero rest length that exerts constraint forces but no constraint torques. A model network
constructed by this procedure is shown in Fig. 1. The filament sections between cross-links are modeled as linear springs with fixed elastic constant per unit length. The nonlinear behavior of semiflexible networks with freely rotating cross-links has been shown to be dominated by semiflexible filament stretching instead of bending, so we neglect filament bending. We anticipate that the results derived here 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 worm-like chain. When an Ig domain unfolds the contour length of the filament 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 $\ell_f$ to be a constant. The critical unfolding force of a domain is $k_f\ell_f$. We neglect the the rate-dependence of this unfolding force. 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.

The network is sheared using Lees-Edwards boundary conditions. At the beginning of each strain step all nodes are moved affinely, then the node positions are relaxed through a conjugate gradient routine to a point of local force equilibrium. Since the cross-linker force extension curve is a sawtooth, there are many possible equilibrium states of the network. In principal, the multiplicity of equilibrium states requires us to use strain steps resulting in displacements smaller than the sawtooth length $\ell_f$ so that equilibrium is achieved at the smallest filamin extension. To reduce computational overhead we use a two step equilibration procedure that finds a state close to desired one, but allows for large strain steps. In the first equilibration step, we replace the sawtooth force law for all cross-linkers by the following force law:

$$f = \begin{cases} k_f x & |x| < \ell_f, \\ k_f \ell_f & |x| \geq \ell_f. \end{cases} \tag{1}$$

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 a second time. 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 present data for networks composed of monodisperse filaments of length $\ell_R = 0.2$ (all lengths are measured in units of the length of the square simulation box) at a filament density such that there are on average 30 cross-links per filament. For these values we find negligible system-size effects. The length of the filament domains is given by $\ell_f = 1.3 \times 10^{-4}$, or $\ell_f/\ell_c = 0.02$. This ratio is about ten times smaller than the expected physiological value [20–21]. We choose the smaller value of $\ell_f/\ell_c$ because it enhances the effects we were measuring: our initial studies at physiological values of $\ell_f/\ell_c$ find qualitatively similar results, but the onset of nonlinear effects occurred at higher strain values [22]. To fix an energy scale we set the extensional modulus $\mu$ of the filaments to unity. The average spring constant for a filament segment can then be determined from the mean distance between cross-links: $k_R = 1/\langle \ell_c \rangle = 150$. The range of cross-linker spring constant values studied here is $10^3 < k_f < 10^4$.

Figure 2 shows the measured equilibrium distributions of cross-link lengths, modulo the sawtooth length $\ell_f$, for a representative set of strained networks with $\ell_f = 1.3 \times 10^{-4}$ and several values of spring constant $k_f$. For values of $k_f < 10 \times \langle k_R \rangle$ the statistical weight for finding a cross-link extension (modulo $\ell_f$) is exponentially enhanced towards length $\ell_f$ where the domains unbind. For values $k_f > 10 \times \langle k_R \rangle$ the statistical weight for finding a cross-link extension (modulo $\ell_f$) grows faster than exponentially near length $\ell_f$. 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

![FIG. 2: (color online) Distribution of normalized cross-linker lengths $\ell/\ell_f$ modulo 1 in equilibrated networks with, from shallowest to steepest slopes respectively, $k_f = 100, 200, 600, 2000, 4000,$ and 10000.](image-url)
distribution appears to be strain independent.

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 saw-tooth 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 is maximum force $k_f \ell_f$ where it is poised at the top of its saw-tooth 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 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 saw-tooth, is given by $d(k) = k_{eff} / (k + k_f)$. 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 saw-tooth 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 $\ell_f$) between $\ell_f - d(k)$ and $\ell_f$. For a given value of the spring constant of the medium we expect that the extensions (modulo $\ell_f$) of the filamin cross-linkers $x_f$ to be uniformly distributed between the bounds given above so that this distribution can be written as

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

where $\Theta$ 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 $\ell_f$) $x_f$:

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

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 $k/k_f$ set to 7.3.

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 effective spring constant representing the medium can be represented as a small number of chains of springs. Each chain of springs represents one path for force propagation through the random network; it 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

$$K(k) \sim H(k) e^{-k/k_f}$$

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 $k_f$ is undetermined. Combining Eqs. 3,4 we find that $P(x_f)$ takes the form

$$P(x_f) \zeta \frac{k_f}{\ell_f} \exp \left( \frac{k_f(x_f - \ell_f)}{kx_f} \right) + \frac{k_f}{\ell_f} \Gamma \left( 0, \frac{k_f(\ell_f - x_f)}{kx_f} \right)$$

as long as $k_f(\ell_f - x_f)$ is large enough that $K(k)$ within the integral in Eq. 3 can be replaced by its high-$k$ asymptotic form. Eq. 5 shows the sought after exponential peak as $x_f \rightarrow \ell_f$.

Using Eq. 5 we may determine the ratio $k_f/k$ using the slope of the numerically measured distribution of $x_f$ shown in Figure 3. From fitting the data for $k_f = 600$ we
find that $k_f/\bar{k} = 7.3$. 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 $k_f$, $K(k)$ appears to have an exponential tail in the stiff spring limit. This data is presented in Fig. 3 for $k_f = 600$. The fitted line demonstrates that the high $k$ tail of this data is consistent with a value of $k_f/\bar{k} = 7.3$. The agreement between the two independently determined values of $\bar{k}$ supports our analysis. In upcoming work, we demonstrate that for higher $k_f$ the distribution function $K(k)$ takes a different form, but it is still related to the distribution function $P(x_f)$ via Eq. 3 [22]. This latter equation should apply for general distributions $K(k)$.

We have found that the strained filamin cross-linked network develops into a highly fragile mechanical state in which a large fraction of the cross-linking filamins reach a critical state where they are poised at the brink of domain unfolding. The presence of fluctuating internal stresses in the cytoskeleton produced by molecular motor activity and/or equilibrium fluctuations can act on this highly fragile state to produce large strain fluctuations due to the correlated failure of numerous critical cross-linkers. Thus, the observation of the formation of this critical state under applied stress may explain a particular feature of the observed low-frequency strain fluctuations as observed by intracellular microrheology.

We have presented a simple, mean-field theory to explain the evolution 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. In addition, the development of a complete model that includes the effect of internally generated random stresses due to the action of molecular motors will be an important step towards the direct calculation of the low-frequency dynamics of this biopolymer gel of fundamental biological importance.

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