# Nonlinear Elasticity of Composite Networks of Stiff Biopolymers with Flexible Linkers

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Motivated by recent experiments showing nonlinear elasticity of *in vitro* networks of the biopolymer actin cross-linked with filamin, we present an effective medium theory of flexibly cross-linked stiff polymer networks. We model such networks by randomly oriented elastic rods connected by flexible connectors to a surrounding elastic continuum, which self-consistently represents the behavior of the rest of the network. This model yields a crossover from a linear elastic regime to a highly nonlinear elastic regime that stiffens in a way quantitatively consistent with experiment.

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The mechanical response of living cells depends largely on their *cytoskeleton*, a network of stiff protein polymers such as filamentous actin (F-actin), along with various associated proteins for cross-linking and force generation. In addition to their importance for cell mechanics, cytoskeletal networks have also demonstrated novel elastic properties, especially in numerous *in vitro* studies [1–6]. The cellular cytoskeleton, however, is an inherently composite structure, consisting of elements with highly varied mechanical properties, and there have been few theoretical or experimental studies of this aspect [7–11]. Recent experiments on F-actin with the physiological cross-linker filamin have demonstrated several striking features; while their linear modulus is significantly lower than for rigidly cross-linked actin systems, they can nonetheless withstand remarkably large stresses and can stiffen by a factor of 1000 with applied shear [7,9,12]. This behavior appears to result from the highly flexible nature of filamin, although the basic physics of such a network, in which the elasticity is dominated by cross-linkers, is not understood. Apart from their physiological importance, such networks suggest new principles that may be extended to new synthetic materials with designed cross-links [8].

Here we develop a theoretical model for composite networks of rigid filaments connected by flexible cross-linkers, in which the macroscopic network elasticity is governed by the cross-links. We examine this model in a limit in which the basic elastic element is a single rigid rod, directly linked by numerous compliant cross-linkers to a surrounding linear elastic medium. We show that such a network stiffens in a manner determined by the mechanics of individual cross-links, which we model both as linear springs with finite extension and also as wormlike chains. We analyze our model in both a fully 3D network as well as a simplified 1D representation, which already captures the essential physics of the nonlinear behavior. The finite extension $\ell_0$ of the cross-links along with the length of the filaments/rods $L$ implies that there exists a characteristic strain $\gamma_c \sim \ell_0/L$ for the onset of the nonlinear response of the network. Indeed, prior *in vitro* experiments, in which the length of the cross-linkers was varied [8], have reported this linear dependence on $\ell_0$. We extend this model in a fully self-consistent manner, replacing the embedding medium by an effective medium whose elastic properties are determined by those of the constituent rods and linkers. This self-consistent model can quantitatively account for the nonlinear response found in prior experiments on actin-filamin networks [7,9].

In a flexibly cross-linked stiff polymer network, randomly oriented stiff filaments or rods are interconnected by relatively short but highly flexible cross-linkers (Fig. 1). The compliance of this network is dominated by the flexible cross-linkers, while the much stiffer filaments act mainly as a scaffold for the cross-linkers, ensuring rigidity of the network as a whole. Recent experiments have demonstrated that flexible biological cross-linkers such as filamin can be described as a semiflexible polymer using the

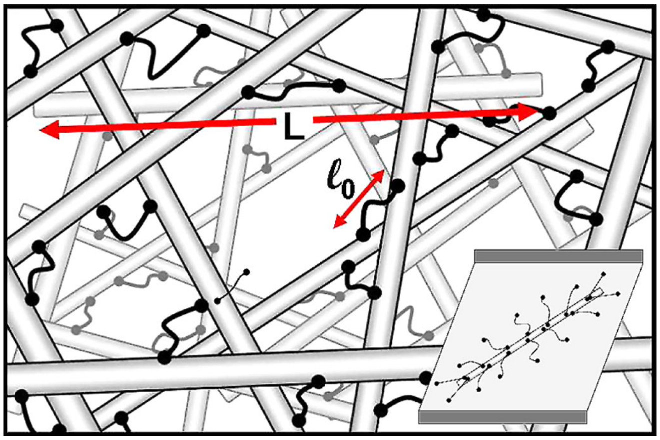

FIG. 1 (color online). Schematic figure of an isotropic stiff polymer network with highly compliant cross-linkers. The inset illustrates the proposed nonuniform deformation of the cross-linkers on a single filament in a sheared background medium.

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wormlike chain (WLC) model [13,14]. The cross-linkers are characterized by their contour length $\ell_0$ and persistence length $\ell_p$ [15]. A realistic force-extension curve of a typical biological cross-linker is shown as a solid black line in the inset in Fig. 2. It is instructive to simplify this curve by assuming linear response with a spring constant $k_{\mathrm{cl}}$ and a finite extensibility $\ell_0$. This simplification retains the essential features and is shown in Fig. 2 as a dashed blue line. We refer to this as *simple* cross-link behavior.

To determine the elasticity of the network, we use an effective medium approach and divide the network into two mechanically connected subsystems. The first consists of a stiff filament of length $L$ decorated by $n$ flexible cross-linkers, which we refer to as a hairy rod (HR). The other is the network connected to it, which we treat as an elastic continuum. Although the medium is assumed to deform affinely, we allow the local strain of the cross-linkers to depend on their position on the HR. By averaging over all orientations, we may express the macroscopic stress in terms of the tension in a single HR connected to a medium, which is subject to a 1D strain $\epsilon$ along its backbone. The tension $\tau$ in the center of this HR is the sum of the forces exerted by all cross-linkers on one-half of the rod. To calculate these forces, we treat the cross-linker as a spring connected in series with the medium, which we describe with a spring constant $K_{\mathrm{EM}}$. We are primarily interested in densely cross-linked networks for which $K_{\mathrm{EM}} \gg k_{\mathrm{cl}}$. The extension of the cross-linker-medium system is given by $\epsilon x$ at a distance $x$ from the center of the rod. If the cross-linkers are homogeneously distributed over the rod with a high density $n/L$, we can write the sum over forces as an integral:

$$\tau(\epsilon) = \frac{n}{L}\int_0^{\ell_0/\epsilon} dx \frac{k_{\mathrm{cl}}K_{\mathrm{EM}}}{k_{\mathrm{cl}}+K_{\mathrm{EM}}}\epsilon x + \frac{n}{L}\int_{\ell_0/\epsilon}^{L/2} dx\left[\frac{k_{\mathrm{cl}}K_{\mathrm{EM}}}{k_{\mathrm{cl}}+K_{\mathrm{EM}}}\ell_0 + K_{\mathrm{EM}}(\epsilon x - \ell_0)\right]. \quad (1)$$

For strains $\epsilon \leq \frac{\ell_0}{L/2}$, only the first integral is present and the integration extends to $L/2$. In this case, the tension depends linearly on the strain. Using Eq. (1), we compute the 1D modulus $G_{\mathrm{1D}} = \tau/\epsilon$, which is shown as a dashed blue line in Fig. 2. For small strains, the system is linearly elastic with $G_{\mathrm{1D}} = \frac{1}{8}n\frac{k_{\mathrm{cl}}K_{\mathrm{EM}}}{k_{\mathrm{cl}}+K_{\mathrm{EM}}}L$. Above a threshold strain $\frac{\ell_0}{L/2}$, a crossover occurs to a second linear regime in which $G_{\mathrm{1D}}$ asymptotically approaches $\frac{1}{8}nK_{\mathrm{EM}}L$.

The nonlinear response of a cross-linker is more realistically modeled with the WLC model [14] (Fig. 2). We calculate the tension in a rod with WLC cross-linkers analogously to Eq. (1). The 1D modulus $G_{\mathrm{1D}}$ is shown as a dashed-dotted purple line in Fig. 2. Though quite similar to the simple cross-linker model, the more realistic force-extension curve has introduced a considerable smoothing of the crossover resulting in a gradual onset of nonlinear behavior of the HR with WLC cross-linkers. Nevertheless, the characteristic strain $\epsilon_c$ for the nonlinear behavior is proportional to $\ell_0/L$ independent of the exact nonlinear response of the linkers.

Using the 1D model presented above, we can compute the macroscopic stress of a network. A 3D isotropic network with a polymer length density $\rho$ is modeled by an effective medium consisting of randomly oriented HRs. We can compute the macroscopic stress $\sigma$ and shear modulus $G = \sigma/\gamma$ by averaging over all orientations [2,6]. The shear modulus is shown in Fig. 2 for the simple cross-linkers and for the WLC cross-linkers. The 3D curves are largely similar to the 1D results, save for a factor of 2 shift, which may be understood by noting that the rods at a 45° angle to the stress plane, which bear most of the stress, experience an extensional strain $\epsilon$ of $\gamma/2$.

At large strains, when many of the cross-linkers are extended well into their nonlinear regimes, it is no longer realistic to assume a linear background medium. To address this, we shall now require the elasticity of the background medium to *self-consistently* represent the nonlinear elasticity of its constituent HRs.

Under strain the cross-links deform the surrounding elastic medium. The resulting longitudinal displacement $\delta\ell$ of the medium leads to a restoring force per unit length along the rod given approximately by the shear stiffness $\frac{d\sigma}{d\gamma} \times \delta\ell$ [16]. The shear modulus of the medium depends on the density $\rho$ of rods and the longitudinal stiffness $\frac{d\tau}{d\epsilon}$, where $\epsilon$ is the 1D extensional strain of the medium along the rod:

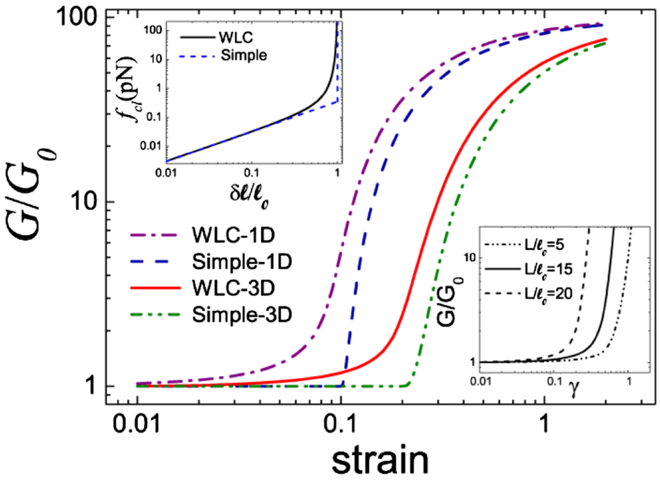

FIG. 2 (color online). The shear modulus $G$ normalized by the linear modulus $G_0$ as a function of strain $\epsilon$ (1D) or $\gamma$ (3D) for simple cross-linkers and WLC cross-linkers in the 1D and 3D versions of the linear medium hairy rod model. In this plot we have chosen $K_{\mathrm{EM}} = 100k_{\mathrm{cl}}$ as an example. The upper left inset shows the force-extension curve of a simple cross-linker (dashed blue curve) and of a WLC cross-linker (solid black curve). The lower right inset shows the normalized shear modulus as a function of strain $\gamma$ for various ratios of $L/\ell_0$ calculated with the self-consistent model.

$$\frac{d\sigma}{d\gamma} = \mathcal{A}\rho\frac{d\tau}{d\epsilon}. \tag{2}$$

Here $\mathcal{A}$ is a dimensionless geometric factor that depends on the architecture of the network. For an isotropic network in 3D, this is $1/15$. (We note that this is a small-strain approximation and that $\mathcal{A}$ will be different for anisotropic networks.) Thus, the effective stiffness $K_{\mathrm{EM}}$ per cross-link is given by

$$K_{\mathrm{EM}} = \mathcal{A}\rho\frac{L}{n}\frac{d\tau}{d\epsilon}. \tag{3}$$

When subject to a shear strain $\gamma$, the resulting stress $\sigma$ within a network of rods can be expressed in terms of the tension $\tau$ in each rod, which depends on its orientation relative to the shear plane. It is given by

$$\tau(\epsilon) = \frac{n}{L}\int_0^{L/2} dx'x' \int_0^{\epsilon} d\epsilon' \frac{k_{\mathrm{cl}}(x'\epsilon')\mathcal{A}\rho\frac{L}{n}\frac{d\tau}{d\epsilon}(\frac{x'\epsilon'}{L/2})}{k_{\mathrm{cl}}(x'\epsilon') + \mathcal{A}\rho\frac{L}{n}\frac{d\tau}{d\epsilon}(\frac{x'\epsilon'}{L/2})}, \tag{4}$$

where $k_{\mathrm{cl}}(\delta\ell)$ is the derivative of the force-extension curve of the cross-linker. Equivalently, we may write for $\tau(\epsilon)$

$$2\frac{d\tau}{d\epsilon} + \epsilon\frac{d^2\tau}{d\epsilon^2} = \begin{cases} \frac{nL}{4}\frac{k_{\mathrm{cl}}(\epsilon L/2)\mathcal{A}\frac{\rho L}{n}\frac{d\tau}{d\epsilon}}{k_{\mathrm{cl}}(\epsilon L/2)+\mathcal{A}\frac{\rho L}{n}\frac{d\tau}{d\epsilon}} & \text{if } \epsilon < \frac{l_0}{L/2}, \\ \frac{1}{4}\mathcal{A}\rho L^2\frac{d\tau}{d\epsilon} & \text{if } \epsilon \geq \frac{l_0}{L/2}. \end{cases} \tag{5}$$

We first investigate the properties of this model using the simple force-extension curve (see inset in Fig. 2). For a densely cross-linked network, we find a linear regime below $\gamma_c = \frac{\ell_o}{L/2}$. For larger strains, the system enters a highly nonlinear regime for which

$$\frac{d\tau}{d\epsilon} \sim \tau^{1-1/[(1/4)\mathcal{A}\rho L^2-1]}. \tag{6}$$

This is in marked contrast with the linear medium model in which there is only a crossover between two distinct linear regimes.

A real network with compliant cross-linkers is more realistically modeled by solving Eq. (5) (numerically) using the WLC force-extension curve for the cross-linkers. The shear modulus in this case, computed exactly as before, is graphed in the lower right inset in Fig. 2. At low strains, $G \sim nk_{\mathrm{cl}}\rho L$, and there is a gradual onset of nonlinear response originating from the nonlinear entropic elasticity of the cross-linkers (see the upper left inset in Fig. 2). At a strain $\sim\ell_0/L$, the cross-linkers at the edges of the rods become effectively rigid, which marks the onset of the nonlinear network behavior.

In view of the nonlinearity of this system, it is more appropriate to use a differential modulus $K = \frac{d\sigma}{d\gamma}$ rather than $G$. The differential modulus is plotted in Fig. 3. Up to a critical stress $\sigma_c$, the elasticity is dominated by WLC cross-linkers placed on a rigid rod connected to a much stiffer medium. At larger stresses, the cross-linkers at the edges of the HR reach full extension and, consequently, couple strongly to the surrounding network. In this limit, the slope in a $\log(K)$ vs $\log(\sigma)$ plot approaches $\approx 1 - 1/(\frac{1}{4}\mathcal{A}\rho L^2 - 1)$, as it does for simple cross-linkers. This exponent is a consequence of the composite nature of the network and its nonlinear constituents, although it is independent of the exact form of the nonlinear response of the cross-linkers. For a dense flexibly cross-linked network, $\rho L^2 \gg 1$, and, therefore, we expect a slope of 1. This is consistent with recent experimental data on actin networks cross-linked by the highly compliant cross-linker filamin in which a slope of 1 was found [7,9] in contrast to a slope of 3/2 found for rigidly cross-linked networks [2]. Interestingly, *in vivo* experiments show that cells also exhibit power-law stiffening with an exponent of 1 [17,18].

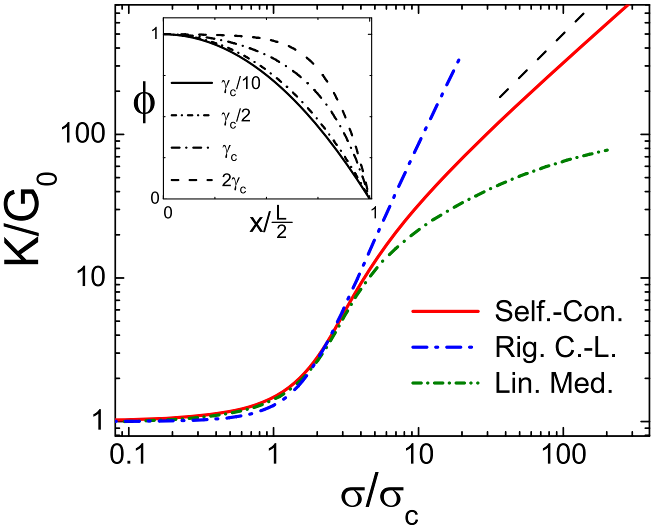

FIG. 3 (color online). Differential modulus $K = d\tau/d\gamma$ normalized by the linear modulus $G_0$ as a function of stress $\sigma$ normalized by $\sigma_c$ for the self-consistent (Self.-Con.) model. We also plot $K/G_0$ for the linear medium (Lin. Med.) model and a model for rigidly cross-linked semiflexible polymer networks (Rig. C.-L.). The black line indicates a slope of 1. The inset shows the reduced tension profile $\phi$ along the rod, normalized by the midpoint tension $\tau$ in Eqs. (4) and (5).

We compare our results to the linear medium model and a model based on the nonlinear response of the semiflexible actin segments between cross-links that has been used successfully to describe rigidly cross-linked actin networks [2,19] in Fig. 3. Although the three curves coincide for small stresses, at intermediate stresses $\sigma \gtrsim \sigma_c$ the linear medium model curve rolls over to a linear regime. Clearly, our self-consistent model and the model for rigidly linked networks begin to differ in the nonlinear regime.

So far, we have considered only the midpoint tension. In networks of elastic filaments of finite length, however, the tension along a single filament is not uniform but decreases towards its ends [20–22]. The inset in Fig. 3 shows the ratio $\phi$ of the tension at point $x$ along the rod to the maximum tension. This maximum occurs at the midpoint $x = 0$ and is given by $\tau$ in Eqs. (4) and (5). The tension at a point $x$ can be obtained by replacing the lower limit of the $x'$ integral in Eq. (4) by $x$. The tension profile is parabolic below $\gamma_c$ and quickly converges to a more flattened out profile in the nonlinear regime. We can use the tension profile to relate the maximum tension in a single HR to the macroscopic stress $\sigma$ [23]. For typical experimental conditions in an actin-filamin gel [12], we estimate a maximum force on a single cross-link to be at most 5 pN for isotropic rods and of order 1 pN or less for oriented rods.

A feature shared by the linear medium model and the self-consistent model is the characteristic strain $\gamma_c \simeq 4\ell_0/L$ for the onset of nonlinear response. The proportionality with $\ell_0$ is consistent with the results of Wagner *et al.*, where cross-linker length was varied, although they observed larger values of $\gamma_c$ than expected either from our model or based on Refs. [7,9]. Recent experiments on actin-filamin networks also show a dependence of the critical strain that is approximately inverse in actin filament length $L$ [24] in agreement with our results. This sensitivity of network response to filament length, both in experiments and in our model, appears to be one of the hallmarks of actin-filamin networks. On the one hand, this may explain the apparent difference between the critical strains reported in Refs. [7–9]. On the other hand, it also suggests that it may be more important to directly measure the filament length distribution in such experiments than in other similar *in vitro* studies. In Wagner *et al.*, for instance, the filament length was not measured but was inferred from prior reports of the length dependence on the capping protein gelsolin [25].

In previous work, DiDonna and Levine have assumed a sawtooth force-extension curve for the cross-linkers to mimic domain unfolding. They report a fragile state with shear softening when an appreciable number of cross-linkers are at the threshold of domain unfolding [10]. Our model is based on the stiffening of the cross-linkers, which occurs at forces far below those required for domain unfolding [13,14]. This leads to strain stiffening at a point where only a fraction of cross-linkers are at their threshold for nonlinear response. Thus in both our model and that of Ref. [10] the network responds strongly to small-strain changes, though in an opposite manner: stiffening in the present case vs softening in Ref. [10]. In related work, Dalhaimer, Discher, and Lubensky show that isotropic networks linked by large compliant cross-linkers exhibit a shear induced ordering transition to a nematic phase [11]. Our model accounts for the architecture of the network through an averaging procedure in a scalar quantity $\mathcal{A}$. We are presently investigating the effect of an ordering transition on the nonlinear response of the network.

We have introduced a model for flexibly cross-linked stiff polymer networks based on cross-linker elasticity. Our model yields an exponent of 1 in the asymptotic power-law behavior of a $K$ vs $\sigma$ curve in agreement with experiments on *in vitro* filamin-actin networks [7,9]. The exact form of the nonlinear response predicted by our model can be tested by further experiments [12].

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