Rheology of Semiflexible Bundle Networks with Transient Linkers

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We present a theoretical and computational analysis of the rheology of networks made up of bundles of semiflexible filaments bound by transient cross-linkers. Such systems are ubiquitous in the cytoskeleton and can be formed *in vitro* using filamentous actin and various cross-linkers. We find that their high-frequency rheology is characterized by a scaling behavior that is quite distinct from that of networks of the well-studied single semiflexible filaments. This regime can be understood theoretically in terms of a length-scale-dependent bending modulus for bundles. Next, we observe new dissipative dynamics associated with the shear-induced disruption of the network at intermediate frequencies. Finally, at low frequencies, we encounter a region of non-Newtonian rheology characterized by power-law scaling. This regime is dominated by bundle dissolution and large-scale rearrangements of the network driven by equilibrium thermal fluctuations.

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The mechanical properties of cells are largely determined by the *cytoskeleton*, a dynamic network of biopolymers, and its bundles, held together by transient linker proteins. The principal constituent of the cytoskeleton is the biopolymer filamentous actin (F-actin) [1]. In F-actin networks, the thermal persistence length of the constituent filaments is much longer than the typical distance between consecutive cross-links along a given filament, leading to the formation of a *semiflexible network*, with mechanical properties distinct from the better known gels of synthetic and highly flexible polymers [2]. In addition to their having implications for cellular mechanics, the study of F-actin networks has served as the standard model for examining the basic physics of such semiflexible networks, in part, because of the ability to reconstitute purified F-actin networks under controlled *in vitro* conditions and precisely measure their mechanical properties. As a consequence, the theoretical understanding of the elastic properties of these networks in thermal equilibrium is well advanced [3–7].

One of the key insights was that small transverse thermal fluctuations dominate the single-filament compliance and the high-frequency rheology of permanently cross-linked networks of single filaments, leading to a universal power-law rheology [5], which was confirmed experimentally [8,9]. Another consequence was that the networks' elastic moduli depend nonlinearly on the filament concentration [3,10,11]. There is, however, increasing evidence that this theory cannot account adequately for the rheology of networks composed of *bundles* of semiflexible filaments cross-linked by transient linkers (e.g., [12]). These are the types of networks that are generally encountered in *in vitro* systems at higher linker concentrations [13,14], and they are also frequently observed in the cytoskeletons of eukaryotic cells. Networks of bundles with higher concentrations of transient linkers are generically in a *nonequilibrium state*: the networks' geometry slowly evolves towards, but is never observed to reach, the equilibrium state of minimum free energy [15]. One would expect such transient networks to have a low-frequency rheology that is history and sample dependent with crossover to Newtonian rheology at very low frequencies. For high frequencies, one would expect to recover the universal single-filament rheology. In this Letter, we present the results of finite element Brownian dynamics simulations of transient bundle networks formed from semiflexible filaments linked by isotropic, transient linkers that show that transient bundle networks are characterized by unique rheological properties that violate these expectations.

The mechanics of the filaments were treated using finite elements to discretize a nonlinear beam model that fully accounts for bending, torsion, shear, and extension of the filaments. Initially straight filaments of equal length $l_f = 4\ \mu$m and persistence length $l_p = 9.2\ \mu$m were inserted into the cubic simulation box with sides of length 6 μm and having periodic boundary conditions. Linkers, introduced at fixed concentrations, were treated as short, stiff, stretchable rods (spring constant $k_\times = 0.122$ pN/nm) of length $\epsilon = 100$ nm, also discretized with finite elements. Each linker terminates at two reactive sites that were allowed to interact with complementary reactive sites spaced periodically along the filaments via chemical reactions modeled by Poisson processes with fixed, force-independent rate

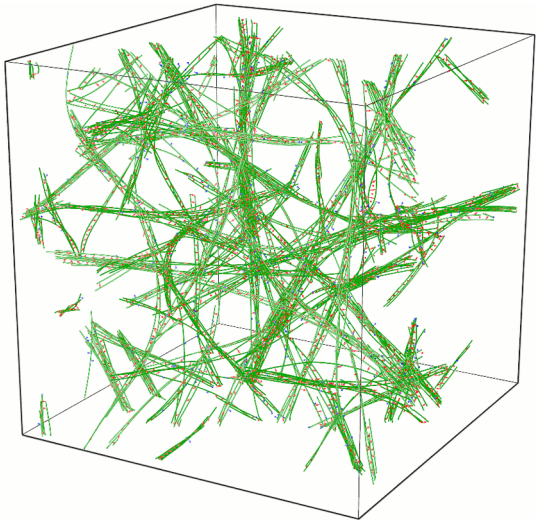

FIG. 1 (color online). Example of a bundle network as prepared for rheological measurements. The simulation cube ($V = 216\ \mu\text{m}^3$) has periodic boundary conditions and 360 filaments ($c_f = 4\ \mu\text{M}$) that are in chemical equilibrium with a solution of linkers at a concentration $c_l \approx 0.07\ \mu\text{M}$. Singly bound linkers (blue) and doubly bound linkers (red) are shown.

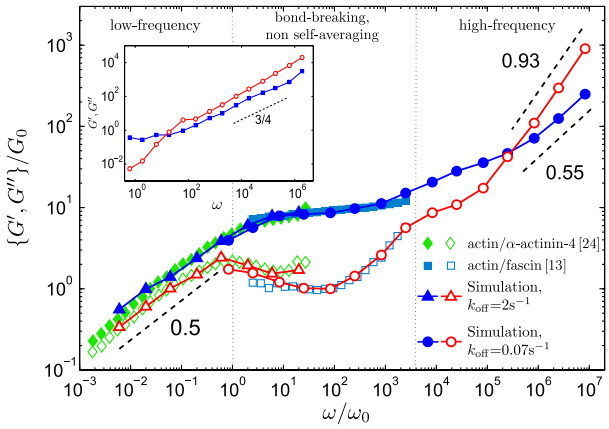

FIG. 2 (color online). Comparison of simulated rheological spectra to experiments. Simulation parameters are $c_f = 4\ \mu\text{M}$, $c_l/c_f = 0.017$, $k_{\text{on}} = 90\ \text{s}^{-1}$, and $k_{\text{off}} = \{2, 0.07\}\ \text{s}^{-1}$. We encounter three distinct regimes. At high frequencies ($\omega > \omega_1$), we see scaling behaviors $G' \sim \omega^{0.93}$ and $G'' \sim \omega^{0.55}$. The inset shows G' and G'' of the same system without linkers. The expected $\omega^{3/4}$ scaling relation is recovered. At intermediate frequencies ($\omega_2 < \omega < \omega_1$), there is a local maximum in G'' and large sample-to-sample variations. At low frequencies, there is again a power-law regime.

constants k_{on} and k_{off}. Including force-dependent unbinding rates [16] did not appreciably alter the results, at least at small imposed strains. The rate constants were given values typical for F-actin linker protein binding or unbinding. The details of the Brownian dynamics finite element code can be found in Refs. [17,18] and in the Supplemental Material [19].

Figure 1 shows a typical network comprised of 360 filaments, corresponding to an F-actin concentration of $c_f = 4\ \mu\text{M}$. Linkers with a concentration of $\sim 0.07\ \mu\text{M}$ are shown as blue and red dots when bound to one filament or to two filaments (green), respectively. Most doubly bound linkers form intrabundle connections, while only a few link two different bundles. The system was initially allowed to relax at a temperature of $T = 293$ K over time intervals corresponding to ~ 25 min in physical F-actin and linker solutions. None of the resulting bundle network systems were in thermal equilibrium, which would be a single large bundle [20]. The observed structures were closely similar to bundled F-actin networks [21], and bundle thicknesses were comparable to the ones found in experiments [22,23].

After this initial evolution, all networks were exposed to a sinusoidal shear strain with an amplitude of 1% and with a frequency range of 10^{-2}–10^{6} rad/s; we verified a linear stress-stress relation for this strain amplitude. The resulting stress was then measured to obtain the rheological spectrum, as shown in Fig. 2, which features two sets of simulation data corresponding to different linker off rates. The higher off-rate simulation agrees well with a rheological study of actin–α-actinin-4 networks [24]. The lower off-rate simulation agrees well with the intermediate-frequency data of an actin-fascin network [13]. For each experimental data set, we made a single adjustment to the modulus scale to account for differences in network density and a single adjustment to the frequency scale to fit the mean off rate of the physiological linkers.

For a given linker off rate, the rheological spectrum may be usefully divided into three frequency ranges corresponding to different dynamical regimes of the network. Beginning at the highest frequencies, one encounters a regime where the rheology is dominated by *single-bundle dynamics* where the cross-linkers may be considered to be fixed because $\omega/k_{\text{off}} \gg 1$. Importantly, the rheology of single-bundle dynamics differs fundamentally from the rheology of single-chain dynamics that is characterized by universal power-law behavior. At intermediate frequencies, dissipation is dominated by shear-induced bond breaking, leading to a peak in $G''(\omega)$ reminiscent of a simple Maxwell fluid [25]. Important features of this intermediate regime are large, nonuniversal, sample-to-sample variations of $G''(\omega)$ for networks having the same filament and cross-linker concentrations [19]. Finally, at even lower frequencies, a new rheology regime is encountered where $G'(\omega)$, $G''(\omega) \sim \omega^{1/2}$. This regime is characterized by bundle dissolution and large-scale collective thermal fluctuations of the network, possibly related to broken orientational symmetry of the network. We now discuss separately these three regimes.

High-frequency regime.—The high-frequency scaling regime of single-filament networks is understood as a form of entropic elasticity with the applied stress stretching out thermally excited transverse undulations on the filaments,

which leads to the $\omega^{3/4}$ scaling relation [3,5,6,9]. Since the same dynamics should apply to bundles, one imagines that the same scaling relation would apply, albeit with a higher-frequency scale due to the longer effective persistence length $l_p(N)$ of N-filament bundles. The bending modulus of such bundles linked by harmonic springs, however, is *scale dependent* [26–28]. When such a scale-dependent bending stiffness is incorporated into the single-filament formalism of Ref. [5], a new high-frequency scaling regime results, reflecting the bundled nature of the network. Following that formalism, we compute the complex modulus $G(\omega)$ of a network of nodes connected by equal-sized composite bundles in terms of the frequency-dependent complex extensional compliance $\alpha(\omega)$ of single bundles. Given these assumptions and a network with mesh size ξ (and length density $\rho \sim \xi^{-2}$), the complex modulus is given by

$$G(\omega) = \frac{\rho\xi}{15\alpha(\omega)} - i\eta\omega, \tag{1}$$

where η is the background solvent viscosity. The extensional compliance of a bundle produced by the stretching out of overdamped transverse bending fluctuations can be written as a sum over modes with wave number $q_n = n\pi/\xi$ [5,26]:

$$\alpha(\omega)/\alpha(0) = \frac{90}{\pi^4}\sum_{n=1}^{\infty}\frac{1}{n^4 - \frac{i\omega}{2\bar{\omega}(n)}}, \tag{2}$$

with $\alpha(0) = (\xi^4/90k_BTl_p(N)^4)$ being the zero-frequency compliance of a bundle with effective persistence length $l_p(N) = N^2 l_p$ and $\bar{\omega}(n)$ the relaxation rate of the nth mode. The specific form of $\bar{\omega}(n)$ is in general contingent upon the model used for the composite bundle but in the limit of large N and for fixed bundle diameter $D = bN^{1/2}$, where b is the filament diameter and $\bar{\omega}(n)$ in general approaches the Timoshenko limit form [29]

$$\bar{\omega}(n)/\omega_1 = \frac{N^2}{1+\Delta n^2}. \tag{3}$$

The frequency scale $\omega_1 = (\kappa\pi^4/\zeta\xi^4)$ is the relaxation rate of the $n = 1$ mode of a single filament with length ξ and friction coefficient per unit length of filament ζ. The dimensionless constant $\Delta = N(\pi^2/12)(Eb^2/\delta k_\times)(\delta/\xi)^2$ depends on the Young's modulus E of the filaments, the filament radius b, the distance δ between linker molecules, and the linker spring constant $k_\times$. The parameter Δ determines the high-frequency rheology. For $\Delta = 0$, $G(\omega)$ reproduces single-filament scaling with $G(\omega) \propto \omega^{3/4}$ (inset of Fig. 2). For $\Delta \gg 1$, the single-filament scaling regime disappears and only the asymptotic high-frequency bundle scaling of $G'(\omega) \simeq \omega^{1/2}$ and $G''(\omega) \simeq \omega$ remains. At intermediate values of Δ, numerical evaluation produces a frequency range with intermediate scaling exponents due to a crossover from the single-filament scaling to the asymptotic high-frequency bundle behavior.

The parameter Δ thus can be viewed as a rheologically accessible measure of the degree of bundling in the network. If we use Δ as a fitting parameter, then the high-frequency dynamics displayed in Fig. 2 would be consistent with $1.5 \geq \Delta \geq 0.4$. An evaluation based on the filament and linker elasticity and an estimate of the network's mesh size yield $\Delta \approx 2$ [19]. The comparison between simulation, experiment, and theory, as shown in Fig. 2, reveals that current rheological studies are in a crossover regime and have not yet reached the true high-frequency power-law regime characteristic of transient bundle networks.

Intermediate-frequency regime.—For frequencies $\omega < \omega_1$, Eq. (2) predicts a frequency-independent plateau modulus $G'(\omega) \simeq (\rho\xi/15\alpha(0))$ and a decreasing "fluid-like" $G''(\omega) \propto \omega$, but neither is observed in Fig. 2. The reason is that even weak oscillatory shear strains have a highly destructive effect on the ability of the transient linkers to establish double bonds. Even at frequencies well above the off rate k_{off}, the interval distribution between binding sites initially bound by cross-linkers is already being affected (see Fig. 3), although the overall network geometry remains intact. As shown in the inset, network bundles do eventually peel apart at sufficiently low ω when the low-frequency regime is approached. In the Supplemental Material [19], we show that the key features of the intermediate regime can be understood on the basis of a simple model of two parallel filaments connected by an array of doubly bound linkers similar to that of Ref. [24]. A striking feature of the intermediate regime is that different

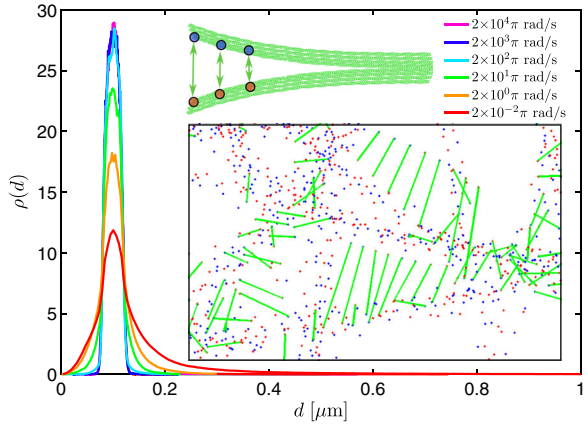

FIG. 3 (color online). The distribution of distances between pairs of initially cross-linked binding sites during oscillatory shear at frequencies of $\omega \in [20\,000\pi; 0.02\pi]$ rad/s showing that the network becomes more sparsely cross-linked at low frequencies. Inset: Binding site pairs (red and blue) with highlighted distances (green) between ruptured cross-links at sites of bundle-bundle detachment for the $\omega = 0.02\pi$ rad/s case.

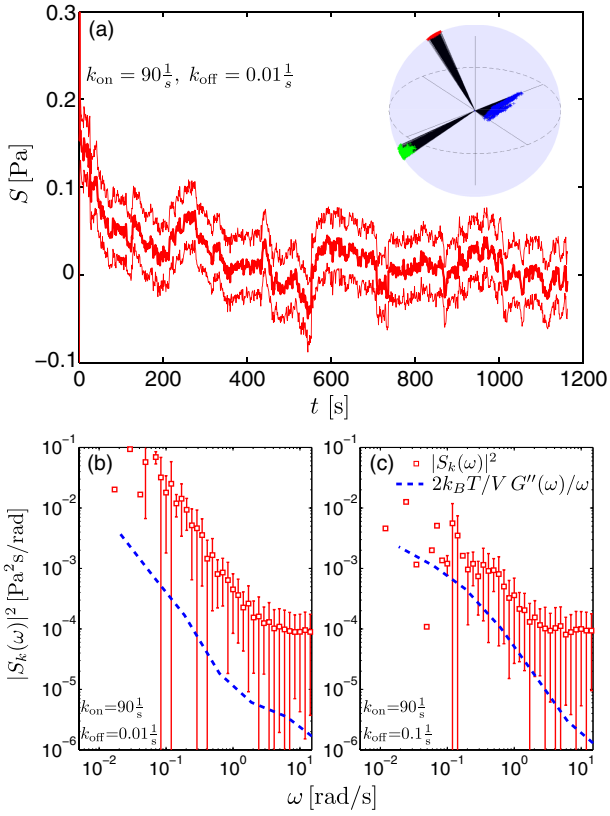

FIG. 4 (color online). (a) Shear stress relaxation and fluctuations after a step strain at $t = 0$ s. The $G' \sim G'' \sim \omega^{1/2}$ scaling relation predicts a $1/t^{1/2}$ stress decay. For $t > 200$ s, spontaneous large-amplitude fluctuations overwhelm the mean stress and are accompanied by subdiffusion of the principle axes of the moment of inertia tensor (inset). (b) Frequency-dependent mean square amplitude of the stress fluctuations (red squares) in the low-frequency regime at $k_{off} = 0.01$ s^{-1} compared to the prediction of equilibrium fluctuation from the fluctuation-dissipation theorem [Eq. (4)] (dashed blue line). (c) The nonequilibrium fluctuation enhancement disappears at an increased $k_{off} = 0.1$ s^{-1}.

samples with the same parameters produce significantly different complex moduli $G(\omega)$, in agreement with the expectation that at lower frequencies, the system becomes increasingly dependent on preparation history.

Low-frequency regime.—In the limiting regime $\omega \ll k_{off}$, we did not observe the expected terminal Newtonian rheology with $G''(\omega) \propto \eta\omega$. Rather, Fig. 2 shows a low-frequency power-law rheology where G', $G'' \sim \omega^{1/2}$. This behavior has been previously observed in simulation [24] as well as in experiment [30,31] and is related to transient cross-linking [32]. It was interpreted as a consequence of single-filament dynamics connected to a background of other filaments by transient linkers [24]. According to this scaling relation, one would expect that, after a step strain, the stress should relax as a power law $1/t^{1/2}$. The computed stress relaxation following a step strain, shown in Fig. 4(a), presented us with a first surprise. After an initial relaxation, *spontaneous* stress fluctuations were so large that it was difficult to ascertain the expected $t^{-1/2}$ decay of the mean stress. To characterize these low-frequency fluctuations, we computed the principle axes of the moment of inertia tensor. If the system were fluid, the principal axes would perform rotational Brownian motion with a power spectrum $|\phi(\omega)|^2 \propto 1/\omega^2$. For an elastic network, $|\phi(\omega)|^2$ would be Lorentzian. The best fit to the measured power spectrum, however, is given by the *subdiffusive* form $|\phi(\omega)|^2 \propto 1/\omega$. These slow orientational fluctuations of structures on the scale of the simulation box indicate that *large-scale collective motion* plays a key role in the low-frequency regime, in contrast to the single-filament interpretation. We speculate that the slow dynamics of the principal axes could be related to thermally excited Goldstone modes associated with orientational symmetry breaking by the bundle network on length scales of the size of the simulation box.

In order to verify whether or not these stress fluctuations are enhanced by the nonequilibrium aging of the network, we evaluated the fluctuation-dissipation theorem (FDT), which provides a connection between the mean square of the amplitude of the stress fluctuations and $G(\omega)$:

$$\langle |S(\omega)|^2 \rangle = \frac{2k_BT}{\omega} G''(\omega). \tag{4}$$

In Fig. 4(b), we see that the stress fluctuation amplitude exceeds the value predicted by the FDT at lower frequencies; we detect nonequilibrium, large-scale network reorientations that are associated with its structural aging and incompatible with equilibrium motion. As expected, if one speeds up the network's structural relaxation by increasing the linker off rate from 0.01 s^{-1} [used in Figs. 4(a) and 4(b)] to 0.1 s^{-1}, the observed stress fluctuations are reduced and, in fact, now consistent with the FDT prediction—see Fig. 4(c). The overall structure of these networks, however, has not yet equilibrated, which is consistent with experiment [33].

In summary, we have presented an analysis of the rheology of transiently cross-linked semiflexible networks made up of bundles. The mechanics of such bundle networks are quite distinct from those composed of individual semiflexible filaments. We propose that the rheology of such systems can be understood in terms of single-bundle dynamics in the high-frequency regime, in terms of dissipative bond-breaking dynamics at intermediate frequencies, and in terms of collective orientational fluctuations and large-scale network rearrangements at low frequencies. The altered rheology of bundle networks will have implications for the mechanics of cells [34] and for novel synthetic materials [28,35].

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