Origin of Slow Stress Relaxation in the Cytoskeleton

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Dynamically cross-linked semiflexible biopolymers such as the actin cytoskeleton govern the mechanical behavior of living cells. Semiflexible biopolymers nonlinearly stiffen in response to mechanical loads, whereas the cross-linker dynamics allow for stress relaxation over time. Here we show, through rheology and theoretical modeling, that the combined nonlinearity in time and stress leads to an unexpectedly slow stress relaxation, similar to the dynamics of disordered systems close to the glass transition. Our work suggests that transient cross-linking combined with internal stress can explain prior reports of soft glassy rheology of cells, in which the shear modulus increases weakly with frequency.

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Biopolymers form the scaffolds of life, providing rigidity to both cells and the extracellular matrix [1–3]. An important characteristic of intra- and extracellular biopolymers [4–6] is their high bending rigidity relative to most synthetic polymers. This feature leads to a competition between entropic and energetic effects that results in a range of material properties not captured by traditional polymer physics. One such property is the highly nonlinear elastic response of biopolymer networks, in which the shear rigidity can increase by orders of magnitude upon strains of only a few percent [7]. For permanently cross-linked semiflexible polymer networks, this phenomenon is well accounted for by the compliance due to transverse bending fluctuations that become suppressed under a load [8,9].

The interactions of biopolymers are also more complex than for traditional polymer materials. An example is the transient cross-linking by specialized crosslinker proteins that takes place in the actin cytoskeleton of the cell, which causes stress relaxation on timescales longer than the typical cross-linker unbinding time [10,11]. The resulting viscoelastic flow does not follow a simple Maxwell model with a single relaxation time, but instead follows power law behavior characteristic of a broad spectrum of relaxation times [12,13].

Here, we show that the nonlinear response of transiently cross-linked actin networks exhibits an unexpectedly slow stress relaxation, resembling the dynamics of soft glassy systems [14]. As reported in Ref. [15] and a large body of follow-up work on cell rheology [16–21], the shear modulus of cells is characterized by a shear modulus that increases as a weak power-law of frequency, with exponents as low as 0.1, for which the term "soft glassy rheology" has been used. Interestingly, in contrast with prior models of soft glassy rheology of cytoskeletal networks [22–24], here we show that exponents below 0.5 are only observed in the nonlinear regime. We show that the time- and stress-dependent response of actin networks is consistent with a model that accounts for both the nonlinear stiffening [7,8] and transient cross-linking [13] of semiflexible polymers. Our results can provide an explanation for the many prior reports of slow relaxation and near solid-like viscoelastic response in reconstituted cytoskeletal networks [22,25] and in living cells [15–21]. While these phenomena have been discussed in the context of phenomenological soft glassy rheology [15,16,19,22,23,25], a more microscopic mechanism has been lacking. The present Letter suggests that the glassy dynamics in the cytoskeleton are a natural consequence of transient cross-links, combined with prestress.

Using small amplitude oscillatory rheology, we measure the storage (crosses) and loss moduli (circles) in the absence of prestress (black data points) as a function of frequency for reconstituted actin networks, cross-linked by the dynamic linker α-actinin-4 (ACTN4), a prominent cross-linker in human cells [26,27] [Fig. 1(c)]. Qualitatively consistent with previous experiments and modeling [13], we find a power law frequency dependence of the moduli at frequencies below 1 Hz (black line) with an exponent close to $\frac{1}{2}$. The $\frac{1}{2}$ exponent reflects the broad spectrum of relaxation times from the unbinding and rebinding of multiple cross-linkers along a filament [Fig. 1(a)] [13]. The viscous modulus becomes less frequency dependent for higher frequencies (>1 Hz), and is expected to peak at the cross-linker unbinding rate (not observed here and therefore likely beyond 10 Hz) as cross-linker unbinding becomes increasingly unlikely [13].

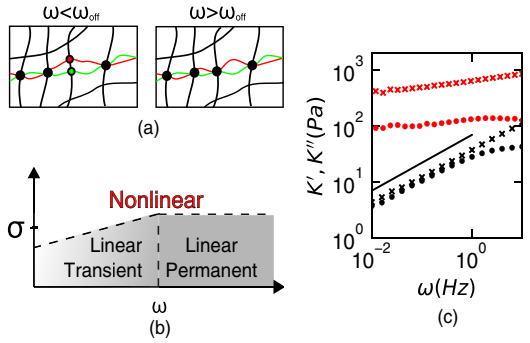

FIG. 1. Time-dependent viscoelastic response of transiently cross-linked semiflexible polymer networks. (a) Schematic showing actin filaments (black lines) connected by cross-linkers (black dots). Left: filament before and after a cross-linker remodeling event (green and red). Right: such events are unlikely for frequencies larger than the cross-linker unbinding rate ω_{off}. (b) Schematic regime diagram showing three different viscoelastic regimes as a function of frequency and applied stress. At low stress, in the linear regime, networks either behave as permanent networks exhibiting a plateau or cross-link kinetics lead to a frequency-dependent transient regime. Our work shows that, beyond an onset stress, a single length scale that is nonlinear in both stress and frequency governs the mechanics. This onset stress decreases for frequencies below the cross-linker unbinding rate as the effective cross-linker distance decreases. (c) The storage (crosses) and loss moduli (circles) of a cross-linked actin network against frequency in the absence of prestress (black) and for 8 Pa prestress (red). The line indicates a $\frac{1}{2}$ power law.

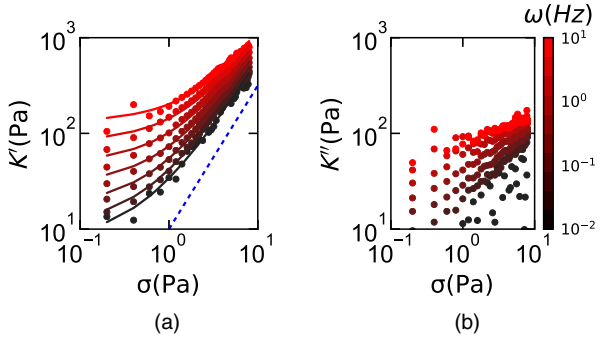

FIG. 2. Time-dependent stiffening. The differential storage (a) and loss (b) moduli of an ACTN4 cross-linked actin network are plotted against the applied stress and color coded as a function of frequency from 0.01 Hz in black to 5 Hz in red in seven logarithmically spaced steps. The solid lines represent fits to Eq. (1). The blue dashed line represents the $\frac{3}{2}$ power law that is characteristic of *permanently* cross-linked networks [7].

Next, to probe the nonlinear response, we measure the differential modulus $\partial\sigma/\partial\gamma$, where σ is the shear stress and γ is strain. We do so by superimposing a small amplitude oscillation on an 8 Pa prestress [red data points in Fig. 1(c)]. We find that both real (storage) and imaginary (loss) moduli are larger in the presence of prestress by 1–2 orders of magnitude than in the absence of prestress over the entire frequency range. We attribute this change to the stress stiffening response of semiflexible polymer networks to suppression of filament bending fluctuations [8,9]. More surprisingly, we find that both the storage and loss moduli are significantly less frequency-dependent in the presence of prestress than the $\frac{1}{2}$ power law observed in the absence of prestress.

To find out the origin of the stress-dependent changes in the time-dependent rheology, we systematically vary the prestress over a range from 0.1 to 8.0 Pa with a superimposed small amplitude oscillation at different frequencies ($\omega = 0.01, \ldots, 10\,\mathrm{Hz}$). We find that both the differential storage and loss moduli increase as a function of prestress over all frequencies [Fig. 2]. This increase is consistent with an asymptotic $\sigma^{3/2}$ power-law stress stiffening (indicated by the blue dashed line), as previously identified both experimentally and theoretically for semiflexible polymer networks at high σ [5,7]. To test the agreement with the model more quantitatively, we fit the differential storage modulus at each frequency to the following cross-over function:

$$K' = G(1 + \sigma/\sigma_{0,\mathrm{tr}})^{3/2}, \tag{1}$$

where $G(\omega)$ is the linear storage modulus and $\sigma_{0,\mathrm{tr}}$ is the characteristic stress for the onset of stiffening at a given frequency. Remarkably, although the model for the $\sigma^{3/2}$ stiffening was developed and confirmed previously for the nonlinear elasticity of *permanently* cross-linked networks, it accurately captures the nonlinear elastic response of transiently cross-linked actin networks as well. However, whereas the onset stress for nonlinearity of permanently cross-linked networks is independent of frequency [5,7], we find that this onset systematically increases with frequency for transiently cross-linked semiflexible polymer networks [Fig. 3(a)].

In order to capture both the frequency and the stress dependence of the shear moduli, we propose a model in

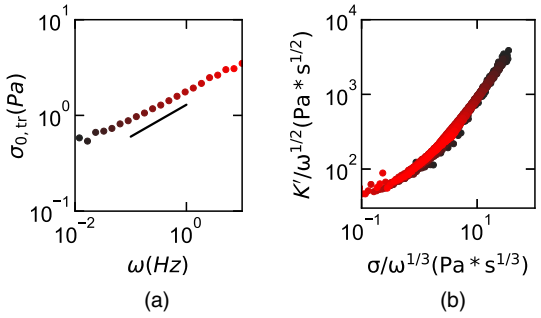

FIG. 3. Mastercurve behavior of the time and stress dependent viscoelastic behavior of actin networks. (a) The onset stress for stiffening of actin networks follows a $\frac{1}{3}$ power law dependence on frequency (black line), consistent with Eq. (6). (b) Stress-stiffening curves over all frequencies can be collapsed onto a single mastercurve using Eq. (9). The color coding is identical to Fig. 2.

which we combine the transient nature of the cross-linkers with the nonlinear force-extension behavior of the semiflexible actin filaments. For permanently cross-linked networks, the storage modulus in the linear regime is dependent on the distance between cross-linkers, l_o [8]:

$$G_0 \sim \rho\kappa l_p / l_0^3, \tag{2}$$

where l_p is the persistence length of the filament, ρ the filament length density per volume, and κ the bending rigidity of the filament. In the nonlinear regime, the modulus becomes independent of the distance between cross-linkers, but is defined by the length scale beyond which bending wavelengths are suppressed due to the filament axial tension τ [2]:

$$l_\tau \sim \sqrt{\kappa/\tau}. \tag{3}$$

As filament axial tension increases with the applied stress, $\sigma \sim \rho\tau$, the relevant bending wavelengths become smaller and the storage modulus increases nonlinearly with the applied stress:

$$K \sim \rho\kappa l_p / l_\tau^3 \sim \frac{\rho\kappa l_p}{l_0^3}(\sigma/\sigma_0)^{3/2}, \tag{4}$$

where $\sigma_0 \sim \rho\kappa/l_0^2$ is the threshold stress at which the network begins stiffening as the typical filament bending wavelength decreases below the typical crosslinker distance.

The important difference between transient networks and permanently cross-linked ones is that the effective cross-link distance increases with time as longer wavelength bending modes relax due to cross-link unbinding and rebinding [13]. This can be captured by an effective cross-link distance

$$l_{\mathrm{tr}} \sim \omega^{-1/6} > l_0 \quad \text{for } \omega < \omega_{\mathrm{off}}, \tag{5}$$

which leads to a $\omega^{0.5}$ dependence of the shear modulus, as reported experimentally and theoretically in Ref. [13]. Here, ω_{off} is the cross-link unbinding rate. As a result, the onset for stress stiffening now depends on frequency according to

$$\sigma_{0,\mathrm{tr}} \sim \rho\kappa/l_{\mathrm{tr}}^2 \sim \omega^{1/3}, \tag{6}$$

consistent with Fig. 3(a). In order to capture both transient and permanent regimes, we let

$$l_{\mathrm{tr}} = l_0(1 + \sqrt{\omega_{\mathrm{off}}/\omega})^{1/3}. \tag{7}$$

Strictly speaking, this is correct in the asymptotic plateau ($\omega \gg \omega_{\mathrm{off}}$) and transient ($\omega \ll \omega_{\mathrm{off}}$) regime, while it is only approximate in the crossover regime at intermediate frequencies.

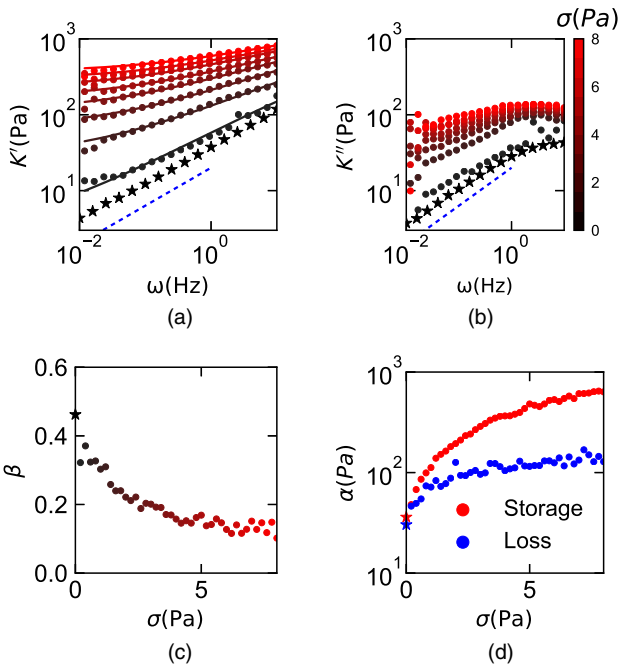

FIG. 4. Frequency dependence of stressed actin networks. The differential storage (a) and loss (b) moduli of an ACTN4 cross-linked actin network are plotted against the applied frequency and color coded as a function of prestress from 0.2 Pa in black to 7.2 Pa in red with 1 Pa steps. The stars are data in the absence of prestress. The blue line represents the $\frac{1}{2}$ power law characteristic of networks *in the absence of stress* [13]. The solid lines represent fits to $K'(\sigma,\omega)$ according to Eq. (9) (see Fig. S1 for the fitting parameters [28]). The differential moduli are fitted between 0.01 and 1 Hz with an empirical power law $\alpha(\sigma)\omega^{\beta(\sigma)}$. The exponent β (c) and prefactor α (d) are shown as a function of stress.

Similarly, in order to approximate the crossover from the linear to the nonlinear regime, we let

$$l_\tau = l_{\mathrm{tr}}[1 + (\sigma/\sigma_{0,\mathrm{tr}})]^{-1/2}. \tag{8}$$

Again, strictly speaking, this is correct for linear ($\sigma \ll \sigma_{0,\mathrm{tr}}$) and highly nonlinear ($\sigma \gg \sigma_{0,\mathrm{tr}}$) regimes, although we show below that it approximates well the behavior of actin networks over the entire experimentally accessible range of stress.

The resulting expression for K' is

$$K' \sim \rho\frac{\kappa l_p}{l_0^3}\frac{[1 + (\sigma/\sigma_{0,\mathrm{tr}})]^{3/2}}{(1 + \sqrt{\omega_{\mathrm{off}}/\omega})}. \tag{9}$$

This model accurately describes the observed trends in the nonlinear rheology of actin networks. First, the theory predicts a $\omega^{1/3}$ scaling of onset stress for nonlinearity [Eq. (6)], consistent with our experimental data [Fig. 3(a)]. Second, using Eq. (9), we successfully collapse all stress-stiffening data [Fig. 3(b)]. Lastly, we use Eq. (9) to accurately fit the differential storage modulus as a function of frequency over all prestresses [Fig. 4(a)]. Interestingly,

using ω_{off} as a free parameter, we find that the characteristic frequency decreases as the applied stress is raised (Fig. S1 in the Supplemental Material [28]). This result is consistent with earlier rheological measurements on networks cross-linked by ACTN4 [31], and suggests catch bonding [32] where the cross-linker unbinding rate decreases with force [33].

We find that the elastic response of transiently cross-linked actin networks is well captured by a simple model that combines prior models for the linear viscoelasticity of transient gels and the nonlinear elasticity of permanent networks. Key to our model is a single length scale defined by Eqs. (7) and (8) that characterizes the upper limit of fluctuating wavelengths. This length depends on both time and stress. Together, these effects result in a frequency dependence that becomes weaker with increasing stress [Figs. 4(a) and 4(b)]. The $K \sim \omega^{1/2}$ power law predicted by Ref. [13] is only observed in the absence of prestress [Fig. 1(c)]. We quantify the dependence of stiffening on stress by fitting the data with an empirical power law $K(\sigma, \omega) = \alpha(\sigma)\omega^{\beta(\sigma)}$ commonly used in the cell rheology literature [15,17–19,21]. We find that the prefactor α increases for the loss modulus and, even more steeply, for the storage modulus as the network stiffens, such that the network becomes more solidlike with increasing stress [Fig. 4(d)]. We also find that the exponent β decreases from 0.5 in the absence of stress to 0.1 at 8 Pa [Fig. 4(c)]. This is in contrast to the mechanics of permanently cross-linked networks [8,9] that are time independent except at very high frequencies, typically beyond 100 Hz, where the viscous drag of the fluid controls filament relaxation [34–37]. In that regime, an exponent β of $\frac{3}{4}$ is expected, but this can decrease to $\frac{1}{2}$ under stress [38]. Recently, Ref. [24] proposed a model for the nonlinear response of transient semiflexible networks, but no specific relationship between the stress and the exponent governing the time dependence was predicted.

Other work on stressed dynamically cross-linked actin networks has focused on the effect of force-induced linker (un)binding [31,39,40], sliding of cross-linkers along filaments [41,42], and the effect of shear-induced filament alignment [43]. While our minimal model does not include such effects, it is able to accurately capture the stress and frequency dependence of the nonlinear elastic response of actin networks. In future work, it would be interesting to include the additional microscopic effects mentioned above [31,39–42,44], as well as to quantitatively understand the differential loss modulus, K'', for example by using detailed network simulations of transiently connected semiflexible polymers [45].

We find that stressed semiflexible polymer networks exhibit power law dynamics with a small exponent ($\beta \sim 0.1$). Remarkably, mechanical experiments on living cells have revealed similar power law dynamics [15–21]. These mechanical properties are reminiscent of observations on a range of disordered systems close to the glass transition [14]. The soft glassy rheological properties found in cells have been phenomenologically described by assuming a broad distribution of microscopic relaxation timescales [15,22,23,25]. Whilst this phenomenological description can account for the experimental data [15,17–22,25], it offers no insight into the microscopic processes governing these dynamics. Here we suggest that the glassy dynamics are a natural consequence of transient cross-links, combined with prestress. This mechanism is different from the microscopic mechanism underlying soft, glassy rheology in systems such as colloidal gels, where particle density, rather than prestress, controls the stress relaxation exponent [46].

Myosin motor-driven contractility is a likely source for such prestress in the cell [38,47]. Consistent with our results, experiments on cells have revealed that the power law exponent of the frequency dependent shear moduli decreases with the internal stress generated by actomyosin contractility [21]. Remarkably though, whereas the reconstituted networks become more solidlike with prestress [Fig. 4(d)], mechanical experiments on cells have shown that the loss modulus increases more rapidly than the storage modulus as a function of myosin-driven tension [21]. We speculate that external stress as imposed in our rheological experiments differs from internal stresses generated by myosin motors, as motors not only cause contractility but also fluidize networks via filament sliding [48,49] and severing [50,51].

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