Cross-link governed dynamics in F-actin gels


  *Nonlinear Viscoelasticity of Actin Cross-linked with Mutant α-Actinin-4* (Under review, 2011)

  *Stress-enhanced Gelation of Biopolymer Networks*, (to be submitted)
CHAPTER 2. CROSS-LINK GOVERNED DYNAMICS

Abstract

Recent experiments show that networks of stiff biopolymers cross-linked by transient linker proteins exhibit complex stress relaxation, enabling network flow at long times. We present a model for the dynamics controlled by cross-links in such networks. We show that a single microscopic timescale for cross-linker unbinding leads to a broad spectrum of macroscopic relaxation times and a shear modulus $G \sim \omega^{1/2}$ for low frequencies $\omega$. This model quantitatively describes the measured rheology of actin networks cross-linked with $\alpha$-Actinin-4 over more than four decades in frequency. Furthermore, we demonstrate an unexpected mechanical behavior in these systems under large external stresses. Applied stress enhances gelation of these networks by delaying the onset of structural relaxation, thereby extending their solid-like behavior to lower frequencies.

2.1 Introduction

Reconstituted biopolymers such as actin are excellent models for semi-flexible polymers, with network mechanics and dynamics that are strikingly different from flexible polymer networks [1–8]. One essential feature setting biopolymer networks apart from rubber-like materials is the intrinsic dynamics of their cross-links. Such systems represent a distinct class of polymeric materials whose long-time dynamics are not governed by viscosity or reptation [9], but rather, by the transient nature of their cross-links. This can give rise to a complex mechanical response, particularly at long times, where the network is expected to flow. Such flow can have important implications for cells, where their internal networks are constantly remodeling, reflecting the transient nature of their cross-links [10]. The simplest possible description of a material that is elastic on short timescales while flowing on long timescales is that of a Maxwell fluid; this exhibits a single relaxation time $\tau$, as depicted in Fig. 2.1. Indeed, some recent experiments on transient networks have been modeled with a single relaxation time [11, 12]; however, those experiments and others [13, 14]—probing longer relative time-scales compared to the linker unbinding time—evidence a more complex viscoelastic behavior, indicative of multiple relaxation times. Thus, the basic physical principles governing transient networks remain unknown. A predictive theoretical model is essential to elucidate the effect of dynamic cross-linking, and to help explain the reported complex viscoelastic behavior.
Figure 2.1 – (color online) A schematic of the frequency dependent shear modulus $G^* = G' + iG''$. Non-permanent networks can exhibit a response ranging from a single timescale ($\tau$) Maxwell-like behavior (blue lines) to a powerlaw regime with an exponent $< 1$ governed by a broad distribution of relaxation times ($> \tau$) (red lines). Upper inset: for times longer than the unbinding time $\tau_{\text{off}}$, large scale conformational relaxation can occur via linker unbinding (open circle) and subsequent rebinding at a new location. Lower inset: for shorter times, only small-scale bend fluctuations between cross-links can relax, resulting in a plateau in $G'$ for frequencies $> 1/\tau_{\text{off}}$. 
Here, we develop a microscopic model for long-time network relaxation that is controlled by cross-link dynamics. This cross-link governed dynamics (CGD) model describes the structural relaxation that results from many independent unbinding and rebinding events. Using a combination of Monte Carlo simulations and an analytic approach, we demonstrate that this type of cross-link dynamics yields power-law rheology arising from a broad spectrum of relaxation rates. Our predictions are in excellent quantitative agreement with experiments on actin networks with the transient linker protein α-Actinin-4.

The CGD model can be qualitatively understood in simple physical terms. We assume each filament is cross-linked to the network, with an average spacing $\ell_c$. Only filament bending modes between cross-links can relax (Fig. 2.1, lower inset), and the thermalization of these modes results in an entropic, spring-like response. To account for transient cross-linking, we assume that the linkers unbind at a rate $1/\tau_{\text{off}}$ (Fig. 2.1, upper inset), which may depend on temperature. This initiates the relaxation of long wavelength ($> \ell_c$) modes, giving rise to a reduced macroscopic modulus. However, the relaxation of successively longer wavelength modes becomes slower, as an increasing number of unbinding events are needed for such a relaxation. This simple physical picture suggests a broad spectrum of relaxation times, as opposed to the single relaxation time of the Maxwell model. As outlined below, both simulations and an analytic treatment of this model yield power-law behavior with $G \sim \omega^{1/2}$ below the characteristic frequency $\omega_0 = 2\pi/\tau_{\text{off}}$ (Figs. 2.2 A and B).

We compare the basic predictions of this model to the rheology of a representative transiently cross-linked actin network. As a cross-linker, we use α-Actinin-4 [14, 15], whose unbinding time $\tau_{\text{off}}$ is reported to be in the range $1 - 10s$. These gels (See section 2.4) [16] exhibit a low-frequency elastic shear modulus $G'$ with a pronounced decay over three decades in frequency, while the viscous modulus $G''$ exhibits a broad local maximum located near the characteristic frequency of cross-link unbinding [11–13,16] (Fig. 2.2B). In the asymptotic low-frequency range, both moduli exhibit power-law rheology with an approximate exponent of $1/2$, in agreement with our predictions. Such behavior clearly indicates a more complex stress relaxation than captured by the Maxwell model, which is governed by a single relaxation time (Fig. 2.1). Taken together, the theoretical and experimental results demonstrate a distinct cross-link governed regime of network dynamics. Finally, we also provide experimental evidence of such dynamics for networks under large external loads. However, the applied stress does affect both the rate of linker unbinding dynamics and the stiffness of the networks, which results in a rich nonlinear viscoelastic response.
2.2 Results and discussion

To develop a predictive microscopic model, we first consider a single polymer within the network, and then extend the description to the macroscopic level. On length-scales longer than $\ell_c$, the motion of the polymer is constrained by its cross-linking to the surrounding network (Fig. 2.1). When a linker unbinds, a local constraint is released, allowing for the relaxation of the freed segment. This thermal relaxation occurs within a time $\tau_{eq}$, which is typically of order milliseconds [3,4,7]. We assume that this process is completed before the segment rebinds to the network at a new location; thus, $\tau_{eq} \ll \tau_{on}$, where $\tau_{on}$ is the rebinding time of the linkers. Furthermore, assuming $\tau_{on} \ll \tau_{off}$, only a small fraction of cross-links will be unbound at any given time, and simultaneous unbinding of neighboring cross-links can be neglected. This suggests a coarse-grained description on length-scales $> \ell_c$, in which independent unbinding events occur at a rate $1/\tau_{off}$. Since the relaxation of wavelengths $< \ell_c$ occurs at a much faster rate $1/\tau_{eq}$, we use the worm-like chain model, where the equilibrated short wavelength fluctuations manifest themselves as an entropic stretch modulus $\mu_{th} \sim \kappa^2 / \ell_c^3 k_B T$ [2,4]. Here, $\kappa$ is the bending rigidity, $k_B$ is Boltzmann’s constant and $T$ is the temperature. In this description the coarse-grained energy is given by

$$H_{CG} = \frac{1}{\ell_c} \sum_n \left[ \frac{\kappa}{2} |\Delta t_n|^2 + \frac{\mu_{th}}{2} \left( |\Delta r_n| - \ell_c \right)^2 \right],$$

(2.1)

where the sum extends over all cross-link positions $r_n$, $t_n$ is the unit tangent vector and, e.g. $\Delta r_n = r_{n+1} - r_n$.

Using the coarse-grained energy $H_{CG}$, we study the dynamics arising from multiple linker unbinding events, by performing 2D simulations of a single polymer. An initial chain conformation with periodic boundary conditions is randomly drawn from a Boltzmann distribution. Cross-link unbinding events are independent and result in the complete thermal equilibration of the two neighboring polymer segments. This is numerically implemented via a Metropolis Monte Carlo algorithm. These simulations allow us to determine the equilibrium fluctuations enabled by linker unbinding of a single polymer embedded in a network that is treated as a rigid medium. According to the fluctuation dissipation theorem (FDT), the linear mechanical response of the polymer is encoded in the fluctuations of the extension, $\delta \ell$, of the polymer. Interestingly, the simulations demonstrate that the power spectrum $C(\omega) = \langle |\delta \ell(\omega)|^2 \rangle$ depends on frequency as a fractional power-law consistent with an exponent $-3/2$, as shown in the inset of Fig. 2.2A, indicating a broad underlying distribution of relaxation times. Although this exponent also arises in the Rouse model for flexible polymers due to the viscous dynamics of longitudinal stretch modes [9], this is not the origin of the behavior found here. Our model does exhibit longitudinal modes; however, their contribution $C_\parallel$ to full spectrum is subdominant (inset of Fig. 2.2A). This demonstrates
Figure 2.2 – (color online) A) The simulated rheology for frequencies below $\omega_0$. The shear modulus is normalized by the elastic plateau value $G_0$. The inset shows the total power spectrum $C(\omega)$ (blue circles) of distance fluctuations, as well as the fraction $C_{||}$ coming from effective stretch fluctuations originating in undulations on length scales shorter than the cross-linking distance. The distance fluctuations are determined over a length $16\ell_c$ of a polymer with a persistence length $\ell_p = 32\ell_c$ and a total length $32\ell_c$. The solid black line represents our analytical mean-field CGD prediction. B) Measured linear rheology of a 23.8 $\mu$M actin network cross-linked with various concentrations of $\alpha$-Actinin-4. The low frequency behavior is consistent with $G \sim (\omega)\langle \omega \rangle^{1/2}$. The solid and dashed lines are global fits utilizing our mean-field CGD model for the low frequency regime together with the known high frequency response [3, 4].
that the polymer’s response to an applied tension is dominated by the dynamics of transverse modes.

The dynamical description of a single polymer can be extended to the network level by assuming that the network deforms affinely. The macroscopic shear modulus $G^*$ is then related to the complex response function $\chi$ of relative length extension of a single polymer in response to a tensile force: $G^* = \rho/(15\chi)$, where $\rho$ is the length of polymer per unit volume [3, 4]. Ignoring end effects, the relative extension $\delta \ell/\ell$ of a polymer segment of length $\ell$ is conjugate to the uniform tension $f$, with $\delta \ell(\omega)/\ell = \chi(\omega)f(\omega)$.

We use the FDT to calculate the imaginary part of the extensional response function $\ell \chi''(\omega) = \omega \langle \delta \ell^2(\omega) \rangle / 2k_B T$. Using the Kramers-Kronig relation, we compute the response function $\chi$ and the network shear modulus $\gamma$. Below $\omega_{\text{off}}$, the shear modulus depends on frequency as a power-law with an exponent of $1/2$ (Fig. 2.2A), consistent with experiments (Fig. 2.2B).

To obtain further insight, we develop a continuum analytical treatment of the CGD model. We calculate the polymer displacement due to the unbinding and subsequent rebinding of a linker to the $n$-th cross-link site. We separate the local equilibration step into a move to the minimum energy position, together with a stochastic thermal contribution set by the form of the energy around the mechanical equilibrium. The mechanical relaxation step $r^{(i)}_n \rightarrow r^{(\text{meq})}_n$, from the initial (i) position to the local equilibrium position (meq), is determined by

$$0 = \frac{\partial H_{CG}}{\partial r_n} \bigg|_{r_n = r^{(\text{meq})}_n}. \quad (2.2)$$

This condition replaces the usual force balance of drag and conservative terms in the low-Reynolds number regime. By performing the discrete calculation solving Eq. (2.2) and taking the continuum long-wavelength limit, the leading order evolution equations are (see section 2.5) [17]

$$\tau_{\text{off}} \partial_t r_\parallel = \frac{\ell^2}{2} \partial_x^2 r_\parallel + \hat{e}_x : \xi_\perp \quad (2.3)$$

$$\tau_{\text{off}} \partial_t r_\perp = \frac{\ell^2}{2} \partial_x^2 r_\perp + \xi_\perp. \quad (2.4)$$

Here $r_\perp$ and $r_\parallel$ are the transverse and longitudinal deflections of the polymer with respect to its average direction $\hat{e}_x$. The noise $\xi_\perp$ captures both thermal effects and local bucking contributions due to thermally-induced compression (see section 2.5) [17]. While thermal contributions can be calculated from a quadratic expansion of $H_{CG}$ around its local mechanical equilibrium, the state of the surrounding polymer influences the form of the Hessian, inducing correlations in the noise. In the inextensible limit, the longitudinal component of $\xi$ is subdominant and is neglected.

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1The Kramers-Kronig relation involves an integral over the whole frequency domain. Since we only simulate the low frequency part, we supplement this with the expected plateau above $\omega_0$. 

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Importantly, the noise $\xi_\perp$ depends nonlinearly on the local state of the polymer and couples Eqs. (2.5,2.6). To explore this coupling, we artificially reduce the stretch modulus $\mu$. In the limit $\mu \ll \mu_{th}$, the evolution equations decouple to leading order and become exactly solvable

$$\tau_{off} \partial_t r_\parallel = \frac{f_c^2}{2} \partial_x^2 r_\parallel + \xi_\parallel \quad (2.5)$$

$$\tau_{off} \partial_t r_\perp = -\frac{f_c^4}{6} \partial_x^4 r_\perp + \xi_\perp \quad (2.6)$$

In this limit, both transverse and longitudinal noise are uncorrected in space and time. The resulting transverse contribution approaches $C_\perp \sim \omega^{-7/4}$. This can also be seen in our simulations with variable $\mu < \mu_{th}$ in Figs. 2.3 A and B. As $\mu$ is reduced below $\mu_{th}$, $C_\perp$ evolves toward $C_\perp \sim \omega^{-7/4}$, which can be seen by the flattening of the normalized spectrum in Fig. 2.3B. In the limit $\mu \ll \mu_{th}$, the transverse bending dynamics are effectively those of a stiff filament fluctuating in a viscous solvent, for which the time-dependent fluctuations are $\langle |\delta \ell(t)|^2 \rangle \sim t^{3/4}$ [3, 4, 18]. Only in this decoupled limit, can one understand the dynamics within the framework of an effective viscosity provided by the transient cross-links [17].

The nonlinear nature of the noise $\xi_\perp$ in the evolution Eqs. (2.5,2.6), in the limit of an inextensible polymer, precludes a full analytical solution of the model. Instead, further insight is gained by approximating the amplitude of the noise term by its mean-field value (see section 2.5) [17]. In the CGD model the noise term captures the width of the thermal distribution associated to a local relaxation event triggered by linker unbinding; in this mean-field approach the local deviations in the width of this thermal distribution are neglected; the width of the distribution used for every local relaxation event is then calculated by averaging over the equilibrium distribution of polymer conformations. In this approximation, the noise contributions are uncorrelated in both time and space, resulting in the response function

$$\chi_{MF}(\omega) \approx 0.0036 \frac{k_B T f_c^3}{\pi \kappa^2} \int dq \frac{1}{q^2 - 2i \omega \tau_{off}}.$$ 

This response function captures the cross-link governed dynamics dominating on timescales $> \tau_{off}$. Furthermore, we calculate the mean-field correlator, $C_{MF} \sim \omega^{-3/2}$, in good agreement with the simulations presented in the inset of Fig. 2.2A. As a further test, we perform simulations over a wide range of $\kappa$ and polymer lengths $L$; the predicted amplitudes of the fluctuation spectrum are in good agreement with the simulated amplitudes, as shown in Fig. 2.3C. This further validates the assumptions made in our analytical approach.

To obtain a complete description of the behavior in the experimentally accessible range we include the viscous polymer dynamics relevant at high frequencies in our
Figure 2.3 – (color online) The power spectrum $C_{\perp}(\omega)$ of end-to-end fluctuations originating from transverse undulations on length scales longer than $\ell_c$, multiplied with $\omega^{3/2}$ (A) and $\omega^{7/4}$ (B) for a range of polymer backbone compliances. C) The simulated amplitude of the power spectrum $C(\omega)/\tau \ell_c^2$ plotted against the 2D mean-field prediction for a range of polymer lengths and bending rigidities.
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model [3, 4]. This extension relies on the separation of timescales of the fast viscous polymer dynamics and the slow cross-link governed dynamics, which implies that their contributions to the fluctuation spectra add in quadrature. The model agrees with the experimental data—over the full range of frequencies—with just three parameters: the plateau modulus, the equilibration time $\tau_{eq}$ and the unbinding time $\tau_{off}$ (see Fig. 2.2). We have globally fitted all data over a decade of cross-linking concentrations with a single value for $\tau_{off} = 2.7s$. This provides strong evidence that the low-frequency rheology of actin networks with the physiological linker $\alpha$-Actinin-4 is governed by the linker-controlled dynamics. Furthermore, the fitting procedure yields $\tau_{eq} < 0.07s$ consistent with $\tau_{eq} \ll \tau_{off}$; this, together with the quality of the fit, lends credence to the separation of timescales assumed in our model. Such a separation of timescales also implies that the fluid viscosity does not affect the rheology in the linker-governed regime, consistent with observations in other experiments [11, 12]. By contrast, for low enough cross-linking densities $\tau_{eq}$ becomes so large that the viscous dynamics and cross-link governed dynamics are no longer expected to be well separated. For an expected diffusive propagation of edge effects, we estimate a terminal relaxation time $\tau_r \approx \tau_{off} (L/\ell_c)^2$ [17]. As few as 10 cross-links per filament can account for the absence of a terminal relaxation in our experiments (Fig. 2.2). The possibility of observing a terminal relaxation for shorter filaments presents an interesting avenue for future experiments.

Many physiological actin cross-linking proteins are dynamic and should induce a $G^* \propto (i\omega)^{1/2}$ behavior at low frequencies. This may enable the cell to regulate its response; on timescales short compared to $\tau_{off}$, the network is effectively permanently connected—thereby providing mechanical resilience—while on longer timescales, dynamic linkers allow for complex network flow. This ability to flow and remodel is required for many vital cellular functions, ranging from motility to division. The extent to which transient cross-linking affects the mechanical properties of the cell is, however, still unknown. Interestingly, some rheological measurements on living cells have suggested a $1/2$ power-law behavior on time-scales ranging from several seconds to hours, consistent with our model for transient networks [19, 20]. Further experiments are needed to determine whether this regime is due to the transient nature of the cross-links.

2.3 Implications for cross-link dynamics at high stress

In this section we explore the effects of large external loads on transiently cross-linked F-actin/$\alpha$-actinin-4 gels. To this end, we examine the nonlinear mechanics using the prestress protocol (see chapter 5). We apply a constant pre-stress, $\sigma_0$, while superpos-
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ing a small amplitude oscillatory stress. We measure the resulting differential strain response, which allows us to determine the differential shear modulus as a function of frequency. Consistent with prior studied on cross-linked actin gels [6,21], the network stiffness increases with applied stress, as shown in Fig. 2.4. Surprisingly however, under applied stress we observe that the elastic plateau extends to significantly lower frequencies, which we characterize as stress-enhanced gelation. Together with the extension of the plateau, we observe a shift to lower frequencies of the local maximum in $G''$, which is identified as the onset of structural relaxation. Upon the removal of the external stress, the network response reverts back to the original linear behavior, indicating the reversible nature of stress enhanced gelation (data not shown). The behavior of these transiently cross-linked actin gels is diametrically opposed to the typical response of most materials, whereby an external stress leads to yielding and fluidization.

We obtain insight into this stress-enhanced gelation by investigating the dependence of the frequency for the onset of structural relaxation, $\omega_r$, as a function of the applied steady stress, as shown in Fig. 2.6. At low stresses $\omega_r \approx 0.5$ Hz, independent of the applied stress. Beyond stresses of $\approx 2$ Pa, however, the relaxation frequency decreases strongly after which it appears to level off at high stress to a value $\omega_r \approx 0.03$ Hz. Despite this dramatic shift in $\omega_r$, the functional form of the nonlinear data appears to be remarkably similar to that of the linear data (Fig. 2.4). In particular, the shear moduli of the prestressed gel exhibit a dependence $G \sim \omega^{1/2}$ at low frequencies, suggesting that the viscoelasticity at large stresses is also governed by cross-link dynamics. Within this picture, the shift of the onset for structural relaxation to lower frequencies is interpreted as an increase of the unbinding time of the linkers $\tau_{off}$ with stress, characteristic of catch-bond behavior. This is in contrast with the expectation in which an applied load reduces the binding affinity of the linker [22]. However, our observations here are analogous to the observed behavior in actin gels cross-linked with the non-physiological linker heavy meromyosin [12].

We hypothesize that the microscopic origin of the apparent catch-bond behavior in the case of the physiological linker $\alpha$-actinin-4 is due to a force-induced change in the protein conformation, which exposes an additional actin binding site with a high binding affinity. The binding head of $\alpha$-Actinin-4 contains three actin-binding sites: ABS, ABS 2, and ABS 3, as illustrated in Fig. 2.5. In the wild-type conformation the actin binding domain is thought to be a closed structure in which the ABS 3 binding site is largely buried [23]. However, under load the actin binding domain may open up enabling accessibility to the ABS 1 binding site. Interestingly, in $\alpha$-Actinin-4 proteins with a point mutation designated K255E, the hinge-like connection between the two CH domains is loosened and the structure is open, which indeed enhances the binding affinity of the linker [13,23]. In this case, the enhanced binding affinity is thought to be a consequence of the exposure of the high-affinity ABS 1 binding
Figure 2.4 – (color online) B) Measured linear rheology of a 23.8 µM actin network cross-linked at an actin:α-Actinin-4 molar ratio of 100:1 (black squares). The differential response under an applied steady prestress of 8.7 Pa is shown in red. In both cases the low frequency behavior is consistent with $G \sim (i\omega)^{1/2}$. Under stress, the plateau modulus of F-actin/α-Actinin-4 increases and the onset of structural relaxation (local maximum in $G''$) shifts to lower frequencies.
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\[ \alpha - \text{actinin-4 binding domain} \]

\[
\begin{array}{c}
\text{CH2} \\
ABS3 \\
ABS2 \\
\text{CH1} \\
\text{ABS1} \\
\text{F-Actin}
\end{array}
\]

Figure 2.5 – (color online) Schematic of the binding domain of wild type \( \alpha \)-actinin-4. In the wild type form the two domains are in a closed conformation and the ABS 2 and ABS 3 binding sites are largely responsible for the binding affinity of the linker [13,23]. We hypothesize that the conformation of the CH domains opens up under load exposing the high-affinity ABS 1 binding site; this mechanism can account for the load-induced reduction of the relaxation frequency we observe in the macroscopic rheological response of F-actin/\( \alpha \)-actinin-4 gels.

Our macroscopic rheological data on actin gels with wild type \( \alpha \)-actinin-4 under prestress has a relaxation frequency that appears to converge to \( \omega_r \approx 0.03 \) Hz at high loads, which is quantitatively consistent with the viscoelastic behavior of actin gels with K255E linkers in the absence of an applied load [13]. This supports the important role of the high affinity ABS 1 binding site in actin gels with wild type \( \alpha \)-actinin-4 under load. Thus, stress-enhanced gelation—characterized by a reduction of the relaxation frequency—can be accounted for by a force-induced exposure of the otherwise sterically hindered ABS 1 binding site.

The stress dependent viscoelastic behavior depicts three distinct regimes as summarized in Fig. 2.6. At the lowest applied stresses, the behavior is linear and thus independent of stress; as stress increases, the differential plateau modulus remains unchanged, while the relaxation frequency decreases substantially. It is only at the highest stresses that differential plateau modulus also exhibits nonlinear behavior. Interestingly therefore, there is an extended range of stress where the network nonlinearity appears to be a consequence of only the force-dependent linker unbinding dynamics. By direct analogy to the structural relaxation observed in networks formed with mutant Actn4 cross-links, the change in relaxation frequencies under stress is consistent with a change in affinity induced by load, a defining feature of catch-bond-like behavior. However, this conclusion is based on macroscopic observations, and
single molecular experiments are needed to confirm this mechanism. Furthermore, a nonlinear extension of the CGD model is required to quantitatively describe the nonlinear viscoelasticity of transiently cross-linked gels.

Our results may have interesting biological implications, relating to the mechanisms of intracellular remodeling and dynamic stress accommodation. In particular, we have identified a mechanism to independently control both the network stiffness and the onset time-scale for structural relaxation. This represents a novel design principle which may allow the cell to maintain structural integrity at longer time scales. While conventional network stiffening increases the elastic modulus, it does not prevent eventual network flow. By contrast however, stress-enhanced gelation may actually enable the cell to substantially delay network flow by extending the frequency range of solid-like behavior.
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Figure 2.6 – The relaxation frequency for the onset of structural relaxation and the differential plateau modulus as a function of applied stress. There are three regimes with qualitatively different behavior. In the grey regime the viscoelasticity is independent of applied prestress, in the orange regime the applied prestress reduces the onset of structural relaxation shift and enhances gelation, and in the blue regime the plateau modulus increases strongly—characteristic of stiffening behavior. The stiffening behavior in which differential plateau modulus scales with stress as $\sim \sigma^{3/2}$, is consistent with prior experiments on cross-linked actin gels [6] and the affine entropic model [2, 21].
2.4 Appendix 1: Materials and methods

Networks of cross-linked actin are formed by mixing 23.8 µM (1mg/ml) G-actin solution with corresponding α-actinin-4 solutions ranging from 0.0238 µM to 0.238 µM. Polymerization was initiated by the addition of 5x polymerization buffer. The mechanical response of the cross-linked actin networks is measured after one hour of polymerization at a temperature of 25°C. We use a stress-controlled rheometer (AR-G2, TA Instruments) using a 20mm diameter 2 degree stainless steel cone plate geometry and a gap size of 50 µm. We utilize a home-made steel bottom plate to ensure that the networks do not slip and a solvent trap to prevent drying. To measure the linear viscoelastic moduli, we apply an oscillatory stress of the form \(\sigma(\omega) = A\sin(\omega t)\), where \(A\) is the amplitude of the stress and \(\omega\) is the frequency. The resulting strain is of the form \(\gamma(\omega) = B\sin(\omega t + \delta)\) and yields the storage modulus \(G'(\omega) = A/B \cos(\delta)\) and the loss modulus \(G''(\omega) = A/B \sin(\delta)\). To determine the frequency dependence of the linear moduli, \(G'(\omega), G''(\omega)\) are sampled over a range of frequencies from 0.001 - 10 Hz.
2.5 Appendix 2: Evolution equations of the CGD model

Here we outline the basic steps used to derive the evolution equations of the CGD model presented in the main text. We describe the local relaxation of a polymer segment, upon unbinding of a cross-linker \( n \) at a position \( x_n \) along the average direction \( \hat{e}_x \) of the relaxed polymer. The release of cross-linker \( n \) lifts a constraint and enables the thermal relaxation of the two surrounding segments. To capture this local equilibration step, we approximate the thermal distribution as Gaussian and centered around the minimum energy position of the coarse-grained chain. This allows us to separate the equilibration step into a move to mechanical equilibrium and a stochastic thermal move.

The mechanical relaxation step of the cross-linker at an initial position \( r^{(i)}(x_n) \) is written as

\[
r^{(i)}(x_n) \rightarrow r^{(\text{meq})}(x_n) = r^{(i)}(x_n) + \Delta r^{(\text{meq})}(x_n) + \Delta r^{(\text{meq})}(x_n)\hat{e}_x. \tag{2.7}
\]

This step is determined through the local minimization condition

\[
0 = \frac{\partial H_{CG}}{\partial r_n} \bigg|_{r_n = r^{(\text{meq})}_n}. \tag{2.8}
\]

A discrete calculation gives a third-order equation for the mechanical relaxation and, using a convenient continuum notation for discrete differences over a length scale \( \ell_c \), we have

\[
\ell_b^2 (\ell_c^4 \partial_x^4 r_\perp + 3 \ell_c^2 \partial_x^2 r_\perp) = -\delta r_\perp \left( 2 \ell_c^2 \partial_x \phi + 6 \ell_b^2 + \delta r_\perp^2 \right), \tag{2.9}
\]

With \( \delta r_\parallel = \hat{e}_x \cdot \delta r_\perp \) and \( \ell_b = \sqrt{\kappa/\mu_{th}} \). Here we have decomposed the relaxation step into two sub-steps,

\[
\Delta r^{(\text{meq})}_{\perp,\parallel}(x_n) = \frac{\ell_b^2}{2} \partial_x^2 r_\perp(x_n) + \delta r_\parallel(x_n), \tag{2.10}
\]

where the first term on the r.h.s. is a step to the midpoint between the still attached flanking cross-linkers, and the second term \( \delta r(x_n) \), which is small in the limit of an inextensible polymer considered here. We have further introduced

\[
\partial_x \phi = \partial_x r_\parallel - 1 + \frac{1}{2} |\partial_x r_\perp|^2, \tag{2.11}
\]

as the differential state of strain along the polymer backbone. When the system evolves around equilibrium, we can estimate the typical size of the relevant terms.
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in the relaxation equations. In the limit of an inextensible fiber, the dominant contribution to $\delta r_\perp$ is of the same order of magnitude as the thermal noise and originates from local bucking,

$$|\delta r_\perp| = \ell_c \sqrt{-2 \partial_x \phi \theta(-\partial_x \phi)} \quad (2.12)$$

where the Heaviside step function $\theta$ is used since a fiber only buckles when under compression.

The full equilibration step, including a thermal contribution can thus be written as

$$\Delta r_\perp = \frac{\ell_c^2}{2} \partial_x^2 r_\perp + \delta r_\perp + \xi_\perp, \quad (2.13)$$

$$r_\parallel = \frac{\ell_c^2}{2} \partial_x^2 r_\parallel + \hat{e}_x \cdot \left( \delta r_\perp + \xi_\perp \right). \quad (2.14)$$

In the parallel equation the transverse fluctuations, projected along $e_x$, dominate over the longitudinal fluctuations, which are thus neglected.

The thermal noise term $\xi_\perp$ can be calculated directly from the inverse Hessian of the coarse-grained energy function in the Gaussian approximation we use here. The dominating contribution to the noise originates in the transverse component of the Hessian,

$$H_\perp \sim k_B T \ell_c^2 \ell_b \ell_b^{-1} \partial_x |\partial_x \phi|. \quad (2.15)$$

This makes explicit the non-linear dependence on the configuration of the polymer which introduces correlations in the thermal noise in both time and space. Since $\partial_x \phi$ depends explicitly on both $\partial_x r_\parallel$ and $\partial_x r_\perp$ (Eq. 2.11), the noise will couple the evolution equations (Eq. 2.14). The direction of the small buckling term is taken randomly from a continuously degenerate set of solutions and thus also acts effectively as a noise contribution.

We can treat this model using a mean field approach. By performing an equilibrium average of the state of the chain, we calculate the average projection on to $\hat{e}_x$ for both the buckling contribution as well as the contributions of the thermal noise.

We can also take the continuum limit in time by replacing the discrete steps $\Delta r_{\perp,\parallel}$ occurring at a rate $\tau_{\text{off}}^{-1}$ with $\tau_{\text{off}} \partial_t r_{\perp,\parallel}$. Here we are primarily interested in the parallel equation, from which the rheological behavior can be calculated. Within the mean-field picture we have

$$\partial_t r_\parallel = \frac{\ell_c^2}{2} \partial_x^2 r_\parallel + \xi_{\text{MF}}, \quad (2.16)$$

where the noise term can be calculated as

$$\langle \xi_{\text{MF}}(x,t) \xi_{\text{MF}}(x',t') \rangle \approx \frac{\tau_{\text{off}} \ell_b^3 \ell_c^2}{12 \ell_b^3} \delta(x-x') \delta(t-t'). \quad (2.17)$$
Here $L$ is the length of the polymer. The unusual—stronger than linear—dependence on temperature originates in the back projection of local fluctuation on to the average direction of the polymer.

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Bibliography


