Active Entanglement-Tracking Microrheology Directly Couples Macromolecular Deformations to Nonlinear Microscale Force Response of Entangled Actin

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ABSTRACT: We track the deformation of discrete entangled actin segments while simultaneously measuring the resistive force the deformed filaments exert in response to an optically driven microsphere. We precisely map the network deformation field to show that local microscale stresses can induce filament deformations that propagate beyond mesoscopic length scales (60 μm, >3 persistence lengths $l_p$). We show that the filament persistence length controls the critical length scale at which distinct entanglement deformations become driven by collective network mechanics. Mesoscale propagation beyond $l_p$ is coupled with nonlinear local stresses arising from steric entanglements mimicking cross-links.

Entangled networks of the archetypical semiflexible polymer actin, which display complex viscoelastic stress responses, are responsible for most mechanically driven cellular processes and exhibit powerful tunable properties for novel biomimetic materials. The complexity of actin networks, compared to flexible polymers, stems from the large persistence length of actin filaments ($l_p \approx 17$ μm) relative to their size ($\sim 5$–20 μm) and the length between entanglements ($l_e \sim 1$ μm). Despite widespread interest in actin network mechanics, the filament dynamics that give rise to stress response, including the propagation of filament deformations surrounding an induced strain, remain controversial. Recently, established microrheology techniques can characterize molecular-level stress response by using passively diffusing or actively driven microscale probes (beads) to sense network dynamics. However, two-point microrheology, which measures correlations between two diffusing beads in the network, is limited to the near-equilibrium regime, and microscope rheometers that image embedded beads during bulk strain cannot determine localized stress propagation. Both techniques also indirectly infer network deformation from foreign probes that can underestimate or disrupt network mechanics.

Previous studies probing actin network mechanics have largely focused on cross-linked networks and linear regimes where filaments are only weakly perturbed. However, steric entanglement dynamics, especially those far from equilibrium, remain relatively unexplored. Cross-linking entangled filaments has been suggested to inhibit nonaffine (nonuniform) network deformation typically exhibited by entangled networks by suppressing filament-bending modes. Resulting affine (uniform) response, driven solely by filament-stretching modes, is predicted to give rise to nonlinear stress response not apparent in entangled networks. However, we recently revealed microscale stress response for entangled actin that exhibited key nonlinear properties such as stress-stiffening and power-law relaxation when strain rates $\dot{\gamma}$ exceeded the disentanglement rate (i.e., rate at which individual deformed entanglement lengths relax, $\tau_{\text{rel}}$). Relaxation dynamics indicated dilation and subsequent healing of the classical reptation model entanglement tube surrounding each filament.

Here, we demonstrate a new widely applicable technique that tracks submicron segments of entangled actin filaments while simultaneously measuring the stress induced in tracked filaments by a bead driven through the network (Figure 1).

We characterize the network deformation field and couple induced microscale stress to network deformations over 3 orders of length scales: from below the network entanglement length ($l_e \approx 0.42$ μm) to >3x the largest length scale of the network (the persistence length $l_p$). We show that the persistence length dictates the distance from the applied strain $R$ at which noncontinuum deformations give way to collective network mechanics. We demonstrate that for strain rates above the disentanglement rate ($\dot{\gamma} \approx \tau_{\text{rel}}^{-1} \approx 3$ s$^{-1}$), reduced nonaffine deformations similar to those of cross-linked networks lead to nonlinear stresses that propagate beyond mesoscale distances.

For all measurements, a trapped bead (radius $r \approx 2.25$ μm) is pulled 10 μm through the network at speeds $v = 1, 5$, and 10

Received: September 15, 2015
Accepted: October 14, 2015
Published: October 15, 2015
Figure 1. Active entanglement-tracking microrheology. (a) Cartoon of tracking labeled segments along an actin filament. (b) Microscope image of filament with interspersed ~0.45 μm labeled segments that are centroid-tracked during experiments. (c) Tracking segments at varying distances R from strain path. Orange circles represent bead position before/after strain and yellow line represents strain path. Dotted lines outline annuli corresponding ensemble average within an annulus of radius R."
find nonzero \( f_{\text{rec}} \) at length scales beyond \( l_p \) for all measured strain rates.

This spatially dependent recovery is also consistent with our measured force relaxations that indicate nonclassical entanglement tube dilation (Figure SII).\(^{22}\) Strain-induced dilation should be strongest near the strain where entanglements are easily forced apart (dilating entanglement tubes), but as the stress propagates along entanglements, the impact of the disturbance on any one entanglement segment is reduced (limiting dilation) and all segments display collective network behavior. Tube dilation leads to faster relaxation dynamics, so for \( \gamma < \gamma_C \), entanglements closest to the strain path can almost completely relax on the time scale of strain (dissipation, small \( f_{\text{rec}} \)). However, during relaxation, these entanglements redistribute induced stress to neighboring entanglements, further from the strain site, in less dilated tubes. These more confined entanglements cannot relax as quickly, resulting in enhanced memory (increased \( f_{\text{rec}} \)). For \( \gamma > \gamma_C \), increased dissipation beyond \( l_p \) indicates a shift from a microscale stress-stiffening regime evidenced by virtually complete recovery to a bulk regime that displays enhanced dissipation and, thus, stress-softening. This spatial dependence on nonlinear stress response can explain why bulk rheology measurements of entangled actin only exhibit stress-softening dynamics (with stress-stiffening reserved for cross-linked networks).\(^{15,30,31}\)

We note that while overall behavior of the two rates above \( \gamma_C \) are quite similar compared to 0.3\( \gamma_C \), the intermediate strain (1.7\( \gamma_C \)) exhibits more dissipative features than for 3.3\( \gamma_C \). Specifically, following the 3.3\( \gamma_C \) strain, filaments close to the applied strain completely recover (\( f_{\text{rec}} \approx 1 \)) dissipating to \( f_{\text{rec}} \approx 0.7 \) in the continuum phase; while at 1.7\( \gamma_C \), filaments only partially recover near the strain (\( f_{\text{rec}} \approx 0.7 \)) reducing to \( f_{\text{rec}} \approx 0.5 \) in the continuum phase. This enhanced dissipation likely arises from the closeness of 1.7\( \gamma_C \) to the crossover rate, allowing for partial entanglement relaxation on the time scale of the strain. In other words, some of the entanglements are mobile, analogous to ruptured cross-links, while others are fixed, providing the partial recovery.\(^{29}\)

Filament recovery distance \( x_{\text{rec}} \) (Figure 3a) corroborates that the persistence length mediates bulk mechanics. Namely, for distances beyond \( l_p \), \( x_{\text{rec}} \) is independent of strain rate and exponentially decays over a length scale \( R \approx 2l_p (x_{\text{rec}} \sim e^{-R/2.4l_p}). \)

Simulations of cross-linked networks subject to local force perturbations have also predicted exponential decay of network deformations beyond a critical distance from the perturbation,
which authors interpret as due to self-averaging behavior.\textsuperscript{22} The corresponding length scale $\lambda$ identified for cross-linked networks is mediated by the distance between cross-links $l_c$ and the bending rigidity of the filaments, scaling as $\lambda \sim (l_c l_p)^{1/2}$\textsuperscript{23,30} Thus, if we remove fixed cross-links from the system, then the most obvious length scale controlling continuum behavior is indeed the persistence length.

Decreasing recovery distance with increasing $R$ is consistent with entanglement tubes becoming less dilated further from the strain site; that is, filaments are more confined, inhibiting retraction to starting positions. Distance-dependent tube dilation is further displayed by measured recovery rates of deformed filaments (Figure 3b). All filament deformations during recovery decay exponentially in time (Figures 1d and SI2) with $\dot{\gamma}$-independent relaxation rates that decrease with increasing $R$. Filaments closest to the bead path decay at a rate of $\sim 0.3$ s\textsuperscript{-1}, corresponding to $\sim 5t_D^{-1}$, indicating that entanglement tubes closest to the strain are dilated $\sim 2.3X$ the equilibrium diameter $d_T$ ($d_T^{-2} \sim t_D^{-1}$). At the furthest point from strain, relaxation slows to $\sim 1.4t_D^{-1}$, corresponding to $\sim 1.1t_D^{-1}$, showing that tubes far from the strain maintain a classically predicted size.

Force measurements show that tube dilation leads to exponential force relaxation for $\dot{\gamma} < \dot{\gamma}_C$ (linear response) versus two-phase power-law relaxation for $\dot{\gamma} > \dot{\gamma}_C$ (nonlinear response; Figure SI1).\textsuperscript{22} While for $\dot{\gamma} < \dot{\gamma}_C$ exponential force decay rates of $5t_D^{-1}$ corroborate the corresponding recovery data near the strain (Figure 3b), the distinct power-law relaxation for $\dot{\gamma} > \dot{\gamma}_C$ is unique to the force response. Two-phase force relaxation is predicted to arise from initial fast tube contraction, followed by slow reptation, coupled with further contraction.\textsuperscript{22,27} This nonlinear force response is coupled with filament deformations that propagate over several $l_p$ with minimal dissipation. Thus, we can understand power-law force relaxation as a second order effect of distant entanglements needing to relax before highly stressed entanglements close to the strain can completely recover. Put differently, nonlinear force response arises from substantial collective mechanics beyond $l_p$ contributing significantly to force exerted at the microscale strain site.

Network deformation is largely dominated by affine (parallel to strain path) entanglement displacements, which have been the focus of the discussion above. However, rate-dependent nonaffine network deformation is also seen in Figures 4 and SI3, which map maximum segment displacements induced by the strain ($\vec{x}_{\text{max}} + \vec{y}_{\text{max}}$) averaged over (2.5 $\mu$m)$^2$ regions of the network, colorized by the directionality of $\vec{y}_{\text{max}}$. By analogy to previous studies characterizing nonaffine response to uniform shear strains,\textsuperscript{15,16,33} we quantify nonaffinity by a normalized nonaffinity parameter $N(R) = \langle \vec{x}_{\text{max}} + \vec{y}_{\text{max}} \rangle / (\langle x_{\text{max}}^2 + y_{\text{max}}^2 \rangle)$ (Figure 4d). We find that, for $\dot{\gamma} < \dot{\gamma}_C$, $N$ is independent of $R$, with an average value that is greater in magnitude than that for $\dot{\gamma} > \dot{\gamma}_C$ deformations. This $R$-independence and increased magnitude have been previously reported for minimally cross-linked actin networks subject to macroscopic shear strain, due to bending modes dominating the response.\textsuperscript{15,16} As cross-linking increases, bending modes are suppressed and $N$ is expected to scale with $R$ as $N \sim R^\alpha$, where $0 < \alpha < 2$ depends on the level of cross-linking. This bending/nonaffine suppression is the source of stress-stiffening and nonlinearity driven principally by stretching modes.\textsuperscript{23,30} In accord with this model, we find that, for $\dot{\gamma} > \dot{\gamma}_C$, $N \sim R^{\alpha_1}$, signifying that strain rates above $\dot{\gamma}_C$ indeed induce cross-linking of entanglements. We further quantify the $\dot{\gamma}$-dependence of nonaffinity by determining the $R$-averaged nonaffine deformations $\langle y_{\text{max}}^2(R) \rangle$ for all strain rates (Figure 4e). We find that $\langle y_{\text{max}}^2(R) \rangle$ decreases with increasing $\dot{\gamma}$, demonstrating that at higher strain rates entangled networks transition to elastically driven dynamics dominated by...
stiffness modes, similar to that of cross-linked networks. Substantial nonaffine response of cross-linked networks is only predicted for length scales below \( \lambda \sim (l_p l_f)^{1/2} \).\(^{23,30–32}\) Strain-induced cross-linking of all entanglements would imply \( l_e \approx l_f \) and \( \lambda \approx 4 \) \( \mu \text{m} \), while our measurement range is \( \sim 2 \lambda < R < 15 \lambda \). Thus, we expect our nonaffinity parameter to scale similarly to bulk deformations as described by the scaling above. Further, \( \lambda \) is predicted to also mediate the crossover to continuum mechanics for cross-linked networks, our results indicate that the length scales controlling nonaffinity and noncontinuum mechanics are two distinct parameters for entangled networks.

As the microscale strain is not as simple as uniform bulk shear strain, we expect to see a footprint of the bead path and geometry in the deformation field. Previous simulations of constant force microsphere perturbations, similar to magnetic tweezer measurements, predict that entangled filaments pile up in front of the bead and vacate the wake behind it, leading to an osmotically controlled restoring force on the bead.\(^{34–36}\) This osmotic pressure difference leads to steric repulsion of filaments away from the leading edge of the bead and filament attraction toward the trailing edge, which is exactly what we see in Figures 4a–c and SI3. The trailing-edge wake is most apparent for higher strain rates due to inability of entanglement segments to rearrange or bend during strain, further corroborating stress-stiffening behavior (Figure SIIb). The repulsion/attraction is most evident for 0.3 \( \mu \text{m} \), as entanglements can easily flow and redistribute in response to the disturbance.

In conclusion, we demonstrate an innovative technique able to directly track individual entanglement segments over mesoscopic length scales while measuring mesoscale forces in response to local strains. We show that for entangled semiflexible polymers, the persistence length mediates the crossover from hierarchical deformations to continuum network mechanics while the disentanglement rate mediates the onset of nonlinearity. The mesoscale spatial crossover at \( l_p \) is likely unique to semiflexible filaments (\( l_p > l_f \) in which individual entanglement segments along a filament are mechanically connected via the persistence length, preventing independent relaxation and continuum mechanics at length scales below \( l_p \)). Strain-induced cross-linking of entanglements for \( \rho > \tau_{\text{rel}}^{-1} \) leads to elastic deformations near the strain path (\( R < l_p \)) coupled with long-range stress propagation; with bulk network deformations beyond \( l_p \) contributing significantly to the force exerted at the strain site. Rigid entanglements < \( l_p \) from the strain give rise to signature nonlinear dynamics, including stress-stiffening and suppressed nonaffinity that are quenched over a length scale \( l_p \) as entanglements soften and become increasingly mobile.

**EXPERIMENTAL METHODS**

**Discrete Filament Labeling.** Multisegmented fluorescent actin filaments were generated via simultaneous annealing and polymerization of labeled and unlabeled actin, forming filaments with interspersed fluorescent-labeled sections \( \sim 0.45 \) \( \mu \text{m} \) in length (Figure 1b).

**Sample Preparation.** Discreetly labeled actin filaments and BSA-treated 4.5 \( \mu \text{m} \) microspheres are embedded in 0.5 mg/mL entangled actin networks.

**Data Collection.** An Olympus IX70 microscope imaged the network and served as the optical trap base.\(^{37}\) We precisely move the trapped bead through the network using a piezoelectric mirror while capturing videos of the network (2.5 fps) and measuring the force exerted on a trapped bead (20 kHz).\(^{22}\)

**Data Processing/Analysis.** We use Crocker and Weeks’ particle tracking algorithms to obtain positions of labeled segments and link positions in time.\(^{36}\) From the resulting tracks, we quantify the displacement of each segment \( i \) in both the parallel/affine \( (s_i(t)) \) and perpendicular/nonaffine \( (y_i(t)) \) directions. We determine the dependence of segment displacement on \( R \) by selecting all tracks within a 4.5 \( \mu \text{m} \) wide annulus of radius \( R \) centered on the bead path (Figure 1c) and calculating the corresponding ensemble average \( \langle (s_i(t)), (y_i(t)) \rangle \). Error bars are determined by bootstrapping over 100 repeating subsets.

All methods are further described in the Supporting Information.

**ASSOCIATED CONTENT**

**Supporting Information**

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsmacrolett.5b00673.

Expanded experimental methods, Figures SI1, SI2, and SI3 (figure content described in text) (PDF).

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**Notes**

The authors declare no competing financial interest.

**ACKNOWLEDGMENTS**

This research was funded by an NSF CAREER Award, Grant No. 1255446.

**REFERENCES**


(16) Basu, A; Wen, Q; Mao, X; Lubesky, T; Janmey, P. A.; Yodh, A. Macromolecules 2011, 44 (6), 1671–1679.

