Ring DNA confers enhanced bulk elasticity and restricted macromolecular diffusion in DNA-dextran blends

Pawan Khanal, Karthik R Peddireddy, Juexin Marfai, Ryan McGorty, Rae M Robertson-Anderson*
Department of Physics and Biophysics, University of San Diego, 5998 Alcala Park, San Diego, CA 92110
*randerson@sandiego.edu

Abstract

Polymer architecture plays critical roles in both bulk rheological properties and microscale macromolecular dynamics in entangled polymer solutions and blends. Ring polymers, in particular, have been the topic of much debate due to the inability of the celebrated reptation model to capture their experimentally observed dynamics. Further, correlating the bulk behavior to the underlying macromolecular dynamics remains a challenge. Macrorheology, microrheology and molecular tracking are powerful methods to determine dynamics of entangled polymers across scales, yet these measurements are typically carried out on different samples under different conditions, preventing direct coupling. Here, we address these issues by both performing macrorheology and imaging fluorescent-labeled DNA molecules in entangled solutions of ring and linear DNA as well as their blends with varying fractions of dextran. Importantly, our different measurements are carried out on the same samples under the same conditions. Our measured bulk viscoelastic moduli show that blending viscoelastic DNA solutions with viscous dextran solutions leads to emergent enhanced elasticity for linear DNA, but this enhanced elastic plateau is still weaker than that for blends with ring DNA. Our differential dynamic microscopy (DDM) and single-molecule tracking analyses corroborate our rheological measurements, revealing nearly halted motion of ring DNA in blends comprising 75% DNA and 25% dextran, and slowing of linear DNA transport in blends compared to solutions of DNA or dextran alone. We argue that threading of ring DNA likely plays a key role in our intriguing results.

Introduction

Ring polymers have been the topic of fervent investigation for decades now due to their intriguing rheological and dynamical properties, biological significance, and industrial applications. For example, DNA naturally occurs in ring formation, and conversion between supercoiled and open circular (ring) topology plays a critical role in DNA replication and repair [1-3]. Further, the addition of ring polymers can tune the rheological properties of polymeric blends for commercial and industrial use [4-6]. While the dynamics of entangled linear polymers is well described by the reptation model developed by de Gennes and Doi and Edwards [7, 8], the extension of this model to ring polymers is not straightforward due to their lack of free ends [9-11]. The extent to which ring polymers form entanglements and corresponding entanglement plateaus, the effect and persistence of threading of one polymer by another, and the relaxation modes available to ring polymers remain topics of debate [12-17].

While previous rheological studies have shown that entangled ring polymers do not display entanglement plateaus that their linear counterparts do, [12, 13, 18-20] indicating weak entanglements, other studies have shown that rings undergo very slow relaxation, compared to
entangled linear chains, preventing the presence of an expected terminal flow regime at low frequencies [12, 17, 21-24].

Further, nearly all synthesis techniques used to produce solutions and melts of ring polymers result in a small percentage of linear polymer ‘contaminants’. Threading of rings by these linear contaminants have been shown to have profound effects on the rheology of entangled rings and blends of linear and ring polymers [12, 21, 22, 25-28]. One such effect is the heightened importance of constraint release, whereby a polymer relaxes stress by the threaded polymer unthreading itself and releasing its constraint [14, 22, 28]. This process, quite slow compared to reptation, is expected to lead to stronger entanglement plateaus, increased viscosity, suppressed relaxation, and hindered diffusion in ring-linear blends as compared to monodisperse systems of linear chains or rings [16, 27, 29-32]. However, direct experimental evidence connecting bulk rheological properties to macromolecular dynamics in systems of entangled ring polymers is lacking.

Further, blends of polymers of distinct sizes, stiffnesses and structures are abundant in biology (e.g., cytoskeleton, mucus, cytoplasm) and industry (e.g., plastics, adhesives, cosmetics), and offer wider dynamic range and increased control over mechanical properties compared to single constituent polymeric materials [33-37]. However, the emergent desirable rheological properties that polymeric blends have been shown to exhibit often cannot be predicted or explained from the properties of the corresponding single-component systems [38-42]. As such, the complexity of polymer blends demands techniques that can directly connect bulk properties to the dynamics of the constituent polymers.

Here, we investigate the rheology and dynamics of concentrated blends of DNA and dextran polymers, elucidating the effect of DNA topology (ring versus linear) and relative fraction of DNA and dextran on the blend properties from the scale of single polymers to that of the macroscopic bulk. Specifically, we couple macrorheology with fluorescence microscopy, differential dynamic microscopy (DDM) and single-molecule tracking to directly connect the bulk rheological properties of blends to the microscale dynamics of the comprising DNA.

While both macro- and micro-rheological techniques have been extensively used to investigate entangled polymers and other soft materials, these distinct measurements are typically performed on different samples with different preparation methods, chamber geometries, and sample volumes [43, 44]. As such, direct connection between the properties at these two scales is non-trivial [45-48]. We overcome these limitations by performing imaging and rheology measurements in the same sample using a rheometer with high-speed fluorescence imaging capabilities. Further, we track DNA molecules comprising the blends, rather than embedded probes (as is typically done in microrheology experiments [49]), to enable a direct report of macromolecular dynamics via differential dynamic microscopy.

Our results reveal a surprising non-monotonic dependence of the elasticity on the DNA blend fraction, with samples comprising 75% DNA and 25% dextran exhibiting the strongest elastic behavior – stronger than single-component samples of DNA or dextran alone. This increased elasticity is coupled with increased DNA fluctuation decay times and subdiffusion. We also show that composites comprising ring DNA exhibit the strongest and most persistence elastic plateau coupled with nearly halted motion of the rings, suggestive of threading.
Fig 1. Experimental platform to investigate bulk rheology and DNA diffusivity in blends of DNA and dextran. (A) Cartoon of labeled 25-kbp ring and linear DNA and 500 kDa dextran polymers along with various blends of DNA and dextran with overall concentration fixed to 11 c∗ and DNA volume fractions ranging from φ = 0 (100% dextran) to φ = 1 (100% DNA). RG denotes the radius of gyration of each polymer. (B) Cartoon of rheology measurement using a cone-plate geometry in a DHR-3 Discovery Hybrid Rheometer (left), and a sample frequency sweep for φ = 1 linear DNA solution. (C) Before and after rheology measurements, we capture time-series of labeled DNA molecules diffusing in the sample using the fluorescence microscopy attachment as described in Methods. We perform DDM analysis on captured time-series to determine the image structure function D(q, Δt) as a function of lag time, Δt, and wavevector, q. By fitting D(q, Δt), we determine the density fluctuation decay times τ as a function of q to describe the DNA dynamics.
Results

In all of our experiments described below, we fix the polymer concentration to $11c^*$, where $c^* = (3/4\pi)(M/N_A)R_g^{-3}$ is the polymer overlap concentration with $M$ the molecular weight [8], to ensure that the molecules are highly overlapping. At this concentration the DNA solutions are $\sim 2x$ above the critical entanglement concentration $c_e \approx 6c^*$ [50]. By fixing the degree to which molecules overlap, we can unambiguously determine the effect of polymer topology (i.e., ring versus linear DNA), as well as interactions between distinct polymers (i.e., DNA and dextran), on the rheological properties and macromolecular dynamics. Specifically, we examine DNA-dextran blends with either purely linear DNA or 90%/10% ring/linear DNA (which we refer to as ‘ring’ throughout the paper) at DNA volume fractions of $\phi = 0$ (pure dextran solution), 0.25, 0.5, 0.75 and 1 (pure DNA solution).

We first examine the bulk rheological properties of entangled solutions of ring and linear DNA and their blends with dextran (Fig 2). Fig 2A compares the elastic modulus $G'(\omega)$ and viscous modulus $G''(\omega)$ for pure solutions of DNA ($\phi = 1$) and dextran ($\phi = 0$). As shown, the viscoelastic responses of ring and linear DNA are distinct. 100% linear DNA ($\phi = 1$) shows a transition from the terminal flow regime at low frequencies to the rubbery plateau regime at high frequencies with a crossover frequency, at which $G'$ exceeds $G''$, of $\omega_c \approx 0.3$ rad/s. In the terminal regime, $G'' > G'$ and both moduli exhibit power-law behavior with $G'' \sim \omega^1$ and $G' \sim \omega^2$. At higher frequencies, the polymer chains do not have sufficient time to free themselves from entanglements causing elastic tension to dominate the rheology, hence $G' > G''$. Different from linear DNA, ring DNA solutions ($\phi = 1$) exhibit an elastic rubbery plateau over the entire frequency range. While the lack of a terminal regime has been reported for entangled rings [17, 21], recall that the ring DNA solutions we study here have $\sim 10\%$ linear contaminants that also likely impact the rheological properties via threading, as described above and further discussed below. While linear and ring DNA solutions show viscoelastic curves, pure dextran solutions exhibit Newtonian properties with $G'' \sim \omega^1$ scaling and undetectable $G'$ values for all but the highest frequencies.

We next examine blends of DNA and dextran with varying volume fractions of DNA $\phi$, while maintaining concentrations of $11c^*$ (Fig 2B). As shown in Fig. 2B, all blends exhibit viscoelastic behavior, with the values of $G'$ and $G''$, as well as the frequency range over which $G' > G''$, increasing with increasing $\phi$. $\phi \leq 0.5$ blends display a high frequency crossover in which $G'' > G'$, which is a measure of the entanglement time $\tau_e$, or the time it takes for an entangled polymer to ‘feel’ its tube confinement [8]. This crossover frequency increases with increasing $\phi$, but is non-existent for $\phi = 0.75$.

How the rheological properties depend on DNA topology is more complex than how they depend on $\phi$. For frequencies above $\sim 10$ rad/s, in which the pure DNA solutions are both in the entangled regime, DNA-dextran blends exhibit negligible dependence on topology, with $G'$ curves for each topology overlapping. However, at lower frequencies, the moduli become topology-dependent. At low $\phi$, blends with linear DNA are more elastic than those with ring DNA (i.e., $G'$ is larger and less $\omega$-dependent). However, as $\phi$ increases this trend switches such that the $\phi=0.75$ ring DNA-dextran blend exhibits the most strongly elastic behavior of all blends and solutions – displaying rubbery regime behavior over the entire frequency range. Of particular interest is the $\phi=0.75$ linear blend because pure DNA solutions show a clear transition to the terminal regime (Fig 2A), while
upon replacing 25% of the volume with dextran, which exhibits Newtonian rheology, the blend displays entanglement behavior for nearly all frequencies.

These intriguing effects can also be seen in Fig 2C,D in which $G'(\omega)$ is plotted for all $\phi$ values for each DNA topology independently. Blending of linear DNA with dextran has an immediate and strong effect on the rheology – increasing the elastic storage considerably even at $\phi=0.25$ and $G'(\omega)$ for $\phi = 0.5$ and 0.75 surpassing that for $\phi =1$. Conversely, for rings, blending with dextran actually decreases $G'(\omega)$ and the corresponding rubbery plateau regime, compared to $\phi=1$, for all blends except $\phi = 0.75$.

Figure 2. Viscoelastic moduli of concentrated blends of DNA and dextran exhibit complex dependence on the topology and volume fraction of DNA. (A) Elastic modulus $G'$ (closed symbols) and viscous modulus $G''$ (open symbols) as function of frequency $\omega$ for 11$c^*$ solutions of linear (purple squares) and ring (purple circles) DNA ($\phi =1$) and dextran (red triangles, $\phi = 0$). Entangled linear DNA exhibits a transition from a rubbery plateau to terminal regime at low $\omega$, while rings show no such transition. Dextran exhibits largely Newtonian viscosity with $G''(\omega) \sim \omega$ (as indicated by the scale bar with exponent 1) and negligible $G'(\omega)$ values for all but the highest frequencies. (B) $G'$ (closed symbols) and $G''$ (open symbols) for 11$c^*$ blends of dextran and linear (squares) and ring (circles) DNA with DNA volume fractions of $\phi = 0.25$ (orange), 0.5 (green) and 0.75 (cyan). Viscoelastic moduli increase and exhibit increasingly elastic behavior as $\phi$ increases. (C,D) $G'$ for solutions and blends with linear (C) and ring (D) DNA. Data and color coding are the same as shown in A and B.

To determine the macromolecular dynamics that give rise to the bulk rheology, we use an optical microscopy attachment to our rheometer to collect time-series of images of diffusing DNA in the blends and solutions shown in Fig 2. Importantly, these data are collected in the exact samples and geometry as bulk rheology data immediately before and after rheology measurements (see Methods). We perform differential dynamic microscopy (DDM) [51, 52] analysis on the time-series to determine the transport properties of ring and linear DNA in all solutions and blends.
Specifically, we determine the characteristic decorrelation time $\tau$ of diffusing DNA polymers versus wavenumber $q$ by fitting the radially-averaged image structure function $D(q,\Delta t)$ to a stretched exponential, as described in Methods. In general, higher $\tau$ values for a given $q$ indicate slower motion, and the scaling of $\tau$ vs $q$ indicates the type of motion (diffusive, halted, etc.). Specifically, by fitting $\tau(q)$ to the power-law function $\tau = 1/(Dq^\alpha)$, one can determine the type of motion. Normal Brownian diffusion is described by $\alpha = 2$ where $D$ is the corresponding diffusion coefficient while restricted motion often results in minimal $q$ dependence ($\alpha \sim 0$) [53, 54]. As shown in Fig 3, for nearly all cases, DNA exhibits approximately diffusive motion ($\alpha \approx 2$) with the obvious exception being ring DNA in $\phi=0.75$ blends which displays minimal $q$-dependence (Fig 3B,D).

![Figure 3](https://example.com/figure3.png)

**Figure 3.** DDM of entangled DNA solutions and DNA-dextran blends show strong non-monotonic dependence on the volume fraction of DNA. (A) Characteristic decay time ($\tau$) versus wave vector ($q$) for ring (circles) and linear (squares) DNA in 1$c^*$ solutions of linear (purple squares) and ring (purple circles) DNA ($\phi = 1$) and dextran (red triangles, $\phi = 0$). (B) $\tau(q)$ for DNA-dextran blends with varying volume fractions $\phi$ of ring (circles) and linear (squares) DNA show that DNA dynamics slow with increasing $\phi$. Rings diffuse faster than linear DNA in $\phi = 0.25$ and 0.5 blends but become markedly slower than linear DNA for $\phi =0.75$, exhibiting restricted non-diffusive motion. (C) Dynamics of linear DNA are faster in entangled DNA solutions ($\phi =1$, purple) compared to DNA-dextran blends, with $\phi = 0.75$ being the slowest. (D) Ring DNA dynamics display little dependence on $\phi$ except for $\phi = 0.75$, which shows highly restricted dynamics. Scaling lines in all plots indicate power-law scaling $\tau(q) \sim q^{-2}$ expected for normal Brownian diffusion.

Comparing $\tau(q)$ curves for the different DNA topologies, we find that ring DNA molecules are faster (smaller $\tau(q)$) than their linear counterparts in pure dextran solutions ($\phi = 0$), while they diffuse more slowly than linear chains in $\phi = 1$ solutions (Fig 3A). Further, while $\tau(q)$ curves for linear DNA are quite similar in solutions of dextran and linear DNA, rings are significantly slowed in entangled ring DNA solutions compared to dextran solutions. This trend is consistent with the rheology over the same timescales evaluated in DDM. Specifically, DDM examines timescales of
~20 to ~2000 s corresponding to $\omega \approx 3 \times 10^{-3}$ to 0.3 rad/s. In this frequency range, linear DNA exhibits largely Newtonian (terminal) flow behavior, similar to dextran, while ring DNA exhibits entanglement dynamics (Fig 2A). As such, we would expect linear DNA dynamics in $\phi = 0$ and $\phi = 1$ solutions to be similar, while rings would be significantly slower in $\phi = 1$ versus $\phi = 0$ solutions. Further, in dilute and Newtonian fluids, it is well understood that rings diffuse faster than linear DNA due to their reduced radius of gyration $R_G$ caused by the conformational constraint of end closure [55, 56], as we see in the dextran solutions. The slowing of rings in ring DNA versus linears in linear DNA, consistent with the rheology, may be due to the ~10% linear contaminants in the ring DNA solution that can thread the rings and slow diffusion [16, 27, 29].

These differences in ring versus linear DNA dynamics, that are dependent on the surrounding matrix polymers, can also be seen in DNA-dextran blends (Fig 3B). In general, DNA is slower in blends compared to pure solutions, evidenced by larger $\tau (q)$ values, and slow down as $\phi$ increases. This result is consistent with the increase in the elastic modulus of the blends with increasing $\phi$ (Fig 2B). Also consistent with bulk rheology results are the observations that ring DNA is faster than linear DNA when blended with dextran at $\phi = 0.25$ and 0.50, while at $\phi = 0.75$ rings are substantially slower than linear DNA and $\tau$ for rings is nearly independent of $q$, signifying highly restricted motion. This restricted motion, only seen for rings, is suggestive of threading of rings by dextran polymers.

The topology-dependence of the relationship between $\tau (q)$ and $\phi$ is further evidenced in Fig 3C,D which shows $\tau (q)$ for all blends and solutions for a given topology. As shown, $\tau (q)$ curves for linear DNA steadily increase with increasing $\phi$, similar to the low-frequency elastic moduli (Fig 2C). Conversely, ring DNA displays minimal $\phi$ dependence except for $\phi = 0.75$, with all other $\tau (q)$ curves overlapping. This trend, different from that of $G'$, indicates that while the bulk viscoelasticity may dominate the rheology, threading events (which are minimal at lower $\phi$ values) have a more apparent effect on macromolecular dynamics. Only once the threaded rings make up the majority of the solution ($\phi > 0.5$) do threading events impact the bulk rheology [16, 27].

To more quantitatively compare bulk rheology to DDM we compare diffusion coefficients determined from DDM to the loss tangent, $\tan \delta = G''(\omega)/G'(\omega)$, computed from bulk viscoelastic moduli (Fig 4). We obtain diffusion coefficients $D$ by fitting $\tau (q)$ to $\tau = 1/(Dq^2)$, noting that for some cases, in particular $\phi=0.75$, $\tau (q)$ does not exhibit $\alpha = 2$ scaling so $D$ should be taken as a rough estimate. We compute the frequency-dependent loss tangent, $\tan \delta$, and average across all $\omega$, with the error bars indicating the frequency dependence. $\tan \delta$ is a measure of the viscous dissipation in the system, such that lower values indicate greater elastic storage [57, 58]. As such, we expect $D$ and $\tan \delta$ to follow similar trends as entanglements and threading events slow diffusion and likewise suppress dissipation. Because the dextran solution ($\phi=0$) exhibits purely viscous behavior it does not have a measurable finite $\tan \delta$. As shown, for both ring and linear DNA, $\tan \delta$ and $D$ decrease as $\phi$ increases from 0 to 0.75 after which they increase again. Further, while $D$ and $\tan \delta$ are greater for rings versus linear chains for $\phi \leq 0.5$ this relationship flips for $\phi > 0.75$. 
Figure 4. DNA diffusion coefficients and loss tangents for DNA-dextran blends with varying volume fractions of DNA. Diffusion coefficient $D$ (closed symbols), determined from DDM, and loss tangent $\tan \delta = G''(\omega)/G'(\omega)$ (open symbols), determined via bulk rheology, for DNA-dextran blends with linear (squares) or ring (circles) DNA at varying DNA volume fractions $\phi$. $D$ and $\tan \delta$ for both topologies show similar $\phi$-dependence, first decreasing with increasing $\phi$ then increasing after reaching a minimum at $\phi = 0.75$.

To further understand this non-monotonic dependence of transport and rheology on $\phi$, and the $\phi$-dependent differences between ring and linear DNA, we perform single-molecule tracking measurements of DNA in $\phi = 0.75$ blends and compare with results for $\phi = 1$ solutions. We focus on $\phi = 0.75$ as it does not exhibit normal Brownian diffusion, so we cannot accurately quantify the transport with DDM. From single-molecule tracking experiments, we measure the center-of-mass mean-squared displacement (MSD) of DNA molecules in the blends. Fig 5 shows the MSDs versus lag time $\Delta t$ for ring and linear DNA at $\phi = 0.75$ and 1. All cases exhibit anomalous subdiffusion, i.e., $MSD \sim \Delta t^\beta$ where $\beta < 1$. For both $\phi$ values, rings display greater deviation from normal diffusion compared to linear DNA, with $\beta \approx 0.34$ compared to $\approx 0.63$ for linear chains. While $\beta$ is only slightly lower for $\phi = 0.75$ compared to $\phi = 1$ for both topologies, the value of the MSD at a given lag time is substantially lower. The strongly anomalous diffusion and concomitantly low MSD for rings at $\phi = 0.75$ corroborate our interpretation of the $\phi = 0.75$ rheology and DDM data as arising from highly restricted motion of the polymers, likely due to threading. We also note that the timescale probed by single-molecule tracking is lower than for DDM, corresponding to $\omega \approx 0.6 - 30$ rad/s. In this range, the linear DNA solution ($\phi=1$) exhibits more elastic-like behavior compared to lower frequencies, probed by DDM, in which it exhibits
terminal flow behavior. As such, DDM measurements show $\phi = 1$ linear DNA obeying normal Brownian diffusion while single-molecule tracking experiments measure subdiffusive transport.

**Figure 5.** Ring and linear DNA exhibit topology-dependent subdiffusion at high DNA volume fractions. MSD versus lag time $\Delta t$ for linear (squares) and ring (circles) DNA diffusing in entangled DNA solutions ($\phi = 1$, purple) and $\phi = 0.75$ blends (cyan). Black lines represent power-law scaling with exponents listed. The exponent $\beta$ from fitting each curve to $MSD \sim \Delta t^\beta$ is listed to the right of each curve. DNA in all cases exhibits subdiffusion ($\beta < 1$) with rings displaying greater deviation from normal diffusion ($\beta = 1$) than linear DNA, in agreement with DDM and bulk rheology results. $MSDs$ are also slower in $\phi = 0.75$ blends compared to $\phi = 1$ solutions for both DNA topologies, consistent with data shown in Figs 2 and 3.

**Conclusions**

In summary, we have coupled bulk rheology measurements with fluorescence imaging and DDM analysis to directly link the bulk viscoelastic properties of entangled DNA-dextran blends to microscale polymer transport within the blends. Importantly, we performed both measurements – at lengths scales that differ by $\sim 4$ orders of magnitude – on the exact same samples, under the same conditions, and within minutes of each other. In this way, we unambiguously connect macroscopic rheological properties of the solution to the underlying macromolecular dynamics.

Using this approach, we investigated the dependence of DNA topology and blend fraction on dynamics, and show that blends exhibit a non-monotonic dependence of both viscoelastic
dissipation and diffusivity on the fraction of DNA comprising the blends. This non-monotonicity is more pronounced for blends with ring DNA versus linear DNA. Notably, blends comprising 75% DNA (25% dextran) exhibit emergent elastic behavior and slowed transport, enhanced beyond those of single-component solutions of DNA and dextran. Further, the anomalous subdiffusion we measure at \( \phi = 0.75 \) is more extreme for rings, which display nearly halted motion not observed for linear chains, with an anomalous scaling exponent of \( \beta \approx 0.31 \). We attribute this emergent behavior to threading of rings by dextran polymers.

More generally, our results demonstrate that for polymer blends, the whole is not always equal to the sum of its parts, rather blending polymers can give rise to emergent dynamics that span from microscopic to macroscopic scales. We further show that polymer end-closure plays an important role in interactions between the different species comprising the blend, with ring polymers conferring uniquely suppressed dissipation and relaxation in blends.

**Methods**

**DNA:** We prepare solutions of double-stranded DNA, 25 kbp in length, via replication of fosmid constructs in *Escherichia coli* followed by extraction, purification and concentrating as described previously [56, 59]. The purified stock solution has a concentration of 3.25 mg/mL and consists of ~90% relaxed circular (ring) and 10% linear DNA, as quantified using gel electrophoresis. We convert half of this stock DNA solution to linear topology via treatment with the restriction enzyme ApaI (New England Biolabs). Both ring and linear DNA stock solutions are suspended in TE10 buffer (10 mM Tris-HCl (pH 8), 1 mM EDTA, 10 mM NaCl). The radius of gyration \( R_G \) for the ring and linear DNA is \(~280 \text{ nm} and \sim 450 \text{ nm}\), respectively [56]. The corresponding polymer overlap concentrations \( c^* = (3/4\pi)(M/N_A)R_G^{-3} \) are \sim 280 and \sim 71 \mu g/mL for ring and linear DNA. To enable imaging of DNA for DDM and particle-tracking, we fluorescent-label a fraction of the DNA molecules with MFP488 (Mirus) using the manufacturer-supplied Label IT Labeling Kit and corresponding protocols (Mirus). The excitation/emission spectrum for MFP488 is 501/523 nm and the dye molecule to DNA base pair ratio is 5:1.

**Dextran:** A solution of 500 kDa dextran (ThermoFisher), with \( R_G \approx 19 \text{ nm} \) [60], is prepared in TE10 at a concentration of 28.9 mg/ml (11\( c^* \)). The solution is homogenized via slow rotation at room temperature.

**Sample Preparation:** We prepare each sample at a volume of 350 \mu L and concentration of 11\( c^* \) for both DNA and dextran. We add 2 \mu L of labeled ring or linear DNA tracers to each sample for microscopy measurements. Each sample is prepared at least 4 days prior to experiments and rotated at 4°C to mix and equilibrate. An oxygen scavenging system (45 \mu g/mL glucose, 43 \mu g/mL glucose oxidase, 7 \mu g/mL catalase, 5 \mu g/mL \( \beta \)-mercaptoethanol) is added to inhibit photobleaching. Blends comprising both DNA (ring or linear) and dextran are prepared by mixing varying volume fractions of 11\( c^* \) DNA and dextran solutions, which we quantify by the volume fraction of the DNA solution \( \phi \). We investigate samples with \( \phi = 0.25 \) (25% DNA, 75% dextran), \( \phi = 0.5 \) (50% DNA, 50% dextran), and \( \phi = 0.75 \) (75% DNA, 25% dextran). For each \( \phi \), we prepare samples with ring DNA and linear DNA.
Rheometry: We use a Discovery Hybrid Rheometer 3 (DHR3, TA Instruments) with a 1° steel cone geometry to perform bulk rheology measurements. We use a glass slide as the bottom plate to enable imaging of fluorescent-labeled DNA in the samples. To prevent evaporation during the experimental cycle we apply mineral oil around the geometry and the sample. To measure linear viscoelastic moduli, \( G'(\omega) \) and \( G''(\omega) \), we perform two identical frequency sweeps from \( \omega = 0.001 \) to 100 rad/s at 5% strain (well within the linear regime as determined by amplitude sweeps). Each frequency sweep lasts ~6.5 hours with individual frequency measurements spaced 30 mins apart.

Fluorescence Microscopy: The DHR3 is outfitted with a Modular Microscope Accessory (TA Instruments) with a 40× 0.6 NA objective (Nikon), blue-light LED source, 490/525 nm excitation/emission filters, and a Hamamatsu ORCA-Flash 2.8 CMOS camera to enable imaging of MPF488-labeled DNA in the blends. Immediately before and after each bulk rheology measurement, we collect three 512×512 pixel videos of 2000 frames at 1 fps.

Differential Dynamic Microscopy (DDM): For DDM analysis (Fig 3), we split the videos into 256 × 256 pixel regions of interest (ROIs) which we analyze separately. We use custom-written scripts (Python) to perform DDM. For standard DDM analysis, one takes two-dimensional Fourier transforms of differences between images separated by a range of lag times \( \Delta t \) in order to quantify how the degree of correlation decays with lag time as a function of the wave vector \( q \). Because this standard correlation function is sensitive to global drift of the sample, we use a slightly modified correlation function referred to as the far-field DDM (FF-DDM) function. Previous work has shown that by using FF-DDM, the DDM correlation function, \( D(q, \Delta t) \), is less sensitive to drift [61, 62]. As with standard DDM analysis, we fit the far-field DDM matrix to \( D(q, \Delta t) = A(q)[1 - f(q, \Delta t)] + B(q) \), where \( f(q, \Delta t) \) is the intermediate scattering function (ISF), \( A(q) \) is the amplitude, and \( B(q) \) is the background. To determine the type of motion and the corresponding rate, we model the ISF as a stretched exponential: \( f(q, \Delta t) = e^{-(\Delta t/\tau(q))^\gamma} \) where \( \tau(q) \) is the decay time and \( \gamma \) the stretching exponent. The use of a stretched exponential as opposed to a simple exponential has been shown to better fit dynamics in confined or entangled systems [63, 64]. Scaling of \( \tau(q) \sim q^{-2} \) is indicative of normal Brownian diffusion (i.e., \( MSD \sim t \)), whereas a decay time that depends less strongly on \( q \) has been associated with more arrested or confined motion [53, 54].

Single-molecule tracking: For single-molecule tracking experiments, we image the MFP488-labeled DNA using an Olympus IX73 inverted fluorescence microscope with a 60× 1.2 NA oil immersion objective (Olympus). We collect five 512×512 pixel videos of 2000 frames at 10 fps for each sample. We use custom particle-tracking scripts (Python) to track the center-of-mass of individual DNA molecules and measure their frame-to-frame \( x \) and \( y \) displacements (\( \Delta x, \Delta y \)) from which we compute the ensemble averaged mean-squared displacements (\( < \Delta x^2 >, < \Delta y^2 > \)). We fit the average of \( < \Delta x^2 > \) and \( < \Delta y^2 > \) (i.e., \( MSD \)) versus lag time \( \Delta t \) to a power-law function \( MSD \sim \Delta t^\beta \) where \( \beta \) is the subdiffusive scaling exponent. For a system exhibiting normal diffusion, \( \beta = 1 \), while \( \beta < 1 \) indicates anomalous subdiffusion.

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Conflicts of Interest

The authors have no conflicts to disclose

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