Novel collective mechanics of actively driven DNA networks

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(Dated: October 30, 2015)

We present direct measurements of fluctuations in the entangled networks of DNA at similar concentration to what is expected in the nucleus, in the presence of an ATP-driven topology-relaxing enzyme. This enzyme catalyzes the passage of one DNA duplex across another [12], an activity essential to resolving tangles (illustrated in Fig. 1 A and B). Without this absolutely essential activity, cell division cannot proceed to successful DNA segregation to the two daughter cells [13–15]. In vertebrate cells, topo II has been shown to be the most abundant metaphase chromosomal scaffold component. DNA entanglement plays a role in other cellular processes, such as relaxation of torsional stress and chromosome condensation [16–18]. Indeed, DNA in mitotic chromatin is heavily self-entangled, and these entanglements stabilize mitotic chromosome structure [19].

Chromosomes need an identity, so they are not randomly positioned in the nucleus. At the same time, they must be able to move through each other as they segregate and undergo homology searches. Chromosome location, movement, and transcriptional accessibility must be related to the physical parameters that define chromosome mechanical behavior. At concentrations approaching that of the cell nucleus [10], strong effects of molecular topology on diffusion of entangled DNA molecules set in, and topological constraints present a problem to flow and segregation [11]. The DNA processing enzyme topoisomerase II (topo II) embodies a specialized mechanism possessed by the eukaryotic cell to eliminate unwanted inter-chromosomal associations or entanglements.

Topo II catalyzes the passage of one DNA duplex across another [12], an activity essential to resolving tangles (illustrated in Fig. 1 A and B). Without this absolutely essential activity, cell division cannot proceed to successful DNA segregation to the two daughter cells [13–15]. In vertebrate cells, topo II has been shown to be the most abundant metaphase chromosomal scaffold component. DNA entanglement plays a role in other cellular processes, such as relaxation of torsional stress and chromosome condensation [16–18]. Indeed, DNA in mitotic chromatin is heavily self-entangled, and these entanglements stabilize mitotic chromosome structure [19].

Topo II binds as a dimer to DNA entanglements and hydrolyses ATP [20], coupling scission of the first duplex strand and capture of the second strand to biasing of the motion of the second strand through the initial strand and re-ligation of the original duplex strand. Subsequently, the enzyme releases both duplex strands exactly as they were before, aside from their relative topology; readying the enzyme for another round of activity at another entanglement [21]. Ultimately this topology-changing activity is related to condensing and decondensing. In a disorganized network, one would expect these DNA processing motors to act in either direction at a single entanglement. For macroscopic-scale relaxation to occur, this symmetry must somehow be broken. How stress and stress-relaxation events propagate across a network of DNA remains poorly understood [22]. Somehow, an interplay between network connectivity and topology-relaxing motor activity must govern the propagation of stresses across long length scales. Since the strand breakage and re-ligation itself is energetically neutral, it is unclear why the enzyme requires ATP.

In this Letter, we study the collective strain fluctuations of an entangled DNA network under the addition of this enzyme that carries out nucleic acid processing reactions and is not ordinarily classified as a motor protein, and investigate how these fluctuations couple to the use of available energy. To investigate motion in such networks in detail, we developed a model system of λ-DNA and topo II, and quantified the time-averaged spectrum of forces imparted by the material to tracer particles. Our experimental data show that these non-processive enzymes actively impart forces to the entangled DNA material, and do so according to a broad spectral distribution, 1/ω noise, which has not previously been observed for ATP-motor driven activity.

The experiments were performed on in vitro samples of λ-DNA at a concentration which simulates the packing of DNA in chromosomes in the cell nucleus. Interestingly, the overall DNA concentration in the yeast nucleus, ~ 3.1 mg/ml, is similar to that measured within the nucleus of mammalian cells (~ 10 mg/ml) [10]. The model system consisted of 1 mg/ml λ-DNA (48,502 b.p.) in physiological salt conditions, which corresponds to a volume fraction of bare DNA of φ_{DNA} ~ 1 × 10^{-3}. The
crossover concentration from dilute to semi-dilute solutions for λ-DNA is 30 μg/ml [23], thus the DNA concentration under study is well above the semi-dilute limit and can be considered an entangled polymer network polymers of a single chain length. At a given concentration in the entangled regime, the chains are well-characterized by 8 entanglements per chain, interpreted from the plateau modulus [24–26]. The enzyme was present at a concentration saturating the entanglements (∼2 enzyme dimers per entanglement, 190 nM), and at saturating starting ATP concentration (2.5 mM) for the enzyme. Full stimulation of topo II by DNA occurs when cDNAbasepairs ≫ 100 ctopoII [27]. In our system of 1 mg/ml λ-DNA and 4 units/μL (190 nM) topo II, cDNAbasepairs = 6442 ctopoII. Therefore, the experimental conditions correspond to full topo II stimulation. Control experiments were performed on DNA with ATP and no enzyme.

We performed passive microrheology measurements [29–31] on this model system, using bright-field microscopy to track the fluctuations in positions of the particles, which couple to the DNA fluctuating movement. One-point (1-pt) passive microrheology uses the autocorrelated motion of many individual beads to obtain the motions as a spectrum over timescales. Two-point microrheology (2-pt) uses the pairwise correlated motions of the microbeads to obtain these mechanical parameters. In equilibrium systems this passive approach also measures the material response $G^*(ω)$ because of the fluctuation-dissipation theorem [32]. The sample was imaged on a Leica DM-IRB inverted microscope in brightfield mode using a 63X 1.4 NA oil immersion objective lens using a CCD camera (Hamamatsu) at 16 fps for 5 min. Data obtained for multiple fields of view were analyzed separately and combined.

Representative trajectories are shown in Fig. 1 C and D, for 1 mg/ml λ-DNA and λ-DNA/topo II/saturating ATP, respectively. The trajectories of probe particles in both systems, in the same concentrated DNA environment, indicate random movements. It can be seen clearly that in the topology-relaxing sample, the probe particles move more freely. Quantifying the trajectories by plotting the 1-pt and 2-pt MSD of these probe particles (Fig. 1 E) reveals that the enzyme activity apparently creates overall fluidization of the entangled DNA environment at the length scales accessed by microrheology.

For the equilibrium response in entangled, concentrated suspensions of DNA at physiological salt conditions, the 1-pt and 2-pt measurements should be in agreement because of the shortness of the persistence length and smallness of the mesh size of entangled DNA relative to the bead size [33]. At the scale of the micrometer-sized tracer particle, the semifilament properties of the DNA do not play a role, and the bead samples a homogeneous microenvironment. 2-pt microrheology measurements have not previously been reported on DNA at such high concentrations of ~30 times $c^*$ [1, 23, 33]: our preliminary data are the first 2-pt measurements reported in this DNA regime. The data in the absence of ATP demonstrate agreement between 1-pt and 2-pt MSD, verifying that there are no ambiguities associated with tracer boundary conditions and medium heterogeneity at this high DNA concentration. This is corroborated by 1-pt and 2-pt MSD agreement if the DNA/enzyme system is permitted to exhaust the ATP supply (data not shown). Any differences between pairwise-correlated motion and autocorrelated individual bead motion in the enzyme’s presence will be due to properties of the system.

By contrast, under the ATP-driven activity, the 1-pt and 2-pt fluctuations do not agree. If the enzyme activity were simply changing the equilibrium material response, increasing the flow properties, then the 1-pt and 2-pt MSD would be expected to be in agreement, as they are in entangled bare DNA at these (our data) and lower

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**FIG. 1:** (A and B) Illustration of the enzyme’s topology-relaxing action at a DNA entanglement. (C and D) Movement of microbeads inside λ-DNA and λ-DNA/enzyme/ATP. 2000-frame, 125-second trajectories of 1 μm PEG-beads in (C) 1 mg/ml λ-DNA and (D) 1 mg/ml λ-DNA + topo II enzyme + ATP. (E) Ensemble-averaged 1-pt and 2-pt MSD.

The λ-DNA (New England Biolabs) duplex strands were blunted using an oligonucleotide complementary to one of the sticky ends (Sigma-Aldrich), then concentrated using ethanol precipitation and subsequent resuspension in buffer (10 mM Tris-HCl, pH 7.9, 50 mM NaCl, 50 mM KCl, 5 mM MgCl₂, 0.1 mM EDTA, and 15 mg/L of bovine serum albumin (BSA) to the desired concentration. DNA concentration was measured spectroscopically (ND-1000 Nanodrop). 1 μm diameter latex microspheres (Polysciences) were used as tracer particles, incorporated in the samples for microrheology. The tracer boundary conditions were first made non-interacting with the DNA/protein networks, by covalent attachment of a block co-polymer, PLL-g-PEG, to all the COOH surface groups on the bead surface [28].

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Beyond this fact, the fact that the 2-pt fluctuations exceed the 1-pt fluctuations, implying greater coupling to relaxations at larger distances, is also counter to expectation for the equilibrium response of entangled polymer biomaterials that do exhibit a discrepancy between the 1-pt and 2-pt fluctuations. In F-actin [31, 34], microtubules [31], a microtubule/F-actin composite [31], and even the same λ-DNA as studied here, at much lower, barely entangled concentrations where the mesh size is comparable to the bead size [33], the 1-pt fluctuations exceed the 2-pt fluctuations, and this discrepancy is explained as arising from bead microenvironments or the semiflexible nature of the filaments [35, 36]. In all those systems, there was no active component, and the fluctuation dissipation theorem was applicable. The disagreement between the 1-pt and 2-pt MSD for the DNA/topo II provides strong evidence that interpretation of the observed fluctuations as being thermally-driven and reporting on the material response [1] is invalid. Instead, our two-point MSD results unambiguously indicate that the DNA/enzyme active network itself has excess strain fluctuations driven by nonthermal forces. Evidently in this system, even though the enzyme is non-processive, acting only on a single entanglement per binding, its activity provides non-thermal “kicks” to the surrounding DNA material.

To measure the material response in the active state directly, we performed measurements using optical tweezers to impose a sinusoidal oscillation as a function of frequency on a 1 μm-diameter probe particle within the material [4, 37]. The enzyme and ATP concentrations were identical to those used in the passive microrheology experiments. The trap stiffness was determined to be 0.018 pN/nm by measuring the Brownian motion of a bead trapped in water and using the equipartition theorem. Using this active microrheology method, we can determine the full frequency-dependent modulus of the DNA/enzyme active material. The data are plotted in Fig. 2. Here, the passive response of the material to small deformation is written here as the ‘spring constant’, \( K(\omega) = 3\pi d G(\omega) \), where \( G(\omega) \) is the viscoelastic shear modulus. Our measurements yielded a viscoelastic behavior that was unchanged within the experimental resolution with the addition of the enzyme and saturating ATP, over the observed frequency range. Thus, our tweezers measurements confirm that the response of the material in the active state cannot account for the enhanced fluctuations.

Our results demonstrate that the individual enzyme molecules exert forces, analogously to prototypical motor proteins whose specialized function is to generate biologically useful force and motion. Hence this DNA/enzyme system is an example of active matter. Since these minimal, stochastic motors clearly generate out-of-equilibrium fluctuations, it is crucial for a complete understanding of processes such as intranuclear transport to ask how the system injects energy, and how this compares to what we expect in light of recent experiments on processive cytoskeletal motors and machines [2–5].

To quantify the network stress fluctuations driven by these nonthermal forces, we measured the bead fluctuations in DNA/enzyme active network at different ATP levels, relative to those in the corresponding network driven only by thermal forces. The ATP levels studied ranged from the mM regime typical of ATP levels in the cell, and where this enzyme’s kinetics are shown to follow Michaelis-Menton kinetics; through the tens of μM regime typical of single molecule experiments, and where this
enzyme dimer on DNA exhibits cooperativity in ATP-binding; to a few nM.

To quantify the spectrum of the nonthermal forces, we adopted a recent theoretical framework for separating the active fluctuations when the forces are stochastic [8]. For a continuum viscoelastic medium characterized by a complex modulus, the displacement fluctuation spectrum \(\langle x^2(\omega) \rangle\) is \(\langle f^2(\omega) \rangle = \langle x^2(\omega) \rangle / |K(\omega)|^2\). In equilibrium systems, \(\langle f^2(\omega) \rangle\) is tied to the viscous response of the material, and the complex modulus can be calculated from the displacement fluctuation spectrum [8, 30, 38]. Conversely, if the stochastic displacement fluctuations are nonthermal in origin, the nonthermal noise spectrum is not tied to the viscous response of the material; but can be determined from separate measurements of the displacement fluctuation spectrum and the complex modulus, via \(\langle f^2(\omega) \rangle = \langle x^2(\omega) \rangle / |K(\omega)|^2\). We used this powerful approach to convert our fluctuation data in the enzyme-driven system at each ATP concentration to \(\langle f^2(\omega) \rangle\); shown in Fig. 3. When the ATP levels are reduced, we observe a marked reduction in the amplitude of particle fluctuations, and consequently in the amplitude of the total force spectrum; and the frequency dependence is subtly changed. When material was completely depleted of ATP, we observed a force spectrum that is consistent with purely thermal fluctuations over the full frequency range measured directly for the material, shown by the black data line in Fig. 3. This colored thermal force/noise spectrum is obviously qualitatively different from the total (active+thermal) force spectrum.

This frequency-dependent thermal noise eventually sets a noise floor for the system. To separate the frequency-dependent active noise from the thermal noise floor, we subtract the zero ATP, thermal spectrum from the total fluctuation spectrum at each ATP concentration, obtaining the active force spectrum \(\langle f^2(\omega) \rangle_{\text{act}}\). We found a nearly \(1/\omega\) spectrum, i.e. \(\langle f^2(\omega) \rangle_{\text{act}} = f_0^2/\omega\). Moreover, we find this \(1/\omega\) spectrum at all the ATP concentrations for which the active fluctuations were observable above the thermal spectrum background – down to a few \(\mu\)M ATP. The amplitude changed but the frequency-dependence did not. Evidently, the details of the ATP hydrolysis do not play a role in the mechanism underlying the \(1/\omega\) color of the active force spectrum.

For direct comparison with our measured active stress spectrum, we also superimpose on Fig. 4 the thermal stress spectrum of probe particles in the corresponding entangled DNA network in the absence of enzyme activity. The data clearly identify qualitative differences between active and thermal driving. The active driving is dominant at low frequencies and has a different time/frequency dependence than thermal driving. The transition to thermal noise dominance at high frequencies depends on overall amplitude of the motor-induced force fluctuations, which in turn depends generally on the density and activity of the ATP-consuming motors [6, 7]. The fluctuations in our system come from DNA scission/ligation forces, which depend directly on ATP. The density of the ATP-consuming motors is fixed, so the active spectrum magnitude should depend only on the motor activity. We plot the active spectrum amplitude \(f_0^2\), which scales the motor activity at the different ATP levels, against \(c_{\text{ATP}}\) in the Fig. 4 inset, and find that \(f_0^2\) increases linearly with \(c_{\text{ATP}}\).

This new \(1/\omega\) noise is far from what is expected for thermal motion, and of a completely different “color” (i.e. frequency spectrum) from non-equilibrium fluctuations in the cytoplasm driven by cytoskeleton motors and machines [2–5], which conform to a simple model of uncorrelated active fluctuations with a characteristic timescale [6–9]. \(1/\omega\) noise means that there is a wide range of processes with different timescales, and that it is not standard gaussian ‘white’ noise. This is in striking contrast with recent results measuring noise due to myosin in acto-myosin networks or microtubule (de)polymerization [2–5], whereby the motion of molecular machines exhibits a well-defined coherence time.

In conclusion, the active force spectrum measured in this new experimental work reflects the ensemble aggregate forces generated by an enzyme that stochastically transports the entangled DNA material inside biological nuclei, and provides knowledge of its collective mechanical modes of action. The nontrivial spectrum of the forces and implied broad distribution of timescales could be related to how network connectivity and topology-relaxing motor activity govern the propagation of stresses across long length scales [22]. Single molecule results suggest
that the rate-limiting step of the topo II enzyme works against an applied force $f$, and is slowed down by an Arrhenius factor related to the typical displacement (1.0 nm) against $f$ during the re-ligation step [39]. The broad distribution of timescales (made broader by the force appearing in the exponent) could arise from a distribution of tensions across the entanglements in the system [40], providing a mechanism by which distal interactions that are affected by the topology of the DNA might be correlated. Future work to extend such measurements in this controllable system to incorporate advanced DNA technologies or proteins that decorate DNA in the cell, should enable testing of possibilities for the mechanism underlying the nontrivial force spectrum of this material, and should be helpful more generally in understanding whether motor/active noise has different “colors” (i.e. frequency spectra) in different biological processes.

The authors thank Peter Olmsted and Fred MacKintosh for helpful discussions.