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Motion of Knots in DNA Stretched by Elongational Fields

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Knots in DNA occur in biological systems, serve as a model system for polymer entanglement, and affect the efficacy of modern genomics technologies. We study the motion of complex knots in DNA by stretching molecules with a divergent electric field that provides an elongational force. We demonstrate that the motion of knots is nonisotropic and driven towards the closest end of the molecule. We show for the first time experimentally that knots can go from a mobile to a jammed state by varying an applied strain rate, and that this jamming is reversible. We measure the mobility of knots as a function of strain rate, demonstrating the conditions under which knots can be driven towards the ends of the molecule and untied.

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Sufficiently long polymers are very likely to contain knots [1], and DNA is no exception. Knots occur naturally in biological DNA, are frequently found in virus capsids [2] and can be tied or untied in mammalian cells by topoisomerase enzymes [3,4]. Knotted DNA serves as a minimal system with which to study polymer entanglement [5] and knot theory as applied to polymer physics [6]. The biophysical study of DNA knots is finding relevance in the development of next-generation genomics technology, for example, in nanochannel mapping assays that are suffering erroneous results due to the stochastic presence of knots in confined DNA molecules [7]. Intentionally knotting DNA has been proposed as a braking mechanism to improve the temporal resolution of nanopore sequencing [8].

In this Letter, we investigate the mechanisms of knot motion in stretched DNA, which is mediated through self-reptation of contour through the knot, and provide clarity on a number of theoretical questions [9]. Knot self-reptation brings together several concepts in polymer physics, including intramolecular friction [10], the effect of topology on polymer behavior [11,12], and the relationship between semiflexibility and excluded volume [13]. We have predicted that knots will move diffusively along uniformly stretched chains, with a diffusivity that decreases with increasing tension [14]. We have also predicted that knots in molecules stretched in an elongational field will translate towards the end of the chain and untie [15]. Experimentally, the picture is less clear. In the only investigation of knot mobility, Bao *et al.* [16] measured the diffusivity of knots tied in DNA using optical tweezers and stretched in a highly viscous buffer, finding slower diffusion in more-complex knots. Preliminary data presented by Metzler *et al.* [17] show a knot moving along a nanochannel-confined DNA molecule until it reaches the end of the chain and unties, although quantitative measurements of the knots' mobility were not made. Plesa *et al.* [18] saw knots in DNA sliding along molecules as they translocated through a nanopore. The previous studies indicate that knots can indeed move along DNA molecules, but they shed little quantitative insight on the conditions under which knots may be mobile.

Here, we investigate two novel phenomena associated with knot motion that have not been previously investigated: we show that knots can be untied by driving them towards the chain ends with elongational fields, and that knot motion can be halted or promoted by controlling the local chain tension. We perform experiments stretching knotted DNA molecules with a divergent electric field in a microfluidic device and imaging them with fluorescence microscopy (see the video in the Supplemental Material [19]). By tracking the knot's position over time when the molecules are stretched at varying strain rates, we can study in detail how knot mobility is affected by stretching forces.

We use a polydimethylsiloxane (silicone) microfluidic device with a T-junction channel geometry [20] [the schematic in Fig. 1(a)]. The channels are 2 μm tall, with an inlet that is 40 μm wide and outlets that are 20 μm wide, with corners smoothed to hyperbolae. A buffer is pipetted into reservoir holes that interface the channels for macroscopic access, and electrodes are placed in each of the reservoirs, with the ground connected to the base of the T and two independently controlled positive leads connected to the two arms. We use T4 DNA stained with YOYO-1 fluorescent dye at a 4:1 base-pair:dye ratio in a 0.5× Tris–borate–ethylenediamine tetra-acetic acid buffer with 4% β-mercaptoethanol and 0.1% polyvinylpyrrolidone (10 kDa). Molecules were illuminated with a filtered light-emitting diode, visualized with a Zeiss-Axiovert microscope with a 63 × objective, and recorded with a Photometrics Prime 95B CMOS camera. The full contour length of stained T4 DNA is estimated to be 77 μm assuming a 38% elongation due to the intercalating dye [21].

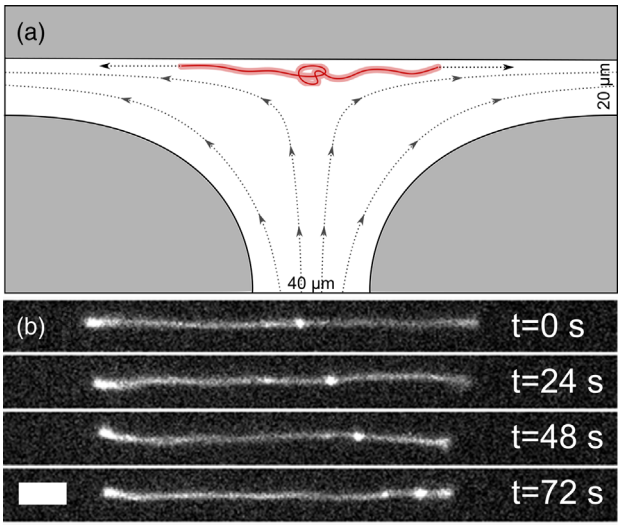

FIG. 1. (a) Drawing of the experimental device. A microfluidic T junction with a diverging electric field stretches knotted DNA at its stagnation point. Field lines show the trajectories of negatively charged test particles. (b) Representative images of a molecule at four time points as two knots translate towards one end of the molecule at Wi = 0.85. The scale bar is 5 μm. Approximately 25% of stretched molecules have two knots.

The electric field in the central region of the T-junction geometry mimics a planar elongational flow [20]. A charged particle accelerating through this region has a velocity in the horizontal direction that is proportional to its position. The effective strain rate $\dot{\epsilon}$ in the divergent region is dependent on the electrophoretic mobility μ of the test particles and the electric field gradient and is on the order of 1 s^{-1} in our setup:

$$v_x = \mu E_x = \dot{\epsilon} x, \qquad v_y = \mu E_y = -\dot{\epsilon} y. \tag{1}$$

When DNA spans the stagnation point, the ends are pulled in opposite directions and the molecule stretches. The molecule's internal elasticity balances the electrophoretic stretching, and a steady state extension is reached that is determined by the Weissenberg number (Wi), the product of the strain rate and the molecule's longest relaxation time τ, which is measured to be 2.2 s [see the Supplemental Material (SM) [19]]:

$$\mathrm{Wi} = \dot{\epsilon}\tau. \tag{2}$$

With fixed geometry, the strain rate is proportional to the applied voltage, which we control to stretch the molecule. The molecule is kept at the unstable stagnation point by small periodic toggles to one of the voltage sources. The voltages used in our experiments are generally between 10 and 30 V, giving a range of Wi in this work between 0.9 and 3.0. To entangle the molecules into knots, we apply a strong alternating electric field, above 1 kV/cm pulsed at a 10 Hz square wave for 0.5 s, to induce a hydrodynamic instability [22], causing the molecules to contract into tight globules. It is very likely for knots to remain trapped in the interiors of these molecules, where they appear as bright spots of excess fluorescence [Fig. 1(b)]. Accumulated evidence from our group [5,12,22,23] supports the fact that molecules undergoing this procedure do indeed form complex knots. The knots produced through this method have a diverse range of topologies that cannot be fully resolved, but previous analysis from our group [5,23] has shown that they contain several microns of contour when highly stretched, an order of magnitude greater than the few-hundred nanometers seen in simple knots [16,18]. To characterize how the electric field induces knot convection, we have used the Weissenberg number of the unknotted chain to characterize the field strength consistently across all molecules. Research by our group [24] suggests that an "effective" Weissenberg number that takes into account the reduction in the molecule's relaxation time may reduce the variance in chain extension as measured across molecules with different topologies.

We record videos of molecules stretched with a fixed voltage and vary the voltage with step changes over the course of several minutes, allowing us to probe the same knot under multiple strain rates. The videos are analyzed using MATLAB scripts that project the linear intensity profile of extended molecules into a kymograph, where the knot is identified as a bright trajectory along the molecule's worldsheet. The location of the knot is taken as the index of the brightest pixel, and the dimensionless knot position is defined as the ratio of the knot's distance from the left end of the molecule to the instantaneous molecular extension.

When molecules are held at a steady strain rate, the knots are seen over minute-long timescales to translate along the molecule via self-reptation, and if a molecule is observed for long enough, it is indeed likely for the knot to reach the end of the chain and untie [Fig. 2(a)]. If the knot's motion is purely diffusive, we expect the mean-square displacement (MSD) of the knot's position to grow linearly over time, but it is seen (in the SM [19]) that, at long time lags, the MSD grows superdiffusively. In Fig. 2(b), an ensemble of knot trajectories and their average are shown as a function of their accumulated strain (the residence time multiplied by the strain rate). For the higher strain-rate trajectories the motion is largely driven, whereas for lower strain rates there is a more apparent diffusive behavior.

There are two limiting cases characterizing the motion of polymer knots in elongational fields. If the knot is experiencing affine deformation, behaving as a particle following the elongational field line without any other interaction, it would have a position growing exponentially in time [25] according to the total strain experienced:

$$\kappa(t) = \kappa(0) e^{\dot{\epsilon} t} = \kappa(0) e^{\epsilon}. \tag{3}$$

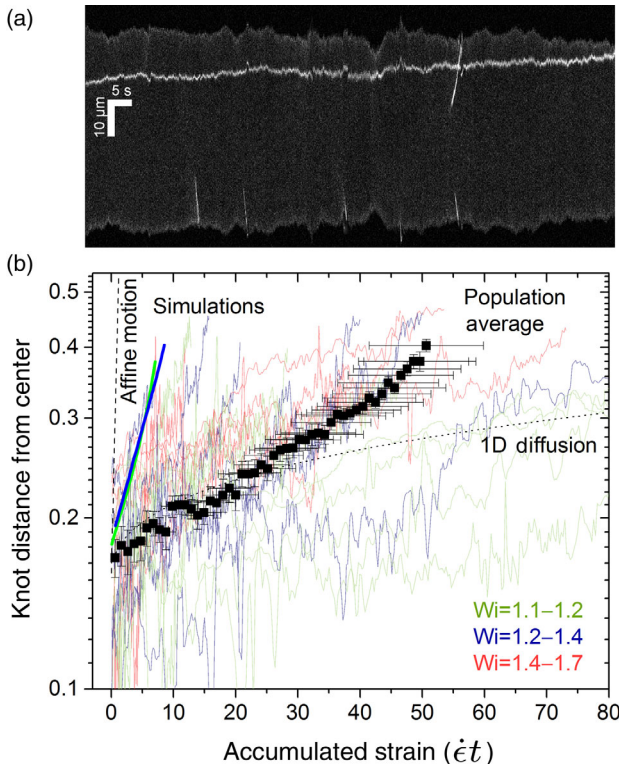

FIG. 2. (a) A kymograph of a knotted molecule stretched at Wi = 1.6. The white streaks are from other molecules passing through the field of view. (b) Averaged trajectory of translating knots along an ensemble of molecules as a function of accumulated strain, with individual trajectories underlaid. Green trajectories occurred at Wi= 1.1–1.2, blue trajectories at Wi= 1.2–1.4, and red trajectories at Wi= 1.4–1.7. The error bars represent standard error over a population average. The nearly vertical dotted line represents affine motion [Eq. (3)], and the curved line represents the hypothetical ensemble trajectory assuming a one-dimensional (1D) random walk with a diffusivity of 0.5 $\mu m^2/s$ [16] [Eq. (4)]. The thick blue and green curves represent simulations of 5_2 and 6_3 knots [15].

While pure affine deformation is not expected, its predicted behavior sets an upper bound on the knot trajectory. Conversely, if there is no bias in the knot's motion, it can be modeled as a particle undergoing one-dimensional Brownian motion, as in the work of Bao *et al.* [16] and Narsimhan *et al.* [14]. In this case, the population-averaged trajectories will have a root-mean-square displacement that grows as the one-half power of time:

$$\kappa(t) = \kappa(0) + \sqrt{Dt} = \kappa(0) + \sqrt{2D\frac{\epsilon}{\dot{\epsilon}}}. \qquad (4)$$

As each knot's position trends towards the chain end, the position of the knot diverges with time in a manner inconsistent with and faster than Brownian motion (the nondimensionalization of the Brownian prediction with the knot's hypothetical diffusivity of 0.5 $\mu m^2/s$ is discussed in the SM [19]). The knot trajectories, though driven towards the chain ends, are considerably slower than the affine prediction, by a factor of 63. This presents a picture of knot motion where the knots tend to follow the lines of the elongational field, but in which motion is significantly hampered by intrachain friction within the knot. Simulations of simple knot topologies (5_2 and 6_3) [15] show that the knots are driven faster than what is typically observed experimentally, but still significantly slower than affine deformation. These results describe the process by which the knot moves towards the chain end, although our analysis halts before the knot reaches the end. When this happens, the molecule often contracts as the knot swells in the lower tension region near the end before spontaneously restretching as the knot unties, a process described in greater detail in our recent publication [12].

The picture of knot mobility is more complex than merely following field lines. A stronger field does not hasten the convection of the knots but instead does the opposite: the motion of knots can be halted or slowed by suddenly increasing the applied strain rate. We performed an assay in which knotted molecules were stretched at Wi = 1.1, and as the knots approached the chain end, the applied voltage was suddenly increased (Fig. 3), with an average post-increase strain rate of Wi = 1.9. Prior to the increase, there was a strong correlation between knot position and time (with a mean Pearson coefficient $r = 0.9$), but afterwards the correlation was weak ($r = -0.35$), and the mean-square knot displacement at a given time lag over the measured period dropped by a median factor of 3. We observed that the endward motion of knots could be halted by a step change in extension, suggesting that the decrease in knot mobility has a

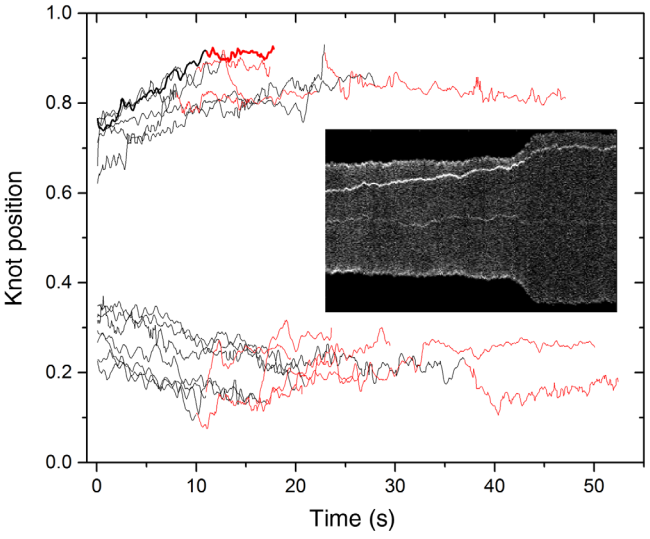

FIG. 3. Time traces of knots moving towards the ends of the chain at Wi = 1.1 (gray) and halting as the strain rate is increased to Wi = 1.9 (red). (Inset) An example of a molecule with two knots, with the larger one moving towards the end and stopping as the molecule is stretched (corresponding to the bold curves), and the smaller one moving about the center of the molecule until halting when the local tension is increased.

stronger effect than the increased convective drive. If the knot is sufficiently close to the end, however, its motion cannot be stopped.

To quantify the relationship between mobility and Wi, we performed an assay [Fig. 4(a)] in which knotted molecules were initially stretched at a high voltage for a minute, then the strain rate was decreased every minute until the knot untied or the molecule relaxed to its coiled state. The range of fields used was between Wi = 0.9 and Wi = 2.9, corresponding to estimated tensions between 0.1 and 0.5 pN at the centers of the molecules (see the SM [19]), but lower towards the ends. Although the knot diffusivity is a natural quantity with which to characterize the knot's motion, the MSD trajectories did not display diffusive behavior but rather were characterized by subdiffusion at short times and superdiffusion at long times. Instead, we choose a fixed time interval of 5 s (long enough to avoid tracking noise [26] but short enough to ensure sufficient statistics) and measure the knot MSD (described in greater detail in the SM [19]) over that interval and divide by the time to characterize the knot's mobility. Figure 4(b) shows the knot mobility as a function of Wi for several molecules as well as the population average. The mobility of the knot decreases with an increasing strain rate. It varies by more than an order of magnitude as a function of strain rate, while the chain extension varies by less than a factor of 2. While there is variation in the mobility with respect to the position of the knot, the observed variation in position-dependent mobility is smaller than the strain-rate dependence, and the variation in knot position throughout the step-down assay is small (see the SM [19]). Comparison between individual knot trajectories is difficult due to both the variation in their initial position and the diverse sampling of topologies, but overall populationwide trends are observed.

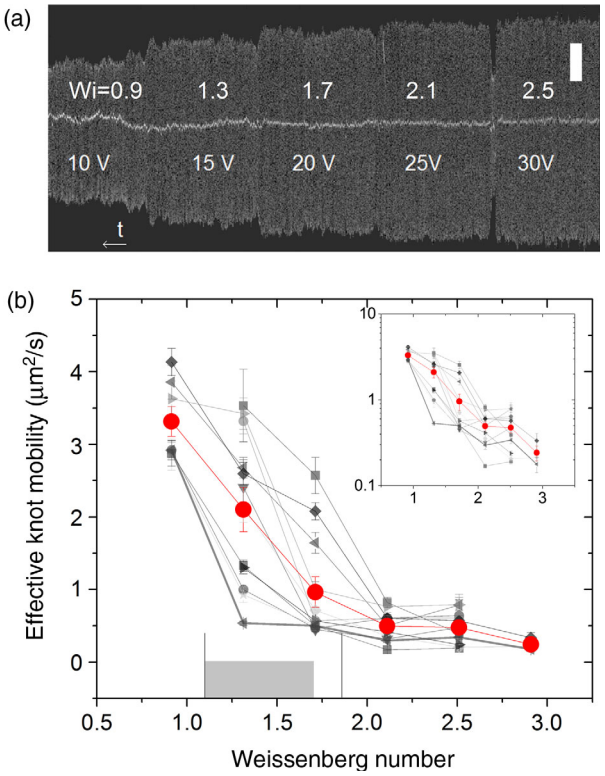

FIG. 4. (a) Kymograph of a molecule held at varying levels of the applied field (Wi= 0.9–2.5, 5–30 V) for a minute each, showing greater mobility at lower Wi. Note that time runs right to left in this image. The scale bar is 10 μm. (b) Relationship between the measured effective knot mobility and the Weissenberg number. Each gray-scale trace represents a different molecule, and the red represents the population average. The vertical lines correspond to the high and low Wi in Fig. 3, and the gray bar corresponds to the range observed in Fig. 2(b). (Inset) The same data with a logarithmic y axis.

Two computational papers from our group [14,15] provide insight on the motion of knots under varying strain-rate conditions. An earlier study by Renner and Doyle [15] predicted that knots would undergo convective motion beyond a critical length scale near the center of the molecule, and indeed such convection was observed in our experiments. However, these simulations used a semiflexible bead-spring model, which was unable to sufficiently probe the role of intrachain friction because of pathological chain crossings at high tensions. A subsequent study by Narsimhan *et al.* [14] overcame these difficulties by using a bead-rod parametrization and by studying flexible as well as semiflexible chains, and it found that friction arising due to molecular corrugation (e.g., corresponding to the grooves along the double helix) can jam the motion of knots as tension increases. The data presented in Fig. 3(b) (of Ref. [14]) exemplify this, showing that although the trend of knot motion is convective, sufficient internal friction (likely occurring when the strands in the knot are bent at subpersistence length scales) can overcome the convective forces and halt the motion of the knot. There is a small dynamic range of strain rates that can be applied such that the molecule does not relax to the coiled state but that the knot is noticeably mobile over minute-long timescales, roughly in the range Wi= 0.9–1.5.

Our results contrast with those of Bao *et al.* [16], who presented knot diffusivity data for molecules stretched at tensions between 0.11 and 0.33 pN as determined by their reported fractional extensions [27] (corresponding to central chain tensions found at Wi $\approx$ 1 to 2 in our work; see the SM [19]), and additionally state that they found no tension dependence between 0.1 and 2 pN. Because they sampled the diffusivity of typically three to four molecules per topology and averaged over tensions spanning a factor of 3, they may not have had sufficient statistics to exclude tension dependence. Furthermore, it is difficult to directly compare our results to those of Bao *et al.* due to the buffer used in their Letter (an overlapping polyethylene glycol solution), the simpler knots they study (seven crossings and fewer), and the difference between uniform tension and the

quadratic tension in an elongational field [28]. Assuming that Bao *et al.* did collect significant but unreported evidence that knot motion is tension independent, we posit that the experiments of Bao *et al.* failed to see tension dependence because they took place in a regime where the strands in the knot are insufficiently close for corrugation-based friction to be relevant [29]. According to the analysis of Bao *et al.*, the strands of DNA within the knot are separated by 26 nm, a factor of 10 greater than the DNA corrugation length scale. By comparison, our experimental buffer gives DNA an effective width of roughly 10.5 nm [30], with 8.5 nm between the strands, less than a factor of 3 above the corrugation length scale.

In summary, we examined the motion of knots in DNA molecules stretched in elongational fields. We observed that knots moved noticeably across the molecule on minute-long timescales until they reached the end and untied. The motion of the knots was driven towards the ends of the chain, the knots reaching the end faster than would be expected from simple one-dimensional diffusion. The motion of the knots can be halted by increasing the strain rate to induce greater intramolecular friction within the knot. We measured the mobility of knots with respect to strain rate and found that knots tend to slow with an increasing Wi. Our findings suggest a systematic way of removing unwanted knots from molecules. This method of removing knots by applying an elongational field may suggest a modification of nanochannel mapping assays [7] to include a cross flow [31] to remove knots.

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