

Steady-State and Transient Behavior of Knotted Chains in Extensional Fields

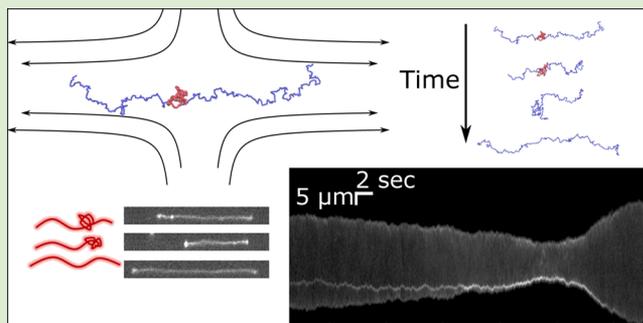
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Supporting Information

ABSTRACT: Recently, there has been a push to understand how molecular topology alters the nonequilibrium dynamics of polymer systems. In this paper, we probe how knotted polymers evolve in planar extensional fields using Brownian dynamics simulations and single-molecule experiments. In the first part of the study, we quantify the extension versus strain-rate curves of polymers and find that knots shift these curves to larger strain-rates. These trends can be quantitatively explained by Rouse-like scaling theories. In the second half of the study, we examine the consequences of knot untying on the time-dependent conformations of polymers in these external fields. We find that knot untying creates significant, transient changes in chain extension. If the topology is complex, the chain undergoes a wide range of time-dependent conformations since knot untying proceeds through many different stages. We provide examples of such untying trajectories over time.



It is unsurprising to physicists that molecular topology plays a vital role in influencing the dynamics of polymers.^{1–7} One such example is a knot on an open chain.⁸ These architectures have been found naturally in DNA^{9,10} and proteins,¹¹ and they have been discovered in nanotechnology applications that perform single-molecule sequencing^{12–15} and genetic mapping¹⁶ of DNA. The removal of self-entanglements is important for the latter technologies as these structures give rise to incorrect readings of base-pair sequences.^{12,16} Since these issues become inevitable as chain sizes become longer,^{17,18} there has been a recent push to understand the physics of knotted polymers in more detail.

Knots can be tied onto DNA by optical tweezers,¹⁹ collisions with nanochannel walls,²⁰ or electric fields.^{21–23} These techniques and coarse-grained simulations allow researchers to study (1) the size and abundance of knots on open chains,^{24–26} and (2) the motion of knots as they traverse along the polymer backbone.^{19,22,27–29} The dynamics of knotted chains far from equilibrium, however, have not been examined as extensively. In this study, we perform Brownian dynamics simulations and single-molecule experiments to quantify (a) the extension versus strain-rate curves of knotted polymer chains and (b) the time-dependent conformations of these chains during knot untying. The chains we examine are highly elongated, which differs from previous works that studied the stretching of self-entangled polymers from a collapsed state.^{30,31} Our study probes how knots shorten the relaxation time of stretched chains and how this phenomenon leads to a variety of large-scale changes in polymer conformation upon untying.

Figure 1a shows a schematic of our Brownian dynamics simulations. We represent our polymer as a flexible, linear string of

N connected beads of size b . We enforce constant bond lengths³² and allow minimal bead overlaps by enforcing a stiff repulsive potential between non-neighboring beads³³ with stiffness $H \gg kT/b^2$. Unless otherwise noted, we neglect hydrodynamic interactions. We tie a knot into the center of the polymer chain, stretch the chain in planar extensional flow $u_x^\infty = \dot{\epsilon}(x - x_{cm})$, $u_y^\infty = -\dot{\epsilon}(y - y_{cm})$, and wait until the chain extension reaches steady-state. When examining steady-state extension, we periodically add reptation moves³⁴ to keep the knot near the center of the chain. When examining time-dependent effects, we do not add this effect. We note that the polymer's center of mass coincides with the flow's stagnation point, which single-molecule experiments are able to maintain for minutes using manual control.²³ We measure the extension $\langle x \rangle_{\text{ext}}$ as the maximum distance subtended by the molecule in the direction of stretching: $\langle x \rangle_{\text{ext}} = \max_{n,m} |x_n - x_m|$ over all beads.

We also measure the knot size L_{knot} as the chain contour stored in the knot, calculated by determining the minimal region on the chain that retains the polymer's overall topology.^{28,35} When measuring steady-state extension, we average all measurables over time $t = 4500 b^2 \zeta / kT$, where ζ is the drag coefficient on each bead and kT is the thermal energy. The knots studied are shown in Figure 1b. More details are in the Supporting Information (SI).

Figure 1c shows the extension versus strain-rate curves of the unknotted and knotted chains for $N = 200$ beads. The x -axis is

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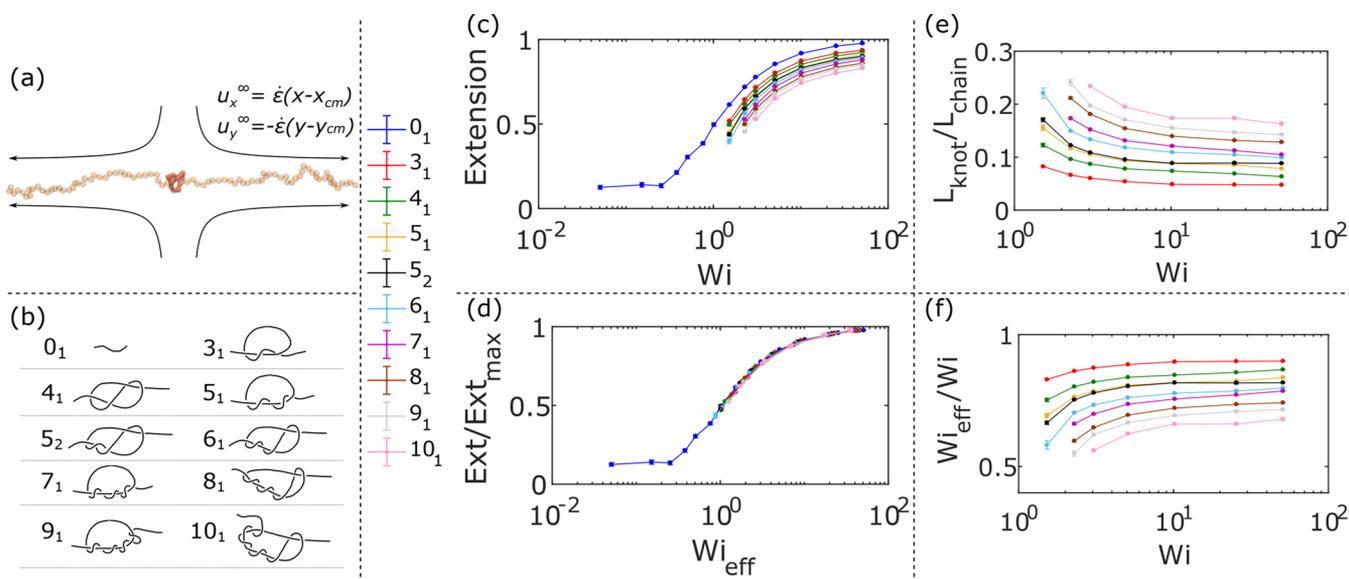


Figure 1. (a) Schematic of setup. (b) Knot topologies studied. (c) Steady-state fractional extension vs Wi for different chain topologies. (d) Rescaling of data in (c). The y -axis is rescaled by the maximum extension the knotted polymer can achieve, approximated as $\langle x \rangle_{\text{ext,knot}} / \langle x \rangle_{\text{ext,unknot}}$ at $Wi = 50$. The x -axis is a renormalized Weissenberg number $Wi_{\text{eff}} = Wi(1 - L_{\text{knot}}/L_{\text{chain}})^{1+2\nu}$, with $\nu = 0.5876$ being the Flory exponent. (e, f) Steady-state knot sizes and Wi_{eff} as a function of topology and strain-rate (i.e., Wi). For all graphs, we examine a freely draining chain of $N = 200$ beads with error bars being standard errors over 5–10 runs. We observe similar scaling for freely draining and non-draining chains of $N = 100$ beads in the SI.

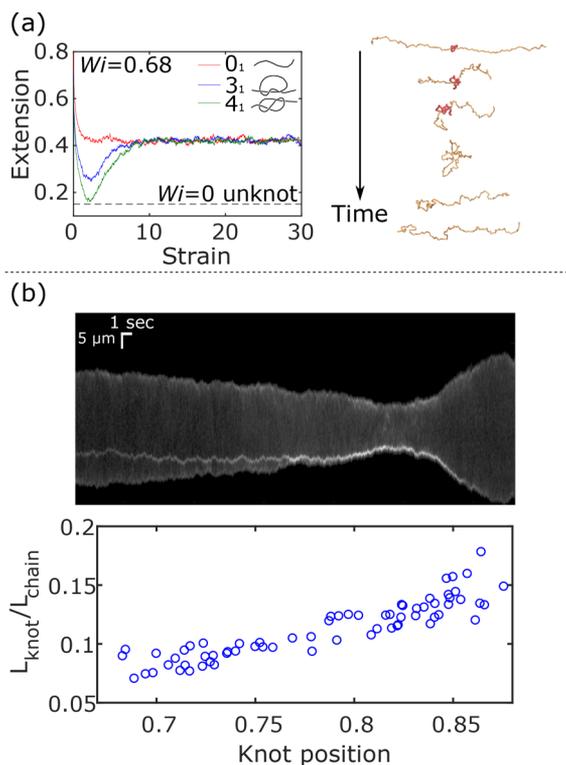


Figure 2. (a) Left: mean fractional extension vs strain for knotted and unknotted polymers at $Wi = 0.68$ and $N = 100$ beads, starting at ~ 70 – 80% extension before being placed in flow. The knotted polymers collapse to near equilibrium ($Wi = 0$) extension and then expand when the knot unties, whereas the unknotted chains proceed directly to their steady-state extension. Curves are averages over 50 runs. Right: snapshots of a 3_1 knotted chain untying. (b) Top: kymograph of a knotted, T4-GT7 DNA molecule ($75 \mu\text{m}$) at $Wi = 1.7$. The knot (i.e., the bright spot on the chain) causes the chain to contract until the knot unties, at which point the chain expands to a much larger steady-state extension. More pictures of this process are in the SI. Below: knot size vs its relative position on the chain (measured from 0 to 1).

the Weissenberg number, $Wi = \dot{\epsilon}\tau_{\text{unknot}}$ of the unknotted chain, where τ_{unknot} is the longest relaxation time of the polymer obtained by fitting the squared end-to-end distance $\langle R^2 \rangle$ to an exponential: $\langle R^2 \rangle - \langle R^2_{\text{equib}} \rangle \sim \exp(-t/\tau_{\text{unknot}})$ (see SI). The y -axis is the fractional extension $\langle x \rangle_{\text{ext}}/L_{\text{chain}}$. As shown in Figure 1c, knotted chains have smaller extensions compared to an unknotted chain for a given Wi . The shift in these curves are reminiscent of the force–extension curves under constant tension by Caraglio et al.³⁶ and the planar extensional experiments by Renner et al.,³⁰ which show that knots stiffen the chain by reducing the chain’s conformational degrees of freedom. We note that we are only able to probe steady-state extensions of knotted polymers for $Wi \gtrsim 1.5$ since knots untie when nearly coiled.

We can understand the altered extension vs strain-rate curves by considering how a knot modifies a chain’s relaxation from a stretched state. Rouse theory predicts that a chain’s relaxation time scales with its length as $\tau \sim L_{\text{chain}}^{1+2\nu}$, where $\nu = 0.5876$ is the Flory exponent for excluded volume interactions.³⁷ For a knotted chain, the amount of free contour available to relax is $L_{\text{chain}} - L_{\text{knot}}$. The ratio of relaxation times is thus given by $\tau_{\text{knot}}/\tau_{\text{unknot}} = (1 - L_{\text{knot}}/L_{\text{chain}})^{1+2\nu}$, and hence, the appropriate Wi to describe knotted chains should be

$$Wi_{\text{eff}} = \dot{\epsilon}\tau_{\text{unknot}} \left(\frac{\tau_{\text{knot}}}{\tau_{\text{unknot}}} \right) = Wi \left(1 - \frac{L_{\text{knot}}}{L_{\text{chain}}} \right)^{1+2\nu} \quad (1)$$

In Figure 1d, we rescale Wi using eq 1. We furthermore rescale the chain’s fractional extension by the maximum extension that the knotted polymer can obtain, that is, $\langle x \rangle_{\text{ext,max}} = \lim_{Wi \rightarrow \infty} \langle x \rangle_{\text{ext,knot}} / \langle x \rangle_{\text{ext,unknot}}$, where the limit is approximated at $Wi = 50$. When we perform these operations, all the data collapse onto a master curve. A similar collapse occurs for different chain sizes and chains with hydrodynamic interactions (see SI). We note that for Wi_{eff} in eq 1, the knot size L_{knot} is a function of the strain-rate (Figure 1e), and hence, Wi_{eff} depends nontrivially on the strain-rate (Figure 1f).

The altered relaxation of the chain occurs because the knot, when tightened, (a) increases the microscopic states available to the free contour, and (b) increases the drag on the portion of the chain that can relax (i.e., contour outside the knot). The conformational-dependent drag occurs even for freely draining chains (Figure 1) and, hence, could have consequences in coil–stretch hysteresis.³⁸ We note that for highly extended polymers with hydrodynamic interactions, we observe the same scaling for Wi_{eff} as eq 1 because the chain's drag and the entropic spring constant scale with its length as $\zeta_{\text{chain}} \sim L_{\text{chain}}$ and $H_{\text{spr}} \sim L_{\text{chain}}^{-2\nu}$. We expect this Rouse-like scaling to fail when the chain is nearly coiled ($Wi \approx 0.5$), but this scaling may hold near the coiled state if the chain is freely draining.

Because knots alter the relaxation of polymer chains, the process of unknotting can greatly modify the time-dependent conformation of polymers in flow. An example is shown in Figure 2a when Wi is slightly above the coil–stretch transition of the unknotted chain ($Wi = 0.68$). Here, we examine the dynamics of three polymer topologies placed in planar extensional flow with initial states at ~ 70 – 80% extension (analogous to a step decrease in strain-rate in experiments). The unknotted chain evolves to its steady-state extension (Figure 2a), while the knotted chains (3_1 and 4_1 knots) first collapse to a nearly coiled state before expanding when the knot unties. This undershoot in extension is significant and is more severe for more complicated topologies. As discussed previously, the presence of a knot decreases the relaxation time of a polymer such that at a constant strain-rate it can be naturally coiled when knotted ($Wi_{\text{eff}} < 0.5$) but extended ($Wi_{\text{eff}} > 0.5$) after the knot unties. Such swings in extension are rarely observed for unknotted chains.

Figure 2b shows an experimental kymograph of how knot untying can induce similar extension undershoots even for Wi well beyond the coil–stretch transition ($Wi = 1.7$). These single-molecule studies follow the protocol in Klotz et al.²³ Briefly, a solution of fluorescently stained, T4-GT7 DNA ($75 \mu\text{m}$) is loaded in a microfluidic T-junction³⁹ with electrodes at the inlets and outlets. The T-junction is $2.0 \mu\text{m}$ tall with one $40 \mu\text{m}$ wide inlet and two $20 \mu\text{m}$ wide outlets. We first pulse a square wave electric field of $\sim 150 \text{ V/cm}$ for $\sim 1 \text{ s}$ at 10 Hz in the straight section of the inlet to collapse the DNA into a compact globule that likely contains one or more knots in its interior.^{21,23,30} We next apply a constant voltage drop to create a planar-extensional field at the center of the T-junction. We stretch the DNA at the field's stagnation point and record its dynamics over time. The knots appear as bright regions of excess fluorescence on the extended chain. See SI for more details.

The experiments demonstrate several key features: as the knot moves toward the end of the chain, the tension in the knot decreases which causes the knot size to swell (Figure 2b). This process decreases Wi_{eff} (eq 1), which in turn reduces the chain extension drastically. When the knot fully unties, the chain stretches back to its steady-state configuration. Other experimental examples of such dynamics are shown in the SI.

In the previous examples, we examined situations where the knot unties in one step. However, it is possible for complex topologies to untie in multiple stages, which adds additional complexities to the chain's dynamics. To understand how such topologies alter untying, we perform simulations of an initially extended 10_1 knot placed in planar extensional flow ($Wi = 0.74$). We observe that the knotted polymers collapse until they untie, after which they extend to a steady-state configuration (Figure 3a).

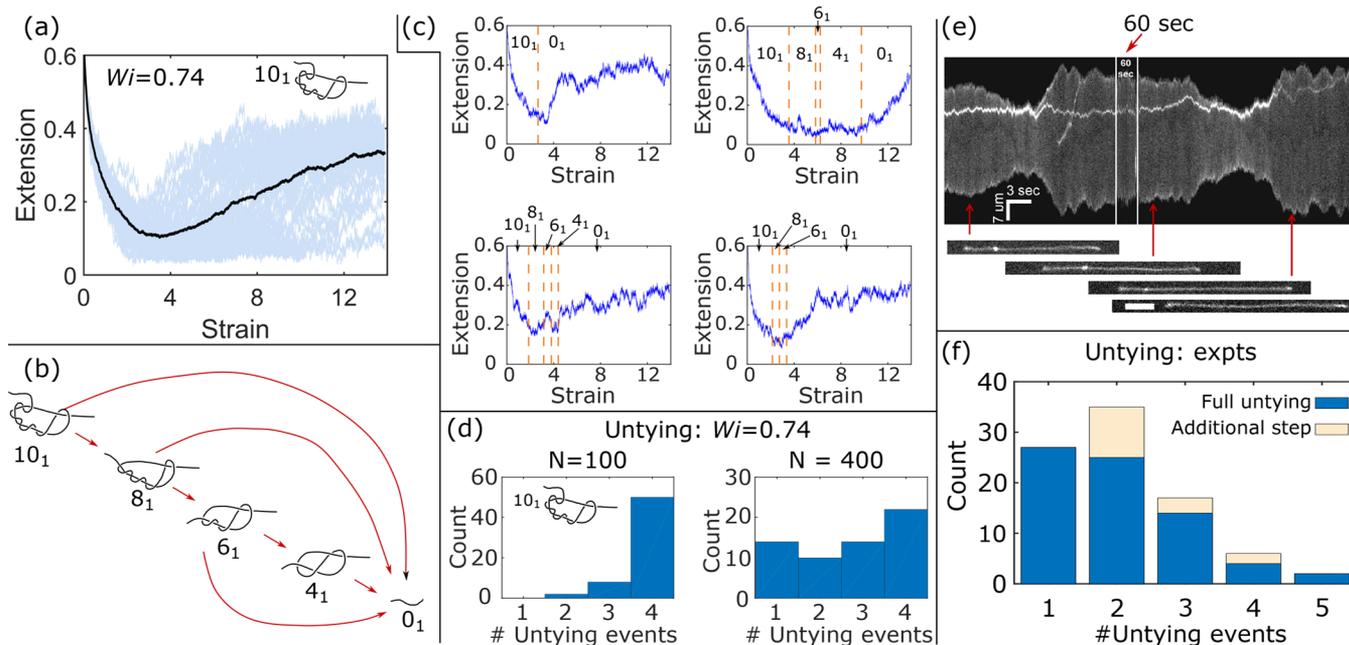


Figure 3. (a) Fractional extension vs strain for a 10_1 knot at $Wi = 0.74$ and $N = 400$ beads when the chain is initially extended. The chain collapses, unties, and stretches toward a steady-state. Blue curves are traces from 60 runs, and the black curve is the average. (b) Possible sequences for a 10_1 knot to unravel. (c) Examples of different untying dynamics of the 10_1 knot from trajectories in (a). (d) Histogram of the number of untying stages for the 10_1 knot at $Wi = 0.74$ for two different chain sizes ($N = 100$ and 400). (e) Kymograph of a knotted DNA molecule untying in multiple stages at $Wi = 1.7$ in experiments. The break in the kymograph is an uneventful minute, and the wayward brightness before the break is another molecule drifting through the field of view (full image in SI). Bottom: snapshots of the molecule (scale bar = $8 \mu\text{m}$) with an initial knot, smaller knots after two stages of partial untying, and no knot. (f) Histogram of the number of observed untying stages for an ensemble of 87 T4-DNA molecules stretched at $Wi = 1$ – 3 . For molecules that only partially untie during the allotted recording period, we assume one additional stage is required for full untying, although this may underestimate the mean number of stages.

However, the individual traces in chain extension (blue) exhibit large variations because the untying process is heterogeneous, that is, demonstrates molecular individualism.⁴⁰ For example, a sampling of individual runs in Figure 3c show that the knot can untie all-or-none or stage-wise. We observe this phenomenon for various chain sizes and it appears that the knot is more likely to untie stage-wise when it occupies a larger fraction of the chain (Figure 3d). We note that in our simulations, we measure topology changes in a strain window of $\dot{\epsilon}t = 0.04$, which means that we underestimate the number of untying events when multiple topology changes occur during this window. This situation occurs for roughly 3% of our simulations.

In our experiments, we do not know the exact topology of the chain but have evidence that the crossing number is greater than 10,²³ which suggests that the multifaceted dynamics in our simulations should be observable. In fact, we do observe different untying pathways experimentally; an all-or-none event is shown in Figure 2b, and an extended three-stage untying is shown in Figure 3e. Just like in our simulations, the diversity in these unraveling dynamics leads to a wide range of knot escape times, intermediate knotted states, and time-dependent chain extensions. In Figure 3f, we show a histogram of experimentally observed untying events for 87 molecules from $W_i = 1-3$. While experiments are more likely than simulations to observe an all-or-none untying, it is likely that the true number of events is greater than what is observed due to our inability to resolve the dynamics within the fluorescent bulb at the chain end. There are two likely mechanisms for the observed shrinking of the knots: a prime knot can partially untie as in Figure 3b, or one member of a compound knot can unravel. While we cannot distinguish these mechanisms visually, we note that on rare occasions a bright knot in the chain has been observed to bifurcate into two smaller knots. These observations of multi-stage untying support our previous claims that the knots produced via electrohydrodynamic collapse are quite complex.^{23,30}

In this study, we examined the dynamics of knotted polymers in extensional fields. We find that a knot shifts the coil-stretch transition of a polymer to a larger strain-rate since the knot hastens the relaxation of the stretched, polymer chain. The modified relaxation time can be described by a Rouse-like scaling theory (eq 1). We then examined how this phenomenon leads to large-scale, transient changes in the chain extension when the knot unties. For complex topologies, we observe that a knot can untie in many different ways, which leads to a diversity of time-dependent polymer conformations in flow. This is another example of molecular individualism⁴⁰ which to our knowledge has been unexplored thus far. In the future, there are several avenues to pursue. For example, the scaling theories developed hold for steady-state configurations, whereas the knot untying is a transient process. Can we extend our theories to situations where the knot size changes dynamically? Lastly, can one use the knot untying pathways to provide greater insight into the topology of the chain?

■ ASSOCIATED CONTENT

■ Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsmacrolett.7b00600.

Simulation and experimental details. Steady-state extensions of freely draining and non-draining chains. Experimental pictures of knot untying (PDF).

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

- (1) Li, Y.; Hsiao, K.-W.; Brockman, C. A.; Yates, D. Y.; Robertson-Anderson, R. M.; Kornfield, J. A.; San Francisco, M. J.; Schroeder, C. M.; McKenna, G. B. When Ends Meet: Circular DNA Stretches Differently in Elongational Flows. *Macromolecules* **2015**, *48*, 5997–6001.
- (2) Mai, D. J.; Marciel, A. B.; Sing, C. E.; Schroeder, C. M. Topology-Controlled Relaxation Dynamics of Single Branched Polymers. *ACS Macro Lett.* **2015**, *4*, 446–52.
- (3) Mai, D. J.; Schroeder, C. M. Single polymer dynamics of topologically complex DNA. *Curr. Opin. Colloid Interface Sci.* **2016**, *26*, 28–40.
- (4) Robertson, R. M.; Smith, D. E. Strong effects of molecular topology on diffusion of entangled DNA molecules. *Proc. Natl. Acad. Sci. U. S. A.* **2007**, *104*, 4824–27.
- (5) Robertson, R. M.; Smith, D. E. Self-Diffusion of Entangled Linear and Circular DNA Molecules: Dependence on Length and Concentration. *Macromolecules* **2007**, *40*, 3373–77.
- (6) Robertson, R. M.; Smith, D. E. Direct Measurement of the Confining Forces Imposed on a Single Molecule in a Concentrated Solution of Circular Polymers. *Macromolecules* **2007**, *40*, 8737–41.
- (7) Kapnistos, M.; Lang, M.; Vlassopoulos, D.; Pyckhout-Hintzen, W.; Richter, D.; Cho, D.; Chang, T.; Rubinstein, M. Unexpected power-law stress relaxation of entangled ring polymers. *Nat. Mater.* **2008**, *7*, 997–1002.
- (8) Meluzzi, D.; Smith, D. E.; Arya, G. Biophysics of Knotting. *Annu. Rev. Biophys.* **2010**, *39*, 349–66.
- (9) Liu, L. F.; Perkocho, L.; Calendar, R.; Wang, J. C. Knotted DNA from bacteriophage capsids. *Proc. Natl. Acad. Sci. U. S. A.* **1981**, *78*, 5498–502.
- (10) Wasserman, S. A.; Cozzarelli, N. R. Biochemical topology: applications to DNA recombination and replication. *Science* **1986**, *232*, 951–60.
- (11) Taylor, W. R. A deeply knotted protein structure and how it might fold. *Nature* **2000**, *406*, 916–19.
- (12) Plesa, C.; Verschuere, D.; Ruitenber, J. W.; Witteveen, M. J.; Jonsson, M. P.; Grosberg, A. Y.; Rabin, Y.; Dekker, C. Observation of DNA Knots Using Solid-State Nanopores. *Biophys. J.* **2015**, *108*, 166a.
- (13) Narsimhan, V.; Renner, C. B.; Doyle, P. S. Translocation dynamics of knotted polymers under a constant or periodic external field. *Soft Matter* **2016**, *12*, 5041.
- (14) Suma, A.; Micheletti, C. Pore translocation of knotted DNA rings. *Proc. Natl. Acad. Sci. U. S. A.* **2017**, *114*, E2991–E97.
- (15) Suma, A.; Rosa, A.; Micheletti, C. Pore Translocation of Knotted Polymer Chains: How Friction Depends on Knot Complexity. *ACS Macro Lett.* **2015**, *4*, 1420–24.
- (16) Reifenger, J. G.; Dorfman, K. D.; Cao, H. Topological events in single molecules of E. coli DNA confined in nanochannels. *Analyst* **2015**, *140*, 4887–94.

- (17) Rensburg, E. J.; Sumners, D. A. W.; Wasserman, E.; Whittington, S. G. Entanglement complexity of self-avoiding walks. *J. Phys. A: Math. Gen.* **1992**, *25*, 6557–66.
- (18) Sumners, D. W.; Whittington, S. G. Knots in self-avoiding walks. *J. Phys. A: Math. Gen.* **1988**, *21*, 1689.
- (19) Bao, X. R.; Lee, H. J.; Quake, S. R. Behavior of complex knots in single DNA molecules. *Phys. Rev. Lett.* **2003**, *91*, 265506.
- (20) Metzler, R.; Reisner, W.; Riehn, R.; Austin, R.; Tegenfeldt, J. O.; Sokolov, I. M. Diffusion mechanisms of localised knots along a polymer. *Europhys. Lett.* **2006**, *76*, 696.
- (21) Tang, J.; Du, N.; Doyle, P. S. Compression and self-entanglement of single DNA molecules under uniform electric field. *Proc. Natl. Acad. Sci. U. S. A.* **2011**, *108*, 16153–58.
- (22) Renner, C. B.; Doyle, P. S. Untying Knotted DNA with Elongational Flows. *ACS Macro Lett.* **2014**, *3*, 963–67.
- (23) Klotz, A. R.; Narsimhan, V.; Soh, B. W.; Doyle, P. S. Dynamics of DNA Knots during Chain Relaxation. *Macromolecules* **2017**, *50*, 4074–82.
- (24) Dai, L.; Renner, C. B.; Doyle, P. S. Metastable Tight Knots in Semiflexible Chains. *Macromolecules* **2014**, *47*, 6135–40.
- (25) Dai, L.; Renner, C. B.; Doyle, P. S. Origin of Metastable Knots in Single Flexible Chains. *Phys. Rev. Lett.* **2015**, *114*, 114.
- (26) Dai, L.; Renner, C. B.; Doyle, P. S. Metastable Knots in Confined Semiflexible Chains. *Macromolecules* **2015**, *48*, 2812–18.
- (27) Matthews, R.; Louis, A. A.; Yeomans, J. M. Effect of topology on dynamics of knots in polymers under tension. *Europhys. Lett.* **2010**, *89*, 20001.
- (28) Narsimhan, V.; Renner, C. B.; Doyle, P. S. Jamming of Knots along a Tensioned Chain. *ACS Macro Lett.* **2016**, *5*, 123–27.
- (29) Di Stefano, M.; Tubiana, L.; Di Ventra, M.; Micheletti, C. Driving knots on DNA with AC/DC electric fields: topological friction and memory effects. *Soft Matter* **2014**, *10*, 6491–98.
- (30) Renner, C. B.; Doyle, P. S. Stretching self-entangled DNA molecules in elongational fields. *Soft Matter* **2015**, *11*, 3105–14.
- (31) Kivotides, D.; Wilkin, S. L.; Theofanous, T. G. Entangled chain dynamics of polymer knots in extensional flow. *Phys. Rev. E* **2009**, *80*, 041808.
- (32) Somasi, M.; Khomami, B.; Woo, N. J.; Hur, J. S.; Shaqfeh, E. S. G. Brownian dynamics simulations of bead-rod and bead-spring chains: numerical algorithms and coarse-graining issues. *J. Non-Newtonian Fluid Mech.* **2002**, *108*, 227–55.
- (33) Heyes, D. M.; Mitchell, P. J.; Visscher, P. B.; Melrose, J. R. Brownian dynamics simulations of concentrated dispersions: viscoelasticity and near-Newtonian behaviour. *J. Chem. Soc., Faraday Trans.* **1994**, *90*, 1133–41.
- (34) Wall, F. T.; Mandel, F. Macromolecular dimensions obtained by an efficient Monte Carlo method without sample attrition. *J. Chem. Phys.* **1975**, *63*, 4592–95.
- (35) Tubiana, L.; Orlandini, E.; Micheletti, C. Probing the Entanglement and Locating Knots in Ring Polymers: A Comparative Study of Different Arc Closure Schemes. *Prog. Theor. Phys. Suppl.* **2011**, *191*, 192–204.
- (36) Caraglio, M.; Micheletti, C.; Orlandini, E. Stretching response of knotted and unknotted polymer chains. *Phys. Rev. Lett.* **2015**, *115*, 188301.
- (37) Clisby, N. Accurate Estimate of the Critical Exponent for Self-Avoiding Walks via a Fast Implementation of the Pivot Algorithm. *Phys. Rev. Lett.* **2010**, *104*, 055702.
- (38) Schroeder, C. M.; Babcock, H. P.; Shaqfeh, E. S. G.; Chu, S. Observation of Polymer Conformation Hysteresis in Extensional Flow. *Science* **2003**, *301*, 1515.
- (39) Tang, J.; Doyle, P. S. Electrophoretic stretching of DNA molecules using microscale T junctions. *Appl. Phys. Lett.* **2007**, *90*, 224103–03–3.
- (40) de Gennes, P. G. Molecular Individualism. *Science* **1997**, *276*, 1999–2000.