Untying of Complex Knots on Stretched Polymers in Elongational Fields

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Supporting Information

ABSTRACT: Knotting is a prevalent phenomenon which occurs in long polymer chains. We perform Brownian dynamics simulations and single-molecule DNA experiments to investigate knot untying in elongational fields that is induced by the knot being convected off the chain. The change in knot size as the knot moves off the chain and unties causes a change in the effective Weissenberg number, which in turn leads to a change in chain extension. Large-scale chain conformational changes are observed in both simulations and experiments for complex knots at low Weissenberg numbers (Wi). We investigate the knot untying time and untying-induced change in extension for a range of knot types and field strengths. Simulations predict a quadratic relationship between the change in extension due to knot untying and initial knot size for Wi ≥ 1.5. Due to the changes in chain extension as a knot unties, the untying process can be diffusion- or convection-driven.

1. INTRODUCTION

Knots are common in our everyday lives, from tangled earphones to sailing knots. In the microscopic world, knots are also of important relevance, having been shown to occur naturally in DNA and proteins. This is not surprising; it has been theoretically proven that the knotting probability of a chain approaches unity as the chain length tends to infinity, hence knots are inevitably present in long polymer chains. The probability of knot formation in polymers has been studied both computationally and experimentally and found to be relevant for length scales of interest. The ubiquity of knots in biomolecules via optical tweezers, compression in nanochannels, and electric fields has generated much interest in studying knots and how knots affect the physical properties of polymers.

While knots are rigorously defined only for circular chains, long chains with free ends can contain localized, unambiguous knots, and the topologies of such chains can be closed and defined algorithmically. Most studies on knotted polymers to date have been computational in nature and have explored a diverse range of topics, such as the equilibrium behavior of polymer knots, translocation of knotted polymers through narrow pores, and spontaneous knotting and unknotting in linear polymers. More recently, researchers have demonstrated experimental methods of studying polymers with knots by introducing knots in biomolecules via optical tweezers, compression in nanochannels, and electric fields. This has enabled an integrated computational and experimental approach to studying knotted polymers, with notable examples being the diffusion of knots along stretched DNA molecules under tension and in elongational fields.

From a polymer physics standpoint, the presence of knots has important consequences for overall polymer properties. Mechanically, simulations have shown that a knot reduces the strength of a polymer chain and that a knotted chain under tension usually breaks at the knot entrance. Dynamically, topological constraints imposed by entanglements in polymer melts restrict chains to reptation along a tubelike region. The effect of knots on polymer dynamics makes it interesting to study not only how molecules with knots behave but also how the knot untying process changes polymer properties. Furthermore, the knot untying process is relevant in the development of next-generation genomic technologies that are impaired by the presence of knots.

Previous computational studies by our group have shown that knots along linear chains in elongational fields can be untied via two methods: convection off the chain beyond a critical length scale from the center of the chain at high field strengths or inducing the chain to undergo a stretch-coil transition, a sharp conformational transition theoretically predicted and experimentally observed near a critical strain rate, upon a step decrease in strain rate. The former can be considered a convection-driven (field-driven) knot untying regime and the latter a diffusion-driven regime. There exists, however, an intermediate regime of knot untying along stretched chains in which single-molecule experiments mostly take place. In this study, we use Brownian dynamics simulations to systematically study the dynamics of knot untying in elongational fields that is induced by the knot being convected off the chain at intermediate field strengths. The knot untying process is amenable to study by simulations because of the ability to...
simulate specific knot types and examine the dynamics with high temporal and spatial resolution. We complement our simulations with single-molecule DNA experiments to demonstrate that the simulations predict a realistic phenomenon. This work focuses on the chain dynamics as the knot unties and considers a range of complex knot topologies and field strengths, which differs from previous studies that examined the knot translation mechanism\(^{37}\) and demonstrated changes in chain conformation during knot untying for simple knots.\(^{48}\) We subject molecules with a range of knot types to elongational fields of different Weissenberg numbers (Wi) and observe the transient dynamics of the chain during and after knot convection off the chain. Specifically, we focus on the change in chain extension induced by knot untying and the time required for a knot to completely untie. We use scaling analysis and empirical results to show that simulations predict a quadratic relationship between the untying-induced change in extension and initial knot size for Wi ≥ 1.5. While a knot is initially convected off the chain by the elongational field, the untying process can be diffusion- or convection-driven depending on the knot topology and field strength.

2. METHODS

2.1. DNA Model. We used a Brownian dynamics approach to simulate double-stranded DNA, which has been parametrized extensively in the literature.\(^{35,49–51}\) The choice of DNA model for our simulations is motivated by the prevalent use of DNA as a model polymer in single-molecule experiments. The DNA molecule is modeled as a linear bead–rod chain of \(N = 300\) beads of diameter \(b\) at positions \(\mathbf{r}_i\), connected by \(N−1\) rigid rods of length \(l = 10\) nm (5 bonds per persistence length \(l_p = 50\) nm). To derive the governing stochastic differential equation, we consider the form of all forces acting on the system: bending, electrostatic, excluded volume, hydrodynamic, constraint, and Brownian.

The semiflexible nature of DNA is enforced by accounting for bending energy, given by

\[
E^b = \frac{g}{2} \sum_{i=1}^{N-2} \theta_i^2
\]

(1)

where \(\theta_i\) is the bending angle between bond \(i\) and bond \(i+1\) and \(g = 4.81\) k\(_b\)T is chosen to set \(l_p = 50\) nm when \(l = 10\) nm.\(^{35}\) Expressions for the resulting bending force are given in Allison et al.\(^{49}\) A screened Debye–Huckel potential is used to model the long-range electrostatics of DNA–DNA interactions and is specified by

\[
E^e = \frac{e^2}{D} \sum_{i,j}^{N} \exp\left(-\frac{|r_{ij}|}{\bar{r}}\right)
\]

(2)

where \(e = 2.43\) e/nm is the effective linear charge density of DNA at an ionic strength of 10 mM, \(D = \kappa^{-1}\) is the Debye constant of water, \(\kappa^{-1}\) is the Debye length, and \(r_{ij}\) is the distance between beads \(i\) and \(j\). At an ionic strength of 10 mM, DNA has an effective width of ~16 nm and a Debye length of ~3 nm.\(^{10,52}\) The excluded volume potential, leading to short-range repulsion interactions between beads, is enforced to prevent self-crossings and is determined as

\[
E^v = -\sum_{i,j} \text{if} r_{ij} < b
\]

(3)

where \(\mu = 35\) pN has been shown to result in a low frequency of chain crossings\(^{35,53}\) and \(b = 2\) nm is the bead diameter. The electrostatic and excluded volume forces (\(F^e\) and \(F^v\)) can be obtained by taking the derivatives of the respective energies. In this work, hydrodynamic interactions between chain segments are neglected, so the drag force on the \(i\)th bead is given by

\[
F_i^d = \zeta \left( u_i(r_i) - \frac{dr_i}{dt}\right)
\]

(4)

where \(\zeta\) is the drag coefficient of a single bead and \(u_i(r_i)\) is the undisturbed solvent velocity. The constraint force is described by

\[
F_i^c = 7b_i - T_i b_{i-1}
\]

(5)

where \(b_i\) is the unit vector of bond \(i\) and \(T_i\) is the tension in rod \(i\) that enforces the constraint of constant bond length. The Brownian forces are random forces that satisfy the fluctuation–dissipation theorem, such that

\[
\langle F_i^c(t) \rangle = 0 \text{ and } \langle F_i^e(t) F_j^e(t') \rangle = \frac{2k_b T \delta_{ij} \delta_{ij}}{\Delta t}
\]

(6)

where \(\delta_{ij}\) is the Kronecker delta, \(I\) is the identity matrix, and \(\Delta t\) is the simulation time step.

2.2. Numerical Simulation. With the neglect of chain inertia, the forces on the beads sum to zero and results in the Langevin equation that describes the motion of each bead:

\[
\frac{d\mathbf{r}_i}{dt} = u_i(r_i) + \frac{1}{\xi} \left( F_i^b + F_i^e + F_i^v + F_i^d \right)
\]

(7)

To evaluate the bead positions at each time step, we performed a predictor-corrector scheme described in detail by Liu et al.\(^{73}\) and used a time step \(\Delta t = 5 \times 10^{-3} t_o\), where \(t_o = L^2/b_k T\) is the characteristic rod diffusion time. Enforcing the rigid rod constraints gives rise to a system of nonlinear equations for the rod tensions \(T_i\) which we solved for using Newton’s method.\(^{35}\)
Simulation conditions for 104 simulation parameters, the knotted chains were equilibrated at the initially stretched chain to a single-exponential decay. For a given set of molecules in elongational parallel to the x and y axes, respectively (Figure 1a). The response of the chain and unties for chains with different knot types in an elongational field (Wi = 1.25). Curves are averaged over 50 simulation runs. The time axis has been shifted such that the knot first reaches the end of the chain at t = 0. The dotted lines in (a) represent the steady-state chain extension for the unknotting chain at Wi = 1.25 (top) and Wi = 0 (bottom). The dotted line in (d) represents the equilibrium unknotted chain extension.

In the simulations, we tied a knot into the center of the polymer chain and stretched the knotted chain in a planar elongational field of the form \( \mathbf{u}(r) = \hat{x}(\hat{x} \cdot \mathbf{y}) \cdot \mathbf{y} \), where \( \hat{x} \) is the strain rate and \( \hat{x} \) and \( \hat{y} \) are unit vectors parallel to the x and y axes, respectively. Figure 1a demonstrates the evolution of the knot under these conditions. During equilibration, the positions of the knots were held at the center of the chain via reaction moves, in which a polymer segment would be cut off from one end of the chain and appended to the other end. We ran the simulations for 2 \( \times 10^6 \) \( \tau_p \), Figure 1b shows snapshots from a representative simulation run, in which the initially centered knot is convected to the end of the chain and proceeds to untie in multiple steps. The knot region was colored based on the topology identified algorithmically, as described in the next section.

### 2.3. Knot Detection

Mathematically, knots are well-defined only in closed, circular chains. To determine the topology of open chains, we first closed the chains into a ring with an auxiliary arc by implementing the minimally interfering closure scheme, in which the auxiliary arc is constructed to minimize additional entanglement that may be introduced during chain closure.\(^{3,14}\) The chain topology was then determined by projecting the chain onto a plane parallel to the extension axis, identifying all chain crossings and calculating the Alexander polynomial.\(^{25}\) The knot position and boundaries of the knot were identified by determining the smallest subset of the chain that retained the topology of the whole chain via calculation of the Alexander polynomial. To ensure that the knot boundaries identified were not biased by the choice of plane projection, we repeated the procedure over many projections along the extension axis and selected the projection that resulted in the median knot size. Identifying the left and right boundaries of the knot allowed for the computation of the number of beads in the knotted region and the central bead of the knot. The knot detection algorithm is also detailed in previous publications from our group.\(^{21,35,48}\) In this work, knot position is defined as the projected distance between the central bead of the knotted region and the first bead on the chain along the primary extension of the field, and knot size refers to the fractional contour stored within the knot.

### 2.4. Experimental Method

The single-molecule DNA experiments follow the general protocol in previous work from our group (see the Supporting Information for detailed experimental method).\(^{29,31,34}\) Briefly, a solution of fluorescently stained, T4 DNA (stained contour = 77 \( \mu \)m) is loaded in a 2 \( \mu \)m tall cross-slot channel with electrodes at the inlets and outlets. The longest relaxation time of T4 DNA was obtained by fitting the last 30% extension of an initially stretched chain to a single-exponential decay and determined to be \( \tau = 2.1 \) s. We first pulsed a square wave electric field of \( \sim 1000 \) V/cm for \( \sim 1 \) s at 10 Hz in the center of the channel to collapse the DNA into a compact globule, which likely contains one or more knots in its interior.\(^{29,31,34}\) We next applied a constant voltage drop to generate a planar elongational field within the channel. We stretched the DNA at the field’s stagnation point and recorded its dynamics over time. The knots appear as bright regions of excess fluorescence on the extended chain. Although we do not know the topologies of the knots generated, we can infer information about knot complexity from the knot size, as determined from the integrated fluorescence intensity in the knotted portion of the chain.\(^{32,34}\) We note that the protocol for inducing knots in our experiments does not allow for precise control over knot type and size, hence we are unable to gather a large ensemble of molecules with the same knot type or size and perform ensemble averaging.

### 3. RESULTS

#### 3.1. Characteristics of Knot Untying Process

As a knot convects off the chain in an elongational field, the knot grows in size and the chain undergoes a decrease in extension (Figure 1b). When the knot reaches the end of the chain, depending on the knot type and orientation, it can untie either completely or...
The knot reaches the chain end at simulation runs and the time axis is shifted such that the knot convects off in elongational extension of the steady-state chain extension for the unknotted chain. Figure 2 (panels a and b) shows the concurrent growth in knot size as the knot convects off the chain, with the knot size attaining a maximum when the knot reaches the chain end, and subsequently stretches back to the steady-state chain extension for the unknotted chain. Figure 2 (panels b and e) shows the concurrent growth in knot size as the knot is convected off a chain in an elongational field of strength \( \text{Wi} = 1.25 \). In Figure 2 (panels d and e), we show plots of chain extension and knot size for chains with an 81 knot in elongational fields from \( \text{Wi} = 0.75 \) to \( \text{Wi} = 3 \) as the knot convects off the chain and unties. All curves are averaged over 50 simulation runs and the time axis is shifted such that the knot reaches the chain end at \( \hat{t} = t/\tau_d = 0 \). Chain extension is determined as the projected distance along the primary axis of extension of the field. As seen from Figure 2 (panels a and d), for all knot types and all \( \text{Wi} \) studied, the chain extension decreases as the knot convects off the chain, reaches a minimum when the knot reaches the chain end, and subsequently stretches back to the steady-state chain extension for the unknotted chain. Figure 2 (panels b and e) shows the concurrent growth in knot size as the knot is convected off the chain, with the knot size attaining a maximum when the knot reaches the chain end and decreasing as the knot partially unties. More complex knots and lower \( \text{Wi} \) give rise to larger changes in knot size and chain extension during the knot untying process. Furthermore, more complex knots require more steps to untie, hence the chains take a longer time to stretch to the unknot steady-state extension compared to simpler knots at the same \( \text{Wi} \).

We can understand the evolution in chain extension during the knot untying process by considering the effective Weissenberg number \( \text{Wi}_{\text{eff}} \) defined for the Rouse regime as \(^{34,48} \)

\[
\text{Wi}_{\text{eff}} = \text{Wi} \left( 1 - \frac{L_{\text{knot}}}{L_{\text{chain}}} \right)^{1+2\nu}
\]

where \( L_{\text{knot}}/L_{\text{chain}} \) is the fractional knot size and \( \nu = 0.588 \) is the excluded volume Flory exponent. \(^{56} \) Figure 2 (panels c and f) shows \( \text{Wi}_{\text{eff}} \) as a function of time during the knot untying process. As an initially centered knot convects off the chain in an elongational field, the decrease in tension along the chain results in an increase in knot size. This leads to a decrease in \( \text{Wi}_{\text{eff}} \) and, consequently, a decrease in chain extension. When the knot reaches the chain end, it begins to untie. Depending on the knot topology and orientation, the knot can untie in either one or multiple steps. As the knot unties, the knot size decreases, leading to an increase in \( \text{Wi}_{\text{eff}} \) and, subsequently, an increase in chain extension. After the knot fully unties, \( \text{Wi}_{\text{eff}} = \text{Wi} \) and the chain stretches to the steady-state extension for the unknotted chain. Such dynamics are not observed in knotted chains that untie while held at constant tension (see Figure S3), affirming that this phenomenon is attributable to a coupling with the elongational field and not purely caused by end effects.

For the more complex knots studied at \( \text{Wi} = 1.25 \), such as the 91 and 101 knots, the knot size is large enough to result in \( \text{Wi}_{\text{eff}} < 0.5 \) throughout the knot untying process, which leads to the large deviation in chain extension from the unknotted steady-state extension. Similarly, for the smaller \( \text{Wi} \) investigated, such as \( \text{Wi} = 0.75 \) and \( \text{Wi} = 1.0 \), the resulting size of the 81 knot leads to \( \text{Wi}_{\text{eff}} < 0.5 \) throughout the knot untying process, hence the chain undergoes a significant change in extension as the knot unties. Essentially, for large knots and low \( \text{Wi} \), the resulting knot size is sufficient to bring the molecule through the coil–stretch transition as the knot unties, hence giving rise to large, transient changes in chain conformation during the untying process.

To gain more insight into the untying process, we consider individual chain trajectories. Figure 3 shows simulated traces of molecules with an initially centered 81 knot convecting off the chain and untying in elongational fields of varying strengths. The knot position is defined as the projected distance between the
Figure 4. Experimental results at a fixed Wi. (a) Chain extension over time of several DNA molecules stretched in an elongational field (Wi = 1.75) as the knots untie. The fractional knot sizes of the molecules, measured as the integrated fluorescence intensity of the knot when the chain was stretched at Wi ∼ 3 divided by the integrated fluorescence intensity of the entire chain, are reported in the legend. The time scale of each trace is centered at the point of minimum extension. The inset shows the same data at a wider range of times. (b) Five representative images of the molecule represented by the black time trace. The scale bar is 5 μm.

Figure 5. Experimentally measured knot position, fractional knot size, fractional extension, and kymograph of chains during the untying process in elongational fields (a) Wi = 1 and (b) Wi = 2. The fractional knot sizes, estimated as the integrated intensity of the knot when the chain is stretched at Wi ∼ 3, are 0.045 and 0.05 for (a) and (b), respectively. The black and red points for knot position and knot size represent the points before and after the knot first becomes indistinguishable from the chain end, respectively. The kymographs plot 1D chain projections vs time.
central bead of the knotted region and the first bead on the chain along the primary extension of the field, with a knot position of zero indicating that the knot is at the left end of the molecule. The knot ends positions are the positions of the beads at the left and right boundaries of the knot.

From Figure 3, we observe distinct qualitative differences in the trajectories of molecules subjected to different field strengths. First, as expected based on Figure 2d, the molecule at $Wi = 1$ undergoes a more significant change in extension during the knot untying process compared to the molecules at $Wi = 2$ and $Wi = 3$. Second, the knot appears to move around the chain in a diffusive manner at low $Wi$, whereas it is clearly convected off the chain in a certain direction at high $Wi$. This can be attributed to the chain being in the coiled versus elongated state at low versus high $Wi$, which is determined by $Wi_{eff}$ and can be visualized from the kymographs, and suggests that the knot untying process can be diffusion- or convection-driven. Third, at low $Wi$, the knot tends to move inward upon partial untying, while at high $Wi$, the knot tends to stay near the chain end as it unties. This can be seen clearly from the plot of knot ends position, with the right boundary of the knot moving inward upon the knot reaching the chain end at $Wi = 1$. The knot tends to move inward after partially untying at low $Wi$ due to the contour released from the relatively large knot upon the first untying step. For the partial knot to fully untie, it then has to move to the chain end again, hence it is unsurprising that the same knot takes a longer time to untie at low $Wi$.

### 3.2. Experimental Observations

In this section, we present individual experimental trajectories of knotted molecules that undergo the untying process when stretched in elongational fields. We highlight the difficulty in gathering large ensembles of molecules with the same knot type and size experimentally, hence experimental observations are based on trajectories of chains with various knot types and sizes. Figure 4 displays experimental results for the change in extension of several DNA molecules with a range of knot sizes as the knot is convected off the chain in an elongational field $Wi = 1.75$. Each trace represents one molecule and the time axis has been shifted such that the chain is at minimum extension at $t = 0$. The fractional knot sizes of the molecules are determined as the integrated fluorescence intensity of the knot when the chain was stretched at $Wi = 3$ divided by the total integrated fluorescence intensity of the chain. As seen from Figure 4a, large-scale changes in extension during the knot untying process are also observed experimentally. The nonmonotonic trend in extent of conformational change with respect to knot size is likely due to the lack of ensemble averaging for experimental results (see the Supporting Information for examples of variations in individual simulated trajectories for the same and different knot types at a constant $Wi$). It is worth noting that the knots observed experimentally can remain in a partially untied state for long periods of time. For example, the molecule represented by the green trace in Figure 4a, which we infer to be the most complex topologically based on knot size, remains in a partially untied, coiled state for $\sim 80$ periods ($\tau = 2.1$ s). The experimental images in Figure 4b highlight the significant conformational changes observed in a DNA molecule as the knot is convected off the chain and partially unties into a smaller knot and qualitatively resemble the simulation snapshots shown in Figure 1b.

Next, we consider individual experimental unknotted trajectories at different $Wi$ (Figure 5). At $Wi = 2$, the molecule experiences fluctuations in extension as the knot convects off the chain and partially unties, although not as drastic as the changes observed at a lower $Wi$. At $Wi = 1$, the continually increasing knot size and resulting $Wi_{eff}$ leads to the chain undergoing a stretch-coil transition as the knot moves off the chain, followed by a coil–stretch transition after the knot partially unties. The knot also appears to diffuse about the chain after partially untying at $Wi = 1$, during which the molecule is in a coiled state, whereas the knot motion looks to be more directed at a higher $Wi$, with the knot remaining close to the chain end during the untying process. We observe qualitatively similar features between the individual experimental and simulated (Figure 3) unknotted trajectories.

On the basis of the individual molecule trajectories, we can define two quantitative metrics: untying time and untying-induced change in extension. The untying time, $t_{untie}$ is defined as the time taken for a knot to completely untie and is measured as the time between when the calculated Alexander polynomial first changes (when the knot first reaches the chain end) and when the Alexander polynomial is equal to 1 for the unknot (Figure 3a). By definition, the simplest knot types $3_1$ and $4_1$, which are typically studied in other unknotted studies, have an untying time of zero as these knots untie in one step. The untying-induced change in extension, $\Delta r_{max}$, is evaluated as the difference in extension when the Alexander polynomial first changes and the steady-state extension after fully untying (Figure 3a). Due to the difficulty in determining when the knot first reaches the chain end in experiments, we use the point of minimum chain extension in place of when the knot first reaches

Figure 6. (a) Plot of $\Delta r_{max}$ as a function of $Wi$ for different knot types. Each point is averaged over 50 simulation runs or 5–10 experimental runs. (b) Normalized extension-$Wi$ curves at $f = 0$ for different knot types. The black line is drawn to guide the eyes. (c) Plot of $\Delta r_{max}$ as a function of initial knot size (fractional) for different knot types, $Wi \geq 1.5$. The dotted line represents the quadratic curve of best fit to the simulation results, given by $y = 1.36x^2 + 0.49x + 0.04$. See the Supporting Information for plots with error bars.
the chain end in obtaining \( t_{\text{untie}} \) and \( \Delta r_{\text{max}} \) from experimental data. If the knot partially unties into a smaller knot, \( t_{\text{untie}} \) is the time between the point of minimum chain extension and when the smaller knot is distinguishable from the chain ends; if the knot appears to untie completely, \( t_{\text{untie}} \) is the time between the point of minimum chain extension and when the intensity of both chain ends are comparable.

3.3. Untying-Induced Change in Chain Extension.

Figure 6a plots \( \Delta r_{\text{max}} \) as a function of \( Wi \) for a range of simulated knot types and experimental data (see the Supporting Information for Figure 6 with error bars). In general, the more complex the knot type and the lower the \( Wi \), the larger the change in chain extension induced by knot untangling, in agreement with both the averaged and individual trajectories presented in Figures 2–5. For the most complex knots studied, we observe a nonmonotonic trend in \( \Delta r_{\text{max}} \). This is a consequence of there being a lower limit to the extension a molecule can attain when the knot reaches the chain end (knotted chain radius of gyration), which the chains with larger knots are likely to have reached within the range of \( Wi \) studied. Although the experimentally generated knot topologies are not known, we can average data for the same \( Wi \) and observe that the trend for the experimental data points is consistent with that from the simulations. We note that the values of \( \Delta r_{\text{max}} \) measured experimentally are much larger than those determined from simulations, which suggests that the knots generated by an electrohydrodynamic instability in our experimental setup are simulations, which suggests that the knots generated by an experimentally are much larger than those determined from known, we can average data for the same \( Wi \) and observe that the knots are likely to have reached within the range of \( Wi \) studied.

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\[ \Delta r_{\text{max}} = r(Wi) - r_{\text{untie}}(Wi_{\text{untie}}) = r(Wi) - r_{\text{untie}}(Wi, L_{\text{knot}}) \quad (9) \]

where \( r(Wi) \) is the steady-state extension after the knot fully unties, and \( r_{\text{untie}}(Wi_{\text{untie}}) \) and \( r_{\text{untie}}(Wi, L_{\text{knot}}) \) are the chain extension, \( Wi_{\text{untie}} \) and knot size, respectively, when the knot first reaches the chain end. Above a certain \( Wi \), the steady-state chain extension does not vary much as a function of \( Wi \). If we assume \( r(Wi) \) to be approximately constant, then \( \Delta r_{\text{max}} \) is driven by \( r_{\text{untie}} \) which is in turn dependent on \( Wi_{\text{untie}} \) or \( Wi \) and \( L_{\text{knot}} \).

In Figure 6b, we plot \( r_{\text{untie}} \) normalized by \( L_{\text{max}} \) as a function of \( Wi_{\text{untie}} \) where \( L_{\text{max}} \) is the maximum extension that the knotted molecule can achieve. Previous publications from our group show that rescaling extension by \( Wi_{\text{untie}} \) and \( Wi \) by \( Wi_{\text{eff}} \) leads to the collapse of extension-\( Wi \) curves for knotted molecules onto that for unknotted molecules. As shown in Figure 6b, the normalized extension-\( Wi \) curves for the knotted molecules are shifted relative to that for the unknot, from which we can infer that \( r_{\text{untie}} \) is not determined by steady-state dynamics. This is to be expected, given that knot untangling is a dynamic process. We can empirically fit a power law to the normalized extension-\( Wi \) curves for the knotted molecules and determine the best fit curve to be \( r_{\text{untie}} \approx Wi_{\text{eff}}^{0.45} \).

Therefore, assuming \( Wi \) to be large enough to approximate \( r(Wi) \) as constant, say \( Wi \geq 1.5 \), we have
\[ \Delta r_{\text{max}} \approx \frac{r_{\text{untie}}}{L_{\text{chain}}} = \frac{L_{\text{knot}}}{L_{\text{chain}}} = \frac{r_{\text{untie}}}{L_{\text{chain}}} \approx \frac{L_{\text{knot}}}{L_{\text{chain}}} \quad (10) \]

Given that \( L_{\text{knot}}/L_{\text{chain}} \) is subject to end effects and difficult to measure experimentally, we choose to investigate \( \Delta r_{\text{max}} \) as a function of the initial knot size \( L_{\text{knot,0}}/L_{\text{chain}} \) which is a more well-defined metric. We find empirically that simulations predict a quadratic relationship between \( L_{\text{knot,0}}/L_{\text{chain}} \) and \( r_{\text{untie}}/L_{\text{chain}} \). Thus, we expect a quadratic relationship between \( r_{\text{untie}}/L_{\text{chain}} \) and \( L_{\text{knot,0}}/L_{\text{chain}} \). We plot \( \Delta r_{\text{max}} \) as a function of \( L_{\text{knot,0}}/L_{\text{chain}} \) for all knot types at \( Wi \geq 1.5 \) in Figure 6c and observe the collapse of simulation results onto a master quadratic curve. We highlight that the existence of a universal curve shows that the untangling-induced change in extension is determined solely by the initial knot size and is not a function of \( Wi \) or knot type. The quadratic relationship between \( \Delta r_{\text{max}} \) and \( L_{\text{knot,0}}/L_{\text{chain}} \) explains why we generally observe two chains with slightly different initial knot sizes undergo noticeably different conformational changes as the knot unties, such as the \( 81 \) knot at \( Wi = 1.5 \) and \( Wi = 1.75 \), depicted in Figure 2 (panels d and e, respectively). See the Supporting Information for more details on \( \Delta r_{\text{max}} \) scaling, determination of \( L_{\text{knot,0}} \) empirical fits to simulation results, and testing of significance of the quadratic regression model.
3.4. Knot Untying Time. Figure 7a shows the untying time as a function of Wi for a range of simulated knot types and experimental data. Generally, the more complex the knot type and the lower the Wi, the longer it takes for the knot to untie. The experimental trend for untying time agrees with that from the simulations, which supports that the model used in our simulations is sufficient for capturing the physics of the untying process despite being simplistic. The experimentally measured untying times relative to chain relaxation time are an order of magnitude larger than those determined from simulations at a given Wi, again suggesting that the knots we generate experimentally have much more complex topologies than the 101 knot. As seen from Figure 7a, the untying times for all knot types appear to plateau at small Wi.

To further investigate the knot untying times, we plot in Figure 7 (panels b–d) the untying times for a 51, 81, and 101 knot as a function of Wi. For reference, we also plot the upper and lower bounds for untying times of each knot, where the upper bound is the untying time for the knotted chain held under constant tension and the lower bound is the untying time for the knotted chain in an elongational field with no Brownian motion. We note that the untying time of a knot in an elongational field generally will not approach that of a knot held under constant tension as Wi tends to zero because the knot size and chain extension do not change appreciably as the knot diffuses off a uniformly tensioned chain. For a knot on a chain held at constant tension to fully untie, the knot has to diffuse the entirety of the distance between the knot boundary and chain end, while a knot in an elongational field at low Wi is aided in the process by a swelling knot and continuously decreasing chain extension. Nevertheless, the untying time of a knot held under constant tension is a useful upper bound for reference. That the untying time of a 51 knot approaches the upper bound at low Wi is likely due to the small knot size involved, which gives rise to a less dramatic change in extension during the untying process.

From Figure 7 (panels b–d), we observe that the knot untying times reach a diffusive limit at low Wi and a convective limit at high Wi, with the untying times for all knots approaching the lower convective bound at high Wi. Furthermore, the more complex the knot, the higher the Wi at which the diffusive and convective limits are reached. Specifically, the untying time for the 51 knot approaches the convective limit for Wi ≥ 1.5, whereas the untying time for the 101 knot does so only for Wi ≥ 3. Similarly, the untying time for the 101 knot evidently approaches a diffusive limit for Wi ≤ 1.25, while the untying time for the 51 knot does so at Wi ≤ 0.6. At a given Wi, more complex or larger knots result in a lower Wi_{eff}, thus the chain is in a less elongated state. The closer the molecule is to a coiled conformation, the more likely for the knot to move along the chain in a diffusive manner (Figures 3a and 5a). Conversely, smaller knots lead to a higher Wi_{eff} and a more stretched conformation. Although the knot untying process is initiated by a knot being convected off the chain, because of the change in knot size and consequent change in chain extension as the knot unties, the untying process can be diffusion- or convection-driven. See the Supporting Information for distributions of untying times and plot of coefficient of variation for untying times as a function of Wi.

4. DISCUSSION

Given that knots are capable of affecting polymer properties,1,13,48,59 there has been growing interest in studying not only the knotting dynamics of polymers but also the unknotting dynamics of chains with free ends. Practically, because knots can reduce the accuracy of next-generation genomics technologies, such as nanochannel genome mapping that relies on uniform stretching of molecules to convert physical distances between markers to genomic distances,43–45 the process of untying knots is relevant for the development of devices that precondition DNA for such applications.

This work delves into the process of untying knots in elongational fields. The varying tension along a polymer chain in an elongational field leads to a dynamic change in knot size as the knot is convected off the chain by the field. Specifically, because tension at the ends of the chain tends to zero, the knot swells as it moves off the chain. This, in turn, gives rise to a change in Wi_{eff}, consequently causing untying-induced changes in chain conformation, the extent of which depends on knot complexity and field strength. For large knots and low Wi, the resulting knot size might be sufficient to bring the molecule through the coil–stretch transition as the knot unties, which brings about large changes in chain conformation during the untying process. We note that our simulation results are independent of the DNA model used and can be generalized to long polymer chains in elongational fields.

It is important to note that flow kinematics strongly influence the knot untying process. As an example, consider knots on polymers confined in nanochannels, which behave similarly to knots along tensioned chains. Due to uniform tension along the chain, the knot size remains relatively constant as the knot moves off the chain and the chain does not undergo appreciable changes in extension as the knot unties.25,60 The dramatic chain conformational changes observed for the knot untying process in elongational fields is a consequence of the varying tension along a chain coupled with the existence of a coil–stretch transition.

Elongational and shear flows are the simplest flow types and hence most commonly studied. A computational study on the dynamics of flexible fibers in steady shear flow found that the flow is capable of knotting and unknotting a 31 knot.61 Although the untying of complex knots in shear flows has not yet been investigated, we can hypothesize what would be observed. The complete untying of a complex knot typically occurs over multiple untying events, with each event requiring a chain end to be pulled through the knot. As shown in the current and previous studies,53,55 the extensional character of elongational fields serves to convect knots off chains. In simple shear flow, for which the extensional and rotational components have equal magnitudes, the rotational component of the flow causes molecules to undergo end-over-end tumbling motion and thus large fluctuations in chain extension.62,63 We expect the stochastic tumbling motion of a knotted polymer in shear flow to prevent the knot from reaching a chain end and frustrate the complete untying of a complex knot. Furthermore, since the rate of extension fluctuations increases with shear rate,62 we postulate that knots will be longer-lived in flows with higher shear rates, in contrast to the decrease in untying time with increasing strain rate observed in elongational fields. Due to the large fluctuations in chain extension and lack of a sharp coil–stretch transition,62 we believe that the untying-induced change in extension seen in elongational fields will not be apparent in shear flows.

It is worth noting that unknotting-knotting transitions in linear polymers, meaning spontaneous tying of a knot following an untying event, have been reported at equilibrium,4,4 in nanochannels,25,28 and in steady shear flow.64 Such transitions in

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equilibrium and in nanochannels reflect the equilibrium incidence of knots for given geometric constraints. The existence of such transitions in simple shear flow, on the other hand, indicates that shear flow is capable of tying knots on molecules. Given that elongational fields serve to convect knots off chains, it is unsurprising that we do not observe the spontaneous formation of knots on molecules in elongational fields. Even if unknotted-knotting transitions do exist in elongational fields, we believe the effects to be fleeting and have an insignificant impact on overall untying dynamics. Since spontaneous knot formation is absent in elongational fields, it is convenient to use such conditions for the study of knot untying processes.

5. CONCLUSION

In this work, we have used Brownian dynamics simulations and single-molecule DNA experiments to systematically study the untying process of knotted chains in elongational fields. Both simulation and experimental results show that the change in knot size during the knot untying process leads to a change in $W_i$, which in turn causes a change in chain extension. The larger the knot and the lower the $W_i$, the more dramatic the transient changes in chain configuration. In some cases, the knot is large enough to induce a stretch-coil transition as the knot convects off the chain, followed by a coil–stretch transition as the knot partially unites. We investigated two metrics defined for the untying process: change in chain extension induced by knot untying and untying time. On the basis of a scaling analysis and empirical results, we showed that simulations predict a quadratic relationship between the change in extension due to knot untying and untying time. On the basis of a scaling analysis and empirical results, we showed that simulations predict a quadratic relationship between the change in extension due to knot untying and untying time. On the basis of a scaling analysis and empirical results, we showed that simulations predict a quadratic relationship between the change in extension due to knot untying and untying time. On the basis of a scaling analysis and empirical results, we showed that simulations predict a quadratic relationship between the change in extension due to knot untying and untying time. On the basis of a scaling analysis and empirical results, we showed that simulations predict a quadratic relationship between the change in extension due to knot untying and untying time.

Given the ubiquity of knots in long polymers of both natural and synthetic origin, it is important to understand how the rheological properties of polymers is impacted not only by the presence of knots but also by the removal of knots. This study has shown that knotted molecules in elongational fields can undergo large, transient conformational changes as a knot moves off the chain and unties. Looking forward, we hope that our work will motivate further mechanistic studies into the unknottting of polymer chains, such as the untying of knots in other flows or fields and the untying pathways of knots.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.macromol.8b01879.

Detailed experimental method, simulation movies details, schematics of simulated knot types, individual simulation trajectories in elongational fields and at constant tension, further analysis of $\Delta r_{\text{max}}$ distribution of untying times and calculation of confidence intervals (PDF) Simulation movies (ZIP)

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