 Untying Knotted DNA with Elongational Flows

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ABSTRACT: We present Brownian dynamics simulations of initially knotted double-stranded DNA molecules untangling in elongational flows. We show that the motions of the knots are governed by a diffusion–convection equation by deriving scalings that collapse the simulation data. When being convected, all knots displace nonaffinely, and their rates of translation are topologically dictated. We discover that torus knots “corkscrew” when driven by flow, whereas nontorus knots do not. We show that a simple mechanism can explain a coupling between this rotation and the translation of a knot, explaining observed differences in knot translation rates. These types of knots are encountered in nanoscale manipulation of DNA, occur in biology at multiple length scales (DNA to umbilical cords), and are ubiquitous in daily life (e.g., hair). These results may have a broad impact on manipulations of such knots via flows, with applications to genomic sequencing and polymer processing.

Knots are commonly encountered and manipulated in everyday experiences such as tying one’s shoelaces or untangling spontaneously knotted strings. Formally defined only for closed rings, the topologies of “open” knots (referred to hereafter simply as knots) are often unambiguous (e.g., shoelaces and neckties) and can be closed and algorithmically defined. At microscopic scales, chromosomal knots are modified by topoisomerases during cell division and are thought to participate in gene regulation. Knots are found in proteins and viral capsid DNA likely with yet to be fully understood functions. It has been mathematically proven that knots become asymptotically likely as the length of a polymer increases, a fact that explains their ubiquity.

Due to the emerging significance of knotted polymers, a growing body of simulation literature is devoted to their study. For instance, while the topology of a ring is fixed, an open polymer can spontaneously form and untie knots. The probability of forming such knots can be nonmonotonic when the polymer is confined in slits or tubes, and increasing the stiffness of a polymer can similarly influence the knotting probability in unintuitive ways. Such knots can substantially affect the mechanical properties and rheological behavior of a polymer, and the probability of forming knots has been used to infer the effective diameter of DNA molecules. Recently, simulations have shown dramatic slowing of processes wherein a knot is driven along a chain such as entropic ejection of DNA from a viral capsid and the translocation of single-stranded DNA through pores.

Common nanofluidic experiments have led to the spontaneous formation of knots in DNA by collision with channel defects or the application of moderate electric fields during electrophoresis. More broadly, the growing library of methods to manipulate DNA molecules in nanofluidic devices has enabled fundamental research about single polymer molecules. These studies inform important applications such as genomic sequencing via nanopore translocation or direct linear analysis. Thus, (un)tying knots in polymers is of interest in its own right. To this end, knots have been intentionally tied with optical tweezers in actin filaments and double-stranded DNA (dsDNA). Impressively, simulations reproduced the sizes and diffusion coefficients of dsDNA knots within a factor of 2.

In this Letter, we use simulations to investigate the transport of a knot on a dsDNA molecule that has been extended by an elongational flow. We show that such flows cause the knot to be driven off the chain and untied, and we elucidate the relevant length and time scales for this process by examining the diffusion–convection equation. We observe that knots of different topologies translocate at different rates when strongly driven by the external flow. We show the different rates of knot translations are explained by a rotational mode of motion, available to torus knots, that facilitates the translation of the knot, providing unique mechanistic insight.

We have used a Brownian dynamics approach to simulate dsDNA, which has been extensively parametrized by others. The dsDNA molecule is represented by a fine-grained bead–spring model with stiff bonds per persistence length, \( I_p = 50 \) nm. Screened Debye–Hückel interactions are used to model the long-range electrostatics of DNA–DNA interactions, and all simulations used an ionic strength of \( I = 10 \) mM, leading to a Debye length \( \kappa^{-1} \approx 3 \) nm.

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and a effective chain diameter of $w \approx 16$ nm.\textsuperscript{23,39,40} These values were chosen to be representative of common low-salt conditions in microfluidic dsDNA experiments. An external planar elongational flow of the form $\mathbf{u}(\mathbf{r}) = \dot{\varepsilon} (\mathbf{x} - \bar{\mathbf{y}}) \mathbf{r}$ was considered, where $\dot{\varepsilon}$ is the strain rate and $\mathbf{r}$ is the position of the $i$th bead. We neglected interbead hydrodynamic interactions in this work, so the drag force on the $i$th bead is simply $F_i = \zeta_i \dot{u} (x_i) (dt/dt)$ where $\zeta_i$ is the drag coefficient of a single bead. The Weissenberg number, $Wi = \dot{\varepsilon} \lambda_i$, is the appropriate dimensionless group for such flows. The DNA longest relaxation time, $\lambda_i$, was determined by fitting the long-time decay of the squared end-to-end distance of an initially fixed DNA molecule at flow strength $Wi = 16$ is shown. The DNA longest relaxation time, $\lambda_i$, was determined by fitting the long-time decay of the squared end-to-end distance of an initially fixed DNA molecule.

Examination of eq 2 reveals the fundamental scale for time to be $\dot{\varepsilon}^{-1}$. A length scale, $l = (D \dot{\varepsilon}^{-1})^{1/2}$, emerges where the probability flux contributions due to diffusion and flow are balanced. To the first order, the motion of the knot will be diffusive for $l \dot{\varepsilon} \ll l$ and follow the deterministic path of the flow for $l \dot{\varepsilon} \gg l$. We independently measured knot diffusivities from chains held at the ends by constant tension (see SI). We found the diffusivity of the knots in this study to be topologically dependent but not strongly influenced by tension (and thus, Wi; see SI). Thus, the scalings depend on knot topology alone.

We simulated the process of a knot, initially centered in the internal chain coordinates ($K(t = 0) = 0$), escaping from a chain extended by elongational flow for a variety of topologies and flow strengths. In Figure 2a, the mean squared displacements of knot position are plotted versus time. The scalings from the

\begin{figure}
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\includegraphics[width=\textwidth]{figure1.png}
\caption{(a) Knotted (red) and unknotted (blue) regions of DNA extended by elongational flow ($Wi = 16$) for the $3_1$ knot. The distance along the contour to the midpoint of the knot, $K$, is shown in green. (b) Simulation snapshots of an initially centered $3_1$ knot untying from DNA at flow strength $Wi = 16$. (c) The midpoint and bounds of the knot pictured in (b) are plotted versus strain. (d) Untying trajectories of 25 initially centered $3_1$ knots at flow strength $Wi = 16$.}
\end{figure}

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure2.png}
\caption{Mean squared displacements of initially centered knots ($K(t = 0) = 0$) in elongational flows. (a) Knot displacement plotted versus dimensionless time ($\zeta = n \zeta_i$). (b) Scaled knot displacement plotted versus strain. The triangle represents the slope of the diffusion-dominated mean squared displacement.}
\end{figure}
This idea is akin to topologically controlled breathing modes, in which the collective mode of motion while being driven along the chain.

The nontorus knots consist of the following: 4_1, 5_1, 6_1, 10_{29}, and 15_{165258}. Displacements from the knot center of mass of the central segment of the knot made dimensionless by \( l_p \) (\( \Delta \bar{K}_y \) and \( \Delta \bar{K}_z \)) for the 3_1 (b) and 4_1 (c) knots in the plane orthogonal to the extensional axis; color changes from blue to red as the knot moves off the chain. Displacements from the knot center of mass of the central segment of the knot (\( \Delta \bar{K}_y \) and \( \Delta \bar{K}_z \)) in the plane orthogonal to the extensional axis plotted versus knot position (\( \bar{K} = K/l_p \)) for the 3_1 (d), 4_1 (e), 5_1 (f), 6_1 (g), 7_1 (h), and 6_0 (i) knots. Color changes from blue to red as the knot moves off the chain. Bottom: snapshots of knots in (d–i) with central segments highlighted in green.

**Figure 3.** (a) Average knot displacements are plotted vs strain for various knots at flow strength \( Wi = 16 \) for knots initialized off-center (\( K(t = 0) = 6_0 \gg 1 \) for all topologies). The nontorus knots consist of the following: 4_1, 5_1, 6_1, 10_{29}, and 15_{165258}. Displacements from the knot center of mass of the central segment of the knot made dimensionless by \( l_p \) (\( \Delta \bar{K}_y \) and \( \Delta \bar{K}_z \)) for the 3_1 (b) and 4_1 (c) knots in the plane orthogonal to the extensional axis; color changes from blue to red as the knot moves off the chain. Displacements from the knot center of mass of the central segment of the knot (\( \Delta \bar{K}_y \) and \( \Delta \bar{K}_z \)) in the plane orthogonal to the extensional axis plotted versus knot position (\( \bar{K} = K/l_p \)) for the 3_1 (d), 4_1 (e), 5_1 (f), 6_1 (g), 7_1 (h), and 6_0 (i) knots. Color changes from blue to red as the knot moves off the chain. Bottom: snapshots of knots in (d–i) with central segments highlighted in green.

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chirality of rotation that coincides with the direction of translation can be predicted from the sketches. The colored trajectories in Figure 4 show the displacements of the central segments from the center of mass of knots of given chirality (right- or left-handed) being convected by an elongational flow off the right or left of the chain. The mechanism in the sketches is in complete agreement with the visualized simulation results. We have found this agreement between knot chirality, direction of knot translation, and knot rotation to hold in all observed simulation trajectories (≈50); i.e., knot rotation always occurs as predicted by the sketches. These results confirm our idea that the translational motion of torus knots is facilitated by knot rotation. Further, this mechanism clearly extends to all of the \((2n + 1)\) family of torus knots; the addition of additional loops in the knot does nothing to prohibit the coupling of knot rotation and translation. The increased mobility of torus knots appears to be quite general, having been demonstrated in macroscopic sharking chain experiments, simulations of DNA ejection from viral capsids, and simulations of tensed electrophoresing DNA. We postulate our mechanism plausibly explains these results.

We have demonstrated the ability of elongational flows to untie knotted dsDNA molecules with computer simulations. Through scaling analysis, we revealed a critical length scale along the molecule that separates diffusive from convective transport of a knot in these flows. We have shown that a subset of knots, torus knots, can move linearly via sustained global rotation, which increases the speed at which they release from the chain. This represents an important mechanistic insight into the motion of driven knots, and since the mechanism is solely a function of topology, we expect it will apply in many other driven processes (e.g., nanopore sequencing or viral ejection of DNA) and for other polymers. We speculate that for longer DNA or for higher flow strengths self-jamming of knots may be observed as has been seen in tensioned knots, and we hope future work will address this notion. Simulations show knots can jam nanopore translocation, and experiments suggest jamming of knots during ultrafiltration of plasmid DNA.

Practically, our results could guide development of microfluidic devices that precondition molecules to unknotted states for such applications. Finally, since the model employed is parametrized to dsDNA at experimentally realizable conditions, we hope future experiments will test our results.

**REFERENCES**


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**Notes**

The authors declare no competing financial interest.

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