Separation of Geometrical and Topological Entanglement in Confined Polymers Driven out of Equilibrium

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ABSTRACT: We use Brownian dynamics simulations and advanced topological profiling methods to characterize the out-of-equilibrium evolution of self-entanglement in linear polymers confined into nanochannels and under periodic compression. By introducing suitable observables, we can distinguish two main forms of entanglement that we term geometrical and topological. The latter is measured by the number of (essential) crossings of the physical knot detected after a suitable bridging of the chain termini. The former is instead measured as the average number of times a linear chain appears to cross itself when viewed under all projections and is irrespective of the physical knotted state. The key discovery of our work is that these two forms of entanglement are uncoupled and evolve with distinct dynamics. While geometrical entanglement is typically in phase with the compression–elongation cycles and it is primarily sensitive to its force \( f \), the topological measure is mildly sensitive to cyclic modulation but strongly depends on both compression force \( f \) and duration \( k \). The findings could assist the interpretation of experiments using fluorescence molecular tracers to track physical knots in polymers. Furthermore, we identify optimal regions in the experimentally controllable parameter space in which to obtain more/less topological and geometrical entanglement; this may help designing polymers with targeted topology.

Although it is generally well established that self-entanglements play vital roles in affecting polymer dynamics, their time evolution under strongly nonequilibrium conditions, such as those achieved via electric fields, convergent flows, or mechanical compression\(^1\)–\(^5\) are still poorly understood. Under such conditions, polymeric filaments cannot return to their equilibrium state until long after the perturbation has ended. For instance, \( \mu \)-long DNA filaments can take seconds to relax after compression by electric pulses\(^3\) or under confinement into narrow slits.\(^6\)

Such unusually slow relaxations have been associated with the emergence of complex forms of self-entanglement that hinder conformational rearrangements of molecules and trap them in long-lived states.\(^7\)–\(^10\) This view is supported by the observation of localized bright spots in fluorescence molecular traces, which can be interpreted as physical knots, that is knots in open chains.\(^2\)–\(^5\)

Several central questions remain unanswered. How exactly does entanglement build up or decrease during nonequilibrium dynamics? What is its inherent degree of variability and spatial inhomogeneity? Is it feasible to control the out-of-equilibrium driving so to produce polymers with a target level of entanglement and heterogeneity?

In this computational study, we address these questions by considering a semiflexible polymer and confining it in a cylindrical channel where it undergoes compression and expansion phases (see Figure 1A). The compression is driven by a piston, a setup inspired by the “nanodozer” arrangement of refs 1 and 11.

By using various topological measures adapted to the case of linear chains,\(^12\) we systematically profile the nature and complexity of self-entanglements that arise in such a system. We show that entanglement created by cyclic compression manifests in two different forms that we term geometrical and topological after the order parameters used to distinguish them. We measure the complexity of topological entanglement in a linear chain, denoted by \( n^{\text{topo}} \), via the number of crossings in the simplest (minimal) diagrammatic representation of the knot trapped in the chain once its termini are suitably bridged.\(^13\)–\(^14\) The complexity of geometric entanglement, denoted by \( n^{\text{geom}} \), is instead defined in terms of the self-crossings in planar projections of the chain (without closure) and is computed as the number of self-crossings averaged over all projections.\(^15\)

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We discover that these two forms of entanglement evolve very differently: while the geometric one responds to compression/relaxation cycles similarly to driven dissipative systems, topological entanglement, reflecting the inherent complexity of physical knots, is only mildly coupled to the instantaneous driving or to the geometrical entanglements and is long-lived. In fact, we show that significant physical knotting sets in only at suitable choices of the compressive force and period. The system can therefore be steered toward the formation of more geometrical or topological entanglements depending on \( \sigma \), with this being suitable to detect knotting.\(^{16,17} \)

The system is evolved via Langevin dynamics simulations using the constant, k, variable \( \kappa \). The average of \( \kappa \) is here additionally taken over the period. Note that time is given in units of cycles, which varies across panels. \( \langle \kappa \rangle \) is fitted with the expression \( \kappa_0(1 - \exp(-t/\tau)) + \kappa_1 \) for the ON phase and \( \kappa_0 \exp(-t/\tau) + \kappa_1 \) for the OFF phase with \( \chi \approx 1 \) for all fits in panels. Unless otherwise specified \( k \) is given in units of 10\(^6\) integration steps or 10\(^4\) \( \tau \).

We consider a general model of a semiflexible polymer confined in a cylinder stopped at one end by a hard planar wall. The polymer is periodically pushed against the wall by a spherical piston that occludes the cylinder cross-section, see Figure 1A. The chain is made of \( N = 1000 \) beads of size \( \sigma \) and has a nominal persistence length of \( \ell_p = 10 \sigma \). The cylinder has a radius \( r = 40 \sigma \), with this being suitable to detect knotting.\(^{16,17} \)

The system is evolved via Langevin dynamics simulations using standard values for the mass and friction coefficient\(^{18} \) (see Supporting Information (SI)).

In this initial study we only consider excluded volume interactions of the polymer with itself and with the piston (WCA repulsion) and the walls of the channel (quadratic repulsion), neglecting hydrodynamic effects. Including these would be computationally expensive and they are not expected to alter the ensuing entanglement properties.\(^{19,20} \)

We focus on the case where the piston is moved by a periodic driving force parallel to the channel axis. The amplitude of the force oscillates between the values 0 and \( f \), following a square wave with period 2\( k \) with each compression/extension phase having duration \( k \) (which in this work we express in units of 10\(^6\) integration steps or 10\(^4\) \( \tau \)); see SI). We typically start from an equilibrated polymer conformation and drive the system for at least 100 compression/extension cycles in order to attain a steady state (see Figure 1C,E).

We take advantage of the structural details available in our simulations to profile entanglements through observables that complement the metric elongation measures, such as the gyration radius (see SI), that are accessible experimentally.\(^{2,3} \) We first measured the geometric entanglement for each sampled conformation by averaging \( \kappa \) at equal times over different realizations of the noise (replicas). We shall denote these averages as \( \langle \kappa \rangle \).

While \( f \) and \( k \) both affect significantly the distribution of \( \langle \kappa \rangle \) depends on the driving force \( f \) but not on the cycle time \( k \) (see Figure 1D,F). Instead, varying \( k \) affects solely the width of the \( \langle \kappa \rangle \) distribution (see Figure 1F). More specifically, we find that, for fixed force \( f \), the distribution of \( \langle \kappa \rangle \) changes from unimodal to bimodal for long enough cycle times \( k \) (Figure 1F). This effect that we shall rationalize below, suggests the presence of characteristic time scales in the system’s dynamics during which a driving force of a certain amplitude must act in order to form entanglements.

That the mean average crossing number is independent of the cycle time \( k \) can also be probed by averaging the \( \langle \kappa \rangle \) over the compression/relaxation cycles at steady state (we consider the last 30 cycles). These data, shown in Figure 1G–I, appear to saturate for large choices of \( k \), while their average over a period (compression and relaxation phases) remains constant. Note that the curves are reminiscent of classic dissipative systems, such as RC circuits or Kelvin–Voigt

Figure 1. (A) Sketch of the system set up. (B) One compression–extension cycle consists of two equal phases, both of duration \( k \): the piston first exerts compressive force \( f \), followed by a zero force recovery phase. (C, E) The measure of geometrical complexity, \( \langle \kappa \rangle \), is here averaged (as denoted by brackets) at equal times across simulation replicas at fixed \( f/k \). (D, F) Distribution of \( \langle \kappa \rangle \) at steady state. Note that the mean value depends on \( f \), but not \( k \), whereas the variance depends on both. (G–I) \( \langle \kappa \rangle \) in the ON and OFF phases for \( f = 5 \times 10^4 \) \( \tau \), \( k = 0.1, 1, 10 \times 10^4 \) \( \tau \). The average of \( \langle \kappa \rangle \) is here additionally taken over the period. Note that time is given in units of cycles, which varies across panels. \( \langle \kappa \rangle \) is fitted with the expression \( \kappa_0(1 - \exp(-t/\tau)) + \kappa_1 \) for the ON phase and \( \kappa_0 \exp(-t/\tau) + \kappa_1 \) for the OFF phase with \( \chi \approx 1 \) for all fits in panels. Unless otherwise specified \( k \) is given in units of 10\(^6\) integration steps or 10\(^4\) \( \tau \).

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The geometric entanglement at steady state (see SI for further manifestation, as captured by details).

Physical knots clearly follow a rather different evolution from \( \langle n_{\text{geom}} \rangle \). After the initial transient (Figure 2A), the \( \langle n_{\text{geom}} \rangle \) varies continuously and in register with compression and expansion phases (Figure 2E), retracing stochastically the loading and unloading curves of Figure 1C,D. Instead, over the same time scales, both the type and the location of physical knots vary discontinuously and can persist across cycles. In fact, physical knots appear, disappear, or change in complexity during both the compression and the expansion phases.

To characterize the entanglement beyond its geometrical manifestation, as captured by \( \langle n_{\text{geom}} \rangle \), we tracked the formation of physical knots using the kymoknot software package. This uses a minimally invasive procedure to close an open chain by joining the termini either directly or through the convex hull of the chain, depending on which of the two provides the shortest route (see SI for more detail). The procedure allows us to assign a topological state (knot) to an open chain, locate the knotted portion along the chain contour and measure its complexity via its minimal crossing number.

Typical time evolutions of the knotted portion are presented in the kymographs of Figure 2. Different colors are used to distinguish the various knot types that form, evolve, and untie during the compression–expansion cycles. The colored region indicates the physical location of the knot on the chain contour. The temporal traces of the associated \( \langle n_{\text{geom}} \rangle \) are also shown. The “ON” phase of the periodic compression (force) are marked by gray shading.

To quantify the external control on the geometrical and topological entanglement, we profiled the dependence of the median values of \( n_{\text{geom}} \) and \( n_{\text{topo}} \) on \( f \) and \( k \) (see SI for details). \( n_{\text{topo}} \) is the so-called crossing number and is a conventional measure of knot complexity. It corresponds to the number of essential crossings (a diagrammatic crossing is essential if its flipping would change the knot type) or, equivalently, to the number of crossings in the minimal diagrammatic representation of a given knot. To establish it, we simplified the crossing pattern of a (closed) chain configuration first geometrically, using topology-preserving Monte Carlo moves and then symbolically via reductions and factorizations of the resulting Dowker code. The latter was finally compared against a lookup table of knots with up to 16 crossings. The procedure allowed for establishing the precise knotted state and hence \( n_{\text{topo}} \) of most of the sampled conformations, and used the lowest number of crossings after simplifications for the rest. These highly complex configurations were a minority of those sampled at any \( \langle f,k \rangle \) and therefore do not significantly bias the

![Figure 2. (A, E) Kymographs for two time windows within the simulations with \( f = 1 \) and \( k = 1 \) showing the formation and evolution of knots. Knots are highly dynamic and appear/disappear, even within a single compression/extension phase. Long-lived knots are less frequent. Note that most of the knots are shallow and spanning a large portion of the polymer (delocalized). The boundaries and topology of the knots are determined as median over a time-window of 11 time points equispaced by \( \Delta t = 10^5 \tau_L \). The traces of \( n_{\text{geom}} \) for this replica (blue) and the average over replicas (red) are shown at the top of the kymographs. (B–D) Three snapshots of two time points indicated with the gray (B) and black (C, D) arrow wedges. (F) Cartoons of knotting by threading (left) and slipknotting (right).](https://dx.doi.org/10.1021/acsmacrolett.0c00366)
length, 26, 30
rings, which vary nonmonotonically with increasing
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The abundance of composite knots is also enhanced at larger
large forces, the population of trefoil knots decreases with
an increase of the cycle time

This is summarized in Figure 4A. The state diagram shows a
dipartite division of parameter space in which different
combinations of geometrical and topological entanglement
can be achieved by tuning \( f \) and \( k \).

Our topological profiling strategy allows for pinpointing
which types of physical knots are preferentially formed at the
different values of \( f \) and \( k \). It also provides a useful term of
reference for future experiments aiming at studying polymers
with a given topological entanglement. In fact, at small forces,
an increase of the cycle time \( k \) leads to an increase of the
simplest types of knots, particularly trefoil knots. However, at
large forces, the population of trefoil knots decreases with \( k \).
The abundance of composite knots is also enhanced at larger
forces, even for small periods while no composites are
observed at small force (Figure 4B). Both aspects are
reminiscent of knot populations in equilibrated chains or
rings, which vary nonmonotonically with increasing
length, 26, 30 with the force playing an analogous role as
rings length; at the same time, the cycle time
is a significant control parameter that has no analogue in equilibrated systems.

It is particularly interesting that most of the knots generated
at \( f = 1 \) and \( k = \{0.01, 0.1\} \) have an unknotting number equal
to 1, see Figure 4C, meaning that they can be untied by a
single suitable strand crossing. At the same time, the balance of
twist and torus knots, shown in Figure 4D, shows a slight
dominance of torus knots, contrary to what has been observed in
unconstrained equilibrated rings.

Innovative single-molecule techniques have made it possible
to explore the out-of-equilibrium behavior of polymers by
monitoring the fluorescence signals and, particularly, the
appearance of bright fluorescence spots, a likely signature of
localized entanglement.\(^1\)\(^5\) At the same time, key aspects of the
out-of-equilibrium entanglement kinetics remains beyond the
reach of such techniques and, hence, are still limitedly
understood. For instance, genuine physical knots and other
types of entanglement can both generate fluorescence spots.\(^3\)\(^4\)
Additionally, the incidence of delocalized physical knots is not
reliably known, as they would be challenging to detect from
fluorescence signals. Accordingly, we still have an incomplete
knowledge of the entanglement that inevitably forms in
polymers driven out of equilibrium and how its evolution affects a polymer’s behavior.

To clarify these questions we modeled a so-called “nodozer”
assay where a semiflexible chain is subject to cyclic compressions inside a cylinder. For this prototypic system, we
precisely monitored the formation and evolution of both
geometrical and topological entanglement, the latter identified
through the algorithmic detection of physical knots, regardless of how “tight” they were.\(^1\)\(^2\)

We discover that these two types of entanglements
(geometrical and topological) are broadly independent and
evolving with different dynamics. For instance, the former
follows closely the compression driving (Figure 1), whereas the
latter does not (Figure 2). We also observe that the interplay
between the driving force and its period gives rise to a rich
phenomenology; for instance, we clearly identify three regions
in the parameter space with different levels of geometrical and
topological (median) entanglement and also heterogeneity
(Figures 3 and 4). Importantly, we find that in some regions of
the parameter space, these two aspects are decoupled and can
therefore be independently tuned to generate of polymer states
with desired combinations of geometrical and topological
entanglement.

Our discoveries ought to be useful both in interpreting
experimental results on out-of-equilibrium polymer entangle-

![Figure 3](https://example.com/figure3.png)

**Figure 3.** (A, B) Median \( n^{geom} \), and median \( n^{topo} \), respectively, observed at steady state for various combinations of \( f \) and \( k \). These plots were obtained as linear interpolation of values obtained for \( f = 1, 5, 10, \) and 20 and \( k = 0.01, 0.1, 1, \) and 10. The black lines are the contours for median equal 120 (case A) and 6.7 (case B).

![Figure 4](https://example.com/figure4.png)

**Figure 4.** (A) Sketch of the state diagram identifying regions of the \((f,k)\) parameter space yielding different combinations of geometric and topological entanglement at steady state, see Figure 3. Region I: Low geometrical and topological entanglement. Region II: High geometrical and low topological entanglement. Region III: high geometrical and topological entanglement. (B) Knot spectrum for different combinations \((f,k)\). The group “others” contains nontrivial knots that, after topology-preserving simplifications, could not be assigned to the labeled groups. (C) Unknotting number and (D) abundance of twist and torus knots. In (C) and (D), we restricted (and normalized) the counting to prime knots with crossing number less or equal than 9 for simplicity. In (D), the trefoil knot contributes to both twist and torus families.
ment and in designing new techniques to generate linear polymers with specific entanglement properties. Finally, the fact that many of the physical knots observed in our simulations are delocalized (i.e., not tight, see Figure 2) raises questions about whether these states can be faithfully detected in today’s experimental assays or whether new experimental approaches are needed.

■ ASSOCIATED CONTENT

* Supporting Information
The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsmacrolett.0c00366.

Additional experimental details and results (PDF)

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