Metastable Tight Knots in a Wormlike Polymer

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Based on an estimate of the knot entropy of a wormlike chain we predict that the interplay of bending energy and confinement entropy will result in a compact metastable configuration of the knot that will diffuse, without spreading, along the contour of the semiflexible polymer until it reaches one of the chain ends. Our estimate of the size of the knot as a function of its topological invariant (ideal aspect ratio) agrees with recent experimental results of knotted dsDNA. Further experimental tests of our ideas are proposed.

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While everyday experience suggests that knots are very common in long linear strings of any kind, and they get quite tight whenever a string is not carefully handled, the study of knots in polymers concentrated almost exclusively on closed loops. It is understandable in the sense that knots are not mathematically well defined for open strings. However, if the string is long enough, while the knot occupies a short fragment of it far from the ends, then distinguishing between different knots, or between knots and no knots, becomes sufficiently unambiguous.

The recent achievement in the theory of knotted loops [1–5] is the idea of knot localization. As simulations show [1,4], when a polymer loop with a knot is placed in a good or θ solvent, it typically adopts a conformation in which most of the polymer forms a long unknotted loop, while the knot gets somewhat tightened in a small part of the contour. Recently, this type of knot localization was also observed experimentally [5]. A theoretical explanation of the knot localization phenomenon is given [3] for the so-called flat knots, confined in a thin slit between two planes. In this case, one can consider a two-dimensional network which corresponds to any configuration of the knot by identifying chain crossings as the cross-links. This mapping allows one to resort to the sophisticated theory of 2D networks with excluded volume [6,7] and conclude that the most entropically favorable network is obtained when one of the knot arcs is made long at the expense of all others, thus localizing the knot. Tightening of knots is also helped by the long range repulsion between monomers [8].

In the present Letter, we argue that at least for a wormlike polymer, there exists a local (metastable) minimum of free energy corresponding to a tight state of the knot even when the chain itself is open (not a loop). That means that if we intentionally tie a sufficiently tight knot somewhere on a very long polymer chain, the knot will spontaneously shrink or expand to a well-defined size and then, on much longer time scales, it will diffuse along the polymer (by polymer self-reptating through the knot) until, finally, the knot is released through the chain end. We expect that this knot will diffuse along the polymer as a soliton, in the sense that its size remains relatively stable as it diffuses over large distances. The size of such a solitary knot depends on the complexity of the knot and on the persistence length of the polymer; we estimate that the knot will tighten to a size smaller than the persistence length of the polymer.

To put forward our argument, we employ knot entropy estimates based on the idea [9], similar to that in reptation theory [10], that noncrossing constraints imposed on the chain in the knot can be self-consistently described by confining the chain in an effective tube. Physically, the most natural construction of such a tube corresponds to making maximally inflated or, equivalently, the shortest length tube consistent with the given knot topology. This approach to estimate knot entropy was invented by us [9]. It was also used in a number of other contexts and widely popularized under the name "ideal knots" [11]. The configuration and aspect ratio, p, of the "ideal" tube represent topological invariants of the knot. This is sketched in the Fig. 1. The values of p were carefully computed for many knots, for instance, $p \approx 10$ for open trefoil and $p \approx 25$ for open knot 7_1 [11,12]. We will assume $p \gg 1$, which means our theory literally applies to the very complex knots.

Within the framework of this approach, we imagine that a tight knot, characterized by ideal aspect ratio p, has been tied in a very long polymer, and that currently the degree of tightening of the knot is such that the diameter of its confining tube is D. The length of the tube is pD and the

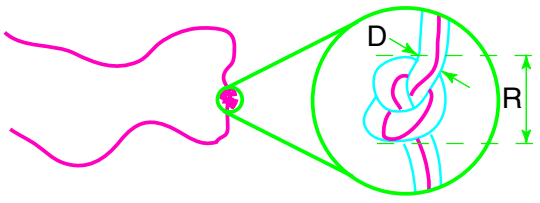

FIG. 1 (color online). A knot is tied on a polymer chain. Knot size in space is R; the chain within the knot is confined to a self-consistent tube of diameter D. Length of this tube is pD, where p is the topological invariant of the knot.

size of the knot in space is R, such that $pD \times D^2 \sim R^3$ or

$$R \sim p^{1/3} D. \tag{1}$$

Throughout this Letter, we drop all numerical coefficients, emphasizing only the scaling aspects of our considerations. We want to estimate the free energy of the knot as a function of D or R.

Although the original estimates of knot entropy were designed for a Gaussian chain [9], they can be readily adapted to a wormlike polymer. Consider a knot with $D < \ell$, where ℓ is the persistence length of the polymer. In this case, the polymer is quite tightly confined in the tube and can only wiggle about its center line. Therefore, the length of polymer within the knot is close to pD, and its free energy consists of bending energy and confinement entropy. We estimate the bending energy as $TpD\ell/R^2$, with temperature expressed T in energy units ($k_B = 1$) and assuming that the radius of curvature of the tube is about the knot size R, which is natural for an ideal knot. We further assume that the confinement entropy is the same as that for a straight tube for which case it was computed by Odijk [13] and turns out to be about unity per every so-called deflection length $\lambda \sim (D^2\ell)^{1/3}$; this results in the free energy contribution about $TpD/\lambda \sim Tp(D/\ell)^{1/3}$. Using (1), we obtain

$$\frac{\Delta F}{T} \sim p^{1/3}\frac{\ell}{D} + p\left(\frac{D}{\ell}\right)^{1/3}, \tag{2}$$

where ΔF is the free energy penalty for forming the knot (taking the reference free energy to be that of the unknotted chain). Obviously, it has a minimum at

$$D^* \sim \ell p^{-1/2}, \quad \text{or} \quad R^* \sim \ell p^{-1/6}, \tag{3}$$

i.e., the knot size decreases with increasing complexity. Notice that the resulting optimal D^* meets the condition $D^* < \ell$, so our estimate is self-consistent in this respect.

Of course, our result applies only as long as $D^* > d$, where d is the thickness of the polymer itself. Furthermore, in the case of a charged polymer such as DNA, we should also require that tube diameter exceeds Debye screening length, $D^* > r_s$. In general, we can write roughly $p < [\ell/(d + r_s)]^2$. More complex knots get so tight that their further collapse is stopped by either the excluded volume or electrostatic repulsion. As an example, for dsDNA, the ratio $\ell/(d + r_s) \approx 20$ under physiological conditions [14], while all knots with 7 or fewer crossings on the projection have p about 30 or less [12]. Therefore, in practice the condition on knot complexity $p < [\ell/(d + r_s)]^2$ is not very restrictive.

Let us now try to understand the physical meaning of our result (3), because at the first glance it might seem counterintuitive. Indeed, the optimal D^* results from the competition of two factors, each of which, as it seems, disfavors tightening. One, chain bending energy, obviously favors more loose states of the knot, or increasing D. The other one, however, related to the confinement entropy, favors tube widening *if* the chain length in the tube is fixed. In our case, when a knot tightens, it reduces the length of the chain, pD, confined in the knot. In other words, the part of the chain that remains in the tube suffers more when D decreases, but the other part of the chain gets completely free of restriction, and that factor wins the whole game. That means: what really tightens the knot is the entropy gain of the chain tails outside of the knot. Alternatively, we can say that the knot gets compressed by the pressure of Rouse modes (or bending phonons) of the outside chain tails. A similar force can be observed during the translocation of a long polymer through a narrow channel across a membrane; while two long polymer ends are outside the membrane, their entropic favorability results in a force stretching the polymer portion inside the channel, and, accordingly, compressing the membrane. Notice that while in such planar geometry this force increases logarithmically with the length of the polymer tails, in our case of two long polymer ends sticking out of a compact knot, the force on the knot is independent of the length of these ends, provided that these tails are bigger than the knot—the condition which is easily met for the tight knot and is practically never met for the membrane.

How tight should the knot be in the first place in order for our mechanism to take over and to bring the knot to its metastable size R^*? In other words, how wide is the basin of attraction of our metastable free energy minimum? We argue that the knot should be initially tightened to the state in which tube diameter is smaller or about chain persistence length ℓ. Indeed, when knot tube is wider, $D > \ell$, the chain inside the tube is roughly Gaussian, with blobs of size D. Each blob contains about D^2/ℓ of polymer contour length, and the entire tube contains polymer length pD^2/ℓ. At the same time, confinement entropy is about unity per blob, which results in overall confinement entropy of about p, independent of D. Thus, our entropic knot tightening effect does not work if the tube is wider than persistence length, and it comes into play only when the knot is prepared in a compact enough state such that $D < \ell$. To achieve this, the knot should be initially prepared by the pulling of the ends with force $f > f^*$, where $f^* \sim T/\ell$.

Let us discuss now the dynamics aspect of the situation. Imagine once again that a sufficiently tight knot was initially tied in the polymer, similar to how it was done in the experimental work [15] with DNA. Suppose now that the chain is released and is free to move. We predict then that the knot tightens spontaneously within a time which is roughly independent of the total chain length L. After that, the knot will diffuse along the chain in pretty much the same way as it was observed experimentally [15] and numerically [16] for the stretched chain (even though in the experiment of Ref. [15] the chain ends were held at fixed separation throughout the diffusion process). As regards the knot diffusion coefficient along the chain, it was shown in the work [15] that it can be quite accurately expressed in terms of the friction coefficient, ζ, of a polymer with length pD and diameter d, moving in the

solvent of bulk viscosity η inside a tube of diameter D: $\zeta = \frac{2\pi\eta}{\ln(D/d)}pD$. The diffusion coefficient is then determined by Einstein's relation as T/ζ, and the diffusion time of knot to the chain end is about $L^2\zeta/T$.

The above description of a solitonlike knot diffusing as a whole along the chain holds as long as diffusion time is shorter than the time of thermally activated loosening of the knot. Indeed, we argued that the knot gets to its metastable size R^* only when it is compact enough to begin with, such that $D < \ell$ and, therefore, a free energy barrier exists at $D \approx \ell$ (knots with $D > \ell$ will loosen up spontaneously). Using Eq. (2), we can estimate the barrier height as $\Delta F|_{D/\ell=1} - \Delta F|_{D=D^*} \sim T(p - p^{5/6})$. As expected, this barrier is very high for complex knots but, even for $p \approx 30$, one gets a barrier of about $10T$. For smaller values of p, the barrier may be too small to stabilize the tight knot and without the applied stretching it will spread due to thermal fluctuations.

As a corollary to our result, let us mention the following rather unexpected prediction. Let us take the polymer with the knot tightened according to our mechanism, and now let us gently pull the chain ends by a weak force. The applied stretching will suppress transverse fluctuations of the chain outside the knot. These fluctuations are the reason why the knot was tightened in the first place and, when they are suppressed by the applied force, the knot swells—instead of further tightening which one could have naively expected. Of course, at larger forces the loosening of the knot stops and normal tightening takes over. We should emphasize that the total distance between chain ends increases monotonically with the pulling force, as it must for stability; however, the knots still get larger at the expense of some extra elongation of the linear chains flanking the knot.

Let us support this physical argument by a little calculation. If the chain of contour length L is stretched by a weak force f, it represents the succession of Pincus blobs, each involving g persistence lengths such that $f\ell g^{1/2} \sim T$; the stretching free energy is about T per blob, or $TL/g\ell \sim L\ell f^2/T$ (see, e.g., [17]). In our case, only the chain outside of the knot is subject to this stretching effect, which yields the overall free energy

$$\frac{\Delta F}{T} \sim p^{1/3}\frac{\ell}{D} + p\left(\frac{D}{\ell}\right)^{1/3} + \frac{L - pD}{\ell}\left(\frac{\ell f}{T}\right)^2, \quad (4)$$

subject to optimization with respect to D [see Eq. (6) below]; here, the term $\propto L$ is large, but can be dropped as independent of D. Equation (4) remains valid as long as Pincus blob is larger than persistence length $f\ell/T < 1$.

If we stretch the outside chain even further, it crosses over to the so-called Marko-Siggia regime [18] in which

$$\frac{\Delta F}{T} \sim p^{1/3}\frac{\ell}{D} + p\left(\frac{D}{\ell}\right)^{1/3} + \frac{L - pD}{\ell}\left(\frac{\ell f}{T}\right)^{1/2}; \quad (5)$$

once again, this has to be optimized with respect to D. Notice that in both Eqs. (4) and (5) we have neglected the effect of force on the chain part inside of the knot. This is justified as long as the amount of transverse fluctuations of the outside chain, $D_{\text{out}}(f)$, which is either the blob size in Pincus regime or the tube diameter in Odijk (or Marco-Siggia) regime, remains larger than the optimized tube diameter inside the knot, D^*. At still larger forces, the chain inside the knot is just as stretched as outside. Summarizing all results of optimization:

$$R^* \sim \begin{cases} \ell p^{-1/6} + \ell p^{-1/2}\left(\frac{f\ell}{T}\right)^2 & \text{for } \frac{f\ell}{T} < 1 \\ \ell p^{-1/6} + \ell p^{-1/2}\left(\frac{f\ell}{T}\right)^{1/2} & \text{for } 1 < \frac{f\ell}{T} < p^{1/3} \\ \ell p^{1/3}\left(\frac{f\ell}{T}\right)^{-3/4} & \text{for } p^{1/3} < \frac{f\ell}{T} \end{cases}. \quad (6)$$

Thus, in accordance with our qualitative argument, the application of weak force loosens the tight knot instead of tightening it further. In practice, for the case of dsDNA, since $\ell \approx 50$ nm and $T \approx 4$ pN $\times$ nm, and taking $p \approx 30$, we get that both crossovers $f\ell/T \sim 1$ and $f\ell/T \sim p^{1/3}$ are within an experimentally feasible force range (about a few tenth and about a few piconewtons, respectively). To observe this effect, sufficiently long DNA should be taken, because the linear DNA outside the knot should be able to stretch more and to release sufficient length to accommodate the knot loosening. Simultaneously, the tails of linear polymer flanking the knot must also each be significantly bigger than the knot in terms of their respective gyration radii in order for the knot compression to become independent of their lengths (under this condition, the entropy increase of a polymer segment that leaves the knot and becomes a part of a free tail does not depend on the length of the tail).

Turning to experimental tests of our theory, we first consider the experiment by Quake *et al.* [15] in which the knots were tied on DNA by the use of optical tweezers and then the knot motion along DNA was observed. Our theory indicates that the optimal tube diameter in the knot, D^*, does depend in a certain way on the knot complexity, p. In the experiment, authors were unable to observe directly the knot and measure its size R; instead, they detected the amount of fluorescence coming from the knot region, in excess of the fluorescence from linear DNA. This means, in our notation, that the authors measured quantity $pD^* - R^*$, because pD^* is the length of DNA within the knot (proportional to the amount of fluorescence), while R is the length of DNA that would have been in that place if there were no knot. Given the normalization condition (1), one could write $pD^* - R^* = (p - p^{1/3})D^*$. Authors of the work [15], assuming *a priori* that D is independent of p, plotted $pD^* - R^*$ against $p - p^{1/3}$, fitted the data to the linear function, and interpreted its slope as the tube diameter D^*. By contrast, our theory predicts that D^* does depend on knot complexity p, such that $pD^* - R^* = (p^{1/2} - p^{-1/6})\ell$. This is illustrated in Fig. 2, where both the linear fit and our theory results are plotted along with the data points reproduced from the

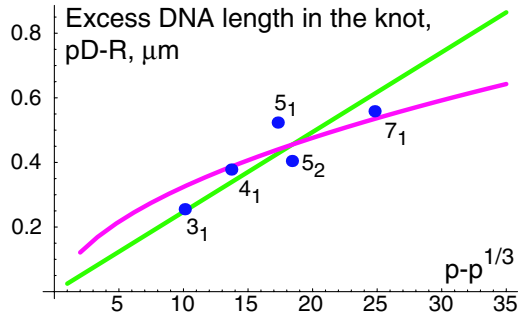

FIG. 2 (color online). The excess DNA length in the knot, $pD^* - R^*$, against $p - p^{1/3}$. The meaning of these variables is explained in the text. Data points are taken from the work [15]. Linear fit corresponds to the assumption that D^* does not depend on the knot type, on p. Curved line corresponds to our theory.

work [15]. At present, it is impossible to decide which theory is a better fit to the data.

To further test our predictions, one should prepare the knot and then consider what happens to it if the force is subsequently switched off or significantly reduced. Another test of our theory would be to see how the knot diffusion time, determined by the friction coefficient ζ, depends on the knot type through D. In all cases, since dsDNA is the most natural candidate for these experiments, one should take care to distinguish the entropic mechanism of knot localization discussed here and knot tightening due to Coulomb forces, even screened ones, described in [8]. One way to discriminate the two effects is to look at the salt dependence: our effect should only become more obvious at very strong screening, while the effect of the work [8] is based on the assumption that polymer stiffness is dominated by the electrostatics. Yet another possibility is to use granular chain experiments [19]. In the movie available in Ref. [20] the knot does not increase in size while moving, although we should put in question the applicability of our arguments based on wormlike model to the granular chain.

To conclude, we have shown that an initially tightened sufficiently complex knot in a wormlike polymer will maintain a well-defined compact size which is smaller than the persistence length. This is fundamentally different from other cases of knot localization in closed loops, where knot size is some power (smaller then unity, but positive) of the total loop length. This configuration is metastable and the knot will diffuse along the chain as a soliton, whether the ends of the chain are subjected to external force or are free to move. The effect is expected in semiflexible polymers such as double stranded DNA for which separation of length scales exists, $L \gg \ell \gg d$, and elastic behavior on length scales smaller than ℓ was demonstrated in single molecule experiments [21,22].

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Note added.—When we submitted this work for publication, we simultaneously sent a copy of our manuscript to A. Vologodskii. With our consent, he performed Brownian dynamics test and his preliminary data do not support our conclusions. We think that it was because his model chain consisted of rigid segments, while our theory relies on the wormlike flexibility of the chain. Numerically, that means one has to take really very short sticks connected by very rigid joints—as short and as rigid as to at least observe the developed Odijk undulations.

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