Twist-bend instability for toroidal DNA condensates

Igor M. Kulić, Denis Andrienko, and Markus Deserno
Max Planck Institut für Polymerforschung, Ackermannweg 10, 55128 Mainz, Germany
(Dated: September 24, 2013)

We propose that semiflexible polymers in poor solvent collapse in two stages. The first stage is the well known formation of a dense toroidal aggregate. However, if the solvent is sufficiently poor, the condensate will undergo a second structural transition to a twisted entangled state, in which individual filaments lower their bending energy by additionally orbiting around the mean path along which they wind. This “topological ripening” is consistent with known simulations and experimental results. It connects and rationalizes various experimental observations ranging from strong DNA entanglement in viral capsids to the unusually short pitch of the cholesteric phase of DNA in sperm-heads. We propose that topological ripening of DNA toroids could improve the efficiency and stability of gene delivery.

PACS numbers: 64.70.Nd, 87.15.He, 61.30.Pq

Single polymers collapse from a random coil conformation to a dense state once the solvent gets sufficiently poor \(1\). For a flexible chain the condition of minimal surface energy yields an approximately spherical globule, but for semiflexible polymers the situation is more complex \(2\): The local structure of the dense phase then consists of essentially straight chains with a basically parallel alignment, in order to minimize bending energy and maximize density, respectively. Such a state can be characterized by a smooth field of tangent vectors, but in the spherical case this field must have at least two energetically unfavorable defects on the surface (you can’t comb a sphere). However, for a torus many defect-free fields are possible. Indeed, DNA, the probably best studied semiflexible polymer, readily forms beautiful nanotori after adding any one of a variety of possible condensing agents (like polyethylenglycol (PEG), multivalent counterions, or bundling proteins) to a dilute solution of DNA chains \(3\). These tori are surprisingly monodisperse, having a radius comparable to the persistence length of DNA \((\approx 50 \text{ nm})\) basically independent of the condensation method \(4\).

Consider such a condensate, in which the chain is wound up like a garden hose to form a torus with axi- 

dal and tubular radii \(r_1\) and \(r_2\), respectively. Since \(r_1\) is the average radius of curvature of the chain, a simple scaling analysis balancing a bending energy \(A/r_1^2\) per unit length of polymer and a surface energy \(\sigma\) per unit area of the torus yields \(2\): \(r_1 \sim (\sigma/A)^{-2/5}V^{1/5}\) and \(r_2 \sim (\sigma/A)^{1/5}V^{2/5}\), where the chain volume \(V \sim r_1 r_2^2\) as well as the packing density are assumed constant. Hence, the aspect ratio \(\xi = r_1/r_2 \sim (\sigma/A)^{-3/5}V^{-1/5}\) shrinks if \(\sigma\) or \(V\) increase (i.e., if the solvent gets poorer or the chain longer), and thus the torus “fattens”. In this case it is no longer justified to calculate the bending energy with some average radius of curvature \(\langle \rho \rangle = r_1\). In fact, since \((\rho^{-2}) \geq \langle \rho \rangle^{-2}\) (by virtue of Jensen’s inequality), the actual curvature energy should be larger. However, the same argument indicates that the condensate can lower its bending energy by redistributing curvature more evenly. In this letter we demonstrate that indeed below a critical aspect ratio \(\xi\) (or above a critical surface tension \(\sigma_c\)) the system spontaneously relaxes bending energy by twisting the bundle of polymer strands.

Indirect indications of such a twisted state can be found in computer simulations \(4\). Analyzing Cryo-EM experiments on DNA toroids \(5\) Hud et al. proposed non-local DNA winding and equidistribution of bending in toroids \(6\). Besides indications for the non-trivial local organization of DNA in toroids there are several lines of evidence for a global, topological, non-triviality coming from in vivo studies: Certain bacteriophages, whose DNA is (due to a genetic modification) no longer attached to their nucleocapsid, display unusually strong knotting of the genome \(7\). And the chirality of the highly confined sperm-chromatin is surprisingly pronounced, with a pitch 10 times shorter than in vitro \(8\). These findings make us wonder whether there is a connection to the topological ripening we will now discuss.

Let us begin our quantitative analysis of the situation by neglecting the connectivity of the chain. More specifically, we will first formulate a local theory which is based on the above mentioned nematic field \(\mathbf{n}\) of unit tangent vectors \(4\). The path of the actual polymer will later be recovered as an integral curve of this flow field, and its global topological properties can then be studied. The elastic energy \(e\) per unit volume, describing the deviation from perfectly parallel alignment, is the Frank-Oseen free energy of a uniaxial nematic liquid crystal \(10\):

\[
e = \frac{K_1}{2} (\nabla \cdot \mathbf{n})^2 + \frac{K_2}{2} (\mathbf{n} \cdot (\nabla \times \mathbf{n}))^2 + K_3 (\mathbf{n} \times (\nabla \times \mathbf{n}))^2,
\]

where the three terms correspond to splay, twist, and bend deformations, respectively. Assuming the condensate to behave like an incompressible liquid the first term (splay) must vanish identically in order to maintain a constant polymer density throughout the condensate \(11\), while the other two terms divide the elastic energy.
The total energy is of course the integral of Eqn. (4) over the torus volume.

It is convenient to discuss this situation in suitable toroidal coordinates \( \{r, \vartheta, \varphi\} \), defined by

\[
x = (r_1 - \cos \vartheta) \cos \varphi, \\
y = (r_1 - \cos \vartheta) \sin \varphi, \\
z = r \sin \vartheta.
\]

The nematic field is now represented as \( \mathbf{n} = n_\vartheta \mathbf{e}_\vartheta + n_\varphi \mathbf{e}_\varphi + n_r \mathbf{e}_r \) (where the \( \mathbf{e}_i = \partial_i r/|\partial_i r| \) are the toroidal unit tangent vectors). When inserting this into Eqn. (4) and integrating over the volume, we obtain the elastic energy as a functional of \( n_\vartheta(r, \vartheta, \varphi) \), \( n_\varphi(r, \vartheta, \varphi) \), and \( n_r(r, \vartheta, \varphi) \). At the surface of the torus the boundary condition \( n_\vartheta(r_2) = 0 \) must hold, and owing to rotational symmetry we will henceforth make the (nontrivial but very reasonable) assumption that none of the coordinate functions depends on \( \varphi \). These steps reduce the task of finding the optimal polymer winding to a two-dimensional variational problem.

Now that the mathematical problem is formulated, let us first have a look at the full solution, which we obtained numerically via a conjugate gradient minimization [12]. The results confirm the suspicions made above: For large enough aspect ratio \( \xi = r_1/r_2 \) the equilibrium nematic field is \( \mathbf{n} = \mathbf{e}_\varphi \), corresponding to simple circumferential winding of the polymer. But as the torus grows sufficiently fat, a continuous transition occurs to a state in which (simultaneously throughout the entire torus) \( \mathbf{n} \) acquires components in \( \mathbf{e}_\vartheta \) and \( \mathbf{e}_r \), i.e., the polymer additionally winds around the tubular circle, see Fig. 1. This winding relaxes bending energy, but only at the expense of the additional twist, which is zero when \( \mathbf{n} = \mathbf{e}_\varphi \). Consequently, this twist instability occurs more readily if the ratio \( \eta = K_2/K_3 \) of twist and bend modulus is small. All this is confirmed in Fig. 2 where we show the maximum twist angle, which is a suitable order parameter, as a function of the aspect ratio \( \xi \) of the torus. Note that the calculation is reliable down to the value \( \xi = 1 \), where the hole in the torus degenerates to a point, the only possible tangent vector is \( \mathbf{e}_\vartheta \) and thus \( \phi = \pi/2 \).

The mathematical task of functional minimization can often be accurately approximated by devising a variational ansatz which is analytically tractable. The following choice turns out to be remarkably good: We will first assume that the nematic field does not have a component in \( \mathbf{e}_r \) direction. The tangential boundary condition \( n_\vartheta(r = r_2) = 0 \) is then automatically taken care of. The remaining two components must satisfy the normalization condition \( n_\vartheta^2 + n_\varphi^2 = 1 \), and it thus suffices to specify one of them, say \( n_\vartheta \). It is easy to check that any ansatz of the form \( n_\vartheta = f(r)/[1 - (r/r_1) \cos \vartheta] \) with an arbitrary function \( f(r) \) yields a divergence free nematic field. We choose a linear \( f(r) = \omega r/r_2 \), i.e.

\[
n_\vartheta(r, \vartheta; \omega) = \omega \frac{r/r_2}{1 - (r/r_1) \cos \vartheta},
\]

where \( \omega \), which we may call the “twisting strength”, is the only free parameter of the ansatz. With this choice we go back into the Frank-Oseen free energy (4), calculate the derivatives, and integrate over the volume of the torus. Since the sign of \( \omega \) only determines the handedness of the twisted structure, it cannot influence the free energy \( E \), which thus must be an even function of \( \omega \) [13]. An expansion in powers of \( \omega^2 \) then yields

\[
\frac{E}{K_3 r_2} = g_0(\xi) + g_2(\xi, \eta) \omega^2 + g_4(\xi, \eta) \omega^4 + \mathcal{O}(\omega^6),
\]

FIG. 1: Illustration of the flow field on a toroidal condensate which features additional twist. The aspect ratio is \( \xi = 1.5 \).

FIG. 2: Order parameter \( \phi := \arccos(n_{\varphi, \text{min}}) \) as a function of the aspect ratio \( \xi = r_1/r_2 \). The curves correspond to different ratios of elastic moduli, \( \eta = K_2/K_3 \in \{0.01, 0.05, 0.1, 0.2, 0.3, 0.4\} \), the gray arrow pointing toward increasing values. The inset illustrates the toroidal coordinate system.
This results in the phase boundary phase transitions with a scalar order parameter, has the form of a Landau free energy as it occurs for localizing the phase transition. The reason is that Eqn. (4) coefficient of the quadratic term vanishes, it predicts a continuous transition at the point where the elastic modulus goes to infinity, and if the ratio of elastic moduli to bending it. For large \( \xi \) this improved ansatz agrees better with the exact answer, basically since the new prefactor cancels the unphysical divergence of the denominator for \( r \to r_1 \) at \( \vartheta = 0 \). The analytical expression for the phase boundary is quite involved and will not be shown here, but it has the same large \( \xi \) asymptotics (see Fig. 4).

Both the ansatz as well as the full numerical solution point to an upper critical ratio of elastic moduli, \( \eta_c \), beyond which the system will no longer spontaneously twist. Even at the lowest possible aspect ratio \( \xi = 1 \) the energetic cost for twisting has become so large that it no longer admits a bending relaxation. The ansatz \( \xi \) gives \( \eta_c = \frac{1}{2} \), the improved ansatz gives \( \eta_c \approx 0.829 \), while the full numerical solution suggests the deceptively simple result \( \eta_c = 1 \). We have no analytical support for the latter, but we also want to remind that the limit \( \xi \to 1 \) is somewhat academic, because our tacit assumption that the condensate shape is strictly toroidal will most likely break down in this case.

We have thus seen that the ratio \( \eta \) and the torus geometry \( \xi \) uniquely specify the twist-state of the condensate. However, while one can easily measure \( \xi \) in an experiment (just by visual inspection), it is hard to specify in advance. In contrast to that, the surface tension \( \sigma \) can be readily changed (for instance via the concentration of condensing agents), but its actual value is hard to measure. In our simple model it is of course not difficult to add a tension term \( \sigma \) times the torus surface \( S \) to the condensate energy. Using \( S \propto \xi^{1/3} r^{2/3} \), one can re-express the twisting transition as being driven by increasing \( \sigma \), and it remains continuous. However, practical considerations would advise to tune \( \sigma \) only for the purpose of modifying \( \xi \), but subsequently use \( \xi \) as the independent variable. This way one needs no longer (neither theoretically nor practically) worry about how a particular concentration of condensing agents gives rise to a particular torus geometry.

After these local considerations it is time to study global aspects of the polymer structure. Let us start with the flow itself. It can be shown that incompressibility, \( \nabla \cdot n = 0 \), together with axial symmetry causes the flow to be Hamiltonian—hence one more conservation law exists. In our case it forces the flow lines to stay on two-dimensional slightly deformed toroidal surfaces, such that the total flow consists of a nested structure of invariant tori. In fact, our ansatz follows readily from the quadratic Hamiltonian \( H = \frac{1}{2} \xi \omega r^2 \), which is constant on circular tubular layers. Of course, the actual polymer has to switch between these layers, reminding us that irrespective of twist none of the above structures can be

![FIG. 3: Structural phase diagram of the toroidally wound complex on a log-log scale.](image)
realized without localized defects \[17\].

There is one global aspect of the polymer structure in which it differs fundamentally from a plainly wound torus: As is visible in Fig. 1, the path of the polymer threads it repeatedly through the middle hole. Moreover, the amount of this looping (as measured \(e.g\), by the average change in \(\theta\) per turn) depends on the layer. This effect implies that the entire polymeric strand is heavily entangled with itself. A rough estimate for \(\xi = 1.5\) and polymer length \(L = 15 \mu\text{m}\) gives about 30 threadings through the hole. In fact, were it not for the two free ends, these knotted states were topologically inaccessible. In other words, unlike the initial collapse, the second stage, the structural ripening, relies on the motion of the free chain ends and is thus a much slower process.

On the other hand this structural ripening meets no kinetic barriers during the relaxation to its twisted ground state, downhill the free energy landscape. The motion of the two free ends is then energetically directed and their local rearrangement does not involve the highly improbable threading through the toroid hole in 3D space. In addition, the weak chiral interaction of DNA molecules neglected above, gives rise to a (small) chiral term in the elastic free energy, which might contribute to the symmetry breaking and “guide” the twisting in a preferred direction.

It is also tempting to explain the unusually short DNA cholesteric pitch (10 times shorter than in vitro) in sperm chromatin assuming that the weak DNA chirality merely determines the handedness of twist whereas its pitch is given by the twisted state of polymer strands after the topological ripening took place. Moreover, the severe knotting in highly dense phage heads, where the 2nm thick DNA has little space left for usual entropic entanglement effects, suggests the same explanation—in particular since it is far more pronounced for genetically modified phages in which the DNA is no longer attached to the capsid and can thus undergo structural ripening.

Finally, is the predicted effect strong enough to be of some relevance for DNA condensation and gene delivery? For typical experimental parameters of DNA length \(L = 15 - 30 \mu\text{m}\), \(\xi \sim 1.5 - 2.5\), bending stiffness \(A \sim 50k_B T\cdot\text{nm}\), and inter-helical distance \(d \sim 3\text{nm}\) we obtain first the elastic constant \(K_3 \sim A/d^2 \sim 20\text{pN}\), which is dominated by the bending stiffness \(B\). The twist constant \(K_2\) can be estimated by the deconcentration force \(\sim 2\text{pN}\) obtained in single molecule experiments with condenser spermidine \[18\]. The difference in elastic energy between the twisted and untwisted states, as bounded below by the the variational ansatz, lies in the range \(15 - 30k_BT\). This indicates that topological ripening stabilizes the condensate. In addition, if the solvent quality abruptly improves, the twisted DNA will unfold more inertly than its untwisted counterpart, due to heavy entanglement with itself. If this stabilization occurs on the typical time scales relevant for gene therapeutical applications, it might prevent a premature digestion of the genetic material by the host organism and influence (positively) the efficiency of the gene delivery process.

We thank H. Schiessel, K. Kremer, I. Pasichnyk, and A. Ryskin for helpful discussions.

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