Writhing Fluctuations of Open DNA

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Motivated by recent experiments on DNA torsion-force-extension characteristics we consider the writhing geometry of open stiff molecules. We exhibit a cyclic motion which allows arbitrarily large twisting of the end of a molecule via an activated process. This process is suppressed for forces larger than femto-Newtons which allows us to show that experiments are sensitive to a generalization of the the Călugăreau-White formula for the writhe. Using numerical methods we compare this formulation of the writhe with recent analytic calculations.

I. INTRODUCTION

Recent experiments where DNA molecules are manipulated [1, 2] with the help of magnetic beads have led to a renewed interest in the statistical mechanical properties of the torsionally stiff wormlike chain [3–7]. In the experiments a bead is attached to the end of a long DNA molecule; the other end remains stuck to a surface. The bead is held in a magnetic trap which allows the simultaneous application of a force, f and couple, Γ; one measures the mean distance of the bead from the surface as a function of f and Γ. In an ingenious theoretical paper is was shown [5] that the statistical mechanics of this problem are related to the problem of a quantum particle in a magnetic field. However, a crucial approximation was made in the formulation of the writhing geometry of the polymer. In this paper we study, the effect of this approximation on the final distribution of angular fluctuations.

The concept of writhe was originally defined in the context of studies on closed curves where it forms one part of a topological invariant, the linking number [8, 9]. In this paper we show how the writhe can be usefully generalized to study the geometry of open DNA molecules. This generalization introduces end corrections to the Călugăreau-White formula for the writhe. We then perform simulations for the writhe distribution of an open chain which we compare with the analytic theory based on the Fuller formula.

Most importantly we show that experiments involving manipulation of DNA with beads in unconfined geometries have unbounded fluctuations in the measured torsional angle. Under an exterior torque a bead can rotate an arbitrarily large angle. When the DNA is under tension this rotation is an activated process with jumps of 4π in the mean angle. Contrary to the calculation performed by Bouchiat *et. al* [5] we find that this unbounded response is not removed by the introduction of an intermediate cut off. However applications of tensions larger than than femto-Newtons suffice to render the problem finite in practice.

II. WRITHE AND LINKING NUMBER

A. Closed curves

The linking number $\mathcal{L}k$ of a closed ribbon is an integer topological invariant [8, 9]. It can be decomposed into two parts the twist, $\mathcal{T}w$ and the writhe, $\mathcal{W}r$.

$$\mathcal{L}k = \mathcal{W}r + \mathcal{T}w \tag{1}$$

This decomposition is useful because the writhe is expressed as function of the centerline $\mathbf{r}(s)$ of the ribbon.

$$\mathcal{W}r = \frac{1}{4\pi}\int ds \int ds' \frac{\mathbf{r}(s)-\mathbf{r}(s')}{|\mathbf{r}(s)-\mathbf{r}(s')|^3}.\frac{d\mathbf{r}(s)}{ds}\times\frac{d\mathbf{r}(s')}{ds'}. \tag{2}$$

The linking number is invariant under deformations of the shape which do not introduce self intersections. If during a deformation the centerline $\mathbf{r}(s)$ crosses itself there is a discontinuity of 2 in $\mathcal{W}r$ and thus $\mathcal{L}k$. Fuller [10, 11] showed the integral of equation (2) could be simplified and introduced the expression

$$\mathcal{W}r^F = \frac{1}{2\pi}\int \frac{\hat{\mathbf{e}}_z.(\mathbf{t}\times\dot{\mathbf{t}})}{1+\mathbf{t}.\hat{\mathbf{e}}_z}.\ ds \tag{3}$$

where $\hat{\mathbf{e}}_z$ is an arbitrary reference direction. In this simplification information is lost so that $\mathcal{W}r$ and $\mathcal{W}r^f$ are related by the equation

$$\mathcal{W}r - \mathcal{W}r^F = 0 \mod 2 \tag{4}$$

For notational convenience let us introduced the angles, $\chi_C = -2\pi\,\mathcal{W}r.$ and $\chi_F = -2\pi\,\mathcal{W}r^F$.

A much more direct approach to Fuller's result is possible [12] by noting that the writhe a stiff polymer is closely related to the geometric anholonomies discovered by Berry [13] in wave phenomena.

B. Bead rotation is given by an extended definition of writhe

The above definition of the writhe, with its emphasis on it being part of an topological invariant hides, to some degree, the interpretation of χ_C as an angle of rotation in many experimental situations. We shall now show that

an extended definition of the writhe, based on equation (2) is the writhe contribution to the bead rotation that is measured in DNA twisting experiments. Rather similar arguments have also been given in [4].

Consider the planar ribbon in figure (1). The linking number, $\mathcal{L}\mathsf{k}$, is zero thus

$$\mathcal{W}\mathsf{r} + \mathcal{T}w = 0. \tag{5}$$

We shall use this closed ribbon as a reference configuration to calculate the writhe of an *open* filament for which the initial and final tangents are parallel ie for $\mathbf{t}(0) = \mathbf{t}(\mathsf{L})$, where L is the polymer length. We do this using the geometry of figure (2). The polymer $\mathcal{P}$ which is also a ribbon is embedded in a construction consisting of two long straight sections $\mathcal{S}_1$, $\mathcal{S}_2$ and a closing loop $\mathcal{C}$. We shall apply equation (2) to the constructions of figures (1,2) then take the length of the straight sections to infinity.

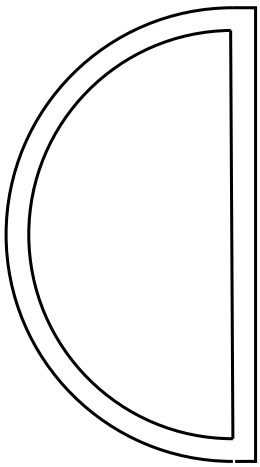

FIG. 1: A flat ribbon with zero linking number.

There are two beads attached to each end of the polymer, joining on to the straight sections. These two beads are our experimental reference. We shall hold the lower bead $\mathcal{B}_1$ stationary and let the upper bead $\mathcal{B}_2$ rotate due to the writhe of the polymer. The final part of our construction is the "twist absorber" $\mathcal{T}$. This is imagined as being a joint, or section of the straight ribbon which twists freely to absorb the writhe generated by the polymer. Let the twist of the polymer be zero so that *all* twist appears in $\mathcal{T}$.

Start from the reference state of figure (1) and deform continuously to an arbitrary state figure (2) without generating a self intersection of the construct. The writhe can be calculated with the classic double-integral. Clearly since $\mathcal{W}\mathsf{r} + \mathcal{T}w = 0$ the writhe which is generated goes into twisting the region $\mathcal{T}$ of the chain. The total twisting angle is just $-2\pi\mathcal{W}\mathsf{r} = \chi_{\mathsf{C}}$. Now let the straight sections go to infinity. In this limit the contribution to the integral of the section $\mathcal{C}$ vanishes. Thus the *experimental* rotation angle is given by the χ_{C} with the addition of a end correction.

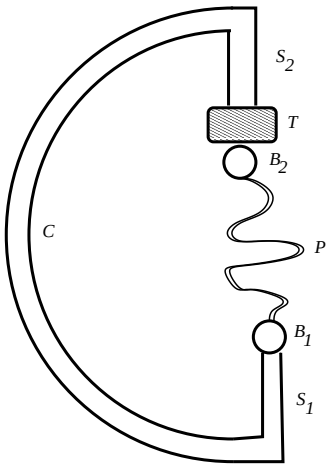

FIG. 2: Deform the ribbon of figure (1) as in the diagram: The initial and final tangents of the polymer section $\mathcal{P}$ are parallel. Long arms $\mathcal{S}_1$ and $\mathcal{S}_2$ attach the polymer to a closing loop C. The experimentally important reference beads $\mathcal{B}_1$ and $\mathcal{B}_2$ are attached to the two ends of the polymer. The twist is absorbed *exclusively* in the section $\mathcal{T}$. Note the beads are NOT in general aligned vertically.

In the light of this construction it is interesting to note that the writhe is a conformal invariant for which the addition of a single point at infinity is topologically "natural". A crucial part of this argument is the restriction of the construct of figure (2) to the sector $\mathcal{L}\mathsf{k} = 0$. We discuss the importance of this restriction in the next section.

C. Choice of linking sector

In the deformation from figure (1) to figure (2) we wish to conserve the linking number $\mathcal{L}\mathsf{k}$. To do so we must firstly forbid self crossing of the polymer configuration. In any experiment with DNA in the absence of topology changing enzymes this restriction in reasonable. However this condition is *insufficient* to conserve the topology of the entire construction. We must also forbid the crossing of the real polymer with the imaginary line from the end of the chain to infinity. In a recent numerical paper [4] the ends of the polymer were considered to be grafted to an external surface rather than beads. Experimentally we would stop this crossing by introducing a steric hindrance: A fine fiber in the neighborhood of the manipulating bead would work perfectly. We show below, however, that this is rarely needed in practice.

If the chain is allowed to bend back in such a way that is passes through the line to infinity there is a discontinuity of $\Delta\,\mathcal{L}\mathsf{k} = \pm 2$. This allows one to construct a cycle of motions in which the shape of the filament undergoes a cycle coming *exactly* back to its original shape while the bead undergoes a rotation of 4π about the vertical axis. This result explains a well known as a demonstration of the utility of spinors in describing three dimensional rota-

tions and is a common amphitheater trick: If one holds a plate horizontally in the palm of one's hand one can spin it about a vertical axis by performing a suitable contortion of the arm. Against all intuition the plate can be turned an arbitrarily large angle; for each single cycle of the arm the plate spins twice. Photographs of Feynman performing this trick are to be found in [14].

We expect that this unbounded torsional fluctuation is an activated process: When the DNA is under tension the bead must move against the magnetic force a distance comparable to its diameter in order to force a loop over the point of attachment. For micron sized beads this introduces a characteristic scale of the force of $k_B T/\mu m \sim fN$. For forces of more than a few femto-Newtons the process is exponentially rare and suppressed. The natural time scale for attempts at crossing the barrier is given by the Zimm time of the bead $\tau \sim d^3\eta/k_B T$ with d the bead size, which is seconds.

Note that the scale of this force is very low, the widely studied crossover from random coil to semiflexible behavior in the force-length characteristics of DNA occur at a force scale of $k_B T/\ell_p$ with ℓ_p the persistence length of DNA. This intrinsic force scale for DNA is orders of magnitude higher than that which is needed to conserve linking number. We conclude that most experiments with tense DNA are performed in a regime where a high energy barrier leads to conservation of the linking number. However, there is *always* a small probability of passage by this barrier so that under torsion the true steady state of a DNA molecule is a state of cyclic motion in which the bead rotates by a series of activated jumps.

D. Twisting

The experiments are actually sensitive to the sum of the the writhing and twisting fluctuations in a polymer. If we now allow the excitation of the torsional modes in the polymer of figure (2) the total rotation between the two ends is the sum of the writhing angle χ_C and rotation due to internally excited twisting motions. Since this twisting mode is unmodified by the writhing geometry (at least in the simplest models of DNA elasticity) we will not consider it further in this article.

E. Fuller formulation

Recent analytic work [3, 5] has been based on the simplified formulation of equation (3). It is much easier to treat than the full double integral in equation (2) due to a direct mapping onto quantum mechanics: The bending energy of a stiff beam in the slender body approximation of elasticity is given by

$$E = \frac{\mathcal{K}}{2}\int \left(\frac{d\mathbf{t}(s)}{ds}\right)^2 \, ds \ , \tag{6}$$

where $\mathcal{K}$ is the bending modulus linked to the persistence length by $\ell_p = \mathcal{K}/k_B T$

To calculate the partition function one must now sum over all paths

$$\mathcal{Z} = \sum_{paths} e^{-E/k_B T} \tag{7}$$

The sum for the partition function is clearly closely related to path integrals studied in quantum mechanics. Formally the energy in equation (6) looks like the kinetic energy of a free particle moving on a sphere. From the sum of paths in equation (7) one derives a Fokker-Planck equation which is entirely analogous to the Schroedinger equation for a particle on a sphere:

$$\frac{\partial P(\mathbf{t}, s)}{\partial s} = \frac{1}{2\ell_p}\nabla^2 P(\mathbf{t}, s) \ , \tag{8}$$

where ∇^2 is the Laplacian operator on the sphere. Here $P(\mathbf{t}, s)$ is the probability of finding the chain oriented in the direction $\mathbf{t}$ at the point s. As a function of s the vector $\mathbf{t}$ "diffuses" with diffusion coefficient which varies as $1/\ell_p$.

Let us now consider the writhe calculated with Fuller's formula for such "diffusing" paths. There a singularity in in equation (3) near $\mathbf{t} = -\hat{\mathbf{e}}_z$. In the neighborhood of this direction the integral measures twice the winding of of the random walk about the point $-\hat{\mathbf{e}}_z$ on a sphere. The winding of a random walk has singular properties in the two dimensional plane and on a sphere [15]. In particular there is logarithmic divergence in the winding properties in the continuum limit. It is this winding number divergence that was picked up in the analytic calculation of [5] and necessitated an intermediate scale cut off in the calculation.

It was not clear to us that these winding number singularities are truly present in the distribution of the writhe. As shown above in the case of constrained linking number it is the Călugăreau-White expression for the writhe which gives the exact twisting angle. The Fuller expression differs by an arbitrary factor of $4n\pi$. One understands that the winding number singularity is linked to the properties of return to the origin of random walks. In both one and two dimensional walks a small disk at the origin is visited an infinite number of times in a diffusion process. The winding occurs predominately during close approaches between the walker and the origin. The properties of return to the origin of a semiflexible walk are very different: already in one dimension return to the origin only occurs a finite number of times. It is thus difficult to imagine how a thermalized DNA molecule could wrap around itself many times in order to form supercoils if, statistically, two pieces of the molecule can not find each other repeatedly.

III. NUMERICAL METHODS

Given the difficulty of treating the full Călugăreau-White expression for the writhe analytically we decided to proceed by numerical exploration of the the distributions of writhe implied by the Călugăreau-White and Fuller formalisms. In particular we study the Cauchy tail predicted analytically [5] in the writhe distribution function. The existence of such a tail would imply the absence of a reasonable continuum limit of the wormlike chain and continuous evolution of the response functions as a function of a microscopic cut off.

A. Numerical Calculation of Writhe

A number of methods are available for the calculation of the writhe of a discretized polymer [16]. We calculate the discretize versions of the integrals of equation (2) and equation (3) by recognizing that they are both areas on a unit sphere. In the Fuller formulation it is the area enclosed by the curve $\mathbf{t}(s)$. For the discretize chain the tangent curve becomes a series of link directions, $\mathbf{t}_i$, corresponding to points on a sphere. One must connect these points by geodesics and then sum over the area of the triangles formed by two successive tangent vectors and $\hat{\mathbf{e}}_z$.

$$\chi_F = \sum_n \mathcal{A}(\hat{\mathbf{e}}_z, \mathbf{t}_n, \mathbf{t}_{n+1}) \tag{9}$$

where $\mathcal{A}$ is the area of the spherical triangle defined by the three vectors.

The full definition, equation (2), of the writhe is treated in a similar manner: The integral is evaluated for a pair of links by noticing that this integral is the spherical area, swept out by the vector

$$\hat{\mathbf{e}}(s, s') = \frac{\mathbf{r}(s) - \mathbf{r}(s')}{|\mathbf{r}(s) - \mathbf{r}(s')|} \tag{10}$$

as s and s' vary. For two straight links this map defines a spherical rectangle. We calculate its area by decomposing it into two spherical triangles.

In both calculations we calculate the area of a spherical triangle by using l'Huilier's expression.

$$\begin{aligned} \mathcal{A} = 4\arctan\Big(\Big(& \tan((a+b+c)/4) \times \\ & \tan((c-a+b)/4) \times \\ & \tan((c+a-b)/4) \times \\ & \tan((a+b-c)/4)\Big)^{1/2}\Big) \end{aligned} \tag{11}$$

where, a, b and c are the lengths of the sides of a spherical triangle. For our purposes the triangle has to be oriented, the area can be either positive or negative.

A useful cross check in the programming is that for any polymer the modulo relation of equation (4) must be satisfied despite very different intermediate results in the calculation. Numerically we found that the equality held to within 10^{-10} when the extended Călugăreau-White definition of writhe was compared with the Fuller formulation.

Since the double integral of equation (2) is reduced to a double summation this step takes takes $O(N^2)$ operations for a chain of N links. For the long chains studied in our simulations it is by far the slowest step in the calculation.

B. Link Sector Choice

Clearly the physical system we are interested in is a self-avoiding DNA chain. However to compare the results of the Călugăreau-White formulation of the writhe with the results with those of [5] we have also performed simulations with a reduced level of topological constraint. As we have shown above there is no unique ground state to the system in the presence of a torque. A minimum model must impose a choice of linking number. Thus in our main series of simulations we use an ensemble of chains with only the constraint $\mathcal{L}k = 0$. This ensemble may contain knots. Clearly any knotted configuration can be studied in the $\mathcal{L}k = 0$ sector since all spatial configurations exist in every sector.

While this ensemble may appear physically "unreasonable" one must not forget that knots are rare in the short chains that we shall study. Grosberg [17] remarks that for a flexible chain unknotted configurations dominate the statistics of chains even several hundred Kuhn lengths long. For the chain lengths that we work with in this paper the contamination coming from such knotted configurations should be weak.

To check the importance of this approximation we also performed some simulations using ensembles in which knotted configurations (here of the whole extended construct) were suppressed by using characteristic polynomials to eliminate some of the simplest knots. Again this ensemble was limited to the sector $\mathcal{L}k = 0$.

C. Chain generation

In order to use the expressions for the writhe given above we are interested in chains in which the initial and final tangents are parallel (though the writhe for non parallel configurations does have a simple generalization in the Fuller description [12]) but for which there is no constraint on the final position of the chain. Rather than using a conventional Monte-Carlo algorithm to generate chains we used a simple "growth algorithm".

We wish to grow chains of length L persistence length ℓ_p using a series of links of length $a = 1/N$, where N is the number of links per persistence length. In the absence of tension we generate chains by starting from a single link in the z direction at the origin. We then successively add links to the chain with small random angle increment $\alpha_0 \sim \sqrt{a/\ell_p}$ to produce a single realization of

an equilibrated semiflexible chain. It is almost certain that this chain does not satisfy the boundary conditions on the tangent thus we continue growing until the final tangent is parallel to the initial tangent to within an angle small compared with α_0. We keep the polymer in our ensemble if the length of the polymer is less than $1.05\times L$, otherwise the whole configuration is rejected and the process restarted from the first link. The configurations are then used to calculate the writhing distributions. The curves that we generate are somewhat "imperfect" since they are due to a mixture of lengths. This admixture of chain lengths plays no role, however, in our analysis of the asymptotic distribution of writhe.

In the presence of an external force the algorithm is slightly more complicated. We proceed by simulating the Fokker-Plank equation

$$\frac{\partial P}{\partial s} = \frac{1}{2\ell_p}\nabla^2 P + f\cos(\theta)\, P \ , \tag{12}$$

where θ is the angle between the direction of the force and the local tangent to the polymer. We use introduce a *pool* of several hundred chains which we grow simultaneously. As each link is added there is angular diffusion as described above and a second process of birth or death of chains in the pool in order to account for the force. If $f\cos(\theta)$ is positive then it is considered to be a growth rate for reproduction of chains in the pool. If $f\cos(\theta)$ is negative the chain is stochastically destroyed with the appropriate probability. The total number of chains in the pool is managed in order to prevent too large fluctuations in the number of members.

There is no self avoidance in this code however it can be shown from a Flory argument that self avoidance is a weak effect in semiflexible chains of moderate length.

IV. ASYMPTOTICS OF WRITHE

In this section we wish to explore the scaling behavior of the writhe of a polymer described as a freely jointed chain with N links. This allows us to understand the length scales and the structures which are important in determining the writhe of a molecule. The argument is generalized to treat the average crossing number.

A. Scaling arguments for flexible chains

We shall start with the interpretation of the writhe as a signed area swept out by the vector $\hat{\mathbf{e}}(s,s')$: Consider two links of length a separated by a distance R_{ij} This area scales as $A_{ij} \sim \pm(a/R_{ij})^2$ when $R_{ij} >> a$. When $a << R_{ij}$ the area is bounded above by 2π. Clearly when one averages over random walks the integral in equation (2) gives zero. The mean squared writhe can be estimated as

$$\langle \mathcal{W}r^2\rangle \sim \sum_{pairs} A_{ij}^2 P(R_{ij}) \tag{13}$$

where $P(R_{ij})$ is the pair distribution function for the polymer. For a three dimensional Gaussian polymer this function scales as

$$P(R_{ij}) \sim 1/R_{ij} \tag{14}$$

for lengths smaller than the radius of gyration, $R_G \sim \sqrt{aL}$ of the polymer. Approximating the summation by integration we find

$$\langle \mathcal{W}r^2\rangle \sim \frac{L}{a^4}\int_a^{R_G} \frac{a^4}{R^4}\frac{a}{R}\quad R^2\, dR. \sim \frac{L}{a} \tag{15}$$

This integral converges at large distances, but is divergent at small distances. We conclude that the writhe integral is dominated by the cut off scale a. For a semiflexible polymer the cut of a corresponds to the persistence length ℓ_p. It is thus structure at this length scale which dominates the writhe of the molecule. The average crossing number is defined in a very similar manner to the writhe except the unsigned area $|A_{ij}|$ is summed over rather than the signed area. This has a non-zero mean and scales in the following way in a Gaussian polymer

$$\langle \mathcal{W}r^X\rangle \sim \frac{L}{a^4}\int \frac{a^2}{R^2}\frac{a}{R}\quad R^2\, dR. \tag{16}$$

leading to a logarithmic divergence $\langle \mathcal{W}r^X\rangle \sim (L/a)\log(L/a)$. Thus in the crossing properties of an arbitrary projection of the polymer we expect all length scales are important.

Finally the end correction to the writhe is a sum of random areas $A_i \sim \pm a/R_i$ where R_i is now the distance between the end of the polymer and the single link i. The corresponding estimate of the mean square writhe is thus

$$\langle \mathcal{W}r^2_{end}\rangle \sim \frac{1}{a^3}\int \frac{a^2}{R^2}\frac{a}{R}\quad R^2\, dR. \tag{17}$$

giving a logarithmic contribution with the structure of the whole molecule being important.

When self avoidance is introduced in the problem we can use the result that $P(R) \sim 1/R^{4/3}$ to show that integral in equation (15) is still dominated by short length scales. The results for the end correction and average crossing number are however modified. They too become sensitive to structure at short wavelengths in the polymer.

Given the importance of this end correction in the interpretation of the experiments we now present a more rigorous study of the end correction order to confirm its subdominant nature compared with the internal contributions to the writhe.

B. Magnitude of End Corrections

In order to understand the scaling behavior of the end corrections we shall examine the limit L/ℓ_p large.

We thus study the problem of a freely jointed chain rather than the semiflexible chain. Numerical results (not shown here) on semiflexible chains lead to the same conclusions.

Consider a chain, $\mathbf{r}(s)$. Place the origin at $\mathbf{r}(0)$. As noted above the integral (2) calculates the area swept out on a sphere by the vector $\hat{\mathbf{e}}(s,s')$ as a function of s and s' considered as curvilinear coordinates on the sphere. Let us now place s in the interior of the polymer and s' on the extension of the polymer to infinity which is directed in the direction $\hat{\mathbf{e}}_z$. Consider, now, $s' = 0$. The curve $\hat{\mathbf{r}}(s) = \hat{\mathbf{e}}(s,0)$ is a spherical curve. As we now let s' vary from 0 to infinity we sweep out the area between $\hat{\mathbf{r}}$ and the point $\hat{\mathbf{e}}_z$. This spherical area can be written as

$$\chi_{end} = \hat{\mathbf{e}}_z . \int ds \, \frac{\hat{\mathbf{r}} \times d\hat{\mathbf{r}}/ds}{1 + \hat{\mathbf{r}}.\hat{\mathbf{e}}_z}. \tag{18}$$

A similar result has been found [18] by direct integration of equation (2). We are rather remarkably back to a problem in statistical mechanics which is very close that of the Fuller formulation of the writhe fluctuations. We convert equation (18) to spherical coordinates and find:

$$\chi_{end} = \int ds \, (1 - \cos\theta) \frac{d\phi}{ds} \tag{19}$$

The singularity at $1 + \hat{\mathbf{r}}.\hat{\mathbf{e}}_z = 0$ corresponds to the limit $\cos\theta = -1$.

Let us now make a hypothesis that the asymptotics are dominated by the largest contributions to the integrand then

$$\chi_{end} \sim \int ds \, \frac{d\phi}{ds} \tag{20}$$

which is the winding number of the polymer about $\hat{\mathbf{e}}_z$. The winding number of a random walk, W, about an infinite line scales as

$$W^2 \sim \log^2 (L/l_p) \tag{21}$$

where L is the length of chain and l_p the persistence length. The new Cauchy singularity appearing in this problem is regularized by the stiffness of the chain, l_p.

We guess that this is also the scaling behavior of the end correction of the writhe. To check this we generated, figure (3), a large number of random walks for a freely jointed chain. We then plot $\sqrt{\langle \chi^2_{end} \rangle}$ as a function of $\log L$ and look for a straight line. We conclude that the end corrections to the writhe are comparable to $\langle \chi^2_{end} \rangle = \log^2 (L/l_p)$. They are negligible compared with the internal contributions which vary as $\langle \chi^2_{int} \rangle \sim (L/l_p)$ for $L \gg \ell_p$.

Despite the similarity between equations (18) and (3) we find very different scaling for $\langle {\chi_F}^2 \rangle \sim L \log(1/\epsilon)$ and $\langle \chi^2_{end} \rangle \sim \log^2 (L/\ell_p)$. At first sight this might seem rather surprising, however in the Fuller formulation one averages over realizations of two dimensional random walks in the surface of the sphere whereas in equation (18) we average over *three* dimensional random walks *projected* onto a sphere. The statistical weights are different.

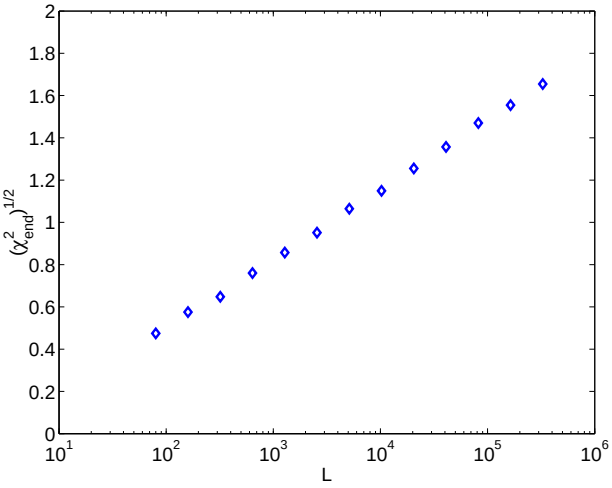

FIG. 3: Variation of end correction, $\sqrt{\langle \chi^2_{end} \rangle}$, with L showing scaling identical to that of the winding of a random walk about an infinite line. Freely jointed chains with up to 320,000 links.

V. RESULTS

A. Short Molecules, zero tension

For short filaments of length $L \sim \ell_p$ the distribution of writhe calculated with the extended Călugăreau-White formula and the Fuller formula can not be distinguished. It is only for filaments of length several times the persistence length that we see a difference between the curves. The writhe distribution of a open polymer with parallel tangents at each end is known in the limit $L/\ell_p \ll 1$; it is given by the Levy [12, 19] formula for the distribution of the area enclosed by random walk in a plane

$$P(\mathcal{W}r) = \frac{\pi \ell_p}{L} \frac{1}{\cosh^2 (2\pi \mathcal{W}r \, \ell_p / L)} \quad . \tag{22}$$

As a final test of the code we generated a large number of configurations of short filaments with $L/\ell_p = 1/2$ and used a binning procedure to find the distribution P. The results are shown in figure (4) where the numerical data is compared with the analytic fit. The agreement is excellent. Note that the curve of figure (4) is plotted on log-linear axes so that the tail of the distribution is exponential in the writhe. This is because the energy barrier needed to wind about $-\hat{\mathbf{e}}_z$ is too high to pick up the extremely weak Cauchy tail expected in this short system.

In numerical work it is not at all convenient to work with probability distributions: Plotting them involves an

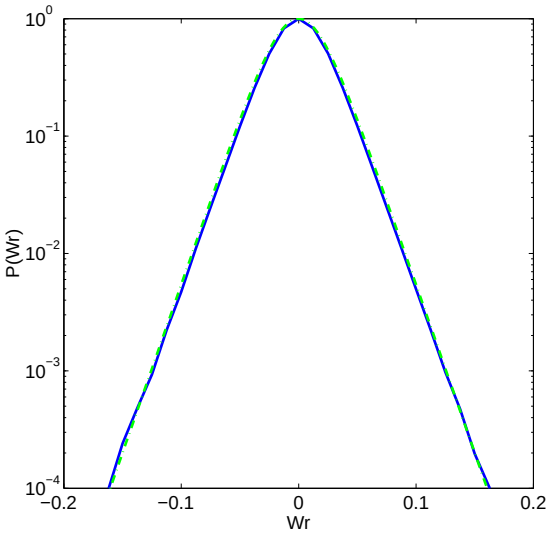

FIG. 4: Writhe distribution, $P(\mathcal{W}r)$ for a very short chain with $L = \ell_p/2$ calculated with the Fuller formula. The chain is formed from 50 links. Solid curve is a binned numerical result from 2×10^6 generated chains. Dashed curve is a plot of equation (22).

arbitrary choice in binning of the data. It is much more convenient to work with the integrated probability function $Q(x)$ defined as

$$Q(x) = \int_{-\infty}^{x} P(x')\,dx' \tag{23}$$

This function is particularly easy to generate from an ensemble of numerical results: For all the distributions that we discuss here $Q(x)$ converges to 0 as $x \to -\infty$ and to 1 as $x \to +\infty$. We shall deduce the asymptotic behavior of P from the analytic form of Q at these two points.

In order to measure the asymptotic distribution of the writhe we have performed simulations on a series of chains of length $L = 8\ell_p$. With chains of this length knots remain rather rare whilst the energetic barrier needed for a chain oriented in the direction $\hat{\mathbf{e}}_z$ to wind about the direction $-\hat{\mathbf{e}}_z$ is only a few k_BT. We are thus sensitive to the winding singularizes of the Fuller formulation. In our simulations we vary the discretization so that there are $N = 10, 30, 100, 300, 900, 2700$ links per persistence length and we generate 200000 independent configurations. The raw data is plotted in figure (5). There is an continuous evolution of the writhe with N when calculated with the Fuller formula, but the Călugăreau-White form converges to a stable value for quite moderate values of N.

B. Long tense molecules

Until now we have ignored the effect of tension on the configuration of the DNA, except to remark that even

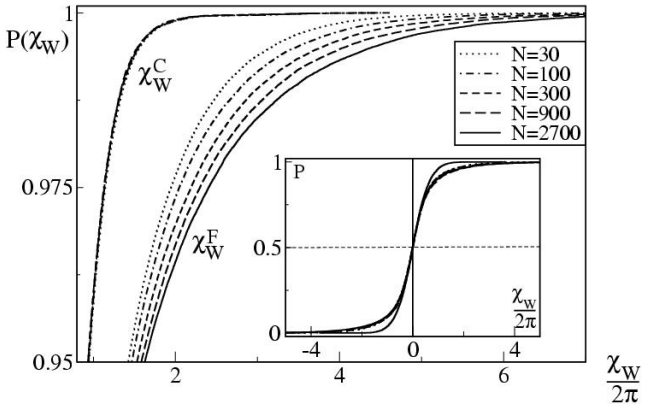

FIG. 5: Inset: The integrated writhe distribution function $Q(\chi)$ computed with two different formulations of the writhe. χ_C not computed for $N = 2700$ due to computer limitations. The main figure is a zoom of the top right corner of the inset. Compare the continuous evolution of χ_F as N grows with the stability of χ_C in the same limit.

very low tensions justify the use of a conserved linking number in the interpretation of the experiments. In this section we indicate how the writhe of DNA varies in the presence of external forces.

When a molecule is under high tension the molecule is largely aligned parallel to the external force. We can use a simplified, quadratic form for the Hamiltonian [3]

$$E = \frac{1}{2}\int \left\{ \mathcal{K}\,(\partial \mathbf{t}_\perp)^2 + \Gamma \mathbf{t}_\perp \wedge \dot{\mathbf{t}}_\perp + f\mathbf{t}_\perp^2 \right\} \quad ds \tag{24}$$

where $\mathbf{t}_\perp$ describes the transverse fluctuations in the direction of the molecule. We can find the mean squared writhing angle using the usual methods of equilibrium statistical mechanics.

$$\langle \chi_F^2 \rangle = \frac{\partial^2 \log \mathcal{Z}}{\partial\,(\beta\Gamma)^2} \tag{25}$$

A short calculation gives

$$\frac{\langle \chi_F^2 \rangle}{L} = \frac{1}{4}\sqrt{\frac{k_BT}{\ell_p^3 f}} \tag{26}$$

This expression is only valid for forces such that $f\ell_p/k_BT > 1$. To estimate the writhe of a molecule at low forces we return to the remark above that the internal contribution to the writhe is dominated by structure occurring at the scale ℓ_p. It is known that under low tensile forces the structure of a polymer is unchanged out to a length scale $\ell_f = k_BT/f$. We conclude that under low tension the typical writhe of a molecule becomes independent of its degree of elongation. It is only under the highest forces when the semiflexible nature of the molecule is sampled that we see an evolution of the writhe with force.

To validate our code for generating tense molecules we performed a series of simulations with a chain of persistence length $N = 250$, fig (6) using the Fuller expression

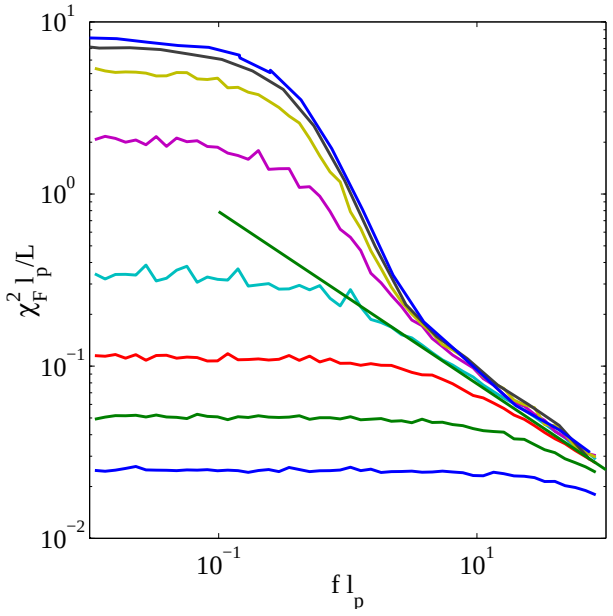

FIG. 6: Log-Log plot of $\chi_F{}^2\ell_p/L$ as a function of the scaled tension. At large forces the result converges to eq. (26). At low tensions the writhe is independent of the force. Simulations for $\ell_p = 250$. Curves for $L = 60$, 120, 250, 500, 1000, 2000, 4000, 8000 (bottom to top). The straight line is eq. (26).

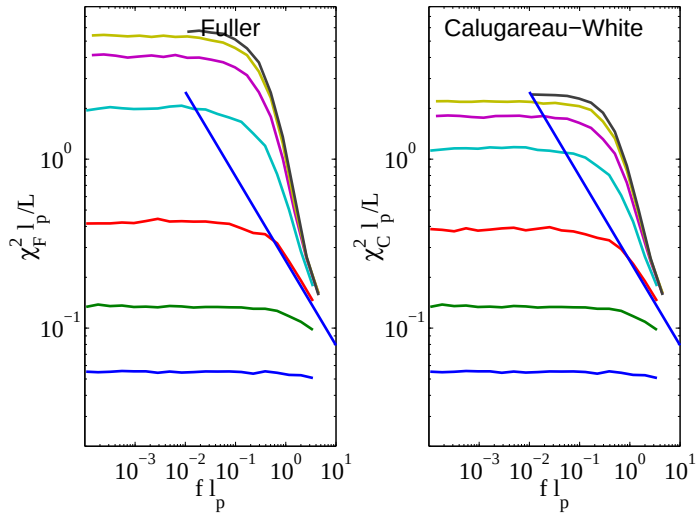

FIG. 7: Log-Log plot of $\chi_F{}^2\ell_p/L$ (left) and $\chi_C{}^2\ell_p/L$ (right) as a function of the scaled tension. For longer chains the expected regime in $1/\sqrt{f}$ is hidden by a large shoulder from the crossover to the low force regime. Simulations for $\ell_p = 15$. Curves for $L = 7$, 15, 30, 60, 120, 250 (bottom to top). The straight line is eq. (26).

for the writhe. At high forces we see that the curves converge towards the law eq. (26) and that at low forces the curves saturate. Somewhat surprising however is the rapid crossover which occurs for forces comparable to $f \sim 1/\ell_p$. In the neighbourhood of this force there is a rapid crossover between the low and high force regimes producing a pronounced "shoulder" on the curve. It it so be noted that convergence to the long chain limit is rather slow. It is not until $L \sim 60\ell_p$ that we see a saturation in writhing curves. This slow convergence can be understood rather easily by noting that any chain within ℓ_p of the surface of the polymer coil is in a region of lower than average density. One expects convergence to the long chain limit only when $R_g^3 \gg R_g^2\ell_p$ and thus relative corrections which decrease as only $\sqrt{\ell_p/L}$.

We have performed a series of simulations on different levels of discretization of the polymers. We find that at the discretization becomes coarser the large shoulder dominates over the law in $1/\sqrt{f}$ for the mean sqaure writhe. This is illustrated in fig. (7) where we use 15 link per persistence length for the discret chain. This is not too far from the optimal fit of Bouchiat *et al.* for DNA. The chain of length $L = \ell_p$ still displays a small regime in agreement with eq. (26). However with longer chains the regime in $1/\sqrt{f}$ is overwhelmed by the crossover to the low tension regime. Eq. (26) substantially underestimates the the writhe fluctuations in the domain where one would expect it to apply. In fig. (7) we have plotted both the writhe due to the Fuller expression and due to the link sector restricted Călugăreau-White formula. We see that at low forces the results are, as expected rather different. The shoulder is somewhat less marked in the Călugăreau-White formulation but the simple prediction of $\chi^2 \sim 1/\sqrt{f}$ is still masked by the crossover.

VI. CONCLUSIONS

In this paper we have shown that the standard experimental geometry does not torsionally confine a DNA strand so that under torque we expect an series of equivalent low energy states separated by a potential barrier. For the usual bead sizes and forces used in the experiments this barrier is very high and the extended linking number is conserved. This allows the use of an extended Călugăreau-White formalism in the calculation of the bead rotations. We find that the Fuller and Călugăreau-White formulas give substantially different distribution functions for the torsional fluctuations due to writhe. In contrast to Bouchiat *et al.* we find that the topologically confined DNA chain does not need an intermediate scale cut off to render the response functions finite.

Experimentally we thus expect three distinct regimes when working with beads of size $d > \ell_p$. For very low forces, $f < k_BT/d$ torsional fluctuations are unbounded and it is not possible to define the torsion-force-extension characteristics. In the regime $k_BT/d < f < k_BT/\ell_p$ tor-

sional fluctuations are bounded but must be calculated using the full double integral representation of the writhe. The Fuller formulation, even with an additional cut off, substantially overestimates the torsional response. For forces larger than $k_B T/\ell_p$ a simple theory based on a Monge representation expanded to quadratic order [20] is unable to fit the data due to strong corrections to scaling.

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