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DNA loop statistics and torsional modulus

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Abstract. – The modelling of DNA mechanics under external constraints is discussed. Two analytical models are widely known, but disagree for instance on the value of the torsional modulus. The origin of this embarrassing situation is located in the concept of *writhe*. This letter presents a unified model for DNA establishing a relation between the different approaches. I show that the writhe created by the loops of DNA is at the origin of the discrepancy. To take this into account, I propose a new treatment of loop statistics based on numerical simulations using the most general formula for the writhe, and on analytic calculations with only one fit parameter. One can then compute the value of the torsional modulus of DNA without the need of any cut-off.

General motivations. – New experimental techniques in single molecule manipulation of DNA and supercoiling control have stimulated improvements in the understanding of DNA mechanics [1–4]. Surprisingly, the measurements have to be interpreted through a rather sophisticated model in order to extract physical constants [5–7]. Different approaches lead to disagreeing values of, in particular, the torsional modulus C of the molecule along its axis. The method used by Moroz and Nelson [6] leads to the value $C/k_B T = 109$ nm, while using the same experimental data the model provided by Mézard and Bouchiat [7] gives 84 ± 10 nm. Recently, another experiment performed two direct measurements [1] with a weighted average of 102 ± 6 nm. In this letter, I will establish the domains of validity of the two mentioned theoretical approaches, clarify the origin of their disagreement and compute a value of the torsional constant C with a new model.

This letter is organized as follows: It starts with a short introduction to two widely used models for elasticity of a polymer. In order to take into account DNA resistance to torsion, one of them, *worm-like chain* model, is improved with a new elastic energy term. As these models only contain a *local* description of the molecule, the following paragraph introduces the global geometrical description of a single DNA molecule. Its three dimensional coiling is described by a quantity called *writhe*, whose fluctuations reduce the effective torsional modulus. I relate

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the models of Ref. [6] and [7] and study their domains of validity. The writhe fluctuations are interpreted in the following paragraph by considering the loops formed by the molecule. The last paragraph describes both a full numerical computation of the writhe fluctuations and a one parameter fit for the interpretation in terms of loops. The different theoretical models are also compared to this new treatment of the writhe, and a value of the torsional modulus is computed.

I will use the notations: $\beta = 1/k_B T$, $F = f k_B T$ the force exerted on the ends of the molecule and θ the angle of a vector with the direction of the force, L the length of the molecule, $A = \ell_p k_B T$ the bending modulus, $C = \ell_t k_B T$ the torsional modulus, $\Gamma = \gamma k_B T$ the torque exerted on the free end of the molecule, z its vertical extension and χ the rotation angle of the free end. The torsion $\omega(s)$ of the DNA molecule is the difference per unit length of the rotation angle of one strand around the other with respect to the unconstrained state in the same conformation. It is a function of the arc length s and can be seen as “twist angle density”: The torsion integrated along the molecule is called *twist angle* and is noted Ω .

DNA elasticity models. – Thanks to its double helix structure, DNA is a very stable polymer. This stability is needed to conserve genetic materials. It gives opportunity to submit a single molecule to mechanical constraints (forces above 0.04 pN) without destroying it and to measure its response. The extension z of a single molecule submitted to a force $F = f k_B T$ is the first quantity that has been studied. It has to be interpreted within a polymer elasticity model.

The *freely jointed chain* (FJC) describes a polymer as a succession of independent sticks of length b . For one stick the elementary partition function is

$$\mathfrak{z} = \int_{-1}^1 \exp(-fb \cos \theta) d(\cos \theta) = \frac{\sinh fb}{fb}. \quad (1)$$

For a whole chain, the free energy is thus $\mathcal{F}_{\text{FJC}} = \frac{k_B T L}{b} \ln \mathfrak{z}$, leading to a relative extension $z/L = fb/3$ when f tends to zero, and $z/L = 1 - (fb)^{-1}$ for large forces.

The *worm-like chain* is on contrary a continuous model based on the bending energy of the axis. The state variable is the tangent unit vector, described by its angle θ with the force, thus the phase space is made of functions $\theta(s)$. The resistance of the molecule to bending creates a correlation between tangent vectors along a curvilinear distance $\ell_p \simeq 50$ nm called the *persistence length*. The Hamiltonian for this model is obtained by analogy with a quantum system (see Ref. [7]):

$$\beta \hat{H}_{\text{worm}}(f \ell_p) = -\frac{1}{2 \sin \theta} \frac{\partial}{\partial \theta} \sin \theta \frac{\partial}{\partial \theta} - f \ell_p \cos \theta. \quad (2)$$

When f tends to zero the worm-like chain extension is $z/L = \frac{2}{3} f \ell_p$. In the large force approximation, the molecule is almost aligned with the force ($\theta \ll 1$) and the Hamiltonian (2) reduces to an harmonic oscillator whose ground state gives the free energy [8]

$$\beta \mathcal{F}_{\text{worm}} = \left(-f \ell_p + \sqrt{f \ell_p} \right) \frac{L}{\ell_p} \quad (f \ell_p \gg 1). \quad (3)$$

From $\beta \partial \mathcal{F}_{\text{worm}} / \partial (f \ell_p) = -z / \ell_p$, when $f \ell_p \gg 1$, one deduces

$$1 - \frac{z}{L} \simeq \frac{1}{2 \sqrt{f \ell_p}} \quad (f \ell_p \gg 1). \quad (4)$$

I now compare the two models. At low force, entropy dominates and the models both describe an object following Hooke's law. Requiring the relation $b = 2\ell_p$ between their parameters makes them equivalent. This is known to be a good approximation when $f\ell_p = fb/2 \leq 1$ or in other terms $F \leq 0.08$ pN. On the contrary at large force, the two models do not have the same asymptotic behaviour. Comparison between experimental data and theoretical models indicate that the worm-like chain provides a more appropriate description of DNA elasticity [2].

The double helix structure of DNA has also for consequence a resistance to torsion along its axis. Mézard and Bouchiat [7] showed that taking into account the local torsion of DNA introduces new elastic terms in the Hamiltonian (2), proportional to γ^2 : A term due to local torsion energy, $\beta\hat{H}_{\text{torsion}} = -\frac{1}{2}\gamma^2 L/\ell_t$, and a term related to the geometry of the molecule

$$\beta\hat{H}_{\text{rod}}(f\ell_p, \gamma) = \beta\hat{H}_{\text{worm}}(f\ell_p) + \beta\hat{H}_{\text{torsion}} - \frac{\gamma^2}{2} \frac{1 - \cos\theta}{1 + \cos\theta}. \quad (5)$$

This improved model is called the *rod-like chain*. In the large force approximation, the denominator $1 + \cos\theta \simeq 2$, thus to second order in θ the Hamiltonian rewrites $\beta\hat{H}_{\text{rod}}(f\ell_p, g) \simeq \beta\hat{H}_{\text{worm}}(f\ell_p - \gamma^2/4) + \beta\hat{H}_{\text{torsion}} - \gamma^2/4$. This approximation is valid when θ is small, thus in a domain where the relation (3) is correct. One deduces the expression of the free energy of the rod-like chain in the large force regime:

$$\beta\mathcal{F}_{\text{rod}}(f\ell_p, \gamma) = \beta\mathcal{F}_{\text{worm}}(f\ell_p - \gamma^2/4) - \frac{1}{2}\gamma^2 \left(\frac{L}{\ell_t} + \frac{L}{2\ell_p} \right). \quad (6)$$

The writhe and its fluctuations. – Experimental devices also allow to change the angle χ , and to measure how the molecule responds to this constraint [3]. One says that the molecule is *supercoiled*. Supercoiling is quantified by the writhing angle of the DNA axis \mathcal{C} [9,10]:

$$\Phi = \frac{1}{2} \oint_{\mathcal{C}} d\mathbf{r} \cdot \oint_{\mathcal{C}} d\mathbf{r}' \times \frac{\mathbf{r} - \mathbf{r}'}{\|\mathbf{r} - \mathbf{r}'\|^3}. \quad (7)$$

This expression is *a priori* valid only for *closed* chains. However, in experimental devices the molecule can not go around its ends [4], which is equivalent to having a very long molecule only manipulated in a small region. The molecule axis can therefore be imaginarily closed and the formula (7) extends to open chains in experimental conditions [11]. Therefore, the fundamental geometrical relation between χ , Ω and Φ [12],

$$\chi = \Omega + \Phi, \quad (8)$$

is exact in experimental conditions. Formula (8) is widely known by DNA specialists, in biology and physics [10]. It explains that a DNA molecule has two ways to deal with an applied torque : to modify its *local torsion* (modify Ω) or to change its shape (modify Φ).

The twist angle Ω is only related to the torsion. The writhe angle Φ , as it is computed from the shape of the axis, is only related to the curvature. A general study of DNA elasticity performed by Marko and Siggia [8] asserts that a coupling between bending and torsion is at least of the third order in strains, and the estimated coupling constant is small [6]. As one wants to focus on regimes close to relaxed state, one shall neglect this coupling and assume that there are no correlations between Ω and Φ . (The same assumption has been made in Ref. [6,7].) Eq. (8) then gives the fluctuations of χ in this approximation:

$$\langle \chi^2 \rangle = \langle \Omega^2 \rangle + \langle \Phi^2 \rangle. \quad (9)$$

The fluctuations of Ω , thanks to the locality of torsional energy and independance of \hat{H}_{torsion} with θ , are proportional to the length L , $\langle \Omega^2 \rangle = L/\ell_t$. If one measures the torsional modulus of a molecule only taking the angle χ into account, one obtains an *effective* value, denoted $k_{\text{B}}T\ell_t^{\text{eff}}$, related to the real torsional constant, $k_{\text{B}}T\ell_t$, through the fluctuations of the writhing angle:

$$\frac{L}{\ell_t^{\text{eff}}} = \frac{L}{\ell_t} + \langle \Phi^2 \rangle. \quad (10)$$

The effect of writhing of the molecule is then to reduce the measured value of the torsional modulus between its ends. To deduce the value of C *along the molecule axis* from measurements, one needs to know the writhe fluctuations.

For the rod-like chain model developed in the preceding section, one remarks that $\langle \chi^2 \rangle$ is deduced from \mathcal{F}_{rod} , Eq. (6), by differentiation $-2\beta\partial\mathcal{F}_{\text{rod}}/\partial(\gamma^2)|_{\gamma=0} = \langle \chi^2 \rangle$, so

$$\langle \Phi^2 \rangle = \frac{1}{2} \left(1 - \frac{z}{L}\right) \frac{L}{\ell_p} \quad (\theta \ll 1). \quad (11)$$

This result, combined with relation (4), was first given by Moroz and Nelson [6] as a correction like in Eq. (10), when the force is large enough:

$$\langle \Phi^2 \rangle = \frac{1}{4\sqrt{f\ell_p}} \frac{L}{\ell_p}. \quad (12)$$

Their model is therefore an approximation of the more general rod-like chain model described by Mézard and Bouchiat. Let us now evaluate the minimum force on their common validity range. Expression (11) is obtained when the molecule is almost straight, namely when $f\ell_p \gg 1$. That might be translated into physical units to $F \gg 0.1$ pN, thus for forces of the order of 1 pN.

While Mézard and Bouchiat's approach is more general, it has been pointed out that it suffers from a pathology related to the writhe formulation [13]. More precisely, the expression for the writhe used in Ref. [7],

$$\int (1 - \cos \theta(s)) d\phi(s), \quad (13)$$

is equal to formula (7) modulo 4π (ϕ is the azimuthal angle of the tangent vector). They are equal only when the molecule can be straightened out without cutting it, nor having a point passing through $\theta = \pi$ at any time. Otherwise the formulæ differ by a multiple of 4π [14, 15]. When the molecule is deformed into a blob, such configurations where the formulæ disagree are numerous. Typically, in this case the molecule has *loops*. The following section is dedicated to an estimation of the number of loops in order to extend the validity range of formula (11).

Estimation of the loops contribution. – Each loop contributes to the writhing angle Φ by an amount of the order of one turn. I will note Δ^2 the mean square value of a loop contribution. This quantity will be numerically estimated in the next section. Under the assumption that loops are uncorrelated, the central limit theorem asserts that the contribution of loops to $\langle \Phi^2 \rangle$ is $n(f\ell_p)\Delta^2$, where $n(f\ell_p)$ is the average number of loops for $f\ell_p$ fixed. In Ref. [6], a treatment of loops was proposed, but only equilibrium loops were considered. This approach does not explicitly provide any loop size. Since the length of one stick being $2\ell_p$, the model avoids the smaller loops, that require much bending energy.

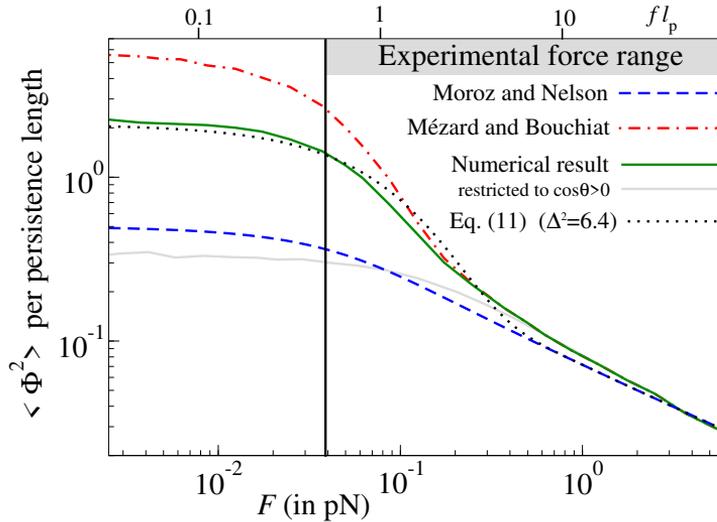


Fig. 1 – Writhe angle fluctuations as a function of the tension. The experimental force range is indicated by the grey bar. The plain line presents the results of the present letter model, compared to modified Moroz and Nelson’s result $\langle \Phi^2 \rangle \ell_p/L = (1 - z/L)/2$ (dashed line) and Mézard and Bouchiat’s (dashed-dotted line). Moroz and Nelson’s result is valid only for large forces ($F > 1$ pN), validity range of Mézard and Bouchiat’s is wider but fails for the lowest experimental force values, around $F = 0.2$ pN. The estimated contribution of loops is displayed as a dotted line (see text). It corresponds to the best fit for Δ^2 in Eq. (15) [16]. The grey line is obtained from the same data set, but after removal of all configurations that have backward facing regions ; therefore there are no loops in this restricted ensemble. The results of Mézard and Bouchiat’s model and the one presented in this letter have been obtained numerically, from equilibrated sets of 50.000 chains of $32 \ell_p$ for each point, with discretisation $\ell_p/30$, error bars are thinner than the line thickness. The two other curves are analytical expressions.

The average number of loops is estimated here by defining a loop as a region where $\cos \theta < 0$, which gives, using Eq. (1) and $b = 2\ell_p$,

$$n(f\ell_p) = \frac{L}{b} \frac{1}{3} \int_{-1}^0 \exp(-fb \cos \theta) d(\cos \theta) \underset{f \rightarrow 0}{\simeq} \frac{L}{4\ell_p} \exp(-f\ell_p). \quad (14)$$

Thus one obtains an estimate for $\langle \Phi^2 \rangle$ in an extended force range by adding the contribution of the loops (regions where $\cos \theta < 0$), given by the preceding formula, to the one of the other regions (where it is supposed that $\theta \ll 1$), given by the expression (11):

$$\langle \Phi^2 \rangle \simeq \frac{1}{2} \left(1 - \frac{z}{L}\right) \frac{L}{\ell_p} + \frac{1}{4} \Delta^2 \exp(-f\ell_p) \frac{L}{\ell_p} \quad (f\ell_p \leq 1). \quad (15)$$

Adding these contributions is allowed here thanks to Fuller’s formula for writhe change during a deformation with fixed boundary conditions (see Ref. [14]). When $f\ell_p$ is small, the last term of Eq. (15) becomes dominant: The writhe is dominated by the loops contribution. Δ^2 is computed in the next section.

Numerical results. – A Monte-Carlo simulation of semiflexible chains was performed (described in details in Ref. [11]) and the writhe angle of each configuration of the simulated

equilibrated ensemble was computed, with both expressions (7) and (13). It has been shown numerically that extra closure terms are negligible [11]. Results are displayed in figure 1. The estimate of Eq. (15) is also displayed for the best value of $\Delta^2 = 6.4$ (one could conjecture that the geometric exact value be 2π). The validity ranges estimated above appear reasonable, and the domains where different model agree are clearly observed. The distribution of writhe angle is *Gaussian*, of width proportional to L/ℓ_p and centered around zero. The estimated loops contribution formula agrees quite well with the results and explains the difference to other models: It is not counted in one case and overestimated in the other one [16]. If one removes the curves that have at least one backfacing region, which corresponds to the definition of a loop (see Eq. (14)), one gets a curve similar to Moroz and Nelson's. The three models agree for $F \geq 1$ pN, so the origin of model disagreement is located at low force.

The numerical results for the writhe angle displayed in figure 1 combined with the experimental data of references [3, 4] obtained at low force, in the elastic regime, give the value

$$\ell_t = C/k_B T = 93 \pm 10 \text{ nm.}$$

This value has to be compared with the ones given in the introduction. The theory of Ref. [7] was applied for forces below this value, in a domain where it overestimates the writhe fluctuations. The value obtained by Moroz and Nelson is the closest to the recent value of Ref. [1]. It was obtained by eliminating data points obtained with a force lower than 0.3 pN therefore in a domain where all models are equivalent. It is suggested in Ref. [1] that applying a large force could lead to an overestimated value for C because of structural modifications of the double helix. This question is still open until now.

Discussion. – Unlike Mézard and Bouchiat's theory, neither Moroz and Nelson's model nor this work needs to be regularized by any cut-off. The value of this cut-off is difficult to relate to an independently measurable quantity. It is suggested in Ref. [6] that a treatment of self-avoidance could be necessary.

In the low force regime, where the freely jointed chain model is valid for DNA, let us consider one molecule as a chain of sticks of length $b = 2\ell_p$ and diameter $d = 2$ nm. The molecule is located in a region of size $R \sim \sqrt{Lb}$. In the low force regime, the sticks have almost random orientations, so excluded volume interactions can be estimated with the second virial coefficient. It follows that excluded volume interactions appear when $\rho^2 R^3 b^2 d \gtrsim 1$ where $\rho = (L/b)R^{-3}$ is the density of sticks. This gives $L/b \gtrsim (b/d)^2 = 2500$. The length of the widely used λ -phage DNA is $16 \mu\text{m} \simeq 300\ell_p$ [3]. For larger forces, the molecule gets an orientation and self-avoidance effects are still smaller. It is established in Ref. [5] that self-avoidance effects can be neglected as long as $z/L \gtrsim 0.25$. This argument does not apply as is in the present study since DNA stores torsion. It is known, for example in the case of a plectoneme, that self-avoidance stabilizes some torque constrained configurations. I focused on the elastic regime, where the torque is small and in presence of a small but non-zero force, then applying a torque diminishes z/L . In this situation, the assumption that has been made consists in considering self-avoidance negligible when $\gamma \rightarrow 0$, or quantitatively when $z/L > 0.25$. As a consequence of self-avoidance the statistical weight of conformations with large writhe increases, in other words Δ^2 is underestimated in our model. Consequently, taking into account self-avoidance effects would result in a small increase of the value of C given above.

In the numerical work, self-avoidance was not taken into account, following the considerations of the preceding paragraph. As a consequence, the studied ensemble contains knotted configurations. I showed from the same numerical simulations that a $8\ell_p$ long worm-like chain has a probability of $(5 \pm 1) 10^{-4}$ to knot [11]. For a force of 0.02 pN the polymer statistic can

be split into independent elastic blobs of size $8\ell_p$. Then the probability to have a knot in at least one blob is around 2.10^{-2} for a $16\ \mu\text{m}$ DNA molecule. If the force is higher, the knot probability decreases, as blobs become smaller, and is negligible in the experimental force range. Knotted configurations are then believed to play no role in the experimental force range.

In this work, I have investigated the connections between different DNA models and showed that Moroz and Nelson's model and Mézard and Bouchiat's are related in a simple way, which depends on how DNA loops are taken into account. The estimates of the writhe fluctuations of the molecule in those models have been compared to numerical results. I have shown that the differences between those models are due to the absence (in Moroz and Nelson's model) or the overestimate (in Mézard and Bouchiat's model) of the contribution of the molecule loops to the writhe. This model takes more accurately writhe fluctuations into account without introducing any cut-off. It also provides a reasonable value for the torsional modulus of DNA under small constraints.

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- [16] The fit for Δ^2 with Mézard and Bouchiat's curve is much worse (not shown).