I. INTRODUCTION

At mesoscopic length scales the elastic response of double stranded DNA to mechanical stresses is usually described by the twistable worm like chain (TWLC), which is characterized by just two elastic stiffnesses corresponding to bend and twist deformations respectively. At length scales of several base pairs, relevant to protein-DNA interactions, the sequence dependent geometry of the double helix leads to a rich spectrum of elastic properties. Even at those length scales, however, it is useful to employ simplified, analytically treatable representations that allow for the identification of generic features that are otherwise masked by sequence dependent variations. A suitable model that takes into account the general features of DNA geometry while still fulfilling the requirement of analytical treatability was put forward in the mid-90’s by Marko and Siggia, who derived it from the analysis of the molecular structure of DNA. These authors showed that the asymmetry between major and minor grooves generates a coupling between bend and twist, supplementing the TWLC with an additional stiffness parameter. Symmetry implies the existence of twist-bend coupling, but it does not yield any information about the magnitude of the associated coupling constant. For a long time, the effect of twist-bend coupling has been ignored, assuming that such coupling would have a minor influence on DNA elastic properties. However, a recent comparative analysis of two very similar coarse-grained DNA models one with symmetric grooves and one with asymmetric groove has shown that the twist-bend coupling constant is comparable in magnitude to the other elastic parameters describing bending and torsional stiffness. A similar conclusion was drawn from the analysis of all atom simulations of DNA.

Twist-bend coupling has been shown to influence elastic properties of DNA both at long and short molecules. For instance, in ≈ 100 base pairs bent DNA fragment twist-bend coupling induces sinusoidal standing waves in the twist. These waves are comparable in shape and magnitude to those observed in nucleosomal DNA, which is wrapped around histone proteins. In longer kilobase pair DNA subject to a stretching force and to a torque, as in single molecule experiments, twist-bend coupling has been shown to lead to a rescaling of the elastic parameter.

The aim of this paper is to focus on minimal energy shapes of DNA minicircles of about $10^2$ base pairs which are overtwisted. We show, by combining analytical and numerical results, that twist-bend coupling induces distinctive shapes of DNA in which the curvature is periodically modulated in an alternance of high and low bending regions. The periodicity is close to that of a straight double helix, but depends on the degree of overtwisting. The resulting shape resembles that of a rounded polygon. We estimate the elastic energy of such a polygon and show that twist elasticity is governed by a rescaled torsional stiffness, rather than the bare one and discuss the consequences of these results for experiments involving minicircles.

II. DNA ELASTICITY

We briefly recall the formalism used to describe a twistable polymer such as DNA (see also e.g. for more details) and review some properties of the model with twist-bend coupling.

Figure 1. The DNA molecule is modeled as a continuum elastic rod in each point of which an orthonormal frame is attached. $e_1$ is tangent to the curve while $e_1$ and $e_2$ lie on the plane of the ideal planar Watson-Crick bases. In the right panel, the rotation around $e_2$ corresponds to a positive $\Omega_2$ (i.e. a bend towards the major groove).
focusing on the bending and torsional persistence lengths. The
collection of an inextensible twistable elastic rod can be
parametrized by three strain fields $\Omega_1(s)$, $\Omega_2(s)$ and $\Omega_3(s)$,
where $0 \leq s \leq L$ is a curvilinear coordinate and $L$ the total
length of DNA. $\Omega_1$ and $\Omega_2$ are bending densities also
referred to as “tilt” and “roll” deformations, while $\Omega_3$ is the excess
twist density. DNA has an intrinsic twist density $\Omega_0 = 2\pi \lambda_0 \approx 1.85$ nm$^{-1}$, with $\lambda_0 \approx 3.4$ nm
corresponding to the distance of one helical turn. Given the $\Omega_i(s)$
the three dimensional shape of the molecule can be obtained by solving
the differential equations $i = 1, 2, 3$:

$$\frac{d\hat{e}_i}{ds} = [\Omega_1 \hat{e}_1 + \Omega_2 \hat{e}_2 + (\Omega_3 + \Omega_0) \hat{e}_3] \times \hat{e}_i,$$ (1)

where $\{\hat{e}_1(s), \hat{e}_2(s), \hat{e}_3(s)\}$ defines an orthonormal triad (the
Darboux frame) where $\hat{e}_3$ is tangent to the curve, while $\hat{e}_1$ and $\hat{e}_2$
lie on the plane of the ideal, planar Watson-Crick base pairs [2], see Fig. 1.

By convention $\hat{e}_1$ is directed along the symmetry axis of the two grooves and points in the direction
of the major groove. Finally $\hat{e}_2 = \hat{e}_1 \times \hat{e}_3$, which yields to a vector
connecting the two DNA backbone [13]. The conformation with all $\Omega_i = 0$ describes a straight twisted rod with an
intrinsic twist density $\Omega_0$.

From the analysis of the molecular symmetry of DNA,
Marko and Siggia [14,15] derived the following free energy
functional to lowest order in $\Omega_i$:

$$\beta E = \frac{1}{2} \int_0^L \left( A_1 \Omega_1^2 + A_2 \Omega_2^2 + C \Omega_3^2 + 2G \Omega_2 \Omega_3 \right) ds,$$ (2)

where $\beta = 1/k_B T$ is the inverse temperature, and $A_1$, $A_2$, $C$ and $G$
is the stiffness parameters. The last term in (2) couples twist ($\Omega_3$) with the bending towards the DNA grooves ($\Omega_2$).
The case of vanishing twist-bend coupling constant $G = 0$
one recovers the twistable wormlike chain (TWLC) which is
the commonly employed model to describe DNA elasticity.
In this paper we focus in particular on effects of twist-bend
interactions. In the case of vanishing twist-bend coupling constant $G = 0$
one recovers the twistable wormlike chain (TWLC) which is
the commonly employed model to describe DNA elasticity.
In this paper we focus in particular on effects of twist-bend
coupling on the minimal energy shapes of bent DNA. Before
entering into the details we recall in this Section some proper-
ties of the model [2], which differ from those of the standard
TWLC.

The bending persistence length $l_b$ of the model [2] has been
computed from the decay of the tangent-tangent correlation and it is given by [13] the following relation

$$\frac{1}{l_b} = \frac{1}{2} \left( \frac{1}{A_1} + \frac{1}{A_2} \right),$$ (3)

where

$$\tilde{A}_2 = A_2 \left( 1 - \frac{G^2}{A_2 C} \right)$$ (4)

can be viewed as a rescaled bending stiffness along the "easy"
bending axis (recall that $A_2 \ll A_1$). In the isotropic TWLC
limit $A_1 = A_2 = A$ and $G = 0$ Eq. (3) reduces to the well-
know results $l_b = A$, i.e. $A$ is both the bending stiffness and the
bending persistence length. In the anisotropic case $A_1 \neq A_2$
and $G = 0$, Eq. (3) shows that $l_b$ is the harmonic mean of $A_1$
and $A_2$, a known result, see [14,15]. In the general case $G \neq 0$
the result can be cast in the form of a harmonic mean of $A_1$
and $A_2$. From the analysis of the twist correlations one can
calculate the twist correlation length $C$ and finds $l_t = 2 \tilde{C}$ with

$$\tilde{C} = C \left( 1 - \frac{G^2}{A_2 C} \right),$$ (5)

which reduces to the well-known result $l_t = 2 \tilde{C}$ in the TWLC
limit $G = 0$. Equations (3) and (5) are exact and have been obtained
from the analysis of thermal fluctuations of the model [2]. These quantities are also relevant in minimal energy DNA
conformations that are permanently bent and twisted, as we
will show in the next Sections.

III. DNA MINICIRCLES

A considerable amount of studies have been devoted to the
analysis of equilibrium and kinetic properties of DNA
microrings [16–26] or to short DNA loops obtained by bend-
ing two ends of DNA [27,28]. Our aim is to describe the effect
that twist-bend coupling has on the minimal energy shape of
overwound minicircles. We employ a homogeneous model
[2], neglecting sequence-dependent variations of the elastic-
ity, which we expect to be valid when averaging over different
sequences. In a minicircle, the two end of the double helix are
covaletly sealed and in order for the loop to close one usually
needs to over or underwind the molecule so that the end point
meet in phase. An excess linking number can also be induced
by using appropriate enzymes that overwist the molecule.
In what follows we discuss separately the torsionally relaxed
and the over and under-twisted cases.

A. Torsionally relaxed minicircles

As this case has been discussed recently in [10] we just briefly
review it here. An analytical shape for anistropic bending
stiffness and $G \neq 0$ cannot be found easily. The constraint to
be imposed to form circular DNA requires that the end points
of the strands meet each other smoothly and that the tangent
vector is continuous. This constraint is typically expressed us-
ing lab-frame quantities, as Euler angles describing the con-
figuration of the DNA with respect to some fixed axes. The
minimal energy shapes could then be calculated numerically.
Here, however, we employ an approximation that allows us
to perform a local energy minimization, expressing the con-
straint in terms of $\Omega_3$. We assume that the exact solution is
a small perturbation of a perfect circle and that the solution
is periodic over helical repeats.

The vector $\hat{\Omega}_b \equiv \hat{\Omega}_1 x_1 + \hat{\Omega}_2 x_2$ is the bending density. Given
a fixed unit vector $\hat{x}$ (see Fig. 1), Ref. [10] introduced the follow-
ing local constraint $-\mu \hat{\Omega}_b \cdot \hat{x}$, with $\mu$ a Lagrange multiplier.
This term favors the alignment of $\hat{\Omega}_b$ along $\hat{x}$, i.e. it forces
the molecule to be bent and to remain close to a plane or-
thogonal to $\hat{x}$ (see Fig. 1). For a straight double helix in the
plane orthogonal to $\hat{x}$ and with constant twist density equal
to \( \omega_1 \), one can choose the curvilinear coordinate such that \( \hat{x} = \sin(\omega_0 s) \hat{e}_1 + \cos(\omega_0 s) \hat{e}_2 \). This relation remains approximately valid if within one helical turn bending is weak and local twist variations are small, conditions which can be respectively expressed as \( |\Omega_0| \ll \omega_1 \) and \( |\Omega_1| \ll \omega_1 \). Within this approximation the constraint takes the following form:

\[
\beta \hat{E} = \beta E - \mu \int_0^L \tilde{\Omega}_b \cdot \hat{x} \, ds
\]

\[
= \beta E - \mu \int_0^L \left[ \Omega_1 \hat{e}_1 + \Omega_2 \hat{e}_2 \right] \cdot \hat{x} \, ds
\]

\[
\approx \beta E - \mu \int_0^L \left[ \Omega_1 \sin(\omega_0 s) + \Omega_2 \cos(\omega_0 s) \right] \, ds ,
\]

with \( E \) given by (2). Minimization with respect to \( \Omega_i \) gives the following solution for a torsionally relaxed minicircle

\[
\Omega_i^{(r)} = \frac{\mu \sin(\omega_0 s)}{A_1} , \\
\Omega_2^{(r)} = \frac{\mu \cos(\omega_0 s)}{A_2} , \\
\Omega_3^{(r)} = -\frac{\mu G \cos(\omega_0 s)}{C A_2} .
\]

In the previous equations \( \mu \equiv \ell_b / R \), where \( R \) is the average radius of the circle and where \( \ell_b \), given by (3), is the bending persistence length of the model (see (10) for more details).

Equations (7) yield a perfect circle only in the case of \( A_1 = A_2 \), corresponding to a constant curvature \( \kappa = \sqrt{\Omega_1^2 + \Omega_2^2} \). In all other cases the above equations describe a quasi-circular shape that exhibits small off-planar and in-planar oscillations, with a modulated total curvature \( \kappa \). The analysis of Eq. (7) shows that the dimensionless quantity \( \kappa R \) (\( R \) is the average radius of curvature of the minicircle) is bounded within the interval

\[
\frac{A_2}{A_1 + A_2} \leq \frac{\kappa R}{2} \leq \frac{A_1}{A_1 + A_2} ,
\]

which reduces to a perfect circle \( \kappa R = 1 \) in the isotropic TWLC limit \( A_1 = A_2 \). Note that \( A_2 \) is the bending stiffness along the easy axis hence \( A_2 < A_1 \) and twist-bend coupling enhances the curvature anisotropy since \( \tilde{A}_2 < \tilde{A}_2 \). Twist-bend coupling induces oscillations in the twist, referred to as "twist waves" in Ref[10] which are in antiphase with the "roll" \( \Omega_2^{(r)} \) wave. Twist oscillations are experimentally observed in crystal structures of DNA wrapped around histone protein[11], however their origin has been so far attributed to an interaction with the underlying histone core proteins, while Eq. (7) shows that these oscillations are a natural feature of bent DNA, directly deriving from the effect of twist-bend coupling[10].

The elastic energy associated with this configuration is obtained by inserting equations (7) into (2). Using \( \mu = \ell_b / R \) and \( L = 2\pi R \), for a torsionally relaxed minicircle one finds

\[
\beta E_{tw} = \int_0^L \left[ \frac{\mu^2 \sin^2(\omega_0 s)}{2A_1} + \frac{\mu^2 \cos^2(\omega_0 s)}{2A_2} \right] \, ds = \pi \ell_b / R ,
\]

which is formally identical to the energy of a TWLC minicircle. The difference being that in the TWLC the persistence length \( \ell_b \) is the harmonic mean of the bending stiffnesses, whereas in model (2) the same quantity is a function of all elastic parameters of the model, see (3) and (4).

### B. Over and undertwisted minicircles

In the case of torsionally constrained minicircles, one should impose a constraint of a fixed linking number \( Lk \). The White theorem states that the linking number is the sum of twist and writhe \( Lk = Tw + Wr \). As we are interested in quasi-planar conformations the writhe is small and \( Lk \approx Tw \). Therefore, we impose a constraint on a twist instead by introducing an additional Lagrange multiplier as follows

\[
\beta \hat{E} \approx \beta E - \int_0^L \left[ \mu \Omega_1 \sin(\omega s) + \mu \Omega_2 \cos(\omega s) + \lambda \Omega_3 \right] \, ds ,
\]

where we have used the same approximation as in (6) but with \( \hat{x} = \sin(\omega s) \hat{e}_1 + \cos(\omega s) \hat{e}_2 \), in order to take into account that the introduction of a constraint in \( \Omega_i \) induces a shift in the intrinsic twist to \( \omega = \omega_0 + \Delta \omega \). Local energy minimization yields the modified equations

\[
\Omega_1^{(ic)} = \frac{\mu \sin(\omega s)}{A_1} , \\
\Omega_2^{(ic)} = \frac{\mu \cos(\omega s)}{A_2} - \frac{\lambda G}{C A_2} , \\
\Omega_3^{(ic)} = -\frac{\mu G \cos(\omega s)}{C A_2} + \frac{\lambda A_2}{C A_2} .
\]

As expected, a non-zero \( \lambda \) introduces an offset in \( \Omega_3 \), i.e. an average excess twist density given by

\[
\Delta \omega = \frac{\lambda A_2}{C A_2} ,
\]

where again we assumed that the writhe has negligible contribution. Because of twist-bend coupling there is a corresponding offset in \( \Omega_2 \) as well, which enhances the inhomogeneity in the curvature. The shape described by Eqs. (11) alternates between high and low curvature regions, but the excess twist induces oscillations in \( \kappa R \) in a wider range when compared to the torsionally relaxed case (6) (more details are shown in Appendix A). The \( \Omega \)’s from Eqs. (11) are in very good agreement with numerical results on coarse-grained DNA models discussed in the next section.

Similarly to what was done for the untwisted case[10] we can show that the Lagrange multiplier \( \mu \) fixes the average radius of curvature, hence \( \mu = \ell_b / R \). Plugging Eqs. (11) into (2) we obtain the following energy for a torsionally constrained minicircle

\[
\beta E_{tc} = \int_0^L \left[ \frac{\mu^2 \sin^2(\omega s)}{2A_1} + \frac{\mu^2 \cos^2(\omega s)}{2A_2} + \frac{C}{2} \Delta \omega^2 \right] \, ds = \frac{\pi \ell_b}{R} + L \frac{C}{2} \Delta \omega^2
\]

(13)
Figure 2. Comparison between deformation parameters ($\Omega$'s) of a minicircle of $N = 104$ bp obtained from MC simulations and analytical results. Left: Torsionally relaxed minicircle with linking number $Lk = 10$ ($\omega = \omega_0 = 1.85$ nm$^{-1}$). Right panels: Overtwisted minicircle with linking number $Lk = 11$ ($\omega = 2.03$ nm$^{-1}$). Each single panel reports $\Omega$ from MC simulations with stiffness parameters from the set $M^1$ (dashed cyan line) and from the set $M^2$ (red solid line), as given in Table I, together with the analytical expressions for the same values of the parameters. MC data for the torsionally relaxed case $Lk = 10$ are in excellent agreement with Eqs. (7), while the torsionally constrained case $Lk = 11$ simulations agree with Eqs. (11). Results of MC simulations are obtained by taking the Fast Fourier Transformation (FFT) of different configurations, shifting all phases in such a way that for each MC configuration $\Omega_1(0) = 0$, taking the inverse FFT and then averaging the reconstructed signal over the different configurations. Averages are done on 250 independent configurations. The bottom horizontal axis shows the local phase $\omega s$ while the top horizontal axis shows the polymer coordinate $s$ in terms of base pairs.

Table I. Values of the stiffness coefficients (in nm) for oxDNA1 and oxDNA2 obtained in Ref[14]. Note that while oxDNA1 has negligible twist-bend coupling $G < 0.3$ nm, this coupling is $G = 30$ nm in oxDNA2. The values in the table define two set of parameters of the triad model referred to as set $M^1 = (A_1, A_2, C, G) = (84, 29, 118, 0)$ and set $M^2 = (A_1, A_2, C, G) = (81, 39, 105, 30)$. The last column gives $\bar{A}_2$ and $\bar{C}$ as obtained from Eqs. (4) and (5), respectively.

|       | $A_1$ | $A_2$ | $C$  | $G$  | $\bar{A}_2$ | $\bar{C}$ |
|-------|-------|-------|------|------|-------------|----------|
| oxDNA1| 84(14)| 29(2) | 118(1)| 0(0.2)| 29          | 118      |
| oxDNA2| 81(10)| 39(2) | 105(1)| 30(1)| 30          | 82       |

where $\bar{C}$ has been defined by Eq. (5) and the term proportional to $\cos(\omega s)$ averages out in the integration. As in the untwisted case the energy is formally identical to that of a TWLC. Again in model (2), the difference with TWLC is that the bending stiffness $b_0$ and torsional stiffness $\bar{C}$ are function of all elastic constant. Naturally, for large excess twist the approximation of negligible write will break down and the minicircle will start supercoiling. This instability is not captured by the present model (10), as it was derived by a local constraint, explicitly neglecting this effect.

IV. NUMERICAL RESULTS

In order to check the theoretical predictions, we performed Monte Carlo (MC) simulations of both relaxed and overtwisted minicircles by discretizing the continuum model (2). In the following, we will refer to this as the triad model (see also[9,10]). We also performed Molecular Dynamics (MD) simulations of oxDNA[20], a coarse-grained model describing DNA as two intertwined strings of rigid nucleotides. In the triad model the stiffness constants $A_1, A_2, C$ and $G$ are input parameters and can be freely chosen, while for oxDNA their values are fixed by the force field parameterization, which was tuned to reproduce known structural, thermodynamical and mechanical properties of DNA[20]. In addition, oxDNA comes in two versions: an achiral implementation with symmetric grooves (oxDNA1) and a version that comprises the chirality of physical DNA manifested in asymmetric grooves (oxDNA2). This feature makes the model suitable to test the implications of twist-bend coupling, which derives precisely from the groove asymmetry[6]. The elastic parameters for oxDNA1 and oxDNA2 were computed in Ref[7] from the analysis of equilibrium fluctuations of a linear molecule and the results are shown in Table II, yielding in particular a value of $G$ which is comparable to that of the other elastic constants.
The White theorem states that the linking number is the sum of the number of times the two strands are wound around each other.

The left panel of Fig. 2 shows a torsionally relaxed minicircle with \( Lk = 10 \) base pairs and linking number \( Lk \). In the simulations we fix the linking number \( Lk \) with \( 10^4 \) base pairs and linking number \( Lk \). The right panel of Fig. 2 shows a comparison of the \( Lk = 10 \) or overtwisted \( Lk = 11 \). Left panel: stiffness parameters from set \( M^2 \), which has a non-zero twist-bend coupling. Right panel: stiffness parameters \( M^1 \). The bottom graph of each panel reports the signed distance \( d = (ax + by + z) / \sqrt{a^2 + b^2 + 1} \) (where \( ax + by + z + c = 0 \) is the plane equation and \((x_i, y_i, z_i)\) the bead coordinates) of the beads from the plane that best fit the beads positions. Colors are consistent with the snapshots shown in the panel.

A. MC simulations with the Triad Model

In the triad model, a double stranded DNA of \( N \) base pairs is represented by \( N \) beads, each carrying a frame of three orthogonal unit vectors \( \{ \hat{e}_1, \hat{e}_2, \hat{e}_3 \} \). The distance between consecutive beads is fixed and equal to \( a = 0.33 \) nm and the vector \( \hat{e}_3 \) always points towards the sequentially adjacent bead. Given two consecutive triads, the deformation parameters are defined by a definition analogous to Eq. (1) valid for finite rigid body rotations (for details see Supplemental Material of Ref[7]), while the energy of a conformation is obtained by discretizing the continuum energy model, Eq. (2). Low temperature MC simulation (sufficiently low so that the circle reaches its ground state configuration) have been carried out using the two sets of parameters \( M^1 \) and \( M^2 \) matching the oxDNA1 and oxDNA2 values (see caption of Table I).

Figure 2 shows a comparison of the \( \Omega_i \) obtained from the triad model simulations colored lines) with the analytical predictions (black lines). In the simulations we fix the linking number \( Lk \), which is a topological invariant measuring the number of times the two strands are wound around each other. The White theorem states that the linking number is the sum of twist and writhe \( Lk = Tw + Wr \). As we are interested in quasi-planar conformations the writhe is small and \( Lk \approx Tw \). Therefore the constraint used in (10) to fix the excess twist density \( \Omega_1 \) is expected to adequately describe closed circular DNA. The left panel of Fig. 2 shows a torsionally relaxed minicircle with 104 base pairs and linking number \( Lk = 10 \), while in the right panel the circle is overtwisted and has \( Lk = 11 \). All analyzed cases display excellent agreement between analytical models and MC data. The horizontal axis shows a single helical turn, corresponding to a phase \( 0 \leq \phi \leq 2\pi \) obtained from the analysis of the Fourier spectrum of \( \Omega_i \), as explained in the caption. In a torsionally relaxed DNA one helical turn corresponds to 10.4 base pairs as shown in the horizontal top scale of the left panel. In the overtwisted case \( Lk = 11 \) one helical turn corresponds to 9.5 base pairs only.

As shown in Fig 3 in the torsionally relaxed case \( Lk = 10 \) and for the set \( M^2 \) \( (G \neq 0) \) the twist oscillates, while these oscillations are absent for the set \( M^1 \) \( (G = 0) \). In the torsionally constrained case \( Lk = 11 \) the twist of both sets is shifted, see bottom right graph of Fig. 2. Overtwisting affects the \( \Omega_2 \) of set \( M^2 \), but not that of set \( M^1 \), as predicted by the analytical model. Is is worth emphasizing that the theoretical predictions of both panels do not contain adjustable parameters. In fact, the Lagrange multipliers \( \mu \) and \( \lambda \) are fixed as follows: \( \mu = l_0 / R \), where \( R \) is the radius of a perfect circular chain of 104 beads with a distance between consecutive beads of 0.33 nm \( (R \approx 5.46 \text{ nm}) \), \( \lambda = CA_2/\Delta \omega \) with \( \Delta \omega = 0.18 \text{ nm}^{-1} \), and the stiffness parameters are given. In the undertwisted case (not shown) very similar profiles for the \( \Omega_i \) are observed, but the shifts in \( \Omega_2 \) and \( \Omega_3 \) carry the opposite sign.

Figure 2 shows the typical shapes of relaxed and over-twisted minicircles. In both sets \( M^1 \) and \( M^2 \), the relaxed configuration \( (Lk = 10) \) is almost completely planar and closely resembles a perfect circle. However, the introduction of additional twist leads to remarkable differences in the behavior of minicircles parameterized by the sets \( M^1 \) and \( M^2 \). The latter starts to exhibit the shape of a rounded polygon or, more pre-
cisely, a rounded hendecagon, where the amount of vertices is induced by the imposed excess linking number. Furthermore, the structure is moderately off-planar, as illustrated by the plot of $d$, the signed distance of each base pair from the best fitting plane vs. base pair position (see left lower panel of Fig. 3). Consistent with the oscillations of the strain fields $\Omega_i$, $d$ fluctuates with a wavelength of $2\pi/\omega$. On the other hand, when one considers the parameter set without twist-bend coupling ($M^1$), depending on the setup of the MC simulation, two typical configurations are found shown in blue and green in Fig. 3 right. If one starts from a perfectly circular overtwisted shape and performs MC updates at low temperature, the simulation relaxes to the shape drawn in blue (Fig. 3 right). From this shape the $\Omega_i$ shown in Fig. 2 were calculated. If, on the other side, one starts from a high temperature simulation and gradually lowers the temperature to reach the ground state a strongly off planar conformation, as that shown in green in Fig. 2 is obtained. The latter shows an onset of supercoiling, which is not found in the simulations with set $M^2$. In that case the polygon shape is always recovered at low temperatures, irrespective from the simulation path followed. This shows that the model $M^1$ is more prone to supercoiling compared to model $M^2$, for which the torsional stress is released in bending and off-planar fluctuations. This is also reflected in Eq. (15), which shows that the torsional energy is controlled by the parameter $\tilde{C}$ [5], rather than the intrinsic twist stiffness $C$. The sets $M^1$ and $M^2$ have comparable twist stiffness $C$, but $\tilde{C} = C = 118$ nm for set $M^1$ as $G = 0$, while $\tilde{C} = 82$ nm and $C = 105$ nm for set $M^2$ which implies a considerable lower twisting energy for the same amount of overtwisting. This explains why model $M^1$ supercoils more easily when compared to $M^2$.

B. MD simulations with oxDNA

Double helical coarse-grained models have become very popular in the recent few years to study a large number of equilibrium and dynamical properties of DNA [29][30][31]. oxDNA [32] provides an effective mesoscopic description of DNA in which each nucleotide is represented by a rigid object equipped with three interactions sites for base pairing, coaxial stacking, electrostatic and steric interactions, that was tuned to reproduce the properties of dsDNA. Langevin dynamics of the system was integrated at low temperature (15 K) with the LAMMPS package [34] using the implementation of Henrich et al [35] and default values for the interaction parameters.

Again, the $\Omega_i$ calculated from oxDNA MD simulations are consistent with those predicted by the analytical model (see Fig. 4). As already pointed out in Ref.10 small deviations can be noticed in the amplitude of $\Omega_i$ oscillations. Interesting enough, also the value of $\Omega_1$ for oxDNA2 slightly deviates from the analytical curve but only in the case in which the minicircle is overtwisted. As for $\Omega_1$, these small deviations probably arise from some additional interactions (e.g. higher-order terms) present in oxDNA, but not considered in the energy functional. Notice that we considered an overtwisted minicircle with small additional twist $\Delta \omega/\omega_0 \simeq 5\%$. Such
Figure 5. Typical shapes of an overtwisted minicircles of 104 bp and \( L_k = 12 \) (20% excess linking number fraction \( \Delta L_k / L_k = 0.2 \)). Shapes are from MD simulations at 15 K of oxDNA1 (right panel) and oxDNA2 (left panel) and minicircles are confined into a slit or outside a cylindrical surface. Blue and red transparent chains represent the two strands while the black solid beads are the center of mass of each base pair. The distance between the two planes of the slit is 3.4 nm while the cylinder radius is 4.4 nm.

choice is due to the fact that for higher additional twist, e.g. of the order of 10% as imposed in the case of Fig. 2, both oxDNA1 and oxDNA2 show a behavior of strong deviations from planarity and a propensity to form supercoils.

Next, we considered strongly overtwisted minicircles in confined geometries so that the DNA cannot easily supercoil. Two cases were analyzed: a DNA minicircle confined between two flat surfaces and a minicircle wrapped around a cylinder, shown in Figure 5. These two situations can be relevant, respectively, in the case of AFM experiments where DNA is confined in 2D by absorption on a mica surface or in the case of nucleosomes where DNA is wrapped around the cylindrically shaped octamer of histone proteins. Figure 5 shows the typical shapes of confined oxDNA1 and oxDNA2 minicircles when an excess linking number of 20% is introduced. Apart obviously from the twist-bend coupling \( G \), oxDNA1 and oxDNA2 have similar elastic parameters (see Table I) but their response to overtwisting and confinement is very different. This is in agreement to what was found in the triad model: as for the set \( M_1 \), also oxDNA1 has a stronger tendency to develop off-planar conformation, indicating that it is easier to supercoil. Furthermore, in the case of oxDNA2, a closer inspection of the base pairs’ center of mass, as shown in the inset of Fig. 5 (left), indicates that the minicircle tends to have a rounded polygonal shape. On the other hand, oxDNA1 has a smoother curvature even if, under planar confinement, the shape of oxDNA1, once projected in 2D, resembles a rounded square.

V. CONCLUSION

In this paper we have studied minimal energy shapes of torsionally constrained circular DNA molecules. As shown earlier \( ^{10} \) the effect of twist-bend coupling is to produce shapes characterized by coupled oscillations in twist (\( \Omega_3 \)) and in the bend (\( \Omega_2 \)) densities. Additional torsional strain induces a net shift in the twist density \( \Omega_3 \), forcing it to oscillate around a non-zero average value. As a consequence of twist-bend coupling this effect is transmitted to the groove-bending strain \( \Omega_2 \). The breaking of the symmetry of \( \Omega_2 \) oscillations results in a shape resembling that of a rounded regular polygon with a periodic alternation of high and low curvature regions. We have shown (extending the theory of Ref. \( ^{10} \)) that a simple analytical model reproduces very well the shapes obtained from simulations. The comparison between theory and simulations is remarkable as there are no adjustable parameters.

The analytical model provides a simple way to estimate the energy of torsionally relaxed and torsionally constrained minicircles. It turns out that, due to the peculiar shapes of the circles induced by twist-bend coupling, bending and twist are not governed by the intrinsic stiffnesses, but by rescaled parameters \( l_b \) and \( \tilde{C} \), given by Eq. \( ^{1} \) and \( ^{5} \) respectively. As a consequence, although the two sets of parameters used in this work have comparable torsional stiffness \( C \) (see Table, 1), their torsional response is very different. In the set \( M_1 \), with vanishing twist bend coupling, the twist energy is \( \frac{1}{2} C L \Delta \omega^2 \) while in \( M_2 \) this is reduced to \( \frac{1}{2} \tilde{C} L \Delta \omega^2 \) (recall that \( \tilde{C} < C \), see \( ^{5} \)). In the set \( M_2 \) the minimal energy shape in-
troduces suitable additional bending to exploit the presence of a cross term $G\Omega_2\Omega_3$ which can become negative if $\Omega_2$ and $\Omega_3$ have opposite signs. The appropriate value of torsional elastic constant for DNA has been debated, with different techniques providing values ranging typically from $C = 75$ nm to $C = 110$ nm. One way of extracting $C$ is from the analysis of dynamical or equilibrium properties of DNA minicircles, see e.g.\textsuperscript{45,51} One of the results of the present work is that cyclization experiments provide $\tilde{C}$ rather than the bare torsional stiffness $C$. Assuming that the parametrization of elastic constant for oxDNA2 approximates well that of real DNA molecules, we conclude that experiments on minicircles should give a value $\tilde{C} = 80$ nm (see Table \textsuperscript{1}), which seems to be consistent with literature values\textsuperscript{15,23}.

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Appendix A: Curvature

From Eqs. (A1) and using $\mu = l_b/R$ one finds

$$\frac{\kappa^2 R^2}{4} = \frac{\tilde{A}_2^2}{(A_1 + A_2)^2} + \Gamma^2 + \frac{A_1 - \tilde{A}_2}{A_1 + A_2} \cos^2(\omega s) - \frac{2\Gamma A_1 \cos(\omega s)}{A_1 + A_2},$$

where we defined

$$\Gamma \equiv \frac{G}{2A_2} R\Delta \omega \approx \frac{G}{2A_2} \Delta Lk$$

(A2)

($\Gamma$ is a rescaled dimensionless excess twist density, or equivalently the linking number if one neglects the contribution of the writhe $2\pi R\Delta \omega \approx \Delta Lk$). The torsionally relaxed case corresponds to $\Gamma = \Delta \omega = 0$, $\omega = \omega_0$. Equation (A1) gives in this case a maximal curvature when $\cos(\omega_0 s) = \pm 1$ and a minimal curvature when $\cos(\omega_0 s) = 0$. These are the bounds given in Eq. (8).

In the torsionally constrained case and for non vanishing twist-bend coupling one has $\Gamma \neq 0$. The analysis of (A1) yields the following bounds

$$\frac{\tilde{A}_2}{A_1 + A_2} \left( 1 - \frac{A_1 - \tilde{A}_2}{A_1 + A_2} \Gamma^2 \right)^{1/2} \leq \frac{\kappa R}{2} \leq \frac{A_1}{A_1 + A_2} + |\Gamma|$$

(A3)

valid for

$$|\Gamma| \leq \Gamma^* \equiv \frac{A_1 - \tilde{A}_2}{A_1}$$

(A4)

and

$$\frac{A_1}{A_1 + A_2} - |\Gamma| \leq \frac{\kappa R}{2} \leq \frac{A_1}{A_1 + A_2} + |\Gamma|$$

(A5)

for $|\Gamma| \geq \Gamma^*$. In the limit $\Gamma = 0$, Eq. (A3) reduces to (8). Comparing (A3) and (A5) with (8) one sees that introducing an excess twist indeed increases the range of values through which the curvature $\kappa$ oscillates.

Figure 6. Top panels: Plots of total curvature $\kappa$ and in-plane curvature $\kappa_//$ for overtwisted and undertwisted minicircles. These plots are obtained from Eqs. (A1) and (A6), respectively, using the stiffness parameters $M^2 = (A_1, A_2, C, G) = (81, 39, 105, 30)$ The torsional constraint and twist-bend coupling enhances curvature anisotropy. Lower panels: Plots of $\Omega_2$ as given by Eqs. (A1). A positive value of $\Omega_2$ corresponds to a bending towards the major groove.
The top panels of Fig. 6 show the total curvature \( \kappa \) and in-plane curvature \( \kappa_\parallel \) as given by (A1) and (A6), respectively. There is a small difference between the two, showing that the contribution of off-planar bending to the total curvature, given by \( \kappa_\perp \), is small. For an isotropic model (\( A_1 = A_2 \)) with \( \Gamma = 0 \) (corresponding to either \( \Delta L k = 0 \) or \( G = 0 \)) one recovers from (A1), (A6) and (A7) a perfect homogeneous and planar circle of radius \( R \): \( \kappa = \kappa_\parallel = 1/R \) and \( \kappa_\perp = 0 \). The introduction of anisotropic bending leads to curvature oscillations, due to the terms proportional to \( \sin(2\omega s) \) and \( \sin(2\omega s) \) in (A6) and (A7). The period of these oscillations is half a helical repeat. Overwinding or underwinding minicircles with non-zero twist-bend coupling (\( \Gamma \neq 0 \)) breaks this symmetry by introducing terms that oscillate with a period of a full helical repeat length. The difference between underwinding (\( \Gamma < 0 \)) and overwinding (\( \Gamma > 0 \)) is that in the former case the region of maximal curvatures \( \kappa \) or \( \kappa_\parallel \) corresponds to a global maximum of \( \Omega_2 \) (\( \geq 0 \)) while in the latter to a global minimum, see lower panels of Fig. 6. The different signs of \( \Omega_2 \) correspond to different modes of groove bendings. Figure 1 (right) shows a positive \( \Omega_2 \), which is a bending towards the major groove.

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