Theory of sequence-dependent DNA elasticity

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The elastic properties of a molecule of duplex DNA are strongly dependent on nucleotide sequence. In the theory developed here the contribution $\psi^n$ of the $n$-th base-pair step to the elastic energy is assumed to be given by a function $\psi^n$ of six kinematical variables, called tilt, roll, twist, slide, and rise, that describe the relative orientation and displacement of the $n$-th and $(n+1)$-th base pairs. The sequence-dependence of elastic properties is determined when one specifies the way $\psi^n$ depends on the nucleotides of the two base pairs of the $n$-th step. Among the items discussed are the symmetry relations imposed on $\psi^n$ by the complementarity of base pairs, i.e., of A to T and C to G, the antiparallel nature of the DNA sugar-phosphate chains, and the requirement that $\psi^n$ be independent of the choice of the direction of increasing $n$. Variational equations of mechanical equilibrium are here derived without special assumptions about the form of the functions $\psi^n$, and numerical solutions of those equations are shown for illustrative cases in which $\psi^n$ is, for each $n$, a quadratic form and the DNA forms a closed, 150 base-pair, minicircle that can be called a DNA o-ring because it has a nearly circular stress-free configuration. Examples are given of non-circular equilibrium configurations of naked DNA o-rings and of cases in which interaction with ligands induces changes in configuration that are markedly different from those undergone by a minicircle of intrinsically straight DNA. When a minicircle of intrinsically straight DNA interacts with an intercalating agent that upon binding to DNA causes local reduction of intrinsic twist, the configuration that minimizes elastic energy depends on the number of intercalated molecules, but is independent of the spatial distribution of those molecules along the minicircle. In contrast, here it is shown here that the configuration and elastic energy of a DNA o-ring can depend strongly on the spatial distribution of the intercalated molecules. As others have observed in calculations for Kirchhoff rods with intrinsic curvature, an o-ring that has its intrinsic twist reduced at a single base-pair step can undergo large deformations with localized untwisting and bending at remote steps, even when the amount $\alpha$ of twist reduction is less than the amount required to induce supercoiling in rings of intrinsically straight DNA. We here find that the presence in the functions $\psi^n$ of cross-terms coupling twist to roll can amplify the configurational changes induced by local untwisting to the point where there can be a value of $\alpha$ at which a first-order transition occurs between two distinct stable non-circular configurations with equal elastic energy.

I. INTRODUCTION

Although there are cases in which one can treat a DNA molecule as an idealized homogeneous elastic rod that is straight when stress-free and has uniform elastic properties, it is known that the genetic information in DNA determines not only the amino acid sequences of encoded proteins and RNA but also the geometry and deformability of DNA at a local level, i.e., at the level of base-pair steps. Recent advances in structural biochemistry have yielded information about the dependence on base-pair sequence of the structure and elastic response of DNA. In this paper we present a method of taking such information into account when one calculates and determines the stability of equilibrium configurations of DNA segments subject to preassigned end conditions.

In a stress-free state, the local bend, twist, and stretch of duplex (double helical) DNA varies from one base-pair step to another. Some steps are wedge shaped with inclination (i.e., bending) strongly dependent on nucleotide composition and occasionally as large as $5^\circ$. Moreover, there are steps that are sites of over- or under-twisting as large as $3.5^\circ$, and there are steps at which the barycenter of a base pair is displaced relative to that of its predecessor and gives rise to a local shear that can be as large as 0.65Å (or 20% of the elevation of a base-pair relative to its predecessor) [1–6].

The chemical architecture of DNA results also in mechanical couplings between kinematical variables (such as those of Fig. 1) that characterize the relative displacement and orientation of adjacent base pairs. Observed

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energies of adjacent base pairs. For each segment is assumed to be the sum of the interaction energies of discrete models in which the elastic energy $\Psi$ of a DNA nucleotide sequence and have, of necessity, a discrete dependence of intrinsic structure and elastic moduli on nucleotide sequence and structure of commonly found forms of duplex DNA including the B, A, and C forms, (iii) the derivation of the variational equations of equilibrium which requires the attainment of a computationally manageable expression for the matrix inverse of the gradient of the orthogonal matrix $[D_{ij}^n]$ relating bases embedded in the $n$-th and $(n+1)$-th base pairs to the parameters tilt, roll, and twist (see Eq. (A.1) and Eqs. (3.7), (3.9)–(3.11)), and (iv) the development of an efficient computational method (of order $O(N)$, with $N$ the number of base pairs) for solving the equilibrium equations and determining the stability of computed solutions.

To illustrate the way in which the theory can be used to explore the influence of nucleotide sequence on configurations of DNA subject to constraints such as ring closure and ligand induced changes in local structure, we give, in Section IV, a few examples in which the theory is employed to calculate equilibrium configurations of closed minicircles which, because they have nearly circular stress-free configurations, are called DNA o-rings. Discussions are given of ways in which equilibrium configurations of naked DNA and DNA that is interacting with ligands can, under appropriate circumstances, show a behavior markedly different from that predicted by the familiar idealized rod model. Among the new topics treated here is the influence of the cross-terms coupling twist to roll on the configurational changes induced by local untwisting.

II. CONSTITUTIVE RELATIONS AND VARIATIONAL EQUATIONS

In the present theory the base pairs are taken to be flat objects that are represented by rectangles. The configuration of a DNA segment with $N+1$ base pairs, and hence $N$ base pair steps, is specified by giving, for each $n$, both the location $\mathbf{x}^n$ of the center of the rectangle $\mathcal{B}^n$ that represents the $n$-th base pair and a right-handed orthonormal triad $\mathbf{d}_1^n, \mathbf{d}_2^n, \mathbf{d}_3^n$ that is embedded in the base pair. (See Fig. 2.) The polygonal curve $\mathcal{C}$ formed by the $N$ line segments connecting the spatial points $\mathbf{x}^1, \ldots, \mathbf{x}^{N+1}$ is called the axial curve.

Each DNA nucleotide in a base pair belongs to one

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1 In engineering practice the term “o-ring” is commonly used for elastic rings that are circular when stress-free.

2 See also the discussion given in Refs. 11–19.
of the two strands that make up the duplex molecule. In a strand the nucleotide bases are laterally attached (through covalent bonds) to a sugar-phosphate chain. The chains of the two strands are antiparallel in the sense that increasing $n$ corresponds to advance in the $5’-3’$ direction on one strand and in the $3’-5’$ direction on the other.\(^3\)

We assume that the elastic energy $\Psi$ of a configuration is the sum over $n$ of the energy $\psi^n$ of interaction of the $n$-th and $(n+1)$-th base pairs, and that $\psi^n$ is a function of the relative orientation and displacement of the two neighboring base pairs, i.e., of the components of the vectors $d_{ij}^n$ and $r^n = x^{n+1} - x^n$ with respect to the basis $d_i^n$. As the $3 \times 3$ matrix $[D_{ij}^n]$ with entries $D_{ij}^n = d_i^n \cdot d_j^{n+1}$ is orthogonal, it can be parameterized by a triplet ($\theta_1^n, \theta_2^n, \theta_3^n$) of angles, with $\theta_1^n$ the tilt, $\theta_2^n$ the roll, and $\theta_3^n$ the twist of $x^{n+1}$ relative to $x^n$. Hence, in the formula

$$\Psi = \sum_{n=1}^{N} \psi^n, \quad (2.1)$$

the elastic energy $\psi^n$ of $n$-th base-pair step is given by a constitutive relation of the type,

$$\psi^n = \tilde{\psi}^n(\theta_1^n, \theta_2^n, \theta_3^n, r_1^n, r_2^n, r_3^n), \quad r_i^n = d_i^n \cdot r^n. \quad (2.2)$$

It is often useful to write this relation in the form

$$\psi^n = \tilde{\psi}^n(\theta_1^n, \theta_2^n, \theta_3^n, r_1^n, \rho_1^n, \rho_2^n, \rho_3^n), \quad (2.3)$$

where the displacement variables $\rho_1^n, \rho_2^n, \rho_3^n$, called shift, slide, and rise are given by coordinate transformation functions, $\rho_i^n$, that are independent of $n$:

$$\rho_i^n = \rho_i(\theta_1^n, \theta_2^n, \theta_3^n, \rho_1^n, \rho_2^n, \rho_3^n). \quad (2.4)$$

As shown in Fig. 2, the vectors $d_i^n$ are defined so that $d_3^n$ is parallel to the major groove of the DNA, and $d_1^n$ is parallel to the minor groove of the DNA.

Each drawing in Fig. 1 illustrates one of the kinematical variables $\theta_1^n, \theta_2^n, \theta_3^n$, $\rho_1^n, \rho_2^n, \rho_3^n$ for a case in which that variable has a positive value and the others (with the exception of $\rho_3^n$) are set equal to zero. That figure is intended to be suggestive rather than precise. To give a precise definition of $\theta_1^n$ and $\rho_i^n$ for cases in which several of these variables are not zero, we follow a procedure (introduced by Zhurkin, Lysov, & Ivanov [9] and further developed by El Hassan & Calladine [8]) in which one employs the Euler-angle system $\zeta^n, \kappa^n, \gamma^n$ for which

$$D_{ij}^n = d_i^n \cdot d_j^{n+1} = Z_{ik} (\zeta^n) Y_{kl} (\kappa^n) Z_{lj} (\gamma^n), \quad (2.5a)$$

and the corresponding matrices $[Y_{ij}(\alpha)],[Z_{ij}(\alpha)]$ are determined from the relations:

$$Y_{ij}(\alpha) = \begin{bmatrix} \cos \alpha & 0 & \sin \alpha \\ 0 & 1 & 0 \\ -\sin \alpha & 0 & \cos \alpha \end{bmatrix}, \quad Z_{ij}(\alpha) = \begin{bmatrix} \cos \alpha & -\sin \alpha & 0 \\ \sin \alpha & \cos \alpha & 0 \\ 0 & 0 & 1 \end{bmatrix}. \quad (2.5b)$$

Each drawing in Fig. 2 illustrates the major and minor groove conditions for which

$$\zeta^n = \frac{1}{2} \theta_3^n - \gamma^n, \quad \kappa^n = \frac{1}{2} \theta_3^n + \gamma^n, \quad (2.6a)$$

$$\tan \gamma^n = \frac{\theta_2^n}{\theta_1^n}. \quad (2.6b)$$

\(^3\) In our numbering of base pairs the choice of the direction of increasing $n$ is arbitrary. Although it is common to number the base pairs so that increasing $n$ corresponds to advance in the $5’-3’$ direction of the strand that (in discussions of transcription) is called the sequence strand, we do not impose such a convention here. Because we study the implications of the requirement that the energy $\psi^n$ be independent of the choice of the direction of increasing $n$, it is necessary for us to consider transformations that change that direction while leaving invariant the orientation and spatial position of the base pairs.

\(^4\) The superposed symbols, $\tilde{\cdot}, \hat{\cdot}, \dot{\cdot}$, etc. here serve to emphasize the distinction between a value of a quantity and a function relating the quantity to other quantities.

\(^5\) In general, $x^n$, the center of the rectangle $x^n$, is not the barycenter (center of mass) of the corresponding base pair. Here, as in Ref. 20, $x^n$, $d_i^n$, and $d_3^n$ are obtained by the following construction: Let $\ell_2$ be the straight line connecting the C1' atoms of the deoxyribose groups corresponding to the $n$-th base pair, and let $\ell_1$ be the line that passes at a right angle through the midpoint of $\ell_2$ and intersects the straight line containing the C8 atom of the purine base and the C6 atom of the complementary pyrimidine base. The point $x^n$ is taken to lie at the intersection of $\ell_1$ and $\ell_2$; the vector $d_3^n$ is parallel to $\ell_1$ and points in the direction of the major groove; $d_1^n$ is parallel to both $\ell_1$ and $\ell_2$ with $d_1^n \cdot r^n > 0$.

\(^6\) We use the Einstein summation convention for subscripts (but not superscripts).
As in Ref. 8, we here use Eqs. (2.5)–(2.6) to define $\rho_i^0$ in terms of $r_i^n$ and $\theta_i^n$:

$$\rho_i^0 = Z_{ij}(-\gamma^n) Y_{jk}(-\frac{1}{2} \kappa^n) Z_{kl}(-\zeta^n) r_j^k.$$  

(2.7)

This last equation renders explicit the functions $\hat{\rho}_k$ in Eq. (2.4) and implies that the numbers $\rho_i^0$ are the components of $r^n$ with respect to an orthonormal basis $\hat{d}_i^n$, which is called the mid-basis for step $n$ and is defined by the relation

$$\hat{d}_i^n \cdot \hat{d}_j^n = Z_{ik}(-\gamma^n) Y_{jk}(-\frac{1}{2} \kappa^n) Z_{lj}(-\zeta^n).$$  

(2.8)

In Section III (i.e., in the discussion containing Eqs. (3.4) and (3.8)) we render precise the following remark: The present definition of $\theta_i^n$ and $\rho_i^0$ is such that, for each configuration of a DNA segment, a change in the choice of the direction of increasing $n$ leaves $\theta_2, \theta_3, \rho_2, \rho_3$ invariant but changes the sign of $\theta_1$ and $\rho_1$.

Several computer programs have been developed to reduce atomic coordinate data (of the type available for DNA crystal structures in the Nucleic Acid Database [21]) to variables $\theta_i^n, \rho_i^0$ which, like those just defined, are compatible with a convention [10] known as the “Cambridge Accord of 1988”. For a survey see Ref. 22.

The function $\psi^n$ of Eq. (2.3) depends on which nucleotide bases are in the $n$-th and $(n+1)$-th base pairs. To discuss the restrictions imposed on $\psi^n$ by the complementarity of A to T and G to C, the antiparallel directions of the sugar-phosphate chains, and the assumption that $\psi^n$ is independent of the composition of base pairs other than the $n$-th and $(n+1)$-th, let us write $\tilde{\psi}^{XY}$ for $\psi^n$, where XY, with X in the $n$-th and Y in the $(n+1)$-th base pair, is a sequence of two nucleotide bases attached to the chain for which $n$ increases in the $5'$–$3'$ direction. In this notation, for the complement of the base X, one writes $\overline{X}$ (i.e., $\overline{X} = T$, $\overline{G} = C$). The complement of a sequence of bases is the sequence of complementary bases in the reverse order: the complement of XY is $\overline{Y}\overline{X}$ (e.g., the complement of AC is GT), because X and Y are attached at the locations $n$ and $n+1$ to the chain for which $n$ decreases in the $5'$–$3'$ direction. In Section III it is shown, that when $\theta_i^n, \rho_i^0$ are defined as here, each function $\tilde{\psi}^{XY}$ determines its complementary function $\tilde{\psi}^{\overline{Y}\overline{X}}$ by the relation

$$\tilde{\psi}^{XY}(\theta_i^1, \theta_i^2, \rho_i^0, \rho_i^0, \rho_i^0) = \tilde{\psi}^{\overline{Y}\overline{X}}(-\theta_i^1, \theta_i^2, -\rho_i^0, \rho_i^0, \rho_i^0).$$  

(2.9)

It follows that, if XY is self-complementary, i.e., if $\overline{XY} = Y\overline{X}$, then $\tilde{\psi}^{XY}$ is an even function of the pair $(\theta_i^n, \rho_i^0)$ for each fixed value of the quadruple $(\theta_2^n, \theta_3^n, \rho_2^n, \rho_3^n)$.

We define an equilibrium configuration to be one for which the first variation of the total energy, i.e.,

$$E = \Psi + P,$$  

(2.10)

vanishes for all variations in configuration that are admissible in the sense that they are compatible with the imposed constraints. (Here $P$ is the potential energy of external forces and moments.) The resulting variational equations, which are derived in Section III, can be written

$$f^n - f^{n-1} = \varphi^n,$$  

$$m^n - m^{n-1} = f^n \times r^n + \mu^n, \quad (2 \leq n \leq N),$$  

(2.11)

where, for each $n$, $f^n$ and $m^n$ are the force and moment that the $(n+1)$-st base pair exerts on the $n$-th base pair and $\varphi^n$ and $\mu^n$ are the external force and moment acting on the $n$-th base pair. The vectors $f^n$ and $m^n$ are the analogues of the vectors $\mathbf{f}(s^*)$ and $\mathbf{m}(s^*)$ that, in Kirchhoff’s theory of elastic rods, are interpreted as the resultant force and moment of the contact forces (i.e., Piola stresses) exerted on the cross-section with the arc-length coordinate $s$ by the material with arc-length coordinates $s > s^*$. The components of $f^n$ and $m^n$ with respect to the local basis $d_i^n$ are given by the relations

$$f_i^n = \frac{\partial \tilde{\psi}^n}{\partial \theta_{i,n}}, \quad m_i^n = \Gamma_{ij}^n \left( \frac{\partial \tilde{\psi}^n}{\partial \theta_j^n} + \frac{\partial \tilde{\psi}^n}{\partial \rho_{k,n}} \frac{\partial \rho_{k,n}}{\partial \theta_j^n} \right),$$  

(2.12)

The numbers $\Gamma_{ij}^n$ form a $3 \times 3$ matrix $[\Gamma_{ij}^n] = [\Gamma_{ij}^n(\theta_1^n, \theta_2^n, \theta_3^n)]$ that depends on the definitions employed for the kinematical variables $\theta_i^n$. When those variables are as in Eqs. (2.5)–(2.6), $[\Gamma_{ij}^n]$ is given by Eq. (A.1). The chain rule permits us to write Eqs. (2.12) in the forms

$$f_i^n = \frac{\partial \tilde{\psi}^n}{\partial \rho_{i,n}} \frac{\partial \rho_{i,n}}{\partial \theta_{i,n}}, \quad m_i^n = \Gamma_{ij}^n \left( \frac{\partial \tilde{\psi}^n}{\partial \theta_j^n} + \frac{\partial \tilde{\psi}^n}{\partial \rho_{k,n}} \frac{\partial \rho_{k,n}}{\partial \theta_j^n} \right),$$  

(2.13)

in which $\tilde{\psi}^n$ and $\hat{\rho}_k$ are as in Eqs. (2.3) and (2.4). When $\theta_i^n$ and $\rho_i^0$ are defined by Eqs. (2.5)–(2.8), for $\hat{\rho}_{ij}/\partial \theta_i^n$ and $\hat{\rho}_{ij}/\partial \theta_j^n$ we have

$$\frac{\partial \hat{\rho}_k}{\partial \theta_i^n} = Z_{jk}(-\gamma^n) Y_{kl}(-\frac{1}{2} \kappa^n) Z_{li}(-\zeta^n),$$  

(2.14)

$$\frac{\partial \hat{\rho}_k}{\partial \theta_j^n} = j A_{kl}^n \rho_i^0,$$  

(2.15)

with $j A_{kl}^n$ as in Eqs. (A.2)–(A.4).

If a DNA segment with $N+1$ base pairs is subject to strong anchoring end conditions at both ends, then $(x_1^1, d_1^1)$ and $(x^{N+1}, d^{N+1})$ have preassigned values. A closed (or “circularized”) DNA segment of $N$ base pairs (bp) can be considered an $N+1$ bp segment that is subject to

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7 Such an external force and moment can arise when the $n$-th base pair is in contact with a protein or with a base pair sequentially distant from it. When electrostatic forces are taken into explicit account, $P$ in Eq. (2.10) will include the potential energy of the interaction of charged residues.
the constraint that its terminal base-pairs coincide, i.e., have the same location and orientation, and hence:
\[ x_i^1 = x_i^{N+1}, \quad d_i^1 = d_i^{N+1}, \quad (i = 1, 2, 3). \] (2.16)

A necessary condition for an equilibrium configuration to be stable is that there be no admissible variation in configuration for which \( \delta^2 E \), the second variation of \( E \), is negative. As a configuration is described in the present theory by giving a finite number of variables, namely the 6\( N \) numbers, \( \theta_i^1, \theta_i^2, \theta_i^3, \rho_i^1, \rho_i^2, \rho_i^3 \), we can say that a sufficient condition for stability is that \( \delta^2 E \) be positive for all admissible variations. A configuration that is stable but does not give a global minimum to \( E \) can be called metastable.

For calculations of the type reported in Section IV, \( \varphi^n = \mu^n = 0 \), i.e., \( P \) is set equal to zero. The Eqs. (2.11) with \( f^n \) and \( m^n \) given by Eqs. (2.12) then are a system \( \mathcal{E} \) of 6\( N \) – 6 nonlinear algebraic equations for the unknown coordinates \( \theta_i^1, \theta_i^2, \theta_i^3, \rho_i^1, \rho_i^2, \rho_i^3 \). That system is weakly coupled in the sense that for each \( n \), 2 \( \leq \) \( n \) \( \leq \) \( N \), the Eqs. (2.11) are a set of 6 equations which are solved numerically using a Newton-Raphson procedure to obtain \( (\theta_i^0, \rho_i^0) \) for given \( (\theta_i^{n-1}, \rho_i^{n-1}) \). This allows one to solve \( \mathcal{E} \) efficiently (using \( O(N) \) computational steps) by a recursive algorithm in which, for each \( n \), \( (\theta_i^n, \rho_i^n) \) is obtained as a function of \( (\theta_i^n, \rho_i^n) \). Equilibrium configurations of segments subject to specified end conditions are calculated using an iterative scheme in which \( (\theta_i^n, \rho_i^n) \) is repeatedly adjusted until the appropriate relations, e.g., Eq. (2.16), hold.

The case in which \( \psi^n \) is given by a quadratic form

To describe quadratic energy functions of the type employed for calculations presented in Section III, we write \( \theta_i^n \) and \( \rho_i^n \) for the values of \( \theta_i^n \) and \( \rho_i^n \) in a stress-free state with \( \psi = 0 \), and put
\[ \Delta \theta_i^n = \theta_i^n - \bar{\theta}_i, \quad \Delta \rho_i^n = \rho_i^n - \bar{\rho}_i^n. \] (2.17)

When \( |\Delta \theta_i^n| \) and \( |\Delta \rho_i^n| \) are small, the assumption that \( \psi^n \) is three-times differentiable implies that, to within an error \( O(\delta^3) \) with
\[ \delta = \max_{i=1,2,3} \{ |\Delta \theta_i^n|, |\Delta \rho_i^n| \}, \] (2.18)
\( \bar{\psi} \) can be taken to be a quadratic function,
\[ \psi^n = \frac{1}{2} F_{ij}^n(\Delta \theta_i^n)(\Delta \theta_j^n) + G_{ij}^n(\Delta \rho_i^n)(\Delta \rho_j^n) + \frac{1}{2} H_{ij}^n(\Delta \rho_i^n)(\Delta \rho_j^n), \] (2.19)
in which \( F_{ij}^n \), \( G_{ij}^n \), and \( H_{ij}^n \) are constants with \( F_{ij}^n = F_{ji}^n \), \( H_{ij}^n = H_{ji}^n \), (i, j = 1, 2, 3). If one wishes, one may write the right-hand side of Eq. (2.19) as a quadratic form in \( (\Delta \theta_i^n, \Delta \theta_j^n, \Delta \rho_i^n, \nu_1^n, \nu_2^n, \nu_3^n) \), where the dimensionless numbers
\[ \nu_i^n = \Delta \rho_i^n / \bar{\rho}_i^n \] (2.20)
are such that \( \nu_1^n \) can be interpreted as a stretching strain and \( \nu_2^n \) and \( \nu_3^n \), as shearing strains.

It follows from Eq. (2.9) that if we replace \( F_{ij}^n \) by \( F_{XY}^n \), etc., and let \( Z \) stand for \( F, G, H \), then in Eq. (2.19)
\[ \bar{\theta}_i^n = Q_{ij} \bar{\theta}_j^n, \quad \bar{\rho}_i^n = Q_{ij} \bar{\rho}_j^n, \] (2.21a)
\[ Z_{ij}^n = Q_{ik} Z_{kl}^n Q_{lj}, \] (2.21b)
where
\[ Q_{11} = Q_{22} = Q_{33} = 1, \quad Q_{ij} = 0 \quad \text{when } i \neq j. \] (2.22)

Equations (2.21) imply that, when \( XY = AT, GC, TA, \) or \( CG \),
\[ F_{ij}^n = F_{XY} = 0, \quad H_{ij}^n = H_{XY} = 0, \] (2.23a)
\[ G_{ij}^n = G_{i1}^n = G_{i2}^n = G_{i3}^n = 0, \] (2.23b)
\[ \bar{\theta}_i^n = \bar{\rho}_i^n = 0. \] (2.23c)

Estimates from X-ray crystal structure data of values of the elastic moduli \( F_{ij}^n, G_{ij}^n, H_{ij}^n \) and the intrinsic parameters \( \theta^n_{XY}, \rho^n_{XY} \) for each distinct combination of a base pair and its successor \([5, 6]\) confirm that these moduli and intrinsic parameters are strongly dependent on the nucleotide composition. If we focus attention on the angular variables \( \theta^n_{XY} \), we can give as follows a rough
summary of the nature of the sequence-dependence of moduli and intrinsic parameters of the B-form of DNA.\textsuperscript{11} It follows from Eqs. (2.21) that the intrinsic tilt $\bar{\theta}_{XY}^n$ obeys the relation $\bar{\theta}_{XY}^n = -\bar{\theta}_{XY}^n$, and the available data indicate that its largest value is $\sim 1.5^\circ$. The data indicate that the intrinsic roll $\bar{\theta}_{XY}^n$ is positive with its largest value $\sim 5^\circ$ and thus more than 3 times the largest value of $\bar{\theta}_{XY}^n$.

The smallest value of $\theta_{XY}^n$, $\bar{\theta}_{XY}^n$ is $\sim 0.5^\circ$. The ratio of the tilt modulus $F_{11}^n$ to the roll modulus $F_{22}^n$ varies in the range $1 \sim 5$, and the ratio of the twist modulus $F_{33}^n$ to the harmonic moment $A_{XY}^n = 2/[\{1/2F_{11}^n\} + (1/2F_{22}^n)]$ of the tilt and roll moduli varies in the range $0.5 \sim 1.8$. The twist-rolling modulus $F_{23}$, in units of $kT/\text{deg}^2$, varies in the range $\sim 0.00 \sim 0.03$.

In Ref. 27, Gonzalez & Maddocks develop a theory that relates the moduli $F_{ij}^n, G_{ij}^n, H_{ij}^n$ and the intrinsic parameters $\bar{\theta}_{ij}^n, \bar{\rho}_{ij}^n$ to statistical averages and correlations between $\bar{\theta}_{ij}^n$ and $\rho_{ij}^n$ for ensembles of configurations obtainable from all-atom molecular dynamics simulations.

**III. DERIVATION OF THE PRINCIPAL RESULTS**

**Symmetry relations**

The underlying assumptions behind Eq. (2.2) is that $\psi^n$ in Eq. (2.1) is given by a function $\bar{\psi}^n$ of $r^n$ and the two right-handed orthonormal triads $d_{1}^n, d_{2}^n, d_{3}^n$ and $d_{1}^{n+1}, d_{2}^{n+1}, d_{3}^{n+1}$; thus

$$\psi^n = \bar{\psi}^n(d_{1}^n, d_{2}^{n+1}, r^n), \quad (i, j = 1, 2, 3). \quad (3.1)$$

The principle of material frame indiscernibility requires that $\bar{\psi}^n$ obey, for each proper orthogonal second-order tensor $J$, the relation

$$\bar{\psi}^n(d_{1}^n, d_{2}^{n+1}, r^n) = \bar{\psi}^n(Jd_{1}^n, Jd_{2}^{n+1}, Jr^n), \quad (3.2)$$

as an identity. It follows from this last relation and Cauchy’s representation theorem for scalar-valued isotropic functions of lists of vectors that there is a function such that\textsuperscript{12}

$$\psi^n = \bar{\psi}^n(D_{ij}^n, r_i^n); \quad (3.3)$$

this relation is equivalent to Eq. (2.2).

Now, the functions $\bar{\psi}^n$ and $\bar{\psi}^n$ are assumed to be determined by the (ordered) pair XY of nucleotide bases located at the $n$-th and $(n+1)$-th positions on the sugar-phosphate chain for which $n$ increases in the 5′-3′ direction. Hence for $\bar{\psi}^n$ and $\psi^n$ we may write $\bar{\psi}_{XY}^n$ and $\psi_{XY}^n$. To derive restrictions that the antiparallel arrangement of the oriented sugar-phosphate chains of duplex DNA impose on these functions, we note that, as the complements of A and G are X = T and G = C, the 16 ordered pairs XY separate into 2 classes. One class contains the 4 self-complementary pairs {AT, GC, TA, CG}, each of which, because it has Y = X, obeys the relation $XY = YX$. The remaining class of 12 is made up of 6 sets of complementary pairs, i.e., pairs {X1Y1, X2Y2} with X2Y2 = Y1X1, namely, {AA, TT}, {AG, CT}, {AC, GT}, {GA, TC}, {GG, CC}, {TG, CA}.

Under the transformation from $d_{1}^n, d_{2}^{n+1}, r^n$ to $d_{1}^m, d_{2}^{m+1}, r^m$, where

$$\begin{align*}
    d_{1}^m &= d_{1}^{n+1}, \\
    d_{1}^{m+1} &= d_{1}^n, \\
    d_{2}^m &= -d_{2}^{n+1}, \\
    d_{2}^{m+1} &= -d_{2}^n, \\
    d_{3}^m &= -d_{3}^{n+1}, \\
    d_{3}^{m+1} &= -d_{3}^n, \\
    r^m &= r^n,
\end{align*} \quad (3.4a)$$

and $m = N - n + 1$, $\psi^n$ remains unchanged, i.e., $\bar{\psi}^n = \bar{\psi}^n$, because the rectangles $\mathfrak{B}^n$, although they are renumbered in reverse order, keep their previous spatial positions and orientations. The transformation takes the vectors $d_{1}^n, d_{2}^{n+1}, d_{3}^n$, into vectors $d_{1}^m, d_{2}^{m+1}, d_{3}^m$, that again point toward the short edges that have corners attached to the chain for which $n$ increases in the 5′-3′ direction (which is the one for which $n$ decreased in that direction before the transformation). As the transformation replaces XY with its complement YX while leaving $\psi^n$ invariant, the functions $\bar{\psi}_{XY}^n$ and $\bar{\psi}_{XY}^n$ must obey the relation

$$\bar{\psi}_{XY}(d_{1}^n, d_{2}^{n+1}, d_{3}^n, r^n) = \bar{\psi}_{XY}(d_{1}^m, d_{2}^{m+1}, d_{3}^m, r^m), \quad \text{i.e.,}$$

$$\bar{\psi}_{XY}(d_{1}^n, d_{2}^{n+1}, d_{3}^n, r^n) = \bar{\psi}_{YX}(d_{1}^{m+1}, -d_{2}^{m+1}, -d_{3}^{m+1}, d_{1}^m, -d_{2}^m, -d_{3}^m, -r^n). \quad (3.5)$$

or, equivalently,

$$\bar{\psi}_{XY}(D_{ij}^n, r_i^n) = \bar{\psi}_{YX}(Q_{ik}D_{ij}^nQ_{kj}, Q_{il}D_{ij}^nQ_{lj}, r_i^n). \quad (3.6)$$

where $Q_{ij}$ is as in Eq. (2.22). If the pair XY is self-complementary, i.e., is AT, GC, TA, or CG, then $\bar{\psi}_{XY} = \bar{\psi}_{XY}$, and Eq. (3.5) becomes an identity for the function $\bar{\psi}_{XY}$.

As the tangent spaces to the manifold $\mathfrak{M}$ of proper orthogonal $3 \times 3$ matrices are of dimension 3, each point of $\mathfrak{M}$ has a neighborhood in which $\mathfrak{M}$ has a smooth 3- coordinate parametrization of the type discussed in Section II, and we can write

$$D_{ij}^n = D_{ij}(\theta_{1}^n, \theta_{2}^n, \theta_{3}^n), \quad (3.7)$$

where the functions $D_{ij}$ are independent of $n$ and locally one-to-one with continuously differentiable inverses. In terms of the kinematical variables $\theta_{i}^n$ and $\rho_{i}^n$ defined in Eqs. (2.5)–(2.7), the Eqs. (3.4) take the form

$$\begin{align*}
    \theta_{1}^n, \theta_{2}^n, \theta_{3}^n, \rho_{1}^m, \rho_{2}^m, \rho_{3}^m &= \theta_{1}^n, \theta_{2}^n, \theta_{3}^n, \rho_{1}^m, \rho_{2}^m, \rho_{3}^m, \\
    \theta_{1}^n, \theta_{2}^n, \rho_{1}^m, \rho_{2}^m, \rho_{3}^m &= \theta_{1}^n, \theta_{2}^n, \rho_{1}^m, \rho_{2}^m, \rho_{3}^m, \quad (3.8)
\end{align*}$$

\textsuperscript{11} The values and ranges we give for the moduli and parameters are based on data reported in Ref. 6.

\textsuperscript{12} In this application of Cauchy’s representation theorem, we make use of the fact that $d_{1}^{n+1} \cdot r^n$, as it equals $D_{ij}^n r_i^n$, is known once $D_{ij}^n$ and $r_i^n$ are known for $i, j = 1, 2, 3$.\[2pt]
and hence Eq. (3.5) is equivalent to the symmetry relation Eq. (2.9).

For use below, we note that because the 3 matrices 
$[V^i_j], \ (i = 1, 2, 3), \ \text{with components}$

$$iV^i_j = \hat{D}_{ik} \frac{\partial \hat{D}_{jk}}{\partial \theta^n_i},$$

de are skew in the sense that $iV^i_j = -V^i_j$ for all $i, j$, there are numbers $\xi_{kl}^n \ (k, l = 1, 2, 3)$, such that\footnote{\varepsilon_{ijk} \text{ is the permutation symbol of Levi-Civita.}}

$$iV^i_j = \varepsilon_{ijk} \xi_{kl}^n. \quad (3.10)$$

The local invertibility of the function $[\hat{D}_{ij}]$ implies that the columns of the matrix $[\xi_{kl}^n]$ form a (not necessarily orthogonal) basis for the tangent space of $\mathcal{M}$ at the point with coordinates $\theta^n_1, \theta^n_2, \theta^n_3$, and hence $[\xi_{kl}^n]$ has a matrix inverse $[\xi_{kl}^{-1}]$. For the transpose of $[\xi_{kl}^{-1}]$ we write $[\Gamma_{kl}^n]$:

$$\Gamma_{kl}^n \xi_{jk}^n = \delta_{ij}. \quad (3.11)$$

**Equations of equilibrium**

A configuration $Z$ of a DNA segment with $N+1$ base pairs is specified by giving the $(N+1)$-tuple $(\{d^n_1, d^n_2, d^n_3, x^n\})_{n=1}^{N+1}$, of ordered sets $(d^n_1, d^n_2, d^n_3, x^n)$ in which $d^n_1, d^n_2, d^n_3$ is a right-handed orthonormal basis and $x^n$ a point in space. The total energy $E$ of the segment is given by Eq. (2.10) in which $P = P(Z)$ is the potential energy of the external forces and moments acting on the segment. A configuration $Z^\#$ is said to be in equilibrium if for each one-parameter family

$$s \rightarrow Z(s), \quad Z(0) = Z^\#, \quad -\varepsilon < s < \varepsilon, \quad (3.12)$$

of configurations compatible with the imposed constraints (e.g., the strong anchoring end conditions or the closure conditions of Eq. (2.16)) there holds $E'(0) = 0$, i.e., in view of Eqs. (2.1) and (2.10),

$$\sum_{n=1}^{N} (\psi^n)'(0) + P'(0) = 0; \quad (3.13)$$

here primes indicate derivatives with respect to $s$.

To derive the variational equations implied by this definition, we employ Eq. (2.2) which yields

$$\psi^n)' = \frac{\partial \psi^n}{\partial \theta^n_i} (\theta^n_i)' + \frac{\partial \psi^n}{\partial r^n_i} (r^n_i)'$$

(3.14)

where

$$(r^n_i)' = (d^n_i \cdot r^n)' \quad (3.15)$$

and, in view of Eq. (3.7), $(\theta^n_i)'$ obeys the relation

$$\frac{\partial \hat{D}_{ik}}{\partial \theta^n_i} (\theta^n_i)' = (P^n_i)' = (d^n_i \cdot d^n_j')'. \quad (3.16)$$

As the vectors $d^n_1, d^n_2, d^n_3$ are orthonormal, there is a vector $w^n$ such that

$$d^n_i' = w^n \times d^n_i. \quad (3.17)$$

By Eqs. (3.9)–(3.11), (3.15), and (3.17),

$$\left(\left(r^n_i\right)'ight)' = d^n_i \cdot [(r^n_i)' - w^n \times r^n], \quad (3.18a)$$

$$(\theta^n_i)' = \Gamma^n_{jk} d^n_j \cdot (w^n + w^n), \quad (3.18b)$$

$$P' = \sum_{n=1}^{N} \left(\left(d^n_i \times \frac{\partial P}{\partial d^n_i}\right) \cdot w^n + \frac{\partial P}{\partial x^n} (x^n)'\right), \quad (3.18c)$$

and, if we put

$$f^n = f^n_i d^n_i, \quad f^n = \frac{\partial \psi^n}{\partial \theta^n_i}, \quad (3.19a)$$

$$m^n = m^n_i d^n_i, \quad m^n_i = \frac{\partial \psi^n}{\partial \theta^n_i}, \quad (3.19b)$$

$$\varphi^n = \frac{\partial P}{\partial x^n}, \quad (3.19c)$$

$$\mu^n = d^n_i \times \frac{\partial P}{\partial x^n}, \quad (3.19d)$$

and write $v^n = (x^n)'$, then Eqs. (3.14) and (3.18c), respectively, can be cast into the forms

$$\psi^n)' = m^n \cdot (w^n + w^n) + f^n \cdot (v^n - v^n + w^n) \cdot r^n, \quad (3.20)$$

$$P' = \sum_{n=1}^{N} (\mu^n \cdot w^n + \varphi^n \cdot v^n). \quad (3.21)$$

In the last two equations, although end conditions affect the choice of the vectors $w^1, v^1, w^{N+1}$, and $v^{N+1}$, the vectors $w^n$ and $v^n$ can be selected arbitrarily for $n = 2, \ldots, N$. Hence, the relation (3.13) holds for the configuration $Z^\#$ if and only if that configuration obeys the following variational equations,

$$f^n - f^{n-1} = \varphi^n, \quad (3.22)$$

and natural end conditions,

$$(m^n - m^{n-1}) = f^n \cdot w^n + \mu^n, \quad (2 \leq n \leq N), \quad (3.22)$$

$$(m^1 - f^1 \cdot w^1) \cdot w^1 - (m^N + \mu^{N+1}) \cdot w^{N+1}$$

$$+ (f^1 - \varphi^1) \cdot v^1 - (f^N + \varphi^{N+1}) \cdot v^{N+1} = 0. \quad (3.23)$$

The relations (3.22) are the analogues in the present theory of the equations of balance of moments and forces in Kirchhoff’s (continuum) theory of elastic rods [28–31] and several recent generalizations of that theory (vid., e.g., the treatise of Antman [32, Section VIII]).
An equilibrium configuration $Z^\#$ is stable if, for each one-parameter family of configurations that obeys (3.12) and is compatible with the imposed constraints, there holds, in addition to Eq. (3.13),

$$0 < E''(0) = \sum_{n=1}^{N} (\psi^n)''(0) + P''(0). \quad (3.24)$$

In practice, to verify stability we employ Eqs. (3.13)–(3.18) to express $E''(0)$ as a quadratic form $Q$ on the $6(N+1)$-dimensional space of the components of $w^1, v^1, \ldots, w^{N+1}, v^{N+1}$ with respect to one fixed basis, e.g., $d_1^i, d_2^i, d_3^i$. If $\lambda$, the smallest proper number of $Q$, is less than zero, the configuration $Z^\#$ is unstable. If $\lambda > 0$, $Z^\#$ is stable. This test was used to verify statements made in Section IV about the stability of calculated configurations.

IV. EXAMPLES OF EQUILIBRIUM CONFIGURATIONS

Much of the research on DNA elasticity employs an idealized elastic rod model, based on the assumption that a duplex DNA molecule is an intrinsically straight, homogeneous, elastic rod obeying the special case of Kirchhoff’s general theory in which there are just two elastic constants: a twisting modulus and a bending modulus. Several recent studies (e.g., Refs. 11–19) of the influence of intrinsic curvature on the elastic response of DNA are based on Kirchhoff’s general theory in which a rod has two bending moduli and, in addition, the possible presence of intrinsic curvature and torsion is taken into account. The constitutive relation for the elastic energy of a rod obeying that theory does not contain cross-terms directly coupling the twisting moment to the local curvature. It is known that in a materially homogeneous region of a rod with intrinsic curvature the density of excess twist is, in general, not uniform in equilibrium. In Ref. 12 it is shown that the equations of equilibrium appropriate to Kirchhoff’s theory imply that when intrinsic curvature is present the derivative of twist with respect to distance along the rod axis is a function of local curvature. See also papers of Bauer, Lund, & White [11, 14, 16] and Garrivier & Fourcade [19] and the calculations therein of the effect of intrinsic curvature on the shape and twist density profile of o-rings [11, 14, 16] and plectonemic loops [19].

We here give examples of calculated equilibrium configurations of DNA minicircles obeying the theory presented in Section II. In order to obtain insight into the implications of our theory, we deliberately treat cases in which known results for intrinsically curved rods obeying Kirchhoff’s theory can yield physical intuition as to what to expect. We therefore treat minicircles that may be called “DNA o-rings” because they are intrinsically bent in such a way that in stress-free configurations their axial curves are nearly circular. The helical nature of DNA makes it impossible to form a stress-free o-ring with the intrinsic kinematical variables the same at each base-pair step, for if they were the same, the bendings occurring in the two halves of each helical repeat length, being then of equal magnitudes but in opposite directions, would cancel. For this reason, the minicircles we treat are composed of 10 bp helical repeat units in which 5 bp form an intrinsically straight segment and the remaining 5 bp form an intrinsically curved segment.\(^{15}\) (The highly curved sequences found in the kinetoplast DNA from the Trypanosomatidae Crithidia fasciculata and Leishmania tarentolae have on one strand stretches of 4 to 6 adenine bases that are separated by stretches of comparable length rich in guanine and cytosine bases [35–37].)\(^{16}\)

In analogy with the familiar assumptions in the continuum theories of DNA elasticity, in our examples $\psi^n$ is taken to be a quadratic function of the kinematical variables. Results are presented for two types of DNA minicircles: For those of type I, the matrix of the quadratic form characterizing $\psi^n$ is diagonal for each $n$. For those of type II, each helical repeat length contains 5 base-pair steps for which the off-diagonal modulus associated with coupling between roll and twist is not zero and hence the minicircle is expected to show chiral phenomena not predicted by Kirchhoff’s classical theory of rods. The configurations shown were obtained by solution of the equations of mechanical equilibrium.

The minicircles we consider have $N = 150$ bp and are

\(^{15}\) Base-pair level models of DNA deformability constructed to account for results of measurements of cyclization kinetics and gel electrophoretic mobility of short DNA segments suggest that highly curved DNA is made up of two types of duplex structure within each helical repeat length: the canonical B-type duplex and a perturbed form in which the base pairs are inclined with respect to the duplex axis [33, 34] as they are in an A-type or C-type duplex.

\(^{16}\) Electron micrographs of a 219 bp segment with such a sequence found in C. fasciculata indicate that the segment has the form of a complete circle [38], and DNA segments with higher intrinsic curvature have been synthesized. Koo et al. [39] have given strong evidence, based a study of cyclization kinetics, to the effect that a 210 bp DNA segment with the sequence (AAAAACGGCAAAAAACGGGC)$_n$ can form a complete circle when stress free. Previously, Ulanovsky et al. [40] had showed that DNA segments with sequences (AAAAATATATAAAAATCTCT)$_n$ can be closed to form minicircles with 105, 126, 147, and 168 bp, i.e., with $n = 5, 6, 7, 8$. They found that the maximum value of the ratio of concentrations at equilibrium of circular and noncircular products occurred when the sequence length was either 126 or 147 bp, and they suggested that such an observation indicates that it may be possible to obtain stress-free minicircles with as few as 137 bp. See also the review of Crothers et al. [41].
composed of 15 repetitions of a 10 bp unit in which the first 5 bp have
\[ (\bar{\theta}_1^n, \bar{\theta}_2^n, \bar{\theta}_3^n) = (0, 0, 36^\circ), \]
\[ (\bar{\rho}_1^n, \bar{\rho}_2^n, \bar{\rho}_3^n) = (0, 0, 3.4 \text{ Å}), \] (4.1)
and hence form an ideal Watson-Crick DNA segment while the remaining 5 bp have\(^{17}\)
\[ (\bar{\theta}_1^n, \bar{\theta}_2^n, \bar{\theta}_3^n) = (0, 7.413^\circ, 35.568^\circ), \]
\[ (\bar{\rho}_1^n, \bar{\rho}_2^n, \bar{\rho}_3^n) = (0, 0, 3.4 \text{ Å}), \] (4.2)
and hence form an intrinsically curved segment. (For the calculations we report, the kinematical variables \( \bar{\theta}_{i,n} \), \( \bar{\rho}_{i,n} \), \( \rho_{i,n} \), \( \bar{\rho}_{i,n} \), \( \rho_{i,n} \), \( \bar{\theta}_{i,n} \) that are schematically illustrated in Figure 1 are taken to be those defined in Ref. 8 and Eqs. (2.5)-(2.7).) A minicircle with this choice of \( N \) and the indicated parameters \( \bar{\theta}_{i,n} \), \( \bar{\rho}_{i,n} \) has a linking number \( \mathcal{L} \) of 15, and in its stress-free configuration its axial curve \( C \) has the appearance of a polygon of 15 sides with rounded vertices that is sufficiently close to a circle to permit us to refer to the minicircle as an o-ring. For both I and II, the moduli \( G_{ij} \) and \( H_{ij} \) are here taken to be independent of \( n \) with \( G_{ij} = 0 \) for all \( i,j \), with \( H_{ij} = 0 \) for \( i \neq j \), and with \( H_{11}, H_{22}, H_{33} \) large enough to suppress deformations that change the displacement variables \( \rho_{i,n} \). The two minicircles differ in the value of the coupling coefficients \( F_{ij} \); for both, \( F_{11}, F_{22}, F_{33} \) are independent of \( n \) and such that \( F_{11} = F_{22} = 4.27 \times 10^{-2} \text{ kT/deg}^2 \); the ratio
\[ \omega = F_{33}/F_{11} \] (4.3)
was set equal to 0.7 for certain calculations and 1.4 for others.\(^{18}\)

**Minicircle I**

Minicircle I is such that, for \( i \neq j \) and \( n = 1, \ldots, 150 \),
\[ F_{ij}^n = 0, \] (4.4)
and hence Eq. (2.19) does not contain off-diagonal terms coupling the kinematical variables.

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\(^{17}\) So as to obtain an o-ring of small size, i.e., with \( N = 150 \) bp, while making the simplifying assumption that the intrinsic shears \( \bar{\rho}_{i,n} \) and \( \bar{\rho}_{i,n} \) are everywhere zero, we chose \( \bar{\theta}_{i,n} \) in Eq. (4.2) to be larger than the available estimates of the upper bound for stress-free values of the roll.

\(^{18}\) The values of \( F_{11} \) and \( F_{22} \) were chosen so that the bending rigidity \( 2/[\{(1/F_{11}) + (1/F_{22})\}] \) of each minicircle equals that of naturally straight DNA with a persistence length of 500 Å. The choice \( \omega = 0.7 \) is then compatible with measurements of fluorescence anisotropy of dyes intercalated in open segments of DNA (see Ref. 42). Measurements of topoisomerase distributions for miniplasmids [43] suggest that \( \omega \) should be close to 1.4. (See also Bouchiat and Mezard [44] who state that single molecule stretching experiments [45] yield a value of 1.6 for \( \omega \).)

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Even when this minicircle is kept free of bound proteins and other chemical agents, it has multiple equilibrium configurations, several of which (each with \( \mathcal{L} = 15 \)) are shown in Fig. 3 where \( \omega = 0.7 \). In that figure, A is the stress-free, nearly circular, minimum energy configuration. The “everted ring” configuration B has the axial curve close to a circle (as does A) and may be described, to a good approximation, as a configuration obtained from A by rotating each base pair in its plane by one half turn (as in the motion called the “smoke-ring rotation”). It has been known for a long time\(^{19}\) that everted ring configurations of o-rings are unstable. The “chair” configuration C is familiar from experience with elastic rings and was treated by Charitat and Fourcade [15] using Kirchhoff’s theory. The stability of chair configurations depends on \( \omega \). We find that (i) when \( \omega < 1 \), it is in the present example, the configuration C is metastable; (ii) C is unstable when \( \omega > 1 \); and (iii) when \( \omega = 1 \), there is a one-parameter family of distinct chair configurations of equal energies.\(^{20}\) The configurations D and E, which to our knowledge have not been discussed before, have less symmetry than C and are unstable; in

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\(^{19}\) See, e.g., the treatise of Thomson and Tait [46]. A modern discussion containing exact solutions of the nonlinear dynamical equations governing the oscillatory motion of an inextensible elastic rod (obeying Kirchhoff’s theory) with uniformly distributed intrinsic curvature and torsion is given in Ref. 13.

\(^{20}\) The (approximate) analysis of Ref. 15 yielded the value \( \omega = 0.9 \) as the critical value of \( \omega \) for loss of stability.
the present example, D lies at a mountain pass for a transition path from C to the globally stable configuration A. The item labeled F, although it corresponds to a transition path from C to the globally stable configuration B, such is not the case for the chair configuration A and approximately zero for the unstable circular configuration B. For that configuration, \( \theta \) has an extremum at \( \alpha, \beta, \gamma, \delta \) and attains its (remarkably large) maximum near the two “apexes” of the chair (labeled \( \alpha, \gamma \)) and the midpoints (\( \beta, \delta \)) of its sides.

FIG. 4: The excess twist \( \Delta \theta_3^n \) (in degrees) as a function of \( n \) for selected configurations of Fig. 3. The points on the graph for the chair configuration C that are labeled \( \alpha, \beta, \gamma, \delta \) have the geometric locations indicated in Fig. 3 and correspond to the apexes (\( \alpha, \gamma \)) of the chair and the midpoints (\( \beta, \delta \)) of its sides.

\[ \Delta \theta_3^n = \theta_3^n - \theta_3^0, \]

for \( 1 \leq n \leq 150 \), which would make the minicircle not only homogeneous in \( n \) but also one that was formed from intrinsically straight DNA (with \( \theta_3^n = \theta_3^0 = 0 \)).\(^{21}\) Then the equilibrium shape and elastic energy of the minicircle would depend on the number of molecules of EtBr intercalated in it but would be essentially independent of their distribution along \( C \).\(^{22}\) We have found, however, that, because Minicircle I is an o-ring formed from DNA that is intrinsically curved in accord with Eqs. (4.1) and (4.2), its equilibrium shape and elastic energy are strongly dependent on both the number and the distribution along \( C \) of the bound molecules.\(^{23}\)

FIG. 5: Influence of the spatial distribution of intercalated molecules on the minimum elastic energy configuration of Minicircle I. Shown are calculated results for cases in which 10 molecules are intercalated into the minicircle with each inducing the local change \( \theta_3^n \rightarrow \theta_3^n - 30^\circ \), \( \rho_3^n = \rho_3^n + 3.4 \AA = 6.8 \AA \). The sites of binding are shown as dark bands. In configurations A and A* these sites are equally spaced along the axial curve; in B and B* they occupy every other base-pair step in two antipodally placed 11 bp subsegments of the minicircle; in C and C* they occupy every other base-pair step in a single 21 bp subsegment. As indicated, \( \omega = 0.7 \) for A, B, C, and \( \omega = 1.4 \) for A*, B*, C*.

It has been known for a long time that one can induce negative supercoiling in closed DNA by reacting it with an intercalating agent, such as ethidium bromide (EtBr), that binds to DNA between base pairs and causes local reduction of the intrinsic twist of the double helix [47]. If, while maintaining the other assumptions we have made about Minicircle I, we were to assume that Eqs. (4.1) hold for \( 1 \leq n \leq 150 \), which would make the minicircle not only homogeneous in \( n \) but also one that was formed from intrinsically straight DNA (with \( \theta_3^n = \theta_3^0 = 0 \)).\(^{21}\) Then the equilibrium shape and elastic energy of the minicircle would depend on the number of molecules of EtBr intercalated in it but would be essentially independent of their distribution along \( C \).\(^{22}\) We have found, however, that, because Minicircle I is an o-ring formed from DNA that is intrinsically curved in accord with Eqs. (4.1) and (4.2), its equilibrium shape and elastic energy are strongly dependent on both the number and the distribution along \( C \) of the bound molecules.\(^{23}\)

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Such a minicircle would be a discrete analogue of a closed idealized elastic rod of a type for which much is known about the symmetry and stability of equilibrium states, with and without self-contact [48, 49].

\[ \theta_3^n = \theta_3^0 + \rho_3^n, \]

for \( 1 \leq n \leq 150 \), which would make the minicircle not only homogeneous in \( n \) but also one that was formed from intrinsically straight DNA (with \( \theta_3^n = \theta_3^0 = 0 \)).\(^{21}\) Then the equilibrium shape and elastic energy of the minicircle would depend on the number of molecules of EtBr intercalated in it but would be essentially independent of their distribution along \( C \).\(^{22}\) We have found, however, that, because Minicircle I is an o-ring formed from DNA that is intrinsically curved in accord with Eqs. (4.1) and (4.2), its equilibrium shape and elastic energy are strongly dependent on both the number and the distribution along \( C \) of the bound molecules.\(^{23}\)

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As the o-ring we are considering here is composed of periodically repeating units of length 10 bp, the minimum energy configuration for a given distribution of sites of intercalation would not be affected if each such site were shifted by 10 bp in the direction of increasing \( n \). Although a shift in the distribution by less than 10 bp can affect the minimum energy configuration, our calculations show that for Minicircle I the corresponding change in \( \Psi \) does not exceed 0.15 \( kT \).

Scatchard plots of EtBr binding activity would be a computationally intensive task that has not yet been undertaken. However, the preliminary results shown here do suggest that, for DNA subject to appropriate end conditions, such as those of the Eqs. (2.16) that characterize ring closure, the spatial distribution of EtBr can be expected to be sensitive to assumed intrinsic curvature.

Under the present assumptions about the o-ring and the intercalating agent, although \( \Delta \theta_3^n \) is positive for all \( n \) when the intercalating molecules are equally spaced along the length of the minicircle, if those molecules are concentrated into a short enough subsegment \( \mathcal{S} \), as they are in the minimum energy configurations \( C \) and \( C^* \), \( \Delta \theta_3^n \) becomes negative in a neighborhood of the antipode of the midpoint of \( \mathcal{S} \). (See Fig. 6.)

To investigate the effect of localization of changes in intrinsic twist, we have calculated configurations for cases in which a single molecule of an agent is bound to an o-ring at one base pair step, say that with \( n = 1 \), and reduces the intrinsic twist of the DNA at that site by a large positive amount \( \alpha \), i.e., induces the transformation \( \theta_3^1 \rightarrow \theta_3^1 - \alpha \) while leaving other intrinsic variables unchanged. In a study of the effect of intrinsic curvature on configurations of DNA minicircles obeying Kirchhoff’s theory (in which, as is the case for Minicircle I, the constitutive relations do not contain cross-terms coupling curvature to twisting moment) Bauer, Lund, & White [11, 14, 16] found that a reduction of the excess link by cutting at a single cross-section (which is roughly equivalent to an increase in intrinsic twist at a single base pair step) can lead to remarkably large deformations even when the magnitude of that reduction is less than 1 (i.e., \( |\alpha| < 360^\circ \)) and hence less than the amount required to induce supercoiling in intrinsically straight rods.

Re-
results for Minicircle I with $\omega$ equal to 1.4 and for rather extreme values of $\alpha$ are shown in Figures 7 and 8.\textsuperscript{26} As seen in Fig. 7, as $\alpha$ increases the o-ring folds up, with the folding becoming appreciable for $\alpha$ near to 180°, and at sufficiently high $\alpha$ the o-ring attains a clamshell configuration. When, as here, the change of intrinsic twist is confined to one base-pair step, configurations (but not their energies) are approximately independent of $\omega$. In Fig. 8 one sees that, as expected from the results shown in Fig. 6, the excess twist is negative in a neighborhood of the antipode to the binding site.\textsuperscript{27} As the constitutive equation for the energy of Minicircle I does not contain off-diagonal terms directly coupling roll or tilt to twist, if the twisting agent were one that raises $\theta_1^3$ (i.e., that has $\alpha < 0$), the resulting configurations would be essentially mirror images of those shown in Fig. 7. See, however, Fig. 11 and comments made below about Minicircle II.

**Minicircle II, an o-ring with off-diagonal terms coupling twist to roll**

In the case of Minicircle II, for those values of $n$ at which Eq. (4.1) holds,

$$(F_{12}^n, F_{23}^n, F_{13}^n) = (0, 0, 0) \quad (4.5)$$

and, for the $n$ at which Eq. (4.2) holds,

$$(F_{12}^n, F_{23}^n, F_{13}^n) = (0, \frac{4}{5} F_{22}^n, 0). \quad (4.6)$$

\textsuperscript{26} There are protein structures that can induce a comparable change in the magnitude of twist, but the changes they induce are generally distributed over several base-pairs steps. See, e.g., Ref. 54 reporting a case in which an 8 bp long region is untwisted by 147° in a crystal structure of the human TFIIB-TBP protein complex bound to duplex DNA.

\textsuperscript{27} See also [11].

Thus, in each repeated 10 bp unit, the first 5 bp form an intrinsically straight segment and do not show coupling between kinematical variables, while the remaining 5 bp form an intrinsically curved segment and are such that the twist-roll coupling modulus $F_{23}^n$ is positive and hence raising the roll tends to lower the twist, i.e., an increase in $\theta_3^2$ results in a twisting moment that tends to decrease $\theta_3$. (The positivity of $F_{23}^n$ is compatible with the coupling of parameters observed in high-resolution DNA crystal structures [4, 6].) The calculations shown here for Minicircle II (Figs. 9–12) were done with $\omega = 1.4$.

Although Minicircles I and II both have minimum energy configurations that are stress-free and nearly circular, the effect of a twisting agent on II is dependent on whether the agent reduces or increases the intrinsic twist of DNA. If subject to the transformation $\theta_3^2 \rightarrow \theta_3^2 - \alpha$, Minicircle II (like I) shows appreciable folding when $|\alpha|$ exceeds 180°. However, as one sees in Fig. 9, in contrast to what the theory yields for I, the minimum energy configurations of II for $\alpha$ and $-\alpha$ are not mirror images. The
FIG. 11: Graphs of the writhe $W$ versus $\alpha$ for minimum energy configurations of Minicircle I (light curve) and Minicircle II (heavy curve) bound to a twisting agent. (In both cases $\omega = 1.4$.) The points $A^+, B^+, A^-, B^-, C^-$ correspond to the configurations of Fig. 9, and the points $S^0, S^1, U$ to the configurations of Fig. 12. The configurations at which $\Psi$ has its global minimum for the specified $\alpha$ lie in the regions drawn as solid curves (light or heavy); there are configurations on the heavy dashed curve that are not even local minimizers of $\Psi$ (see Figs. 12 and 13). The $\times$ marks the largest $\alpha$ for which the minimum energy configuration of each minicircle is free from self-contact.

FIG. 12: The stable configurations $S^0, S^1$ and the unstable configuration $U$ of Minicircle II of Fig. 11 with $\alpha = 215.0^\circ$.

The graphs of $\Delta \theta^3_n$ versus $n$ in Fig. 10 for the configurations of Minicircles I and II with $\alpha = 240^\circ$ tell us that in the 5 bp subsegments of II in which coupling is present $\Delta \theta^3_n$ is much lower than it is in the corresponding subsegments of I, which is a consequence of the fact that in those subsegments $\theta^3_n$ (i.e., roll) is larger than its stress-free value. The presence of coupling here induces untwisting by as much as $2.7^\circ$, with the most untwisting attained at the base-pair steps with $n = 49$ and 106 which are located roughly 1/3 of the circumference away from the binding site of the untwisting agent.

The graphs in Fig. 11 showing the dependence on $\alpha$ of the writhe $W$, which is a measure of the folding of the $\alpha$-ring [55, 56], illustrate well the difference in the response of the two minicircles to the binding of a twisting agent. While the response of I is nearly antisymmetric, in the sense that the configurations corresponding to $\alpha$ and $-\alpha$ are close to mirror images and hence $W(-\alpha) \approx 2W(0) - W(\alpha)$, the response of II is chiral and such that, for negative $\alpha$ (i.e., for an increase in intrinsic twist), $W$ changes monotonically with $\alpha$, but for each (positive) $\alpha$ in the interval $211.9^\circ < \alpha < 220.7^\circ$ there are three equilibrium configurations, with two stable and one unstable. When $\alpha = 215.0^\circ$, the two stable configurations (labeled $S^0, S^1$) have equal elastic energy (Fig. 12). As seen in Fig. 13, the configurations with preassigned $\alpha \leq \alpha(S^0)$ that give a global minimum to $\Psi$ form a continuous one-parameter family of configurations which terminates at $S^0$ and is not connected to the family of minimum energy configurations with $\alpha \geq \alpha(S^1)$. At $\alpha = 215.0^\circ$ infinitesimal changes in $\alpha$ result in first-order transitions between the stable equilibrium states $S^0$ and $S^1$. The unstable configuration $U$ with $\alpha(U) = \alpha(S^0) = \alpha(S^1)$ lies at a mountain pass for these transitions. The graph of $\Psi$ versus $\alpha$ seen in Fig. 13 tells us that there is an essentially negligible energy barrier, namely 0.13 $kT$, for the transitions $S^0 \rightarrow S^1$ and $S^1 \rightarrow S^0$ between the “open” configuration $S^0$ and the “partially folded” configuration $S^1$, both of which give to $\Psi$ its global minimum in the class of all configurations that are accessible without change in $\alpha$.  

FIG. 13: Graphs of $W$ versus $\alpha$ and $\Psi$ versus $\alpha$ for equilibrium configurations of Minicircle II with $\alpha$ in a neighborhood of $215.0^\circ$. $S^0$ and $S^1$ are points of exchange of global stability, $U$ determines the energy barrier for the transition $S^0 \rightarrow S^1$. Stability of configurations is indicated as follows: (——) globally stable, (–·–·–) locally stable, (····) unstable.
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APPENDIX

For the matrix $[\Gamma^n_{ij}]$ of Eq. (2.12), the Eqs. (2.5)–(2.6) and (3.9)–(3.11) yield,

$$[\Gamma^n_{ij}] = \begin{bmatrix}
-\frac{\theta^n}{\kappa^n} \sin \zeta^n + \frac{\theta^n \cos \zeta^n}{2 \tan \left( \frac{1}{2} \kappa^n \right)} & -\frac{\theta^n}{\kappa^n} \sin \zeta^n - \frac{\theta^n \cos \zeta^n}{2 \tan \left( \frac{1}{2} \kappa^n \right)} & \tan \left( \frac{1}{2} \kappa^n \right) \cos \zeta^n \\
\frac{\theta^n}{\kappa^n} \cos \zeta^n + \frac{\theta^n \sin \zeta^n}{2 \tan \left( \frac{1}{2} \kappa^n \right)} & \frac{\theta^n}{\kappa^n} \cos \zeta^n - \frac{\theta^n \sin \zeta^n}{2 \tan \left( \frac{1}{2} \kappa^n \right)} & \tan \left( \frac{1}{2} \kappa^n \right) \sin \zeta^n \\
-\frac{\theta^n}{2} & \theta^n & 1
\end{bmatrix} \quad (A.1)$$

For each $j$, the numbers $\Lambda^n_{kl}$ of Eq. (2.15) are the components of a skew matrix $[\Lambda^n_{kl}]$. When $j = 1$,

$$1 \Lambda^n_{12} = \frac{\theta^n (1 - \cos \left( \frac{1}{2} \kappa^n \right))}{(\kappa^n)^2}, \quad 1 \Lambda^n_{13} = \frac{\theta^n \theta^n (2 \sin \left( \frac{1}{2} \kappa^n \right) - \kappa^n)}{2(\kappa^n)^3}, \quad 1 \Lambda^n_{23} = \frac{1}{2} + \left( \frac{\theta^n}{\kappa^n} \right)^2 \frac{2 \sin \left( \frac{1}{2} \kappa^n \right) - \kappa^n}{2 \kappa^n}. \quad (A.2)$$

When $j = 2$,

$$2 \Lambda^n_{12} = \frac{\theta^n \left( \cos \left( \frac{1}{2} \kappa^n \right) - 1 \right)}{(\kappa^n)^2}, \quad 2 \Lambda^n_{13} = \left( \frac{\theta^n}{\kappa^n} \right)^2 \frac{\kappa^n - 2 \sin \left( \frac{1}{2} \kappa^n \right)}{2 \kappa^n} - \frac{1}{2}, \quad 2 \Lambda^n_{23} = \frac{\theta^n \theta^n (\kappa^n - 2 \sin \left( \frac{1}{2} \kappa^n \right))}{2(\kappa^n)^3}. \quad (A.3)$$

When $j = 3$,

$$3 \Lambda^n_{12} = \frac{1}{2} \cos \left( \frac{1}{2} \kappa^n \right), \quad 3 \Lambda^n_{13} = -\frac{\theta^n}{2 \kappa^n} \sin \left( \frac{1}{2} \kappa^n \right), \quad 3 \Lambda^n_{23} = -\frac{\theta^n}{2 \kappa^n} \sin \left( \frac{1}{2} \kappa^n \right). \quad (A.4)$$


