Direct Observation of Azimuthal Correlations between DNA in Hydrated Aggregates

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This study revisits the classical x-ray diffraction patterns from hydrated, noncrystalline fibers originally used to establish the helical structure of DNA. We argue that changes in these diffraction patterns with DNA packing density reveal strong azimuthally dependent interactions between adjacent molecules up to ~40 Å interaxial or ~20 Å surface-to-surface separations. These interactions appear to force significant torsional "straightening" of DNA and strong azimuthal alignment of nearest neighbor molecules. The results are in good agreement with the predictions of recent theoretical models relating DNA-DNA interactions to the helical symmetry of their surface charge patterns.

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Interactions between densely packed DNA are involved in packaging of meters of genetic material within cells, genetic recombination, gene silencing and activation, etc. One unanswered question of the physics of these interactions is whether juxtaposition of DNA is accompanied by alignment and correlations in azimuthal orientations of neighboring molecules (Fig. 1). In nematic liquid crystals, azimuthally dependent interactions (through molecular asymmetry) lead to a biaxially correlated phase [1], observed in lyotropic and thermotropic systems [2]. In DNA aggregates, azimuthal correlations are involved in the formation of the cholesteric phase [3]. A model based on an explicit description of the double helical motif of surface charge distributions in DNA predicts strong azimuthally dependent interactions [4]. It appears to rationalize the observed features of the cholesteric phase [5] and many other phenomena [6]. Still, simpler models with no [7] or weak azimuthal correlations [8] are more commonly used for DNA.

We now present direct experimental evidence of strong azimuthal correlations. We revisit x-ray diffraction patterns of highly hydrated DNA fibers [9]. Such patterns have been known since the celebrated papers of Wilkins *et al.* [10] and Franklin and Gosling [11]. However, the possibility of using them to extract information about correlations in mutual alignment of adjacent molecules has not been realized.

Diffraction theory.—To reveal azimuthal alignment of adjacent helices in *liquid-crystalline* DNA mesophases, the classical diffraction theory [12,13] must be adapted to account for short-range azimuthal order. The scattering intensity from an aggregate of N molecules parallel to the z axis (Fig. 1) is given by

$$I(\mathbf{k}) = \sum_{n=-\infty}^{\infty} \delta_{k_z,gn} I_n(\mathbf{K}, n). \tag{1}$$

Here, $\mathbf{k} \equiv (\mathbf{K}, k_z) \equiv (K, \Phi_{\mathbf{k}}, k_z)$ is the scattering vector; $2\pi/g$ is the helical pitch of DNA;

$$I_n(\mathbf{K}, n) = N\langle |F_M(K, n)|^2\rangle + \langle |F_M(K, n)|\rangle^2 \sum_{i\neq j} \langle e^{-in(\phi_i-\phi_j)} e^{i\mathbf{K}(\mathbf{R}_i-\mathbf{R}_j)}\rangle; \tag{2}$$

ϕ_i is the azimuthal orientation and $\mathbf{R}_i$ is the (x, y) coordinate of the main axis of each molecule i;

$$F_M(\mathbf{K}, n) = \sum_{\nu} f_\nu^a J_n(Kr_\nu^a) \exp[in(\Phi_{\mathbf{k}} - \phi_\nu^a + \pi/2 + gz_\nu^a)]; \tag{3}$$

$\langle \cdots \rangle$ indicates statistical averaging; and f_ν^a and $(r_\nu^a, \phi_\nu^a, z_\nu^a)$ are the scattering amplitude and coordinates of each atom ν of DNA in an *ideal* helical conformation [14]. The first and second terms in Eq. (2) describe *intramolecular* and *intermolecular* scattering correspondingly.

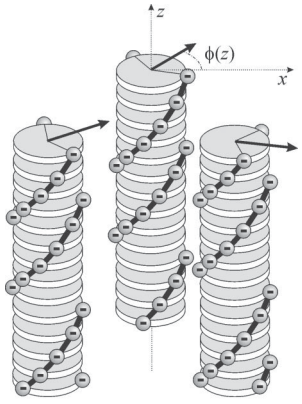

FIG. 1. Azimuthal alignment of adjacent DNA in aggregates. Each base pair is represented by a disk with two negatively charged phosphate groups (spheres) attached to it. The phosphates are connected into the sugar-phosphate backbone. Bold vectors depict altitude-dependent azimuthal orientations $\phi(z)$ of the middle of the minor groove.

For qualitative analysis, the scattering amplitudes of all atoms except phosphates and the difference between $\langle|F_M|^2\rangle$ and $\langle|F_M|\rangle^2$ due to atomic vibrations can be neglected so that

$$\langle|F_M(K,n)|^2\rangle \approx \langle|F_M(K,n)|\rangle^2 \propto \cos^2(n\tilde{\phi}_s)J_n^2(Ka), \quad (4)$$

where $\tilde{\phi}_s$ is the azimuthal half-width of the minor groove and a is the average distance of phosphate centers from the central axis of DNA (in B-DNA, $\tilde{\phi}_s \approx 0.4\pi$ and $a \approx 10$ Å).

The classical Eq. (2) was derived for ideal helices and used to describe *crystalline* arrays with *fixed* or aggregates with random azimuthal orientations of the molecules. In either case, $\langle\exp[-in(\phi_i - \phi_j)]\rangle$ did not have to be calculated and it was often omitted from explicit expressions. These two limits were successfully used to reveal the helical symmetry of DNA. However, such approximation does not account for the nonideality of DNA structure, thermal disorder, and the effect of correlated orientations in noncrystalline aggregates. The latter factors must be considered for a full understanding of the observed diffraction patterns and extracting information about DNA interactions and arrangement in assemblies.

While the difference in azimuthal orientations $(\phi_i - \phi_j)$ and lateral positions $(\mathbf{R}_i - \mathbf{R}_j)$ of *rigid*, ideal, and identical helices does not depend on the "altitude" z, this is not true for real DNA. Specifically, azimuthal coordinate $\phi_i(z)$ of any fixed point on an ideal helix (e.g., the center of its minor groove) depends on the axial coordinate as $\phi_i(z) = \psi_i + gz$ so that $\phi_i(z) - \phi_j(z) = \psi_i - \psi_j$, where we define ψ_i as the azimuthal coordinate at $z = 0$. For real DNA,

$$\phi_i(z) = \psi_i + \delta\phi_i(z) + \bar{g}z, \quad (5)$$

where $2\pi/\bar{g}$ is the *average* helical pitch and $\delta\phi_i(z)$ is the deviation from the ideal helix due to the sequence dependence of the twist angle between adjacent base pairs [15] and torsional fluctuations ($\langle\delta\phi_i(z)\rangle = 0$). Similarly, one can speak of $\delta\mathbf{R}_i(z)$ due to bending fluctuations. As long as the z-dependent fluctuations are small ($\Delta_\phi^2 \equiv \langle\delta\phi_i(z)^2\rangle \ll 1$ and $\Delta_R^2 \equiv \langle\delta\mathbf{R}_i(z)^2\rangle \ll 1$), Eq. (2) can still be used. It applies regardless of the amplitude of azimuthal fluctuations $\delta\psi_i$ caused by rotation of the whole molecule about its long axis, although the calculation of the exact form of $\langle\exp[-in(\phi_i - \phi_j) + i\mathbf{K}(\mathbf{R}_i - \mathbf{R}_j)]\rangle$ might be nontrivial.

For independent, Gaussian $\delta\phi_i$, $\delta\psi_i$, and $\delta\mathbf{R}_i$, in crystalline arrays,

$$\langle e^{-in(\phi_i-\phi_j)+i\mathbf{K}(\mathbf{R}_i-\mathbf{R}_j)}\rangle = e^{-n^2(\Delta_\phi^2+\Delta_\psi^2)}B(K^2\Delta_R^2) \times e^{-in(\bar{\psi}_i-\bar{\psi}_j)+i\mathbf{K}(\bar{\mathbf{R}}_i-\bar{\mathbf{R}}_j)}, \quad (6)$$

where $\bar{\psi}_i$ and $\bar{\mathbf{R}}_i$ are the average azimuthal and positional coordinates of each helix at $z = 0$ and $\Delta_\psi^2 \equiv \langle\delta\psi_i^2\rangle$ is the mean square amplitude of thermal rotation of the whole molecule. The first factor in the right-hand side of Eq. (6) originates from Gaussian averaging of $\exp[-in(\phi_i - \phi_j)]$ over $\delta\phi_i$ and $\delta\psi_i$. It is associated not only with thermal torsional and azimuthal fluctuations but also with quenched torsional disorder due to differences in twist angles between different base pairs [15]. Similarly, $B(K^2\Delta_R^2)$ is a factor associated with positional and bending fluctuations. Its explicit form is not essential for the present analysis and will be presented elsewhere.

For short-range azimuthal or positional order, we can approximate the dependence of the scattering amplitude on the alignment of molecules by

$$I_n(\mathbf{K},n) = N\langle|F_M(K,n)|^2\rangle + e^{-n^2(\Delta_\phi^2+\Delta_\psi^2)}B(K^2\Delta_R^2) \times \langle|F_M(K,n)|\rangle^2\sum_{i\neq j}G(n,\mathbf{K},\bar{\mathbf{R}}_i - \bar{\mathbf{R}}_j) \times e^{-in(\bar{\psi}_i-\bar{\psi}_j)+i\mathbf{K}(\bar{\mathbf{R}}_i-\bar{\mathbf{R}}_j)}. \quad (7)$$

Here the correlation function $G(n,\mathbf{K},\bar{\mathbf{R}}_i - \bar{\mathbf{R}}_j)$ describes the loss of azimuthal and positional order with increasing $|\bar{\mathbf{R}}_i - \bar{\mathbf{R}}_j|$. Outside the correlation range as well as in the absence of any positional or azimuthal order, $G = 0$ and $\bar{\psi}_i - \bar{\psi}_j$ and $\bar{\mathbf{R}}_i - \bar{\mathbf{R}}_j$ do not have to be defined. In a perfect crystal, $G = 1$. In an aggregate with crystalline positional order and no azimuthal correlations, $G = \delta_{n,0}$. Model-dependent calculations of $G(n,\mathbf{K},\bar{\mathbf{R}}_i - \bar{\mathbf{R}}_j)$, based on the statistical mechanical theory of columnar DNA assemblies [16], will be reported elsewhere.

Equation (7) captures the main qualitative features of the effect of azimuthal correlations and correctly describes the limiting cases. It is likely to give a reasonable description of possible diffraction patterns, from which one may extract admissible upper bounds of Δ_ϕ^2 and Δ_ψ^2 even without the knowledge of $G(n,\mathbf{K},\bar{\mathbf{R}}_i - \bar{\mathbf{R}}_j)$. At weak or no azimuthal correlations ($\Delta_\psi^2 \gg 1$), it predicts that intermolecular scattering contributes only to the equatorial Bragg spots at $n = 0$, whose positions are determined by the DNA packing density (Fig. 2). All other diffraction spots on the helical layer lines with $n \neq 0$ originate from intramolecular scattering and depend only on the structure of DNA. As long as the structure remains the same, their positions and intensities should be independent of the aggregate density.

In contrast, strong azimuthal correlations ($\Delta_\psi^2 < 1$) might lead to significant contribution of intermolecular scattering *to nonzero layer lines*, resulting in dependence of the whole diffraction pattern on the aggregate density. However, the intermolecular contribution decreases exponentially with $n^2(\Delta_\phi^2 + \Delta_\psi^2)$ and disappears at higher layer lines even at perfect azimuthal order ($\Delta_\psi^2 = 0$) due to the effect of torsional distortions (Δ_ϕ^2).

Analysis of diffraction data.—We reanalyzed ~60 diffraction patterns from highly oriented, wetted fibers of salmon sperm DNA, initially used to study effects of hydration on the structure of DNA [9]. Preparation of the fibers, the measurement technique, and the equipment were described in the original publications. A succession of x-ray pictures in Fig. 2 illustrates changes in the diffraction pattern caused by decreasing the packing density of DNA. The decreasing distance between the equatorial Bragg spots is related to increasing intermolecular spacing. The meridional (k_z) positions of the layer lines appear to be

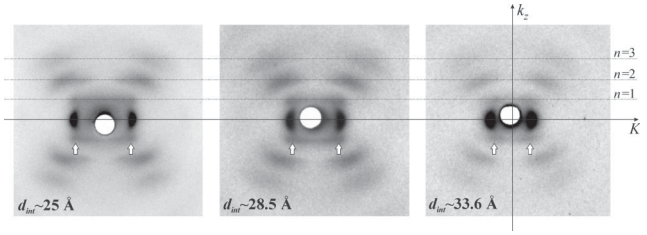

FIG. 2. Dependence of x-ray diffraction patterns from oriented DNA fibers on the interaxial distance d_{int}. The Bragg peaks at the equator (two dark spots at $k_z = 0$, $K = k_{\mathrm{Bragg}}$) originate from the positional order of the molecules ($k_{\mathrm{Bragg}} = 4\pi/\sqrt{3}d_{\mathrm{int}}$ in a hexagonal lattice). Other diffraction maxima along the dotted layer lines ($n = 1, 2, 3$) originate from the helical structure of DNA. Their meridional coordinates (k_z) are determined by the helical pitch of DNA, and K are determined by Eqs. (4) and (7). The white arrows depict the varying locations of the diffraction maxima at the first helical layer line indicating strong azimuthal correlations between adjacent molecules.

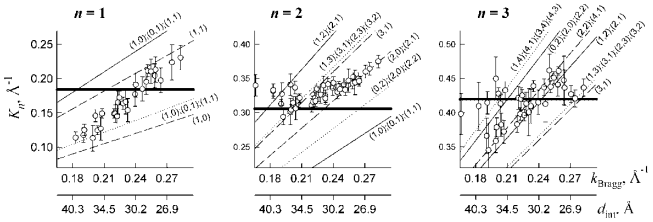

FIG. 3. Variation in the locations K_n of the diffraction maxima on the first ($n = 1$), second ($n = 2$), and third ($n = 3$) layer lines with separation between DNA. The bold lines show expected locations of the *intramolecular* maxima [Eq. (4)]. Thin solid lines show the locations of the diffraction peaks in *hexagonal crystals* with the same azimuthal orientation of all molecules; dashed lines, in crystals with alternating orientations of molecules in consecutive rows; dotted lines, in crystals with different azimuthal orientations of all three molecules in the unit cell. The corresponding Miller indices are shown near each line. *Intermolecular* scattering appears to determine K_1 at all separations. K_2 are dominated by intramolecular scattering at $d_{\mathrm{int}} > 35$ Å, resulting in flattening of the observed dependence. K_3 are consistent with intramolecular scattering at all d_{int}.

unaffected by the packing density, suggesting that the helical pitch, the number of base pairs per pitch, and, thereby, the structure of DNA remain the same [9,17]. However, the positions of the spots at the first helical layer line ($n = \pm 1$) appear to change significantly, tracking the positions of the equatorial Bragg spots. Such changes point to a contribution of intermolecular scattering at least at the first layer line, which is expected only in the presence of strong azimuthal correlations between adjacent molecules.

For further analysis, we digitized the photographs on AGFA Arcus II or FUJI FLA5000 (Fuji Medical Systems) scanners and calibrated them by utilizing the diffraction from calcite crystals added as an internal standard to every sample [9]. We generated several density profiles for each helical layer line, systematically averaged them to reduce the inherent noise, and determined the locations of the diffraction maxima with respect to the meridian. The dependencies of the locations of the diffraction maxima for the first three helical layer lines on the interaxial spacing between DNA are shown in Fig. 3 [18]. The uncertainty in the measurement of the interaxial spacing was estimated as ~0.2–0.5 Å. The horizontal, bold lines show the positions of the maxima for intramolecular scattering expected in the absence of azimuthal correlations between adjacent molecules. Thin solid, dashed, and dotted lines show locations of diffraction maxima for intermolecular scattering expected for three possible types [19] of crystalline arrangement of ideal helices on a hexagonal lattice.

The observed locations of the diffraction maxima at the first and second layer lines are clearly more consistent with intermolecular scattering from azimuthally correlated DNA arrays than with intramolecular scattering in the absence of correlations. Only the third layer line maxima are consistent with the intramolecular scattering. Exactly this behavior is predicted by Eq. (7) for liquid-crystalline aggregates of nonideal helices with strong azimuthal correlations between adjacent molecules. Moreover, a comparison of relative intensities of the intermolecular maxima at the equatorial line and intramolecular maxima at the third helical line with those expected from Eqs. (4) and (7) suggests that $\Delta_\phi^2 + \Delta_\psi^2 < 1$ rad. Our previous estimates [20] indicate that Δ_ϕ^2 accounts for at least a considerable fraction of this value ($0.1 \leq \Delta_\phi^2 \leq 1$ rad), implying that adjacent DNA exhibit strong azimuthal alignment even in the most hydrated aggregates.

Discussion and conclusions.—This study is based on classical x-ray diffraction patterns. Previously, the focus was on the DNA structure [10,11] and its dependence on hydration [9]. The patterns from hydrated fibers were interpreted as diffraction on uncorrelated helices [13], assuming that DNA were "relatively free from the influence of neighboring molecules, each unit being shielded by a sheath of water" [11]. Our analysis suggests *exactly the opposite*, i.e., significant azimuthal alignment of adjacent molecules separated by up to ~20 Å of water (much further apart than estimated [21] in recent computer simulations).

The only alternative explanation of the observed variation in the locations of the diffraction maxima at the first and second layer lines would be a change in the DNA radius, but such a change would affect all of the layer lines and not just the first two. The radius estimated, e.g., from the third layer line appears to be independent of DNA packing density within ~10% accuracy of the measurement. This is consistent with no observed significant changes in the pitch and number of base pairs per turn (which would be affected by radius variation) [17]. However, a twofold radius change would be required to explain the diffraction maxima on the first layer line or an ~50% change to explain the maxima on the second layer line.

Strong azimuthally dependent interactions between DNA in hydrated aggregates were predicted by analysis

of electrostatic forces associated with the helical nature of DNA surface charge [4]. The alignment becomes favorable because it minimizes direct apposition of negatively charged phosphate strands and maximizes their interaction with counterions bound on opposite surfaces. The cost of deviation from the optimal alignment for 500 Å-long DNA fragments was estimated as $E_\psi = k_\psi(\delta\psi)^2/2$, where $k_\psi \sim 1$–$5k_BT$ at 40 to 30 Å interaxial separations [5]. The corresponding $\Delta_\psi^2 = k_BT/k_\psi \sim 0.2$–$1$ rad^2 gives an upper bound for Δ_ψ^2 expected for longer DNA [22], in good agreement with the diffraction data.

Strong azimuthal alignment of adjacent helices is also consistent with and appears to be the driving force of the change in the helical repeat upon DNA aggregation reported in [23]. Indeed, the sequence-dependent variation in the twist angle between adjacent base pairs [15] would make the alignment of long DNA impossible without reducing Δ_ϕ^2 through torsional deformation [24]. The energetic advantage of the alignment appears to be strong enough to overcome the torsional rigidity of DNA and reduce Δ_ϕ^2 below 0.1–1 rad^2, in agreement with theoretical estimates [20].

In conclusion, the evidence for significant azimuthal correlations between adjacent molecules in hydrated DNA aggregates is quite strong, at least for the experimental data discussed here.

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[1] M. J. Freiser, Phys. Rev. Lett. **24**, 1041 (1970).
[2] L. J. Yu and A. Saupe, Phys. Rev. Lett. **45**, 1000 (1980); L. A. Madsen, T. J. Dingemans, M. Nakata, and E. T. Samulski, Phys. Rev. Lett. **92**, 145505 (2004); B. R. Acharya, A. Primak, and S. Kumar, Phys. Rev. Lett. **92**, 145506 (2004).
[3] A. B. Harris, R. D. Kamien, and T. C. Lubensky, Phys. Rev. Lett. **78**, 1476 (1997).
[4] A. A. Kornyshev and S. Leikin, J. Chem. Phys. **107**, 3656 (1997); **108**, 7035(E) (1998).
[5] A. A. Kornyshev, S. Leikin, and S. Malinin, Eur. Phys. J. E **7**, 83 (2002).
[6] A. A. Kornyshev and S. Leikin, Biophys. J. **75**, 2513 (1998); Proc. Natl. Acad. Sci. U.S.A. **95**, 13 579 (1998); Phys. Rev. Lett. **82**, 4138 (1999); **86**, 3666 (2001).
[7] See, e.g., G. S. Manning, Q. Rev. Biophys. **11**, 179 (1978); Y. Levin, Rep. Prog. Phys. **65**, 1577 (2002).
[8] A. B. Harris, R. D. Kamien, and T. C. Lubensky, Rev. Mod. Phys. **71**, 1745 (1999).
[9] S. B. Zimmerman and B. H. Pheiffer, Proc. Natl. Acad. Sci. U.S.A. **76**, 2703 (1979); Cold Spring Harbor Symposia on Quantitative Biology **XLVII**, 67 (1982).
[10] M. H. F. Wilkins, A. R. Stokes, and H. R. Wilson, Nature (London) **171**, 738 (1953).
[11] R. E. Franklin and R. G. Gosling, Nature (London) **171**, 740 (1953).
[12] W. Cochran, F. H. C. Crick, and V. Vand, Acta Crystallogr. **5**, 581 (1952); A. Klug, F. H. C. Crick, and H. W. Wyckoff, Acta Crystallogr. **11**, 199 (1958).
[13] B. K. Vainshtein, *Diffraction of X-rays by Chain Molecules* (Elsevier, Amsterdam, 1966).
[14] For small torsional and bending fluctuations, the deviation from an ideal helical conformation can be described in terms of local fluctuations in ϕ_i and $\mathbf{R}_i$ [see Eqs. (5) and (6)].
[15] W. Kabsch, C. Sander, and E. N. Trifonov, Nucleic Acid Res. **10**, 1097 (1982); A. A. Gorin, V. B. Zhurkin, and W. K. Olson, J. Mol. Biol. **247**, 34 (1995); W. K. Olson, A. A. Gorin, X.-J. Lu, L. M. Hock, and V. B. Zhurkin, Proc. Natl. Acad. Sci. U.S.A. **95**, 11 163 (1998).
[16] A. Wynveen, D. J. Lee, and A. A. Kornyshev, Eur. Phys. J. E **16**, 303 (2005).
[17] A small (~3%), continuous variation in the helical pitch of DNA in hexagonal aggregates was reported [D. Durand, J. Doucet, and F. Livolant, J. Phys. II (France) **2**, 1769 (1992)] and discussed in the context of line-hexatic packing of the molecules [H. H. Strey, J. Wang, R. Podgornik, A. Rupprecht, L. Yu, V. A. Parsegian, and E. B. Sirota, Phys. Rev. Lett. **84**, 3105 (2000); F. Manna, V. Lorman, R. Podgornik, and B. Zeks, in *Proceedings of the 8th European Conference on Liquid Crystals, Sesto, Italy, 2005* (Taylor & Francis, Philadelphia, 2005), p. 72]. However, we found no pitch variation in the samples analyzed here, as originally described in Refs. [9,23]. Regardless, such variation would be negligibly small in the context of the present study.
[18] Uncertainties in the positions of the maxima are shown by the error bars, which do not include systematic error at $n = 1$ potentially caused by stronger background scattering near the beamstop. However, this systematic error should not affect the slope of the measured dependence of K_1 on k_{Bragg}.
[19] V. Lorman, R. Podgornik, and B. Žekš, Phys. Rev. Lett. **87**, 218101 (2001); H. M. Harreis, A. A. Kornyshev, C. N. Likos, H. Löwen, and G. Sutmann, Phys. Rev. Lett. **89**, 018303 (2002); H. M. Harreis, C. N. Likos, and H. Löwen, Biophys. J. **84**, 3607 (2003).
[20] A. G. Cherstvy, A. A. Kornyshev, and S. Leikin, J. Phys. Chem. B **108**, 6508 (2004); D. J. Lee, A. Wynveen, and A. A. Kornyshev, Phys. Rev. E **70**, 051913 (2004).
[21] E. Allahyarov, G. Gompper , and H. Löwen, Phys. Rev. E **69**, 041904 (2004).
[22] The increase in k_ψ with the length of the molecules (expected for rigid, ideal helices) is limited by torsional fluctuations and structural defects in DNA. For instance, at least one of every 1000 DNA base pairs is believed to be always open [W. Saenger, *Principles of Nucleic Acid Structure* (Springer-Verlag, New York, 1984)], resulting in torsional decoupling and independent azimuthal fluctuations of the fragments on each side of the defect.
[23] D. Rhodes and A. Klug, Nature (London) **286**, 573 (1980).
[24] In solution, $\Delta_\phi^2 = L/4\lambda_c$, where L is the length and $\lambda_c \sim 300$–700 Å is the helical coherence length of DNA [20].