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DNA Structural Changes Under Different Stretching Methods Studied By Molecular Dynamics Simulations

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We present a molecular dynamics simulation study of 22-mer DNA conformational variations obtained by stretching both 3′-termini and both 5′-termini. Stretching 3′-termini by 3.5 nm required 142 kJ mol^{-1} and the force plateau was ~80 pN, whereas stretching 5′-termini by the same length required 190 kJ mol^{-1} and the force plateau was ~100 pN. Stretching 3′-termini led to a larger untwisting of the double helix and the successive base pairs rolled to the side of the DNA minor groove, while stretching 5′-termini resulted in the base pairs rolling to the major groove side and reducing of the diameter of DNA molecule. The most distinctive difference between stretching 3′-termini and 5′-termini was that at the force plateau region stretching the 5′-termini resulted in breakage of the base pairs, which considerably disturbed the structure of the DNA double helix. All of the variations of base rotation and translation for both stretching methods took place when the relative length of DNA l was longer than 1.2, which was the point the force plateau appeared.

1. Introduction

DNA-protein interactions are essential to numerous biological functions and play an important role in replication, transcription, translation, and DNA repairing.[1] Stretching of DNA and RNA molecules has been a method that was used to probe their interactions with proteins.[2,3] Recently, with the development of single molecule manipulation, the mechanical properties of DNA have been found to play an important role in DNA–protein interactions.[4–6] For example, changes in DNA tension have been shown to greatly influence the interaction between DNA and DNA gyrase.[4] The mechanical properties of DNA also apparently influence the interaction between DNA and restriction endonucleases.[5,6] Clearly, studying the structure of stretched DNA can benefit the understanding of protein recognition and interaction with DNA molecules.

With the development of micromanipulation techniques, the experimental studies on the behaviour of single DNA molecules under stretching became possible since 1990s.[7] In 1996, two groups from Europe and America found B- to S- form phase transitions occuring at the stretching force about 70 pN.[8,9] Lager et. al found the qualitatively different structural transitions of DNA molecules depending on whether the DNA winding is allowed to relax, or held fixed during the stretching process.[10] Rief and co-workers found that both the B–S transition force plateau and the melting transition occur at significantly lower forces in poly(dA–dT) compared to poly(dG–dC)[11] and they investigated the melting transition at a load between 35 and 300 pN, where the value depends on the loading rate.[12] The melting forces of short DNA molecules varies from 20–50 pN depending on the number of base pairs as reported by Strunz et al.[13] Two years later, Pope and co-workers found that the energy barrier of melting was about 9–13 kcal mol^{-1} in a 12-mer duplex.[14] In 2003, Bustamante gave a very good review on this topic.[15] Later, Prentiss and co-workers found that the DNA structures of stretched 3′-termini differed from the structure of stretched 5′-termini via observing the force hysteresis with glyoxal appearing in the solvent.[16] Goh et al. showed that the contribution to melting forces from stacking interactions was more important than that from hydrogen bonding.[17] Very recently, Gaub et al. found that the DNA molecules were easily ruptured by stretching the 5′-termini rather than stretching the 3′-termini.[18]

Molecular dynamics (MD) simulations are useful to describe single molecules at the atomic level.[19–23] By engaging the MD simulation, Mackerel et al. found the molecular dynamics simulations of the DNA stretching process were agreed with experiment results in quality.[24] Zhou et al. found that the dipole variations of water molecules caused a B–A transition.[25] In addition, van der Maarel showed that multivalent counter-ions between two parallel DNA double strands can form ion bridges within a few nanoseconds.[26] Lavery et al. studied the stretching of DNA molecules by different ways with molecular mechanics calculations,[27,28] which suggested that the S-form had a ladder structure where the base pairing was retained, but the nucleobases were tilted with respect to the axis of the helix and base stacking was interstrand, rather than intrastrand.[29] In 2005, the stability of the ladder S-DNA structure was questioned by some MD simulations of DNA stretch-

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ing,[30,31] where the entropy contributions to the stability were analyzed and the ladder structure was proven to be unstable. In 2006, the elongation of a overwinding DNA molecule was observed by Lionnet and co-workers.[32]

In this paper, we present a molecular dynamics study of DNA structural changes induced by gradually increasing the distance between the opposing 3′-termini (O3′, O3′) and the opposing 5′-termini (O5′, O5′) of a 22mer DNA (22 base pairs) molecule. The simulation shows that the two methods causes different variations in the DNA conformation. Stretching both 3′-termini by 3.5 nm requires 142 kJ mol^{-1} and the force plateau is at ~80 pN, whereas stretching both 5′-termini by the same length requires 190 kJ mol^{-1} and the force plateau is at ~100 pN. Stretching 3′-termini leads to a larger untwisting of the double helix and the successive base pairs rolled to the side of DNA minor groove. In contrast, stretching 5′-termini results in the base pairs rolling to the major groove side and reduction of the diameter of the DNA molecule. The most important difference between stretching both 3′-termini and both 5′-termini is that, at the force plateau region, stretching of the 5′-termini results in breakage of the base pairs, which considerably disturbs the structure of the DNA double helix. All of the variations of base rotation and translation for both stretching methods take place when the relative length *l* of the DNA molecule is longer than 1.2, which is the point the force plateau appears. These conformational changes give us an insight into DNA melting transitions occurring during the stretching process. The findings from this study may also help to further understand the DNA–protein interaction.

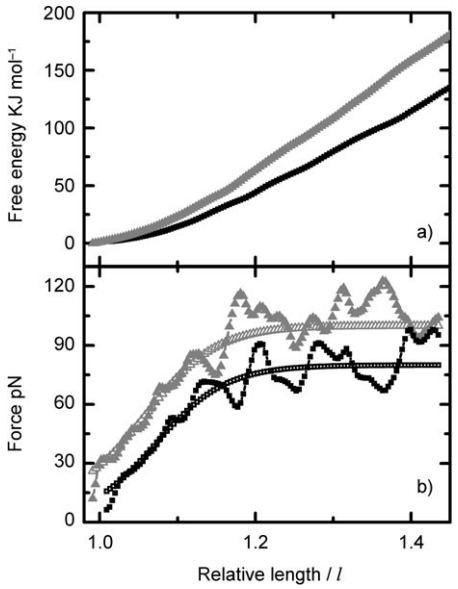

Figure 1. a) Plot of the free energy versus relative extension *l* as determined by a weighted histogram analysis of the umbrella sampling simulations of stretching 3′-termini (■) and stretching 5′-termini (▲). b) Plot of force (pN) versus relative extension *l*. Forces have been obtained as the derivation of the free energy profile stretching 3′ termini (■) and stretching 5′ termini (▲). Fitting curve of forces in stretching 3′-termini (□) and the plateau is 80 pN; (△) is the fitting curve of forces in stretching 5′-termini and the plateau is 100 pN.

2. Results and Discussion

2.1. Free Energy and Forces

The free energy with respect to the DNA relative length *l* is shown in Figure 1 a. It is interesting that the stretching 5′-termini requires more energy than stretching the 3′ termini. The difference becomes more significant as the length of the DNA molecules increases. At $l=1.45$, the free energy of stretching both 5′-termini is about 40 kJ mol^{-1} higher than that of stretching both 3′-termini. In Figure 1 b we present the forces derived from the free energy profiles. Stretching 3′-termini requires a smaller force than stretching the 5′-termini. The following Boltzman function shown in Equation (1)

$$\text{force} = \frac{A_1 - A_2}{1 + e^{\frac{l-l_0}{dl}}} + A_2 \qquad (1)$$

is used to fit the force profiles. For stretching 3′-termini, the fitting parameters are $l_0=1.07$, $A_1=0.0$, $A_2=80.0$; for stretching 5′-termini, $l_0=1.06$, $A_1=10.0$, $A_2=100.0$. There were clear plateaus in the fitting lines at about 80 pN and 100 pN for stretching 3′-terimini and stretching 5′-terimini respectively, which are consistent with Piana's simulation results of the force plateau at about 100 pN in stretching both 5′-termini.[30] We note that there were still nonzero forces at the beginning of the stretching. This is because the DNA molecules usually curve in the native state,[33] while the initial state of the DNA molecule for our simulations was assumed to be straight. We believe that the forces of about 10 pN at the initial state results from the requirement for straightening the DNA molecule. As shown in Figure 1 b, our simulation of stretching processes can be divided into two parts: the force increasing region and the plateau portion with consistent force. The transition point of the force occurs at about relative extension $l=1.2$.

2.2. Base Rotation

In order to understand the free energy difference between the two stretching methods, we present a systemic study of the DNA deformations in base rotation and translation. The parameters in Figure 2 describe the variations of the base orientations.[34,35] As the DNA was stretched, the fluctuation of all of the parameters became large, especially for stretching both 5′-termini. Other than the dihedral buckle (Figure 2 f), the other rotation parameters were affected by different stretching methods.

The inclination (Figure 2 c) and the roll (Figure 2 h) did not change with stretching either both 5′-termini or both 3′-termini prior to $l\approx1.2$. The two parameters increased or decreased after the point where the force plateau began. It is easy to understand the variations of the inclination: picturing the DNA molecule as a ladder, the "ladder" will incline to different sides when stretched at different termini. The inclined base pairs during stretching are favorable with the calculation of Lavery and Lebrun using molecular mechanics.[28] Stretching both 3′-termini causes the angle roll to decrease slightly, while stretch-

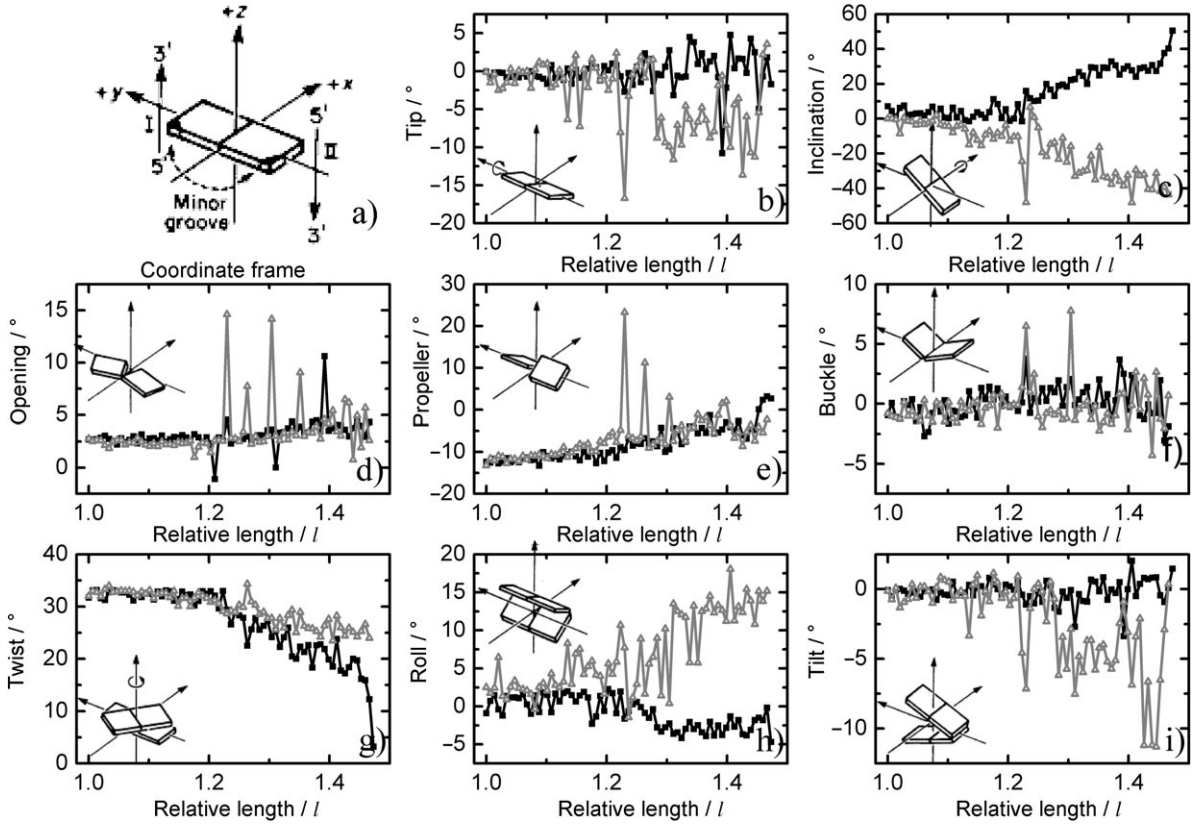

Figure 2. Plot for the variables of base rotation versus relative extension *l*. a) The standard coordinate frame; b) Tip is the angle of a base pair rotating around *y* axis; c) Inclination is the angle of a base pair rotating around the *x* axis; d) Opening is the angle of two bases in a base pair rotating in opposite directions about the *z* axis; e) Propeller is the dihedral angle of two bases in a base pair about the *y* axis; f) Buckle is the dihedral angle of two bases in a base pair about the *x* axis; g) Twist is the angle between two successive base pairs about the *z* axis; h) Roll is the dihedral angle of two successive base-pairs about the *y* axis; i) Tilt is the dihedral angle of two successive base-pairs about the *x* axis.[39,43] In every image, stretching both 3′-termini: (■); stretching both 5′-termini: (○).

ing both 5′-termini increases the roll. The angles of tip and tilt did not show considerable changes on stretching the 3′-termini; these decreased at $l = 1.3$ on stretching the 5′-termini, then they returned at $l \approx 1.45$. The opening and the propeller increased more slowly by stretching the 3′-termini than by stretching the 5′-termini. The angle twist decreases more quickly by stretching the 3′-termini than by stretching the 5′-termini. The variations of the twist corroborate the findings of Lavery and Lebrun that stretching the 5′-termini leads to less unwinding than stretching the 3′-termini.[28]

Overall, stretching both 3′-termini untwisted the double helix and kept the base pairs parallel to each other, except that the successive base pairs slightly rolled to the side of the DNA minor groove. The stretching 5′-termini usually caused larger fluctuations, showing that the method of stretching the 5′-termini disturbs the DNA base structure.

2.3. Base Translation

As seen in Figure 3, stretching both 3′-termini did not result in significant changes in the variables of base translations, except for the rise (Figure 3g). In contrast, stretching both 5′-termini causes the base pairs to move away from the original axis of the double helix in both the *x* and *y* direction. The small decrement of the stagger (Figure 3d) showed that stretching 5′-termini tends to make the base pair plane break into two different planes. The small decrement of the stretch (Figure 3e) implies that the two bases move closer to each other. Combining the variations of the stagger and the stretch, the bases attempt to insert into the opposite strand on stretching the 5′-termini. Stretching 5′-termini increases the distances between successive base pairs more than stretching the 3′-termini. As the variable of base rotations, stretching 5′-termini causes larger fluctuations compared to stretching 3′-termini. The variations in these variables occurr from $l = 1.2$ where the force plateau is initiated.

Stretching both 3′-termini does not induce significant effects on the base pairs translation until $l = 1.4$, except for the distance of successive base pairs (the rise), while stretching both 5′-termini considerably disturbs the stability of base pairs.

2.4. Number of Hydrogen Bonds

The number of hydrogen bonds (H-bond) of the middle 18 base pairs between the opposite strands is shown in Figure 4. Under both methods, the numbers of H-bonds decreases, but by stretching the 5′-termini more H-bonds are broken than stretching the 3′-termini. This implies that stretching the 5′-ter-

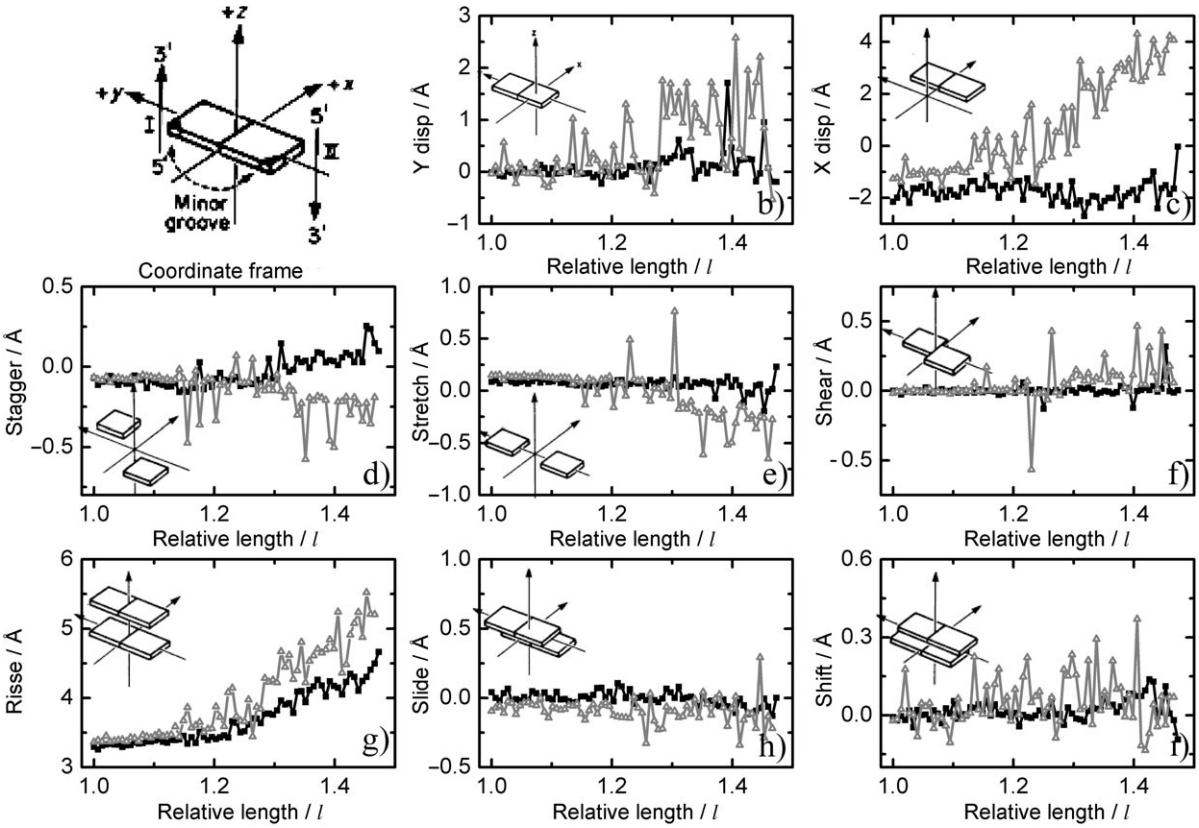

Figure 3. Plots for the variables of base translation versus relative extension *l*. a) The standard coordinate frame; b) Ydisp is the translation of a base pair moved along *y* axis in its mean plan; c) Xdisp is the translation of a base pair moved along the *x* axis in its mean plan; d) Stagger is the relative displacement of one base compared to the other one in a base pair along the *z* axis; e) Stretch is the relative displacement of one base compared to the other one in a base pair along the *y* axis; f) Shear is the relative displacement of one base compared to the other one in a base pair along the *x* axis; g) Rise is the relative displacement of one base pair compared another along the *z* axis; h) Slide is the relative displacement of one base compared to the other one in a base pair along the *y* axis; i) Shift is the relative displacement of one base compared to the other one in a base pair along the *x* axis. Stretching 3′-termini (■); stretching 5′-termini (△).

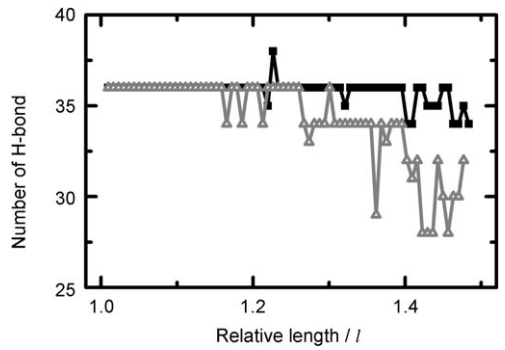

Figure 4. Plot for variations of hydrogen bonds between the two complementary strands of a DNA molecule upon stretching 3′-termini (■) and stretching 5′-termini (△) versus the relative extension (*l*). The geometrical criterion is used to define a hydrogen bond (H-bond), when the donor-acceptor distance is less than 3.6 Å and the angle is less than 30°.

mini results in more disturbances of the stability of base pairs than would those by stretching the 3′-termini; this is consistent with the observation of base rotation and base translation.

Very recently, Danilowicz et al.[16] found that stretching 5′-termini and stretching 3′-termini had the same force plateau at about 65 pN, which was smaller than the force at the plateau obtained in our numerical simulations. Moreover, our simulation showed that the force at the plateau had a bigger value by stretching 5′-termini, different from the experimental observation by Danilowicz et al.[16] We note that the force at the plateau of stretching 5′-termini in a recent molecular dynamics simulation is about 100 pN,[30] consistent with our simulation result in stretching 5′-termini. We think that the discrepancy between the results from the simulations and the experiments mainly result from the high stretching velocity in the simulation (the velocity is 6.25 mm s^{-1} in our simulation and ~20 μm s^{-1} in experiments). It should also be noted that the experiment of Gaub[18] showed that the difference of the rupture behavior becomes significant by stretching 3′-termini from stretching 5′-termini at high velocities. The discrepancy may also result from the choice of an irregular sequence in this study, combined with the different degrees of deformation along the two pathways. We will continue the molecular dynamics simulation study on a regular sequence together with the rupture of DNA in a separate paper. Due to the limited computer capacity, we cannot obtain a simulation result with a

stretching velocity comparable to the experiments. We hope that this simulation can be renewed in the future when both the simulation method and the computer capacity are considerably improved.

The enzyme DNase I bends the DNA to the major groove at the binding site.[36] Our demonstration that stretching 3′-termini make successive base pairs roll to the minor groove would restrict the activity of enzyme DNase I. In contrast, stretching both 5′-termini would favor the activity of enzyme DNase I. Smith and co-workers[5,6] have found that the reaction rate of DNase I with the stretched DNA was slowed down by stretching both 3′-termini of the DNA molecule. Their finding is favorable with our observation. The most distinctive difference between stretching 3′-termini and 5′-termini is that stretching 5′-termini causes breakage of the base pairs. This breakage disturbs the structure of the DNA double helix. All of the variations of base rotation and translation for both stretching paths took place when the relative length of DNA *l* was longer than 1.2, which also was the point at which the force plateau appeared. These findings may be helpful in furthering the understanding of the behavior of DNA at the atomic level, as well as its interaction with proteins.

3. Conclusions

We present a molecular dynamics simulation study of 22-mer DNA conformational variations caused by stretching both 3′-termini and both 5′-termini. Stretching 3′-termini by 3.5 nm required 142 kJ mol^{-1} and the force plateau was at ~80 pN, whereas stretching 5′-termini by the same length required 190 kJ mol^{-1} and the force plateau was at ~100 pN. Stretching 3′-termini led to greater untwisting of the double helix and the successive base pairs rolled to the side of DNA minor groove. Stretching 5′-termini resulted in the base pairs rolling to the major groove side and reduction in the diameter of DNA molecule.

Experimental Section

All of the molecular dynamics (MD) simulations were performed with Gromacs-3.3.1[37] with force field AMBER-94.[38] Starting conformations for the DNA double helix were generated by the *nucgen* program in Amber8,[39] and the B-DNA[40] was used as the native conformation. The DNA segment with sequence of (GTCTGAATTC-TAATGTAGTATA)$_2$, which is a part of λ DNA extensive used in experiments.[11,18] was immersed in a periodic box of TIP3P water[41] having a size of 4.5 nm × 4.5 nm × 18.0 nm. A total of forty-two Na^+ ions were added to the box to neutralize the negative charges of the DNA molecule (there are 42 O–P–O atom groups in the 22-mer [two O–P–O atom groups missing at the terminal] and almost 1e negative charge resides on each group). A cut-off of 1 nm was applied for the Lennard–Jones interaction and the real space portion of electrostatic interactions, while the PME method[42,43] was used to calculate the reciprocal space portion of electrostatic interactions. The water molecules and counter ions were relaxed for 0.5 ns with the position of the DNA fixed. Subsequently, the system was equilibrated for 2 ns with constant pressure and temperature conditions (NPT) of 300 K and 1 bar. The NPT condition was obtained by coupling the system to a Berendsen thermostat[44] and barostat, with relaxation times of 0.5 ps.

In the preparation of the structure for the umbrella sampling simulations, stretching the B-DNA duplex at NPT conditions were performed by connecting a harmonic spring, with original length of zero, to one 3′-terminus or 5′-terminus of the B-DNA duplex while the other end (3′-terminus or 5′-terminus) of the DNA was fixed. The force constant of the spring was 833 pN nm^{-1}. The other end of the spring moved away from the DNA with a constant velocity of 0.5 nm ns^{-1}, and consequently the DNA was stretched. The structures (namely, the starting structures) were stored for umbrella sampling simulation when the distance between the two 3′-termini (or two 5′-termini) of the DNA reached a length *L*. $L = 7.4\ \text{nm} + n \times 0.05\ \text{nm}$, $n = 0,1,2,\ldots,69$, with a maximal value of 10.85 nm.

For each starting structure, we performed an 8 ns umbrella sampling simulation with the distance of *L* restrained during storage, and the data of the last 6 ns were collected for analysis. The force constant of the spring for umbrella sampling was 833 pN nm^{-1}. These structures of the DNA molecule from each simulation were first clustered and averaged in each cluster by the program g_cluster in GROMACS. The criterion for clustering the structures was the root mean square deviation of these structures within 2 Å. The two base-pairs at each end of the averaged structures were ignored, and only 18 base-pairs in the middle were analyzed by the program Curves.[35] The unbroken base pairs are defined as the distance between the atom N1 in purine bases and the atom N3 in pyrimidine bases is smaller than 5 Å. The parameters of all the 18 base pairs are provided in the Supporting Information. The forces were calculated as the derivation of the free energy profiles, while the free energy profiles were reconstructed with the weighted histogram analysis method, as implemented in the WHAM[45,46] program by Alan Grossfield.

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