DNA Unzipping under a periodic force: Scaling behavior of hysteresis loop

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We propose a dynamic transition, which has potential application in understanding non-equilibrium processes such as transcription and replication of DNA, unfolding of proteins and RNA etc. Here, we demonstrate that without changing the physiological condition, a DNA may be brought to a new dynamic state from the zipped or unzipped state by varying the frequency of the applied force. We report the value of various critical exponents associated with this transition, which are amenable to verification in the force spectroscopic experiments.

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The mechanism involved in the separation of a double stranded DNA (dsDNA) into two single stranded DNA (ssDNA) is a prerequisite for understanding the processes like replication and transcription. In vitro, opening of DNA is generally achieved either by increasing the temperature (85-90°C) termed as thermal melting or by changing the pH value of the solvent (< 3 or > 9) called DNA denaturation [2]. However, such a drastic change in the physiological conditions is not possible in living systems. The mechanism of opening of dsDNA in vivo is quite complex and is initiated by helicases, DNA and RNA polymerase, ssb proteins etc, which exert force of the order of pN and as a result DNA unwinds. It is now possible to unzip the two strands of a DNA using techniques like optical tweezers, atomic force microscopy, magnetic tweezers etc [3, 4]. Theoretical understandings of DNA unzipping are mostly based on equilibrium condition [5–8].

However, living systems are open systems and never at equilibrium. Understanding the separation of DNA in the equilibrium is one approach, but another route is to perform the analysis in a situation, which closely resembles the living systems i.e. in non-equilibrium condition. Moreover, DNA helicases are ATP driven molecular motors. The periodic hydrolysis of ATP to ADP can generate continuously push and pull kind of motion, which spans a wide range of length and time scales. In the catalytic sites, it is localized (∼ 1Å) and has fast chemical reaction (∼ fs), whereas, it can extend to the large scale (up to 10 nm) and has much slower conformational motions (up to ns) in physiological functions [1, 9]. This along with other studies suggest that the force acting on DNA is oscillatory in nature of varying frequency (ν) and amplitude (F) rather than constant [10–12]. The typical time scale of fluctuations involved in unzipping varies from ns to µs, and therefore, it is necessary to understand the influence of oscillatory force on the unzipping. Surprisingly, in most of the studies (experiments, theories and simulations), the force or loading rate is kept constant [13].

In DNA unzipping, the equilibrium response of reaction coordinate (extension (y)) to the constant force is well understood in terms of simple models amenable to statistical mechanics [13–15]. However, when a dsDNA is driven by an oscillatory force, y will also oscillate and lag behind the force due to the relaxation delay. This relaxation delay induces hysteresis in the force-extension (f − y) curve of DNA unzipping, which has been recently observed in simulations and experiment [10–12]. The nature of hysteresis and its dependence on the F and ν is well studied in context of the spin systems [13, 21]. It is found that the area under the hysteresis loop A_loop scales as F^n ν^β. Values of α and β differ from system to system [21]. However, for DNA unzipping, the non-equilibrium response of y to the oscillatory force remains elusive.

In this Letter, we shall probe how a living system not in equilibrium remains structurally stable for an extended period of time. More specifically, we show that under a certain physiological condition, a dsDNA remains in the steady and stable (zipped or open) state for an extended period of time. Furthermore, without any change in temperature (T) or pH of the solvent, by varying ν alone, a dsDNA may be brought from the time averaged open or the zipped state to a new dynamic state (hysteric), oscillating between the zipped and the unzipped states, which is dynamical in origin and vanishes in the quasi static limit [21]. We evaluate the scaling exponents α and β associated with A_loop, which are amenable to verification in the force spectroscopic experiments. We also show that using the work theorem [22], it is possible to extract the equilibrium f − y curve from the non-equilibrium pathways.

In order to study the dynamical stability of DNA under the oscillatory force, one may fix ν and vary F or keep F constant and vary ν. Since, time scale involved here is quite large, therefore, an all atom simulation of longer base sequence is beyond our computer resource. In view of this, we adopted minimal off-lattice coarse grained model of DNA. Here, a bead represents few bases associated with sugar and phosphate groups. We consider a sequence in such a way that the first half of the chain is complementary to the other half. This gives the possibility of the formation of a dsDNA at low temperature [22]. The energy of the model system, we adopted, is defined as

\[ E = \sum_{i=1}^{N-1} k(d_{i,i+1} - d_0)^2 + \sum_{i=1}^{N-2} \sum_{j>i+1}^{N} 4 \left( \frac{B}{d_{i,j}^8} - \frac{A_{ij}}{d_{i,j}^6} \right), \quad (1) \]
where \( N (=32) \) is the number of beads. The distance between beads \( d_{ij} \), is defined as \(|\vec{r}_i - \vec{r}_j|\), where \( \vec{r}_i \) and \( \vec{r}_j \) denote the position of bead \( i \) and \( j \), respectively. The harmonic (first) term with spring constant \( k (=100) \) couples the adjacent beads along the chain. The remaining terms correspond to Lennard-Jones (LJ) potential. The first term of LJ potential takes care of the "excluded volume effect", where we set \( B = 1 \). We assign the base pairing interaction \( A_{ij} = 1 \) for native contacts and 0 for non-native ones [10]. This choice corresponds to the Go model [24]. By native, we mean that the first base forms pair with the \( N^{th} \) (last one) base only and second base with \((N-1)^{th} \) base and so on as shown in Fig.1. The parameter \( d_0 (=1.12) \) corresponds to the equilibrium distance in the harmonic potential, which is close to the equilibrium position of the average L-J potential. In the Hamiltonian (Eq. 1), we use dimensionless distances and energy parameters. We obtained the dynamics by using the following Langevin equation [25, 26]

\[
m d^2 \vec{r}_i \over dt^2 = -\zeta d\vec{r}_i \over dt + F_c + \Gamma
\]  

where \( m (=1) \) and \( \zeta (=0.4) \) are the mass of a bead and friction coefficient, respectively. Here, \( F_c \) is defined as \( -\frac{dE}{dt} \) and the random force \( \Gamma \) is a white noise i.e., \(<\Gamma(t)\Gamma(t')>=2\zeta T \delta(t-t'). \) This keeps temperature constant throughout the simulation. The equation of motion is integrated using \( 6^{th} \) order predictor corrector algorithm with a time step \( \delta t = 0.025 \) [25]. We add an energy \(-f(t).y(t)\) to the total energy of the system given by Eq.1 The value of \( f \) increases to its maximum value \( F \) in the \( m_s \) steps at the interval \( \Delta f (=0.01) \) and then decreases to 0 in the same way. Since, we are interested in the non-equilibrium regime, we allow only \( n \) LD time steps (much below the equilibrium time) in each increment of \( \Delta f \). Here, \( y(f) \) is the distance between two ends of the chain after the \( n \) LD time steps, where the force \( f \) is applied. We keep sum of the time spent \( (\tau=2nm_s) \) in each force cycle constant to keep \( \nu = (1/\tau) \) constant.

We have recently shown that the equilibrium force-temperature diagram of a short DNA is in good agreement with the two state model in the entire range of the \( f \) and \( T \) [16]. In the following, we keep \( T = 0.1 \) and \( F > 0.25 \) [27]. In Fig.2, we plot the value of \( y(f) \) (averaged over \( C=1000 \) cycles) with \( f \) for different values of \( \nu \). It is interesting to note that the average value of \( y(f) \) for different initial conformations remains almost the same, showing that the system is in the steady state [28]. All plots show hysteresis, however, the dynamic order parameter \( A_{loop} = \oint y df \) depends upon \( F \) and \( \nu \). The melting temperature is usually defined, when half of the base-pairs of the chain gets open [2]. If \( y(f) \) is less than 5, we call the system is in the zipped state, where as if \( y(f) > 5 \), it is in the open state or unzipped state. At high \( \nu \), it is evident that for small \( F \), dsDNA remains in the zipped state (Fig. 2a), whereas at high \( F \), it is in the unzipped state (Fig. 2b), irrespective of initial conformation. Moreover, the path of \( y(f) \) for the force 0 to \( F \) is different than that of the path for the force \( F \) to 0, which constitutes a hysteresis loop. Decrease in \( \nu \) leads to a bigger path of the hysteresis loop. Depending on the amplitude, system starts from the zipped conformation as shown in Fig. 2a (or open conformations shown in Fig. 2b) and then gradually approaches to the open state (or the zipped state) and back to the initial state.

One may note that even though \( f \) decreases from \( F \) to 0 (Fig. 2a), \( y(f) \) increases and there is some lag, after which it decreases. One may recall that the relaxation time is much higher compare to the time spent at each interval of \( \Delta f \), and hence, increase in \( y(f) \) with decrease in \( f \) indicates that system gets more time to relax. As a result \( y(f) \) approaches to a path, which is close to the equilibrium. Once the system gets enough time, the lag disappears. Similar lag one can expect, when the system starts from the open state at high \( \nu \). However, in this case as \( \nu \) decreases, \( y(f) \) decreases with increase in \( f \) (Fig. 2b). In both cases , whether dsDNA starts from the zipped or open state, as \( f \) decreases, \( A_{loop} \) increases (Fig. 3a). At some value of \( \nu \), \( A_{loop} \) is found to be maximum and then it approaches to

**FIG. 1:** DNA in a zipped and open conformation. One end is fixed and the other end is subjected to a pulling force.

**FIG. 2:** \((y)\) of DNA as a function of the cyclic force of amplitudes (a) 0.4 and (b) 1.0 at different \( \nu \).
(a) At high \( \nu \), DNA remains in the zipped state with a small hysteresis loop. As \( \nu \) decreases, the system extends from the zipped state to the open state with a bigger loop. For \( \nu \to 0 \), the hysteresis loop vanishes and the system approaches to the equilibrium path.
(b) DNA remains in the open state at high \( \nu \) and approaches the equilibrium path from below as \( \nu \to 0 \).
In Fig. 3, we have plotted $A_{\text{loop}}$ with $\nu$ at different $F$. In this case also, $A_{\text{loop}}$ increases with $F$ and above a certain amplitude, it starts decreasing. However, one can observe here that $A$ is the slow variant of $F$ and never approaches to zero for all $F$.

The other dynamic order parameter is the slow variant $Q = \frac{1}{\tau} \int x(t)dt$, studied in context of magnetic system, has recently been applied to obtain the force-frequency ($F - \nu$) diagram of DNA hairpin [29]. The explicit loop of hairpin captures essential features of a long DNA with implicit bubbles, and $f - T$ diagram is found to be qualitatively similar to the experiment [4]. In Fig. 4, we plot the value $Q$ with cycles for different $\nu$ and $F$. The distribution shows that the path remains in zipped state or in open state or in dynamic state, depending on $F$ and $\nu$. In Contrast to DNA hairpin, a short DNA shows a continuous transition from the zipped state to the new dynamic state as frequency decreases.

We now focus our study on the scaling of the area of hysteresis loop. In Fig. 5a, we have plotted $A_{\text{loop}}$ as a function of $(F\nu)^{0.5}$. For low $\nu$, we observe that all plots for different $F$ collapse on a straight line. This gives the value of $\alpha = 0.5 = \beta$, which are consistent with the mean field values for a time dependent hysteretic response to periodic force in case of the isotropic spin [20]. At high $\nu$, depending on the amplitude, the system remains either in the zipped state (low $F$) or in the open state (high $F$). In contrast to the spin system, in this case two states are asymmetric and hence, one should not expect the same scaling. In fact, at low $F$, we find that $A_{\text{loop}}$ scales as $\nu^{-1}f^5$, which is different than the scaling predicted for the spin system, where it scales as $\nu^{-1}f^2$ [20]. To explain this difference, one may recall that in the spin system two states (up and down) are degenerate and there is no net gradient potential, which drives system from the up state to the down state or vice versa. However, in the case of DNA, there is a net gradient potential term arising due to the attractive interaction between complimentary nucleotides, which always drives the system from the open state to the closed state during each cycle. At high amplitude, we did not get reliable scaling, where all curves collapse on a single curve. In single molecule experiments, measurements have been done at non-equilibrium condition. It is always desirable to infer the equilibrium properties of the system from these data to have better understanding of the system. In order to do so, generally measurements have been taken in the quasi static [4] so that techniques involved in thermodynamics can be employed. In recent years, there have been considerable work to extract equilibrium properties from the non-equilibrium data e.g. Jarzynski equality, which relates free energy differences between two equilibrium states through non-equilibrium processes [30]. In another approach dominant reaction pathway algorithm is developed to compute the most probable reaction pathways between two equilibrium states [31]. Here, we use the work theorem to derive the equilibrium path between the two states [22].

The underlying assumption behind the work theorem relies on the fact that the initial state of the system should be in thermal equilibrium.

Instead of repeating the force cycle $C$ times, we now randomly choose any $C$ conformations, which belong to equilibrium conformations at that $T(F = 0)$. We follow the similar protocol described above to reach the final state from the initial state. No attempt is made to achieve equilibrium during this process. The total work performed on the system going from the zipped state to open state (forward path) is $w_m = -\Delta f \sum_{i=1}^{m} y_i$. When the applied force decreases (backward path), the work done by the system from the open state to the zipped state can be written as $w_1 = \Delta f \sum_{i=m}^{1} y_i$. The equilibrium distance $y_k$ for the force $f_k$ for the forward path can be obtained by assigning the weight $\exp(-\beta w_k)$ to all forward $C$ paths [22] at that instant $k$, which can be written as

$$y_k = \frac{\sum_{C} y_k \exp(-\beta w_k)}{\sum_{C} \exp(-\beta w_k)}.$$
Similarly, $y_k$ for the reverse path can also be obtained.

In Fig.5, we have plotted the simple average of extension over many ($C = 1000$) forward paths as well as backward paths ($n = 10^5$ LD time steps). One can see the existence of hysteresis. In this figure, the solid line corresponds to the equilibrium path, which is the same, whether we start from the zipped or open conformations. In our simulation, the data has been sampled over four times of the equilibration time. We have used $2 \times 10^4$ time steps out of which the first $5 \times 10^3$ steps have not been taken in the averaging. The results are averaged over many trajectories, which are almost the same within the standard deviation. The weighted average of the extension for the forward and backward paths have also been depicted in this plot. One can see from these plots that the weighted average even for $n = 10^4$ LD steps is quite close to the equilibrium value ($5 \times 10^8$ LD steps). We further note that the weighted average of the backward path almost overlaps with the equilibrium path. This may be because of the fact that in a reverse path, two strands of DNA are in the open state and the system can access more configurational space. This gives the higher probability of choosing the rare conformations, which have dominant contributions in Eq. 3.

Force induced transition in bio-molecules is the subject of interest for the last two decades [13]. However, there is almost no effort to study the dynamic transition, which can be induced by the oscillatory force. For the first time, we show the existence of dynamic transition in DNA unzipping, which can be observed in vitro as well as in other systems e.g. protein unfolding and RNA unfolding. In low frequency limit, the value of exponents $\alpha$ and $\beta$ associated with this transition are in agreement with the values obtained for the isotropic spin case. Because of the asymmetry in the present case, in high frequency limit, the scaling obtained here differs significantly from the isotropic spin case. This is because the open state is always under the drive towards the zipped state under the potential gradient term, which is absent in the spin system. We also showed that using the work theorem, it is possible to extract the equilibrium properties of the system from the non-equilibrium data, which have potential application in single molecule force measurements.

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[1] Alberts, A. et al. Molecular Biology of the Cell, (Garland, New York, 1994).

[2] Wartell, R. & Benight, A. Thermal denaturation of DNA molecules: A comparison of theory with experiment Phys. Rep., 126 67-107 (1985).

[3] Bockelmann, U., Essevaz-Roulet, B. & Heslot, F. Molecular Stick-Slip Motion Revealed by Opening DNA with Piconewton Forces Phys. Rev. Lett., 79 4489-4492(1997).

[4] Danilowicz, C. et al. Measurement of the Phase Diagram of DNA Unzipping in the Temperature-Force Plane Phys. Rev. Lett., 93 078101 (2004).

[5] Bhattacharjee, S. M. Unzipping DNAs: towards the first step of replication J. Phys. A, 33 L423 (2000).

[6] Lubensky, D. K. & Nelson, D. R. Pulling Pinned Polymers and Unzipping DNA Phys. Rev. Lett., 85 1572-1575 (2000).

[7] Kafri, Y., Mukamel, D. & Pelti, L. Why is the DNA Denaturation Transition First Order? Phys. Rev. Lett., 85 4988-4991 (2000).

[8] Marenduzzo, D., Bhattacharjee, S. M., Maritan, A., Orlandini, E. & Sono, F. Dynamical Scaling of the DNA Unzipping Transition Phys. Rev. Lett., 88 028102 (2001).

[9] Dittrich, M., Yu, J. & Schulten, K. PcrA Helicase, a Molecular Motor Studied from the Electronic to the Functional Level Top. Curr. Chem, 268 319-347 (2007); Dittrich, M. & Schulten, K. PcrA helicase, a prototype ATP-driven molecular motor Structure, 14 1345-1353 (2006).

[10] Donmez, I. & Patel, S. S. Mechanisms of a ring shaped helicase NAR, 34 4216-4224 (2006).
[11] Velankar, S. S., Soultanas, P., Dillingham, M. S., Subramanya, H. S. & Wigley, D. B. Crystal Structures of Complexes of PcrA DNA Helicase with a DNA Substrate Indicate an Inchworm Mechanism *Cell*, **97** 75-84 (1999).

[12] Williams, M. F. & Jankowsky, E. Unwinding Initiation by the Viral RNA Helicase NPH-II *JMB*, **415** 819-832 (2012).

[13] Kumar, S. & Li, M. S. Biomolecules under mechanical force *Phys. Rep.*, **486** 1-74 (2010).

[14] Marko, J. & Siggia, E. Stretching DNA *Macromolecules*, **28** 8759-8770 (1995).

[15] Kumar, S., Jensen, I., Jacobsen, J. L & Guttmann, A. J. Role of Conformational Entropy in Force-Induced Biopolymer Unfolding *Phys. Rev. Lett.*, **98** 128101 (2007).

[16] Mishra, G., Giri, D., Li, M. S. & Kumar, S. Role of loop entropy in the force induced melting of DNA hairpin *J. Chem. Phys.*, **135** 035102 (2011).

[17] Hatch, K., Danilowicz, C., Coljee, V. & Prentiss, M. Measurements of the hysteresis in unzipping and rezipping double-stranded DNA *Phys. Rev. E*, **75** 051908 (2007).

[18] kapri, R. Hysteresis and nonequilibrium work theorem for DNA unzipping arXiv: 1201.3709 (2012).

[19] Rao, M. & Pandit, R. Magnetic and thermal hysteresis in the O(N)-symmetric (2)3 model *Phys. Rev. B*, **43** 3373-3386 (1991); Rao, M., Krishnamurthy, H.R. & Pandit, R. Magnetic hysteresis in two model spin systems *Phys. Rev. B*, **42** 856-884 (1990).

[20] Dhar, D & Thomas, P. Hysteresis and self-organized criticality in the O(N) model in the limit N to infinity *J. Phys. A*, **25** 4967 (1992).

[21] Chakrabarti, B. K. & Acharyya, M. Dynamic Transitions and Hysteresis *Rev. Mod. Phys.*, **71** 847-859 (1999).

[22] Sadhukhan, P. & Bhattacharjee, S. M. Thermodynamics as a nonequilibrium path integral *J. Phys.*, **43** 245001 (2010).

[23] Kumar, S. & Mishra, G. Force-induced stretched state: Effects of temperature *Phys. Rev E*, **78** 011907 (2008).

[24] Li, M. S. & Cieplak, M. Folding in two-dimensional off-lattice models of proteins *Phys. Rev E*, **59** 970-976 (1999).

[25] Allen, M. P. & Tildesley, D. J. *Computer Simulations of Liquids* (Oxford Science, Oxford, UK, 1987).

[26] Frenkel, D. & Smit, B. *Understanding Molecular Simulation* (Academic Press UK, 2002).

[27] For $T = 0.1$, critical force $F_c$ was found to be 0.25 [16].

[28] We have analyzed 80 conformations but in Fig. 2, only 10 conformations are shown.

[29] Mishra, G., Sadhukhan, P., Bhattacharjee, S. M., & Kumar, S. Dynamical phase transition of a periodically driven DNA arxiv:1204.2913.

[30] Jarzynski, C. Nonequilibrium Equality for Free Energy Differences *Phys. Rev. Lett.*, **78** 2690-2693 (1997).

[31] Faccioli, P., Lonardi, A. & Orland, H. Dominant reaction pathways in protein folding: A direct validation against molecular dynamics simulations *J. Chem. Phys.*, **133** 045104 (2010).