A Letters Journal Exploring the Frontiers of Physics

November 2012

EPL, **100** (2012) 48001
doi: 10.1209/0295-5075/100/48001

www.epljournal.org

Nucleosome response to tension and torque

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received 26 July 2012; accepted in final form 31 October 2012
published online 27 November 2012

PACS 87.14.gk – DNA
PACS 87.16.Sr – Chromosomes, histones
PACS 87.15.ad – Analytical theories

Abstract – We investigate the combined effect of torque and force on a nucleosome, the most abundant DNA-protein complex in eukaryotic cells. Using the worm-like chain model (WLC) we show how low positive torques ease the unwrapping of the DNA from the nucleosome. Remarkably a combination of high forces and high negative torques favors DNA unwrapping as well. The theory is also applicable beyond nucleosomes, namely whenever DNA spools are involved.

Introduction. – In every cell of our organism there is enough DNA that, if stretched, would be longer than most of us. Even more remarkably, some ten thousand cells, stacked one over the other, are needed to be as long. Nature had thus to find a clever way to compact the DNA, a negatively charged, semi-flexible polymer, into every cell.

To accomplish such a high degree of compaction, DNA molecules are wrapped into nucleosomes. In each nucleosome DNA is wrapped ≈ 1.7 turns along a left-handed superhelical wrapping path of 4.3 nm radius around an octamer of histone proteins. In this configuration, with highly bent DNA, equilibrium is reached through 14 binding sites at the nucleosome surface providing electrostatic attraction and hydrogen bonding. The resulting string of nucleosomes with short pieces of bare linker DNA in between condenses further, presumably into chromatin fibers [1,2], finally packing into a whole chromosome.

To allow replication and transcription the DNA molecule needs to unbind —at least locally— from the octamer. To learn about the nucleosomal stability and energetics, experiments have been designed to pull on its DNA with various tensions [3,4] and the findings have been analyzed using theoretical models [5–7]. Here we choose a different route by exploring the response of a nucleosome to a combination of tension and torque. We note that, with the due modifications, this theory can also be applied to other DNA spools widely found in Nature, like the Lac1 repressor [8], DNA gyrase [9] and RNA polymerase [10].

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General model. – We consider a nucleosome, with DNA legs at its ends, under tension f and torque, see fig. 1. In our model the DNA is described as a worm-like chain being wrapped around a cylinder that represents the histone octamer. The wrapped section of the DNA molecule is described by the space curve (n stands for *nucleosome*) $\mathbf{r}_n(s) = r(\pi s \tan\alpha, \cos\pi s, \sin\pi s)$ with $r = 4.3\,\mathrm{nm}$ and $\alpha = -0.085$ and thus a pitch of $2\pi r \tan\alpha$. A nucleosome with s^*-turns of DNA adsorbed is described by $\mathbf{r}_n(s)$ with $s \in [-s^*, s^*]$. To unwrap its DNA the nucleosome has to rotate around the $y-$axis by an angle β (fig. 1) resulting in $\mathbf{r}_n(s,\beta) = O_y(\beta)\mathbf{r}_n(s,0)$ with O_y denoting the corresponding rotation matrix [5] and $\mathbf{r}_n(s,0) \equiv \mathbf{r}_n(s)$.

In the torsionless case the conformation of the free DNA is planar with its ends aligned with the force as it has been worked out in detail in [5] using the Kirchhoff kinetic analogy [11]. Adding torsion causes the DNA legs to bend out of plane. Since a non-planar homoclinic loop is only favored when the inserted number of turns is between -1 and 1, and since most of the non-planarity is contained within the wrapped part of the DNA, we simplify our analysis by describing the legs by the planar homoclinic orbit with the tangent vector $\mathbf{t}_l(s,\delta) = O_z(\delta)(0, \sin\theta(s), \cos\theta(s))$, with $\cos\theta(s) = 1 - 2\,\mathrm{sech}^2(s/\lambda)$; here $\lambda = \sqrt{A/f}$ with A being related to the DNA persistence length $l_p = A/k_BT \approx 50\,\mathrm{nm}$. From $-s_0$ to $+s_0$ we replace this curve with the wrapped nucleosomal DNA (see fig. 1). The δ-rotation of the DNA legs ensures continuity at the insertion point, without affecting the energy. In addition continuity

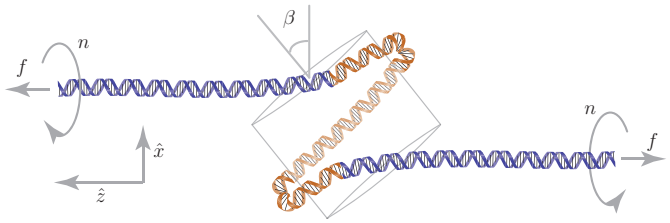

Fig. 1: (Colour on-line) The nucleosome under tension and torque. In our model the histone octamer is represented by a cylinder. A part of the DNA molecule is wrapped around it (in an orange hue), the rest forms the legs (in a blue hue).

requires

$$0 = \mathbf{t}_l(s_0, \delta) + \dot{\mathbf{r}}_n(-s^*, \beta)$$

from which follows

$$s_0(s^*, \beta) = \frac{\lambda}{t} \operatorname{arcsech} \frac{t_{\min}}{t} \tag{1}$$

with

$$t_{\min} = \sqrt{\frac{1 + \cos\alpha \cos \pi s^* \cos\beta - \sin\alpha \sin\beta}{2}} \tag{2}$$

and $t = 1$. In eq. (1) t represents the homoclinic parameter, which quantifies how "planar" the legs are. In this work we assume the legs to be perfectly planar ($t = 1$). This approximation is good for several reasons: first of all the domain of arcsech limits t to $[t_{\min}, 1]$. When $s^* \not\approx 0, 1, 2$, the β that minimizes the energy leads to $t_{\min} \approx 1$. On the other hand, when $s^* \approx 0, 1, 2$, the contribution of the legs to the energy is almost 0, since s_0 is very high (see eq. (7)). As convention we assume that the point $\pm s^*$ of the adsorbed DNA is attached to the point $\pm s_0$ of its free counterpart so that the path of the DNA is described by

$$\mathbf{r}(s, s^*, \beta) = \begin{cases} \int \mathbf{t}_l(s, \delta_1) \mathrm{d}s, & \text{if } s \in [-L_l(s^*, \beta), -s_0], \\ \int \mathbf{t}_l(s, \delta_2) \mathrm{d}s, & \text{if } s \in [+s_0, +L_l(s^*, \beta)], \\ \mathbf{r}_n(s, \beta), & \text{if } s \in [-s^*, s^*]. \end{cases} \tag{3}$$

In the integrals one of the integration boundaries is the length $L_l(s^*, \beta) = (L + 2s_0 - L_n(s^*))/2$, where $L_n(s^*) = 2\pi r s^* \sec\alpha$ is the length of the DNA adsorbed by the nucleosome. The two angles δ_1, δ_2 are important to ensure continuity at the boundary between legs and nucleosome, but they do not influence the energy. Therefore, we drop the δ argument of $\mathbf{t}_l$ from now on. Once s^* and β are known, the energy of the system can be computed from $\mathbf{t}_l(s_0)$ and $\mathbf{r}_n(s, \beta)$. The resulting structure is depicted in fig. 1.

Writhe. – The torsional energy of the DNA is proportional to the square of its twist, Tw. The twist is related to the number of turns externally inserted into the system, the linking number n, through White's relation $n = Tw + Wr$ [12]; the writhe of the curve, Wr, is in principle only

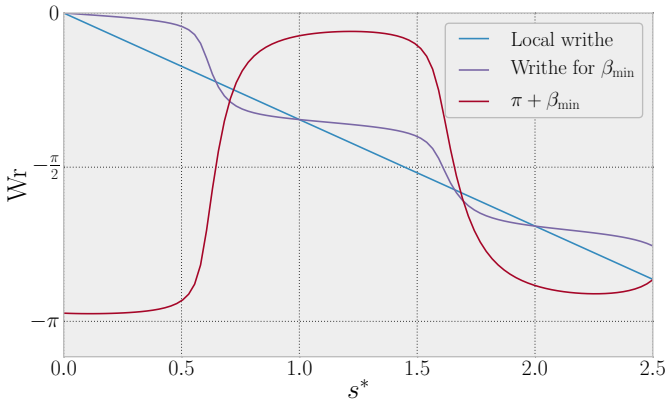

Fig. 2: (Colour on-line) A comparison between the local writhe, *i.e.*, using the helix axis in eq. (4), and the writhe for the β that minimized the energy, $\beta_{\min}$. For reference also $\pi + \beta_{\min}$ is plotted.

defined for a closed loop as its Gauss integral with itself. However, thanks to Fuller's relation under certain assumptions [13], the writhe of the nucleosome is

$$\frac{1}{2\pi} \int_0^s \frac{-\hat{z} \times \mathbf{t}_n(s, \beta)}{1 + (-\hat{z}) \cdot \mathbf{t}_n} \cdot \frac{\mathrm{d}\mathbf{t}_n(s, \beta)}{\mathrm{d}s} \mathrm{d}s, \tag{4}$$

where $\mathbf{t}_n$ is the unit tangent vector of the wrapped DNA path $\mathbf{r}_n$. Applying eq. (4) gives

$$Wr^i(s, \beta) = \frac{\arctan\left(\cos\frac{\alpha-\beta}{2} \csc\frac{\alpha+\beta}{2} \tan\frac{\pi s}{2}\right)}{\pi} - \frac{1}{2} s \sin\alpha - \Delta(s, \alpha). \tag{5}$$

This means that the total writhe of a nucleosome with s^* turns adsorbed is

$$Wr(s^*, \beta) = Wr^i(s^*, \beta) - Wr^i(-s^*, \beta). \tag{6}$$

The function Δ eliminates the (finite-size) discontinuities of the trigonometric function and it is -1 for $s \in [-3, -1]$, 0 for $s \in [-1, 1]$ and 1 for $s \in [1, 3]$ etc. The legs should also, in principle, be counted in the integral in eq. (4); however, since they are in a plane "parallel" to the $\hat{z}$-axis, their net contribution to the writhe is 0. Note that eq. (6) deviates from the intuitive writhe of the helix, *i.e.*, $-s^*(1 + \sin\alpha)$ that can be derived from eq. (4) by using the axis of the helix (that rotates with β) instead of $-\hat{z}$. The different behavior of the writhe is presented in fig. 2, while in fig. 3 we present the nucleosome with a varying amount of DNA wrapped around it.

As required for the use of Fuller's relation, there is a homotopy between the straight $\hat{z}$-axis and any of the states (partially or fully wrapped nucleosome plus rotated legs) considered here. The continuity of the homotopy follows from the fact that the chain continuously changes from $s^* = 0$ (*i.e.*, the $\hat{z}$-axis) to any subsequent state thanks to eq. (3).

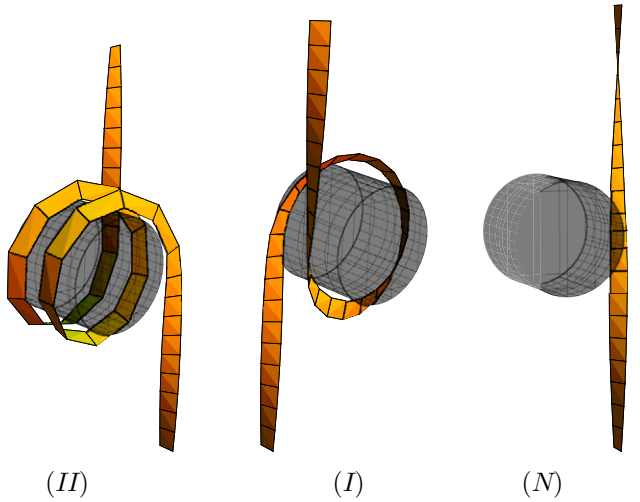

Fig. 3: (Colour on-line) Schematic representation of the various stages of nucleosome unwrapping. The roman numerals indicate how many turns are approximately wrapped (N stands for 0). In order to show the effect of torque, the DNA double helix is represented here as a ribbon that is untwisted in the torsionless case.

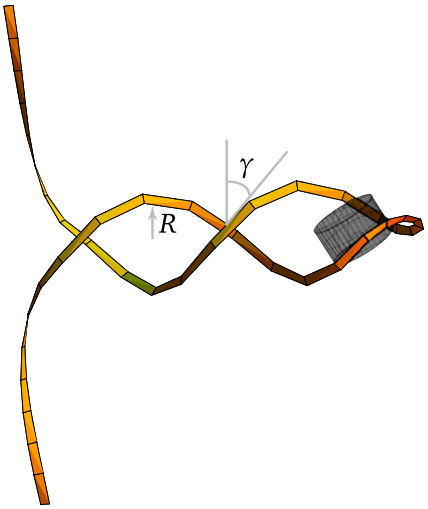

Fig. 4: (Colour on-line) A plectoneme with a nucleosome at its end. R and γ are the plectoneme parameters.

Energy. – The total energy of a DNA chain of length L with s^* bound turns inside the nucleosome is the sum of the bending, potential, adsorption and torsional energy:

$$\begin{aligned} E_t(s^*,\beta) \;=\; & 2\times\frac{A}{2}\int_{s_0}^{L_l(s^*,\beta)}\dot{\mathbf{t}}_l^2(s)\mathrm{d}s \\ & +f\Delta z(s^*,\beta)-2\int_0^{s^*}\frac{\mathrm{d}E_{\mathrm{ads}}(s)}{\mathrm{d}s}\mathrm{d}s \\ & +\frac{2\pi^2 C}{L-L_n(s^*)}(n-Wr(s^*,\beta))^2. \end{aligned} \tag{7}$$

Here

$$\begin{aligned} \Delta z(s^*,\beta) \;=\; & L_n(s^*)+(\mathbf{r}_n(-s^*,\beta)-\mathbf{r}_n(s^*,\beta))\cdot\hat{z} \\ & +2\times\int_{s_0}^{L_l(s^*,\beta)}(1-\mathbf{t}_l(s)\cdot\hat{z})\,\mathrm{d}s \end{aligned} \tag{8}$$

is the shortening of the DNA end-to-end distance in the $\hat{z}$-direction due to the bending of the legs and the wrapping around the octamer. Following ref. [7] we assume that the net adsorption energy density is given by

$$\frac{\mathrm{d}E_{\mathrm{ads}}}{\mathrm{d}s}=\begin{cases}\varepsilon-\varepsilon_b, & \text{if } |s^*|\leqslant 1,\\ \varepsilon-\varepsilon_b-\varepsilon_{\mathrm{el}}, & \text{if } 1.67\geqslant|s^*|>1,\\ -\varepsilon_b-\varepsilon_{\mathrm{el}}, & \text{if } |s^*|>1.67.\end{cases} \tag{9}$$

Here ε is the pure adsorption energy density whereas ε_b accounts for the DNA bending cost and $\varepsilon_{\mathrm{el}}$ for the electrical repulsion between the two wrapped turns. We choose $\varepsilon=1.51k_BT/\mathrm{nm}$, $\varepsilon_b=0.75\,k_BT/\mathrm{nm}$ and $\varepsilon_{\mathrm{el}}=0.2k_BT/\mathrm{nm}$ [7]. Finally, in the last term of eq. (7) the quantity C is related to the torsional persistence length l_t via $l_t=C/k_BT$; we assume here $l_t=100\,\mathrm{nm}$ [14].

To find the optimal configuration for given values of f and n the energy, eq. (7), needs to be minimized with respect to s^* and β. Since we neglect in our theory entropic contributions our results are only reliable for large enough forces, $f\gtrsim 0.5\,\mathrm{pN}$ [6].

Plectoneme. – The unwrapping of the nucleosome is eased for moderate positive torques or, as shown later, for high negative torques. However, depending on the force the DNA can also form a structure called plectoneme (see fig. 4) that adsorbs approximately all the linking number inserted in the system [14,15]. We do not expect nucleosome unwrapping in the presence of a plectoneme as the plectoneme can adsorb torsional stress more efficiently once formed. To estimate the parameter range where the plectoneme occurs, we give here the energy of a DNA molecule of length L that contains a plectoneme of length $p\geqslant 0$ with radius R and angle γ (fig. 4):

$$E(p)=\frac{2\pi^2 C}{L}(n-\mathrm{d}Wp)^2+(f+\mathrm{d}E_b)p. \tag{10}$$

Here $\mathrm{d}W=\cos\gamma\sin\gamma\,\mathrm{sign}\,n/2\pi R$ and $\mathrm{d}E_b=A\cos^4\gamma/(2R^2)$ are, respectively, the writhe density, which changes sign with n, and the bending energy density of the plectoneme [15].

In eq. (10) we ignore the energetic contribution of the end loop [15] assuming that the nucleosome sits at the end of the plectoneme (fig. 4). In principle a plectoneme could also appear somewhere else but the high bending energy of an end loop makes it highly improbable.

By minimizing eq. (10) for p one finds that a plectoneme is expected, *i.e.*, $p>0$, for all values of n with

$$n\notin\left[-\frac{(f+\mathrm{d}E_b)L}{4\pi^2 C\mathrm{d}W}+Wr(2),\frac{(f+\mathrm{d}E_b)L}{4\pi^2 C\mathrm{d}W}\right]. \tag{11}$$

Here $Wr(2)=-2.14$ is the writhe for 2 fully wrapped turns that for $s^*=2$ is independent of β (see fig. 2).

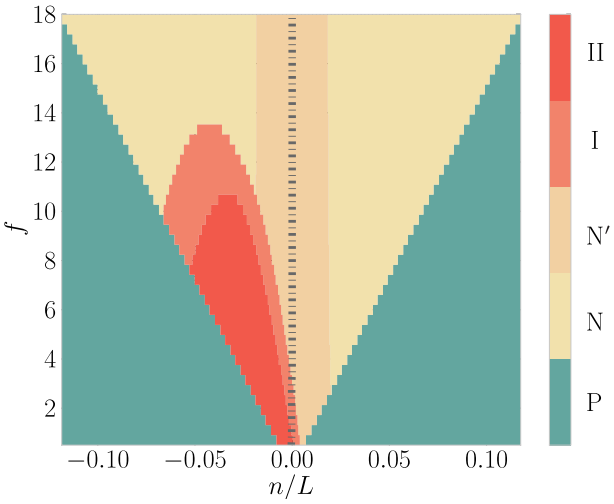

Fig. 5: (Colour on-line) Diagram of state showing the configurations with the lowest energy for $L = 3500\,\mathrm{nm}$ in the $(f, n/L)$-plane. (II) (I) and (N) corresponds to the states shown in fig. 3, while (N') and (P) represent, respectively, the almost unwrapped configuration and the plectoneme state characterized by eq. (11). The grey dash-dotted line represents the writhe of the nucleosome when the legs are free to release torsional stress.

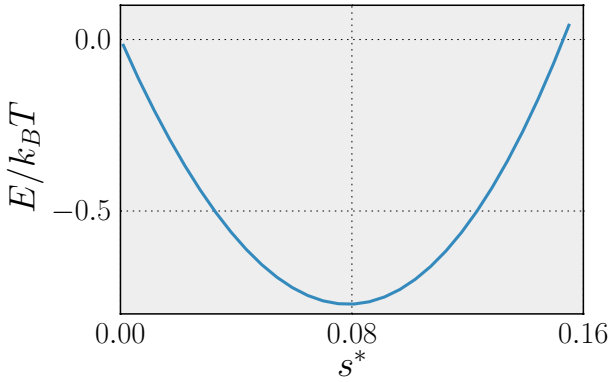

Fig. 6: (Colour on-line) The energy landscape near $s^* = 0$ for $f = 10\,\mathrm{pN}$, $n = 0$. The minimum of energy is very close to the $s^* = 0$ case which makes it easy for the nucleosome to "evaporate."

With this term we account for the writhe absorbed by the nucleosome that has around two fully wrapped turns for $n > 0$ and not too large forces. Following ref. [15] we use $\gamma \approx 1$ and $R \approx 1.8\,\mathrm{nm}$ when the salt concentration is about 150 mM. We stress that when a plectoneme forms, it forms on top of the state the system was in before its formation. For instance, if we start in fig. 5 from a nucleosome with two-turns (state II) and decrease n we obtain a plectoneme with a fully wrapped nucleosome at its tip once eq. (11) is fulfilled. As mentioned above we do not expect the nucleosome state to change with n inside the plectonemic region.

Twist defects. – Apart from the plectoneme, twist defects [16,17] can influence the nucleosome stability. A twist defects is present in a nucleosome when one DNA basepair is added or removed between two consecutive nucleosome binding sites, resulting in a local under- or overtwisting of the DNA. We can write an equation similar to eq. (10) for the twist defects if we replace $p \to m\Delta l$, $\mathrm{d}W \to k \operatorname{sign} n/\Delta l$, $\mathrm{d}E_b \to \mathrm{d}E_d/\Delta l$ and $f \to f \operatorname{sign} n$. m is an integer between 0 and 13 denoting the number of defects. $\Delta l = 0.34\,\mathrm{nm}$ and $k = 1/10$ are, respectively, the length and twist lost or gained by a defect. Finally $\mathrm{d}E_d = 9k_BT$ is the energetic cost of a defect [16]. Since $m \leqslant 13$ the shift in turns will be up to 1.3; a quick computation reveals that the 13 defects form before a plectoneme occurs. This changes the boundaries where the plectoneme forms, namely we need to subtract 1.3 from the left side of eq. (11).

The 1.3 turns per nucleosome are found in experiments where chromatin fibers are put under positive torsional stress [18]. It was suggested that this can be explained by a chiral transition of the nucleosome. Unfortunately a comparison of our model to these experiments is not possible as it involves a multinucleosome geometry and forces where thermal fluctuations cannot be neglected. It would be crucial to perform single nucleosome experiments to see whether the observed strong asymmetry in the response to positive and negative torsion is still present which favors the picture of a chiral transition.

Results. – In fig. 5 we present the optimal nucleosome configurations for a wide range of forces and n/L-values of a DNA molecule with $L = 3500\,\mathrm{nm}$ length. This diagram of states is nearly identical for all experimentally reasonable values of L, say for all $L > 500\,\mathrm{nm}$. We find 5 different states, 3 of which are depicted in fig. 3 ((II) fully wrapped, (I) one turn wrapped and (N) unwrapped) and one in fig. 4 ((P) fully wrapped plus plectoneme). In addition, we indicate with (N') almost unwrapped configurations. That state is, however, not stable against thermal fluctuations as the global minimum is only tenths of k_BT away from the totally unwrapped state. A typical example is shown in fig. 6. We therefore expect that the nucleosome typically falls apart once it has unwrapped its last turn.

The negative writhe of the wrapping path makes the nucleosome unwrapping highly asymmetric since the factor $(n - Wr(s^*, \beta))^2$ in the torsional energy, eq. (7), favors wrapping, $s^* > 0$, for $n < 0$ and unwrapping, $s^* = 0$, for $n > 0$. For large enough negative values of n, however, the nucleosome unwraps to have more twistable DNA available, see fig. 5. The factor $1/(L - L_n)$ in the twist energy dominates then the behavior. In the diagram of states, fig. 5, we indicate also by a dash-dotted line the torsionless case where the unbound DNA is free to rotate. This situation has been studied in ref. [5].

So far we have only determined the optimal configurations via energy minimization. Of special experimental importance is, however, also to know the energy barrier between different states, especially at common boundaries

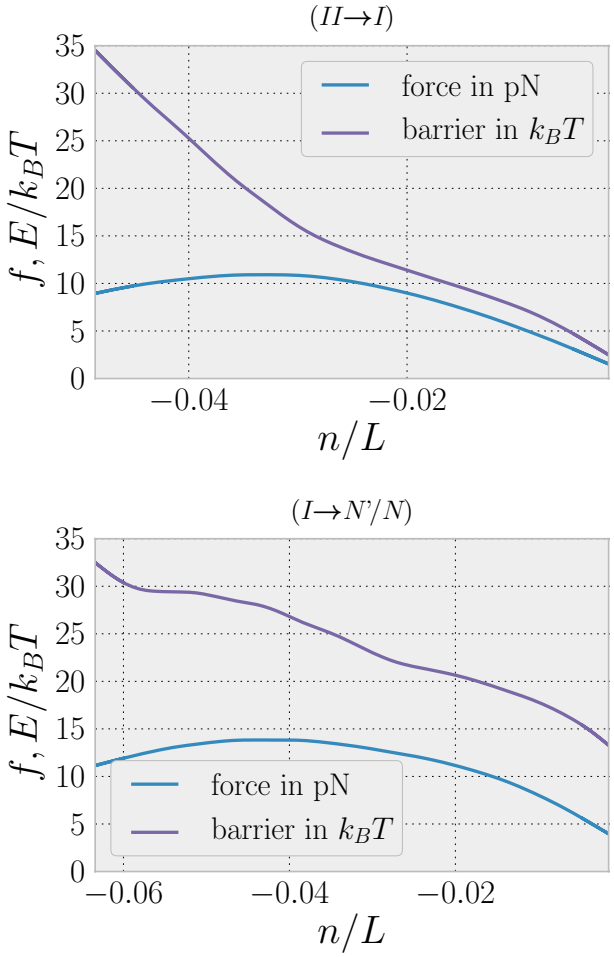

Fig. 7: (Colour on-line) The force at which the minimum of the energy around $s^* = 2$ (1) and the one around $s^* = 1$ (0) have the same value, and the energy barrier necessary to cross from one state to the other, as a function of n/L. Here $L = 3500$ nm.

in the diagram of states, fig. 5. Choosing experimental parameters such that one has two minima between a large barrier, one can observe the hopping dynamics between them. This has been indeed observed in the torsionless case where a fast hopping between states (II) and (I) was observed at a certain force value manifesting itself in a change of the end-to-end extension [4,19]. The boundaries and corresponding barriers between (II) and (I) and between (I) and (N'/N) are shown in fig. 7. Note that the system under torsion provides a much wider range of parameters where one can observe hopping as compared to the torsionless case. Especially for a wide range of forces we predict two values of n/L where hopping should be observed. It might be challenging to observe the branch with the transitions at the more negative n/L-values as these transitions are associated with much higher barrier values (see fig. 5).

* * *

We thank Marc Emanuel and Nima Hamedani Radja for helpful discussions. This work is part of the research program "DNA in action: Physics of the genome" of the "Stichting voor Fundamenteel Onderzoek der Materie (FOM)", which is financially supported by the "Nederlandse Organisatie voor Wetenschappelijk Onderzoek (NWO)".

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