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## **DNA Mechanics Inside Plectonemes, Nucleosomes and Chromatin Fibers**

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# Unwrapping

If you ask me now, I cannot prove it, but I'm sure it's true.

GIUSEPPE DE MARCO

In this chapter we investigate the effect of torque and force on a *nucleosome*. Using the worm-like chain model (WLC) we show how low negative torques eases the unwrapping of the DNA from the nucleosome. In some case a combination of high forces and high positive torques, surprisingly, favors the unwrapping as well. The theory presented provides an interesting insight on how to access the genetic code with tensions smaller than what previously thought.

To study the response, we consider a molecule of DNA bound to a single nucleosome. However, with due modifications, this applies to more general DNA spools, widely found in nature, as the Lac1 repressor [67], DNA gyrase [29] and RNA polymerase [54].

## 3.1 General model

We consider a nucleosome, with DNA legs at its ends, under tension  $f$  and torque, see fig. 3.1. 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  $\mathbf{r}_n(s) = r(\pi s \tan \alpha, \cos \pi s, \sin \pi s)$  with  $r = 4.3$  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<sup>1</sup> 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  $\beta$  (fig. 3.1) resulting in  $\mathbf{r}_r(s, \beta) = O_y(\beta)\mathbf{r}_n(s)$  with  $O_y$  denoting the corresponding rotation matrix [36]. To avoid collision between DNA and nucleosome we impose  $\beta \in [-\pi + \alpha, -\alpha]$ .

In the torsion-less case the shape of the planar DNA, where its ends are aligned with the force, has been worked out in [36] using the Kirchhoff kinetic analogy [51]. 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 (see the section at the end of this chapter), we simplify our analysis by describing the legs by the planar homoclinic orbit with the tangent vector

$$\mathbf{t}_l(s) = O_z(\delta)(0, \sin \theta(s), \cos \theta(s))$$

with  $\cos \theta(s) = 1 - 2 \operatorname{sech}^2(s/\lambda)$ ; here  $\lambda = \sqrt{A/f}$  with  $A$  being related to the DNA persistence length  $l_p = A/k_B T \approx 50$  nm. From  $-s_0$  to  $+s_0$  we replace this curve with the wrapped nucleosomal DNA (see fig. 3.1). The  $\delta$ -rotation of the DNA legs ensures continuity at the insertion point, without affecting the energy. In addition continuity requires

$$0 = \mathbf{t}_l(s_0) + \mathbf{t}_n(-s^*) \quad (3.1)$$

therefore

$$s_0(s^*, \beta) = \frac{\lambda}{t} \operatorname{arcsech} \frac{t_{\min}}{t} \quad (3.2)$$

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

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<sup>1</sup>Not counting an eventual translation, irrelevant for the energy.

and  $t = 1$ . In eq. (3.2)  $t$  represent 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  $s^* \approx 0, 1, 2$ , the  $\beta$  that minimizes the energy leads to  $t_{\min} \approx 1$ . On the other hand, when  $s^* \approx 0, 1, 2$ , the contribution by the legs to the energy is almost 0, since  $s_0$  is very high (see eq. (3.7)). In principle eq. (3.1) determines  $\delta$  as well, but the parameter is not relevant for our analysis.

As convention we assume that the point  $\mp s^*$  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

$$\mathbf{r}(s, s^*, \beta) = \begin{cases} \int \mathbf{t}_l(s, \delta_1) ds & \text{if } s \in [-L_l(s^*, \beta), -s_0] \\ \int \mathbf{t}_l(s, \delta_2) ds & \text{if } s \in [+s_0, +L_l(s^*, \beta)] \\ \mathbf{r}_n(s, \beta) & \text{if } s \in [-s^*, s^*]. \end{cases} \quad (3.3)$$

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  $\delta_1, \delta_2$  are important to ensure continuity at the boundary between legs and nucleosome, but otherwise they do not influence the energy. Therefore we drop the  $\delta$  argument of  $\mathbf{t}_l$  from now on. Once  $s^*$  and  $\beta$  are known, the energy of the system can be computed from  $\mathbf{t}_l(s_0)$  and  $\mathbf{r}_r(s, \beta)$ . The resulting structure is depicted in fig. 3.1.

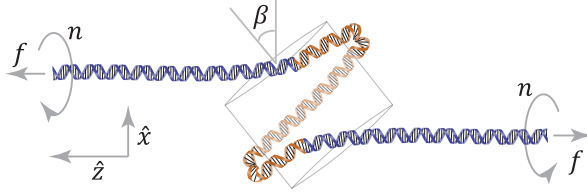


Figure 3.1: The nucleosome under tension and torque. In our model the histone octamer is represented by a cylinder. A part of the DNA molecule is wrapped around it (in an orange hue), the rest forms the legs (in a blue hue).

## 3.2 Writhe

To compute the writhe of the molecule, we use eq. (1.39) with respect to the  $-\hat{z}$ -axis:

$$\begin{aligned}
 Wr_{\text{DNA}} &= \frac{1}{2\pi} \int_{-s_0}^{-L/2} \frac{-\hat{z} \times \mathbf{t}_l(s)}{1 + (-\hat{z}) \cdot \mathbf{t}_l} \cdot \frac{d\mathbf{t}_l(s)}{ds} ds \\
 &+ \frac{1}{2\pi} \int_{L/2}^{s_0} \frac{-\hat{z} \times \mathbf{t}_l(s)}{1 + (-\hat{z}) \cdot \mathbf{t}_l} \cdot \frac{d\mathbf{t}_l(s)}{ds} ds \\
 &+ \frac{1}{2\pi} \int_0^s \frac{-\hat{z} \times \mathbf{t}_r(s, \beta)}{1 + (-\hat{z}) \cdot \mathbf{t}_r} \cdot \frac{d\mathbf{t}_r(s, \beta)}{ds} ds. \tag{3.4}
 \end{aligned}$$

The first two integrals give no contribution, while the third gives

$$Wr(s^*, \beta) = Wr^i(s^*, \beta) - Wr^i(-s^*, \beta); \tag{3.5}$$

$$\begin{aligned}
 Wr^i(s, \beta) &= \frac{\arctan\left(\cos \frac{\alpha-\beta}{2} \csc \frac{\alpha+\beta}{2} \tan \frac{\pi s}{2}\right)}{\pi} \\
 &- \frac{1}{2} s \sin \alpha - n_{\text{sol}}(s, \alpha). \tag{3.6}
 \end{aligned}$$

The function  $n_{\text{sol}}$  eliminates the (here of 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. Note that eq. (3.6) deviates from eq. (1.41), computed using the axis of the helix (that here rotates with  $\beta$ ). The different behavior of the writhe is presented in fig. 3.2.

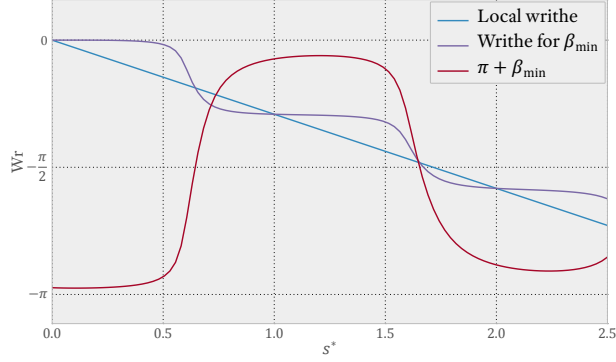


Figure 3.2: A comparison between the local writhe, i.e. using the helix axis in eq. 3.4, and the writhe for the  $\beta$  that minimized the energy,  $\beta_{\min}$ . For reference also  $\pi + \beta_{\min}$  is plotted.

In eq. (3.4) we use  $-\hat{z}$  instead of  $\hat{z}$  to avoid a vanishing denominator for some values of  $\beta < 0$ . 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.

### 3.3 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:

$$\begin{aligned}
 E_t(s^*, \beta) &= 2 \times \frac{A}{2} \int_{s_0}^{L_l(s^*, \beta)} \mathbf{t}_l^2(s) ds \\
 &+ f \Delta z(s^*, \beta) - 2 \int_0^{s^*} \frac{dE_{\text{ads}}(s)}{ds} ds \\
 &+ \frac{2\pi^2 C}{L - L_n(s^*)} (n - Wr(s^*, \beta))^2. \tag{3.7}
 \end{aligned}$$

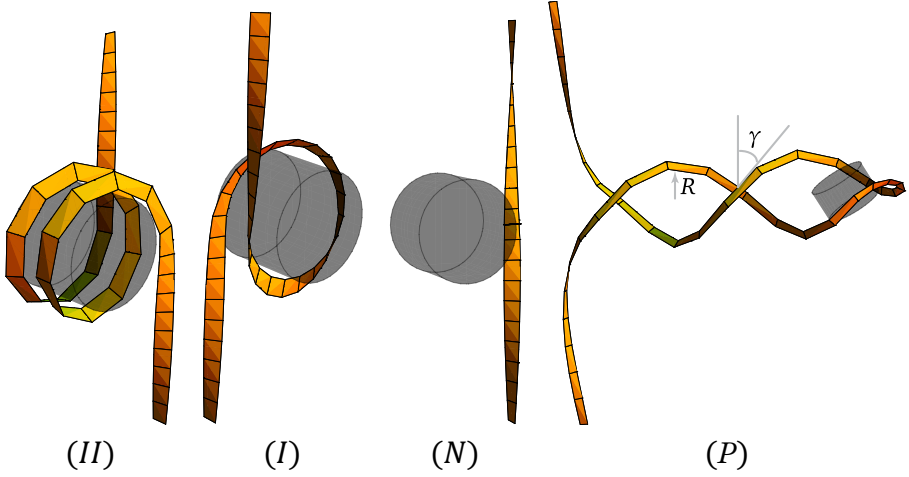


Figure 3.3: Schematic representation of the various stages of nucleosome unwrapping. The roman numerals indicate how many turns are approximately wrapped ( $N$  stands for 0,  $P$  stands for plectoneme). In order to show the effect of torque, the DNA double helix is here represented as a ribbon that is untwisted in the torsion-less case.

Here

$$\begin{aligned} \Delta z(s^*, \beta) = & L_n(s^*) + (\mathbf{r}_r(-s^*, \beta) - \mathbf{r}_r(s^*, \beta)) \cdot \hat{\mathbf{z}} \\ & + 2 \times \int_{s_0}^{L_l(s^*, \beta)} (1 - \mathbf{t}_l(s) \cdot \hat{\mathbf{z}}) ds \end{aligned} \quad (3.8)$$

is the shortening of the DNA end-to-end distance in the  $\hat{\mathbf{z}}$ -direction due to the bending of the legs and the wrapping around the octamer. The adsorption energy is given, with the relevant details, by eq. (1.56) and in the last term of eq. 3.7 the quantity  $C$  is related to the torsional persistence length  $l_t$  via  $l_t = C/k_B T$ ; we assume here  $l_t = 100$  nm [24].

To find the optimal configuration for given values of  $f$  and  $n$  the energy, eq. 3.7, needs to be minimized with respect to  $s^*$  and  $\beta$ . Since we neglect in our theory entropic contributions our results are only reliable for large enough forces,  $f \gtrsim 0.5$  pN [65].



## 3.4 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. 3.3(P)) that adsorbs approximately all the linking number inserted in the system (see [24] and chapter 4). 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  $p \geq 0$  with radius  $R$  and angle  $\gamma$  (fig. 3.3(P)):

$$E(p) = \frac{2\pi^2 C}{L} (n - dWp)^2 + (f + dE_b)p. \quad (3.9)$$

Here  $dW = \cos \gamma \sin \gamma \text{sign } n/2\pi R$  and  $dE_b = A \cos^4 \gamma/(2R^2)$  are, respectively, the writhe density and the bending energy density of the plectoneme (see chapter 4).

As specified in chapter 4, in eq. 3.9 we ignore the energetic contribution of the end loop assuming that the nucleosome sits at the end of the plectoneme (fig. 3.3(P)). In principle a plectoneme could also appear somewhere else. However the high bending energy of an end loop makes it highly improbable.

By minimizing eq. 3.9 for  $p$  one finds that a plectoneme is expected, i.e.  $p > 0$ , for all values of  $n$  such that

$$n \notin \left[ -\frac{(f + dE_b)L}{4\pi^2 C dW} + Wr(2), \frac{(f + dE_b)L}{4\pi^2 C dW} \right]. \quad (3.10)$$

Here  $Wr(2) = -2.14$  is the writhe for 2 fully wrapped turns that for  $s^* = 2$  is independent of  $\beta$  (see fig. 3.2). 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 chapter 4 we use  $\gamma \approx 1$  and  $R \approx 1.8$  nm when the salt concentration is about 150 mM. We stress that when the plectoneme forms, it forms on top of the state the system had before the formation. E.g.: if the system has approximately two

turns wrapped, when we, for example, decrease  $n$  so that it is outside the interval describe by eq. (3.10), then a plectoneme will form with at its end a nucleosome wrapped twice. In this sense fig. 3.3( $P$ ) is only indicative of what really happens.

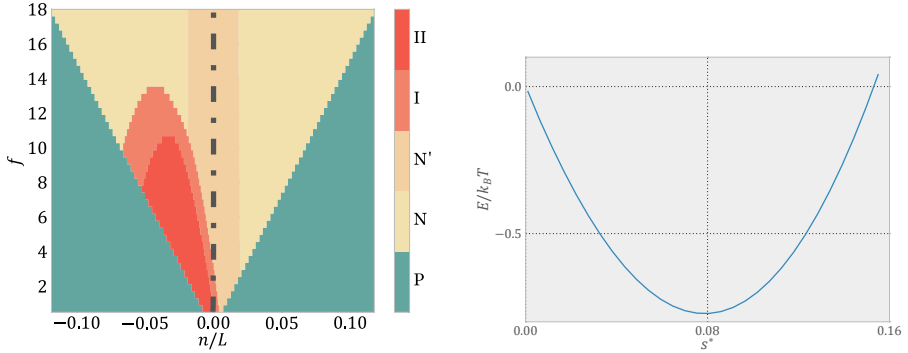
## 3.5 Twist defects

Apart from the plectoneme, twist defects [35, 45] can influence the nucleosome stability. A twist defects is present in a nucleosome when one DNA base pair 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. 3.9 for the twist defects if we replace  $p \rightarrow m\Delta l$ ,  $dW \rightarrow k \text{ sign } n/\Delta l$ ,  $dE_b \rightarrow dE_d/\Delta l$  and  $f \rightarrow f \text{ sign } n$ .  $m$  is an integer between 0 and 13 denoting the number of defects.  $\Delta l = 0.34 \text{ nm}$  and  $k = 1/10$  are, respectively, the length and twist lost or gained by a defect. Finally  $dE_d = 9k_B T$  is the energetic cost of a defect [35]. Since  $m \leq 13$  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. 3.10.

The 1.3 turns per nucleosome are found in experiments where chromatin fibers are put under positive torsional stress [2]. 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.

## 3.6 Results

In fig. 3.4a we present the optimal nucleosome configurations for a wide range of forces and  $n/L$ -values of a DNA molecule with  $L = 3500 \text{ nm}$



(a) Diagram of state showing the configurations with the lowest energy for  $L = 3500$  nm in the  $f$ - $n/L$ -plane. The grey dashed-dotted line represents the writhe of the nucleosome when the legs are free to release torsional stress.

(b) The energy landscape near  $s^* = 0$  for  $f = 10$  pN,  $n = 0$ . The minimum of energy is very close to the  $s^* = 0$  case which makes it easy for the nucleosome to “evaporate.”

Figure 3.4: Various results from the computations.

length. This diagram of states is nearly identical for all experimentally reasonable values of  $L$ , say for all  $L > 500$  nm. We find five different states, four of which are depicted in fig. 3.3 (*II*) fully wrapped, (*I*) one turn wrapped, (*N*) unwrapped and (*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  $k_B T$  away from the totally unwrapped state. A typical example is shown in fig. 3.4b. 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. 3.7, 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. 3.4a. The factor  $1/(L - L_n)$  in the twist energy dominates then the behavior. In the diagram of states, fig. 3.4a, we indicate also by a dashed-dotted line the torsion-less case where the unbound DNA is free to rotate. This situation has been studied in Ref. [36].

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 in the diagram of states, fig. 3.4a. 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 [43, 33]. The boundaries and corresponding barriers between (*II*) and (*I*) and between (*I*) and (*N'/N*) are shown in fig. 3.5. 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 transition are associated with much higher barrier values (see fig. 3.5).

## Appendix: why can we assume the legs to be planar?

The bending and force contribution of the legs in the  $t = 1$  case basically always smaller than when  $t < 1$ . This leaves the writhe as the other possible source of error when excluding the non-planar solutions. However looking at the contribution of the legs to the torsional energy

$$E_T \propto (n - Wr_l)^2$$

$$Wr_l = \frac{2}{\pi} \arcsin t \quad (3.11)$$

we see that when  $n \notin [-1, 1]$ , the energy is minimized by  $t = 1$ . Considering the large amount of turns investigated (see figure 3.4a) we can safely ignore the non-planarity of the loop. Moreover eq. (3.11) is only partially true: when the legs are cut at  $s_0$ , which is generally high, at least for the case (*II-I-N*) of figure 3.3, the writhe contribution would decrease,

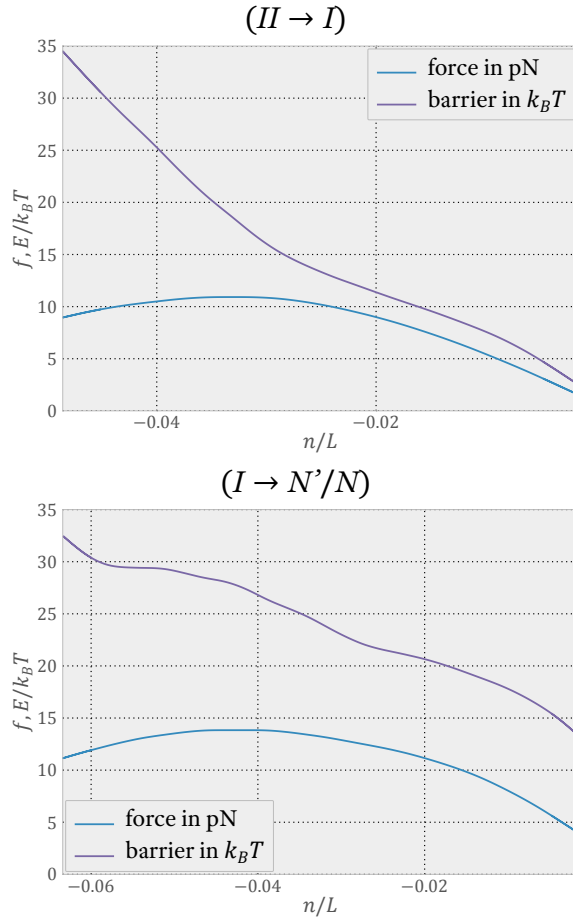


Figure 3.5: The force at which the minimum of the energy around  $s^* = 2$  (1) and the one around  $s^* = 1$  (0) have the same value, and the energy barrier necessary to cross from one state to the other, as a function of  $n/L$ . Here  $L = 3500$  nm.

reducing even further the region in which a non-planar homoclinic loop matters.

