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Magnetic tweezers based force spectroscopy studies of the structure and dynamics of nucleosomes and chromatin

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Citation

Kruithof, M. C. (2009, October 1). *Magnetic tweezers based force spectroscopy studies of the structure and dynamics of nucleosomes and chromatin*. Retrieved from <https://hdl.handle.net/1887/14030>

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CHAPTER 5

Thermal Fluctuations of Neighbouring Nucleosomes increase Nucleosome Stability¹

Abstract

Nucleosomes play a fundamental role in DNA compaction. For a greater understanding of processes that involve protein DNA interaction it is therefore imperative to determine physical properties such as the interaction energy between the DNA and the histone core. These properties are generally studied using force spectroscopy. DNA unwraps in two steps from the histone core. Experiments on single nucleosomes and nucleosomes in a fiber revealed an unexpected difference of the unwrapping force of the unwrapping of the first turn and unwrapping of the second turn. The unwrapping forces for a single nucleosome were much smaller, 3 pN for the first turn and 6 pN for the second turn, than those for a nucleosome in a fiber, 6 pN and 18 pN respectively. Here we modeled a nucleosome-DNA-bead system, used in force spectroscopy experiments, as spheres and springs. We found that the thermal fluctuations of neighbouring nucleosomes stabilized the nucleosome thereby increasing the unwrapping force for a nucleosome in a fiber. This effect shows that results obtained for single nucleosomes cannot simply be extrapolated to a system containing more than 1 nucleosome.

¹A manuscript based on this chapter has been submitted for publication.

5.1 Introduction

Chromatin structure plays a fundamental role in the regulation of nuclear processes such as DNA transcription, replication, recombination, and repair. The basic repeating unit of chromatin, the nucleosome core particle, organizes 147 bp of DNA in 1.65 left-handed superhelical turns around an octamer of four core histones [1–3]. The nucleosome is a dynamic entity in which the DNA transiently unwraps from the histone core. *In vitro* linear nucleosome arrays fold into a fiber of 30 nm diameter [4, 5]. The mechanical stability of single nucleosomes [6] (Chapter 3 of this thesis) as well as chromatin fibers [7–16] (Chapter 4 of this thesis) have been intensively studied by force spectroscopy. Such measurements on single nucleosomes show unwrapping of the first turn of DNA, occurring at 3 pN, and unwrapping of the second turn at 6 pN [6] (Chapter 3 of this thesis). In contrast, experiments on chromatin fibers did not show unwrapping of the first turn of DNA until the force is increased to 6 pN (Chapter 4 of this thesis). Similarly, unwrapping of the second turn of DNA from a nucleosome embedded in chromatin occurs at forces much higher ($F > 15$ pN) than for a single nucleosome ($F \sim 6$ pN) [9]. Since some of these experiments were performed at constant force, the striking difference between the unwrapping forces of single nucleosomes and fibers cannot be explained by the difference in loading rate, which determines the unwrapping force in non-equilibrium conditions [17]. The large differences in unwrapping forces warrants a closer inspection of the (un)wrapping dynamics of nucleosomes under force before extrapolation to the *in vivo* DNA organisation of chromatin fibers can be made.

To investigate the apparent discrepancies in unwrapping forces between the different structures we will first analyse the unwrapping of single nucleosomes with and without a tether to a surface. From this analysis we will see that a nucleosome tethered to a surface is subject to force fluctuations. To calculate the effect of the force fluctuations it is necessary to include the geometry of the fiber and the mechanical coupling generally used in these experiments: a DNA molecule attached to a fixed surface on one end and a bead on the other end. With the description of this system we fit experimental data obtained by Mihardja *et al.* [6] and find that the energy landscape is asymmetric. Which, in combination with the force fluctuations and the large change in stiffness of the DNA during unwrapping, drives the nucleosome into the wrapped state. When we compare single nucleosomes to nucleosomes in fibers we find that the force fluctuations that the nucleosome experiences in the fibers is much larger than the fluctuations in a force spectroscopy experiment on a single nucleosome. Since larger force fluctuations drive the nucleosome towards the wrapped state, a nucleosome in a fiber will unwrap at a higher force. This phenomenon is similar to the mechanism of thermal ratchets in which stochastic fluctuations can drive an object in a specific direction if the energy landscape would be asymmetric.

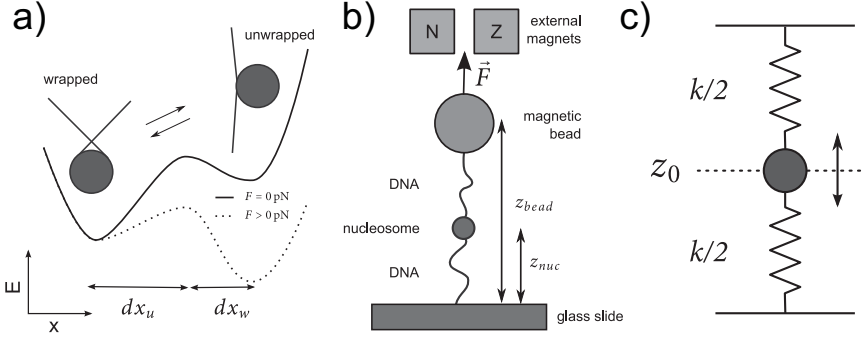


Figure 5.1: (a) Schematic overview of the energy landscape (black line) shows the distances in reaction coordinates between the wrapped and unwrapped state and the transition state. Increasing the force tilts the energy landscape (dashed line) and changes the height of the energy barrier. (b) A schematic overview of a mononucleosome in a magnetic tweezers setup. (c) A model of a nucleosome tethered to a surface. The nucleosome (dark grey sphere) fluctuates around a position z_0 and is attached on both ends to two springs with spring constant $k/2$.

5.2 A Model for a Single Nucleosome Under Force

Let us first consider a single nucleosome in solution. A simple model for the energy landscape for unwrapping of the nucleosome is depicted by the solid line in Fig. 5.1a, where a nucleosome in the unwrapped state is separated from the wrapped state by an energy barrier E_b . The lifetime, τ_0 , of a state (wrapped or unwrapped) of the nucleosome in free solution is [17, 18]

$$\tau_0 = \frac{1}{\omega_0} \exp\left(\frac{E_b}{k_b T}\right), \quad (5.1)$$

with ω_0 a frequency factor and $k_b T$ the thermal energy. Because the DNA ends are free to move, the motion of the nucleosome does not affect the energy landscape of unwrapping. This changes, however, when the nucleosome is tethered to a fixed surface. Any movement of the nucleosome will change the tension in the tether, thereby applying a force to the DNA exiting from the nucleosome. This force will tilt the energy landscape (Fig. 5.1a dashed line) and changes the height of the energy barrier. To quantify the effect of the change in height of the energy barrier on the dynamics of the DNA of a nucleosome, we describe the dependence of the lifetime of a state i of the nucleosome on a force F by an Arrhenius-like expression

$$\tau_i(F) = \tau_{0,i} \exp\left(-\frac{F a_i}{k_b T}\right), \quad (5.2)$$

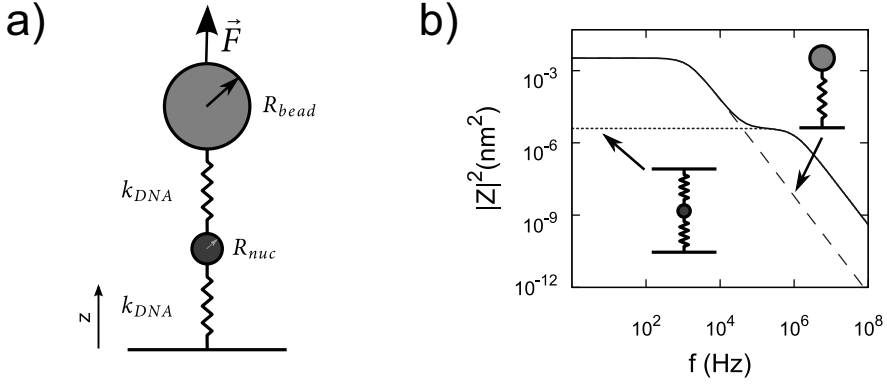


Figure 5.2: Modeling nucleosome thermal motion. (a) The magnetic bead and the nucleosome are modeled as spheres, the radius of the bead is $1\ \mu\text{m}$ and the radius of the nucleosome $11\ \text{nm}$. The DNA is modeled as a spring, with a spring constant calculated from Eq. 5.10 using a contour length of $500\ \text{nm}$, a persistence length of $52\ \text{nm}$ at a force of $3\ \text{pN}$. (b) The model for the power spectral density (PSD) of the nucleosome (solid line) can be approximated by the sum of the PSD of the bead (dashed line) and the PSD of the nucleosome if it was attached between two fixed points (dotted line).

with a_i the distance in reaction coordinates between state i and the transition state.

During a force spectroscopy experiment the force on the nucleosome is generally applied by tethering the nucleosome between a bead and a surface and then applying a force to the bead using a focused laser (optical tweezers) or a pair of magnets (magnetic tweezers) as depicted in Fig. 5.1b. The bead will be subject to thermal fluctuations, changing the tension in the tether and contributing to the force fluctuations on the nucleosome. A full description of the mechanics of the system should take the motion of the bead during a force spectroscopy experiment into account. Let us consider the equations of motion of a DNA-nucleosome-bead system as shown in Fig. 5.1b. We model both the magnetic bead and the nucleosome as spheres and the connecting DNA as springs, as depicted in Fig. 5.2a. The equations of motion of the whole system become

$$\begin{aligned} m_{nuc} \frac{d^2 z_{nuc}}{dt^2} &= -z_{nuc} k_{DNA} - \gamma_{nuc} \frac{dz_{nuc}}{dt} + (z_{bead} - z_{nuc}) k_{DNA} + F_{nuc} \\ m_{bead} \frac{d^2 z_{bead}}{dt^2} &= -(z_{bead} - z_{nuc}) k_{DNA} - \gamma_{bead} \frac{dz_{bead}}{dt} + F_{bead} + F_{ext}, \end{aligned} \quad (5.3)$$

with m the respective masses, z the positions, and γ the drag coefficients of the nucleosome and the bead. F_{bead} and F_{nuc} are the Brownian forces on the bead and the nucleosome. k_{DNA} is the spring constant of the DNA and F_{ext} the external force on that the tweezers apply on

the bead. In case of magnetic tweezers F_{ext} is a constant force, in case of optical tweezers $F_{ext} = k_{trap} (z_{bead} - z_{trap})$. The system is overdamped ($Re \ll 1$) thus inertia can readily be neglected, $m \frac{d^2 z}{dt^2} = 0$. Since we are interested in fluctuations around a steady state, we solve the Fourier transform of the equations of motion

$$\begin{aligned} 0 &= -Z_{nuc} k_{DNA} - 2\pi i \gamma_{nuc} Z_{nuc} f + (Z_{bead} - Z_{nuc}) k_{DNA} + \mathcal{F}_{nuc} \\ 0 &= -(Z_{bead} - Z_{nuc}) k_{DNA} - 2\pi i \gamma_{bead} Z_{bead} f + \mathcal{F}_{bead} + \mathcal{F}_{ext}, \end{aligned} \quad (5.4)$$

with Z_{bead} and Z_{nuc} the Fourier transform of z_{bead} and z_{nuc} , $\mathcal{F}$ the Fourier transform of the Brownian force, and f the frequency. The Fourier transform of the external force, $\mathcal{F}_{ext}$, is constant for magnetic tweezers and only contributes at $f = 0$: $\mathcal{F}_{ext} = F_{ext} \delta(f)$. For optical tweezers in position clamp mode, the position of the trap remains constant leading to $\mathcal{F}_{ext} = k_{trap} (z_{trap} \delta(f) + Z_{bead})$. The drag coefficients of the bead and the mononucleosome depend on their radius, R , and the kinematic viscosity, η , as defined by Stokes' law

$$\gamma = 6\pi\eta R. \quad (5.5)$$

From fluctuation dissipation theorem, it follows that the Brownian force appears as white noise in the spectrum [19]:

$$|\mathcal{F}|^2 = 24\pi k_b T \eta R. \quad (5.6)$$

The stiffness of a tether can be calculated from

$$k(F) = \left. \frac{dF(s)}{ds} \right|_{s=z(F)}, \quad (5.7)$$

with $F(s)$ the force needed to stretch the tether to an extension s and $z(F)$ the extension of the tether at a constant force F , which is the inverse of $F(s)$. If DNA is taken to be the tether, the force needed to extend a DNA molecule with a persistence length p and a contour length L is given by the Worm-Like-Chain (WLC) model [20]

$$F_{WLC}(z) = \frac{k_b T}{p} \left[\frac{1}{4 \left(1 - \frac{z}{L}\right)^2} - \frac{1}{4} + \frac{z}{L} \right]. \quad (5.8)$$

At large forces ($F > 5$ pN) the elastic stretching of the DNA molecule cannot be neglected [21]. The elastic stretching spring constant of a DNA molecule is

$$k_{ES} = \frac{K_0}{L}, \quad (5.9)$$

with K_0 the stretching modulus of DNA. Thus the spring constant of a DNA molecule for the

full force range is given by a entropic spring (Eq. 5.7) and an elastic spring (Eq. 5.9) in series

$$k_{DNA}(F) = \frac{k_{ES}k_{WLC}(F)}{k_{ES} + k_{WLC}(F)}, \quad (5.10)$$

with $k_{WLC}(F)$ and $k_{elastic}(F)$ from Eqs. 5.7 and 5.8. The spring constant of a DNA molecule depends on the external force, the contour length and the persistence length of the molecule.

We first compute the power spectral density (PSD) of the motion of the nucleosome-bead system. The PSD shows that in the case of a constant external force of 3 pN, at low frequencies ($f < 10^4$ Hz) the bead dominates the motion of the nucleosome (Fig. 5.2b). At high frequencies ($f \gg 10^4$ Hz) the motion of the bead gets dampened out due to its larger radius and the nucleosome behaves like a sphere attached between two fixed points.

The solutions for the PSD of the nucleosome of Eq. 5.4 can be split into two contributions, the bead without nucleosome, $|Z_{bead,sol}(F)|^2$, and the nucleosome without bead, $|Z_{nuc,sol}(F)|^2$ (Fig. 5.2b)

$$|Z_{nuc,tot}(F)|^2 = |Z_{bead,sol}(F)|^2 + |Z_{nuc,sol}(F)|^2. \quad (5.11)$$

The average fluctuations in position of the nucleosome are calculated from the PSD using

$$\sigma_z^2 = \int_{-\infty}^{\infty} |Z(F)|^2 df. \quad (5.12)$$

Leading to the contribution of the bead to the thermal motion of the nucleosome

$$\sigma_{z,bead}^2(F) = \frac{k_b T}{k_{DNA}(F) + k_{trap}}, \quad (5.13)$$

with $k_{DNA}(F)$ the stiffness of the entire DNA molecule that links the nucleosome to the bead and the surface and k_{trap} the stiffness of an optical trap (for magnetic tweezers and an optical trap in constant force mode $k_{trap} = 0$). For the PSD contribution of the nucleosome we get

$$\sigma_{z,nuc}^2 = \frac{k_b T}{k_{DNA,1}(F) + k_{DNA,2}(F)}, \quad (5.14)$$

with $k_{DNA,1}(F)$ the stiffness of the DNA below the nucleosome, and $k_{DNA,2}(F)$ the stiffness of the DNA above the nucleosome.

5.3 The Effect of Thermal Motion on Wrapping Dynamics

When the energy landscape is rocked back and forth the lifetimes change continuously, leading us to calculate the average lifetime of state i from the average rate constant, $k = 1/\tau$, using Eq. 5.2 [22, 23]

$$\begin{aligned}
 \langle \tau_i (F(t)) \rangle &= \langle k_i (F(t)) \rangle^{-1} \\
 &= \tau_{0,i} \left\langle \exp \left[\frac{F(t)a_i}{k_b T} \right] \right\rangle^{-1} \\
 &= \tau_{0,i} \left(\int_{-\infty}^{\infty} P(F_0, \sigma_F^2) \exp \left[\frac{Fa_i}{k_b T} \right] dF \right)^{-1} \\
 &= \tau_i (F_0) \exp \left[-\frac{a_i^2}{2(k_b T)^2} \sigma_F^2 \right],
 \end{aligned} \tag{5.15}$$

with $P(F_0, \sigma_F^2)$ the probability distribution of the force fluctuations, which is normally distributed around the average force F_0 with a variance of σ_F^2 . From Eq. 5.15 we see that the lifetime of each state is decreased due to the force fluctuations. More importantly, if the energy landscape is asymmetric ($a_u \neq a_w$), the lifetime of the state with the shortest distance to the transition state decreases less than the lifetime of the other state, driving the nucleosome towards this state. Furthermore, we see from Eq. 5.15 that this effect becomes stronger with increasing force fluctuations. Since the nucleosome is subject to thermal motion, the energy landscape of the nucleosome will constantly tilt back and forth. Thus the (un)wrapping of a nucleosome can be described as a rocked thermal ratchet [24–26].

As we have seen the thermal motion of the nucleosome can be split into two components, each component results in a force to the DNA of the nucleosome. The average contributions to the force caused by each component is calculated from Eqs. 5.13 and 5.14

$$\sigma_F^2 = k^2 \sigma_z^2 = k_b T k. \tag{5.16}$$

Thus, the stiffness of the tether, k , is the only variable which determines the force fluctuations. Using Eq. 5.10 we find that the force fluctuations grow with increasing force and decreasing contour length.

Combining Eqs. 5.13, 5.14, 5.15, and 5.16, we get for the average lifetime of a state of the nucleosome

$$\begin{aligned}
 \langle \tau_i (F, t) \rangle &= \tau_i (F) \exp \left[-\frac{a_i^2 (k_{DNA,1,i}(F) + k_{DNA,2,i}(F))}{2k_b T} \right] \\
 &\times \exp \left[-\frac{a_i^2 (k_{DNA,i}(F) + k_{trap})}{2k_b T} \right].
 \end{aligned} \tag{5.17}$$

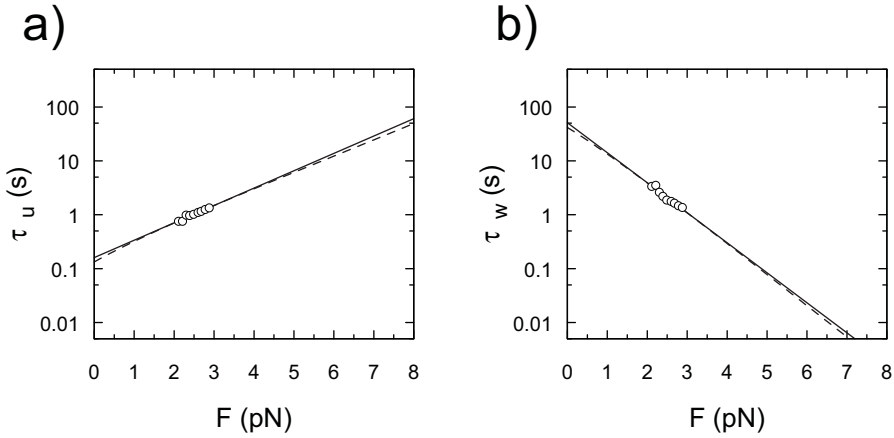


Figure 5.3: (a) The unwrapped lifetime fit with a simple exponential (solid line) and the force-fluctuation corrected model (dashed line). (b) The wrapped lifetime fit with a simple exponential (solid line) and the force-fluctuation corrected model (dashed line).

Eq. 5.17 describes the lifetime of the wrapped or unwrapped state of a single nucleosome tethered between a surface and a bead as depicted in Fig. 5.1b, which we can use to fit the force-lifetime curve obtained from single molecule force spectroscopy experiments.

5.4 Comparison With Experiments on Mononucleosomes

Let us use Eq. 5.17 to extract the distance between the wrapped and unwrapped state and the transition state from single molecule force spectroscopy experiments. Mihardja *et al.* [6] measured mononucleosome (un)wrapping dynamics under constant force using optical tweezers. Since the lifetime depends exponentially on the stiffness which in turn depends on the contour length and the apparent persistence length of the tethering DNA we need to carefully consider the DNA-nucleosome-bead geometry. In the experiments of Mihardja *et al.* [6], the length of DNA, before unwrapping, on one side of the nucleosome was 375 nm and on the other side 785 nm leading to a total contour length of 1160 nm. Furthermore, it was shown that a kink or bend in the DNA, induced by a nucleosome, reduces the apparent persistence length of the DNA [27–30] (Chapter 3 of this thesis). The dimensions in this particular experiment result in a reduced apparent persistence length of 48 nm (Chapter 3 of this thesis) compared to 52 nm for DNA that does not contain a kink.

The fits of the data to Eq. 5.17 are depicted in Fig. 5.3a and b. In this case the corrected model (Eq. 5.15, blue line) deviates only marginally from a simple exponential model (red line) which

does not take the compliance of the tethers and the Brownian motion of the bead and nucleosome into account. The fit resulted in a distance between the unwrapped state and the transition state $a_u = 4.19 \pm 0.06$ nm, and a lifetime of the unwrapped state at zero force $\tau_u = 0.135 \pm 0.004$ s. For the distance between the wrapped state and the transition state we got $a_w = 4.02 \pm 0.04$ nm and a wrapped lifetime of $\tau_w = 41.6 \pm 1.5$ s, resulting in an equilibrium constant of $K = \frac{\tau_w}{\tau_u} = 380$ for wrapping in absence of force, this large equilibrium constant in absence of force confirms that the nucleosome is a very stable structure. Mihardja *et al.* found a lifetime of the unwrapped state of $\tau_u = 0.017$ s and a lifetime of the wrapped state of $\tau_w = 2600$ s. They fit their data with an equation that accounts for the decreased hop size they see with increasing force, which explains the difference between their results and our results. However we did not see this behavior in our experiments on mononucleosomes (see Chapter 3 of this thesis). This counter intuitive approach results in an improbable large lifetime of the wrapped state. Li *et al.* [31] measured the zero-force lifetimes of nucleosome breathing directly using bulk FRET. They observed a wrapped lifetime of 0.25 s and an unwrapped lifetime of 0.01 s. Our lifetimes are much larger, which might be explained since we extrapolate force induced full turn unwrapping events whilst the FRET experiments look at smaller unwrapping lengths.

Though the data show a slight asymmetry in the energy landscape of unwrapping, the difference is only marginal. Similar to a rocked thermal ratchet, the force fluctuations decrease the lifetimes of each state but due to the asymmetry in the energy landscape the lifetime of one state will decrease more than the other which will drive the nucleosome towards the state with the smallest distance to the transition state, in this case the wrapped state. The larger these fluctuations, the more the nucleosome gets driven towards the wrapped state, showing that force fluctuations actually stabilize the nucleosome.

5.5 Contributions of Neighbouring Nucleosomes

Now that the distances between the wrapped, the unwrapped and the transition state are known for a single nucleosome, let us consider the motion of a single nucleosome within an array of nucleosomes tethered between a glass surface and a bead. The presence of neighbouring nucleosomes will result in additional force fluctuations. These additional force fluctuations can be calculated from the relative motion of a neighbour and the stiffness of the DNA between the nucleosome and its neighbour. Fig. 5.4 shows the relative contribution of the neighbours on the force fluctuations of a nucleosome. Since a nucleosome at the end of a fiber has a different configuration of neighbours as a nucleosome at the center of a fiber, the force fluctuations

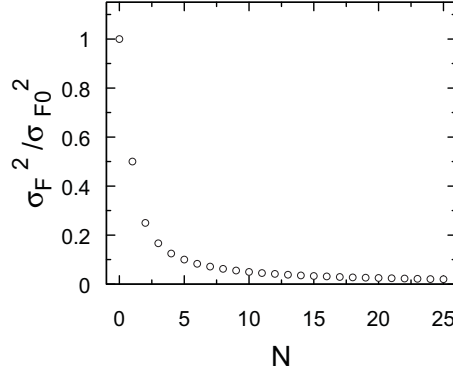


Figure 5.4: *The contribution of neighbour N to the force fluctuations of a nucleosome relative to the fluctuations of the nucleosome.*

the nucleosomes feel will be different. This situation is similar as described before (Eq. 5.11), but now the fluctuations of neighbouring nucleosomes add to the motion of the nucleosome resulting in a sum over all neighbours

$$\sigma_{z,neigh,i}^2 = \sum_j \frac{k_b T}{k_{DNA,neigh,j}(F)}, \quad (5.18)$$

where we estimate the stiffness of the DNA between the nucleosomes, $k_{DNA,neigh}(F)$, using Eq. 5.10.

Using Eqs. 5.13, 5.14, 5.15, 5.16, and 5.18 we get for the average lifetime of a state of the nucleosome in a fiber

$$\begin{aligned} \langle \tau_i(F, t) \rangle &= \tau_i(F) \exp \left[-\frac{a_i^2 (k_{DNA,1}(F) + k_{DNA,2}(F))}{2k_b T} \right] \\ &\times \exp \left[-\frac{a_i^2 k_{DNA}(F)}{2k_b T} \right] \exp \left[-\frac{a_i^2}{2k_b T} \sum_j k_{DNA,neigh,j}(F) \right]. \end{aligned} \quad (5.19)$$

We need to carefully look at the contour and apparent persistence length of the DNA in the fiber. The fibers used in our experiments (Chapter 4 of this thesis) contain 25 nucleosomes. The distance between the nucleosomes is 17 nm and the total contour length of the fiber is 782 nm. The distance between the two neighbours of a nucleosome is therefore 34 nm. After unwrapping, the length of DNA between the nucleosome and its neighbour becomes longer (29 nm) as well as the distance between the neighbours (58 nm). As shown before for the mononucleosome, the loop induced by the nucleosome in the DNA causes a change in apparent persistence length. We expect this effect to be much larger for a fiber since more nucleosomes are present.

The change in apparent persistence length due to a loop in the DNA can be calculated from the exit angle of the DNA wrapped around the nucleosome [30]. If we take for the exit angle $\alpha = 81^\circ$ (Chapter 3 of this theses) we get for the apparent persistence length $p = 5$ nm. However, in measurements on chromatin fibers in the beads-on-a-string configuration we see a apparent persistence length of 10 nm (Chien *et al.* unpublished work). This difference might be explained because the relation between the apparent persistence length and the exit angle assumes freely rotating DNA ends which is not the case for a fiber. We took the apparent persistence length found in our previous measurements of 10 nm before unwrapping in an array of nucleosomes. After unwrapping, the exit angle is much larger $\alpha = 144^\circ$ resulting in a apparent persistence length of 50 nm. Due to the difference in opening angle, the apparent persistence length before unwrapping is smaller than the apparent persistence length after unwrapping which, combined with the larger contour length between nucleosomes after unwrapping, leads to a change in the compliance of the fiber.

5.6 Second Turn Unwrapping

The lifetimes of the second turn unwrapping can also be calculated using Eq. 5.17 for a single nucleosome and Eq. 5.19 for a nucleosome in a fiber. Again we need to consider the the contour and apparent persistence length of the DNA in the fiber. For the second turn, the distance between the nucleosomes is 41 nm. After unwrapping, the length of DNA between the nucleosome and its neighbour becomes longer (66 nm). Again the loop induced by the nucleosome in the DNA causes a change in apparent persistence length. For the second turn the change in persistence length is much less than for the first turn, since the angle is closer to 180° . If we take for the exit angle $\alpha = 144^\circ$ (see Chapter 3 of this thesis) we get for the apparent persistence length $p = 48$ nm. Furthermore, we need to know the distance between the wrapped and unwrapped state and the transition state. Brower-Toland *et al.* [9] calculated the distance between the wrapped state and the transition state to be 3.2 nm. Because the second turn of the nucleosome does not rewrap during force spectroscopy experiments [6, 9, 12] the distance between the unwrapped state and the transition state is not known. We chose the distance between the unwrapped state and the transition state such that it would lead to an unwrapping of the second turn of a single nucleosome at 6 pN as reported by Mihardja [6] resulting in a distance of 4.3 nm.

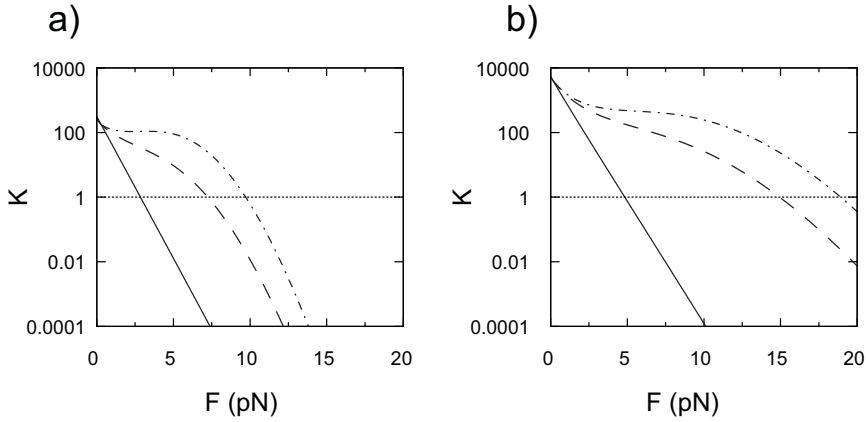


Figure 5.5: (a) The ratio between the corrected wrapped lifetime and the corrected unwrapped time of the first turn for a mononucleosome (solid line), a nucleosome in the center a fiber (dash dotted line) and a nucleosome at the end of a fiber (dashed line). The transition between wrapped and unwrapped state occurs near the force where the ratio crosses 1. (b) The ratio between the corrected wrapped lifetime and the corrected unwrapped time of the second turn for a mononucleosome (solid line), a nucleosome in the center of a fiber (dash dotted line) and a nucleosome at the end of a fiber (dashed line). The transition between wrapped and unwrapped state occurs near the force where the ratio crosses 1.

5.7 Rupture Forces Depend on Force Fluctuations

Now that we can calculate the lifetimes of a nucleosome in a fiber, we can compare the lifetimes in a fiber to the lifetimes in a mononucleosome. We define the unwrapping force as the force at which the wrapping equilibrium constant is 1. When this equilibrium constant is larger than 1, the nucleosome spends most of its time in the wrapped state. The reverse holds when the equilibrium constant is smaller than 1. When the equilibrium constant is 1, wrapping and unwrapping occurs at the same rate. These wrapping and unwrapping events of the first turn of DNA can directly be observed in constant force time traces. The wrapping equilibrium constant, as calculated from Eq. 5.17 is depicted in Fig. 5.5a for a mononucleosome and for a fiber. The figure shows unwrapping occurring at a force of 3 pN for mononucleosome and 6 pN for a nucleosome embedded in a fiber. This exactly reproduces values reported in previous measurements [6, 9, 12] (Chapter 3 and 4 of this thesis). Furthermore it is interesting to see that a nucleosome in the center of the fiber has an even larger rupture force than a nucleosome at the end of the fiber.

The quantitative model shows that when the energy landscape of the unwrapping is asymmetric, with $a_u > a_w$, force fluctuations drive the nucleosome towards the wrapped state and

therefore increase the unwrapping force. Because the lifetimes depend exponentially on the distance between the state and the transition state, even a small asymmetry can have a large effect on the unwrapping force. The change in stiffness of the DNA during unwrapping also pushes the nucleosomes into the wrapped state and for the unwrapping of the first turn this effect dominates the change in unwrapping force. Since a nucleosome in a fiber is much closer to its neighbours the coupling with the fluctuations of the neighbouring nucleosomes causes the force fluctuations to be much higher, stabilizing the nucleosome and thereby increasing the unwrapping force.

The same argument holds for the unwrapping force of the second turn, as is depicted in Fig. 5.5b where the equilibrium constant of a single nucleosome is equal to 1 at 6 pN and the equilibrium constant for a nucleosome in a fiber is equal to 1 at a much higher force (20 pN), which explains the large differences between the unwrapping force of fibers [9] and mononucleosomes [6] in force spectroscopy experiments. Here the change in stiffness is much less and the asymmetry plays the dominant role. Again a nucleosome in the center is more stable than a nucleosome at the end of the fiber.

This effect implies that the nucleosomes unwrap cooperatively because if a nucleosome is fully unwrapped the length of DNA between it and a neighbouring nucleosome suddenly increases. This will decrease the force fluctuations for that nucleosome, which in turn will destabilizes the next nucleosome and so forth. Furthermore, as mentioned before, since a nucleosome at the end of a fiber is less stable than a nucleosome in the center, unwrapping is much more likely to start at the ends of the fiber.

The change in equilibrium constant due to the stiffness of the DNA attached to a nucleosome is important in any experiment where a nucleosome is attached to a surface with a small tether. Since the size of the tether is directly related to the extend of the force fluctuations, small tether lengths may drive the nucleosome into the wrapped state, even in absence of force. The lifetimes found in such experiments are underestimates of the lifetime of a nucleosome in solution, though the equilibrium is shifted towards the open state.

Although such tethering seems artificial, they give a more accurate description of the behavior of nucleosomes *in vivo*, since there the distance between a nucleosome and its neighbour is generally small, in the order of 20 nm [32]. It is imperative to take the effect of the force fluctuations into account when general conclusions are made about the *in vivo* situation from measurements on single nucleosomes. Recent theory and simulations on the unfolding of RNA hairpins shows a similar trend where an increased stiffness of the tether decreases the lifetime of the folded state [33]. The arrest force of RNA polymerase, a typical DNA interacting motor protein in a cell, is 15 pN at room temperature [34], Fig. 5.3c shows that at those forces the wrapping equilibrium constant for a fiber differs significantly from the equilibrium constant

of a mononucleosome. This effect stabilizes the nucleosome but at the same time makes it more open, since the fluctuations between the conformations increase due to the decreased lifetimes.

5.8 Conclusion

We have shown that force induced unwrapping of a tethered nucleosome should be described by a rocked energy landscape in analogy with a thermal ratchet. This rocking decreases the lifetimes of both the wrapped and the unwrapped state. Furthermore, the asymmetry in the energy landscape of nucleosome unwrapping drives the nucleosome towards the wrapped state. The asymmetry and the change in stiffness during unwrapping results in the large difference in unwrapping force that is observed between force spectroscopy experiments on fibers and mononucleosomes. It also shows that careful consideration is in order if one wants to extrapolate information from experiments on mononucleosomes to the *in vivo* case where nucleosomes are always embedded in chromatin. The neighbouring nucleosomes pick up thermal fluctuations since the length of the DNA between the nucleosome and its neighbours is an important parameter.

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