Chromatin dynamics: Nucleosomes go mobile through twist defects

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(Dated: February 2, 2008)

We study the spontaneous “sliding” of histone spools (nucleosomes) along DNA as a result of thermally activated single base pair twist defects. To this end we map the system onto a suitably extended Frenkel-Kontorova model. Combining results from several recent experiments we are able to estimate the nucleosome mobility without adjustable parameters. Our model shows also how the local mobility is intimately linked to the underlying base pair sequence.

PACS numbers: 87.15.He, 36.20.Ey

The genetic information of all higher organisms is organized in huge beads-on-a-chain arrays consisting of centimeters to meters of DNA wrapped around globular aggregates of so-called histone proteins. The basic unit of chromatin, the nucleosome, is a tiny 10 × 5 × 6 nm sized spool composed of 147 base pairs (bps) DNA tightly wrapped around an octamer made from 8 histone monomers. Each nucleosome is connected via a stretch of “linker” DNA to the next such protein spool. The wrapped DNA, being coiled in ~ $1 \frac{3}{4}$ turns of a left handed helix with radius ~ 4.2 nm, is strongly distorted from its preferred straight ground state due to strong interactions with the histone octamer, namely short range electrostatics (between the negatively charged DNA sugar-phosphate backbone and the positively charged octamer surface) and through extensive hydrogen-bonding – both localized at 14 discrete interaction patches helically arranged along the octamer surface.

Higher order structures, from the 30nm-chromatin fiber up to the highest level of DNA condensation, the fully folded chromosome, are designed to achieve a huge DNA volume fraction. They all rely on the significant stability of the nucleosome complex. On the other hand, fundamental life processes like transcription (making RNA offprints from the underlying DNA) and DNA replication seem to be in conflict with the picture of a stable nucleosome, as they are all performed by protein machines that track the DNA helix. The latter inevitably implies that every DNA bound obstacle (protein) has to be penetrated or even completely removed from its DNA target. In fact, the numbers are quite dramatic: A typical gene extends over hundreds of nucleosomes, each contributing 30 – 40 $k_B T$ net adsorption energy. Also other mechanisms like the activation of genes rely on regulatory protein binding to specific DNA sequences that are often covered by nucleosomes making them inaccessible.

A key to the understanding of these seemingly contradictory features might be the physical phenomenon of thermally driven nucleosome “sliding” along DNA (also called nucleosome repositioning) which has repeatedly observed in well-defined in vitro experiments, reviewed in Ref. 5. Spontaneous repositioning is strongly temperature dependent; at room temperature nucleosomes move a few tens of bps within an hour. Despite clear evidence for repositioning the underlying mechanism has been the matter of longstanding controversy, especially due to the lack of any quantitative theoretical treatment of nucleosome statics and dynamics that has to rely on the detailed knowledge of the molecular structure and its underlying parameters.

Only very recently – since the documentation of the high resolution X-ray structure and the presentation of other new experiments – this has become possible. First theoretical models of nucleosome repositioning assume that it is based on the formation of DNA loop defects that form on either end of the nucleosomal DNA followed by their thermal diffusion around the octamer, similar to the de Gennes-Edwards reptation mechanism. This model seems to be successful in explaining the apparent 10 bps quantization of the nucleosome “jump” length and it also reproduces the observed diffusion constants. Recent experiments, however, indicate a more local 1 bp-step mechanism that cannot be understood within this model. This lead us here to consider an alternative mechanism: twist diffusion. The carrier of motion in this case is a twist defect that contains one missing or one extra bp.

The possibility of twist defects was demonstrated as soon as the high resolution crystal structure of the core particle (the octamer plus wrapped DNA) was resolved. In that study the core particles were reconstructed from palindromic 146 bp DNA and histones assuming that this would result in a complex with perfect two-fold symmetry. However, it turned out that one bp is localized at the dyad axis, the rest being divided into a 73 bp half and a 72 bp half. The missing bp of the shorter half is, however, not localized at its terminus but instead at a 10 bp stretch close to the dyad axis (cf. Fig. 4d in Ref. 1). This is due to the attraction between the DNA termini of adjacent particles in the crystal that come close to mimic a bp step at the cost of forming a twist defect far inside the wrapped chain portion. This allows us to estimate the energy for a single defect to be smaller than the stacking energy that is $\sim 10 – 20 k_B T$.

In order to model the twist diffusion mechanism we
map the nucleosomal DNA on a Frenkel-Kontorova (FK) chain of particles connected by harmonic springs in a spatially periodic potential (cf. Fig. 1). The original FK model was introduced more than sixty years ago to describe the motion of dislocations in crystals [11]. In the meantime variants of this model were applied to many different problems including charge density waves [12], sliding friction [13,14], ionic conductors [13,16], chains of coupled Josephson junctions [17] and adsorbed atomic monolayers [18,19]. Here, in the context of DNA adsorbed on the octamer, the beads represent the base pairs. The springs in between have an equilibrium distance $b = 0.34$ nm and a constant that reflects the coupled DNA twist-stretch elasticity. Specifically

$$E_{\text{elastic}} \left( \{x_n\} \right) = \sum_k C \left( \frac{x_{k+1} - x_k}{b} - 1 \right)^2$$

(1)

Here the conformation of the wrapped DNA is given by the set $\{x_n\}$ where $x_n$ is the position of the $n$th bp measured along the helical backbone; $C \approx 70 - 100 k_BT$ is the combined twist and stretch spring constant including the (here unfavorable) twist-stretch coupling [20] and the summation goes over all bp associated with the wrapped DNA. In addition there is the external potential of the 14 contact points to the octamer with neighboring points being 10 bp apart [1] that we model as follows

$$E_{\text{ads}} \left( \{x_n\} \right) = -U_0 \sum_k \sum_{l=1}^{14} \left( \frac{x_k - 10lb}{a} - 1 \right)^2 \times \theta (a - |x_k - 10bl|)$$

(2)

with $\theta$ being the Heaviside step function. The two parameters of the external potential, its depth $U_0$ and its width $a$, can be estimated as follows. $U_0$ represents the pure adsorption energy per point contact which follows from competitive protein binding [2] to be of order $6k_BT$. The other parameter, $a$, can be estimated from the fluctuations of the DNA in the crystal measured by the B-factor (cf. Fig. 1b in [1]) at different nucleosome positions. The ratio of DNA helix fluctuations $R_{\text{fluct}} = \langle x_{\text{middle}}^2 \rangle / \langle x_{\text{bond}}^2 \rangle \approx 3$ at positions between the binding sites and at the bound sites is a measure of DNA localization. Using a quadratic expansion of Eq. 2 one finds from a straightforward normal mode analysis that $a = (5U_0/(R_{\text{fluct}} - 1)C)^{1/2} b \sim b/2$, i.e., the adsorption regions lead to a strong localization of the DNA. Knowing all involved parameters the total energy of the DNA chain confined in the nucleosome can be written down

$$E_{\text{tot}} = E_{\text{elastic}} + E_{\text{ads}} + E_{\text{sd}}$$

(3)

The last term $E_{\text{sd}}$ is the sequence dependent part of the total energy which we will neglect first. In the following we study the mechanism for thermal motion of DNA governed by $E_{\text{tot}}$. Generally two scenarios are possible: (i) The generation of kink-antikink pairs inside the nucleosome and (ii) a kink (or antikink) injection at either nucleosome end. Since the first mechanism is energetically roughly twice as costly than the second one, we will focus here on the (anti)kink injection mechanism only.

How and how fast does the kink step around the nucleosome? Due to the strong DNA localization at the binding sites $a/b < 1$ for a realistic range of parameters $U_0$ and $C$ the kink is localized either between two adsorption positions, i.e., smeared over 10 bp (denoted by the $K_{10}$ state), or between three of them, i.e., smeared out over 20 bp (the $K_{20}$ state). It is obvious that the motion of a (anti)kink will consist of an alternation between $K_{10}$ and $K_{20}$ states similarly to an earthworm creep motion. To model this process we introduce the effective kink coordinate $x_K$ describing the coordinate of the DNA bp being pinned/depinned during a single kink step, so that $x_K \approx 0$ and $x_K \approx b/2$ correspond to $K_{10}$ and $K_{20}$, respectively, whereas $x_K \approx b$ means that the kink moved by one bp step. The Peierls-Nabarro potential experienced by the kink is then given by $U_{PN}(x_K) = C_{\text{eff}} \left( \frac{x_K}{b} - 1/2 \right)^2 - U_0 \left( \frac{x_K}{a} - 1 \right)^2$ for $0 < x_K < b/2$ and $U_{PN}(x_K) = U_{PN}(b - x_K)$ for $b/2 \leq x_K < b$. Here $C_{\text{eff}} = \frac{10k_BT}{10A_C}$ with the “+” sign referring to a kink (1 bp missing) and the “-” sign to an antikink (1 additional bp). Depending on the ratio of parameters $U_0$ and $C$, the state $K_{20}$ corresponds to a local minimum or maximum of $U_{PN}$ whereas $K_{10}$ is always stable for the relevant parameter range. The rate for the kink step process is then given by the expression $f_{\text{step}} = k_B T \int_0^{b/2} \frac{1}{\xi_{\text{eff}}} ds$ with $\xi_{\text{eff}}^{-1} = \int_0^{b} e^{-U_{PN}(sb)/k_BT} ds \int_0^{b} e^{+U_{PN}(sb)/k_BT} ds$ and $\xi_{\text{eff}} = \frac{4\pi}{106} \mu_{\text{spin}}^2$, the effective kink friction constant. Here $\mu_{\text{spin}} = 1.3 \times 10^{-20}$Ns is roughly the rotational friction for a single basestep [21]. To determine the rate at which twist defects are formed at the entry/exit points of

![FIG. 1: The twist-diffusion mechanism for nucleosome repositioning. a) A concerted translational and rotational motion of DNA leads to injection of twist-defects (kinks) which migrate between the octamer adsorption sites (black triangles) leading to a "creep" motion of DNA. b) The corresponding Frenkel-Kontorova model for twist diffusion and its characteristic parameters (cf. text for details).](image-url)
of magnitude faster than the ones observed in experiments. We find $T \approx 580$ bp.

The average lifetime of a defect related to $t_{\text{life}}$ is the energetic cost for a single kink (cf. above). How is the average life time $t_{\text{life}}$ of a defect related to $t_{\text{step}}$, the typical time needed for one step? This can be determined from the mean first passage times $\tau_{\text{left}}$ and $\tau_{\text{right}}$ for a defect that forms, say, at the left end to leave the nucleosome at the same or at the other end, respectively. From Ref. \cite{25} one finds $\tau_{\text{left}} = (25/6) t_{\text{step}}$ and $\tau_{\text{right}} = 28 t_{\text{step}}$. Furthermore, the probability to leave at the left end is $P_{\text{left}} = 12/13$ and at the right end $P_{\text{right}} = 1/13$ \cite{22} which gives the life time as the weighted average $t_{\text{life}} = 6 t_{\text{step}}$. Only a fraction $P_{\text{right}}$ of the defects reaches the other end and will lead to a repositioning step, i.e., the time of a $1\text{bp}$ diffusion step of the nucleosome along the DNA is given by $T = t_{\text{inj}} + P_{\text{right}}$. Putting all this together we arrive at $T \approx 6b^2 \varepsilon_{\text{eff}} \lambda_0^{-1}/k_BT \exp(C/10k_BT)$. For realistic parameter values $C = 100$ and $U_0 = 6k_BT$ and $R_{\text{fluct}} = 3$ we find $T \approx 10^{-3} \text{s}$ implying a nucleosome diffusion constant $D = 580 \text{bp}^2/\text{s} = 6.6 \times 10^{-17} \text{m}^2/\text{s}$.

Hence we find repositioning rates that are orders of magnitude faster than the ones observed in experiments \cite{3}. Even worse, the experimental observation of an apparent $10\text{bp}$ jump length \cite{3} seems to be inconsistent with our predictions. We show now how these facts can be explained by the existence of additional barriers with a $10\text{bp}$ periodicity. To do so we have to extend our simple model to deal with the quenched disorder stored in the DNA bp sequence. The sequence dependent anisotropic bendability, i.e., the propensity of DNA to bend in different directions with different elastic constants turns out to be essential. It has been known for a long time \cite{23, 24} that (A/T) rich dinucleotide steps (dns) prefer to face the octamer in the minor groove (i.e., at the octamer contact points) whereas (G/C) rich dns prefer to face the octamer in the major groove (i.e., between contact points). This reflects different propensities of the dinucleotides to widen or compress towards the DNA minor groove. To incorporate these anisotropic effects into our model we first note that the bending state of the DNA molecule is fully constrained by its helical path on the octamer surface. Moving a DNA sequence via twist diffusion by a few bp ($< 10\text{bp}$) along that path changes the relative rotational setting of the bent DNA with respect to its preferred bending direction causing an energetic penalty, whereas a motion by $10\text{bp}$ restores the initial rotational setting. We address this by introducing a $10\text{bp}$ periodic "bending field" $F_{\text{bend}}(x) = -\cos[2\pi x/(10\text{b})]$ attached to the octamer surface. We assume the DNA sequence to couple linearly to that field through "bending charges" $q_k$ attached to each of the dns. This gives us finally the third term in Eq. \ref{eq:3.1}.

$$E_{\text{sd}} = \sum_k q_k F_{\text{bend}}(x_k) + m_k$$

In addition to the anisotropic term we also introduced here the isotropic bending parameters $m_k$ to include isotropic flexibility effects (which become important when the $q_k$’s vanish or average out). The summation involved is again over all base pairs incorporated in the nucleosome. $q_k$ and $m_k$ both have units of energy and can be extracted from competitive protein binding experiments \cite{24} for each of the $10$ dns (AA, AT, GC...). To obtain a rough estimate we distribute the dns into three classes: 1) (G/C) containing dns, 2) (A/T) containing dns and 3) mixed dns (like AG, CT etc.) and treat the dns in each class as identical. Using the available experimental data \cite{24} we then arrive at $q_{\text{G/C}} \approx 95$, $q_{\text{A/T}} \approx -85$, $m_{\text{mixed}} \approx 0$ and $m_{\text{G/C}} \approx 20$, $m_{\text{A/T}} \approx -3$, $m_{\text{mixed}} \approx 7$, where all energies are in cal/mol per dns.

It turns out that the nucleosome mobility depends strongly on the underlying bp sequence. When shifting the position of all beads by $l$ bp steps, $x_k \rightarrow x_k + l$, we find $E_{\text{sd}}(l) = (A/2) \cos(2\pi l/10 - \phi)$ to vary as a cosine function of $l$ with phase $\phi$ and amplitude $A$ determined by the DNA sequence, which is assumed to be appropriately periodic here. Arranging G/C and A/T tracts properly and taking the sequence dependent $q$ and $m$
values given above we can easily reach amplitudes \( A \) (i.e.
barriers to repositioning) that exceed 10 – 12 kcal/mol!
A very effective sequence arrangement called the "TG"-
sequence which leads to a strong nucleosome stability and
localization was experimentally constructed in Ref. [24]
by putting G/C tracts around positions \( k = 0, 10, 20...\)
and A/T tracts around \( k = 5, 15, 25,... \). In our picture
this means to put the "bending charges" \( q \) along the
DNA such that they couple favorably to the bending field
\( F_{\text{bend}} \) for a distinct rotational setting whereas a 5 bp shift
is extremely costly (cf. Fig. 2). The 5S-RNA sequence
which was used in most nucleosome mobility experiments
shows also the effect of an optimal rotational setting. It
is less pronounced than in the "TG" case, yet it is still detec-
table. More involved theoretical computations relying
on molecular sequence dependent deformability parameters
[27] reveal barriers \( A \approx 5 – 6 \text{ kcal/mol} \) for this par-
ticular sequence. The sequence dependent barrier height
\( A \) exponentially suppresses the bare (sequence indepen-
dent) diffusion constant \( D \) obtained above leading to the
sequence dependent diffusion constant \( D_{sd} \):

\[
D_{sd} = D I_0^{-2} (A/2k_BT) \approx \frac{\pi j_0 A}{12k_BT e^{(A+q/C)/k_BT}}
\]

with \( I_0 \) being the modiﬁed Bessel function.

Equation 5 predicts that mobility experiments with
highly anisotropic sequences like "TG" (instead of the
standard "5S-RNA") would find hardly any appreciable
repositioning on the one hour timescale if it would be
solely mediated via twist defects \( (D_{sd} = 10^{-6} – 10^{-7} \times
D = 10^{-8} – 10^{-5} \text{ bp}^2/s) \). The typical path for a nu-
ucleosome to escape from such a rotational trap goes very
likely via the previously considered loop formation mecha-
nism [2, 10] that allows "tunneling" over sequence bar-
riers, thus dominating over twist-diffusion for extremely
anisotropic sequences. An experimental test for this pre-
diction would be to increase the free DNA segment length
which in this regime should strongly enhance the loop
mediated mobility [10] whereas it would leave the twist
diffusion unaffected. Going to the other extreme, in the
most relevant case of random isotropically bendable se-
quences which make up more than 95\% of the eucaryotic
gene one should observe that the twist diffusion mecha-
nism is strongly enhanced by 2-3 orders of magnitude
as compared to the \textit{in vitro} measurements on "5S-RNA".

In conclusion the following picture is implied: On physi-
ological timescales the majority of genomic nucleosomes
seems to be intrinsically highly mobile. However, only
a small fraction (< 5\%) of all nucleosomes has strongly re-
duced mobility due to anisotropic DNA sequences which
they populate. We speculate that only the latter re-
quire the action of active (ATP consuming) remodelling
mechanisms [28] making them hotspots and switching el-
ments for global chromatin rearrangements.

We thank R. Bruinsma, K. Kremer, K. Luger, F.
Müller-Plathe and J. Widom for helpful discussions.

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