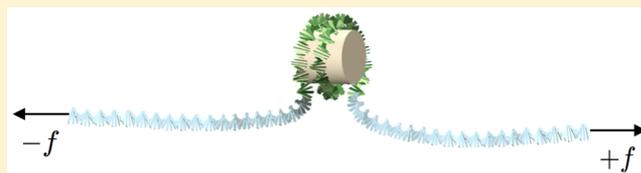


# Why Do Nucleosomes Unwrap Asymmetrically?

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**ABSTRACT:** Nucleosomes, DNA spools with a protein core, engage about three-quarters of eukaryotic DNA and play a critical role in chromosomal processes, ranging from gene regulation, recombination, and replication to chromosome condensation. For more than a decade, micromanipulation experiments where nucleosomes are put under tension, as well as the theoretical interpretations of these experiments, have deepened our understanding of the stability and dynamics of nucleosomes. Here we give a theoretical explanation for a surprising new experimental finding: nucleosomes wrapped onto the 601 positioning sequence (the sequence used in most laboratories) respond highly asymmetrically to external forces by always unwrapping from the same end. Using a computational nucleosome model, we show that this asymmetry can be explained by differences in the DNA mechanics of two very short stretches on the wrapped DNA portion. Our finding suggests that the physical properties of nucleosomes, here the response to forces, can be tuned locally by the choice of the underlying base-pair sequence. This leads to a new view of nucleosomes: a physically highly varied set of DNA–protein complexes whose properties can be tuned on evolutionary time scales to their specific function in the genomic context.



## 1. INTRODUCTION

DNA molecules in eukaryotic cells are wrapped into a series of DNA spools which are known as nucleosomes. For about every 200 base-pairs (bp), a 147-bp-long stretch of DNA is wrapped in  $1\frac{3}{4}$  superhelical turns around a disk-shaped octameric protein core that is composed of two molecules each of histones H2A, H2B, H3, and H4.<sup>1</sup> Linker DNA of a typical length of 50 bp connects the nucleosomes.

Many questions still surround the energetics of nucleosome–DNA interaction and the dependence of this affinity on DNA sequence. A new avenue of enquiry was opened by micromanipulation experiments where DNA unpeeling can be induced by applying a tension to the nucleosome. The first experiment that observed the unwrapping of individual nucleosomes was performed in 2002 by the Wang lab.<sup>2</sup> In an optical tweezer setup, an array of nucleosomes was put under increasing tension and individual rupture events were observed. Remarkably, nucleosomes unwrapped sequentially, not in parallel, and at forces which were much higher than expected from earlier experiments.<sup>3,4</sup> These observations—together with the fact that the rupture forces increased with the pulling rate—suggested the presence of an energetic barrier against unwrapping. The height of this barrier was estimated via force spectroscopy to be  $36\text{--}38 k_B T$ .<sup>2</sup>

What could cause such a high barrier? Brower-Toland et al.<sup>2</sup> suggested that this could reflect two strong binding sites that stabilize the last turn of the nucleosome and that need to be broken for full unwrapping. However, the crystal structure does not provide evidence for such strong sites.<sup>1</sup> Moreover, in a theoretical study,<sup>5</sup> it was shown that the spool design of the nucleosome and the elasticity of the DNA molecules are

enough to lead to a large barrier, even if no strong binding sites are present. This study suggested that the barrier is in fact a response to the external force, reflecting the strong deformation of the DNA as the nucleosome flips by  $180^\circ$  during unspooling.

This view was supported by newer micromanipulation experiments on *single* nucleosomes.<sup>6,7</sup> In fact, as a nucleosome has two turns, two such flipping transitions were observed, one at low forces (two to one DNA turn) and one at high forces (one to zero turns). It was that latter transition that had been observed in the earlier study on a nucleosome array.<sup>2</sup>

The nucleosome manifests a twofold symmetry, where the symmetry axis, i.e., the nucleosome dyad, passes through the central DNA base-pair. Consequently, most modeling studies assumed that the nucleosome unwraps symmetrically or at least that it does not care from which end it unwraps first. Surprisingly, in a recent micromanipulation experiment, the Ha lab demonstrates that nucleosomes can respond highly asymmetrically to an external force, unwrapping almost always from one and the same end.<sup>8</sup> To show this, the experiment combines a micromanipulation setup with a FRET measurement where pulling experiments are performed on single nucleosomes, each having a pair of dyes at a strategic position. By looking at FRET data of nucleosomes with pairs of dyes at different positions, it becomes clear that the nucleosome

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practically always unwraps from one end. What can cause this asymmetric response?

Ngo et al.<sup>8</sup> claim that the asymmetry is caused by the fact that the wrapped DNA portion (the 601 Widom nucleosome positioning element,<sup>9</sup> a non-natural bp sequence used nowadays in many laboratories<sup>6,7,10–27</sup>) is nonpalindromic. They speculate that one-half of the wrapped portion is stiffer than the other and that it is the stiffer half that unwraps first. They test this idea by measuring the propensity for ring formation for two pieces of DNA, one having the sequence of one-half of the wrapped portion and the other having the sequence of the other half. They test the idea further by flipping the inner two quarters of the sequence which leads to a nucleosome that unwraps now from the other end. Finally, slightly symmetrizing the wrapping sequence by adding three soft TA dinucleotide steps to the stiffer inner quarter leads to a situation where the nucleosome responds symmetrically to tension, unwrapping stochastically from either end.

The finding that nucleosomes typically unwrap asymmetrically is not completely unexpected, as it is known that DNA shows sequence-dependent mechanical properties<sup>28,29</sup> which have been observed to manifest themselves in a sequence dependence of nucleosome affinity,<sup>9</sup> of nucleosome positioning,<sup>30</sup> and of nucleosome breathing.<sup>10</sup> What is really surprising is that the order of the unwrapping of the *outer* sections is determined by the sequence of the *inner* two wrapped quarters. This seems counterintuitive. One would rather expect that what determines the order of unwrapping are the stiffnesses of the outer two quarters with the stiffer one unwrapping first, and that the bp sequence of the inner quarters would be irrelevant to that order.

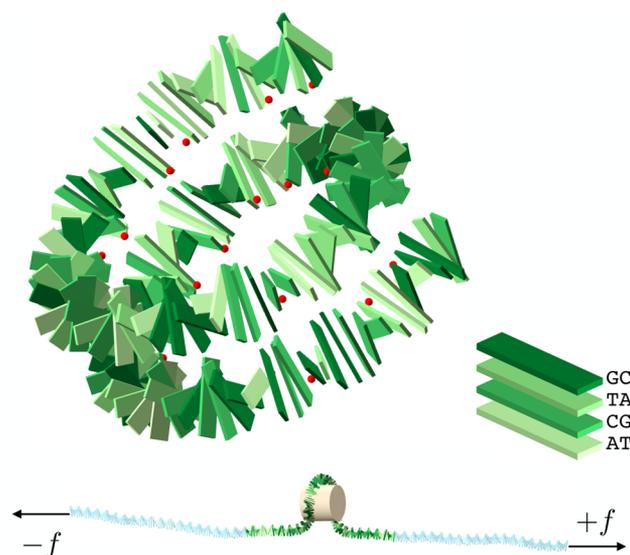
In the current paper, we introduce a sequence-dependent nucleosome model that allows us to explain this surprising finding. We show that it is only a very small fraction of the wrapped DNA that causes the asymmetry of the response. As it will become clear, this curious behavior may reflect the procedure by which the 601 sequence (on which these experiments are based) was produced.<sup>9</sup> The fact that the physical properties of nucleosomes are to such a large extent determined by the wrapped sequence (and here even just a short fraction of it) suggests the possibility that the mechanical properties of DNA molecules might have evolved not only to position a substantial fraction of nucleosomes but also to equip them with special physical properties according to their genomic context.

Our paper is structured as follows. In the next section, we introduce the computational nucleosome model that is based on the rigid base-pair description of DNA molecules. Section 3 contains our results and a discussion of how our model can explain the experimental data. Finally, the last section provides some conclusions and discusses more general implications of our findings.

## 2. MODEL

Our nucleosome model consists of a 147-bp-long DNA molecule represented by the rigid base-pair model that is forced into a superhelical conformation through constraints that mimic the binding of 28 DNA phosphates to the protein core; see Figure 1 (top). We first describe the coarse-grained DNA model and then explain how we constructed the constraints.

We represent the DNA by the rigid base-pair model which describes the conformations of the DNA double helix solely by



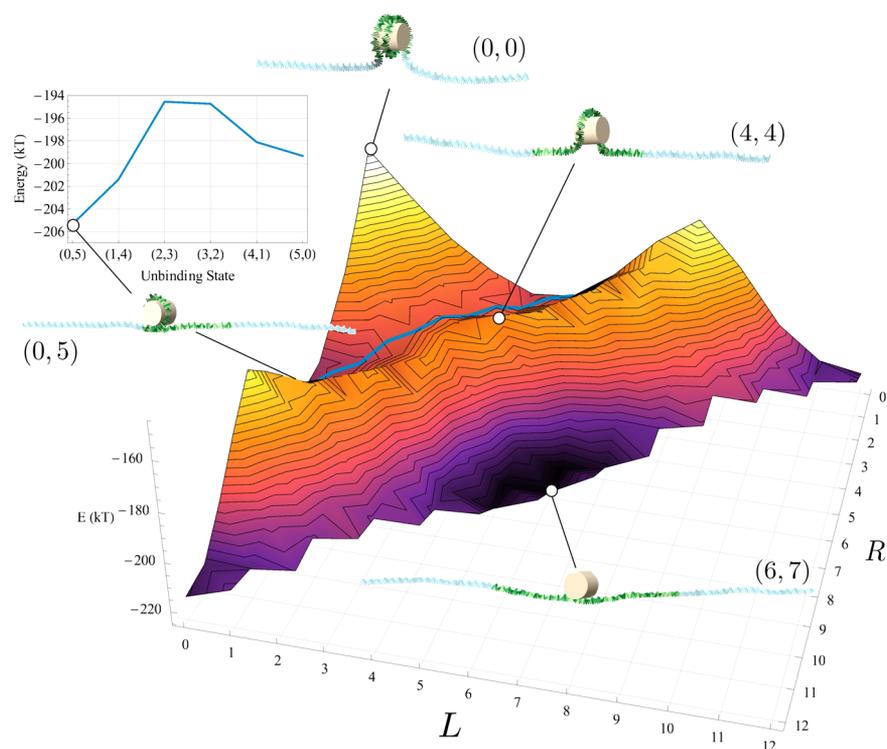
**Figure 1.** Nucleosomal DNA model with bp step dependent mechanical properties. (Top) The rigid base-pair model is forced, using 28 constraints (indicated by red spheres), into a left-handed superhelical path that mimics the DNA conformation in the nucleosome crystal structure.<sup>1</sup> Shown here is the 601 sequence. The four different colors indicate the two different base-pairs in two different orientations. (Bottom) Nucleosome (with two tails, each consisting of 100 bp) under a force of 14 pN. Here 8 binding sites have opened symmetrically, releasing some of the nucleosomal DNA.

the positions and orientations of its base-pairs that are represented by rigid plates.<sup>31,32</sup> This leaves six degrees of freedom per bp step, three translations—shift, slide, rise—and three rotations—twist, roll, tilt. To explain the sequence-dependent response of the nucleosome to external tension, we also need the energy cost of deforming the DNA away from its preferred conformation. We assume that the six degrees of freedom of a given bp step have preferred intrinsic values (dependent on its chemical composition) and that deviations from these values incur a mechanical energy cost quadratic in this deformation:

$$E_{\text{el}} = \frac{1}{2}(q - q^0)^T Q (q - q^0) \quad (1)$$

Here  $q$  is a six-component vector that contains the six degrees of freedom whose intrinsic values are given by  $q^0$  and which are coupled by the  $6 \times 6$  stiffness matrix  $Q$ . Each dinucleotide has its own intrinsic values and stiffnesses that are fully parametrized in the literature.<sup>28,29</sup> We use here the hybrid parametrization<sup>33</sup> in which intrinsic deformations are derived from protein–DNA crystals and the stiffnesses from atomistic molecular simulations.

The DNA is forced into a superhelix by constraining the positions and orientations of 28 middle-frames of consecutive bp that mimic DNA phosphates bound to the histone octamer. We identified these 28 strongly bound phosphates from local minima in the crystallographic B-factor in the NCP147 structure.<sup>34</sup> They give rise to 14 distinct nucleosome binding sites, each containing two bound phosphates. We studied several nucleosome crystal structures and found that each pair of DNA phosphates connecting two successive base-pairs, whether bound to the octamer or not, is stationary in the so-called midframe, the coordinate system whose position and orientation is exactly in between those of the two base-pairs.



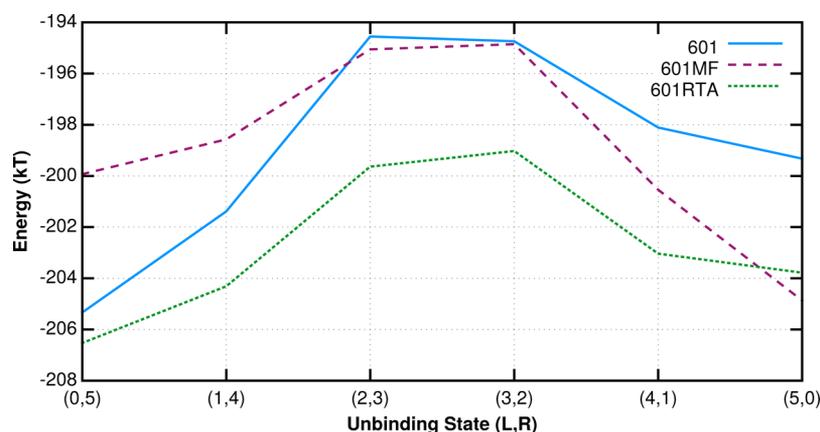
**Figure 2.** Energy landscape of the 601 nucleosome under a 14 pN force as a function of the number of open binding sites from the left,  $L$ , and from the right,  $R$ . Also shown is a cut through this landscape for metastable states with five open binding sites that sit at the bottom of the valley. Snapshots of the nucleosome are given at  $(L, R) = (0, 0)$  (fully wrapped), at  $(0, 5)$  (unwrapped from the right, leaving one turn wrapped, a metastable state), at  $(4, 4)$  (a state on top of the ridge), and at  $(6, 7)$  (unwrapped state, global minimum).

This allows us to implicitly take bound phosphates into account, even though our model consists only of rigid base-pairs. A more detailed discussion of the construction of the constraints is provided elsewhere. Compared to other similar models in the literature,<sup>35–39</sup> the benefit of our model is that it does not contain free parameters and allows for efficient Monte Carlo sampling.

According to a computational study by de Pablo and co-workers,<sup>40</sup> there are two main features that determine the sequence affinity to nucleosomes: intrinsic curvature and minor groove width. Both features are manifestations of the equilibrium shape of the unbound DNA which is accounted for in the rigid base-pair model by the intrinsic values of the bp steps, namely, the quantities  $q^0$  in eq 1. Whereas the role of intrinsic curvature to alleviate the cost of bending DNA into nucleosomes is obvious (and is accounted for also by other studies like refs 36 and 38), the role of the minor groove width is more subtle and is neglected in those studies. Our model, however, automatically takes the minor groove width into account. Each binding site actually consists of two points where the DNA is fixed to the histone core, one on either side of the minor groove. A mismatch in minor groove width (as prescribed by the equilibrium shape of the DNA) thus automatically leads to a frustrated molecule, increasing the energetic cost.

As we are studying the force-induced unwrapping, we need two more elements. (1) We add 100-bp-long DNA tails at each terminus of the wrapped portion and pull the two ends apart with a given force; see Figure 1 (bottom). We are not interested in the (small) effects arising from specific base-pair sequences of the two DNA tails; we therefore model that part of DNA as homogeneous DNA where we chose all elastic and

geometric constants to represent the average over all possible bp steps. Our simulations are performed at a tension of 14 pN. This will allow us to demonstrate the remarkable fact that even at such a high force the nucleosome is stuck in a metastable state with a substantial barrier to the global minimum, the unwrapped nucleosome. (2) We study the nucleosome in various states of unwrapping from the left and from the right. To then compare energies of different unwrapping states, we need to assign adsorption energies for the 14 binding sites. Due to the dyad symmetry of the nucleosome, this leaves us with seven independent values. We follow here an earlier study<sup>39</sup> where the energies were assumed to have different values for each of the seven independent sites. The energies were estimated, up to a constant offset, from an experiment where DNA was unzipped into a nucleosome,<sup>41</sup> and the offset was derived from a nucleosome breathing experiment.<sup>3</sup> This led to the values presented in Table 1 in that study.<sup>39</sup> However, the newer experimental setup allows us to determine the offset much more precisely. Specifically, Figure 1E of Ngo et al.<sup>8</sup> gives the FRET signal for small forces for the weak end of the nucleosome. At a force of about 4 pN, this signal is half as big as that for a vanishing force, meaning this part of the DNA spends equal time being bound and being free. We thus choose the offset in our model such that at this force the energies of the fully wrapped state and that of the cheapest singly wrapped state (a metastable state, as explained below) have the same energy. This is achieved when we add an extra offset of  $1.87 k_B T$  to the values provided by Fathizadeh et al.<sup>39</sup> in their Table 1. This is the only adjusted parameter in our model. Importantly, however, the results of our analyses are not very sensitive to the choice of the adsorption energies as long as they remain within reasonable limits. Even naively choosing all the



**Figure 3.** Energy of the singly wrapped nucleosomes under a 14 pN tension as a function of the unwrapping state ( $L, R$ ) with  $L + R = 5$ . Three different sequences, 601, 601MF, and 601RTA, are shown, the sequences studied experimentally.<sup>8</sup> These energy landscapes correspond to the bottom of the metastable valley; see also Figure 2 for the 601 case.

binding sites to have the same energy, rather than the measured values, has been seen to have little effect and does not change the conclusions of this paper.

For each possible state of unwrapping, we produce random samples of nucleosome conformations using the standard Metropolis algorithm. Our Monte Carlo moves consist of local moves of base-pairs (making sure that constraints on fixed middle-frames are not violated) and, for the nonwrapped DNA, additional pivot moves.

### 3. RESULTS AND DISCUSSION

**An Asymmetric Set of Metastable States.** The energy landscape of the 601 nucleosome under a force of 14 pN as a function of the unwrapping state is shown in Figure 2 together with snapshots of four different states. The unwrapping state ( $L, R$ ) is defined by two integers,  $L$  and  $R$ , which correspond to the number of opened binding sites from the left,  $L$ , and from the right,  $R$ . As can be seen in this figure, the fully wrapped state (0, 0) is the most expensive state, whereas fully unwrapped states, i.e., states with  $L + R = 13$  (assuming that the DNA is still bound at one site), are highly favored. However, to go from the fully wrapped nucleosome to the fully unwrapped states, the system has to cross over a substantial barrier. This barrier corresponds to a nucleosome that still has half a turn of DNA wrapped,  $L + R = 8$ , and extends along a straight ridge that connects state (0, 8) with state (8, 0). The presence of the ridge leads to a metastable valley, extending from (0, 5) to (5, 0) which corresponds to nucleosomes with one full turn of wrapped DNA.

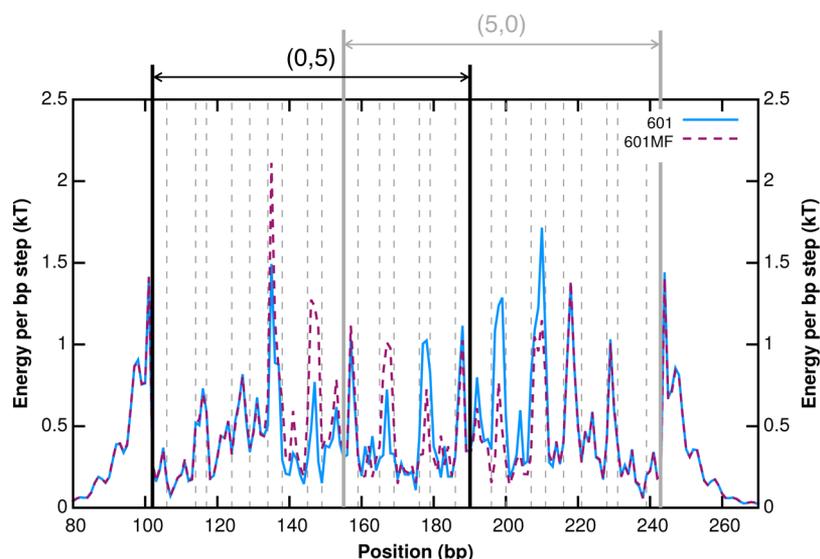
The general shape of the unwrapping landscape has been predicted on purely theoretical grounds.<sup>5</sup> In that model, the nucleosome was represented by a cylinder wrapped by a DNA molecule, modeled by the wormlike chain (a homogeneous elastic rod with bending modulus  $A$ ). The energy landscape of this model nucleosome with a force  $f$  applied to the ends of the wormlike chain was calculated analytically (as detailed in a recent textbook;<sup>42</sup> extensions of the model can be found in various studies<sup>43–50</sup>). It was found that, for the last turn of the DNA to unpeel, the system has to cross a high energy barrier. This barrier is the result of a 180° flip of the nucleosome which is a geometrical necessity for the DNA unwrapping to occur. Halfway, when the nucleosome has turned by 90° and only one-half DNA turn remains wrapped, the transition state is reached. Most of the energy in this state is focused in two

strongly bent DNA portions where the straight DNA arms connect to the wrapped section by 90° bends. The height of the barrier scales like  $\sqrt{Af}$ . In other words, the barrier is only present and is caused by the applied force and becomes higher when one pulls harder, reflecting a sharpening of the two 90° bends. Only because the overall landscape is tilted by the force, the barrier eventually shrinks and disappears at very high forces.

Going back to the simulated landscape, Figure 2, we can indeed see that, even at 14 pN, there is still a substantial barrier against unwrapping. The cause of this barrier can be seen in the example configuration (4, 4) where the two strongly bent DNA portions can be clearly detected. Likewise, the fully wrapped nucleosome (0, 0) is highly unfavorable, as the DNA arms have to be bent substantially as well. On the other hand, the two DNA arms for the nucleosome inside the valley at (0, 5) and for the fully unwrapped nucleosome at (6, 7) feature nearly straight DNA arms, even close to the wrapped portion. The barrier is substantial; e.g., the difference between the highest point in the valley and the pass over the ridge is still about 10  $k_B T$  for  $f = 14$  pN.

In the earlier theoretical work,<sup>5</sup> it was indeed speculated that nucleosomes “are strongly kinetically protected from mechanical disruption upon applied tension”. We claim here that it is this kinetically protected set of states that is observed between 5 pN  $< f < 15$  pN in the new experiment.<sup>8</sup> The rupture force of about 15 pN might seem low given that the pulling rate of 466 nm/s is substantial. However, the force clamp is applied on a long DNA tether (lambda DNA). This results in loading rates on the nucleosome which are comparable to the rates used in previous experiments using shorter tethers<sup>2,6</sup> and where similar rupture forces were observed.

We are now in the position to test whether our model predicts the same asymmetries as observed for the three experimental sequences. We start with the 601 sequence. Figure 3 shows a cross section of the energy landscape taken along the metastable valley (see also the inset in Figure 2). As can be seen, the nucleosome strongly prefers to have the single DNA turn wrapped at either end. This reflects the fact that the DNA is less bent close to the termini of the nucleosome; if the single wrap were in the middle, none of the termini would be involved. Most importantly, it can be seen in Figure 3 that there is a strong asymmetry in this set of metastable states: according to the model, state (0, 5) is 6.0  $k_B T$  more favorable than state



**Figure 4.** Elastic energy per bp step along a 190 bp long DNA molecule with sequence 601 (blue) and 601MF (dashed purple). The DNA is fully wrapped into a nucleosome that extends from position 100 to 246, and a force of 14 pN is applied to its ends. Twenty-eight dashed vertical lines indicate constraints, two per binding site. The extents of wrapping in states (0, 5) and (5, 0) are also indicated.

(5, 0). Assuming that the system has time to equilibrate between all of the states in the valley on the time scales of this experiment, we expect to find the nucleosome in state (0, 5) for more than 99% of the time. We performed this calculation for 14 pN, but the relative energies inside the valley are hardly affected by the force, so that this statement holds for the whole relevant experimental range of forces  $5 \text{ pN} < f < 15 \text{ pN}$ . This is in remarkable agreement with the experimental observation that the label (called ED2) at the left end shows high FRET up to 15 pN, whereas the label at the right end, ED1, already loses its FRET signal around 5 pN (see Figure 2 of Ngo et al.<sup>8</sup>).

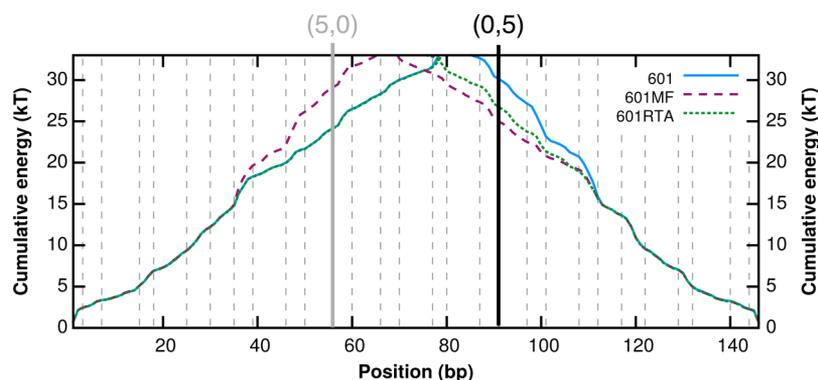
Also shown in Figure 3 are the energy landscapes for the metastable valley for the other two sequences. Sequence 601MF has the inner two quarters of the 601 sequence swapped. As can be seen in the plot, the lowest energy state in the metastable valley is now at the other end, (5, 0). The state at the other end is  $4.9 k_B T$  more expensive. If the system has time to equilibrate within the valley, the nucleosome is in state (5, 0) about 98% of the time. It has indeed been observed in the experiment that the FRET signal is flipped with respect to that of the 601 nucleosome.<sup>8</sup> Finally, Figure 3 also depicts the energies for sequence 601RTA, a sequence which is derived from 601 by adding three extra TA steps at the stiffer of the two inner quarters. The experimentalists speculated that these soft extra steps make the 601 sequence more symmetric and indeed found a much more symmetric FRET signal. In our model, the difference between the left and right ends has shrunk to  $2.6 k_B T$ , less than half the difference found for the original 601 nucleosome. On the basis of this, we predict that the nucleosome spends about 81% in state (0, 5) but also a substantial fraction, about 7%, in state (5, 0).

To have a nucleosome that unwraps stochastically from both sides, there needs to be a near-cancellation of two huge energies. In our model, the bending energy of the 601RTA DNA is reduced by  $26.8 k_B T$  when the right five sites are opened and by  $24.2 k_B T$  when the left five sites are opened, leaving just the  $2.6 k_B T$  difference. The experimental FRET signals are even more symmetric, pointing to an energy difference between these two states that might be smaller than one  $k_B T$ . This small but noticeable difference between our

model and the experimental data gives a hint of the level of accuracy of our model. We noticed also an error of the same order, about  $2.5 k_B T$ , when comparing our predicted difference in affinities between the 601 and the 5S rDNA nucleosome positioning sequence to experimental data. However, we can conclude that our model is precise enough to explain the different scenarios found for the three different nucleosomes.<sup>8</sup>

To summarize, our computational nucleosome model predicts that a nucleosome under a (quickly) increasing tension will be trapped in metastable states which correspond to singly wrapped nucleosomes with various degrees of wrapping asymmetry. The asymmetries observed in our model agree well with the experimentally observed ones. As there are three sequences (601, 601MF, 601RTA) and three possible outcomes (unwrapping from the left, from the right, or from both sides), we predict the correct scenario out of 27 different possible scenarios. What remains to be explained is the actual underlying mechanism that leads to the puzzling effect that the unwrapping of the outer DNA sections is governed by the mechanical properties of the inner sections.

**Peculiarities of the 601 Nucleosome.** Before comparing the two sequences, 601 and 601MF, in detail, let us analyze first what causes the asymmetric response of the 601 sequence. In Figure 4, we plot the deformation energy for each bp step along a 190-bp-long stretch of DNA containing the 601 sequence (continuous blue curve). The nucleosome is fully wrapped, at state (0, 0), under an external force of 14 pN. The 601 sequence corresponds to the 146 wrapped bp steps (marked by positions 100–246 along the  $x$ -axis); the rest of the DNA is homogeneous (see the Model section). Our system is at the ground state which we obtained by simulated annealing. To understand the asymmetric response, we need to compare states (0, 5) and (5, 0) (both indicated in Figure 4; the positions of constraints are indicated by dashed vertical lines). Inspection with the naked eye suggests that the energies stored in the right half are higher, which means that more energy is released when the nucleosome unwraps from the right to state (0, 5) than when it unwraps from the left to state (5, 0). This can be seen more directly by inspecting the cumulative elastic energy in Figure 5. The blue curve coming up from the left



**Figure 5.** Cumulative energy for nucleosome unwrapping. This plot gives the total released elastic energy for unwrapping DNA from the left or from the right to the corresponding bp positions for the three different sequences, 601, 601MF, and 601RTA. These plots follow from Figure 4 by summing up the energies starting from the left or from the right ends of the wrapped portion (compared to Figure 4, we renumber here the positions such that the wrapped portion starts at bp 1 instead of bp 101). A comparison between the energies in states (5, 0) and (0, 5) for each sequence reveals which are the preferred states in the metastable valley.

shows the elastic energy released as a function of the position up to which the left side of the 601 DNA has unwrapped. At the gray vertical line, one can read off the released energy when one unwraps into state (5, 0) to be about  $24 k_B T$ . The blue curve coming up from the right of Figure 5 shows the released elastic energy for unwrapping from the right. The released energy in state (0, 5) is about  $30 k_B T$ ,  $6 k_B T$  more than the value for (5, 0). This is indeed the energy difference between these two states that we found in Figure 3 (blue curve). A similar line of arguments explains the response with opposite asymmetry of the 601MF nucleosome (see dashed purple curves in Figures 4 and 5).

After having explained the asymmetric response of the 601 and 601MF nucleosome separately, we are now in the position to answer the question of which property of the 601 sequence causes the flipping of the force response when going to 601MF. For this, we need to compare the two sequences directly. To do so, compare the elastic energy per bp step in Figure 4 for both sequences (blue, 601; purple, 601MF). As can be seen clearly, the energies of the two sequences are identical up to about position 134 from the left and up to about position 212 from the right. The elastic energies for the inner region of the wrapped nucleosomes, extending from position 134 to position 212, are vastly different from each other. They are in fact mirror images of each other, reflecting the symmetry operation that relates the two sequences. In other words, the elastic energy is in a first approximation local and just depends on the position and chemical identity of each bp step. This is, however, not strictly true as, e.g., a soft step can take up more deformation, allowing stiffer steps close by to relax slightly. This effect manifests itself at the interfaces between the inner and outer quarters (positions 136 and 210) where this symmetry does not hold and deviations that extend over about five bp steps can be detected.

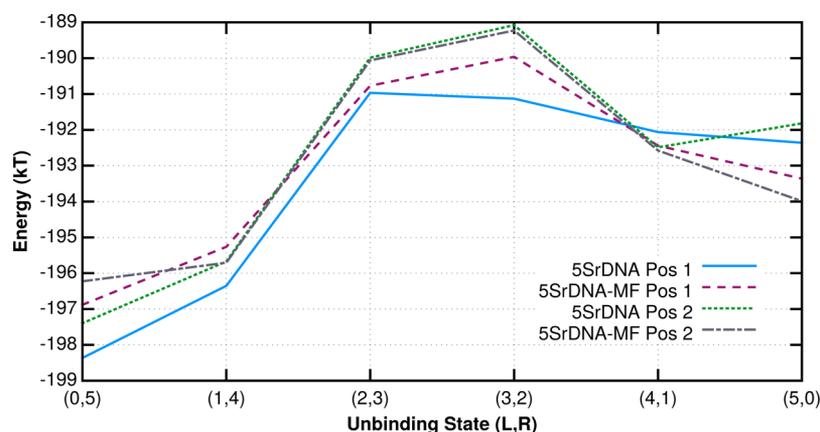
We need now to compare in Figure 4 the two states of interest, (0, 5) (black) and (5, 0) (gray), for the two sequences. Consider first state (0, 5). For both nucleosomes, 601 and 601MF, there is a stretch, from position 100 to 134 (corresponding to the left outer quarter minus the interfacial region), where the elastic energies of these two nucleosomes are identical. Moreover, most of the rest of the wrapped DNA can also not contribute to the energy difference between the 601 and 601MF nucleosome, as the energies of the 45-bp stretch from position 155 to 190 are mirror images of each

other; see Figure 4. The large difference in energy between these two nucleosomes (more than  $5 k_B T$ , see Figure 3) must therefore stem from DNA outside these portions. In principle, DNA from outside the wrapped portion could contribute to this difference. However, since the DNA arms are nearly straight for nucleosomes with a single DNA wrap, this contribution is negligible (see the example configuration (0, 5) in Figure 2). This leaves as the only source of the large difference in energy between 601 and 601MF the 20-bp stretch from position 135 to 155. A similar line of reasoning for state (5, 0) singles out the region from position 191 to 211 as the cause of the more than  $5 k_B T$  elastic difference between the 601 and 601MF nucleosomes. In short, logic dictates that the dramatic differences in the asymmetric response to tension between the 601 and 601MF nucleosomes are caused by the 20 outmost bp of the inner quarters.

This can indeed be seen when comparing the cumulative elastic energy in Figure 5. Unwrapping 601MF (purple) from the left to state (5, 0) (gray line) releases about  $5 k_B T$  more energy than for the 601 and 601RTA sequences (which are identical on that side of the nucleosome). The difference between the sequences starts to build up only when the inner quarter is unwrapped, i.e., from position 35 onward. On the other hand, for unwrapping from the right to positions beyond (i.e., smaller than) 111, the three sequences deviate from each other: the unwrapping of 601 releases about  $3 k_B T$  more energy than 601RTA and about  $5 k_B T$  more than 601MF.

To make it possible that the preferred asymmetry changes when going from 601 to 601MF, one needs two properties of the 601 sequence: (1) The outer 20-bp stretches of the inner half need to have a substantial asymmetry in the stored elastic energy (about  $9 k_B T$  for the left vs  $14 k_B T$  for the right stretch), and (2) the elastic energy difference between the two outer quarters must be much smaller ( $15 k_B T$  vs  $16 k_B T$ ).

Is there something special about the 601 sequence that could explain why two 20-bp stretches determine the “fate” of the whole nucleosomes? There are good reasons to believe that this is the case. The 601 sequence was discovered via an experimental scheme where a huge pool of 5 trillion short random sequences underwent selection under pressure.<sup>9</sup> Histone octamers were assembled onto these sequences, but far fewer octamers were available than there were sequences, causing them to bind mainly to high-affinity sequences. The 601 sequence, being the winner in this scheme, performs



**Figure 6.** Energy of the singly wrapped nucleosomes under 14 pN of tension as a function of the unwrapping state ( $L, R$ ) with  $L + R = 5$ , i.e., along the bottom of the metastable valley. The nucleosome is positioned on the 5S rDNA sequence at two major positions, 19 bp apart.<sup>52</sup> In both cases, a flipping of the inner two quarters of the bp sequence does not change the asymmetry, unlike for the 601 nucleosome, cf. Figure 3.

especially well in the specific protocol that was employed in this experiment. The histone proteins in solution consist of  $(H3-H4)_2$  tetramers and  $(H2A-H2B)$  dimers.<sup>51</sup> The tetramer binds first, followed by the dimers (after lowering the ionic strength). It is thus the tetramer that “reads out” the affinity of the DNA. The 601 DNA molecule therefore contains a sequence optimized for tetramer binding, whereas the DNA portions involving the dimers (the outer quarters) are less strongly selected for. Moreover, it is the outermost stretches of the tetramer part that are most important, as they secure the binding of the tetramer in the middle of the DNA molecules, leaving space for the dimers. Indeed, the two stretches of 20 bp contain the outermost two binding sites of the six binding sites that are associated with the globular part of the histone tetramer.

We therefore speculate that the curious response of the 601 nucleosome might be the exception rather than the rule. To test this, we also simulated the force response of a nucleosome that is associated with a natural nucleosome positioning sequence, the *Lytechinus variegatus* 5S rDNA sequence, the only other high-affinity sequence that has been used in a nucleosome unwrapping experiment.<sup>2</sup> This sequence does not bind the nucleosome at a unique position but has two prominently favorable positions that have been mapped with single bp resolution.<sup>52</sup> These two positions are 19 bp apart from each other. For both positions, we find a strong asymmetry in the set of metastable states (similar to 601) with the state  $(0, 5)$  being the preferred state; see Figure 6. We then performed for each case the same symmetry operation as the one that produces the 601MF sequence from the 601 sequence, namely, swapping the inner two quarters. We found that this operation does not change the asymmetry of the landscape; see Figure 6.

Why do the two sequences respond differently? We expect that the flipping behavior of nucleosomes with asymmetric force response typically reflects the way the corresponding sequence has been experimentally isolated. Sequences like 601 that compete well against others during salt dialysis reconstitution<sup>9</sup> might typically change the order of unwrapping when their DNA segment associated with the tetramer is flipped, since the tetramer needs to be stably bound before a nucleosome can form. On the other hand, stable nucleosome positioning sequences that survive micrococcal nuclease digestion of native chromatin<sup>30,53</sup> are especially stably bound

close to the termini, where then also the largest asymmetry is to be expected.

Such effects thus have an impact on the results of a flipping experiment such as that performed by Ngo et al.,<sup>8</sup> and one should be careful when drawing general conclusions from such an experiment. We suggest a general approach to examining the flipping behavior of sequences that show an asymmetric force response. By studying how the response changes when sequences of various lengths (centered around the dyad) are flipped, one can determine the distance from the dyad up to which the bulk part of the asymmetry between the left and right is located. In such a scheme, once the flipped segment length reaches half the nucleosome wrapping length, the 601 nucleosome has already flipped its response to force, whereas the 5SrDNA nucleosome is still largely unaffected. In this way, force-induced unwrapping asymmetries could be characterized in more detail.

#### 4. CONCLUSION AND OUTLOOK

For the experimentally most favored nucleosome positioning sequence, the Widom 601 sequence,<sup>9</sup> the order in which the outer DNA quarters unwrap from a nucleosome under tension depends on the orientation of the inner two quarters. In this paper, we have shown that this surprising dynamic behavior, observed experimentally,<sup>8</sup> can be explained through a static analysis of a computational nucleosome model with sequence-dependent mechanical properties. We were able to show that this asymmetry reflects an asymmetry in a set of metastable states in which the nucleosome gets stuck once it is put under tension. (The stabilizing energy barrier comes from the elasticity of the DNA itself.<sup>5,44</sup>) This asymmetry is due almost entirely to an asymmetry in the termini (about 20 bp long) of the inner DNA quarters.

We draw three main conclusions from this work. (1) The new experimental insights into nucleosome dynamics provide a novel testing ground for nucleosome models based on rigid base-pair elasticity as studied by us and others.<sup>35–39</sup> We find that such a model can indeed predict and help interpret the asymmetric force response of nucleosomal sequences. (2) Our findings indicate that the surprising experimental observations reflect peculiarities of the 601 sequence that result from the specific method by which it was selected from a pool of many sequences. This serves as a warning: experiments that deal with this very special sequence (which is convenient to use and is

thus found in many experiments<sup>6,7,10–27</sup>) might find very special results that do not reflect the behavior of typical nucleosomes. We hope this insight will motivate research into the dynamical properties of nucleosomes that are wrapped into other sequences as well, a suggestion also made by some of us in a recent review.<sup>54</sup> (3) We found that a small fraction of the wrapped DNA portion (here two 20-bp stretches) can have a dramatic effect on the dynamics of the nucleosome (in this case to make the nucleosome about 150 times more likely to unwrap from one end than from the other). This suggests that the mechanical properties of base-pair sequences can be used not only to position nucleosomes<sup>30,55,56</sup> or repel them from certain DNA stretches<sup>57,58</sup> but also to strongly affect the physical properties of nucleosomes.

These three conclusions open a range of exciting possibilities. We speculate that bp sequences might have evolved over evolutionary time scales to equip positioned nucleosomes with physical properties depending on their genomic context. Additionally, there is the tantalizing possibility that such sequences could be engineered, starting from a nucleosome model such as the one we employed. In a preview on the Ngo et al. experiment,<sup>8</sup> Moevus and Greene<sup>59</sup> speculated that the observed “unexpected mechanical asymmetry may offer a path of least resistance, allowing RNA polymerases to traverse nucleosomes if they approach from the correct direction”. We speculate further that nucleosome sequences before and after transcription start sites might have evolved to absorb a twist in the form of one-bp-twist-defects,<sup>37,39</sup> helping RNA polymerases to initiate elongation. On more general grounds, one might imagine that a whole range of nucleosomal “species” has evolved, each reflecting its genomic context, just as the 601 sequence has been selected for its special mechanical properties in an in vitro scheme.

In the specific context of the unwrapping of nucleosomes under force, we speculate that nucleosomal sequences can be designed where the unwrapping happens along a prescribed path that has been “programmed” into the bp sequence. Of special importance are the two strongly bent DNA portions in the transition state. We speculate, for example, that one could design their sequences such that the energy cost of this state is reduced substantially, lowering the barrier against unwrapping through a specific configuration.

Finally, we mention that it might be interesting to create more detailed nucleosome models to study their force response. On one hand one might replace the rigid constraints representing binding sites by soft ones (e.g., by using a coarse-grained representation of the protein core<sup>40</sup>) and check whether the elasticity of the buried DNA segments also affects the unwrapping landscape. On the other hand, one could implement a less coarse-grained DNA model like the rigid base model<sup>60</sup> where geometrical frustration between bases leads to longer ranged effects along the sequence.

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### Notes

The authors declare no competing financial interest.

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