Inferring coarse-grain histone-DNA interaction potentials from high-resolution structures of the nucleosome

Sam Meyer\textsuperscript{1,2,3} and Ralf Everaers\textsuperscript{1}

\textsuperscript{1} Université de Lyon, Laboratoire de Physique and Centre Blaise Pascal, Ecole normale supérieure de Lyon, UMR CNRS 5672, Lyon, France
\textsuperscript{2} Université de Lyon, INSA-Lyon, INRIA, LIRIS, CNRS UMR 5205, Lyon, France
\textsuperscript{3} Université de Lyon, Microbiologie Adaptation et Pathogénie, INSA-Lyon, CNRS UMR 5240, Lyon, France

E-mail: sam.meyer@ens-lyon.org

Received 22 March 2014, revised 15 July 2014
Accepted for publication 18 July 2014
Published 7 January 2015

Abstract

The histone-DNA interaction in the nucleosome is a fundamental mechanism of genomic compaction and regulation, which remains largely unknown despite increasing structural knowledge of the complex. In this paper, we propose a framework for the extraction of a nanoscale histone-DNA force-field from a collection of high-resolution structures, which may be adapted to a larger class of protein-DNA complexes. We applied the procedure to a large crystallographic database extended by snapshots from molecular dynamics simulations. The comparison of the structural models first shows that, at histone-DNA contact sites, the DNA base-pairs are shifted outwards locally, consistent with locally repulsive forces exerted by the histones. The second step shows that the various force profiles of the structures under analysis derive locally from a unique, sequence-independent, quadratic repulsive force-field, while the sequence preferences are entirely due to internal DNA mechanics. We have thus obtained the first knowledge-derived nanoscale interaction potential for histone-DNA in the nucleosome. The conformations obtained by relaxation of nucleosomal DNA with high-affinity sequences in this potential accurately reproduce the experimental values of binding preferences. Finally we address the more generic binding mechanisms relevant to the 80\% genomic sequences incorporated in nucleosomes, by computing the conformation of nucleosomal DNA with sequence-averaged properties. This conformation differs from those found in crystals, and the analysis suggests that repulsive histone forces are related to local stretch tension in nucleosomal DNA, mostly between adjacent contact points. This tension could play a role in the stability of the complex.

Keywords: nucleosome, crystallographic structures, coarse-graining, protein-DNA interaction, electrostatics

(Some figures may appear in colour only in the online journal)

[Online supplementary data available from stacks.iop.org/JPCM/27/064101/mmedia]

1. Introduction

The nucleosome is the basic unit of DNA compaction in the eukaryotic nucleus \cite{1}. Its central part, the nucleosome core particle (NCP), incorporates around 147 base-pairs (bp) of negatively charged DNA, helically wrapped around an octamer of cationic histone proteins, forming an approximately two-fold symmetric complex \cite{2}. The physical mechanisms involved in this wrapping play an important role in the organization of the genome \cite{3} and the regulation of its expression \cite{4}, and they have been studied over several decades.

Significant progress arose with the crystallization of the NCP, which revealed its structural details at almost atomic resolution \cite{5}, in particular the existence of 14 regularly spaced
histone-DNA contact points, which were interpreted as the sites of non-specific interaction [6]. However, structural information alone is insufficient to infer the location of strong interactions, let alone their physical nature. How do mechanical forces distort the DNA at the contact points? Is there an electrostatic attraction in the intermediate regions where the DNA is more distant from the proteins? What is the elastic penalty of wrapping the DNA in the core? It is also unclear whether the structures currently available reveal artifacts of strongly binding DNA sequences or universal features of DNA wrapping in nucleosome core particles.

To answer these questions, it is useful to include physical models in the analysis of the structural data. The stability of the NCP is due to at least two key factors: (i) the mechanical energy of wrapping the DNA around the octamer, and (ii) the histone-DNA interaction, which is mostly of an electrostatic nature, and where the solvent plays a crucial role. These factors have been described with very different models. The recently reported first fully-atomic molecular dynamics simulations of the entire nucleosome [7, 8] were made possible by improvements in computational power. These simulations take all atomic details into account and are very promising, but they are limited to sampling times of less than 100 ns, which are probably not long enough to accurately describe the subtle effects of the ionic environment on the complex [9], not to mention large-scale rearrangements of the nucleosome such as breathing (\(\sim 10\) ms) [10]. At the other extreme, many theoretical studies have described DNA as a uniform semi-flexible polymer, and have employed continuous solvent models, such as the Debye–Hückel [11–13] or Poisson–Boltzmann [8] potentials. These continuous treatments are accurate at distances of a few nanometers, but they ignore e.g. the finite size of the ions and water molecules, which are probably crucial at locations where the two oppositely charged molecules come into close contact. Even if the combination of these models with more detailed descriptions of the macromolecules is a promising line of research [8], their predictive power in terms of sequence-dependent conformation of the DNA molecule, at least at nanometer-scale resolution, remains to be validated. This study is located at this intermediate, nanoscale level of description, which we consider a good compromise for the study of the internal mechanics of the NCP. The sequence-dependent structure and internal elasticity of DNA are described using the rigid base-pair model, commonly used in mechanical studies of the nucleosome [14–17], and parameterized from a combination [18] of experimental [19] and simulation data [20].

In order to overcome the limitations of the theoretical models of histone-DNA interaction at this scale, several authors have proposed a local harmonic approximation of the potential, relevant to the wrapped conformation, and incorporating a level of empirical knowledge (in this case, from the crystallographic structural information). Most of these studies used a drastic approach, simply assuming that all sequences wrap the histone core in exactly the same conformation (infinite stiffness of the potential well), which was then taken from the crystals or from smoother models [12, 14, 16, 17, 21]. The rationale for this scheme was the non-specificity of nucleosome binding, which cover as much as \(\sim 80\%\) of the genome, suggesting that all sequences accommodate (more or less easily) the same wrapping constraints that are imposed by the histones. This absence of specificity was further supported by the absence of direct contact between the histones and the bases in the high-resolution structures [6, 22]. However, the non-specificity indicates that the histone-DNA interaction potential, rather than the conformation, is sequence-independent. It is important to note that, even in this case, both the internal DNA mechanical energy and the interaction energy depend on the DNA sequence-dependent structure and elasticity, and we thus still expect that different sequences bind the histones with different affinities (indirect readout). For example, if the primary interactions involve the charged phosphate groups of the DNA backbone [23, 24], these sequence preferences will reflect the propensities of the different sequences to accommodate (i) the bending of the DNA around the octamer and (ii) the attraction of the phosphate groups to the maxima of the (sequence-independent) electrostatic potential, close to the cationic amino acids [23], even though indirect specificity cannot be ruled out (mediated e.g. by the ionic environment of the base-pairs).

Assuming an identical conformation for all sequences is therefore a very crude treatment. This was further emphasized by the observation of significant differences among the crystallographic conformations [22], with the likelihood of even greater differences with respect to those relevant in solution. Only two studies [15, 23] (to our knowledge) refined the models by introducing quadratical potentials with a finite stiffness, thereby crucially improving the description of the mechanics by allowing the DNA to adopt a sequence-dependent conformation. Morozov et al [15] considered an idealized model, with homogeneous quadratic springs directed toward the regular superhelical path of the DNA. However, considering that the experimental structures present substantial deviations from the ideal helix [25], it is unlikely that the forces exerted by the histones follow an ideal homogeneous pattern. In contrast, Fathizadeh et al [24] took these knowledge-based deviations into account, imposing elastic springs on those phosphate groups of the DNA that were in contact with histones, which were parameterized from the analysis of a crystallographic structural model. In this case, the potential thus still relies on the ad hoc choice of a particular input model and a given combination of interaction sites. We note however that, as much effort has been expended on resolving an increasing number of crystallographic structures, it is desirable to incorporate all the experimental knowledge contained in this growing database into a single interaction model. This construction requires an appropriate framework for the analysis of the structural database, the proposal of which is the object of the present paper. The resulting force-field does not rely on arbitrary assumptions about the location and strength of the forces, and enables estimation of the properties of nucleosomes incorporating DNA of arbitrary sequences.

This kind of structural analysis is not new: Olson et al followed the same rationale to extract the internal elastic energy function of (naked) DNA at the nano scale [19]. Here we propose a self-consistent extension based on the analysis of the forces and torques experienced by the double helix in NCP structures. As shown in a previous study [26], this information
can be inferred from experimental input given a mechanical model of DNA. The present study repeats the exercise for the available high-resolution NCP crystal structures and records the forces and torques in different crystal structures as a function of the DNA position and orientation relative to the histone spool. The idea is then to reconstruct a sequence-independent histone-DNA interaction potential from the information on its derivative for a representative set of sequence-specific conformations. The resulting interaction potential is specific to (and optimally adjusted for use in combination with) the DNA mechanical model from which it is derived. While we illustrate the approach for the rigid base-pair model, the self-consistent parameterization procedure can be applied to arbitrary coarse-grain elastic models of DNA [27–29].

The paper is organized in three sections. The models and methods section presents the background and theoretical framework for the analysis of an ensemble of NCP structural models, in a formulation that may easily be adapted to other non-specific DNA-binding proteins. The structural models used in the analysis are also described, including not only most published crystallographic structures, but also a collection of snapshots from MD simulations of the entire nucleosome, which are systematically compared with the experiment-derived models. The Results section compares the forces and torques in different crystal structures as a function of DNA position and orientation relative to the histone-DNA contact points. The conformation of sequence-averaged nucleosomal DNA is analyzed to show the forces at the histone-DNA contact points. The conformation is given by the derivative of the free energy function at the observed deformed conformation.

### 2. Models and methods

#### 2.1. Background

##### 2.1.1. The rigid base-pair model of DNA

This section describes the mechanical properties of the rigid base-pair (RBP) model of DNA. A base-pair, ignoring its internal fluctuations, is described as a rigid body with 6 degrees of freedom, \( \xi = \{\omega, r\} \), where \( \omega \) is the orientation and \( r \) is the position of the bp in the reference frame. The fluctuations of a bp-step are then described by the 6-vector \( \xi \) representing the relative orientation and position of the successive bp in the local frame (tilt, roll, twist, shift, rise, slide), as defined by the conventional axes [30]. The fluctuations of the successive steps are regarded as independent: the free energy contribution of a given step depends only on its conformation \( \xi \) and sequence \( s \). Finally, this free energy is treated in the linear elastic approximation, i.e. the free energy function is harmonic:

\[
F(s, \xi) = (\xi - \xi_0(s))^T K(s) (\xi - \xi_0(s))
\]

where \( s \) is the step sequence, \( \xi_0(s) \) is the equilibrium conformation, and \( K(s) \) is the \( 6 \times 6 \) symmetric stiffness matrix associated to the step.

##### 2.1.2. Extraction of parameters from DNA structures

The RBP model involves 21 + 6 parameters for each step sequence \( (\xi_0, K) \), which makes a total of 270 (taking into account self-symmetric steps). These parameters have been obtained from MD simulations [20, 31], from the analysis of a database of high-resolution crystallographic DNA or DNA protein structures [19], or from a combination of both [18]. Structural databases hold a large number of conformations \( \{\xi\}_{i=1,N} \) for each step sequence, from which it is possible to compute an average conformation and a covariance matrix. It is then assumed that these conformations follow a canonical distribution at an effective temperature, i.e. the external constraints of the crystal packing and the bound proteins act as the random forces of a thermal bath. The parameters of equation (1) are then simply given by the average conformation and the inverse of the covariance matrix computed on the dataset.

##### 2.1.3. Nanomechanical analysis of nucleosomal DNA

The RBP model describes internal DNA elasticity. The deformation of DNA under external potential must now be considered. In the NCP, the DNA is tightly wrapped around a histone octamer [6]. The aim of the nanomechanical analysis [32] is to infer, from the deformed shape of the DNA, the forces experienced by the molecule. Conceptually, this is as simple as estimating a person’s weight from the state of deformation of a scale.

For the sake of simplicity, the method is illustrated on a unidimensional, spring-like model of the nucleosome in figure 1. The free energy function of the DNA RBP model (dashed parabola) is a potential of mean force: in the regime of linear response, the mean force required for a given mean conformation is given by the derivative of the free energy [32] (dashed straight line). According to the mechanical equilibrium hypothesis, the deformed conformation \( x_c \) observed in the nucleosome is the result of the balance between this force \( \mu_d \) and the external force \( \mu_b \) exerted by the histone core (grey straight line). Knowledge of the DNA mechanical properties \( (l, b) \) and of the structure \( (x_c) \) implies that we can compute the force \( \mu_b = -\mu_d \) responsible for the deformation. In practice, the coordinates of the atomistic model are first mapped onto 6D RBP coordinates. The appropriate derivative of the DNA free energy function at the observed deformed conformations \( \hat{\xi} = [\omega, r] \) of the successive steps then enables the corresponding forces \( f \) and torques \( t \) to be computed.

Note that the method does not require the potential to be harmonic; the condition of validity is that the regime of linear response is still valid in the deformed system. Whether this condition holds for the RBP in nucleosomal DNA remains an open question [33]: the base-pairs are strongly deformed, with possible changes of the backbone states and subtle electrostatic effects [6]. Note, however, that this model was successful in predicting the position of twist defects in the NCP [26] and is widely used to estimate the sequence-dependent nucleosome association free energies [15, 34]. Here, it is also justified...
the total free energy.

\[ \mu_d(x_e) = \frac{-k(x-a)}{d|x|} \]

by the histone core (left, grey) and the neighbouring base-pairs (right, dashed) balance each other at the experimental conformation \( x_e \).

**Upper panel:** schematic depiction: the opposing forces \( \mu_d \) exerted by the histone core (left, grey) and \( \mu_d \) by the neighbouring base-pairs (right, dashed) balance each other at the experimental conformation \( x_e \). **Lower panel:** corresponding schematic energy landscape in the simple case where the springs are linear (straight lines of mean force). Both free energy contributions are indicated by a parabola, and the equilibrium conformation \( x_e \) is the minimum of the total free energy.

*a posteriori* by the relatively accurate predictions of sequence binding preferences (see the results section).

### 2.2. Extraction of the coarse-grained force-field in a biomolecular complex

The nanoscale elastic potential of internal DNA deformations was parameterized from statistical analysis of an ensemble of high-resolution structural models [19]. This method is only applicable if the number of structural datapoints available is much larger than the number of parameters in the potential, so its application is limited to the RBP (rather than, e.g. the rigid base) model of DNA. A simple estimation of the independent coordinates shows that the same method cannot be simply transposed to the more complex NCP system. The total potential experienced by a given DNA bp is the sum of (i) the internal DNA elastic potential and (ii) the external potential exerted by the histones. They depend (i) on its position relative to its neighbours (2 \( \times \) 6 = 12 degrees of freedom) and (ii) on its position relative to the histone core (6 dof): if the latter are fixed in the laboratory frame, these are the absolute coordinates. Even in the harmonic approximation, the fluctuations of the bp positions are therefore described by 18 \( \times \) 18 stiffness matrices. Their parameters depend on the sequence of the bp being studied and its neighbours: altogether, a harmonic model thus involves 4752 parameters. For comparison, there are currently \( \sim \)50 published NCP crystal structures.

![Figure 1. Equilibrium conformation of a nucleosomal DNA base-pair, in a unidimensional spring-like model of the nucleosome.](image)

**Figure 1.** Equilibrium conformation of a nucleosomal DNA base-pair, in a unidimensional spring-like model of the nucleosome. **Upper panel:** schematic depiction: the opposing forces \( \mu_h \) exerted by the histone core (left, grey) and \( \mu_d \) by the neighbouring base-pairs (right, dashed) balance each other at the experimental conformation \( x_e \). **Lower panel:** corresponding schematic energy landscape in the simple case where the springs are linear (straight lines of mean force). Both free energy contributions are indicated by a parabola, and the equilibrium conformation \( x_e \) is the minimum of the total free energy.

Note, however, that this considerable parameter set is largely redundant, with the *internal* DNA elasticity already determined from independent experiments on naked DNA. In the nanomechanical analysis described in the previous paragraph, we showed that the combination of this prior knowledge with one NCP high-resolution structure already allows us to compute the static forces acting on the DNA. Now we go one step further, and show that the application of this analysis to a limited ensemble of structural models yields the potential of mean force.

The method is illustrated in figure 2 with the unidimensional model described in the previous paragraph. The force inferred from each individual structure is the local derivative of the histone potential: \( \mu_h = -\odot (dV_h/dx)|_{x_e} \). Importantly, while the DNA potential depends on the sequence and conformation of the structure analyzed, the histone potential \( V_h \) is assumed to be non-specific. Each structure analyzed thus provides an independent point \( (x_e, (dV_h/dx)|_{x_e}) \). Even a limited number of such datapoints allows us to reconstruct the curve \( (dV_h/dx)(x) \) and the potential of mean force from which it derives. Figure 2 shows that, for a harmonic potential, the points are aligned along a linear force curve, enabling the two parameters \( \{a, k\} \) to be fitted. The same construction can be generalized to any functional form, provided the number of datapoints is sufficient. Note that even though the interaction potential is sequence-independent, the interaction force and energy associated with each conformation depends on its sequence, because of the sequence-dependent internal DNA structure and elasticity.

In the RBP model, each bp is described by a 6D vector \( \xi \) representing its position and orientation. The histone potential must therefore be fitted into the 6D configuration space. In the
particular case of a harmonic potential,
\[ V_h(\xi) = (\xi - \xi_0)^T K (\xi - \xi_0), \]  
the force \( \mu_h \) depends linearly on the conformation \( \xi \):
\[ \mu_h(\xi) = -K (\xi - \xi_0) \]  
Here, the unknown histone potential is described by the 6D equilibrium conformation \( \xi_0 \), and the \( 6 \times 6 \) symmetric stiffness matrix \( K \), i.e. 27 parameters per base-pair. These parameters are determined by fitting the datapoints \( \{\xi_0, \mu_i\}_{i=1,N} \) according to equation 3, where each of the \( N \) structural models analyzed contributes 6 points. For the entire 79 bp-long internal turn of the NCP which is the focus of this study, there are \( 27 \times 79 = 2133 \) elastic parameters, and each structure contributes \( 6 \times 79 = 474 \) datapoints. Algebraically, 5 structures are therefore sufficient to solve the minimization problem. However, considering that the 6 coordinates of each inferred force are not obtained independently, it is desirable to have at least 27 independent structures (here we have 118).

2.3. NCP structures dataset

The proper sampling of the interaction potential depends crucially on the available dataset, which should include a variety of conformational states representative of different regions of the energy landscape. Here, our dataset is composed of three families of structural models (the detailed list is given in table S1 in the supplementary material (stacks.iop.org/JPCM/27/064101/mmedia)). Most crystallographic structures [45] include derivatives of the same human \( \alpha \)-satellite sequence [2]; 4 structures are based on the 601 sequence [35]. Throughout this article, we mostly refer to a few well-resolved structures, NCP147 (PDB 1kx5) and NCP146 (PDB 1kx3) [5], 601 (PDB 3 mvd) [35] and 601L (PDB 3ut9) [37]. A part of the dataset includes modified or variant histones, and in these cases the corresponding ‘excited’ structure cannot be used to infer the ‘regular’ nucleosome potential. However, this is true only in the region that is in contact with the perturbation, which is generally limited. We assumed that in the remaining part of the complex, the external potential is not modified. If the forces resulting from the perturbation partially propagate to remote locations through DNA mechanics, they might even allow sampling of new (and higher) parts of the energy landscape of the nucleosome.

Finally, the dataset includes snapshots from molecular dynamics runs of the entire nucleosome (excluding the histone tails) with the NCP147 sequence: 5 snapshots where the thermal fluctuations give access to other excited states, and 5 ‘relaxed’ versions obtained through short energy minimization. The snapshots were separated by 2 ns in the MD trajectory. In the nanomechanical analysis of these snapshots, it is assumed that they represent local (metastable) mechanical equilibria (see figure S3).

The histone octamer has an axis of symmetry passing through the central NCP bp (the dyad axis), and it is therefore assumed that the histone potential should be symmetric with respect to this axis. The structural models are not symmetric, however, either because the DNA sequence employed is non-palindromic (e.g. 601), or because the two halves have crystallized in different conformations. We take advantage of this effect by considering the DNA conformation along either strand as independent data. This operation makes the dataset symmetric with respect to the dyad axis, while doubling the number of datapoints at each position (hence a total of 118 data-points). The atomic coordinates of the different structures were mapped into RBP coordinates using the 3DNA program [38], and superposed on the reference NCP147 structure by minimizing the sum of distances between the base-pair centres. Note that some additional crystallographic structures were removed from the dataset in cases when the atomic coordinates could not be properly mapped into RBP coordinates, probably because of a higher level of noise.

3. Results

The nanomechanical analysis [32] of individual high-resolution structural models of the Nucleosome Core Particle (NCP) [5] showed that strong forces concentrate at the \( \sim 10 \)-bp periodic contact points with the histones, where stereotypical force motifs result in strong distortions of the DNA base-pairs [6]. In this section, we generalize this analysis to an ensemble of high-resolution structural models of the NCP, following the method described in detail in the models and methods section.

The dataset analyzed includes 49 structural models obtained from high-resolution x-ray crystallographs (see Models and Methods and the complete list in supplementary material (stacks.iop.org/JPCM/27/064101/mmedia), table S1). Most of these structures are based on the same \( \alpha \)-satellite sequence [2] or related ones, including the NCP147 structure [5], which is the best resolved nucleosomal structure and is used as a reference in what follows. Because of this sequence similarity, the ability of this dataset to represent the whole conformational ensemble of nucleosomal DNA is questionable. Recently, 4 additional crystallographic structures [36, 37 39] were obtained with the strongly positioning 601 sequence [35], whose structural features were found to differ from the previous ones [22]: their inclusion in the dataset therefore increases the variety of the sample, enabling new parts of the energy landscape of the nucleosome to be sampled.

In order to further increase this variety, we have included a set of structural models obtained from snapshots of all-atomic MD simulations of the entire nucleosome, including the NCP147 sequence. Such simulations are becoming computationally tractable [7] and present conformations that are different from the knowledge-based models (see below). Whether these conformations are representative of actual nucleosomes remains open to question: possible bias includes the limited sampling time (10 ns) and the force-fields used. We therefore systematically compare the features observed in these models with those based on experimental data. The reader should however also keep in mind that the latter could be equally biased according to the sequences used, and by the crystallization process, with most structures crystallized in the same orthorombic geometry (albeit with different unit cell sizes).
The relatively good agreement between the experimental and simulated models, and e.g. the 601 structure that crystallized in a different geometry, suggests that these possible biases are not the dominant effects [40].

### 3.1. Base-pairs are shifted outwards at the contact points

Figure 3 shows four superhelical locations (SHL) (~1/2 turn) of the NCP, for the 'canonical' NCP147 structure (PDB ID 1kx5, grey), which will be considered a reference in the remaining analysis, and for (i) one of the MD snapshots (MD1, cyan), (ii) the NCP146 structure (PDB 1kx3, orange), which has the same sequence as NCP147 except at SHL −2.5 where it exhibits a twist defect, and (iii) a 601 crystal structure (PDB 3mvd, magenta). The contact points are located at semi-integral SHL, and the primary bound phosphates in the NCP147 structure are shown as grey spheres.

Overall, the bp in the MD snapshot remains remarkably close to those of the experimentally derived structures. The deviations from the original structure are larger than in the NCP146 crystal, where they are hardly visible except on the extreme left side where the twist defect of this structure [5, 26] begins to appear. On the other hand, they are apparently not larger than in the 601 crystal [22], and present no obviously aberrant feature. If these discrepancies are indicative of actual alternative nucleosomal conformations rather than artifacts of the simulation, they may thus constitute a valuable source of structural information.

For a more detailed analysis, a reduced depiction of the bp of the NCP147 structure is shown in figure 4. Remarkably, the bp located at the contact points appear slightly shifted outwards with respect to the average superhelical path (grey line), a counter-intuitive feature given that the contact points are generally regarded as the points of attractive interaction with the core histones. This feature can be seen even more clearly on the more detailed figure 6, at bp 5 (SHL 0.5) and bp 14−16 (SHL 1.5), and is confirmed by the distance profile with respect to the superhelical axis (figure S1).

### 3.2. Strong and repulsive forces at the interaction sites

While the crystallographic data provide detailed structural information on the complex, physical models are needed to understand the underlying physical mechanisms. In the nanomechanical analysis (see Models and Methods) we combined the rigid base-pair (RBP) model of DNA with high-resolution data, in order to infer the nanoscale forces acting on DNA in protein-DNA complexes [26, 32], hypothesising mechanical equilibrium at the nanoscale. This process has now been repeated with the entire dataset. To regularize the force profiles and for ease of comparison, the structures were allowed to slightly ‘prerelax’ in this case [26] (see technical details in supplementary material (stacks.iop.org/JPCM/27/064101/mmedia)). The NCP147 structural forces are shown in 3D in figure 4. They are strongest at the contact points, where they exhibit characteristic patterns [26]: at SHL ±0.5, a strong radial force acts on the central bp, and two approximately opposed forces act 2 bp away on either side, resulting in a global torque on the chain. At SHL ±1.5, two strong, approximately radial forces are separated by 2 bp. Importantly, figure 4 shows that the major forces point outwards, consistent with the associated bp being pushed away from the core at the contact points. This is reflected in the radial component of the force profile, figure 5, which indicates that the contact points are the locations of repulsive forces. These characteristics of the contact point forces are generic, and are common to all families of the structures under analysis, even though their detailed profiles differ (see also figure S3). A more useful comparison is to inspect these forces directly on the 3D structures, as shown in figure 6. In most cases, the base-pairs in contact (e.g. 5 and 16) are indeed shifted outwards by repulsive forces (upper panel). In the transverse direction (lower panel), these base-pairs are also attracted in the direction of the primary bound phosphates. There are some exceptions however: for example, in one of the MD snapshots (cyan) bp 5 is shifted inwards by a strong negative radial force.

These qualitative observations already allow the separation of the most generic features of nucleosome binding, which will be the object of the upcoming discussions, from the specificities and irregularities inherent to each particular model. Anticipating the discussion, we note that the unexpected repulsive forces are perfectly compatible with the stability of the complex, provided they are compensated by inwards internal forces resulting from a (local) stretch tension in the DNA. A common macroscopic example is a stretched rubber band holding together a group of pencils. It follows that the large
Figure 4. Force profile of the NCP147 structure (grey arrows): view along the superhelical axis (upper image) and along the dyad axis (lower image). At the contact points, the base-pairs are pushed outwards by strong forces, with a different pattern at SHL ±0.5 and ±1.5 (see text). Arrow size proportional to the force magnitude. Dyad axis indicated by a black line, and primary bound phosphates as grey spheres. Base-pair depiction scale 0.25.

Figure 5. Radial force profile, averaged among the different groups of structural models. A positive value indicates a force directed outwards: the contact points are therefore the locations of repulsive forces, whereas attractive forces are weaker and less localized. Contact regions are indicated by grey rectangles.

Figure 6. Base-pair conformations and inferred forces at the histone contact points SHL 0.5 (left) and 1.5 (right), in NCP147 (grey), the 601 structure 3mvd (magenta), and two MD snapshots: MD1 (cyan) and MD2 (green). View along the superhelical axis (upper panel) and in the direction of the NCP (lower panel). The upper panel shows that the contact bp are pushed away from the core, consistent with a force directed outwards (see details in text).

The number of available structures provides the statistical power to quantify the differences between the structures under analysis. Figure 7 shows the radial component of the force at the anchor points 0.5 (a) and 1.5 (b), and in an intermediate region (c), for all the models analyzed. While the forces are weak and noisy in the latter case, they are stronger and better defined in the former. Note that while most models exhibit repulsive forces, at bp 5 the negative force observed previously in a single MD
Figure 7. Relation between the bp radial position and the external force, (a) at the SHL 0.5: example of the central bp +5, (b) at SHL 1.5 (bp 16) and (c) outside the contact regions: SHL 0 (bp 0). For the 3D plot, same colours and conventions as in figure 6. View along the superhelical axis. Symbols: α-satellite-based crystallographic structure with regular (circular) or variant (square) histones (see models and methods), 601-based crystallographic structure (triangle), MD snapshot (star). At bp +5, the datapoints divide into two groups with either strong repulsive radial forces (most crystallographic structures, and some snapshots) or negative forces (most MD snapshots, and a single crystallographic structure). The same positive correlation is found for the whole dataset, consistent with a repulsive quadratic potential centred at \( x \approx 3.75 \) nm.

The snapshot is in fact representative of a whole alternative group of datapoints (including a crystallographic structure). It may therefore constitute an alternate binding mode, rather than an artifact of the analysis.

3.3. Sequence-dependent forces derive from a sequence-independent histone-DNA potential

Figure 7 shows that the forces inferred from completely independent structures are not randomly distributed; rather, they align themselves along well-defined linear force-extension curves at the expected sites of histone-DNA interaction (a–b). The physical interpretation of this correlation is that these various forces derive from a unique histone-DNA potential of mean force: each inferred datapoint is therefore the derivative of this potential at the relevant position (see figure 2 in models and methods section). Importantly, we note that the datapoints obtained with the 601-sequence (triangles) are aligned along approximately the same curve as the other points obtained with very different sequences (the deviations do not exceed those observed with similar sequences). This observation indicates that the interaction potential experienced by the DNA is sequence-independent (non-specific). This property has often been proposed because there is no direct molecular contact with the bases, but indirect specificity could not be ruled out. It could, for example, be mediated by the solvent, if different base-pairs had very different ionic environments; different datapoints located at the same position in the curve of figure 7 would then be expected to experience very different electrostatic forces from the cationic amino acids, but this effect was not observed in this case. Even though this conclusion is limited (i) to the dataset analyzed, and (ii) by the fact that the DNA model used does not explicitly represent the primary interaction sites (the backbone phosphates) [23], the alignment of the datapoints provides strong evidence for a non-specific potential.

The slope defined by the datapoints gives the stiffness of the local harmonic approximation of the histone potential (equation (3) in models and methods). This potential was then computed by fitting the datapoints. Importantly, while figure 7 shows only one particular dimension (radial coordinate) of the datapoints, the non-radial features are known to play an important role in the mechanics of the nucleosome [25]. Our fitting procedure therefore involves the full 6D conformations of the base-pairs analyzed. We compared the quality of the fit with a randomly generated control sample, which mimics a situation where the variation in basepair positions is due to pure noise (figure S5); the analysis confirms the presence of detectable histone forces in the inner turn of the nucleosome, while the latter are less well defined in the outer parts of the complex. This may indicate that the key interactions with the core histones are concentrated in the inner turn, consistent with the ∼80 bp definition domain of high-affinity sequences [35], while the external parts might be involved in more subtle and less generic interactions with the tails [7]. In the following, we focus our analysis on the internal turn of the NCP. The fit is also more precise in the contact point regions where the bp are more localized and the forces are stronger than in the internal turn.
intermediate regions where the relative noise is important, as examination of figure 7 would suggest. Still, even in these regions, many inferred forces contain a detectable non-random component (see all radial plots in figure S8), indicating that the histones also interact with the DNA at a distance, probably through electrostatics [12].

The first nanoscale potential of the nucleosome has thus been obtained from experimental data, not from ideal models. For consistency, we computed the model-derived force profile along the 6D, as computed on the input structures NCP147 and the palindromic 601-derived structure 601L [37]; their agreement with the original profiles validates the interpolation scheme used (figure S7). In the next section, this potential is used to estimate the sequence-dependent nucleosome-wrapping elastic energy.

3.4. Sequence-dependent conformation of nucleosomal DNA

Our model describes the small harmonic fluctuations of nucleosomal DNA around its equilibrium position: it is therefore not directly applicable to large-scale rearrangements such as unwrapping or contact breaking. Rather, its immediate principal application is to predict the sequence-dependent equilibrium conformation, which gives access to the energy of the wrapped DNA conformation. By relaxing the DNA with different sequences in the extracted force-field, we compute the sequence-dependent wrapping energy, which can then be compared with the binding free energies, as measured in competitive binding experiments [15, 41–43].

Several studies have looked at predicting these energies from mechanical models of the nucleosome, but in the absence of a reliable nanoscale histone-DNA potential, most of them simply threaded the DNA sequences onto fixed nucleosomal conformations, either the crystallographic models [34] or ideal superhelical templates [16]. We addressed the question of the dependence of predictions in these models on the chosen nucleosomal template. For the crystallographic models, the first difficulty is the presence of experimental noise affecting all the energies and forces being computed, as noted by [26, 44]: they are considerably larger than the experimental values (table S2). An estimate of this global factor was made and all energies and forces rescaled accordingly (by 0.2, see supplementary material (stacks.iop.org/JPCM/27/064101/mmedia)).

The coloured curves of figure 8 compare the resulting predictions using different nucleosomal templates (NCP147, NCP146, 601L, ideal superhelix), for a small number of high-affinity sequences where the position of the NCP is well defined. The variations between most rescaled datapoints are of the correct order of magnitude ~10k_BT. However, and interestingly, each crystallographic structural template strongly underestimates the particular sequence with which it was obtained, and is a poor predictor of the affinity of other sequences (e.g. all coloured datapoints on the right side of the plot have the wrong sign). Two conclusions can be drawn from these observations. First, the DNA (rigid base-pair) elastic model used reliably predicts the sequences employed in the crystals, i.e. it accurately describes the deformations present in these structures. This important observation justifies the choice of this model for the present study. Second, no single structural template can properly describe the variety of conformations of even our limited set of sequences. This is true also for the ideal superhelix (blue), where some of these problems are absent, but which incorrectly predicts 5S to have less affinity than a random sequence, suggesting that the binding mode of 5S differs from an ideal helix. Altogether, these observations support our attempts to combine the different known structures into a single mechanical model.

The thick black curve in figure 8 shows the predictions that were obtained in our model by relaxing the different sequences in the histone potential, the computation of which was described in the previous section. In contrast to the previous computations, the relative preferences of all sequences with respect to 5S are correctly predicted. This qualitative success may indicate a better description of the binding mode of 5S than in any previous template. Some properties of this unknown binding mode may be reflected in the structural profiles of the relaxed structures (figures S12 and S13), which are different from all input structures.

Interestingly, our results also agree relatively closely with the non-rigid mechanical model DNABEND [15], where nucleosomal base-pairs are subject to forces directed toward the ideal superhelical path. Both models accurately rank the sequences discussed, but fail to predict the quantitative values of the relative energies. However, the validity of such a quantitative comparison is questionable: the measured free energies involve an ensemble of unwrapped and translated nucleosomal states, which are not considered in our model. These experiments have also only been conducted on a few sequences, and the results depend to a great extent on which protocol is used [41, 43]. In conclusion, at the qualitative level of comparison available, our model successfully predicts the relative affinity of high-positioning sequences, and is consistent with the best existing mechanical models.
4. Discussion

Two opposite approaches to the mechanics of DNA in the nucleosomes have previously been described. Either a very specific conformation deduced from crystallographic data has been extrapolated to all genomic sequences [14, 16, 17], or the histone-DNA interaction has been derived from ideal models with limited experimental justification [15]. We adopted an intermediate approach [24], combining an extensive set of sequence-specific high-resolution NCP conformations with a mechanical model of DNA to infer a sequence-independent nanoscale potential of the interaction. The proposal of this scheme is the central message of the present article. By construction, the potential extracted reflects current knowledge, and results will certainly evolve with the refinement of the elastic model of DNA (for instance using a recently proposed rigid base model [29, 31]) and particularly with the availability of additional NCP structures, which can then be incorporated into the input database. This type of structural model may be obtained experimentally, but also from MD simulations of entire nucleosomes, which are just beginning to be computationally tractable [7]. A particularly relevant extension of the present work would involve a coarse-grained DNA model that explicitly describes the backbones, and in particular the phosphate groups that dominate the electrostatic interactions with the histones [27, 28]. One example would be the relation of the base-pair dispersion (figure 7) and the constraint of placing these groups near the cationic amino acids of the core [23], which might involve sequence dependent structural transitions of the backbones [31] that the present model does not describe accurately. But more detailed description of the electrostatics will also rely crucially on accurate treatments of the solvent [9], as well as detailed monitoring of how the cationic amino acids, in addition to the DNA, are displaced among the different structures, in a common reference frame.

Previous nanomechanical models of the nucleosome have been used to predict new high-affinity sequences, in particular the DNABEND model [15] which gives similar predictions to ours. Experiments were in relatively poor agreement with the predictions, suggesting that the description of the 1st-order sequence-dependent variations will still require improvement, either of the DNA model used [29] or of the variety of the crystallographic structures that are analyzed. In the next section, we focus, rather, on the 0th-order sequence-independent features of nucleosome binding that were revealed by the analysis.

The strongest forces involved in DNA wrapping are localized at the sites of histone-DNA contact, but, maybe surprisingly, these sites are not the location of radial attraction. Rather, in the structures under analysis, DNA base-pairs often appear to be shifted outwards by repulsive forces, which align themselves along well-defined force-extension curves (figure 7). Equally surprising is the positive slope exhibited by these curves, $f_x = k_{xx}(x - x_0)$, which is the indication of a repulsive quadratic potential, $F_{xx} = -k_{xx}(x - x_0)^2/2$ (negative stiffness), in contrast to a regular spring where the slope would be negative (corresponding to a positive stiffness—see for instance figure 1). Note that this does not depend on the particular choice of coordinates; in the 6D configuration space where our analysis is conducted, most fitted stiffness matrices have negative eigenvalues. Importantly, this repulsive potential is compatible with the local mechanical stability of the base-pairs, provided it is compensated by the internal DNA elastic force (see figure S9).

4.1. Repulsive forces are compatible with a stable complex involving locally stretched DNA

The existence of these repulsive potentials seems counterintuitive, considering the expected electrostatic attraction between the positively charged histones and the negatively charged DNA backbone. We now suggest that these features result from the cylindrical geometry of the nucleosome, and may provide important information on the role of DNA stretch in the physical mechanism of wrapping. To illustrate this point, we need momentarily to leave aside the complex multidimensional model of DNA that we have been using, where such effects are difficult to interpret. Figure 9 shows that even the simplest ideal models of the nucleosome can give rise to them. DNA is described as a uniform semi-flexible polymer (worm-like chain) with bending and stretching rigidities, and the histone-DNA interaction is first described as a uniform Lennard–Jones-like potential accounting for the electrostatic attraction and short-range steric repulsion (left-hand column). The wrapping mechanism thus depends crucially on two length-scales: (i) the interaction range $\sigma$, which corresponds to the added molecular radii of the histones and DNA, and (ii) the natural radius $r_0$ of the wrapped DNA length, imposed by the mean distance between successive base-pairs (rise). Importantly, if the actual radius differs from this spontaneous value, the resulting stretch, rather than bending, dominates the elastic energy variations (figure S10). Even in this oversimplified description, the grey curve of the left-hand column ($\sigma > r_0$) illustrates a case where the mechanically stable solution (minimizing the total energy) is a stretched DNA conformation held by repulsive histone forces (positive value in the force panel). This scenario corresponds to the familiar example of a stretched rubber band holding together a group of pencils: at each contact point, the force exerted by the pencils is indeed repulsive, and compensated by the internal forces of the elastomer.

While this simple analogy is useful to rationalize the observations, including additional details in the models shows that they strongly affect the local shape of the interaction potential. In the middle panel of figure 9, we consider the approximate 10-bp oscillatory structure of the nucleosome (sinusoidal $\sigma(n)$). For simplicity, the stiff DNA is assumed to keep a uniform radius, which minimizes the total energy integrated along the superhelical path. As a result, at the contact points, the protruding amino acids hold the DNA by repulsive forces, while imposing a stretch tension on the molecule. This tension also affects the intermediate parts, which experience a weaker attractive force, in qualitative agreement with many of our observations. The exception is the observation of a negative stiffness $k_8$ (positive slope of figure 7) at the contact points, i.e. in the convex left region of the Lennard–Jones-like curve. This behaviour can be expected to arise from the atomic-scale features that are crucial in these regions. We suggest in particular that the molecular packing of ions and water molecules...
Figure 9. Elastic models of the nucleosome of increasing complexity (left to right, upper panel), with the profiles of the histone-DNA potential energy, radial force, and radial stiffness (upper to lower panels) along two helical turns of the DNA. These simplified models show that the stability of the complex is compatible with very different force and stiffness patterns, and in particular with repulsive forces, corresponding to a locally stretched DNA ($\sigma > r_0$). $\sigma$ is the effective DNA+histone radius, $r_0$ is the natural radius of wrapped DNA (defined by the average rise), and $n$ refers to the basepair index along the superhelical path (dyad at 0). A negative stiffness (lower right panel) can be expected to arise from the atom-scale irregularities at the contact points, in particular the layering of ions and water molecules. Functional form of the potential adapted from [45], with an arbitrary oscillation amplitude.

may play an important role, as it is known to strongly influence macromolecular interaction in solution at short distances [45]. This is especially relevant in those histone-DNA contact regions where DNA is probably in direct competition with the first, strongly bound layer of counter-ions, and therefore subject to so-called hydration or solvation forces [46]. In this case, the effective interaction typically contains an oscillatory element, which reflects the ordered layering of ions and water molecules ($\sim 3$ Å periodic), and decays exponentially with distance [45, 46], as shown in the right-hand column of figure 9. The rugged potential leads to a stable complex, globally very similar to the previous case, but exhibiting very irregular local features: all combinations of force and stiffness signs are possible, depending on the molecular details. Negative values of the stiffness correspond to local (concave) maxima of the potential that are characteristic of the ionic or molecular shells. Interestingly, we have already noted that in the datapoints of SHL 0.5 (figure 7(a)), not all structural models exhibit repulsive forces; in a second, well-separated group of inwards-shifted base-pairs, the force is attractive. We suggest that these groups could be located at the two sides of a peak in the solvent density function, corresponding to a positive and negative radial force respectively. They are indeed separated by a distance of $\sim 2$ Å, remarkably close to the expected value if the inwards-located base-pairs correspond to an alternate binding mode where the internal ion or water molecule is absent or displaced. This observation indicates that our coarse-grained DNA model might be able to detect atomic-scale features if many structures are analyzed simultaneously.

4.2. Sequence-averaged nucleosomal DNA

In contrast to the usual view, the examples presented in the previous paragraph suggest that DNA stretching, in addition to bending, could play a key role in the mechanics of nucleosome wrapping. However, it is not easy to directly detect this effect on the crystallographic structural models, where not only the wrapping but also the very specific sequences that are used contribute to the observed deviations from a regular B-DNA conformation. Since we focus instead on the non-specific binding mechanisms relevant to the $\sim 80\%$ genomic sequences that are incorporated in nucleosomes most of the time, we propose to analyze in more detail the conformation of sequence-averaged (neutral) DNA, as obtained after relaxation in the histone potential, which avoids these problems thanks to the uniform generic mechanical features.

Figure 10 shows the profiles of the 6-step helical parameters (a) and force components (b) along the central turn of the
nucleosome, for the sequence-averaged structure (blue line, the palindromic sequence makes it symmetric with respect to the dyad axis by construction). We see that this conformation is closer to the regular superhelix (grey) than to any family of crystallographic structures that we have analyzed (red and green), but still exhibits substantial deviations which are particularly apparent in the three-dimensional depiction of the structure (c). We checked that these deviations do not depend on the initial state used in the relaxation procedure, but indeed reflect the properties of the extracted force-field (figure S11). Interestingly, while the local irregularities of the two sequence-specific families of crystallographic structures are quite different, those of the generic relaxed conformation generally differ from both, suggesting that their sequences may play an important role in these structural features. These non-superhelical features, perhaps indicative of an alternate (and more generic) binding mode of the nucleosome, stem from the forces exerted by the histones, which are generally lower in the relaxed structure than in the input models, except at the contact points (particularly SHL 1.5). In the latter case, where the steps are strongly deformed, some of the structural features may, however, also reflect the limitations of the quadratic models used (either for the internal DNA mechanics or for the histone-DNA interaction) [33]. For instance, the strong peak at bp 16, which is absent from the original structures, might be due to the quadratic coupling with tilt, where a peak is indeed present.

To investigate the possible role of internal mechanical tension in the energetics of the complex, figure 10(c) shows not only the structure and the external forces (green arrows) exerted on the base-pairs, but also the internal forces which result from the deformations of the basepair steps. These forces are depicted as coloured bars, with the sign of the forces indicated by the colour. A red (resp. cyan) bar corresponds to a compressed (resp. stretched) step. In each case, two opposite forces act on the base-pairs of the step, and tend to separate (resp. attract) them. These forces have very different directions and intensities along the path owing to the local deformations of the steps, but most of them do in fact correspond to stretched steps. More precisely, there are strong irregular forces at the contact points (especially at SHL 1.5 where the steps are compressed) resulting from strong external (mostly repulsive) forces. In contrast, outside these regions, nearly all steps are stretched, while the external forces are weak. The internal forces have a strong radial component only in the vicinity of the contact points, where they become mostly tangential (i.e. pure stretch) in the intermediate parts. The qualitative picture emerging from these observations is in agreement with the suggestions of the nucleosome models: the histones impose not
only strong local (and irregular) deformations on the contacted steps, but also distance and orientation constraints between the successive contact points, resulting in a state of tension of the helix in the intermediate regions where the external forces are weak. These observations are quantified in figure 11, which shows the stretch component of the internal tension together with the radial external forces. Interestingly, the level of tension is very different for the different locations. The low repulsive forces at the contact points ±0.5 correspond to a low intermediate average tension of ~2 pN.nm. But this tension may result from the propagation of the much stronger forces at SHL ±1.5, which would distribute into the whole central region between bp −15 and bp +15 (average level 2 pN.nm). On the other side, conversely, the tension is concentrated between SHL ±1.5 and SHL ±2.5, with a strong level of ~10 pN.nm.

These observations demonstrate that DNA tension could play an important role in the mechanics of wrapping. Controversy over this topic is of long standing [47], but perhaps partly due to experimental difficulties the presence of a global stretch tension has never been demonstrated [48]. In particular, the tension was only assayed at the scale of the entire nucleosome, and for specific sequences which can substantially influence the properties of the DNA. Our results suggest, however, that the tension could affect only or mostly some portions of the complex, with a relevant extension scale of 10 bp corresponding to the distance between the successive contact points, and might thus be detected only at this resolution. Interestingly, if the net histone-DNA force is indeed repulsive at least at some contact points, then the DNA also ‘pushes’ and holds the histones together by reaction, a feature qualitatively compatible with the observation that the octamer dissociates in the absence of wrapped DNA [3].

As our framework only describes the internal part of the complex, further investigation will be necessary to resolve these features along the entire superhelical path. In particular, while the details of the force patterns we analyzed at the contact points may partly reflect the limitations of the DNA model we employed, the net direction of the force as well as the stretch profiles in the intermediate regions could be tested with molecular dynamics simulations of the nucleosome incorporating mutated or truncated histones (e.g. deleted or neutralized DNA-contacting amino acids). In the meantime, our observations underline the strength of combining nanoscale mechanical models with structural data. They suggest a re-evaluation of the role of DNA elasticity in the physics of the nucleosomal complex: far from opposing the wrapping, it could contribute to its stability.

5. Conclusion

The physical mechanisms of histone-DNA interaction in the nucleosome are a key ingredient of the genomic compaction in the nucleus, and yet they remain largely unknown. This study has proposed a new method for the extraction of effective nanoscale potentials in DNA-protein complexes from the analysis of high-resolution structural data, and it has been applied to the NCP. Forty-nine crystallographic structural models based on two families of high-positioning sequences were analyzed. Because both types of conformations may be significantly different from the dynamic structures present in solution, this database was increased by the introduction of 10 snapshots from MD simulations of an entire nucleosome: we verified that those properties analyzed were generally consistent with those of knowledge-based models. In some cases however, they present differences, which may be indicative of alternate binding modes and were considered in the analysis.

Within the rigid base-pair description of DNA, we find that the base-pairs are locally repelled rather than attracted at the 10 bp periodic histone contact points. This behaviour can be described, for the whole dataset analyzed, by a repulsive quadratic force-field at these locations. To test the validity of this knowledge-derived nanoscale potential, we compared the computed wrapping energy with measured binding free energies for a few high-positioning sequences, and found qualitative agreement, comparable to the best available estimates based on ideal models. The conformations obtained after relaxation often differ from both input families, and might indicate alternate conformations. The extracted repulsive potential diverges from the most common view of nucleosomal stability, where the electrostatic attraction opposes the bending stiffness of the stiff molecule. In the Discussion, we suggest reconsideration of the role of DNA elasticity, which, if the DNA is constrained not only in the bending but also in the coupled twist/stretch degrees of freedom, may contribute to this stability instead of simply opposing the wrapping.

Acknowledgments

We thank Richard Lavery for fruitful discussions, and Agnes Noy and Modesto Orozco (IRB Barcelona) who kindly provided the nucleosome snapshots. This work was supported by the Agence Nationale de la Recherche grant ‘FSCF’ [ANR-12-BSV5-0009-01].
References

[1] Kornberg R D 1974 Science 184 868–71
[2] Luger K, Mader A W, Richmond R K, Sargent D F and Richmond T J 1997 Nature 389 251–60
[3] Schiessel H 2003 J. Phys.: Condens. Matter 15 R699
[4] Flaus A and Owen-Hughes T 2001 Curr. Opin. Genet. Dev. 11 148–54
[5] Davey C A, Sargent D F, Luger K, Maeder A W and Richmond T J 2002 J. Mol. Biol. 319 1097–113
[6] Richmond T J and Davey C A 2003 Nature 423 145–50
[7] Ettig R, Kepper N, Stehr R, Wedemann G and Rippe K 2011 Biophys. J. 101 1999–2008
[8] Biswas M, Langowski J and Bishop T C 2013 Wiley Interdisciplinary Reviews: Computational Molecular Science 3 378–92
[9] Lavery R, Maddocks J H, Pasi M and Zakrzewska K 2014 Nucleic Acids Res. 42 8138–49
[10] Li G, Levitus M, Bustamante C and Widom J 2005 Nature 436 1–10