

Nucleosome wrapping energy in CpG islands and the role of epigenetic base modifications

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eLife Assessment

This **valuable** simulation study proposes a new coarse-grained model to explain the effects of CpG methylation on nucleosome wrapping energy and nucleosome positioning. The evidence to support the claims in the paper looks **solid** and this work will be of interest to the researchers working on gene regulation and mechanisms of DNA methylation.

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Abstract

The majority of vertebrate promoters have a distinct DNA composition, known as a CpG island. Cytosine methylation in promoter CpG islands is associated with a substantial reduction of transcription initiation. We hypothesise that both atypical sequence composition, and epigenetic base modifications may affect the mechanical properties of DNA in CpG islands, influencing the ability of proteins to bind and initiate transcription. In this work, we model two scalar measures of the sequence-dependent propensity of DNA to wrap into nucleosomes: the energy of DNA required to assume a particular nucleosomal configuration and a measure related to the probability of linear DNA spontaneously reaching the nucleosomal configuration. We find that CpG density and modification state can alter DNA mechanics by creating states more or less compatible with nucleosome formation.

Introduction

CpG islands (CGIs) are regions in vertebrate genomes with a higher frequency of CpG dinucleotide steps [Bird et al. \(1985\)](#) ; [Gardiner-Garden and Frommer \(1987\)](#)  than surrounding DNA. This is a reflection of the general depletion of CpGs outside CGIs, where CpGs are observed at around one fifth of the randomly expected frequency [International Human Genome Sequencing Consortium \(2001\)](#) . Most vertebrate, including human, genes often have associated CGIs [Cooper et al.](#)

(1983) [Larsen et al. \(1992\)](#) typically coinciding with sites of transcription initiation and likely contributing to the regulation of gene activity [Deaton and Bird \(2011\)](#). One way CGIs function is by attracting chromatin proteins with the CxxC domain, which recognise epigenetically unmodified CpGs and are instrumental for the establishment of characteristic chromatin modification profiles at CGIs [Long et al. \(2013a\)](#).

The general consensus is that the majority of CGIs are epigenetically unmodified, whereas in the regions outside CGIs most cytosines in the CpG dinucleotides are methylated [Ioshikhes and Zhang \(2000\)](#); [Hannenhalli and Levy \(2001\)](#); [Bock et al. \(2007\)](#); [Han and Zhao \(2008\)](#). Recently, [Long et al. \(2013b\)](#) have experimentally identified regions with non-methylated DNA in seven diverse vertebrates. They called those regions non-methylated islands (NMIs). [Long et al. \(2013b\)](#) demonstrated that in some instances NMIs do not coincide with computationally classified CGIs (**Table 1**). Furthermore, they showed that NMIs, and not CGIs, are central to the definition of gene promoters in the vertebrates that they studied.

For understanding how CGIs and NMIs impact the local chromatin structure and contribute to gene regulation, it is important to know how DNA mechanics is influenced by its sequence and epigenetic modifications. (In this work we are solely concerned with double-stranded or dsDNA, which we therefore just hereafter refer to as DNA.) One of the widely studied properties of DNA are the sequence-dependent effects on nucleosome positioning. A nucleosome comprises 147 base pairs of DNA wrapped around the histone core, and is the elementary unit of DNA packing into chromatin. The positions and dynamics of nucleosomes contribute to DNA transcription, replication, and repair [Andrews and Luger \(2011\)](#); [Yasuda et al. \(2005\)](#); [Chen et al. \(2010\)](#). Various computational models have been developed for predicting nucleosome positioning based on DNA sequence [Ioshikhes et al. \(2006\)](#); [Segal et al. \(2006\)](#); [Gupta et al. \(2008\)](#); [Struhl and Segal \(2013\)](#), physical properties [Gabdank et al. \(2009, 2010\)](#) and deformation free energy [Ruscio and Onufriev \(2006\)](#); [Battistini et al. \(2010\)](#); [Chen et al. \(2016\)](#); [Eslami-Mossallam et al. \(2016\)](#); [Liu et al. \(2018\)](#). It has been shown that methylation and hydroxymethylation change DNA mechanical properties [Pérez et al. \(2012\)](#); [Battistini et al. \(2021\)](#) and nucleosome forming affinity [Buitrago et al. \(2021\)](#); [Choy et al. \(2010\)](#); [Lee and Lee \(2012\)](#); [Lee et al. \(2015\)](#); [Jimenez-Useche and Yuan \(2012\)](#); [Li et al. \(2022\)](#). For example, [Ngo et al. \(2016\)](#) demonstrated that methylation of DNA decreases the mechanical stability of a nucleosome, as measured by a fluorescence-force spectroscopy assay. Whereas, multiple studies reveal that DNA methylation induces a more compact and rigid nucleosome structure [Choy et al. \(2010\)](#); [Lee and Lee \(2012\)](#); [Lee et al. \(2015\)](#). Another computational study by [Yoo et al. \(2021\)](#) showed that DNA methylation of CpG sites can significantly increase the bending energy.

In this work, we compute the free energy, required for DNA to reach a configuration in a nucleosome, as well as the probability density, associated with the optimal nucleosomal configuration of DNA, for ensembles of sequence fragments drawn from different regions across the human genome, and compare with analogous computations on sequence ensembles generated artificially. To model sequence-dependent DNA mechanics we use the *cgNA+* model (<https://cgdnaweb.epfl.ch>) [Sharma et al. \(2023\)](#); [Bruin and Maddocks \(2018\)](#).

In previous work we presented a method for predicting a sequence-dependent configuration and associated free energy of DNA wrapped on a nucleosome [Giniūnaitė and Petkevičiūtė-Gerlach \(2022\)](#). The method is based on minimisation of the *cgNA+* model free energy for a given sequence while constraining the positions of phosphates bound to the histone core. The indices and allowed positions of bound phosphates were identified from the cylindrical coordinates of 30 experimental PDB structures of nucleosomes.

	NMI	Not NMI	Total
CGI	20257	7413	27670
Not CGI	16855	42349	59204
Total	37112	49762	86874

Table 1.

Total number of considered sequences from different regions of the human genome.

Sequence listings available at <https://github.com/rginiunaite/CGI-NMI-sequences> [↗](#).

In this article we use an improved version of this method to explore the differences in nucleosome wrapping energies and the probability densities for nucleosomal configurations between sequences drawn from inside and outside both CGIs and NMIs. We first show that the nucleosome wrapping energy increases with increasing concentration of CpG dinucleotide steps only when the cytosines in those steps is methylated or hydroxymethylated. Then we investigate intersections and disjunctions of CGI and NMI regions and demonstrate that the intersection of these two sequence ensembles ensures the lowest probability densities of nucleosomal configurations. We also show that the probability densities of nucleosomal configurations decrease with increasing CpG numbers. Finally we investigate the relation between wrapping energies and experimentally observed nucleosome occupancy scores [Schwartz et al. \(2019\)](#); [Yazdi et al. \(2015\)](#).

Methods

The cgNA+ model

cgNA+ is a coarse-grain model of double-stranded nucleic acids (dsNA). A linear dsNA is modeled as a system of rigid bases and phosphates and its configuration is described by a coordinate vector $w \in \mathbb{R}^N$. Given an arbitrary n base pair sequence $\mathcal{S}$ and a model parameter set $\mathcal{P}$, *cgNA+* constructs the expected, or ground, or minimum energy configuration $\mu(\mathcal{S}, \mathcal{P}) \in \mathbb{R}^N$ and the (banded) stiffness, or inverse covariance, matrix $K(\mathcal{S}, \mathcal{P}) \in \mathbb{R}^{N \times N}$ with $N = 24n - 18$, scaled such that

$$U(w; \mathcal{S}, \mathcal{P}) := \frac{1}{2} (w - \mu) \cdot K (w - \mu) \quad (1)$$

is the energy (or the free energy difference between the configurations w and μ) expressed in units of kT. Then

$$\rho(w; \mathcal{S}, \mathcal{P}) := \frac{1}{Z} \exp \{-U(w; \mathcal{S}, \mathcal{P})\} \quad (2)$$

is an equilibrium distribution on coordinates w in the Gaussian, or multidimensional normal, form. Here Z is the normalising constant, or partition function,

$$Z = (2\pi)^{\frac{N}{2}} \det(K)^{-\frac{1}{2}}. \quad (3)$$

In this presentation we restrict the parameter set $\mathcal{P}$ to cases describing DNA with arbitrary sequences in the alphabet $\{A, T, C, G, \text{MpN}, \text{HpK}\}$, where MpN and HpK are CpG dinucleotide steps in which the cytosines are either both methylated or both hydroxymethylated, respectively.

The *cgNA+* model is an extension in two directions of the precursor *cgDNA* model [Gonzalez et al. \(2013\)](#); [Petkevičiūtė et al. \(2014\)](#) in which the configuration coordinate w was restricted to rescaled versions of the standard intra and inter base-pair Curves+ [Lavery et al. \(2009\)](#) coordinates which determine the relative rigid body displacements of all the bases in a DNA (and which respect the Tsukuba convention [Olson et al. \(2001\)](#)). For our purposes, the first critical extension of *cgDNA* was to *cgDNA+* [Patelli \(2019\)](#) in which the coordinate vector w was extended to explicitly include the relative rigid body displacements between bases and adjacent phosphate groups, also assumed to be rigid, but only with a parameter set $\mathcal{P}$ allowing sequences $\mathcal{S}$ in the standard $\{A, T, C, G\}$ alphabet. The second crucial extension from *cgDNA+* to *cgNA+* [Sharma et al. \(2023\)](#); [Sharma \(2023\)](#) was to estimate, and test, parameter sets for other dsNAs and with extended alphabets including epigenetically modified bases. In this presentation we consider only the case of DNA but with a parameter set that distinguishes between unmodified CpG

dinucleotide steps, methylated CpG dinucleotide steps (symmetrically so that both cytosines are modified, denoted MpN), and hydroxymethylated CpG dinucleotide steps (again symmetrically and denoted HpK).

cgNA+ parameter sets are estimated by fitting model predictions for first and second moments (or respectively $\mu(\mathcal{P}, \mathcal{A})$ and $K^{-1}(\mathcal{P}, \mathcal{A})$) for a training library of sequences $\mathcal{S}_i$ to statistics drawn directly from large scale, fully atomistic molecular dynamics (or MD) simulations. The MD simulation protocol reflects both assumed physical solvent conditions, such as counter ion species and concentration, and the choice of atomistic MD simulation potentials. The parameter set $\mathcal{P}$ adopted here was based on simulations with 150mM KCl ions and the AMBER software Pearlman et al. (1995) [Case et al. \(2005\)](#) [Ivani et al. \(2013\)](#) [explicit TIP3P water Jorgensen et al. \(1983\)](#) [and the Joung and Cheatham ion model Joung and Cheatham III \(2008\)](#) [The additional MD force field parameters for modified cytosines were taken from Pérez et al. Pérez et al. \(2012\)](#) [and Battistini et al. Battistini et al. \(2021\)](#) [MD simulations of twelve 24 base-pair length sequences were used for training model parameters for methylated DNA. These sequences contained methylated CpG steps and combinations of methylated CpG steps in diverse sequence contexts. An analogous training library was used to train hydroxymethylated DNA parameters. The model parameters for unmodified DNA were separately trained on diverse and comprehensive library of 16 sequences containing all possible tetranucleotides at least once.](#)

The predictions of the *cgNA+* model were found to be extremely accurate compared to an extensive set of test MD simulations and in good agreement with limited experimental protein-DNA X-ray crystallography data [Sharma \(2023\)](#) [Above all, the *cgNA+* model is computationally so efficient that predictions of statistics for hundreds of thousands of sequences can be easily handled, which is not feasible with direct MD simulation. Thus, we used *cgNA+* free energy for linear fragments as the starting point for developing a method for computing sequence-dependent nucleosome wrapping energies.](#)

Nucleosome wrapping energy for a DNA sequence

A sequence-dependent configuration w_{opt} of 147 bp of DNA wrapped into a nucleosome is modelled by minimising the *cgNA+* energy $U(w; \mathcal{S}, \mathcal{A})$ (1):

$$w_{\text{opt}}(\mathcal{S}, \mathcal{P}) = \arg \min_w \left(U(w; \mathcal{S}, \mathcal{P}) + \sum_{i=1}^{28} C_i(w) \right) \quad (4)$$

where

$$C_i(w) = c_i ||(p_i(w) - \bar{p}_i)||^2, \quad i = 1, \dots, 28, \quad (5)$$

is a set of elastic constraints on the positions $p_i(w)$ of the 28 DNA phosphates, that are closest to the histone core. $\mathcal{S}$ is any given DNA sequence of length 147 bp and $\mathcal{P}$ is our *cgNA+* model parameter set. The reference positions $\bar{p}_i$ were obtained from a set of 100 experimental PDB structures of nucleosomes by averaging, and the indices of the 28 phosphates, closest to the nucleosome core, are identified as in our previous work [Giniūnaitė and Petkevičiūtė-Gerlach \(2022\)](#) [The penalty coefficients \$c_i\$ are set through numerical experiments to keep the distances \$||\(p_i\(w_{\text{opt}}\) - \bar{p}_i\)||\$ within the ranges observed in the PDB structures, while avoiding steric clashes between the two turns of DNA in a nucleosome.](#)

The energy minimisation (4) is performed numerically using the *fminunc* function of Matlab with provided gradient and Hessian values. An averaged configuration of 100 experimental structures of DNA in nucleosomes is used as the initial or starting configuration for the optimisation procedure for all sequences. The optimisation for a 147 bp DNA sequence takes approximately 30 seconds.

The energy minimisation algorithm used in this work improves its previous version [Giniūnaitė and Petkevičiūtė-Gerlach \(2022\)](#) [↗](#) which was sensitive to the starting configuration and reached to minimum energies with inflated magnitude while keeping the trends similar to experimental observations. This development improves those shortcomings by incorporating two main changes. Firstly, the constraints (5) are elastic, in contrast to previously used hard intervals. In addition, rather than performing the optimisation (4) in the *cgNA+* coordinates and computing the absolute positions of the constrained phosphates after every minimisation step, we use a mixed coordinate vector, with absolute positions of constrained phosphates, absolute positions and orientations of their adjacent bases and all the base pairs, while the rest of the configuration is described in the *cgNA+* coordinates. Although the conversion to the full *cgNA+* coordinates for evaluating the energy is still necessary after every optimisation step, this approach provides the possibility to derive the gradient vector and the Hessian matrix for the constrained optimisation problem (4), which significantly improves the performance of the algorithm. As a consequence of these modifications, nucleosome wrapping energies are similar in magnitude as well as in trends to those observed in experiments (as discussed in results).

In this work we compare sequence-dependent energy values $U(w_{\text{opt}}; \mathcal{S}, \mathcal{A})$ (1) with units kT as well as the natural logarithms of the optimal nucleosomal configuration probability density (2)

$$\ln \rho(w_{\text{opt}}; \mathcal{S}, \mathcal{P}) = -U(w_{\text{opt}}; \mathcal{S}, \mathcal{P}) + \frac{1}{2} \ln \det(K) - \frac{N}{2} \ln(2\pi) \quad (6)$$

for different sequences $\mathcal{S}$. The probability densities can be regarded as proportional to probabilities of DNA spontaneously reaching the configuration w_{opt} , when these probabilities are estimated in a small domain around w_{opt} , with the same domain volume for all the sequences. The negative log probability density, $-\ln \rho(w_{\text{opt}}; \mathcal{S}, \mathcal{A})$, is equivalent to the free energy associated with the configuration w_{opt} . It is also worth noting that

$$\ln \rho(w_{\text{opt}}; \mathcal{S}, \mathcal{P}) = -U(w_{\text{opt}}; \mathcal{S}, \mathcal{P}) - H(\mathcal{S}, \mathcal{P}) + \frac{N}{2}, \quad (7)$$

where $H(\mathcal{S}, \mathcal{A})$ is the entropy.

A more detailed mathematical description of the computational method will be published separately, and the Matlab code is available at https://github.com/daivaaviad/optDNA_nucleosome [↗](#).

Experimental data

Computationally predicted CGI regions from the human genome are obtained from the UCSC genome browser [Kent et al. \(2002\)](#) [↗](#), whereas experimentally identified NMIs for human liver cells are taken from [Long et al. \(2013b\)](#) [↗](#). The human genome version used in these studies is Genome Reference Consortium Human Build 37 (GRCh37). Note that to make the necessary computations feasible, for each specific sequence in an ensemble such as CGIs, NMIs or their intersections or complements, we only consider one specific central 147 bp sequence per region. The exact sequences used in our analysis are available at <https://github.com/rginiunaite/CGI-NMI-sequences> [↗](#). Data for nucleosome occupancy scores for HeLa cells was taken from [Schwartz et al. \(2019\)](#) [↗](#) and for human genome embryonic stem cells from [Yazdi et al. \(2015\)](#) [↗](#).

Results

The spread of predicted DNA nucleosomal configurations is similar to that of experimental structures

We first compare our predicted sequence-dependent optimal DNA nucleosomal configurations (4) for 100 human genome sequences with 100 experimental configurations from the Protein Data Bank [Berman et al. \(2000\)](#). The human genome sequences are a random subset of our sequence sample for the CGI and NMI intersection in the Chromosome 1, but the following observations remain unchanged for sequence samples from different genomic regions.

In [Figure 1a](#) we observe an orderly positioning of phosphates in the aligned experimental structures. Note that, because the structures are aligned, the phosphates adjacent to the nucleosome dyad fall into the same spatial cluster despite the variation in sequence length across the PDB structures. For each helical turn, we choose one phosphate cluster that is closest to the nucleosome center (points coloured in red) and use the index of that cluster to define the constraints in (5). [Figure 1c](#) shows the analogous scatter plot for the configurations $w_{\text{opt}}(\mathcal{P}, \mathcal{A})$ (4) predicted for the first 100 non-methylated CGI sequences in our human genome sample. The positioning of phosphates in [Figure 1c](#) is rather similar to the one in [Figure 1a](#), and the clusters of phosphates are of comparable sizes in both plots, even though there seems to be more variation in the experimental structures. This difference can be explained by the diversity of experimental settings, such as differences in ion concentration, the presence of histone modifications, additional ligands and other experimental conditions, that are not captured in our model. The variation in predicted structures could be increased by reducing the penalty coefficients in (5). However, this would require additional constraints in (4) to avoid the self-overlap of DNA (steric clashes between the two DNA turns in the nucleosome), which is not present with the current setting. Another difference between the two plots is the unwrapping of approximately five base-pairs at each end of the predicted configurations. While in our model there are no restrictions for this behaviour, in the experimental setting there could be other factors, such as histone tails, keeping the DNA ends closer to the nucleosome core. This issue could be solved by adding additional constraints at the ends of the 147 bp sequence. Such a modification would increase the nucleosome wrapping energy only marginally, as it would affect about 10 of the 147 base pairs. Two side views of the experimental and predicted nucleosome structures are displayed in [Supplementary Figure S8](#).

The spread of phosphate positions in each cluster is quantified by the standard deviations of phosphate distances to the nucleosome central axis, plotted in [Figure 1b](#) and [Figure 1d](#). As already seen in the scatter plots on the left, the spread of the predicted configurations is smaller and more regular than that of the experimental structures. The main conclusion here is that the standard deviation reaches its local minima for the phosphate clusters closest to the nucleosome core (indices marked by solid red vertical lines, corresponding to the red points on the left plots). Interestingly, the local minima of standard deviations is also reached for positions corresponding to the histone touching phosphates on the complementary (Watson) strand (marked by dashed black vertical lines). This observation holds for both experimental and predicted nucleosomal configurations and indicates that the phosphates chosen to be constrained in our optimisation method are also constrained (bound to the histone core) in the experimental nucleosomes.

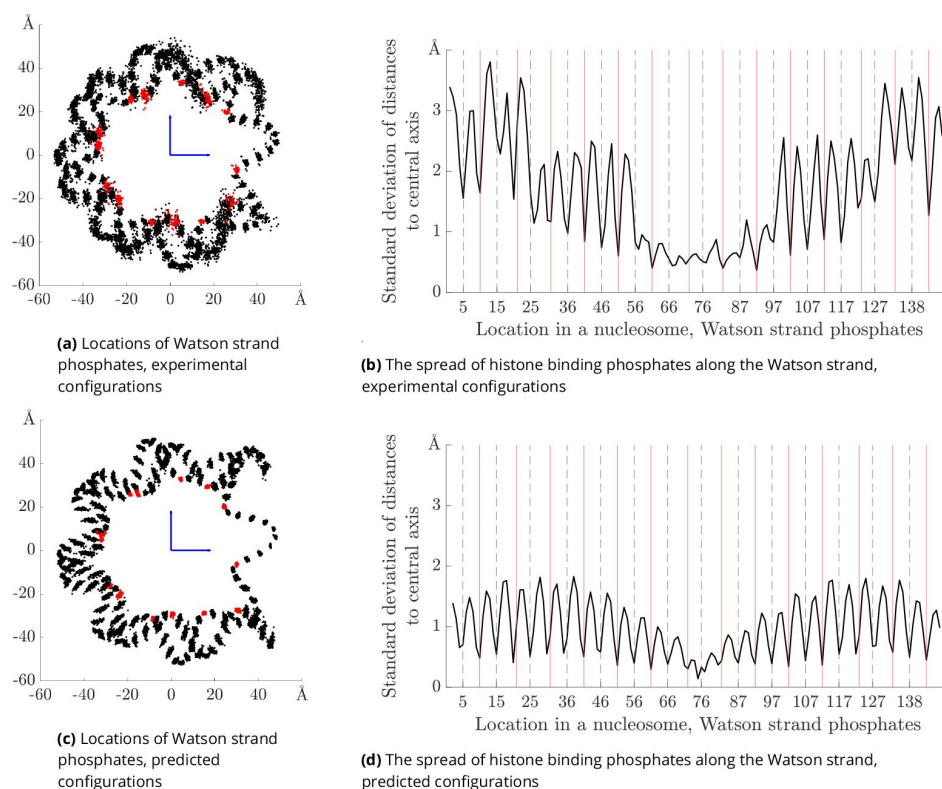


Figure 1.

Left column: locations of the Watson strand phosphates for 100 aligned nucleosome structures, projected to a plane perpendicular to the nucleosome central axis. Top row corresponds to 100 experimental PDB nucleosome structures (not all with independent sequences). Red points are phosphates with local minima of radial distance used to identify bound indices. Bottom row analogous data over 100 predicted minimal energy nucleosomal configurations for sequences drawn from human genome CpG islands. The phosphates with bound indices that are constrained during the optimisation are coloured in red. Right panels: standard deviations over sequence of radial distance of all phosphates against index along the Watson strand. Top PDB structures, bottom model computations. Bound indices are marked with solid red vertical lines. Dashed black vertical lines mark indices of bound complementary (Crick) strand phosphates.

CpG step (hydroxy)methylation affects DNA nucleosome wrapping energy and the probability density of nucleosomal configuration

To assess the sequence dependence of the nucleosome wrapping energy and of the probability density of the optimal nucleosomal configuration, we initially perform a computational experiment in which we generate four sets of sequences of length 147 bp, each containing a thousand sequences with a varying number of CpG dinucleotide steps, ranging from 0 to 4, from 5 to 14, from 15 to 24 and from 25 to 34. Each sequence is first generated with equal probabilities for each base, and then if the desired density of CpG steps needs to be increased, dinucleotide steps in random positions are replaced by CpGs. Similarly, if the density needs to be decreased, a base in a CpG dinucleotide is replaced by another, all in a randomised way. From these sequence ensembles, we also create another eight sets of sequences, first by symmetrically methylating (MpN), and second by hydroxymethylating (HpK), both cytosines in all the instances of the CpG dinucleotides.

We then use our optimisation algorithm to compute the energies required for these sequences to wrap onto nucleosomes. The resulting energy values are shown in **Figure 2**. The average of the predicted nucleosome wrapping energy over all the 4K unmodified random sequences is 86.12 kT. As expected, this value is higher than the energy prediction for the synthetic nucleosome positioning sequence Widom 601 [Lowary and Widom \(1998\)](#) (76.23 kT) and the naturally occurring sequence 5S, known to have a high nucleosome forming affinity [Simpson and Stafford \(1983\)](#) (83.76 kT). An opposite extreme, the 147 bp poly-A sequence, has a high predicted wrapping energy of 95.08 kT. Above examples illustrate that the modeling matches expectations for some known DNA sequences. When we vary unmodified CpG density, only minor differences of wrapping energy are observed (**Figure 2a**). However, the average energy increases substantially when cytosines are methylated or hydroxymethylated to obtain MpN or HpK steps. These results can be well-associated with the findings that suggest that methylation increases DNA stiffness [Lee and Lee \(2012\)](#); [Pérez et al. \(2012\)](#); [Ngo et al. \(2016\)](#). The effects of hydroxymethylation and methylation are quite similar.

The changes in nucleosome wrapping energy due to CpG methylation or hydroxymethylation can be explained not only by altered DNA stiffness, but also by modifications in its equilibrium configuration. For example, the roll, twist and slide inter base-pair coordinates are strongly affected when DNA wraps onto a nucleosome [Giniūnaitė and Petkevičiūtė-Gerlach \(2022\)](#) and they are all substantially modified in the linear ground state when cytosines are methylated or hydroxymethylated (**Figure 3a**). The linear ground state coordinates of the phosphates also change both when wrapping onto a nucleosome and with cytosine modification (**Figure 3b**), but this change is more dependent on the sequence context [Sharma \(2023\)](#). The same observation holds for intra base-pair coordinates (**Supplementary Figure S7**). The ground state changes resulting from cytosine modifications – primarily characterized by an average increase in roll and a decrease in twist – may be linked to steric hindrance caused by the cytosine 5-substituent [Battistini et al. \(2021\)](#). Notably, the negative coupling between twist and roll has already been observed in X-ray crystallography data [Olson et al. \(1998\)](#).

We then compare the values of the logarithm of the probability density of the optimal nucleosomal configurations (6). The probability density is proportional to probability of DNA spontaneously acquiring its optimal nucleosomal configuration, estimated in a small domain around that configuration. It can also be regarded as a measure of DNA mechanical affinity to form nucleosomes, which includes the (negative) nucleosome wrapping energy and also approximates entropic effects or thermal fluctuations.

For our set of random sequences, the log probability density decreases with the growing number of unmodified CpG steps (**Figure 2c**). Cytosine methylation weakens the trend while also increasing the average log densities within each range of CpG count. In contrast, cytosine

Figure 2.

Spectra of nucleosome wrapping energies and logarithms of probability densities for the optimal nucleosomal configurations for 147 bp sequences (a,c) generated randomly and (b,d) drawn from the human genome, grouped by the indicated ranges of numbers of CpG dinucleotide steps: dots averages, bars standard deviation in sequence.

For methylated and hydroxymethylated data all CpG steps are symmetrically modified.

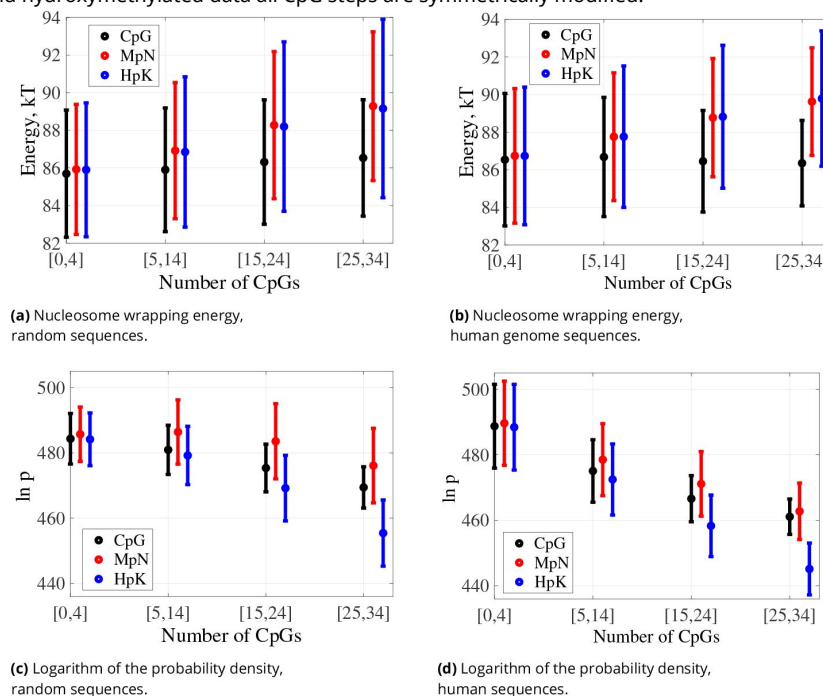
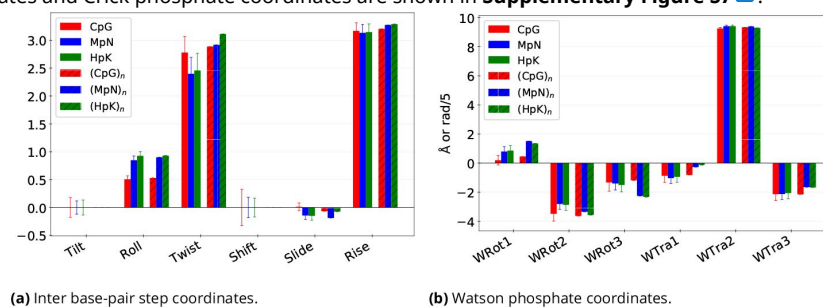


Figure 3.

Effects of sequence context and epigenetic base modifications on the cgNA+ model predicted ground state shape of CpG steps.

Statistics over $4^8 = 65,536$ sequences of 22 bps length, constructed around the central CpG step as GCGTCG $X_4X_3X_2X_1$ C G Y $_1Y_2Y_3Y_4$ GTCGGC, with all the possible X_j and $Y_j \in \{A, T, C, G\}$, $\forall j \in \{1, 2, 3, 4\}$. Bar plots show the ground state values of (a) six inter base-pair step and (b) six Watson phosphate coordinates for CpG steps i) averaged over sequence context with standard deviations in thin lines and ii) the extreme case of poly(CpG) (in hatch). In each case three versions corresponding to unmodified, methylated and hydroxymethylated steps. The standard deviations highlight the crucial role of non-local sequence dependence in the equilibrium structure of CpG/MpN/HpK steps. Analogous plots for the remaining intra base-pair coordinates and Crick phosphate coordinates are shown in **Supplementary Figure S7**.



hydroxymethylation leads to a faster decrease in log densities with the growing CpG count.

To verify whether our observations for randomly generated sequences also hold for biologically more realistic sequences, we perform the same analysis for sequences obtained from the human genome. We consider four sub-ensembles of our human sequence fragments grouped by their numbers of CpG dinucleotides falling in the intervals that correspond to constrained numbers of CpG steps in our randomized sequence ensembles. **Figure 2b** demonstrates that for the human sequence ensembles, just as for the random sequence ensembles (**Figure 2a**), the nucleosome wrapping energy is not strongly affected with the number of unmodified CpG dinucleotide steps. Cytosine (hydroxy)methylation also increases the nucleosome wrapping energy for human genome sequences. However, some differences can be observed between the two ensembles. For most of the human sequence sub-ensembles, there are somewhat higher nucleosome wrapping energies and a sharper drop in log probability densities than for the comparable random ensembles. This observation remains unchanged after sub-sampling human genome sequences to have 1K data points in each CpG range, the same number as for random sequences (**Supplementary Figure S1**). We first hypothesised that different clustering features of CpG dinucleotides might explain these differences. To investigate this hypothesis, we looked at the distances along the sequences between CpG dinucleotides. But we did not observe any significant differences in the distributions of these distances between human genome and random sequence ensembles (**Supplementary Figure S2**).

These differences might instead be associated with non-random distribution of other dinucleotide steps in the two sets of sequence ensembles. **Figure 4** gives the average number of all of the 16 possible dinucleotide steps in the random and human ensembles in the case of 10 CpGs for random and [5, 14] CpGs for human ensembles (other cases are provided in **Supplementary Figures S3-S5**). The distribution can be seen to be highly non-uniform for the human genome sequences. For example, one striking feature of the [5, 14] human sequence ensemble is the small number of ApT and TpA dinucleotides. In fact, ApT and TpA are found to be the most stiff and flexible dimer steps in both experiments and simulations [Young et al. \(2022\)](#); [Sharma \(2023\)](#). It may well be that this depletion is a result of promoter sequences avoiding mechanistically extreme dimer steps.

We further tested whether the non-uniform dinucleotide counts, as opposed to the specific arrangement of dinucleotides, is the key reason for the difference in energies and nucleosomal configuration probability densities between the human and random sequences. To this end, we explored the scenario in which we keep the same count of each dinucleotide step in each sequence in the [5, 14] human genome sequence ensemble, but we reordered the dinucleotide steps using the Altschul-Erickson dinucleotide shuffle algorithm [Altschul and Erickson \(1985\)](#). We observe that in this scenario the resulting distributions of nucleosome wrapping energies and of nucleosomal configuration log probability densities remain significantly more similar to that of the unshuffled human ensemble than to the analogous random sequence ensemble (**Supplementary Figure S6**). This observation suggests that the non-uniform count of dinucleotides is central in explaining the differences in wrapping energies and log probability densities between random and human genome sequence ensembles.

In fact the ground state configuration of the DNA in each junction has a quite strong dependence on sequence context beyond the junction dinucleotide. This phenomenon has been observed in MD simulation [Pasi et al. \(2014\)](#); [Balaceanu et al. \(2019\)](#) and crystallography experiments [Young et al. \(2022\)](#). It is also encapsulated in the *cgNA+* model. It has further been observed [Sharma \(2023\)](#) that epigenetic base modifications lead to larger changes in the ground state configuration within CpG junctions when the two flanking bases in the tetramer context are C/G rich (also **Figure 3**). For instance, in an average context, hydroxy(methylation) of CpG step reduces its twist significantly. In contrast, when a poly-CpG sequence is hydroxy(methylated), the predicted twist of the CpG steps increases (**Figure 3**). Therefore, for assessing the effect of

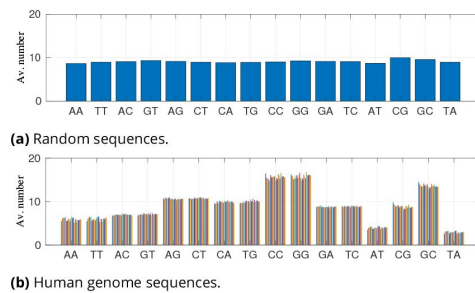


Figure 4.

Average number of instances of the 16 different dinucleotide steps for (a) 1000 random 147 bp sequences and for (b) our 147 bp human genome sequence ensemble, with [5, 14] CpGs (different colours correspond to fragments taken from different chromosomes).

Dinucleotide steps are ordered next to their complements, with self-complementary steps listed on the right.

sequence shuffling on the ground shape of DNA, it is of interest to investigate the flanking context of the CpG dinucleotides. The tetranucleotide sequence logos over all CpG steps in three of our four sub-ensembles of human sequences are in fact rich in C/Gs, as shown in the sequence logos of **Figure 5a**, where the amount of the flanking enrichment depends on the four cases of ranges of numbers of CpG dinucleotide steps. It is also the case that the constraints on the elevated number of CpG steps in the fragments are strong enough that the tetranucleotide sequence logos remain essentially unaltered in each of the four cases for the sequence ensembles that arise after the dinucleotide step sequence shuffling algorithm is applied, **Figure 5b**. Nevertheless when comparing the logos in panels a) and b) in detail, there is a signal indicating that the flanking C/G enrichment is slightly stronger in the original human sequence ensemble, than it is after shuffling.

Overlap of CpG islands and NMIs leads to the lowest probability densities of nucleosomal configurations

In this section we split the human genome into four regions based on data from [Long et al. \(2013b\)](#): A) Intersection of CpG islands (CGIs) and NMIs; B) NMIs that do not intersect with CGIs; C) CGIs that do not intersect with NMIs; D) Regions that intersect neither with CGIs nor with NMIs. The numbers of sequences in each sub-ensemble listed in **Table 1**.

The data presented in **Figure 6a** reveals that the nucleosome wrapping energies have similar distributions in all four regions, if we do not include methylation (round dots and solid error bars). If we include methylation everywhere in not NMIs (i.e. respecting the definition of NMI), there is an increase in the wrapping energy for sequences that are CGIs that are not NMIs (triangle dots and dashed error bars in **Figure 6a** red). Wrapping energies for sequences that belong neither to CGIs nor to NMIs, do not exhibit such a significant change upon methylation (green).

The log probability density of the optimal nucleosomal configuration has the lowest average value for sequences in the intersection of CGIs and NMIs. Even though methylation of the sequences that are CGIs but not NMIs increases the log probability density values, the highest densities are for sequences that are not CGIs but are NMIs (blue) or in the regions outside CGIs and NMIs (green).

It is important to note that the number of sequences drawn from the four different regions is not equidistributed. **Table 1** shows that there are fewer sequences that are CGIs but not NMIs, i.e. they are methylated CGIs, than in the other three categories. Nevertheless, approximately 30% of CGIs are methylated, so it is reasonable to consider methylated CGIs as a separate category. Note that for practical restrictions on total computational resources we compute wrapping energies for only one 147 bp representative sequence drawn from each occurrence of each of the four types of regions over the entire genome. **Table 1** reports the resulting numbers of fragments, i.e. the number of instances of each of the four types of regions. But the numbers in **Table 1** do not reflect the total number of bp covered by each of the four types of region. In reality the number of base pairs in each occurrence of the regions that are neither CGI nor NMI is much higher than in the other three types, so that the union of all not CGI and not NMI regions covers by far most of the genome.

Nucleosome wrapping energies and probability densities of the optimal nucleosomal configurations compared with nucleosome occupancy scores

We now compare our wrapping energy predictions and DNA nucleosomal configuration log probability density predictions with experimentally measured nucleosome occupancy scores as reported in [Schwartz et al. \(2019\)](#) (an experiment with HeLa cells) human genome and [Yazdi et al. \(2015\)](#) (an experiment with embryonic stem cells). We have extracted their reported

Figure 5.

Sequence logos for tetramer flanking context of CpG dinucleotide steps for (a) all four sequence ensembles from the human genome with varying numbers of CpG junctions, and (b) all four sequence ensembles from the human genome after dinucleotide shuffling (but respecting the numbers of dinucleotide steps).

Just specifying the numbers of CpG dinucleotide steps is a strong enough constraint to leave the tetramer sequence context logos largely unchanged after shuffling. The sequence logos in panel a) for the human sequence ensemble before sequence shuffling, suggest a slightly stronger C/G flanking enrichment than after shuffling.

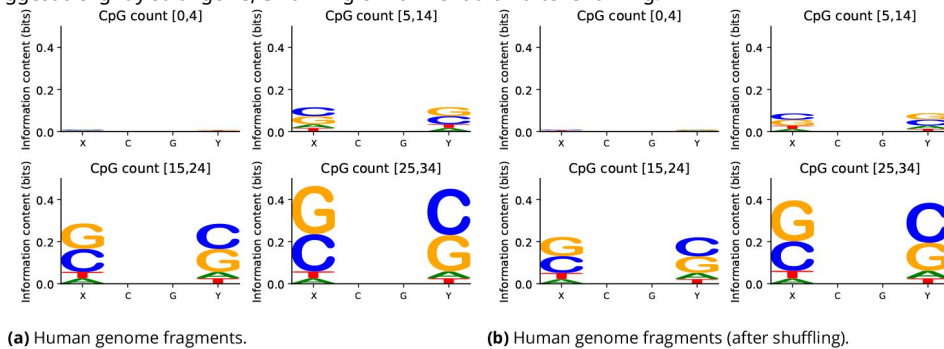
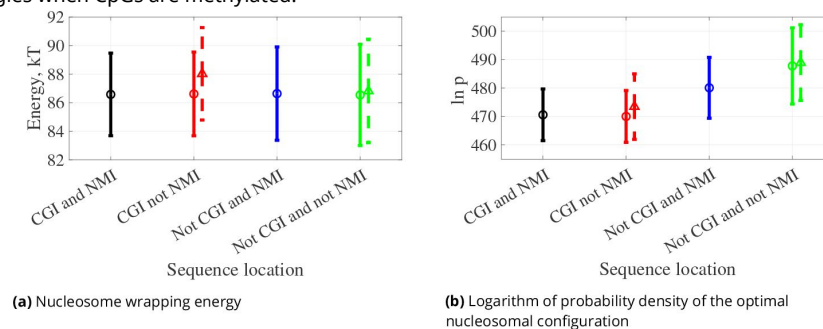


Figure 6.

Spectra of (a) nucleosome wrapping energies and (b) log probability densities of the optimal nucleosomal configurations for 147 bp sequences drawn from four different regions of the human genome: (A) intersection of CGI and NMI, (B) NMI and not CGI, (C) CGI and not NMI, (D) not CGI and not NMI (Table 1) [\[1\]](#).

Dots averages, error bars standard deviation over sequence, solid and circles when CpG dinucleotides are not methylated, dashed and triangles when CpGs are methylated.



occupancy scores for each of our selected 147 bp fragments and first grouped the data by the methylated and nonmethylated regions (NMIs and not NMIs), then within each region according to the number of CpGs in the corresponding sequences.

Figure 7 [↗](#) shows that nucleosome occupancy is decreasing with increasing CpG count for both NMI and not NMI regions, with one exception of passing from [0;4] to [5;14] in the Yazdi et al. (data. This trend is compatible with the increase of nucleosome wrapping energy for methylated sequences in **Figure 2b** [↗](#) and the decrease of log probability density for nucleosomal configurations in **Figure 2d** [↗](#).

According to Yazdi et al. (data, for the CpG count falling into the middle intervals, from 5 to 14 and from 15 to 24, methylated sequences have a higher average occupancy than unmethylated sequences. This difference is also observed in our log probability density predictions in **Figure 2d** [↗](#). For the remaining two CpG count intervals and all the Schwartz et al. (data, the occupancy for methylated sequences is lower than or very similar to unmethylated ones.

We then grouped our sequences according to the four genomic sub-regions. **Figure 8a** [↗](#) reveals that for both sets of data the average of nucleosome occupancy scores is lowest for the intersection of CGIs and NMIs (black). For the data extracted from Schwartz et al. (2019) [↗](#), methylated CGIs (red) have a higher average nucleosome occupancy than unmethylated CGIs, but smaller than the non-CGI regions. For Yazdi et al. (2015) [↗](#) data, the distribution of nucleosome occupancy scores is highest for the intersection of CGIs and not NMIs (red), i.e. both the lowest and highest occupancy distributions arise for sequences drawn from CpG islands, with the lowest occupancies in unmethylated fragments and the highest in methylated fragments.

Boths sets of experimental data indicate that in CGIs the highest occupancies arise for the fragments that have methylated CpG dinucleotides and therefore higher nucleosome wrapping energies. This conclusion, in particular, apparently runs counter to the (perhaps naive) intuition that high nucleosome forming affinity should arise for fragments with low wrapping energy. Instead, a higher log probability density seems to be a better indicator of higher occupancy scores: the lowest average of log probability densities corresponds to the unmethylated CGIs (**Figure 6** [↗](#) panel (b)).

In order to further probe this observation we selected a 50K run of bp in the human genome. (Specifically from chromosome I, between genomic positions 850K and 900K, as this range contains the largest number of CGI and NMI intersections.) We then computed the probability density of an optimal nucleosomal configuration for every possible 147 bp window at the resolution of 1 bp shifts. (These computations are quite intensive, requiring around 900 hours of CPU time for the relatively short 50K bp segment, which is why longer subsequences were not considered.) The resulting data is plotted in **Figure 9** [↗](#) panel (a), with the CGIs indicated with magenta underlining and NMIs in cyan. On average, the lowest log probability densities arise at the intersections of CGIs and NMIs: the mean value of log probability density is 468.61 kT over the intersection of CGI and NMI regions, and 476.10 kT in the complementary regions.

Panels (b) and (c) of **Figure 9** [↗](#) provide analogous plots for occupancy scores, again taken from Schwartz et al. (2019) [↗](#) and Yazdi et al. (2015) [↗](#) respectively. Again the lowest average values arise for sequences in the intersection of CGIs and NMIs: the average scores are 2.62 and 139.53 in the intersection of CGIs and NMIs, versus 5.89 and 212.53 outside of the intersection regions.

The observations about nucleosome occupancy should be regarded as preliminary, and be treated with caution, as they are based on experimental data obtained for the cancerous HeLa cells Schwartz et al. (2019) [↗](#) and human genome embryonic stem cells Yazdi et al. (2015) [↗](#), while for the classification of NMI and not NMI we use the data of Long et al. (2013b) [↗](#) obtained from human liver cells. Although comparing data from different cell lines is not optimal, to the best of

Figure 7.

Spectra of nucleosome occupancy scores for our 86,874 selected sequences, grouped by the genomic regions (NMI and *not* NMIs) and by indicated ranges of numbers of CpG dinucleotide steps: dots averages, error bars standard deviation in sequence.

The number of sequences in each group is listed in [Table 2](#). See also [Figure 2d](#).

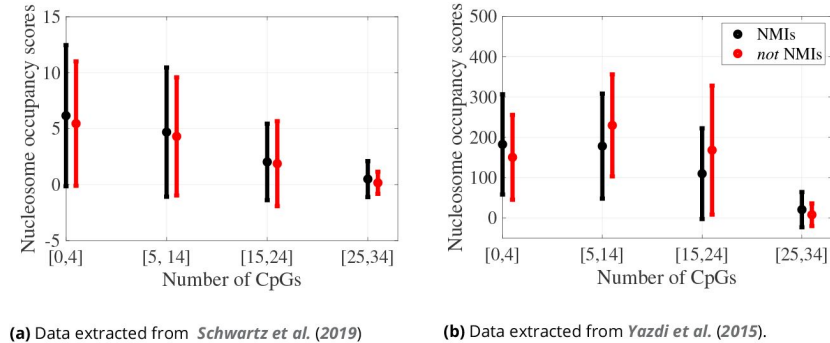


Table 2.

Numbers of human genome sequence fragments of length 147 bp taken from CGIs, non CGIs, NMIs and non NMIs grouped by the number of CpG dinucleotide steps in each of four intervals.

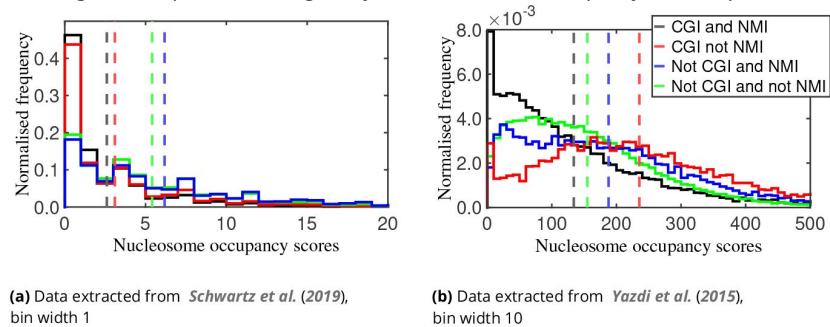
As expected CGI fragments have relatively more CpG junctions than non CGI fragments. NMIs also have more CpGs than non NMIs.

CpG count	[0, 4]	[5, 14]	[15, 24]	[25, 34]
CGI	216	16392	10248	814
Not CGI	43512	15272	340	76
NMI	7624	19383	9308	796
Not NMI	36104	12281	1280	94

Figure 8.

Normalised frequencies (each of the four histograms in each plot normalised independently) of experimental nucleosome occupancy scores for our 86,874 selected sequences grouped by each of the four types of regions in the genome ([Table 1](#)).

Average score for each region is indicated by a vertical dashed line of appropriate colour. The black and red (but not blue or green) histograms have significant spikes reflecting many instances of zero occupancy in the experimental data.



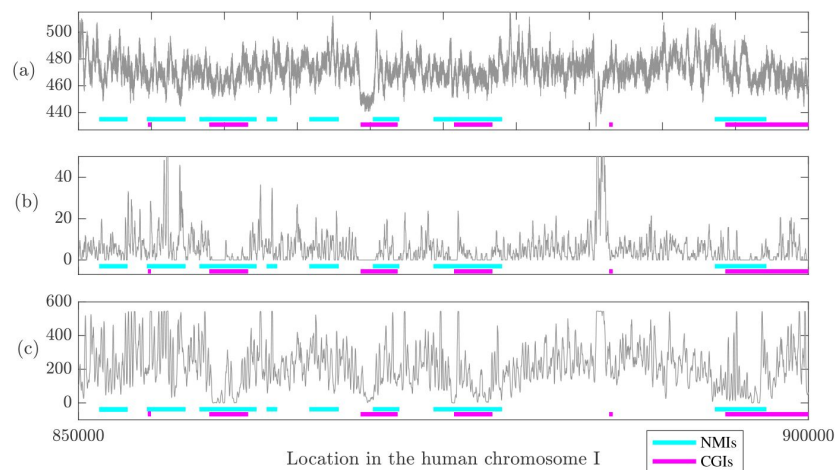


Figure 9.

(a) Predicted log probability density for an optimal nucleosomal configuration (b) nucleosome occupancy scores from Schwartz et al. (2019) and (c) nucleosome occupancy scores from Yazdi et al. (2015) for sequence positions 850K-900K of human chromosome I. In the regions corresponding to the intersection of CGIs and NMIs, both the mean log probability density (468.61) and mean scores (2.62 and 139.53) are smaller than outside of the intersection regions (476.10, 5.89 and 212.00 respectively).

our knowledge, no publicly available methylation and nucleosome occupancy data exist for the entire human genome within the same cell type. Nevertheless, since the lowest log probability densities in the human genome are predicted for CpG-rich sequences regardless of their methylation state (**Figure 2d**) [↗](#), and the same holds for both sets of the nucleosome occupancy scores (**Figure 7**) [↗](#), we conclude that the lowest occupancies occur for sequences with the lowest log probability densities.

Conclusions and Discussion

Conclusions

In this work, we studied the computed sequence-dependent mechanical nucleosome wrapping energy, required to deform a linear 147 bp DNA fragment to a configuration, where the appropriate 28 phosphates can bind to the histone core, as well as the probability density function, that can be regarded as proportional to the probability of linear DNA spontaneously reaching the nucleosomal configuration.

We explored sequence dependence of the energy and the probability density corresponding to our predicted optimal nucleosomal DNA configurations. Our analysis includes the effects of both methylation and hydroxymethylation epigenetic modifications of CpG dinucleotides. To achieve this, we used the newly developed computational method to solve the constrained minimisation problem (4) in terms of the *cgNA+* energy (1) subject to constraints on the phosphates binding to histones in given ranges of configurations. The fact that the *cgNA+* model includes an explicit description of the phosphate group configurations allows for a comparatively simple description of the DNA-histone binding site constraints, which we believe to be a significant improvement over prior rigid base-pair coarse grain DNA models used for nucleosome wrapping energy prediction [Eslami-Mossallam et al. \(2016\) ↗](#); [Chen et al. \(2016\) ↗](#); [Liu et al. \(2018\) ↗](#); [Neipel et al. \(2020\) ↗](#). We believe that our minimisation algorithm delivers an accurate ordering of sequence dependent wrapping energies and probability densities, given the accuracy of the *cgNA+* energy (1). The *cgNA+* probability density function (2) is itself known to deliver highly accurate sequence-dependent statistics of linear fragments compared to MD simulations carried out with the same protocol as the *cgNA+* parameter set training data. However, a MD protocol perfectly emulating experimental conditions (which are often different in different experiments) is challenging and therefore, some approximations must be made. For example, the parameter set used here models DNA in 150mM KCl solution, whereas both ion type and concentration might be different in both experiment and *in vivo*.

Nucleosome wrapping energies, the corresponding optimal configurations and their probability densities could also be computed via approaches that adopt MD simulations directly, e.g. [Ruscio and Onufriev \(2006\) ↗](#); [Ngo et al. \(2016\) ↗](#); [Battistini et al. \(2021\) ↗](#). Along with accurate treatment of sequence-dependent mechanics of DNA, the key advantage of our coarse-grained approach is that it is computationally much more efficient, so that large numbers of sequences can be considered. For example, when epigenetic sequence variants are included, the data described in this article involves approximately 400K solves of the minimisation problem (4). And analogous numbers of MD simulations are currently unfeasible.

The minimisation principle (4) delivers not only a wrapping energy and a probability density, but also the detailed configuration w_{opt} realising the minimal wrapping energy. We compared our computed optimal configurations of DNA in a nucleosome with the experimental PDB structures and found significant similarities between the two configuration ensembles. Further and more detailed analysis is both feasible and interesting. For example, the roll and slide (inter base-pair coordinates) are strongly affected when DNA wraps onto a nucleosome [Giniūnaitė and Petkevičiūtė-Gerlach \(2022\) ↗](#), and they are both substantially modified in the linear ground state

when cytosines are methylated or hydroxymethylated [Sharma \(2023\)](#). The linear ground state coordinates of the phosphates also change with cytosine modification, but this change is more dependent on the sequence context [Sharma \(2023\)](#).

We then computed spectra of wrapping energies and the nucleosomal configuration probability densities for ensembles of 147 bp fragments with differing numbers of CpG dinucleotides, with sequences both generated artificially and drawn from the human genome. We concluded that for increasing numbers of CpG steps the wrapping energies increased substantially, but only for epigenetically modified CpGs. The effects on the wrapping energies of the two epigenetic modifications of methylation and hydroxymethylation are very similar. The nucleosomal configuration probability densities decreased with increasing CpG counts both for unmodified and (hydroxy)methylated DNA. However, for each CpG count interval, methylation increased and hydroxymethylation decreased the average probability densities.

As discussed fully in the main text, these trends were similar in both the artificial and human genome sequence ensembles, although there are perceptible differences, perhaps because of local and non-local sequence dependence in DNA. Notably, the two data sets have different flanking contexts, for example, the human genome sequences have a small bias towards having more C/G flanking bases in the tetramer context to central CpG dinucleotides, along with some highly nonuniform distributions of other dinucleotides, e.g. very low occurrences of ApT and TpA steps.

We then compared nucleosome wrapping energies, in both epigenetically unmodified and modified versions, for ensembles of DNA sequences constructed by drawing one representative from each instance in the human genome of the four region types CGI and NMI, CGI and not NMI, not CGI and NMI, and finally not CGI and not NMI. We were motivated to consider four types of region by the work of [Long et al. \(2013b\)](#) who demonstrated that NMIs cannot be reliably identified by CGIs algorithms and NMIs may have more biological significance. They also found that NMIs are consistent across species, and in warm-blooded organisms these regions coincide with transcription initiation sites. The assumption that CGIs never have epigenetically modified CpG dinucleotides is often made when analysing CGIs [Ioshikhes and Zhang \(2000\)](#); [Hannenhalli and Levy \(2001\)](#); [Bock et al. \(2007\)](#); [Han and Zhao \(2008\)](#), although the current definitions of CGIs do not actually entail this information, so that the studies often lack detail in this respect [Long et al. \(2013b\)](#). Accordingly we considered all four possibilities of intersections and disjunctions between CGIs and NMIs. Our main conclusion from studying wrapping energy spectra from the four types of region is that the lowest probability densities of nucleosomal configurations arise precisely for unmodified CGI sequences, that is sequences that are both CGI and NMI.

The restriction to drawing one representative from each instance of each of the four types of region was dictated merely to limit the necessary computations to a feasible magnitude. We did verify that our results were not sensitive to precisely how we chose the 147 bp representative from each region. Another limitation dictated by available computational resources is the focus on human genome data only. It would be interesting to explore the same data (CGIs and NMIs) for other warm and cold-blooded organisms which were also provided by [Long et al. \(2013b\)](#). That data might provide deeper insights because the regions of interest and their intersections differ vastly across different organisms.

Discussion

We believe that our predictive computational model of nucleosome wrapping energies and the nucleosomal configuration probability densities is (subject to the aforementioned caveats) both sufficiently accurate and efficient to explore biologically pertinent ensembles of sequences and compare model predictions with experimental observations. It is presumably the case that nucleosome wrapping energy will make a significant contribution to predicting nucleosome binding affinities at a particular site. Both stiffness and groundstate of DNA fragment (which are accurately captured in the *cgNA+* model [Sharma \(2023\)](#); [Sharma et al. \(2023\)](#)) contribute to

the sequence dependence of wrapping energy. At the same time, differences in stiffness also contribute to sequence dependent differences in fluctuations about the minimal energy wrapped configuration w_{opt} . Thus we believe that sequence (including epigenetic modifications) dependent entropy-like corrections are necessary to be able to accurately predict binding affinities from wrapping energies, and computing the probability densities of the optimal nucleosomal configurations is a way to account for those corrections.

Furthermore, the process of comparing the predicted densities with the nucleosome occupancy scores is fraught with many potential sources of inaccuracy. Firstly, any computation involving only the DNA takes no account of the possibly sequence-dependent contributions of the histone tails, epigenetically modified or not. Secondly, the probability densities are not probabilities of DNA wrapping into a nucleosomal configurations, but could be regarded as proportional to such, assuming that these probabilities can be approximated by a one-point integral over a small domain of the same volume for all the sequences. The validity of this assumption is not completely obvious. Generally there have been opposing views in the literature about the relationship between nucleosome occupancy scores and sequence induced mechanical properties of DNA. Yoo et al. [Yoo et al. \(2021\)](#)  claimed that nucleosome occupancy scores anticorrelate with the wrapping energy. Perez et al. [Pérez et al. \(2012\)](#)  found that methylation increases DNA deformation energy and decreases nucleosome formation. They also showed that genomic regions with high wrapping energy are nucleosome-depleted. Whereas, Collings et al. [Collings and Anderson \(2017\)](#)  demonstrated that methylated regions are among the highest nucleosome occupied elements in the genome. It has also been shown that CGIs are five-fold depleted for observed nucleosome coverage [Valouev et al. \(2011\)](#) . These two results suggest a positive correlation between nucleosome binding energy and nucleosome occupancy scores.

In this work, we contribute to this discussion by investigating the relations between our probability density predictions and the experimentally observed nucleosome occupancy scores from [Schwartz et al. \(2019\)](#)  and [Yazdi et al. \(2015\)](#) . Our predictions agree with both sets of data in concluding that methylation of CpG islands increase the probability of nucleosome formation. However, the precise ordering of the four genomic regions of CGI and NMI groups by nucleosome occupancy is different in all three cases (two experimental data sets and our predictions). This might be due to different methylation patterns for cancerous HeLa cells in [Schwartz et al. \(2019\)](#) , human embryonic stem cells in [Yazdi et al. \(2015\)](#)  and liver cells in [Long et al. \(2013b\)](#) , used for identifying non methylated regions for our computations. Matched DNA modification to nucleosome occupancy experimental data and investigation of different cell-types will likely reveal more accurately how cells evolve nucleotide composition and modification patterns to reach optimal nucleosome occupancy in different genomic regions.

Supplementary Information

FIG S1

Spectra of nucleosome wrapping energies and logarithms of probability densities for the optimal nucleosomal configurations for 147 bp human genome sequences, grouped by the indicated ranges of numbers of CpG dinucleotide steps: dots averages, error bars standard deviation in sequence.

For the two plots on the right, we used all the sequences in our ensemble; numbers of sequences falling into each CpG range are given in Table II of the main article. For the plots on the left, we took a random 1K sub-sample of sequences for each CpG range. There are no visible differences between plots on the left and on the right.

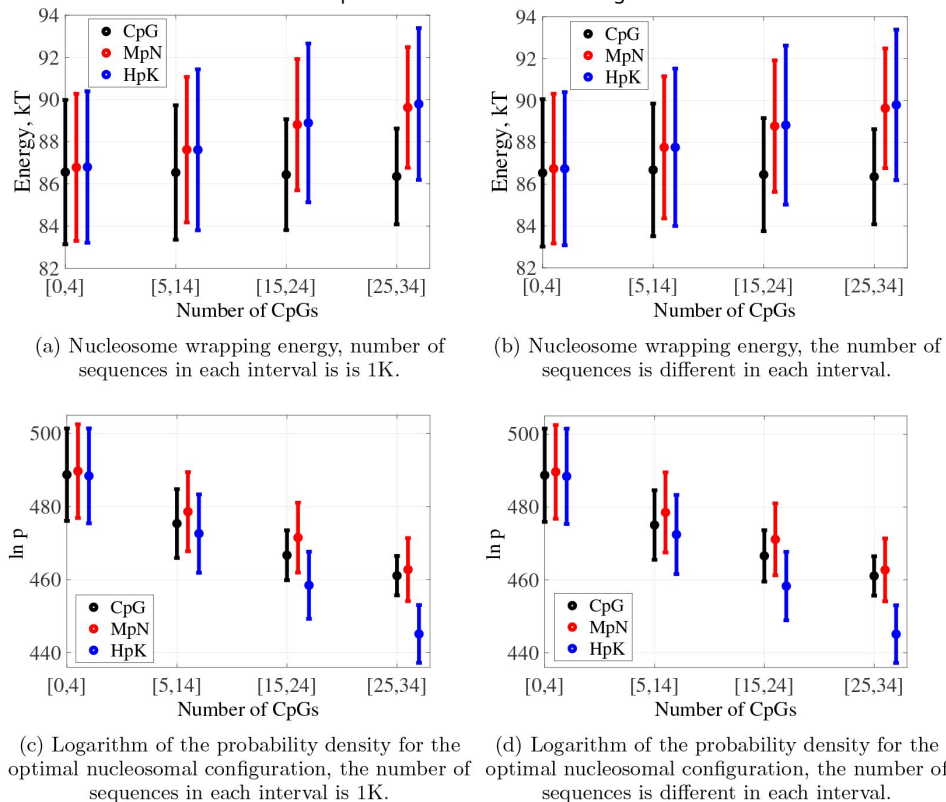


FIG S2

Distances between CpG dinucleotides when there are 10 CpG dinucleotides in sequences of length 147. Left - randomly generated DNA sequences (1000).

Right - sequences from human genome (2341).

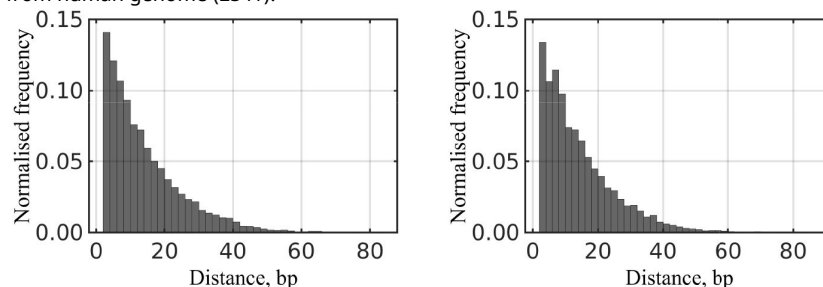


FIG S3

Average number of the 16 different dinucleotide steps for (a) 1000 random sequences and for (b) our human genome sequence ensemble, with [0, 4] CpGs (different colours correspond to fragments taken from different chromosomes).

Dinucleotide steps are ordered next to their complements, with self-complementary steps listed on the right.

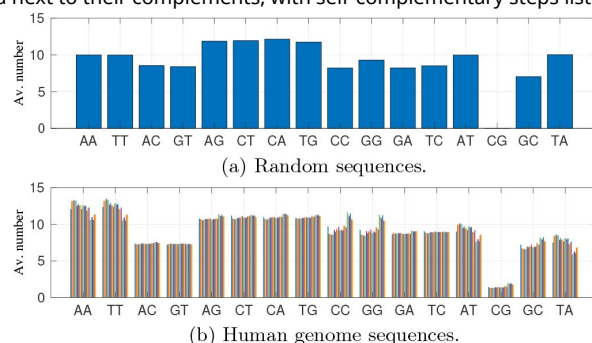


FIG S4

Average number of the 16 different dinucleotide steps for (a) 1000 random sequences and for (b) our human genome sequence ensemble, with [15, 24] CpGs (different colours correspond to fragments taken from different chromosomes).

Dinucleotide steps are ordered next to their complements, with self-complementary steps listed on the right.

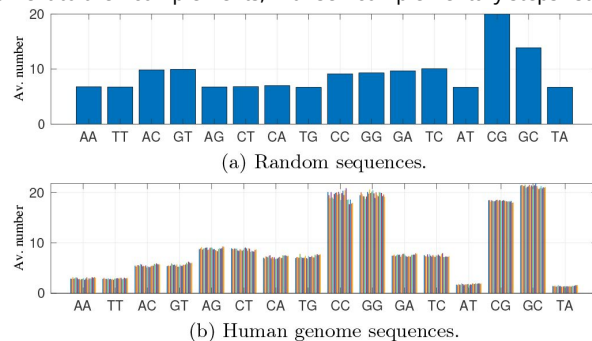


FIG S5

Average number of the 16 different dinucleotide steps for (a) 1000 random sequences and for (b) our human genome sequence ensemble, with [25, 34] CpGs (different colours correspond to fragments taken from different chromosomes).

Dinucleotide steps are ordered next to their complements, with self-complementary steps listed on the right.

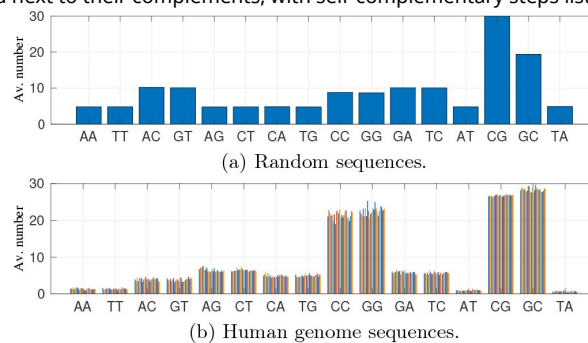
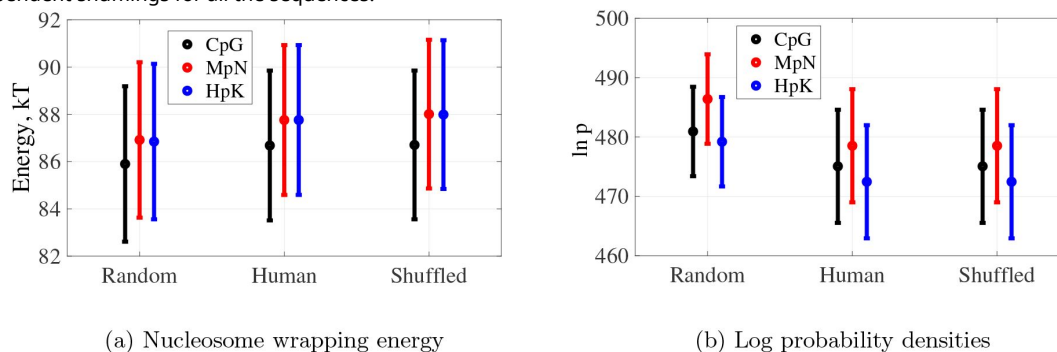


FIG S6

Spectra (a) nucleosome wrapping energy and (b) natural logarithms of probability densities for DNA nucleosomal configurations for unmethylated (CpG), methylated (MpN) and hydroxymethylated (HpK) DNA sequences with CpG dinucleotide count from 5 to 14.

Random corresponds to randomly generated sequences and Human to sequences from the human genome. Shuffled corresponds to sequences with the same count of dinucleotides as in the human genome sequences but shuffled. The difference between random and human sequences is significantly larger than the difference between human and shuffled (human) sequences. The results are consistent for different independent shufflings which we verified by performing three independent shufflings for all the sequences.



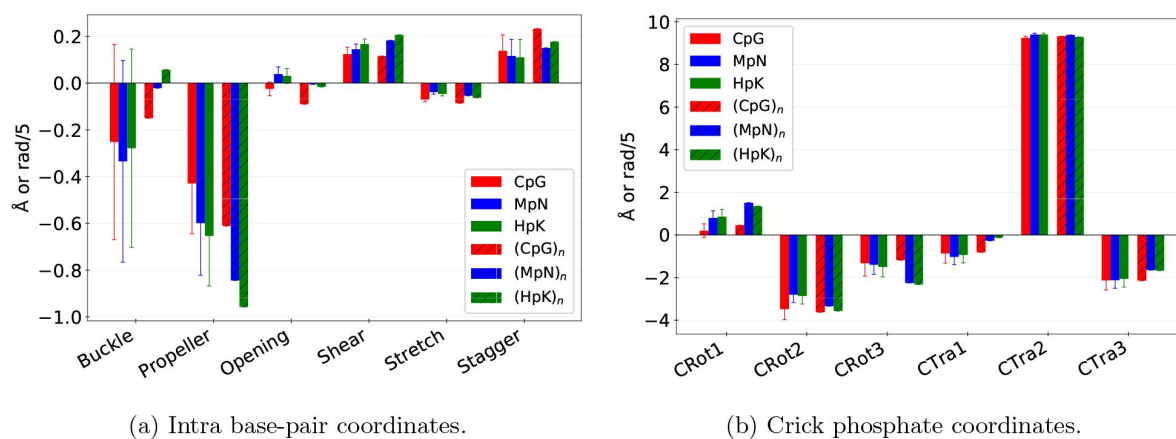


FIG S7

Effects of sequence context and epigenetic base modifications on the ground state shape of CpG steps.

Bar plots of the ground state values of (a) six intra base-pair and (b) six Crick phosphate coordinates for CpG steps i) averaged over sequence context with standard deviations in thin lines and ii) the extreme case of poly(CpG) (in hatch). In each case three versions corresponding to unmodified, methylated and hydroxymethylated steps. The standard deviations highlight the crucial role of non-local sequence dependence in the equilibrium structure of CpG/MpN/HpK steps. (Also see

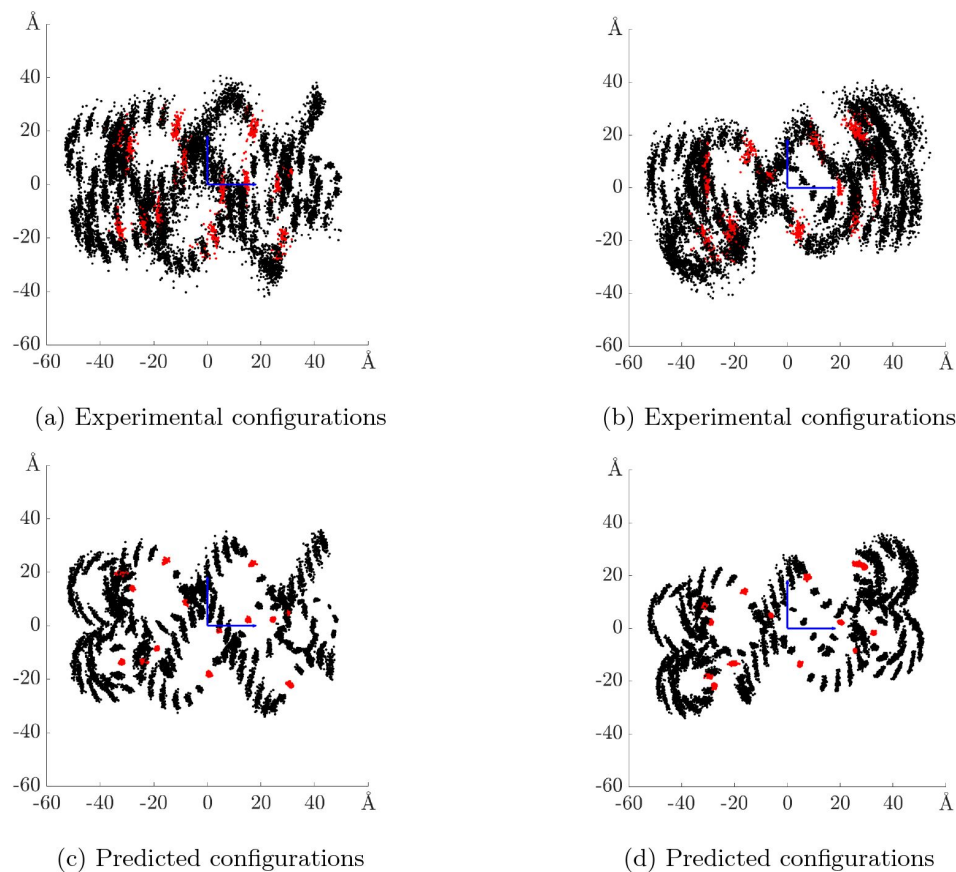


FIG S8

Locations of the Watson strand phosphates for 100 aligned nucleosome structures, projected to planes parallel to the nucleosome central axis (side views of the nucleosomes).

Top row corresponds to 100 experimental PDB nucleosome structures (not all with independent sequences). Red points are phosphates with local minima of radial distance used to identify bound indices. Bottom row analogous data over 100 predicted minimal energy nucleosomal configurations for sequences drawn from human genome CpG islands. The phosphates with bound indices that are constrained during the optimisation are coloured in red. Left panels: horizontal axis is pointing to the nucleosome dyad, right panels: horizontal axis is perpendicular to the dyad axis and to the nucleosome central axis. For analogous plots showing the top view of the nucleosomes, see [Figure 1](#) in the main text.

Data availability

CGI regions from the human genome can be obtained from the UCSC genome browser [Kent et al. \(2002\)](#) [↗](#), whereas experimentally identified NMIs for liver cells are provided in the SI of [Long et al. \(2013b\)](#) [↗](#). The human genome version used in these studies is Genome Reference Consortium Human Build 37 (GRCh37). The full listing of sequences used in our analysis are available in the github repository, at <https://github.com/rginiunaite/CGI-NMI-sequences> [↗](#). The raw experimental occupancy data can be accessed from the SI of [Schwartz et al. \(2019\)](#) [↗](#) and [Yazdi et al. \(2015\)](#) [↗](#).

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Note

This reviewed preprint has been updated to correct level headings.

Additional information

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Ludwig Cancer Research

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Reviewer #1 (Public review):

Summary:

In this manuscript, the authors used a coarse-grained DNA model (cgNA+) to explore how DNA sequences and CpG methylation/hydroxymethylation influence nucleosome wrapping energy and the probability density of optimal nucleosomal configuration. Their findings indicate that both methylated and hydroxymethylated cytosines lead to increased nucleosome wrapping energy. Additionally, the study demonstrates that methylation of CpG islands increases the probability of nucleosome formation.

Strengths:

The major strength of this method is the model explicitly includes phosphate group as DNA-histone binding site constraints, enhancing CG model accuracy and computational efficiency and allowing comprehensive calculations of DNA mechanical properties and deformation energies.

Weaknesses:

A significant limitation of this study is that the parameter sets for the methylated and hydroxymethylated CpG steps in the cgNA+ model are derived from all-atom molecular dynamics (MD) simulations that use previously established force field parameters for modified cytosines (Pérez A, et al. *Biophys J.* 2012; Battistini, et al. *PLOS Comput Biol.* 2021). These parameters suggest that both methylated and hydroxymethylated cytosines increase DNA stiffness and nucleosome wrapping energy, which could predispose the coarse-grained model to replicate these findings. Notably, conflicting results from other all-atom MD simulations, such as those by Ngo T in *Nat. Commun.* 2016, shows that hydroxymethylated cytosines increase DNA flexibility, contrary to methylated cytosines. If the cgNA+ model were trained on these later parameters or other all-atom MD force fields, different conclusions might be obtained regarding the effects of methylated and hydroxymethylation on nucleosome formation.

Despite the training parameters of the cgNA+ model, the results presented in the manuscript indicate that methylated cytosines increase both DNA stiffness and nucleosome wrapping energy. However, when comparing nucleosome occupancy scores with predicted nucleosome wrapping energies and optimal configurations, the authors find that methylated CGIs exhibit higher nucleosome occupancies than unmethylated ones, which seems to contradict the expected relationship where increased stiffness should reduce nucleosome formation affinity. In the manuscript, the authors also admit that these conclusions "apparently runs counter to the (perhaps naive) intuition that high nucleosome forming affinity should arise for fragments with low wrapping energy". Previous all-atom MD simulations (Pérez A, et al. *Biophys J.* 2012; Battistini, et al. *PLOS Comput Biol.* 2021; Ngo T, et al. *Nat. Commun.* 2016) show that the stiffer DNA upon CpG methylation reduces the affinity of DNA to assemble into nucleosomes or destabilizes nucleosomes. Given these findings, the authors need to address

and reconcile these seemingly contradictory results, as the influence of epigenetic modifications on DNA mechanical properties and nucleosome formation are critical aspects of their study.

Understanding the influence of sequence-dependent and epigenetic modifications of DNA on mechanical properties and nucleosome formation is crucial for comprehending various cellular processes. The authors' study, focusing on these aspects, definitely will garner interest from the DNA methylation research community.

Comments on revised version:

The authors have addressed most of my comments and concerns regarding this manuscript.

<https://doi.org/10.7554/eLife.98468.2.sa3>

Reviewer #2 (Public review):

Summary:

This study uses a coarse-grained model for double stranded DNA, cgNA+, to assess nucleosome sequence affinity. cgNA+ coarse-grains DNA on the level of bases and accounts also explicitly for the positions of the backbone phosphates. It has been proven to reproduce all-atom MD data very accurately. It is also ideally suited to be incorporated into a nucleosome model because it is known that DNA is bound to the protein core of the nucleosome via the phosphates.

It is still unclear whether this harmonic model parametrized for unbound DNA is accurate enough to describe DNA inside the nucleosome. Previous models by other authors, using more coarse-grained models of DNA, have been rather successful in predicting base pair sequence dependent nucleosome behavior. This is at least the case as long as DNA shape is concerned whereas assessing the role of DNA bendability (something this paper focuses on) has been consistently challenging in all nucleosome models to my knowledge.

It is thus of major interest whether this more sophisticated model is also more successful in handling this issue. As far as I can tell the work is technically sound and properly accounts for not only the energy required in wrapping DNA but also entropic effects, namely the change in entropy that DNA experiences when going from the free state to the bound state. The authors make an approximation here which seems to me to be a reasonable first step.

Of interest is also that the authors have the parameters at hand to study the effect of methylation of CpG-steps. This is especially interesting as this allows to study a scenario where changes in the physical properties of base pair steps via methylation might influence nucleosome positioning and stability in a cell-type specific way.

Overall, this is an important contribution to the questions of how sequence affects nucleosome positioning and affinity. The findings suggest that cgNA+ has something new to offer. But the problem is complex, also on the experimental side, so many questions remain open. Despite of this, I highly recommend publication of this manuscript.

Strengths:

The authors use their state-of-the-art coarse grained DNA model which seems ideally suited to be applied to nucleosomes as it accounts explicitly for the backbone phosphates.

Weaknesses:

The authors introduce penalty coefficients c_i to avoid steric clashes between the two DNA turns in the nucleosome. This requires c_i -values that are so high that standard deviations in the fluctuations of the simulation are smaller than in the experiments.

<https://doi.org/10.7554/eLife.98468.2.sa2>

Reviewer #3 (Public review):

Summary:

In this study, authors utilize biophysical modeling to investigate differences in free energies and nucleosomal configuration probability density of CpG islands and nonmethylated regions in the genome. Toward this goal, they develop and apply the cgNA+ coarse-grained model, an extension of their prior molecular modeling framework.

Strengths:

The study utilizes biophysical modeling to gain mechanistic insight into nucleosomal occupancy differences in CpG and nonmethylated regions in the genome.

Weaknesses:

Although the overall study is interesting, the manuscripts need more clarity in places. Moreover, the rationale and conclusion for some of the analyses are not well described.

<https://doi.org/10.7554/eLife.98468.2.sa1>

Author response:

The following is the authors' response to the original reviews.

Reviewer #1 (Public Review):

Summary:

In this manuscript, the authors used a coarse-grained DNA model (cgNA+) to explore how DNA sequences and CpG methylation/hydroxymethylation influence nucleosome wrapping energy and the probability density of optimal nucleosomal configuration. Their findings indicate that both methylated and hydroxymethylated cytosines lead to increased nucleosome wrapping energy. Additionally, the study demonstrates that methylation of CpG islands increases the probability of nucleosome formation.

Strengths:

The major strength of this method is that the model explicitly includes elastic constraints on the positions of phosphate groups facing a histone octamer, as DNA-histone binding site constraints. The authors claim that their model enhances the accuracy and computational efficiency and allows comprehensive calculations of DNA mechanical properties and deformation energies.

Weaknesses:

A significant limitation of this study is that the parameter sets for the methylated and hydroxymethylated CpG steps in the cgNA+ model are derived from all-atom molecular dynamics (MD) simulations that suggest that both methylated and hydroxymethylated cytosines increase DNA stiffness and nucleosome wrapping energy (Pérez A, et al.

Biophys J. 2012; Battistini, et al. *PLOS Comput Biol.* 2021). It could predispose the coarse-grained model to replicate these findings. Notably, conflicting results from other all-atom MD simulations, such as those by Ngo T in *Nat. Commun.* 2016, shows that hydroxymethylated cytosines increase DNA flexibility, contrary to methylated cytosines. If the cgNA+ model was trained on these later parameters or other all-atom force fields, different conclusions might be obtained regarding the effects of methylated and hydroxymethylation on nucleosome formation.

Despite the training parameters of the cgNA+ model, the results presented in the manuscript indicate that methylated cytosines increase both DNA stiffness and nucleosome wrapping energy. However, when comparing nucleosome occupancy scores with predicted nucleosome wrapping energies and optimal configurations, the authors find that methylated CGIs exhibit higher nucleosome occupancies than unmethylated ones, which seems to contradict their findings from the same paper which showed that increased stiffness should reduce nucleosome formation affinity. In the manuscript, the authors also admit that these conclusions “apparently runs counter to the (perhaps naive) intuition that high nucleosome forming affinity should arise for fragments with low wrapping energy”. Previous all-atom MD simulations (Pérez A, et al. *Biophys J.* 2012; Battistini, et al. *PLOS Comput Biol.* 202; Ngo T, et al. *Nat. Commun.* 2016) show that the stiffer DNA upon CpG methylation reduces the affinity of DNA to assemble into nucleosomes or destabilizes nucleosomes. Given these findings, the authors need to address and reconcile these seemingly contradictory results, as the influence of epigenetic modifications on DNA mechanical properties and nucleosome formation are critical aspects of their study. Understanding the influence of sequence-dependent and epigenetic modifications of DNA on mechanical properties and nucleosome formation is crucial for comprehending various cellular processes. The authors’ study, focusing on these aspects, will definitely garner interest from the DNA methylation research community.

Training the cgNA+ model on alternative MD simulation datasets is certainly of interest to us. However, due to the significant computational cost, this remains a goal for future work. The relationship between nucleosome occupancy scores and nucleosome wrapping energy is still debated, with conflicting findings reported in the literature, as noted in our Discussion section. Interestingly, we find that our predicted log probability density of DNA spontaneously acquiring a nucleosomal configuration is a better indicator of nucleosome occupancy than our predicted DNA nucleosome wrapping energy.

Reviewer #2 (Public Review):

Summary:

This study uses a coarse-grained model for double-stranded DNA, cgNA+, to assess nucleosome sequence affinity. cgNA+ coarse-grains DNA on the level of bases and accounts also explicitly for the positions of the backbone phosphates. It has been proven to reproduce all-atom MD data very accurately. It is also ideally suited to be incorporated into a nucleosome model because it is known that DNA is bound to the protein core of the nucleosome via the phosphates.

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It is thus of major interest whether this more sophisticated model is also more successful in handling this issue. As far as I can tell the work is technically sound and properly accounts for not only the energy required in wrapping DNA but also entropic effects, namely the change in entropy that DNA experiences when going from the free state to the bound state. The authors make an approximation here which seems to me to be a reasonable first step.

Of interest is also that the authors have the parameters at hand to study the effect of methylation of CpG-steps. This is especially interesting as it allows us to study a scenario where changes in the physical properties of base pair steps via methylation might influence nucleosome positioning and stability in a cell-type-specific way.

Overall, this is an important contribution to the question of how the sequence affects nucleosome positioning and affinity. The findings suggest that cgNA+ has something new to offer. But the problem is complex, also on the experimental side, so many questions remain open.

Strengths:

The authors use their state-of-the-art coarse-grained DNA model which seems ideally suited to be applied to nucleosomes as it accounts explicitly for the backbone phosphates.

Weaknesses:

(1) According to the abstract the authors consider two “scalar measures of the sequence-dependent propensity of DNA to wrap into nucleosomes”. One is the bending energy and the other, is the free energy. Specifically in the latter, the authors take the difference between the free energies of the wrapped and the free DNA. Whereas the entropy of the latter can be calculated exactly, they assume that the bound DNA always has the same entropy (independent of sequence) in its more confined state. The problem is the way in which this is written (e.g. below Eq. 6) which is hard to understand. The authors should mention that the negative of Eq. 6 is what physicists call free energy, namely especially the free energy difference between bound and free DNA.

We have included the necessary clarifications in the revised manuscript, below Eq. 6.

(2) In Eq. 5 the authors introduce penalty coefficients c_i . They write that values are “set by numerical experiment to keep distances ... within the ranges observed in the PDB structure, while avoiding sterical clashes in DNA.” This is rather vague, especially since it is unclear to me what type of sterical clashes might occur. Figure 1 shows then a comparison between crystal structures and simulated structures. They are reasonably similar but standard deviations in the fluctuations of the simulation are smaller than in the experiments. Why did the authors not choose smaller c_i -values to have a better fit? Do smaller values lead to unwanted large fluctuations that would lead to steric clashes between the two DNA turns? I also wonder what side views of the nucleosomes look like (experiments and simulations) and whether in this side view larger fluctuations of the phosphates can be observed in the simulation that would eventually lead to turn-turn clashes for smaller c_i -values.

The side view plots of the experimental and predicted nucleosome structures are now added to Supplementary material (Figure S8). Indeed, smaller c_i values lead to steric clashes between the two turns of DNA – this is now specified in the Methods section. A possible improvement of our optimisation method and a direction of future work would be adding a penalty which prevents steric clashes to the objective function. Then the c_i values could be

reduced to have bigger fluctuations that are even closer to the experimental structures. We added this explanation to the Results section.

Reviewer #3 (Public Review):

Summary:

In this study, the authors utilize biophysical modeling to investigate differences in free energies and nucleosomal configuration probability density of CpG islands and nonmethylated regions in the genome. Toward this goal, they develop and apply the cgNA+ coarse-grained model, an extension of their prior molecular modeling framework.

Strengths:

The study utilizes biophysical modeling to gain mechanistic insight into nucleosomal occupancy differences in CpG and nonmethylated regions in the genome.

Weaknesses:

Although the overall study is interesting, the manuscripts need more clarity in places. Moreover, the rationale and conclusion for some of the analyses are not well described.

We edited the manuscript according to the reviewer's suggestions and hopefully improved its readability.

Reviewer #1 (Recommendations For The Authors):

(1) The cgNA+ model parameters are derived from all-atom molecular dynamics (MD) simulations, yet there is no consensus within all-atom MD simulations regarding the impact of CpG methylation on DNA mechanical properties. The authors could consider fitting the coarsegrained model with a different all-atom force field to verify whether the conclusions regarding the effects of methylation and hydroxymethylation on DNA nucleosome wrapping energies still hold. For further details on MD simulations related to CpG methylation effects, the authors are advised to consult the review paper by Li et al. (2022) titled "DNA methylation: Precise modulation of chromatin structure and dynamics" published in Current Opinion in Structural Biology.

Parametrizing the cgNA+ model using MD simulations with various force fields is certainly of interest to us. However, due to the computational cost involved, it remains a goal for future work.

(2) Beyond DNA mechanical properties, which are directly linked to nucleosome wrapping energies in this study, the authors might also consider other factors such as geometric properties that could influence nucleosome formation. This approach might help the authors to reconcile the observed higher nucleosome occupancy scores for methylated CpGs. The authors are encouraged to review the aforementioned paper for additional experimental and MD simulation studies that could support this perspective.

Geometric properties of DNA are directly incorporated into our method through the cgNA+ model equilibrium shape prediction μ . We compute the mechanical energy needed deform μ to a nucleosomal configuration. Notably, the equilibrium shape μ is sensitive to methylation, as demonstrated in Figure 3.

(3) There are some issues with citation accuracy in the manuscript. For instance, in the Discussion section, the authors attribute a statement to Collings et al. and Anderson (2017), claiming that "methylated regions, known to have high wrapping energy, are

among the highest nucleosome occupied elements in the genome.” However, upon reviewing this paper, it appears that it does not make any claims about the high wrapping energy of methylated regions.

The paragraph is now edited and a separate citation, Pérez et al. (2012), is given for the statement that methylation regions have high wrapping energy.

Reviewer #2 (Recommendations For The Authors):

Please improve the readability by:

(1) making clear that $-\ln p$ in Eq. 6 on page 4 is actually the free energy. Also, the word entropy comes too late (on page 7) where the best explanation of Eq. 6 is presented.

We added a comment about $-\ln p$ being the free energy after Eq. 6 and also included an equation, relating $\ln p$ and entropy.

(2) page 12 and 13 show two sets of experimental data. They are quite different from each other. When reading this, I wondered why there is this difference. But only on page 16, you explain that these are different cell types. The difference should be explained already when the papers are introduced on page 12.

A corresponding sentence already appeared in page 12: “The observations about nucleosome occupancy should be regarded as preliminary, and be treated with caution, as they are based on experimental data obtained for the cancerous HeLa cells Schwartz et al. (2019) and human genome embryonic stem cells Yazdi et al. (2015)”. Now we also added this information to the first paragraph of the subsection for clarity.

Finally, I add here some general thoughts that came up when reading the paper, comparing your findings with earlier findings in the field. This is not a strict one-to-one comparison and thus does not have to find its way into this manuscript but might give ideas for future studies. Experiments suggest that nucleosomes prefer DNA with a high content of C's and G's. Figure 2 does not look at the GC content but at the number of CpG's. But in any case, let's use this as a proxy for GC content. Figure 2a suggests that there is not a strong dependence of the bending energy on the number CpG steps. This is consistent with earlier work with the rigid basepair model which shows the same behavior for GC content (for both MD and crystal parametrizations). Figure 2c (related to the negative free energy) shows that with an increasing number of CpG steps the propensity to bind goes down. This suggests that the entropic cost to confine CpG-rich DNA increases, which in turn reflects that these DNA stretches are softer. This is rather interesting since in the case of the rigid basepair model this effect is observed only when stiffnesses are extracted from crystal data not MD data (however, this refers again to CG content). This might indicate a difference between the rigid bp model and cgNA+ which will be interesting to study in the future. Interesting is also the effect of CpG methylation. The stiffer methylated steps lead to an increase in the energy with the number of such steps (Figure 2a). The entropic cost for binding is thus expected to be smaller and this is indeed observed in Figure 2c when compared to the non-methylated steps.

We thank the reviewer for this comment. As for the GC content, the energy and $\ln p$ plots are indeed very similar to those in Figure 2.

Reviewer #3 (Recommendations For The Authors):

(1) The formulation of the cgNA+ model in the method section was not easy to follow and can be described better to improve clarity.

We have revised the model description and hope that its clarity has been improved

(2) The authors mention utilizing 100 human genome sequences with 100 configurations from DB. It would be helpful to clarify the source of these 100 human genome sequences. Are these 100 distinct regions on the human reference genome, or are they from a specific dataset or database?

We now include an explanation about the origin of sequences: “The human genome sequences are a random subset of our sequence sample for the CGI and NMI intersection in the Chromosome 1, but the following observations remain unchanged for sequence samples from different genomic regions.”

(3) The authors mention the lack of tail unwrapping in their model. It would be beneficial to understand the magnitude of this issue and its potential impact on the overall results. How significant is the lack of unwrapping events in their current model?

We observed the unwrapping of approximately five base-pairs at each end of our predicted nucleosome configurations, in comparison to the experimental configurations (Figure 1). This issue could be solved by adding additional constraints at the ends of the 147 bp sequence. The wrapping energy would increase marginally, as only about 10 of 147 bp would be affected. We added this remark to the main text.

(4) Observations from Figure 3 are not described properly. Are these differences statistically significant? Why is twist higher for CpG sites but lower for a roll?

We added an explanation of how the statistics was computed into the caption of Figure 3. In fact, we didn’t use statistical estimates here, but generated all the possible cases and computed the exact statistics (for the given set of our model parameters). Regarding the changes in twist and roll, we have added the following comment on page 7: “The ground state changes resulting from cytosine modifications – primarily characterized by an average increase in roll and a decrease in twist – may be linked to steric hindrance caused by the cytosine 5-substituent (Battistini et al. (2021)). Notably, the negative coupling between twist and roll has already been observed in X-ray crystallography data (Olson et al. (1998)).”

(5) Figure 4 does not clarify the authors’ conclusion of higher stiffness for ApT and TpA dinucleotides. The authors should provide further explanation for this observation.

We revised the text to clarify that the statement regarding ApT and TpA being the most stiff and the most flexible dinucleotides is not a conclusion derived from Figure 4, but rather from earlier work that we cite.

(6) In Figure 7, the authors note that methylated CGIs have higher nucleosome occupancy on average than unmethylated sequences. Is this observation statistically significant?

We observe that methylated sequences have a higher average occupancy than unmethylated sequences in Yazdi et al. data, when the CpG count falls into the intervals from 5 to 14 and from 15 to 24. For each of the two intervals this difference is statistically significant: the permutation test, used due to the lack of normality, yields a p-value of 0.0001 for both cases. The differences in mean scores shown in Figure 8 are also statistically significant. Such test results are expected, given the large sample sizes and the observed differences in means, therefore we prefer not to include this discussion in main text.

(7) The authors note that their analyses to correlate nucleosome occupancy profile with the methylation state of underlying sequences are preliminary, as different cell lines were used to perform these analyses. Given this inconsistency, it needs to be clarified why this analysis was performed and what the takeaway is.

We added the following comment at the end of the Results section: “Although comparing data from different cell lines is not optimal, to the best of our knowledge, no publicly available methylation and nucleosome occupancy data exist for the entire human genome within the same cell type. Nevertheless, since the lowest log probability densities in the human genome are predicted for CpG-rich sequences regardless of their methylation state (Figure 2d), and the same holds for both sets of the nucleosome occupancy scores (Figure 7), we conclude that the lowest occupancies occur for sequences with the lowest log probability densities.”

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