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Explicit Ion Modeling Predicts Physicochemical Interactions for Chromatin Organization

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Abstract

Molecular mechanisms that dictate chromatin organization *in vivo* are under active investigation, and the extent to which intrinsic interactions contribute to this process remains debatable. A central quantity for evaluating their contribution is the strength of nucleosome-nucleosome binding, which previous experiments have estimated to range from 2 to 14 k_BT. We introduce an explicit ion model to dramatically enhance the accuracy of residue-level coarse-grained modeling approaches across a wide range of ionic concentrations. This model allows for *de novo* predictions of chromatin organization and remains computationally efficient, enabling large-scale conformational sampling for free energy calculations. It reproduces the energetics of protein-DNA binding and unwinding of single nucleosomal DNA, and resolves the differential impact of mono and divalent ions on chromatin conformations. Moreover, we showed that the model can reconcile various experiments on quantifying nucleosomal interactions, providing an explanation for the large discrepancy between existing estimations. We predict the interaction strength at physiological conditions to be 9 k_BT, a value that is nonetheless sensitive to DNA linker length and the presence of linker histones. Our study strongly supports the contribution of physicochemical interactions to the phase behavior of chromatin aggregates and chromatin organization inside the nucleus.

eLife assessment

The authors have developed a **compelling** coarse-grained simulation approach for nucleosome-nucleosome interactions within a chromatin array. The data presented are **solid** and provide new insights that allow for predictions of how chromatin interactions might occur in vivo. The tools presented herein will be **valuable** for the chromosome biology field.

Introduction

Three-dimensional genome organization plays essential roles in numerous DNA-templated processes.[1–5] Understanding the molecular mechanisms for its establishment could improve our understanding of these processes and facilitate genome engineering. Advancements in high-

throughput sequencing and microscopic imaging have enabled genome-wide structural characterization, revealing a striking compartmentalization of chromatin at large scales.[6–9] For example, A compartments are enriched with euchromatin and activating post-translational modifications to histone proteins. They are often spatially segregated from B compartments that enclose heterochromatin with silencing histone marks.[3,4,10–12]

Compartmentalization has been proposed to arise from the microphase separation of different chromatin types as in block copolymer systems.[13–25] However, the molecular mechanisms that drive the microphase separation are not yet fully understood. Protein molecules that recognize specific histone modifications have frequently been found to undergo liquid-liquid phase separation,[23,26–31] potentially contributing to chromatin demixing. Demixing can also arise from interactions between chromatin and various nuclear landmarks such as nuclear lamina and speckles,[11,15,25,32] as well as active transcriptional processes.[33–36] Furthermore, recent studies have revealed that nucleosome arrays alone can undergo spontaneous phase separation,[37–39] indicating that compartmentalization may be an intrinsic property of chromatin driven by nucleosome-nucleosome interactions.

The relevance of physicochemical interactions between nucleosomes to chromatin organization *in vivo* has been constantly debated, partly due to the uncertainty in their strength.[40–43] Examining the interactions between native nucleosomes poses challenges due to the intricate chemical modifications that histone proteins undergo within the nucleus and the variations in their underlying DNA sequences.[44,45] Many *in vitro* experiments have opted for reconstituted nucleosomes that lack histone modifications and feature well-positioned 601-sequence DNA to simplify the chemical complexity. These experiments aim to establish a fundamental reference point, a baseline for understanding the strength of interactions within native nucleosomes. Nevertheless, even with reconstituted nucleosomes, a consensus regarding the significance of their interactions remains elusive. For example, using force-measuring magnetic tweezers, Kruithof et al. estimated the inter-nucleosome binding energy to be $\sim 14\ k_BT$.[40] On the other hand, Funke et al. introduced a DNA origami-based force spectrometer to directly probe the interaction between a pair of nucleosomes,[43] circumventing any potential complications from interpretations of single molecule traces of nucleosome arrays. Their measurement reported a much weaker binding free energy of approximately $2\ k_BT$. This large discrepancy in the reported reference values complicates a further assessment of the interactions between native nucleosomes and their contribution to chromatin organization *in vivo*.

Computational modeling is well suited for reconciling the discrepancy across experiments and determining the strength of internucleosome interactions. The high computational cost of atomistic simulations[46–48] have inspired several groups to calculate the nucleosome binding free energy with coarse-grained models.[49,50] However, the complex distribution of charged amino acids and nucleotides at nucleosome interfaces places a high demand on force field accuracy. In particular, most existing models adopt a mean-field approximation with the Debye-Hückel theory[51] to describe electrostatic interactions in an implicit-solvent environment,[49,50,52,53] preventing an accurate treatment of the complex salt conditions explored in experiments. Further force field development is needed to improve the accuracy of coarsegrained modeling across different experimental settings.[54–57]

We introduce a residue-level coarse-grained explicit-ion model for simulating chromatin conformations and quantifying inter-nucleosome interactions. We validate our model's accuracy through extensive simulations, demonstrating that it reproduces the binding affinities of protein-DNA complexes [58] and energetic cost of nucleosomal DNA unwinding.[59] Further simulations of chromatin at various salt concentrations reproduce experimentally measured sedimentation coefficients.[60] We also reveal extensive close contacts between histone proteins and DNA across nucleosomes, the perturbation of which explains the discrepancy among various experimental studies. Finally, we determined the binding free energy between a pair of nucleosomes under

physiological salt concentrations as $\sim 9\ k_BT$. While longer linker DNA would reduce this binding energy, linker histones can more than compensate this reduction to mediate inter-nucleosome interactions with disordered, charged terminal tails. Our study supports the importance of intrinsic physicochemical interactions in chromatin organization *in vivo*.

Results

Counterion condensation accommodates nucleosomal DNA unwrapping

Various single-molecule studies have been carried out to probe the stability of nucleosomes and the interactions between histone proteins and DNA. [41,59,61–63] The DNA-unzipping experiment performed by Hall et al.[59] is particularly relevant since the measured forces can be converted into a free energy profile of DNA unwinding at a base-pair resolution, as shown by Forties et al. with a continuous-time Markov model.[64] The high-resolution quantification of nucleosome energetics is valuable for benchmarking the accuracy of computational models. We introduce a coarse-grained explicit-ion model for chromatin simulations (**Fig. 1**).

The model represents each amino acid with one coarse-grained bead and three beads per nucleotide. It resolves the differences among various chemical groups to accurately describe biomolecular interactions with physical chemistry potentials. Our explicit representation of monovalent and divalent ions enables a faithful description of counter ion condensation and its impact on electrostatic interactions between protein and DNA molecules. Additional model details are provided in the *Materials and Methods* and the Supporting Information.

We performed umbrella simulations[65] to determine the free energy profile of nucleosomal DNA unwinding. The experimental buffer condition of 0.10M NaCl and 0.5mM $MgCl_2$ [59] was adopted in simulations for direct comparison. As shown in **Fig. 2B**, the simulated values match well with experimental results over a wide range. Furthermore, we computed the binding free energy for a diverse set of protein-DNA complexes and the simulated values again match well with experimental data (Fig. S1), supporting the model's accuracy.

Counterions are often released upon protein-DNA binding to make room for close contacts at the interface, contributing favorably to the binding free energy in the form of entropic gains.[66] However, previous studies have shown that the histone-DNA interface in a fully wrapped nucleosome configuration is not tightly sealed but instead permeated with water molecules and mobile ions. [67,68] Given their presence in the bound form, how these counterions contribute to nucleosomal DNA unwrapping remains to be shown. We calculated the number of DNA-bound cations and protein-bound anions as DNA unwraps. Our results, shown in **Fig. 2C**, indicate that only a modest amount of extra Na^+ and Cl^- ions becomes associated with the nucleosome as the outer DNA layer unwraps. However, significantly more ions become bound when the inner layer starts to unwrap (after 73 bp). These findings suggest that counterion release may contribute more significantly to the inner layer wrapping, potentially caused by a tighter protein-DNA interface.

Charge neutralization with Mg^{2+} compacts chromatin

In addition to contributing to the stability of individual nucleosomes, counterions can also impact higher-order chromatin organization. Numerous groups have characterized the structures of nucleosome arrays, [60,69–73] revealing a strong dependence of chromatin folding on the concentration and valence of cations.

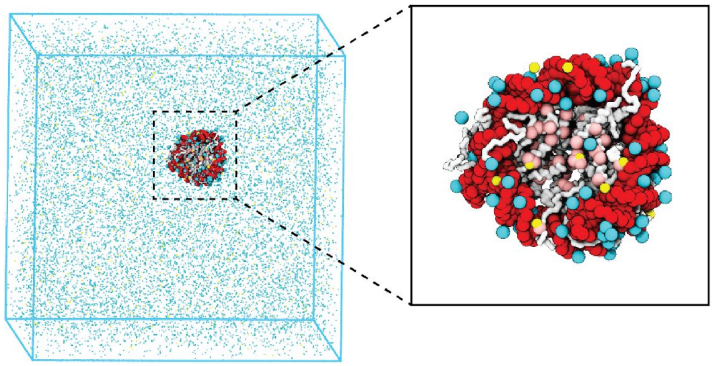

Figure 1

Illustration of the residue-level coarse-grained explicit-ion model for chromatin simulations.

The left panel presents a snapshot for the simulation box of a 147-bp nucleosome in a solution of 100 mM NaCl and 0.5 mM $MgCl_2$. The nucleosomal DNA and histone proteins are colored in red and white, respectively. The Zoom-in on the right highlights the condensation of ions around the nucleosome, with Na^+ in cyan and Mg^{2+} in yellow. Negative residues of the histone proteins are colored in pink.

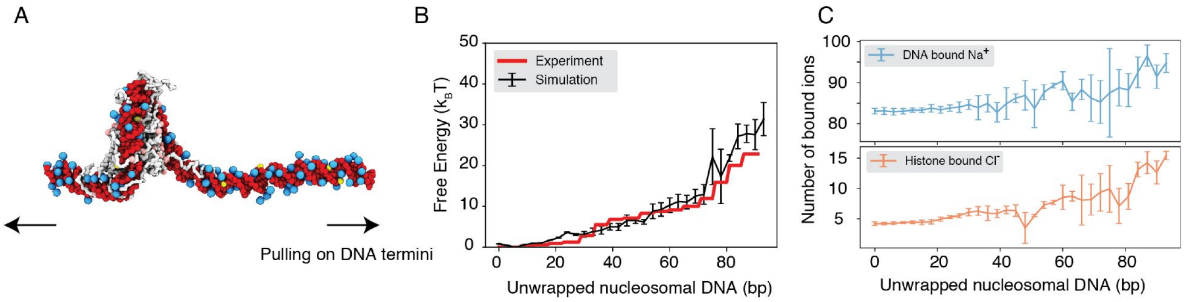

Figure 2

Explicit ion modeling reproduces the energetics of nucleosomal DNA unwrapping.

(A) Illustration of the umbrella simulation setup using the end-to-end distance between two DNA termini as the collective variable. The same color scheme as in **Fig. 1** is adopted. Only ions close to the nucleosomes are shown for clarity. (B) Comparison between simulated (black) and experimental (red) free energy profile as a function of the unwrapped DNA base pairs. Error bars were computed as the standard deviation of three independent estimates. (C) The average number of Na^+ ions within 10 $^{\circ}$A of the nucleosomal DNA (top) and Cl^- ions within 10 $^{\circ}$A of histone proteins (bottom) are shown as a function of the unwrapped DNA base pairs.

To further understand the role of counterions in chromatin organization, we studied a 12-mer with 20-bp-long linker DNA under different salt conditions. We followed the experiment setup by Correll et al.[60] that immerses chromatin in solutions with 5mM NaCl, 150mM NaCl, 0.6mM $MgCl_2$, or 1mM $MgCl_2$. To facilitate conformational sampling, we carried out umbrella simulations with a collective variable that quantifies the similarity between a given configuration and a reference two-start helical structure. Simulation details and the precise definition of the collective variable are provided in Supporting Information. Data from different umbrella windows were combined together with proper reweighting[74] for analysis. As shown in **Fig. 3A**, the average sedimentation coefficients determined from our simulations match well with experimental values. Specifically, the simulations reproduce the strong contrast in chromatin size between the two systems with different NaCl concentrations. Chromatin under 5 mM NaCl features an extended configuration with minimal stacking between 1-3 nucleosomes (**Fig. 3B**). On the other hand, the compaction is evident at 150 mM NaCl. Notably, in agreement with previous studies,[75–78] we observe tri-nucleosome configurations as chromatin extends. Finally, the simulations also support that divalent ions are more effective in packaging chromatin than NaCl. Even in the presence of 0.6 mM $MgCl_2$, the chromatin sedimentation coefficient is comparable to that obtained at 150 mM of NaCl.

We further characterized ions that are in close contact with DNA to understand their impact on chromatin organization. Our simulations support the condensation of cations, especially for divalent ions (**Fig. 3A** bottom) as predicted by the Manning theory.[79,80] Ion condensation weakens the repulsion among DNA segments that prevents chromatin from collapsing. Notably, the fraction of bound Mg^{2+} is much higher than Na^{+}. Correspondingly, the amount of neutralized negative charges is always greater in systems with divalent ions, despite the significantly lower salt concentrations. The difference between the two types of ions arises from the more favorable interactions between Mg^{2+} and phosphate groups that more effectively offset the entropy loss due to ion condensation.[80] While higher concentrations of NaCl do not dramatically neutralize more charges, the excess ions provide additional screening to weaken the repulsion among DNA segments, stabilizing chromatin compaction.

Close contacts drive nucleosome binding free energy

Encouraged by the explicit ion model's accuracy in reproducing experimental measurements of single nucleosomes and nucleosome arrays, we moved to directly quantify the strength of inter-nucleosomes interactions. We once again focus on reconstituted nucleosomes for a direct comparison with *in vitro experiments*. These experiments have yielded a wide range of values, ranging from 2 to 14 k_BT.[40,41,43] Accurate quantification will offer a reference value for conceptualizing the significance of physicochemical interactions among native nucleosomes in chromatin organization *in vivo*.

To reconcile the discrepancy among various experimental estimations, we directly calculated the binding free energy between a pair of nucleosomes with umbrella simulations. We adopted the same ionic concentrations as in the experiment performed by Funke et al.[43] with 35 mM NaCl and 11 mM $MgCl_2$. We focus on this study since the experiment directly measured the inter-nucleosomal interactions, allowing straightforward comparison with simulation results. Furthermore, the reported value for nucleosome binding free energy deviates the most from other studies. In one set of umbrella simulations, we closely mimicked the DNA origami device employed by Funke et al. to move nucleosomes along a predefined path for disassociation (**Fig. 4A**, A1 to A3). For example, neither nucleosome can freely rotate (Fig. S3); the first nucleosome is restricted to the initial position, and the second nucleo-some can only move within the Y-Z plane along the arc 15 nm away from the origin. For comparison, we performed a second set of independent simulations without imposing any restrictions on nucleosome orientations. Additional simulation details can be found in the Supporting Information.

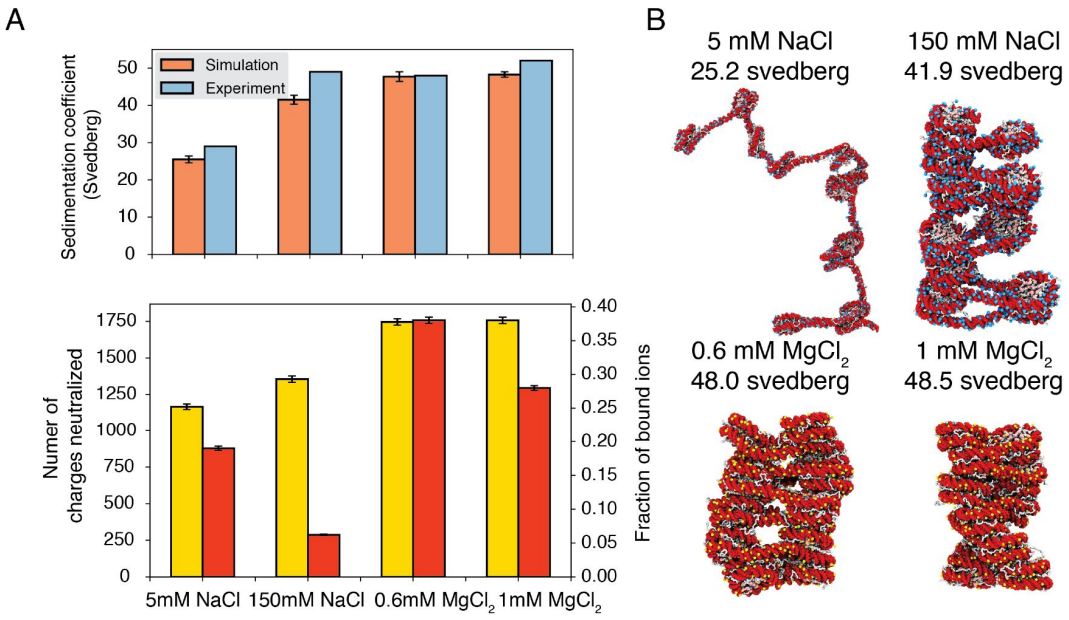

Figure 3

Explicit ion modeling predicts salt-dependent conformations of a 12-mer nucleosome array.

(A) Top: Comparison of simulated and experimental[60] sedimentation coefficients of chromatin at different salt concentrations. Bottom: Number of DNA charges neutralized by bound cations (yellow, left y-axis label) and the fraction of ions bound to DNA (red, right y-axis label) at different salt concentrations. The error bars were estimated from the standard deviation of simulated probability distributions (Fig. S2) (B) Representative chromatin structures with sedimentation coefficients around the mean values at different salt concentrations.

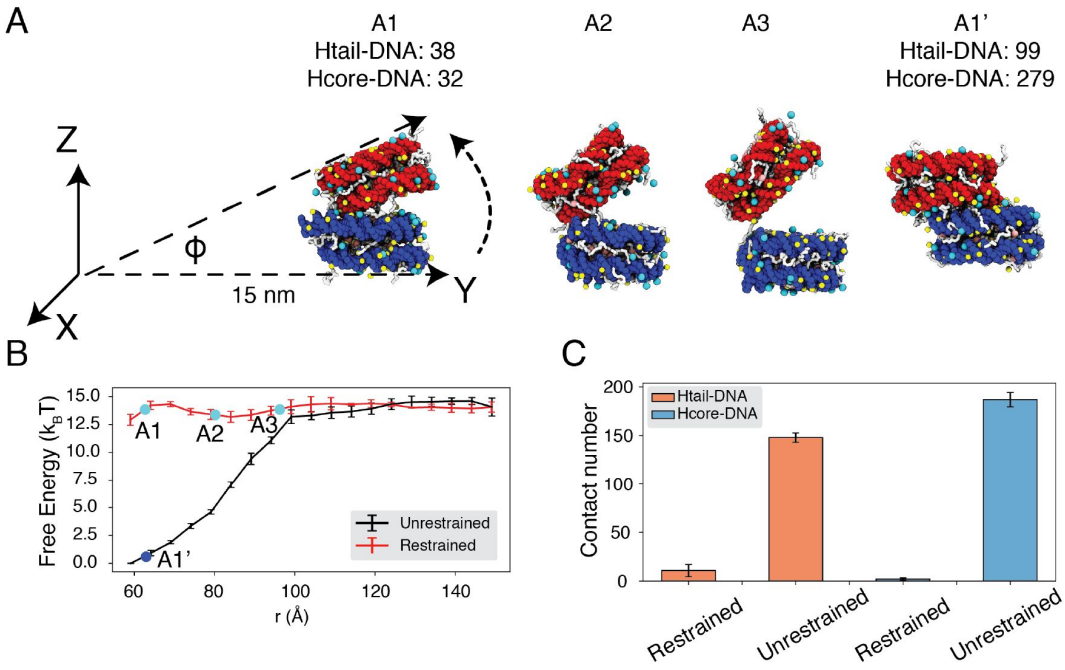

Figure 4

Close contacts give rise to strong internucleosomal interactions.

(A) Illustration of the simulation protocol employed to mimic the nucleosome unbinding pathway dictated by the DNA origami device.[43] The three configurations, A1, A2, and A3, corresponding to the three cyan dots in part B at distances 62.7, 80.2, and 96.3 Å. For comparison, a tightly bound configuration uncovered in simulations without any restraints of nucleosome movement is shown as A1'. The number of contacts formed by histone tails and DNA (Htail-DNA) and by histone core and DNA (Hcore-DNA) from different nucleosomes are shown for A1 and A1'. (B) Free energy profile as a function of the distance between the geometric centers of the two nucleosomes, computed from unrestrained (black) and DNA origami-restrained simulations (red). Error bars were computed as the standard deviation of three independent estimates. (C) Average inter-nucleosomal contacts between DNA and histone tail (orange) and core (blue) residues, computed from unrestrained and DNA origami-restrained simulations. Error bars were computed as the standard deviation of three independent estimates.

Strikingly, the two sets of simulations produced dramatically different binding free energies. Restricting nucleosome orientations produced a binding free energy of ~ 2k_BT, reproducing the experimental value (**Fig. 4B**, S4). On the other hand, the binding free energy increased to 15 k_BT upon removing the constraints. Further examination of inter-nucleosomal contacts revealed the origin of the dramatic difference in nucleosome binding free energies. As shown in **Fig. 4C**, the average number of contacts formed between histone tails and DNA from different nucleosomes is around 150 and 10 in the two sets of simulations. A similar trend is observed for histone core-DNA contacts across nucleosomes. The differences are most dramatic at small distances (Fig. S5) and are clearly visible in the most stable configurations. For example, from the unrestricted simulations, the most stable binding mode corresponds to a configuration in which the two nucleosomes are almost parallel to each other (see configuration A1' in **Fig. 4A**), with the angle between the two nucleosome planes close to zero (Fig. S6C). However, the inherent design of the DNA-origami device renders this binding mode inaccessible, and the smallest angle between the two nucleosome planes is around 23° (see configuration A1 in **Fig. 4A**). Therefore, a significant loss of inter-nucleosomal contacts caused the small binding free energy seen experimentally.

Modulation of nucleosome binding free energy by *in vivo* factors

The predicted strength for unrestricted inter-nucleosome interactions supports their significant contribution to chromatin organization *in vivo*. However, the salt concentration studied above and in the DNA origami experiment is much higher than the physiological value.[42] To further evaluate the *in vivo* significance of inter-nucleosome interactions, we computed the binding free energy at the physiological salt concentration of 150 mM NaCl and 2 mM of $MgCl_2$.

We observe a strong dependence of nucleosome orientations on the inter-nucleosome distance. A collective variable, θ, was introduced to quantify the angle between the two nucleosomal planes (**Fig. 5A**). As shown in two-dimensional binding free energy landscape of internucleosome distance, r, and θ (**Fig. 5B**), at small distances (~60 °A), the two nucleosomes prefer a face-to-face binding mode with small θ values. As the distance increases, the nucleosomes will almost undergo a 90° rotation to adopt perpendicular positions. Such orientations allow the nucleosomes to remain in contact and is more energetically favorable. The orientation preference gradually diminishes at large distances once the two nucleosomes are completely detached. Importantly, we observed a strong inter-nucleosomal interaction with two nucleosomes wrapped by 147-bp 601-sequence DNA (~ 9 k_BT).

Furthermore, we found that the nucleosome binding free energy is minimally impacted by the precise DNA sequence. For example, when the 601 sequence is replaced with poly-dA:dT or poly-dG:dC, the free energy only varied by ~2 k_BT (Fig. S7). However, the poly-dA:dT sequence produced stronger binding while poly-dG:dC weakened the interactions. The sequence specific effects are potentially due to the increased stiffness of poly-dA:dT DNA,[81] which causes the DNA to unwrap more frequently, increasing cross nucleosome contacts at larger distances (Fig. S8).

In addition to variations in DNA sequences, *in vivo* nucleosomes also feature different linker lengths. We performed simulations that extend the 601 sequence with 10 extra base pairs of poly-dA:dT sequence at each end, reaching a nucleosome repeat length (NRL) of 167 bp. Consistent with previous studies,[60,82,83] increasing the NRL weakened inter-nucleosomal interactions (**Fig. 5C** and Fig. S9), reducing the binding free energy to ~ 6 k_BT.

Importantly, we found that the weakened interactions upon extending linker DNA can be more than compensated for by the presence of histone H1 proteins. This is demonstrated in **Fig. 5C** and Fig. S9, where the free energy cost for tearing part two nucleosomes with 167 bp DNA in the presence of linker histones (blue) is significantly higher than the curve for bare nucleosomes (red). Notably, at larger inter-nucleosome distances, the values even exceed those for 147 bp nucleosomes (black). A closer examination of the simulation configurations suggests that the disordered C-terminal tail of linker histones can extend and bind the DNA from the second

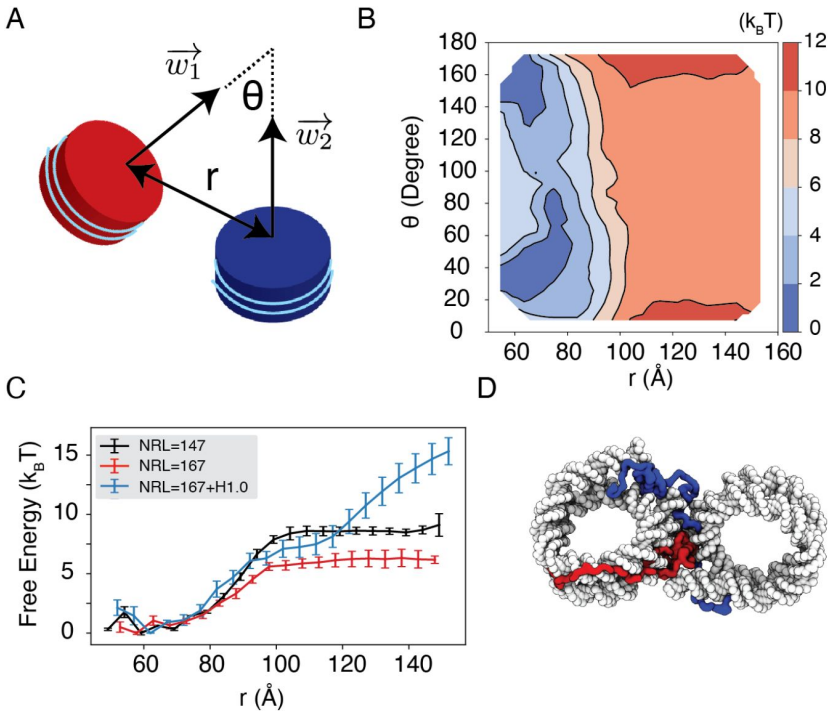

Figure 5

Simulations predict significant internucleosome interactions at physiological conditions.

(A) Illustration of the collective variable, θ defined as the angle between two nucleosomal planes, and r defined as the distance between the nucleosome geometric centers. $\vec{w}_1$ and $\vec{w}_2$ represent the axes perpendicular to the nucleosomal planes. (B) The 2D binding free energy profile as a function of θ and r at the physiological salt condition (150mM NaCl and 2mM $MgCl_2$) for nucleosomes with the 601 sequence. (C) Dependence of nucleosome binding free energy on nucleosome repeat length (NRL) and linker histone H1.0. (D) Representative structure showing linker histones (orange and green) mediating inter-nucleosomal contacts.

nucleosome, thereby stabilizing the internucleosomal contacts (as shown in **Fig. 5D**). Our results are consistent with prior studies that underscore the importance of linker histones in chromatin compaction,[84,85] particularly in eukaryotic cells with longer linker DNA.[78,86]

Discussion

We introduced a residue-level coarse-grained model with explicit ions to accurately account for electrostatic contributions to chromatin organization. The model achieves quantitative accuracy in reproducing experimental values for the binding affinity of protein-DNA complexes, the energetics of nucleosomal DNA unwinding, nucleosome binding free energy, and the sedimentation coefficients of nucleosome arrays. It captures the counterion atmosphere around the nucleosome core particle as seen in all-atom simulations[68] and highlights the contribution of counterions to nucleosome stability. The coarse-grained model also succeeds in resolving the difference between monovalent and divalent ions, supporting the efficacy of divalent ions in neutralizing negative charges and offsetting repulsive interactions among DNA segments.

One significant finding from our study is the predicted strong inter-nucleosome interactions under the physiological salt environment, reaching approximately 9 k_BT. We showed that the much lower value reported in a previous DNA origami experiment is due to the restricted nucleosomal orientation inherent to the device design. Unrestricted nucleosomes allow more close contacts to stabilize binding. A significant nucleosome binding free energy also agrees with the high forces found in single-molecule pulling experiments that are needed for chromatin unfolding.[40,42,87] We also demonstrate that this strong inter-nucleosomal interaction is largely preserved at longer nucleosome repeat lengths (NRL) in the presence of linker histone proteins. While post-translational modifications of histone proteins may influence inter-nucleosomal interactions, their effects are limited, as indicated by Ding et al. [75], and are unlikely to completely abolish the significant interactions reported here. Therefore, we anticipate that, in addition to molecular motors, chromatin regulators, and other molecules inside the nucleus, intrinsic inter-nucleosome interactions are important players in chromatin organization *in vivo*.

We focused our study on single chromatin chains. Strong inter-nucleosome interactions support the compaction and stacking of chromatin, promoting the formation of fibril-like structures. However, as shown in many studies,[39,88–90] such fibril configurations can hardly be detected *in vivo*. It is worth emphasizing that this lack of fibril configurations does not contradict our conclusion on the significance of inter-nucleosome interactions. In a prior paper, we found that many *in vivo* factors, most notably crowding, could disrupt fibril configurations in favor of inter-chain contacts.[76] The inter-chain contacts can indeed be driven by favorable inter-nucleosome interactions.

Several aspects of the coarse-grained model presented here can be further improved. For instance, the introduction of specific protein-DNA interactions could help address the differences in non-bonded interactions between amino acids and nucleotides beyond electrostatics.[31] Such a modification would enhance the model's accuracy in predicting interactions between chromatin and chromatin-proteins. Additionally, the single-bead-per-amino-acid representation used in this study encounters challenges when attempting to capture the influence of histone modifications, which are known to be prevalent in native nucleosomes. Multiscale simulation approaches may be necessary.[91] One could first assess the impact of these modifications on the conformation of disordered histone tails using atomistic simulations. By incorporating these conformational changes into the coarse-grained model, systematic investigations of histone modifications on nucleosome interactions and chromatin organization can be conducted. Such a strategy may eventually enable the direct quantification of interactions among native nucleosomes and even the prediction of chromatin organization *in vivo*.

Methods and Materials

Coarse-grained modeling of chromatin

The large system size of chromatin and the slow timescale for its conformational relaxation necessitates coarse-grained modeling. Following previous studies,[5,31,75,76,92] we adopted a residue-level coarse-grained model for efficient simulations of chromatin. The structure-based model[93,94] was applied to represent the histone proteins with one bead per amino acid and to preserve the tertiary structure of the folded regions. The disordered histone tails were kept flexible without tertiary structure biases. A sequence-specific potential, in the form of the Lennard Jones (LJ) potential and with the strength determined from the Miyazwa-Jernigan (MJ) potential,[95] was added to describe the interactions between amino acids. The 3SPN.2C model was adopted to represent each nucleotide with three beads and interactions among DNA beads follow the potential outlined in Ref. 96, except that the charge of each phosphate site was switched from −0.6 to −1.0 to account for the presence of explicit ions. The Coulombic potential was applied between charged protein and DNA particles. In addition, a weak, non-specific LJ potential was used to account for the excluded volume effect among all protein-DNA beads. Detail expressions for protein-protein and protein-DNA interaction potentials can be found in Ref. 75 and the Supporting Information.

We observe that residue-level coarse-grained models have been extensively utilized in prior studies to examine the free energy penalty associated with nucleosomal DNA unwinding,[97–99] sequence-dependent nucleosome sliding,[100,101] binding free energy between two nucleosomes,[49] chromatin folding,[75,76] the impact of histone modifications on tri-nucleosome structures,[102] and protein-chromatin interactions.[92,103] The frequent quantitative agreement between simulation and experimental results supports the utility of such models in chromatin studies. Our introduction of explicit ions, as detailed below, further extends the applicability of these models to explore the dependence of chromatin conformations on salt concentrations.

Coarse-grained modeling of counter ions

Explicit particle-based representations for monovalent and divalent ions are needed to accurately account for electrostatic interactions.[54–57,104–106] We followed Freeman et al.[54] to introduce explicit ions (see **Fig. 1**) and adopted their potentials to describe the interactions between ions and nucleotide particles, with detailed expressions provided in the Supporting Information. Parameters in these potentials were tuned by Freeman et al. [54] to reproduce the radial distribution functions and the potential of mean force between ion pairs determined from all-atom simulations.

This explicit ion model was originally introduced for nucleic acid simulations. We generalized the model for protein simulations by approximating the interactions between charged amino acids and ions with parameters tuned for phosphate sites. Parameter values for ion-amino acid interactions are provided in Table S1 and S2.

Details of molecular dynamics simulations

We simulated various chromatin systems, including a single nucleosome, two nucleosomes, and a 12-mer nucleosome array. The initial configurations for the molecular dynamics simulations were constructed based on the crystal structure of a single nucleosome with PDB ID: 1KX5[67] and 3LZ1,[107] or a tetranucleosome with PDB ID: 1ZBB.[108] We used the 3DNA software[109] to add additional DNA, connect and align nucleosomes, and extend the chain length as necessary. Further details on constructing the initial configurations are provided in the Supporting Information. Chromatin was positioned at the center of a cubic box with a length selected to avoid interactions

between nucleosomes and their periodic images. Counterions were added on a uniformly spaced grid to achieve the desired salt concentrations and to neutralize the system. The number of ions and the size of simulation boxes are provided in Table S3.

All simulations were performed at constant temperature and constant volume (NVT) using the software package LAMMPS.[110] The electrostatic interactions were implemented with the particle-particle particle-mesh solver, with the relative root-mean-square error in per-atom force set to 0.0001.[111] A Nośe-Hoover style algorithm[112] was used to maintain the system temperature at 300K with a damping parameter of 1 ps. We further modeled the histone core and the inner layer of the nucleosomal DNA together as a rigid body to improve computational efficiency. This approximation does not affect the thermodynamic properties of chromatin.[75,76] Umbrella simulations were used to enhance the sampling of the conformational space,[65] and details of the collective variables employed in these simulations are provided in the Supporting Information. All the results presented in the main text are reweighted from the biased simulations by the weighted histogram algorithm.[74]

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Data availability

Implementation of the residue-level coarse-grained model and examples for setting up and performing simulations can be found on our GitHub repository.

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Editors

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Joint Public Review:

In this manuscript, the authors introduced an explicit ion model using the coarse-grained modelling approach to model the interactions between nucleosomes and evaluate their effects on chromatin organization. The strength of this method lies in the explicit representation of counterions, especially divalent ions, which are notoriously difficult to model. To achieve their aims and validate the accuracy of the model, the authors conducted coarse-grained molecular dynamics simulations and compared predicted values to the experimental values of the binding energies of protein-DNA complexes and the free energy profile of nucleosomal DNA unwinding and inter-nucleosome binding. Additionally, the authors employed umbrella sampling simulations to further validate their model, reproducing experimentally measured sedimentation coefficients of chromatin under varying salt concentrations of monovalent and divalent ions.

The significance of this study lies in the authors' coarse-grained model which can efficiently capture the conformational sampling of molecules while maintaining a low computational cost. The model reproduces the scale and, in some cases, the shape of the experimental free energy profile for specific molecule interactions, particularly inter-nucleosome interactions. Additionally, the authors' method resolves certain experimental discrepancies related to determining the strength of inter-nucleosomal interactions. Furthermore, the results from this study support the crucial role of intrinsic physicochemical interactions in governing chromatin organization within the nucleus.

The authors have successfully addressed the majority of my key concerns. I appreciate the clarification regarding the parameterization from Pablo's lab and the addition of comparisons of energy profiles as a function of inter-nucleosome distances.

However, the statement "The agreement is evident" may not sufficiently capture the essence of Figure S4, as there is a shortage of substantial agreement. The authors rightly acknowledge it but should delineate the nature of the observed discrepancies.

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Author Response

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

> ***eLife assessment***
>
> *The authors have developed a compelling coarse-grained simulation approach for nucleosome-nucleosome interactions within a chromatin array. The data presented are solid and provide new insights that allow for predictions of how chromatin interactions might occur in vivo, but some of the claims should be tempered. The tools will be valuable for the chromosome biology field.*

Response: We want to thank the editors and all the reviewers for their insightful comments. We have made substantial changes to the manuscript to improve its clarity and temper necessary claims, as detailed in the responses, and we performed additional analyses to address the reviewers' concerns. We believe that we have successfully addressed all the comments, and the quality of our paper has improved significantly.

In the following, we provide point-to-point responses to all the reviewer comments.

> ***RESPONSE TO REFEREE 1:***
>
> *Comment 0: This study develops and applies a coarse-grained model for nucleosomes with explicit ions. The authors perform several measurements to explore the utility of a coarse-grained simulation method to model nucleosomes and nucleosome arrays with explicit ions and implicit water. 'Explicit ions' means that the charged ions are modeled as particles in simulation, allowing the distributions and dynamics of ions to be measured. Since nucleosomes are highly charged and modulated by charge modifications, this innovation is particularly relevant for chromatin simulation.*

Response: We thank the reviewer's excellent summary of the work.

> *Comment 1: Strengths: This simulation method produces accurate predictions when compared to experiments for the binding affinity of histones to DNA, counterion interactions, nucleosome DNA unwinding, nucleosome binding free energies, and sedimentation coefficients of arrays. The variety of measured quantities makes both this work and the impact of this coarse-grained methodology compelling. The comparison between the contributions of sodium and magnesium ions to nucleosome array compaction, presented in Figure 3, was exciting and a novel result that this simulation methodology can assess.*

Response: We appreciate the reviewer's strong assessment of the paper's significance, novelty, and broad interest, and we thank him/her for the detailed suggestions and comments.

> *Comment 2: Weaknesses: The presentation of experimental data as representing in vivo systems is a simplification that may misrepresent the results of the simulation work. In vivo, in this context, typically means experimental data from whole cells. What one could expect for in vivo experimental data is measurements on nucleosomes from cell lysates where various and numerous chemical modifications are present. On the contrary, some of the experimental data used as a comparison are from in vitro studies. In vitro in this context means nucleosomes were formed 'in a test tube' or under controlled conditions that do not represent the complexity of an in vivo system. The simulations performed here are more directly compared to in vitro conditions. This distinction likely impacts to what extent these simulation results are biologically relevant. In vivo and in vitro differences could be clarified throughout and discussed.*

Response: As detailed in Response to Comment 3, we have made numerous modifications in the Introduction, Results, and Discussion Section to emphasize the differences between reconstituted and native nucleosomes. The newly added texts also delve into the utilization of the interaction strength measured for reconstituted nucleosomes as a reference point for conceptualizing the interactions among native nucleosomes.

> *Comment 3: In the introduction (pg. 3), the authors discuss the uncertainty of nucleosome-tonucleosome interaction strengths in vivo. For example, the authors discuss works such as Funke et al. However, Funke et al. used reconstituted nucleosomes from recombinant histones with one controlled modification (H4 acetylation). Therefore, this study that the authors discuss is measuring nucleosome's in vitro affinity, and there could be significant differences in vivo due to various posttranslational modifications. Please revise the introduction, results section "Close contacts drive nucleosome binding free energy," and discussion to reflect and clarify the difference between in vitro and in vivo measurements. Please also discuss how biological variability could impact your findings in vivo. The works of Alexey Onufriev's lab on the sensitivity of nucleosomes to charge changes (10.1016/j.bpj.2010.06.046, 10.1186/s13072-018-0181-5), such as some PTMs, are one potential starting place to consider how modifications alter nucleosome stability in vivo.*

Response: We thank the reviewer for the insightful comments and agree that native nucleosomes can differ from reconstituted nucleosomes due to the presence of histone modifications.

We have revised the introduction to emphasize the differences between in vitro and in vivo nucleosomes. The new text now reads

"The relevance of physicochemical interactions between nucleosomes to chromatin organization in vivo has been constantly debated, partly due to the uncertainty in their strength [cite]. Examining the interactions between native nucleosomes poses challenges due to the intricate chemical modifications that histone proteins undergo within the nucleus and the variations in their underlying DNA sequences [cite]. Many in vitro experiments have opted for reconstituted nucleosomes that lack histone modifications and feature wellpositioned 601-sequence DNA to simplify the chemical complexity. These experiments aim to establish a fundamental reference point for understanding the strength of interactions within native nucleosomes. Nevertheless, even with reconstituted nucleosomes, a consensus regarding the significance of their interactions remains elusive. For example, using force-measuring magnetic tweezers, Kruithof et al. estimated the inter-nucleosome binding energy to be ~ 14 kBT [cite]. On the other hand, Funke et al. introduced a DNA origamibased force spectrometer to directly probe the interaction between a pair of nucleosomes [cite], circumventing any potential complications from interpretations of single molecule traces of nucleosome arrays. Their measurement reported a much weaker binding free energy of

approximately 2 kBT. This large discrepancy in the reported reference values complicates a further assessment of the interactions between native nucleosomes and their contribution to chromatin organization in vivo."

We modified the first paragraph of the results section to read

"Encouraged by the explicit ion model's accuracy in reproducing experimental measurements of single nucleosomes and nucleosome arrays, we moved to directly quantify the strength of inter-nucleosomes interactions. We once again focus on reconstituted nucleosomes for a direct comparison with in vitro experiments. These experiments have yielded a wide range of values, ranging from 2 to 14 kBT [cite]. Accurate quantification will offer a reference value for conceptualizing the significance of physicochemical interactions among native nucleosomes in chromatin organization in vivo."

New text was added to the Discussion Section to emphasize the implications of simulation results for interactions among native nucleosomes.

"One significant finding from our study is the predicted strong inter-nucleosome interactions under the physiological salt environment, reaching approximately 9 kBT. We showed that the much lower value reported in a previous DNA origami experiment is due to the restricted nucleosomal orientation inherent to the device design. Unrestricted nucleosomes allow more close contacts to stabilize binding. A significant nucleosome binding free energy also agrees with the high forces found in single-molecule pulling experiments that are needed for chromatin unfolding [cite]. We also demonstrate that this strong inter-nucleosomal interaction is largely preserved at longer nucleosome repeat lengths (NRL) in the presence of linker histone proteins. While posttranslational modifications of histone proteins may influence inter-nucleosomal interactions, their effects are limited, as indicated by Ding et al. [cite], and are unlikely to completely abolish the significant interactions reported here. Therefore, we anticipate that, in addition to molecular motors, chromatin regulators, and other molecules inside the nucleus, intrinsic inter-nucleosome interactions are important players in chromatin organization in vivo."

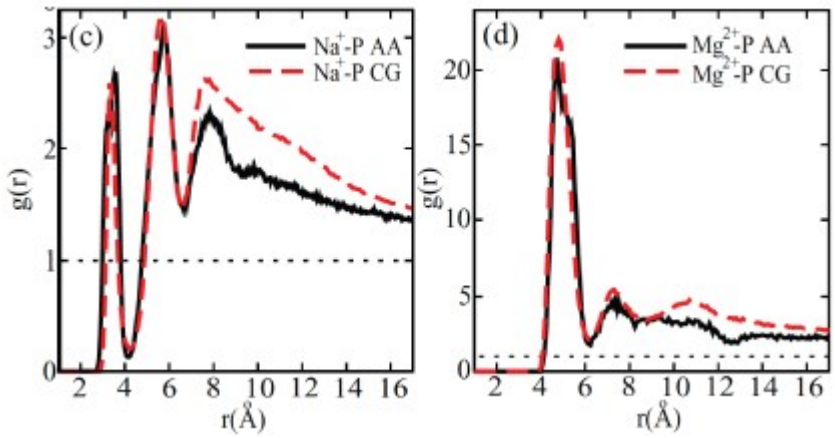
The suggested references (10.1016/j.bpj.2010.06.046, 10.1186/s13072-018-0181-5) are now included as citations # 44 and 45.

> *Comment 4: Due to the implicit water model, do you know if ions can penetrate the nucleosome more? For example, does the lack of explicit water potentially cause sodium to cluster in the DNA grooves more than is biologically relevant, as shown in Figure 1?*

Response: We thank the reviewer for the insightful comments. The parameters of the explicit-ion model were deduced from all-atom simulations and fine-tuned to replicate crucial aspects of the local ion arrangements around DNA (1). The model's efficacy was demonstrated in reproducing the radial distribution function of Na+ and Mg2+ ion distributions in the proximity of DNA (see Author response image 1). Consequently, the number of ions near DNA in the coarse-grained models aligns with that observed in all-atom simulations, and we do not anticipate any significant, unphysical clustering. It is worth noting that previous atomistic simulations have also reported the presence of a substantial quantity of Na+ ions in close proximity to nucleosomal DNA (refer to Author response image 2).

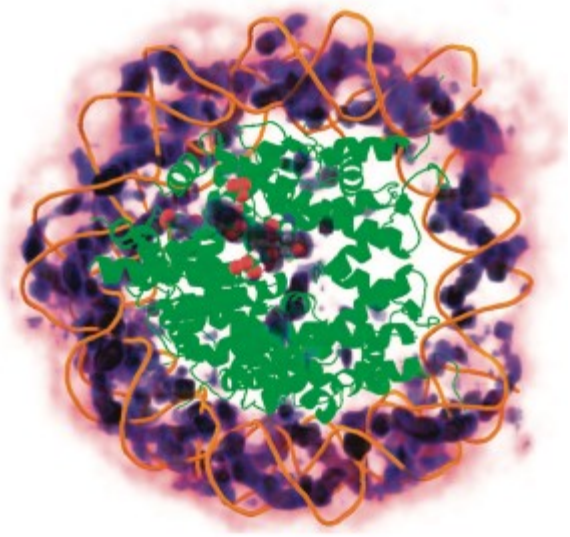
Author response image 1.

Comparison between the radial distribution functions of Na+ (left) and Mg2+ (right) ions around the DNA phosphate groups computed from all-atom (black) and coarse-grained (red) simulations. Figure adapted from Figure 4 of Ref. 1. The coarse-grained explicit ion model used in producing the red curves is identical to the one presented in the current manuscript.



Author response image 2.

Three-dimensional distribution of sodium ions around the nucleosome determined from all-atom explicit solvent simulations. Darker blue colors indicate higher sodium density and high density of sodium ions around the DNA is clearly visible. The crystallographically identified acidic patch has been highlighted as spheres on the surface of the histone core and a high level of sodium condensation is observed around these residues. Figure adapted from Ref. 2.



> *Comment 5: Histone side chain to DNA interactions, such as histone arginines to DNA, are essential for nucleosome stability. Therefore, can the authors provide validation or references supporting your model of the nucleosome with one bead per amino acid? I would like to see if the nucleosomes are stable in an extended simulation or if similar dynamic motions to all-atom simulations are observed.*

Response: The nucleosome model, which employs one bead per amino acid and lacks explicit ions, has undergone extensive calibration and has found application in numerous prior studies. For instance, the de Pablo group utilized a similar model to showcase its ability to accurately replicate the experimentally measured nucleosome unwinding free energy penalty (3), sequence-dependent nucleosome sliding (4), and the interaction between two nucleosomes (5). Similarly, the Takada group employed a comparable model to investigate

acetylation-modulated tri-nucleosome structures (6), chromatin structures influenced by chromatin factors (7), and nucleosome sliding (8). Our group also employed this model to study the structural rearrangement of a tetranucleosome (9) and the folding of larger chromatin systems (10). In cases where data were available, simulations frequently achieved quantitative reproduction of experimental results.

We added the following text to the manuscript to emphasize previous studies that validate the model accuracy.

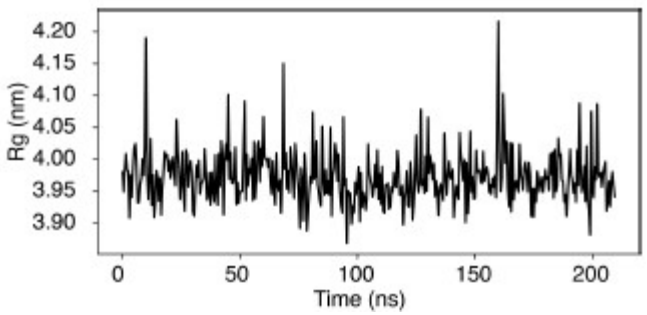
"We observe that residue-level coarse-grained models have been extensively utilized in prior studies to examine the free energy penalty associated with nucleosomal DNA unwinding [cite], sequence-dependent nucleosome sliding [cite], binding free energy between two nucleosomes [cite], chromatin folding [cite], the impact of histone modifications on tri-nucleosome structures [cite], and protein-chromatin interactions [cite]. The frequent quantitative agreement between simulation and experimental results supports the utility of such models in chromatin studies. Our introduction of explicit ions, as detailed below, further extends the applicability of these models to explore the dependence of chromatin conformations on salt concentrations."

We agree that arginines are important for nucleosome stability. Since we assign positive charges to these residues, their contribution to DNA binding can be effectively captured. The model's ability in reproducing nucleosome stability is supported by the good agreement between the simulated free energy penalty associated with nucleosomal DNA unwinding and experimental value estimated from single molecule experiments (Figure 1).

To further evaluate nucleosome stability in our simulations, we conducted a 200-ns-long simulation of a nucleosome featuring the 601-sequence under physiological salt conditions–100 mM NaCl and 0.5 mM MgCl2, consistent with the conditions in Figure 1 of the main text. We found that the nucleosome maintains its overall structure during this simulation. The nucleosome's radius of gyration (Rg) remained proximate to the value corresponding to the PDB structure (3.95 nm) throughout the entire simulation period (see Author response image 3).

Author response image 3.

Time trace of the radius of gyration (Rg) of a nucleosome with the 601-sequence along an unbiased, equilibrium trajectory. It is evident the Rg fluctuates around the value found in the PDB structure (3.95 nm), supporting the stability of the nucleosome in our simulation.



Occasional fluctuations in Rg corresponded to momentary, partial unwrapping of the nucleosomal DNA, a phenomenon observed in single-molecule experiments. However, we advise caution due to the coarse-grained nature of our simulations, which prevents a direct mapping of simulation timescale to real time. Importantly, the rate of DNA unwrapping in our simulations is notably overestimated.

It's plausible that coarse-grained models, lacking side chains, might underestimate the barrier for DNA sliding along the nucleosome. Specifically, our model, without differentiation between interactions among various amino acids and nucleotides, accurately reproduces the average nucleosomal DNA binding affinity but may not capture the energetic variations among binding interfaces. Since sliding's contribution to chromatin organization is minimal due to the use of strongly positioning 601 sequences, we imposed rigidity on the two nucleotides situated at the dyad axis to prevent nucleosomal DNA sliding. In future studies, enhancing the calibration of protein-DNA interactions to achieve improved sequence specificity would be an intriguing avenue. To underscore this limitation of the model, we have included the following text in the discussion section of the main text.

"Several aspects of the coarse-grained model presented here can be further improved. For instance, the introduction of specific protein-DNA interactions could help address the differences in non-bonded interactions between amino acids and nucleotides beyond electrostatics [cite]. Such a modification would enhance the model's accuracy in predicting interactions between chromatin and chromatin-proteins. Additionally, the single-bead-per-amino-acid representation used in this study encounters challenges when attempting to capture the influence of histone modifications, which are known to be prevalent in native nucleosomes. Multiscale simulation approaches may be necessary [cite]. One could first assess the impact of these modifications on the conformation of disordered histone tails using atomistic simulations. By incorporating these conformational changes into the coarse-grained model, systematic investigations of histone modifications on nucleosome interactions and chromatin organization can be conducted. Such a strategy may eventually enable the direct quantification of interactions among native nucleosomes and even the prediction of chromatin organization in vivo."

> *Comment 6: The solvent salt conditions vary in the experimental reference data for internucleosomal interaction energies. The authors note, for example, that the in vitro data from Funke et al. differs the most from other measurements, but the solvent conditions are 35 mM NaCl and 11 mM MgCl2. Since this simulation method allows for this investigation, could the authors speak to or investigate if solvent conditions are responsible for the variability in experimental reference data? The authors conclude on pg. 8-9 and Figure 4 that orientational restraints in the DNA origami methodology are responsible for differences in interaction energy. Can the authors rule out ion concentration contributions?*

Response: We thank the reviewer for the insightful comment. We would like to clarify that the black curve presented in Figure 4B of the main text was computed using the salt concentration specified by Funke et al. (35 mM NaCl and 11 mM MgCl2). Furthermore, there were no restraints placed on nucleosome orientations during these calculations. Consequently, the results in Figure 4B can be directly compared with the black curve in Figure 5C. The data in Figure 5C were calculated under physiological salt conditions (150 mM NaCl and 2 mM MgCl2), which are the standard solvent salt conditions used in most studies. It is worth noting that the free energy of nucleosome binding is significantly higher at the salt concentration employed by Funke et al. (14 kBT) than the value at the physiological salt condition (9 kBT). Therefore, comparing the results in Figure 4B and 5C eliminates ion concentration conditions as a potential cause for the the almost negligible result reported by Funke et al.

> *Comment 7: In the discussion on pg. 12 residual-level should be residue-level.*

Response: We apologize for the oversight and have corrected the grammatical error in our manuscript.

> ***RESPONSE TO REFEREE 2:***
>
> *Comment 0: In this manuscript, the authors introduced an explicit ion model using the coarse-grained modelling approach to model the interactions between nucleosomes and evaluate their effects on chromatin organization. The strength of this method lies in the explicit representation of counterions, especially divalent ions, which are notoriously difficult to model. To achieve their aims and validate the accuracy of the model, the authors conducted coarse-grained molecular dynamics simulations and compared predicted values to the experimental values of the binding energies of protein-DNA complexes and the free energy profile of nucleosomal DNA unwinding and inter-nucleosome binding. Additionally, the authors employed umbrella sampling simulations to further validate their model, reproducing experimentally measured sedimentation coefficients of chromatin under varying salt concentrations of monovalent and divalent ions.*

Response: We thank the reviewer's excellent summary of the work.

> *Comment 1: The significance of this study lies in the authors' coarse-grained model which can efficiently capture the conformational sampling of molecules while maintaining a low computational cost. The model reproduces the scale and, in some cases, the shape of the experimental free energy profile for specific molecule interactions, particularly inter-nucleosome interactions. Additionally, the authors' method resolves certain experimental discrepancies related to determining the strength of inter-nucleosomal interactions. Furthermore, the results from this study support the crucial role of intrinsic physicochemical interactions in governing chromatin organization within the nucleus.*

Response: We appreciate the reviewer's strong assessment of the paper's significance, novelty, and broad interest, and we thank him/her for the detailed suggestions and comments.

> *Comment 2: The method is simple but can be useful, given the authors can provide more details on their ion parameterization. The paper says that parameters in their "potentials were tuned to reproduce the radial distribution functions and the potential of mean force between ion pairs determined from all-atom simulations." However, no details on their all-atom simulations were provided; at some point, the authors refer to Reference 67 which uses all-atom simulations but does not employ the divalent ions. Also, no explanation is given for their modelling of protein-DNA complexes.*

Response: We appreciate the reviewer's suggestion on clarifying the parameterization of the explicition model. The parameterization was not carried out in reference 67 nor by us, but by the de Pablo group in citation 53. Specifically, ion potentials were parameterized to fit the potential of mean force between both monovalent and divalent ion pairs, calculated either from all-atom simulations or from the literature. The authors carried out extensive validations of the model parameters by comparing the radial distribution functions of ions computed using the coarse-grained model with those from all-atom simulations. Good agreements between coarse-grained and all-atom results ensure that the parameters' accuracy in reproducing the local structures of ion interactions.

To avoid confusion, we have revised the text from:

"Parameters in these potentials were tuned to reproduce the radial distribution functions and the potential of mean force between ion pairs determined from all-atom simulations."

to

"Parameters in these potentials were tuned by Freeman et al. [cite] to reproduce the radial distribution functions and the potential of mean force between ion pairs determined from all-atom simulations."

We modified the Supporting Information at several places to clarify the setup and interpretation of protein-DNA complex simulations.

For example, we clarified the force fields used in these simulation with the following text

"All simulations were carried out using the software Lammps [cite] with the force fields defined in the previous two sections."

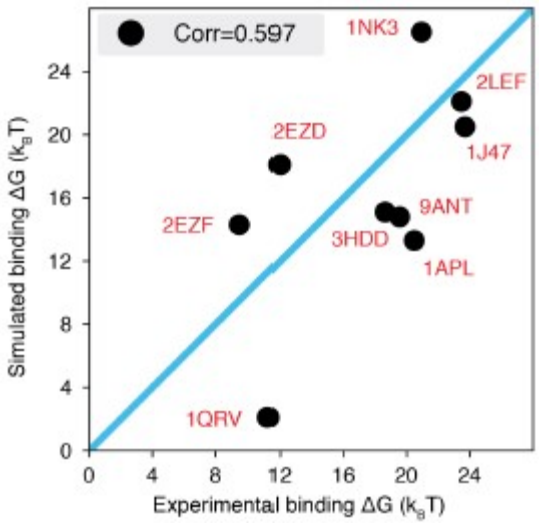
We added details on the preparation of these simulations as follows

"We carried out a series of umbrella-sampling simulations to compute the binding free energies of a set of nine protein-DNA complexes with experimentally documented binding dissociation constants [cite]. Initial configurations of these simulations were prepared using the crystal structures with the corresponding PDB IDs listed in Fig. S1."

We further revised the caption of Figure S1 (included as Author response image 4) to facilitate the interpretation of simulation results.

Author response image 4.

The explicit-ion model predicts the binding affinities of protein-DNA complexes well, related to Fig. 1 of the main text. Experimental and simulated binding free energies are compared for nine protein-DNA complexes [cite], with a Pearson Correlation coefficient of 0.6. The PDB ID for each complex is indicated in red, and the diagonal line is drawn in blue. The significant correlation between simulated and experimental values supports the accuracy of the model. To further enhance the agreement between the two, it will be necessary to implement specific non-bonded interactions that can resolve differences among amino acids and nucleotides beyond simple electrostatics. Such modifications will be interesting avenues for future research. See text Section: Binding free energy of protein-DNA complexes for simulation details.



> *Comment 3: Overall, the paper is well-written, concise and easy to follow but some statements are rather blunt. For example, the linker histone contribution (Figure 5D) is not clear and could be potentially removed. The result on inter-nucleosomal interactions*

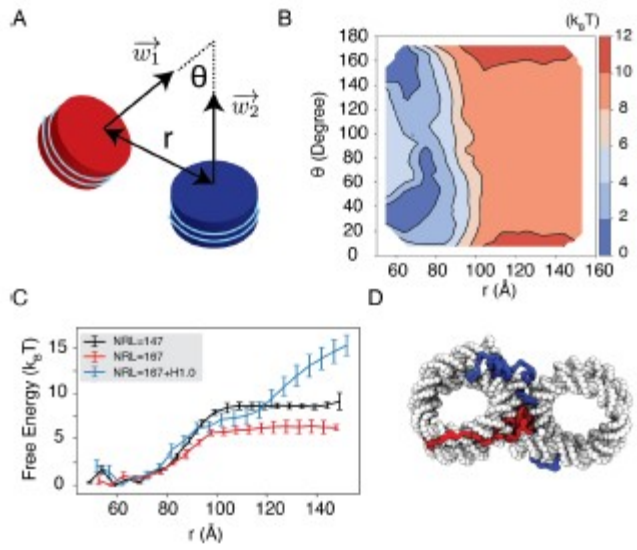
> *and comparison to experimental values from Ref#44 is the most compelling. It would be nice to see if the detailed shape of the profile for restrained inter-nucleosomal interactions in Figure 4B corresponds to the experimental profile. Including the dependence of free energy on a vertex angle would also be beneficial.*

Response: We thank the reviewer for the comments and agree that the discussion on linker histone results was brief. However, we believe the results are important and demonstrate our model's advantage over mesoscopic approaches in capturing the impact of chromatin regulators on chromatin organization.

Therefore, instead of removing the result, we expanded the text to better highlight its significance, to help its comprehension, and to emphasize its biological implications. The image in Figure 5D was also redesigned to better visualize the cross contacts between nucleosomes mediated by histone H1. The added texts are quoted as below, and the new Figure 5 is included.

Author response image 5.

Revised main text Figure 5, with Figure 5D modified for improved visual clarity.



"Importantly, we found that the weakened interactions upon extending linker DNA can be more than compensated for by the presence of histone H1 proteins. This is demonstrated in Fig. 5C and Fig. S8, where the free energy cost for tearing part two nucleosomes with 167 bp DNA in the presence of linker histones (blue) is significantly higher than the curve for bare nucleosomes (red). Notably, at larger inter-nucleosome distances, the values even exceed those for 147 bp nucleosomes (black). A closer examination of the simulation configurations suggests that the disordered C-terminal tail of linker histones can extend and bind the DNA from the second nucleosome, thereby stabilizing the internucleosomal contacts (as shown in Fig. 5D). Our results are consistent with prior studies that underscore the importance of linker histones in chromatin compaction [cite], particularly in eukaryotic cells with longer linker DNA [cite]."

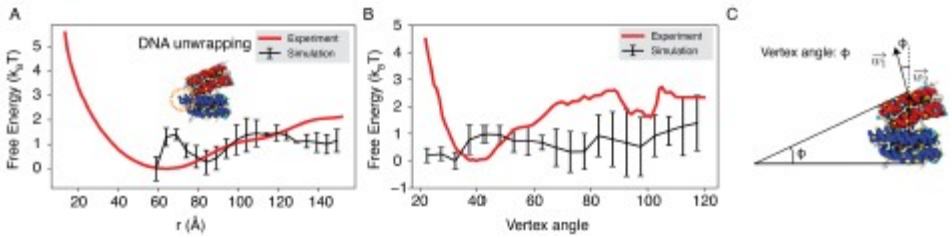
We further compared the simulated free energy profile, depicting the center of mass distance between nucleosomes, with the experimental profile, as depicted in Author response image 6. The agreement between the simulated and experimental results is evident. The nuanced features observed between 60 to 80 Ain the simulated profile stem from DNA unwinding˚to accommodate the incoming nucleosome, creating a small energy barrier. It's worth noting

that such unwinding is unlikely to occur in the experimental setup due to the hybridization method used to anchor nucleosomes onto the DNA origami. Moreover, our simulation did not encompass configurations below 60 A, resulting in a lack of data in˚that region within the simulated profile.

We projected the free energy profile onto the vertex angle of the DNA origami device, utilizing the angle between two nucleosome faces as a proxy. Once more, the simulated profile demonstrates reasonable agreement with the experimental data (Author response image 6). Author response image 6 has been incorporated as Figure S4 in the Supporting Information.

Author response image 6.

Explicit ion modeling reproduces the experimental free energy profiles of nucleosome binding. (A) Comparison between the simulated (black) and experimental (red) free energy profile as a function of the inter-nucleosome distance. Error bars were computed as the standard deviation of three independent estimates. The barrier observed between 60A and 80˚ A arises from the unwinding of nucleosomal DNA when the two nu-˚cleosomes are in close proximity, as highlighted in the orange circle. (B) Comparison between the simulated (black) and experimental (red) free energy profile as a function of the vertex angle. Error bars were computed as the standard deviation of three independent estimates. (C) Illustration of the vertex angle Φ used in panel (B).



> *Comment 4: Another limitation of this study is that the authors' model sacrifices certain atomic details and thermodynamic properties of the modelled systems. The potential parameters of the counter ions were derived solely by reproducing the radial distribution functions (RDFs) and potential of mean force (PMF) based on all-atom simulations (see Methods), without considering other biophysical and thermodynamic properties from experiments. Lastly, the authors did not provide any examples or tutorials for other researchers to utilize their model, thus limiting its application.*

Response: We agree that residue-level coarse-grained modeling indeed sacrifices certain atomistic details. This sacrifice can be potentially limiting when studying the impact of chemical modifications, especially on histone and DNA methylations. We added a new paragraph in the Discussion Section to point out such limitations and the relevant text is quoted below.

"Several aspects of the coarse-grained model presented here can be further improved. For instance, the introduction of specific protein-DNA interactions could help address the differences in non-bonded interactions between amino acids and nucleotides beyond electrostatics [cite]. Such a modification would enhance the model's accuracy in predicting interactions between chromatin and chromatin-proteins. Additionally, the single-bead-per-amino-acid representation used in this study encounters challenges when attempting to capture the influence of histone modifications, which are known to be prevalent in native nucleosomes. Multiscale simulation approaches may be necessary [cite]. One could first assess the impact of these modifications on the conformation of disordered histone tails using

atomistic simulations. By incorporating these conformational changes into the coarse-grained model, systematic investigations of histone modifications on nucleosome interactions and chromatin organization can be conducted. Such a strategy may eventually enable the direct quantification of interactions among native nucleosomes and even the prediction of chromatin organization in vivo."

Nevertheless, it's important to note that while the model sacrifices accuracy, it compensates with superior efficiency. Atomistic simulations face significant challenges in conducting extensive free energy calculations required for a quantitative evaluation of ion impacts on chromatin structures.

The explicit ion model, introduced by the de Pablo group, follows a standard approach adopted by other research groups, such as the parameterization of ion models using the potential of mean force from atomistic simulations (11; 12). According to multiscale coarse-graining theory, reproducing potential mean force (PMF) enables the coarsegrained model to achieve thermodynamic consistency with the atomistic model, ensuring identical statistical properties derived from them. However, it's crucial to recognize that an inherent limitation of such approaches is their dependence on the accuracy of atomistic force fields in reproducing thermodynamic properties from experiments, as any inaccuracies in the atomistic force fields will similarly affect the resulting coarse-grained (CG) model.

We have provided the implementation of CG model and detailed instructions on setting up and performing simulations GitHub repository. Examples include simulation setup for a protein-DNA complex and for a nucleosome with the 601-sequence.

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