

Molecular dynamics simulations reveal subtle consequences of H3K9 and H3K27 tri-methylation on chromatin constituents

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ABSTRACT Epigenetic modifications of histone tails are key mechanisms of genome regulation. In particular, tri-methylation of lysines (K) 9 and K27 of the histone H3 tail is important for genome silencing. In this work, we explore, using all-atom molecular dynamics simulations, the effect of these two epigenetic marks on the structure and interactions of the H3 tail in several contexts: isolated tails, nucleosomes, chromatosomes, and stacked nucleosomes. Overall, we find that although the isolated tails do not show significant conformational changes upon methylation, a more flexible and extended H3 tail compared to the native tail results in the nucleosome systems, with K9 methylation effects more pronounced. This change could facilitate the interaction of the tail with protein readers like heterochromatin protein 1 or Polycomb group. We also observe that both methylations increase the interactions of the H3 tail with the linker DNA in the context of the chromatosome, producing a chromatosome with tighter linker DNA, which could favor chromatin compaction. For stacked nucleosomes mimicking $i \pm 2$ zigzag interactions, methylation of either K9 or K27 reduces the interactions of one of the H3 tails with its parental nucleosome and increases its interactions with the nonparental nucleosome, which could also help compact the chromatin fiber. In the three nucleosome-containing systems, we observe an asymmetry between the two tails, especially in the chromatosome, where one tail extends to interact with the linker DNA. This asymmetry modulates the effect that methylation has on each tail. Thus, overall, methylations of K9 and K27 have a subtle but notable impact on the H3 tail structure and its interactions within the chromatin fiber. These results help explain how this epigenetic modification compacts chromatin fibers and promotes longer-range interactions; these changes also guide how to approximate these effects in coarse-grained chromatin models.

SIGNIFICANCE The epigenetic regulation of gene expression is crucial for controlling many cellular functions, including development, differentiation, and aging. Among the many epigenetic modifications, tri-methylation of lysine residues on positions 9 and 27 of the core histone H3 tail is associated with gene silencing. We apply all-atom molecular dynamics simulations to explore the structural effect of these modifications on the H3 tail and its interactions with nucleosomal and linker DNA. Overall, we find that although these epigenetic changes impact the structure of the H3 tail in a subtle way, they produce a tail that is more flexible and extended than the native tail. This in turn increases the interactions of the tail with neighboring nucleosomes and linker DNA. Importantly, we find that the effect of lysine methylation is asymmetric; depending on the extent of tail folding (e.g., extended versus more compact), methylation increases tail flexibility, extension, and interactions with linker DNA. Overall, our results highlight how epigenetic modifications can regulate the chromatin architecture and thus gene expression.

INTRODUCTION

In the eukaryotic nucleus, the DNA is wrapped around histone proteins in the form of chromatin. The chromatin fiber

allows a tight packing of DNA as well as a controlled unraveling when gene transcription is initiated. Such packing and unpacking can be regulated by epigenetic modifications of the histone proteins (posttranslational modifications [PTMs]) or the DNA, representing a second level of DNA coding. This is because such modifications, implicated in many cellular functions (1), affect the chromatin structure and regulate its interaction with several chromatin

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“readers,” such as transcription factors, remodelers, and repressors.

One of these epigenetic modifications is the methylation of lysine (K) residues on the N-terminal intrinsically disordered tails of the histone core proteins H3 and H4 (2). Methylation does not alter the charge of the residue, but it creates a bulkier residue with different hydrophobic properties. In addition, lysines can be mono-, di-, or tri-methylated (me1, me2, or me3, respectively), conferring high variability to this epigenetic mark.

Histone methylation can be associated with the repression or activation of genes (3). For example, the tri-methylation of H3 on K9 or K27 is associated with silenced chromatin and is involved in many cellular functions, such as the aging process (4,5), X-chromosome inactivation (6), and cancer (7). However, their mechanisms of action are different. Whereas H3K9me3 recruits the heterochromatin protein 1 (HP1) to silence genes, H3K27me3 is recognized by Polycomb group (PcG) proteins that mediate long-range interactions. On the other hand, methylation of H3 on K4, K36, or K79 is mostly recognized by remodeling enzymes and acetyltransferases and are thus related to the activation of gene expression, controlling processes like cancer and development (8), cognitive diseases (9), and cardiomyopathies (10).

These observations highlight the versatility of the K methylation mark, working mostly through the interaction with chromatin readers. This feature also makes it difficult to introduce these nuanced methylation effects into chromatin or chromosome models.

Biomolecular simulations have helped decipher the role of K methylation on genome architecture and regulation. At the histone tail level, molecular dynamics (MD) simulations have shown that methylation produces a change in the backbone dihedral angles ψ and ϕ of the K residue (11), but the overall structure of the methylated tail remains similar to the native tail (12). At a larger scale, MD simulations of nucleosomes without linker DNA and linker histone and with native and tri-methylated H3K4 tails have shown that methylation only affects residues around the methylation site, whereas the rest of the tail remains mostly unaffected (13). Another MD study further showed that tri-methylation of H3 in several K residues reduces the number of contacts between the H3 tail and the DNA near the nucleosome dyad but increases the contacts with the DNA near the entry/exit sites (14). Thus, although major structural changes are absent, specific contacts between the tail and DNA might be affected by K methylation, and these could impact chromatin architecture. Such changes are important to understand and also necessary for introducing the K methylation effect into chromatin mesoscale models.

In this work, we aim to discern patterns regarding the effect of methylation of H3 residues K9 and K27 on the tail structure and its interactions with its parental and nonparental nucleosomes.

Further, we aim to use the information learned at the all-atom level to modify our chromatin mesoscale model to introduce histone methylation effects (15). To this end, we perform MD simulations of isolated H3 tails as well as nucleosomes, chromatosomes, and stacked nucleosomes with native H3 and with H3K27me3 and H3K9me3 tails (Fig. 1).

Overall, we find that lysine methylation has a subtle effect on the structure of the H3 tail but a more significant effect on the flexibility and extension of the tail, especially for K9 methylation. Methylation also affects the interaction of the tail with the linker DNA in chromatosomes, an interaction we find to be asymmetric, and the nucleosomal DNA of nonparental nucleosomes in stacked nucleosomes. These interactions could affect fiber compaction and internucleosome contacts. Such effects could be significant on a global scale, especially when occurring in tandem on several nucleosomes.

MATERIALS AND METHODS

All-atom MD simulations of isolated histone tails

MD simulations were conducted using the AMBER 22.03 software package. We use the ff19SB force field (16) to describe the protein and the TIP3P water model to describe solvation effects (17). The parameters for the tri-methylated lysine are obtained from the literature (18).

The initial structure of the histone H3 tail (residues 1–43) was obtained from the crystal structure of the nucleosome solved at 1.9 Å (PDB: 1KX5) (19). This structure was used to create the initial structures of the H3K9me3 and H3K27me3 tails by modifying the corresponding lysine residue using tleap. Each system, H3, H3K9me3, and H3K27me3, was neutralized with chloride ions (Cl^-), and sodium (Na^+) and Cl^- ions were also introduced to establish physiological ionic conditions (150 mM NaCl). Each system was solvated in a cubic water box with a 10.0 Å buffer of TIP3P water molecules.

After the system setup, an energy minimization was performed with Cartesian restraints (force constant of 500 kcal/mol/Å²) applied to the solute to relieve any unfavorable interactions. We used 10,000 cycles of energy minimization, where the first 5000 cycles are with the steepest descent and the rest with a conjugate gradient. A second round of energy minimization to fully relax the systems was conducted without Cartesian restraints using the same protocol.

Following energy minimization, the systems were gradually heated from 0 to 300 K using the Langevin thermostat and 50,000 MD steps with a time step of 2 fs. The systems were coupled to a constant volume ensemble with periodic boundary conditions and a nonbonded cutoff of 8.0 Å.

After the heating phase, the systems underwent an equilibration process to stabilize them under constant pressure. The equilibration was performed over 500,000 steps with a time step of 2 fs. Temperature (300 K) was controlled with the Langevin thermostat with a collision frequency of 2 ps⁻¹. Constant pressure was maintained using the Berendsen barostat with a pressure relaxation time of 5 ps. Periodic boundary conditions were applied, and the system was equilibrated with a nonbonded cutoff of 8.0 Å.

After equilibration, we ran the production simulations over 500 million steps with a time step of 2 fs, corresponding to a total simulation time of 1 μs. The simulations were conducted at a constant temperature of 300 K using the Langevin thermostat with a collision frequency of 2 ps⁻¹. Periodic boundary conditions were applied, and the SHAKE algorithm was used to constrain bonds involving hydrogen atoms (20). We ran 50 replicas per system starting from different random seeds.

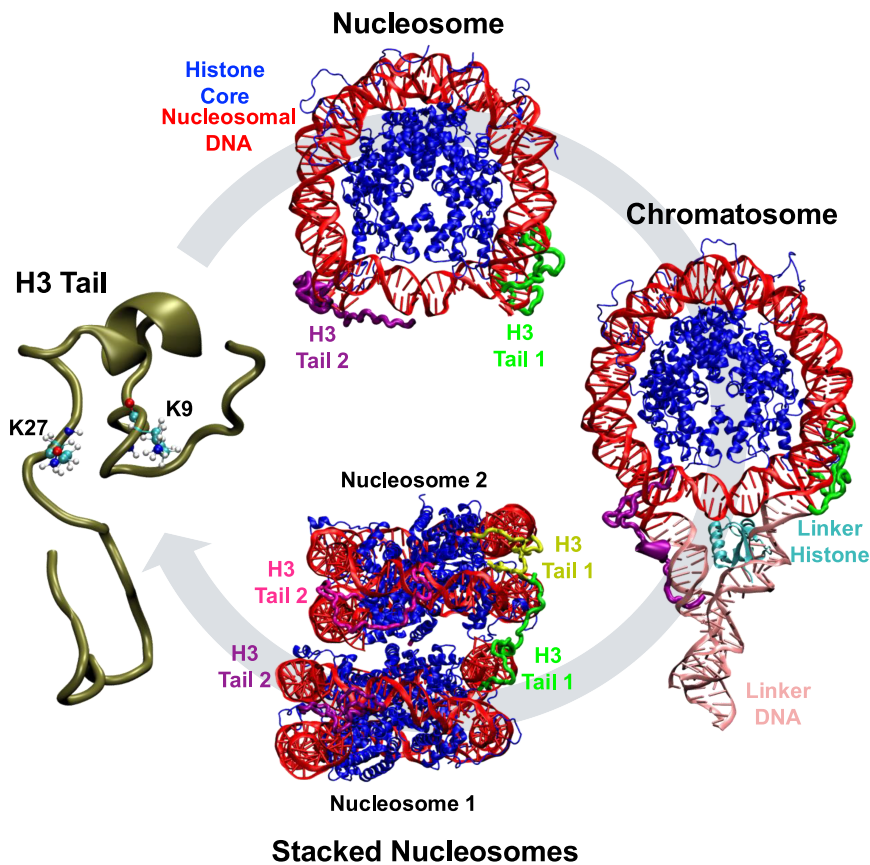


FIGURE 1 All-atom systems simulated in this work. We simulate (clockwise direction) isolated H3 tails, nucleosomes, chromatosomes, and two stacked nucleosomes with native H3 tails and with K9 and K27 tri-methylated H3 tails. The systems are not drawn in the same scale.

All-atom MD simulations of nucleosomes, chromatosomes, and stacked nucleosomes

Similar to the MD simulations of the isolated histone H3 tails, we perform MD simulations of nucleosomes, chromatosomes, and two stacked nucleosomes containing the native H3 tail and the H3K9me3 and H3K27me3 tails (Fig. 1).

As initial structures, we used the crystal structure of the nucleosome solved at 1.9 Å (PDB: 1KX5) (19); the cryo-electron microscopy structure of the chromatosome containing the globular domain of human H1.4 linker histone and linker DNAs of 25 bp solved at 2.7 Å (PDB: 7K5Y) (21) plus the histone tails from the nucleosome (PDB: 1KX5) (19); and two nucleosomes (PDB: 1KX5) (19) stacked based on the crystal structure of a tetra-nucleosome (PDB: 1ZBB) (22).

We use the same force fields as before for the proteins, tri-methylated lysine, and water and the bsc1 force field for the DNA (23). Each system is neutralized with Na⁺ ions, and the salt concentration is set to 150 mM by adding Na⁺ and Cl⁻ ions based on the volume of the TIP3P water solvation box with a 10 Å buffer.

We follow the same energy minimization protocol as for the isolated tails (described above) but increase the number of cycles to 20,000, where 10,000 are steepest descent and 10,000 conjugated gradient. We then heated the system to 298 K over 1 ns using the Langevin thermostat with a collision frequency of 1 ps⁻¹ and a time step of 2 fs. We apply weak Cartesian restraints (100 kcal/mol/Å²) to the protein and DNA and SHAKE to bonds involving hydrogen (20).

After heating, we equilibrate the systems at constant pressure using six stages of 1 ns each to reduce the restraints gradually from 100 kcal/mol/Å² to 0. In the first stage, a restraint of 100 kcal/mol/Å² is

applied to protein + DNA; in the second stage, we reduce the restraint to 10 kcal/mol/Å²; in the third stage, we apply the 10 kcal/mol/Å² restraint only to the protein + DNA backbone atoms; in the fourth stage, we reduce the restraint on backbone atoms to 1 kcal/mol/Å²; in the fifth stage, we reduce it to 0.1 kcal/mol/Å²; and in the sixth stage, we eliminate the restraint. In all stages, pressure is controlled with the Monte Carlo barostat and a relaxation time of 1 ps, and temperature is controlled by the Langevin thermostat with collision frequency of 1 ps⁻¹.

We then perform the production MD simulations. For each system with native and methylated H3 tails, our trajectories cover from 0.3 to 1 μs at constant pressure and 298 K using the Monte Carlo barostat and Langevin thermostat with the same parameters as above. We simulate nine replicas per system starting from different random seeds to set up the random velocity vector.

To assess the role of the initial tail configurations on the overall results, we also conduct simulations of nucleosomes, chromatosomes, and stacked nucleosomes starting from initial native tails that are completely extended. Specifically, similar to the approach used by (14), we use the nucleosome crystal structure PDB: 1KX5, clip the N- and C-terminal tails of all histones, and then generate the tails using the module called “building structure” in Chimera (24) with default peptide dihedral parameters $\omega = 180^\circ$ and $\phi = -120^\circ$ (Fig. S1). After equilibrating the tails in the nucleosome with the same six-step protocol as described above, we use two copies of the equilibrated nucleosome structure to construct the stacked nucleosomes system. To construct the chromatosome, we substitute the coordinates of the histone proteins in the 7K5Y structure with the histone coordinates of the equilibrated nucleosome structure with extended tails. We run four replicas per system using the same protocol as described above and compare the behavior of H3 tail 1 versus that of tail 2 (Fig. S2).

Analysis

We use the CPPTRAJ module (25) of AMBER for structural analysis of the histone H3 tail in each system. Based on the root-mean-squared deviation (RMSD) values (Figs. S3–S6), we use all frames for the analysis of the H3 isolated tails, and we use all frames minus the first 0.1 μ s for the analysis of the H3 tails in the context of the nucleosome, chromatosome, and stacked nucleosomes.

For each system, we calculate the following.

1) RMSD

To monitor the convergence of the simulations, we calculate the RMSD of each system (tail, nucleosome, chromatosome, and stacked nucleosomes) using the coordinates of all heavy atoms along each production trajectory. The RMSD is calculated as

$$\text{RMSD} = \sqrt{\frac{1}{N} \sum_{k=1}^N (x_k - x_0)^2 + (y_k - y_0)^2 + (z_k - z_0)^2}, \quad (1)$$

where N is the total number of atoms; x_k , y_k , and z_k are the atomic coordinates of atom k ; and x_0 , y_0 , and z_0 are the atomic coordinates of atom k at the beginning of the simulation.

2) Root-mean-squared fluctuation (RMSF)

To analyze the flexibility of the H3 tail in each system, we calculate the RMSF per residue as follows:

$$\text{RMSF}_i = \sqrt{\frac{1}{T} \sum_{t=1}^T \langle (r'_i(t) - r_i(t_{\text{ref}}))^2 \rangle}, \quad (2)$$

where T is the trajectory time, t_{ref} is the reference time, r_i is the position of the residue i , r' is the position of atoms in residue i at the beginning of the trajectory, and the angle brackets designate the average of the squared distance of atoms in residue i .

3) Radius of gyration (Rg)

We calculate the Rg as a measurement of H3 tail compaction and folding as follows:

$$\text{Rg}^2 = \frac{1}{N} \sum_{i=1}^N |r_i - r_{\text{mean}}|^2, \quad (3)$$

where N is the total number of atoms, r_i is the position vector of the i -th atom, r_{mean} is the center of mass of the tail, and $|r_i - r_{\text{mean}}|$ is the Euclidean distance between each atom and the center of mass.

4) Solvent-accessible surface area (SASA)

We measure the H3 tail SASA as an indicator of its folding and accessibility state. We use the linear combination of pairwise overlaps (LCPO) algorithm (26), which uses linear combinations of pairwise overlaps of hard spheres to approximate the accessible molecular surface. The SASA of a molecule is calculated by determining the accessible surface area A of each atom i as follows:

$$\text{SASA} = \sum_{i=1}^N A_i, \quad (4)$$

where N is the total number of atoms of the tail, and

$$A_i = P_1 S_1 + P_2 \sum_{j \in N(i)} A_{ij} + P_3 \sum_{\substack{j \in N(i) \\ k \in N(j) \\ k \neq j}} A_{jk} + P_4 \sum_{j \in N(i)} A_{ij} \left(\sum_{\substack{j,k \in N(i) \\ k \in N(j) \\ k \neq j}} A_{jk} \right), \quad (5)$$

where P_1 – P_4 are parameters obtained from multiple linear regression, $N(i)$ contains the list of spheres that overlap with sphere i , A_{ij} describes the overlap of sphere i with its neighbors, and A_{jk} describes the overlap of neighbors of i with each other.

5) Secondary structure

To analyze the secondary structure content of the H3 tail, we use the dictionary of secondary structure in proteins (DSSP) algorithm (27), which assigns the secondary structure to amino acids based on geometrical features and hydrogen bond (H-bond) patterns. DSSP identifies parallel and antiparallel β structures; 3–10, α , and π -helical structures; and turns and bends. In our analysis, we combine the occurrence of both β structures, the occurrence of the three helical structures, and the occurrence of turns and bends. We also record the absence of these structural features.

6) H-bonds

We measure the number of H-bonds between the H3 tail and the linker DNA and nucleosomal DNA. An X–H ... Y H-bond, where X is the H-bond donor and Y the H-bond acceptor, is defined by the distance between X and Y and the angle X–H ... Y. An H-bond is defined when the X–Y distance is less than 3.0 Å and the X–H ... Y angle is at least 135°.

7) End-to-end distance

We calculate the Euclidean distance between the α C of the H3 tail residue 1 and residue 40.

8) Linker DNA entry/exit angle and angle between linker DNA and nucleosome plane (β)

To measure the entry/exit angle, we create two vectors that align with each linker DNA. To define vector 1, we use the center of mass of residues 1–5 and residues 20–25 of the 25 bp linker DNA. To define vector 2, we use the same residues of linker DNA 2 (Fig. S7). We then calculate the angle formed by these two vectors as

$$\text{entry/exit}_{\text{angle}} = \text{acos}(|V1| * |V2|). \quad (6)$$

To calculate the angle β , the linker DNA axis/nucleosome plane angle, we first calculate the angle between the last bp of the linker DNA 2 and the nucleosome plane defined by the central nucleotide of the 146 bp of DNA wrapped around the nucleosome core and the center of the nucleotides at +38 and –38 bp of this central nucleotide. We then subtract this angle to 180° to obtain β and determine how much the linker DNA offsets from the nucleosome plane (Fig. S7).

9) Statistical significance

We assess the statistical significance between the native H3 tail and the methylated tails in the nucleosome, chromatosome, and stacked nucleosomes by performing the Student's t -test and reporting the p values in Table S1.

RESULTS

K9 and K27 methylation on genome components

Isolated H3 tail

To investigate the structural effects of lysine methylation on the H3 tail, we perform 50 replicas of 1 μ s all-atom MD simulations of the H3 isolated tail in native form and with K9me3 and K27me3.

We first study the secondary structure content of the H3 tail in each condition. As shown in the stacked column plots in Fig. 2 A depicting the secondary structure content of each residue across all 50 trajectories, methylation of K9 or K27 does not significantly affect the secondary structure of the tail. Residues remain without any defined secondary struc-

ture or display small bends and turns. In all three cases, we see that residues 17–27 have around 50% content of α helix, but this content does not change much with methylation of the lysine residue (see *red and yellow arrows* in Figs. 2 and S8; Table S2). Representative structures of the tails also show that they mostly lack secondary structure features or contain low percentages of bends and turns. The average Rg slightly increases upon methylation of K9 and K27, from an average of 15.8 to 16.3 and 17.0 Å, respectively (Fig. S9). Thus, MD simulations of isolated tails show that the secondary structure of the tail is not significantly affected by methylation, in agreement with previous MD results on dimethylation of H3K4 and H3K9 (12).

The Ramachandran plots in Fig. 2 B for residues K9 and K27 in the native H3 tail and the H3K9me3 and H3K27me3

Tail

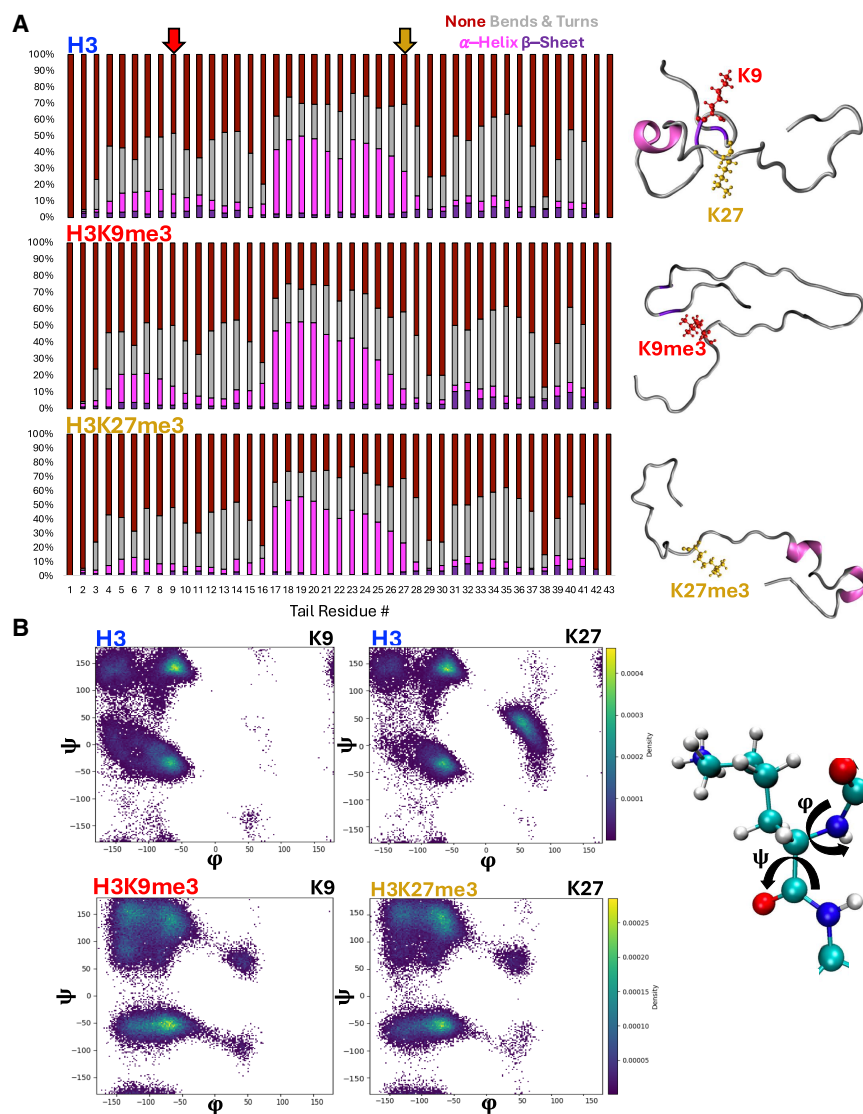


FIGURE 2 Lysine methylation effects on secondary structure of histone H3 tail and on the methylated lysine residue. (A) Stacked column plots showing, for each residue, the percentage of the occurrence of the following secondary structure elements: α helix (pink), β sheet (violet), and bends and turns (gray) for the H3, H3K9me3, and H3K27me3 tails. The arrows indicate the positions of the residues K9 and K27. (B) Ramachandran density-based plots for the residues K9 and K27 in the H3, H3K9me3, and H3K27me3 tails.

tails reveal that methylation affects the ψ and ϕ backbone dihedral angles of the lysine residue. Upon methylation, both K9me3 and K27me3 favor a wider range of conformations on the lysine residue, independently of the residue position along the polypeptide chain. Thus, methylation of K9 and K27 stabilizes certain conformations that are less populated in the native tail. These results agree with previous MD simulations showing that tri-methylation of H3K4 affects the torsional angles of the residue significantly (11).

These trends indicate that methylation induces or stabilizes specific conformations of the lysine residue, possibly by influencing the local environment through steric effects or by altering the interaction with surrounding residues or solvent molecules. The stabilization of these specific conformations in the methylated histone tails could have functional consequences, possibly altering the way the tails interact with other histones or DNA, or could lead to more favorable interactions with chromatin-associated proteins like HP1 or PcG, which might influence gene expression or chromatin compaction.

In sum, histone methylation induces very subtle changes on the secondary structure of the lysine residue but has potential for altering neighboring interactions.

The nucleosome

Next, we simulate nucleosomes with native and K9me3 and K27me3 H3 tails to understand the effect of methylation on tail folding when interacting with DNA, a context more similar to that of the native chromatin fiber.

Fig. S10 shows the RMSF for each H3 tail of the nucleosome (see nucleosome representation in Fig. 1). Residues at the N-terminal end of each H3 tail have high fluctuations, but fluctuations are reduced as the tail residues approximate the folded histone domain, from residue 31 to 40. For tail 1, we see a higher RMSF for residues 10–36 and no major differences for residues 1–9 between the native tail and the two methylated tails. For tail 2, we see that the native and K27me3 tails show higher RMSFs in the N-terminal end (residues 1–20) than the K9me3 tail. Regarding the configurations of the K9 and K27 residues, Ramachandran plots show that, similar to isolated tails, methylation affects the ψ and ϕ backbone dihedral angles (Fig. S11). These adopt wider values upon methylation.

As observed in Fig. 1, tail 1 appears to be slightly more folded, whereas tail 2 appears to be more extended. Although a slight asymmetry is found, previous reports indicate that the asymmetry of the two sister H3 tails is much more important in nucleosomes that have lost the H2A/H2B dimer (28).

When analyzing compaction parameters like the Rg (Fig. 3 A) and SASA (Fig. 3 B), the asymmetry between tail 1 and tail 2 also emerges. Namely, for all three systems, tail 1 has a smaller Rg than tail 2. In agreement, simulations with completely unfolded and extended initial tail configurations also reveal a slight asymmetry in the H3 tails of

the nucleosome, especially with respect to the Rg values (Fig. S2).

For tail 1, we see that both methylations of K9 and K27 increase Rg (Fig. 3 A), from an average of 12.9 to 13.4 and 13.3 Å, respectively, indicating a more extended tail. Similar results are obtained for the SASA. Methylation of K27 increases tail 1 SASA from an average of 3224 to 3288 Å², and methylation of K9 increases it to a larger extent, 3326 Å². Thus, overall, both methylations of K9 and K27 produce a more extended and flexible tail 1, with the effect of K9me3 more pronounced. This is also reflected by the cumulative positional distribution of tail 1 (Fig. 3 E), showing wider distributions upon methylation of both K9 and K27. Results along the same lines were reported by NMR experiments, showing that mono-methylation increases the conformational dynamics of the H4 tails (29).

On the other hand, for tail 2, methylation of K9 increases Rg to a lesser extent than for tail 1, and methylation of K27 does not increase the Rg. Thus, for this intrinsically more extended and flexible tail, methylation seems to have less of an impact on its folding. Indeed, the cumulative positional distribution of tail 2 (Fig. 3 E) shows that the H3K27me3 tail extends less from the nucleosome center than the native tail (yellow instead of orange-red color), in agreement with a small reduction of the Rg, from 14.9 on average to 14.1 Å (Fig. 3 A). Thus, depending on the position of each sister H3 tail, buried within the nucleosome or more solvent exposed, methylation would have a stronger or weaker effect on the tail structure and dynamics.

We next analyze the interactions between each H3 tail and the nucleosomal DNA (Fig. 3 C). We see that the number of H-bonds between the tails and the nucleosomal DNA does not change much upon methylation of either K9 or K27 (see average values in Fig. 3 C). These results agree with MD simulations of H3K4 tri-methylated nucleosomes showing that methylation does not affect the binding energy of H3 to nucleosomal DNA (13). When we analyze the end-to-end distance of the H3 tail to measure its extension (Fig. 3 D), we see that for tail 1, both methylations on K9 and K27 increase this distance, from an average of 23.4 to 28.9 and 26.6 Å, respectively, and again, the effect of K9 methylation is more pronounced. Similar results have been seen in MD simulations of nucleosomes containing dimethylation of K9 and K27, plus phosphorylation of S10 and S28, all repressive marks (30). This change in the global tail folding is expected to favor interactions with protein readers, such as HP1 in the case of K9me3 or PcG proteins in the case of K27me3. For example, it has been shown that different PTMs or mutations of the H3 tail expose the tail to solution, increasing its accessibility to protein domains that bind to histones, like the PHD of the nucleosome-remodeling factor subunit (13). Thus, although the number of H-bonds of the H3 tail with the DNA does not change much upon methylation, the induced structural changes can have a direct effect

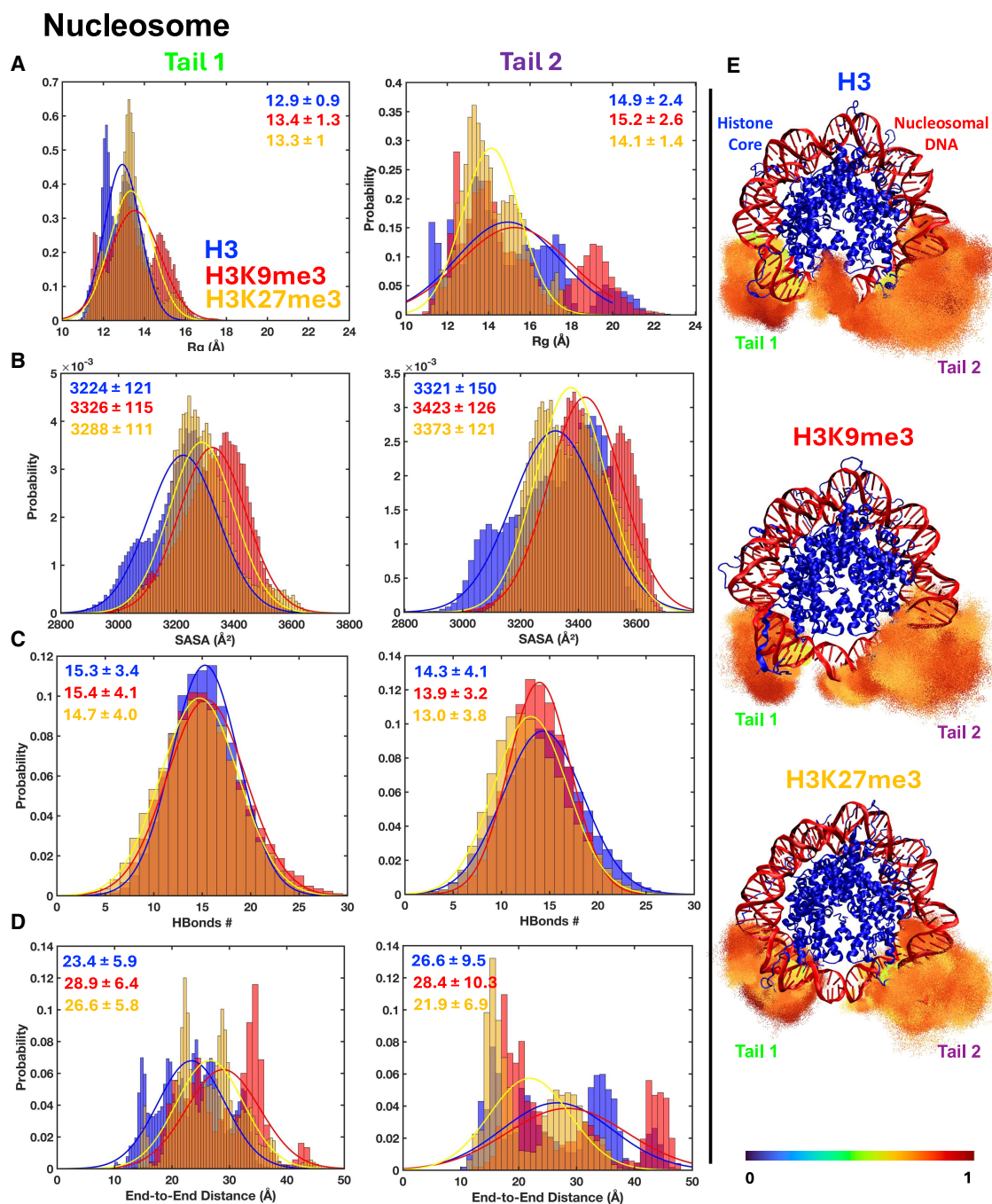


FIGURE 3 Lysine methylation effects on H3 tails in the context of the nucleosome. For each H3 tail of the nucleosome in native and methylated conditions (K9 and K27), we show probability distribution plots for (A) radius of gyration (Rg), (B) solvent-accessible surface area (SASA), (C) number of hydrogen bonds (H-bonds) with the nucleosomal DNA, and (D) end-to-end distance. In (E), we show the H3 tail's cumulative positional distribution across all trajectories, with atoms represented as dots and colored based on the radial distance to the nucleosome center of mass.

on the fiber architecture by facilitating the interaction of the tail with other proteins that modulate the chromatin fiber.

The chromatosome

Because the H3 histone tails are located close to the linker DNA in the chromatin fiber, we study chromatosomes of

25 bp linker DNA containing the LH H1.4 (Fig. 1) with native H3 tails and with K9me3 and K27me3 H3 tails.

Fig. S10 shows the RMSF for each tail on the chromatosome. Similar to the results observed for the nucleosome, we see a clear but more marked asymmetry between tail 1 and tail 2. The native system shows a folded tail 1 with low RMSF and an expanded tail 2 with higher RMSF. This is

also evident in the contact matrices of $C\alpha$ - $C\alpha$ distances across all residues (Fig. S12), showing distances between residues of up to 60 Å for tail 2 but only up to 30 Å for tail 1. Although the asymmetry effect might be exaggerated due to the initial tails configurations of the PDB: 1KX5 structure, simulations that started from extended tails also reveal asymmetry (Figs. S1 and S2).

When we introduce the methylation, we see that K9me3 increases the flexibility of tail 1, and K27me3 slightly increases it (Fig. S10). Besides being more flexible, the methylated tail 1 also shows larger Rg (Fig. 4 A) and SASA (Fig. 4 B). The values increase for K9me3 and K27me3 from an average of 13.7 to 15.2 and 14.9 Å and from an average of 3600 to 3672 and 3661 Å², respectively. Thus, similar to the results obtained for the nucleosome, methylation of K9 or K27 produces a more flexible and extended tail 1. This effect is also shown by the cumulative positional distribution of tail 1 (Fig. 4 E). Namely, H3K9me3 and

H3K27me3 tails 1 present much wider distributions than the native H3 tail 1.

Tail 2, on the other hand, is less sensitive to the effect of methylation. For example, the $C\alpha$ - $C\alpha$ distance matrix of the tail 2 shows smaller changes than tail 1 upon methylation of either K9 or K27 (Fig. S12). Specifically, the Rg of tail 2 is slightly smaller upon methylation, with the average value decreasing from 22.1 to 21.6 and 20.6 upon K9 and K27 methylation, respectively (Fig. 4 A). However, a higher SASA (Fig. 4 B) than the native tail is obtained for both methylations. Additionally, the positional distribution shows that the methylated tail 2 extends further from the center of the chromosome; see the red color instead of yellow in Fig. 4 E. Ramachandran plots also show a larger effect of methylation on tail 1 than on tail 2 (Fig. S11). Thus, overall, although changes are not as important as in tail 1, methylation of tail 2 also produces a slightly more extended tail.

Chromosome

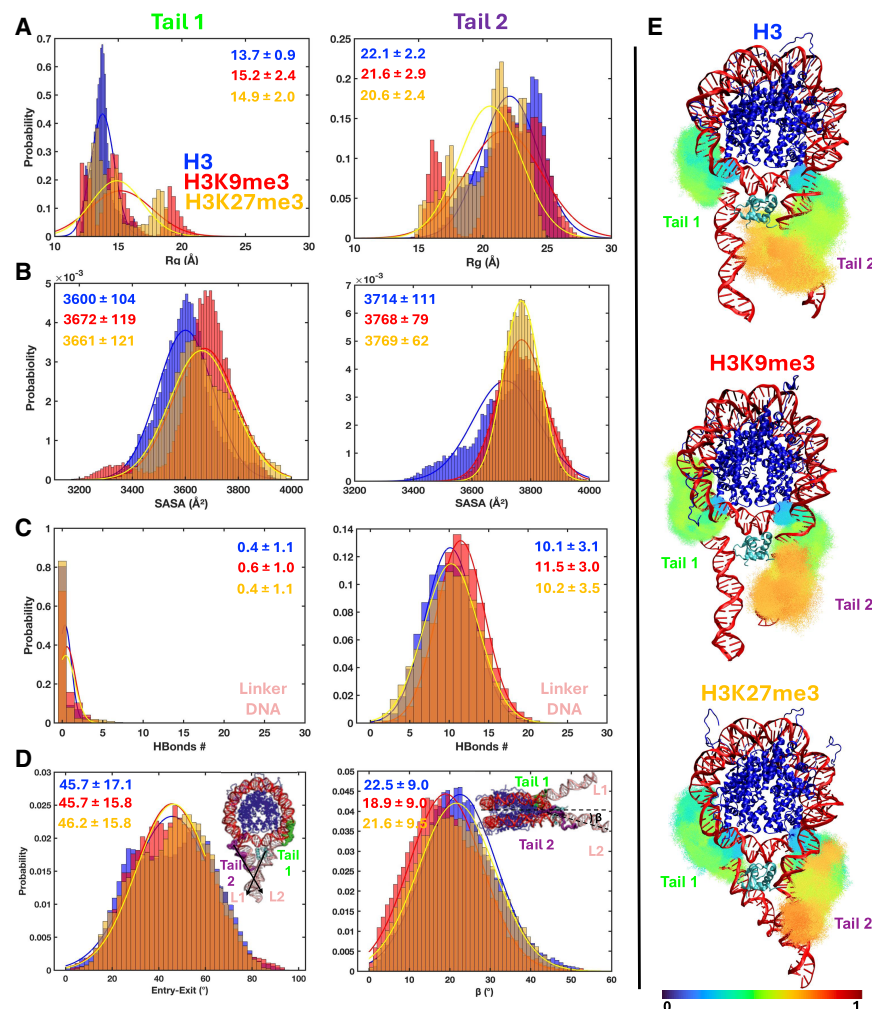


FIGURE 4 Lysine methylation alters H3 tail flexibility and extension in the chromosome context. For each H3 tail of the chromosome in native and methylated conditions (K9 and K27), we show probability distribution plots for (A) radius of gyration (Rg), (B) solvent-accessible surface area (SASA), and (C) the number of hydrogen bonds (H-bonds) with the linker DNA. In (D), we show probability distribution plots for the linker DNA entry/exit angle and β angle formed between linker 2 and the nucleosome plane. In (E), we show the cumulative positional distribution of each H3 tail, with atoms represented as dots and colored based on their distance to the chromosome center of mass.

The asymmetry of the H3 histone tail in native chromatosomes has been previously reported (31). It was shown that whereas one tail interacts with the nucleosomal DNA, the other tail interacts mostly with the linker DNA, and this was attributed to the presence of linker histone, which increases the flexibility of the tail. These trends are also supported by our simulations of chromatosomes, with extended initial tail configurations showing that one of the tails has larger Rg and SASA (Fig. S2). However, how this asymmetry modulates the effect of lysine methylation has not been described previously.

Because of its proximity to the linker DNA, we next study the interactions of the H3 tail with nucleosomal and linker DNA (Figs. 4 C and S13). Interestingly, only tail 2 establishes H-bonds with the linker DNA, and the number slightly increases upon methylation of the K9 residue (Fig. 4 C), which agrees with the extension from the nucleosome center seen in the cumulative positional distribution (Fig. 4 E). This finding is in line with results from MD simulations of nucleosomes with tri-methylated H3 showing an increase in the contacts with the DNA near the entry/exit site (14). Tail 1, on the other hand, mostly interacts with the nucleosomal DNA, and the number of H-bonds is not affected by methylation of K9 or K27 (Fig. S13).

We additionally measure the linker DNA entry/exit angle (Fig. 4 D, left) and the β angle (Fig. 4 D, right) formed between the linker DNA that interacts with tail 2 (L2) and the nucleosome plane. We capture a reduction of the β angle upon methylation, where the effect of K9 methylation is more marked. Thus, methylation affects the interaction of tail 2 with the linker DNA, which has consequences on the structure of the chromatosome. A smaller β angle could implicate a higher chromatin compaction, further contributing to the repressive role of this epigenetic mark.

Dinucleosome

The H3 tails have been shown to contribute to chromatin compaction by evidence that their neutralization results in the unfolding of the chromatin fiber (32). In particular, they mediate internucleosome interactions (32). Thus, we study a pair of stacked nucleosomes that mimic $i \pm 2$ zigzag interactions and investigate how the interaction of H3 tails with parental and nonparental DNA is affected by the methylation of K9 and K27.

Similar to the isolated tails, nucleosome, and chromatosome systems, Ramachandran plots for stacked nucleosomes show that methylation affects the lysine residue (Fig. S11). The residue adopts a less-defined structure, exploring more configurations, evidenced by wider distributions of ϕ and ψ backbone dihedral values. Fig. S10 shows the RMSF for each tail (tails 1 and 2) of each nucleosome (nucleosomes 1 and 2). For the four H3 tails, methylation of either K9 or K27 does not increase tail fluctuation compared to the native tail. When analyzing the Rg of each tail, we see that tail 2 of nucleosome 1 and tail 1 of

nucleosome 2 show a larger Rg upon K9 methylation (Fig. 5 A). For the other tails, a small reduction or no changes are observed. The positional distribution of the tails show wider distributions for tail 2 of nucleosome 1 upon methylation of both K9 and K27 and for tail 1 of nucleosome 1 upon methylation of K27 (Fig. 5 C), indicating that the methylated tails might adopt more varied positions than the native tail.

For the H-bonds established by each tail with the DNA of its parental nucleosome and its nonparental nucleosome, we do not observe major changes in the interactions for tails 1 and 2 of nucleosome 1 upon the methylation of either K9 or K27 (Fig. S14). For nucleosome 2, however, we see that tail 2 (last row of Fig. 5 B) interacts less with the DNA of its parental nucleosome and more with the DNA of its nonparental nucleosome upon methylation of K9 or K27. This is also evident in the positional distribution of the tail that shows how its position changes toward nucleosome 1 upon K9 methylation (Fig. 5 C). A similar redistribution of contacts upon histone tail methylation has been previously observed in MD simulations of nucleosomes containing linker DNA (14). Thus, for tail 2, methylation increases internucleosome interactions. Such an increase of interactions could help compact the chromatin fiber.

DISCUSSION

Our all-atom MD simulations have explored the effect of tri-methylation of the K9 and K27 residues on the H3 histone tail, as summarized in Fig. 6. These epigenetic modifications are related to gene repression, but their mechanisms of action are different. Whereas K9me3 is mostly recognized by the HP1 protein, K27me3 is recognized by PcR proteins. To explore different chromatin contexts, we performed simulations of isolated tails, nucleosomes, chromatosomes, and stacked nucleosomes, all in the presence of native or K9me3 or K27me3 H3 tails.

In general, we conclude that K9 and K27 methylation produces H3 tails that are more flexible and extended than native tails, which could help their recognition by HP1 and PcR proteins. As we have found previously for tail acetylation, a key consequence of more rigid and folded tails is that they impede internucleosome interactions and thereby hamper fiber folding (33). Correspondingly, the higher flexibility and extension upon methylation could have the opposite effect and thereby favor the interaction with binding proteins. Additionally, HP1 binding to H3K9me3 is driven by flexible and dynamic electrostatic interactions (34), and NMR studies show that the HP1-H3K9me3 nucleosome complex is highly flexible (35). Thus, it is expected that a more flexible and extended tail will facilitate interactions with HP1 and other proteins.

However, details are context dependent (Fig. 6). For isolated tails, changes upon methylation are very small. This

Stacked Nucleosomes

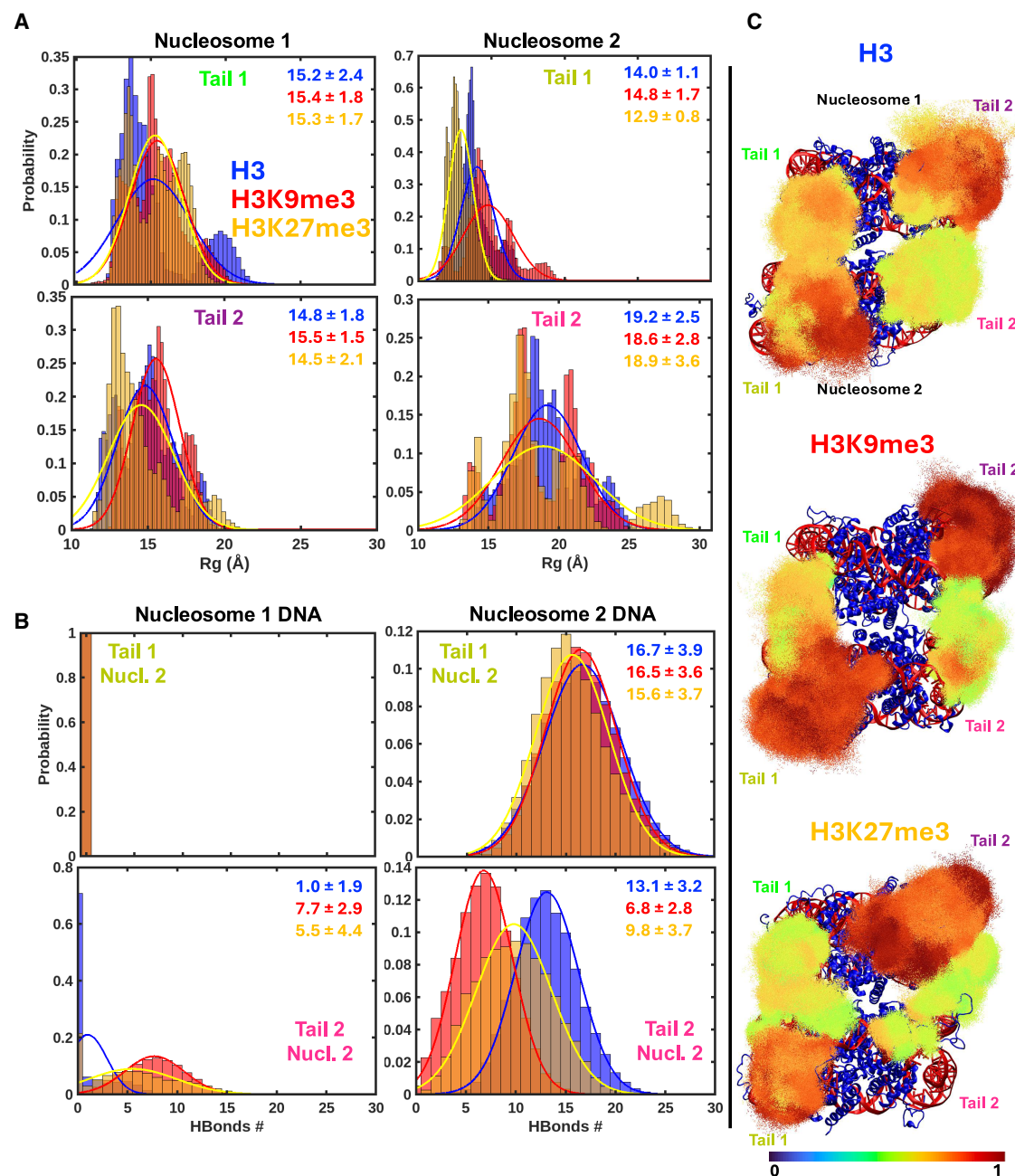


FIGURE 5 Lysine methylation effects on H3 tails in the context of stacked nucleosomes. For each H3 tail of the two stacked nucleosomes in native and methylated conditions (K9 and K27), we show probability distribution plots for (A) radius of gyration (Rg) and (B) number of hydrogen bonds (H-bonds) between the tails of nucleosome 2 and the DNA of nucleosome 1 and 2. In (C), we show the cumulative distribution of each tail position, with atoms represented as dots and colored based on their distance to the center of mass of the stacked nucleosomes.

was somehow expected as the tails are intrinsically disordered, and in solution, they behave as unstructured and flexible. This behavior is evident in the secondary structure analysis and tail configurations (Fig. 2). Although some studies have reported the presence of more α helix content in some regions of the H3 tail (36,37), we only observe a small portion of the tail with helical content, from residues

17–26 (Fig. 2 A), and this content does not surpass 50% of occurrence during the simulations. Similar unstructured tails have been observed by NMR and MD simulations in more recent studies (13). Importantly, no changes are observed in the structure upon methylation, and tails remain mostly in coil conformation. But a slight increase in Rg is observed.

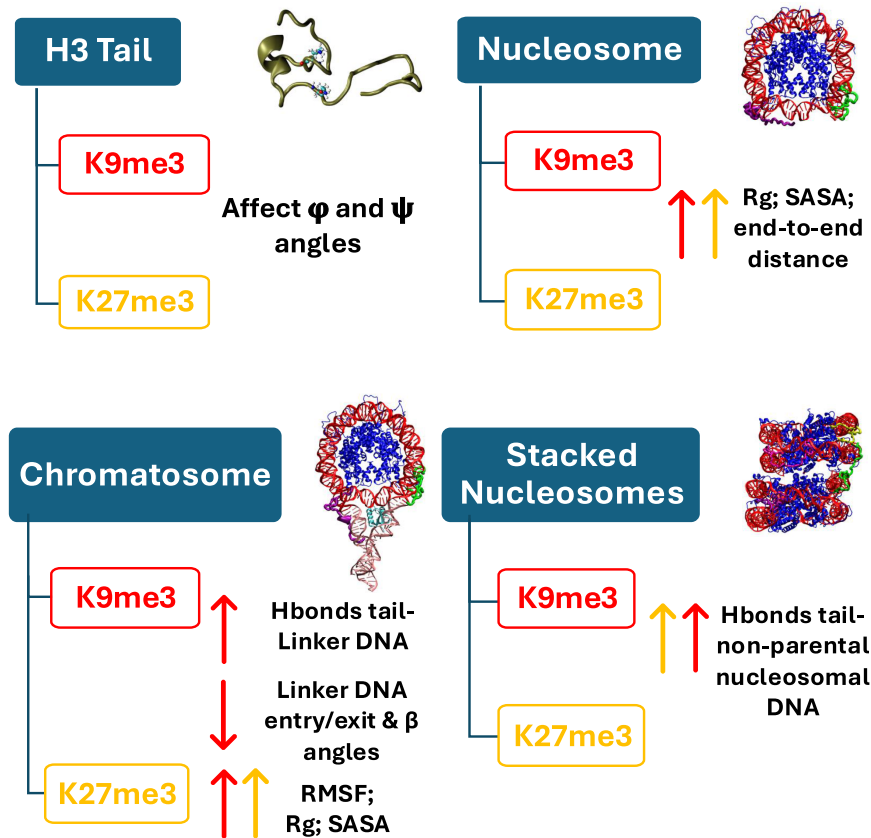


FIGURE 6 Summary of main findings. In isolated tails, methylation of K9 and K27 affects the lysine backbone dihedral angles ϕ and ψ . In the nucleosome context, in general, methylation increases the root-mean-squared fluctuation (RMSF), Rg, SASA, and end-to-end distance indicating a more flexible and extended tail, which might help its interaction with chromatin readers. In the chromatosome context, in general, methylation increases the Rg, SASA, and the interactions with linker DNA, and it reduces the angle between the linker DNA and nucleosome plane. Thus, methylation affects interactions of the tail with the linker DNA, which has direct effects on the chromatosome structure. For stacked nucleosomes, in general, methylation increases interactions with nonparental nucleosomal DNA, which might aid chromatin repression.

When we analyze H3 tail behavior in the context of the nucleosome and chromatosome, where the tails can interact with nucleosomal and linker DNA, some structural differences and effects on the interaction with the DNA are noted. In both cases, asymmetry emerges, where one of the methylated H3 tails become more extended and flexible (Figs. 3 and 4), and, in the case of the chromatosome, one of the tails interacts more with the linker DNA, reducing the β angle (Fig. 4 C). An increase in solvent exposure has also been observed for H3 tails marked with a combination of four repressive marks (30). This could be related to the fact that both epigenetic marks, K9 and K27 methylation, work through binding to repressive proteins.

The marked asymmetry observed in the native tails of the chromatosome and the different effect of methylation on each copy of H3 could be related to the fact that, *in vivo*, nucleosomes carry repressive and activating methylation marks on opposite H3 tails (38). Thus, this intrinsic asymmetry might help the differential epigenetic marking of the two tails. Although this asymmetry might be exaggerated by the initial tail configurations of the PDB: 1KX5 experimental structure, our simulations starting from extended tails also reveal such tail asymmetry (Figs. S1 and S2).

Our simulations of stacked nucleosomes mimicking zigzag interactions show an increase of tail nonparental

nucleosome interactions for one tail of one nucleosome (e.g., tail 2 of nucleosome 2 in Fig. 5) upon methylation of either K9 or K27. Thus, besides compacting chromatin by binding to repressive proteins, methylation might help compact chromatin by changing the interactions of the H3 tail with nonparental nucleosomes. The important role of H3 in mediating internucleosome interactions has already been demonstrated by early mesoscale modeling (32).

Overall, the insights obtained here at the all-atom level (Fig. 6) have helped us incorporate local methylation effects into chromatin fibers modeled at the mesoscale level (15). In particular, modified force constants on the H3 tail stretching and bending terms reflect the more extended and flexible tail upon methylation (15). For other methylation modifications like K4me3, K36me3, and K79me3, which are mostly related to transcriptional activation (3), similar all-atom MD simulations can be used to infer structural changes and help develop modified parameters for mesoscale chromatin models. Because the mechanisms involved may be different, as involved in gene activation or suppression, the strategies applied may vary in each case. For example, a switch from activating to repressive methylation marks accompanies the inactivation of the X chromosome (39). Thus, to incorporate the effect of these methylations, a case-by-case study will be needed to mimic their activating and

repressive effects. For example, as done for repressive marks, methylation can be incorporated into mesoscale chromatin models by introducing contacts between methylated regions (15). For activating methylation marks, a repulsive interaction potential could be introduced between methylated regions. As our knowledge of the epigenetic effects of various PTMs increases through experiments and modeling, further studies of gene regulation and its effects on human disease are becoming possible. Methylation, for example, is implicated in many human diseases and conditions, including aging (5,40).

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AUTHOR CONTRIBUTIONS

S P.-L. designed research, performed research, analyzed data, and wrote the paper. Z.L. designed research, performed research, and analyzed data. T.S. designed research, reviewed the data and result analysis, wrote the paper, and acquired funding.

DECLARATION OF INTERESTS

The authors declare no competing interests.

SUPPORTING MATERIAL

Supporting material can be found online at <https://doi.org/10.1016/j.bpj.2025.08.002>.

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Supplemental information

**Molecular dynamics simulations reveal subtle consequences of H3K9
and H3K27 tri-methylation on chromatin constituents**

Stephanie Portillo-Ledesma, Zilong Li, and Tamar Schlick

Supporting Information for “Molecular Dynamics Simulations Reveal Subtle Consequences of H3K9 and H3K27 tri-methylation on Chromatin Constituents”

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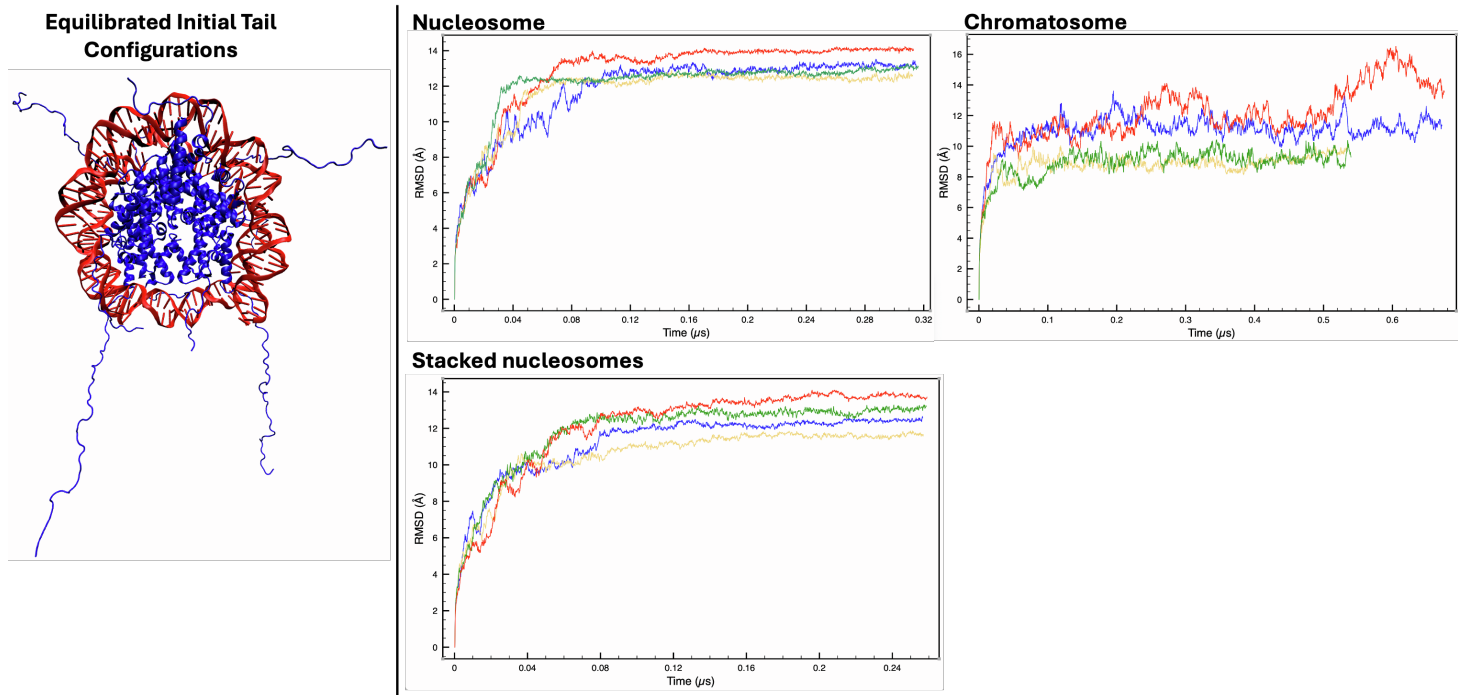


Figure S1. Initial tail configurations used in simulations of nucleosomes, chromosomes, and stacked nucleosomes to test effect of initial structure on overall results.

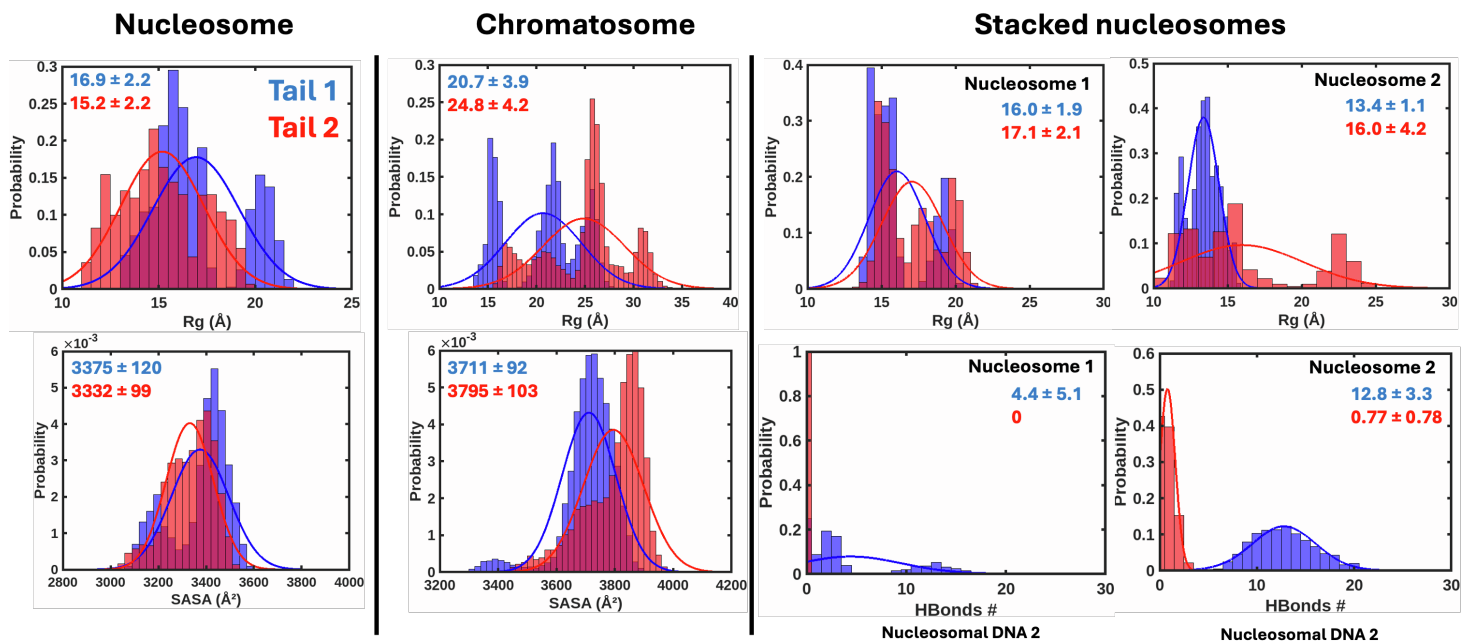


Figure S2. Comparison of behavior of native H3 Tail 1 versus Tail 2 in the nucleosome, chromosome, and stacked nucleosome systems when starting from extended tail configurations.

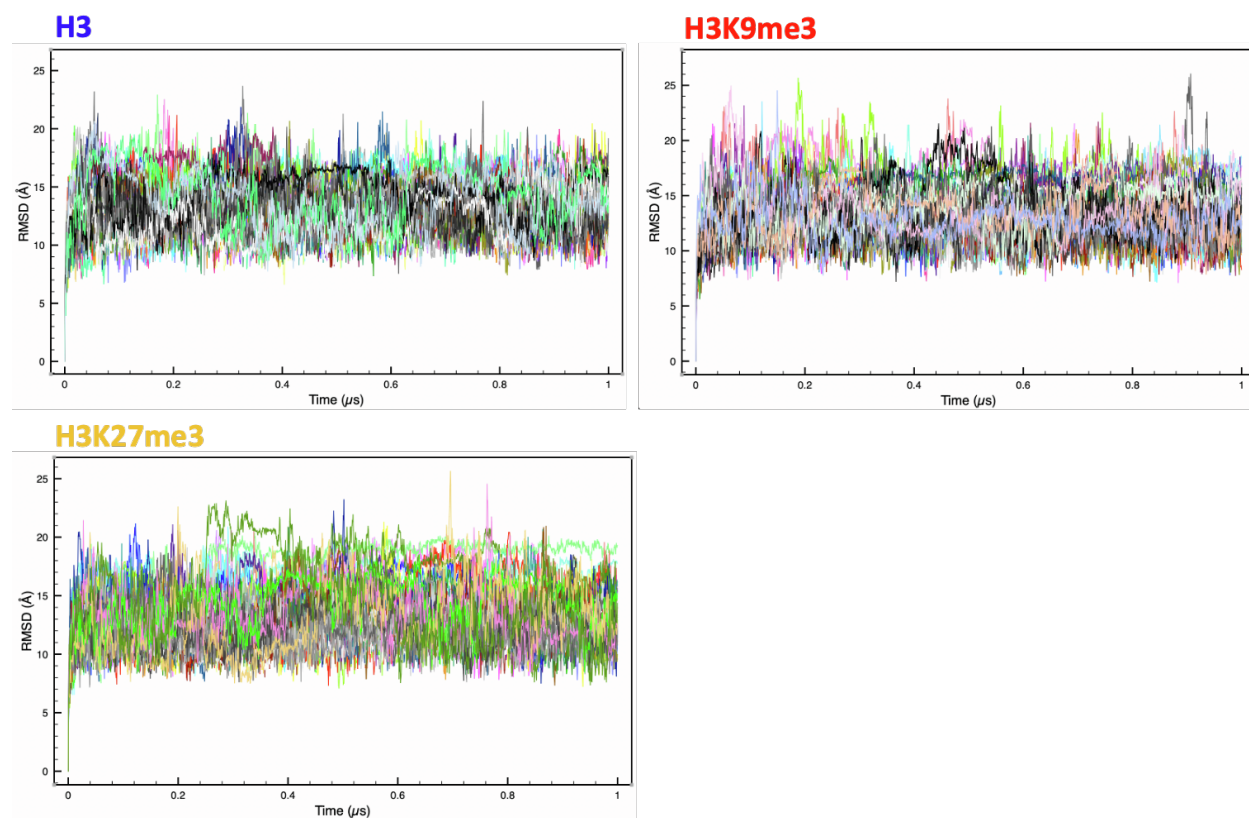


Figure S3. RMSD for the 50 replicas of native H3 tail and the K9me3 and K27me3 H3 tails.

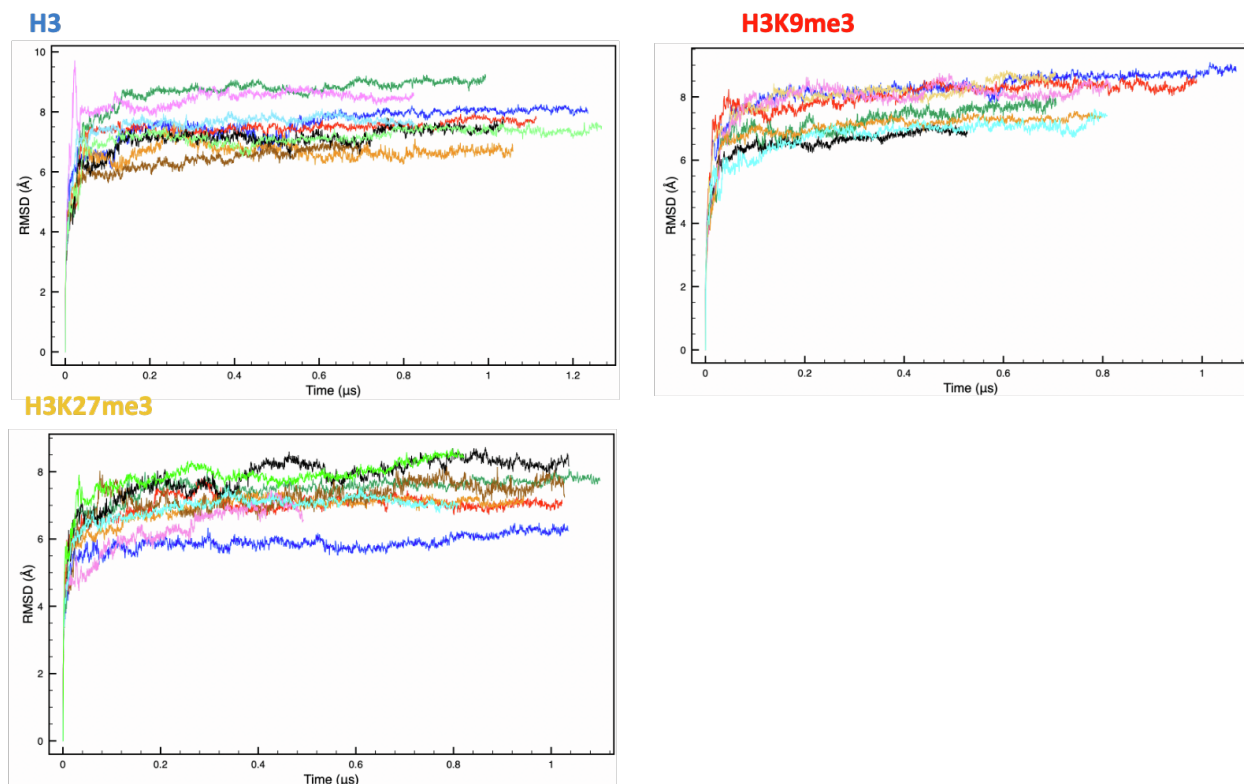


Figure S4. RMSD for the 9 replicas of the nucleosome with native H3 tails and the K9me3 and K27me3 H3 tails.

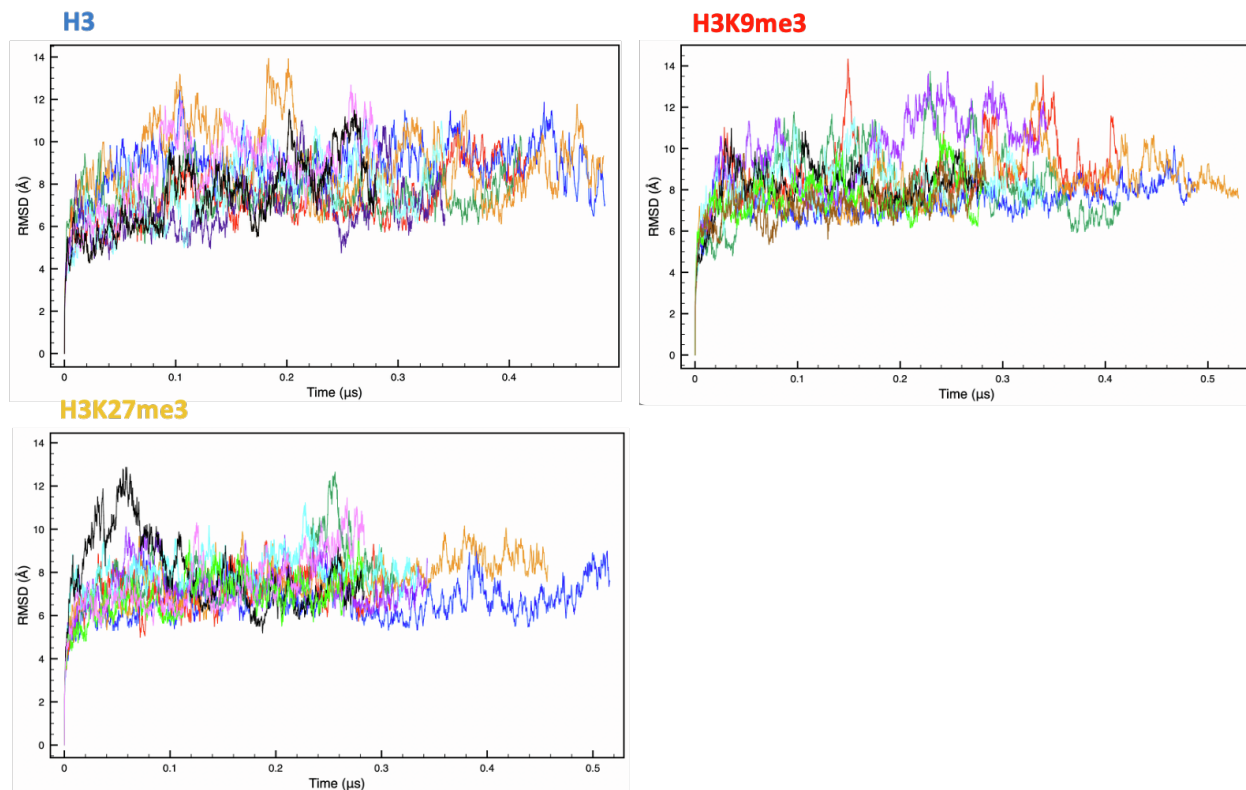
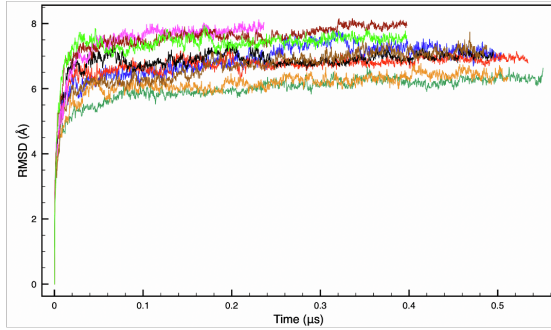


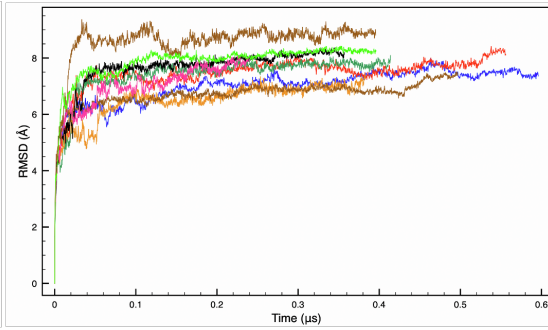
Figure S5. RMSD for the 9 replicas of chromosomes with native H3 tails and with K9me3 and K27me3 H3 tails.

Stacked nucleosomes

H3



H3K9me3



H3K27me3

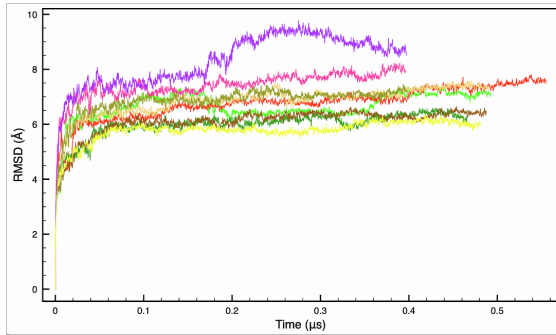


Figure S6. RMSD for the 9 replicas of two stacked nucleosomes with native H3 tails and with K9me3 and K27me3 H3 tails.

Table S1. p-values for Student's test comparing compaction properties of the native H3 versus K9 and K27 methylated H3.

Chromatosome					Nucleosome					Stacked Nucleosomes				
System 1	System 2	Property	p-value	NULL hypothesis	System 1	System 2	Property	p-value	NULL hypothesis	System 1	System 2	Property	p-value	NULL hypothesis
Native	K9me3	Entry-Exit	0.95	not rejected	Native	K9me3	Tail1			Native	K9me3	Rg (tail1; nuc1)	7.68E-39	rejected
Native	K27me3	Entry-Exit	2.81E-04	rejected	Native	K27me3	Rg	0.00E+00	rejected	Native	K27me3	Rg (tail1; nuc1)	1.65E-08	rejected
Native	K9me3	Beta angle	0	rejected	Native	K27me3	Rg	0.00E+00	rejected	Native	K9me3	Rg (tail2; nuc1)	0.00E+00	rejected
Native	K27me3	Beta angle	2.37E-25	rejected	Native	K9me3	SASA	0	rejected	Native	K27me3	Rg (tail2; nuc1)	4.87E-50	rejected
					Native	K27me3	SASA	0	rejected	Native	K9me3	Rg (tail1; nuc2)	0.00E+00	rejected
Native	K9me3	Rg	0	rejected	Native	K9me3	HBond	1.63E-05	rejected	Native	K27me3	Rg (tail1; nuc2)	0.00E+00	rejected
Native	K27me3	Rg	0	rejected	Native	K27me3	HBond	5.58E-78	rejected	Native	K9me3	Rg (tail2; nuc2)	2.61E-126	rejected
Native	K9me3	SASA	0	rejected	Native	K9me3	End-to-End	0.00E+00	rejected	Native	K27me3	Rg (tail2; nuc2)	5.01E-28	rejected
					Native	K27me3	End-to-End	0.00E+00	rejected					
Native	K27me3	SASA	0	rejected						Native	K9me3	Hbonds (tail1;nuc2;DNAnuc2)	2.92E-08	rejected
										Native	K27me3	Hbonds (tail1;nuc2;DNAnuc2)	1.64E-214	rejected
Native	K9me3	Hbonds	4.69E-176	rejected						Native	K9me3	Hbonds (tail2;nuc2;DNAnuc1)	0.00E+00	rejected
Native	K27me3	Hbonds	1.29E-12	rejected	Native	K9me3	Rg	9.60E-50	rejected	Native	K27me3	Hbonds (tail2;nuc2;DNAnuc1)	0.00E+00	rejected
					Native	K27me3	Rg	0.00E+00	rejected	Native	K9me3	Hbonds (tail2;nuc2;DNAnuc2)	0.00E+00	rejected
					Native	K9me3	SASA	0.00E+00	rejected	Native	K27me3	Hbonds (tail2;nuc2;DNAnuc2)	0	rejected
Native	K9me3	Rg	2.41E-87	rejected	Native	K27me3	SASA	0.00E+00	rejected					
Native	K27me3	Rg	0	rejected	Native	K9me3	HBond	1.66E-31	rejected					
Native	K9me3	SASA	0	rejected	Native	K27me3	HBond	8.3003e-322	rejected					
Native	K9me3	SASA	0	rejected	Native	K9me3	End-to-End	2.80E-101	rejected					
Native	K27me3	Hbonds	9.56E-06	rejected	Native	K27me3	End-to-End	0	rejected					

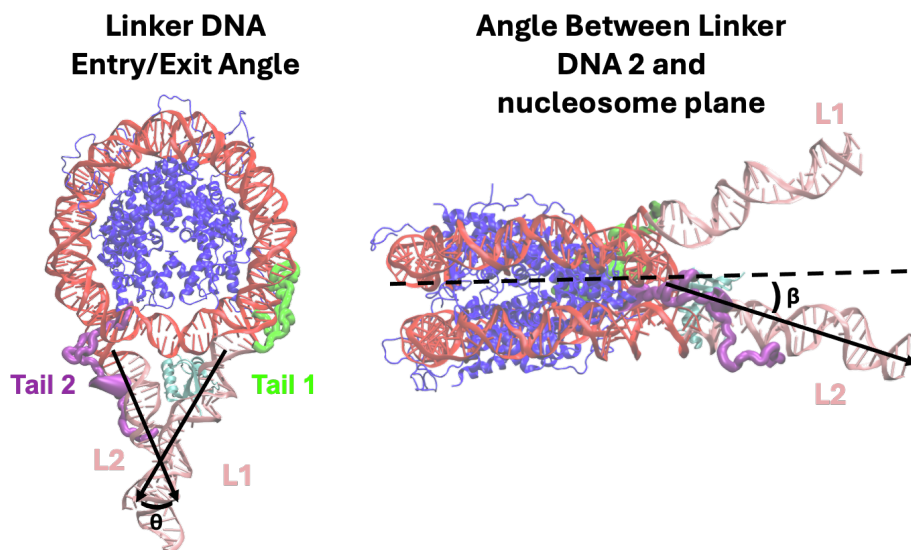


Figure S7. Sketch showing the calculation of the linker DNA entry/exit angle and β angle.

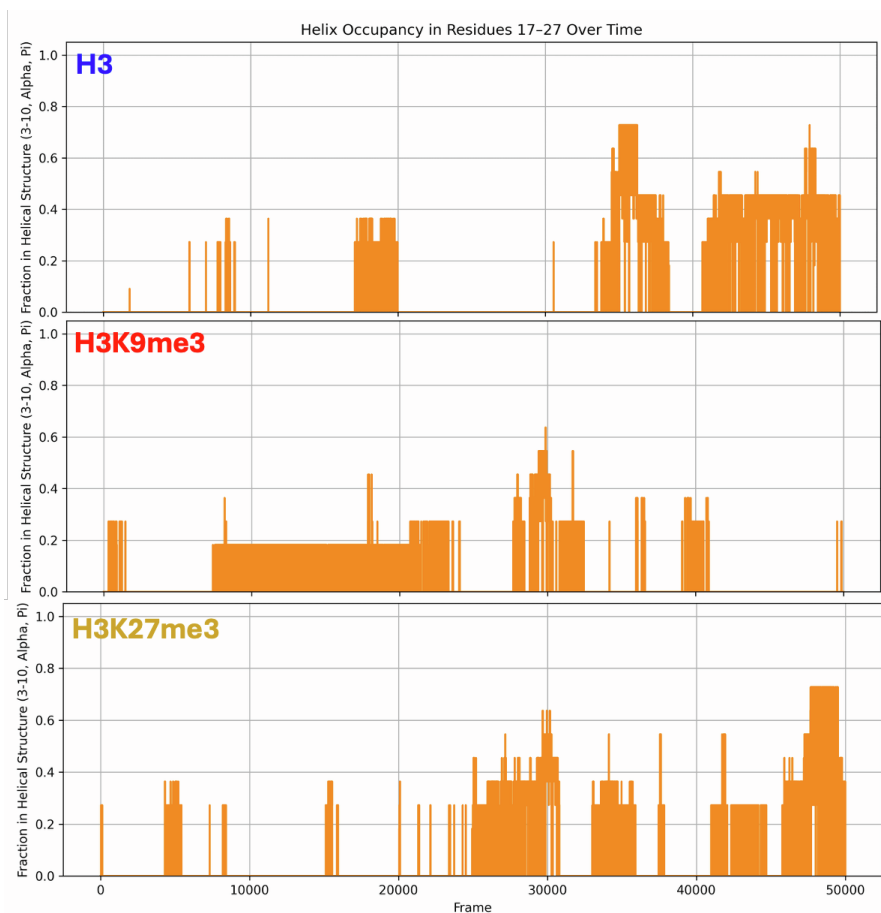


Figure S8. Alpha helix content across a single trajectory for native and methylated H3 tails. We determine the fraction of residues 17 to 27 that are in helix conformation across each frame of a single trajectory.

Table S2. Probability of secondary structure motifs of residues 17 to 27 of native and methylated isolated H3 tails across the 50 MD replicas.

H3residue #	Alpha helix	3--10	Pi	Turn	Bend	Extended	Bridge
17	0.3716	0.0206	0	0.1036	0.1043	0.0077	0.0153
18	0.4236	0.0385	0	0.1043	0.155	0.0077	0.0096
19	0.4283	0.0439	0	0.0939	0.107	0.0082	0.021
20	0.4336	0.0382	0	0.1063	0.1011	0.0067	0.0076
21	0.3692	0.0239	0	0.1206	0.1674	0.0096	0.0035
22	0.3142	0.0124	0	0.0745	0.2171	0.0139	0.0202
23	0.4293	0.028	0	0.1196	0.1656	0.0137	0.0072
24	0.415	0.0291	0	0.125	0.1671	0.0068	0.0032
25	0.3728	0.0355	0	0.1323	0.1233	0.0029	0.0084
26	0.3227	0.0306	0	0.1512	0.1536	0.0097	0.0134
27	0.2279	0.0218	0	0.1964	0.2156	0.0165	0.0172
H3K9me3 residue #	Alpha helix	3--10	Pi	Turn	Bend	Extended	Bridge
17	0.4358	0.0258	0	0.0935	0.0744	0.0126	0.0166
18	0.4763	0.0297	0	0.0999	0.1011	0.014	0.0158
19	0.499	0.0352	0.0001	0.0968	0.0787	0.0099	0.0133
20	0.4888	0.028	0.0001	0.1209	0.0933	0.0053	0.0038
21	0.432	0.026	0.0001	0.1273	0.1514	0.004	0.0056
22	0.3724	0.018	0	0.0965	0.1922	0.006	0.0095
23	0.4254	0.0269	0.0001	0.1661	0.1361	0.0068	0.0057
24	0.3965	0.0257	0.0001	0.1318	0.1581	0.0064	0.0089
25	0.338	0.0307	0.0001	0.1321	0.1301	0.0035	0.0064
26	0.2766	0.0277	0	0.1529	0.1574	0.0047	0.0086
27	0.1797	0.0213	0	0.2239	0.2311	0.0152	0.0164
H3K27me3 residue #	Alpha helix	3--10	Pi	Turn	Bend	Extended	Bridge
17	0.4092	0.0265	0	0.1083	0.0856	0.0213	0.0129
18	0.4475	0.0346	0	0.1008	0.1322	0.0226	0.0128
19	0.4577	0.0464	0	0.1026	0.0954	0.006	0.0115
20	0.4648	0.0373	0	0.1278	0.1023	0.0049	0.0092
21	0.4019	0.0278	0	0.1293	0.1618	0.0124	0.007
22	0.3474	0.0143	0	0.0685	0.1708	0.0256	0.0229
23	0.3526	0.0337	0	0.1345	0.1557	0.0257	0.0132
24	0.3038	0.0367	0	0.1548	0.1741	0.0104	0.014
25	0.2352	0.0364	0	0.1597	0.1497	0.0059	0.0182
26	0.1709	0.0151	0	0.1163	0.2295	0.0099	0.0089
27	0.0889	0.0051	0	0.1642	0.3022	0.0155	0.0092

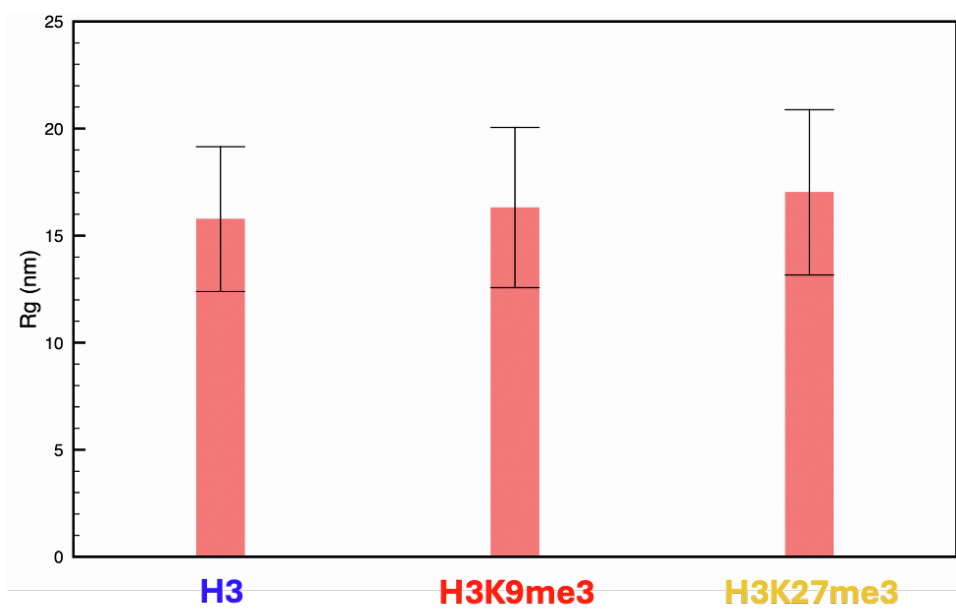
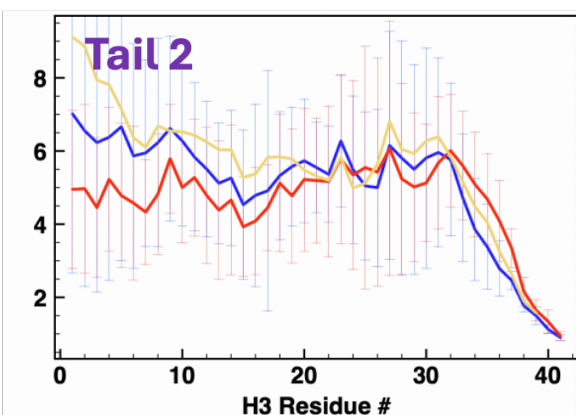
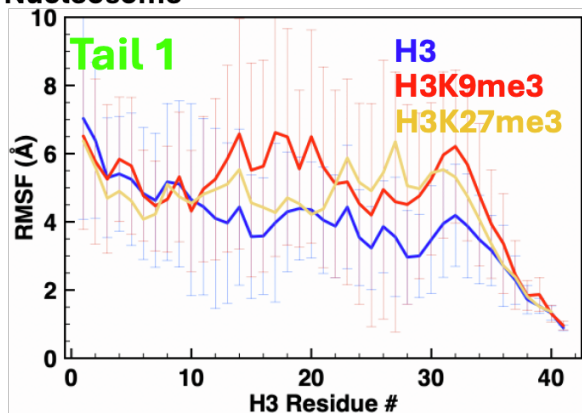
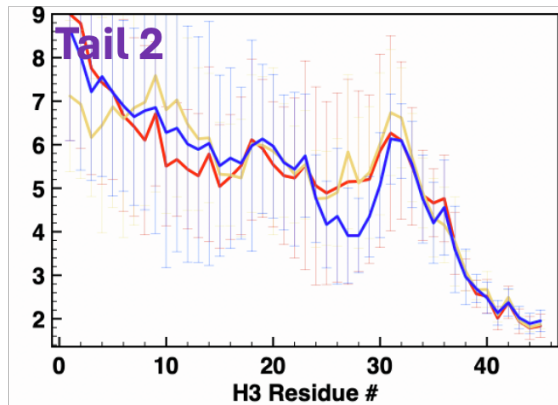
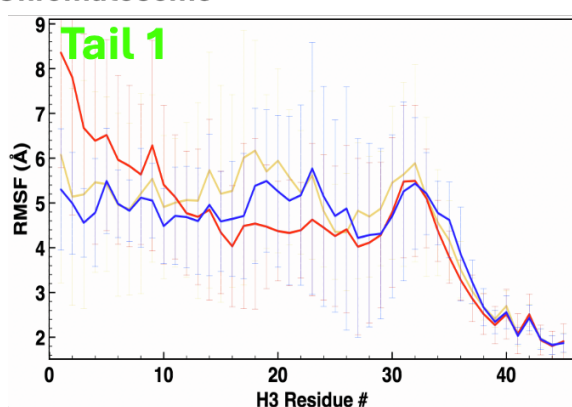


Figure S9. Average Rg for isolated H3 tails in native form and methylated on K9 or K27.

Nucleosome



Chromatosome



Stacked nucleosome

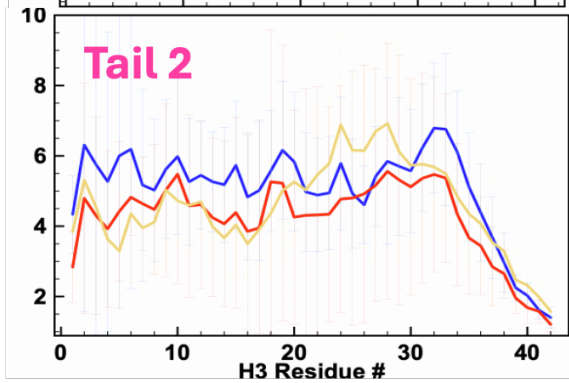
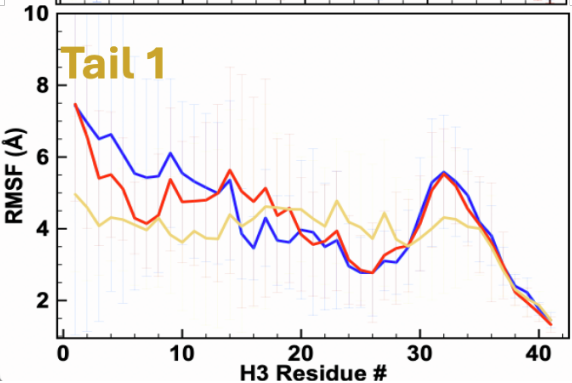
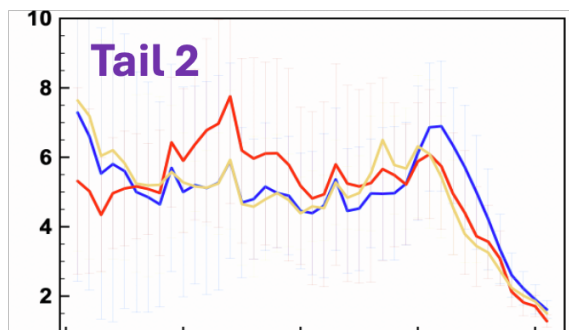
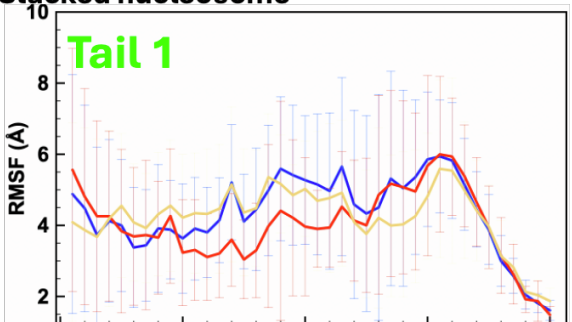
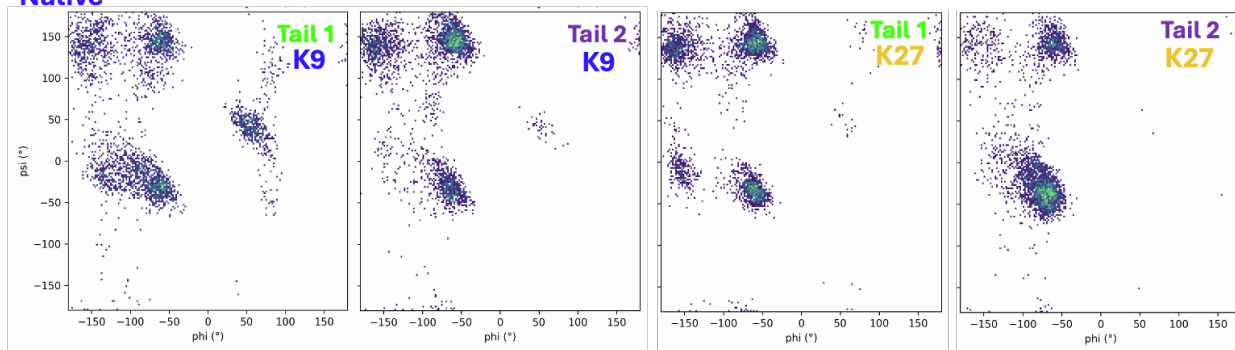


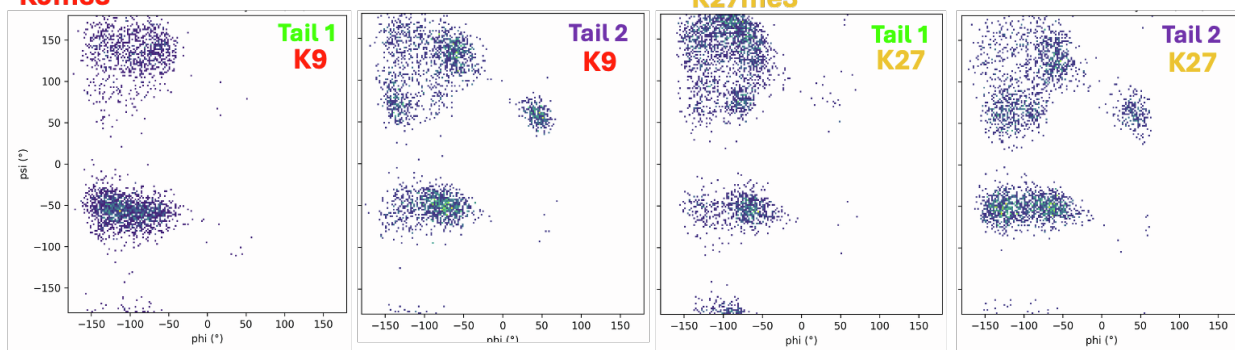
Figure S10. Average RMSF calculated for 6 replicas for the nucleosome, chromatosome, and stacked nucleosome systems.

Nucleosome

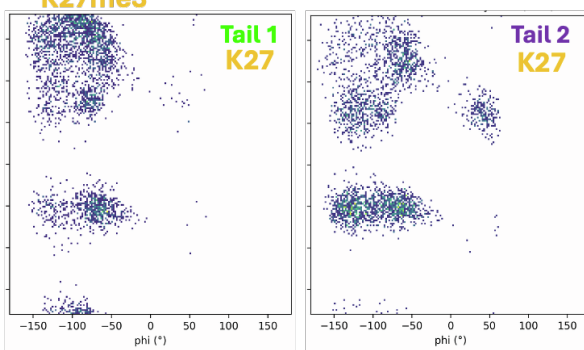
Native



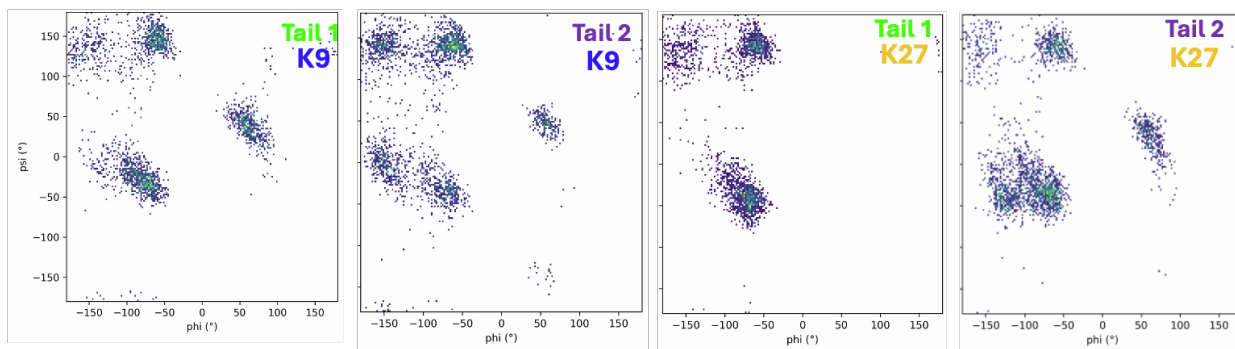
K9me3



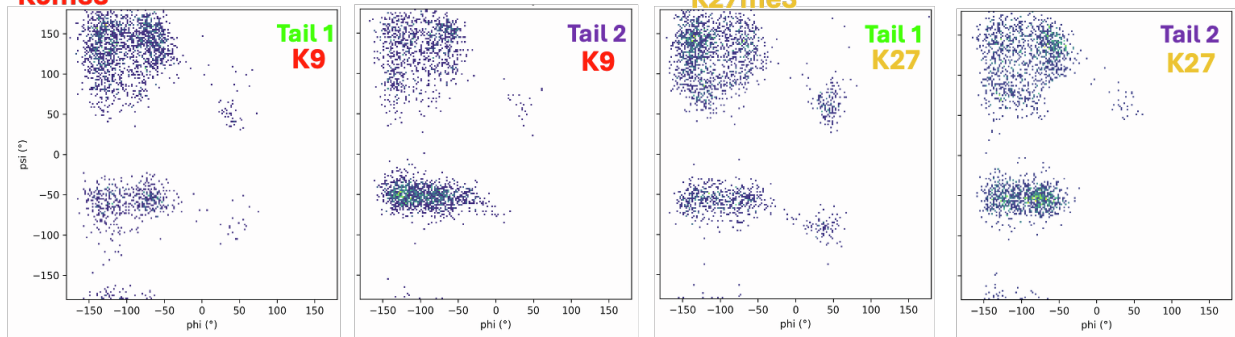
K27me3



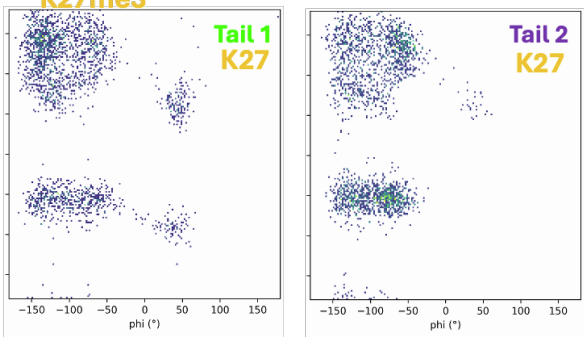
Chromatosome



K9me3



K27me3



Stacked Nucleosomes

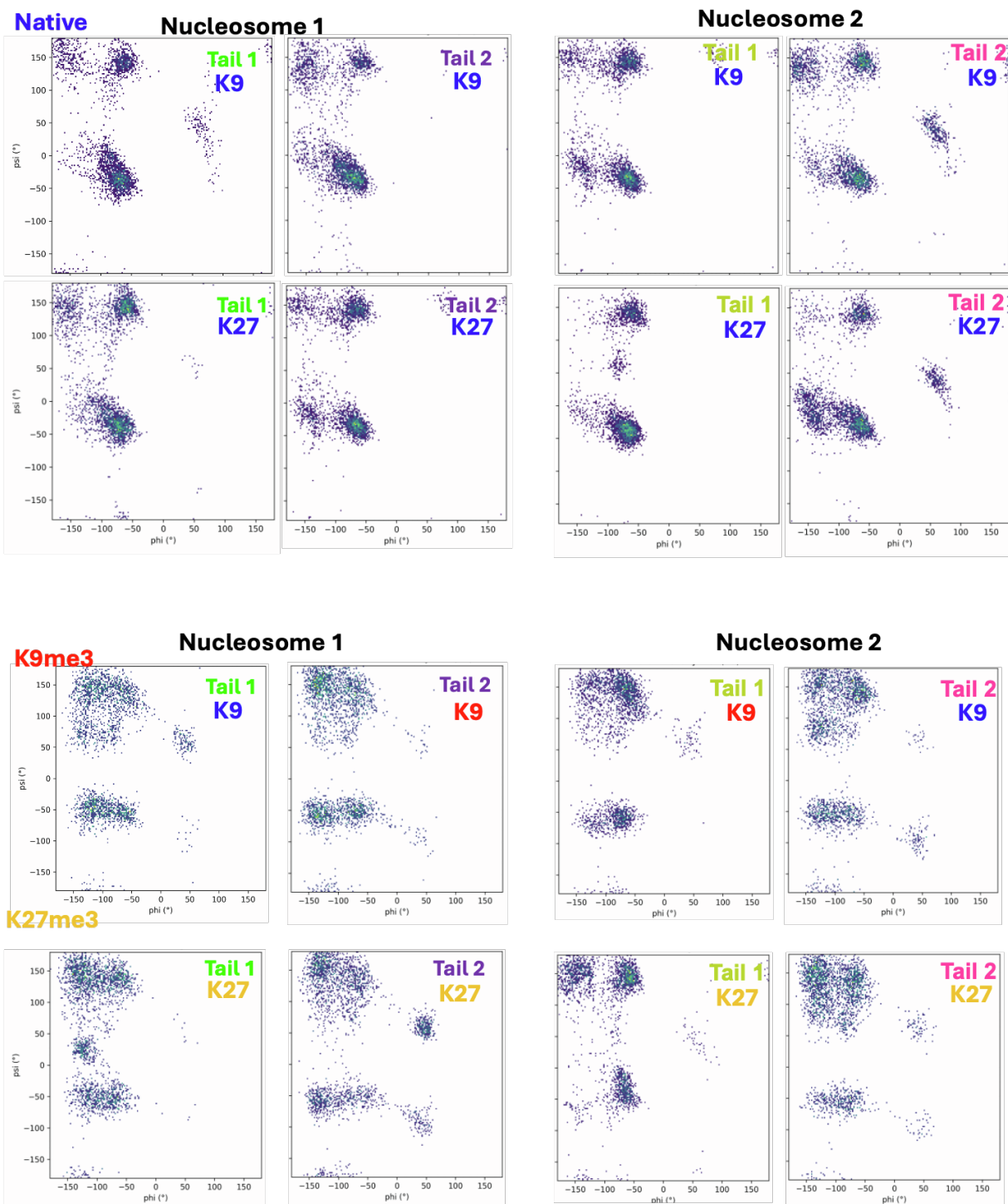


Figure S11. Ramachandran plots for residues K9 and K27 in the in the nucleosome, chromatosome, and stacked nucleosomes with native and K9me3 and K27me3 H3 tails.

Chromosome

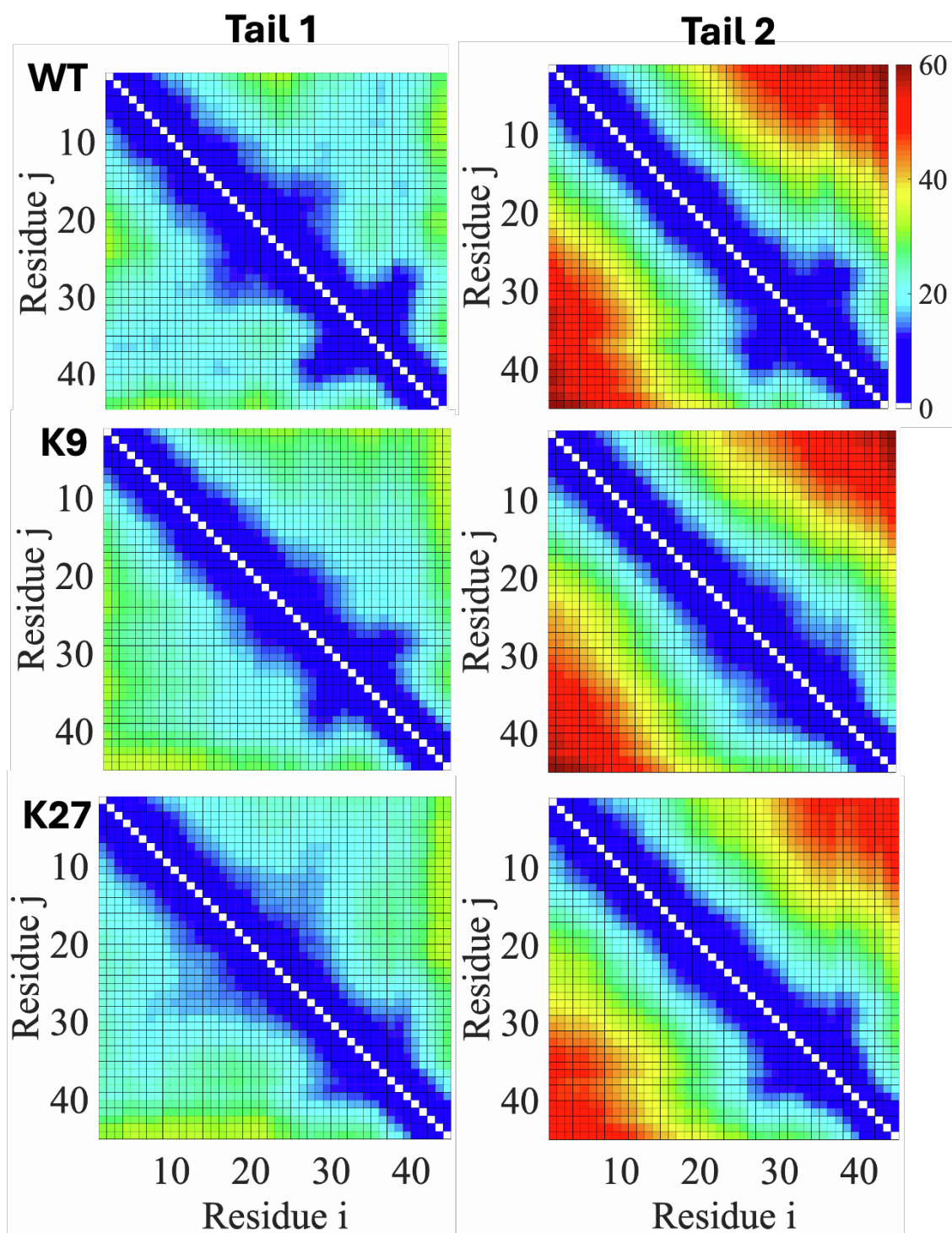


Figure S12. Contact matrices depicting $\text{C}\alpha$ - $\text{C}\alpha$ distances across all residues for the Tail1 and Tail2 of native and methylated chromosomes.

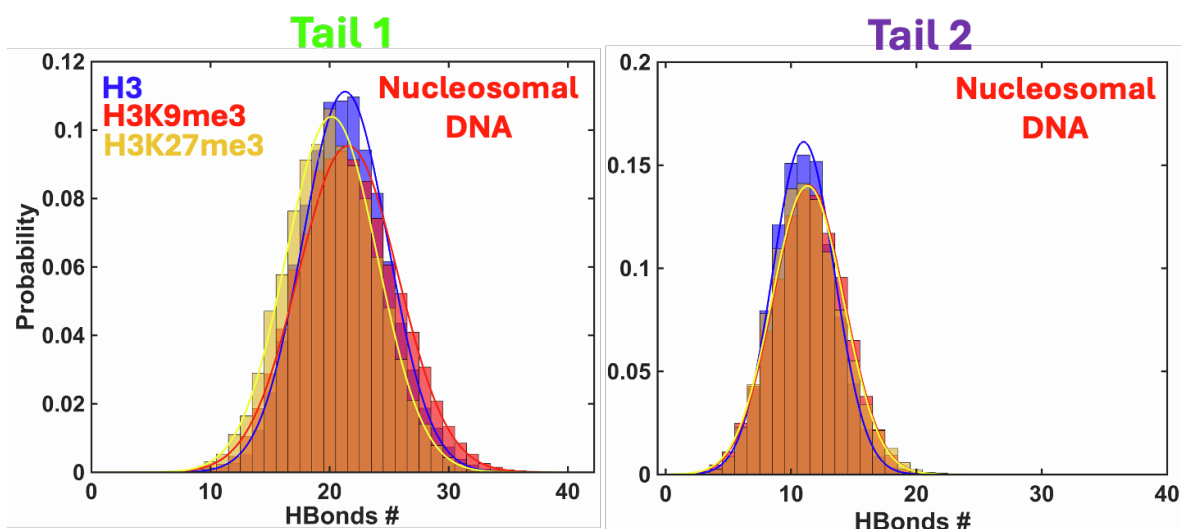


Figure S13. Number of hydrogen bonds (HBonds) between the tail 1 or tail 2 and the nucleosomal DNA of the chromosome.

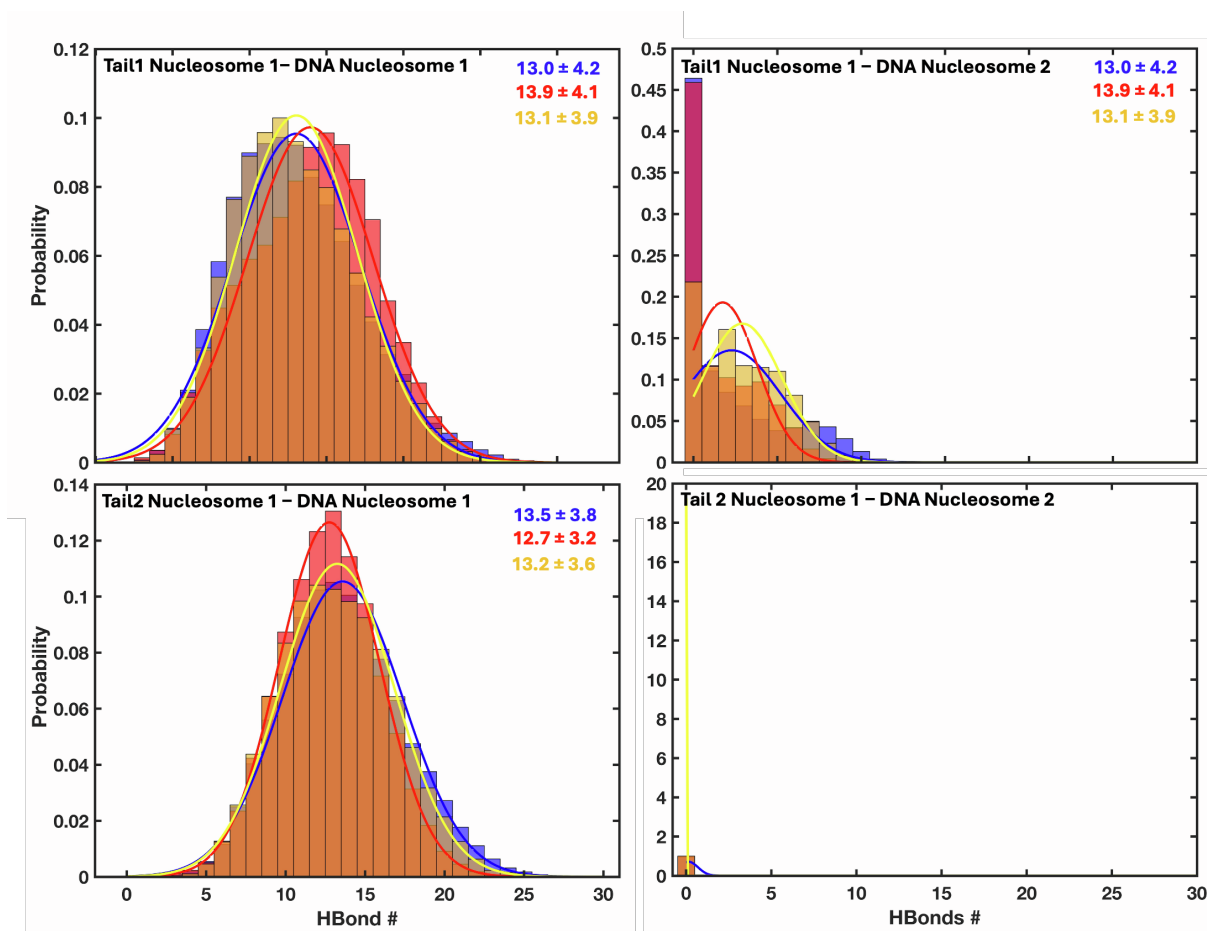


Figure S14. Number of hydrogen bonds (HBonds) between the tail 1 or tail 2 of nucleosome 1 of the stacked nucleosome system and the nucleosomal DNA of nucleosome 1 and nucleosome 2.