Determination of the Oligonucleosome Free Energy Landscape through Molecular Simulation and Enhanced Sampling

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Abstract

Understanding the mechanisms by which genetic and epigenetic changes influence gene expression through changes in chromatin structure requires accurate spatial models for chromatin across multiple length scales. Our simulations of nucleosome arrays using enhanced sampling techniques have revealed two distinct, metastable structural states present in chromatin fibers, separated by a free energy difference on the order of 1 kbT per nucleosome. The relative stability of these states, and the energy penalty of transitioning between them, is revealed to be highly sensitive to the presence of a linker histone, the nucleosome repeat length, and the presence of histone acetylation on the nucleosome H4 tails. These metastable structures both shed light on the historical difficulty of determining the structure of chromatin at the 30-nanometer scale, and extends the recent findings of stable tetranucleosome structural motifs to longer fibers. These structures may play major roles in understanding the rearrangement of chromatin during active biological processes, and in the behavior of chromatin domains on larger length scales.

Background

The discovery of the double-helical structure of successive nucleic acids along the backbone by Rosalind Franklin et al. enabled the modern field of genetics, through its insights that genetic information was sequentially coded in the A,T,C, and G sequence which could be “read” by RNA polymerases. It has also become increasingly apparent that the higher-order structure of chromatin influences gene expression by affecting the availability of DNA to the nucleoplasm and selectively allowing binding of transcription factors, and that epigenetic modifications such as histone post-translational modifications may code for changes in this higher order structure.

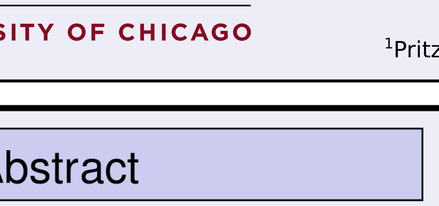


Figure 1. (a) The zig-zag model for the 30-nm fiber. In this model, linker DNA segments between nucleosomes are straight and pass through the central axis, and nucleosomes are stacked in two helices which spiral around the central axis. (b) The solenoid model fo rthe 30-nm fiber. In this model, the nucleosomes spiral around the outside of the fiber with a helical angle of roughly 36 degrees, with the linker DNA bent in the core between successive nucleosomes.

Unfortunately, experiments to determine the paradigms of higher-order chromatin folding have been largely inconclusive to date. Figure 1 shows two of the most well-studied models for chromatin structure past the nucleosome, the zig-zag fiber[1] and the solenoid[2], each of which is consistent with a portion of experimental data in the field but not all[3,4,5]. In this study, we propose using molecular simulation to assess the free energy landscape of these structures and intermediates to elucidate the possibility of heteromorphic and disordered structures between these ideal endpoints[6,7].

Results

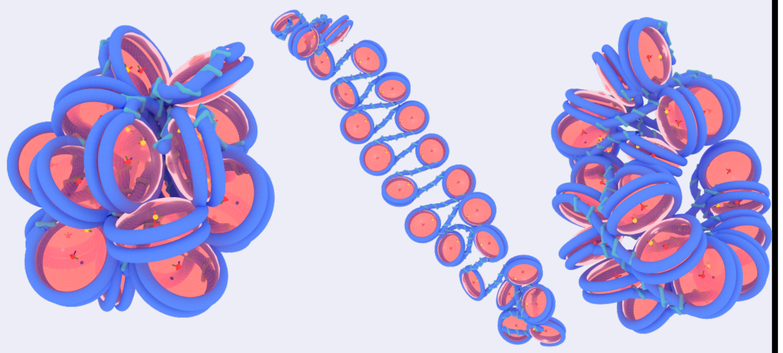


Figure 3. Right to left: Representative structure of the equilibrated solenoid structure, the midpoint of the reaction coordinate, and the zig-zag structure. Red ellipsoids surrounded by a cylindrical spiral represent the nucleosome core particle, and the intervening blue cylinder represents linker DNA.

Enhanced sampling along the path between the solenoid and zig-zag produced the endpoint structures shown in Figure 3. The zig-zag structure remained largely cohesive, maintaining I +- 2 contacts throughout the ensemble. The solenoid structure remained condensed with many I +- 1 contacts, but the interdigitation of the fiber was not stable under thermal motion. The potential of mean force along the reaction coordinate is shown in Figure 4a, along with the PMF for NRL 207 with mark H4K16Ac. These same PMFs, but with the linker histone present, are shown in Figure 4b.

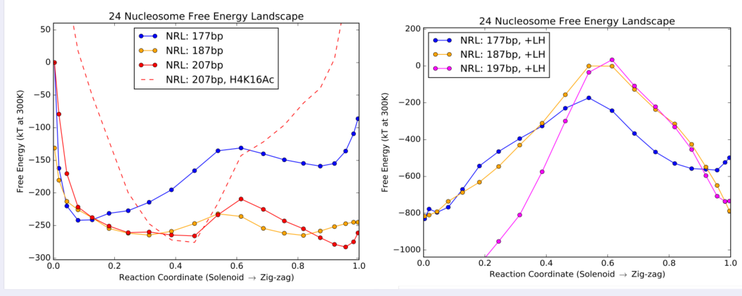


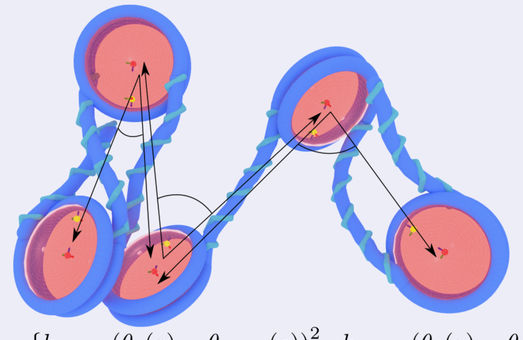
Figure 4. (a) The free energy landscape of the solenoid to zig-zag transition without linker histone or epigenetic modification. Vertical alignment of different curves are arbitrary and performed for visual effect. The crystalline solenoid structure is unstable for all NRLs, but the zig-zag and near-zig-zag structures become increasingly stable with growing NRL. (b) The free energy landscape of this transition with linker histones. The solenoid-like condensed structures are now always more stable than zig-zag-like structures, but now with an energetic barrier larger than 20kT per nucleosome.

Our simulations clearly show the presence of two metastable states along the coordinate, one similar to the solenoid structure, and one similar to the Zig-zag structure. Without a linker histone, the barrier between these states is less than 1.5 kT per nucleosome for any NRL, which can be easily overcome with thermal motion. By contrast, in the presence of a linker histone this barrier grows to tens of kT per nucleosome, likely preventing any transitions between these states. Lastly, histone H4 acetylation destabilizes the zig-zag-like state entirely, leading to only one stable disordered fiber structure.

Methods

All simulations were performed in the LAMMPS molecular dynamics package. Umbrella sampling was performed using the SSAGES package for enhanced sampling to fix the system at multiple points along the proposed reaction coordinate[8]. The reaction coordinate was defined as a combination of the fiber radius of gyration, and all nucleosome triplet angles, as shown in Figure 1. For any position s along the coordinate, biasing was applied to fix the configuration of the system to a specific set of triplet angles and radius of gyration. The mean biasing force was recorded for positions along s according to legendre-gauss quadrature, and these forces were integrated with the trapezoidal rule to produce potentials of mean force.

This free energy sampling was performed for a span of nucleosome repeat lengths: 177bp, 187bp, 197 bp, and 207bp. Additionally, all systems were simulated with and without a linker histone on each nucleosome, and for the system modified by the H4K16Ac epigenetic mark.



$$\Delta(\mathbf{r}, s) = \{k_{angle}\left(\theta_1(\mathbf{r}) - \theta_{1,ref}(s)\right)^2,\ k_{angle}\left(\theta_2(\mathbf{r}) - \theta_{2,ref}(s)\right)^2,\ ...,\ \left(R_g(\mathbf{r}) - R_{g,ref}(s)\right)^2\}$$

Figure 3. The collective variable describing the system is composed of the fiber radius of gyration, and all interior triplet angles between nucleosome centers shown here. As in Maragliano et al. (2006)[9], the biasing forces applied to the system are a multiple of the vector of squared residuals of each component variable.

Acknowledgements

We gratefully acknowledge helpful discussions with Josh Moller, Artem Rumyantsev, Dan Mendels, Ashley Guo and Cody Bezik in the development of this work.
This material is based upon work supported by the National Science Foundation Graduate Research Fellowship Program under Grant No. (DGE-1746045), and by National Science Foundation Grant Nos. (MCB-1818328), (CEE-1830969) and (MCB-180078). Any opinions, findings, and conclusions or recommendations expressed in this material are those of the author(s) and do not necessarily reflect the views of the National Science Foundation.

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