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Characterizing the mechanism of chromatin remodeling by molecular dynamics simulations

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Chromosomes inside our nucleus are not just a passive containers of genetic information: their structure and dynamics have great effect on many cellular processes. Our research used computer simulations to reveal how subtle molecular motions of chromosomes control the access to the genetic material.

The DNA inside our nucleus is wrapped around proteins to form complexes called nucleosomes, whose structure and dynamics greatly affect the processing of genetic information. In this research, we performed computer simulations that allow us to visualize the molecular dynamics of nucleosomes at the level of individual atoms. Our simulations revealed the molecular mechanism of nucleosome motion along the genome, an important process that regulates the access to DNA, highlighting the high plasticity of protein-DNA interactions within nucleosomes. Importantly, we also showed that DNA sequence can affect the motion of nucleosomes, and therefore act as a new layer of information to regulate the genome on top of the one used to express proteins.

chromatin

nucleosome sliding all-atom MD simulation string method twist defects

Nucleosomes are the fundamental units of Eukaryotic chromatin organization. Each of them is composed of ~147 base pairs of DNA wrapped almost 2 times around a core made of 8 histone proteins. This configuration enables the compaction of long genomes into nuclei and limits the access to DNA by other proteins such as transcription factors, regulating key genomic activities such as transcription. Chromatin remodelers are ATP-dependent molecular motors in charge of modulating the organization of chromatin along the genome. They comprise of a large family of proteins performing a diverse range of activities: sliding DNA around histones to generate regularly spaced nucleosomes, ejecting nucleosomes from promoters to facilitate the binding of the transcriptional machinery, and exchanging histone variants at target genomic regions. Despite the diversity of the observed remodeling activities, experiments suggest that all remodeler mechanisms are based on their shared ability to slide the nucleosomal DNA around histones.

We were the first laboratory to study nucleosome sliding by remodelers using coarse-grained molecular dynamics (MD) simulations. Specifically, we showed that the conserved translocase domain of remodelers can actively slide nucleosomal DNA by generating small DNA deformations called twist defects via its inchworm motion occurring during ATP hydrolysis (Brandani and Takada 2018). The nucleosome is stabilized by 14 strong contact points formed every DNA helical turn with the histone octamer. Twist defects are changes in DNA twist enabling to accommodate one extra or one missing base pair between two neighboring contact points. DNA sliding can occur when an interaction between a histone residue and a DNA phosphate group breaks and then reforms with a neighboring phosphate, with a concomitant diffusion of a twist defect. In our simulations we found that the translocase domain can actively induce the formation of twist defects at its binding location, and their spontaneous diffusion up to the opposite side eventually results in a net sliding of the entire nucleosomal DNA by 1 bp relative to the initial conformation. Over the past two years, various labs published results from cross-linking (Winger et al. 2018), single-molecule FRET (Sabantsev et al. 2019), and cryo-EM (Li et al. 2019) experiments in agreement with the mechanism of action proposed from our MD simulations.

While the community has now recognized the key role of twist defects for chromatin remodeling, major open problems remain. In particular, the key scientific questions we want to address are: 1. What is the energy cost of twist defects within nucleosomes? Currently the nature of the intermediate conformations and bottlenecks of the sliding process is still debated, and a detailed characterization of the free energy landscape of the process would be highly valuable. 2. How do specific histone-DNA interactions determine the dynamics of nucleosome sliding? Experimentally, some histone mutations have large effects on the kinetics of nucleosome sliding, and it would be of great interest to understand the molecular origin of such dependence. 3. How does the intrinsic sequence-dependent DNA elasticity affect sliding? The activity of many remodelers depends on the sequence of the underlying nucleosomal DNA. For instance, the RSC remodeler has been shown to recognize long poly-A tracts to displace nucleosomes specifically from yeast promoter regions. Such dependence may be due to the differences in DNA elasticity.

Our results will elucidate many currently unclear aspects of nucleosome sliding and remodeling activities: 1. We will provide a quantitative understanding of the energetics of the process, characterizing both intermediate metastable conformations and bottlenecks of the dynamics. 2. We will be able to identify the specific roles of key histone residues in either facilitating or inhibiting the sliding process. 3. Finally, we will be able to characterize sequence-dependent effects by repeating part of our simulations for different DNA sequences.

Due to limitations in the resolution and accuracy of the previously used computational and experimental methods, such key open questions could not be addressed so far. However, all-atom MD simulations are ideally suited to investigate these problems. The purpose of the proposed project is to use all-atom MD to provide a quantitative understanding of the energetics and dynamics of nucleosome sliding. All-atom MD was previously employed to study nucleosome conformational changes such as unwrapping, but, due to the slow dynamics of twist-defect formation, they were never applied to investigate nucleosome sliding. In spite of the complexity of the system, specific computational methods can be used to speed-up the simulations. In particular, we plan to reconstruct the

minimum-energy energy path of twist defect diffusion through a nucleosome during sliding using the string method (Matsunaga et al. 2018; Kobayashi et al. 2017; Maragliano et al. 2006). A minimum-energy path represents the most likely trajectory that a system employs to go from an initial state to a final one, providing a simple understanding of the underlying dynamics.

To obtain the minimum-energy path between two states A and B, we firstly define an initial path connecting A and B using a discrete number of reference conformations, called "images", equally spaced in a space of collective variables, for instance a set of distances. The first and last images in the path correspond to states A and B respectively. This initial pathway is usually obtained by steered-MD simulations. Then, the path is optimized by updating the image positions in the chosen collective-variable space until the path follows the gradient of the free energy landscape. One update step requires computing the mean forces on the path image by restrained MD simulations. Several cycles of restrained MD and image updates are repeated until convergence. Finally, after convergence of the minimum-energy path, we will also be able to reconstruct the full free-energy profile of the sliding transition via a series of restrained MD simulations, identifying the main intermediate states and bottlenecks of the process.

In our case, the starting state of the transition path, A, consists of the nucleosome conformation observed when bound to the ATP-free translocase domain (Li et al. 2019). This conformation already contains a 1-bp twist defect/bulge at the remodeler binding location. The final state B consists of a canonical nucleosome conformation where the initial bulge moved to the opposite end of the nucleosomal DNA, completing a 1-bp sliding event. Our study will focus on the diffusion of the initial bulge defect, but not on its generation by the translocase domain, since this step was already observed by cryo-EM (Li et al. 2019). Furthermore, our simulation system will likely not include the translocase domain itself, since its limited contacts with the nucleosomes are not expected to affect the diffusion of defects.

We now completed running our planned MD simulations to investigate the molecular mechanism of nucleosome sliding, and are preparing a manuscript that will describe our achievements. First, we could complete microseconds-long all-atom MD simulations of full nucleosomes and smaller histone disomes comprising of one H3/H4 histone dimer wrapping 35 bp of DNA. In both these systems we could observe the spontaneous motion of DNA at several contact points with the histone, accompanied by the formation and propagation of twist defect deformations. These simulations established that twist defects, which are believed to be exploited by chromatin remodelers to actively slide nucleosomes, can also form spontaneously due to thermal fluctuations. Then, we used biased MD simulations to observe the complete sliding of nucleosomal DNA by 1 full base pair over the entire interface with the histones. These simulations were the basis for computationally expensive string method calculations that allowed us to estimate the potential of mean force (or free energy) of the system as sliding progresses. These calculations allowed us to understand the molecular details of the sliding dynamics: in particular, we showed that DNA sliding is facilitated by concerted motions of the histone octamer, for example involving the re-orientation of certain amino acid side chains close to the DNA, and that the key barrier to sliding is represented by the breaking of several hydrogen bonds that stabilize the overall nucleosome structure.

Finally, our simulations of nucleosomes with different DNA sequences suggested that indeed DNA sequence can have a great effect on sliding. We focused our attention on poly-A tracts, which some previous studies suggested to affect the organization of nucleosomes and the activity of remodelers. Specifically, using a combination of all-atom and coarse-grained MD simulations, we showed that poly-A tracts destabilize defect deformations used by remodelers to actively slide DNA from their binding site. Our simulations predict that poly-A tracts would inhibit remodeling activity and act as barriers to active sliding, which could be confirmed in experiments done in the laboratories of Gregory Bowman and Taekjip Ha at Johns Hopkins University. A manuscript describing the sequence-dependent activity of remodelers has already been submitted to a journal for publication.

Overall, we made important advancements in characterizing the detailed molecular mechanism of nucleosome sliding, which, aside from being an important fundamental question on its own, will also aid our understanding of other processes that occur on our genome, most notably chromatin remodeling, but also transcription and gene regulation.

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