View metadata, citation and similar papers at core.ac.uk

brought to you by CORE

provided by Juelich Shared Electronic Resources

John von Neumann Institute for Computing

DNA Packaging and Electrostatic Interactions

N. Korolev, L. Nordenskiöld

published in

From Computational Biophysics to Systems Biology (CBSB07),
Proceedings of the NIC Workshop 2007,
Ulrich H. E. Hansmann, Jan Meinke, Sandipan Mohanty,
Olav Zimmermann (Editors),
John von Neumann Institute for Computing, Jülich,
NIC Series, Vol. **36**, ISBN 978-3-9810843-2-0, pp. 189-194, 2007.

© 2007 by John von Neumann Institute for Computing
Permission to make digital or hard copies of portions of this work for personal or classroom use is granted provided that the copies are not made or distributed for profit or commercial advantage and that copies bear this notice and the full citation on the first page. To copy otherwise requires prior specific permission by the publisher mentioned above.

http://www.fz-juelich.de/nic-series/volume36

DNA Packaging and Electrostatic Interactions

Nikolay Korolev and Lars Nordenskiöld

School of Biological Sciences, Nanyang Technological University,
60 Nanyang Drive, 637551 Singapore
E-mail: korolev@ntu.edu.sg

In cell nucleus, all DNA exists as a highly packed nucleoprotein complex called chromatin. DNA is highly negatively charged polyelectrolyte and as consequence DNA-DNA repulsion must be overcome to form chromatin. The first level of DNA packaging is the nucleosome core particle (NCP), composed of 150 bp of DNA wrapped around a histone octamer core and flexible positively N-terminal domains (histone tails) protruding out from the NCP. Linear arrays of the NCP are further folded into higher level chromatin structures with the histone tails playing important roles in their formation. Theoretical models from Poisson Boltzmann (PB) approximation to all-atom molecular dynamics simulations are used to describe chromatin/nucleosome statics and dynamics. Applying the PB theory we show that free energy of NCP formation is extremely favorable that challenges established opinion about marginal stability of the NCP. To describe the influence of the histone tails on aggregation and dynamics of the NCPs, we carried out molecular dynamics simulations with coarse-grained approximation of the NCP. Our results are in good agreement with experimental neutron scattering and X-ray diffraction data. To reveal molecular details of the histone tail-DNA binding and dynamics, all-atom MD simulations were undertaken in a system comprising several DNA oligomers and fragments of the histone tails. Correlation between DNA-DNA distance and binding the histone tails to DNA is clearly observed. At the same time, binding of the tails does not restrict internal dynamics of the DNA.

In eukaryotic cell, long DNA (roughly 2 m in humans) is packed about 400,000 times inside a cell nucleus of 10-20 μm in diameter. DNA packaging is performed by nuclear proteins and this specific nucleoprotein complex called chromatin performs contradictory functions keeping DNA compact and protected while remaining selectively accessible and dynamic. About 85% of DNA in chromatin is represented by uniform units, the nucleosomes, which are the complex of 160-230 base pairs (bp) of DNA double helix with five histone proteins (H2A, H2B, H3, H4, and H1). The most regular central part of the nucleosome is called the nucleosome core particle (NCP) and consists of 147 bp DNA wrapped as a 1.75 turn superhelix around the histone octamer formed from one $(H3/H4)_2$ tetramer and two H2A/H2B dimers.[1,2] Variable length (10-80 bp) of the DNA (called linker DNA) connects the NCPs to each other and binds to the linker histone H1. Linear arrays of the NCP are further folded into higher level chromatin structures with the histone tails playing important roles in their formation. The histones are responsible for the first step of DNA compaction in chromatin and pose a major obstacle for direct access to the DNA of proteins responsible for DNA replication, transcription, repair, and recombination. Variable length double stranded linker DNA connects the NCPs with each other to form nucleosomal arrays which condense into the 30-nm chromatin fibers.[3,4] Each of the core histones has unstructured N-terminal domain called "histone tail" protruding through the DNA superhelix. The tails are able to interact with DNA of the NCP, linker DNA and variety of nuclear proteins. The structure of the histone tails largely escapes detection by X ray crystallography and by other experimental methods, implying that they are highly flexible and dynamic. The histone tails are essential for maintenance of the higher order compact folded structures of chromatin and for regulation of transcription and replication.[2,5] These

functions of the histone tails are regulated by covalent modifications of the amino acids, which may change the net charge and distribution of the charged groups in the tails.

DNA is highly negatively charged polyelectrolyte and therefore huge repulsive force between the DNA molecules must be overcome to form chromatin. Positively charged amino acids Lys^{+} and Arg^{+} of the "histone-fold" domains form a distinct charged surface which direct DNA wrapping in the NCP. The histone tails are highly basic. One might imagine that huge forces of electrostatic origin are involved in all transitions of chromatin. However, the role of electrostatic interactions contributing to chromatin/nucleosome structure and dynamics, has received less attention compared to the other phenomena (e.g. DNA bending properties, topological problems related to the DNA unwinding and twisting). Our work analyses and models contribution of electrostatic forces to formation of the nucleosome and ability of the nucleosomes to aggregate and mediation of the charged groups of the histone tails in close DNA-DNA contacts.

1 Estimation of the NCP Stability Using Poisson Boltzmann Polyelectrolyte Model

In the process of unwrapping DNA from histones two strongly charged polyelectrolyte entities are formed: the negatively charged DNA and positively charged histone core. Electrostatic interactions arising in such a case must have a profound influence on the course of whole process and on equilibrium between the two phases. In a single-molecule experiments,[6,7] the electrostatic forces resist the detachment of the DNA from the histones during the single-chromatin fiber stretching under the influence of a mechanical pulling force. The degree of this resistance is dependent not only on the strength of the individual histone core – DNA contacts but also on the ionic conditions in the solution as well as on the balance between the positively and negatively charged groups in the chromatin. Other important contributions to the detachment process, the mechanical bending force and specific short-range DNA-histone interactions, are not dependent on the ionic conditions. Despite the fact that the electrostatic interactions must have a significant influence on DNA unwrapping, their role is rarely discussed in the literature. Recently, we analyze the electrostatic component of the DNA-histone interactions within the NCP.[8,9] Using the mean theory (Poisson Boltzmann) approximation, the contribution of the electrostatic forces to a formation of the elementary structural unit of chromatin, the nucleosome was estimated. Results of the modeling were discussed in relation to the recent DNA stretching experiments.[6,7]

Figure 1 shows contribution of the electrostatic forces involved in gradual removal of the DNA from the histone proteins in chromatin.[8,9] Results from the PB theory challenge an established opinion that the free energy of the NCP formation is small and that the forces related to the bending of the DNA double helix plays make the major contribution to the NCP energetics. Instead, calculations within the PB model reveal that of the electrostatic free energy of the NCP formation is extremely favorable (see Fig. 1). Remarkably, simple polyelectrolyte model is in a semi-quantitative agreement with recent optical tweezers stretching experiments measuring the force necessary to unwrap DNA from the histone core.[6,7] Our analysis shows that the electrostatic interactions between the highly negatively charged polymeric DNA and the positively charged histones play a determining role in stabilizing the nucleosomes at physiological conditions.

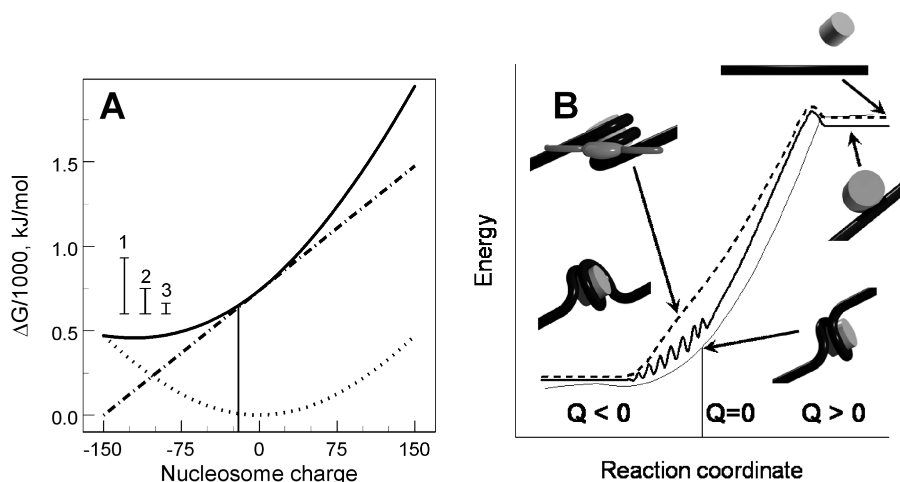

Figure 1. (A) Estimation of electrostatic free energy of the NCP dissociation. Degree of the nucleosome unwinding (abscissa) is measured as change of the total charge of the NCP (histone octamer + bound DNA). Dash-dotted line shows the free energy contribution from DNA, released from the octamer; dotted line is the electrostatic free energy of the NCP, approximated as a sphere of 50 Å radius; solid line is a sum of the two terms. Vertical bar shows the position when one full turn of DNA is wrapped around the histone core. PB cell model has been applied with conditions modeling chromatin stretching experiments. Vertical bars 1-3 are estimations of the DNA bending energy given in the literature and are drawn to highlight the importance of the electrostatic interactions relative to the DNA bending. (B). Change of the energy of the NCP in the process of the chromatin fiber unwinding. The thin line displays a hypothetical equilibrium DNA unwrapping from the histone core; solid and dashed curves are estimations of energy passes in single-fiber-pulling experiments from refs. and, respectively. Cartoons illustrate the NCP state at different stages of the DNA fiber stretching. See ref. 8 for details.

2 Coarse-Grained Molecular Dynamics for Salt-Dependent NCP-NCP Interaction

Molecular dynamics (MD) computer simulations of charged histone tail–DNA interactions in systems mimicking nucleosome core particles (NCP) have been conducted using coarse-grained model of the NCP.[10] The nucleosome is approximated as a negatively charged spherical particle with flexible polycationic histone tails attached to it in a dielectric continuum with explicit mobile counterions and added salt. The size, charge and distribution of the tails relative to the core were built mimicking real NCP. In this way, attractive ion-ion correlation effects due to fluctuations in the ion cloud and the attractive entropic and energetic tail bridging effects were incorporated. MD simulations in a dielectric continuum model containing explicit mobile counterions and various amount of added salt describe the effect of changing experimental conditions were carried out.[10] In agreement with experimental data,[11,12] increase of monovalent salt content from salt-free to physiological concentration leads to formation of NCP aggregates; and likewise in the presence of $MgCl_2$ the NCPs form condensed systems via histone tail bridging and accumulation of counterions (Fig. 2). The simulations give insight into the tail mediated bridging between core particles and are also of relevance for the mechanism of secondary and tertiary condensation of nucleosomal arrays. To our knowledge this is the first theoretical demonstration of

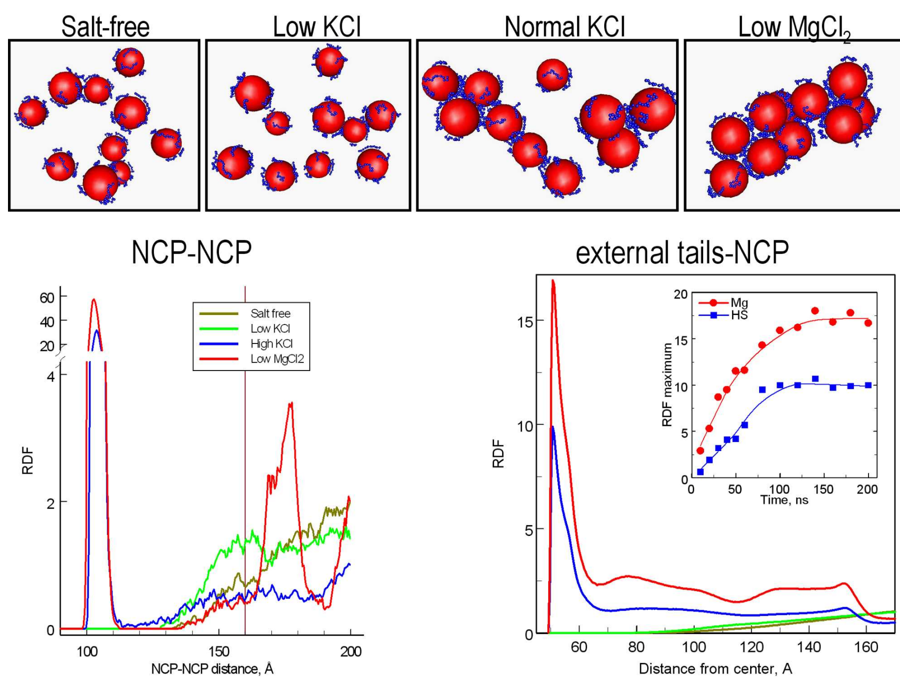

Figure 2. Top: Snapshots showing distribution of the NCP particles at the end of the four 200 ns coarse-grained MD simulations with increasing concentration of K^+ (3 cartoones to the left) and in the presence of Mg^{2+} (right). Bottom: RDFs calculated from the last 25% of the four coarse-grained MD simulations. Left: NCP-NCP correlation function. Maxima between 100-110 Å indicate NCP-NCP aggregation. Right: Intermolecular tail-NCP RDF. Appearance of maximum below 60 Å reveals crosslinking of the histone tails in the aggregated NCPs. Insert displays the dependence of the maximum of the RDF on simulation time. See ref. 11 for details.

nucleosome-nucleosome tail bridging attraction within a statistical mechanical treatment which (within the model) gives the equilibrium description (NVT ensemble simulations) for a system whose parameters model the NCP and which explicitly includes all charged mobile particles taking into account salt dependent ion-ion correlation and flexible charged tails.

3 All Atom MD Simulation of DNA-DNA Attraction Mediated by the Histone Tails

To investigate atomic details of the histone-tail mediated DNA-DNA interaction, two series of all-atom MD simulations were performed during 30-50 ns for a system of 3-4 identical DNA 22-mers, 14-20 short fragments of the charged H4 histone tail peptide fragments (amino acids 5-12, $[KGGKGLGK]^{3+}$,[10] and 13-19, $[GGAKRHRK]^{4+}$ (unpublished work)) with K^+ counterions and explicit water. The simulation setup mimics the crowded conditions of DNA in eukaryotic chromatin. To assess the influence of tail fragments on DNA structure and dynamics, 'control' MD simulations were carried for systems with the

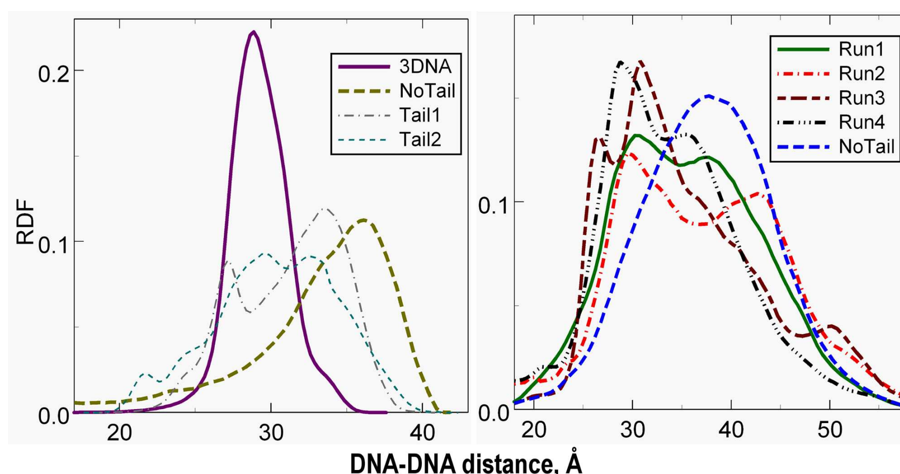

Figure 3. Distribution of the average DNA-DNA distance in MD simulations with 3 (left) and 4 (right) DNA 22-mers in the simuation cell. Short-dashed lines show DNA-DNA distance in the absence of the H4 histone tail fragments. Thin dotted lines (Tail1, Tail2) in the left graph display DNA-DNA distance in the presence of the 5-12 a.a. $[KGGKGLGK]^{3+}$ fragment. Solid line in the left graph ("3DNA") and Run1-Run4 curves in the right graph show DNA-DNA distance in the presence of the 13-19 a.a. $[GGAKRHRK]^{4+}$ fragments. In the absence of the tail fragments DNA-DNA distance is determined by the dimension of the simulation cell; presence of the histone tail fragments makes DNA-DNA distance 8-10 Å shorter.

same DNA and water content but in the absence of oligopeptides. DNA structure and dynamics, DNA-DNA interaction and its interplay with the histone tail fragments binding are described.[10,13] MD simulations allow capturing typical features of the histone tail-counterion-DNA structure, interaction and dynamics.

Binding of the tail fragments to the DNA is dynamic. The charged side chains of the lysines and arginines play a major role in mediating DNA-DNA attraction by forming bridges and coordinating to phosphate groups and electronegative sites in the minor groove. Correlation of the DNA-DNA distance with the presence and association of the histone tail between the DNA molecules is observed. Figure 3 compares distance between DNA molecules in the absence (dashed lines with maximum at 35-40 Å) and in the presence of the tails (all other data). In the systems without tails DNA molecules tend to distribute as far as possible from each other. In the systems with the histone tail fragments, the most populated DNA-DNA distances are in the range 24-34 Å (Fig. 3).

Detailed investigation of the histone tail-DNA interaction shows that tail bridging is performed through numerous contacts of Lys^{+} and Arg^{+} with the phosphate groups combined with penetration of the charged lysine side chains into the minor groove of the DNA. Experimental and computational work is in progress which investigates interplay between covalent modification of the histone tails and compaction of chromatin, NCP-NCP and DNA-DNA interaction.

References

1. K. Luger, A. W. Mader, R. K. Richmond, D. F. Sargent, T. J. Richmond, *Crystal structure of the nucleosome core particle at 2.8 Å resolution,* Nature **389**, 251-260, 1997.
2. A. P. Wolffe, *Chromatin: Structure and function* 3rd Edition, pp. 447, Academic Press, San Diego, CA, 1998.
3. C. L. Woodcock, S. Dimitrov, *Higher-order structure of chromatin and chromosomes,* Curr.Opin.Genet.Dev. **11**, 130-135, 2001.
4. J. C. Hansen, *Conformational dynamics of the chromatin fiber in solution: determinants, mechanisms, and functions,* Annu.Rev.Biophys.Biomol.Struct. **31**, 361-392, 2002.
5. P. J. Horn, C. L. Peterson, *Chromatin higher order folding: Wrapping up transcription,* Science **297**, 1824-1827, 2002.
6. B. D. Brower-Toland *et al.*, *Mechanical disruption of individual nucleosomes reveals a reversible multistage release of DNA,* Proc.Natl.Acad.Sci.U.S.A. **99**, 1960-1965, 2002.
7. M. L. Bennink *et al.*, *Unfolding individual nucleosomes by streyching single chromatin fibers with optical tweezers,* Nature Struct.Biol. **8**, 606-610, 2001.
8. N. Korolev, A. P. Lyubartsev, A. Laaksonen, *Electrostatic background of chromatin fiber stretching,* J.Biomol.Struct.Dyn. **22**, 215-226, 2004.
9. N. Korolev, O. V. Vorontsova, L. Nordenskiöld, *Physicochemical analysis of electrostatic foundation for DNA-protein interactions in chromatin transformations,* Prog.Biophys.Molec.Biol. **in press**, 2007.
10. N. Korolev, A. P. Lyubartsev, L. Nordenskiöld, *Computer modeling demonstrates that electrostatic attraction of nucleosomal DNA is mediated by histone tails,* Biophys.J. **90**, 4305-4316, 2006.
11. S. Mangenot, E. Raspaud, C. Tribet, L. Belloni, F. Livolant, *Interactions between isolated nucleosome core particles. A tail bridging effect?,* Eur.Phys.J.E **7**, 221-231, 2002.
12. A. Bertin, A. Leforestier, D. Durand, F. Livolant, *Role of histone tails in the conformation and interaction of nucleosome core particles,* Biochemistry **43**, 4773-4780, 2004.
13. N. Korolev, L. Nordenskiöld, *H4 histone tail mediated DNA-DNA interaction and effects on DNA structure, flexibility and counterion binding. A molecular dynamics study,* Biopolymers **in press**, 2007.