Role of histone tails in NCP stability and chromatin assembly

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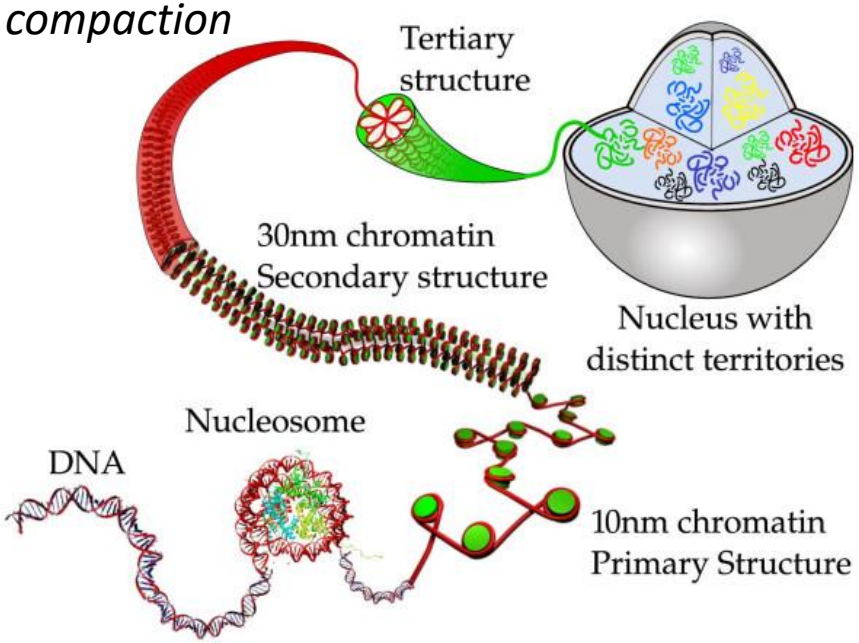
Outline of the talk

- Structure of Nucleosome
- Survey of the current atomistic and coarse-grained simulations
- Atomistic simulations of stacked NCPs
- Understanding role of different tails
- Parallel vs anti-parallel stacking
- Elasticity of NCP DNA
- Conclusion

Nucleosomes *in vivo* and *in vitro*

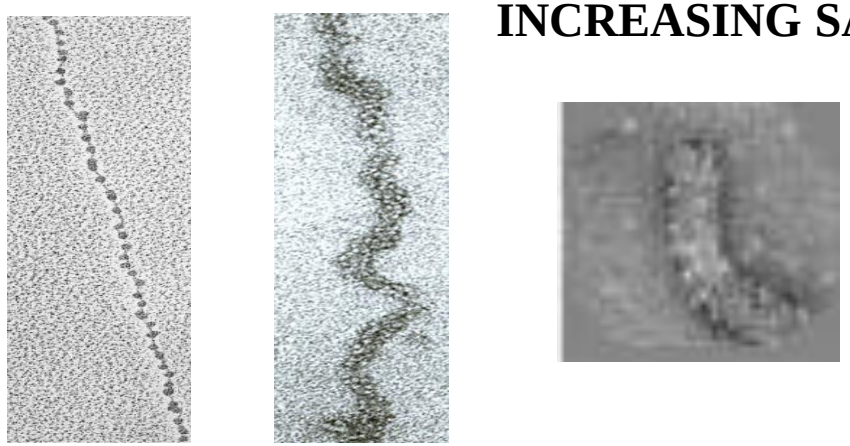
Hierarchical levels of DNA compaction

In vivo



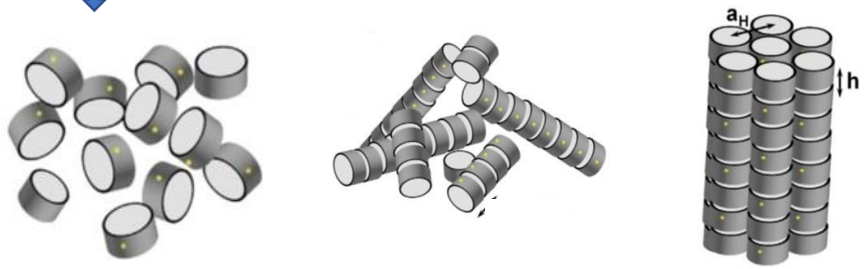
In vitro

INCREASING SALT



J.R. Daban, Micron, 42, 2011, 733-750.

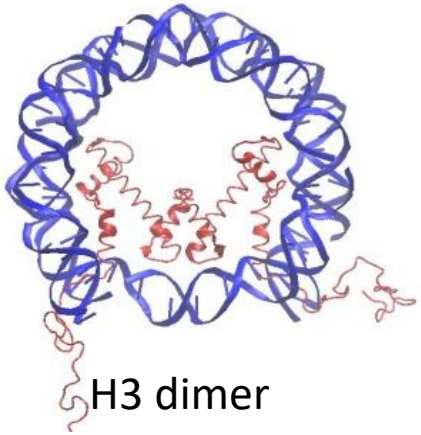
LINKERS DIGESTED



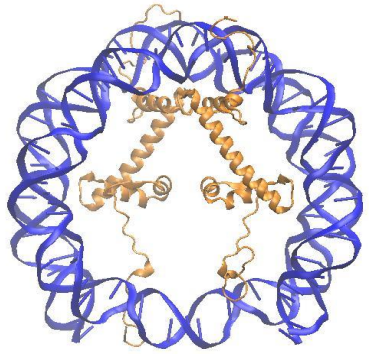
INCREASING SALT AND OSMOTIC PRESSURE

Nordenskiold et. al., Biophysical Journal 110, 1720–1731, 2016;
Livolant et. al., Biophysical Journal ,81, 2414–21, 2001; J.R. Daban, Micron, 42, 2011, 733-750.

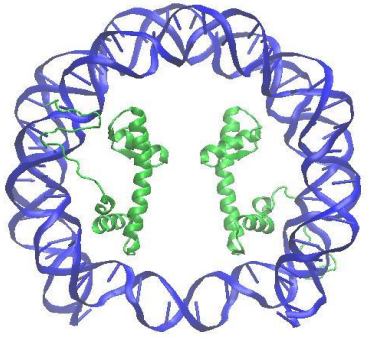
Structure of Nucleosome Core Particle



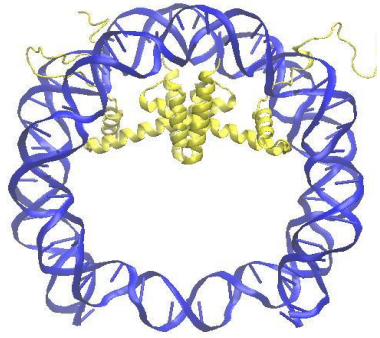
H3 dimer



H2A dimer

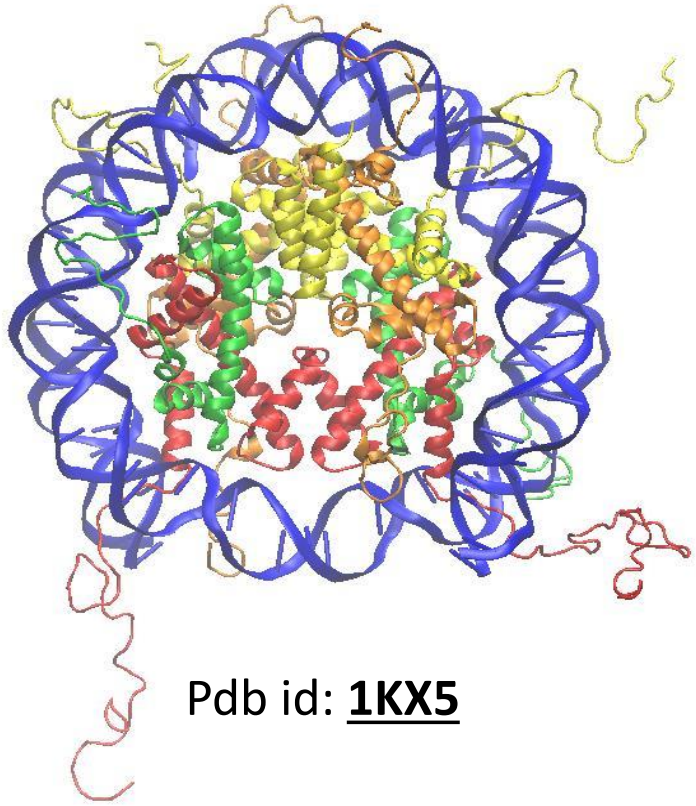


H4 dimer



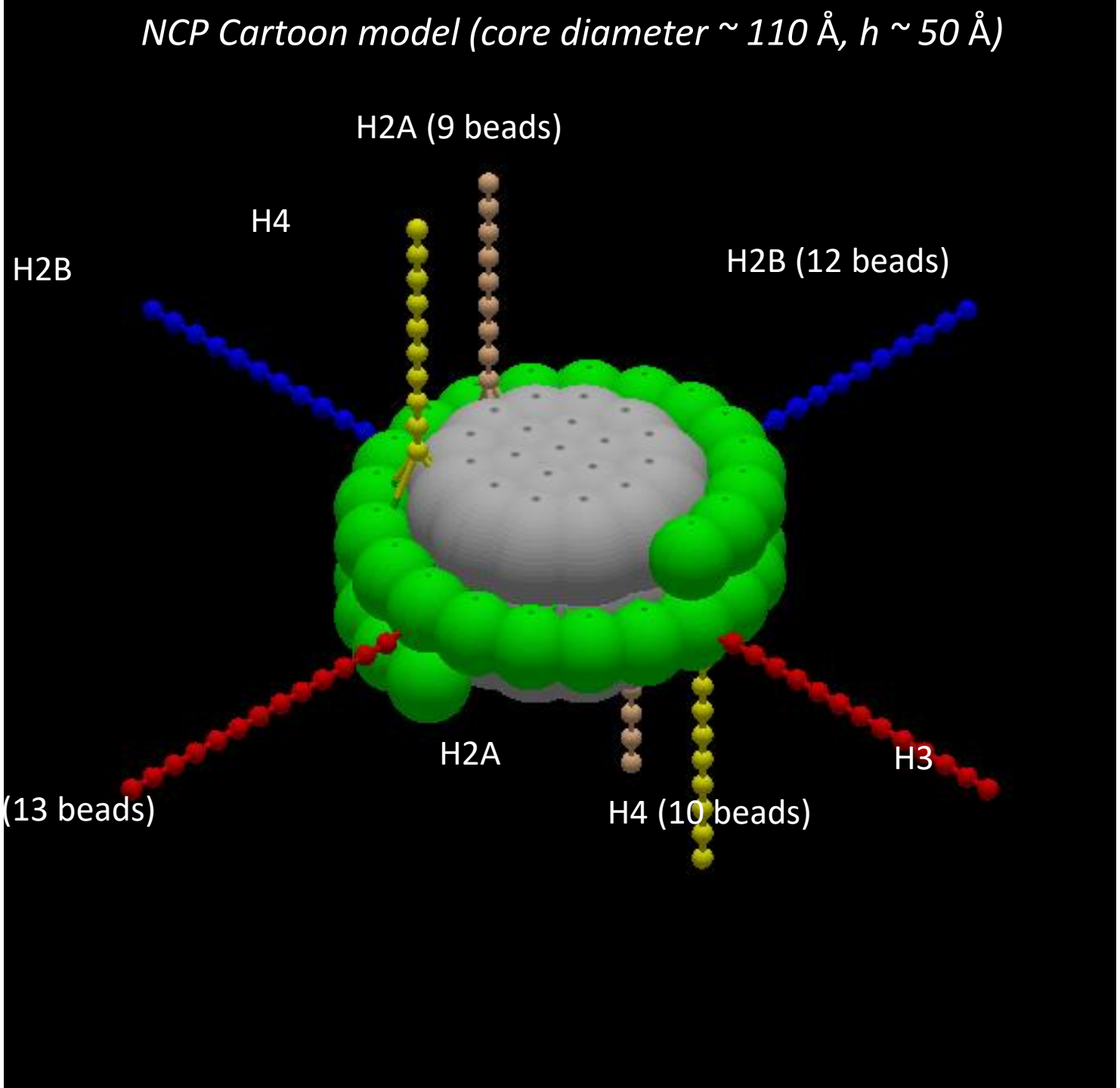
H2B dimer

Nucleosome Core Particle

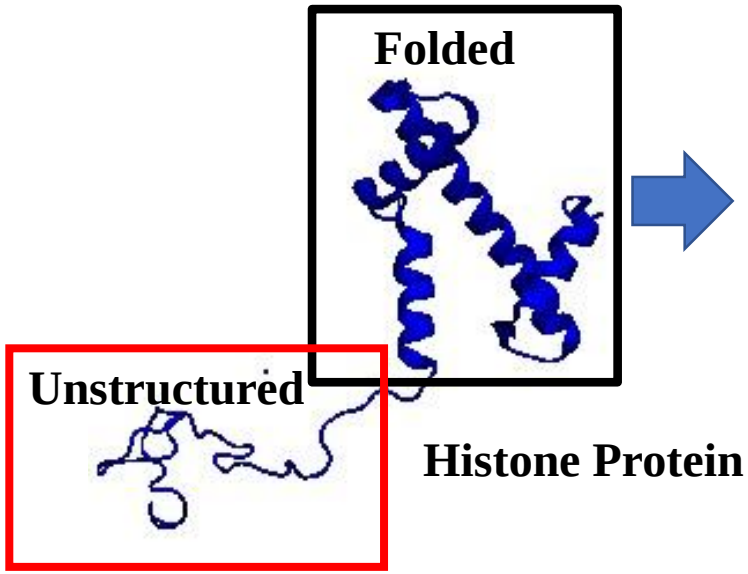


Pdb id: **1KX5**

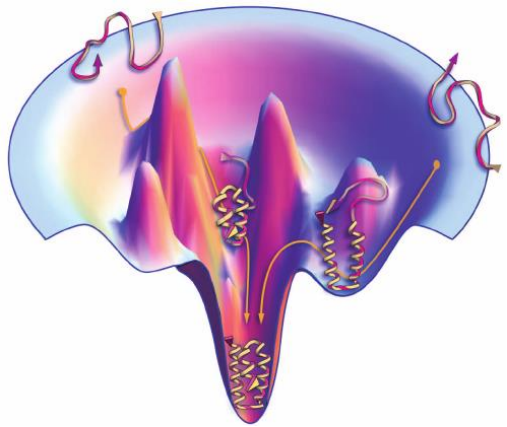
Davey, C.A., Sargent, D.F., Luger, K., Maeder, A.W., Richmond, T.J., ***Solvent mediated interactions in the structure of the nucleosome core particle at 1.9 a resolution***, J. Mol. Biol., **319:** 1097-1113, 2002.


NCP Cartoon model (core diameter ~ 110 Å, h ~ 50 Å)
H2A (9 beads)
H4
H2B
H2B (12 beads)
H2A
H3
(13 beads)
H4 (10 beads)

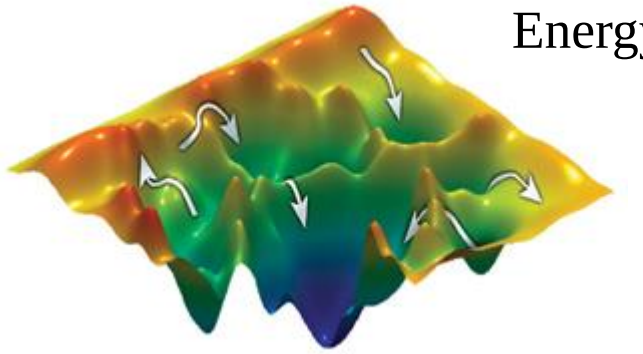
Histone Tails



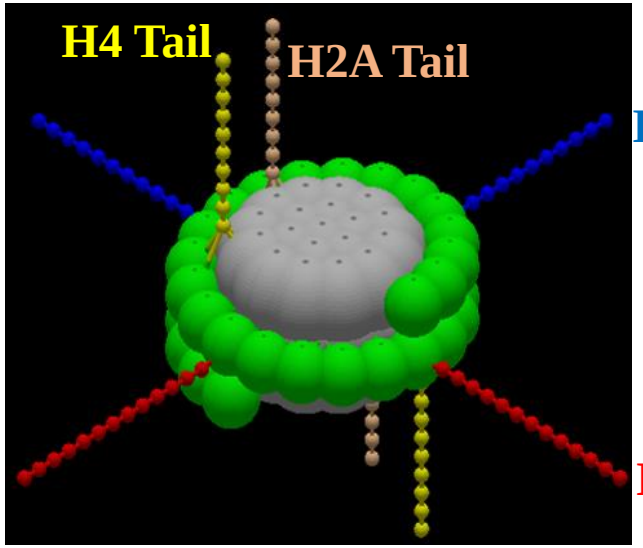
Histone Protein



Energy landscape of the folded proteins



Energy landscape of IDPs



Unstructured regions are known as tails. Categorized as Intrinsically Disordered Proteins (IDPs). They are unstructured and do not fold.

Role of Histone Tails

Histone Tails affect the conformation of the nucleosome and also the chromatin fibre.

Ultra-centrifugation experiment demonstrates that the removal of the H4 tail leads to a decompacted chromatin fibre.

Dorigo et. al., J Mol Biol., 2003, 327, 85-96.

SAXS experiments show that the inter-nucleosome interaction in the absence of histone tails are repulsive.

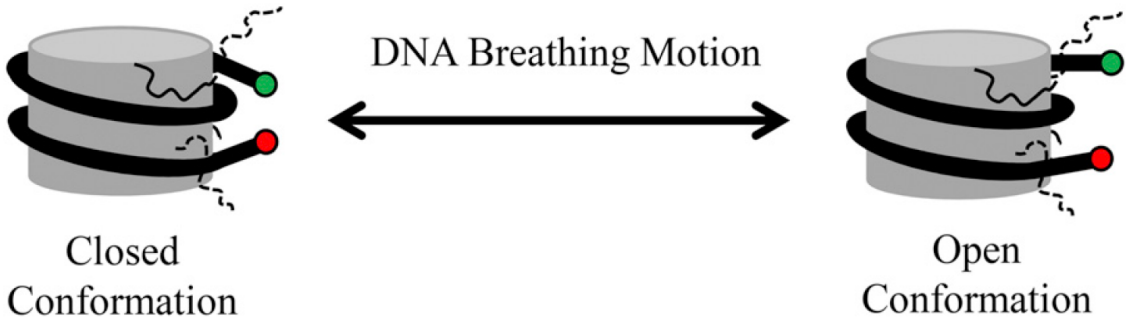
Bertin et al., Biochemistry, 2004, 43 (16), 4773–80.

Effect of histone tail deletions on inter-nucleosome interaction was studied using small angle x-ray scattering. H4 tail deletion was seen to affect the interaction more severely as compared to H3 tail deletion.

Howell et al., Biophys J, 2013, 105(1), 194-99.

Removal of H3 and H2B histone tails was found to affect histone-DNA interaction and hence the stability of the nucleosome.

Iwasaki et al., FEBS Open Bio, 2013; 3, 363-69.



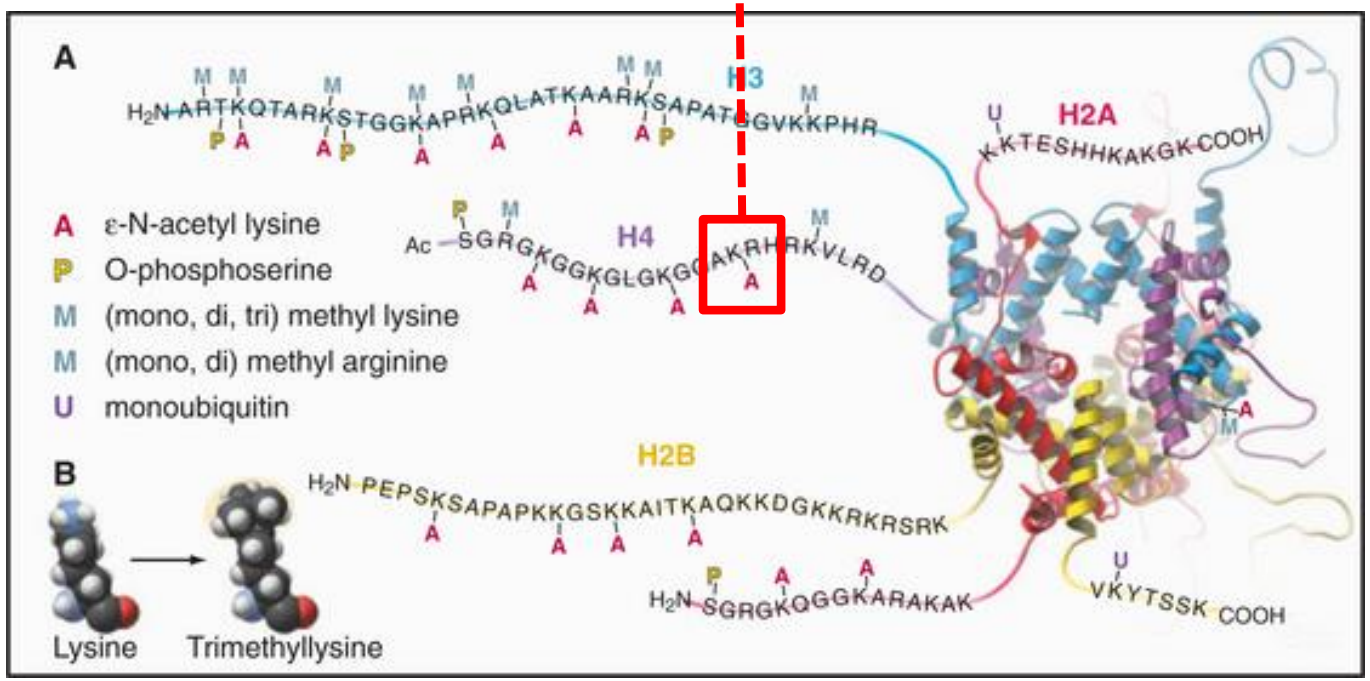
Nurse et al., Biophys J, 2013, 104(5), 1081-88.

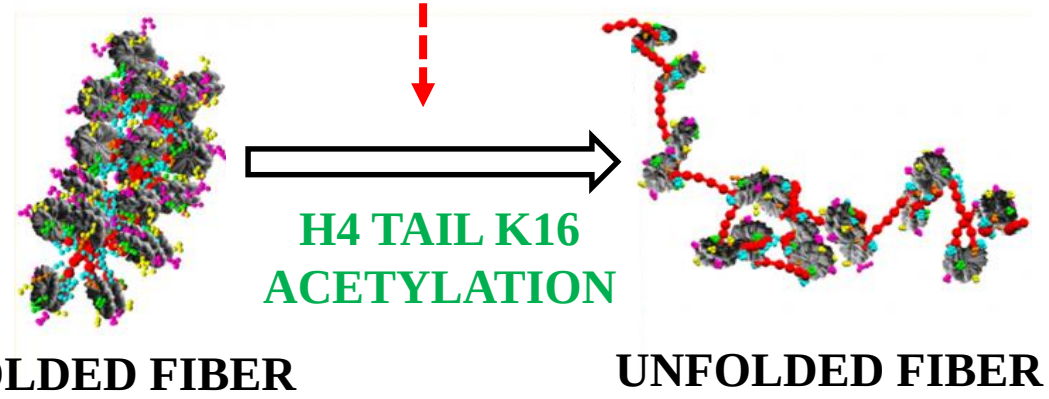
FRET experiments demonstrate that H3 histone tails control the breathing motion and hence the accessibility of the NCP DNA in a salt-independent manner. The H4 tail truncation has a much lower effect on the breathing motion but the effect is salt dependent.

Post Translational Modifications (PTMs)

PTMs are covalent modifications in the histones.

A set of combination of such modifications is called a **HISTONE CODE** and determines the state of the chromatin.





FOLDED FIBER **UNFOLDED FIBER**

PTMs

PTMs determine the state of the chromatin and certain PTMs are correlated with transcriptionally active and inactive phases of chromatin.

Vettese-Dadey et al., EMBO J, 1996, 15(10), 2508-18.

Experiment demonstrates that the affinity of binding of transcription factors to nucleosomal DNA was enhanced by acetylating H4 tail to higher and higher degree.

Schrogen-Knaak et al., Science, 2006, 311(5762), 844-7.

Incorporation of K16 acetylated histone H4 into nucleosomal arrays prevents the formation of the 30-nm fibre.

Kaimori et al., Scientific Reports 6, 2016.

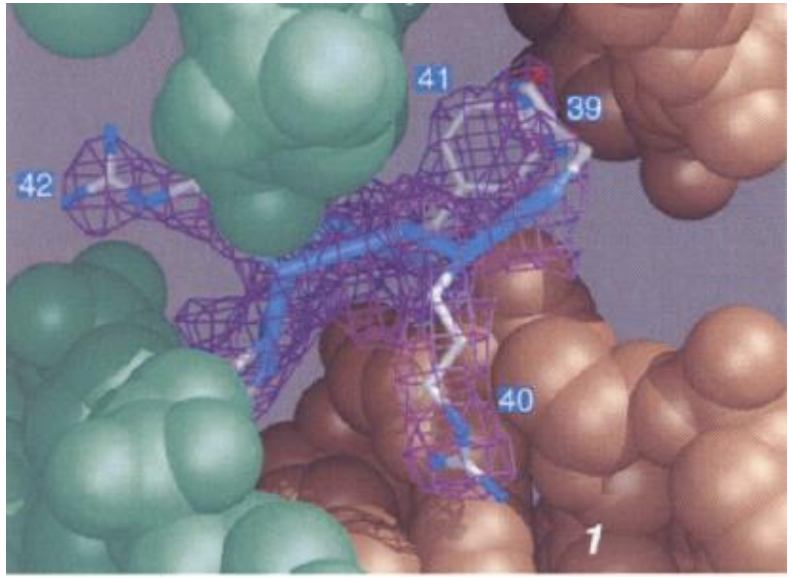
Acetylation of H4 tail at K20 acts as a gene repressor.

Insight from NCP Crystal Structure

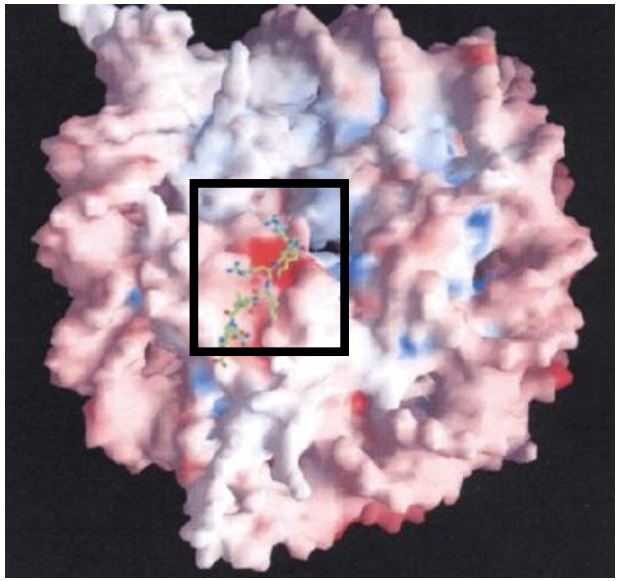
First crystal structure of NCP was determined in 1997 by Luger et. al. at a resolution of 2.8 Å. The structure contained a 146 bp DNA.

[Luger et. al., Nature, (1997), 387, 252-260]

The highlight of this structure was the H4-tail mediated interaction between neighbouring NCPs. **The H4 tail of one NCP was seen to contact a negatively charged acidic patch on the H2A/H2B core histone of the near-by NCP.**



H3 tail in the DNA groove.

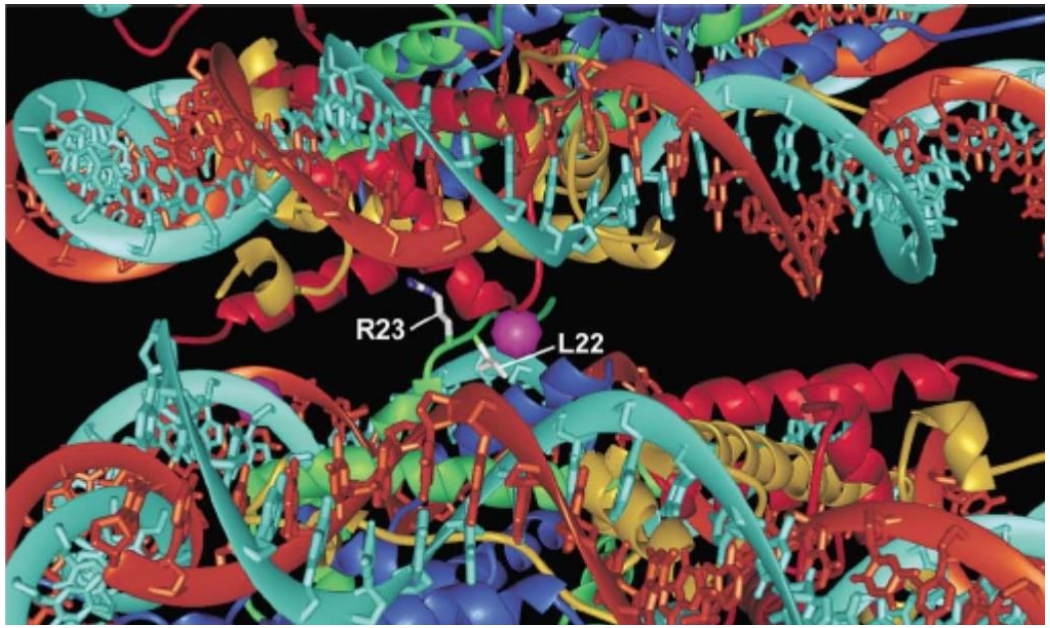


H4 tail mediating inter-NCP interaction. The red region is the acidic patch.

Insight from NCP Crystal Structure

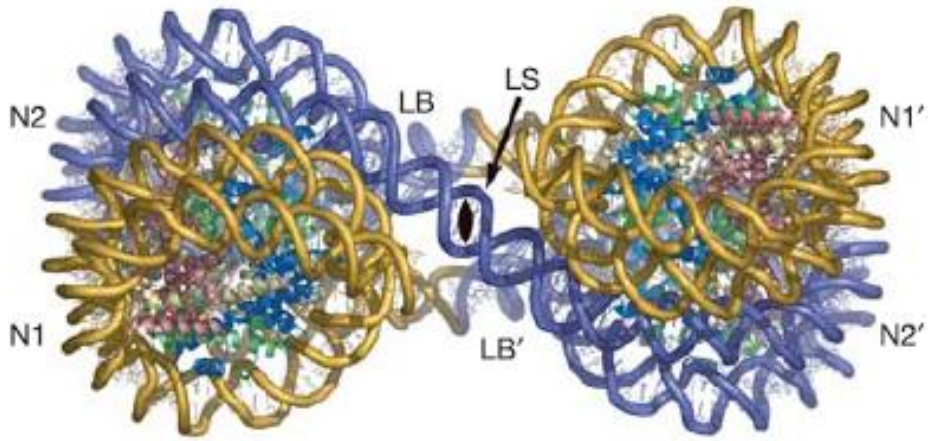
A higher resolution structure (1.9 Å) was determined in 2002 and contained 147 bp long DNA.
[Davey et. al., J. Mol. Biol., (2002), 319(5), 1097-113]

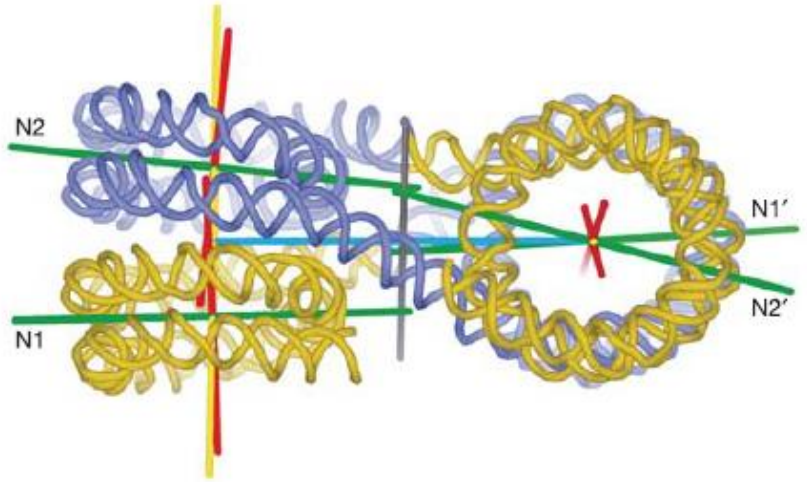
Due to higher resolution, water-mediated interactions between histone core and DNA could be located and shown to be important in stabilizing the NCP.



The structure also depicted the H4 tail in a bridging conformation, forming contacts between two NCPs via an interaction with the acidic patch.

The Tetranucleosome





[Schalch et al. Nature, 2005, 435, 138-41]

An oligonucleosome crystal structure generated using molecular replacement technique.

The linker DNA zigzags back and forth between two stacks of NCPs.

The tetranucleosome was assembled *in vitro* and provided some hints about the possible structure of the chromatin fibre.

Atomistic Simulation of NCPs

Determination of crystal structure of the NCP inspired the atomistic simulation based studies of the properties of the NCP.

Atomistic MD simulations enabled zooming in into microscopic phenomena and determination of

- **Qualitative features like the tail conformation.**
- **Interaction modes between tails and NCP DNA.**
- **Conformational changes as a function of mutations.**
- **Microscopic features of NCP DNA dynamics.**

Survey of current all-atom NCP simulation

TABLE 1 | Table of Published All-Atom MD Simulations of the Nucleosome Arranged by Publication Date.

References	PDB	MD/F.F./Solvent	Simulation
Bishop,[5]	1KX3	N/P99/NaCl@150 mM, 9 Å	2 × 10 nanoseconds, PCS, woT
Ruscio and Onufriev[9]	1KX5	A/P99/Neutral Na^+, 9 Å	0.5–1 nanoseconds, 1 nanosecond GB
Roccatano et al. [10]	1KX5	A/P99/Mg,KCl@100-150 mM, 10 Å	1 × 21 nanoseconds,1 × 18 nanoseconds
Materese et al.[11]	1ID3	N/C27/NaCl@150 mM, 15 Å	200 nanoseconds, PB, woT
Ponomarev et al.[12]	1ID3	N/P99,BSC/NaCl@150 mM, 12 Å	1 × 50 nanoseconds, 2 × 20 nanoseconds, woT
Biswas et al.[13]	1KX5	N/C27/NaCl@150 mM, 10 Å	8 × 100 nanoseconds
Ettig et al.[14]	1ZBB, 2FJ7	N,A/P99/NaCl@150 mM, 15 Å	2 × 20 nanoseconds, 1 × 120 nanoseconds, 2 × SMD
Mukerjee and Bishop[15]	1KX5	N/P99,BSC/NaCl@150 mM, 12 Å	16 × 20 nanoseconds, woT, SS
Voltz et al.[16]	1KX5-1ZBB	N/C27/NaCl@150 mM, 15 Å	2 × 100 nanoseconds, CG
Yakubovich et al.[17]	3LEL	N/C22/NaCl@150 mM, 10.5 Å	10 picoseconds, NVE-HS, woT
Korolev et al. [31]	NA	Mixed/NA/NA	Tail–DNA interactions
Sharma et al.[30]	1KX5	CG/NA/NA	DMD
Potoyan and Papoian[29]	Model	A/P99SB/NaCl@150 mM, 12 Å	3μs, REMD, oT
Mukherjee et al.[22]	1KX5	N/P99, BSC/NaCl@150 mM, 12 Å	336 × 20 nanoseconds, woT, SS

Atomistic simulations of nucleosomes

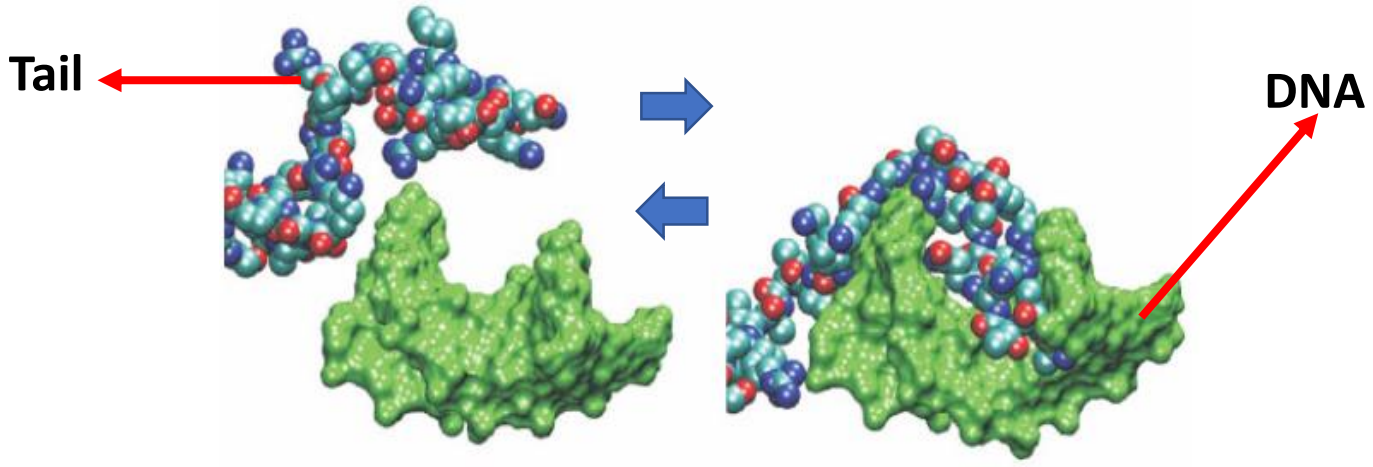
WIREs Comput Mol Sci 2013, 3: 378–392 doi: 10.1002/wcms.1139

Mithun Biswas,[1] Jörg Langowski[2] and Thomas C. Bishop

Insight from atomistic MD simulation

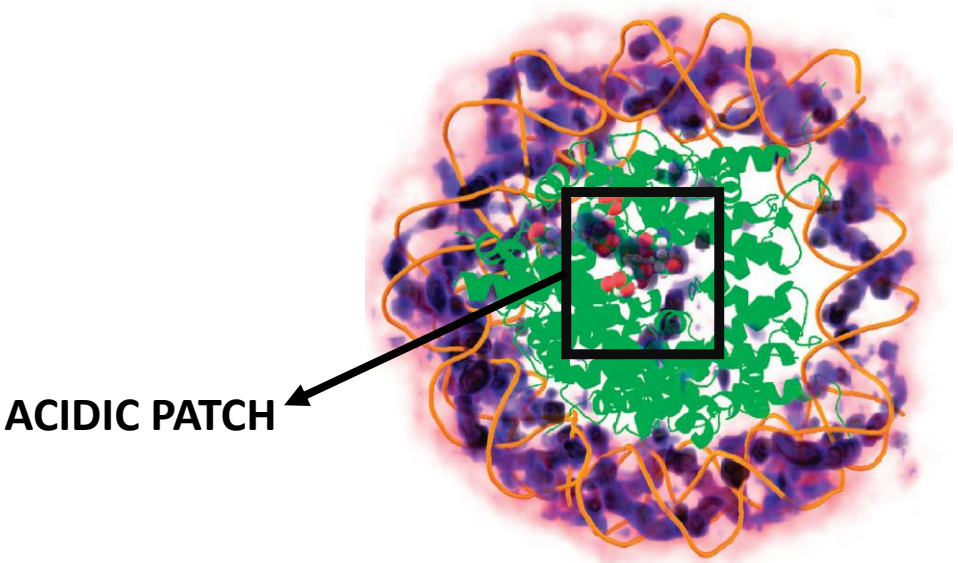
20 ns long simulation in explicit solvent. Studied the dynamics of histone tails and NCP DNA. Tails were seen to be very dynamic while the dynamics of the NCP DNA was restricted. Tail conformation shuttled between DNA grooves and solvent-dissolved conformations

Roccatano et al., Biophys J, 2007, 85, 407-21

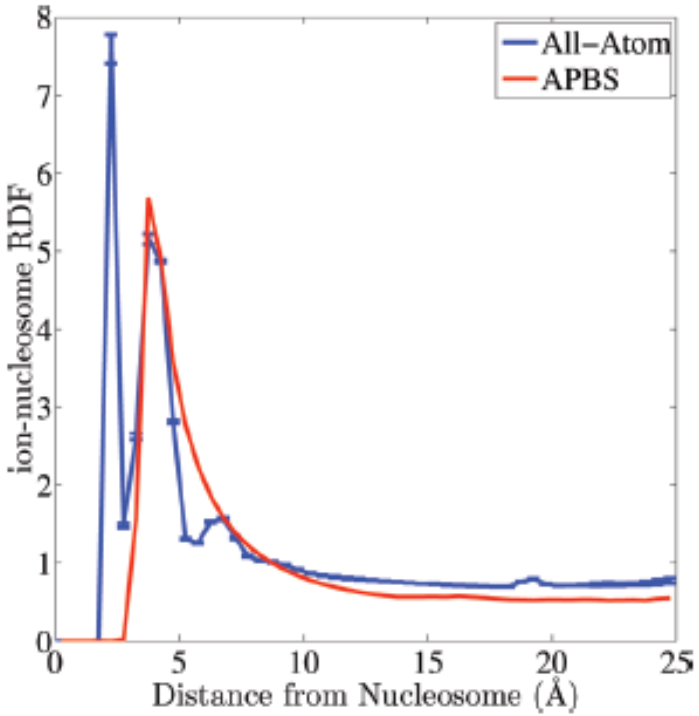


200 ns long simulation in explicit solvent. Studied the ionic environment around the NCP. Inferred that the NCP DNA is more neutralized than a free DNA under similar ionic conditions.

Materese et al., JACS, 2009, 131, 15005-13



Na^+ distribution around the NCP



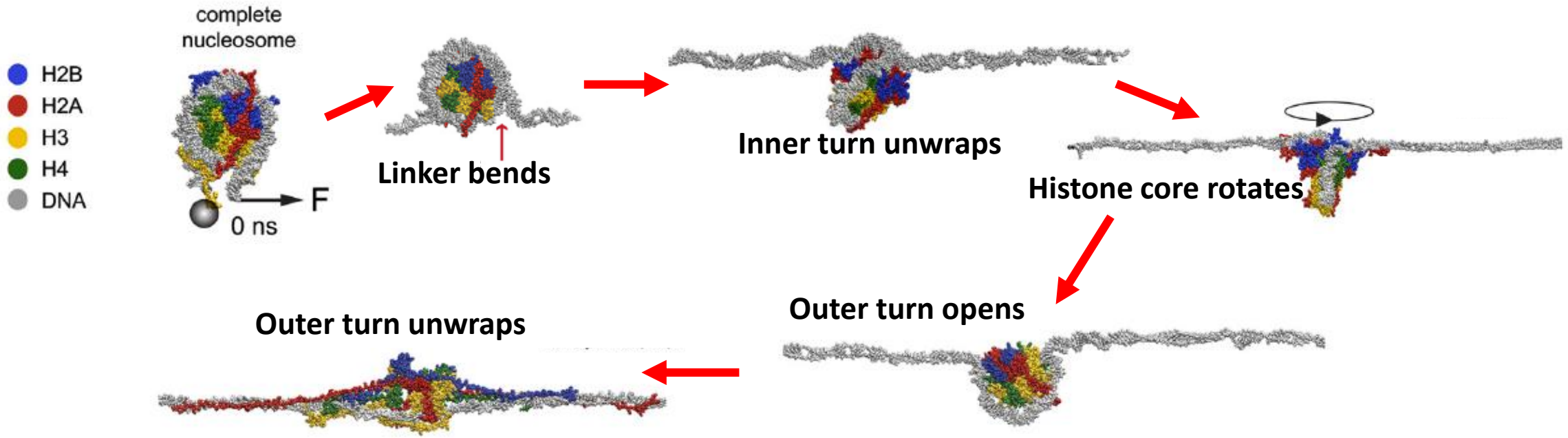
Ion-NCP RDF showed a sharp peak corresponding to Na^+ condensation on the DNA. The peak was absent for the mean-field PB calculations.

Multiple 100 ns long simulations in explicit solvent. Studied the role of histone tails in keeping the NCP stable. Inferred that the removal of H3 and N2A histone tails and mutations along their sequence induces structural changes in the NCP.

Biswas et al., PLoS Comput Biol, 2011, 7, e1002279

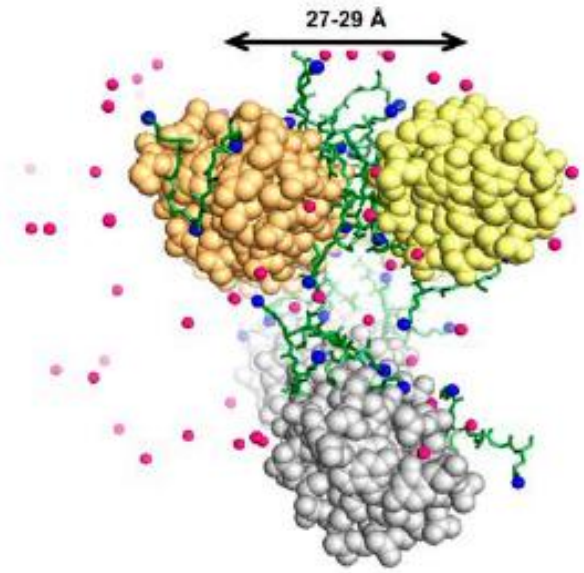
Multiple 100 ns long simulations in explicit solvent and non-equilibrium **SMD simulations**. Studied the intra-nucleosomal DNA-histone interactions. Attempt to understand the unwrapping pathway of the NCP DNA.

Ettig et al., Biophys J, 2011, 101, 1999-2008

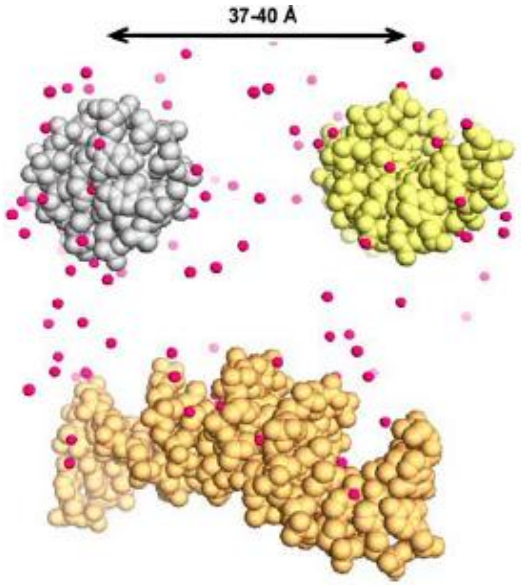


Simulation of a collection of histone tails and DNA in explicit solvent. Demonstrated that the attraction between NCP DNA would be mediated by the histone tails.

Korolev et al., PLoS Comput Biol, 2011, 7, e1002279



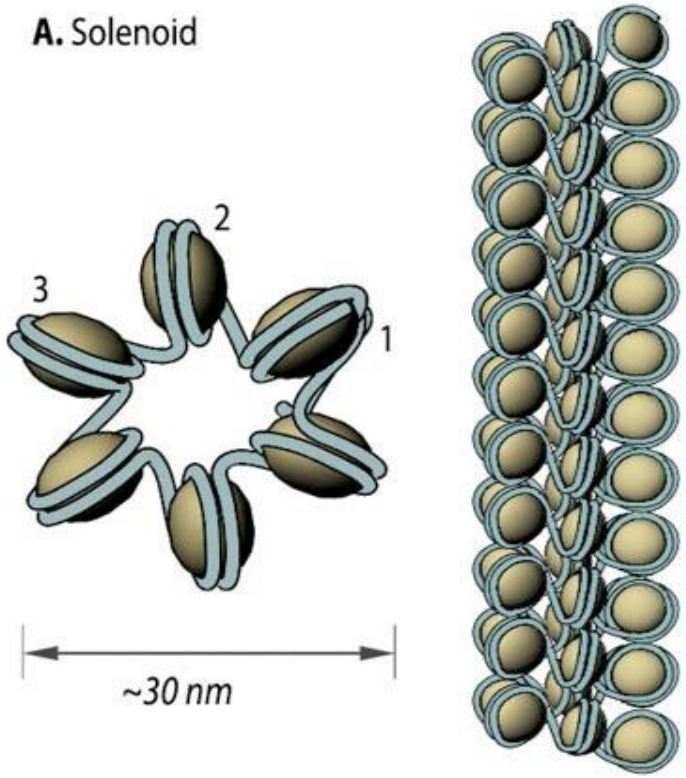
With Tails



No Tails

Chromatin Models

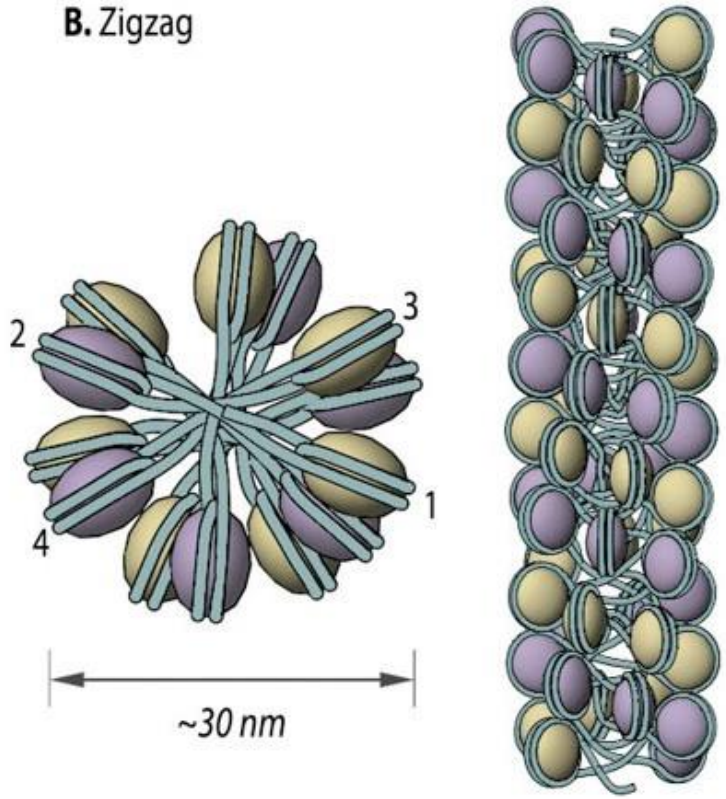
Based on experimental results like a measure of the Nucleosome repeat Length (NRL), various models of the chromatin have been suggested.



Finch and Klug. Proc. Natl. Acad. Sci. U.S.A. 1976; 73(6):1897-901

Dorigo et al., Science 2004; 306(5701):1571-3.

Variations of this model are the N-start (N>1) models, where the nth and (n+N)th NCPs are in contact with a straight linker DNA. Figure below is a 2-start zig-zag model.



The solenoidal model depicts NCPs connected by bent linker DNA, with the nth and (n+1)th NCPs in contact.

Experimental Results

Cross-linking experiments

Grigoryev et. al., PNAS, 2009, 106, 13317-22.

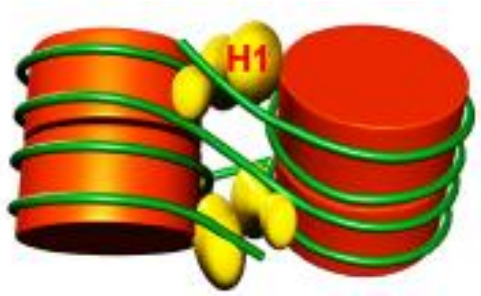
In the absence of divalent cations, the structure is zigzag… In the presence of ions some fraction of solenoidal structure is also present.

Cryo-EM experiments

Song et. al., Science, 2014, 344, 376-80.

A tetranucleosome repetitive unit was observed, supporting a zig-zag structure.

Cryo-EM image of the repeating unit of a reconstituted chromatin fibre shown with atomistic DNA model.

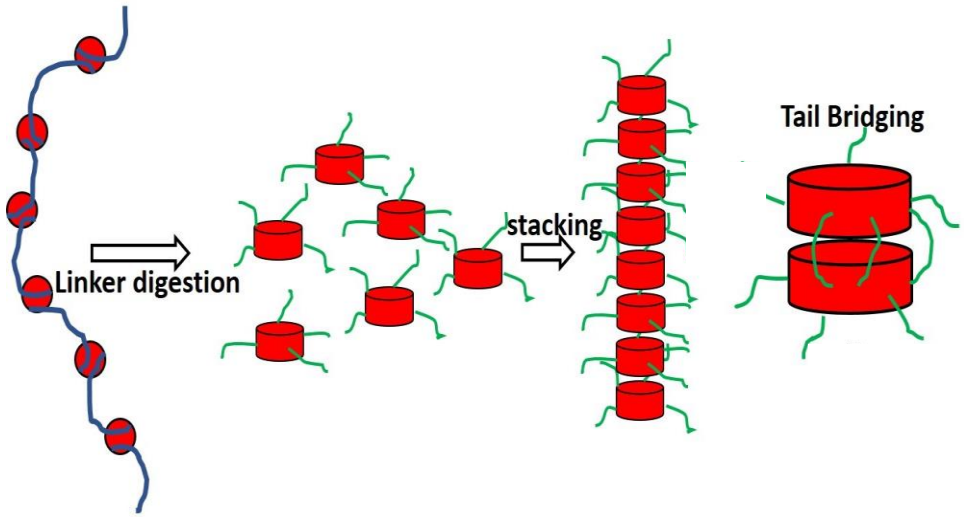


Schematic of the repeating unit.

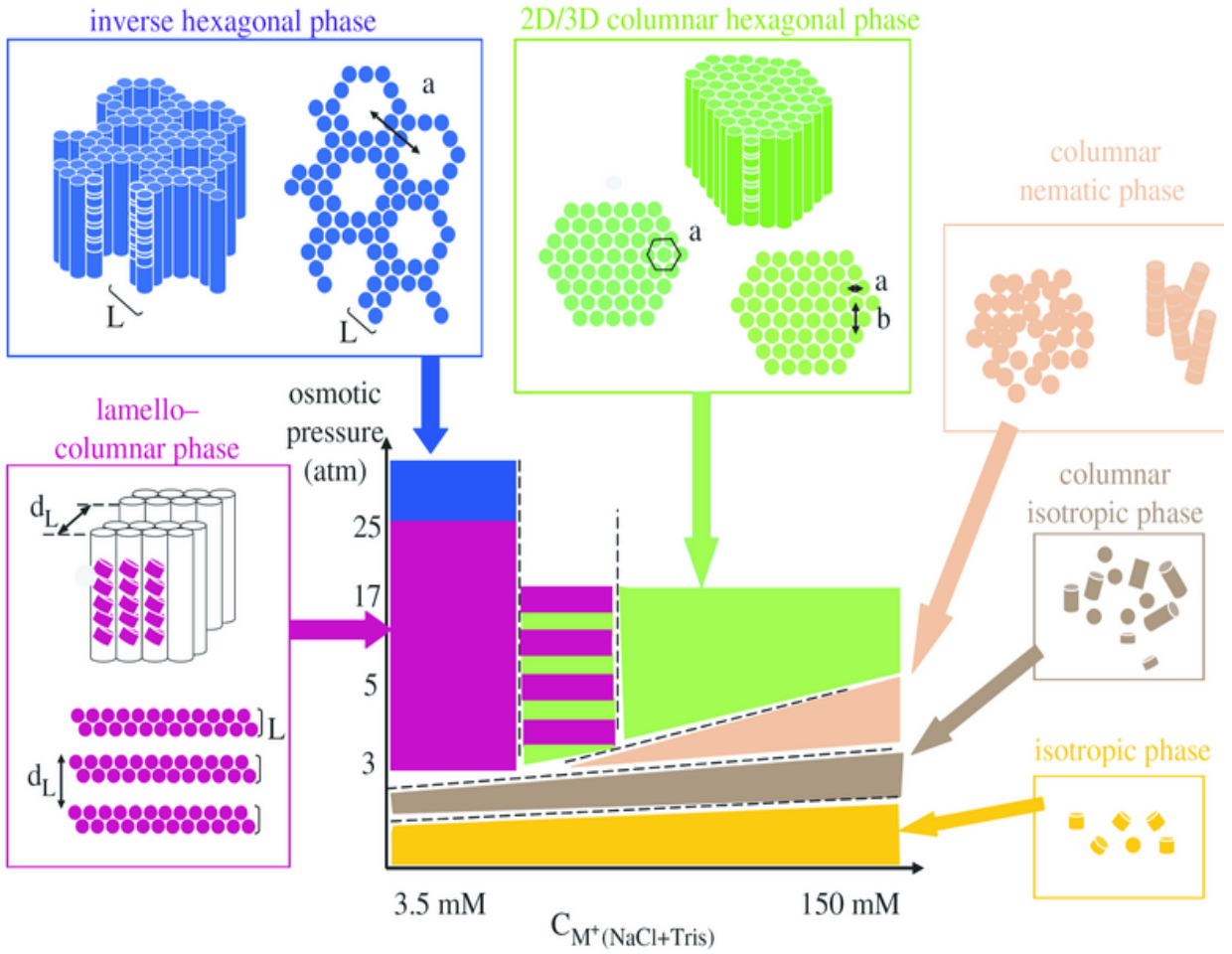
Most of the experiments have been performed on reconstituted NCP arrays. Nucleosome Repeat Length, histone variants etc. induce nonuniformity in the actual chromatin unlike the reconstituted experimental arrays.

LC Phase of Nucleosome Core Particles

Mechanism



Phase diagram



Mangenot et. al., J Mol Biol., 2003, 327(1):85-96

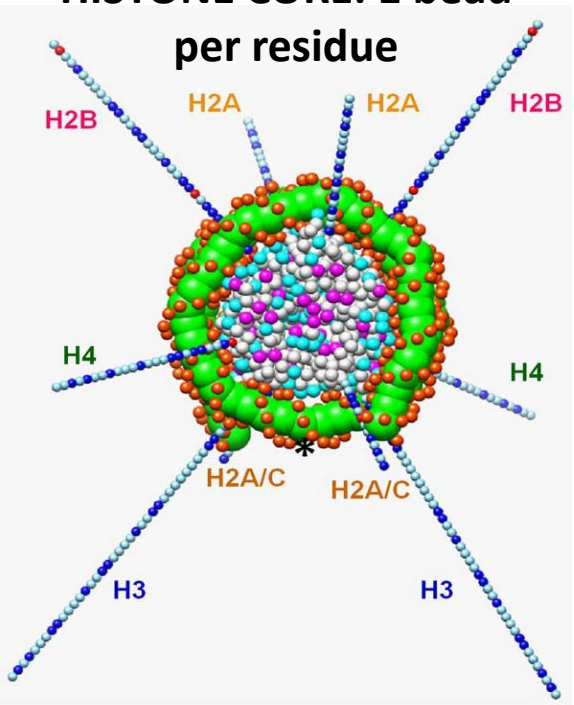
Coarse-grained models of the NCP and Chromatin

Nucleosomes are large molecules and it is difficult to simulate phenomena like NCP aggregation and LC ordering at an atomistic level. Many coarse grained (CG) models have been developed that make the simulation of such phenomena possible.

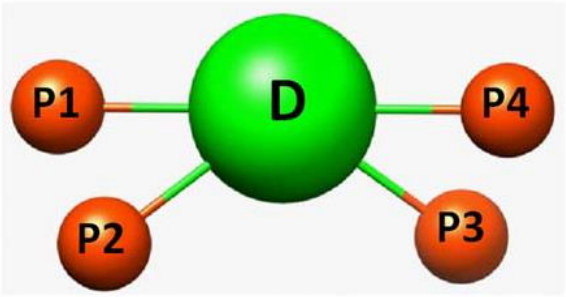
Many CG models of chromatin have also been developed to study the effect of environmental factors and histone tails on the structure of the chromatin fibre.

CG NCP

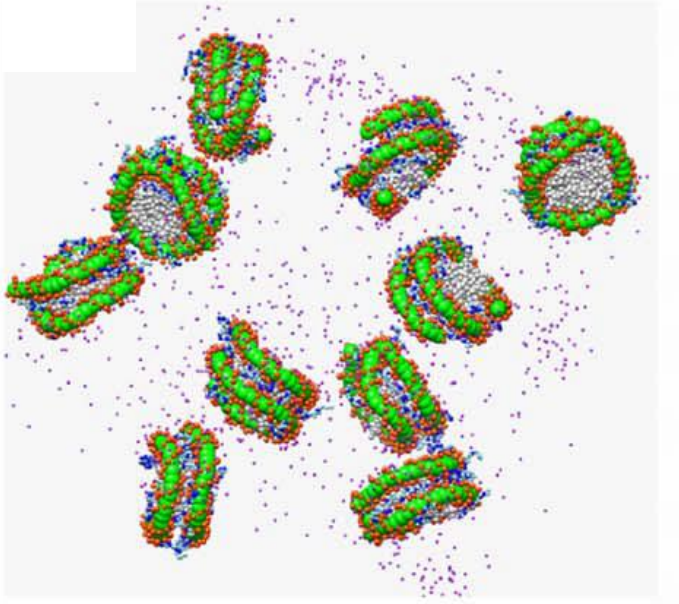
HISTONE CORE: 1 bead per residue



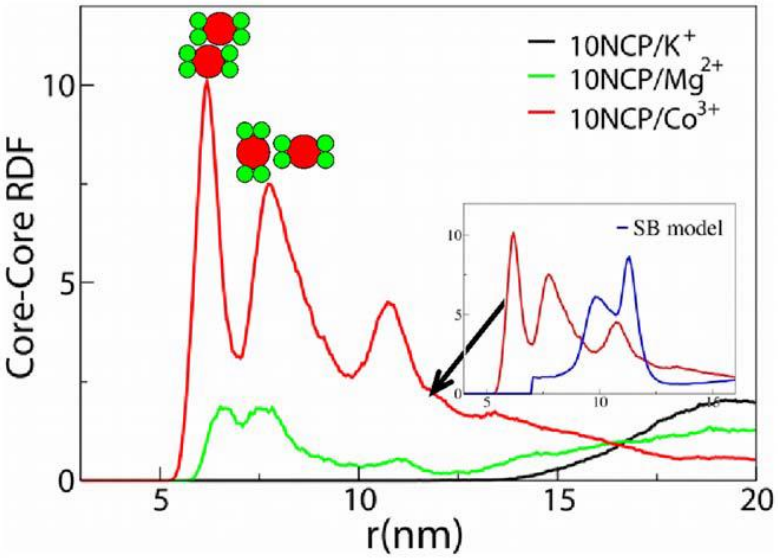
CG DNA base-pair



Fan et al., PLoS one, 2013, 8(2), e54228



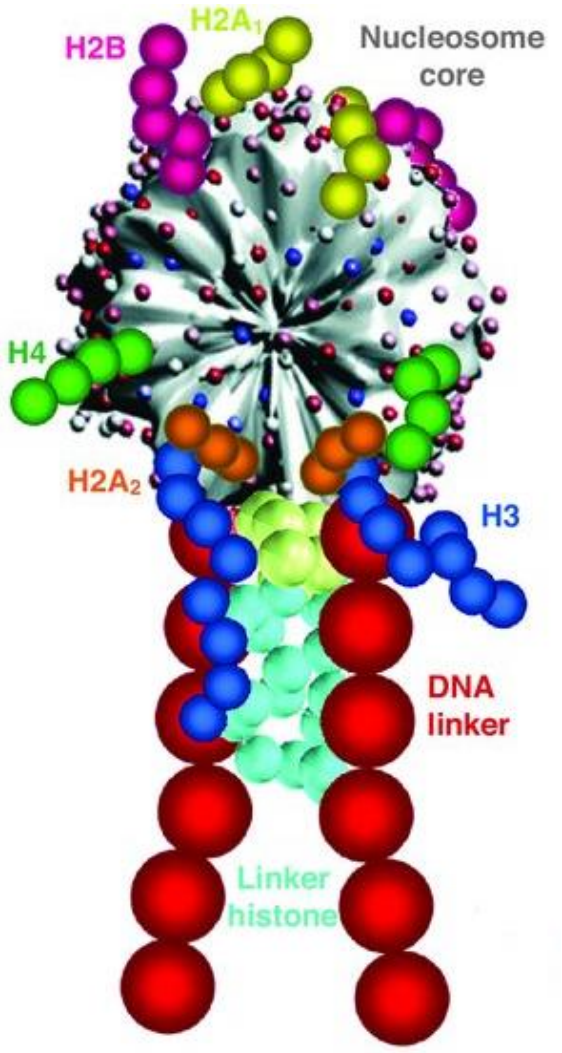
A collection of the CG NCPs were simulated.



NCPs aggregate in a salt dependent manner.

The NCPs start forming stacks with an increase in salt concentration. An examination of the stacks revealed that the NCPs stack with their dyads parallel.

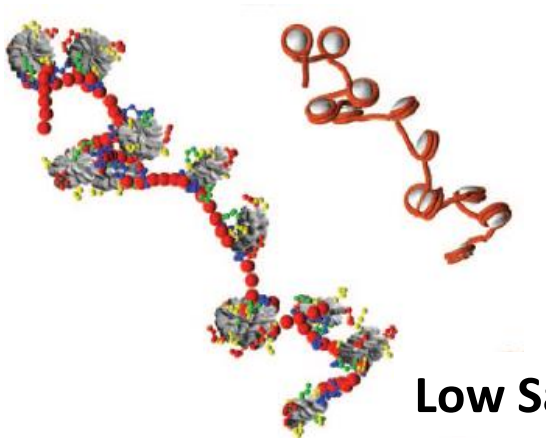
Arya et al., JPC A, 2009, 113, 4045-59



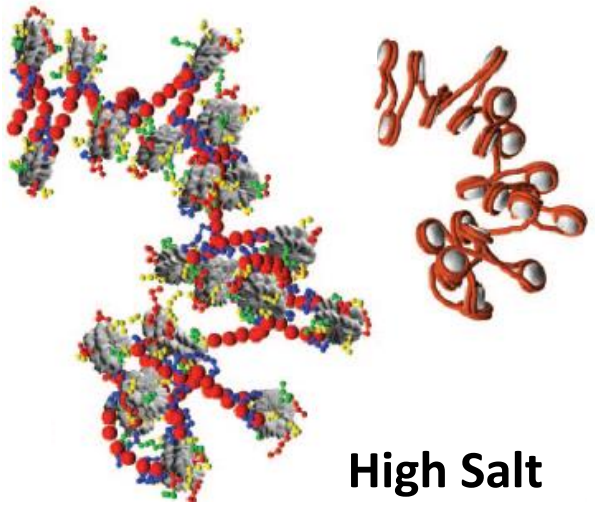
A CG model of chromatin was developed.

The constituent nucleosome contained a core represented by a large disc .

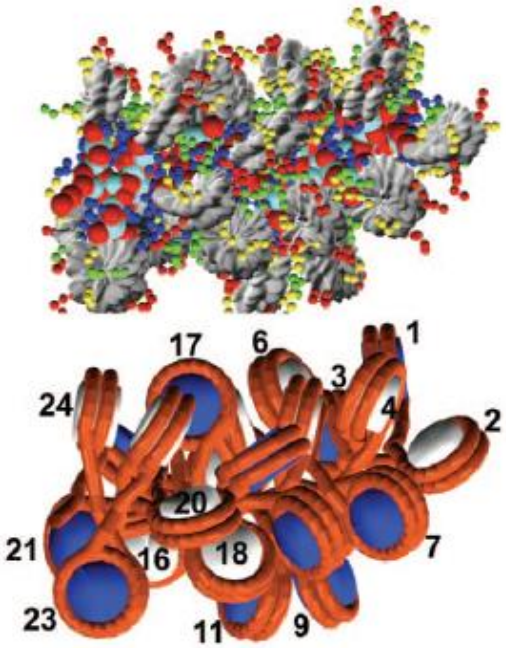
A CG linker histone was also present with the C-terminal domain of the protein represented by 22 CG beads.



Low Salt



High Salt



High Salt + Linker histone

Structures obtained using this model support the zig-zag model of chromatin fibre.

Questions…

Stacking is the key step for LC ordering…. It is also a feature of chromatin compaction… What kind of interactions stabilize NCP-NCP stacking??

What are the relative contributions of different histone tails in the staking interaction??

The organization of NCPs in the chromatin fibre is still unclear… Different experiments lead to different outcomes… Can we use atomistic MD simulations to draw inference about organization of NCP in the chromatin fibre??

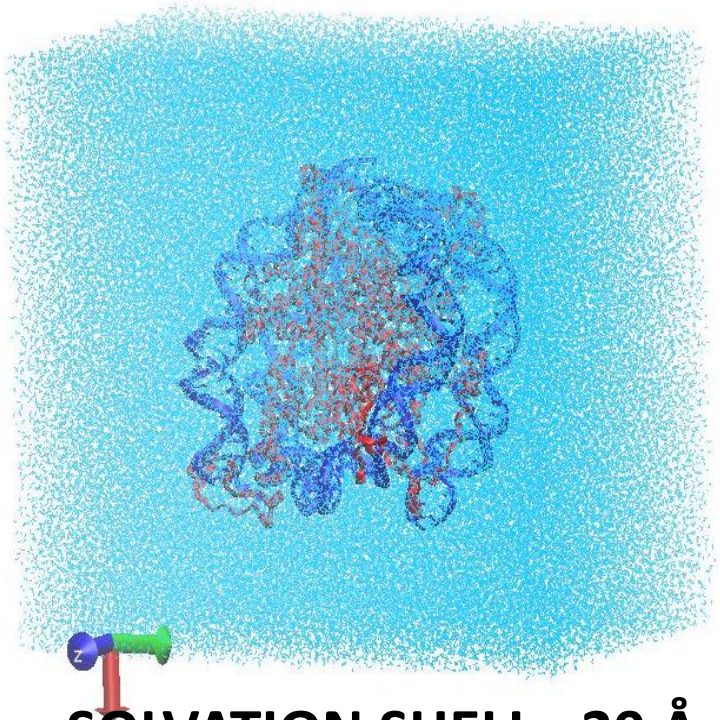
SIMULATION OF THE PARALLEL STACK

To study interactions that stabilize stacks.

To study the relative role of different histone tails in stacking interactions.

NCPs solvated in water

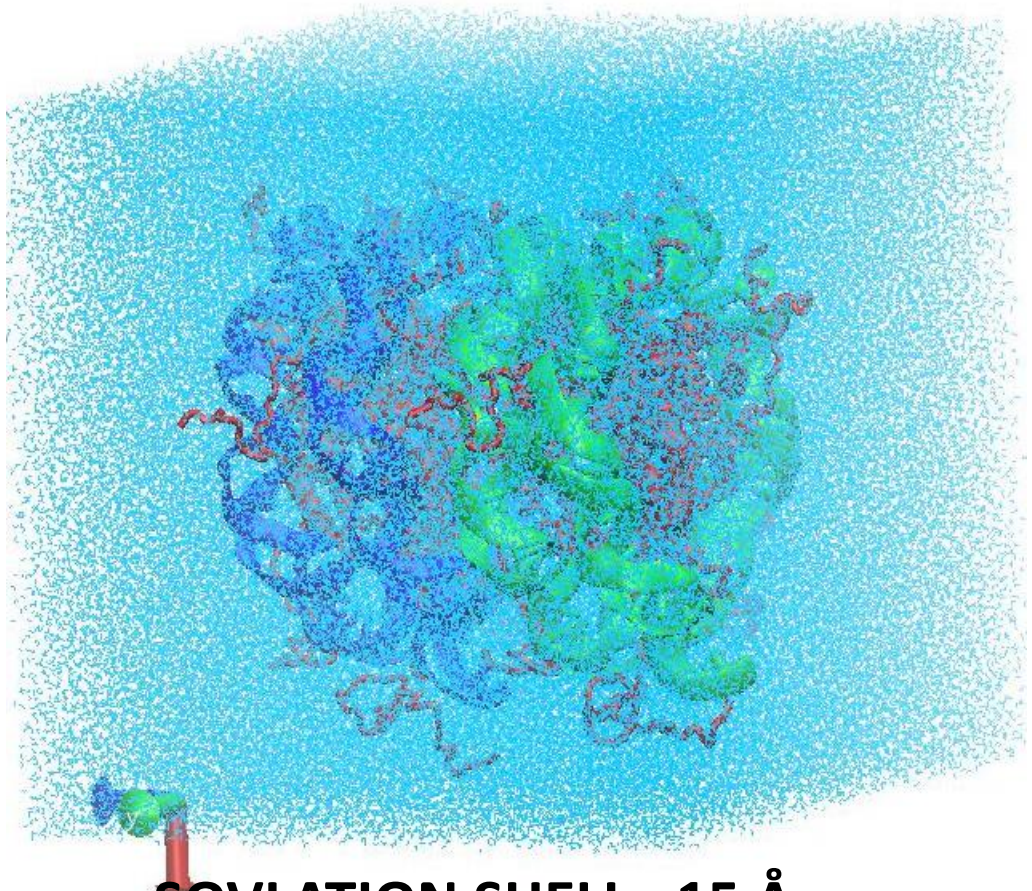
1KX5



SOLVATION SHELL : 20 Å

# of Atoms : 362412

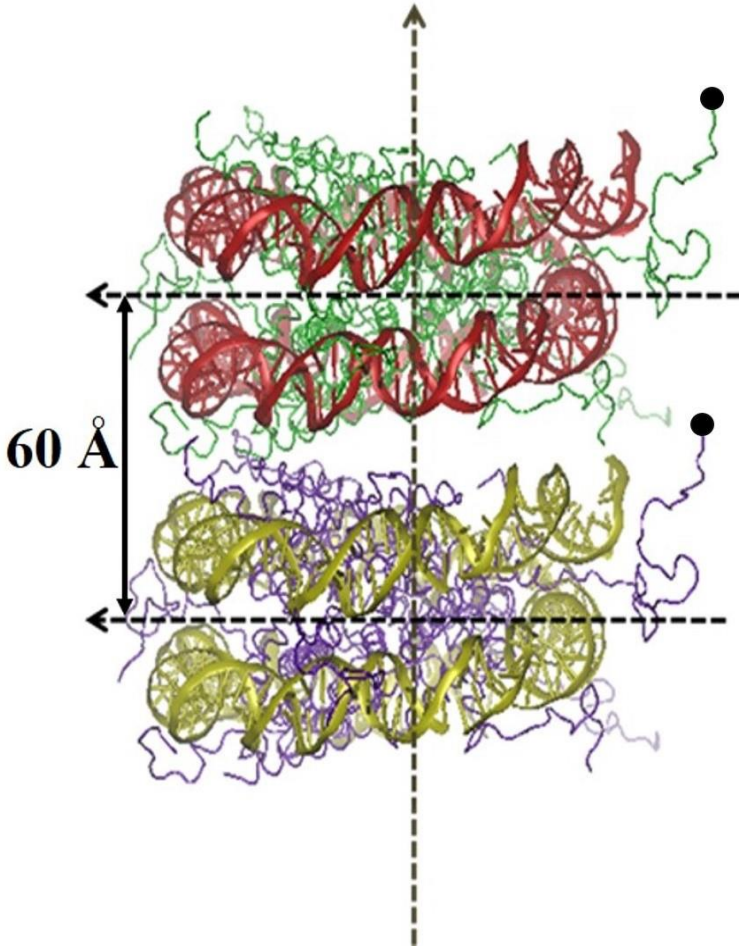
2KX5



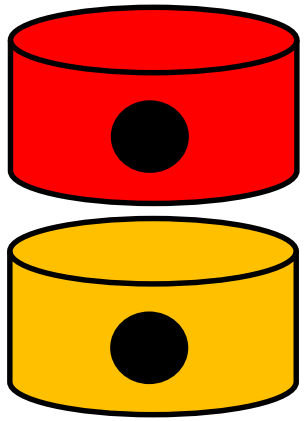
SOVLATION SHELL : 15 Å

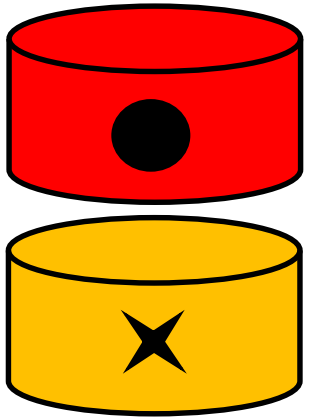
# of Atoms : 470226

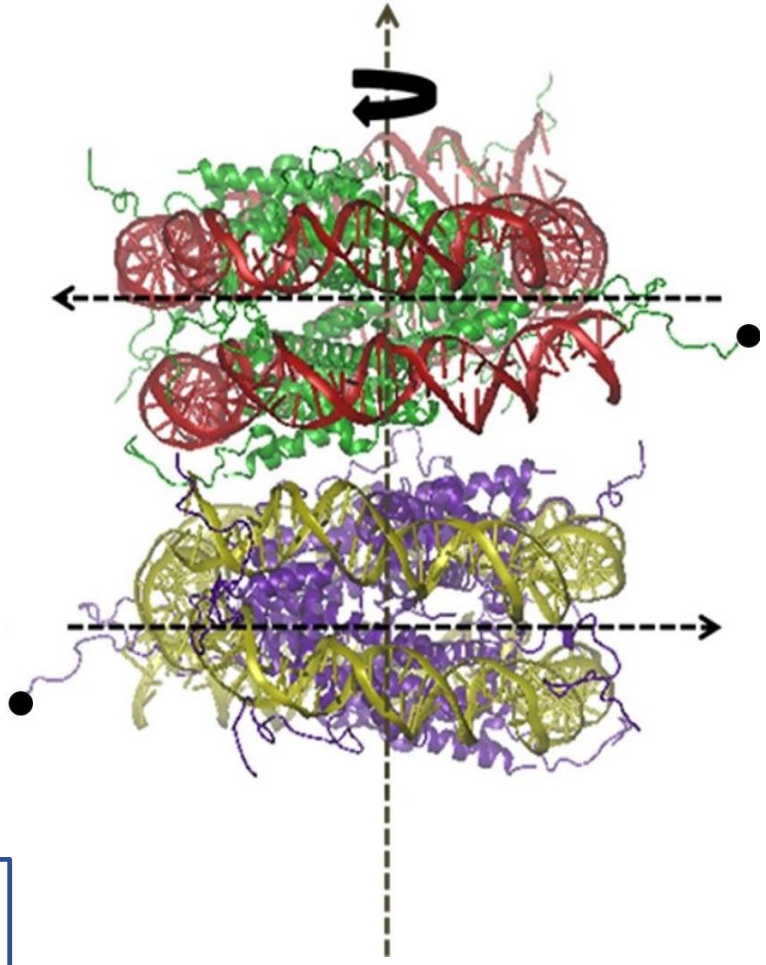
NCP STACKS



PARALLEL





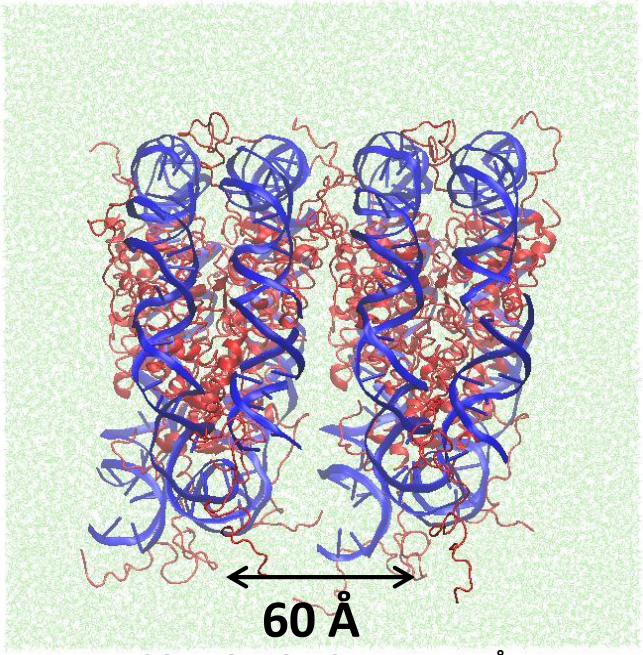


ANTIPARALLEL

DOTS AND CROSS ON THE NCP CARTOONS REPRESENT THE DIRECTION OF THEIR DYAD AXIS.

Simulation of stacked NCPs

2 NCPs stacked



System size : 470226 atoms

Simulation Details:

System size: 470226 atoms.

Force field: ff99SB for proteins and bsc0 for DNA. Joung and Cheatham parameter set for ions.

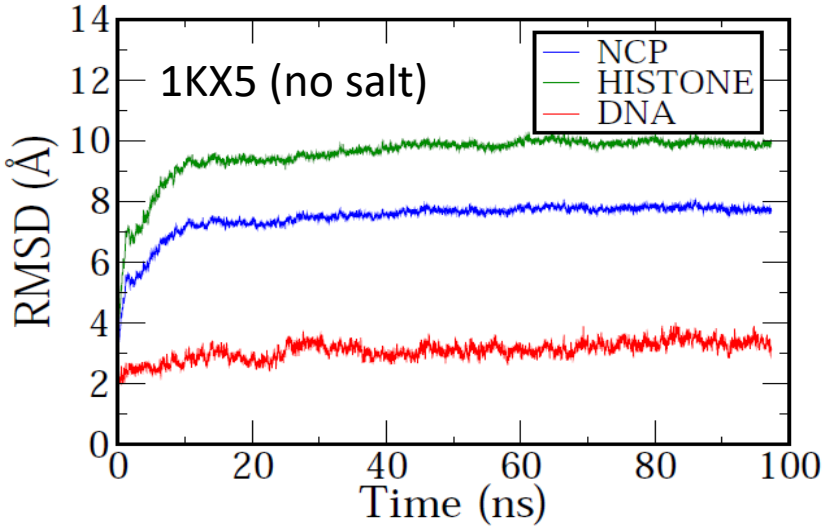
Thermostat: Berendsen.

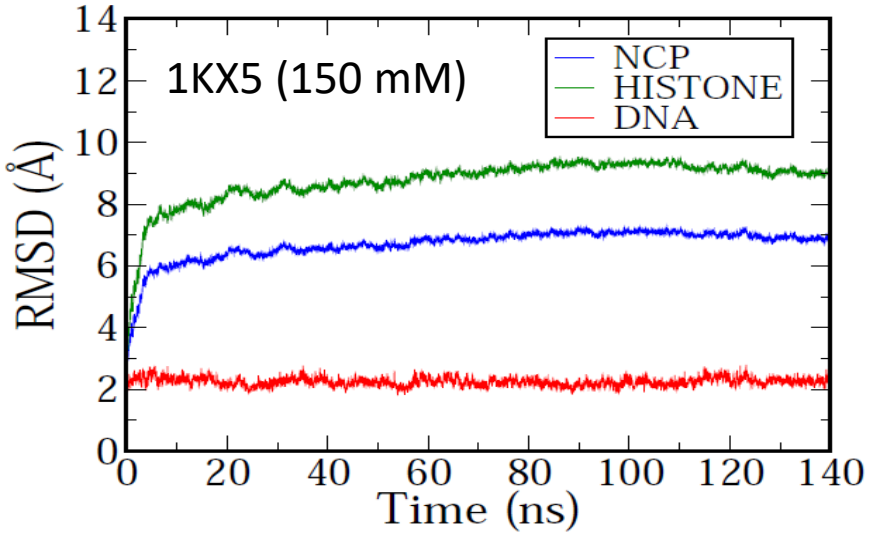
Barostat: Berendsen.

Temperature: 300 K.

Saurabh et. al., *J. Phys. Chem. B*, 2016, 120 (12), pp 3048–3060

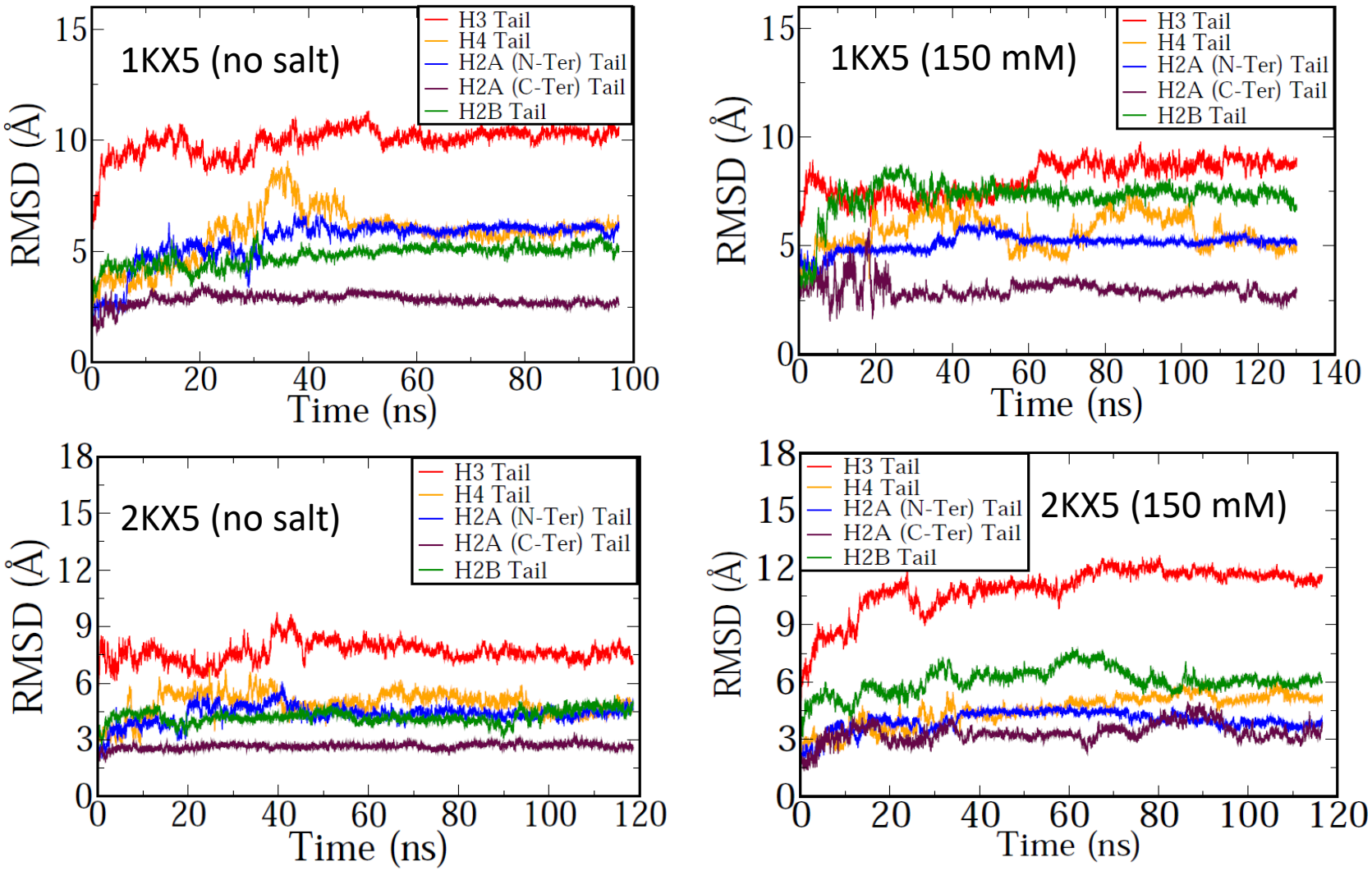
Structural stability





- Protein shows the largest RMSD. This can be attributed to the histone tails. They are Intrinsically disordered and change a lot from their initial crystal structure conformation.
- DNA conformation remain close to their crystal structure

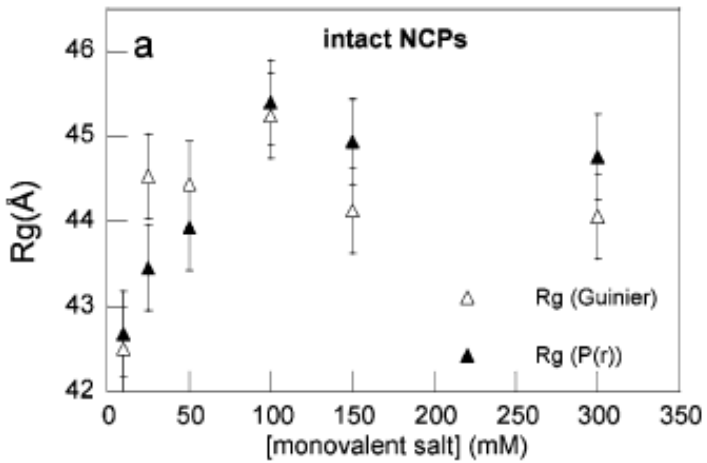
Tail RMSD

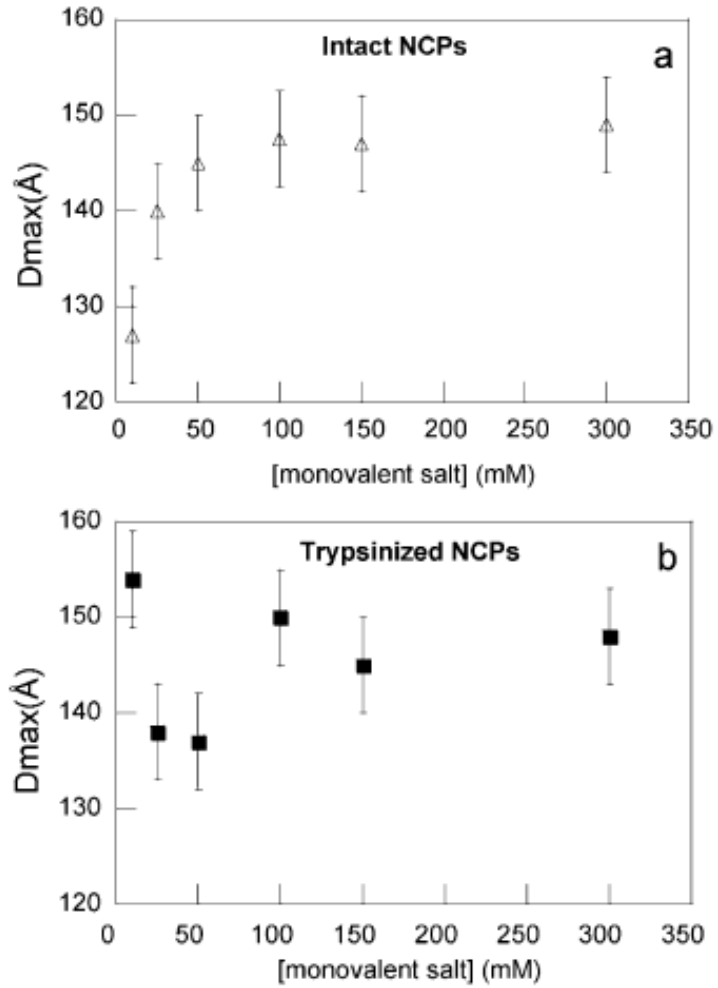


1. H3 tail has the largest RMSD. 2. H2A (C-Ter) which is buried inside the histone core and is hence kinetically trapped, has the least RMSD.

Role of Histone Tails in the Conformation and Interactions of Nucleosome Core Particles†

Aurélie Bertin,‡ Amélie Leforestier,‡ Dominique Durand,§ and Françoise Livolant*,‡





Experimental Results

IN PRESENCE OF HISTONE TAILS:

and maximum extension increase with salt concentration.

(R_g :42 Å to 44 Å ; extension:127 Å to 147 Å)

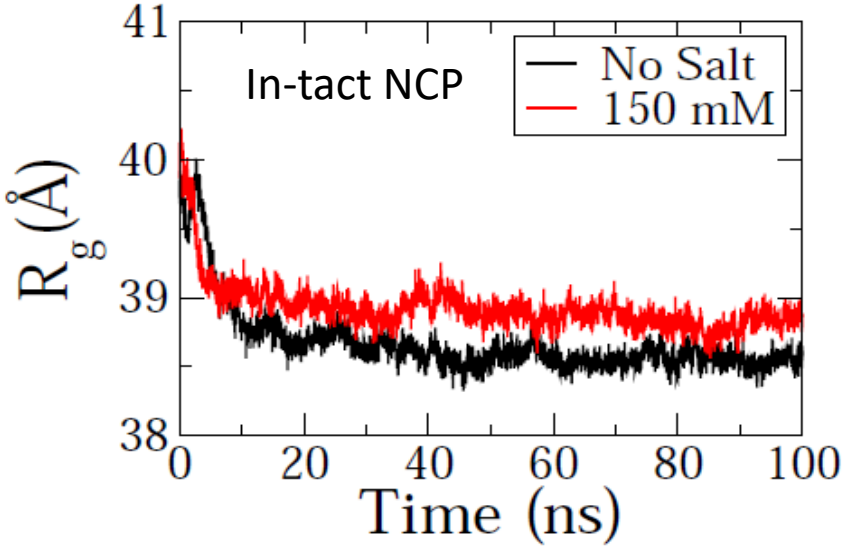
IN ABSENCE OF HISTONE TAILS:

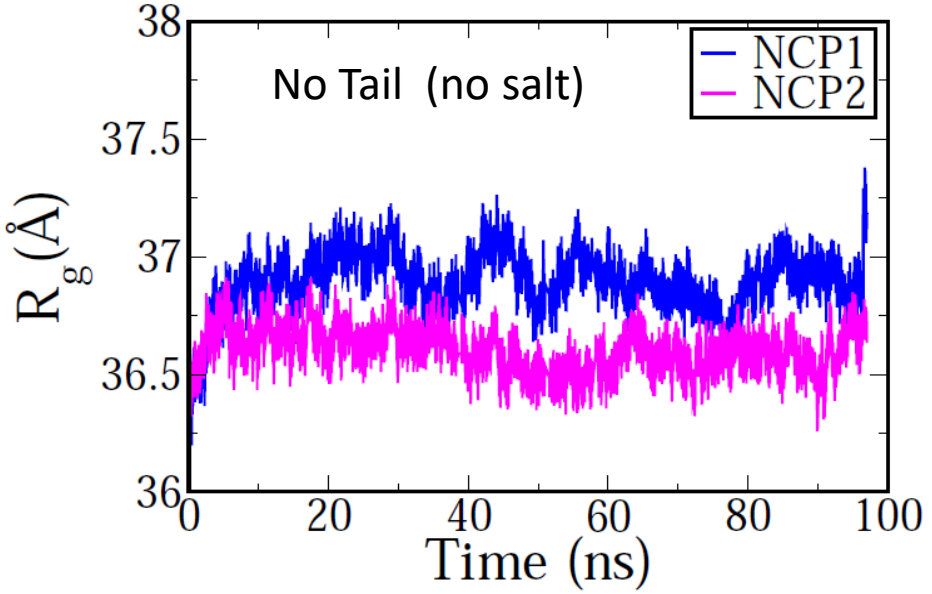
R_g *and maximum extension become maximum at 10 mM salt. Then decrease with conc. and finally increase to a value smaller than that at 10 mM.*

(R_g :46 Å -42 Å -44 Å; extension : 154 Å-138 Å-148 Å)

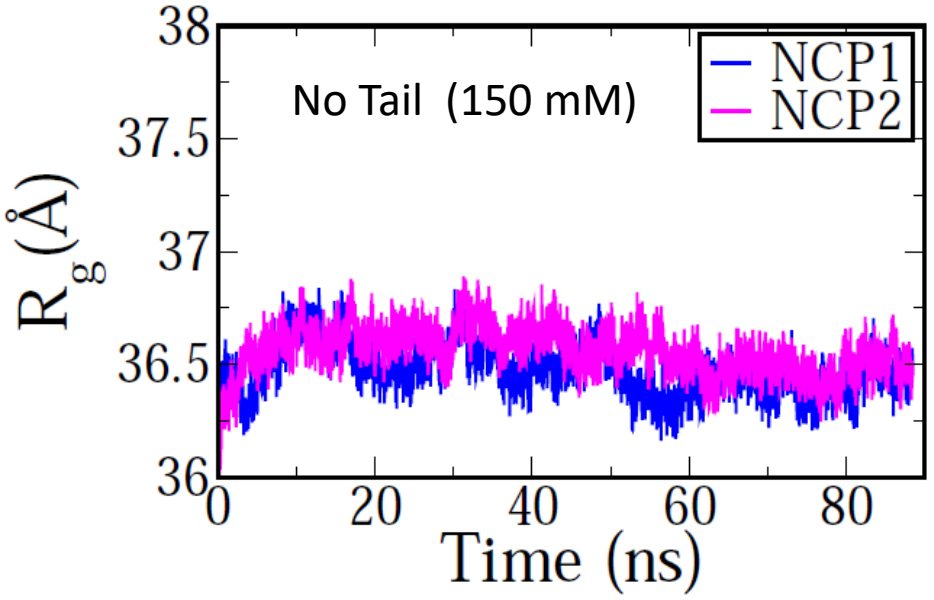
Role of Histone Tails in the Conformation and Interactions of Nucleosome Core Particles

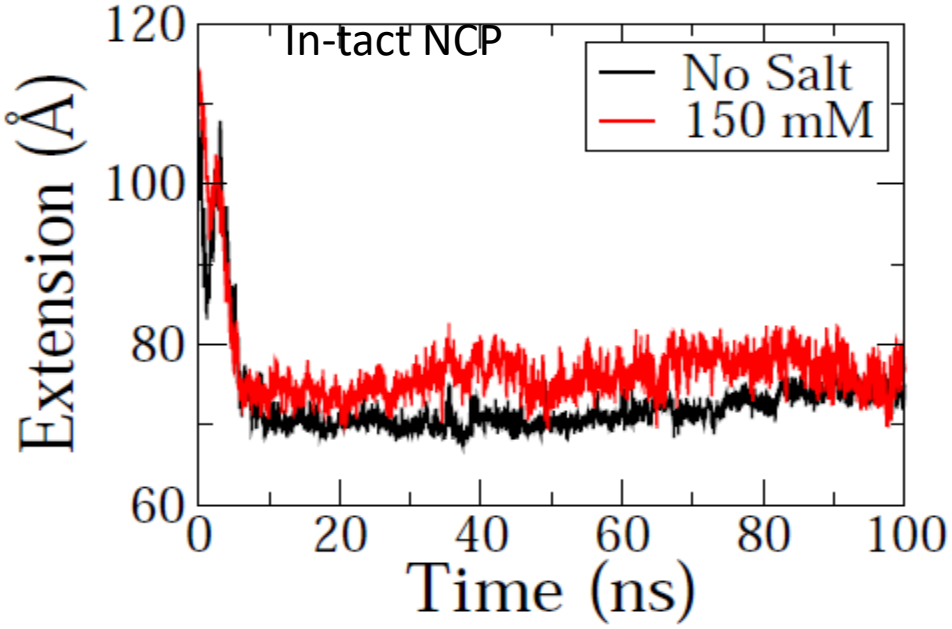
Aure´lie Bertin, Ame´lie Leforestier,Dominique Durand, and Francüoise Livolant, Biochemistry, 43,4773 (2004)

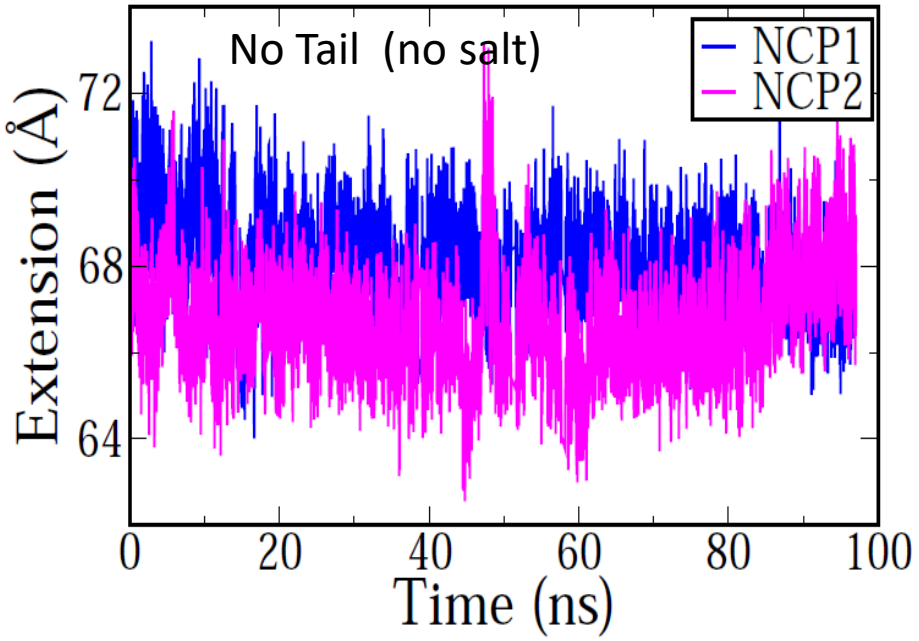




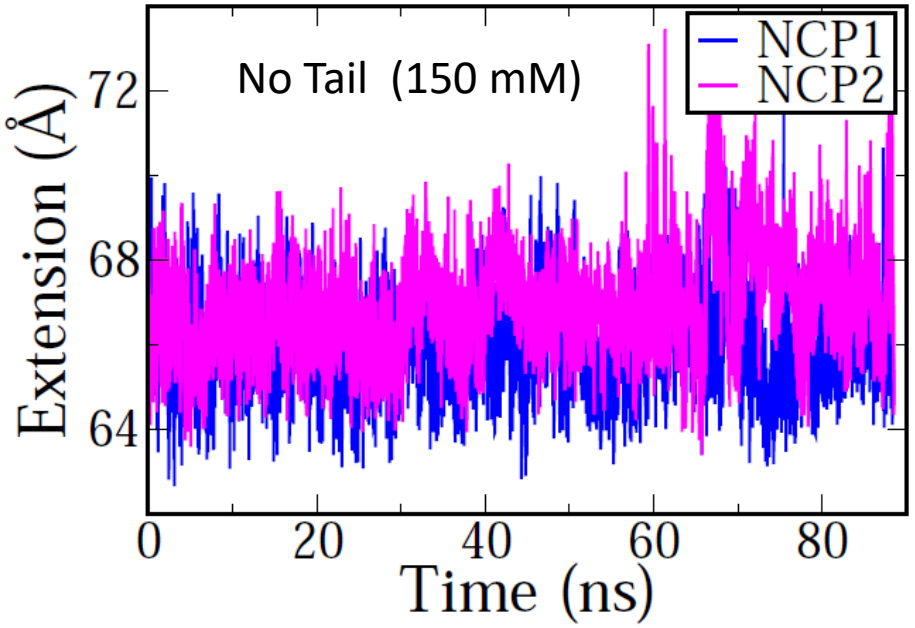
In the absence of tails, the R_g is lesser for higher salt concentration. A trend opposite to that shown by intact NCPs





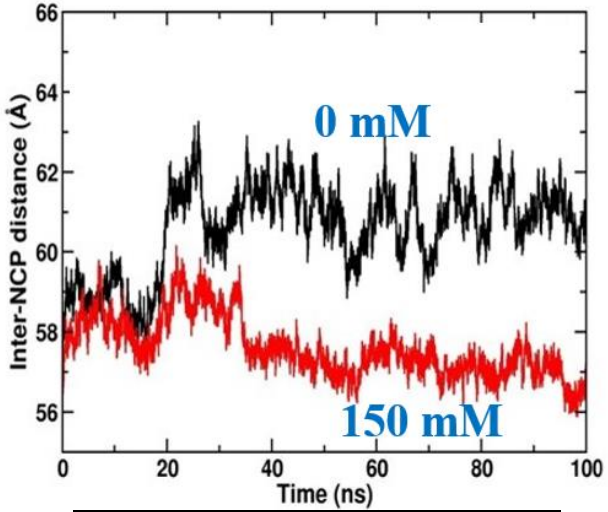


In the absence of tails, the extension is lesser for higher salt concentration. Again opposite to what is seen for intact NCPs

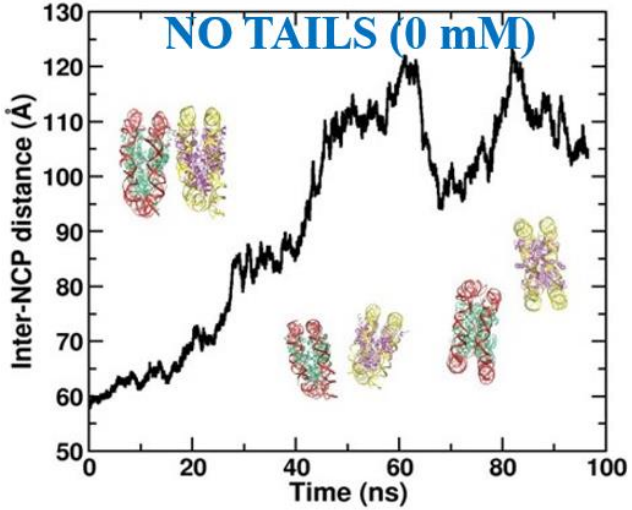


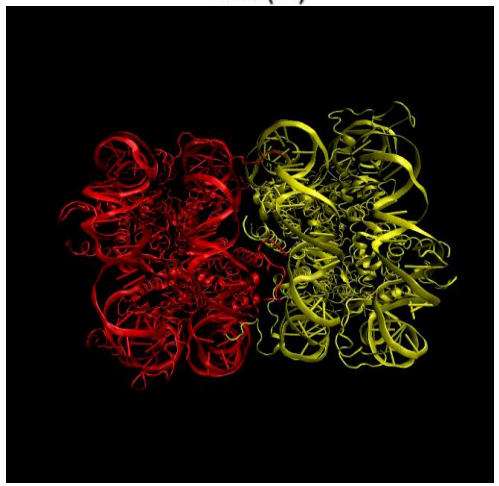
Effect of Salt and Tails

Effect of Salt

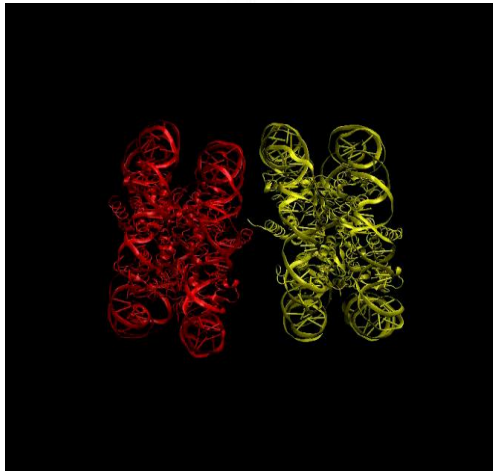


Effect of histone tails





All Tails present (0mM salt)



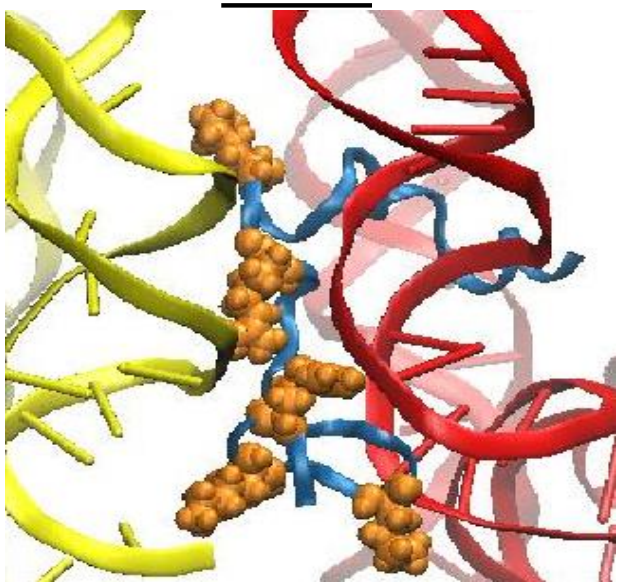
No Tails present (0mM salt)

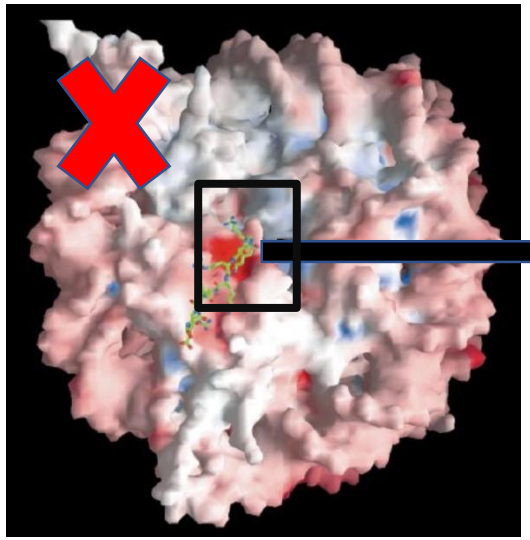
When all the histone tails are removed, the NCPs did not stay together.

Modes of Inter-NCP Interaction

TAIL-BRIDGING

H4 Tail

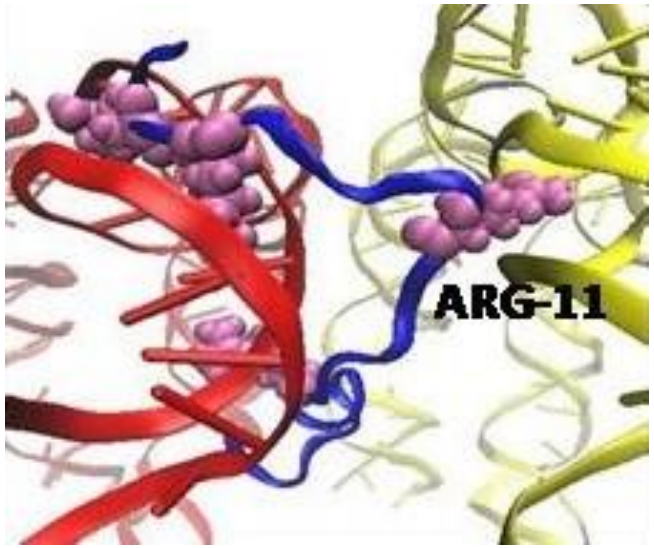




Davey et.al., J. Mol. Biol., 2002, 319(5), 1097-113

Residue K16, R19, K20, R23 of H4 tail of one NCP interacting with the Acidic Patch (E56, E61, E64, D90, E91, D92 of core histone H2A and E110 of core histone H2B) of adjacent NCP.

H2A Tail



In our Simulations *the H4 tail was seen to form most of the bridging contacts*. Apart from the H4 tail, the *H2A tail formed some bridging contacts* while the *H3 and H2B tails remained attached to the DNA of their NATIVE NCP.* H4 tail did not bind to the Acidic Patch.

TAIL	NUMBER OF INTER-NCP CONTACTS
H2A	109
H2B	2
H3	0
H4	142

Inter-NCP contacts were defined to exist when a tail atom was within 3 Å of any atom belonging to the adjacent NCP.

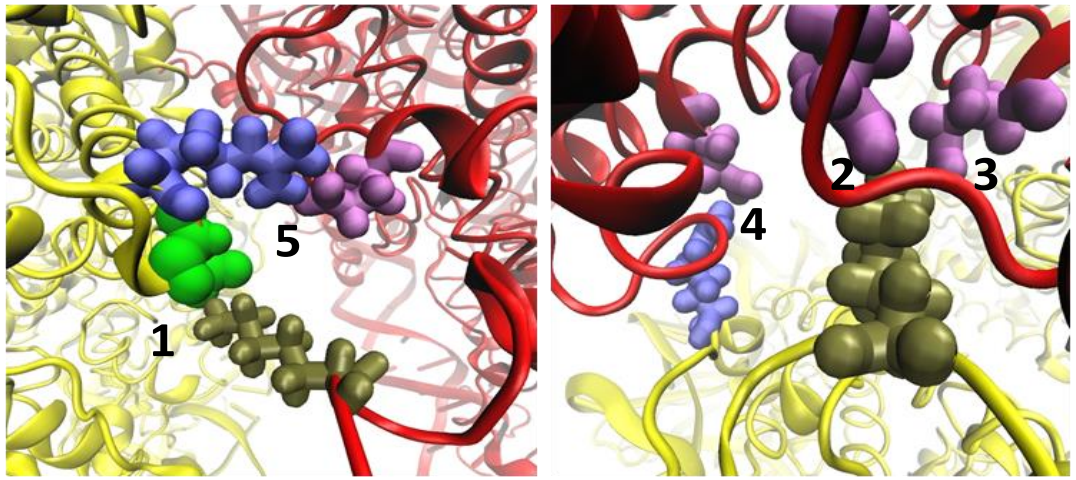
Modes of Inter-NCP Interaction

SALT BRIDGE

A SALT BRIDGE IS A NON-COVALENT INTERACTION BETWEEN POSITIVELY AND NEGATIVELY CHARGED PROTEIN RESIDUES. A COMBINATION OF HYDROGEN BONDS AND ELECTROSTATIC INTERACTION.

A SALT BRIDGE IS DEFINED TO EXIST BETWEEN TWO OPPOSITELY CHARGED RESIDUES IF THEY ARE LESS THAN 3.5 Å APART.

SALT BRIDGE

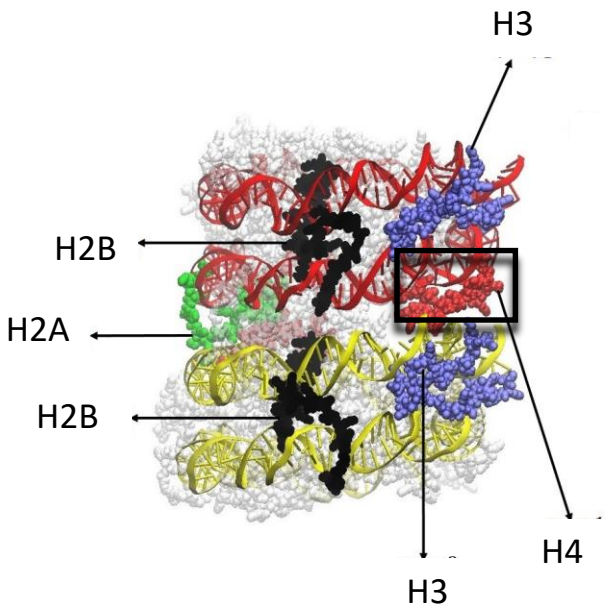


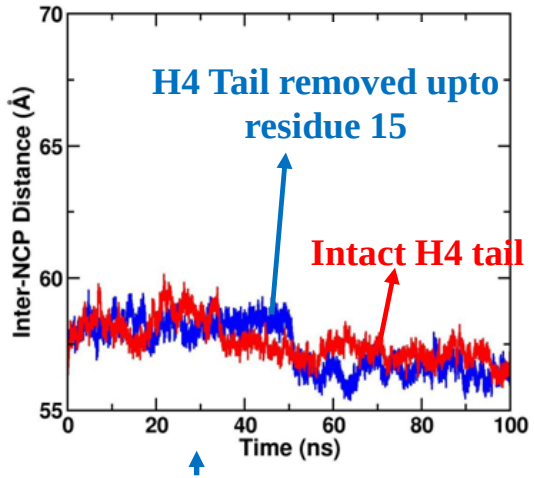
Saltbridge	HISTONE	Core/Tail	Residue	Contact with	Histone	Core/Tail
1	H4	Tail	K	D	H2A	Core
2	H4	Tail	K	E	H2A	Core
3	H4	Tail	K	E	H2B	Core
4	H4	Tail	R	E	H2A	Core
5	H2A	Core	R	E	H3	Core

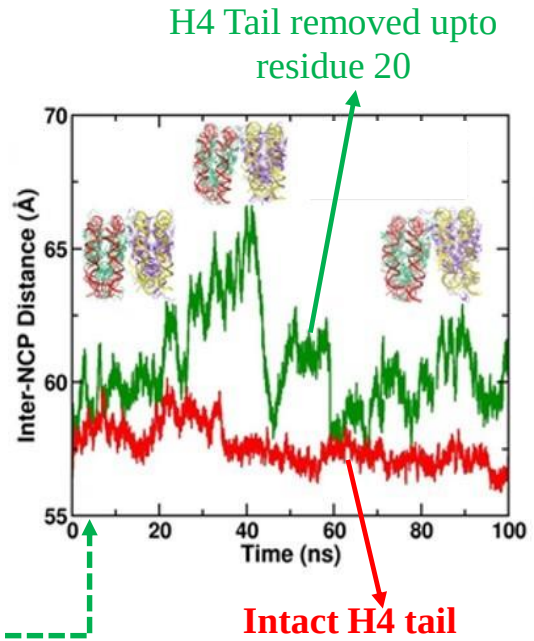
Tail-clipped Simulations (H4 Tail, 150 mM)

Experiment: **The removal of H4 tail decompacted the chromatin fiber .**

Dorigo et. al., J Mol Biol., 2003, 327(1):85-96







H4 Tail amino acid sequence

N-Terminus ---SGRGKGGKGLGKGGA | KRHRK | VLRDN**---HISTONE CORE**

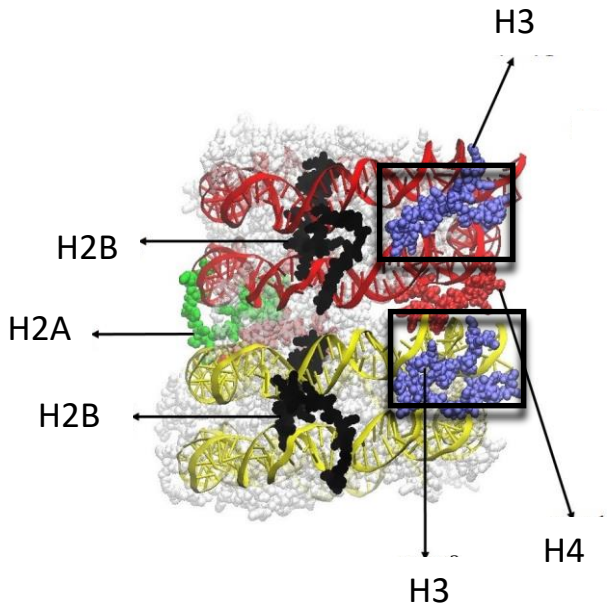
Region between K16 to K20 of the H4 tail is important for tail bridging.

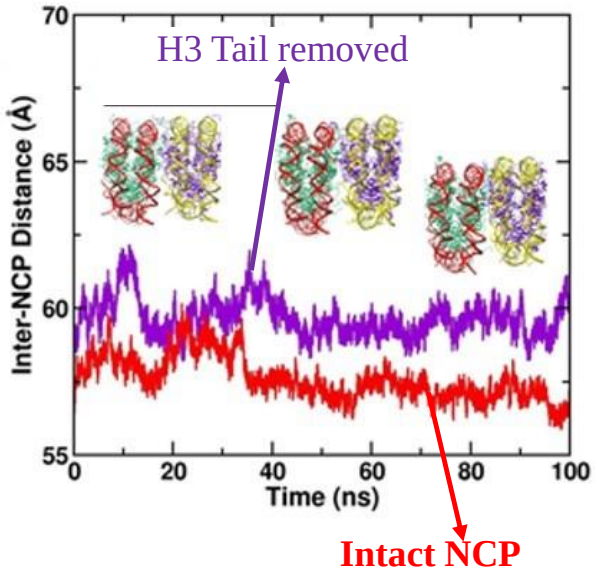
Saurabh et. al., *J. Phys. Chem. B*, 2016, 120 (12), pp 3048–3060

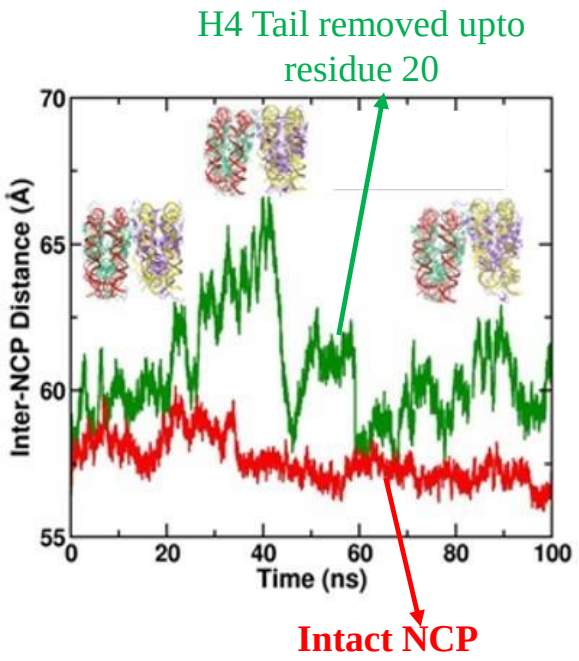
Tail-clipped Simulations (H3 Tail, 150 mM)

Experiment: **The removal of H4 tail decompacted the chromatin fiber while the removal of H3 tail did not have a severe effect.**

Dorigo et. al., J Mol Biol., 2003, 327(1):85-96



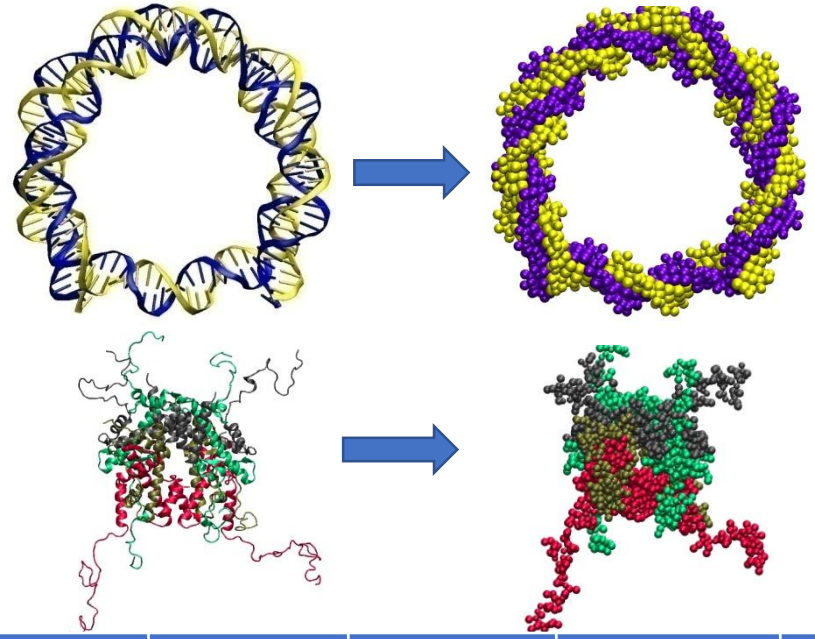


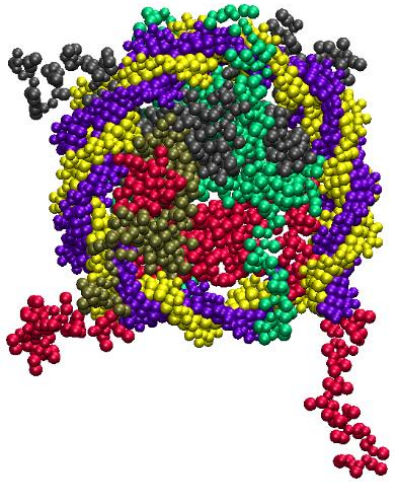


Effect of truncation of the H3 tail on the stability of the stack is much less pronounced in comparison to the effect of truncation of the H4 tail.

MARTINI Simulation of NCP Stack

To look at the stability of the stack and the behavior of histone tails over a longer period of time.





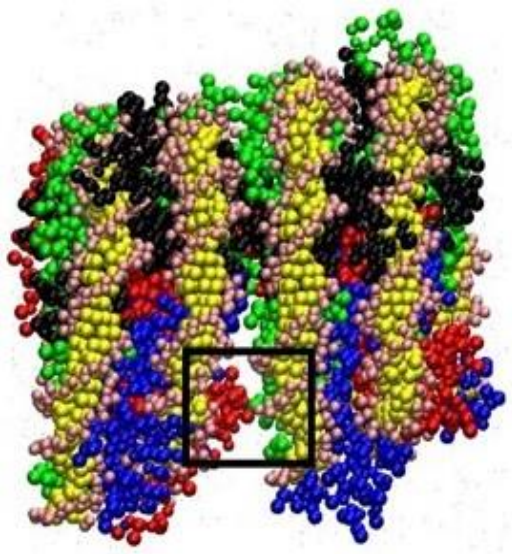
25086 atoms

3991 beads

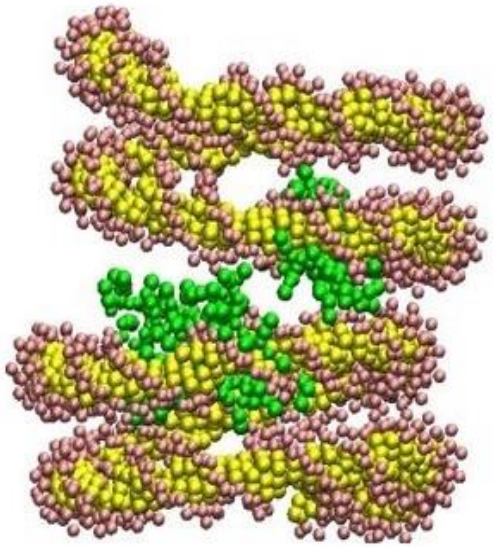
4 water molecules clubbed into 1 bead.

Unit	Histone H3	Histone H4	Histone H2A	Histone H2B	DNA	Histone Core	NCP
No. of Atoms (Atomistic)	2225	1647	2032	1966	9346	15740	25086
No. of particles (MARTINI)	294	221	267	272	1883	2108	3991

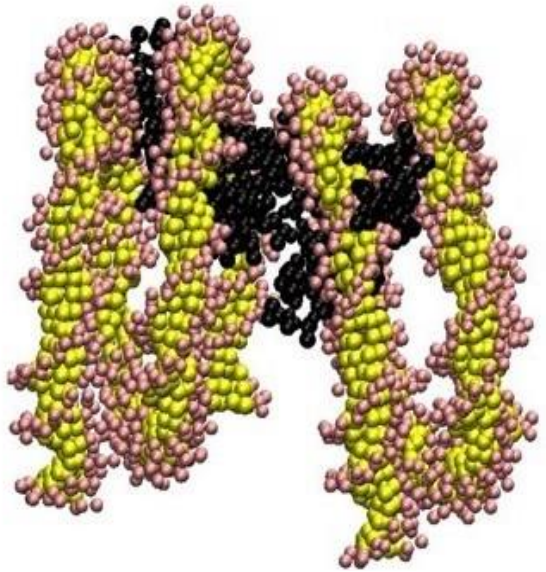
Coarse Grained Simulations



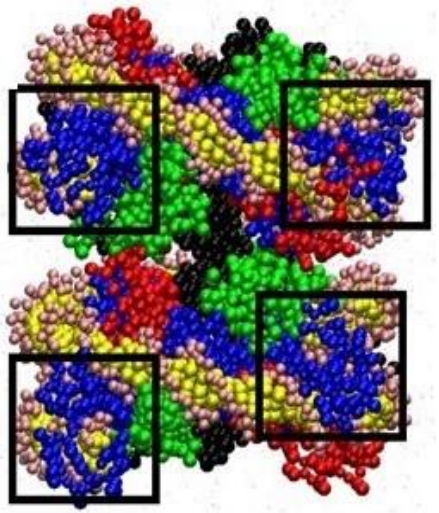
RED: H4 TAIL



GREEN: H2A Tail



BLACK: H2B Tail



BLUE: H3 Tail

MARTINI SIMULATIONS SHOW SIMILAR BEHAVIOR OF HISTONE TAILS AS IN THE ATOMISTIC SIMULATIONS

Summary of Results

Stacking interactions are salt-dependent and the presence of histone tails are necessary for NCP stacking

H4 tail is more Important in maintaining a stacked conformation.
Removal of H4 tail affected the stack more than the removal of H3 tail.

Positively charged region between K16 to K20 of the H4 tail is important.

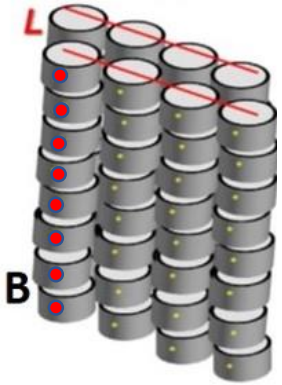
Salt bridges and H-bonds form a significant portion of contacts between stacked NCPs

COMPARISON BETWEEN PARALLEL AND ANTIPARALLEL STACK

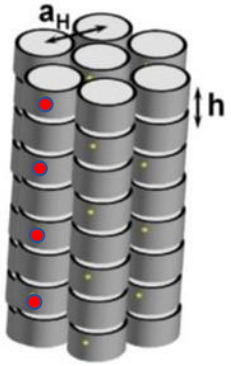
To explain the behaviour shown by LC phases in presence or absence of histone tails.
To draw inferences about the NCP organization in the chromatin fibre.

MOTIVATION

BILAYER



HEXAGONAL

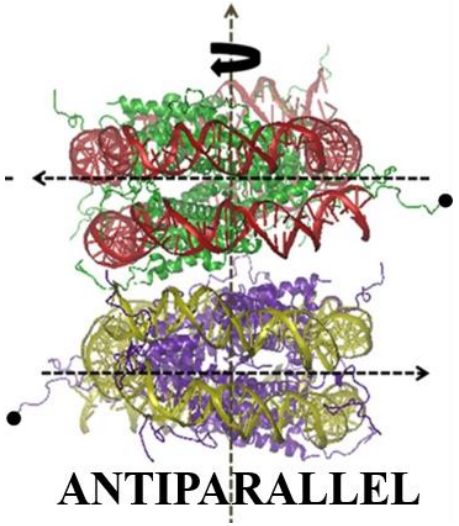


Different ordered phases observed *in vitro* have different relative orientations of adjacent NCPs in a column.
RED DOTS ON THE NCPs REPRESENT THE DIRECTION OF THEIR DYAD AXIS.

Removal of different sets of histone tails precludes the formation of different LC phases.
Nordenskiold et. al., Biophysical Journal 110, 1720–1731, 2016

Tail mediated interactions leading to the formation of columns via NCP-NCP stacking thus seem to depend on inter-NCP orientation.

ANTIPARALLEL STACK



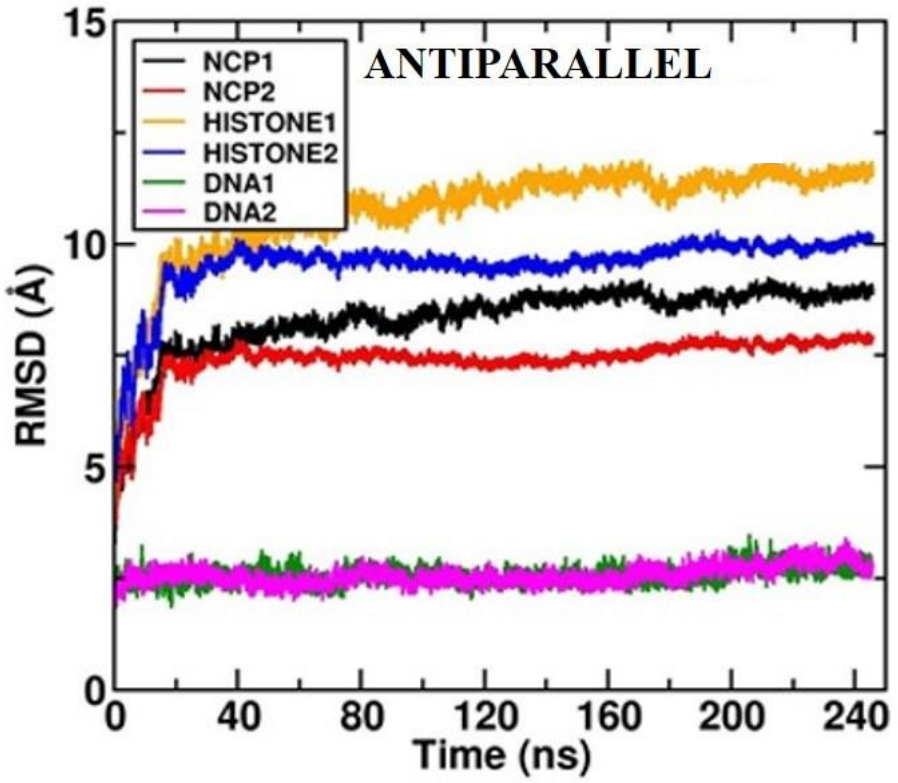
Similar to the parallel stack, no H4 tail-acidic patch interaction was observed.

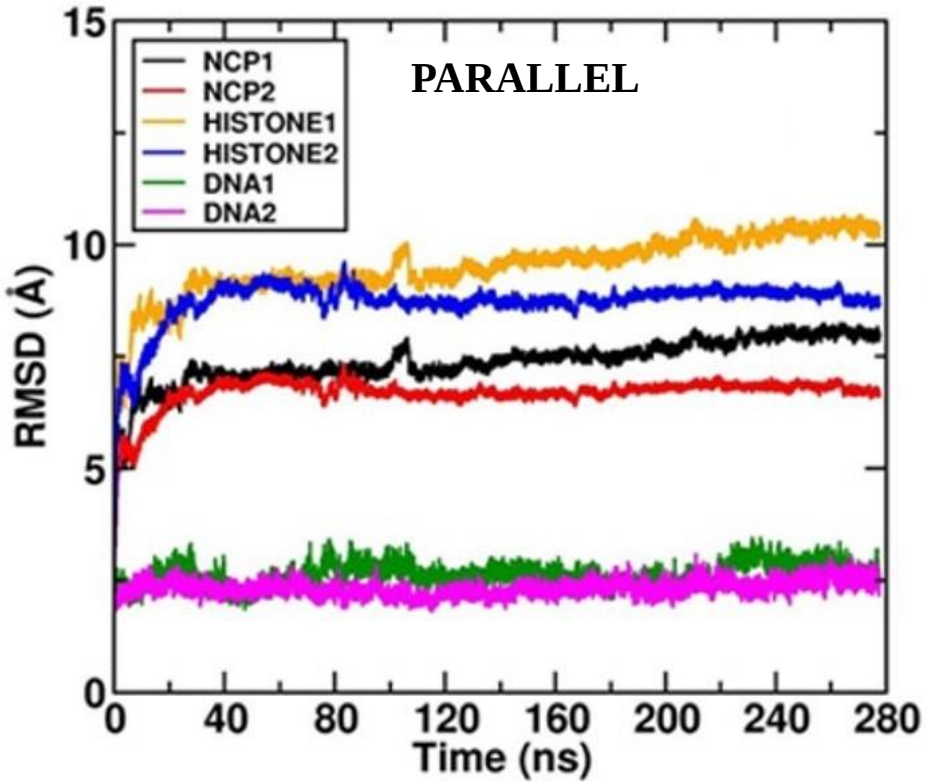
Unlike the parallel orientation, the H4 tail did not form direct inter-NCP contacts.

TAIL	NUMBER OF INTER-NCP CONTACTS	
	PARALLEL	ANTIPARALLEL
H2A	109	10
H2B	2	72
H3	0	105
H4	142	77

Most of the inter-NCP contacts in the parallel orientation were formed by the H4 and H2A tails while the H3 and H2B tails form most of the contacts in the antiparallel orientation.

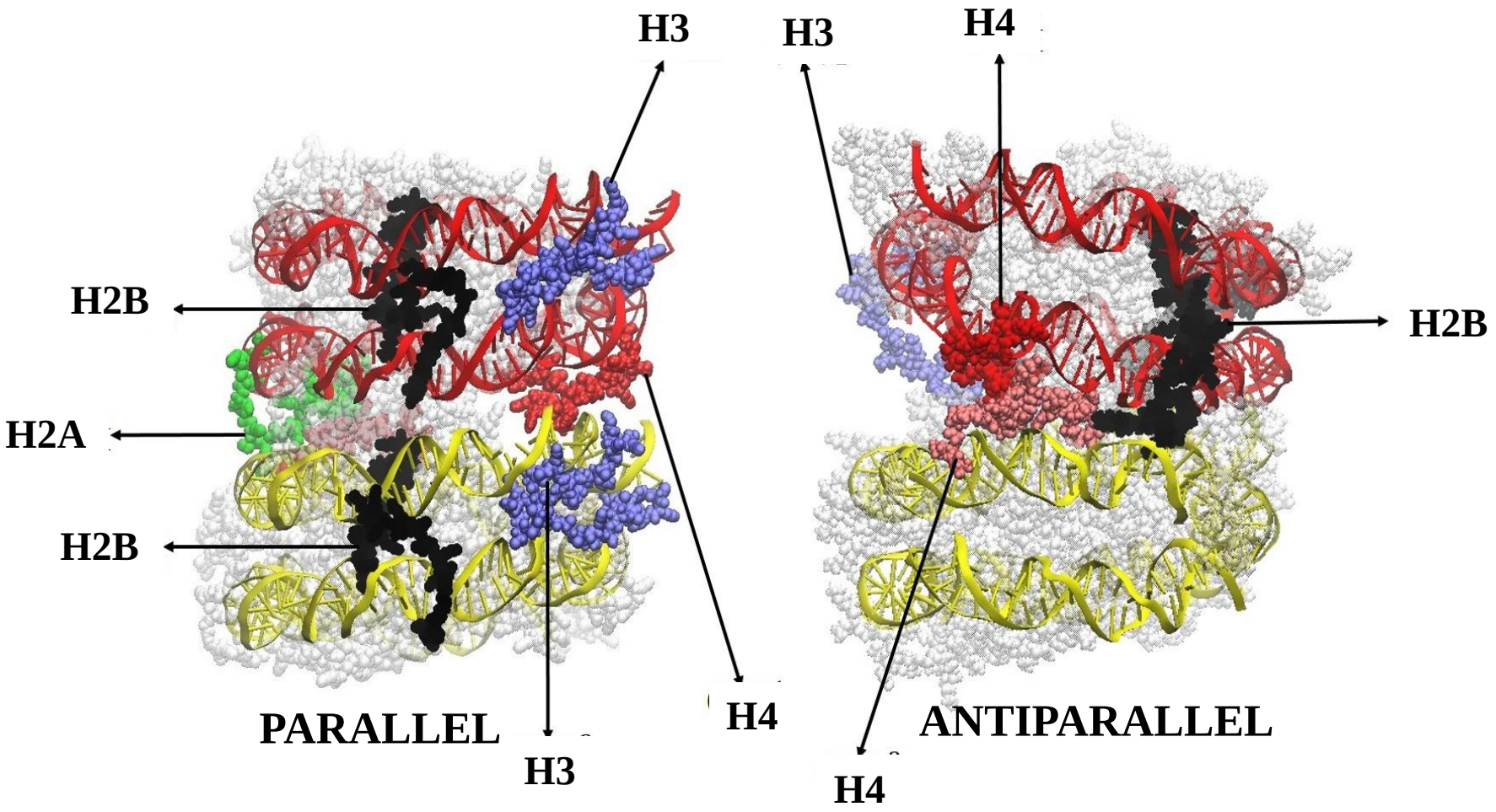
Structural Stability





THE NCPs IN THE TWO STACKS SHOW SIMILAR BEHAVIOUR

TAIL CONFORMATION IN THE PARALLEL AND ANTIPARALLEL ORIENTATION



TAIL COLOR CODE

RED: H4 TAIL

BLACK: H2B TAIL

GREEN: H2A TAIL

BLUE: H3 TAIL

THE SNAPSHOTS SHOW DIFFERENT SETS OF TAILS FORMING INTER_NCP CONTACTS IN THE TWO ORIENTATIONS. IN ADDITION THE H4 TAIL IS SHOWN FORMING TAIL-TAIL CONTACTS RATHER THAN DIRECT BRIDGING CONTACTS.

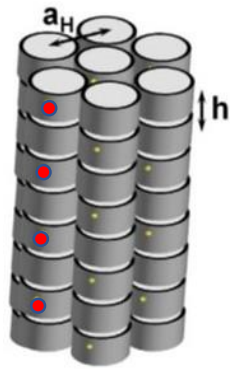
Comparison with Experimental results

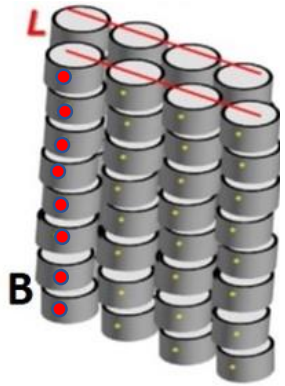
EXPERIMENT

Hexagonal (*with antiparallel orientation of NCPs along a stack*) phase not affected by the truncation of H4 tail.*

On Truncation of H3 or the H2B tail, hexagonal phase (*with antiparallel NCP orientation*) is not formed.*

The bilayer phase (*with parallel NCP orientation*), is observed under specific conditions. The assembly of the bilayer is thought to be mediated by the H2B tail.**





SIMULATION

H4 tail does not form inter-NCP contacts in the antiparallel orientation.

H3 and H2B tails are the dominant contributors of inter-NCP contacts in the antiparallel orientation.

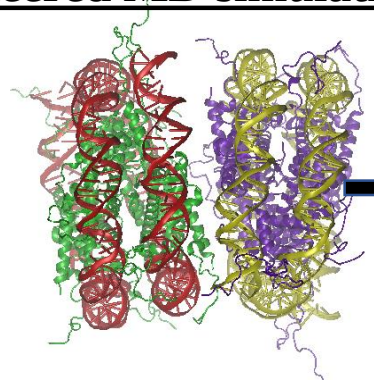
H2B tails do not form any inter-NCP contacts along the stack in the parallel orientation and thus are free to mediate inter-array contacts

** Nordenskiold et. al., Biophysical Journal 110, 1720–1731, 2016*

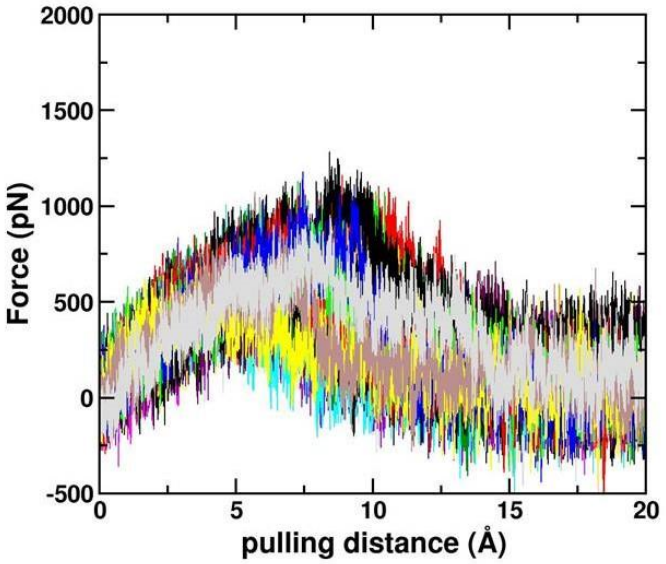
*** Livolant et. al., Biophysical Journal ,81, 2414–21, 2001*

Comparison of Stability of the Stack in the two orientations

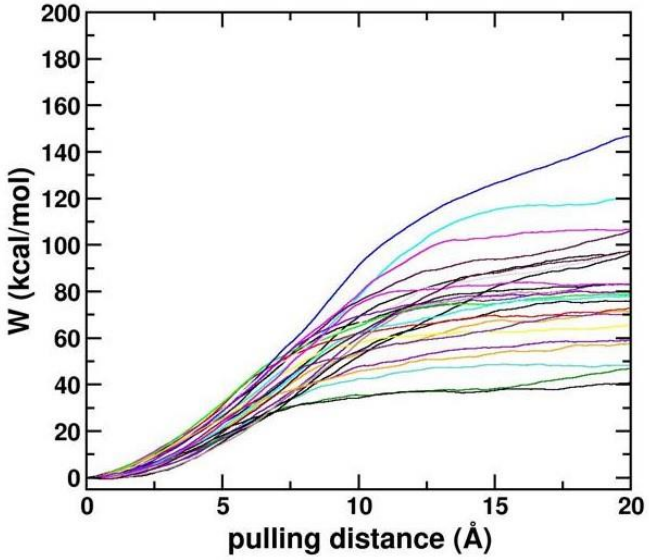
Steered MD simulations



V= 0.001 Å/ps



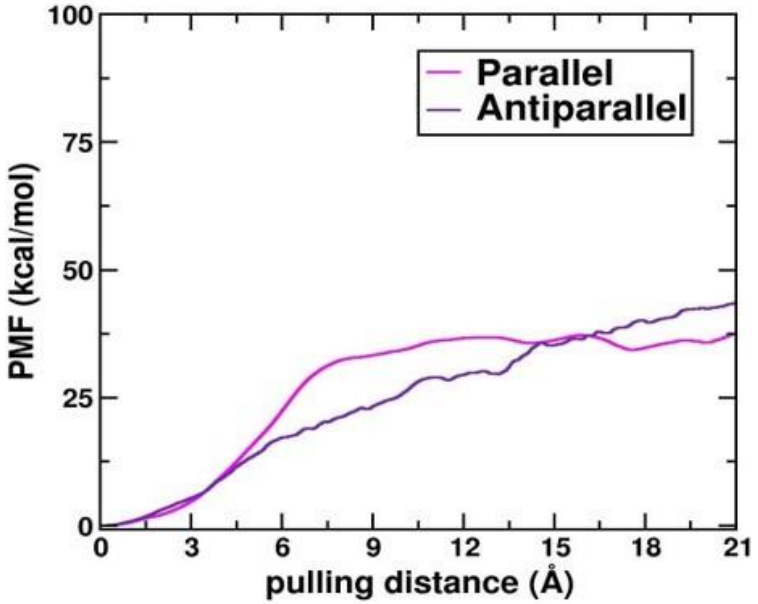
INTEGRATION



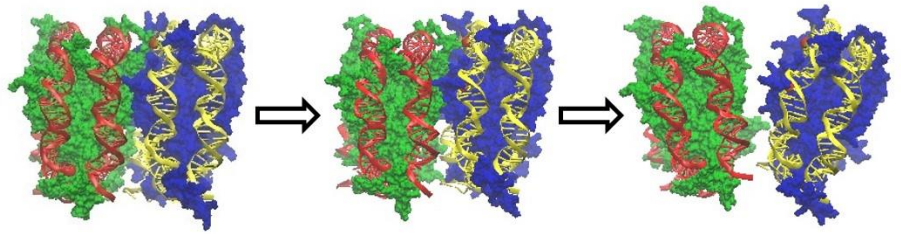
JARZYNSKI EQUALITY

$$\Delta F_{A\to B} = -\frac{1}{\beta}\ln\sum_{i=1}^{N}\frac{1}{N}\exp(-\beta W_{i,A\to B})$$

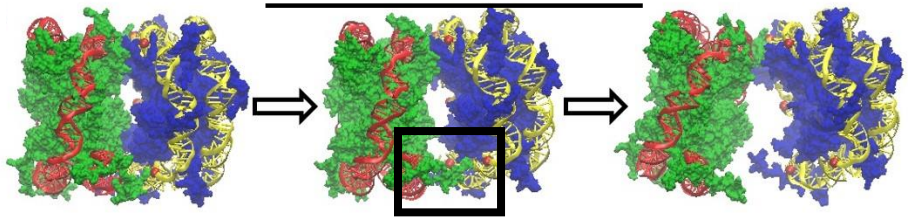
A and B are initial and final states.
N is the number of pulling runs.
W is the work done.



PARALLEL



ANTIPARALLEL



The Parallel and Antiparallel Orientations lead to similar stabilization energy. The difference lies in the way the stacks de-assemble.

Similar stabilization for the parallel and antiparallel orintation indicate towards a polymorphic chromatin.

Experiments show that the removal of H4 tail decompacts chromatin fibre.

But, in our simulation, the Antiparallel stack shows no direct inter-NCP contacts formed by the H4 tail between the two stacked NCPs.

The above two points indicate towards an absence of antiparallel stacking in the chromatin fibre.

Summary of Results

The behavior of the LC phase as a function of presence or absence of particular histone tails can be attributed to a difference in tail-conformation as a function of relative orientation of stacked NCPs

The parallel and antiparallel stacks show similar stability.

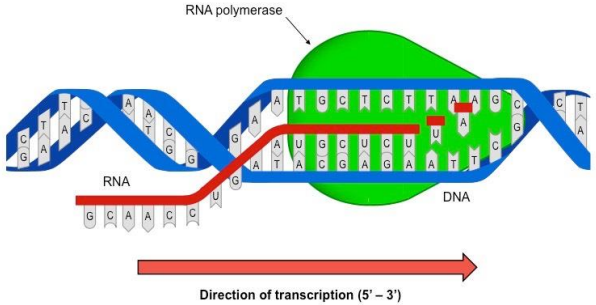
A comparison of the simulation results with experimental results predicts an absence of antiparallel or near-antiparallel orientation of consecutive NCPs in the chromatin.

Dynamics and Elasticity of NCP DNA

Introduction

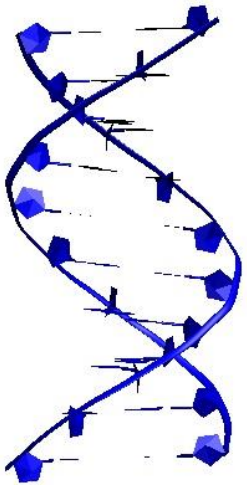
Genetic disorders in humans (Huntington's disease, fragile X syndrome, myotonic dystrophy etc.) are characterized by an increase in DNA repeats corresponding to lower than normal DNA elasticity.

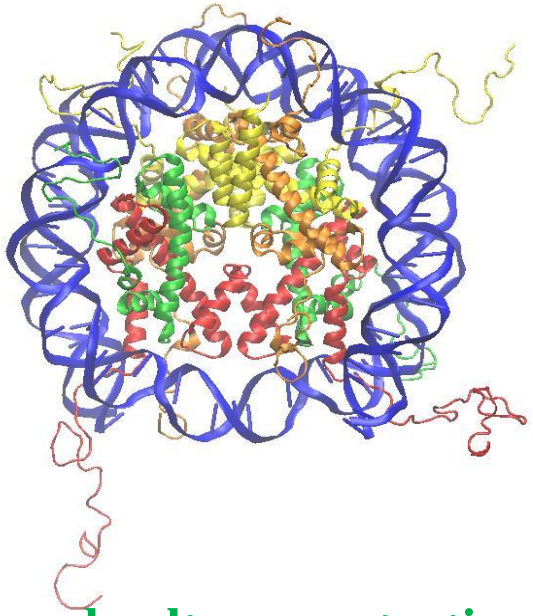
Understanding the effect of various environmental factors on the elasticity of free and histone-bound DNA is thus important to predict and find a cure to genetic disorders.



Short fragments of DNA are involved in interaction with the transcription machinery.
Understanding the nature of DNA elasticity is thus important also to understand the intra-cellular processes.

Aim



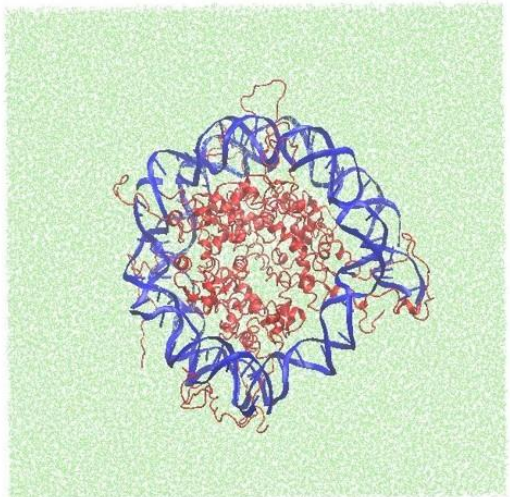


Studying the dependence of sequence length and salt concentration on DNA persistence length which is a measure of flexibility (correlation of local tangents along a polymer).

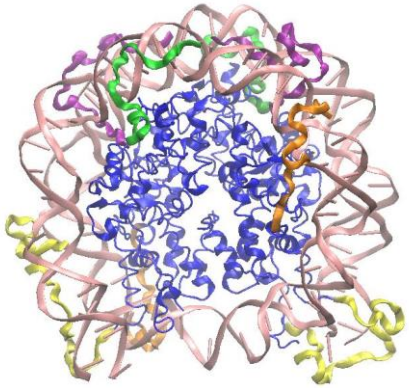
The transcription machinery inside the cell encounters a protein-bound DNA. The stiffness of the nucleosomal DNA is expected to be different from bare DNA.

To compare the persistence length of the nucleosomal DNA with that of the bare DNA under different salt concentrations.

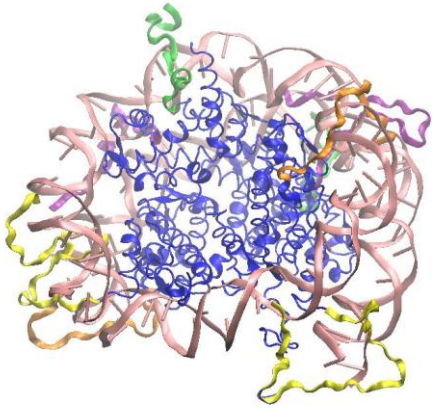
Simulation of Single NCP



Single NCP in a Water Box



0 mM



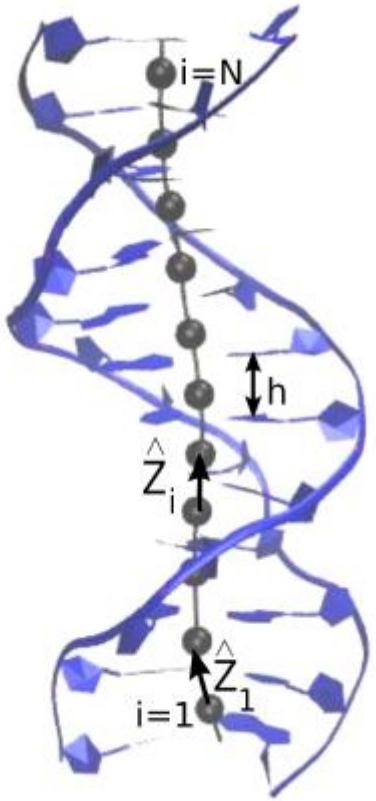
150 mM

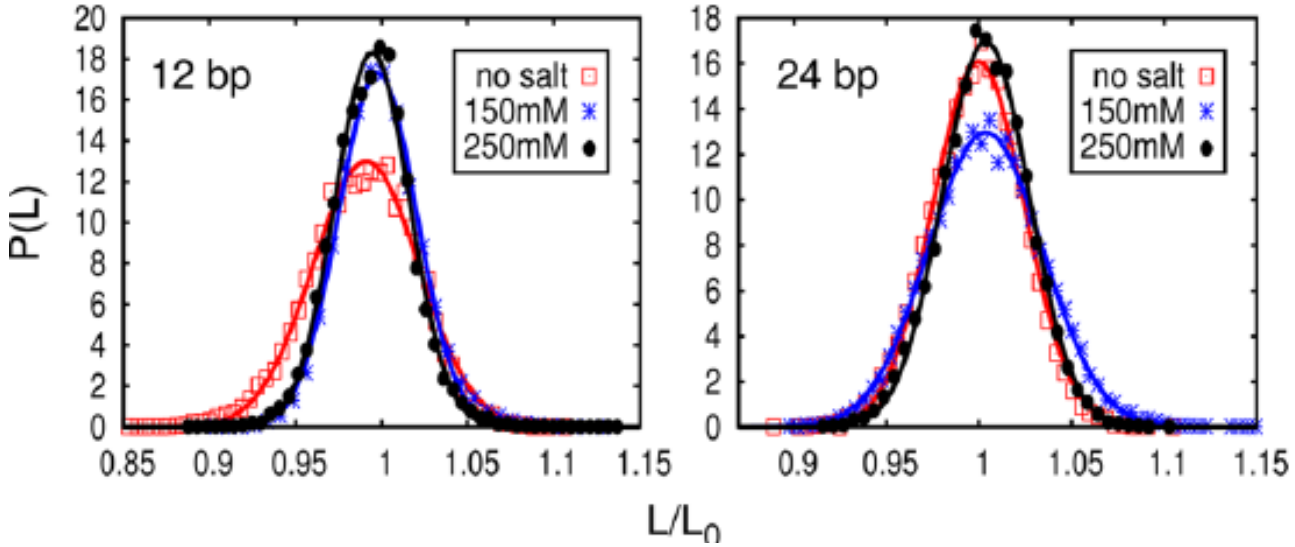
In the presence of salt, the tails were more extended. In the absence of salt they stayed collapsed onto the DNA.

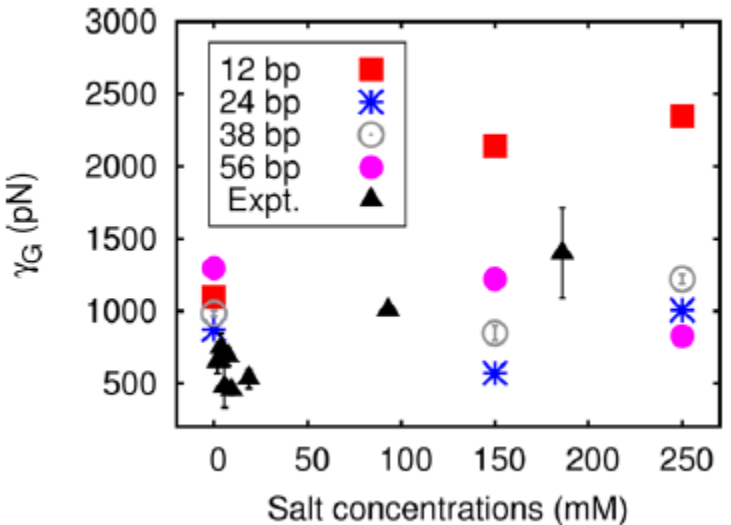
Contour length distribution

$$P(L) = \sqrt{\frac{\gamma}{2\pi k_B T L_0}} \exp\left[-\frac{\gamma L_0}{2 k_B T} (L/L_0 - 1)^2 \right]$$

Maiti et al., EPJE, 2013, JPCB 2015



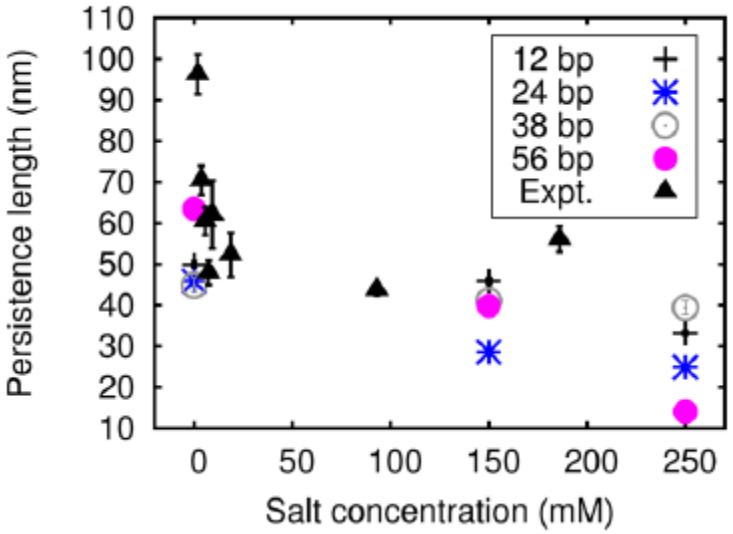


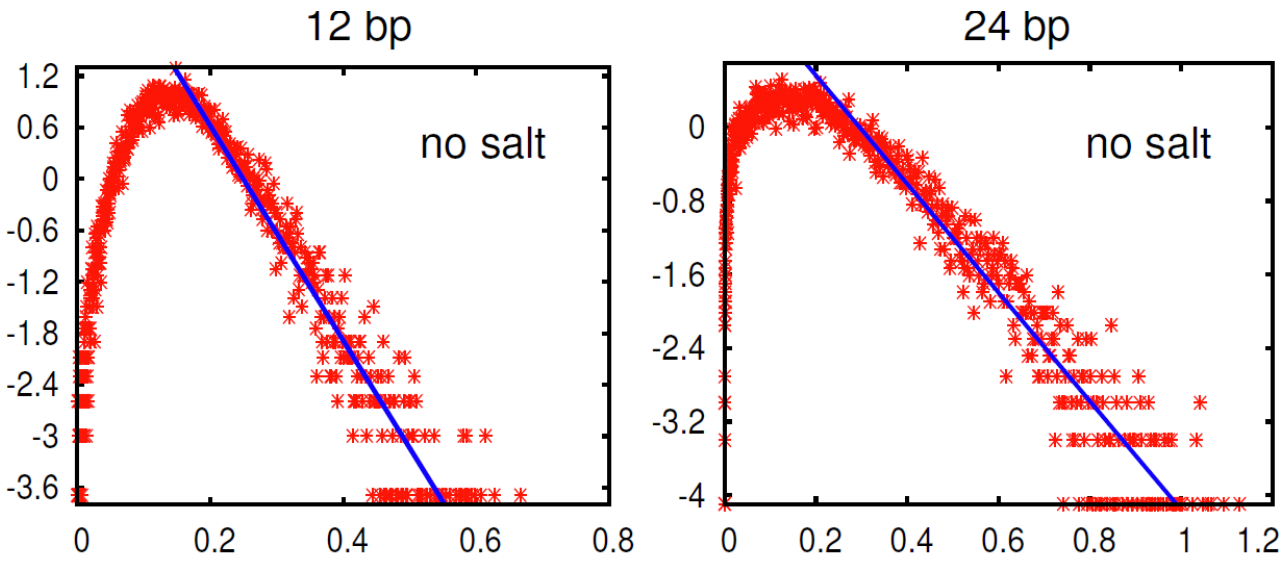


Bending angle distribution

$$P(\theta) = \sqrt{\frac{\kappa}{2\pi L_0 k_B T}} e^{-\frac{\kappa}{2L_0 k_B T}\theta^2}$$

$$\ln P(\theta) \approx -\frac{l_p}{L_0}(1-\cos\theta) + \frac{1}{2}\ln\left(\frac{l_p}{2\pi L_0}\right);$$

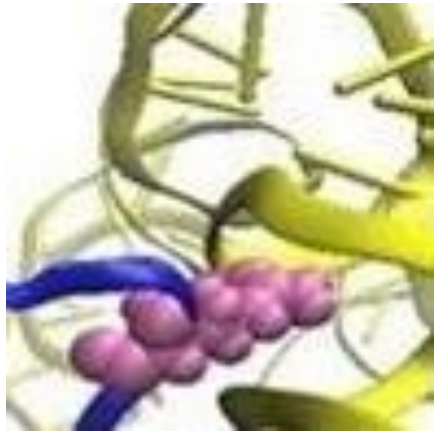


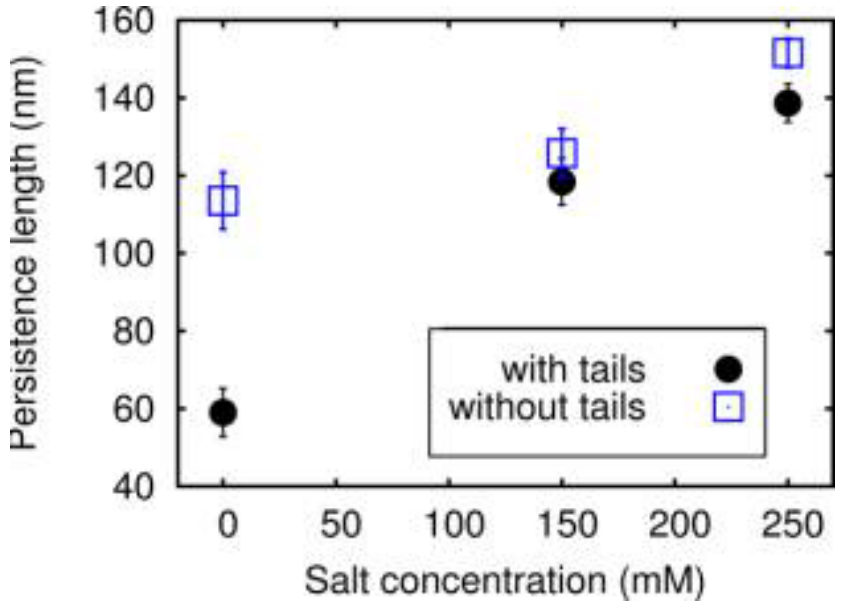


Maiti et al., EPJE, 2013, JPCB 2015

NCP DNA elasticity

l_p (nm)	24 bp (bare)	NCP DNA
0 mM	46 ± 0.9	59 ± 6
150 mM	29 ± 0.9	118 ± 6
250 mM	25 ± 0.7	139 ± 5





Persistence length of bare DNA decreases with salt concentration while that for NCP DNA increases with increasing salt. This trend can be attributed to the behavior of histone tails as a function of salt.
NCP DNA is stiffer than the bare DNA.

Garai et. al., J Phys Chem B. 2015, 119(34), 11146-56

Summary of Results

The Persistence length of DNA is dependent on the length of the DNA.

Nucleosomal DNA is stiffer than bare DNA.

While the persistence length of bare DNA decreases with increase in salt concentration, the trend is opposite for the nucleosomal DNA.

The variation of the persistence length of nucleosomal DNA is dictated by the behavior of histone tails.

Future Direction

Simulating the effect of post-translational modifications on the stability of the stacks.

Studying the self-aggregation properties of the histone tails and how tail-tail interactions contribute to the stability of the NCP-NCP stack.

As water models are known to have a considerable affect on the conformations that disordered proteins attain, to study how different water models would affect tail conformationis relevant.

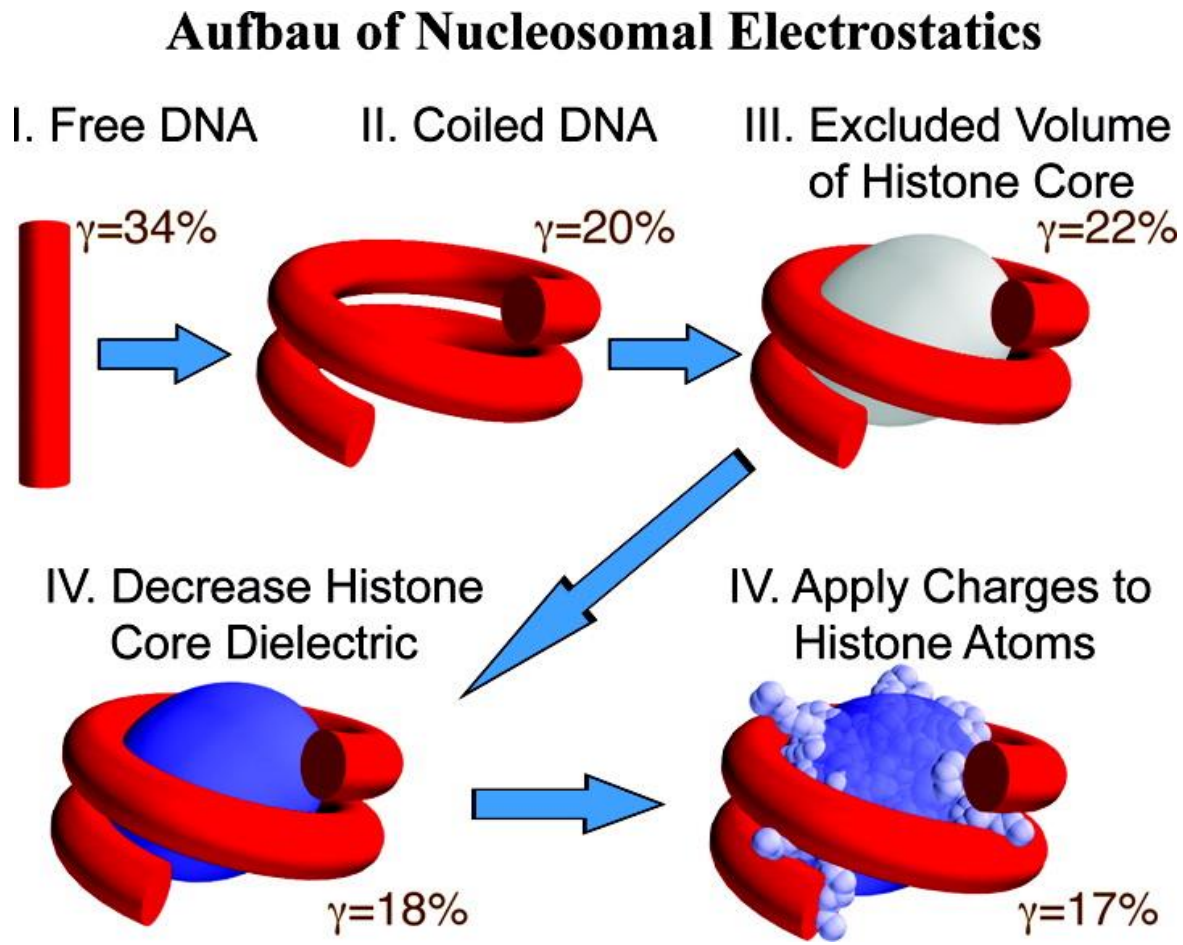
MARTINI level Coarse-grained simulations of systems like 3-NCP stacks and the tetranucleosome.

Thank you

Nucleosomal Electrostatics

Materese et.al., JACS, 2009, 131, 15005-13

The DNA charge is neutralised better in the nucleosome than it's free state in an ionic environment. This helps in chromatin stability.



γ : Percentage of the residual DNA charge after accounting for counterions and histone charges within 1 nm of DNA surface.

THANK YOU