Modelling Chromatin with explicit ions: ion correlation effects and like charge attraction.
Comparison with new experiments

Lars NORDENSKIOLD
School of Biological Sciences Nanyang Technological Univ.
60 Nanyang Drive
SINGAPORE 637551
“Modelling chromatin with explicit ions: ion correlation effects and like charge attraction. Comparison with new experiments.”

Electrostatic Interactions in Compaction and Aggregation of Chromatin

From DNA-Inspired Physics to Physics-Inspired Biology, (1 - 5 June 2009)

Lars Nordenskiöld

School of Biological Sciences, Nanyang Technological University-Singapore
OVERVIEW:

1. INTRODUCTION:
   DNA Condensation, Nucleosome and Chromatin

2. Nucleosome Core Particle (NCP) aggregation:
   Experiments & coarse-grained modelling of NCP- NCP interaction

3. Electrostatics in Chromatin condensation:
   Experiments & coarse-grained computer modelling of chromatin folding/aggregation

4. A novel DNA/protein/lipid self assembly system?
DNA condensation by oligocations:
An established polyelectrolyte effect!

Critical concentration of oligocation for condensation of DNA solution depends dramatically on cation charge

\[
\begin{align*}
\varepsilon\text{-Lys}_7^{7+} & : 1 \mu M \\
\text{Spermine}^{4+} & : 0.3 mM \\
\text{CoHex}^{3+} & : 3 mM
\end{align*}
\]

Scattering

Ligand added, M

Light Scattering (SLS)

\[
\begin{align*}
\text{Spd}^{3+} & : +3 \\
\text{CoHex}^{3+} & : +3 \\
\text{Spm}^{4+} & : +4 \\
\varepsilon\text{K5} & : +5 \\
\varepsilon\text{K6} & : +6 \\
\varepsilon\text{K7} & : +7 \\
\varepsilon\text{LYRK10} & : +20 \\
\varepsilon\text{K31} & : +31
\end{align*}
\]

\varepsilon\text{-oligolysines:}

branched \varepsilon\text{-oligolysines}

\[
\begin{align*}
\varepsilon\text{-oligolysines:} & : n = 3-10, 31 \\
branched \varepsilon\text{-oligolysines} & : n = 10; R' = L, Y, R, YR, LYR\end{align*}
\]
DNA & biopolyelectrolytes compact and/or aggregate to ordered phase induced by multivalent counterions:

**Counterions:**  $\text{Mg}^{2+}, \text{Ca}^{2+}, \text{Co}^{3+}$, Polyamines (3+, 4+), $\text{L}^{n+}$

**Aggregation** of charged biopolymers:
- DNA, f-Actin, charged virus particles, ...

Aggregation depends on:
- Biopolymer charge density
- Counterion valency
- Solvent dielectric medium

**Implies:** A mechanism governed by electrostatic attraction between polyelectrolytes! Induced by ion-ion correlations
Attraction between like-charged polyelectrolytes can be induced by correlated charge fluctuation.

Correlated fluctuation in the mobile ion charge distribution induces attractive configurations.

Compare attractive London Dispersion force between atoms/molecules.

Larger counterion charge valency and larger polyion surface charge density gives larger attraction.

Electrostatics of the nucleosome in chromatin

The Nucleosome Core Particle (NCP):

DNA, ~147 bp
Z = −294

Histone protein octamer core:
2xH2A, 2xH2B, 2xH3, 2xH4

NCP Total charge: ~ -148

N-Histone tails Z = ~ +88
Histone tail modifications of chromatin *in vivo*

Chromatin folding likely regulated by acetylation/deacetylation.

Acetylation quench lysine positive charge and correlates with transcriptional activity.

Histone tail bridging
Recombinant approach: Biophysical studies of well defined nucleosome and chromatin systems:

Assembly of recombinant histone octamer with:

1: 147 bp DNA to NCP:

2: The 12 tandem 177 bp repeat DNA to chromatin array with exactly 12 histone octamers:
Production of chromatin arrays

Recombinant histones
Histones expression plasmids

Histone expression
H3 H4 H2A H2B

Gel filtration
ion exchange
(denaturing)

H3 H4 H2A H2B

Histone octamer

Tandem array of a strong positioning sequence

15-147-15

12x177 bp DNA

“601” DNA

Nucleosome

Histone octamers

Salt

Higher Order Structure (HOS)

Atomic Force Microscopy image

12x177 bp DNA

177bp nucleosome

177bp DNA

array

12x177bp DNA

array

12x177bp DNA

177bp DNA
2. Nucleosome Core Particle (NCP) aggregation

Cation induced aggregation of NCP (By SLS):

Effects of tail charge:

4 lys$^+$ → glut mutations on H4 tail:
Small increase of EC$_{50}$ to higher conc.

Tailless gNCPs don’t (fully) condense with Mg$^{2+}$/ Ca$^{2+}$. 
Continuum Langevin simulation of NCP

- NCP sphere ($r = 50$ Å).
- 8 strings of +1 charges.
- Primitive dielectric model.
- Explicit mobile ions (salt).

10 “spiders in a can”

Result from simulations:
NCP aggregation in the presence of Mg\(^{2+}\):

- Only Na\(^{+}\) counterions
- Presence of divalent salt: (Mg\(^{2+}/4\) mM\(^{+}\))
- Reduced tail charge & presence of M\(^{2+}\)

• Magnesium induce attraction, as with experiments
• Aggregation includes bridging of tails between NCP
• Tail charge quenching (”acetylation”) reduce attraction
• More detailed NCP model gives similar results:
More Detailed NCP Model: Effects of tails

With tails:
Attraction for Co$^{3+}$ and Mg$^{2+}$

Tail-less:
Weaker attraction for Co$^{3+}$
no attraction for Mg$^{2+}$

Yang, Y., Korolev, N., Lyubartsev, A. P. & Nordenskiöld, L., (2009), Biophys.J. 96, 2082
3. Electrostatics in chromatin condensation

Addition of Mg\(^{2+}\) ions to buffer solution of arrays in extended form, induce two transitions:

1) Folding:

\[
35S \rightarrow 55S
\]

2) Aggregation:
Analytical ultracentrifugation

Measurement of sedimentation S-value:
Change from 35 (extended array) to 55 (compact folded array)

Cation concentration for max folding:  
Co\(^{3+}\): 15\(\mu\)M  Mg\(^{2+}\): 1mM

Effect of H4- a.a. K16 acetylation: compare H4- a.a. K16 mutation (Q)

(Acetylation by chemical ligation method, C-F Liu et. al.)

H4-K16 Acetyl. S=43↑  ↑ H4-K16 Q-mut: S=50
Dynamic Light Scattering (DLS):

Cation concentration needed for *inter-array aggregation*:

Scattering

---

Particle size
“Snake-Spider” model of chromatin array in coarse-grained Langevin simulations

**MODEL:** HO core/charged beads-DNA/Charged flexible tails/Dielectric continuum/ *Explicit ions*

1 HO core, \( r=33 \) \( \text{A} \), \( q=+0 \) or +64
25 DNA core beads, \( r=8 \) \( \text{A} \), \( q= -9.4 \) or -12
5 linker DNA beads, \( r=8 \) \( \text{A} \), \( q= -12 \)
8 linker tails, bead \( r= 3 \) \( \text{A} \), \( q=+1 \), variable #beads on each linker
Explicit ion simulations shows compaction of chromatin in the presence of Mg$^{2+}$/Ca$^{2+}$ and Co$^{3+}$

Mean end-to-end distances:

- 0.8 mM MgCl$_2$: 51 nm
- 0.8 mM KCl: 112 nm
- 0.2 mM [Co(NH$_3$)$_6$]$^{3+}$Cl$_3$: 37 nm
Tail-less array compacts in the presence of $\text{Co}^{3+}$:

$\uparrow \text{Co}^{3+}$: Somewhat weakened compaction

Little compaction for $\text{Mg}^{2+}$:

$\uparrow$
Simulations in Debye-Hückel approximation (no explicit ions) inadequate for multivalent ions:

Debye-Hückel (w tails)

Explicit ions

$I = 4.1 \text{ mM (ionic strength)}$

$I = 100 \text{ mM (ionic strength)}$

$I = 16.6 \text{ mM (ionic strength)}$
Debye-Hückel: \( I = 2 \text{ mM} \)

Debye-Hückel: \( I = 100 \text{ mM} \)

Debye-Hückel: Evolution of the gyration radius as a function of ionic strength

Gyration radius

Uncharged chromatin array

\( I = 100 \text{ mM} \)
Debye-Hückel: No Tails – No compaction:

Mean end-to-end distances:

Conclusions:
# Compaction in Debye-Hückel is due to screening of electrostatic interactions and tail interactions.
# Mobile ion-ion correlations contribute to array compaction.
Summary on NCP and chromatin condensation:

Conc. of multivalent counterion conc. for compaction or aggregation of NCP and chromatin follow:

\[(Na^+ \sim K^+) > Mg^{2+} \sim Ca^{2+} > spermidine^{3+} > Co(NH_3)_6^{3+} > spermine^{4+} > M^{4+n}\]

Condensation caused by:

- Salt screening
- Attractive ion-ion correlation (mobile ions)
- Attractive correlation (tails)
- Tail bridging

Described in theoretical modelling with continuum models and explicit mobile ions (not Debye-Hückel/PB).
4. A novel DNA/protein/bilayer self assembly system?

*DNA and cationic phospholipid liposomes are known to self-assemble to ordered complexes:

Driven by electrostatic interaction and entropy of ion release.

Application: Delivery systems.

What about NCP or Chromatin array – lipid complex formation?

(See e.g.: Cyrus Safinya et al.)
NCP (Chromatin) complexes with cationic lipids?

**Fluorescence labelling:** Histone protein (green), DNA (blue), Lipid (red)

DOTAP-NCP at Lipid/NCP= CR=0.5: *Co-localization of lipid, DNA and histones*

Dissociation of histones from DNA to Lamellar complex with repeat, $d_L \sim 6$ nm, with (dis)ordered DNA ($d \sim 3.5$ nm) between. But where are histones?

**Cryo-EM, DOTAP-NCP, CR=0.3**

**Synchrotron X-Ray (SAXS), DOTAP-NCP**
ACKNOWLEDGEMENTS

Nikolay Korolev (NTU)
Chenning Lu (NTU)
Nikolay Berezhnoy (NTU)
{Yu Hang} (NTU)
Yan Jiang (NTU)
{Dandan Huang} (NTU)
Ying Liu (NTU)
Abdollah Allahverdi (NTU)
Ye Yang (NTU)
Sasha Lyubartsev (Stockholm U)

Chuan-Fa Liu (NTU)
Yuguang Mu (NTU)
James Tam (NTU)
Curt Davey (NTU)

Liang Dai (NUS)
Johan van der Maarel (NUS)
Jie Yan (NUS)

Singapore Biomedical Research Council (A*STAR)
Singapore Ministry of Education (MOE)

Dan Lundberg (Coimbra)
Maria Miguel (Coimbra)
Björn Lindman (Lund)
Viveka Alfredsson (Lund) (Lund)
1. Compaction: DNA, Nucleosome and Chromatin

**Human genome**

- $= 2 \text{ m DNA}$
- $= 46 \text{ chains of length } \approx 4 \text{ cm}$

$46 \text{ Random coil: } \varnothing = 100 \mu m$

**Cell nucleus**: $\varnothing \sim 10 \mu m$

**Chromatin** is condensed nucleosomes

**Nucleosome** is DNA wrapped around histone octamer protein

The central part of the nucleosome is the *Nucleosome Core Particle (NCP)*
Histone tail modifications of chromatin *in vivo*

Chromatin folding likely regulated by acetylation (quench lysine positive charge) / deacetylation.

Acetylation correlates with transcriptional activity.

Histone tail bridging
Malfunction of acetylation enzyme machinery is implicated in cancer and ageing
Production of large quantities of pure and well defined Nucleosome Core Particles (NCP)

Fig. 13. Precipitation of 177 NCP and 147 NCP with ligands in 10 mM Tris-HCl pH 7.5. C_{DNA} are 162 μM for 177 NCP and 150 μM for 147 NCP. C_{ligand} plotted versus remaining absorbance in NCP-ligand mixture after centrifugation.
Partial aggregation of tail-less NCP by divalent ions:
Salt concentration at maximum $s_{20,w}$ values for various cations.

<table>
<thead>
<tr>
<th>Cation</th>
<th>Conc. (mM)</th>
<th>$s_{20,w}$ (S)</th>
</tr>
</thead>
<tbody>
<tr>
<td>K$^+$</td>
<td>100</td>
<td>46</td>
</tr>
<tr>
<td>Na$^+$</td>
<td>100</td>
<td>56</td>
</tr>
<tr>
<td>Mg$^{2+}$</td>
<td>1</td>
<td>53</td>
</tr>
<tr>
<td>Ca$^{2+}$</td>
<td>0.8</td>
<td>56</td>
</tr>
<tr>
<td>Co(NH$_3$)$_6^{3+}$</td>
<td>0.015</td>
<td>53</td>
</tr>
<tr>
<td>Spermidine$^{3+}$</td>
<td>0.06</td>
<td>53</td>
</tr>
<tr>
<td>Spermine$^{4+}$</td>
<td>0.002</td>
<td>53</td>
</tr>
</tbody>
</table>

![Graph showing $S_{20,w}$ values vs. salt concentration for various cations.](image)

The graph illustrates the relationship between salt concentration (mM) and $S_{20,w}$ values for different cations, showing how the $S_{20,w}$ values increase with increasing salt concentration.
Precipitation Assay Chromatin Arrays

![Graph showing the relationship between absorbance (260 nm) and salt concentration (mM) for various ions: Na^+ (red circles), K^+ (purple squares), Ca^{2+} (green triangles), Mg^{2+} (blue diamonds), Co(NH_3)_6^{3+} (red triangles), Spermidine^{3+} (pink inverted triangles), and Spermine^{4+} (light blue squares).]
Preparation of H4K16Ac

General strategy: Arg19-Lys20 was chosen as the ligation junction
1st step: Ligation to form an Arg19-Cys20 junction
2nd step: Conversion of Cys20 to sLys20 by treatment with bromoethylamine
(Note: sLys is a functionally equivalent analog of Lys).

Reference:
Modelling DNA-DNA attraction: MC Simulation of aggregation of DNA by polyamines

Grand Canonical Monte Carlo simulations of Osmotic Pressure, $P_{\text{osm}}$, at different Spermidine conc.

Flexible polymeric cations also mediate attraction by bridging mechanism!

Spermidine: $\text{NH}_3^+(\text{CH}_2)_3\text{NH}_2^+(\text{CH}_2)_4\text{NH}_3^+$

Spermidine causes attraction at submillimolar concentrations in agreement with experiments!
Polyamine bridging is general: MD simulations in full atomic model

Attraction induced by polyamine bridging between two DNA molecules in atomic simulation with explicit water.

FIG. 1. (a): Top view of the simulation box with two parallel DNA decamers and ten Sm counterions in the initial configuration. The box has a transverse dimension of 7×7 nm$^2$ and 3.4 nm height. (b): Snapshot illustrating ion bridge formation.

Tail bridging between NCP

Mechanism:
Mg-Induced screening of repulsion and attractive ion-ion correlations and entropic gain of tail bridging
Tail-less NCP: RDF Debye-Hückel (DH), I = 33 mM

In DH there is never attraction for tail-less system and aggregation can not occur.

Conclusion: Ion correlation drives attraction in tail-less NCP and must also contribute in the presence of tails.
All-atom Molecular Dynamics (MD) simulations of DNA - DNA interactions Mediated by Histone tails

DNA 22-mer: 

|      | G | A | T | G | C | A | G | T | C | A | C | C | G | C | G | A | A | T | T | G | G | C |
| L    | C | T | A | C | G | T | C | A | G | T | G | C | G | C | G | C | T | T | A | A | C | C | G |

Tail-1: 

Tail-2: 

No-Tail: 

H4 tail: 

K+ 84 (126 in the absence of tails)

H2O 9430
All-atom explicit water MD Simulations of DNA - DNA Interactions Mediated by Histone Tails

DNA and KCl only

DNA and Histone H4 peptide tails fragments (3+ charge)

Histone Tails Make DNA-DNA bridges!

Distribution of average DNA-DNA distances in the simulations demonstrates aggregation in the presence of tails, but repulsion without tails.

Particles:
\[ 1 + 88 + 25 + 5 = 119 \]

Core: \( r=33\, \text{Å}, \ q=+62\, e/0\, e \)
Tail: \( q=+1\, e \)
First bead of tail is constrained to the core.
\( Q(\text{Core+Tail}) = 62 + 88 = +150e \)

DNA: \( r=8\, \text{Å}, \ q=-12\, e \) (or
25 beads are constrained to the core. 5 beads are outside NCP.
\( Q(\text{DNA}) = (25+5) \times (-12) = 30 \times -12 = -360e \)

Weak bonds linking tail beads or DNA beads.
Outside-NCP DNA beads subjects bending potential to keep non-electrostatic persistence length of DNA and make linker close to straight.
No torsion potential between beads.
Charge per NCP
\[-360 + 150 = -210e \]
Total charge of the array
\[-210 \times 12 = -2520e \]
Gy: 20.754
H2T: 51.54nm
0.77mM MgCl2

Gy: 36.0776
H2T: 112.486
0.77mM KCl

Gy: 15.49
H2T: 37nm
70% titration of 0.256mM [Co(NH3)6]Cl3
Sphere-Bead and Sphere-Bead-Array Model (Current)

Energy

\[ U_{short}(r_{ij}) = 4\varepsilon \left( \frac{\sigma}{r_{ij} - \text{offset}} \right)^{12} - \left( \frac{\sigma}{r_{ij} - \text{offset}} \right)^{6} + \text{shift} \]  \quad \text{if } (r_{ij} - \text{offset}) \leq \text{cutoff}

\varepsilon = 1
\sigma = 4
\text{cutoff} = 1.2246 \times \sigma
\text{shift} = 0.25 \times \varepsilon

No repulsive: when \( r - \text{offset} = \text{cutoff}, U = 0 \).

Effective Radius of Particles

- Core = 33
- DNA Bead = 8
- Tail Bead = 3
- Cl = 2
- K = 2
- Mg = 2.5
- Co = 3.5
Polyelectrolyte - Lipid/Surfactant self assembly is general:

*Charged rod-like f-actin, virus particles, etc form ordered complexes with lipids.

Neg. charged liposomes also form aggregates (need M^{2+}).

*Gerard C. L. Wong et al.
Relevance:
Chromatin organization and dynamics in the nucleus

At interphase *chromatin* is separated from the double membrane *Nuclear Envelope (NE)* by the *lamina* protein network.

The NE and lamina breaks down during cell division. NE is reassembled after mitosis. *Direct membrane – chromatin interaction* is then implicated.
NCP (Chromatin) complexes with cationic lipids?

**Fluorescence Labelling of:**
(C) Histone protein (green), (D) DNA (blue), (E) Phospholipid (red)

100% DOTAP-NCP at Lipid/NCP CR = 1 (isoelectric):

Co-localization of lipid, DNA and histones

Disintegration of histones and DNA to form Lamellar complex with repeat, \( d_L \sim 6 \) nm, with (dis)ordered DNA \( (d \sim 3.5 \) nm \) in between. But where are histones?

Cryo-EM, 100% DOTAP-NCP, CR=0.3

Synchrotron X-Ray (SAXS), DOTAP- NCP