HOW TO MODEL THE CHROMATIN FIBER?
IN VITRO AND IN VIVO PERSPECTIVES

Castro Urdiales, July 2007

Equipe M3V :
Modélisation Multi-échelles de la Matière Vivante

LPTMC université Paris 6

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THE **NUCLEOSOME**: 1.65 turn of DNA (145-7 pb) wound in a left handed superhelix.

Cristallographic structure *Luger et al.*, 1997

Chromatin *in vivo*

- **NCP** ~150 bp
- **linker** ~50 bp

2R~10nm  P~2.4nm
Chromatin \textit{in vivo}

Array of regularly spaced nucleosomes

Cryoelectron microscopy

the 30 nm fiber
Chromatin \textit{in vivo}

A multiscale organization, partitioned and dynamic

The fiber compaction tunes DNA accessibility to the \textit{transcription} machinery

Byrd Corces J Cell Biol 2003
A biophysical approach: Magnetic Tweezers

- Force (\(\text{cte}\)) (pN)
- Rotation (turn)
- Length (~10 nm)
Magnetic Tweezers: DNA response under torsion

rotation – extension

$L (\mu m)$

2.02 pN
0.92 pN
0.45 pN
0.22 pN

Rotation ($Lk$)

Strick et al, 1996
Magnetic Tweezers: DNA response under torsion

Rotation = \( Lk = Tw + \ldots \)
Magnetic Tweezers: DNA response under torsion

**Fuller theorem**
Rotation = $Lk = Tw + Wr$

Strick et al, 1996
Magnetic Tweezers:
DNA+ nucleosome under torsion

+ Histones

?
what is expected:
nucleosome compaction and topology

- Packing ratio of ~7
  - Reduction of the fiber length $z$
    - $L \sim 50$ nm
    - $D \sim 7$ nm

- DNA unwinding of \(~-1\) turn
  - Contribution to the rotation $L_k$
    - Writhe
    - Corresponding twist
      - Signature extensively used in molecular biology
Magnetic Tweezers: DNA+ nucleosome under torsion

+ Histones

⇒ DNA, ΔLk~+1

Nucleosome, ΔLk~ -1
Magnetic Tweezers: DNA+ nucleosome under torsion

+ Histones ➞ DNA, ΔLk=0

Nucleosome, ΔLk~ -1
Magnetic Tweezers:
DNA+ nucleosome under torsion

+ Histones

Nucleosome, ΔLk~ -1
Chromatin fiber: experimental construct

Force

Rotation

Nucleosome array reconstituted on a 36 tandemly repeated nucleosome positioning sequence (208 bp)

...with \( n \) of the \( N=36 \) positioning sites occupied
Fiber under torsion

Buffer: Tris-HCl (10 mM) + EDTA (1 mM)

\[ \Delta L \approx -1.3 \, \mu m \]
\[ \Delta L k \approx -24 \, \text{turns} \Rightarrow \approx 24 \, \text{nuc.} \]

\[ \Delta L k \approx 24 \, \text{nuc.} \]

\[ \Delta L \approx 55 \, \text{nm/turn} \]

ok: 1 nucl. \( \sim 150 \, \text{pb} \)
\[ \sim 50 \, \text{nm} \]
Result: the fiber is resilient under torsion

b. **Torsional resilience**

In red: Torsional response of a DNA molecule of the same length as the fiber

**chromatin**: ±20 turns at ~ constant length

bending persistence length 28 nm
torsion persistence length **5 nm**!

(resp. ~60 nm et ~80 nm for DNA)

Challenge: how to model this behaviour?
Modeling tools: the 2 angle model

The Woodcock 2 angle model

- straight (elastic) linkers: $L < L_p$
- rigid nucleosomes
- regular fibers

- The fiber as a composite spring
  - elastic properties:
  - fiber persistence lengths

- Calculation of DNA twist and writhe
  - topological properties of the fiber
  - topological contribution of each nucleosome to $\Delta L_k$

The Model – 1. canonic fibers are not resilient

1. fiber with canonic nucleosomes (crystallographic structure)

In black: torsional response of a fiber equivalent elastic rod

Impossible to fit the torsional response curve
The Model – 2. the three states of the nucleosome

equilibrium coexistence of **three nucleosome configurations**

<table>
<thead>
<tr>
<th></th>
<th>C-</th>
<th>O</th>
<th>C+</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\Delta Lk$ (turns)</td>
<td>-1.4</td>
<td>-0.7</td>
<td>0.4</td>
</tr>
<tr>
<td>$\Delta G^\circ$ (kT)</td>
<td>0.8</td>
<td>0</td>
<td>3.6</td>
</tr>
</tbody>
</table>

Negatively crossed $\alpha=45^\circ$

open $\alpha=-34^\circ$

Positively crossed $\alpha=30^\circ$+inversion

Biochemical experiments and cryomicroscopy on minicircles

(De Lucia et al., 1999, Sivolob et al., 1999)
The Model – 3. A composite fiber

<table>
<thead>
<tr>
<th>state</th>
<th>Negative</th>
<th>Open</th>
<th>Positive</th>
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</thead>
<tbody>
<tr>
<td>α</td>
<td>54°</td>
<td>-30°</td>
<td>30°</td>
</tr>
<tr>
<td>β</td>
<td>115°</td>
<td>90°</td>
<td>115°</td>
</tr>
<tr>
<td>Δl (nm)</td>
<td>4.1</td>
<td>8.3</td>
<td>2.8</td>
</tr>
<tr>
<td>ΔLk minicircle (turn)</td>
<td>-1.4</td>
<td>-0.7</td>
<td>-0.4</td>
</tr>
<tr>
<td>ΔEnergy minicircle (kT)</td>
<td>0.8</td>
<td>0</td>
<td>3.6</td>
</tr>
<tr>
<td>ΔLk model (turn)</td>
<td>-1.4</td>
<td>-0.5</td>
<td>-0.4</td>
</tr>
<tr>
<td>ΔEnergy model (kT)</td>
<td>0.7</td>
<td>0</td>
<td>2</td>
</tr>
</tbody>
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A composite fiber:

computed
2 fitting parameters
The role of the applied torque

applied torque favors one of the states by a work contribution $-\Gamma Lk$ to the energy

Free enthalpy = $\Delta G = \Delta F - \Gamma Lk$

torque = 0: negative has minimal $\Delta G$

torque > 0: positive has minimal $\Delta G$
The Model – 4. Statistical Mechanics

coexistence of 3 states:  \( n = n_o + n_n + n_p \)

3 lengths \( z \)

3 numbers of turns \( L_k \)

3x2 persistence lengths
The Model – 4. Statistical Mechanics

coexistence of 3 states: \( n = n_o + n_n + n_p \)

Constraints:
constant torque (the “pression”) and temperature,
varying \( L_k \) (the “volume”)

3 lengths \( z \)
3 numbers of turns \( L_k \)
3x2 persistence lengths
The Model – 4. Statistical Mechanics

**coexistence of 3 states:** \( n = n_o + n_n + n_p \)

Constraints:
- constant torque (the "pression") and temperature,
- varying \( L_k \) (the "volume")

Free enthalpy:
\[
G = F(n_n, n_o, n_p, \Gamma, T) - \Gamma \cdot L_k
\]

\( \Gamma \) = torque, \( L_k \) = torsion

\( F = kT \ln(Z) \) with \( Z \) the standard partition function
The Model – 4. Statistical Mechanics

Coexistence of 3 states: \[ n = n_o + n_n + n_p \]

Constraints:
- Constant torque (the “pression”) and temperature,
- Varying \( L_k \) (the “volume”)

Free enthalpy:
\[ G = F(n_n, n_o, n_p, \Gamma, T) - \Gamma \cdot L_k \]

\( \Gamma = \text{torque, } L_k = \text{torsion} \)

Minimization of \( G \) (at \( T \) and \( \Gamma = \text{cts} \)) \( \Rightarrow \) \( n_n, n_p, n_o \)

\[ \begin{align*}
  z &= z_n + z_o + z_p + z_{\text{DNA}} = z(n, T, \Gamma) \\
  L_k &= L_{k_n} + L_{k_o} + L_{k_p} + L_{k_{\text{DNA}}} = L_k(n, T, \Gamma)
\end{align*} \]

\( \Rightarrow \) Elimination of \( \Gamma \) \( \Rightarrow \) \( z(L_k) \) \( \Rightarrow \) \text{fit}(n)
The Model – 5. fitting the curves

Internal structure reorganization induced by torsional constraints at nearly constant length

\( n \) is the single fitting parameter (once fixed the 2 energy parameters)
back to biology: a functional fiber?

the chromatin fiber can absorb huge torsional constraints while reorganizing its architecture

what functional advantage?

Torque prediction during the three state rearrangement:

The torque remains $< 4 \text{ pN} \cdot \text{nm}$, lower than exerted by RNA polymerase ($\geq 5 \text{ pN} \cdot \text{nm}$, Harada 2001)
back to biology: a functional fiber?

A topological buffer:

100 bp transcribed ⇒ ~±10 tours ⇒
⇒ absorbed by a fiber of ~50 nucleosomes
initially at equilibrium, with low torque, clamped ends
An interdisciplinary collaboration!

<table>
<thead>
<tr>
<th>Institut Curie</th>
<th>Institut J. Monod</th>
<th>LPTMC (Paris 6)</th>
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</table>
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Annick Lesne |
Further torsion… plectonemes

Fiber defects $\rightarrow$ a new mechanism for plectonemes initiation:

the “mini-fibers” inversion
Further torsion... hysteresis!

fibers display an hysteretic behavior

• not explained by nucleosome-nucleosome interactions

• Compatible with a nucleosome internal rearrangement:
  transition to a metastable altered state with
  a similar $\Delta L$, a larger (positive) $\Delta L_k$
Further torsion... hysteresis!

a new multiscale simulation tool:

- local DNA curvature/rigidity induced by bind proteins
- DNA path satisfying external geometrical constraints
- rebuilding of the detailed molecular structure ($E_{\text{min}}$)

Thanks to the young people!
⇒ nucleosome is not a tuna can!

inverse kinematic (+ some additional experimental results on “tetrasomes”)

nucleosome to reversome

$\Delta R \approx -0.4$ to $0.9$

Bancaud et al., Mol Cell 2007
⇒ nucleosome is not a tuna can!

Extended model: torque dependent activation barrier from pos. to reversome including breaking of intermolecular interactions + mechanical instability effects...

Free enthalpy = $\Delta F - \Gamma$

$\text{Lk}$

$\text{torque} = 0$: reversome is metastable

$\text{torque} > 0$: reversome is stable
⇒ nucleosome is not a tuna can!

toward a fit: statistical equilibrium between the 3 states + rate equations for the passage to reversome:

\[ \text{eq. } k_1 \text{ eq. } k_{-1} \]

neg. ⇔ open ⇔ pos. ⇔ reversome

\[ \Delta \]
②. Further torsion induces a nucleosome “opening”  

(transition to a metastable reversome where intermolecular interactions are broken)

many models exist on how the polymerase passes through nucleosomes during transcription..
Further torsion induces a nucleosome “opening” where intermolecular interaction are partially broken, this can help polymerase to pass through:

reversome as a torsion driven activated nucleosome?
the α angle depends on the DNA wrapping onto NCP

~ 54° in the crystallographic “canonical” structure

the β angle depends on the number of bp and torsion of the linker

~ 360° / 10.5 bp at Tw=0