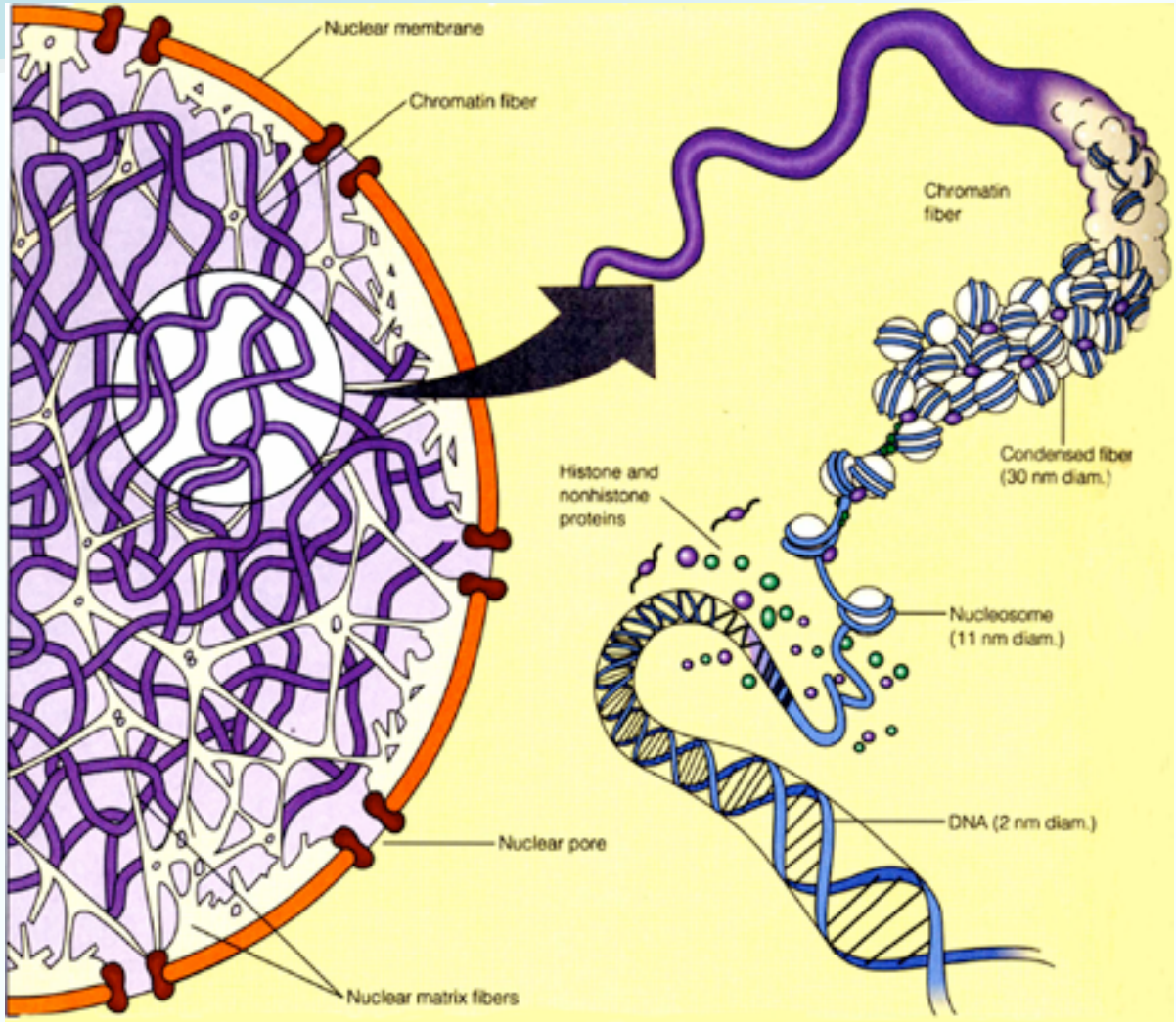
HOW TO MODEL THE CHROMATIN FIBER?
IN VITRO AND *IN VIVO* PERSPECTIVES



Castro Urdiales, July 2007

Equipe M3V : Modélisation Multi-échelles de la Matière Vivante

LPTMC université Paris 6

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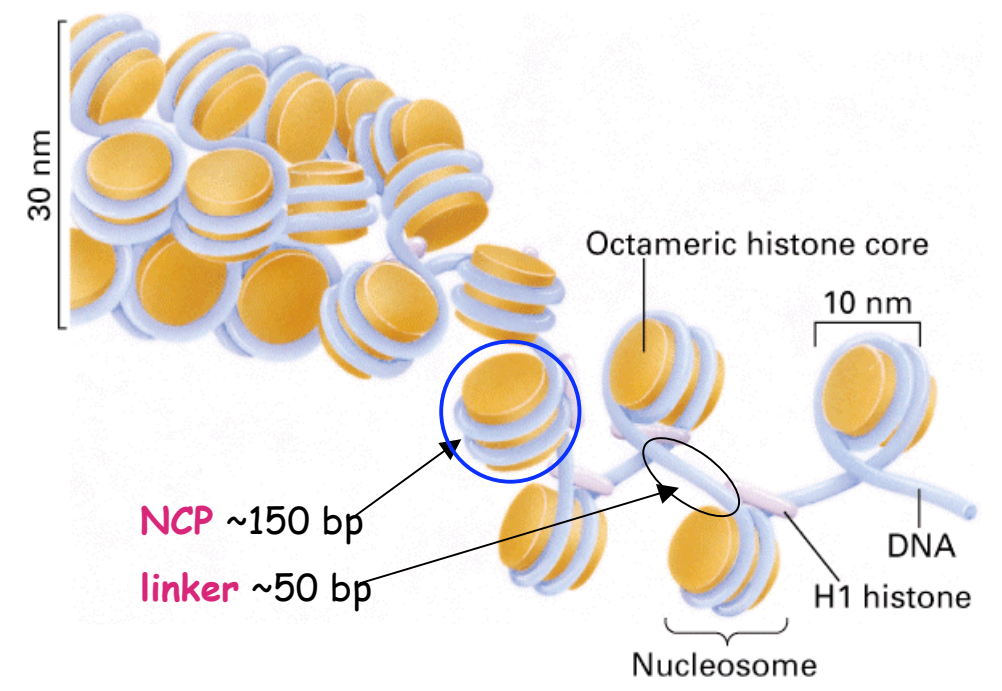
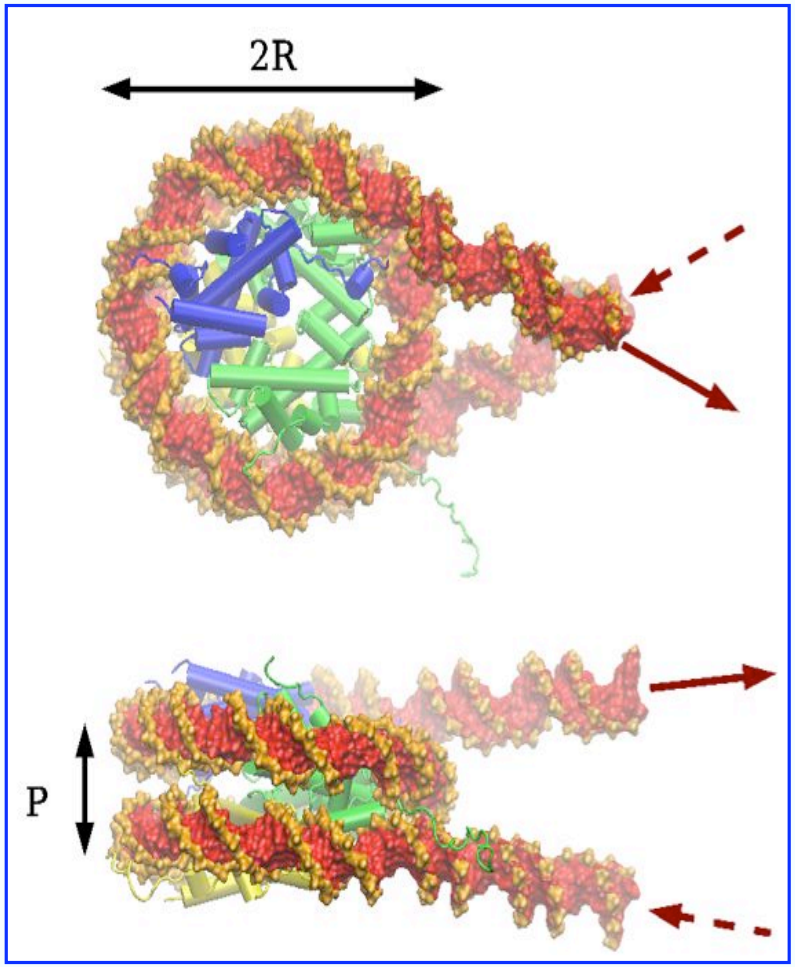
Chromatin *in vivo*

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



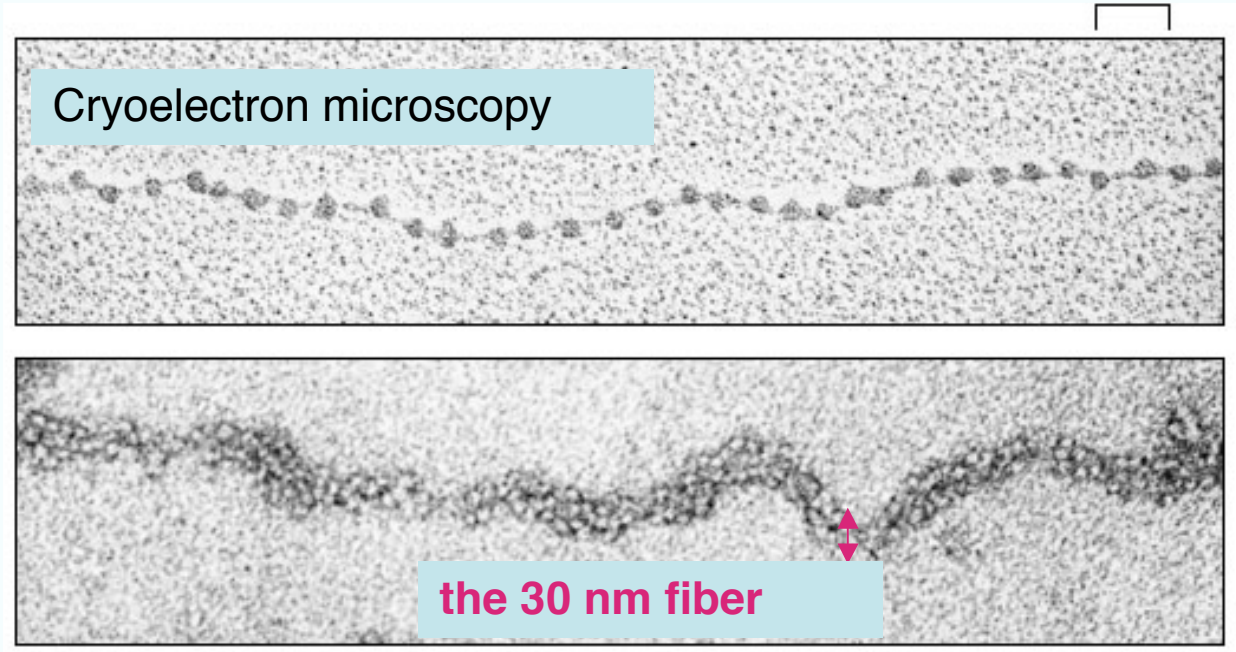
2R~10nm P~2.4nm

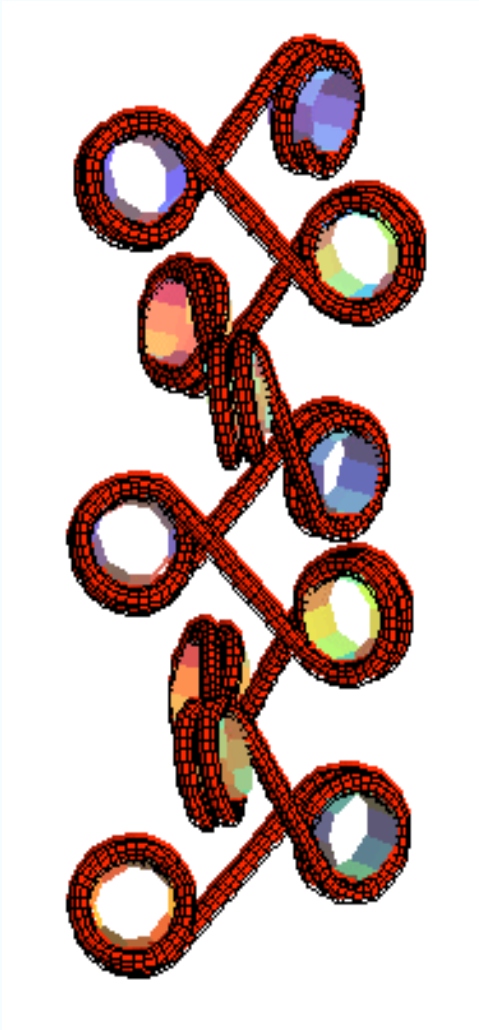


Cristallographic structure Luger et al., 1997

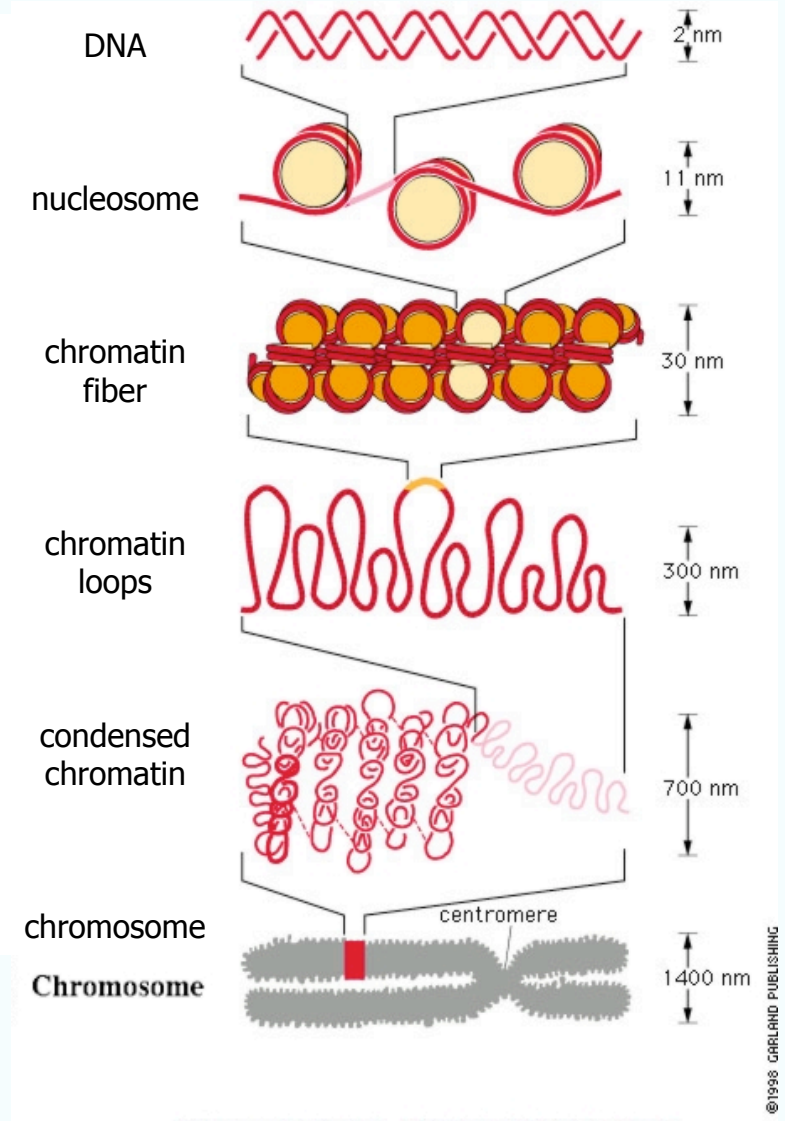
Chromatin *in vivo*

Array of regularly spaced nucleosomes



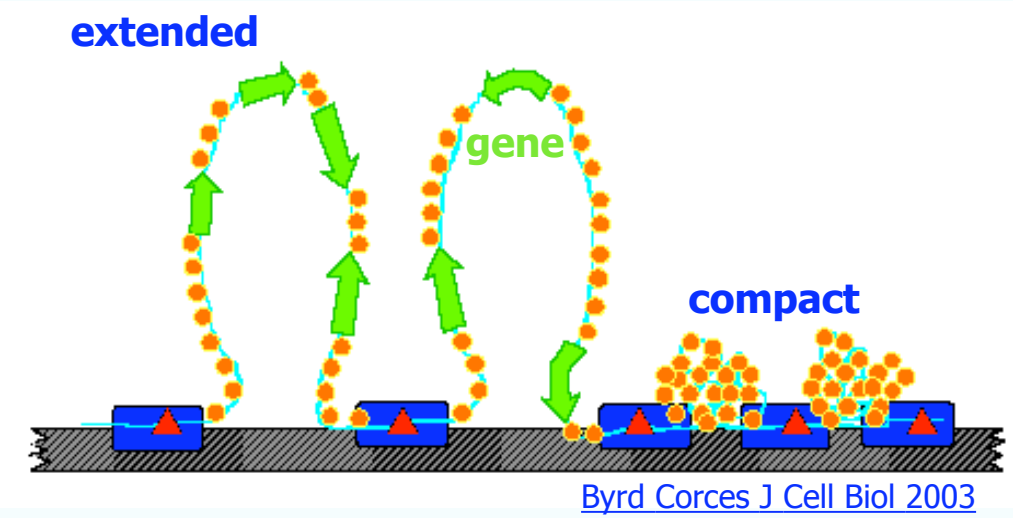


Chromatin *in vivo*



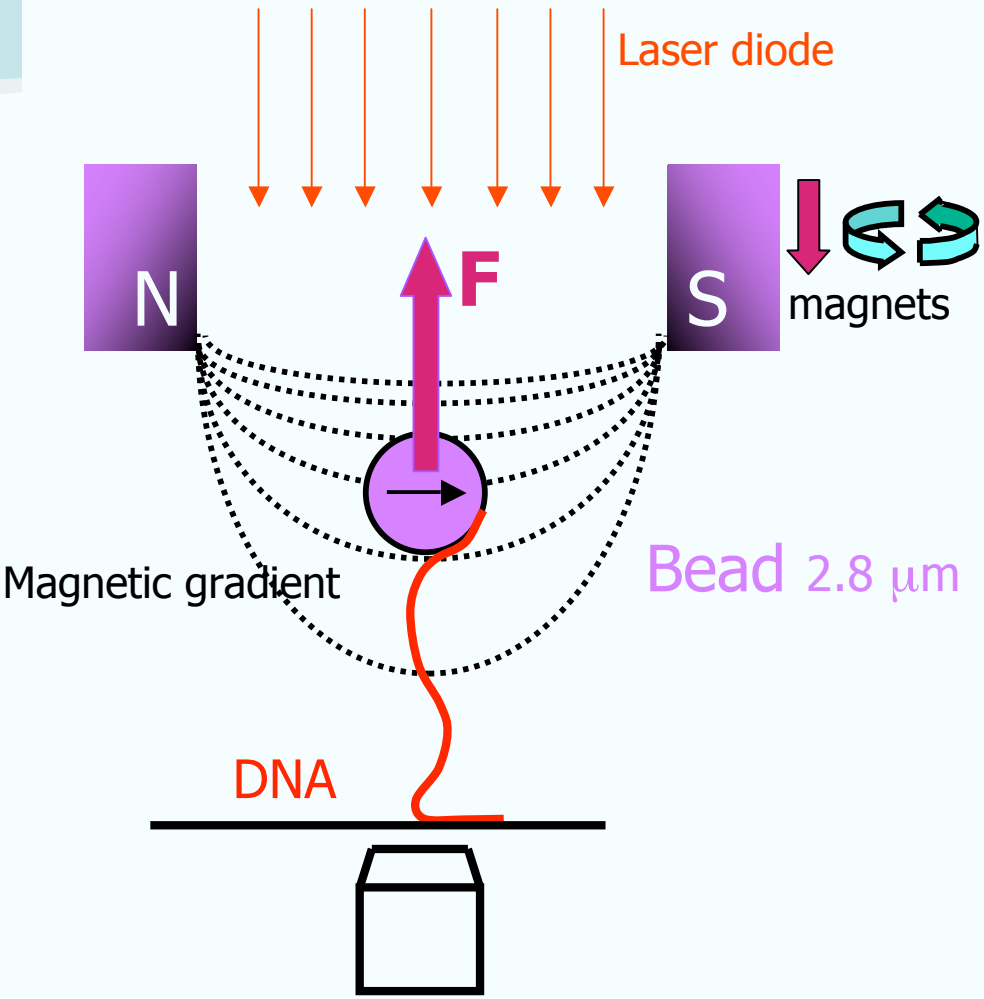
A multiscale organization,

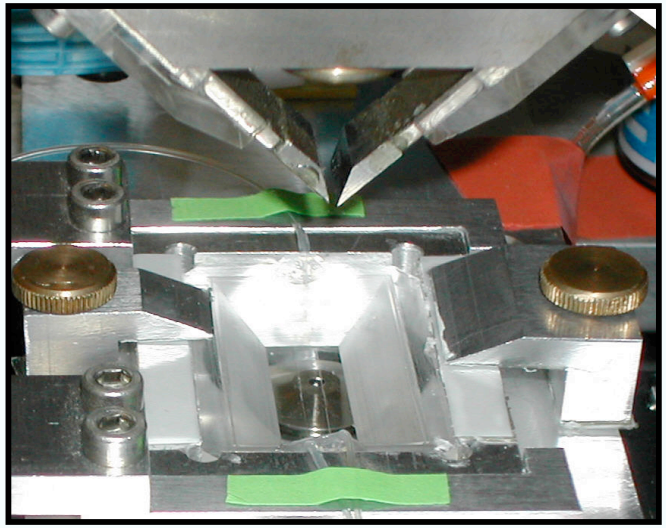
partitioned and dynamic



The fiber compaction tunes
DNA accessibility
to the **transcription** machinery

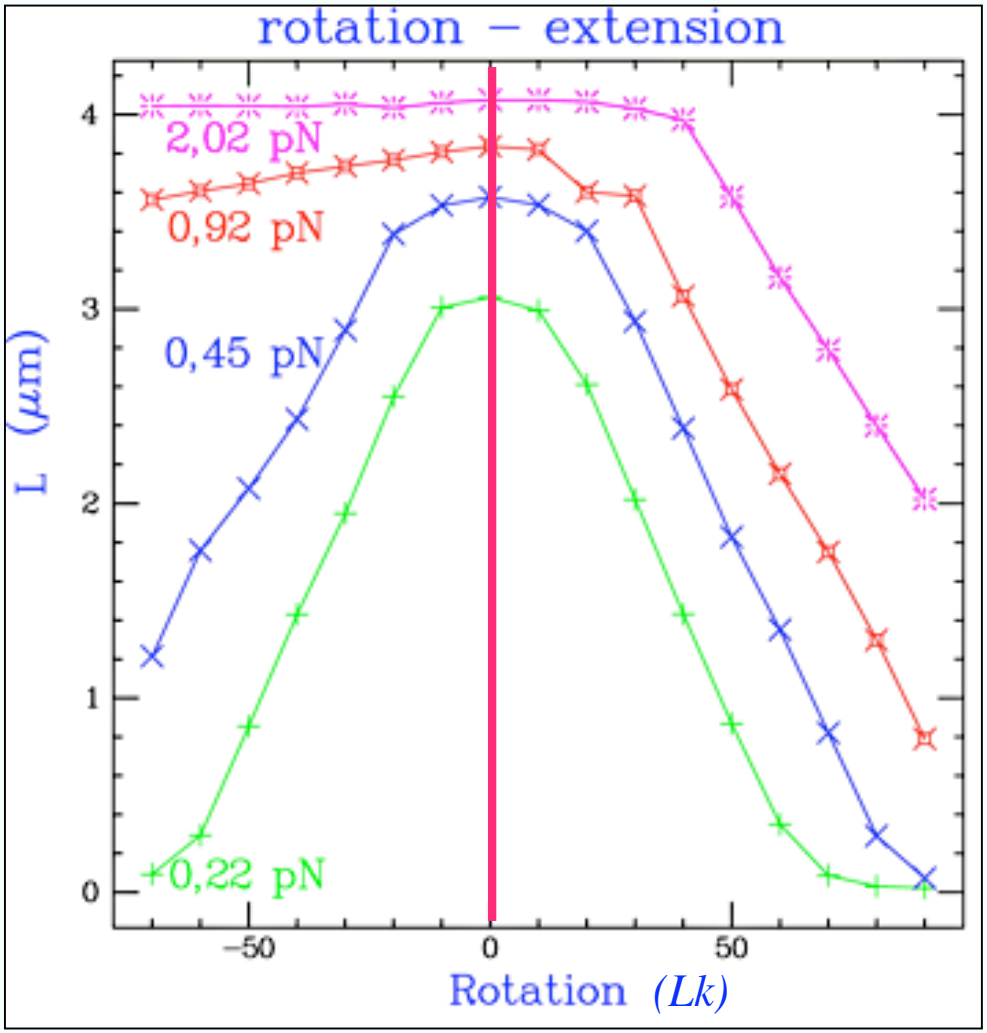
A biophysical approach: Magnetic Tweezers

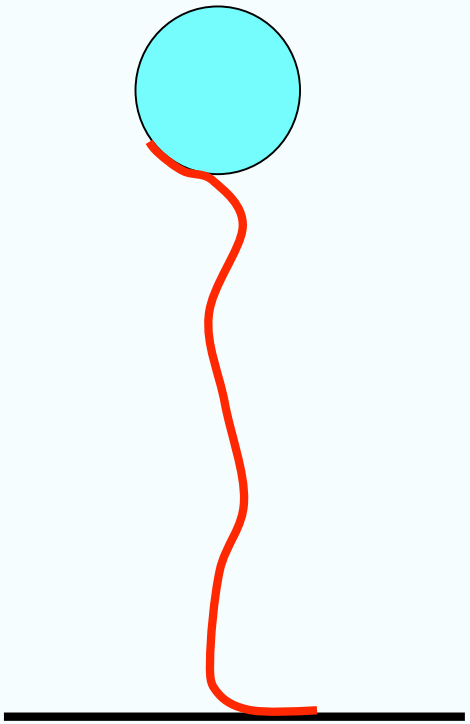




- Force (cte) (pN)
- Rotation (turn)
- Length (~10 nm)

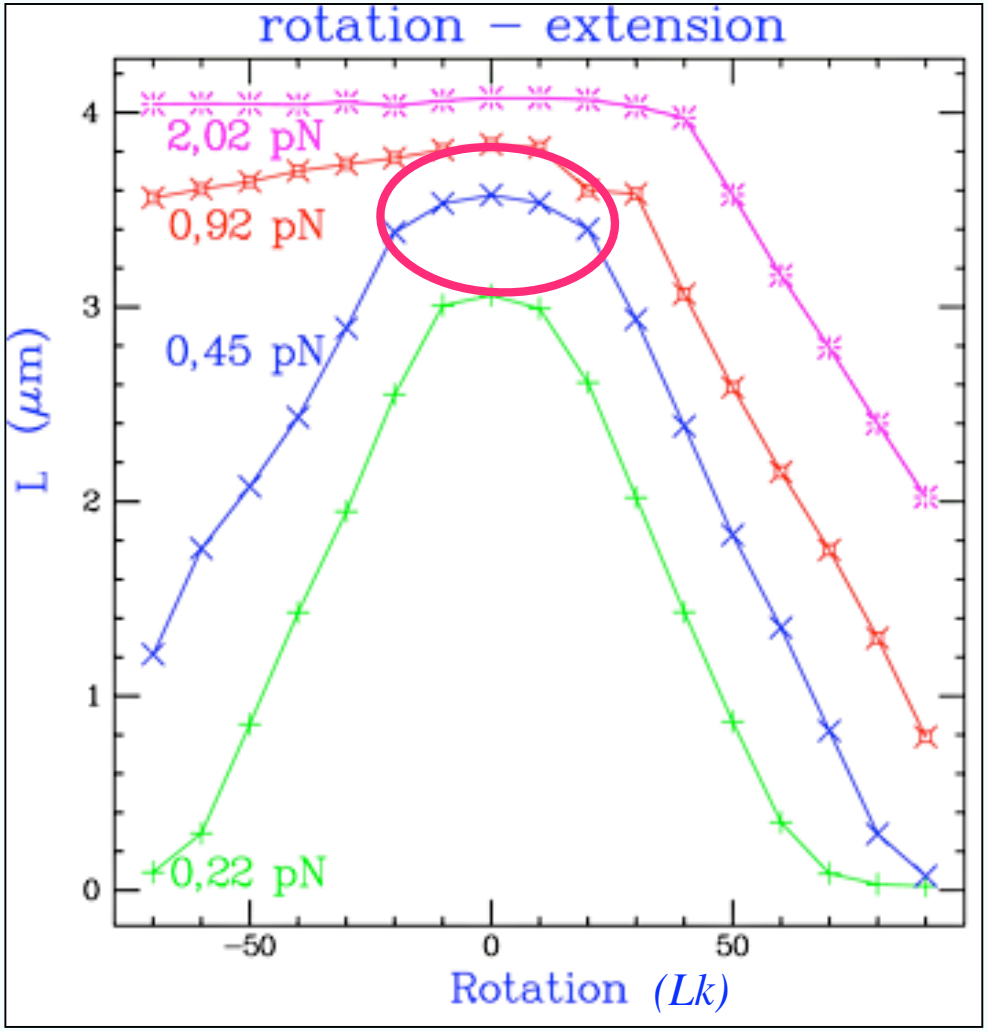
Magnetic Tweezers : DNA response under torsion



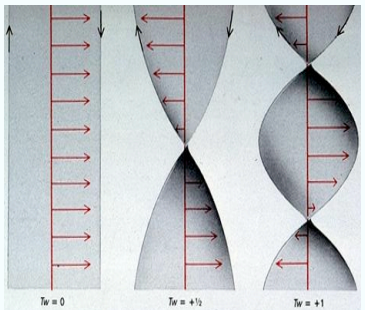


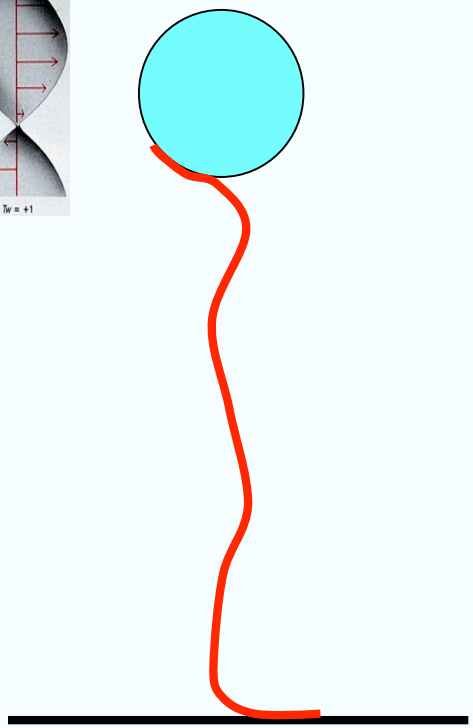
Strick et al, 1996

Magnetic Tweezers : DNA response under torsion



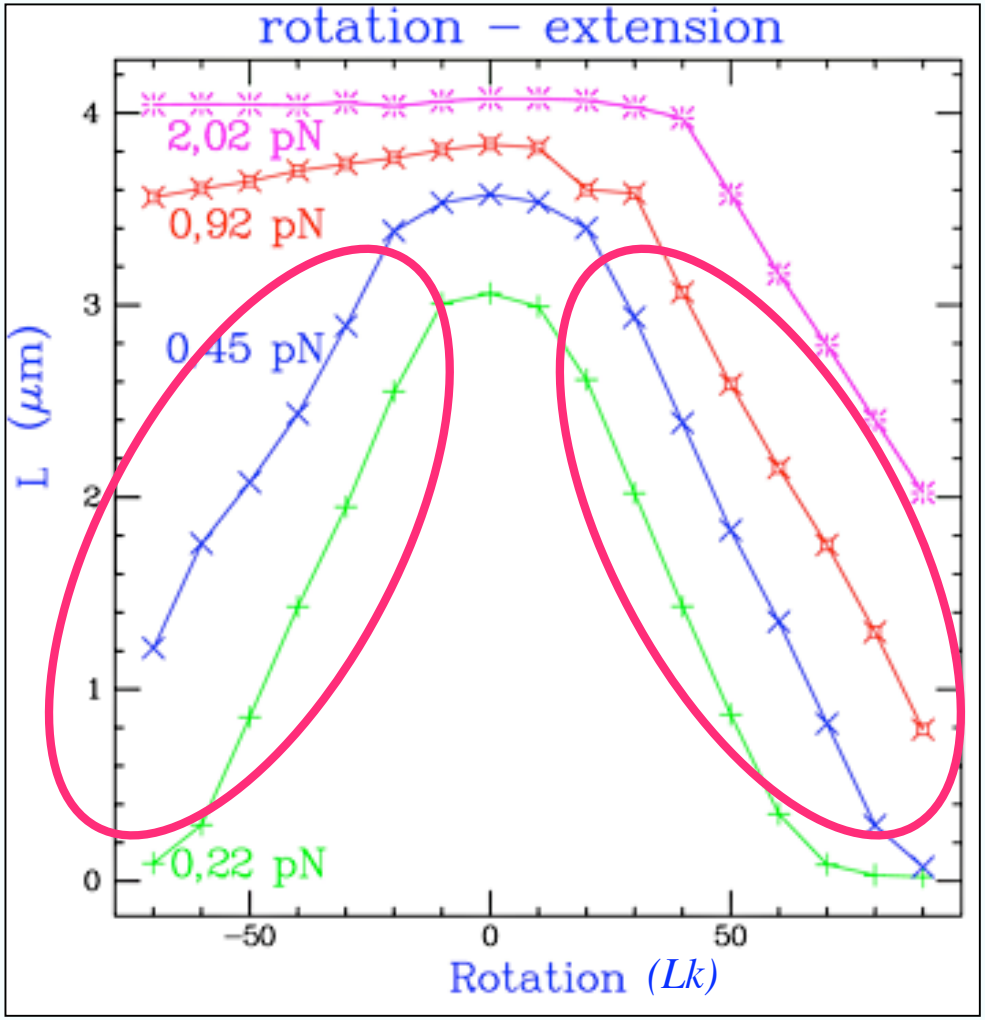
Rotation = ***Lk*** = ***Tw*** + ...





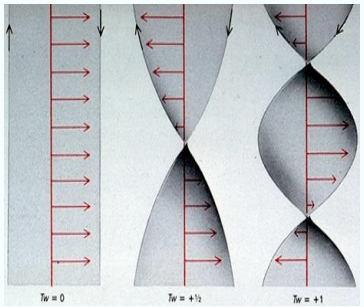
Strick et al, 1996

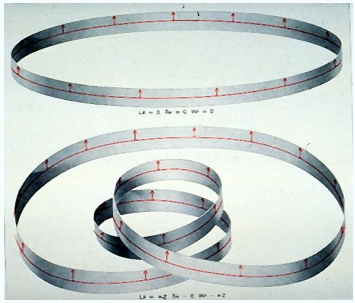
Magnetic Tweezers : DNA response under torsion

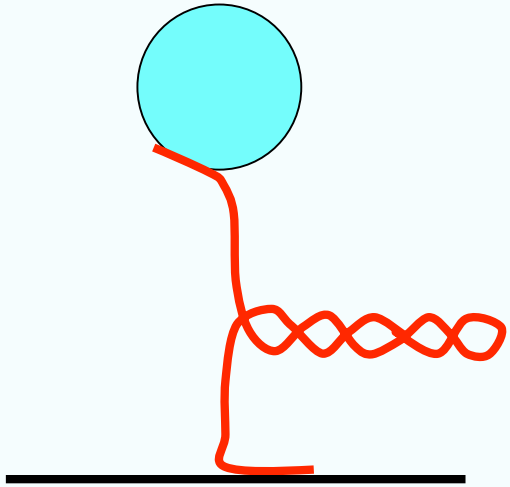


Fuller theorem

Rotation = ***Lk*** = ***Tw*** + ***Wr***

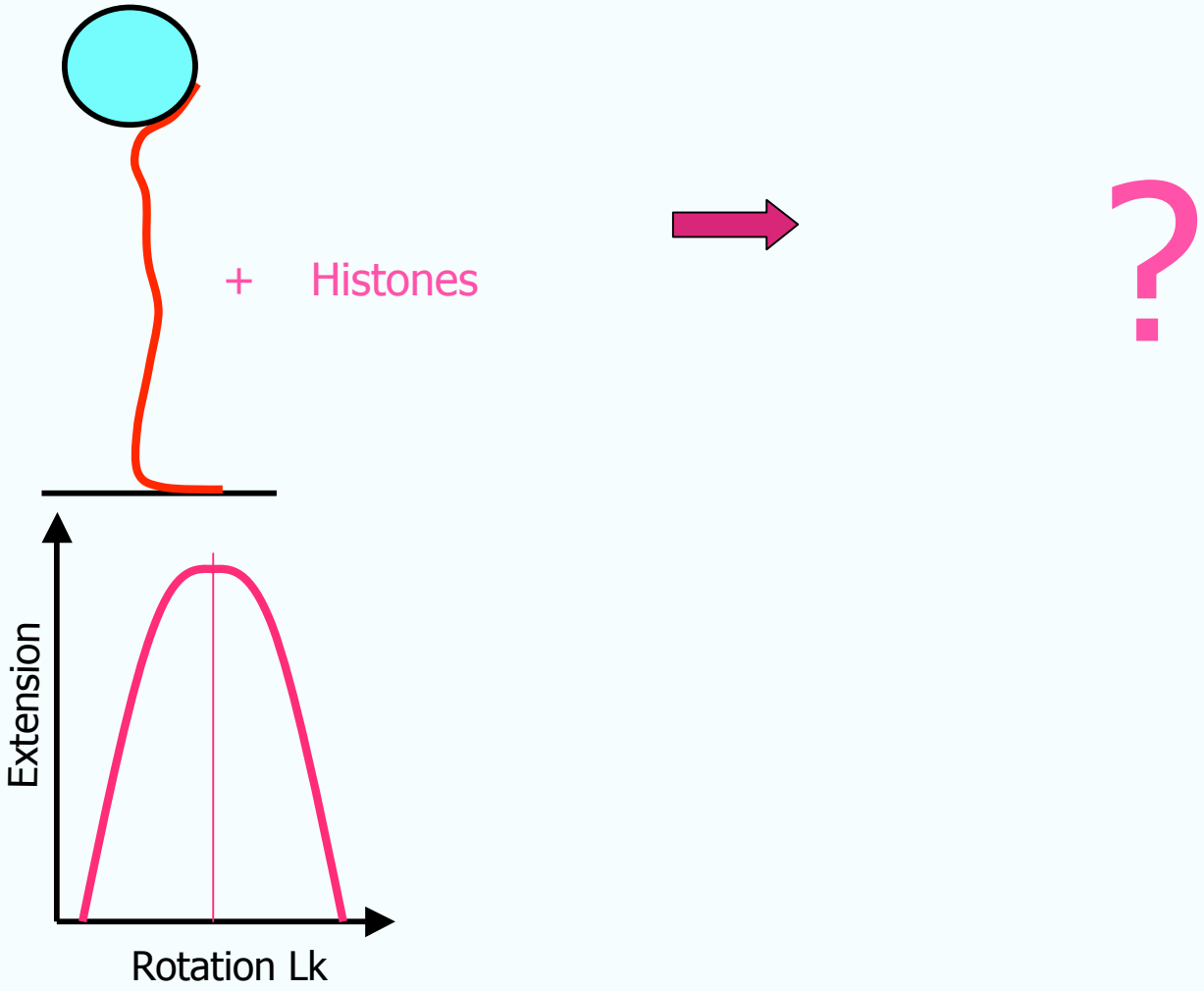






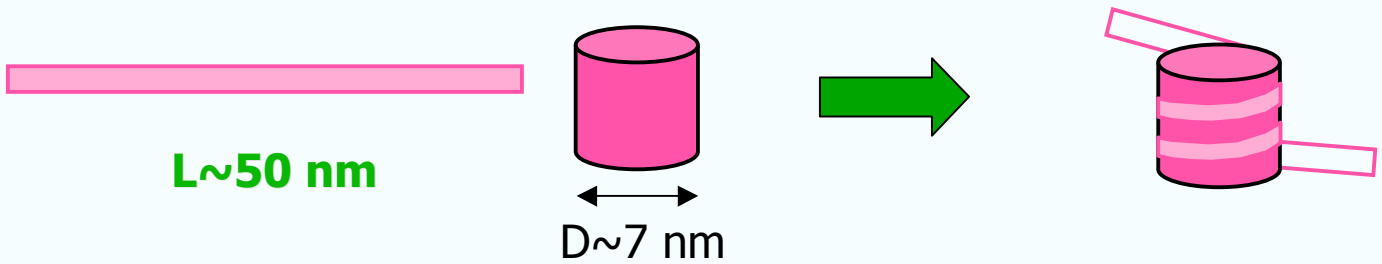
Strick et al, 1996

Magnetic Tweezers :
DNA+ nucleosome under torsion

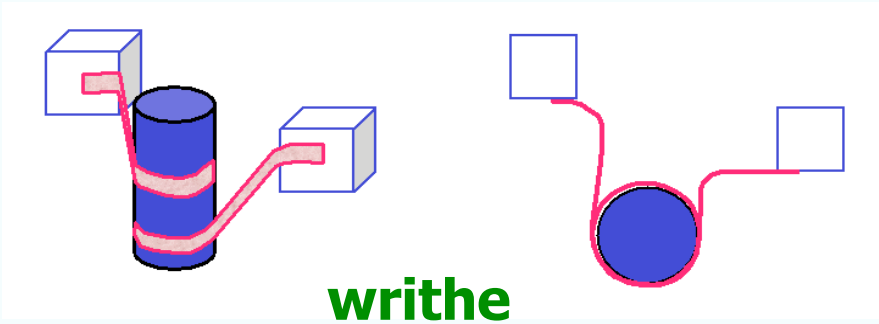


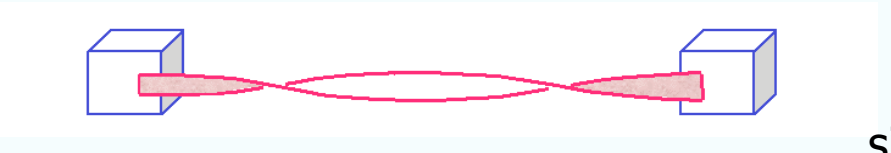
what is expected: nucleosome compaction and topology

- Packing ratio of ~7 ➡ reduction of the fiber length z



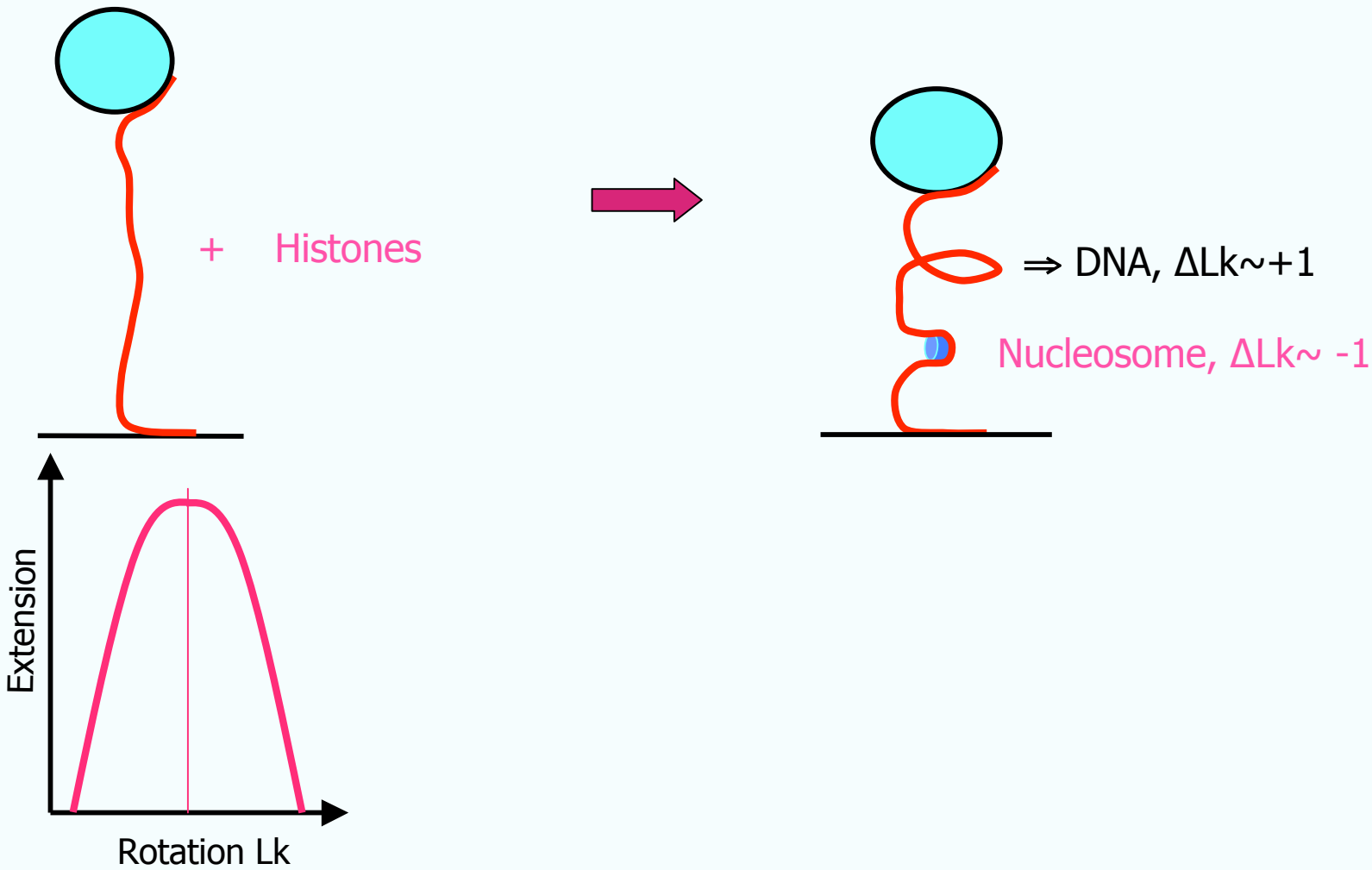
- DNA unwinding of **~-1 turn** ➡ contribution to the rotation Lk



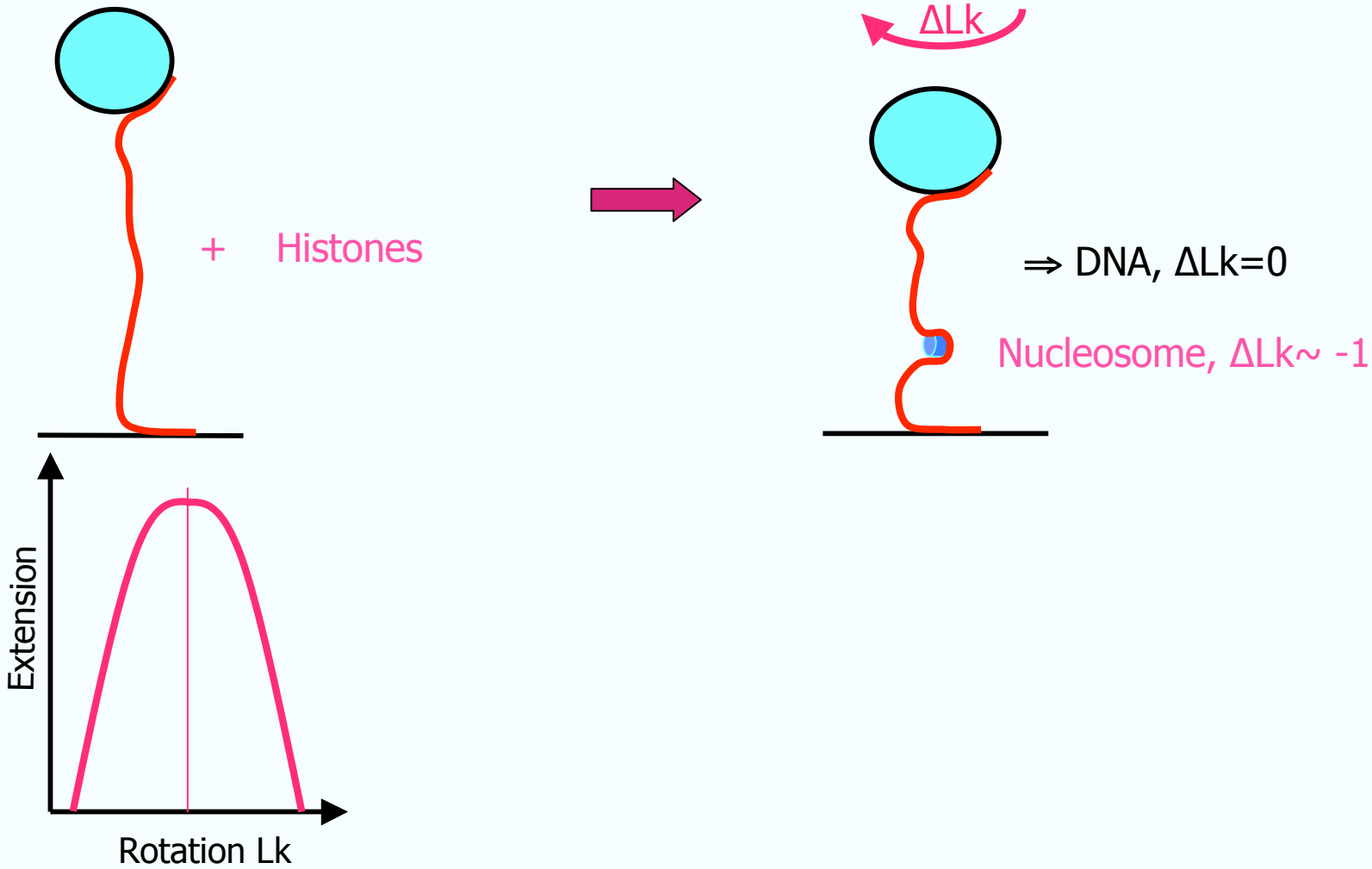


signature extensively used in molecular biology

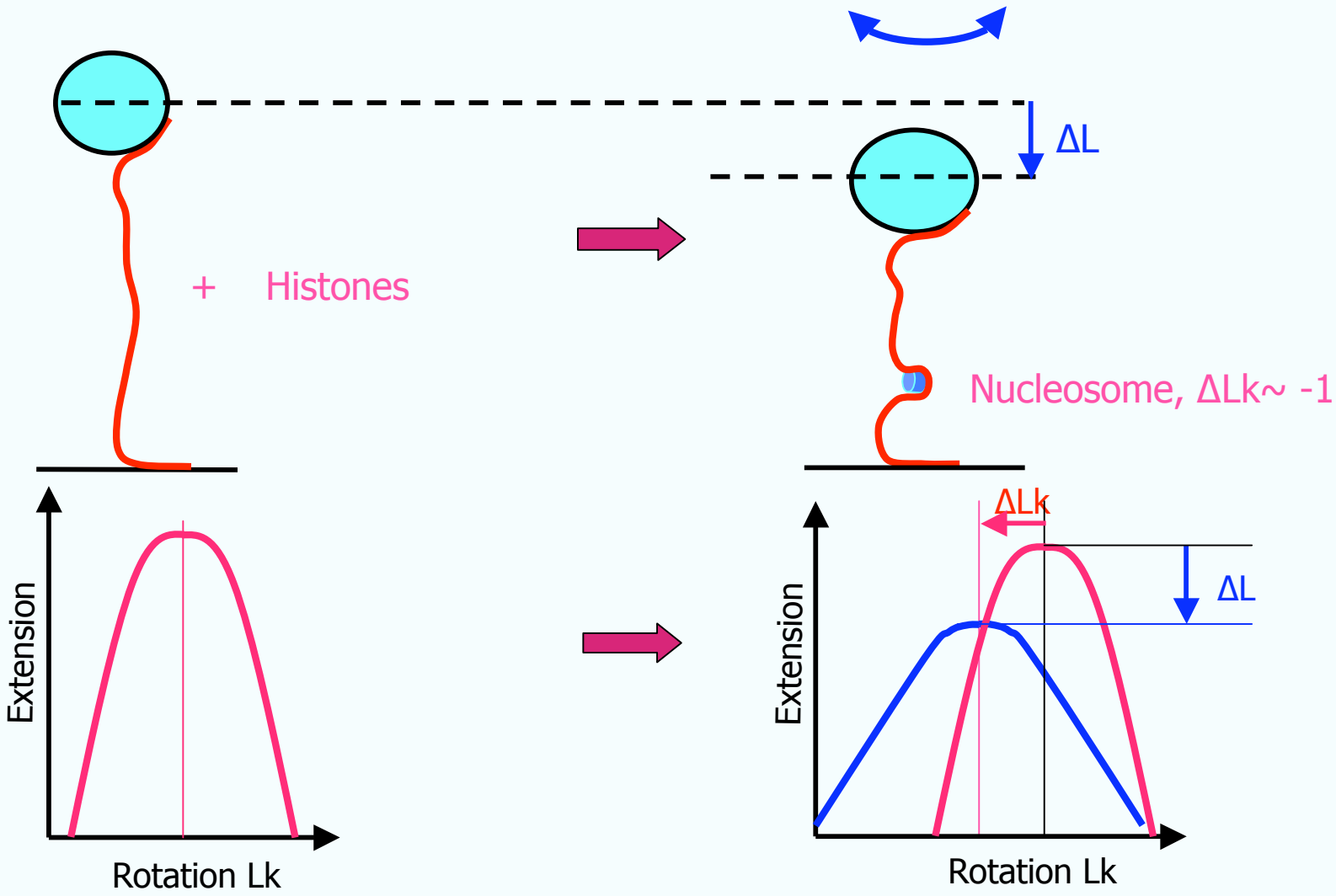
Magnetic Tweezers :
DNA+ nucleosome under torsion



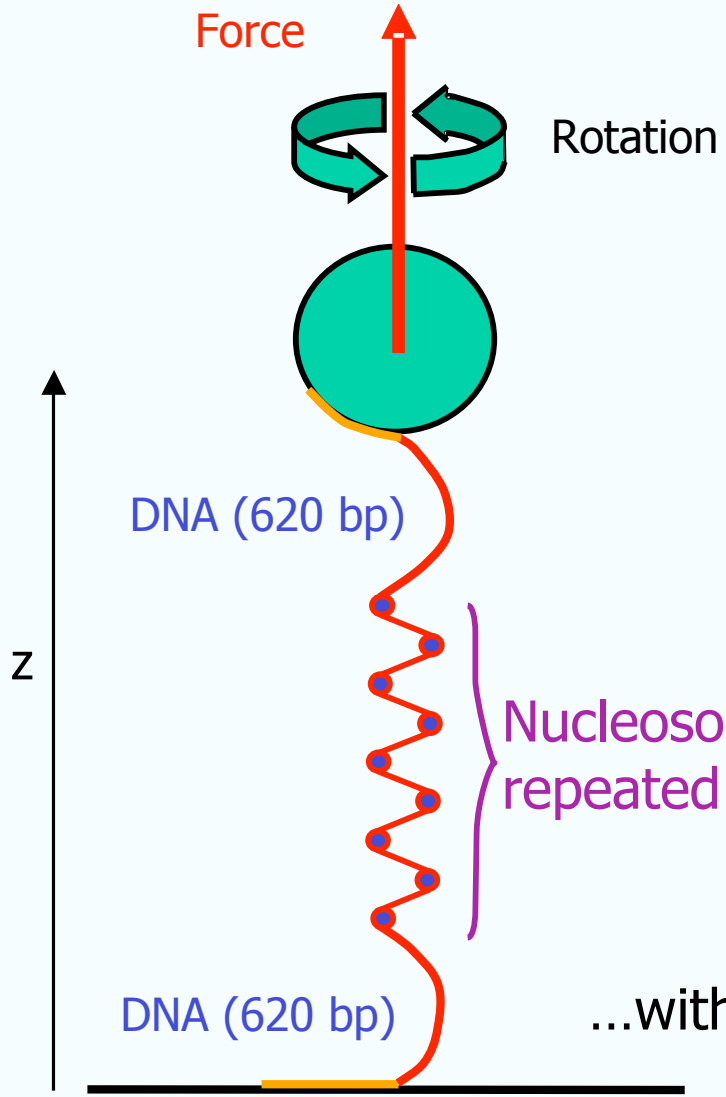
Magnetic Tweezers :
DNA+ nucleosome under torsion



Magnetic Tweezers :
DNA+ nucleosome under torsion



Chromatin fiber : experimental construct

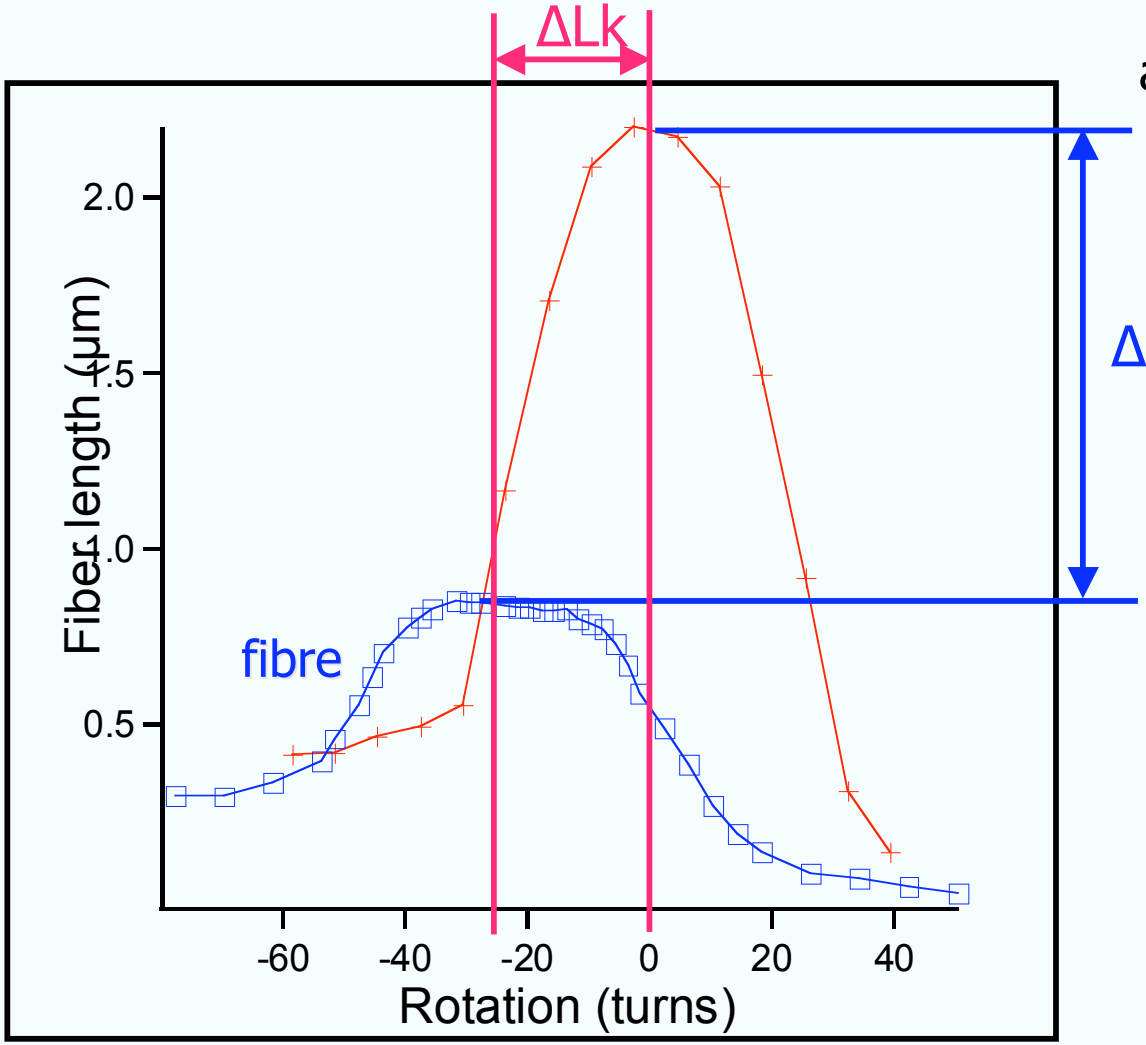


torsional response of a chromatin fiber

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



a. Nucleosomes signature

ΔL

ΔLk ≈ -24 turns ⇒ ≈ 24 nuc.

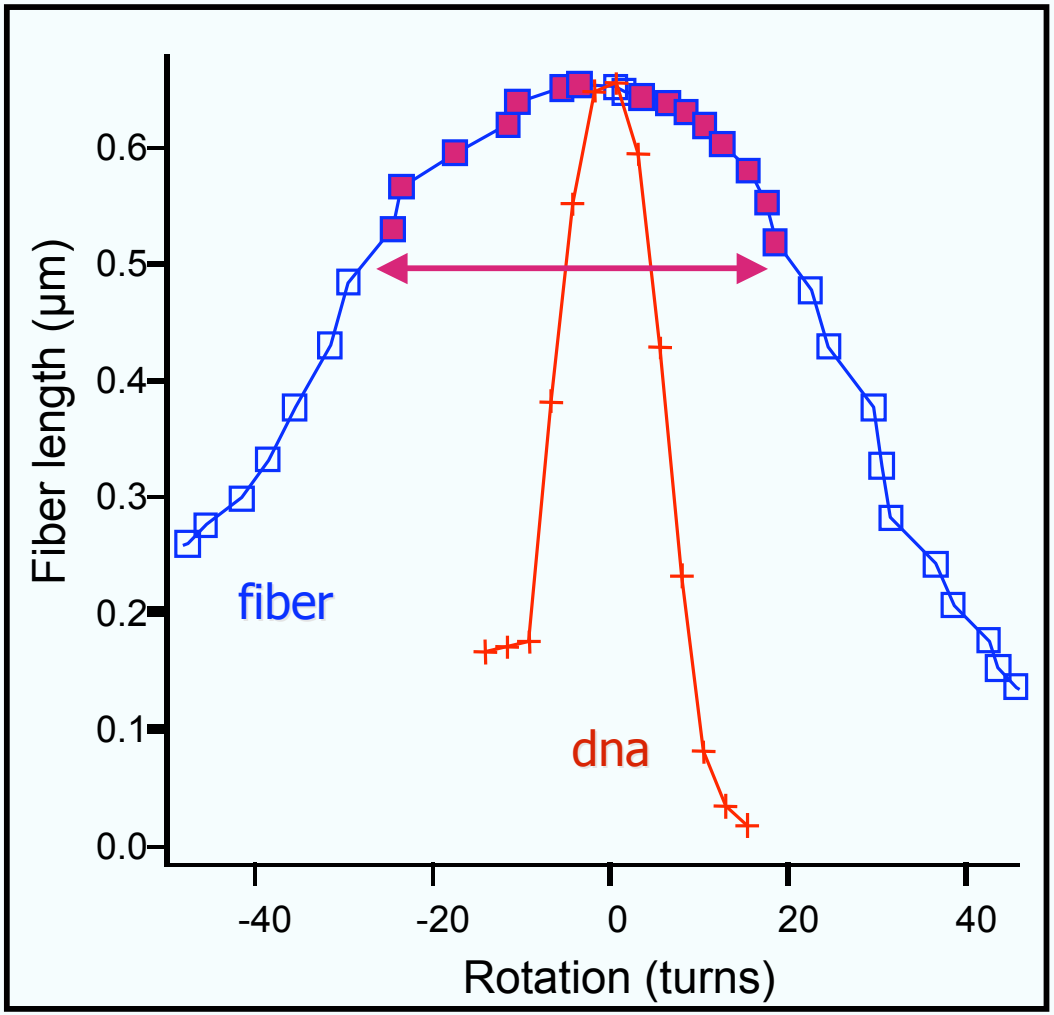
ΔL ≈ -1.3 µm

≈ 55 nm/turn

ok: 1 nucl. ~150 pb
~50 nm

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

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 !**

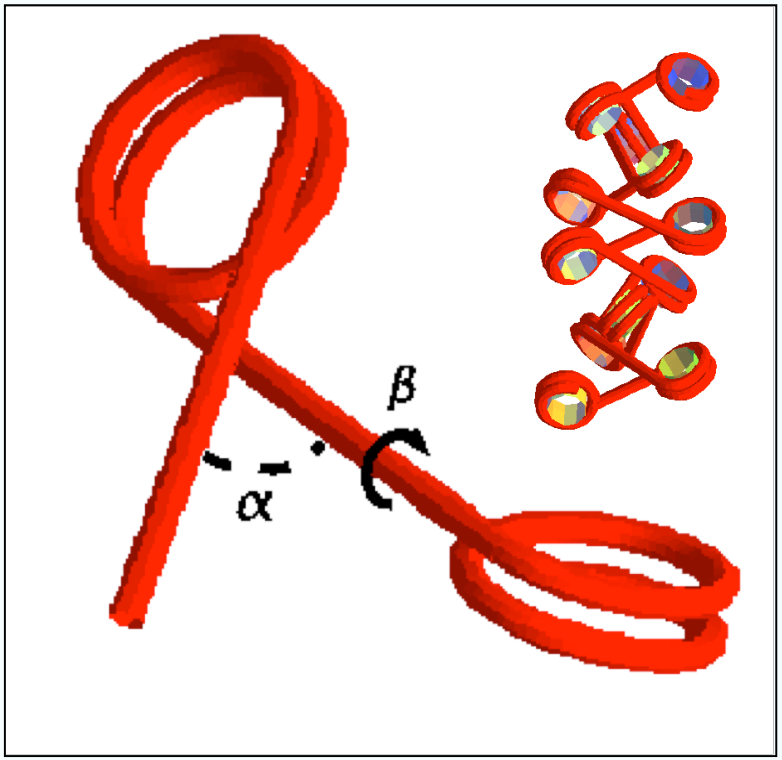
(resp. ~60 nm et ~80 nm for DNA)

Challenge : how to model this behaviour?

Modeling tools: the 2 angle model

The Woodcock 2 angle model

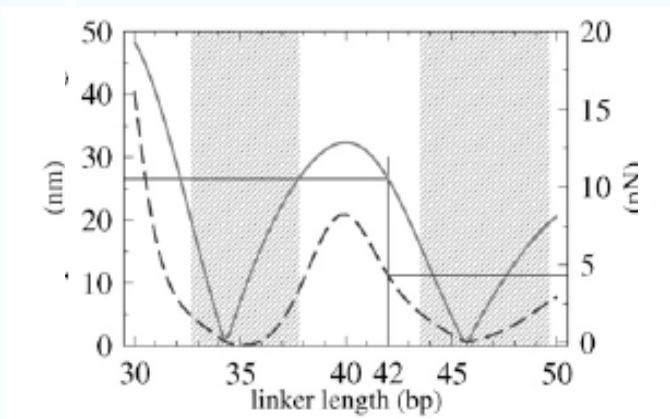
- straight (elastic) linkers : $L<L_P$
- rigid nucleosomes
- regular fibers



• The fiber as a **composite** spring

⇒**elastic properties:**

⇒**fiber persistence lengths**



Ben-Haim, Lesne, Victor, Phys Rev E 2001

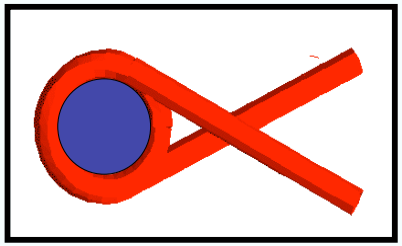
• Calculation of dna **twist** and **writhe**

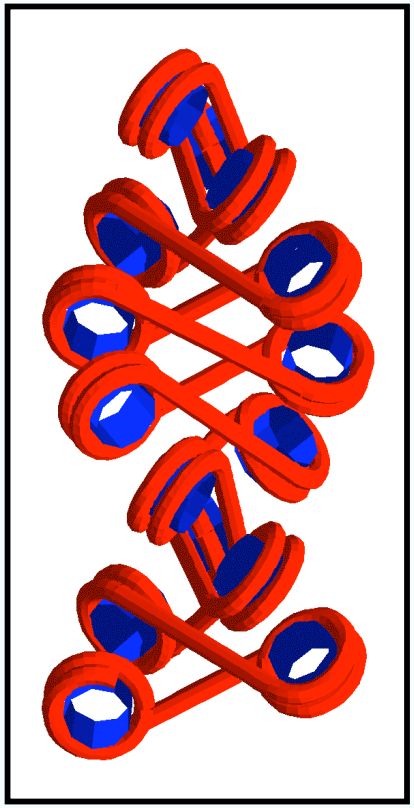
⇒topological properties of the fiber

⇒**topological contribution of each nucleosome to ΔLk**

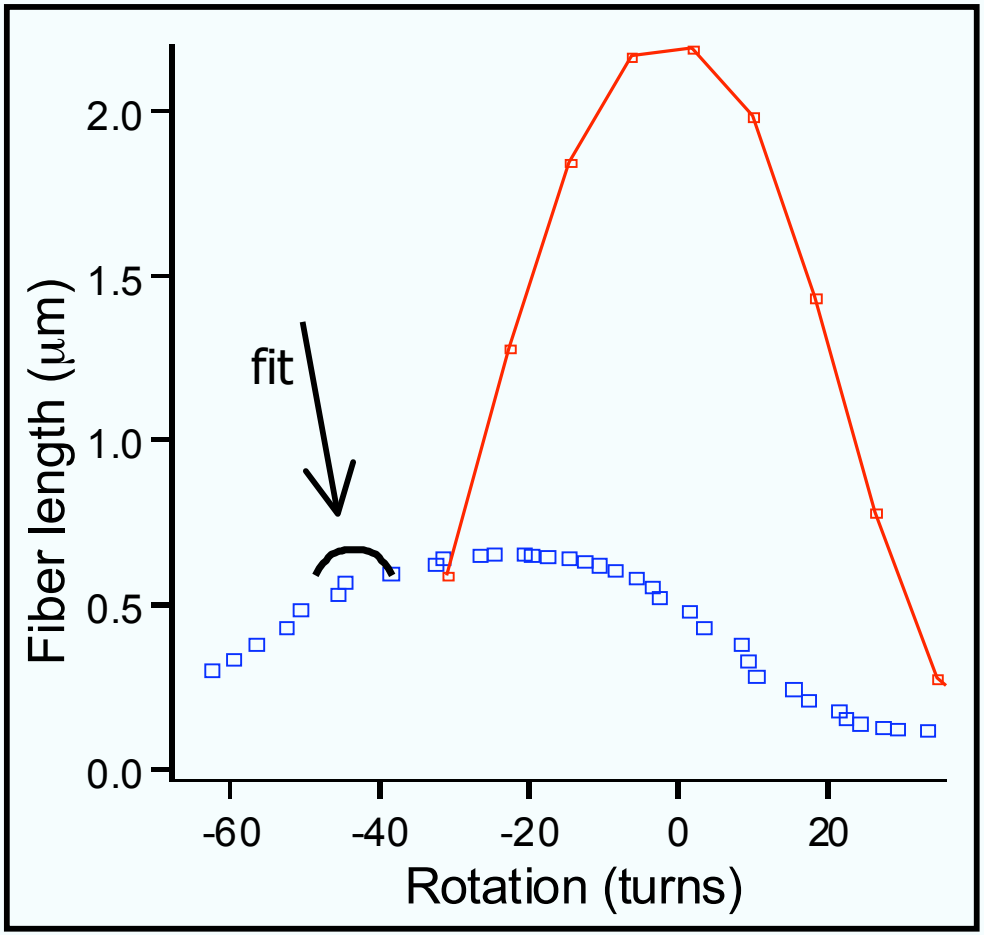
Barbi et al, Phys. Rev. E **71**, 031910 (2005)

The Model – 1. canonic fibers are not resilient





1. fiber with canonic nucleosomes
(cristallographic structure)

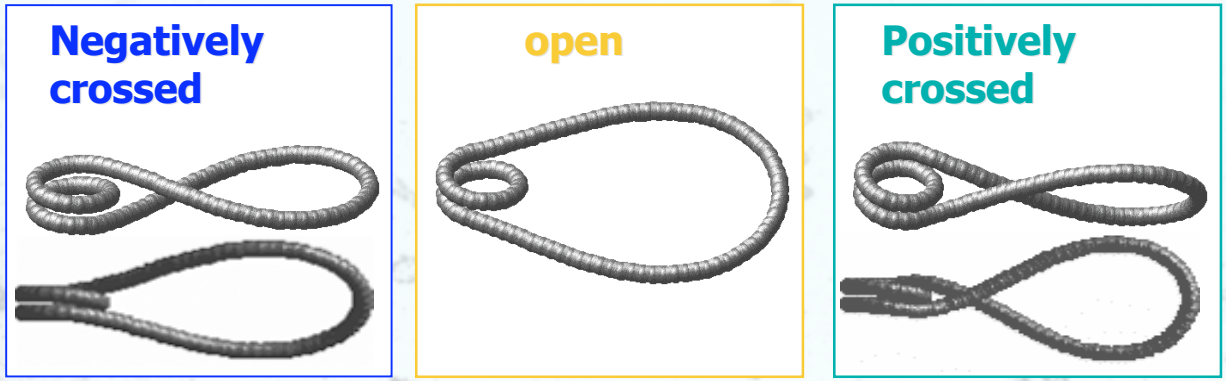


In black: torsional reponse of a fiber equivalent **elastic rod**

Impossible to fit the torsional response curve

The Model – 2. the three states of the nucleosome

equilibrium coexistence of **three** nucleosome **configurations**

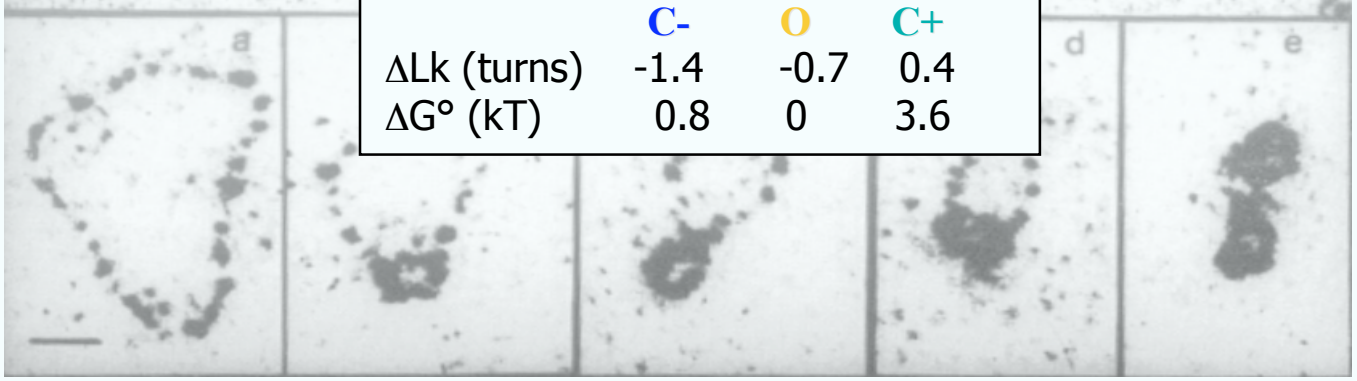


α=45° α=-34° α=30°+inversion

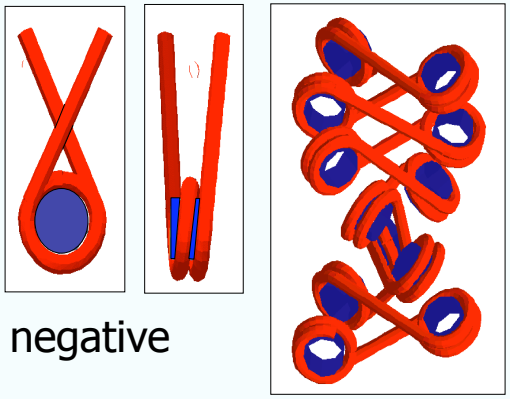
Biochemical experiments and cryomicroscopy on minicircles

(De Lucia et al., 1999, Sivolob et al, 1999)

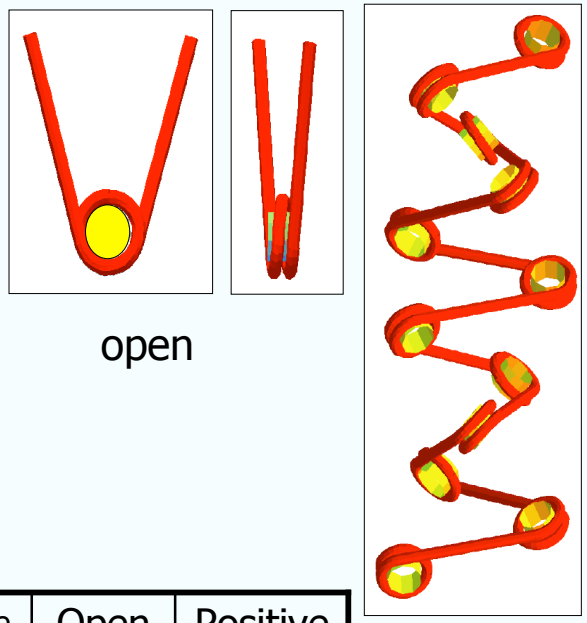
	C-	O	C+
ΔLk (turns)	-1.4	-0.7	0.4
ΔG° (kT)	0.8	0	3.6



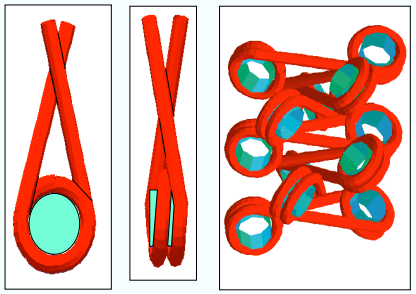
The Model – 3. A composite fiber



negative



open



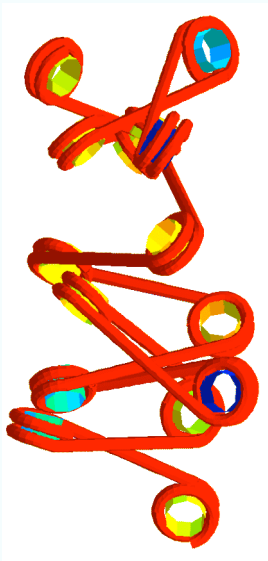
positive

state	Negative	Open	Positive
α	54°	-30°	30°
β	115°	90°	115°
Δl (nm)	4.1	8.3	2.8
ΔLk minicircle (turn)	-1.4	-0.7	-0.4
ΔEnergy minicircle (kT)	0.8	0	3.6
ΔLk model (turn)	-1.4	-0.5	-0.4
ΔEnergy model (kT)	0.7	0	2

← computed

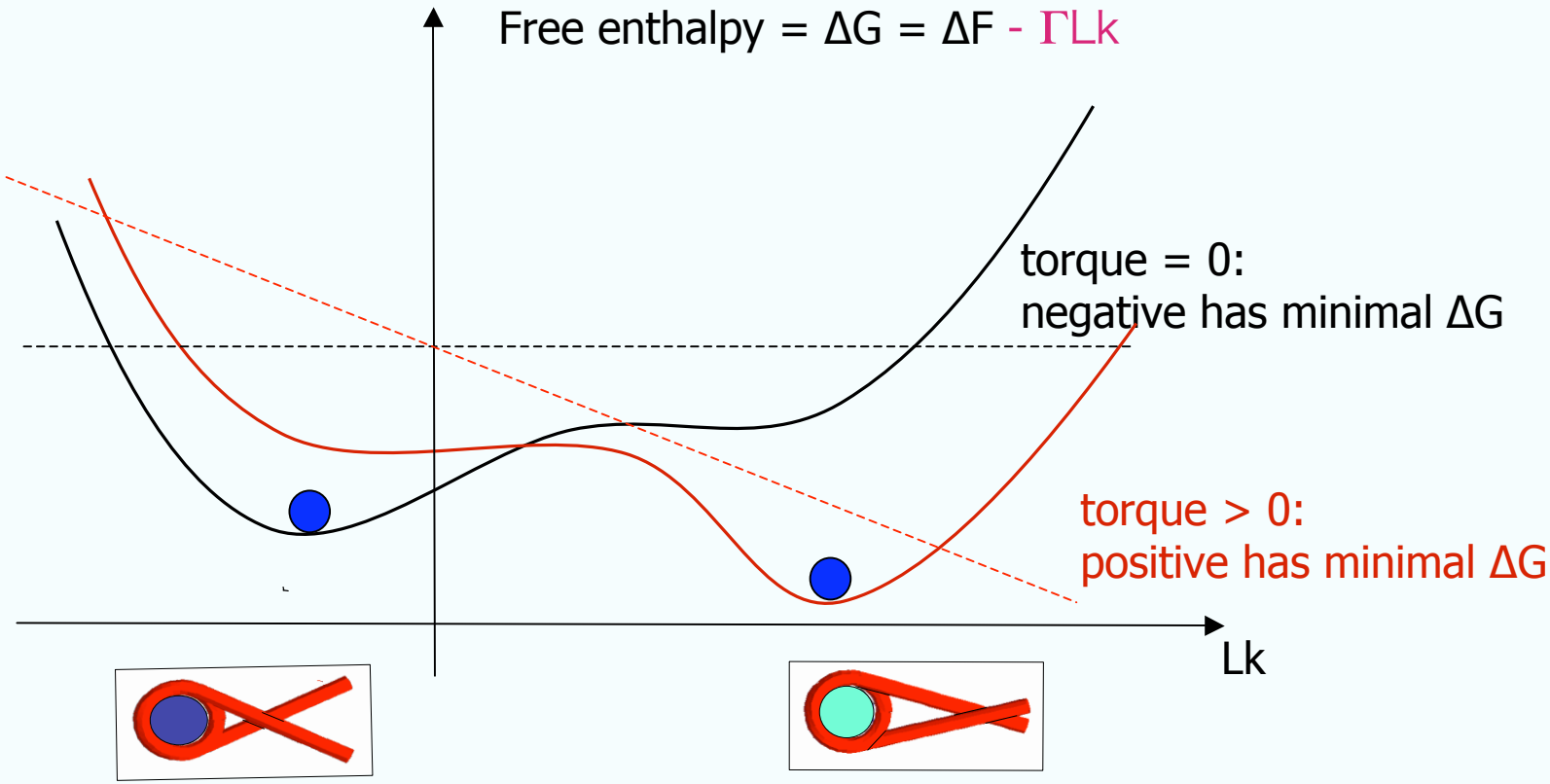
← 2 fitting parameters

A composite fiber :

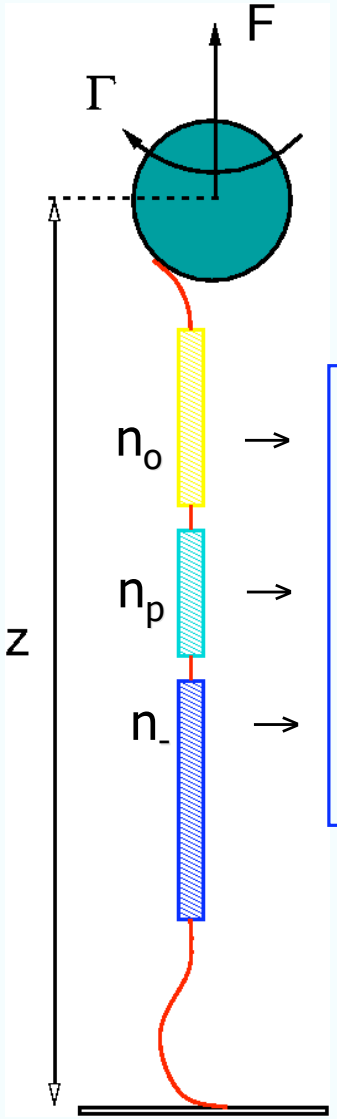


The role of the applied torque

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



The Model – 4. Statistical Mechanics



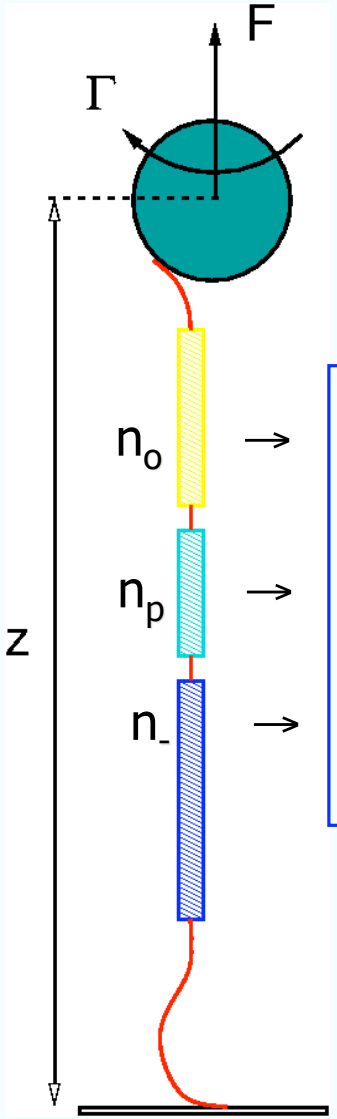
3 lengths z

3 numbers of turns Lk

3x2 persistence lengths

coexistence of 3 states : $n = n_o + n_n + n_p$

The Model – 4. Statistical Mechanics



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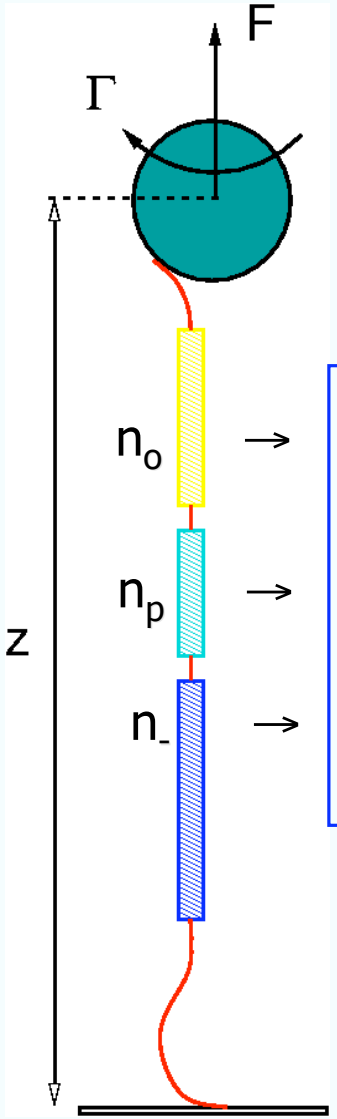
Constraints:
constant torque (the "pression") and temperature,
varying Lk (the "volume")

The Model – 4. Statistical Mechanics



3 lengths z

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coexistence of 3 states : $n = n_o + n_n + n_p$

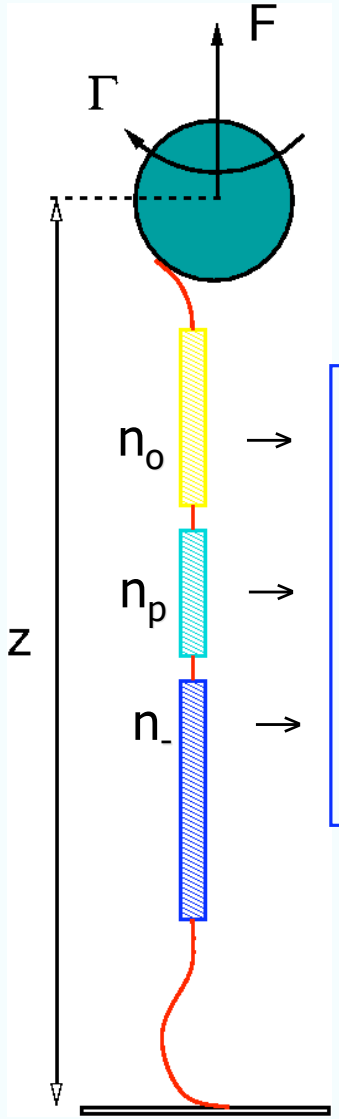
Constraints:
constant torque (the "pression") and temperature,
varying Lk (the "volume")

Free enthalpy: $G = F(n_n, n_o, n_p, \Gamma, T) - \Gamma \cdot Lk$

Γ=torque, Lk=torsion

F = kT ln(Z) with Z the standard partition function

The Model – 4. Statistical Mechanics



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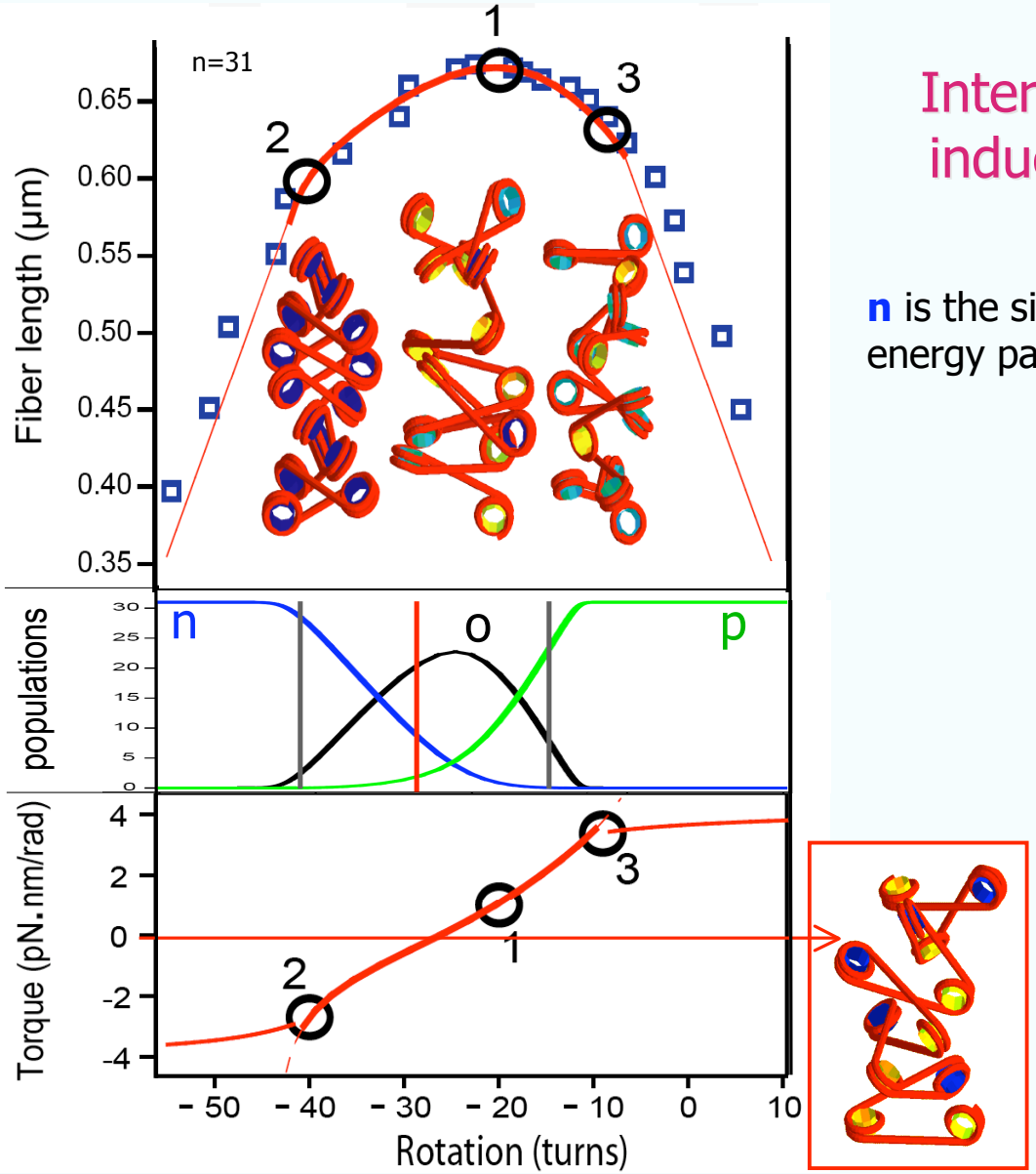
Γ=torque, Lk=torsion

minimization of G (at T and Γ=cts) $\Rightarrow$ $\mathbf{n_n, n_p, n_o}$

$$\Rightarrow \begin{cases} z = z_n + z_o + z_p + z_{DNA} = z(n,T,\Gamma) \\ Lk = Lk_n + Lk_o + Lk_p + Lk_{DNA} = Lk(n,T,\Gamma) \end{cases}$$

$\Rightarrow$ elimination of Γ $\Rightarrow$ **z(Lk)** $\Rightarrow$ **fit (n)**

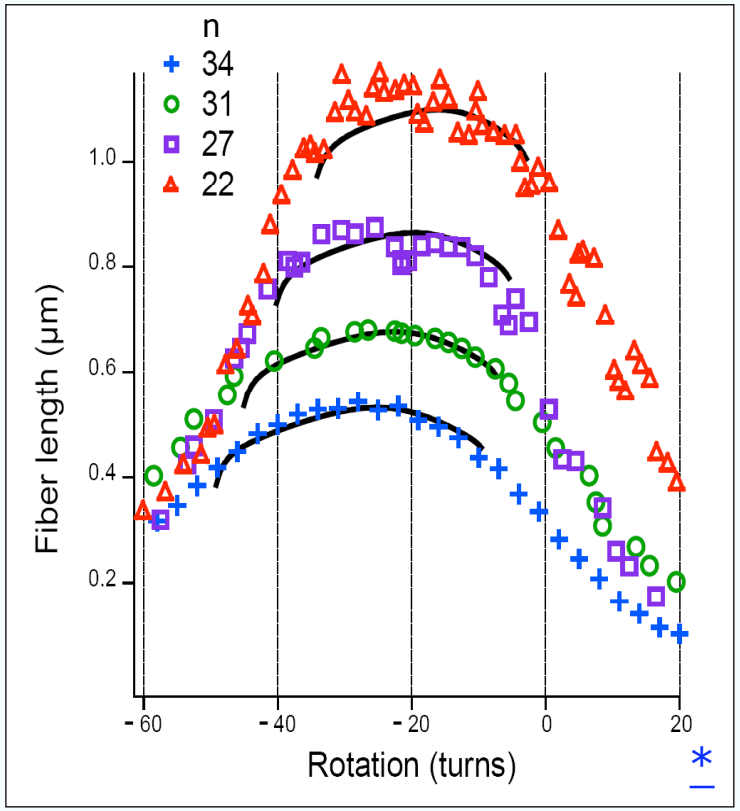
The Model – 5. fitting the curves



Internal structure reorganization induced by torsional constraints

at nearly constant length

n is the single fitting parameter (once fixed the 2 energy parameters)



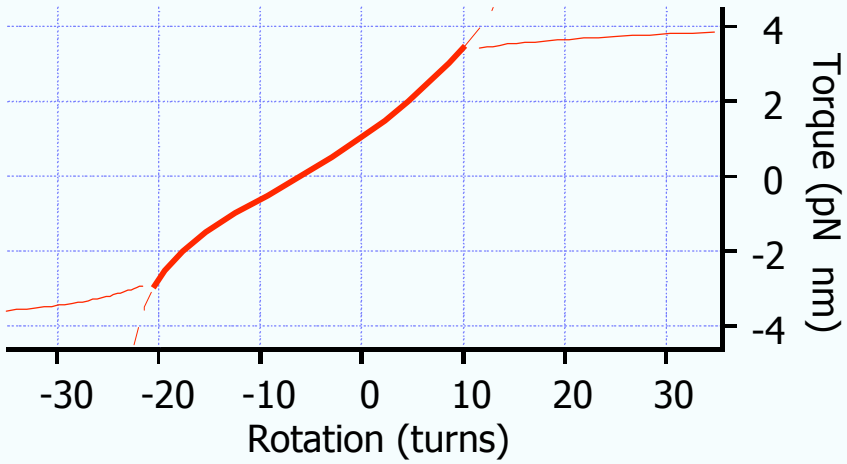
back to biology: a functional fiber?

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 pN·nm, lower than exerted by **RNA polymerase** (≥ 5 pN·nm, Harada 2001)



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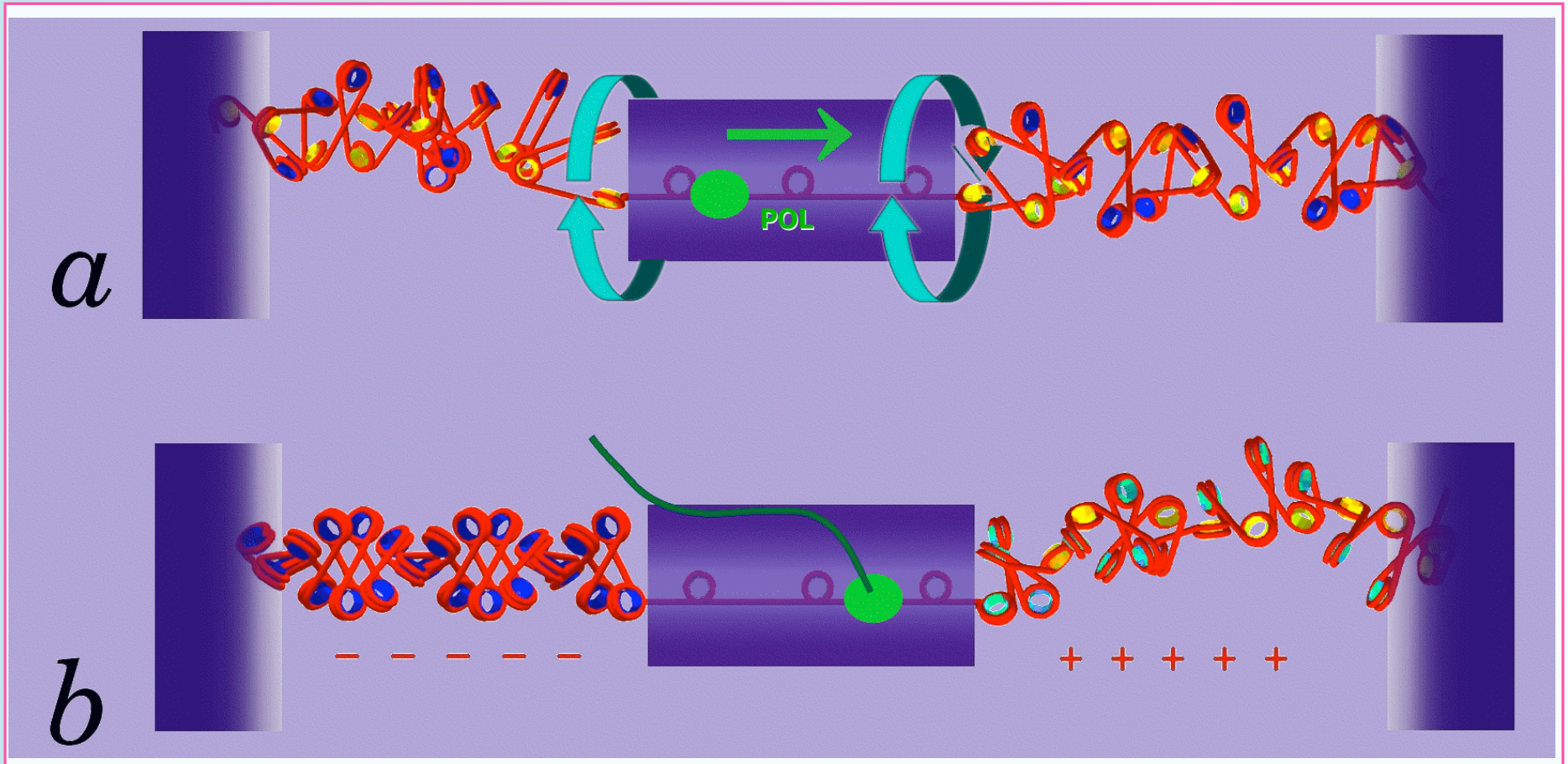

A topological buffer:

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

An interdisciplinary collaboration !

Institut Curie :

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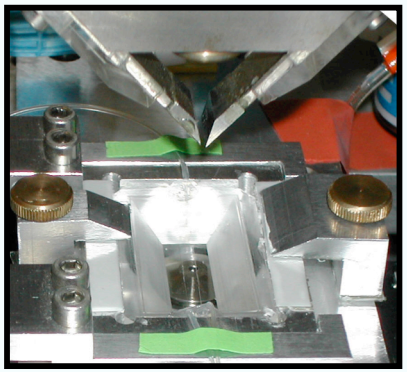

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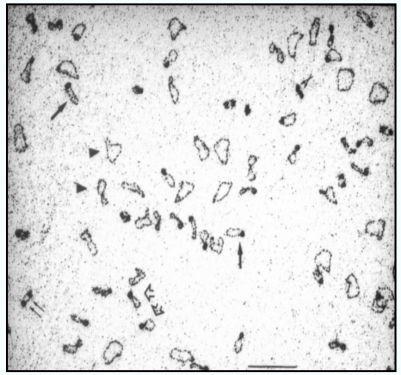

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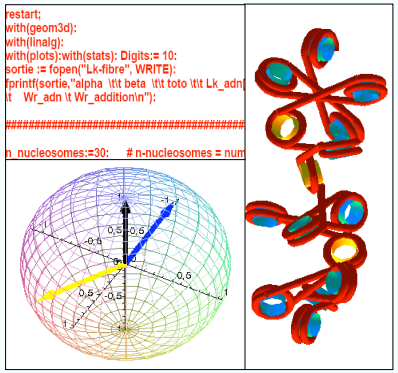
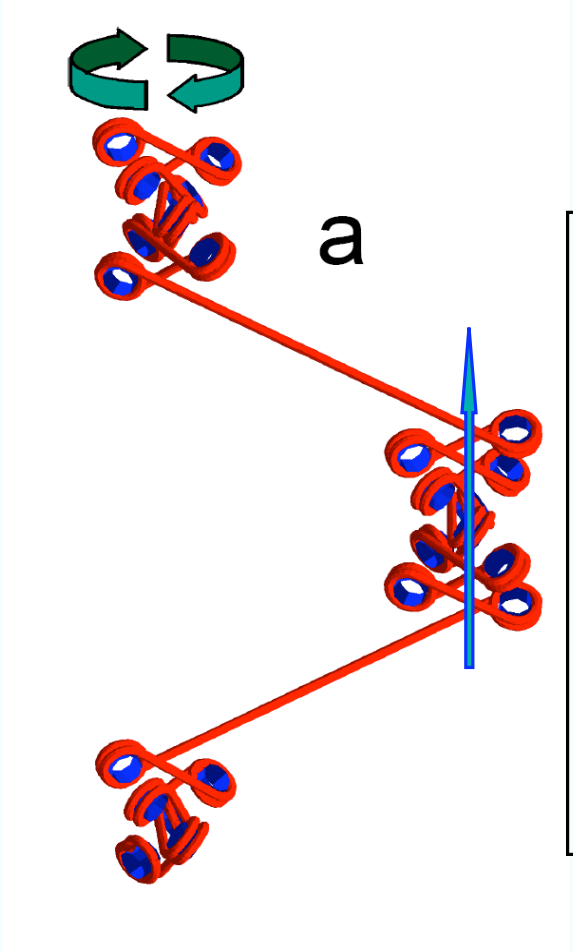
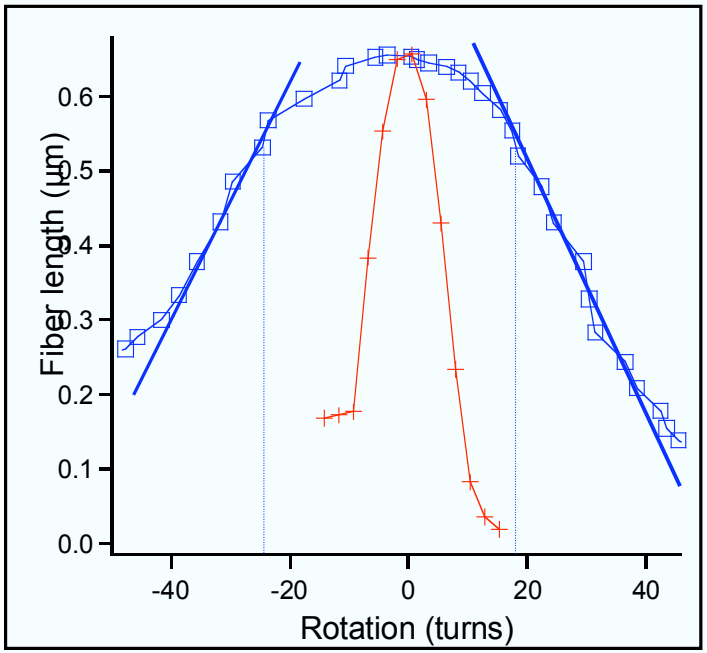


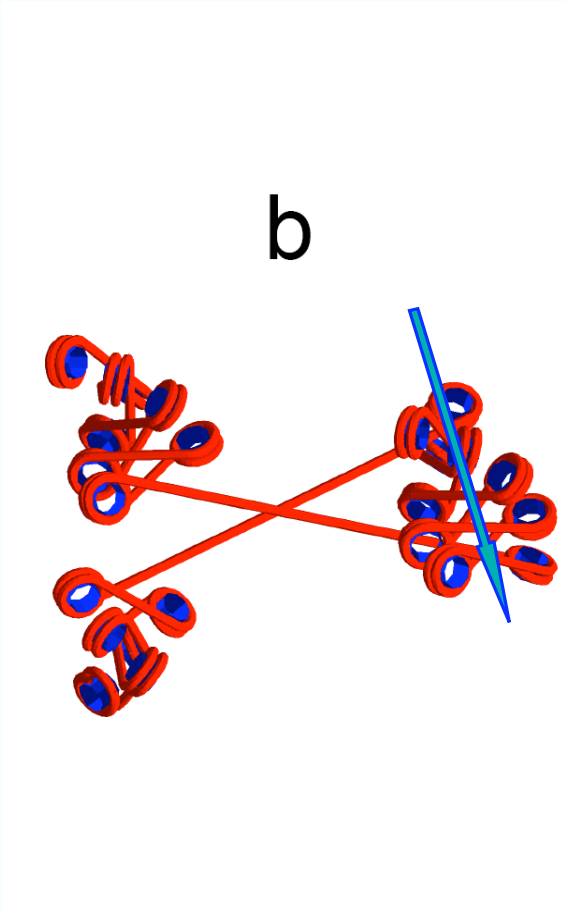
Further torsion… plectonemes

Fiber defects → a new mechanism for plectonemes initiation:

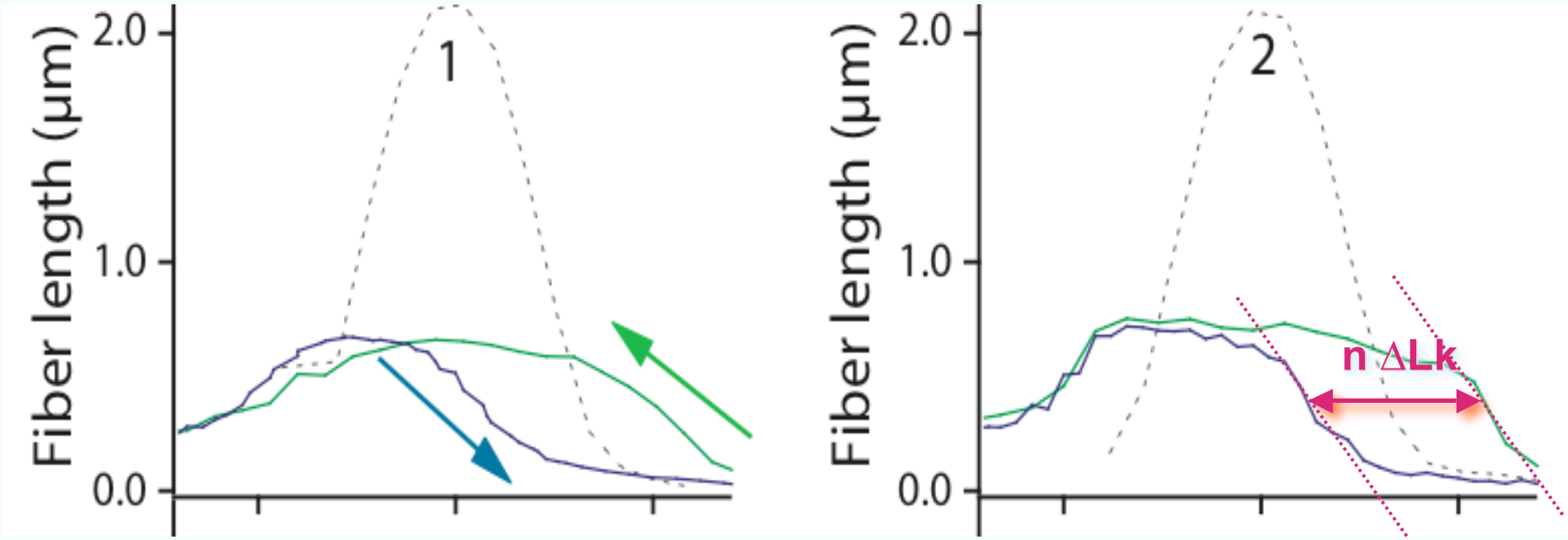
the "mini-fibers" inversion







Further torsion… hysteresis!



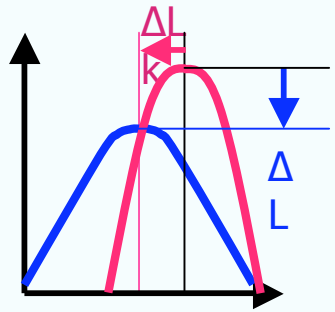
fibers display an hysteretic behavior

- not explained by nucleosome-nucleosome interactions

- Compatible with a nucleosome internal rearrangement:

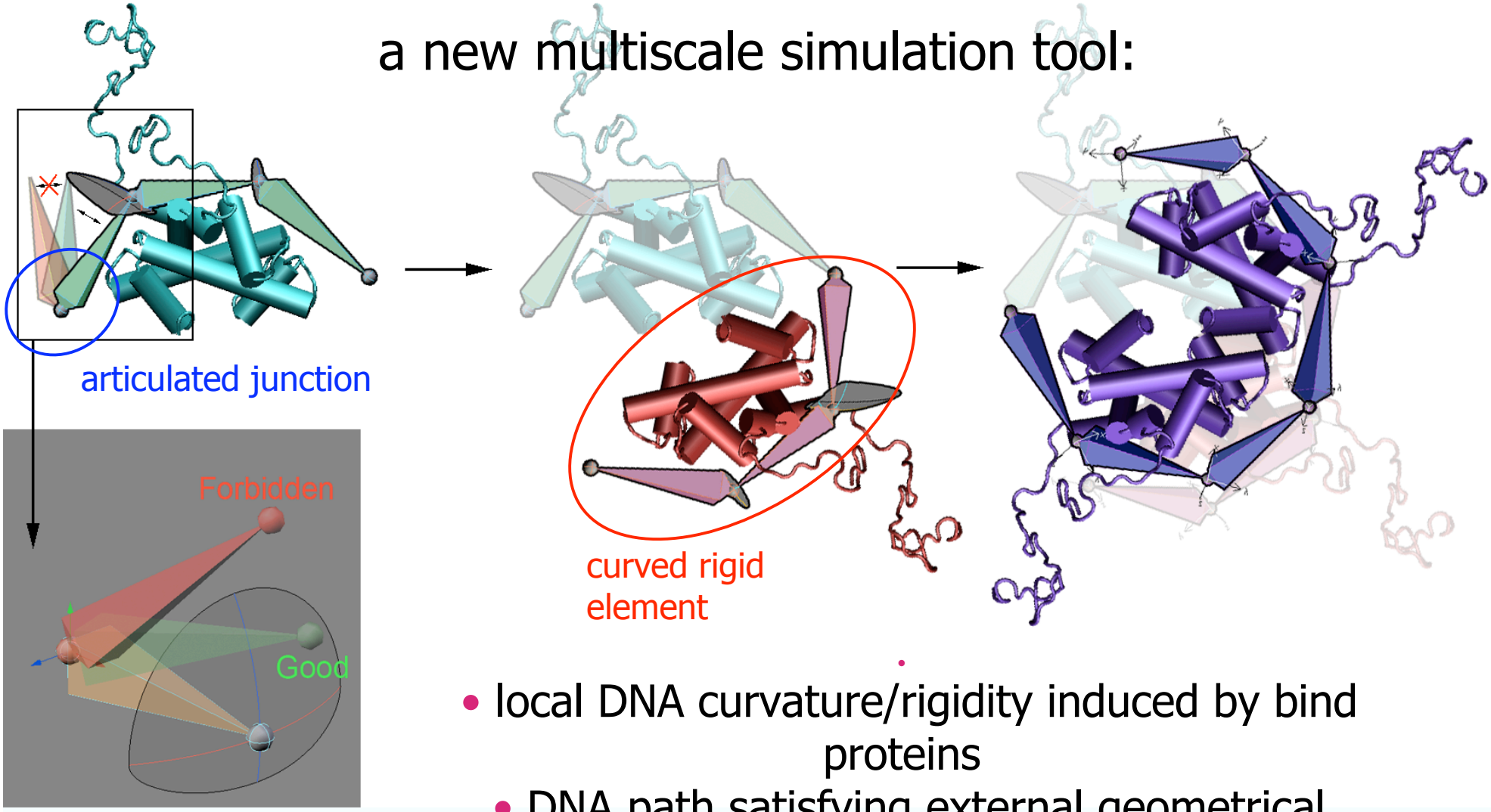
transition to a metastable altered state with

a similar ΔL, a larger (positive) ΔLk



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 min)

Thanks to the young people!

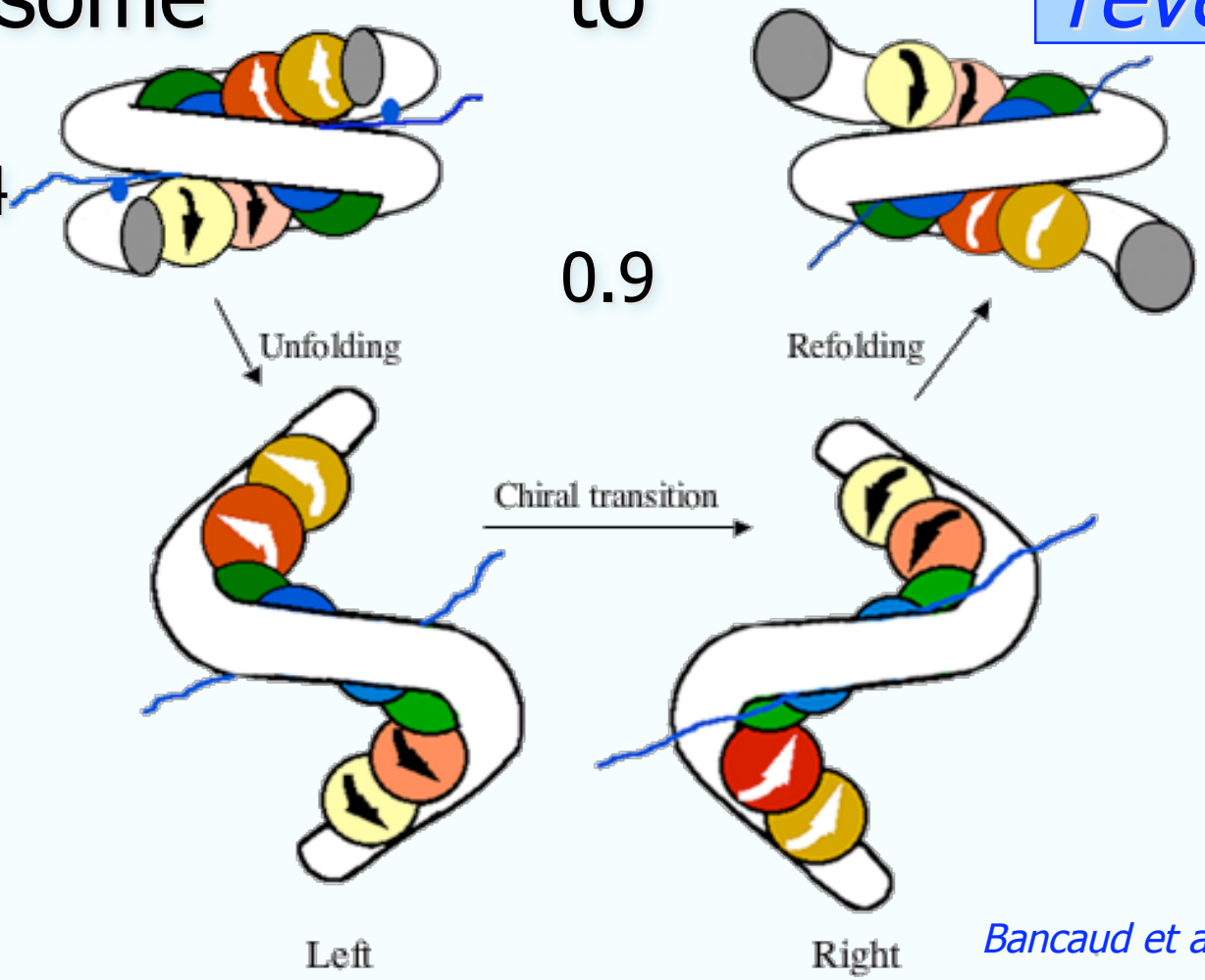
⇒ nucleosome is not a tuna can!

inverse kinematic (+ some additional experimental results on "tetrasomes")

nucleosome to *reversome*

ΔR≈-0.4

ΔR≈ 0.9



Bancaud et al., Mol Cell 2007

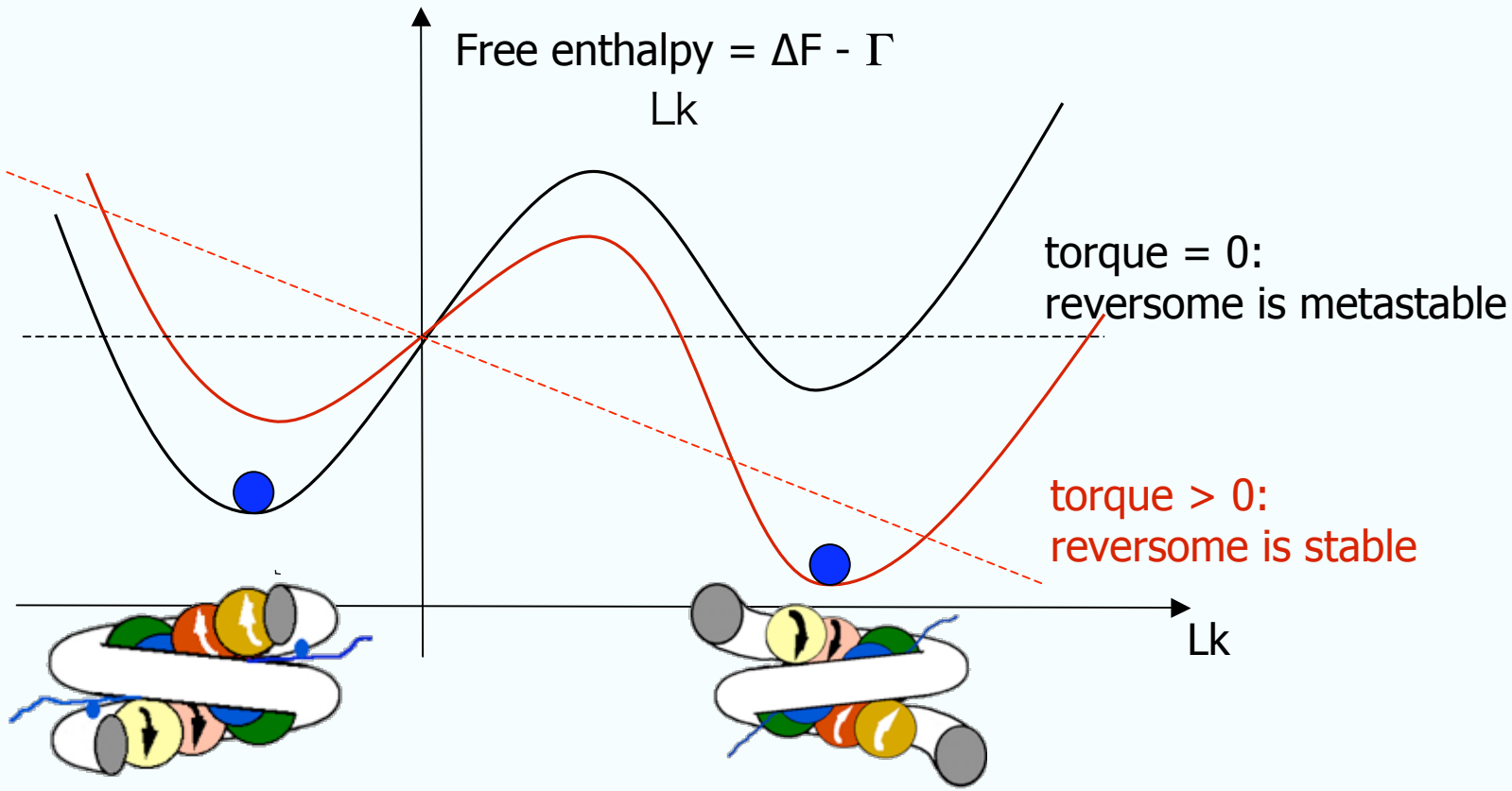
⇒ nucleosome is not a tuna can!

Extended model:

torque dependent **activation barrier** from pos. to reversome

including

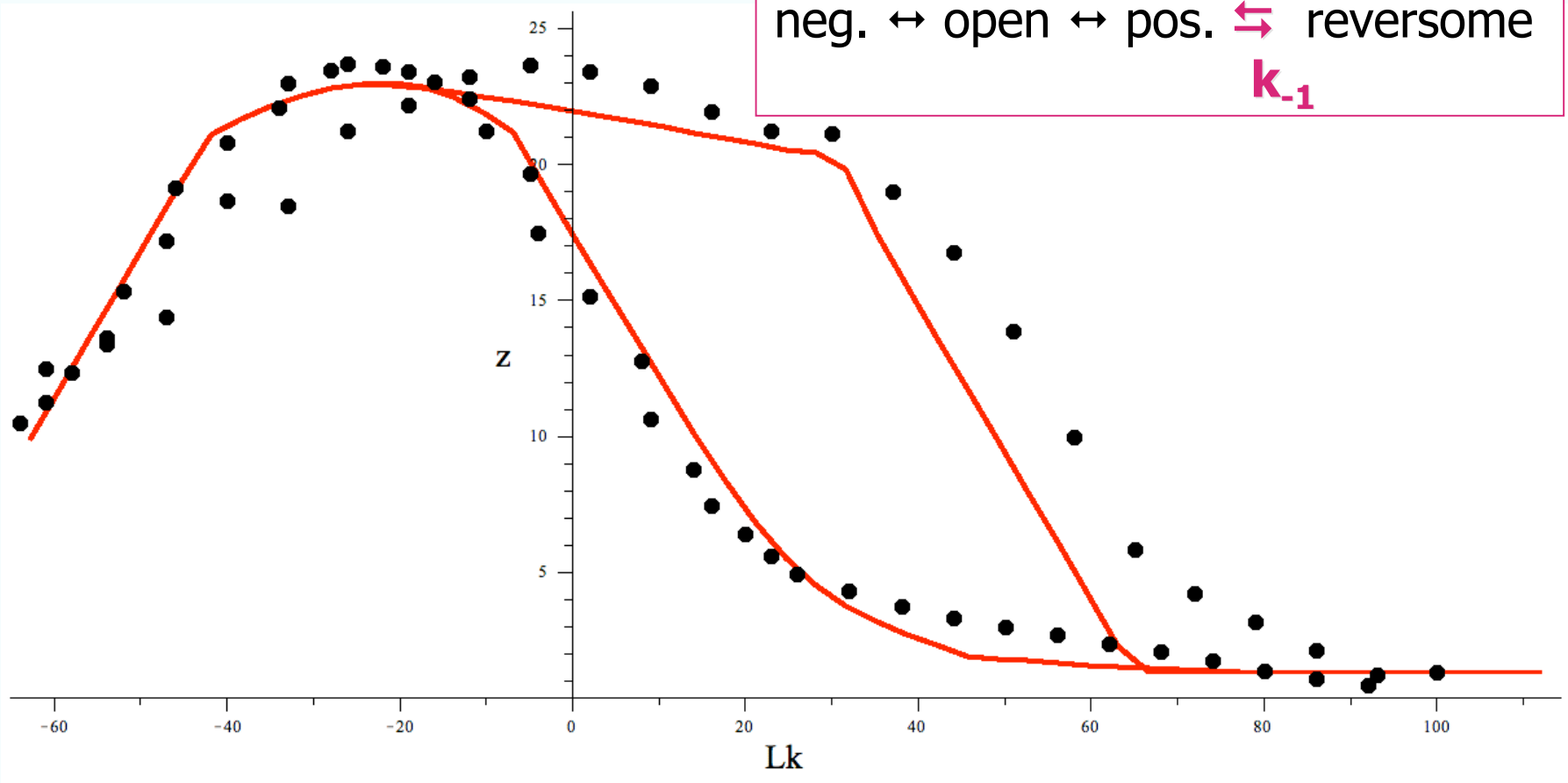
breaking of intermolecular interactions + mechanical instability effects…



⇒ nucleosome is not a tuna can!

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

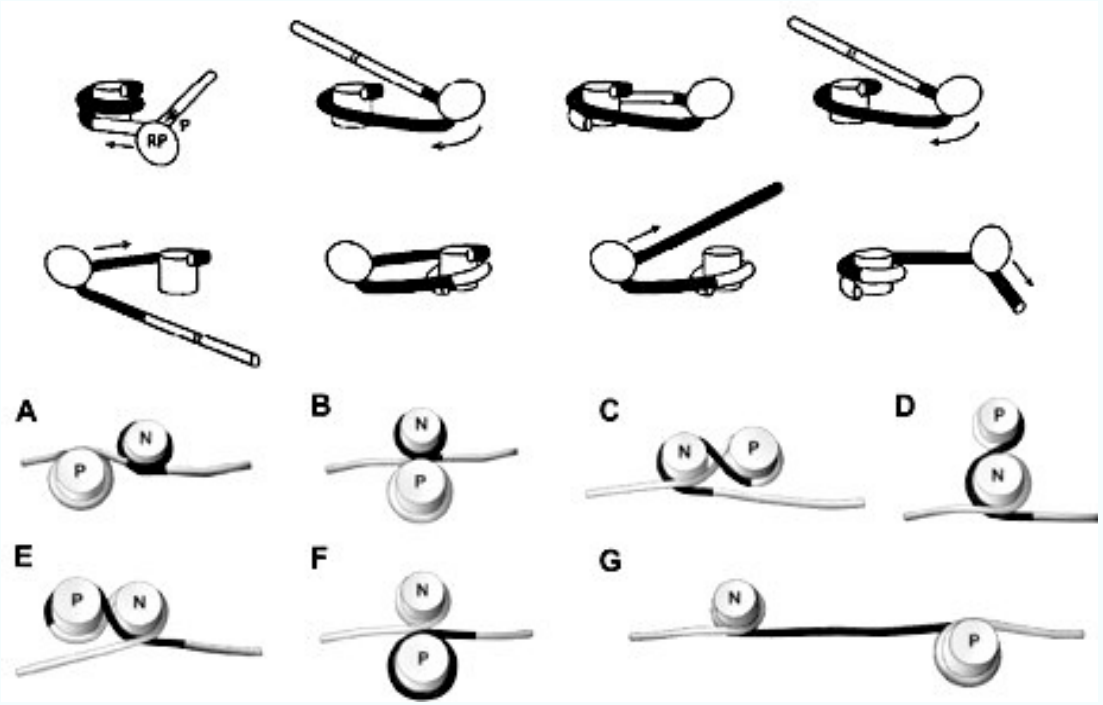
$$\text{neg.} \overset{\text{eq.}}{\leftrightarrow} \text{open} \overset{\text{eq.}}{\leftrightarrow} \text{pos.} \underset{k_{-1}}{\overset{k_1}{\leftrightarrows}} \text{reversome}$$



back to biology: a functional fiber?

②. Further torsion induces a nucleosome "opening"

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



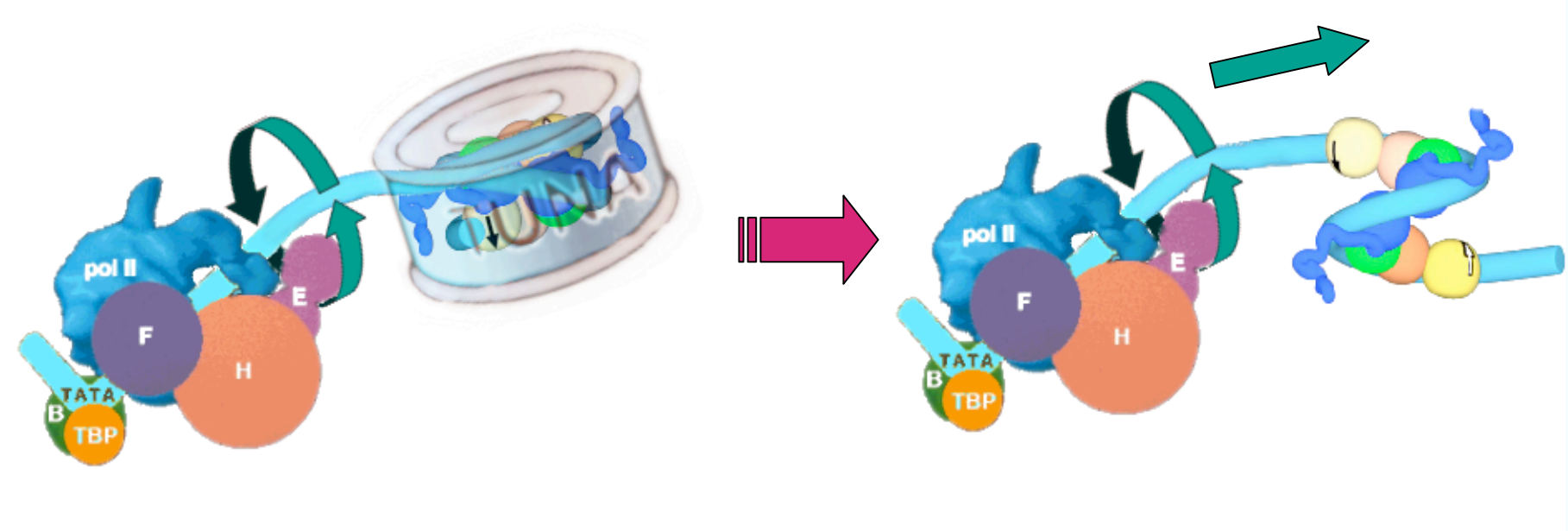
Lavelle, Biochimie 89 (2007)

many models exist on how the polymerase passes trough nucleosomes during transcription..

back to biology: a functional fiber?

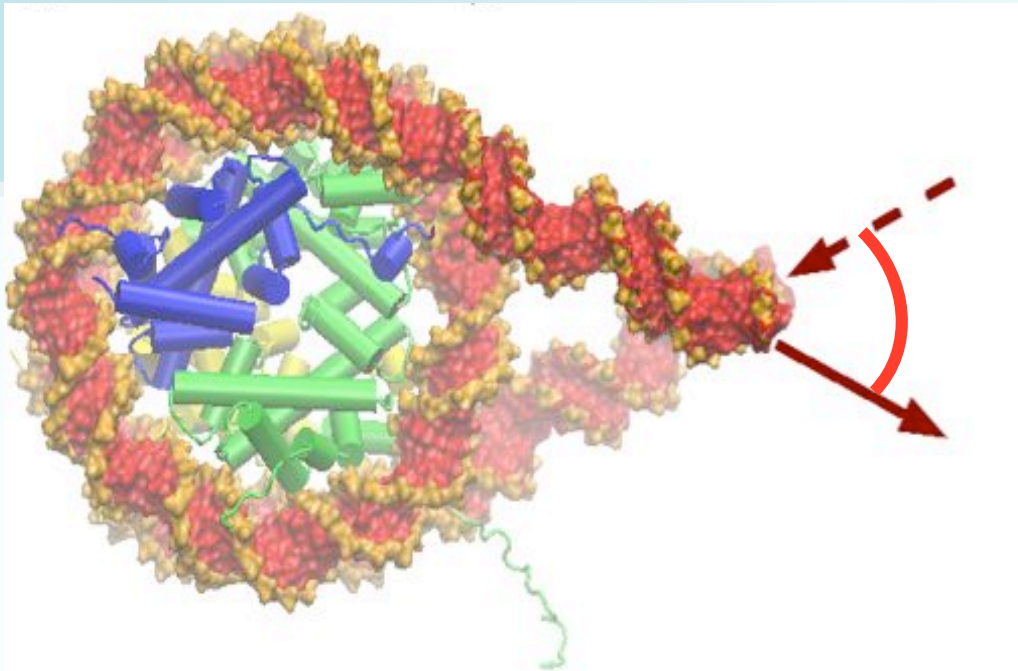
②. Further torsion induces a nucleosome "opening"

where intermolecular interaction are partially broken,
this can help polymerase to pass through:



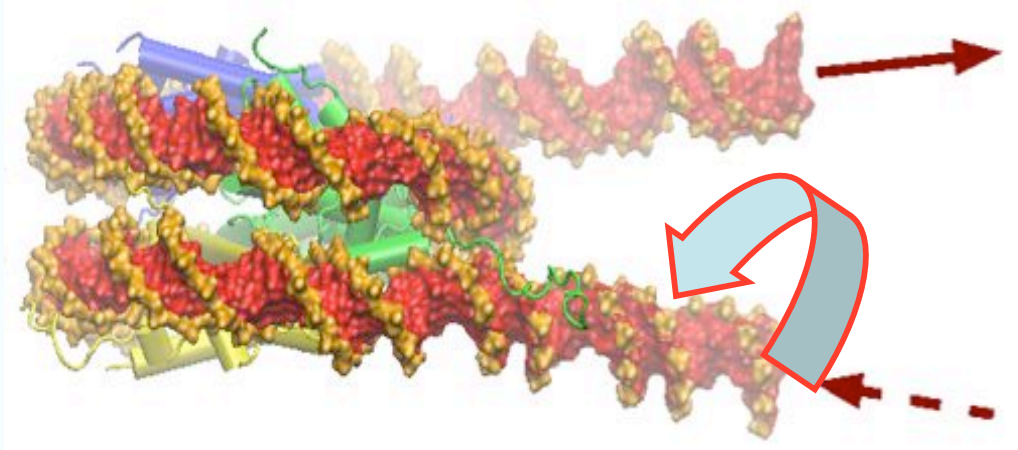
reversome as a
torsion driven activated nucleosome?

Modeling tools: the 2 angle model



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