Dealing with supercoiling during transcription elongation in a chromatin loop:

the reversome wave

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collaboration *experimental group:*

Jean-Louis Viovy, Institut Curie, Paris

1. transcription elongation

2. …in eukaryotic chromatin

3. transcription in condensed chromatin?

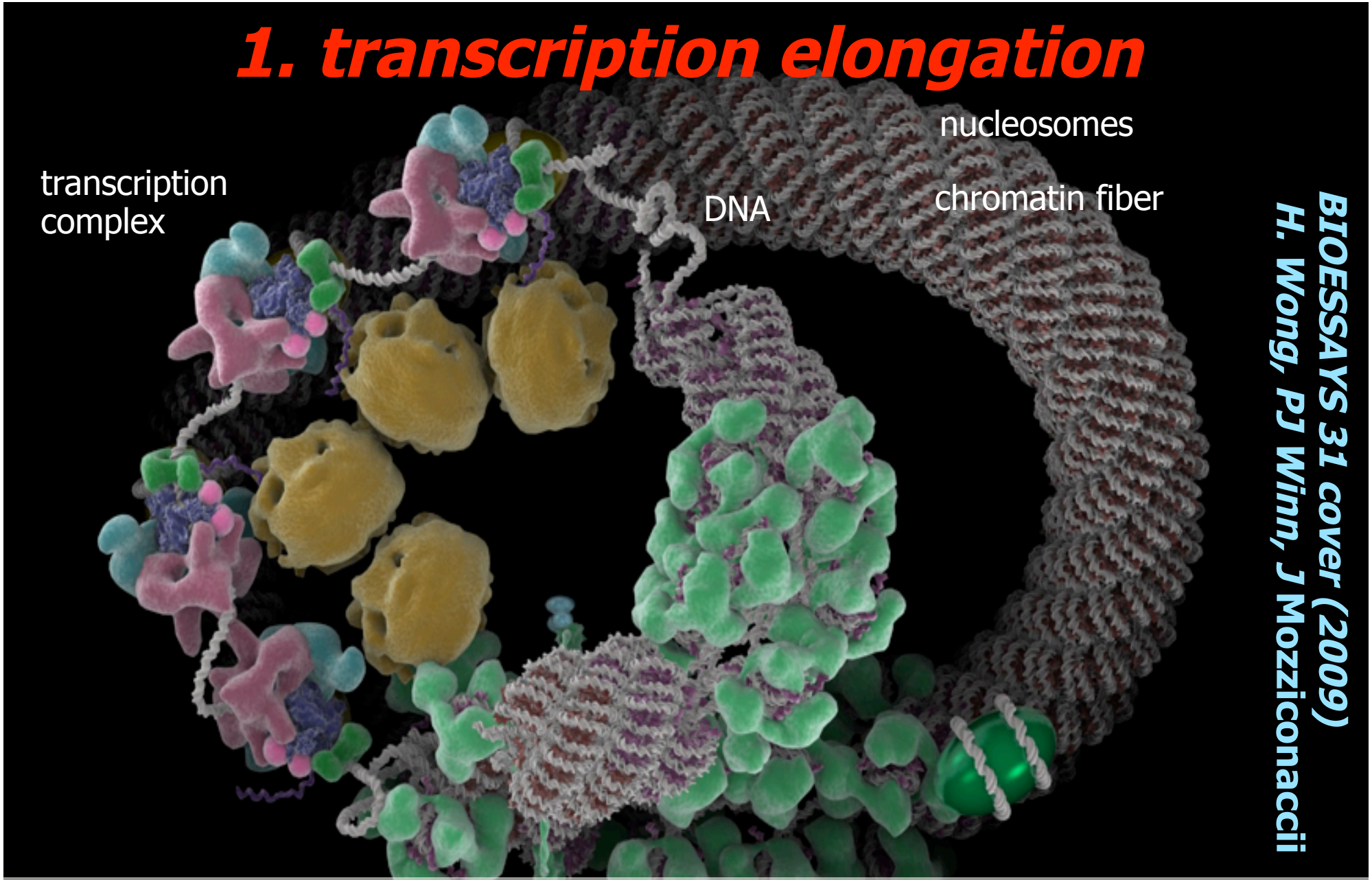
4. what about nucleosomes?

5. probing nucleosomes under torsion

6. back to transcription

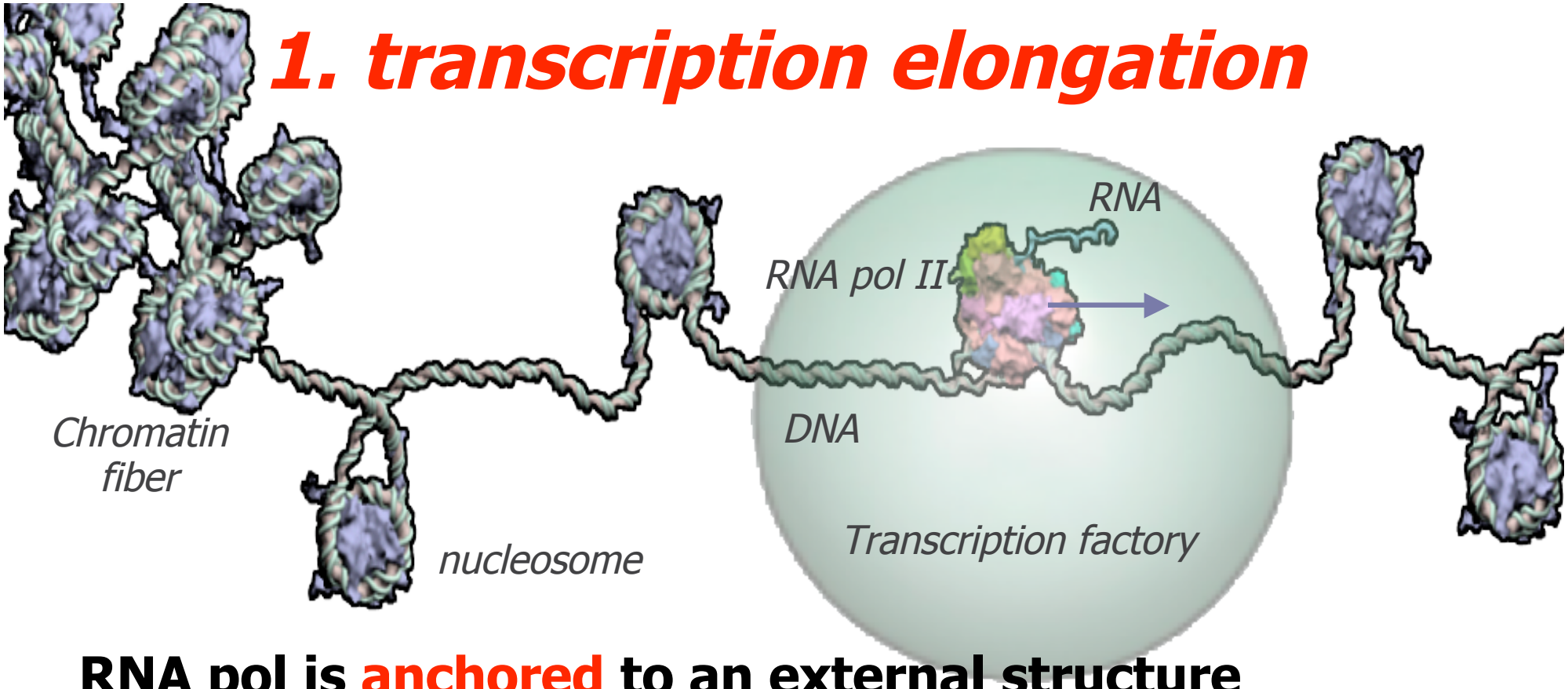
7. resulting scenario

1. transcription elongation



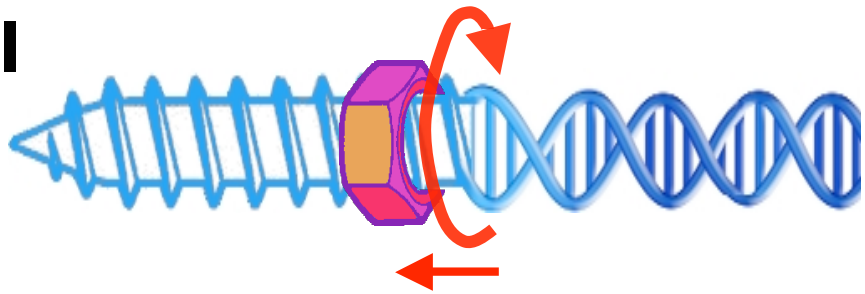
- **Are nucleosomes significant obstacles for RNA pol?**
- **How is the chromatin fiber modified during transcription?**

1. transcription elongation



RNA pol is anchored to an external structure

⇒ **DNA *screws* through RNA pol**



rate: ω_0 **~ 2 turns/sec**
(V ~ 20 bps/sec)

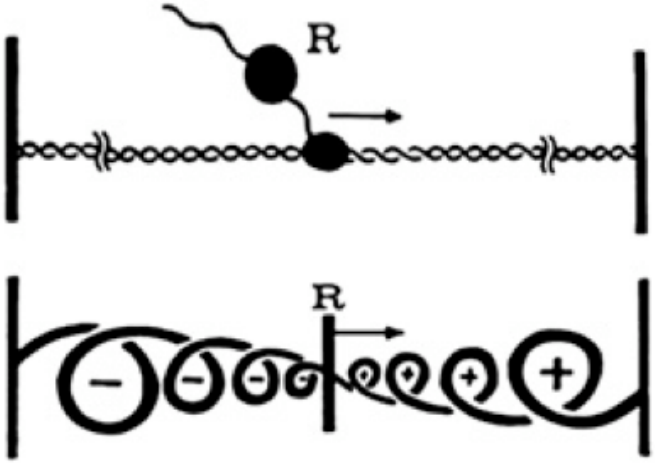
↔ **RNA pol II exerts a torque > 5 pN·nm** (Harada 2001)
<40 pN·nm (kT~4 pN·nm)

1. transcription elongation

⇒ **topological consequences:**

generation of positive/negative supercoiling ahead/behind the RNA pol (~2 turns/sec)

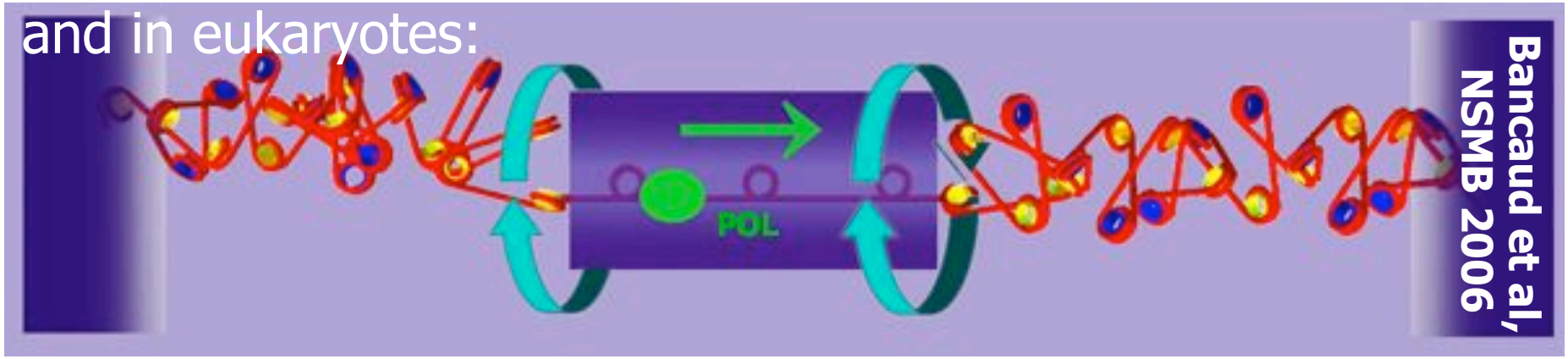
in prokaryotes:



Twin-supercoiled domain model

(Liu & Wang, PNAS 1987)

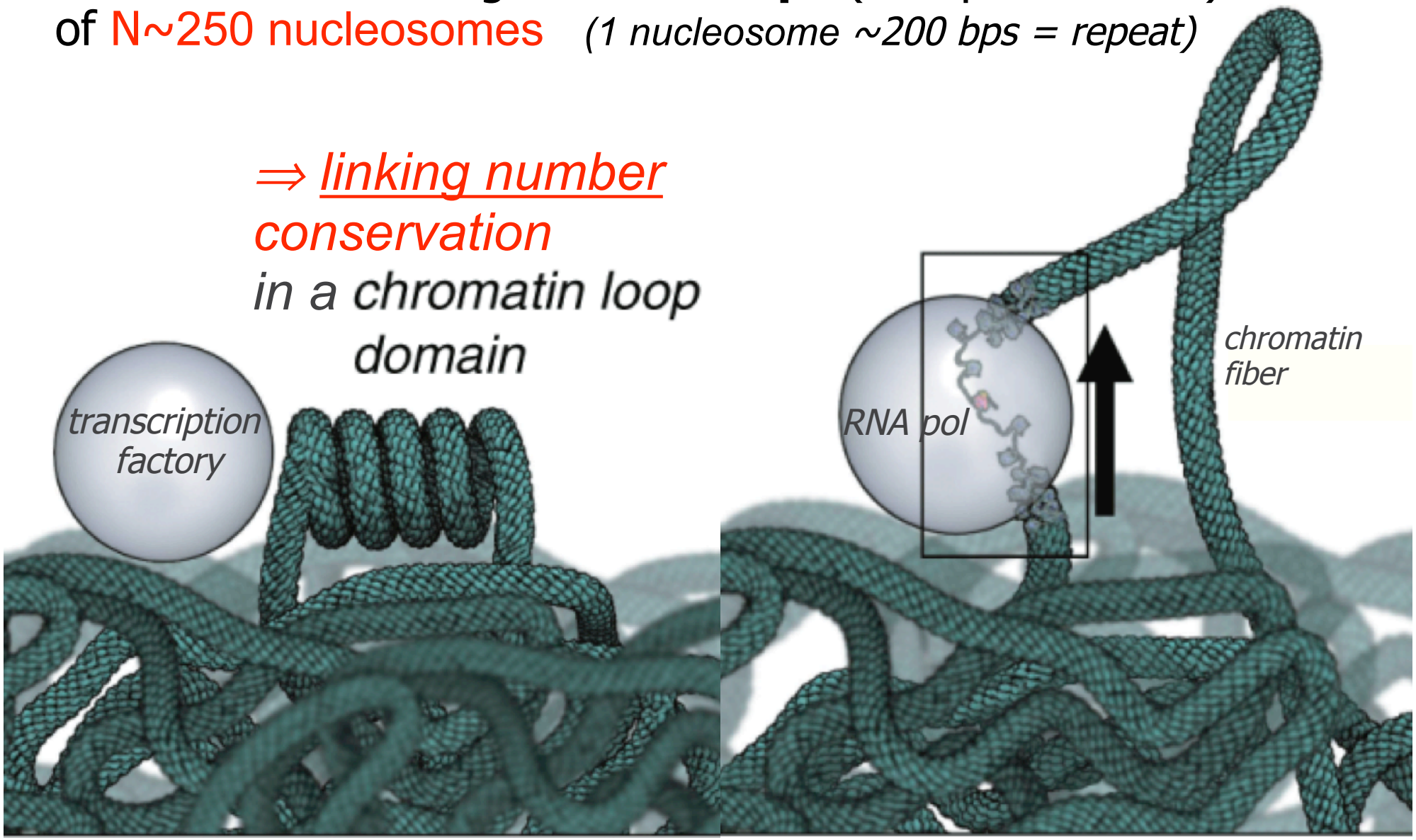
and in eukaryotes:



2. ...in eukaryotic chromatin

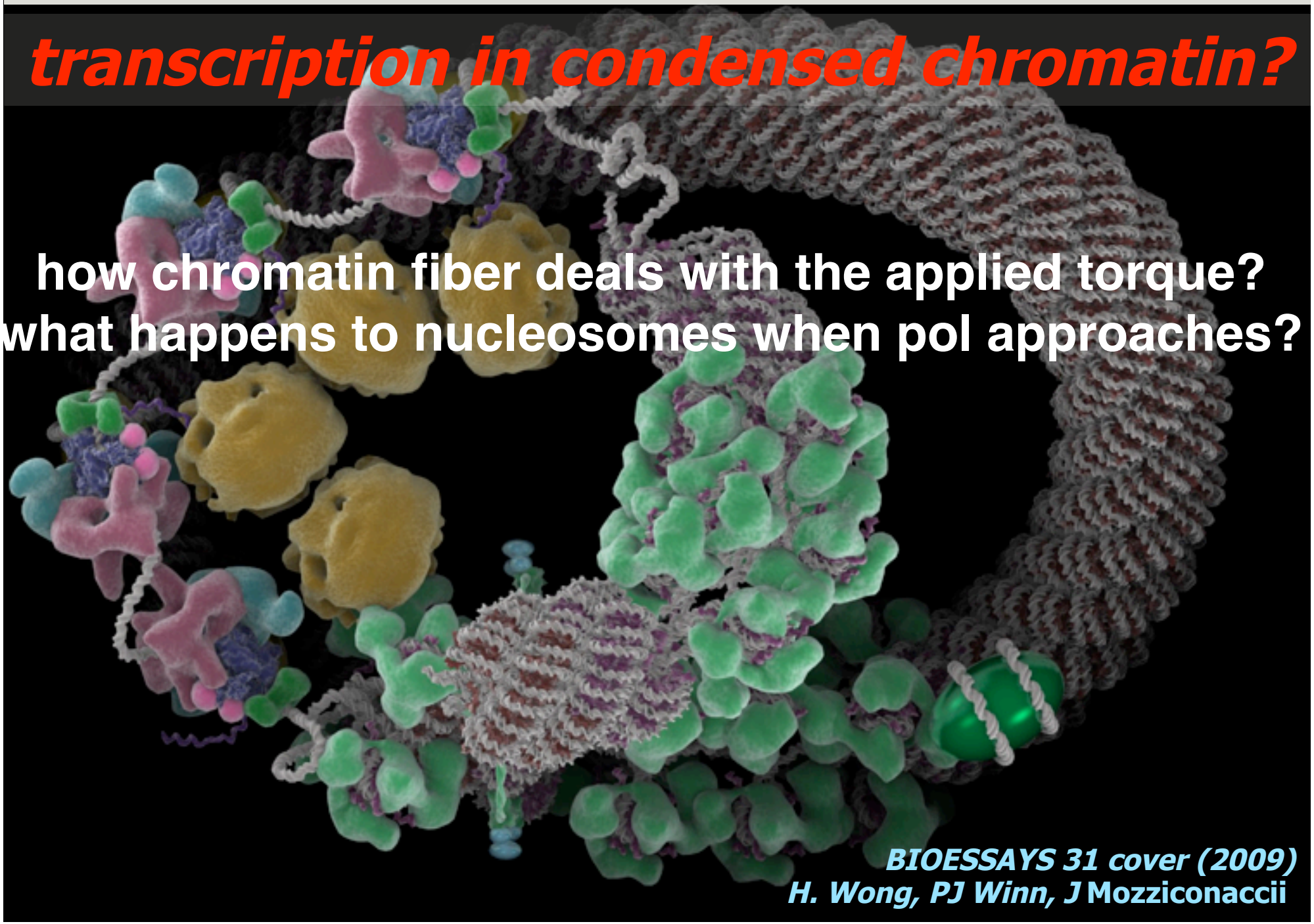
- chromatin fiber is organized in **loops** (clamped at ends) of N~250 nucleosomes *(1 nucleosome ~200 bps = repeat)*

⇒ *linking number conservation in a chromatin loop domain*

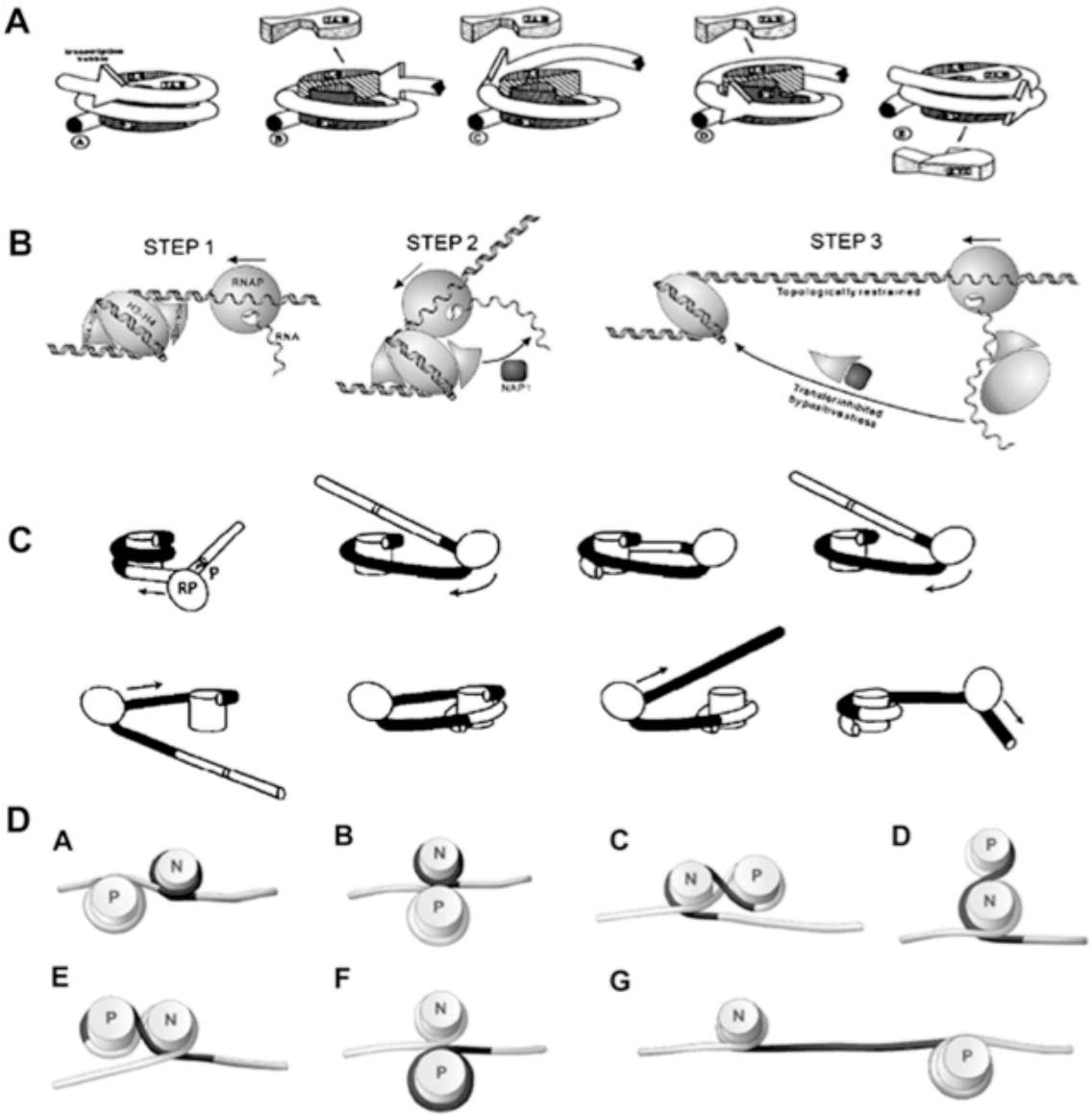


Figures by H. Wang for C. Lavelle, Biochem Cell Biol 87:307-322 (2009) .

3. no evidence for decondensation prior to transcription:



4. what about nucleosomes?



how RNA pol get trough nucleosomes during transcription?

→ **many models!**

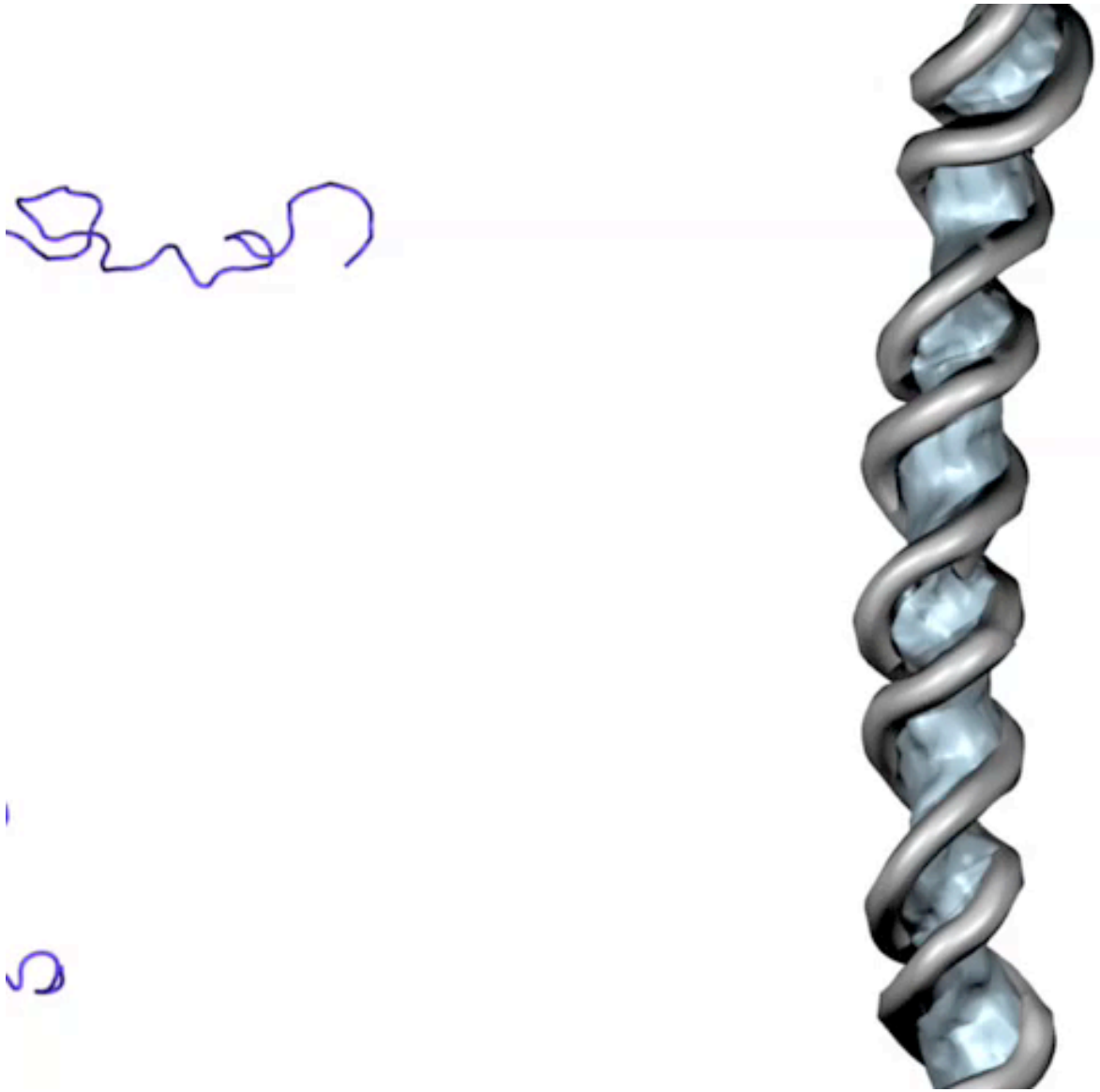
→ **need for an *destabilized state* of the nucleosome**

- histone release,
- nucleosome translocation,
- DNA unwrapping,
- nucleosome distortion…

RNA pol II :
H2A-H2B destabilization
no octamer translocation

Lavelle, Biochimie 89 (2007)

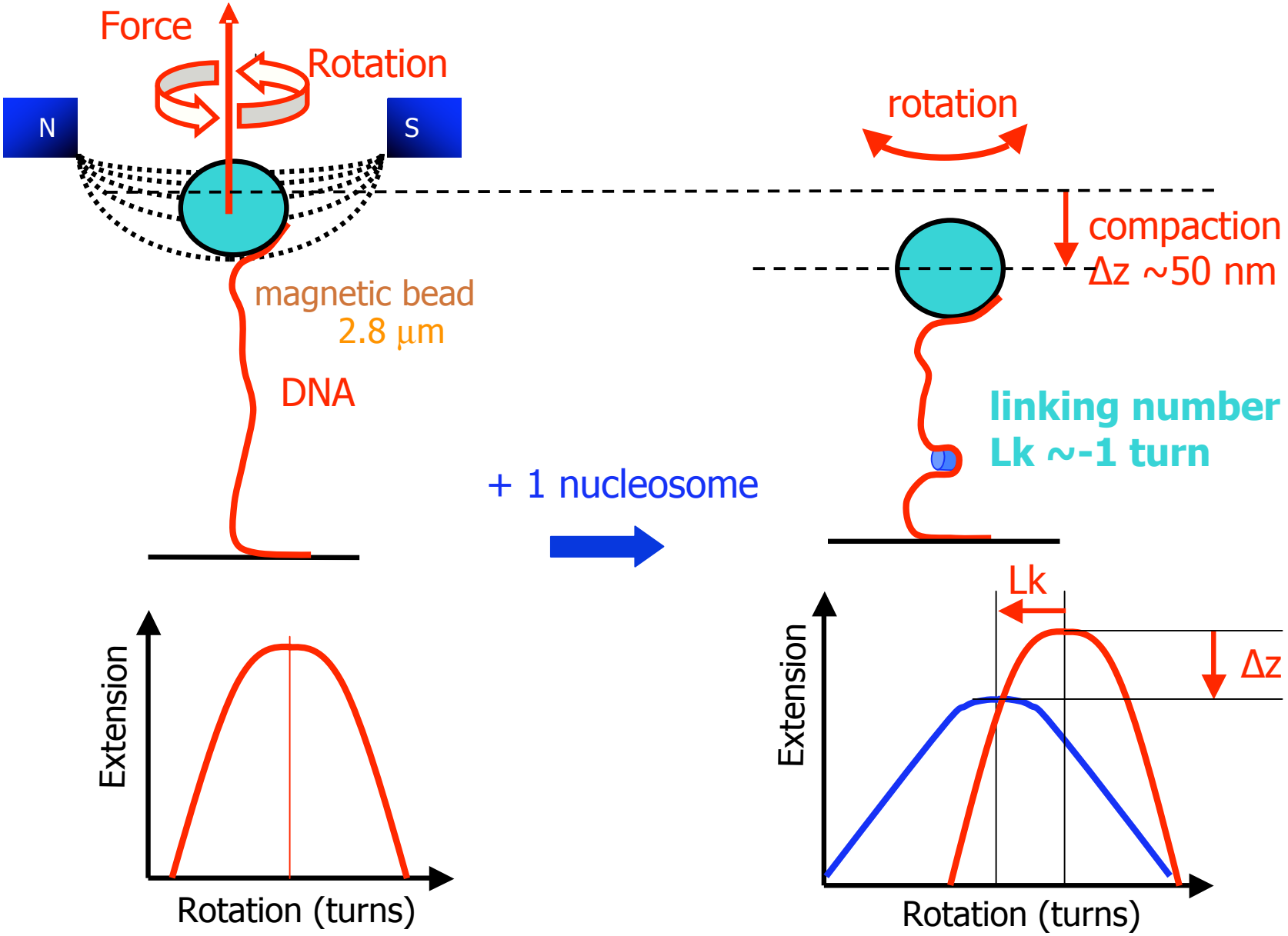
4. what about nucleosomes?



Animation by Hua Wong

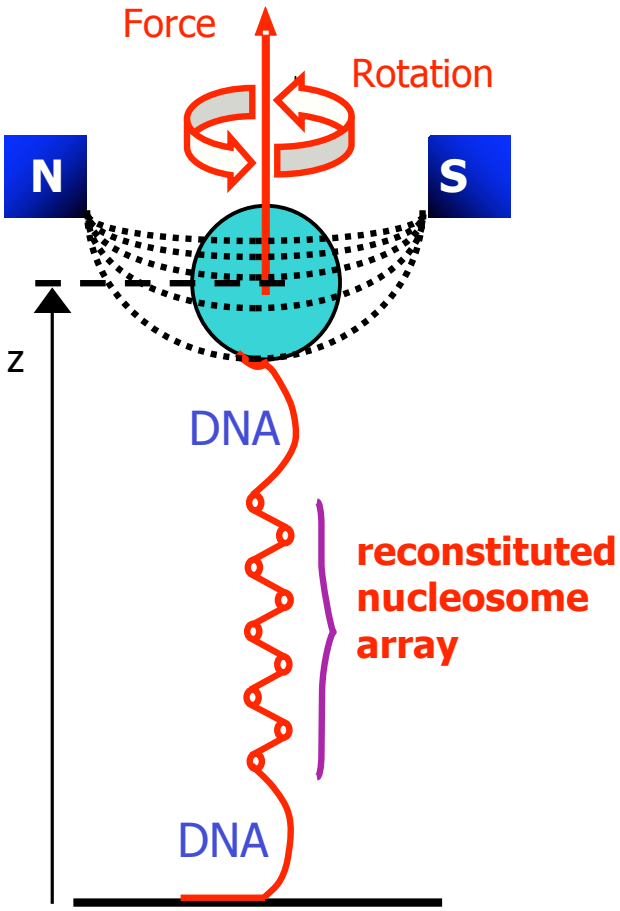
5. probing nucleosomes under torsion

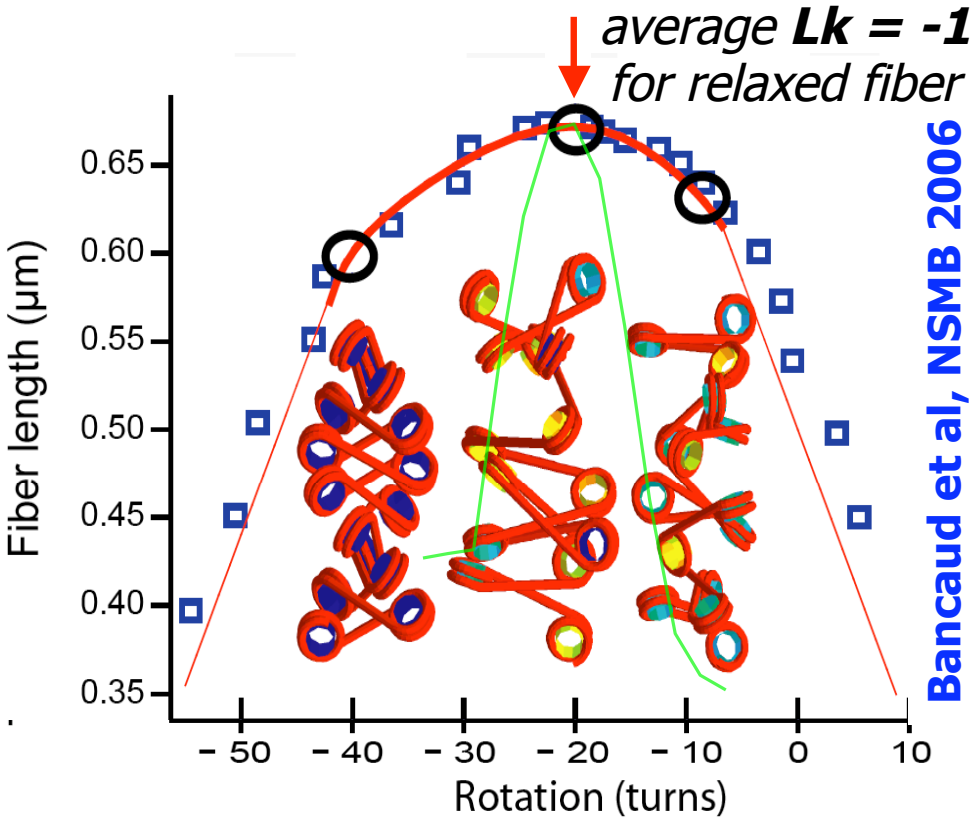
by magnetic tweezers

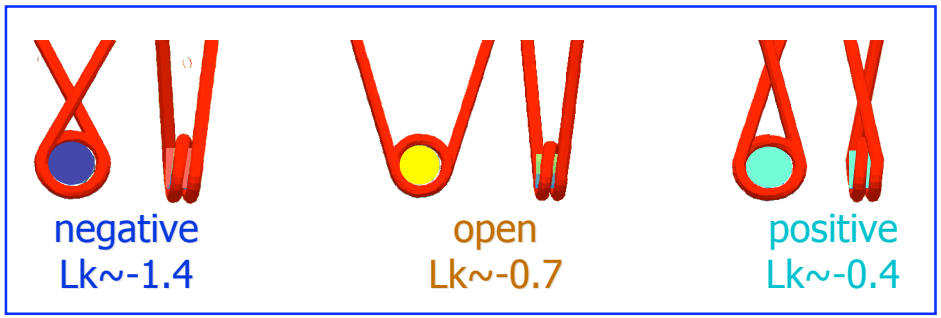


5. probing nucleosomes under torsion

torque < 3 pN nm



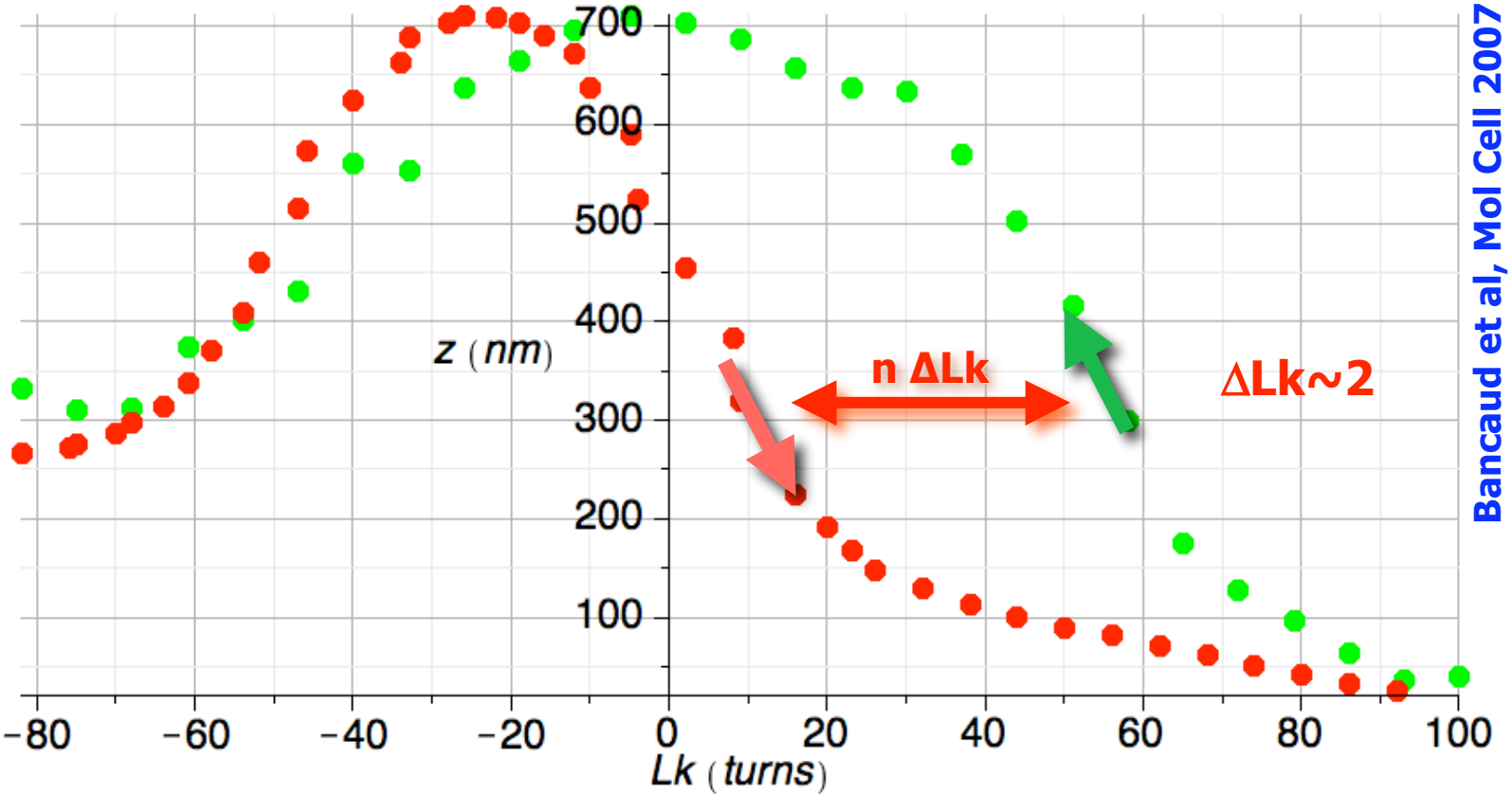




fiber = topological buffer:
torque-dependent equilibrium between the 3 states of nucleosome

5. probing nucleosomes under torsion

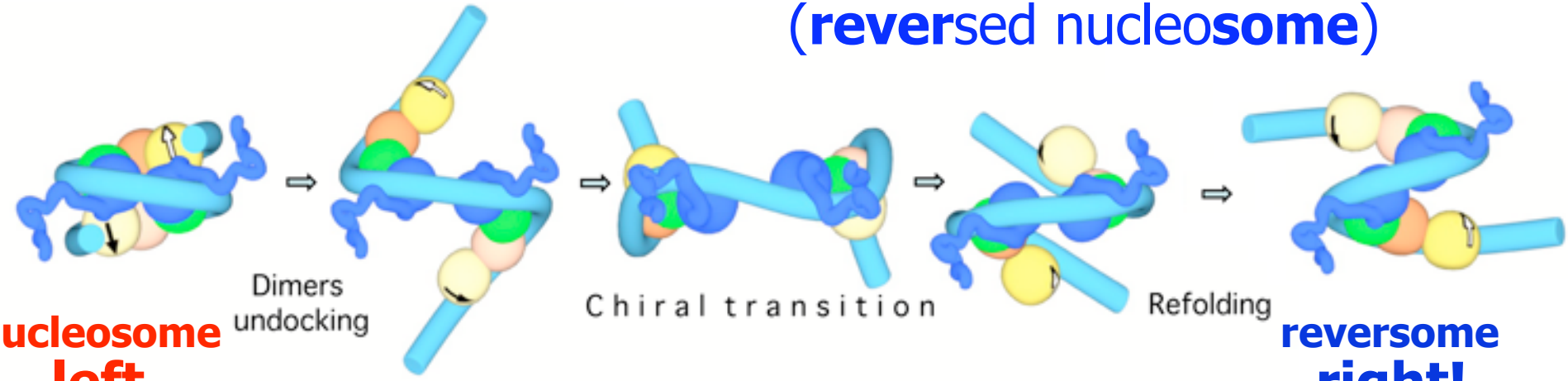
RNA pol II torque > 5 pN nm: histeretic behavior



- not explained by nucleosome-nucleosome interactions
- compatible with a nucleosome internal rearrangement: **transition to a metastable altered state** with a larger (positive) ΔLk

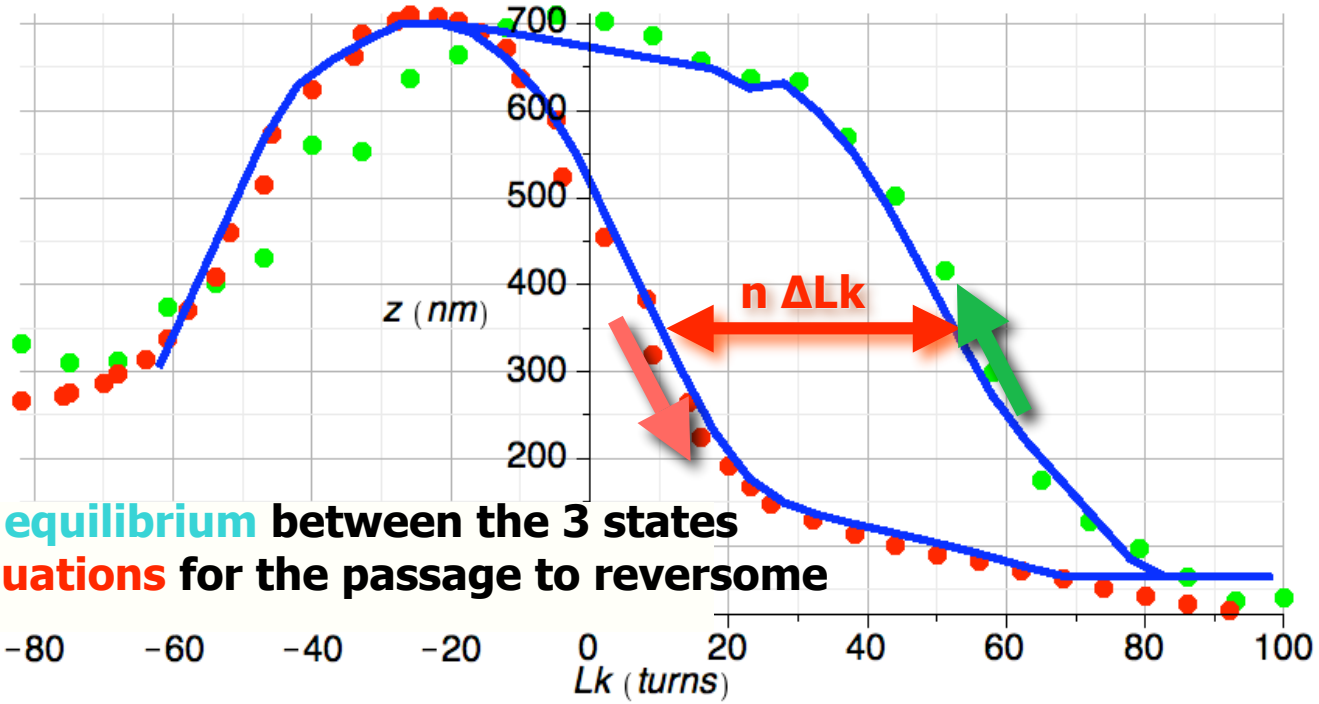
5. probing nucleosomes under torsion

torque > 5 pN nm: **transition to reversome**
(**rever**sed nucleo**some**)



nucleosome
left

reversome
right!



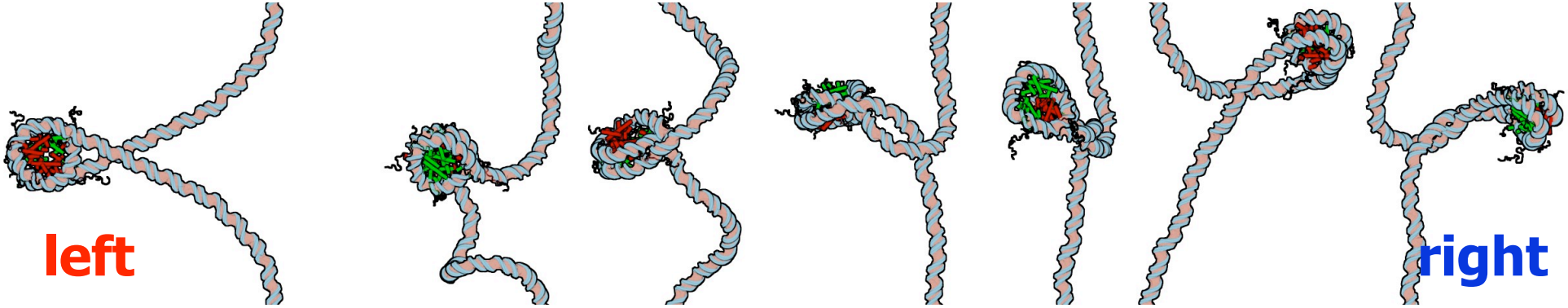
fit:

statistical equilibrium between the 3 states
+ kinetic equations for the passage to reversome

Bancaud et al, Mol Cell 2007

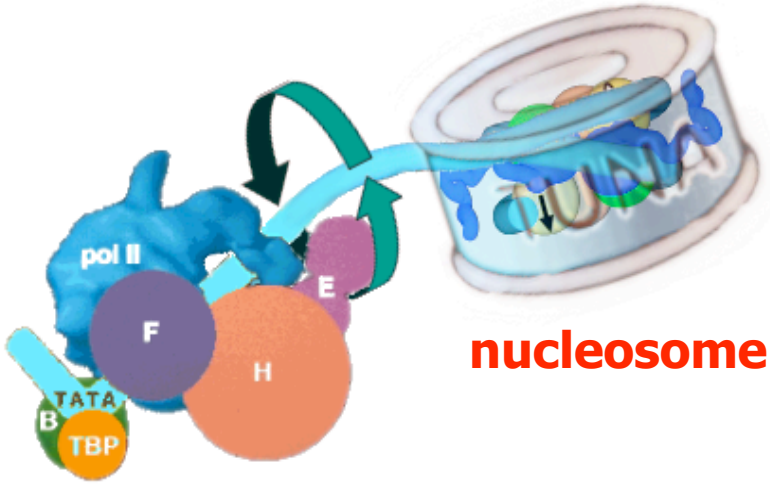
6. back to transcription

coarse grained (rigid body) numerical simulation

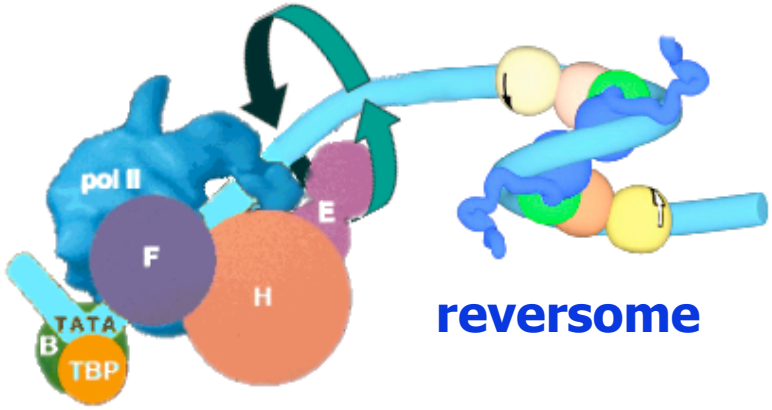


⇒ *need for the undocking of the H2A-H2B dimers*

⇒ **reversome as a "transparent" state of nucleosome?**

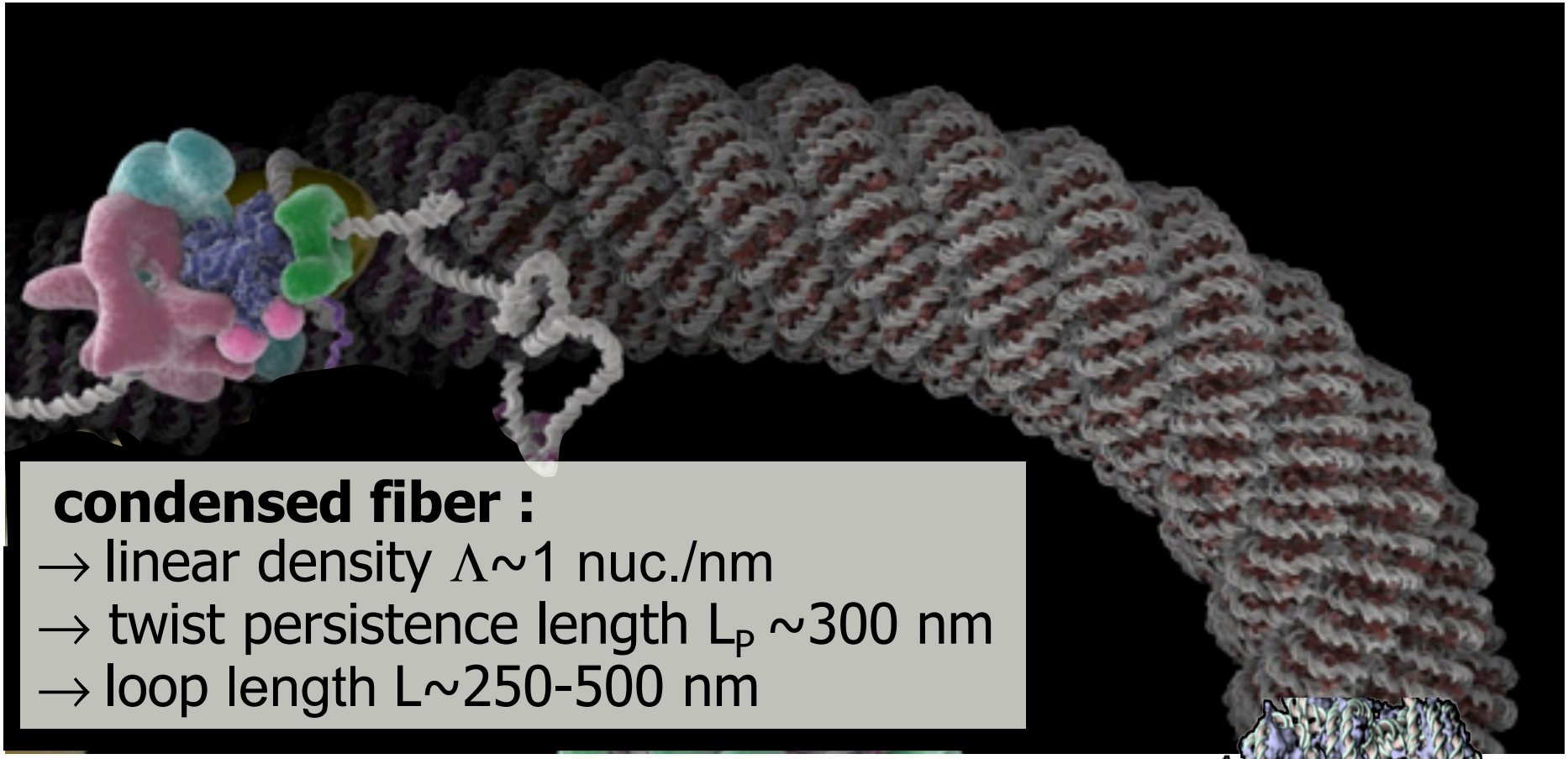


nucleosome



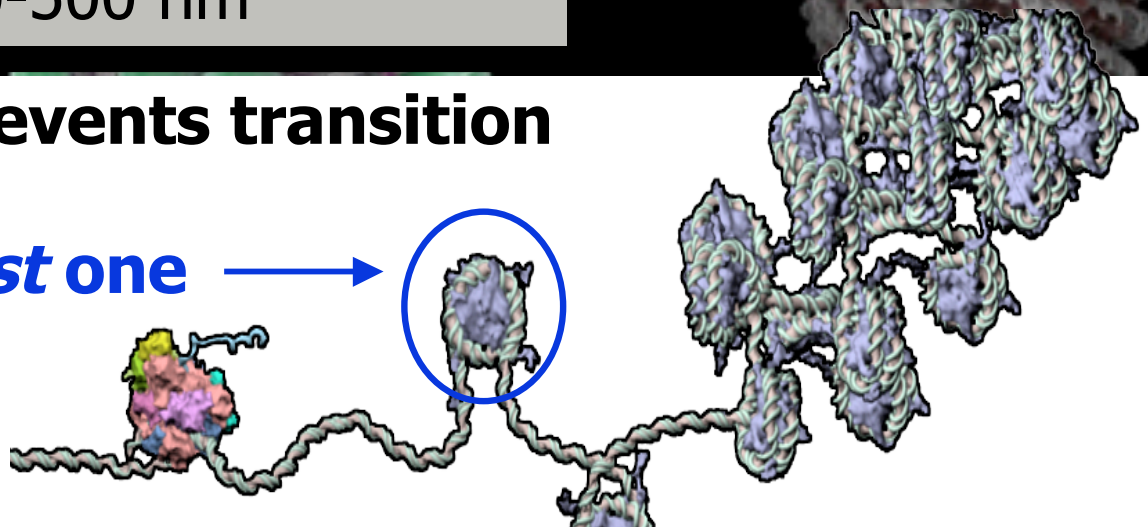
reversome

6. back to transcription



condensed fiber :
→ linear density Λ~1 nuc./nm
→ twist persistence length L_p ~300 nm
→ loop length L~250-500 nm

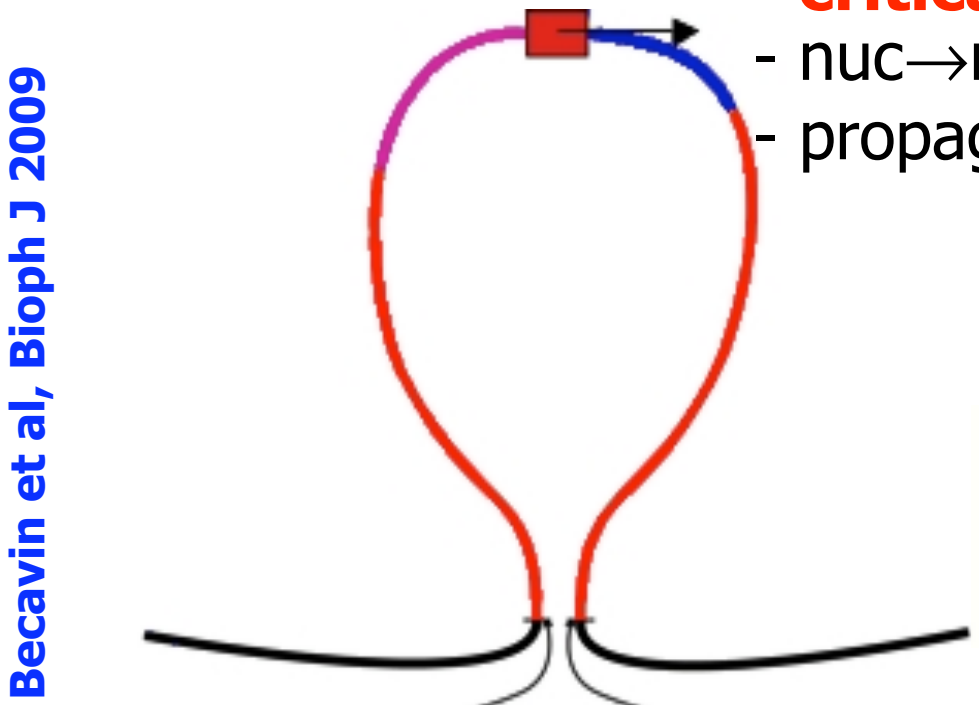
Steric hindrance prevents transition to reversomes
…except for the *first* one



6. back to transcription

→ **"domino effect":**

- **critical torque** for the transition
- nuc→rev transition **one by one**
- propagation of a **reversome front**

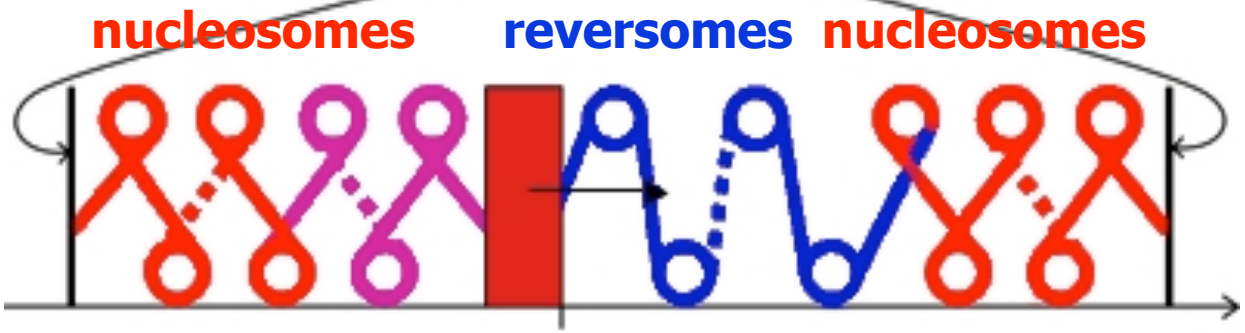


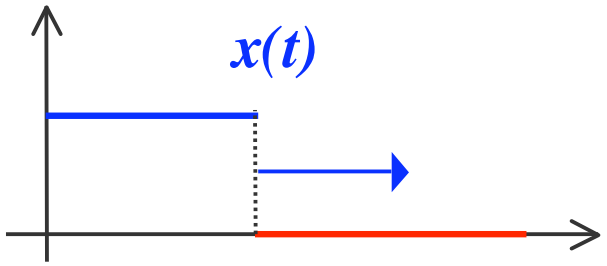
Becavin et al, Bioph J 2009

reversome fiber :

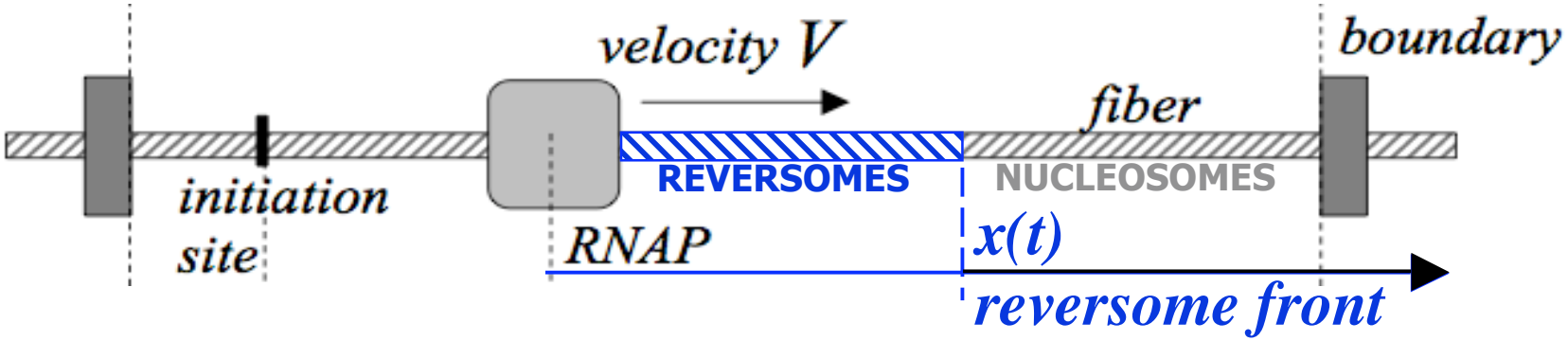
→ linear density $\Lambda \rightarrow \sim 1/2$

→ twist p. length $L_P \rightarrow \sim 1/10$





6. back to transcription



Becavin et al, Bioph J 2009

linking number conservation:

$$\frac{\omega_0 t}{2\pi} = \left[\Lambda\ \Delta Lk + \frac{\tau}{2\pi}\right]\cdot x(t)$$

RNA pol rotation | **reversome fiber internal twist (ΔLk nucl→rev)** | **reversome fiber torsion (<<)**

Λ *linear density*

Γ *applied torque*

L_P *twist pers. length*

$\tau = \dfrac{\Gamma}{L_P k_B T}$ *fiber torsion*

τ_c *critical torsion*

reversome front progression:

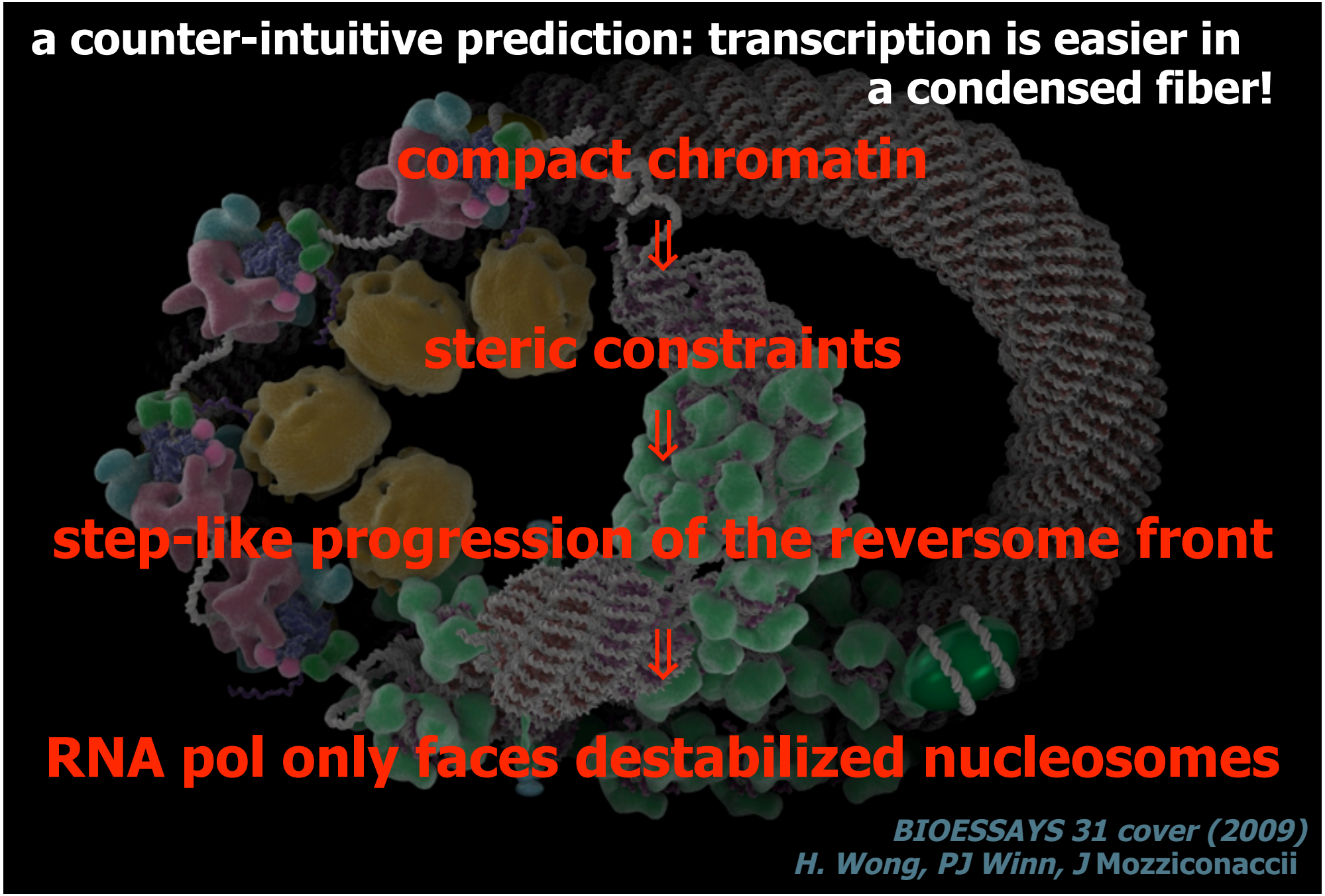
$$x(t) = v\,t + c, \quad v = \frac{\omega_0}{2\pi\ \Lambda\ \Delta Lk + \tau_c} \simeq \frac{\omega_0 / 2\pi}{\Lambda\ \Delta Lk}$$

→ **v = 1 nucl./s**

= 200 bps/s

7. resulting scenario

a counter-intuitive prediction: transcription is easier in a condensed fiber!



compact chromatin

⇓

steric constraints

⇓

step-like progression of the reversome front

⇓

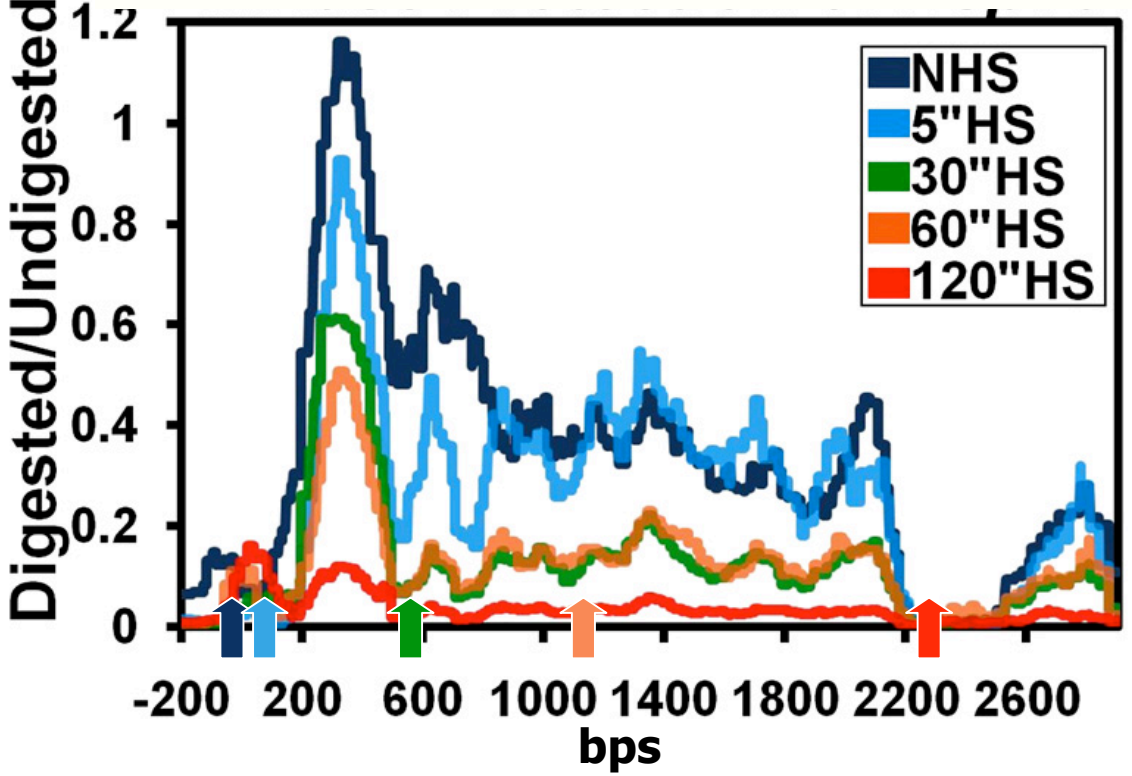
RNA pol only faces destabilized nucleosomes

BIOESSAYS 31 cover (2009)
H. Wong, PJ Winn, J Mozziconaccii

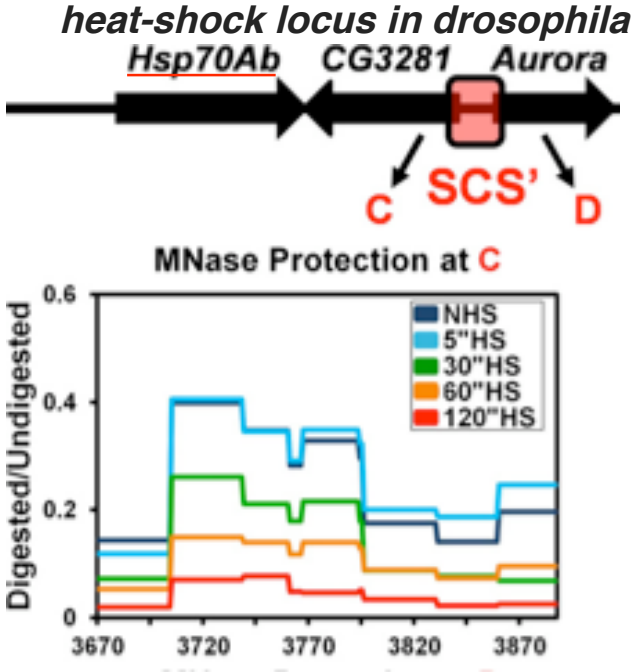
7. resulting scenario

first comparison with experiments !

a wave of nucleosome disruption



(Petesh and Lis, Cell 2008)



30'' : all the loop and only the loop

reversome-wave interpretation:

- RNA pol at start
- 100 bps elongation → 10 turns, 5 rev., 1000 bps
- 600 bps elongation → 60 turns, 30 rev., 6000 bps
- 1200 bps elongation → 120 turns, 60 rev., 12000 pbs
- ...

(Zlatanova and Victor, HFSP J 2009)

Laboratoire de Physique Théorique de la Matière Condensée, Paris

M3V Group

- **Maria Barbi** (UPMC)
- **Annick Lesne** (CNRS)
- **Jean-Marc Victor** (CNRS)
- **Julien Mozziconacci** (UPMC)
- **Hua Wong** (doc 2005–2008)
- **Fabien Paillusson** (doc 2007–2010)
- **Christophe Bécavin** (doc 2007–2010)
- **Pascal Carrivain** (doc 2008–2011)
- **Christophe Lavelle** (postdoc 2003–2004)

- **A. Bancaud et al. Structural reorg. of chromatin fibers revealed by torsional nanomanipulation NSMB 2006**
- **A. Bancaud et al. Torsional manipulation of chromatin fibers reveals a highly flexible structure Mol Cell 2007**
- **C. Lavelle Transcription elongation through a chromatin template Biochimie 2007**
- **C. Lavelle Forces and torques in the nucleus: … Biochem. Cell Biol. 2009**
- **J. Zlatanova & JM. Victor How are nucleosomes disrupted during transcription elongation? HFSP J. 2009**
- **C. Bécavin et al. Transcription within Condensed Chromatin: … Biophys J. 2009**

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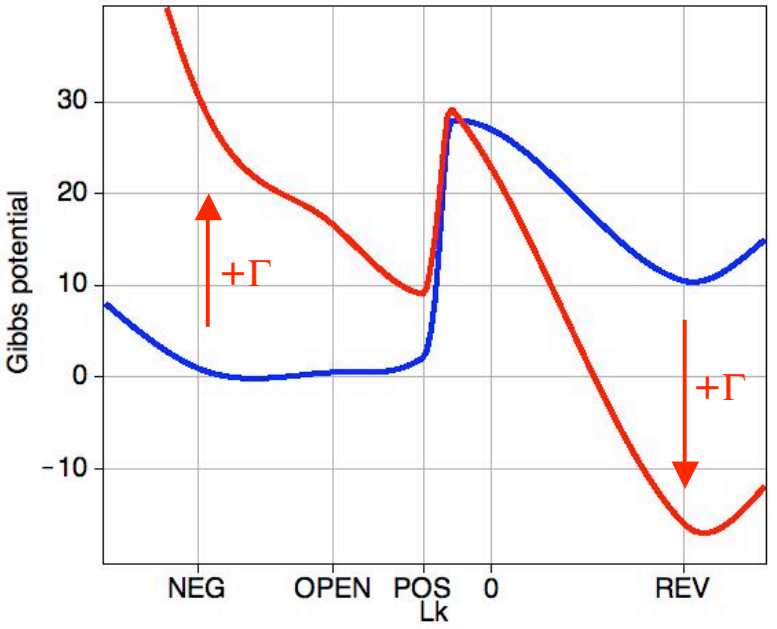
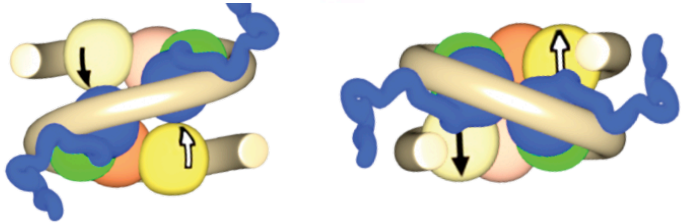
Reversome : a **right handed** metastable state…

stabilized by the applied torque

data fit: **statistical equilibrium between the 3 states**
+ kinetic equations for the passage to reversome:

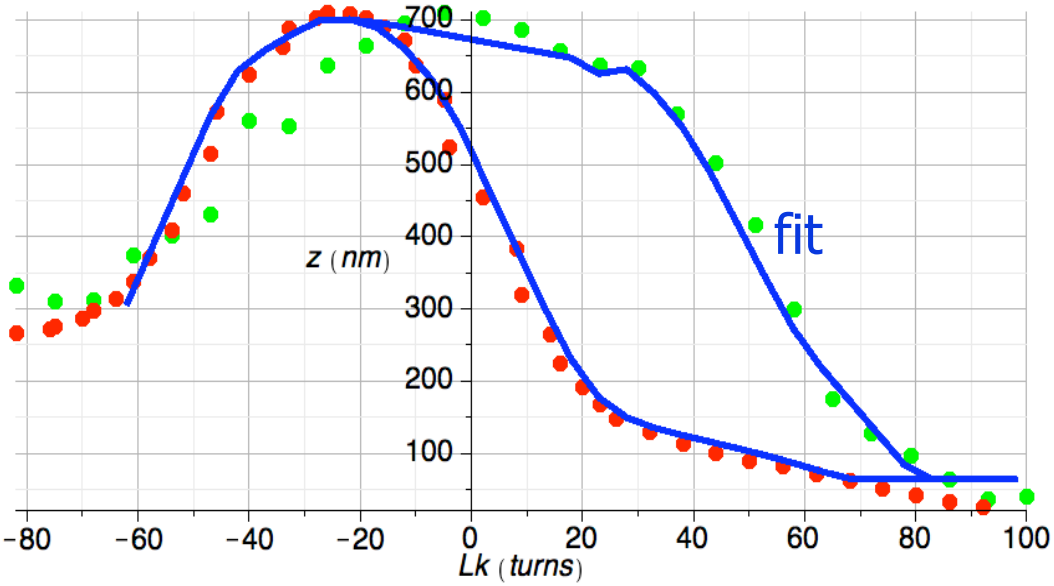




left $\xrightarrow{+\Gamma}$ **right**

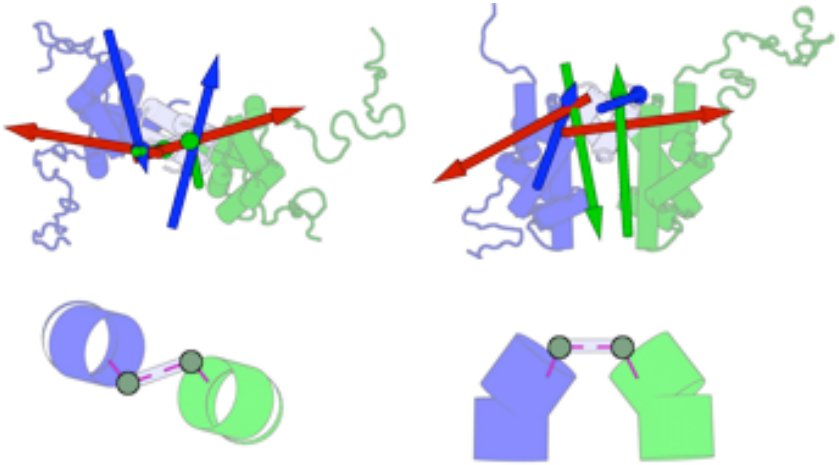
ΔLk~-0.4 ΔLk>~+1

eq. eq. k_1
neg. ↔ open ↔ pos. ⇆ reversome
k_{-1}

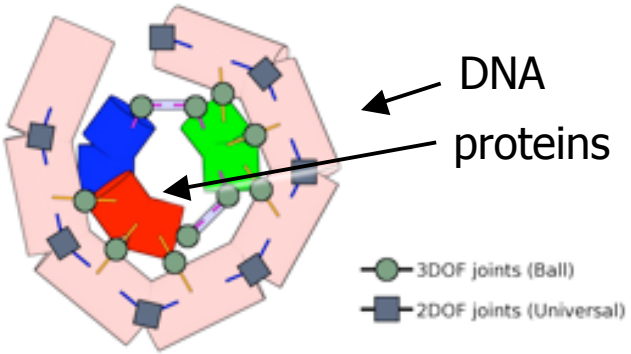


Rigid Body Dynamics with Skeletal Animation

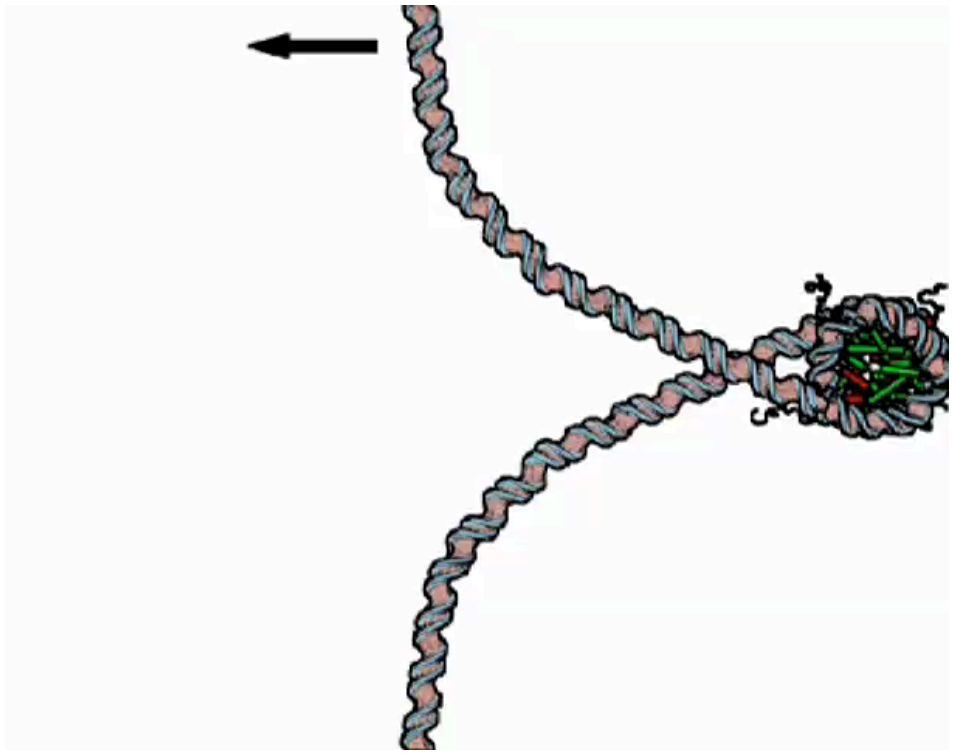
1. normal modes analysis



2. nucleosome structuration

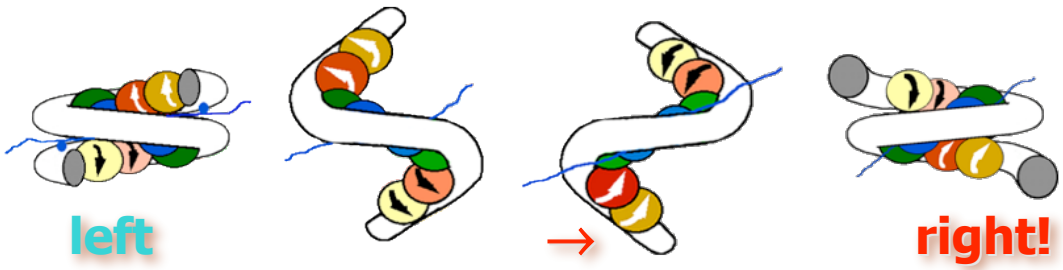


3. Brownian dynamics at constant torque

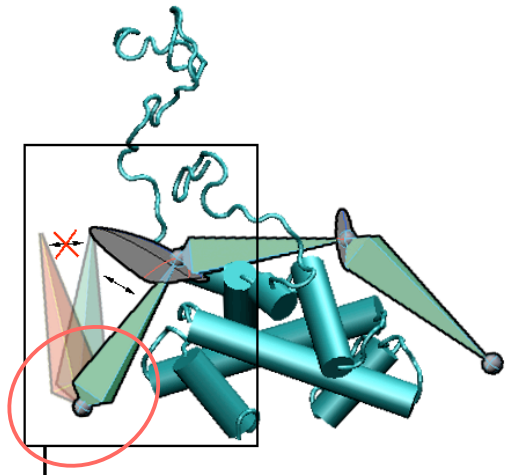


Animation by Hua Wong

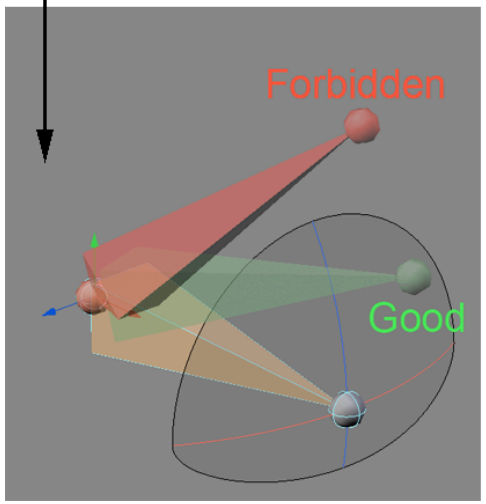
result: chiral transition:

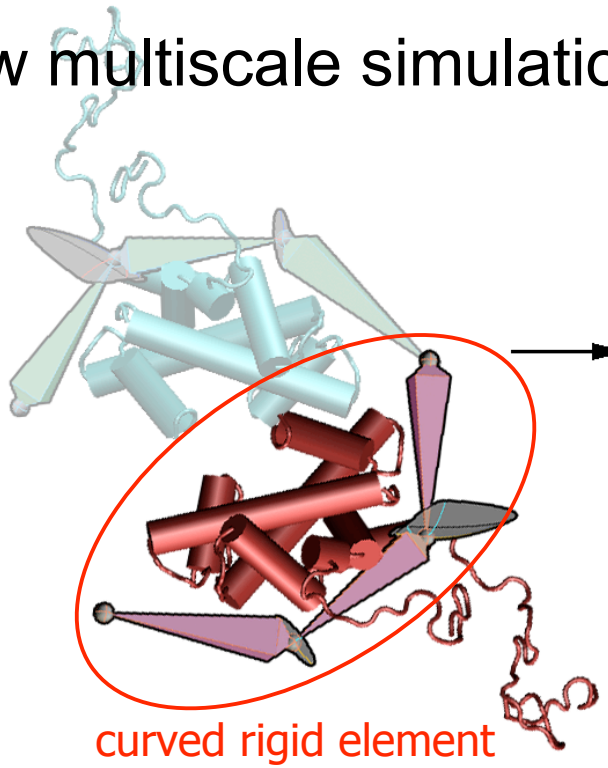


a new multiscale simulation tool:

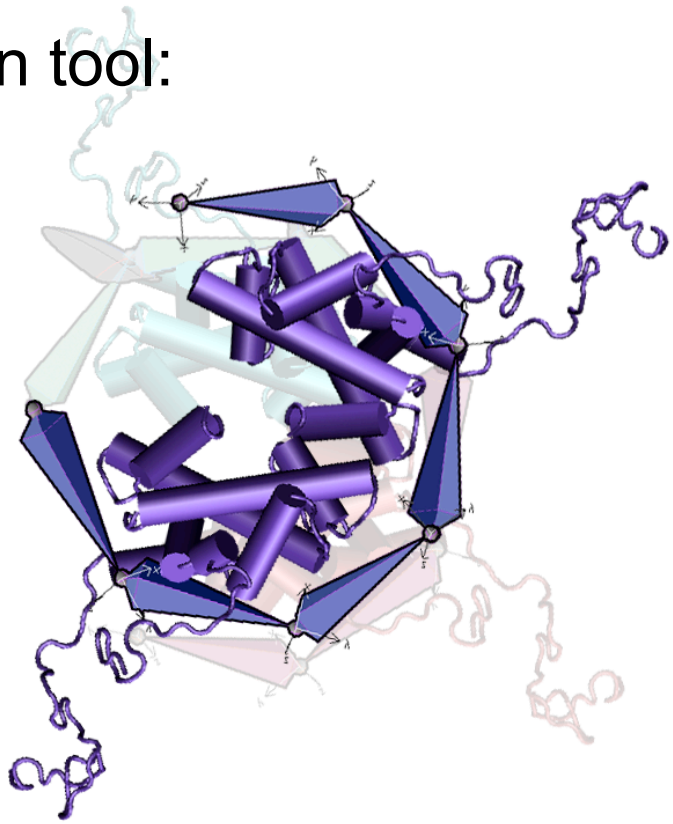


articulated junction

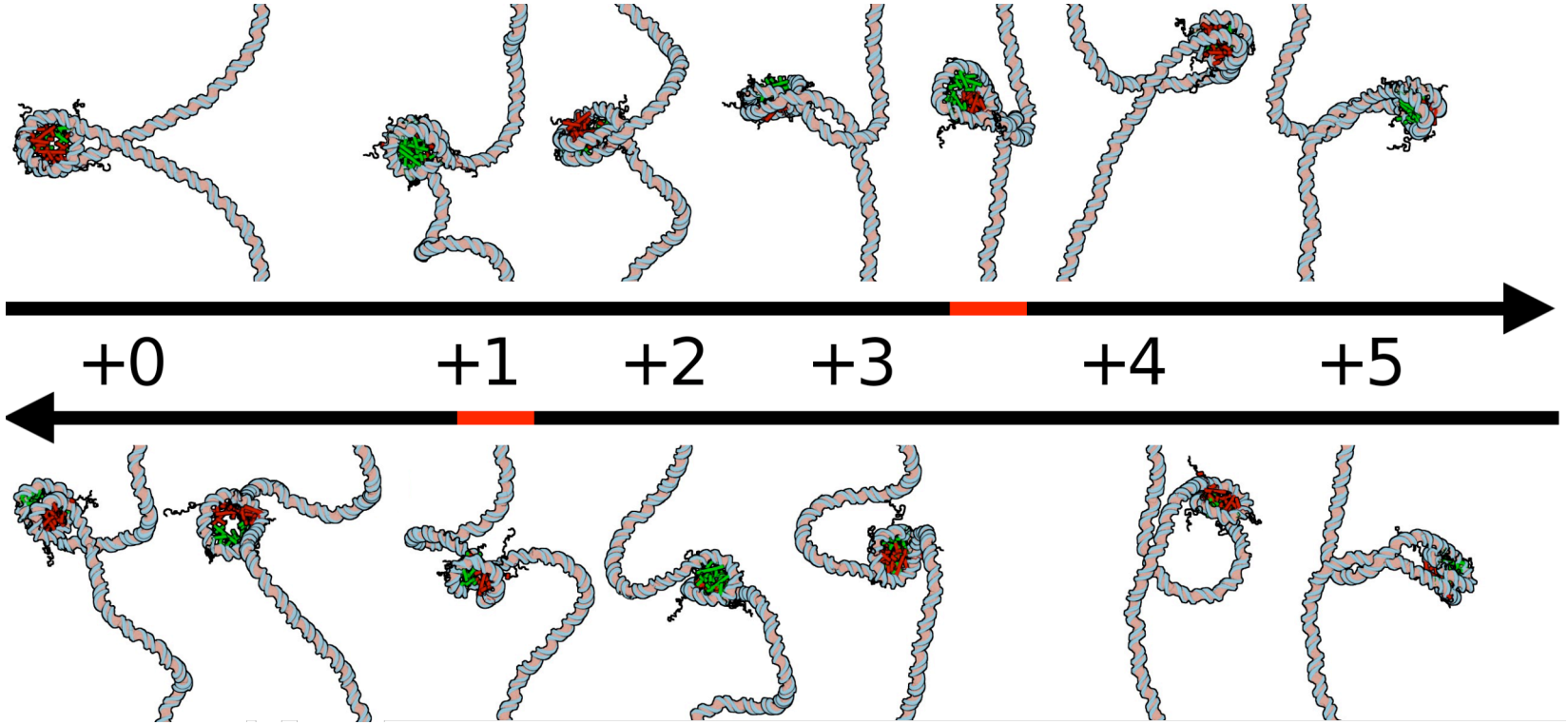




curved rigid element



- 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!

Looking at the transition path