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Conference: From DNA-Inspired Physics to Physics-Inspired Biology

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Dynamics of supercoiled DNA: some recent insights from single-molecule experiments

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Dynamics of supercoiled DNA: some recent insights from single-molecule experiments

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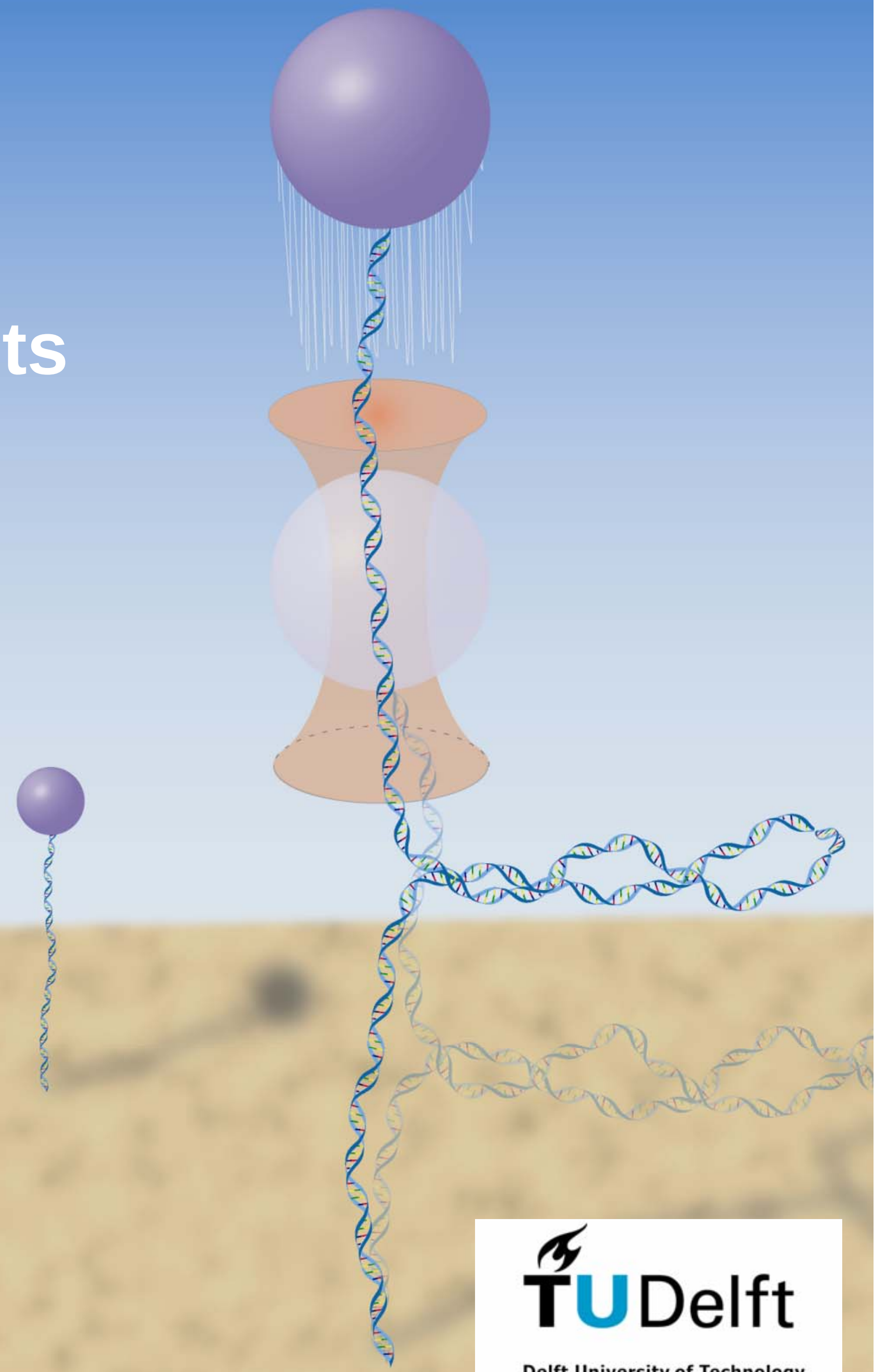


Outline

Introduction

DNA supercoiling and its biological importance

1) Physical removal of supercoils

a) force-induced supercoil removal

b) nick-induced supercoil removal

2) Enzymatic removal of supercoils

a) topoisomerases

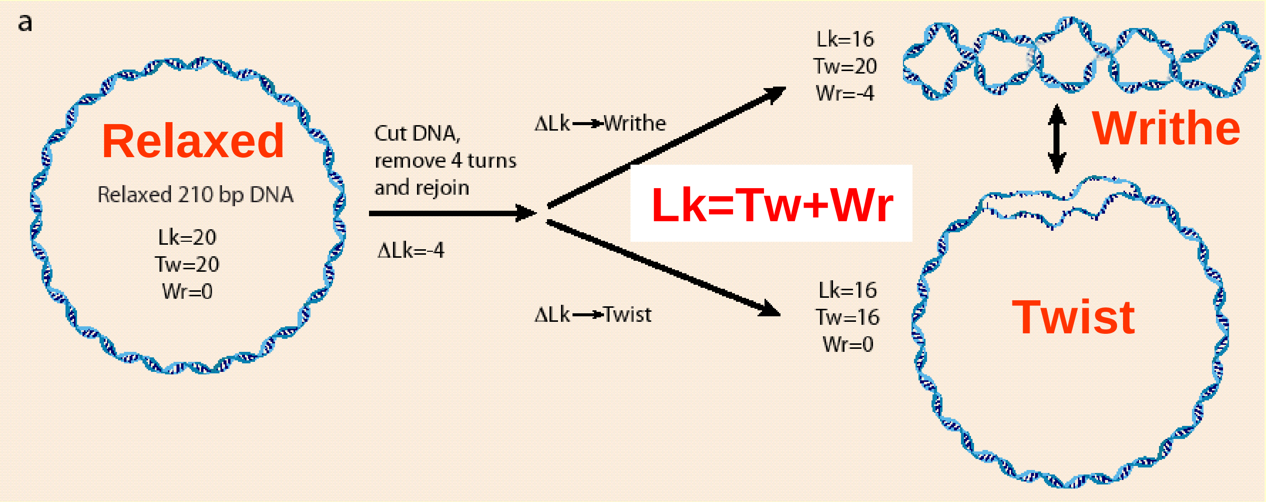
b) ligases

Conclusions

- timescales associated to supercoil removal
- future prospects

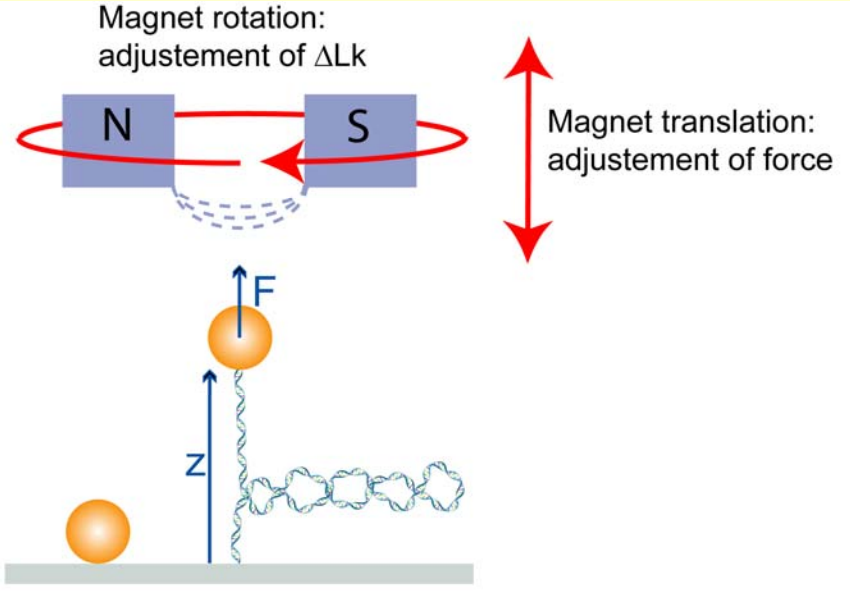
DNA topology in a nutshell

Description of DNA topology



Manipulation of a single DNA molecule with magnetic tweezers

- Lk directly controlled by magnet rotation
- Access to Wr from DNA apparent length



T.R. Strick, J.F. Allemand, D. Bensimon,
A. Bensimon, V. Croquette Science **271** (1996)

Biological relevance of DNA topology

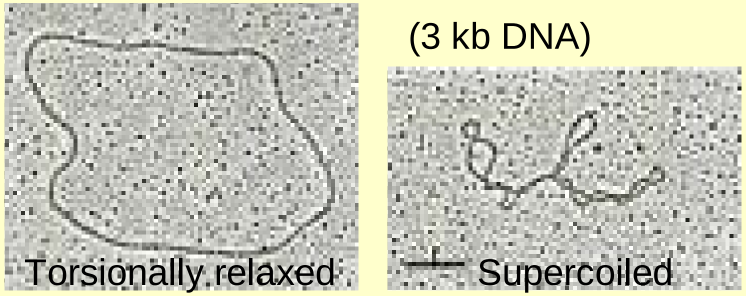
. Control of gene expression

Binding of RNA polymerases to DNA is generally affected by supercoiling

L.M. Fisher, Nature **307** (1984).
M.R. Gartenberg and J.C. Wang, PNAS **89** (1992).

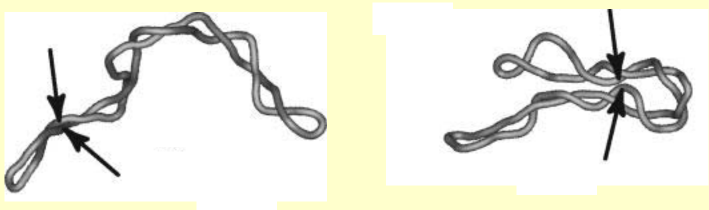
. DNA compaction

Supercoiled DNA is more compact



. Site juxtaposition

Essential for site-specific recombination



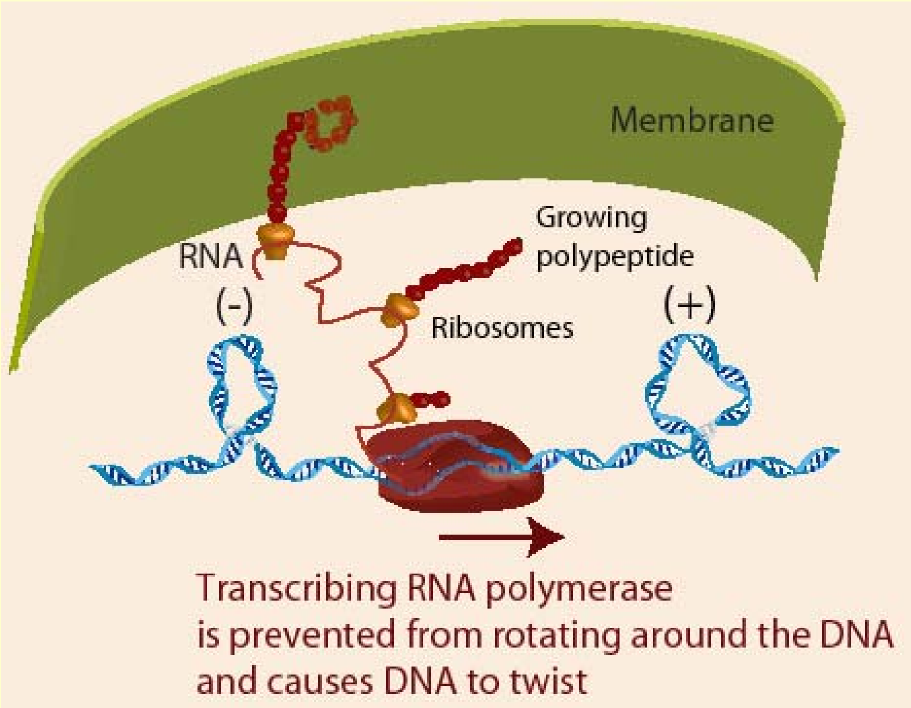
⇒ DNA topology needs to be strongly regulated !

- Cellular processes generate supercoiling.
- Supercoiling is dissipated by both “physical” and enzymatic processes.

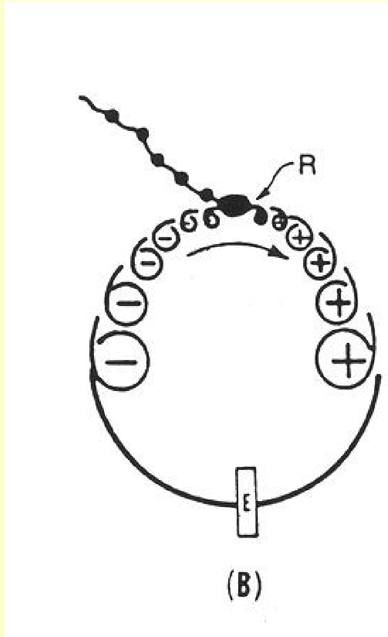
Example: transcription

DNA supercoiling during transcription

Transcription-induced supercoiling of DNA



Supercoil waves on a circular DNA



Liu and Wang, *PNAS* **84** (1987)

Supercoil waves are relaxed by two processes in kinetic competition:

- by **propagation and merging** (no change in Lk)
- by action of **topoisomerases** (permanent change in Lk)

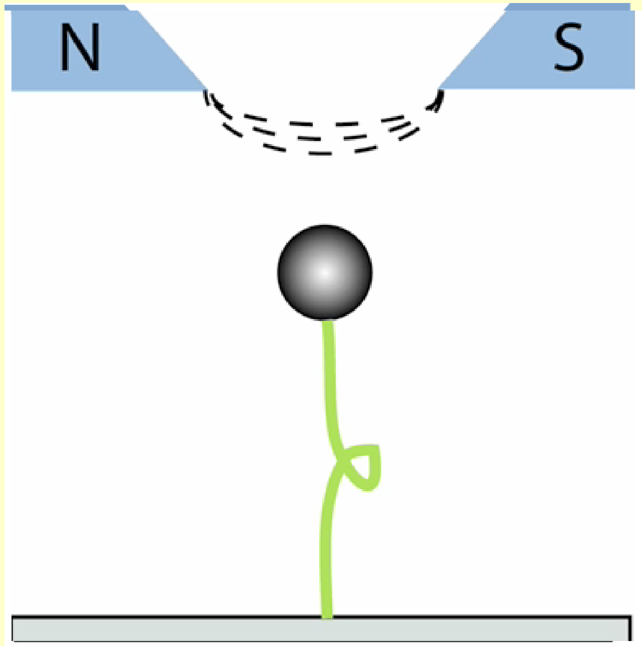
A quantitative description of the dynamics of these processes is required !

Part 1:

Physical removal of supercoils

a) Force-induced supercoil removal

Experimental strategy



- 22 kb DNA
- ∅ 3 microns magnetic beads

Experimentally accessible parameters:
- stretching force
- bead position

Variations of DNA topology during stretching phase

$$\Delta Lk = \Delta Tw + Wr$$

Initial bead rotation *(constant)*

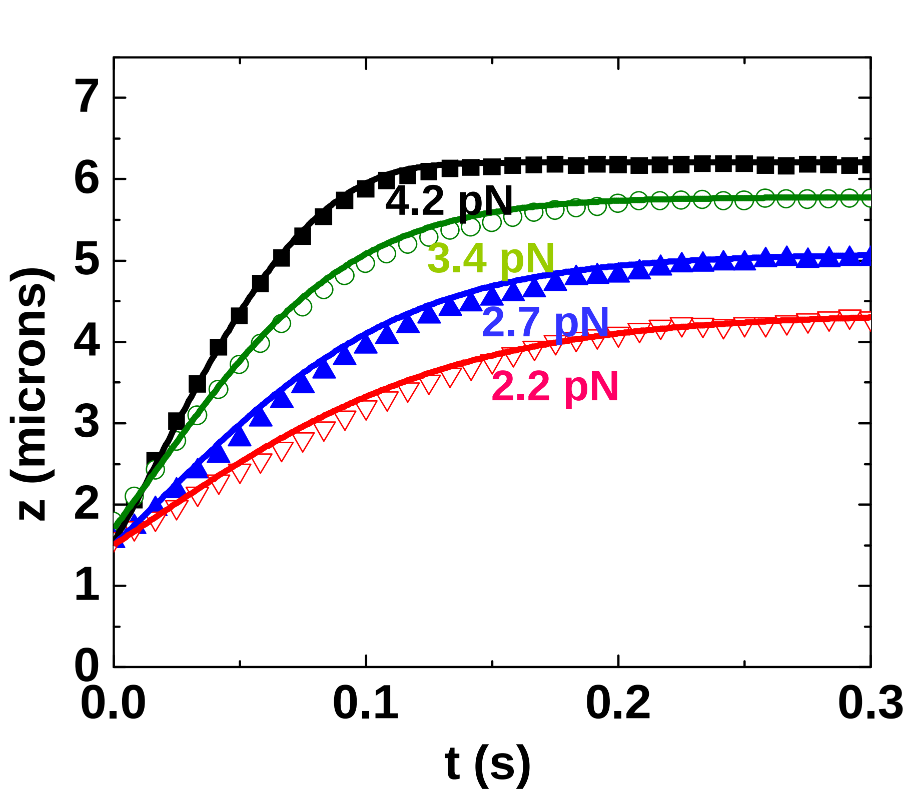
Twist *(increases)*

Plectonemes *(decreases)*

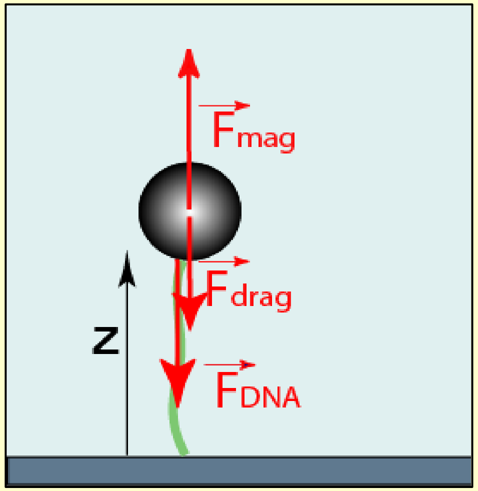
movie

Results: quasistatic dynamics

Experimental results



Modeling



Equation of motion:

$$F_{mag} = \zeta_{bead}\,(z)\ dz/dt + F_{DNA}\,(z)$$

Quasistatic model:
same as at equilibrium

Conclusion

No need to include a friction term associated to plectoneme removal

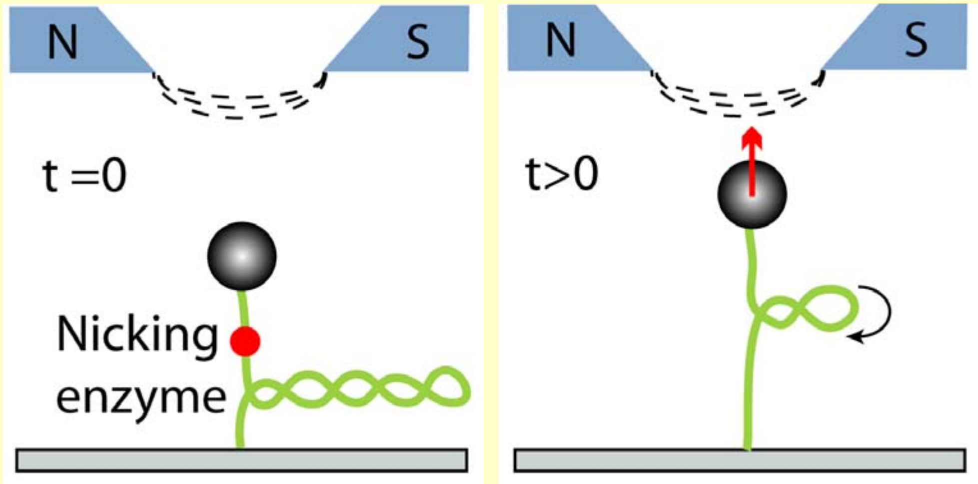
⇒ **Supercoil removal is "fast" !**

Experiments set an **upper bound on rotational drag**.

A. Crut, D.A. Koster, R. Seidel, C. Wiggins & N.H. Dekker, PNAS **104** (2007).

b) Nick-induced supercoil removal

Experimental strategy



Variations of DNA topology during the stretching phase

$$\boxed{\Delta Lk = \Delta Tw + Wr}$$

All relax to 0

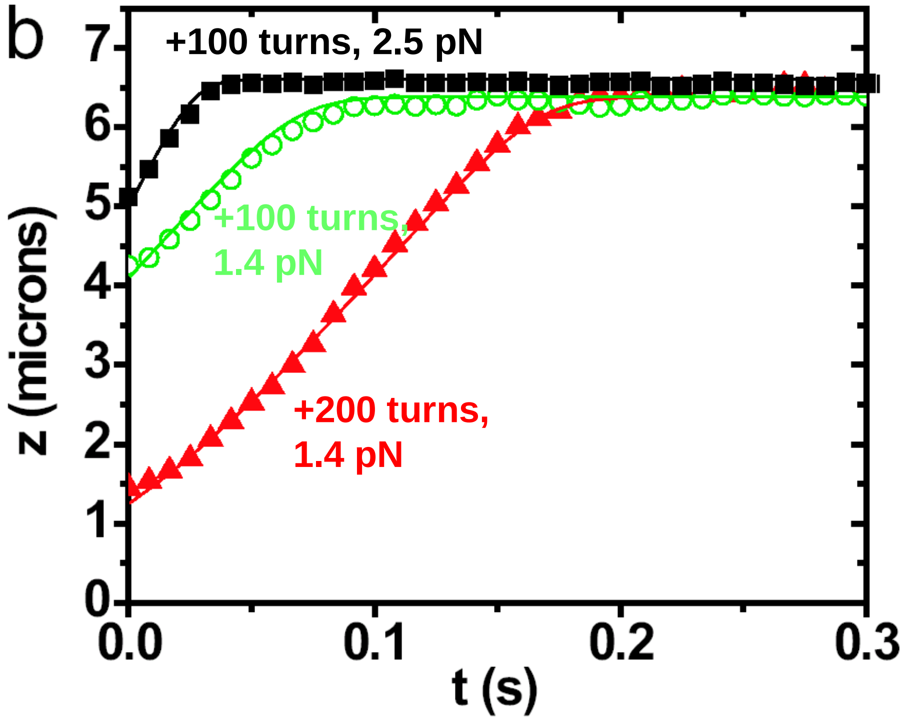
Possible scenarios:

1) Dynamics limited by DNA stretching

Immediate supercoil removal followed by stretching of torsionally relaxed DNA

2) Dynamics limited by supercoil removal

Results



Modeling (plain lines): quasistatic model for **torsionally relaxed DNA.**

Conclusion

- **Supercoil removal occurs within ~10 ms or less**
- **Consistent with the conclusions drawn from force-induced supercoil removal experiments!**

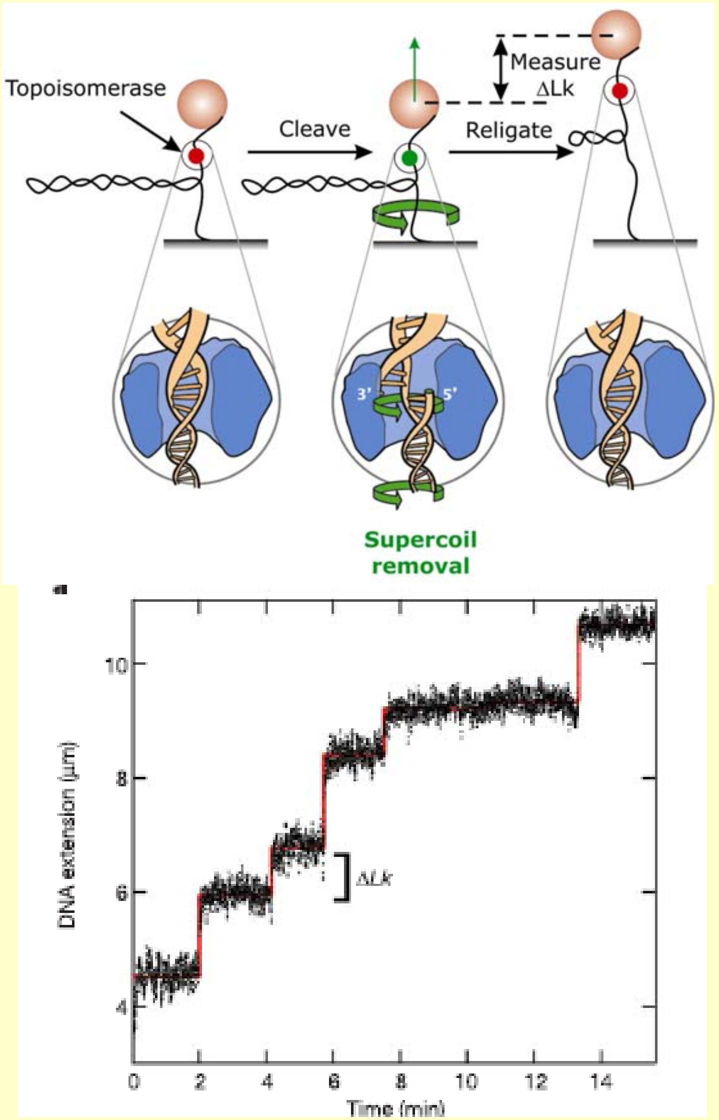
A. Crut, D.A. Koster, R. Seidel, C. Wiggins & N.H. Dekker, PNAS **104** (2007).

Part 2:

Enzymatic removal of supercoils

a) Previous work: supercoil removal by topoisomerase IB

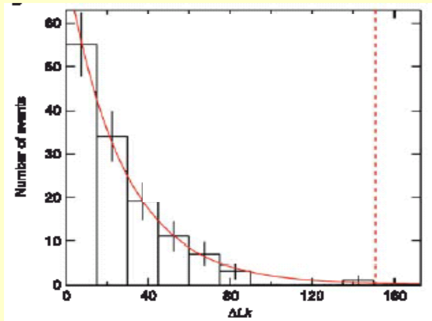
Experiments



D.A. Koster et al., Nature **434** (2005)

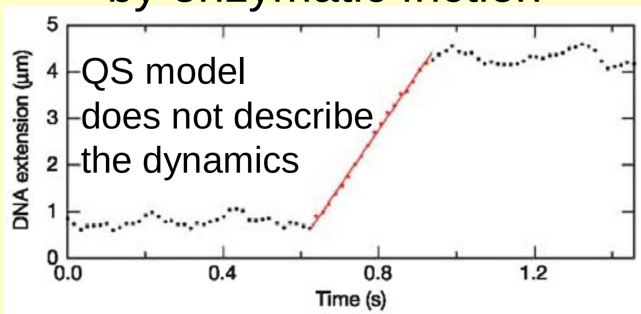
Result 1

Exponential distribution of step sizes



Result 2

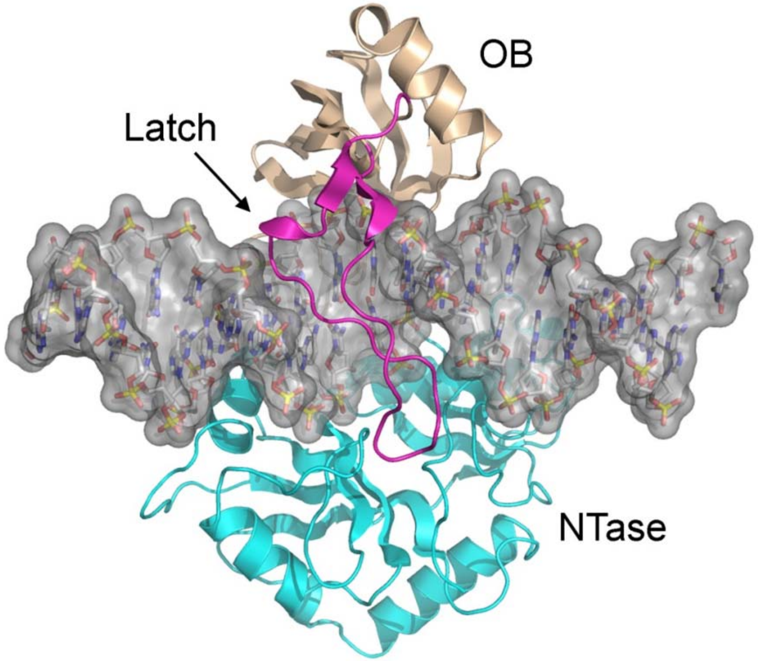
Dynamics are influenced by enzymatic friction



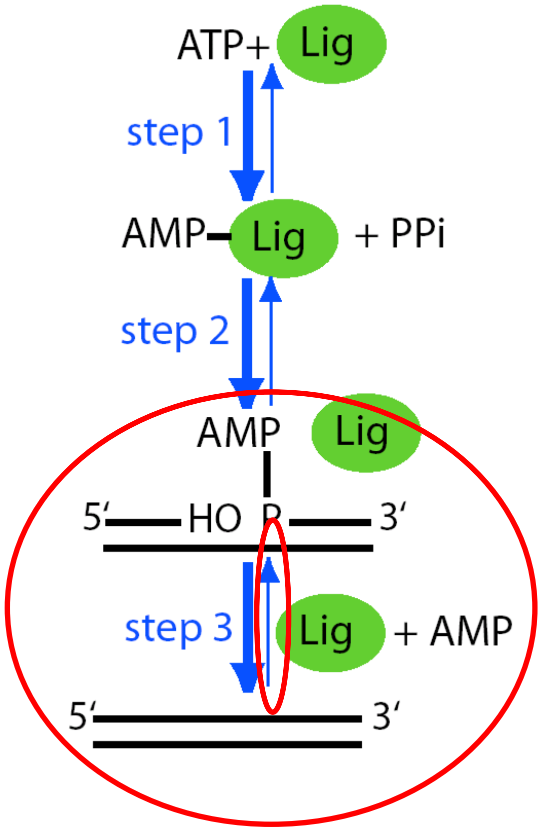
How general are these results??

b) Chlorella Virus Ligase: structure & reaction scheme

Structure of CVLig

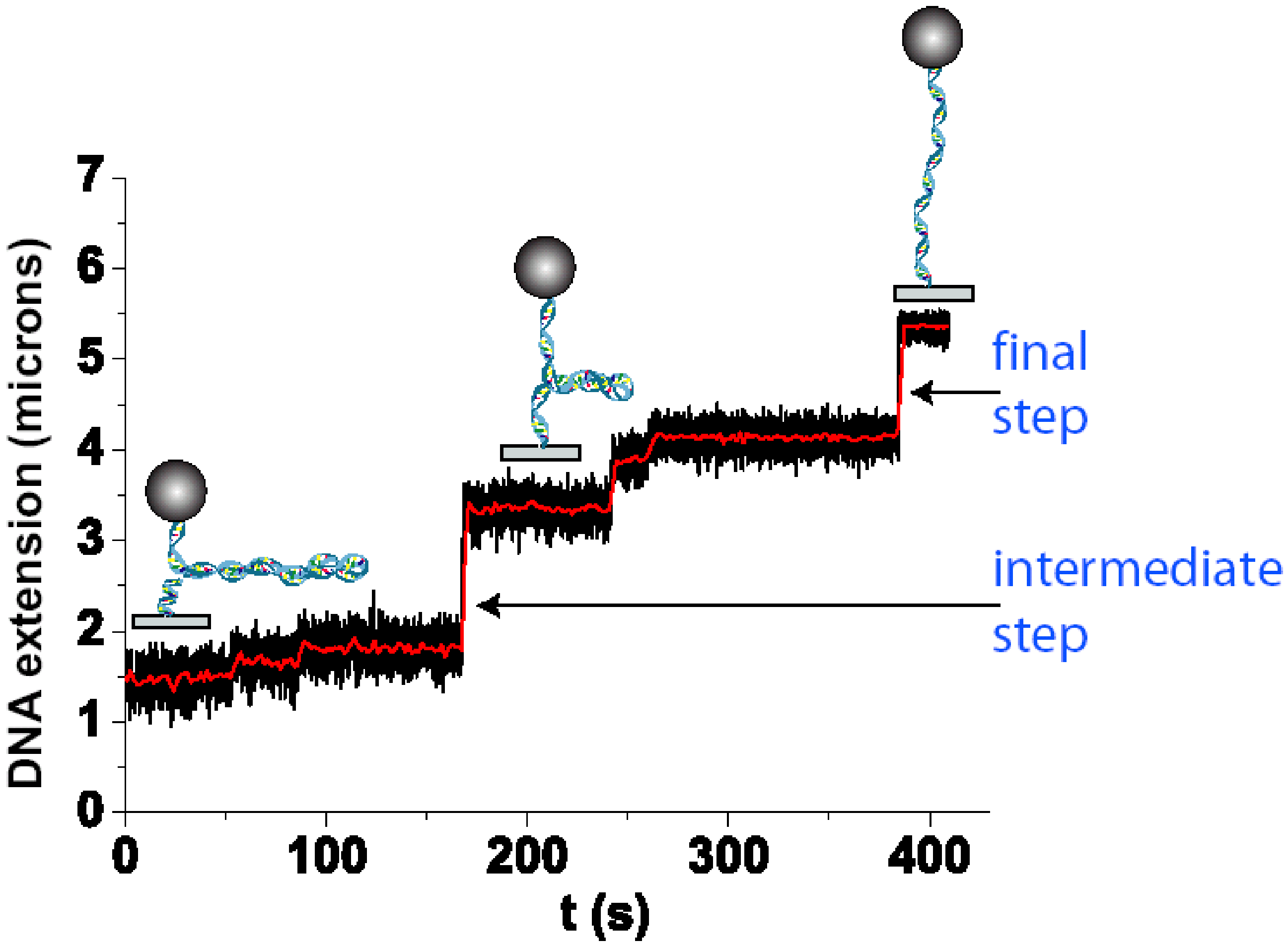


Reaction scheme



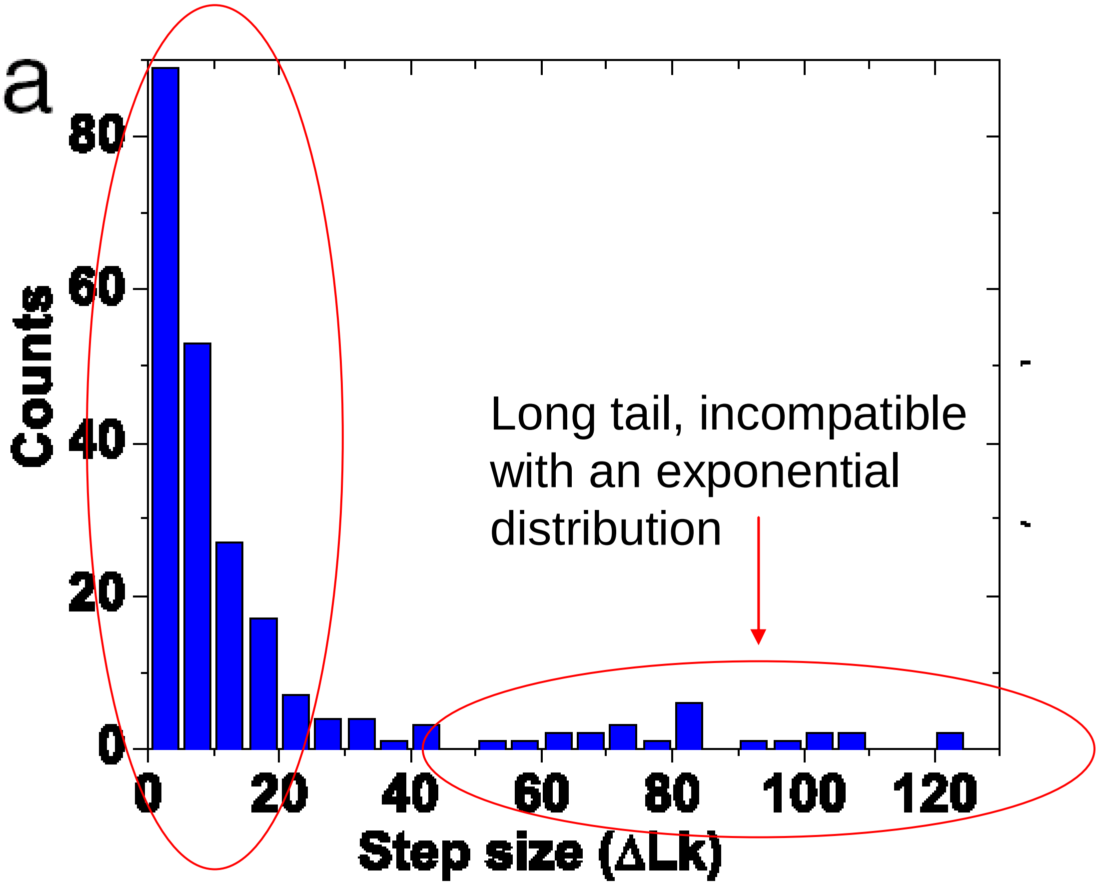
CVLig has a topoisomerase-like activity on supercoiled DNA in the presence of AMP.

Relaxation of a single DNA molecule by CVLig



A. Crut, P. Nair, D.A. Koster, S. Shuman & N.H. Dekker, PNAS **105** (2008).

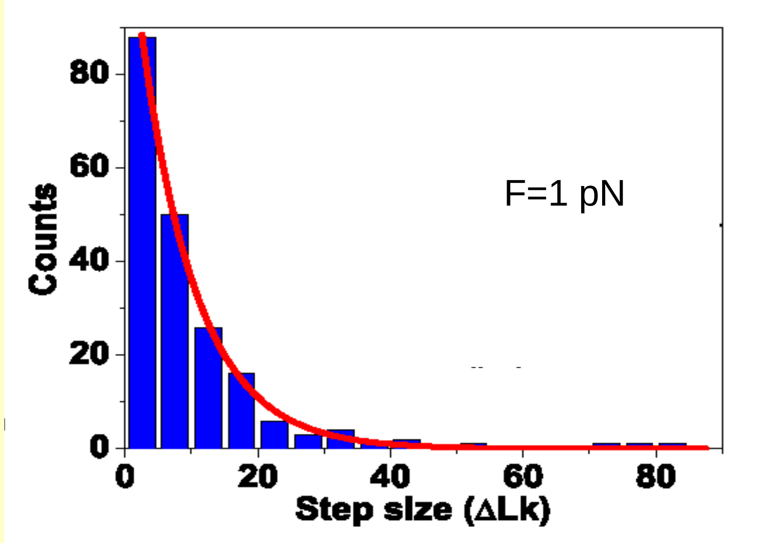
Step size distribution: bimodal distribution



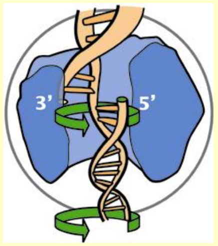
Two distinct populations?
⇒ Separate analysis of intermediate and final steps

Intermediate steps: analysis of step sizes

Step size distribution

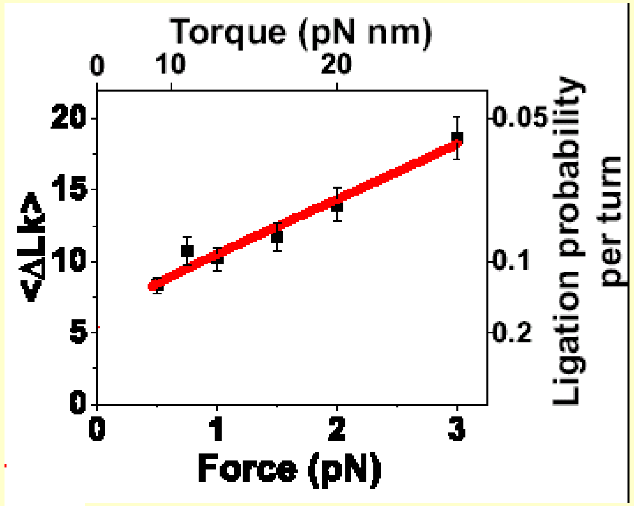


Interpretation:
constant ligation probability per DNA turn.

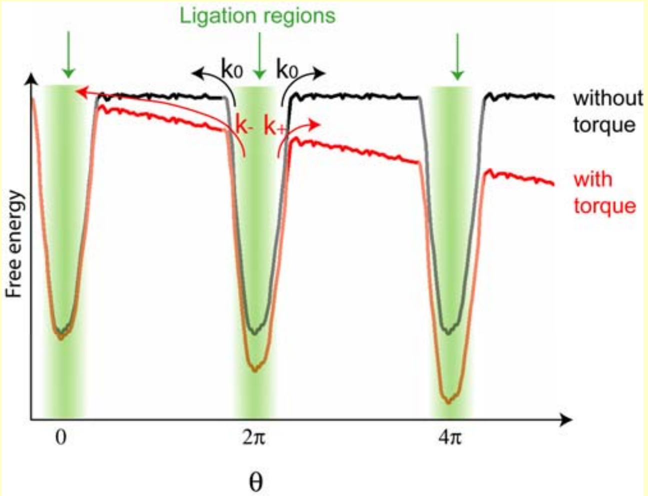


Similar to TopIB
(cf Koster et al., Nature 434 (2005))

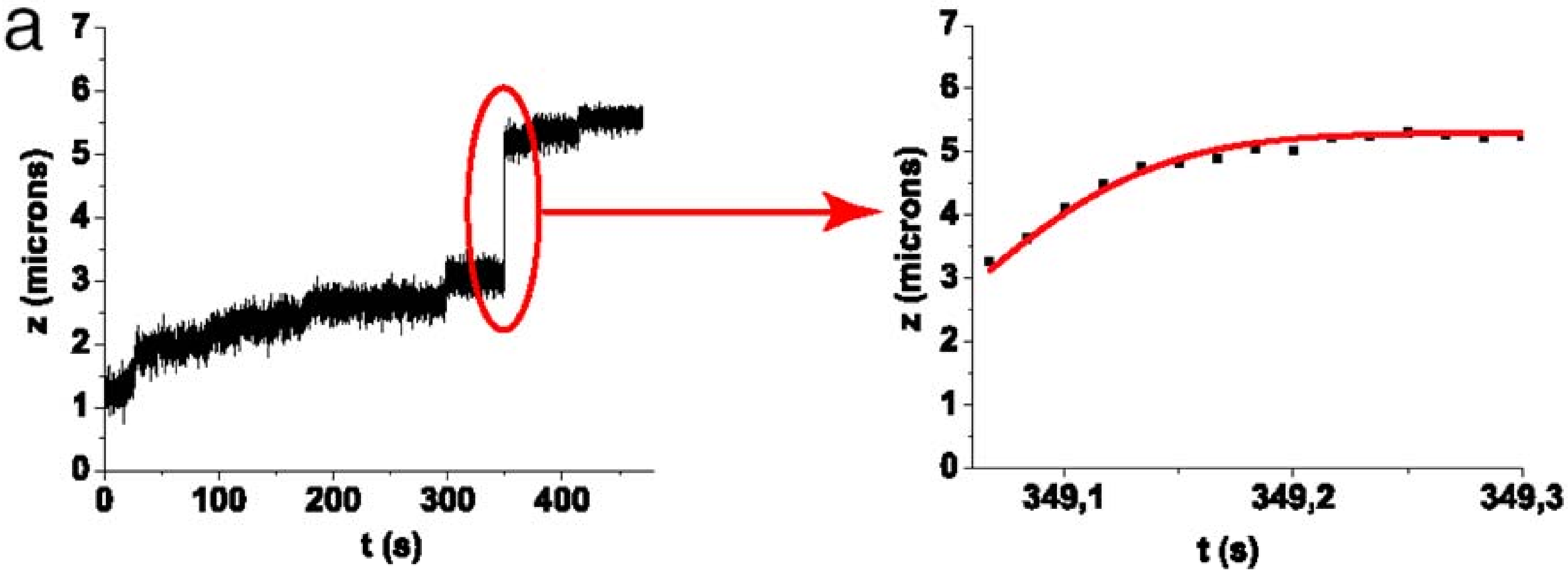
Average step size vs force



Interpretation: diffusion in an energy landscape biased by force



CVLig does not cause observable enzymatic friction



Interpretation:

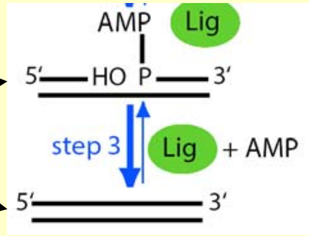
Accurate description of dynamics by the quasistatic model used for bare DNA: CVLig does not induce a significant enzymatic friction.

Difference with TopIB!

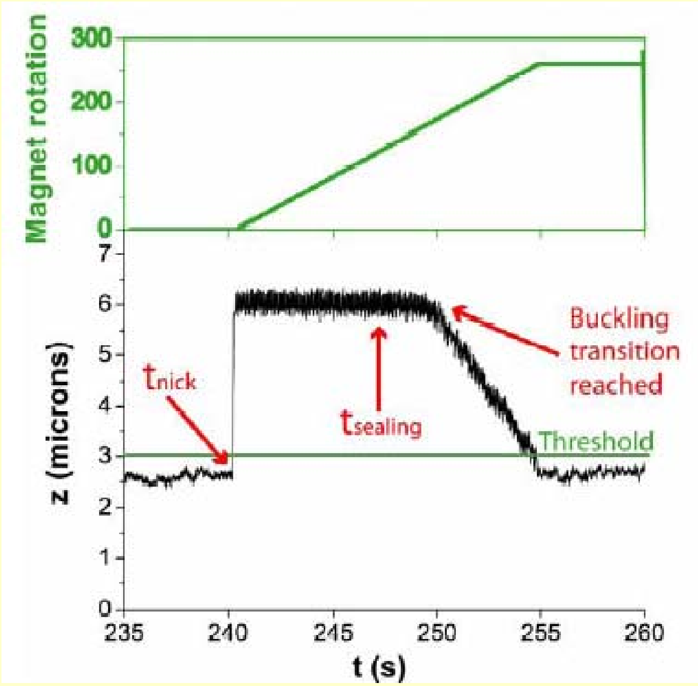
This result also implies that DNA ligation occurs at high rates ($k_{lig} \geq 400\ s^{-1}$)

Evidence for an occasional dissociation of ligase

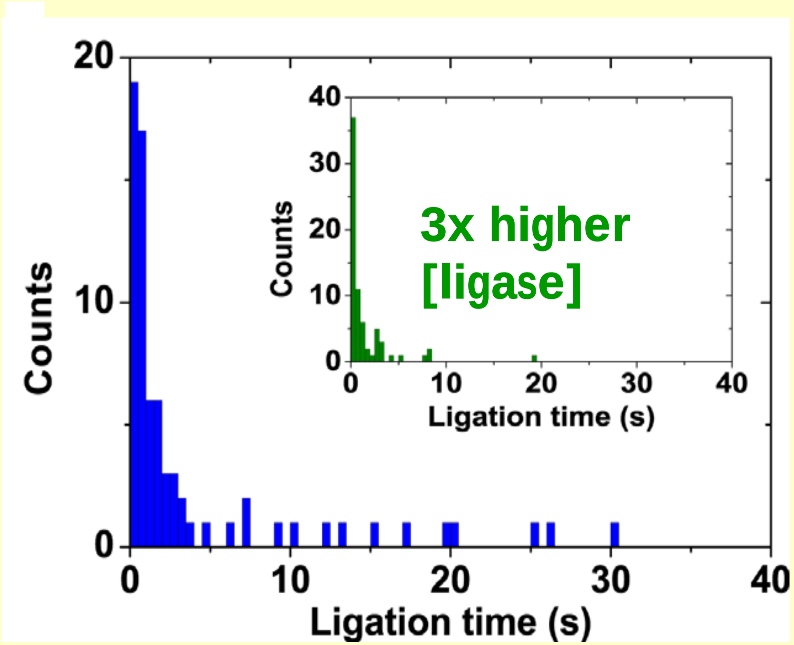
Question: after final steps, is DNA - nicked?
- intact?



DNA remains nicked after most large final steps



Religation time is concentration-dependent

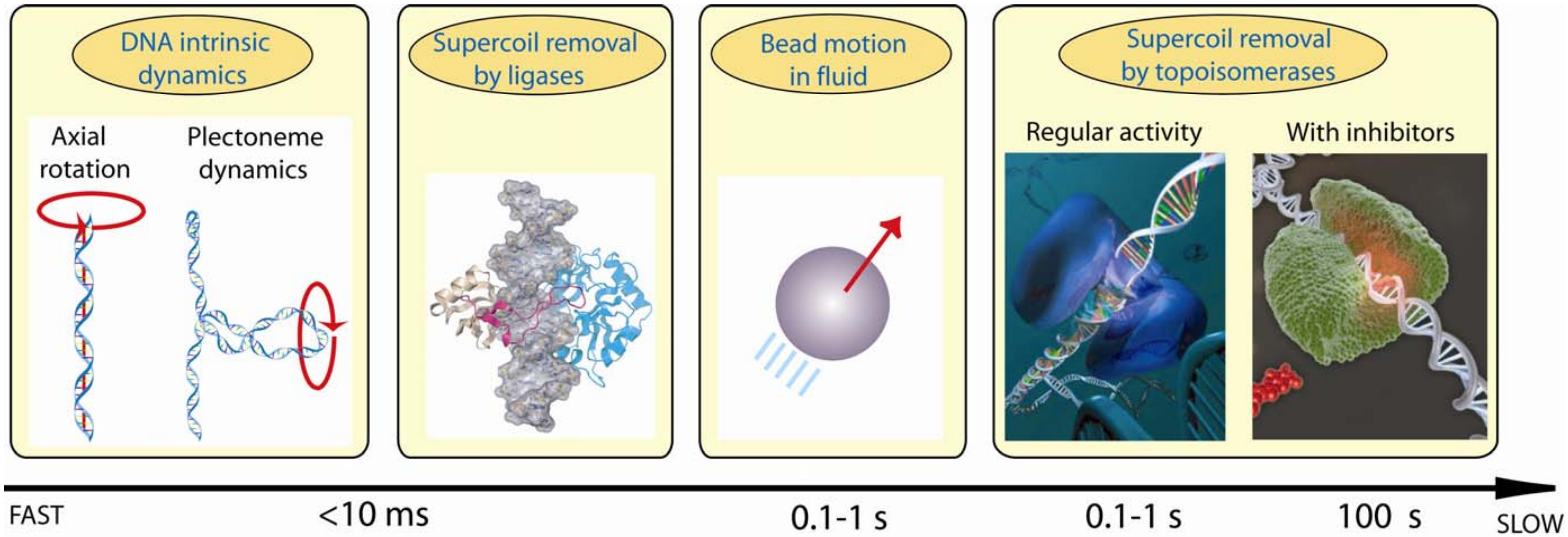


⇒ These observations suggest an **occasional dissociation** of ligase without DNA religation (~10% of all events)

Conclusions: overview of time scales

- **Single-molecule techniques provide access to the dynamics of DNA and DNA-protein interactions in real-time**

- **Time scales associated to various processes involving supercoil dynamics:**



(Typical times for the removal of ~100 supercoils)

- **Interesting points still to be addressed:**
  . Dynamics of DNA in the presence of bound proteins
  . Friction by other enzymes
  . Correlation with DNA imaging…

Acknowledgments

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External Collaborators

Theory

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(Columbia)

Ligase

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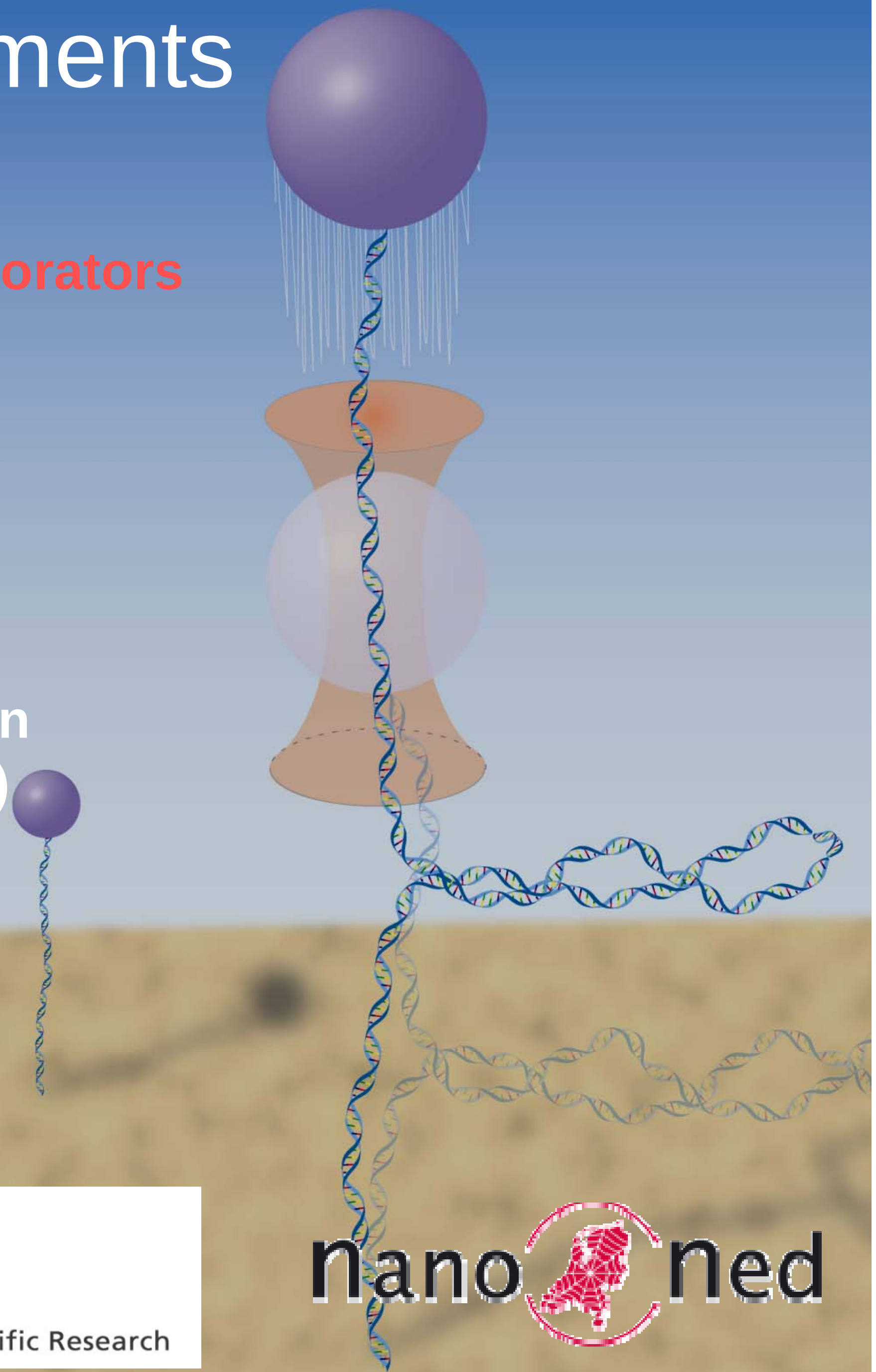
Stewart Shuman
(SKI, New York)

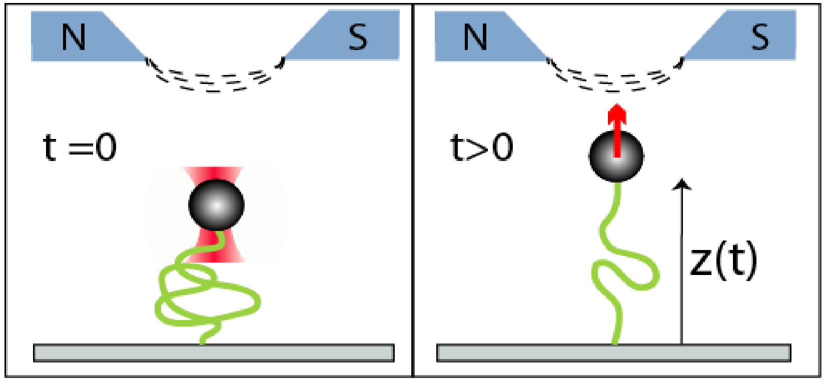


FOM

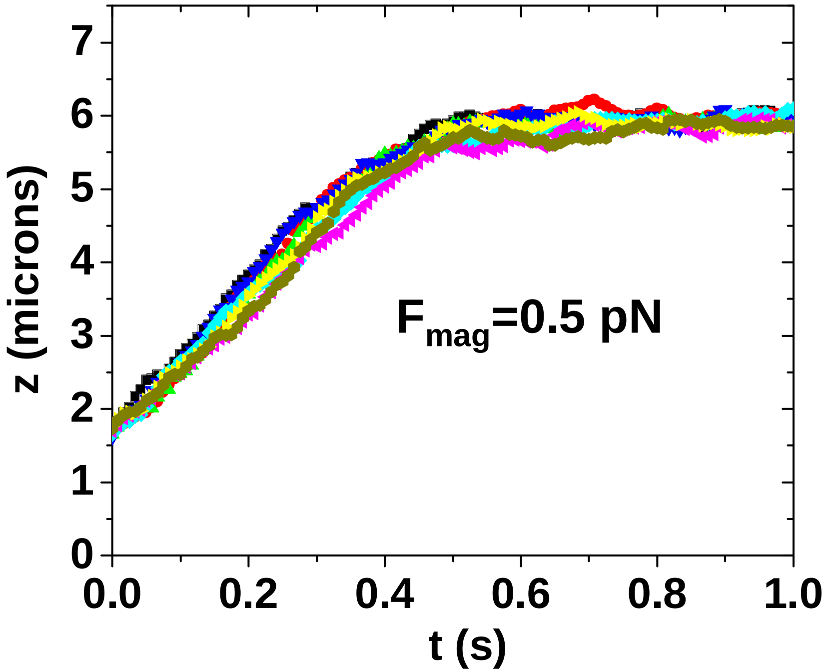
NWO
Netherlands Organisation for Scientific Research

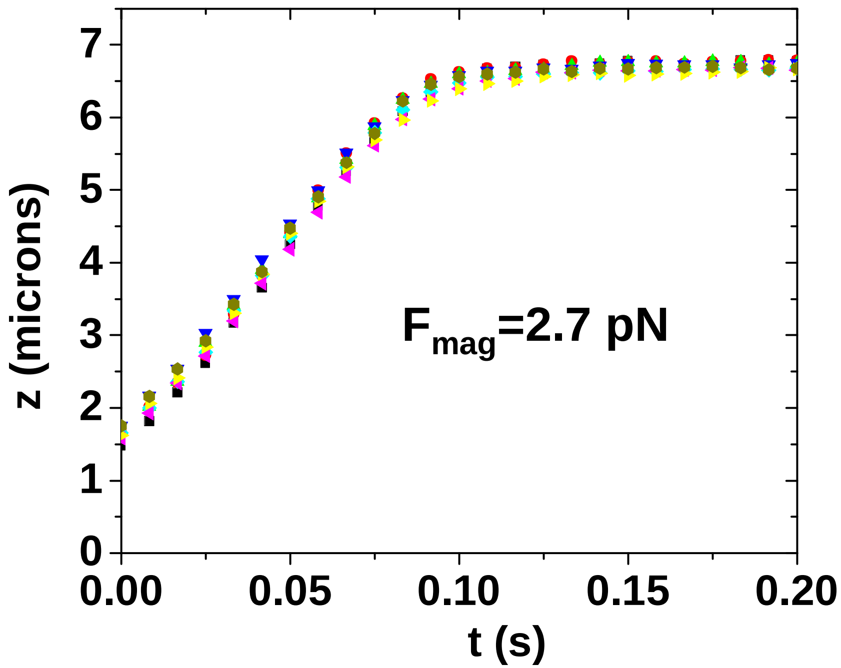
nano ned

Dynamics of torsionally relaxed DNA



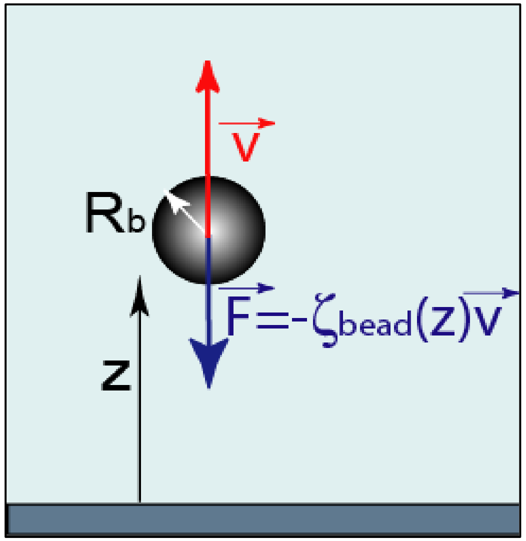
- 8 successive runs
- Later averaged





Ingredients of the analysis

Surface effects

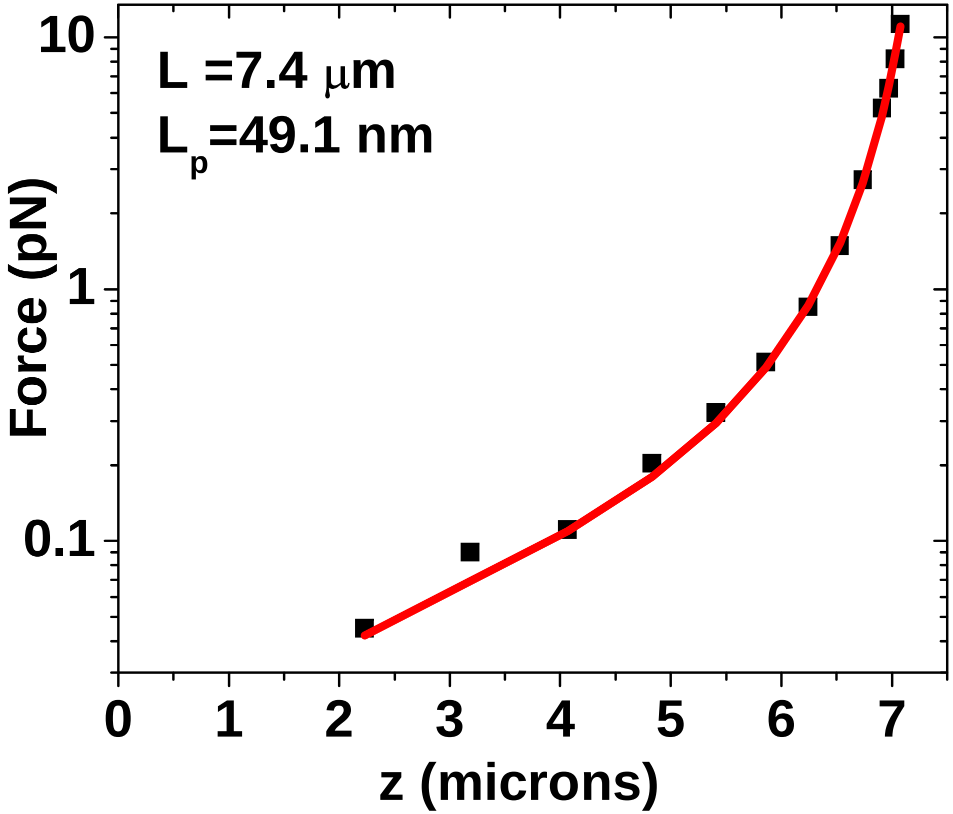


$$\zeta_{bead}(z) \approx \zeta_{Stokes}\,\left(1+\frac{R_b}{z}+\frac{R_b}{2R_b+6z}\right)$$

Correction factor

Bevan and Prieve, *J. Chem. Phys* **113** (2000)

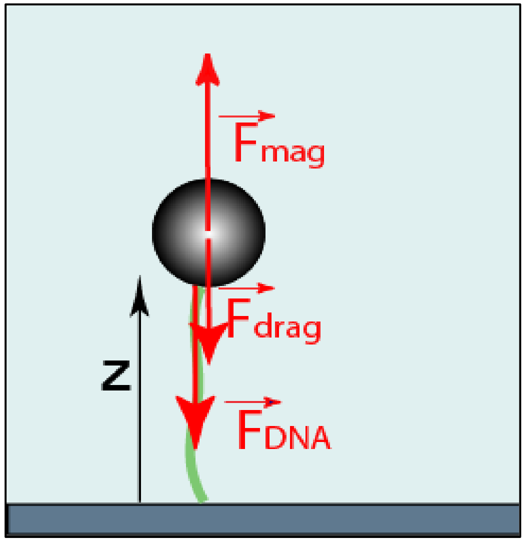
Equilibrium force-extension curve



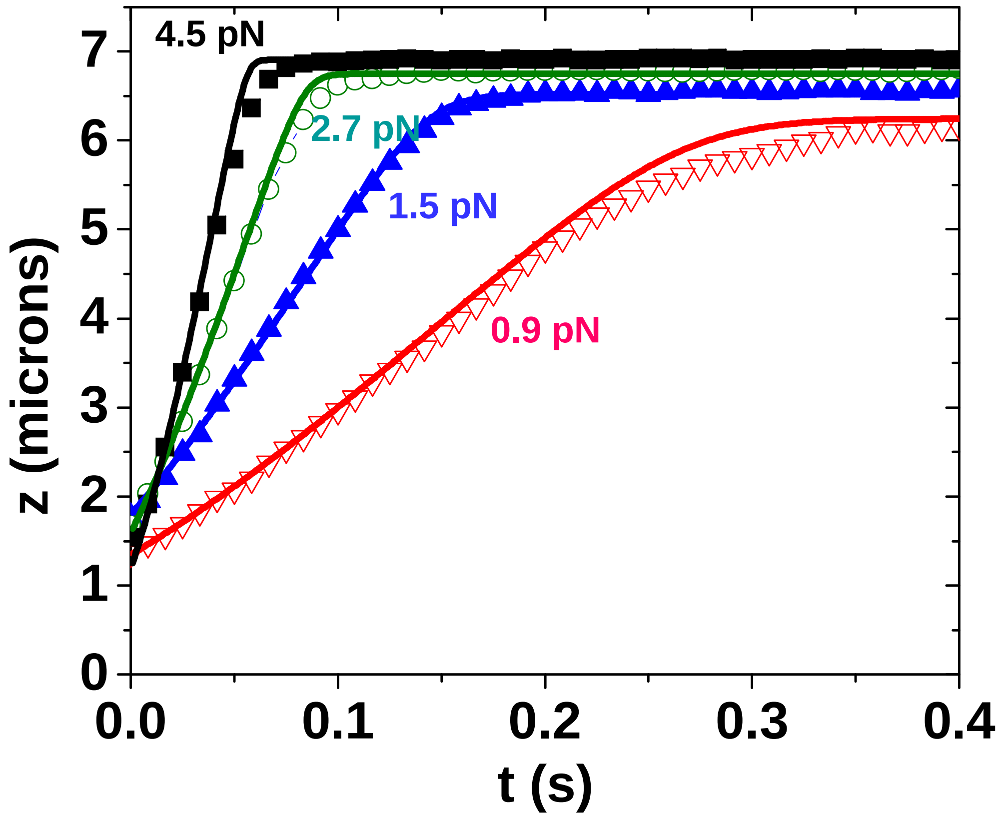
Accurate description
with the **Worm-like Chain model**

The dynamics are quasistatic

Quasistatic model $F_{mag} = \zeta_{bead}(z)\, dz/dt + F_{DNA}(z)$ ← value at equilibrium



Forces involved



Conclusion: **internal dynamics of DNA are fast enough so that DNA is constantly at equilibrium**

Experiments with supercoiled DNA

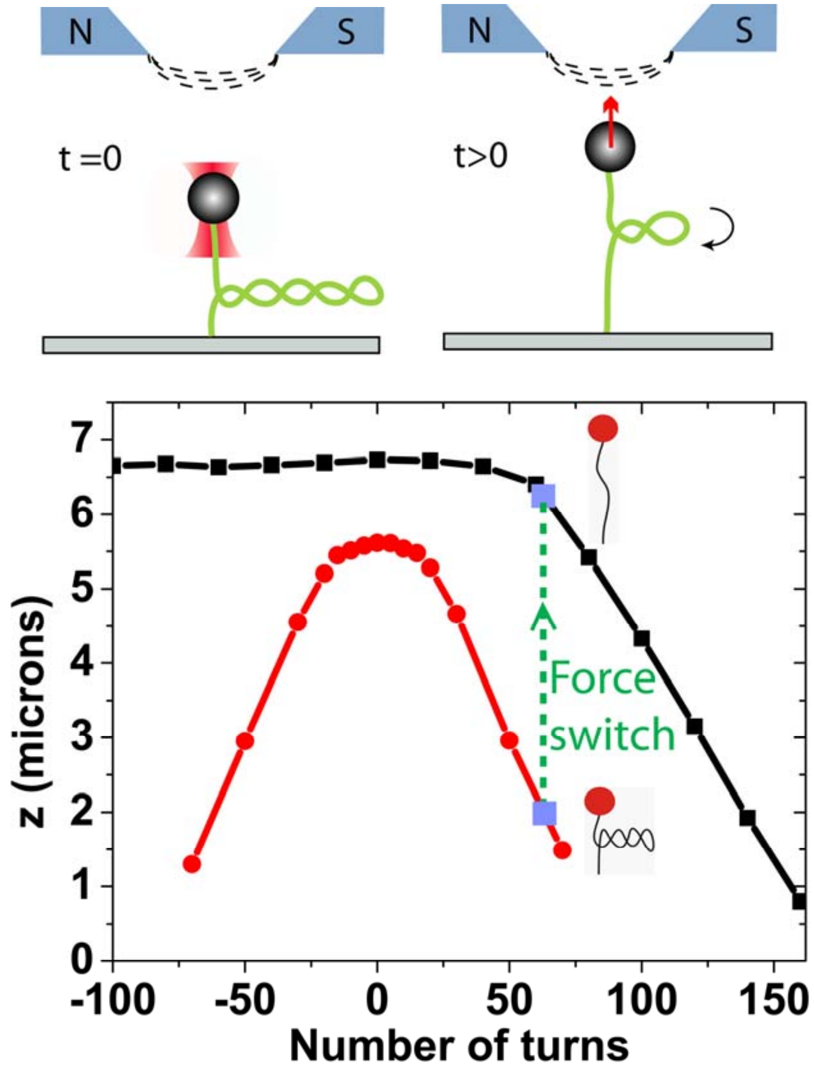
Topology of a DNA molecule

$$\boxed{\Delta Lk = \Delta Tw + Wr}$$

Bead
Initial rotation

Twist

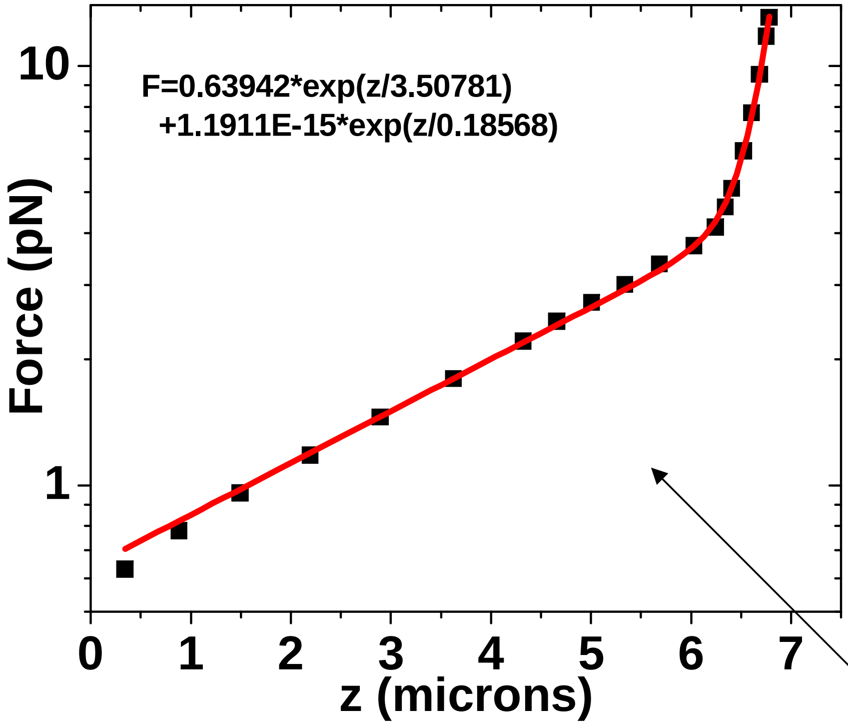
Plectonemes



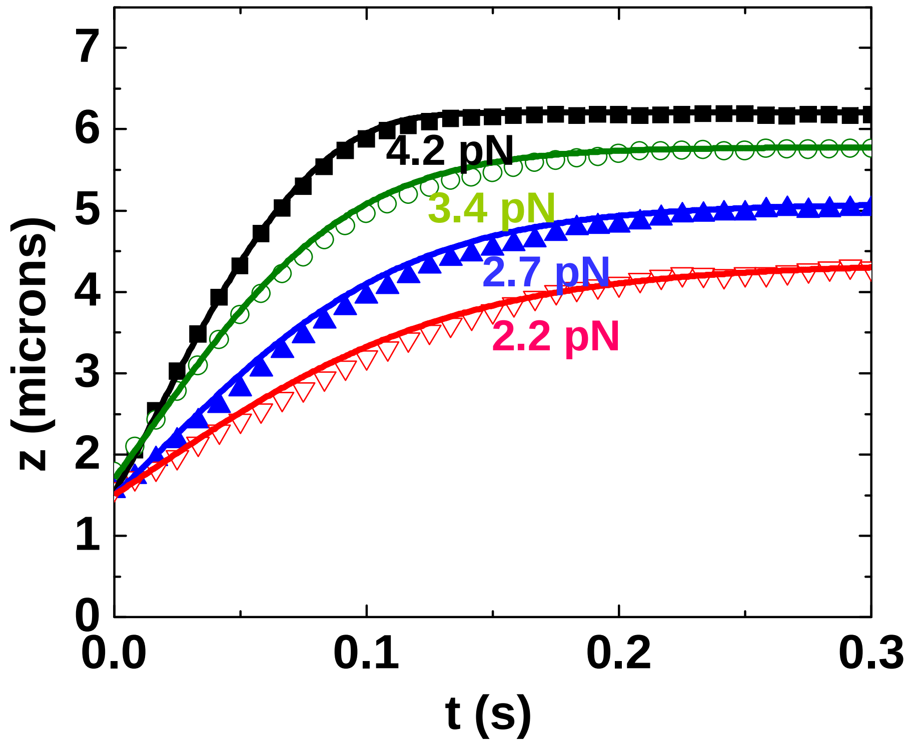
DNA stretching converts plectonemes into twist

The dynamics are quasistatic too

Equilibrium force-extension curve (with +100 turns)

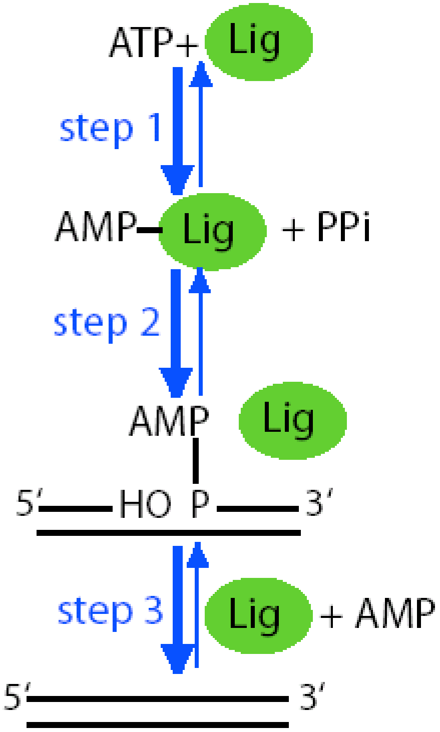


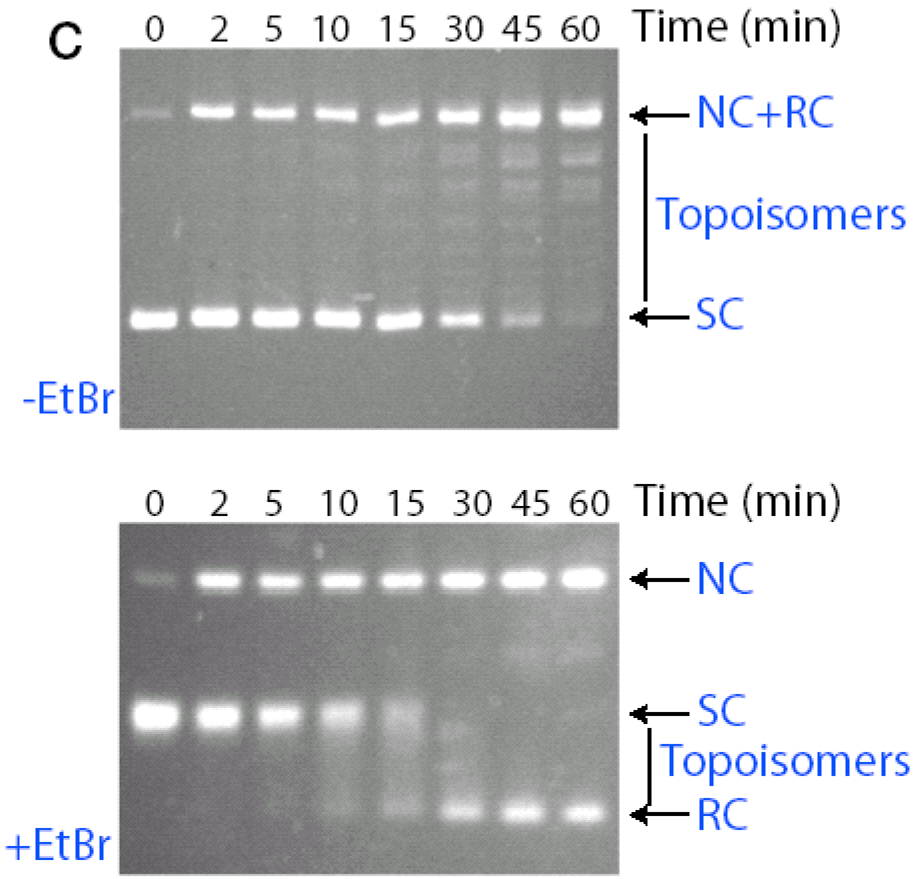
Stretching experiments



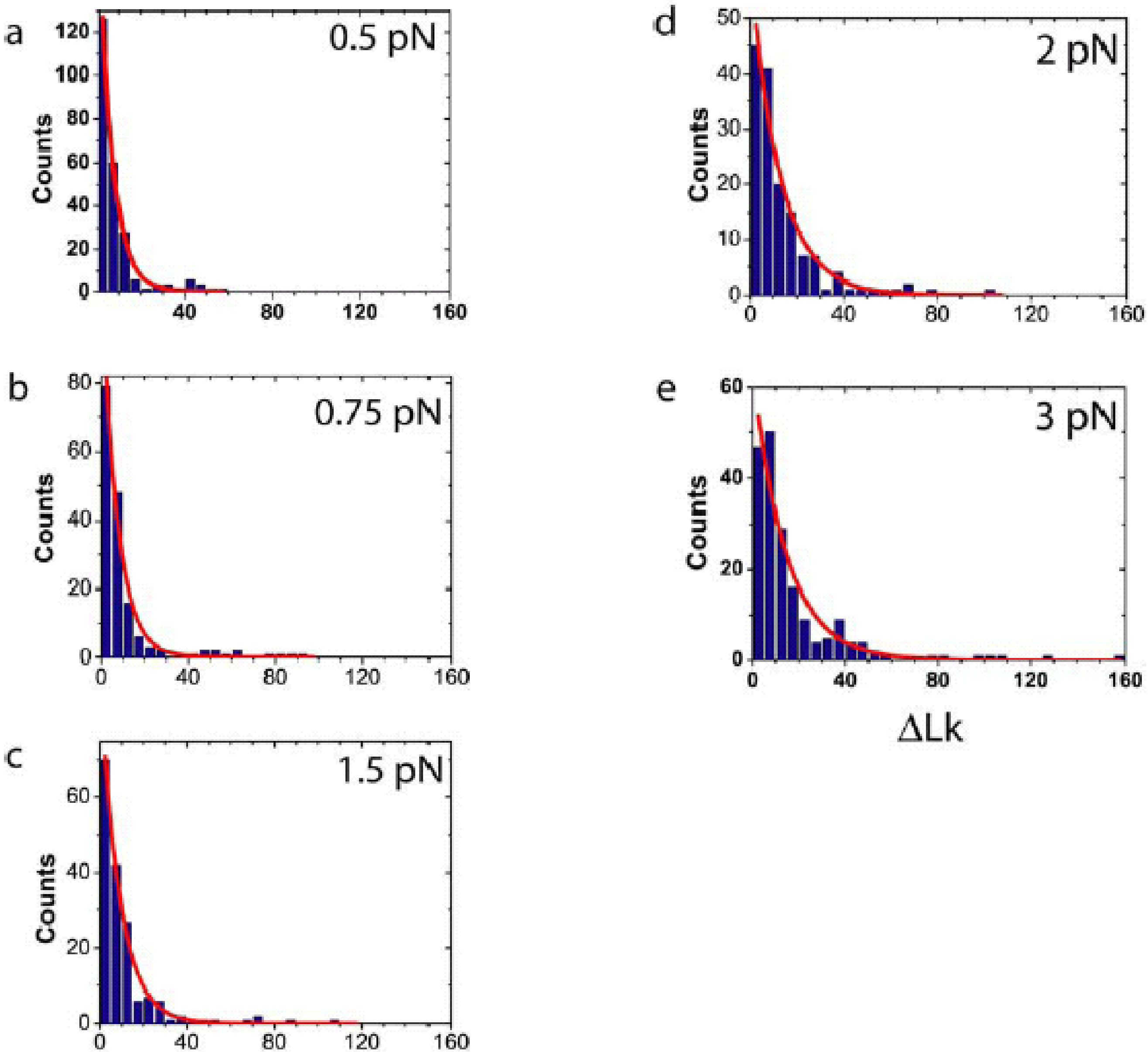
Quasistatic model $F_{mag} = \zeta_{bead}(z)\, dz/dt + F_{DNA}(z)$

DNA relaxation by CVLig: bulk experiments





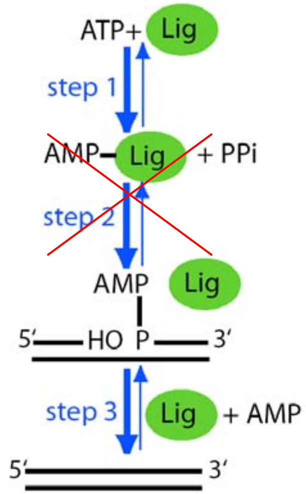
WT CVLig: step size distributions



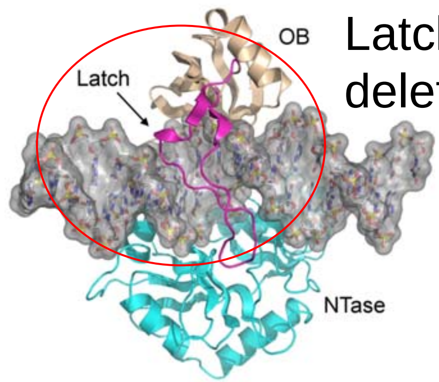
Study of two CVLig mutants

K27A

This mutant cannot bind AMP !

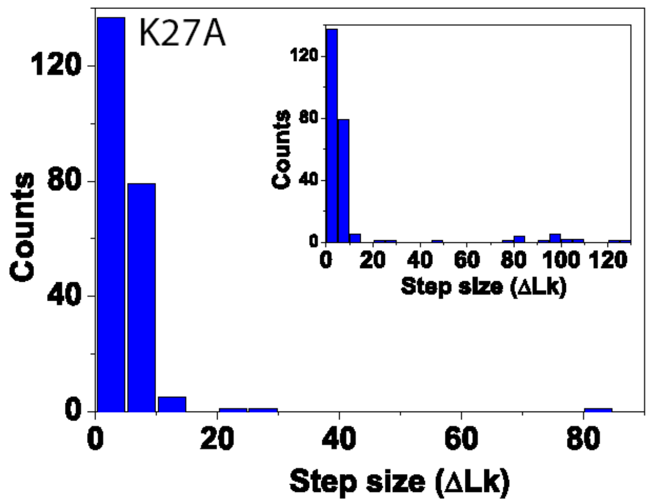


Δlatch



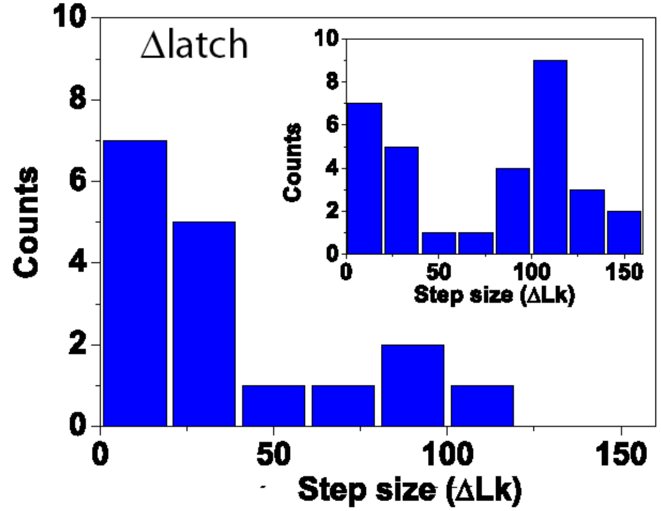
Latch module deleted

c



F=1pN

d



Shorter step size (because reversal of step 2 cannot take place?)

Relaxation often occurs in a single step:
- decreased ligation efficiency
- &larger dissociation probability