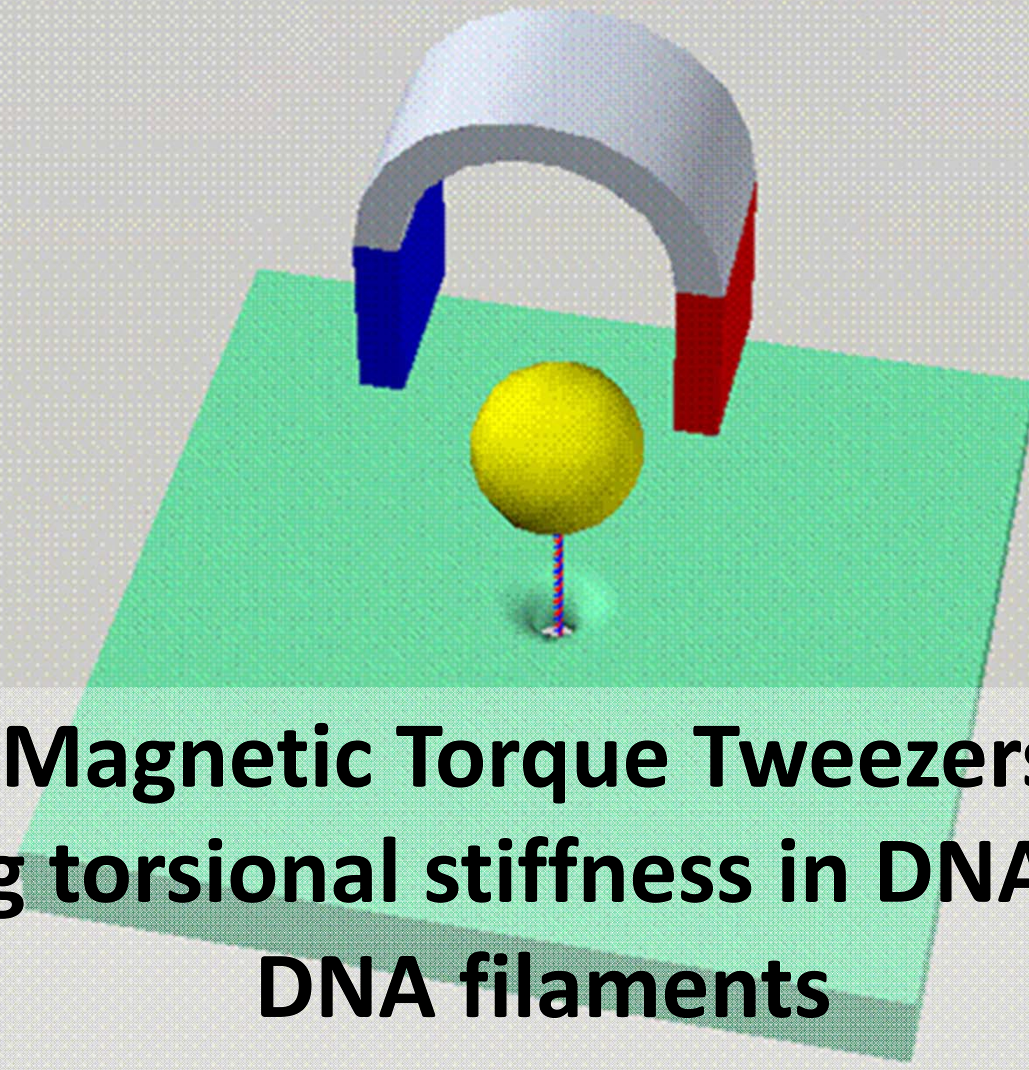


Magnetic Torque Tweezers: measuring torsional stiffness in DNA and RecA-DNA filaments

Lipfert, J., Kerssemakers, J. W., Jager, T. & Dekker, N. H. Magnetic torque tweezers: measuring torsional stiffness in DNA and RecA-DNA filaments. *Nat. Methods* **7,** 977–980 (2010).

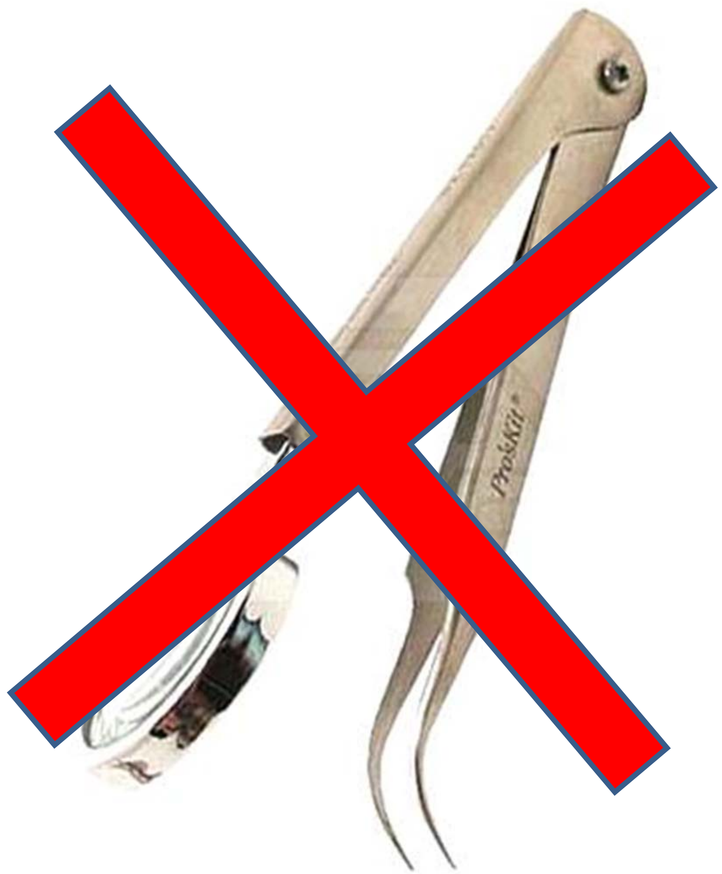
Outline

- Introduction of optical and magnetic tweezers
- Experimental setup and theory of magnetic torque tweezers
- Result and discussion
- Critique and citation analysis

Tweezers

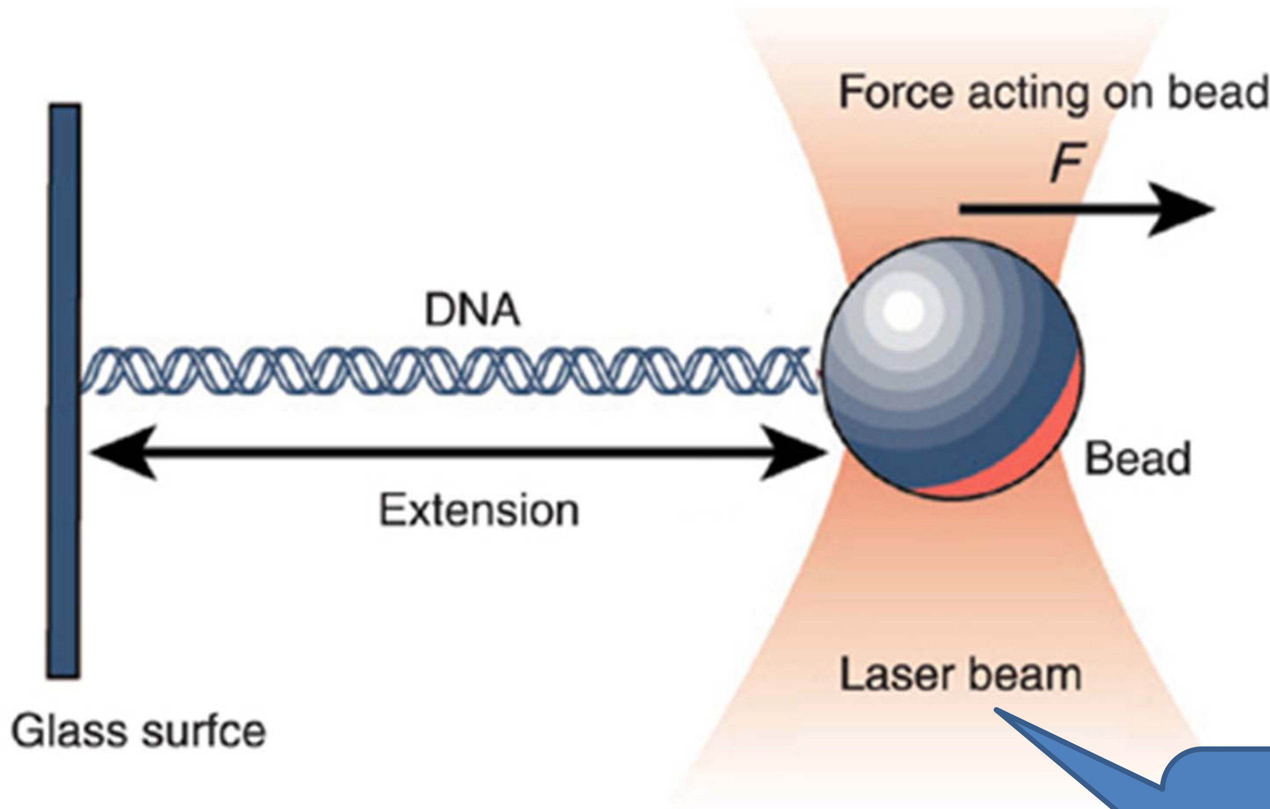
http://www.bio-world.com/categories/4_76_604/Tweezers.html

Optical tweezers



http://jolisfukyu.tokai-sc.jaea.go.jp/fukyu/mirai-en/2007/10_3.html

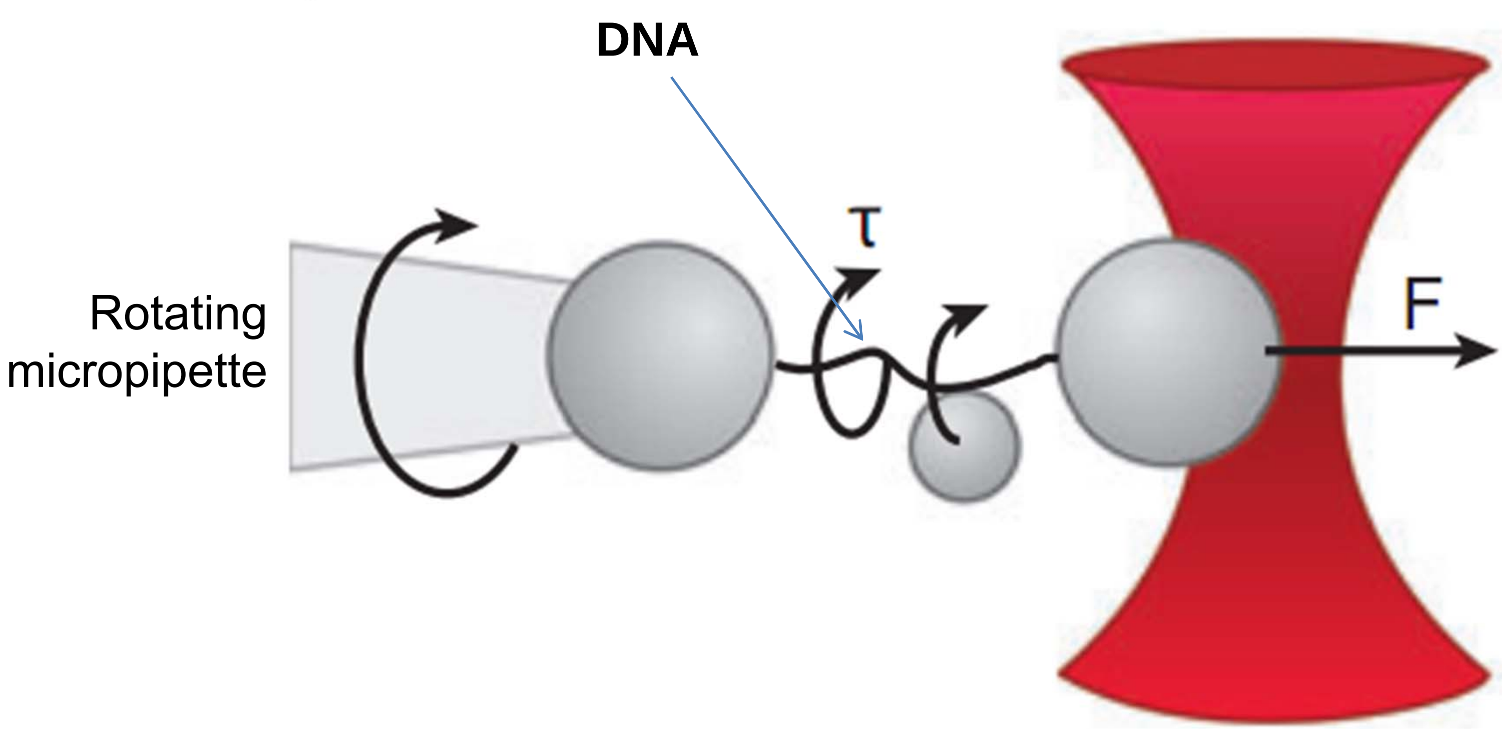
Pulling DNA with optical tweezers



The laser is the optical tweezers

http://jolisfukyu.tokai-sc.jaea.go.jp/fukyu/mirai-en/2007/10_3.html

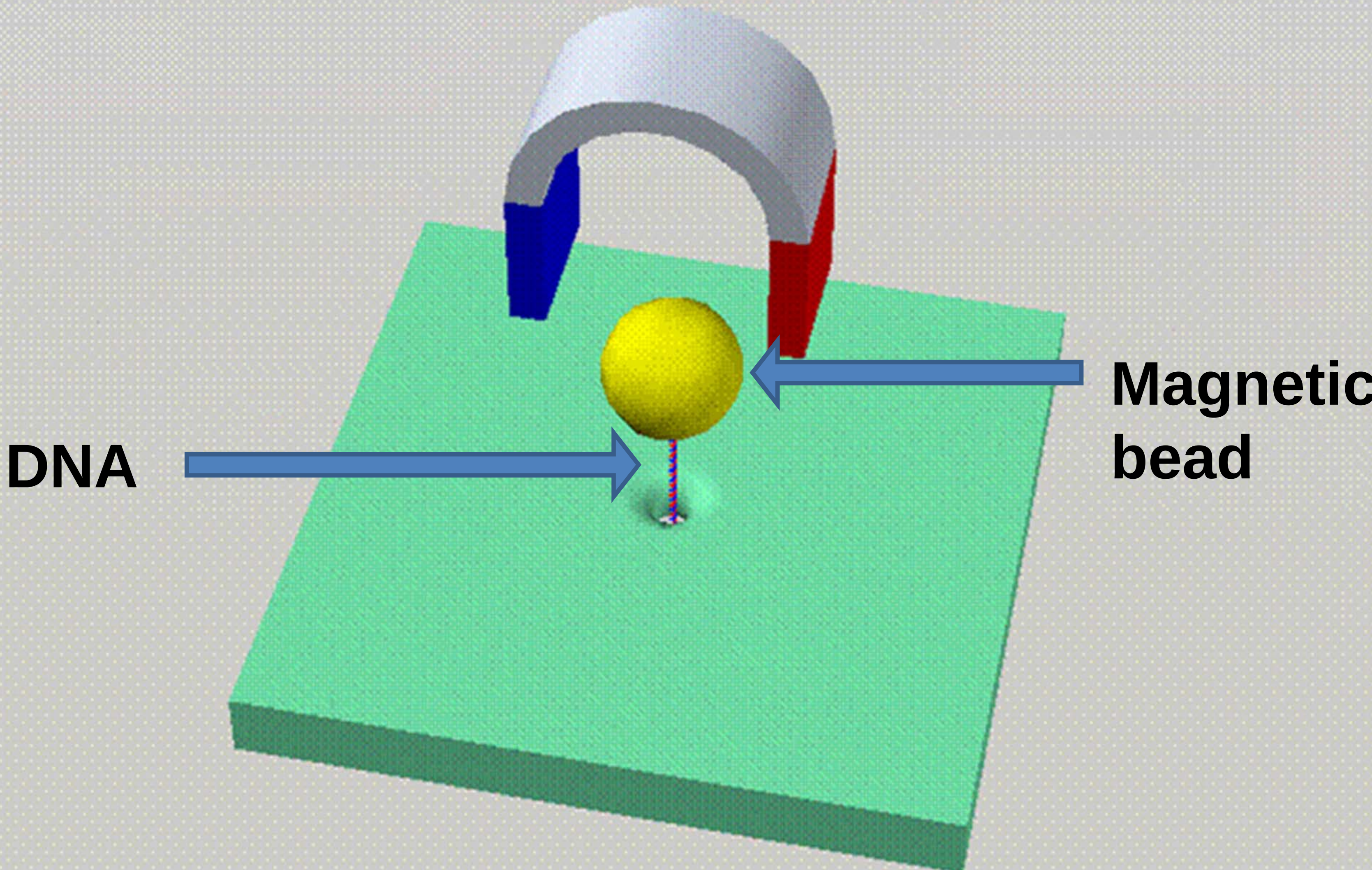
How to measure torque of DNA?



But prolonged heating might denature DNA

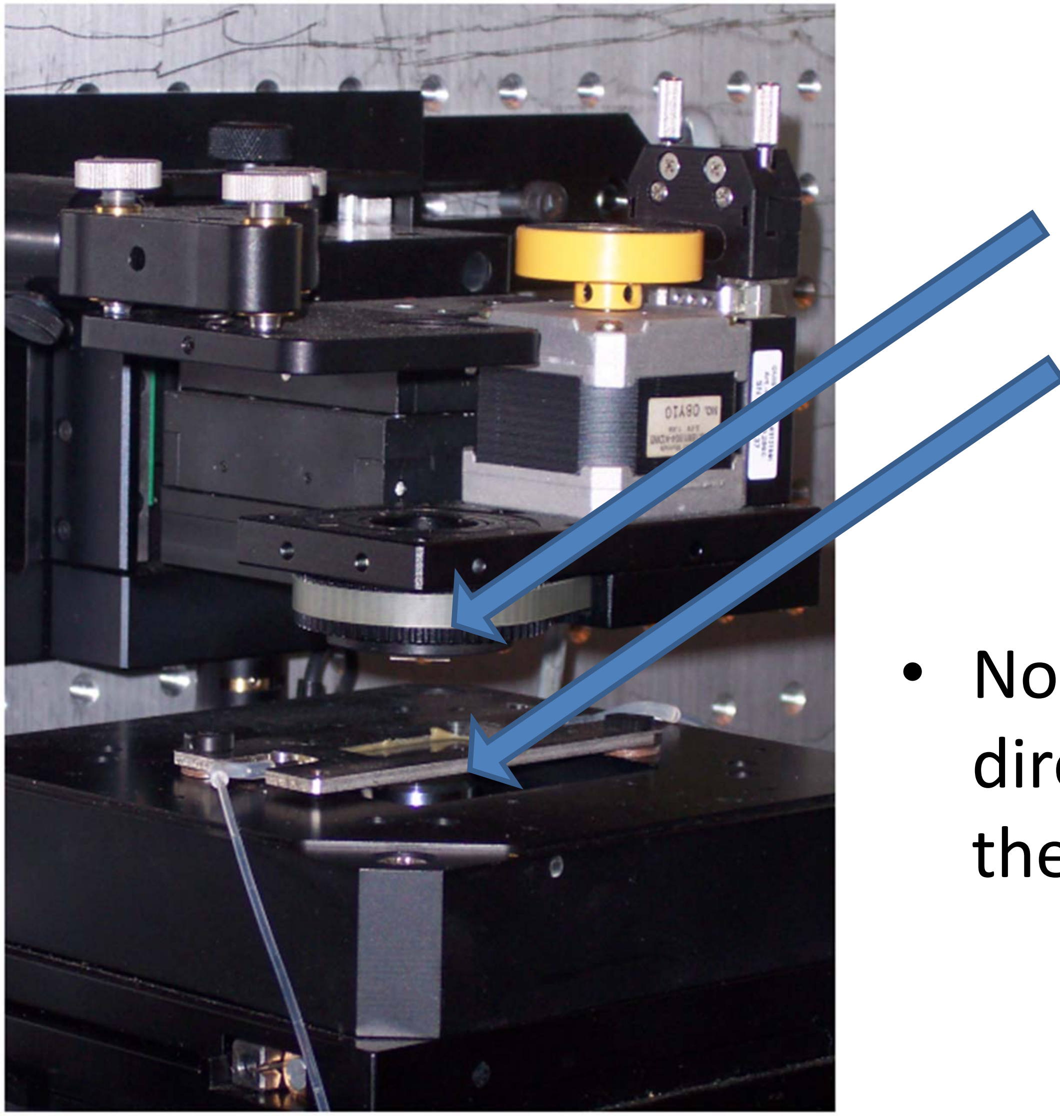
J. R. Moffitt, Y. R. Chemla, S. B. Smith, and C. Bustamante, Annu. Rev. Biochem. 77, 205 (2008)

Magnetic tweezers



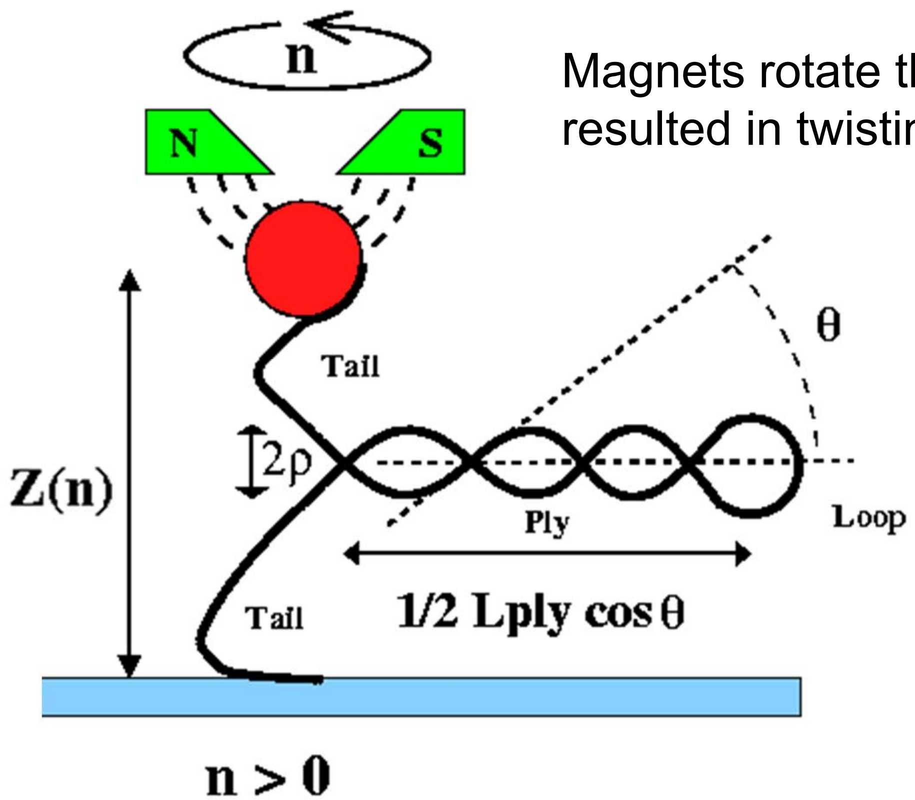
http://news.brown.edu/pressreleases/2009/04/sequencing

Actual magnetic tweezers in lab



- Note that the magnet directly pulls or rotates the bead, not the DNA

Conventional Magnetic Tweezers



Magnets rotate the bead, which resulted in twisting of DNA

However, torque applied is unsuitably large for DNA

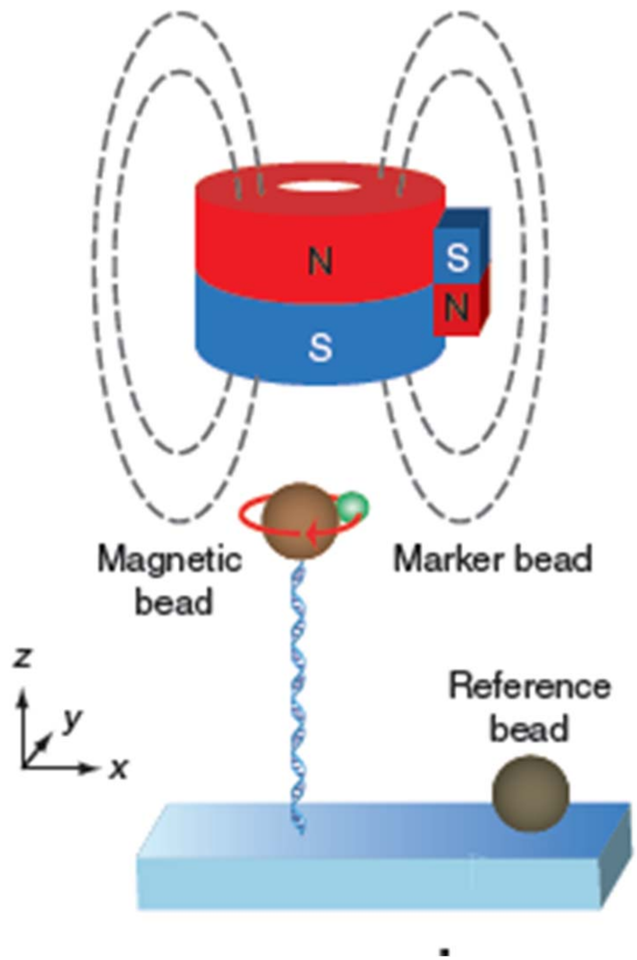
http://www.lmm.jussieu.fr/~neukirch/single_mol.html

Magnetic Torque Tweezers (MTT)

Experimental setup and theory

Basic Setup of Magnetic Torque Tweezers

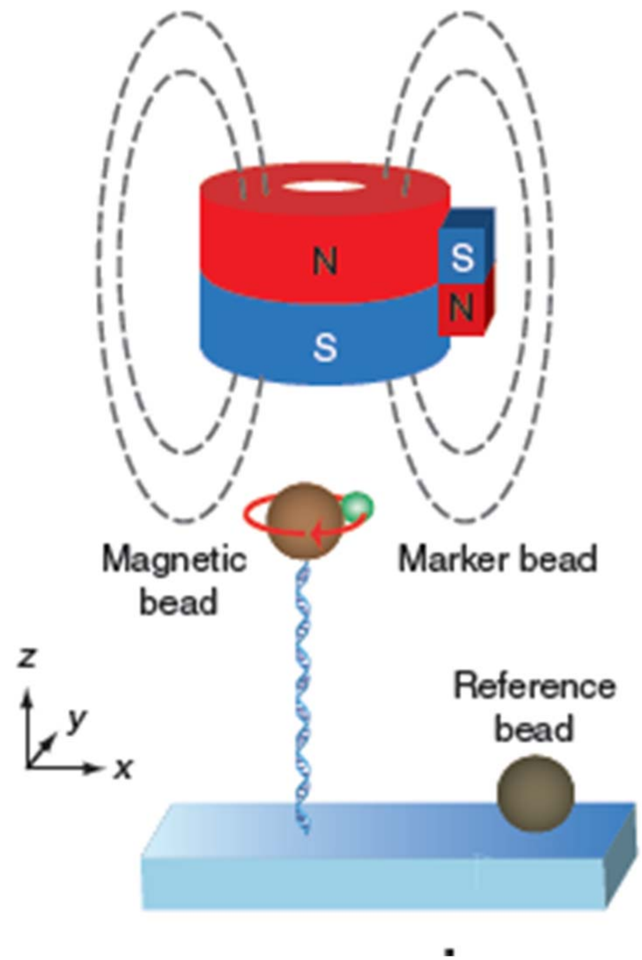
- Strand of DNA linked to magnetic bead, which is pulled upward by a large permanent magnet



Basic Setup of Magnetic Torque Tweezers

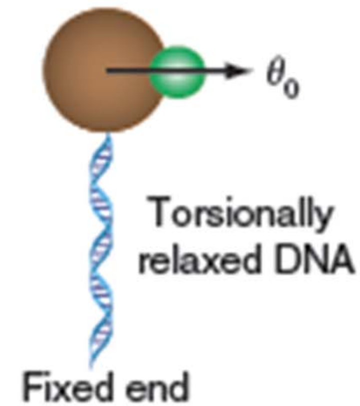
- Strand of DNA linked to magnetic bead, which is pulled upward by a large permanent magnet

- Small secondary magnet can provide torque on the magnetic bead. Acts as a low-stiffness angular trap



Torque and Equilibrium Angle

- Winding DNA causes it to produce a restoring torque τ_{DNA}, opposed by the τ_B due to the small magnet.



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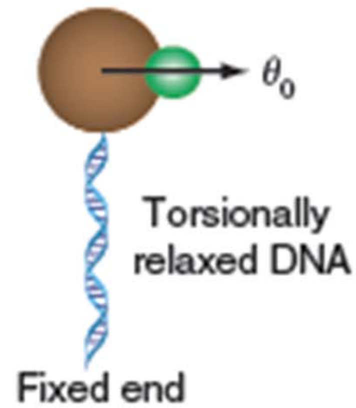
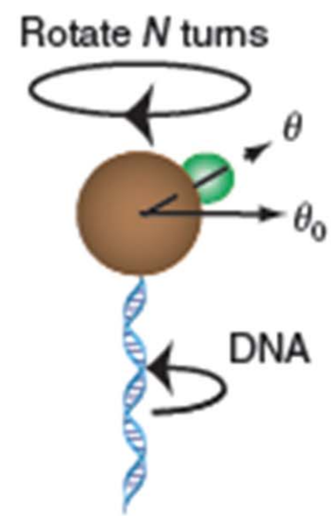


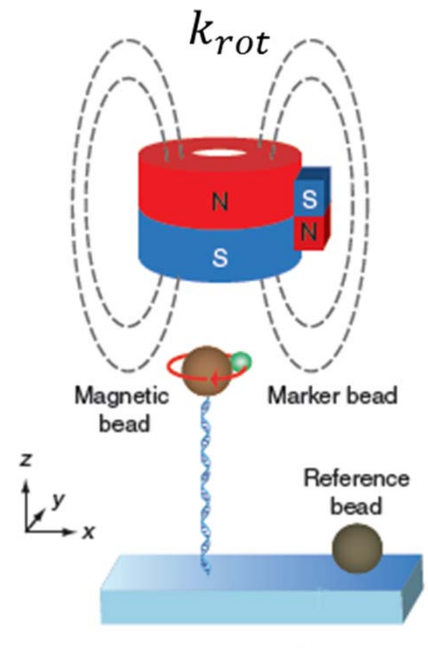
- Average angle shifts from equilibrium with increasing τ_{DNA}



$$\tau_{DNA} = -k_{rot}\langle\theta - \theta_0\rangle$$

Measuring torsional trap stiffness (k_{rot})

- k_{rot} is a measure of the stiffness of the magnetic trap used.



Measuring torsional trap stiffness (k_{rot})

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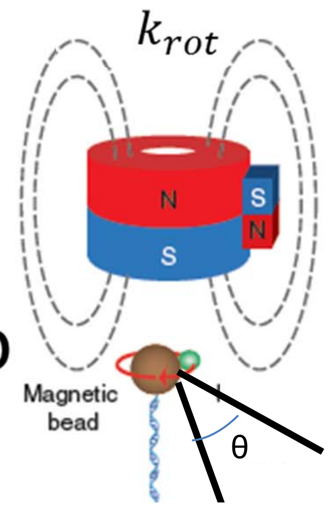
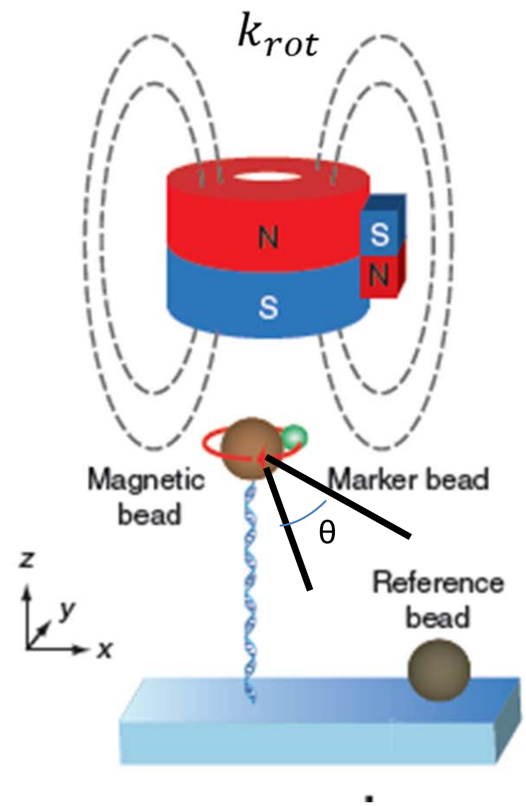


- Can measure fluctuations $\langle \delta\theta^2 \rangle$ due to thermal effects and apply the equipartition theorem to obtain

$$k_{rot} = \frac{k_B T}{\langle \delta\theta^2 \rangle}$$

CCD Cameras, angular shift

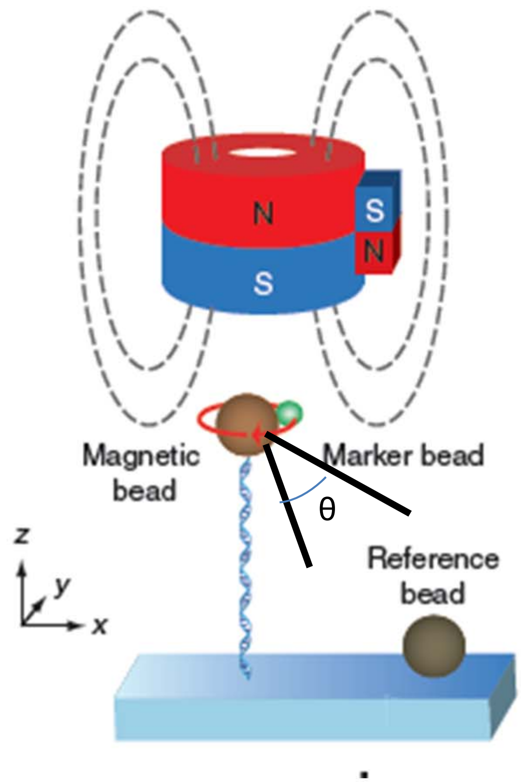
- If k_{rot} is known, τ_{DNA} can be found as a function of $\langle\theta - \theta_0\rangle$.

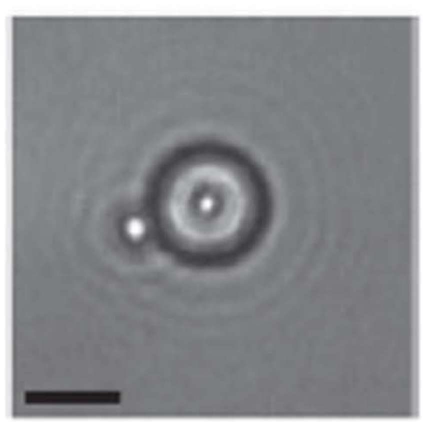


CCD Cameras, angular shift

- If k_{rot} is known, τ_{DNA} can be found as a function of $\langle\theta - \theta_0\rangle$.

- Unmagnetized bead used as marker, tracked by CCD camera to determine angular shift



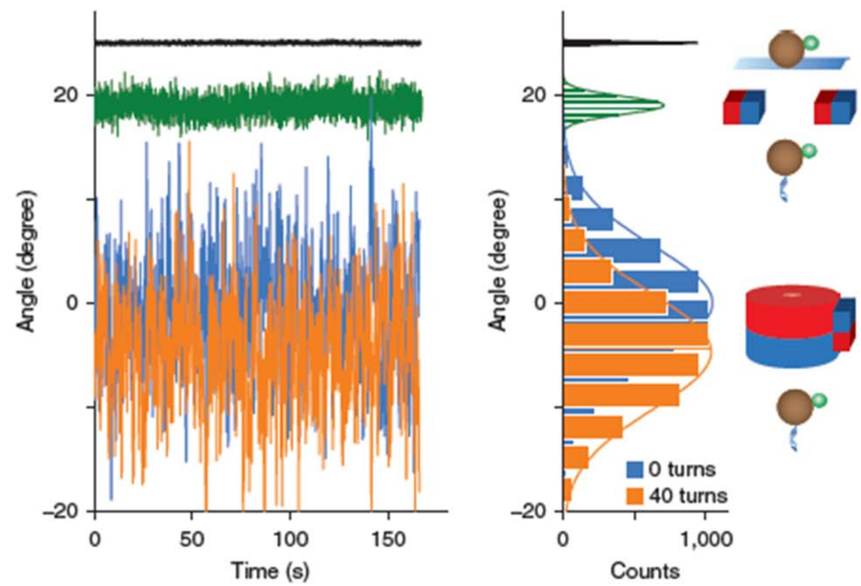


Measurement resolution

$$\tau_{DNA} = -k_{rot}\langle\theta - \theta_0\rangle$$

- High angular resolution (~0.1°) attained in τ_{DNA} measurement due to low angular trap stiffness.

- Such precision is useful for measuring small τ effects

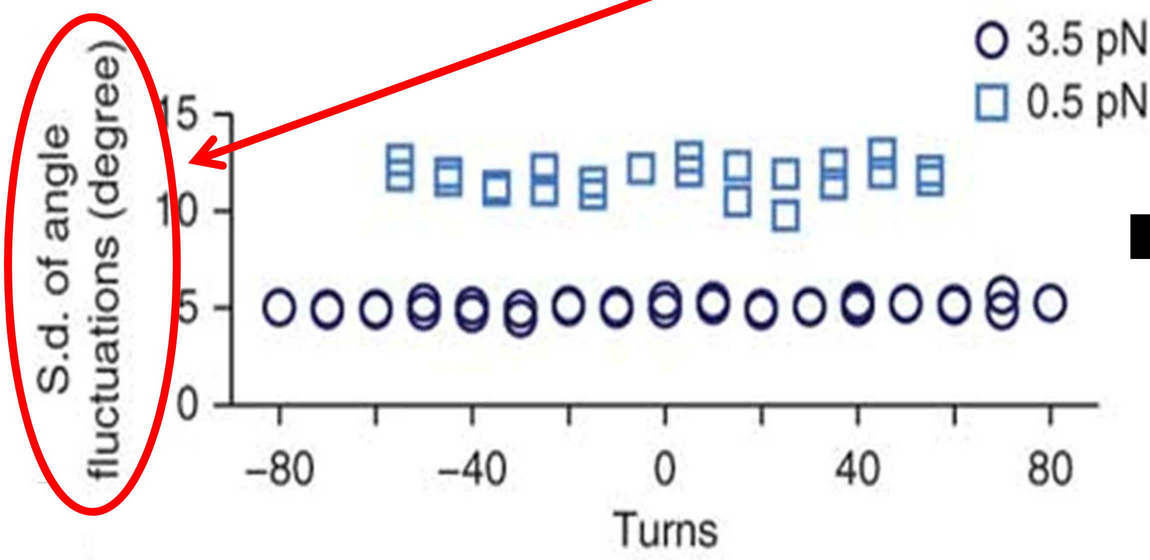


Result and Discussion

How to measure the torque

$$\tau_{DNA} = -k_{rot} < \theta - \theta_0 >$$

$$k_{rot} \propto < \delta\theta^2 >^{-1}$$



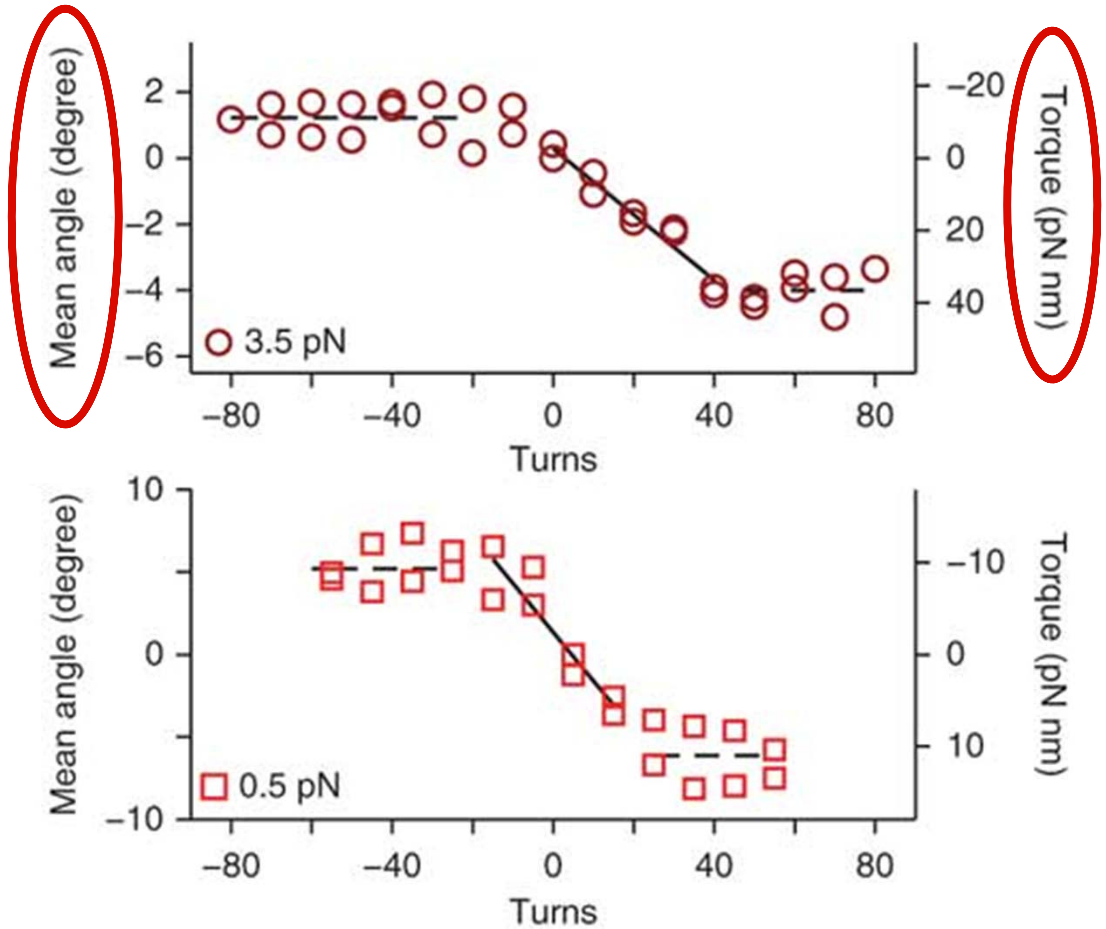
$\delta\theta^2$ is constant

$$\tau_{DNA} \propto < \theta - \theta_0 >$$

So they can be shown together:

$$\tau_{DNA} \propto < \theta - \theta_0 >$$

$< \theta - \theta_0 >$



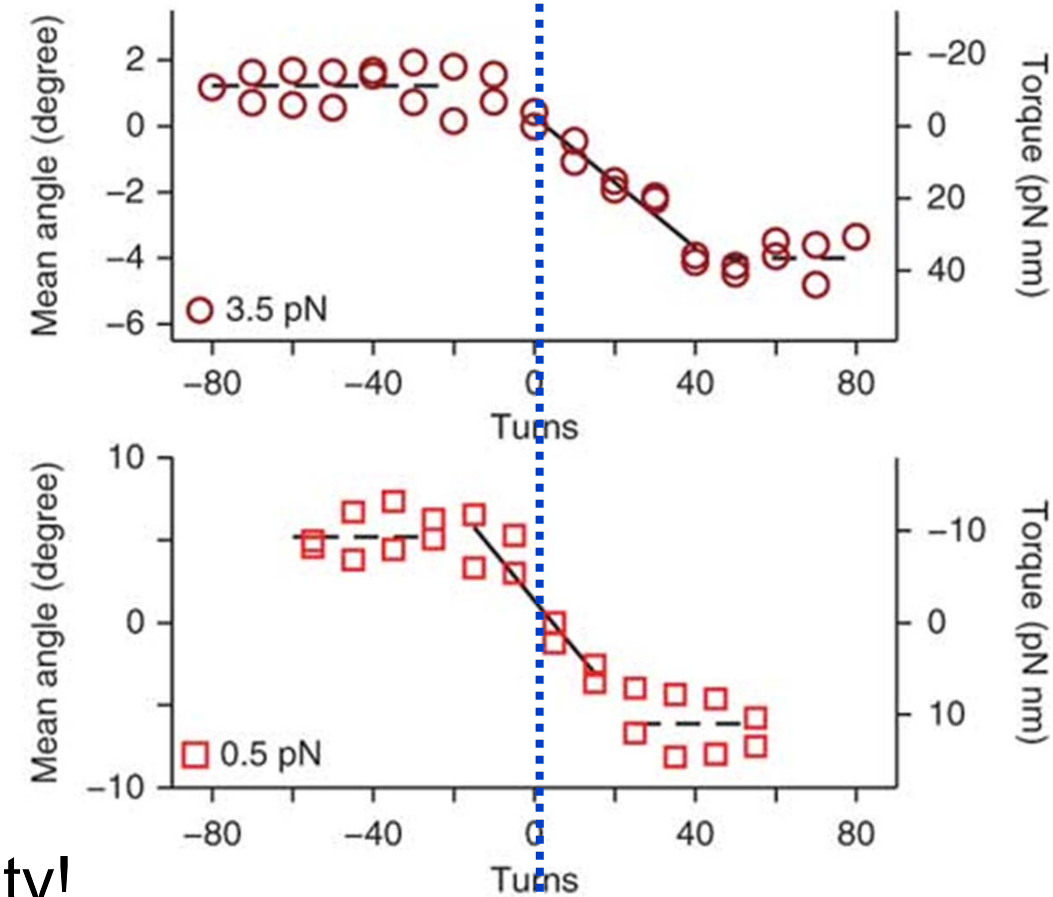
τ_{DNA}

Two cases to rotate DNA

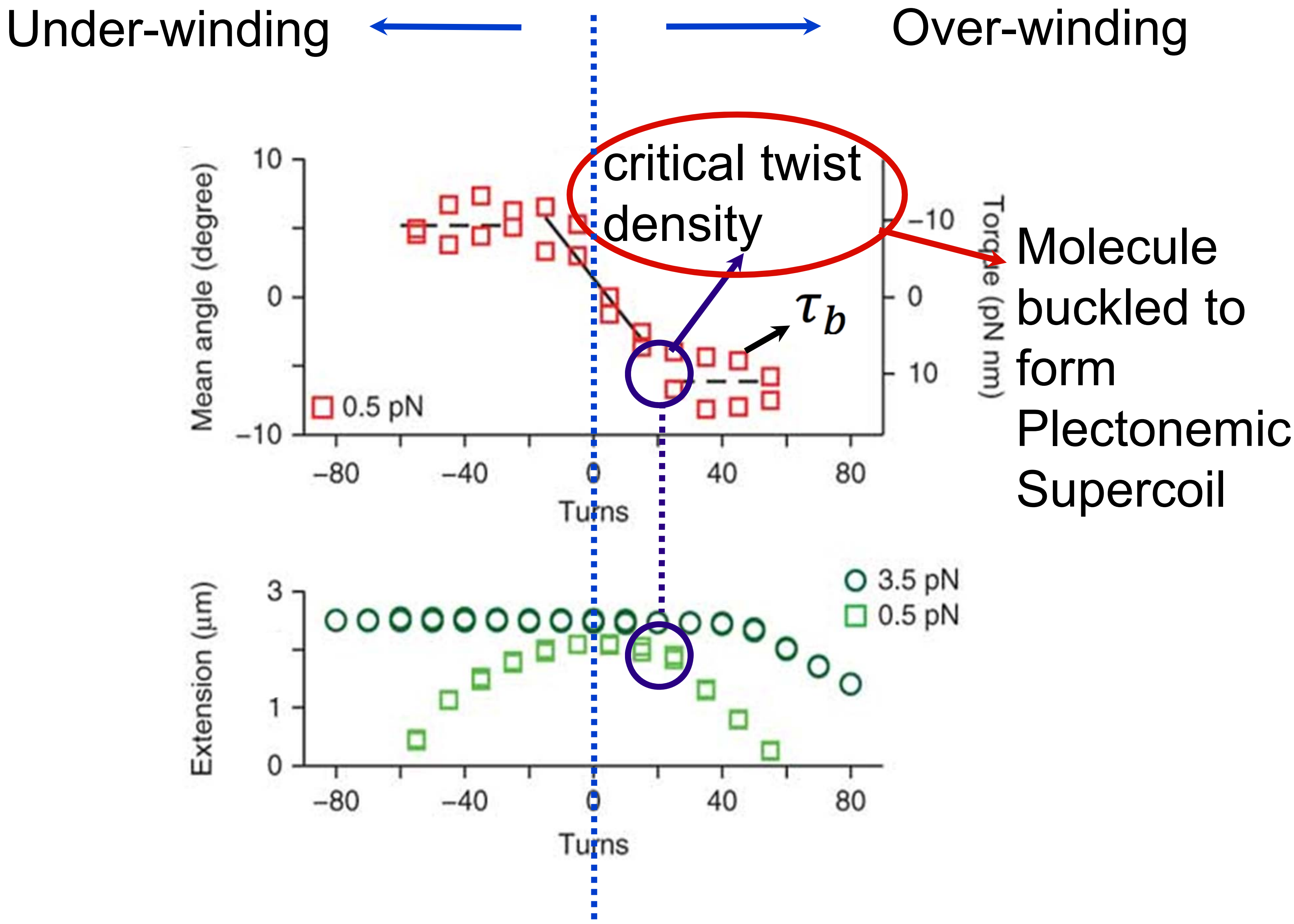
Under-winding ← → Over-winding

Structure of DNA

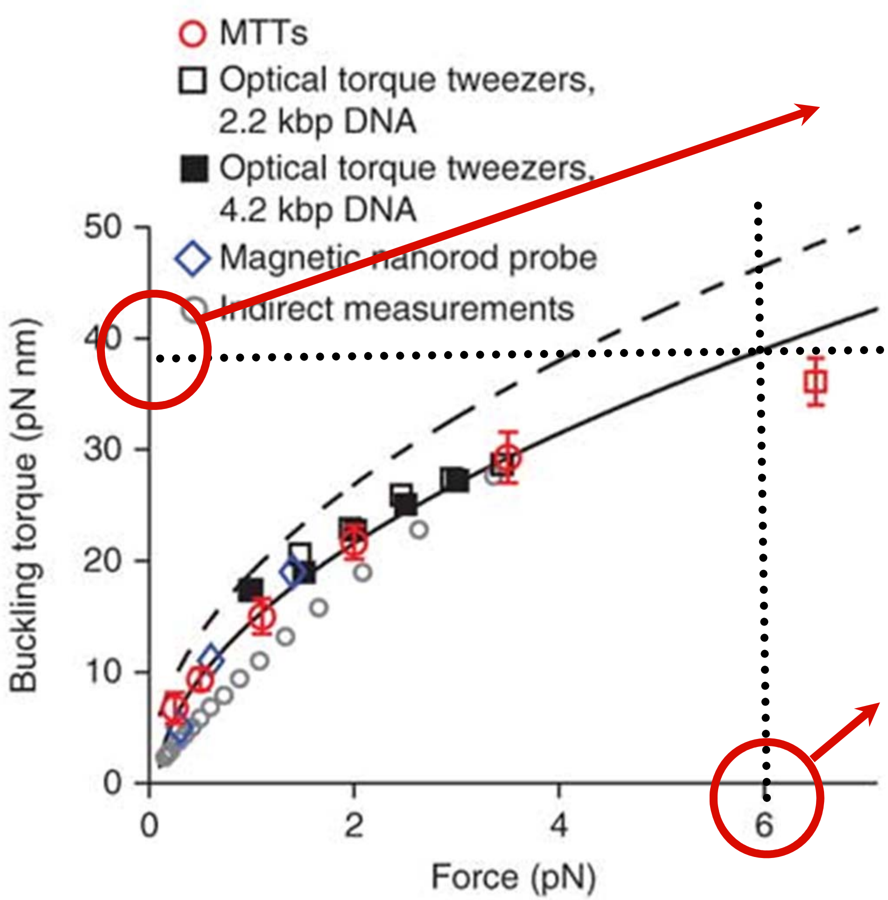
It has preferred helicity!



dsDNA Torque and Extension



dsDNA Buckling Torque τ_b



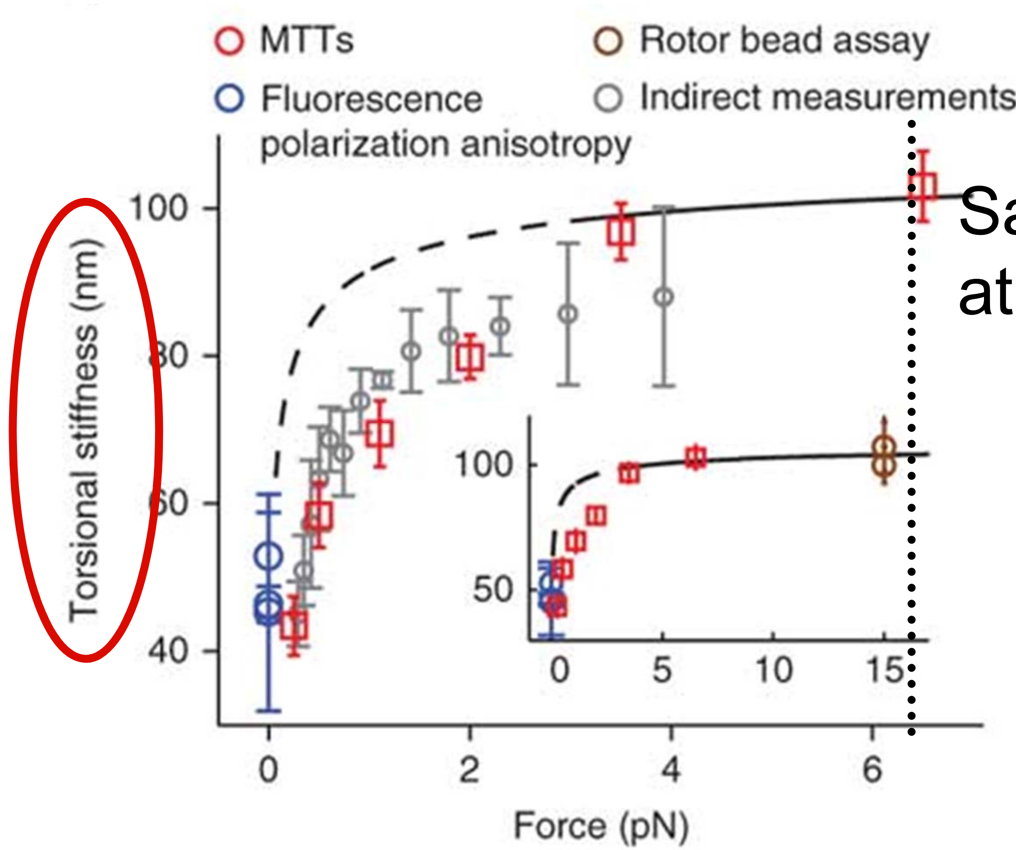
Instead underwent a transition from B- to P-DNA at 36 ± 2 pN nm (Buckling Torque)

At forces above 6 pN, DNA no longer formed Plectonemic Supercoil

dsDNA Torsional Stiffness C

$$\tau_{DNA} = 2\pi N k_B T C / L_C$$

Effective Torsional Stiffness C

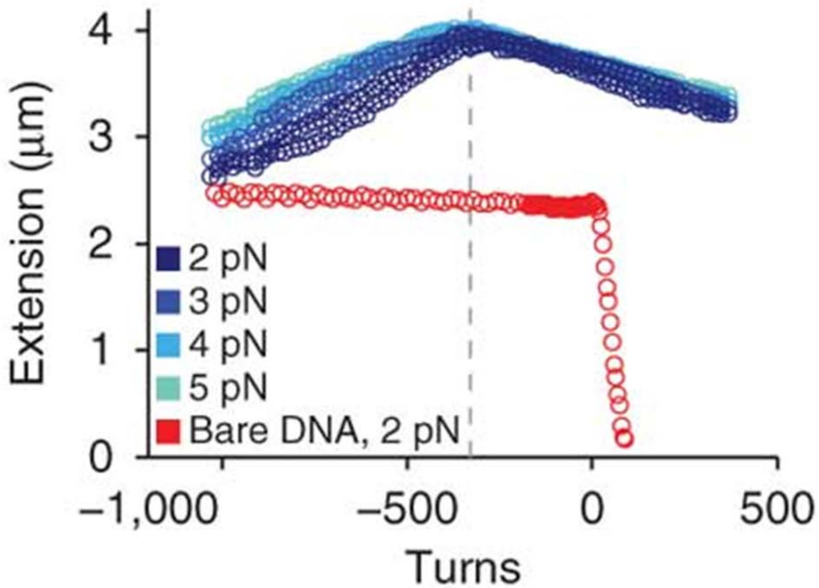


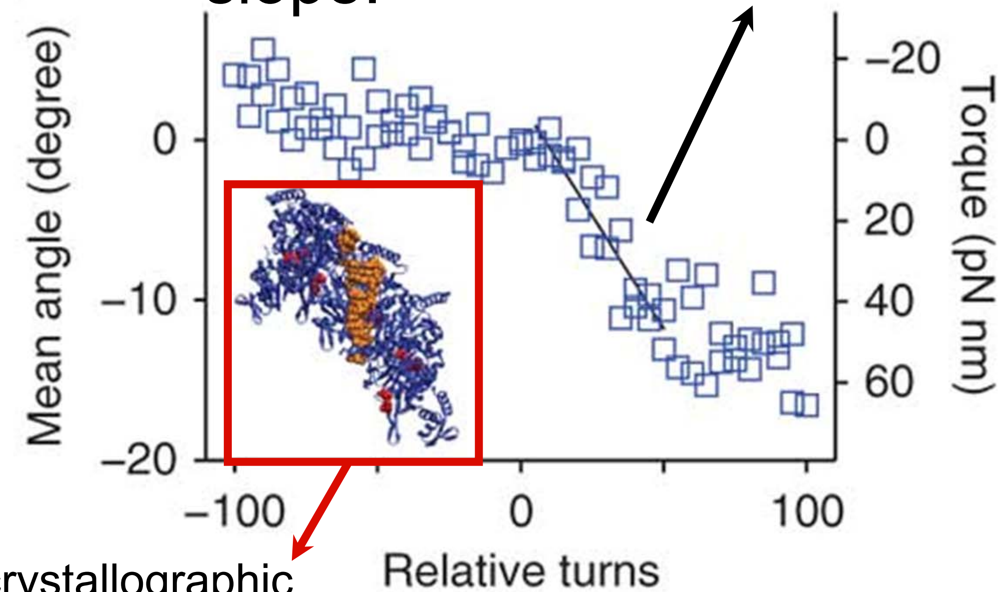
Saturation at 6.5 pN

RecA-dsDNA Measurement

$$\tau_{DNA} = 2\pi N k_B T C / L_C$$

Effective Torsional Stiffness C is determined by the slope!





crystallographic structure of RecA-DNA

Compare with dsDNA:

1. Higher Effective Torsional Stiffness C
2. Combination of RecA unbinding and supercoiled P-DNA formation upon overwinding and DNA observed saturation of the torsional strain

Conclusion

- dsDNA’s extension remains approximately constant under tensions of 1pN or smaller;
- dsDNA’s extensions remains constant and torque increases linearly with increasing turns until it reaches the critical twist density at which DNA buckled to form Plectonemic Supercoil;
- dsDNA’s Effective Torsional Torque (torque difference per turn) reaches saturation at tensions of 6.5 pN.

Critique and Citation analysis

Critiques of this paper

- Compare MTT with optical tweezers and conventional magnetic tweezers.
- Many figures to aid understanding.
- Little explanation on rotation caused by the small side magnet.
- Lack of detailed explanations in certain figures.

Citing Articles

- 8 citing articles in 2011:

  - 2 theoretical papers

  - 2 review papers

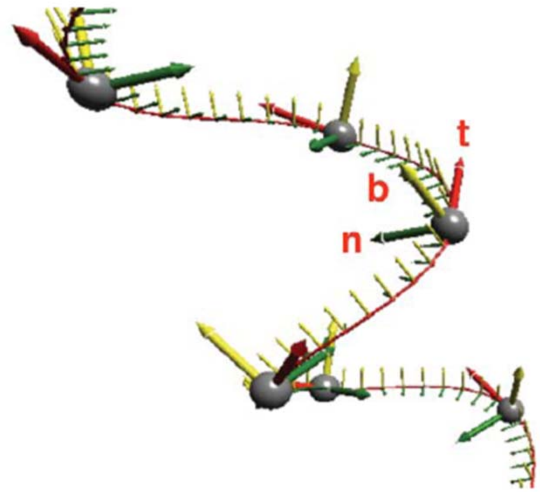
  - 4 experimental papers

- How they describe this paper:

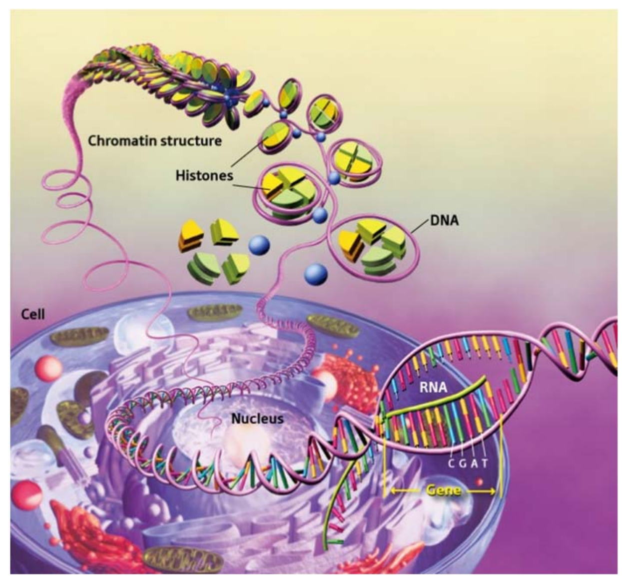
  An elegant way to solve the problem of traditional MT

The paper has had some impact

- Paper caused chemical physicists to model the torsional elasticity of biopolymers *



- Biologists think paper is a supplement to our understanding of DNA and chromatin remodeling **

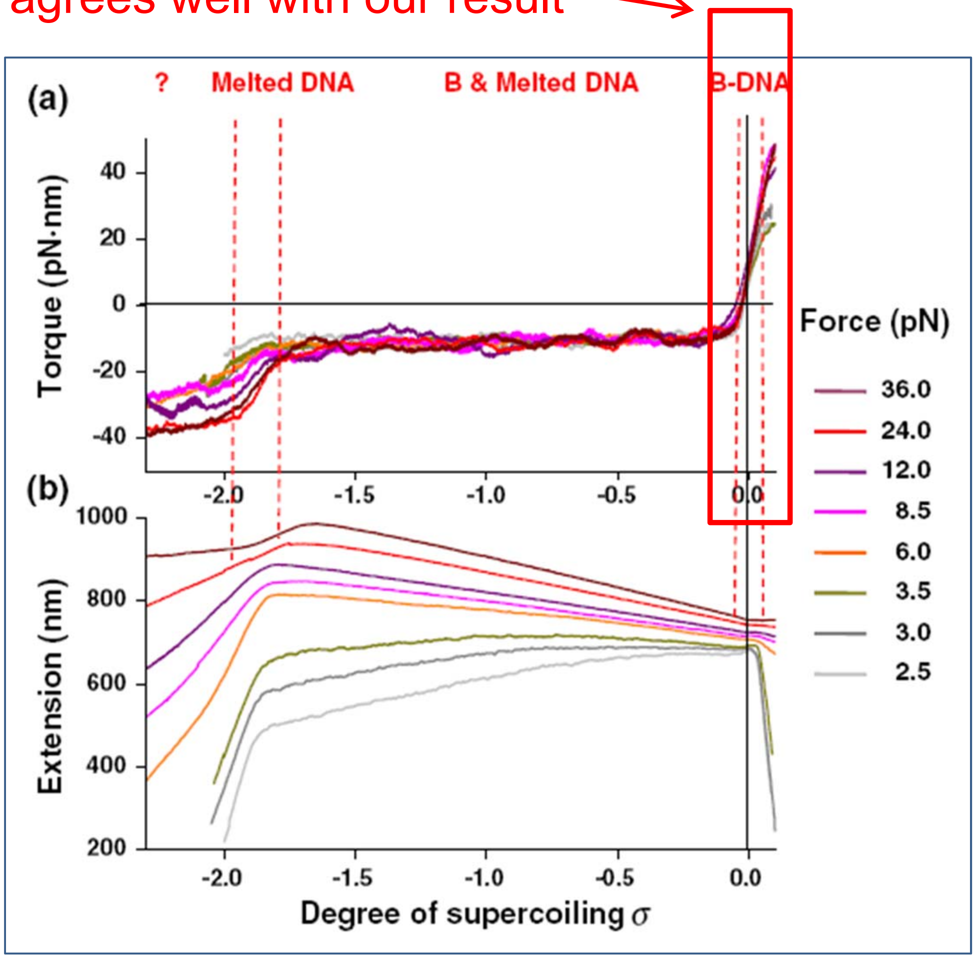


* Ya Liu, et al, J. Chem. Phys. **134**, 065107(2011)

** Christophe Lavelle, et al, FEBS Journal **278**, 3578(2011)

The paper has had some impact

In this region it agrees well with our result

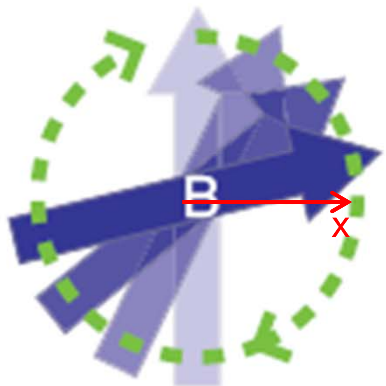


- Optical trap group is also doing similar measurements.

Maxim Y.Sheinin, et al, Phys. Rev. Lett. **107**, 108102(2011)

Work to improve MTT

(a) Fast rotation



- Synchronous: viscous drag causes the phase difference
- Asynchronous: a complicated motion called "slippage"(fast oscillation with slow rotation)

  (average torque decreases → sensitivity increases)

Francesco Mosconi, et al, Review of Scientific Instruments **82**, 034302(2011)

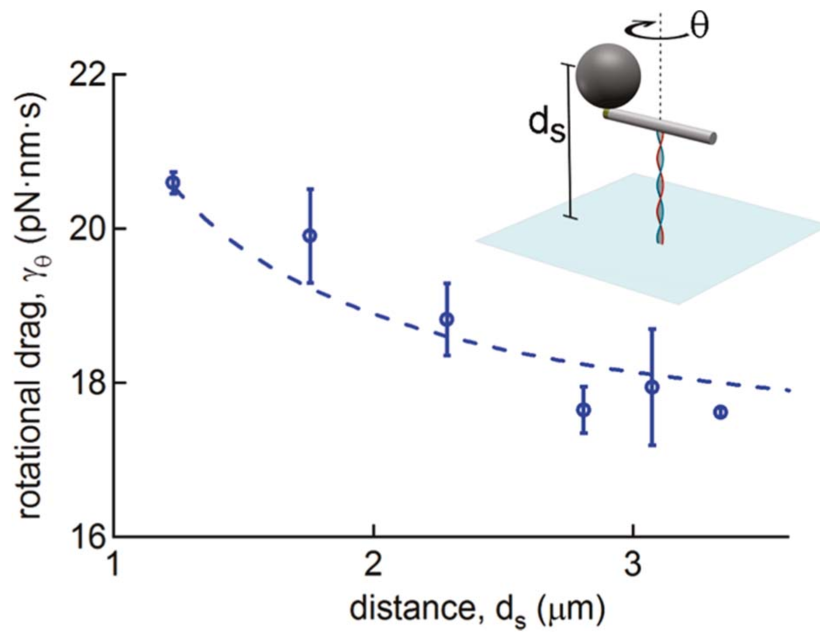
Work to improve MTT

(b) Viscous drag should be taken into account

•This affects the fluctuation of the bead

$$k_{rot} = \frac{k_B T}{\langle \delta\theta^2 \rangle}$$

•The viscous drag increases near the surface

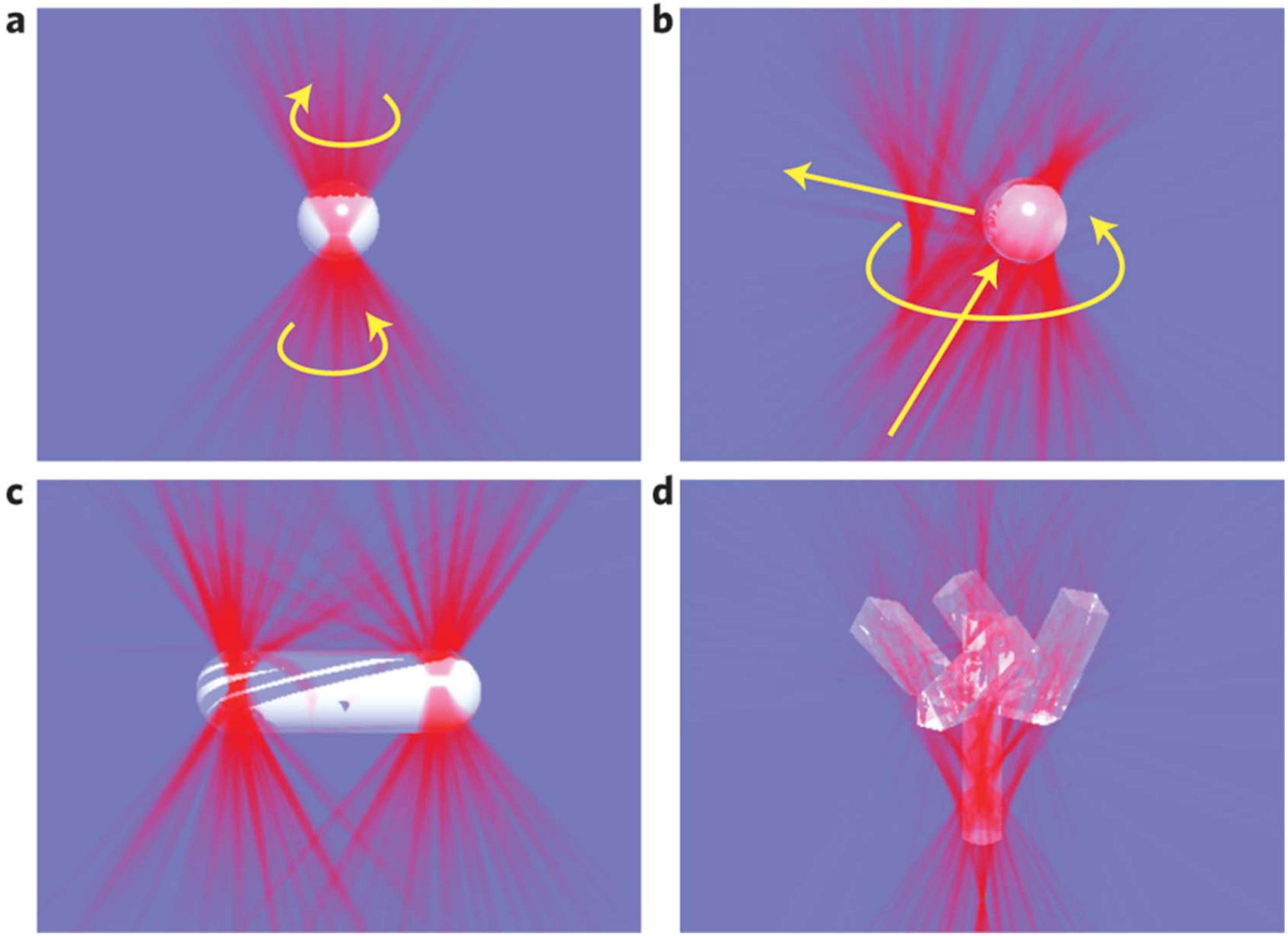


Sebastian Lobo, et al, Langmuir, **27**, 2142(2011)

Thanks for your attention

Questions?

transferring angular momentum



http://www.nature.com/nphoton/journal/v5/n6/fig_tab/nphoton.2011.81_F3.html