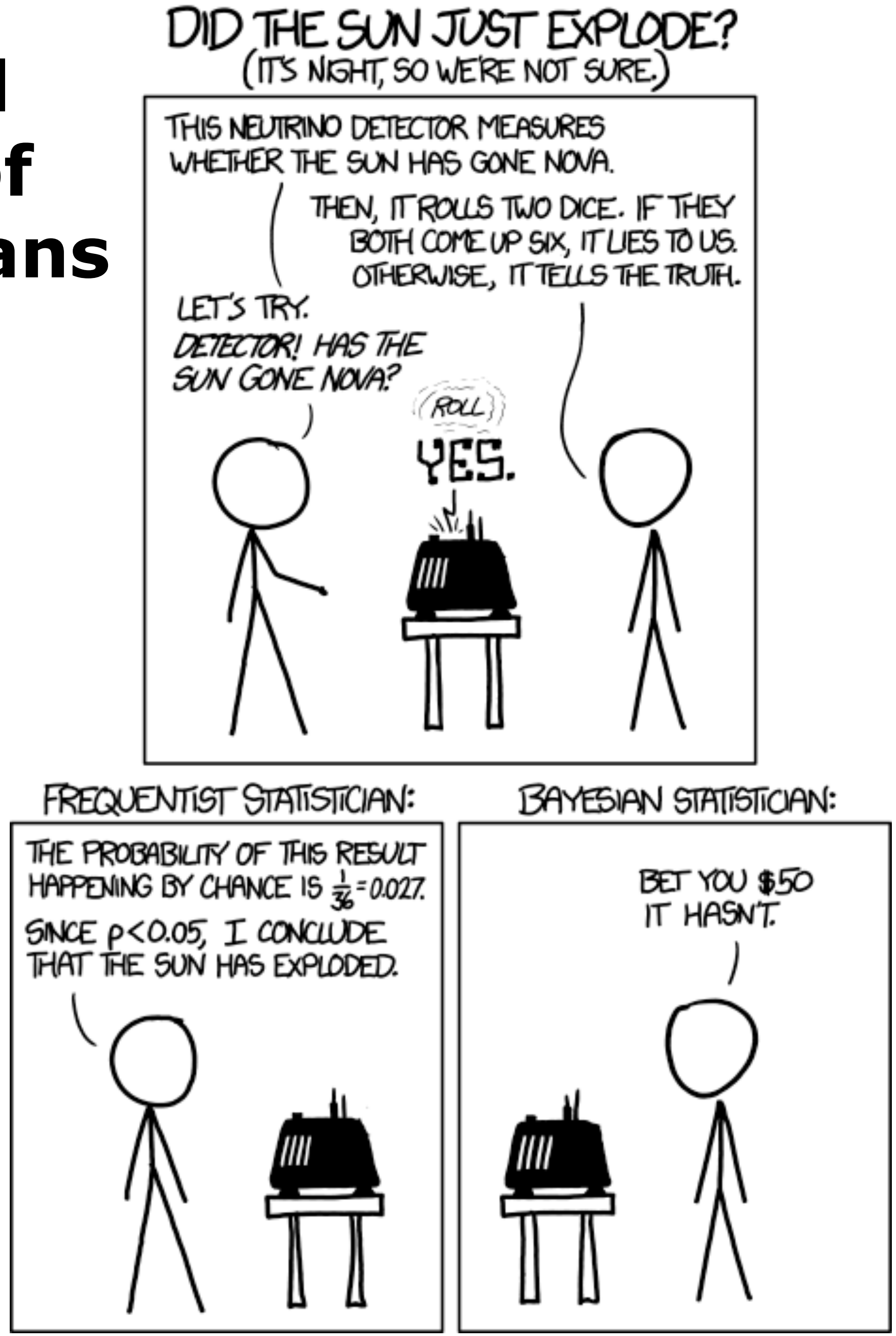
Coming back to Phil Nelson's comment of frequentists vs. baysians



Magnetic tweezers: how do they work, what are they good for, and how can their capabilities be expanded?

XX INTERNATIONAL SUMMER SCHOOL "NICOLAS CABRERA »

Biomolecules and Single Molecule Techniques

July 22, 2013

TU Delft

Delft University of Technology

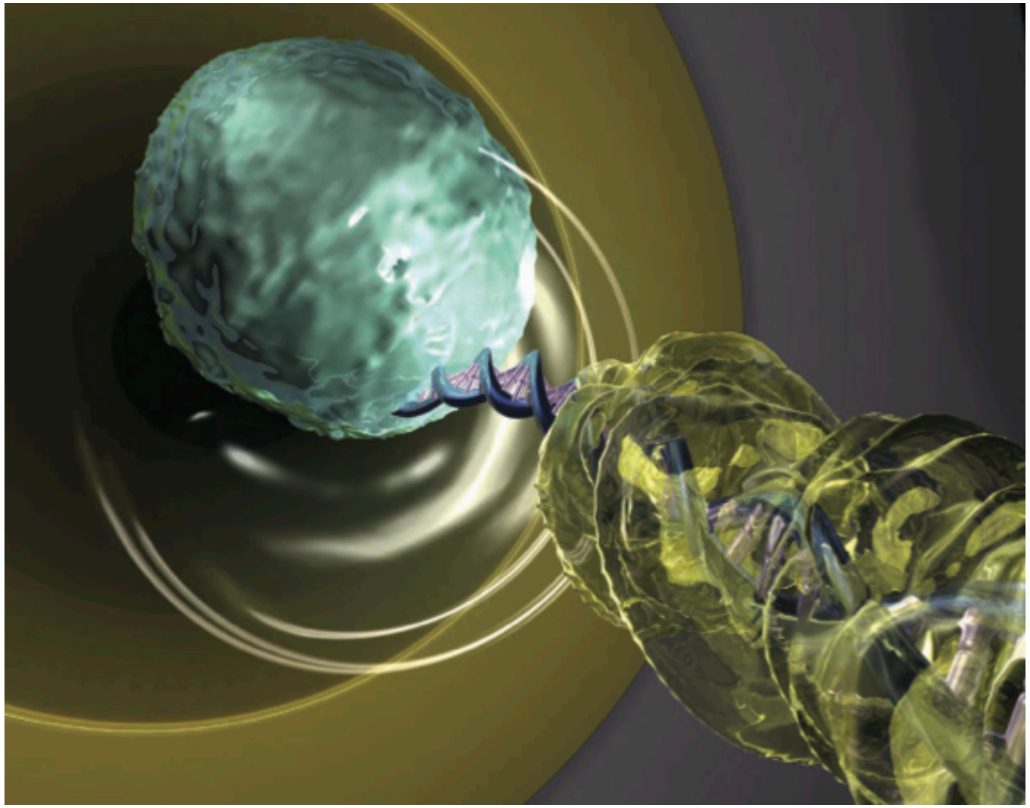
NWO

Netherlands Organisation for Scientific Research

Moving this summer to Munich:

LMU

LUDWIG-MAXIMILIANS-UNIVERSITÄT MÜNCHEN

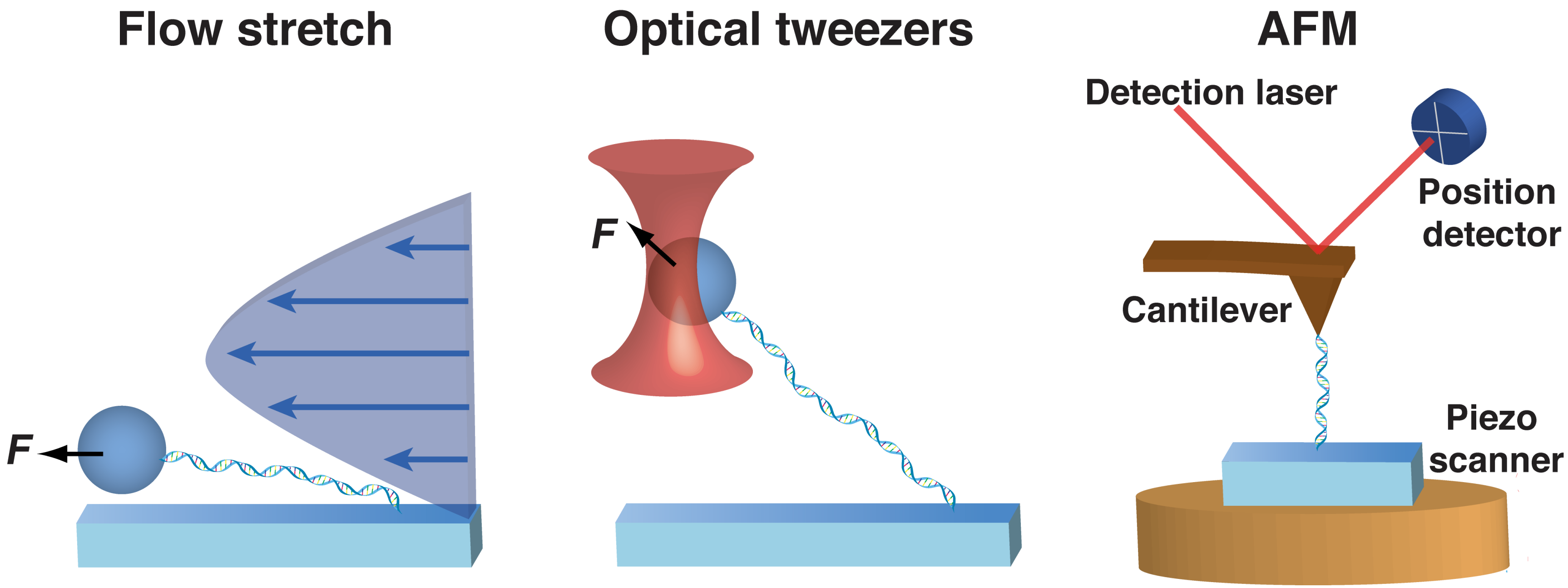


Lee, Lipfert, Sanchez, Wyman & Dekker *NAR* (2013)

Jan Lipfert
(Nynke Dekker Lab, Delft)
`j.lipfert@tudelft.nl`

Fernando’s legacy in Delft lives on…

Single Molecule Manipulation Techniques



Adapted from: Dulin, Lipfert, Moolman & Dekker *Nature Reviews Genetics* (2013)

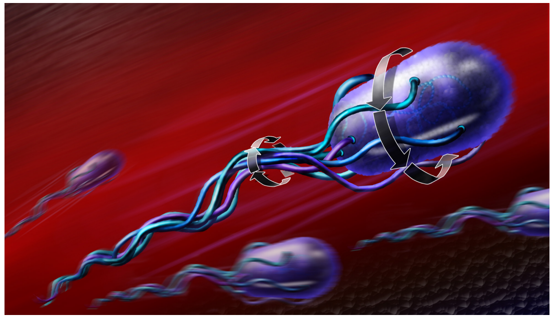
• In particular optical tweezers (OT) and atomic force microscopes (AFM) are commonly used to pull on single molecules

• Force manipulation has provided unique insights into mechanical properties and enzymatic activities

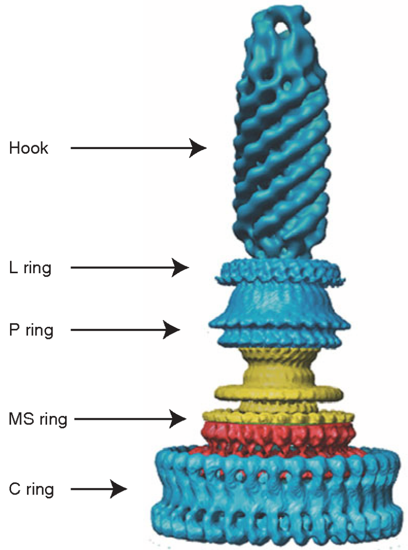
OT, AFM, and flow stretch measurements naturally operate in the space of **force and extension.**

Torque in Biology

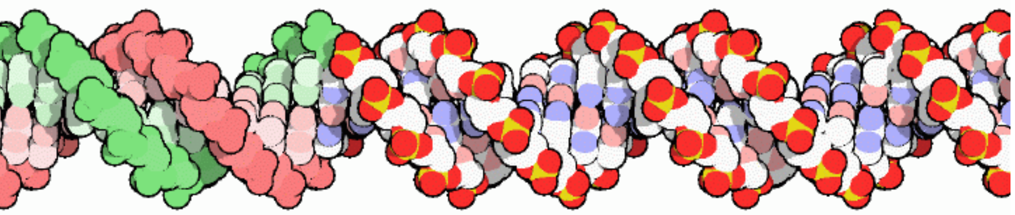
A classic example:
Bacterial flagella are rotary motors



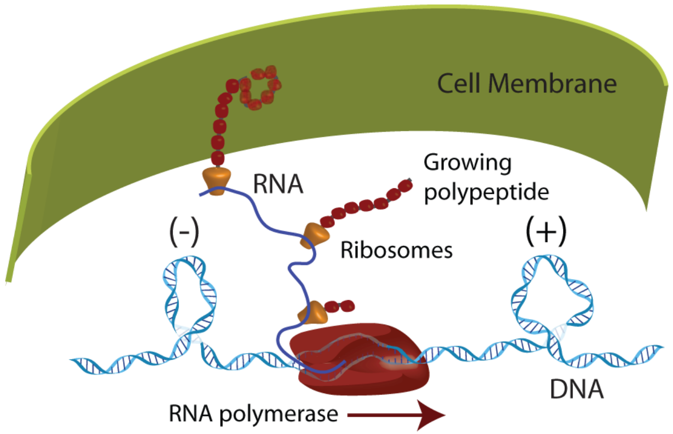
Sowa & Berry, *Quat, Rev. Biophys* (2008)



The helical nature of DNA has important consequences for topology and torque generation in the cell

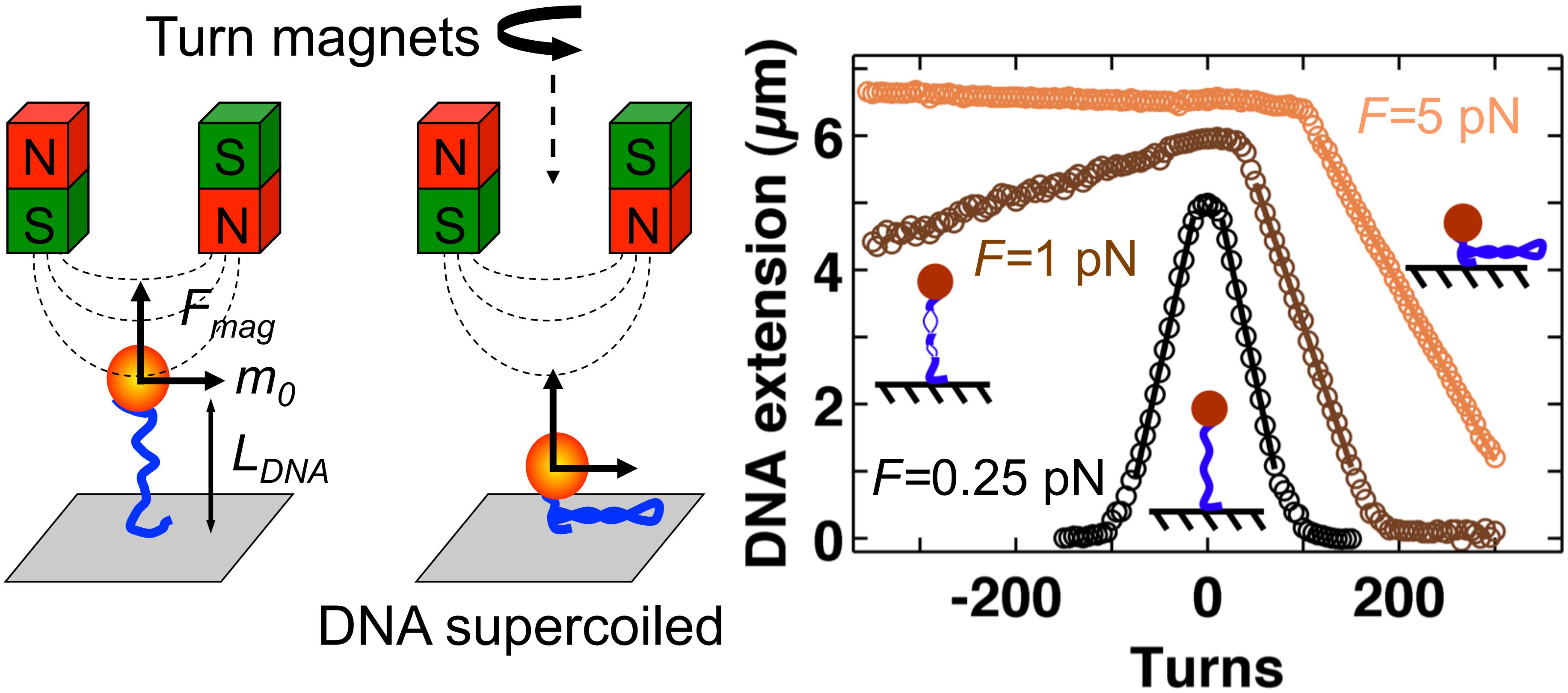


Liu & Wang, *PNAS* (1987)



Koster, Crut, *et al.*, *Cell* (2010)

(Super-)coiling DNA with Magnetic Tweezers



Magnetic tweezers allow us to apply both **forces and torques** to biological macromolecules.

Conventional MT: Strick, *et al. Science* (1996)
Figure adapted from: Lipfert, Koster, Vilfan, Hage & Dekker, *Methods Mol. Biol.* (2009)

Outline

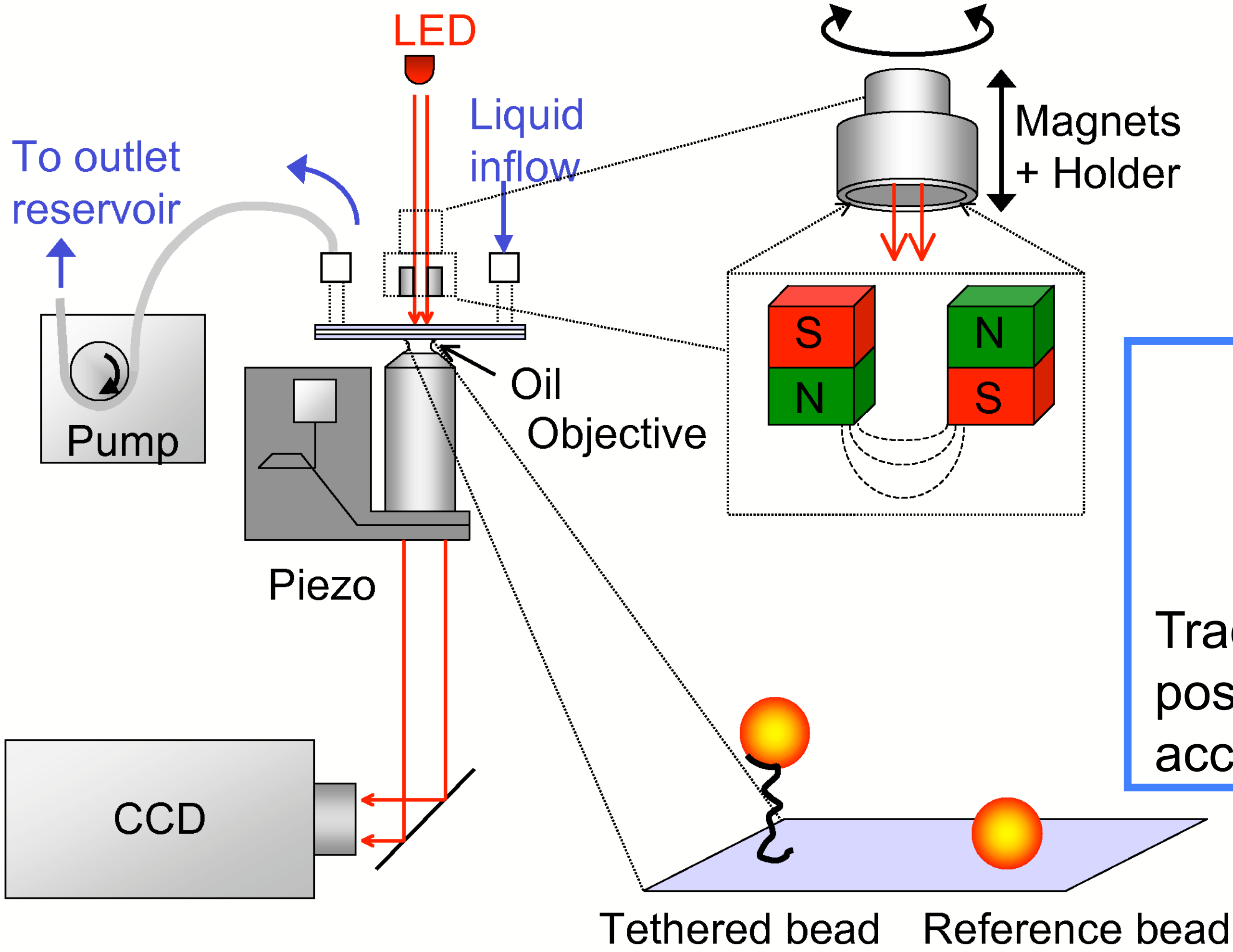
- How do (conventional) magnetic tweezers work?
  - *How do they apply forces?*
  - *How do they apply torques?*

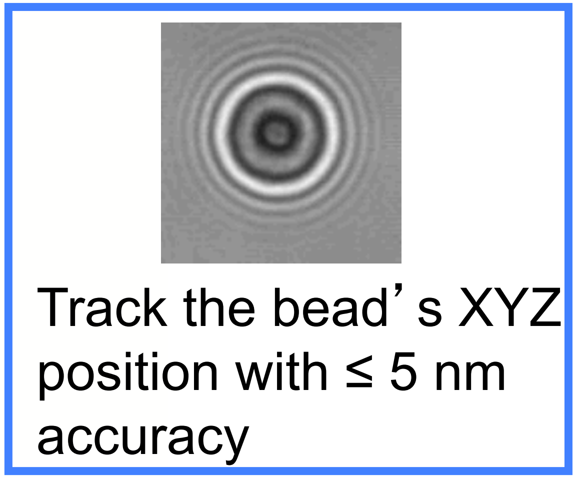
- Novel magnetic tweezers for direct measurements of single-molecule torque and twist
  - *Magnetic torque tweezers (MTT)*
  - *Freely-orbiting magnetic tweezers (FOMT)*

The trouble with people is not their ignorance, but that they know so much that ain't so.

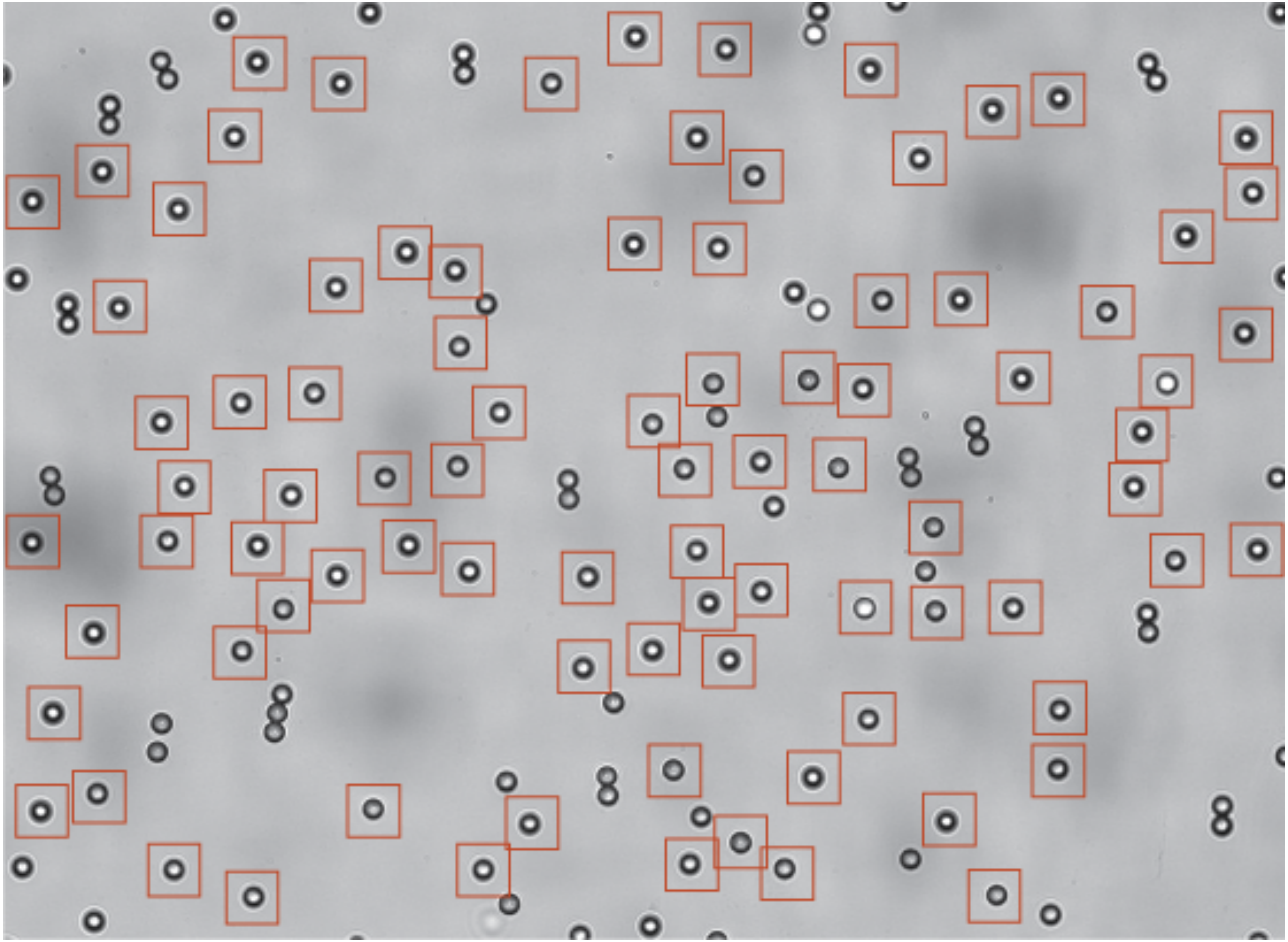
Mark Twain

Single Molecule Measurements with Magnetic Tweezers





Vilfan, Lipfert, Koster, Lemay & Dekker, *Springer Handbook of Single-Molecule Biophysics* (2009)

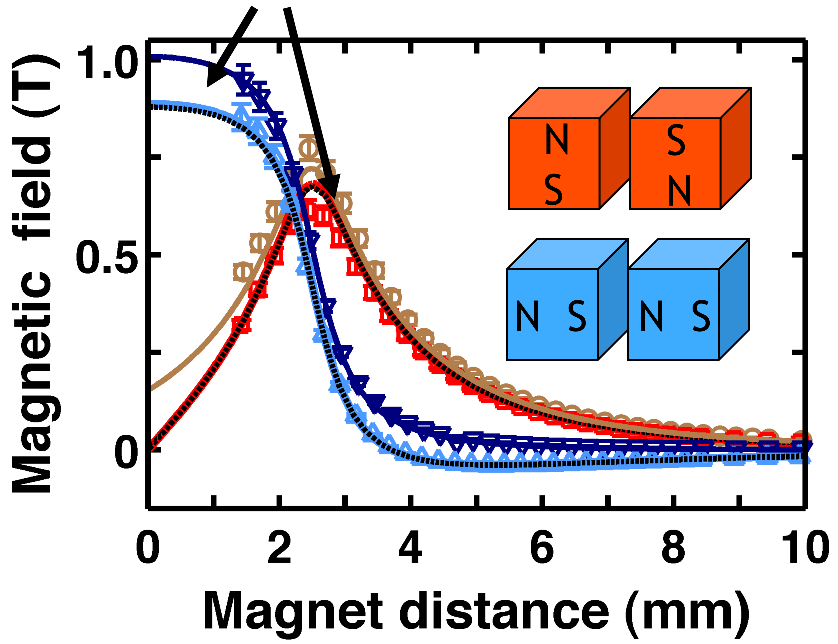


Modeling the force exerted by magnetic tweezers

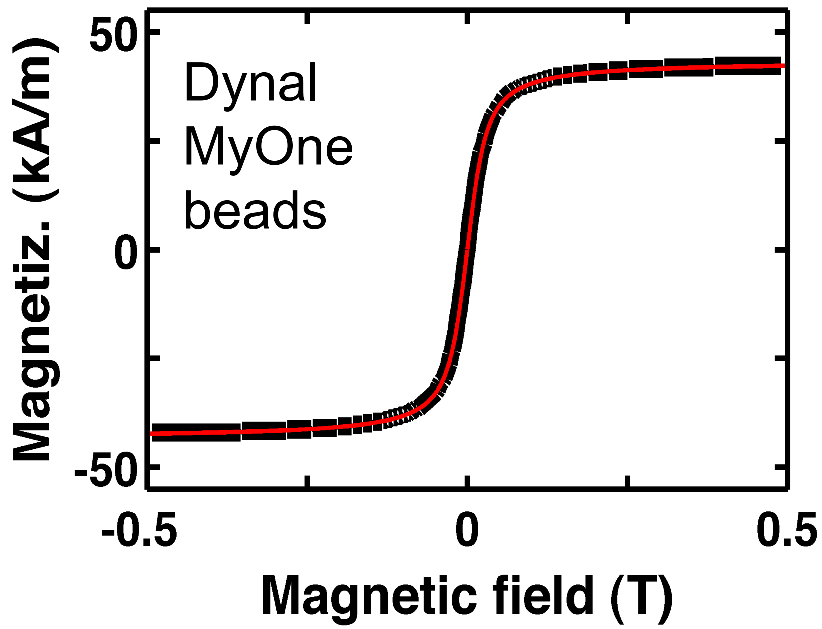
The force on the beads is given by the negative gradient of the magnetic energy: $$\vec{F}_{\mathrm{mag}} = -\vec{\nabla} U_{\mathrm{mag}} = \frac{1}{2}\vec{\nabla}\left(\vec{m}(\vec{B})\cdot\vec{B}\right)$$

1) Calculate the magnetic field

With and without iron yoke



2) Consider the field dependent magnetization of the beads

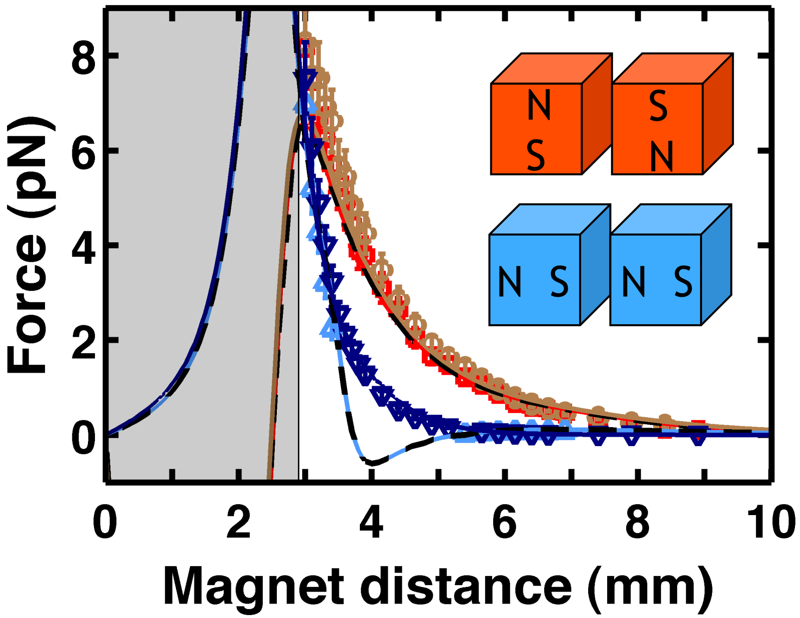


Lipfert, Hao & Dekker, *Biophys. J.*, (2009)

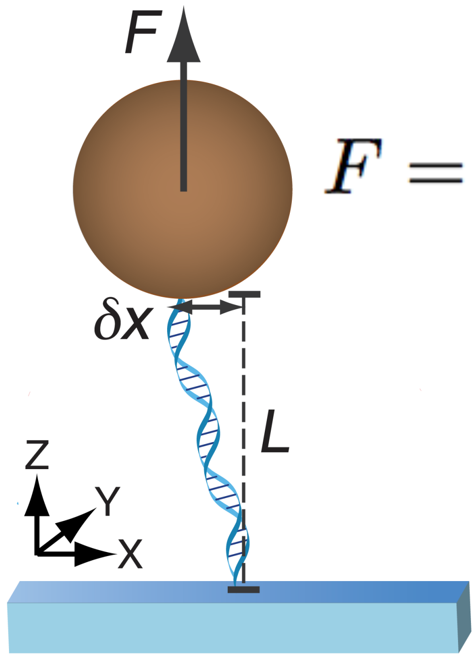
Measuring & modeling the forces in magnetic tweezers

3) Calculate the force exerted on the beads

$$\vec{F}_{\text{mag}} = \frac{1}{2}\vec{\nabla}\left(\vec{m}(\vec{B})\cdot\vec{B}\right)$$



4) Compare to forces determined from fluctuations*



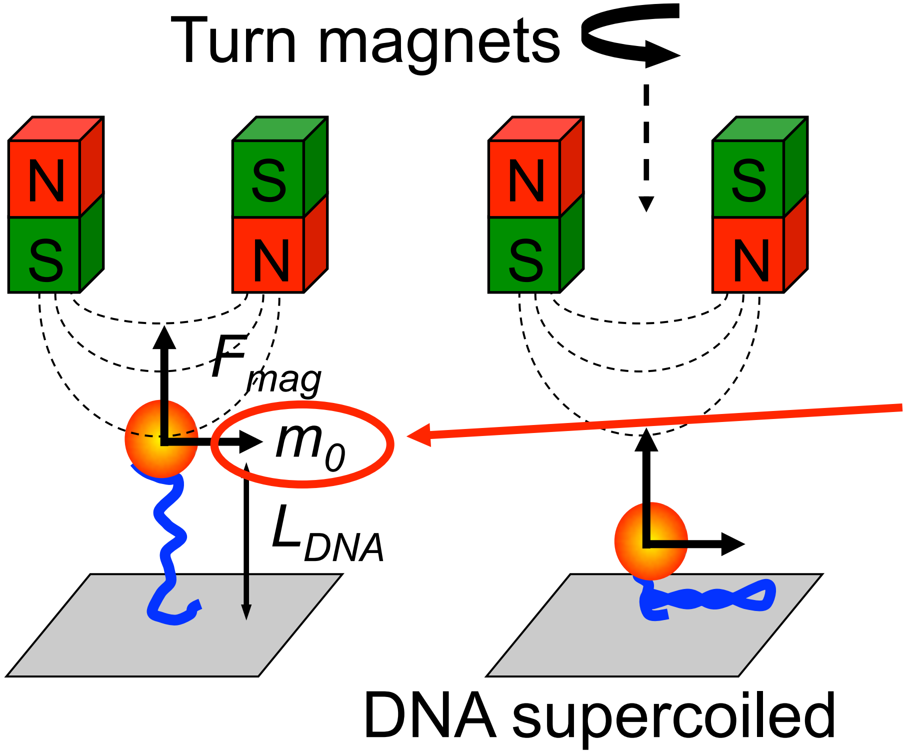
$$F = \frac{k_B T L}{\langle \delta x^2 \rangle}$$

Predictive theory for MT from first principles

Lipfert, Hao & Dekker, *Biophys. J.*, (2009)

*Force calibration with spectral corrections:
Te Velthuis†, Kerssemakers†, Lipfert & Dekker
Biophys. J. (2010)

How do magnetic tweezers exert torque?



- For a perfectly paramagnetic bead, rotating the magnets would not apply any torque!
- There has to be a "special" or preferred direction in the bead to apply torques

Two models:

- Small constant & permanent magnetization component?

$$\vec{m}_0 = const.$$

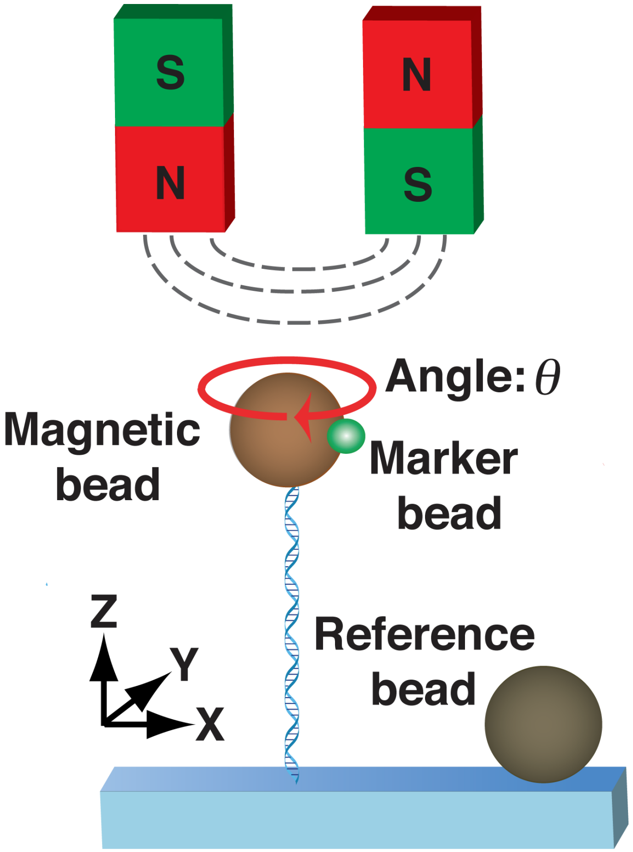
Janssen, Schellekens, Ommering, IJzendoorn & Prins *Biosens. Bioelectr.* (2009)

- Soft or "easy" magnetization axis?

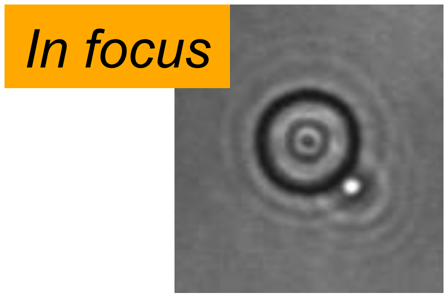
$$\vec{m}_0 = \vec{m}_0(\vec{B})$$

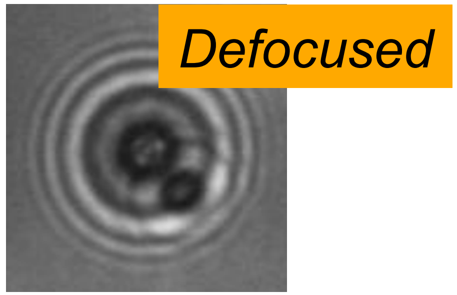
Theory: Normanno, Capitanio & Pavone *PRA* (2004)
Indirect experimental evidence: Klaue & Seidel *PRL* (2009)

Directly monitor bead rotation in MT



- Attach a (non-magnetic) fiducial marker bead to the magnetic bead

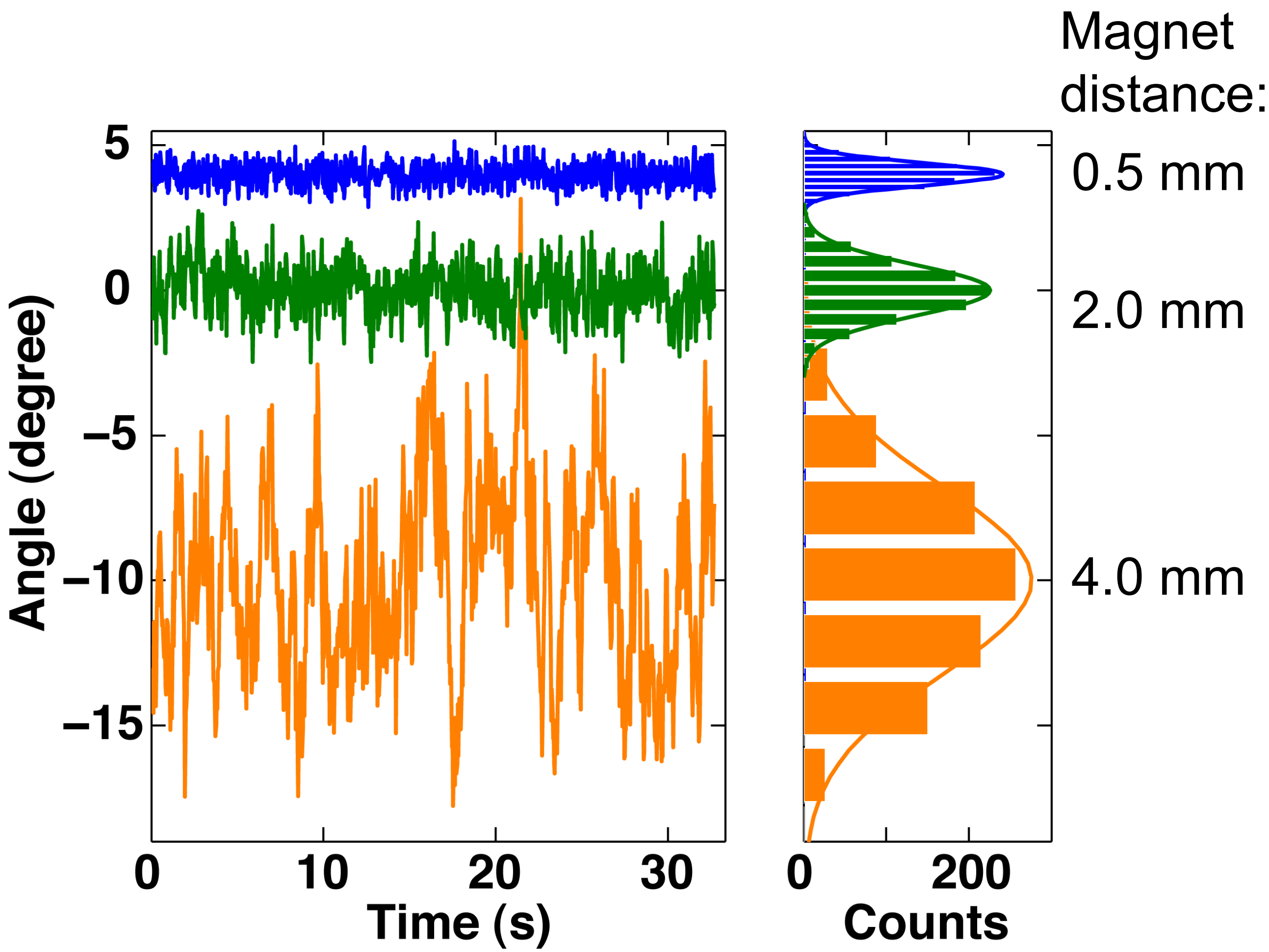




- Implement angular tracking protocol to monitor bead's rotation as well as (*x*,*y*,*z*)-position

Lipfert, Kerssemakers, Jager & Dekker, *Nature Methods* (2010)
Lipfert†, Kerssemakers†, Rojer & Dekker, *Rev. Sci. Instrum.* (2011)

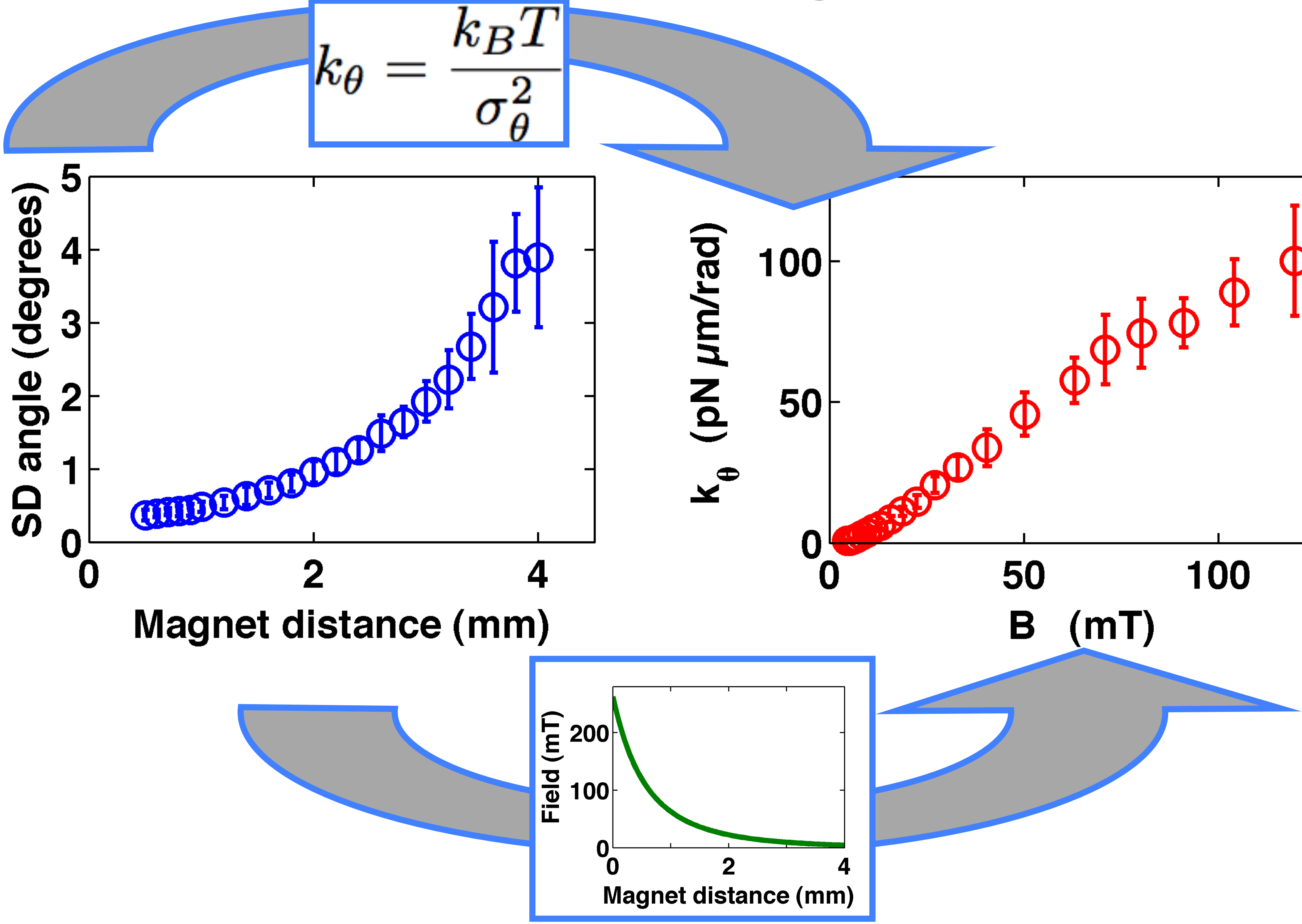
Measure the rotational fluctuations as a function of magnet position



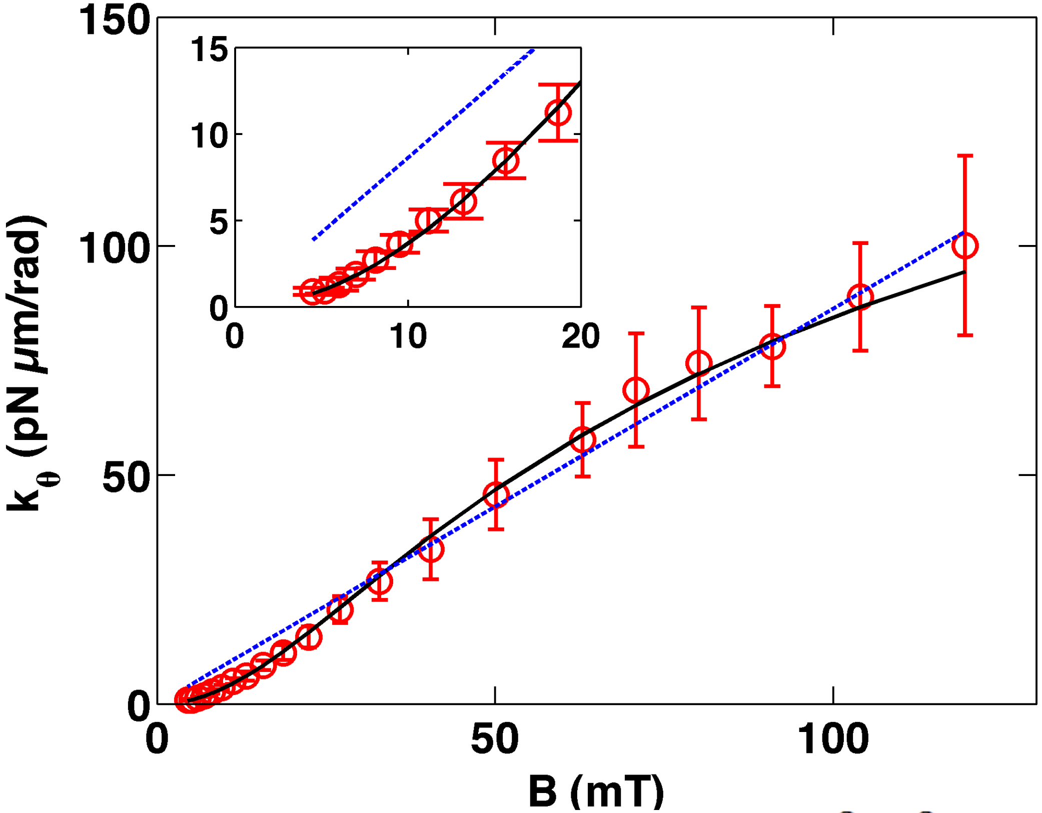
Traces offset for clarity. M270 beads tethered to 8 kbp DNA in PBS buffer.
Horizontal magnets, 1 mm gap, with iron yoke.

Rotational trap stiffness as a function of magnetic field

$$k_\theta = \frac{k_B T}{\sigma_\theta^2}$$



The experimental data support a "soft axis" model



Model with permanent magnetization component:

$$k_\theta = m_0 \cdot B$$

- Fit m_0 (= dipole moment)
- reduced χ^2 = 59, p<<0.01

Soft magnetization axis model:

- reduced χ^2 =0.13, p=0.99

$$k_\theta = N \cdot V \cdot \left(BM \left(1 + \frac{B^2 M^2}{(C+BM)^2} - \frac{2BM}{(C+BM)} \right) + \frac{B^2 C M^2}{(C+BM)^2} \right)$$

Theory: Normanno, Capitanio & Pavone *PRA* (2004)

- Fit: *C* = 2990 J/m^3 (crystalline anisotropy constant)
- and *(N·V)* = 0.06 µm^3 (~effective size of magnetic grains)

Lipfert, *et al.*, *in preparation*

The trouble with people is not their ignorance, but that they know so much that ain't so.

~~Mark Twain~~

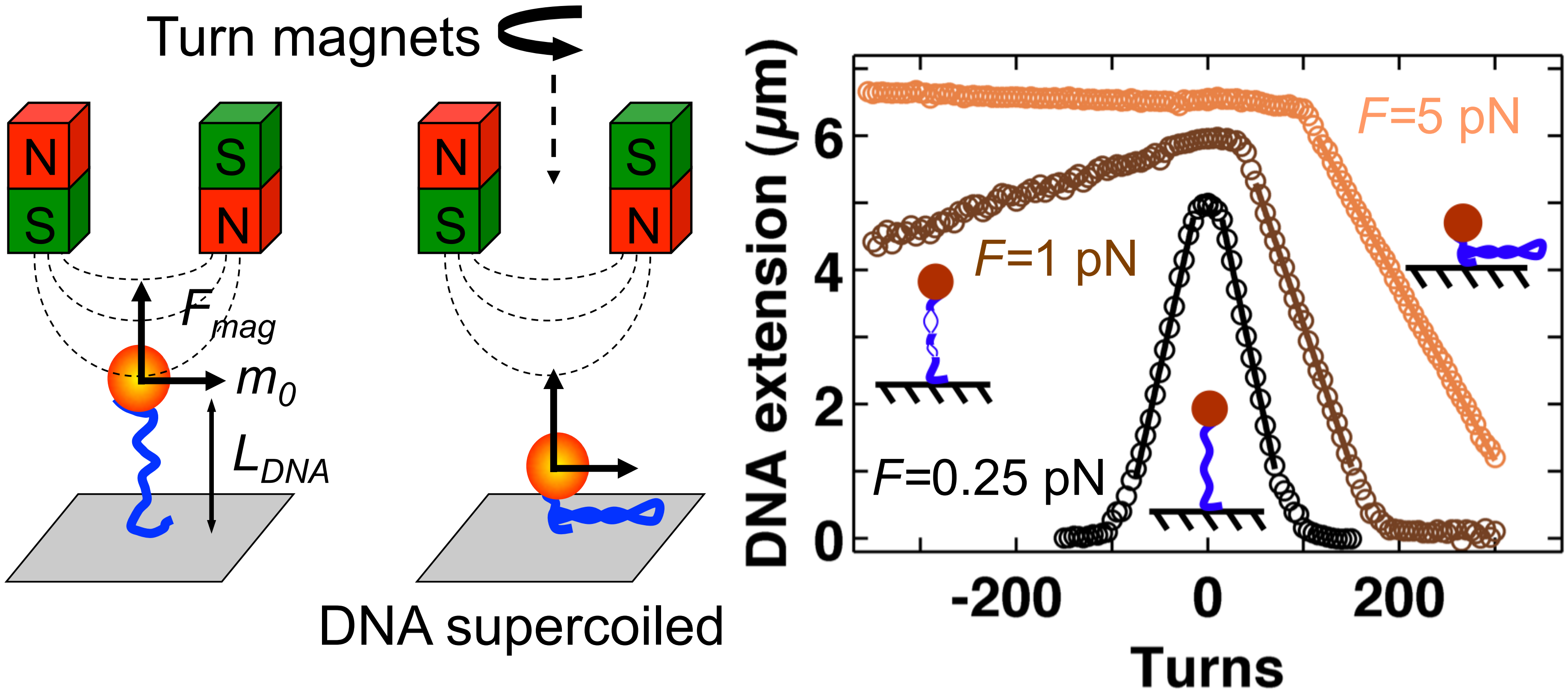
Josh Billings

Outline

- How do (conventional) magnetic tweezers work?
    - *How do they apply forces?*
    - *How do they apply torques?*

- Novel magnetic tweezers for direct measurements of single-molecule torque and twist
    - *Magnetic torque tweezers (MTT)*
    - *Freely-orbiting magnetic tweezers (FOMT)*

Conventional Magnetic Tweezers

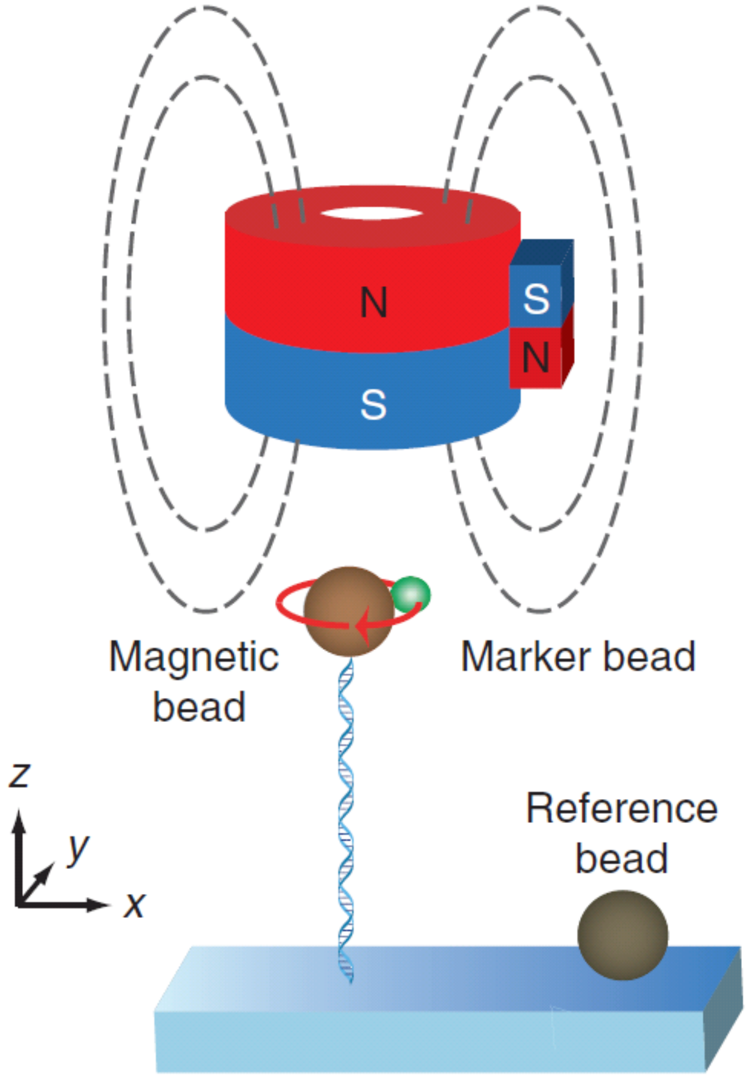


However, current MT

- **Do not track** the bead's rotation directly
- **Do not** permit to measure the applied torque

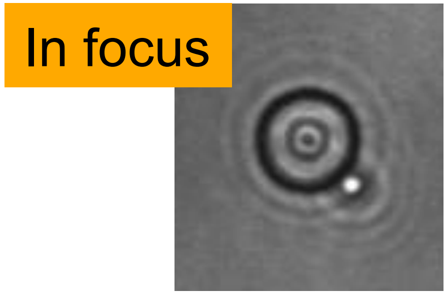
Conventional MT: Strick, *et al. Science* (1996)
Figure adapted from: Lipfert, Koster, Vilfan, Hage & Dekker, *Methods Mol. Biol.* (2009)

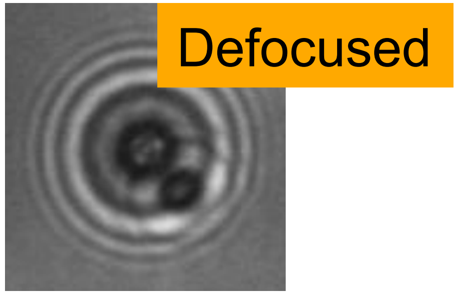
Magnetic Torque Tweezers



Two changes compared to “conventional” MT:

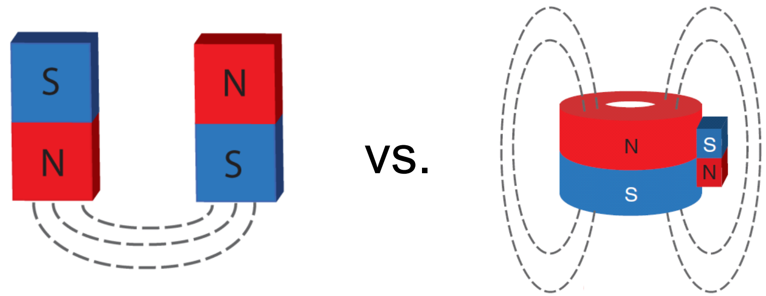
1) Attach a fiducial marker bead and implement angular tracking





Simultaneously track X, Y, Z, and angle (& force and torque)

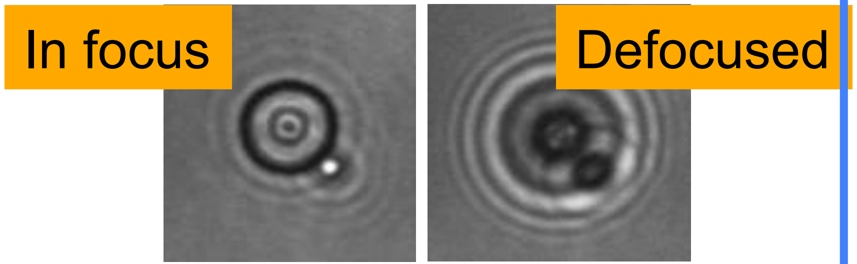
2) Modify the magnet geometry to lower the torsional trap stiffness

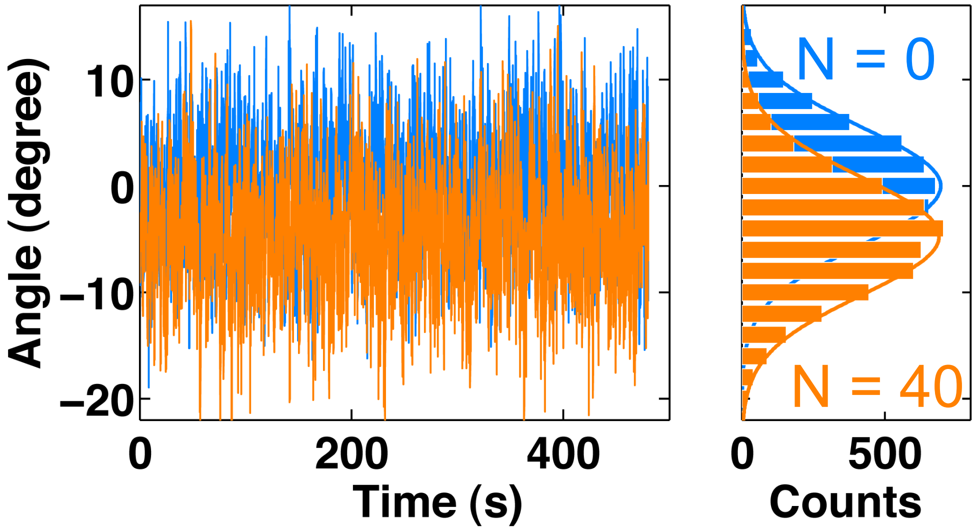


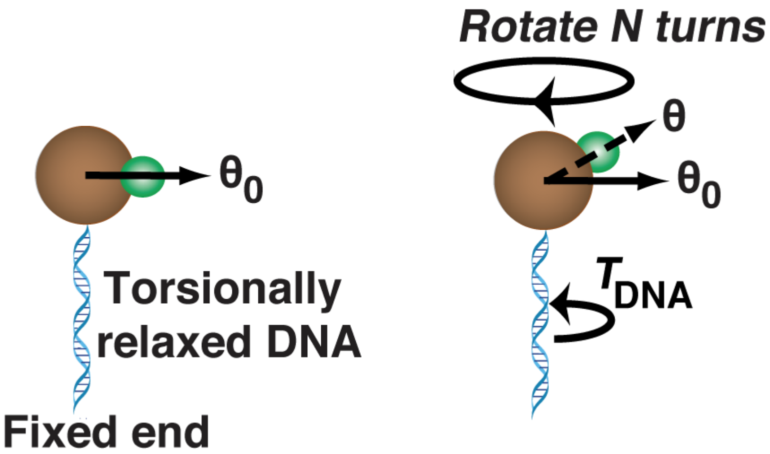
Lipfert, Kerssemakers, Jager & Dekker, Nature Methods (2010)
Lipfert†, Kerssemakers†, Rojer & Dekker, Rev. Sci. Instrum. (2011)

Principle of Magnetic Torque Tweezers

Fiducial marker beads facilitate angle tracking in addition to (x,y,z)







$$\langle \theta \rangle = \theta_0$$

Mean (equilibrium) angle

$$k_\theta = \frac{k_B T}{\langle (\theta - \theta_0)^2 \rangle}$$

Calibrate the trap stiffness from the fluctuations, using equipartition

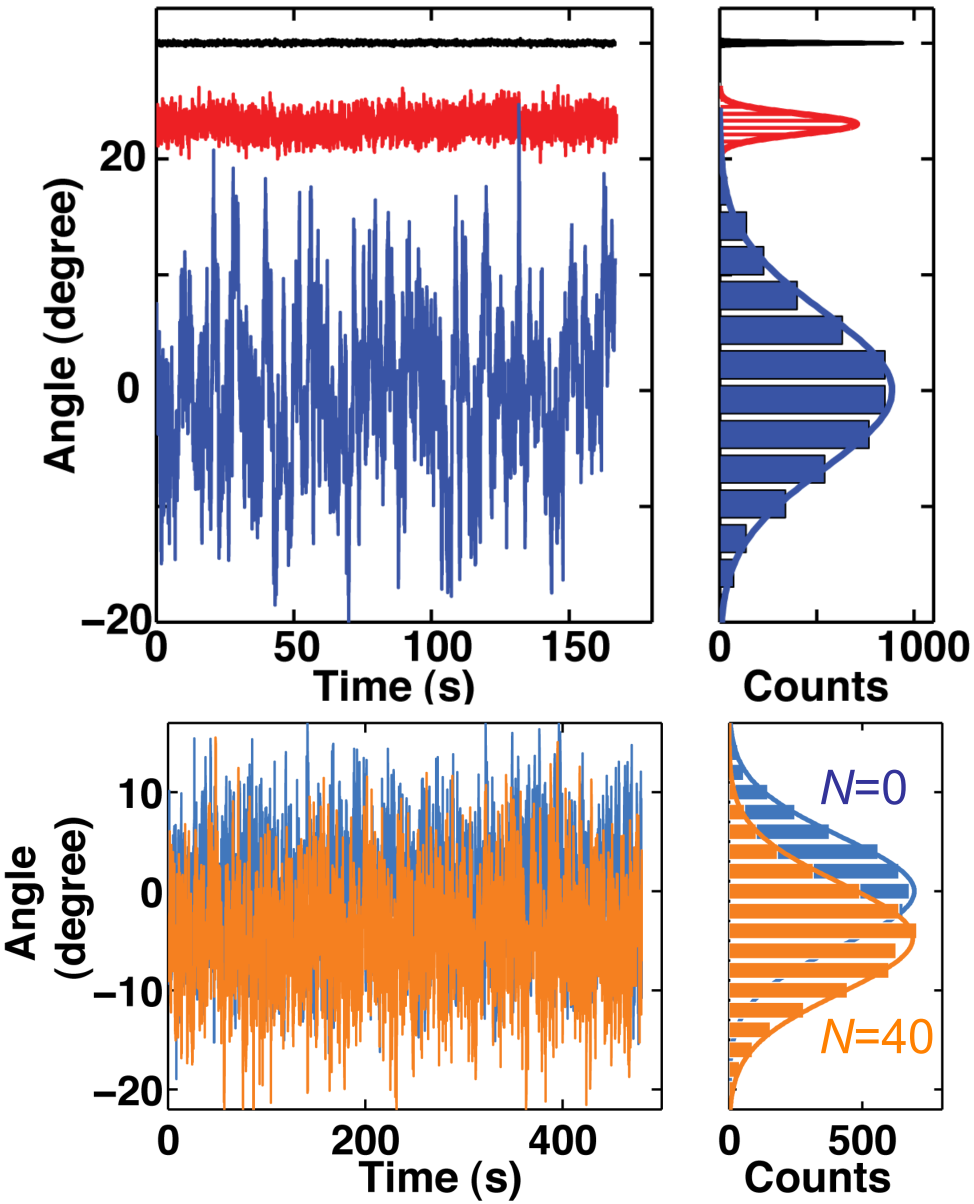
Restoring torque of the tether gives a shift in the equilibrium position:

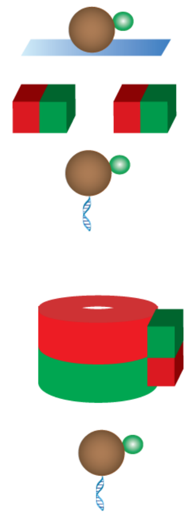
$$\tau_{\mathrm{DNA}} = k_\theta \langle \theta - \theta_0 \rangle$$

Lipfert, *et al., Nature Methods* (2010) and Lipfert†, Kerssemakers†, *et al.*, *Rev. Sci. Instrum.* (2011)

Angular Resolution and Magnet Geometry

Angular traces from image analysis





Surface attached bead:
$\sigma_\theta \approx 0.1^\circ$

Conventional magnetic tweezers:
$\sigma_\theta \approx 1^\circ$

$\Rightarrow$Trap stiffness k_θ is 10^4-10^5 pN·nm/rad

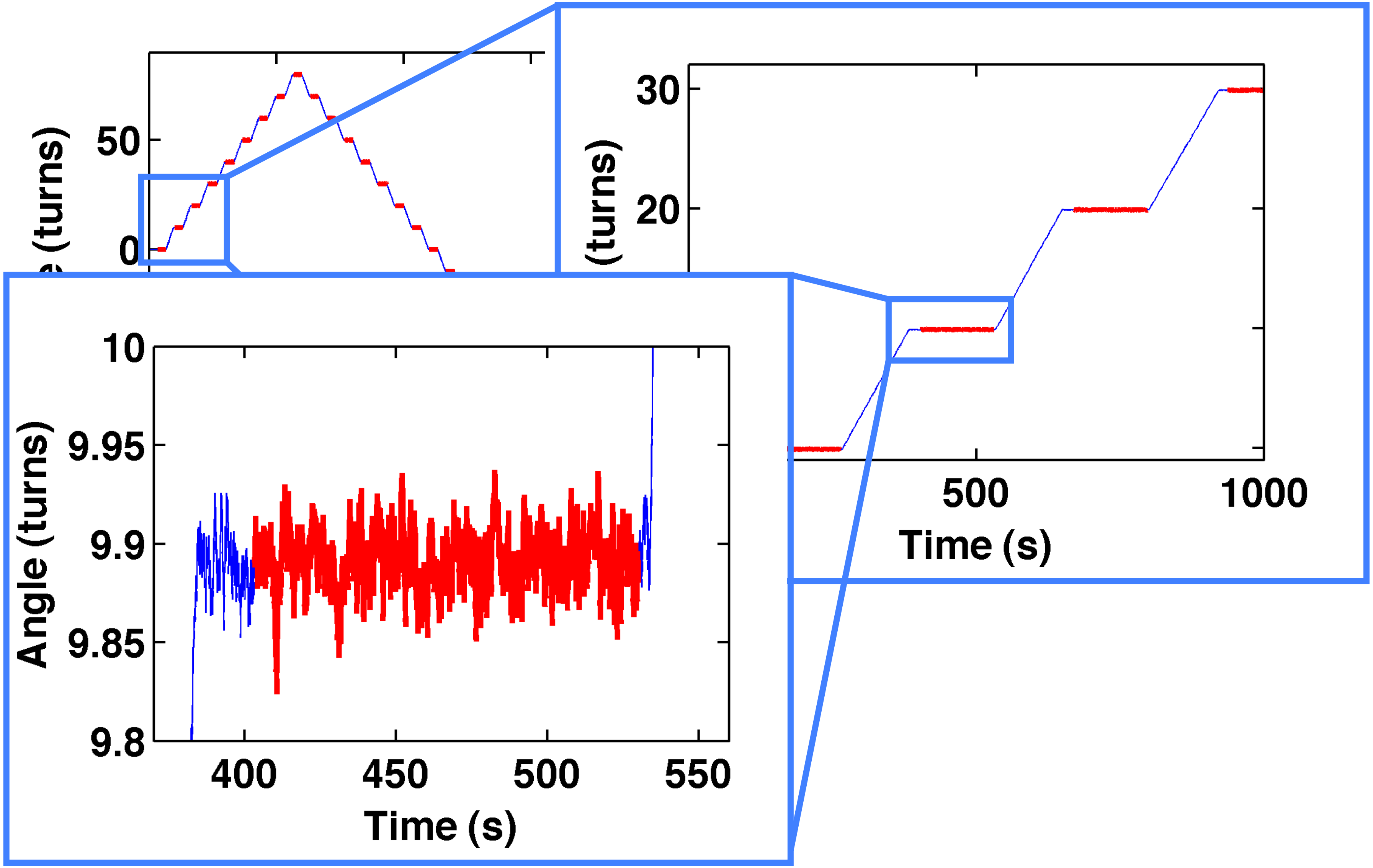
$\Rightarrow$Expected DNA signal $\langle \theta - \theta_0 \rangle \leq 0.1^\circ$

Torque tweezers:
$\sigma_\theta \approx 5\text{-}10^\circ$

$\Rightarrow$Trap stiffness k_θ is 100-500 pN·nm/rad

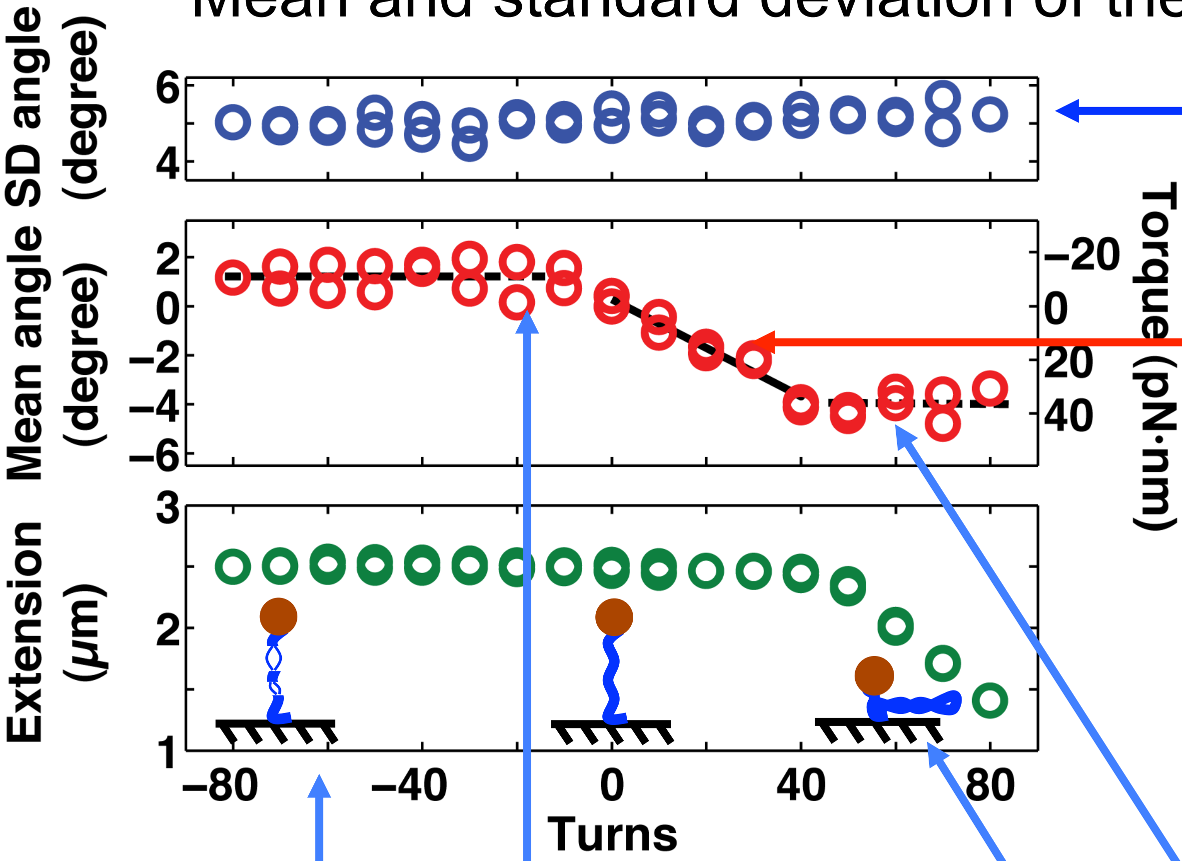
“Twist curve” measurement

Our strategy for measuring the torsional stiffness of DNA



Torque Measurement for DNA

Mean and standard deviation of the angle plateaus



Constant trap stiffness

Elastic DNA response

$$C = \frac{L_c}{2\pi\sigma_\theta^2} \cdot \left(\frac{\Delta\theta}{N}\right)$$

The slope gives the torsional stiffness *C*

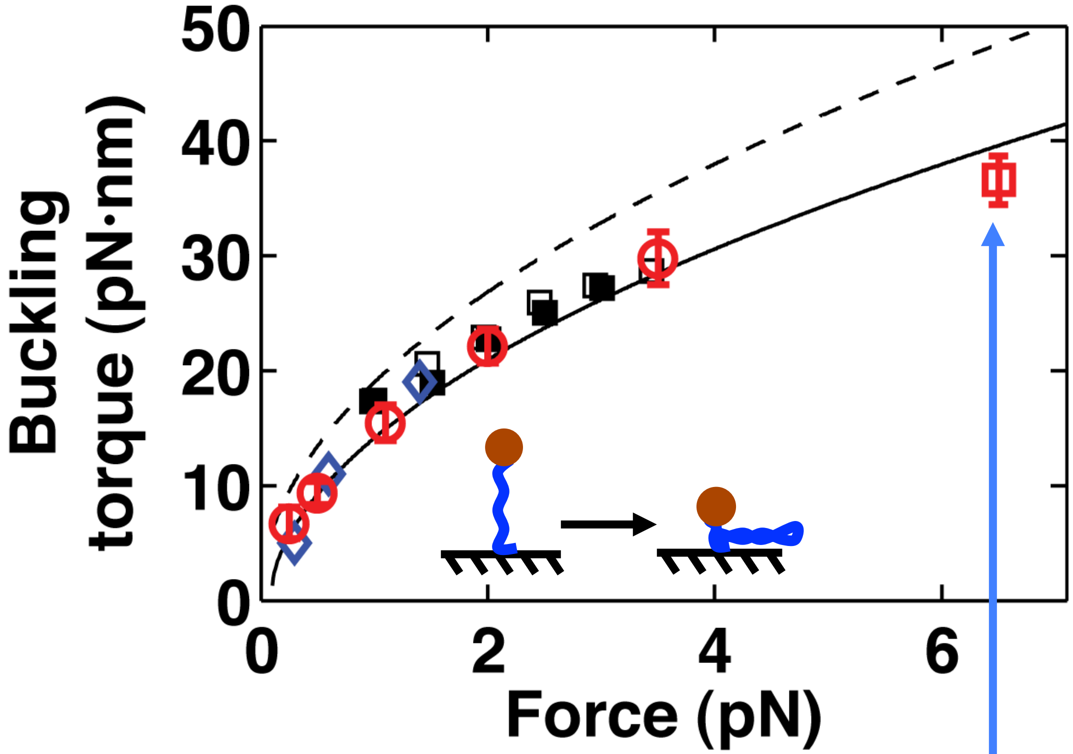
@ negative turns, the torque saturates as DNA denatures

τ_{denat} = -10 ± 1 pN·nm

@ positive turns, the torque saturates as the DNA buckles and plectonemes form

Measurement for 8 kbp DNA in PBS buffer; *F* = 3.5 pN

DNA Buckling Torque



Magnetic torque tweezers (this work)

Optical torque tweezers (Wang lab)

Open squares: 2.2 kbp DNA

Filled squares: 4.2 kbp DNA

Forth, *et al. PRL* (2008)

Nanorod probe (Sun lab)

Celedon, *et al. Nano Lett.* (2009)

- - - Simple elastic model

Strick, *et al. Rep. Prog. Phys.* (2003)

—— Marko model

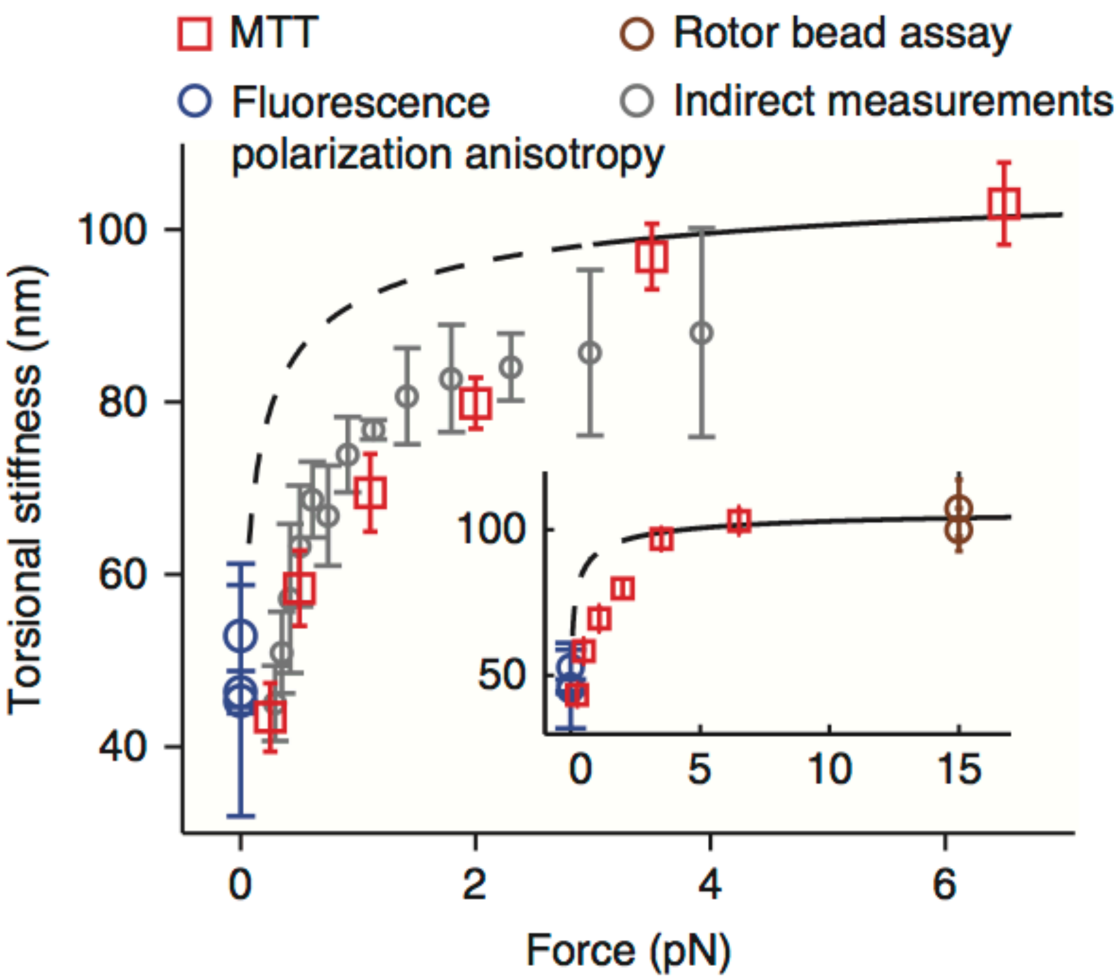
Marko *PRE* (2007)

@ forces > 6 pN, (B-form) DNA does not buckle, but undergoes a transition to P-DNA. We measure: $\tau_{B \rightarrow P}$ = **36 ± 2 pN·nm**

See also: Sheinin *et al. PRL* (2008) and Bryant *et al. Nature* (2003)

MTT measurements are for 8kbp DNA in PBS buffer. Red symbols are the mean and SEM of 5-10 independent molecules; Ref: Lipfert, Kerssemakers, Jager & Dekker, *Nature Methods* (2010)

DNA Torsional Persistence Length



Magnetic torque tweezers

Rotary bead tracking @ high F (Bustamante lab)

Bryant *et al. Nature* (2003)

FPA measurements on linear DNA ⇒ zero force

Fujimoto & Schurr *Nature* (1990)

Selvin *et al. Science* (1992)

Heath *et al. JMB* (1996)

Indirect measurement by thermodynamic integration

Mosconi *et al. PRL* (2009)

Model by Moroz & Nelson

Moroz & Nelson *PNAS* (1997)

MTT measurements cover the entire force range from “high” to effectively zero

Lipfert, Kerssemakers, Jager & Dekker, *Nature Methods* (2010)

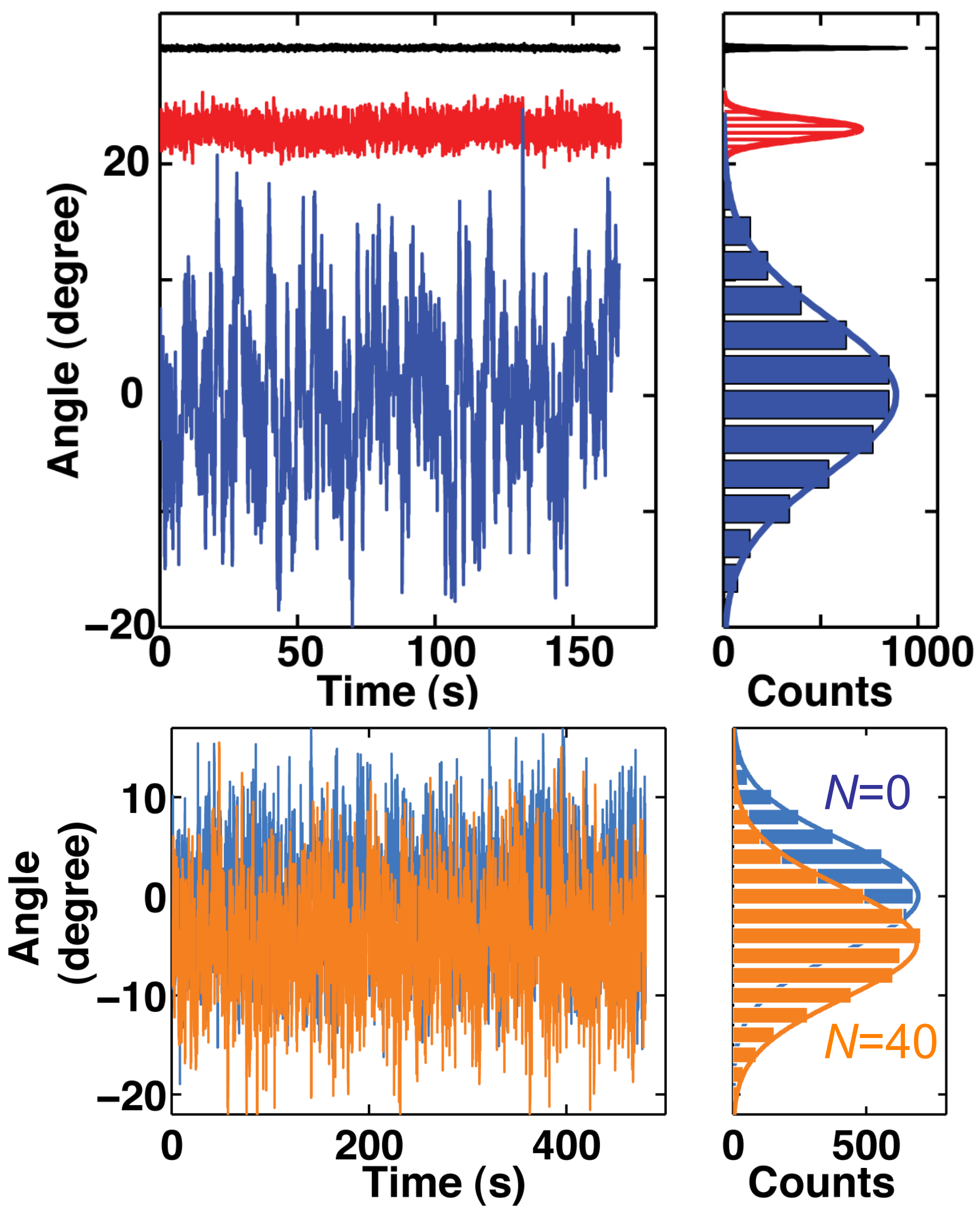
Outline

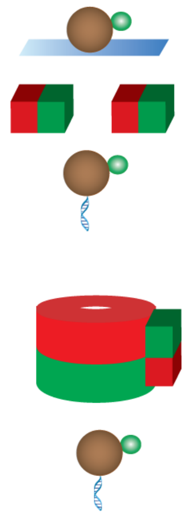
- How do (conventional) magnetic tweezers work?
  - *How do they apply forces?*
  - *How do they apply torques?*

- Novel magnetic tweezers for direct measurements of single-molecule torque and twist
  - *Magnetic torque tweezers (MTT)*
  - *Freely-orbiting magnetic tweezers (FOMT)*

Angular Resolution and Magnet Geometry

Angular traces from image analysis





Surface attached bead:

$\sigma_\theta \approx 0.1°$

Conventional magnetic tweezers:

$\sigma_\theta \approx 1°$

⇒Trap stiffness k_θ is 10^4-10^5 pN·nm/rad

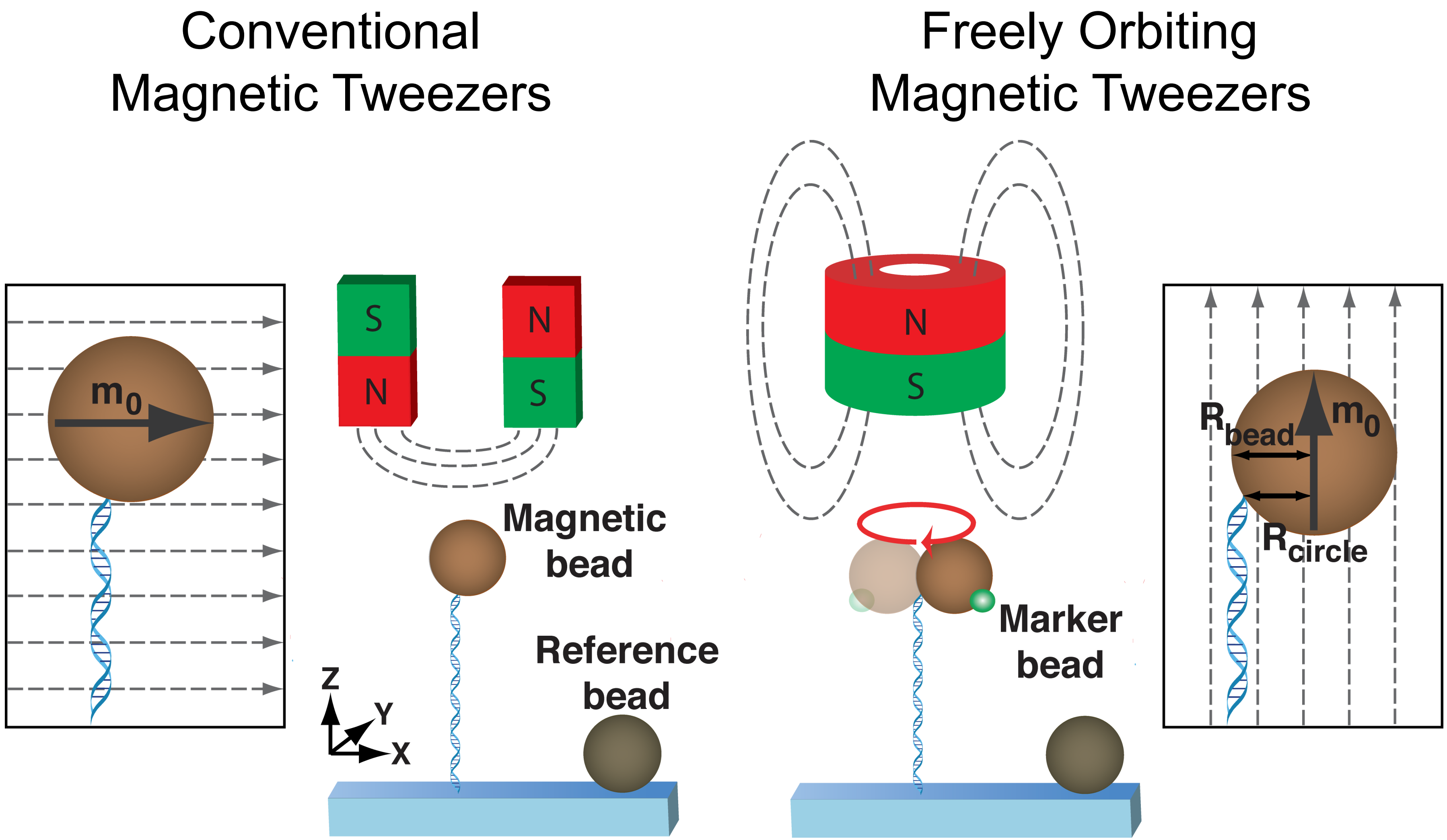
⇒Expected DNA signal $<\theta - \theta_0> \leq 0.1°$

Torque tweezers:

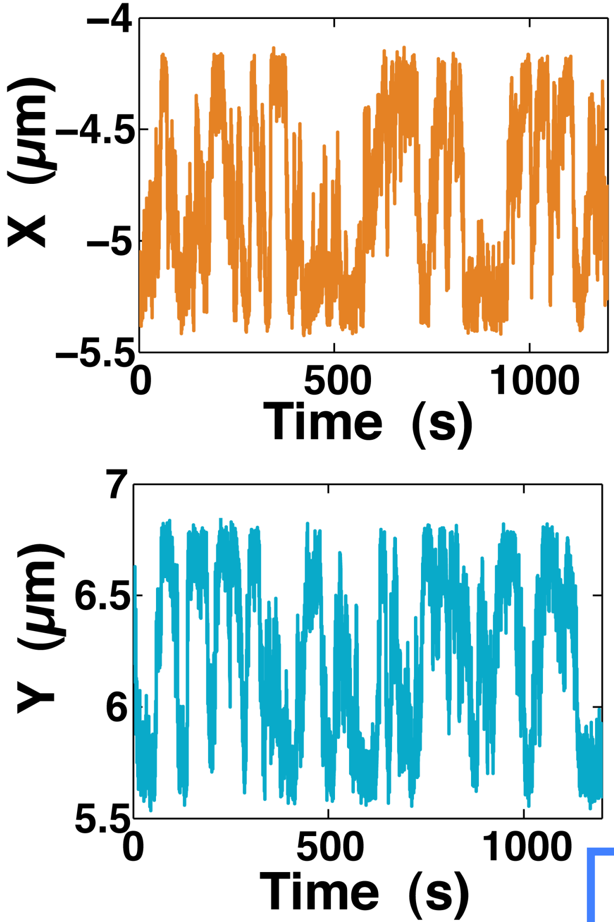
$\sigma_\theta \approx 5$-$10°$

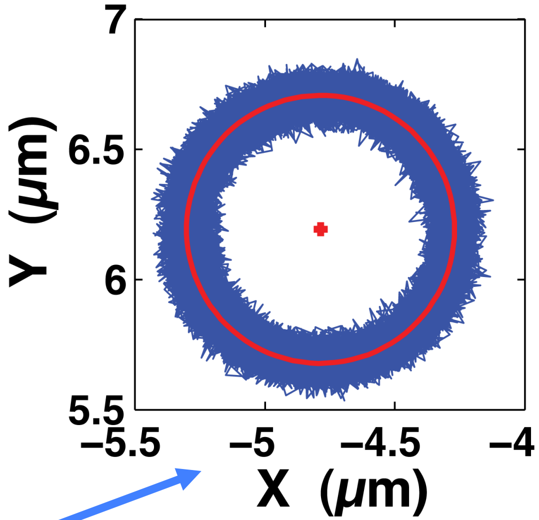
⇒Trap stiffness k_θ is 100-500 pN·nm/rad

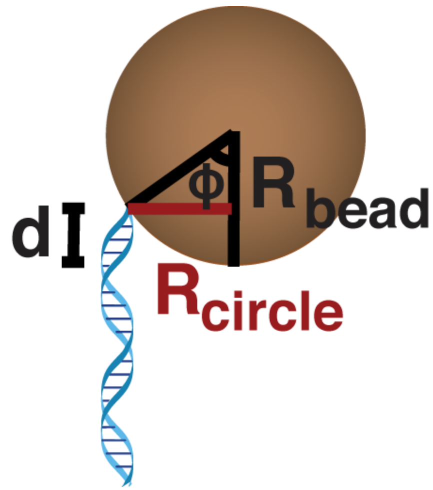
Can we reduce the torsional trap of the magnets to zero?



For a perfectly aligned cylindrical magnet, the fluctuations lie on a circle



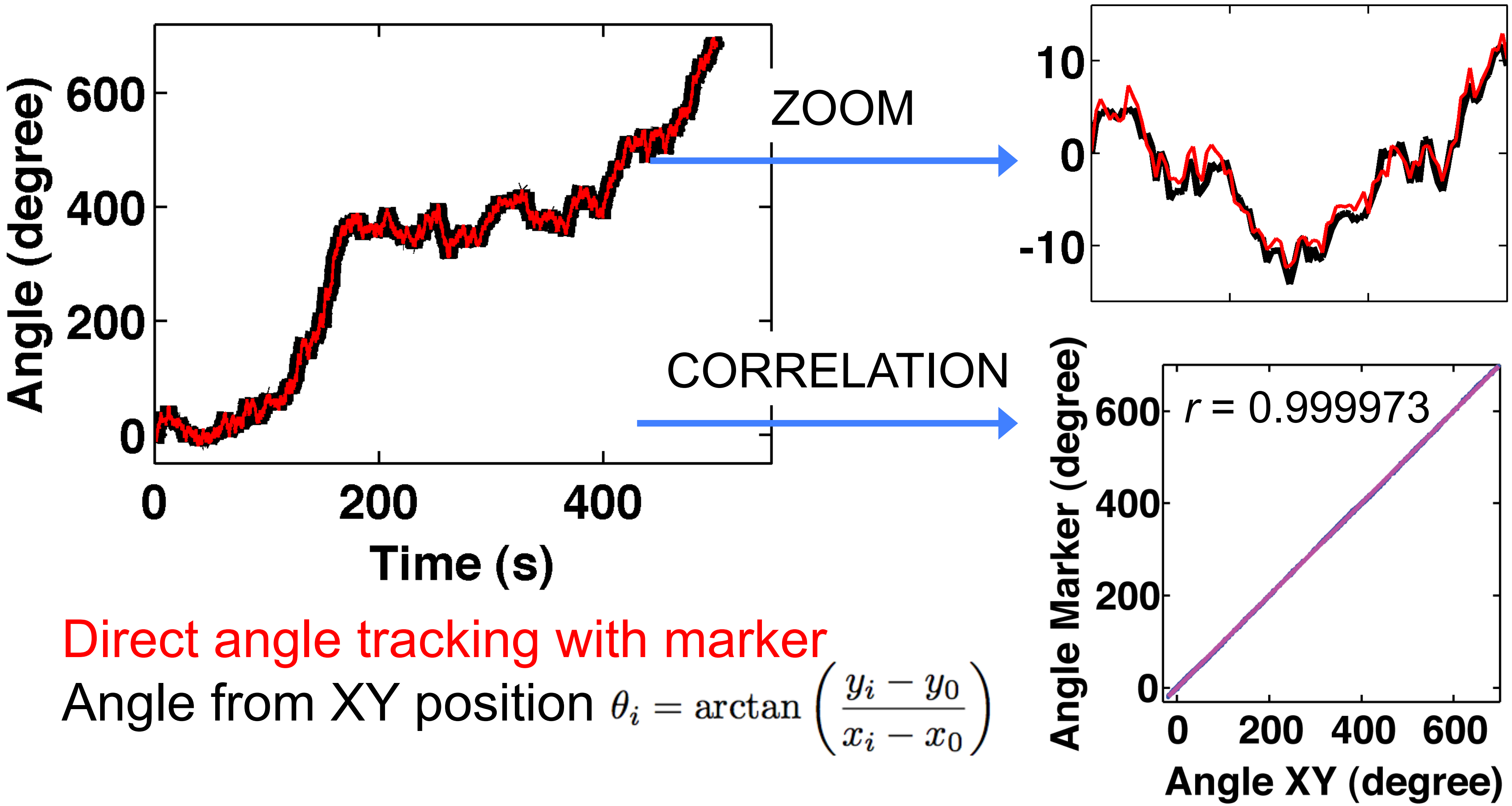




Fit a circle with radius R_{circle} and center (x_0,y_0)

$$\min_{x_0,y_0,R} = \sum_{i}^{N} \left((x_i - x_0)^2 + (y_i - y_0)^2 - R^2\right)^2$$

Tracking bead rotation from XY-position



Direct angle tracking with marker

Angle from XY position $\theta_i = \arctan\left(\frac{y_i - y_0}{x_i - x_0}\right)$

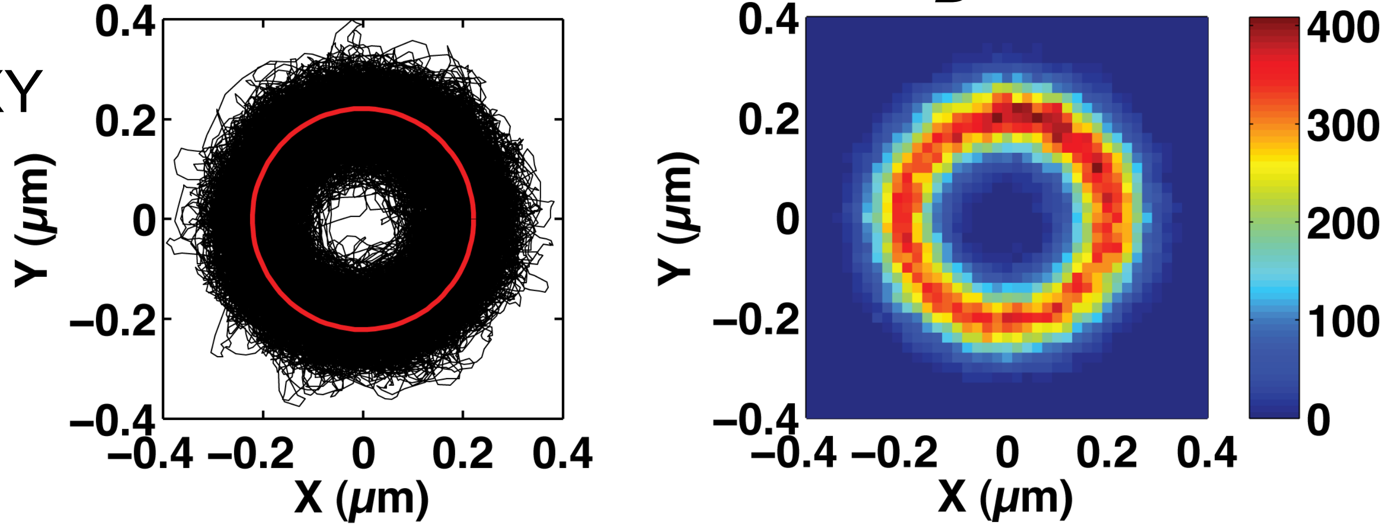
"Tracking angle without tracking angle"

“The art of fighting without fighting”

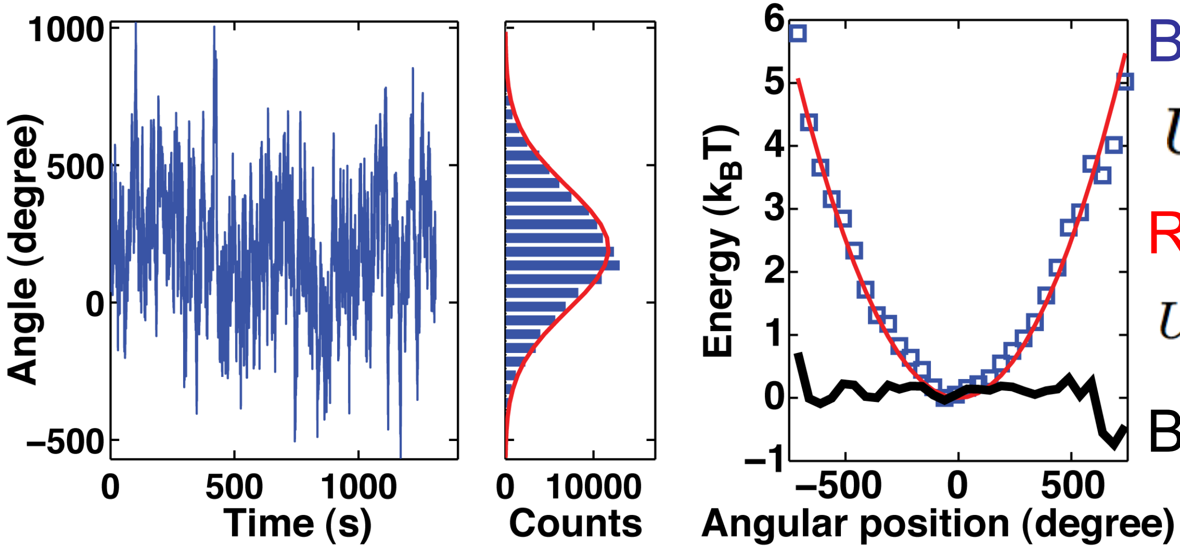
Bruce Lee, *Enter the Dragon* (1973)

Torsional trap of the magnets is small compared to k_BT

Convert XY to angle



Fluctuations imply a harmonic energy landscape



Blue symbols:

$$U \sim -k_B T \ln(P_\theta)$$

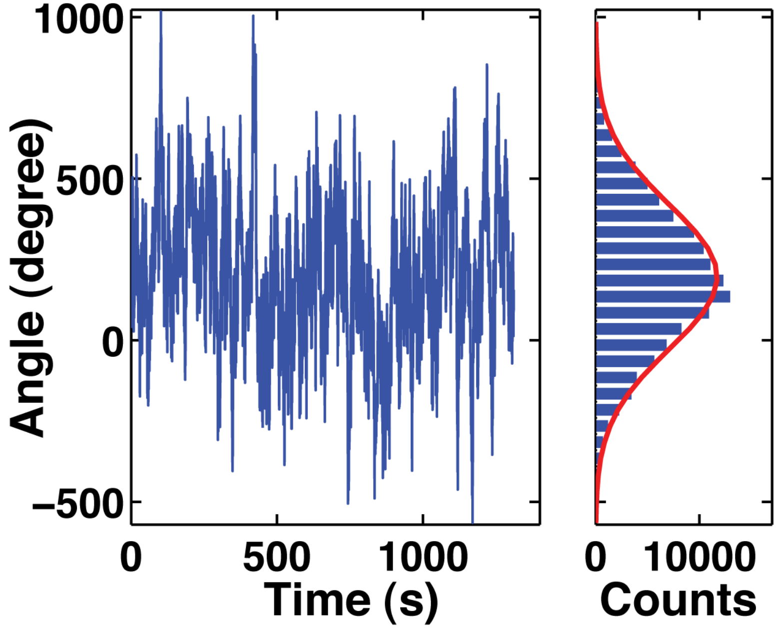
Red line:

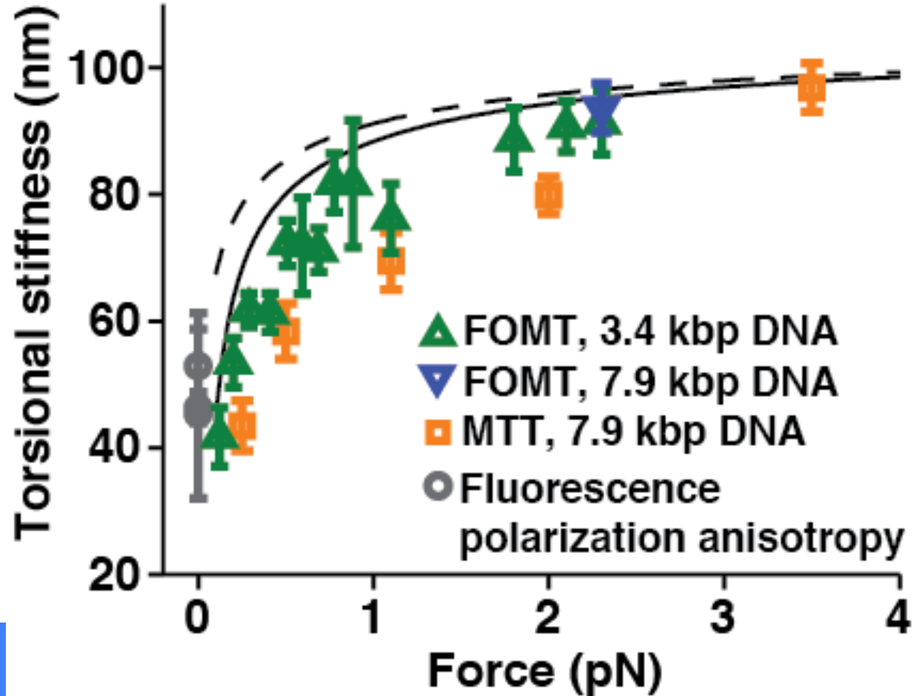
$$U_{harm} = \frac{1}{2}\frac{k_B T}{\sigma_\theta^2}(\theta - \theta_0)^2$$

Black: Difference

Lipfert†, Wiggin†, Kerssemakers, Pedaci & Dekker, *Nature Communications* (2011)

DNA torsional stiffness determined from angular fluctuations





Torsional stiffness C_{DNA}:

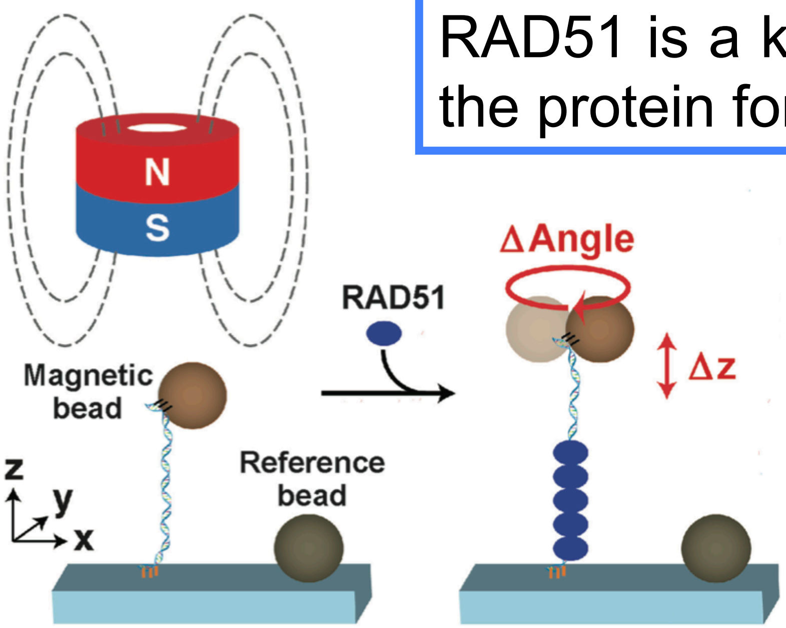
$$C_{\mathrm{DNA}} = \frac{L_c}{\sigma_\theta^2}$$

Contour length L_C = 1.15 µm
Fluctuations σ_θ = 223° = 3.9 rad
⇒ Measured C_{DNA} = 76 nm

Overall, we find consistent *C(F)* values from FOMT and MTT measurements.

Lipfert†, Wiggin†, Kerssemakers, Pedaci & Dekker, *Nature Communications* (2011)

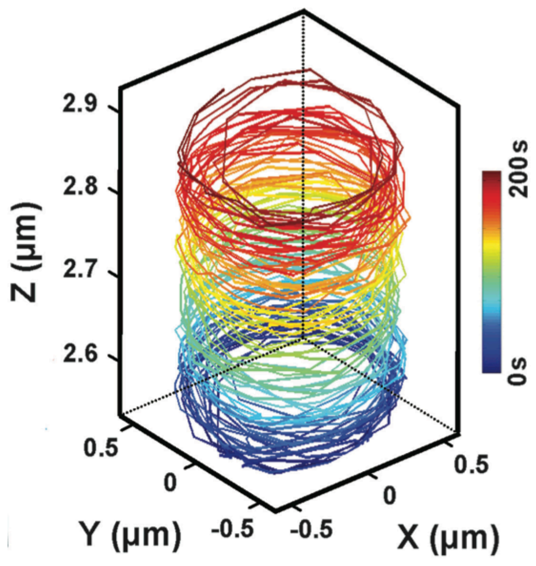
Studying the assembly and mechanical properties of RAD51-dsDNA filaments

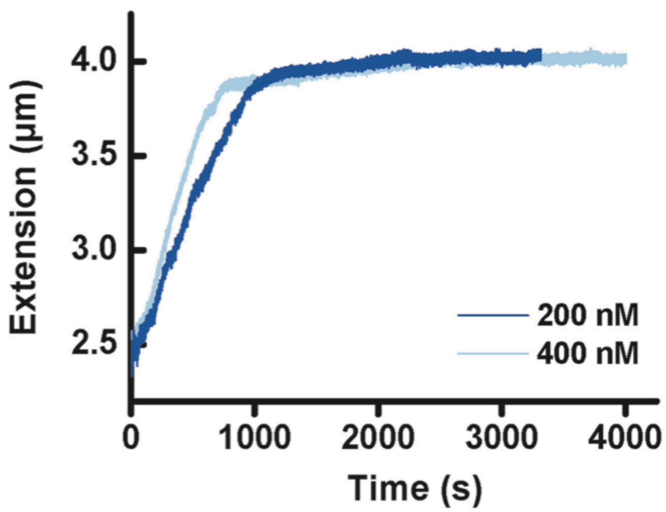


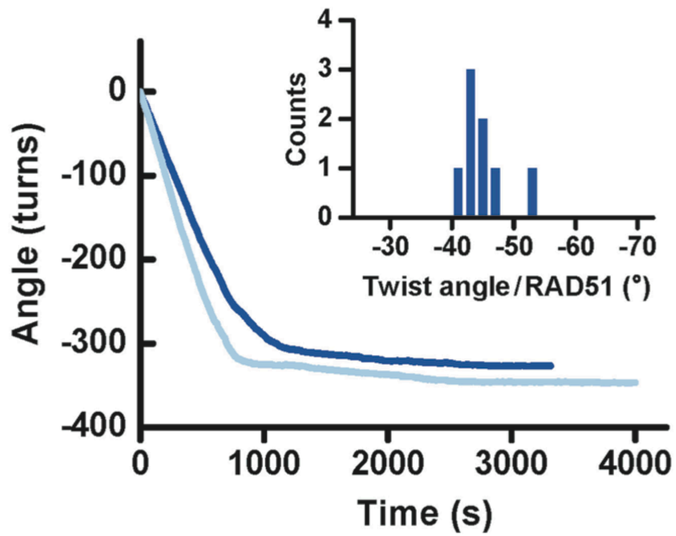
RAD51 is a key factor in homologous recombination; the protein forms filaments on ss and ds DNA

Monitor filament assembly in real time both in extension and angle; deduce the change in angle per monomer binding event.

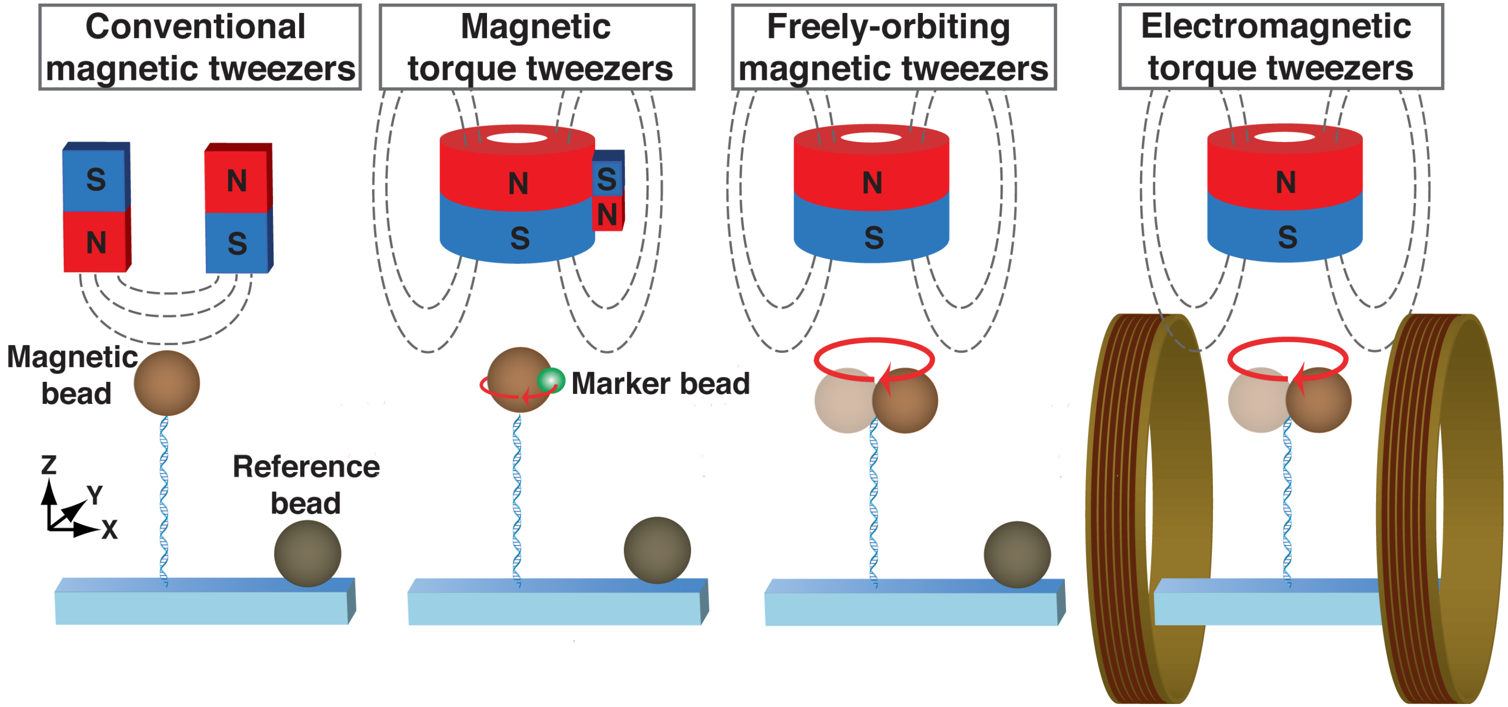
Lee, Lipfert, Sanchez, Wyman & Dekker *NAR* (2013)







Novel Magnetic Tweezers for Direct Torque and Twist Measurements



	Conventional magnetic tweezers	Magnetic torque tweezers	Freely-orbiting magnetic tweezers	Electromagnetic torque tweezers
Torque	***apply only***	***apply & measure***	***free rotation***	***apply & measure or free***
Angle tracking		✓	✓	✓
Force (apply & measure)	✓	✓	✓	✓
(x,y,z)-tracking	✓	✓	✓	✓
	Strick, et al., Science (1996)	Lipfert, et al., Nature Methods (2010) Lipfert, et al., Rev. Sci. Instrum. (2011)	Lipfert†, Wiggins†, et al. Nature Commun. (2011) Lipfert, et al., Patent (2010), Licensed (2012)	Janssen†, Lipfert†, et al. Nano Letters (2012)

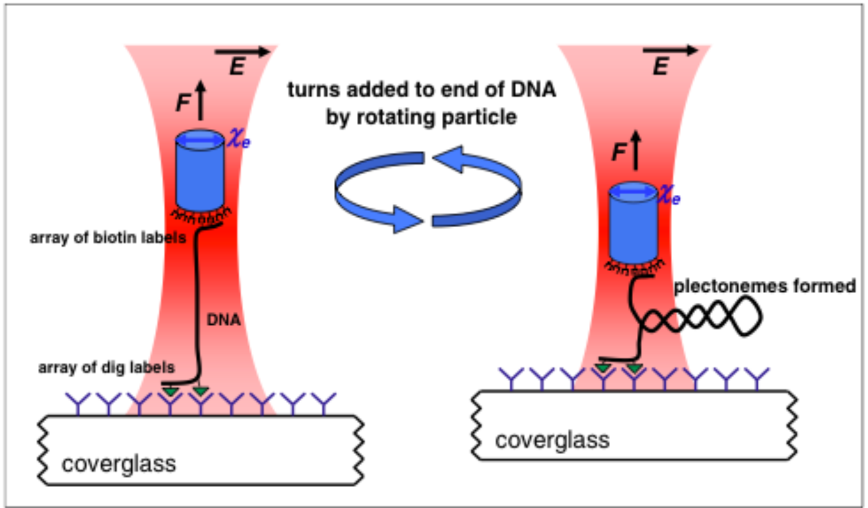
(†Equal contribution)

One sentence summary:

Magnetic tweezers can apply (and measure) both forces and torques to individual macromolecules

Back up slides

Angular Trapping Strategies:
Optical vs. Magnetic Torque Tweezers



Optical torque weezers:

\+ High-bandwidth (kHz)

\+ Better position resolution

– Hard to apply forces < 1 pN:
No negative supercoils!

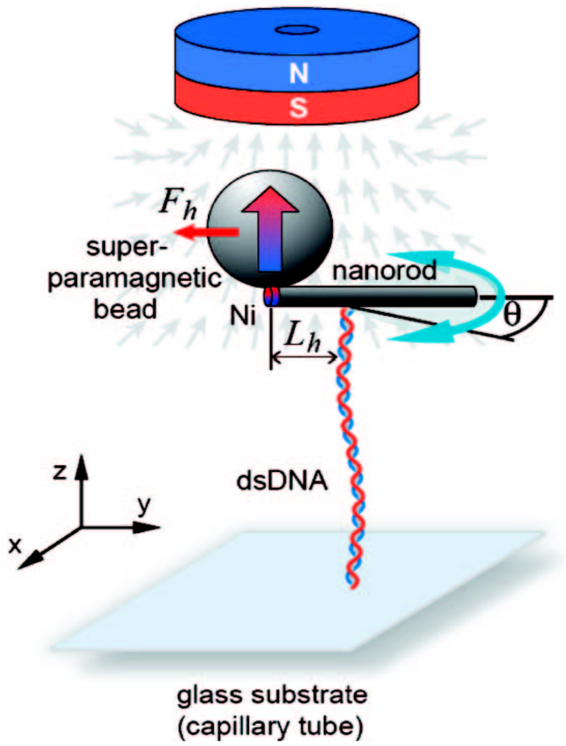
– Relative complicated set up
and samples (nanofabrication)

– Difficult to multiplex

La Porta & Wang *PRL* (2004)
Deufel et al. *Nature Methods* (2007)
Forth, et al. *PRL* (2008)

Similar approach:
Oroszi, Galajda, Kirei, Bottka & Ormos *PRL* (2006)

Angular Trapping Strategies: Nanorod Torque Tweezers



Overall similar to our scheme
Disadvantages of nanorod tweezers:

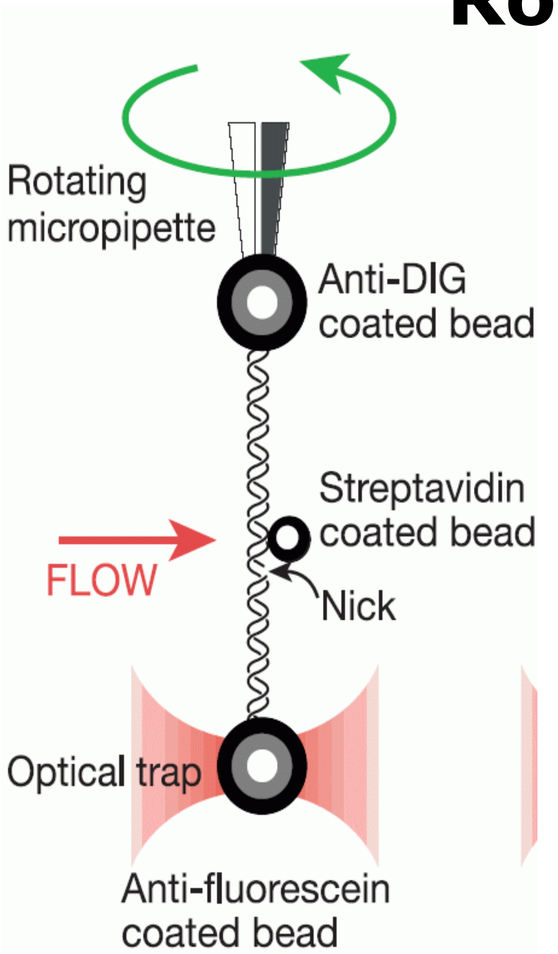
– Complicated sample assembly (nanofabricated rods + beads)

– Rotate by rotating the stage!

– Currently achieve only fairly poor resolution (~ 50 nm) and low forces (< 1.5 pN)

Celedon, et al. *Nanoletters* (2009)

Angular Trapping Strategies: Rotor Bead Tracking



Rotor bead tracking:

+ Can use small rotor beads, achieve good time resolution

– Difficult to multiplex

– Relies in specifically nicked DNA: Difficult for filaments, RNA, etc.

Bryant, *et al. Nature* (2003)

Using magnetic tweezers to rotate:
Gore, *et al. Nature* (2006), Oberstrass, *et al. PNAS* (2012)