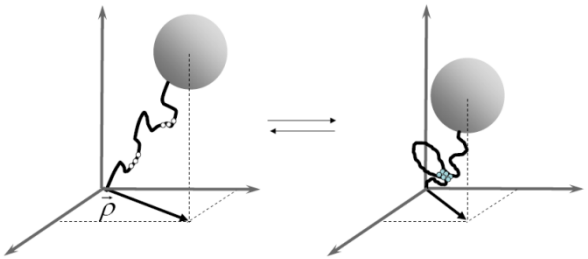
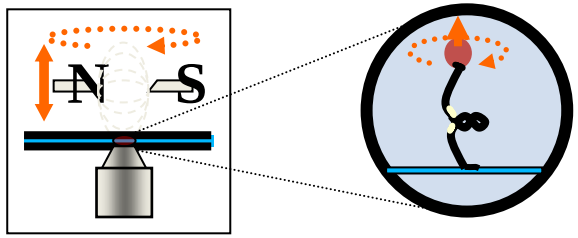
International School of Physics "Enrico Fermi"
Course 210 - Multimodal and Nanoscale Optical Microscopy
Varenna, 10 - 15 July 2022

Tethered particle motion (TPM) technique & magnetic tweezers (MT)

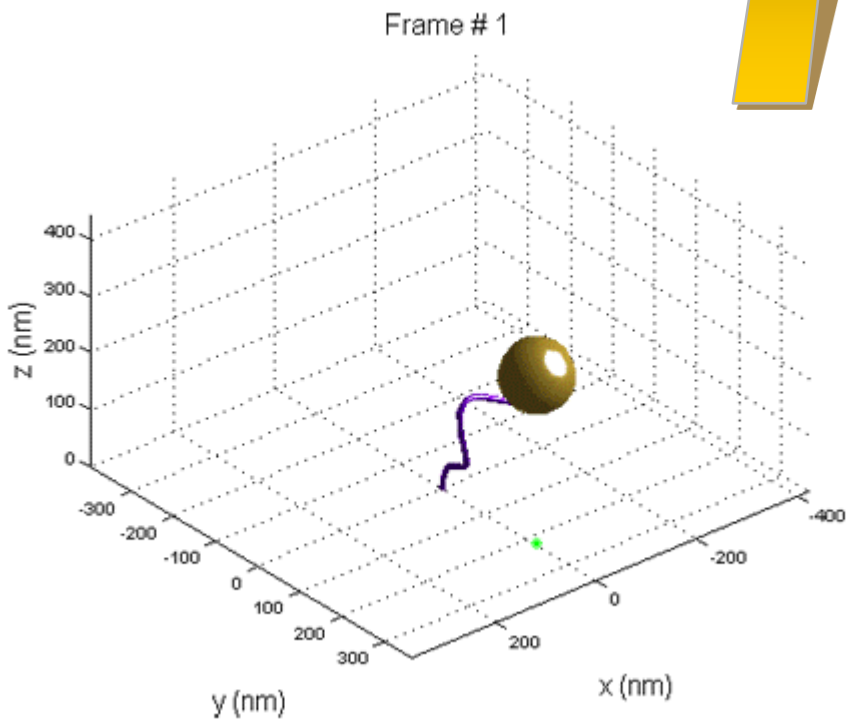


Tethered Particle Microscopy



Magnetic tweezers

TPM

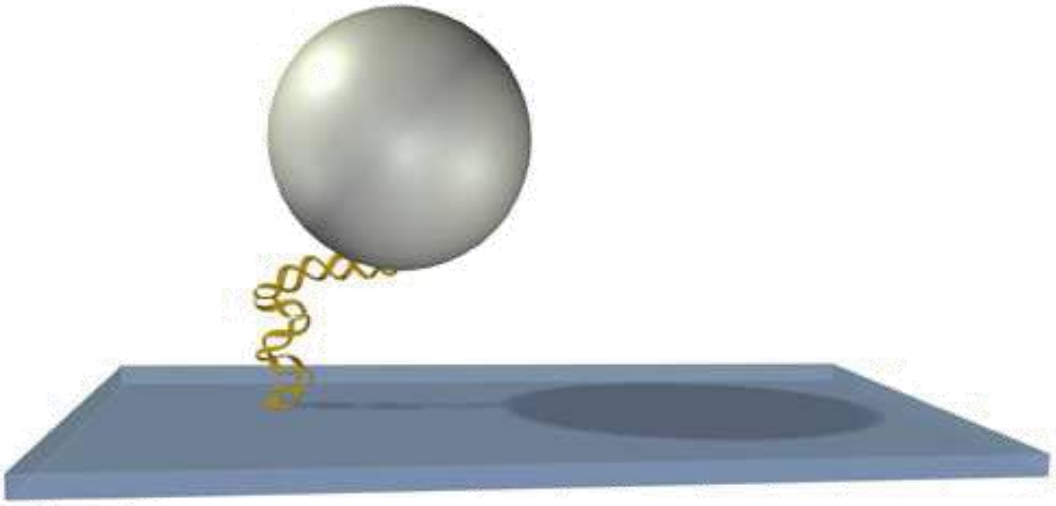


Introduction

Jeff Gelles (Brandeis),
Phil Nelson (Upenn),
Gijs Wuite (Vrej Un.),
Keith Weningen (NCSU),
Rob Phillips (Caltech),
Francesco Pavone (LENS),
Yuval Garini (Bar Ilan U.),
Lawrence Salome' (CNRS),
……

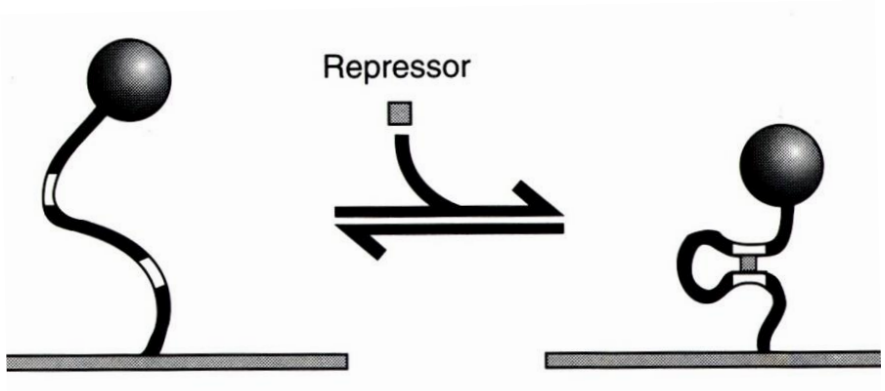
The tethered particle motion technique is a single particle tracking method

- A macromolecule is anchored at one end to a surface, while the other end is attached to a bead. The observed motion of the bead serves as a reporter of the underlying macromolecular motion.

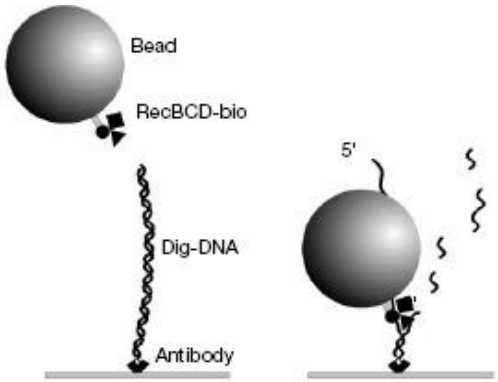


The bead is a reporter of the tether fluctuations which reflect changes in molecule conformation

Measure gene Repression by DNA loop formation



Measure Helicase/nuclease activity



Tracking Kinesins-driven movements and motor strength

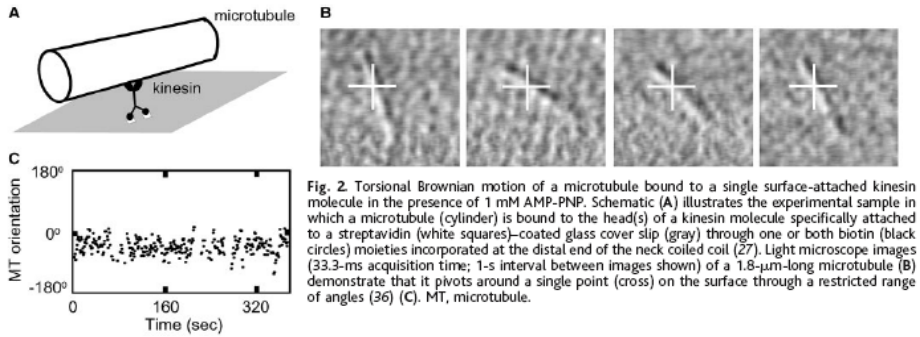
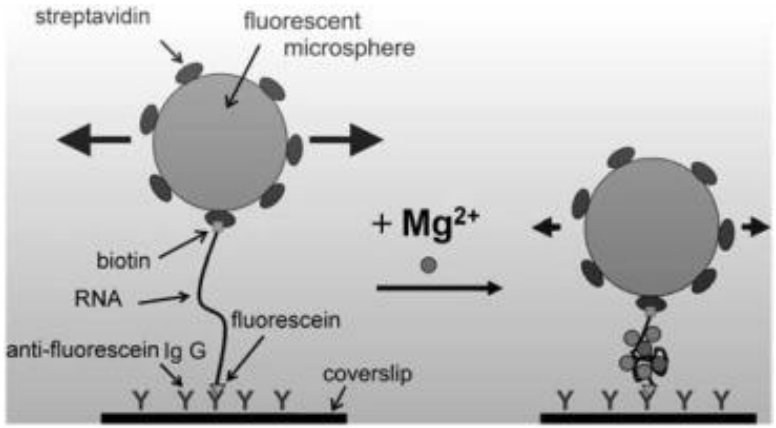
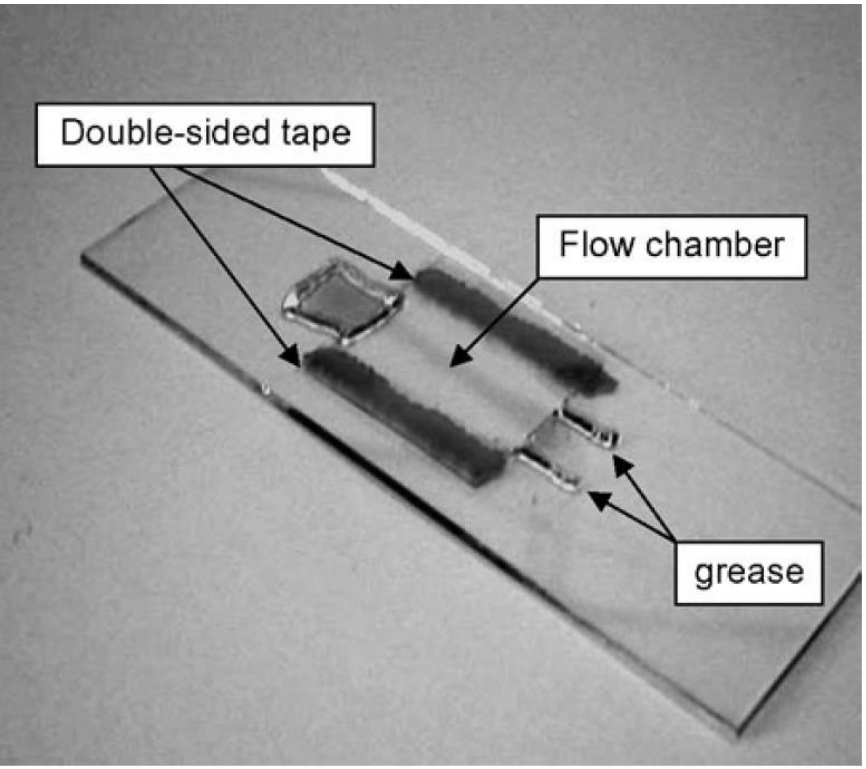


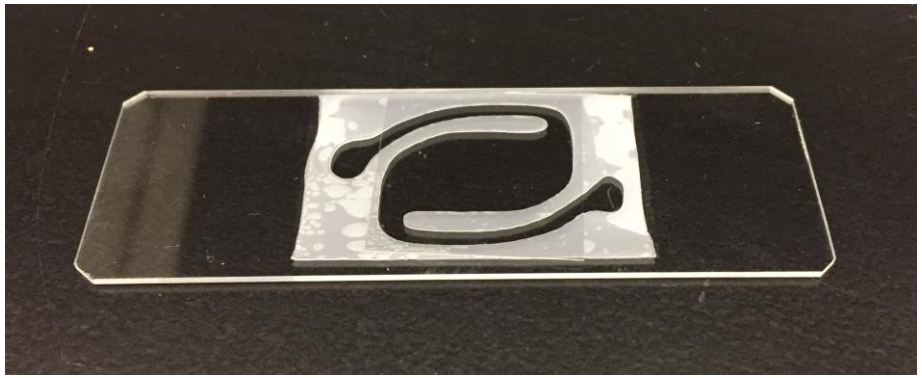
Fig. 2. Torsional Brownian motion of a microtubule bound to a single surface-attached kinesin molecule in the presence of 1 mM AMP-PNP. Schematic (A) illustrates the experimental sample in which a microtubule (cylinder) is bound to the head(s) of a kinesin molecule specifically attached to a streptavidin (white squares)–coated glass cover slip (gray) through one or both biotin (black circles) moieties incorporated at the distal end of the neck coiled coil (27). Light microscope images (33.3-ms acquisition time; 1-s interval between images shown) of a 1.8-μm-long microtubule (B) demonstrate that it pivots around a single point (cross) on the surface through a restricted range of angles (36) (C). MT, microtubule.

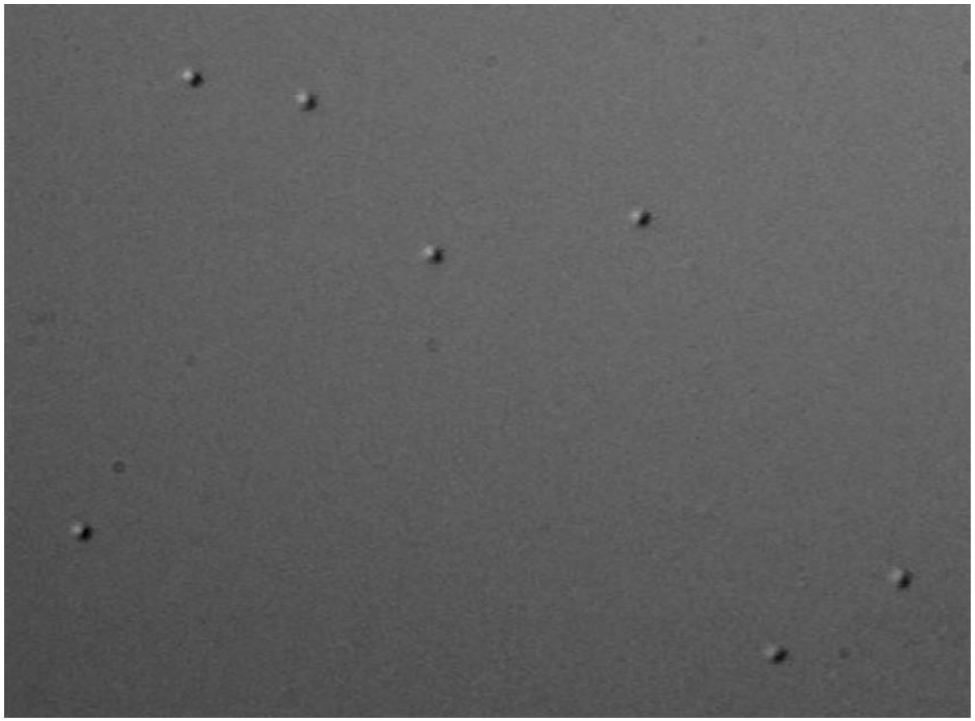
Measuring Mg2+ binding of RNA



The sample is assembled in a microchamber



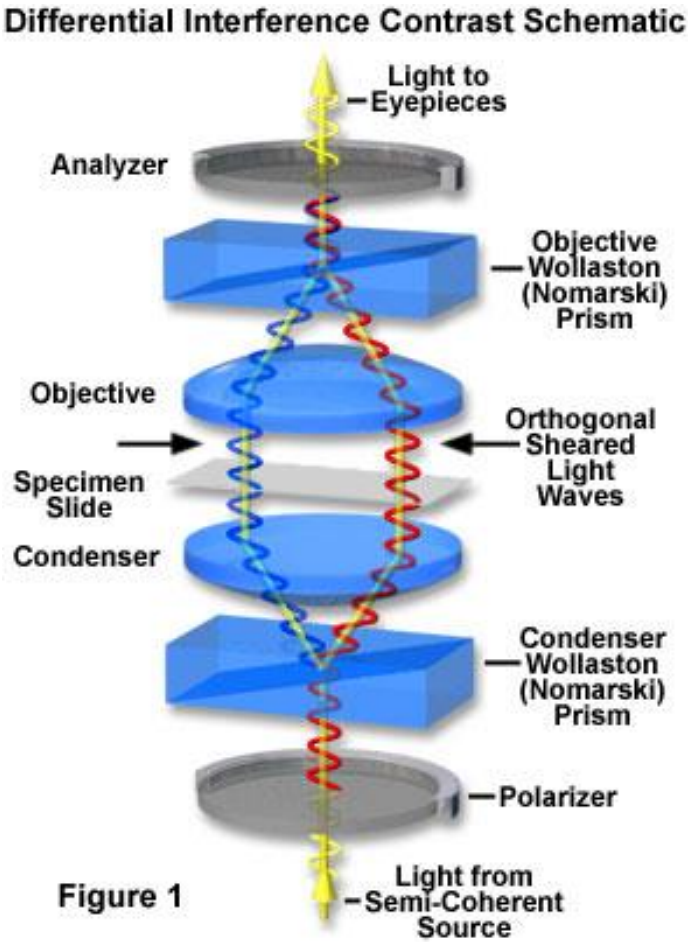


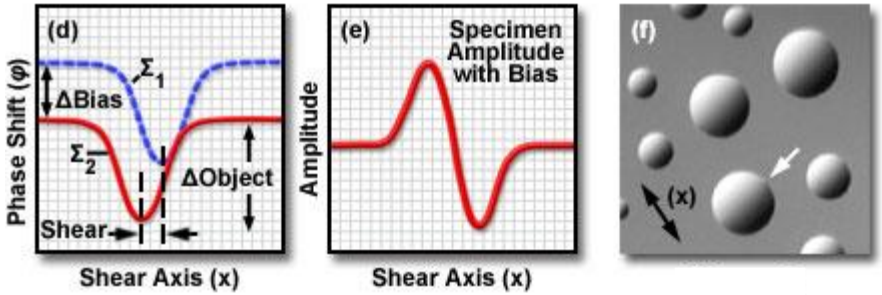


The instrument used is a standard microscope

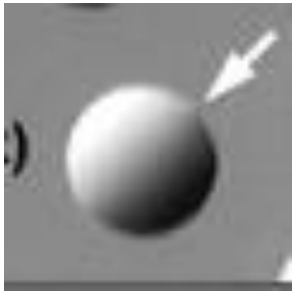
- A telescopic lens and digital camera allow digital imaging of field of view.
- A stable base allows stable alignment, rapid buffer exchange and precise tracking.
- Diferential Interference Contrast optics allows nm-precision location of bead centroid.

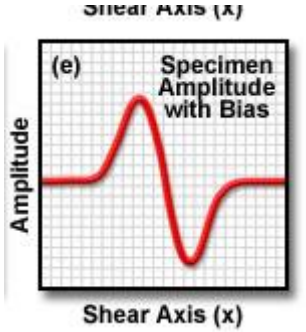
Differential Interference Contrast (DIC) leverages optical path differences within the sample





Real-time bead position tracking requires less data management



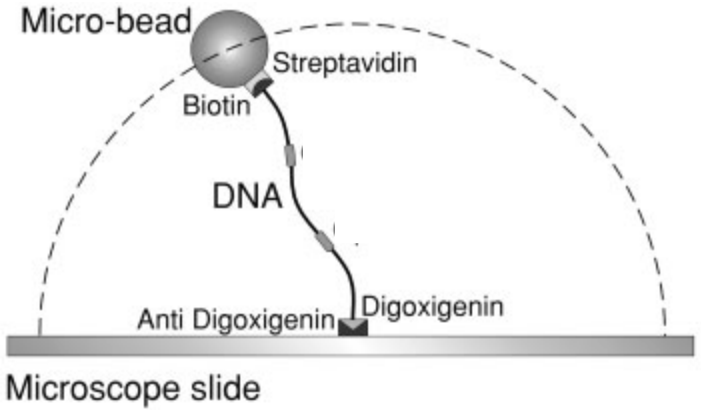


1. Search for the maximum and background intensity
2. Define a threshold 30%
3. Select the contiguous points with intensity over the threshold
4. Evaluate the center of mass (centroid)
5. Repeat the operation for the dark region
6. Average the two centers.

This algorithm is applied to each frame.

Daniel Kovari Yan Yan, Laura Finzi & David Dunlap, Single Molecule Analysis: Methods and Protocols, *Methods in Molecular Biology*, Springer, 2018

TPM involves constrained Brownian Motion

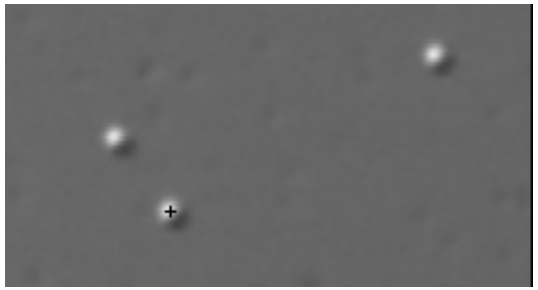


Two main factors establish the limits for studying a given macromolecular system using TPM:

1. the expected scale of changes in tether length, and

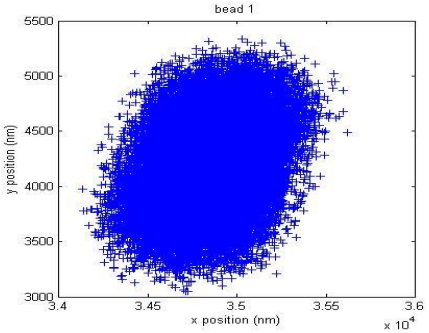
2. the time scale of those changes. As a rough approximation, the length and stiffness of a molecule can be used to estimate it.

Data acquisition and analysis



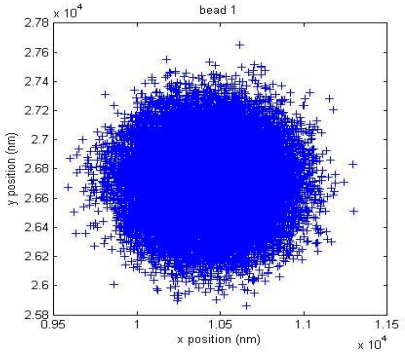
DIC image

Labview routine



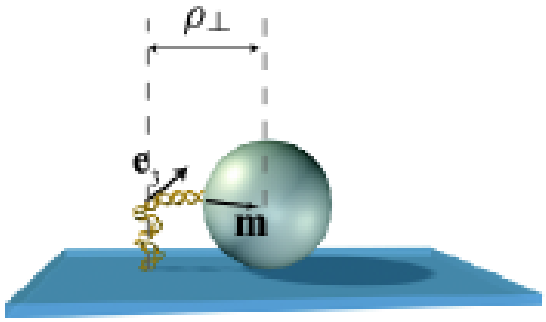
x and y coordinates

Drift subtraction

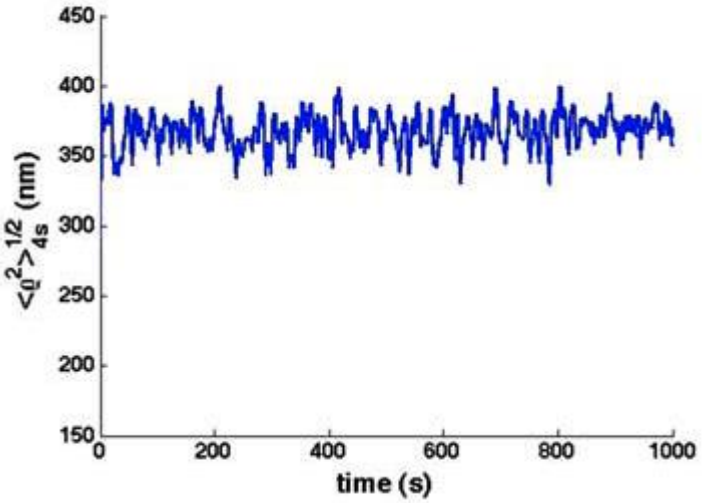


x' and y' coordinates of the anchor point

$$< \rho_{\perp} > = <[(x - x')^2 + (y - y')^2]^{1/2}>$$



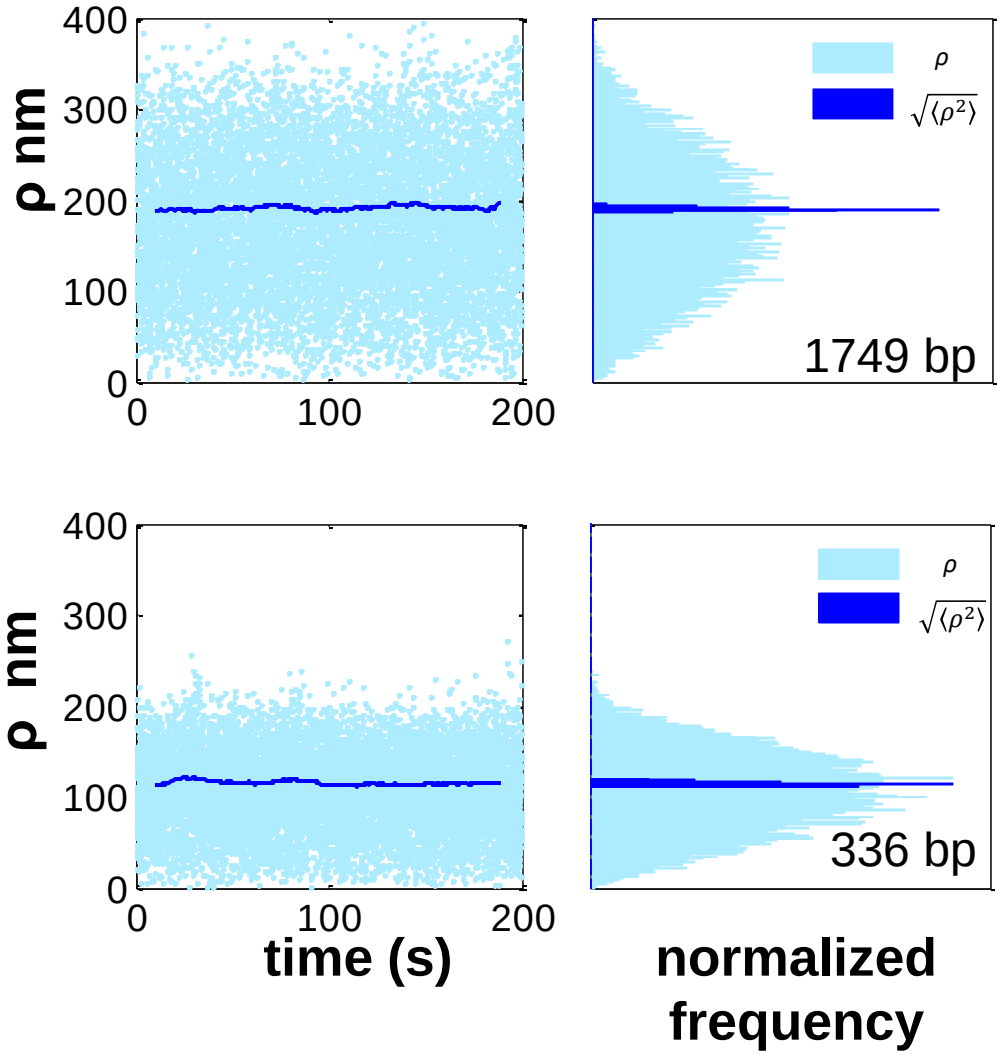
Plot of $\rho_{\perp}$ over time



P. Nelson, *et al*., J. Physical Chem. B, **110**, 17260, 2006

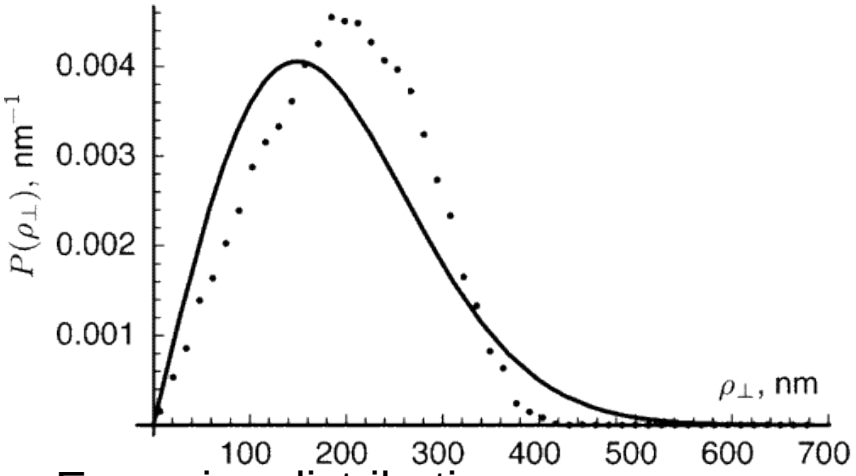
D. Dunlap, Zurla, Manzo and L. Finzi, "Probing DNA topology with Tethered particle Motion" in "*Methods in Molecular biology: Single-Molecule Analysis: Methods and Protocols*", eds. E.J.G. Peterman and G Wuite, Humana Press. Chapter 16, pp. 295-313, 2011

Dependence of TPM data on tether length



Bead diameter: 320 nm

Tether or bead dominated excursions

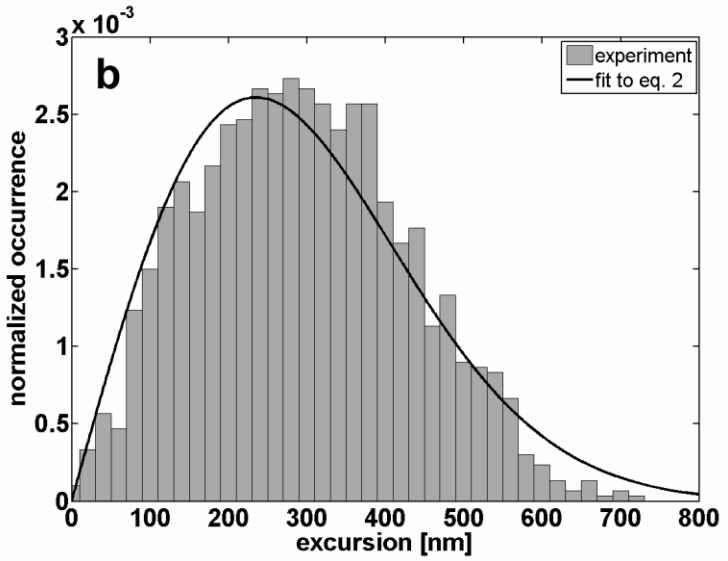


Excursion distribution:

- 480 nm dia. Polystyrene bead
- 1096 bp DNA

N = 3.04

Nelson, J PhysChemB 2006



Excursion distribution:

- 80 nm dia. gold colloid
- 4882 bp DNA

N = 0.24

Deitrich,J Nanophotonics 2009

Excursion number, $N = \frac{R}{\sqrt{L\varepsilon/3}}$

Segall, PhysRevLett 2006

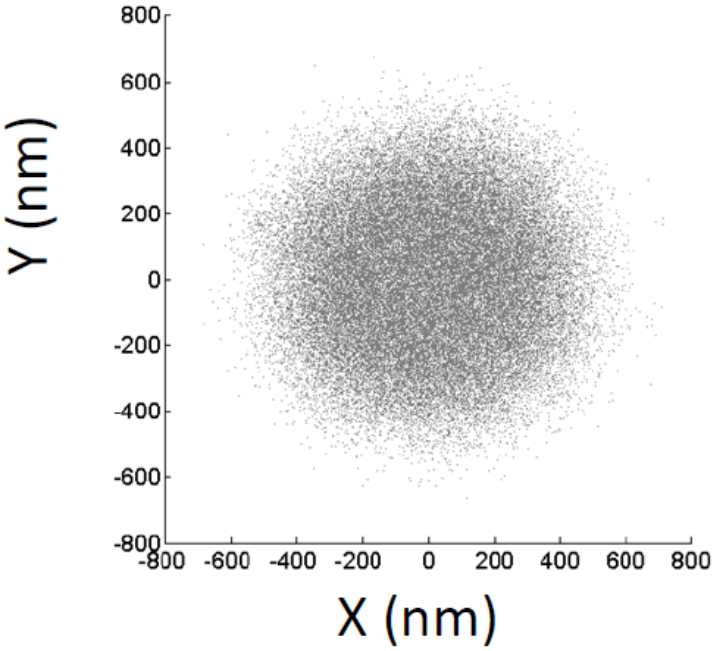
If $N>1$, excursions are bead dominated

If $N<1$, excursions are tether dominated

- R is the bead radius
- L is the contour length
- ε is the persistence length

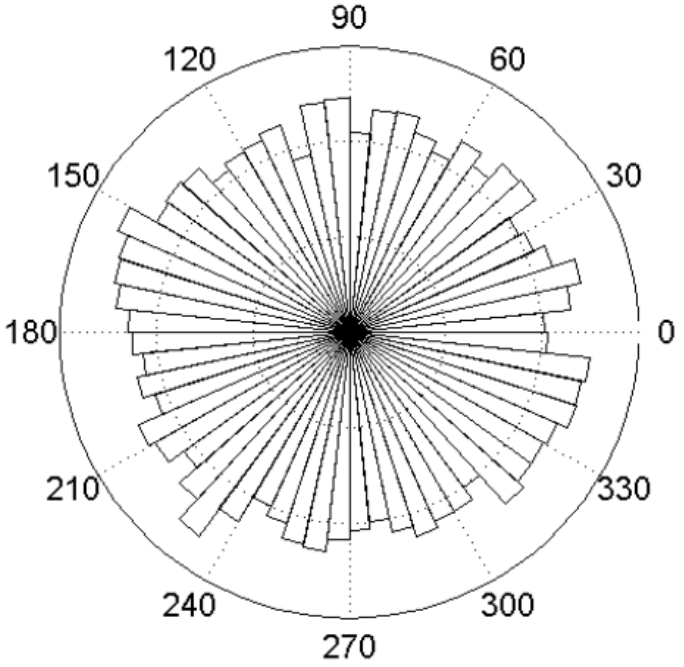
Symmetry is a selection criterion

A

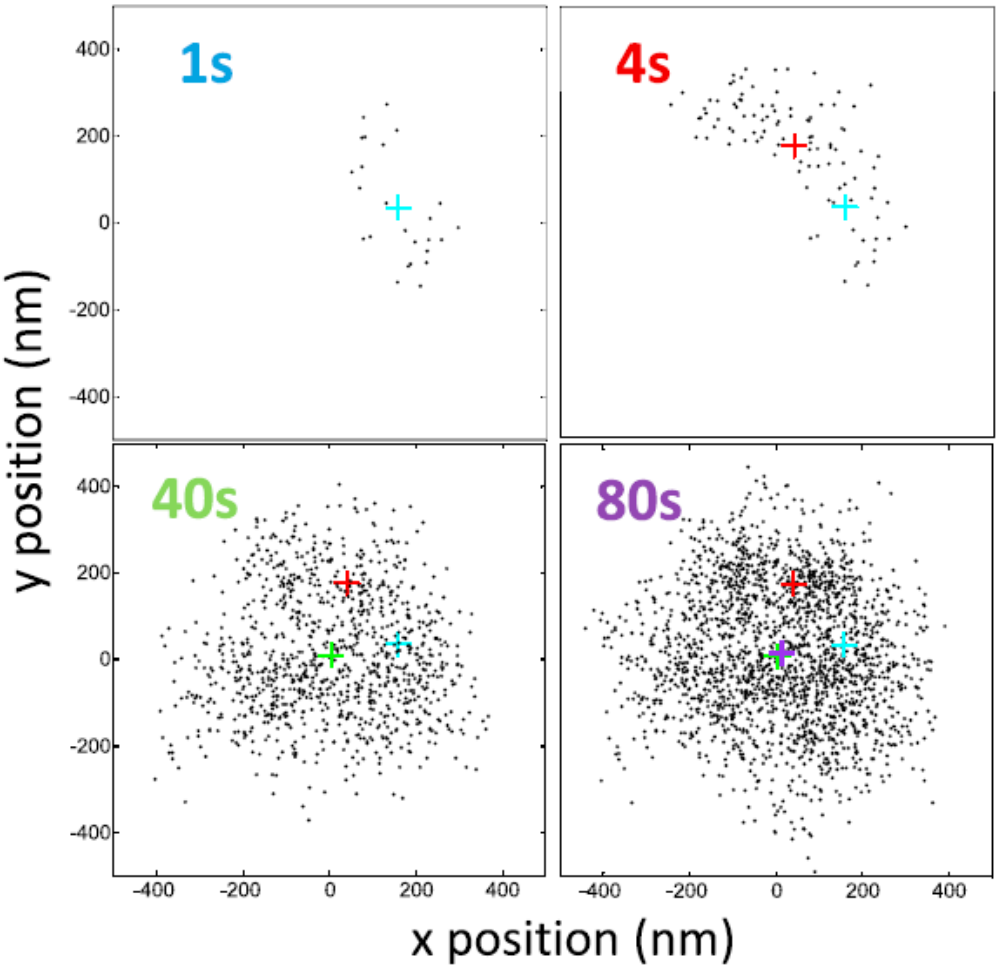


Diagonal ratio 1.0436

B



Determination of anchoring point

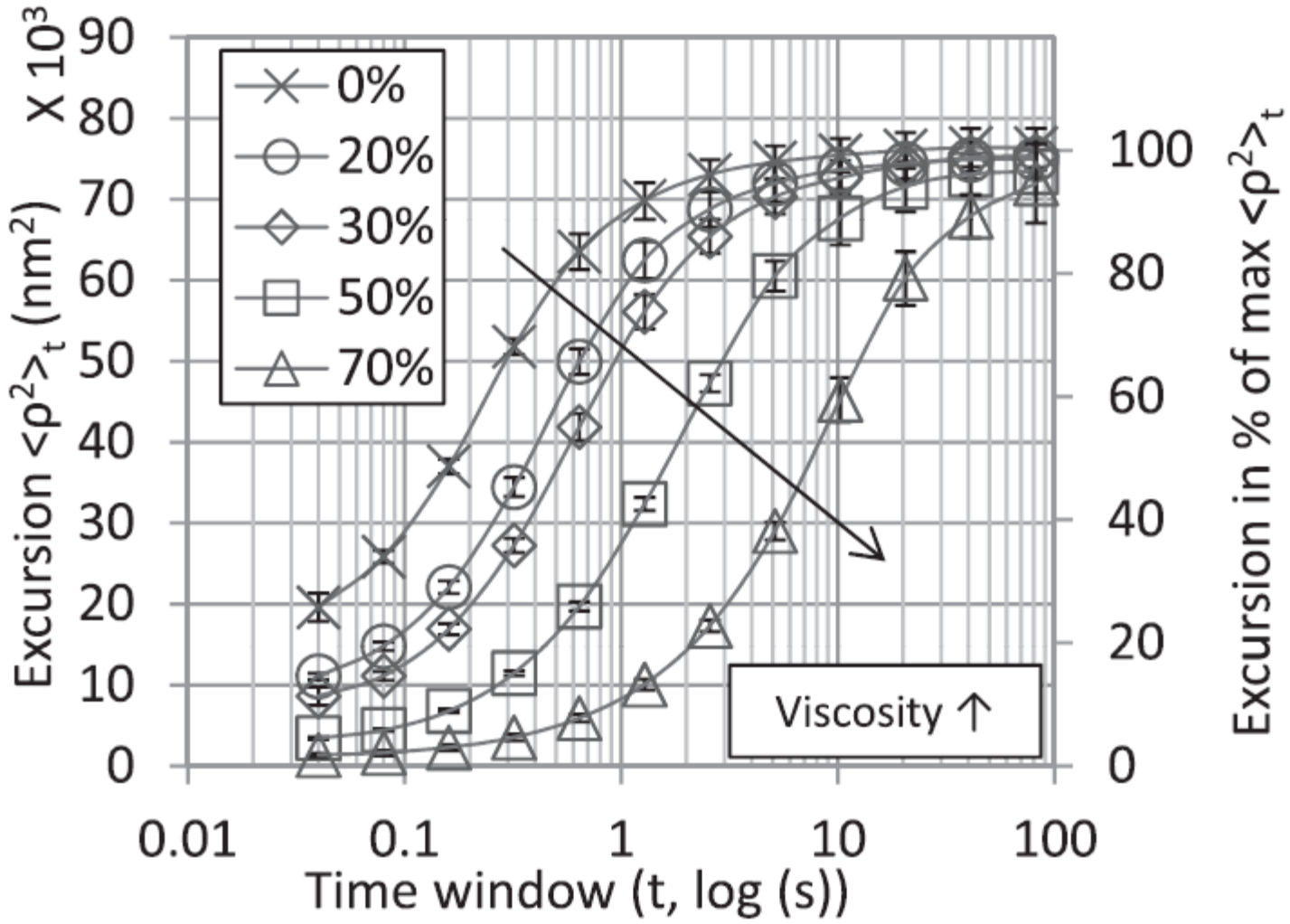


These measurements were conducted in a highly viscous solution to enhance the effect. In saline buffers, 4s or less are sufficient to establish the anchoring point.

Kumar et al, Biophys. J., 2014

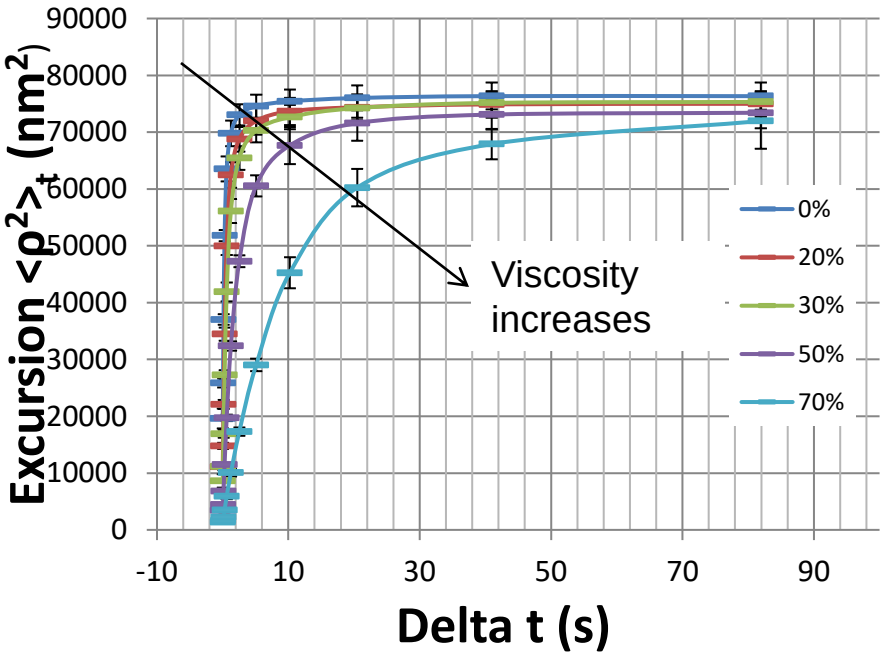
Viscosity affects the TPM measurement

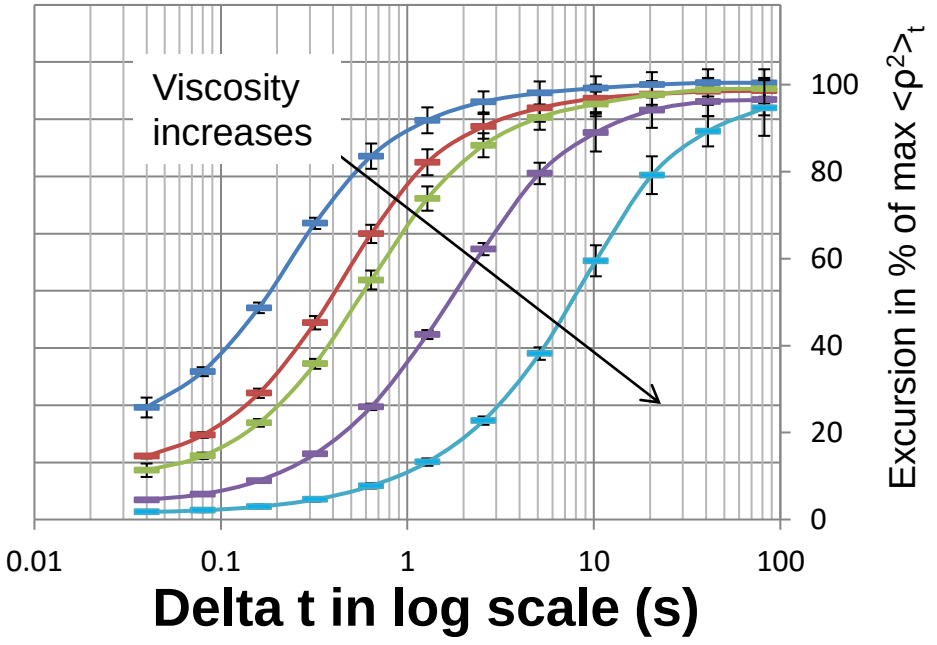
Kumar et al, Biophys. J., 2014



Max Excursion is independent of viscosity – DNA elasticity not changed!

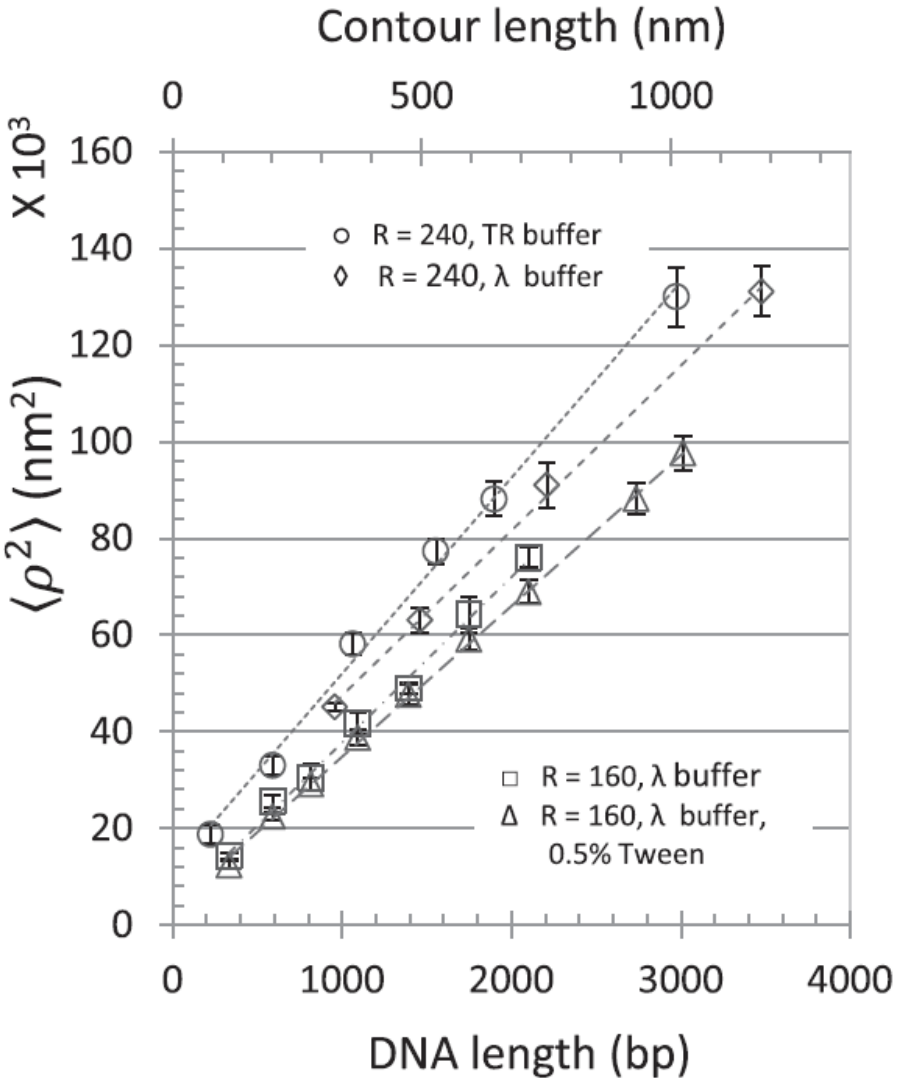
Effect of viscosity





Bead R=160 nm, DNA L = 2103b

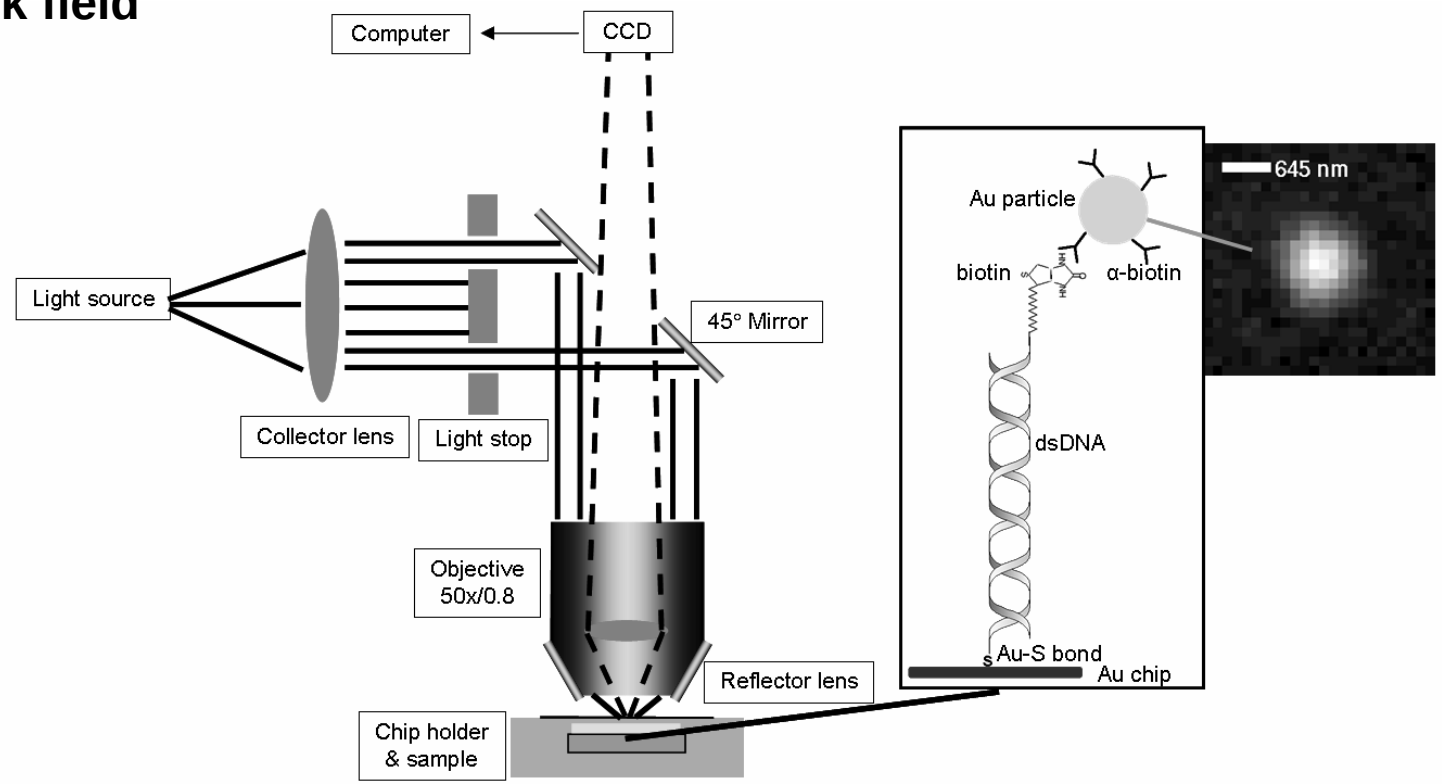
Bead size and buffer also affect TPM



Kumar et al, Biophys. J., 2014

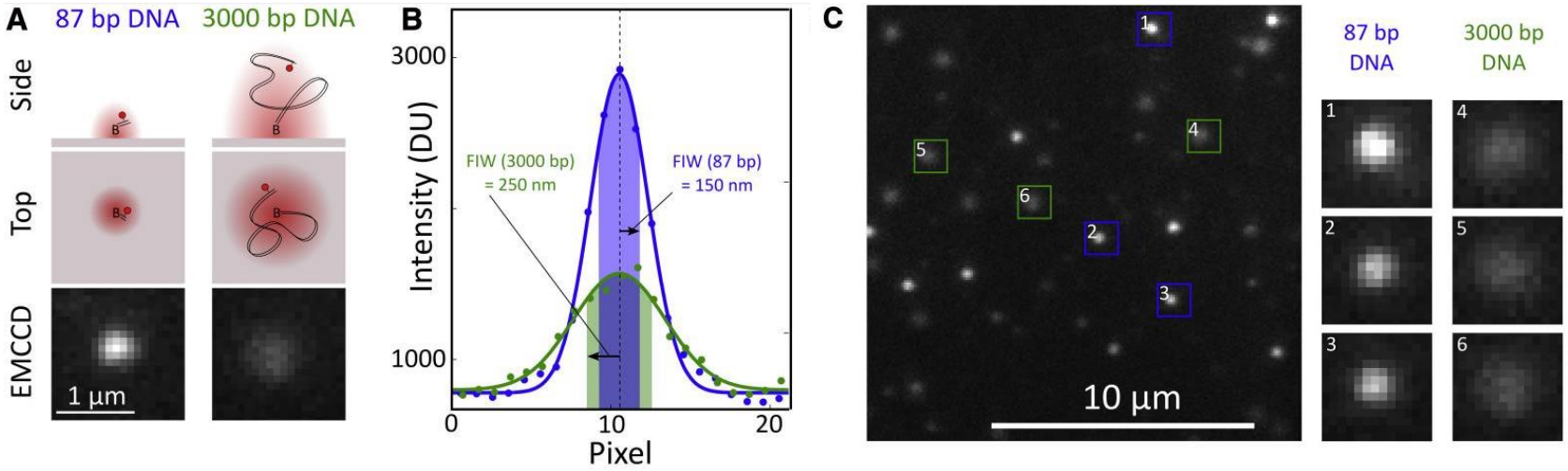
Alternative TPM configurations

Dark field

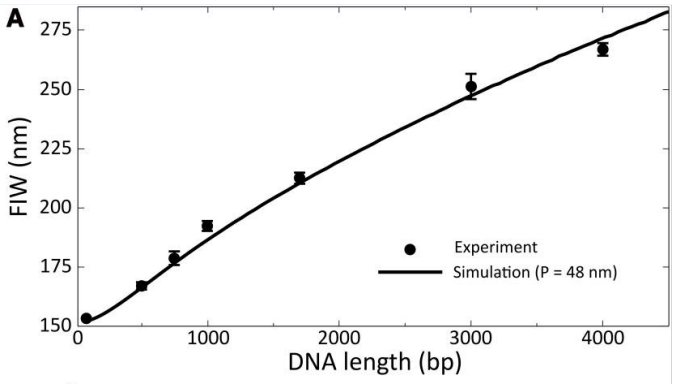


Guy Nir, Moshe Lindner and Yuval Garini (2012)
Laurens, N., Bellamy, S.R., Harms, A.F., Kovacheva, Y.S., Halford, S.E. and Wuite, G.J. NAR (2009)
Ucuncuoglu, S. et al., Methods in Enzymology, , vol. “Single-molecule Enzymology”, https://doi.org/10.1016/bs.mie.2016.08.008.

Derivative of Basic TPM: TFM



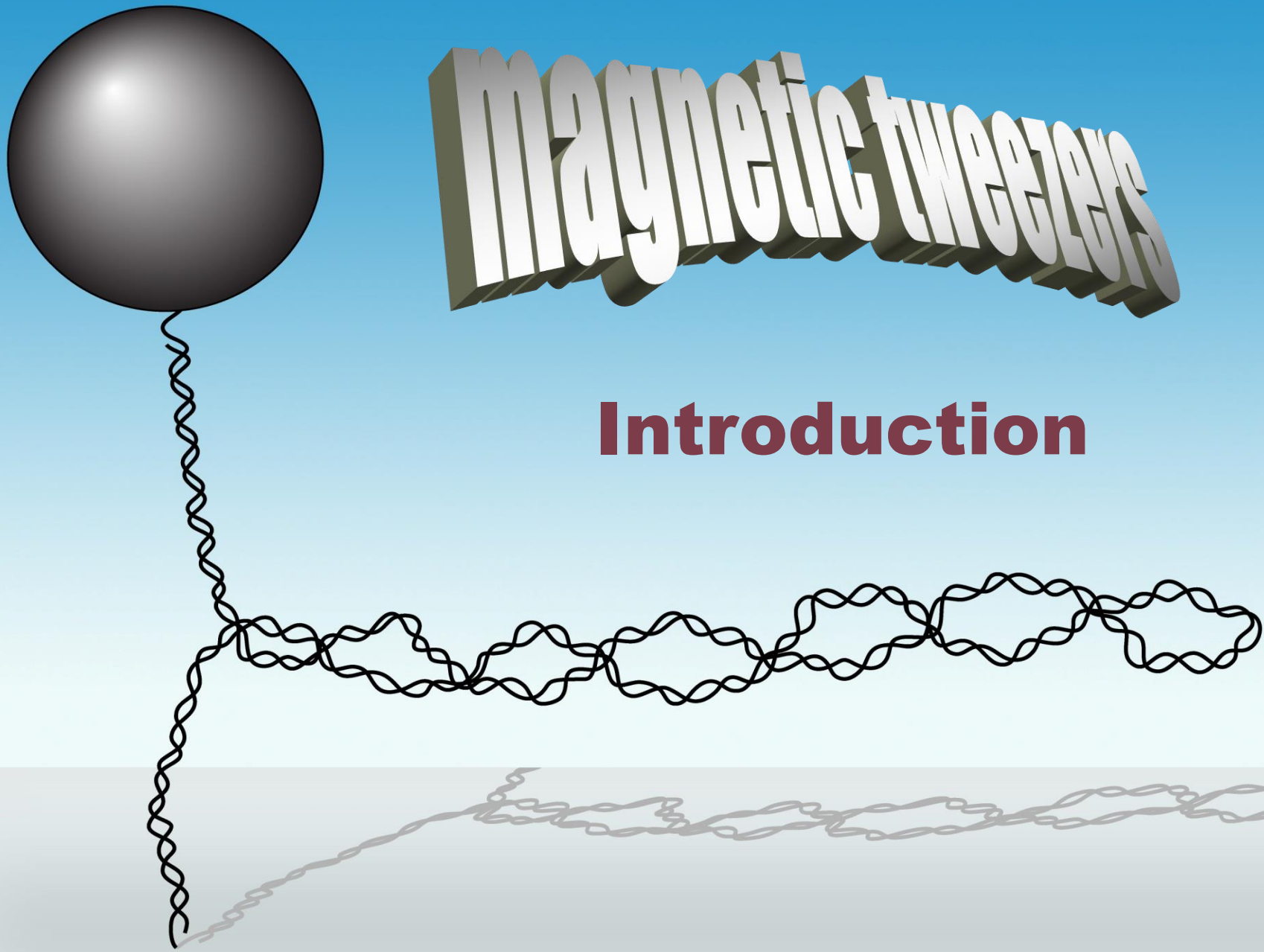
- Longer DNA allows the fluorophore to diffuse farther about the tether point during a frame, causing the image to appear broader.
- FIW (fluorophore image width) can be used to measure tether length.
- Resolution is about 100bp.
- TFM can be combined with other fluorescent technique, such as PIFE and FRET.



May & Kapanidis_BJ 2014

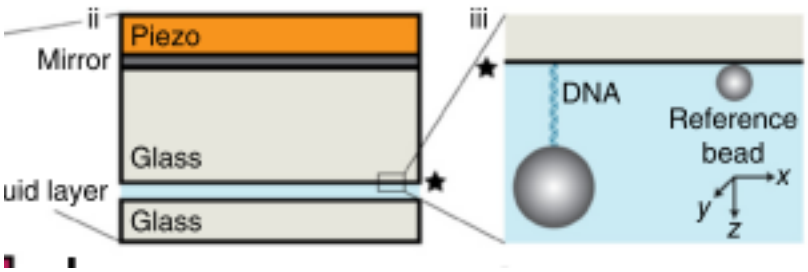
Magnetic tweezers

Introduction

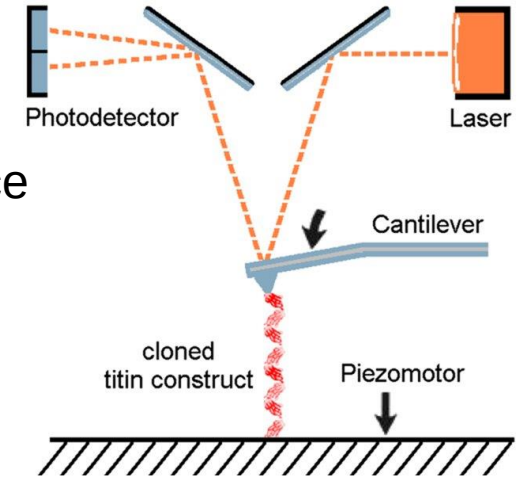


Techniques for Exerting Forces

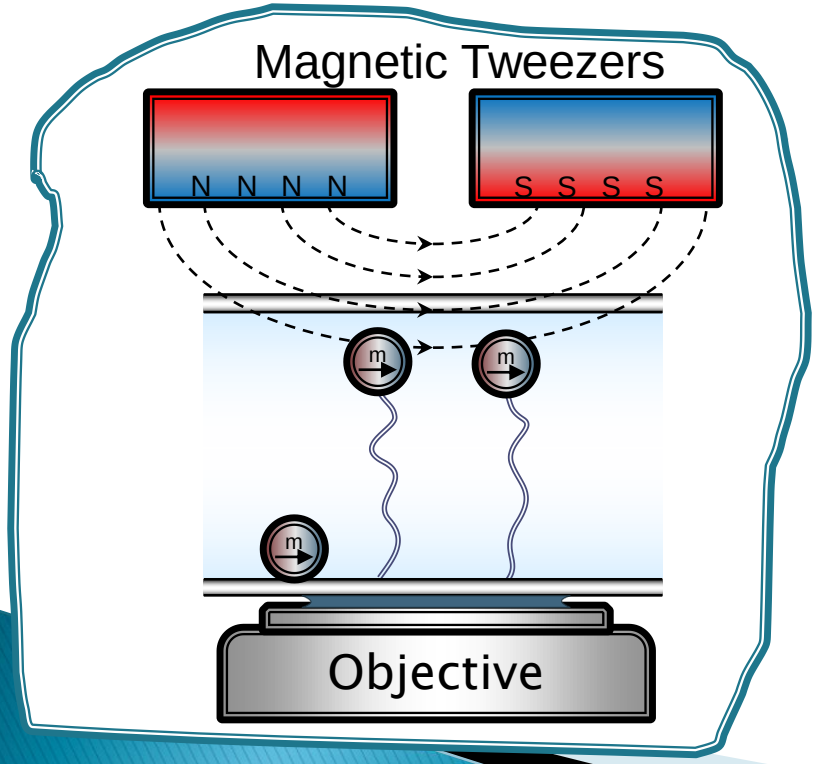
Acoustic Force Microscopy



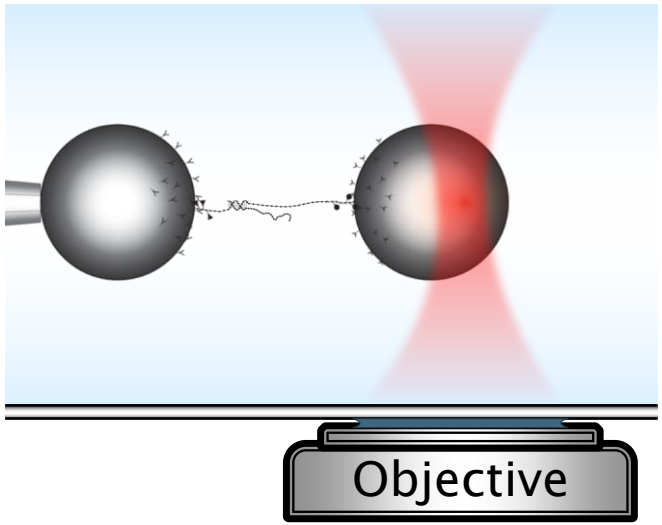
Atomic Force Microscopy



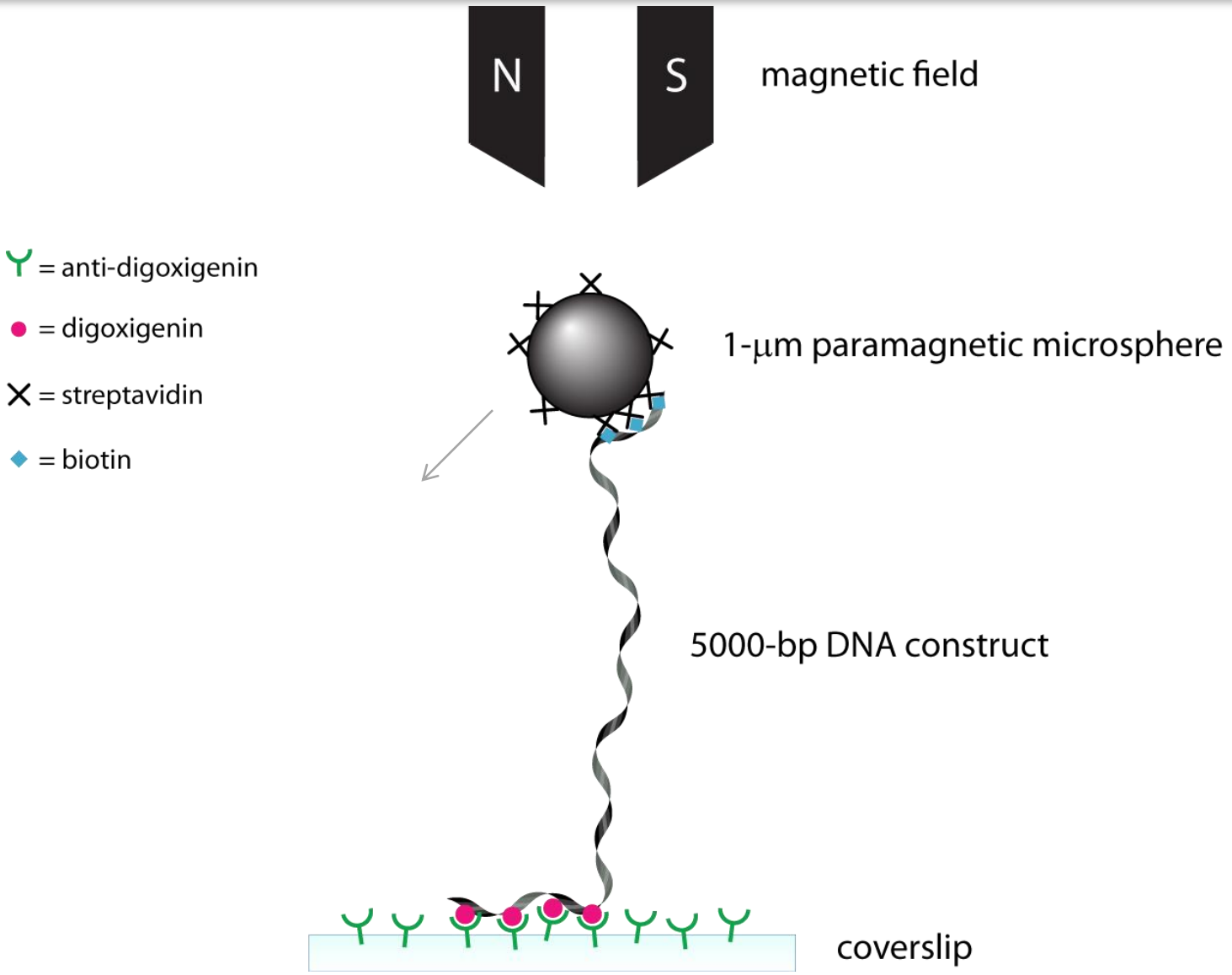
Magnetic Tweezers



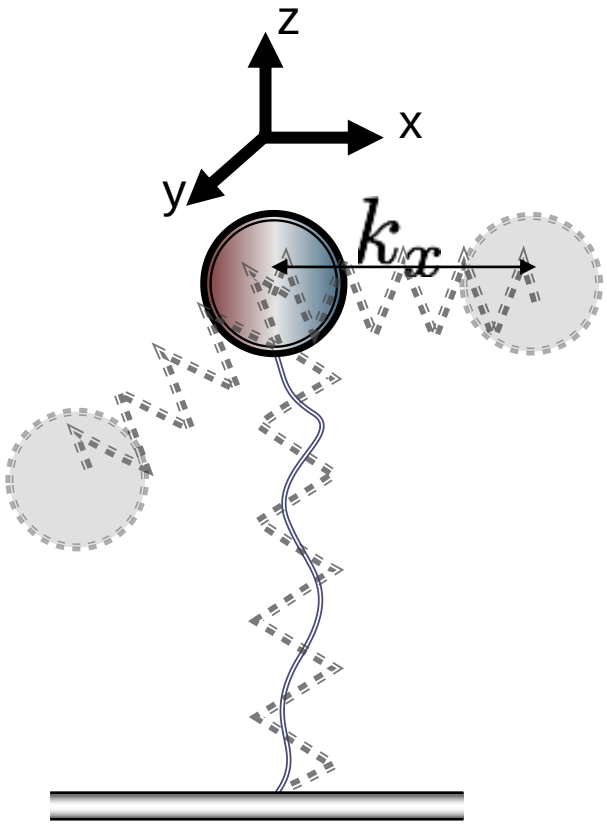
Optical Traps



DNA construction



Pulling on Molecules: Springs in a Thermal Bath



Particles in a Trapping potential can be approximated by a 3D harmonic

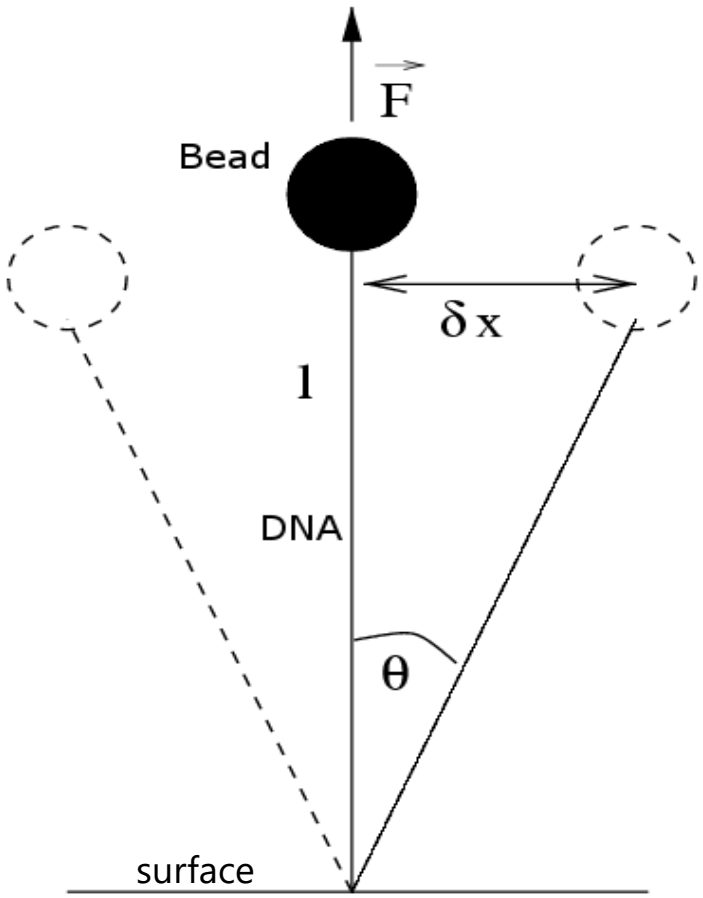
$$E = \frac{1}{2}k_x x^2 + \frac{1}{2}k_y y^2 + \frac{1}{2}k_z z^2$$

From the **Equipartition Theorem**:
Average of ½kT per degree of freedom

$$\frac{1}{2}k_x \left\langle x^2 \right\rangle = \frac{1}{2}k_B T$$
$$\frac{1}{2}k_y \left\langle y^2 \right\rangle = \frac{1}{2}k_B T$$
$$\frac{1}{2}k_z \left\langle z^2 \right\rangle = \frac{1}{2}k_B T$$

Brownian Motion Analysis

The DNA-bead system behaves like an **inverted pendulum** pulled along the vertical direction above its anchoring point and **subjected to Brownian fluctuations**:



Tethered bead in a harmonic potential

$$F_x = F\sin\theta \sim F\theta \sim F\frac{\delta x}{l}$$

$$F_x = \frac{F}{l}\delta x = \overline{k_x}\delta x$$

Equipartition of energy:

$$\frac{1}{2}k_x\langle\delta x^2\rangle = \frac{1}{2}k_BT$$

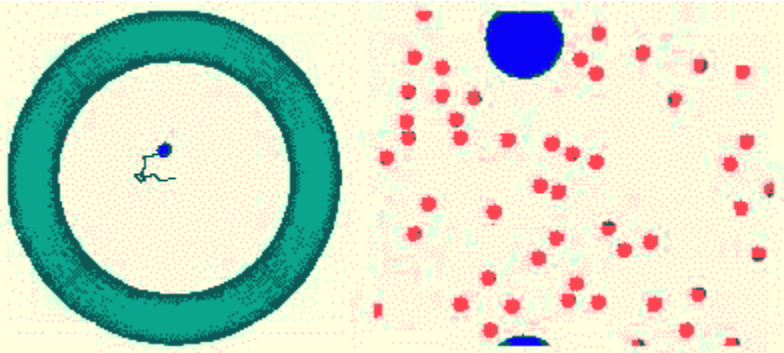
$$\frac{F}{l}\langle\delta x^2\rangle = k_BT$$

$$F = \frac{k_BTl}{\langle\delta x^2\rangle}$$

T. Strick et al., *J. Stat. Phys*. **93**, 648-672 (1998)

Magnitude of the stretching force

- The force that the magnetic field exerts on the sphere is determined measuring its Brownian motion.



http://galileo.phys.virginia.edu/classes/109N/more_stuff/Applets/brownian/brownian.html

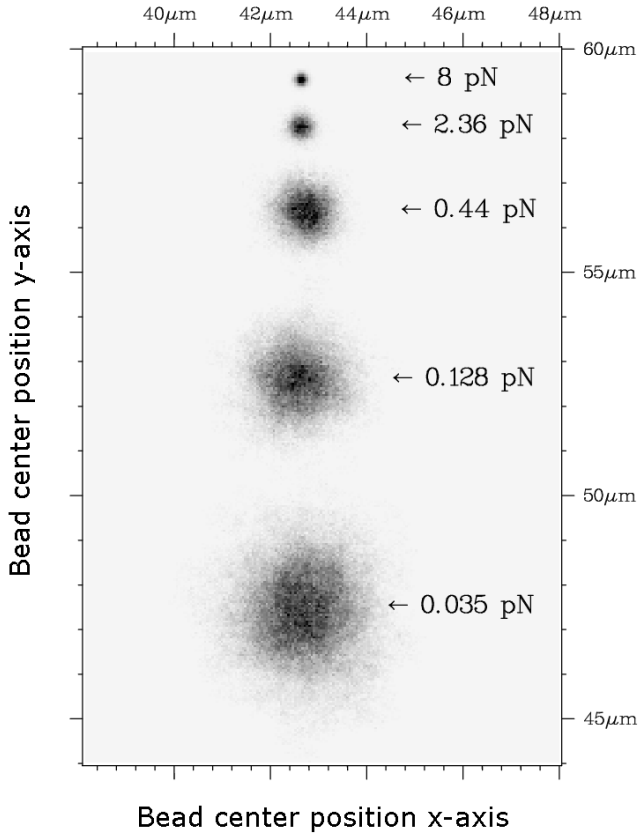
- And applying the equipartition theorem in one dimension:

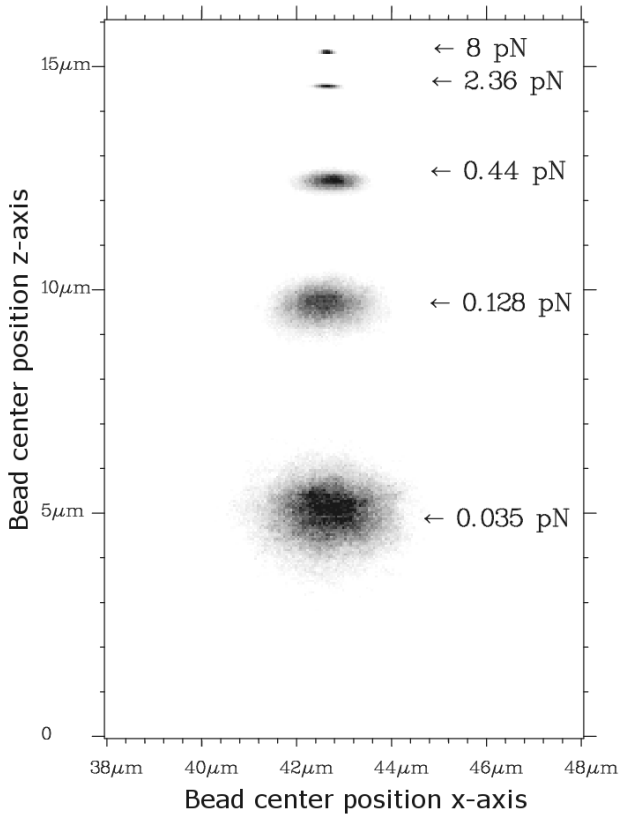
$$\langle F \rangle = \frac{k_B T L_{DNA}}{\langle \Delta x^2 \rangle}$$

Smith S.B.; Finzi, L.; Bustamante, C. *Science*. **1992**, 258, 1122-1126

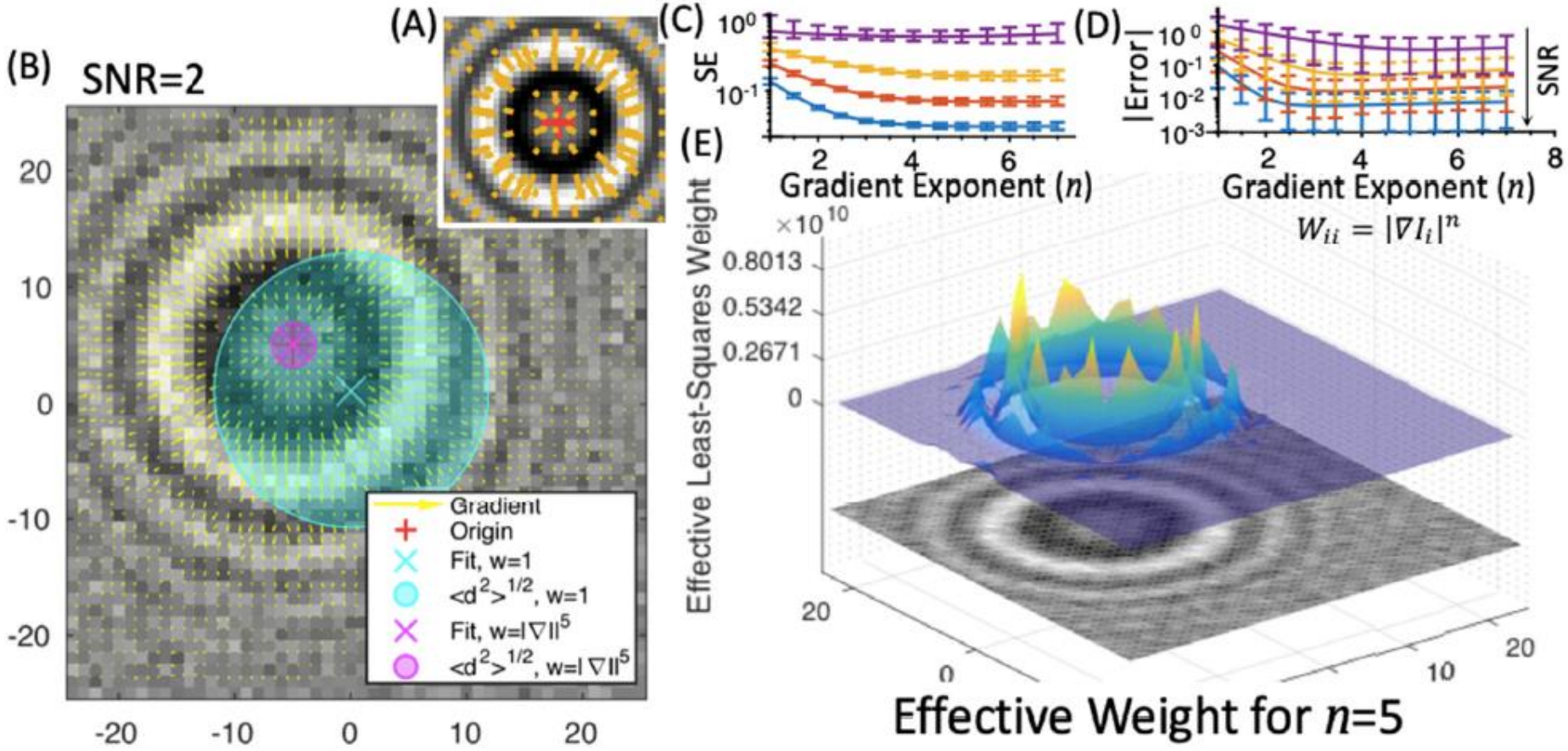
Strick, T.R.; Allemand, J. F.; Bensimon, D.; Bensimon, A.; Croquette, V. *Science*. **1996**, 271, 1835-1837

MT is an excellent low-force measurement technique





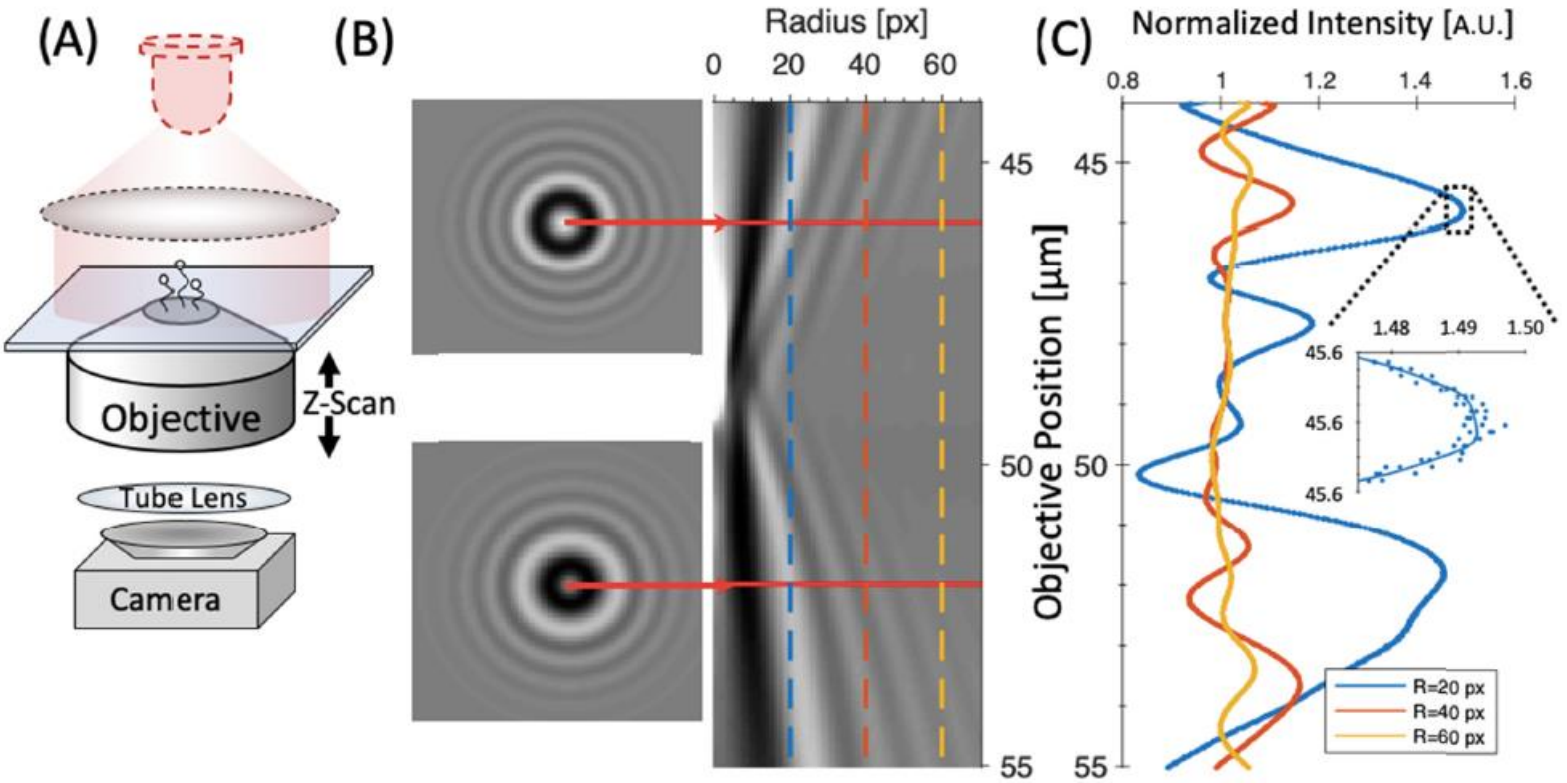
Measuring DNA extension in MT



$W_{ii} = |\nabla I_i|^n$

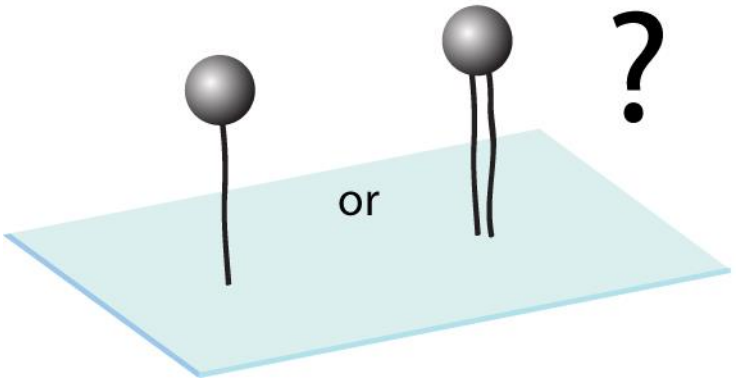
Kovari *et al*., “Model-free 3D localization with precision estimates for brightfield-imaged particles”, *Optics Express*, 2019

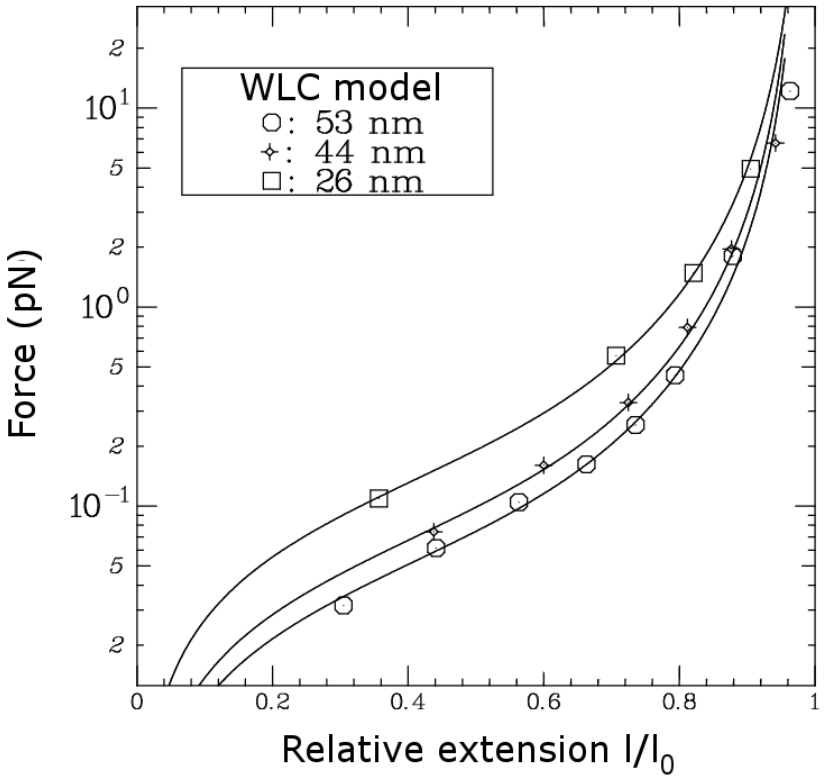
Measuring DNA extension in MT



Kovari *et al*., “Model-free 3D localization with precision estimates for brightfield-imaged particles”, *Optics Express*, 2019

One or two DNA tethers?





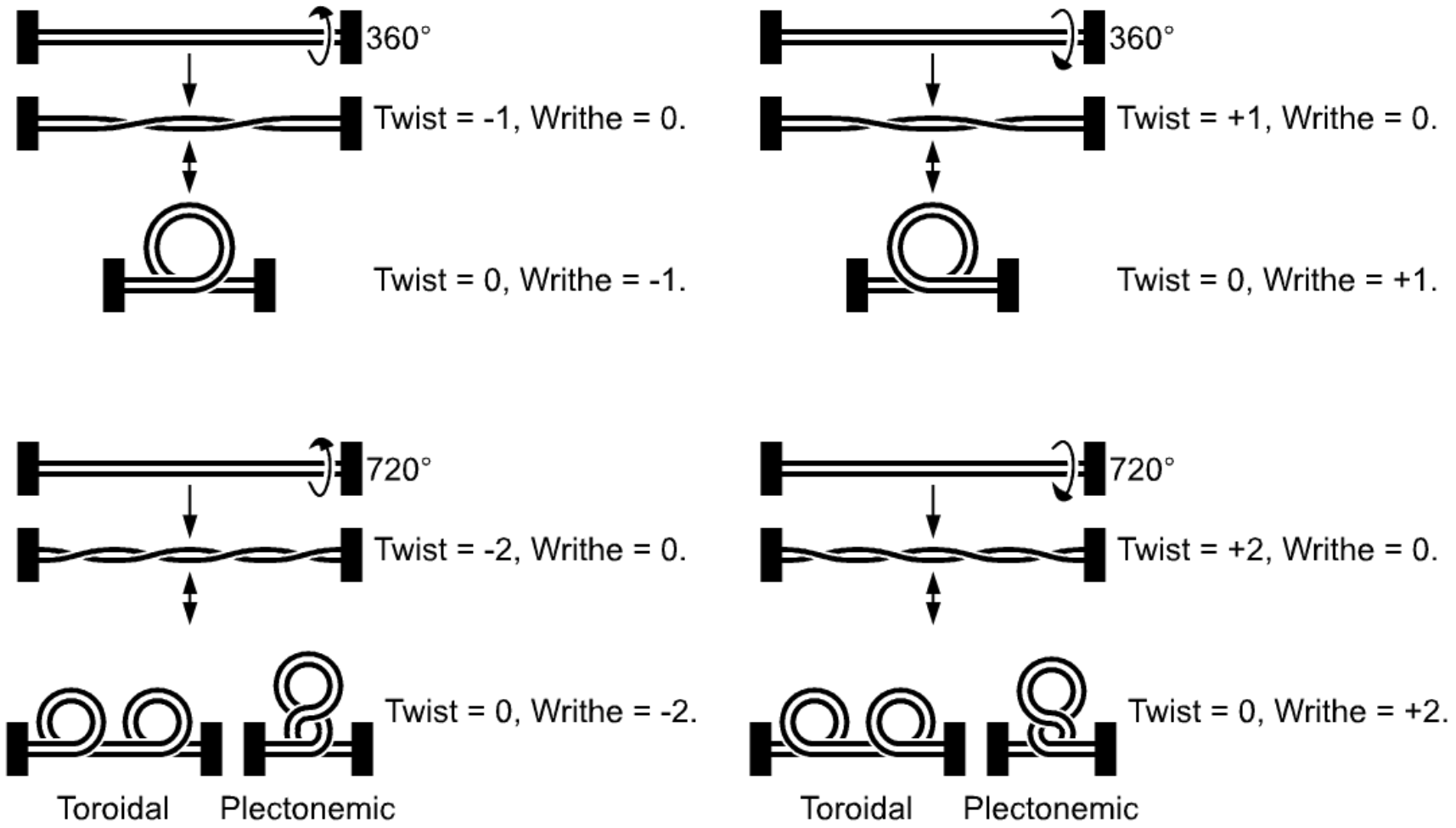
Single DNA tether has P = 53 nm
Double DNA tether, P = 26 nm
Double tether with one L DNA and the other 2L DNA, P = 44 nm (L: contour length)

Strick *et al., J. Stat Phys*, **93**, 1998

DNA Supercoiling *in vivo* and *in vitro*

- In most organisms, DNA is negatively supercoiled ($s \approx -0.06$)
- Actively regulated by **topoisomerases**, a ubiquitous and essential family of proteins
- Supercoiling is involved in DNA packaging around histones, and the initiation of transcription, replication, repair & recombination
- Known to induce structural changes in DNA
- Traditional means of study (gel electrophoresis, sedimentation analysis, cryo-EM, etc.) have the following disadvantages:
  1. no real time observation of interaction
  2. not reversible
  3. distributions of topoisomers,
  4. only $-0.1 < \sigma < 0.1$

Supercoiling of linear constrained DNA



http://en.wikipedia.org/wiki/DNA_supercoil

Topological formalism for torsionally constrained DNA

Tw ⇨ **Twist** (the number of helical turns of the DNA)
\+ **Wr** ⇨ **Writhe** (the number of loops in the DNA)
Lk ⇨ **Linking number** (total number of crossings between the 2 strands)

Linking number for torsionally relaxed DNA:

$Lk_o = Tw_o$ (Tw_o = 1 per 10.5 bp of B-DNA, Wr_o= 0)

Linking number for torsionally strained DNA:

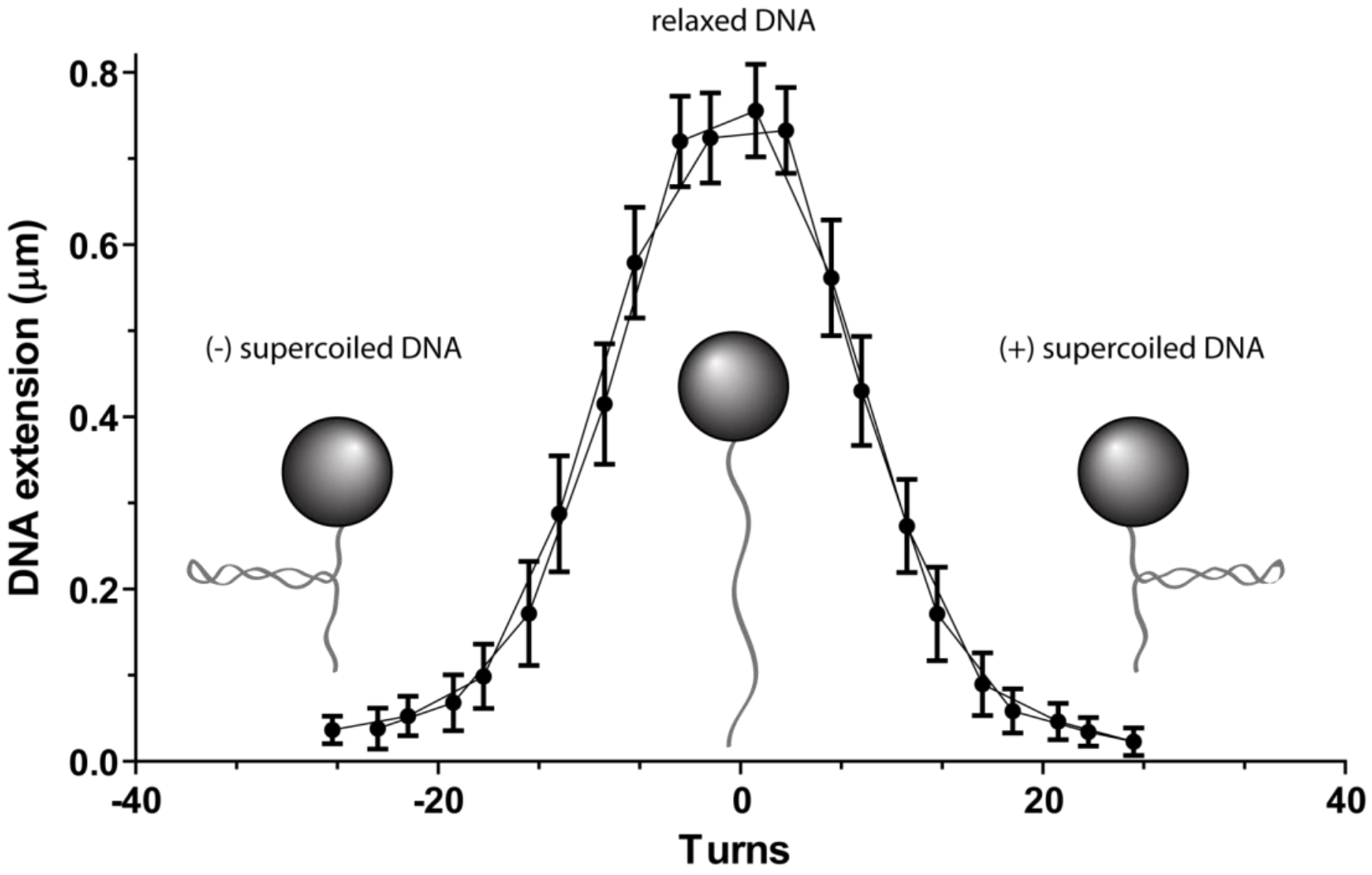
$\Delta Lk = Lk - Lk_o = \Delta Tw + Wr$

Normalized linking number difference:

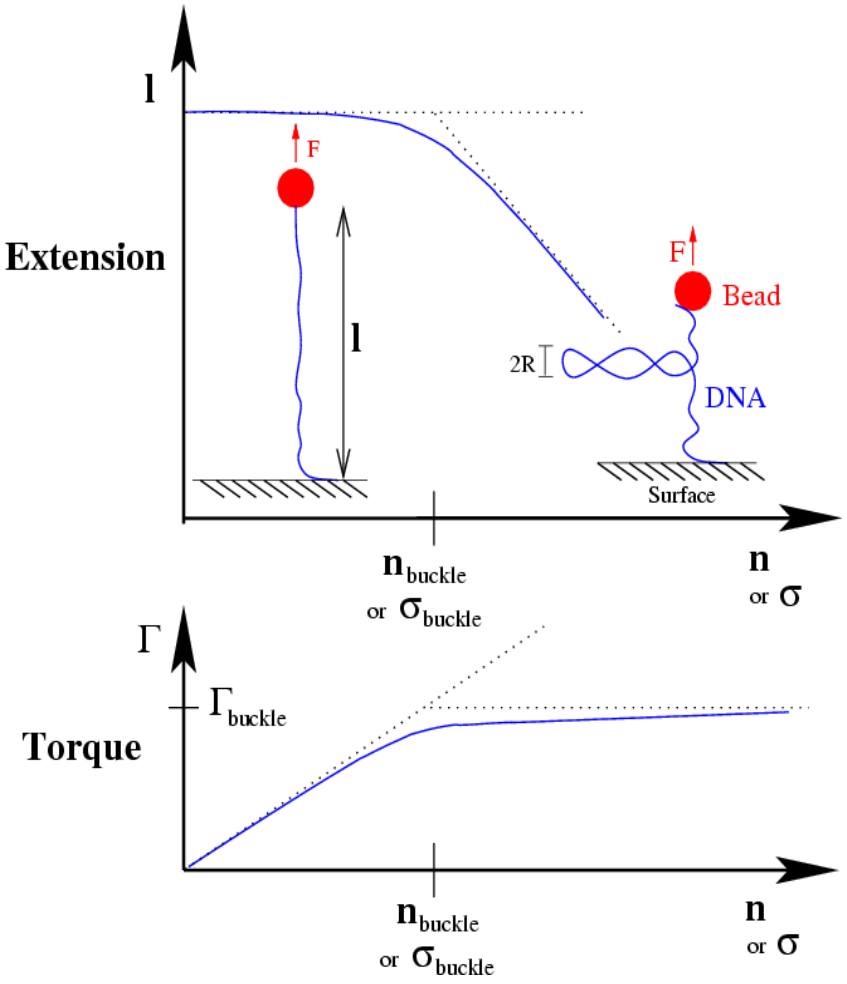
Supercoiling density = $\sigma = \Delta Lk / Lk_o$

http://en.wikipedia.org/wiki/DNA_supercoil

DNA supercoiling compacts DNA tethers – Hat Curves



Supercoiling and the buckling transition



Balancing the torsional energy against the work done and the increase in bending energy:

$$2\pi\Gamma_{buck.} = 2\pi RF + 2\pi R \cdot \frac{B}{2R^2} \qquad (B = k_B T \xi)$$

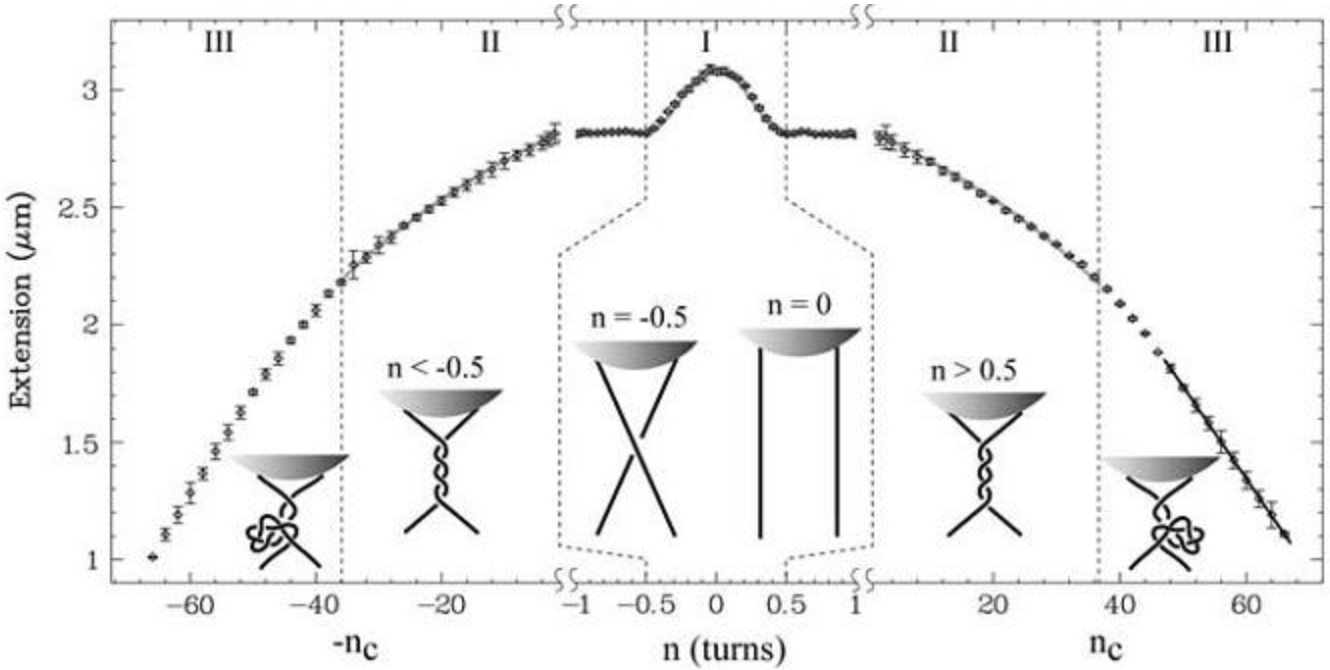
twist energy — potential energy — bend energy — Persistence length

$$R = \sqrt{\frac{B}{2F}} \qquad \Gamma_{buck.} \sim \sqrt{BF}$$

Strick et al., Rep. Progr. Phys., 66, 2003

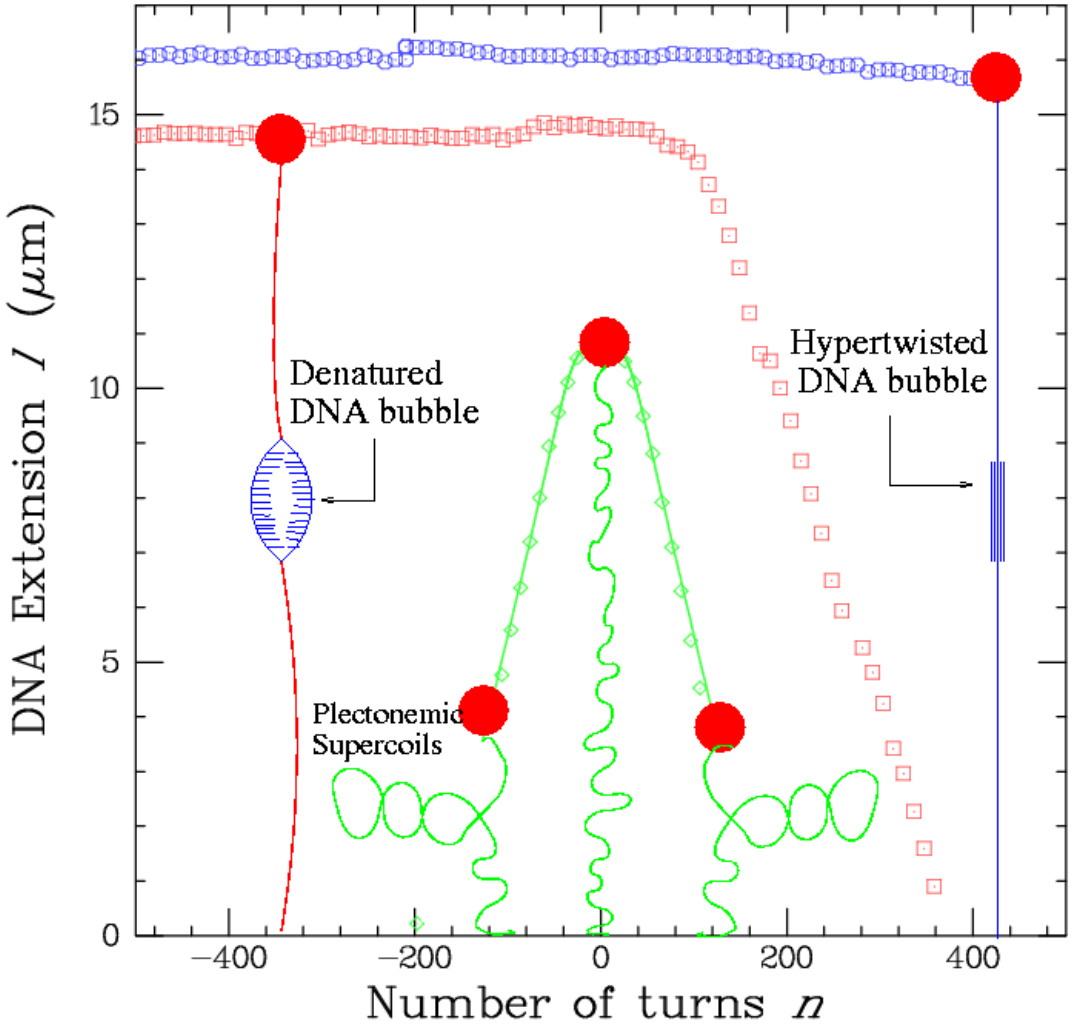
If there are two tethers: DNA braiding

Extension vs. supercoiling at constant force



G. Charvin, A. Vologodski, D. Bensimon, V. Croquette, *Biophys. J.* **88**, 2005

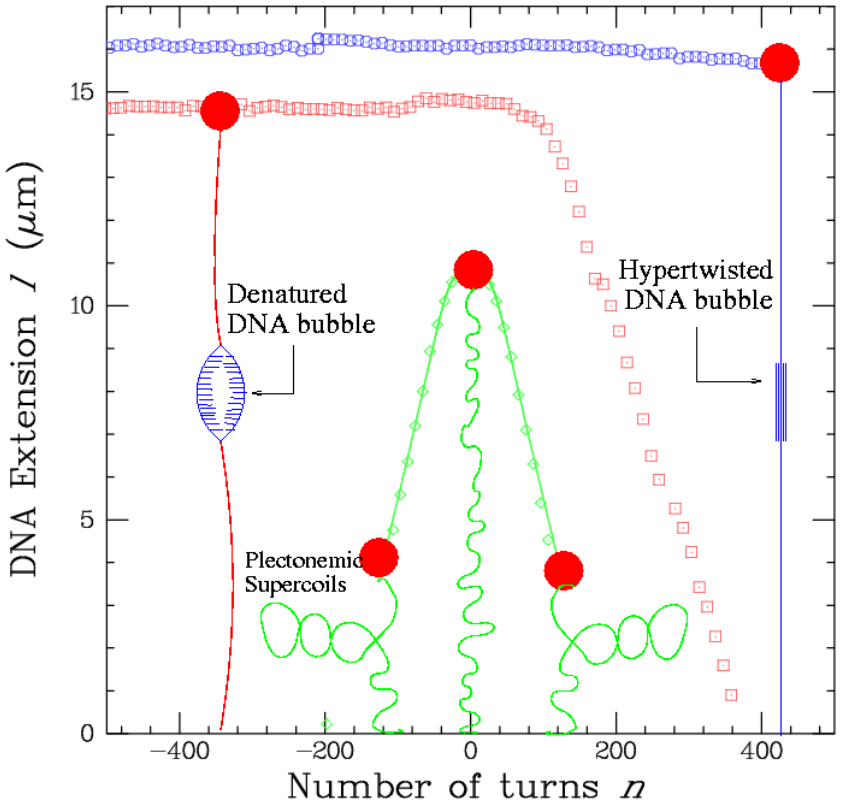
DNA extension vs. Supercoiling

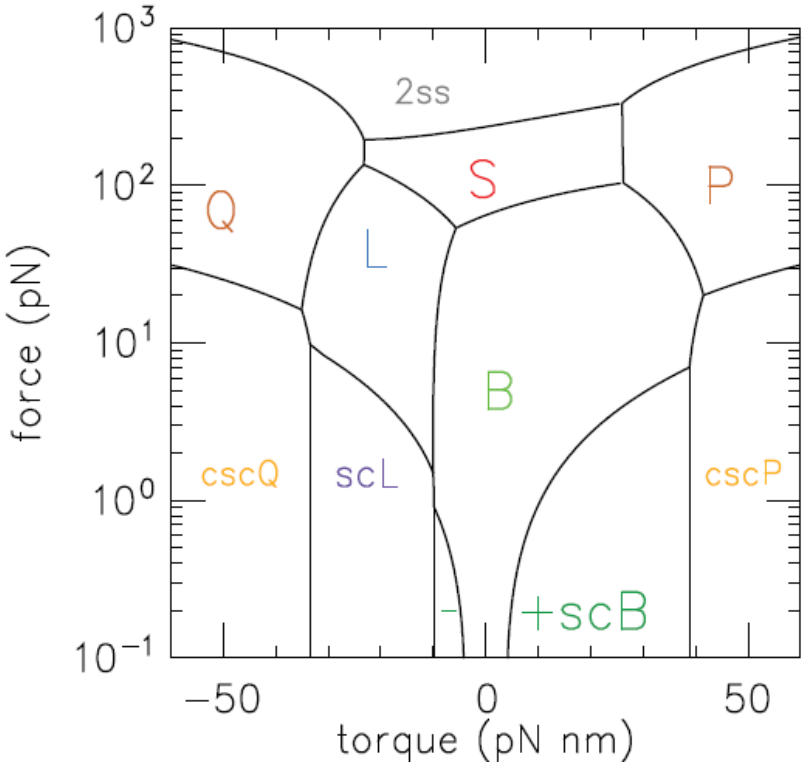


- **Low force regime**
  - (-)sc and (+)sc molecules behave similarly under stretching.
  - Excess or deficit of Lk is transferred into (+) or (-) writhe, increasing the portion of plectonemic supercoils.
- **Intermediate force regime**
  - (-)sc DNA locally denatures to relax the torsional stress due to the increased twist.
  - (+)sc DNA forms plectonemes.
- **High force regime**
  - Supercoiled DNA behaves like a torsionless molecule
  - sufficiently overwound molecules ($\sigma >$ 0.1) yield to the increased torsional stress, possibly forming local regions of highly overwound DNA

Strick et al., Biophysical Journal, Vol. 74, 2016 –2028 (1998)

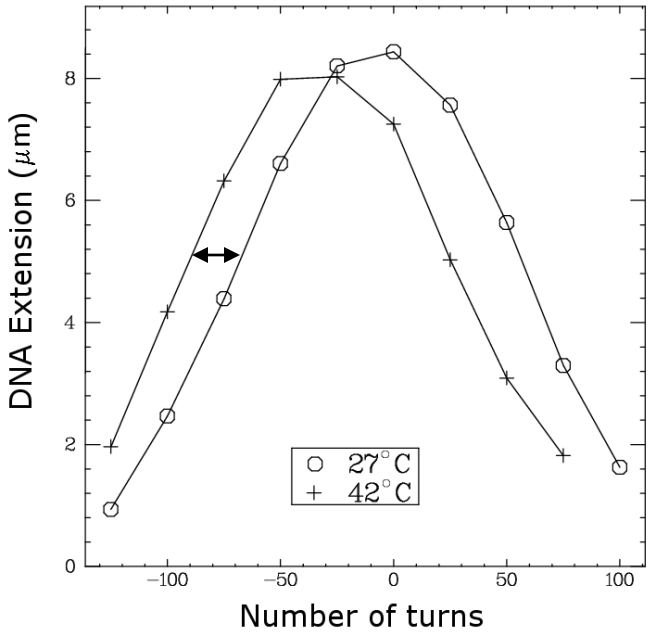
Torsion-induced DNA phase changes





John Marko, Sebastian Neukirch,
2013 Phys Rev E, 88(6), 062722

Temperature-dependence of DNA helicity



Raising the temperature by 15°C causes λ-DNA to unwind by ~ 25 turns

DNA unwinds by ~0.012°/°C/bp