magnetic tweezers

2/29/12

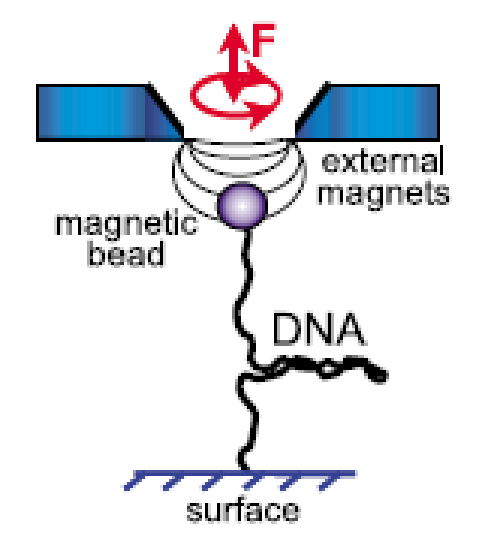
"MT"

Main Points

- How Magnetic Trap Works
- DNA acts like Worm Like Chain
- DNA has Linking Number = Twist and Writhe
- DNA acts as a tiny wind-up toy

Magnetic Tweezers and DNA

Can be conveniently used to **stretch** and **twist** DNA.



With Super-paramagnetic bead, no permanent dipole.

Dipole moment induced, and $\mu \alpha B$. $\tau = \mu \times B = 0$

$U = -\mu \cdot B$

$F = \nabla(\mu \cdot B) : U \sim -\mu B^2$.

It is the gradient of the force, which determines the direction, the force is up. (i.e. where B is highest)

- DNA tends to be stretched out if move magnet up.
- DNA also tends to twist if twist magnets (since μ follows B).

(either mechanically, or electrically move magnets)

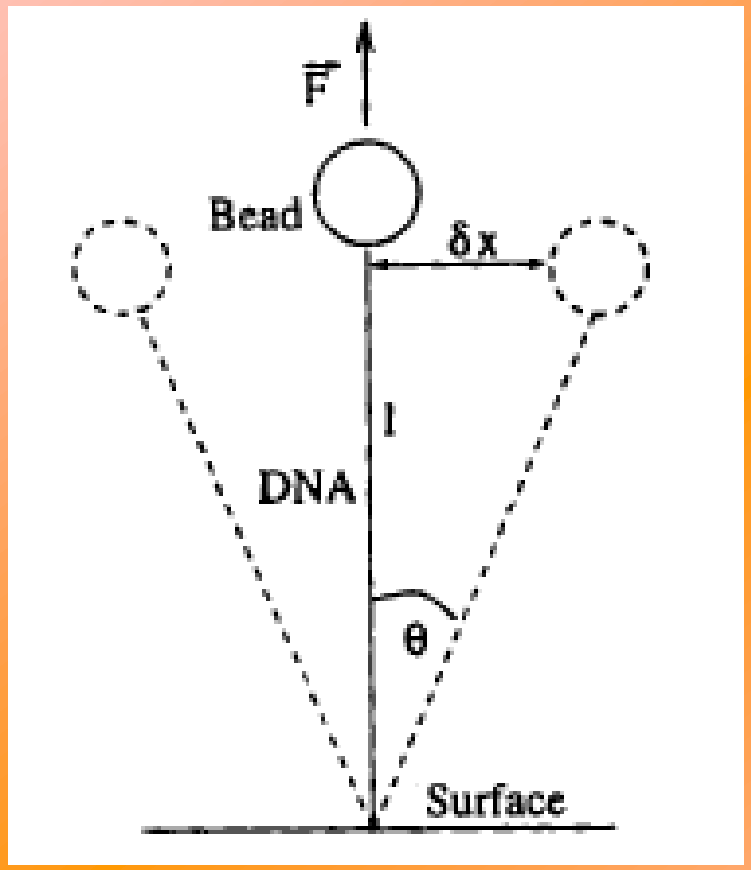
Forces ranging from a few fN to nearly 100 pN: Huge Range

Watch as a function of protein which interacts with DNA (polymerases, topoisomerases), as a function of chromatin: look for bending, twisting.

Force measurement- Magnetic Pendulum

The DNA-bead system behaves like a small pendulum pulled to the vertical of its anchoring point & subjected to Brownian fluctuations

Do not need to characterize the magnetic field nor the bead susceptibility, just use Brownian motion



Equipartition theorem:

Each degree of freedom goes as x^2 or v^2 has $\frac{1}{2}k_BT$ of energy.

Derive the Force vs. side-ways motion.

$$\frac{1}{2}\, k \langle \delta x^2 \rangle = \frac{1}{2}\, k_B T$$

$$F = k\, l$$

Note: $U_{\text{vert. disp}} = \frac{1}{2}\, k l^2$

$U_{\delta x \text{ displacement}} = \frac{1}{2}\, k(l^2 + \delta x^2)$

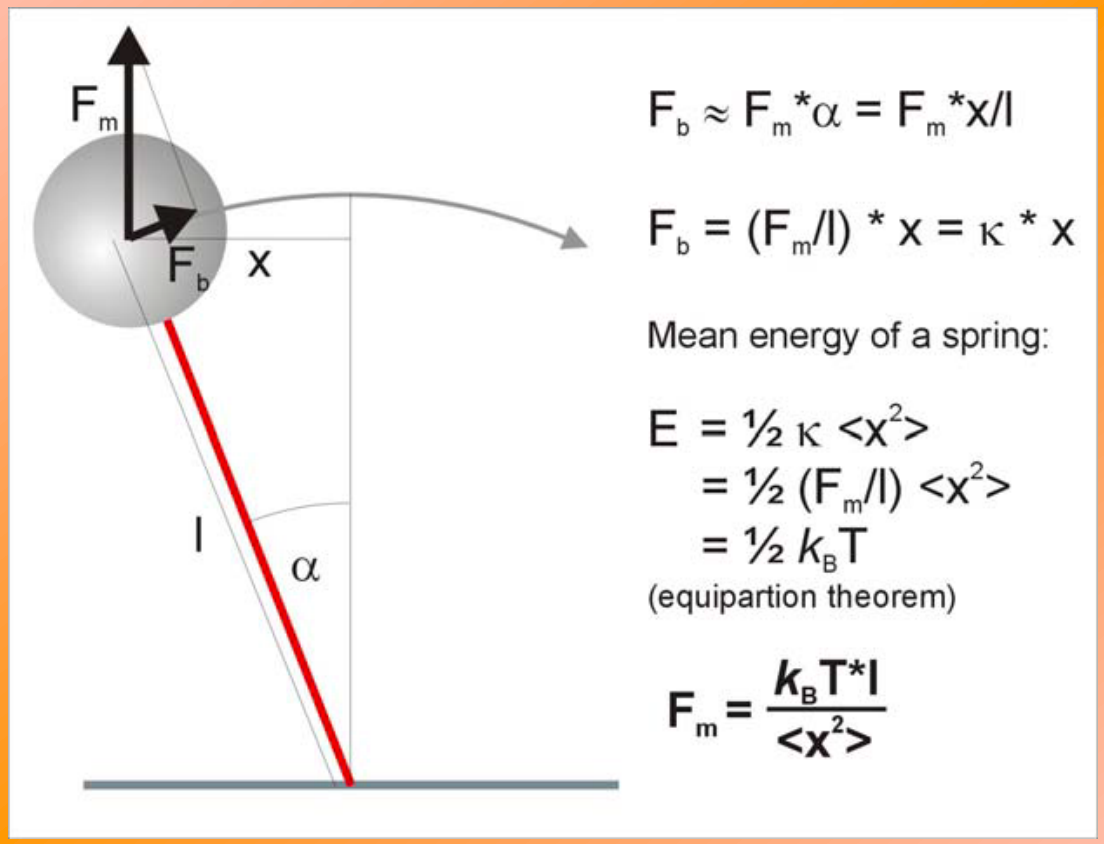
Therefore, same k applies to δx .

$$\frac{1}{2}\, (F/l) \langle \delta x^2 \rangle = \frac{1}{2}\, k_B T$$

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

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

Calculating the Force of the magnet using Equipartition Theorem



$$F_b \approx F_m * \alpha = F_m * x/l$$

$$F_b = (F_m/l) * x = \kappa * x$$

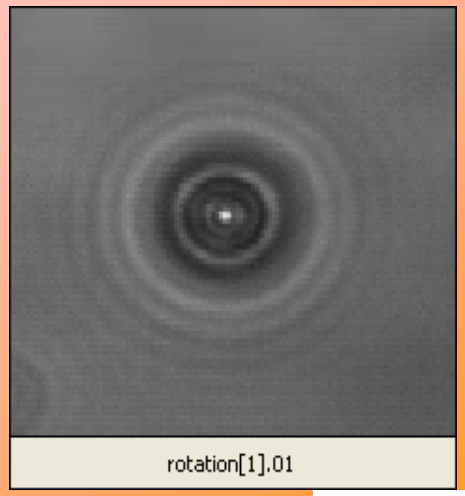
Mean energy of a spring:

$$E = \tfrac{1}{2}\,\kappa \langle x^2 \rangle$$
$$= \tfrac{1}{2}\,(F_m/l) \langle x^2 \rangle$$
$$= \tfrac{1}{2}\,k_B T$$

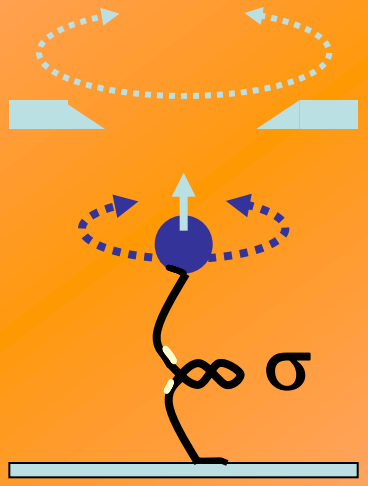
(equipartion theorem)

$$F_m = \frac{k_B T * l}{\langle x^2 \rangle}$$

Magnetic Traps: Measuring twist



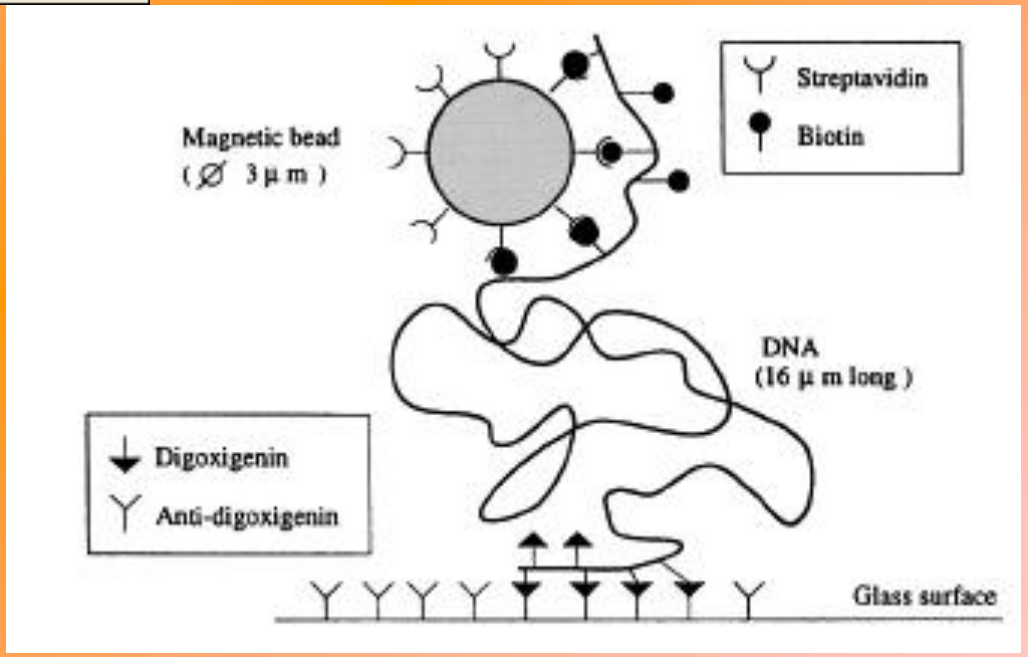
DNA twisting



Twisting leads to motion in x-y plane

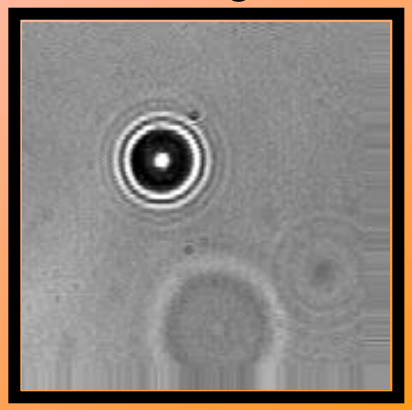
Antibody-ligand

Antibody

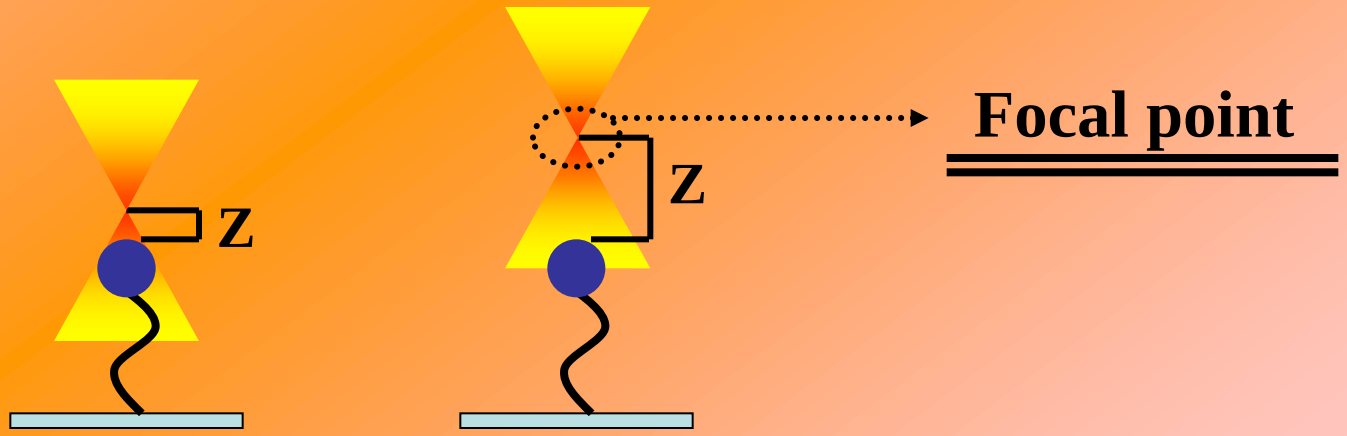


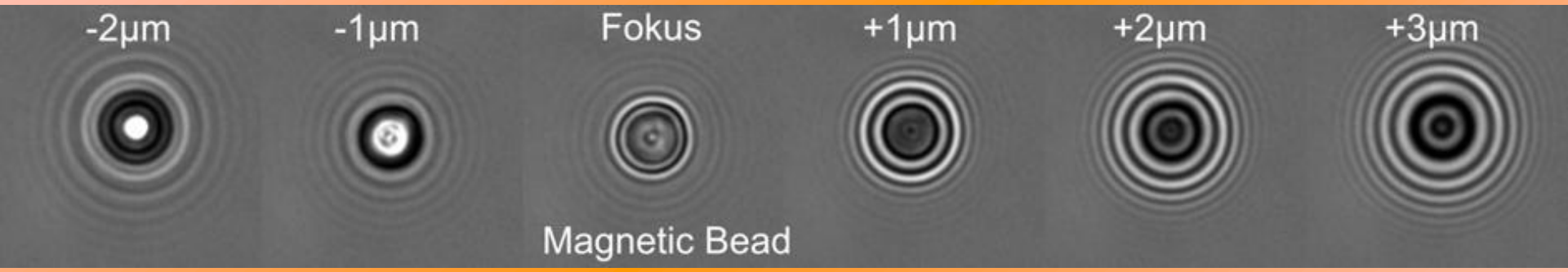
Magnetic Traps: Measuring DNA stretch

Bead rings.avi



Diffraction rings



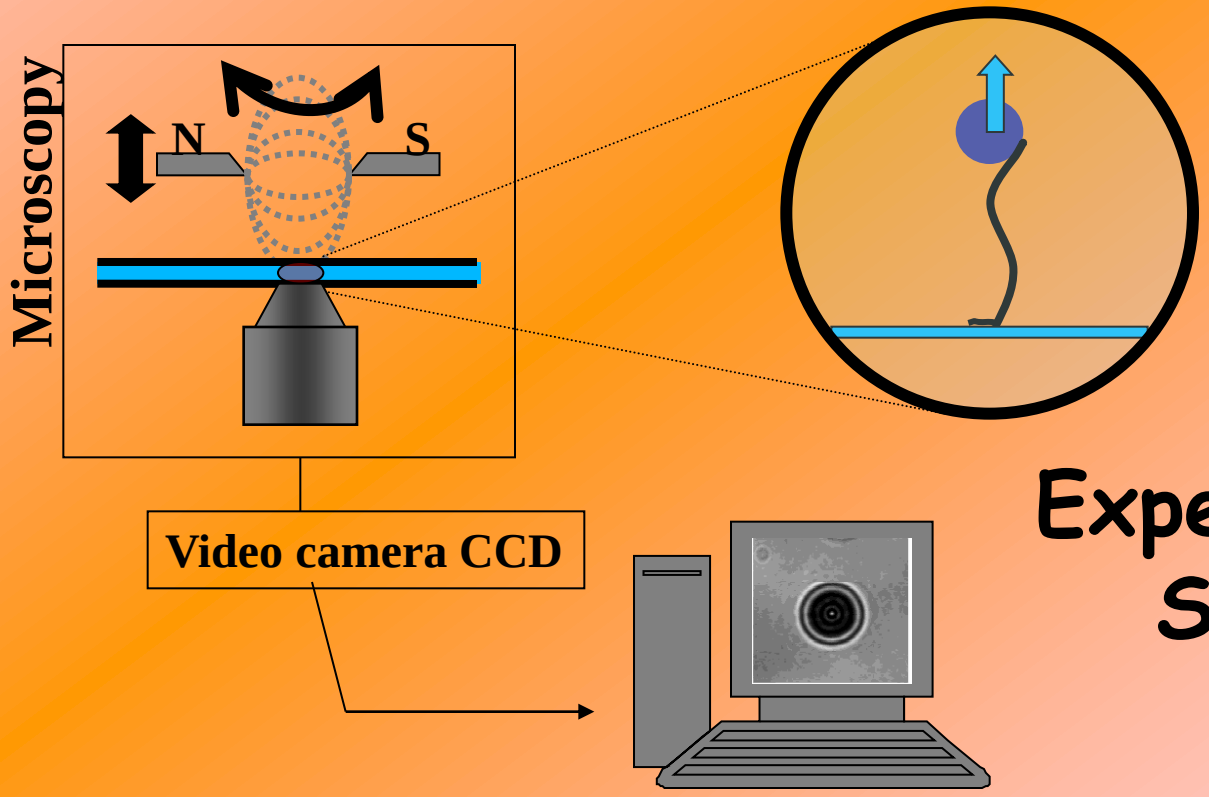

-2µm
-1µm
Fokus
+1µm
+2µm
+3µm
Magnetic Bead

Magnetic Trap movie (ADN.SWF)

How to attach DNA: to glass; to paramagnetic bead

Set-up of Experimental system

Detect nanometer displacements with visible light



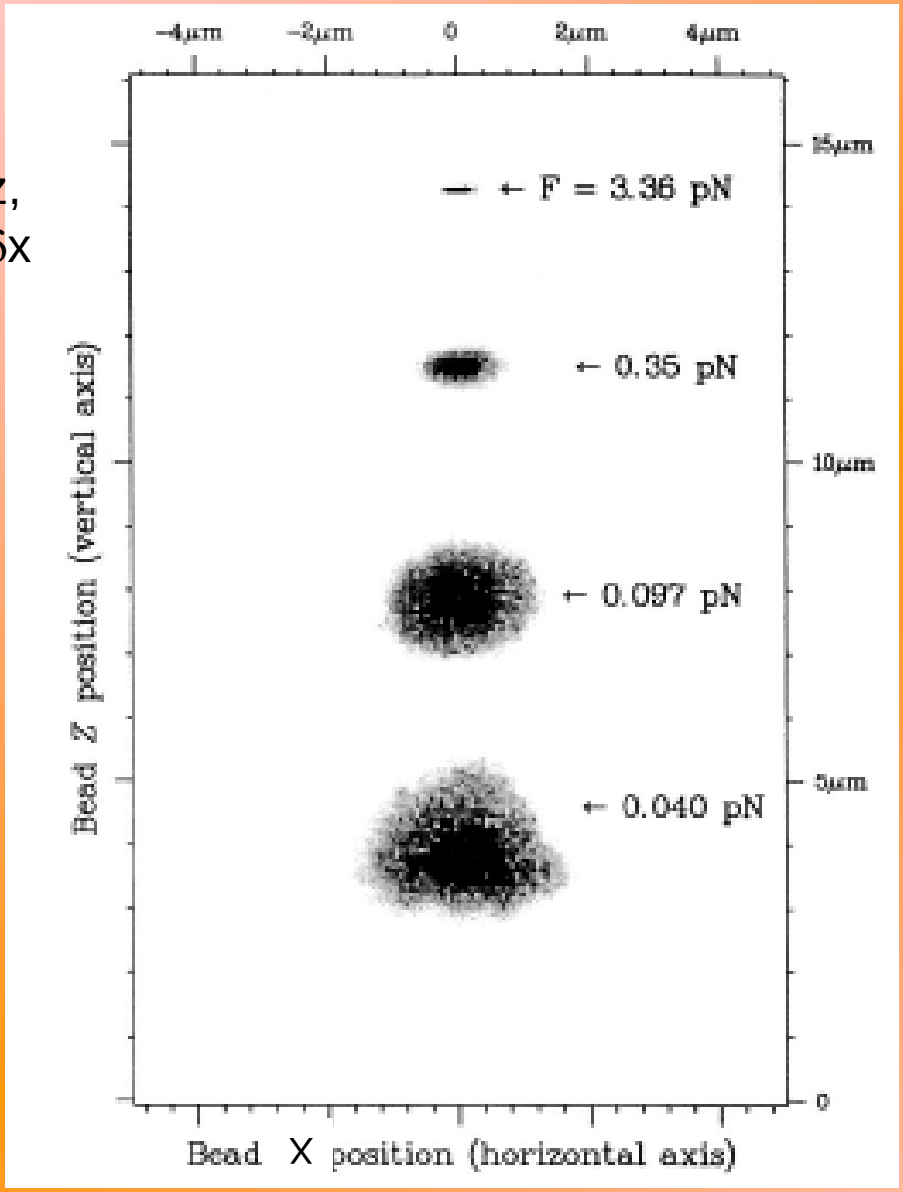
Experimental Set-up

Force measurements- raw data

Measure z, measure δx

Find F by formula.

$Z = l$



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

Measure $< \delta x^2 >$, l and have F!

$$F = \frac{k_B T l}{< \delta x^2 >}$$

Example: Take l = 7.8 μm

(4.04 pN-nm)(7800nm)/ 577^2 nm = 0.097 pN

At higher F, smaller δx; so does δz.

Lambda DNA = 48 kbp = 15 μm

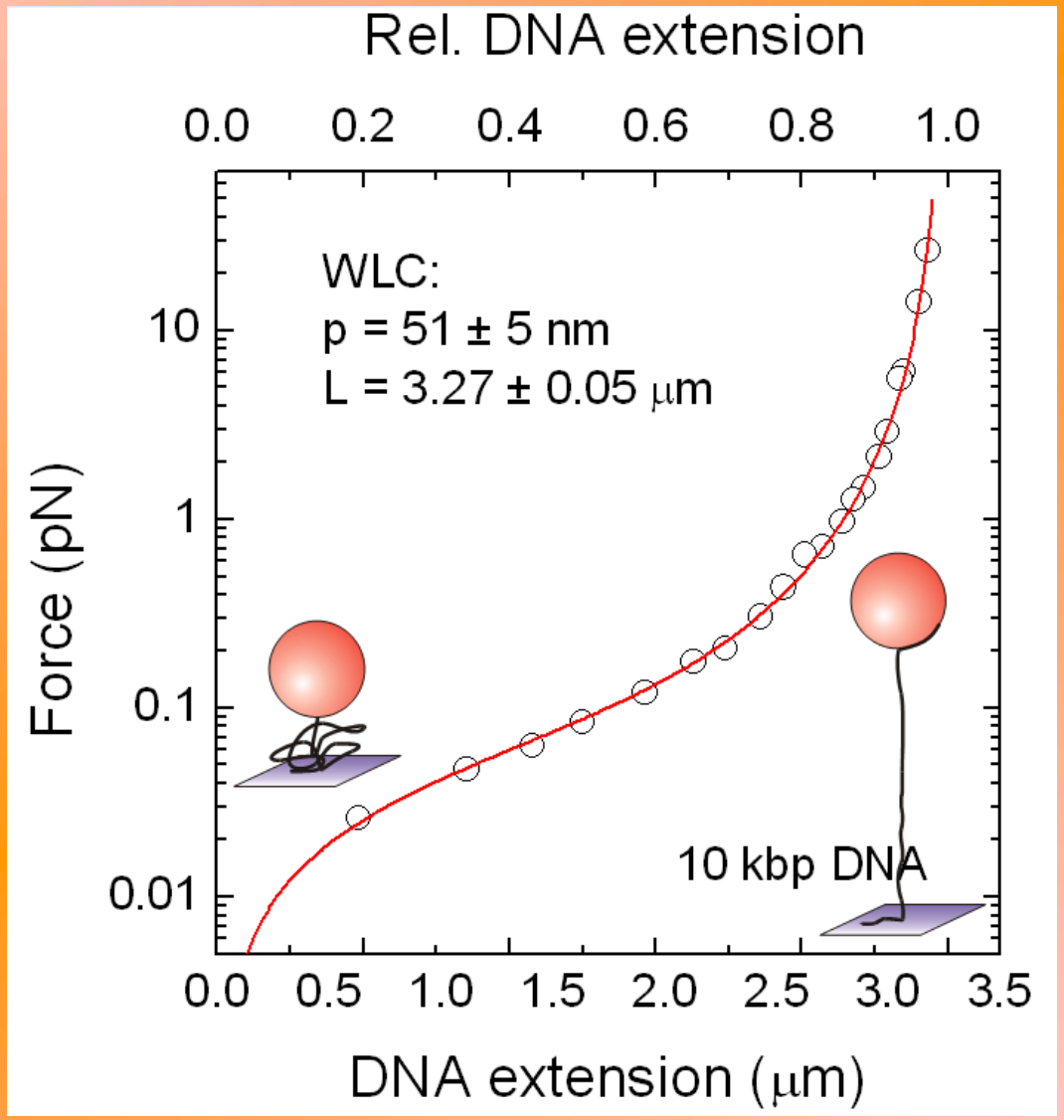
At low extension, with length doubling, δx ~ const., F doubles.

At big extension (l: 12-14 μm), Δx decrease, F ↑10x.

Spring constant gets bigger. Hard to stretch it when almost all stretched out!

Movie measureforce.

WLC Fits very well at all stretches

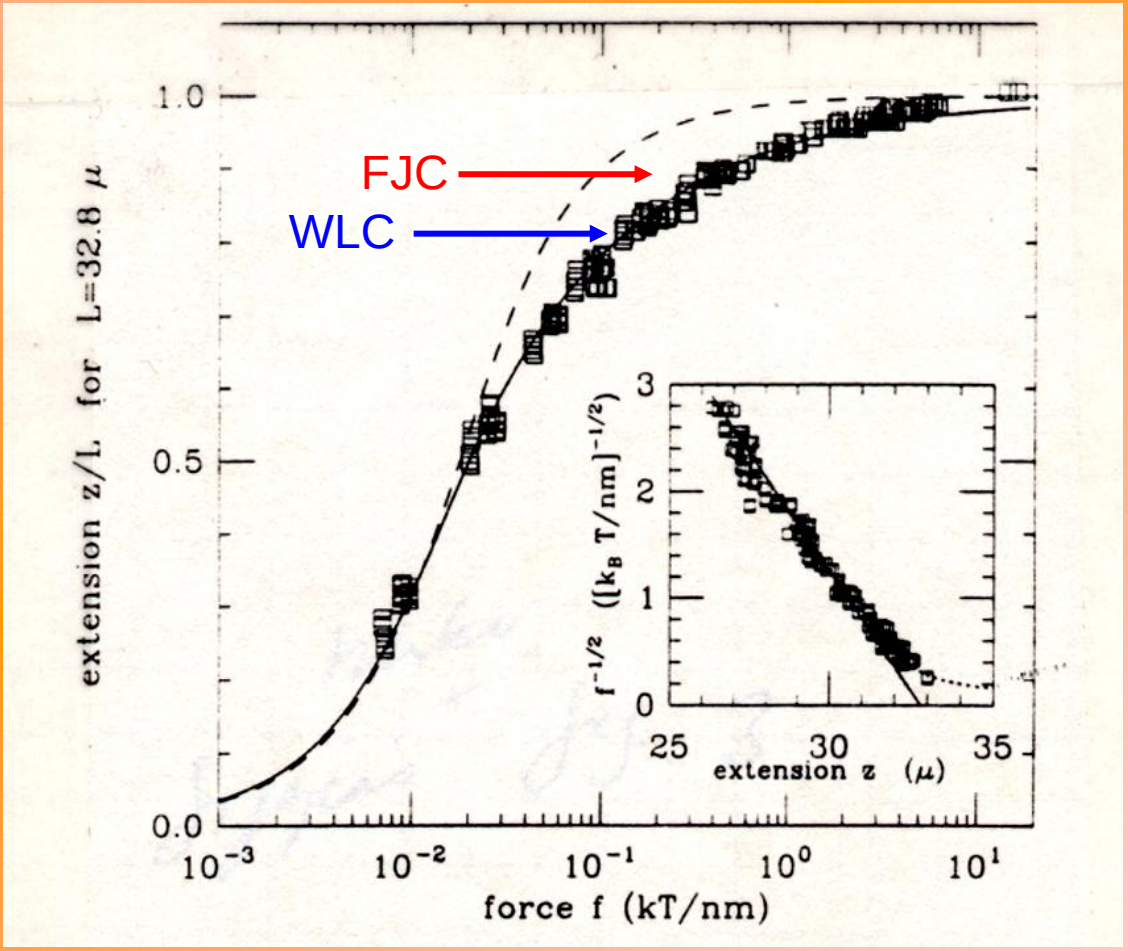


FJC not as good as WLC

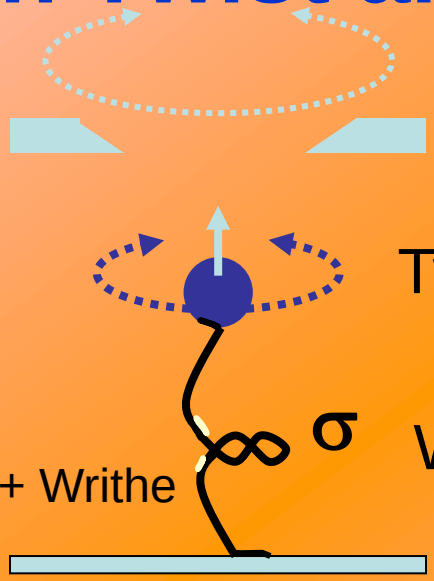
At very low (< 100 fN)
and at high forces (> 5 pN),
the FJC does a good job.

In between it has a problem.

There you have to use WJC.



DNA may be Supercoiled: Has both Twist and Writhe



Linking Number = Twist + Writhe
Lk = Tw + Wr

Relaxed DNA: Twist =1 turn/10.4 bp. Writhe = 0. ΔTw=0

Can't just twist up DNA and have it all go into twist.

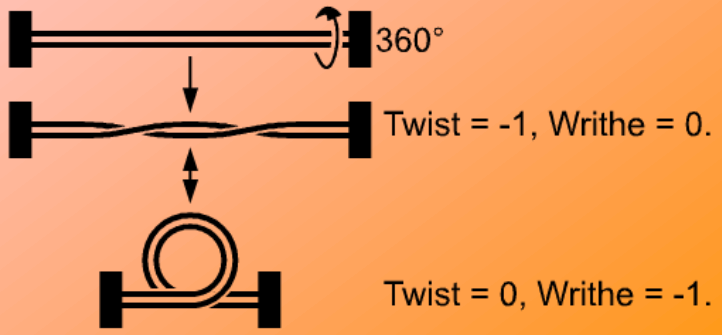
Example: Phone cord.

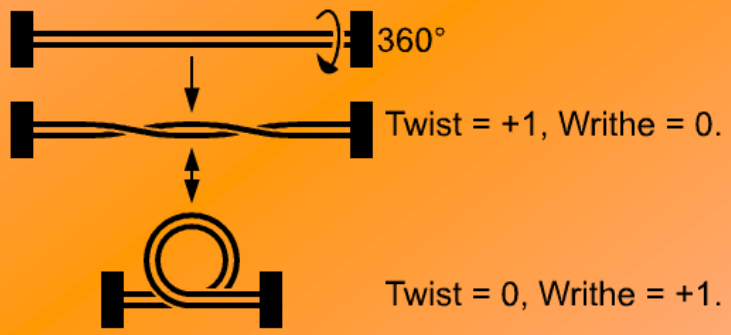
Pull on DNA and writhe comes out.

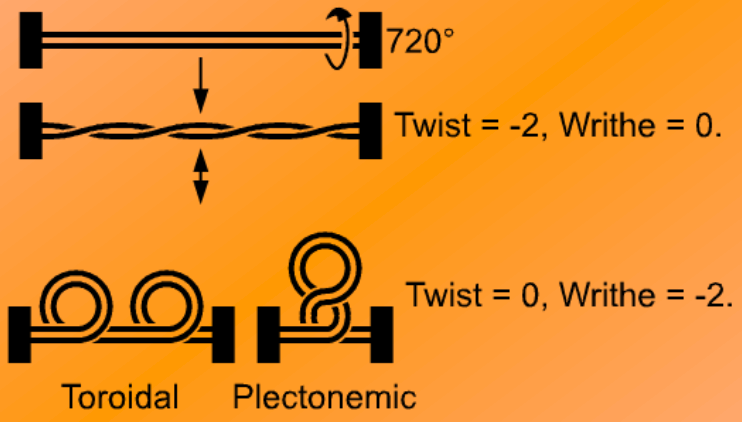
Measure relaxed (non super-coiled) DNA and figure out length vs. force.

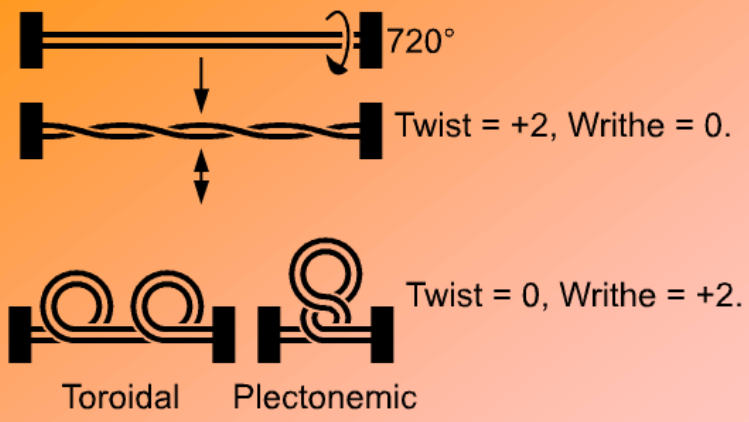
Some Examples of Tw and Wr

Linear DNA with Constrained Ends





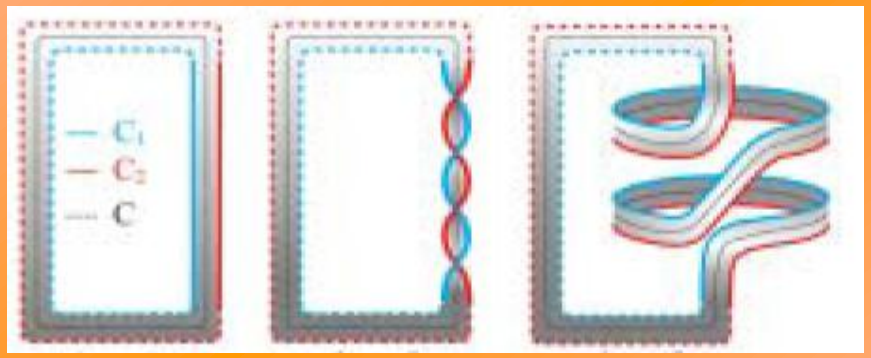




Twist (T_w), Writhe (W_r), Linking Number (Lk)

T_w: # of times the two strands wrap around each other

W_r: # of times C crosses itself.



T_w=2 **T_w=0**

W_r=0 **W_r=2**

Charvin, Contemporary Physics,2004

Linking Number = Twist + Writhe
Lk = Tw + Wr

Supercoiled DNA (σ): is the deviation from relaxed linking number.

$\sigma = (Lk - Lk_o)/Lk_o = \Delta Lk / Lk_o$

Ex: Hold DNA out straight so that it has no Writhe, add of take out twist, then let fold up (Twist goes into Writhe).

Normal DNA is negatively supercoiled, -0.06 = 6 turns for every 100 taken out. Why?

Helps unwind DNA– makes it easier to uncoil, separate strands. Enzymes which do this called Topoisomerases.

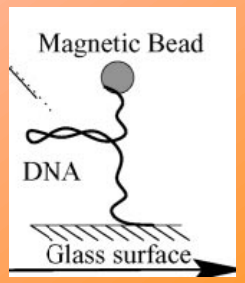
Why? What about archebacteria that lives in hot springs? Positively supercoiled

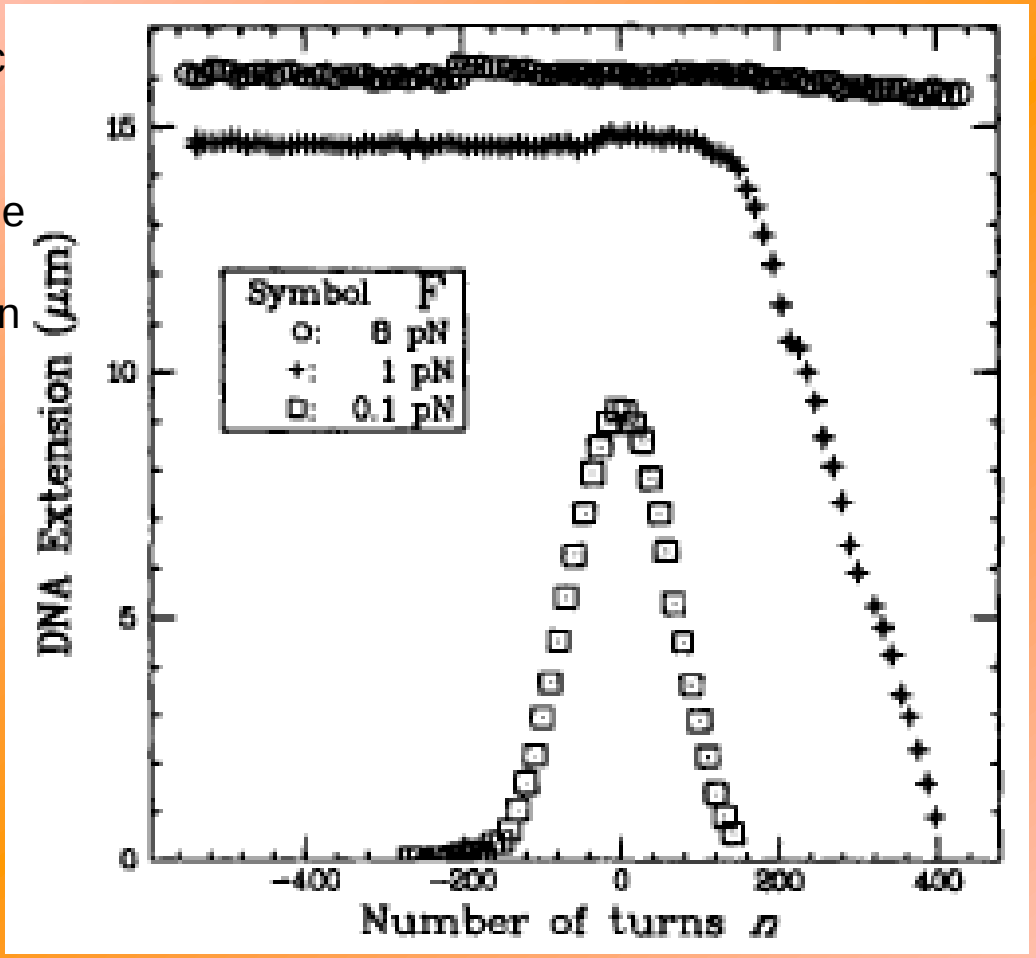
Makes DNA more stable

Torsionally stressed single DNA molecule

Playing with phone cord: can you explain graphs?

Low F: symmetric under $\sigma \rightarrow -\sigma$
The shortening corresponds to the formation of plectonemes upon writhing.





When the force is increased above 0.5 pN, the curve becomes asymmetric: supercoils still form for positive coiling while local denaturation adsorbs the torsional stress for negative σ.

At forces larger than 3 pN no plectonemes are observed: the torsional stress is adsorbed not by writhe but in local structural changes of the molecule.

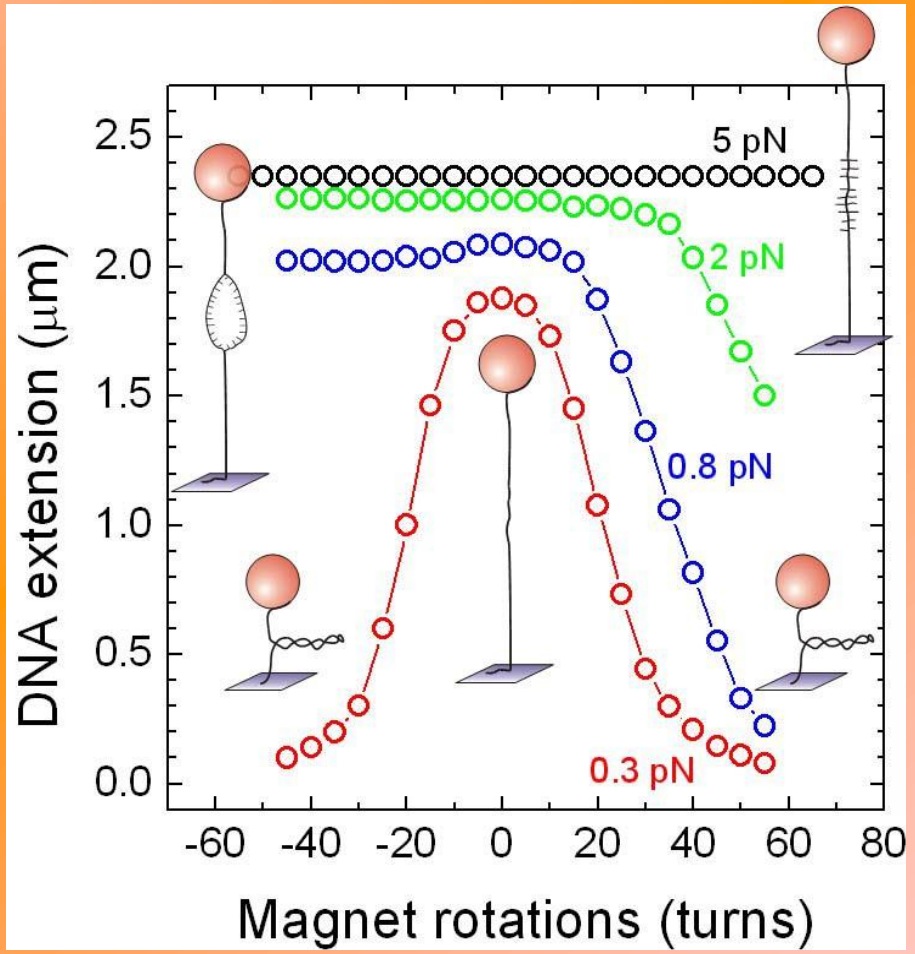
Extension vs. supercoiling at constant force

Three regimes

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

Over- and under-stretching

Upon twisting a DNA molecule it takes a number of turns, before the DNA length reduces significantly and plectonemes are formed. The point ($N_{buckling}$) where DNA starts to form plectonemes with a constant length reduction per turn is called buckling instability



Rotation extension curves for different forces. At higher forces one cannot induce supercoils but denature the DNA molecule.

$N_{buckling}$ & $T_{buckling}$

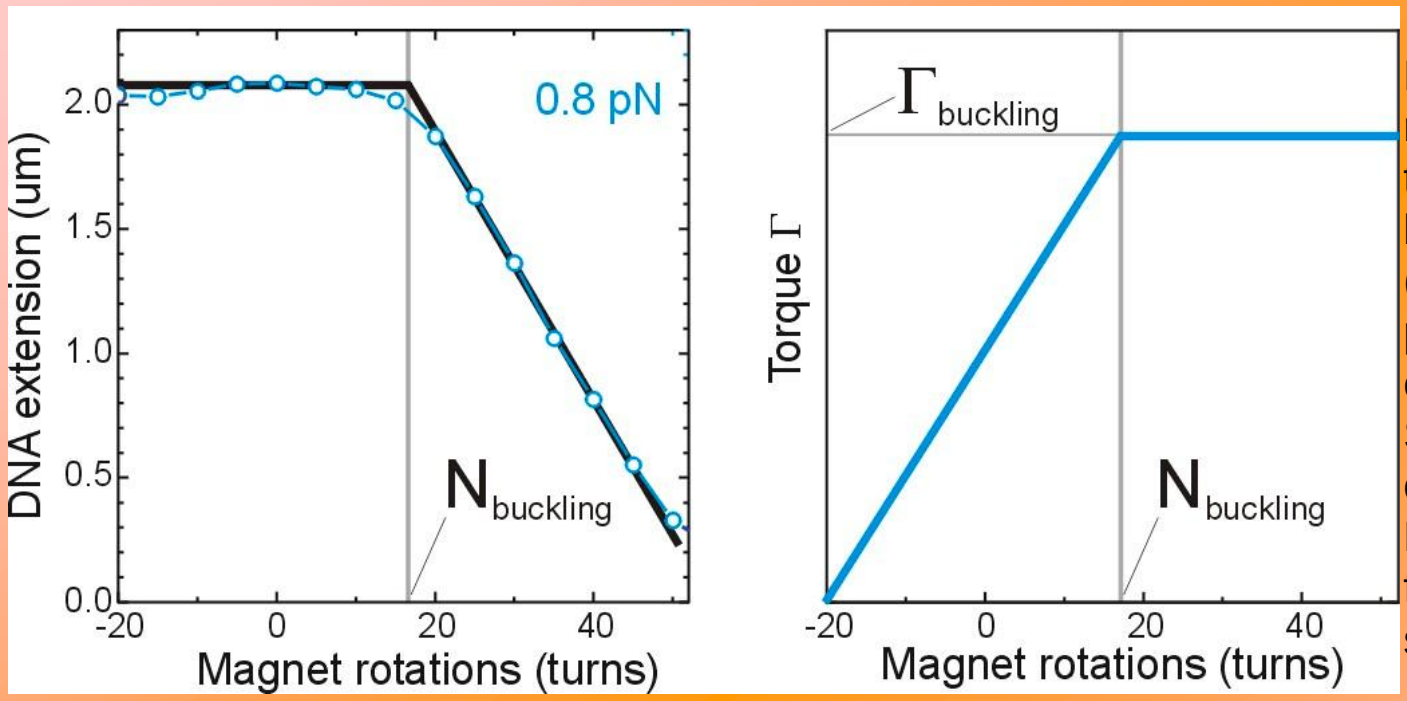
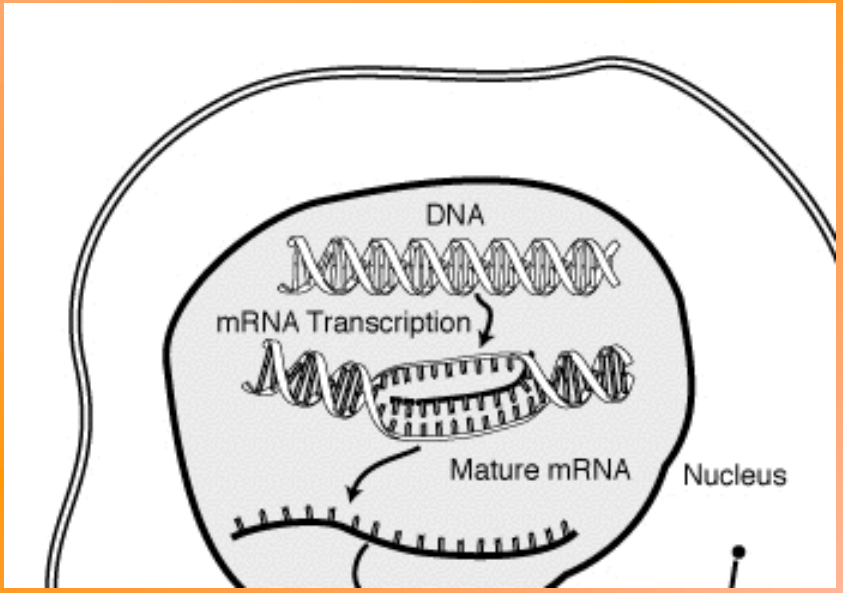


Fig. 7: a) From fitting a rotation-extension curve the position of the buckling instability (Nbuckling), at which plectonems start to form, can be determined. b) Schematic representation of the torque within the DNA molecule as a function of the induced supercoiling.

Up to that point the twist or torque builds up linearly with the number of turns (Fig. 7b). Beyond the buckling instability the torque stays constant, as all supercoils which are generated is now transformed into plectonemes (Fig. 7a). This is because the energetic cost to further twist the DNA is now higher than the cost to move the bead down against magnetic force and to bend the DNA into a plectoneme. Calculating the competition between DNA twisting and plectoneme formation within a simplified model allows to estimate the critical torque (**Γ**buckling) where plectonemes are start to form.

Application to Eukaryotic Cells

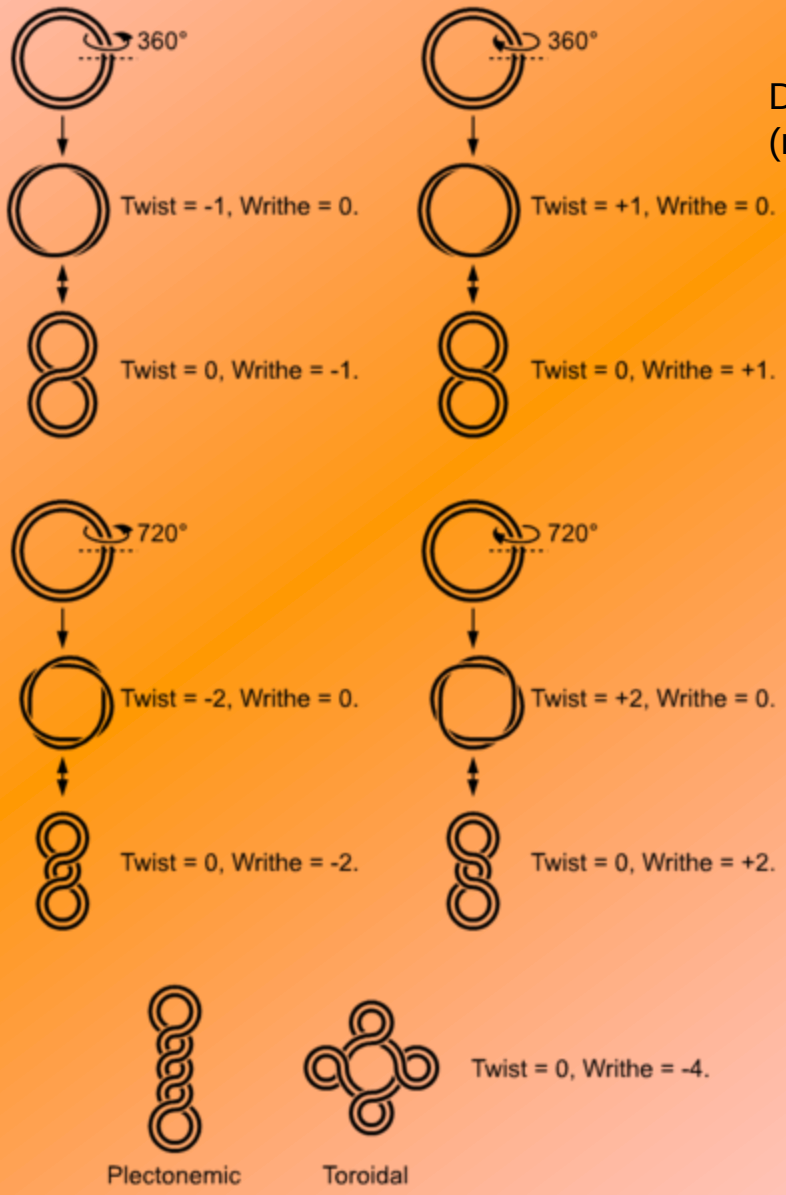
In Eukaryotic cells: When a chromosome wants to express a particular gene, it becomes single stranded. Requires a tremendous amount of energy (remember partition function and genes, typically 10-100k a.a., require many H-bonds to be broken: DNA is a very stable structure). Requires enzymes– topoisomerases.



In response to supercoiling, they will assume an amount of writhe, just as if their ends were joined.

More Examples of Writhe and Twist

Circular DNA



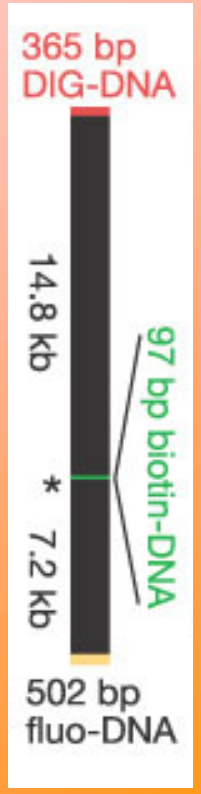
DNA's helical nature (natural twist) not shown.

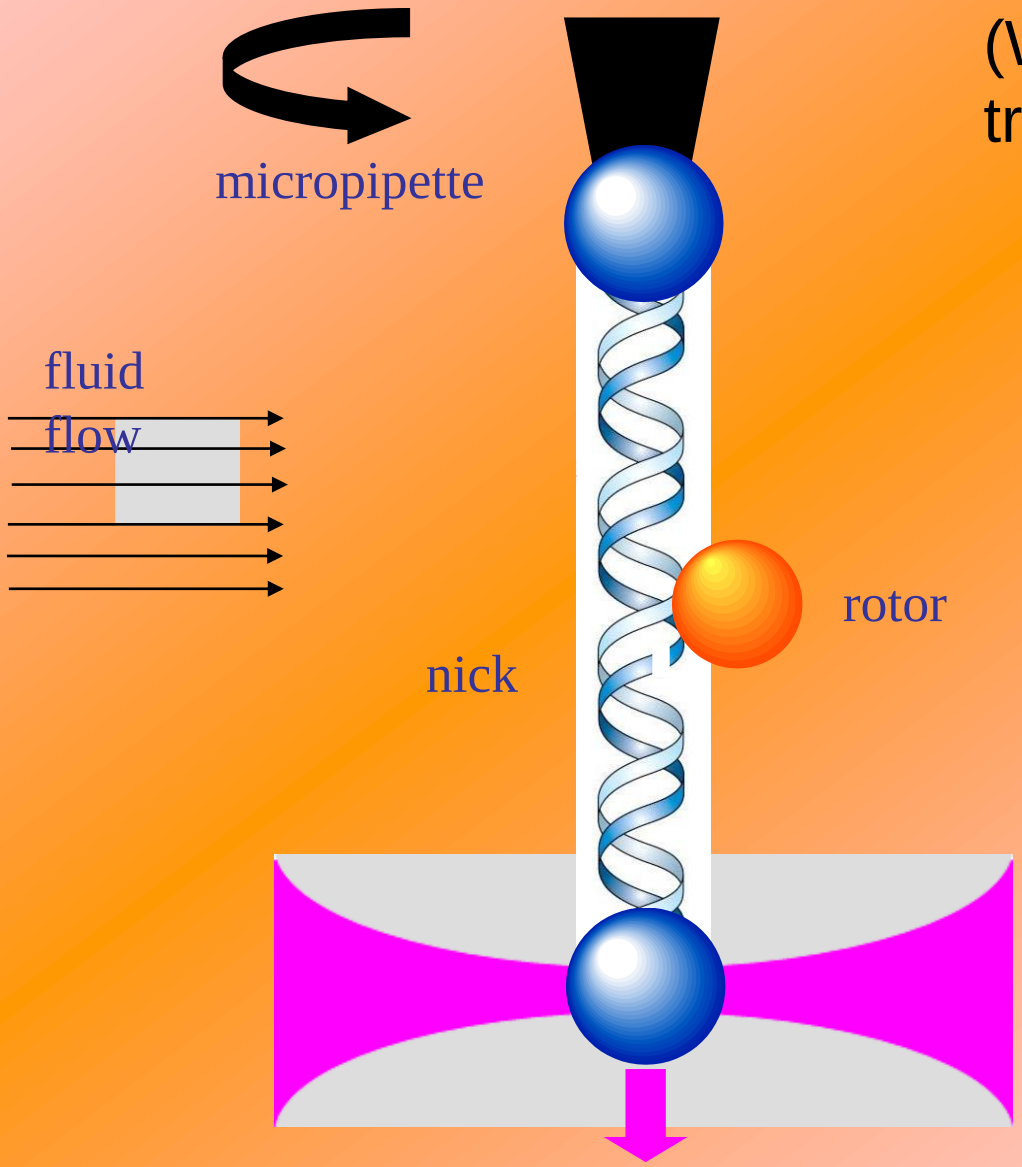
Plectonemes more common: shape bacterial plasmids take.

http://commons.wikimedia.org/wiki/Image:Circular_DNA_Supercoiling.png

Optical Trap Set-up: Similar to MT, but uses Optical Trap and micropipette

(Will discuss optical traps in detail later)

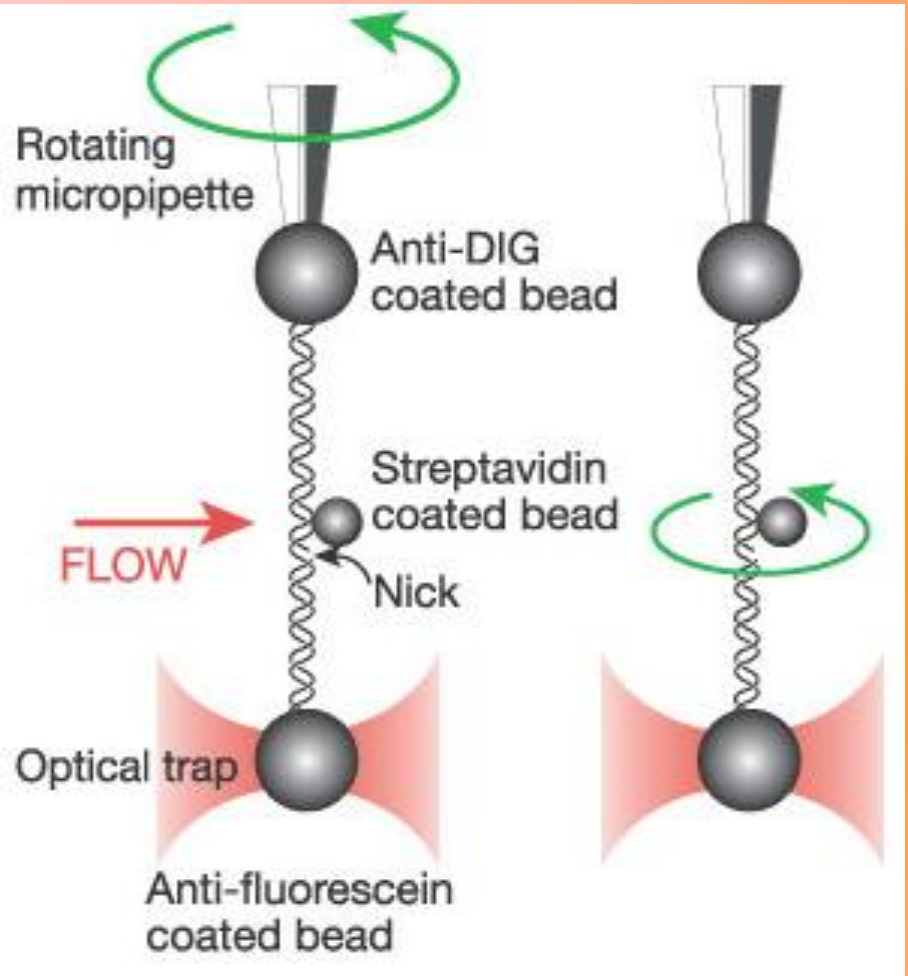


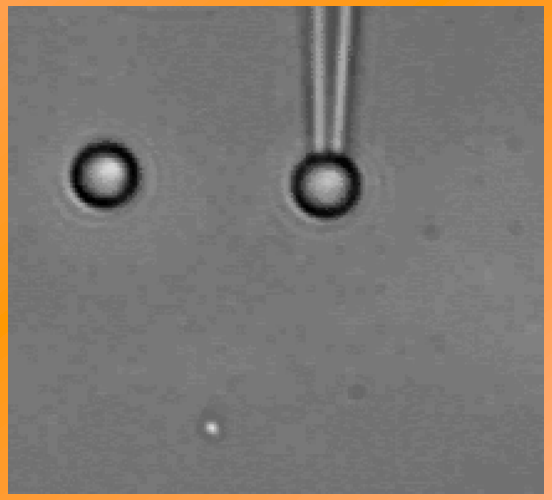


Beads: 2.8 µm
Rotor 520-920 nm

Bryant, 2004, Nature

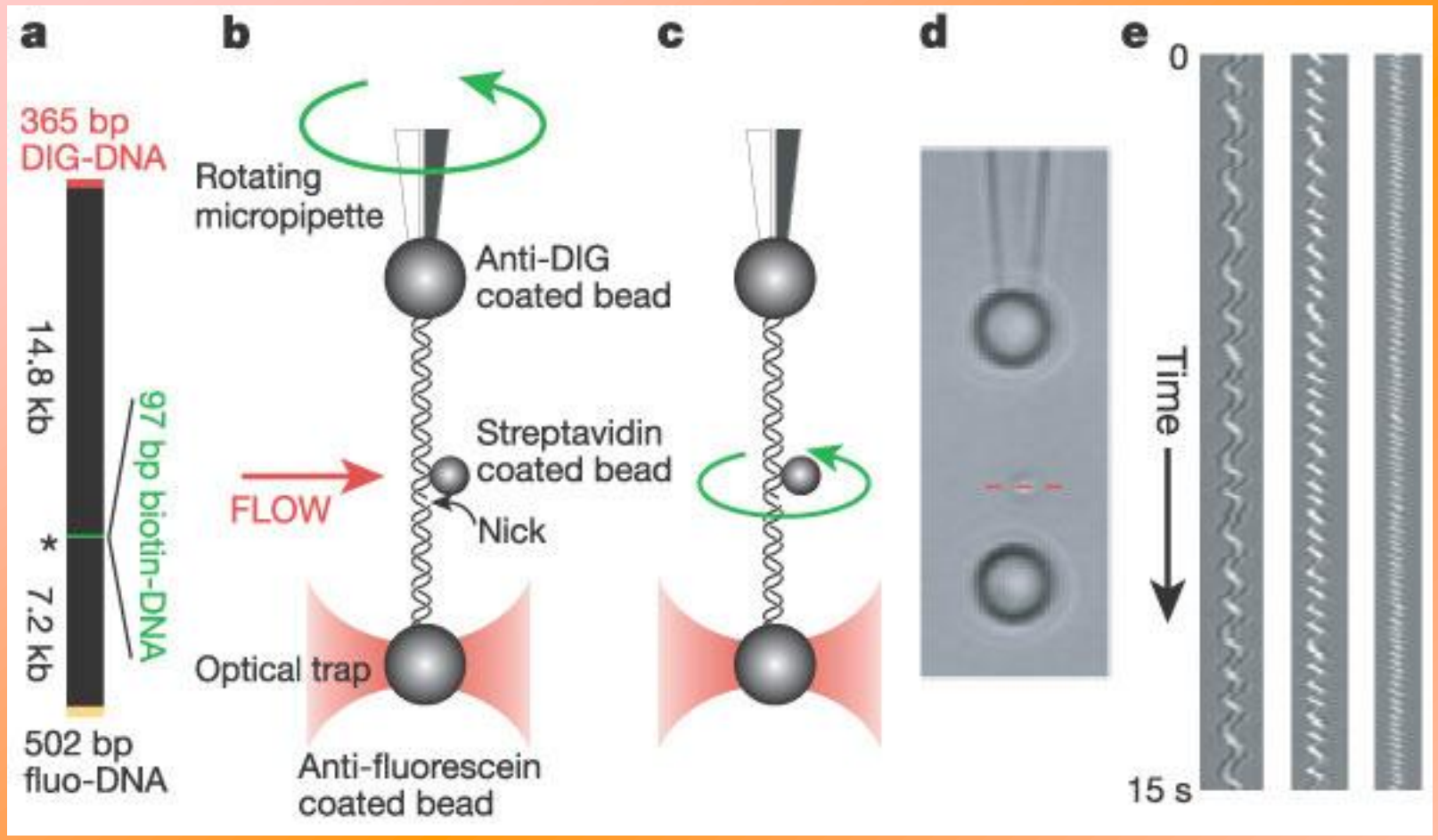
Experimental Method





See Supplemental Movie 1 of Bryant et al, Nature, 2003

$$\tau = \gamma \omega$$

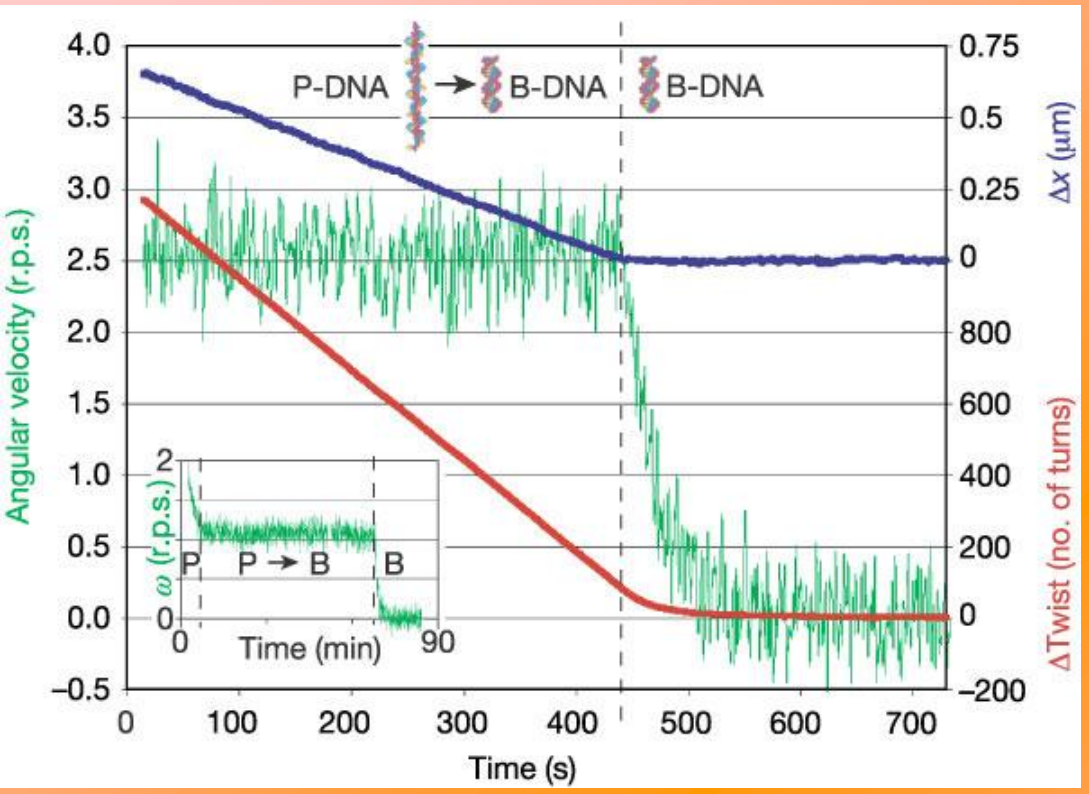


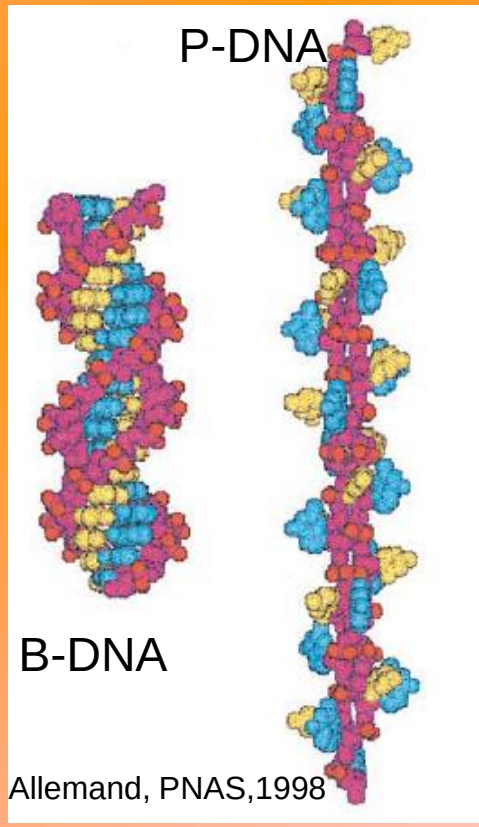
a, The molecular construct contains three distinct attachment sites and a site-specific nick (asterisk), which acts as a swivel. **b**, Each molecule was stretched between two antibody-coated beads using a dual-beam optical trap[11]. A rotor bead was then attached to the central biotinylated patch (see Supplementary Movie 1). The rotor was held fixed by applying a fluid flow, and the micropipette was twisted to build up torsional strain in the upper segment of the molecule. **c**, Upon releasing the flow, the central bead rotated to relieve the torsional strain. **d**, Video of the rotating bead (Supplementary Movies 2 and 3) was analysed to track cumulative changes in angle. Horizontal sections (red dashed line) of successive video frames can be stacked (**e**) to allow visualization of the helical path of the bead in space and time. Left to right, traces of 920 nm, 760 nm and 520 nm rotor beads. (See Bryant, Bustamante, Nature, 2003)

Bryant, Bustamante Nature article

We begin each assay by assembling the DNA molecule and rotor between two antibody-coated beads held in a micropipette and a force-measuring optical trap11 (Fig. 1b and Supplementary Movie 1). Typically, we build up torsional stress in the molecule by holding the rotor bead stationary using fluid flow, and rotating the micropipette by 300 turns. When the flow is released, torque stored in the upper DNA segment causes the central bead to rotate continuously about its edge (Fig. 1c–e, and Supplementary Movies 2 and 3) until the torsional stress has been relieved, after which the bead rotates slightly back and forth under the influence of thermal fluctuations. Constant tension is maintained using force feedback, preventing buckling of the molecule2 during the experiment, so that the observed dynamics reflect changes in twist and not writhe. At low Reynolds numbers, the magnitude of the torque can be determined as the product of the angular velocity (**q**) of the rotor and its rotational drag (**g**).

Structural Changes B-DNA↔P-DNA





Allemand, PNAS,1998

- B-DNA: regular stranded DNA
- P-DNA: over-extended, high helicity, 50% longer than B-DNA
- Constant torque: B→P transition is cooperative.
- Full conversion of 14.8 kb into P-DNA requires ~4,000 turns, ~2.7 bp per turn.

Class evaluation

1. What was the most interesting thing you learned in class today?
2. What are you confused about?
3. Related to today's subject, what would you like to know more about?
4. Any helpful comments.

Answer, and turn in at the end of class.

Notes from Gilles Gaven?

Hi Paul,

here are some flash animations I made that Laura has probably seen on an old web page :

ADN.swf is a quick description of the magnetic trap setup from the DNA construct to the microscope (use "space" key to move forward) . I have the same with white background but in french (tell me if you d prefer that one)

force.swf is intended to explain 1) qualitatively bead fluctuation and DNA extension as a function of force (drag the magnets to change the force) . The simul is actually generating monte-carlo steps for an FJC model with several segments
2) the way extension is measured in the Croquette-Bensimon kind of apparatus : a movie on the left is synchronized to the the simulation on the right , so that one sees how diffraction rings change as the bead move in the z direction.

mesureforce.swf is a slight variation of force.swf.

.swf files can be embedded in ppt (it's a bit tricky...) , otherwise these files can be read in a web browser or a standalone flash player. Don't hesitate if you need other stuff ....

Hope it helps !