Introduction II and applications

2/4/08

magnetic tweezers

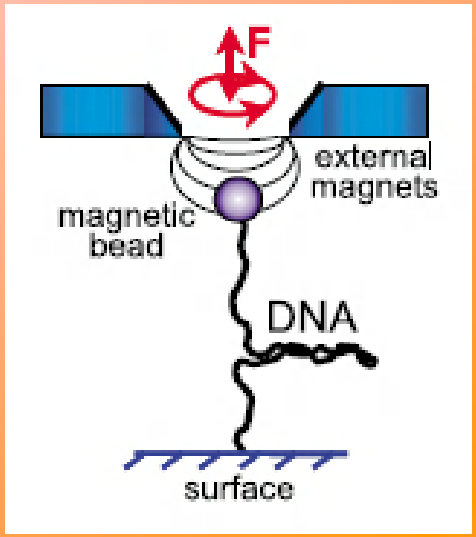
"MT"

Many slides came from Laura Finzi at Emory University. Thanks!

Some came from Majid Minary-Jolandan, grad. student at UIUC. Thanks!

MT and DNA

Example of **External field manipulators** — optical tweezers (OT), magnetic tweezers (MT), and flow fields — the molecule is acted upon from a distance, by application of external fields (photonic, magnetic, or hydrodynamic) either to the molecule itself or to an appropriate handle to which the molecule is attached.



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

Correction:

F= qv x B = m x B = torque! → vertical (z-axis) (not net force.

With Super-paramagnetic bead, no permanent dipole. Dipole moment induced, and $\mu \alpha B$.
$U = -\mu \cdot B$ (why negative sign?)

Definition: if μ, B parallel, lowest energy.

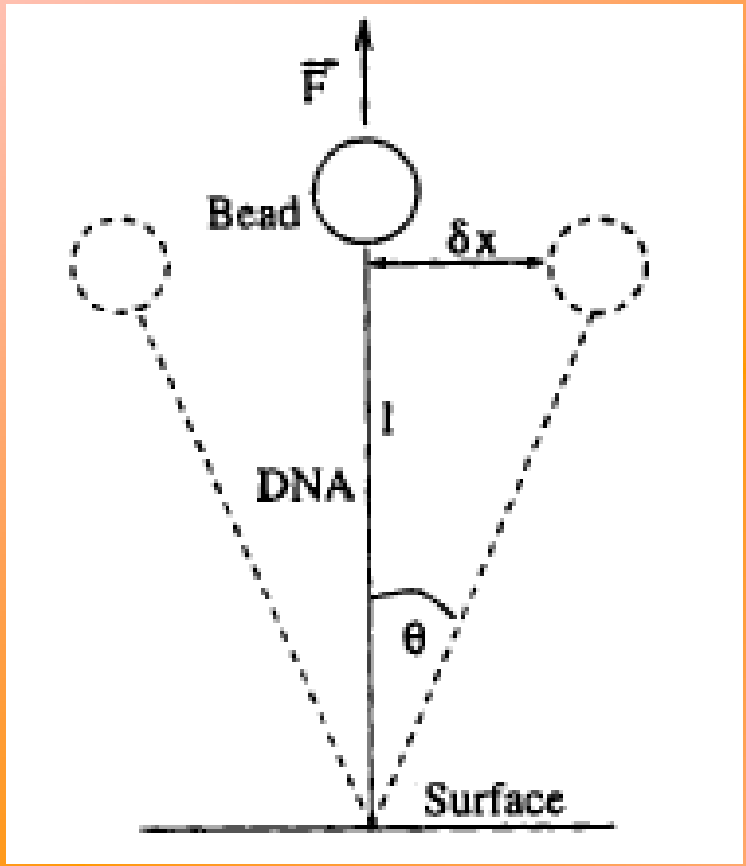
How does energy depend on B?

$U \sim -\mu B^2$. So goes to region where B is highest intensity: up.

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. (HW this week)

$$\tfrac{1}{2}\, k < \delta x^2 > = \tfrac{1}{2}\, k_B T$$

$$F = k\, l \quad \text{: but note}$$

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

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

Therefore same k applies to δx .

$$\tfrac{1}{2}\,(F/\,l) < \delta x^2 > = \tfrac{1}{2}\, k_B T$$

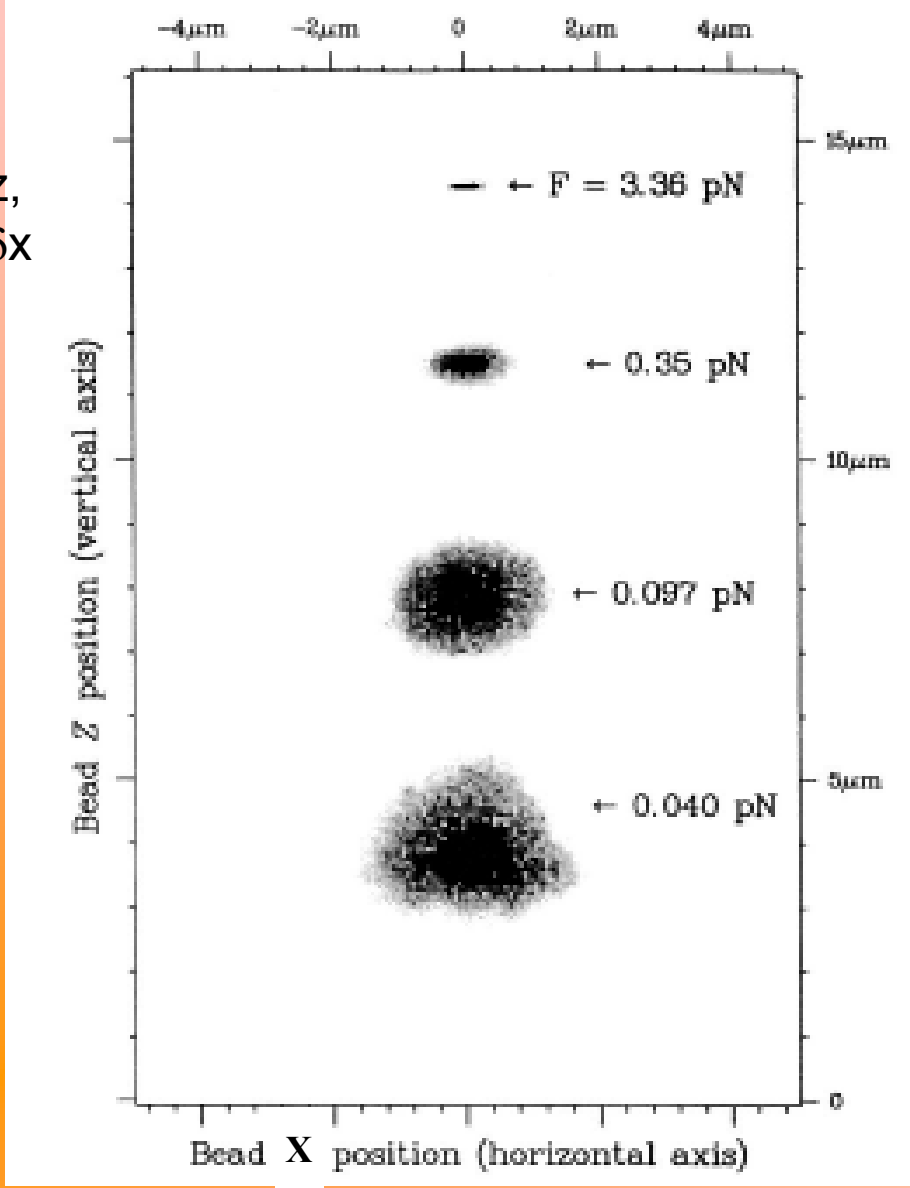
$$\boxed{F = \frac{k_B\, T\, l}{< \delta x^2 >}}$$

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

Force measurements- raw data

Measure z, measure δx

Find F by formula.



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

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

Ex: (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.

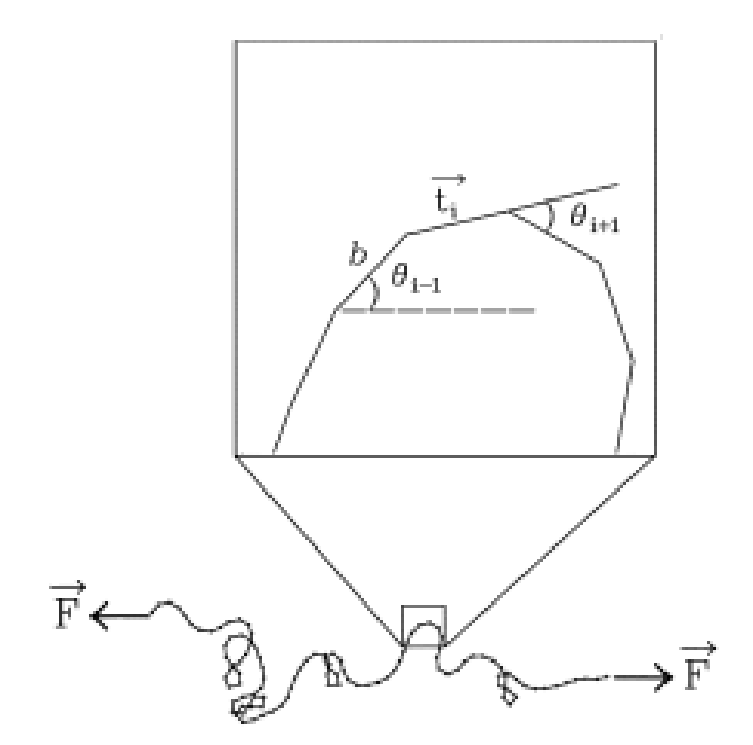
At big extension (l: 12-14 μm),

Δx decrease, F ↑10x.

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

Two Models of DNA

Freely Jointed Chain & Worm-like Chain

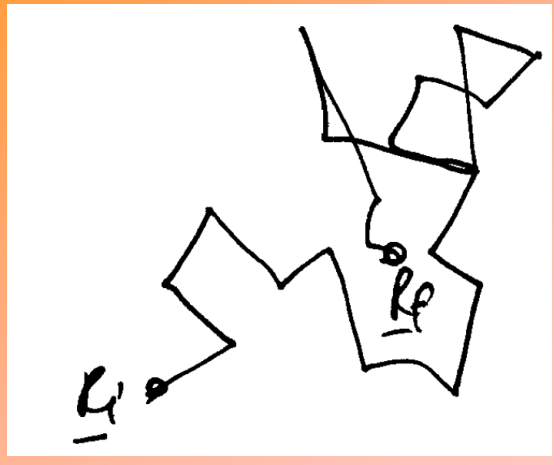


Strick, 2000, Prog. In Biophysics & Mol. Bio.

FJC: A continuous polymer chain can be simulated by a chain of freely rotating segments of size b (Kuhn length = 2 x Persistence length) and orientation vector t_i. N steps of length b. $<|R_f-R_i|^2> = \#R_g^2 .= Nb^2$, where R_g = radius of gyration.

Orientation of the next step is completely independent of the previous step → FJC.

b for DNA ~ 50 nm ~ 150 bp. (Depends on [salt].)

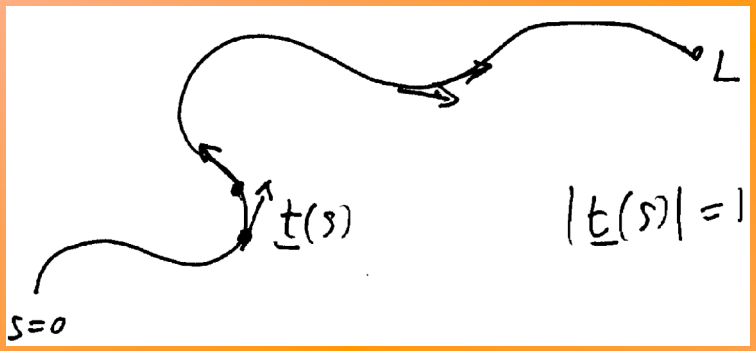


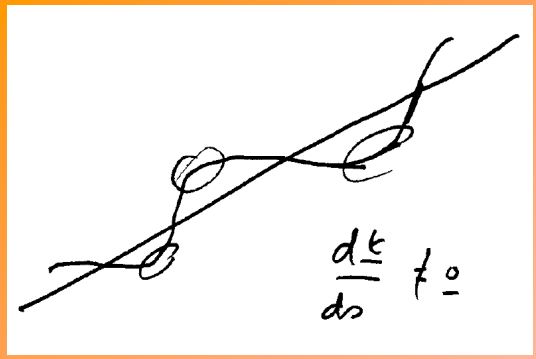
Worm-Like Chain

Fixed contour length, L. It has a tangent (of fixed length) which varies as you proceed along a contour (s)

$$U = C\int_{s=0}^{s=L} ds\ |dt/ds|^2 \qquad |t(s)=1|$$

If used with Boltzmann weight, gives these decaying correlations—non-trivial



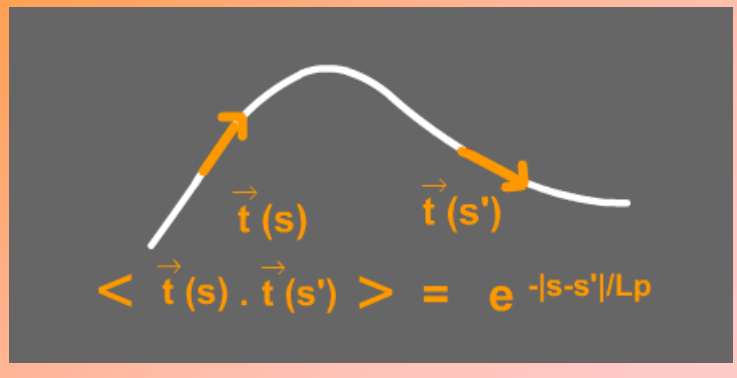


If dt/ds large, curves a lot.

If C big, persistence length is big.

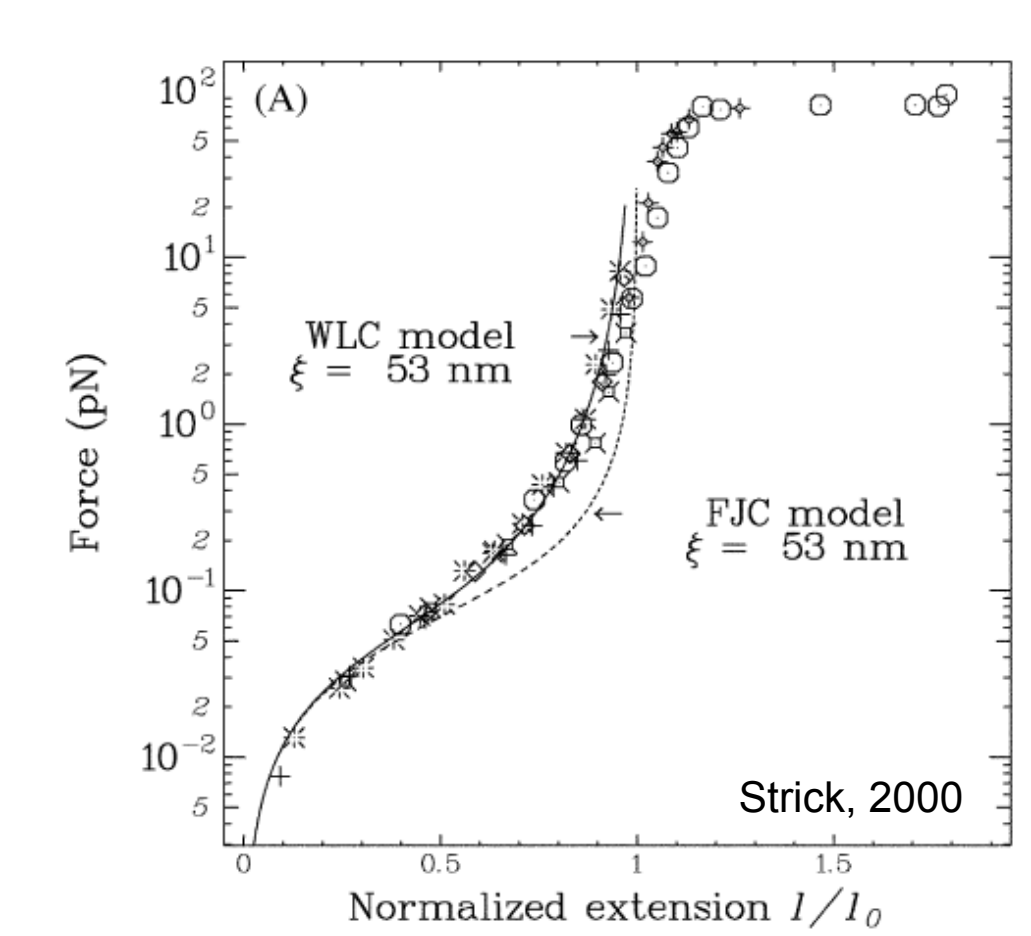
$C = Lp \cdot T \cdot k_B$.

(Large C means large penalty for bending – and this means large Lp.)



Lp=persistence length

FJC vs. WLC Model of DNA



Huge range of Force important in experiment

The FJC model is too crude and is not a good approximation of the elastic behavior of a DNA molecule at large extensions ($l > R_g$).

A much more precise description is afforded by the worm like chain (WLC) model.

DNA's persistence length: $\xi_T = 52 \pm 2$ nm in physiological conditions (10 mM phosphate buffer, pH . 7.5, 10 mM NaCl)

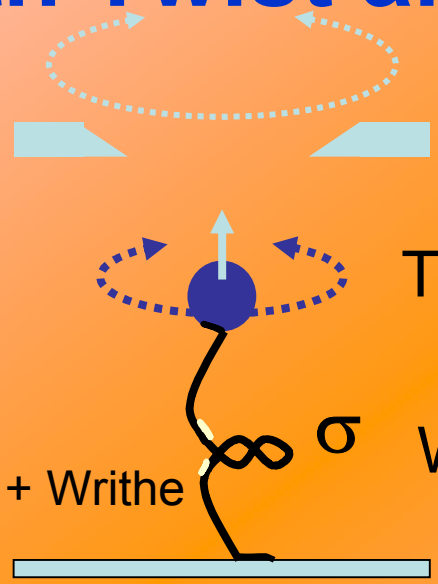
FJC saturates at $l=l_o$ (all segments aligned straight out.) You're fighting entropy → need huge Force.

FJC & WJC fits pretty well up to $0.5l_o$.

WLC is better, particularly at large extension.

Movie measureforce.

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.

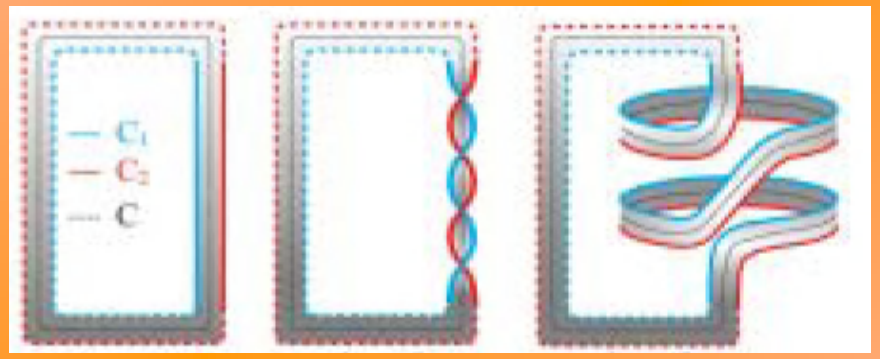
Pull on DNA and writhe comes out.

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

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.

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

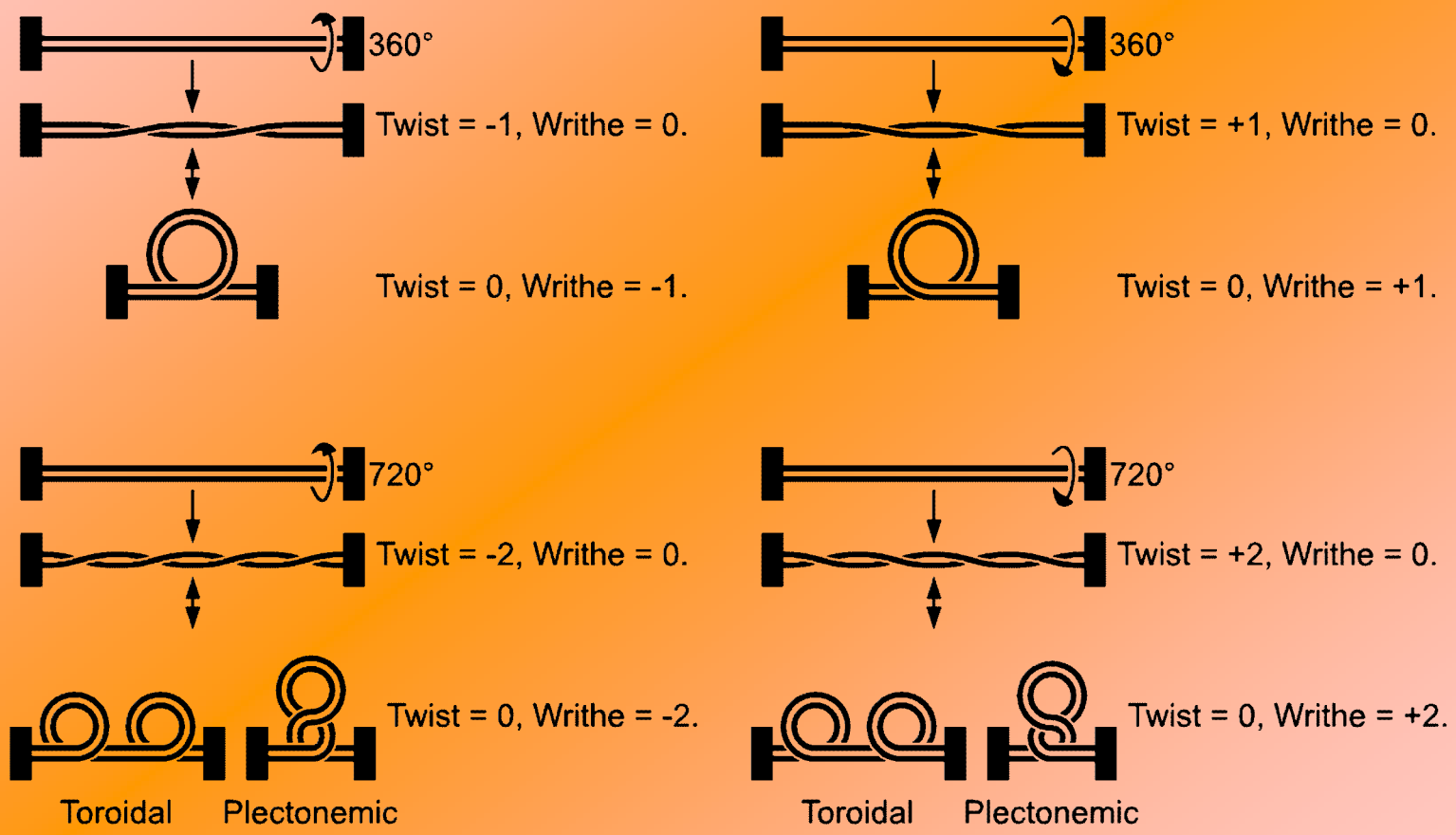
Ex: Hold DNA out straight so that it has no Writhe, add of take out twist, then let fold up (Twist goes into Writhe).

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

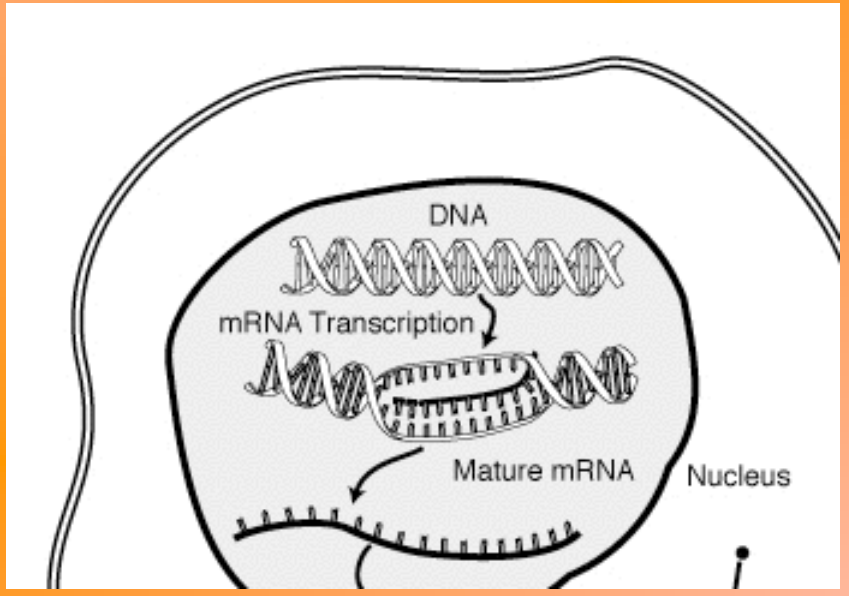
Some Examples of Tw and Wr

Linear DNA with Constrained Ends



Application to Eukaryotic Cells

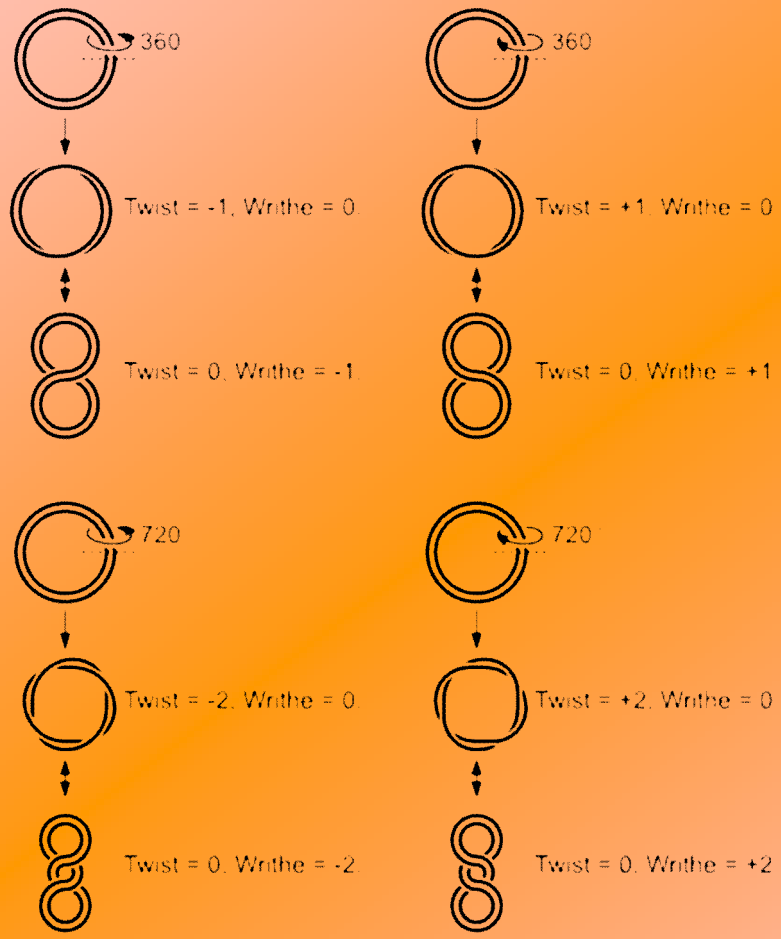
In Eukaryotic cells: When a chromosome wants to express a particular gene, it becomes single stranded. Requires a tremendous amount of energy (remember partition function and genes, typically 10-100k a.a., require many H-bonds to be broken: DNA is a very stable structure). Requires enzymes– topoisomerases.



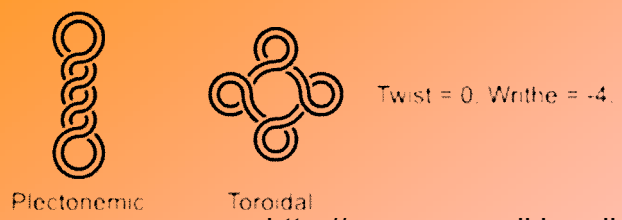
In response to supercoiling, they will assume an amount of writhe, just as if their ends were joined.

More Examples of Writhe and Twist

Circular DNA



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



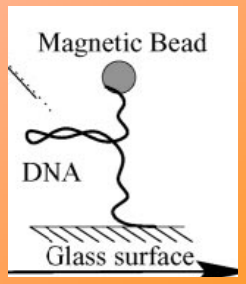
Plectonemes more common: shape bacterial plasmids take.

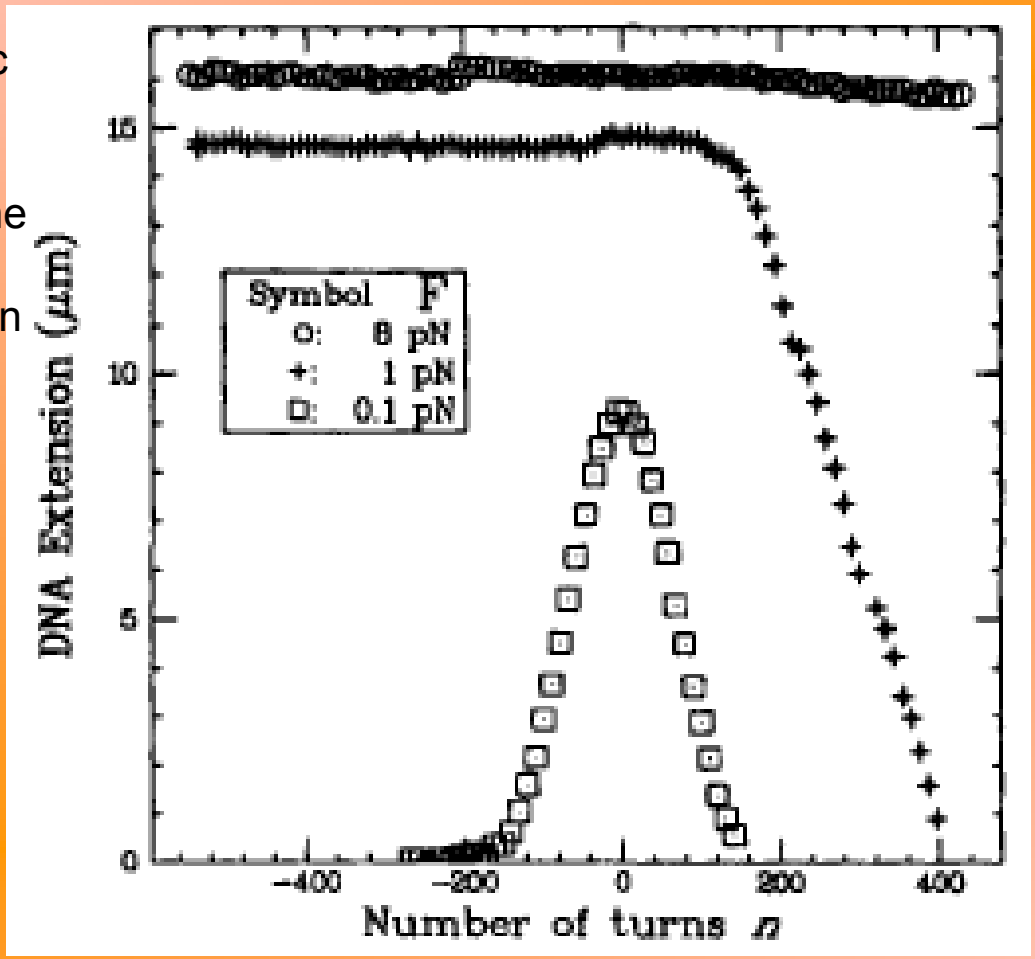
http://commons.wikimedia.org/wiki/Image:Circular_DNA_Supercoiling.png

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.

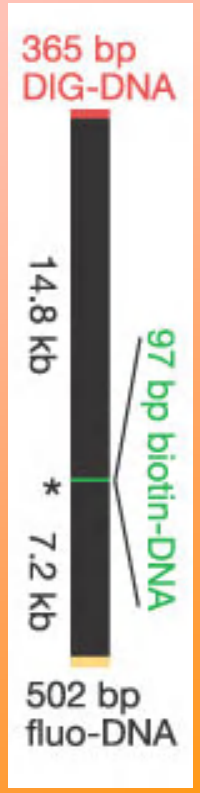
At forces larger than 3 pN no plectonemes are observed: the torsional stress is adsorbed not by writhe but in local structural changes of the molecule.

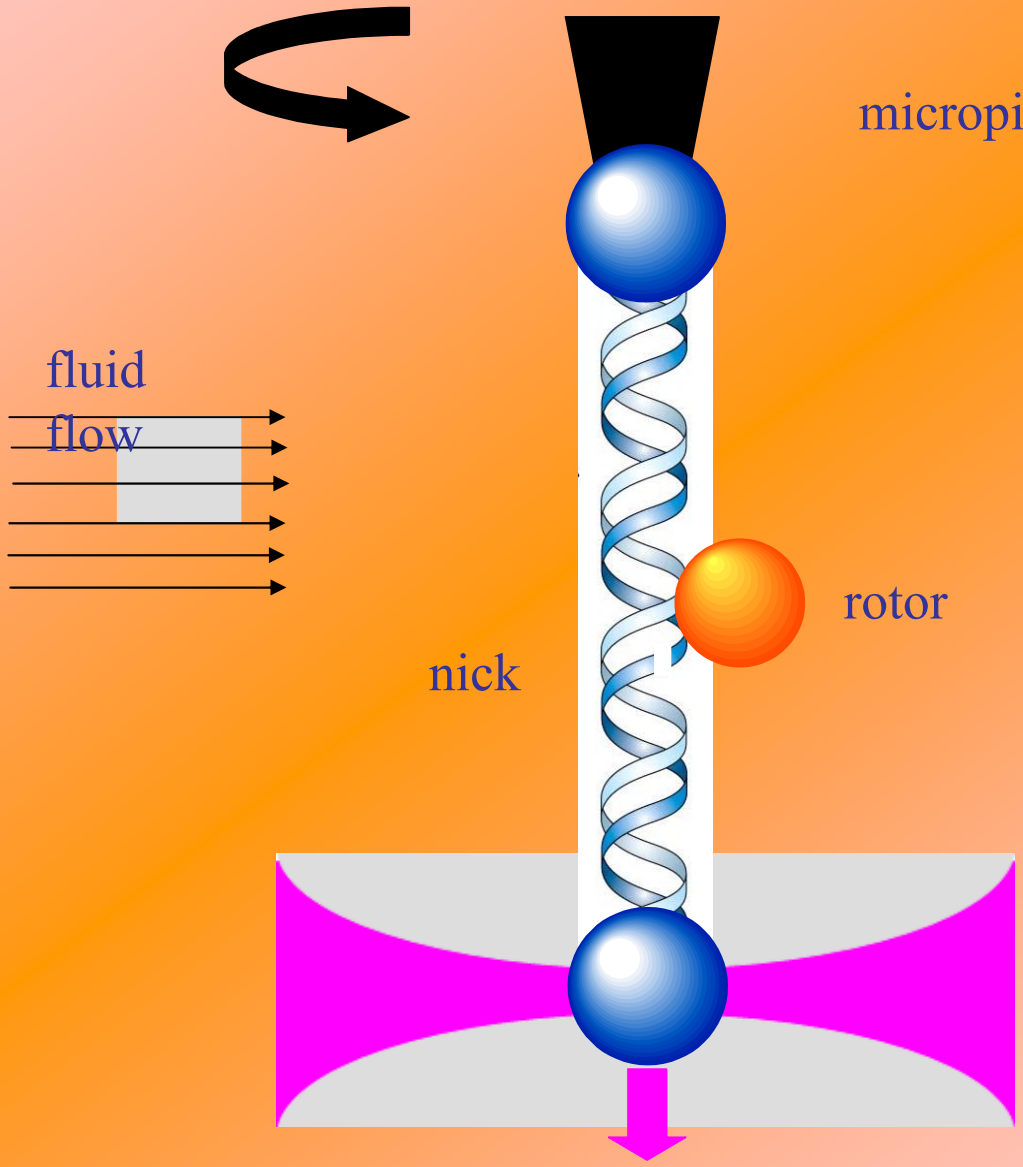
Extension vs. supercoiling at constant force

Three regimes

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

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

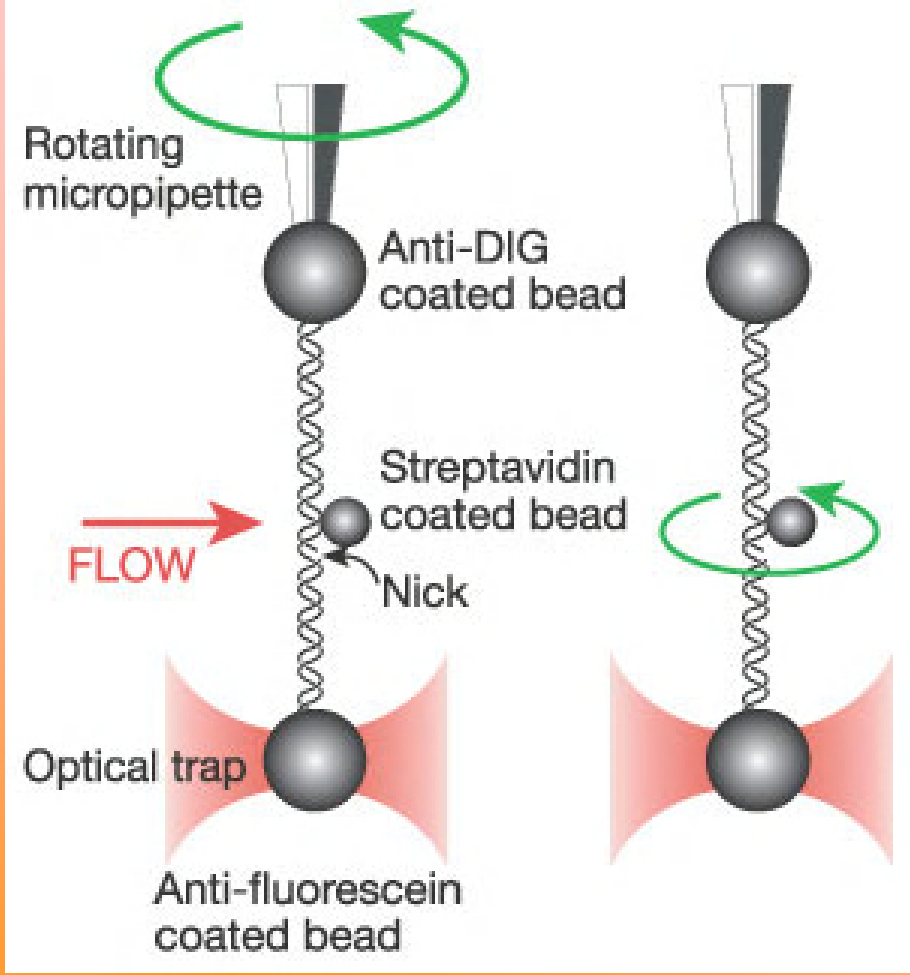


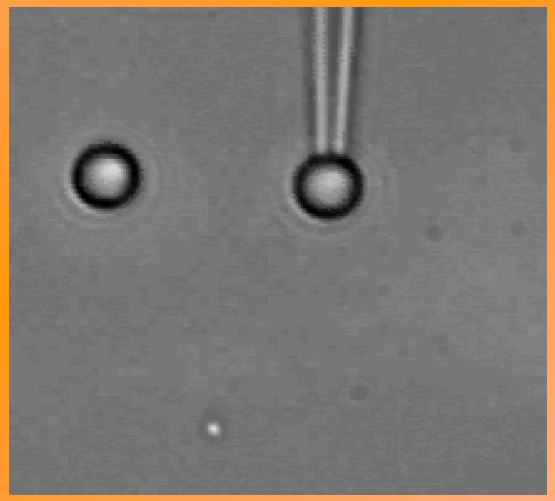


Beads: 2.8 µm
Rotor 520-920 nm

Bryant, 2004, Nature

Experimental Method



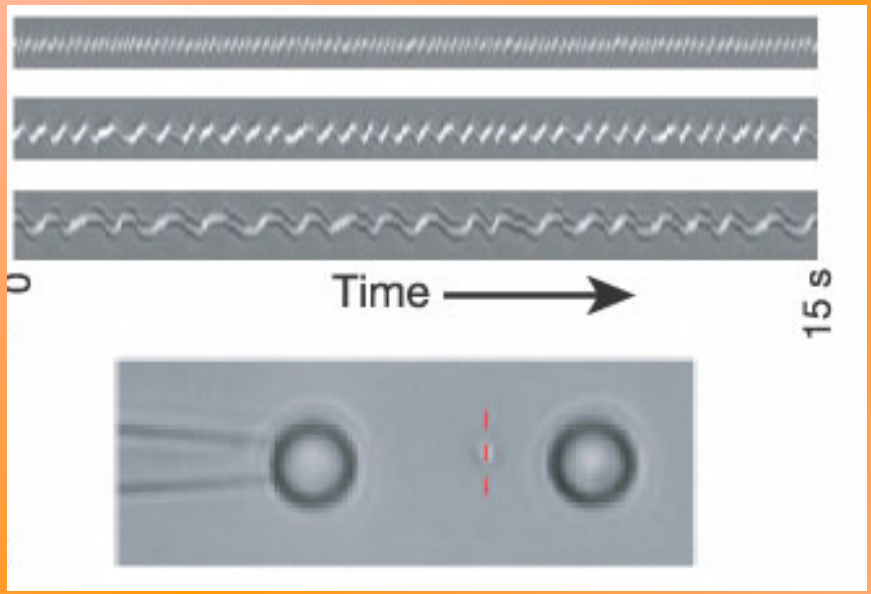


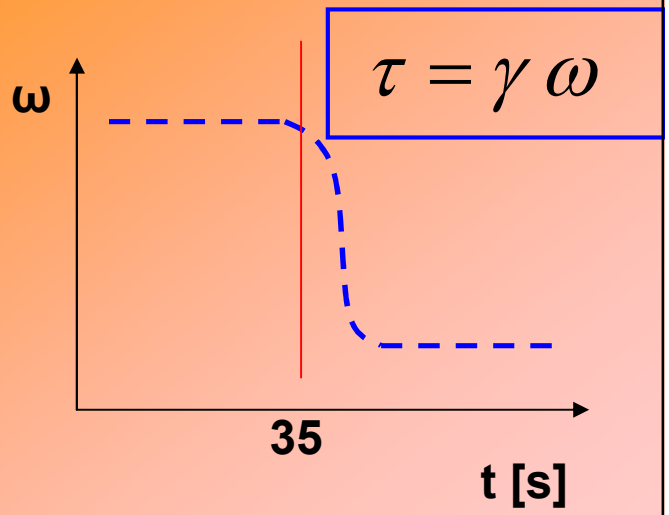
$$\tau = \gamma \, \omega$$

DNA is a wind up motor

An over- or under-wound DNA molecule behaves as a constant torque wind up motor.

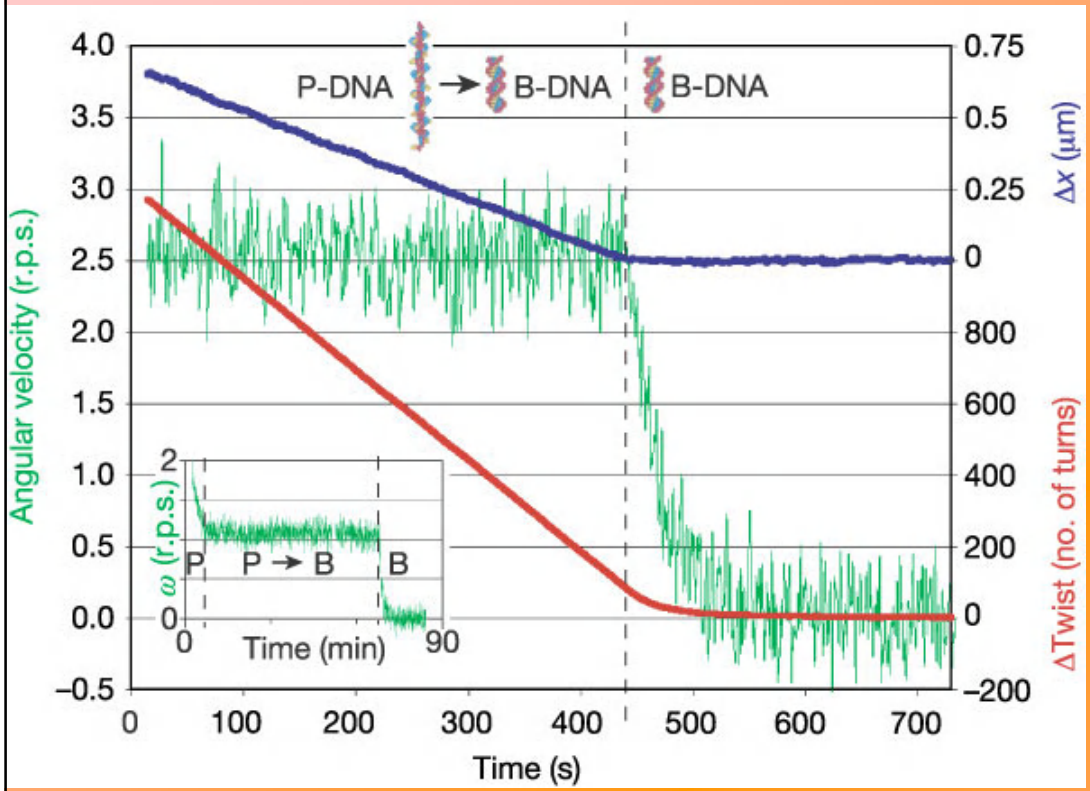
Constant force feedback to avoid buckling (twist and not writhe).

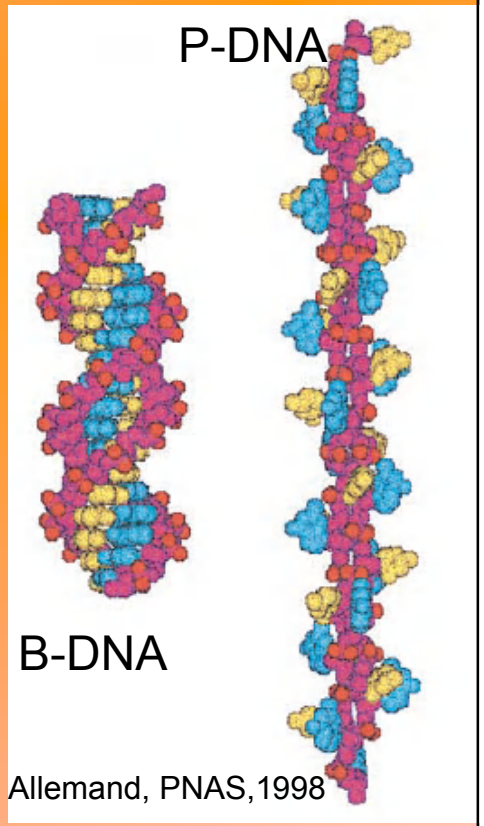




$$\tau = \gamma\,\omega$$

Structural Changes B-DNA↔P-DNA





- 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.