Lecture 6 Elasticity

Entropic property of polymers is only half the story. We need to consider elastic energy. Commonly discussed in engineering / physics as properties of "beams". (not X-ray).
Mostly structural: cytoskeleton, cell walls (plants)
also functional: flagella, cilia of inner ear.

1. Beams.
3 dimensions → 3 modes of deformation
"stretch, bend, twist"
slide for simplicity, assume uniform profile.
i) stretch by Hooke's Law. [ignore Poisson ratio]

$$E \frac{\Delta L}{L} = \frac{\text{Force}}{\text{Area}}$$

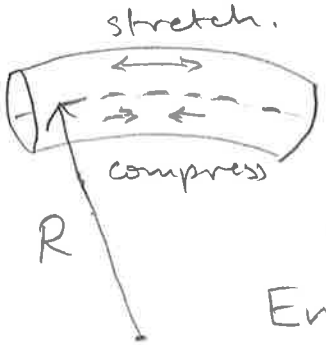
E = Young's modulus
= 200 GPa steel.
= 1-3 GPa polymers

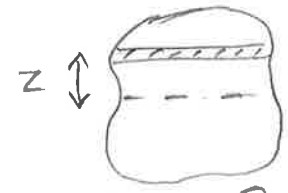
[1GPa = 10^9 N/m^2 = 10^4 Bars
large because $\Delta L \ll L$.]

"Resistance" to stretch ∝ area of beam.

ii) twist
can be important as a complication to bending, but often ignored..
"Supercoiling" of DNA is important factor, still largely unexplored in biology, which can affect: bending, coiling, packing. replication, gene expression, repressors etc
Topoisomerase: enzyme relieves or drives supercoiling. Type I: single strand cut
movies (II needs ATP). II: double strand / passage

1/2 iii) Bend.

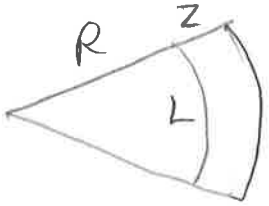




Ignoring Poisson effect + twisting.

Energy $dW = \frac{1}{2} E \left(\frac{\Delta L}{L}\right) dA \cdot \Delta L$.

Work = Force x Distance, integrate 0 to ΔL. → $\frac{1}{2}$



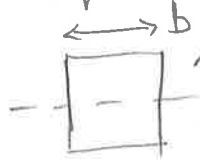
$$\frac{L+\Delta L}{L} = \frac{R+z}{R} \Rightarrow \frac{\Delta L}{L} = \frac{z}{R}.$$

Total bending energy:

$$E_{bend} = \frac{L}{2} \int_A E \frac{z^2}{R^2} dA = \frac{L E I}{2R^2}$$

where $I = \int_{Area} z^2 dA$ is the moment of inertia of cross section

eg square / rectangle



$$I = b \frac{(a/2)^3}{3} = \frac{b a^3}{24} \quad \text{unit} = L^4$$

resistance to bend $\propto$ (area)^2

[difference btw actin (8nm dia) & μtubules (25nm)].

3x bigger diam → $3^4 = 81$ x stiffer

Now we need to encode the quantities E and I into useful, measurable parameters.

2. Persistence Length.

$\xi_p = 50$ nm for DNA. Definition from before:

$$g(s) = \langle \vec{t}(s) \cdot \vec{t}(0) \rangle = e^{-s/\xi_p}$$

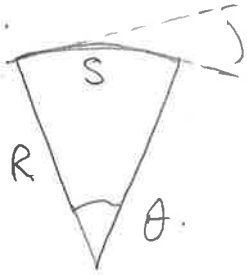
[exponential form comes from $g(s_1+s_2) = g(s_1)\,g(s_2)$]

$g(s)$ is a correlation function.

If $\theta(s)$ is angle btw $\vec{t}(s)$ and $\vec{t}(0)$

$$g(s) = \langle \cos\theta(s) \rangle \simeq 1 - \left\langle \frac{\theta(s)^2}{2} \right\rangle \quad \text{thermal avge.}$$

Distribution of θ given by E_{bend}.



$\theta(s) = \frac{s}{R}$ $\quad R = s/\theta(s)$.

$$E_{bend} = \frac{sEI}{2}\left(\frac{\theta}{s}\right)^2 = \frac{EI}{2s}\theta^2.$$

Average over configurations:

$$Z = \int_0^{2\pi} d\phi \int_0^{\pi} d\theta \sin\theta\, e^{\underbrace{-EI\theta^2/2sk_BT}_{-x}}$$

$$\langle \theta^2 \rangle = \frac{1}{Z}\int_0^{2\pi} d\phi \int_0^{\pi} d\theta \sin\theta \cdot \theta^2 \cdot e^{} = \frac{1}{Z}\,\frac{-2sk_BT}{I}\,\frac{\partial Z}{\partial E}$$

usual trick of rewriting integral.

In energy range of interest, θ is small, $\int \to \int_0^{\infty} d\theta$

$$Z = 2\pi \int_0^{\infty} \underbrace{dx}_{EI\,2\theta\,d\theta\,/\,2sk_BT \text{ from above.}} e^{-x} \cdot \frac{2sk_BT}{EI} \cdot \frac{1}{2} = \frac{2\pi sk_BT}{EI}$$

$$\langle \theta^2 \rangle = \frac{EI}{2\pi sk_BT} \cdot \frac{-2sk_BT}{I} \cdot \frac{2\pi sk_BT}{I} \cdot \frac{-1}{E^2} = \frac{2sk_BT}{EI}$$

$$g(s) = e^{-s/\xi_p} \simeq 1 - \frac{s}{\xi_p} \simeq 1 - \frac{\langle\theta^2\rangle}{2} \simeq 1 - \frac{sk_BT}{EI}$$

Hence $\xi_p = \frac{EI}{k_BT}$ or $EI = \xi_p k_BT$ desired conds.

Total Bending Energy, $E_{bend} = \frac{LEI}{2R^2} = \frac{L\xi_p k_BT}{2R^2}$.

3. Loop energy.

We want to complete our discussion of the lac operon which forms a loop. $L = 2\pi R$.

$$E_{loop} = \frac{2\pi R \xi_p k_B T}{2R^2} = \frac{\pi \xi_p}{R} k_B T$$

quite high.

Nucleosome $R = 4.5\,nm$, $\xi_p = 50\,nm$ $E_{loop} \simeq 35\,k_BT$

Lac operon $500\,bp \times 0.34\,nm = 2\pi R$

$R = 27\,nm$, $E_{loop} = 6\,k_BT$ more reasonable

4. J-factor / Cyclization

Notation used by Jon Widom to combine energy & entropy of "cyclization".

Experiments consist of making DNA of a range of lengths and varying the concentration

indep of [DNA].

probability / rate increases with [DNA].

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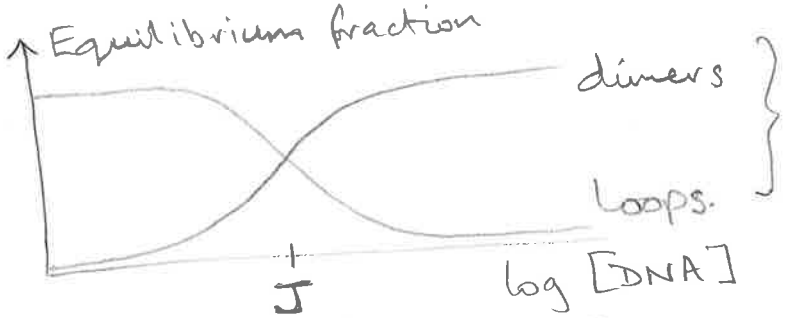

seen on gel.

Crossover concentration, J, varies with length.

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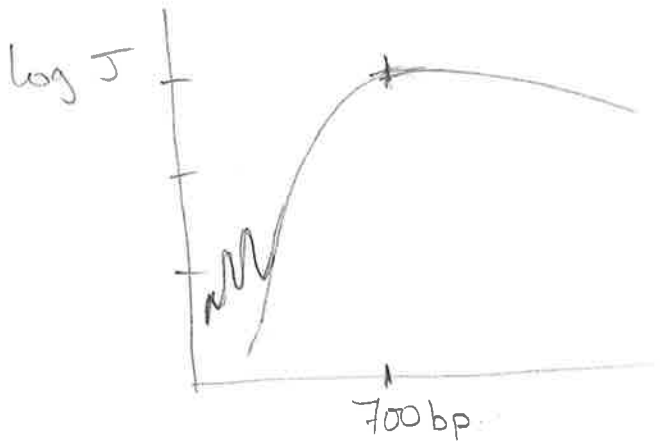


Small sizes depends periodically on N_{bp}:

$\Delta N_{bp} = 10$ = turns of helix

DNA fragment length

5. Free energy of loop:

$p_0 = \left(\frac{2}{\pi N}\right)^{3/2}$ in 3D from lecture 4

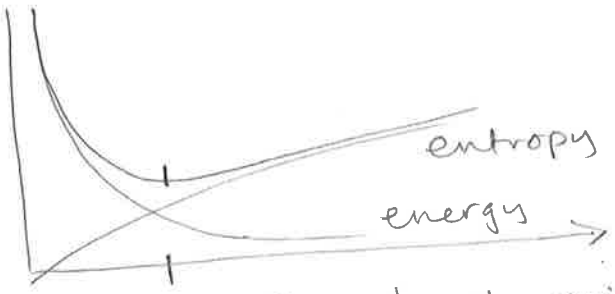
$\Delta S_{loop} = k_B \ln p_0 = -\frac{3}{2} k_B \ln N + \text{const.}$

$\Delta E_{loop} = \frac{\pi \xi_p}{0.34\, N_{bp}/2\pi} k_B T \triangleq \frac{3000}{N_{bp}} k_B T.$

$\Delta G_{loop} = \Delta E_{loop} - T\Delta S_{loop}$

$= \left(\frac{3000}{N_{bp}} + \frac{3}{2} \ln N_{bp} + \text{const}\right) k_B T.$

$\frac{\partial \Delta G}{\partial N} = -\frac{3000}{N^2} + \frac{3}{2}\frac{1}{N} = 0$



$N_{bp} = 2000$ bp at minimum.

If $J \propto e^{-\Delta G_{loop}/k_B T}$ for concentration

$\ln J = -\Delta G_{loop}/k_B T + \text{const.}$

$= -\frac{3000}{N_{bp}} - \frac{3}{2} \ln N_{bb} + \text{const.}$

log-log plot of J vs N_{bp}:

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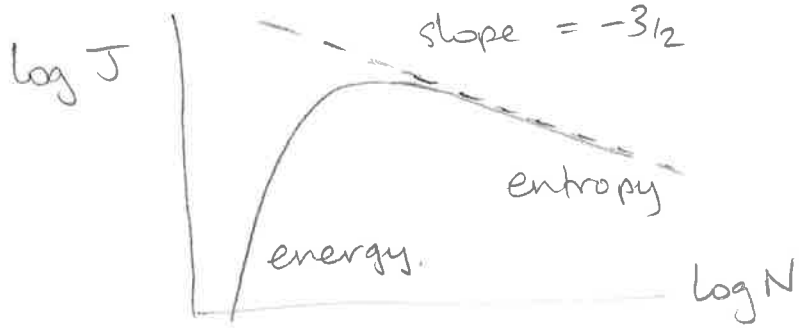

6. DNA packing.

We study two examples : viruses + nucleosomes
Problem is self-organisation
Lowest level : space consideration

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i) λ-phage – looks like T4
5×10^4 bp ds DNA inside a capsid of $R = 27\,nm$

$$\text{Filling fraction } \nu = \frac{5 \times 10^4 \times 1\,nm^3}{\frac{4}{3}\pi (27\,nm)^3} = 0.6.$$

ii) E. Coli
DNA condensed in a "nucleoid" $R = 0.25\,\mu m$
5×10^6 bp:

$$\nu = \frac{5 \times 10^6\,nm^3}{\frac{4}{3}\pi (250\,nm)^3} = 0.1$$

Virus needs active packing mechanism, while E. Coli does not.

iii) Sperm
$\sim 5\,\mu m$ diam 3.5×10^9 bp human genome

$$\nu = \frac{3.5 \times 10^9\,nm^3}{\frac{4}{3}\pi (2500\,nm)^3} = 0.02$$

iv) Human chromosomes in metaphase
Our own data show chr 1 has $1.7 \times 10^9\,nm^3$

slides

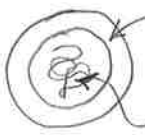
$$\nu = \frac{2.5 \times 10^8\,nm^3}{1.7 \times 10^9\,nm^3} = 0.15$$

This high fraction is achieved by highly organised packing into nucleosomes

All 4 examples are critical packing ratios at extreme states of the life cycle
All impressively large numbers.

7. Caspar - Klug paradox (1962 paper CSH)

23.02.15 Minimum size of life :



} RNA has to code for coat.

Quasi-equivalent packing of proteins
slide. based on icosahedron = geodesic dome.
532 point symmetry · 12 × 5-fold axes.
60 identical repeating units (T=1 particle)
[T=3, T=7 quasi-equivalent superstructures]
Minimum protein strong enough ?

i) NTL9 ms-folder (2HBB) 2×α 3×β
2.4 × 1.7 nm 51 amino acids.
Area = $60 \times 4\,nm^2 = 245\,nm^2 = 4\pi R^2$ $R = 4.4$ nm.
Volume = $360\,nm^3$ = 360 bp.] on surface of sphere
this codes for 120 aa, 2× more than needed.

ii) TIM protein. 8-strand β-barrel + α 248 aa (dimer)
3.5 × 3.5 nm area × 60 subunits
Volume of sphere coated by this area :
V = $1870\,nm^3$ codes for 620 a.a.

iii) Real virus : Tomato Bushy Stunt (TBSV)
T=3 180 copies of 387 a.a.
Inner radius = 11 nm (5nm thick coat).
Volume = $5600\,nm^3$
Actual genome = 4700 bases + } [ν = 0.8]
But single stranded RNA.
Complexed with R+K-rich tail of protein
Solves electrostatic problem of packing.

+ contains other genes, reverse transcriptase

movies Self-assembly : Art Olson
Assembly of RNA : Stephen Harrison

8. DNA packing

Move constrained than RNA because of bending energy + high pack fraction ν.

TEM images show layered loops around interior of capsid.

slides

Data for lambda & T7 show that the DNA spacing changes according to how much DNA is packed.

electrostatics

DNA spreads out — very simple model

$$\text{Volume} = L \times d_s^2 \frac{\sqrt{3}}{2} = \text{constant}.$$

vary L, expect $d_s = \sqrt{2V/\sqrt{3}L}$ $\propto L^{-1/2}$

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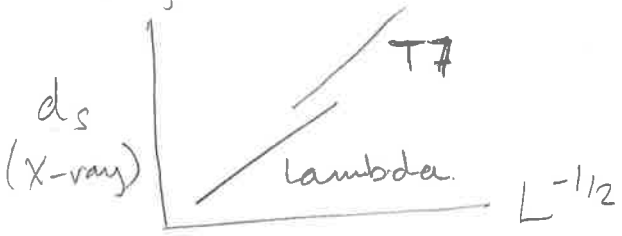
X-ray measurements of d_s confirm this.



9. Circular Hoops.

$$E_{loop} = \frac{\pi \xi_p k_B T}{R} \quad \text{elastic, due to } \xi_p$$

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If $N(R_i)$ loops are packed at radius R_i

$$G_{bend} = \pi \xi_p k_B T \sum_i \frac{N(R_i)}{R_i}$$

Close packing at spacing d_s → layers of $\frac{\sqrt{3}}{2} d_s$

$$G_{bend} = \left(\frac{2}{\sqrt{3} d_s}\right) \pi \xi_p k_B T \int_{R_1}^{R_2} \frac{N(R)}{R} dR$$

height

Simple cylinder packing $N(R) = Z/d_s$

$$G_{bend} = \frac{Z}{d_s}\left(\frac{2}{\sqrt{3} d_s}\right) \pi \xi_p k_B T \ln \frac{R_2}{R_1}$$

$R_2 = R_{out}$ = outside radius of cylinder.

R_1 determined by length of DNA = L.

$$L = \left(\frac{2}{\sqrt{3} d_s}\right) \int_{R_1}^{R_{out}} 2\pi R \cdot \frac{z}{d_s} dR = \frac{2\pi z}{\sqrt{3} d_s^2} \left(R_{out}^2 - R_1^2\right)$$

$$R_1 = R_{out} \sqrt{1 - \sqrt{3} d_s^2 L / 2\pi z R_{out}^2}$$

$$G_{bend} = - \frac{\pi \xi_p k_B T z}{\sqrt{3} d_s^2} \ln\left(1 - \frac{\sqrt{3} d_s^2 L}{2\pi z R_{out}^2}\right)$$

slide Experiments (laser tweezers) measure the force needed to pack the DNA in, or pull it out:

$$F(L) = - \frac{dG_{bend}}{dL} = - \frac{\xi_p k_B T / 2 R_{out}^2}{1 - \sqrt{3} d_s L / 2\pi z R_{out}^2}$$

fraction filled. L / L_0.

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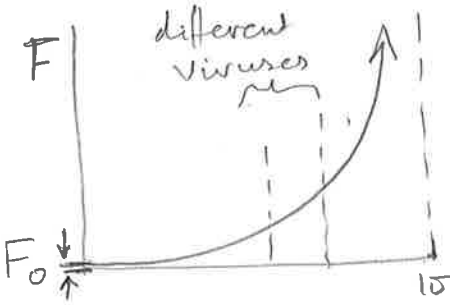


Agrees quite well with tweezer experments on $\phi 29$. DE Smith et al Nature (2001) p404.

Magnitude of force: $\xi_p = 50$ nm.

$R_{out} \simeq 40$ nm.

$k_B T = 4.1$ pN-nm

$$F_0 = \frac{\xi_p k_B T}{2 R_{out}^2} = 0.06 \text{ pN}$$

Discrepancy with expt is accounted for by G_{charge} estimated on p406.

10. Nucleosome

Eukaryotic solution to the DNA organisation problem. 2 turns of DNA around 8 small proteins forming a cylinder "octamer".

Lysines in H3 tail are common methylation sites: "epigenetic markers" used to control gene expression etc etc.

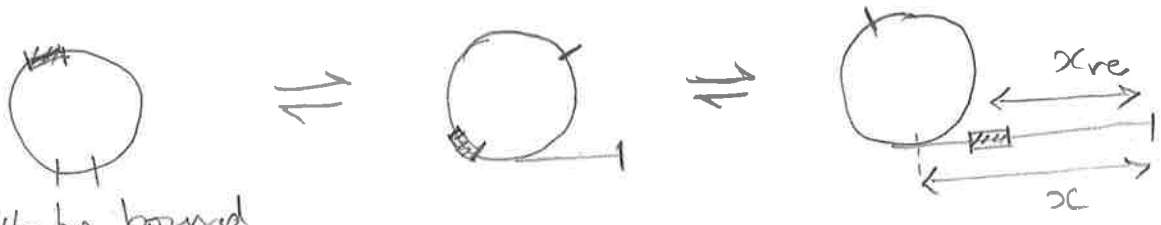
slide. DNA packed into "10-nm fibres" is 10× more compact than naked DNA.

But it costs energy: $R = 4.5\,nm$

$$E_{loop} = \frac{\pi \xi_p}{R} k_B T \sim \frac{\pi \times 50}{4.5} k_B T = 35\, k_B T \text{ per turn}$$

The 70 $k_B T$ has to be offset by attractive protein-DNA interactions, about 10 H-bonds. Experiments (below) show $E_{bind} \sim 48 k_B T$ / turn

11. Binding Experiment. (Widom 1995)



When $x > x_{re}$ the restriction enzyme site is exposed and the RE will cut DNA

Expt:

i) Make synthetic 146 bp DNAs with different x_{re}

ii) expose each to a range of RE concentrations

[RE] for 50% cutting is K_{eq}^{conf}

Analyse with Grand Partition Function

$$Z = \int_0^L e^{\beta \Delta\gamma (L-x)} \frac{dx}{a} + e^{\beta\mu} e^{\beta \varepsilon_{bind}} \int_{x_{re}}^{L} e^{\beta \Delta\gamma (L-x)} \frac{dx}{a}.$$

where $\beta = 1/k_B T$

ε_{bind} is the binding energy of RE

$\Delta\gamma = \gamma_{ad} - \gamma_{bend}$ ← $E_{Loop}/2\pi R$

↑ binding / site

L = length = 146 a.

a = spacing per base pair.

Second term in Z counts the states in which the RE is bound to its site:

$$P_{bound} = \frac{1}{Z} e^{\beta\mu} e^{\beta \varepsilon_{bind}} \left(e^{\beta \Delta\gamma (L - x_{re})} - 1 \right) \cdot \frac{1}{\beta \Delta\gamma a}$$

This can be converted into an expression for the measured K_{eq}^{conf} (p412)

Fit to data gives $\Delta\gamma = 0.47\ k_B T/nm$.

slide

$$\Delta\gamma = \gamma_{ad} - \gamma_{bend}$$

$$\gamma_{bend} = E_{Loop}/2\pi R = 35\ k_B T / 2\pi \times 4.5\ nm$$
$$= 1.24\ k_B T/nm$$

$$\Rightarrow \gamma_{ad} = 0.47 + 1.24 = 1.71\ k_B T/nm.$$
$$= 48\ k_B T / turn \text{ as above.}$$

12. Cytoskeleton beams

Universal role of two protein polymer structures which form rigid filaments.
Structures preserved over 3 byr of evolution.
Analogs present in archea + prokaryotes (but names vary).

i) microtubule.

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13 filaments of polymerised tubulin
Circular, tube structure, 25nm diam
Slight spiral. GTP bound.
Directional (+/- ends) as used as motor element to move cargo one way.
Bundles. eg 30x in mitotic spindle.

ii) actin filaments

2x filaments wrapped in 8nm diam fibre.
ADP bound to polymer; ATP to monomer
Widely used in motor systems also.

slide movies

eg propulsion of HL-60 cell line
actin "speckles" = retrograde flow "treadmilling"
Lamellipodium cells.

iii) intermediate filaments

between actin + tubules in size
coiled rope-like structure
resistant to stretching.
unlike actin + mt, no treadmilling.
myosin and lamin examples
α-helical parallel structure
no ATP in assembly.