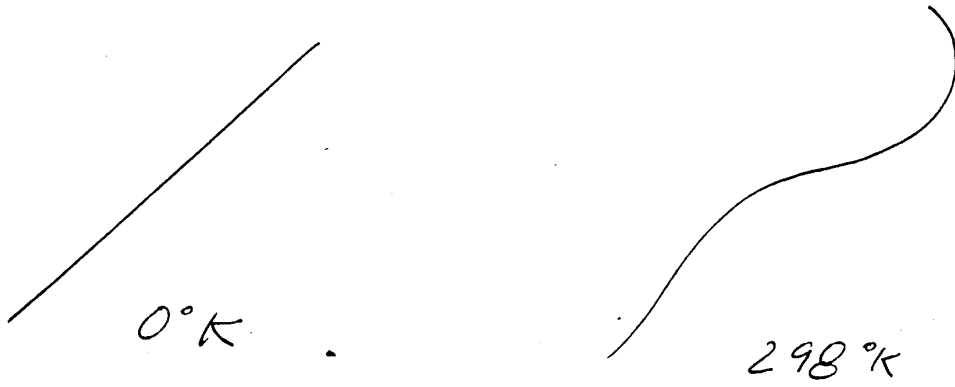


Chain Statistics II

The Worm-like chain, or Kratky-Porod

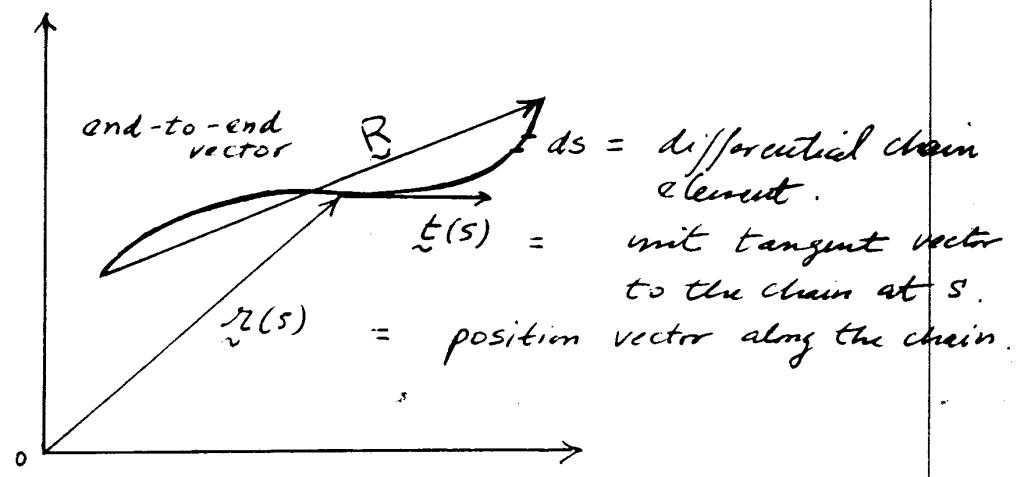
- Alternative description of chain flexibility, particularly useful for stiff chains.
- The molecule is treated as a continuum elastic rod.
- The molecule, if intrinsically straight, i.e. not bent, will adopt a perfectly aligned conformation at $T = 0^\circ\text{K}$. But at room temperature, the thermal energy will induce a deviation from the straight conformation, as the molecule will curve smoothly.



- How much the molecule curves and bends, i.e., how flexible the molecule is to bending, depends on the inner structure of the molecule. But if the local deviations in structure are not too large, then the molecule can be assumed to obey Hooke's law locally and behave as a persistent or worm-like chain.

A few mathematical definitions: A polymer of length l .

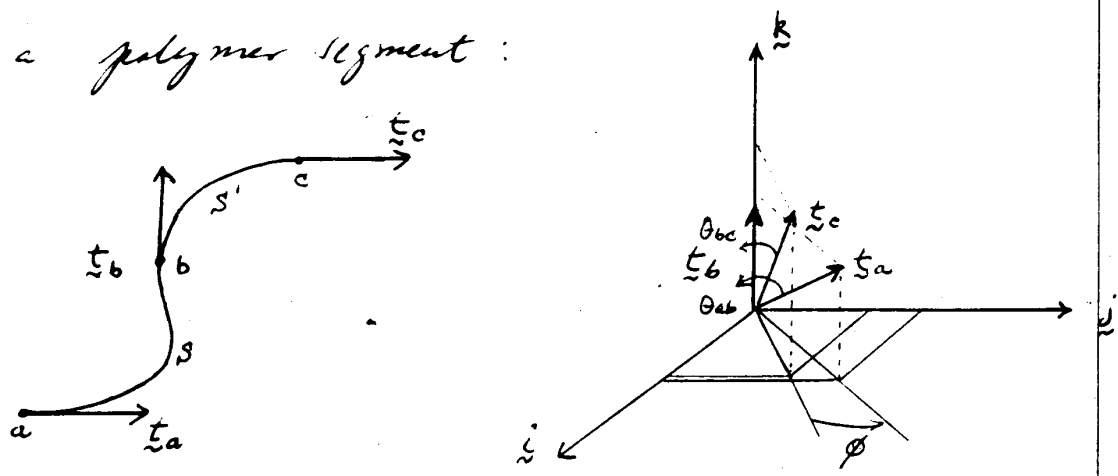
Arbitrary coordinate frame.



$$\underline{t}(s) \equiv \frac{\partial \underline{r}(s)}{\partial s} \quad \text{and} \quad \underline{R} = \int_0^l \underline{t}(s) ds$$

The orientational correlation function:-

Take a polymer segment:



We want to obtain $\langle \underline{t}_a \cdot \underline{t}_c \rangle = \langle \cos \theta(sts') \rangle$

Then from the diagram:

$$\underline{t}_c = \cos \theta_{bc} \hat{k} + \sin \theta_{bc} \hat{i-j}$$

$$\underline{t}_a = \cos \theta_{ab} \hat{k} + \sin \theta_{ab} \hat{i-ja}$$

Then:

$$\underline{t}_b \cdot \underline{t}_a = \cos \theta_{ab} \cdot \cos \theta_{bc} + \sin \theta_{ab} \cdot \sin \theta_{bc} \cdot \cos \phi$$

For an isotropic chain, $\langle \cos \phi \rangle = 0$, since for any given orientation of segment bc there is an equally probable configuration with the opposite orientation.

Moreover, since the chain is assumed ideal, then the bending of the ab segment is independent of the bending of the bc segment. Therefore:

$$\langle \underline{t}_a \cdot \underline{t}_c \rangle = \langle \cos \theta_{ab} \rangle \langle \cos \theta_{bc} \rangle = \langle \underline{t}_a \cdot \underline{t}_b \rangle \langle \underline{t}_b \cdot \underline{t}_c \rangle$$

because the magnitude of the orientational correlation function between any two segments of the polymer should only depend on the distance between the segments, we can write:

$$\langle \cos \theta(s+s') \rangle = \langle \cos \theta(s) \rangle \langle \cos \theta(s') \rangle$$

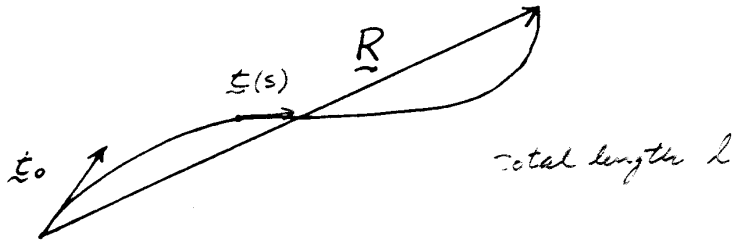
The function that has this 'multiplicative' property is:

$$\langle \cos \theta(s) \rangle = e^{-s/P}$$

where P is a constant for each polymer. It is the decay length through which the memory of the initial orientation of the polymer chain persists. It is called the PERSISTENCE LENGTH.

An alternative definition of P can be obtained by calculating:

$$\langle \underline{r}_0 \cdot \underline{R} \rangle$$



i.e. the ave. projection of the end-to-end vector on the initial segment of the polymer:

$$\begin{aligned} \langle \underline{r}_0 \cdot \underline{R} \rangle &= \langle \underline{r}_0 \cdot \int_0^l \underline{r}(s) ds \rangle = \langle \int_0^l \underline{r}_0 \cdot \underline{r}(s) ds \rangle \\ &= \int_0^l \langle \underline{r}_0 \cdot \underline{r}(s) \rangle ds = \int_0^l e^{-s/P} ds \end{aligned}$$

$$\langle \underline{r}_0 \cdot \underline{R} \rangle = P (1 - e^{-l/P})$$

Then:

$$\lim_{l \rightarrow \infty} \langle \underline{r}_0 \cdot \underline{R} \rangle = P$$

i.e.: The persistence length is the ave. projection of the end-to-end vector on the initial segment of the chain, in the limit of infinite length.

Another useful quantity is the mean square end-to-end distance of the chain:

$$\langle R^2 \rangle = \langle \underline{R} \cdot \underline{R} \rangle = \langle \int_0^l \underline{r}(s) ds \cdot \int_0^l \underline{r}(s') ds' \rangle$$

$$\begin{aligned}\langle R^2 \rangle &= \int_0^l ds \int_0^l \langle \underline{t}(s) \cdot \underline{t}(s') \rangle ds' \\ &= \int_0^l ds \int_0^l e^{-|s-s'|/P} ds'\end{aligned}$$

$$\langle R^2 \rangle = 2Pl \left[1 - \frac{P}{l} (1 - e^{-l/P}) \right]$$

In the limit of $l \gg P$:

$$\langle R^2 \rangle = 2Pl$$

Remember that in the freely jointed chain,

$$\langle R^2 \rangle = Nb^2 = \underbrace{N}_l b \cdot b = l \cdot b$$

where N is the # of Kuhn segments of length b in the chain. Therefore:

$$\underline{b = 2P}$$

the Kuhn segment is equal to twice the persistence length of the chain.

In the small deformation limit in which the worm-like chain is valid, the molecule behaves locally as a hookean spring. Then:

$$\langle \cos \theta \rangle = e^{-s/P} \Rightarrow \text{expanding both sides for small angles and distances.}$$

(6)

$$\left\langle 1 - \frac{\theta^2}{2!} + \dots \right\rangle = 1 - \frac{S}{P} + \dots$$

i.e.:

$$\boxed{\langle \theta^2(s) \rangle = \frac{2S}{P}}$$

The next term in the expansion is:

$$\left\langle \frac{\theta^4(s)}{4!} \right\rangle = \frac{1}{2!} \left(\frac{S}{P} \right)^2$$

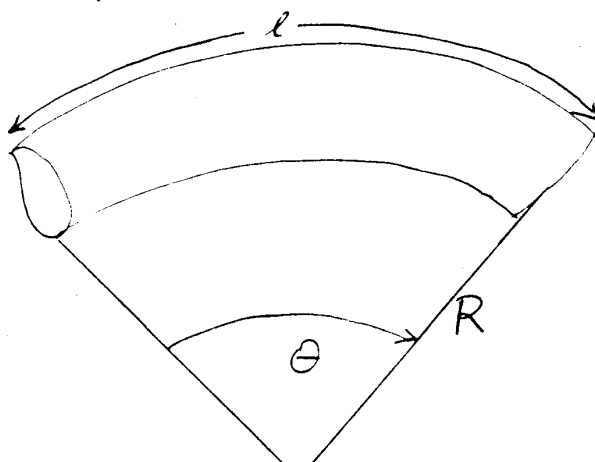
$$\langle \theta^4 \rangle = 12 \frac{S^2}{P^2}$$

$$\langle \theta^4(s) \rangle = 3 \langle \theta^2(s) \rangle^2$$

$$\boxed{\frac{\langle \theta^4(s) \rangle}{\langle \theta^2(s) \rangle^2} = 3}$$

The elastic energy of the chain:

Bending in the plane (2D):



Take a segment
of length l :

$$R\theta = l$$

In the small deformation limit, we can write the energy of the molecule per unit length as:

$$E = \frac{1}{2} A R^2$$

↑
constant of bending rigidity.

where $R^2 =$ is the local curvature of the chain, and:

$$R^2 \equiv \frac{\partial^2 R(s)}{\partial s^2} \equiv \frac{1}{R^2}$$

Therefore:

$$E_{\text{tot}} = \frac{1}{2} \frac{A \theta^2}{l}$$

Now, the prob. of finding a segment of the polymer making an angle between θ and $\theta + d\theta$ at room temperature is then:

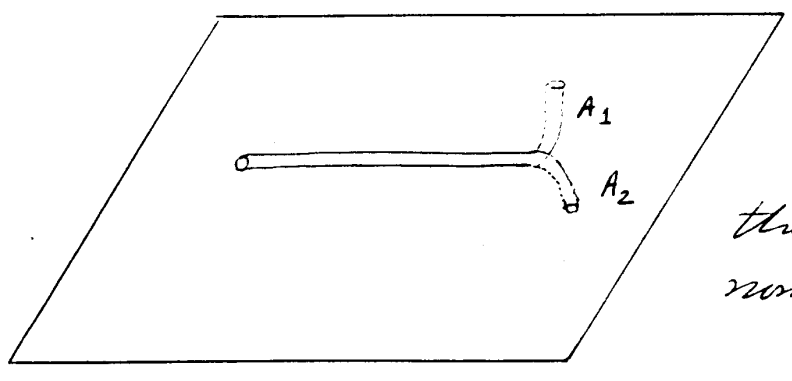
$$P(\theta) d\theta \propto e^{-\frac{A \theta^2}{2l k_B T}} d\theta$$

which can be normalized to obtain:

$$P(\theta) d\theta = \frac{1}{\sqrt{2\pi \langle \theta^2 \rangle}} e^{-\frac{\theta^2}{2 \langle \theta^2 \rangle}} \quad (\text{in 2D})$$

where $\langle \theta^2 \rangle = \frac{k_B T l}{A}$ is the variance of the distribution.

In 3D :



If $A_1 \neq A_2$
the chain is
non-isotropic

Then:

$$E_{tot} = \frac{1}{2} \frac{A_1 \theta_1^2}{l} + \frac{1}{2} \frac{A_2 \theta_2^2}{l}$$

As before:

$$\langle \theta_1^2 \rangle = \frac{k_B T l}{A_1} \quad \text{and} \quad \langle \theta_2^2 \rangle = \frac{k_B T l}{A_2}$$

For sufficiently small polar deflections, i.e., as $l \rightarrow 0$

$$\langle \theta^2 \rangle \approx \langle \theta_1^2 \rangle + \langle \theta_2^2 \rangle = \frac{k_B T}{l} \left(\frac{1}{A_1} + \frac{1}{A_2} \right) l^2$$

For isotropic chains: $A_1 = A_2 = A$

$$\langle \theta^2 \rangle = \frac{2 k_B T l}{A} \quad \text{in 3D}$$

Since: $P = \frac{2l}{\langle \theta^2 \rangle}$: then:

Formula for bending in 3D

$$\left\{ \begin{aligned} P &= \frac{A}{k_B T} ; \quad E_{tot} = \frac{P k_B T \theta^2}{2l} \\ P(\theta) d\theta &= \sqrt{\frac{P}{2\pi l}} e^{-\frac{P\theta^2}{2l}} \end{aligned} \right.$$

I.e.: This last result allows us to connect the persistence length, a statistical quantity describing the random nature of the elasticity of the chain, with the corresponding constant of bending rigidity, strictly speaking a macroscopic quantity that is a property of materials.

Notice that $P = \frac{A}{k_B T}$ implies that P is independent of the choice of l . P is thus an intensive property of the molecule.

Finally, we have seen that:

$$\langle \theta^2 \rangle_{2D} = \frac{k_B T l}{A}$$

$$\langle \theta^2 \rangle_{3D} = \frac{2 k_B T l}{A}$$

$$\therefore \langle \theta^2 \rangle_{3D} = 2 \langle \theta^2 \rangle_{2D}$$

Now, we saw that:

$$\langle \theta^2 \rangle_{2D} = \frac{2l}{P_{2D}} \quad \text{and} \quad \langle \theta^2 \rangle_{3D} = \frac{2l}{P_{3D}}$$

Then:

$$\underline{P_{2D} = 2 P_{3D}}$$

i.e. The persistence length of the chains in 2D double relative to those in 3D

Experimental determination of persistence length.

→ Most work done on DNA

Methods:

1) Light scattering $\rightarrow \langle R_G^2 \rangle \rightarrow \langle h^2 \rangle \rightarrow \langle b \rangle \rightarrow P = \frac{\langle b \rangle}{2}$
 (Eisemberg, Acc. Chem. Res. 1987)

2) Sedimentation Velocity (Kovacic + van Holde, Biochemistry 14: 1490 (1976))

Requires use of a pre-averaged hydrodynamic int. tensor. Zimm (Macromolecules, 13, 592, 1980) has shown that this can lead to serious errors on P .

3) Dynamic light scattering: investigate bending modes of the molecule.

All these approaches predict that DNA of a few hundred base pairs are rod-like. This suggests that they may not be sensitive enough to small bending fluctuations.

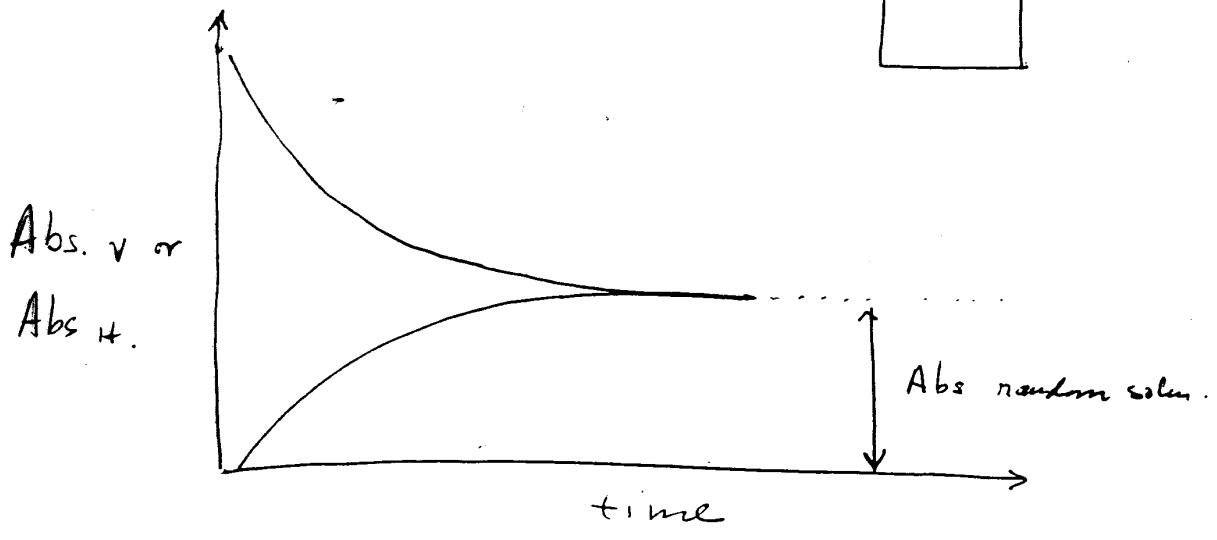
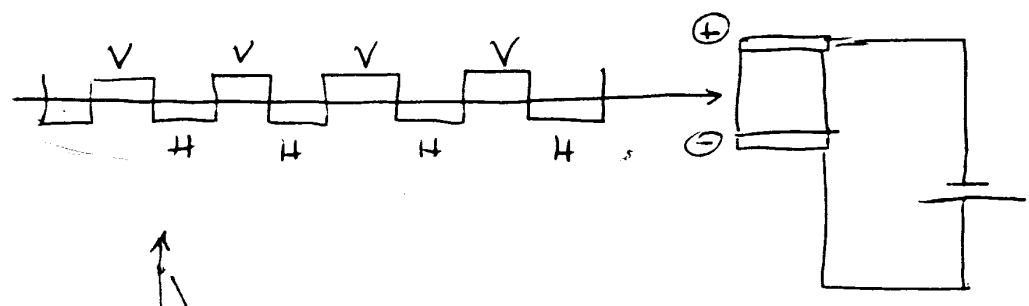
Better Experimental approaches:-

1) TEB: Transient electric birefringence
TED: " " dichroism

Orient DNA molecules with a strong Λ E-field (short pulses)

Observe the decay of the optical anisotropy with time: \rightarrow follow the randomization of the molecular orientation.

T E D:



From these studies: $D_{rot} \rightarrow \begin{matrix} P \\ b \end{matrix}$

Generally values of $P \rightarrow 500 - 640 \text{ \AA}$ but lean more towards 500 \AA in $\sim 10 \text{ mM Na}^+$

2) Electron Microscopic Approach.

Basis: Curvature of DNA molecules in EM \rightarrow can be related to intrinsic flexibility of the chain.

Two assumptions:

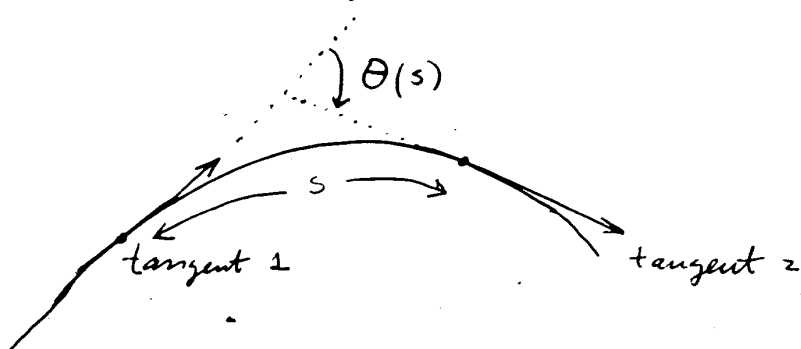
- (a) Two-D contour on the grid is obtained by re-equilibration of the contour as the DNA is laid down on the grid, not by projection of the three dim. contour on the grid.
- (b) That the int. of DNA w/ cytochrome for example does not influence the intrinsic flexibility

Bettini* + Fratani** obtained:

* (Biopolym. 19, 1689, 1980) ** (Biopolym. 12, 1353, 1979)

$$\langle \cos(\theta(s)) \rangle = e^{-\frac{s}{2P}}$$

$s < P$



the average is taken over a large # of points

Results $\rightarrow P \approx 540 \pm 50 \text{ \AA}$ in 100 mM Na⁺
but it appears to be dependent on the method of deposition.

Several advantages: - Single molec. analysis
- Pull out aggregation

- Rule out size heterogeneity
- Detect differences in P for other sequences?

Recently this approach has been re-examined by Claudio Rivetti using SFM (Scanning Force Microscopy). Rivetti et al. JMB (1996).

showed that for DNA molecules of various lengths:

$$\langle R^2 \rangle = 4Pl \left[1 - \frac{2P}{l} \left(1 - e^{-\frac{l}{2P}} \right) \right]$$

where $P_{20} = 2P_{30}$, for a variety of conditions of deposition.

Obtained a $P_{20} = 106 \text{ nm}$ i.e. $P_{30} = 53 \text{ nm}$.

Ligase-catalyzed Cyclization of DNA -

Shore + Baldwin

Rate of catalyzed cyclization of DNA is determined by the inherent flexibility of the molecule.

Define the ring closure probability, j , related to the polymer flexibility.

- Does not involve hydrodynamics
- Use low DNA conc. \rightarrow avoid aggregation

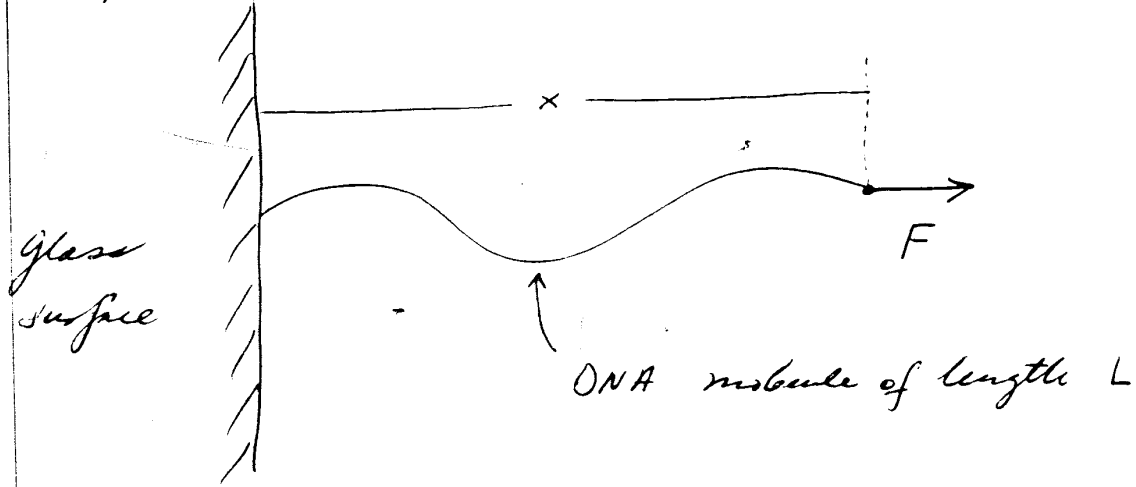
$$P \rightarrow 475 \text{ \AA} - 507 \text{ \AA}$$

Somewhat limited to ionic strengths compatible with enzyme activity.

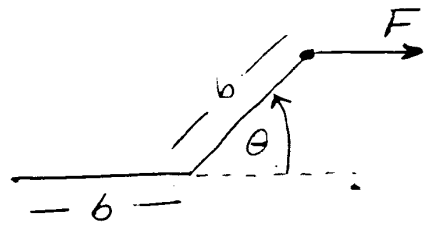
Elasticity Studies -

Single molecule methods can be used to extract the elasticity of biopolymers:

Exps:



A) Using the Freely Jointed Chain Model -



the constant tension along the molecule tends to align the Kuhn segments against entropy, that tends

to misalign them. The energy of a segment of length b in a force field F is:

$$U = Fb \cos \theta$$

then, the ave. alignment of the dipoles is:

$$\langle \cos \theta \rangle = \langle \frac{x}{L} \rangle = \frac{\int_{-1}^1 \cos \theta e^{-\frac{Fb \cos \theta}{k_B T}} d(\cos \theta)}{\int_{-1}^1 e^{-\frac{Fb \cos \theta}{k_B T}} d(\cos \theta)}$$

$$\left\langle \frac{x}{L} \right\rangle = \coth a - \frac{1}{a} = L(a)$$

where $a \equiv \frac{Fb}{k_B T}$ and $L(a)$ is the Langevin function.

Limiting behavior: 1) when $a \rightarrow 0$ (at low forces):

$$\coth(a) = \frac{e^a + e^{-a}}{e^a - e^{-a}} \approx$$

$$\approx \frac{1}{a} + \frac{a}{3} - \dots \quad \text{for } a \ll 1$$

\therefore

$$\left\langle \frac{x}{L} \right\rangle = \frac{a}{3} = \frac{Fb}{3k_B T}$$

$$F = \underbrace{\frac{3k_B T}{Lb}}_{\uparrow \text{ spring constant}} \langle x \rangle \quad \text{Hookes' law}$$

2) When $a \gg 1$, i.e. at high forces:

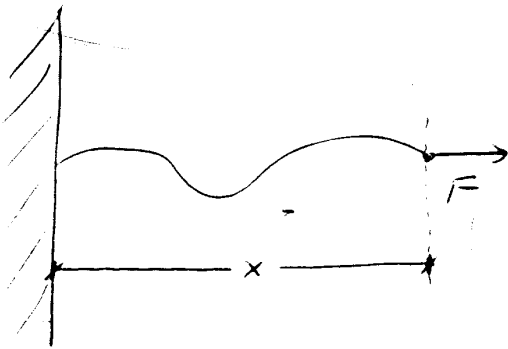
$$\coth a \rightarrow 1$$

$$\left\langle \frac{x}{L} \right\rangle = 1 - \frac{1}{a}$$

$$\frac{Fb}{k_B T} = \frac{1}{\left(1 - \frac{x}{L}\right)}$$

B) Using the Worm-like-chain model
 (Bustamante et al, Science, 1994; Marko + Siggia
 Macromolecules (1994)).

In the presence of force; the energy of the chain under tension is:



$$\frac{E}{k_B T} = \int_0^L \frac{A}{2} R^2 ds - Fx$$

1) At low forces, we expect Hooke's law behavior:

$$\frac{FA}{k_B T} = \frac{\langle x \rangle}{L}$$

2) At the other extreme, i.e. at large forces, $\langle x \rangle \rightarrow L$ and the tangents to the chain fluctuate only slightly about \hat{x} . In this limit:

$$\left\langle \frac{x}{L} \right\rangle = 1 - \frac{1}{\sqrt{\frac{4FA}{k_B T}}}$$

$$\frac{FA}{k_B T} = \frac{1}{4\left(1 - \frac{x}{L}\right)^2}$$

← Notice the inverse quadratic behavior of the force on extension of the chain in this limit

3) Can summarize the overall behavior in an interpolation formula:

$$\frac{FA}{k_B T} = \frac{x}{L} + \frac{1}{4\left(1 - \frac{x}{L}\right)^2} - \frac{1}{4}$$

so that $\langle x \rangle \rightarrow 0$ when $F \rightarrow 0$.

Experimental test:

Smith et al., Science 92

Smith et al. Science 96

