

Announcements

Homework 3 (Klaus Schulten's Lecture): Due Wednesday at noon.

Next homework assigned. Due Wednesday March 1.

No lecture next Monday, Feb. 27th!

(Homework is a bit longer.)

Marco will have office hrs this Friday from 7-8pm and via Skype (voice-only) Monday 9-10pm. www.skype.com: id: tjioe2

Today's Lecture:

AFM, WLC, Magnetic Traps

Main Points

- Different types of AFM: Stationary vs. Non-stationary
 - For non-stationary: Contact, non-contact.
- Titin—pull on it with AFM, get repetitive unfolding
- Worm Like Chain— model for extended biopolymer.
 - Protein Folding, DNA.
- Magnetic Trap
 - DNA has Linking Number = Twist and Writhe

Imaging vs. Pulling/Probing AFM

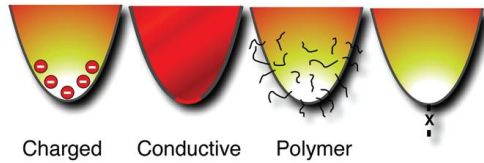
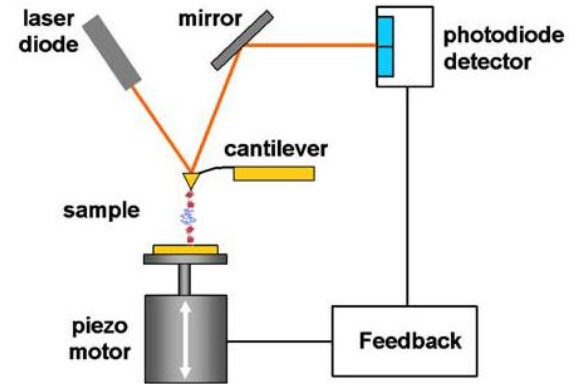
Contact between Sample & Tip: **scanning** (<1 Å) vs. **non-scanning**

Imaging
Sub-nm resolution!

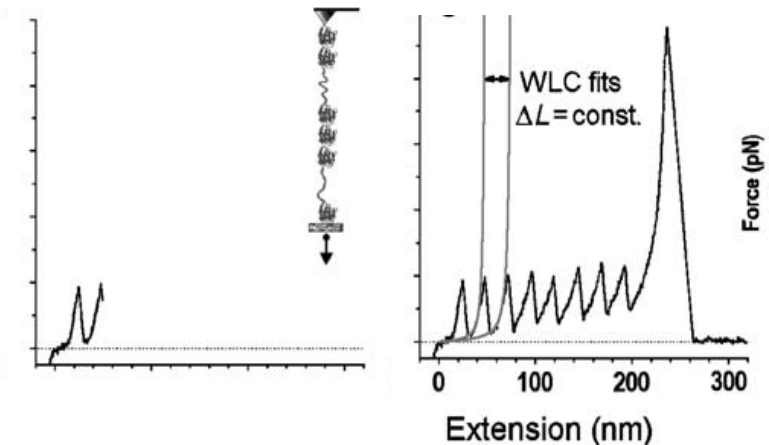
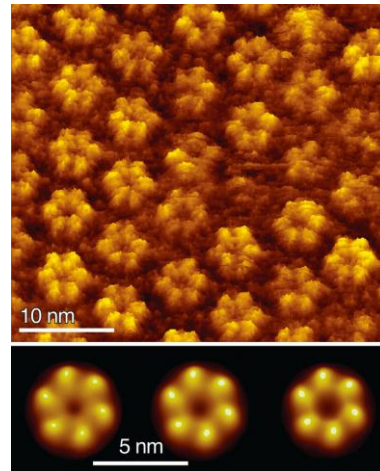
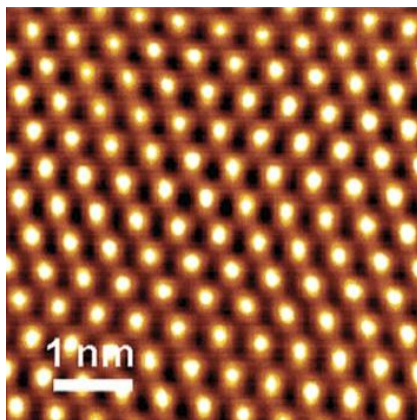
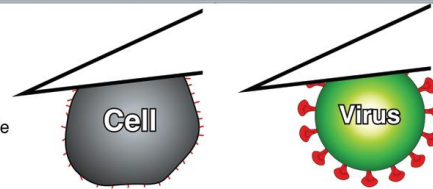
- High-resolution
- Oligomeric state
- Functional assembly
- Time-lapse imaging
- Conformational state
- Elasticity/flexibility
- Electrostatic potential
- Electric current
- Ion conductance
- Multimodal imaging
- Functional imaging
- Molecular recognition imaging
- ...

Probing
Unfolding single molecules

- Manipulation at molecular precision
- Molecular interactions
- Energy landscape
- Molecular toolbox
- Molecular probes
- Unfolding/folding
- Ligand binding
- Inhibitor binding
- Reaction pathways
- Epitope mapping
- Chemical groups
- Cells as probes
- Molecular adhesion
- ...

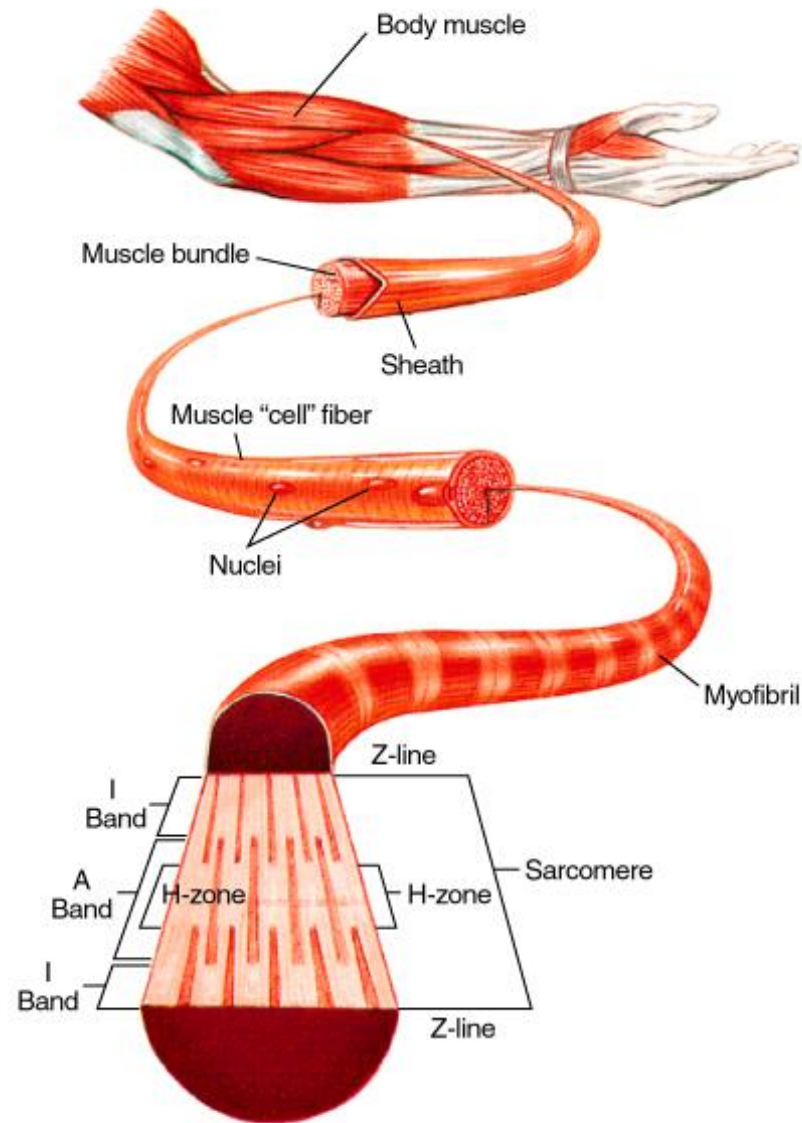


- x = Ligand
- Receptor
- DNA
- RNA
- Peptide
- Oligosaccharide
- Lipid
-

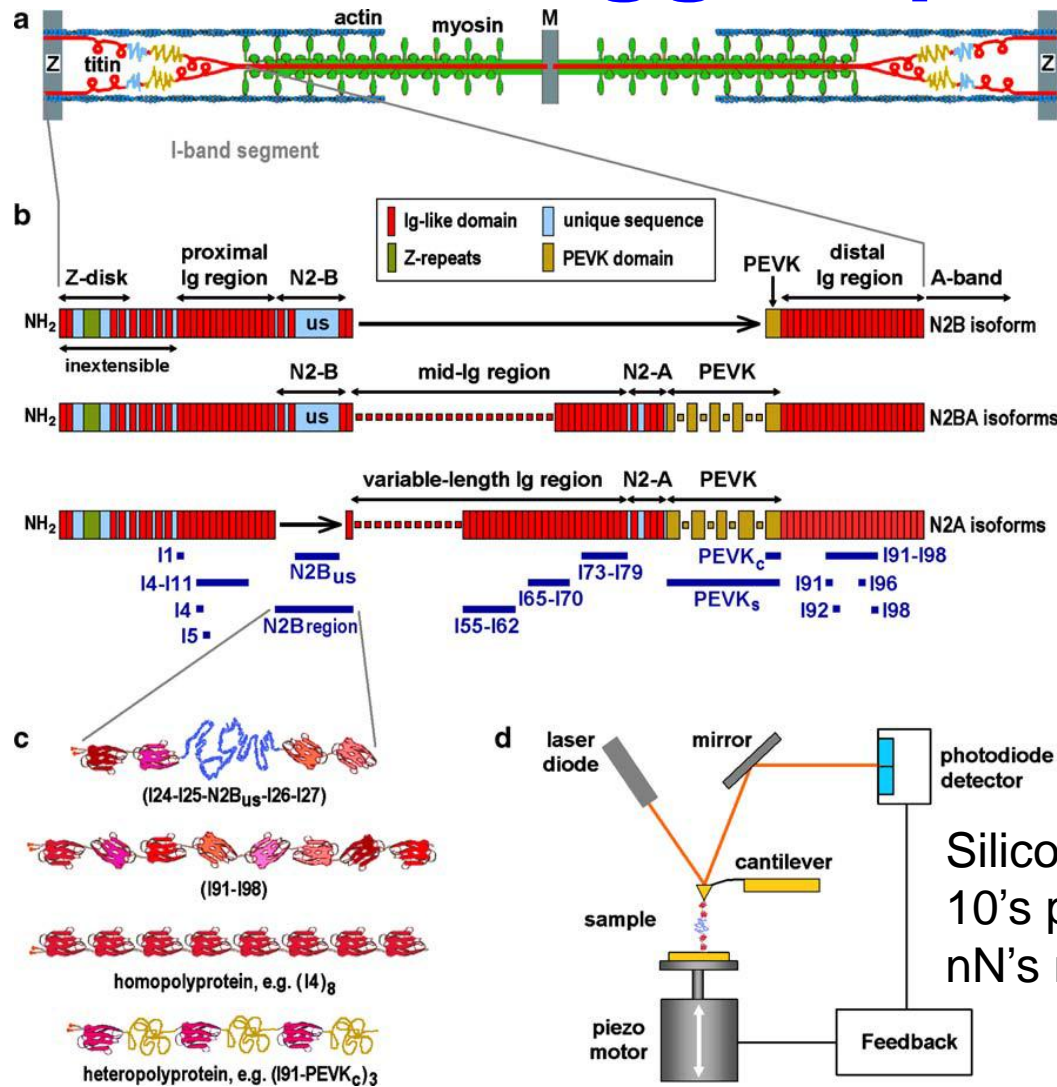


Extracellular surface of Cx26 gap junction hemichannels.
Muller, Biochemistry, 2008

Biological Example of Pulling AFM: Muscle & Titin



Titin: Human's Biggest protein



Each domain IgG

Silicon Nitride lever:
10's pN – several
nN's measurable

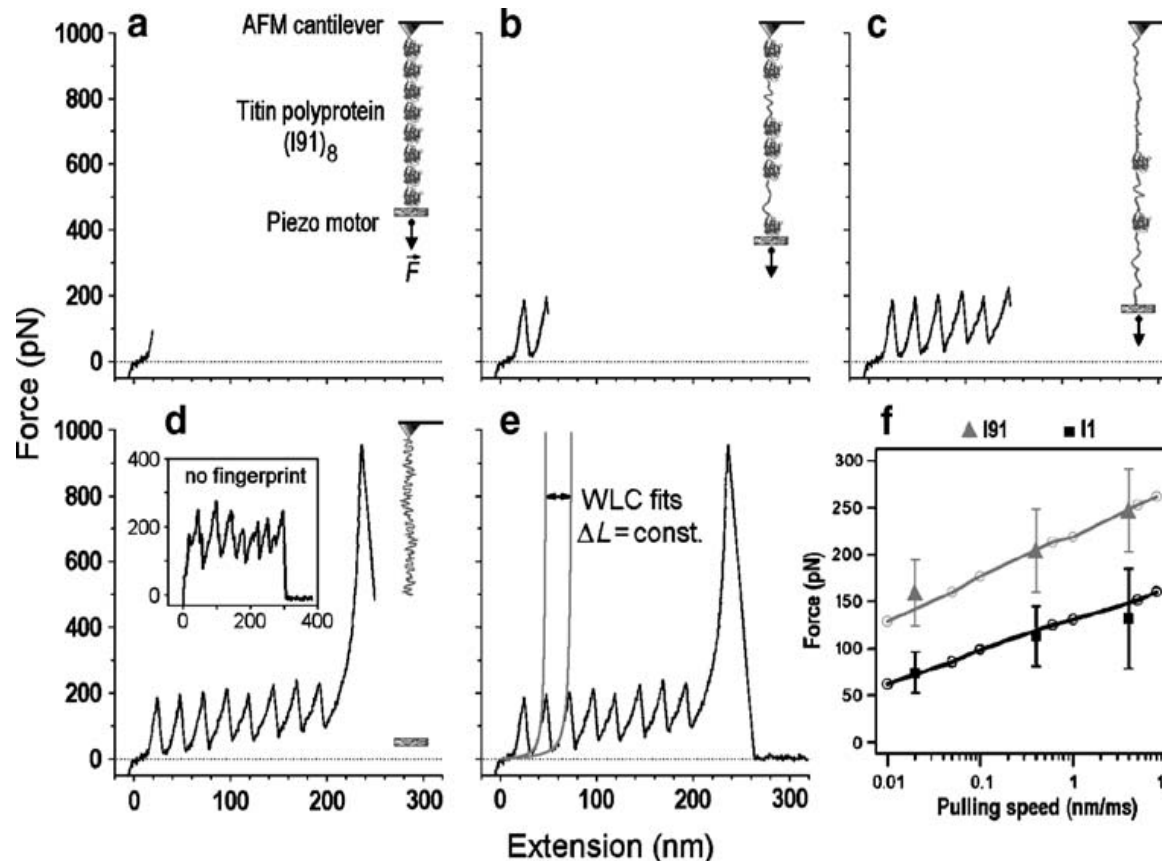
Titin: 4.2MDa; Gene (on # 2) = 38,138 aa: Goes from Z-disk to Center; stretchy = I Band

Cardiac (N2B & N2BA). Skeletal (N2A). Smooth all have different regions.

Picking up a single protein

“needle in a haystack”: usually pick up > 1 protein

“Fingerprint” of e.g. (I91)₈: by using identical repeats, unfolding forces are nearly identical with peaks equally spaced. (see Fig d)



Protein stretched at constant velocity

Titin: $\approx 1 \mu\text{m}/\text{sec}$
Physiological range

Worm-like Chain (WLC) is very good approximation to F vs. x of individual unit (protein, DNA) expansion.

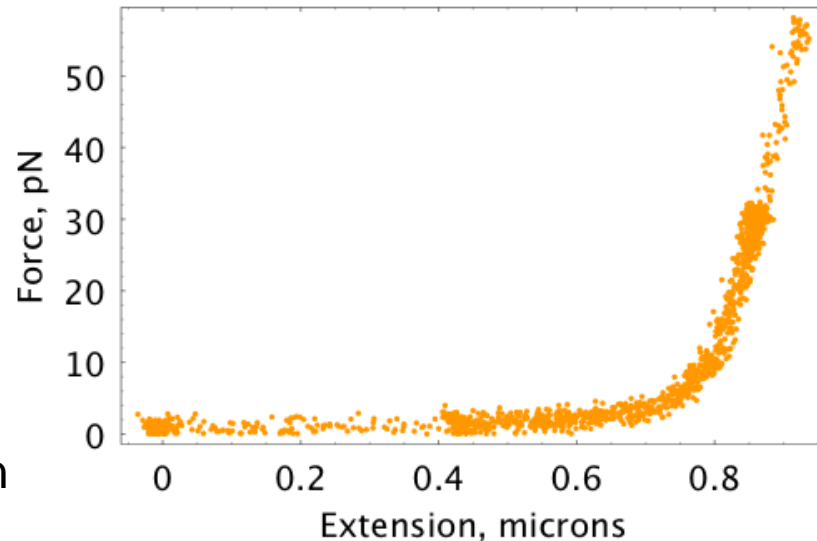
Analysis: The Worm-Like Chain for elasticity

$$F = -kx$$

Hooke's Law: You apply a force on something and it increases in length linearly. Proportional constant = k . Minus sign because it's a restoring force.

Force related to fractional increase (x/L). What if force isn't proportional to distance?

DNA is much longer than it is wide – λ DNA (virus DNA) is about $16.5 \mu\text{m}$ upon full extension, but the molecule's diameter is only about 2 nanometres, or some four orders of magnitude smaller. Can expect some simplification where don't need to get into details of molecular bonding.

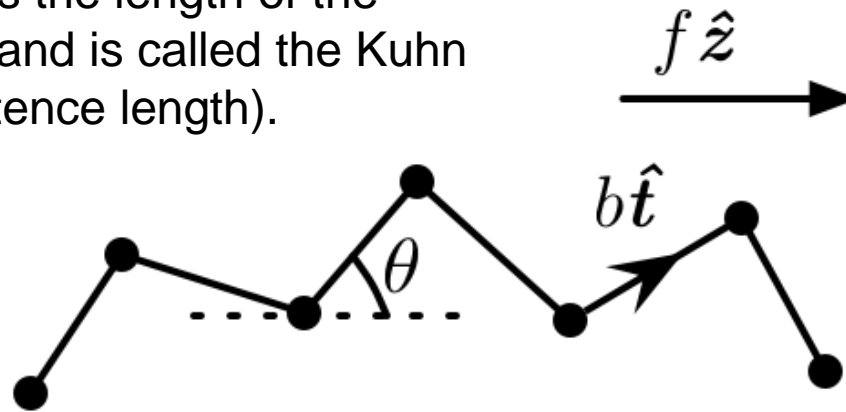


Towards the Worm-Like Chain for elasticity

try: **The Freely jointed Chain (FJC):**

The molecule as a chain of perfectly rigid subunits of length b joined by perfectly flexible hinges. Segment-to-segment angle = θ

You can think of b as the length of the repeating subunits, and is called the Kuhn length (= 2 x Persistence length).



If the molecule is under an applied force f as above, the effective energy for the chain is given by

$$\frac{E}{k_B T} = \sum_{i=1}^N \frac{fb}{k_B T} \hat{t}_i \cdot \hat{z}$$

If there were no applied force, all configurations have equal energy (and therefore the system has large configurational entropy), and the chain orients itself in any which way—analogous to a random walk.

Towards WLC: The FJC

When a force is applied, the molecule is elongated and the work being done is that to remove entropy in the system. An analytic force-extension relation for the freely jointed chain:

$$\left\langle \frac{z}{L_{tot}} \right\rangle = \coth \left(\frac{fb}{k_B T} \right) - \frac{k_B T}{fb}$$

Hyperbolic cotangent: $\coth(x) = \cosh(x)/\sinh(x) = (e^x + e^{-x})/(e^x - e^{-x})$

In the limit of low stretching (expand hyperbolic cotangent about $f = 0$)

$$\coth(x) = 1/x + (1/3)x - 1/45 x^3 + \dots$$

we get back Hooke's law (show!)

(Indeed, all polymer models reduce to a linear relation in the limit of low force, and DNA acts Hookean at very low forces).

In the high-force limit the model predicts z approaches the contour (total) length L_{tot} as $1/f$. Does not follow the force extension curve data for dsDNA or proteins at all.

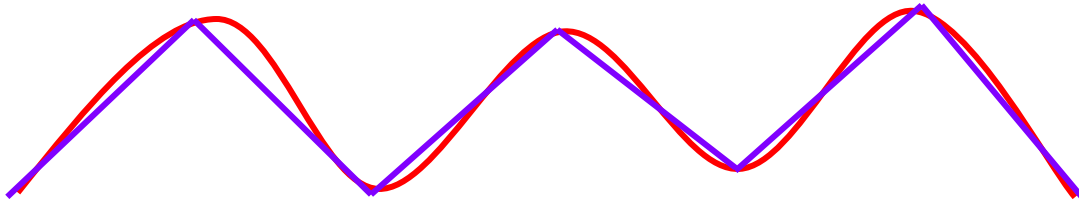
$$\coth(x) = 1 + e^{-2x}; \text{ as } x \rightarrow \text{infinity, } \coth \text{ goes to } 1; zL_{tot} = 1 - f$$

Two Models of DNA

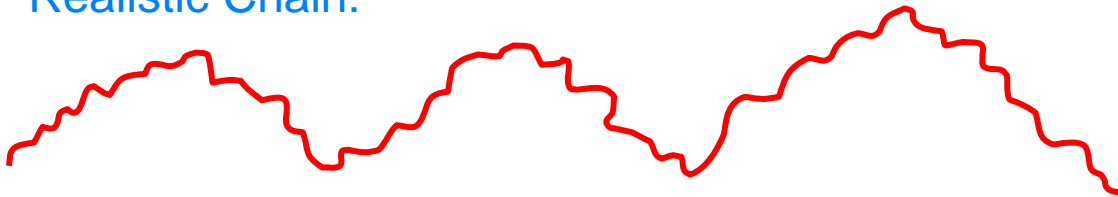
(simple) **Freely Jointed Chain (FJC)**

& (more complicated) **Worm-like Chain (WLC)**

Idealized FJC:



Realistic Chain:



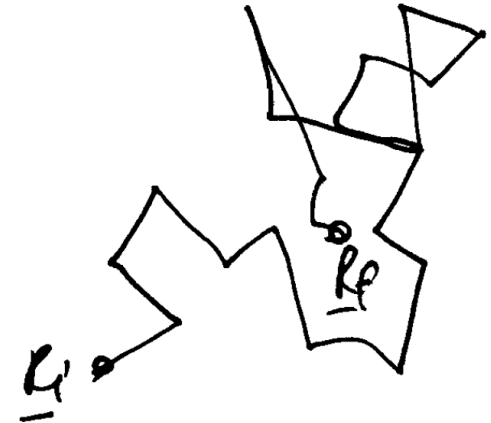
FJC: Completely straight, unstretchable. No thermal fluctuations away from straight line are allowed

The polymer can only disorder at the joints between segments

WLC: Have a correlation length

FJC: Head in one direction for length b , then turn in any direction for length b .

[$b = \text{Kuhn length} = \frac{1}{2} P$, where $P = \text{Persistence Length}$]

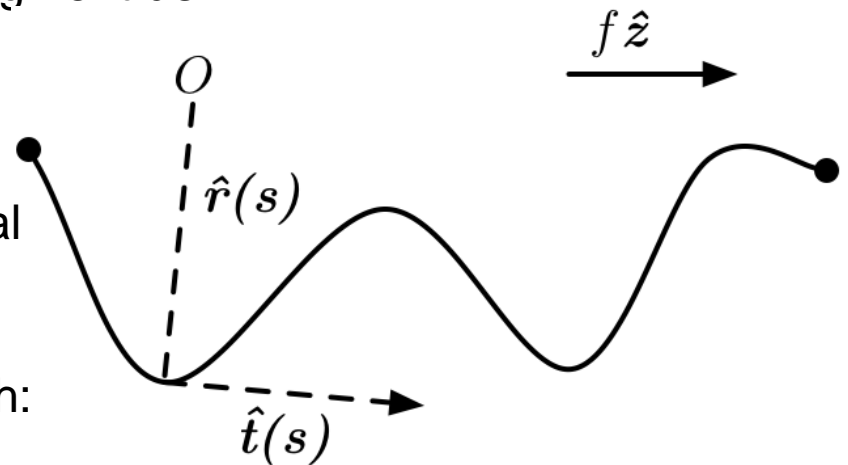


FJC: Can think of DNA as a random walk in 3-D.

WLC: A slight extension of FJC

A more realistic model for dsDNA is actually quite similar to the freely jointed chain, but changes from discrete segments to a continuous elastic medium. This can be done because DNA is actually a rather stiff molecule, with successive segments displaying a sort of cooperativity—all pointing in roughly the same direction. The figure below is a cartoon of the wormlike chain (WLC) model, where now we define $\mathbf{r}(\mathbf{s})$ as the position as a function of the relaxed-state contour length, s . Also shown is the tangent vector $\mathbf{t}(\mathbf{s})$, which is the first derivative of $\mathbf{r}(\mathbf{s})$ with respect to a line segment ds .

There is now another added term to the effective energy of the chain which is related to the curvature (itself proportional to the square of the tangent vector), and the summation is now replaced by integration along the entire contour length:



$$\frac{E}{k_B T} = \int_0^{L_{tot}} \left[\frac{A}{2} \left| \frac{d \hat{\mathbf{t}}(s)}{ds} \right|^2 - \frac{f}{k_B T} \hat{\mathbf{t}}(s) \cdot \hat{\mathbf{z}} \right] ds$$

WLC: A slight extension of FJC

A new phenomenological parameter has entered the energy term, A , which is a measure of the *persistence length* of the chain, or how long a segment of the chain will have tangent vectors all pointing in nearly the same direction. Indeed, the tangent-tangent correlation function for the wormlike chain at zero stretching force is given by

$$\langle \hat{\mathbf{t}}(0) \cdot \hat{\mathbf{t}}(s) \rangle = e^{-|s|/A}$$

or, the similarity in directionality for the chain decays as an exponential in the persistence length.

An analytic solution to WLC is not currently known, but the above equation has been solved numerically.

At low force it again displays a Hookean linear relation, but as the extension nears the contour length of the molecule, it scales not as $1/f$ as predicted by freely jointed chains, but **as $1/f^{1/2}$, in significantly better agreement with the data.**

Analysis: The Worm-Like Chain for elasticity

Increase in end-to-end length (x) of polypeptide when one IgG domain unfolds
Worm Like Chain (WLC) of entropic elasticity.

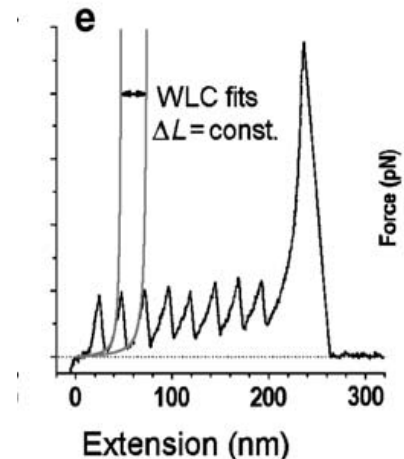
Force related to fractional increase (x/L)

$$f = \frac{k_B T}{L_p} \left(\frac{1}{4 \left(1 - \frac{z}{L}\right)^2} - \frac{1}{4} + \frac{z}{L} \right)$$

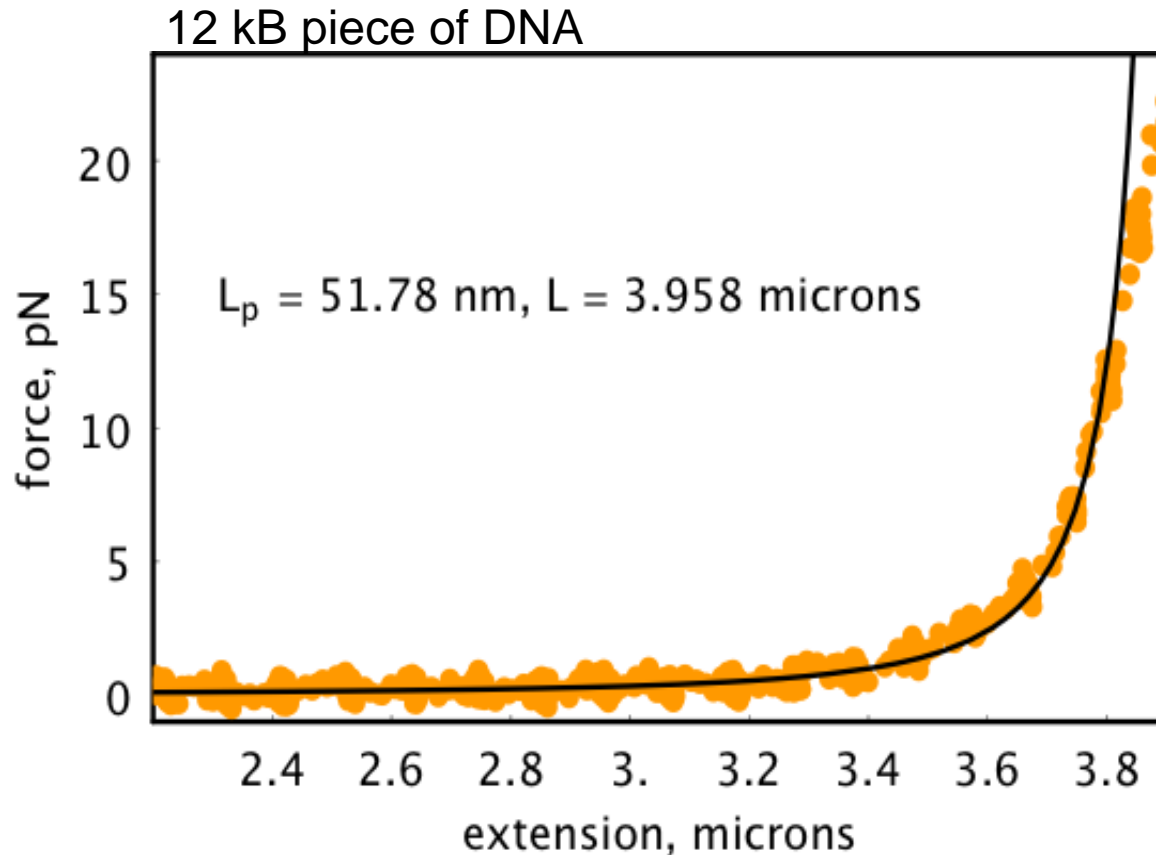
where $A = L_p$: persistence length, a measure of the chains bending rigidity
= 2x Kuhn Length
 L = contour length
 x = extension

Each unfolding event increases the contour length of the homopolymer by a constant value, ΔL . For $(I91)_8 = 28.1$ nm.
(For other domains, it varies, typically from 27-31 nm.)

Folded Ig domain length = 4.5 nm; 7x increase upon unfolding,
which explains why the force goes down dramatically after each unfolding



The Worm-Like Chain for Elasticity



What's happening at forces $> 20 \text{ pN}$?

$>10\text{-}15 \text{ pN}$, model keeps increasing quicker than the experimental data.

Because the WLC model is an *inextensible* model, where the chain contour length is constant. If make the applied force is no longer simply extending the molecule, but has started doing work on the structure itself, deforming it from its regular B-DNA form. This addition makes the model valid up to approximately 60 pN .

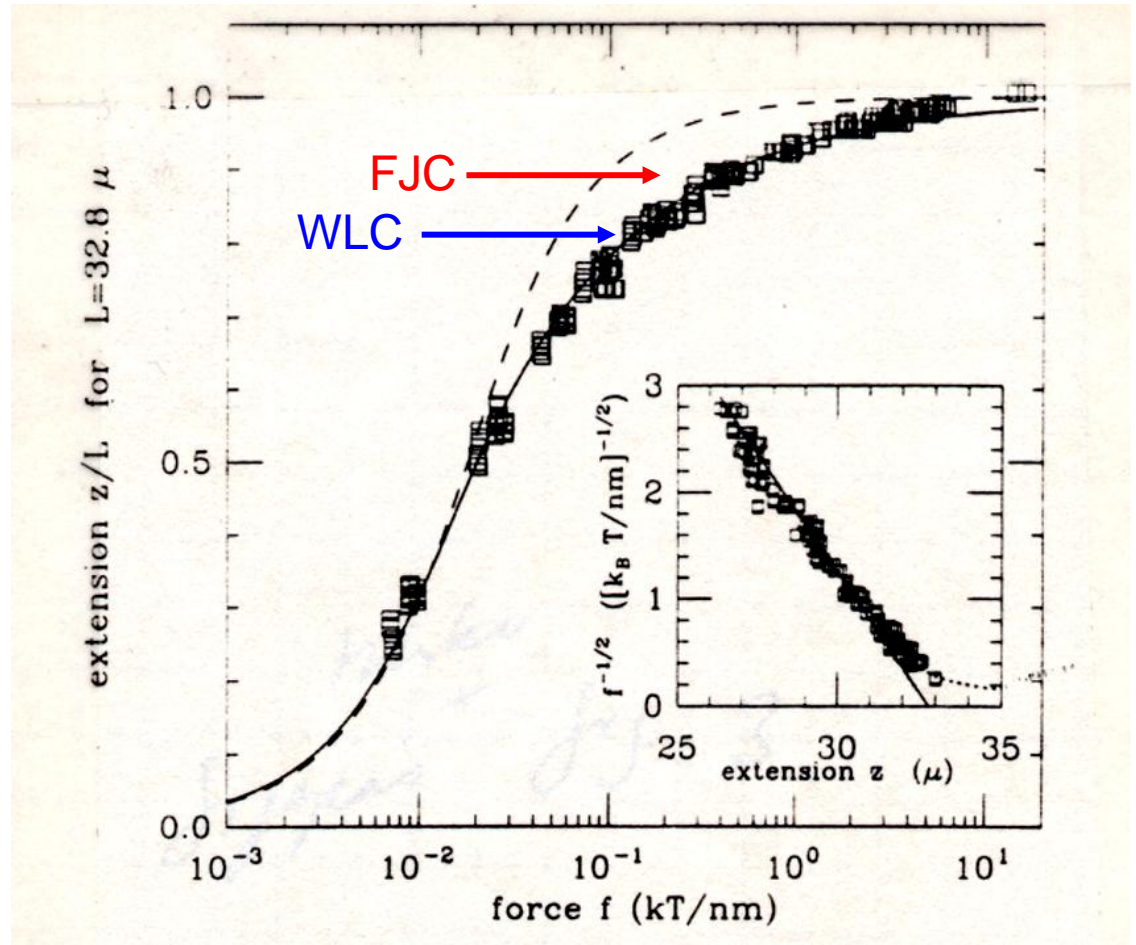
FJC vs. WLC

For DNA, WLC works amazingly well!

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.



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.