Announcements & Lecture Points

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

Quiz returned, next homework on Wednesday.

Today's Lecture: Protein Folding, Misfolding, Aggregation.
Experimental Approach

Protein Folding Summary

(From last 2 lectures)

- Proteins can fold and do say fairly fast (< second).
- Protein Funnel is a good model. Extending beyong nearest neighbor interaction: Molecular Dynamic Simulations sometimes do a better job (with a lot of $$).
- ΔG is almost always small: (5-10 kT—few H-bonds). E goes down; S goes down. They compensate.
- Kinetics – fast cause not huge barriers. (Detailed calculations necessary.)
- In most cases, don't need help. In complicated cases (big proteins, very crowded conditions such as in a cell) proteins get help: proteins called chaperones.

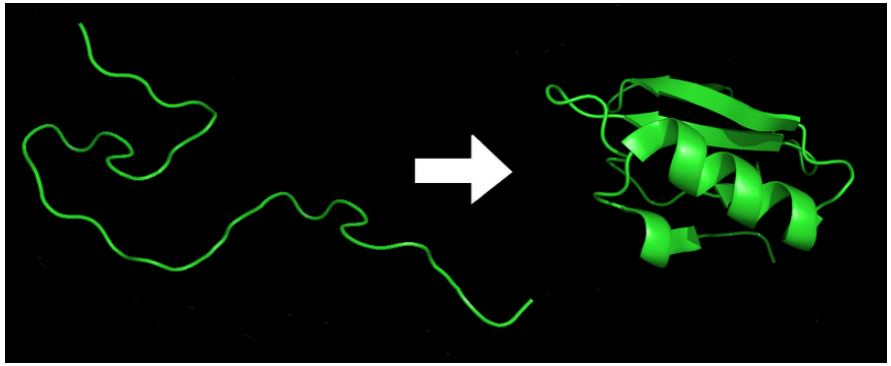
Today's Points

- To avoid problems with folding due to either kinetic traps or protein interactions, sometimes need chaperones.
- Amyloid Diseases
- Experimental Protein Folding. Atomic Force Microscopy
- Equipartition Theorem (simple but important).

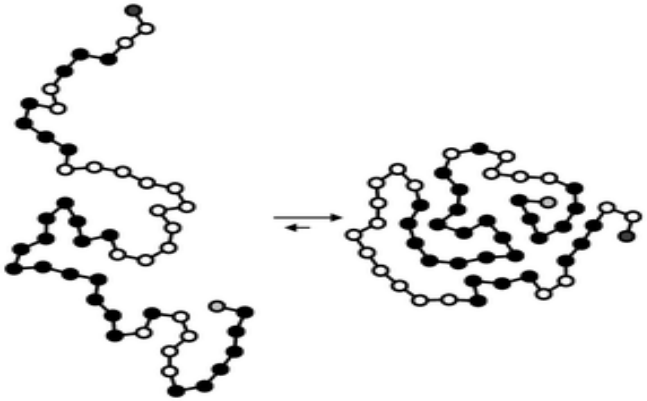
  --Can see Angstrom scale changes!
- Worm Like Chain model of Protein Folding (and DNA)

Protein folding: the energy landscape theory
Levinthal's Paradox

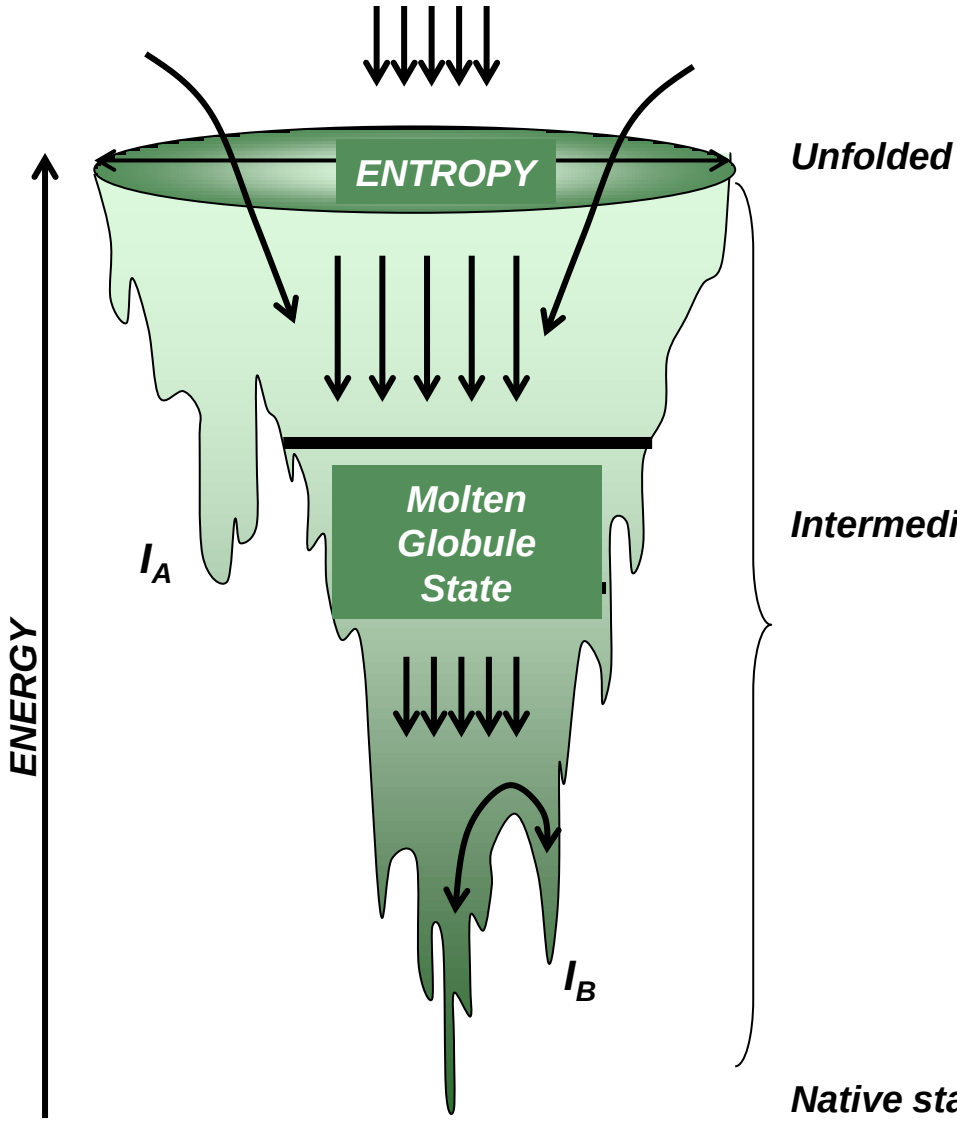
Unfolded → Folded



Inactive → Active



Lattice Model: only worry about nearest neighbor interactions. Hydrophilic and hydrophobic interactions



Molecular Dynamics: write F=ma for everything

Misfolding of Proteins

Most proteins can spontaneously refold

Some proteins do not: boil and egg, and bring temp back down and won't re-form. Commonly the hydrophobic residues get exposed. When concentration of protein is high, they can fold up with other proteins instead of with itself and remain unfolded and aggregated.

Wide variety of proteins; similar structure, bad outcomes!

Amyloid fibers & plaques: Mad Cow diseases, Alzheimer Disease, Parkinson Disease, maybe some forms of diabetes

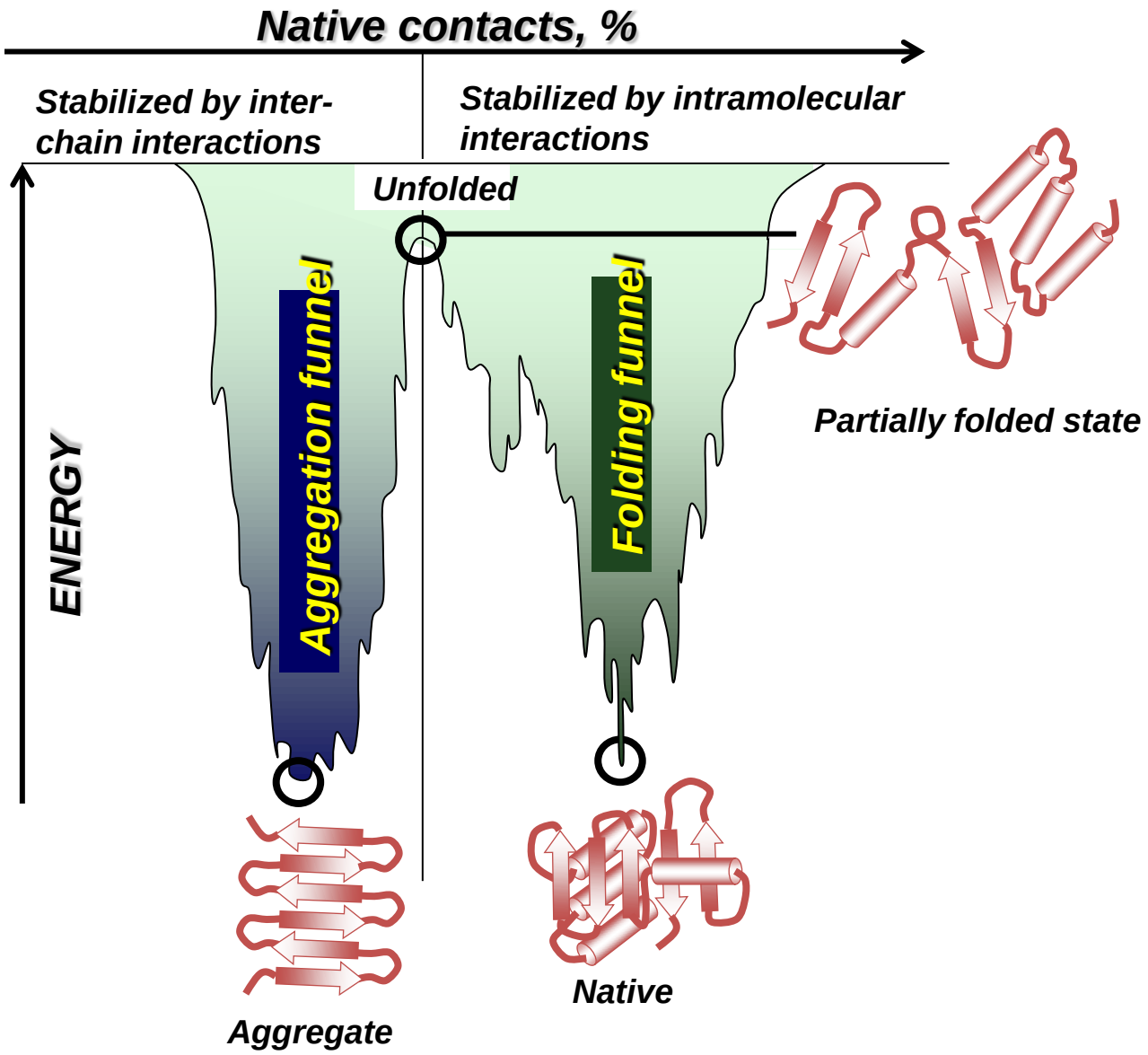
Protein Folding in the Cell

- It is hard to predict a protein's structure from its primary structure
- Most proteins probably go through several stages on their way to a stable structure
- **Chaperonins** are protein molecules that assist the proper folding of other proteins
- Misfolded protein- Either stuck in kinetic traps, or interact with other proteins (which may be partially unfolded).
- Diseases such as Alzheimer's, Parkinson's, and mad cow disease are associated with misfolded proteins

When intermolecular contacts are significant, need to modify energy funnel

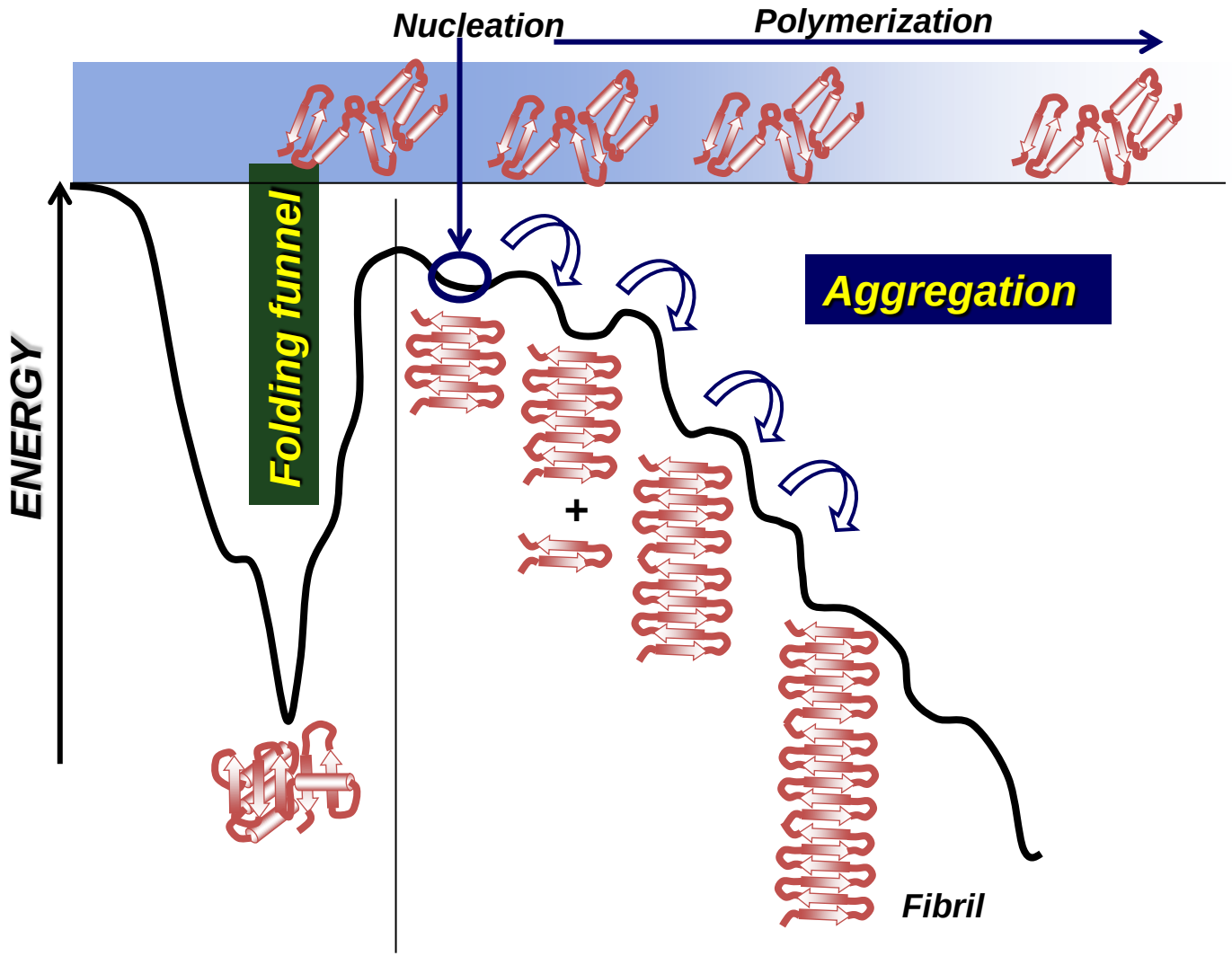
Idealized case:

Assume that 50% of contacts are formed, then can either fold normally (i.e. in dilute conditions), or it can react with another partially unfolded protein and fall down the aggregation funnel.



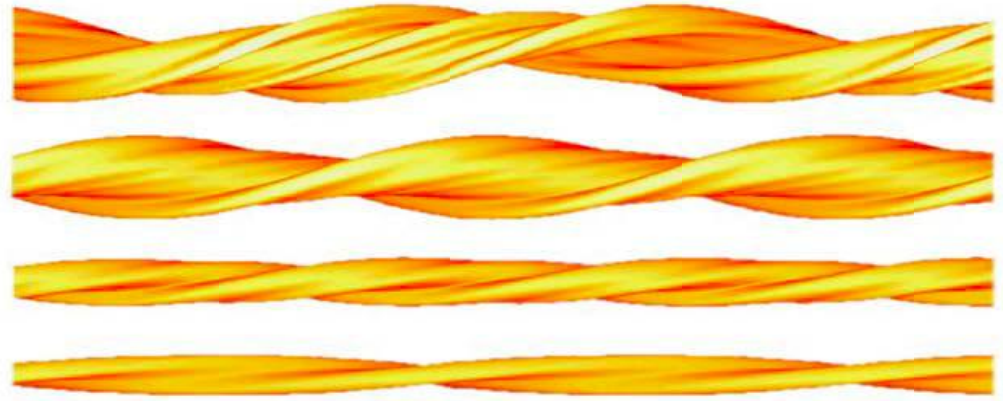
Slightly More Realistic Scenario

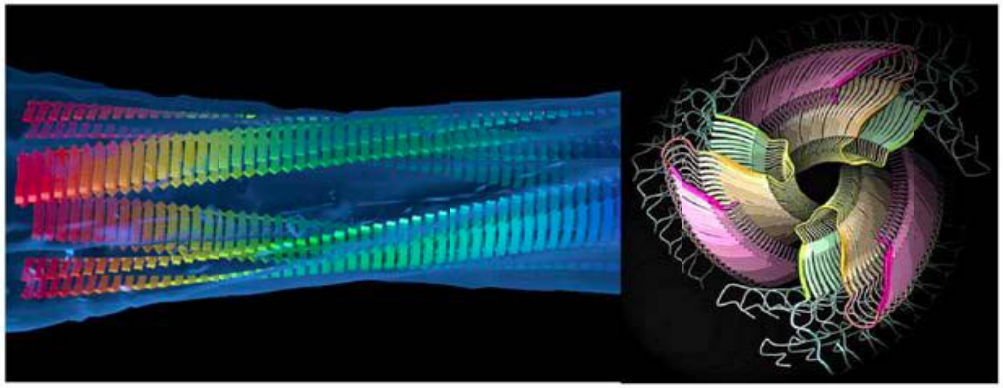
(allow for formation of long fibrils)



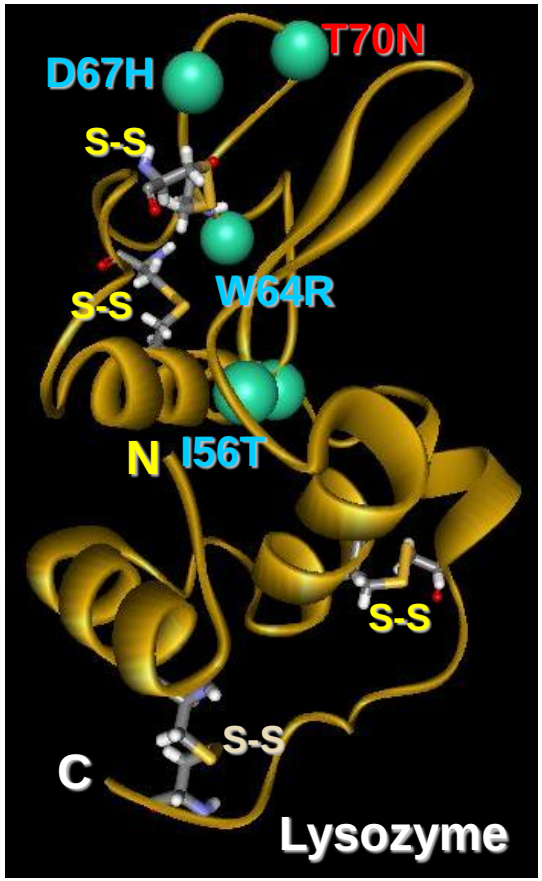
Possibly similar structures

The fibrillar structures formed ex vivo (outside cell) are usually long, un-branched and often twisted; the core of the organized structure is composed of **β**-sheets having strands positioned perpendicular to the fibril axis. The portion of a polypeptide chain that is incorporated into fibril core may vary substantially for different proteins; in some cases only a handful residues may be involved in the core structure, with the remainder of the chain associated in some other manner with the fibrillar assembly.



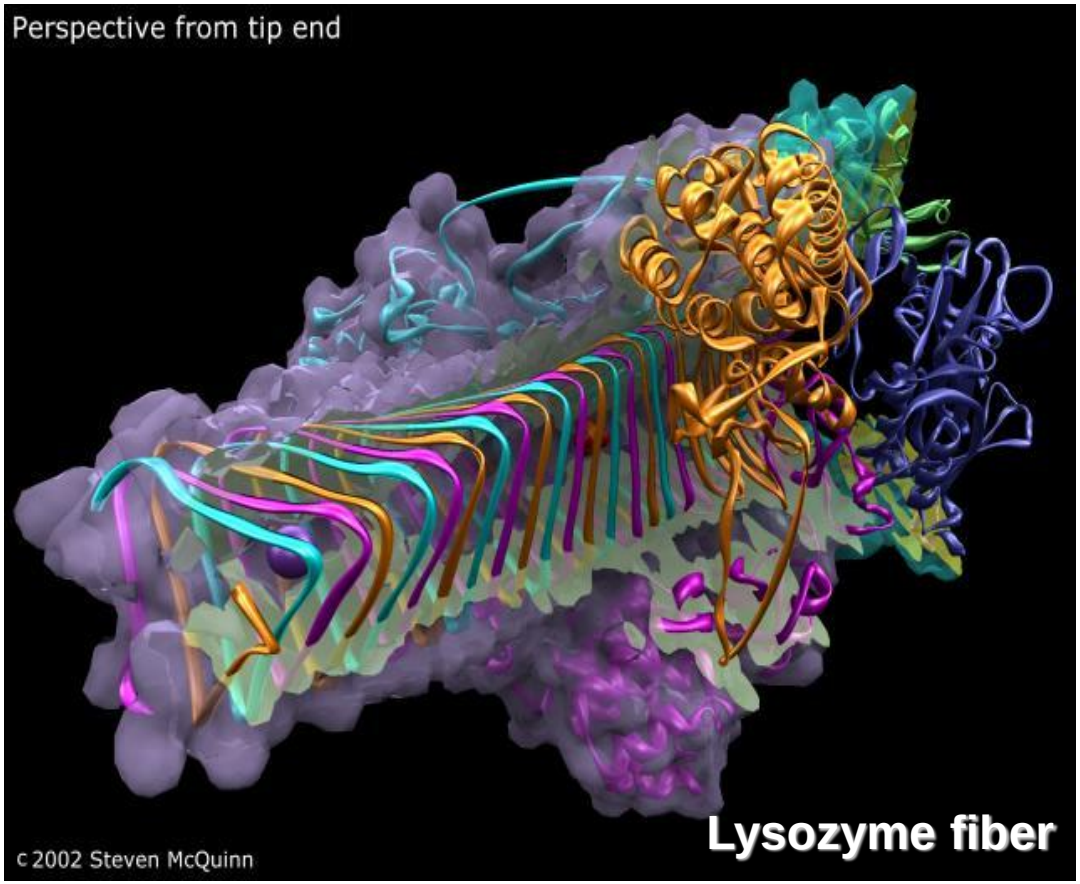


Lysozyme: Well Studied example of mis-folding



β-domain

α-domain



form amyloid deposits in the viscera

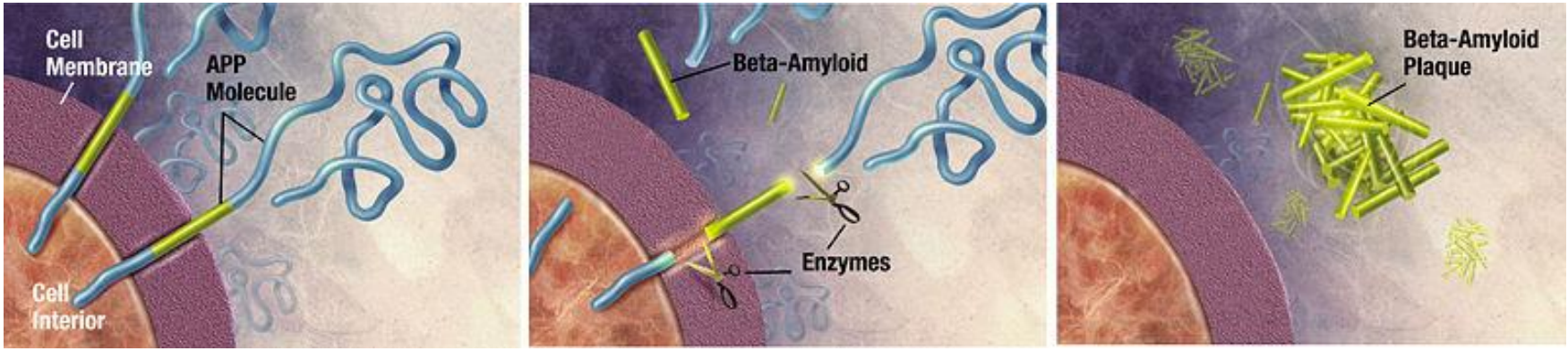
Lysozyme is abundant in a number of secretions, such as tears, saliva, human milk, and mucus. Also in egg white. (Well studied.) Damage bacterial cell walls through catalyzing. Forms amyloid fibers.

Amyloid Fibers…involved in Alzheimers

Protein amyloid fibers are often found to have a β-sheet structure regardless of their sequence, leading some to believe that it is the molecule's misfolding that leads to aggregation.

There is a lower energy state which is fibers—e.g. ameloid fibers–multiple states!

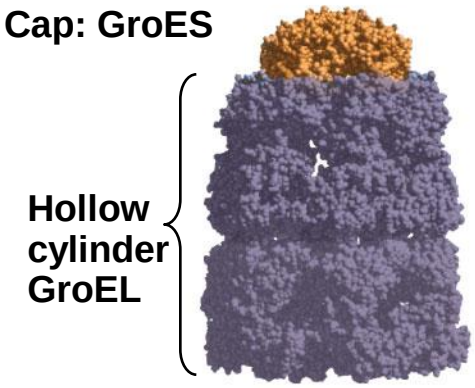
http://www.informaworld.com/smpp/content~content=a779685983~db=medi~order=page



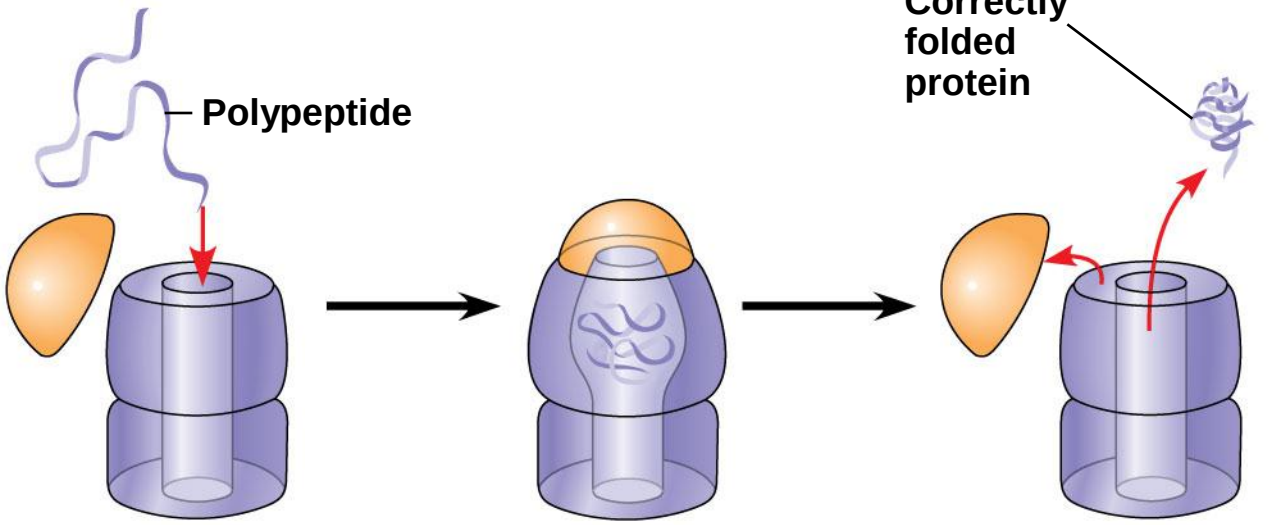
Enzymes act on the APP (Amyloid precursor protein) and cut it into fragments of protein, one of which is called beta-amyloid and its crucial in the formation of senile plaques in Alzheimer

Figure 5.23

Chaperones



Chaperonin (fully assembled)

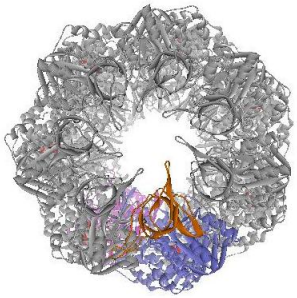


Steps of Chaperonin Action:

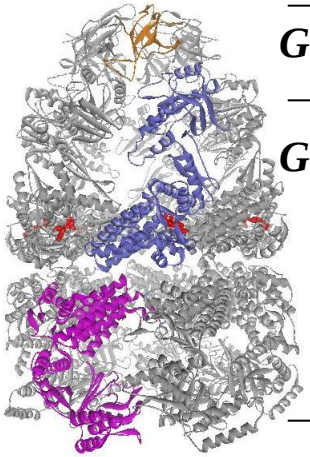
1. An unfolded polypeptide enters the cylinder from one end.
2. The cap attaches, causing the cylinder to change shape in such a way that it creates a hydrophilic environment for the folding of the polypeptide.
3. The cap comes off, and the properly folded protein is released.

Chaperones (Bacterial GroES-GroEL)

With ATP, environmental conditions suitable for proper folding



135Å

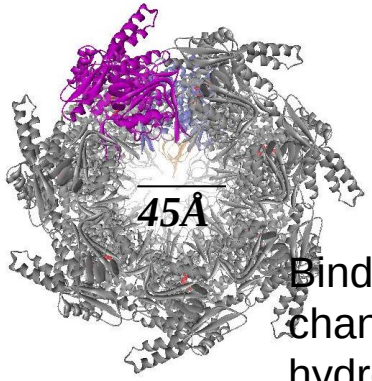


GroES

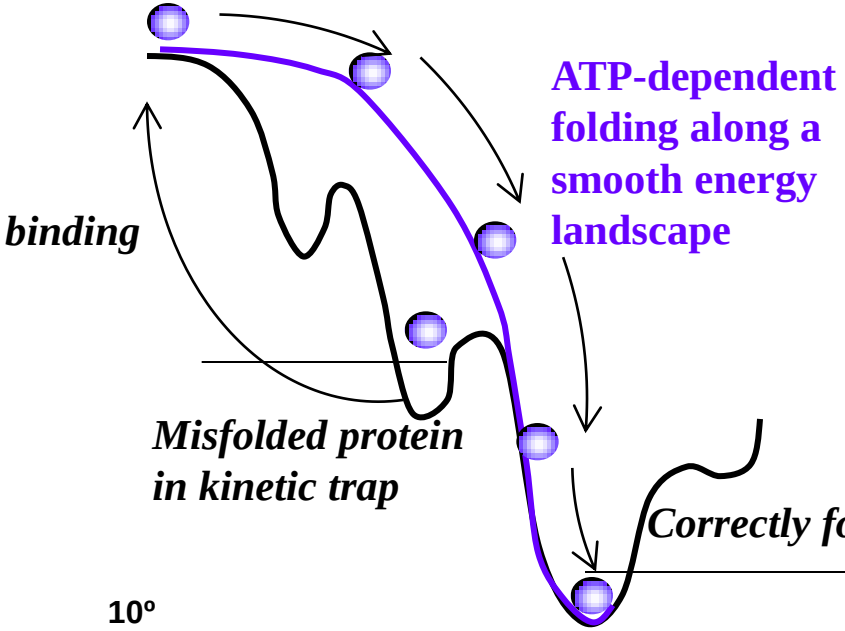
GroEL

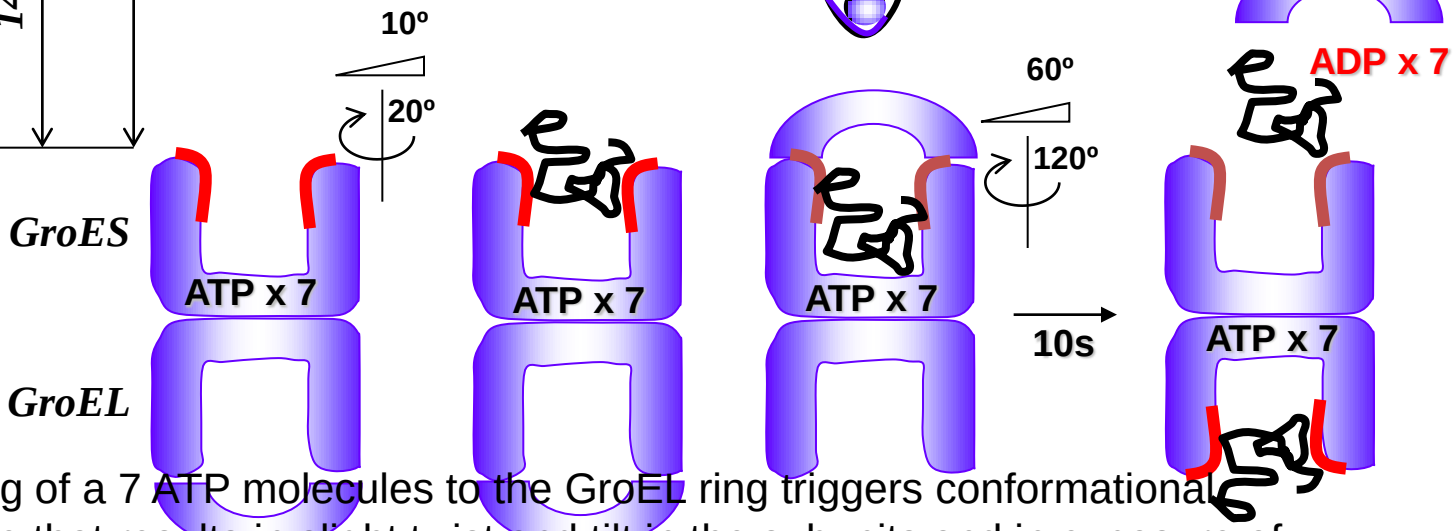
145Å

184Å



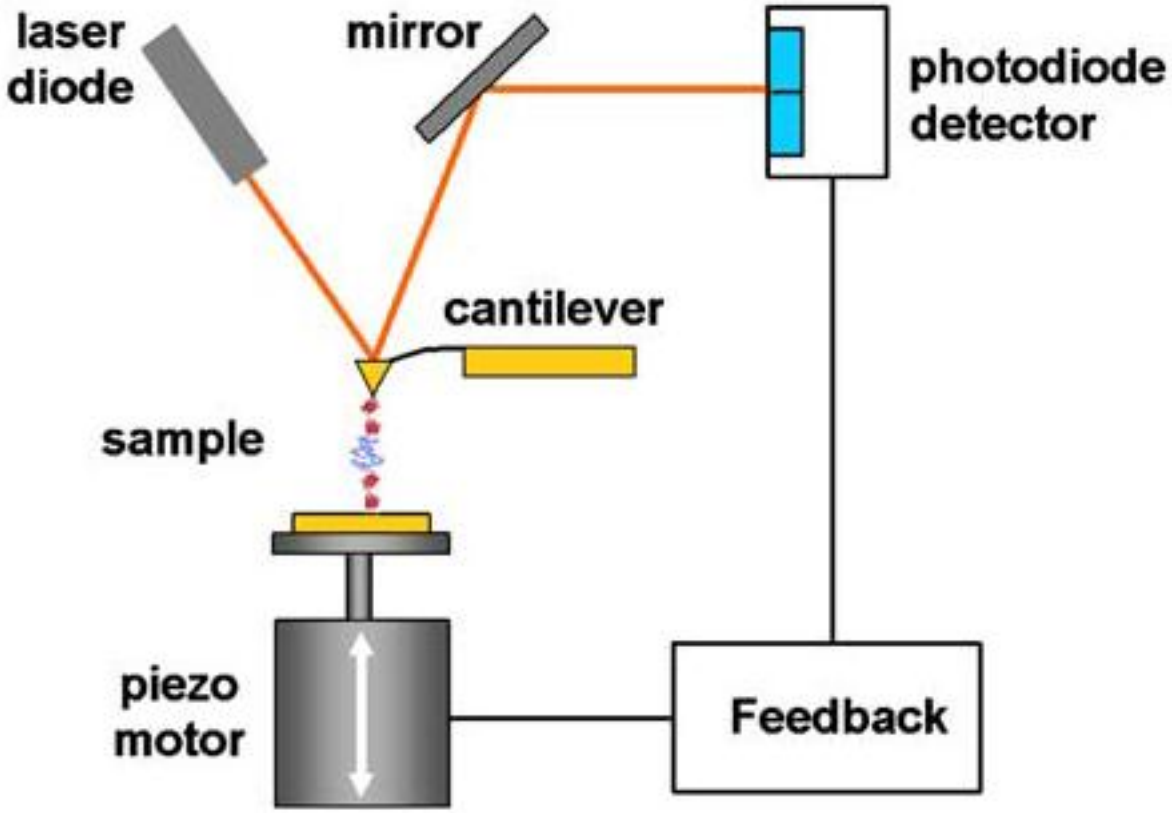
45Å

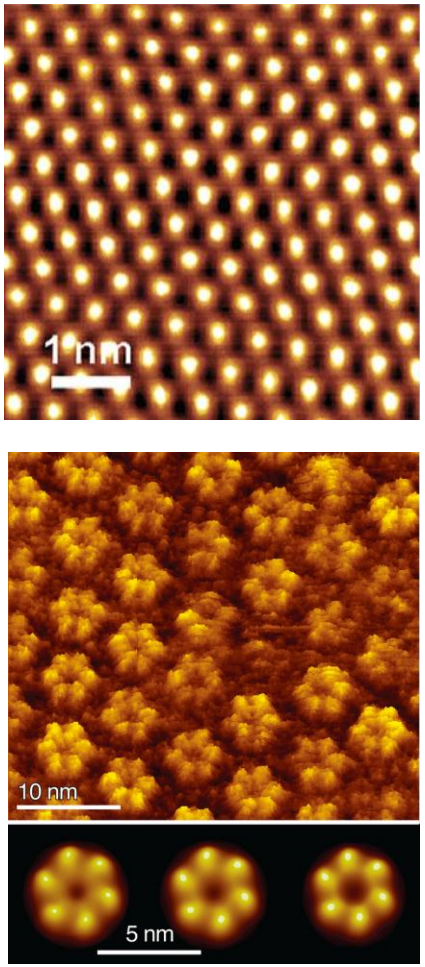




Binding of a 7 ATP molecules to the GroEL ring triggers conformational change that results in slight twist and tilt in the subunits and in exposure of hydrophobic patches that interact with and help to unfold misfolded protein.

Experimental Protein Folding
Atomic Force Microscopy

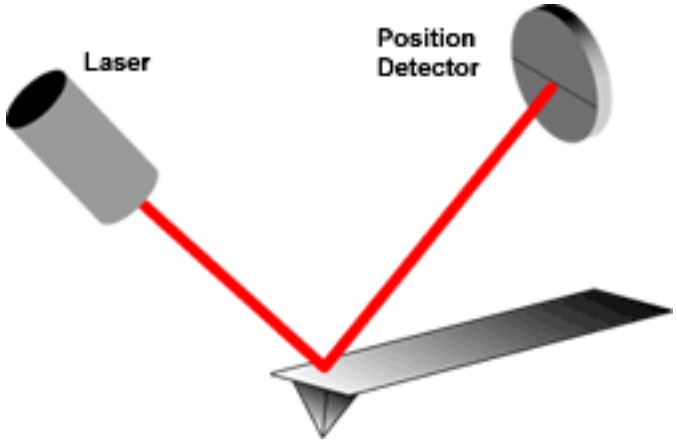


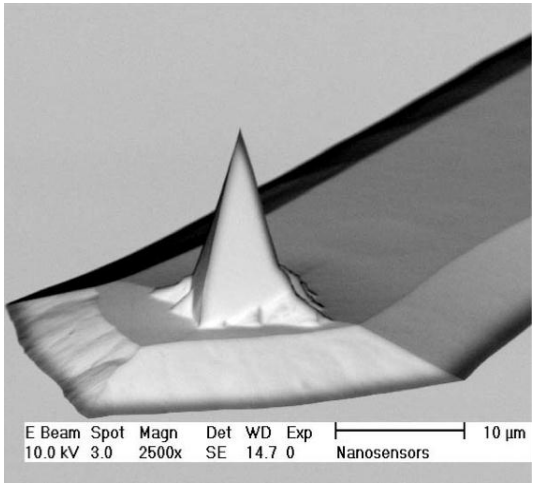


The precursor to AFM, called the Scanning Tunneling Microscope, won the Nobel Prize, 1986.
Can see fraction of a nanometer, >1000x better than optical techniques

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

Hook's Law and AFM





Most AFM probes are made from silicon and/or silicon nitride (Si_3N_4) wafers using semiconductor- based etching processes.

Measuring forces

Because the atomic force microscope relies on the forces between the tip and sample, knowing these forces is important for proper imaging. The force is not measured directly, but calculated by measuring the deflection of the lever, and knowing the stiffness of the cantilever. Hook's law gives F = -kz, where F is the force, k is the stiffness of the lever, (in Newtons/meter) and z is the distance the lever is bent.

http://cp.literature.agilent.com/litweb/pdf/5990-3293EN.pdf

Important Aside: Equipartition Theorem

Relevant to Magnetic Tweezers, Optical Tweezer, Diffusion
Anytime you have thermal noise (which is always!)

Average energy = (1/2)$k_B T$
for every variable which energy depends on quadratic,
e.g. if E α x^2, or E α v^2

(In classical statistical mechanics), the **equipartition theorem** is a general formula that relates the temperature of a system with its average energies. In thermal equilibrium, energy is shared equally among all of its various forms; for example, the average kinetic energy in the translational motion of a molecule should equal the average kinetic energy in its rotational motion.

Note: There are quantum corrections (such at very low temperatures).
(But in biophysics, don't have to worry about.)

For example:
Simple Harmonic Oscillator (in 1D) at temperature T.
What is average displacement-squared? $\frac{1}{2} k_B T = \frac{1}{2} k\langle x\rangle^2 = \langle x\rangle^2 = k_B T/k$
What is average velocity? $(k_B T/m)^{1/2}$ $\langle x\rangle = \sqrt{\langle x\rangle^2} = (k_B T/k)^{1/2}$

For monotonic gas, what is average translational kinetic energy: $(3/2)k_B T$

http://en.wikipedia.org/wiki/Equipartition_theorem

AFM Cantilever

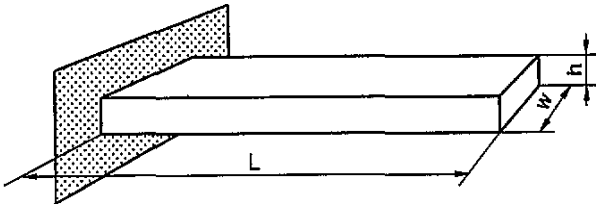
How small of a motion can you measure?

Bend a cantilever (in z-direction): ½ kz^2

½ k_BT = ½ kz^2

(z^2 is the mean square deflection
of the cantilever caused by thermal vibrations)

What is k?



$k = 0.25Ewh^3/L^3$, where E = modulus of Elasticity
(how stiff the material is).

$z^2 = k_BT/k = 0.64Å/\sqrt{k}$ at 22˚C (where k is in N/m)

k between 0.001 to 100 N/m
(Huge range! Very useful for measuring large Δz, F: 1 pN - nN)
Say, typical: 1 N/m = 1nN/nm: 1 nN causes deviation of 1 nm
[1 nN usually really large→0.01 N/m = 10 pN/nm: 1 pN would cause a deviation of 1 nm)

Can measure an Angstrom or less!!

How Strong is a Covalent Bond?

(recall we said it was about 100-200 kT)

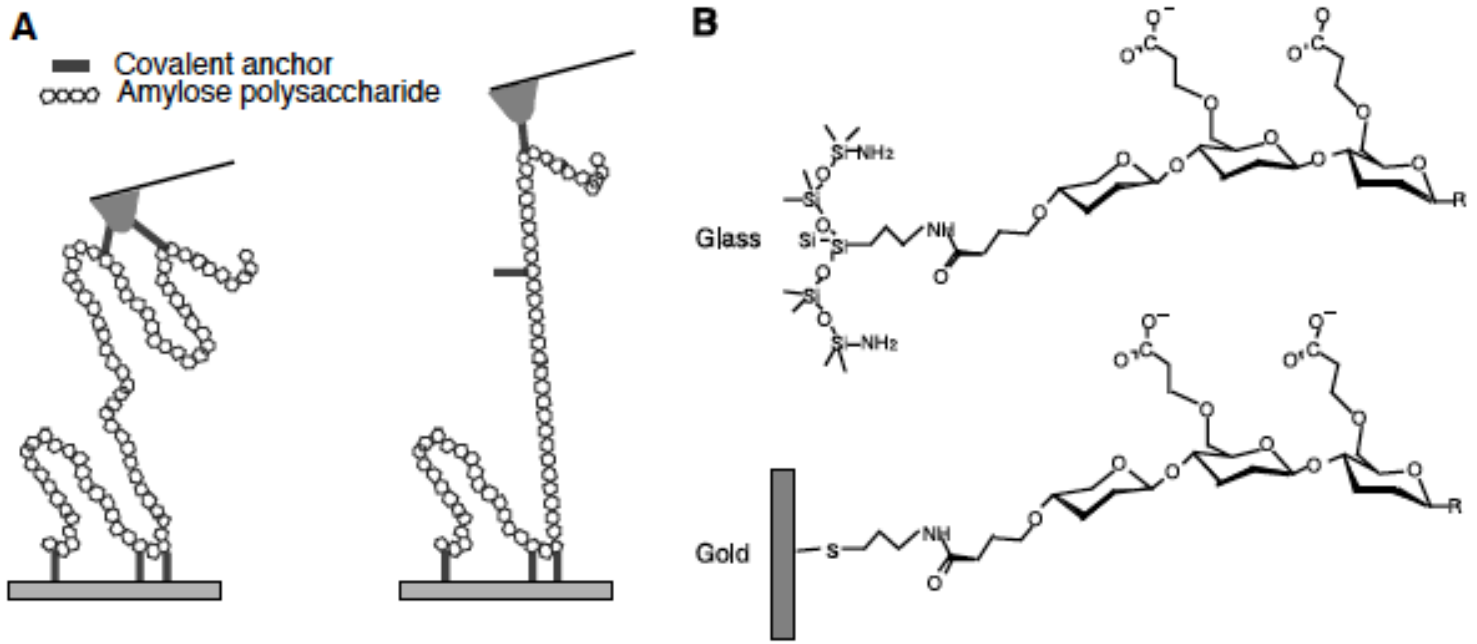


Fig. 1. (**A**) Stretching of a single polysaccharide chain that is covalently attached to the AFM tip and the substrate. (**B**) Schematics of the covalent attachment of the amylose by carbodiimide chemistry to glass or Au surfaces, which were both functionalized with amino groups.

Note: It's actually the C-Si which breaks!

How Strong is a Covalent Bond?
Gaub, Science, 1999

A Single Covalent bond

F = 2.0 nN = 2000pN
C-Si: 0.185 nm
(2000pN)(0.185 nm) =
370 pN-nm

$1k_BT = 4pN\text{-}nm$
$E = 92.5\ k_BT$

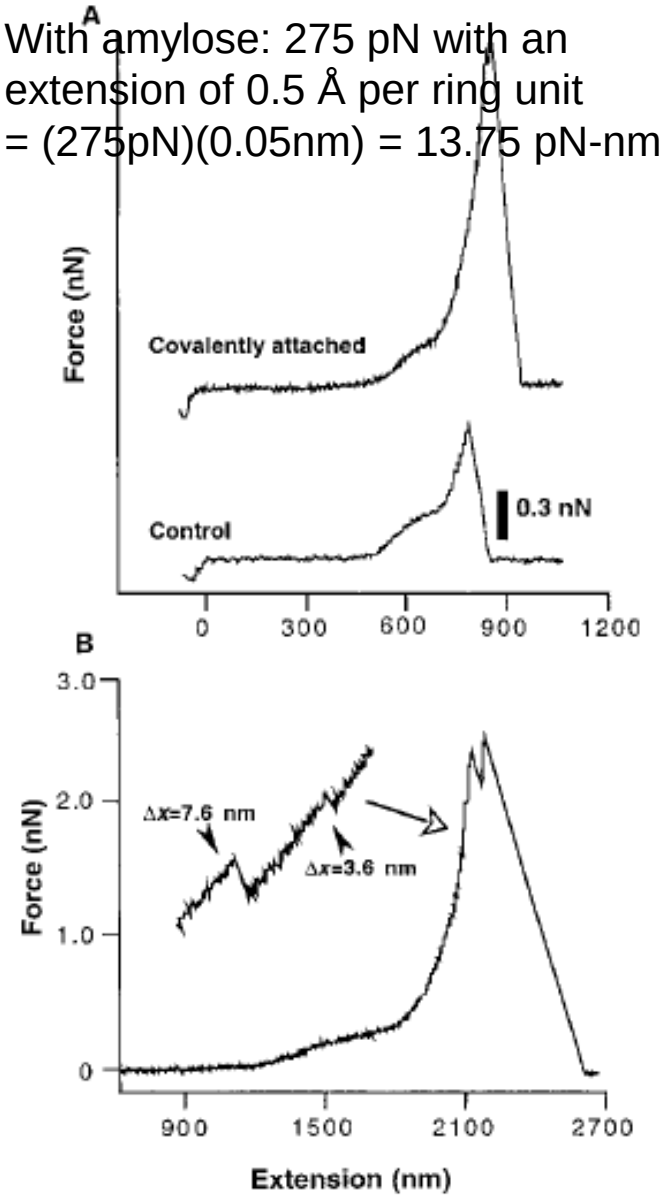
Example Rupture Force
Breaking of a covalent bond
C-C ≡ 1600 pN
Breaking of a non-covalent bond.
Biotin/streptavidin ≡160 pN (strongest known)

Breaking of a weak bond.
Hydrogen bond ≡ 4 pN

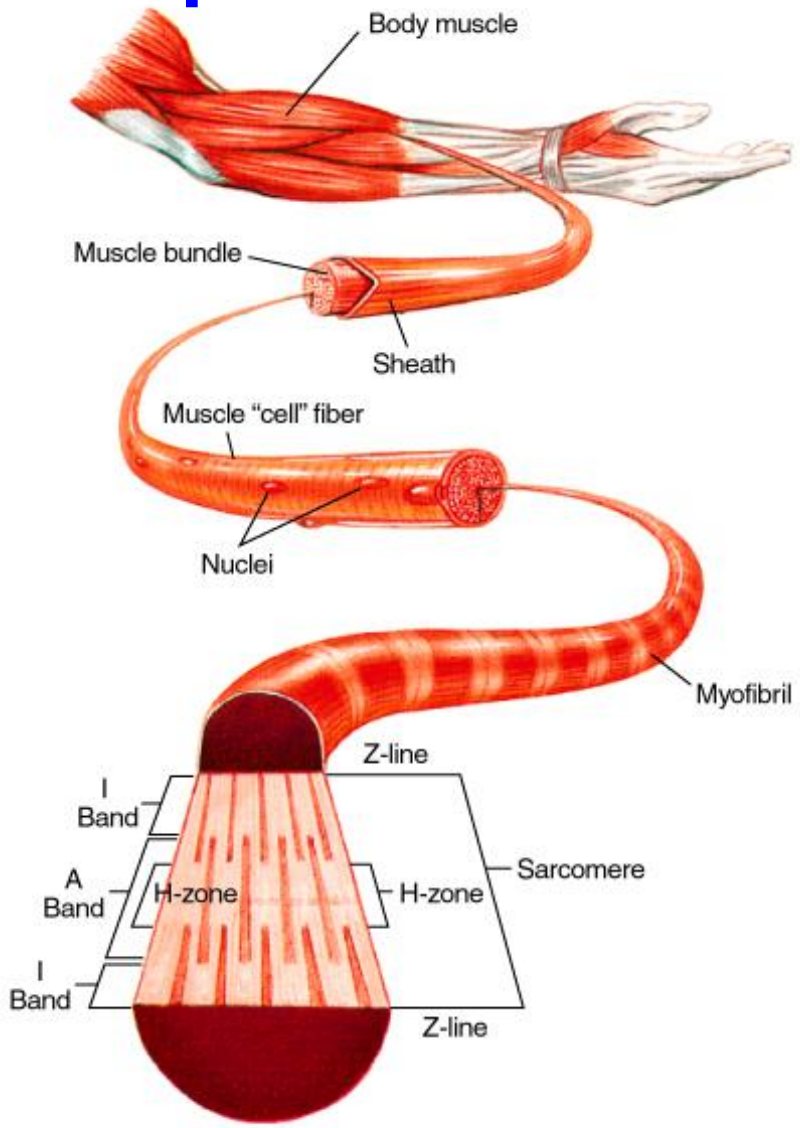
Biotin-Streptavidin= 160 pN

H-bond: 1-4 pN

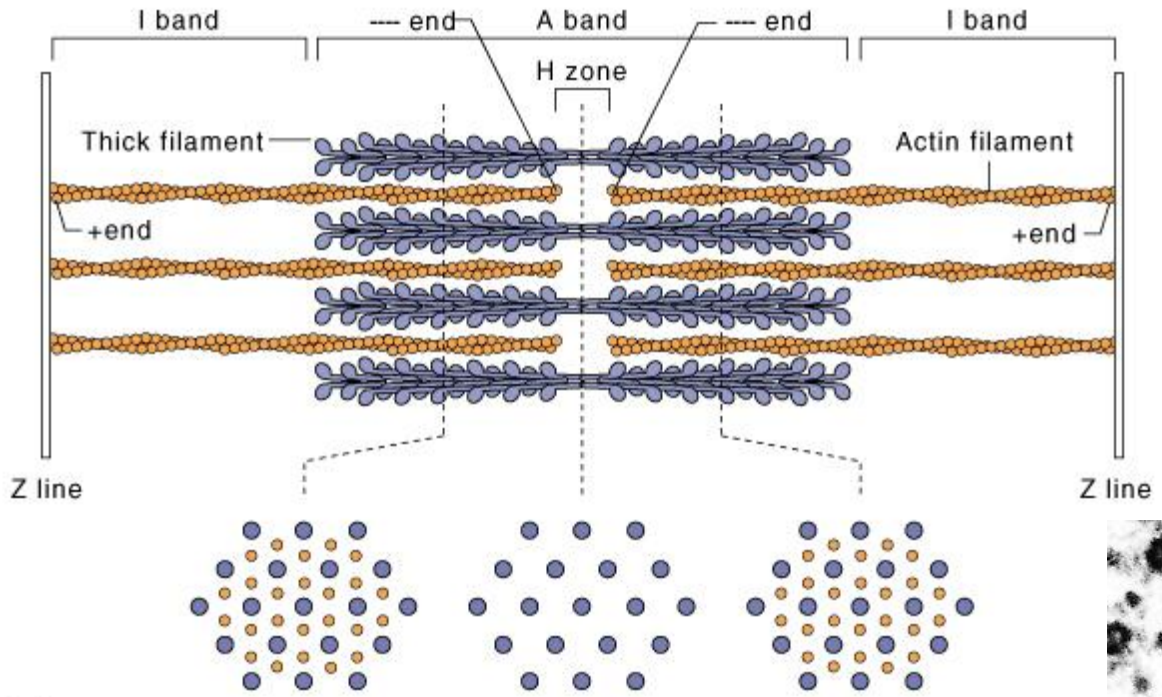
With amylose: 275 pN with an extension of 0.5 Å per ring unit
= (275pN)(0.05nm) = 13.75 pN-nm = $3k_BT$



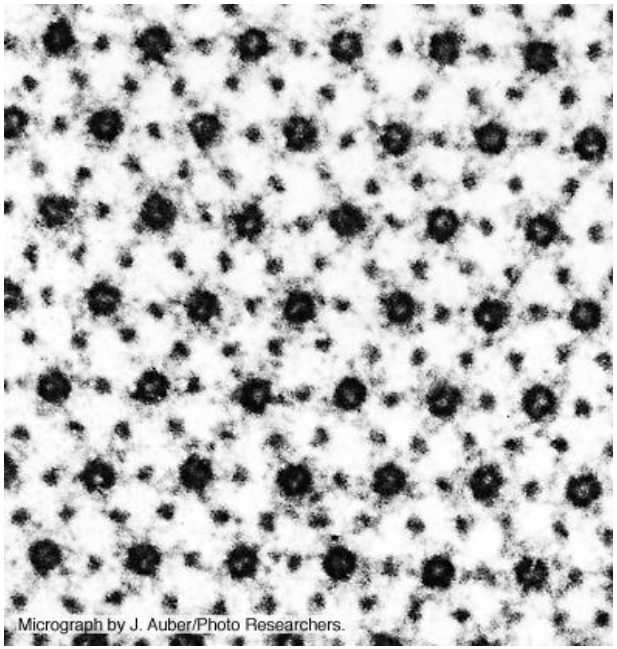
Biological Example of AFM: Muscle & Titin



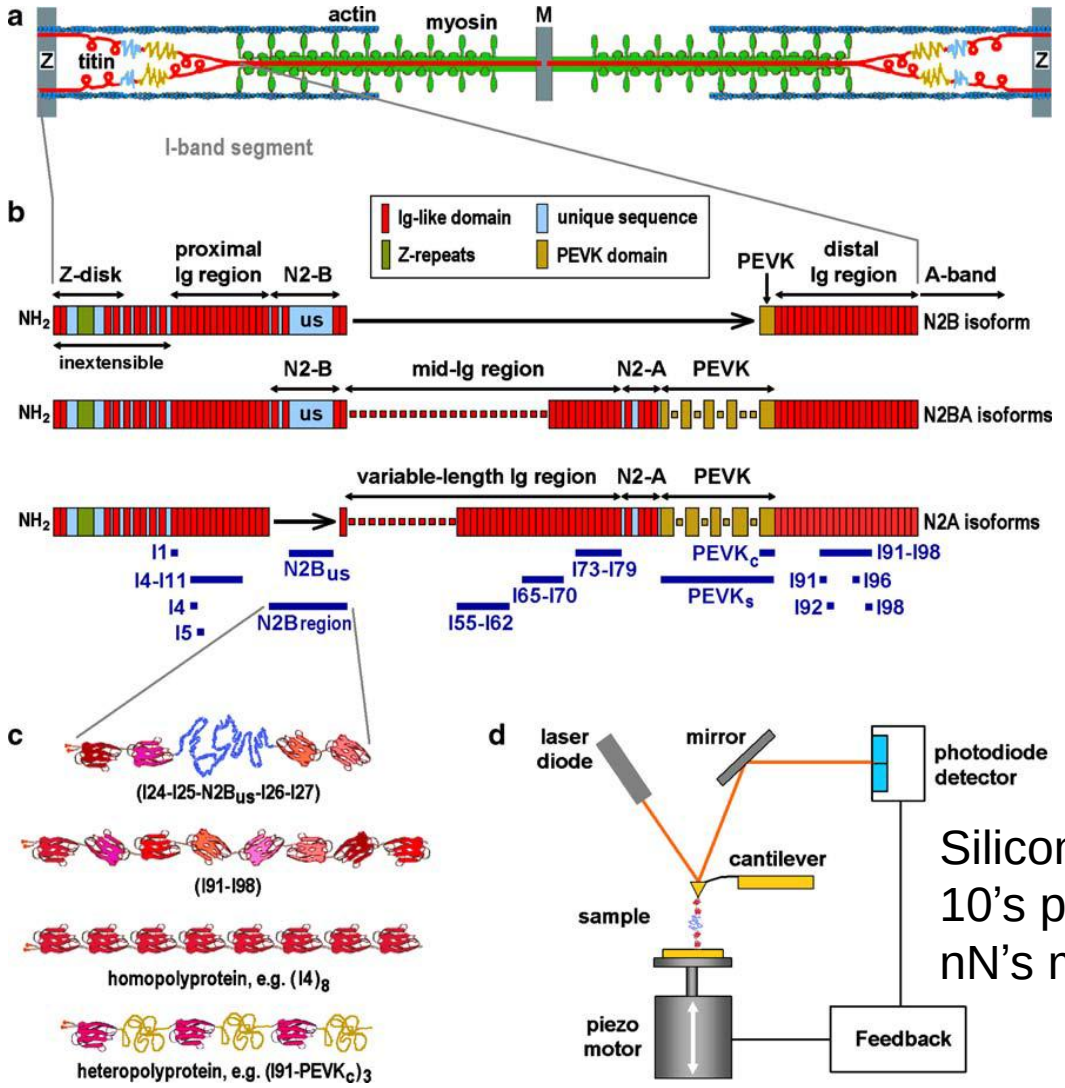
The Sarcomere



(a)



Titin: Human's Biggest protein



Each domain IgG

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

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.

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.