3.052 Nanomechanics of Materials and Biomaterials: Spring 2007
Assignment #5
Due Date: Tuesday 05.01.07
You are encouraged to use additional resources (e.g. journal papers, internet, etc.) but please cite them (points will be deducted for not doing so). You will need to research additional sources to answer some questions. Everything must be answered in your own words; also, do not copy phrases or sentences from the paper or podcast.
+ 5 extra credit for posting a message on one of the podcast message boards.

1. Nanomechanics of Chondrocytes. Ng, et al. *J. Biomech.*, Vol. 40, Issue 5, 1011-1023, 2007. (Note : nanoindentation will be covered in more detail later on the course, but this podcast was included at this time to correlate with the cartilage lectures).

> **a. The data presented in this paper has alot of relevance to understanding the fundamentals of cartilage tissue engineering. Why doesn't injection of cartilage cells into a damaged tissue result in regeneration? Why is it more advantageous to use young immature chondrocytes for tissue engineering? Look up one journal article published in the last year on cartilage tissue engineering, post the PDF for this journal article along with your assignment, give the full citation in your assignment solutions, and summarize what you feel was the most important /interesting discovery in this paper in a few sentences / short paragraph (do not copy abstract). You are not allowed to use any papers posted by classmates on the class message boards (i.e. you need to look up your own).**

Individual answers will vary.

> **b. The original AFM image for Fig. 2d has been posted on the MIT Server. Using WSXM software (http://www.nanotec.es/imagenes/titu_wsxm.gif), measure the banding periodicity and diameter of 5 different individual fibrils (show an image and section profile for one of these) and report a mean and standard deviation for the measured values. Comment on the relationship of these experimentally measured values to the specific type of collagen expected in the pericellular matrix (cite appropriate references).**

From Lodish et al., *Molecular Cell Biology*, W.H. Freeman and Company, NY, 2000. pp 981-985:
Collagen molecules pack together side-by-side to form collagen fibrils; the structures of these fibrils vary depending on the type of collagen (type I, II, III, etc) that forms them. Collagen II fibrils are generally around 50 nm in diameter and found in collagen. Offsets in the side-by-side associations give rise to a banding periodicity (see discussion on class message boards). For collagen II, the periodicity is about 67 nm.
These values should match the averages measured from 5 different fibrils in the AFM images from Figure 2d.
Note that the 67 nm period of a band is made up of a "gap" region of about 0.6*67 nm = 40.2 nm and an "overlap" region of about 0.4*67 = 26.8 nm. One could also measure one of these characteristic features to identify the type of collagen.

c. Name 5 major experimental differences between the AFM-based single cell mechanics experiments presented in this paper compared to the previous podcast by Suresh, et al. *Acta Biomaterialia* 2005 1, 15 using optical tweezers.

1) In Suresh, et al. the stress state was tension while in Ng, et al. it was primarily compression.
2) In Suresh, et al. the stress strate was uniaxial while in nanoindentation the stress state is multiaxial with stress concentrations near the probe tip.
3) The forces probed in Ng, et al. were much higher range (up to ~ 1.5 nN) compared to Suresh,et al. (up to ~ 200 pN).
4. In Suresh, et al. the cell was in contact with two bead surfaces while in Ng, et al contact was made in a pyramidal well.
5. In Suresh, et al. a continuum like-response was obtained for the entire cell while for Ng, et al. the nanosized probe tip deformed localized areas. The micron-size probe tip was more similar to the deformation in Suresh, et al.

d. The data in Figure 6 have been digitized and are posted on the MIT Server. Calculate and compare the energy dissipated for the nanosized and micron-sized probe. Suggest a hypothesis for any similarities / differences in terms of the deformation of the cell.
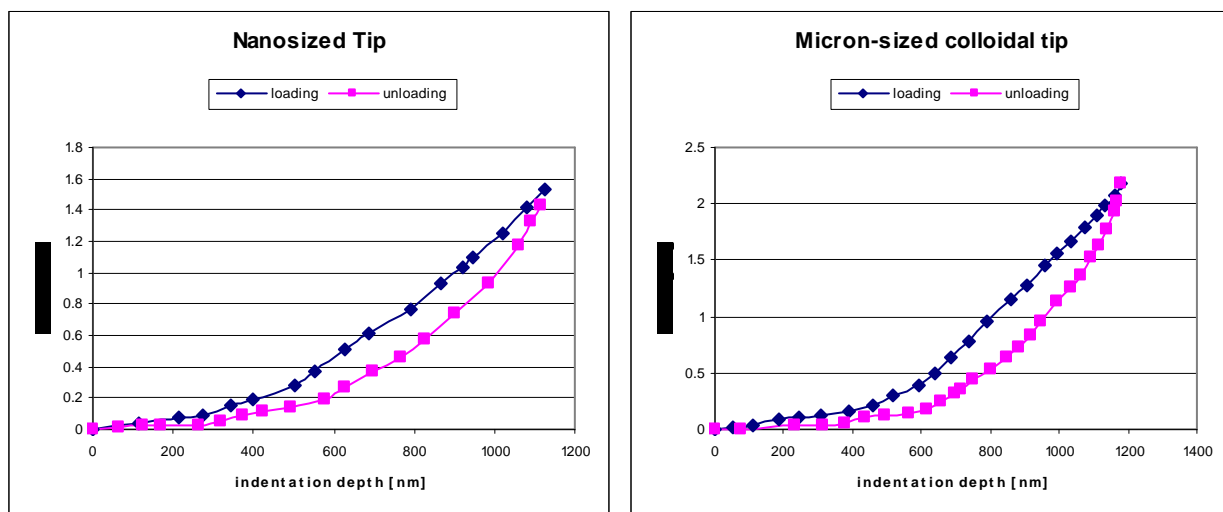
Energy dissipated by each probe is given by the area between the loading and unloading curves. One option for this calculation is to fit each curve to a polynomial and then integrate under the area, subtracting loading-unloading. The energy dissipated values are 1.88×10^{-16} J for the nanosized tip and 2.61×10^{-16} J for the micron-sized tip (see Excel spreadsheet on the MIT Server for exact calculations). For the nanosized tip (50 nm), more localized viscoelastic and permanent deformations involving the cell membrane, transmembrane proteins, and cytoskeleton lead to the energy dissipated while for the micron-size colloidal probe, dissipative components include the cytosol, nucleus, and intracellular organelles.

e. What is the explanation given in this paper/podcast for why the chondron (Day>0) is less stiff than the Day 0 chondrocyte?

The chondron is a composite of the chondrocyte and the matrix of collagen, etc., which has developed around it. If the matrix has a lower modulus that the chondrocyte itself, then the

properties of the matrix which are probed after day 0 will show that the chondron is less stiff than the Day 0 chondrocyte, which has no matrix.

2. Nanomechanics and Biocompatibility. Halperin, *Langmuir* 1999, 15, 2525-2523.

> **a. Explain what the following parameters defined in the paper are physically: U_{eff}, U^*, U_{brush}, U_{in}, U_{out}, U_{bare}, U_{ads}**
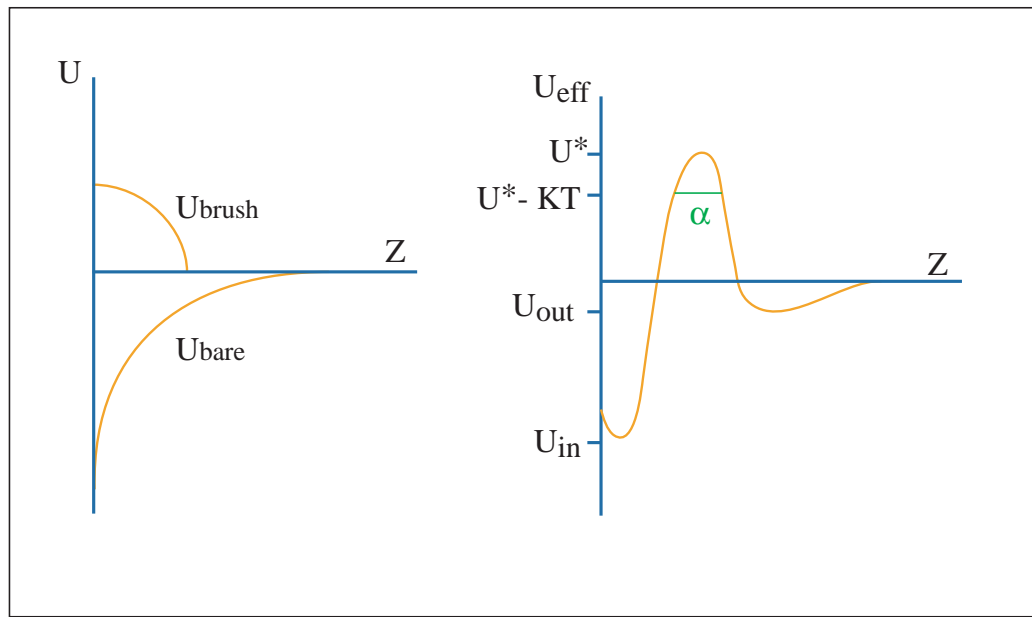

Figure by MIT OCW.

Figure 1. The effective potential U_{eff} experienced by a protein approaching a brush-coated surface (c), which is the result of the superposition of two contributions: (b) the purely attractive interaction potential between the bare surface and the protein U_{bare} and (a) the purely repulsive interaction between the protein and the swollen brush U_{brush}.

U_{eff}: total "effective potential experienced by a protein approaching a brush-coated surface"

U_{brush}: "purely repulsive interaction between the protein and the swollen brush"; it arises from the entropic penalties involved in displacing the brush molecules in order to bring the protein closer to the surface

U_{bare}: "purely attractive interaction potential between the bare surface and the protein"; it can arise from several sources, including van der Waals and hydrophobic interactions

U^*: energy maximum of the effective total potential U_{eff}; it is the energy barrier that keeps the protein from penetrating farther into the brush layer

U_{out}: first energy minimum seen by the protein as it approaches the surface. If the protein cannot get over the energy barrier presented by U^*, it will tend to settle at the position corresponding to U_{out}.

U_{in}: energy minimum seen by the protein if it overcomes the energy barrier presented by U^*; if it gets this far into the brush layer it will tend to settle at the position, close to the surface, corresponding to U_{in}.

U_{ads}: depth of the energy minimum at the surface; $U_{ads} = U_{in} - U_{brush}$; this is the attraction that would be felt by the protein at the surface if there were no polymer brush between it and the surface.

b. Plot U_{eff}(z) in units of k_BT for the compressive mechanism for a typical large protein (cite references for protein property values needed) using the following polymer brush properties; monomer size, a = 1 nm, equilibrium brush height, L_o = 5 nm, grafting density σ^{-1} = 0.2 nm^{-2}, N=10 monomers per polymer chain, and Hamaker constant, A = 0.1×10^{-19} J. Explain how the form U_{eff} changes with variations in the Hamaker constant.

Consider the case of a large protein complex from inside the cell, the ribosome, which actually made of a mix of RNA and protein. The ribosome carries out the process of translation, where mRNA sequences are translated into polypeptides. Ribosomes are about 20 nm in diameter.(http://en.wikipedia.org/wiki/Ribosome).

According to the compression mechanism outlined in Halperin's paper, U_{eff} takes the form below, which is the sum of U_{brush} [eq27] and U_{bare} [eq28].

$$U_{eff} \approx \frac{F_0 R L_0}{\sigma} \left[\left(\frac{L_0}{L} \right)^{-1/4} - \left(\frac{L_0}{L} \right)^{11/4} \right] - \frac{AR}{L}$$

Here, R= radius of protein, A= Hamaker constant, L_0= initial uncompressed brush height, σ (m^2/chain) = area per chain, σ^{-1} = grafting density = chains/ m^2, F_0 = free energy per chain (J), N= numbers of monomers/polymer chain, a= size of monomer, and L = D = the protein - surface separation distance perpendicular to the surface (x/y) plane.
From [eq10] :

$$F_o \approx N k_B T \left(\frac{a^2}{\sigma} \right)^{5/6}$$

The ratio L_0/L is constrained to be less than one; in other words, the contribution from U_{brush} is only valid over the length of the polymer brush. At lengths past the edge of the polymer brush, U_{brush}=0 and the attractive potential U_{bare} is the only contributor to U_{eff}. A plot of this equation (see Excel spreadsheet) shows the shape of U_{eff}:
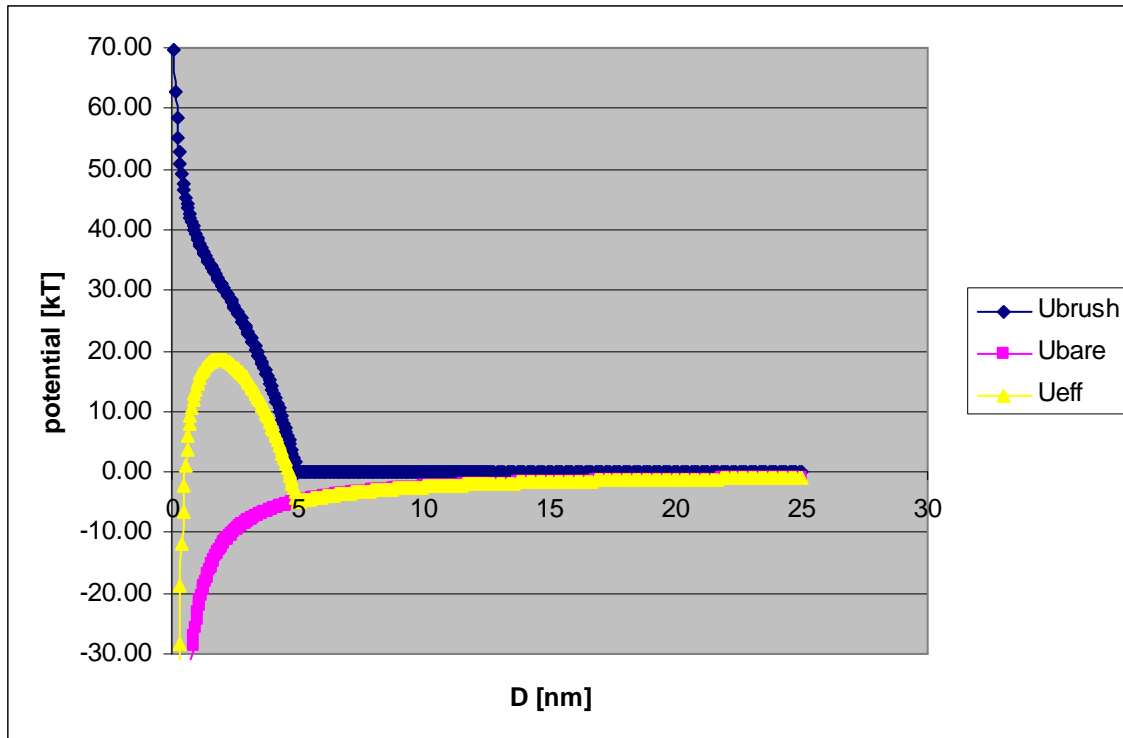
As the Hamaker constant (**A**) changes, the shape of the curve changes overall because the attractive contribution from van der Waals forces is different. If **A** increases, the attraction is stronger and the value for U* will drop. If **A** decreases, the attraction is less and the energy barrier U* will be higher.

c. Calculate a numerical value for k_{ads} for the same protein chosen in part b using values extracted from your plot in part b. Explain what k_{ads} means physically.

As discussed in Halperin's paper, Kramers rate theory defines k_{ads}:

$$k_{ads} \approx \exp\left(\frac{-U^*}{kT}\right)\frac{D}{\alpha L_0}$$

Here, D = diffusion coefficient = $kT/(6\pi\eta R)$; L_0 = uncompressed height of polymer brush; α = width of U_{eff} at U* - kT. We can read the values of α and U* from the plot of U_{eff} in part b. From the plot, α = (2.45 − 1.4) = 1.05 nm and U* = $46k_BT$ (see Excel spreadsheet) (note: U* = $46k_BT$ is calculated from equation 31; you could also use U* = $18.6k_BT$ as read from the graph. The answer is actually very sensitive to this value. We have two different answers because Halperin has not considered all of the necessary prefactors in his scaling relationships). The value of k_{ads} is then

$$k_{ads} \approx \exp(-46)\frac{(5\times10^{-11}m^2/s)}{(1.05\times10^{-9}m)(5\times10^{-9}m)} = 1.0029\times10^{-13}\,s^{-1}$$

For U* = $18.6\,k_BT$, k_{ads} ~ 0.08 s^{-1}.

However, for a large spherical protein, under the compression mechanism, Kramers rate theory has a large error due to the fact that the protein displacements are << R so a description of this as diffusive motion is tenuous.

k_{ads} is the adsorption rate constant and $1/k_{ads}$ gives the characteristic time for a large protein molecule to traverse the length of the brush layer from L_0 down to the surface (page 2528). For BSA, $1/k_{ads} = \sim 10^{13}$ s. This is very slow! It indicates that the protein will prefer to adsorb at the edge of the brush layer (secondary adsorption) rather than at the primary surface. The number can be put into perspective by comparing it to the time required for BSA to move the same distance in bulk aqueous solution. This characteristic transfer time is given by $L_0^2/D = (5 \text{ nm})^2/(5 \times 10^{-11} \text{ m}^2/\text{s}) = 5 \times 10^{-7}$ s. The protein is slowed by at least 19 orders of magnitude in approaching the surface through the brush versus diffusing freely in solution.

3. Elasticity of Fibronection. Abu-Lail, et al. *Matrix Biology* 2006 25 175.
   a. Locate a reliable source for the 3D structure of fibronectin, copy it into your assignment solutions, research and explain in ~1 paragraph the important details of this structure. Professor Zauscher refers to the fibronectin modules as "homologous." Explain what is meant by this.

The protein data bank (www.pdb.org) is a reliable source for 3D protein structures. Structure 1X3D shows the "Solution structure of the fibronectin type-III domain of human fibronectin type-III domain containing protein 3a" as solved by nuclear magnetic resonance; the ribbon image of the molecule is given below.
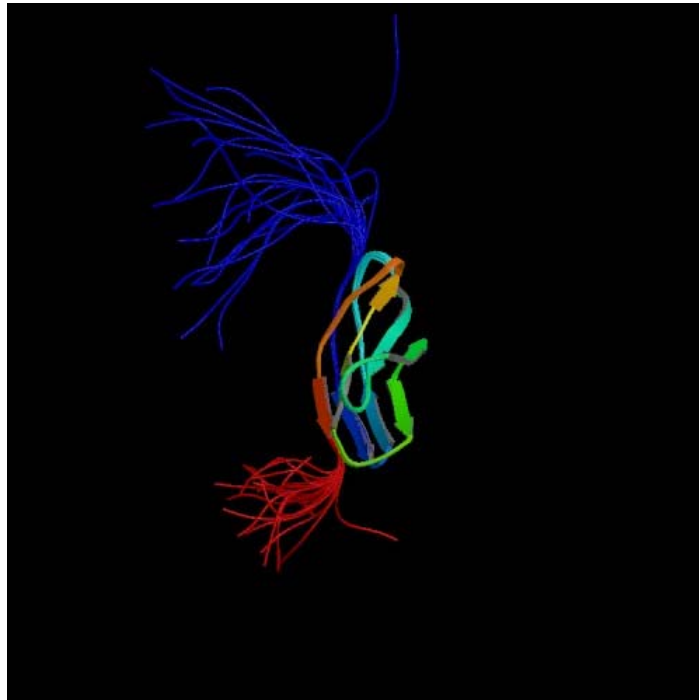
Courtesy of RCSB Protein Data Bank.

The FN-III domain is one of several homologous domains which link together to form the fibronectin molecule. The domains are termed "homologous" because they are largely the same in sequence and structure, with a few differences that give them slightly different properties (such as different contour lengths and unfolding forces, as seen in the curves from figure 1c). Each FN-III domain contains seven β-strands that form anti-parallel β-sheets, also known as β-sandwiches, stabilized by hydrogen bonds, which are able to pop apart upon application of force

(see movie posted on the MIT Server). The fibronectin molecule overall can exist as a soluble protein in the plasma, or as insoluble fibrils in the ECM. The FN-III domain is able to bind membrane-bound receptors on cell surfaces, thereby enabling adhesion of cells to their extracellular matrix.

b. The data in Figure 1c (pulling rate of 580 nm/s) has been digitized and is posted on the MIT Server as an Excel file. Prof. Zauscher stated that one proof that single molecules are being probed is that when the data from each individual peak in a force-separation curve is normalized by the contour length estimated by the worm-like chain (WLC) model, all of the data should overlay and produce a single master curve representing the elasticity of the molecule. Prove this by taking the following steps.

i) Fit each peak in this digitized dataset to the WLC model, as Zauscher has done, plotting all of the model fits on a single graph. Make a table summarizing the fitting parameters for each simulation (corresponding to each peak) and the corresponding contour lengths calculated from these fitting parameters, as well as the mean and standard deviations for the fitting parameters and contour length for FN-III and GFP. Is any difference observed in the estimated persistence length for FN-III and GFP?

The WLC model gives force (f) as a function of extension (r) (r is equivalent to separation distance in Figure 1c):

$$f(r) = \frac{k_B T}{p}\left[\frac{r}{L_{contour}} - \frac{1}{4\left(1 - \dfrac{r}{L_{contour}}\right)^2} - \frac{1}{4}\right]$$

$L_{contour}$ is the fully extended length of the worm-like chain; it is equal to n*p, where n is the number of links per chain and p is the persistence length of the chain.

In order to fit the series of domain unfoldings shown in Figure 1c of the fibronectin paper, the value of $L_{contour}$ has to vary for each domain. This comes from the fact that each domain can be a different size, depending on whether it is a FN-III domain or a GFP domain. Also, the contour length for the WLC fit to any one domain must depend on the contour length of the domain plus the summed contour length of all the domains that have unfolded before it. This leads to the definition of two different contour lengths in the attached spreadsheet: Lc and Ldomain. Lc is the contour length which is used to fit the WLC model to the f vs. r data for each domain. Ldomain is the contour length of the domain itself. Therefore, Lc will be equal to Ldomain plus the Ldomain values for all the domains that are already unfolded before it; therefore it is also equal to Ldomain + Lc from the previous domain.

Following Zauscher's protocol, the persistence length was kept constant at p = 0.42 nm and the number of links per chain was varied to obtain a varying $L_{contour}$. However, one could vary p along with n in order to get better fits to the curves below.

Zauscher data with WLC fits
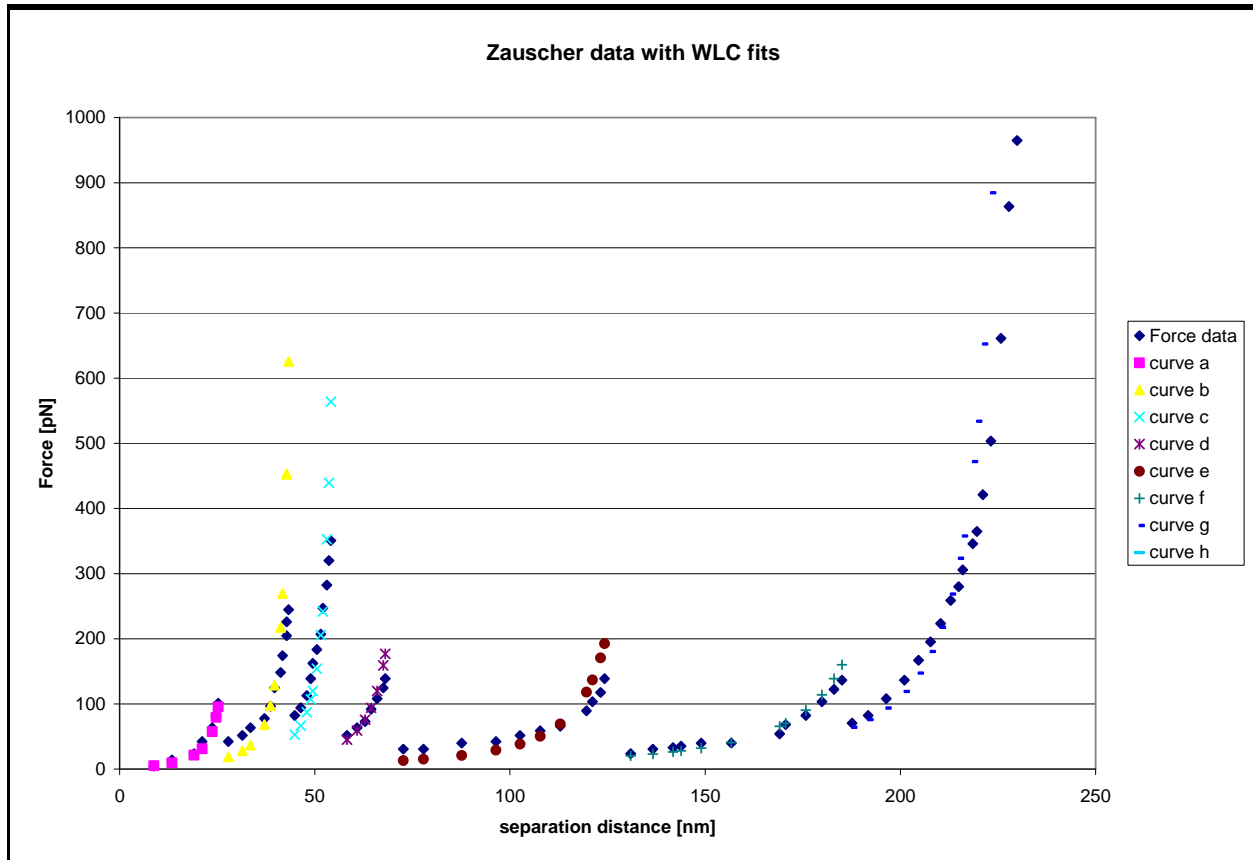
curve	Domain type	N	p	Lc fit	L domain
A	FN III	72	0.42	30.24	30.24
B	FN III	38	0.42	46.2	15.96
C	FN III	28	0.42	57.96	11.76
D	FN III	46	0.42	77.28	19.32
E	GFP	150	0.42	140.28	63
F	GFP	170	0.42	211.68	71.4
G	FN III	57	0.42	235.62	23.94

	Mean n	Stdev n	mean Ldomain	stdev Ldomain
FN III	48.2	17.035	20.244	7.154808174
GFP	160	14.142	67.2	5.939696962

The data show that the contour lengths of FN III and GFP domains are markedly different, as stated in the paper. On average, FN III domains have a contour length of about 30 nm, while GFP domains have a much longer contour length around 67 nm.

> **ii) From the values obtained in i), create two separate master elasticity curves, i.e. two plots; one for FN-III and one for GFP. Does this analysis support Zauscher's assertion that single molecules are being probed?**

Master Curve for FN-III

Master Curve for GFP
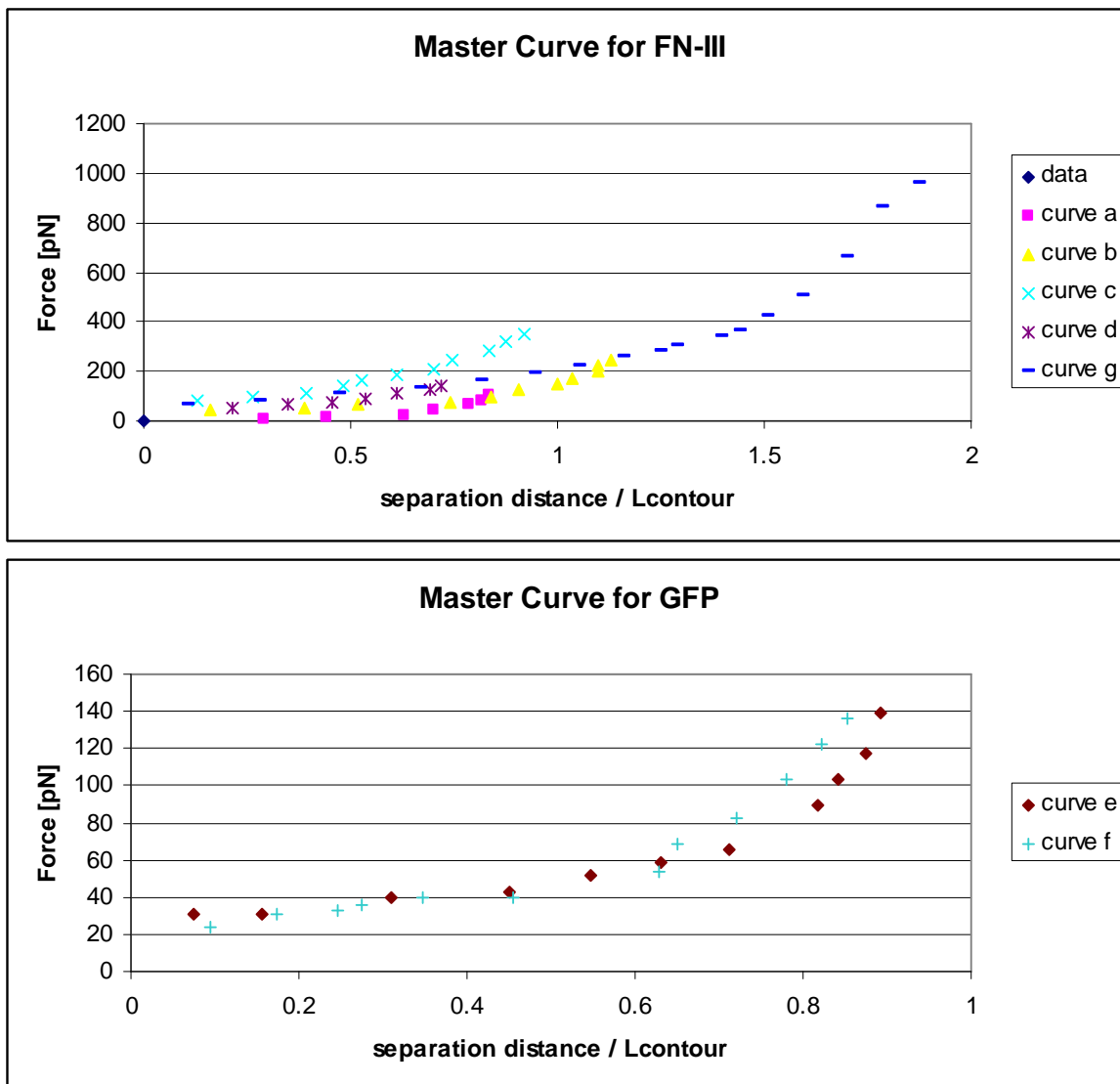
These master curves were created by normalizing the separation distance for each curve by its contour length, and off-setting the values by the separation distance of the preceding force peak. See attached Excel file for details. The curves are fairly consistent and support Zauscher's hypothesis that a single molecule is probed, because multiple molecules would skew the contour length of any given curve such that it would no longer line up with the master curve after normalization.

c. Calculate the theoretical contour lengths based on known peptide bond angles for FN-III and GFP and compare to the values obtained in 3a.

As given in Section 3.4 of the paper, FN-III domains have 90 amino acids and GFP has 239. The tetrahedral geometry of the peptide bond in a fully-extended β-sheet gives a length of about 0.34 nm per residue. For FN-III, $L_{contour}$ = (0.34)*90 = 30.6 nm. The contour length seen in the experiments is actually the fully unfolded length, 30.6 nm, minus the folded diameter of the protein, 3.6 nm, which gives a contour length of 27 nm in close agreement with the data. For GFP, $L_{contour}$ = (0.34)*239 = 81.26 nm. After subtracting the unfolded protein diameter, about 2

nm, this gives a contour length of 79 nm, in the same range as the data but not as closely aligned.

d. Section 2.2 on page 177 is entitled "Fingerprints of FN-III and GFP Domains"- what do the authors mean by "fingerprints"?

Molecular "fingerprints" mean that each protein should have its own unique elasticity profile, unique persistance length and Lcontour. For example, when the authors analyzed the unfolding lengths of the different unknown domains in their force vs. separation distance curves, they found that the lengths fell into two distinct groupings, one around 28 nm, and one around 75 nm. These groupings corresponded well to the expected unfolded lengths of FN-III and GFP domains, respectively, and so the authors termed the lengths the "fingerprints" of FN-III and GFP domains.

e. How did Prof. Zauscher link his single molecule data presented in the paper to the two possible mechanisms of fibronectin deformation? Did you find his argument convincing? Why or why not?

If the force to unfold GFP is much lower than the force required to unfold a fibronectin domain, then if the GFP-based FRET signal is still present at a given stretch, indicating an intact GFP structure, the fibronectin domains must also be intact. In reality, the forces are roughly equal for GFP and fibronectin domain unfolding. The authors concluded that since their fibronectin constructs could undergo a 400% stretch while maintaining the FRET signal, a compact-to-extended fibronectin conformation change was more likely than domain unfolding. For further explanation, listen from ~ minute 40 on the podcast.