

### 3.052 Nanomechanics of Materials and Biomaterials: Spring 2007 Assignment #6

**Due Date:** Friday 05.11.07

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 any papers or podcast.

+ 5 extra credit for posting a message on one of the podcast message boards.

#### 1. Sacrificial Bonding in Biological Materials Podcast. Fantner, et al. *Biophys. J.* 2006 90, 1411.

**a. Dr. Fantner comments that a modular structure with folded domains is not necessary for sacrificial bonding. Explain why. Where are the sacrificial bonds in Figure 3D?**

Modular domains are not required; sacrificial bonding refers to any situation where breaking the bond frees up additional contour length that was otherwise “hidden” by the bond. For example, in Figure 3D, as in Figure 3C, the sacrificial bonds are those connecting the molecules to the substrate. When the shortest molecule breaks first, it frees up the hidden length of the longer molecules still attached to the substrate.

**b. In Figure 2, plots 2 and 3, Dr. Fantner explained that to create these simulated WLC curves, he used the same forces and same spacing between sacrificial bonds (i.e., A and B are always the same linear distance apart as you travel along the molecule). Why are the rupture distances for case 3 farther apart than those for molecule 2?**

When a sacrificial bond breaks in molecule 2, the hidden length revealed is the linear distance between bonds A and B. When a sacrificial bond breaks in molecule 3, the hidden length revealed is actually twice that distance, due to the parallel strand structure of the molecule. This leads to the longer spacing between ruptures as the molecule is pulled apart.

**c. Is the increased energy dissipated in the systems described by Dr. Fantner with sacrificial bonding due to enthalpic contributions? Why or why not?**

No, the increased energy dissipation is primarily entropic in origin. There is some enthalpic energy dissipated in breaking the sacrificial bonds, but most work in stretching the systems described by Dr. Fantner goes into stretching out the hidden length. This work is against the entropic recoil of the hidden length of the polymers.

**d. Consider a modular polymer with a fully unfolded contour length  $L_{\text{contour}}(\text{unfolded})$  of 20 nm and a persistence length of 1 nm. Calculate and plot the normalized energy dissipated upon single chain stretching versus number of domains,  $N$ , from 0-20 where the contour length of each unfolded domain is  $L_{\text{contour}}(\text{unfolded})/N$  and the rupture force = 200 pN. The energy normalization factor in the denominator should be for  $N = 1$ . The most feasible way to complete this problem is by writing a code in Mathematica or Matlab.**

See next page for answer.

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%% WLCpset6.m
%% for pset6, Q1
%% dcfrance 05.03.07 for 3.052 Spring 2007

clear all

% contour length of entire molecule [nm]
Lc = 20;
% persistence length [nm]
p = 1;

% N = number of domains making up molecule;
% Lcd = fitting contour length for domain n out of N domains in a chain
% forces calculated in units of kBT/nm
% rupture force = 200 pN = 49 kBT/nm

EN = [];
figure;
for N = 1:20
    % calculate force according to WLC model
    f = []; Edn = []; r = []; rrupture = [];
    for n = 1:N
        Lcd = n*Lc/N;
        rn = [Lcd/1000:Lcd/1000:Lcd-Lcd/1000];
        fn = (rn./Lcd + 1./(4*(1 - rn./Lcd).*(1 - rn./Lcd)) - 0.25) ./ p ;
        % cut off force at rupture
        fnzeroes = [];
        for i = 1:length(fn)
            if fn(i) < 49
                fn(i) = fn(i);
            else
                fn(i) = 0;
                fnzeroes = [fnzeroes i];
            end
        end
        r = [r; rn];
        % save rvalue where force ruptured
        rrupture = [rrupture rn(min(fnzeroes))];

        % to calculate energy of domain deformation,
        % first set range of r values over which to sum (only the r values
        % corresponding to that domain); do this by setting force of the domain

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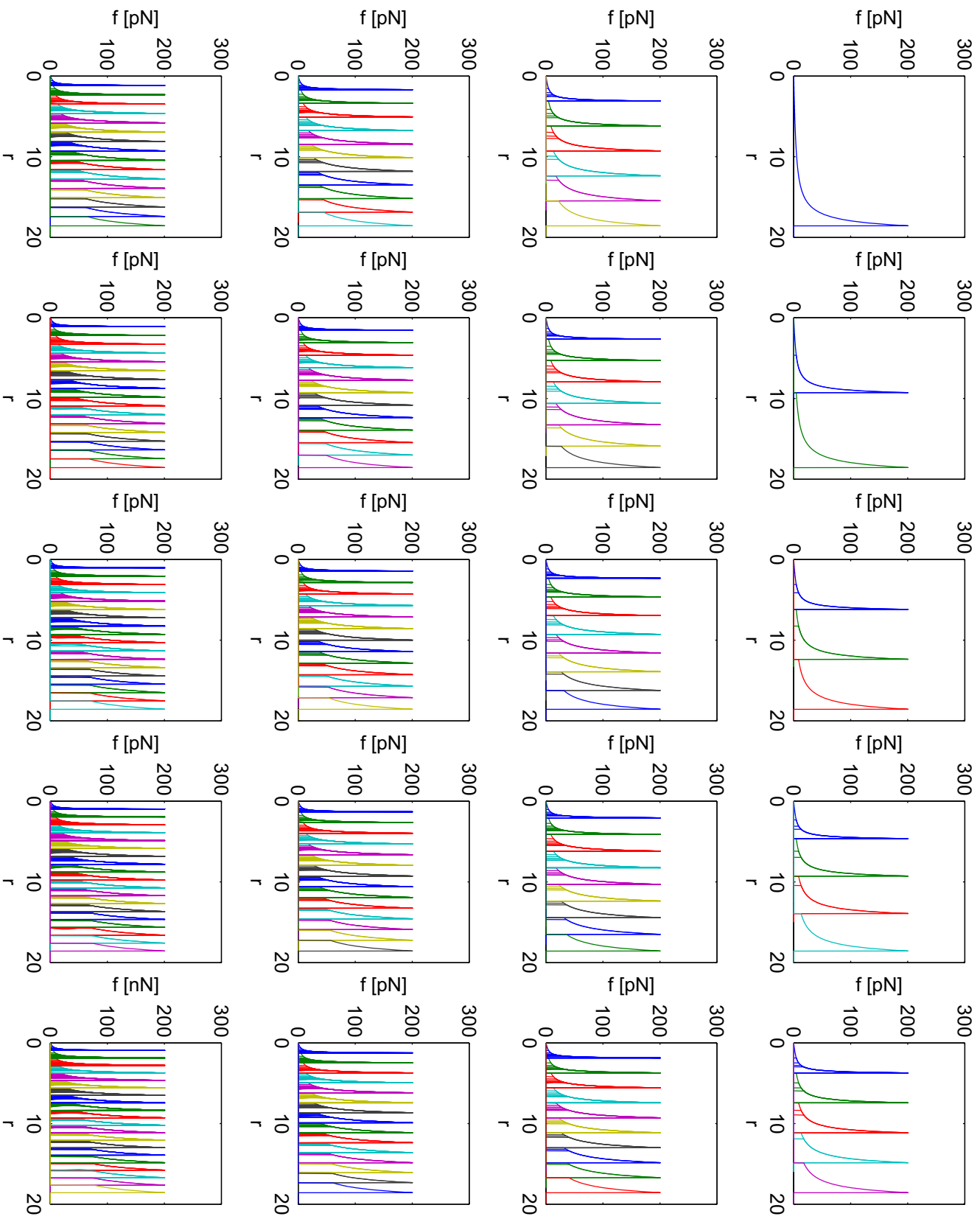
% equal to zero over the r values when it is "hidden" by the domain
% before it
if n > 1
    for i = 1:length(rn)
        if rn(i) < rrupture(n-1)
            fn(i) = 0;
        end
    end
end
end

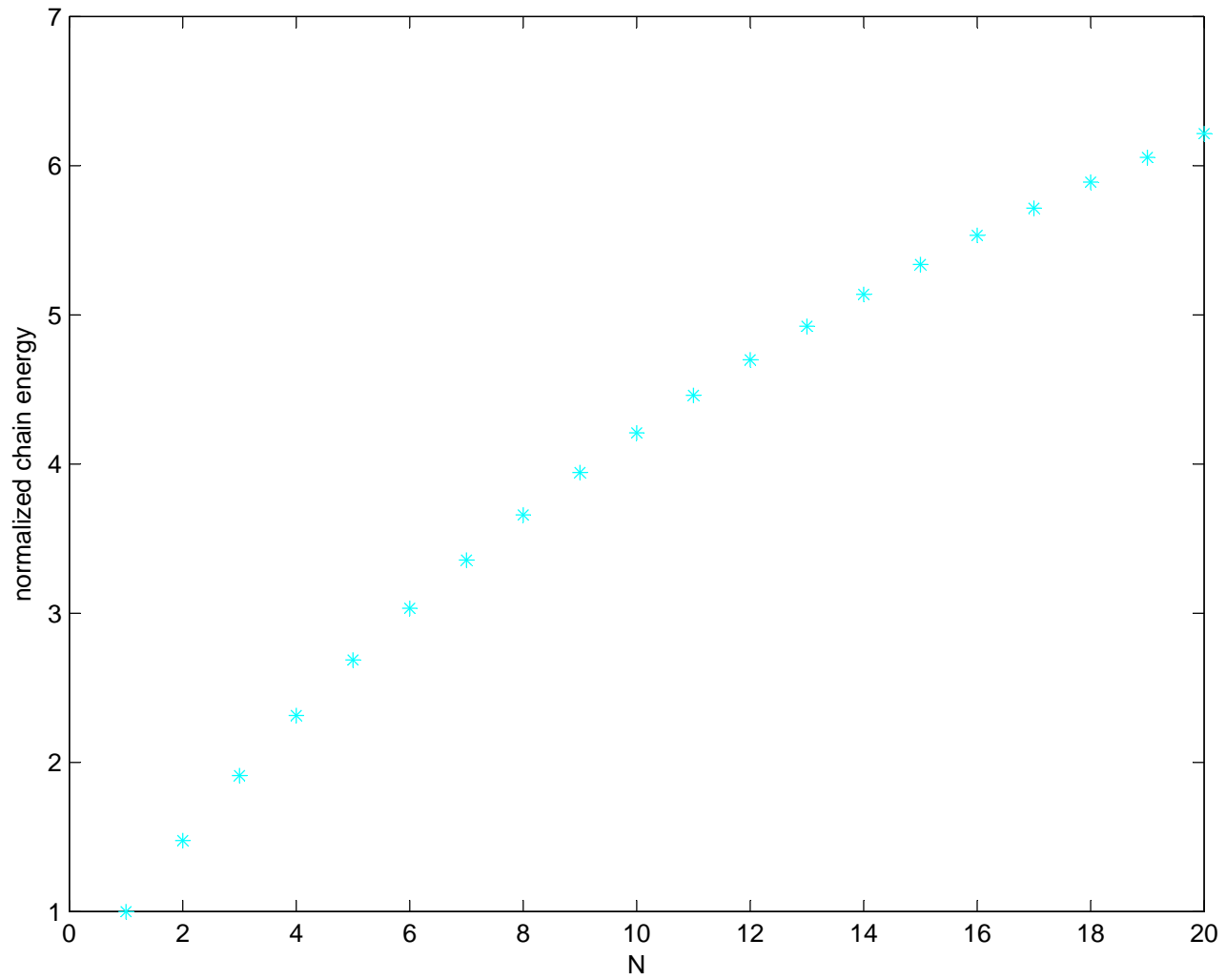
% calculate energy of domain deformation (=sum(fn.*rn)) and add to array
% "Edn" containing domain energies for each domain within chain N
Edn = [Edn sum(fn.*rn)];
% plot force
subplot(4,5,N);
plot(r,fn.*(4.1e-21).*(10^21)); hold on
end
xlabel('r'); ylabel('f [pN]');

% calculate stretching energy per chain as the sum of all domain
% energies in the chain (EN) and add to array
EN = [EN sum(Edn)];
end
xlabel('r'); ylabel('f [nN]');

% plot chain energies normalized by energy of chain with N = 1 domain
figure
plot([1:N],EN./EN(1),'c*')
xlabel('N'); ylabel('normalized chain energy');

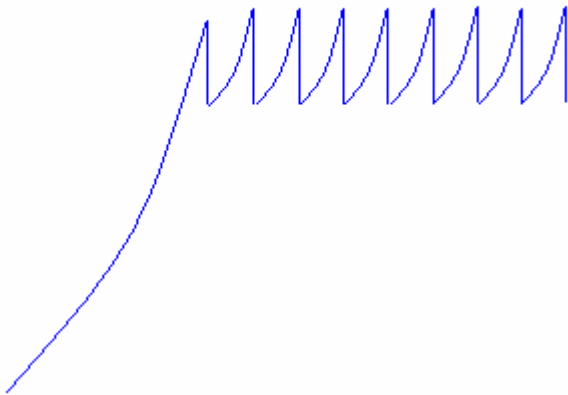
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**e. Draw a schematic of an "ideal" force vs. distance curve for maximum energy dissipation involving sacrificial bonding, point out the different features of your schematic including where the sacrificial bonds rupture, and explain how they lead to increased energy dissipation.**

An "ideal" force vs. distance curve will start with a high global entropic chain stiffness, so that the area under the curve (equivalent to the energy dissipated) will quickly increase. A plateau with high extensibility makes up the rest of the curve, so that the cell dissipates as much energy as possible at the high forces required to reach the plateau.



This type of curve can be achieved by a long, stiff domain making up most of the length of the molecule; if it has a lower rupture force than the other smaller domains making up the rest of the molecule, it will absorb all of the energy at first and create the rise to the plateau. Then the rest of the molecule will be made up of small domains that don't release much contour length as their sacrificial bonds rupture, so that the force never has a chance to really drop back down to zero.

The image above shows a schematic of what the curve might look like, but if taken to the limit of small domains, it would look a more continuous "squeakle" of high extensibility along the plateau. Each additional domain adds to the total amount of energy which can be dissipated by the chain. A limit to the number of domains in an ideal curve will come when the domain length comes too close to the persistence length and folding of a domain is actually not realistic due to the stiffness of the chain.

**f. Professor Zhibin Guan is a chemist at UC Irvine who has been designing synthetic polymers using the sacrificial bonding concept. Look up one of his papers on this topic and write a one paragraph summary (do not copy abstract).**

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**g. Sacrificial bonding is thought to be particularly important to the fracture resistance of bone. Research the proposed molecular origin in the literature (i.e. which molecules and structures could be involved).**

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- 2. Nanomechanics of DNA (\*data taken from Bustamante, et al., *Nature*, 2000, 404,103).**
- a. Based on your knowledge of the structure of DNA, the electrostatic double layer, and of theoretical models for single molecule elasticity, how would you expect the global entropic chain stiffness (slope of force vs. distance curve) for stretching of a single DNA molecule to vary with salt concentration and why?**

DNA is negatively charged, so that as the salt concentration increases, the positive salt ions will act to screen the negative charges on the DNA. This leads to a reduction in the intramolecular electrostatic repulsion of fixed charge groups along the chain, and allows the DNA chain to access a greater number of random, thermally-driven conformations. A higher number of conformations is the basis of higher elasticity, which means that the chain will be more difficult to stretch out from its equilibrium position. The increased difficulty would manifest as increased global entropic chain stiffness in the force vs. distance curve; i.e., the slope will increase with increased salt concentrations.

- b. Below is a schematic and plot of optical tweezers data for stretching of double-stranded (ds) compared to single-stranded (ss)DNA. Explain all of the differences and similarities between the two curves.**



Figure removed due to copyright restrictions. See Figure 1 in Bustamante et al, *Nature* 404 (2000): 103.

Differences: Single-stranded DNA behaves as a typical worm-like chain, with monotonically increasing force and stiffness as extension increases. Double-stranded DNA, on the other hand, has various different stiffnesses, and a sudden transition from one stiffness around a fractional extension of one to a much lower stiffness that takes over until a fractional extension of around 1.7. Double-stranded DNA also starts out at a much lower stiffness in low extensions; it requires much less force to stretch dsDNA at low extensions that it does for ssDNA. The difference can be attributed to the different persistence lengths of the two molecules: ssDNA has a lower persistence length so it leads to higher forces.

Similarities: Both curves tend towards the same stiffness at very high extensions, after dsDNA has undergone its form transition.

**c. The x-axis of the force vs. extension curve is given as “fractional extension” for dsDNA. How is it that “fractional extension” can be greater than one? Use this information to calculate the distance between adjacent base pairs in dsDNA in the two different structural forms depicted in the plot.**

Fractional extension can be greater than one because the dsDNA actually changes form, from B-form to S-form. Extension of one is equivalent to stretching out the full contour length of B-form dsDNA, which has a lower contour length than the same chain in S-form. From the graph, we can read off two approximate contour lengths for the two different forms (B-form and S-form) of dsDNA; for B-form, (fractional) contour length is set at the fractional extension  $\sim 1$ ; for S-form,

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the (fractional relative to B-form) contour length is around 1.8. This means that spacing between base pairs in S-form is 1.8x that in B-form. The rise per base pair, equivalent to linear spacing between base pairs, in B-form dsDNA is 0.34 nm [Stryer, *Biochemistry*, 4<sup>th</sup> Ed, 1998, p791]. This means that the spacing increases to  $\sim 0.34 \times 1.8 = 0.612$  nm.

### 3. Nanoindentation of Seashell Nacre: Bruet, et al. *J. Mater. Res.* 20(9), 2005. 2400-2419.

- a. Create two tables; one for fresh nacre and one for hydrated nacre. In each table, create columns with numerical values obtained from and calculated from data in the paper for  $P_{\max}$ ,  $h_{\max}$ , and  $A_{\max}$ .  $P_{\max}$  is the maximum load upon indentation,  $h_{\max}$  is the maximum depth upon indentation, and  $A_{\max}$  is the projected contact area at  $P_{\max}$  and  $h_{\max}$ . You can assume that the Berkovich probe tip has an ideal shape.

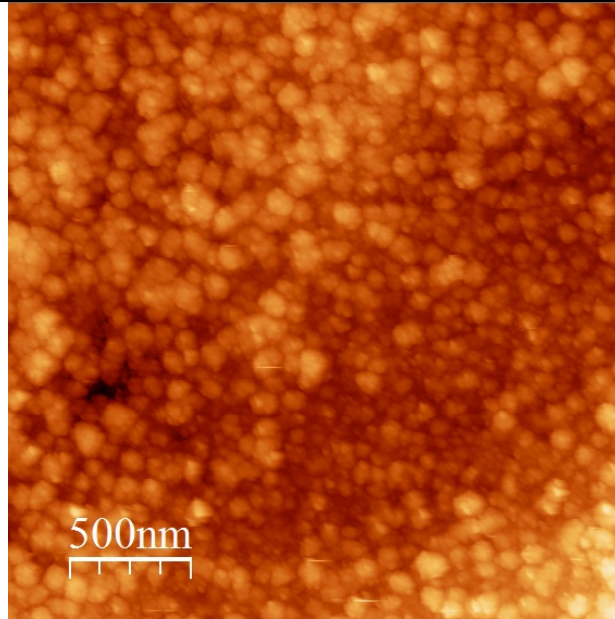
For an ideal Berkovich tip,  $A_{\text{projected}} = 24.5 h^2$ . We can measure the max depths from Fig 11 a and b. Then we find the following areas for the two experiments:

Experiment ( $\mu\text{N}$ )	Fresh nacre		Hydrated nacre	
	max depth (nm)	max A ( $\text{nm}^2$ )	max depth (nm)	max A ( $\text{nm}^2$ )
50	9.5	2211.13	16.8	6914.88
100	15.1	5586.25	25.8	16308.2
250	27.3	18259.6	32	25088
500	42.4	44045.1	51.5	64980.1
750	54.5	72771.1	63.7	99413.4
1000	66.7	108998	72.8	129846

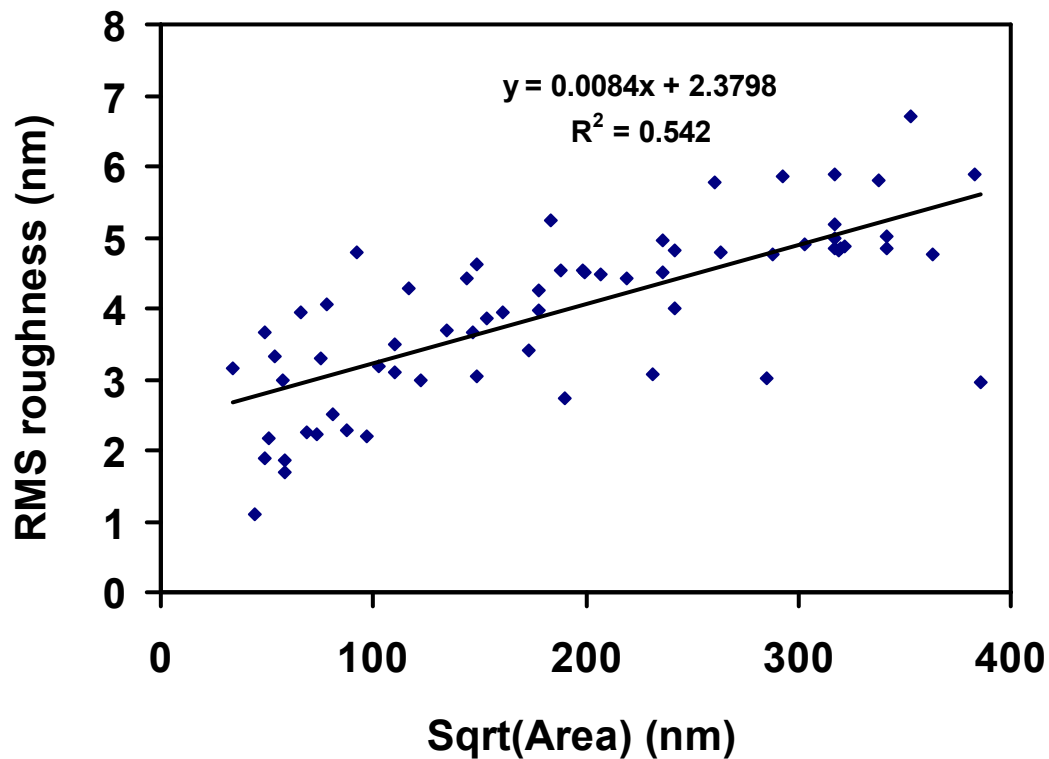
We see that the areas are all within the range  $2 \times 10^3 - 1.3 \times 10^5 \text{ nm}^2$  so we want to measure the RMS roughness of areas within this range on the AFM image.

- b. Measure the RMS surface roughness of a real nacre sample using the AFM image posted on the MIT Server which spans the full range of  $A_{\max}$  you obtained in 3a. Perform at least 50 measurements of different sizes. Plot RMS surface roughness (nm) vs.  $\sqrt{A}$  ( $\sqrt{\text{nm}}$ ) and carry out a linear regression of this data.

AFM image provided:

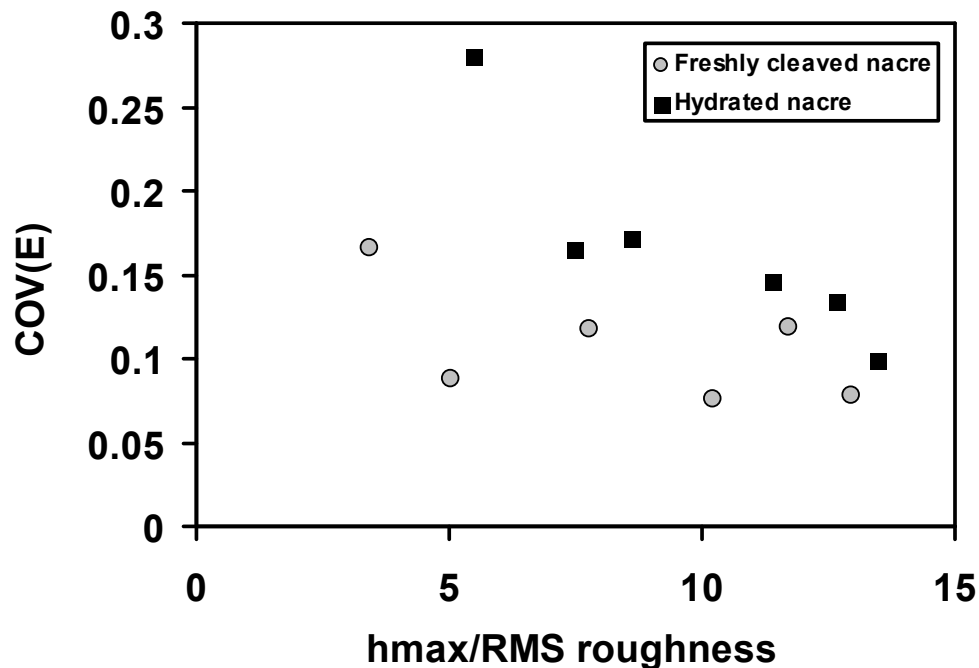


Plot of the RMS measurements (~70 data points) using WsXM:



- c. Datasets of modulus values for each maximum load (calculated from the Oliver-Pharr method) are posted on the MIT Server. Calculate and plot the coefficient of variation  $COV(E) = \text{standard deviation}/\text{mean}$  for both fresh and hydrated nacre vs. the ratio  $h_{\text{max}}/\text{RMS roughness}$ . Explain the resulting trends.

Experiment ( $\mu\text{N}$ )	Fresh nacre			Hydrated nacre		
	max depth	RMS	ratio	max depth	RMS	ratio
50	9.5	2.77479	3.423683	16.8	3.078308	5.457543
100	15.1	3.007626	5.020571	25.8	3.452509	7.472826
250	27.3	3.514876	7.766988	32	3.710292	8.624658
500	42.4	4.142702	10.23487	51.5	4.521061	11.39113
750	54.5	4.645794	11.73104	63.7	5.028311	12.66827
1000	66.7	5.153045	12.9438	72.8	5.40667	13.46485



The COV(E) has high values for 50 $\mu\text{N}$  indents (with max depth of  $\sim 10\text{nm}$ ) and then decreases toward 10%. Most likely this is due to the roughness of the sample (we've seen that the max height of the nanoasperities is 7.4nm). For the hydrated sample in Fig 12 the nanoasperities appear more swollen (greater roughness) so this can explain why the decrease for the COV is slower for the hydrated sample.

The nanoscale heterogeneity plays a role in the equilibrium value for the COV (about 10% here). For very homogeneous samples such as Fused Silica ( $\text{SiO}_2$ ) typical values are 2%, that is, 5 times smaller. The shape of the indenter also plays a role in the sensitivity to small scale heterogeneity. A rather flat tip such as the Berkovich tends to be quite sensitive to surface roughness and less to the material heterogeneity because it probes large areas. A very pointy tip will be less sensitive to surface roughness and more to material heterogeneity, but its tip area function will be more difficult to characterize accurately and it will tend to wear (change shape) faster.

**d. Describe the advantages and disadvantages of the finite element analysis (FEA) approach compared to Oliver-Pharr (O-P) approach.**

The Oliver-Pharr method assumes an isotropic, elastic, continuum formulation that works for any geometry. Since it is an analytical solution, an enormous amount of data can be processed very rapidly. It assumes sink-in which may not be valid for certain types of plastic deformation. FEA is a computational method, much slower than O-P, that can take into account full constitutive models such as yield stress, hardening, etc., to more accurately capture the material behavior. However, this adds more fitting parameters and it can be difficult to obtain a unique solution.

**e. Explain the difference between Hardness values calculated from the Oliver-Pharr approach compared to AFM imaging measurements of residual indent area.**

Hardness calculated using O-P is calculated from the contact area at maximum depth and hence, includes both elastic and plastic deformation; i.e. it is total resistance to deformation. Hardness calculated using AFM imaging of the residual indent is analogous to traditional hardness and represents resistance to plasticity only.