

### 3.052 Nanomechanics of Materials and Biomaterials : Spring 2007 Assignment #2 Solutions

**Due Date** : Tuesday 03.06.07

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.

#### 1. Piezos :

**a. Monolithic z-tube scanners operate on the "transversal piezo effect." Explain what this means.**

ANS. Lecture 4, Slide 2-3 describes the function of the z-piezo tube scanner. An additional reference is the corresponding reading "*Basic introduction to nanopositioning with piezoelectric technology*", Physik Instruments, Inc., Course Reader Document #9. The general idea is that an electric voltage is applied between the outer and inner diameters of the tube, the tube contracts axially and radially (by a relatively small amount) and due to a positive Poisson's ratio expands much more in z-direction due to high aspect ratio. In other words, a transversal activation (voltage) across the tube translates into dimension/displacement changes along the length of the tube.

**b. Would you be able to quantitatively measure the height of the rabies virus adsorbed on a surface using AFM imaging if you were using a piezo tube scanner with the following properties ( $d_{31}=-1.73 \text{ \AA/V}$ ,  $d = 0.5588 \text{ mm}$ ,  $L_o=12.7 \text{ mm}$ ) and a voltage range of +18V to -18V?**

ANS. From Lecture 4: Slide 2 the equation for the z-piezo extension of a tube scanner,  $\Delta L$ , is given by

$$\Delta L = \frac{d_{31} \times U_3 \times L_o}{d}$$

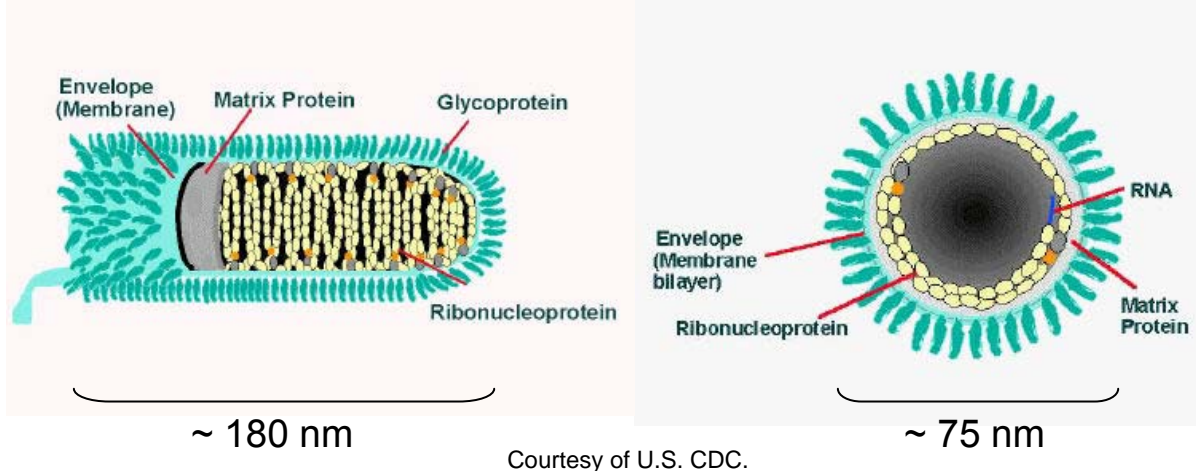
where :  $d_{31}$  = strain coefficient [ $\text{\AA}/\text{volt}$ ];  $U_3$  = voltage applied across direction 3 [Volts];  $d$  = wall thickness [mm]; and  $L_o$  = initial tube length [mm].

The negative value of the strain coefficient indicates that the tube contracts when a positive voltage is applied. Substituting in values one obtains :

$$\Delta L = \frac{(-0.173 \frac{\text{nm}}{\text{V}})U_3(12.7\text{mm})}{(0.5588\text{mm})} = -3.93 \frac{\text{nm}}{\text{V}} U_3$$

This defines the z-scan range; the tube can deflect from -70.8 nm at +18 V to +70.8 nm at -18V; the range is therefore  $\approx 142 \text{ nm}$ .

The dimensions of the rabies virus are shown below (<http://en.wikipedia.org/wiki/Rabies>).



The piezo tube would not be able to accommodate the height (~180 nm) of a virus which happens to absorb to the surface with its long axis at exactly  $90^\circ$  to the imaging plane. However, because the viruses would deposit on the surface in a variety of orientations, if one took a large sample of images, it would become apparent that the virus is roughly cylindrical with a length and diameter than can be reliably measured.

c. A new 3-axis nanositioner has been designed by *Asylum Research, Inc.* for their 3D nanomechanics instrument called the "Molecular Force Probe," as detailed in the "Introduction to AFM" movie you were assigned to watch. Label and explain the function of the following components shown in the photo below; x- and y- piezo stacks, x- and y-axes flexure levers, x- and y-axes flexure fulcrums, x- and y-axes central stages. Explain what a "nested design" is and what are its advantages compared to the tube piezo.

ANS. Figure 1. 3-axis nanositioner in Asylum Research, Inc. Molecular Force Probe.

Photo removed due to copyright restrictions.

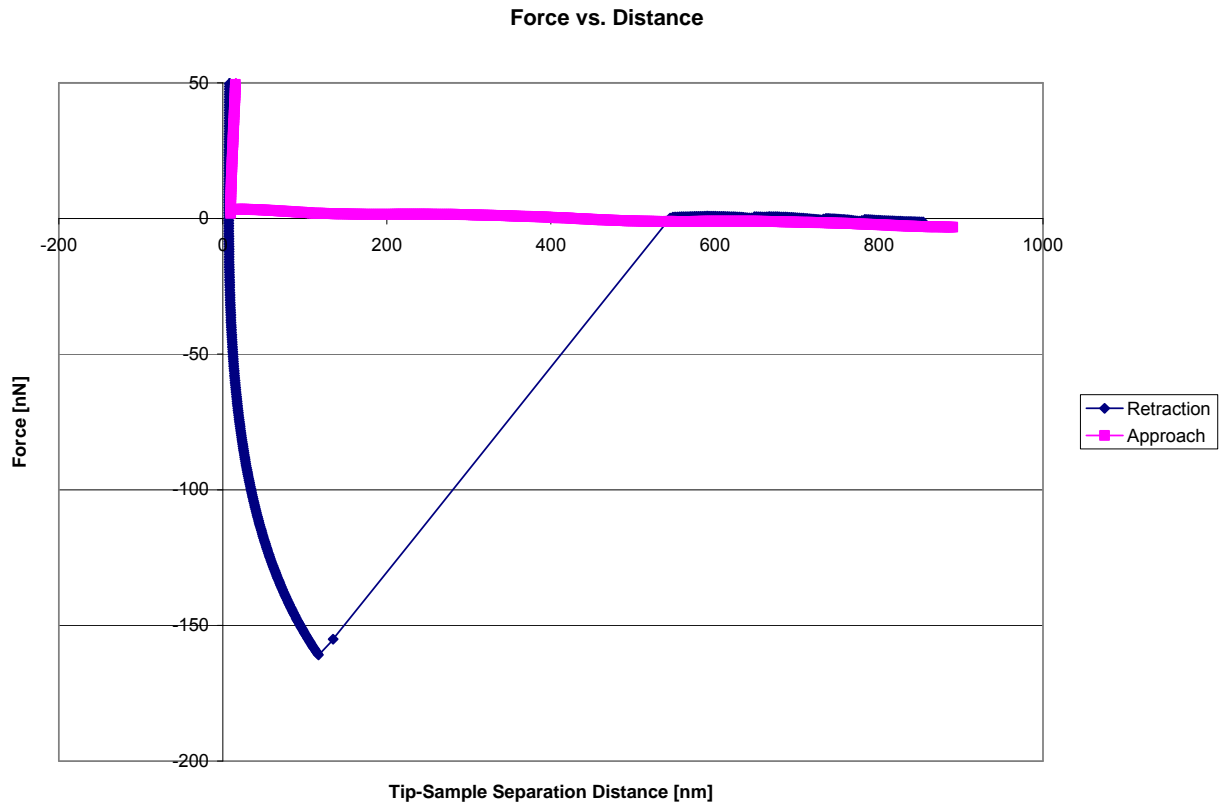
The flexure design uses piezo stacks to drive motion in one dimension at a time. The piezo stack in x or y expands longitudinally in response to an applied voltage. This pushes through the flexure fulcrum and on the flexure lever to move the x or y central stage. Because the x stage is wholly seated within the y stage, the design is called "nested". The largest advantage of the flexure design over a tube piezo is that the motion in any one direction is completely independent and uncoupled from movement in other directions.

**2. Force-Distance Curves. A high resolution force spectroscopy experiment was conducted normal to a mica surface (z-direction) using an atomic force microscope with a  $\text{Si}_3\text{N}_4$  cantilever ( $k = 0.38 \text{ N/m}$ ) and probe tip in air. The sample was first lowered to  $\approx 1000 \text{ nm}$  below the probe tip, data acquisition was initiated, and then the piezo incrementally moved the sample towards the tip in the z-direction relative to the fixed probe tip position, i.e. "approach." Upon contact with the surface, the piezo then reversed direction and incrementally moved away from the tip in the z-direction, i.e. "retract." The raw datafile consists of z-piezo deflection, z (nm) (column 1) versus sensor output, s (V).**

**a. Convert this data into force, F (nN), versus tip-sample separation distance, D (nm), and plot (feel free to use any software program you would like, e.g. it can be done in an Excel spreadsheet).**

ANS. See attached excel file for full conversion and graphing procedure.

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**b. Explain the origin of the adhesion force given its magnitude and range, as well as the known surface chemistry of mica.**

ANS. In Lecture 4, Professor Ortiz commented that in air a thin layer of water exists on the surface of materials. Mica has many silanol groups on the surface and hence, is hydrophilic and will possess a large amount of water on the surface. Hence, the large force here is due to capillary action of a water meniscus between the tip and surface. All of the other noncovalent mica- $\text{Si}_3\text{N}_4$  interactions are small compared to the capillary effect.

**c. Can you calculate an accurate value of the adhesion energy, why or why not?**

ANS. No, you cannot calculate an accurate value for the adhesion energy because upon retraction, the cantilever exhibits a mechanical instability on pull-off whereby it is moving too fast to record any data. This makes it impossible to accurately integrate under the adhesion force curve to obtain adhesion energy.

**d. Looking at the data, can you tell if the mica is being indented by the probe tip? How?**

ANS. If mica was being indented, the force-distance curve would never reach a region of apparent infinite slope at large compressions. The curve would instead continue to have a finite negative slope. In the given experiment, the mica does not appear to be indented by the tip.

### 3. AFM Imaging : Artifacts.

An artifact called "tip broadening" sometimes occurs in AFM images whereby the width of a feature appears larger than the true size. As you will see, this phenomenon can actually be used to measure the probe tip radius. Consider the case of a sharp probe tip of triangular geometry imaging a larger nanoparticle fixed to a planar substrate where the tip radius is much less than the feature curvature radius (*Figure 2*).

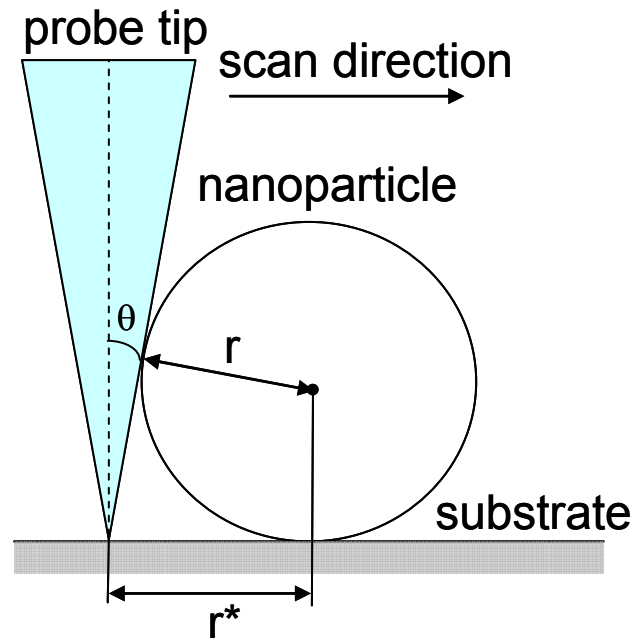
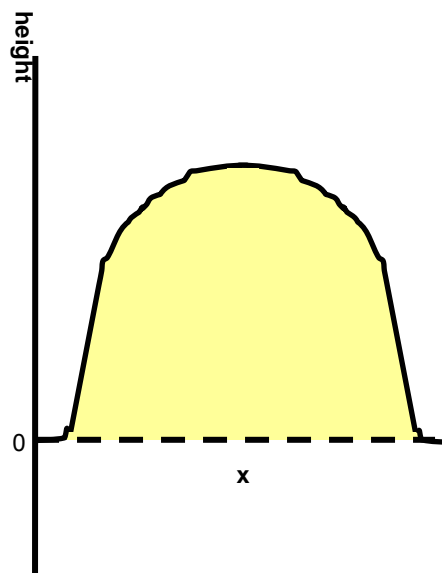


Figure 2. Geometry of a sharp probe tip imaging a spherical nanoparticle where  $r$  = nanoparticle radius,  $r^*$  = apparent nanoparticle radius,  $\theta$  = probe tip angle.

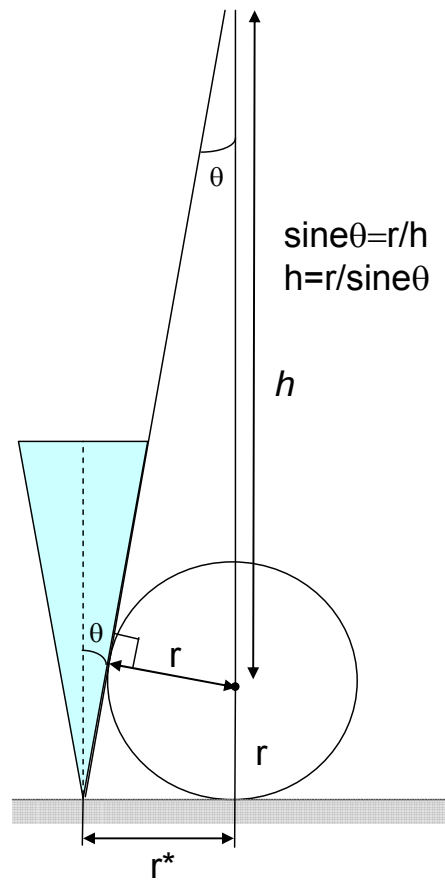
a. When the nanoparticle is imaged, it does not appear spherical in shape. Draw schematically the 2-dimensional height profile of what the nanoparticle will look like and explain why.

ANS.



The 2D height profile appears this way because of the geometry of the tip – it has a finite size and shape so it is not able to fit into all of the negative space not filled by the particle being imaged. Some negative spaces are inaccessible and will contribute to the appearance of the height profile.

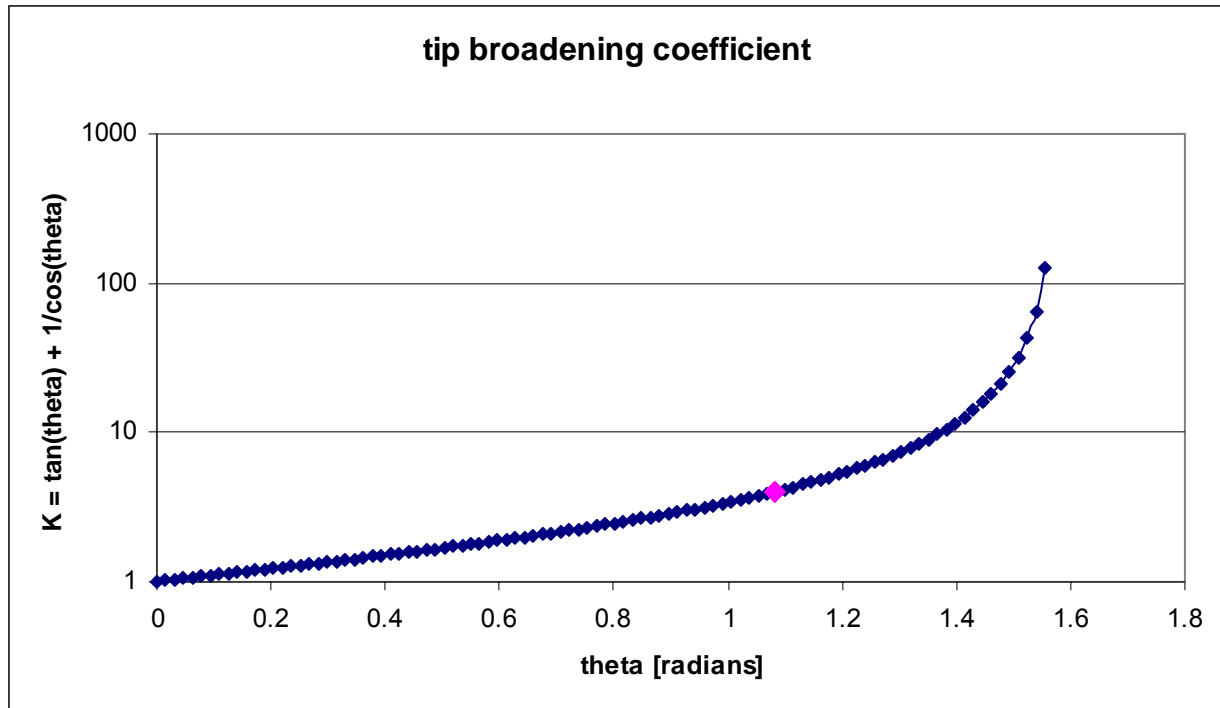
**b. Calculate  $r^*$  in terms of  $r$  and  $\theta$  and then estimate the broadening correction coefficient  $K$  where  $r^*=Kr$ . Plot  $r^*/r$  versus  $\theta$ .**



$$\tan\theta = \frac{r^*}{r + \frac{r}{\sin\theta}} \rightarrow \frac{r^*}{r\left(1 + \frac{1}{\sin\theta}\right)}$$

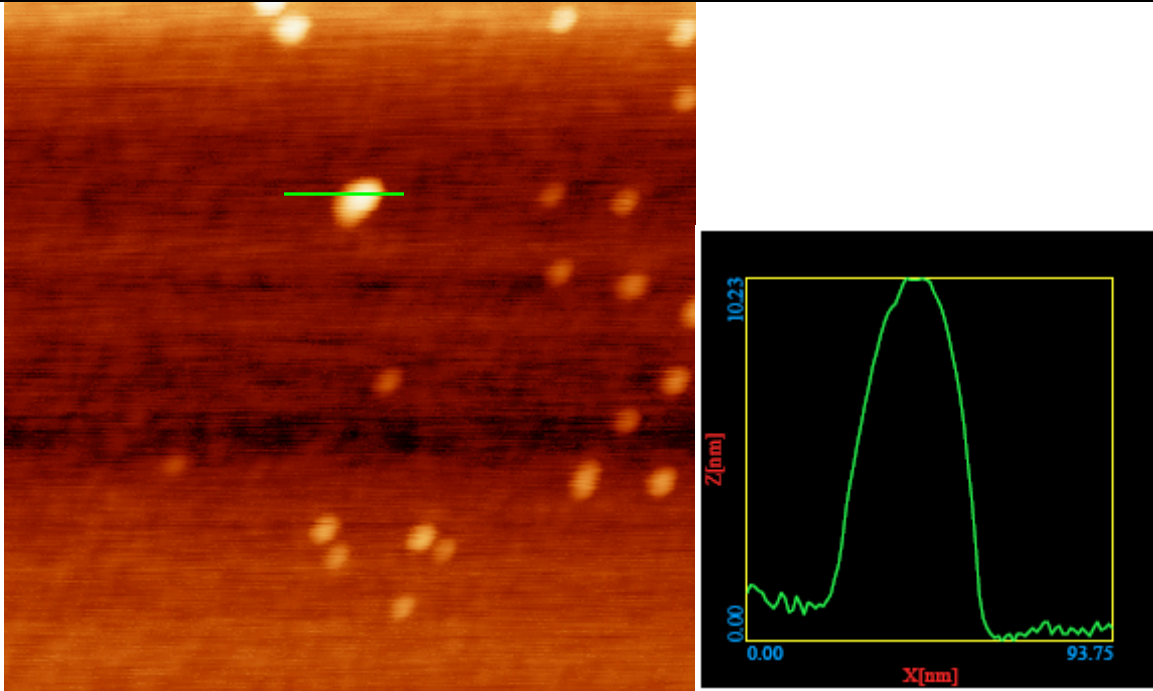
$$r = \frac{r^*}{\tan\theta\left(1 + \frac{1}{\sin\theta}\right)}$$

$$K = \frac{r^*}{r} = \tan\theta\left(1 + \frac{1}{\sin\theta}\right) = \tan\theta + \frac{1}{\cos\theta}$$

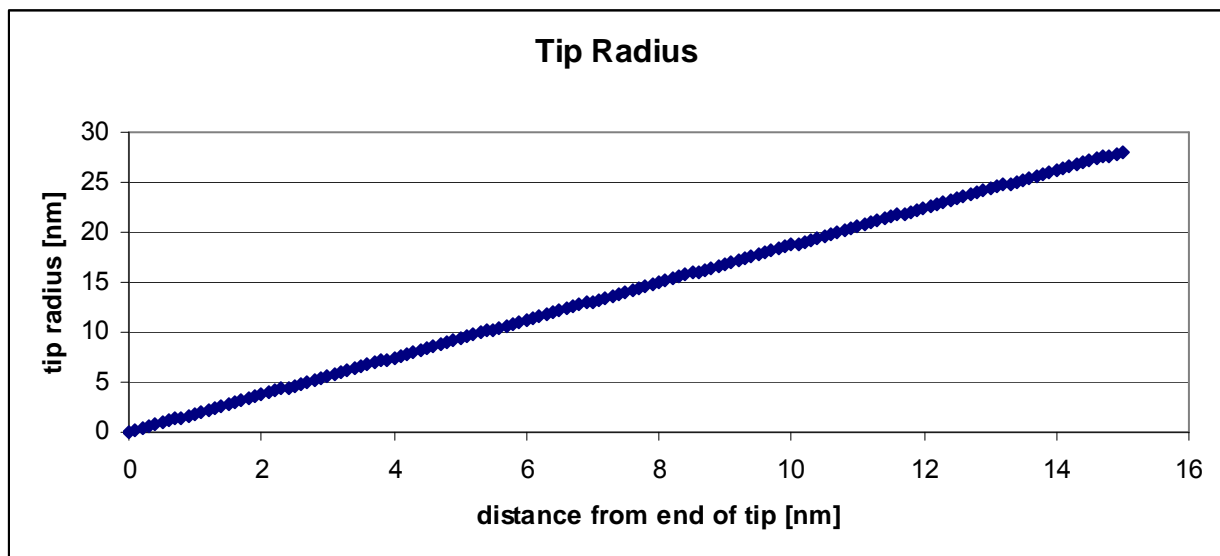


c. A tapping mode AFM image is posted on the MIT Server for this problem of 5 nm gold nanoparticles imaged on atomically flat mica. Download the following software which can open this file ([http://www.nanotec.es/wsxm\\_download.html](http://www.nanotec.es/wsxm_download.html)) WSxM v4.0 Develop 10.1. Open the file and based on the image appearance select the height image. Select "Process" from the top drop down menu and then "Profile." Draw a line over a nanoparticle and a height profile plot will appear in another box. Include the image (with the line showing which nanoparticle was used for the calculation) and the height profile in your assignment solutions. Use this data to estimate the probe tip radius.

ANS. The height from the image gives  $d=2r \sim 10$  nm while the width is gives  $2r^* \sim 40$ nm.



From the graph in part b, we can estimate the angle  $\theta$  of the tip: at  $r^*/r \sim 4$ ,  $\theta \sim 1.08$  radians. The radius of the tip at any point  $d$  from the end of the tip is then given by  $r_{\text{tip}} = d \cdot \tan\theta$ :



**d. Another imaging artifact may occur whereby the height is underestimated; explain one possible reason for this and the conditions under which it would be likely to occur.**

ANS. If the stiffness of the sample is soft enough compared to the probe tip, the constant force necessary for imaging will compress/deform the sample, thereby leading to an underestimation in the height.



**e. A number of papers have reported sub-nanometer scale resolution using relatively large probe tips (50-100 nm radius). Explain how this could be possible.**

ANS. As mentioned in lecture, the geometry of as-received probe tips are highly variable and hence, it is possible that a much smaller protrusion ("nanoasperity") cluster of a few atoms is actually doing the imaging.

**4. Podcast 1: Higgins, M.J. et al. "Structured water layers adjacent to biological membranes." *Biophysical Journal* 91, 2532-2542 (2006).**

**a. In this paper, the samples were prepared by the "solution spreading technique." In the podcast, Dr Higgins mentioned that since the paper was published he now has switched to using the "vesicle fusion technique." Research what this technique is and write up a paragraph summary. Define all terms used and cite references appropriately.**

ANS. The vesicle fusion technique is a way to deposit lipid layers on surfaces; one first mixes amphiphilic molecules in water (or other polar solvent) and shakes or sonicates the solution, so that the lipids form small unilamellar vesicles. These are spherical bodies where a lipid bilayer surrounds a core of polar solvent. When the solution is exposed to a glass (or other material) surface under the right conditions (e.g., low pH, high ionic strength to diminish electrostatic repulsion from the surface) attractive van der Waals forces will cause a vesicle to spread out along the surface in a single bilayer.

See Cremer and Boxer, "Formation and spreading of lipid bilayers on planar glass supports", *J. Phys. Chem. B*, 1999, 103, 2554-2559.

**b. Dr. Higgins stated that the sample imaged by AFM in Figure 2C was an ideal sample for force measurements. Why is that?**

ANS. In Figure 2C, one can tell that there is only a single lipid bilayer formed (in patches) over the mica substrate. The other images show either too little bilayer coverage (2A,B) or too much (2D,E show multiple layers in places).

**c. Estimate the height of a bilayer based on the chemical structure of the lipids and known bond angles. Then, use this information in conjunction with Figure 6A to explain how Dr. Higgins could conclude that the force oscillations he observed were due to movement through layers of water molecules and not through the lipid bilayer.**

The chemical structures of the lipids (POPC, DPPC, DMPC etc) are not exactly linear in shape (DMPC has exactly same chemical structure as DPPC except that there is one less C-C bond on the tail group and the tail is saturated). The height calculation for the long tail group can be done from the end of the tail to the phosphate group in the same manner for all three lipids. The transaction of the length of each bond can be simply calculated as follows

$$L_{trans} = \cos\left(\frac{A}{2}\right) \times L$$

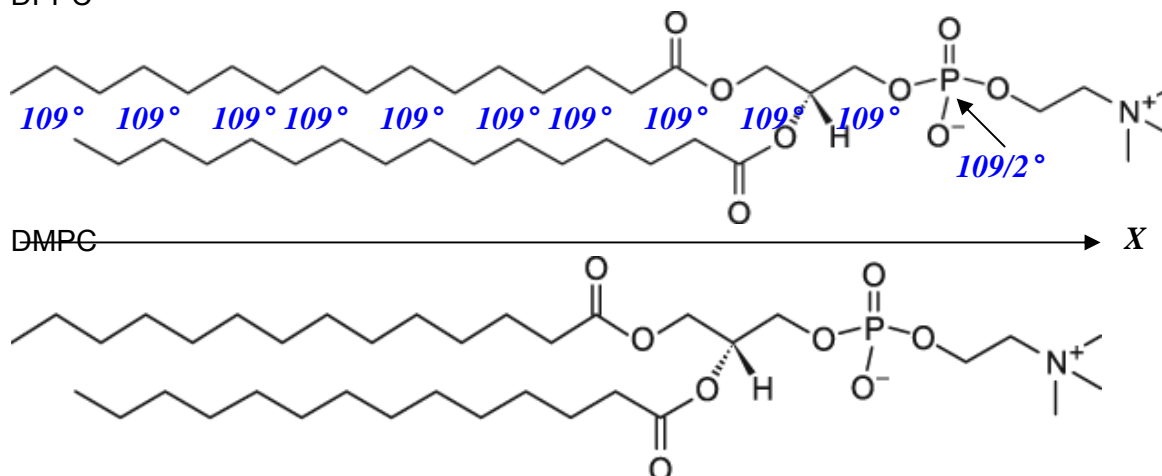
$L_{trans}$  : Transacted length of each bond

$A$  : angle between each bond

$L$  : length of each bond

By adding these projected lengths for each bond, we can come up with a maximum length of the lipid in the liquid; other factors, such as tilt of the head group in water, or fluctuation of the lipid layer, would lessen the lipid length. Detailed calculations for the full lipids are in Table 1.

DPPC



**Figure 2.** 2D structure of lipids and angles

**Table 1.** Detailed calculation of the each bond for DPPC, DMPC and POPC,

**DPPC**

Bond	Unit Bond Length (pm)	Number Of Bonds	A*=Angle (degree)	cos (A/2)	Total Bond Length (pm)
C-C	154	17	109	0.58	1518.44
C-O	143	3	109	0.58	248.82
C-N	143	0	109	0.58	0
O-P	163	1	109	0.58	94.54
C-H	107	1	109	0.58	62.06
				Long tail total	1923.86

**DMPC**

Bond	Unit Bond Length (pm)	Number Of Bonds	A=Angle (degree)	cos (A/2)	Total Bond Length (pm)
C-C	154	15	109	0.58	1339.8
C-O	143	3	109	0.58	248.82
C-N	143	0	109	0.58	0
O-P	163	1	109	0.58	94.54

C-H	107	1	109	0.58	62.06
				Long tail total	1745.22

\* The values of angle between each bond were taken from

[http://www.wpbschoolhouse.btinternet.co.uk/page06/molecule\\_shapes.htm](http://www.wpbschoolhouse.btinternet.co.uk/page06/molecule_shapes.htm)

In addition to the calculation of the one single lipid, the thickness of the water layer between upper and lower tails and the interaction between upper & lower lipids and lipids & water, etc., should be considered, but it can not be calculated exactly by hand. Recent analyses from molecular dynamics (2) and X-ray studies (3) show the height of lipid bilayer is about 4 nm, which is still roughly twice the length of one lipid.

### References

Lopez, C. F. et. al. *J. Phys. Chem.*, B, 108, p. 6603-6610, 2004

de Vries, A.H. et. al. *J. Phys. Chem.*, B, 109, p. 11643-11652, 2005

Nagle, J. F. *Biochimica et Biophysica Acta*, 1469, p159-195, 2000

In Figure 6A, the histogram shows the spacing between oscillation peaks for a set of curves like those shown in Figure 5B. Peak spacing is at 2.9 angstroms, which is consistent with the approximate thickness of a layer of water molecules. The thickness of the lipid bilayer is much larger and would have shown oscillation peaks spaced much farther apart.

**d. Dr. Higgins presented another piece of evidence for the hypothesis mentioned in Question 4c, which was that the tip-normalized punch-through force for a lipid bilayer was much higher than the possible range of normalized forces at which he observed force oscillations to occur. Do this calculation and include an explanation of all of numerical values used.**

ANS. Extra information from podcast: Nanotubes at the cantilever tips were about 5-30 nm in diameter, but could be interacting over a smaller area due to the shape of the nanotube tip after it breaks off from its razor blade support during tip construction. It is reasonable to assume a minimum tip diameter of 0.5 nm and a maximum tip diameter of 30 nm. Tip-normalized forces are calculated as force/tip radius (as described in the paper). Therefore the low limit is calculated from the lowest force oscillation peak in Figure 5B and the highest tip radius:

$$F_{norm} = \frac{80 pN}{15 nm} = 5.333 \frac{pN}{nm} \times \frac{mN}{10^9 pN} \times \frac{10^9 nm}{m} = 5.333 \frac{mN}{m}$$

The high limit is calculated from the highest force oscillation peak in Figure 5B and the lowest tip radius:

$$F_{norm} = \frac{200 pN}{2.5 nm} = 80 \frac{mN}{m}$$

Both of these are lower than the typical 230 mN/m tip-normalized punch-through force for a lipid bilayer which is cited in the paper.

5. Podcast 2: Suresh, S. *et al.* "Connections between single-cell biomechanics and human disease states: Gastrointestinal cancer and malaria." *Acta Biomaterialia* 1, 15-30 (2005).

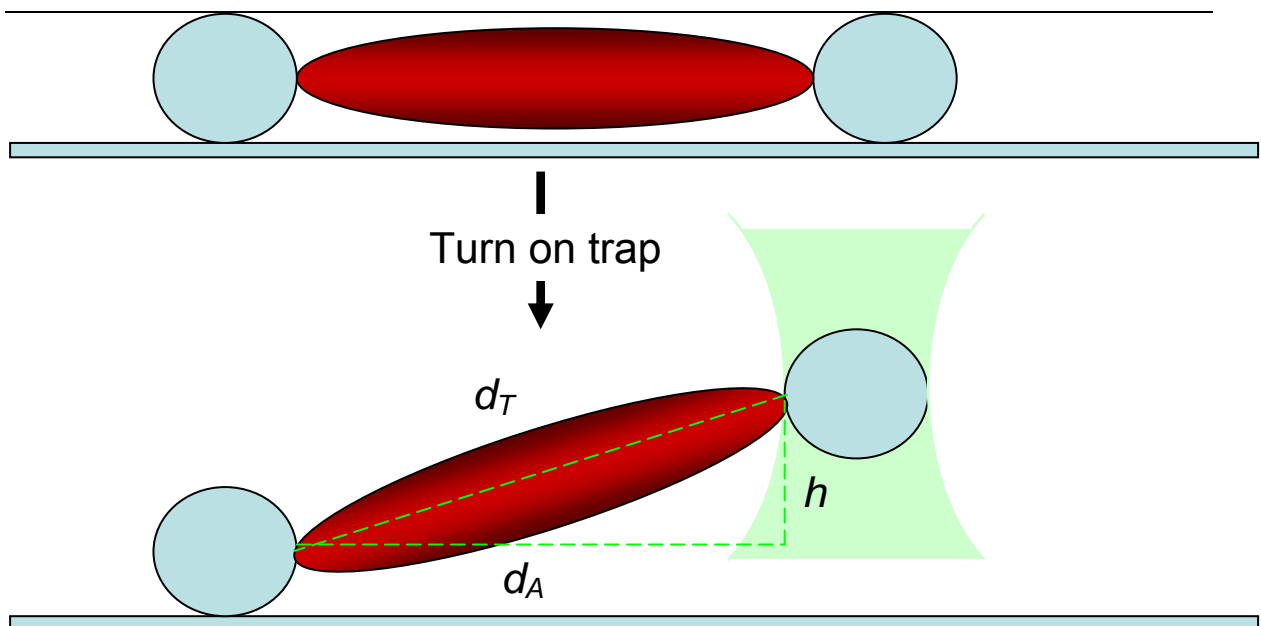
a. Why did Suresh and Mills use a microplate stretcher for their cancer cell experiments instead of the optical tweezer apparatus used to stretch red blood cells infected by malaria parasites?

ANS. Optical tweezers are limited in the maximum force they can apply; John Mills cited a maximum force of 200 pN on their system in Singapore and 600 pN on the system at MIT. These forces were not high enough to investigate the cancer cells, which were stretched with hundreds of nN of force.

b. In Figure 6, why does the appearance of the bead attached to the left side of the cell appear different in the 68 pN and 151 pN frames? What effect does this have on measurement of diameter changes? Calculate the actual axial diameter change of the cell in terms of the axial diameter change observed under the microscope. (No numerical values are necessary; use symbolic quantities and define your variables).

ANS. In the "Original Shape" images in Figure 6, both beads are resting on the coverslip surface and appear in the same focal plane. When the optical trap is turned on to exert the 68 pN and 151 pN forces pictured in subsequent columns, the bead attached to the left side of the cell is pulled away from the coverslip surface. This leads it to appear out of focus in the images. The cell is therefore tilted at an angle to our plane of observation, but the tilt can be easily corrected with the following calculation. Let  $h$  be the distance that the bead is displaced from the coverslip surface by the optical trap. Let  $d_A$  be the apparent axial diameter measured from images, and let  $d_T$  be the true axial diameter of the cell.

$$\text{Then } d_T = \sqrt{(d_A^2 - h^2)}$$



**c. Rank the following in order of increasing elastic modulus: a healthy red blood cell, a red blood cell infected by the Plasmodium falciparum parasite, and a red blood cell infected by the Plasmodium vivax parasite. How do the different elastic moduli of these red blood cells affect their ability to remain in circulation throughout the body? Which disease leads to more fatalities? How might fatality be related to these disease-induced mechanical changes?**

ANS. A P. vivax-infected RBC has a lower elastic modulus than a healthy red blood cell, which has a lower elastic modulus than a P. falciparum-infected RBC. Healthy RBCs have sufficient deformability to pass through the smallest circulatory vessels as well as cellular gaps in the spleen and continue on through the circulation. P. falciparum stiffens the RBC so that becomes caught in the microcirculation, blocking adequate blood flow. P. vivax, on the other hand, makes the RBC more deformable, so that it circulates freely throughout the body, but also avoids sequestration in the spleen. Although the body has a way to “catch” P. falciparum-infected RBCs, fatalities from the parasite are higher than those from P. vivax, likely because of problems caused in the microcirculation.

**d. What did you find to be the most interesting part of the podcast/paper?**

**Extra Credit:** +5 points for posting a comment on either of the two message boards.