

LECTURE 13: MIDTERM #1 SOLUTIONS REVIEW

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Objectives: To review the solutions to Midterm #1 in detail with your corrected exams to see where questions were answered correctly and incorrectly.

Readings: Dupres, et al. *Nature Methods* 2, 7, **2005**, 515; and Midterm Solutions.

Multimedia : none

HRFS : GENERAL EXPERIMENT DESCRIPTION

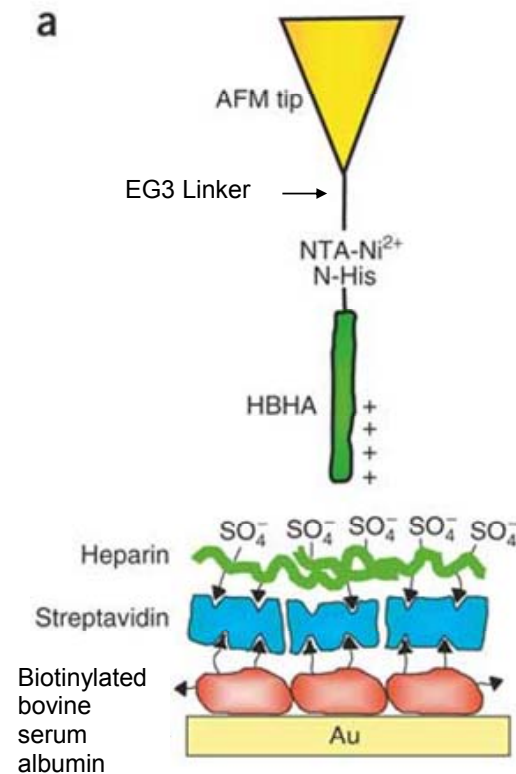


Figure 1a is a schematic of a HRFS experiment to measure the interaction between a protein found on the surface of *M. tuberculosis* bacterial cell called HBHA (heparin-binding haemoglutinin adhesion) and heparin. The experiments are performed in aqueous solution.

→ the latter statement indicates that capillary forces are minimal, hence one can measure accurately intersurface forces (e.g. van der Waals, bioadhesion, ionic, etc.)

→ Functionalized probe tip and surface (i.e. attaching chemical groups/molecules to a surface)

→ since the authors draw one molecule on the probe tip, can assume they are suggesting this is a single molecule experiment

→ EG3 linker is 1) to increase mobility molecule on tip to allow easier binding with heparin, 2) to allow HBHA-Heparin binding when the tip is far away from surface to avoid tip-surface nonspecific interactions

→ 4 monolayers on substrate :

1) biotinylated-BSA which is physisorbed to Au (remember we talked about what happens to proteins when adsorbed to surfaces (biocompatibility), they can denature due to hydrophobic interactions)

2) Streptavidin binds to biotin with ligand-receptor lock and key mechanism

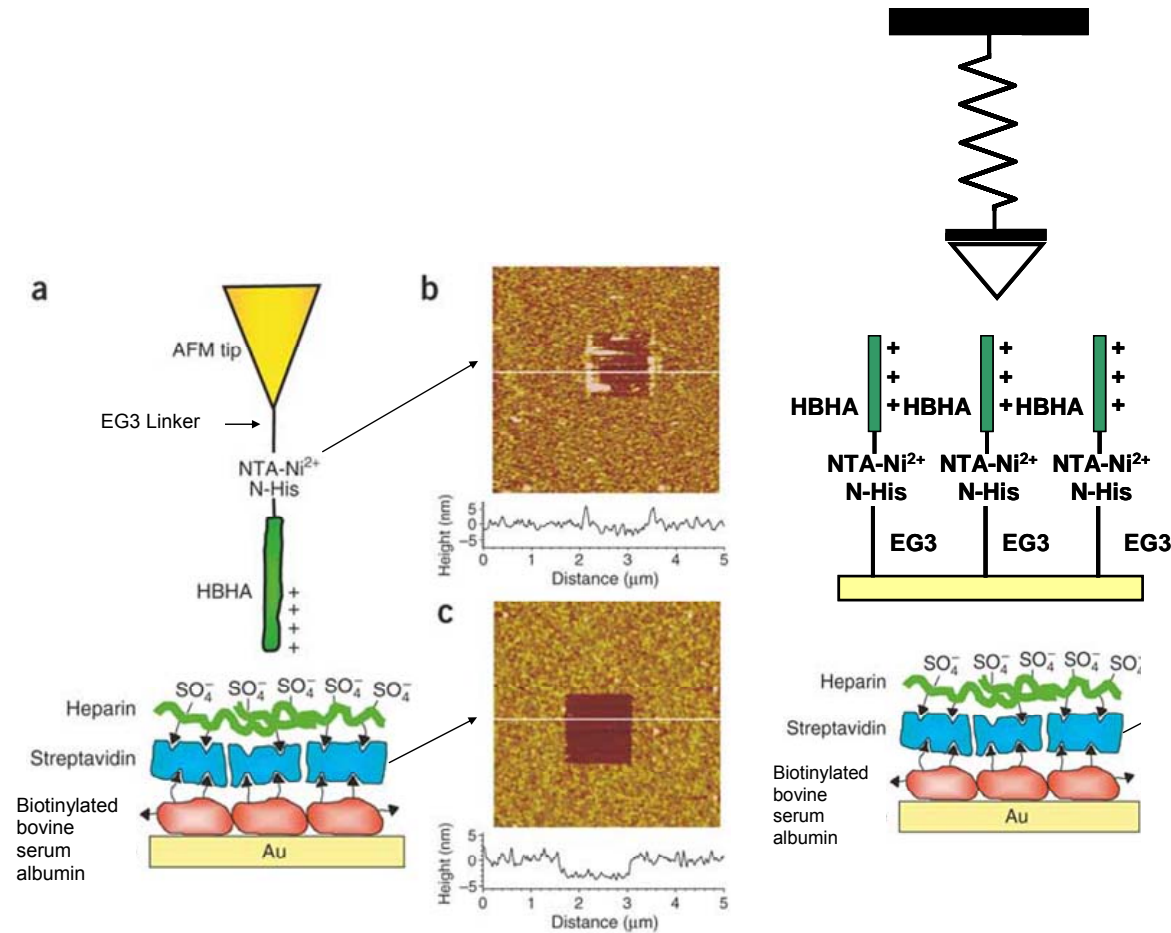
3) Biotinylated-Heparin binds to streptavidin

4) Heparin- negatively charged sulfate groups, since HBHA + charged, assume ionic interactions dominant

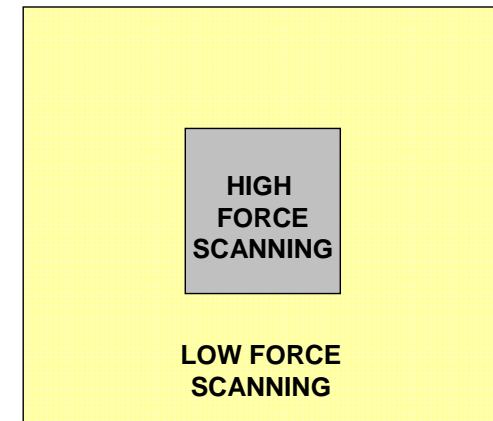
→ Each layer can potentially contribute to the net intersurface interaction

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HRFS : VERIFICATION OF SURFACE FUNCTIONALIZATION : IMAGING OF PLANAR SUBSTRATES

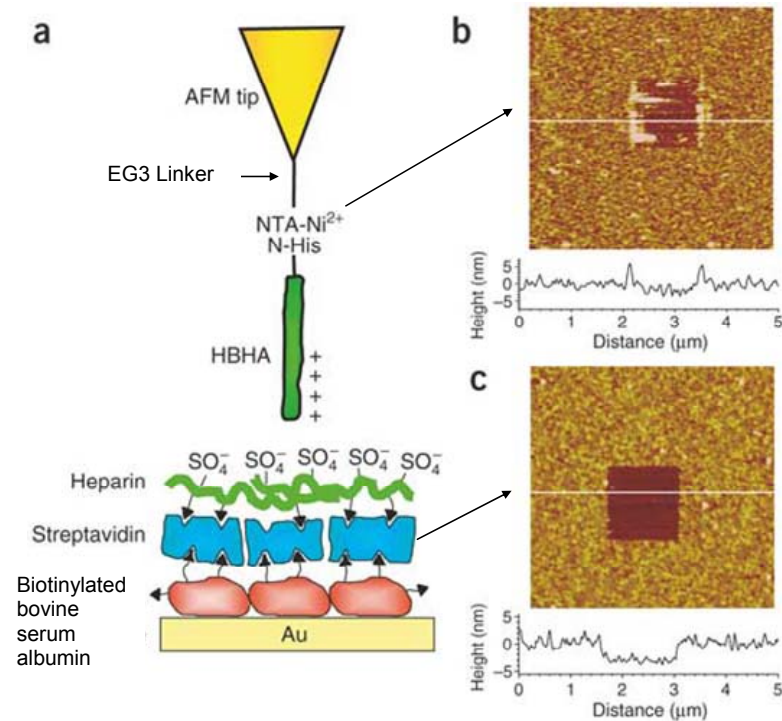


1. The authors state that they have verified their surface chemistry by first carrying it out on planar surfaces (Figure 1b = tip functionalization scheme, Figure 1c = substrate functionalization scheme) and then AFM imaging these functionalized surfaces at very high force (within the internal square regions shown in Figure 1b,c), and then zooming out and imaging at very low force.



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HRFS : QUANTIFIED VERIFICATION OF SURFACE FUNCTIONALIZATION 2



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1. Explain what the data is in Figures 1b,c (both images and plots) and where it comes from in the instrument.

-The images in Figure 1b, c are AFM height images as seen from the 2D section profile plots (1D line scans along the white line in each image) underneath each image which are labeled "height" on the vertical axis.

-AFM height images come from the movement of z-piezo (z=direction perpendicular to the sample x/y plane) controlled by the feedback loop.

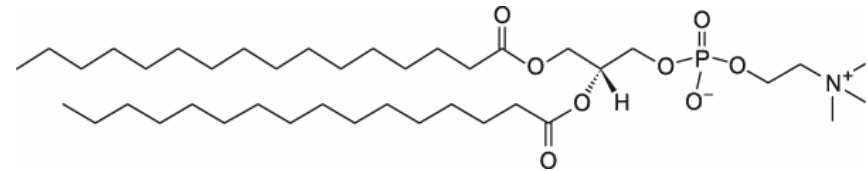
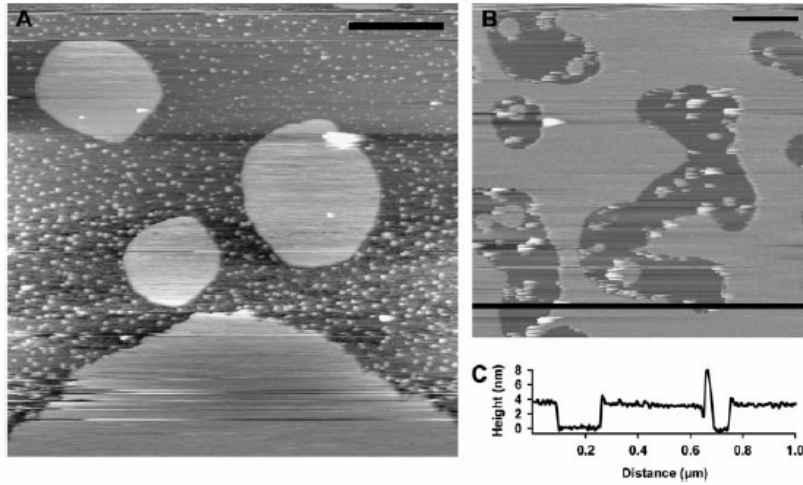
What the observed features are.

-When the tip scans at high force within the smaller square region in the center, of the image, the imaging force is high enough to scratch off and remove all of the attached molecules to the surface revealing the atomically flat underlying gold substrate (notice there is no recordable roughness in the 2D section profile). In the larger low force scan, one can see the functionalized region with molecules attached around the "bare" internal square.

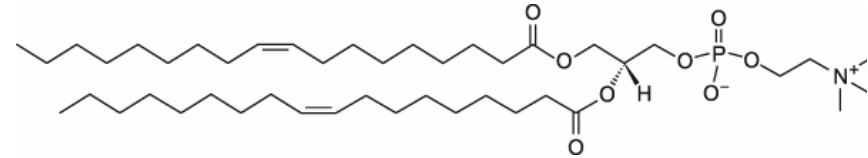
How these images quantitatively verify the success of the surface chemistry.

The 1D section profile gives us the height of the functionalized molecular layer as the tip scans from the functionalized to bare region. This height should correlate well with the known sizes of the molecules being attached to the surface. This is how the surface chemistry is verified quantitatively.

HRFS : VERIFICATION OF SURFACE FUNCTIONALIZATION 3 : LIPID BILAYERS

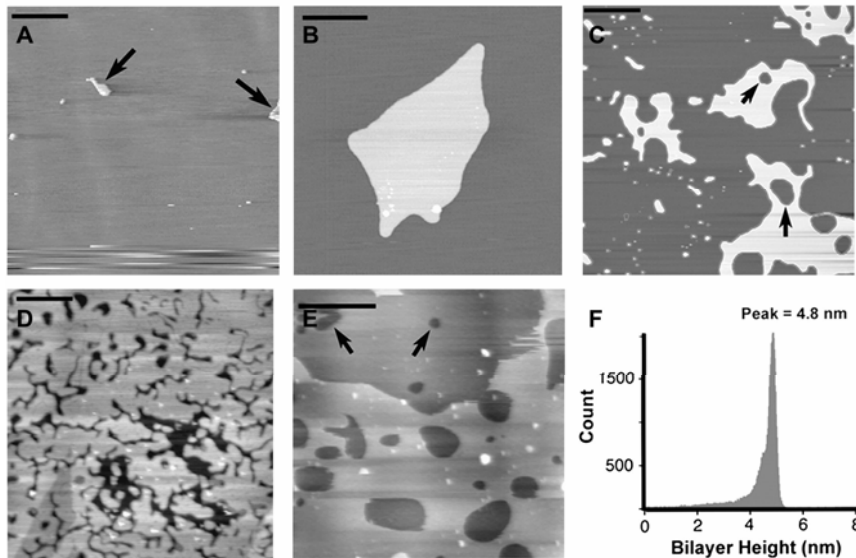


DPPC



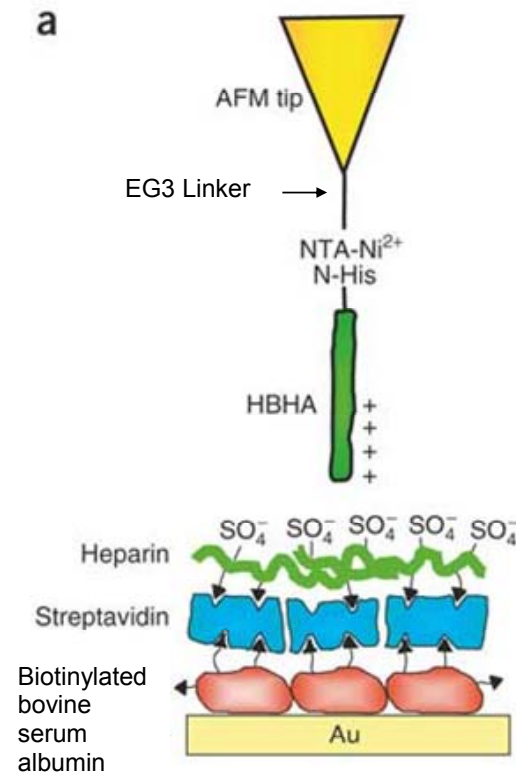
DOPC

Higgins, et al. Biophys. J. 2006 91, 2532.



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REQUIREMENTS FOR SINGLE MOLECULE FORCE SPECTROSCOPY

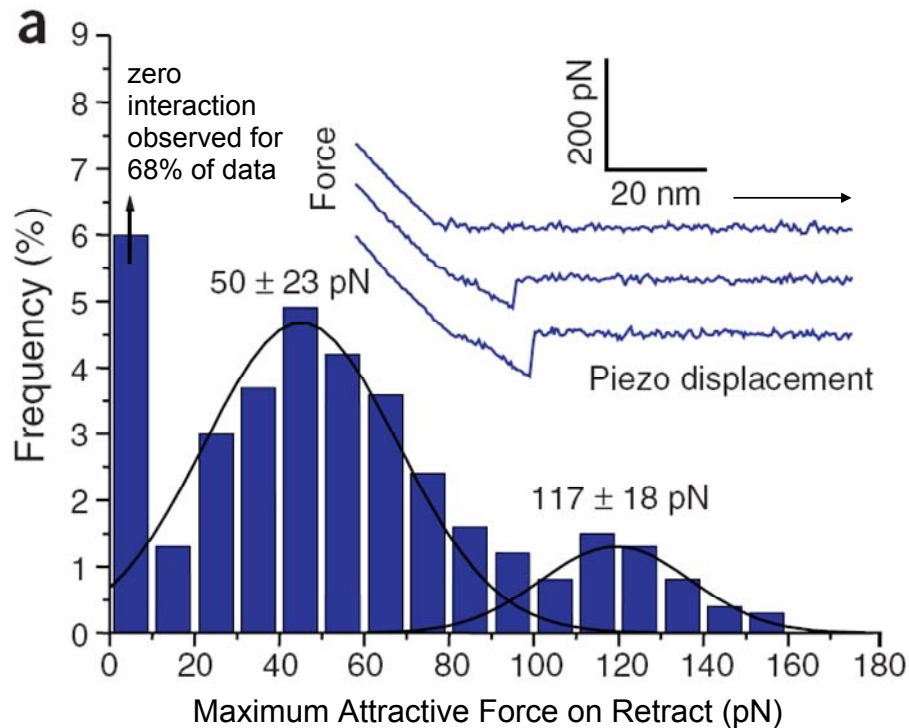


2. Assuming the surface and tip functionalization schemes shown in Figure 1a, what five requirements for the experimental setup/surface chemistry must be met in order to measure the single molecule interaction between HBHA and heparin?

- 1) The HBHA-heparin binding interaction must be weakest compared to other surface functionalization linkages (e.g. biotin-streptavidin, BSA-Au, etc).
- 2) A sufficiently low density of HBHA must be present on the tip and/or a small enough probe tip radius to avoid attachment of multiple molecules
- 3) a flexible linker provides enough mobility for binding
- 4) the binding force must be greater than limit of force detection
- 5) all of the protein molecules attached to the surface need to be maintained in their native state since if they denature the internal hydrophobic groups will become exposed and add an unwanted hydrophobic interaction to the experiments
- 6) each layer should be a well-defined monolayer.
- 7) Small enough forces on approach that you don't damage surface functionalization

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HIGH RESOLUTION FORCE SPECTROSCOPY : RAW DATA CONVERSION



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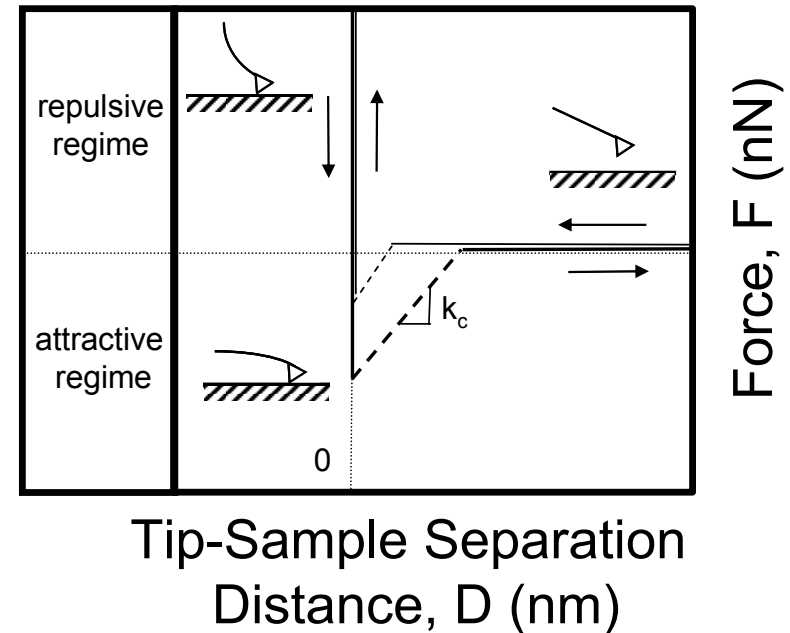
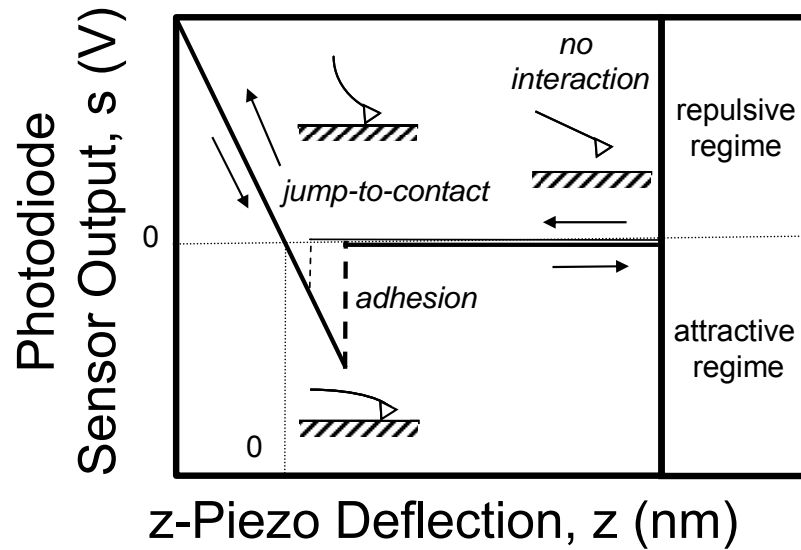
3. High resolution force spectroscopy data from ~ 1000 of these experiments is shown in Figure 2, which is a histogram of the maximum attractive forces observed on retract (pN) with insets showing some typical data for three individual experiments on retract.

-3 individual force curves, explain scale bars, adhesion force

a. In the inset curves, why don't we observe a region of apparent infinite slope? Is the constant compliance regime reached?

- The reason we don't see a region of apparent infinite slope is because the x-axis data has not been converted to tip-sample separation distance, it is the raw z-piezo displacement data. Yes we reach the constant compliance regime where the force appears linear with the piezo displacement.

HIGH RESOLUTION FORCE SPECTROSCOPY EXPERIMENT (HRFS): RAW DATA CONVERSION TO FORCE-DISTANCE



- Measure sensor output (Volts) vs. z-piezo displacement/deflection

- **Conversion of raw data**; sensor output, s (Volts) vs. z-piezo displacement/deflection, δ (nm) to Force, F , versus tip-sample separation distance, D :

$$\delta = s/m$$

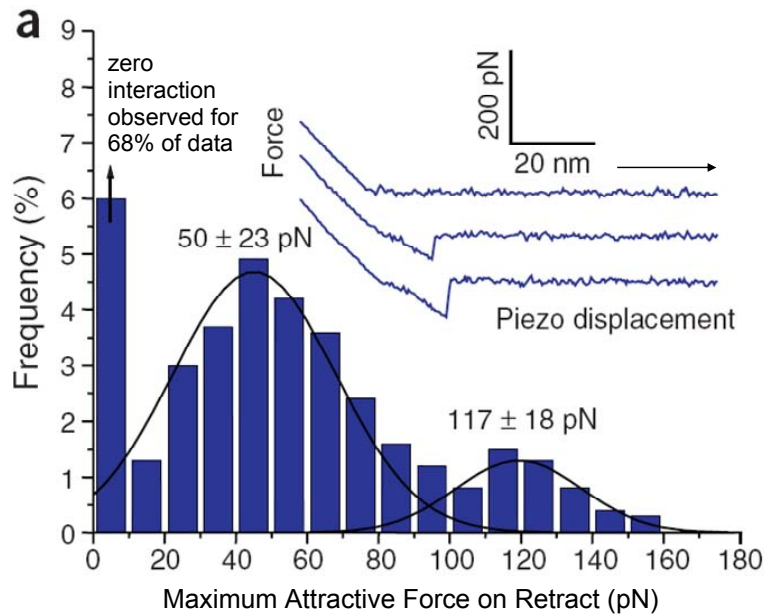
$$m = \text{slope in constant compliance regime} = \Delta s / \Delta \delta \text{ (V/nm)}$$

$$F = k\delta$$

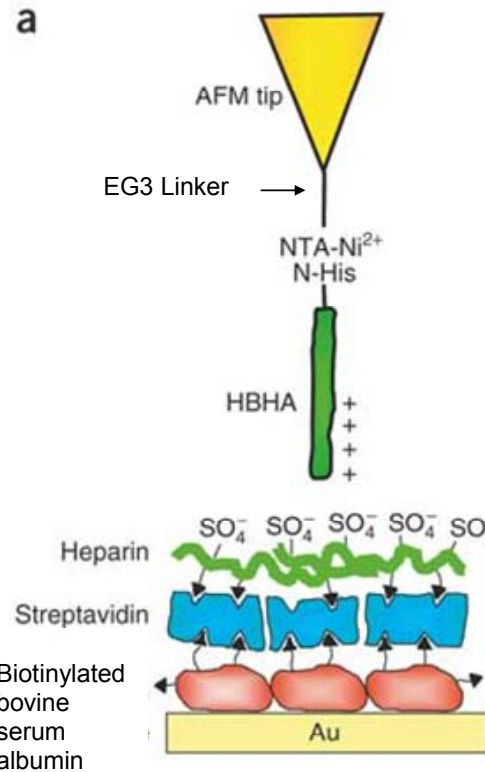
$$D = z + \delta$$

-zeroing the baseline

HIGH RESOLUTION FORCE SPECTROSCOPY : DATA INTERPRETATION



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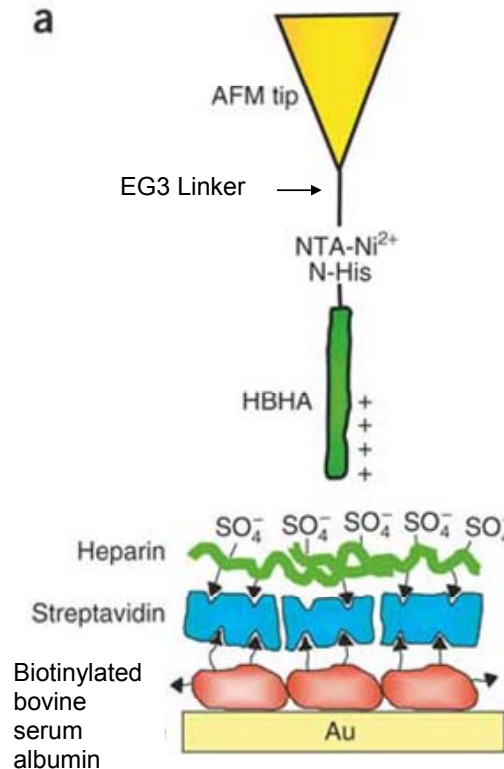
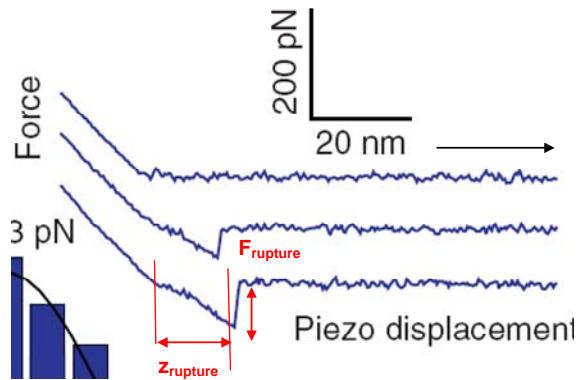
3.b. What could be the origin of the bimodal distribution?

ANS. The proposed origin of the bimodal distribution is that the 50 pN force is the HBHA-heparin single molecule rupture force and the 117 pN force corresponds to two HBHA-heparin molecules in parallel rupturing simultaneously.

c. Think of a control experiment one could do to verify that these attractive forces were in fact those measured between HBHA and heparin and not interactions between the underlying tip and substrate.

ANS. The same exact system would need to be used except where the active functional groups were "blocked" either on the tip or the substrate. One could potentially flow in HBHA into the heparin functionalized surface to have them bind to the heparin and then measure HBHA-HBHA or vice versa, or one could omit the heparin functionalization step and measure HBHA-streptavidin.

HIGH RESOLUTION FORCE SPECTROSCOPY : CALCULATION OF RUPTURE DISTANCE



3d. From the inset data given in Figure 2, calculate the typical experimentally observed rupture distance given a cantilever spring constant of 0.01 N/m.

ANS. The experimentally observed rupture piezo displacement, $z_{rupture}$, is measured from the position of maximum attractive force to the beginning of the constant compliance regime (See Figure to the left).

$D_{rupture} = z_{rupture} - \delta_{rupture}$ where δ is the cantilever deflection (negative for attractive displacement).

$$\delta_{rupture} = F_{rupture} / k = 100 \text{ pN} / 0.01 \text{ N/m} = 10 \text{ nm}$$

$$D_{rupture} = 20 \text{ nm} - 10 \text{ nm} = 10 \text{ nm}$$

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e. List three potential intermolecular interactions that exist between HBHA and Heparin and explain the molecular origins of each one. 1) ionic interactions between HBHA cationic groups and heparin anionic chemical groups, 2) van der Waals interactions from induced dipole-induced dipole interactions, and 3) hydrophilic hydration repulsion.

INTRA- AND INTERMOLECULAR POTENTIALS

4. Calculate the theoretical separation distance corresponding to the HBHA-heparin rupture force using appropriate mathematical forms for the intermolecular potentials. Use an attractive prefactor $A = 10^{-77} \text{ J}\cdot\text{m}$ and a repulsive prefactor $= 10^{-134} \text{ J}\cdot\text{m}^{12}$. Compare this theoretical distance to the experimentally determined rupture distance in (3(d)). Explain any differences.

ANS. A $\sim r^{-12}$ short range repulsive potential can be assumed to be additive with a Coulombic interionic $\sim r^{-1}$ attractive potential :

$$w(r) = w(r)_{\text{repulsive}} - w(r)_{\text{attractive}} = Br^{-12} - Ar^{-1}$$

The intermolecular force is given by the negative first derivative with respect to r .

$$F(r) = -\frac{\partial w(r)}{\partial(r)} = 12Br^{-13} - Ar^{-2}$$

The rupture force is found at the separation distance (r_s) where force is at a minimum; i.e., rupture occurs as :

$$\frac{\partial F(r)}{\partial r} \rightarrow 0: \quad \frac{\partial F(r)}{\partial r} = -156Br_s^{-14} + 2Ar_s^{-3} = 0$$

Therefore :

$$r_s = \left(\frac{156B}{2A} \right)^{1/11} = \left(\frac{156 \times 10^{-134} \text{ J} \times \text{m}^{12}}{2 \times 10^{-77} \text{ J} \times \text{m}} \right)^{1/11} = 0.36 \text{ nm}$$

This value is much, much smaller than that recorded in 3(d) because of the presence of the EG3 linker which extends the HBHA away from the probe tip.

IN VITRO VERSUS IN VIVO

5. In a number of the podcasts, we discussed how all nanomechanics experiments so far are model *in vitro* systems which are very different from the *in vivo* physiological environment. Name 3 differences between this model *in vitro* system and binding of a living *M. tuberculosis* bacterial cell.

ANS.

- 1) The cell will have a different compressibility due to the underlying cytoskeleton.
- 2) Many other molecules (lipid bilayer, other transmembrane proteins, and proteoglycans) will be present on the cell surface
- 3) The heparin generally will be free in solution with a higher translational mobility.