Getting to the Root of Bacterial Hair

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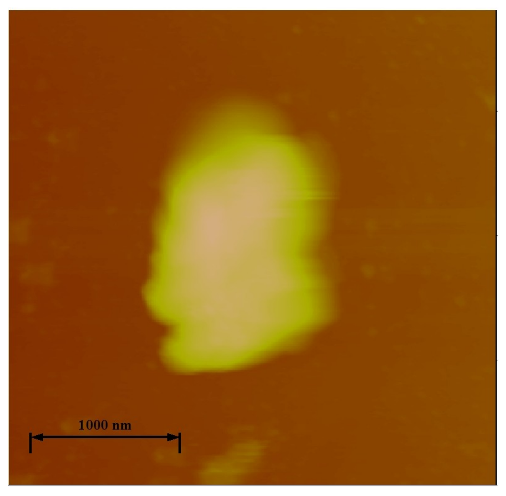
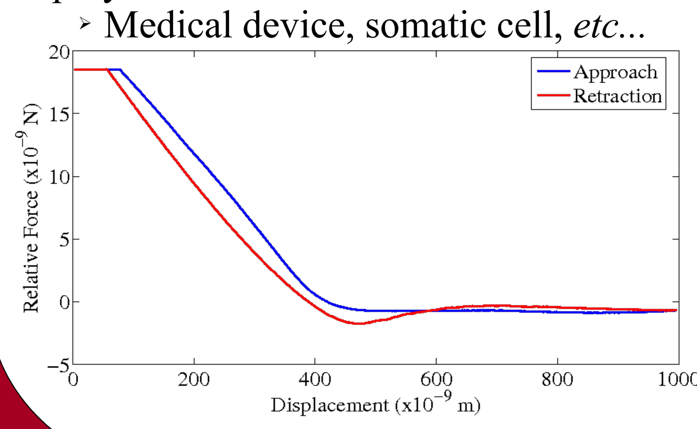
Abstract

- Many bacteria use their extracellular polymers to attach to surfaces
  - Leads to industrial biofouling, medical device infections
- Physicochemical and physicomechanical properties of these polymers may be deduced from AFM force curves
  - For these data to be useful, it is necessary to define a zero of separation and to understand what that zero represents
- Previous researchers have defined the point of zero separation as the cell wall, and assumed the constant compliance region of the force curve to be representative of that location[1,3,5]
  - The force at the cell wall has been used to calculate the equilibrium length of the brush, as well as the grafting density of the polymers at the point of zero separation
- Here, we show:
  - I. That the constant compliance region frequently occurs in the middle of the layers
  - II. How the layer thickness may be more accurately quantified
  - III. A quantitative method of establishing the position of the "roots of the hairs" at the cell wall
  - IV. The more appropriate use of the mesh density of the polymer brush, in lieu of the polymer grafting density

Experimental System

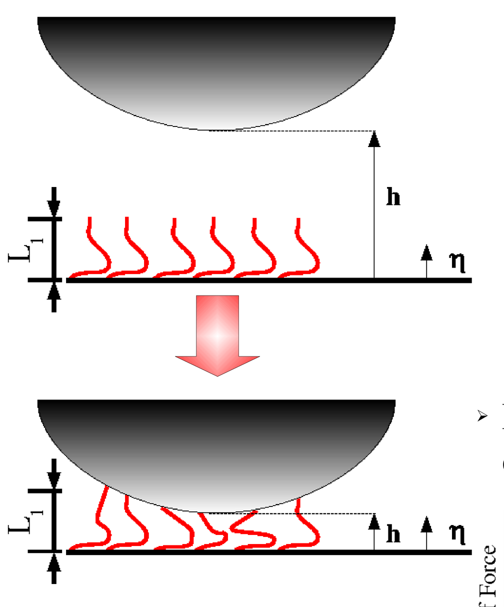
- Common environmental pathogen
- Responsible for multiple infection types
  - Keratitis of the ocular cornea
  - Burn wound infections
  - Bronchoscopic pulmonary infections
  - Main persister strain in cystic fibrosis infections
- Each infection pathway may be attributed to polymer-mediated adhesion to a substrate
  - Medical device, somatic cell, *etc...*



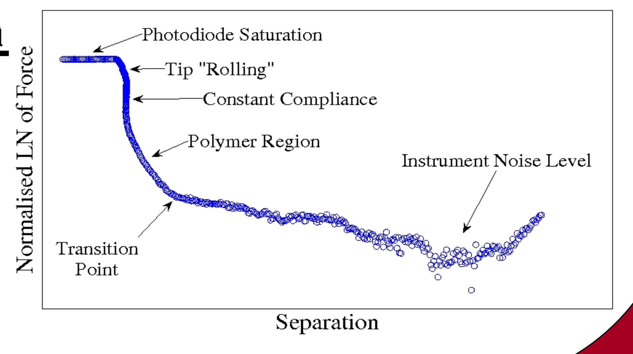


- AFM approach curves show no attractive minima
- Interactions are dominated by steric compression of the polymer brush
- Presents significant difficulty in assigning a coordinate system

System Schematic



- On approach, the probe passes through several phases:
  - *Zero Interaction* – Deflection is dominated by instrument noise
  - *Polymer Interaction* – Deflection is dominated by compression of the polymer brush
  - *Constant Compliance* – Deflection is equal to PZT displacement
  - *Tip "Rolling"* - Under high load, deflection is due to in-plane forces
- Plotting the data series in semilogarithmic coordinates amplifies these phases



Derivation of the Model

- From de Gennes[2] expression for the energy per unit volume, we may integrate across the surface of the probe to determine the force as a function of tip-sample separation

$$F(h)=100\frac{k_B T}{\xi_1^3}\int_h^{L_1}\exp\left[\frac{-2\pi}{L_1}\eta\right]dA_p$$

$$F(h)=100\frac{k_B T}{\xi_1^3}a_p L_1\exp\left[\frac{-2\pi}{L_1}h\right]$$

$$F(h)|_{0\le h\le L_1}\equiv F_{L1}(0)\exp\left[\frac{-2\pi}{L_1}h\right]$$

- Where:
  - $F(h)\equiv$ *Force as a function of tip−sample separation*
  - $k_B\equiv$ *Boltzmann's constant*
  - $T\equiv$ *Absolute temperature*
  - $\xi_1\equiv$ *Mesh spacing of the polymer brush*
  - $L_1\equiv$ *Equilibrium thickness of the polymer brush*
  - $a_p\equiv$ *Radius of curvature of the probe*

- The zero of separation will be defined according to the intersection of the function previously derived with the noise level of the instrument
  - It is therefore necessary to filter the raw data sets to remove interference artifacts
- This is modeled as an offset sine wave, according to:

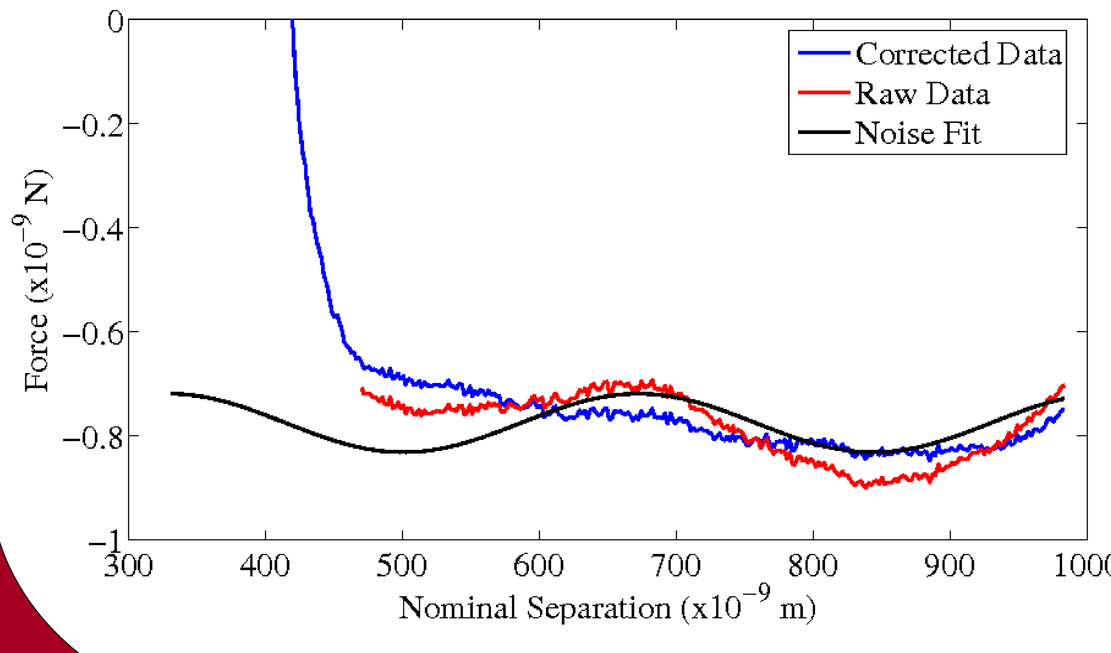
$$F(h)=P_A\sin\left(\frac{\pi}{P_B}h-P_C\right)+P_D$$

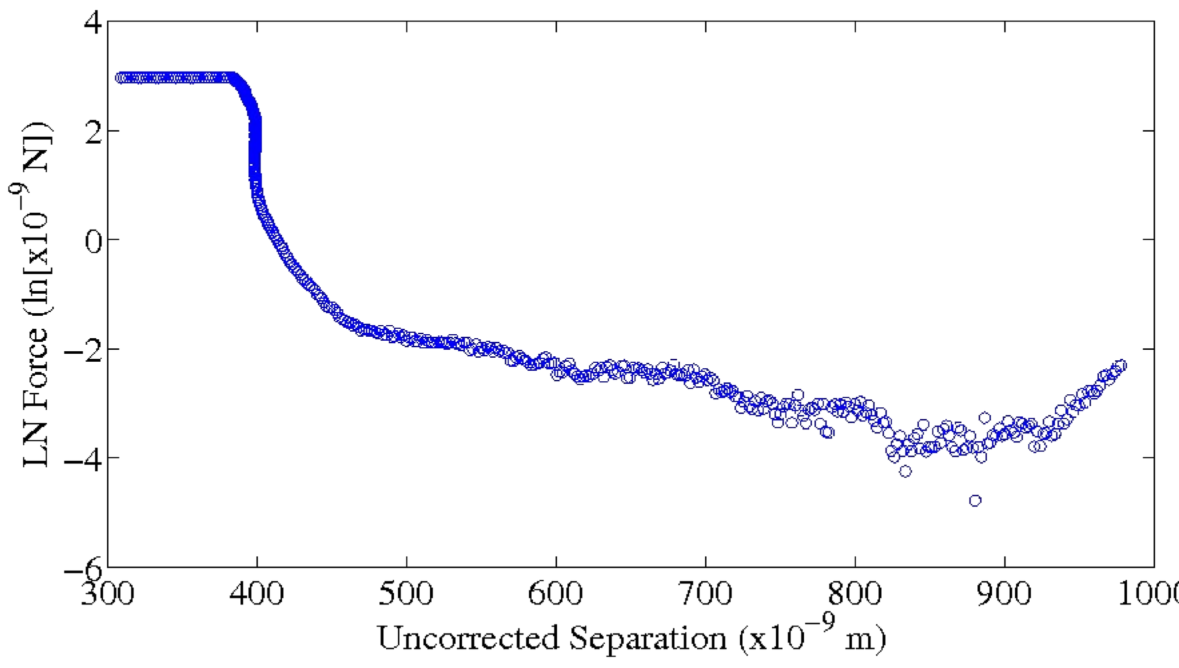
- Where:
  - $P_A\equiv$ *Amplitude of the interference pattern*
  - $P_B\equiv$ *Period of the interference pattern*
  - $P_C\equiv$ *Phase offset of the interference pattern*
  - $P_C\equiv$ *Vertical offset of the interference pattern*

Model Application and Results

Removal of Noise Artifacts

- Applying the noise filter defined above, we see that a periodic interference pattern provides a significant signal at large separations
- The period of the noise corresponds to interference between incident light at the back of the cantilever and extraneous light reflecting from the AFM stage into the photodetector
- Subtraction of this signal allows us to use the actual instrument noise level as a reference point for defining the zero of separation

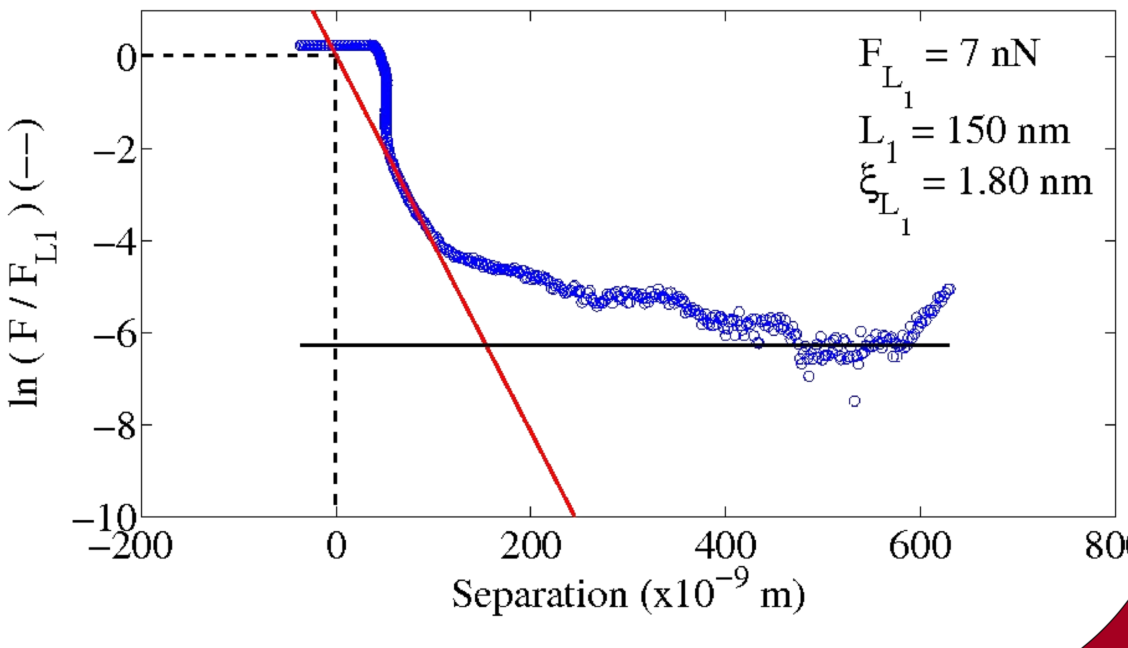




		Mean	Deviation	% Precision	% Accuracy
$F_{L1}(0)$	(nN)	6	2	30	25
L_1	(nm)	160	8	5.0	10
ξ_1	(nm)	1.80	0.04	2.0	10
Ξ_1	(nm^{-3})	0.19	0.01	5.0	30

Identification of the Zero of Separation

- To define the noise level, a horizontal line is drawn through the median value of the points at large separations
- The intersection of this line with the expression describing the polymer brush interaction is then located
  - Taking the limit of the polymer brush expression, we see that the intersection must rest at (L_1, -2π)
  - The polymer brush function must also intersect the origin of the plane



Discussion and Conclusions

- Model parameters derived from the experimental data are consistent with data obtained from empirical methods
  - Polymer lengths and populations have been measured using TEM cryo-sectioning techniques[4]
- In all cases, the point of zero separation rests within the constant compliance region, and often beyond the point at which the photodiode becomes saturated
  - Indicates that the polymer brush is compressible to a finite degree, but that the surface of the cell can never be reached
  - Further, since the diode is saturates at force values of +20 nN, that the forces exerted by the polymer brush are larger than those previously reported
- The linear region bounded by the two limits used to determine the zero of separation may represent an additional interaction
  - Potentially, these may be interactions with a second, less dense polymer brush, or an electrostatic double-layer interaction
  - More likely, where the signal is so small, it is an additional noise source that has not been removed in the filtering algorithm
- The quantitative identification of the point of zero separation for a brushed surface exhibiting purely repulsive interactions is now possible

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Acknowledgments

- Funding Sources
  - National Science Foundation Grant BES-0238627
  - American Chemical Society Petroleum Research Fund Grant 38988-G2
- Laila Abu-Lail, Arzu Atabek, Yatao Liu, Paola Pinzon-Arango, Joshua Strauss (Biological Interaction Forces Lab, Dept. of Chemical Engineering, WPI)