Summer Nano Workshop Series

Atomic Force Microscopy
Guangzhao Mao and Peter Hoffmann
June 7, 2011
Atomic Force Microscopy (AFM)

- Spatial resolution ~ 1 nm
- Force sensitivity ~ 1 pN
- Imaging in solution
- Roughness, heterogeneity, forces, etc.

http://www.youtube.com/watch?v=1kbAHPCakHA
Contact (CM) v.s. Tapping Mode (TM)

Image Force Minimization

375nm  405nm  250nm

Image force increase
Data Types

OTHER AFM MODES
Force Modulation Mode in CM AFM
Thermal AFM

X: isopropylacrylamide
Y: 1-vinylimidazole
Z: ethylene glycol

**Electrochemical AFM**

Figure. AFM images of electrochemically reduced AuNPs on HOPG in (a) 1 mM, (b) 0.5 mM, and (0.1 mM) gold salt solution. Scan size = 5 μm. Z range = 50 nm for (a) and 20 nm for (b) and (c). (d) The CV curves on HOPG (solid line) and AuNP-decorated HOPG (dotted line) in 1 mM K₃Fe(CN)₆ and 0.1 M KCl electrolyte solution.
Electrochemical AFM

Ring counter electrode (Pt wire 0.25mm x 10cm)

AgCl/Ag Reference Electrode
Colloidal Force AFM

Mao et al.
An example of collaboration between Mao and Oupicky

AFM IN GENE DELIVERY
Bioreducible Polyplexes for Gene Delivery

AFM study of DNA release in simulated physiological environment:
10-20 mM DTT
0.1-0.2 M NaCl
pH 5-8

DTT: Dithiothreitol

Peptide and DNA Polyplexes

Polyplex Nanostructure

Incubation time = 30 min

Polyplex Disassembly

Decondensation
Aggregation/fusion
Toroid
Frozen particle

Chains held by a compact core

Scan size = 600 nm.
Z-range = 8 nm.

Polyplex Characterization

- Nuclear localization signal (NLS) (CGAGPKKKRKVC)
- Histidine-rich peptide (HRP) (CKHHHKHHHKC)

<table>
<thead>
<tr>
<th></th>
<th>Mw</th>
<th>Hydrodynamic diameter of polyplex (nm)</th>
<th>Zeta potential of polyplex (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PNLS</td>
<td>100,000</td>
<td>105 ± 3</td>
<td>47 ± 1</td>
</tr>
<tr>
<td>PHRP</td>
<td>200,000</td>
<td>96 ± 1</td>
<td>55 ± 1</td>
</tr>
<tr>
<td>PEI</td>
<td>25,000</td>
<td>115 ± 1</td>
<td>47 ± 5</td>
</tr>
</tbody>
</table>

NLS Polypeplex

HRP Polypeplex

Force-distance measurements

Approach & retract tip vertically towards/away from the surface.

Measure observable (force, dissipation, force gradient etc) while the tip is moved to obtain a “force-distance curve”.

Static, DC or contact mode:
Signal: Deflection of cantilever (Force, \( F = -kLd \))

Dynamic mode:
Oscillate lever at resonance, below resonance, or at higher resonance mode

Possible signals/observables:
• Amplitude (AM)
• Phase (PM)
• Resonance frequency (FM)
Force-distance measurements

Push: Elastic modulus

Pull: Adhesion Force
Indentation measurements to measure local (visco-) elastic properties

Hertz Model Relationship between displacement, contact radius and applied force:

\[
\delta = \frac{a^2}{R} = \left( \frac{9F^2}{16RE^2} \right)^{\frac{1}{3}}
\]
Colloidal probes
Nanoindentation by AFM

Nanomechanical analysis of cells from cancer patients

SARAH E. CROSS$^{1,2†}$, YU-SHENG JIN$^{3†}$, JIANYU RAO$^{3†}$ AND JAMES K. GIMZEWSKI$^{1,2†}$

Nature Nanotechnology | Vol 2 | December 2007
Cell adhesion measurements

Streptococcus viridans – grown in 0% or 2% xylitol
Single molecule force spectroscopy

Measurable parameters:

- Rupture forces
- Bond lengths
- Kinetic rates: dissociation, association
- Activation barriers
- Bond stiffness
- Energy landscapes
Single molecule AFM

Effect of applied force: Lowering of activation barrier
Protein Interactions

Mechanics, dynamics and regulation of biological macromolecules and molecular assemblies

\[ f(pN) \]

\[ \text{distance(nm)} \]
Dissociation Kinetics of an Enzyme–Inhibitor System Using Single-Molecule Force Measurements

Essa Mayras,† Marisarda Bernardo,‡ Lindsay Runyan,† Anium Sohail,† Venkatesh Subba-Rao,† Mircea Pantea,† Raisel Fridman,‖ and Peter M. Hoffmann*†

Figure 7. Histograms and fits to two Gaussian distributions of unbinding forces of TIMP1–proMMP9 at different pulling speeds, \( \nu = 29, 296, 685, \) and \( 1380 \) nm/s.

| Table 1. Extracted Values of the Bond Length, Kinetic off Rate and Activation Barrier for the Complex Bond TIMP1-proMMP9 Using the Three Different Models Discussed in the Text* |
|---|---|---|---|
| model | \( x^* \) (nm) | \( k_{off}^{\nu} \) (s\(^{-1}\)) | \( E_0^{\nu} \) (kJ/mol) |
| BE | \( 0.79 \pm 0.01 \pm 0.02 \) | \( 0.025 \pm 0.002 \pm 0.005 \) | \( 65.8 \pm 0.2 \pm 0.6 \) |
| BE-FJC | \( 0.82 \pm 0.01 \pm 0.17 \) | \( 0.017 \pm 0.002 \pm 0.002 \) | \( 66.8 \pm 0.3 \pm 0.3 \) |
| BE-WLC | \( 0.83 \pm 0.01 \pm 0.17 \) | \( 0.010 \pm 0.001 \pm 0.002 \) | \( 68.1 \pm 0.3 \pm 0.6 \) |

* Two errors are given for each parameter: the first error is the error of the fit and the second error is the propagated error from uncertainties in the various parameters, especially the lever stiffness and linker parameters.
Single Molecule Force Spectroscopy

Building advanced instrumentation

... at Wayne State...
… to measure the mechanics of 0.15 nm water molecules

AFM cantilever = same scale as eye of an ant

with 0.003 nm precision

Sensitivity of AFM: 3 pm

0.1 nm

Measurement amplitude

Hydrogen atom

Water molecules
Viscoelastic properties measured at the nanoscale

FIG. 2 (color online). Left column: Stiffness (blue) and damping coefficients (red) measured for the last 3–4 layers adjacent to the mica substrate versus sample displacement. Substrate is located to the right in each case. Right column: Stiffness and mechanical relaxation time (green) versus sample displacement. (a), (b) Approach speed 2 Å/s, cantilever stiffness 2.4 N/m; (c), (d) 8 Å/s, 1.4 N/m; (e), (f) 14 Å/s, 1.4 N/m. Inset in Fig. 1(b): Stiffness after subtraction of repulsive background.
Shared instrument in the Mao Lab (Engineering):

- VEECO Dimension 3100
- Hourly user fees:
  - Non-profit $25 ($75 if Mao lab does the measurements)
  - For-profit $100 ($200 if Mao lab does the measurements)
- Make sure to acknowledge NSF MRI grant CTS-0216109 when publish AFM results obtained from the Dimension AFM.
- Contact person: Dr. Guangzhao Mao

Instruments available in P. Hoffmann Lab (Physics):

- Agilent AFM for ambient/liquid/biological imaging and force measurements
- WSU built small amplitude AFM for nanomechanical force measurements in liquids
- WSU built combined AFM/FCS system
- WSU built ultra-high vacuum based STM/small amplitude AFM
- NOTE: These are not open access instruments, but can be accessed if part of a collaboration.