High-speed Atomic Force Microscopy Shows Dynamic Molecular Processes in Photo-activated Bacteriorhodopsin


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Outline

• Principle of AFM
• Bacteriorhodopsin (bR)
• High-speed Atomic Force Microscopy
• Results
  - Conformational Changes
  - Decay Kinetics
• Conclusions
Introduction

1982, H. Rohrer (left) and G. Binnig (right) with atomic resolution
STM, Nobel Prize 1986

1985, Binnig, Quate and Gerber, Atomic Force Microscopy (AFM)
In Touch with Atoms

Limitation of Optical Microscope
1. Magnification 1600X
2. Wavelength 400~700 nm

Resolution limit: 0.2 µm

AFM: 1 nm

Binnig G. and Rohrer H. Rev. of Mod. Phys. 71(2), 324, 1999.
Elements of AFM Setup

Hooke's law: $F = k\Delta X$

**cantilever**
- spring which deflects as probe tip scans sample surface

**computer**
- controls system
- performs data acquisition, display, and analysis

**position sensitive photodetector**
- measures deflection of cantilever

**probe tip**
- senses surface properties and causes cantilever to deflect

**feedback loop**
- controls $z$-sample position

**laser diode**

**mirror**

**sample**

**piezoelectric scanner**
- positions sample ($x, y, z$) with Å accuracy

**sensor output, $\delta c, F_c$**
FIGURE 2-5 Block diagram showing the components in an AFM. The image is created by monitoring the voltage that drives the Z piezoelectric ceramic.
Modes of AFM

1. **Contact mode**: Constantly makes contact with the sample and the repulsive force is monitored to provide the topographic image of the sample.

2. **Non-contact mode**: Keeps a certain range from the sample and the attractive force is monitored to provide the topographic image of the sample.

3. **Tapping mode**: Driven to vibrate with large amplitude close to its resonant frequency. The tip only makes a temporary contact with the sample and the amplitude change is monitored to provide the topographic image of the sample (Good for bio-materials).
Interactions Between the Tip and Sample

Short range forces:
1) Chemical bonding
2) Coulomb repulsive

Long range forces:
1) Van der Waal
2) Capillary
3) Magnetic
4) Electrostatic

Lennard-Jones potential $\phi(r) = -\frac{A}{r^6} + \frac{B}{r^{12}}$
Approach-retract Cycles
What Information of Bio-materials Can be Obtained by AFM?

- **Sample morphology**
  - Sample shape, sample size, height, surface roughness, and total volume

- **Mechanical Properties**
  - Elasticity and viscoelasticity

- **Interactions between Tip and Sample**
  - Binding of ligand and receptor, specific chemical adhesive force, surface frictional force, electrostatic force, and magnetic force
Why Are Membrane Proteins So Important?

• Comprise approximately 30% of the proteome of most organisms. They mediate energy conversion, signal transduction, solute transport and secretion.

• More than 50% of all known drugs interact specifically with membrane proteins.

• To date, there are over 50,000 entries in the Protein Data Bank (PDB) repository of protein structures, but less than 1% of these entries represent membrane proteins.
## Comparison of Structural Determination Techniques

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<tr>
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<th>X-ray</th>
<th>NMR</th>
<th>AFM</th>
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<tbody>
<tr>
<td><strong>Advantages</strong></td>
<td>1. Atomic resolution</td>
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<td>1. Capability of imaging biological samples in physiological conditions</td>
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<td></td>
<td>2. No limitations of protein sizes</td>
<td>2. Similar to native status (solution phase)</td>
<td>2. Resolve structure and monitor interactions</td>
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<tr>
<td><strong>Disadvantages</strong></td>
<td>1. Challenge in crystallization</td>
<td>1. Hard to get complex structure information</td>
<td>1. Cannot compete with X-ray techniques in terms of resolution</td>
</tr>
<tr>
<td></td>
<td>2. Large amount of sample required</td>
<td>2. Size limitation of sample (35 kDa)</td>
<td>2. Cannot see inside of structure</td>
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Bacteriorhodopsin

1. Light-driven proton pump
2. Contains 7 transmembrane alpha helices
3. Covalently linked to Lys216 in the chromophore by Schiff base, giving protein itself purple color
4. Known as "purple membrane"
5. Retinal molecule that changes its conformation when absorbing a photon, resulting in a conformational change
Limitation of Image Acquisition Time

Conventional AFM: 30~60s to capture an image.

• Bandwidth of the local interaction between the tip and sample,

• Rate at which the tip can scan the surface of the sample in an $x, y$ plane

• *How quickly the tip* can follow the contours of the sample.
Challenge

• Most dynamic biological processes occur within milliseconds time scale or less.

e.g.
Photocycle of bR
WT: \( \sim 10 \) ms
D96N bR mutant: \( \sim 10 \) s
High-Speed AFM

1. Small cantilever of high resonant frequency (>1MHz in air; conventional: 100kHz) in tapping mode
2. High scan frequency and resonance frequency of the scanner
3. High bandwidth of photo-detector
4. Wide bandwidth of feedback loop control system

Image acquisition time is improved from ~30s to ~20ms
Successive AFM Images of D96N bR

Photocycle
D96N bR: ~10 s
WT: ~10 ms

Adsorbed onto a mica surface in 10 mM Tris-HCl (pH 7) and 300 mM KCl.

Movie 1
Traces of Mass Center (Position of Monomer)

“Mass centre” for each bR monomer was calculated from the corresponding surface area and height distribution in the image.

The photo-induced movement of bR includes counterclockwise rotation (7.4±2.28) around the trimer centre.
Decay Kinetics of Trefoils

$M_1$ to $M_3$ (Mn1–Mn3; n: indicates different trefoils)

0.007 $\mu$W

0.5 $\mu$W
Decay Kinetics of bR-bR in Trefoils
Conclusion

• High-Speed AFM can be used to visualize dynamic changes in stimulated proteins under realistic conditions.

• Direct and dynamic visualization using high-speed AFM should provide a better overview of the conformational changes of bR.

• bR-bR interaction in the transiently formed assembly engenders both positive and negative cooperative effects in the decay kinetics as the initial bR recovers and, as a consequence, the turnover rate of the photocycle is maintained constant, on average, irrespective of the light intensity.
Future Applications

• Investigate how physiological parameters influence membrane protein conformation.
  1. Binding of a Ligand or Inhibitor
  2. Oligomerization
  3. Supramolecular Assembly
  4. Functional State of Protein