Investigation of Protein-Protein Interaction Using Atomic Force Microscopy

PhD Dissertation Defense

by

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OUTLINE

• MOTIVATION
• EXPERIMENTAL SETUP
• THEORY AND DATA ANALYSIS
• FORCE MEASUREMENTS
• DISSOCIATION RATE OF ENZYME-INHIBITOR SYSTEM
• RUPTURE FORCE OF COMPLEX BOND ON THE MEMBRANES OF LIVING CELLS
• CONCLUSIONS & FUTURE DIRECTIONS
Can the AFM be used to investigate the proteins interaction?

\[ K_{eq} = \frac{k_{on}}{k_{off}} \]
Experimental Setup

The head of the AFM

SEM images for AFM probes

Schematic diagram showing the operating principle of the AFM.
Experimental Setup

Golden surface

Mica

Avidin

Biotin

PEG linker

AFM probe

Golden surface

Golden surface

Mica

Liquid cell
Theory

Standard Theory

- \( \frac{dS(t)}{dt} = -k_{off}(f)S(t) \)

- \( k_{off}(f) = k_{off}^0 \exp(fx^*/k_BT) \)

- \( r_f = \frac{df}{dt} = k_l \nu = \text{const} \tan t \)
### Theory

1. \( P_d(f) = \frac{k_{\text{off}}^0}{r_f} \exp \left( \frac{f}{f_c} \right) \exp \left[ \frac{k_{\text{off}}^0 f_c}{r_f} \left( 1 - \exp \left( \frac{f}{f_c} \right) \right) \right] \)

2. \( f_p = f_c \ln \left( \frac{r_f}{k_{\text{off}}^0 f_c} \right) \)
• Three examples of typical force curves, which read rupture forces and predict different scenarios of molecules interaction.
Force Measurements (Biotin-Avidin)

- Rupture force histograms, and Gaussian fitting.

- Pdf: probability distribution function.

\[ \text{Probability} = \int_{f_1}^{f_2} \text{pdf}(f) df \]
Results (Biotin-Avidin)

- Extracted values of the bond length ($x^*$), kinetic off rate ($k_{off}^0$) and activation barrier ($E_b^0$) for the complex bond Biotin-Avidin using the six different models discussed in the text.

<table>
<thead>
<tr>
<th>Model</th>
<th>$x^*(\text{nm})$</th>
<th>$k_{off}^0 (\text{s}^{-1}) \times 10^{-3}$</th>
<th>$E_b^0$ (kJ/mole)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard theory</td>
<td>0.42±0.07</td>
<td>8.32±2.6</td>
<td>72.70±0.77</td>
</tr>
<tr>
<td>BE-Effective</td>
<td>0.42±0.07</td>
<td>1.6±0.5</td>
<td>72.70±0.77</td>
</tr>
<tr>
<td>Cusp</td>
<td>0.43±0.06</td>
<td>1.2±0.3</td>
<td>73.41±0.62</td>
</tr>
<tr>
<td>Cubic</td>
<td>0.43±0.06</td>
<td>1.4±0.4</td>
<td>73.03±0.70</td>
</tr>
<tr>
<td>BE-FJC</td>
<td>0.40±0.70</td>
<td>2.4±1.2</td>
<td>71.70±1.23</td>
</tr>
<tr>
<td>BE-WLC</td>
<td>0.41±0.07</td>
<td>1.1±0.7</td>
<td>73.63±1.50</td>
</tr>
</tbody>
</table>
Force Measurements (TIMP-MMP)

MMP: Matrix metalloproteinases

TIMP: Tissue inhibitor matrix metalloproteinases

Results (TIMP1-MMP)

- Kinetic Off Rates, bond lengths and activation energies of TIMP1 and MMP as given by BE-WLC.

<table>
<thead>
<tr>
<th>Complex bond</th>
<th>$k_{off}^0$ (s$^{-1}$) $\times 10^{-3}$</th>
<th>$x^*$ (nm)</th>
<th>$E_b^0$ (kJ/mole)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TIMP1-MMP2</td>
<td>1.6±0.1</td>
<td>1.2±0.20</td>
<td>72.7±0.5</td>
</tr>
<tr>
<td>TIMP1-ProMMP2</td>
<td>No binding</td>
<td>No binding</td>
<td>No binding</td>
</tr>
<tr>
<td>TIMP1-MMP9</td>
<td>1.4±0.1</td>
<td>1.4±0.30</td>
<td>73.0±0.5</td>
</tr>
<tr>
<td>TIMP1-ProMMP9</td>
<td>14.0±1.7</td>
<td>0.83±0.10</td>
<td>67.4±0.9</td>
</tr>
</tbody>
</table>
Results (TIMP2-MMP)

- Kinetic Off Rates, bond lengths and activation energies of TIMP2 and MMP as given by BE-WLC.

<table>
<thead>
<tr>
<th>Complex bond</th>
<th>$k_{off}(s^{-1}) \times 10^{-3}$</th>
<th>$x^*(nm)$</th>
<th>$E_b^0$ (kJ/mole)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TIMP2-MMP2</td>
<td>9.7±1.1</td>
<td>0.72±0.11</td>
<td>68.3±0.8</td>
</tr>
<tr>
<td>TIMP2-ProMMP2</td>
<td>19.4±2.5</td>
<td>1.25±0.13</td>
<td>66.5±1.0</td>
</tr>
<tr>
<td>TIMP2-MMP9</td>
<td>18.7±2.7</td>
<td>0.75±0.11</td>
<td>66.6±1.1</td>
</tr>
<tr>
<td>TIMP2-ProMMP9</td>
<td>40.0±5.4</td>
<td>0.72±0.06</td>
<td>64.8±1.0</td>
</tr>
</tbody>
</table>
Force Measurements (TIMP-MT1-MMP) Living Cells

![Diagram showing protein molecules, carbohydrate chains, and a living cell with labeled parts like outside cell, inside cell, protein channel, and a lipid bilayer.]

![Graph showing force measurements in pN versus distance in nm, with two curves: Approaching (black) and Retracting (red). Points (a) and (b) are indicated on the graph.]
Force Measurements (TIMP-MT1-MMP) Living Cells

(a) - Force vs. distance for TIMP-MT1-MMP interaction with living cells, showing approaching and retracting phases.

(b) - Force vs. distance for a different interaction, also showing approaching and retracting phases.
Results (TIMP-MT1-MMP) Living Cells

- Binding probability of TIMP1, TIM2 to the receptors MT1-MMP using two types of cells, GPI and EV.
Results (TIMP-MT1-MMP)
Living Cells

- Gaussian fitting

Rupture force of TIMP1-MT1-MMP
Pdf (pN⁻¹)
Rupture force (pN)

Gaussian fitting

Rupture force of TIMP2-MT1-MMP
Pdf (pN⁻¹)
Rupture force (pN)
Conclusions

• The AFM can investigate proteins interaction and provides useful parameters.

• Using PEGs as cross linkers promotes the dissociation of the proteins bond.

• The TIMP2 inhibitor binds all MMP enzymes including MMP2, ProMMP2, MMP9 and ProMMP9. The TIMP1 inhibitor binds MMP9, ProMMP9 and MMP2, but it does not bind ProMMP2. The complex bond TIMP-MMP slowly dissociates with rate $10^{-3}$ s$^{-1}$, which indicates to high affinity.

• Unlike TIMP1, TIMP2 binds the receptors MT1-MMP on the membrane of living cell. The strength of the bond TIMP2-MT1-MMP is several hundreds of pico-Newton
And last but not the least, THANKS TO the Nanomechanics group:

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