The Effects of Different Linkers on Methotrexate-DHFR Interactions

Brandon Cen
University of Iowa

Background
Atomic Force Microscopy (AFM):
- One of the most effective tools for high-resolution imaging and force spectroscopy in biophysics.
- Laser focuses on the back of a cantilever containing a very sharp tip and reflects onto a photodiode.
- Tip raster scans across the sample surface, creating an image from the movement of the laser.
- Molecular Recognition Force Spectroscopy (MRFS): chemically attaching molecules on the tip and measuring interaction forces with them and the sample surface.

Diagram of AFM and picture taken from University of Iowa Dept. of Chemistry

- Polyethylene glycol (PEG) linker: a short polymer between a molecule and the tip it is attached to, designed to reduce nonspecific forces between the surface and tip.
- Dihydrofolate reductase (DHFR) is an enzyme important for its role as a target for anti-cancer drugs, specifically methotrexate (MTX).

Objective
To explore different linking techniques to more accurately and efficiently quantify biomolecular forces.

Hypothesis: The addition of DNA and PEG linkers will allow separation of nonspecific interactions from data and provide more accurate force analysis.

Methods
- Test a new linker method involving the addition of DNA on to the sample surface for DHFR to bind on top of it.
- Better ensures only interactions between MTX and single DHFR molecules and reduces nonspecific forces between the tip and the surface.
- Ethanol amine to block DHFR from binding on any surface besides tops of DNA.

AFM-scanned image with white circles showing vertical DNA along the sample surface

- 3 setups interacting:
  - DHFR on top of DNA bound to mica and directly tip-fixed MTX
  - DHFR homogenously spread as a monolayer on gold and MTX tip-fixed via PEG linker
  - DHFR on top of DNA bound to mica and MTX tip-fixed via PEG linker

Data compared to previous study using homogenous DHFR layer on gold and directly tip-fixed MTX
- Force plots generated with respect to tip-sample separation distance.
- Control measurements made by blocking active sites with free MTX or measuring DHFR-free surface spots

Results

<table>
<thead>
<tr>
<th>Method</th>
<th>Average Rupture Force (pN)</th>
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<tbody>
<tr>
<td>No linker/DNA</td>
<td>245 ± 110</td>
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<tr>
<td>PEG linker/DNA</td>
<td>194 ± 64</td>
</tr>
<tr>
<td>PEG linker/1 Monolayer</td>
<td>230 ± 85</td>
</tr>
<tr>
<td>PEG linker/2 Monolayer</td>
<td>120 ± 40</td>
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Conclusions
- Setups using DNA show a smaller deviation in average rupture forces compared to that of experiments using a monolayer.
- Produced histograms show a significant symmetry, suggesting a higher accuracy in MRFS.
- Overall decrease in average rupture values in experiments utilizing linkers implies a removal of nonspecific interactions previously increasing force readings.
- Significant decrease in deviation and average rupture forces in experiment using both PEG linker and DNA confirms the ability for the two linker types to work simultaneously to ensure single-molecule adhesion events.

Implications
- Developed a new approach to utilize AFM for sensitive and selective measurements of microscopic forces on a single molecule level.
- The methodology is expected to be utilized for the direct measurement of biomolecular dynamics through the analysis of force fluctuations due to enzymatic motions.
- Advancements in viewing enzyme conformation changes when proteins interact with specific ligands.
- For example, could further investigation of the kinetics of the moving loop of DHFR over methotrexate during binding complex, possibly leading to new research in enzyme catalysis dynamics.

References

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