HIV RT Binding to DNA Primer-Template Tethered to a Quantum Dot

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Introduction

Using kinetically resolved single molecule microscopy, we have monitored the binding of DNA primer-template to the catalyzing enzyme HIV reverse transcriptase (HIV RT) molecules tethered to a biotinylated layer of bovine serum albumin (BSA).

We could identify and mark the 5’ end primer DNA with fluorescent quantum dots in a solution at 1nM. The 5’ primer DNA was linked to the quantum dot through a covalently bound streptavidin (SA) molecule. Each quantum dot had an average of seven binding sites for SA to attach. On the 3’ prime end of the DNA, a biotinylated HIV RT molecule was linked to an SA molecule at 5E-6 mg/ml which was in turn bound to BSA at .25% that was bound to a cover slip. The goal of the experiments was to measure the association and disassociation rates by observing the “on” and “off” times exhibited with different concentrations of deoxyguanine diphosphate (dGDP), directly observing the fluorescent glow of the quantum dot/DNA complex solutions on an intensified charged coupled device. The “on” times, or those where the quantum dots are visible are used to measure the dissociation rate while the “off” times show the association rates. To reduce the amount of blinking by the quantum dots from photo-oxidation that occurs, we added 10mM beta mercaptoethanol, a known oxygen “scavenger.”

Before the main experiment, characterization was needed to determine what concentration of biotinylated BSA (bBSA) would be needed. Experiments were made using an electron multiplying charged coupled device. Using bBSA linked to SA on a fluorescent dye called AlexaFluor555, an array of different percentages of bBSA at .1 mg/ml in a 50ul solution were used: 100%, 70%, 40% 10% 5% .5% .25% and .1% to determine the saturation point of molecules in each viewing window on the EMMCD. This was also a way to find where the easiest percentage of bBSA in order to have a reasonable number of molecules that weren’t blurred together in each viewing area. The final percentage of bBSA chosen to go with was .25% bBSA at .1mg/ml.

Goals

- Observe and characterize the ideal concentrations of bBSA in order to create a sample where there were around 5-12 quantum dots in each viewing window.
- Observe the association and dissociation rates of the quantum dot with different amounts of dGDP

Experiment

- 0 µM dGDP association rate
  - k_{apparent, association} = 0.166 ± 0.002 sec⁻¹

- 300 µM dGDP association rate
  - k_{apparent, association} = 0.227 ± 0.003 sec⁻¹

Results

Biotinylated BSA Surface Characterization

- Tested range of 0 - 100% bBSA bound to AlexaFluor 555
- Optimal number of fluorophores per window was 5-12

One Color Single Molecule Fluorescence Microscopy

- Thermally Stable, Endoscopic Oil immersion Objective
- Neutral Density Filter
- Mirror
- ICCD Camera
- Dicronic Mirror
- Neutral Density Filter
- Laser Beam

References

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