Fluorescent DNA-Binding Proteins for Single Molecule Visualization

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Abstract:

Single molecule manipulation techniques are powerful tools to examine the physical properties of nucleic acid biopolymers. Although the width of DNA is well below the diffraction limit, many remarkable studies have been achieved by monitoring microsphere handles attached to the nucleic acids. Combining single-molecule manipulation techniques with fluorescence visualization methods may open new pathways for future nucleic acid studies. Novel fluorescent DNA-binding proteins have recently been developed and could serve as a reversible method of visualizing DNA directly [1]. This study aims at complimenting single molecule manipulation techniques with fluorescent DNA-binding proteins. *Escherichia coli* (*E. coli)* cells were transformed in order to purify fluorescent DNA-binding proteins of interest. These proteins were then used in simple single molecule experiments to verify functionality. Fully characterized proteins will be incorporated into future single-molecule manipulation studies to enhance measurement capabilities.

Introduction:

Deoxyribonucleic acid (DNA) is a vital polymer in every known organism, which contains all of the genetic information of those organisms. DNA is essential in the biological processes of transcription and replication. These processes require molecular motors, which both recognize and temporarily alter the mechanical configuration of DNA [2]. Functional DNA topology is maintained through various enzymatic pathways, indicating the importance of torsional strain on individual DNA strands. If the DNA structure is not maintained, processes like transcription will fail or produce mutated products [3], which can cause death or disease to multicellular organisms.

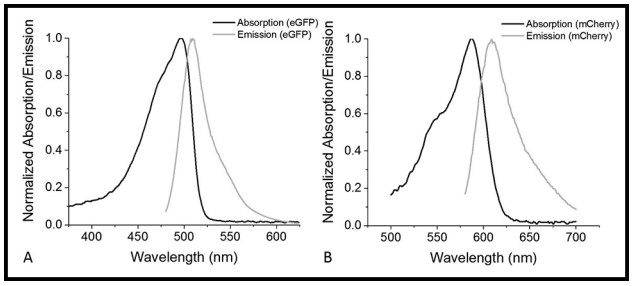

Figure 1: Measured spectra of eGFP-DBP (A) and mCherry-DBP (B) at their excitation and emission wavelengths.

This research aims at using fluorescent DNA-binding proteins (FDBP) to enhance these techniques by allowing visualization of individual strands of DNA.

Methods:

Protein Purification. *E. coli* cells (BL21 DE3) were transformed to express two FDBPs: eGFP DNA-binding protein (eGFP-DBP) and mCherry DNA-binding protein (mCherry-DBP). These proteins were purified using a Ni-NTA column followed by dialysis. The absorption and emission spectra of the purified proteins were characterized (Figure 1) and were in agreement with published values.

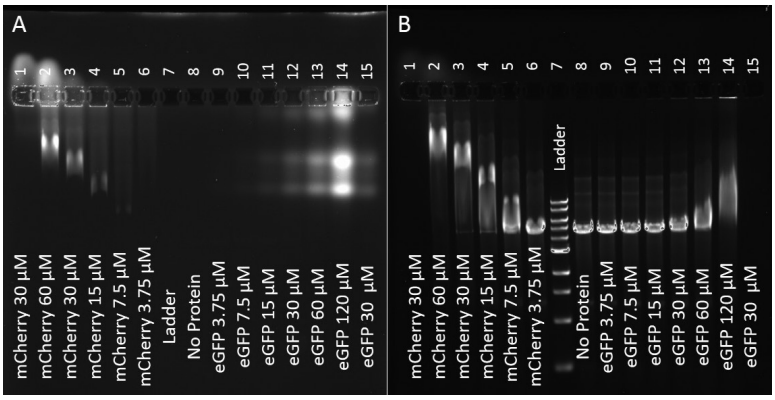

Figure 2: Gel mobility assay for determining DNA-Binding ability of both FDBPs. All lanes (except lanes 1 and 15) include 150 ng of 4.7 kbp DNA. Contents are labeled on each lane. (A) mCherry (left) and eGFP (right) fluorescence. (B) DNA stained with ethidium bromide.

DNA-Binding Characterization. The ability of the proteins to bind DNA was characterized by gel electrophoresis. Bound proteins are expected to slow the travel of the DNA through the gel, varying with concentration. Figure 2 shows that mCherry-DBP bound DNA, as indicated by the reduction of DNA travel with increasing protein concentration. eGFP-DBP bound DNA less efficiently, and furthermore, two strong bands appear on Figure 2a on eGFP-DBP lanes, reflective of a degraded protein. This behavior may result from the DNA-binding region coming apart from the eGFP domain in the FDBP.

Single-Molecule Visualization. Single-DNA visualization was attempted by fluorescence microscopy after incubating FDBP with surface-anchored DNA in a single-molecule flow chamber. Single-molecule chambers were established between two microscope coverslips fastened by double-sided tape. Chambers were first incubated with anti-digoxigenin (5 ng/µl), and then passivated with either casein from bovine milk (10 ng/µl) or bovine serum albumin (BSA, 5 ng/µl). DNA template (48 kbp, 8.3 nM), end-labeled with digoxigenin, was then incubated in order to anchor to the surface. After thorough rinsing with PBS, FDBPs were flowed through the chamber. Due to strong non-specific adsorption by FDBPs, variations in chamber preparation were attempted. Notably, pre-incubation of FDBP with DNA, along with patterning of the anti-digoxigenin were attempted without significant reduction in non-specific surface attachment of FDBP. Expected DNA attachment was confirmed using SYTOX Green DNA stain. All of these attempts appeared nearly identical to Figure 3. The protein appeared to readily adsorb to the surface, even at concentrations as low as 0.5 nM. The resulting background fluorescence prevented visualization of anchored DNA.

Future Work:

Binding of eGFP-DBP to DNA needs to be further characterized, and new protein may need to be expressed and purified. Non-specific adsorption of FDBPs to surfaces in single-molecule flow chambers is currently a significant limitation.

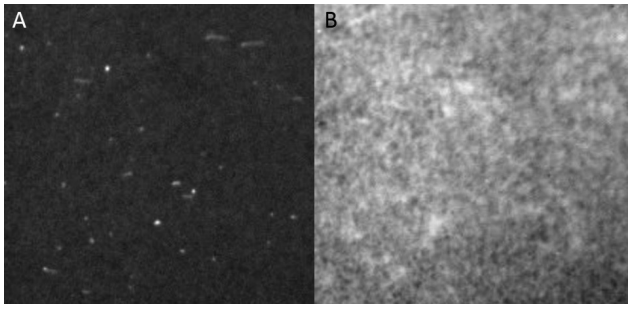

Figure 3: Representative fluorescence microscopy images. (A) SYTOX Green DNA stain fluorescence. (B) mCherry fluorescence.

Passivation can be improved with additional protein characterization (e.g. isoelectric point), concentration tuning, and alternative approaches (e.g. lipid bilayer passivation). Protein concentration for single-molecule visualization also needs further optimization. After optimization, we expect to conduct single-molecule DNA studies with the added visualization functionality provided by FDBPs.

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