

Application Note

Featuring BiOptix'
Surface Plasmon Enhanced (SPE)
Interferometry Technology for
highly sensitive multiplexed biodetection

Characterization of Single Stranded Binding Protein's Nucleic Acid Binding Requirements using the BiOptix ACCOLADE™ Instrument

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Introduction

Protein-nucleic acid interactions are critical during all phases of the life of a cell. Among the proteins that bind nucleic acids are those that preferentially bind to single stranded DNA. This not only protects the DNA molecule, but can stabilize or modify access to genetic information for subsequent cellular processes. One such protein, the single-stranded DNA binding protein from *E. coli* (SSB) is involved in cellular reproduction and maintenance of genetic information (Krauss et al. 1981).

In this application note we present data that demonstrate BiOptix' proprietary Surface Plasmon-Enhanced common path interferometry (SPE) is capable of analyzing protein-oligonucleotide interactions in real time and without the need of prior molecular labeling. Using the BiOptix ACCOLADE™ instrumentation and NeutrAvidin SensorChips, SSB's single stranded and base-length dependent binding specificities were evaluated and corroborated with previous reports (Meyer and Laine, 1990; Kozlov and Lohman, 2002).

Materials

Biotinylated and non-biotinylated oligonucleotides were purchased from Integrated DNA Technologies. Oligonucleotide sequences are listed in Table 1. SSB protein was purchased from Epicentre Biotechnologies. BiOptix' NeutrAvidin SensorChips (part number C-40403) were used. Phosphate-buffered saline (PBS, 10x) was supplied by Mediatech, Inc.

Table 1: Oligo names and sequences

| Oligonucleotide Name | Sequence* |
|----------------------|---|
| 4511 (Ligand Oligo) | B-TCTCTCACAAACACCATTGTCCACTCCACTCT |
| 7434 (Analyte Oligo) | AGAGTGGAGTGTGACAATGGTGTGGT |
| 12-mer | GGTAACGATGT |
| 22-mer | TAACACTGTCTGGTAACGATGT |
| 50-mer | GCATTTGTTATCATCATCCCTGAATTCAGAGATGAAATTTGGCCACTCA |

* B indicates oligonucleotide is biotinylated at the 5' end

Methods

BiOptix NeutrAvidin SensorChips were rinsed thoroughly with PBS to remove the surface preservative. The SensorChips were prepared for insertion into the ACCOLADE™ per manual instructions. All binding studies were performed in PBS at 20°C.

Experiments And Results

Comparing SSB's binding affinity for double stranded and single stranded oligonucleotides

An experiment was performed to demonstrate the binding specificity of SSB for single stranded DNA versus double stranded DNA. Biotinylated oligonucleotide 4511 was first immobilized by injection onto a NeutrAvidin-coated SensorChip. Double stranded (ds) DNA was generated by injecting the complementary 7434 oligonucleotide over the same SensorChip. As expected, when SSB was injected onto a SensorChip containing dsDNA, no significant binding was observed (<30 RUs). Denaturation of the dsDNA with 20mM NaOH for 20 seconds, followed by a subsequent injection of SSB resulted in an instrument response of around 2000 RUs. (Figure 1).

Competitive binding experiment

Researchers have previously reported that SSB binding requires a DNA strand that is at least 35 nucleotides in length (Kozlov and Lohman, 2002). A competitive inhibition binding experiment was performed to confirm this observation using the ACCOLADE™ and the same sensor chip as described above. Serial 25 min injections, 15 min washes and 20 sec regenerations were performed. Each injection consisted of 20nM SSB pre-mixed with 200nM of an oligonucleotide of varying length, ranging from 12 to 50 nucleotides. A positive control was also run in which SSB was injected alone. The results shown in Figure 2 are consistent with a previous report that concluded a minimal DNA length (between 22 and 50 nucleotides) is required for efficient SSB binding.

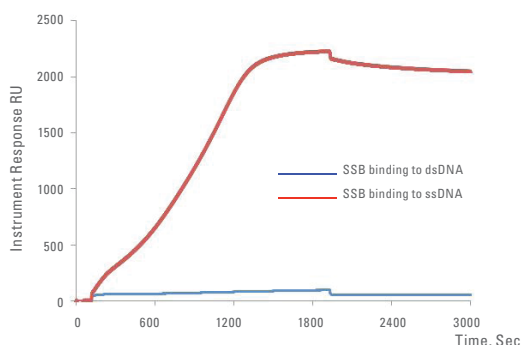


Figure 1. Demonstration of preferential ssDNA binding. No binding was detectable when SSB was injected over the SensorChip charged with dsDNA (Blue Curve). After ssDNA regeneration, SSB binding was observed with a response that showed a sigmoidal association curve indicative of a cooperative binding event (Red Curve).

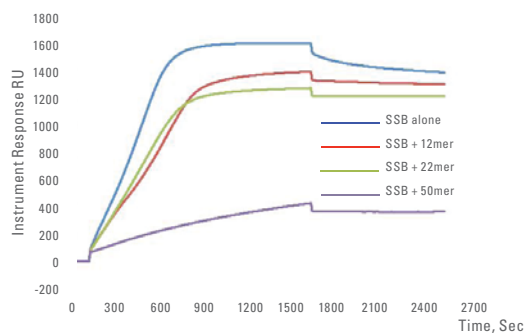
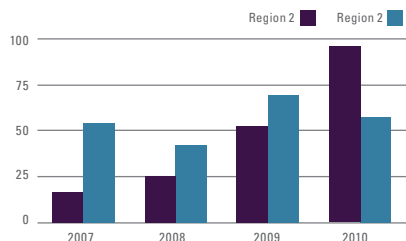


Figure 2. Competitive binding experiment to evaluate single stranded sequence requirements of SSB. Each curve represents a different injection followed by a wash step, superimposed in one graph. The experiment's injection sequence was as follows: SSB alone, SSB + 12mer, SSB + 22mer, and SSB + 50mer with regeneration steps between each injection. Each curve shown is an average of the four channels. The responses of the four channels were within 5% of the average.

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Experiments And Results (continued)

Kinetic analysis of SSB binding to single stranded DNA

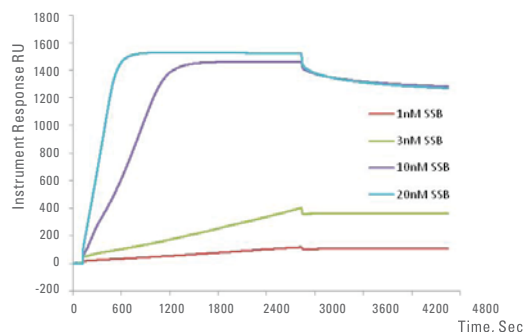


Figure 3. SSB binding kinetic analysis. SSB at the various concentrations was injected for 45 min onto a NeutrAvidin-coated SensorChip containing immobilized oligonucleotide 4511, followed by a 30 min buffer wash.

An experiment was performed to evaluate the changes in SSB binding rates relative to protein concentrations. A SensorChip with immobilized oligonucleotide 4511 was exposed to a series of SSB concentrations, from 1 nM to 20 nM (Figure 3). These data illustrate the importance of choosing the proper analyte concentrations when evaluating binding kinetics. The sigmoidal-like curve shape observed at the higher concentrations is consistent with the cooperative binding modality previously reported for this protein (Meyer and Laine, 1990). The linear association and dissociation curves at the lowest concentration (1 nM) suggest the existence of mass transport issues, and require more complex analysis to determine the correct kinetic constants (Myszka et al., 1998).

Conclusions

These experiments demonstrate the advantages of using the BiOptix ACCOLADE™ instrument to characterize binding modalities of a protein-nucleic acid molecular interaction. Using SPE technology, it was possible to quickly confirm SSB binding preferences for single stranded oligos and the cooperative nature of this interaction.

References

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