APPLICATION NOTE

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Binding Kinetics of Complementary Oligonucleotide Sequences using OpenSPR™

Summary

- DNA-DNA interactions were analyzed using OpenSPR-XT Instrument.
- Biotinylated oligonucleotide was immobilized with streptavidin capture.
- Kinetic analysis was used to determine on-rate, off-rate, and affinity constant of the interaction between the biotinylated oligonucleotide and a reverse complement analyte sequence.

Overview

Oligonucleotides are an important area of biotechnological and pharmaceutical research, as demonstrated by the recent success of mRNA-based COVID-19 vaccines. Oligonucleotides, namely siRNA and RNAi, are also used to treat a number of diseases related to gene expression, due the ability of complementary oligonucleotides to block other molecules from accessing relevant portions of a DNA or mRNA sequence.

Characterizing such molecules is critical for the continued development of these important vaccines and therapeutics. Surface plasmon resonance (SPR) is a well-suited technique for analyzing nucleic acid binding interactions as it can accurately and precisely measure affinities and on/off-rates. In this application note, OpenSPR was used to measure binding of a biotinylated DNA sequence to another DNA sequence containing the reverse complement.

Materials & Equipment

- OpenSPR-XT Instrument
- TraceDrawer™ Kinetic Analysis Software
- OpenSPR Biotin Sensor
- OpenSPR Streptavidin Kit
- PBS-T running buffer (137 mM NaCl, 2.7 mM KCl, 10mM Phosphate, pH 7.4, 0.1% Tween 20)
- Biotinylated Oligo Ligand (Bt-Oligo)
- Surface Oligo Analyte (S-Oligo)

Procedure

Following the start-up procedure in the software, the OpenSPR-XT instrument was set up, using PBS-T as the initial running buffer. Streptavidin was then immobilized on the biotin sensor surface at a concentration of 20 µg/mL and a flow rate of 20 µL/min.

The ligand, Bt-oligo, was immobilized on the streptavidin surface at a concentration of 300 nM and a flow rate of 20 µL/min on channel 2 only (designated as the response channel). The analyte, S-oligo, was diluted in running buffer to 200 nM and further diluted in 5-fold series.

Each analyte sample was analyzed in order of low to high concentration at 20 µL/min with an association period of 180 seconds, and dissociation period of 600 seconds. The sensor surface was regenerated between each analyte injection with an injection of 1mM NaOH at 150 µL/min.

Binding kinetics were measured using the TraceDrawer™ Analysis Software using a one-to-one diffusion corrected fit model.

Results and Discussion

The immobilization of streptavidin on the biotin sensor had a response of over 2500 RU as shown in Figure 1. Figure 2 shows that approximately 125 RU of ligand was captured by streptavidin.

In Figure 3, the kinetic fit of S-oligo to Bt-oligo is shown, along with the fit obtained from the one-to-one diffusion corrected model. This experiment was run nine times across multiple batches of OpenSPR biotin sensors, and the average of the calculated kinetic constants are presented in Table 1 along with the standard deviation and coefficient of variation. This system has an affinity constant of 5.72 nM and the observed Rmax of the analyte was 299 RU. These results show remarkable precision and highlight the reproducibility and reliability of the OpenSPR instrument and sensors.

The specificity of the interaction is confirmed by the reference channel response shown in Figure 4. No non-specific binding was observed.

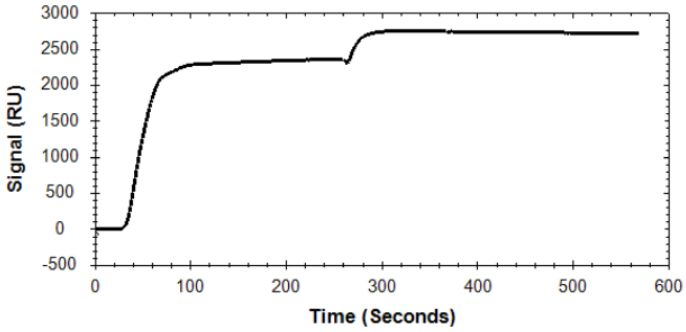

Figure 1: *Immobilization of streptavidin to the OpenSPR Biotin Sensor.*

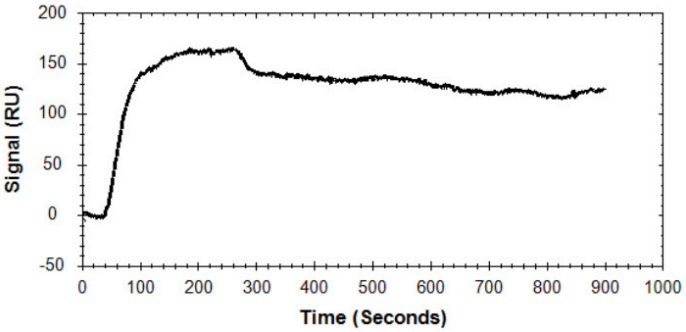

Figure 2: *Capture of Bt-oligo ligand by the streptavidin surface.*

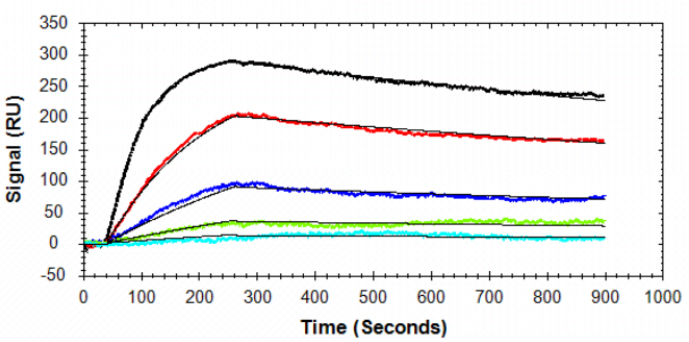

Figure 3: *Binding of S-oligo at 200 nM, 67 nM, 22 nM, 7.5 nM and 2.4 nM to the immobilized Bt-oligo. The solid black lines represent the one-to-one diffusion corrected kinetic model fits.*

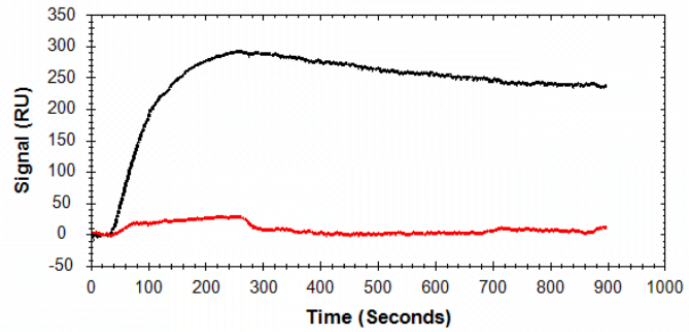

Figure 4: *Specific and non-specific binding of 200 nM S-oligo to Bt-oligo. Specific binding in the response channel is shown in black and non-specific binding in the reference channel is shown in red.*

Table 1: Kinetic values measured using OpenSPR data with the TraceDrawer analysis software.

n=9	k_a (1/(M*s))	k_d (1/s)	K_D (nM)	R_{max} (RU)
Average	$7.73 \times 10^4 \pm 1.32 \times 10^4$	$4.33 \times 10^{-4} \pm 6.60 \times 10^{-5}$	5.72 ± 1.09	299 ± 22.6
% CV	17.09	15.23	19.11	7.57

Conclusion

In this application note, OpenSPR was used to precisely and specifically measure kinetics and affinity between an oligonucleotide and its complementary sequence. These results demonstrate the value of surface plasmon resonance in oligonucleotide research, particularly in sense-antisense interactions which are used in several important applications such as siRNA and RNAi.