Binding Kinetics of Aptamer-Protein Interactions using OpenSPR

Summary

- Aptamer-protein interactions were analyzed on the OpenSPR™ instrument
- Kinetic analysis was used to determine the on rate, off rate, and affinity constant of the interaction between a lysozyme and its aptamer
- The K_D was found to be 15 nM

Overview

OpenSPR™ is a powerful instrument providing in-depth label-free binding kinetics for a variety of different molecular interactions. One of the most common applications of surface plasmon resonance is the analysis and quantification of the interactions between proteins and other molecules. In this application note, OpenSPR™ is used to analyze the k_{on}, k_{off}, and K_D of an aptamer–lysozyme interaction. Lysozymes are enzymes that damage bacterial cell walls via glycoside hydrolysis.

Materials and Equipment

- OpenSPR™ Instrument
- OpenSPR Sensor Chip
- TraceDrawer Kinetic Analysis Software
- Con A & Glycan Samples
- Running Buffer
- Regeneration Buffer

Procedure

1. Following the start-up procedure found in the OpenSPR manual, setup the OpenSPR instrument and software.

2. Immobilize biotinylated lysozyme aptamer on an OpenSPR Streptavidin Sensor Chip via loading 200 µL of 2.5 µM aptamer for a 5 minute interaction time

3. Once the immobilization is complete, continue pumping running buffer for 5 minutes until a stable baseline is achieved. Rinse the sample loop with running buffer and purge with air.

4. Prepare 200 µL of lysozyme analyte dilutions into the running buffer at 100, 33, 11, and 1.23 nM.

5. Inject analytes at a flow rate of 30 µL/min with an association time of 150 seconds and a dissociation time of 300 seconds.

6. Regenerate the surface with the regeneration buffer (2 M NaCl) in between each injection.

7. In a separate experiment, the level of non-specific binding of the analyte is tested using a plain streptavidin sensor to confirm a specific response.

8. Data from OpenSPR is analyzed using TraceDrawer™ using a one to one binding model with diffusion correction.

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Results and Discussion

Aptamer immobilization is shown in Figure 1, with approximately 250pm of aptamer loading on the streptavidin surface. Results from the lysozyme analyte binding to the aptamer coated surface are shown below in Figure 2 for the four concentrations tested. The association and dissociation phases are clearly evident from the real-time sensorgrams. The binding models are shown as solid black lines overlaid onto the raw data. The data fits very well with the theoretical binding model as the residuals are low and random errors small. The kinetic constants are given below with the errors in brackets:

OpenSPR™

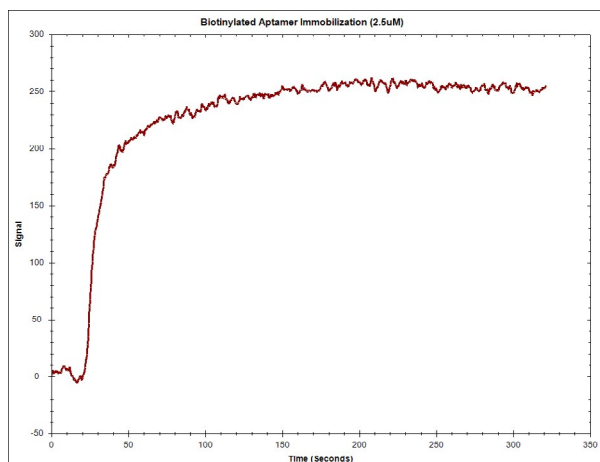|	
k_D	14.6 nM (+/- 0.093)
k_{on}	$1.76e^5$ 1/M · sec (+/-$1.0e^3$)
K_{off}	$2.58e^{-3}$ 1/sec (+/- $2.6e^{-6}$)

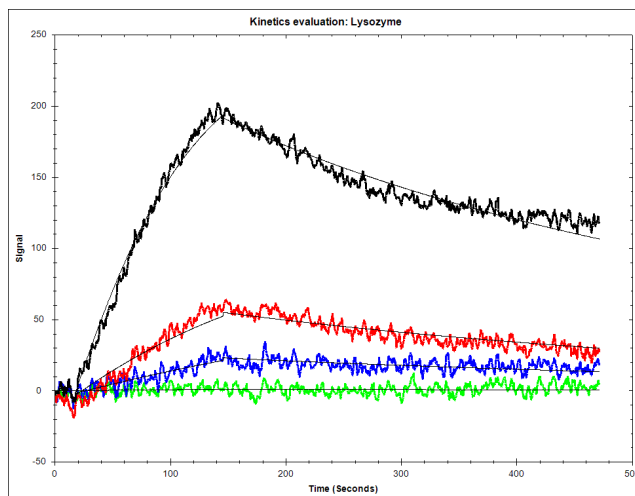Figure 1. Immobilization of biotinylated aptamer to Streptavidin Sensor Chip on OpenSPR

Figure 2. Binding curves and kinetic analysis of lysozyme binding to its aptamer on OpenSPR (100, 33, 11, and 1.23 nM from top to bottom)

Conclusions and Summary

This study demonstrates how OpenSPR can be used to determine the binding kinetics between aptamers and proteins. Similar procedures can be used to evaluate a wide number of aptamer–protein interactions. Simple experiments that use minimal sample were conducted to extract powerful data and insight into the binding nature of this biomolecular system.