Binding Kinetics of Glycoprotein Interactions using OpenSPR

Summary

- Glycan-Con A interactions were analyzed on the OpenSPR™ instrument
- Kinetic analysis was used to determine the on rate, off rate, and affinity constant of the interaction
- The K_D was found to be 0.26 µM

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 a glycan – concanavalin A (Con A) interaction. Con A is a lectin (carbohydrate binding protein) that binds to a number of sugars, glycans, glycoproteins, and glycolipids.

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. Glycan (sugars) are immobilized onto OpenSPR sensor chips.
3. Once the immobilization is complete, continue flowing running buffer for 5 mins until a stable baseline is achieved. Rinse the sample loop with running buffer and purge with air.
4. Prepare 200 µL analyte dilutions into the running buffer at 10 µM, 5 µM, and 2 µM.
5. Inject analytes at a flow rate of 40 µL/min with an association time of 150 secs and a dissociation time of 800 secs.
6. Regenerate the surface with the regeneration buffer in between each injection.
7. In a separate experiment, the level of non-specific binding of the analyte is tested using a non-glycan coated sensor to confirm a specific response.
8. Data from OpenSPR is analyzed using TraceDrawer with a bivalent binding model as Con A has 4 binding sites.

Results and Discussion

Results from the Con A analyte binding to the glycan coated surface are shown below in Figure1 for the three concentrations tested. The association and dissociation phases are clearly evident from the real-time sensorgrams. The binding model is a bivalent model and is 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 and the errors small.

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	OpenSPR™
k_D	0.26μM (+/- 0.0087)
k_{on}	$8.25e^{3}$ 1/M · s (+/-1.8)
K_{off}	$2.13e^{-3}$ 1/s (+/- $2.12e^{-7}$)

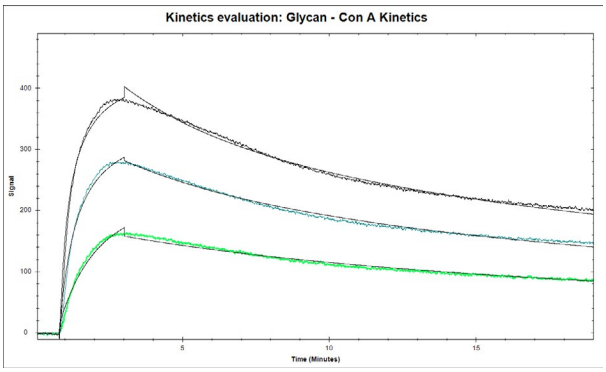

Figure 1. Binding curves and kinetic analysis of Con A on OpenSPR (10 μM, 5 μM, and 2 μM from top to bottom)

Conclusions and Summary

This study demonstrates how OpenSPR can be used to determine the binding kinetics between glycans and proteins. Similar procedures can be used to evaluate a wide number of glycan related interactions. Simple experiments that use minimal sample were conducted to extract powerful data and insight into the binding nature of this biomolecular system. OpenSPR is an essential tool for studying carbohydrate-protein interactions, examining the effects of protein glycosylation, and studying many other interactions involving glycoproteins and glycans.