Binding Kinetics of Protein–Protein Interactions using OpenSPR™

Summary

- A protein-protein interaction was analyzed on the OpenSPR™
- Kinetic analysis was used to determine the on rate, off rate, and affinity constant of the interaction of a protein ligand with a protein analyte
- The K_D was found to be 1.53nM with OpenSPR™

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. In this application note, OpenSPR™ is used to analyze the kon, koff, and K_D of a protein-protein interaction.

Materials and Equipment

- OpenSPR™ Instrument
- OpenSPR™ COOH Sensor Chip & Amine Coupling Kit
- TraceDrawer Kinetic Analysis Software
- Ligand Protein (MW 30kDa)
- Analyte Protein (MW 15kDa)
- Running Buffer: PBS + 150mM NaCl + 0.05% Tween 20, pH 7.4
- Regeneration Buffer: 10mM Glycine HCl pH 2.5
- CM4 Sensor Chip
- Immobilization buffer: Sodium Acetate

Procedure

OpenSPR Experiment

1. Following the start-up procedure found in the OpenSPR™ manual, setup the OpenSPR™ instrument and load a COOH Sensor Chip.
2. Following the instructions included in the Amine kit, activate the COOH surface. Dilute ligand at concentration of 82.5ug/ml into immobilization buffer, and inject 100uL at 20uL/min for a 5 minute interaction time.
3. Block the surface with an injection of 100uL of blocking buffer.
4. Increase the flow rate to 30uL/min, and inject analyte at the following concentrations: 6.25, 12.6, 25, and 50nM. Use an association time of 150 seconds and a dislocation time of 400 seconds.
5. Regenerate the surface with an injection of regeneration buffer at a speed of 150ul/min in between each analyte injection.
6. Data was single referenced with blank injections.

Results and Discussion

Results from the protein-protein interaction measured on the OpenSPR™ instrument can be found in *Figure 1*. The data fits very well with the theoretical 1:1 binding model as the residuals are low and random and the errors small. The kinetic constants determined from the fit are shown in *Table 2*. OpenSPR™ determined a K_D of 1.53nM.

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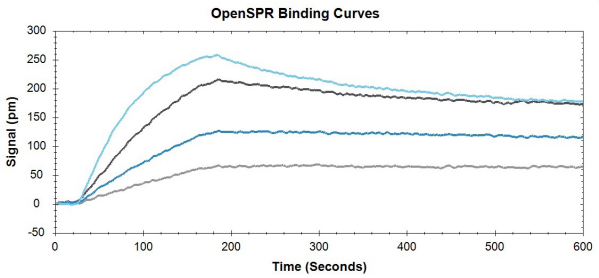

Figure 1. Protein-protein interaction analyzed using OpenSPR™ with analyte protein concentrations of 6.25, 12.6, 25, 50nM

Table 1. Kinetic and affinity constants of protein-protein interaction measured on OpenSPR™

	OpenSPR™
k_{on} [1/M*s]	8.18e5
k_{off} [1/s]	1.25e-3
K_D [nM]	1.53

Conclusions and Summary

This study demonstrates how OpenSPR can be used to determine the binding kinetics between proteins. A simple experiment that uses minimal sample was conducted to extract powerful data and insight into the binding nature of this biomolecular system.

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