Binding Kinetics of Protein–Small Molecule Interactions using OpenSPR™

Summary

- Protein-small molecule interactions were analyzed on the OpenSPR™ instrument
- Kinetic analysis was used to determine the affinity constant of the interaction between arginine kinase and ATP+Creatine
- The K_D was found to be on the order of 0.303 mM

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 small molecules. This is of significant importance in the pharmaceutical industry to help researchers identify and characterize new drugs. In this application note, OpenSPR™ is used to analyze the K_D of the arginine kinase enzyme with ATP and Creatine.

Materials and Equipment

- OpenSPR™ Instrument
- OpenSPR NTA Sensor Chip
- TraceDrawer Kinetic Analysis Software
- Ligand Arginine Kinase (his-tagged, 40 kDa)
- Analyte: ATP and Creatine
- Running Buffer: HBS-EP

Procedure

1. Following the start-up procedure found in the OpenSPR manual, setup the OpenSPR instrument and software.
2. Prime the NTA sensor chips with imidazole followed by $NiCl_2$ as outlined in the technical guide.
3. Immobilize his-tagged arginine kinase on the NTA sensor chip by injecting 100 µL of 10 µM arginine kinase at a flow rate of 20 µl/min for a 5 mins incubation period.
4. Prepare 150 µL of Creatine and ATP dilutions into the running buffer at the following concentrations:
   a. ATP, Creatine: 1 mM, 0.5 mM, 0.25 mM
5. Inject the analytes above individually at a flow rate of 40 µL/min with an association time of 150 secs and a dissociation time of 150 secs.
6. Data from OpenSPR is analyzed using TraceDrawer™.

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Results and Discussion

The immobilization of the enzyme onto the NTA sensor chip is shown in *Figure 1* with over 700 pm of response units for the immobilization. The immobilization was stable with a minor background drift, as is typically seen with his-tagged immobilization methods and is easily compensated for by subtracting a blank injection. *Figure 2* shows the binding of the ATP+Creatine analyte at 3 different concentrations. The association phase and dissociation phases are clearly evident as is the concentration dependence. The data is fit to a 1:1 binding model in TraceDrawer. The kinetic constants are shown in *Table 1*, and the fits are overlaid in *Figure 1* as solid black lines. The fit quality is excellent with a low Chi squared value of 1.94. The K_D value is determined to be 0.303 mM for this interaction.

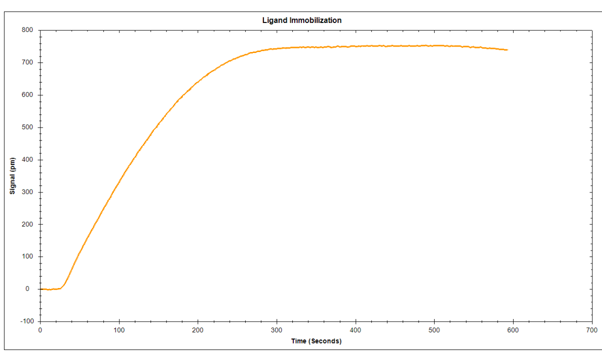

Figure 1. Ligand immobilization of arginine kinase enzyme onto NTA sensor chip

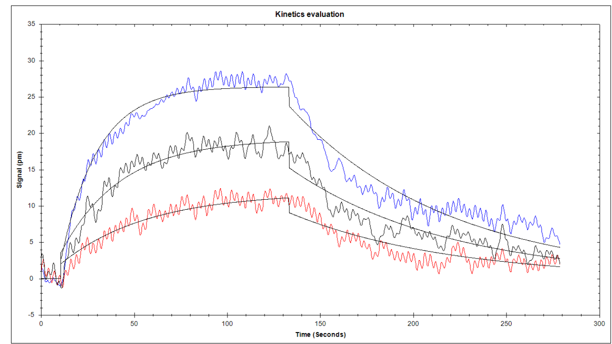

Figure 2. Binding of ATP+Creatine to enzyme at concentrations of 1 mM, 0.5 mM and 0.25 mM (blue, black and red curves). Solid black lines are the 1:1 binding model fits.

Table 1. Binding kinetics and affinity measured using OpenSPR between kinase enzyme and ATP+Creatine

	OpenSPR™
k_{on} [1/M · s]	3.88e1
k_{off} [1/s]	1.18e-2
K_D [mM]	0.303

Conclusions and Summary

This study demonstrates how OpenSPR can be used to determine the binding kinetics between proteins and small molecules. Similar procedures can be used to evaluate a wide number of protein-small interactions using OpenSPR.

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