

Binding Kinetics of Glutathione-S-Transferase (GST) Tagged Proteins Using OpenSPR™

SUMMARY

- GST-tagged Ubiquitin was immobilized onto a GST specific sensor.
- Kinetic binding of Anti-Ubiquitin antibody to Ubiquitin was measured using the OpenSPR™ instrument
- A bivalent kinetic interaction model was used to determine the affinity constant of the interaction between GST-Ubiquitin and Anti-Ubiquitin Antibody
- The K_D was determined to be 95.3 nM
- OpenSPR™ GST sensors can be used for GST tagged protein immobilization

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 tags used for protein separation is glutathione-S-transferase (GST). Proteins containing this tag bind freely to glutathione. Nicoya Lifesciences has created a glutathione functionalized sensor that makes immobilization of GST tagged proteins simple. In this application note, OpenSPR™ is used to analyze the K_D of the GST-Ubiquitin with Anti-Ubiquitin Antibody.

Materials and Equipment

- OpenSPR™ Instrument
- OpenSPR™ GST Sensor Chip
- OpenSPR™ GST Reagent Kit
- TraceDrawer™ Kinetic Analysis Software
- Ligand: GST-Ubiquitin
- Analyte: Anti-Ubiquitin Antibody
- Running Buffer: PBS-T pH 7.4

Procedure

1. Following the start-up procedure found in the OpenSPR™ manual, setup the OpenSPR™ instrument and software.
2. Clean the GST Sensor with 10mM NaOH as outlined in the technical guide.
3. Prime the GST Sensor with GSH solution provided in the GST Reagent Kit as outlined in the technical guide.
4. Immobilize GST-Ubiquitin at a flow rate of 20 μ L/min for a 5 minute incubation period.
5. Prepare 200 μ L Anti-Ubiquitin Antibody

dilutions into the running buffer at the following concentrations: 66.7 nM, 22.3 nM and 7.4 nM

6. Inject the analytes above individually at a flow rate of 20 $\mu\text{L}/\text{min}$ with an association time of 300 s and a dissociation time of 500 s.
7. Data from OpenSPR™ is analyzed using TraceDrawer™.

Results and Discussion

The immobilization of the GST-ubiquitin onto the GST sensor chip is shown in Figure 1 with over 600 pm of response for the immobilization. Figure 2 shows the binding of the Anti-Ubiquitin Antibody analyte at 3 different concentrations. The association phase and dissociation phases are evident as is the concentration dependence. The data is fit to a bivalent binding model in TraceDrawer™. The bivalent model accounts for the 2 binding sites found on the analyte surface and is perfect for antibody based analyte interactions. 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 12.5. The K_D value is determined to be 95.3 nM for this interaction.

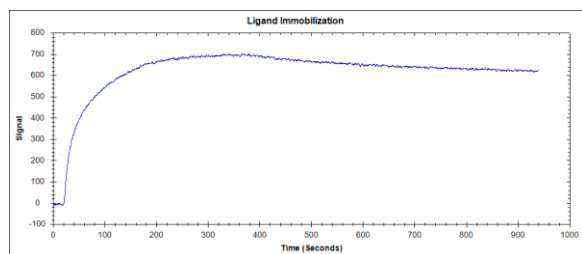


Figure 1. Ligand immobilization of GST-ubiquitin to the GST Sensor Chip

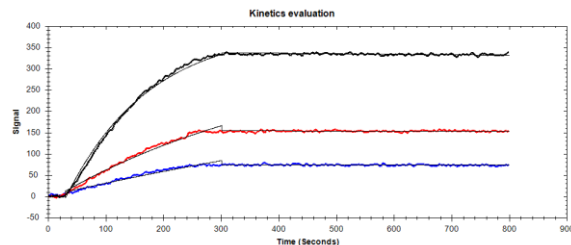


Figure 2. Binding of Anti-ubiquitin antibody at concentrations of 7.4 nM, 22.3 nM and 66.7 nM (blue, red and black curves). Solid black lines are the bivalent binding model fits.

Table 1. Binding kinetics and affinity measured using OpenSPR™ between GST-Ubiquitin and anti-ubiquitin antibody.

	OpenSPR™
k_{on} [1/M*s]	5.18×10^4
k_{off} [1/s]	4.94×10^{-3}
K_D [M]	9.53×10^{-8}

Conclusions and Summary

This study demonstrates how OpenSPR™ can be used to immobilize GST-tagged proteins and determine the binding kinetics between those proteins and their binding partners. As many proteins are GST-tagged, GST sensors allow users to have the flexibility to study various applications via OpenSPR™.