

Binding Kinetics of Ubiquitin to Ubiquitin Antibody Immobilized with Protein A using OpenSPR

Summary

- Anti-Ubiquitin Antibody was immobilized onto a Protein A sensor
- Binding of Ubiquitin to Anti-Ubiquitin antibody was measured using the OpenSPR™ instrument
- A one to one kinetic interaction model was used to determine the affinity constant of the interaction between Ubiquitin and Anti-Ubiquitin Antibody
- The KD was determined to be 40.0 nM
- OpenSPR™ Protein A Sensors can be used for simple antibody 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 methods of antibody immobilization is through the use of Protein A. Protein A is a protein that will bind to the Fc region of many antibody isoforms. The advantage of Protein A facilitated antibody immobilization is that the orientation of their epitope binding regions is projected outward from the sensor surface, which will guarantee their availability for analyte binding. When antibodies are immobilized directly to a sensor via amine coupling, much of the binding availability may be limited due to improper orientation of the antibody. Nicoya Lifesciences has created a Protein A kit that makes immobilization of antibodies simple. In this application note, OpenSPR™ is used demonstrate the use of the Protein A kit by analyzing the KD of the interaction between Ubiquitin and Anti-Ubiquitin Antibody.

Materials and Equipment

- OpenSPR™ Instrument
- OpenSPR™ COOH Sensor Chip
- OpenSPR™ Protein A Reagent Kit
- TraceDrawer™ Kinetic Analysis Software
- Ligand: Anti-Ubiquitin Antibody
- Analyte: Ubiquitin
- Running Buffer: HBS-EP pH 7.4

Procedure

1. Follow the start-up procedure found in the OpenSPR™ manual.
2. Clean the COOH Sensor with 10 mM HCl as outlined in the technical guide.
3. Inject EDC/NHS solution from the OpenSPR™ Amine Kit to prime the COOH surface at 20 $\mu\text{L}/\text{min}$.
4. Immobilize Protein A at a flow rate of 20 $\mu\text{L}/\text{min}$ for a 5 minute incubation period.
5. Inject Blocking Solution from the OpenSPR™ Amine Kit to complete the immobilization.
6. Immobilize 50 $\mu\text{g}/\text{mL}$ Anti-Ubiquitin Antibody diluted in running buffer at a flow rate of 20 $\mu\text{L}/\text{min}$ for a 5 minute incubation period.
7. Prepare 150 μL Ubiquitin dilutions into the running buffer at the following concentrations: 1 μM , 333 nM and 111 nM.
8. Inject the analytes above individually at a flow rate of 20 $\mu\text{L}/\text{min}$ with an association time of 300 seconds and a dissociation time of 500 seconds.
9. Data from OpenSPR™ is analyzed using TraceDrawer™.

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Results and Discussion

The immobilization of the Protein A onto the COOH sensor chip via amine coupling is shown in Figure 1 with over 200 pm of response for the immobilization. The binding of anti-ubiquitin antibody, also shown in Figure 1, produced over 1100 pm of response. Figure 2 shows the binding and kinetic fits of the ubiquitin analyte at 3 different concentrations. The association phase and dissociation phases are evident as is the concentration dependence. The data is fit to a one to one binding model in TraceDrawer™. The kinetic constants are shown in Table 1, and the fits are overlaid in Figure 2 as solid black lines. The KD value is determined to be 40.0 nM for this interaction.

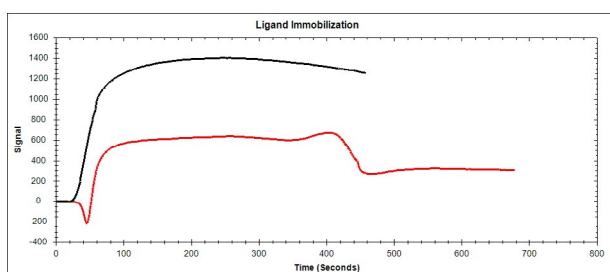


Figure 1. Ligand immobilization levels of Protein A (red curve) and anti-ubiquitin antibody 50 µg/mL (black curve).

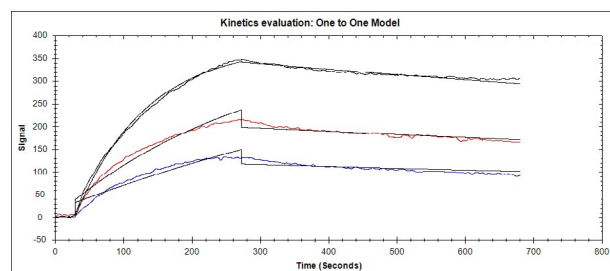


Figure 2. Binding of Anti-ubiquitin antibody at concentrations of 111 nM, 333 nM, and 1 µM (blue, red, and black curves). Solid black lines are the binding model fits.

Table 1. Binding kinetics and affinity measured using OpenSPRTM between ubiquitin and anti-ubiquitin antibody

	OpenSPRTM
On Rate (k_{on})	9.24×10^3
Off Rate (k_{off})	3.68×10^{-4}
Equilibrium Constant (K_D)	3.99×10^{-8}

Conclusions & Summary

This study demonstrates how the OpenSPRTM Protein A Reagent Kit used in conjunction with COOH sensor chips can be used to immobilize antibodies and determine the binding kinetics to their binding partners. As there are many antibody-antigen combinations to study, this reagent kit provides a simple method to study them via OpenSPRTM.

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