OpenSPR 2-Channel Instrument for Protein Interaction Analysis

Summary

- In this application note, we demonstrate how OpenSPR's 2-channel operation can be used to improve kinetic analysis of protein interactions
- A 1:1 kinetic interaction model was used to determine the kinetics of the interaction between Protein A and Human IgG
- The affinity constant of the interaction was determined to be 0.76 nM
- OpenSPR™ provides an affordable benchtop solution for kinetic anaysis of protein interactions and many other important applications

Overview

OpenSPR™ is a powerful instrument providing in-depth label-free binding kinetics for a variety of different molecular interactions. The determination of kinetic binding constants for antibody-antigen interactions is critical in many research and development applications. These are often high-affinity interactions which can make them challenging for analysis.

A well-known example of a high affinity interaction is that between Protein A and Human IgG. Protein A is commonly used as a capture molecule for IgG antibody immobilization, providing strong and reliable capture with a low dissociation rate. In this application note, OpenSPR™ is used to measure the affinity and kinetics between Protein A and Human IgG. We demonstrate how the 2-channel OpenSPR is able to quickly and easily generate high quality data by simultaneously removing any drift and/or non-specific binding via the reference channel.

Materials and Equipment

- OpenSPR™ 2-Channel Instrument
- OpenSPR Carboxyl Sensor Chip and Amine Coupling Kit
- TraceDrawer™ Kinetic Analysis Software
- Ligand: Protein A
- Analyte: Human IgG
- Running Buffer: HBS-EP, pH 7.4
- Regeneration solution: 10 mM Glycine-HCl, pH 2.5

Procedure

1. Perform the OpenSPR instrument setup procedure following the software guides.
2. Load the Carboxyl Sensor into the OpenSPR instrument.
3. Run the Ligand Wizard to immobilize 20 µg/ml of Protein A onto Channel 2 of the Carboxyl senor chip. Channel 1 is activated and blocked and serves as the reference.
4. Prepare 150 µL Human IgG analyte dilutions into the Running Buffer at the following concentrations: 111 nM, 37 nM, 12.1 nM, and 4.1 nM.
5. Inject the analyte solutions individually at a flow rate of 40 µL/min with an association time of 120 s and a dissociation time of 600 s.
6. Between each analyte measurement, perform an injection of the Regeneration Solution (10 mM glycine-HCl, pH 2.5) at a flow rate of 150 µL/min. This will remove the bound analyte and regenerate the Protein A ligand surface.
7. Finish the test and import the processed data into the TraceDrawer analysis software. Calculate the binding kinetics with a 1:1 binding model.

Results and Discussion

In this experiment, Channel 1 acts as the reference channel with a blocked Carboxyl surface, while Channel 2 acts as the sensing channel with Protein A ligand bound. The immobilization of Protein A onto the Carboxyl Sensor via amine coupling resulted in approximately 650pm of immobilization on Channel 2 (and 0pm on Channel 1). Figure 1 presents the binding curves of the Human IgG analyte at the 4 different concentrations on both Channel 1 and Channel 2. On Channel 2, the binding curves demonstrate clear concentration dependence with evident association and dissociation phases. Minimal binding of the analyte is observed on Channel 1. Even before processing the data, the raw binding curves on Channel 2 look excellent with minimal artefacts. However, using the data from Channel 1 as a reference and subtracting it from the data in Channel 2, an improvement in data quality is observed. The processed data is shown in Figure 2. The kinetic constants are summarized in Table 1, and the fits are overlaid in Figure 2 as solid black lines. The KD is determined to be 0.76 nM for this interaction, with a ka of 1.3 x 105 M-1s-1, kd of 9.4 x 10-5 s-1. These values compare very well with other studies performed with Protein A and IgG.

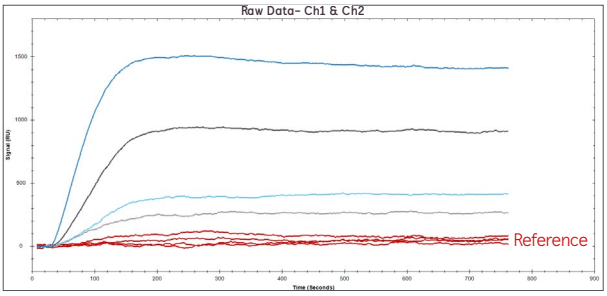

Figure 1. Raw data of IgG binding to Protein A at 111 nM, 37 nM, 12.1 nM, 4.1 nM on Channel 1 (red lines) and Channel 2 (blue/grey lines).

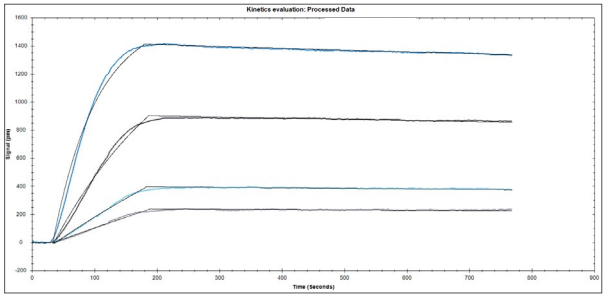

Figure 2. Processed data of IgG binding to Protein A at concentrations of 111 nM, 37 nM, 12.1 nM, and 4.1 nM. Solid black lines overlaid are the binding model fits.

	OpenSPR™
k_a [1/M*s]	1.3 x 10^5 (± 2.1 x 10^0)
k_d [1/s]	9.4 x 10^{-5} (± 3.5 x10^{-6})
K_D [M]	0.76 x 10^{-9} (± 2.0 x 10^{-11})

Table 1. Binding kinetics and affinity measured using OpenSPRTM between Protein A and Human IgG.

Conclusions and Summary

The OpenSPR measured a KD of 0.76 nM for the interaction between Protein A and Human IgG. The OpenSPR's 2-channel capabilities allow for removal of artefacts such as non-specific binding and bulk shifts to produce high quality and accurate data in the toughest applications.