# Binding Kinetics of a High Affinity Binding Interaction Using OpenSPR™


## Summary

- The high affinity binding interaction between Human IgG and Protein A was measured using the OpenSPR™ instrument
- A one to one kinetic interaction model was used to determine the kinetics and affinity of the interaction between Protein A and Human IgG
- The $K_D$ was determined to be 0.52 nM
- OpenSPR™ can be used to accurately measure high affinity binding interactions


## 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. Antibody-antigen interactions can often be of high affinity. OpenSPR allows users to easily and accurately measure the binding kinetics of numerous ligand-antigen systems, including high affinity interactions.

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 high affinity kinetics between Protein A and Human IgG.

## Materials and Equipment

- OpenSPR™ Instrument
- OpenSPR™ Protein A Sensor Kit
- TraceDrawer™ Kinetic Analysis Software
- Ligand: Protein A
- Analyte: Human IgG
- Running Buffer 1: HBS-EP, pH 7.4
- Running Buffer 2: HBS-EP, pH 7.4 + 0.1% BSA
- Regeneration solution: 10 mM Glycine-HCl, pH 2.5

## Procedure

1. Perform the OpenSPR™ instrument setup procedure following the software guides. Begin the experiment using Running Buffer 1, which does not contain BSA.
2. Load a Carboxyl Sensor from the Protein A Sensor Kit into the OpenSPR™ instrument.
3. Clean the sensor with an injection of 10 mM HCl as outlined in the Technical Guide.
4. Inject EDC/NHS solution to activate the Carboxyl Sensor at a flow rate of 20 µL/min.
5. Immobilize Protein A at a flow rate of 20 µL/min for a 5-minute incubation period.
6. Inject the Blocking Solution to block any remaining active carboxyl groups to complete the immobilization.
7. Change the running buffer to Buffer 2, containing 0.1% BSA and allow the sensor response to baseline. The added BSA is used to prevent non-specific binding of the Human IgG.
8. Prepare 200 µL Human IgG analyte dilutions into Running Buffer 2 at the following concentrations: 111 nM, 37 nM, 12.1 nM, 4.1 nM and 0.45 nM.


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9. 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 600s.
10. Between each analyte measurement, perform an injection of the Regeneration Solution (10 mM glycine-HCl, pH 2.5) at a flow rate to 150 μL/min. This will remove the bound analyte and regenerate the ligand surface.
11. Finish the test and import the data into the TraceDrawer analysis software. Measure the kinetics with a 1:1 binding model.

## Results and Discussion

The immobilization of Protein A onto the Carboxyl Sensor via amine coupling is shown in Figure 1 with approximately 500 pm of immobilization response. Figure 2 presents the binding curves and kinetic fits of the Human IgG analyte at the 5 different concentrations. The binding curves demonstrate clear concentration dependence with evident association and dissociation phases. The data is fit to a one to one binding model in TraceDrawer™. The kinetic constants are summarized in Table 1, and the fits are overlaid in Figure 2 as solid black lines. The $K_D$ is determined to be 0.52 nM for this interaction, with a $k_a$ of $2.1 \times 10^5\ M^{-1}s^{-1}$, and $k_d$ of $1.1 \times 10^{-4}\ s^{-1}$.

With high affinity binding interactions with slow off-rates, it is important to collect sufficient dissociation data to extract a confident kd value. The precision of the kd value can be determined by the measured error, which you want to be at least an order of magnitude lower than that of the measured value. To obtain this, the dissociation phase of at least one binding curve should decrease in signal intensity by at least 5-10%. For this measured interaction, a 600 s dissociation phase was sufficient to obtain an 8% decrease in the dissociation response. This amount of time series data resulted in an accurate dissociation rate with an error 2 orders of magnitude less than the measured value.

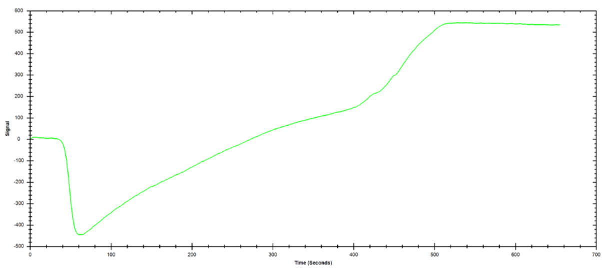

*Figure 1. Ligand immobilization of Protein A onto Carboxyl Sensor Chip*

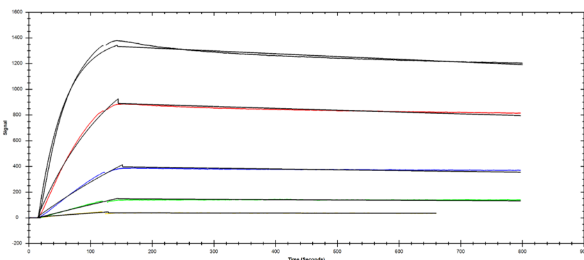

*Figure 2. Binding of Human IgG at concentrations of 111 nM, 37 nM, 12.1 nM, 4.1 nM and 0.45 nM. Solid black lines overlaid are the binding model fits.*

*Table 1. Binding kinetics and affinity measured using OpenSPR™ between Protein A and Human IgG.*

| | OpenSPR™ |
|---|---|
| $k_a$ [1/M*s] | $2.1 \times 10^5$ ($\pm 1.9 \times 10^0$) |
| $k_d$ [1/s] | $1.1 \times 10^{-4}$ ($\pm 1.9 \times 10^{-6}$) |
| $K_D$ [M] | $0.52 \times 10^{-9}$ ($\pm 9.3 \times 10^{-12}$) |

## Conclusions & Summary

The OpenSPR™ was able to measure a KD of 0.52 nM for the interaction between Protein A and Human IgG. This study demonstrates how the OpenSPR™ can be used to determine the binding kinetics of high affinity binding partners.