# Binding Kinetics of Protein-Protein Interactions using OpenSPR

## Summary


- Protein-protein interactions were analyzed on the OpenSPR™ instrument
- Kinetic analysis was used to determine the on rate, off rate, and affinity constant of the interaction of two different ligands (M1 & M2) with an analyte
- The average $K_D$ for M1 was 5.7 nM and for M2 was 0.54 nM


## 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. In this application note, OpenSPR™ is used to analyze the $k_{on}$, $k_{off}$, and $K_D$ of two different protein ligands (M1 and M2) to one protein analyte. The biomolecular system and conditions were developed by Dr. Olan Dolezal from CSIRO.

## Materials and Equipment

- OpenSPR Instrument
- OpenSPR Streptavidin Sensor Chip
- TraceDrawer Kinetic Analysis Software
- Biotinylated Ligand Protein M1 and M2 (27 kDa each)
- Analyte protein A1 (50 kDa)
- Running buffer (HBS, 0.005% Tween20, 0.1 mg/ml BSA)
- Regeneration buffer (HCl pH 2.0)

## Procedure

1. Following the start-up procedure found in the OpenSPR manual, setup the OpenSPR instrument and software.
2. Set the pump speed to 20 µl/min and fill the 100 µL sample loop with 200 µL of ligand M1 diluted in the running buffer to a concentration of 1/20 the stock. The same procedure is used for ligand M2 using a new sensor chip.
3. The ligand should be immobilized to a level of 150-200 pm.
4. Once the immobilization is complete, continue pumping running buffer for 5 mins until a stable baseline is achieved. Rinse the sample loop with running buffer and purge with air.
5. Prepare 200 µL analyte dilutions into the running buffer at 100, 33.3, 11.1, 3.7, 1.2, and 0.4 nM.
6. Inject analytes at a flow rate of 20 µL/min with an association time of 200 secs and a dissociation time of 700 secs.
7. For M1 ligand no regeneration is needed due to the high off rate, so once the baseline is reached the next analyte concentration can be injected.
8. For the M2 ligand, once the dissociation phase is complete the pump speed is increased to 140 µL/min and the loop filled with regeneration buffer and injected (40 secs regeneration time). Once the signal returned to baseline, the next analyte injection is performed.
9. During each experiment buffer blanks are also injected to be subtracted out as references.
10. In a separate experiment, the level of non-specific binding of the analyte is tested using a streptavidin coated sensor without any ligand. The analyte is injected up to a concentration of 200 nM to check for non-specific binding.
11. Data from OpenSPR is analyzed using TraceDrawer with a 1:1 diffusion corrected binding model with global fitting. Buffer blanks are also referenced out.

# Results and Discussion

Results from the M1 and M2 ligand on the OpenSPR instrument are shown below in Figure 1 and Figure 2. The 1:1 binding models are shown as solid black lines overlaid onto the raw data. The residual plots are also included and show uniform residuals throughout the association and dissociation phases. The data fits very well with the theoretical 1:1 binding model as the residuals are low and random and the errors small. Visually, it is clear that the off rate of the M1 ligand is much higher than that of the M2. The results from the non-specific test are shown in Figure 3, and no binding of the analyte and no background response was observed.

The results of the kinetic analysis for each ligand are summarized in Table 1. The error is given in brackets. The $K_D$ for M1 was found to be 5.7 nM, and the $K_D$ for M2 was found to be 0.54 nM. These results indicate that the modifications made to mutant 1 had a much more significant effect on the binding affinity to the analyte than mutant 2, giving insight into the binding site/ structure of the ligand and analyte.

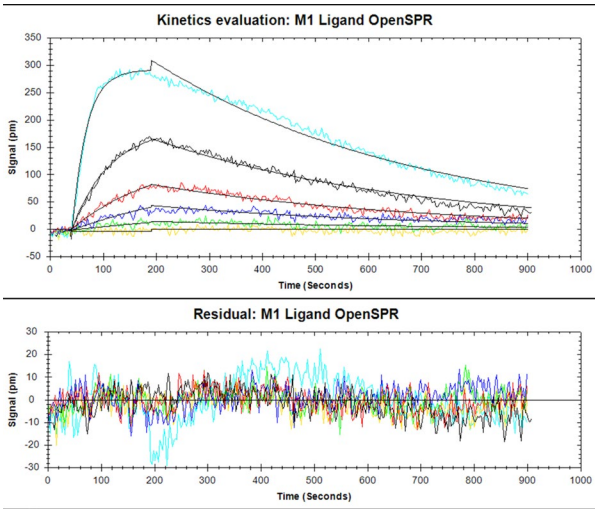


Figure 1. Binding curves and kinetic analysis of M1 ligand on OpenSPR

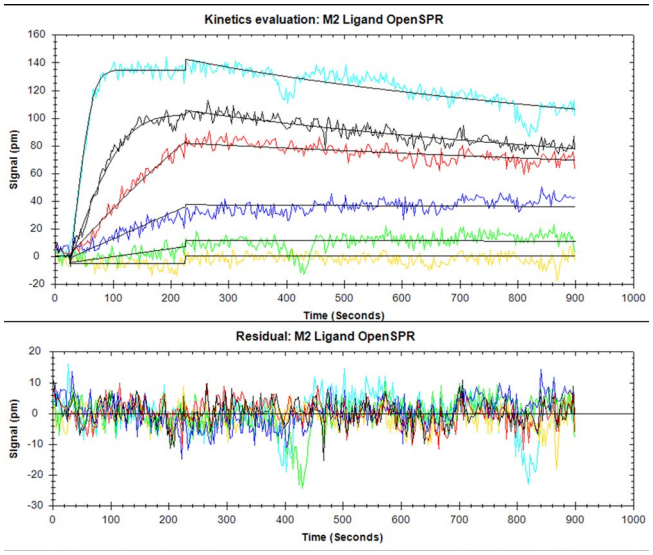


Figure 2. Binding curves and kinetic analysis of M2 ligand on OpenSPR

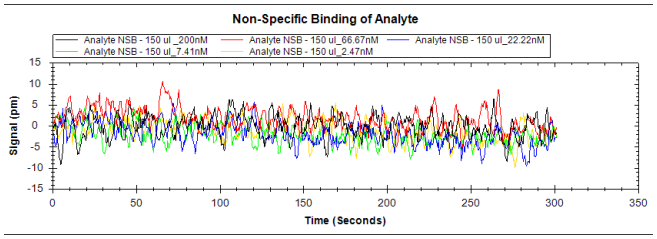


Figure 3. Non-specific binding of analyte to streptavidin coated sensor on OpenSPR

| | $k_a(x10^6)$ | $k_d(x10^{-3})$ | $k_D(x10^{-9})$ |
|---|---|---|---|
| **M1** | 0.353(9) | 2.02(4) | 5.7(2) |
| **M2** | 1.09(6) | 0.60(6) | 0.54(9) |

Table 1. Binding kinetics and affinity of M1 and M2 ligand

# Conclusions and Summary

This study demonstrates how OpenSPR can be used to determine the binding kinetics between proteins. Simple experiments that use minimal sample were conducted to extract powerful data and insight into the binding nature of this biomolecular system.