Comparison Study of Binding Kinetics on OpenSPR™, Biacore™ & IBIS™

SUMMARY

- **Protein-protein interactions were analyzed on OpenSPR™, Biacore™ and IBIS instruments™**
- **Kinetic analysis was used to determine the on rate, off rate, and affinity constant of the interaction of 2 different ligands (M1 & M2) with an analyte**
- **The average K_D for M1 was 6.61nM (+/-2.01nM) and for M2 was 0.27nM (+/-0.23nM).**
- **All three instruments produced acceptable kinetic constants within the expected error range for these experiments, validating the OpenSPR™ instrument against other commercial SPR equipment**

Overview

OpenSPR™ is a powerful instrument providing in-depth label-free binding kinetics for a variety of different molecular interactions. With any new technology, it is important to compare the performance of various tools and techniques to establish their consistency and accuracy. In this study, binding kinetic results of protein-protein interactions are compared between the OpenSPR™, Biacore™ and IBIS™ MX96 surface plasmon resonance instruments. The biomolecular system and conditions were developed by Dr. Olan Dolezal from CSIRO. The k_{on}, k_{off}, and K_D of two different ligands (M1 and M2) to one analyte are determined and compared.

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, 0.1mg/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 20µl/min and fill the 100µL sample loop with 200µL 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 150-200pm.
4. Once the immobilization is complete, continue pumping running buffer for 5 minutes until a stable baseline is achieved. Rinse the sample loop with running buffer and purge with air.
5. Prepare 200µL analyte dilutions into the running buffer at 100, 33.3, 11.1, 3.7, 1.2, and 0.4nM.
6. Inject analytes at a flow rate of 20µL/min with an association time of 200s and a dissociation time of 700s.
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 140µL/min and the loop filled with regeneration buffer and injected (40s 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 200nM 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.

Data was collected for the same M1 and M2 ligands and same analyte using a Biacore and IBIS instrument. Conditions were similar to those run on the OpenSPR except an association time of 300 seconds and dissociation time of 600 seconds were used. The analyte concertation range was 3 fold dilutions from 100nM to 0.04nM and the flow rate used was 50µl/min. The IBIS, an imaging SPR, was run with 7 different ligand densities and the average kinetic values from all surfaces reported. Data was fit with Biacore or IBIS software with 1:1 global fit binding models with diffusion correction.

Results and Discussion

Results from the M1 and M2 ligand on the OpenSPR instrument are shown below in Figure 1 and Figure 2, and the kinetic curves from the other instruments are shown in the Appendix. 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 the appendix (Figure 7), and no binding of the analyte was observed.

The results of the kinetic analysis for each ligand from each instrument are summarized in Table 1 and Table 2 below along with the average and standard deviation. The error is given in brackets where available. In general the results agree well between all three instruments. The K_D for M1 was found to be 5.7nM, 5.2nM, and 8.9nM on the OpenSPR, IBIS, and Biacore, respectively. The K_D for M2 was found to be 0.54nM, 0.12nM, and 0.16nM on the OpenSPR, IBIS, and Biacore, respectively. The average K_D was 6.61nM +/- 2.0

for M1 and 0.27 +/-0.23nM for M2. The binding curves between the instruments all look comparable with similar signal to noise levels.

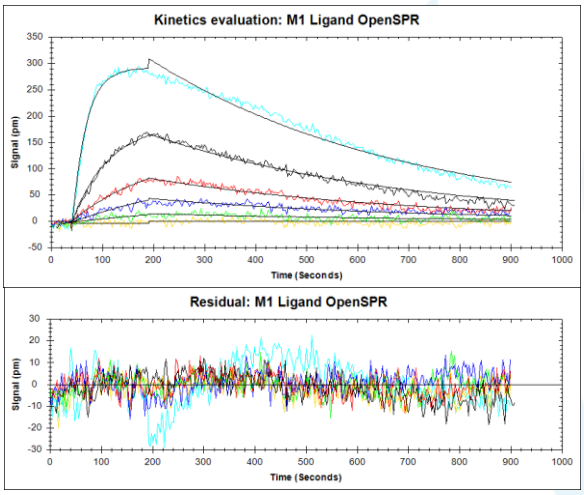

Figure 1. Binding curves and kinetic analysis of M1 ligand on OpenSPR

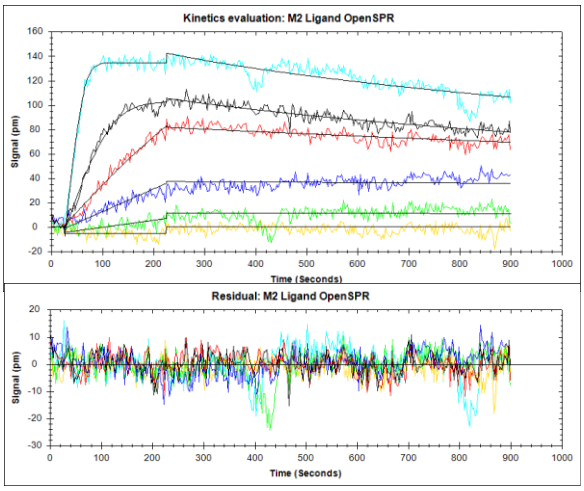

Figure 2. Binding curves and kinetic analysis of M2 ligand on OpenSPR

In general the OpenSPR and IBIS constants were closer together compared with the Biacore. Small differences in procedure, temperature, the age of the sample, and instrument differences are likely responsible for these variations. Comparing to a global benchmark study of affinity biosensors that used over 150 different users showed that for the same protein-antibody interaction the K_D was found to be 0.620nM +/-0.980nM [1]. Therefore, the results of this study show that all three instruments produce results that are consistent with the errors that can be expected in such experiments across different instruments, conditions, and users. This study validates the OpenSPR against other commercial SPR equipment.

Table 1. Binding kinetics and affinity of M1 ligand

M1	k_a ($x10^6$)	k_d ($x10^{-3}$)	K_D ($x10^{-9}$)
OpenSPR	0.353(9)	2.02(4)	5.7(2)
IBIS	0.572	3.00	5.22
Biacore	1.614(7)	14.37(3)	8.91(4)
Average	0.85	6.46	6.61
StdDev	0.67	6.86	2.01

Table 2. Binding kinetics and affinity of M2 ligand

M2	k_a ($x10^6$)	k_d ($x10^{-3}$)	K_D ($x10^{-9}$)
OpenSPR	1.09(6)	0.60(6)	0.54(9)
IBIS	2.44	0.292	0.119
Biacore	5.90(2)	0.9502(7)	0.1611(6)
Average	3.14	0.61	0.27
StdDev	2.48	0.33	0.23

Conclusions and Summary

This study demonstrates that accurate and high quality kinetics that are comparable to other commercial SPR instruments can be obtained with OpenSPR. The results are all within reasonable error ranges between all instruments.

[1] R. Rich et al., "A global benchmark study using affinity-based biosensors," Analytical Biochemistry, vol. 386, pp. 194-216, 2009.

Appendix

Representative kinetic curves from the IBIS (Figure 3 and Figure 4) and Biacore (Figure 5 and Figure 6) instruments are shown below.

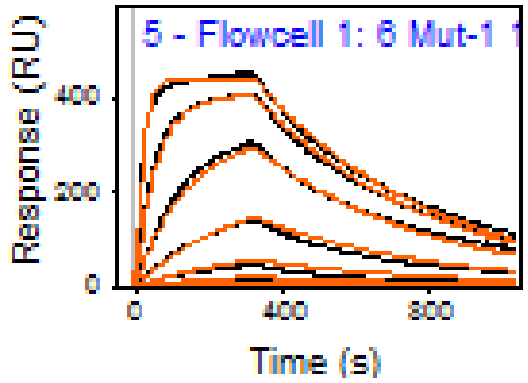

Figure 3. Binding curves and kinetic analysis of M1 ligand on IBIS

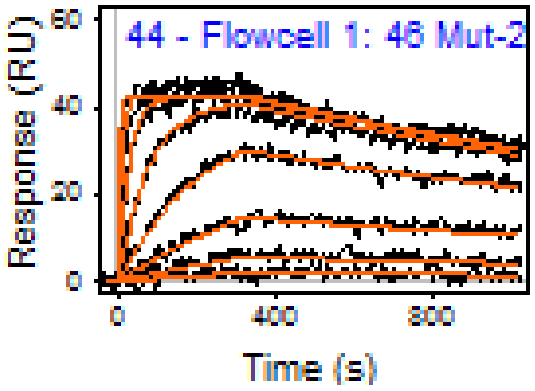

Figure 4. Binding curves and kinetic analysis of M2 ligand on IBIS

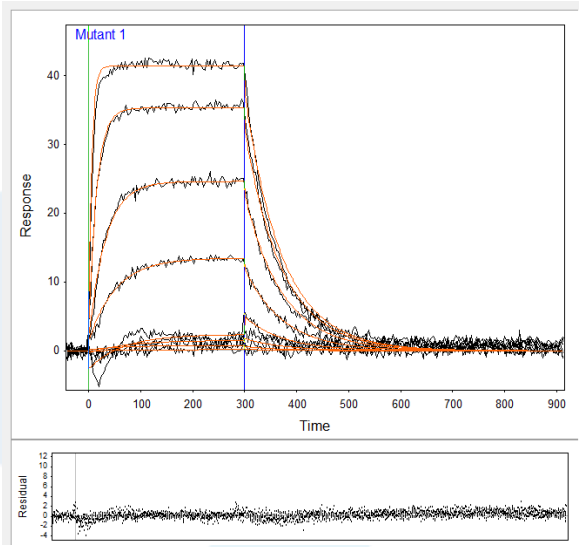

Figure 5. Binding curves, kinetic analysis, and residuals of M1 ligand on Biacore

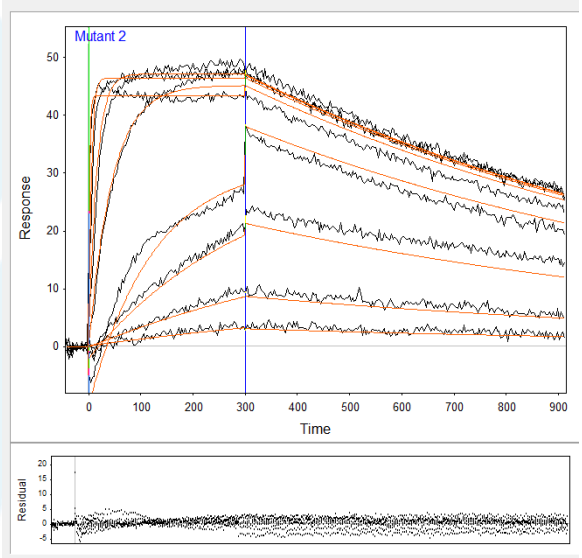

Figure 6. Binding curves, kinetic analysis, and residuals of M2 ligand on Biacore

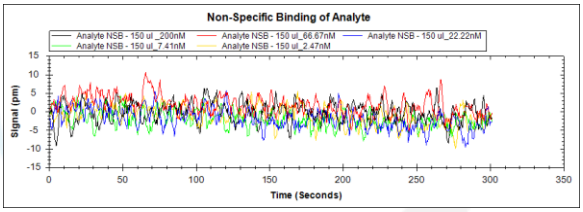

Figure 7. Non-specific binding of analyte to streptavidin coated sensor on OpenSPR