Comparison Study of Label-Free Biosensors Using Commercially Relevant Binding Systems

Summary

- Binding kinetics of biotinylated CD16a and IgG were characterized by comparing OpenSPR™ with a standard SPR instrument and a commonly-used BLI instrument.
- K_D values measured with the OpenSPR™, the standard SPR instrument and the commonly-used BLI instrument were found to be 74.7 nM, 48.7 nM and 226 nM, respectively.
- This study supports the equivalency of OpenSPR™ with other commercial instruments for the analysis of binding kinetics and affinity.

Overview

The OpenSPR™ is a powerful instrument providing researchers with in-depth label-free binding kinetics for a variety of different molecular interactions. A side by side comparison study was performed with OpenSPR™, a standard SPR instrument, and a commonly-used BLI instrument to demonstrate that OpenSPR™ can provide the same high-quality data as commonly-used instruments on the market. The standard SPR and BLI instruments are used for analyzing binding kinetics, but cost hundreds of thousands of dollars, making them inaccessible to many researchers who need this data. An Fc-FcR interaction was analyzed using the three instruments under similar conditions to show that the OpenSPR™ can generate comparable results to both the standard SPR and commonly-used BLI systems for a fraction of the cost.

Samples and Reagents

- **Ligand:** Human CD-16a (FcγRIIIa) (biotinylated), SinoBiological, Cat# 10389-H27H1-B
- **Analyte:** Human IgG FC fragment, Abcam, Cat# Ab90285
- **Running Buffer** (OpenSPR™ and standard SPR instrument): HBS-EP (0.005% Tween-20)
- **Assay Buffer** (BLI instrument): PBS (0.01% BSA and 0.002% Tween-20)

Instruments and Sensors

- **OpenSPR™ Instrument:** OpenSPR™ Streptavidin Sensor
- **Standard SPR Instrument:** High-affinity Streptavidin Sensor
- **BLI Instrument:** High Precision Streptavidin Sensor

Experimental Procedures

OpenSPR™

1. Following the OpenSPR™ start-up procedure in the software, the Streptavidin Sensor was loaded into the instrument.
2. The Streptavidin Sensor was cleaned at 75 µL/min using solutions of 50 mM NaOH and 10 mM HCl.
3. The CD16a was immobilized in channel 2 at a concentration of 4.5 µg/mL at 5 µL/min (total contact time ~20 min).
4. Kinetic analysis of analyte (IgG Fc) was performed at a flow rate of 30 µL/min at concentrations of 31.25, 62.5, 125, 250, 500 and 1000 nM (prepared in HBS-EP).
5. The analyte was allowed to dissociate fully over a minimum of 15 minutes at 30 µL/min or until response returned to the pre-injection baseline.
6. The unmodified streptavidin in channel 1 of the sensor was used as the surface reference and HBS-EP was used as the blank for double referencing.
7. Kinetic data was processed using TraceDrawer Kinetic Analysis Software using a 1:1 kinetic model.

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Standard SPR Instrument

1. Following the SPR instrument's startup procedure in the software, the High-Affinity Streptavidin Sensor was loaded into the instrument.
2. The sensor chip was activated by injection of 1 M NaCl/50 mM NaOH for 1 min at 20 µL/min, repeated 3 times.
3. The CD16a was immobilized at a concentration of 30 ng/mL at 5 µL/min.
4. Unoccupied functional sites on the sensor were blocked by injecting 10 µg/mL biocytin at 10 µL/min for 3 minutes.
5. Kinetic analysis of Analyte (IgG Fc) was performed at a flow rate of 30 µL/min at concentrations of 31.25, 62.5, 125, 250, 500 and 1000 nM (prepared in HBS-EP).
6. The analyte was allowed to dissociate fully over a minimum of 15 minutes at 30 µL/min or until response returned to the pre-injection baseline.
7. Kinetic data was processed with the instrument's built-in software using a 1:1 kinetic model.

BLI Instrument

1. Following the BLI systems startup procedure, the High Precision Streptavidin Sensor was loaded into the instrument.
2. Biotinylated CD16a was captured on the sensor at 5 µg/mL in PBS.
3. Unoccupied functional sites on the sensor were blocked with 50 µg/mL biocytin in PBS.
4. The probes were dipped in assay buffer for baselining. Subsequently, the probes were dipped into wells containing varying concentrations of IgG Fc (31.25, 62.5, 125, 250, 500 and 1000 nM) to measure the association rate (6 minute contact time). The probes were then dipped into wells containing assay buffer to measure the dissociation rate. Referencing was completed by having sensors with no immobilized ligand dipped into analyte.
5. Kinetic data was processed with BLI software using a 1:1 kinetic model. The 31.25 nM and 1000 nM concentrations were omitted from the fitting.

Results and Discussion

Results from the Fc-FcR interaction measured on the OpenSPR™, standard SPR and BLI instruments can be found in Figures 1, 2 and 3, respectively. Data was fit in each experiment with a 1:1 binding model. The OpenSPR™, and the two other instruments determined KD values of 74.0 nM, 48.7 nM and 226 nM, respectively. The calculated KD values between OpenSPR™ and the other SPR instrument are highly-comparable, while the value reported by the BLI instrument differs by ~5X. The kinetic constants determined from the fits are shown in Table 1. The off-rates are similar across all three instruments, however, the BLI-derived on-rates differs by over an order of magnitude. The quality of the fits is comparable for OpenSPR™ and the standard SPR instrument, but there is more deviation from the model seen in the BLI data.

It is common to see variations in measured affinity and kinetic values even when running assays on the same instrument. The closeness of the values between both SPR instrument experiments demonstrate the comparability of these instruments for biomolecular interaction analysis. The relatively large difference seen in the BLI data could be due to the higher immobilization level used, fitting differences, or fundamental differences in the dip and read technique used with BLI.

Table 1. Kinetic and affinity constants of the CD16a-Fc interaction measured on OpenSPR™, standard SPR instrument, and BLI instrument.

	OpenSPR™	Standard SPR Instrument	BLI Instrument
k_{on}	5.57e4	1.42e5	2.68e4
k_{off}	4.16e-3	6.89e-3	6.08e-3
K_D	7.47e-8	4.87e-8	2.26e-7

Conclusions

In summary, both OpenSPR™ and the other SPR instrument reported similar kinetics and affinity values, but differed more significantly from the BLI system for the interaction studied. The results support the use of OpenSPR™ as the best affordable alternative to traditional SPR and BLI instruments in generating accurate binding kinetics and affinity data.

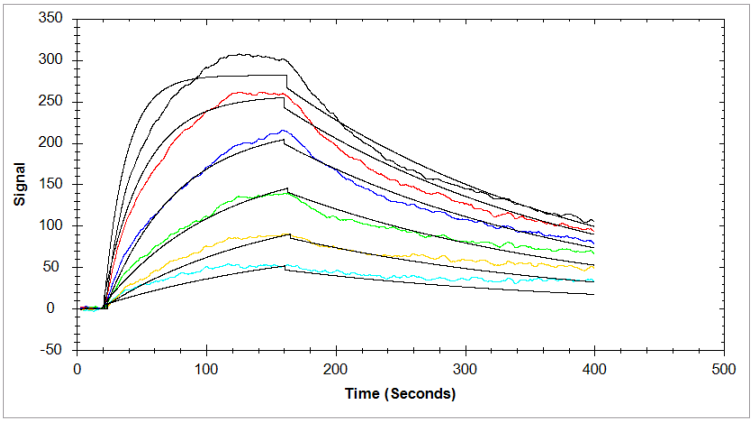

Figure 1: CD16a-Fc interaction analyzed using the OpenSPR™ with analyte concentrations of 31.25, 62.5, 125, 250, 500 and 1000 nM.

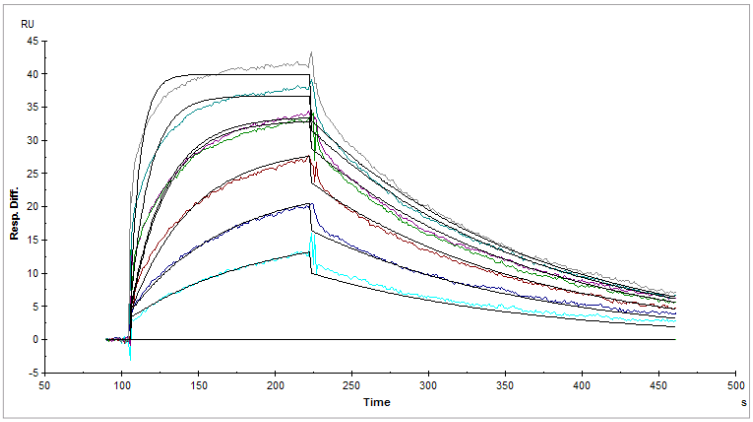

Figure 2: CD16a-Fc interaction analyzed using the standard SPR instrument with analyte concentrations of 31.25, 62.5, 125, 250, 500 and 1000 nM.

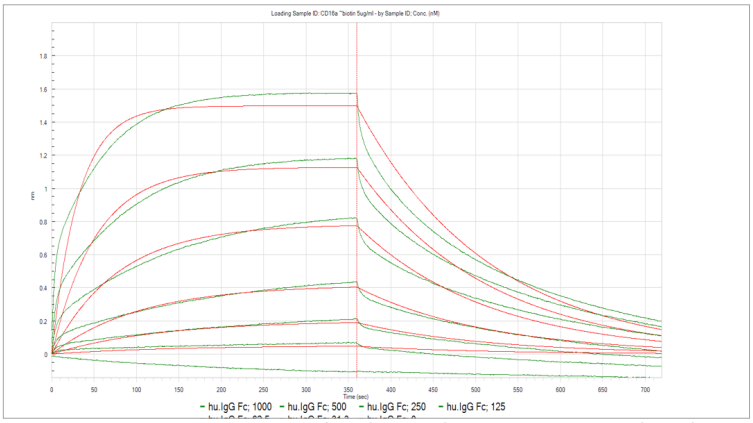

Figure 3: CD16a-Fc interaction analyzed using the BLI instrument with analyte concentrations of 31.25, 62.5, 125, 250, 500 nM and 1000 nM. Green lines represent measured curves and red lines represent 1:1 fits.