OpenSPR™ vs Biacore™: Protein–Protein Binding Kinetics Comparison Study

Summary

- A protein-protein interaction was analyzed on the OpenSPR™ and Biacore™ T200
- The K_D was found to be 1.53nM with OpenSPR™ and 0.686nM with the Biacore™, confirming the accuracy of the OpenSPR instrument

Overview

OpenSPR™ is a powerful instrument providing in-depth label-free binding kinetics for a variety of different molecular interactions. In order to demonstrate the powerful capabilities and accuracy of the OpenSPR™, a side by side experiment was conducted against a Biacore™ T200. The Biacore™ T200 costs hundreds of thousands of dollars, but is considered a standard in the pharmaceutical industry. In order to show that OpenSPR is able to generate comparable results to the Biacore™ but at a fraction of the cost, a protein-protein interaction was analyzed using both instruments under similar conditions. The Biacore™ was run by a trained technical in a testing lab while the OpenSPR™ was run by a Nicoya scientist.

Materials and Equipment

- OpenSPR™ Instrument
- OpenSPR™ COOH Sensor Chip & Amine Coupling Kit
- TraceDrawer Kinetic Analysis Software
- Ligand Protein (MW 30kDa)
- Analyte Protein (MW 15kDa)
- Running Buffer: PBS + 150mM NaCl + 0.05% Tween 20, pH 7.4
- Regeneration Buffer: 10mM Glycine HCl pH 2.5
- Biacore™ T200
- CM4 Sensor Chip
- Immobilization buffer: Sodium Acetate pH 5.25

Procedure

OpenSPR Experiment

1. Following the start-up procedure found in the OpenSPR™ manual, setup the OpenSPR™ instrument and load a COOH Sensor Chip.
2. Following the instructions included in the Amine kit, activate the COOH surface. Dilute ligand at concentration of 82.5ug/ml into immobilization buffer, and inject 100uL at 20uL/min for a 5 minute interaction time.
3. Block the surface with an injection of 100uL of blocking buffer.
4. Increase the flow rate to 30uL/min, and inject analyte at the following concentrations: 6.25, 12.6, 25, and 50nM. Use an association time of 150 seconds and a dislocation time of 400 seconds.
5. Regenerate the surface with an injection of regeneration buffer at a speed of 150ul/min in between each analyte injection.
6. Data was single referenced with blank injections.

Biacore™ T200 Experiment

1. Follow the Biacore™ startup procedure, loading the CM4 sensor chip in the instrument.

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2. Follow instructions included in the Amine kit to activate the COOH surface. Dilute ligand at concentration of 100ug/ml into acetate buffer at pH 5.25, and inject 100uL.
3. Block the surface with ethylenediamine.
4. Using the Single Cycle Kinetics mode, increase the flow rate to 45uL/min, and inject analyte at the following concentrations: 6.25, 12.6, 25, and 50nM. Use an association time of 100 seconds and a dissociation time of 100 seconds. No regeneration is needed with this mode.
5. Data was double referenced with blank injections and a deactivated reference channel.

Results and Discussion

Results from the protein-protein interaction measured on the OpenSPR™ instrument can be found in *Figure 1* and the Biacore™ instrument in *Figure 2*. Data was fit in each with a 1:1 binding model. Both data sets show excellent fits to the theoretical models with very low errors and chi squared values. The OpenSPR™ produced larger analyte signals due to the 4x higher amount of ligand density and higher sensitivity. The kinetic constants determined from the fit are shown in *Table 2*. The calculated on-rates are identical, while the off-rates differ by approximately 50%, which causes the K_D to differ by the same amount. OpenSPR™ determined a K_D of 1.53nM while the Biacore™ determined a K_D of 0.686nM. These are extremely close considering the Biacore™ experiment was done with a much lower ligand density, used single cycle without regeneration and used a significantly shorter off rate period compared to the OpenSPR™.

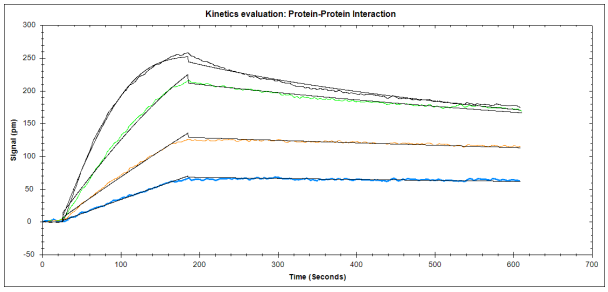

Figure 1. Protein-protein interaction analyzed using OpenSPR™ with analyte protein concentrations of 6.25, 12.6, 25, 50nM

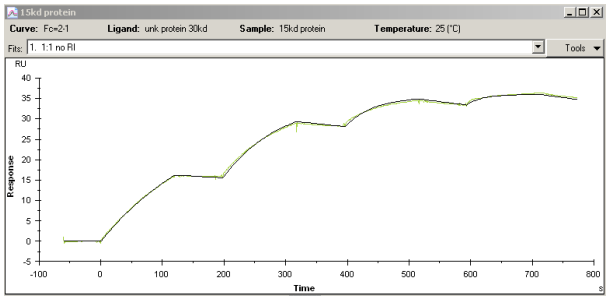

Figure 2. Protein-protein interaction analyzed using Biacore™ with analyte protein concentrations of 6.25, 12.6, 25, 50nM

Table 1. Kinetic and affinity constants of protein-protein interaction measured on OpenSPR™ and Biacore™

	OpenSPR™	Biacore™
k_{on} [1/M*s]	8.18e5	8.18e5
k_{off} [1/s]	1.25e-3	5.61e-4
K_D [nM]	1.53	0.686

Conclusions and Summary

This study demonstrates that OpenSPR™ can generate kinetic and affinity data that is comparable to data that can be obtained from a Biacore™ T200 instrument.

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