Application Note

*Featuring BiOptix'
Surface Plasmon Enhanced (SPE)
Interferometry Technology for
highly sensitive multiplexed biodetection*

Kinetic Characterization of Antibody-Antigen Interactions using the BiOptix ACCOLADE™ Instrument

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Introduction

Antibody-based therapeutics is currently the fastest growing area of drug development with more than 20 approved compounds and hundreds more in preclinical and clinical trials. During drug discovery, researchers rank various antibody candidates based on their affinity interaction with the target antigen. Typically the most efficacious compounds are very tight binders which make the accurate characterization of their affinity interactions more difficult. In this application note we describe the kinetic characterization of several high affinity protein-protein model systems using the BiOptix ACCOLADE™ Instrument. We also provide experimental protocols and useful suggestions for researchers who need high quality affinity characterization for both research grade and therapeutic antibodies.

A. Protocol for Non-Biotinylated Antibody Affinity Measurements

A BiOptix NeutrAvidin SensorChip (part no. C-40403) was first soaked in phosphate buffered saline (PBS) to remove the preservative prior to being placed in the BiOptix Chip Charger. Forty microliters of biotinylated Protein A (Sigma Aldrich) at 5 µg/ml in 10 mM HEPES, pH 7.3, 150 mM NaCl and 0.15% polysorbate 20 (HBS-P) was spotted onto two separate channels with the remaining two channels serving as reference channels. After 20 min, the chip was washed with HBS-P, removed from the charger, and loaded into the BiOptix Accolade™ instrument that was previously equilibrated in HBS-P. A 50 µg/ml solution of adalimumab (Humira®, Abbott Laboratories, an antibody that binds TNF) was injected into the instrument with the immobilization being monitored.

At a signal of 800-900 RUs the injection was stopped and buffer was run through the flow cell. The captured antibody was then chemically crosslinked to Protein A by treatment with an injection of 1 mg/ml bis(sulfosuccinimidyl)suberate (BS3, Thermo Scientific) over 5 minutes. Residual active sites were quenched with 1 M ethanolamine (pH 8.0).
For the kinetic analysis studies of TNF binding to adalimumab, TNF-α (Preprotech) at various concentrations was injected at a flow rate of 60 µl/min for 15 minutes followed by running buffer for 15 min. After each assay, the chip's surface was regenerated with a brief exposure (20 sec) to 50 mM NaOH, 1 M NaCl. Kinetic analyses were performed using the BiOptix software package.

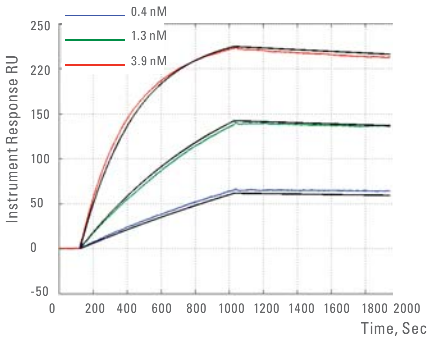

Figure 1. Binding responses of TNF-α for adalimumab. The black curves represent the kinetic model fit overlaid with the data. Background noise was subtracted using the data from the reference channels. The second channel that had immobilized adalimumab showed an identical response (data not shown). The calculated kinetic constants are shown in Table 1

Results

Adalimumab was captured on a sensor chip using immobilized Protein A which binds to the Fc region of the antibody.
This approach provides optimal orientation for efficient binding of the antigen to the variable region of the antibody. The complex was further stabilized using a chemical crosslinker BS3, which minimizes the possibility of the antibody leaching from the surface of the chip during the kinetic measurements.
The sensorgrams for TNF-α binding are shown in Figure 1.
The kinetic rate constants determined by the ACCOLADE™ are in reasonable agreement with the literature reported values that were obtained using a BIAcore 3000 instrument (Table 1) (Kaymakcalan et al. 2009 Clin Immunol 131:308)

Protein	k_a, M^{-1} s^{-1}	k_d, s^{-1}	$K_D=k_d/k_a$, M
BiOptix	7.64 e+005	4.27 e-005	5.58 e-011
Biacore	1.69 e+006	4.71 e-005	3.04 e-011

B. Protocol for Biotinylated Antibody Affinity Measurements

A NeutrAvidin SensorChip was loaded with biotinylated anti-human GRO-β polyclonal antibody (Preprotech) (10 μg/mL in PBS, 60 min) using the BiOptix Chip Charger. Two channels were left un-derivatized to serve as references channels. The sensor chip was rinsed with 10 mM HEPES, 150 mM NaCl, 0.15% polysorbate 20, 0.05% CHAPS, pH 7.3 (HBS-CP) prior to being inserted into the ACCOLADETM. The instrument was equilibrated with HBS-CP. A series of injections of GRO-β (Preprotech) were performed with the association and dissociation phases being monitored for 500 sec each. The sensor surface was regenerated using three sequential 60 second injections of 100 mM glycine, pH 2.8.

Results

Biotinylated biomolecules are idea candidates for immobilization on BiOptix NeutrAvidin SensorChips. The high affinity biotin NeutrAvidin complex provides a stable surface that is resistant to extremes in pH, detergents, high salt and temperatures up to 45oC. Biotinylated anti-GRO-β was loaded onto the NeutrAvidin SensorChip using the BiOptix Chip Charger. Again two channels were left open to serve as references. Initial studies indicated that GRO-β was hydrophobic which resulted in non-specific binding to the chip's surface. Given that polyclonal reagents contain antibodies with heterogeneous affinities, fitting a 1:1 iteration kinetic model is significantly more challenging. In order to minimize antibody subsets with lower affinity interactions, CHAPS detergent was added to the running buffer. The sensorgrams recorded for the antibody-antigen interaction are shown in Figure 2 with the calculated kinetic constants in the table. The polyclonal mixture's approximate binding constant was determined to be in the picomolar range.

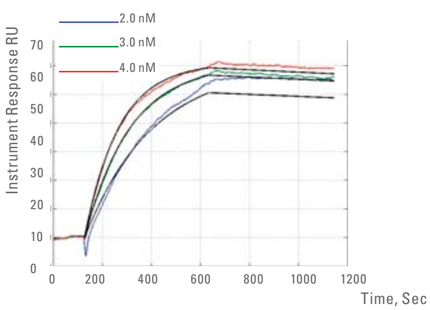

Figure2. Binding responses of GRO-β for anti-GRO-β polyclonal antibody.

The black curves represent the kinetic model fit overlaid with the data. Background noise was subtracted using the data from the reference channels. The calculated kinetic constants are shown in the table

Protein	k_a, M^{-1} s^{-1}	k_d, s^{-1}	$K_D = k_d/k_a$, M
GRO-β	1.77 e+006	7.06 e-005	3.99 e-011

C. Protocol for Direct immobilization of an Antibody-like Biomolecule

A BiOptix mercaptoundecanoic acid (MUA) monolayer SensorChip (part no. C-40401) was amine-activated with a mixture of N-(3-Dimethylaminopropyl)-N?-ethylcarbodiimide hydrochloride (EDAC) and N-Hydroxysuccinimide (NHS) prior to being placed in the BiOptix Chip charger. Fifty microliters of etanercept (reconstituted according to the manufacturer's instructions and buffer exchanged into PBS) was spotted onto separate channels on the chip at a concentration of 100 µg/mL in PBS. After 60 min, the chip was washed with PBS and blocked with a solution of BSA (100 µg/mL) followed by 1 M ethanolamine, pH 8 for 10 min each. The SensorChip was loaded into the BiOptix Accolade™ instrument. Assay running buffer consisted of 10 mM HEPES, pH 7.3, 150 mM NaCl, 0.05% CHAPS, 0.15% polysorbate 20 (HBS-CP). During the analyses, TNF-? in concentrations ranging from approximately 0.1 nM to 20 nM was injected at a flow rate of 120 µL/min for 10 minutes followed by running buffer for 10 min. After each assay, the chip's surface was regenerated with a brief exposure (20 sec) to 50 mM NaOH, 1 M NaCl.

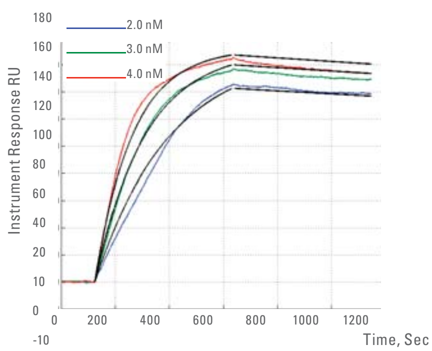

Figure 3. Binding responses of TNF-α for Etanercept.

The black curves represent the kinetic model fit overlaid with the data. Background noise was subtracted using the data from the reference channels. The calculated kinetic constants are shown in the table.

Results

This example evaluated the TNF-binding properties of etanercept using the ACCOLADE™. (Etanercept is currently approved for the treatment of a rheumatoid arthritis and psoriasis.) The TNF antagonist was captured on the SensorChip surface by direct chemical random coupling via the protein's surface amine groups. The chip was loaded into the ACCOLADE™ and various concentrations of TNF were introduced into the solution phase. The rates of dissociation and association were measured.
The results are shown in Figure 3.
The calculated affinity constants again matched previously reported data that were generated by the Biacore instrument (Kaymakcalan et al. 2009). Overall, these findings also suggest that TNF binding properties of adalimumab and etanercept are similar.

Protein	k_a, $M^{-1} s^{-1}$	k_d, s^{-1}	$K_D = k_d/k_a$, M
BiOptix	1.15 e+006	8.35 e-005	3.29 e-011
Biacore	5.64 e+006	6.66 e-005	1.18 e-011