

Application Note

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

Affinity Differences for Antibodies and Fc-fusion Proteins when Binding to Protein A or an Anti-IgG Affibody using the BiOptix ACCOLADE™ Instrument

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Introduction

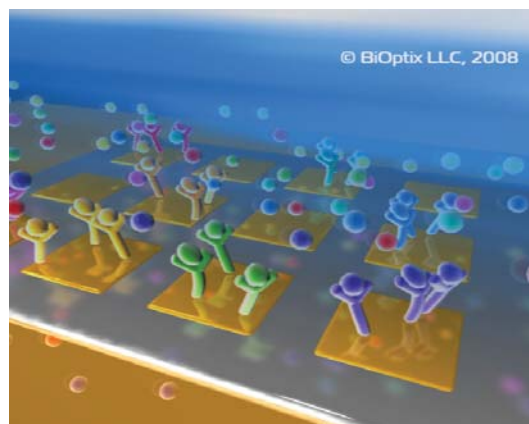
Ligands leaching from purification columns continue to be a significant and costly problem for producers of therapeutic proteins. Leachates can lead to toxicity and regulatory sanction. With the increasing use of affibodies in affinity columns (Ronnmark et al. 2002 and Ramstrom et al. 2009), the present study was conducted to assess the relative utility of an anti-IgG affibody versus Protein A by comparing the affinity of each to two monoclonal antibodies and two Fc-fusion proteins. The affibody used in this study was a small protein (6 kDa) composed of a three-helix bundle based on the scaffold of one of the IgG-binding domains of Protein A. Functionally, this affibody should mimic Protein A with similar binding affinities; this study was designed to determine if this is indeed the case.

The two monoclonal antibodies (Mab-A and Mab-B) and two Fc-fusion protein (Fc-A and Fc-B) that were chosen for this study have different molecular weights and unique molecular structures. Mab-A and Mab-B are IgG1 monoclonal antibodies (fully human and chimeric, respectively) with molecular weights of ~150 kDa. In contrast, the fusion proteins are comprised of an extracellular receptor domain and the hinge and Fc domains of human Ig. The molecular weights of Fc-A and Fc-B are 150 kDa and 92 kDa, respectively.

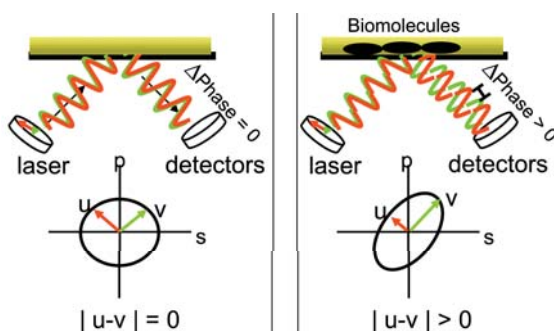
Binding affinities were measured using Surface Plasmon Enhanced common path interferometry (SPE). SPE measures the relative change in phase of the P and S components of polarized light when reflected off a surface coated with a transducer material. This phase change is caused by a local change in refractive index due to specific binding of an analyte to the surface. Figure 1 illustrates the concept of SPE detection.



Figure 1. Principle of SPE-CPI



Panel A Principle of SPE. Panel A shows the covalently linked antibodies present on the gold surface of the SensorChip. As the sample is exposed to the SensorChip surface, accumulation of biomass at the surface due to specific affinity interactions causes an optical phase shift of the P and S components of the laser, which is measured with the photo detectors. The response is then analyzed and the output delivered as a voltage measurement proportional to changes in refractive index at the surface, which in turn is proportional to concentration of the target molecule on the gold surface.



Panel B illustrates the phase differential induced by refractive index change at surface.

Materials

The recombinant monoclonal antibodies and Fc-fusion proteins were obtained from corporate collaborators in powder form and reconstituted according to the manufacturers' instructions. The biotinylated Anti-IgG affibody molecule (ab31901) and biotinylated Protein A were purchased from Abcam (Cambridge, MA) and Sigma-Aldrich (St. Louis MO), respectively. Surface plasmon-based measurements were obtained at 20°C with a BiOptix ACCOLADE™ instrument (BiOptix Diagnostics, Boulder, CO) using BiOptix MUA SensorChips, (part no. C-40401)

Methods

Each mercaptoundecanoic acid-coated SensorChip was first activated by a 7 min exposure to EDC/NHS reagent (EDC: 0.23 M 1-ethyl-3-(3-dimethylpropyl)-carbodiimide + NHS: 0.275 M N-hydroxysuccinimide in water). The chip was washed with ultrapure water and dried with clean nitrogen. The chip was next loaded into the Chip Charger and NeutrAvidin (0.2 mg/ml in 30 mM NaCl) was loaded onto channels 1 and 3. Reference channels 2 and 4 were treated with BSA (1 mg/ml). The immobilization was allowed to proceed for 60 minutes. The chips were then removed from the chip charger and treated with 1 M ethanolamine to neutralize any remaining active groups. The chips were loaded into the ACCOLADE™ previously equilibrated with the assay running buffer (10 mM HEPES, pH 7.3, 150 mM NaCl, 3 mM EDTA, and 0.15% Tween 20). Biotinylated anti-IgG affibody (0.2 µg per ml) or biotinylated Protein A (0.2 µg per ml) was loaded onto the SensorChip while in the instrument for 60-90 secs with a target loading of 100-150 RUs. In separate experiments, Mab-A, Mab-B or Fc-fusion proteins at concentrations ranging from approximately 1 nM to 5 nM were injected at a flow rate of 130 µl/min for 10 minutes followed by running buffer for 10 min. After each assay, the SensorChip's surface was regenerated with 50 mM NaOH, 1 M NaCl. Kinetic analyses were performed using the BiOptix software package. The protein analyte concentrations and flow rates that were used for the binding studies were prescreened to determine a range where mass transport artifacts would be minimal (data not shown).

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Results

The sensorgrams for Mab-A, Mab-B or the Fc-fusion proteins binding to the anti-IgG affibody or Protein A are shown in Figures 2 and 3. Calculated kinetic constants for a global fit of the biomolecular protein-protein interaction based on three concentrations of analyte are shown in Tables 1 and 2.

Figure 2. Binding responses for three model Fc containing proteins to an Anti-IgG Affibody. The black curves represent the kinetic model fit overlaid with the data.

The minor inflection at the top of the curves is a bulk effect that is accounted for by the BiOptix KinetiX software during analysis.

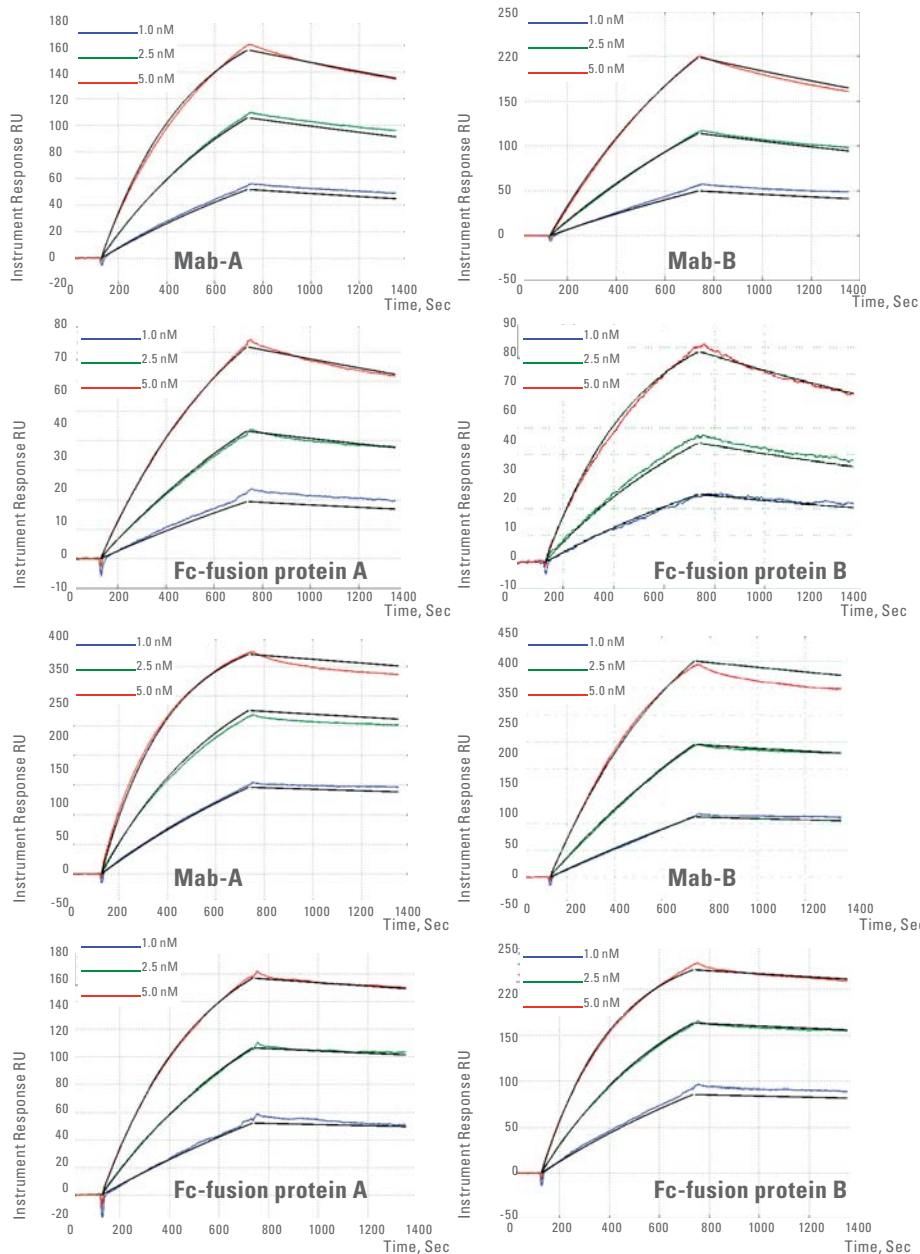


Figure 3. Binding responses for three proteins to Protein A. The black curves represent the kinetic model fit overlaid with the data.

Table 1. Anti-IgG Affibody Affinity Data

Protein	$k_a, M^{-1} s^{-1}$	k_d, s^{-1}	$K_D = k_d/k_a, M$
Mab-A	4.59 e+005	2.75 e-004	5.99 e-010
Mab-B	2.02 e+005	3.09 e-004	1.53 e-009
Fc-fusion protein A	2.77 e+005	2.27 e-004	8.17 e-010
Fc-fusion protein B	4.76 e+005	3.63 e-004	7.62 e-010

Table 2. Protein A Affinity Data

Protein	$k_a, M^{-1} s^{-1}$	k_d, s^{-1}	$K_D = k_d/k_a, M$
Mab-A	7.15 e+005	1.03 e-004	1.56 e-010
Mab-B	3.07 e+005	1.12 e-004	3.66 e-010
Fc-fusion protein A	4.95 e+005	8.07 e-004	1.63 e-010
Fc-fusion protein B	6.89 e+005	7.67 e-005	1.11 e-010

Conclusion

Protein A chromatography is commonly used in the purification of monoclonal antibodies and Fc fusion proteins. Due to possible toxicity issues, the presence of Protein A in the final product is an ongoing concern for protein therapeutic manufacturers. To test the performance characteristics of an alternative affinity ligand, the BiOptix ACCOLADE™ instrument was used to probe the dynamic aspects of the interactions of several Fc containing proteins with an anti-IgG affibody versus Protein A.

As shown in Tables 1 and 2, the Protein A and anti-IgG affibody ligands displayed picomolar affinities for all of the monoclonals and Fc-fusion proteins tested with the Protein A-Fc interaction being slightly tighter (3-6 fold). The preliminary data also indicate that there may be a secondary weaker interaction with the Protein A ligand as evidenced by an initial drop in the signal during the early dissociation time period. These data do suggest that anti-IgG affibody may be a viable alternative for Protein A for affinity purification of Fc-containing proteins.

This study also demonstrates that the BiOptix ACCOLADE™ SPE technology is a highly sensitive, label-free method for real-time detection of protein-protein interactions. Additionally, the BiOptix KinetiX Software conveniently provides data analysis and kinetic parameter determinations.



References

- Ramstrom et al., (2009) Biotech. Appl. Biochem. 52:159-166.
Ronmark et al., (2002) Eur. J. Biochem 269: 2647-2655.

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