



● High-throughput SPR Screening and Characterization of Biotherapeutics

The growing demand and acceptance of the use of biotherapeutic drugs has created the need to quickly and accurately measure their activity and binding characteristics throughout the entire therapeutic development and production cycle.

Introduction

Real-time, label-free (RT-LF) analysis utilizing SPR detection is a powerful tool for the biophysical characterization of protein therapeutics. The next generation RT-LF analysis platform, Sierra SPR-32,

was evaluated for its assay flexibility and rapid kinetic characterization of binding interactions. Equipped with a 4 x 8 array of 32 individually addressable detection spots, the Sierra SPR-32 system is uniquely configured to provide active and control binding data for

each sample injection. Simultaneous injection of eight samples facilitates high-throughput assay development and optimization, as well as rapid quantitative analysis of crude and purified samples. For antibody screening or epitope mapping/binning,

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up to 32 samples can be evaluated in single assay cycle. In the present study, high-throughput kinetic screening data obtained for a panel of antibody samples will be discussed.

Materials and Methods

Instrumentation

All real-time, label-free (RT-LF) assays were completed using Sierra SPR-32, from Bruker Daltonics SPR (Hamburg, Germany). The Sierra SPR-32 system contains 32 detection spots arranged in a 4 x 8 array. Eight samples are delivered simultaneously to the detection spots via a continuous flow microfluidic device. A high sensitivity surface plasmon resonance imaging detector, SPR⁺, was used to measure binding interactions in real time.

Surface Preparation

The target proteins were attached to high-capacity (HCA) sensors (Bruker Daltonics SPR) using standard primary amine immobilization chemistry. Thirty-one (31) detection spots in eight (8) channels were immobilized with target proteins. Detection Spot A in flow cell 1 was used as the control surface. See Figure 1a for the arrangement of active and control surfaces. The final average immobilization response for all target proteins on all detection spots is shown in Table 1.

Assay Conditions

Immobilization of the target proteins and all binding assays were conducted using PBS buffer containing 0.05% Tween 20, pH 7.4. All assays were completed at 25°C. For the screening assay, 92 antibody samples were tested without purification from hybridoma supernatants. Samples were diluted ~1.4-fold by adding 70 µl of supernatant to 30 µl of PBS buffer containing 0.05% Tween 20. Antigen samples were diluted into PBS buffer containing 0.05% Tween 20 to a final concentration of 60 nM for the screening assay. Antigen samples were injected for 2 min followed by dissociation in buffer for 8 min. The bound material was removed from the surface with 2 x 1-min injections of 10 mM glycine, pH 2.0.



Table 1. Immobilization Consistency Across Eight Flow Cells

Protein (Spot)	Immobilization Level (RU)
Target 1 (2-8A)*	166 ± 11
Target 2 (1-8B)	83 ± 6
Target 3 (1-8C)	2752 ± 72
Target 4 (1-8D)	2233 ± 110

* Spot 1A was used as a control surface for the experiments.

Results

Surface Preparation

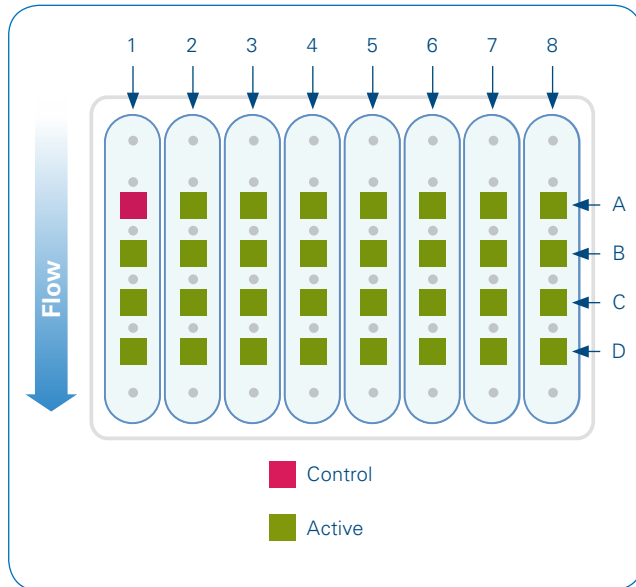


Figure 1a: Flow Cell Schematic and Immobilization Pattern. Four different target proteins were immobilized onto the four detection spots within each flow cell. Spot 1A (shown in red) was used as the control surface while Spots 2A – 8D (shown in green) are active surfaces.

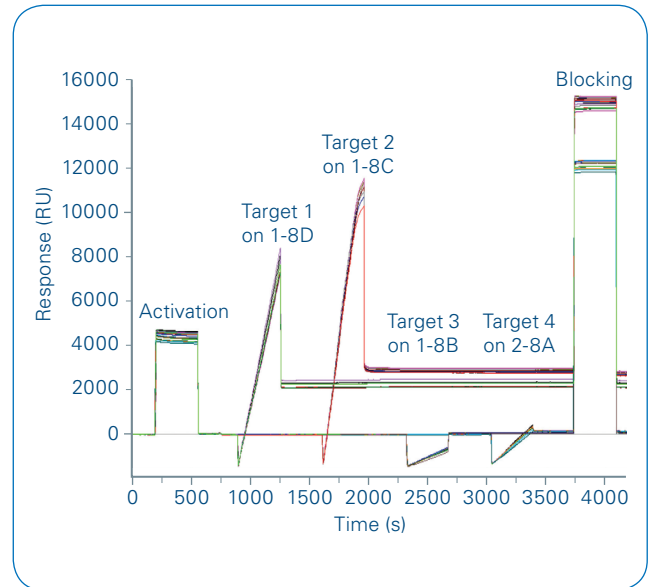


Figure 1b: Immobilization of 1 Control Surface and 31 Active Surfaces. Using a standard primary amine coupling protocol, 4 different target proteins were attached to 31 detection spots across 8 flow channels. One detection spot was prepared for use as the control surface. The target proteins were injected sequentially across the desired spot locations beginning with Spot D and finishing with Spot A (see schematic Figure 1a). All detection spots were activated and blocked using a single injection. Preparation of 32 surfaces (1 control + 31 active) was completed in ~ 1 hour. Average immobilization levels are reported in Table 1.

Screening and Kinetic Assays

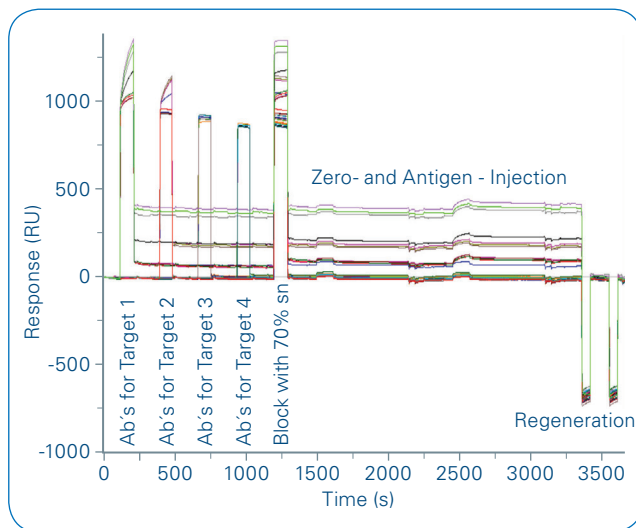


Figure 2a: Complete Assay Cycle — Capture, Binding, Regeneration. Eight (8) different antibodies were captured in each of four injections. A blocking injection of 70% supernatant without antibody was applied to control for nonspecific binding. A buffer injection (0 nM) preceded the antigen injection (60 nM). The association time was 2 min followed by dissociation of the complex in buffer for 8 min.

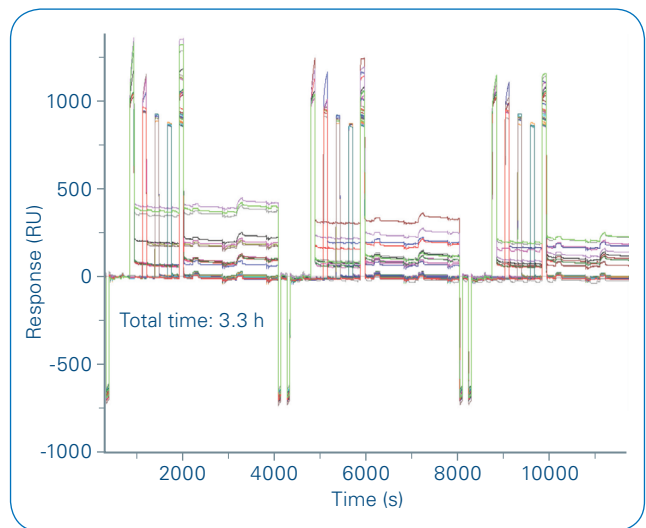


Figure 2b: Screening Assay for 93 Antibodies. Ninety-two (92) antibodies in 70% hybridoma supernatant were screened in 3.3 hours. Using 2 min for association and 8 min for dissociation it was possible to pre-characterize the interactions and select antibody candidates for full kinetic characterization.

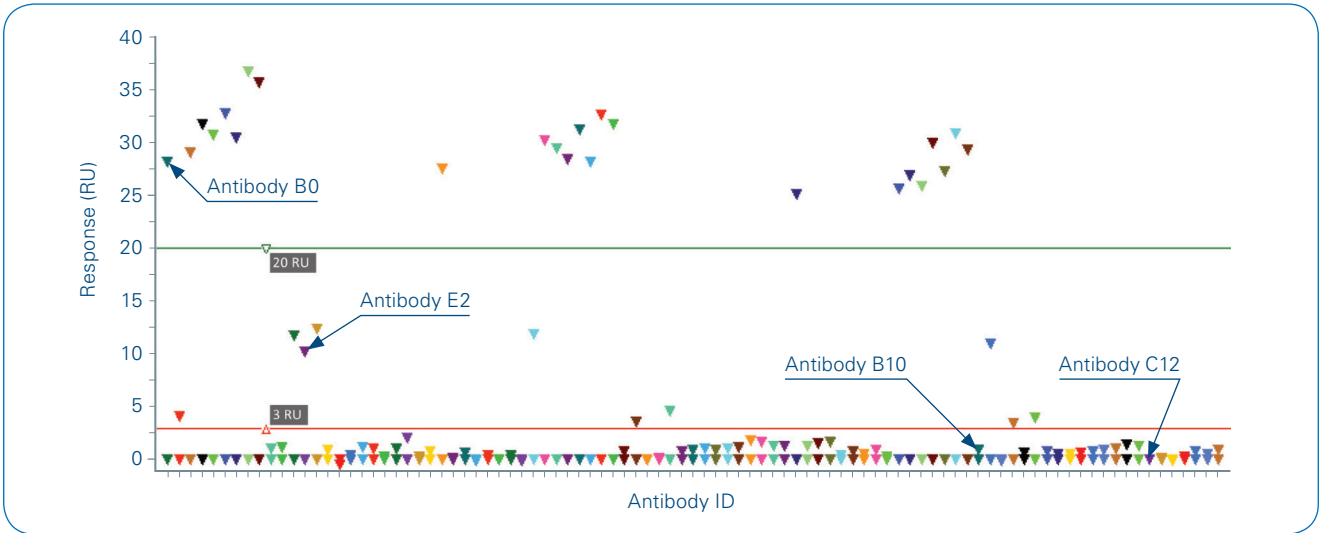


Figure 3: Binding Response for Antibody-Antigen Complexes. The measured binding response for the antibody-antigen complexes was presented in a scatter plot (Response [RU] vs Antibody ID) for rapid hit identification. Binding level thresholds were set at Low = 0-3 RU, Medium = 3-20 RU, and High = 20+ RU. Representative sensorgrams for each binding level are shown in Figure 4.

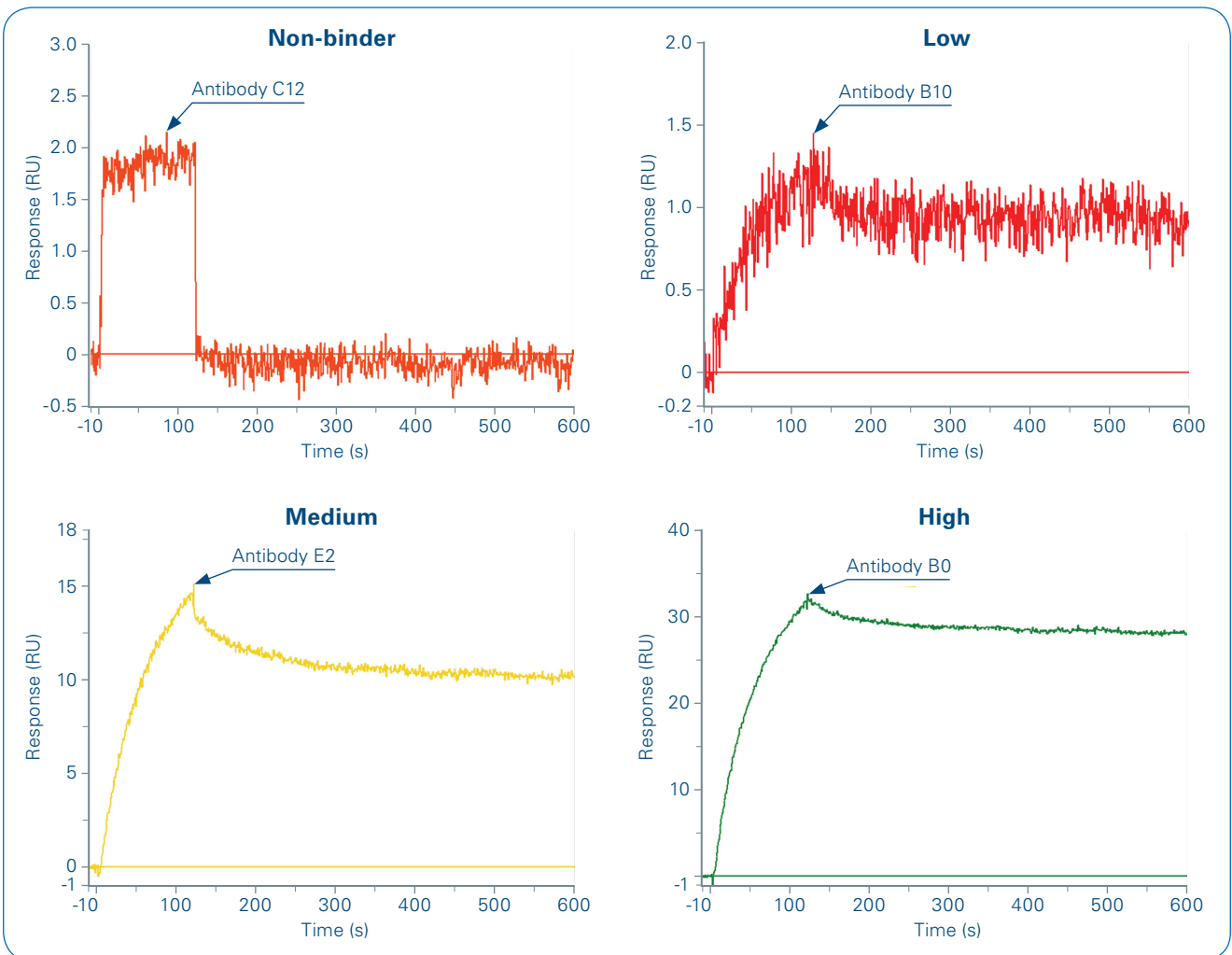


Figure 4: Sensorgrams for Ab-Ag Binding Interactions at Different Binding Levels. The double-referenced sensorgrams are examples of the varied binding levels and kinetics for the Ab-Ag complexes. Although some of the binding responses are low, the binding kinetics reveal the formation of high affinity complexes (e.g. Ab B10).

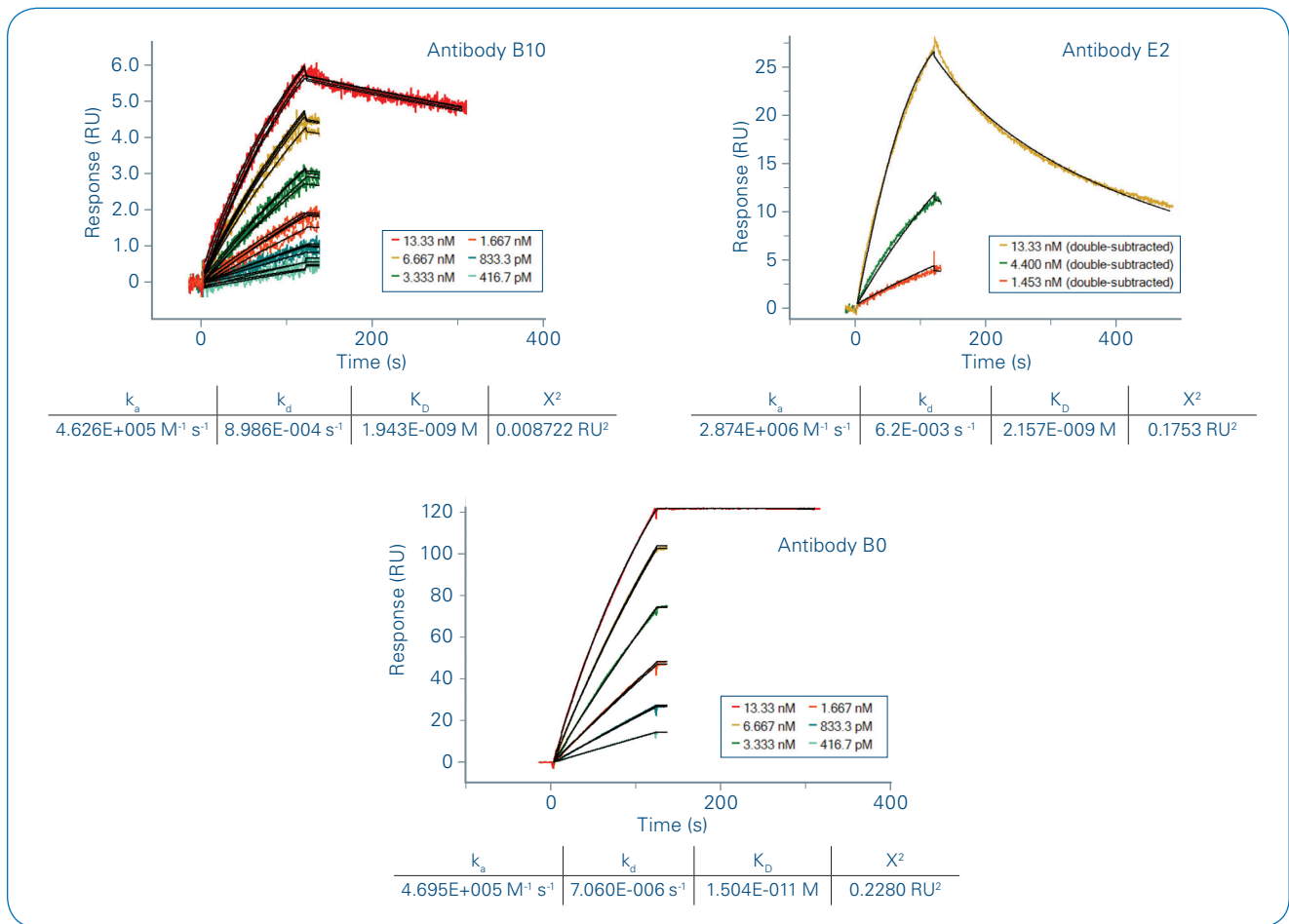


Figure 5: Kinetic Analysis of Selected Hits from Antibody Screen. Selected hits from the antibody screen were evaluated further in a rapid kinetics analysis experiment. A concentration series (0-13 nM) of the antigen was injected over the antibody surfaces. Dissociation data were collected for the lowest (0 nM) and the highest (13 nM) concentration samples in the series. Kinetic fitting results are reported for the replicate data sets. Ab B10 was analyzed in quadruplicate and Ab B0 was subject to duplicate analysis.

Conclusions

- Several unique features of the Sierra SPR-32 system, including the eight quad-sensor detection flow cells, integrated eight-needle sample delivery unit, SPR+ detection technology and Hydrodynamic Isolation™ microfluidics, enabled the high-performance and highly sensitive real-time, label-free (RT-LF) analysis of 92 antibodies in 3 hours.
- Owing to consistent detection and measurements across all 32 sensor spots, the antibody screening assay was completed using 1 control surface and 31 active surfaces. All 32 surfaces were prepared in 1 hour. This assay configuration increases throughput while yielding high quality data and results.
- The Sierra SPR-32 system rapidly screens and pre-characterizes plates of antibody samples in crude matrices, such as hybridoma supernatant or serum, without clogging. In the current study, antibody samples were diluted to a final concentration of 70% supernatant for analysis.
- Hit validation was accomplished using a rapid kinetic assay format. For several antibody samples, replicate kinetic measurements showed good agreement when the data were analyzed using a Langmuir 1:1 model.



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