High-throughput SPR Screening and Characterization of Small Molecules Binding to Protein Targets

The high cost, lengthy time line and low success rate for small molecule drugs has created the need to quickly and accurately measure their activity and binding characteristics throughout the entire discovery and development cycle.

Introduction

Real-time, label-free (RT-LF) analysis utilizing SPR detection is a powerful tool for the biophysical characterization of small molecule drugs and drug candidates. 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 4x8 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 samples in complex matrices and organic solvents,

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such as DMSO. For kinetic screening or detailed kinetic characterization of slowly dissociating or covalent complexes, up to eight different sample concentrations can be evaluated in a single injection cycle. In the present study, high-throughput kinetic screening data obtained for a panel of small molecule samples will be discussed.

Materials and Methods

Instrumentation

All real-time, label-free (RT-LF) assays were completed using the Sierra SPR-32 system, from Bruker Daltonics SPR (Hamburg, Germany). The Sierra SPR-32 system contains 32 detection spots arranged in a 4x8 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 protein was attached to high-capacity (HCA) sensors (Bruker Daltonics SPR) using standard primary amine immobilization chemistry. Eight (8) detection spots, one per channel (Spot D), were immobilized with the target protein. Detection Spot C in each flow cell was immobilized with a reference protein for use as a control surface. To create a secondary control surface, detection Spot B in each flow cell was activated and blocked. See Figure 1a for the arrangement of active and control surfaces. Spot A in each flow cell was not used for the experiments. The final average immobilization response for all target proteins on all detection spots is shown in Table 1.

Assay Conditions

Immobilization of the target and reference proteins was conducted using PBS buffer containing 0.05% Tween 20, pH 7.4. Binding assays were completed using PBS buffer containing 0.05% Tween 20 and 3% DMSO, pH 7.4. Compound samples were injected across detection spots B, C, and D (in series). All assays were conducted at 25°C.

Screening and Kinetic Characterization

A 384-well plate containing compound samples at 100 µM was evaluated for binding to the target and reference proteins. Samples were injected for 30 sec at 30 µl/min; dissociation was monitored for 30 sec. Kinetic characterization was completed for selected compound samples using a flow rate of 30 µl/min. Compound samples ranging in concentration from 0 – 20 µM or 0 – 35 µM were tested in duplicate across all 8 channels yielding 16 replicate measurements for each concentration. Association data were collected for 1 min for each concentration and complex dissociation was followed for 2 min after the end of the injection. Three (3) DMSO calibration curves were prepared (beginning, middle, end) per 384-well plate of samples.

Data Analysis

Data analysis was completed using Analyser (Bruker Daltonics SPR) software. All sensorgrams were double- reference subtracted and DMSO-corrected prior to data analysis. A Langmuir 1:1 binding model was used to fit the kinetic data.

Table 1: Immobilization Consistency Across Eight Flow Cells

Protein (Spot)	Immobilization Level (RU)	M%CV
Reference (1-8C)	4689 ± 136	2,9
Target (1-8D)	2838 ± 95	3,3

Results

Surface Preparation

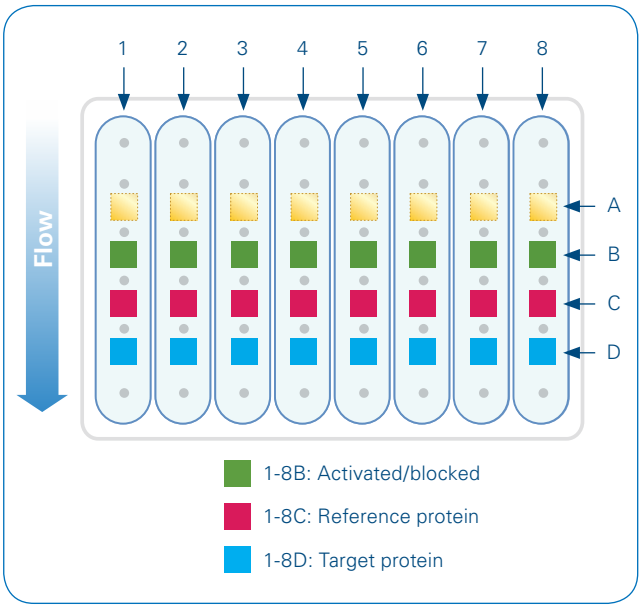


Figure 1a: Flow Cell Schematic and Immobilization Pattern. One target protein and one reference protein were immobilized onto detection Spots D and C, respectively, within each flow cell. A secondary control was created by activating and blocking Spot B in each flow cell.

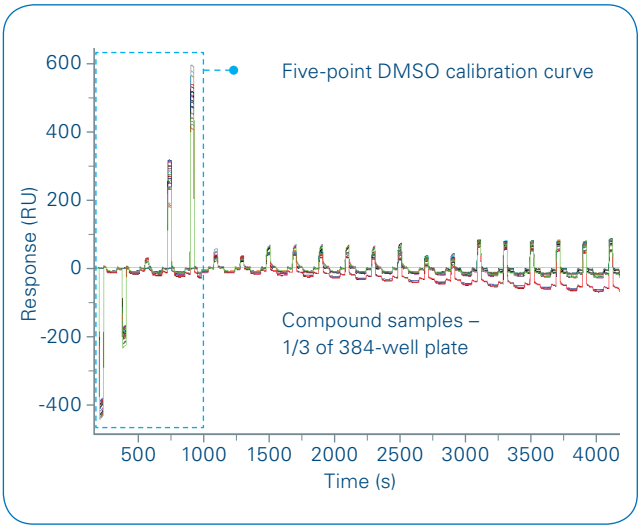


Figure 2: DMSO Calibration and Compound Screen. The compound screen was preceded by a five-point DMSO calibration curve. Compound samples (100 µM) were injected over the active (Spot D) and control (Spot B and C) surfaces for 30 sec; complex dissociation was monitored for another 30 sec. Only one-third of the compounds tested is shown. The entire 384-well plate of compounds was screened in ~3.5 hours.

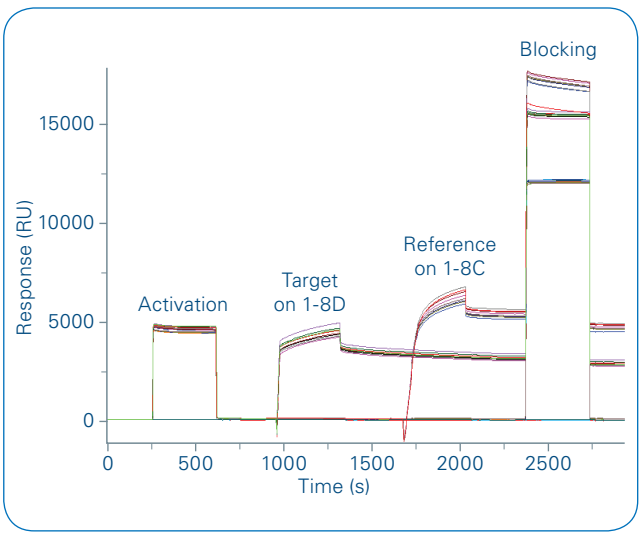


Figure 1b: Immobilization of Control and Active Surfaces. Using a standard primary amine coupling protocol, the target and reference proteins were attached to 16 of 32 detection spots across 8 flow channels. One row of detection spots (Spot B) was activated and blocked for use as the control surface. The proteins were injected sequentially across the desired spot locations beginning with Spot D and finishing with Spot C (see schematic Figure 1a.). All detection spots were activated and blocked using a single injection. Preparation of 24 surfaces (16 controls + 8 active) was completed in ~ 1 hour. Average immobilization levels are reported in Table 1.

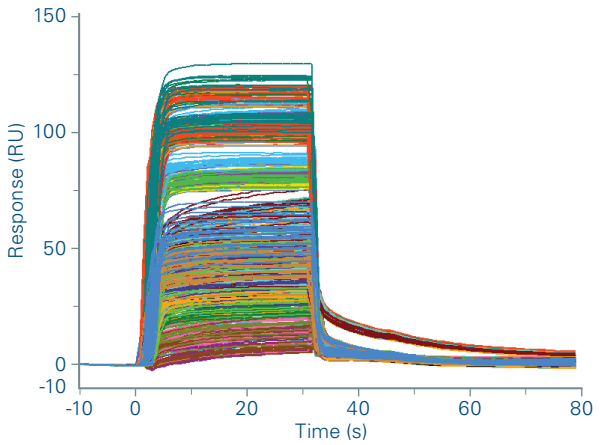


Figure 3: Screening Assay for LMW Compounds. Compound samples diluted into PBS buffer containing 3% DMSO were screened for binding to the target protein. Using 30 sec for association and 30 sec for dissociation the entire 384-well plate was analyzed in ~3.5 hours, generating over 1100 interactions plus 3 x DMSO calibration curves. It was possible to pre-characterize the interactions and select antibody candidates for full kinetic characterization.

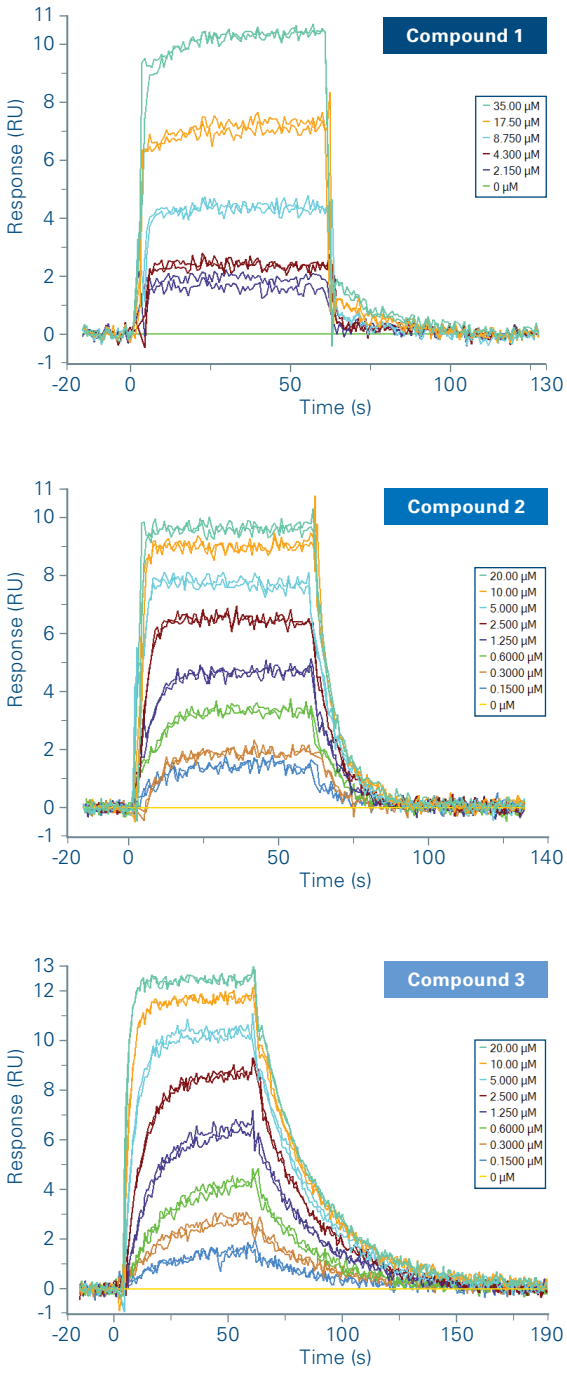


Figure 4: Dose-dependent Binding for Selected Compounds. The double-referenced and DMSO-corrected sensorgrams are examples of the varied binding levels and kinetics for the LMW compound complexes formed with the target protein. Dose-dependent binding was measured in duplicate for selected compounds.

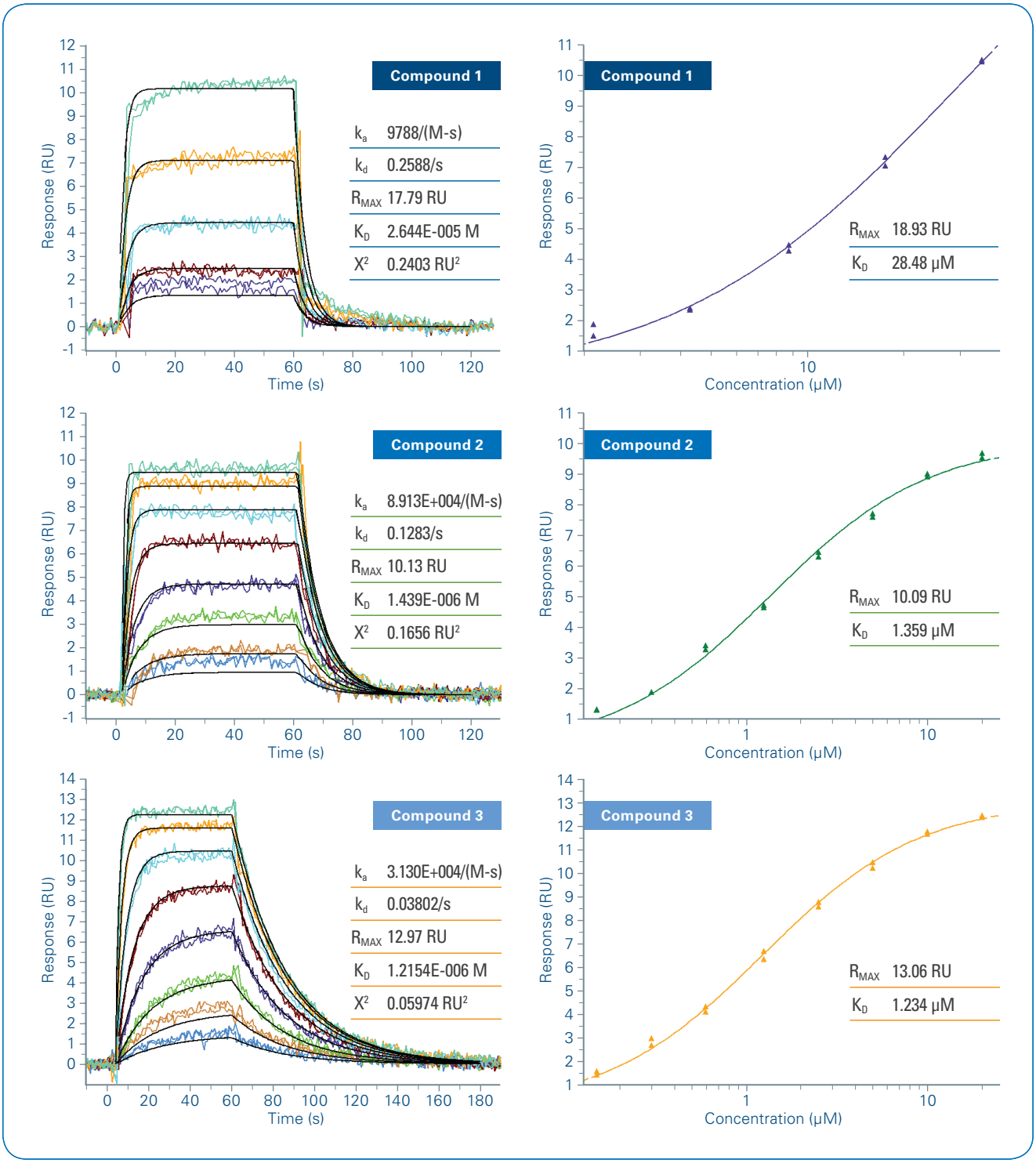
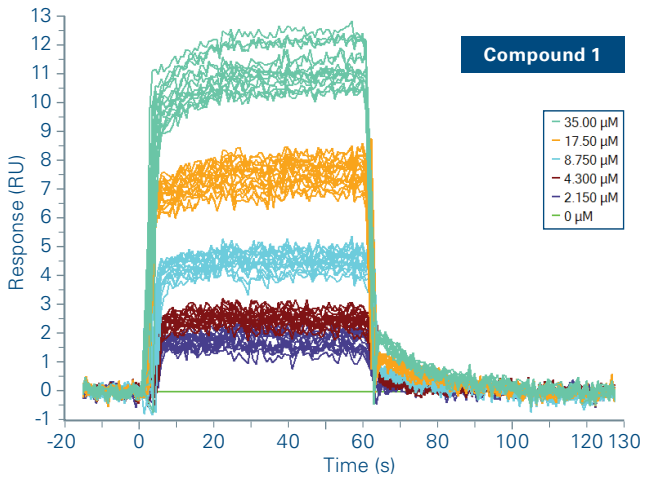
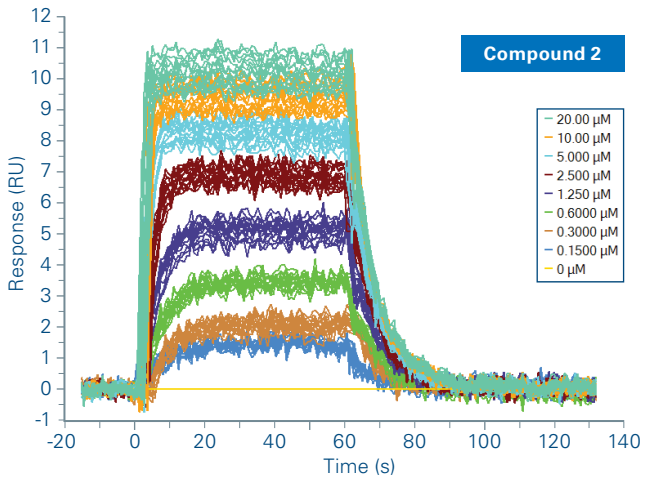


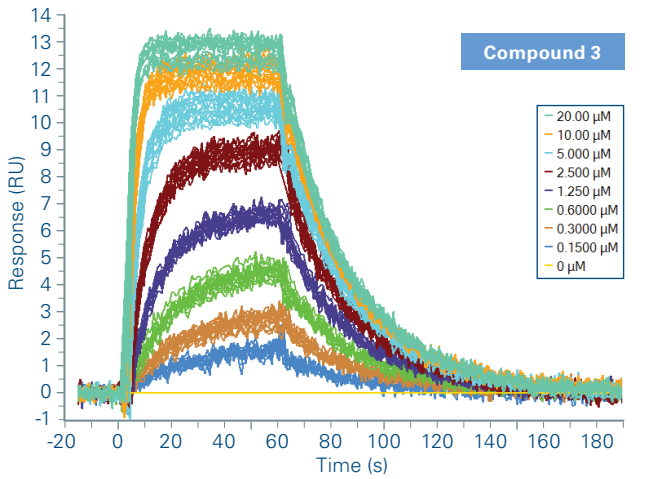
Figure 5a,b,c: Kinetic Analysis of Selected Hits from Compound Screen. Selected hits from the compound screen were evaluated further in a kinetics analysis experiment. A concentration series (0–20 or 0–35 µM) of the compound was injected over the target protein surfaces. Data were double-reference subtracted and DMSO-corrected prior to fitting using a 1:1 Langmuir model. Kinetic and affinity fitting results are reported for duplicate data sets.



Compound 1	
Concentration (µM)	Reponse (RU)
2.15	1.89 ± 0.13
4.3	2.62 ± 0.23
8.75	4.49 ± 0.29
17.5	7.35 ± 0.39
35	11.25 ± 0.65



Compound 2	
Concentration (µM)	Reponse (RU)
0.15	1.49 ± 0.04
0.3	1.96 ± 0.20
0.6	3.42 ± 0.17
1.25	5.08 ± 0.27
2.5	6.81 ± 0.28
5	8.21 ± 0.27
10	9.37 ± 0.41
20	10.09 ± 0.43



Compound 3	
Concentration (µM)	Reponse (RU)
0.15	1.52 ± 0.14
0.3	2.72 ± 0.23
0.6	4.46 ± 0.21
1.25	6.60 ± 0.18
2.5	8.93 ± 0.27
5	10.61 ± 0.32
10	11.94 ± 0.35
20	12.67 ± 0.37

Figure 6: Reproducibility of Replicate Binding Measurements. Three compound samples were selected for replicate binding measurements in order to evaluate the system reproducibility. A concentration series (0-20 or 0-35 µM) of each compound was injected in duplicate over the 8 target protein surfaces, resulting in 16 replicate measurements for each concentration. The %CV is ~3% for most of the sample concentrations tested.

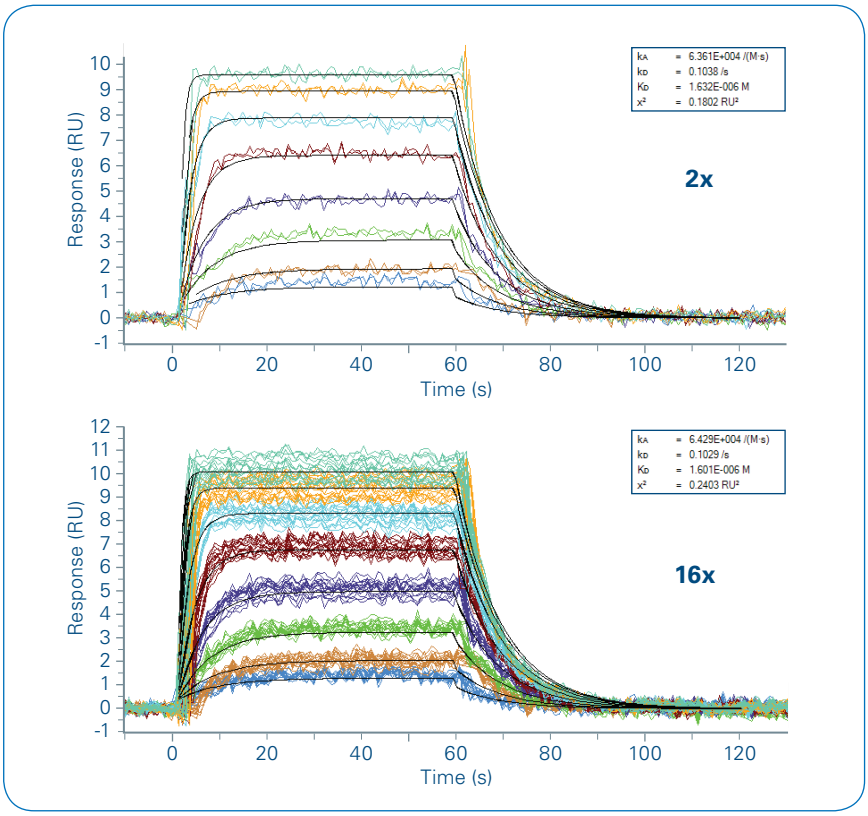


Figure 7: Comparison of Kinetics Using Replicate Data Sets. Kinetic analysis of duplicate vs. 16x replicate data sets for Compound 2 show excellent agreement in the fitted results. The low signal-to-noise of the Sierra SPR-32 system in the 0-10 RU range is clearly demonstrated in the binding data and fitted results.

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) screening of 384 compounds in ~3.5 hours.
- Owing to consistent detection and measurements across all 32 sensor spots, binding measurements were highly reproducible and displayed low %CV for 16 replicates. This performance feature of the Sierra SPR-32 system allows for flexible assay configuration, and increases throughput while yielding high quality data and results.
- The Sierra SPR-32 system rapidly screens and characterizes plates of compound samples in complex matrices, such as buffer + DMSO. In the current study, compound samples were diluted to a final concentration of 3% DMSO for analysis. Double-referencing and DMSO correction were applied to all compound samples.
- Kinetic and affinity characterization for selected compounds binding to a target protein was accomplished using a traditional multi-cycle kinetic assay format. For several compound samples, replicate binding measurements showed good agreement when the data were analyzed using a Langmuir 1:1 model.

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