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This was a truly collaborative effort, in which Genedata and Bruker really stepped up and enabled us to push the limits of SPR. By setting up robust automation and a comprehensive data processing pipeline, we were able to screen and analyze our data with lightning speed.

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INDUSTRY

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ABOUT NOVARTIS

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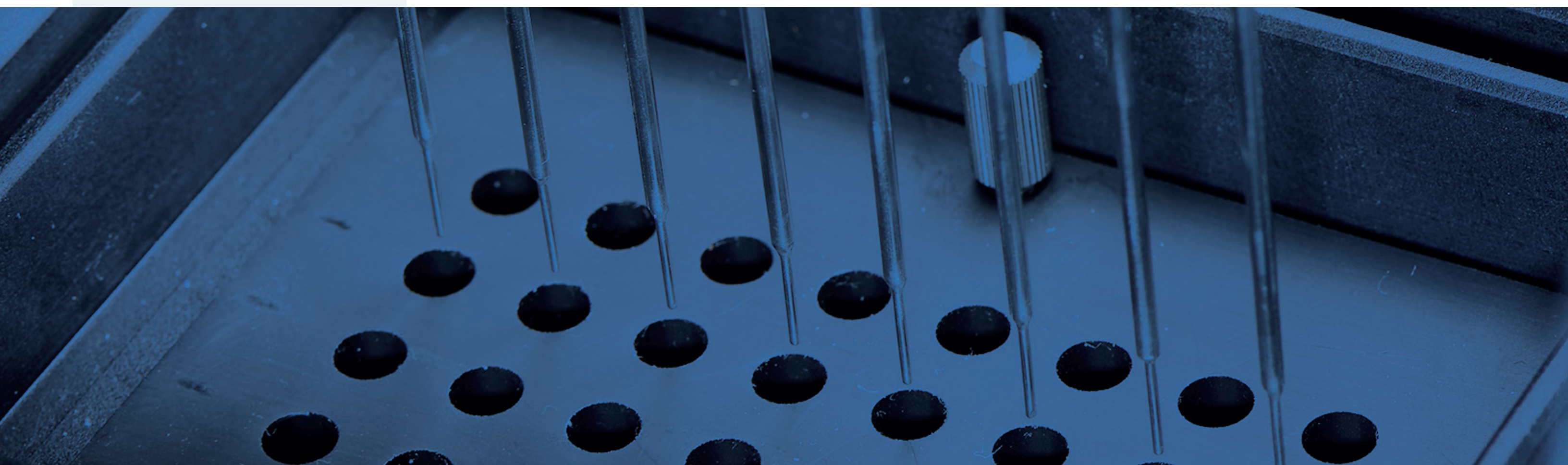
GENEDATA SOLUTION

Tackling Challenging Targets with High-Throughput Biophysical Screening at Novartis

Scaling Up Surface Plasmon Resonance-Based Screens to Increase Novel Hit Finding

This application note describes how Novartis successfully identified novel drug candidates for challenging protein targets by pioneering high-throughput SPR screening. To accomplish this, screens were performed on a Sierra SPR®-32† instrument and data analyzed using Genedata Screener®. This made it possible to:

- Scale up biophysical screening and employ a multiplexed assay design for initial hit-finding campaigns. This endeavor allowed for primary screening against mutant forms of the protein target, resulting in the discovery of novel chemical scaffolds.
- Rapidly process complete campaigns by automating sensorgram processing and calculations, while giving scientists full control over result review and ability to adapt the analysis to their workflows.
- Unify data analysis into a single software platform, increasing efficiency and data traceability.



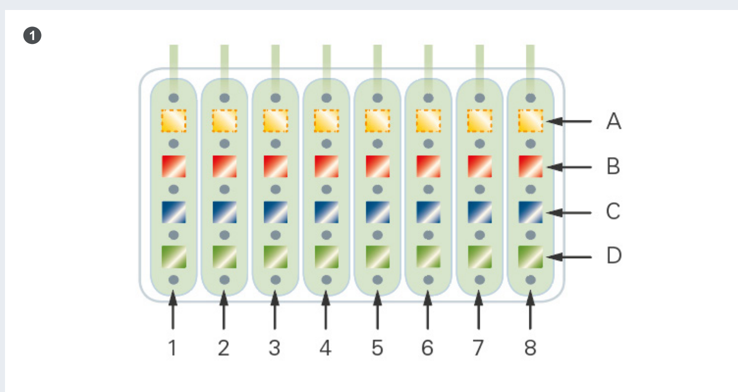
Introduction

Surface plasmon resonance (SPR) is a powerful method that allows sensitive, real-time, label-free measurement of binding and kinetics, including of moderate to low binding affinities.[1,2] Scaling SPR for medium to high throughput screens, on the order of thousands or tens of thousands of compounds, enables its application early in the drug discovery pipeline. By doing so, one can triage or select hits based on detailed biophysical and direct target-binding information. This is especially useful when traditional biochemical and functional assay readouts are unable to detect these binding interactions. As such, a biophysical-based approach can be advantageous in unveiling new hits and broadening the chemical space for challenging targets.

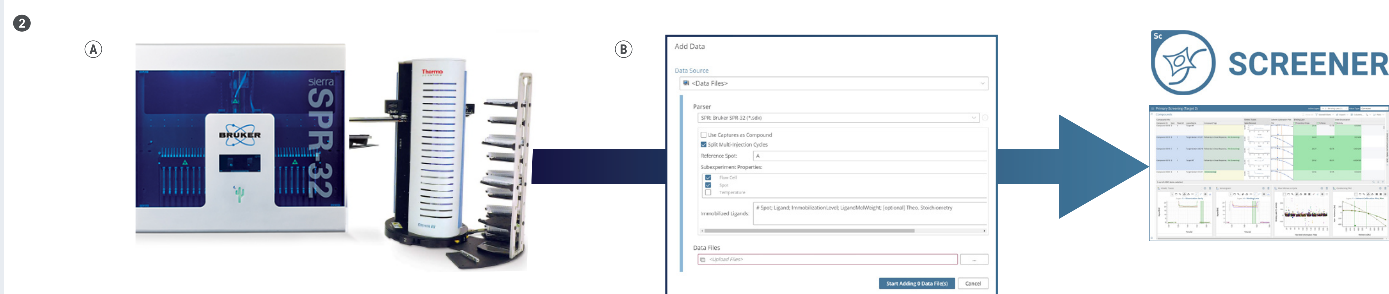
At Novartis, we sought to perform high throughput small-molecule biophysical screening campaigns for two challenging targets of different protein classes. Furthermore, the library needed to be screened against additional forms of the targets, as this can be useful in selecting the most promising chemical material with which to inhibit disease-causing mutants, variants, or target isoforms (such as for oncology targets). Finally, because the Novartis SPR portfolio includes many different SPR instruments,[3] we wanted to replace existing processes for SPR analysis—which required shuffling data between the instrument and an amalgam of softwares and spreadsheets—with a single solution.

In order to achieve these goals, our team worked closely with Bruker and Genedata to create a highly efficient workflow for high throughput SPR screening, including automated plate loading, multiplexed assay design, and rapid data analysis.

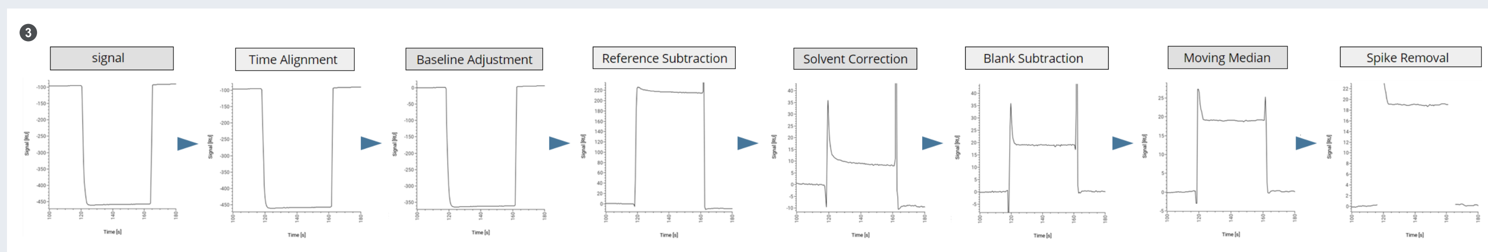
❶ ***Flow-cell layout.*** *The Sierra SPR®-32† has eight flow channels, each of which contains four measurement spots: spot A is used as a reference spot, while spots B, C, and D can be used for different targets. Together, this multiplexed design allows one to obtain an interaction profile of up to eight compounds against three targets with a single injection.*



❷ ***Automated plate loading and direct import of raw data from the SPR-32 into Screener.*** *Ⓐ Automated plate loading enabled continuous screening overnight, such that 4 to 5 384-well plates could be run in a 24-hour period. Ⓑ The standard integration between the Sierra SPR®-32† and Screener includes automatic import of ligand information and immobilization levels.*



❸ ***Automated Sensorgram Processing.*** *For SPR screens, Novartis applied the following, built-in processing steps: Time Alignment, Baseline Adjustment, Reference Subtraction, Solvent Correction, Blank Subtraction, Moving Median, and Spike Removal.*



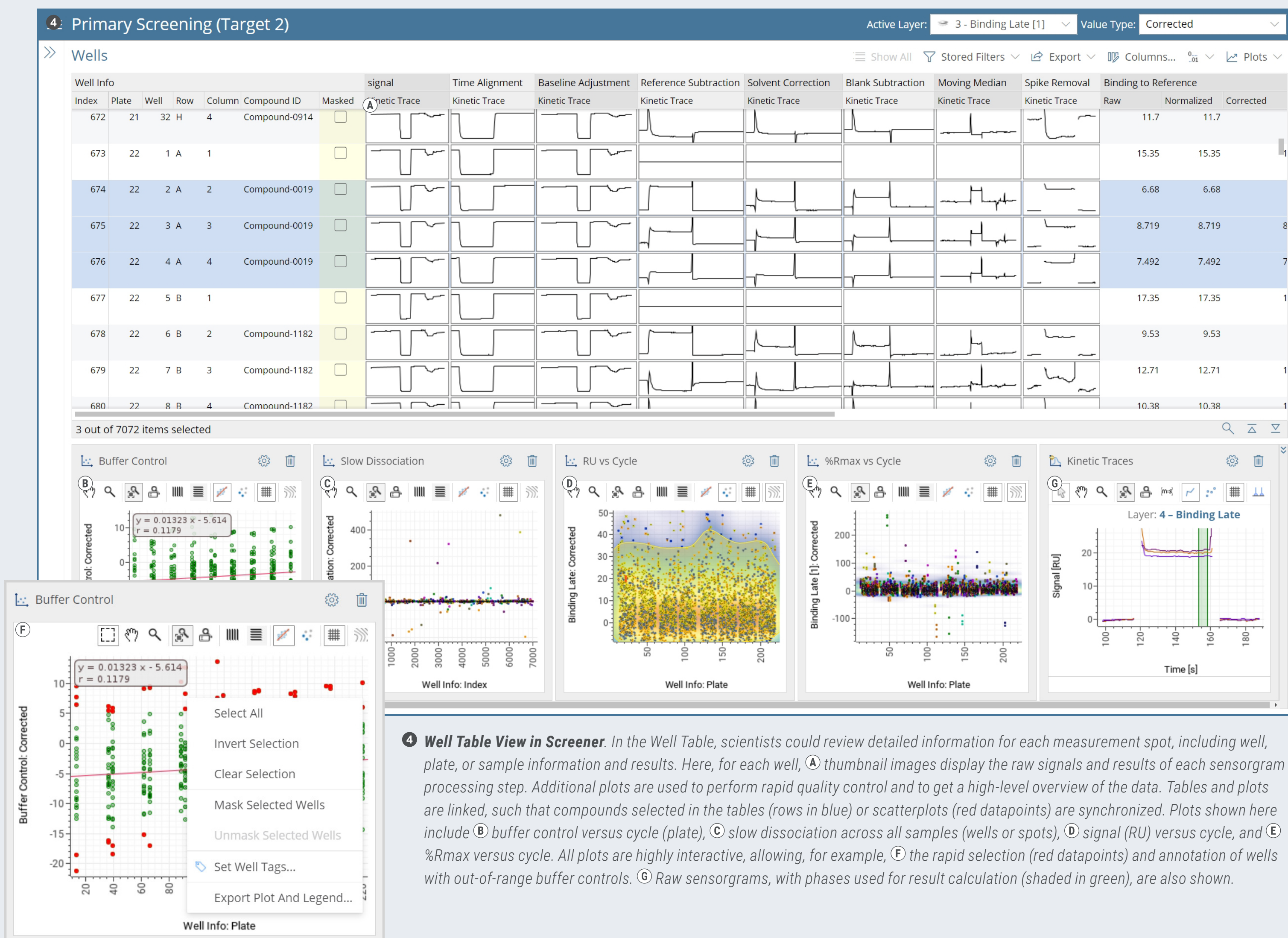
Experimental Setup

Screening campaigns were run on two different targets from different disease areas: Target 1 (Molecular weight ~20kDa) and Target 2 (Molecular weight ~120 kDa). For this application note, data is shown for Target 2, which was tested along with two of its disease-relevant mutants. All experiments were performed on a Sierra SPR®-32† using Xantec SAD200M chips. The Sierra SPR®-32† has a flow-cell setup of eight channels each with four measurement spots, providing 32 individual sensor spots overall. This flexible set-up allows scientists to screen either multiple analytes against up to three target proteins (plus one reference) or a single analyte against up to 31 target proteins. We chose an experimental approach with three target proteins and one reference spot, an approach optimally suited for small molecule screens.

For primary screening, the target and its mutants were run against 10,000 compounds (28 × 384-well plates) at a single concentration of 180µM, for a total of ~34,200 datapoints when including solvent correction data. Compounds were preselected from a novel compound library by physical parameters such as molecular weight and solubility.

Chips were preconditioned and ligands immobilized in pre-defined runs. Control compounds were injected periodically using a single concentration, while solvent correction calibration solutions were injected intermittently in between compound plates.

Plate loading was automated using an Orbitor plate changer (Thermo Scientific). A fresh chip was loaded or new immobilization was run every 19 to 24 hours. Assays were performed at 15 °C, with 45-second association and



❹ ***Well Table View in Screener.*** *In the Well Table, scientists could review detailed information for each measurement spot, including well, plate, or sample information and results. Here, for each well,* Ⓐ *thumbnail images display the raw signals and results of each sensorgram processing step. Additional plots are used to perform rapid quality control and to get a high-level overview of the data. Tables and plots are linked, such that compounds selected in the tables (rows in blue) or scatterplots (red datapoints) are synchronized. Plots shown here include* Ⓑ *buffer control versus cycle (plate),* Ⓒ *slow dissociation across all samples (wells or spots),* Ⓓ *signal (RU) versus cycle, and* Ⓔ *%Rmax versus cycle. All plots are highly interactive, allowing, for example,* Ⓕ *the rapid selection (red datapoints) and annotation of wells with out-of-range buffer controls.* Ⓖ *Raw sensorgrams, with phases used for result calculation (shaded in green), are also shown.*

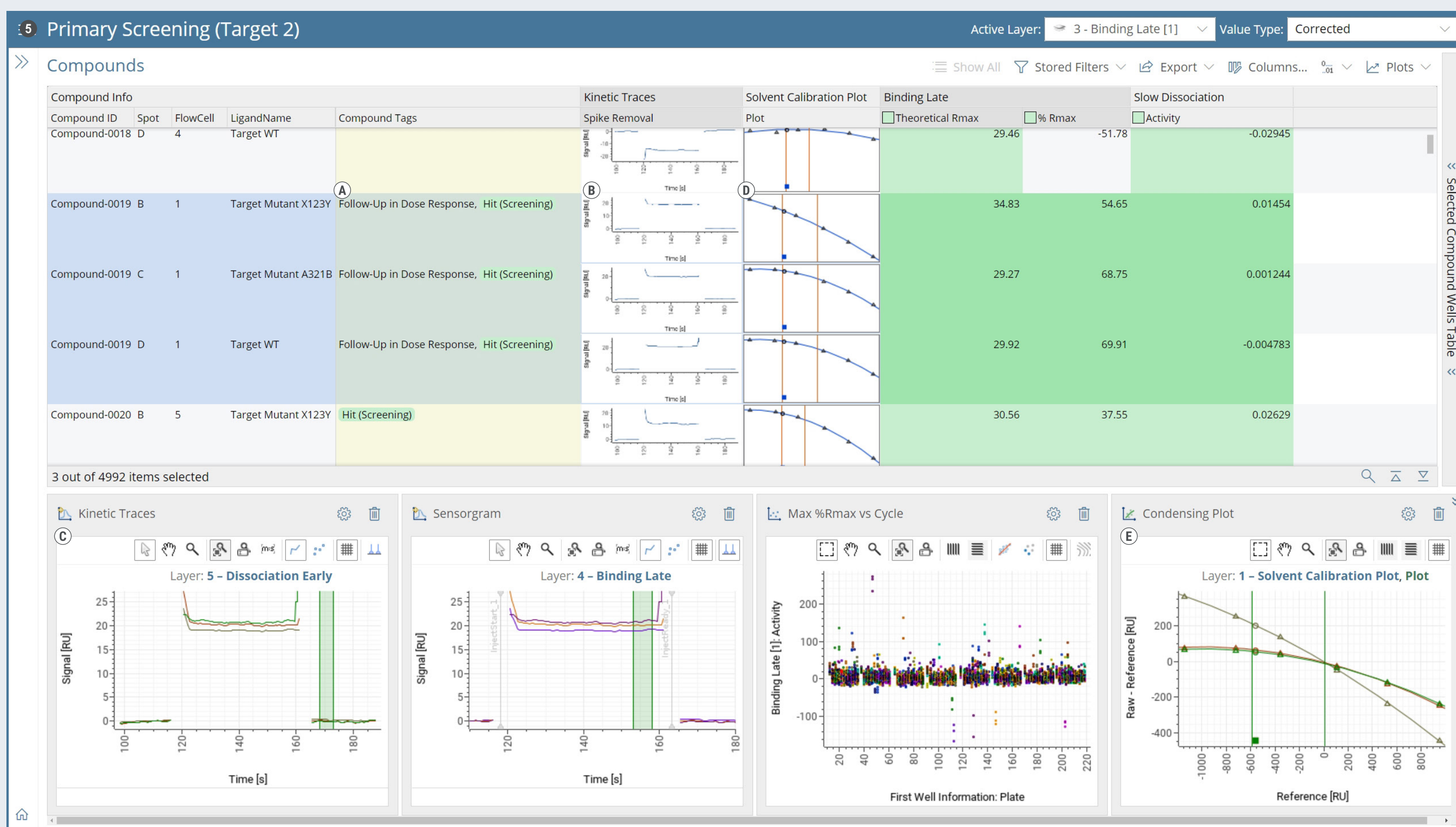
45-second dissociation phases. Buffer included 2% DMSO.

Finally, in follow-up validation screens, hits were further tested in 8-point dose response, with a 60-second association and 120-second dissociation phase.

Principle & Workflow

The goal of the Target 2 screening campaign was to find novel chemical starting points that can be tuned to modulate only the mutant, oncogenic forms of this protein target. In addition, Target 2 possesses many isoforms in the cell, which heightens the challenges of medicinal chemistry follow-up. Therefore, the target was a good candidate for a multiplexed, SPR-based screen: this permits triage of compounds with undesirable binding properties—such as aggregation or superstoichiometric binding—upfront,[2] and provides an easy method for prioritizing compounds for follow-up with NMR and X-ray crystallography. The Sierra SPR®-32† enables multiplexed assays, by allowing parallel injection of eight compounds in one shot and simultaneous testing of both the wildtype and two mutant forms of the target (*Figure* ❶). An open design also allows integration with a plate changer, so that experiments could run autonomously through the night. With this automation in place, 4 to 5 384-well plates could be run over 24 hours, limited in speed only by target stability (*Figure* ❷).

Raw data was imported from the instrument through a standard integration between the Sierra SPR®-32† and Screener (*Figure* ❷). Responding to input from our team, Bruker and Screener also expanded the integration to include automatic import of ligand information and immobilization levels. These important parameters were used to calculate key results and incorporated into visualizations later in the workflow.



❺ ***Selecting Hits in the Compound Table.*** *In the Compound Table view of Screener, scientists can view calculated results for each compound or sample. In the screenshot, compounds with a theoretical Rmax, %Rmax, and slow dissociation ratio within the desired ranges have been automatically flagged in green, and compounds for which all three criteria are fulfilled are annotated as hits. Here, results for Compound-0019's interaction with both the wildtype target and its mutant forms have been selected in blue. Compound-0019 has been* Ⓐ *annotated as a hit that should be followed-up in validation screening.* Ⓑ-Ⓒ *Processed sensorgrams and* Ⓓ-Ⓔ *solvent calibration plots (provided as a customization for Novartis) are both shown as thumbnails within table or as more detailed, interactive plots.*

Using templates tailored to the Novartis team's analysis needs, Screener automatically processed (*Figure* ❸) and quantified raw traces, such that key numerical results and plots were instantly viewable. For the primary screen, Screener computed the following results across the entire dataset:

- **Theoretical Rmax**, or the theoretical maximum possible signal generated by interaction between the target and compound. It is calculated from imported information as follows: *(Molecular Weight of Analyte/Ligand Molecular Weight) × Ligand Immobilization Level (RUs) × Theoretical Stoichiometry*
- **%Rmax**, calculated as *(Rmax/Theoretical Rmax) × 100*
- **Slow Dissociation Ratio**, calculated as *Early Dissociation/ Late Binding*, where Early Dissociation and Late Binding are the median values of selected early dissociation and late binding phases, respectively.

In subsequent validation screens, Novartis scientists made use of automated sensorgram fitting with available steady-state and 1:1 global fit models, as well as dose-response curve fitting. Finally, beyond its built-in capabilities, Screener provided the flexibility to implement the Novartis group's own normalization methods, analysis calculations, visualizations, and automation through open APIs.

Scientists performed quality control in the Well Table (*Figure* ❹) view in Screener. Here, summary visualizations made it easy to get a high-level overview of the dataset and perform quality control. Visualizations and tables are interactive and dynamically linked. For example, scientists could select a given compound to display fitted raw sensorgrams alongside the results. Poor-quality data could be flagged or excluded by directly clicking on the plots.

Hit selection occurred in the Compound Table (*Figure* ❺),

❻ ***Flagging Inconclusive Results.*** *In contrast to Compound-0019, Compound-0708 (selected in blue) is flagged as "inconclusive" because while its theoretical Rmax is within the desired range, %Rmax is over 50-fold higher than expected, suggesting superstoichio-metric binding.*

❼ ***Custom Hit-Calling Plot.*** *Genedata also developed a custom hit-calling plot to help Novartis scientists more easily visualize compound activity in relation to theoretical Rmax.*

where compounds can be sorted and filtered based on numerical results. Specifically, scientists could quickly select hits within a desired range of activity or %Rmax, while excluding those compounds with slow dissociation, indicative of possible aggregation or otherwise unwantedly strong binding to the target. Scientists further flagged compounds for which the %Rmax far exceeds the theoretical Rmax, indicating superstoichiometric binding (Figure 6). Genedata also worked with Novartis to provide a custom hit-calling plot to enable further visualization of %Rmax in relation to theoretical Rmax. All compounds were automatically annotated as "hit," "not hit," or "inconclusive" and tagged with a more detailed explanation.

A key benefit of bringing all SPR analysis at Novartis into Screener was that it allowed us to upload all results, sensorgram images, plots, and annotations more efficiently and seamlessly into the corporate data warehouse. The improved reporting workflow ensures future availability of this information as compounds are progressed to downstream confirmation assays and medicinal chemistry teams.

Conclusions

With the above solution, Novartis was able to perform each full screen within one week and cut down analysis times from 5 days to 2.5 hours. The combination of Genedata Screener and the Sierra SPR®-32† afforded us the speed, scale, and sophistication of automated screening, analysis and multiplexed experimental design with SPR. Crucially, unifying all SPR analysis into a single software streamlined our reporting workflow, improving efficiency, facilitating data transparency and accessibility, and positioning us to apply AI-based methods to data collected across multiple campaigns

Underlining the power of the approach enabled here, Novartis recovered hits which were later confirmed undetectable in a biochemical assay, yet detectable by NMR. Moreover, we were able to eliminate hits with undesirable binding profiles early on, addressing particular challenges associated with this target. Overall, through this collaboration with Bruker and Genedata, Novartis has elevated the capabilities of SPR screening and demonstrated the value of high throughput biophysical approaches in revealing new chemical starting points for high-potential but difficult targets. We now look forward to expanding this strategy to discover exciting new therapeutics.

† The Sierra SPR®-32 has already been discontinued and replaced by Bruker´s Sierra SPR®-32 Pro. The Sierra SPR®-32 Pro offers the same automation capabilities at an even higher throughput level.

References

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GENEDATA SOLUTION

SCREENER

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