# AlphaLISA® Technology and the SciStream™ Add-on for TIBCO Spotfire® Provide a Quick Analysis of Various Analytical Assays

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## Introduction

Immunoassays are commonly used in the development and characterization of therapeutic proteins, vaccines, and monoclonal antibodies (TAbs). Bioprocess groups developing lot release QC assays are subject to strict regulations, so there is a need for novel techniques that speed drug development, while still meeting regulatory demands.

An increasing need for robust, efficient, and sensitive assays to detect and characterize these biologics during various stages of development has led many researchers to AlphaLISA assays. PerkinElmer EnVision® multimode plate reader AlphaLISA assay data was analyzed and batch-imported with SciStream, an add-on for TIBCO Spotfire. SciStream is a generic data loader to import plate-based data from most commercially available instruments.

By directly importing raw data from multiple plates, readouts/channels, time points, as well as all the relevant metadata, it is possible to analyze and compare multi-parametric data from multiple assays and experiments. Plate layout properties can be visually represented in a plate map in TIBCO Spotfire, enabling scientists to interact with their data, and perform curve fit modeling. We present how AlphaLISA technology and the SciStream add-on for TIBCO Spotfire provide a versatile workflow of various analytical assays in the modern biotherapeutic development laboratory.
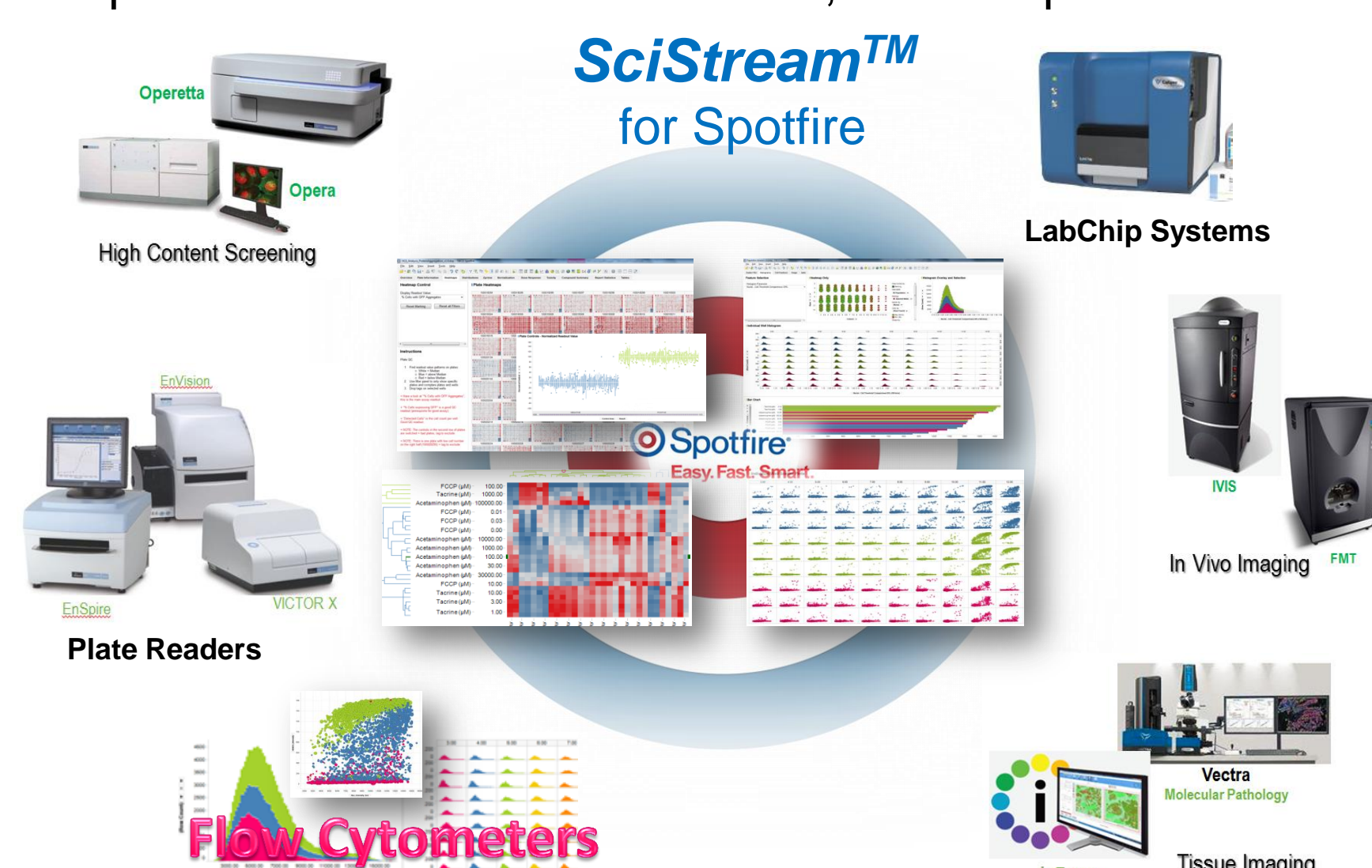
## Solution

### CHALLENGE

Many scientists face a challenge when analyzing plate-based assays. When high through-put screening experiments are performed, the data is most often exported in block or columnar files, and this proves as a challenge when analyzing the data.

### SOLUTION

SciStream is an add-on to TIBCO Spotfire which enables scientists using plate readers to transform their data from block, block columnar, and columnar data file formats to:

- Single readout value for each well
- Multiple readout values for each well
- Multiple readout values, time points, and plates in a single file
- Support for both well level and cell level data import into Spotfire



## Experimental Protocol & Data Analysis Workflow

Prepare stock solutions of all required reagents (analyte, AlphaLISA acceptor beads, biotinylated antibody, AlphaLISA donor beads).



JANUS® automated workstation combines 5 µL of analyte with 10 µL of AlphaLISA anti-analyte acceptor beads and 10 µL of biotinylated anti-analyte antibody.

JANUS® automated workstation adds 25 µL streptavidin AlphaLISA donor beads to each well in a single step.
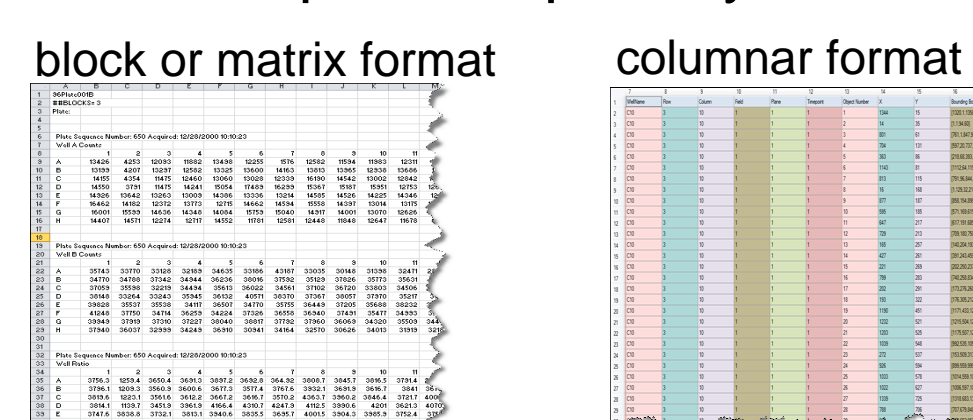
Remove completed plate and read using EnVision or EnSpire® multimode plate readers.

Import data using SciStream for TIBCO Spotfire.

Batch imports entire screening data in one step into TIBCO Spotfire.

- Batch import multiple plate data files into a single file
- Attach or create plate map or layout information

block or matrix format    columnar format

Preview data according to templates and define plate layout.

Combine complex data from multiple assay types for instant insight. Correlate data from high throughput screening (HTS), high content analysis, in vitro and in vivo imaging campaigns into a single analysis to visualize hits, leads and responses to achieve robust, sharable and interactive results.

## Results & Reports

**Alpha Data**

| Well name | Well number | row | column | Meas /Result |
|---|---|---|---|---|
| A01 | 1 | 1 | 1 | 214906 |
| A02 | 2 | 1 | 2 | 216501 |
| A03 | 3 | 1 | 3 | 206381 |
| A04 | 4 | 1 | 4 | 15870 |
| A05 | 5 | 1 | 5 | 15630 |
| A06 | 6 | 1 | 6 | 15400 |
| A07 | 7 | 1 | 7 | 17780 |
| A08 | 8 | 1 | 8 | 18500 |
| A09 | 9 | 1 | 9 | 19170 |
| A10 | 10 | 1 | 10 | 160080 |
| A11 | 11 | 1 | 11 | 157700 |
| A12 | 12 | 1 | 12 | 157036 |
| B01 | 13 | 2 | 1 | 206733 |
| B02 | 14 | 2 | 2 | 205172 |
| B03 | 15 | 2 | 3 | 204562 |
| B04 | 16 | 2 | 4 | 157036 |
| B05 | 17 | 2 | 5 | 987 |
| B06 | 18 | 2 | 6 | 900 |
| B07 | 19 | 2 | 7 | 182100 |
| B08 | 20 | 2 | 8 | 192000 |
| B09 | 21 | 2 | 9 | 193100 |
| B10 | 22 | 2 | 10 | 160060 |
| B11 | 23 | 2 | 11 | 67255 |
| B12 | 24 | 2 | 12 | 85870 |
| C01 | 25 | 3 | 1 | 160060 |
| C02 | 26 | 3 | 2 | 157709 |
| C03 | 27 | 3 | 3 | 157036 |
| C04 | 28 | 3 | 4 | 903 |
| C05 | 29 | 3 | 5 | 823 |
| C06 | 30 | 3 | 6 | 909 |
| C07 | 31 | 3 | 7 | 2905 |

### DATA IMPORT

With SciStream we can import plate-based and other forms of instrument data into TIBCO Spotfire, thus taking advantage of the power of visualizations and drill-down capabilities. Users of plate readers, high content imaging systems and other instruments, can easily and dynamically visualize their experimental data, metadata and plate layouts.

Screening analysis can include evaluation of plate quality control, assay performance, dose response curve fit. toxic side effects, compound classification

### PLATE LAYOUTS AND HEATMAPS

Heatmaps can be used to display the plates in the screen. This is a convenient way of getting a bird's eye view of screening results and identify potential plate effects, trends, as well as assess the overall quality of the screen.
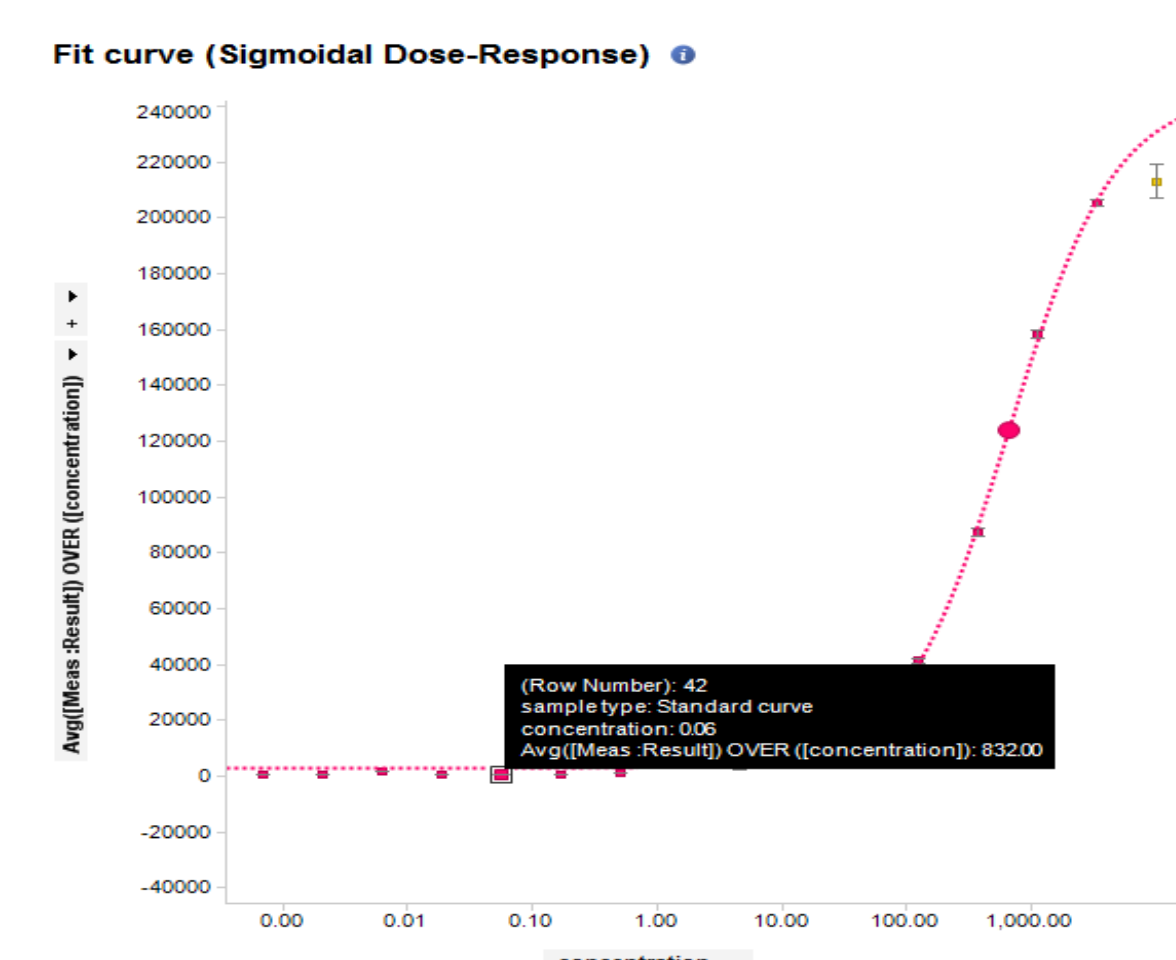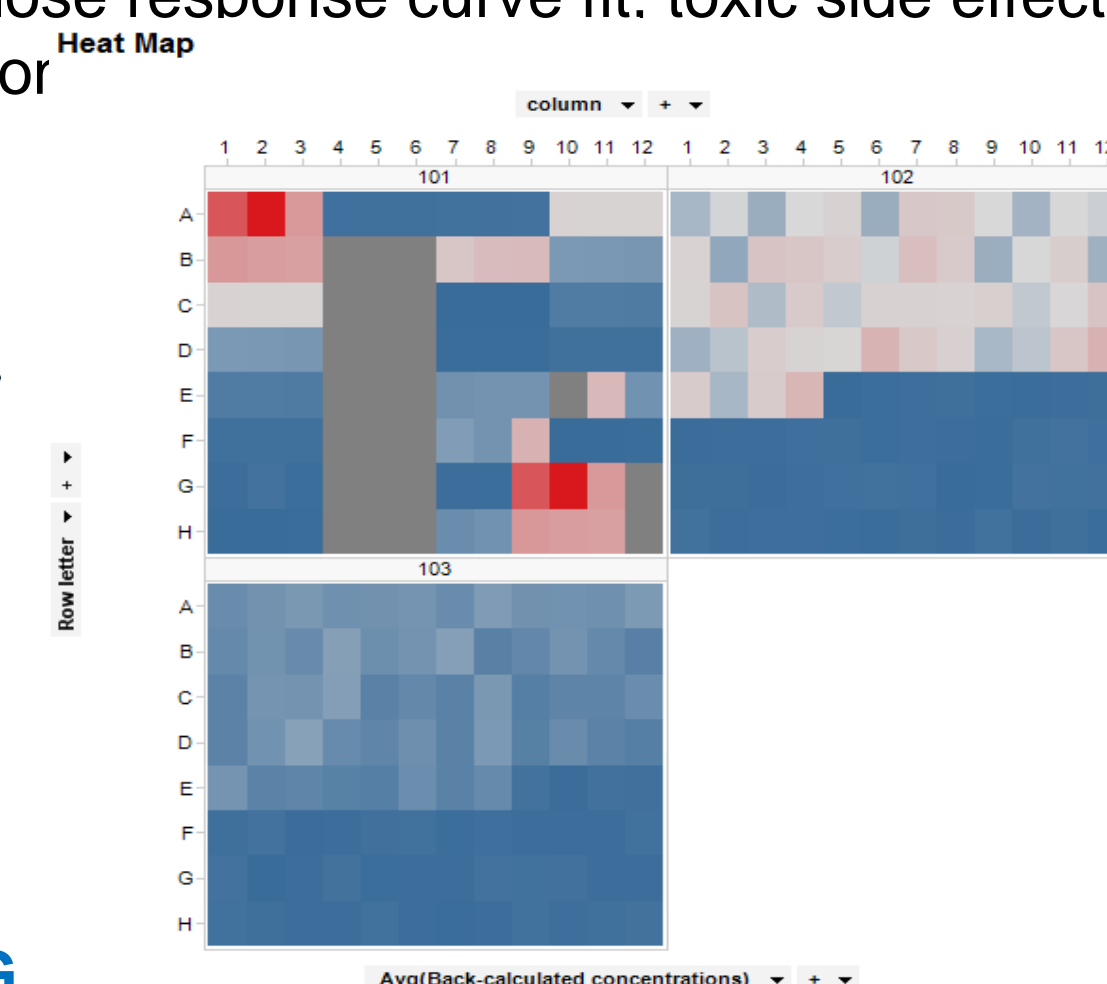


### CURVE FITTING

To characterize a compound or entity of interest, titration or dose response experiments are commonly performed after an initial screen on few selected hits.

Using the plate layout, it is possible to identify wells where the responses are very low or high at different concentrations, as well as to highlight and select the wells to include in dose response curves.

### COMPOUND EVALUATION

The use of HCS/HTS techniques allow scientists to perform chemical, genetic or pharmacological tests in a large scale.

Through this process one can rapidly identify active compounds, antibodies or genes which modulate a particular biomolecular pathway.
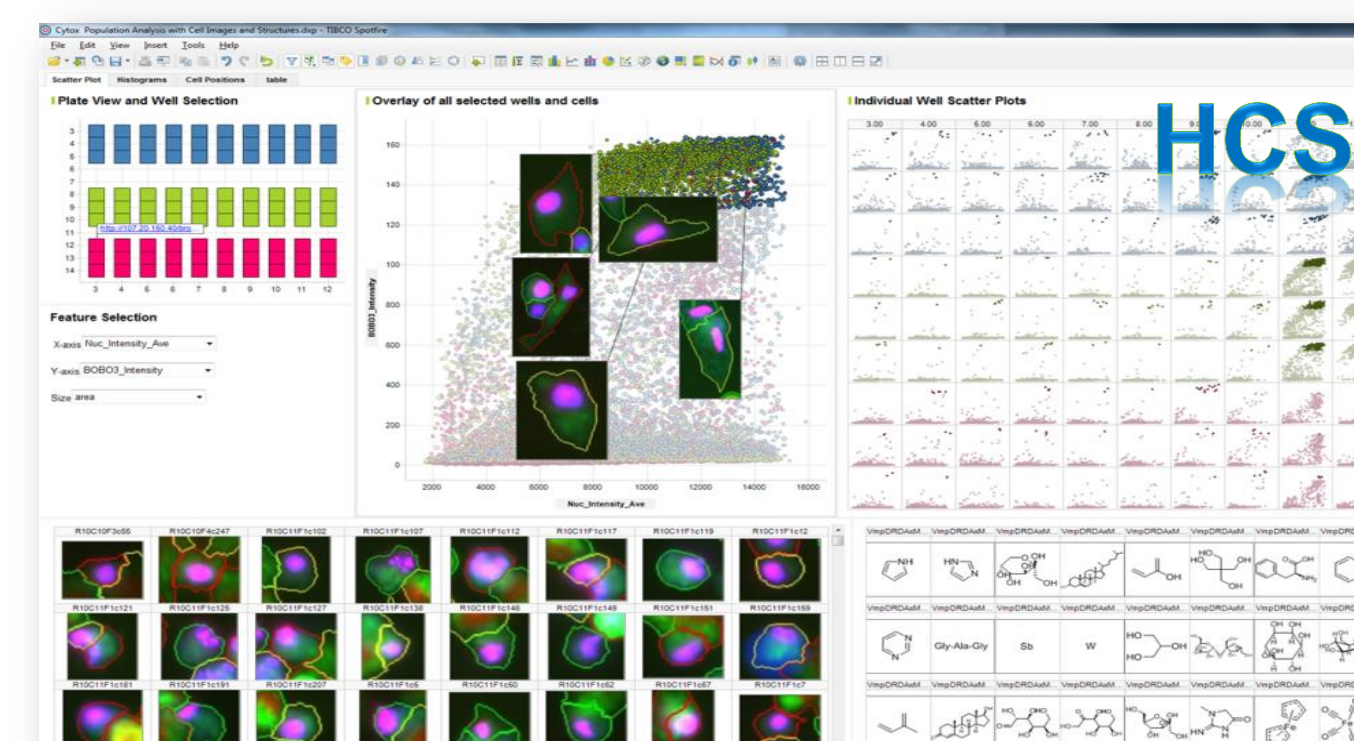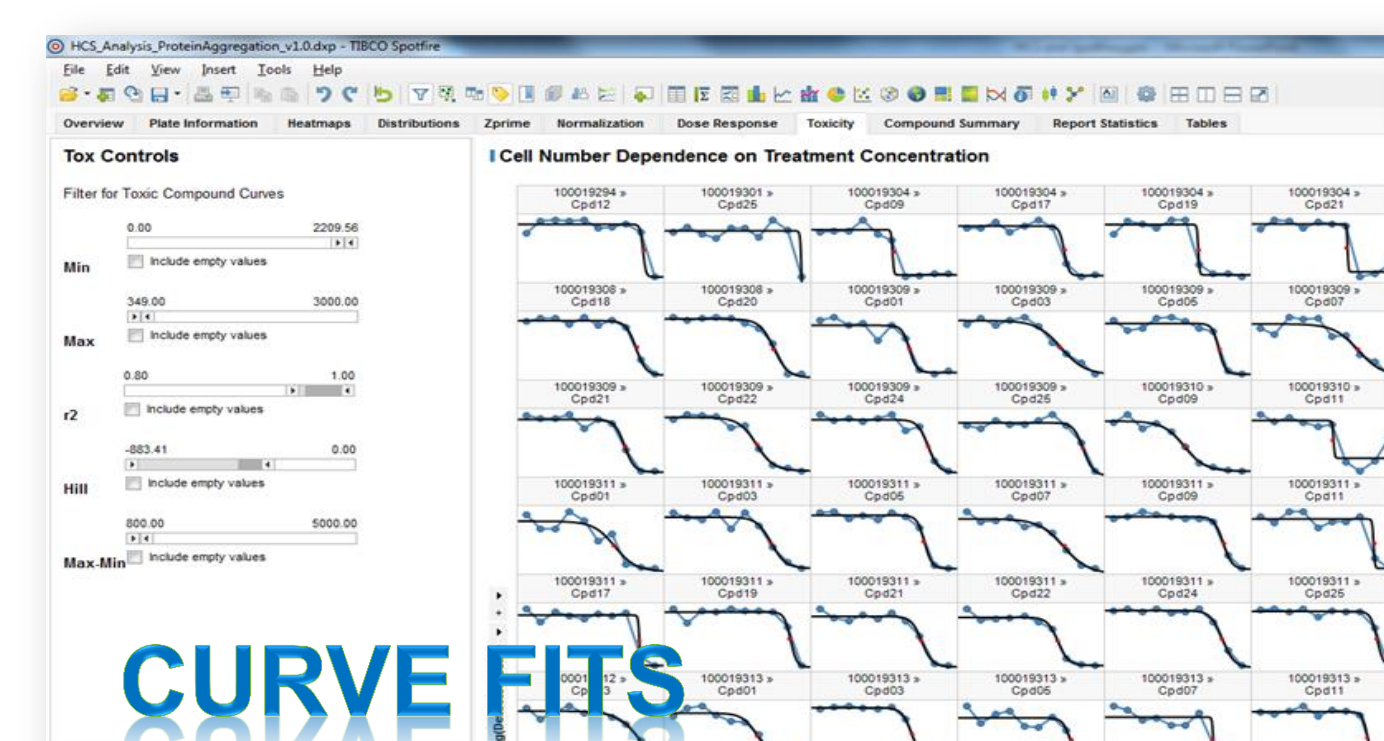
The results of these experiments provide starting points for drug design and for understanding the interaction or role of a particular biochemical process in biology.
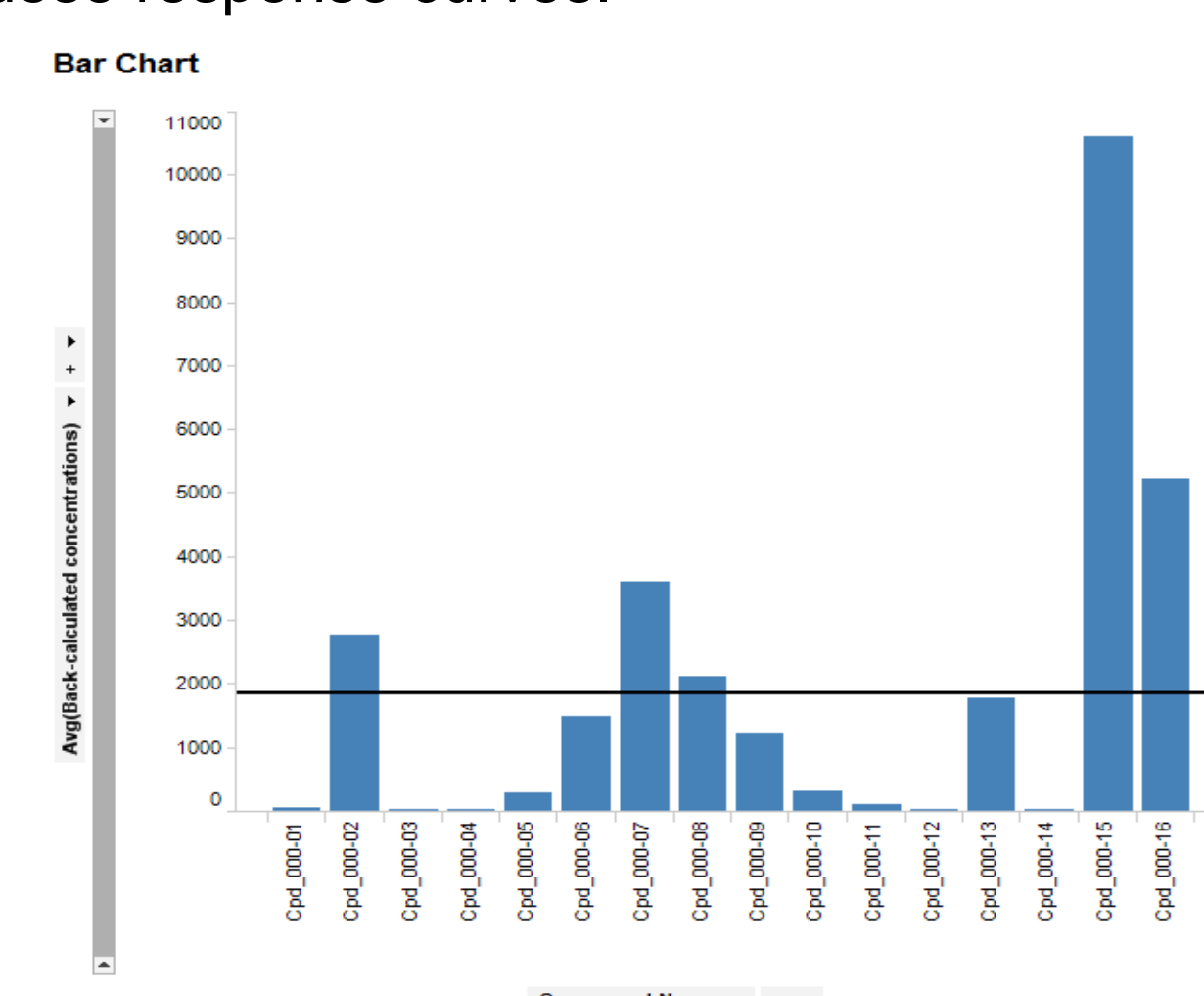
## Conclusion

SciStream enables I the mport, aggregation, and arrangement of raw instrument data and metadata files in TIBCO Spotfire software. This allows scientists to quickly and easily import various text-based instrument data files in batch including files from plate readers and high content screening instruments along with their corresponding metadata and experimental information or plate layout. SciStream accommodates a variety of data and file formats in high volume to benefit scientists in accelerating time to insight during assay development as well as large screening campaigns.

Faster visualization and analysis in TIBCO Spotfire software leads to scientific insights much more quickly than using traditional tools for assay development as well as large screening campaigns.



LABEL-FREE KINETICS    CURVE FITS

HCS    CORRELATIONS