SPR screening at CRL: Drug Discovery Applications and Data Processing

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SLAS 2016, SAN DIEGO
01/2016
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Overview

Biacore™ surface plasmon resonance (SPR) instruments are used in the drug discovery sector for studying the affinity and kinetics of protein:analyte interactions. At CRL, these optical biophysical screening tools are routinely use for hit identification (fragment screening), orthogonal hit confirmation and hit-to-lead activities.
Complex data analysis is required to extract full value from an SPR dataset; this process can be resource intensive and time consuming which can be prohibitive when using the technology to generate larger volumes of data. To address this, we have developed customized ActivityBase™ protocols to support our data analysis/storage requirements for the Biacore™ 4000 platform. Using bespoke Excel templates as the interface between the Biacore™ raw data output and a refined ActivityBase™ data set, the raw data can be efficiently processed and formatted into an output suitable for data interpretation.

Introduction

The Biacore™ 4000 platform is designed for large-scale, parallel interaction analyses, such as fragment screens or orthogonal screens to facilitate hit confirmation post-HTS.
This poster details the Biacore™ 4000 data analysis workflows configured at CRL using example data from an orthogonal hit confirmation screen.

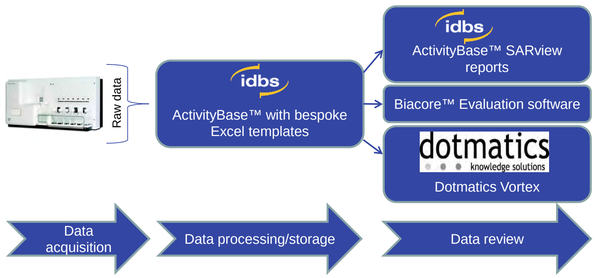


Figure 1: Biacore™ 4000 analysis workflow. Excel templates process Biacore™ output files from binding level and affinity screens.

Methods

Data processing

- Biacore™ RPT file (reference flow cell-subtracted, solvent-corrected data) used for ActivityBase™ analysis
- ActivityBase™ Excel template processes data: analyte MW adjustment, blank subtraction (“double referencing”), surface activity adjustment and numerical binding behaviour assessment (slope, adherence, stoichiometry)

(A)

Processing step	Calculation
Analyte MW adjustment *“normalized response”*	((binding late response – avg blank response) / compound MW))* 250
Surface activity adjustment “normalized scaled response”	normalized response * ((nominal ref. response / (avg Calibrator response – avg blank response))
Stoichiometry measure	((binding late response – avg blank response) / (TRmax – avg blank response)* 100
Slope measure	100-(((binding early response – avg blank response) / (binding late response – avg blank response))*100)
Adherence measure	((stability late response – avg blank response) / (binding late response – avg blank response))*100

(B)

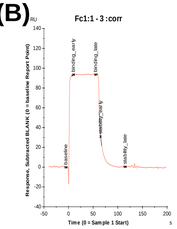


Figure 2: ActivityBase™ data processing.
(A) Excel template binding calculations. Average Calibrator/Blank responses taken from controls flanking compound data.
(B) Example sensorgram showing binding level report points contained in Biacore™ RPT file.

Results

Orthogonal SPR hit confirmation case study

A biochemical fluorescence polarization (FP) HTS was undertaken to identify compounds able to inhibit a protein-peptide interaction; with subsequent confirmation for target interaction using SPR.

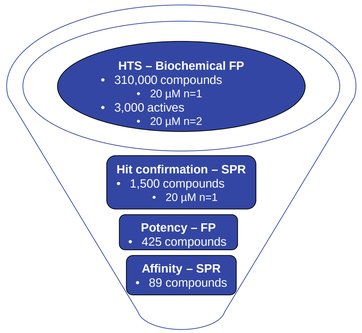
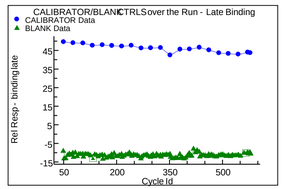


Figure 3: Biacore™ 4000 platform used to validate HTS hits. FP hits were triaged through binding level and affinity screens to confirm target engagement. Binding affinity, specificity and stoichiometry results were used to validate and prioritise HTS hits for active-to-hit progression.

Results (cont.)

(A)

Late Binding	Average	Median	St. Dev.	%CV
CALIBRATOR	46.054	46.283	2.121	4.6
BLANK	-11.063	-11.040	0.961	-8.7
Signal: Background	4.2			
Z' Factor	0.84			



(B)

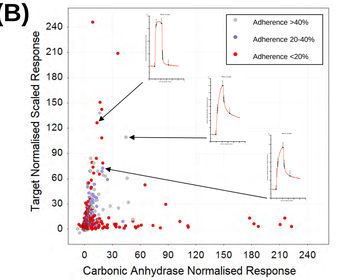


Figure 4: Biacore™ 4000 hit confirmation screen.
(A) Representative Calibrator/Blank performance statistics and associated binding level plot for target surface.
(B) ActivityBase™ processed compound binding level data visualized in Vortex for target and specificity (Carbonic anhydrase) proteins. Colour coded to reflect target protein adherence score; representative sensorgram plots inset to validate accuracy of the adherence score.

(A) **(B)**

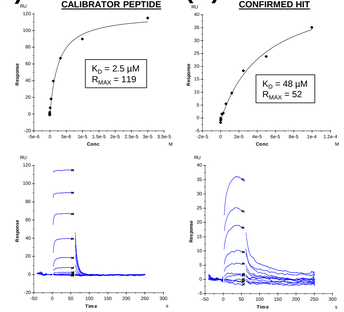


Figure 5: Biacore™ 4000 affinity screen. Representative steady state fit and associated sensorgrams for peptide calibrator control **(A)** and a representative confirmed hit **(B)** binding to target protein.

From the hits progressed to affinity determination 50 compounds with a measurable K_D & reasonable stoichiometry were prioritized. Several chemotypes were contained within these hits.

Conclusions

- **Biacore™ 4000 is a proven high-throughput SPR platform for both fragment and orthogonal screening at CRL**
- **Biacore™ 4000 used in combination with customized ActivityBase™ protocols enables efficient and traceable processing of large screening data sets**