School of Medicine

FACULTY OF MEDICINE AND HEALTH

UNIVERSITY OF LEEDS

Surface Plasmon Resonance: SensiQ Pioneer as a means to screen drug discovery fragments for hits

C. Wishart[1], L. Hardy[1], E. Hethershaw[1], A. Cilia La Corte[1], R. Ariens[1], C. Revill[2], R. Taylor[2], C. Fishwick[2], R. Foster[2], H. Philippou[1]

Background

Common approaches to the identification of hits from small molecules or fragments are via high-throughput screening (HTS) assays and/or surface plasmon resonance (SPR). The aims of this study were to validate the hits from a high-throughput (HT) SPR assay with a well-characterised HTS enzymatic assay against an undisclosed target.

Method

HTS Enzymatic Assay

The undisclosed enzyme target was incubated with 500µM fragment‡ in TBST* at 37°C for 10 minutes in a 96-well flat-bottom plate. Hits were screened in duplicate (48 fragments maximum per run at a single concentration). Chromogenic substrate was added and absorbance read at 405nm using a microtitre plate reader at 12 second intervals for 3 hours. The V_0 was calculated using the slope from the first 14 points in Excel. Positive hits were defined as having a V_0 of 90% or less of the vehicle alone, indicating enzyme inhibition. Graphs below were created with GraphPad 6.0.

* 50mM Tris, 137mM NaCl, 0.05% Triton-X-100, pH 7.4.

** 10mM HEPES, 140mM NaCl, 1.5mM $CaCl_2$, 5% DMSO, pH 7.4.

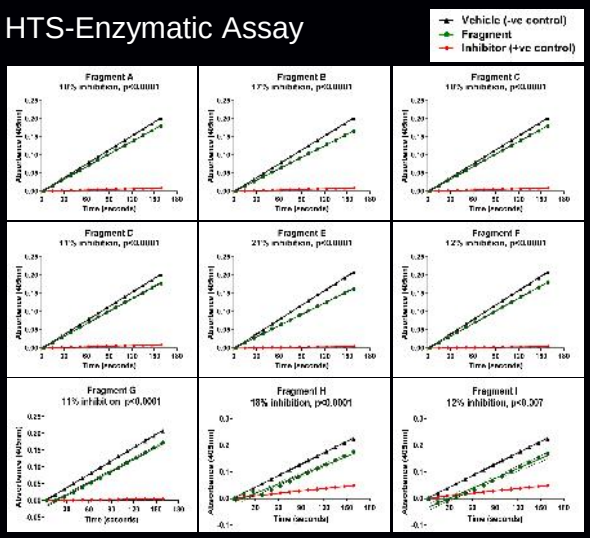
‡ All fragments were purchased from Domainex.

High-throughput SPR Assay

Two undisclosed enzyme targets were immobilised to the surface of a COOH-5 chip to an R_{MAX} of 50RU using the EDC/NHS coupling technique. The OneStep® gradient injection type at 50% loop volume was used to analyse 192 fragment‡ analytes from a 384-well plate against the two target ligands in a single run. The instrument automatically generated a continuous concentration gradient of each fragment in running buffer** at a flow-rate of 125µL/min, and a 20 second dissociation time. Therefore, only one binding curve of each fragment was necessary to calculate the one binding site Ka/Kd and the equilibrium dissociation constant, following the export of data to Qdat, the software employed for data analyses. The data below were created with Qdat.

Results

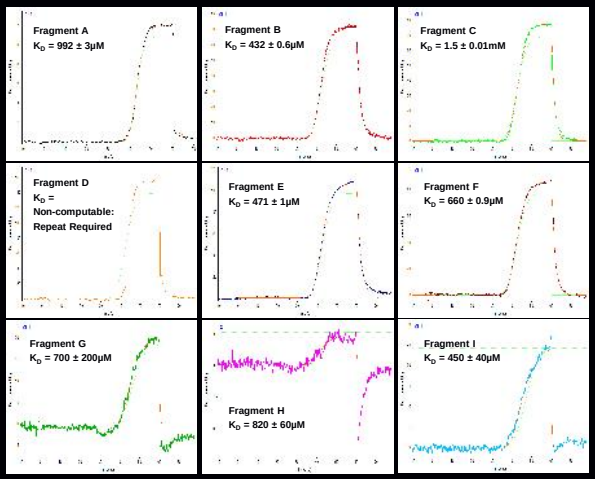
HTS-Enzymatic Assay



Graphical representation of V_0 of fragment hits.

Fragments were screened in duplicate alongside a DMSO vehicle (black) and a well-characterised enzyme inhibitor (red). Data were analysed in Excel and graphs made in GraphPad 6.0.

High-throughput SPR Assay



HT-SPR binding profiles of the fragment hits.

Data were analysed in Qdat: the binding profile during the titration of fragment against the target is shown as the coloured line, the 1 site binding model is illustrated as a smooth red line.

Conclusions

Using HT-SPR, 598 fragments were screened using 3 plates against 2 target ligands simultaneously, which identified not only hits but the K_D of those hits as the instrument titrates the analyte over 3-4 orders of magnitude in concentration within minutes; whereas the HTS-Enzyme Assay (HTS-EzA) required 14 plates to give **only the hits** at one concentration of fragment. It should be noted that the binding profiles of fragments A and D fell outside of the DMSO micro-calibration range, thus although these fragments are clearly hits, the SPR needs repeating to give an accurate K_D. The 9 hits identified by HTS-EzA were also identified by HT-SPR, with an additional 60 hits identified by HT-SPR giving a total of 69 hits out of 598 fragment; an 11.5% hit rate. It is likely the additional 59 hits given by HT-SPR were not identified by the HTS-EzA because those fragments did not bind at the active site. There was an additional fragment identified by HTS-EzA as having an 18% inhibitory effect, which was not identified by HT-SPR, giving a false negative hit rate of 0.0017%.The volume of fragment used is comparable between the methods used, but the HTS-Enzyme Assay has an additional on-going cost of substrate and the fragment hits require further analysis to yield Ki, thus more sample is required. We conclude that SPR using the SensiQ Pioneer instrument is a time and cost-effective method for fragment/compound screening.

1. Division of Cardiovascular and Diabetes Research, Faculty of Medicine and Health, School of Medicine, Leeds Institute of Cardiovascular and Metabolic Medicine (LICAMM), University of Leeds, Leeds, LS2 9JT.
2. School of Chemistry, University of Leeds, Leeds, LS2 9JT
All correspondence should be directed to Dr. Helen Philippou: Tel: 0113 343 7768; Fax: 0113 343 7738; Email: H.Philippou@leeds.ac.uk