



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

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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 500 μ M 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 CaCl₂, 5% DMSO, pH 7.4.

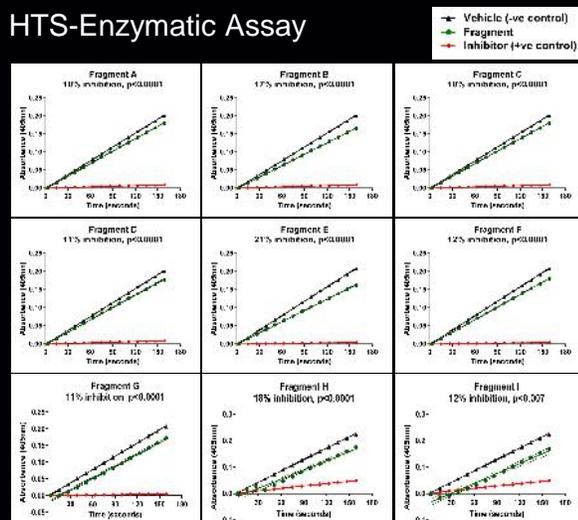
All fragments were purchased from Domainex.

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 125 μ L/min, and a 20 second dissociation time. Therefore, only one binding curve of each fragment was necessary to calculate the one binding site K_a/K_d 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

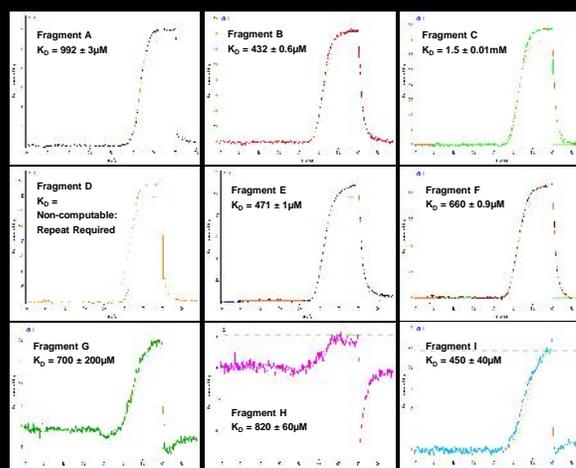
HTS-Enzymatic Assay



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 K_i , 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.

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