Using SPR to drive integrated screening projects

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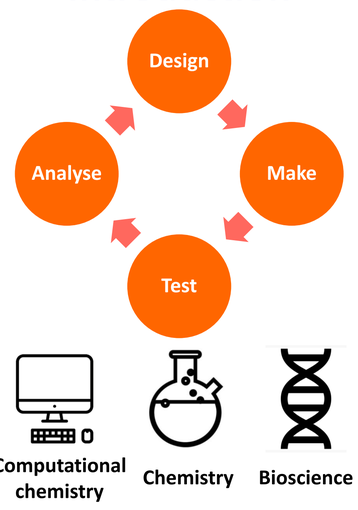
Introduction



An existing client was interested in an additional, integrated project with Charnwood Molecular to learn more about their lead compound. In collaboration with a third-party external **computational chemist** our chemistry department arranged a **virtual screen** of tens of thousands of compounds against their lead compound's protein target. From the list of docking hits a set of ~ 100 compounds was identified that would help confirm our ideas about the binding mode of the lead compound and improve our confidence in the model that had been generated for future docking work. This set was purchased and brought in-house.

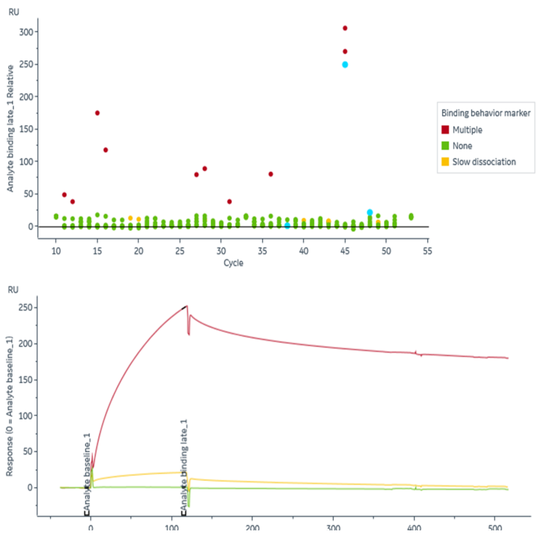
Separately, a team of **Charnwood chemists** were tasked with synthesising a set of analogues that shared the same **difficult core** as the lead compound. They wanted to see whether an easier to synthesise core could be found that retained activity and avoided infringing on existing IP-space.

The joint set of computational and synthetic chemistry compounds were passed to our **Bioscience** colleagues who had **transferred and developed several different assays** to assess the binding and activity of the lead compound against the protein target and several related isoforms.

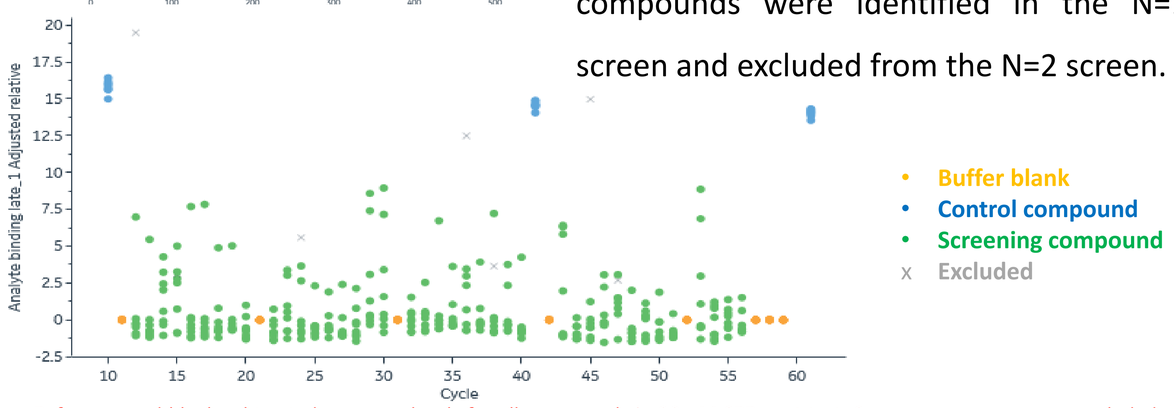
Primary Spot-Test Screen

At the start of the project we developed *de novo* SPR assays for the client's principal protein target and several related proteins to check for specificity of binding. All four different SPR assays were validated with tool compound affinity measurements.

We screened all compounds using **SPR** in a **spot-test format (20 µM, n=3, N=2)**, excluding poorly-behaved PAINS-like compound results from analysis.

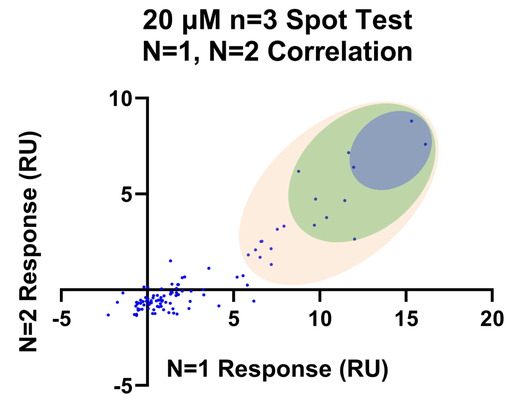


The Insight software highlights sensorgrams that it thinks show sub-optimal behaviour (slow dissociation, superstoichiometric binding, etc.). As we ran every compound in triplicate we could detect whether flagged sensorgrams indicated an unsuitable compound or a one-off artefact. A number of troublesome compounds were identified in the N=1 screen and excluded from the N=2 screen.



Reference and blank-subtracted response levels for all compounds in 20 µM SPR spot test. Some sensorgrams were excluded after failing Insight Evaluation software's QC checks

Hit Threshold Definition

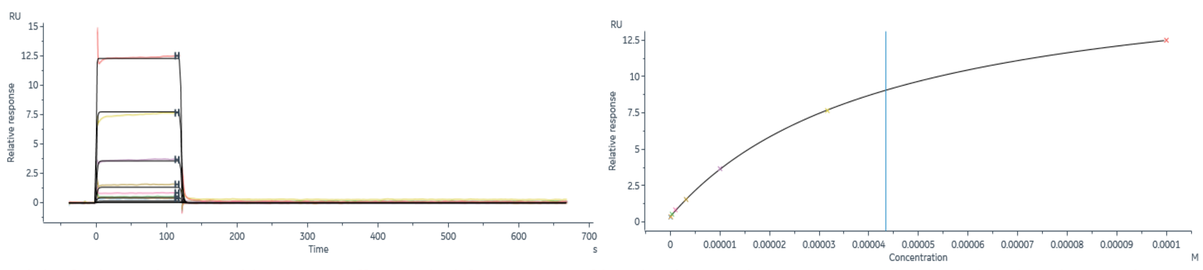


Correlation plot showing the response levels measured in the N=1 and N=2 SPR spot test screens.

Different levels of hit threshold definition – and which compounds cross each threshold - are shown as shaded ovals

For this project, the client requested that we define our hit threshold based on the **average response level (RU)** of all screening compounds (with control compound responses excluded). We measured the mean RU and the standard deviation of all responses and defined three hit thresholds – the mean RU plus 1, 2 or 3-fold the standard deviation. We used the least stringent threshold to **identify ~ 20 hit compounds** that we progressed to the dose-response, affinity-determination step.

Affinity Determination

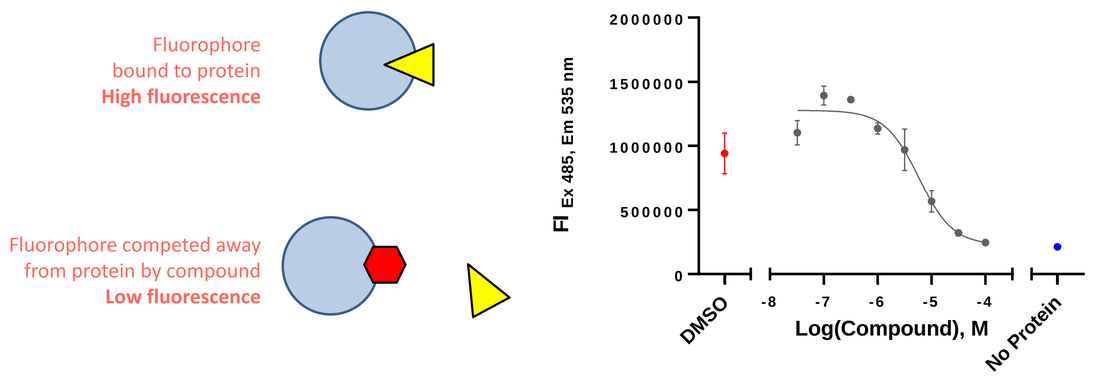


Whilst fairly weak, we could measure affinities (K_D) for majority of hits (such as in the sensorgrams/binding plot above). Some however were too weak to measure or appeared to be non-specific binders (not shown)

We used **SPR** to measure the **affinities** of the hit compounds and see how well spot-test response translated to affinity against our protein target. We included a non-hit compound as a negative control.

For the majority of the compounds we could measure **weak affinities (~ 50 µM)** – too weak for robust measurement of the kinetics however. Distinguishing between weak and non-specific binding was a challenge with these hits and for this protein target in particular.

Orthogonal Fluorophore-Displacement Assay



We always advise the use of an **orthogonal assay** to both confirm the results from the primary assay as well as provide additional insights into the functional effect of a compound. In this case we used a fluorophore-displacement assay to check whether the affinities of our hits measured by SPR translated to an IC_{50}. We see that our compounds do effectively compete with our protein-specific fluorophore to cause a reduction in fluorescence. We therefore have **high confidence** in the **specificity** and **activity** of our hits.

Summary

Our work here illustrates the power of SPR to quickly drive drug discovery projects forward and yield critical compound binding data. The ability to triage compounds based on their affinities to a protein target is key for understanding the SAR of a new series and identifying new chemical space to explore.

In this work we demonstrate several of our capabilities here at Charnwood Molecular:

- **SPR assay development** for difficult targets
- **Small molecule screening** in multiple different assay formats
- **Integrated drug discovery** between different disciplines and departments

Find out about our integrated approach to drug discovery and how we can help you achieve the best outcomes for your project.

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