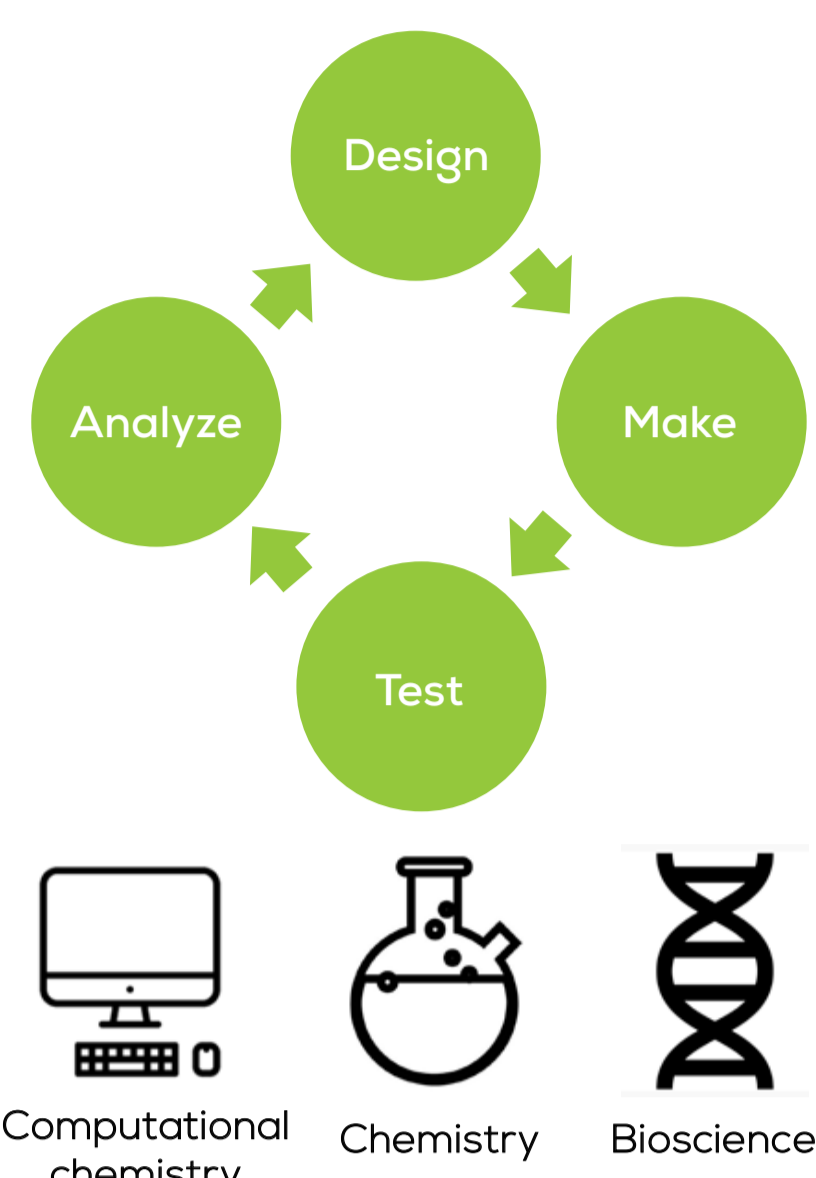


# Using SPR to Drive Integrated Screening Projects

Patrick McIntyre, Tom Miller, Alex Scollan, Phil Clarke, John King-Underwood, Craig Avery, James Hitchin, Kathy Dodgson and Gary Allenby  
Charnwood Discovery, Charnwood Campus, Summerpool Road, Loughborough LE11 5RD

## Introduction

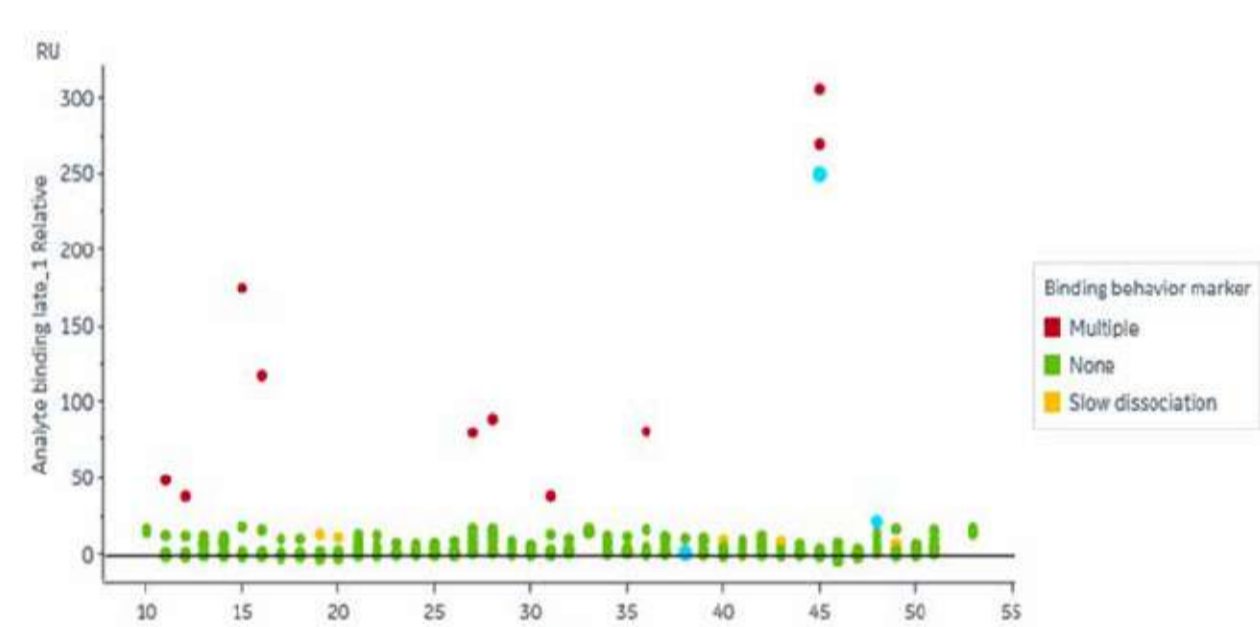


An existing client was interested in an additional, integrated project with Charnwood Discovery to learn more about their lead compound. In collaboration with a third-party external **computational chemist** our chemistry department arranged a **virtual screen** of tens of thousands of compounds against 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 Discovery chemists** were tasked with synthesising a set of analogues that shared the same **difficult core** as the lead compound. The client wanted to identify whether an easier to synthesize core could be found that retained activity and avoided infringing on existing IP-space.

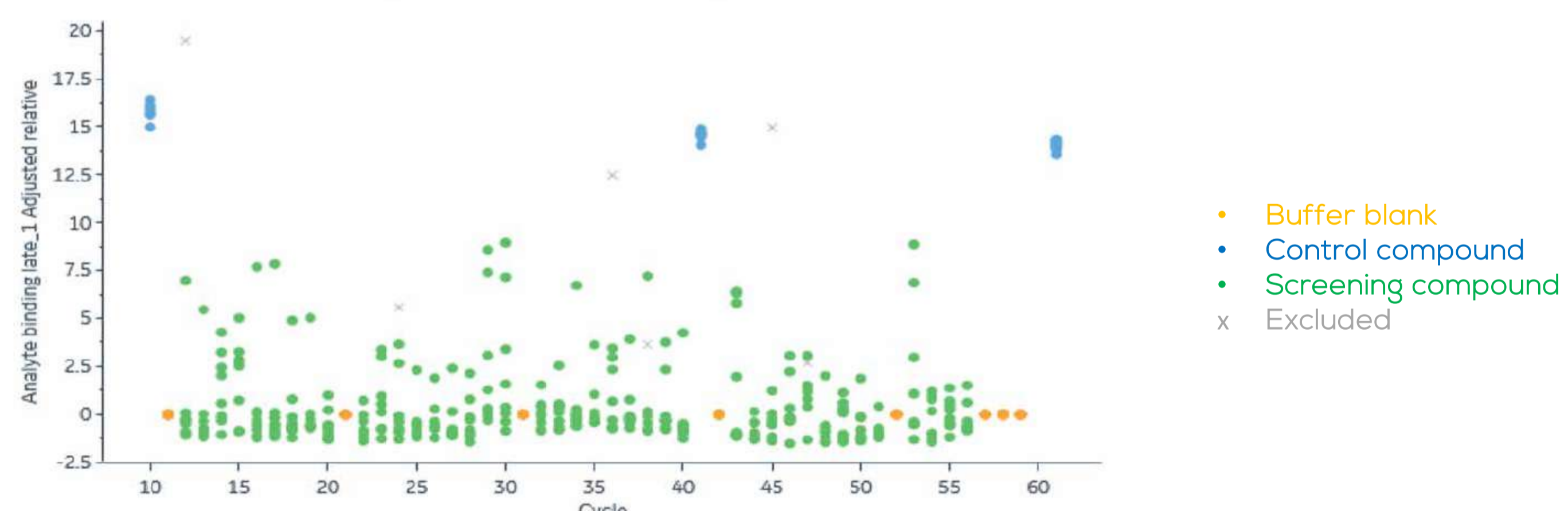
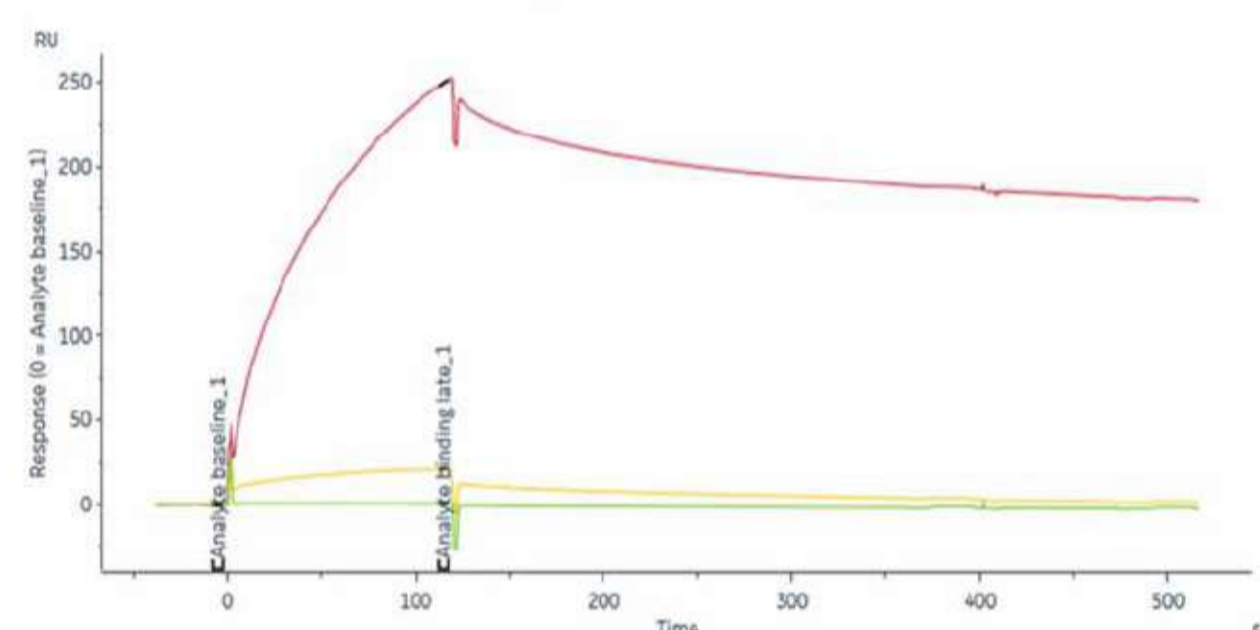
The joint set of computational and synthetic chemistry compounds were passed to our **bioscience** team, who had **transferred and developed several different assays**, to assess the binding and activity of the lead compound against the protein target and several related isoforms.

## 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  $\mu$ M, n=3, N=2), excluding poorly-behaved PAINS-like compound results from analysis.

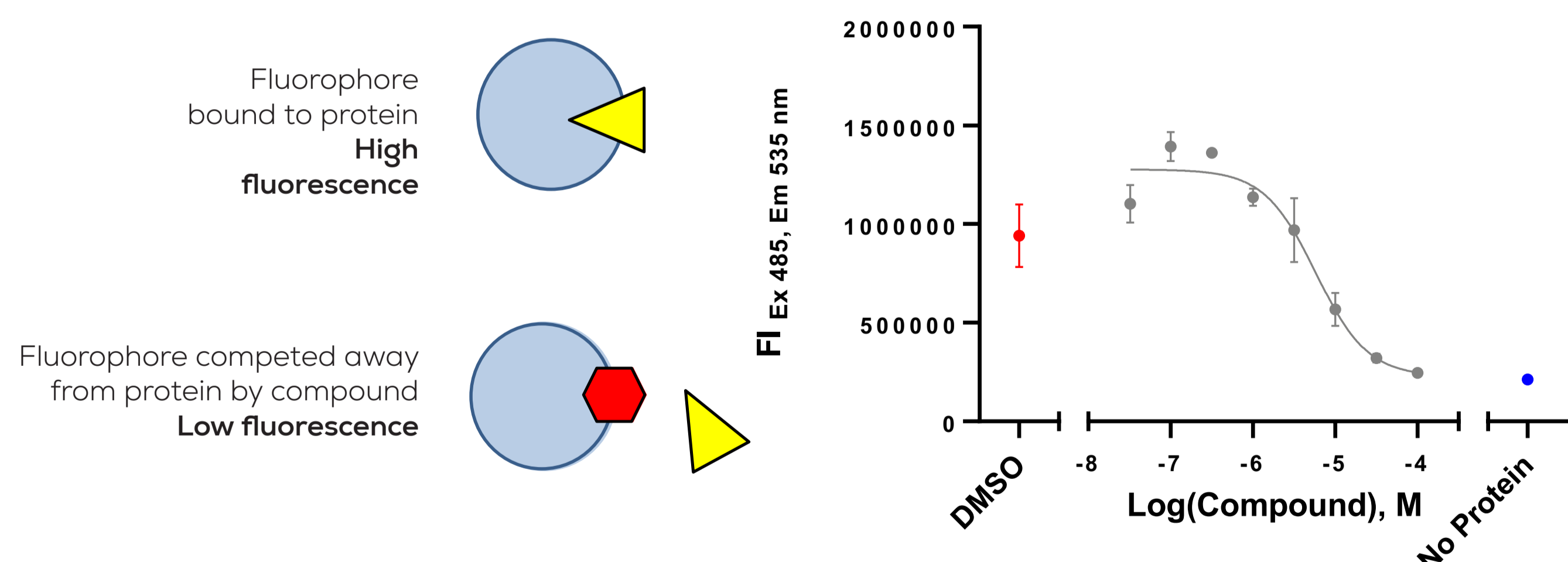


The Insight software highlights sensorgrams showing sub-optimal behaviour (slow dissociation, superstoichiometric binding, etc.). Screening every compound in triplicate we could detect whether flagged sensorgrams indicated an unsuitable compound or a one-off artefact. A number of troublesome compounds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



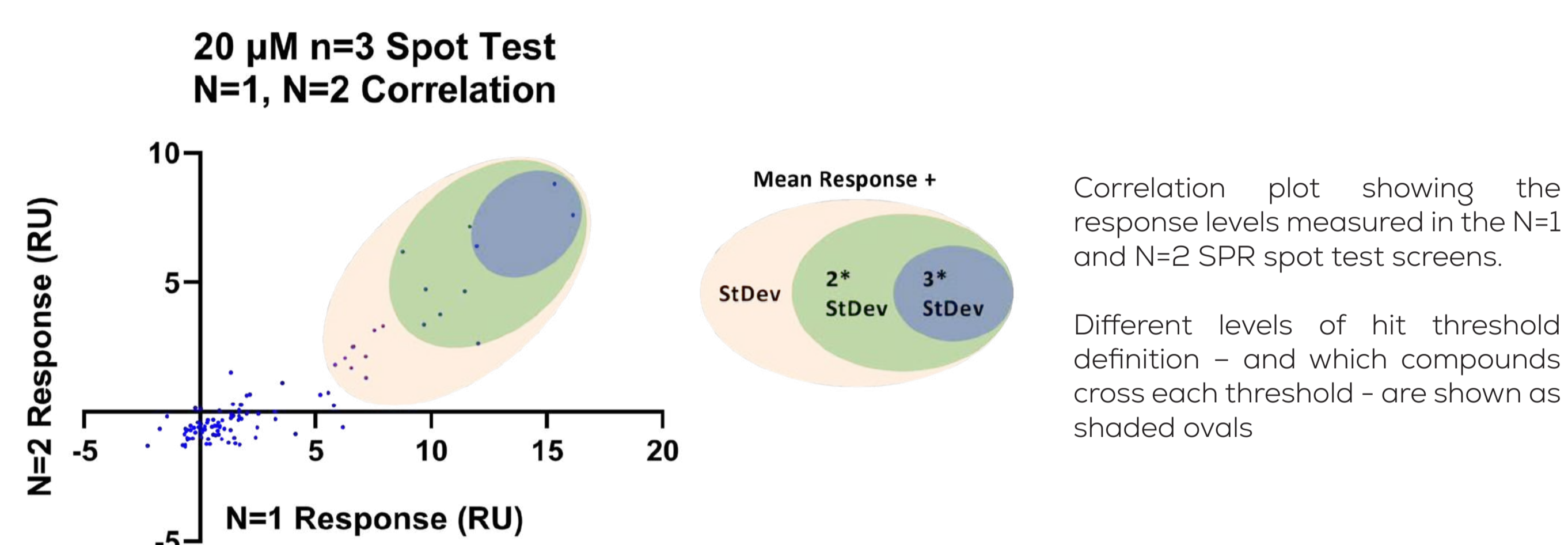
Reference and blank-subtracted response levels for all compounds in 20  $\mu$ M SPR spot test. Some sensorgrams were excluded after failing Insight Evaluation software's QC checks

## Orthogonal Fluorophore-Displacement Assay



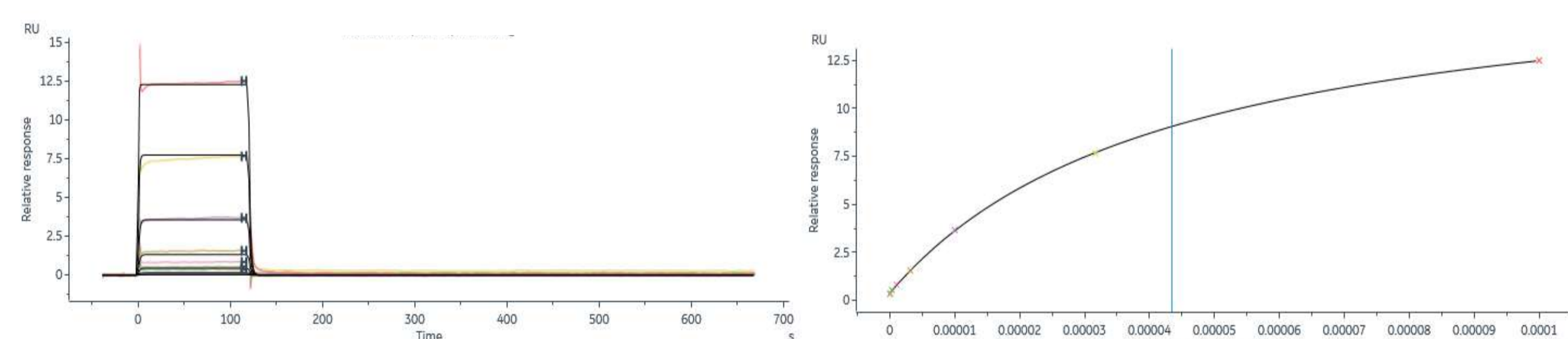
We always advise the use of an **orthogonal assay** to both confirm the results from the primary assay as well as provide additional insights into the functional effect of a compound. In this case we used a fluorophore-displacement assay to check whether the affinities of our hits measured by SPR translated 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.

## Hit Threshold Definition



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 (KD) 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  $\mu$ M), however these were too weak for robust measurement of the kinetics. Distinguishing between weak and non-specific binding was a challenge with these hits and for this protein target in particular.

## 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 Discovery:

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

