Advantages of MicroCal ITC for secondary screening in a FBDD campaign

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Introduction

Recent developments in ITC instrumentation, namely MicroCal™ PEAQ-ITC and MicroCal PEAQ-ITC Automated, have led to an increase in the throughput and decrease in the protein consumption of the technique. In addition, there have been recent methodological advancements1 that have extended the affinity range that ITC can measure into the mM range. The combination of all these factors has made the technique ideal for fragment based drug discovery campaigns (FBDD).

This work outlines the role of ITC instrumentation, in a fragment based drug discovery program of Sprint Bioscience, to identify and optimise potential drug candidates that will inhibit the activity of Vps34. This class 3 phosphatidylinositol 3-kinase is central to autophagy and has been shown to play an important role in resistance to cancer drugs 2,3. As such it has been identified as a target for therapeutic intervention.

ITC i a generic assay without the need for assay development and, as such, the affinity of 50 compounds was measured in less than three days after receiving the purified protein. This approach was fast and proved very successful for identifying fragments that co-crystallised with the target protein. Of the 14 compounds chosen, based on the ITC data, 12 formed crystals that could be used in the optimisation process.

Figure 1. MicroCal PEAQ-ITC Automated

THE FBDD APROACH USED BY SPRINT BIOSCIENCE

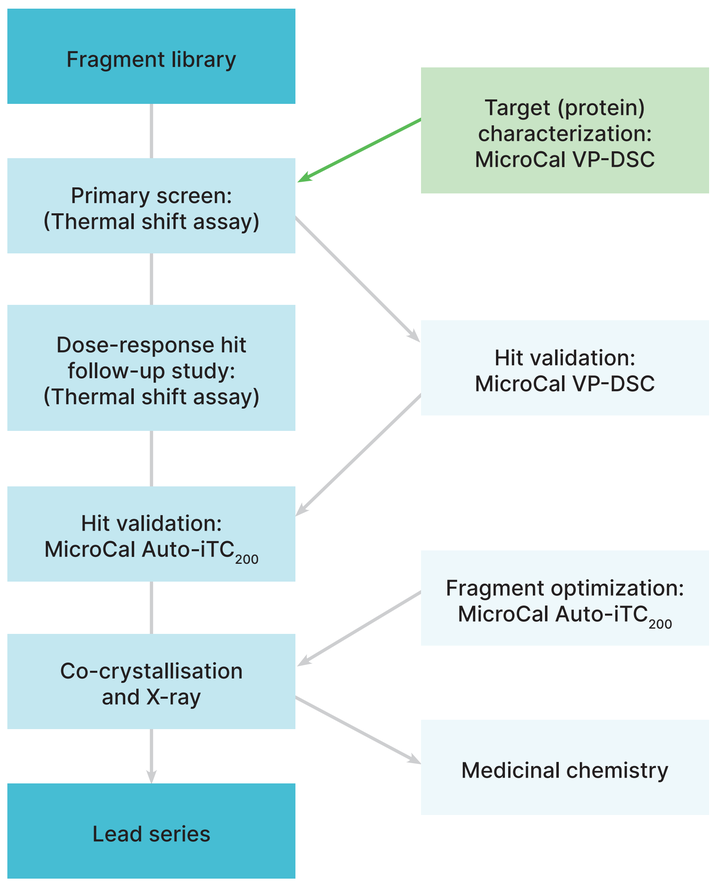


Figure 2. Overview of the FBDD campaign workflow.

THE FBDD APROACH USED BY SPRINT BIOSCIENCE

MicroCal Auto-iTC$_{200}$ data was used to validate hits from a thermal shift based primary screen and to accurately rank order the affinities of this chemically diverse group. The tightest binders were put forward for co-crystallisation trials and the structure based medicinal chemistry program. The fragments ranged from 149 to 333 in molecular weight.

Of the 47 fragments selected from the primary screen, 33 provided good ITC binding data that could be used to quantify the affinity. An example of which is shown in Figure 2A which represents a 'low C' measurement. A low C measurement differs from a typical ITC binding isotherm (Figure 2B) in that the ratio of fragment to protein is raised. This reduces the protein requirement of the assay but requires that the stoichiometry of the binding is fixed to 1:1 in the data analysis and not fitted as an independent variable. 14 showed either no heat of binding or unusual isotherms. The top 20 binders were screened a second time to test the robustness of the assay. 14 of these 20 fragments were successfully crystallized with the Vps34 target.

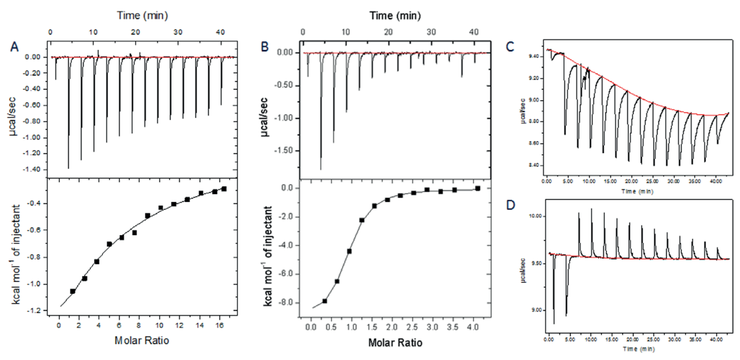
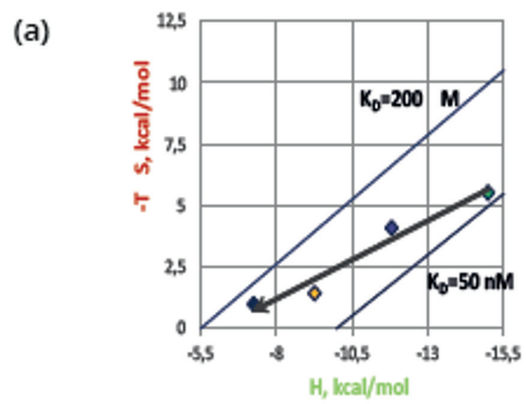


Figure 3. Examples of the data generated by MicroCal Auto-iTC$_{200}$ for the binding of various fragments to the Vps34 target protein. The left and middle graphs show the raw data (upper panels) and binding isotherms (lower panels) of fragments with affinities of (A) 0.9 mM and (B) 4 mM. (C) and (D) show complex isotherms. These fragments were rejected as false positives.

LEAD OPTIMISATION

MicroCal™ Auto-iTC$_{200}$ was used for the direct determination of binding affinities and mode of action studies of the lead candidates. Of the 21 compounds selected from the lead-optimization programme, 17 provided good quality binding data that could be used to confidently rank them. The affinities ranged from 1 nM to 50 µM, with 4 compounds showing no evidence of binding.

In addition, inspection of the data flagged potential issues with stability and/or solubility of a number of compounds.



	cmpdA	cmpdB	cmpdC	cmpdD
Kd, µM	20	2	2	0,095
XLogP	1,0	1,5	1,6	2,1
MW, g/mol	179	214	256	291
Ligand efficiency	0,48	0,49	0,41	0,43

Figure 4. Thermodynamic map (A) and signatures (B) for a series of four compounds evolving through the lead optimization programme. The arrow on Fig. 4 (a) indicates the optimization vector beween isoaffinity lines. With careful control of compound lipophilicity and ligand efficiency, it was possible to successfully overcome entropy-enthalpy compensation Fig. 4 (c).

CO-CRYSTALLISATION TRIALS

The ability of this approach to successfully predict hits that would form co-crystal complexes with the target was demonstrated by the successful co-crystallisation of 12 out of the 14 hit:protein complexes that were put into trials (see Figure 6). The instrument was also used to measure the affinity of subsequent lead optimisation iterations to assess the success of the structure based medicinal chemistry program.

During the lead-optimisation work careful patchwork-type iterations of the compound design were verified by binding data and structural information. Special care was taken to maintain high-ligand efficiency with favourable lipophilicity. The success of this approach is illustrated by the thermodynamic map for a series of related compounds (Fig. 4b).

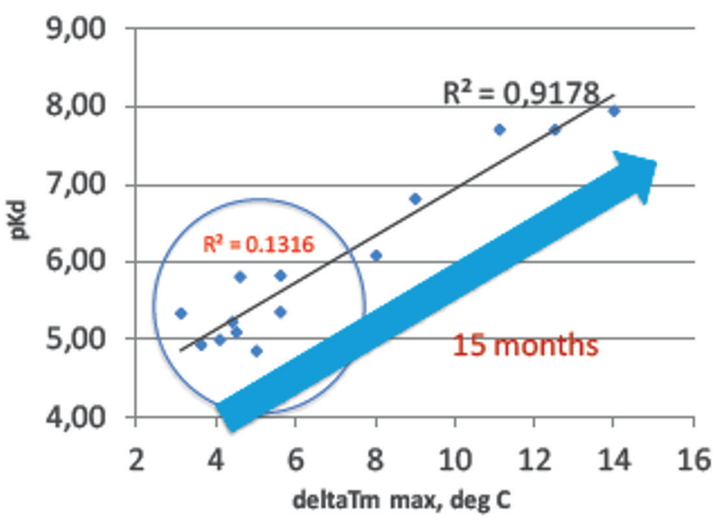


Figure 5. Correlation between pKd from direct binding assay (ITC) and Tm shifts obtained in differential scanning fluorimetry (DSF) throughout the FBDD project.

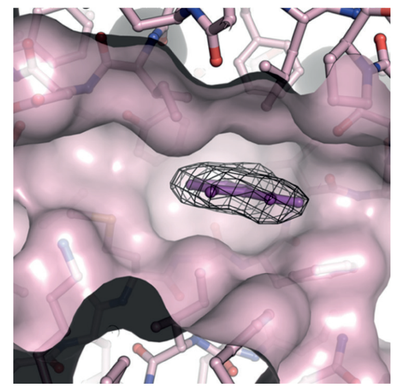


Figure 6. Crystal Structure of a fragment binding to Vps34.

"We have used ITC to verify binding in our fragment screening cascade between primary screening and X-ray crystallography. The correlation between compounds that are verified binders in ITC and the successful determination of a co-crystal structure has been very high."

Kenth Hallberg, PhD Director,
Structural Chemistry,
Sprint Bioscience

CONCLUSIONS

MicroCal-Auto-iTC$_{200}$ has been incorporated into a drug discovery campaign with the following experiences:

- ITC requires minimal assay development and sample preparation
- ITC can be used as a secondary screening tool in hit validation for proteins with limited stability in orthogonal assays
- The rapid experimental setup and execution enabled screening of approximately 50 fragment compounds in less than 3 days
- ITC can be used to identify fragments that will form high resolution co crystal with the target protein
- ITC allowed early determination and elimination of false positives produced by the primary screen
- ITC is a SAR assay which can deliver KD values that can be used to prioritize fragment series and to guide the chemical development of the next iterations of fragments early stage with MicroCal DSC
- Orthogonal use of differential scanning calorimetry (DSC) validates DSF primary screen findings for early elimination of false negatives and positives
- Although an overall correlation can be seen between pKd from direct binding assay (ITC) and Tm shifts obtained in DSF the correlation is significantly more poor for the weaker binders in the early stage of FBDD and med chem iterations

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