Complementary biophysical approaches to assess fragment binding to the epigenetic target bromodomain-containing protein 3 (BRD3)

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INTRODUCTION

- Fragment-based approaches have been applied successfully to the discovery and optimization of ligands for a wide range of targets
- Fragment-based lead discovery (FBLD) is proving particularly valuable for bromodomain (BRD)-containing proteins which are potential therapeutic targets for cancer, inflammation and multiple sclerosis[1]
- There are several BRD isoforms, and compounds selective for individual BRDs would be useful pharmacological tools, with the potential to be therapeutic leads[2,3,4]
- BRD3/BD1 was chosen as a target of interest and subjected to a screening and characterisation work-flow:

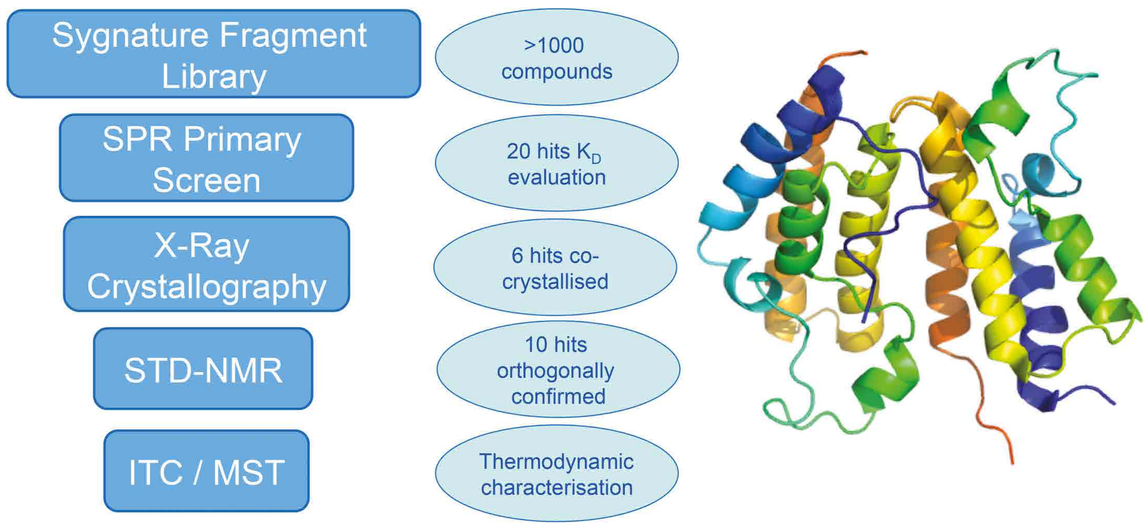

Figure 1: PDB rendering based on human BRD3 (2nxb)

SPR SCREEN

The Sygnature fragment library contains > 1000 diverse and well-characterised compounds:
- each fragment containing at least one point of diversity for elaboration
- a small number of analogues (1-4) are present for each fragment

The library was screened by SPR at 250 µM, 1% DMSO against BRD3/BD1, BRD4/BD1 (for selectivity/confirmation) and human Carbonic anhydrase II (for specificity).

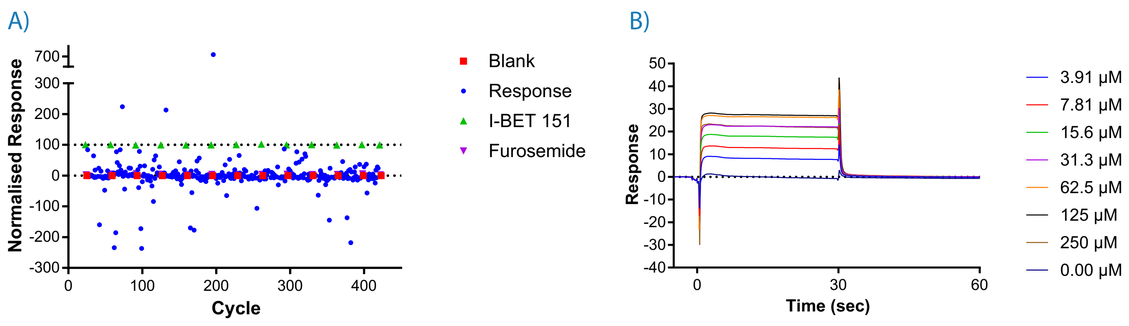

Figure 2:
A) Example SPR data showing relative responses across 320 fragments
B) Concentration response titration to a typical hit fragment

1. Fragment actives defined as response of ≥2x standard deviation of blank injections — 275 hits
2. Interrogate hit further for sensorgram shape (i.e. square wave), effects on baseline and potential for superstoichiometric binding — 148 hits
3. Perform concentration response curves to calculate K_D for each of these hits — 20 hits

X-RAY CRYSTALLOGRAPHY

X-ray crystallography was performed by Peak Proteins and data collected at the Diamond Light Source (Oxford, UK).

- 9 SPR fragment hits were soaked with BRD3/BD1
- 6 fragments co-crystallised with BRD3/BD1

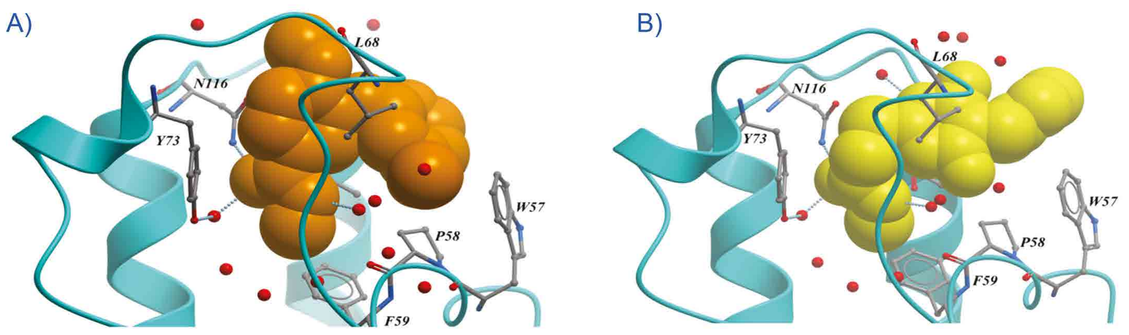

Figure 3: Fragments bound to BRD3/BD1 A)SYG-00003236, 1.67Å, 42µM K_D B)SYG-00000127, 1.69Å, <10µM K_D

LIGAND-OBSERVED NMR

Ligand-observed NMR was used to provide tag-free solution state orthogonal confirmation of a selection of SPR hits:
1. Reference ^{1}H spectra recorded of fragments with target protein
2. Sample selectively irradiated (protein signal only) and ^{1}H spectra recorded again
3. Calculate STD spectra (reference – irradiated spectrum)

If a fragment interacts with target, irradiation is transferred causing the attenuation of the corresponding signals, giving a difference signal in the STD spectrum.

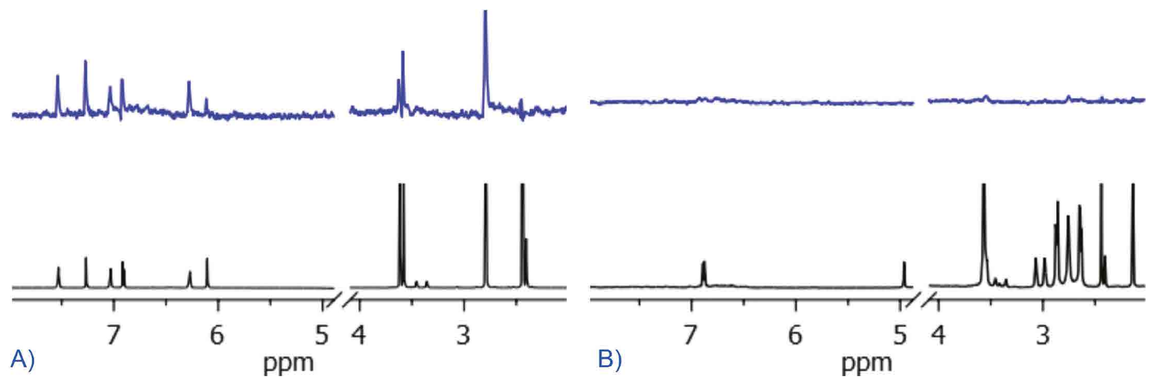

Figure 4: A) Example of fragment SYG-10001520 + BRD3 spectrum prior to irradiation (black) and resulting STD spectrum (blue) 50x scale zoom, evidence of peaks at ligand chemical shifts indicating this is a BDR3/BD1 binder. B) Example of fragment SYG-10000006 + BRD3 spectrum prior to irradiation and resulting STD spectrum, absence of ligand chemical shifts in this spectrum is evidence that no binding interaction has occurred, identifying this fragment as a non-binder of BRD3

- 10 fragments were screened at 250 µM in 5% D_2O, ligand excess 20:1 BRD3/BD1 at 278 K
- 8 actives reconfirmed, including one ambiguous SPR hit
- 2 SPR negatives also confirmed

ITC & MST

- Both ITC and MST allow calculation of binding affinities in the untethered solution state
- ITC also enables the thermodynamic evaluation of the binding parameters
- ITC measures changes in heat as the fragment is injected into a sample of the untagged target
- MST measures the movement of the protein, covalently attached to the fluorescent label NT-647 over a temperature gradient, in the presence and absence of the ligand

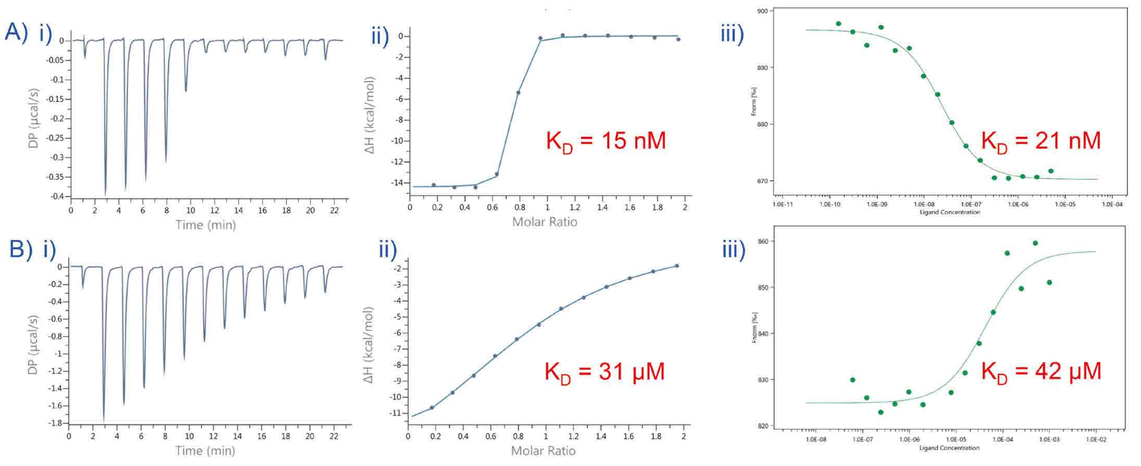

Figure 5: In-solution analysis for the BRD3 interaction with A) tool compound JQ1 and B) fragment SYG00000127. i) ITC thermogram, ii) ITC fitted curves showing calculated affinity, iii) MST binding isotherms showing calculated affinity.

- For ITC, the fragments were screened at 800 µM against 80 µM BRD3/BD1
- For MST, the fragment concentration range was 30 nM – 1 mM against 5 nM BRD3/BD1

MST data courtesy of NanoTemper Tecnologies GmbH.

CONCLUSIONS

- A fragment screening programme to identify BRD3/BD1 binders was successfully completed using our proprietary fragment library, SPR screening and X-ray crystallography
- Of the nine fragments identified as binders from the SPR screen, six gave liganded structures when soaked with BRD3/BD1 construct
- These hits were orthogonally confirmed by ligand observed NMR experiments, which identified binding events for eight SPR hits and clarified one ambiguous result as a weak binder
- ITC and MST were utilised to determine in-solution KD values for several of the identified hits and demonstrated excellent agreement with each other and the SPR

References

1 Muller et al (2011), Expert Rev Mol Med., 13: e29 **2** Zhao et al (2014), Bioorg & Med Chem Lett., 24, 2493-2496 **3** Chung et al (2012), J Med Chem, 55(2), 576-86 **4** Arrowsmith et al (2012), Nat Rev Drug Discov., 11, 384-400