Fragment Screen on BRD4 using NMR Spectroscopy

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Introduction

The Saturation Transfer Difference (STD)[1] NMR is tested as a screening method to identify binding and non-binding fragments against Bromodomain-containing protein 4 (BRD4).[2,3] The results were compared to Surface Plasmon Resonance (SPR)[4] and X-ray[5] data previously acquired in BioFocus.[6] The degree of agreement was assessed.

STD NMR[1]

- STD NMR is one of the most sensitive techniques in FBLD.
- It utilises the saturation of macromolecule resonances through a train of frequency selective pulses. Saturation is then transferred to bound ligand via ^{1}H-^{1}H cross-relaxation. In the end, free ligand relaxation is detected following the dissociation.
- Due to the saturation it experienced, the binder signals have lower intensity than non-binder's, allowing the distinction between the two in a single experiment.
- Usually NMR screen involves several fragments in a cocktail (5-7) aimed at testing as many compounds as possible using minimum time and material.
- A deconvolution NMR screen can be performed in order to avoid competition and verify the hits.

BRD4[2]

- BRD4 is a known target for various forms of carcinoma.
- Acetylation of histone lysines has a key role in regulating chromatin architecture and transcription.
- BRD4 „reads" lysine acetylation state through binding to it. BRD4 is a member of BET family of bromodomains.
- The goal of studies on BRD4 is to find a small molecule which will selectively prevent the interaction of BRD4 with acetylated lysine without affecting the function of other bromodomain-containing proteins.

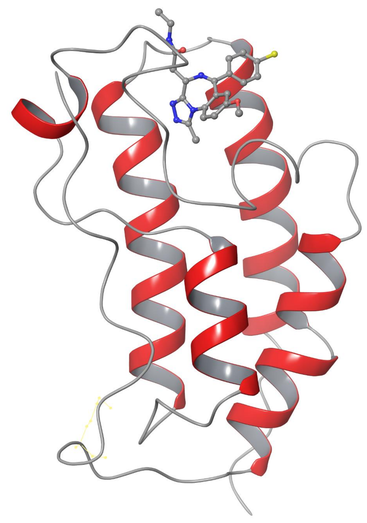

Figure 1. X-ray structure of BRD4 with known binder I-BET762 (3P5O.pdb)[3]

Experimental

- All NMR spectra were recorded at 25 °C on Bruker Avance III 600 spectrometer using the inverse 5 mm room temperature probe.
- A known binder I-BET762 was used to optimise the experimental conditions of the screen.
- The DMSO-d_6 stock solutions of the fragment library were at a concentration of 100 mM. The final fragment concentration in TRIS buffer was 3.25 mM and the protein 6.5 µM (the ratio of 1 : 500).
- The STD NMR spectra were acquired using a sequence provided by Bruker BioSpin. Selective saturation of BRD4 was performed using Gauss G3 cascade pulse in duration of 3 s and on-resonance frequency of -1 ppm. The excitation sculpting scheme was used to suppress the HDO signal while relaxation filtering was employed to suppress protein resonances. An on-resonance frequency and pulse selectivity check was performed on each sample by acquisition of STD NMR spectra in absence of protein in the sample.
- 18 fragments (Table 1) were screened, the results interpreted as binding or non-binding events. Figure 2 shows the examples of STD NMR spectra belonging to a binder and a non-binder.

Results

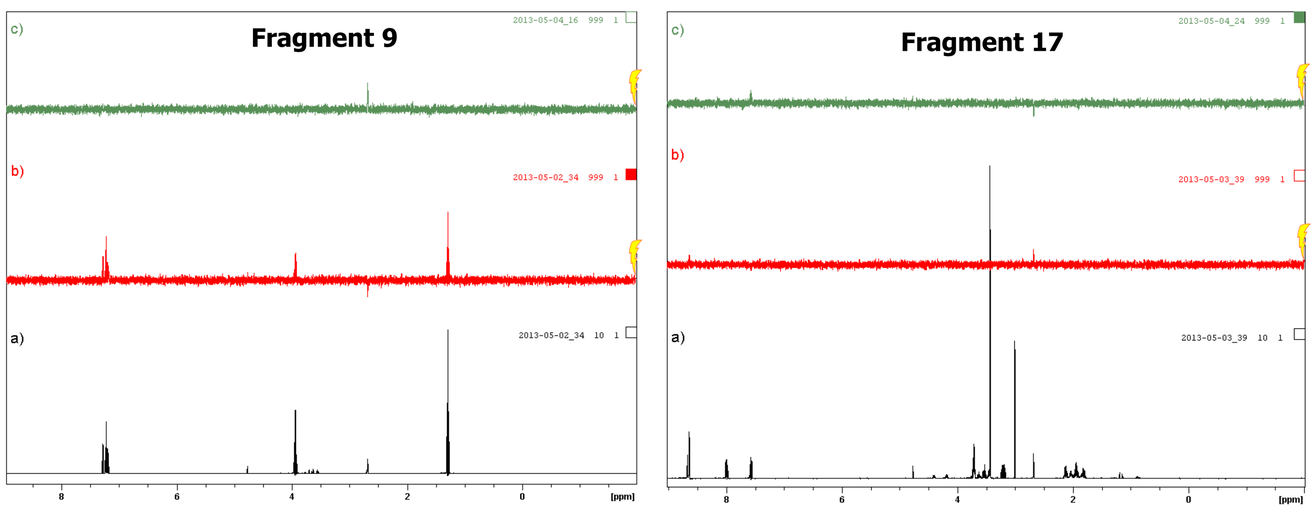

Figure 2. A binder (fragment 9) vs. non-binder (fragment 17): a) ^{1}H spectra; b) STD NMR spectra with BRD4; c) STD NMR spectra in absence of BRD4; all in TRIS buffer at 25 °C

Comparison: STD NMR, SPR & X-ray

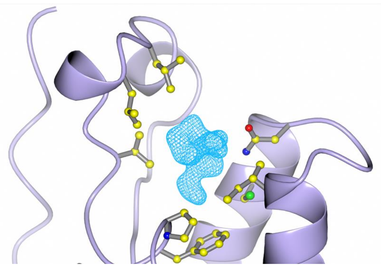

Table 1. Comparison of STD NMR results (Fidelta) with SPR[4] and X-ray[5] data (BioFocus)[6]

	NMR	SPR	X-ray
I-BET762	YES	YES	YES
FRG 2	NO*	YES	NT
FRG 3	YES*	YES	NT
FRG 4	NT**	YES	NT
FRG 5	NO	Non specific	NT
FRG 6	NO	NO	NT
FRG 7	POSSIBLE	Non specific	NT
FRG 8	NO*	YES	NO
FRG 9	YES	YES	YES
FRG 10	YES	YES	NT
FRG 11	NT**	Non specific	YES
FRG 12	YES	NO	NO
FRG 13	YES	NO	NO
FRG 14	NT**	Non specific	YES
FRG 15	NT**	Non specific	NT
FRG 16	POSSIBLE	NT	YES
FRG 17	NO	NT	NO
FRG 18	NO	NT	NO

*low solubility under experimental conditions
**not soluble under experimental conditions
NT=not tested

Conclusions

- Structure integrity and solubility check was performed under screening conditions prior to the screen, resulting in four discarded fragments.
- STD NMR was tested as a fragment screening method against BRD4. A known binder I-BET762 was used to adjust the experimental conditions of the screen. The result of the screen were five unambiguous and two possible binders.
- Comparison with SPR data revealed a good correlation between the techniques identifying three fragments which qualified as orthogonalized binders: fragments 3, 9 and 10.
- Experimentally determined protein-ligand crystal data was obtained for two out of four NMR hits.
- STD NMR screen revealed an additional two weak binders (fragments 12 and 13) undetected by other techniques that warrant further investigation in fragment-to-lead chemistry.

References

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Poster available online at:
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