Using SPR to Characterise the Binding Modes of PROTAC Molecules

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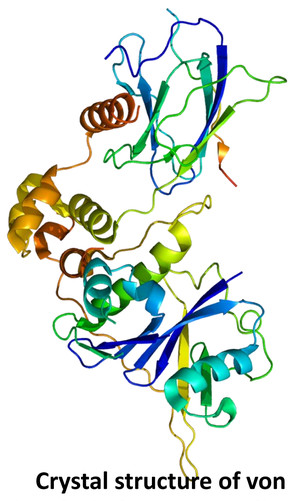
Introduction

PROteolysis-TArgeting Chimeras (PROTACs) are an exciting new development in drug discovery that have the potential to modulate protein targets once considered 'undruggable'.

A small molecule is designed with two "warheads", one binds to the protein target of interest (TOI) and the second binds to an E3 ligase such as VHL (right). The two are joined by a flexible linker which allows the E3 ligase to ubiquitinate the TOI - targeting it for degradation by the intracellular ubiquitin proteasome system.



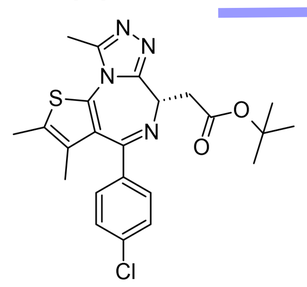
Crystal structure of von Hippel–Lindau (VHL) E3 ligase[1]

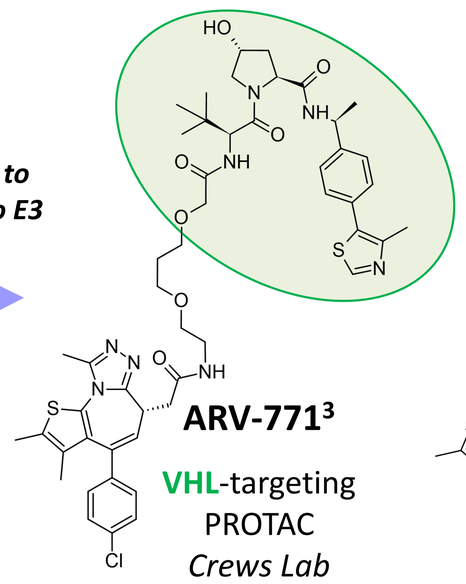
More information on the binding mode of one PROTAC compared to another can be invaluable in drug discovery projects when deciding which lead to pursue.
At Charnwood Molecular, we use our Biacore 8K system to measure important insights into the binding behaviour of PROTAC – and other – molecules.

Creating PROTAC molecules from Target Of Interest (TOI)-binding small molecules

Use chemical linker to join TOI 'warhead' to E3 ligase 'warhead'

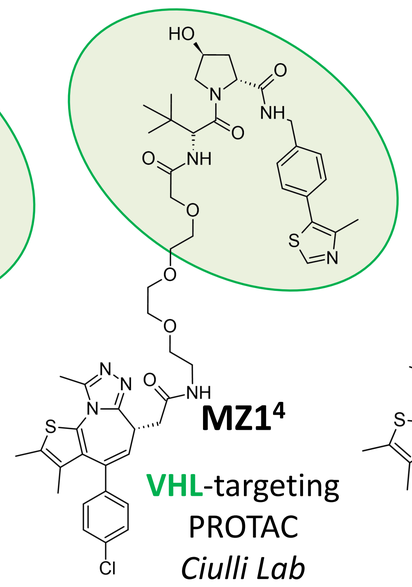
JQ1(+)[2]





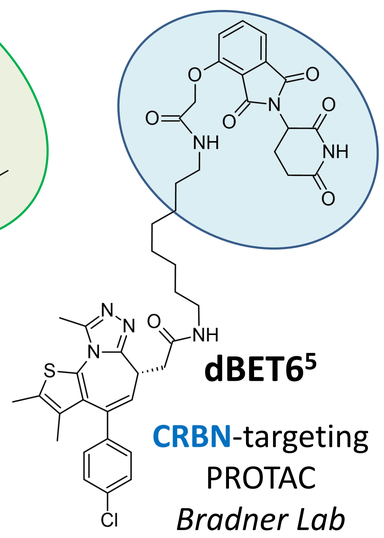
ARV-771[3]

VHL-targeting PROTAC
Crews Lab



MZ1[4]

VHL-targeting PROTAC
Ciulli Lab



dBET6[5]

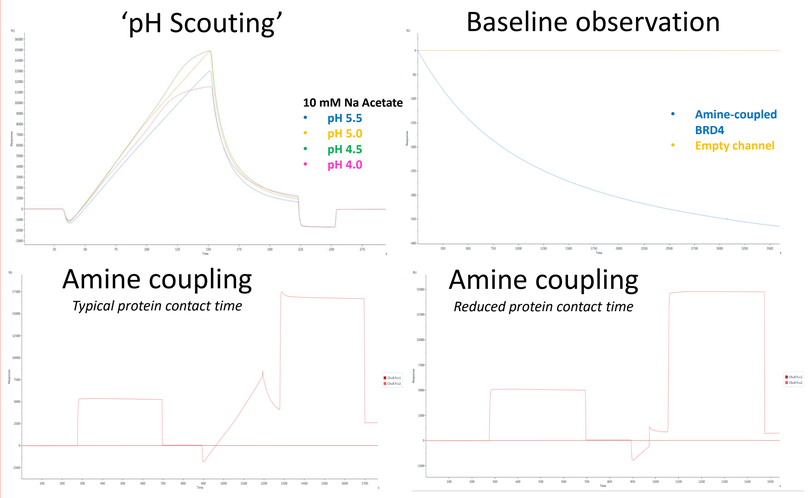
CRBN-targeting PROTAC
Bradner Lab

After having identified small molecules that bind to the TOI – the next step is to link this first warhead to a second warhead that binds to one of the multiple E3 ligases that function in the proteasomal degradation system.

JQ1(+) is one such warhead that has been used by several different groups to successfully develop PROTACs capable of strong in-cell degradation of BRD4 (and other BET family members). It is selective to the BET family over non-related proteins and demonstrates nanomolar affinity and potency against the different BRD human isoforms. Discovering such a useful probe compound is an important step for any PROTAC discovery project.

As demonstrated above a variety of chemical linkers and E3 ligase-warheads have been joined to JQ1(+) to generate an effective and varied panel of PROTACs. The choice of linker and E3-ligase is not a simple one and requires multiple rounds of testing in different cell lines to find the optimum combination.

BRD4 Immobilisation for SPR



We found that BRD4 immobilised very readily to the surface of CM5 chips using the amine-coupling method. We had to drastically cut short the protein contact time to ensure we didn't capture too much protein. The surface was very robust – fully stabilising around 1 hour post-immobilisation.

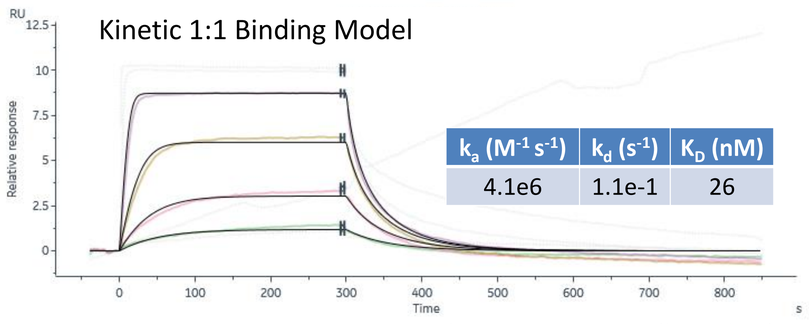
Summary and Conclusion

Our work here illustrates the insights that SPR can bring to any drug discovery project. The ability to characterise an interaction can be critical for deciding how to progress a project.

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

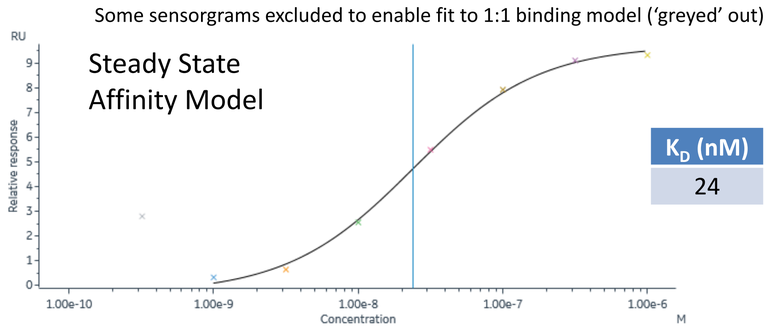
- **Assay development for SPR experiments** – using literature precedent (where possible) to design and develop SPR screening assays for new protein targets.
- **Mechanistic insights into interactions between small molecules and protein** – SPR is a powerful technique that can help us infer subtle details about a biomolecular interaction.
- As well as **PROTAC drug discovery**, these techniques can be applied to other more general aspects of drug discovery and screening.

Characterising binding modes of different PROTAC molecules using SPR



k_a (M^{-1} s^{-1})	k_d (s^{-1})	K_D (nM)
4.1e6	1.1e-1	26

Some sensorgrams excluded to enable fit to 1:1 binding model ('greyed' out)



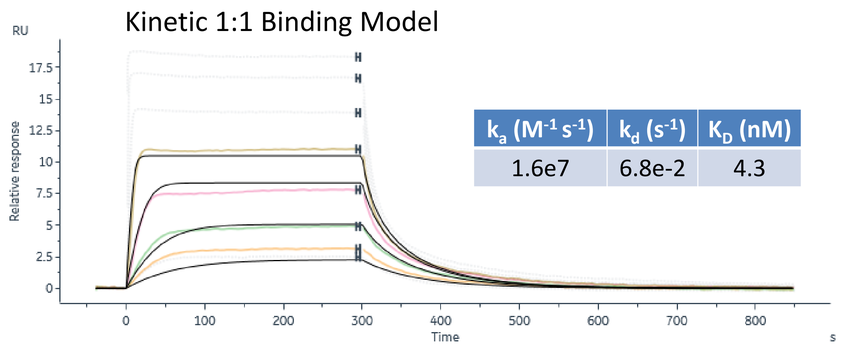
K_D (nM)
24

ARV-771

For the VHL-targeting PROTAC molecule ARV-771 we can also measure very high quality sensorgrams for its interaction with BRD4. We see a greater deviation away from 1:1 binding as seen with JQ1(+) on its own – presumably due to the linker/VHL-warhead moieties - and some sensorgrams have to be excluded to allow even a reasonable fit of the model to the data.
The subsequent kinetic fit (K_D = 4.3 nM) shows relatively poor agreement with the affinity fit (K_D = 26 nM) – likely due to the fact the interaction doesn't entirely match the 1:1 binding model.1

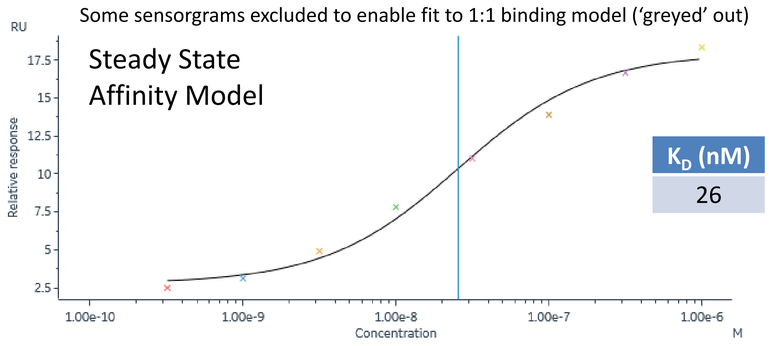
JQ1(+)

In the case of the BRD4-targeting warhead JQ1(+) we can measure very high quality sensorgrams for its interaction with BRD4. At some of the higher concentrations of compound we see a deviation away from 1:1 binding and these sensorgrams have to be excluded to allow a good fit of the model to the data.
The subsequent kinetic fit (K_D = 26 nM) shows good agreement with the affinity fit (K_D = 24 nM).



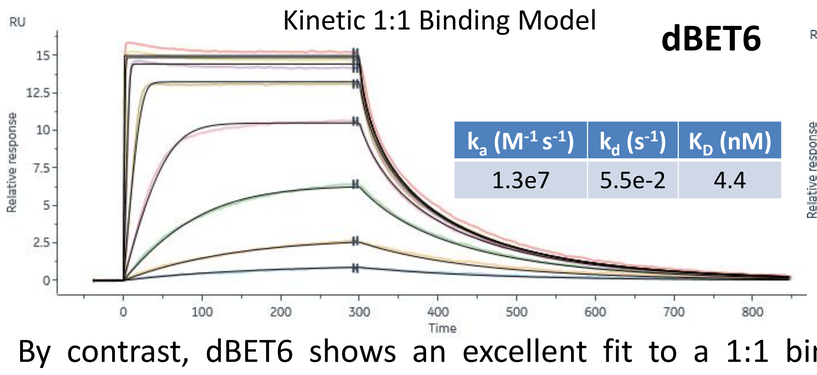
k_a (M^{-1} s^{-1})	k_d (s^{-1})	K_D (nM)
1.6e7	6.8e-2	4.3

Some sensorgrams excluded to enable fit to 1:1 binding model ('greyed' out)

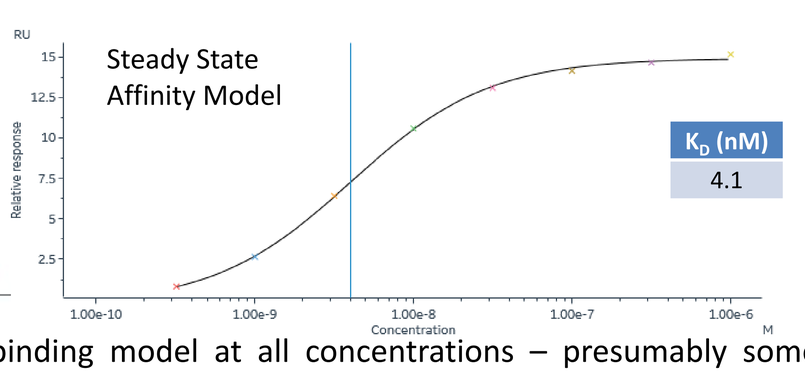


K_D (nM)
26

dBET6



k_a (M^{-1} s^{-1})	k_d (s^{-1})	K_D (nM)
1.3e7	5.5e-2	4.4



K_D (nM)
4.1

By contrast, dBET6 shows an excellent fit to a 1:1 binding model at all concentrations – presumably some properties conferred on the whole PROTAC molecule from either the linker region or the Cereblon-targeting warhead have positively influenced the molecule's binding to BRD4.
The subsequent kinetic fit (K_D = 4.4 nM) shows excellent agreement with the affinity fit (K_D = 4.1 nM).

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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4 - Zengerle, M. *et al.* (2015), *ACS Chem. Bio.*, **10** (8): 1770–17775
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