

Speeding up PROTAC Development: A Direct-to-Biology approach for Rapid Optimization of PROTACs. Comparing HiBiT and Transfected NanoLuc® Approaches

Joshua McArthur, Kathy Dodgson, Gary Allenby, Ralph Kirk

Charnwood Discovery, Charnwood Campus, 9 Summerpool Road, Loughborough, LE11 5RD

Introduction

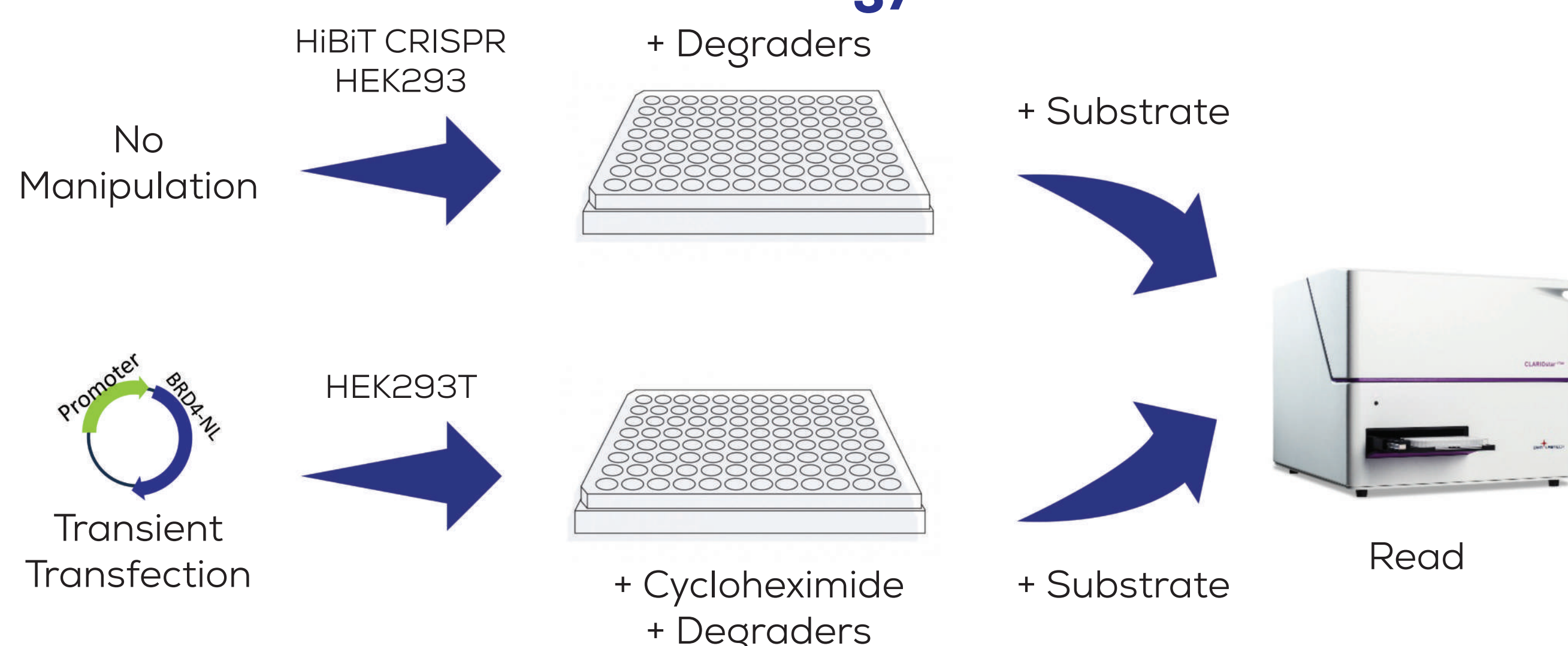
PROteolysis-Targeting Chimera (PROTAC) development is a challenging process. Multiple aspects of the molecule can be optimized, but conventional synthesis and purification of iterations of PROTACs would be highly time-consuming and resource-heavy. Linker optimization can have a profound effect on PROTAC efficiency and potency with significant influences on the cooperativity of ternary complex formation, as well as the overall physical chemical properties and metabolic liabilities. Plate-based chemistry approaches can speed this process up by synthesizing many versions of PROTACs (with varied warheads, linkers and vector points) and, with minimal clean up, these crude mixtures can be added directly to a suitable cell assay to generate degradation data, a process known as direct-to-biology (D2B) [1]. There are a plethora of reasons why a HiBiT system is the gold standard for PROTAC screening, primarily due to the natural expression levels of the protein of interest (POI). However, we demonstrate here that similar results can be achieved with transiently expressed NanoLuc® tagged POI system as a proof of concept.

1

HiBiT

NanoLuc®

Methodology



2

NanoLuc® Method Development

When faced with highly expressed targets (such as BRD4) the introduction of more POI using an expression vector with a CMV promoter, meant that too much POI was being produced to achieve a good signal-to-noise ratio, (dBET6 a known BRD4 tool PROTAC [2], could not overcome this system). Addition of cycloheximide (CHX), just prior to PROTAC addition, prevented further protein synthesis during the incubation period. By introducing CHX we vastly improved the S:N from ~1.5 to ~10 fold.

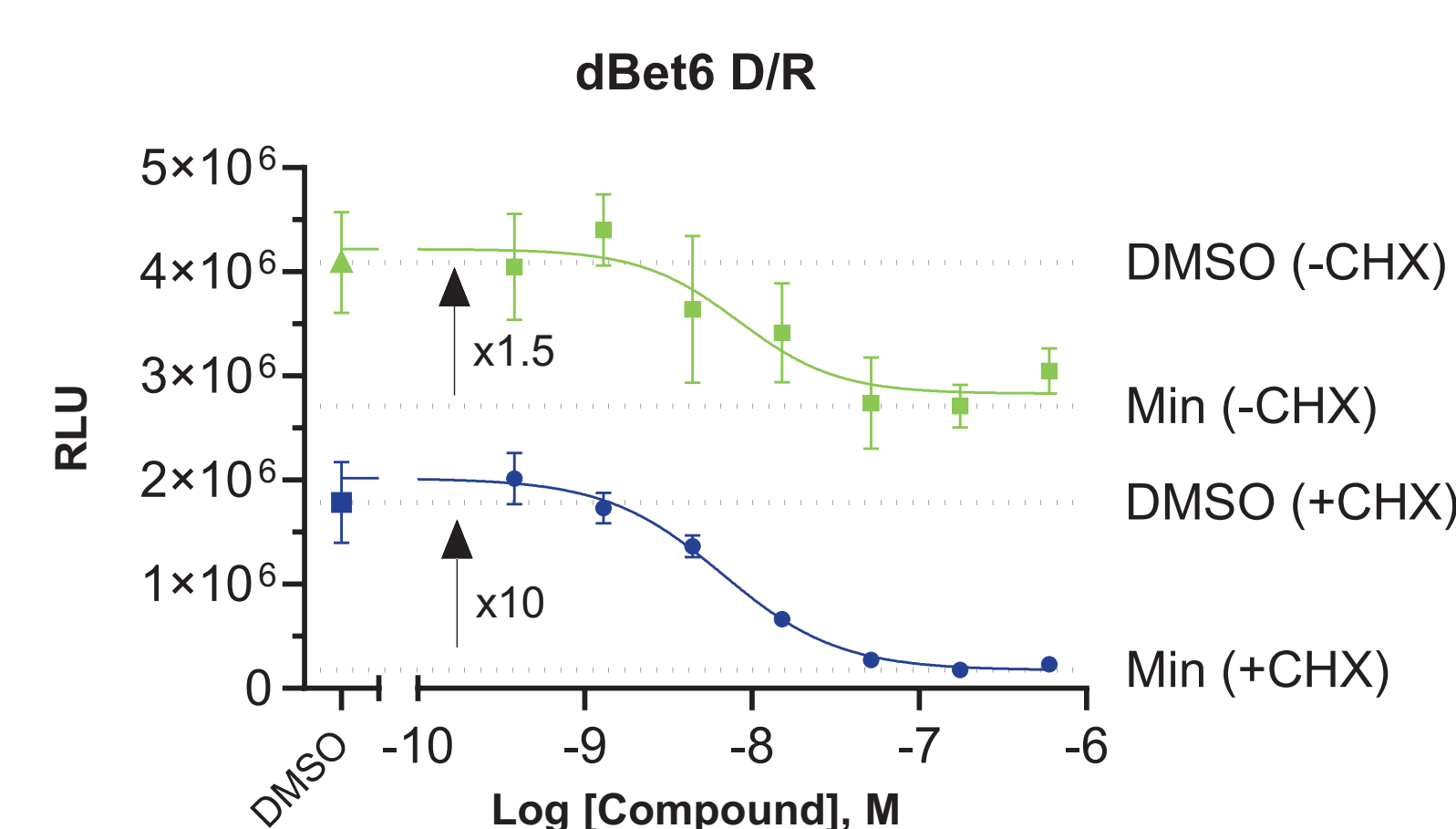


Figure 1: Dose responses of dBET6 with and without CHX addition

When working with PROTACs it is critical to understand the mechanism of action behind any observed degradation. Here we added JQ1+ or thalidomide in an effort to saturate the binding sites of BRD4 or CRBN respectively, doing this demonstrated a 20x decrease in potency (DC₅₀) with thalidomide; JQ1+ competition showed an even greater effect.

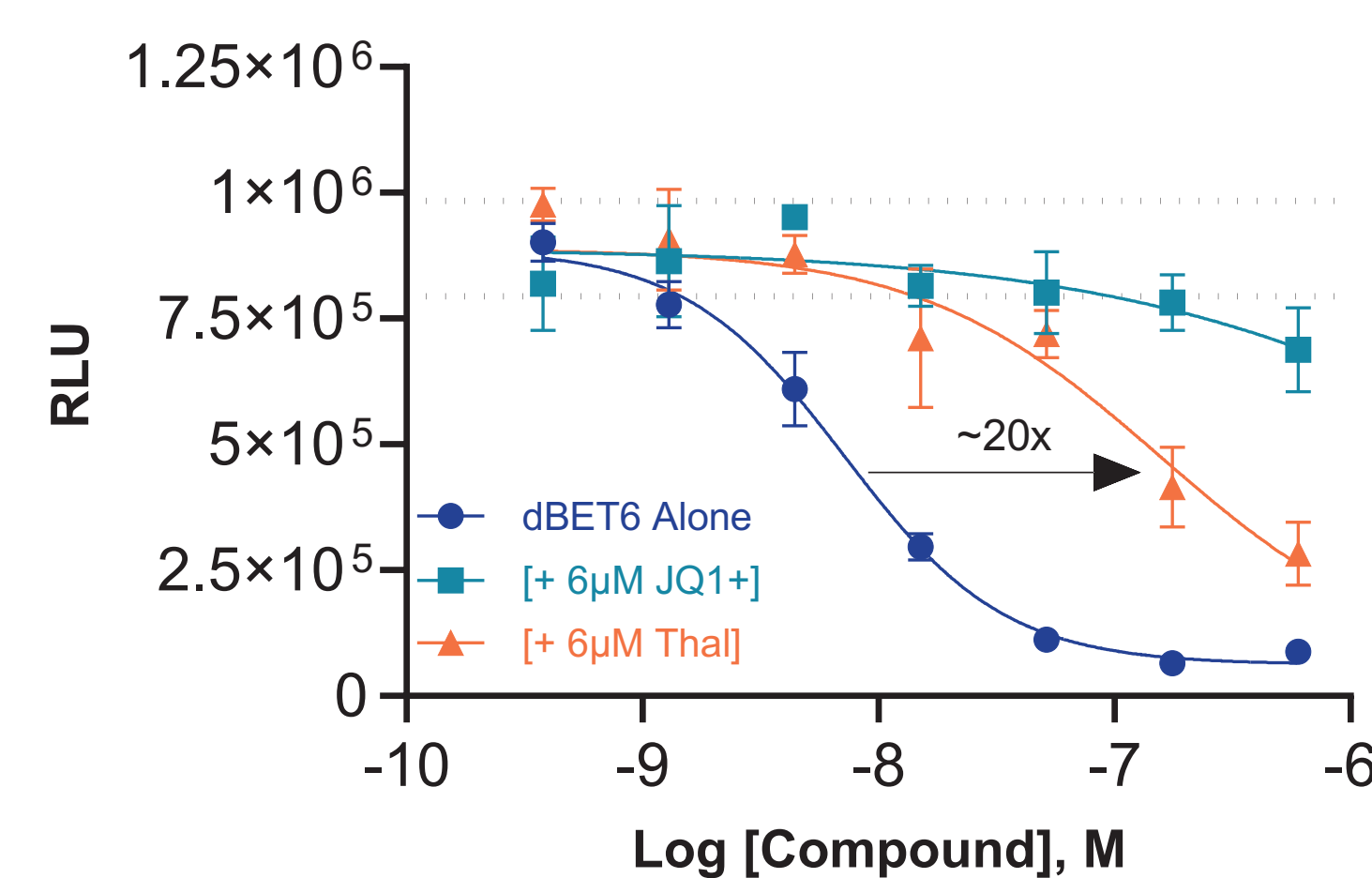


Figure 2: Dose responses of dBET6 alone or in the presence of a BRD4 or an E3-Ligase binder (JQ1+ and thalidomide, respectively)

3

Direct-to-Biology (Spot Test to Dose Response)

A 96-well plate with crude compound mixtures was provided by colleagues from our Chemistry department. For initial spot test screening, using liquid handling solutions, a series of dilutions were performed to produce a 384-well assay plate of 1 µM and 0.1 µM in duplicate. Screening at two concentrations reduces false negatives, often derived from the hook effect, a known phenomenon of PROTAC molecules. Where we used the NanoLuc® transfected system, cycloheximide was to prevent protein synthesis during compound treatment (not required for HiBiT).

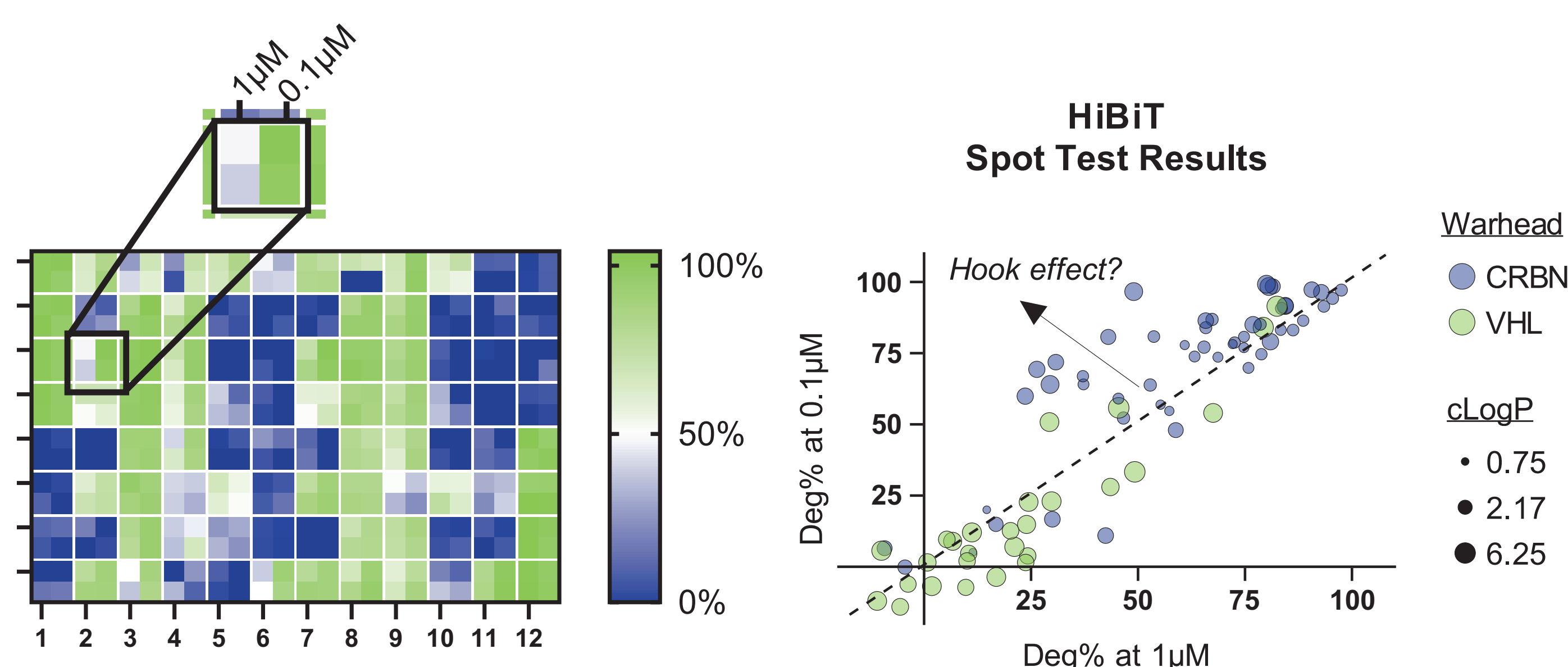


Figure 3: Heat map and correlation plots for the spot test degradation data

Degradation data (RLU) was normalized to the dBET6 control response and plotted as "Deg%". Heat maps (Figure 3) are useful to efficiently discriminate between hits, inactives and compounds with unusual profiles. This information can then be used to construct a correlation plot (Figure 3) showing degradation at 1 µM vs 0.1 µM, with each point's size representing a proportional cLogP value. We see an interesting trend showing that the less lipophilic CRBN-based PROTACs are better degraders than the VHL-based PROTACs in this particular case.

Once the data has been interrogated, we can then follow up the most interesting compounds with extended dose response experiments (Figure 4).

D2B PROTACs D/R

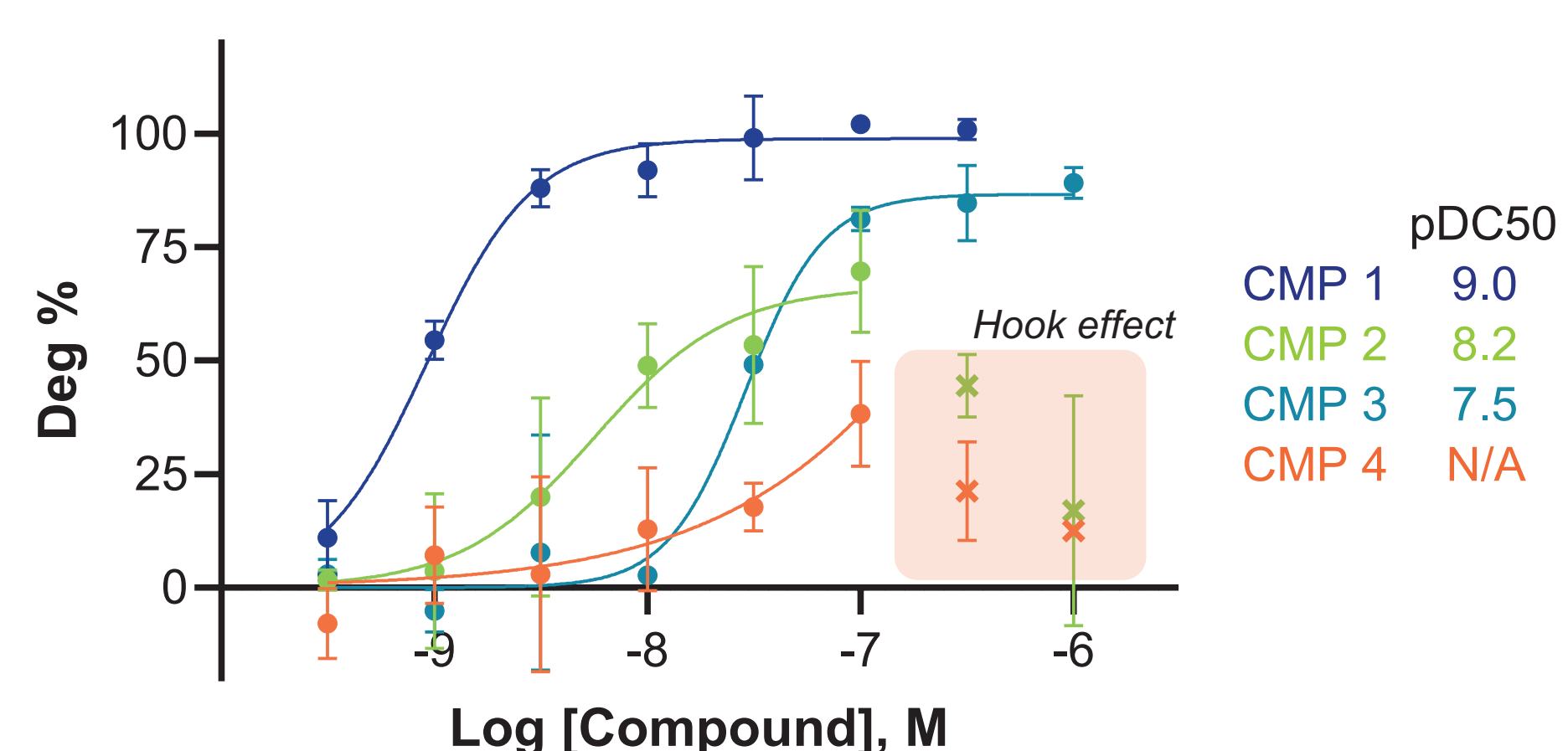


Figure 4: Follow-up on the spot test result data with dose responses. A range of compounds were characterized, some with known hook liabilities.

Further screening options can then also be performed such as a Western Blotting, or characterization of ADME properties via our DMPK department.

4

Conclusion and Summary

As a fully integrated drug discovery research partner Charnwood Discovery's small molecule development expertise dovetails with our well-established experience of targeted protein degradation (TPD) and the development of PROTACs, molecular glues and other similar degrader molecules.

Here we showcase data from our direct-to-biology (D2B) platform which can be used to rapidly screen for PROTAC-caused degradation of any biological target. Our continually expanding library of partial PROTAC molecules can be quickly and efficiently combined with novel warheads which allows swift progress from initial hit identification through to PROTAC optimization and lead compound selection.

HiBiT Vs NanoLuc® (1µM data)

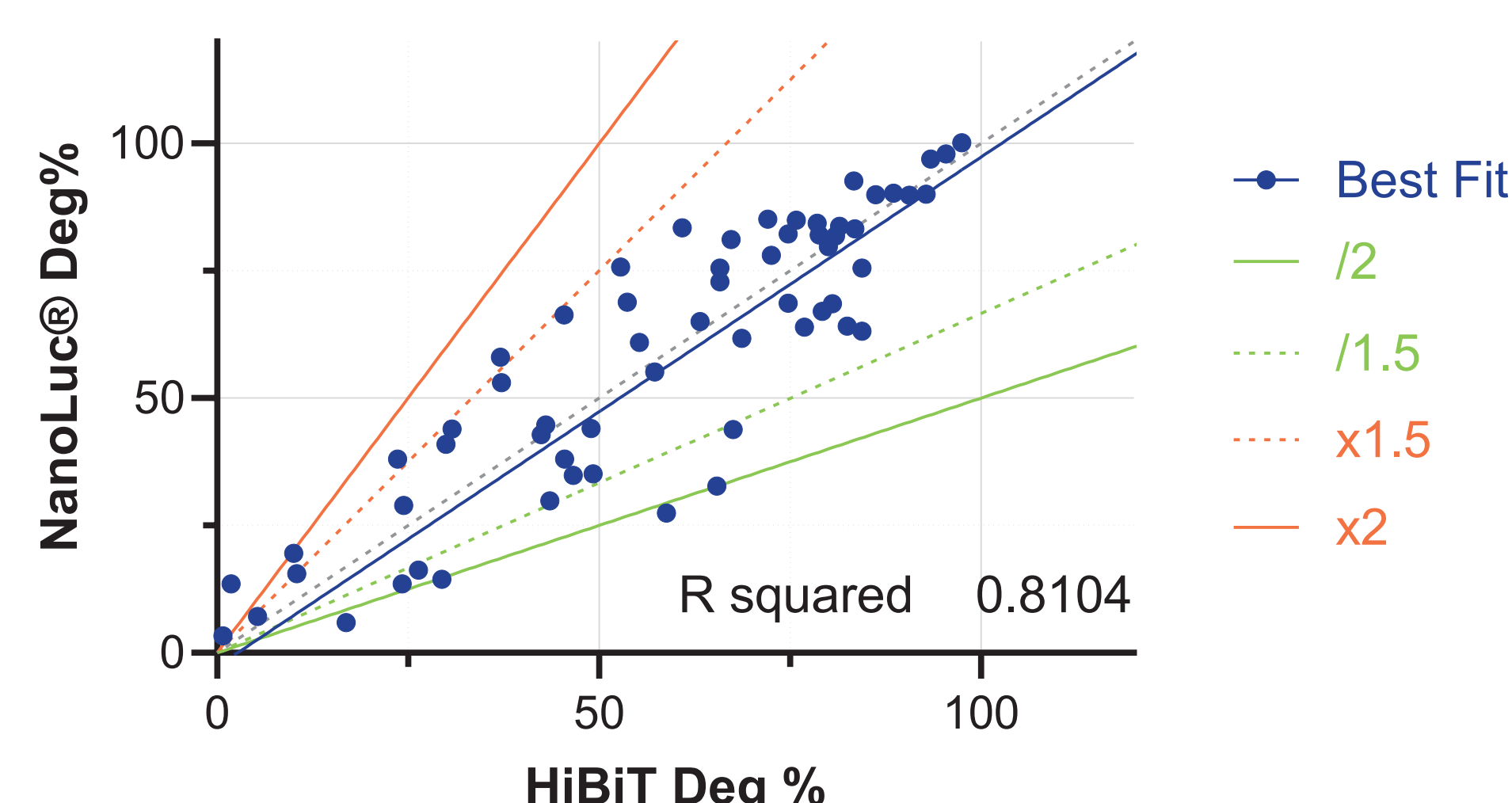


Figure 5: Data showing that BRD4-NanoLuc® degradation data correlates well with the BRD4-HiBiT equivalent assay. In both instances the PROTACs were dosed in the same manner.

