An Integrated Multi-Modal Platform for Comprehensive Analysis of Targeted Protein Degraders

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Introduction

Drug modalities facilitating targeted protein degradation (TPD) have driven the development of analytical platforms which facilitate efficient and translational drug discovery. TPDs can target proteins previously considered 'undruggable', but there are challenges associated with their development. Deploying an appropriate drug discovery strategy and applying key technological and computational drug design platforms can help de-risk the process, identify early and address some of the difficulties associated with TPD development. Here we discuss a fully developed high throughput analytical platform to study and optimise protein degrader candidates. It consists of multiple orthogonal technologies that generate major mechanistic information to advance TPD drugs rapidly through the preclinical development stages.

Toolbox for TPD Drug discovery

Selvita has an established toolbox for TPD drug discovery. Here we describe in detail the biological aspects of the toolbox for the development of degraders, focussing on POI, ligase and MG screening, target engagement, cell permeability ternary complex formation and efficacy measurements.

Biology
- Screening and optimisation of POI and ligase binders
- E3 ligase identification, engagement and appropriate cellular expression / activity Cellular permeability
- Target turnover
- Ternary complex formation and target ubiquitination
- Disease appropriate cellular models

Medicinal Chemistry
- Linker design
- SAR optimisation to improve solubility, reduce metabolism, and increase cell permeability. Based on synthetic tractability, structure drug-likeness and linker attachment possibilities

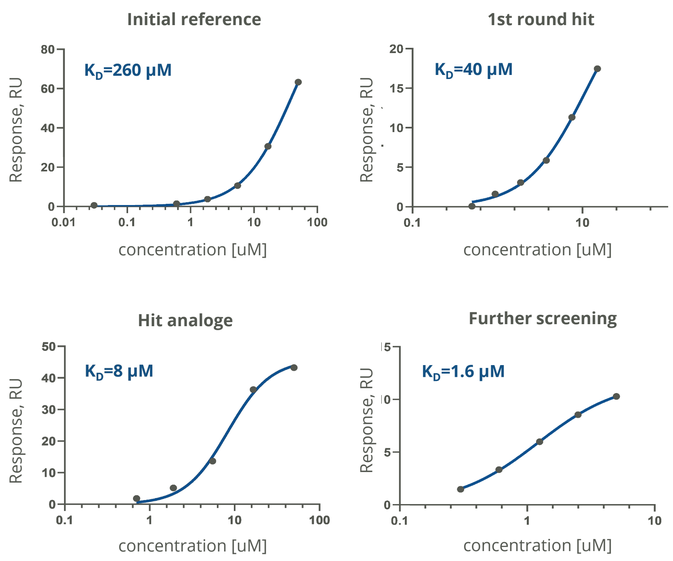
CADD/AI/ Modelling
- Structure and ligand-based molecule optimization
- Analysis of ternary complex structures
- Bifunctionals modelling and linker optimisation and macrocycle design
- PK/PB modelling for cascade optimization and prediction of human dose

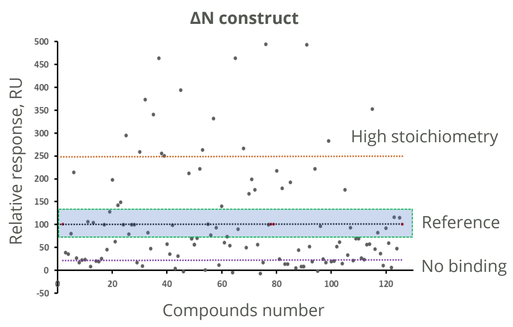
DMPK
- Optimise in vitro assays that correlate with in vivo PK
- Target engagement
- Issue-driven ADME screening cascade
- In-depth PK characterization (mechanistic studies)
- Appropriate PK/PD study design and data integration

Safety/toxicity
- Identification of off target effects and neo-substrate degradation
- Assess key drug metabolism enzymes which could enhance the risk of DDIs
- In vitro toxicology assays and safety panels explored to highlight liabilities

Biophysical screening for warhead and ligase binders using **SPR**

Example: Medium-throughput (MT) hit finding for a novel E-3 ligase

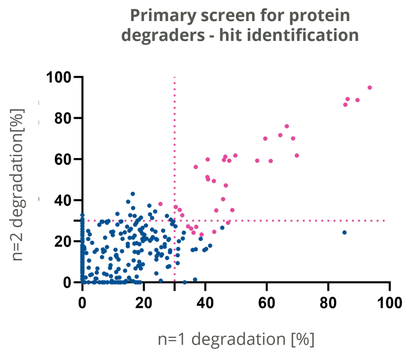


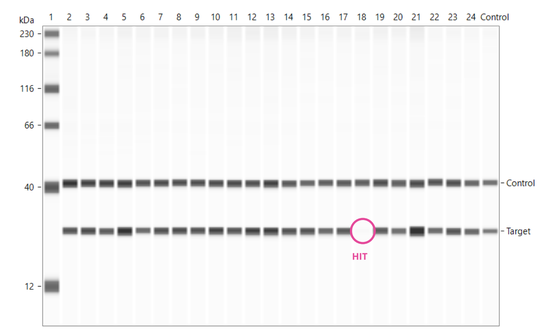


SPR has been implemented across multiple targets to identify and characterise POI and ligase binders. Here an SPR assay was optimised for the screening of *in silico* selected potential binders of a novel E3 ligase. Following HitID, hits were subject to iterative SAR. The KD was improved from 40 µM 1.6 µM after rounds of iterative screening

Cell-based screening for TPDs using **Simple Western (Jess)**

Example: MT hit finding for protein degraders

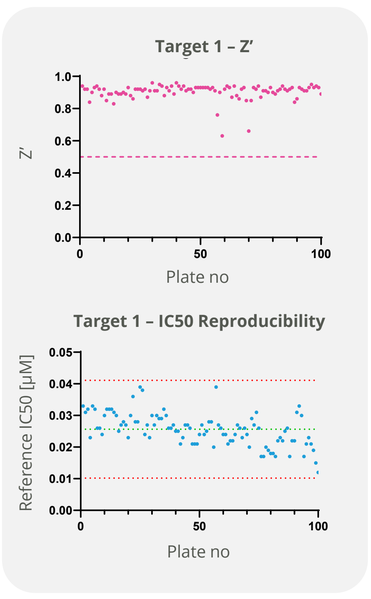


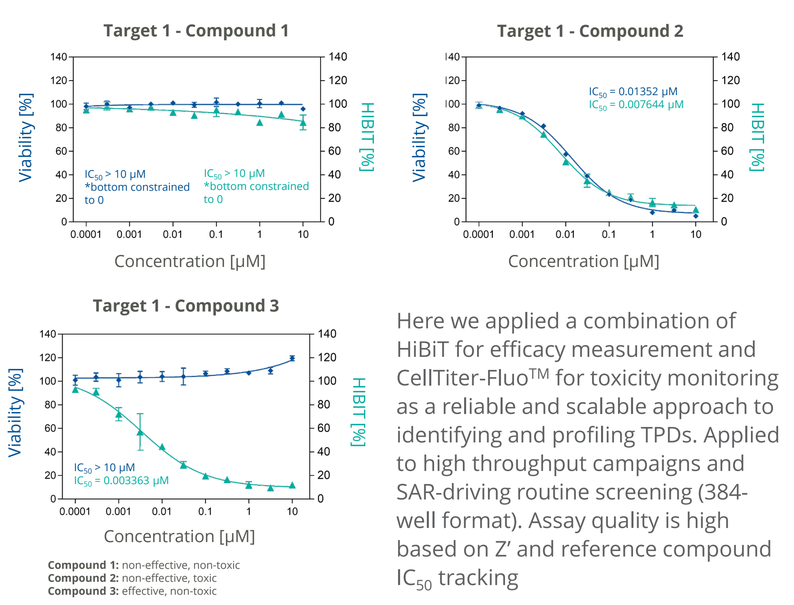


In addition to implementation of capillary Western blot in screening cascades, it can be applied in hit finding. A medium-throughput single concentration PROTAC screen was executed using an automated capillary Western blotting system. Over 600 compounds were screened in two biological replicates, with excellent concordance. The system allows for size-based multiplexing as well as colour-based multiplexing due to parallel detection in two fluorescence channels. Jess is also deployed to measure of-target neo-substrate degradation in TPD projects

Application of the HiBiT technology in **screening for TPDs**

Example: Combined efficacy and cytotoxicity screening for molecular glues



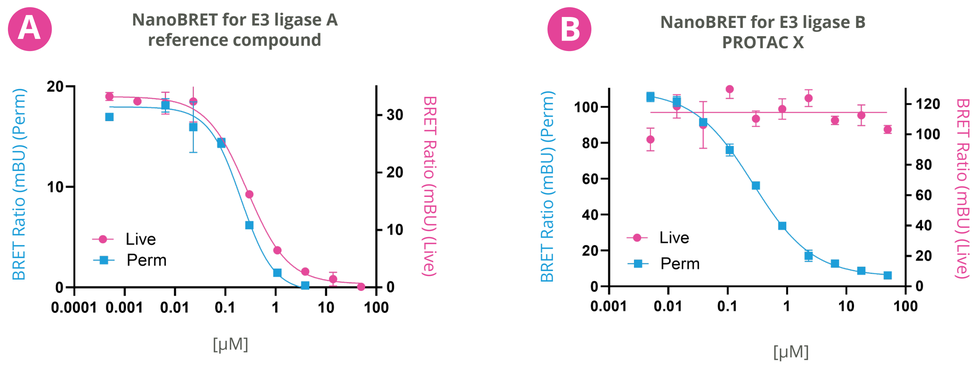


Compound 1: non-effective, non-toxic
Compound 2: non-effective, toxic
Compound 3: effective, non-toxic

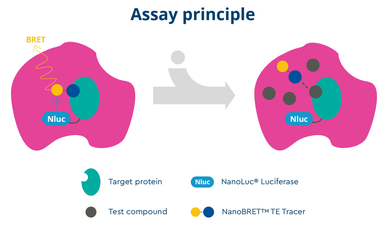
Here we applied a combination of HiBiT for efficacy measurement and CellTiter-Fluo™ for toxicity monitoring as a reliable and scalable approach to identifying and profiling TPDs. Applied to high throughput campaigns and SAR-driving routine screening (384-well format). Assay quality is high based on Z' and reference compound IC_{50} tracking

Cellular **Target Engagement** of TPDs to CRBN Using NanoBRET

Example: Measurement of TPD efficacy and cellular permeability

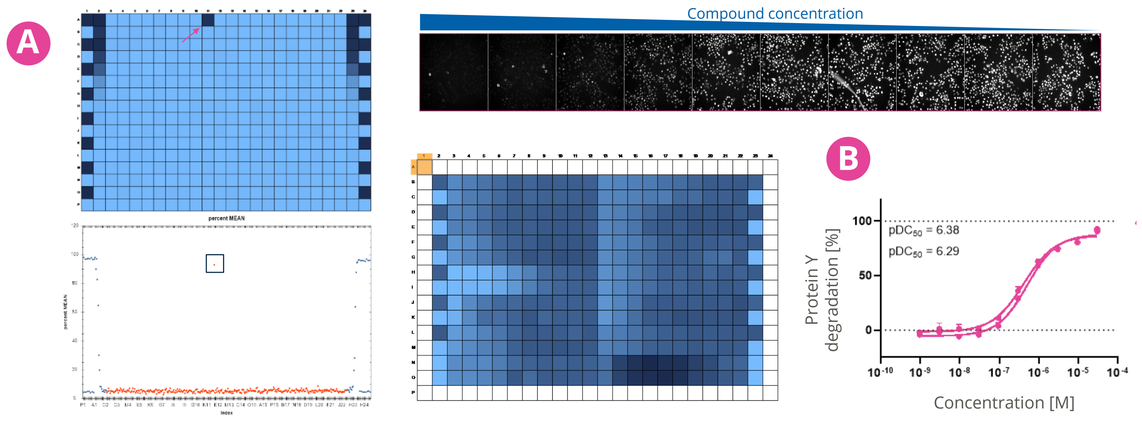


A cell-based NanoBRET assay is developed to measure target engagement and membrane permeability. It is performed on intact and permeabilized cells. **(A)** The apparent IC50 of a reference compound is consistent between live and permeabilised cells (indicating a highly permeable compound). **B)** Good target engagement of PROTAC X is only observed in permeabilized cells (indicating a good binder that is poorly cell permeable)



Cell-based efficacy screening using **High Content Imaging**

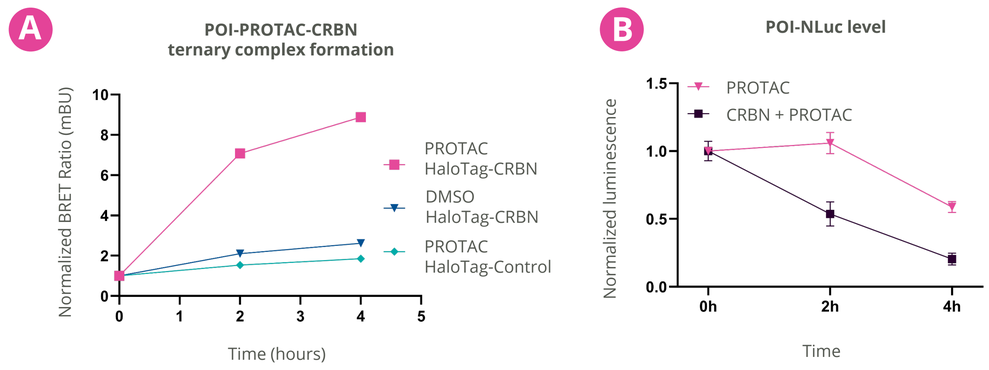
Example: Cellular target detection using immunofluorescence



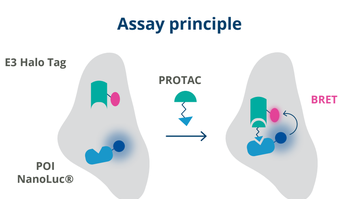
Quantitative immunofluorescence screening enables quick and efficient progression from TPD high throughput single-concentration screening **(A)** to hit confirmation and concentration-response studies **(B)**. This efficacy assay has been multiplexed with additional readouts (cellular morphology, cytotoxicity, cellular location, biomarkers etc) and applied to disease relevant cell lines and patient derived cells.

Measurement of **Ternary Complex** formation

Example: Cell-based ternary complex formation using NanoBRET



The cell-based Ternary Complex Formation NanoBRET assay measures TPD-induced proximity of an E3 ligase and a POI. **(A)** PROTAC treatment induced POI – CRBN interaction. **(B)** overexpression of CRBN leads to accelerated POI degradation, as quantified by POI-NLuc luminescence.



Summary

- In order to address research needs specific for Targeted Protein Degrader discovery, a suite of assays appropriate to a given target are developed and deployed.
- These methods cover all stages of compound development, and evaluation of key events leading to POI degradation: cell penetration by the compound, E3 ligase engagement, formation of ternary POI-PROTAC-E3 complex, direct measurement of ubiquitin transfer and proteasome engagement (not shown), as well as a number of methods for POI quantification
- In addition, full Medicinal Chemistry, Computer Aided Drug Design and DMPK support – critical in TPD development – are utilized in a fully integrated approach to degrader drug discovery