PROTAC Platform: Enabling Rapid Design, Characterisation and Assessment

Domainex
Enrich your Medicines Pipeline

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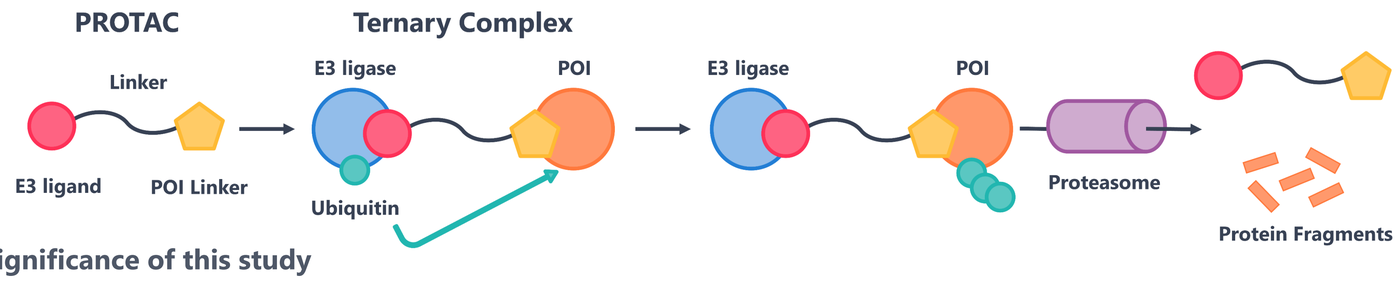
Introduction & Significance



What are PROTACs?

- **Pro**teolysis **ta**rgeting **c**himeras (**PROTACs**) are heterobifunctional molecules that induce proximity between an E3 ligase and protein of interest (POI) to facilitate ubiquitination and degradation of the target protein
- Ternary complex formation is required to induce proximity-driven ubiquitination
- Degradation of targeted protein is achieved by hijacking the ubiquitin-proteasome system which can offer numerous advantages versus traditional inhibitors

Significance of this study

- ✓ High-throughput parallel synthesis enables rapid cycle times and faster SAR
- ✓ An integrated platform promotes holistic understanding of chemical matter and aids smarter compound design in an area where property prediction is challenging
- ✓ Quick coverage of chemical space, establishment of SAR and hit ID, is better suited to earlier stage projects than traditional PROTAC platforms

Traditional PROTAC Platform

Phase I

Curation of POI ligand

Structural information
POI ligand SAR

Set of 10-20 designed PROTACs synthesised

- Different POI ligands
- Linker length & attachment group
- Choice of E3 ligases

Phase II

Optimise "best" PROTAC

Further SAR exploration around linker and linker attachments

- Potency
- Selectivity
- ADME

Nomination of PROTAC for *in vivo* studies

ADME Physchem Profiling

Assays

- Hepatocyte turnover
- Biorelevant solubility
- Media cell stability
- Permeability
- Cell accumulation

Biological Profiling

Inhibition assays

Degradation assays

- Nano-Glo® HiBiT detection system
- Automated western blotting

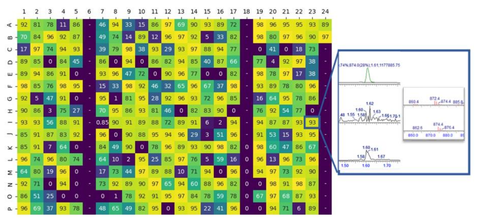
Evaluation of binary and ternary complex formation

- MST
- GCI
- FP
- Alpha screen/HTRF
- Nano DSF

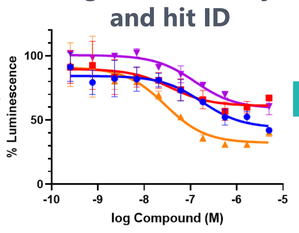
Direct-to-Biology (D2B) PROTAC Platform

Plate-based synthesis of PROTACs

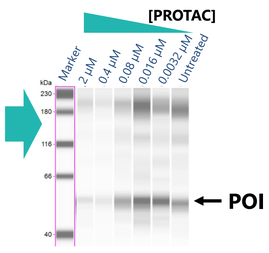
Automated UPLC analysis



Evaluation in degradation assays and hit ID



Hit Validation



- Up to 384 compounds synthesised, analysed and screened in single plate format. Throughput of 1000 compounds/month
- Minimal reagent and solvent use (0.25 µmol POI ligand, 10 µL solvent/ reaction)
- Crude mixtures used in hit identification using cell-based Nano-Glo HiBiT assays
- Hit validation performed using automated western blotting system
- Validated hits to be resynthesized and purified for evaluation of binary/ternary complex formation and ADME

Library of linker-E3 ligase ligands prepared in tube racks

Day 1: Set up reactions in 96 or 384 well plates using automation

Day 2: Dilute to assay concentration, generate QC plate

Day 3: Automated UPLC analysis, plates shipped for hit identification

Day 4-7: Cell based screening assay (Hit ID)

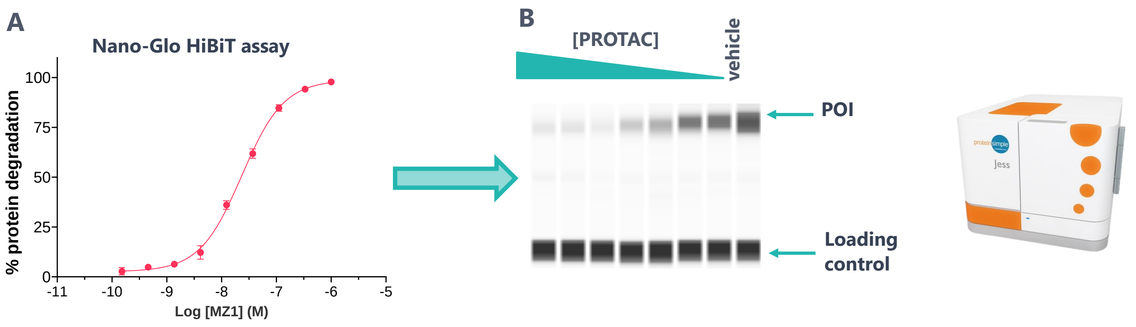
Day 7-8: Screening results available

Typical workflow and timelines for D2B approach

Biological Profiling

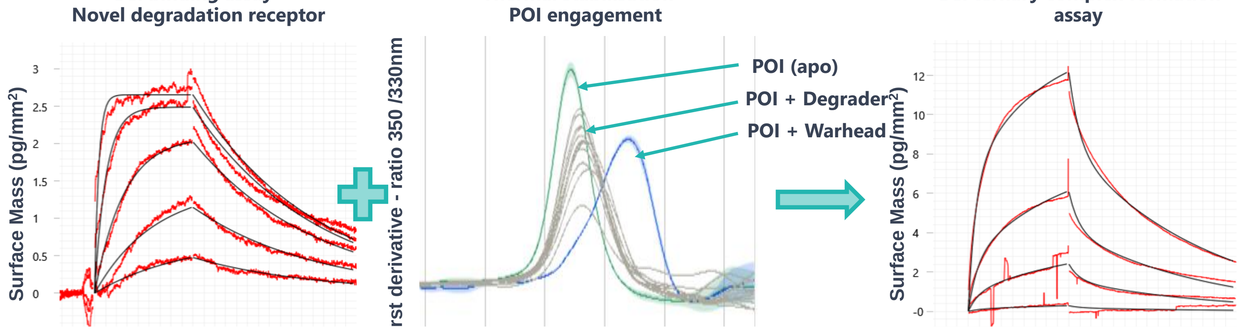
Degradation assays:

- Nano-Glo HiBiT assays can be used to quantify cellular protein and assess PROTAC potency (A)
- Native POI-specific protein degradation can be re-confirmed in an orthologous screen, using an automated, capillary-based immuno-assay system (Simple Western™) (B). Endogenous-tagging of POI is not required



Biophysical evaluation of binary and ternary complex formation:

- Biophysical suite (MST, GCI, NanoDSF, FP) for development of binding assays
- Characterise ligand and degrader binding to degradation receptor (A), POIs (B), and ternary complex formation (C)



Advancing the Field of PROTACs

- ✓ High-throughput plate-based chemistry (1000 compounds per month) and direct-to-biology to accelerate cycle times and rapidly build SAR
- ✓ Computational chemistry to direct initial PROTAC design phase (e.g. growth vectors for linkers, novel E3 ligase binding motifs and attachment points)
- ✓ Bespoke linker-E3 ligase ligand toolbox with high diversity (linker type, length and rigidity, E3 ligase type and attachment points, etc)
- ✓ Comprehensive biochemical, biophysical, and cellular profiling, integrated with chemical design & synthesis
- ✓ Rapid ADME and physchem profiling to accelerate property optimization

Domainex welcomes interest from any potential collaborators, industrial or academic. If you would like to learn more about our drug-discovery platform, please contact: **enquiries@domainex.co.uk**

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