A platform to harness new E3 ligases for targeted protein degradation

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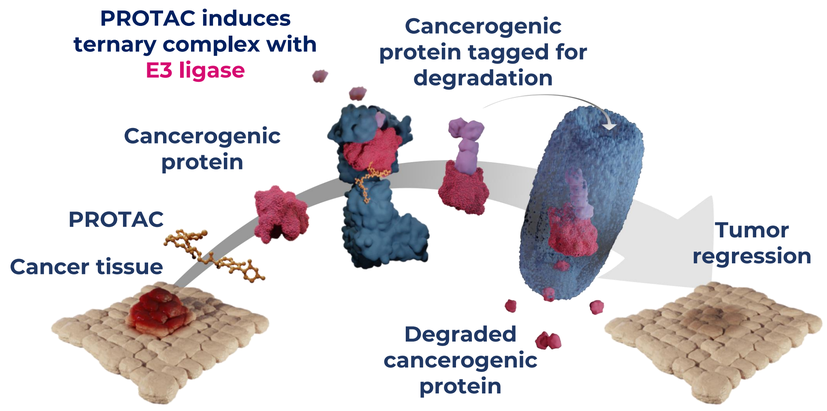
Captor Therapeutics S.A.

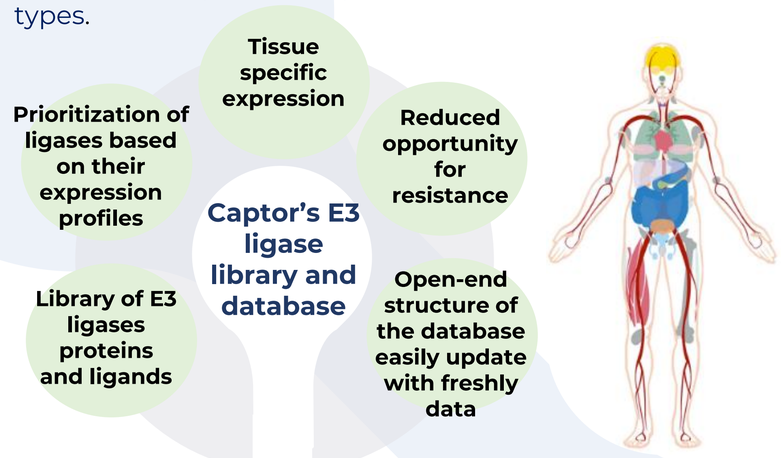
Introduction

Targeted protein degradation (TPD) has become a widely used strategy to discover novel drugs. It is based on removing pathogenic proteins from the cell, including "undruggable" proteins. Targeted protein degraders can be designed as proteolysis-targeting chimera (PROTAC) or molecular glue. PROTAC comprises an E3 ligase recruiter, linker, and target protein ligand. This heterobifunctional molecule leads to the proximity of the E3 ligase complex to the target protein and its subsequent degradation in the proteasome. Currently, most biologically functional degraders use E3 CRBN or VHL ligases. However, over 600 E3 ligases are active in human cells, suggesting a large pool of E3 ligases that could potentially be used in targeted protein degradation technology. Here, we give an overview of the platform's components and outline the processes that led to identifying of novel PROTAC handles for unprecedented E3 ligases.



Target selection: E3 ligase databsae

SQL database to identify E3 ligases with the desired biological properties. The database contains expression profiles of ubiquitination proteins in various tissues and cell types.



Production of recombinant E3 proteins

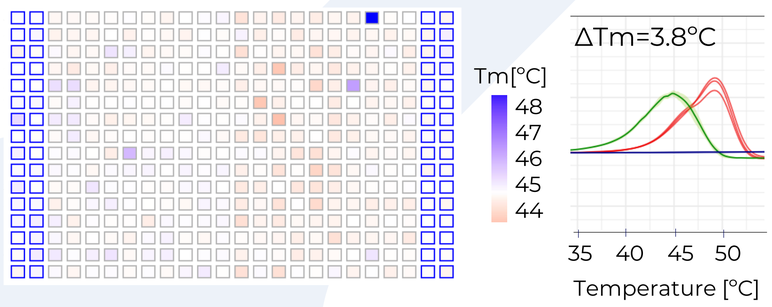
Production of recombinant proteins is carried out in bacteria or insect cells. QC and activity data of every protein sample are stored in the dedicated repository.

Screening assays

DSF for hit selection

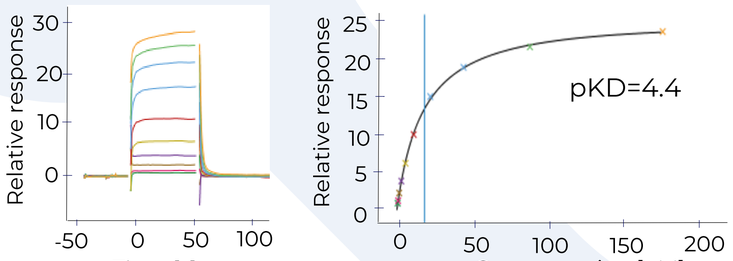
- Fragment screening to early assessment of the chemical tractability of the E3 through a collection of 2500 Ro3-compliant fragments
- Promising hits identified, sharing common pharmacophore features
- Screened 10 E3 prioritized by biological profile

Heatmap showing Tm values



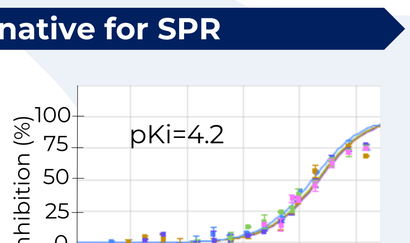
SPR for determining affinity

- Testing multiple immobilization strategies and assay conditions
- Running fragment screen and dose-response experiment for selected hits.
- Successful determination of pKD for compounds



FP as an alternative for SPR

- Establishing an FP assay with labelled control peptide
- Dose response experiment for selected hits
- Successful determination of pKi for hit compounds

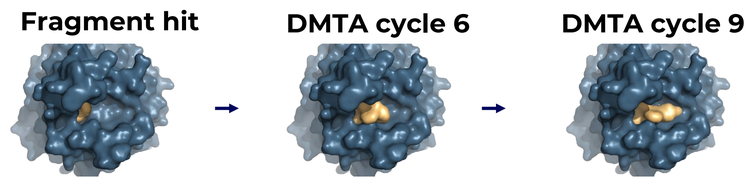


Ligases that yield robust fragment hits are progressed further into crystallization trials.

Structural biology of E3s

We developed X-ray crystallography systems for eight ligases, for which we have generated >100 X-ray structures. Knowledge of fragments binding poses facilitates structure-based drug discovery and enables progress of the hit-to-lead phase for some E3 targets.

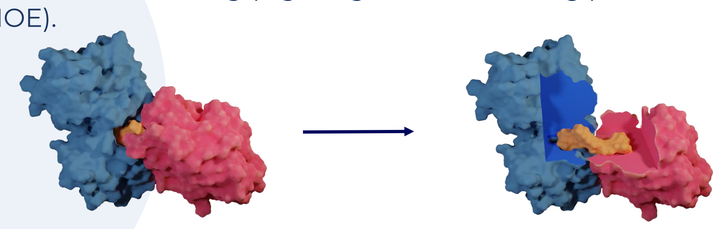
Structure guided hit-to-lead process for KLHDC2



Discovery of PROTACs

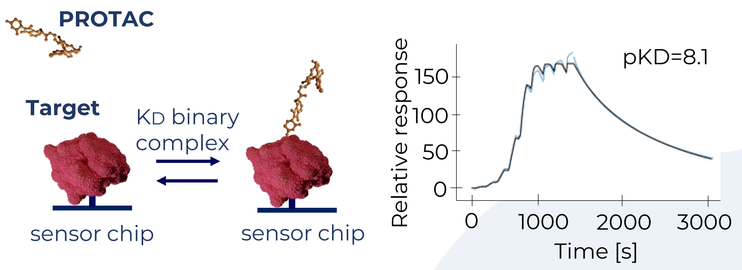
We employ the synthesis of bifunctional degraders against model proteins (including BRD4 and Halo-tag) and promiscuous kinase inhibitor warheads.

Design of model bifunctional molecules is based on molecular modeling (e.g., degraders modeling protocol of MOE).

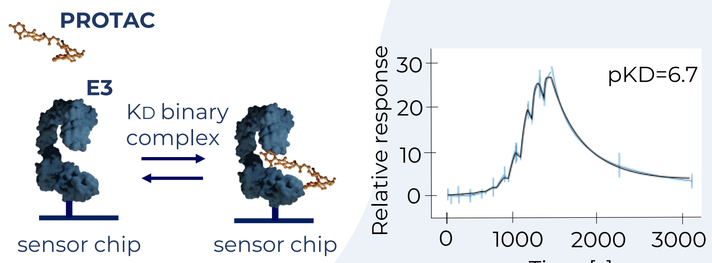


In vitro binary complex can be measured by SPR by protein or ligase immobilization, then the ternary complex formation can be confirmed by HTRF.

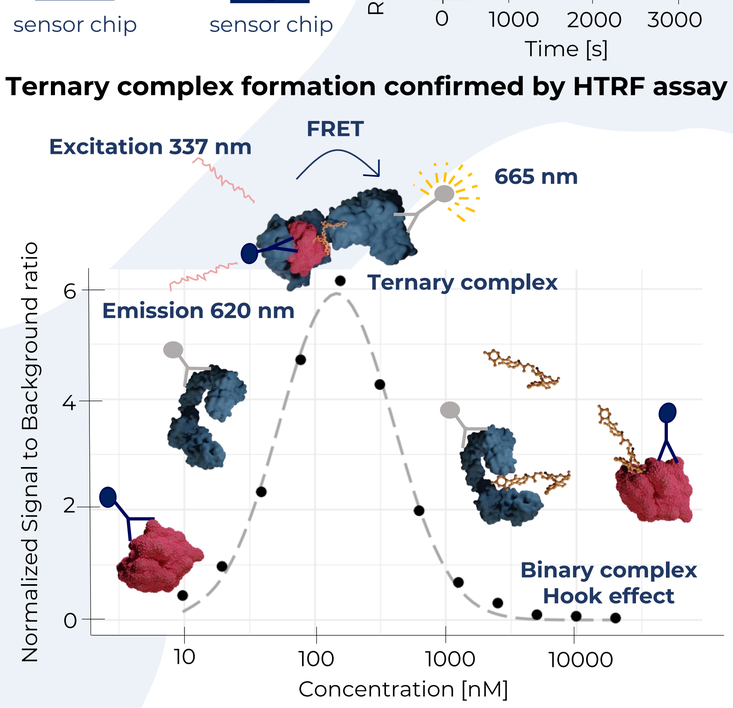
Binary complex with immobilized target on sensor chip



Binary complex with immobilized ligase on sensor chip

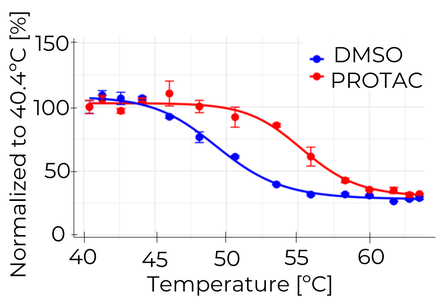


Ternary complex formation confirmed by HTRF assay



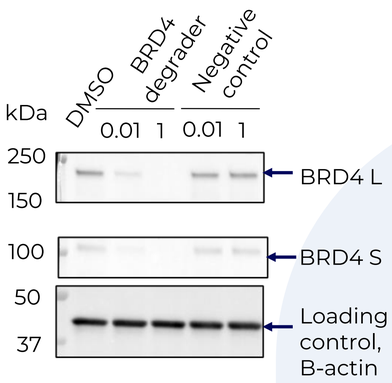
Cellular assays

Intercellular stabilization assay

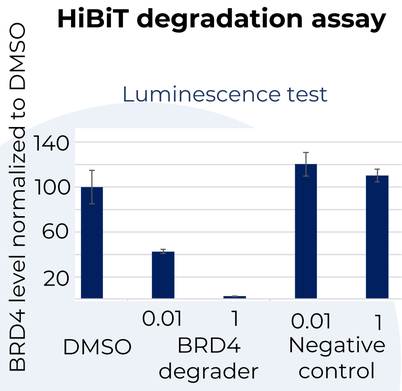


- developed cellular assays such as intercellular thermal stabilization and bioluminescence test to confirm E3 ligase engagement and cell-membrane permeability
- various methods to establish the degradation of model substrates, such as Western blot-based assays, luminescence tests, or proteomics

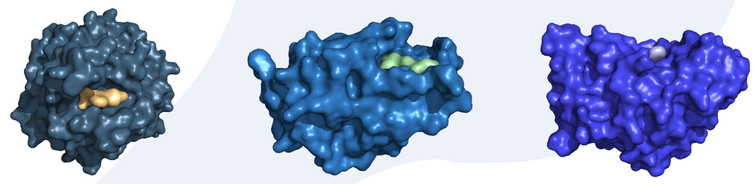
Western blot-based degradation assay



HiBiT degradation assay



Captor Therapeutics' ligase portfolio



Current best ligand for KLHDC2 :	Current best ligand for ligase A:	Current best ligand for ligase C:
KD=12 nM	KD=25 nM	KD= 500 nM
LE = 0.37, MW = 430Da, logP=1.6, TPSA= 101 Å2	LE = 0.33, MW=440 Da, logP= 4.1, TPSA=75 Å^2	LE = 0.59, MW=201 Da, logP= 1.0, TPSA=68 Å2
✓ over 30 X-ray structures ✓ 3 chemotypes identified ✓ 5 different EVs identified ✓ target engagement in cells	✓ 49 X-ray structures ✓ in cell target engagement ✓ good solubility, chemical and plasma stability ✓ molecular glue opportunity	✓ 24 X-ray structures ✓ shallow binding site ✓ attachment SAR ✓ solvent exposed ligand

Conclusions

Our library of E3 ligase proteins and ligands has established the drug discovery pipeline to generate new E3 ligase binders and transform these into PROTACs. The new ligases are desirable from a therapeutic point of view because they reduce the likelihood of resistance and provide tissue-specific expression.

Acknowledgements

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