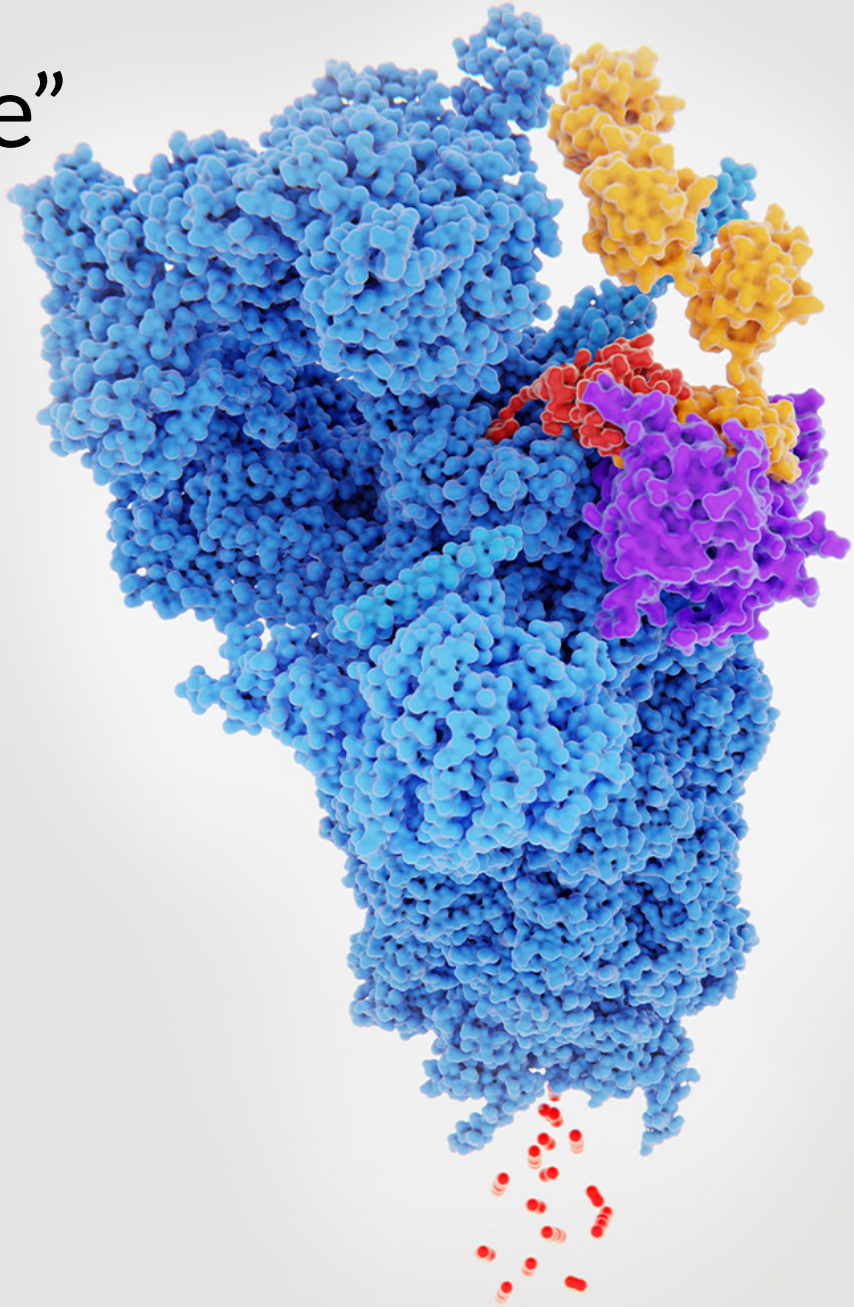
VIEW POINT

Accelerating **PROTAC programs** to drug the “undruggable”



Syngene

Putting Science to Work

Overview

PROteolysis TArgeting Chimeras (PROTACs) have garnered a lot of attention in recent times for their ability to exploit the cell's own degradation machinery to irreversibly degrade target proteins.

In this point of view, we discuss how Syngene can help you move your PROTAC programs from hit to lead to the clinic with speed. We are a one-stop solution for all targeted protein degradation (TPD) research requirements across a variety of molecules, including heterobifunctional degraders and molecular glue degraders. Our experience allows us to create a roadmap for X-TACs research programs, supported at each stage by our experienced team of scientists across chemistry and biology.

Introduction

PROTACs are heterobifunctional molecules comprising a ligand targeting a protein of interest (POI) and a ligand targeting an E3 ligase with a connecting linker. This novel design utilizes the organism's own ubiquitin-proteasome system and induces proximity between E3 ligases and POI, leading to its proteasomal degradation.

PROTACs, differ from conventional small molecules in their catalytic mechanism by way of inducing the degradation of the target protein. PROTACs have demonstrated success and remarkable biological responses across challenging targets, including non-druggable targets. Not only is this novel strategy enabling the development of more potent and selective treatments for cancer and other intractable diseases, but it is also unlocking parts of the proteome that have traditionally been considered "undruggable". As a result, targeted protein degradation (TPD) therapeutics have emerged as a relatively new modality with the potential to revolutionize the field of drug discovery.

With 200+ dedicated TPD scientists and more than 15 global clients, Syngene has a strong track record in accelerating PROTAC programs for its clients. To date, Syngene's Discovery team has successfully identified Hits and leads across different projects, including programs moving into preclinical development through a strong collaborative effort (Figure 1).

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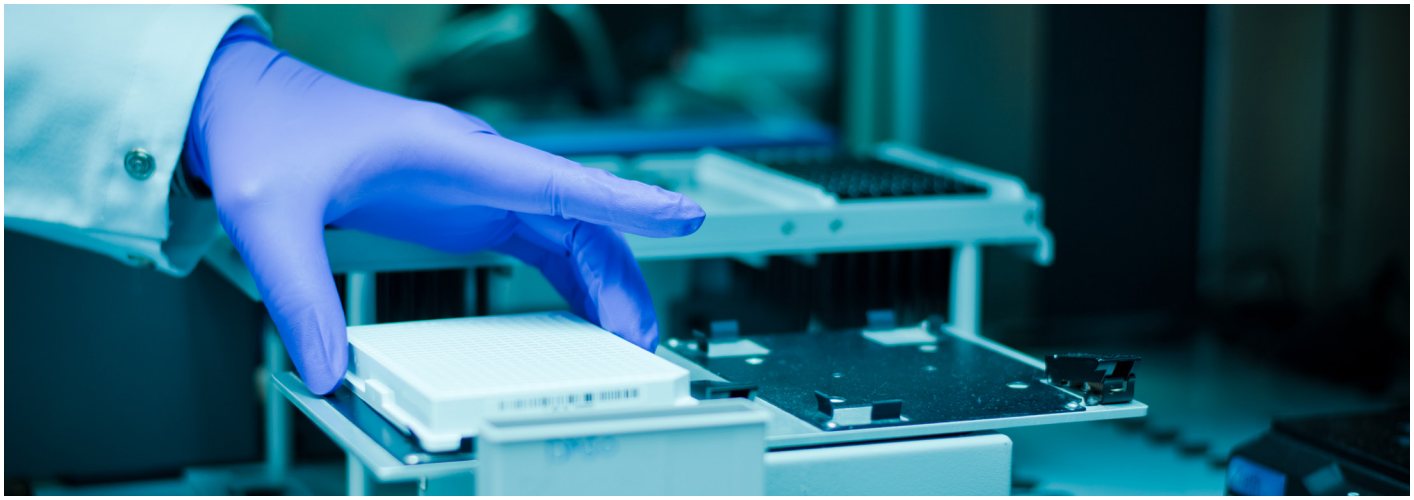
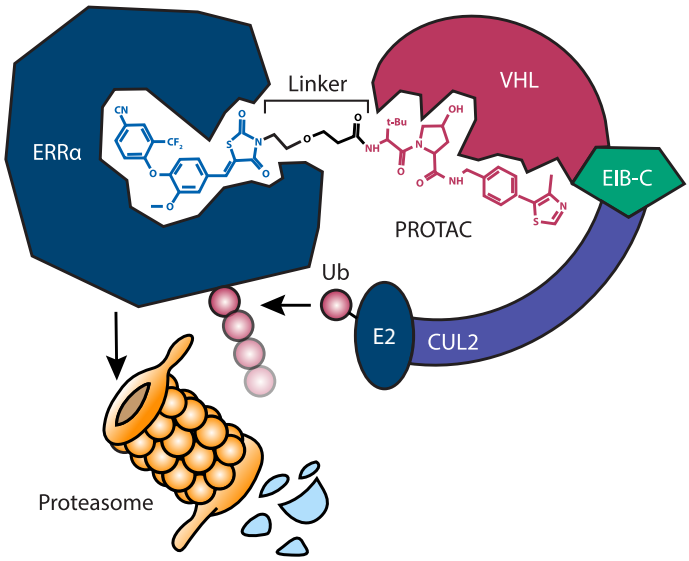
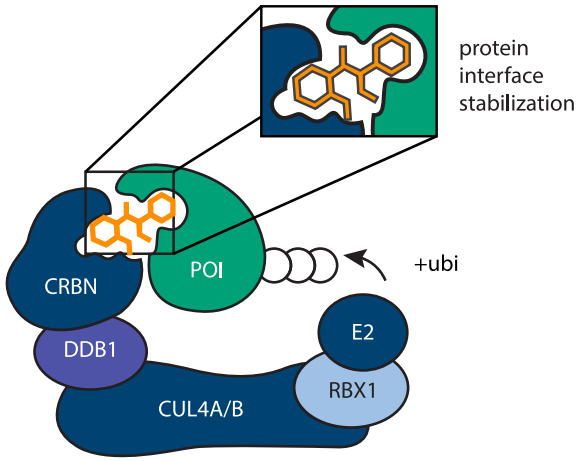


Proteolysis Targeting Chimeras (PROTACs)



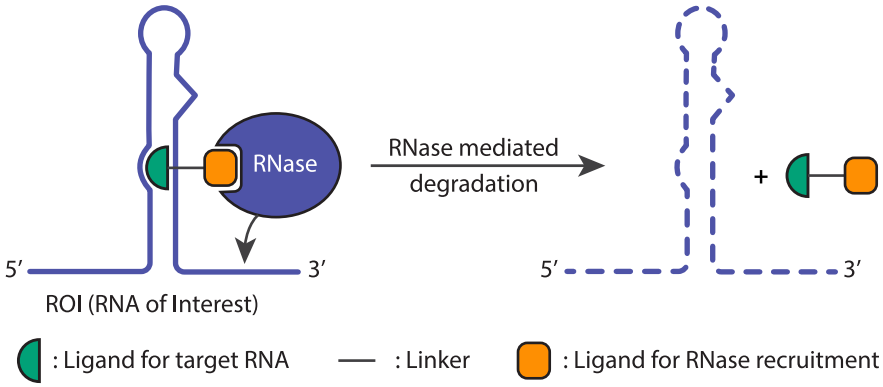
Nature Chemical Biology
volume 11, pages 634–635(2015)

Molecular Glue



Current Opinion in Chemical Biology
Volume 56, June 2020, Pages 35-41

Ribonuclease Targeting Chimeras (RIBOTACs)



Cell chemical Biology, Volume 26, Issue 8, 15 August 2019, Pages 1047-1049

Figure 1: Syngene's capability in Targeted Degradation (XTACs)

Key challenges in developing novel degraders for TPD

Heterobifunctional degraders such as PROTACs offer a number of advantages over traditional small molecules. These include:

- Providing a step-up in regulating protein levels as PTOTACS can also affect the non-enzymatic function of the protein
- Providing an alternative to targeting drug-resistant proteins for overcoming tumor drug resistance,
- Effective against "undruggable" targets such as transcription factors
- Highly efficient against scaffolding molecules which cannot be targeted by the traditional small molecule inhibitors

However, several aspects need to be considered before developing these degraders and translating them from bench to clinic. Only a careful analysis will ensure that the degrader is efficient, specific, and optimized for its target protein (Figure 2)

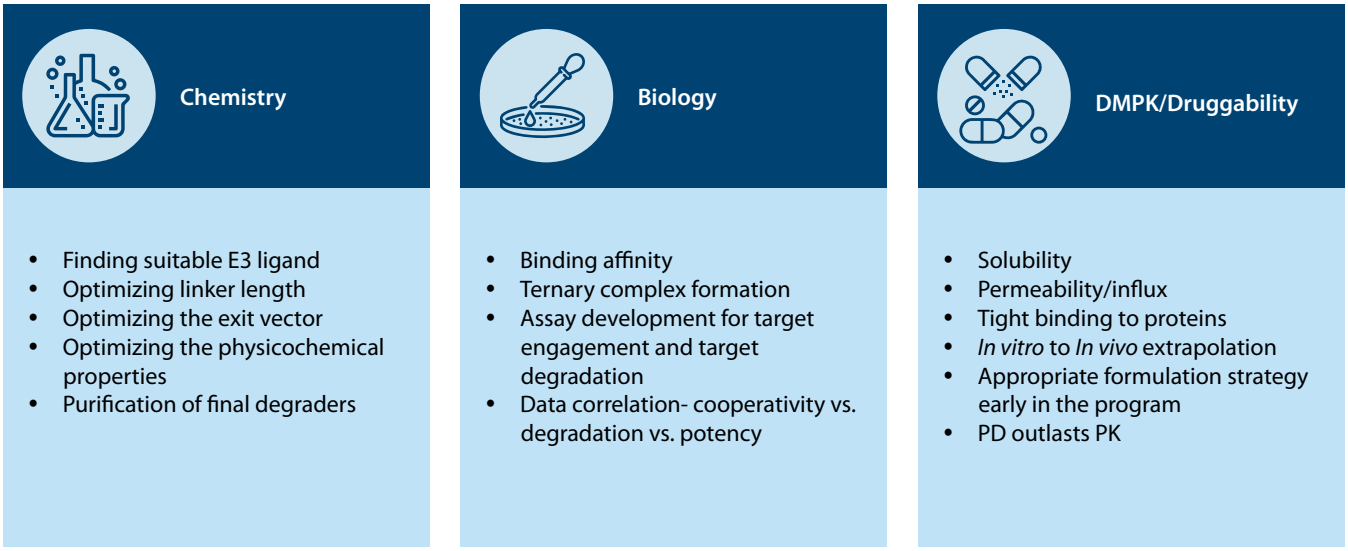


Figure 2: Syngene's capabilities in addressing key challenges in heterobifunctional degrader discovery

Although PROTACs are considered revolutionary, to date, the design and optimization of PROTACs remain empirical. This is due to the complicated mechanism of induced protein degradation and molecular weight and size, leading to solubility, permeability, and bioavailability issues. This limitation calls for an efficient workflow with assay development that helps enhance our understanding of the structure-activity relationship facilitating the rational design of PROTACs.

Heterobifunctional degraders consist of two different subunits performing distinct functions. Several properties need to be standardized for synthesizing a heterobifunctional molecule before it can be made to effectively induce the degradation of the target protein.

Mammalian cells consist of around 600 different ubiquitin ligase proteins, all suited to bind different proteins. The initial chemistry challenge to designing a degrader molecule is to choose a suitable E3 ubiquitin ligase and a stably binding exit vector. The two motifs then need to be attached using a linker of appropriate length so that the target protein and the E3 ligase are at the optimum orientation for ubiquitination.

Once such a degrader is designed, its physicochemical properties must be tested to ensure the maximum degrading effect, followed by its synthesis and purification for application to biological systems.

The success of the degrader in biological systems must be assessed on the following grounds:

- Binding affinity to target protein *in vivo*
- Formation of a stable ternary complex
- Efficient engagement of target protein
- Absence of non-specific binding
- Potent degradation of the protein of interest

Finally, the potential to use the degrader as a therapeutic drug, or its 'druggability,' must be analyzed, based on the following:

- Solubility in an aqueous medium,
- Permeability into mammalian, especially human cells
- Stability and ability to bind tightly to the target protein
- Pharmacodynamic (PD) and pharmacokinetic (PK) correlations

A heterobifunctional molecule that ticks all these boxes will have strong translational potential and clinical applications.

Syngene's capability in developing degraders

A concerted approach to designing PROTACs that consider all the above challenges is the need of the hour. TPD strategies that can be used to tackle the growing number of undruggable targets are crucial for coming up with novel therapeutic approaches for treating real-world patients.

Syngene's PROTAC capabilities put it at the forefront of developing protein degraders across a range of diseases. Syngene has been working right from establishing the proof-of-concept (POC) studies for the target of interest to hit identification and lead optimization until the nomination of a clinical candidate for TPD. We have supported our clients through our collaborative programs from early discovery to development and candidate selection for multiple targets in this segment.

Syngene is expanding its capabilities to support programs based on molecular glues, ribonuclease targeting chimeras (RIBOTACs), and lysosome-targeting chimeras (LYTACs), generally termed as X-TACs. Our experience in chemistry, biology, and DMPK allow us to create a roadmap for X-TACs research programs.

We have expertise in the following:

- Hit identification and Targeted library synthesis
- Computational drug designs for X-TACs
- Synthesis, optimization, and purification
- Screening and target degradation assay
- DMPK characterization
- Assessment of druggability

Hit identification and Targeted library synthesis

Syngene can offer top-of-the-line PROTAC chemistry allowing fast-track validation and proof of concept. We can do this by identifying PROTACs for targets through a rapid synthesis of the libraries, spanning small molecule binders with different exit vectors, linkers, and E3 ligands. This includes cereblon (CRBN), von hippel–lindau ligase (VHL), and inhibitors of apoptosis protein (IAP).

To date, Syngene has completed the synthesis of a large number of libraries with a very high success rate (>99%). Each library consists of a diverse set of linkers, exit vectors, and E3 ligands, which are processed through rapid purification using automated systems.

Computational Drug Designs for X-TACs

We provide computational drug design solutions for X-TACs by establishing the binding mode of warhead/ligand in the target protein, identifying exit vectors for attachment of linkers, and enabling library enumeration of target compounds with appropriate linkers.

Following the molecular dynamics simulations, the molecules' functionality is tested by generating initial models of the ternary complex of target protein, ligand, and E3 ligase. Additionally, the insights from molecular simulations of the ternary complex provide a good understanding of the binding modes of ligands.

Medicinal Chemistry and Optimization

Syngene has strong expertise in X-TAC synthesis and optimization for TPD programs. We offer solutions that aid in the standardization of suitable E3 ligand, linker length, exit vector, physicochemical, and ADME (absorption, distribution, metabolism, and elimination) properties of the assembled molecules.

We have expertise in synthesizing linker acids and amines with E3 ligases in multigram quantities and PROTAC degraders on a scale for advanced studies. Our experience in this class of molecules gives us an advantage in providing clients with services from concept to preclinical candidates.

We have successfully optimized the different regions of a novel small molecule inhibitor and achieved sub-nanomolar potency with excellent metabolic stability and PK profile. We have also generated and modified various E3 ligase ligands such as CRBN, VHL, IAP, and MDM2 (mouse double minute 2 homolog) as a part of our discovery efforts (Figure 3). Using these ligands, we have synthesized and optimized different PROTAC degraders with specific E3 ligands.

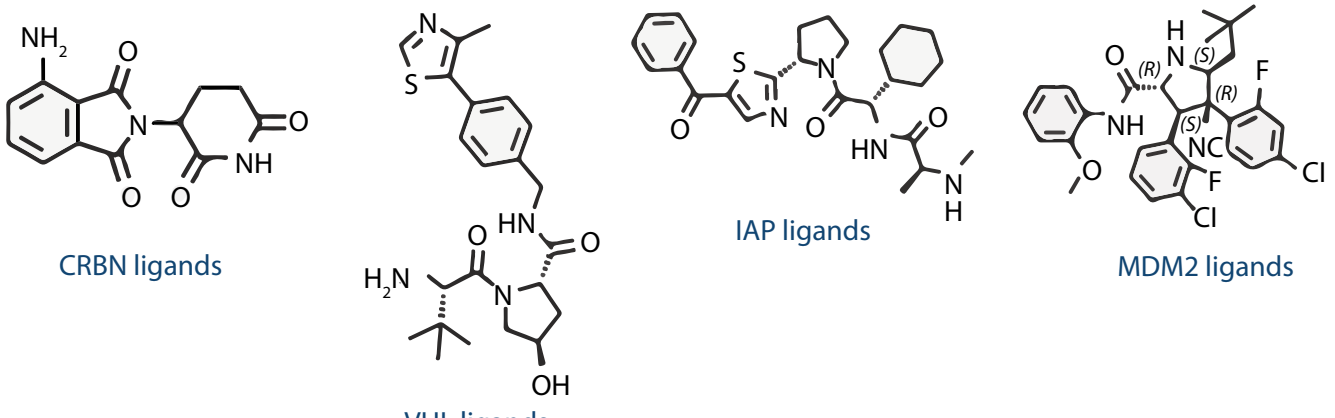


Figure 3: E3 ligase ligands optimized by Syngene

Biology

Despite rapid progress, PROTAC discovery remains challenging and laborious, and largely defined by an empirical process. Hence, it is important to have a set of techniques and assays that can characterize and rank such heterobifunctional compounds rapidly and efficiently for different properties and provide valuable feedback to guide medicinal chemists during the lead discovery and lead optimization process.

The compound profiling for PROTACs involves a step-wise approach engaging the ubiquitin-proteasome degradation pathway using biochemical, biophysical, and cellular assays. This is to ensure that the molecules are not only penetrating the cells but also forming a ternary complex amongst POI, PROTAC, and E3 Ligase followed by target ubiquitination degradation (Figure 4).

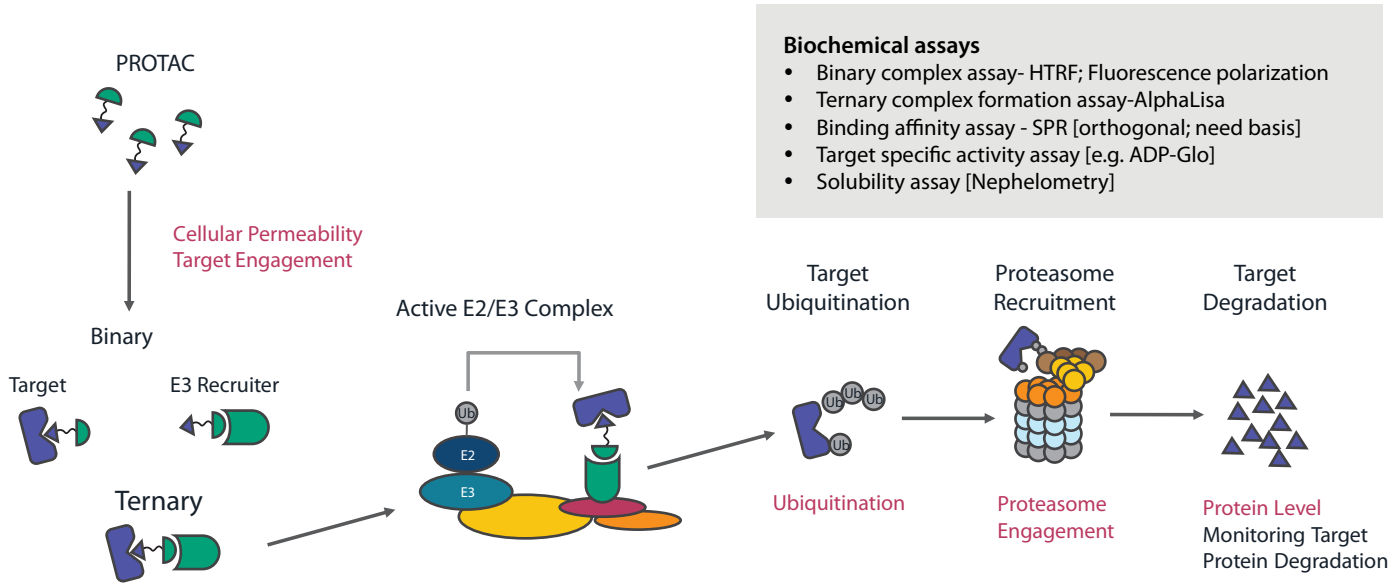


Figure 4: Syngene's list of biochemical and cell-based assays to query PROTACs

The Syngene Biology team has developed fluorescence polarization (FP), surface plasma resonance (SPR), and amplified luminescent proximity homogeneous assay (ALPHA) technology-based binding assays in the cell-free system for binding affinity characterizations. Additionally, our Assay Biology team has provided support in developing a cell-based target engagement assay using Nano-BRET and an in-house derived ALPHA-LISA platform for determining the binding of compounds to E3 ligases. The target degradation in the cellular setting is further evaluated using different approaches, including regular Western blot, WES, HiBiT assay, and using In-Cell Western (ICW).

HiBiT assay is a sensitive, faster, and high throughput assay using Nano-Glo technology and is extensively used for studying the targeted protein degradation intracellularly. We are currently conducting HiBiT assays for more than 15 targets in cell lines supplied by a partner or generated at Syngene (Figure 5).

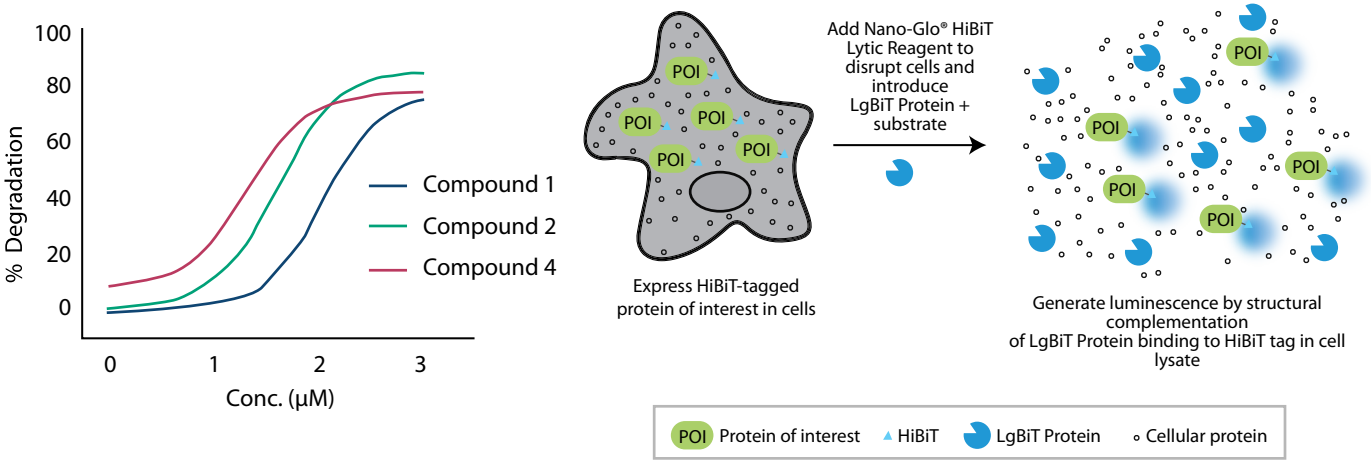


Figure 5: Cellular Target Degradation assay using HiBiT Nano-Glo technology

The combination of these technologies and selection of the right assay platforms helps the chemistry team understand the mechanism of action of the PROTAC compounds.

Syngene is currently running several integrated drug discovery research programs with multiple targets to screen and identify molecules that are superior in terms of solubility, cellular permeability, and degradation capabilities.

Currently, we are engaged in expanding our existing biophysical, biochemical, and cellular assay toolbox by introducing new technologies or new assays to provide new insights into the mode of action of PROTAC degraders.

Assessment of Druggability

Optimized degrader molecules have fast rates of degradation and relatively short exposure to therapeutic doses that can result in the complete elimination of the target protein. This helps in obtaining a durable and sustained effect, subject to protein turnover rate. Our optimized technology and assay platform can be utilized for any soluble target and bring discovery efforts on a fast track for research programs of interest in multiple disease areas.

Syngene has the capability to optimize a number of properties for any degrader to enhance its druggability. This includes its solubility, permeability, ability to bind tightly to proteins, ensure efficient functioning *in vivo*, and thermodynamic stability.

We use a battery of assays to analyze these properties before pronouncing a new heterobifunctional degrader ready for biological use. These include:

In vitro ADME assays

- Solubility (kinetic);
- LogD
- Plasma and whole blood stability
- Metabolic stability in hepatocytes (mouse (most preferred), rat, rabbit, dog, monkey, and human)
- Bidirectional Caco-2 permeability- specially designed for PROTACs
- UC-PPB (custom-based with stability assessment) and RED
- Cocktail CYP inhibition
- Time-dependent CYP inhibition (need-based)
- Metabolite identification (value-added services) in hepatocytes- specific to PROTACs
- Preclinical formulation development and stability studies

PK studies

- Cassette PK studies in mice
- Single-dose oral and IV PK studies
- Tissue exposure studies
- Blood and plasma PK studies

PK-PD studies are challenging for PROTACs for the reasons described above and to establish a correlation. Syngene team is using different approaches for drawing the PK-PD correlation followed by testing the molecule in the animal efficacy models based on disease relevance.

Conclusion

The degrader toolbox is growing rapidly, with many pharmaceutical companies investing in this revolutionary modality, especially for diseases like cancer. By partnering with CROs/CDMOs like Syngene, who have expertise in TPD and a deep understanding of the ubiquitin system, biotechs can scale operations quickly and cost-effectively. Syngene has significantly optimized the various steps involved in taking a degrader from bench to clinical development, thereby cutting down the time and effort needed to identify the right TDP approaches successfully.

While inhibitors need to achieve a high degree of target engagement over an extended period of time to exert their sustained pharmacodynamic effects, PROTACs act catalytically and are ready to bind to another molecule of a target protein after a limited engagement with it. This mechanism, along with target specificity, can help in improving the therapeutic index and minimizing safety liabilities.

Syngene's drug discovery efforts which involve step-by-step profiling of compounds in sync with platforms associated with Ubiquitin proteasome system and pathway, help medicinal chemists design better molecules with physiologically relevant effects.

In a nutshell, we are a one-stop solution for all TPD research requirements across various molecules, including heterobifunctional degraders and molecular glues.

About the authors

Atul Tiwari

Atul Tiwari, Director – Discovery Biology, Syngene International Ltd.

Atul is a Ph.D. in Biochemistry with 21+ years of relevant drug-discovery industrial experience in Small and Large Molecules based on in-house and collaborative research. This includes IND filing of six drugs that underwent different stages of clinical trials (Phase I and Phase II) and nomination of multiple clinical candidates. The experience lies in multiple therapeutic areas, namely Oncology/IO, Metabolic Disorders, Autoimmune Disorders, CNS (Parkinson, Narcolepsy), and Urology (BPH & UI). Prior to joining Syngene in 2012, he worked with Ranbaxy Research Laboratories, Jubilant Biosys, and Advinus Therapeutics. Currently, Atul is associated with integrated drug discovery programs and leading the Discovery X-TAC strategy at Syngene.

Santosh Kulkarni

Deputy Research Director – Discovery Chemistry, Syngene International Ltd.

Santosh is a Ph.D. in Chemistry with 21+ years of experience in medicinal chemistry and drug discovery. He has extensive experience in small molecule drug discovery, Hit to lead, lead optimization, and candidate selection. His medicinal chemistry experience spans enzymes, GPCRs, PPIs, transporters, and nuclear receptors. He has experience working with novel modalities such as heterobifunctional degraders and molecular glues. He has successfully led teams to deliver preclinical candidates for partners, which have transitioned into clinical development. He has also authored over 60 patents and publications.

To know more about our PROTAC services or to contact our experts, click here

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About Syngene

Syngene International Ltd. (BSE: 539268, NSE: SYNGENE, ISIN: INE398R01022) is an integrated research, development and manufacturing services company serving the global pharmaceutical, biotechnology, nutrition, animal health, consumer goods and specialty chemical sectors. Syngene's more than 4700 scientists offer both skills and the capacity to deliver great science, robust data management and IP security and quality manufacturing at speed to improve time-to-market and lower the cost of innovation. With a combination of dedicated research facilities for Amgen, Baxter and Bristol-Myers Squibb as well as 2 Mn sq. ft of specialist discovery, development and manufacturing facilities, Syngene works with biotech companies pursuing leading-edge science as well as multinationals, including GSK and Merck KGaA.

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