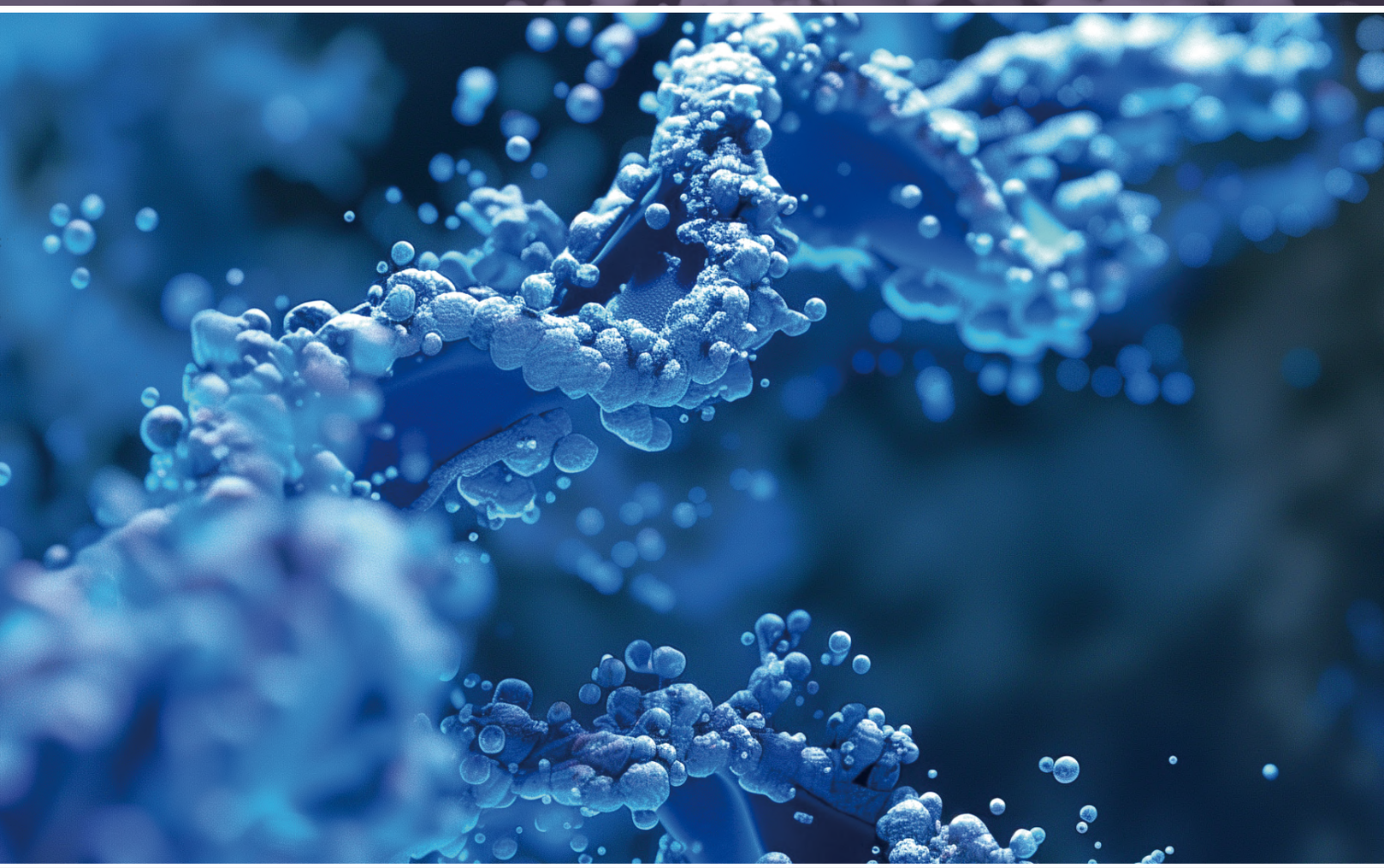
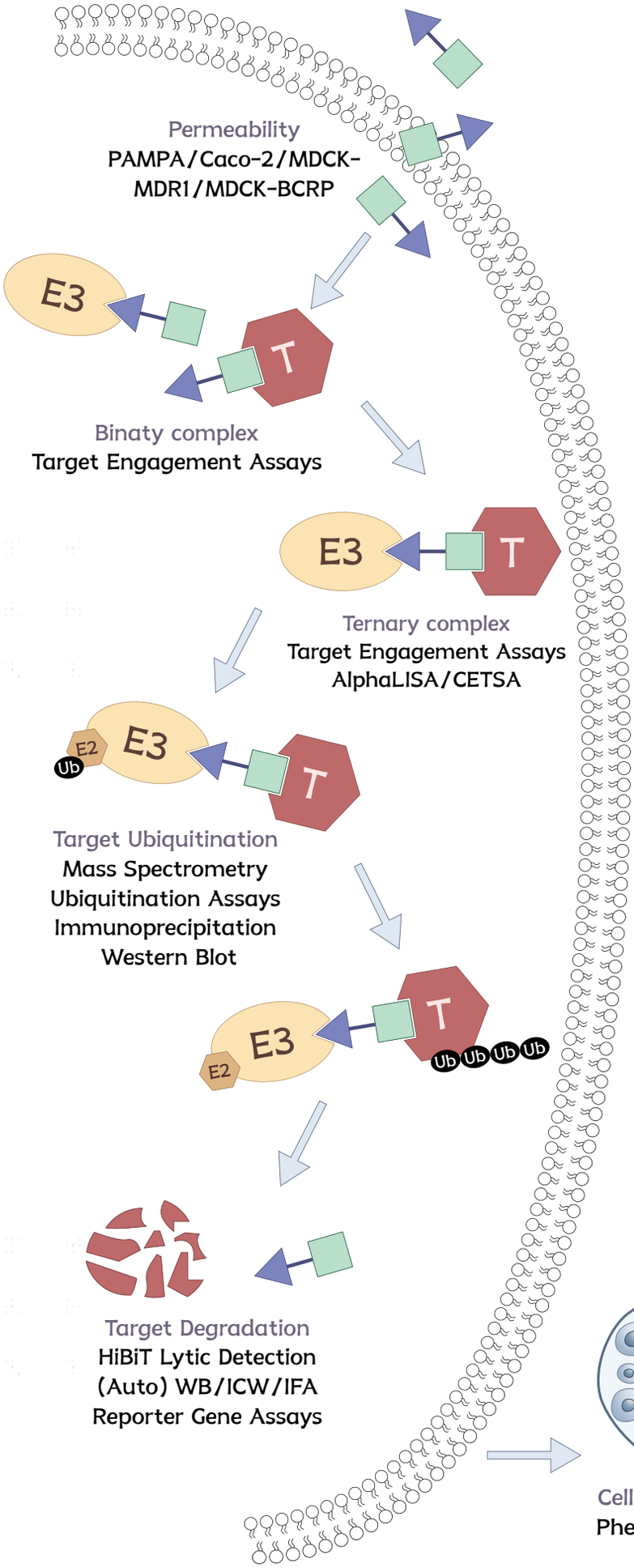
Targeted Protein Degradation
Drug Discovery Solutions

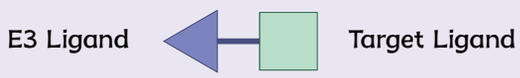


Innovative CRO+Explorer
ICE Bioscience

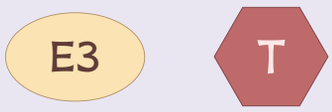
Drug Discovery Solutions for PROTACs



PROTAC



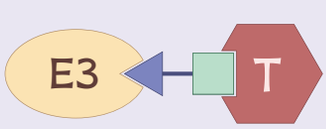
Solubility
Stability



Protein Expression & Purification

Binary Complex: E3 ligase binding

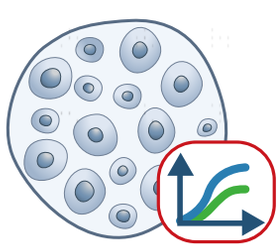
Binary Complex: Target binding



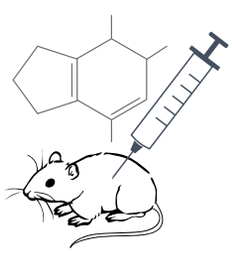
Ternary Complexes: Target protein binding

Biophysical Assays
SPR/DSF/MST

Tagged HiBiT Fusions with CRISPR/Cas9
E3 Overexpression (OE) / Knockdown (KD)
Target Knockdown (KD) / Knockout (KO)



Cell Panel Screening
Phenotypic Profiling



In Vivo Study
PK/PD
Biomarker study

Targeted Protein Degradation

Targeted protein degradation has emerged as a transformative approach in drug discovery, offering promising avenues for addressing challenging therapeutic targets. Traditional drug discovery strategies have predominantly focused on modulating protein function through inhibition or activation. However, these approaches may be limited by factors such as target accessibility, specificity, and the emergence of resistance mechanisms. Targeted protein degradation represents a paradigm shift by directly removing disease-causing proteins, offering advantages in terms of target diversity and selectivity.

Two prominent modalities within targeted protein degradation are PROTACs (Proteolysis Targeting Chimeras) and molecular glues. PROTACs function by recruiting an E3 ubiquitin ligase to a target protein, leading to its ubiquitination and subsequent degradation by the proteasome. This strategy enables the degradation of proteins considered "undruggable" by traditional small molecule inhibitors. On the other hand, molecular glues induce protein-protein interactions that result in the degradation of specific targets, providing an alternative approach to PROTACs.

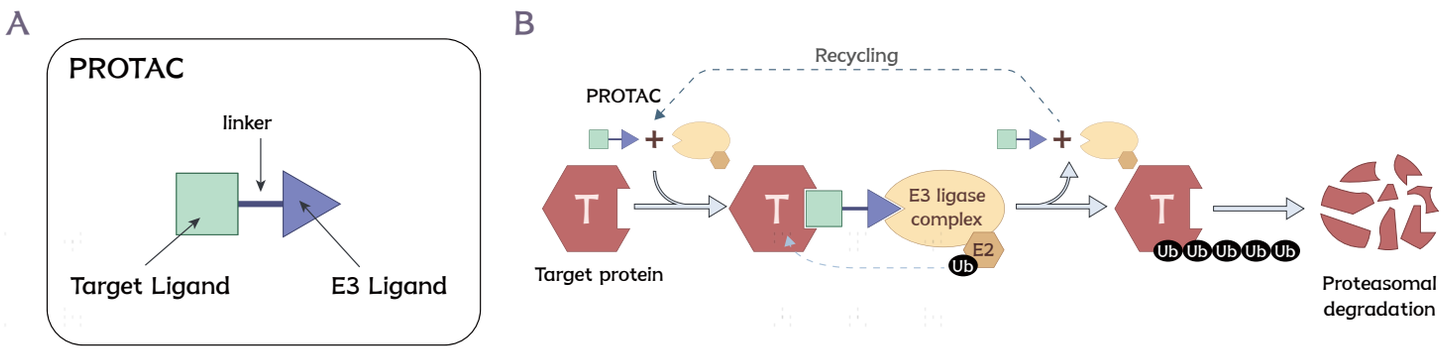
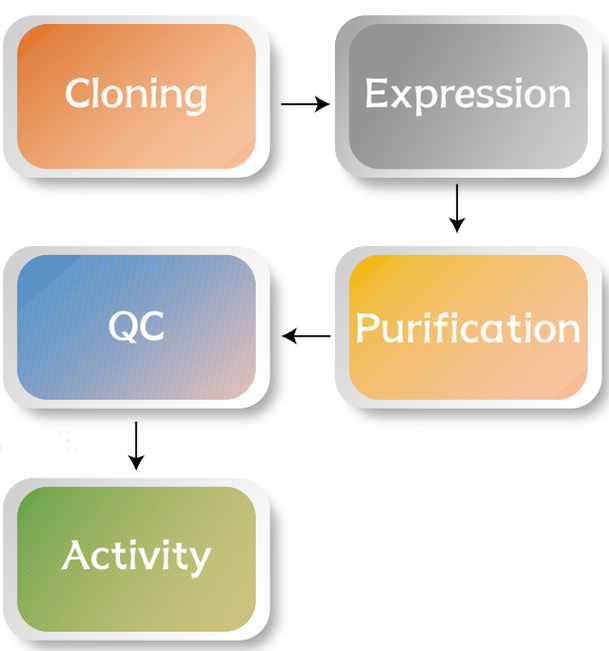


Figure 1. (A) Basic components of a PROTAC – featuring a small molecule ligand, a linker, and an E3 ubiquitin ligase recruiting moiety. (B) The mechanism of action (MOA) of PROTACs involves linking a small molecule ligand with an E3 ubiquitin ligase recruiting moiety to achieve targeted protein degradation. When a PROTAC forms a ternary complex with the target protein (T) and the E3 ligase (E3), it induces ubiquitination of the target protein, marking it for degradation by the ubiquitin-proteasome system, thereby facilitating the specific degradation of the target protein.

Protein Production Expertise for TPD



- **Versatile Expression Systems:** Bacterial, insect, and mammalian systems for optimal protein yield and functionality.
- **High-Purity Purification:** Advanced chromatographic techniques ensure proteins of the highest purity and activity.
- **TPD Research Support:** Essential proteins for PROTAC development, binding studies, and functional assays.
- **Collaborative Development:** Tailored support from gene to protein, accelerating TPD projects from concept to reality.
- **Quality Commitment:** Dedicated to delivering research-grade proteins, facilitating breakthroughs in TPD therapy development.

Ternary Complex Formation Assays

Our services are equipped with advanced assays essential for the development of effective PROTACs, ensuring precise detection of PROTAC binding to target proteins as well as E3 ligases. These assays are critical for monitoring the interactions that are fundamental to the PROTAC mechanism, providing reliable data to inform the optimization of PROTAC design and function.

- Biochemical assays, such as time-resolved fluorescence energy transfer (TR-FRET) assays, and amplified luminescent proximity homogeneous assays (ALPHAs);
- Biophysical assays, such as surface plasmon resonance (SPR);
- Cell-based assays, such as nanoBRET assays;
- Analytical methods, such as mass spectrometry; and other methods, such as coimmunoprecipitation.

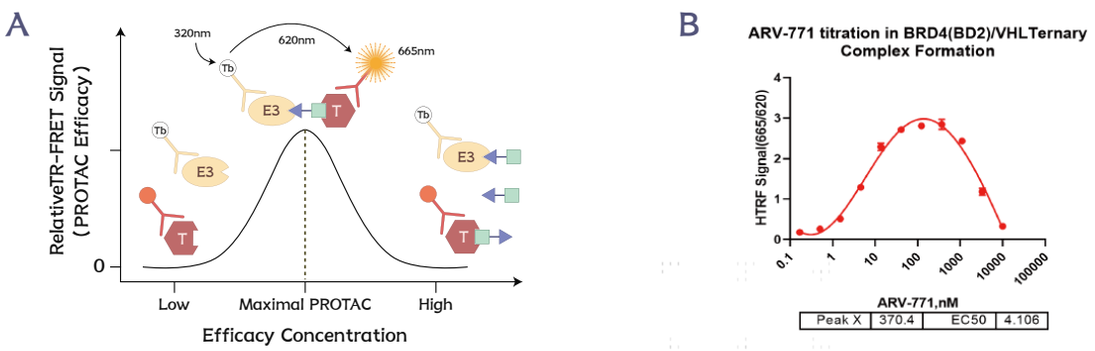


Figure 2. (A) TR-FRET ternary complex formation assay. Complexes are formed among target–PROTAC-E3 ligase, with Tb as the donor fluorophore and AF488 as the acceptor fluorophore. A typical bell-shaped dose–response curve is shown. (B) Pharmacology data of BRD4(BD2)/ARV-771/VHL ternary complex formation.

The research on molecular glues targeting KRAS, particularly in combination with cyclophilin A (CYPA), has shown promising progress. Scientists have developed small molecules that can remodel CYPA to interact with the active, GTP-bound state of KRAS. This interaction forms a ternary complex that inactivates oncogenic signaling, which has led to tumor regression in human cancer models.

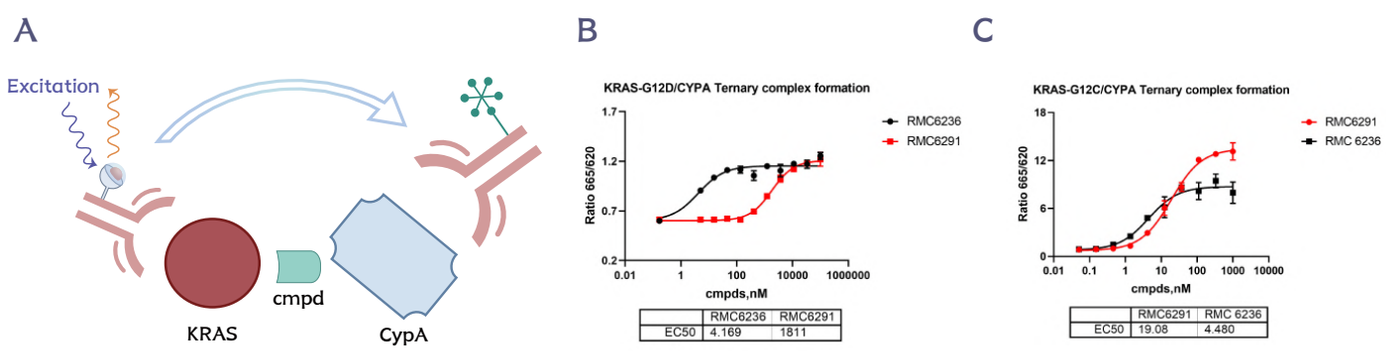


Figure 3. (A) TR-FRET ternary complex formation assay for molecular glues. (B, C) The graphical data illustrate the interaction of two compounds, RMC6236 and RMC6291, with the KRAS-G12D/CYPA and KRAS-G12D/CYPA complex.

SPR

Surface Plasmon Resonance (SPR) is used in TPD research to characterize the formation of ternary complexes. SPR can provide valuable data on the kinetics of how a PROTAC interacts with both a target protein and an E3 ligase. This advanced technology, known for its sensitivity and reliability, delves into the mechanisms underlying ternary complex formation. It achieves this by precisely determining parameters such as the affinity constants (Kd), kinetics (Kon, Koff), and residence time (t1/2) associated with the formation of these complexes.

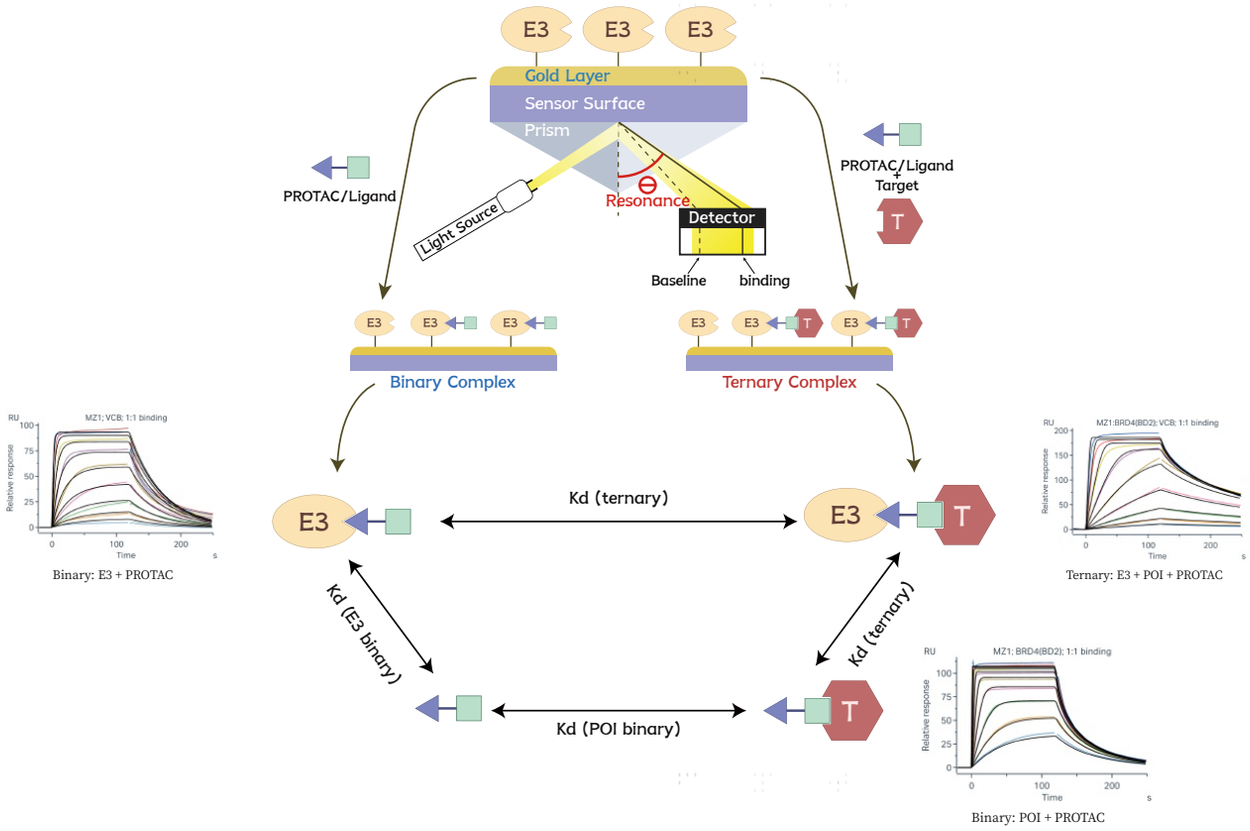
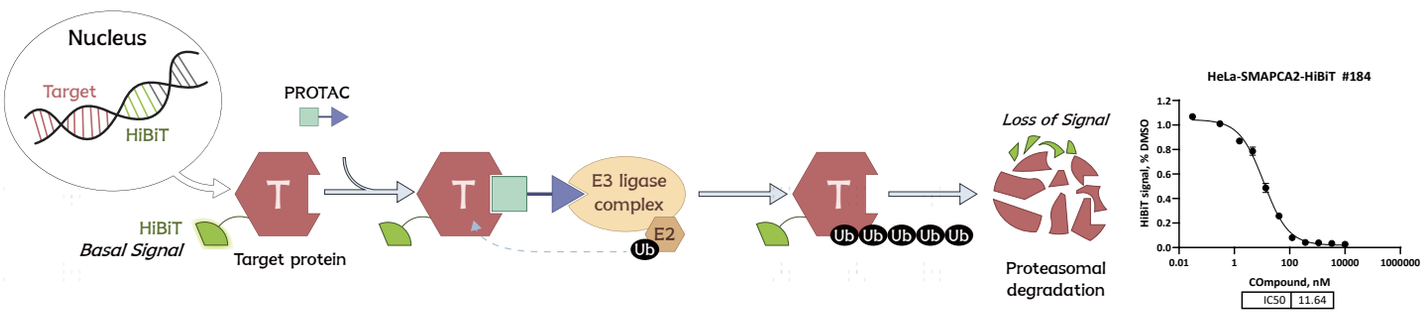


Figure 4. Graph depicting the cooperative binding affinity in a ternary complex formation, where the calculated cooperativity factor (α) of 18.7 indicates a strong positive interaction between E3 ligase, PROTAC, and the protein of interest (POI). Affinity constants (Kd), kinetics, and cooperativity (α) values are crucial factors for comprehending the target ubiquitination and degradation process. Cooperativity plays a significant role in the occurrence of the 'hook effect', wherein high TPD concentrations compete with effective ternary complexes.

HiBiT-Based Protein Degradation Assays

HiBiT is a small 11-amino acid piece from NanoLuc® luciferase. It can easily bind to another part called LgBiT, forming a complete NanoBiT® luciferase. This combined luciferase emits light, allowing us to detect tagged proteins using a special substrate called furimazine. The HiBiT-encoding sequence is integrated into the desired genomic locus using CRISPR/Cas9 technology. Subsequent to the addition of PROTAC, degradation of the recombinant HiBiT fusion protein can be evaluated either through a lytic endpoint assay or real-time kinetic experiment within live cells.

We've developed HiBiT-based protein degradation assays enabling precise quantification of bifunctional degrader-induced degradation of target proteins in physiologically relevant cell models.

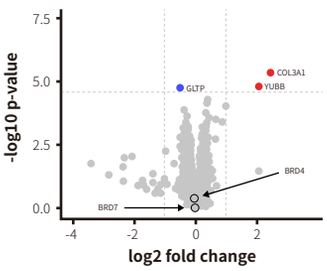


- Targeted Protein Degradation Assays Portfolio: BRD4, cMYC, IKZF1, STAT3, SMARCA2, MAPK7, AR-V7, MAPKAK2, EZH2, GSPT1, P21, WRN, KRT80, PRMT5, HPK1, AR, P300, CPB, and more.
- Reliable and Sensitive: Evaluate the degradation kinetics of endogenous proteins to discover disease-relevant therapeutic candidates.
- Rapid Kinetics: With heightened sensitivity, our assay platform detects target protein degradation induced by bifunctional degraders at a pace faster than conventional phenotypic endpoint assays, such as cell proliferation studies.
- Efficient Turnaround: Utilizing a homogeneous format and superior sensitivity, our assay offers swift and direct quantification of drug-induced alterations in endogenous protein levels, making it ideal for medium to high throughput screening processes.

Other Protein Degradation Assays

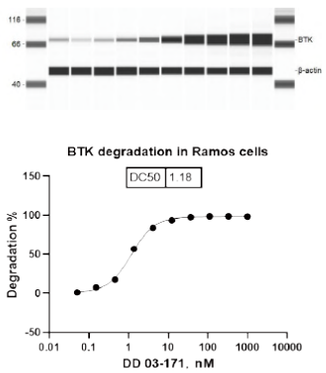
Proteomics:

Proteomics quantifies global protein changes post-PROTAC treatment, identifying degraded targets and assessing drug specificity and off-target effects.



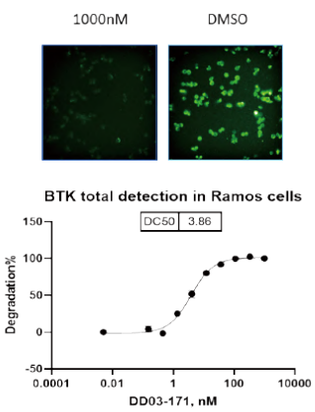
Jess (Automated Western System):

Automating protein separation and immunodetection. Provides reproducible and quantitative analysis of protein expression levels.



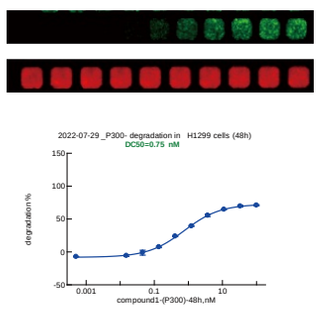
High-Content Screening (HCS):

Observing changes in cellular events, such as protein localization and expression levels in response to TPD agents.



In-Cell Western (ICW):

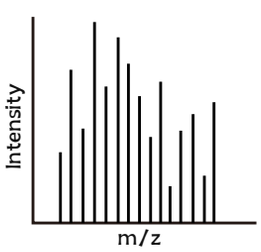
Combines the specificity of WB with cell imaging, allowing for the quantification of protein levels directly in cells.



Mechanism of Action (MOA) Studies for PROTACs

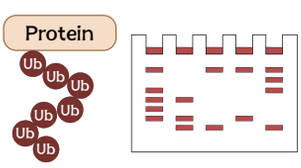
Mass spectrometry:

Uncover the mechanism of action (MOA) of PROTACs through the identification, quantification, and characterization of protein interactions, ubiquitination patterns, and degradation products.



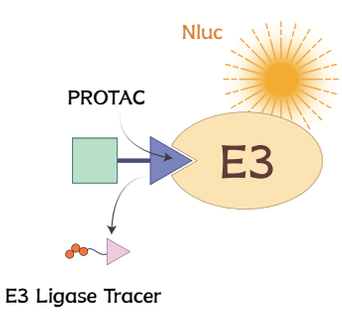
Immunoprecipitation/Western Blot (IP/WB):

IP/WB techniques are used to pull down the target protein and detect its ubiquitination and subsequent degradation.



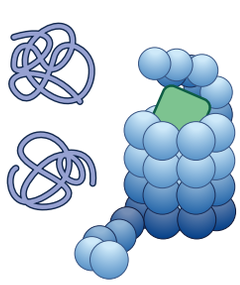
NanoBRET:

NanoBRET assays allow for the real-time monitoring of protein-protein interactions in living cells, particularly between the PROTAC, target protein, and E3 ligase.



Proteasome Inhibitors:

Proteasome inhibitors are used to confirm that the degradation of the target protein is proteasome-dependent, as accumulation of ubiquitinated proteins would indicate blockade of their degradation by the proteasome.

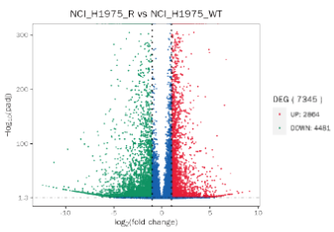


Cancer Cell Panel Screening

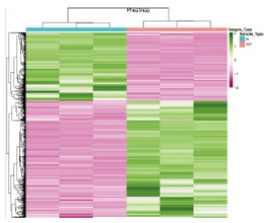
Cancer cell panel screening is integral to Targeted Protein Degradation (TPD) drug discovery, allowing for the assessment of TPD compound efficacy across diverse cancer cell lines. Through this screening process, sensitive cell lines are identified, indicating potential therapeutic efficacy of TPD agents, while resistant cell lines offer insights into intrinsic or acquired resistance mechanisms.

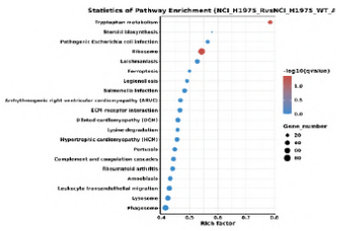
Additionally, this approach contributes to biomarker discovery, enabling the identification of predictive biomarkers associated with TPD compound sensitivity or resistance, thus informing patient stratification strategies and personalized medicine approaches for targeted cancer therapy.

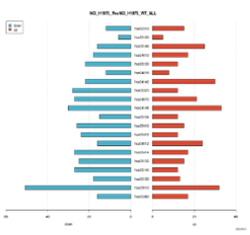
- Robust, flexible, and tailored profiling of test agents on total 500+ cancer cell lines.
- Various assay formats such as 2D proliferation, 3D proliferation, colony formation, and apoptosis, with no assay timeline constraints.
- For generated drug resistant cell lines, we perform RNA-seq-based bioinformatic analysis to investigate the mechanism and provide detailed information about gene expression, enriched pathway and featured gene profiling.



Volcano Plot


Heatmap Analysis


Scatters of Enriched KEGG Terms


Top 20 Features by Score

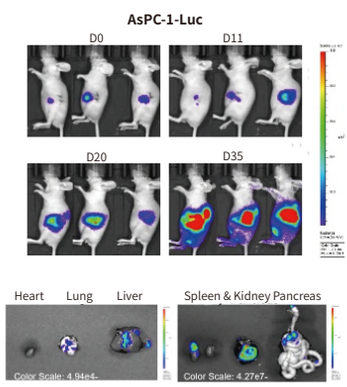
DMPK and In Vivo Pharmacology

Our DMPK service assays provide comprehensive support for PROTAC discovery, lead optimization, clinical candidate selection, and IND filing processes.

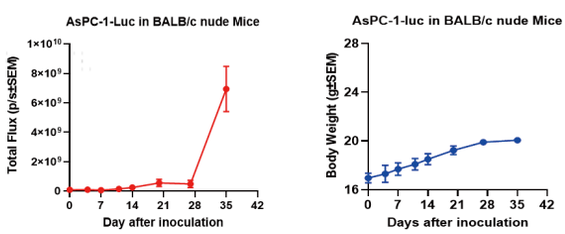
	Lead Discovery and Optimization	Clinical Candidate Selection	Clinical Candidate Characterization and IND Filing
Biotransformation	▪ Metabolic Soft Spot Analysis ▪ Reactive Metabolite Screening ▪ Special Metabolizing Enzyme Phenotyping	▪ Metabolism in Hepatocytes Across Species ▪ ADME in BDC Rats ▪ Radiolabeled Tissue Distribution Study in Rats	▪ Radiolabeled ADME Study in Animals ▪ Radiolabeled Tissue Destruction Study ▪ Metabolizing Enzyme Mapping
In Vitro DDI/ADME	▪ Solubility and Permeability ▪ P-gp/BCRP Substrate Analysis ▪ Plasma Protein Binding ▪ Metabolic Stability	▪ CYP Inhibition and Induction ▪ Transporter Inhibition	▪ Transporter Substrate Analysis
Bioanalysis/PK	▪ PK Screening in Animals ▪ PK/PD in Pharmacological Model	▪ PK/TK in Animals ▪ Biomarker Exploration ▪ Tissue Distribution Studies of the Parent ▪ Excretion Studies of the Parent	▪ Full PK Studies in Animals ▪ Tissue Distribution Studies of the Parent ▪ Excretion Studies of the Parent

- **Absorption:** We streamline PROTAC absorption prediction, refining solubility and permeability correlations from lab to clinic.
- **Distribution & DDI:** Our tailored PPB assays and DMPK insights guide PROTAC distribution and DDI risk profiling.
- **Clearance:** Utilizing hepatocyte and microsome platforms, we expedite in vitro metabolism screening for PROTACs.
- **Metabolite Profiling and Identification:** Collaborating with XenoFinder, we utilize unique and untargeted LC-HRMS approach to study in vitro and in vivo biotransformation of PROTAC.

CDX models are commonly used to evaluate the efficacy of TPD compounds in inhibiting tumor growth and inducing protein degradation in vivo. Our 150+ CDX models enable researchers to assess the antitumor activity, pharmacokinetics, and safety profile of TPD drugs in a preclinical setting, facilitating the selection of lead compounds for further development.



Survival cycle:38day



Pancreatic Orthotopic tumor model

ICE Bioscience

Innovative CRO+ Explorer

ICE Bioscience was founded in 2010 as an Innovative CRO+ Explorer company. We specialize in early drug discovery services, spanning from target validation to the identification of pre-clinical candidates. We stand out for our collaborative spirit and expertise in boldly exploring new therapeutic target research. Our commitment to drug discovery services, delivered with enthusiasm and professionalism, empowers clients to overcome challenges, address scientific puzzles, and fulfill our promises to clients, communities, the environment, and global health.

To learn more, please visit: en.ice-biosci.com