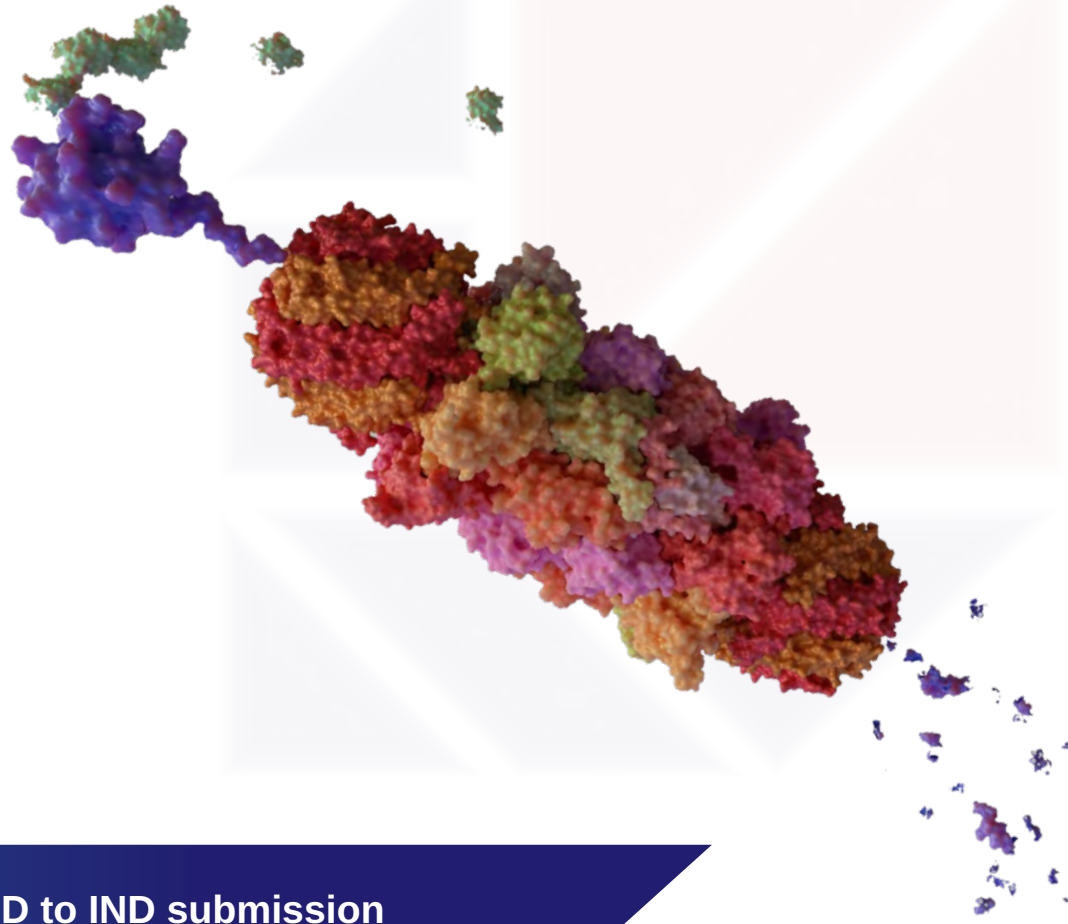
康龙化成
PHARMARON

Services for Protein Degrader Discovery



End-to-end services from target ID to IND submission

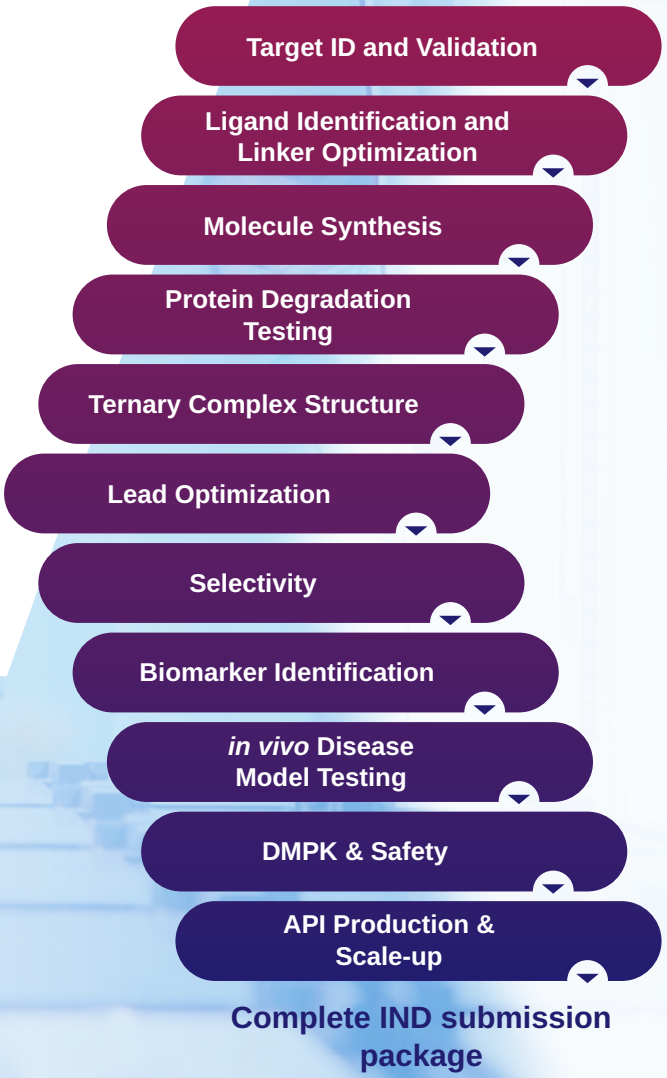
Advance your Protein Degrader with Pharmaron

Pharmaron's comprehensive protein degrader services provide the full coverage for preparation and testing of protein degrading medicines.

Our expertise spans different modalities, including

- Proteolysis-Targeting Chimeras
- Hydrophobic Tagging Agents
- Molecular Glues, such as IMiDs

Pharmaron's commitment to innovation in this area advances the field and brings new treatment options to patients.



Why work with Pharmaron?

Pharmaron is perfectly set up to support protein degrader drug discovery programs

Extensive Experience

in leading protein degrader projects and reaching key milestones (inc. *in vivo* degradation of POI)

Track Record

Extensive track record, having supported many protein degrader projects for > 8 years

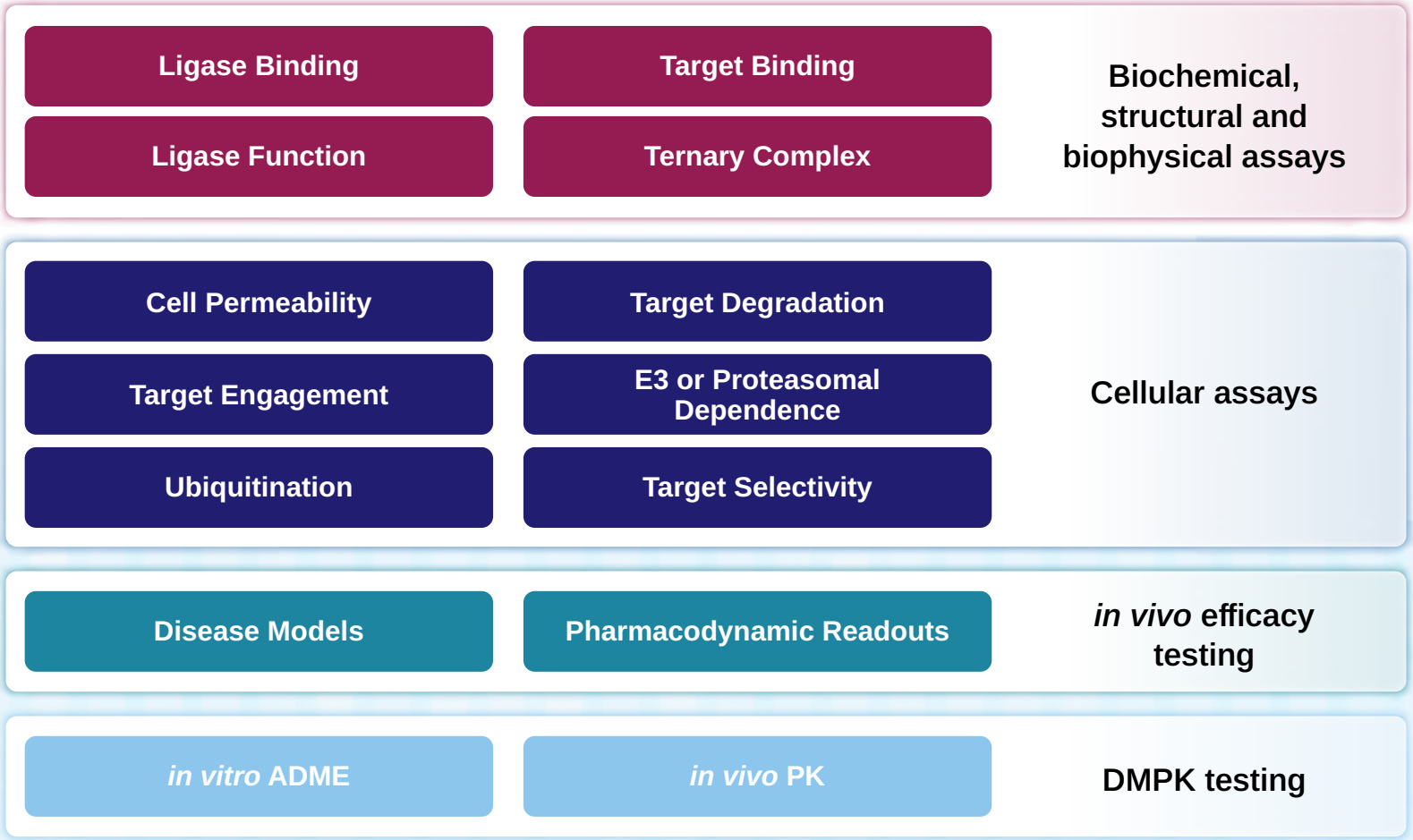
Modern Facilities

Protein degrader-specific suites of assays in place, e.g., PhysChem, DMPK, Biology

Scientific Excellence

A toolbox of key building blocks (E3 warhead and linker combinations) is in development to efficiently deliver the first sets of active protein degraders.

Due to their unique mechanism of action, testing of protein degraders require specialized assays and readouts.



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Synthesis toolbox

Libraries of building blocks, including linkers and ligases, are readily available to accelerate the synthesis of new protein degraders.

Ligase and linker libraries • Drug assembly

2

Computer-aided drug discovery

Structure-based drug design and virtual screening identify compounds most likely to interact with target and ligase. *In silico* modeling helps select drugs with favorable ADMET properties.

Structure-based • Statistics-based • AI-based

3

Structural biology

Our structural biologists help elucidating the binary and ternary structure of the complex for understanding the interaction of the drug with ligase and target.

Crystallography • CryoEM (via partner)

4

Target validation

Ligand-induced genetic degradation mimics degrader treatment by depleting a tag-fusion protein in the presence of a specific ligand.

dTAG KnockIn System • HaloTag KnockIn System

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Binding

The interaction of the degrader with the ligase and the target are investigated via fluorescence-based and biophysical techniques. Options range from high-throughput screening to identify suitable target-binding moieties to ternary complex formation.

Surface Plasmon Resonance • TR-FRET • AlphaLISA • MST • Thermal shift assay

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Ubiquitination

The confirmation that the degrader molecule ubiquinates the target protein ensuring the functionality of the protein degrader.

TR-FRET • MSD • IP/Western Blot

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Cellular binding

Real-time measurement of the binary and ternary complex formation in cells can be performed with BRET from NanoLuc-target protein and HaloTag-E3 ligase. Cellular thermal shift assays detect binding of the drug to endogenous proteins.

NanoBRET • Cellular thermal shift assay

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Target protein degradation

Measuring the degradation of the endogenous target in cells is a readout for the potency of the drug.

Western Blot (ECL/Fluorescence) • JESS Western Blot MSD/ELISA • Intracellular Western Blot/High-content Imaging AlphaLISA/HTRF • Hibit

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Protein degradation kinetics

The kinetics of the drug's action are determined with the HiBit assay system which can be run in high-throughput mode and coupled with imaging. Pharmaron creates the cells necessary for tracking real-time target degradation in-house.

HiBit/LgBit Assay

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Degradation mechanism

Pharmaron tests the mechanism of degradation by inhibition of the proteasomal and E3 ligase pathway.

Proteasomal inhibition • E3 Ligase KO cells

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Target selectivity

Drug selectivity is examined via AirID, a proximity biotinylation-based approach to identify neo-substrates by IP-MS. Quantitative mass spec is used to identify induced changes in protein levels by measuring the relative abundance of each protein.

TMT labelling followed by mass spec • AirID followed by immunoprecipitation and mass spec (IP-MS)

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Efficacy testing in animals

Protein degraders can be tested for efficacy in animals at Pharmaron in a variety of disease models. Pharmacodynamic readouts are used to track protein degradation including kinetics and reversibility. Our protocols are optimized for testing of complex *ex vivo* samples.

Flow cytometry • IHC • Western blotting • ELISA / MSD / AlphaLISA • fluorescence tagging of target • qPCR • proteomics

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in vitro DMPK strategies for protein degraders

Optimizing DMPK properties of protein degraders can be challenging due to non-specific binding, solubility issues and assay dynamic range. Pharmaron offers comprehensive DMPK services and modified *in vitro* assays that are specifically designed to characterize protein degraders.

Solubility • Log D • Protein Binding • Permeability • Metabolism • Transporter Involvement • Metabolite ID

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in vivo PK/PD Modelling

When protein degraders are tested for PK/PD parameters, the complex mechanism of action which employs the cellular degradation machinery, the dynamic nature of the targets and the ternary nature of the drug adds variability and unpredictability which must be addressed by advanced modelling strategies.

Computational modelling • Formulation • Small and large animals

Let us know how we can help propel your protein degrader discovery project.

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PHARMARON

www.pharmaron.com • bd@pharmaron.com

Laboratory Services

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