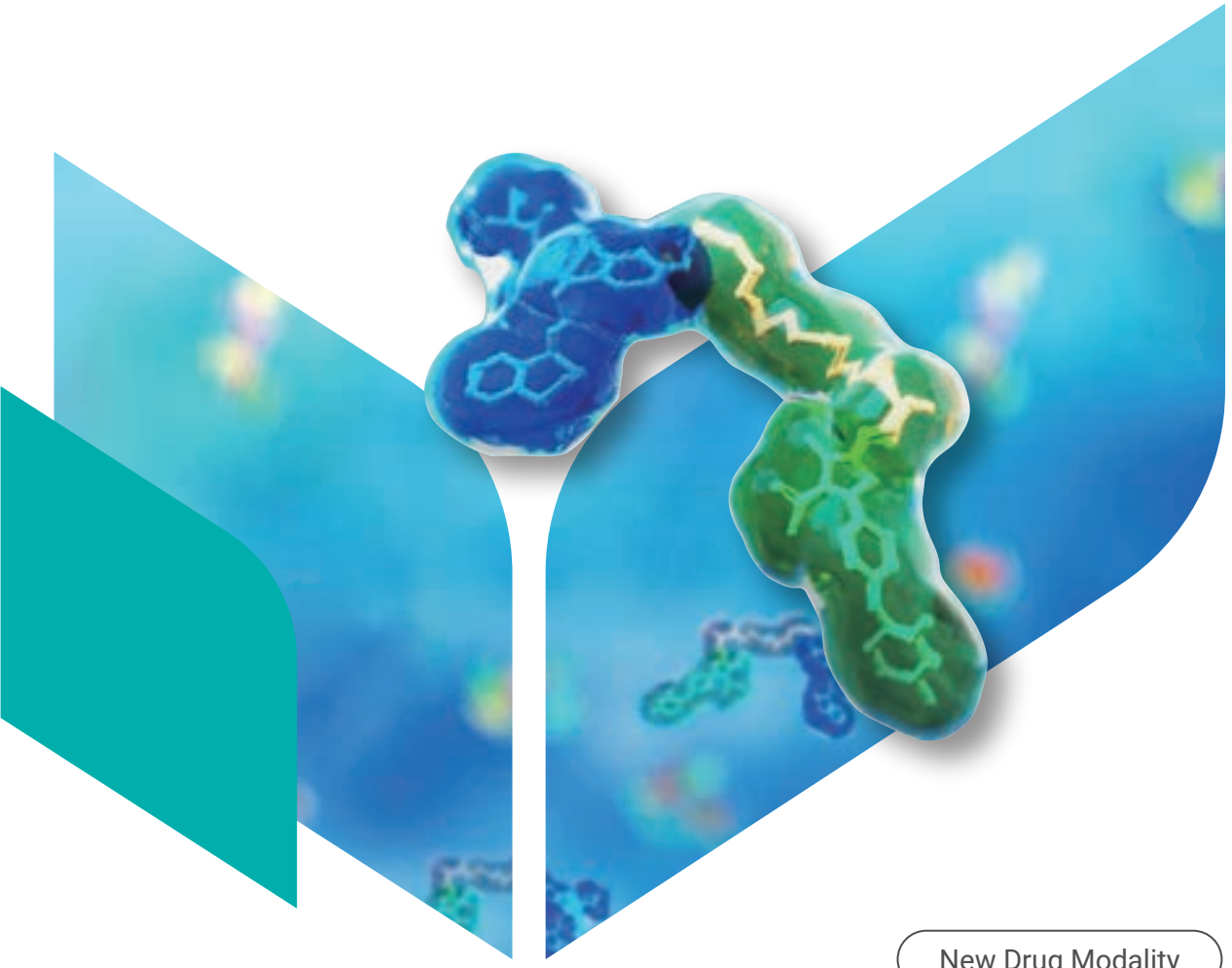
Laboratory Testing Division
Bioanalytical Services

WuXi AppTec

Proteolysis Targeting Chimeras (PROTACs)
Bioanalysis



New Drug Modality

Bioanalytical Services

Global Integrated Bioanalytical Platform

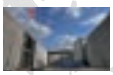
WuXi AppTec Bioanalytical Services operates globally and has leading integrated laboratories in China and the U.S. We adhere to GLP and GCP principles and strictly follow high industry regulatory standards. Our comprehensive platform, equipped with advanced instruments and staffed by experienced experts, provides professional one-stop services in preclinical/clinical mass spectrometry, immunochemistry, molecular/cellular, and central laboratory services to global clients.

Shanghai, China
Headquarters
Bioanalytical Services (Clinical)
Central Lab

Suzhou, China
Bioanalytical Services (Preclinical)
Antibody Service

New Jersey, USA
Bioanalytical Services (Preclinical/Clinical)
Central Lab

Chengdu, China
Bioanalytical Services (Preclinical)



Nantong, China
Bioanalytical Services (Preclinical)

18+ Years lab operations in China and the U.S.
24,300 m² lab space

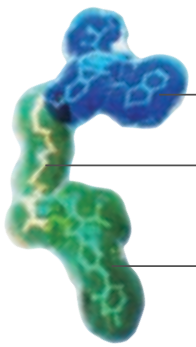
5,000+ small molecule validated methods
2,800+ large molecule validated methods
2M+ samples analyzed per year

Global Regulatory Standards

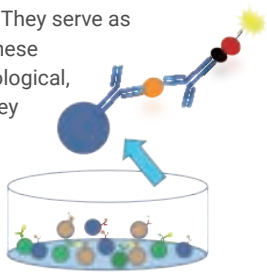
Successfully passed global regulatory inspections, comply with GLP, GCP, CAP quality standards
200+ China NMPA
30+ International regulatory agencies (U.S. FDA, OECD, EMA, PDMA, CAP)

Proteolysis targeting chimeras (PROTACs) are innovative small molecules with an E3 ligase binding component, a target protein ligand, and a connecting linker. This unique structure facilitates the degradation of target proteins via the ubiquitin-proteasome system.

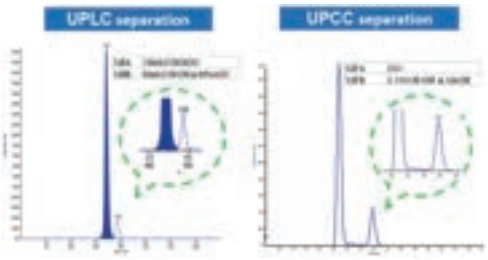
At WuXi AppTec's Bioanalytical Services (BAS), we have supported over 100 preclinical and clinical studies involving PROTAC bioanalysis. Our extensive bioanalytical method development expertise enables us to address various analytical challenges effectively. We offer comprehensive bioanalytical services for PROTACs at any stage of preclinical and clinical development, with the goal of helping our partners expedite global IND and NDA filings.



Bioanalytical Solutions for Proteolysis Targeting Chimeras (PROTACs)

Study Type	Platforms	Study Design
PK/TK	HPLC Chiral Analysis 	▪ Develop chiral analytical method at an early stage to investigate *in vitro/in vivo* interconversions ▪ Take appropriate approaches and develop bioanalytical method for PK/TK ▪ Validate the method according to ICH M10 ▪ After validation, perform sample analysis
	Ultra-Performance Convergence Chromatography (UPCC) Chiral Analysis 	▪ Can be applied to enantiomers, which are difficult to separate by HPLC ▪ Provide superior solutions for prolonged/difficult separations by HPLC ▪ Provide superior efficiency of separation and shorten analytical run time
	MS Instruments Triple Quad MS/MS 	▪ Triple Quad MS/MS can be used for quantitation of PROTAC with high selectivity and specificity ▪ The concentrations of PROTAC are monitored in plasma and tissue samples by validated method ▪ The concentrations of each enantiomer can be monitored simultaneously
	Q-TOF HRMS 	▪ Q-TOF HRMS can be used for identification of PROTAC metabolites ▪ Different strategies can be used for complicated metabolic processes of PROTACs
PD/Biomarker	ELISA/MSD/ Luminex/ELLA/ Simoa/Gyros/ SMCxPRO, etc.	Biomarkers are objective measures used to evaluate biological processes. They serve as valuable tools for comparing these processes under normal, pathological, and therapeutic conditions. They are widely utilized in the pharmaceutical industry to monitor clinical outcomes, pharmacodynamics (PD), and safety profiles. 

Bioanalytical Challenges for Proteolysis Targeting Chimeras (PROTACs)

	Challenges	Solutions
PK/TK	Matrix stability A PROTAC candidate showed poor plasma stability at ambient temperature as reported by Nguyen, et al.[3]	▪ Strict temperature/light control during sample collection and treatment. ▪ Develop chiral analytical method at an early phase to investigate interconversions. ▪ Mitigate the impact of non-specific binding by blocking and passivating treatments. ▪ Optimize MS method to avoid in-source fragmentation. ▪ The SFC system significantly improves the efficiency for the analysis of enantiomers. 
	Chiral analysis Among enantiomers possessing at least one chiral center of a typical VHL ligand, only one is active while the others are generally inactive.[1]	
	Non-specific binding	
	In-source fragmentation	

	Assays	Platform Selection/Challenges
PD/ Biomarker	Biomarkers for PD/Toxicity	▪ Robustness of analytical method ▪ Multiplexed assay ▪ Correlation between species/cross-reactivity ▪ Flow cytometry/ELISA/MSD/Luminex
	Determination of target protein degradation	▪ Assay sensitivity ▪ Robustness of analytical method ▪ Analysis in different cell population: flow cytometry ▪ Tissue sample analysis: LC-MS/MS, ELISA/MSD

Proteolysis Targeting Chimera (PROTAC) Case Study

Background:

Consider a PROTAC drug currently in Phase I clinical trials aimed at degrading the IRAK4 protein. This drug has demonstrated clinical benefits for patients with hidradenitis suppurativa (HS) and atopic dermatitis (AD), significantly reducing inflammation in those with moderate to severe symptoms.

For pharmacokinetic (PK) bioanalysis, LC-MS/MS was utilized to measure drug concentrations in plasma and skin biopsy samples from patients with HS and AD. The findings revealed a dose-dependent increase in plasma drug concentrations, with drug levels in skin biopsy samples being 10 to 14 times higher than in plasma.[2]

	Dosing Route	System Clearance	Cell Penetration	Accumulations in Target
Small Molecule Inhibitors	Orally available	Fast to moderate	Yes	No
Biotherapeutics	*i.v. or s.c.* for systemic delivery	Slow	Need special designs	Yes, due to protein-protein interaction
PROTACs	Orally available	Moderate	Yes	Yes, due to the target-binding ligand

Table 1. Comparison of PK characteristics of small molecule inhibitors, biotherapeutics, and PROTACs

Flow cytometry and MSD immunoassay platforms were employed for biomarker analysis to assess IRAK4 protein degradation and inflammation-related cytokine release in PBMC samples from treated patients. The results indicated that IRAK4 protein concentrations decreased by over 90% compared to baseline levels. Additionally, the levels of inflammation-related cytokine release were significantly lower in patients receiving the treatment than those on placebo.[2]

	Mechanism of Target Inhibition	Degree of Inhibition
Small Molecule Inhibitors	Usually non-covalent inhibition	Varied, prone to drug resistance
Molecular Glues	Monovalent, i.e., bind to E3 ligase	Moderate, due to existing protein-protein interaction
PROTACs	Bivalent, target oriented	Potentially strong inhibition

Table 2. Comparison of target inhibition mechanisms of small molecule inhibitors, molecular glues, and PROTACs

Study design:

We provide customized services in accordance with global regulatory guidance, supporting IND submission.

1. Preclinical studies
   PK/TK, tissue distribution, excretion, immunogenicity and immunotoxicity.
2. Clinical studies
   PK, excretion, DDI, immunogenicity and immunotoxicity.

Our advantages

Global Integrated Platform: We provide one-stop services to global sponsors, from preclinical to clinical stages, supported by multiple BAS sites worldwide.

Customized Services: Our sponsor-centric professional project management team delivers tailored services to meet your specific needs.

Experienced Team: Our core technical team members boast over 10 years of experience, with our management team having more than 20 years.

With extensive experience from over 100 studies in Proteolysis Targeting Chimeras (PROTACs) bioanalysis, we are dedicated to accelerating your PROTAC R&D projects and addressing a wide range of analytical challenges.

Citations

1. PROTAC'ing Oncoproteins: Targeted Protein Degradation for Cancer Therapy, Molecular Cancer, 2023
2. IRAK4 Degrader in Hidradenitis Suppurativa and Atopic Dermatitis: a Phase 1 Trial, Nat Med, 2023
3. Development of an LC-MS/MS Method for ARV-110, a PROTAC Molecule, and Applications to Pharmacokinetic Studies, Molecules, 2022

Common Industry References

1. Method Validation for Quantitation in Biological Samples, Chinese Pharmacopoeia (2020)
2. Bioanalytical Method Validation and Study Sample Analysis M10, International Council for Harmonization
3. Bioanalytical Method Validation, U.S. Food and Drug Administration

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