# Pre-clinical Discovery Using Simple Western™ for Protein Detection – A CRO Perspective

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## Introduction

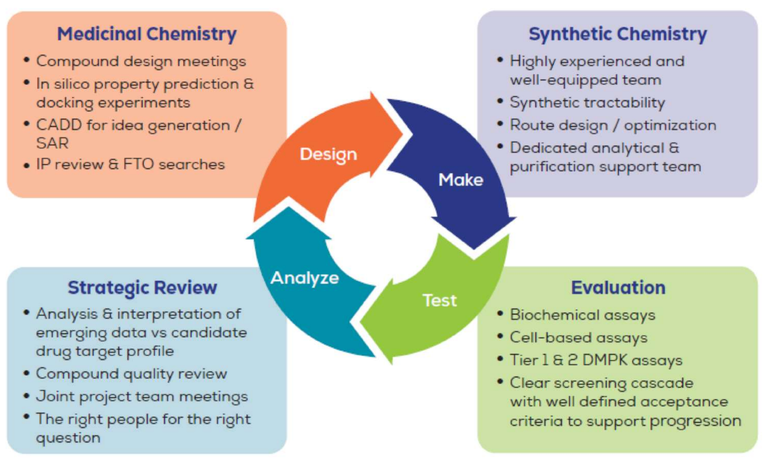
Proteins are key molecules in the maintenance of many complex processes within the human body. The dysregulation of key proteins can lead to diseases including cancer, auto immune disease, inflammation and neurological diseases. Targeting of these proteins is common practise within the world of drug discovery, making accurate determination of protein quantity and monitoring of signalling pathways within a cell background a powerful tool to further understand mechanism of action.

Charnwood Discovery is a CRO and leading provider of drug discovery services, from standalone customized solutions through to fully integrated programs that enhance the experience and success of complex drug discovery. Offering chemistry, bioscience, and ADME/DMPK we are an established partner of choice for many biotech and pharmaceutical companies.

Immunoblotting has been the gold standard method for detection of protein within a biological sample for decades; at Charnwood Discovery we have utilized the Simple Western™ Jess to increase throughput, accuracy, and quantitation of protein targets, driving forward our client's discovery programmes from target validation to pre-clinical candidates. Here we demonstrate how the Simple Western technology has played an important part in therapeutic development.


## Charnwood Discovery

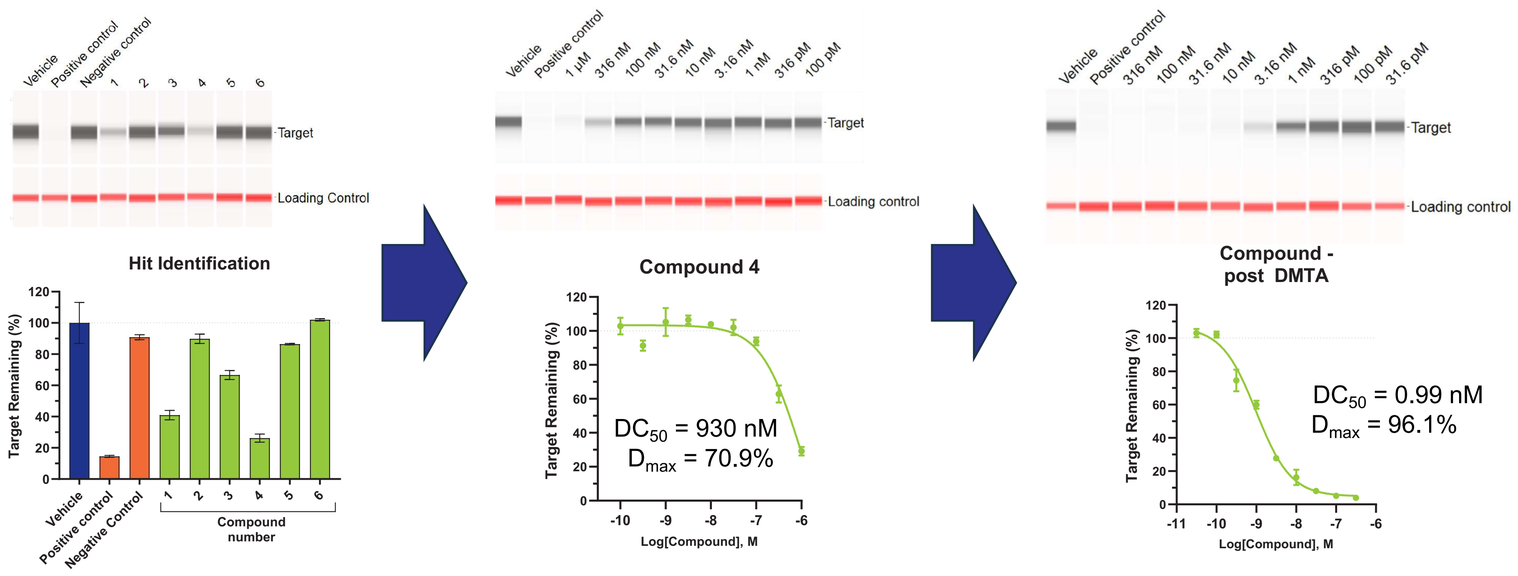
Our approach to integrated drug discovery is driven from the co-location of scientists, combined with a culture of collaboration and knowledge-sharing. This facilitates data driven feedback and decisions to be communicated quickly.




*The design-make-test-analyze (DMTA) cycle (Figure 1), a well-known central process in drug discovery is all carried out under one roof at our cutting-edge campus facility.*

## Hit Identification and Development

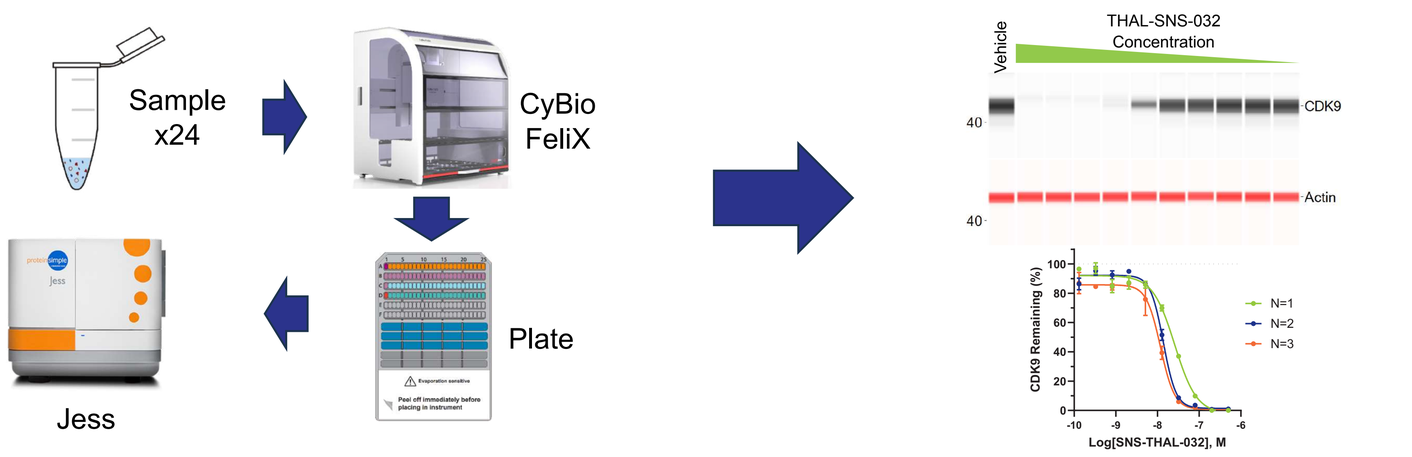
Development of a targeted protein degradation molecule using the Simple Western technology. Hit identification is carried out by examining numerous molecules at a fixed concentration and comparing these to a known degrader molecule. Once a hit is identified, development of the molecules can be carried out using the DMTA cycle using constants such as $DC_{50}$ and $D_{max}$ to understand the structure-activity-relationship (SAR) and aid hit development.




*Hit identification was carried out by examining potential degrader molecule activity at a fixed 1 µM concentration. Once identified, hit development was carried out, comparing and ranking molecules using $DC_{50}$ and $D_{max}$ constants. Following multiple rounds of DMTA to understand the molecules SAR, the data shows a 3-magnitude order improvement in potency and 26% improvement in maximum degradation.*

## Automation to Enhance Detection Capability

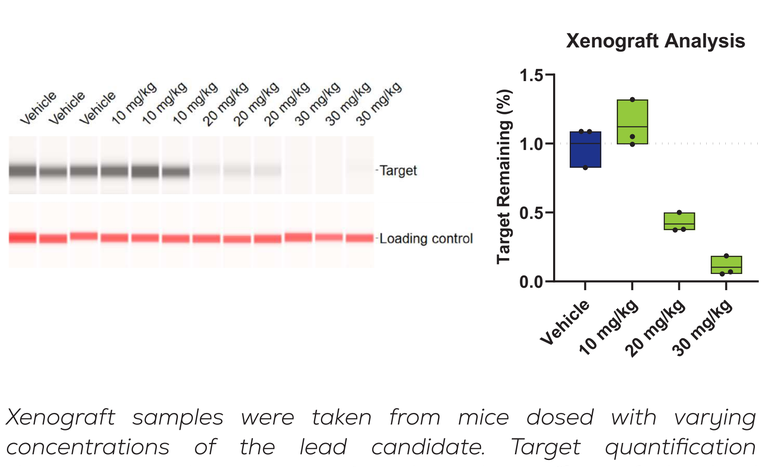
To increase throughput, maintain consistency, and facilitate simple assay transfer, the process of preparing the Simple Western™ Jess plate has been automated at Charnwood Discovery in collaboration with Analytik Jena. To test the system, known PROTAC molecule THAL-SNS-032 and degrader of CDK9, was examined for reproducibility and potency. THAL-SNS-032 demonstrated a reproducible concentration dependent response with comparable degradation constants to those seen within the industry.




*THAL-SNS-032 demonstrated a concentration dependent depletion of CDK9 with an average $DC_{50}$ of 16.1 nM ± 7.6 nM and $D_{max}$ of 99%.*

## Lead Optimization - *Ex Vivo*

As drug discovery program develop, efficacy pharmacokinetics, and pharmacodynamics become important for lead optimization. Biomarker protein levels can be examined accurately in physiologically relevant models such as patient samples and xenografts.




*Xenograft samples were taken from mice dosed with varying concentrations of the lead candidate. Target quantification demonstrates the concentration dependant effect of the lead compound*

## Summary and Conclusion

Charnwood Discovery has been a leading provider of drug discovery services for over 25 years, offering bioscience, DMPK/ADME, and chemistry services to support any project. Our experience of the Wes and Jess platforms has helped drive forward and develop pipelines for numerous projects, including:

- Monitoring cell signalling pathways such as phosphorylation for kinase inhibition and ubiquitination in molecule degradation.
- Determining remaining protein for targeted protein degradation-based projects for PROTAC and molecular glue therapeutic development.
- Biomarker absolute quantitation in human tissue for efficacy, pharmacokinetics, and pharmacodynamic development using standard curves.