Protein Detection Capabilities – How to Push Forward your Hit to Lead Program

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Introduction

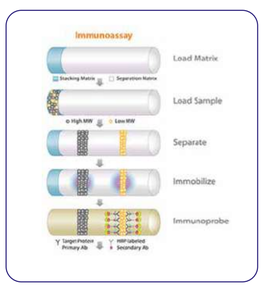
Accurate determination of protein quantity and monitoring of signalling pathways within a cell background is a powerful tool to further understand mechanisms of action within the drug discovery field. Western blotting has been the gold standard method for detection of protein within biological samples for decades, however, this method is time consuming, suffers from poor reproducibility, and only allows for the collection of qualitative data.

At Charnwood Discovery, we have pioneered the use of Simple Western technology for drug discovery purposes. This modern alternative to traditional Western blotting is high-throughput, truly quantitative, easily adaptable, and only takes 10% of the time.

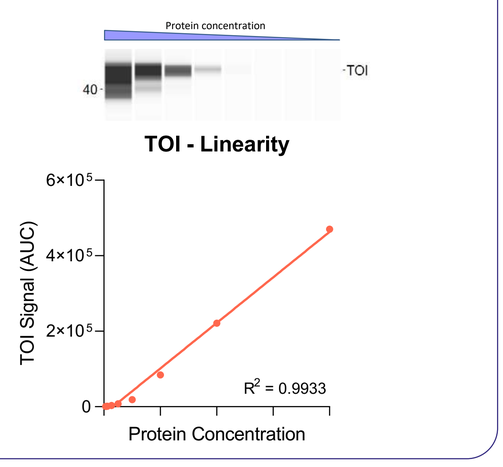
Our experts have used the Simple Western technology to validate drug discovery targets, monitor signalling pathways, and perform Structure-activity-relationship (SAR) Proteolysis targeting chimera (PROTAC) screens.

Simple Western Jess

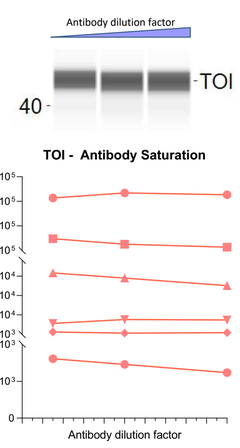
Simple Western Jess is a capillary based, high-throughput Western blotting technology that allows fast, accurate, and quantitative determination of a target within a biological sample. Due to its sensitive nature, linear range determination and saturation of the antibody is essential to ensure accurate detection of the target of interest (TOI).



Linear range is determined by examining the relationship between signal and protein concentration.

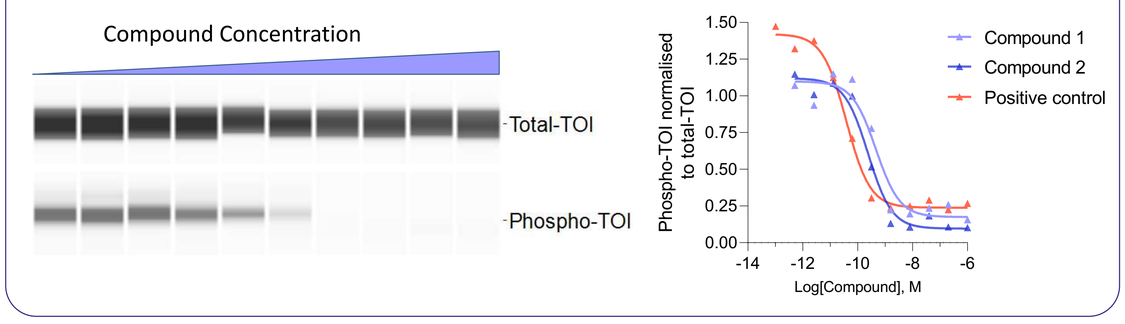


Antibody saturation is examined by ensuring signal remains consistent in a cell lysate with differing antibody dilution factors.



Cell Signalling Pathways

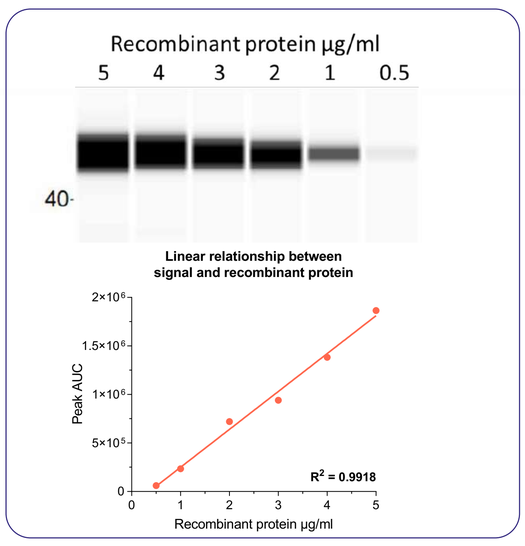
Small molecule inhibitors are organic compounds that interact selectively and potently with their target proteins, often to modulate downstream signalling pathways. As such it is important to monitor these signals, often phosphorylation, to understand the mechanism of action and potency of novel small molecules.



Absolute Quantification

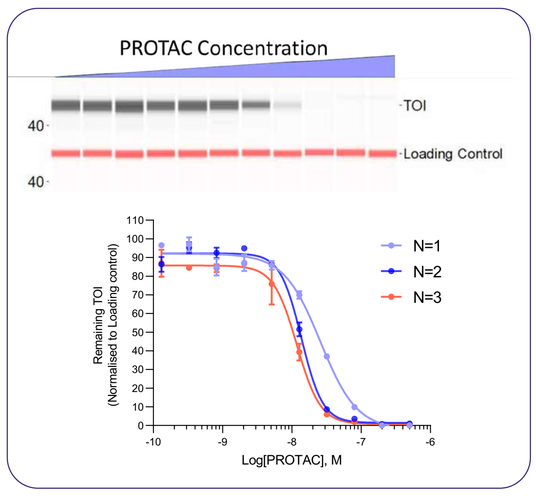
Absolute quantification of a TOI can be achieved using a standard curve generated with recombinant protein as demonstrated. The limit of detection is <0.5ug/ml.

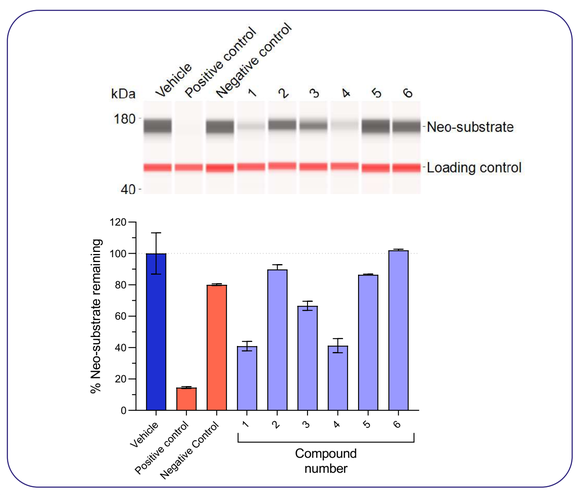
This allows interpolation of unknown quantities and can be useful when looking at secreted peptides or proteins or localisation to specific cellular compartments.



Proteolysis-targeting Chimera (PROTAC) and Neo-substrates

PROTAC molecules are specific and targeted protein degraders. The molecule consists of a ligand directed towards the TOI, covalently linked to a ligand of an E3 ubiquitin ligase. PROTACs allow co-recruitment of the TOI and the E3 ligase, the TOI is then ubiquitinated and degraded via the proteosome[1]. Quantitation of the TOI is therefore important for understanding and developing new molecules.

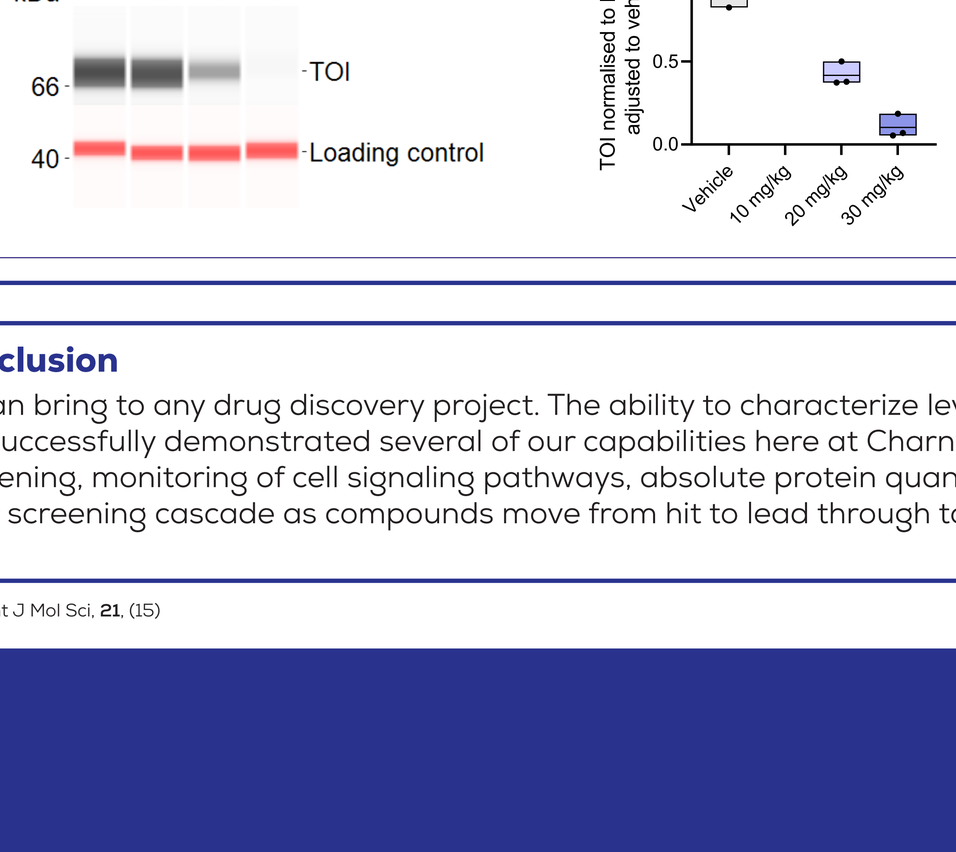




The CRBN E3 ligase ligands are commonly derived from a class of immunomodulatory drugs (iMiDs) that possess a number of unwanted neo-substrates, these include IKZF1, IKZF3, CK1, GSPT1, and SALL4[2]. For this reason, counter-screening is important to understand the structure-activity-relationship and optimize new PROTAC molecules.

Tissue Analysis

As drug discovery programs develop, analysis of efficacy, pharmacokinetics, and pharmacodynamics become important for lead optimization. Signalling pathways and protein levels of biomarkers from patient samples and xenograft models can be examined accurately in physiologically relevant model systems as demonstrated below.



Summary and Conclusion

Our work here illustrates the insights that protein detection using Simple Western Jess can bring to any drug discovery project. The ability to characterize levels of protein targets can be critical in the progression of a drug discovery project. We have successfully demonstrated several of our capabilities here at Charnwood Discovery, including TOI optimization, PROTAC screening and neo-substrate counter screening, monitoring of cell signaling pathways, absolute protein quantification, and analysis of targets within tissues. These capabilities are powerful tools to have in any screening cascade as compounds move from hit to lead through to lead optimization and beyond.

1 – Song, Y. *et al* (2020), J Hematol Oncol, **13**, (50): 1756-8722 2 – Gao,S. *et al*. (2021), Biomark Res, **9**, (43): 2050-7771 3 – Kang, C. *et al*. (2020), Int J Mol Sci, **21**, (15)