bio-techne®

Scientific Review

Simple Western Hits the Bullseye of Targeted Protein Degradation

Western Blot Misses the Mark

Finding the right degrader is like finding a needle in a haystack. Researchers in targeted protein degradation (TPD) need speed, precision, and throughput to find the sweet spot of degrader efficiency, screening many samples of varying concentrations and time points.

Researchers generally run dose-response curves using traditional SDS-PAGE Western blotting methods to characterize the efficacy of degradation molecules. However, the lengthy, manual workflow and resulting low reproducibility make it unreliable for determining DC_{50} values.

Instead, the ideal solution would be highly reproducible, allow for easy quantitation, and have a short time for results. Simple Western™ is just that, letting you separate and analyze proteins by size from 2 kDa to 440 kDa in just 3 hours. With the push of a button, researchers can process up to 96 samples in a hands-free Simple Western run, with quantitative results and reproducibility for accurate dose-response measurements.

Plus, Simple Western is an open platform and conventional Western blot antibodies may be used for detection, eliminating the need for epitope tags that can interfere with degrader activity. Choose from thousands of Simple Western validated antibodies or validate your own antibody.

The Simple Western Advantage

Time to Result
- Fully automated Westerns
- Fully analysed results in 3 hours

Quantitation
- Built in analysis software
- Absolute and relative protein quantitation

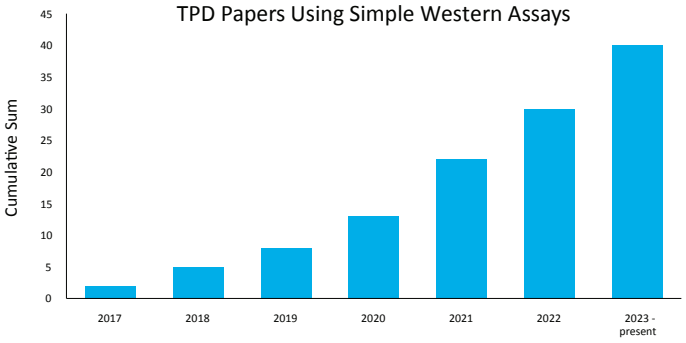
Throughput and Flexibility
- Up to 96 samples per run
- Multiplex by chemiluminescence and/or fluorescence

Reproducibility
- Low inter- and intra-assay CVs

Low Sample Volume
- Start from as little as 0.3 µg or 3 µL per well to get pg-level sensitivity

Simple Western in the Hands of Your Peers

Leading TPD researchers across the globe are turning to Simple Western to monitor protein degradation activity and advance cutting-edge targeted protein degradation research, supported by the steady increase in publications in premier journals like *Cell*, *Nature Chemical Biology*, and *PNAS*. This review highlights vital publications that represent Simple Western's high-throughput screening of degrader activity with reproducible quantification, flexible multiplex strategies, and fast-time to results, from drug discovery of novel degraders through translation to the clinic.



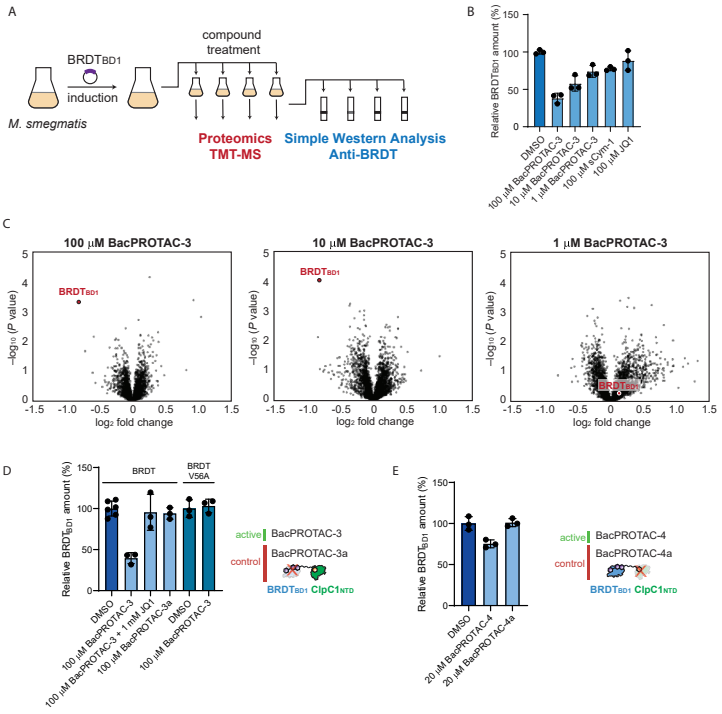
Discovery of New Degraders

A Novel Approach to Antibiotics

The global increase in antibiotic resistance is becoming a human health crisis by hindering combatting microbial infections. So far, it has not been possible to reprogram bacterial degradation machinery to interfere with microbial infections. With the help of Simple Western, researchers developed bacterial-based proteolysis-targeting chimeras (BacPROTAC) that bind to the substrate receptor of the Clp protease, targeting new substrates, or neosubstrates, for degradation. Simple Western was essential to monitor the degradation activity of BacPROTACs directly in the lysates of Mycobacterium cells (**FIGURE 1**). The high-throughput analysis provided by Simple Western enabled 96-well plate-based screening of many different BacPROTAC candidates and concentrations. The researchers also leveraged multiplex detection to measure cytosolic amounts of neosubstrate BRDTBD1 simultaneously with housekeeping protein RpoB to normalize protein expression data.

- **Targets Analyzed by Simple Western:** BRDTBD1 and RpoB
- **Sample Type:** Lysates of Mycobacteria
- **Experimental Design:** Protein levels in response to treatment with titrations of BacPROTACs relative to untreated controls
- **Read More At:** Morreale FE, Kleine S, Leodolter J, et al. BacPROTACs mediate targeted protein degradation in bacteria. Cell. 2022 Jun 23;185(13):2338-2353.e18.

FIGURE 1. BacPROTACs mediate selective POI degradation in mycobacteria



1A. Schematic outline of BRDTBD1 degradation analysis in mycobacteria.

1B. Simple Western analysis of BacPROTAC-3-mediated effects on BRDTBD1 after 30 min of incubation. BRDTBD1 levels relative to DMSO control treatment (dark blue) are plotted as mean ± SD of experimental triplicates. BacPROTAC-3 induces BRDTBD1 degradation in a concentration-dependent manner, while sCym-1 or JQ1 treatments did not significantly alter protein levels.

1C. Tandem mass spec analysis of the BacPROTAC-3 effect after 2 h of incubation. The volcano plots show the fold-change (log2) in protein abundance in comparison with DMSO treatment, plotted against p value (log10) (two-tailed Student's t test; triplicate analysis). The BRDTBD1 protein is highlighted in red.

1D. Simple Western analysis of BRDTBD1 levels after 2 h of incubation with the indicated compounds. DMSO was used as control.

1E. Simple Western analysis of BRDTBD1 levels after 2 h of incubation with BacPROTAC-4 and BacPROTAC-4a, with the latter compound carrying a binding-deficient dCymM distomer. All Simple Western data are represented as mean ± SD.

BAF Complex Vulnerabilities in Cancer Demonstrated Via Structure-based PROTAC Design

Boehringer Ingelheim and University of Dundee researchers developed PROTAC degraders of the BAF ATPase subunits SMARCA2 and SMARCA4. Protein degradation levels of SMARCA2 and SMARCA4 were assessed using Simple Western.

- **Targets Analyzed by Simple Western:** SMARCA2 and SMARCA4
- **Sample Type:** Human acute myeloid leukemia MV-4-11 cells
- **Experimental Design:** Dose response curves
- **Read More At:** Farnaby W, Koegl M, Roy MJ, et al. BAF complex vulnerabilities in cancer demonstrated via structure-based PROTAC design. Nat Chem Biol. 2019 Jul;15(7):672-680.

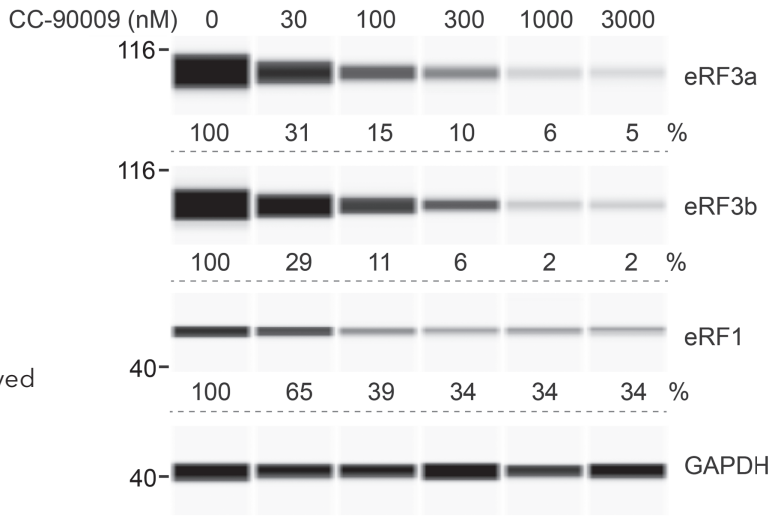
Translation to the Clinic

Pharmacodynamics in Patient Cells and Xenograft Models

Simple Western needs only 3 µL of a sample while offering pg-level sensitivity. It is ideal for clinical applications for PK/PD studies in patients where patient-derived samples available for analysis are critically limited in supply. Therefore, researchers studying CC-20009, a degrader in Phase 1b clinical trials, used Simple Western, including antibodies from Bio-Techne, to investigate the effect of small molecule eRF3a degraders on PTC readthrough in several genetic disease models from patient-derived cells (**FIGURE 2**).

- **Targets Analyzed by Simple Western:** eRF3a, eRF3b, eRF1, UPF1, SMG1, GAPDH, Vinculin, p53, Dystrophin, and TPP1
- **Sample Type:** Patient-derived cells
- **Experimental Design:** Dose response
- **Read More At:** Baradaran-Heravi A, Balgi AD, Hosseini-Farahabadi S, Choi K, Has C, Roberge M. Effect of small molecule eRF3 degraders on premature termination codon readthrough. Nucleic Acids Res. 2021 Apr 19;49(7):3692-3708.

FIGURE 2. Effect of CC-90009 on COL17A1 PTC readthrough

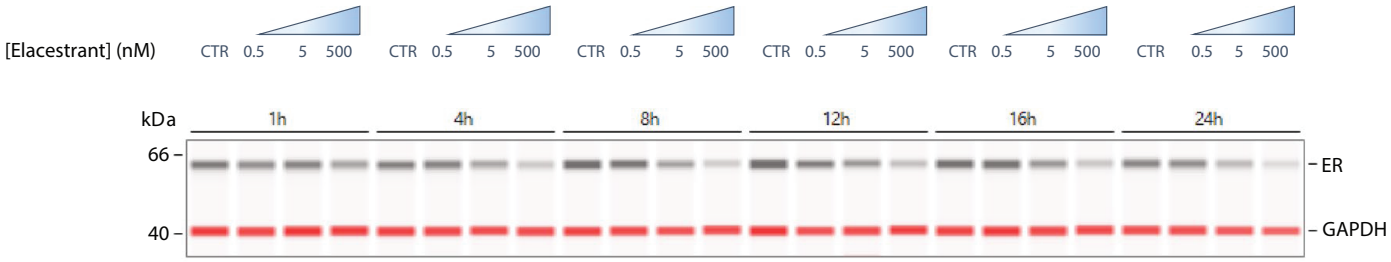


JEB01 keratinocytes derived from a JEB patient with a COL17A1 nonsense mutation (R688X/R688X) were exposed to the indicated concentrations of CC-90009 for 72 h and eRF3a, eRF3b and eRF1 (NBP2-52552) levels were determined using automated capillary electrophoresis western analysis. GAPDH was used as loading control.

In another translational study, Harvard researchers tested the efficacy of elacestrant on ex vivo circulating tumor cell (CTC) cultures using multi-channel chemiluminescence and NIR detection only availably on Jess™ to provide a novel therapeutic intervention for patients with metastatic HR+ breast cancer (**FIGURE 3**).

- **Targets Analyzed by Simple Western:** ER protein and housekeeping protein GAPDH were multiplexed and detected with CHEMI and NIR channel, respectively.
- **Sample Type:** Ex vivo CTC cultures
- **Experimental Design:** Time and dose-response
- **Read More At:** Dubash TD, Bardia A, Chirn B, et al. Modeling the novel SERD elacestrant in cultured fulvestrant refractory HR-positive breast circulating tumor cells. Breast Cancer Res Treat. 2023 Aug;201(1):43-56.

FIGURE 3. Elacestrant suppresses ER protein and ER-targets in hormone- refractory metastatic CTCs cultured ex vivo



MCF7 cells were treated with increasing doses of elacestrant (DMSO, 0.5, 5 and 500 nM) for various times and analyzed by Simple Western, showing suppression of ER protein expression by elacestrant, in a time and dose-dependent manner. GAPDH expression is shown as control.

Customers Agree: Simple Western is a Superior TPD Assay

Simple Western provides critical information to run quantitative DC_{50} curves with a high throughput protein degradation western blot workflow and reproducible and fully quantitative data. Simple Western assays with automated Western blotting capabilities enhance accuracy, ensure data quality, and deliver results. Our systems integrate easily in PROTAC and Molecular Glue degrader screening assays while providing you the flexibility of seamless transferability of methods and an open platform.

I work for a fast-paced drug discovery CRO where our clients expect high-quality data with rapid turnarounds. Jess allows me to assess drug compound potency accurately, and with its high throughput ability, I can screen multiple compounds quickly and efficiently.

Dr. Rachel Doidge
Senior Research Scientist,
Aurelia Bioscience

Learn More About Simple Western in Targeted Protein Degradation on Bio-Techne.com

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