PELAGO BIOSCIENCE
The home of CETSA®

ARE YOUR DEGRADERS DELIVERING?

Biologically Relevant Insights for Protein Degraders with CETSA®

Targeted protein degraders represent a promising opportunity in drug discovery. They enable modulation of the proteome by harnessing the cell's natural protein degradation systems to eliminate specific disease-related proteins, paving the way for treating diseases with high unmet medical needs.

Pelago Bioscience's patented CETSA technology provides a powerful platform for studying protein degrader target specificity and mechanism of action (MoA), which is crucial for optimizing these molecules for clinical success. This application note highlights recent examples of Pelago Bioscience providing critical insights into protein degraders.

Case 1: CETSA Profiling of Thalidomide and IMiDs

Thalidomide, one of the first molecular glue degraders (MGDs), exemplifies the therapeutic potential of small molecules that modulate E3 ligase-neosubstrate interactions. Developed initially as a sedative, thalidomide and its analogs have been repurposed as potent anticancer agents, targeting Cereblon (CRBN) as their primary E3 ligase.

CETSA profiling of pomalidomide in lysed induced pluripotent stem cells (iPSCs) confirmed CRBN as a selective target. GLRX3 was also specifically stabilised by pomalidomide, suggesting its antioxidative effects may involve GLRX3 binding, aligning with reports of neuroprotection (Figure 1).

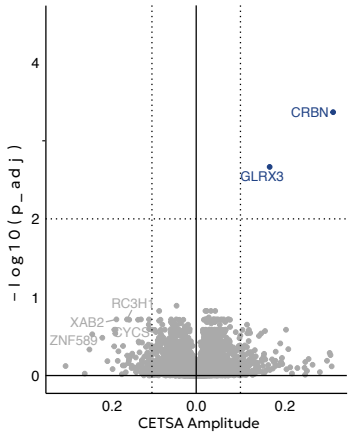

Figure 1. Volcano plots of significantly stabilized proteins in lysed iPSCs.

Proteome-wide CETSA concentration response profile of pomalidomide in live iPSCs, demonstrating time-dependent decreases in abundance or increases in the stability of known and novel protein targets of the E3 ubiquitin ligase complex. Highlighted in Figure 2 are SALL4, ZFP91, RAB28, CSNK1A1, FAM83F and DTWD1, which had been discovered previously as substrates of the IMiD-activated E3 ubiquitin ligase.

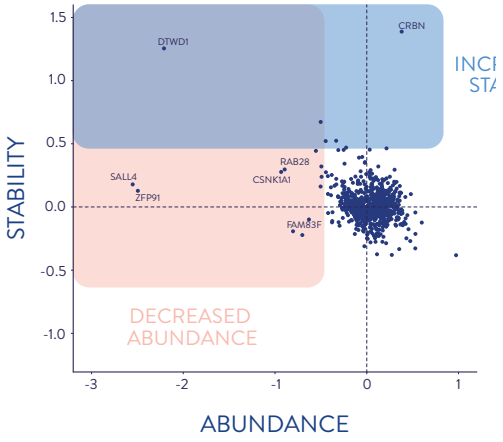

Figure 2. Proteome-wide CETSA concentration response profile of pomalidomide in live iPSCs.

CETSA, combined with quantitative proteomics, provides a holistic approach to elucidating both direct target engagement and functional downstream effects, underscoring its value in IMiD research. This case also demonstrates how unbiased CETSA profiling can accelerate drug repurposing by rapidly and reliably identifying new targets, off-target effects, and mechanisms of action.

Case 2: CETSA Identifies an Unknown E3 Ligase

FIMECS, a phenotypic-first approach company focused on developing innovative drugs for previously undruggable targets through targeted protein degradation, approached Pelago with a specific challenge.

Levering their RaPPIDS™ platform, they developed a novel bifunctional degrader molecule that demonstrated potent degradation of their POI, IRAK-M. However, the E3 ligase responsible for mediating this process remained unidentified. Traditional E3 ligases such as VHL, CRBN, and XIAP did not show binding, suggesting the involvement of an unknown E3 ligase. Proteome-wide CETSA profiling confirmed the primary POI target and successfully identified a novel E3 ligase, E3-ligase B, as a likely mediator of the degradation.

These findings were validated through a pull-down experiment. Based on this discovery, novel E3-ligase B-based degraders demonstrating cell—and tissue-specific profiles have been generated, and optimization studies are currently underway.

These insights were crucial in understanding off-target interactions and allowed the project to advance with a much clearer understanding of the degrader's mechanism of action.

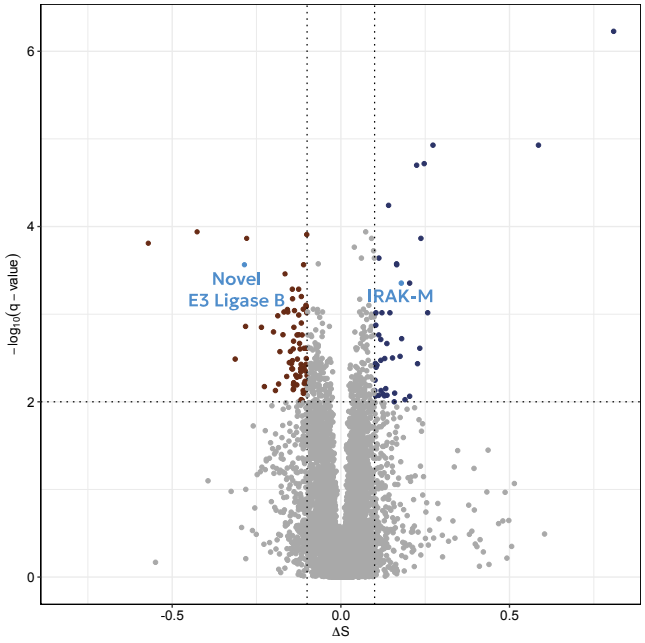

Figure 3. Proteome-wide CETSA profiling confirming the primary POI target and the successful identification of a novel E3 ligase.

References

Chernobrovkin et al. SLAS Discovery 2021

Conclusion

CETSA technology offers a comprehensive toolkit for studying the mechanisms, specificity, and downstream effects of protein degraders. From elucidating the molecular interactions of thalidomide and IMiDs to uncovering novel E3 ligases and dissecting the dual mechanisms of PROTACs, CETSA provides biologically relevant insights that drive drug discovery. By bridging target engagement with functional outcomes, CETSA empowers researchers to optimize degraders with greater precision and confidence, ultimately increasing the likelihood of clinical success.

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