INTEGRATING DEGRADATION, ENGAGEMENT, AND SELECTIVITY: CETSA® AS A FRAMEWORK FOR DEGRADER VALIDATION

PELAGO BIOSCIENCE AB, SWEDEN

PELAGO BIOSCIENCE
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INTRODUCTION

Targeted Protein Degradation (TPD) offers a powerful strategy for eliminating disease-relevant proteins through the ubiquitin–proteasome system. Target engagement and downstream degradation must be verified directly in physiologically relevant systems to interpret degrader activity confidently.

The Cellular Thermal Shift Assay (CETSA) is a label-free biophysical method that measures ligand-induced changes in protein stability in live cells and tissues. This study uses CDK9-directed degraders as a model system and demonstrates how CETSA combines live-cell degradation data, target engagement analysis, and proteome-wide selectivity profiling to reveal the degrader's mechanism of action.

ADVANTAGES OF CETSA IN DEGRADER RESEARCH

- **Physiological relevance:** performed in intact, unmodified cells for clinically translatable results
- **Native proteins:** assesses endogenous targets without tagging or overexpression
- **Quantitative:** provides time- and dose-dependent kinetic readouts
- **Scalable:** compatible with targeted, high-throughput, and proteome-wide studies
- **Translational utility:** applicable to patient-derived PBMCs for ex vivo validation

1. DEGRADE – MEASURING TARGET LOSS IN LIVE CELLS

Degradation of CDK9 was quantified in K562 cells using AlphaLISA™ assays following treatment with CDK9-targeting degrader. Protein levels declined in a dose- and time-dependent manner over 60–180 minutes, confirming measurable target depletion in live cells.

To extend these findings, assays were applied to human PBMCs from two donors. The experiments demonstrated degrader activity in primary cells, illustrating the workflow's translational potential when moving from cell lines to patient-derived material.

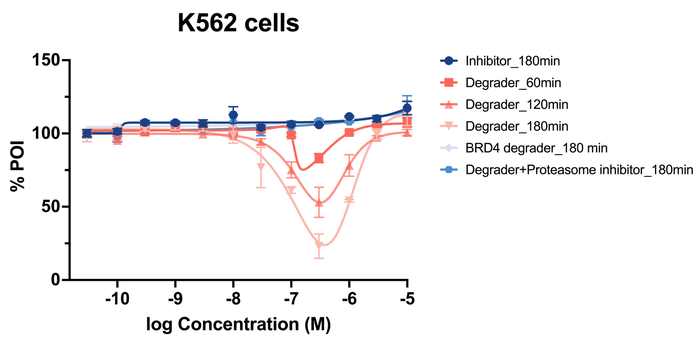

Figure 1. AlphaLISA™ time-course and dose-response curves for CDK9 degradation in K562 cells.
Degradation progresses over 60–180 minutes, confirming measurable target loss and showing consistent time dependence and hook effect in degradation kinetics.

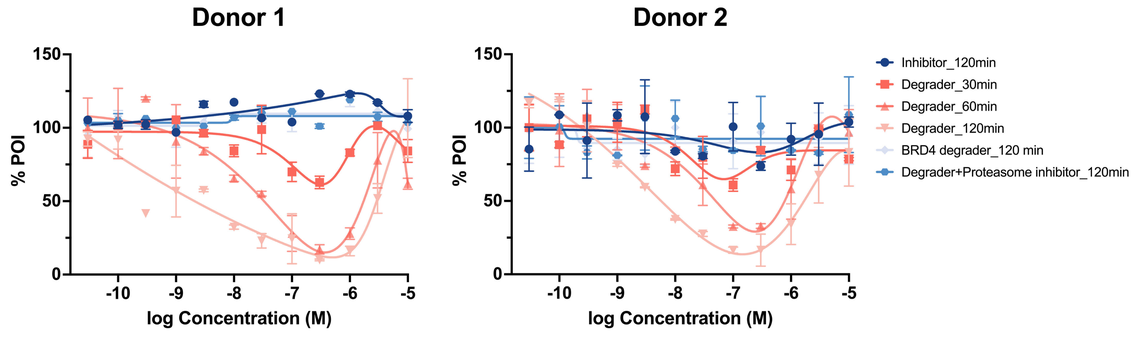

Figure 2. CDK9 degradation in PBMCs from two donors.
Degrader activity observed in primary cells supports the translational applicability of the workflow.

2. ENGAGE – CONFIRMING PROTEIN BINDING IN INTACT CELLS

AlphaLISA measurements in K562 cells were performed to assess degrader-induced target engagement. Both the degrader and the inhibitor stabilized CDK9, confirming binding in intact cells.

Parallel MS analysis in K562 lysates showed correlated engagement behavior for CDK9, together with CRBN engagement only with the degrader, establishing that binding precedes degradation. The data shown including other proteins highlight how this approach distinguishes on-target binding from non-productive interactions and also gives insight about the shared modulation of signaling pathways.

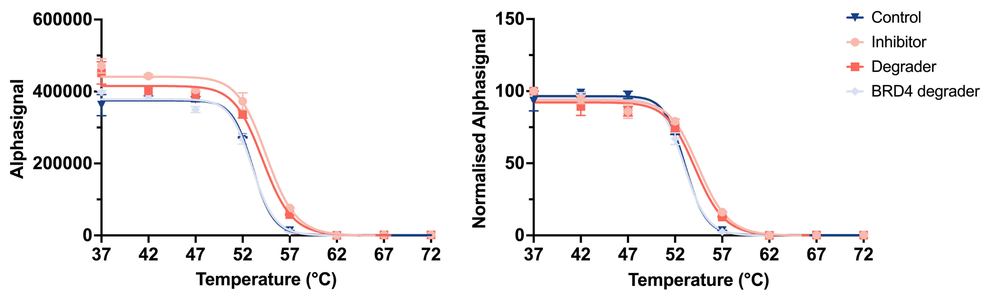

Figure 3. Melt curves of CDK9 in intact K562 cells.
Thermal stabilization profiles shows inhibitor- and degrader-induced engagement.

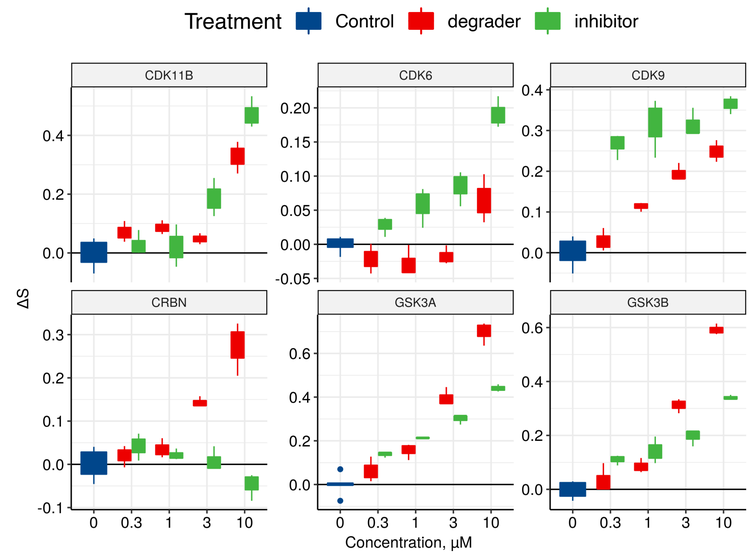

Figure 4. Comparison of concentration response curves of degrader and inhibitor in lysate.
Both, degrader and inhibitor, show target engagement of CDK9, but only degrader shows target engagement of CRBN.

3. PROFILE – PROTEOME-WIDE SELECTIVITY AND OFF-TARGET EFFECTS

Mass spectrometry-based profiling of the degrader showed degradation of CDK9 and its associated cyclin, CCTN1, in intact cells. Also, thermal shifts in kinases, GSK3A/B, and downstream substrate FOXK1 were observed with both degrader and inhibitor, suggesting modulation of the GSK3–FOXK1 signaling axis independent of degradation.

Degradation of CRBN neo-substrates (IKZF1, ZFP91) was detected, consistent with the IMiD-derived moiety in the degrader. STRING network analysis of shared hits revealed enrichment in RNA processing and splicing factors, reflecting downstream consequences of CDK9 pathway perturbation.

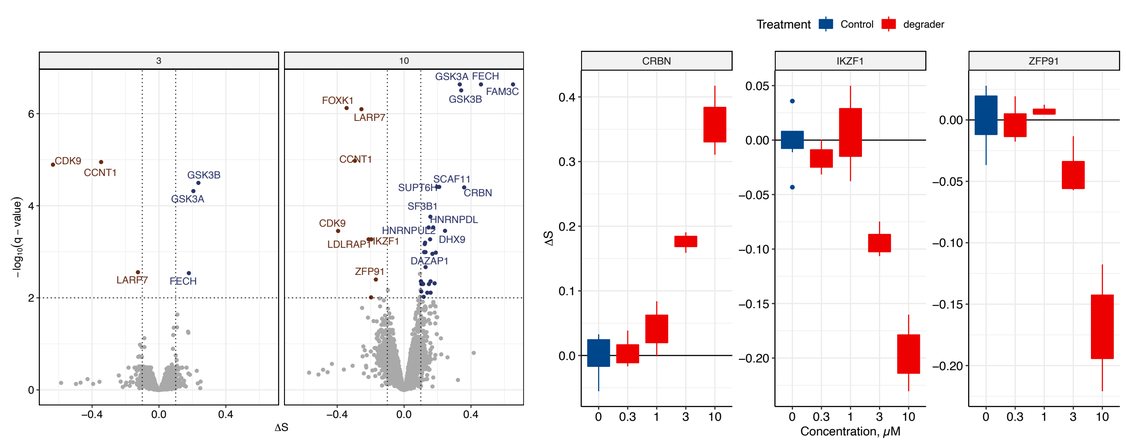

Figure 5. MS-based volcano plots comparing different concentrations of degrader.
Degrader specific thermal shifts for CRBN and its Neo-substrates in intact cells.

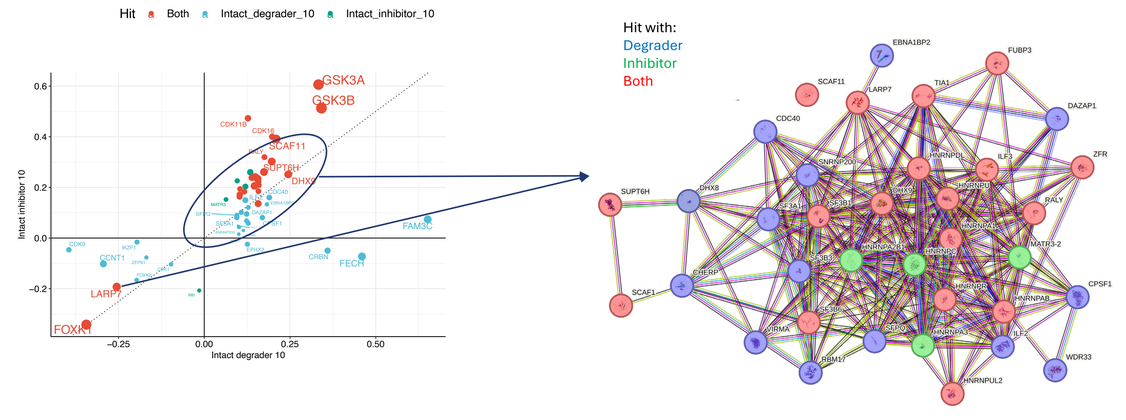

Figure 6. STRING network of commonly shifted proteins.
RNA metabolism-related proteins affected by both compounds indicate pathway-level modulation beyond direct degradation targets.

CONCLUSIONS

- CETSA ENABLES INTEGRATED DEGRADER VALIDATION BY LINKING CELLULAR DEGRADATION, ENGAGEMENT, AND SELECTIVITY DATA.
- THE APPROACH DISTINGUISHES ON-TARGET DEGRADATION FROM NON-DEGRADATIVE INTERACTIONS.
- PROTEOME-WIDE PROFILING PROVIDES MECHANISTIC INSIGHT INTO PATHWAY AND OFF-TARGET EFFECTS.
- THE WORKFLOW SUPPORTS CONFIDENT INTERPRETATION OF DEGRADER ACTIVITY FROM SCREENING TO TRANSLATIONAL STUDIES.

REFERENCES

1. Martinez Molina, D. et al. Science 341, 84–87 (2013).
2. Chernobrovkin, A. et al. SLAS Discovery 26, 534–546 (2021).