MAPPING DRUG DISCOVERY DECISIONS WITH CETSA

Target

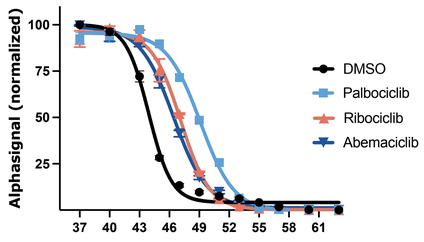
CDK4 as a use case

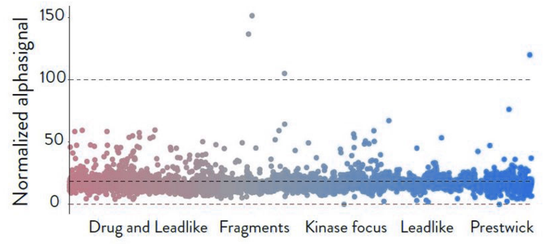
Early drug discovery is often limited by poor insight into compound behavior in biological systems and by reliance on label-based technologies, increasing the risk of advancing the wrong molecules. CETSA® enables unbiased, label-free assessment of compound-protein interactions directly in biologically relevant systems. Here, we illustrate how CETSA can be applied across the drug discovery pipeline, using CDK4 as an example.

Hit

Hit identification in cells

CETSA library screening enables early confirmation of on-target activity under physiological conditions. By prioritizing chemotypes with genuine cellular engagement, CETSA helps shift HTS from hit quantity to hit quality and reduce downstream attrition.





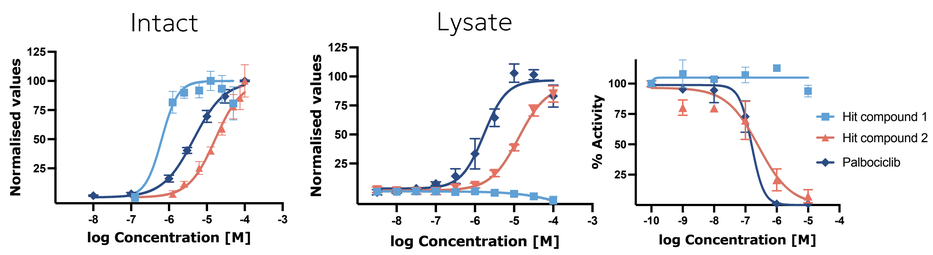
Targeted Profiling

A CDK4 CETSA assay was established in THP-1 cells. Screening conditions were optimized by evaluating melt- and shift behavior with known reference compounds (left panel) and by assessing assay performance across different screening temperatures. A primary screen of a 10K diverse library was conducted at 50 µM, yielding a hit rate of 1% based on a cutoff of 3SD (right panel).

Hit

Hit validation and ranking

Key questions in hit validation are whether a compound engages its target in intact cells in a concentration-dependent manner, and whether that engagement is direct or dependent on cellular context. By comparing sample matrices and correlating with orthogonal functional assays, CETSA confirms on-target binding and provides early insight into mechanism of action. In the example shown, one series exhibits engagement only in intact cells, suggesting that lysate does not fully recapitulate the relevant biology.



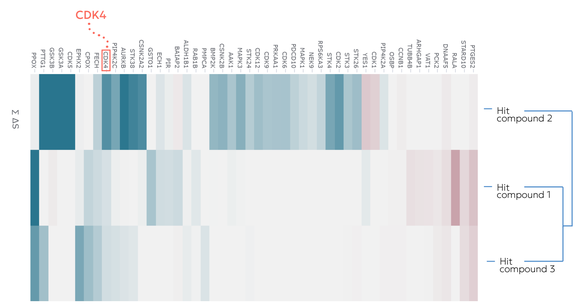
Targeted Profiling

Concentration response curves were generated to quantify CETSA target engagement potencies on CDK4 in intact (left panel) or lysed (middle panel) cells. Data for Palbociclib and two representative confirmed screening hits from different chemical series (Hit compound 1 and Hit compound 2) are shown. In parallel, activity was evaluated using a functional ADP-Glo kinase assay with recombinant CDK4 (right panel).

Lead

Selectivity Profiling

CETSA-based selectivity profiling reveals unexpected off-target interactions in an endogenous lysate setting, enabling more confident optimization of the best leads. The example shown highlights diverging interaction patterns between compound series and supports the finding that CDK4 engagement in lysate is observed only for one chemical series.



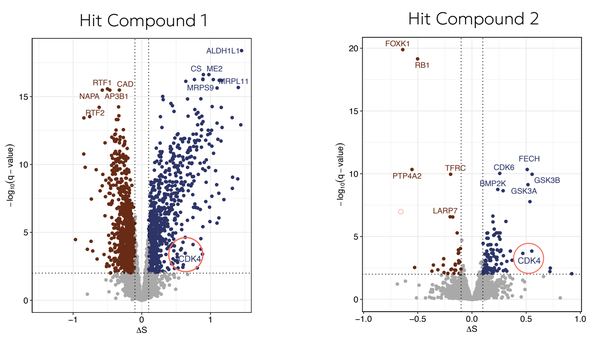
Selectivity Profiling

Heatmap illustrating how three representative confirmed screen hits from different chemical series compare based on unbiased interaction profiling in a standard cell lysate . (Hit compound 1 and Hit compound 3 from series A, Hit compound 2 from series B). The heatmap color scale represents shift sizes compared to control, where blue indicates stabilizing response and red indicates destabilizing response.

Lead

Lead optimization readouts

Proteome-wide profiling in live cells provides a deeper understanding of compound interaction profiles in a relevant cellular environment. The intact cellular setting enables identification of both direct interactions and downstream events, providing insight into mode of action. The profiles shown indicate a clear difference between the chemical series. In the rightmost plot, destabilization of RB1 and FOXK1 reflects a decrease in phosphorylation caused by kinase inhibition rather than direct compound interaction.



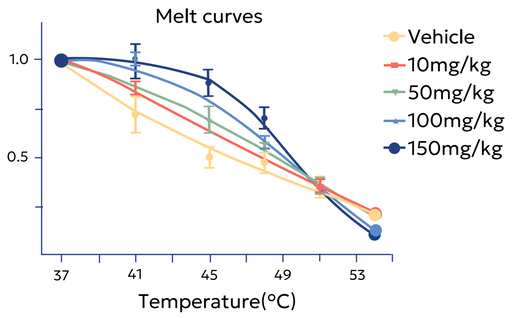
Targeted Profiling and Proteome Wide

Volcano plots showing changes in soluble protein abundance after heat treatment induced by incubation of intact K562 cells with 30 µM of two representative confirmed screening hits, Hit compound 1 (left panel) and Hit compound 2 (right panel). Proteins stabilized by the compound (ΔS>0.1, q<0.01) are shown in blue, destabilized proteins (ΔS<-0.1, q<0.01) are shown in red.

Translational

Translation toward preclinical studies

As a label-free assay, CETSA is readily transferable to different cell lines and tissues. CETSA shows dose-dependent CDK4 target engagement in dosed animal tissue after palbociclib treatment, providing direct evidence of target engagement in vivo. These results bridge cellular findings to a tissue-level endpoint, strengthening confidence that the observed cellular activity can translate into a physiologically meaningful response.



Targeted Profiling

Melt and shift curves of CDK4 in mouse tissue from control animals or animals treated with 10, 50, 100 or 150 mg/kg of Palbociclib.

Conclusion

Drug Discovery with Pelago Bioscience

- Confirm that your compound engages its target inside live cells.
- Explain the molecular mechanism and help select a better series.
- Map off-targets early to reduce the risk of late-stage attrition.
- Link engagement to function and to system-level effects before further investment in studies.
- Increase confidence that the dosed drug reaches and engages the target, helping bridge preclinical work to clinical samples.

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