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THE POWER OF PROTEOME-WIDE CETSA®

Unveiling Drug Targets Through Proteome-Wide CETSA Profiling

Proteome-wide CETSA (Cellular Thermal Shift Assay) addresses these challenges by enabling unbiased compound profiling across the entire proteome in disease-relevant, unmodified cell systems. This approach provides a comprehensive view of both direct target engagement and downstream pathway effects, offering critical insights into compound mechanisms of action.

Our CETSA technology enables high-confidence target identification, supporting drug discovery by deorphanizing targets and providing physiologically relevant, proteome-wide evidence of target engagement.

Identification of Cancer Drug Targets

During a small-molecule screening campaign, researchers identified a131, a novel compound capable of selectively eliminating cancer cells while sparing healthy ones.

Proteome-wide CETSA profiling revealed that a131 targets phosphatidylinositol-5-phosphate 4-kinases (PIP4Ks), with PIP4K2A and PIP4K2C showing significant drug-induced thermal shifts. Replicated experiments confirmed these interactions, validating PIP4Ks as key mediators of a131's dual-inhibitory antitumor activity.

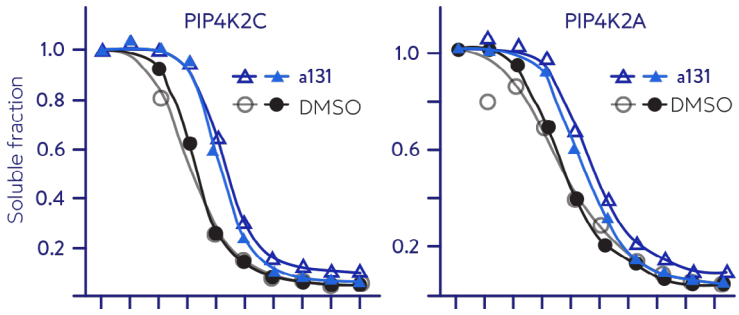

Figure 1: Melt curves for PIP4K isoforms after a131 treatment compared to vehicle control

Follow-up studies further established the compound's potent and broad anticancer efficacy, highlighting its potential as a novel therapeutic strategy for Ras-driven cancers.

Deconvolution of Antimalarial Drug Targets

Despite their potent activity, the mechanisms of action for many antimalarial drugs remain poorly understood. Using proteome-wide CETSA, researchers identified novel drug targets for quinine and mefloquine, two widely used antimalarials.

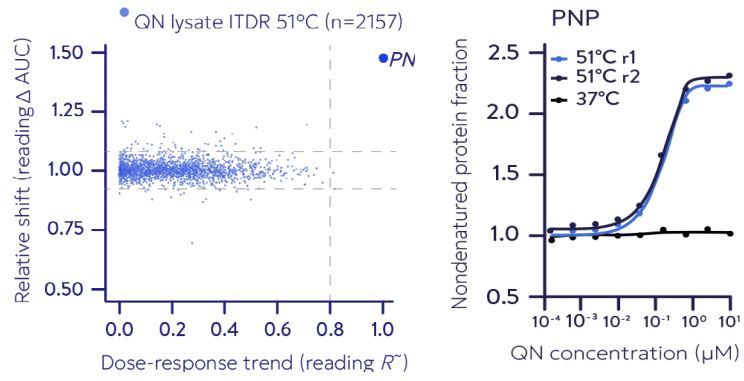

Figure 2: Identification of quinine and mefloquine protein target PNP

The study revealed that both drugs bind to P. falciparum purine nucleoside phosphorylase (PNP)—marking the first time this interaction has been observed. Further whole-proteome concentration-response analyses confirmed PNP as the sole target of quinine, demonstrating that its binding is essential to therapeutic efficacy.

Mechanistic Characterization of Brefeldin A

LifeMine conducted a CETSA profiling of the fungal polyketide lactone Brefeldin A (BFA) to explore fungal-derived small molecules. The study confirmed BFA´s established mechanism of action as an inhibitor of GFB1-mediated activation of the small GTPase ARF1 through a molecular glue mechanism. CETSA profiling validated the Golgi-associated primary target and identified a high degree of functional and physical connectivity among ten hit proteins associated with the Golgi apparatus. These findings demonstrate the utility of CETSA in uncovering mechanistic insights for fungal small molecules and accelerating the development of novel therapeutics.

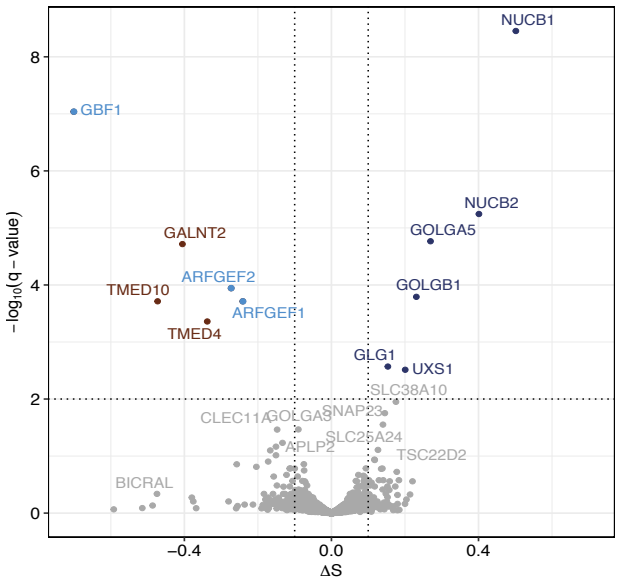

Figure 3: Volcano plot illustrating target hit profile of Brefeldin A in intact cells.

References

Jafari et al. Nature Protocols 2014
Rowlands et al. SLAS Discovery 2023
Shaw et al. SLAS Discovery 2018

Figures in this application note are modified from original.

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Advantages of CETSA for Proteome-Wide Profiling

Proteome-wide CETSA offers a unique platform for understanding drug mechanisms by enabling the unbiased identification of targets, pathways, and off-target effects. Its physiologically relevant, cell-based approach improves the likelihood of clinical success by revealing critical interactions often missed by traditional biochemical assays. This comprehensive profiling reduces the risk of late-stage failures, and supports the efficient development of first-in-class drugs.