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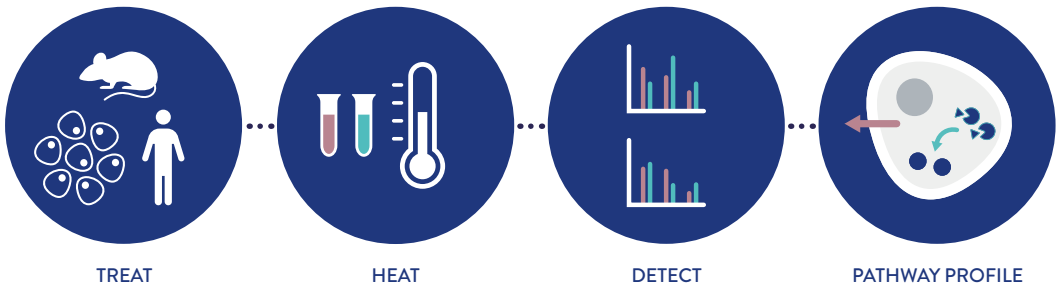
# Explore the proteome with CETSA®

## CETSA® MS for hit evaluation and target deconvolution

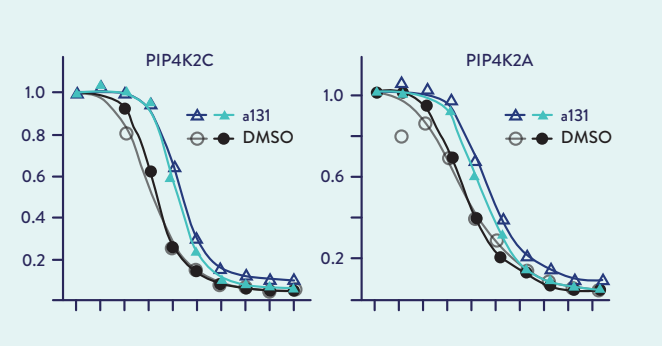
Recent years have seen a resurgence in phenotypic drug discovery owing to its potential for delivering first-in-class treatments for diseases with unmet clinical needs[1]. However, one of the major challenges to phenotypic approaches is the need for target deconvolution and hit confirmation. CETSA MS offers unbiased proteome-wide thermal profiling that can provide key insights for phenotypic drug discovery in a disease-relevant, label-free system. The method allows the entire proteome to be simultaneously monitored for changes in protein thermostability during drug treatment.




## Unbiased CETSA MS identifies targets of a novel compound that selectively kills cancer cells

The novel compound a131 was identified during a small molecule screening campaign for selective elimination of transformed cancer cells but not healthy cells[2]. a131 was found to have dual-inhibitory properties, effectively eliminating Ras-activated cancer cells while protecting normal cells and allowing them to retain their proliferative capacity.

CETSA MS was used to explore cellular targets and signaling pathways that contribute to the dual-inhibitory anti-tumor properties of a131 on a full proteome level. Members of PIP4Ks stood out as the most prominent protein target hits. The graphs show melting curves for PIP4K isoforms in duplicated experiments of a131 and DMSO treatment. a131 induced a thermal shift in PIP4K2A and PIP4K2C. PIPKs have been previously




shown to be involved in tumorigenesis[3]. Follow up experiments confirmed PIPKs as responsible for the a131 mechanism of action. The potent and broad anticancer efficacy of a131 provides novel therapeutic strategies against a vast majority of human cancers.

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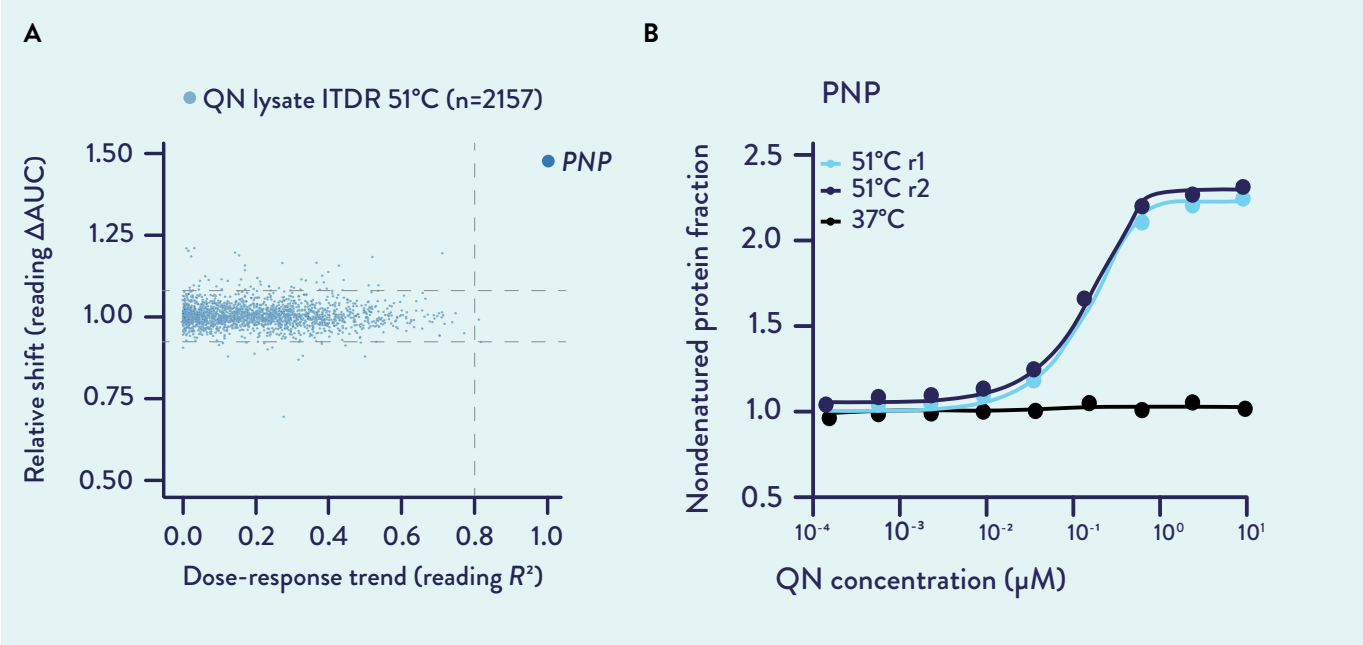
## Deconvolution of anti-malarial drug targets

The majority of anti-malarial drugs in clinical use have been identified by their potent anti-malarial properties in phenotypic screening [4]; however, their mechanisms of action remain largely elusive. This knowledge gap serves as a barrier for optimal implementation and usage of anti-malarial drugs, particularly as they begin to show decreasing efficacy. CETSA MS was applied to investigate the targets of quinine and mefloquine, two important anti-malaria therapies[4]. The study effectively identified the direct targets of the two drugs for the first time. This provides critical knowledge for understanding resistance mechanisms and developing new, effective drugs.




**A.** Whole proteome concentration response analyses of the *P. falciparum* proteome treated with quinine in cell lysate at 51°C. The CETSA MS experiment identifies PNP as the sole target of quinine (QN), suggesting that this binding provides a substantial contribution to its therapeutic effect.

**B.** Protein stabilisation profile of PNP CETSA MS has been used to elucidate this drug target for the first time, despite use of quinine treatment dating back hundreds of years.

## CETSA increases the likelihood for drug success

Gaining a clear understanding of how a drug works, which targets it hits, what processes it alters, and which of these actions are required for therapeutic efficacy can help guide drug development and prevent late-stage failures. CETSA MS is a valuable tool to deorphanize drug targets and provide an unbiased proteome-wide measure of target engagement in a physiologically-relevant setting.

### References:

1. Moffat et al. Nature 2017
2. Kitagawa et al. Nature Communications 2017
3. Emerling et al. Cell 2013
4. Dziekan et al. Science Translational Medicine 2019

Figures in this application note are modified from original.