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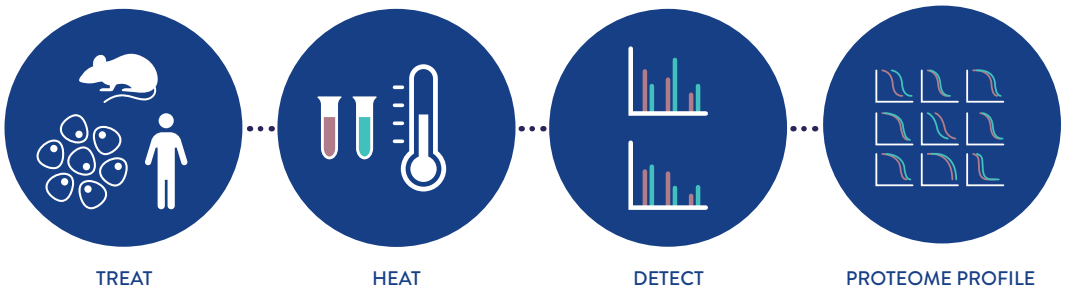
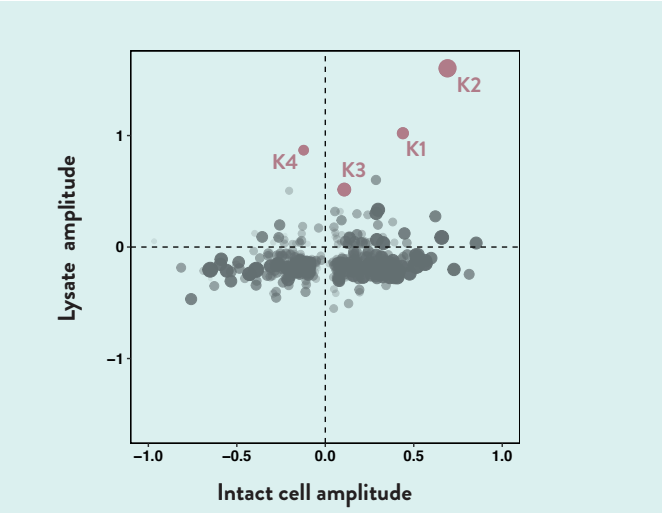
Is your target a hit?

Assessing the selectivity of candidate drugs is crucial in order to reduce drug development attrition rates. Conventional screening methods using target panels are not sufficient to fully profile the selectivity since drug candidates commonly have multiple physiological targets far beyond the limited scope of these panels. CETSA® MS has been developed to effectively assess both on- and off-target protein binding through unbiased proteome-wide profiling. The cornerstone of the CETSA method is the fact that a protein bound to a ligand has a different thermal stability than the unbound protein. CETSA principles are then combined with LC-MS/MS protein quantification in order to measure the selectivity of compounds by assessing thousands of proteins in parallel. It allows researchers to determine the impact on both individual proteins and entire pathways targeted by bioactive molecules. When performed in whole cell experiments, CETSA MS can also provide an analysis of a compound's mechanism of action in a disease-relevant setting.



CETSA MS confirms direct and downstream targets

In the newly developed two dimensional (2D) CETSA MS set up, the compound is incubated in a range of concentrations and heated to multiple temperatures followed by full proteome analysis by mass spectrometry. This experimental expansion provides enhanced sensitivity for identifying small thermal shifts, allowing identification of every compound target and an estimate of effect size. By evaluating data from intact cells in addition to cell lysate, compound selectivity can be assessed in the relevant matrix and natural environment of the protein, while also allowing analysis of downstream effects. Data from cell lysate can confirm direct ligand binding.



Treating Jurkat cell lysate and live cells with kinase inhibitors, 2D CETSA MS revealed several novel targets. K1, a previously known target for the profiled kinase inhibitor, served as a positive control. The inhibitory compound binds K1 and K2 in the intact cell as well as lysate matrix. The compound only binds K3 and K4 in lysate, indicating that the binding site is unavailable or occupied in its natural environment in the intact experiment.

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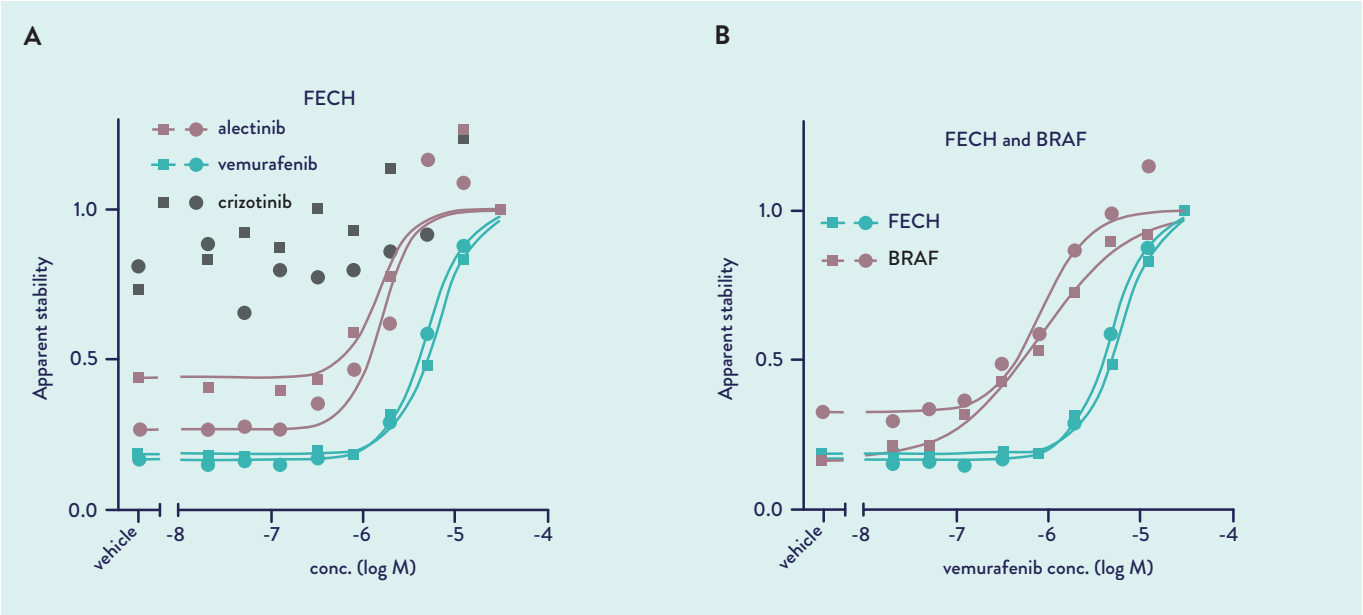
Uncovering adverse drug effects with CETSA MS

The kinase inhibitors vemurafenib and alecitinib are used in cancer treatment. However, both drugs are known to cause phototoxicity and skin rashes as a side effect, due to increased tissue levels of protoporphyrins. CETSA MS was used to study the thermal response of the proteome under increasing drug concentrations and found that alongside the primary drug target BRAF, the two compounds also bind to the heme biosynthesis enzyme FECH – inhibition of which causes increased protoporphyrin levels[1].



Concentration response profile

A. Concentration response profile demonstrating stability shifts of FECH induced by vemurafenib, alecitinib and crizotinib over a range of concentrations. Alecitinib affected FECH more potently than vemurafenib. Crizotinib, another oncology drug, has no known photosensitivity side effects and here showed no effect on FECH stability[1].

B. Concentration response profile with vemurafenib-treated K562 cells shows concentration-dependent thermal stabilization of BRAF (target binding) and FECH (off-target binding).

Unbiased proteome-wide assessment of compound selectivity

CETSA MS is a versatile tool to address challenges throughout the drug discovery pipeline. The method can be applied to any cell and tissue, enabling a label-free assessment of a compound on up to 6000 proteins in a physiologically relevant matrix. It measures not only the direct binding of compound to proteins but also subsequent downstream effects of the initial target engagement. Unbiased proteome-wide profiling makes CETSA MS uniquely positioned to help avoid failures in clinical development, by identifying issues with selectivity earlier in the process.

References:

1. Savitski et al. Science 2014
2. Becher et al. Nature Chemical Biology 2016

Figures in this application note are modified from original.