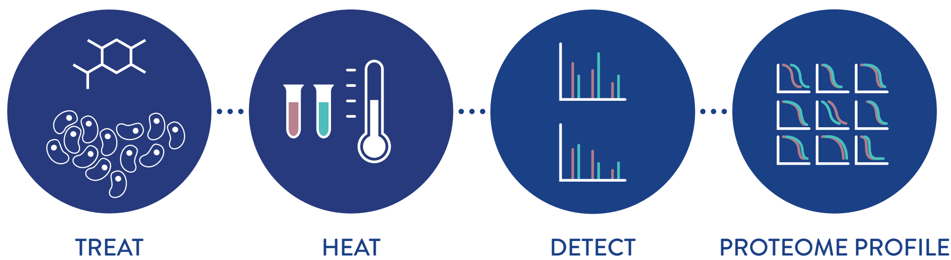
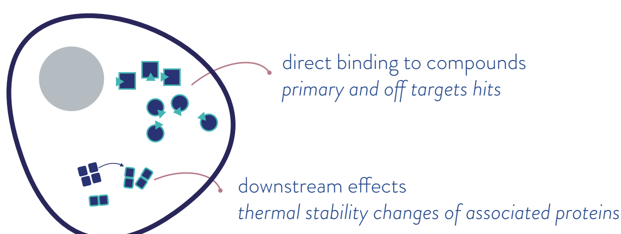
Is your target a hit?

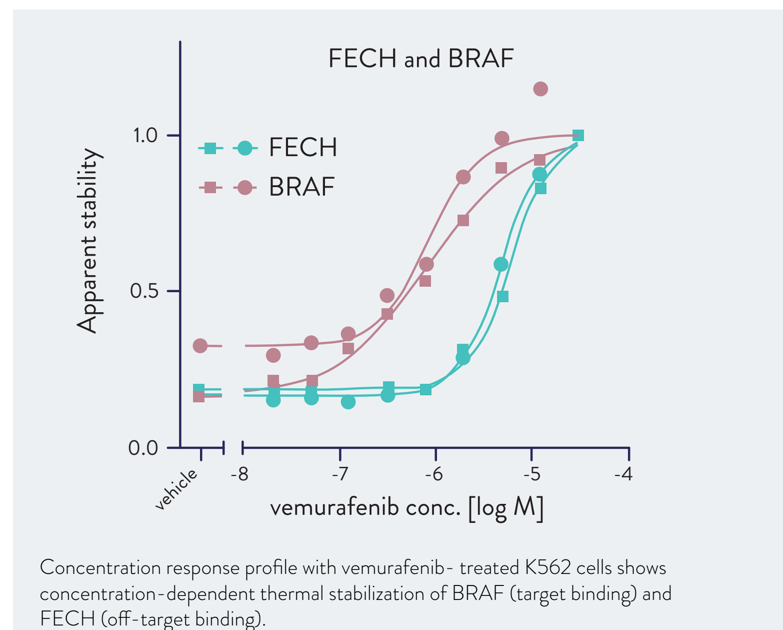
Assessing the selectivity of candidate drugs is crucial in order to reduce drug development attrition rates. Conventional screening methods using target panels are not sufficient to fully profile the selectivity since drug candidates commonly have multiple physiological targets far beyond the limited scope of these panels. CETSA® Explore 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® Explore can also provide an analysis of a compound's mechanism of action in a disease-relevant setting.



Uncovering adverse drug effects with CETSA® Explore

CETSA® Explore was used to study the proteome thermal response under increasing drug concentrations of vemurafenib, and found that alongside the primary drug target BRAF, this cancer drug also binds to the heme biosynthesis enzyme FECH- inhibition of which causes increased protoporphyrin levels[1], responsible for the side effects of phototoxcity and skin rashes described for vemurafenib.





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

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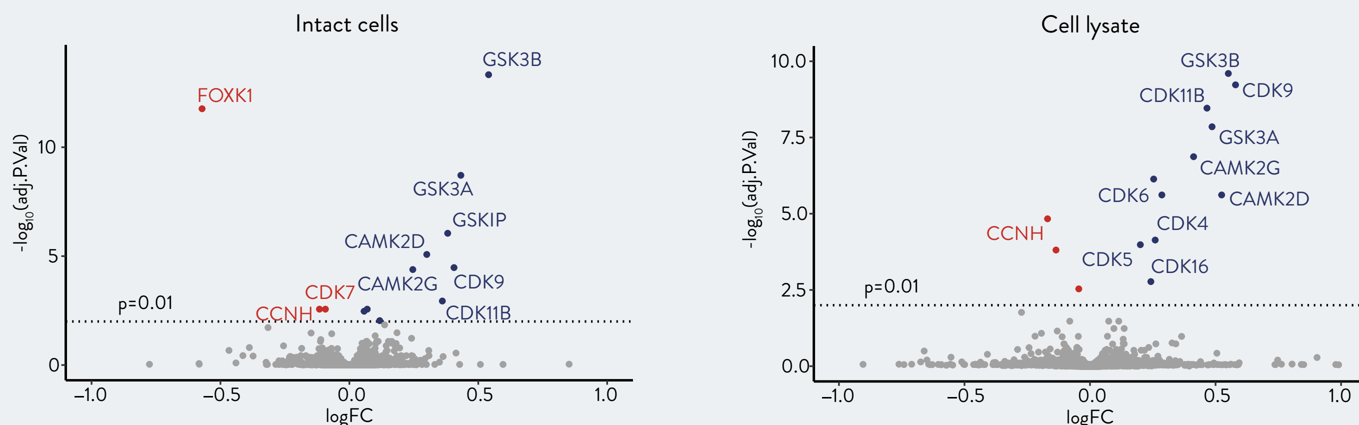
CETSA® Explore confirms direct and downstream targets

CDK9 is an attractive oncology therapeutic target, indirectly targeting short-lived proteins regulating proliferation and/or apoptosis by transient inhibition of gene transcription. CETSA® Explore was used to profile compound 1, designed as a potent and selective CDK9 inhibitor.[2] Using the compressed format, samples from different temperatures are pooled across a range of concentrations to efficiently reduce sample material and shorter running times on the MS instrument, while providing wide concentration response information based on differences in protein stability across a full melt curve.

A great advantage of CETSA® Explore is the unlabelled nature but also the power of comparing intact cell profile, where the proteins are in their native cellular compartments and protein complexes are maintained, with cell lysate profile, where direct ligand-protein interactions are still present, but there are not pathway and downstream effects, i.e biology is off. In the lysate matrix, CDK9 was thermally stabilised by compound 1, while other parts of the pTef complex were not, highlighting the power of CETSA® identifying direct binding.

In intact cell matrix, secondary downstream effects were identified: GSKIP, which binding partner is GSK3β. FOXK1 - a transcriptional regulator implicated in metabolism, cell proliferation and apoptosis- was found destabilized in the intact cell. Since GSK3 proteins mediate phosphorylation of FOXK1, Compound 1 Inhibition of GSK3 proteins might cause hypophosphorylation of FOXK1 and subsequent destabilisation. Several CDKs did not shift in intact cell while they were clearly stabilised in lysate, suggesting that despite of strong affinity to other CDKs, no target engagement occurs in physiologically relevant conditions.



Vulcano plot representing proteins thermally stabilized or de-stabilized using cellular lysates or intact cells

Unbiased proteome-wide assessment of compound selectivity

CETSA® Explore 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® Explore 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. Hendricks et al. ACS Chem Bio (accepted)

Figures in this application note are modified from original.