PELAGO BIOSCIENCE
The home of CETSA®

Selectivity Profiling

unique unbiased off target assessment

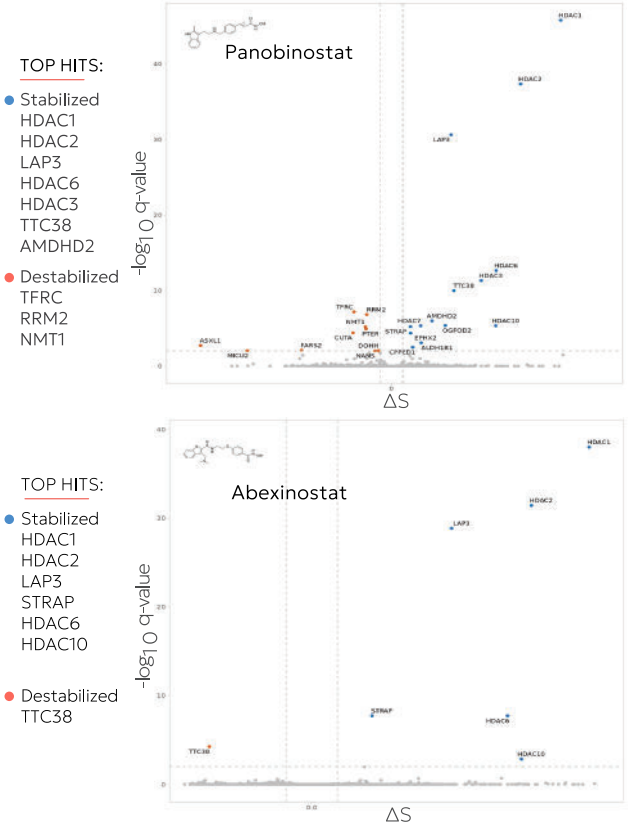
Standard Selectivity Profiling in lysate is a CETSA® based service with Mass Spectrometry (MS) detection, providing unbiased compound–protein interaction assessment for more than 5,000 proteins simultaneously. The method was developed for small molecules but is also suitable for target interaction with peptides, antibodies and other biologics, as it reports on changes in apparent protein thermal stability. Profiling in cell lysate allows for bypassing potential cell penetration issues.

Method description
This method utilizes a proprietary lysate preparation stemming from a human suspension cancer cell line. After CETSA[1] , the supernatant is subjected to quantitative MS analysis. Compound-induced changes in apparent thermal stability on the global proteomes are reported, with each compound treatment performed in triplicate.

Statistical analysis
Protein intensities are log2-transformed and normalized across treatments and replicates. For every treatment, any apparent thermal stability shifts (ΔS) are estimated as log2-transformed fold change relative to the average vehicle control. Moderated t-test implemented in the limma R-package[2] is used to estimate significance (p-value) of observed thermal stability changes. The q-values are then calculated using Benjamini and Hochberg (BH)'s method to account for multiple testing (statistical test applied for every quantified protein). While using q<0.01 cut-off for hit selection one would expect 1% probability to have a random (false) hit among the selected proteins. However, in many cases it is advisable to consider different significance cut-offs (i.e. q<0.05) for the hit selection, especially when few significant protein hits are observed.

Example data
Volcano plots for two HDAC inhibitors of hydroxamate type: Panobinostat and Abexinostat. Both compounds induce thermal stability changes of annotated targets as well as off-targets at the investigated saturating concentration of 30 µM. Selectivity profiling at saturating concentrations allows for simultaneous detection of both low and high affinity interactions. Both Panobinostat and Abexinostat are pan-HDAC inhibitors, and induce thermal stability changes of several HDAC proteins. Their proteome thermal stability change profiles differ, both with regards to which HDAC proteins they bind, but also which off-targets they affect.

Data Interpretation
In these volcano plots, thresholds for a considered hit has been set to: |ΔS| >0.1 | q<0.01, meaning that a hit can be both thermally stabilized and destabilized. Thresholds can be adjusted to better suit individual compounds (here empirically chosen to give a low rate of false positives). A more profound understanding about biology and pharmacology associated with the individual compounds may warrant investigators to adjust thresholds accordingly. In these examples, HDAC1, HDAC2, and LAP3 can be considered as clear hits, for both HDAC inhibitors, with very small q-values and rather large ΔS. The size of ΔS, on its own, does not indicate significance of a hit, as the size of ΔS is intrinsic to any compound–protein interaction. Some proteins have intrinsically small shift sizes, but these can still be significant. A plausible explanation for why some hit proteins has a higher q-value is that they have an interaction with lower affinity, compared to the hits with lower q-value. To resolve a difference in affinity with CETSA, a concentration–response experiment is needed.



These examples suggest that Abexinostat has a cleaner selectivity profile. Compared to Panobinostat it binds to fewer proteins at the tested concentration and hence has less suspected off-targets.

1 Martinez Molina, D. et al. Monitoring Drug Target Engagement in Cells and Tissues Using the Cellular Thermal Shift Assay. Science 341(84-87) (2013)
2 Ritchie, ME. et al. limma powers differential expression analyses for RNA-sequencing and microarray studies, Nucleic Acids Research, 43(7) (2015)