UNBIASED SELECTIVITY PROFILING, CETSA WITH MS DETECTION

TOLVANEN T, FRIMAN T, BOSTRÖM T, CHERNOBROVKIN A, MARTINEZ MOLINA D
PELAGO BIOSCIENCE AB SWEDEN

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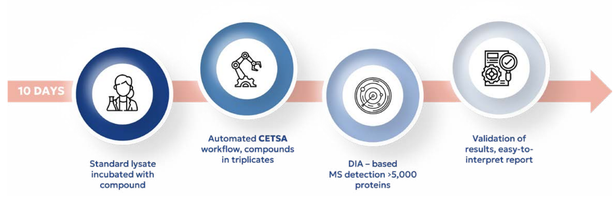
The home of CETSA®

INTRODUCTION

- Confirming selective binding reduces off-target effects and improves drug safety.
- Pelago Bioscience offers a proteome-wide, unbiased Selectivity Profiling Service.
- Assesses binding and detects off-target interactions across proteins often missed by traditional screening—beyond conventional panels.
- Supports large-scale compound testing early in drug discovery to identify potential liabilities early.
- Help to prioritize lead compounds and support informed decision-making for further development.

METHOD

- We have applied our proprietary Cellular Thermal Shift Assay (CETSA®)[1,2] coupled to mass spectrometric readout in a standardised lysate from a human cancer suspension cell line.
- The use of compressed, integrated melt curve format (also known as PISA) ensures a high throughput protocol.
- A semi-automated sample handling platform in combination with DIA analysis allows fast, precise and reproducible profiling of compound with a turn-around time of 10 days.
- Induced thermal stability changes with a protein coverage of over 5,000 proteins.



RESULTS

We profiled a set of well-characterized probes and marketed drugs to demonstrate the utility of our Selectivity Profiling service.

Figure 1a displays concentration-dependent responses for key protein hits for Abemaciclib and Palbociclib.

- Abemaciclib appears more promiscuous than Palbociclib.
- Shared targets include CDK4, CDK6, and the complexing partner, cyclin D3 - CCND3.
- Examples of targets engaged by Abemaciclib are CAMK2G, GSK3A/B, IRAK1, and PRKCB and ALDH1B1.
- Palbociclib uniquely engages PLK1.
- The data highlights the value of broad unbiased selectivity profiling based on relative selectivity and potency in understanding compound specificity and off-target potential.

Figure 1b shows the kinase selectivity profiles of Abemaciclib, Palbociclib (CDK inhibitors), and Dasatinib (BCR-Abl inhibitor) at 30 µM.

- As expected, the CDK inhibitors show overlapping profiles, while Dasatinib engages a distinct kinase set.
- Figure 1c includes example non-kinase hits for all three inhibitors.

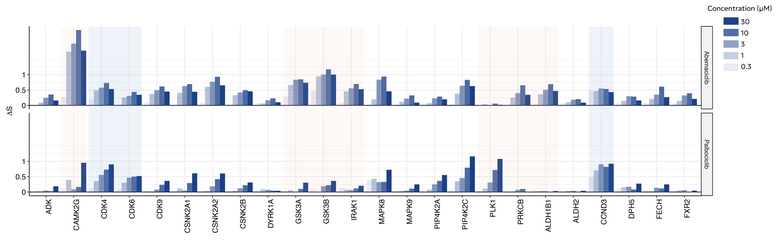
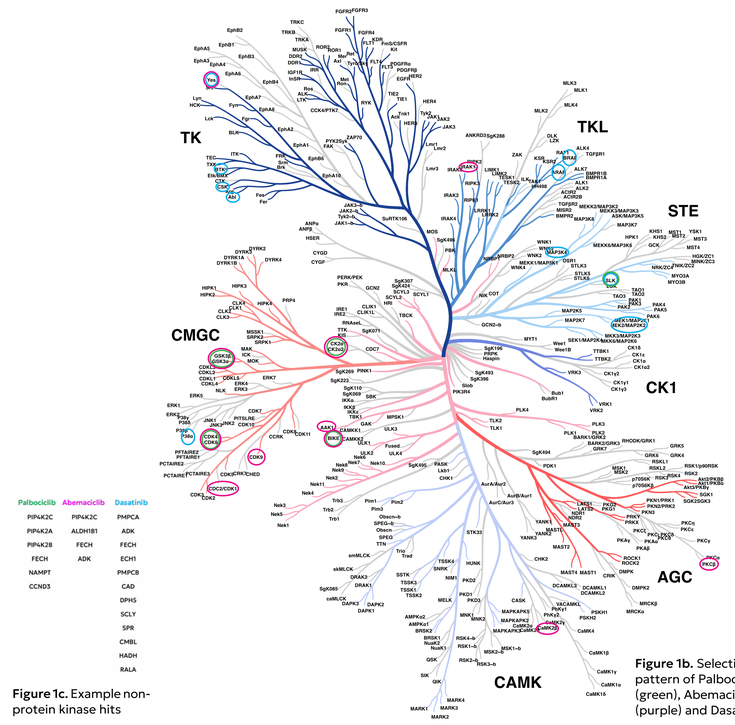


Figure 1a. Top kinase and non-kinase hits (examples of common hits highlighted in blue, and examples of hits that are not shared between the two targets are highlighted in apricot) for Abemaciclib and Palbociclib tested at five different concentrations



Palbociclib	Abemaciclib	Dasatinib
PIP4K2C	PIP4K2C	PMPCA
PIP4K2A	ALDH1B1	ADK
PIP4K2B	FECH	FECH
FECH	ADK	ECH1
NAMPT		PMPCB
CCND3		CAD
		DPH5
		SCLY
		SPR
		CMBL
		HADH
		KALA

Figure 1c. Example non-protein kinase hits

Figure 1b. Selectivity pattern of Palbociclib (green), Abemaciclib (purple) and Dasatinib (blue)

Histon deacetylates (HDACs) are key enzymes regulating important cell processes such as cell-cycle progression and apoptosis. Figure 2 delineates the CETSA selectivity profile of several HDAC inhibitors.

- Clear differences in selectivity profile observed across compounds tested at 30 µM (both HDAC and non-HDAC targets).
- Entinostat (non-hydroxamate) appears to have cleanest profile.
- Panobinostat has the most off-targets.

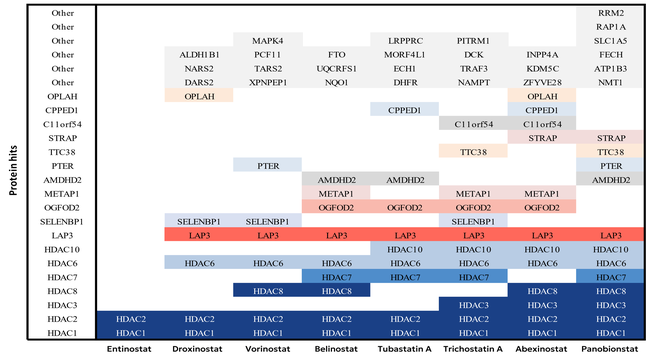


Figure 2. Selectivity profiling of HDAC inhibitors

The applicability of standard selectivity profiling in lysate extends beyond kinase and HDAC inhibitors. Figure 3 show more examples of different target classes that are of interest for drug development in oncology.

- The DUB (deubiquitinating enzymes) targeting probe RA-9 show target engagement of several USPs (Ubiquitin Specific Proteases) as well as some off-targets.
- Two different probes targeting the EZH2 methyl transferase show very different off-target profile, however both appear to engage with the intended target.
- PI3 kinase inhibitor Idelalisib engages with its primary target PIK3CD and also withPIK3CB and the regulatory subunit PIK3R1 as well as some off-targets.
- Both PARP1 inhibitors Olaparib and Talazoparib show target engagement of PARP1, whereas few other proteins are affected.

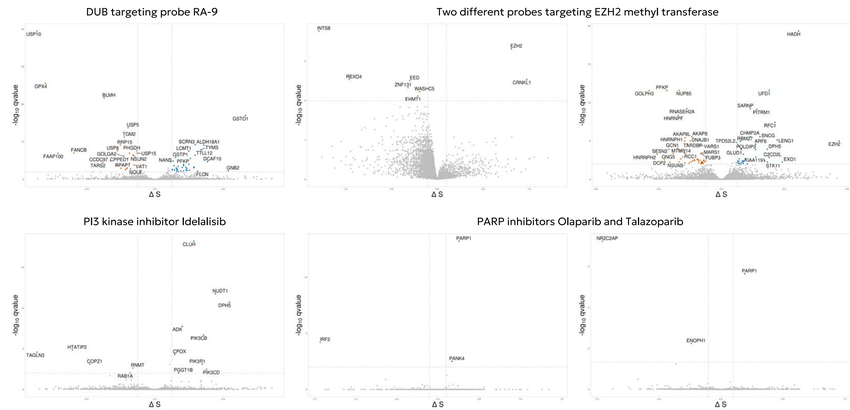


Figure 3. Selectivity profiling of the DUB inhibitor RA-9, two EZH2 methyltransferase inhibitors, the PIK3CD inhibitor Idelalisib and the PARP1 inhibitors Olaparib and Talazoparib

SUMMARY

- A new Standardized Selectivity Service, based on CETSA with MS detection, allows for target engagement assessment of >5,000 proteins.
- In contrast to other selectivity panels, this Standardized Selectivity Service is unbiased, as all detected proteins are probed for compound interactions.
- The service is well suited to compare relative selectivity of compounds with similar mode of action and/or target. The service can also be used for comparison of selectivity of a compound at different concentrations.
- The service is also well suited to be used in the lead generation/lead optimization phase to assess selectivity of different compounds or compound series.
- The Standardized Selectivity Service enables early prioritization and assessment of compound promiscuity, potential liabilities, and identification of critical off-target proteins.

References

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. Chernobrovkin, A. et al. In-depth characterization of Staurosporine induced proteome thermal stability changes. bioRxiv (2020)