PELAGO BIOSCIENCE

REVEAL BROAD COMPOUND SELECTIVITY EARLY

What proteome-wide CETSA® Selectivity can reveal in lysate and intact cells

LYSATE • INTACT CELLS • PAIRED PROFILING

Executive summary

A compound's selectivity profile can differ between an isolated biochemical assay, cell lysate, and intact cells. Cellular access, metabolism, protein organization, and treatment responses all influence which interactions and effects are observable. These differences matter when comparing compounds or deciding which findings to investigate further.

CETSA Selectivity compares compound-associated protein responses across endogenous proteins without limiting the experiment to predefined target panels. A lysate matrix provides an interaction-focused view, removing cellular entry as a barrier. An intact-cell matrix retains the conditions that shape compound behavior during cellular exposure. Studying both matrices provides complementary information on compound–protein interactions and how these manifest in a cellular environment.

Three examples illustrate these uses. Staurosporine analogs show how related structures differ in the breadth and identity of their protein responses. A four-compound CDK comparison connects lysate interaction profiles with cellular responses and a question about cellular exposure. An HDAC inhibitor comparison focuses on selecting compounds with different coverage within the same target family. The cases illustrate when lysate alone is informative and when intact-cell profiling adds useful biological information.



Why selectivity can look different across experimental systems

Biochemical assays and focused panels establish activity against defined targets and compare compounds across selected proteins. Proteome-wide profiling extends that comparison to endogenous proteins detected under the experimental conditions. It can reveal differences beyond the intended target, including responses that emerge as the chemistry changes.

Compounds with similar biochemical potency can produce different cellular profiles. A compound may engage a protein in lysate but encounter different access, competing ligands, or protein states in an intact cell. Conversely, an intact-cell response may depend on metabolism or cellular processes that are not preserved in lysate. The two matrices therefore provide different information about compound behavior.[1–3]

Useful questions for a proteome-wide comparison include:

- Did a chemical modification alter the wider protein-response profile?
- When compounds show similar activity in an existing target assay, which additional protein responses distinguish them in CETSA profiling?
- Which protein responses appear in lysate, intact cells, or both?
- Which additional targets or cellular responses should guide the next experiment?

From target engagement to proteome-wide comparison

When this readout is coupled to quantitative mass spectrometry, responses can be measured across thousands of endogenous proteins. A compound-associated change in the measured soluble protein signal after heat treatment is called a CETSA response. At the proteome level, these responses can reflect target engagement, changes in protein interactions, localization or abundance, and other cellular responses to treatment.[3-5]

Each protein response is assessed alongside the experimental matrix, concentration, and responses across the rest of the proteome. This allows comparison of compounds by responding protein identity, the concentrations at which responses appear, and the biological relationships among the findings.

What lysate and intact-cell profiling contribute

Lysate profiling
In cell lysate, compounds are exposed to endogenous proteins without first crossing the cell membrane. This makes lysate useful for comparing interaction profiles across compounds or chemotypes under standardized exposure conditions. It can reveal shared and compound-specific protein responses, including responses beyond the expected target profile.

Intact-cell profiling
In intact cells, uptake, efflux, metabolism, compartmentalization, protein assemblies, and active pathways remain part of the experiment. Profiling can therefore reveal target engagement during cellular exposure alongside broader cellular responses. These findings support compound comparison and can provide initial clues about the cellular processes a compound affects.

Combined profiling
Comparing the matrices shows which proteins respond in both settings and which responses are detected mainly in one. A target response in lysate may help interpret associated cellular changes even when that target does not produce a detectable response in intact cells. Likewise, an intact-cell profile can reveal effects that are not apparent from the lysate interaction profile.

The value of combining the datasets is the additional biological information. Overlap supports the consistency of an observation across matrices; differences identify where cellular context affects the measured profile. Neither pattern alone establishes the cause of every response.

Published examples of context-dependent CETSA responses

Published CETSA studies provide a basis for these comparisons. Almqvist and colleagues screened 10,928 compounds against thymidylate synthase in live K562 cells. Decitabine produced a cellular target-engagement response but did not stabilize thymidylate synthase in treated lysate or a recombinant-protein thermal shift assay. Intracellular phosphorylation and deamination generated an active metabolite, explaining why the intact-cell experiment was informative.[2]

Proteome-wide CETSA also detects treatment-associated cellular changes. Ramos and colleagues compared five families of apoptosis-inducing cancer drugs and identified distinct early response patterns that converged on biochemical changes at the nuclear periphery. The work demonstrates how intact-cell profiling can distinguish cellular responses across compounds while also identifying shared biology. [5]

The following Pelago Bioscience examples apply these principles to compound comparison: identifying similarities across a chemical family, interpreting findings across matrices, and distinguishing compounds within a target class.

Compound comparisons in lysate and intact cells

Comparing staurosporine analogs beyond their reported targets

Staurosporine inhibits a broad range of kinases, and several structurally related compounds also affect multiple members of this protein family.[6] Comparing them therefore requires looking beyond their commonly reported targets to understand where their profiles overlap and differ.

Six compounds were profiled in K562 cell lysate and intact cells across a concentration range. Although they shared some protein responses, they differed in how many proteins responded and at what concentrations. Figure 1 shows these differences across the proteome and for selected individual proteins.

The individual protein results add information that a total response count cannot provide. Responses involving protein kinase A and protein kinase C, commonly reported targets for this group, appeared mainly in lysate at higher concentrations. Other kinases responded at lower concentrations, identifying additional interactions that could be relevant when interpreting the compounds' effects.

The comparison distinguishes related compounds by the number and identity of responding proteins. This helps researchers assess which candidates show the desired target responses and which additional protein responses warrant further investigation.

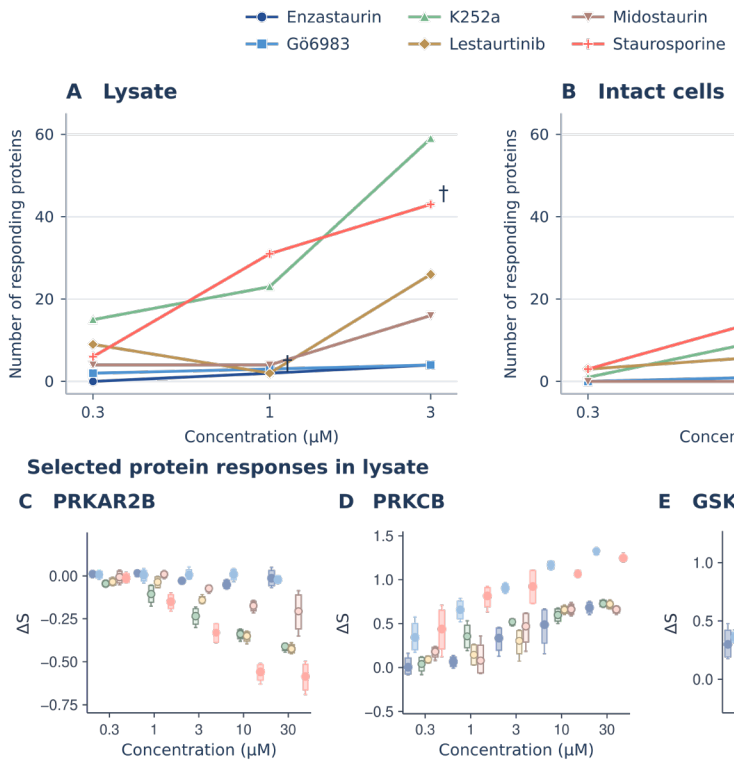


Figure 1. Concentration-dependent protein-response profiles in lysate and intact cells. Numbers of responding proteins at 0.3, 1 and 3 µM are shown for six compounds in lysate (A) and intact cells (B). Selected lysate profiles show ΔS responses for PRKAR2B (C), PRKCB (D) and GSK3B (E) at 0.3, 1, 3, 10 and 30 µM. Colors identify compounds throughout the figure. Panels C–E retain their individual vertical scales.

Connecting CDK interaction profiles with cellular responses

Olomoucine, (R)-roscovitine, purvalanol A, and purvalanol B are related inhibitors of cyclin-dependent kinases (CDKs), which include regulators of cell division. The purvalanols were developed through chemical changes intended to improve CDK inhibition.[7] Comparing these four compounds provides an opportunity to examine how those changes affected both protein interactions and cellular responses.

The compounds were profiled retrospectively in K562 lysate and intact cells at 1, 10, and 30 µM. Two findings are particularly useful: changes in the lysate interaction profile across the four compounds, and a difference between how purvalanol A and B behaved in cells.

How the interaction profiles differed

The lysate results revealed an interaction outside the kinase family. Olomoucine produced a response for methionine aminopeptidase 2 (METAP2) at a lower concentration than any detected CDK response. METAP2 also responded to (R)-roscovitine, alongside a broader set of CDKs.

Neither purvalanol produced a METAP2 response. Both produced CDK2 and CDK5 responses, with purvalanol B responding at a lower tested concentration than purvalanol A. Other CDKs and non-CDK kinases also responded, however, so neither compound's profile was restricted to CDK2. Table 1 summarizes these differences. It shows how a chemical change can remove one additional interaction while retaining others that remain relevant to compound selection.

A.

Protein or protein group	Olomoucine	(R)-Roscovitine	Purvalanol A	Purvalanol B
CDK2	NS	30	10	1
CDK5	NS	10	10	1
CDK9	30	10	30	10
CDK12	NS	NS	30	30
METAP2	10	10	NS	NS
Non-CDK Kinases*	30	30	1	1

B.

Protein or protein group	Olomoucine	(R)-Roscovitine	Purvalanol A	Purvalanol B
RB1	NS	NS	30	NS
PTP4A2	NS	NS	10	NS
LUC7L2/LARP7	NS	30	30	30

Table 1. Selected responses in the four-compound CDK comparison.
A. Lysate: lowest tested response concentration, µM.
B. Intact cells: lowest tested response concentration, µM.

Values indicate the lowest tested concentration at which a significant response was reported in K562-derived lysate (A) or intact K562 cells (B). Tested concentrations were 1, 10, and 30 µM. NS: no significant response. *The non-CDK kinase row reports the lowest response concentration within this group.

What the cellular results added

Purvalanol A and B provided the clearest contrast. Although purvalanol B produced CDK2 and CDK5 responses at lower concentrations in lysate, purvalanol A produced a wider set of cellular responses associated with cell-cycle regulation and RNA processing.

For purvalanol A, these cellular changes were observed even though the CDKs themselves did not show significant responses in intact cells. The lysate results therefore helped interpret the cellular findings without requiring the same proteins to respond in both matrices.

Purvalanol B's more limited cellular profile raised a question about whether enough compound reached the relevant proteins inside the cell. The original characterization described purvalanol A as more membrane-permeable than purvalanol B, making cellular exposure a reasonable explanation to investigate.[7]

A separate experiment in MCF-7 cells also showed a smaller reduction in viability with purvalanol B than with purvalanol A after 72 hours.

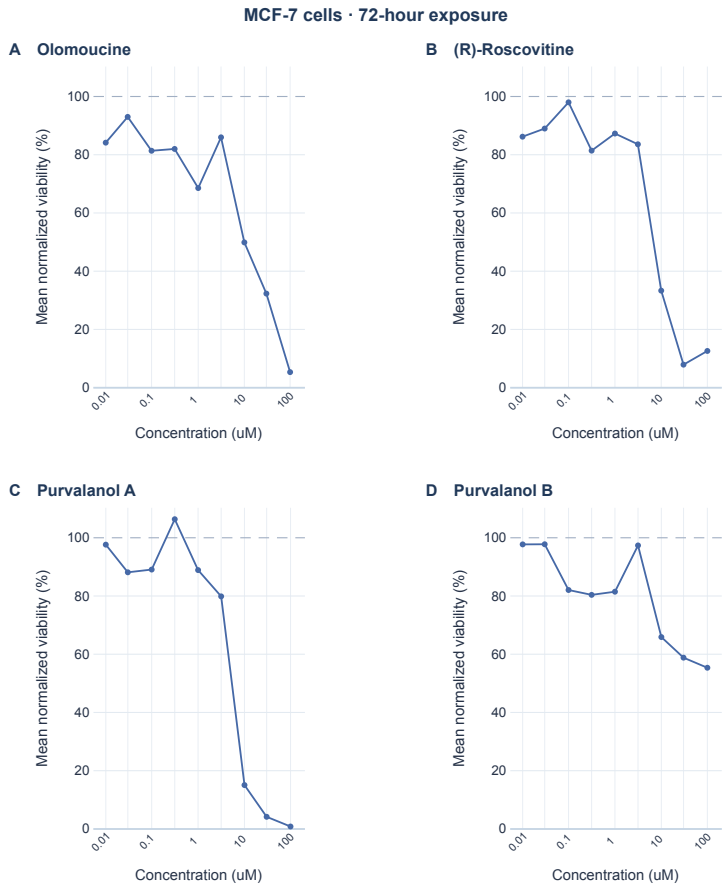


Figure 3. Viability responses to four CDK inhibitors in MCF-7 cells. Mean normalized viability after 72 hours of exposure to olomoucine (A), (R)-roscovitine (B), purvalanol A (C), or purvalanol B across the tested concentration range. Concentration is shown on a logarithmic scale. Dashed horizontal lines indicate 100% normalized viability.

These findings highlight the need to investigate whether differences in cellular exposure explain this contrast. The additional kinase responses also identify interactions to examine when assessing which might contribute to desired or unwanted cellular effects.

Comparing target coverage within the HDAC family

Histone deacetylases (HDACs) regulate protein acetylation and contribute to gene regulation and other cellular processes. HDAC inhibitors differ in which members of the family they affect.[8] Choosing a compound therefore involves deciding which HDACs are relevant to the biological question and whether activity against additional proteins could influence the results.

The lysate comparison illustrates these differences using tubastatin A, panobinostat, and entinostat. Tubastatin A induced an HDAC6 response at the lowest tested concentration, while HDAC1 and HDAC2 responses appeared at higher concentrations. Panobinostat showed broader HDAC coverage, whereas entinostat's responses were focused on HDAC1 and HDAC2.

The comparison also extended beyond the HDAC family. LAP3 responded to tubastatin A and panobinostat, providing an additional distinction from entinostat. Table 2 brings these findings together by showing the lowest tested concentration at which each response was recorded.

Protein	Tubastatin A HCl	Panobinostat	Entinostat
HDAC1	10	3	3
HDAC2	30	3	3
HDAC3	NS	3	NS
HDAC6	3	3	NS
HDAC7	30	30	NS
LAP3	3	3	NS

Table 2. HDAC inhibitor response profiles in K562 cell lysate.

Values indicate the lowest tested concentration (µM) at which a CETSA response was reported for each protein following treatment with tubastatin A, panobinostat, or entinostat. Compounds were tested at 3, 10, and 30 µM. Selected HDAC proteins show differences in target coverage, while LAP3 illustrates an additional response outside the HDAC family. NS: no significant response recorded.

Adding the protein-complex context

HDAC1 and HDAC2 work within protein complexes involved in gene regulation. Published studies show that this surrounding protein environment can influence inhibitor binding.[8] Intact-cell profiling can add information about responses in these associated proteins.

Panobinostat and entinostat shared some responses in proteins associated with HDAC-containing complexes, while panobinostat produced additional responses (Figure 4). These findings provide another way to distinguish the compounds, beyond the HDAC coverage observed in lysate.

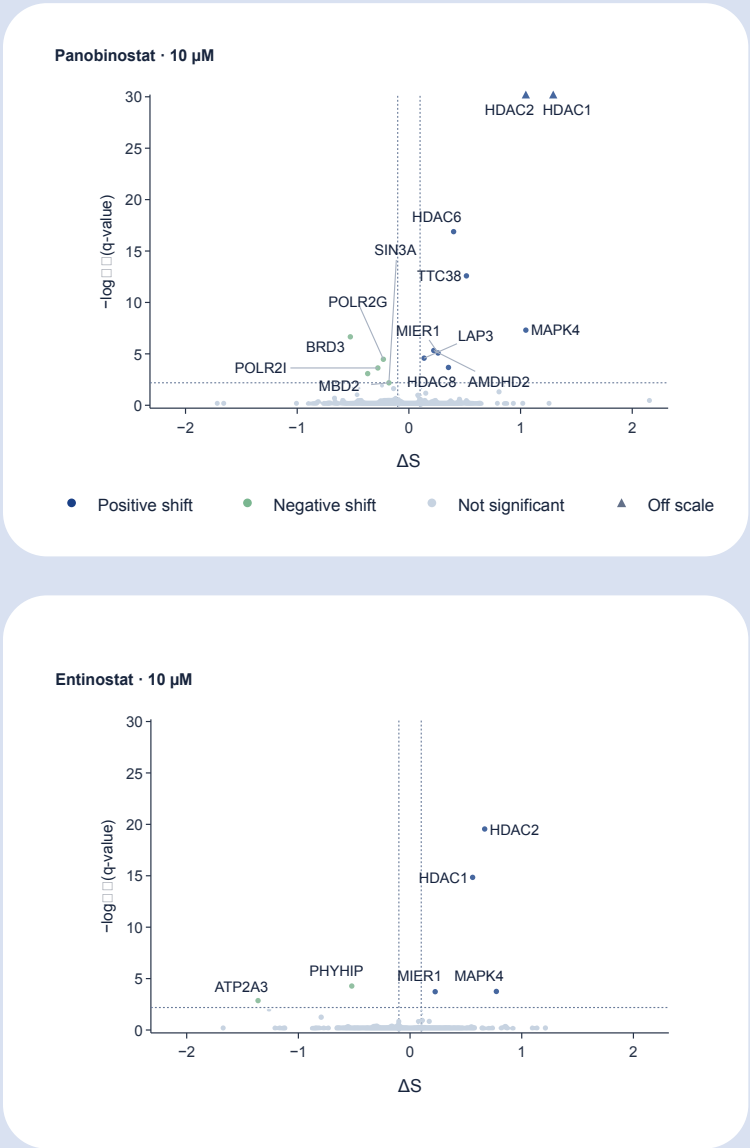


Figure 4. Intact-cell response profiles distinguish panobinostat and entinostat. Volcano plots show protein-level CETSA responses in intact K562 cells treated with 10 µM panobinostat (A) or entinostat (B). The horizontal axis shows ΔS, and the vertical axis shows $-\log_{10}$(q value). Blue and green points indicate positive and negative ΔS responses meeting both reporting criteria: q < 0.01 and |ΔS| > 0.1. Grayed-out points do not meet the minimum ΔS or q-value. Selected proteins are labeled. Bold labels highlight the shared MIER1 response and the additional MBD2 and SIN3A responses observed with panobinostat.

Lysate profiling addresses the main selection question in this example: which HDACs and additional proteins respond to each compound? Intact-cell profiling adds information when the project also needs to compare the cellular responses associated with those interactions.

Using the profiles to guide decisions

Across the three cases, the useful differences concern the identity, concentration dependence, and cellular context of protein responses. Hit counts provide a summary of profile breadth, but the targets and associated biology determine what those differences mean for a project.

The staurosporine comparison distinguishes related compounds by profile breadth and target identity. In the CDK comparison, combining lysate and intact-cell profiles helped distinguish the compounds and identify cellular exposure as a question for follow-up.

The HDAC example focuses on target-family coverage and additional responses, with intact-cell responses available when the cellular consequences are part of the question.

These findings can focus follow-up on specific targets, pathways, or exposure questions. They can also identify additional proteins for counter-screening and computational modeling during subsequent compound development.

Choosing the matrix

Match the matrix to the scientific question. A project may need an interaction-focused comparison, an assessment of cellular behavior, or an explanation of differences between the two.

Scientific question	Matrix	Information contributed
How do compound interaction profiles compare when cellular entry is removed as a barrier?	Lysate	Responses across endogenous proteins under controlled exposure conditions.
Which protein responses occur during cellular exposure?	Intact cells	Responses shaped by cellular access, protein organization, and active biology.
How do target responses relate to wider cellular effects?	Lysate and intact cells	A comparison of interaction-focused findings and associated cellular responses.
Did a chemical modification alter the wider compound profile?	Lysate, intact cells, or both	A consistent comparison matched to the intended use of the compounds.

Matrix choice can change as the project's questions develop. Lysate may be sufficient for an initial comparison of interactions; intact cells may be the appropriate starting point when cellular behavior is the main concern. Combined profiling is useful when the relationship between those views is itself the question.

CETSA Selectivity at Pelago Bioscience

CETSA Selectivity is a proteome-wide service for comparing compounds in lysate, intact cells, or both, without predefined target panels. The standard workflow compares three concentrations and combines protein-level results, compound clustering, a structured report, and results discussion. Standard results are delivered in 10 working days.

Study design begins with the compounds under consideration and the question the team needs to answer. Pelago Bioscience can help select the matrix and comparison that will make the findings useful for the next stage of the project.

Conclusion

Lysate and intact-cell CETSA provide complementary information about compound selectivity. Lysate supports comparison of interaction profiles across endogenous proteins. Intact cells reveal responses during cellular exposure, including effects associated with protein organization and downstream biology.

The case studies show how these views contribute to specific decisions: selecting among related structures, distinguishing additional targets, and relating interaction-focused findings to cellular responses. Choosing the matrix around those questions makes the resulting profiles more useful for compound selection and follow-up.

References

1. Jafari R, Almqvist H, Axelsson H, et al. The cellular thermal shift assay for evaluating drug target interactions in cells. Nature Protocols. 2014;9(9):2100–2122. DOI: 10.1038/nprot.2014.138

2. Almqvist H, Axelsson H, Jafari R, et al. CETSA screening identifies known and novel thymidylate synthase inhibitors and slow intracellular activation of 5-fluorouracil. Nature Communications. 2016;7:11040. DOI: 10.1038/ncomms11040

3. Dai L, Prabhu N, Yu LY, Bacanu S, Ramos AD, Nordlund P. Horizontal cell biology: monitoring global changes of protein interaction states with the proteome-wide Cellular Thermal Shift Assay (CETSA). Annual Review of Biochemistry. 2019;88:383–408. DOI: 10.1146/annurev-biochem-062917-012837

4. Martinez Molina D, Jafari R, Ignatushchenko M, et al. Monitoring drug target engagement in cells and tissues using the cellular thermal shift assay. Science. 2013;341(6141):84–87. DOI: 10.1126/science.1233606

5. Ramos AD, Liang YY, Surova O, et al. Proteome-wide CETSA reveals diverse apoptosis-inducing mechanisms converging on an initial apoptosis effector stage at the nuclear periphery. Cell Reports. 2024;43(10):114784. DOI: 10.1016/j.celrep.2024.114784

6. Anastassiadis T, Deacon SW, Devarajan K, et al. Comprehensive assay of kinase catalytic activity reveals features of kinase inhibitor selectivity. Nature Biotechnology. 2011;29(11):1039–1045. DOI: 10.1038/nbt.2017

7. Gray NS, Wodicka L, Thunnissen AM, et al. Exploiting chemical libraries, structure, and genomics in the search for kinase inhibitors. Science. 1998;281(5376):533–538. DOI: 10.1126/science.281.5376.533

8. Bantscheff M, Hopf C, Savitski MM, et al. Chemoproteomics profiling of HDAC inhibitors reveals selective targeting of HDAC complexes. Nature Biotechnology. 2011;29(3):255–265. DOI: 10.1038/nbt.1759

PELAGO
BIOSCIENCE

Pelago Bioscience helps drug discovery teams make faster, better-informed decisions. Our founders began developing proprietary CETSA® (Cellular Thermal Shift Assay) in 2010 and published the method in Science in 2013. CETSA measures drug-target interactions directly in physiologically relevant cells and tissues, showing target engagement while preserving native biology.

In 2026, Pelago Bioscience acquired RG Discovery, adding medicinal chemistry, peptide chemistry, custom synthesis, ADME, and bioanalysis. The combined organization connects biology and chemistry across the design-make-test-analyze cycle, linking compound design and synthesis with cellular target engagement through a single specialist partner.

With Pelago Bioscience, you have a partner who understands the pressures and complexities of drug discovery. We're here to bring confidence, speed, and reliability to your early-stage research, helping you advance with precision and clarity on the road to therapeutic breakthroughs.

www.pelagobio.com

© Pelago Bioscience 2026. All intellectual property rights, including copyrights and trademarks, relating to CETSA are owned by Pelago Bioscience AB. The Pelago Bioscience logotype is a registered trademark of Pelage Bioscience AB.