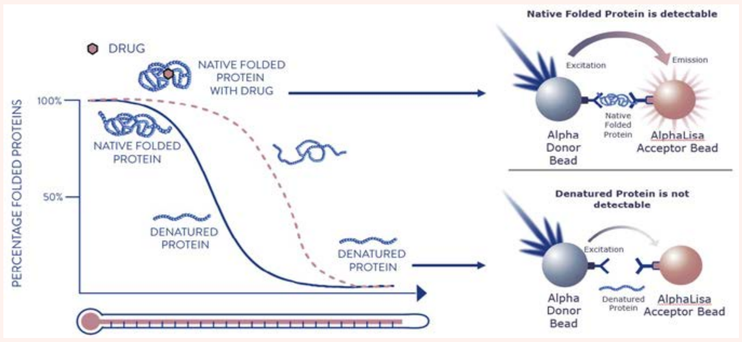
ENHANCING PRIMARY SCREENING WITH CETSA®

PELAGO BIOSCIENCE
The home of CETSA®

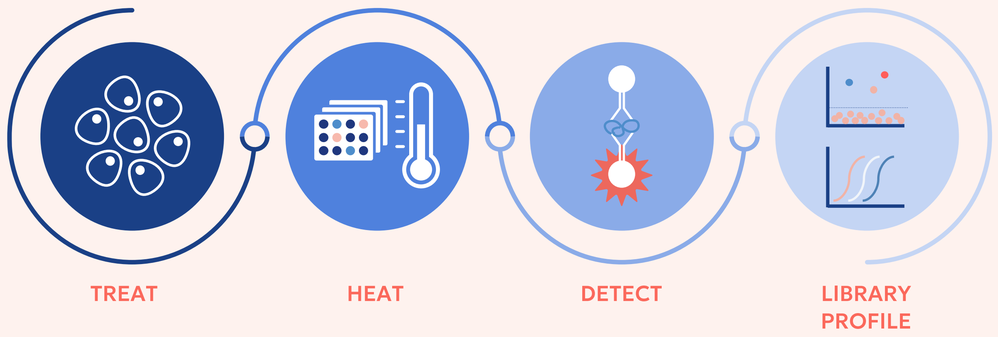
INTRODUCTION TO CETSA

- Cellular Thermal Shift Assay (CETSA) is a patented label-free technology that assesses protein-ligand interactions in the native cellular environment.
- Provides target engagement (TE) confirmation in physiological settings, aiding decision-making in drug discovery.



High-throughput CETSA format enables:

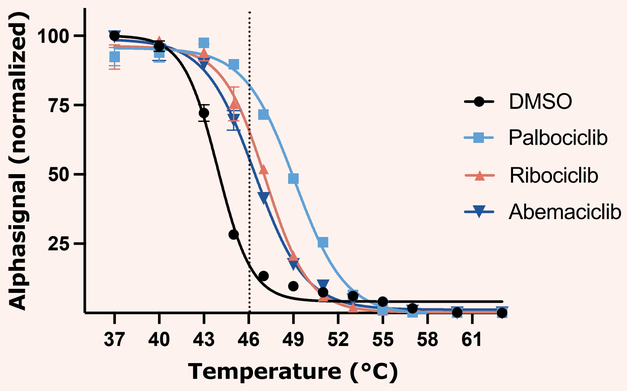
- Screening and profiling large sets of drug molecules.
- Single-point (SP) data for large library screening.
- Concentration-response (CR) data to determine TE potency.
- Rapid and efficient identification of high-quality hits.



CASE STUDY

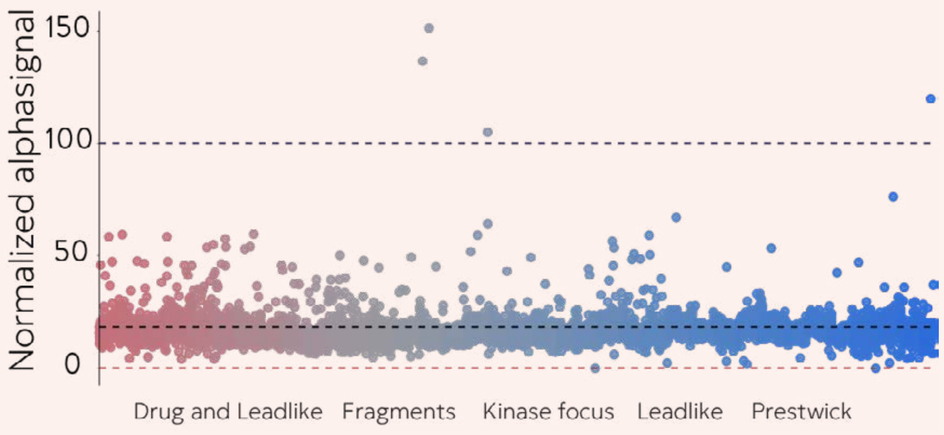
Primary screening applied to CDK4, a known target of interest in several cancers, demonstrating CETSA's effectiveness in hit identification and validation.

- CETSA assay developed in intact THP1 cells and AlphaLISA® SureFire® Ultra™ detection kit.
- Assay validation using three tool compounds by generation of melt and shift curves.
- Clear thermal shifts observed, leading to the selection of 46°C as screening temperature.



SCREENING STRATEGY

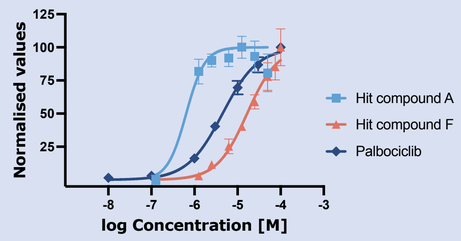
- 12,000 structurally diverse compounds tested at 50 µM.
- Hit rate: 1.2% with Z'-factor > 0.8.
- >90% of preliminary hits confirmed in secondary screening at three concentrations.



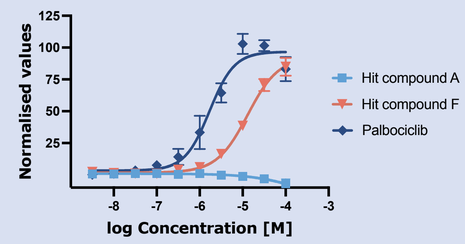
HIT CONFIRMATION

- Full concentration-response (CR) curves generated in intact cells for Palbociclib (reference) and selected hits (data for compounds A and F shown below).
- CR curves also generated in cell lysates to assess direct CDK4 binding.
- Data suggests identified hits represent distinct chemotypes with different mechanisms of action.

CR curves in intact cells



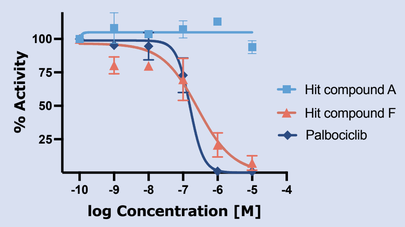
CR curves in lysed cells



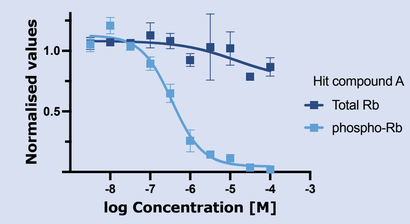
FOLLOW-UP STUDIES

- Functional studies performed to confirm biochemical and cellular impact of hits.
- Recombinant CDK4 activity expected to decrease.
- CDK4 inhibition expected to decrease phosphorylation of Rb (retinoblastoma protein).

Biochemical activity readout



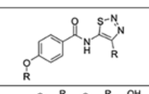
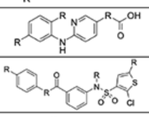
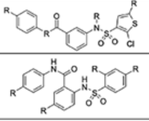
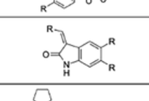
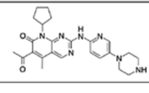
Functional readuot in intact cells



Results:

- No inhibition of recombinant CDK4 activity observed with hit compound A
- Rb phosphorylation decreased without affecting total Rb protein levels
- Functional validation strengthen the hypothesis of different mechanism of actions

SUMMARY TABLE OF EXAMPLE HITS

Compound ID	Structure	CETSA® EC_{50} (µM) Intact cells	CETSA® EC_{50} (µM) Lysate	p-Rb inhib. IC_{50} (µM)	Calculated properties				
					MW	clogP	HBA	HBD	PSA
A		0.7	Inactive	0.4	400	5.3	5	1	92
B		15	Inactive	>50	361	3.8	5	2	65
C		4.6	Inactive	31	445	4.6	5	2	111
D		0.9	Inactive	11	483	3.1	8	2	129
E		30	39	n.d.	270	0.9	5	2	63
F		3.6	1.3	n.d.	241	0.4	5	2	67
G		3.2	1.5	n.d.	211	0.4	4	2	57
Palbociclib		5	1.7	1	447	2.0	9	2	103

CONCLUSIONS

- CETSA ASSAY SUCCESSFULLY ESTABLISHED FOR CDK4 HIT IDENTIFICATION.
- BOTH KNOWN AND NOVEL CHEMOTYPES WITH CDK4-DRIVEN CELLULAR EFFECTS.
- PLANNED NEXT STEPS:
  - MECHANISM-OF-ACTION DECONVOLUTION (E.G., SELECTIVITY PROFILING VIA PROTEOME-WIDE CETSA).
  - BROAD APPLICABILITY OF CETSA TO NATIVE, FULL-LENGTH PROTEIN TARGETS IN LIVE-CELL ENVIRONMENTS.

ADVANTAGES OF CETSA

- IDENTIFIES HIGH-CONFIDENCE HITS WITH HIGHER SUCCESS RATES IN LATER DEVELOPMENT.
- ENABLES SCREENING OF A WIDE RANGE OF PROTEINS, INCLUDING HARD-TO-DRUG TARGETS.
- POTENTIAL FOR NOVEL CHEMISTRY DISCOVERY IN PREVIOUSLY UNDRUGGABLE PROTEINS.

References

1. Martinez Molina, D. et al. Science 341, 84-87 (2013)
2. Almqvist, H. et al. Nat Commun. 7 (2016)
3. Shaw, J. et al. SLAS Discovery 24, 121-132 (2019)
4. Goel, S. et al. Nat Rev Cancer 22, 356–372 (2022)