CETSA®EXPLORE: Target Engagement Profiles to Study SAR, Off-Targets, and Toxicity at Proteome Wide Level

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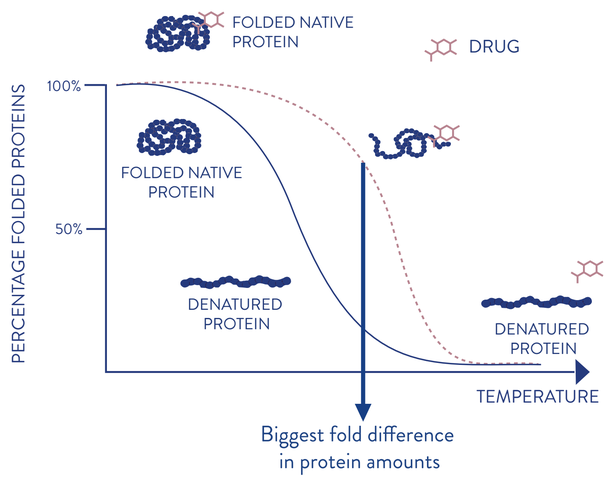
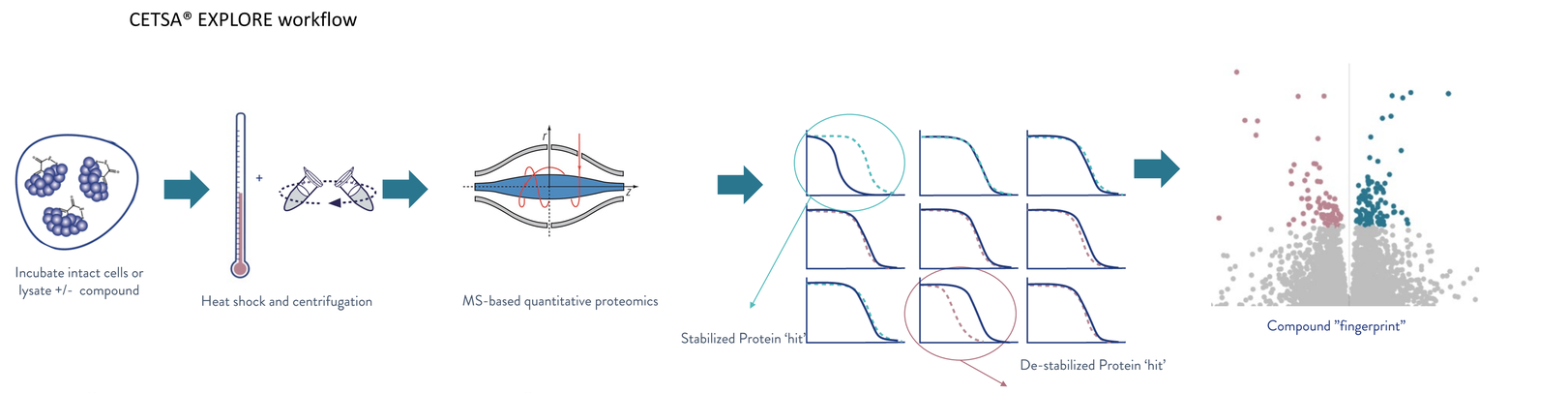




CETSA® EXPLORE, also known as CETSA®MS compressed or PISA, provides direct detection of compound binding using Mass Spectrometry as readout, assessing ligand-induced changes in protein thermal stability at the proteome-wide level.

TARGET ENGAGEMENT ATLAS OR TEA

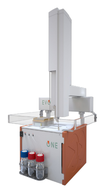
The objective of this project is to collect compound target engagement profiling, or CETSA® fingerprints for a wide spectrum of compounds representing different parts of the chemical, pharmacological and biological space.

Positive unintended effects, as well as liabilities can be discovered at an early phase of drug development.

TARGET ENGAGEMENT ATLAS PIPELINE

intact cells	• 1 hour incubation
throughput	• Single compound concentration 30 µM • TMT 16-plex
reproducibility	• Biological triplicates • Positive/negative controls*
compounds	• Commercially available compounds • 6h/compound process time

Agilent Bravo
Pipette station



Evosep One
Nano LC system

High-resolution MS

Visualization of the results with tSNE in HepG2 cells, an unsupervised non-linear reduction model that group related compound fingerprints close to each other. Colour coded by pharmacology, compounds tend to cluster together within their pharmacological effect.

CONCLUSIONS

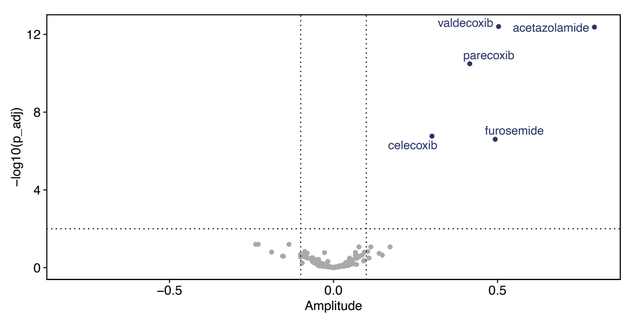
CETSA® EXPLORE compound profiling of hundred of known drugs and probes in three different cell lines reveals:

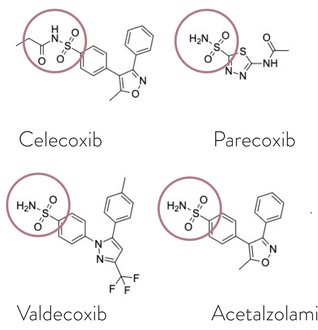
- Specific chemical motifs influence compound CETSA® fingerprints.
- Compound protein hits response differently dependent on the cellular background.

TARGET ENGAGEMENT PROFILES FOR SAR STUDIES

Some chemical motifs have a strong impact on the CETSA fingerprint, which can be applied to SAR studies.

Carbonic Anyhdrase 13 profiling showns its inhibitor Acetazolamide as a hit, as well as some coxibs, which are NSAID class, and MoA reported to act through inhibition of cyclooxygenase 2. All these compounds had a sulfonamide group in common, and this chemical feature was not found in a Coxib which didn't shift Carbonic anhydrase 13.



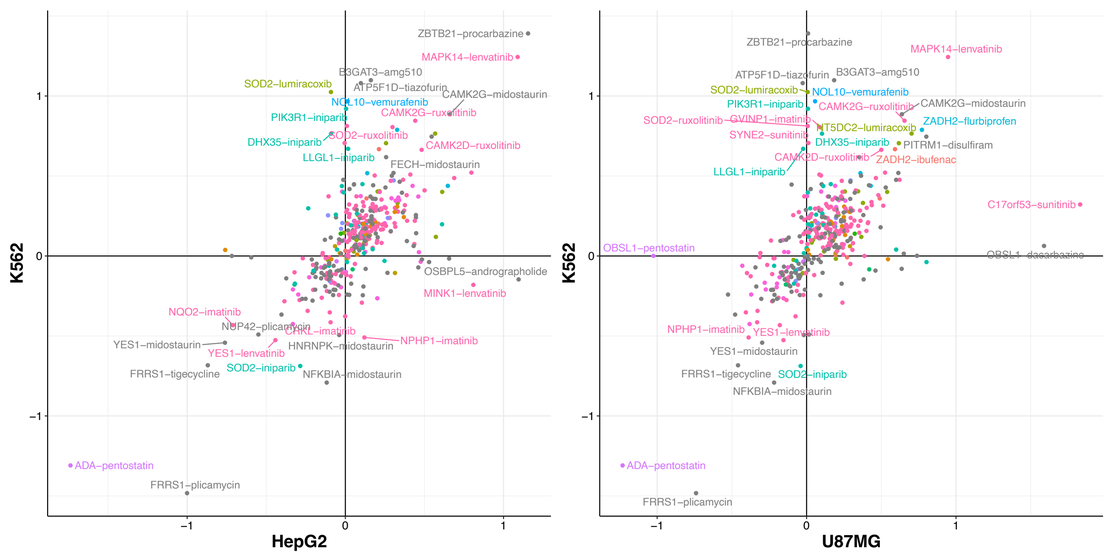


TEA AND THE CELLULAR CONTEXT

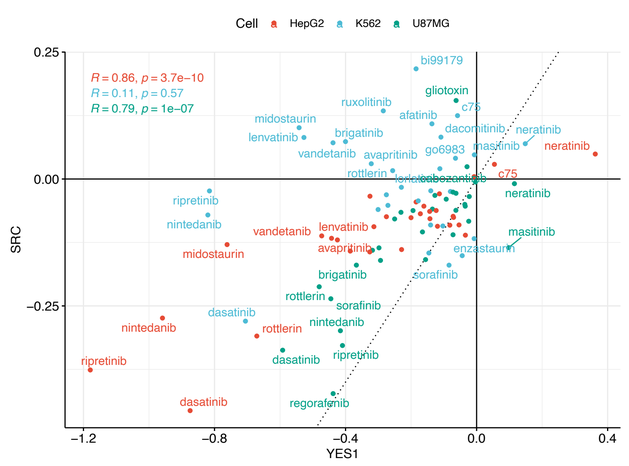
Target engagement atlas identifies hallmarks of cellular responses to different types of drugs and assigns biomarkers beyond specific target engagement.

We chose three different cell lines that cover most of the human proteome: HepG2, (human hepatocellular carcinoma), U87MG (primary glioblastoma), and K562 (myeloid leukemia, lymphoblast).

A large number of compounds proteomic profiles correlate with each other, primary target in particular, but other targets are cell line-specific. We observe clear cellular discrepancies in response patterns to a large number of drugs



Scatter plot to correlate proteomic responses in K562 and HepG2 or K562 and U87MG with 100 compounds .



Different Src type kinases response differently to treatment in different cell types.

Yes1 and Src responses correlated between HepG2 and U87MG but not in K562.

Dasatinib, a specific SRC inhibitor, is effective in the three cell lines. Interestingly, Dasatinib is used in clinics for bcr-abl Leukemia, the same source tumor as K562.

References:
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3. Chernobrovkin BirRx 2020
4. Friman Bioorg. Med Chem 2020
5. Friman SLAS Discovery 2021
6. Chernobrovkin SLAS Discovery 2021
7. Hendricks ACS Chemical Biology 2022