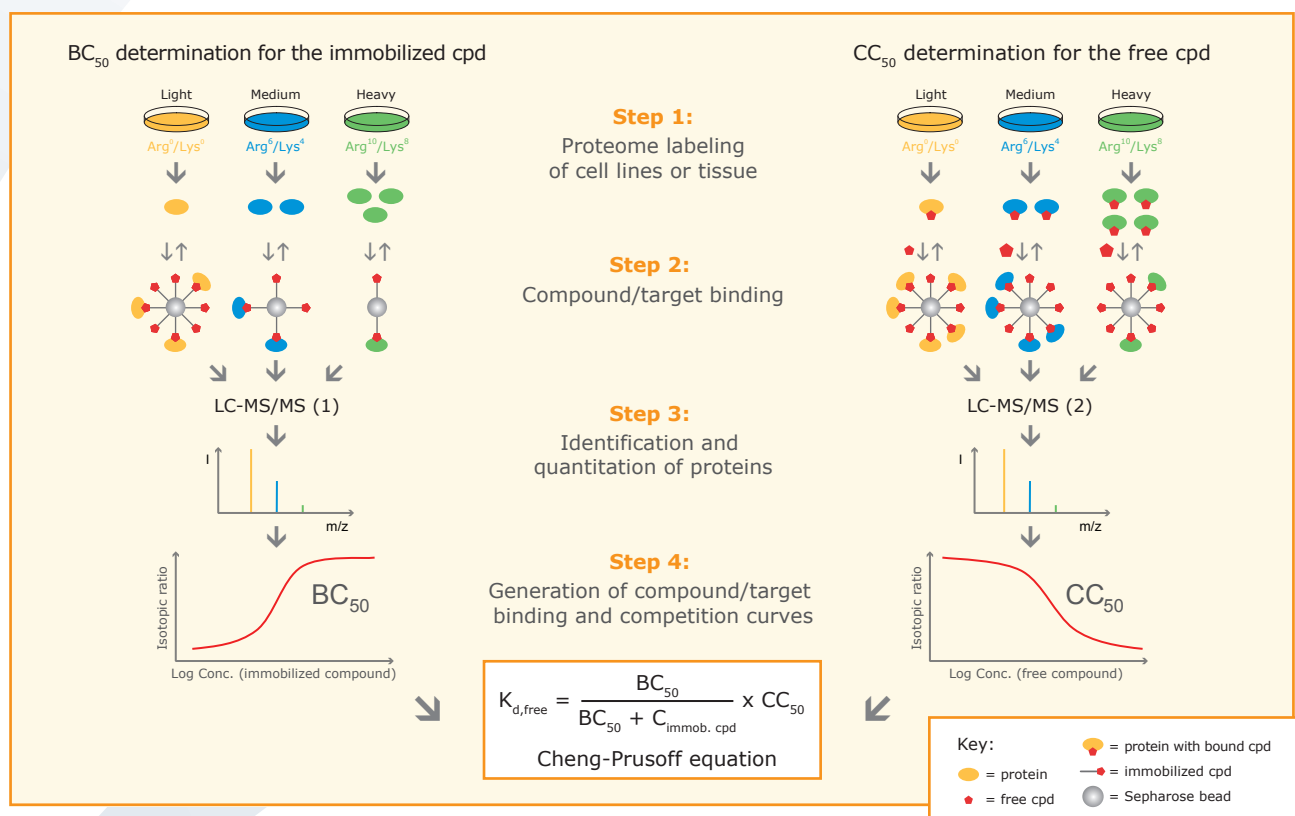


KINAXO's Cellular Target Profiling®: Service Methodology and Reproducibility

Summary

- KINAXO's Cellular Target Profiling® is a proprietary affinity separation and mass spectrometry service platform for identifying small molecule protein binding targets across selected cellular proteomes and for defining the affinities of these interactions.
- The compound's protein binding targets are identified and ranked according to their binding affinities ($K_{d, free}$ values).
- The robustness and reproducibility of Cellular Target Profiling® was demonstrated through measuring of only a 2-fold mean variance in $K_{d, free}$ values for Dasatinib binding to common targets in HeLa and K562 cell line lysates.
- A $K_{d, free}$ dynamic range over 4 orders of magnitude was determined across critically relevant compound concentrations.

KINAXO's Cellular Target Profiling® Methodology



Step 1: Cells are grown in the presence of normal and two different forms of isotopically labeled amino acids using Stable Isotope Labeling with Amino acids in Cell culture (SILAC) labeling technology (Ong *et al.* 2002). Similarly, covalent tags like iTRAQ (isobaric tag for relative and absolute quantitation) or TMT (tandem mass tags) can also be used to label the proteome of any given cell line or tissue. Using

this technology, proteomes can be distinguished in a mass spectrometer by their mass difference.

Step 2: *Left panel BC₅₀ determination:* Differentially labeled cell or tissue lysates are incubated with the test compound immobilized at different densities. *Right panel CC₅₀ determination:* Differentially labeled cell lysates are incubated with increasing concentrations of the free

compound in the presence of the immobilized compound. Five and seven data points are determined to generate the BC₅₀ and CC₅₀ curves, respectively.

Step 3: Proteins that bind to the immobilized compound are eluted and combined. The proteins are then separated by SDS-PAGE and digested. The resulting proteolytic peptides are analyzed and identified by LC-MS/MS.

Step 4: Isotopic peptide signals allow the calculation of relative ratios of proteins that were either enriched at different immobilized compound concentrations (left panel) or remain bound upon competition with the free compound (right panel). The $K_{d, free}$'s for the free compound are calculated using the Cheng-Prusoff equation.

KINAXO's Cellular Target Profiling® Service Reproducibility

Service reproducibility is illustrated below through the Cellular Target Profiling® of Dasatinib against K562 and HeLa cell lines.

Based on the literature and accumulated chemical proteomics expertise at KINAXO, a strategy was developed to conjugate Dasatinib to sepharose beads while not affecting its target binding activities. The cell lines K562 and HeLa were SILAC labeled as required according to the methodology illustrated overleaf.

The measurement of only a 2-fold mean variance in $K_{d, free}$ values for Dasatinib binding to targets common to HeLa and K562 cell line lysates was determined from the Cellular Target Profiling® experiments (*see below*). Furthermore, these targets displayed a broad range of affinities for Dasatinib across 4 orders of magnitude (~ 0.001 to $10 \mu\text{M}$). This result demonstrates the strong experimental reproducibility and robustness of the Cellular Target Profiling® service platform across a wide range of relevant small molecule/drug target affinities.

For further information about its applicability, please download [AN1](#).

KINAXO's Cellular Target Profiling® Control Experiments

In order to ensure the highest service data quality and reproducibility, certain control experiments are performed alongside the core methodology outlined overleaf.

Four aspects are addressed by these control experiments:

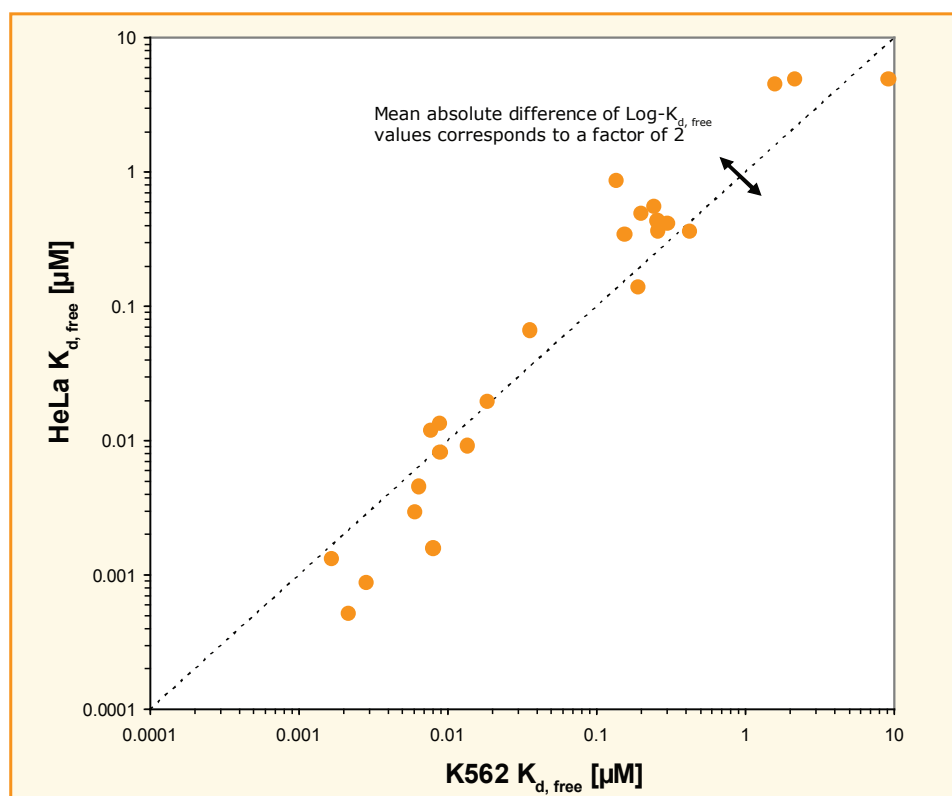
Background binding: Non-specific binding proteins are identified and rejected.

Matrix saturation: Non-saturation of the affinity resin by the target proteins is ensured.

Equilibrium conditions: The equilibrium binding status of the experiment is monitored.

Target binding: The consistency of target binding is validated by 'label switch' experiments.

Scatter plot of the target $K_{d, free}$ values from the Cellular Target Profiling® of Dasatinib versus K562 and HeLa cell lysates



Each spot represents a Dasatinib target identified in both the K562 and HeLa Cellular Target Profiling® experiments. The position on the plot is determined according to the $K_{d, free}$ value derived in each of those experiments.

Reference

Ong, Blagoev, Kratchmarova, Kristensen, Steen, Pandey, Mann (2002) Stable isotope labeling by amino acids in cell culture, SILAC, as a simple and accurate approach to expression proteomics. *Mol. Cell. Proteomics*. **1(5)**:376-86.

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