

## CHEMICAL PROTEOMICS

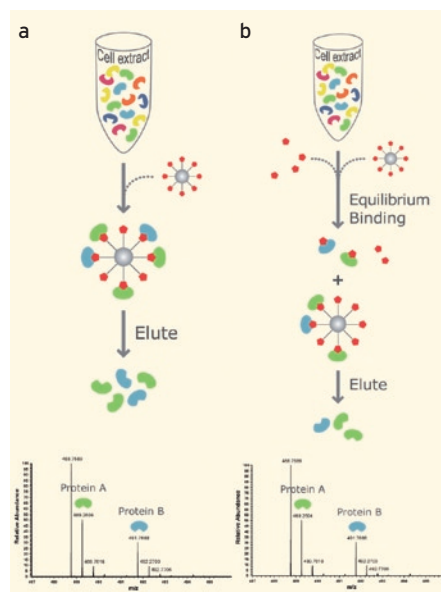
# Small molecule selectivity testing

Dr. Klaus Godl, KINAXO Biotechnologies GmbH, Martinsried, Germany

➤ Novel chemical proteomics technologies deliver valuable insights into small molecule target interactions and binding affinities across the proteomes of cell lines or tissue samples, allowing researchers to reliably identify a compound's cellular interaction partners and uncover the mechanisms of drug efficacy and potential off-target toxicities.

Comprehensive knowledge of the cellular proteins targeted by small molecules helps us to understand their molecular modes of action in both animal models and humans. To help foster that knowledge, Kinaxo Biotechnologies GmbH has developed a new chemical proteomics strategy called Cellular Target Profiling™. This technology platform identifies the targets of small molecule drugs in biologically relevant systems, and also delivers the binding affinities of these interactions. By combining quantitative mass spectrometry with carefully designed compound affinity purification and competition experiments, the platform determines the dissociation constants ( $K_D$  values) for compound-target interactions. Internal quality-control experiments validate the identified target proteins and respective binding affinities. The  $K_D$  values are derived in a two-step process. Drug-protein binding partners are first captured on a solid support that is coupled to the compound of interest. The affinities of each of the protein targets for this bead-coupled compound are then determined (Fig. 1a). In the second step, competition experiments take place with the free compound displacing targets bound to the bead-coupled compound (Fig. 1b). Both of these experiments require performance under multiple test conditions in order to generate data to derive the critical binding

and competition curves. Metabolic (SILAC) or chemical (iTRAQ) labeling of the cell line or tissue sample proteomes, combined



**Fig. 1: Cellular Target Profiling Methodology** (a) Cellular targets are captured by the bead-coupled compound, eluted, then identified and quantified by mass spectrometry. (b) An equilibrium binding reaction is established between the cell lysate, bead-coupled compound and 'free' compound. The targets that remain bound to the bead-coupled compound are eluted, then identified and quantified by mass spectrometry.

with proprietary experimental and analytical methods, enable the quantitation of the drug-protein target affinities.  $K_D$  values of the target(s) for the free compound in solution are derived by applying algorithms to the above two data sets.

## Dasatinib target profile

The application of this technology to the analysis of a human myeloid leukemia cell line (K562) has confirmed the tyrosine kinase ABL as the highest affinity cellular target of dasatinib (Sprycel®, Bristol-Myers Squibb), a drug that is approved for the treatment of chronic myeloid leukemia (CML) and Philadelphia chromosome-positive acute lymphoblastic leukemia (ALL). Dasatinib is prescribed to patients with resistance to prior therapy – including imatinib (Gleevec®, Novartis) – resulting from BCR-ABL kinase domain mutations. Low nanomolar target proteins of dasatinib include tyrosine kinases closely related to ABL kinase (e.g. ARG, CSK, BTK and TEC) as well as members of the SRC kinase family (SRC, LYN, FRK, YES), suggesting drug activity in cells with activated SRC signaling pathways. In addition, receptor tyrosine kinase targets (EphB4, KIT, DDR1) indicate dasatinib efficacy in angiogenesis and cell migration. Further, the activity of dasatinib targets like RIPK2, TNK2, MAP3K4, QSK, GAK and QIK, which have poorly understood physiological roles, may well be modified during dasatinib treatment, as their  $K_D$  values indicate dasatinib binding at therapeutically relevant drug concentrations.

## Applications for target profiling

Cellular target profiling enables the comparison of a drug's target-binding spectra across a variety of biological tissues and cell types. This provides insights into the drug's mode of action with respect to different proteomes of therapeutic relevance, such as drug-sensitive/insensitive cell lines or healthy/disease tissues. For example, PDGFR $\beta$  is not expressed in K562 cells, and thus was not identified when probing dasatinib in this cell line. In contrast, dasatinib was found to bind additional tyrosine kinases (EPHA2, EPHB2, FYN, DDR2, BRK) in HeLa cells

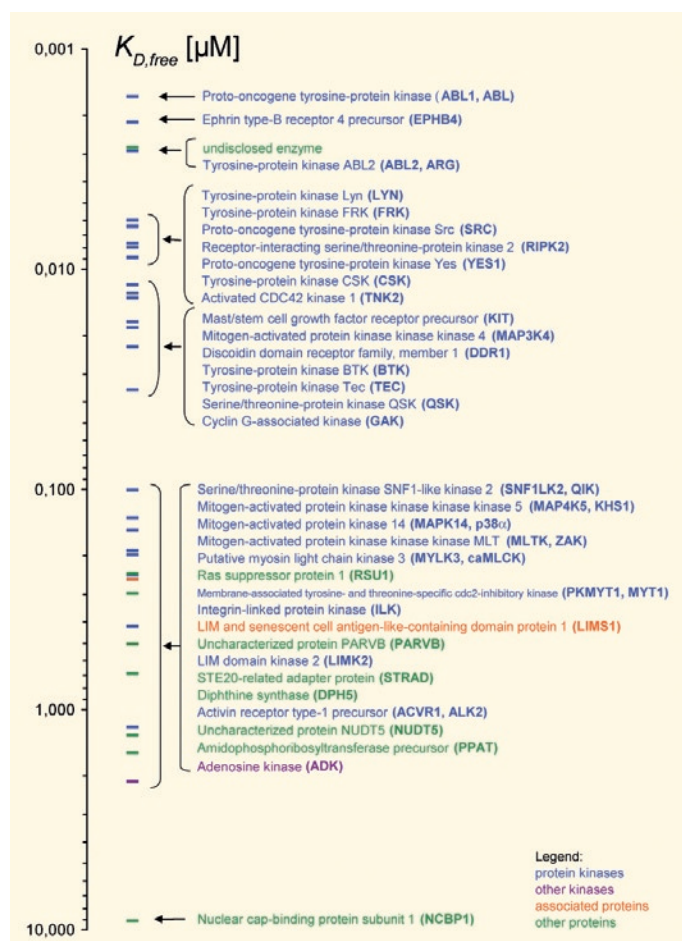


Fig. 2: Cellular Target Profiling™ demonstrates the possibility of compound-protein affinity quantitation across four orders of magnitude. Shown are the targets identified from a K562 cell lysate probed with dasatinib (Sprycel®, BMS). Targets are ranked in order of affinity.

with low nanomolar affinities. Further key applications that may be addressed with Kinaxo’s Cellular Target Profiling™ platform include drug repositioning and lead discovery. Many drug discovery screening campaigns are based on phenotypic screens designed to identify compounds with a desired cell-based assay profile. This approach requires the subsequent application of target deconvolution strategies to identify the compound’s cellular targets, which is a prerequisite for knowledge-driven lead optimization. Drug development programs can be effectively supported by comparing candidate interaction profiles and target affinities to enhance lead compound selection for (pre)-clinical studies. That will not only allow the discovery of potential off-target related side effects early in the development process, but could also open up novel target proteins and new indications for approved or failed compounds. ▼

**Contact**

Dr. Klaus Godl, Kinaxo Biotechnologies GmbH  
 Am Klopferspitz 19a, 82152 Martinsried, Germany  
 Tel.: +49-(0)89-461336312, k.godl@kinaxo.de, www.kinaxo.com