

DRIVE YOUR DRUG DISCOVERY TO A NEW LEVEL OF SUCCESS!

KINAXO Biotechnologies and Proteros Biostructures now offer combined services to identify all of your small molecule's cellular protein targets including comprehensive kinetic characterization of these interactions.

Combine KINAXO's Cellular Target Profiling® and Proteros' Kinetic Screening to

- Discover all your compound's targets in a physiological setting
- Identify potential off-target toxicity effects
- Explore your compound's kinetic selectivity
- Use residence time as new dimension for lead optimization
- Gain essential insights into your compound's cellular mode of action

Assessment of a compound's cellular mode of action and its potential off-target liabilities requires knowledge about all targeted proteins, their corresponding affinities and binding kinetics. A critical factor for sustained drug efficacy *in vivo* is the residence time a small molecule spends on its molecular target. Measurement of cellular K_d values and subsequent evaluation of a compound's target residence time therefore helps to predict a drug's efficacy, *in vivo* pharmacological activity and potential side-effects.

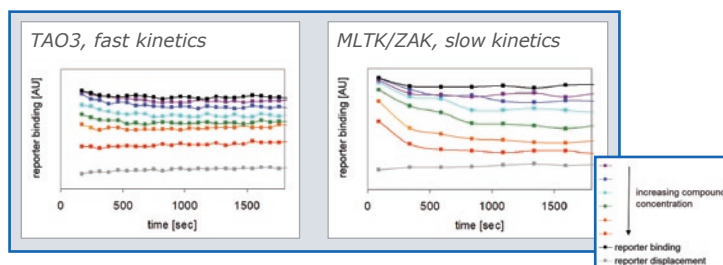
KINAXO's Cellular Target Profiling® was applied to profile Sorafenib (Nexavar®) in prostate cancer cells (PC3), a multi specific kinase inhibitor marketed by Bayer HealthCare AG.

13 protein targets with K_d values below 4 μM were identified, among them previously unknown Sorafenib targets such as MAP3K1, MNK1, TAO3 kinase, and several non-kinase proteins. Furthermore, 16 protein kinases with K_d values higher than 4 μM were detected. Known receptor tyrosine kinase targets of Sorafenib, such as KDR, are not expressed in PC3 cells and therefore not detected.

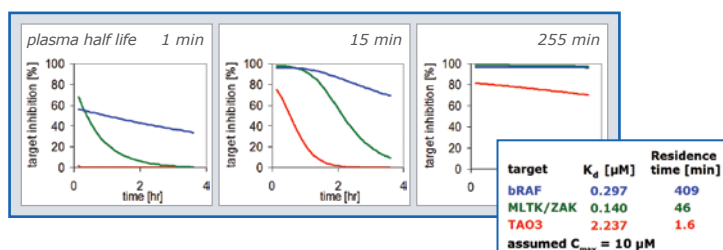
Targets identified by Cellular Target Profiling®	K_d [μM] (by Cellular Target Profiling®)	K_i [μM] (by Kinetic Screening)	k_{on} [$1/\text{s}$ $1\mu\text{M}$]	k_{off} [$1/\text{s}$] ($K_d \times k_{on}$)	Residence time [min]
MLTK / ZAK	0.032	0.140	0.00259	0.00036	46
DDR2	0.058	0.014	0.02639	0.00037	45
DDR1	0.13	0.072	0.00963	0.00069	24
MAP3K1	0.422	> 3	n.d.	n.d.	n.d.
MAPK14/ p38 α	1.9	0.282	0.00029	0.00008	202
bRAF	n.d.	0.297	0.00014	0.00004	409
TAO3	2.92	2.237	0.00478	0.01070	1.6
MNK1	3.510	> 3	n.d.	n.d.	n.d.
MAPK11/ p38 β	3.527	2.787	n.d.	> 0.009	< 2

n.d. = not determined

A selection of high-affinity protein kinase targets were further characterized using **Proteros' Kinetic Screening**. This measured the kinetically relevant parameters k_{on} and k_{off} as well as the residence time of Sorafenib at the target protein. The residence times between Sorafenib targets varied greatly, ranging from 1.6 min (TAO3) to 46 min (MLTK/ZAK) and up to 409min (bRAF).



Simulation of the *in vivo* target inhibition for three of the targets shows the dependency between compound parameters such as plasma half-life, residence time and inhibition of the various drug targets. As demonstrated for three hypothetical compound plasma half-life times (see figure to the right), especially for drugs with short or mediocre plasma half-lives the residence time, rather than K_d , is the dominant compound property that dictates target selectivity over time.



KINAXO's Cellular Target Profiling® combines chemical proteomics with up-to-date quantitative mass spectrometry to determine the target profile of small molecules across proteomes of selected cells or tissues and compound-target affinities are determined.

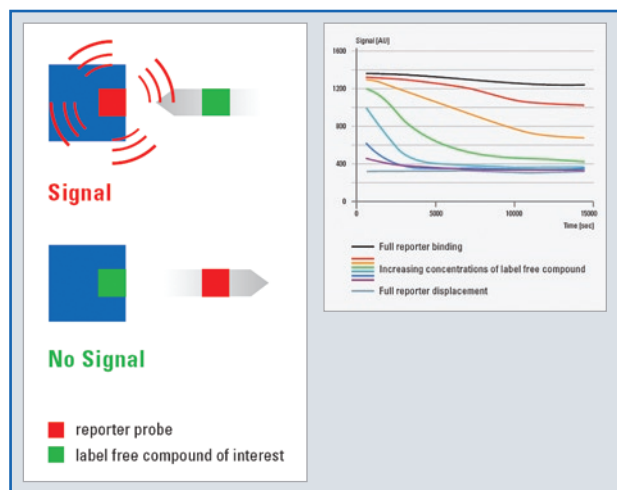
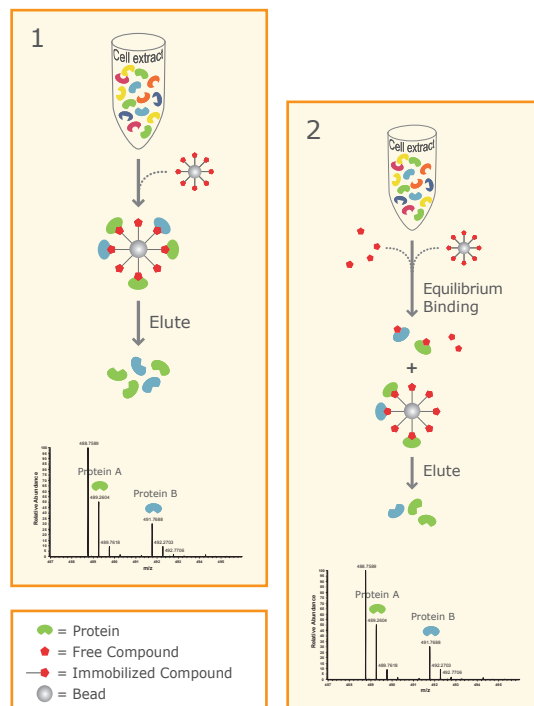
These profiles significantly support decision making at various stages of the drug discovery process, e.g. lead compound selection, target deconvolution, drug reprofiling, and off-target toxicity assessment.

1. Identification of target proteins

Cellular targets are captured by the bead-coupled compound, eluted and identified by mass-spectrometry

2. Compound/target affinity measurement

Compound/target affinities (K_d values) are determined by combining quantitative mass spectrometry with competition experiments



Proteros' Kinetic Screening Technology enables reliable determination of the characteristics K_d , k_{on} , k_{off} and residence time of small molecule inhibitors and thus delivers valuable insight into the drug target interaction behavior *in vivo*.

The knowledge of kinetic data is the key for pharmacodynamics, and toxicity issues. The identification of compounds with slow k_{off} rates has been proven to be a successful strategy for the discovery of novel drugs.

The *reporter probe* binds to the target protein and generates a specific biophysical signal. Displacement of the reporter probe by a competing compound of interest results in signal loss. By following the kinetics of signal loss at various compound concentrations values such as k_{on} , k_{off} , K_d and residence time can be calculated for the *compound of interest*.

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