GREATER PRECISION FOR TRANSFORMATIVE DRUG DISCOVERY

Identify and Validate Drug Targets with Structural Proteomics

IDENTIFY DRUG BINDING SITES AND ON- AND OFF-TARGET EFFECTS

Small molecule target identification and validation are critical challenges in drug discovery. Exclusively licensed to Biognosys, TrueTarget™ is the only platform that offers label-free and targeted probing of structural changes across the entire proteome with peptide-level resolution, providing unique insights into target identification and compound binding.

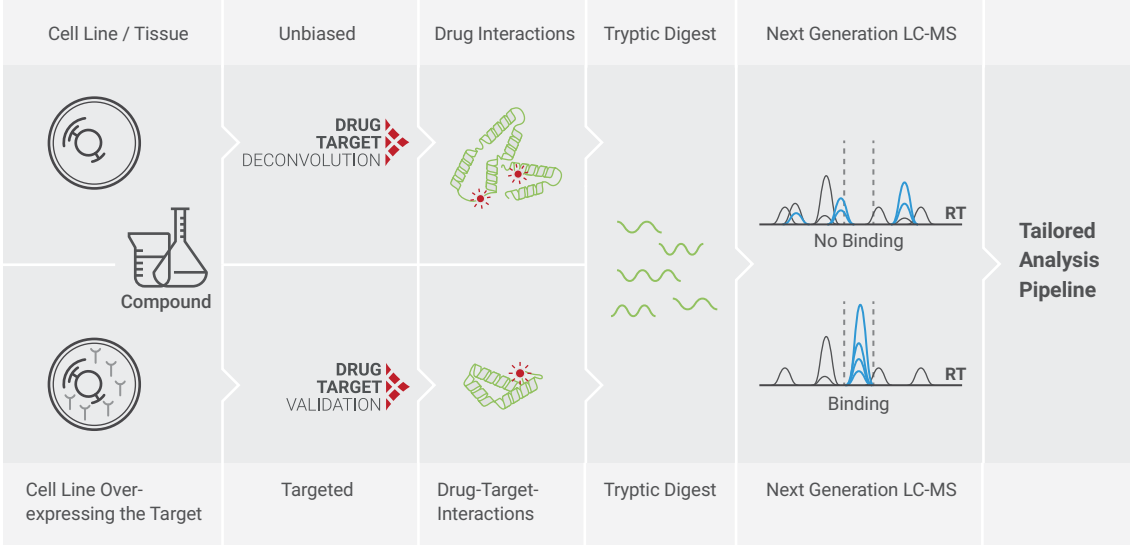
DRUG TARGET DECONVOLUTION

- Powered by limited proteolysis-coupled mass spectrometry (LiP-MS)
- Intuitive report structure with ranked protein target candidates
- Identifies on- and off-target binding effects
- Reveals mechanism of action and potential toxicities
- Detects structural and surface changes
- Proteome coverage of up to 9,000 proteins in human cells
- High confidence of target identification

DRUG TARGET VALIDATION

- Powered by High Resolution limited proteolysis-coupled mass spectrometry (HR LiP)
- Characterizes binding sites
- Effective for difficult-to-characterize proteins, such as membrane and high molecular mass proteins
- Provides insights to aid compound optimization
- Simple to run, requiring only protein overexpression in cells and a stock of your compound

Schematic Representation of Drug Target Deconvolution and Drug Target Validation Workflows



TRUE TARGET™

«Biognosys' TrueTarget™ platform was instrumental in identifying our target protein's binding site and provided valuable mechanistic insights that helped us to understand the underlying biology of our observed phenotype.»

Prof. David Rubinsztein
Professor of Molecular Neurogenetics, UK Dementia Research Institute Group Leader at the University of Cambridge, and Deputy Director of the Cambridge Institute for Medical Research

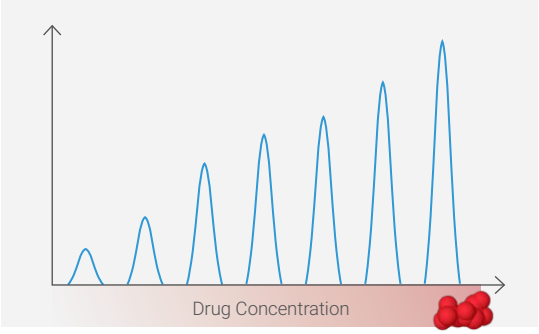
BENEFITS	FEATURES
Unique	The only platform that can probe protein structural alterations with peptide-level resolution
Label-free	Does not require protein and compound labeling
Insightful	Whole proteome approach reveals mechanism of action, off-target effects and binding sites
Versatile	Applicable to complex biological matrices, including a variety of cell lines and organisms
Efficient	Established robust pipeline provides a ranked list of protein targets for rapid target deconvolution and validation

PRECISE DRUG TARGET DECONVOLUTION

Identifying the target of a therapeutic compound is key to gaining deeper insights into its mechanism of action and potential toxicities. By applying LiP-MS, our Drug Target Deconvolution service offers a unique way to optimize and de-risk your drug discovery journey. The service offers a depth of coverage of up-to-9,000 proteins with best-in-industry depth of target identification.

LiP-MS Uncovers On- and Off-Targets throughout the Entire Proteome

A Streamlined Workflow for Small Molecule Target Identification



LiP-MS Resolves the Binding Site of a Kinase Inhibitor to Its Target

How Does Drug Target Deconvolution Work?

Drug-treated and control cell extracts are digested with proteases, creating distinctive peptide fingerprints depending on where and how the drug is bound. Peptides are identified and quantified with mass spectrometry, and a comparison of treated and control extracts reveals on- and off-target binding effects throughout the entire proteome.[1,2]

A Robust Pipeline for Target Identification

Label-free mass spectrometry enables proteome-wide coverage to support target-based and phenotypic drug discovery. LiP-MS offers a unique analytical workflow for target scoring based on dose-response curves and machine learning algorithms, resulting in an efficient and robust target deconvolution approach.[3]

Deep Insights for Drug Discovery

TrueTarget™ has peptide-level resolution, enabling robust target identification across the whole proteome. Exploiting drug-induced structural changes, TrueTarget™ highlights potential off-target effects early in the drug discovery journey and can reveal a compound's mechanism of binding and action.

1) **Feng et al.,** Nat Biotech 32, 1036 (2014)
2) **Piazza et al.,** Cell 172,358 (2018)

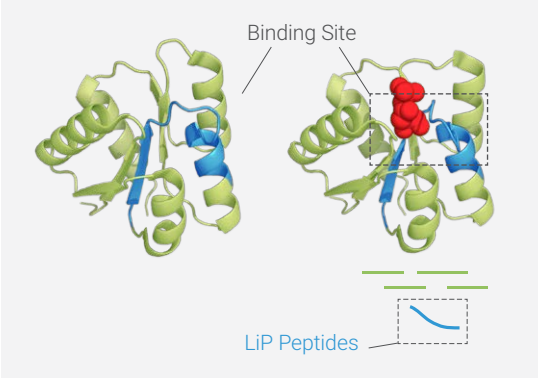
CONFIDENT DRUG TARGET VALIDATION

Using HR-LiP, our Drug Target Validation service can characterize your drug candidate's binding site with confidence and without the need for compound tags, protein modifications or the use of recombinant proteins. Evidence of target engagement, binding site mapping and IC50 can support further compound optimization.

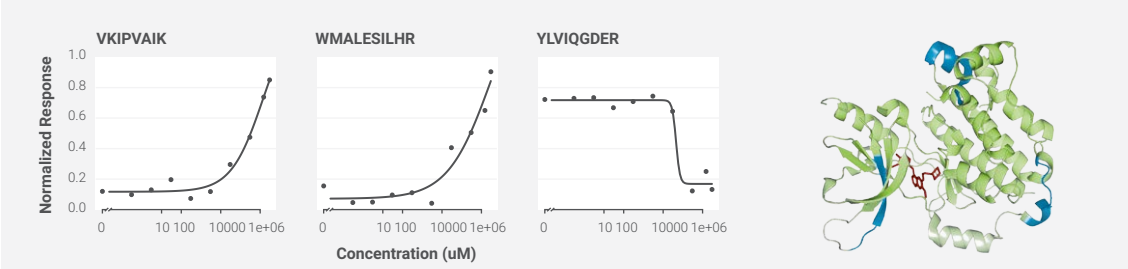
How Does Drug Target Validation Work?

Native cell lysates overexpressing the target of interest are treated with the active compound and vehicle. Compound binding triggers differential peptide cleavage during limited proteolysis (LiP), which is more pronounced in regions closer to the binding site. Unique LiP peptides can then be identified via high-resolution mass spectrometry and used to identify compound binding sites.

Differentiation of the Structural Fingerprints of Bound versus Free Targets



EGFR Binding Site Mapping for Gefitinib Using HR-LiP [3]



A Powerful and Flexible Tool

Biognosys' Drug Target Validation service can be performed on any protein from any species in a near-native environment. Furthermore, proteins with a high molecular mass or membrane localization can be analyzed in full length. Compared to conventional structural biology techniques such as crystallography, Drug Target Validation offers a cost- and time-efficient alternative.

Drug Target Deconvolution and Validation

This service is a valuable tool for confirming compound binding and mapping binding sites of targets discovered via our Drug Target Deconvolution studies.

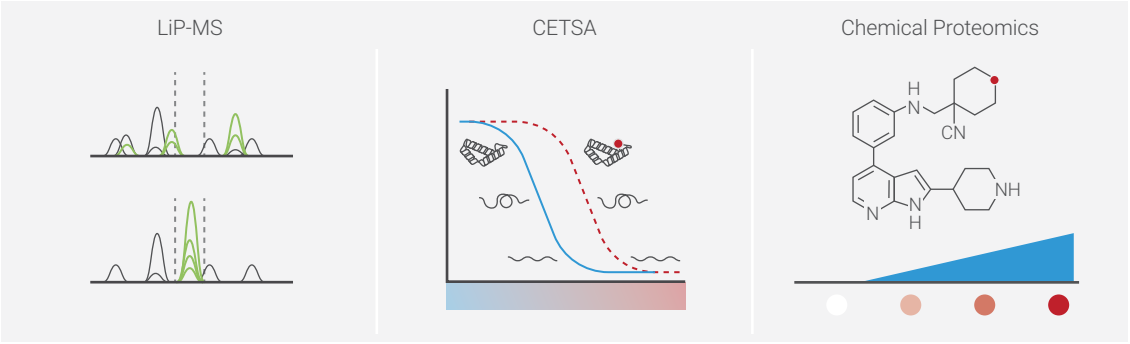
3) Piazza & Beaton et al., Nat Commun 11, 4200 (2020)

Case Study

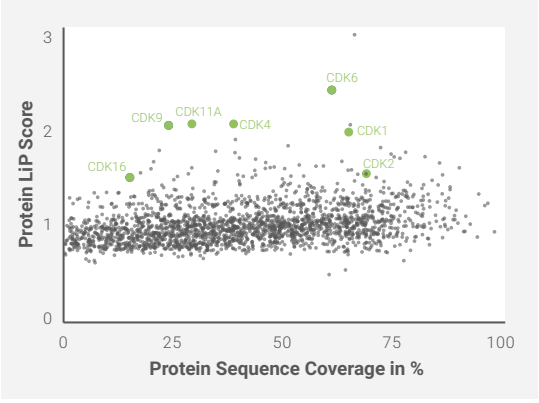
MECHANISTIC INSIGHTS ON CDK VIA ORTHOGONAL PROTEOMICS METHODS

Loss of cell cycle control caused by deregulation of CDK is a key feature of cancer. However, many CDK inhibitors have shown mixed outcomes in clinical trials and challenging tolerability issues. Here, we applied orthogonal proteomics approaches to quantitatively profile the selectivity of a CDK9 inhibitor.

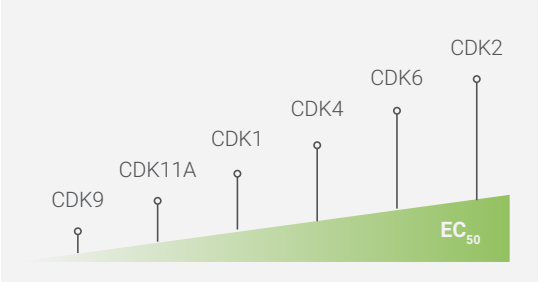
Different Proteomics Methods Used in the Study



Scatter Plot Depicting LiP Score in Correlation to Sequence Coverage for Each Protein



Dose-Response Curves of Representative LiP-Peptides from Each of the CDK Proteins Identified



Study Design

Although attractive targets for inhibition, compounds targeting the CDK family often have complex polypharmacological effects. We used several different proteomics methods to quantitatively profile the selectivity of the predicted binding site of a novel CDK inhibitor.

Deeper Insights

TrueTarget™ was used to screen the entire proteome and identify potential targets via structural alterations. Binding affinities across the family could also be estimated and, by exploiting the technology's peptide-level resolution, we could also identify the putative binding site of the CDK inhibitor.

Conclusion

We enabled target identification, binding affinity estimation, and binding site localization for the compound. This study highlights the synergistic value of using multiple quantitative proteomic techniques in conjunction with proteome-wide target identification and selectivity profiling — a critical step during drug discovery.

Hendricks et al., ACS Chemical Biology 2022 17 (1), 54-67

Case Study

ACTIVATION OF VCP ENHANCES NEUROTOXIC PROTEIN CLEARANCE

Enhancing the removal of aggregate-prone, toxic proteins is a rational therapeutic strategy for many neurodegenerative diseases. However, high-resolution profiling of drug-protein interactions and binding mechanisms remains a major hurdle during lead selection and optimization. In this study, we use HR-LiP to characterize drug-protein interactions for a compound that boosts autophagy.

Study Design

SMER28 activates autophagy and neuronal clearance by interaction with VCP, an 800 amino acid protein that forms a hexamer. To validate the impact of SMER28 on VCP, our Drug Target Validation service was used to map the compound's binding site with peptide-level resolution.

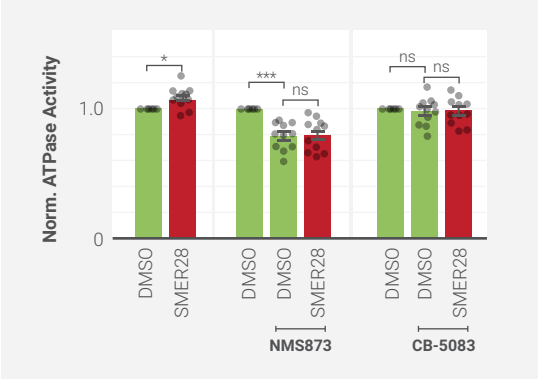
Prediction of Binding Site

Less than 1% of VCP peptides showed robust dose-response correlations when treated with SMER28. The top three peptides clustered based on IC50. The center of mass of these peptides suggests binding in the cleft between VCP's substrate binding domain and ATPase domain 1, a previously demonstrated activation site within VCP.

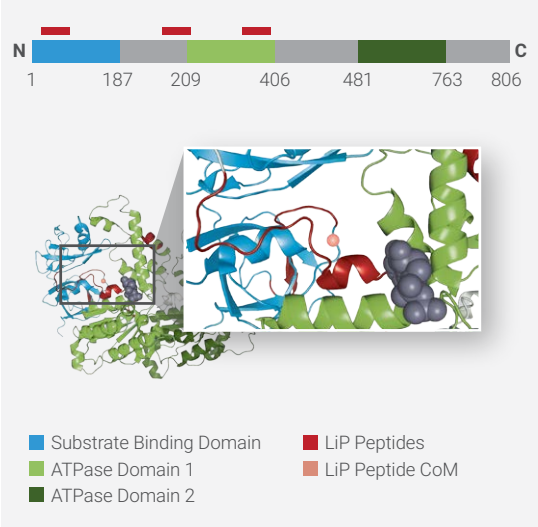
Conclusion

Drug Target Validation predicts that SMER28 binds in the cleft formed between VCP's substrate binding domain (blue) and ATPase domain 1 (green). Collectively, our data provide strong evidence that SMER28 binds in this cleft on VCP and increases its ATPase activity.

SMER28 Activates VCP ATPase Activity



Identified Binding Site of SMER28 on VCP



Wrobel, L., Hill, S.M., Djajadikerta, A. et al., Nat Commun 13, 4146 (2022)

BIOGNOSYS: A LEADING INNOVATOR IN PROTEOMICS

As the leading inventor and innovator of mass spectrometry-based proteomics solutions, we offer industry-leading technology and actionable insights that you can trust to accelerate your research.

> 14	> 50%	> 55	> 3,000	> 10,000	> 800
years of innovation in Proteomics	PhD scientists on staff	granted international patents	publications on Biognosys' technology	sqft lab and LC-MS facilities	global customers

YOUR PARTNER THROUGHOUT THE RESEARCH JOURNEY

Biognosys is your trusted partner throughout the discovery and development journey with attentive pre-study consultation, high quality and timely data delivery, and insightful reporting.

Contact us at **services@biognosys.com** to discuss your specific study needs with one of our scientific or technical consultants.

At Biognosys, we believe that deep proteome insights hold the key to breakthrough discoveries that transform science for better lives. We make the proteome actionable to empower research, drug development, and clinical decision-making with our versatile portfolio of mass spectrometry-based proteomics research services, software, and kits. These solutions provide a multi-dimensional view of protein expression, function, and structure in all biological species and sample types.

biognosys.com

BIOGNOSYS
NEXT GENERATION PROTEOMICS