LIP-QUANT, AN AUTOMATED CHEMOPROTEOMIC APPROACH TO IDENTIFY DRUG TARGETS IN COMPLEX PROTEOMES

Nigel Beaton[1], Ilaria Piazza[2], Roland Bruderer[1], Yuehan Feng[1], Paola Picotti[2], Lukas Reiter[1]

1) Biognosys AG, Schlieren (Zurich), Switzerland 2) Institute of Molecular Systems Biology, ETH Zurich, Zurich, Switzerland

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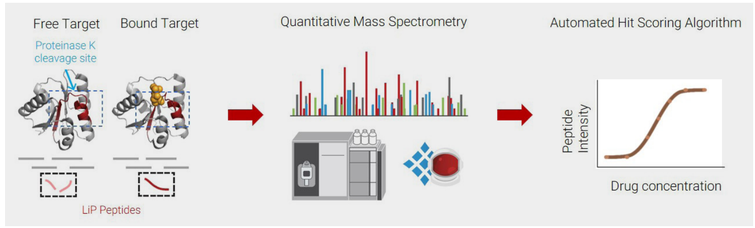
Yuehan Feng, PhD
Business Development Manager

yuehan.feng@biognosys.com
www.biognosys.com

INTRODUCTION

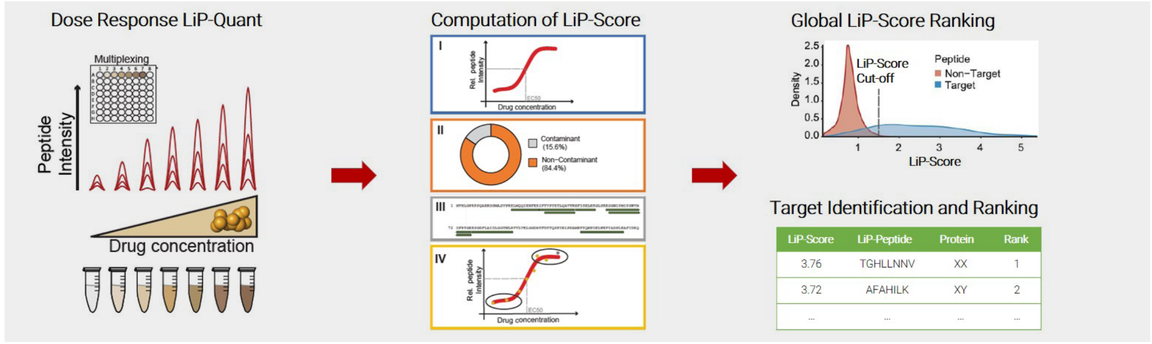
- Target identification plays a key role in both target-based drug discovery and phenotypic drug discovery
- In order to refine a promising drug candidate, extensive knowledge of its target protein(s), including undesirable off-target binding events, is essential
- A multidisciplinary approach combining genetic (e.g. CRISPR-based screening), biochemical (e.g. CETSA) and biophysical techniques is needed to meet this challenge
- We present a novel approach of the target ID tool kit based on limited proteolysis (LiP) and quantitative mass spectrometry (MS)



CONCLUSIONS

- **We present an integrated machine learning-based chemoproteomic workflow which can be applied to identify drug targets in complex proteomes**
- **LiP exploits orthogonal biophysical principles of compound binding - conformational changes and steric hindrance - for target deconvolution**
- **The peptide-centric approach enables estimation of relative binding affinities and mapping of potential binding sites**
- **LiP-Quant is an addition to the target deconvolution toolbox that enables the probing of compound-target interactions, off-target binding, and can potentially provide crucial information regarding binding site prediction**

RESULTS



LiP-Score	LiP-Peptide	Protein	Rank
3.76	TGHLLNNV	XX	1
3.72	AFAHILK	XY	2
...	...	...	...

Figure 1: Machine Learning-based Chemoproteomic Pipeline for Small Molecule Target Deconvolution
Mechanically sheared cell/tissue lysate is incubated with compound at multiple concentrations (left panel) followed by LiP and subsequent trypsin digestion. Resulting peptides are analyzed via data-independent acquisition mass spectrometry (DIA-MS). A machine learning-based model is employed to discern features indicative of drug binding (middle panel) and an integrated LiP score is assigned to each peptide. Global LiP score distribution (right panel) for known target and non-target proteins shows clear separation (in positive control training data sets). LiP score ranks potential candidates in a target deconvolution experiment without bias.

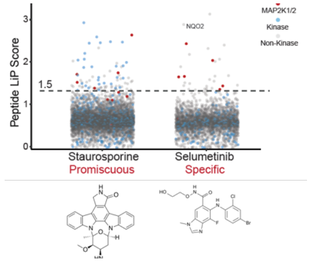
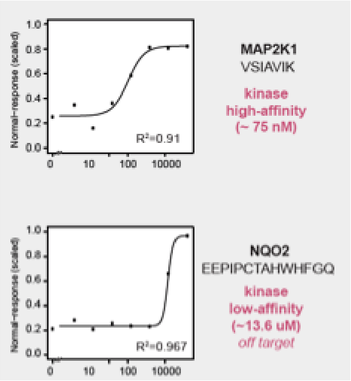


Figure 2: Global Target Deconvolution for Kinase Inhibitors based on Ranking of LiP-peptides
In two 7-series dose response experiments in HeLa lysate, the targets of a highly specific kinase inhibitor, **selumetinib** and a broad specificity inhibitor, **staurosporine** are identified. LiP scores of all ranked peptides (q-value < 0.01 and absolute $\log_2$ fold change > 0.46) are shown where peptides from kinases and MEK1/2 are colored in blue and red respectively.

Figure 3: Dose Response Curves for High-ranking LiP Peptides of MAP2K1 (Target of Selumetinib) and NQO2 (Off Target)



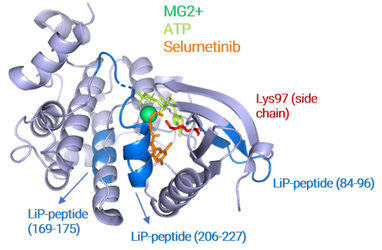


Figure 4: Peptide Mapping on MEK1 Protein
Mapping of three LiP-peptides (in blue, denoted with first and last amino acid residue) to the previously reported crystal structure (Robarge et al. 2014) of human MEK1 bound to selumetinib (in orange).