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SHORT COMMUNICATION
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Combined approach to analysis of protein-protein interactions

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Abstract

In this work combined approach for protein-protein interactions analysis is described.

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Introduction

Although it is now possible to sequence the genome or characterize the proteome of an organism, it is still challenging to identify protein-protein interaction networks and study their individual components. Moreover, interactomic research is often the only way to elucidate unknown functions of certain proteins and deorphanize a wide range of cell signaling pathways mediators.

Materials and Methods

Our approach consists of a protein pull-down assay (molecular “fishing”), which is the most convenient method to date, but to some extent is limited to the identification of the most abundant and high-affinity binding proteins, combined with a reverse interactomics approach, which is free from this limitation.

The crucial step of a pull-down assay is obtaining highly pure “bait” protein of interest. In our work we utilized heterologous expression of double-tagged cytochrome enzymes in *E.coli* cells. 10xHis-tag is used for IMAC affinity purification and biotinylated Avi-tag is used for immobilization of the bait molecule in pull-down assays. Most proteins required additional purification steps such as IEX chromatography and gel-filtration.

Reverse interactomics approach in turn involves phage peptide library screening and determination of consensus binding sequence as well as database searching.

Results

Due to the nature of the strong (Kd ~ 10^{-15} M) biotin-streptavidin interaction, it is possible to capture stable or transient strong specific interaction between bait and its putative functionally competent partners (“prey” molecules) present in natural biological systems. Biotinylation occurs in vivo during protein expression and can be carried out in vitro with BirA biotin ligase enzyme as well. Tags can be removed, using the introduced proteolysis site for TEV protease, in order to release protein complexes from the matrix or obtain proteins with intact sequence for further kinetic experiments.

A new range of “prey” proteins of cytochrome b5, which may be potentially involved in the interaction, was then identified using shotgun proteomics mass spectral methods (1).

In experiment with CYP5A1 (thromboxane synthase) we found a sequence motif with a high homology to THPO (thrombopoietin), that assumes its role in thromboxane synthesis regulation. Peptide was then synthetized and subjected to a biolayer interferometry kinetic assay, which indicated high affinity ($9{,}56 \cdot 10^{-8}$ M) binding with CYP5A1 (**Fig. 1**).

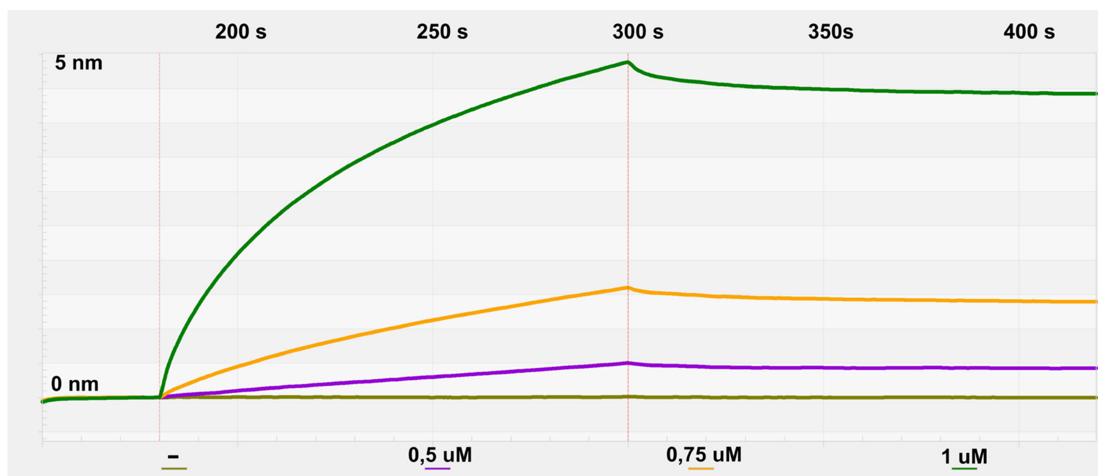

Figure 1. Biolayer interferometry kinetic assay of CYP5A1 binding to immobilized peptide.

Reverse interactomics methods serves as complementary approach for identifying the protein targets of a binding domain or enzyme and the peptide motif(s) responsible for the interaction.

Discussions

A combined approach for revealing specific protein-protein interactions with a wide range of binding affinities has been developed. This approach may be universally applied to a number of protein-protein interactions preserving "nativeness" of the targets.

References

1. Ivanov AS, Ershov PV, Gilep AA. Protein interactomics based on direct molecular fishing on paramagnetic particles: practical realization and further SPR validation. Proteomics 2014; 14(20): 2261-74.