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SPR biosensors in direct molecular fishing: implications for protein interactomics

Key words: SPR biosensors, molecular fishing of proteins, bait proteins

There is increasing evidence that in living systems proteins exist and function within stable or dynamic molecular complexes [16]. Protein–protein interactions (PPIs) determining formation and lifespan of such complexes attract much interest; they are extensively studied by using various bioinformatic, genomic, and biochemical technologies [1, 10, 14]. In this context, biochemical methods are the most reliable ones: using these methods researchers investigate PPIs under conditions close to physiological.

Biochemical methods employ the strategy of molecular fishing for isolation of protein complexes and subsequent mass spectrometry identification of potential protein partners.

Molecular fishing is a variant of affinity-based isolation of target proteins from a lysate of the biological material due to specific interaction between the immobilized ligand (a bait molecule) and its putative (one or several) functionally competent partners (prey molecules) [10, 13, 15]. Various compounds have been used as the bait molecules; these include small organic molecules [2, 12], proteins and nucleic acids [9].

In this report, we have summarized results of our studies on the use of SPR-based approach for direct molecular fishing of proteins from lysates of biological materials and identification of prey proteins by mass spectrometry [3 – 9]. We initially consider a general strategy for the use of SPR biosensors at the particular experimental stages of molecular fishing with special attention to the SPR-based optimization of experimental protocols (including immobilization of bait proteins on a carrier, evaluation of intactness of the immobilized bait protein, and optimization of protocols for preparation of tissue/cell culture lysates, etc.).

After that we consider the role of the SPR biosensor technology in the SPR-based analytical fishing. Finally, we demonstrate applicability of the SPR biosensor technology for analysis of ligand protein interactions using non-peptide small molecules as baits [11].

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