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Techniques for detecting protein-protein interactions in living cells: principles, limitations, and recent progress

Yaning Cui[1,2†], Xi Zhang[1,2†], Meng Yu[1,2], Yingfang Zhu[3], Jingjing Xing[3] & Jinxing Lin[1,2*]

[1]*Beijing Advanced Innovation Center for Tree Breeding by Molecular Design, Beijing Forestry University, Beijing 100083, China;*
[2]*College of Biological Sciences & Biotechnology, Beijing Forestry University, Beijing 100083, China;*
[3]*Institute of Plant Stress Biology, State Key Laboratory of Cotton Biology, Department of Biology, Henan University, Jinming Street, Kaifeng 475001, China*

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Detecting protein-protein interactions (PPIs) provides fundamental information for understanding biochemical processes such as the transduction of signals from one cellular location to another; however, traditional biochemical techniques cannot provide sufficient spatio-temporal information to elucidate these molecular interactions in living cells. Over the past decade, several new techniques have enabled the identification and characterization of PPIs. In this review, we summarize three main techniques for detecting PPIs *in vivo*, focusing on their basic principles and applications in biological studies. We place a special emphasis on their advantages and limitations, and, in particular, we introduced some uncommon new techniques, such as single-molecule FRET (smFRET), FRET-fluorescence lifetime imaging microscopy (FRET-FLIM), cytoskeleton-based assay for protein-protein interaction (CAPPI) and single-molecule protein proximity index (smPPI), highlighting recent improvements to the established techniques. We hope that this review will provide a valuable reference to enable researchers to select the most appropriate technique for detecting PPIs.

protein-protein interactions, *in vivo*, techniques, RET, PCA, co-localization

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Introduction

Proteins typically interact with each other to form stable or transient complexes with a range of vital functions. Detecting these protein-protein interactions (PPIs) is therefore crucial for uncovering the regulatory mechanisms underlying individual cellular processes. The importance of PPIs means that their detection has received considerable attention, with traditional techniques being supplemented by emerging, improved methods.

In principle, the detection of PPIs is dependent on the shape and chemistry of the protein surface, differences that enable these molecules to perform a huge range of biological functions, from development to cell-environment communication (Reichmann et al., 2007). Several techniques have been developed to directly or indirectly detect PPIs *in vitro* (Lalonde et al., 2008). Among them, researchers commonly use immunoprecipitation, pull-down assays, and yeast two- or three-hybrid assays. Immunoprecipitation requires an antibody that can recognize an epitope on the protein of interest. The interacting antibody-protein complex in the cell lysate can be captured and detected using specialized antibodies in a Western blot, or identified using mass spectrometry (Conrad, 2008). Immunoprecipitation can be used for large-scale screening (Fan et al., 2016; Lin and Lai, 2017); however, this technique also has several disadvantages; most

†Contributed equally to this work
*Correspondence author (email: linjx@ibcas.ac.cn)