



STUDY OF DECIPHERING PROTEIN-PROTEIN INTERACTIONS USING MOLECULAR TECHNIQUES

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Abstract:

Protein-protein interactions (PPIs) are involved in almost every major cellular process. Proteins interact with one another to regulate signalling, gene expression, metabolism, trafficking, and the formation of multiprotein complexes. Understanding these interactions is therefore important for explaining normal cell function and the molecular basis of disease. A wide range of methods is available for studying PPIs, but each method provides a different type of information. Classical approaches such as yeast two-hybrid, co-immunoprecipitation, and pull-down assays are useful for screening and validation. More recent approaches, including affinity purification-mass spectrometry, cross-linking mass spectrometry, FRET, surface plasmon resonance, proximity ligation, and proximity labeling with BioID, TurboID, and APEX, allow interactions to be examined at larger scale or in more native cellular settings. This review summarizes the principles, applications, strengths, and limitations of these molecular techniques. Particular attention is given to transient and spatially restricted interactions, which are difficult to capture using conventional purification methods. The review also discusses current trends in spatial proteomics, single-cell interactomics, and computational analysis. Overall, the literature supports the use of complementary methods rather than reliance on a single assay when establishing biologically meaningful protein interactions.

Keywords Protein-Protein Interactions, Molecular Techniques, Protein Interactome, Mass Spectrometry, Proximity Labeling, Protein Interaction Networks.

1. Introduction

Proteins rarely work as isolated molecules. Inside a cell, they associate with other proteins to form complexes, regulate enzymatic activity, transmit signals, control gene expression, and maintain cellular organization. These physical associations are known as protein-protein interactions (PPIs). Because many cellular pathways depend on coordinated protein interactions, studying PPIs has become an important part of modern molecular and

cellular biology (1, 2). The complete collection of protein associations in a biological system is often described as the protein interactome. Interactomes are not fixed maps: interactions can change with cell type, subcellular location, developmental stage, post-translational modification, or external stimulus. A protein may therefore have different interaction partners under different conditions. This context dependence is one reason why PPI studies are technically challenging (2, 3).

Early PPI research relied mainly on genetic and biochemical approaches. The yeast two-hybrid (Y2H) system provided a way to identify possible binary interactions by reconstituting transcriptional activity when two proteins interact (4, 5). Co-immunoprecipitation (Co-IP) and affinity-based pull-down assays became useful for testing whether proteins occur together in cellular or biochemical complexes. These methods remain widely used because they are practical and can provide strong evidence when appropriate controls are included (6).

Large-scale proteomics has changed the scale of PPI research. Affinity purification coupled to mass spectrometry (AP-MS) can identify many components of a protein complex, while cross-linking mass spectrometry (XL-MS) can provide additional information about proteins that are physically close within a complex. Recent MS-based work emphasizes that enrichment strategy and experimental context strongly influence which interactions are detected (7). (7–10). Imaging approaches such as FRET, BRET, and BiFC provide another type of evidence because they can show protein proximity or interaction within cells. FRET, for example, can be used to investigate molecular proximity at nanometre-scale distances and can be combined with live-cell imaging (11, 12). Surface plasmon resonance (SPR) is useful for a different question: it measures binding behaviour and can provide association and dissociation kinetics using purified molecules (14). (11–13)

Proximity labeling has further expanded the field. BioID uses a promiscuous biotin ligase to label proteins near a protein of interest, while TurboID was engineered to perform this labeling much faster. APEX-based methods use a peroxidase to label nearby proteins and can provide spatially resolved proteomic information in living cells (15–17). These approaches are especially useful for transient or spatially restricted protein environments that may be lost during conventional purification. (15–19). The purpose of this review is to describe major molecular techniques used to decipher PPIs, compare the type of information they provide, discuss their advantages and limitations, and highlight how combining complementary methods can improve confidence in PPI studies.

2. Materials and methods

2.1 Literature search strategy

A narrative literature review was conducted using peer-reviewed literature related to the identification, validation, characterization, and mapping of PPIs. Searches were performed using scientific databases and publisher-indexed records, with PubMed and PubMed Central used as major sources. Search terms included “protein–protein interactions”, “PPI detection”, “yeast two-hybrid”, “co-immunoprecipitation”, “pull-down assay”, “affinity purification mass spectrometry”, “cross-linking mass spectrometry”, “FRET”, “surface plasmon resonance”, “proximity labeling”, “BioID”, “TurboID”, and “APEX”.

2.2 Literature selection

Foundational publications were included when they introduced or established an important PPI method. Recent reviews and methodological papers were given greater weight for emerging areas such as mass-spectrometry-based interactomics, proximity labeling, spatial proteomics, and computational interpretation. Literature was selected for relevance to molecular PPI detection and for the clarity of the methodological information provided.

2.3 Data extraction and synthesis

Information was organized according to the principle of each technique, experimental context, type of interaction detected, major applications, advantages, and limitations. The methods were then compared according to the biological questions they can address, including binary interaction screening, complex identification, binding kinetics, spatial localization, transient interactions, and large-scale interactome mapping.

3. Results

3.1 Overall trends identified from the literature

Table 1: Comparison of major molecular techniques used for protein-protein interaction analysis

Technique	Main principle	Useful for	Major strength	Important limitation
Y2H	Reporter activation after bait-prey interaction	Binary interaction screening	Simple genetic screening	Non-native cellular environment can affect results
Co-IP	Antibody-based isolation of protein complexes	Cellular protein association	Can examine complexes from cells	Does not by itself prove direct binding
Pull-down	Affinity capture of interacting proteins	Biochemical validation	Controlled assay conditions	Often requires tagged or purified proteins
AP-MS	Affinity purification followed by MS	Protein complexes and interactomes	Identifies many proteins in one experiment	Weak interactions may be lost during purification
XL-MS	Chemical cross-linking followed by MS	Complex architecture and proximity	Can provide structural constraints	Technically and computationally demanding
FRET	Energy transfer between fluorophores	Live-cell proximity and dynamics	Spatial information in cells	Requires careful labeling and controls
BiFC	Fluorescent protein fragment complementation	Interaction localization	Direct visual readout	Signal can persist after interaction
SPR	Optical measurement of binding at a sensor surface	Binding affinity and kinetics	Quantitative and label-free	Usually requires purified proteins
PLA	Antibody-based proximity detection	Cell/tissue validation	Spatial information	Proximity is not equivalent to direct binding
BioID/ TurboID/APEX	Enzyme-mediated labeling of nearby proteins	Transient/spatial protein environments	Works in living cells	Nearby proteins may not be direct interactors

Table 2: Matching PPI techniques to common research questions

Research question	Useful approaches
Is there a possible binary interaction?	Y2H, FRET, BRET
Does a protein occur in a cellular complex?	Co-IP, AP-MS
Which proteins are associated with a protein of interest?	AP-MS, proximity labeling
Are transient or weak associations important?	TurboID, APEX, XL-MS
Where does an interaction occur in a cell?	FRET, BiFC, PLA
What is the binding affinity or kinetic behaviour?	SPR
Which residues or regions may be close within a complex?	XL-MS and structural methods
How does the interactome change with cellular conditions?	Quantitative AP-MS, proximity labeling, imaging
Can experimental data be supported computationally?	Network analysis, structural modelling, machine-learning approaches

Table 3: Common sources of error in PPI studies and possible solutions

Problem	Possible cause	Useful response
False-positive interaction	Nonspecific binding or overexpression	Use negative controls and independent validation
False-negative result	Weak or transient interaction	Consider cross-linking or proximity labeling
Indirect association interpreted as direct binding	Proteins occur in the same complex	Use purified-protein assays or orthogonal validation
Interaction lost during preparation	Cell lysis or stringent washing	Optimize extraction and consider cross-linking
Tag-related artefact	Fusion tag affects protein function	Test tag position and expression level
High proximity-labeling background	Nonspecific labeling	Use matched controls and quantitative filtering
Context-dependent result	Cell type or cellular state	Validate in the biologically relevant system
Large or noisy dataset	High-throughput MS output	Use statistical filtering and independent confirmation

The literature shows a clear progression from methods designed mainly to identify individual protein pairs toward approaches that can describe protein interaction networks in more native cellular environments. Y2H remains useful for binary interaction screening, while Co-IP and pull-down assays are still important for biochemical confirmation. AP-MS has expanded the ability to identify multiple components of protein complexes,

and XL-MS adds structural information that is difficult to obtain from conventional affinity purification alone (6, 7). A second major trend is the increasing use of live-cell and proximity-based methods. FRET and related fluorescence approaches can provide spatial and temporal information, whereas BioID, TurboID, and APEX can capture nearby proteins without requiring the interaction to survive cell lysis and purification (11, 15–17). The 2026 review by Yang and colleagues also highlights the movement toward methods that capture the spatial and dynamic nature of PPIs, including proximity labeling, advanced imaging, spatial proteomics, and single-cell interactomics (3). (11, 15–19)

Overall, the reviewed literature supports a complementary strategy. A candidate interaction identified by a screening method can be followed by biochemical validation, quantitative binding analysis, cellular imaging, or proximity-based mapping. Using more than one independent approach is particularly useful when the biological question involves transient, weak, indirect, or context-dependent interactions. Table 1, Table 2 and Table 3 shows the comparison of major molecular techniques, matching PPI techniques to various research questions and common sources of error in PPI studies.

4. Discussion

No single PPI technique can answer every biological question. This is probably the most important point when selecting a method. Y2H is well suited to screening possible binary interactions, but the assay is performed in a heterologous system and may not reproduce all features of the original cellular environment (4, 5). Co-IP is more closely connected to the cellular context, but proteins detected together may be part of the same complex without directly contacting each other (6).

AP-MS has become a central approach for interactome studies because it allows many associated proteins to be identified in one experiment. Its main limitation is that the purification process can disturb weak or transient associations. XL-MS can partly address this problem by covalently stabilizing proteins that are close to one another and can also provide information useful for studying complex architecture (7). (7, 9, 10)

Fluorescence-based approaches answer a different set of questions. FRET can provide information about molecular proximity in living cells and can be adapted to study changes in interaction state over time (11, 12). BiFC can show where an interaction occurs, although the stable fluorescent complex means that it should not be treated as a perfect measurement of rapid association and dissociation. SPR is particularly useful when the goal is quantitative characterization of purified protein binding, including association and dissociation behaviour (14). (11–13)

Proximity labeling is especially useful for interactions that are difficult to preserve during conventional biochemical purification. BioID demonstrated that a promiscuous biotin ligase could label proteins close to a target protein in living cells, while TurboID substantially increased labeling speed and extended the approach to model organisms (15, 16). APEX introduced a complementary peroxidase-based strategy for spatially restricted labeling and demonstrated how enzymatic tagging could combine the spatial advantages of microscopy with the scale of mass spectrometry (17). (15–19)

An important limitation shared by proximity-based approaches is interpretation. A labeled protein is located near the protein of interest, but it is not necessarily a direct binding partner. The same caution applies to PLA and, in a different way, Co-IP. Strong conclusions therefore come from combining evidence. For example, AP-MS can be used for discovery, Co-IP or pull-down for validation, SPR for binding behaviour, and FRET or PLA for cellular

localization. Such a workflow reduces the chance that an artefact from one method will be mistaken for a biological interaction. (20)

Recent literature suggests that the field is moving toward spatially and temporally resolved interactomes. The 2026 review by Yang et al. describes this shift from classical interaction mapping toward proximity labeling, advanced imaging, spatial proteomics, and single-cell interactomics (3). Likewise, recent MS-focused work emphasizes that different enrichment strategies capture different classes of interactions, making method selection an important part of experimental design (7). (3, 10, 19)

Computational analysis is likely to become increasingly important as these methods generate larger datasets. Network analysis can help identify highly connected proteins and functional modules, while structural modelling can help interpret possible interaction interfaces. Computational predictions, however, should be treated as complementary evidence and ideally tested experimentally.

5. Conclusion

Protein–protein interactions are central to cellular organization and function, and their disruption can contribute to disease. Molecular techniques have progressed from classical binary interaction assays to large-scale proteomics, live-cell imaging, proximity labeling, and integrated computational analysis. Each method has a particular strength: Y2H is useful for screening, Co-IP and pull-down assays support biochemical validation, AP-MS maps protein complexes, XL-MS provides structural constraints, imaging approaches reveal spatial or dynamic information, and proximity labeling can capture proteins in their native cellular neighbourhood.

The evidence reviewed here indicates that the most reliable PPI studies are usually based on complementary methods rather than a single assay. This is especially important for transient, weak, indirect, and context-dependent interactions. Future PPI research is likely to place greater emphasis on spatial proteomics, single-cell approaches, improved mass spectrometry, advanced imaging, and computational integration. Together, these developments should provide a more realistic picture of the dynamic interactome and may help identify new mechanisms of disease and opportunities for therapeutic intervention.

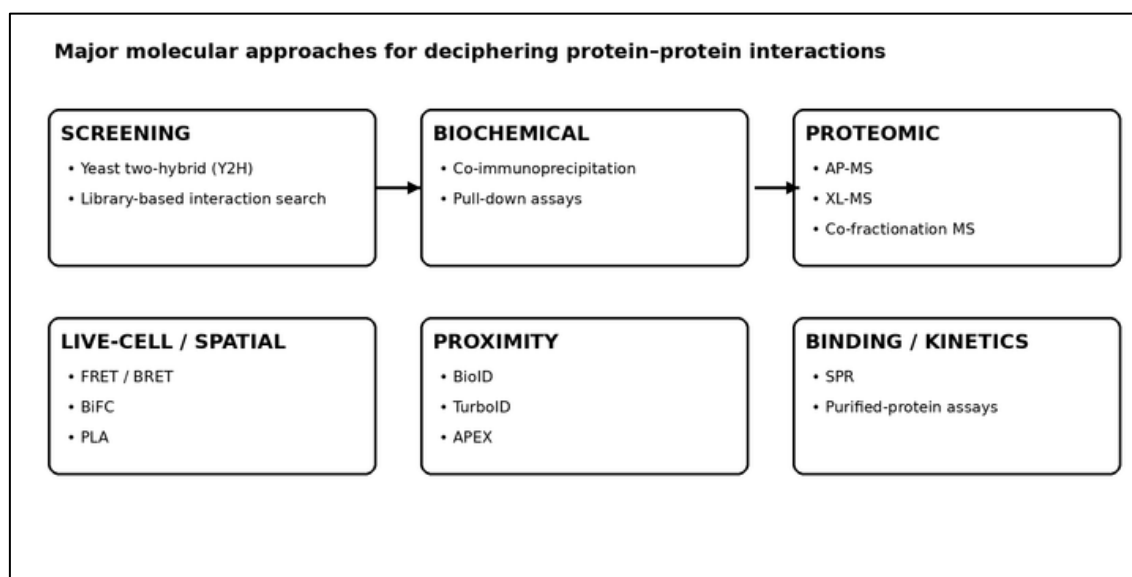


Figure 1: Overview of major molecular approaches used to decipher protein–protein interactions

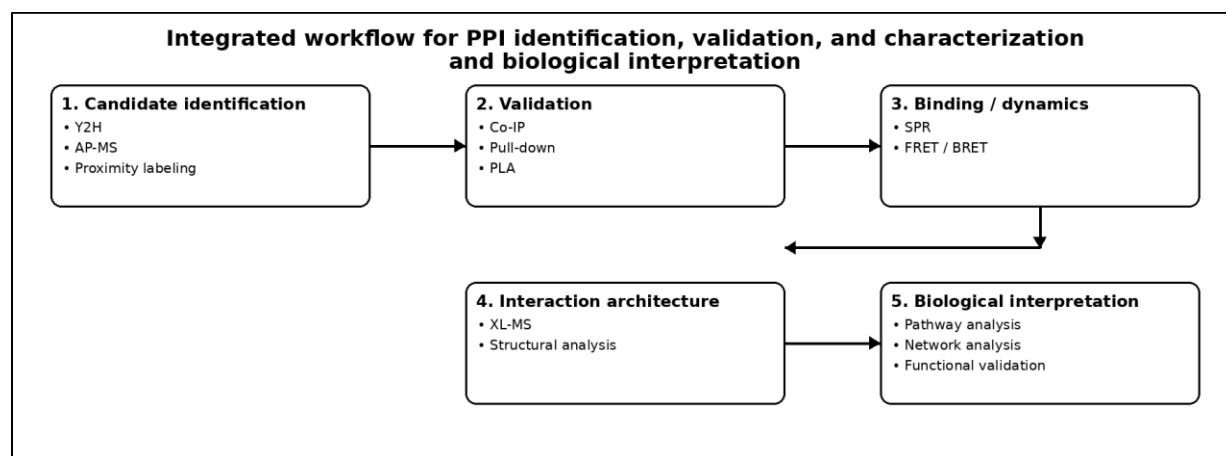


Figure 2: Integrated workflow for identification, validation, characterization, and biological interpretation of protein–protein interactions.

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