Research Techniques Made Simple: Emerging Methods to Elucidate Protein Interactions through Spatial Proximity

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Interactions between proteins are essential for fundamental cellular processes, and the diversity of such interactions enables the vast variety of functions essential for life. A persistent goal in biological research is to develop assays that can faithfully capture different types of protein interactions to allow their study. A major step forward in this direction came with a family of methods that delineates spatial proximity of proteins as an indirect measure of protein-protein interaction. A variety of enzyme- and DNA ligation-based methods measure protein co-localization in space, capturing novel interactions that were previously too transient or low affinity to be identified. Here we review some of the methods that have been successfully used to measure spatially proximal protein-protein interactions.

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Description: This article, designed for dermatologists, residents, fellows, and related healthcare providers, seeks to reduce the growing divide between dermatology clinical practice and the basic science/current research methodologies on which many diagnostic and therapeutic advances are built.

Objectives: At the conclusion of this activity, learners should be better able to:
- Recognize the newest techniques in biomedical research.
- Describe how these techniques can be utilized and their limitations.
- Describe the potential impact of these techniques.

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Interaction between proteins underlies a significant amount of the mechanical, structural, and signaling processes that are necessary to support various functions of living cells. The variety of protein-protein interactions is highly diverse and heavily context dependent. Methods to study protein-protein interactions like affinity-capture complex purification (LaCava et al., 2016), surface plasmon resonance (Schuck, 1997), isothermal titration calorimetry (Velazquez-Campoy, 2006), yeast-two-hybrid screening (Miller et al., 2004), and florescence resonance energy transfer (Heim et al., 1996) among many others have provided key insights to understanding function, but each method suffers from limitations. Most existing methods require stable protein interactions to survive the harsh processing steps necessary to extract proteins from cells, and many others query interactions outside the native cellular context, relying on empirically reconstituted conditions (Table 1).

Proximity-based protein labeling attempts to address some of these limitations through a different approach. Instead of purifying protein complexes or searching for evidence of

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SUMMARY

- Proximity protein labeling allows detection of spatially proximal proteins.
- BirA, Apex, and HRP are enzymes that generate spatially confined reactive biotin intermediates, providing the mechanism by which spatial labeling is possible.
- Proximity labeling can also be used to label cellular compartments using localization sequences or to perform conditional proteomics with split versions of the enzymes.
- DNA-based proximity ligation is an alternative, low-throughput method that also assays spatial proximity.

interactions between recombinant proteins in vitro, proximity labeling allows identification of proteins that reside within a 10- to 20-nm radius of an introduced labeling enzyme. Such a labeling strategy allows surveillance of transient and weak interactions. Because proximity labeling can be done in living cells, it can also detect interactions that require fragile macromolecular assemblies, intact subcellular structures, lipid or nucleic acid cofactors, and posttranslational modifications that are difficult to retain or reconstitute in vitro. This idea of using spatial information to expand and inform the networks of interactions inside a cell is increasingly being applied in new techniques and applications. This review focuses on how proximity labeling has been successfully used thus far, the limitations of the data it provides, and the potential for further development.

ENZYMATIC PROXIMITY LABELING (BirA, APEX, HRP)

At the heart of proximity labeling are enzymes that produce distance-constrained reactive biotin molecules. Although spatial proximity of proteins within a cell could to some extent be measured by modern microscopy techniques, the reagents and time necessary make microscopy impractical as a high-throughput discovery method. In contrast, labeling followed by protein isolation allows for unbiased discovery through mass spectrometry. The discovery of several enzymes that generate short-lived, reactive biotin intermediates have made the latter approach possible. These enzymes generate reactive biotin that can covalently link to proteins but are also quickly quenched within 10–20 nm of travel through the cell. If reactive biotin reaches a protein before being quenched, it can covalently link to the protein, providing a permanent biotin handle for purification. Three types of enzymes successfully used for this purpose are promiscuous variants of *Escherichia coli* and *Aquifex aeolicus* biotin ligases (BirA) (Kim et al., 2016; Roux et al., 2012), an engineered ascorbate peroxidase (APEX/APEX2) (Lobingier et al., 2017), and horseradish peroxidase (HRP) (Honke et al., 2012), although ongoing efforts promise to diversify and improve on this existing toolset. BirA enzymes are usually responsible for biotinylation of an acetyl-CoA carboxylase subunit, but mutations that cause them to prematurely release their reactive biotin intermediates have made them useful for proximity labeling. HRP and ascorbate peroxidase are both peroxidases that use hydrogen peroxide to perform oxidation reactions. HRP has been a staple enzyme in activity assays, finding wide applications from ELISAs to Western blotting long before its application in proximity labeling. HRP is unfortunately inactive in the cytosol, necessitating investigation of other peroxidases. Ascorbate peroxidase was therefore engineered as an HRP substitute and given the new name APEX (Martell et al., 2012). APEX was first applied to electron microscopy but quickly found a role in proximity-dependent protein labeling because of its ability to rapidly generate short-lived, spatially confined reactive biotin intermediates.

When a construct of these labeling enzymes fused with a protein of interest is expressed within a cell, the fusion protein can properly localize and perform its usual biological functions (Figure 1a–c). Proteins that stay within the labeling radius of the enzyme longer than would be expected by random motion become enriched in the total biotinylated subset of proteins. During this time, cells can be exposed to biochemical and genetic perturbations appropriate for the given experiment. At the end of the biotin-labeling period, cells can be lysed to isolate total protein (Figure 1d). Because biotin labels are covalently linked to proteins themselves, lysis conditions are unlikely to introduce artifacts, providing a significant advantage to affinity purification methods where lysis conditions must be carefully chosen to preserve interactions and where lysis itself can compromise the separation of cellular compartments and lead to false positive and false negative findings (Table 2). A key advantage of the biotin handle is biotin's strong but reversible affinity for streptavidin (Chivers et al., 2011). The high affinity of the biotin-streptavidin complex allows biotinylated proteins to be efficiently captured from the lysed solution and then stringently washed to remove nonspecific interactions (Figure 1e). After protein elution and digestion, samples can be subjected to Western blotting to query specific interactions or mass spectrometry to broadly map all of the spatially proximal proteins detected (Figure 1f). The fidelity of resulting candidate interactions can be tested by determining if previously known protein interactions were detected and by using gene ontology annotations to cross-check for functions known to be associated with the protein of interest (Figure 1g). Novel associations between proteins can frequently be shown in such data. However, the veracity of each detected association invariably requires further validation by orthogonal technical approaches. An example of how proximity proteomics can be used to investigate the novel interactors is illustrated by Perez-White et al. (2017) in their study of the receptor tyrosine kinase EphA2 (Figure 1h and i).

The choice of labeling enzyme must be carefully considered for a number of practical experimental reasons. BirA converts biotin into reactive biotin–adenosine monophosphate, whereas APEX and HRP create free radicals of biotin as intermediates. BirA can be used in living tissue, as has been successfully applied within organotypic skin models (Perez-White et al., 2017). Unlike the simple biotin used for BirA, the biotin-phenol reagent used for APEX and the hydrogen peroxide reagent used for HRP are not suitable for organoid or in vivo studies because of the tissue toxicity of