Protein-Protein Interactions: Methods & Applications

AP-MS Workflows

- Affinity purification-mass spec
- Pull-down interacting partners
- Identify protein complexes

Proximity Labeling

- BioID, APEX, TurboID
- Spatial proteomics
- In vivo labeling

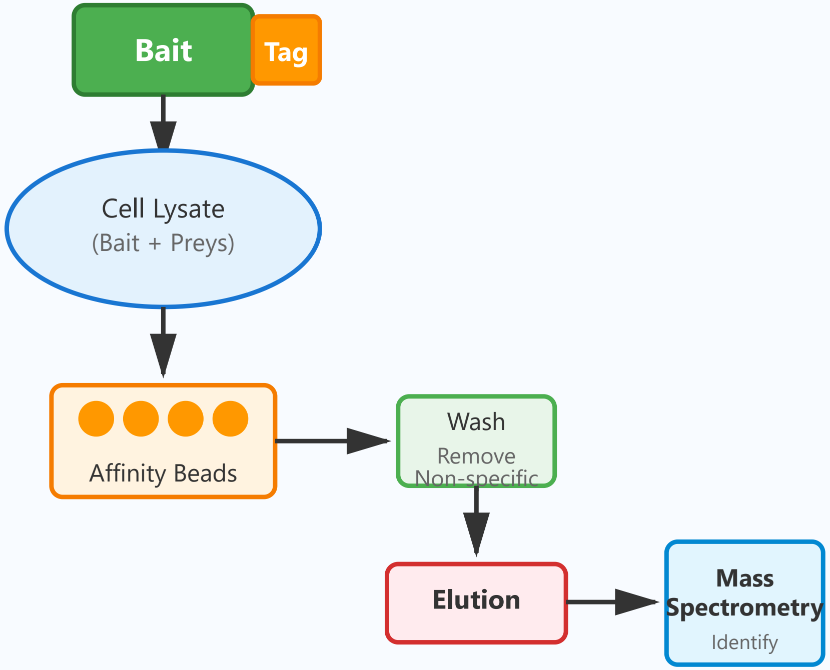
Cross-linking MS

- Chemical cross-linkers
- Distance constraints
- Protein structure information

Network Construction

- Interaction databases
- Scoring significance
- Pathway analysis

1. Affinity Purification Mass Spectrometry (AP-MS)



Overview

Affinity Purification Mass Spectrometry (AP-MS) is a powerful technique for identifying protein-protein interactions in complex biological samples. This method combines the specificity of affinity purification with the analytical power of mass spectrometry.

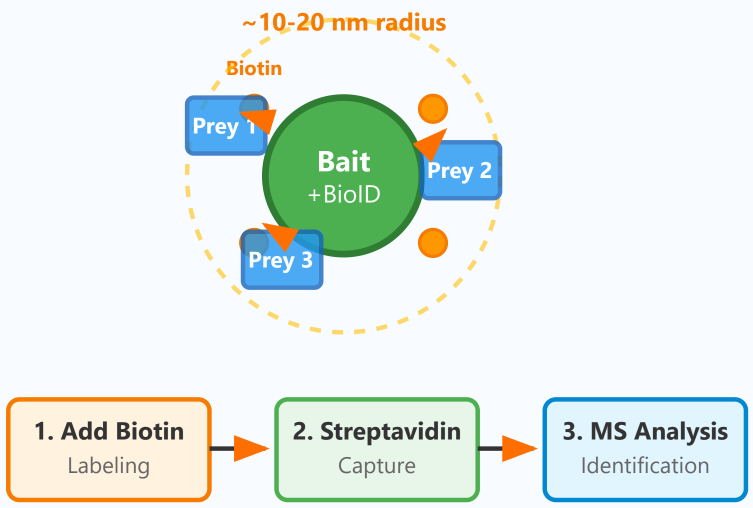
Workflow

The bait protein is tagged (FLAG, HA, or other epitope tags) and expressed in cells. After cell lysis, the tagged protein and its interacting partners are captured using affinity beads coated with antibodies or other binding molecules. Following stringent washing steps to remove non-specific binders, the protein complex is eluted and analyzed by mass spectrometry to identify all bound proteins.

Key Advantages

- ✓ High specificity for direct and indirect interactions
- ✓ Can identify entire protein complexes
- ✓ Quantitative analysis possible with labeled methods
- ✓ Works with native or near-native conditions

2. Proximity Labeling (BioID/APEX/TurboID)



Overview

Proximity labeling methods use engineered enzymes (BioID, APEX, or TurboID) fused to a bait protein to biotinylate neighboring proteins within a defined radius. This approach captures both stable and transient interactions in living cells.

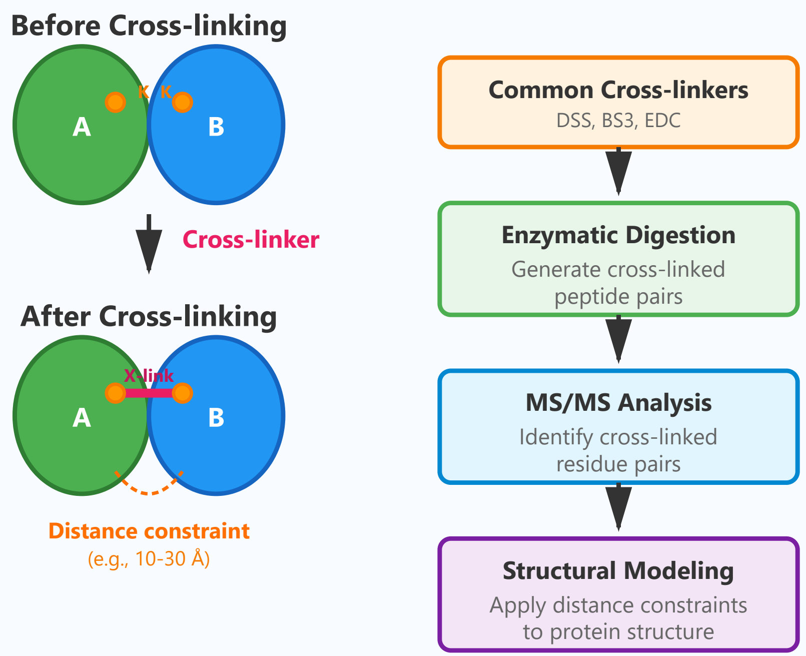
Mechanism

The fusion protein localizes to its native cellular location, where the enzyme catalyzes biotin conjugation to nearby proteins (within ~10-20 nm). After labeling, cells are lysed, and biotinylated proteins are captured using streptavidin beads and identified by mass spectrometry. This method is particularly powerful for studying membrane proteins, transient interactions, and subcellular compartments.

Key Advantages

- ✓ Captures transient and weak interactions in vivo
- ✓ No need to maintain protein complexes during purification
- ✓ TurboID offers rapid labeling (10-60 minutes)
- ✓ Effective for difficult-to-access cellular compartments

3. Cross-linking Mass Spectrometry (XL-MS)



Overview

Cross-linking Mass Spectrometry (XL-MS) uses chemical cross-linkers to covalently connect amino acids that are in close spatial proximity. This technique provides valuable structural information about protein complexes and protein-protein interfaces.

Methodology

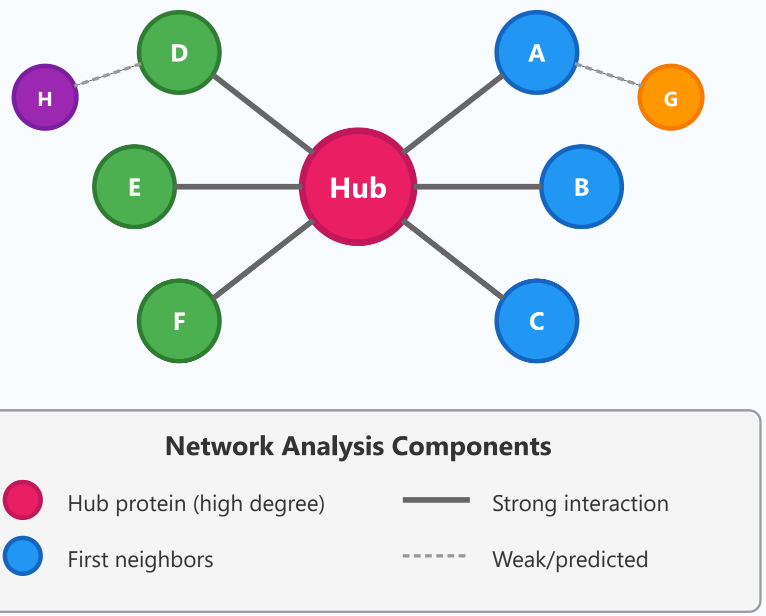
Proteins or protein complexes are treated with bifunctional cross-linkers (such as DSS or BS3) that react with specific amino acid residues, typically lysines. The cross-linker spans a defined distance (usually 10-30 Ångströms), creating covalent bonds between nearby residues. After enzymatic digestion, cross-linked peptide pairs are identified by mass spectrometry, revealing which parts of proteins are in close proximity and providing distance constraints for structural modeling.

Key Advantages

- ✓ Provides distance constraints for protein structure
- ✓ Maps protein-protein interaction interfaces
- ✓ Can study large multi-protein complexes
- ✓ Complementary to cryo-EM and X-ray crystallography

4. Protein Interaction Network Construction & Analysis

Protein Interaction Network



Overview

Protein interaction network construction integrates experimental data from various sources to create comprehensive maps of cellular protein interactions. These networks reveal the organization of cellular processes and help identify key regulatory proteins and pathways.

Network Analysis Approaches

Network construction combines data from multiple sources including AP-MS experiments, yeast two-hybrid screens, and curated databases like STRING, BioGRID, and IntAct. Statistical methods assign confidence scores to each interaction based on experimental evidence. Network topology analysis identifies hub proteins, protein complexes, and functional modules. Advanced algorithms perform pathway enrichment analysis, predict protein function, and identify disease-related subnetworks.

Key Applications

- ✓ Identify protein complexes and functional modules
- ✓ Predict protein function through guilt-by-association
- ✓ Discover drug targets and disease mechanisms
- ✓ Integrate multi-omics data for systems biology