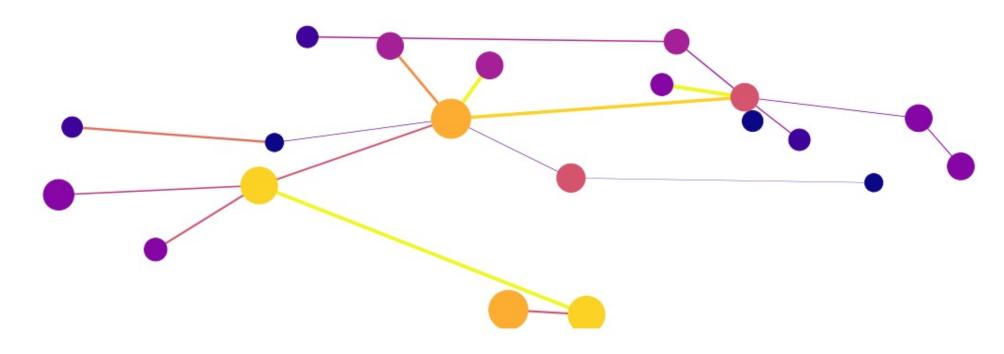
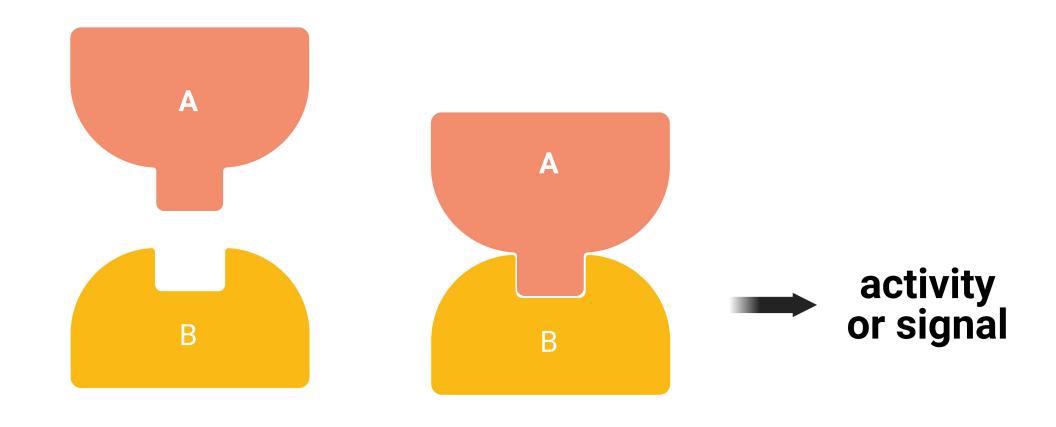
Methods for detecting protein-protein interactions

Danielle Schmidt

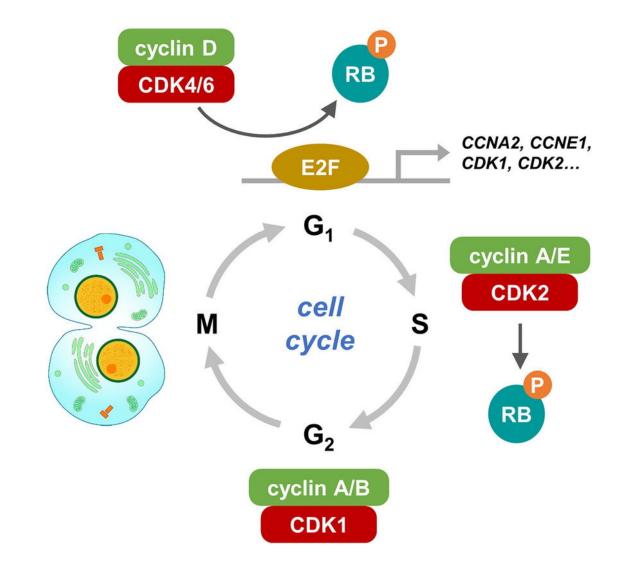


What are protein-protein interactions (PPIs)?

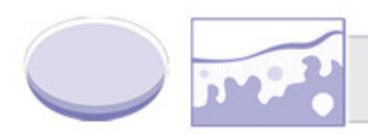


Communications between proteins that are involved in many cellular processes

Why are PPIs important?



Integral for communication in biological processes like the cell cycle



CELL CULTURE OR TISSUE

Proteins for bottom-up analyses come from a variety of sources.



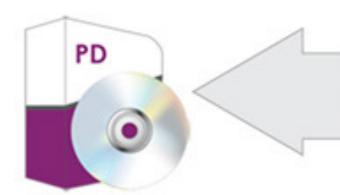
EXTRACTION OF PROTEINS

Proteins are extracted and, in some cases, fractionated to reduce complexity.

How are proteins identified?

AUTOMATED DATA ANALYSIS

Peptides are identified using Proteome Discoverer software, an automated program capable of analyzing CID, HCD, EThcD, and ETD spectra.



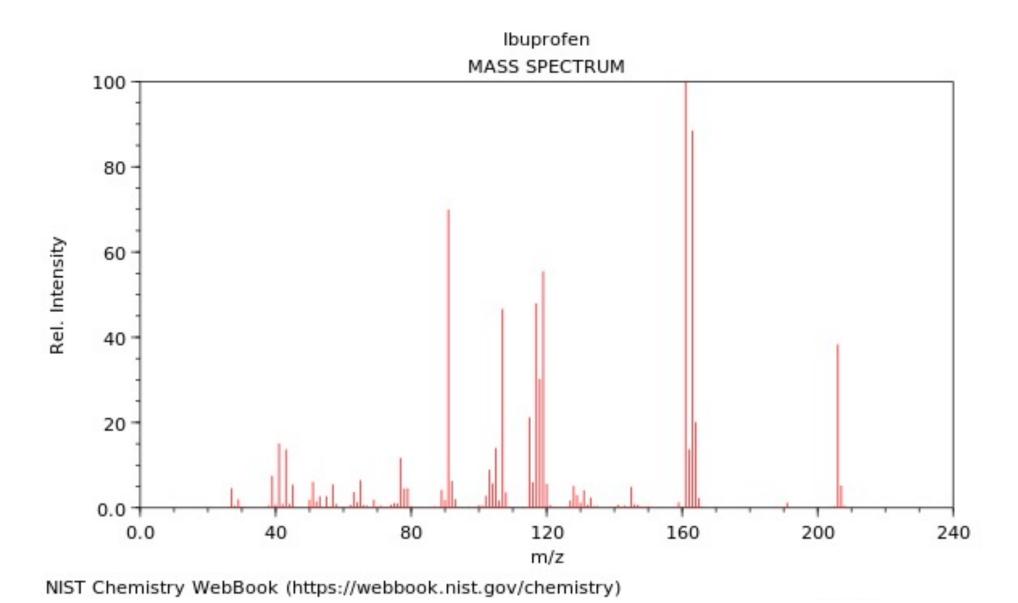
Peptides are analyzed by LC-MS/MS on Orbitrap-based mass spectrometers.



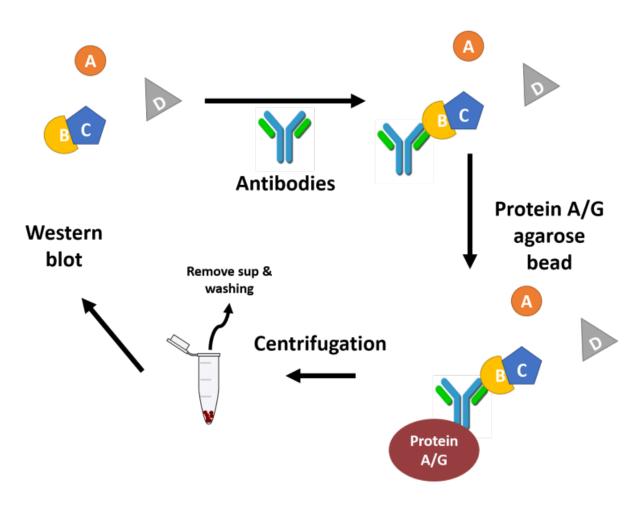
Proteins are denatured, reduced, alkylated, and digested into peptides. Peptides are, in some cases, fractionated to reduce complexity.



How does mass spectrometry work?



What are ways to isolate protein interactions?

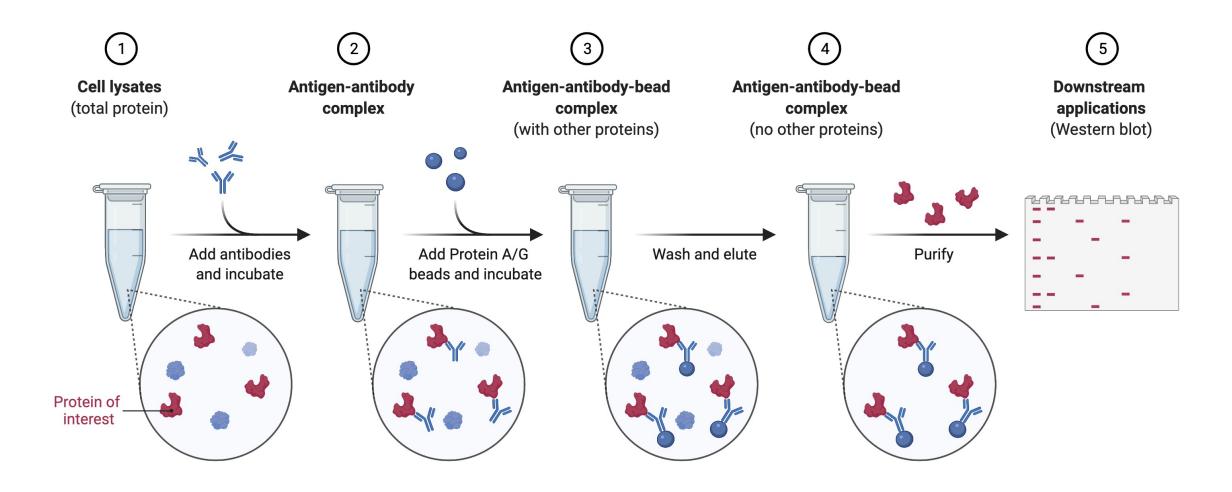


Immunoprecipitation (IP)

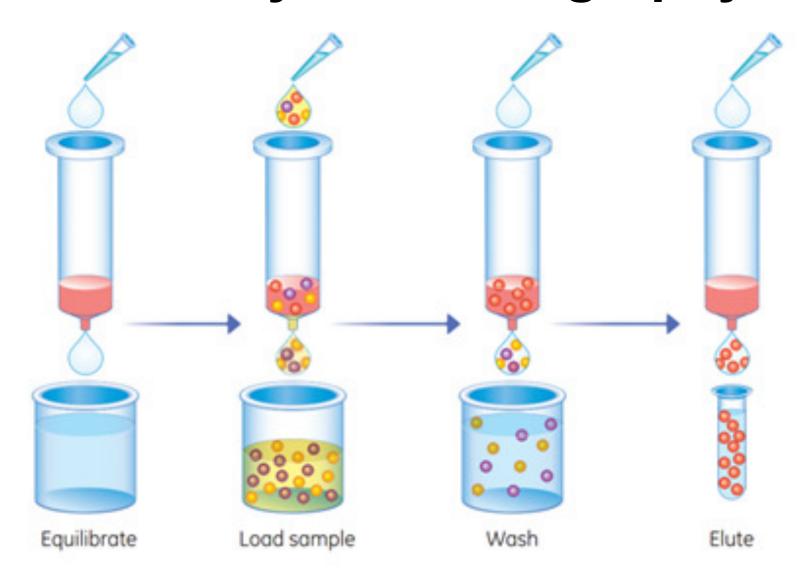


Affinity Chromatography

How does an immunoprecipitation experiment work?

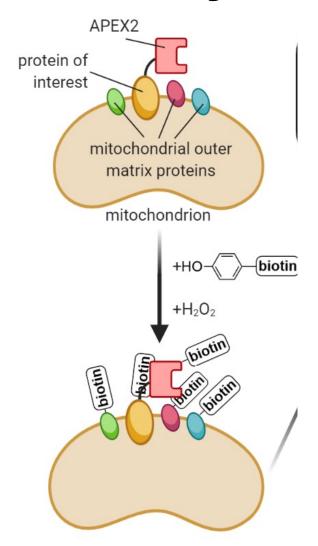


How does affinity chromatography work?



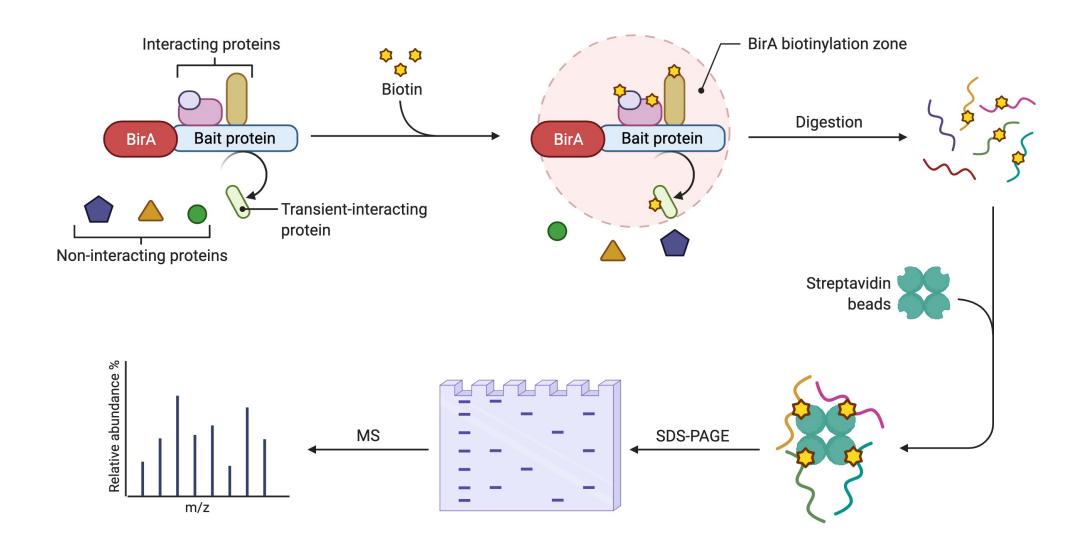
What in the past has limited the study of PPIs?





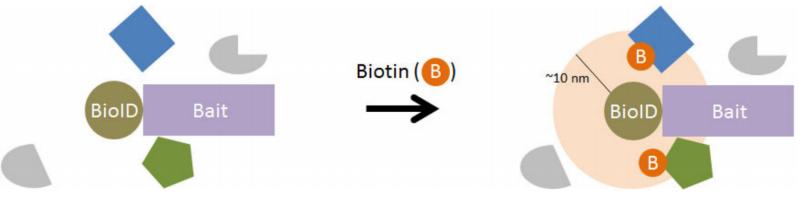
Constricted to high affinity proteins and nonphysiological conditions

What is proximity-dependent labeling?

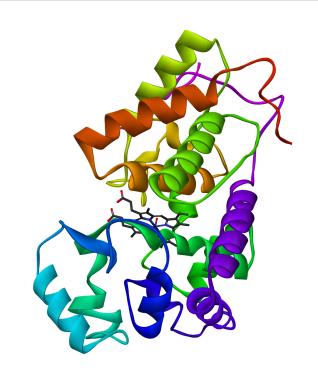


What are the two common proximity-dependent labeling methods?

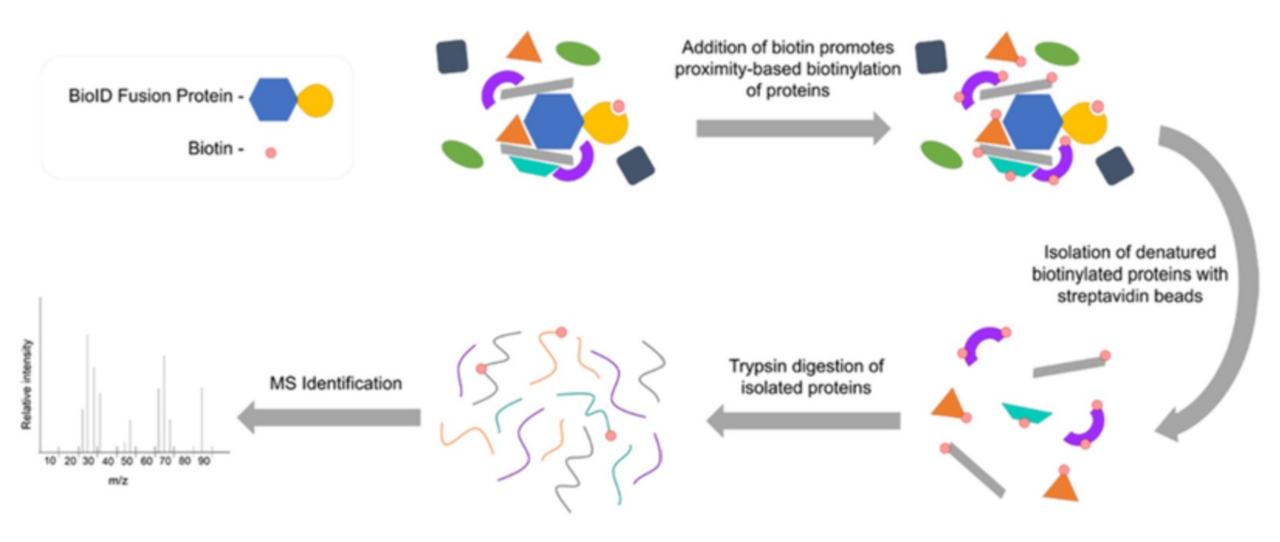
Biotin Identification (BioID)



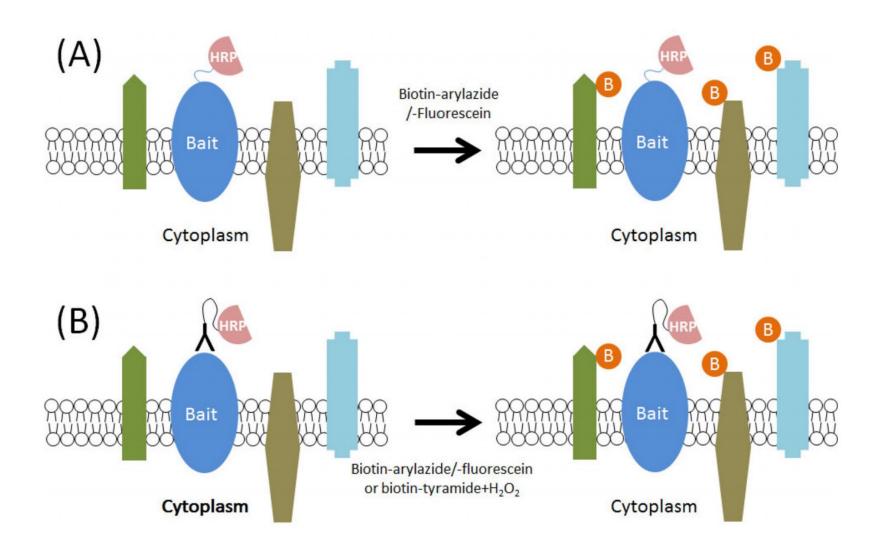
Horseradish Peroxidase (HRP)



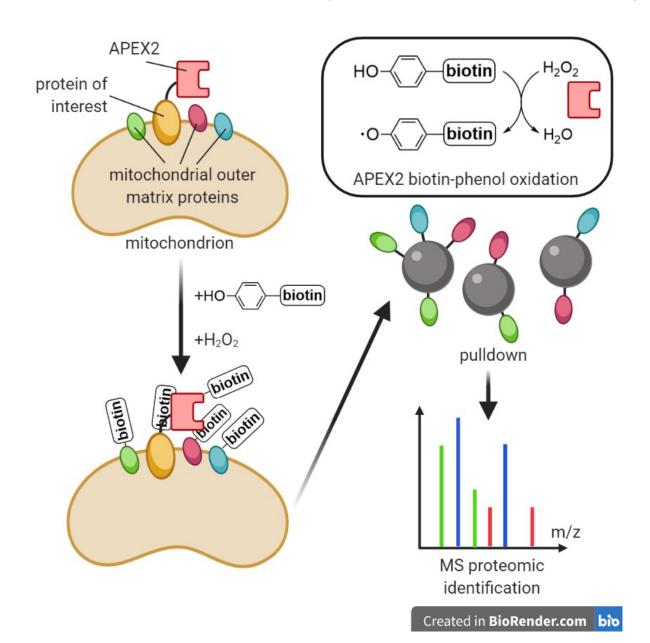
How does BioID work?



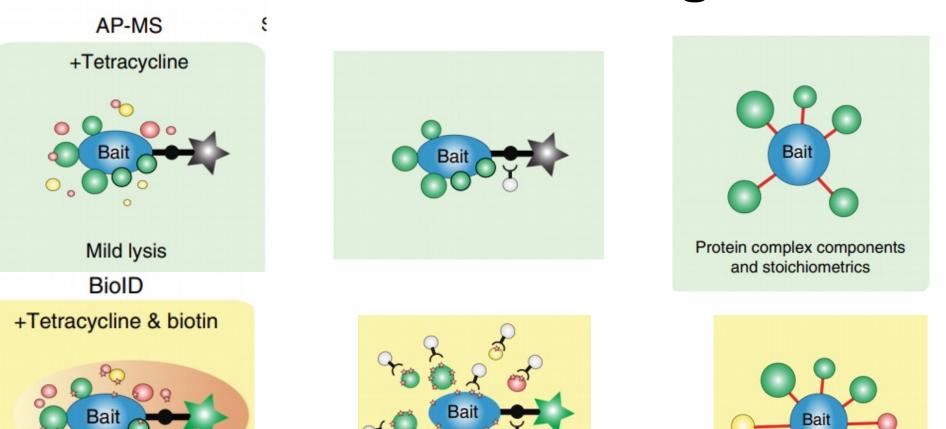
How does the HRP-based approach work?



What is an example of how proximity-dependent labeling can be used?



What is MAC-tag?



Combination of BioID and Affinity Purification-MS

Harsh lysis

Transient or proximal

interactions

What are the advantages and disadvantages of using these techniques?

Detects low affinity PPIs

Used in complex model systems



in vitro and in vivo



BioID cannot be used in secretory pathways

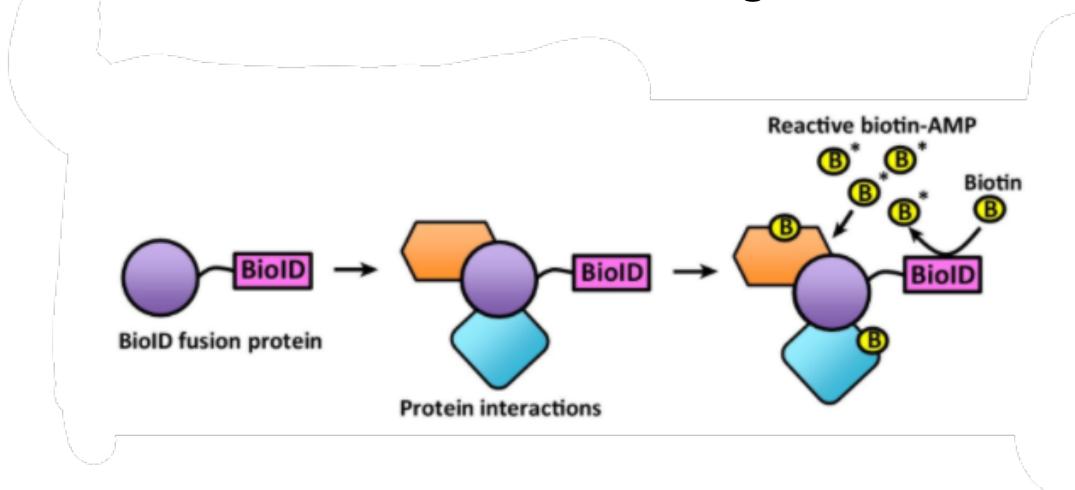
Time



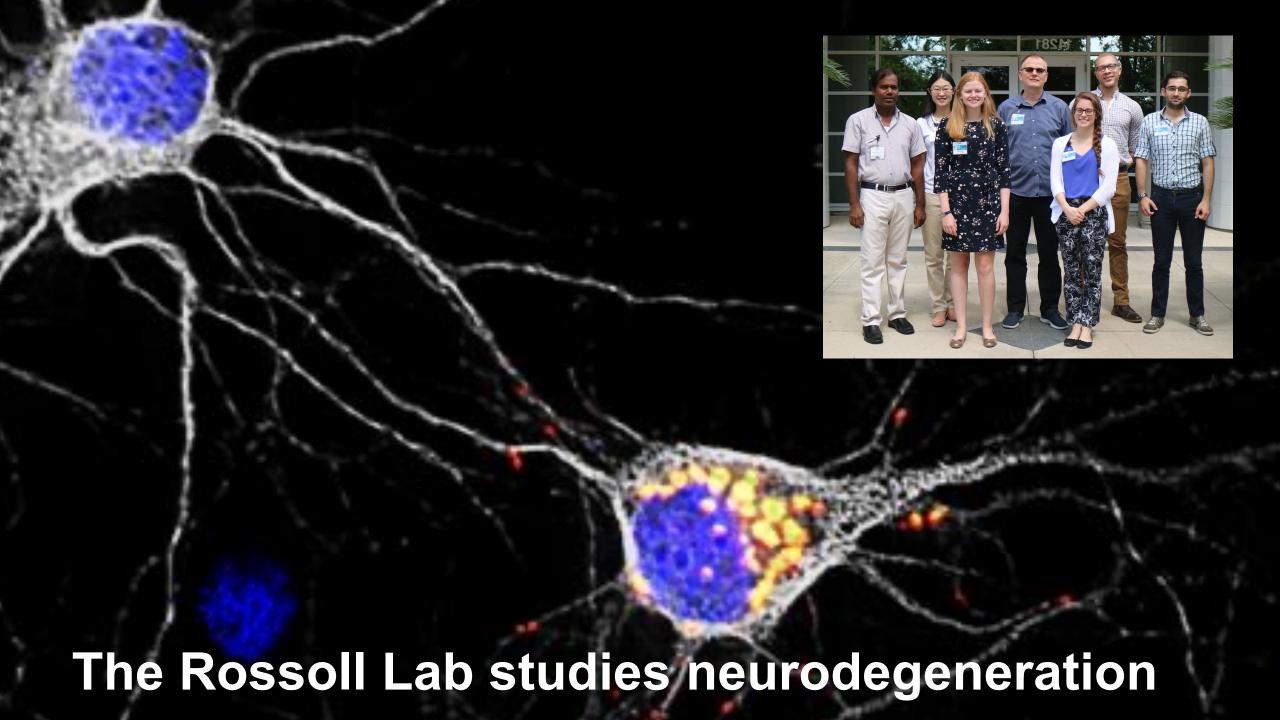
Advantages

Disadvantages

How can BioID be used in neurodegenerative research?



To study the proteome of TDP43 aggregates within the cytoplasm

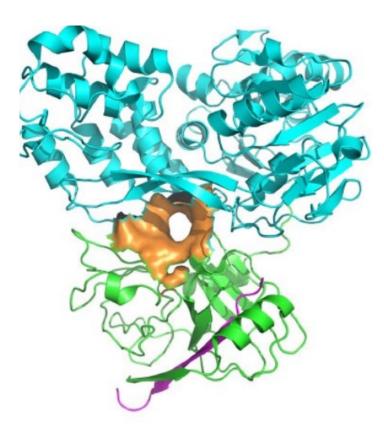


Review

Older PPI identification techniques have limitations in detecting low affinity proteins and replicating physiological conditions

Proximity-dependent labeling can overcome previous limitations by using techniques such as BioID and HRP

These new techniques can be used in researching neurodegenerative conditions



Questions?

References

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