

# **Protein-Protein Interaction**

Detection



#### What are Protein-Protein Interactions (PPIs)?



### Proteins do not typically function alone

## Why are PPIs important



Protein-protein interaction play a role in protein function which is important for research and the development of drugs

#### How are proteins identified?



### Affinity Purification (AP) is one way to isolate a protein



#### How else can you isolate a protein?



Immunoprecipitation is another protein purification strategy like AP

#### How does mass spectrometry (MS) work?



Affinity purification mass spectrometry (AP-MS) is a combination of the two techniques



### What are the limitations of these techniques?



Previous techniques are limited to high affinity proteins and can have false results

### What is Proximity dependent labeling?



Proximity dependant labeling using enzymes that produce reactive molecules to bind and label neighboring proteins

## What are different kinds of proximity dependent labeling?





Bir A\*, a mutated biotin ligase protein, is fused to protein of interest and incubated in biotin



After being incubated in biotin the interacting proteins are tagged, the closer the protein to the bait protein the more likely it is to be tagged with biotin

Denature proteins



The protein complex is exposed to harsh lysis which denatures the proteins

Denature proteins



Biotin affinity is used to capture the tagged proteins using beads

Denature proteins



# How does APEX – Based proximity dependant labeling work?



#### APEX, a peroxidase, is fused to the protein of interest

# How does APEX – Based proximity dependant labeling work?



Apex catalyzes the oxidation of biotin-phenol, leading to the biotinylation of nearby proteins

# How does APEX – Based proximity dependent labeling work?



Biotin labeled proteins are pulldown using beads and isolated

# How does APEX – Based proximity dependant labeling work?





### What is MAC-tag?

# MAC-tag is a combination of AP-MS and BioID pathways

# Why us Mac-Tag?

Advantages to Mac-Tag

In vivo and in vitro

Can be used in multiple model systems

Detects low affinity PPIs



#### What are disadvantages To Mac-Tag? Time

Can't be used in all pathways

Doesn't mean proteins interact, just that they are proximal to the bait protein



#### How can Mac-tag be used to study neurodegeneration?



Studying PPIs of TDP43 in the cytoplasm may help us understand the pathway

#### The Rossoll Lab uses PPIs to study neurodegeneration



# Summary

There are many different ways to look at protein-protein interactions

Older techniques are less frequently used because they face limitations and can only look at high affinity PPIs

New techniques like proximity dependant labeling can better look at low affinity interactions and overcome other previous limitations

Proximity dependant techniques like BioID can be combined with APMS in new techniques like Mac-tag



### References

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