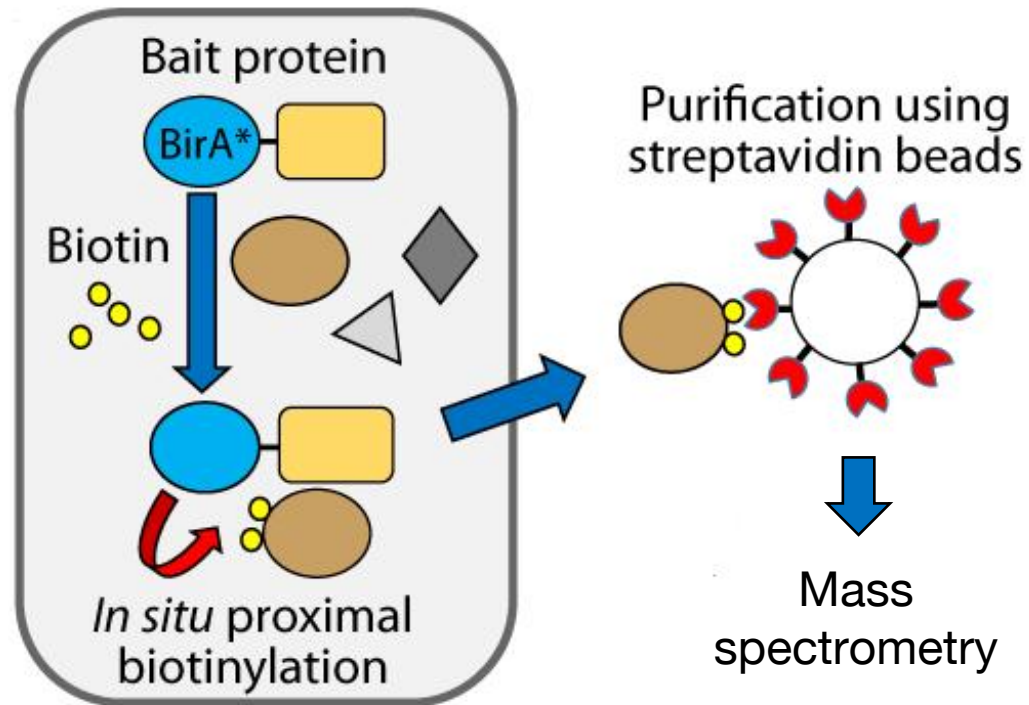


# BioID: proximity-dependent Biotin IDentification

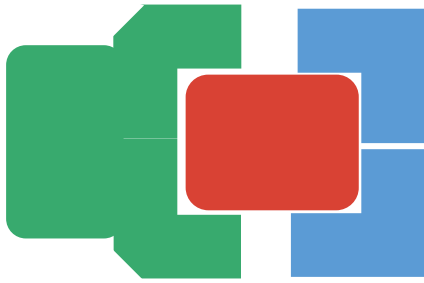


**Technique to study the potential interactome of a protein of interest**

Substantial use in the last 10 years

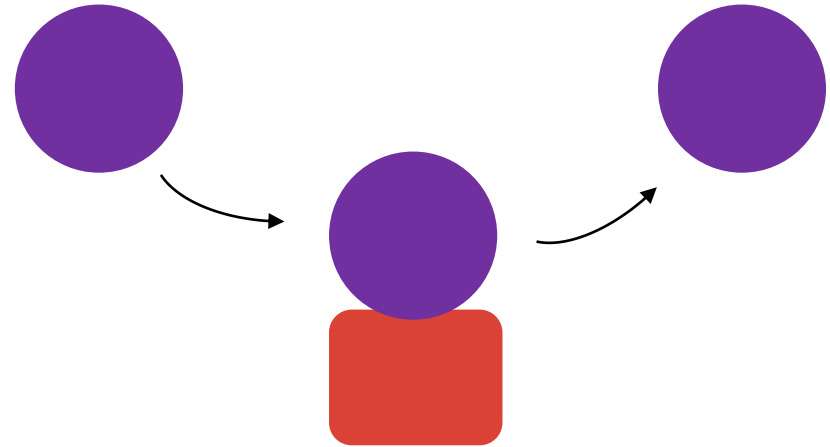
# Protein-protein interactions

To truly understand the functions of a protein one must explore its interactions



**Stable interactions**

Multi-subunit complexes

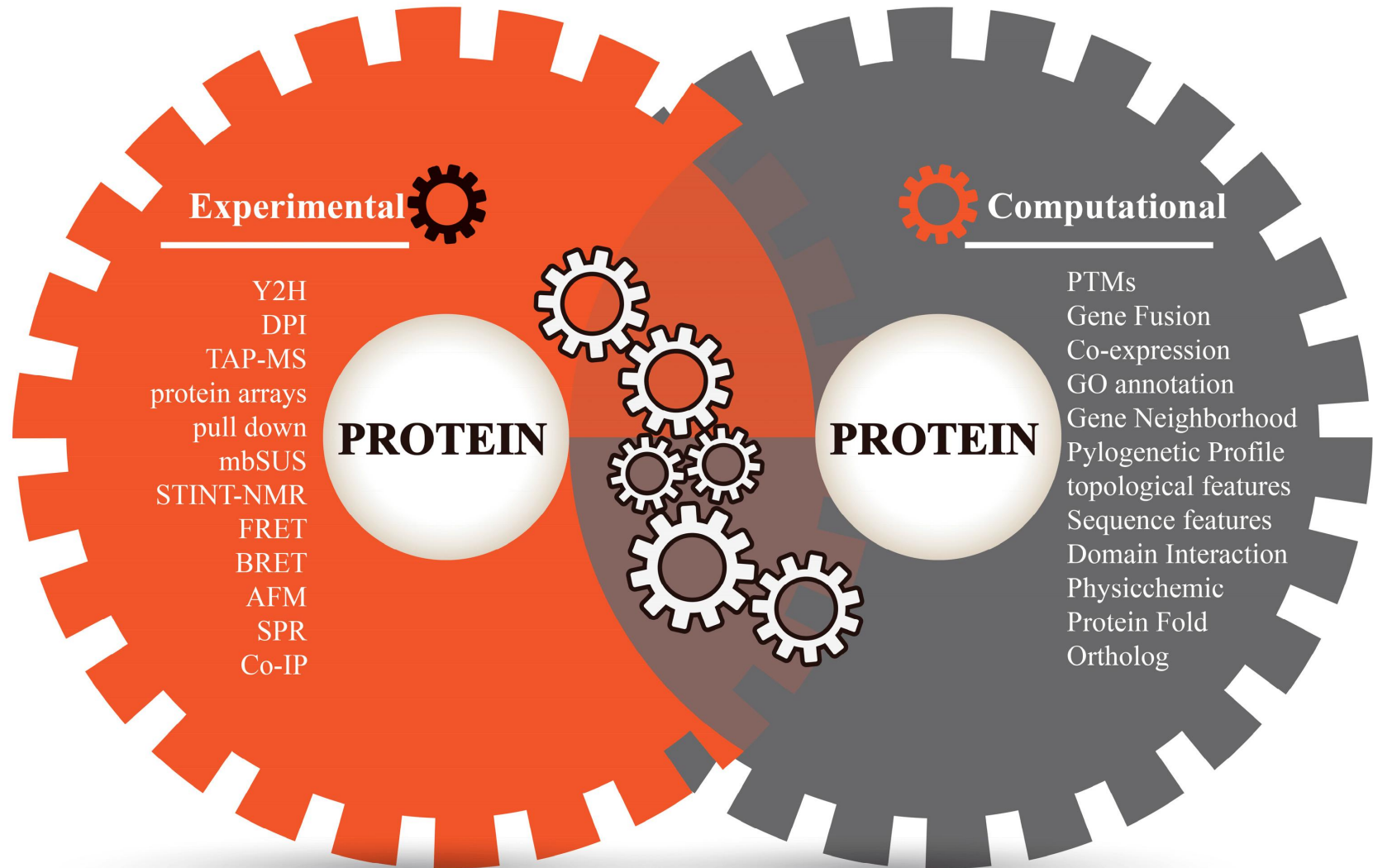


**Transient interactions**

Temporary

**Different methods exist to study protein-protein interactions**

# Methods to explore protein-protein interactions



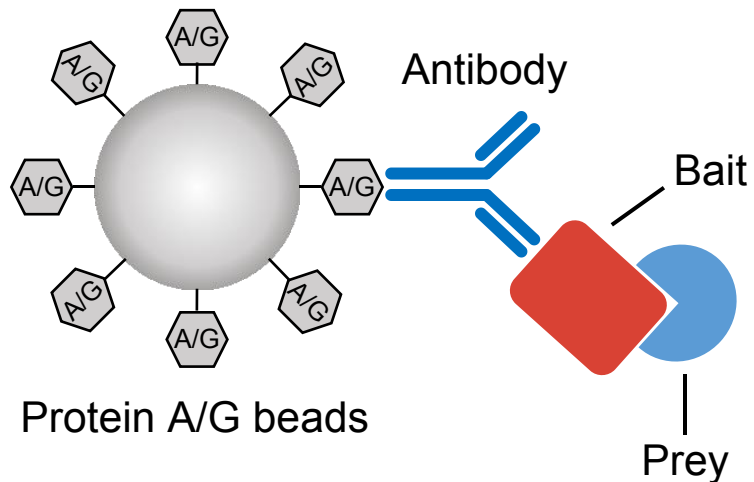
# Candidate approach vs exploratory approach

## Candidate approach

Requires knowledge of proteins to observe

Co-IP or Pulldown  
+  
Immunoblot against proteins

## Co-immunoprecipitation



# Candidate approach vs exploratory approach

## Candidate approach

Requires knowledge of proteins to observe

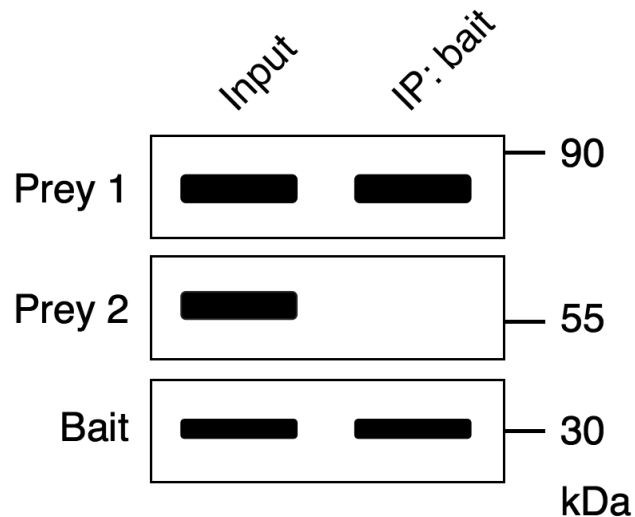
Co-IP or Pulldown  
+  
Immunoblot against proteins

## Exploratory approach

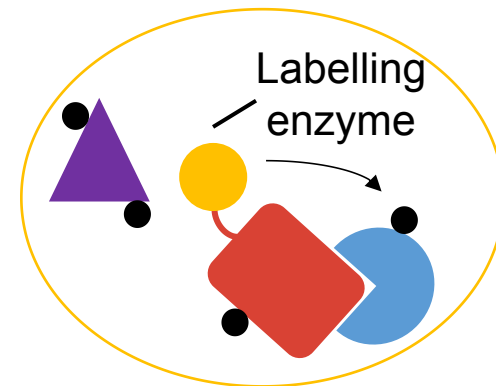
No prior knowledge of proteins required

Mass spectrometry-based

## Co-immunoprecipitation



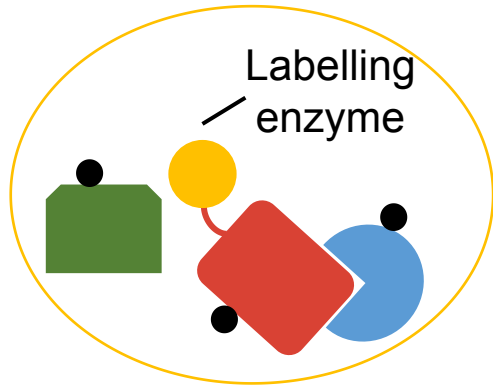
## Proximity labelling approaches:



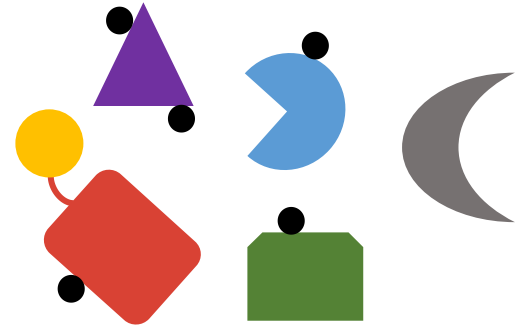
- Rare/absent tag in cells
- Tag should facilitate protein recovery

# Key steps of proximity labelling proteomics

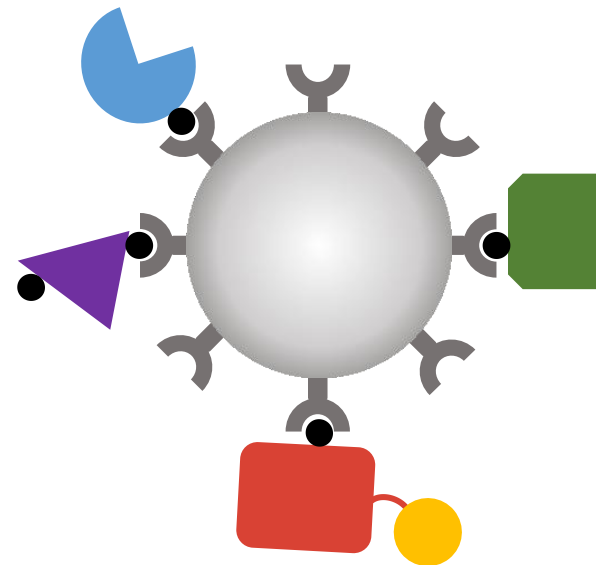
In cellulae:



Cell lysis

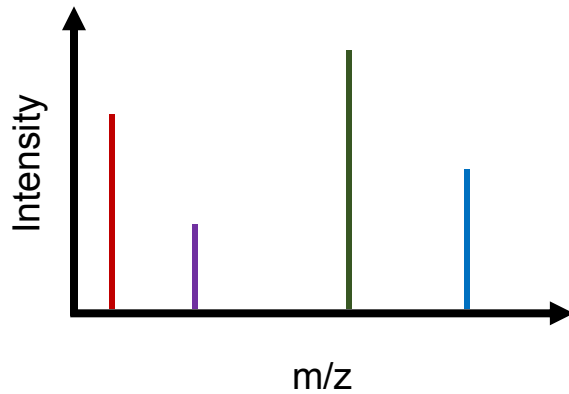


Recovery of tagged proteins



Elution


Identification by MS



Tools | March 12 2012

# A promiscuous biotin ligase fusion protein identifies proximal and interacting proteins in mammalian cells

In Special Collection: JCB65: Methods

Kyle J. Roux , Dae In Kim, Manfred Raida, Brian Burke

+ Author and Article Information

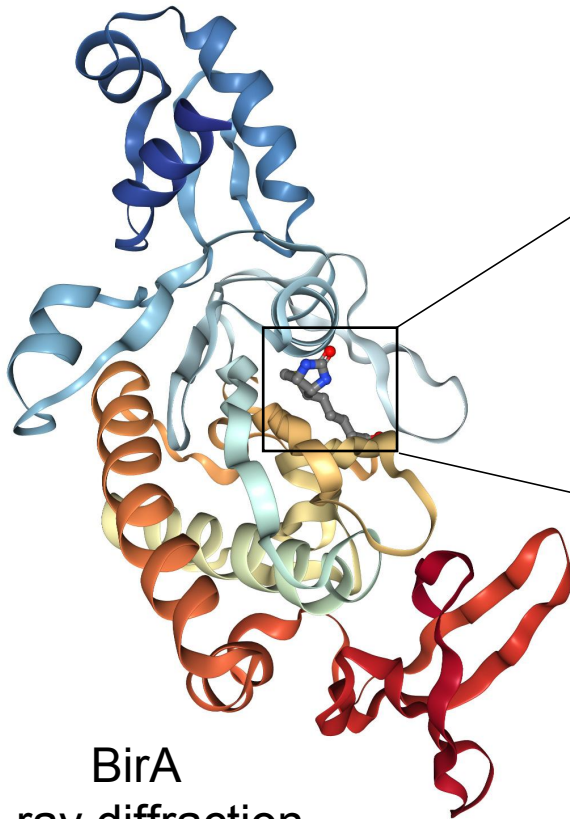


*J Cell Biol* (2012) 196 (6): 801–810. | <https://doi.org/10.1083/jcb.201112098> | **Article history** 

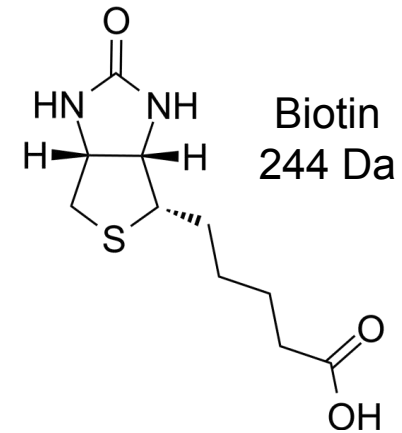
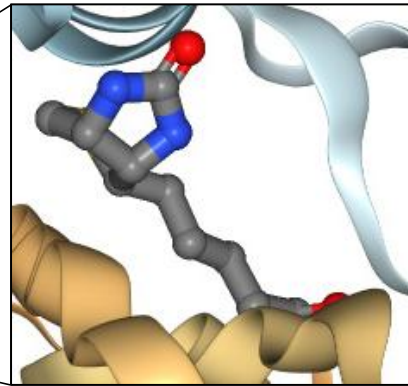
1. Identification of a promiscuous biotin ligase (BirA\*) in *E. coli*
2. Fusion of BirA\* to protein of interest -> proximal biotinylation (rare modification)
3. Pulldown of biotinylated proteins (streptavidin beads) -> Mass spectrometry
4. Generation of “interaction” network

# Background on the BirA biotin ligase

BirA, 35 kDa, biotin ligase in *E. coli* that has its own specific target



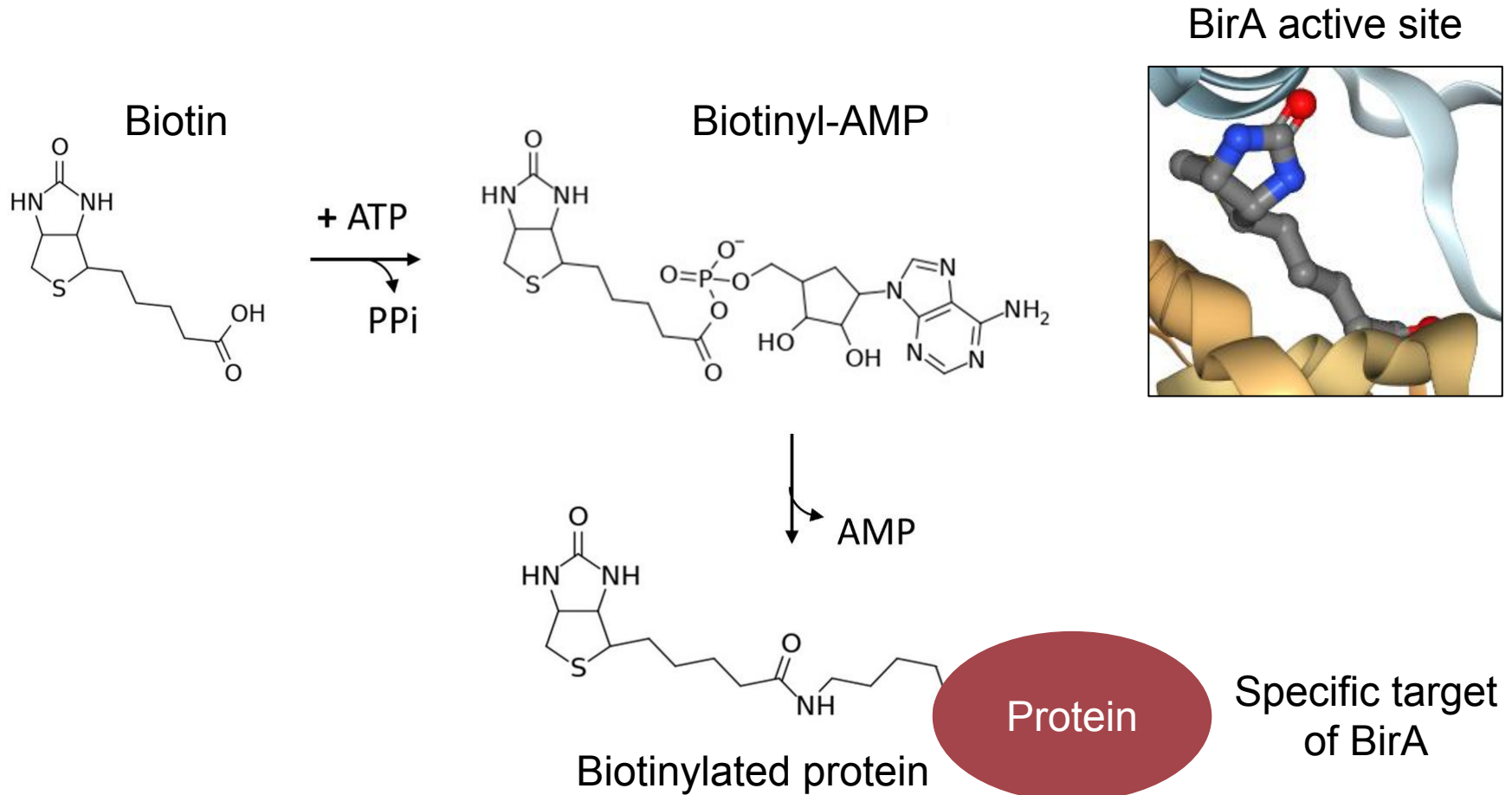
BirA  
X-ray diffraction  
(PDB: 1HXD)



**Biotinylation occurs through a two step reaction**



# Mechanism of action of BirA wild-type

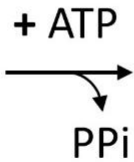
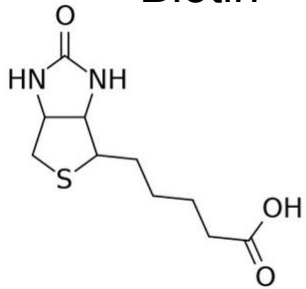


**Specific biotinylation of a target protein**

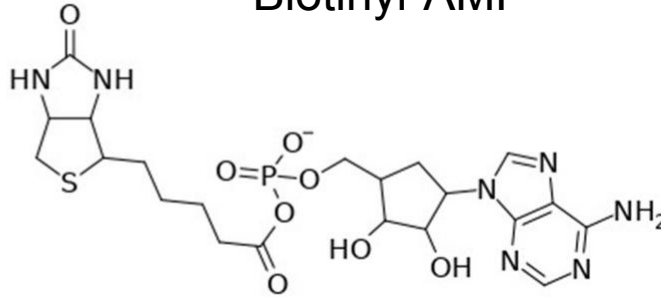
**Identification of mutant BirA (Arg118Gly), releases biotinyl-AMP prematurely**

# Mechanism of action of BirA mutant (BirA\*)

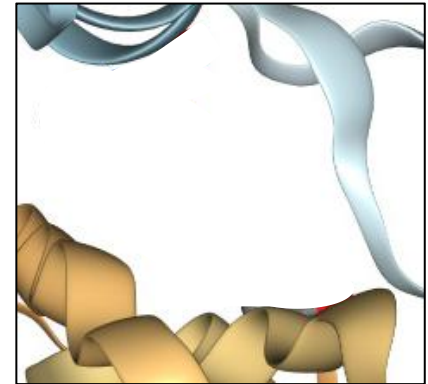
Biotin



Biotinyl-AMP



BirA\* active site



↓  
Release

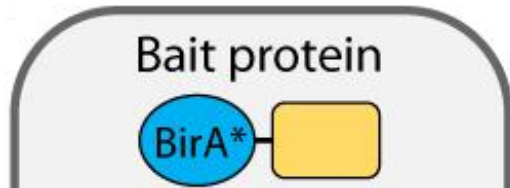
Highly reactive reaction intermediate

Biotinylation of primary amines (N-term and lysines)

**Promiscuous biotinylation of neighbouring proteins**

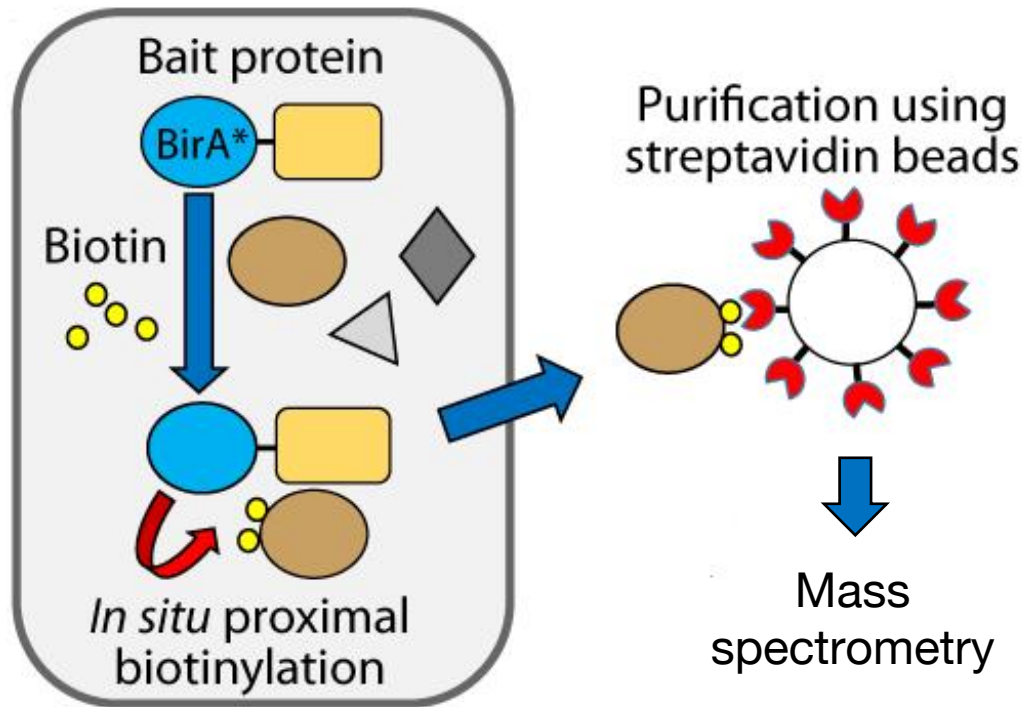
# BioID: proximity-dependent Biotin IDentification

Fusion of protein of interest with BirA\*  
Overexpression in cells



# BioID: proximity-dependent Biotin IDentification

Fusion of protein of interest with BirA\*  
Overexpression in cells



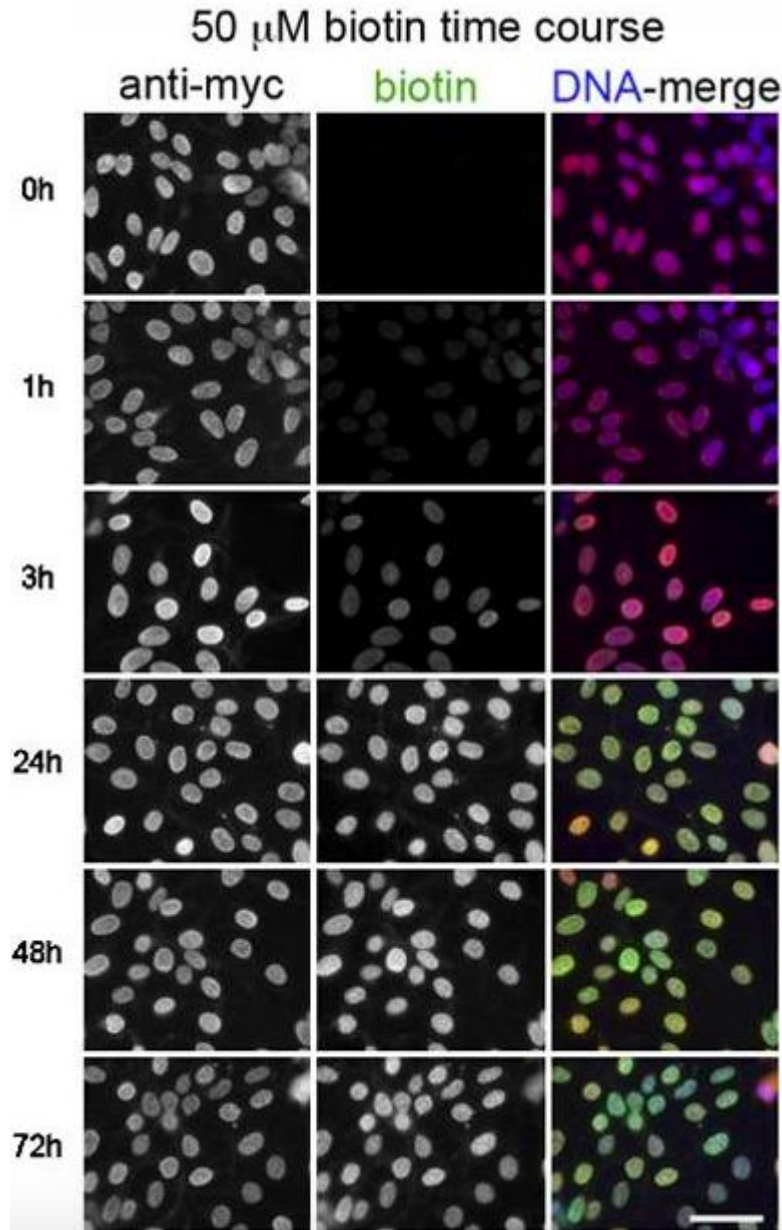
Biotinylation radius: 10-15 nm

## Controls to do:

- Functional fusion protein
- Functional BirA\*
- Similar or lower expression compared to endogenous protein
- Stable cell line is recommended (more uniform expression levels)
- Use BirA\*-only cell line as a control

**What do we expect to see during a BioID experiment?**

# BioID experiment: 18-24h of biotin incubation



Cells expressing Myc-BirA\*-Protein

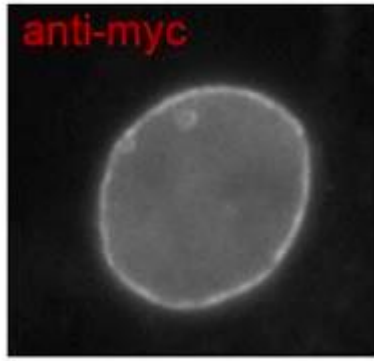
50  $\mu$ M biotin in cell culture medium

Maximal biotinylation after 24h

## Drawback

Long labeling time may hinder the study of protein interactions during transient biological processes

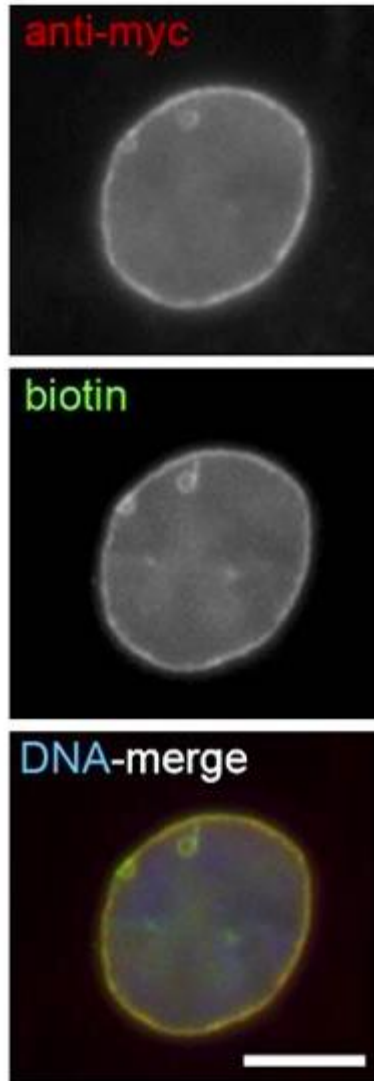
# Biold experiment: checking the localisation and activity of BirA\* fusion



## Myc-BirA\*-Lamin A

Lamin A is found at the nuclear periphery

# BioID experiment: checking the localisation and activity of BirA\* fusion



## Myc-BirA\*-Lamin A

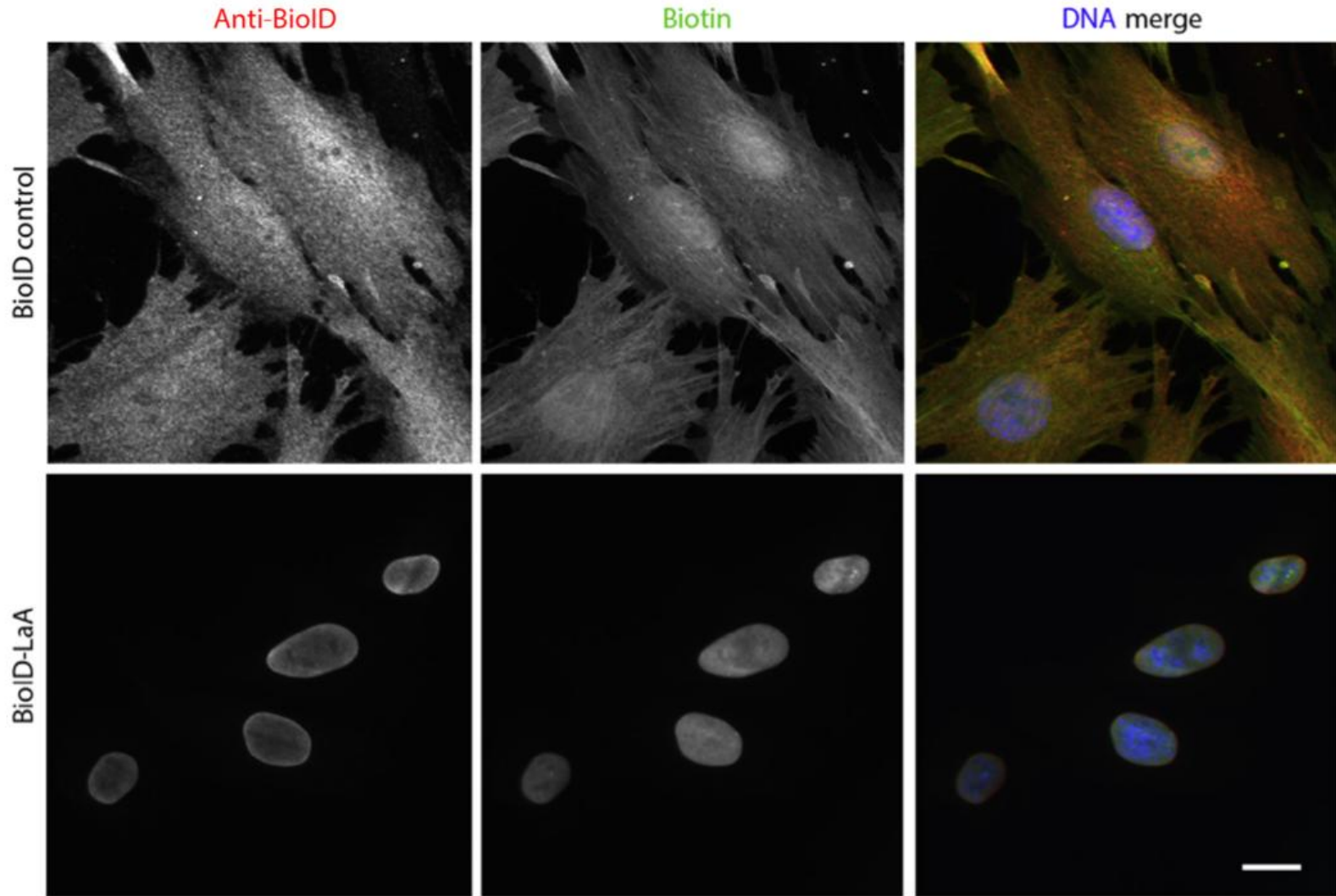
Lamin A is found at the nuclear periphery

Biotin signal is detected at the nuclear periphery, good indication that BirA\*-Lamin A fusion protein is functional

Proceed with the BioID experiment:

- Myc-BirA\*-Lamin A cells
- Myc-BirA\*-only cells (background)

# BioID experiment: the importance of the BirA\*-only cell line

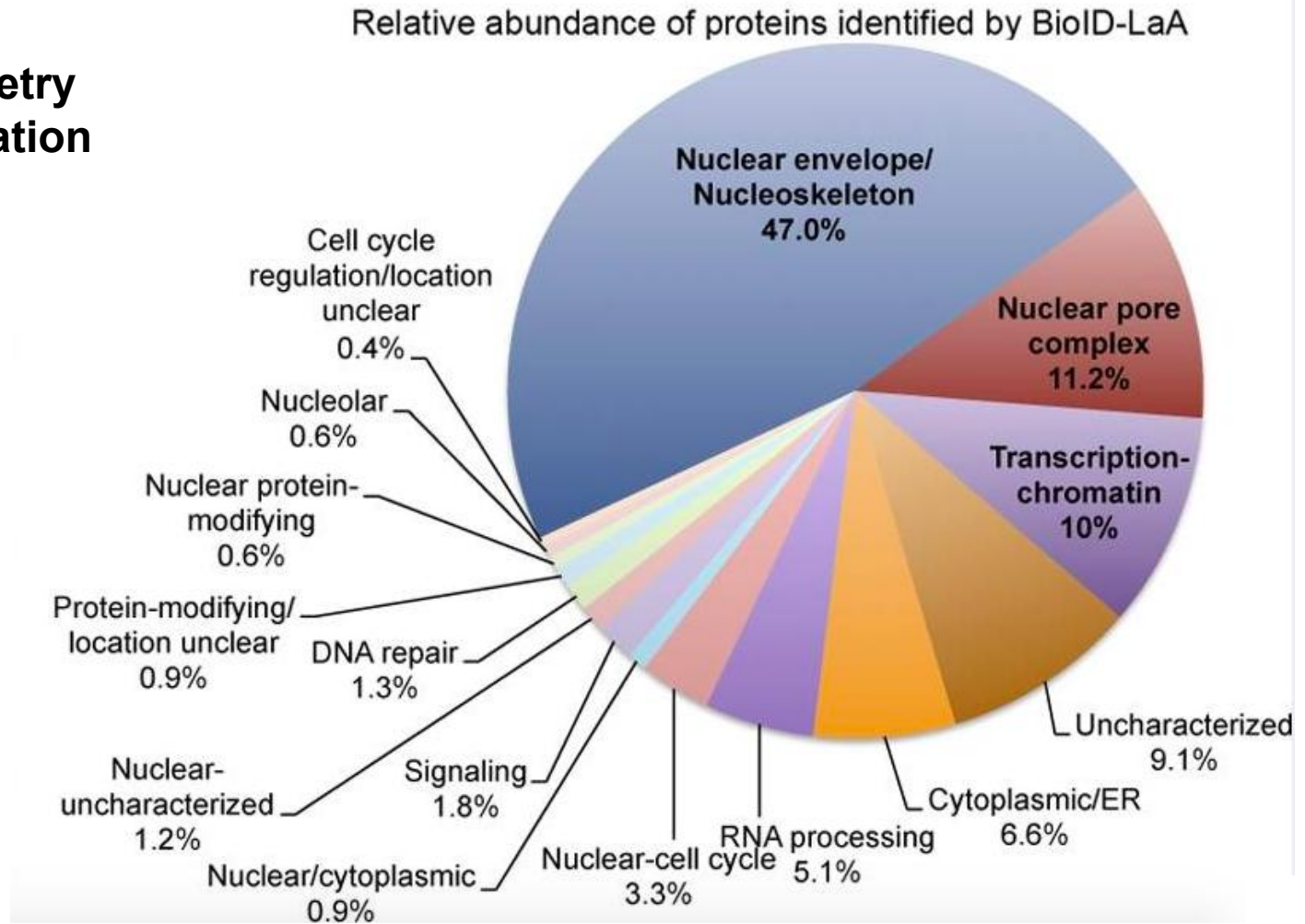
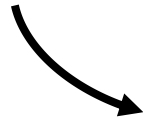


**Necessary to use a BirA\*-only cell line to account for background biotinylation**



# BioID: an exploratory proximity labelling approach

**Pulldown +  
mass spectrometry  
protein identification**



**Exploratory approach that generates a history of protein associations  
(be it interactions or not)**

# Biold: considerations

## Advantages

- Easy to label and recover proteins
- Identification of multiple “interactions”
- Recovery of transient interactions

## Disadvantages

- Proximity  $\neq$  interaction -> validation
- Amount of biotinylated protein  $\neq$  strength or abundance of interaction
- BirA\* fusion may alter localisation/function
- Long labeling time

**Biold2:** smaller version of biotin ligase from *Aquifex aeolicus* that requires less biotin

Proximity labelling enzyme	Biold	APEX2	Turbold/miniTurbo
Substrates	biotin, ATP	biotin phenol, H <sub>2</sub> O <sub>2</sub>	biotin, ATP
Labelling time	18-24 hours	0.3-1 minute	10 minutes

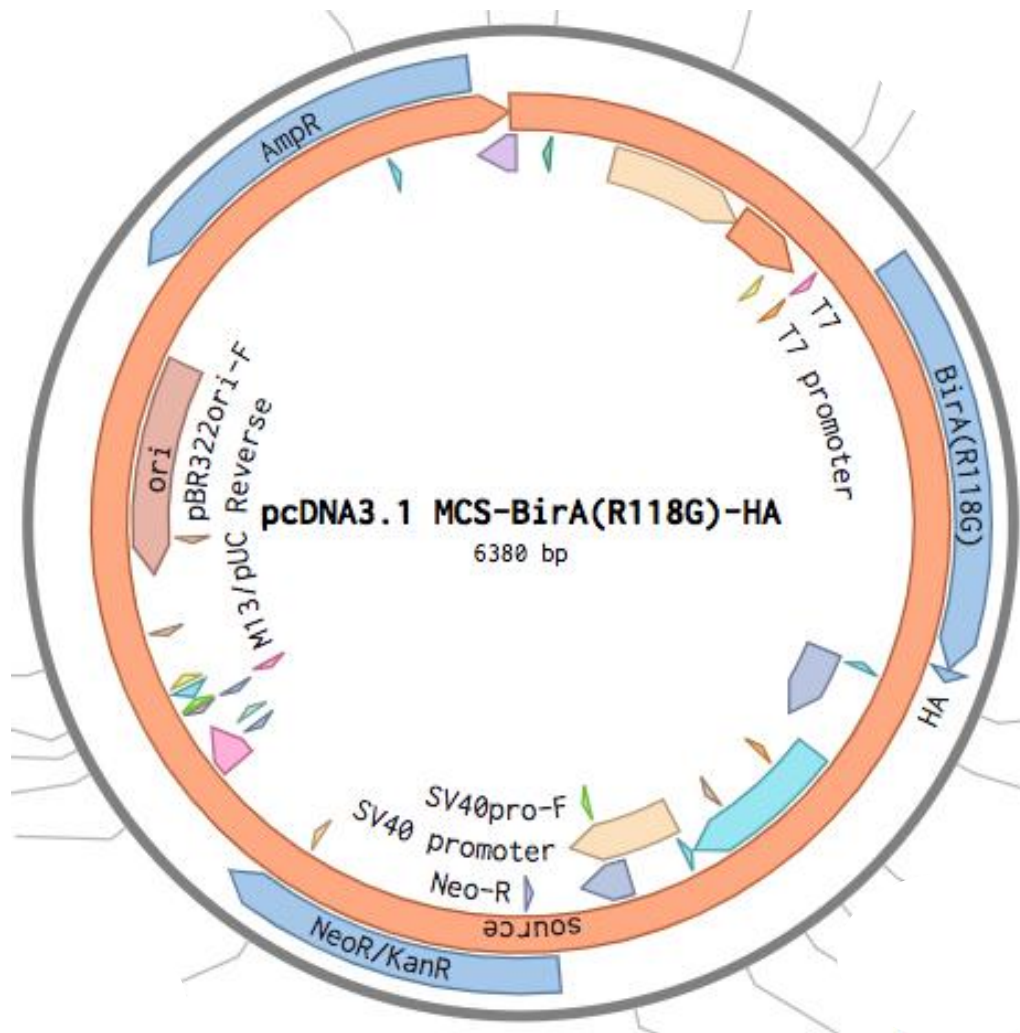
### **APEX2**

Modified ascorbate peroxidase  
Potentially toxic substrates

### **Turbold/miniTurbo**

Mutated BirA\* to be faster  
No toxic substrates

# Biold: accessibility to the scientific community



## BirA\* plasmid from Addgene

For fusion to the C-terminal of a protein of interest

More than 100 requests

# Papers on BioID

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- Roux KJ, Kim DI, Raida M, Burke B. **A promiscuous biotin ligase fusion protein identifies proximal and interacting proteins in mammalian cells.** J Cell Biol. 2012;196(6):801-810. doi:10.1083/jcb.201112098
- Sears RM, May DG, Roux KJ. **BioID as a Tool for Protein-Proximity Labeling in Living Cells.** Methods Mol Biol. 2019;2012:299-313. doi:10.1007/978-1-4939-9546-2\_15