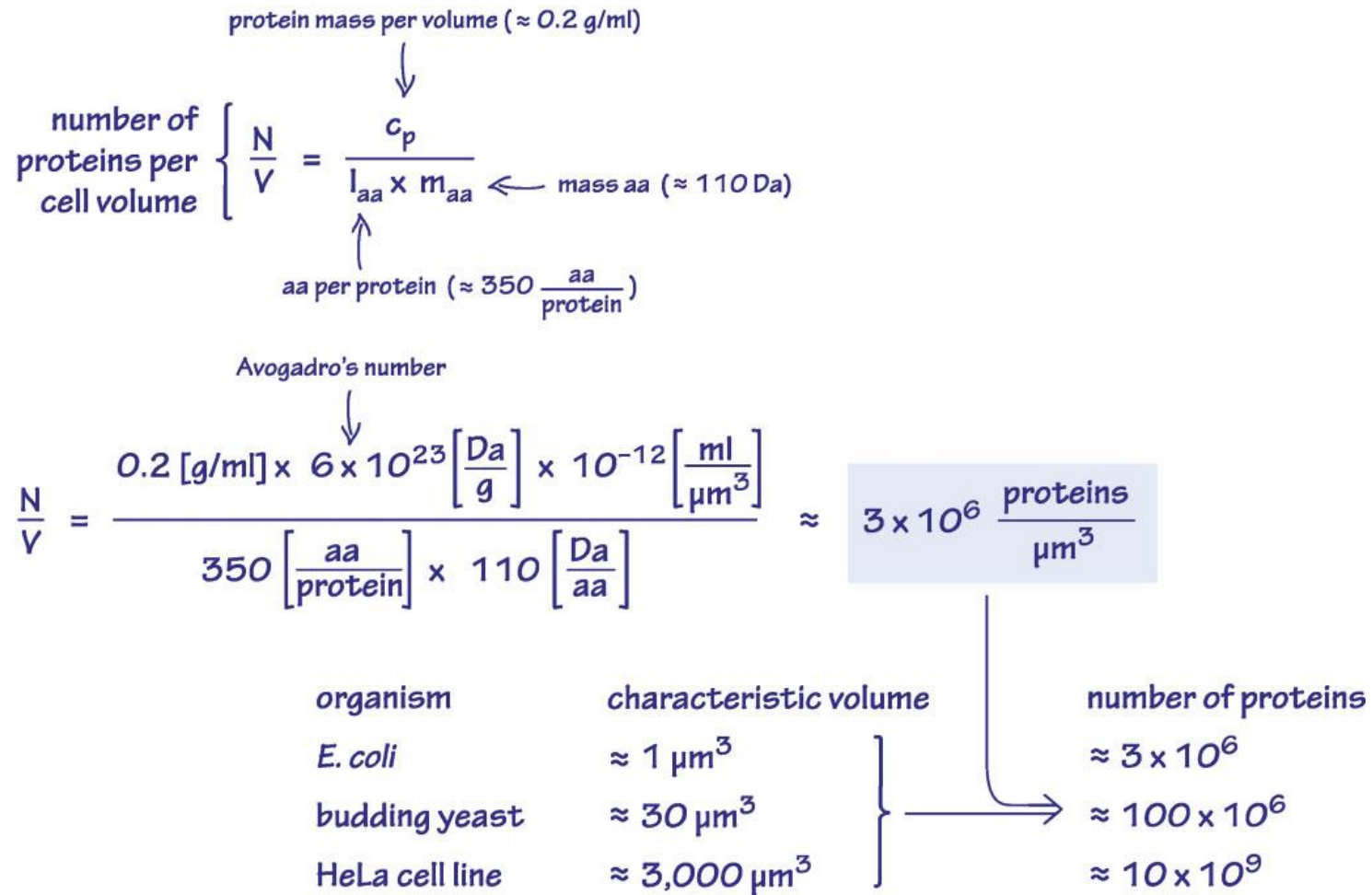


Proximity Labeling: A Powerful Tool for Protein Complex Purification and Proteomic Mapping

Liu Wenjuan
2021.03.04

estimating the number of proteins per cell volume



Bioessays_2013_1050-1055

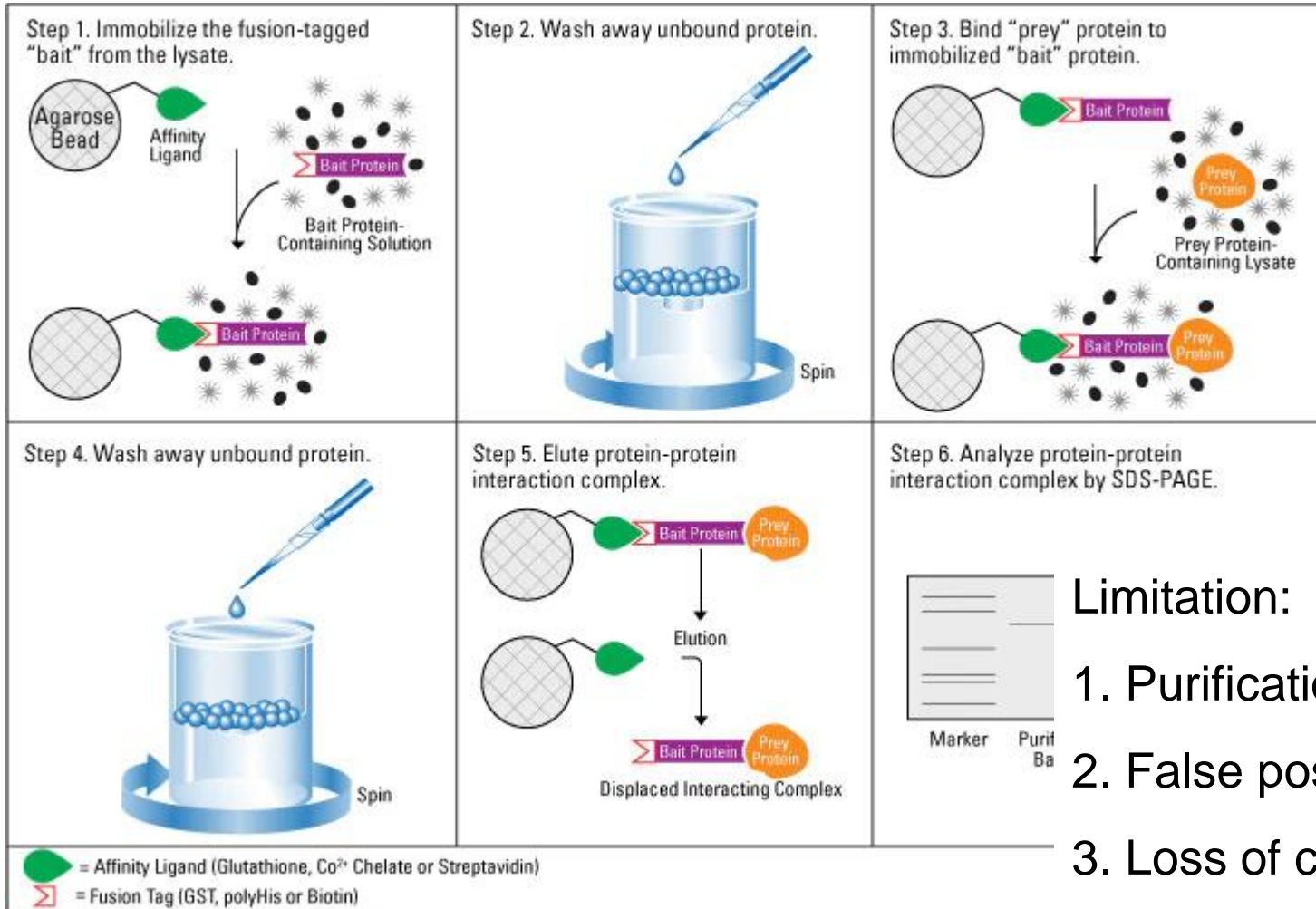
1. Subcellular regions located

2. Interactional

→ Identification of interacting proteins within spatially defined cellular domains is key

Traditional:

Antibody based affinity purification and mass spectrometry approaches



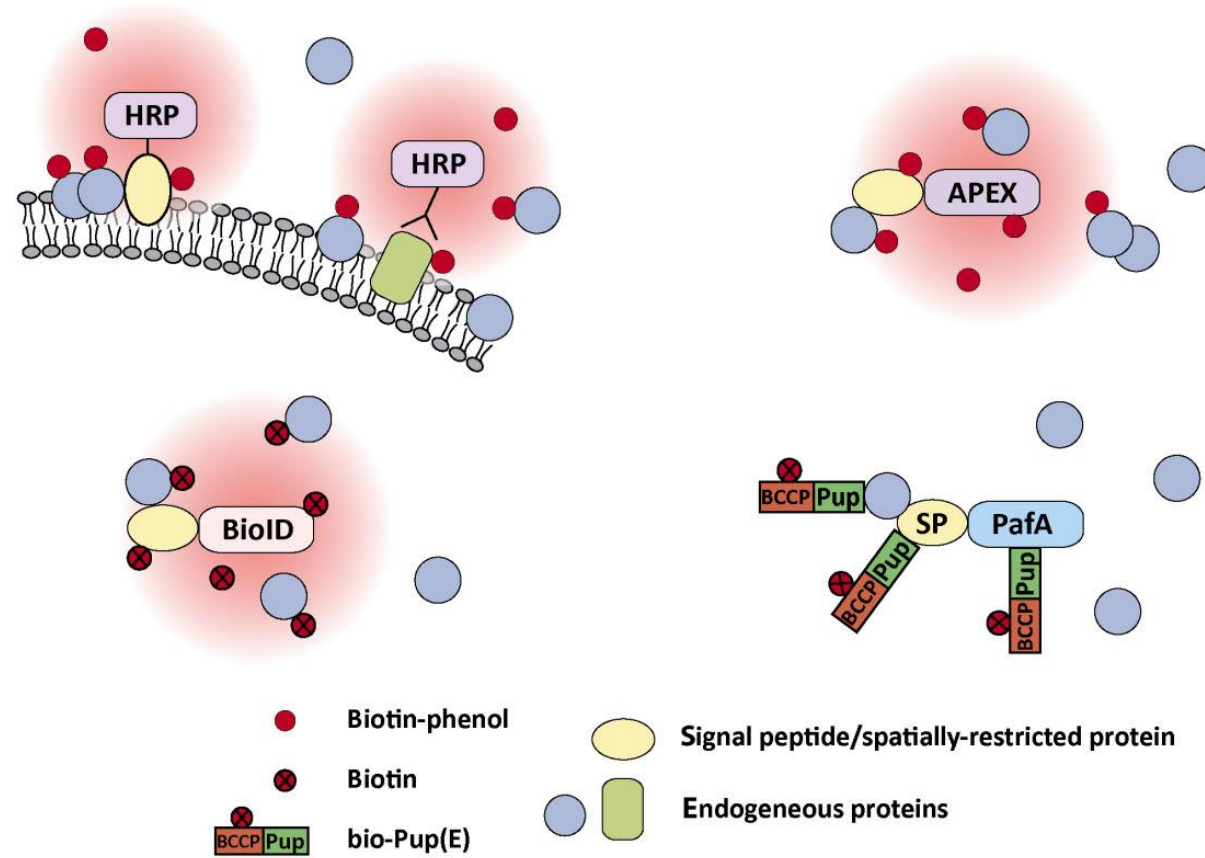
Limitation:

1. Purification methods
2. False positives introduced by cellular disruption
3. Loss of components caused by disruption
4. Discrete cellular regions cannot be purified by centrifugation

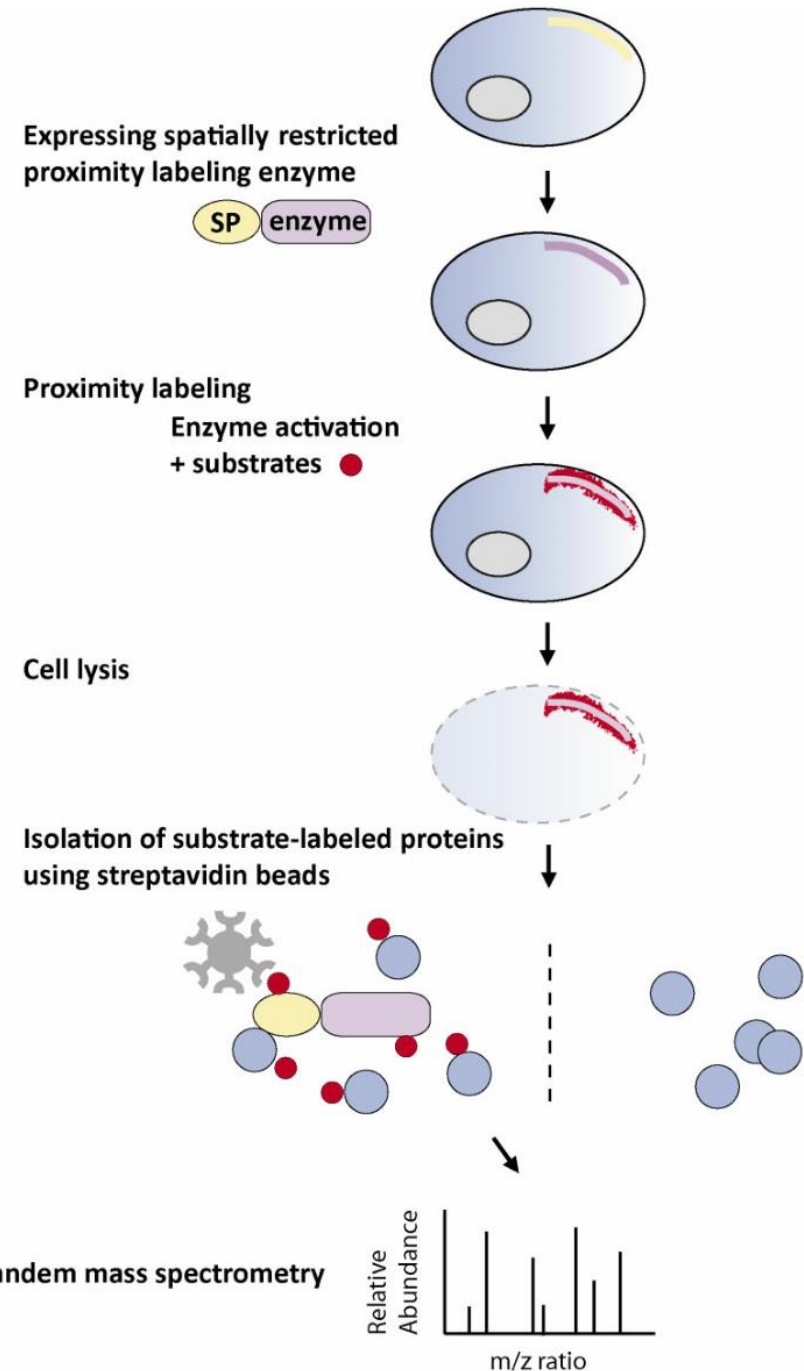
Now:

Proximity labeling

Enzymes catalyze the conversion of a substrate into a reactive radical that covalently tags neighboring proteins



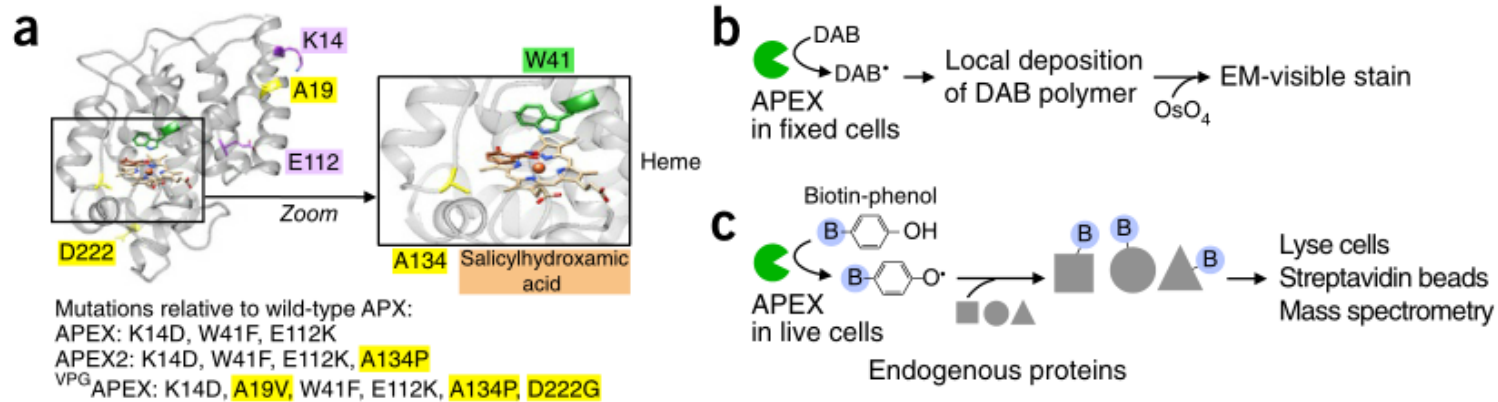
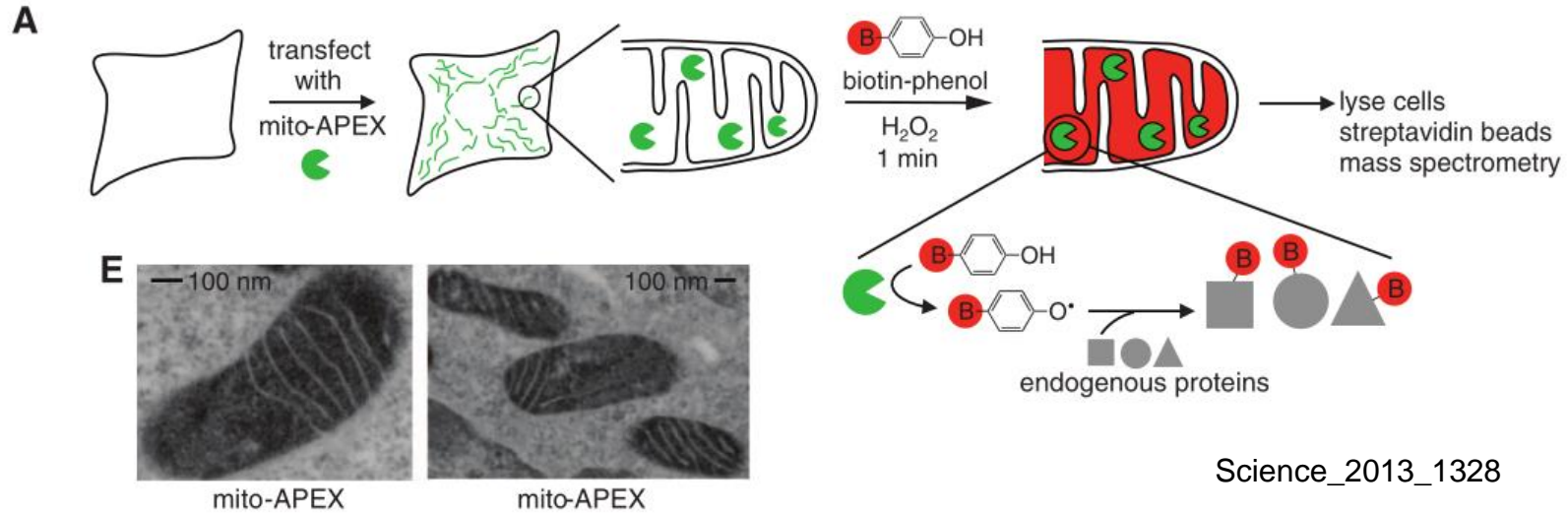
	HRP	APEX	BioID	Pup-IT
Enzymatic Activity	Peroxidase	Peroxidase	Biotin ligase	Pup ligase



Key Role: Ascorbate peroxidase

基于过氧化物酶催化二氨基联苯胺 (Diaminobenzidine, DAB) 染色标记方法是在过氧化氢 (H_2O_2) 存在的条件下, 过氧化物酶可以催化DAB形成DAB 聚合物, 进而被 OsO_4 染色实现标记。

HRP, APEX



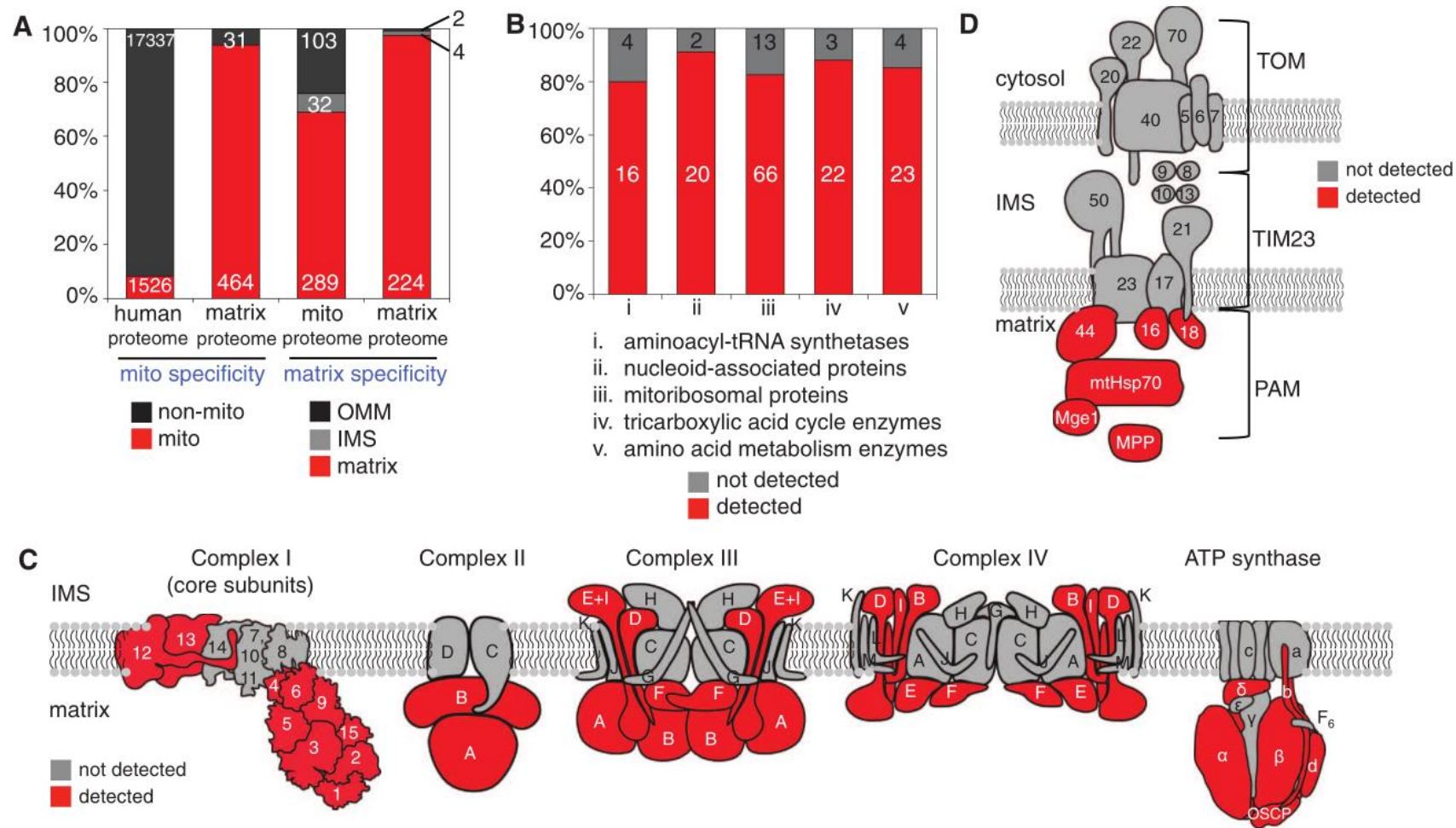
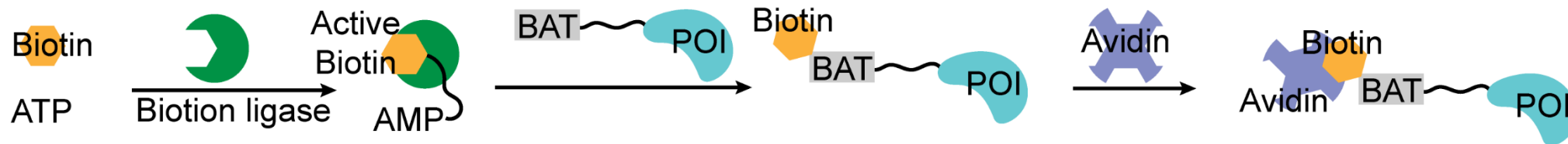


Fig. Specificity and depth of coverage of the mitochondrial matrix proteome.

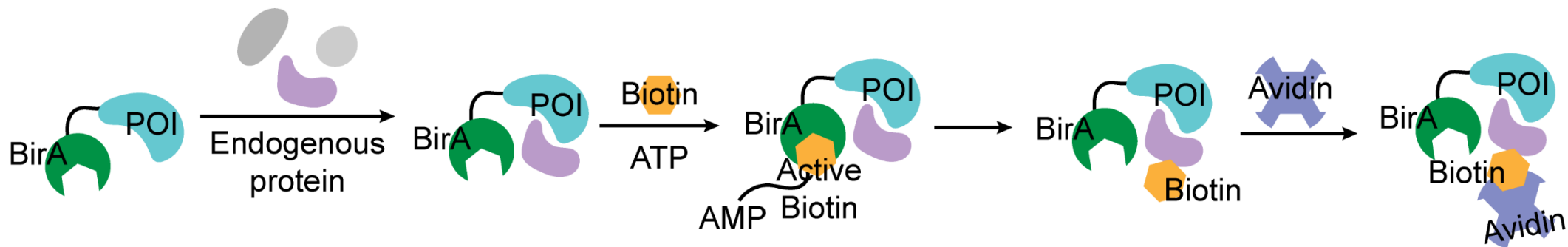
Limitation:

1. Low sensitivity
2. Toxicity of Biotin-phenol reagent

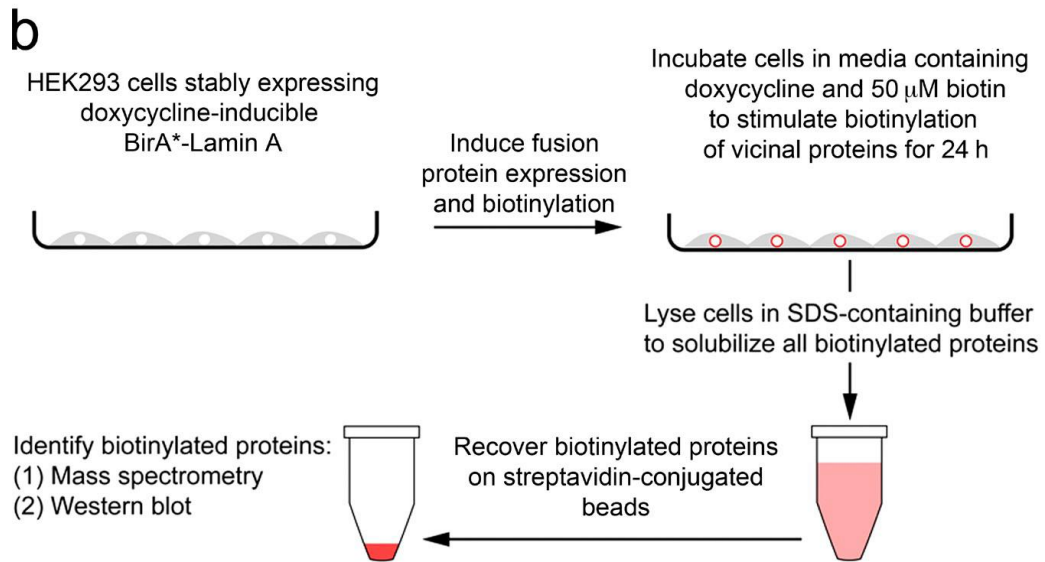
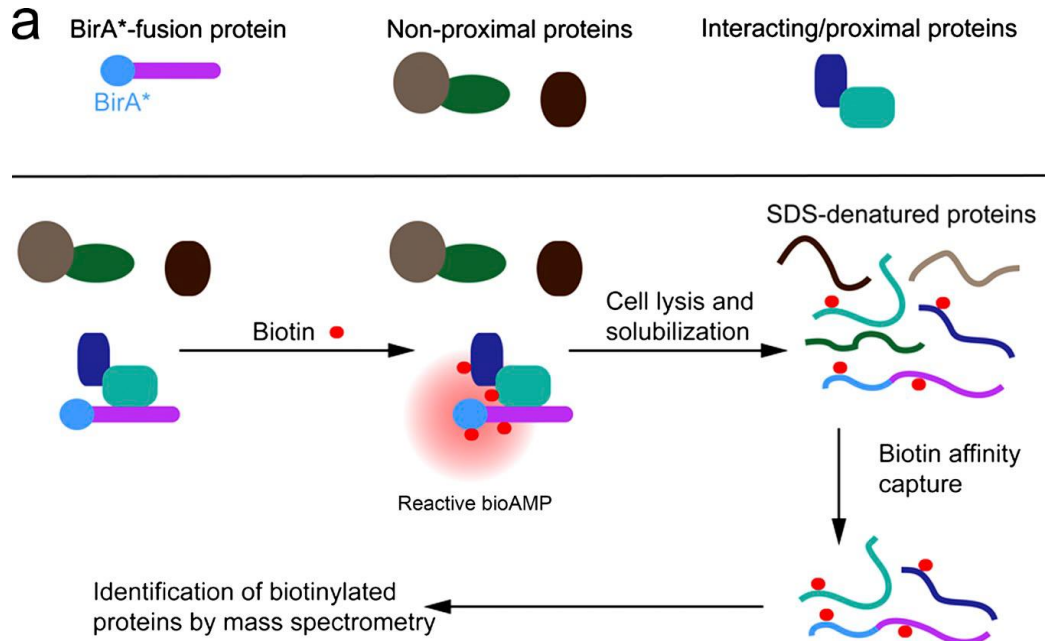
Key Role: Biotin ligase



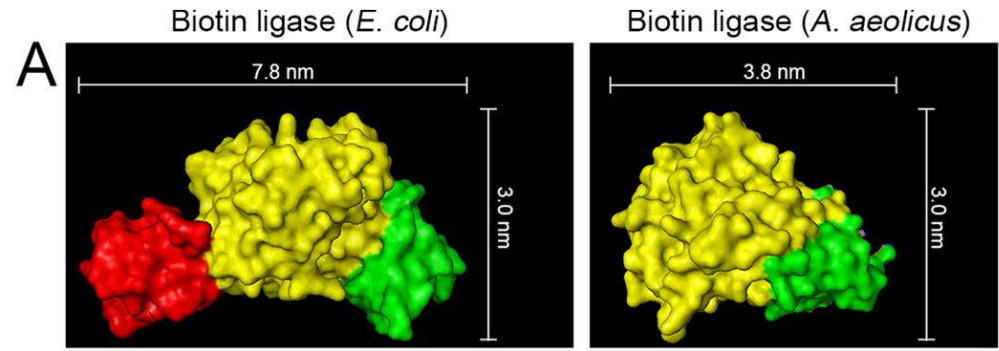
BioID



BioID

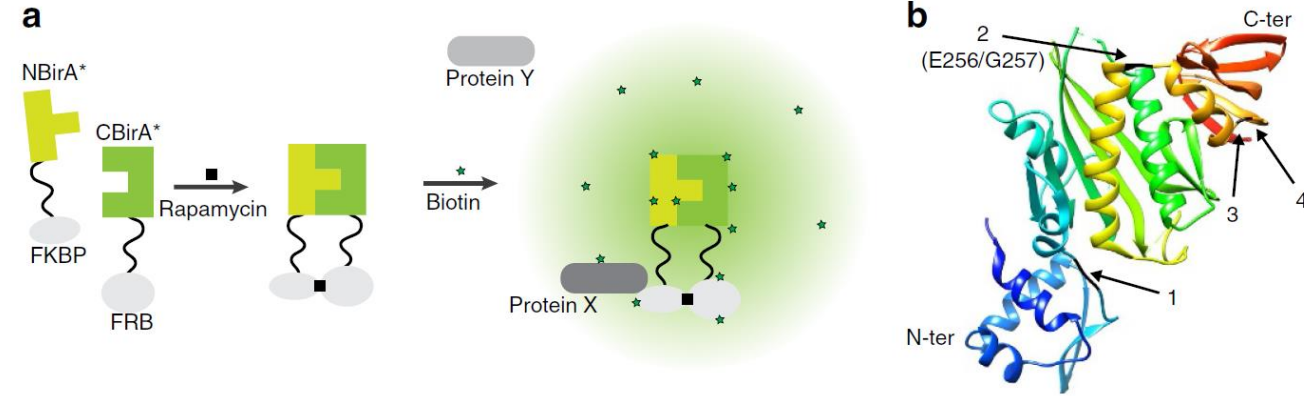


BioID2



Molecular Biology of the Cell_2016_1188

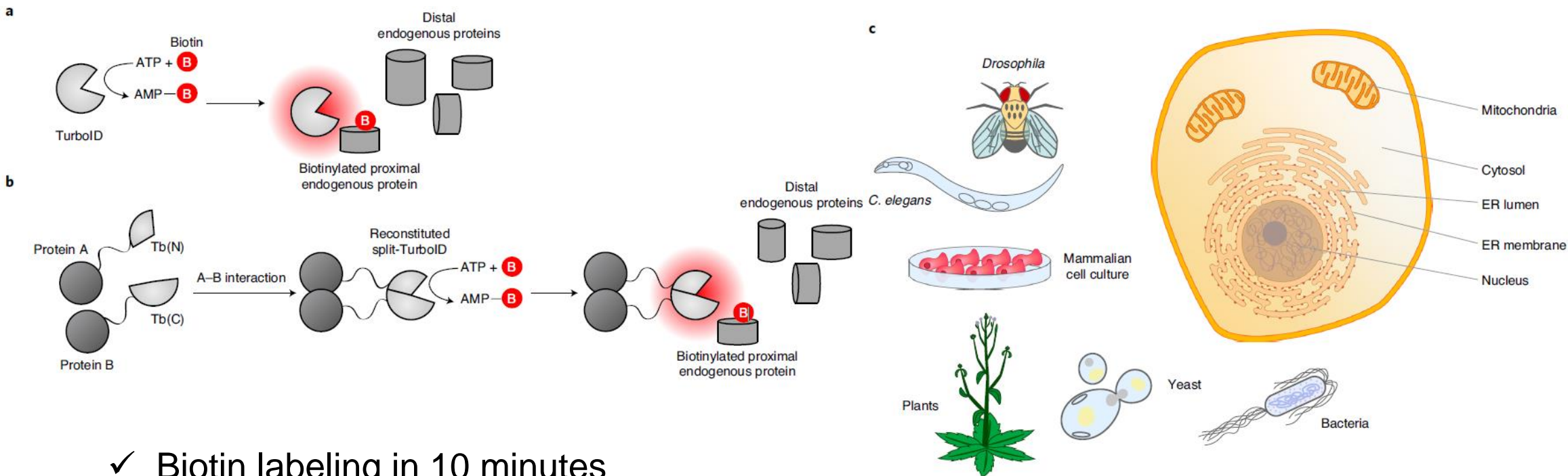
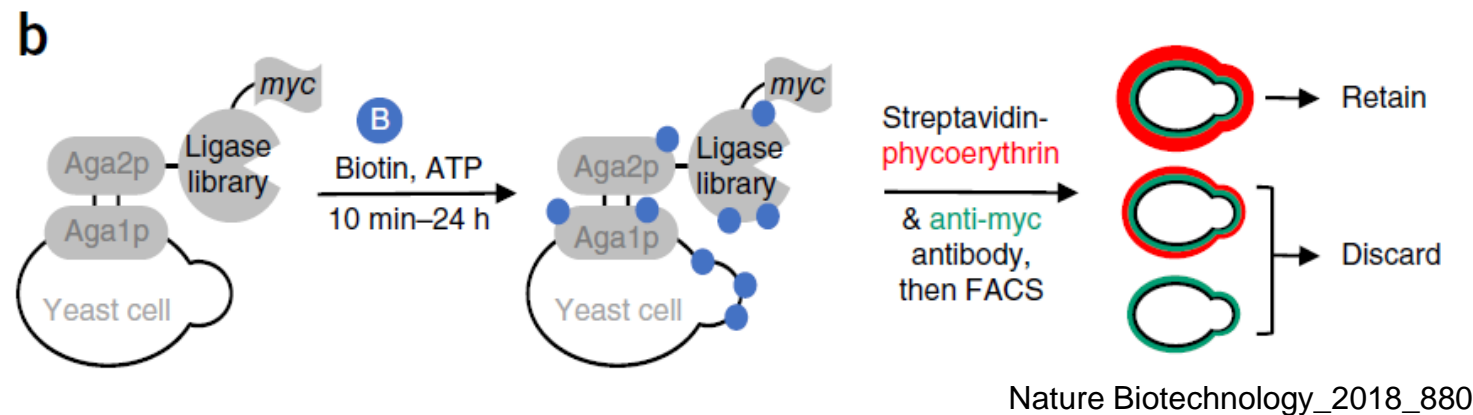
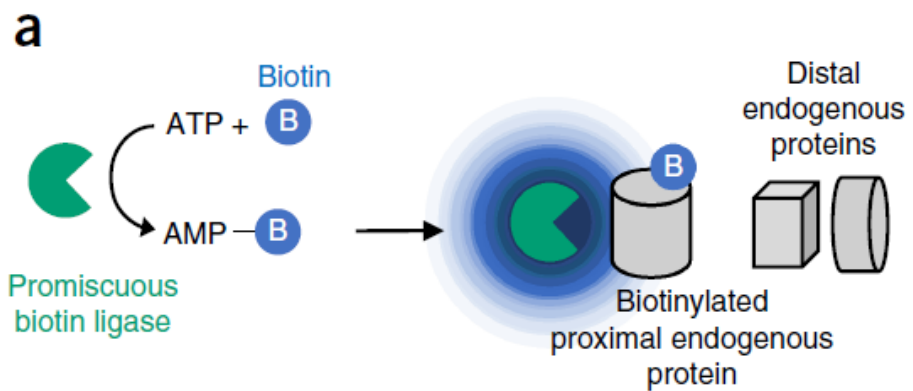
Split-BioID



NATURE COMMUNICATIONS_2017_15690

Limitation: Long time needed

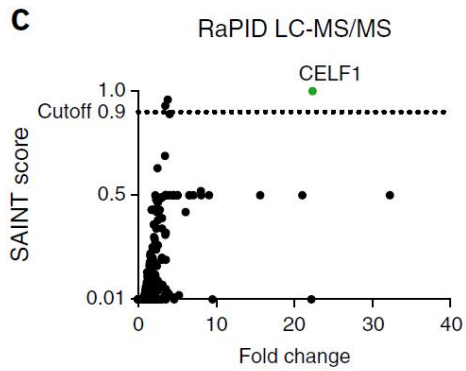
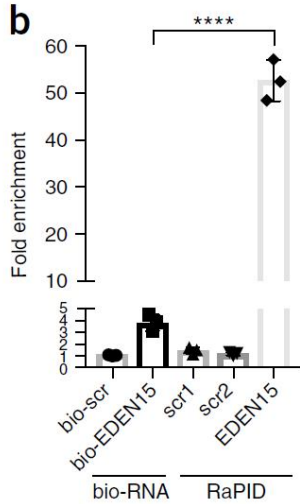
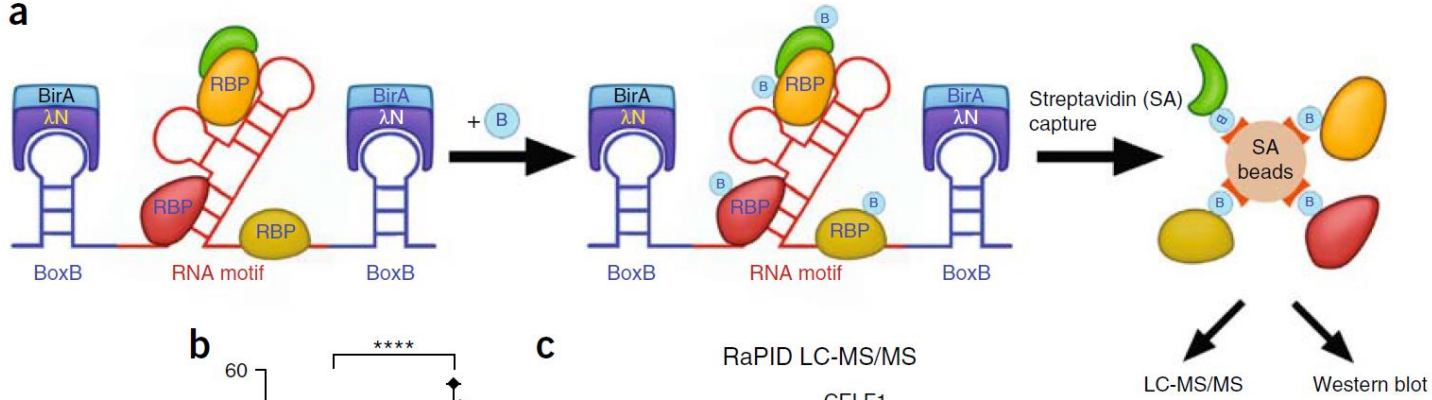
TurbID and split-TurbID



- ✓ Biotin labeling in 10 minutes
- ✓ Without any issues in toxicity.

RaPID

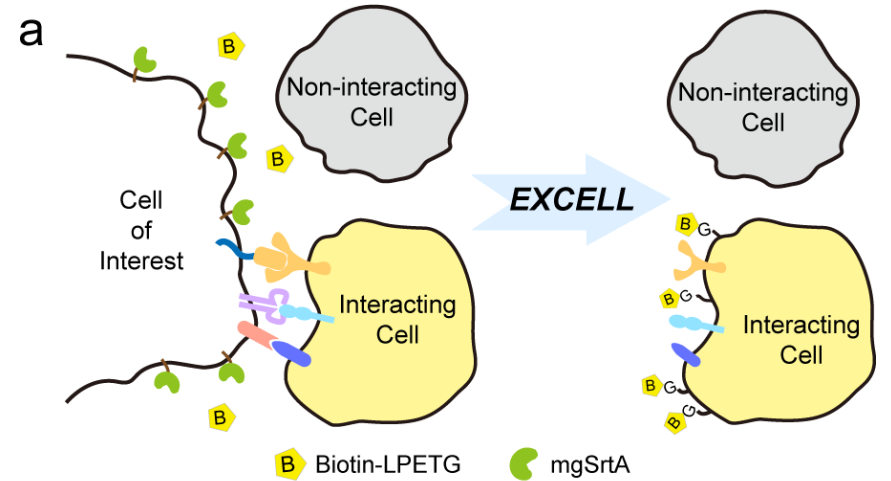
a



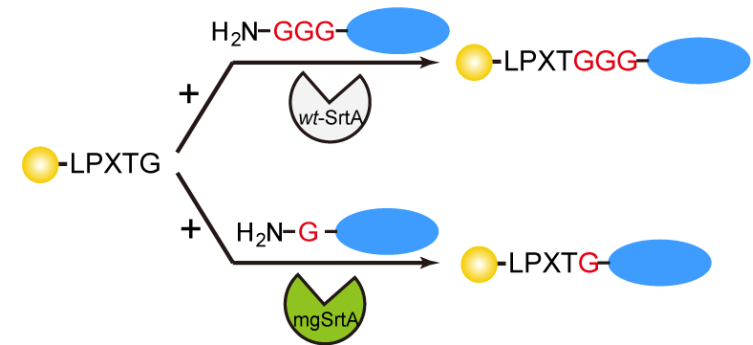
NATURE METHODS_2018_207

EXCELL

a



b

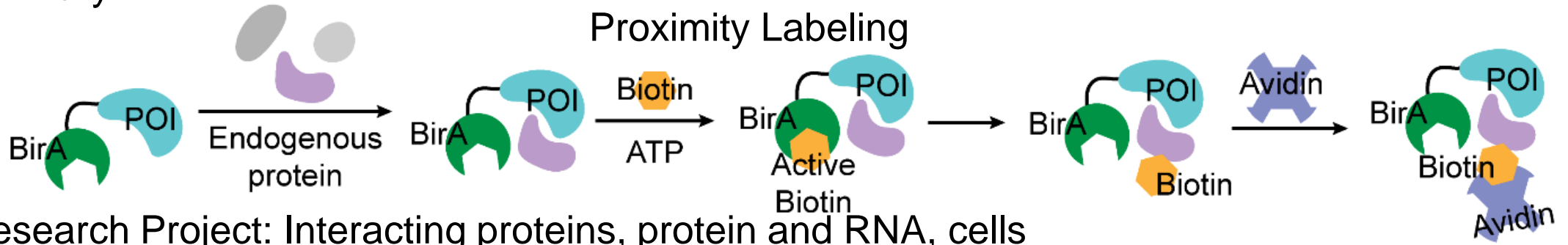


J. Am. Chem. Soc._2019_1833

➤ Summary

	HRP	APEX	BioID
Enzymatic Activity	Peroxidase	Peroxidase	Biotin ligase
Labeling Target	Electron-rich amino acids	Tryrosine and potentially other electron-rich amino acids	Lysine
Size	44 kD	27 kD	BioID, TurboID: 35kD BASU: 29kD miniTurbo: 28kD BioID2: 27kD
Labeling time	5-10min	1min	BioID: 15-24hr TurboID: 10min
Incubation time with substrate	5-10min	30-60min	BioID: 15-24hr TurboID: 10min
Activation by H ₂ O ₂	Yes	Yes	No
Substrates for protein labeling	biotin-phenol (and biotin- or fluorescein-acylazide)	biotin-phenol	biotin
Half-life of generated radicals	<1ms	<1ms	mins
Active region	extracellular, secretory pathway (inactive in cytosol)	intracellular	intracellular
Notes	Can be used as EM tag; HRP-conjugated antibodies available	Can be used as EM tag	BioID: reduced activity below 37°C TurboID: evolved in yeast at 30°C

➤ Summary



Research Project: Interacting proteins, protein and RNA, cells

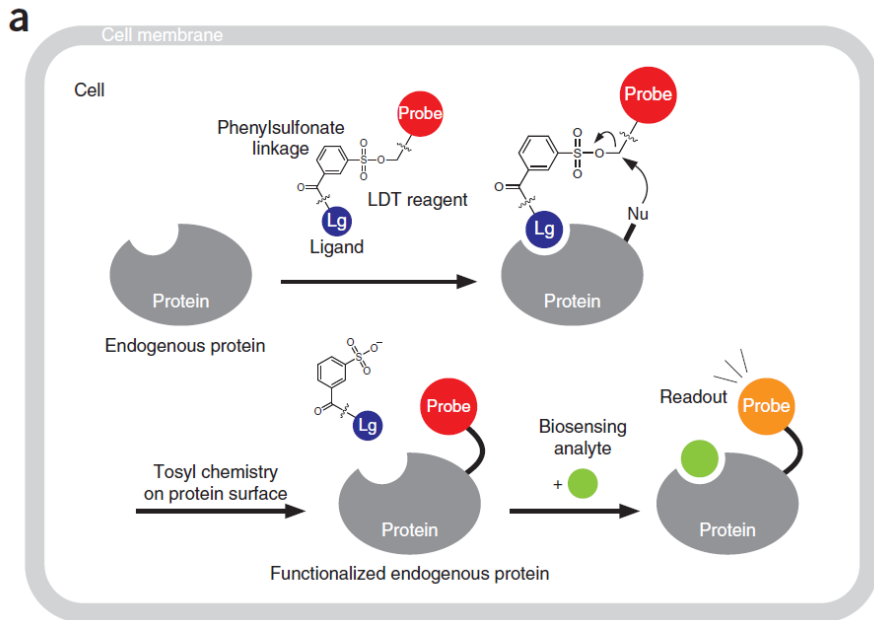
Key Factor: Enzyme catalysis

Suitable object: Live cells

Labeling Time: 5-10 min

Combination Techniques: LC/MS/MS, SDS-PAGE, Western Blot, Fluorescence image, EM

Affinity labeling



Research Project: Interested proteins, organelles

Key Factor: Substrate

Suitable object: Live cells

Labeling Time: Several minutes to hours

Combination Techniques: LC/MS/MS, SDS-PAGE, Western Blot, Fluorescence image