

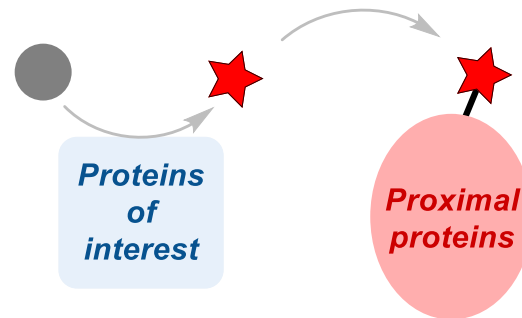
XU GROUP
Department of Chemistry, Peking University

Selected Weekly Literature Presentations

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Proximity Labeling: Development and Applications

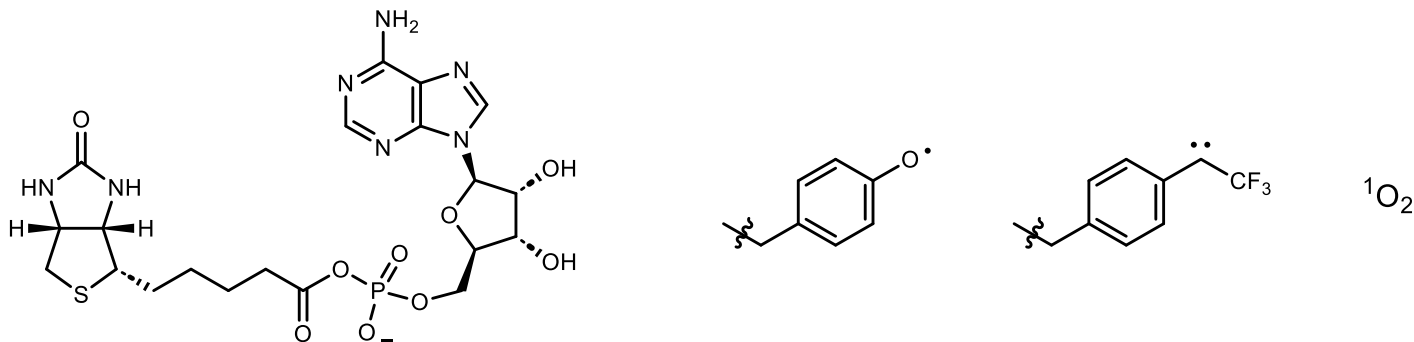


Reporter: Tianxing Jiang

Supervisor: Yan Xu

2026.03.14

1. Explain the labeling selectivity of the following intermediates:



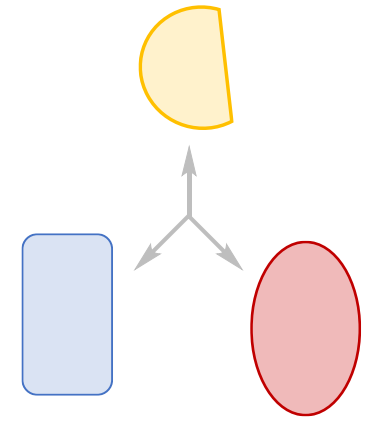
2. Generally, how do we colocalize enzyme / photocatalyst with POIs?

3. What is the upper limit of labeling accuracy (low limit of labeling radius)?

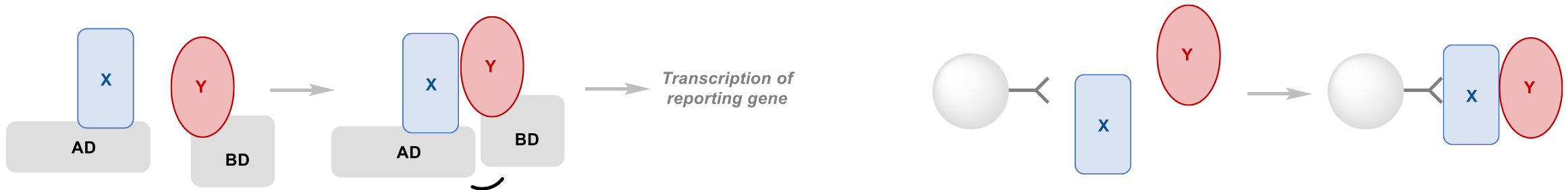
- *Introduction*
- *Enzyme-based proximity labeling*
- *Photocatalytic proximity labeling*
- *Measurement & mechanistic discussion*
- *Summary*

Protein-protein interactions (PPIs)

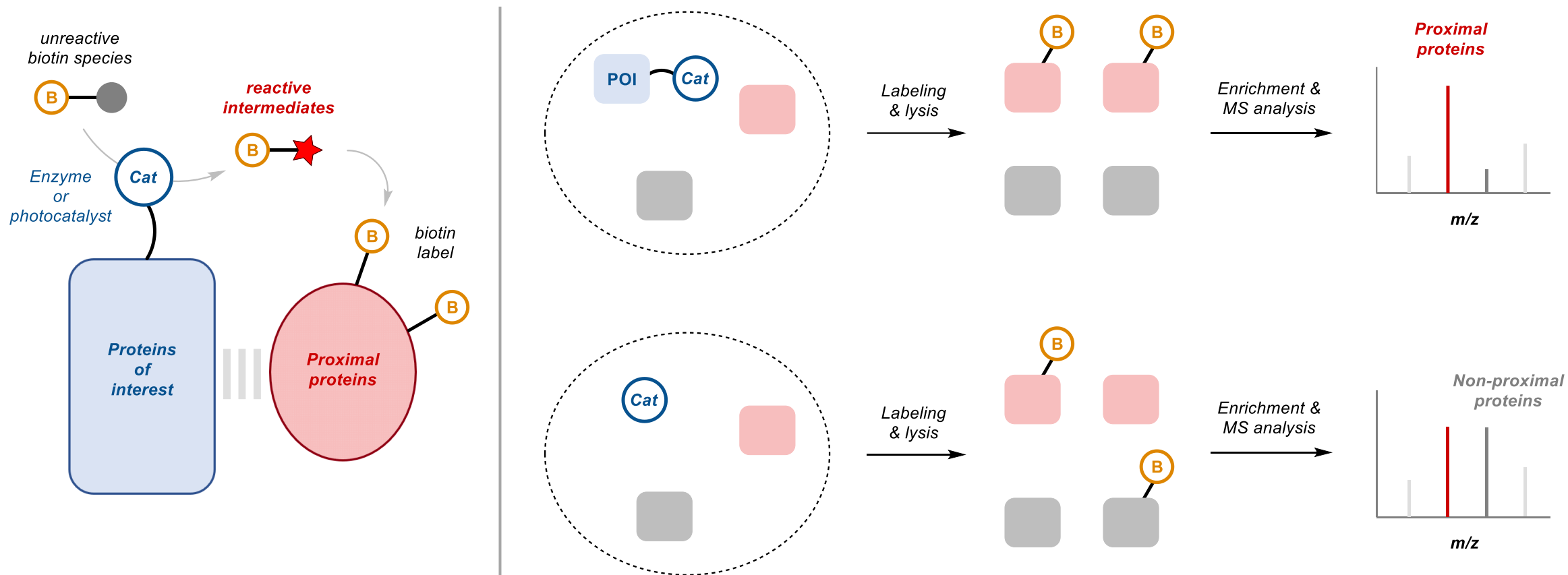
- essential for biological processes
- cellular organization, signal transduction, immunological responses, ...



traditional methods: yeast 2 hybrid, co-immunoprecipitation, ...



Proximity labeling (PL)



Proximity labeling (PL)

Advantages

- Detection of *weak or transient* interactions
- Able to process in *live cells* or *in vivo*
- Capable for *insoluble proteins*

➤ Enzyme-based PL 

BioID, TurboID, APEX, ...

➤ Photocatalytic PL 

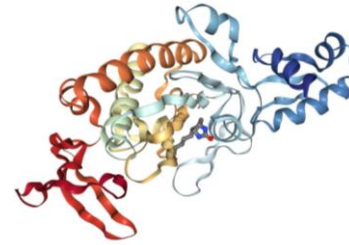
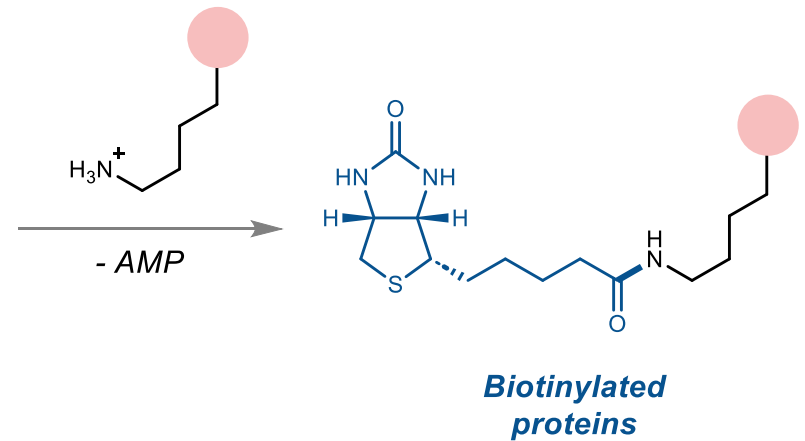
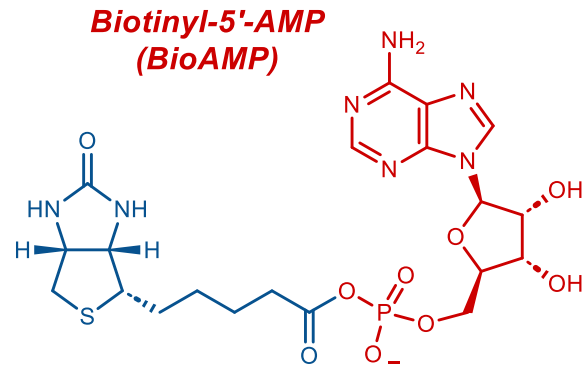
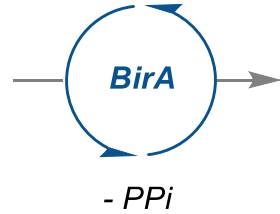
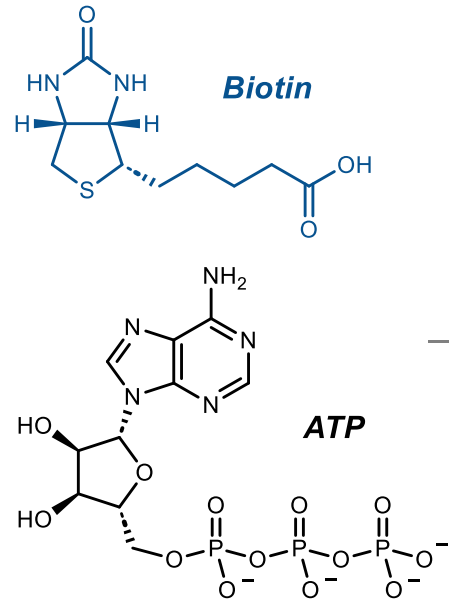
μMap, PhoTag, CAT-S, ...

- *Introduction*
- ***Enzyme-based proximity labeling***
- *Photocatalytic proximity labeling*
- *Measurement & mechanistic discussion*
- *Summary*

Enzyme-based proximity labeling



Biotin ligase-based PL

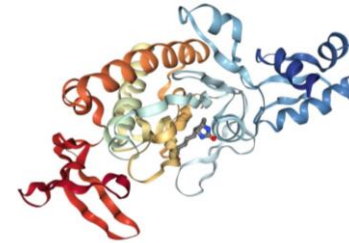
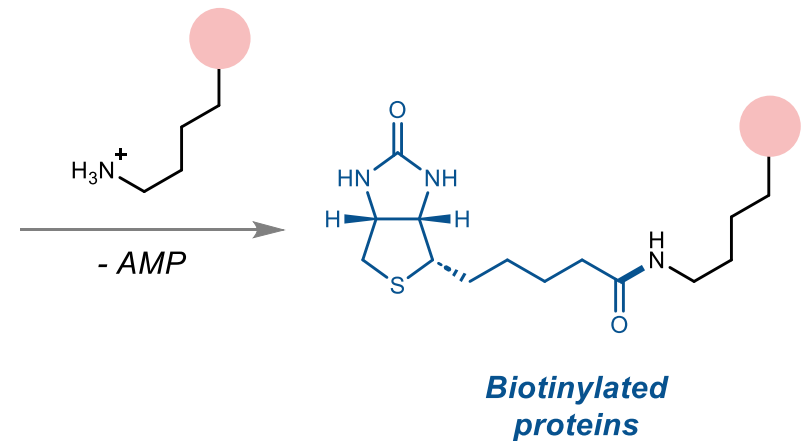
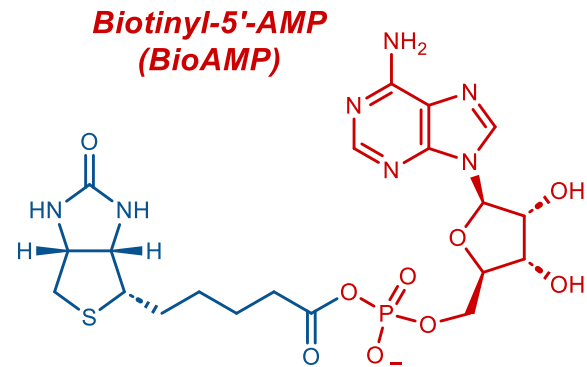
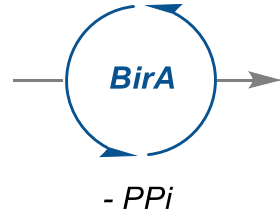
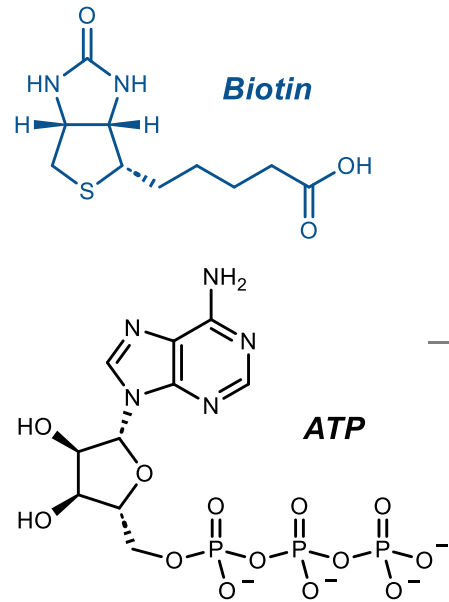


problem: intermediate kept in BirA active site
not diffusible

Enzyme-based proximity labeling



Biotin ligase-based PL



BirA from *E.coli*
~ 35 kDa

BirA
 $K_D = 4.5 \times 10^{-11}$



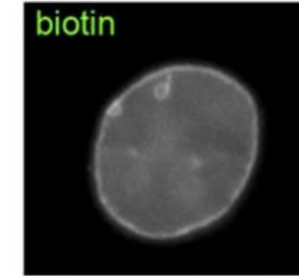
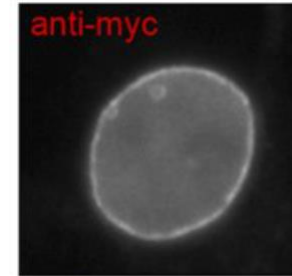
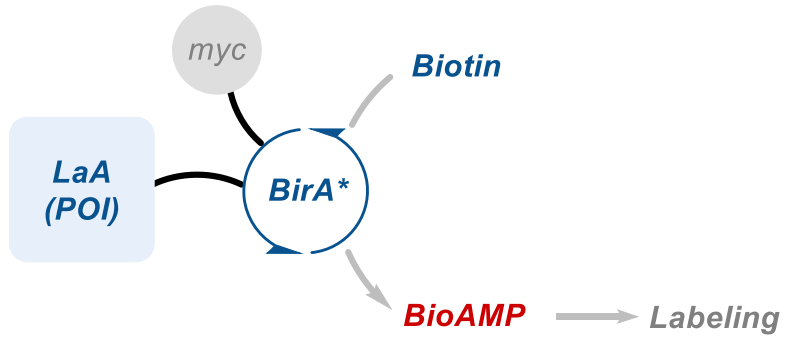
*BirA** (R118G)
 $K_D = 2.0 \times 10^{-8}$

capable for BioAMP release and diffusion

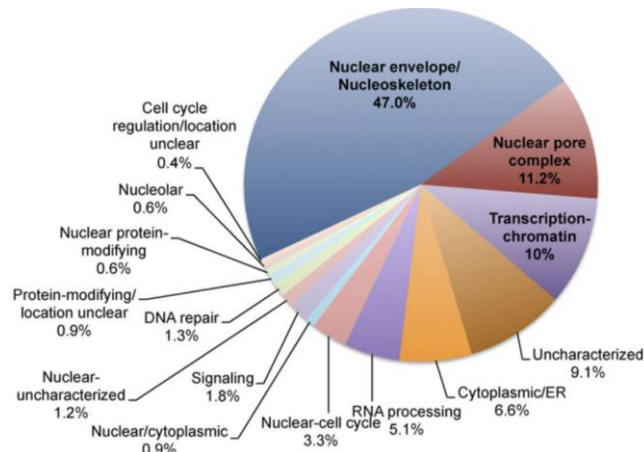
Enzyme-based proximity labeling



“BioID” method

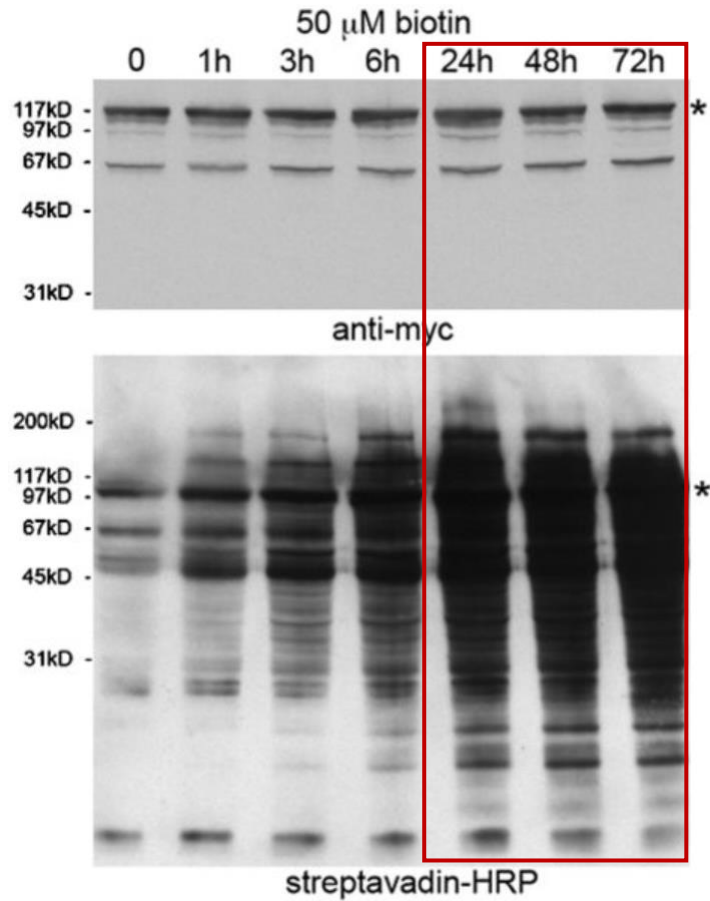


colocalized POI and biotin-labeled proteins



122 proteins was identified, including 10 uncharacterized

Enzyme-based proximity labeling



labeling saturated within *24 h*

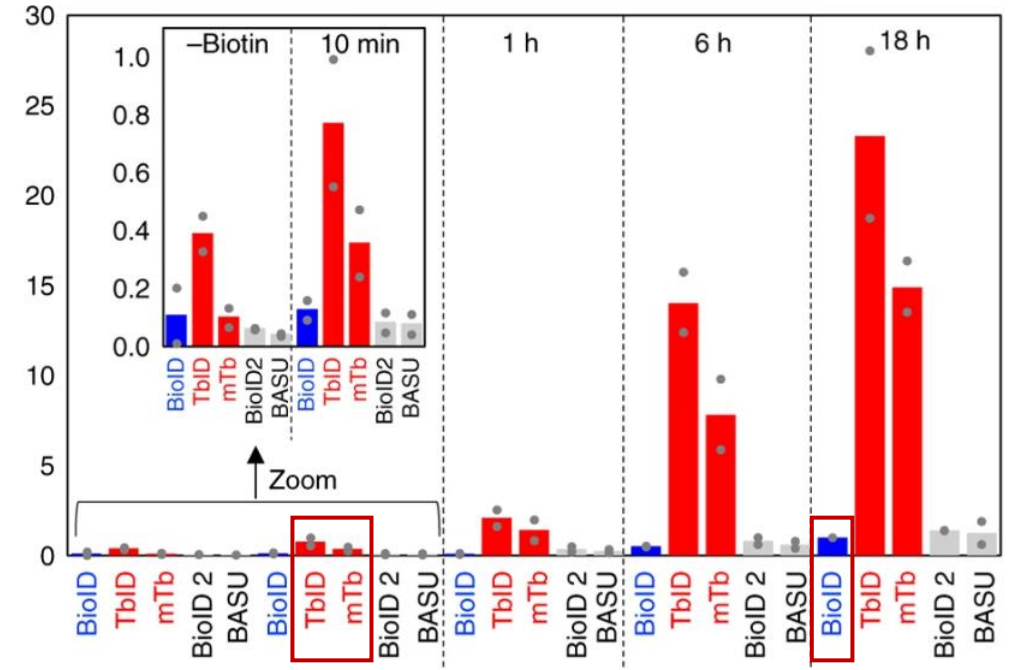
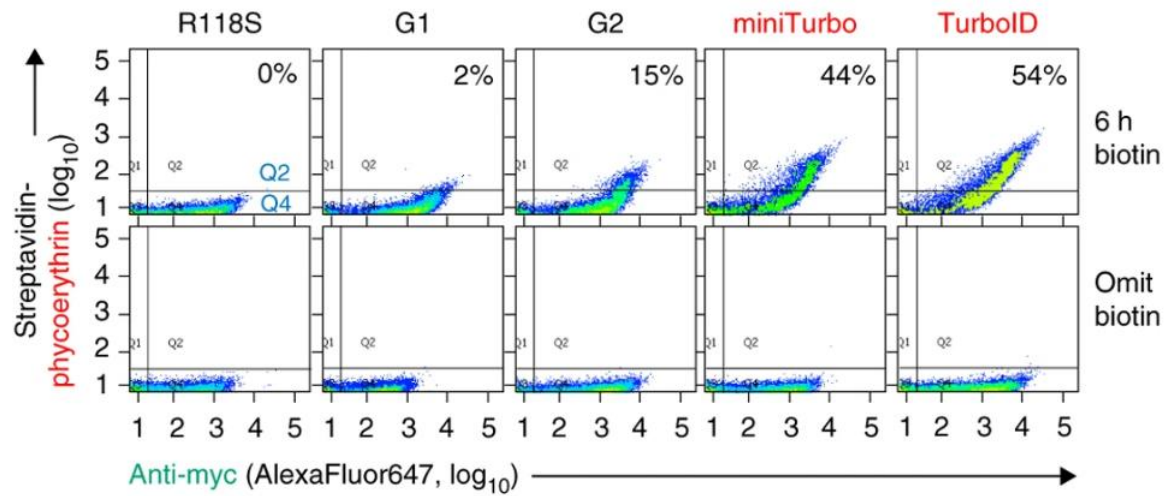
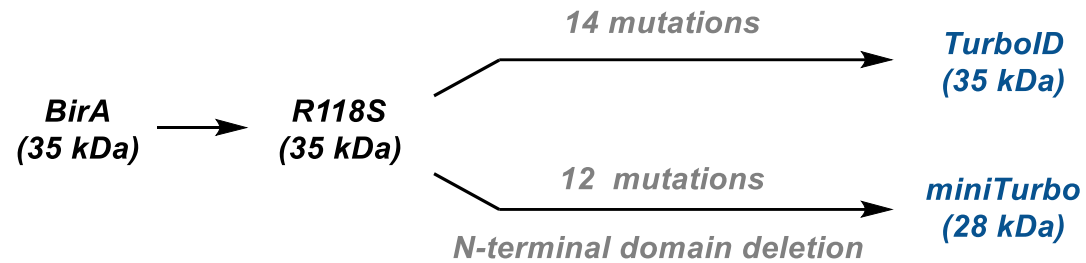


improvement of *labeling efficiency*?

Enzyme-based proximity labeling

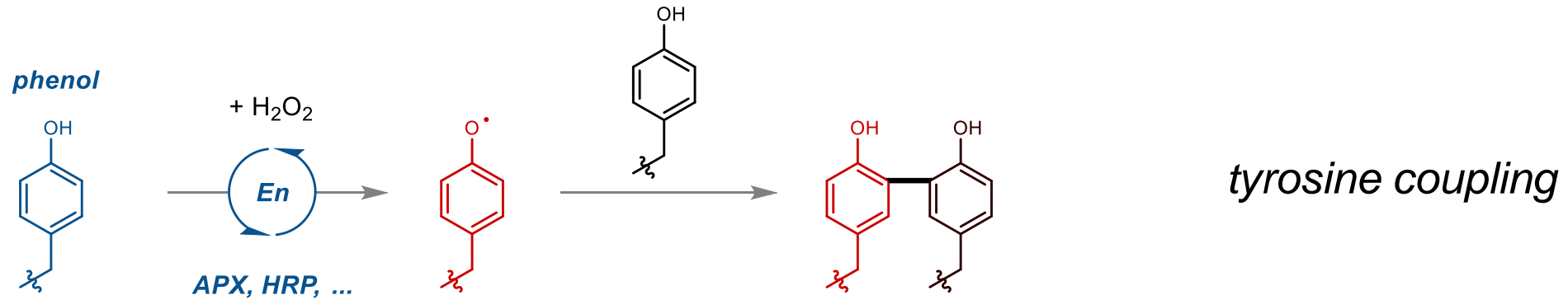


TurboID & miniTurbo



labeling time: 18 h → 10 min

Peroxidase-based PL

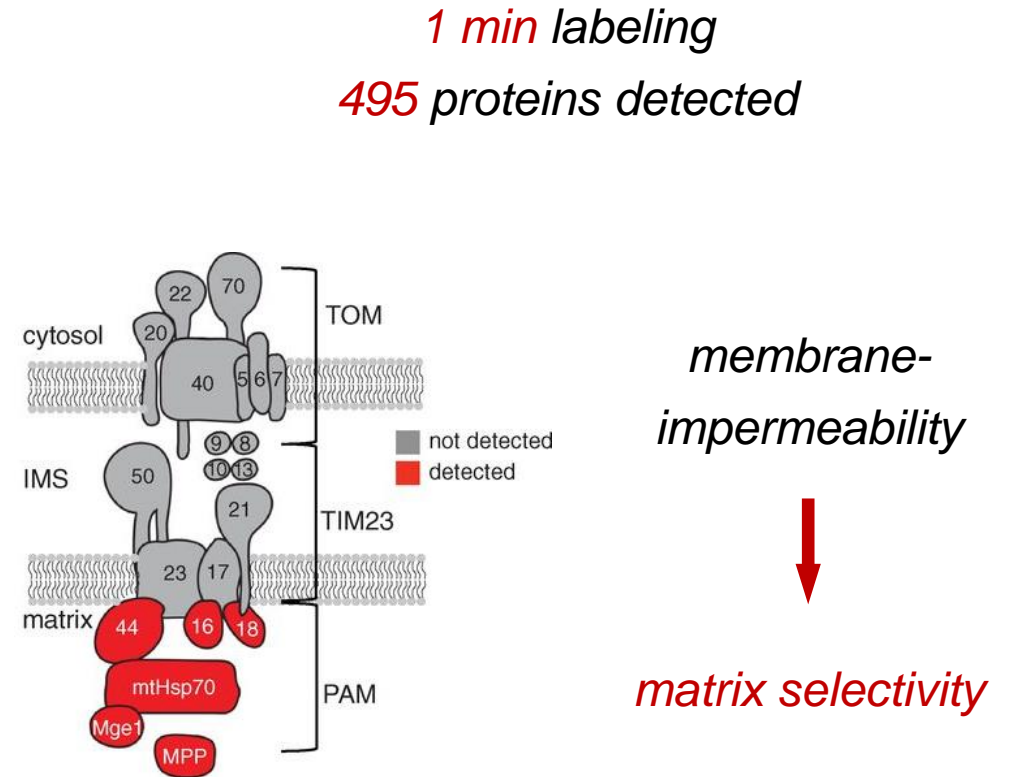
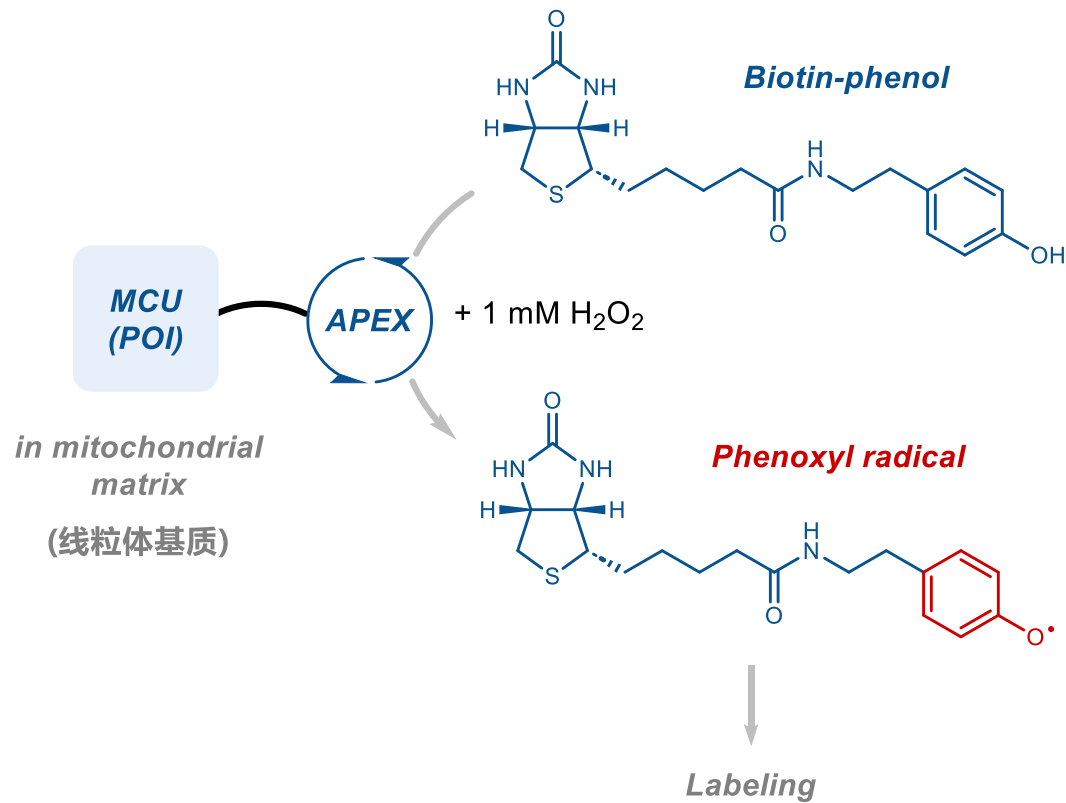


Kamiya, N.; *et al. Bioconjug. Chem.* **2011**, *22*, 2332-2338.
Ting, A, Y.; *et al. Nat. Biotechnol.* **2012**, *30*, 1143-1148.

Enzyme-based proximity labeling



“APEX” method



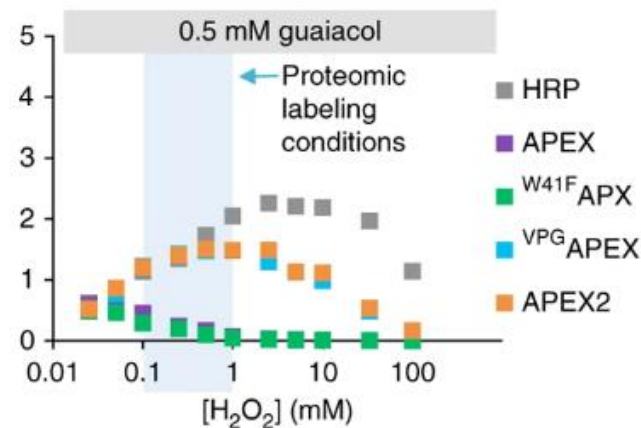
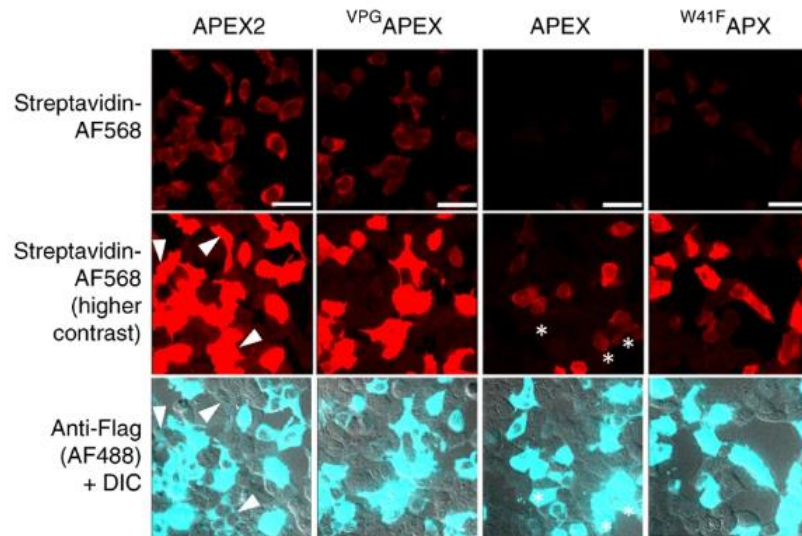
Ting, A, Y.; *et al. Nat. Biotechnol.* **2012**, 30, 1143-1148.

Ting, A, Y.; *et al. Science* **2013**, 339, 1328-1331.

Enzyme-based proximity labeling



“APEX2” method



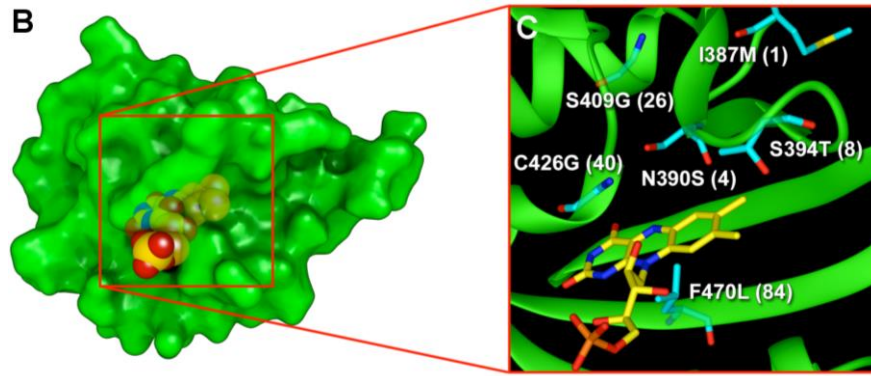
enhanced *catalytic efficiency*

higher *H₂O₂ resistance*

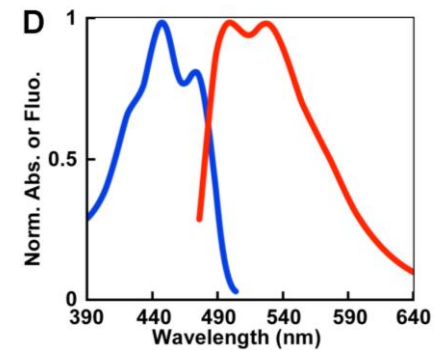
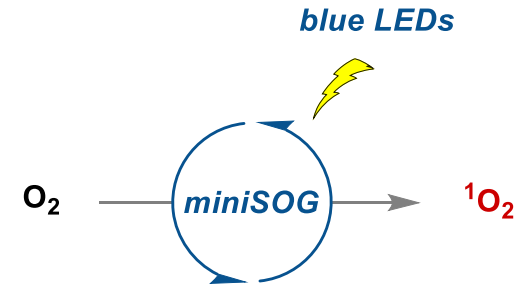
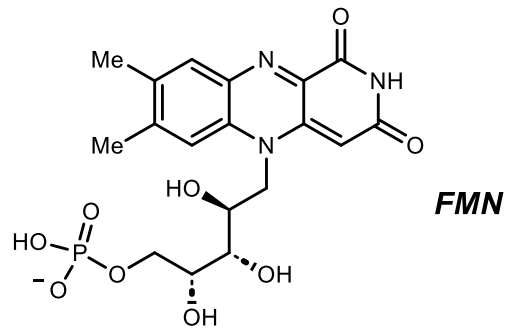
Enzyme-based proximity labeling



miniSOG-based PL



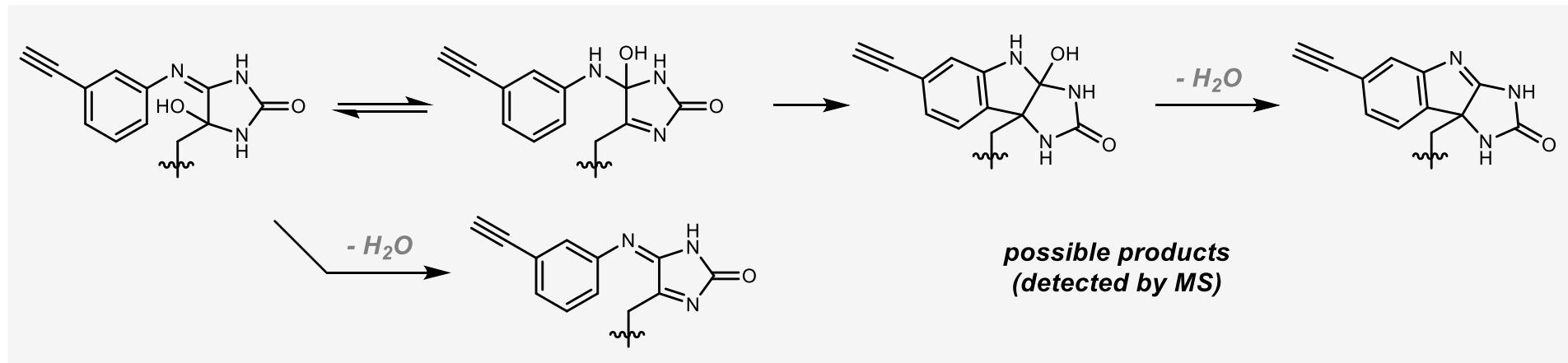
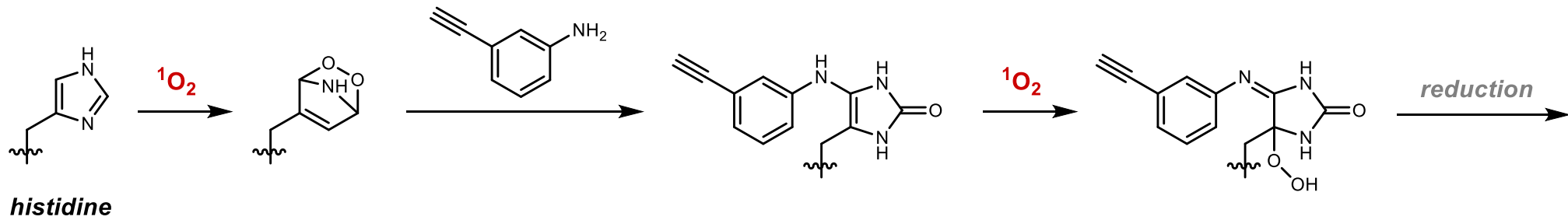
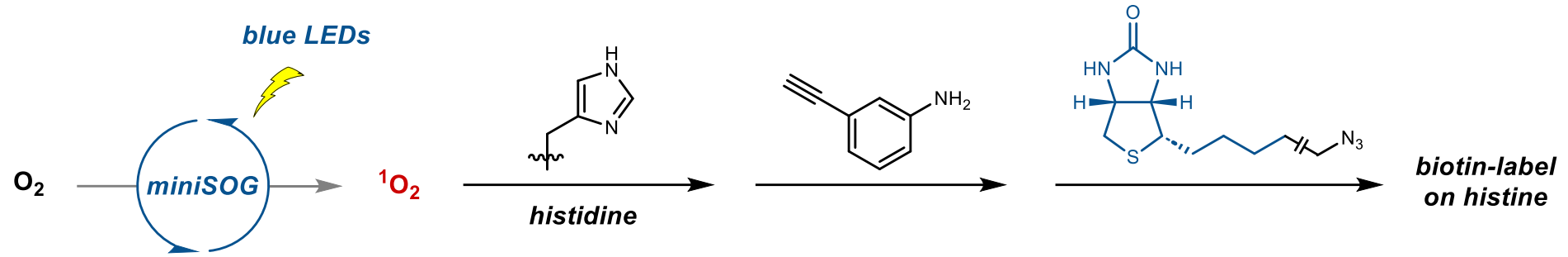
~ 12 kDa



$$\lambda_{\max} = 448 \text{ nm}$$

$$\phi(^1O_2) = 0.47$$

Enzyme-based proximity labeling



Summary

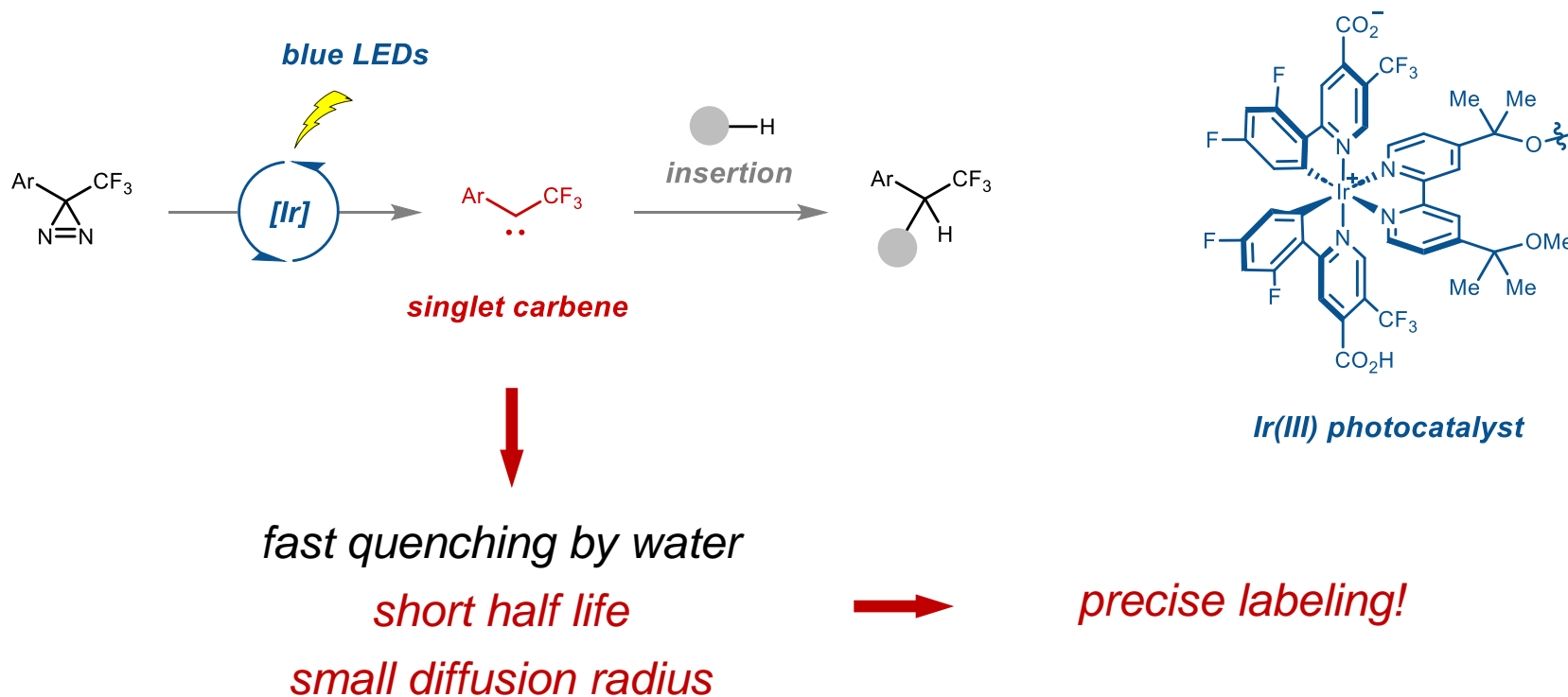
- *Genetically encodable*
 - *High specificity of POIs*
 - *Easy modification & evolution*
- *Limited labeling species*
 - *Obstruction of native PPIs*

- *Introduction*
- *Enzyme-based proximity labeling*
- ***Photocatalytic proximity labeling***
- *Measurement & mechanistic discussion*
- *Summary*

Photocatalytic proximity labeling



“ μ Map” method

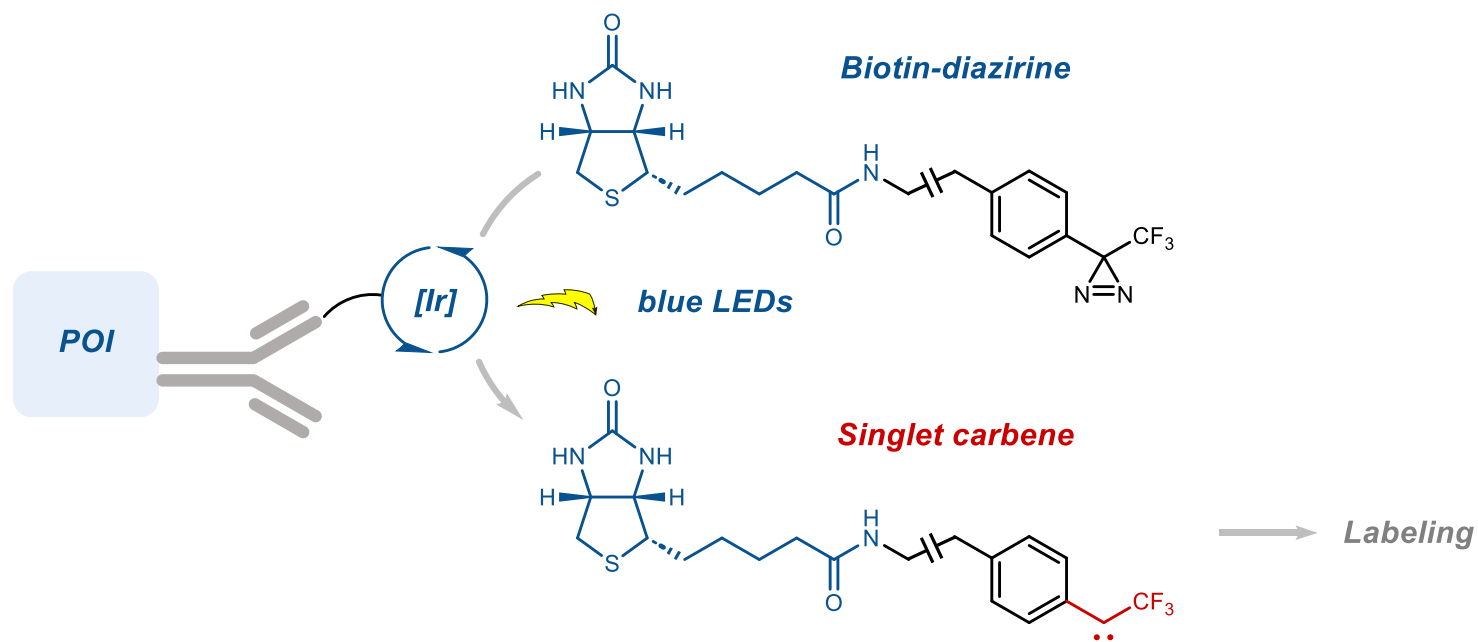
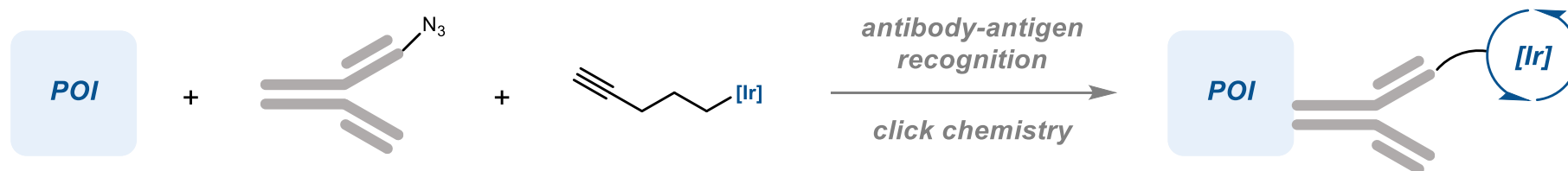


MacMillan, D. W. C.; et al. *Science* **2020**, 367, 1091-1097.

Photocatalytic proximity labeling



“ μ Map” method

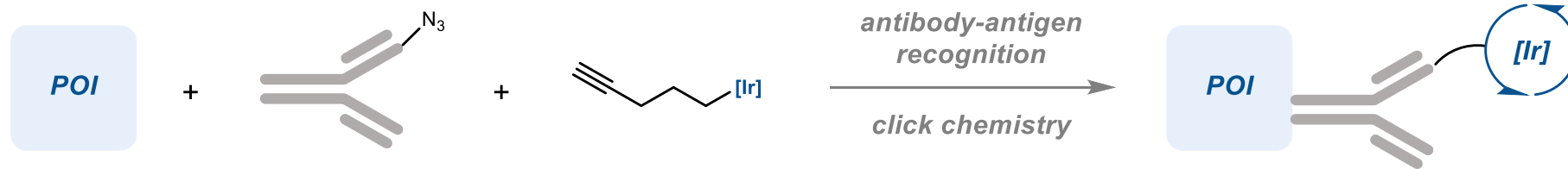


MacMillan, D. W. C.; et al. *Science* **2020**, 367, 1091-1097.

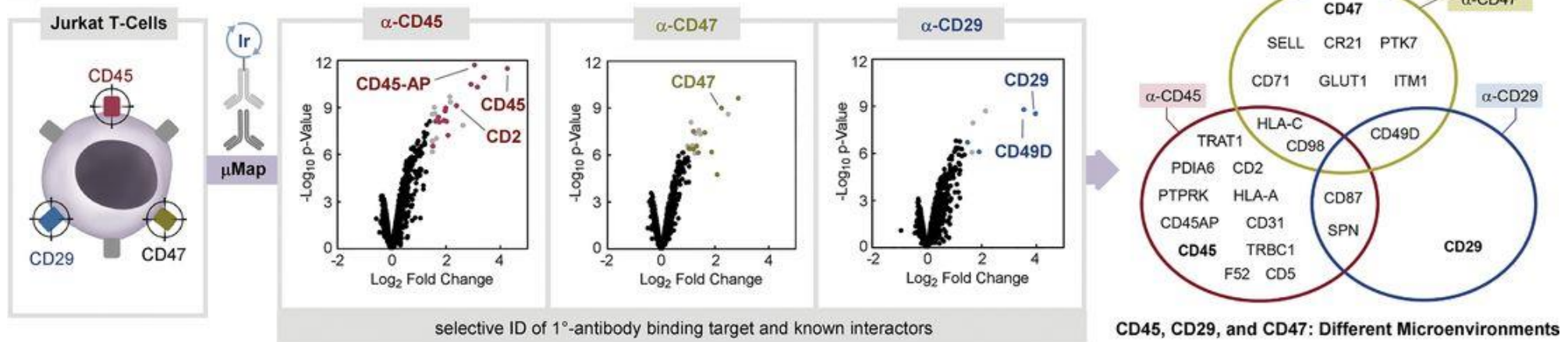
Photocatalytic proximity labeling



“μMap” method



C Validation: CD45, CD47, and CD29 Targeted μMap Proteomics

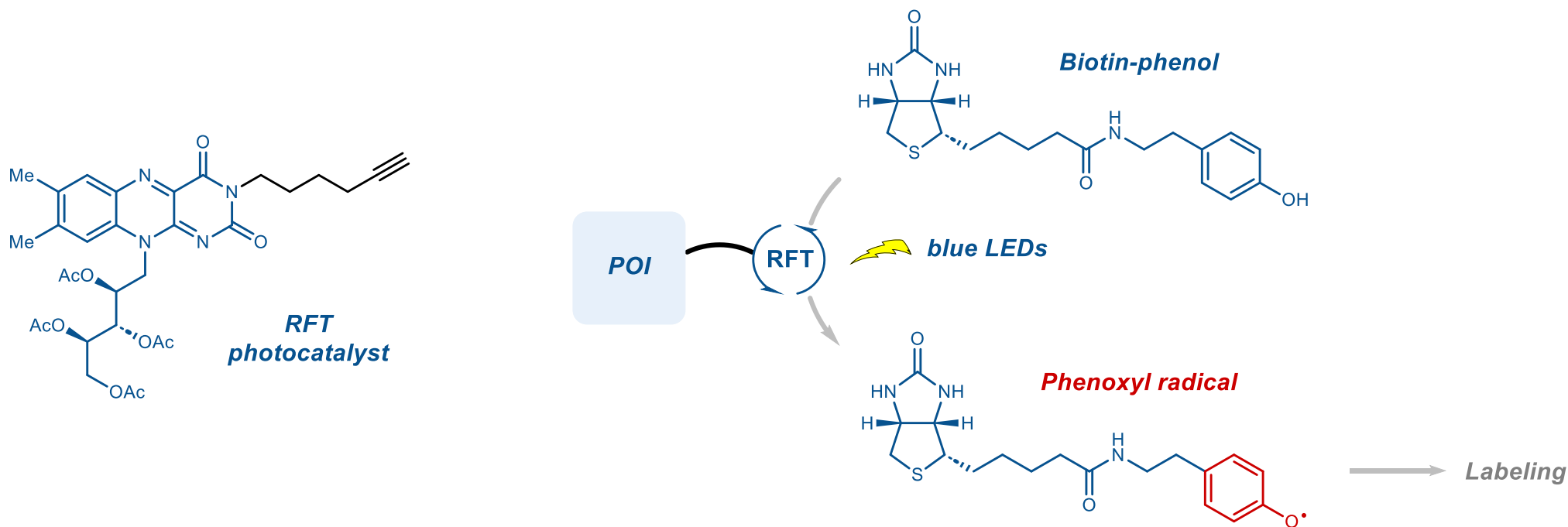


MacMillan, D. W. C.; *et al. Science* **2020**, 367, 1091-1097.

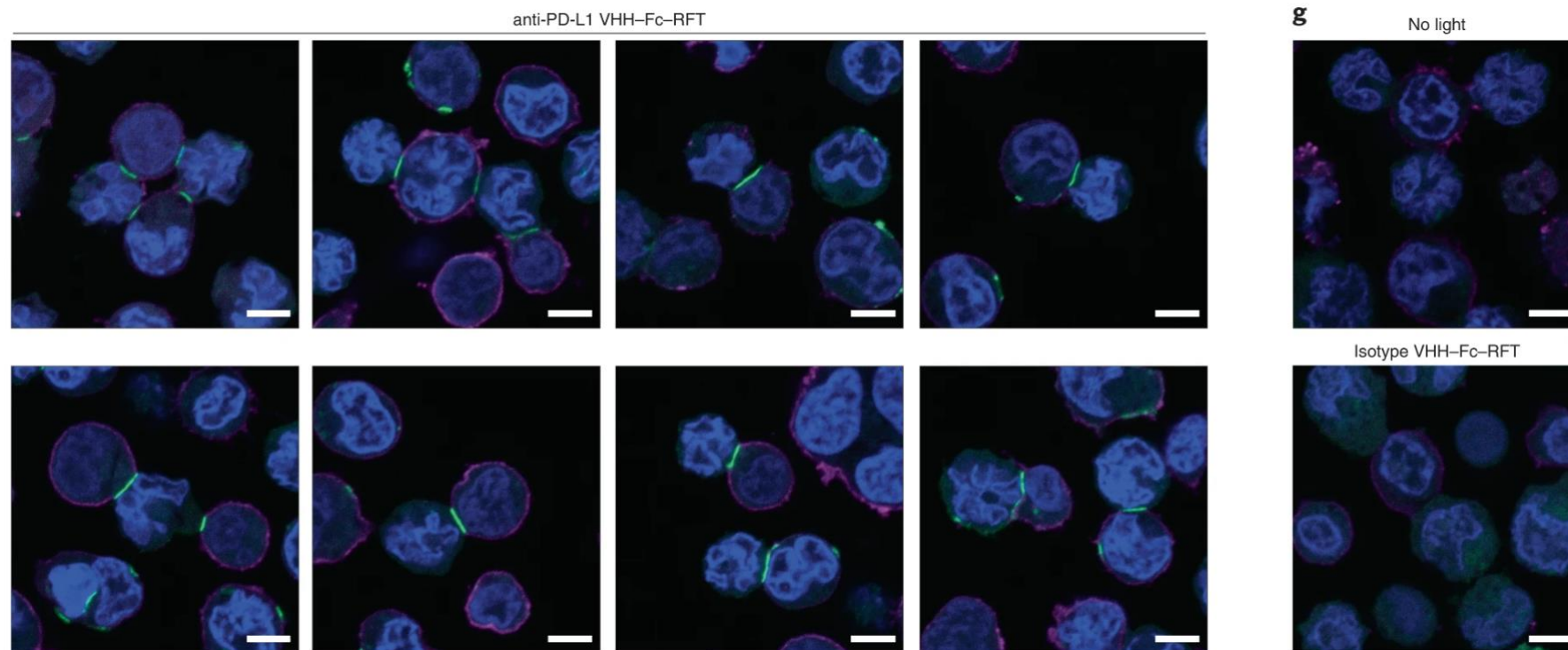
Photocatalytic proximity labeling



“PhoTag” method



“PhoTag” method

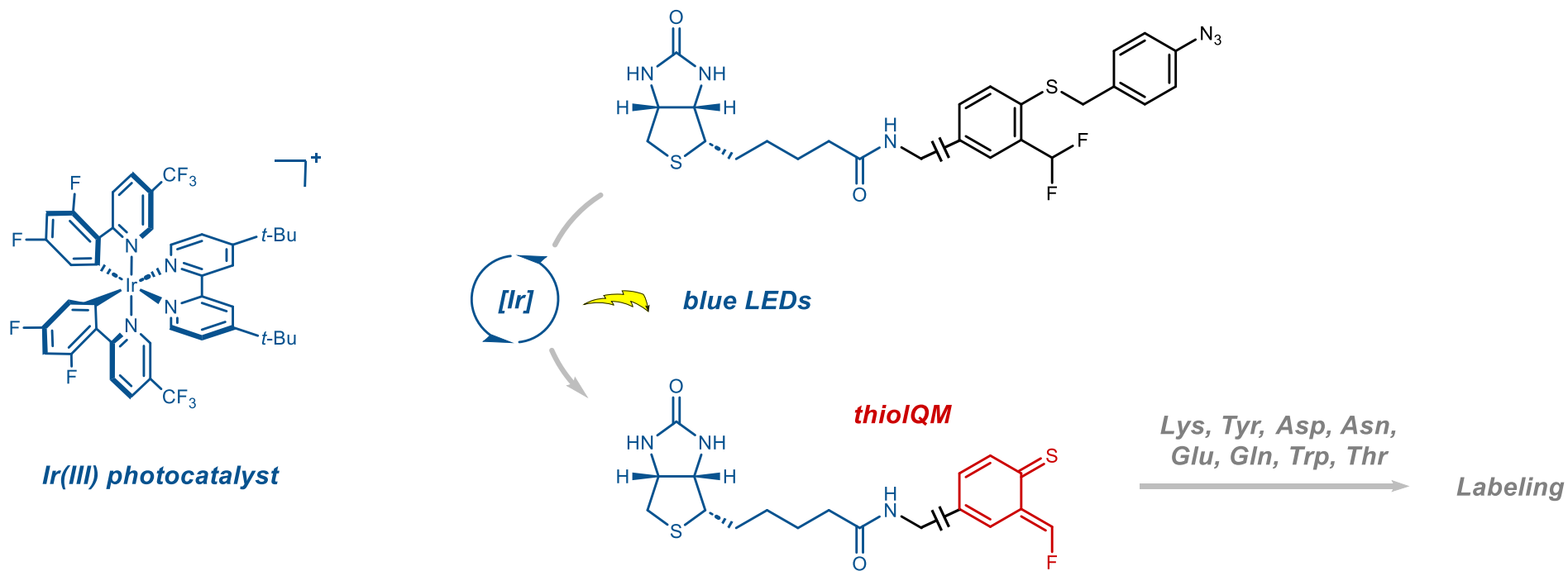


selective labeling of cellular immune synapses

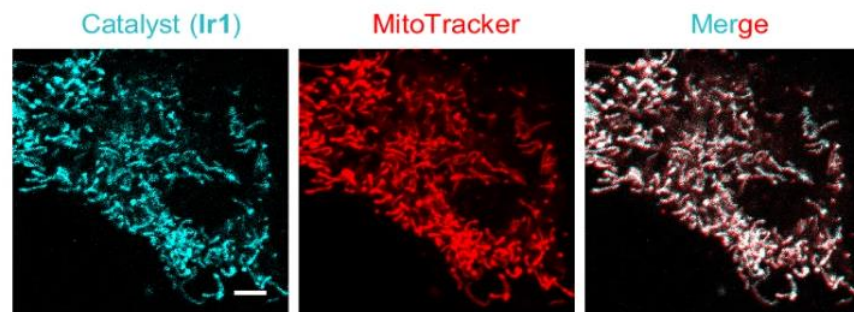
Photocatalytic proximity labeling



“CAT-S” method



“CAT-S” method



cationic [Ir] localized in mitochondria (线粒体)

no conjugation between PC and proteins

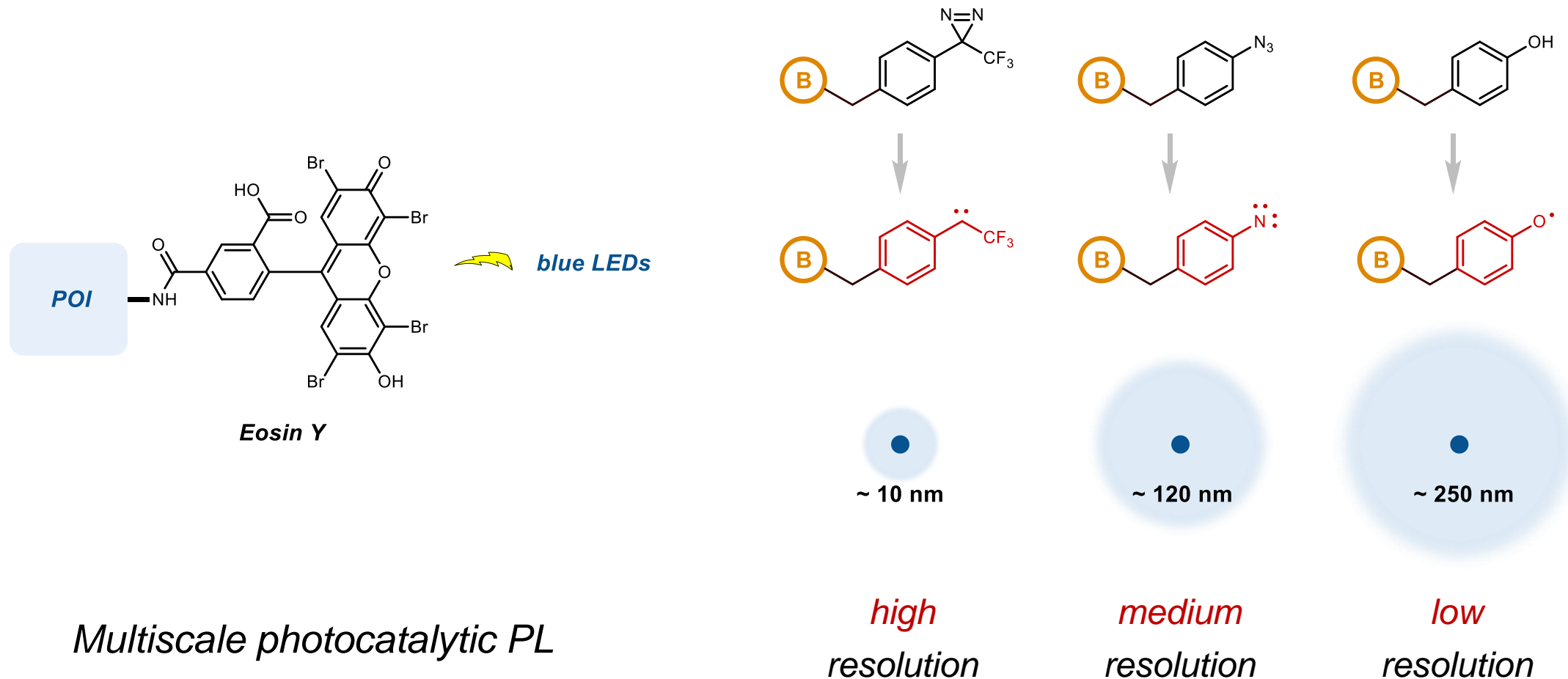


membrane-permeability

Photocatalytic proximity labeling



“MultiMap” method

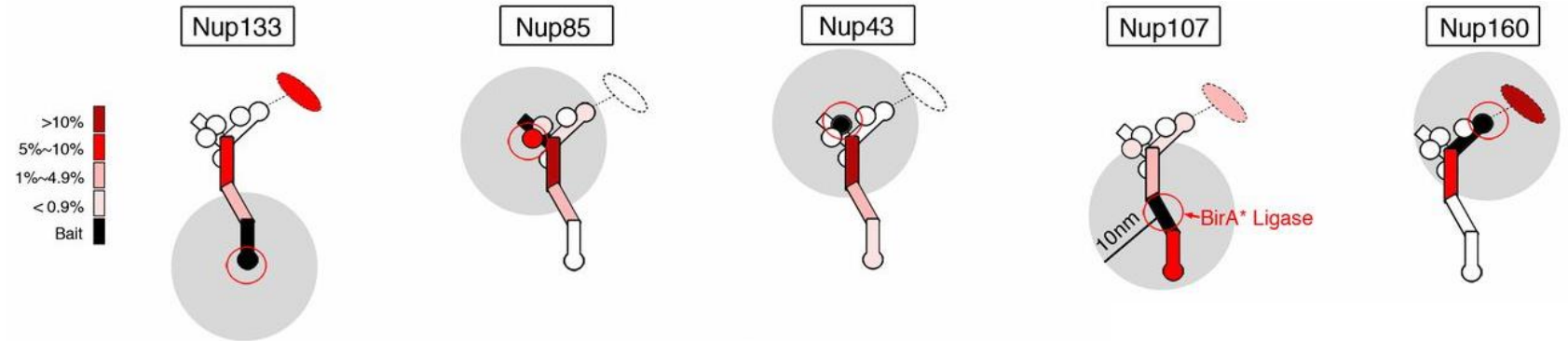
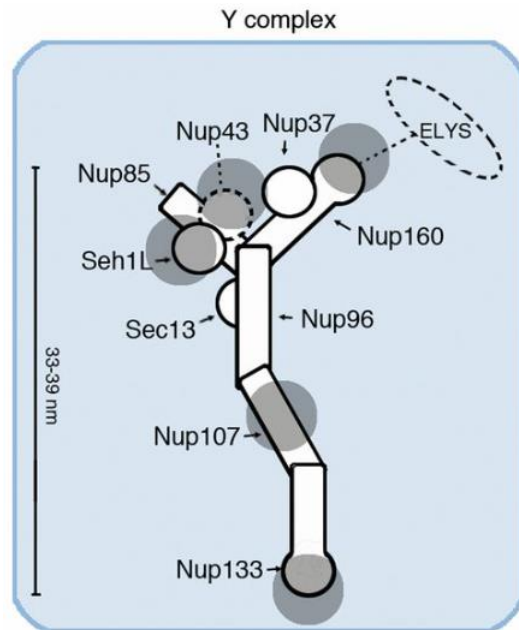


Summary

- *Photoswitch, high temporal resolution*
 - *Various photoreactions available*
 - *Easy to process on multiple POIs*
-
- *Challenging catalyst delivery*
 - *Obstruction of native PPIs*
 - *Additional structural design*

- *Introduction*
- *Enzyme-based proximity labeling*
- *Photocatalytic proximity labeling*
- ***Measurement & mechanistic discussion***
- *Summary*

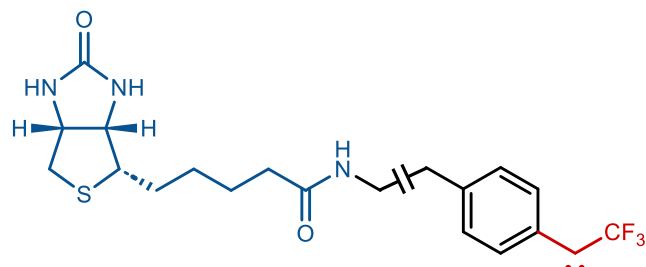
Measurement of labeling radius



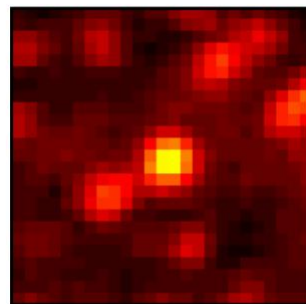
labeling radius of BioAMP: ~ 10 nm

labeling efficiency *varies* depending on proteins

Measurement via super-resolution microscopy



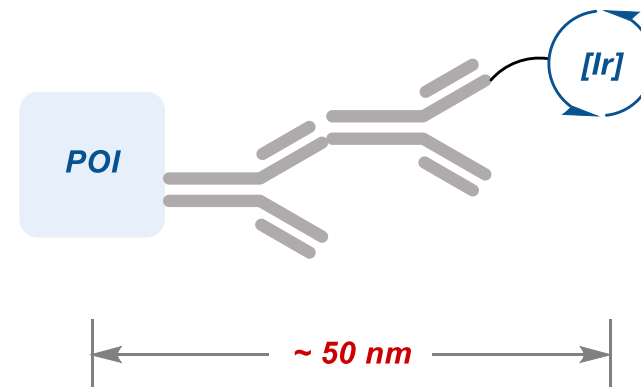
representative cluster
(μ Map)



0.5 μ m
mean FWHM:
 54 ± 12 nm (n = 100)

theoretical: 4 nm

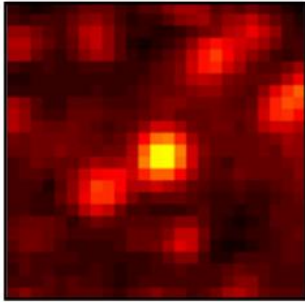
??



extended labeling radius

Measurement via super-resolution microscopy

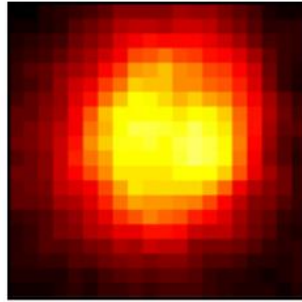
representative cluster
(μ Map)



0.5 μ m

mean FWHM:
 54 ± 12 nm (n = 100)

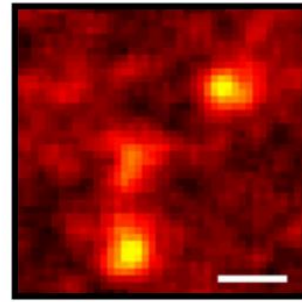
representative cluster
(peroxidase)



0.5 μ m

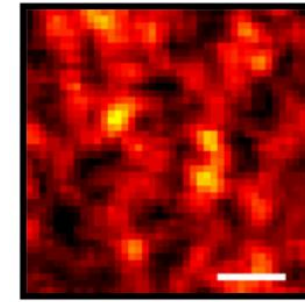
mean FWHM:
 269 ± 41 nm (n = 100)

PEG3 PhN₃

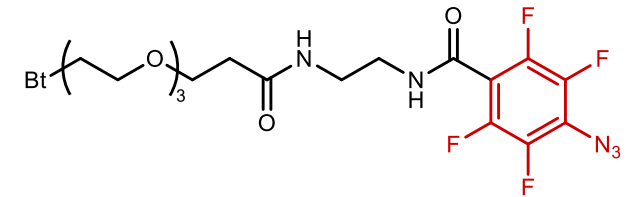
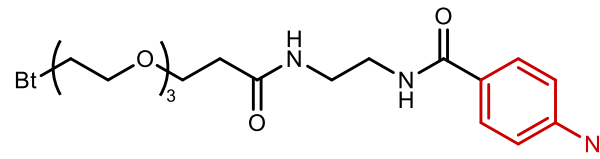


mean FWHM:
 119 ± 33 nm (n = 100)

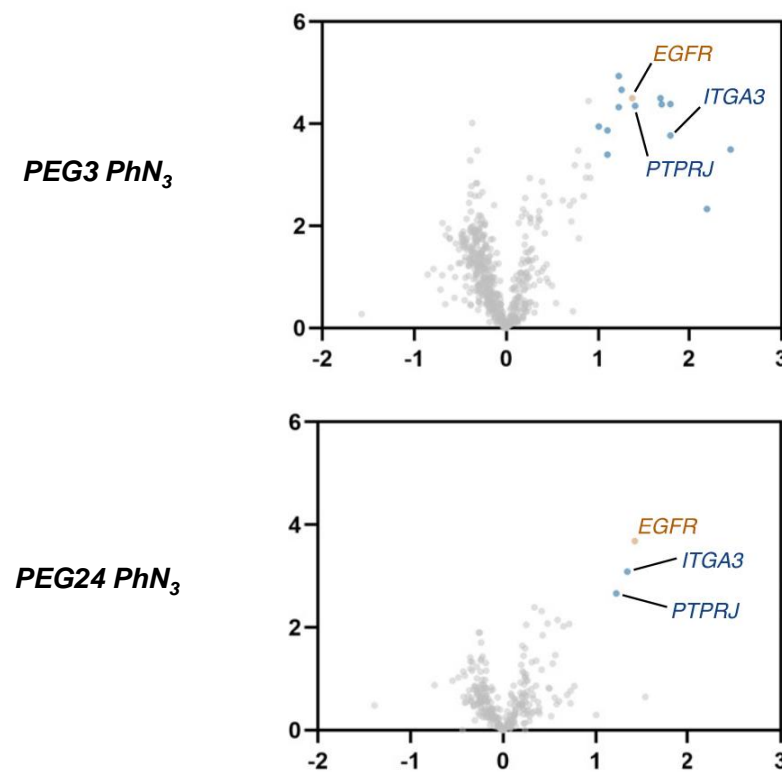
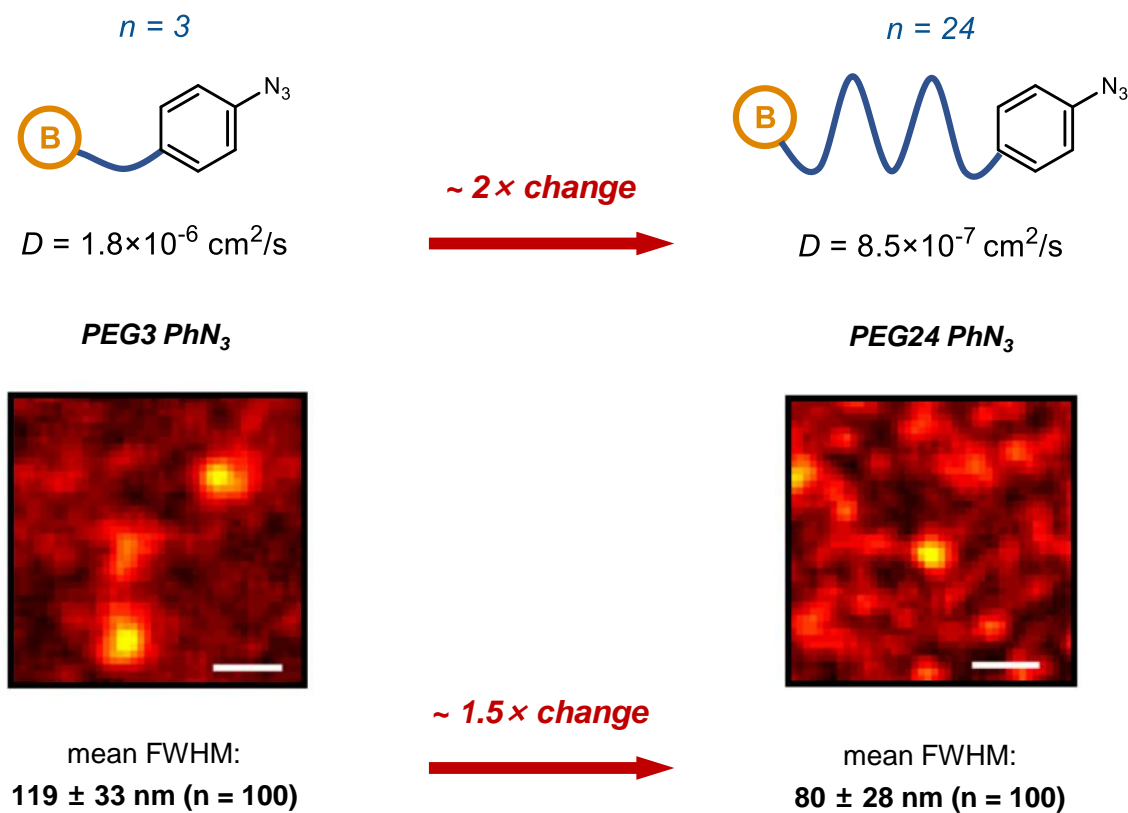
PEG3 PhF₄N₃



mean FWHM:
 68 ± 17 nm (n = 100)

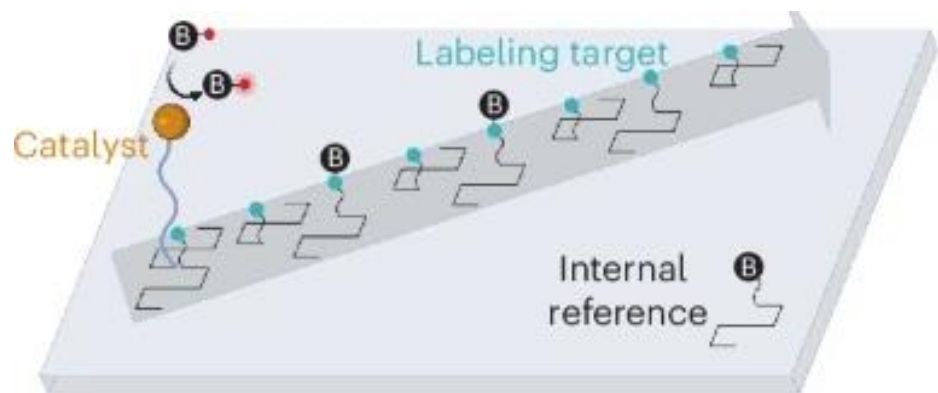


Radius control via diffusion coefficient modulation

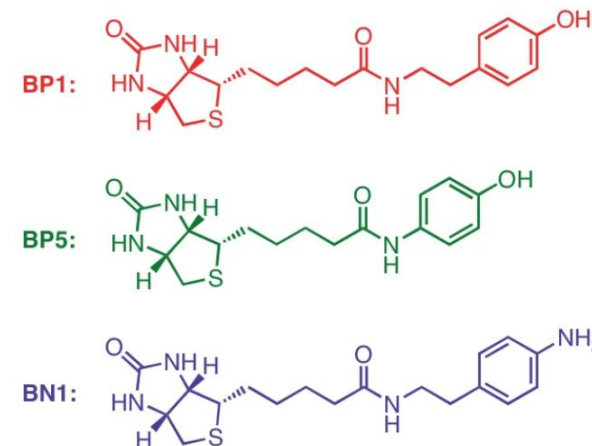
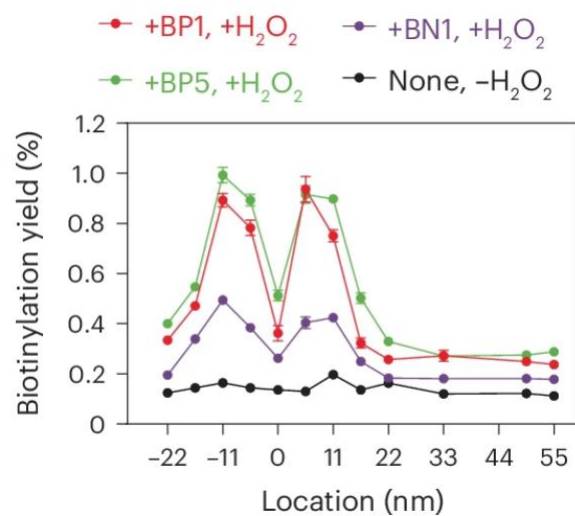


enhanced selectivity

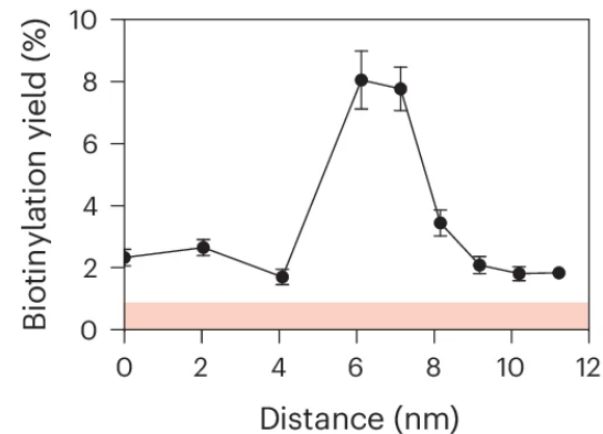
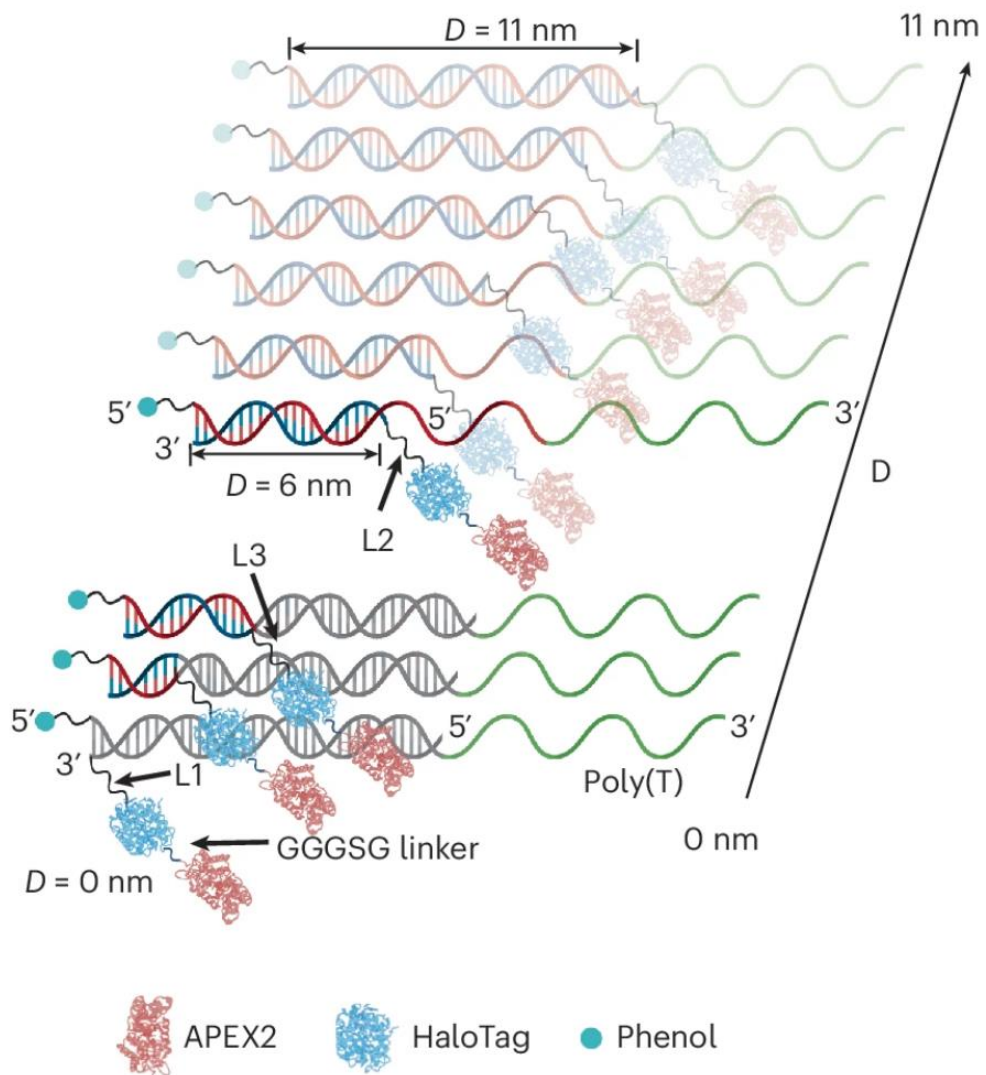
Measurement via DNA nanoruler



for APEX:



notable labeling occurs within **55 nm**
highest labeling occurs within **11 nm**

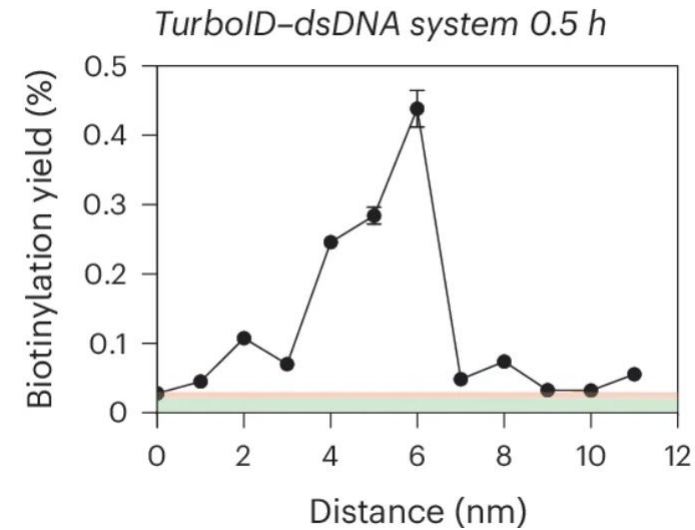
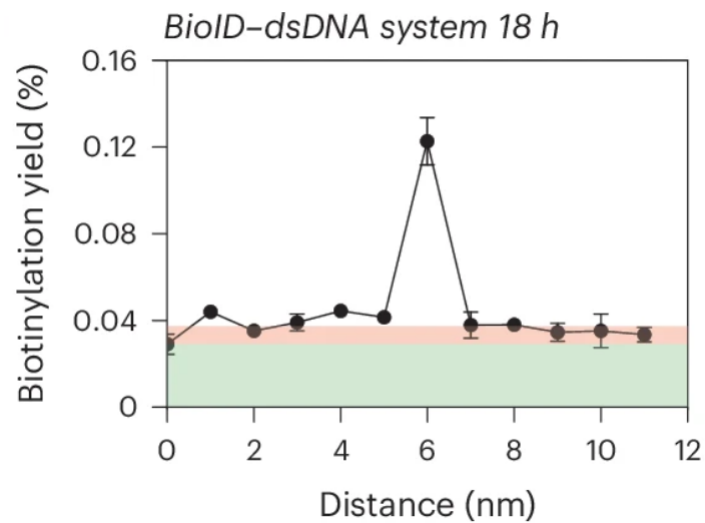


dual labeling mechanism of APEX:

strong contact labeling within 10 nm

diffusive labeling within 250 nm

BioAMP system: BioID & TurboID



mostly *contact* labeling mechanism

- *Introduction*
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- *Photocatalytic proximity labeling*
- *Measurement & mechanistic discussion*
- **Summary**

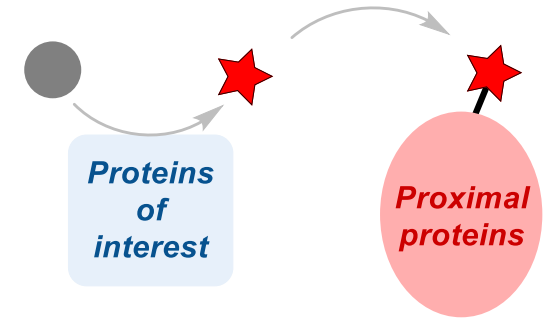
Abundant toolkit

Various accuracy

Nice biocompatibility



Powerful tool for PPI studies



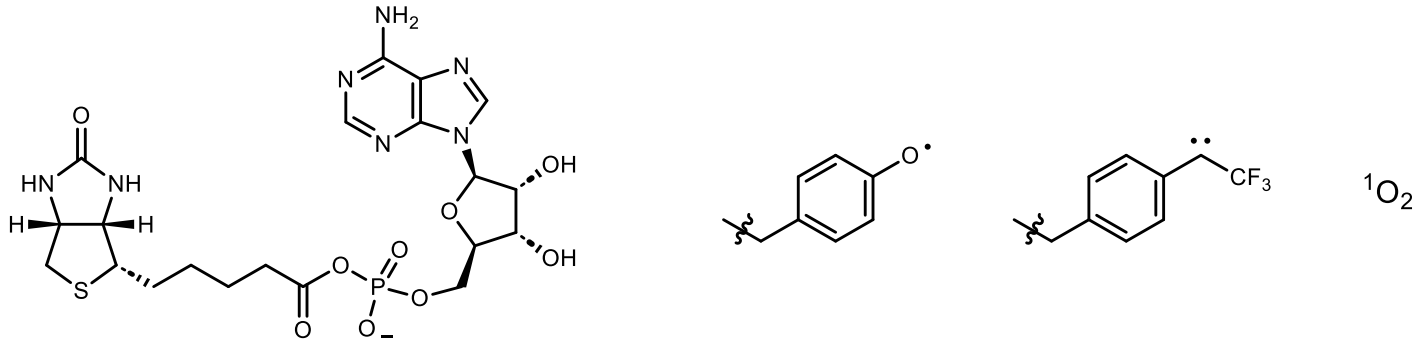
- *Further development of catalytic systems*
- *Optimization of catalyst introducing methods*
- *Acute resolution control*

Acknowledgements



- *Prof. Yan Xu*
- *All members in Xu Group*
- *Prof. Yang Jiao*
- *Everyone here*

1. Explain the labeling selectivity of the following intermediates:



2. Generally, how do we colocalize enzyme / photocatalyst with POIs?

3. What is the upper limit of labeling accuracy (low limit of labeling radius)?