

*Proximity- and affinity- based labeling methods
for interactome mapping*

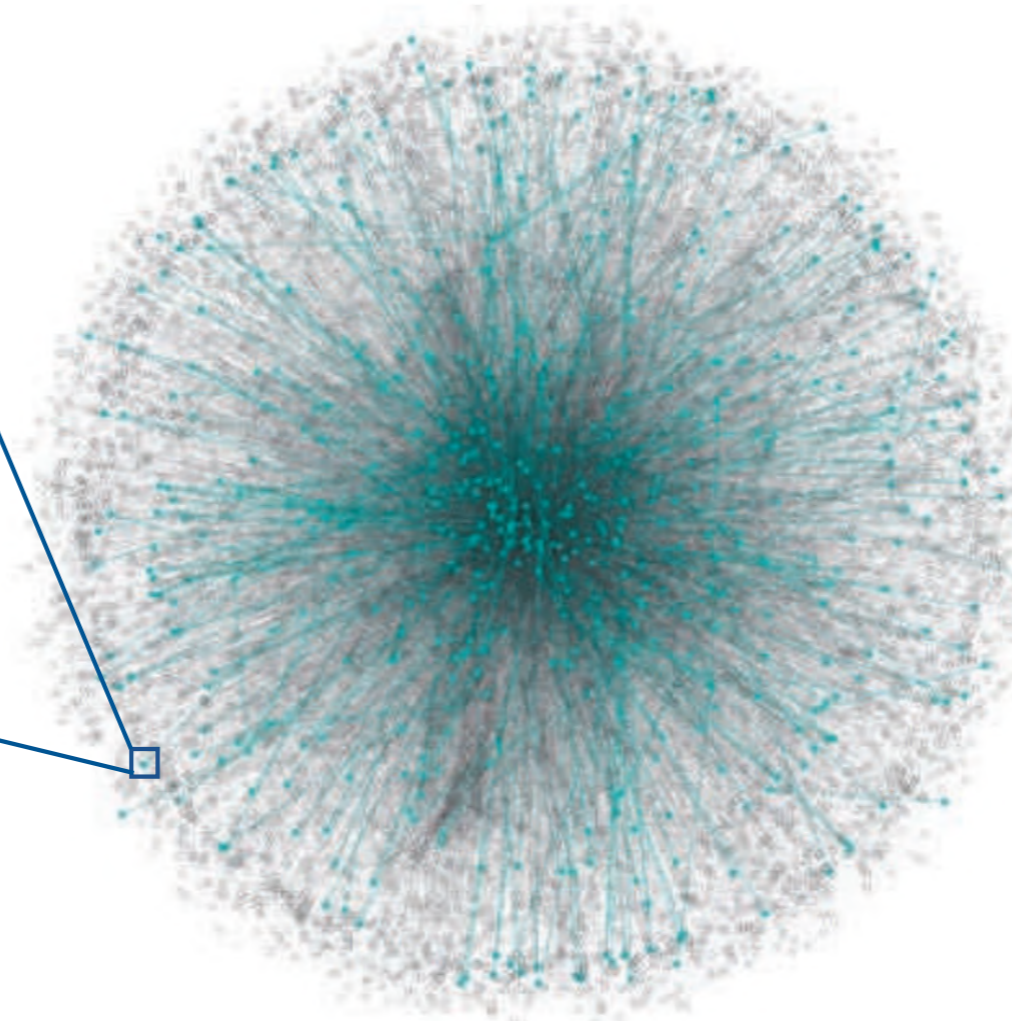
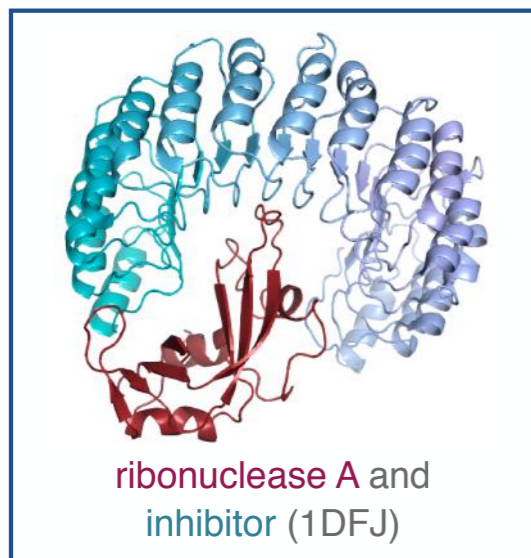


Beryl X. Li
March 13th, 2019

Proximity- and affinity-based labeling methods

significance

Human protein-protein interactome
contains a quarter million interactions
between 22,000 proteins



Classical methods

- yeast two-hybrid assay
host incompatibility
low through-put
- affinity-complex purification
non-physiological conditions
limited to high affinity interactions



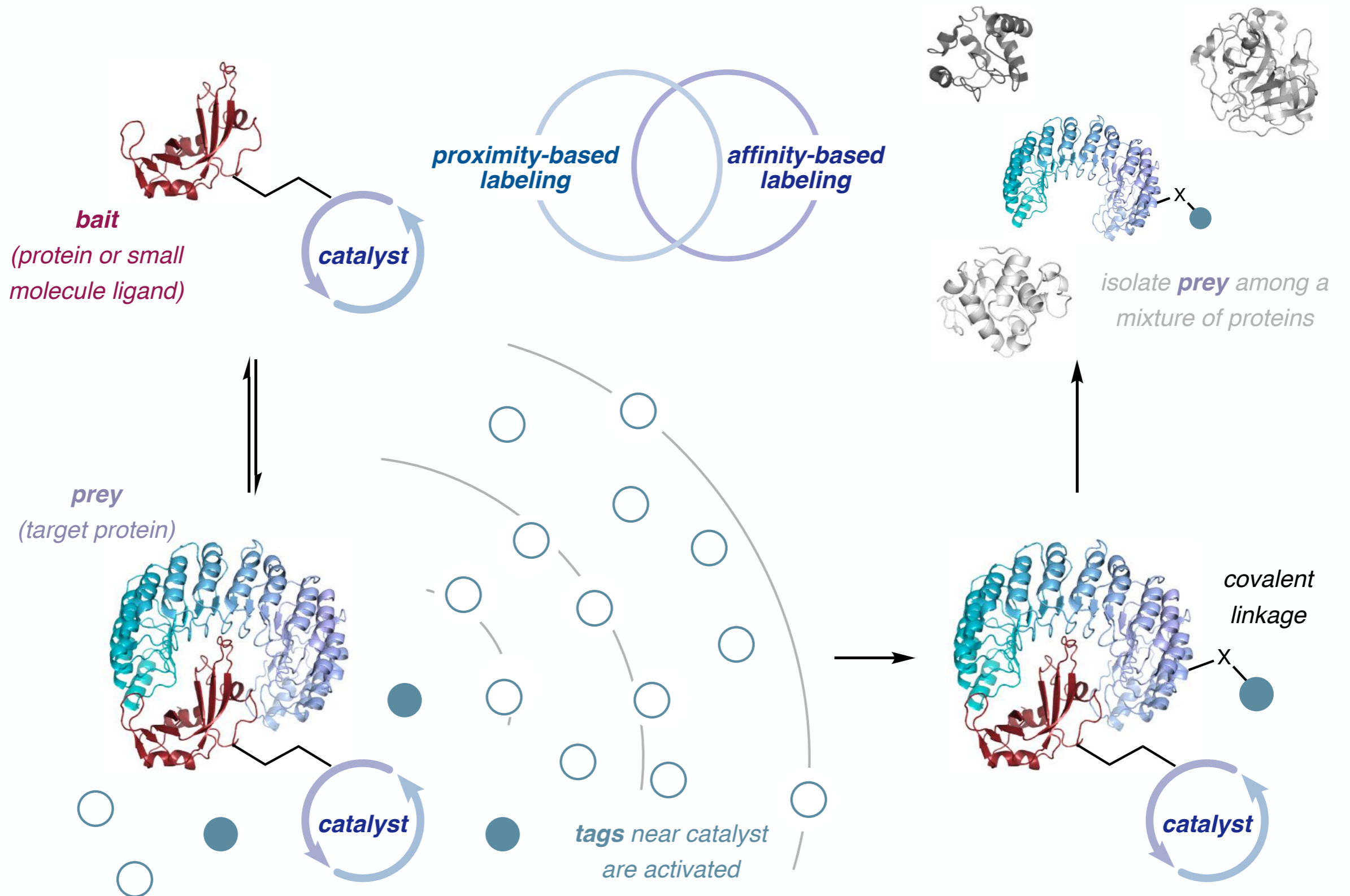
Proximity labeling

- spatial resolution
- temporal resolution
- native environment

Protein-protein and protein ligand interactions elucidate biological processes and facilitate drug-target identification

Proximity- and affinity-based labeling methods

general mechanism



Proximity- and affinity-based labeling methods

outline

Prevalent enzymatic methods

- BiolD: (Biotin IDentification)

 - Split BiolD

 - BiolD2, BASU, TurboID

- APEX (Enhanced Ascorbate PeroXidase)

 - temporal resolution

Trinkle-Mulcahy, L. *F1000 Research* **2019**, *8*, 135.

Kim, D.I.; Roux, K.J. *Trends Cell Biol.* **2016**, *26* (11), 804–7.

Affinity-guided catalysts

- Ligand-tethered DMAP

- MoAL method (MOdular Affinity Labeling)

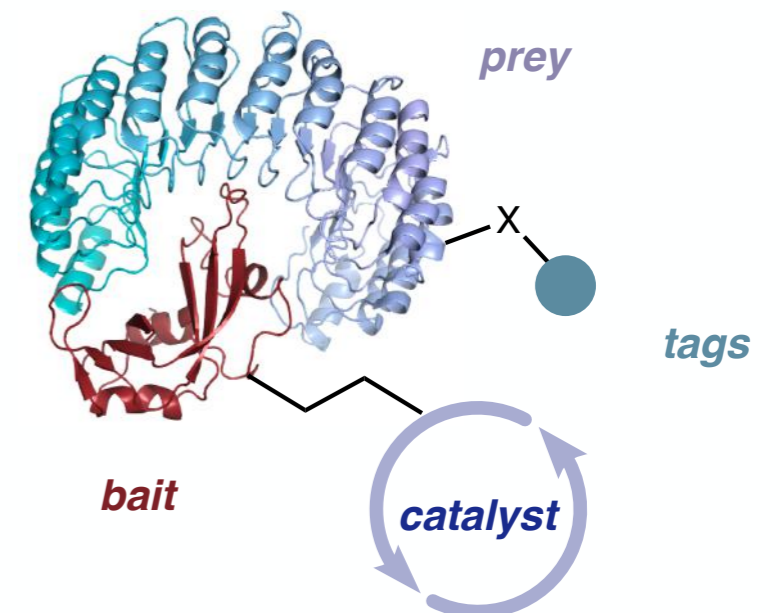
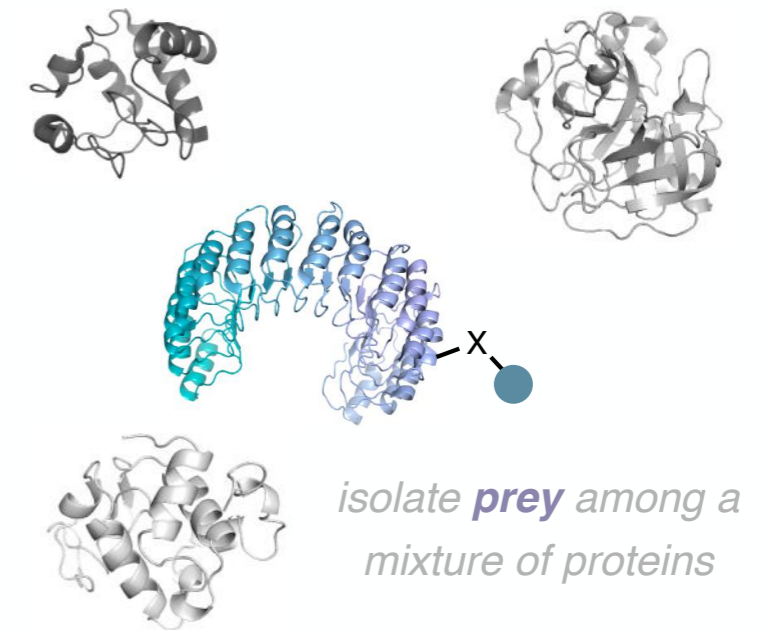
 - Local SET catalysis

- Survey of photo-affinity labeling agents

Chen, Y.; Topp, E.M. *J. Pharm. Sci.* **2019**, *108* (2), 791–7.

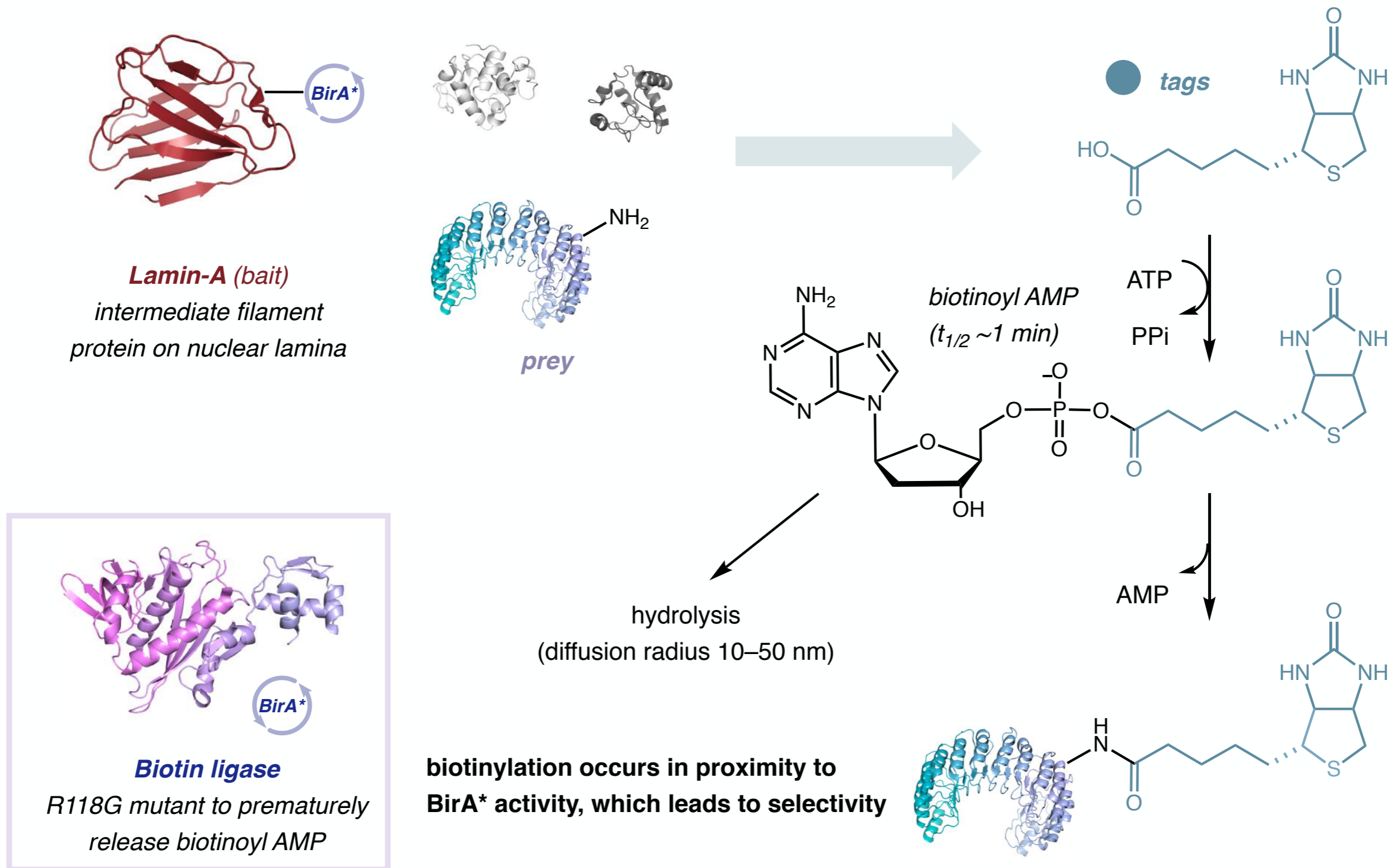
Murale, D.P.; Hong, S.C.; Haque, M.; Lee, J.-S. *Proteome Science* **2017**, *15*:14.

Chen, Y.; Hu, C. *Tetrahedron Lett.* **2015**, *56*, 884–8.



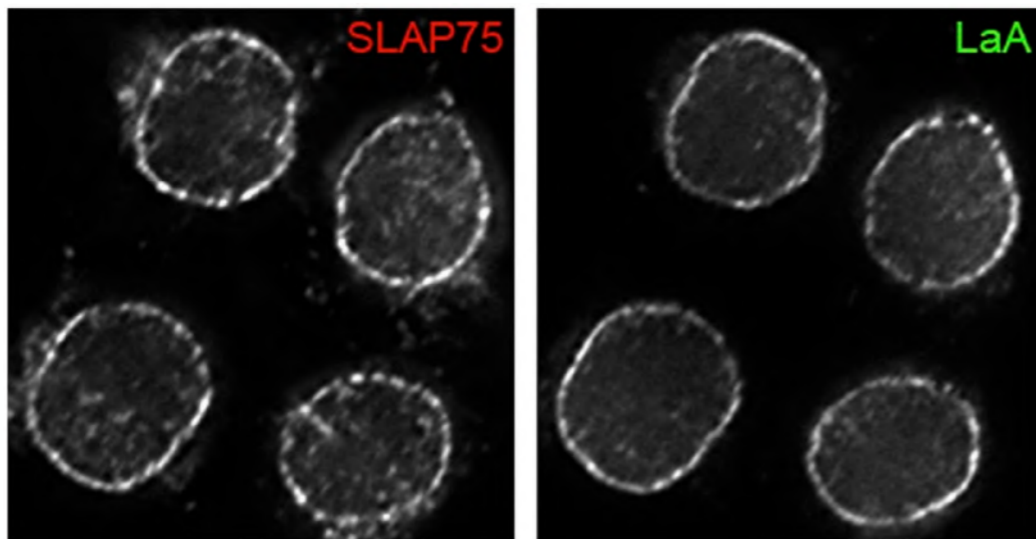
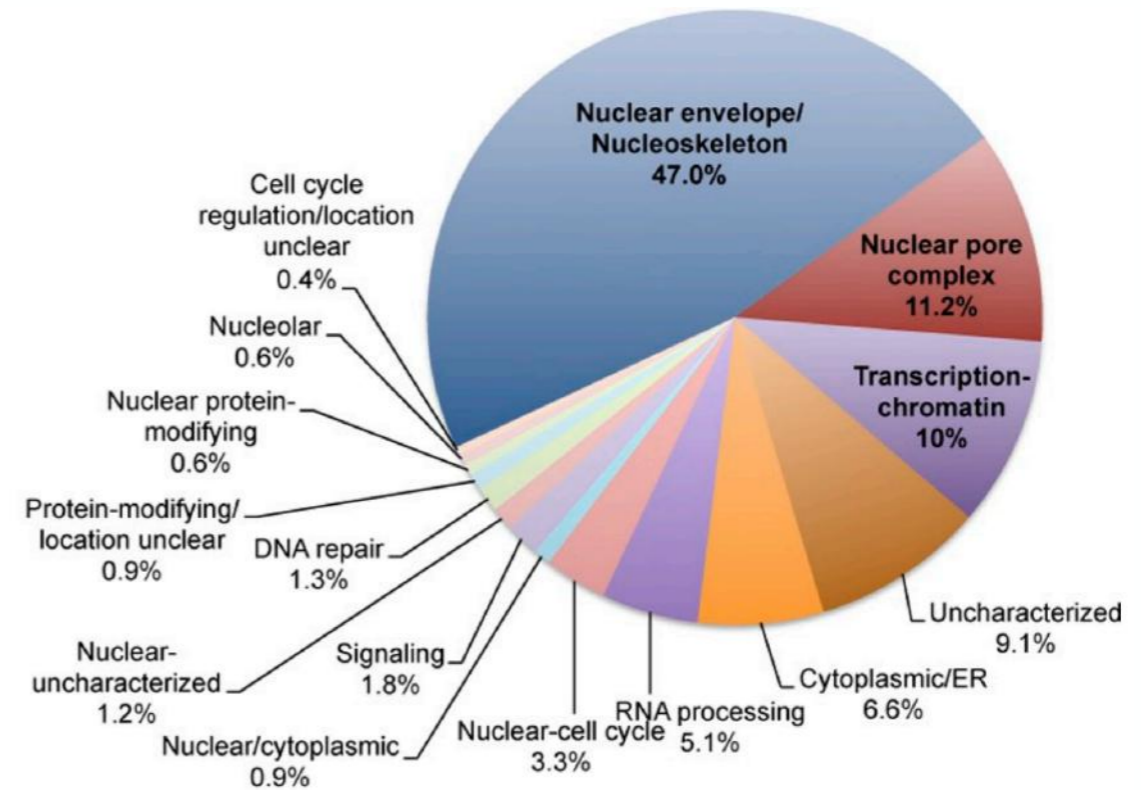
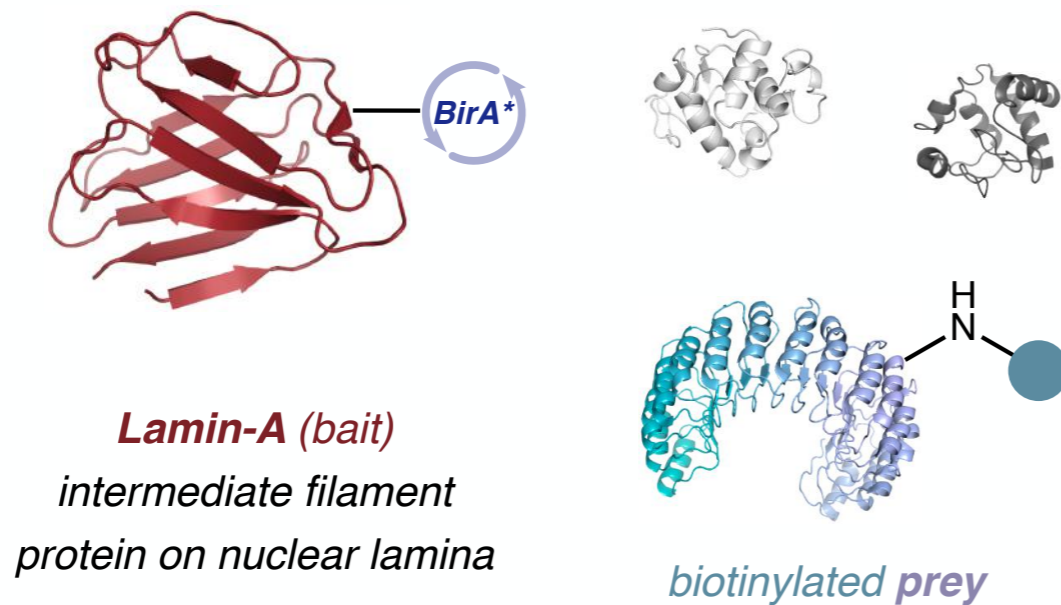
Proximity- and affinity-based labeling methods

Biotin IDentification (BioID)



Proximity- and affinity-based labeling methods

Biotin IDentification (BioID)



identify **SLAP75**, novel nuclear envelope constituent



no predicted transmembrane domain
no clues in sequence motif

- BirA* is 35 kDa, might impact native binding
- long incubation times lack temporal resolution
- cells can express bait–ligase conjugate *in vivo*

Proximity- and affinity-based labeling methods

variations of BioID

Split-BioID

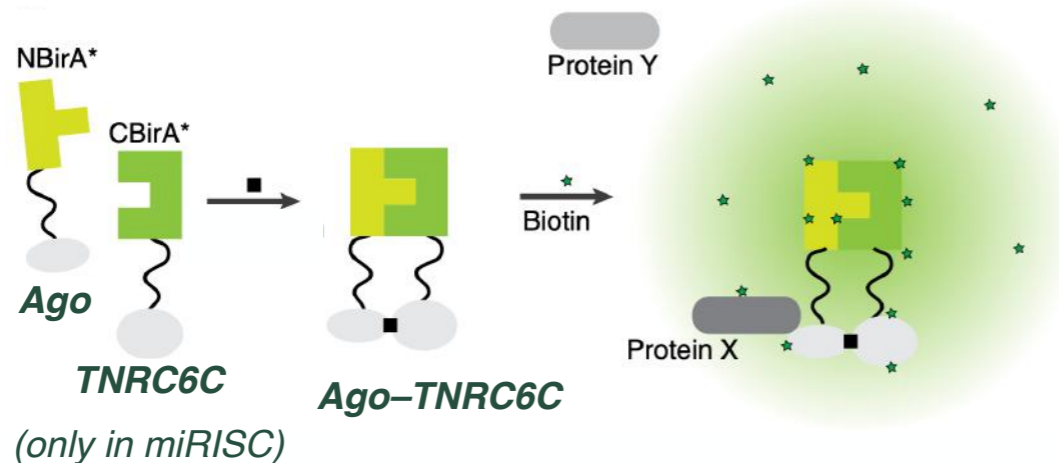
Ago protein plays at least two roles in gene-silencing

- 1) represses mRNA in miRISC complex
- 2) load miRNA in RISC-loading complex

how to assign novel identified proteins to a specific step?



protein fragment complementation assay



identified novel regulator **GIGYF2** with split BioID system, which previously AP/MS did not show association with Ago or TNRC6C

Bethune, J. *Nat. Commun.* **2017**, *8*, 15690.

BioID2

- biotin ligase from *Aquifex aeolicus* which naturally lacks N-terminus
- 27 kDa (vs. original 35 kDa)

Roux, K.J. *et al. Mol. Biol. Cell* **2016**, *27* (8), 1188–96.

BASU

- biotin ligase from *Bacillus subtilis*
- removing N-terminus did not impact activity; 28 kDa

Khavari, P.A. *et al. Nat. Methods* **2018**, *15* (3), 207–212.

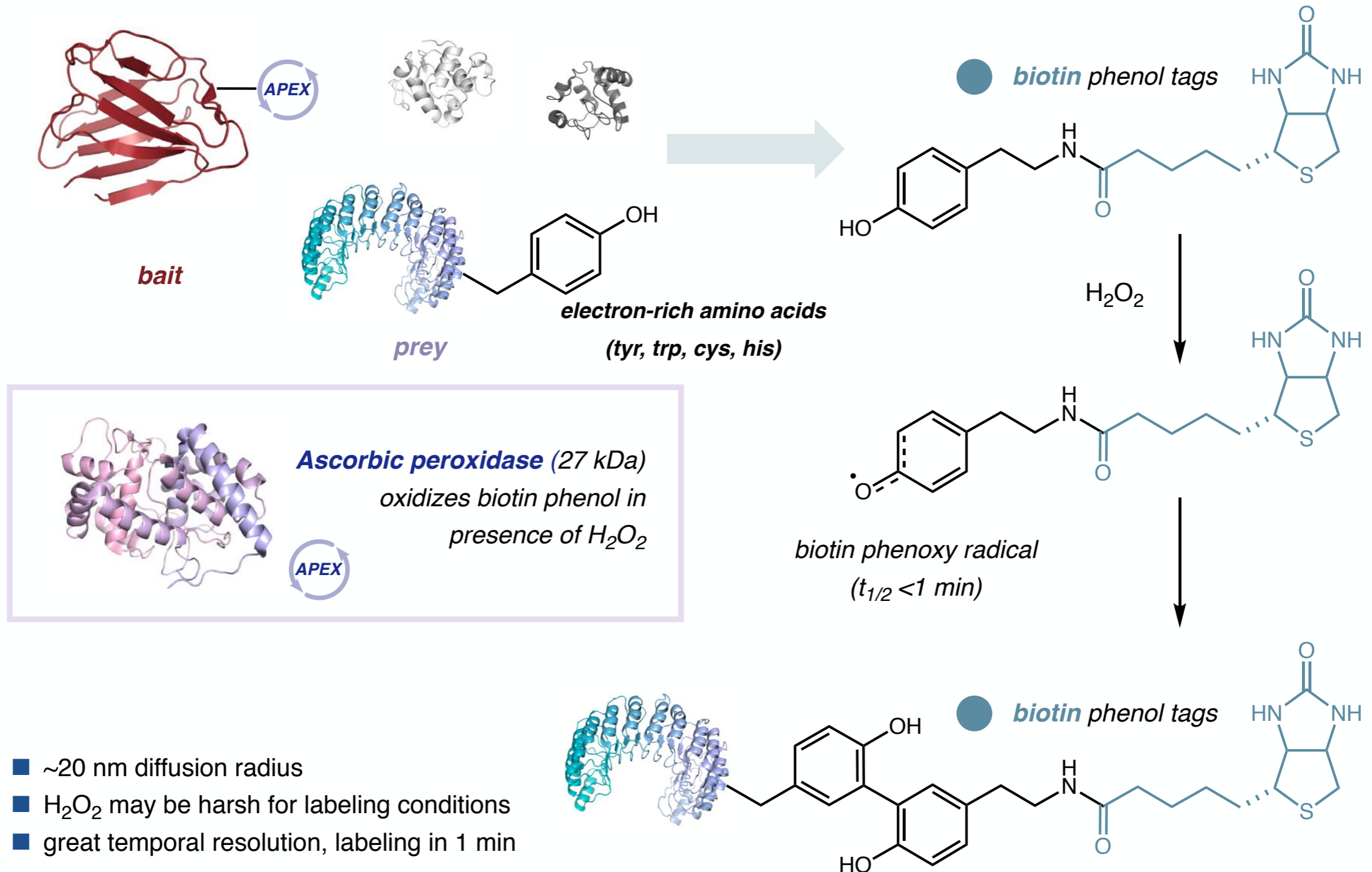
TurboID and MiniTurbo

- 14 and mutations from original BirA*
- MiniTurbo is 28 kDa
- original efficient labeling in minutes instead of hours
- ideal combination of *temporal resolution* and *non-toxicity*

Ting, A.Y. *et al. Nat. Biotechnol.* **2018**, *36* (9), 880–7.

Proximity- and affinity-based labeling methods

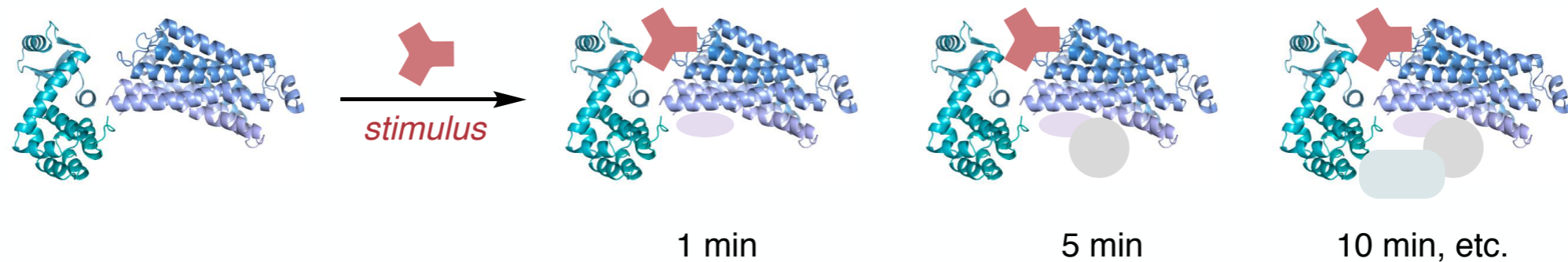
APEX (Ascorbic peroxidase)



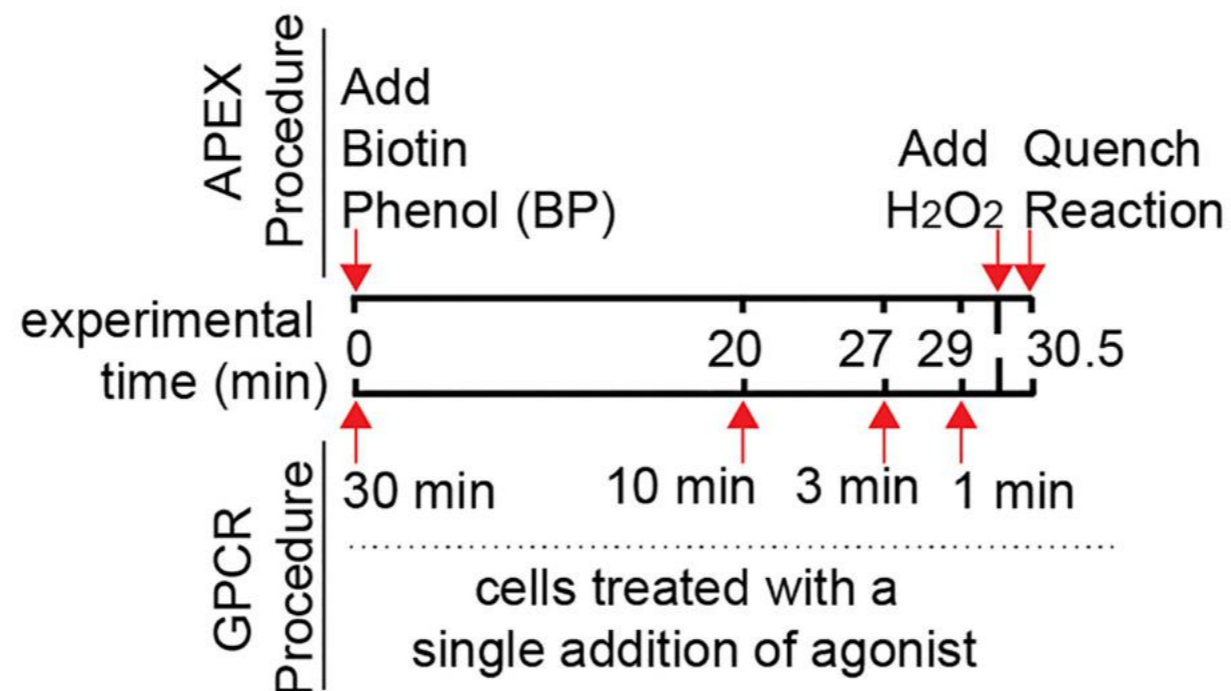
Proximity- and affinity-based labeling methods

APEX resolves GPCR networks in vivo

G-protein-coupled receptors (GPCRs) mediates physiological responses to many *stimuli* (e.g., hormones, neurotransmitters, light, etc.)



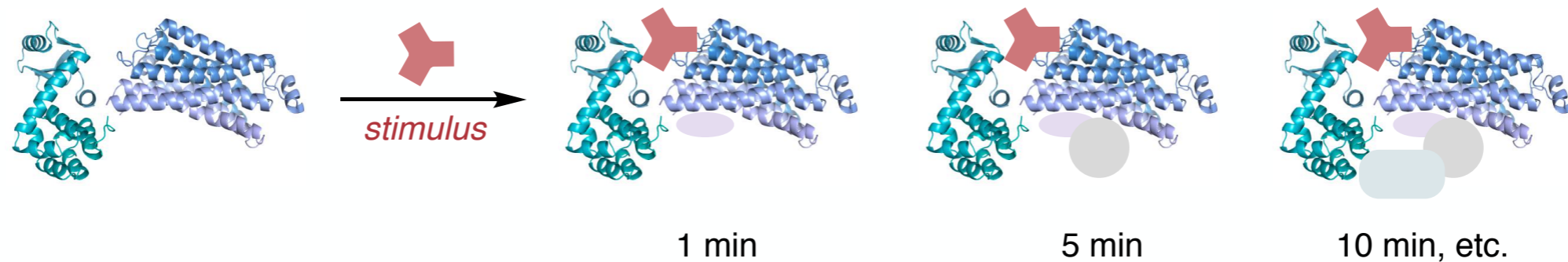
How to track the series of cascading protein-protein interactions following agonist binding?



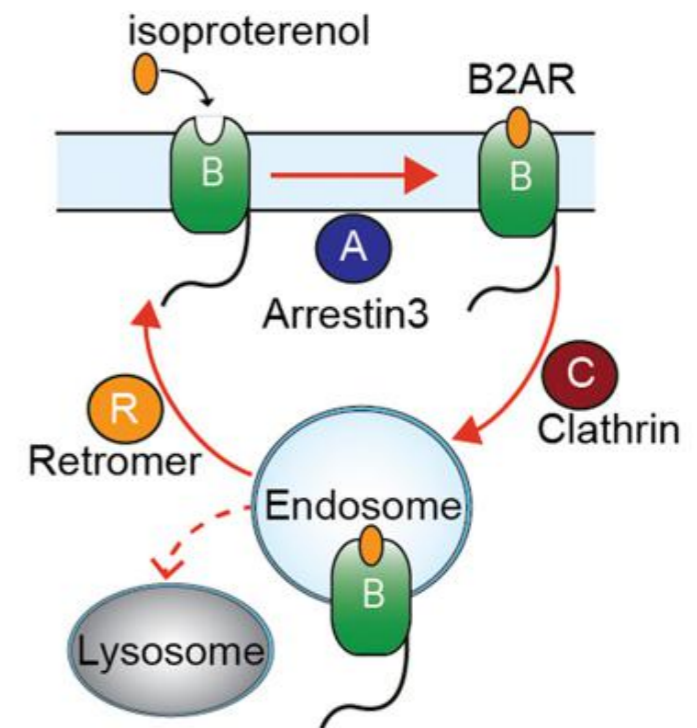
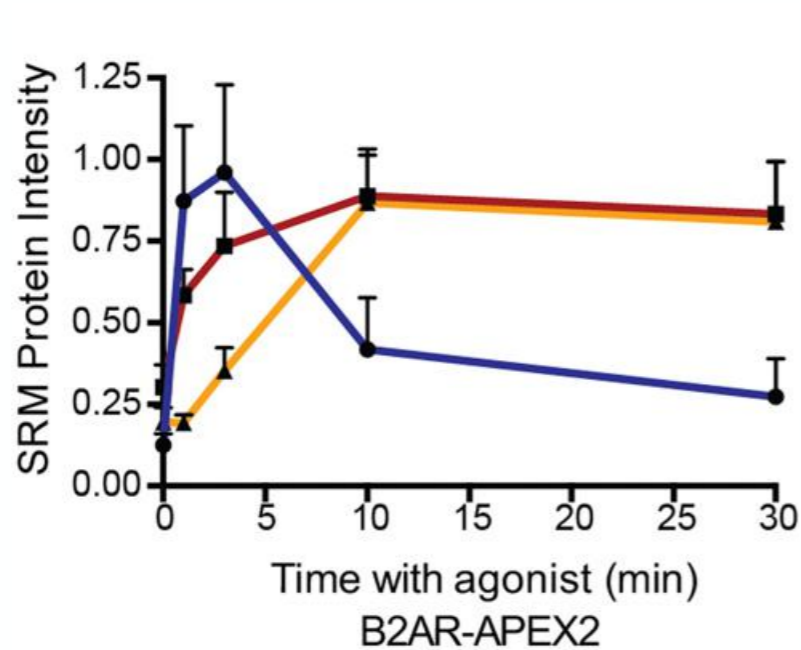
Proximity- and affinity-based labeling methods

APEX resolves GPCR networks in vivo

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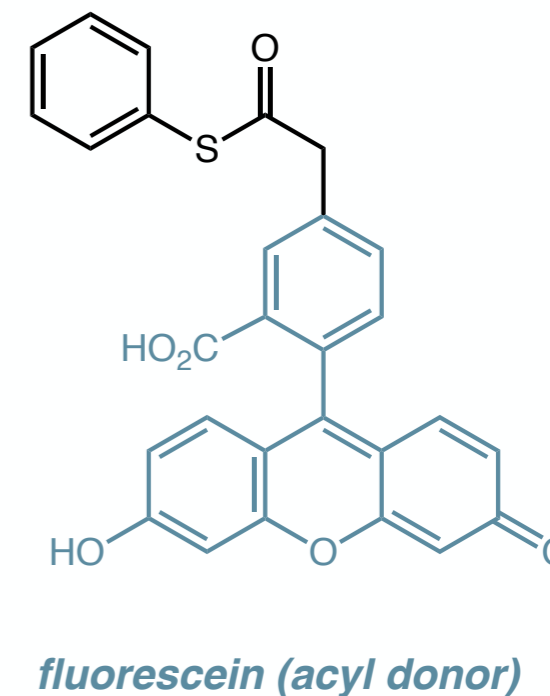
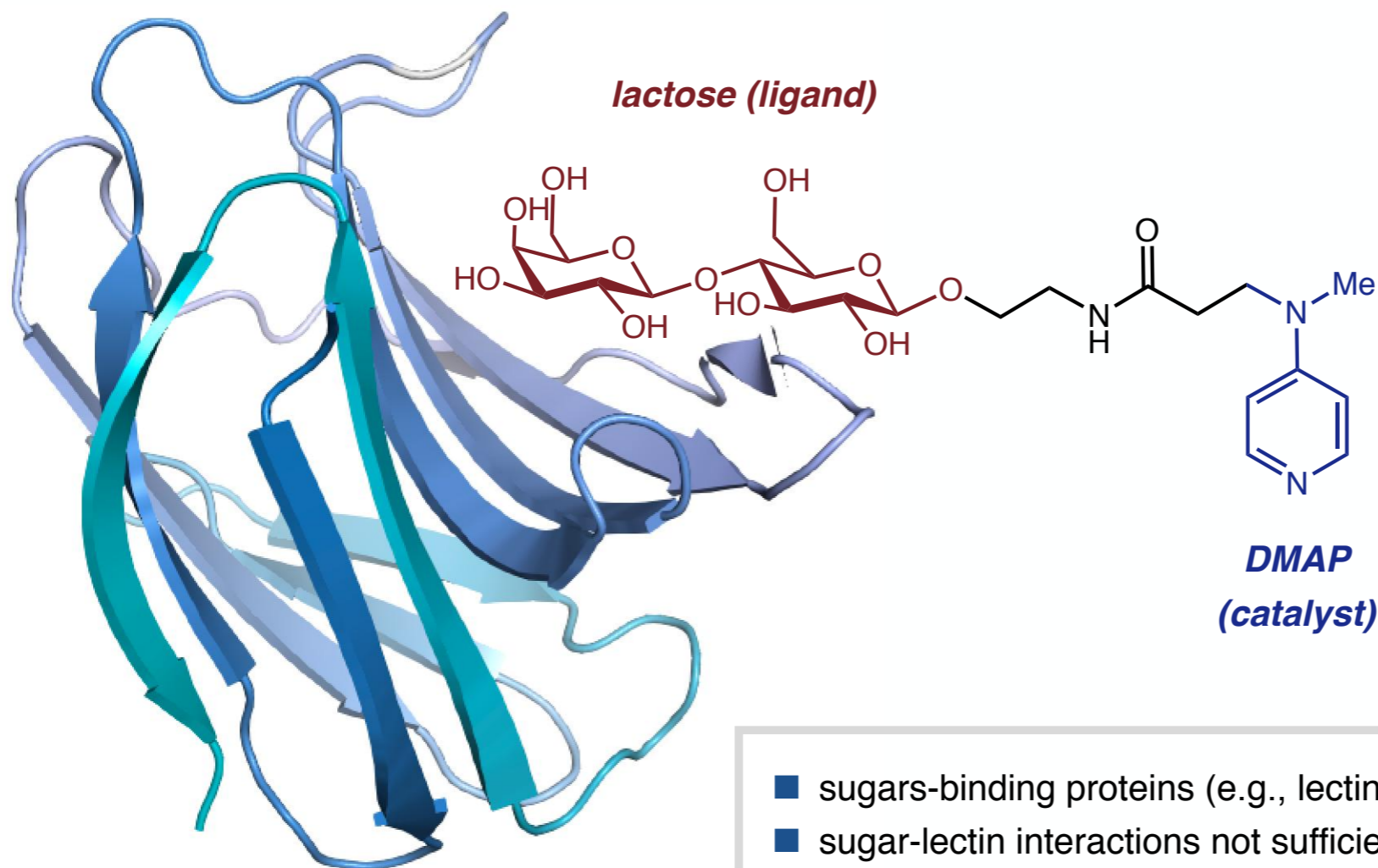


How to track the series of cascading protein-protein interactions following agonist binding?



Proximity- and affinity-based labeling methods

acylation of lectins by ligand-tethered DMAP catalysts



Congerin II (animal lectin with high lactose affinity)

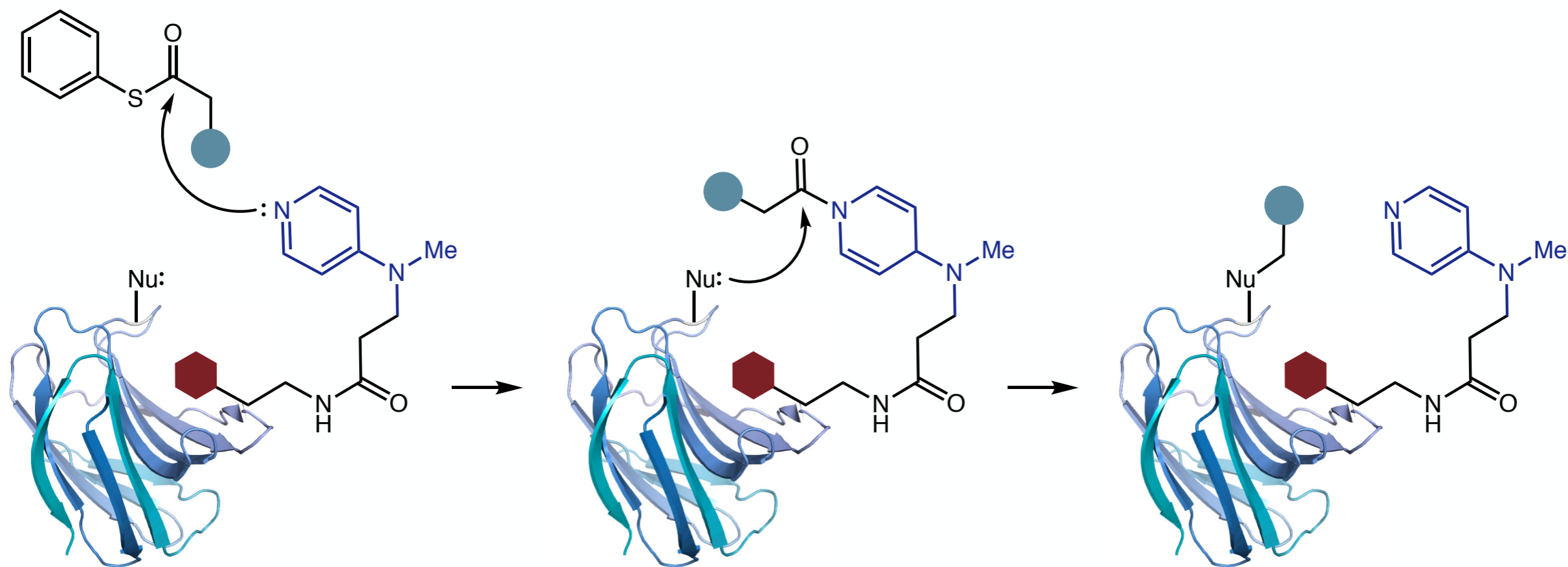
- sugars-binding proteins (e.g., lectins) play important roles in glycobiology
- sugar-lectin interactions not sufficiently strong for affinity purification



use **DMAP** as acyl-transfer catalyst to *covalently* tag sugar-binding proteins

Proximity- and affinity-based labeling methods

acylation of lectins by ligand-tethered DMAP catalysts



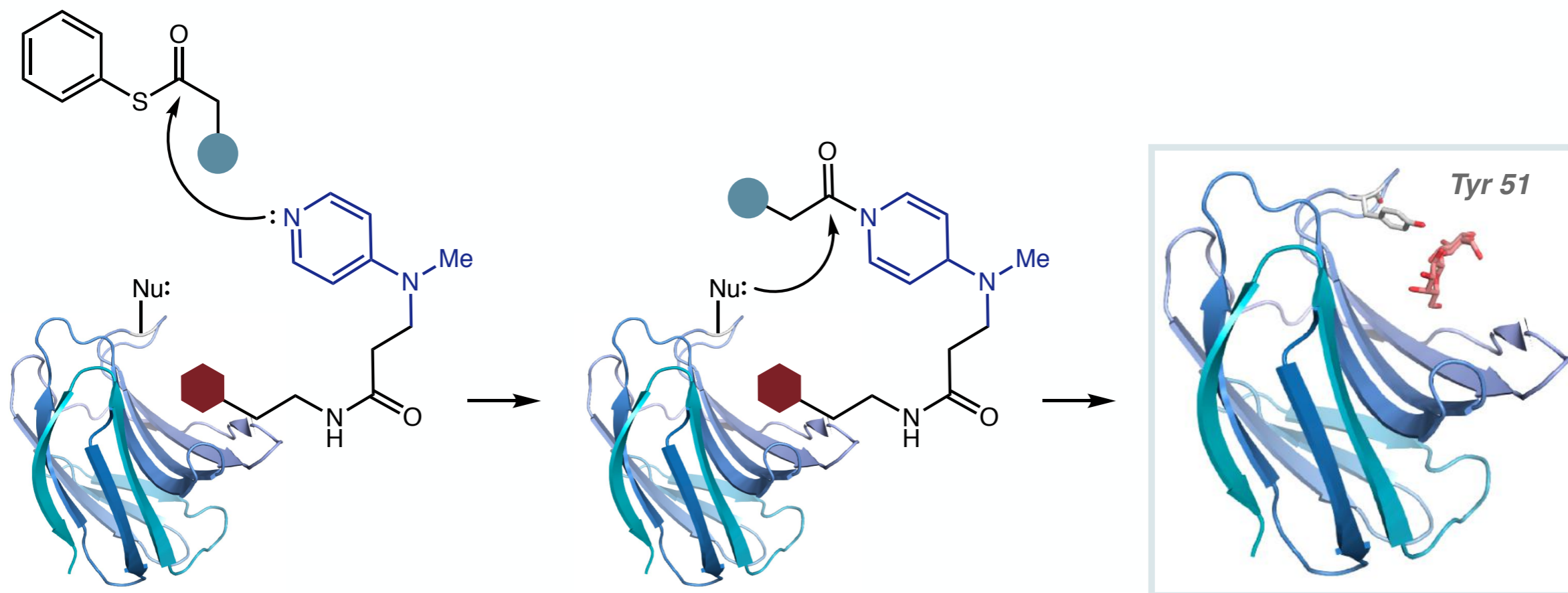
35% yield in 3 h

 ***lactose (ligand)***

 ***fluorescein (acyl donor)***

Proximity- and affinity-based labeling methods

acylation of lectins by ligand-tethered DMAP catalysts



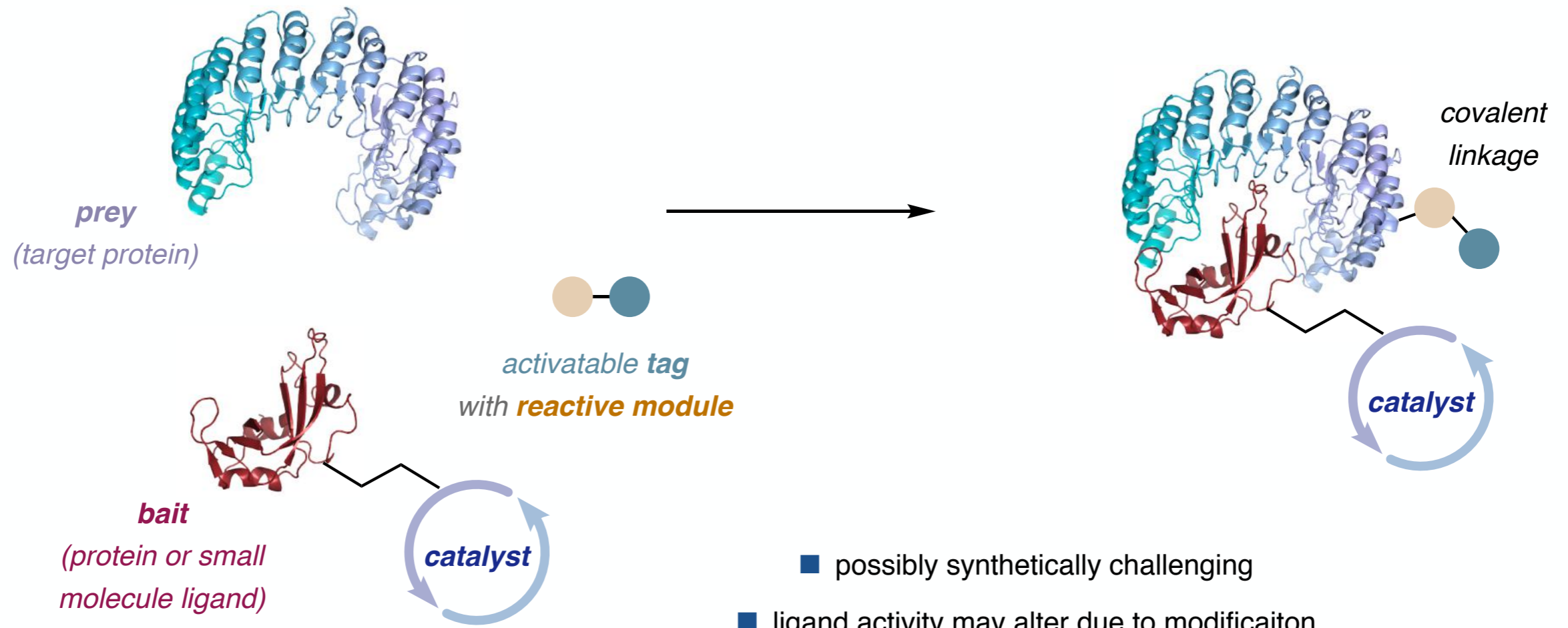
35% yield in 3 h
single product

 **lactose (ligand)**

 **fluorescein (acyl donor)**

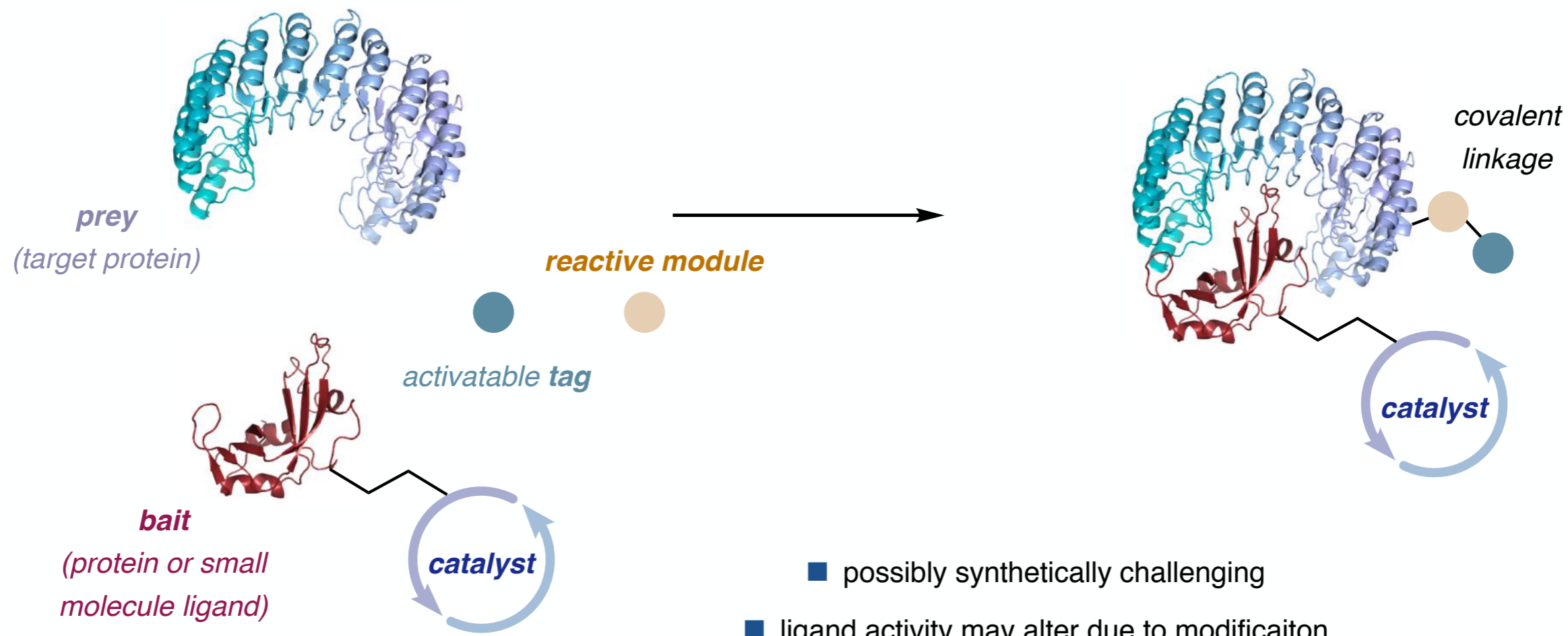
Proximity- and affinity-based labeling methods

modular affinity labeling based on catalytic amidation



Proximity- and affinity-based labeling methods

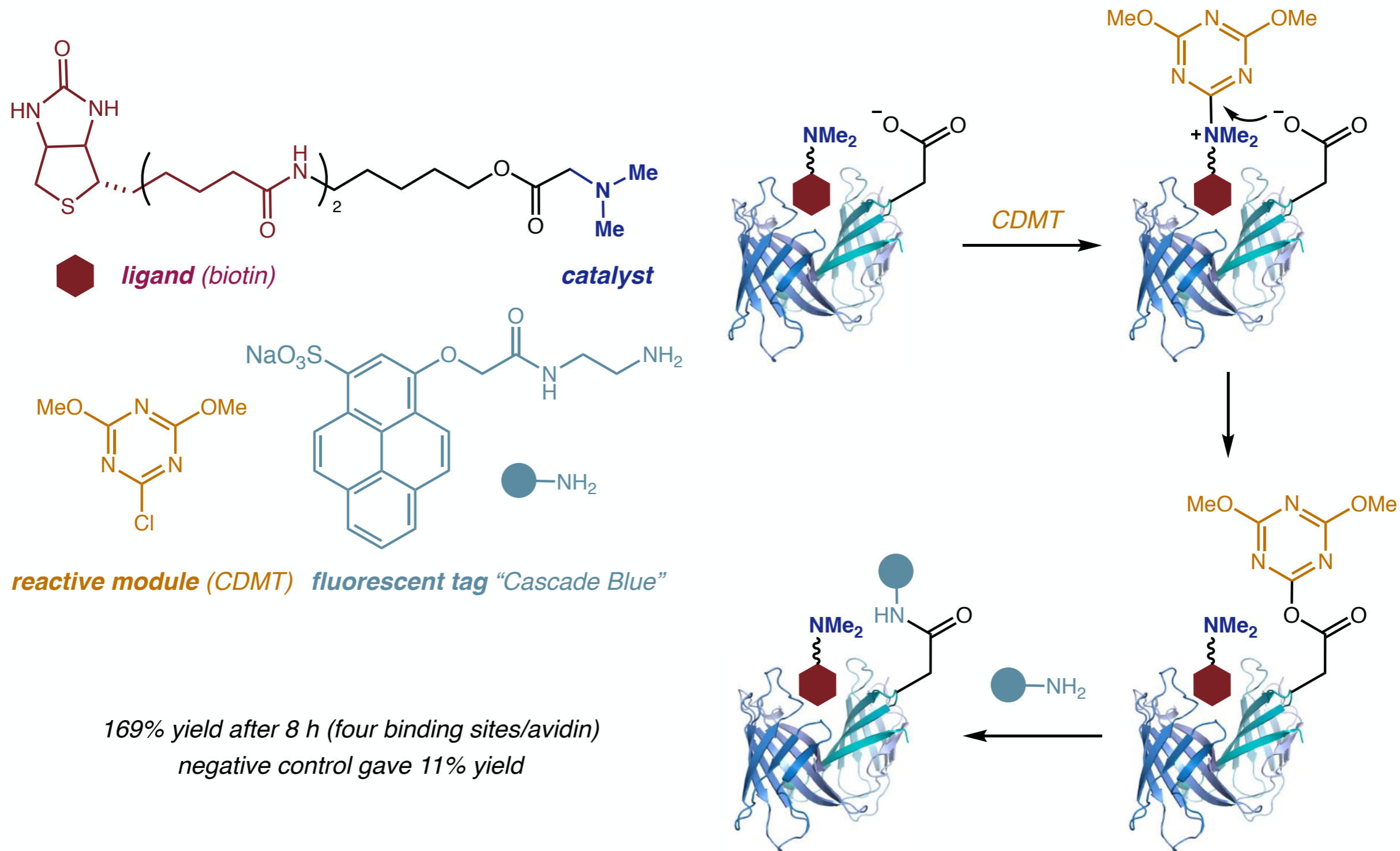
modular affinity labeling based on catalytic amidation



modular approach, where **ligand**, **tags**, and **reactive module** are completely separated

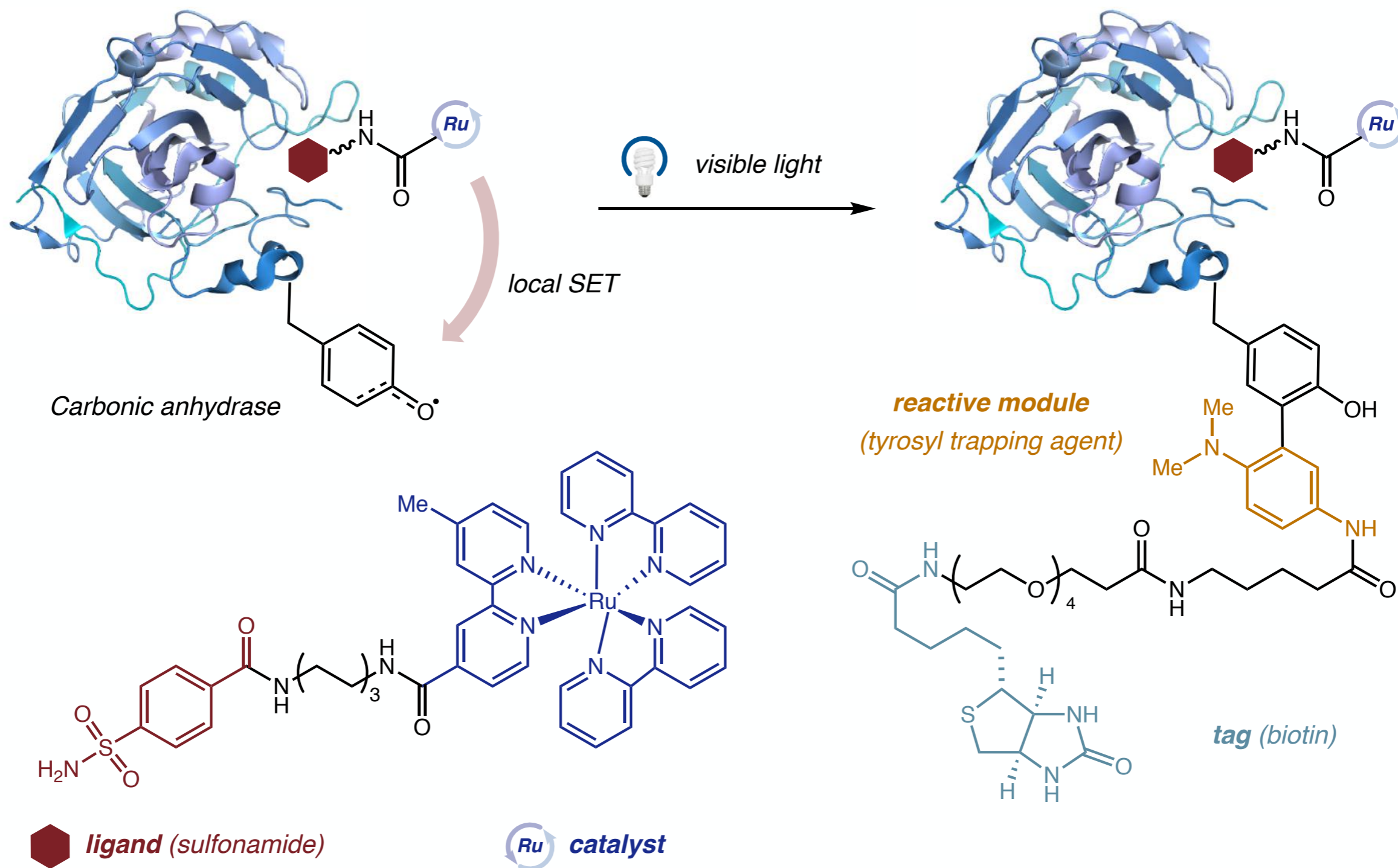
Proximity- and affinity-based labeling methods

modular affinity labeling based on catalytic amidation



Proximity- and affinity-based labeling methods

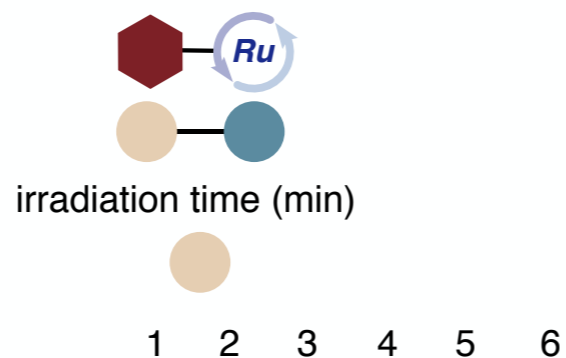
photoredox proximity-based labeling



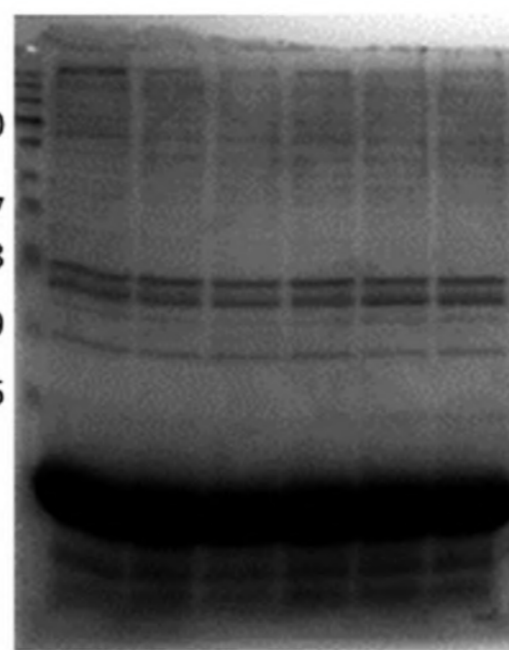
Proximity- and affinity-based labeling methods

photoredox proximity-based labeling

| | | | | | |
|---|---|----|---|----|----|
| - | - | - | + | + | + |
| - | + | + | + | + | + |
| - | - | 15 | - | 15 | 15 |
| - | - | - | - | - | + |
| 1 | 2 | 3 | 4 | 5 | 6 |

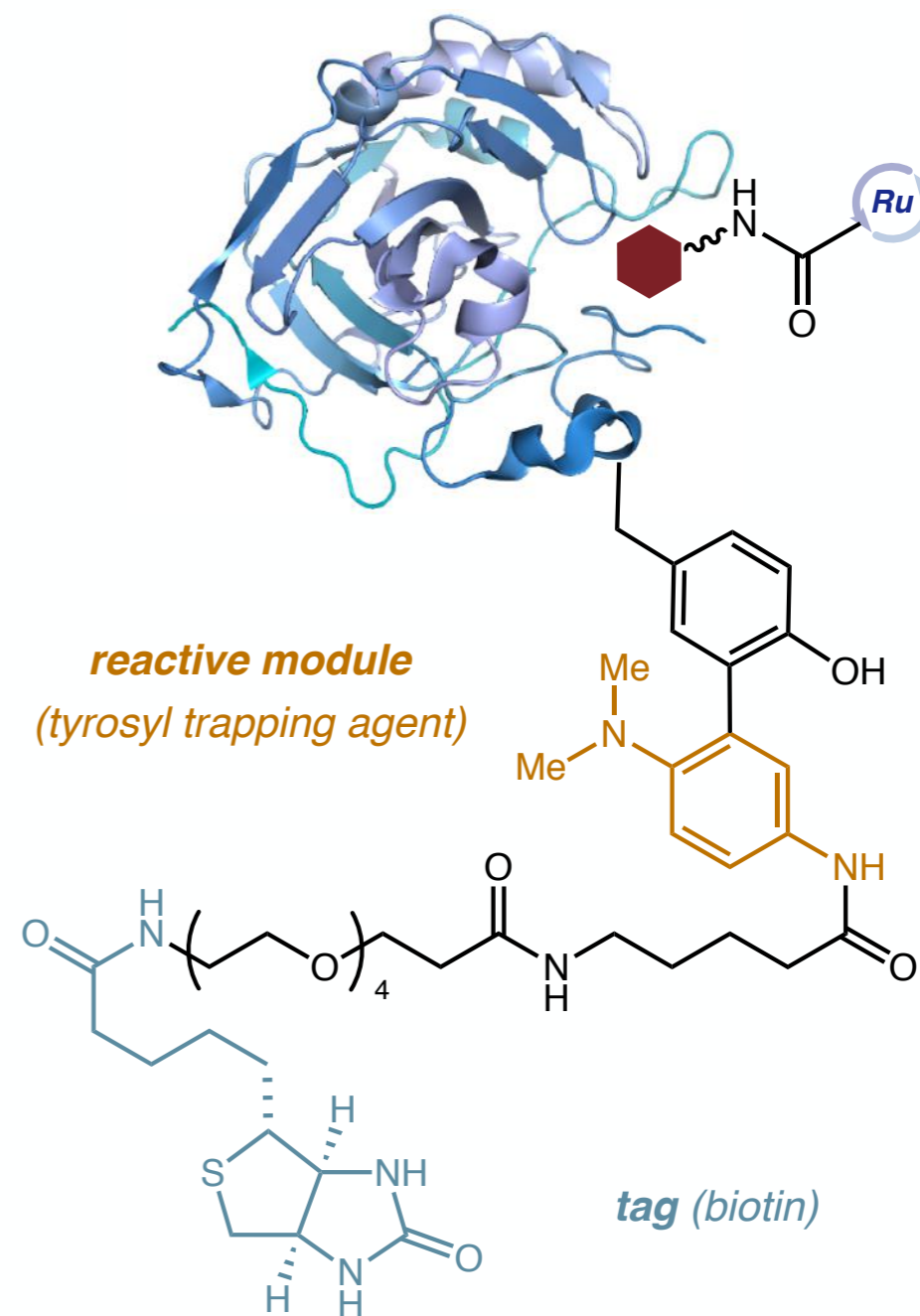


streptavidin-HRP



Coomassie brilliant blue

selectively label carbonic anhydrase in mouse erythrocyte lysate



Proximity- and affinity-based labeling methods

photo-affinity labeling

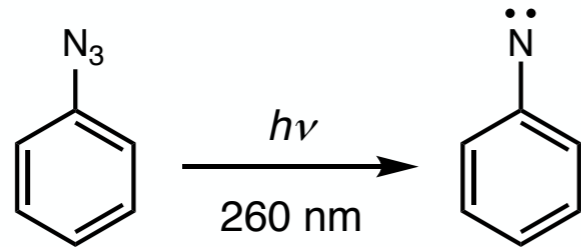
Advantages

- reactive under specific activation conditions
 - (potentially) mild conditions
- covalently modify targets for easy purification

Applications

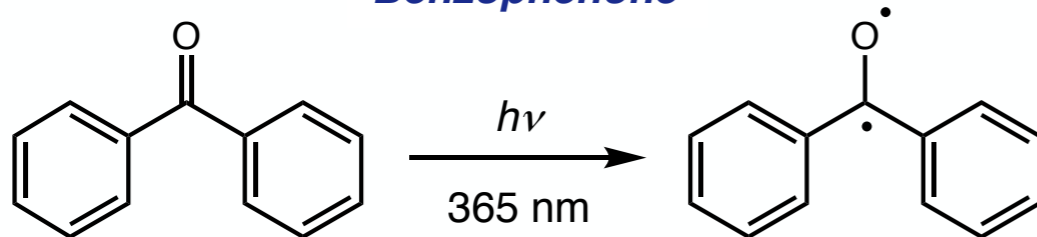
- identification of membrane protein targets
- elucidation of protein structure in solution
- characterization of proteins in pharmaceutical solids

Nitrenes



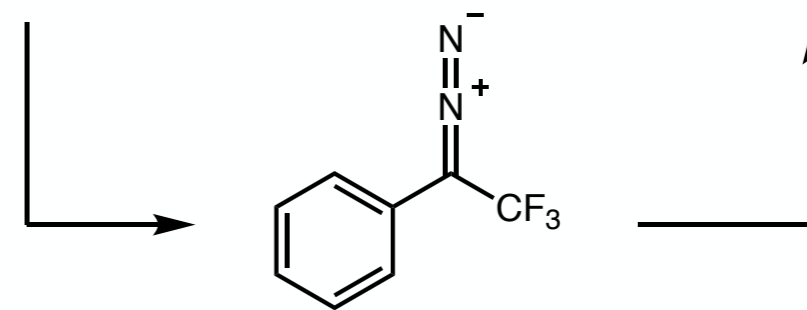
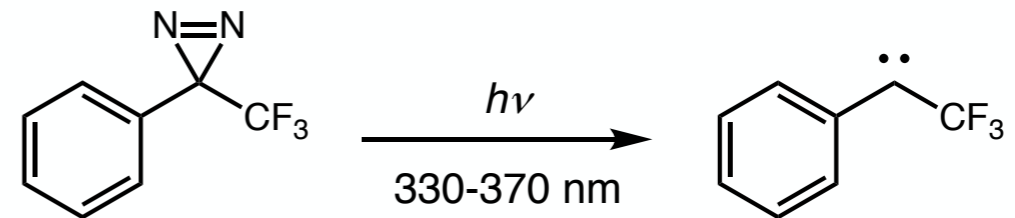
- insertion into C–H, N–H, or O–H bonds
- ring-expansion with nucleophilic addition
- preference for cysteine and aromatic residues

Benzophenone



- triplet ketyl biradical
- high affinity towards methionine
- relatively long wavelength activation:
less damaging to proteins

Carbenes



stable diazo isomer

- insertion into C–C and X–H bonds (X = C, O, N, S)
 - addition to C=C bonds
- preference for cysteine and aromatic residues