

9. Identifying Unknown Proteins

Photoaffinity labeling

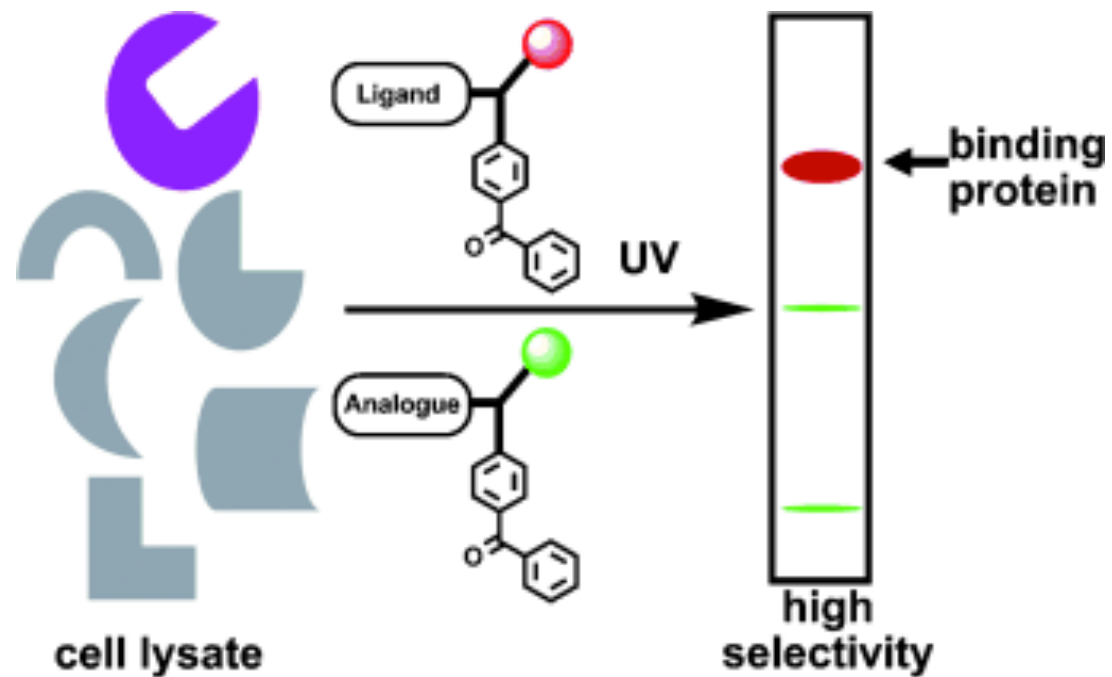
1. Serine protease inhibitors
2. Enzyme inhibitor discovery

Nakanishi et al. *Chem. Comm.* **1999**, 365

Niphakis, Cravatt *Annu. Rev. Biochem.* **2014**, 341–377

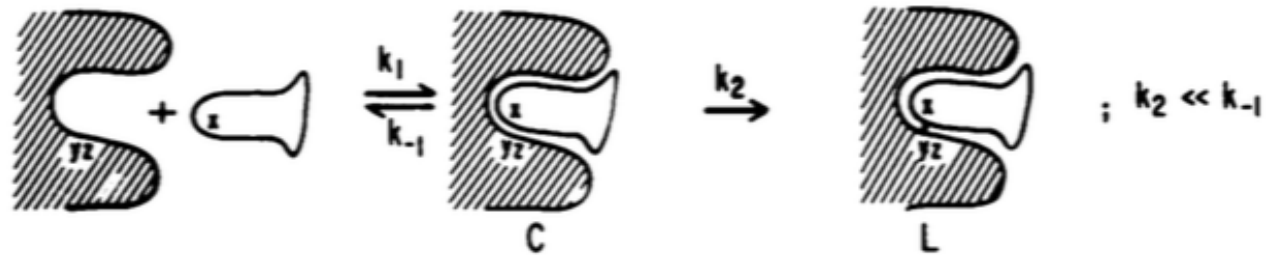
Photoaffinity labeling

Covalent binding of a ligand to its associated protein **triggered by light**

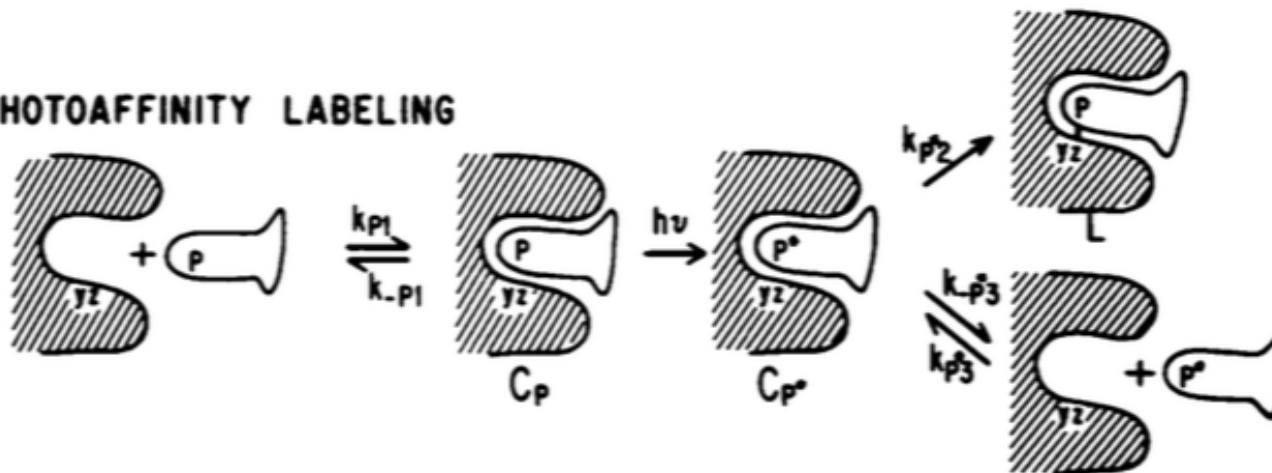


Photoaffinity labeling

AFFINITY LABELING

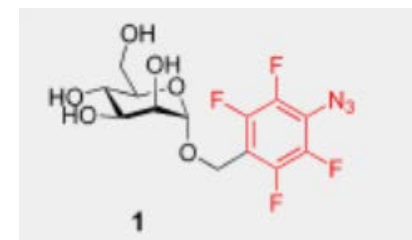
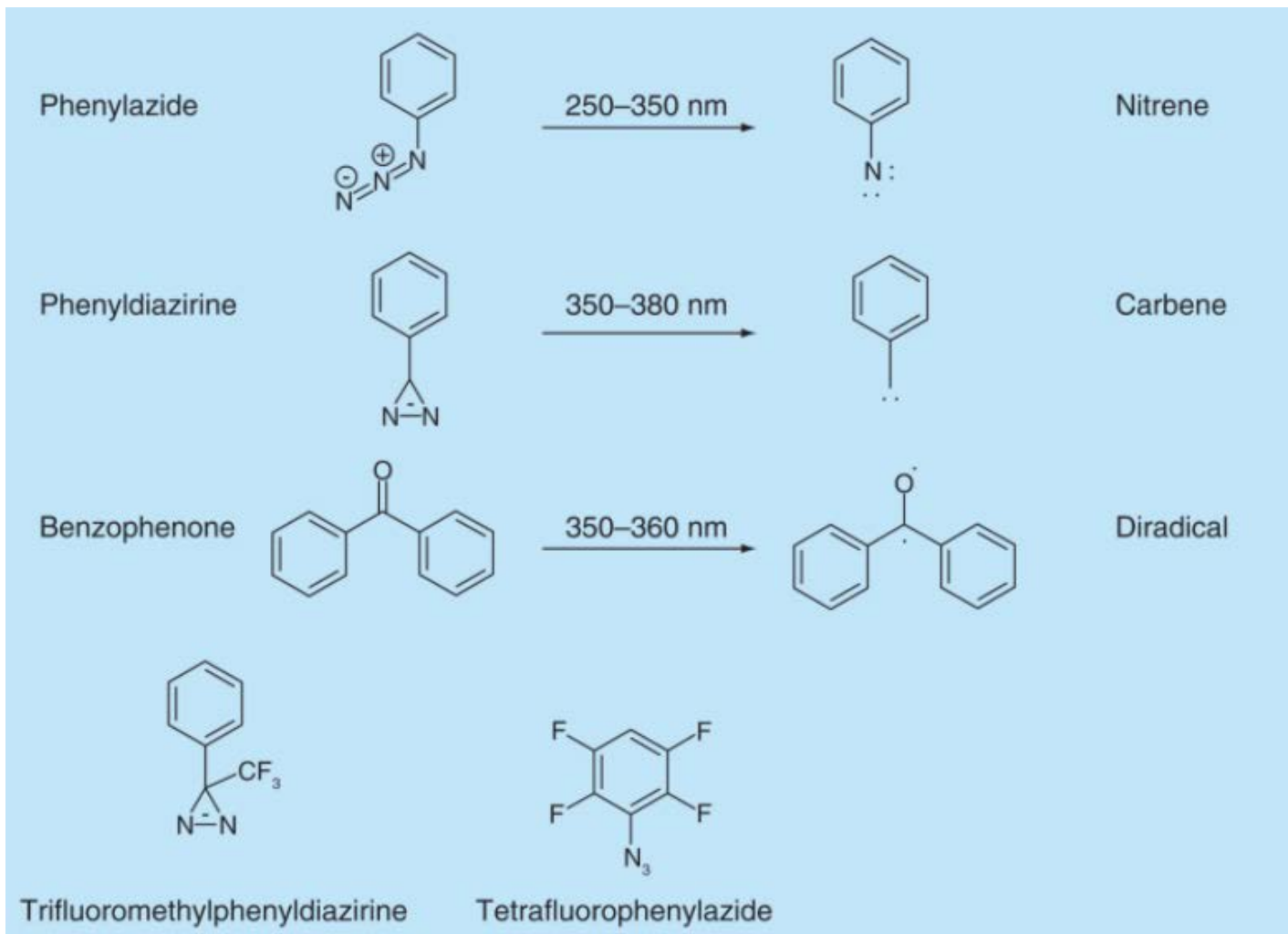


PHOTOAFFINITY LABELING



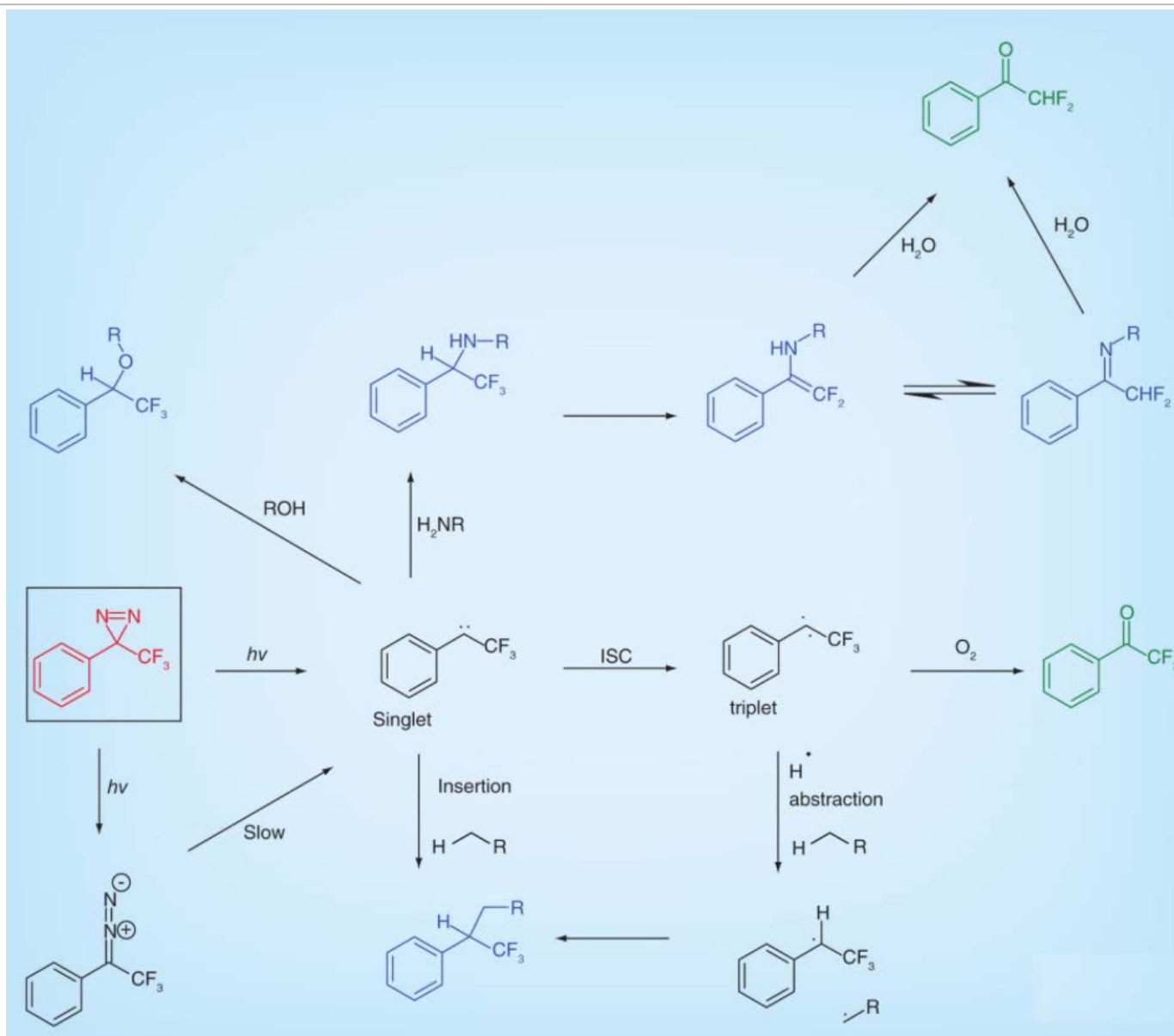
Photoaffinity labeling

Major photoaffinity functional groups and their reactive intermediates



much better than phenyl

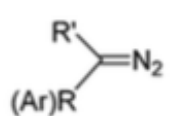
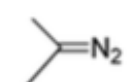
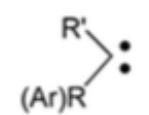
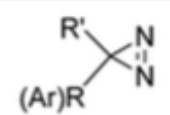

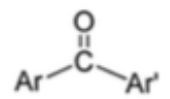
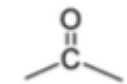
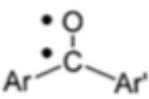
Photoaffinity labeling



Productive and nonproductive reactions of carbenes.

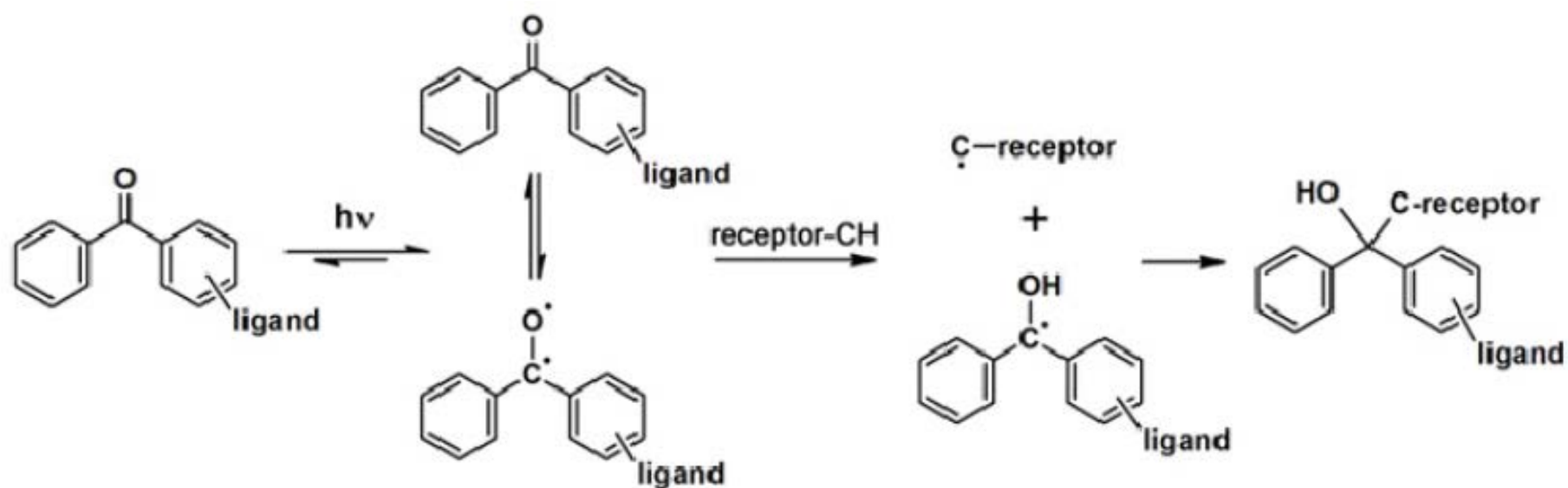
The carbene species generated by photolysis of trifluoromethylphenyldiazirine (red) undergoes many possible reactions to give productive capture of targets (blue) or destructive side reactions (green).

Photoaffinity labeling

Compound	Photoactivatable group	Reactive species	General features
$\text{Ar}-\text{N}_3$ Azides	$-\text{N}_3$	$\text{Ar}-\text{N}^\bullet$ Nitrenes	<ul style="list-style-type: none"> - Small in size; - Easy for synthesis; - Chemical stability in dark; - Maximal absorption less than 300 nm; - Form C-N or labile X-N bonds.
 Diazo compounds		 Carbenes	<ul style="list-style-type: none"> - Small in size; - Easy to synthesize; - Maximal absorption around 360 nm; - More active than nitrene; - Form more stable C-C or C-O bonds; - React with diverse nucleophiles; - More efficient in hydrophobic environments than in aqueous medium.
 Diazirines			<ul style="list-style-type: none"> - Diazirines share similar properties as diazo compounds; - Aryl diazirines display improved thermal and chemical stability.
$\text{Ar}-\text{N}_2^+ \text{X}^-$ Diazonium salts	$-\text{N}_2^+ \text{X}^-$	Ar^+ Carbocations	<ul style="list-style-type: none"> - Highly reactive and form stronger cross-linkage than nitrene and carbene; - Usually less stable; - Electron-donating substituents confer stability and absorption greater than 300 nm.
 Diaryl ketones		 Radicals	<ul style="list-style-type: none"> - Increased chemical stability; - Relatively easier synthesis; - Reduced sensitivity to ambient light; - Activated at above 350 nm; - Preferential reactivity with C-H bonds; - Lower reactivity in water or with bulky nucleophiles.

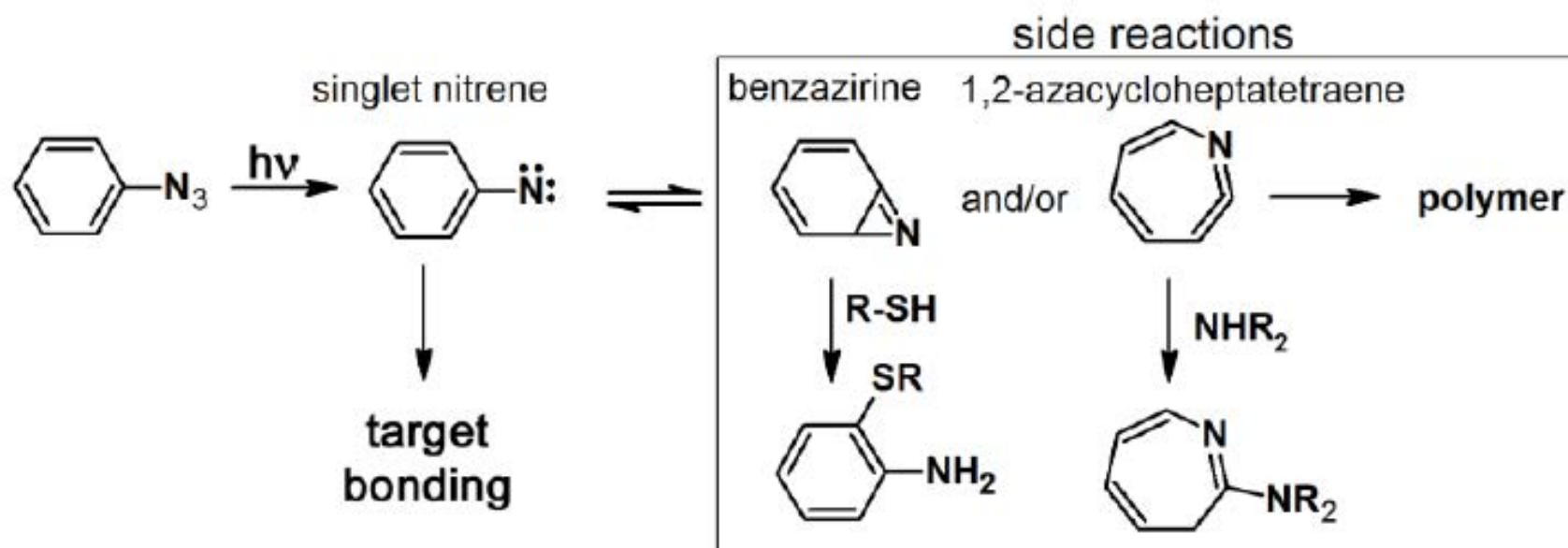
Photoaffinity labeling

Mechanism: photoactivation of **benzophenones**



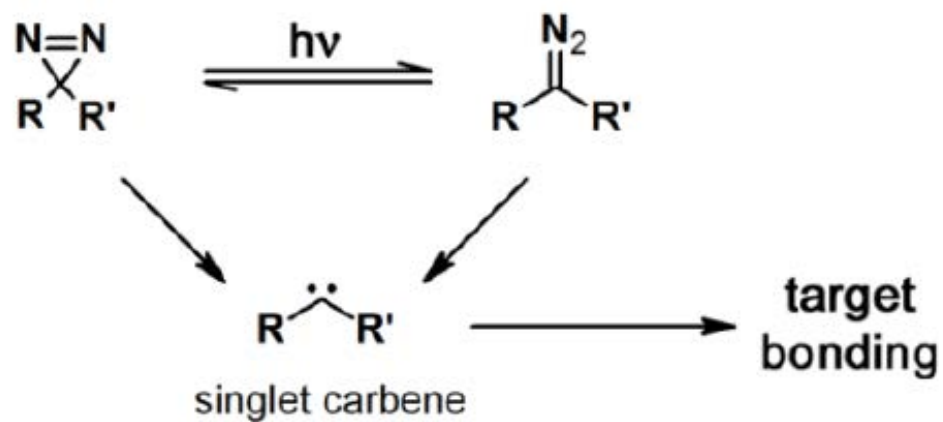
Photoaffinity labeling

Mechanism: photoactivation of **aryl azides**



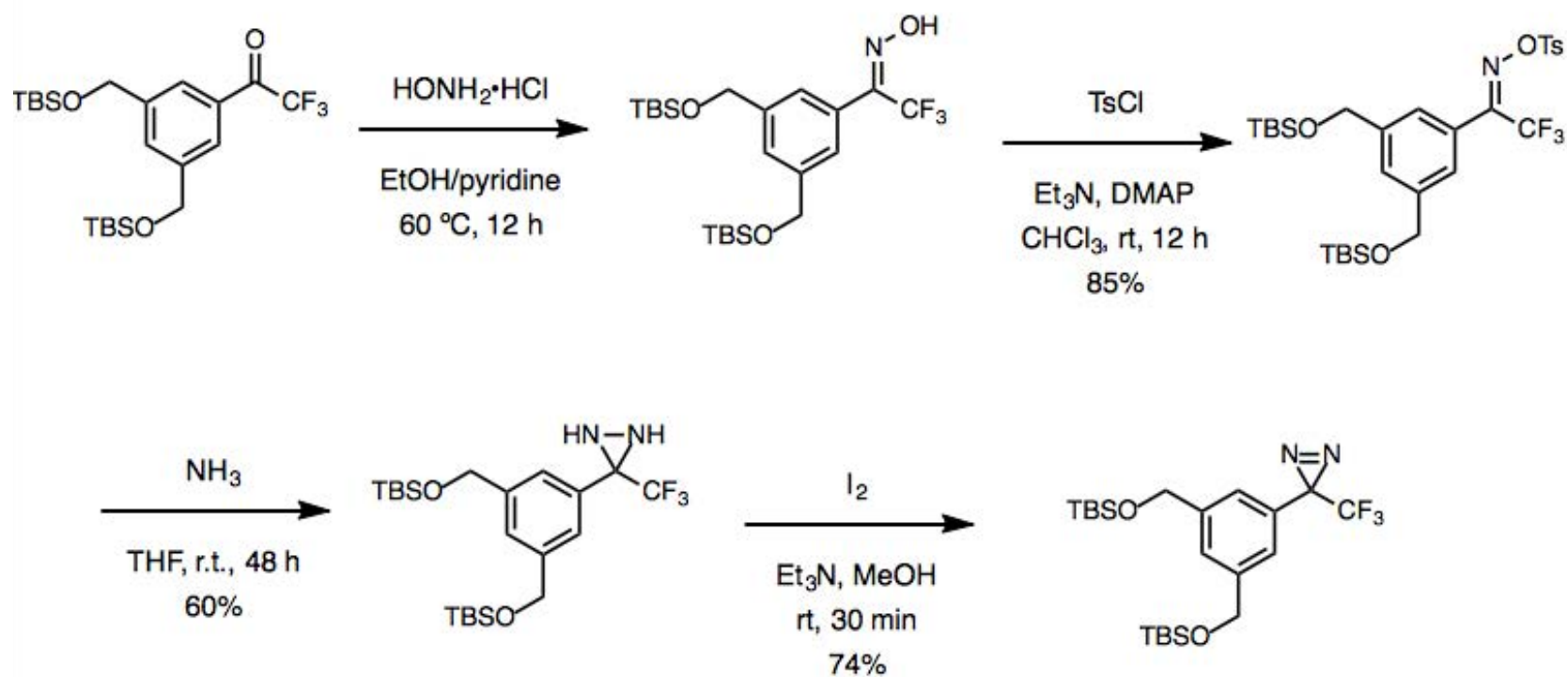
Photoaffinity labeling

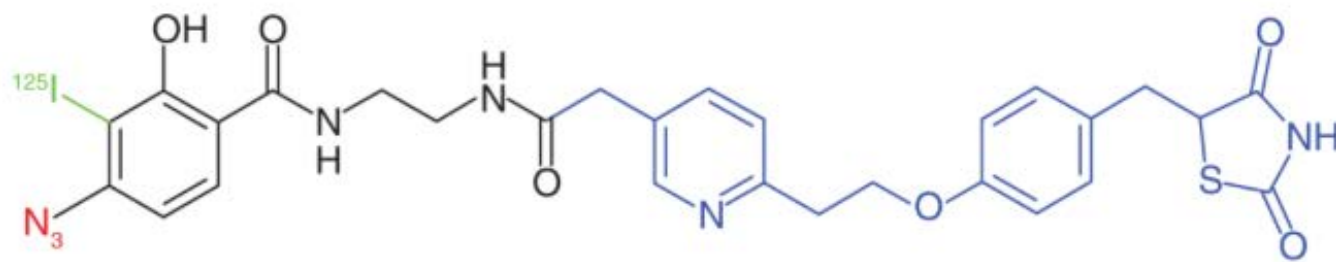
Mechanism: photoactivation of **diazirines**



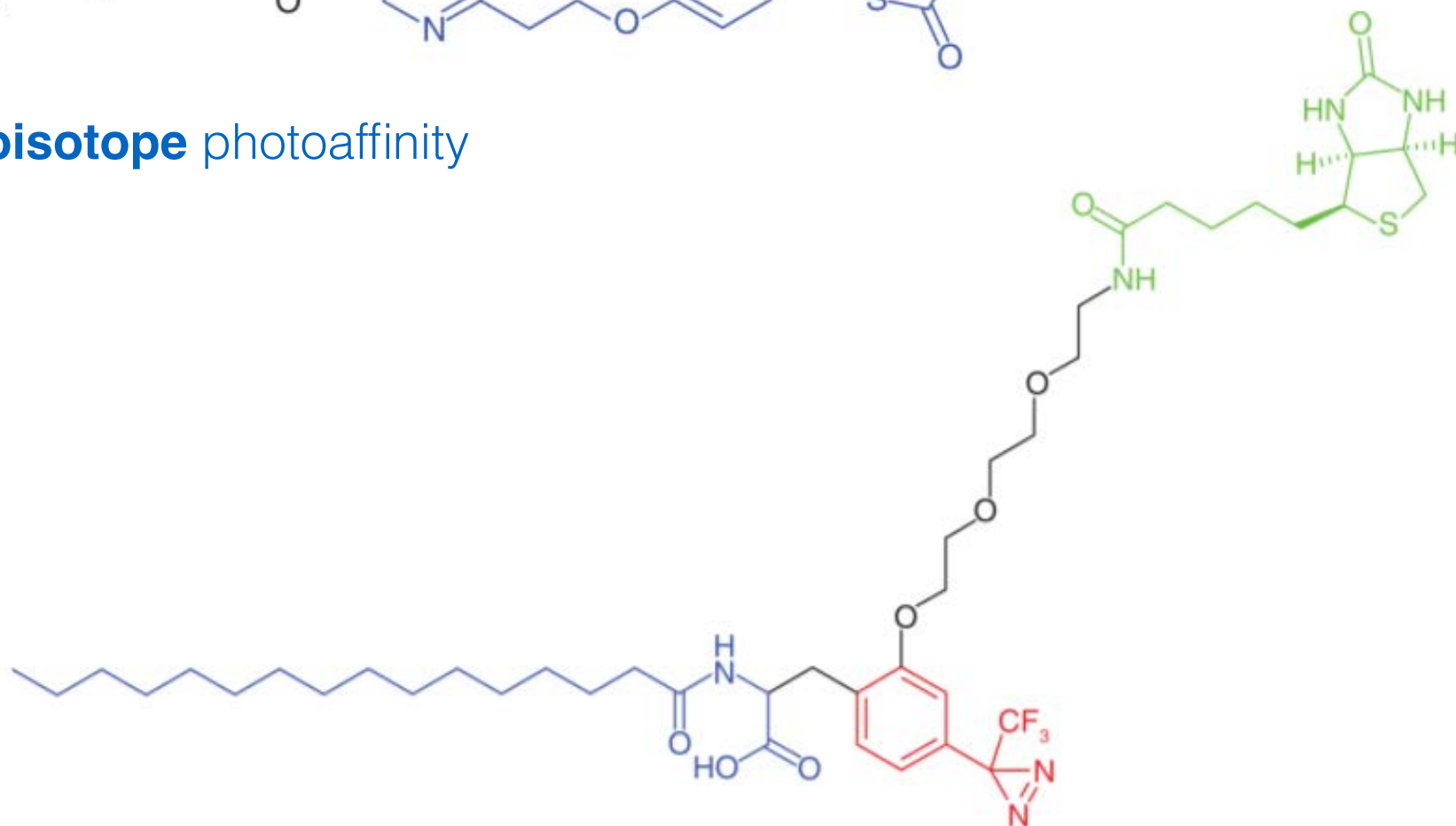
Photoaffinity labeling

Diazirines synthesis





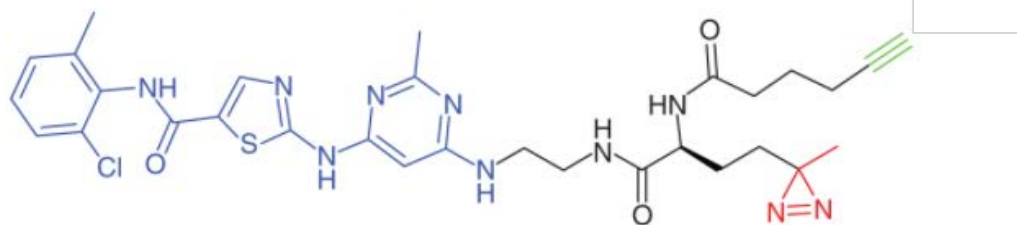
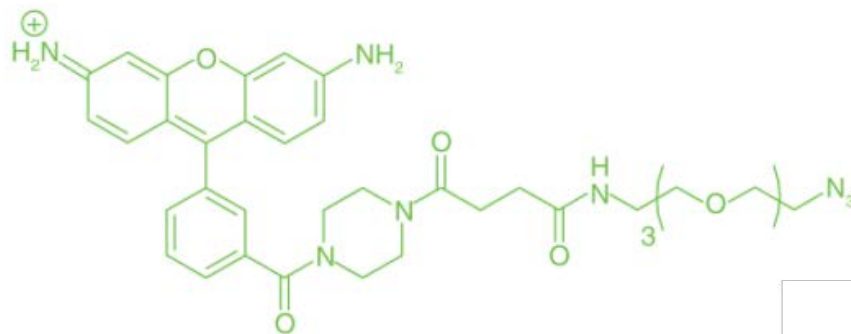
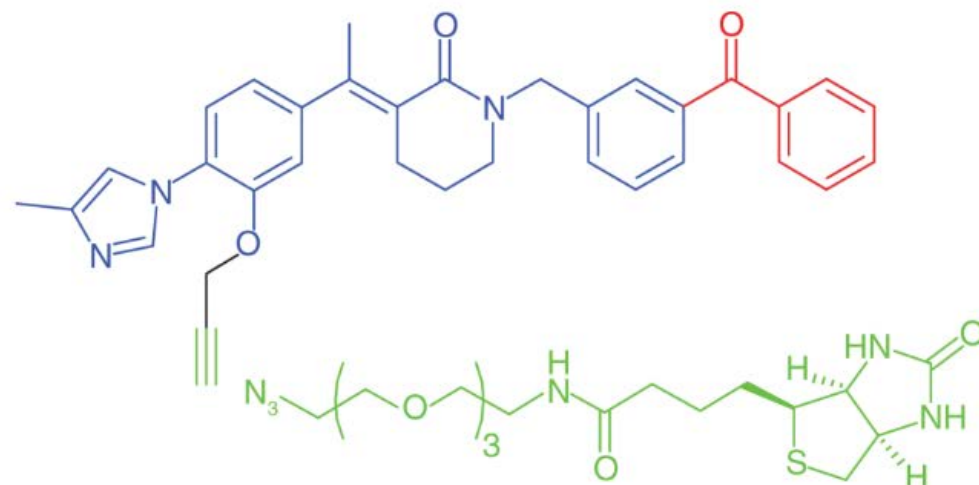
Azide-radioisotope photoaffinity probe



Diazirine-biotin photoaffinity probe

Benzophenone-biotin click

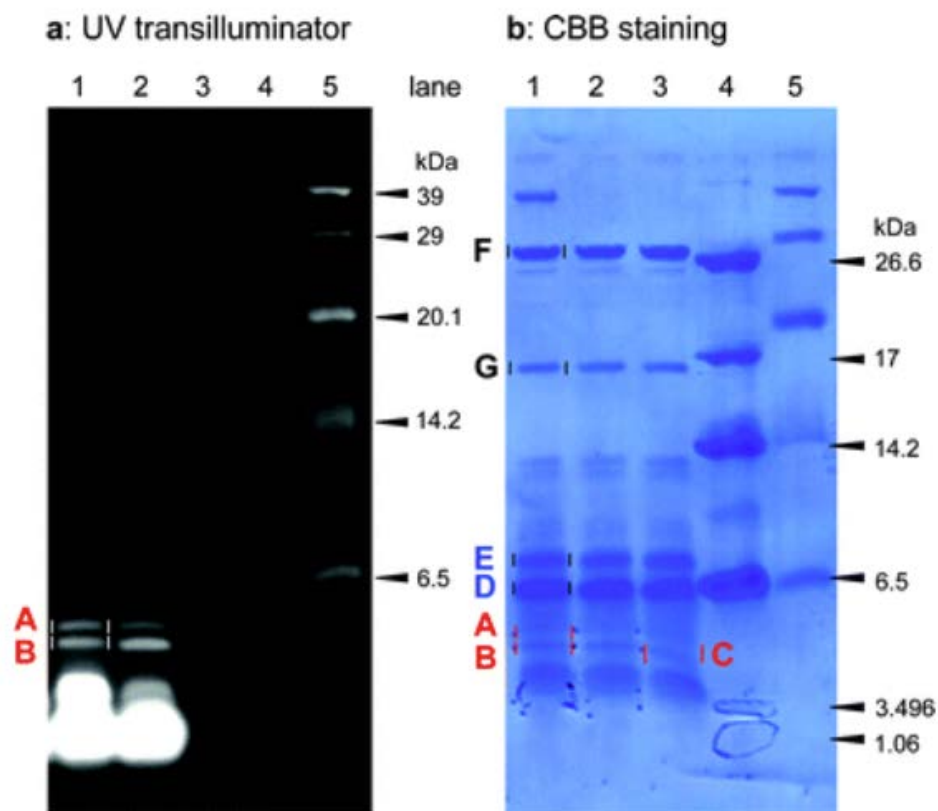
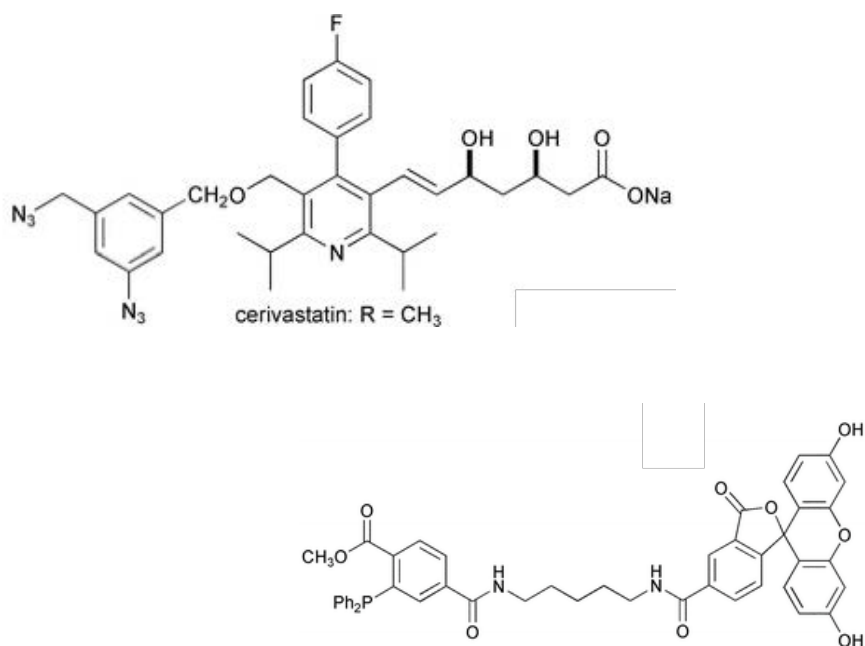
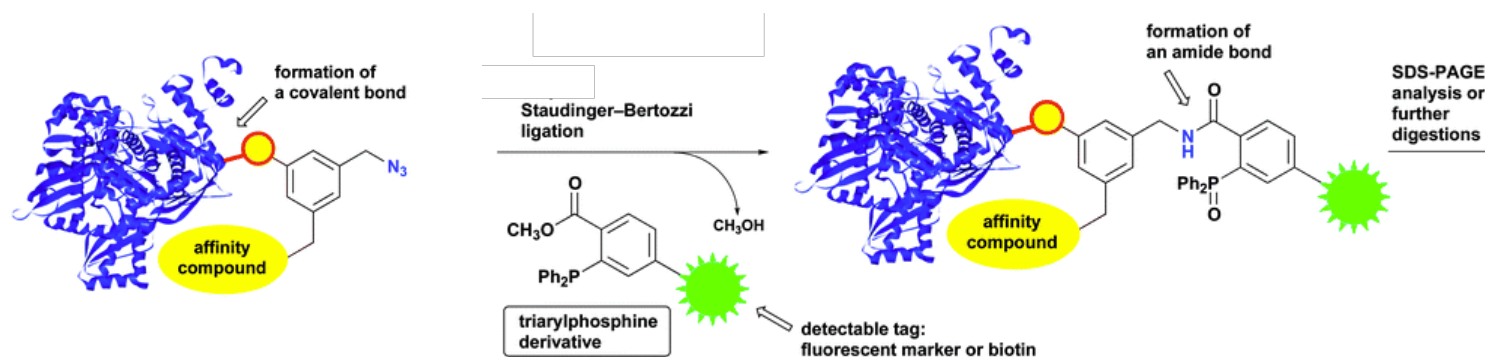
photoaffinity probe



Diazirine-fluorescent click

photoaffinity probe

Photoaffinity labeling



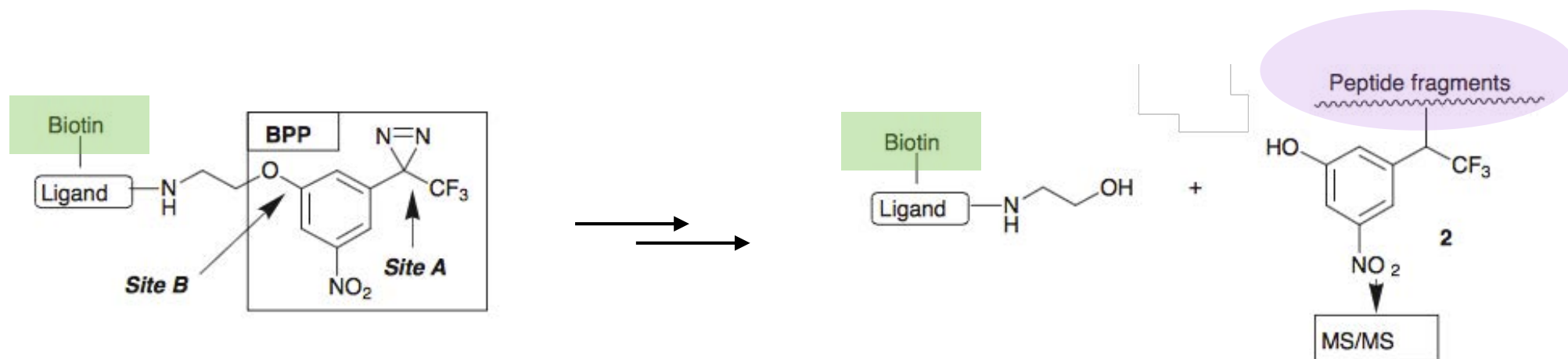
SDS-PAGE analysis of digested peptide fragments.

A simple photo-affinity labeling protocol

Hong-yu Li, Ying Liu, Kan Fang, and Koji Nakanishi*

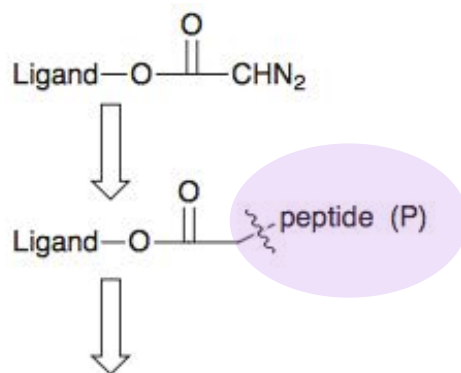
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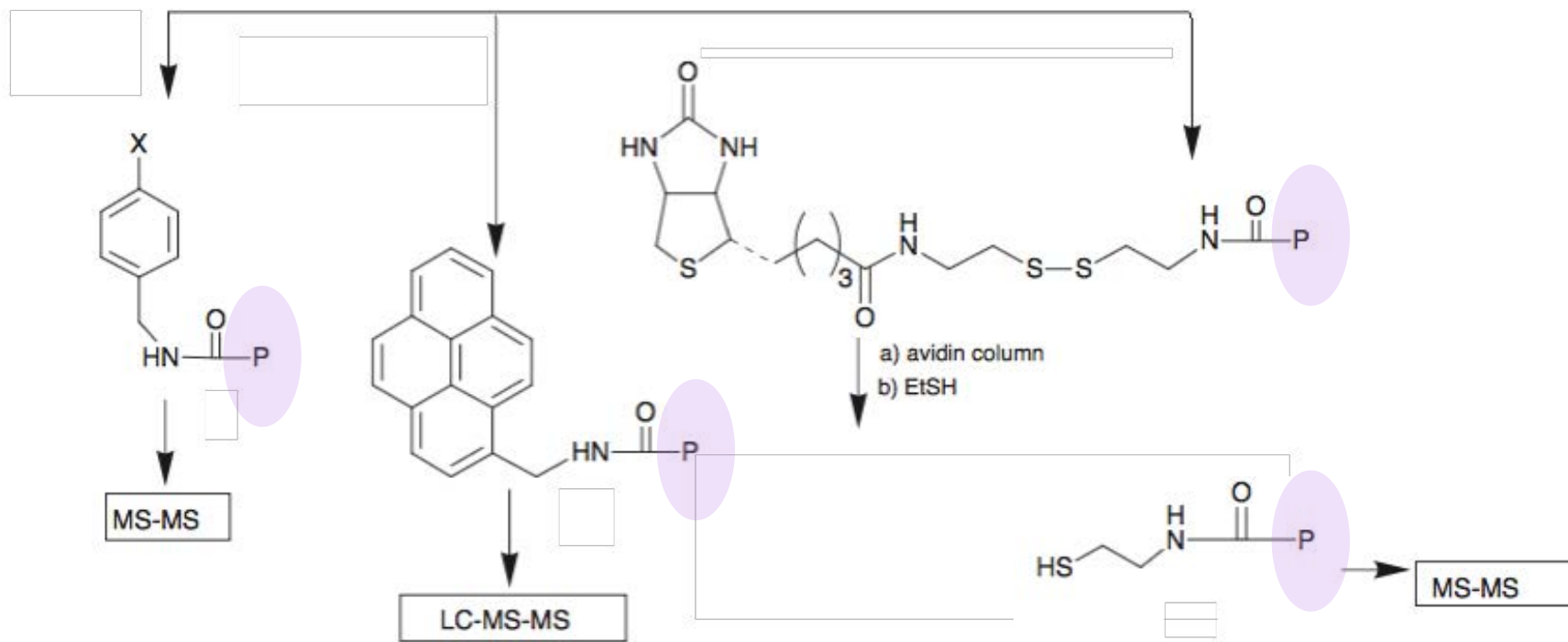


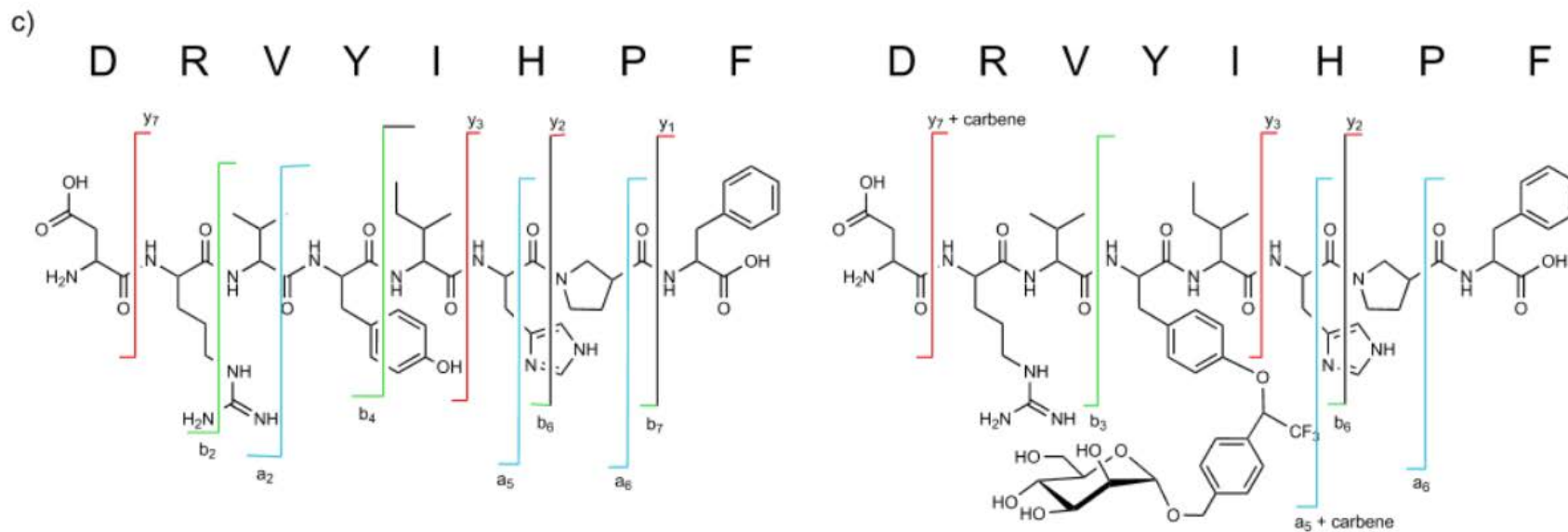
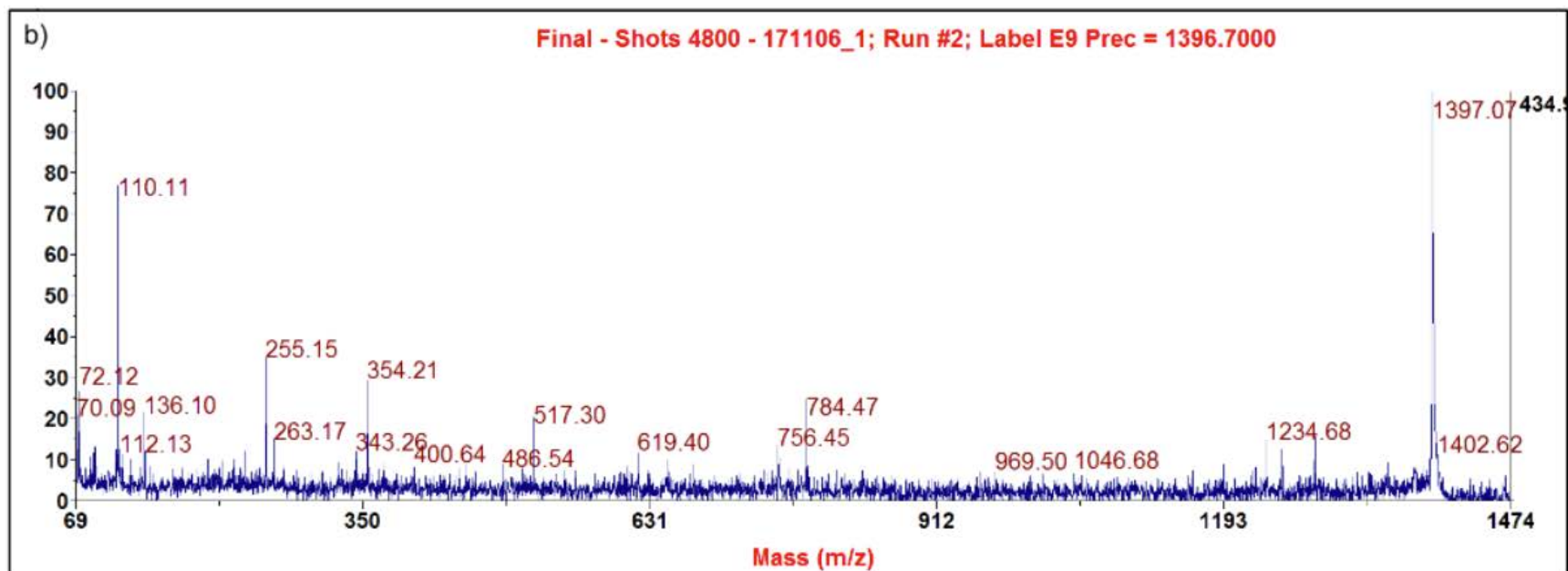
Bifunctional photoaffinity probe

- Irradiation simultaneously **label + fragments** the probe



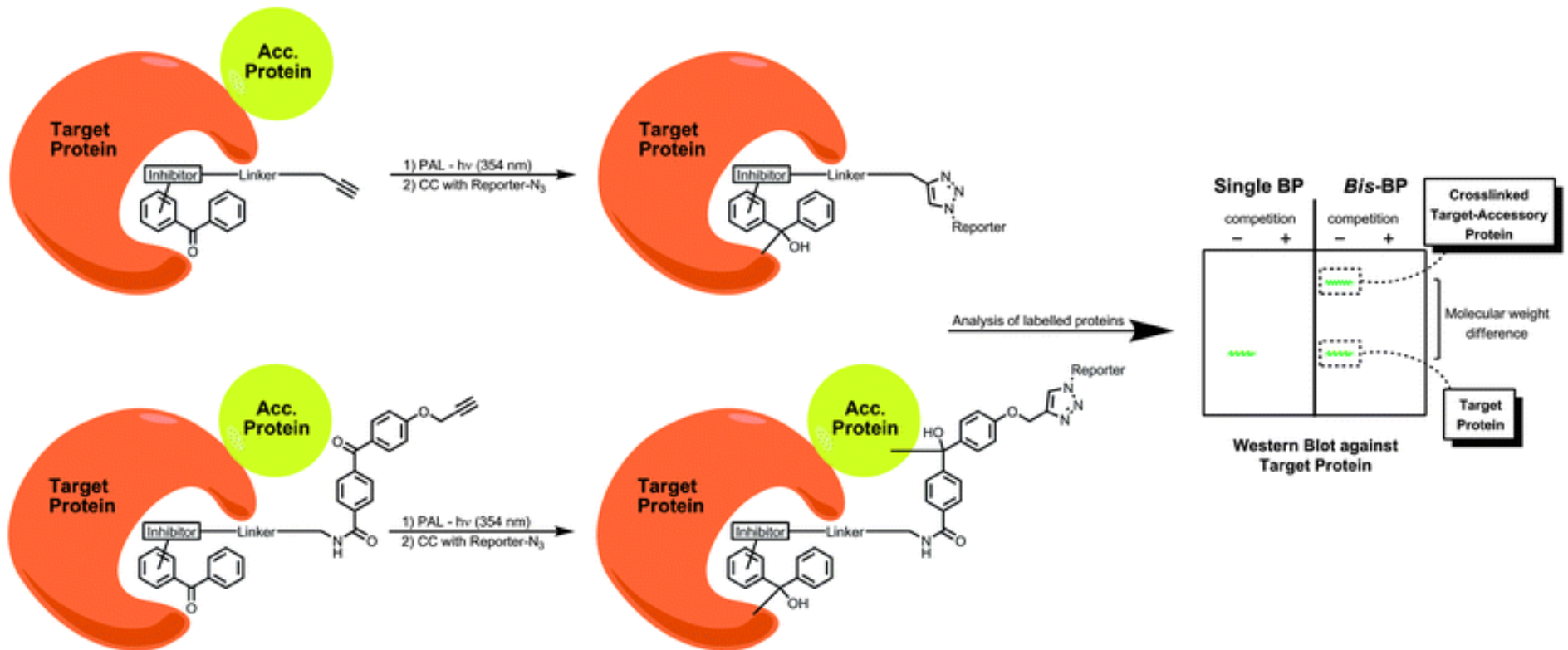
1. ester coupling
2. incubation
3. irradiation
4. proteolysis
5. amine reaction





Photoaffinity labeling

Covalent binding of a ligand to its associated protein **triggered by light**



Can also be used to study **protein-protein interactions**