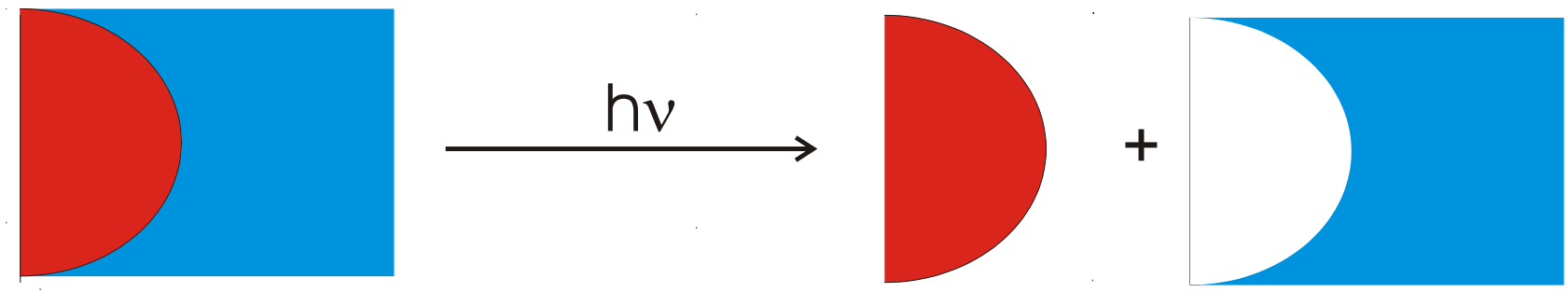
Caged compounds in time-resolved macromolecular crystallography

Andy Pacheco
University of Wisconsin-Milwaukee
Chemistry-Biochemistry

Outline

- Introduction
- *o*-alkylated nitrophenyl compounds
- Potential problems and their possible solutions

What is a “caged compound”?

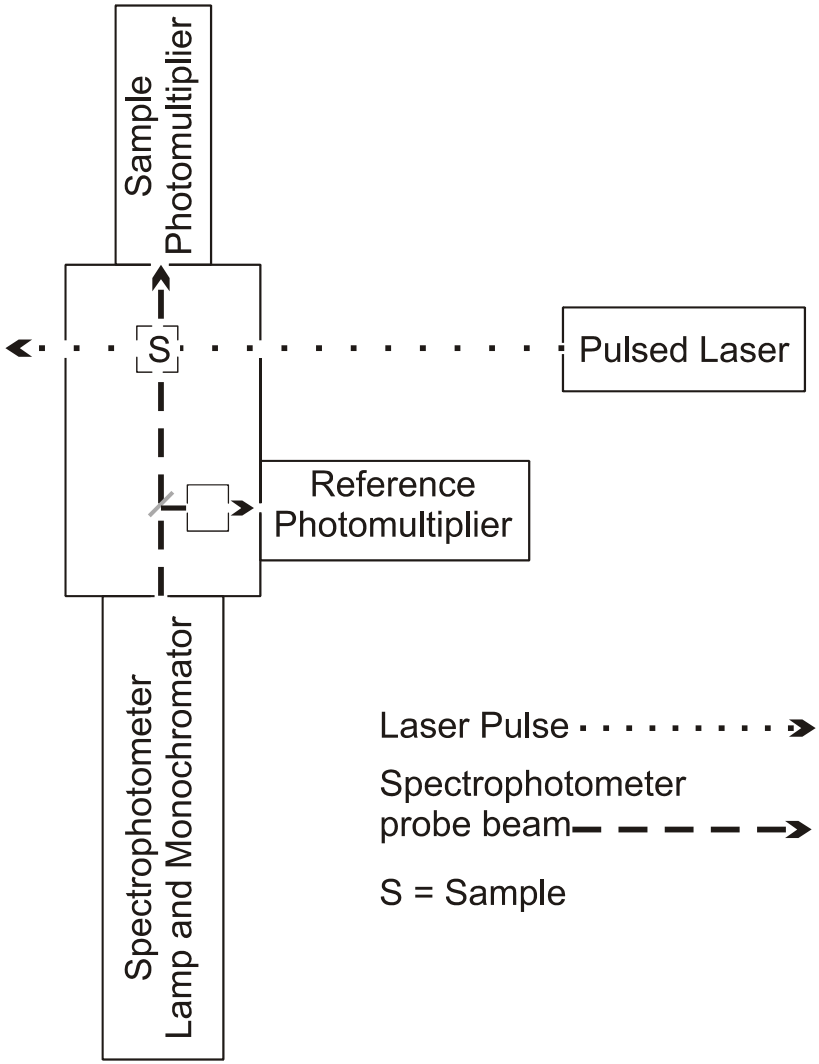


Inactive precursor

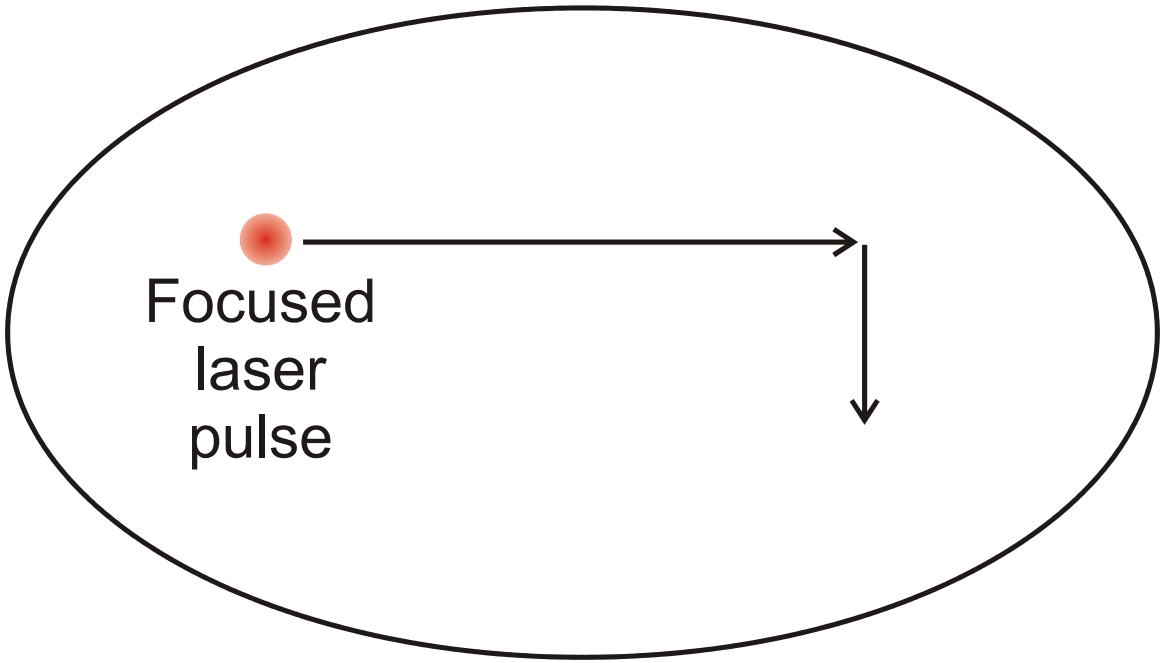
Biologically active reagent

Blocking group

Typical setup for photoinitiating a fast reaction and monitoring spectroscopically



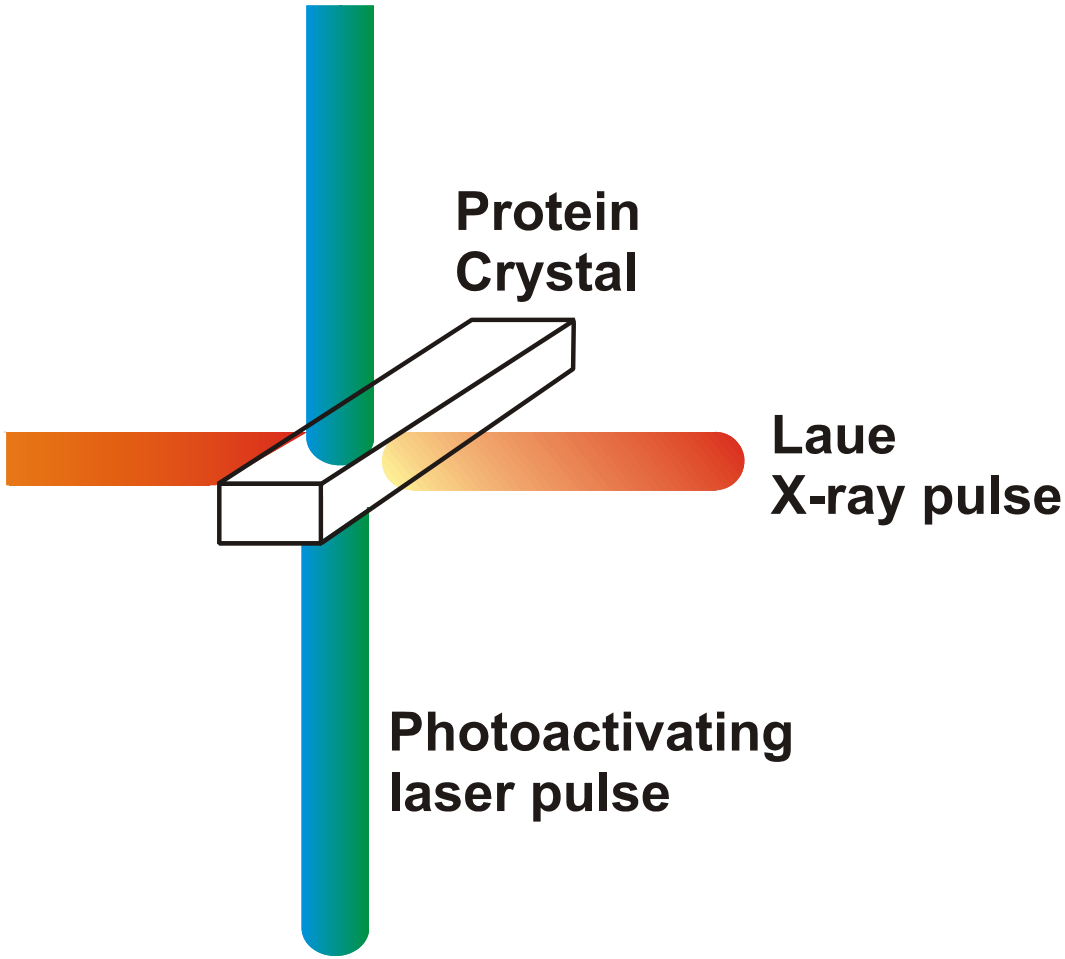
Applications to physiology



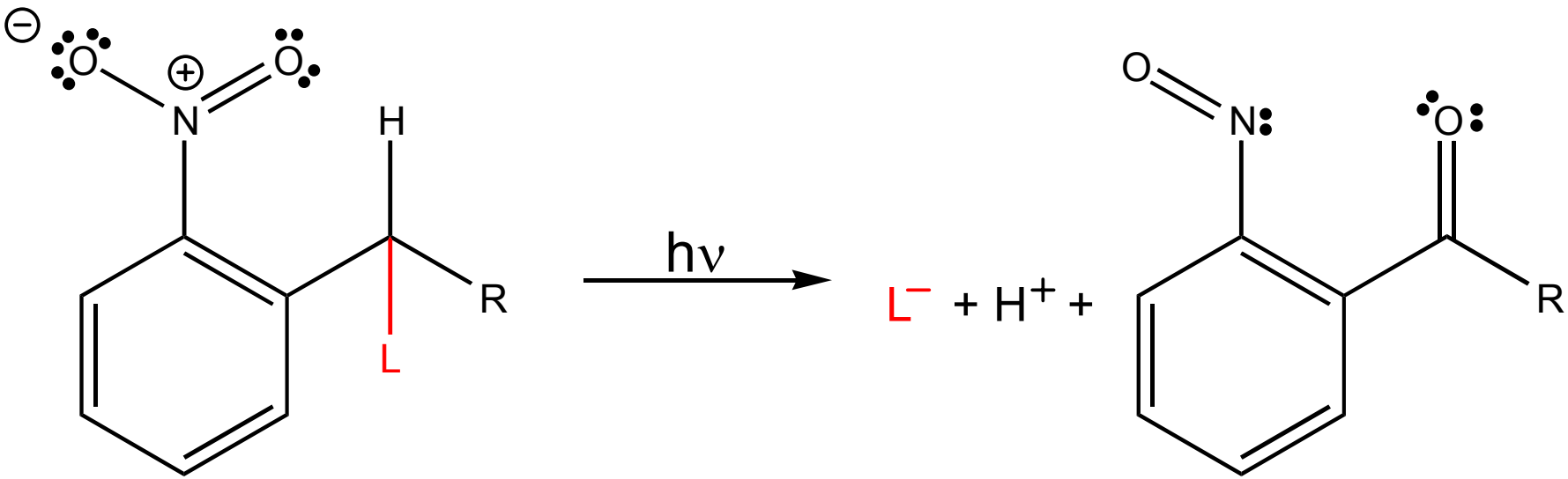
Living cell

Observe changes: f(x,y)
f(t)

Application to time-resolved X-ray crystallography

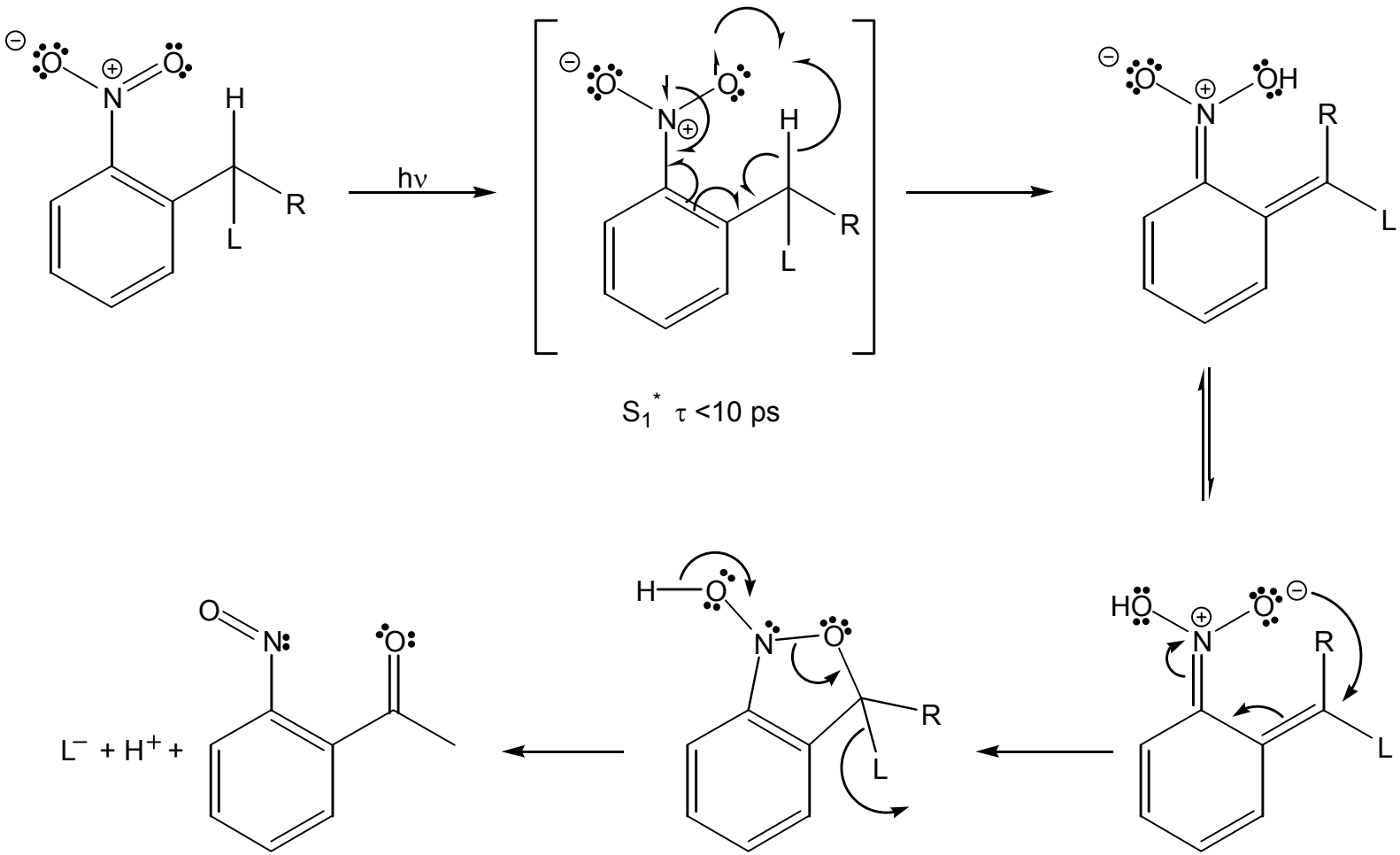


In-depth treatment:
o-alkylated nitrophenyl compounds



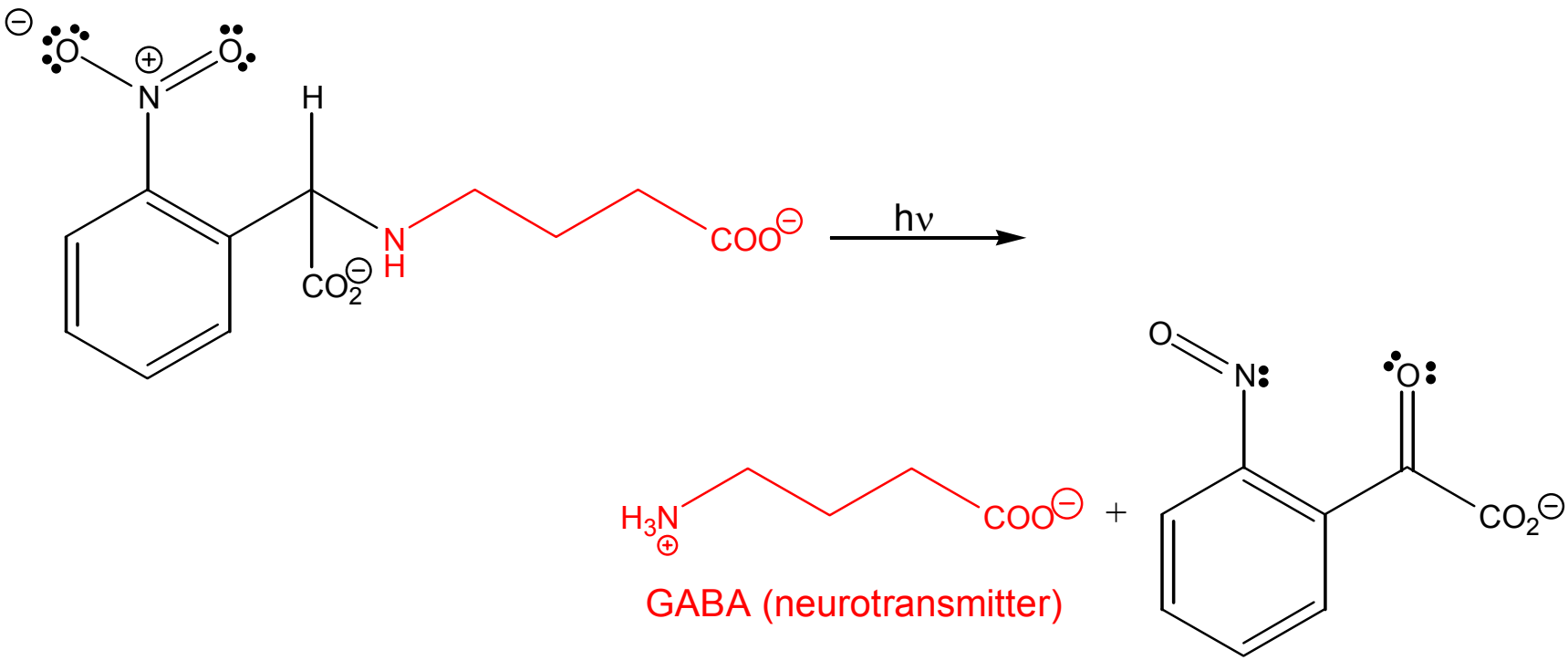
Mechanism for substrate release from *o*-alkylated nitrophenyl compounds

Pellicioli and Wirz *Photochem Photobiol Sci* **2002**, *1,* 441-458



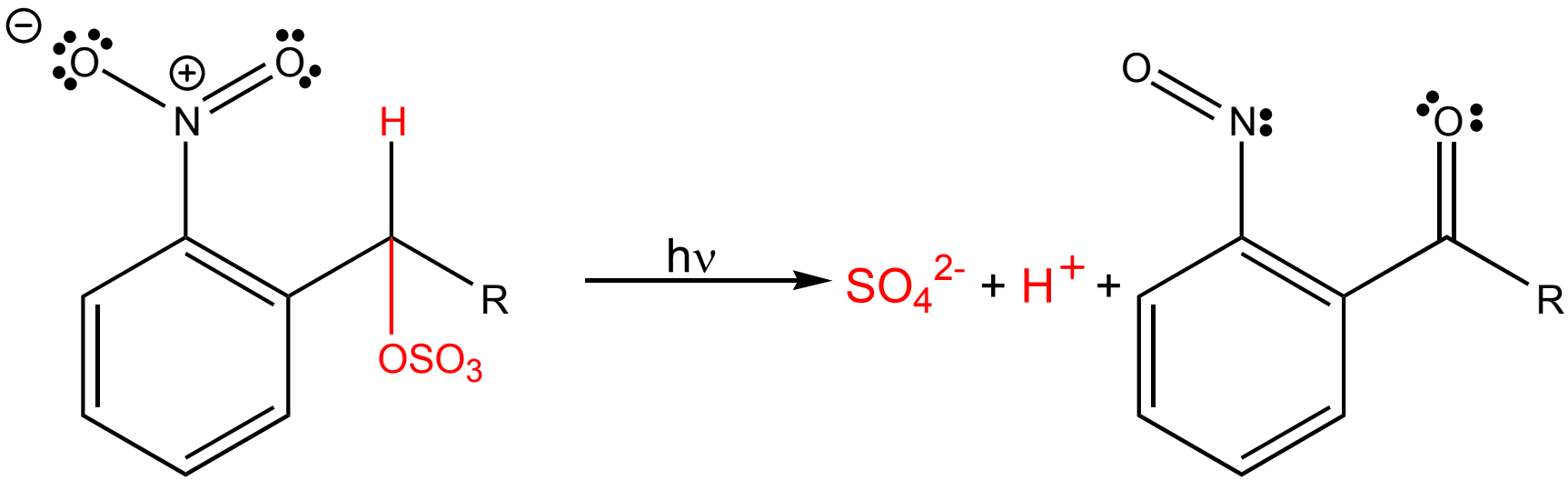
Example: photorelease of the neurotransmitter GABA

Wiebold et al. *Biochemistry*, **1994**, *33*, 1526-1533



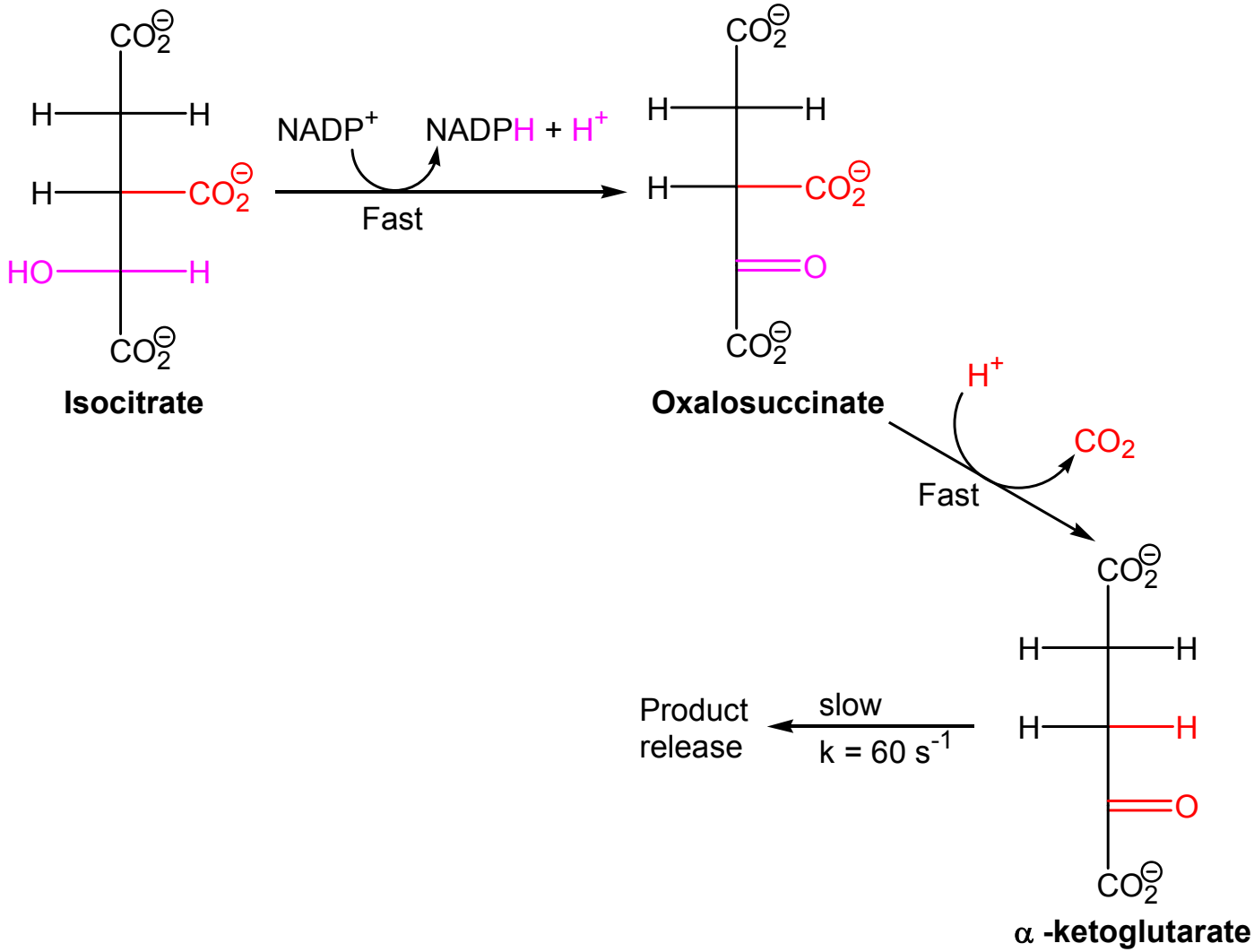
Example: photogenerated pH jump

Barth and Corrie *Biophys J.* **2002**, ***83*,** 2864-2871

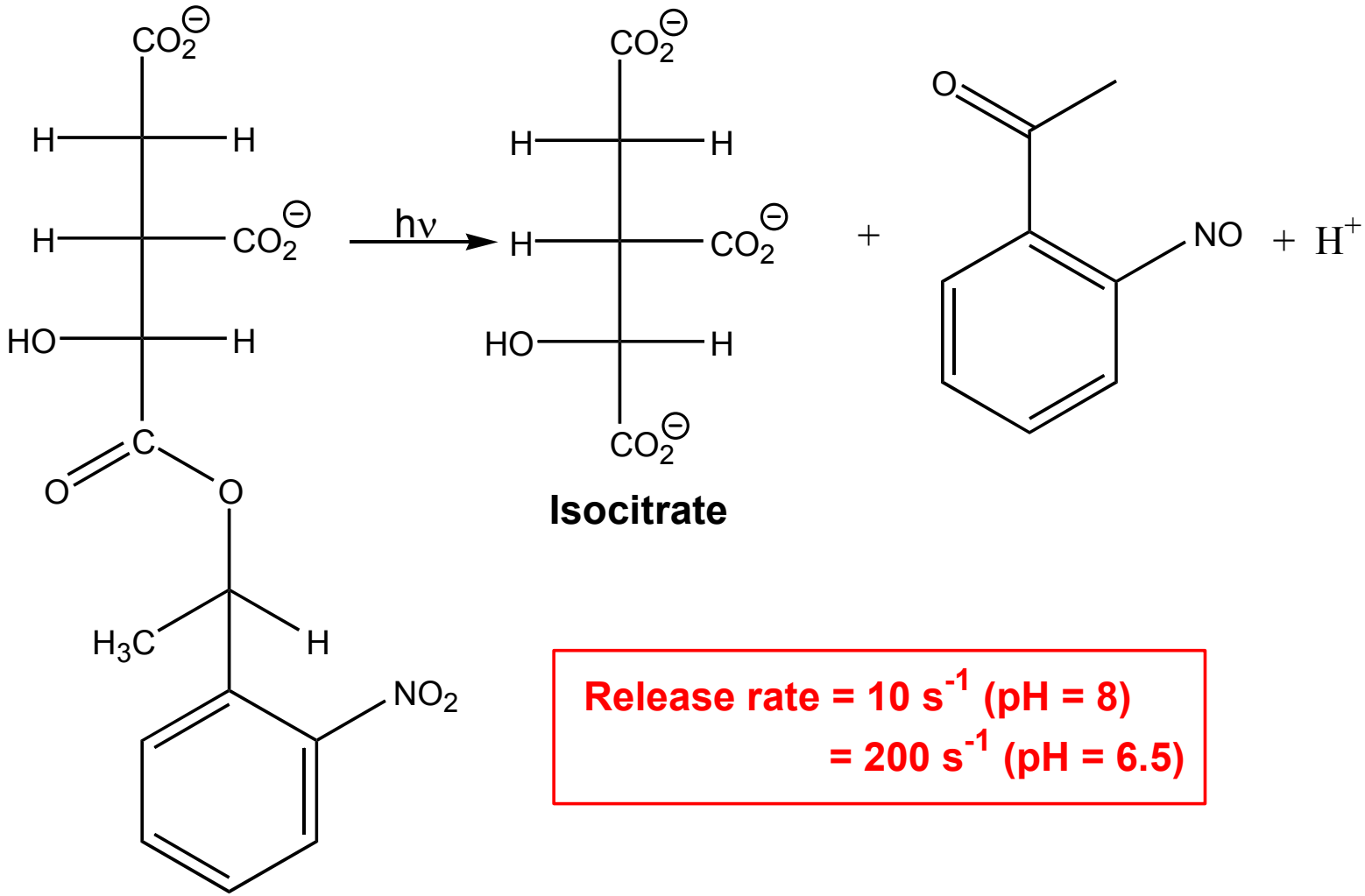


Isocitrate dehydrogenase: reaction intermediate observed crystallographically on the ms timescale

Stoddard et al. *Nat. Struct. Biol.* **1998**, ***10***, 891-897



Strategy # 1



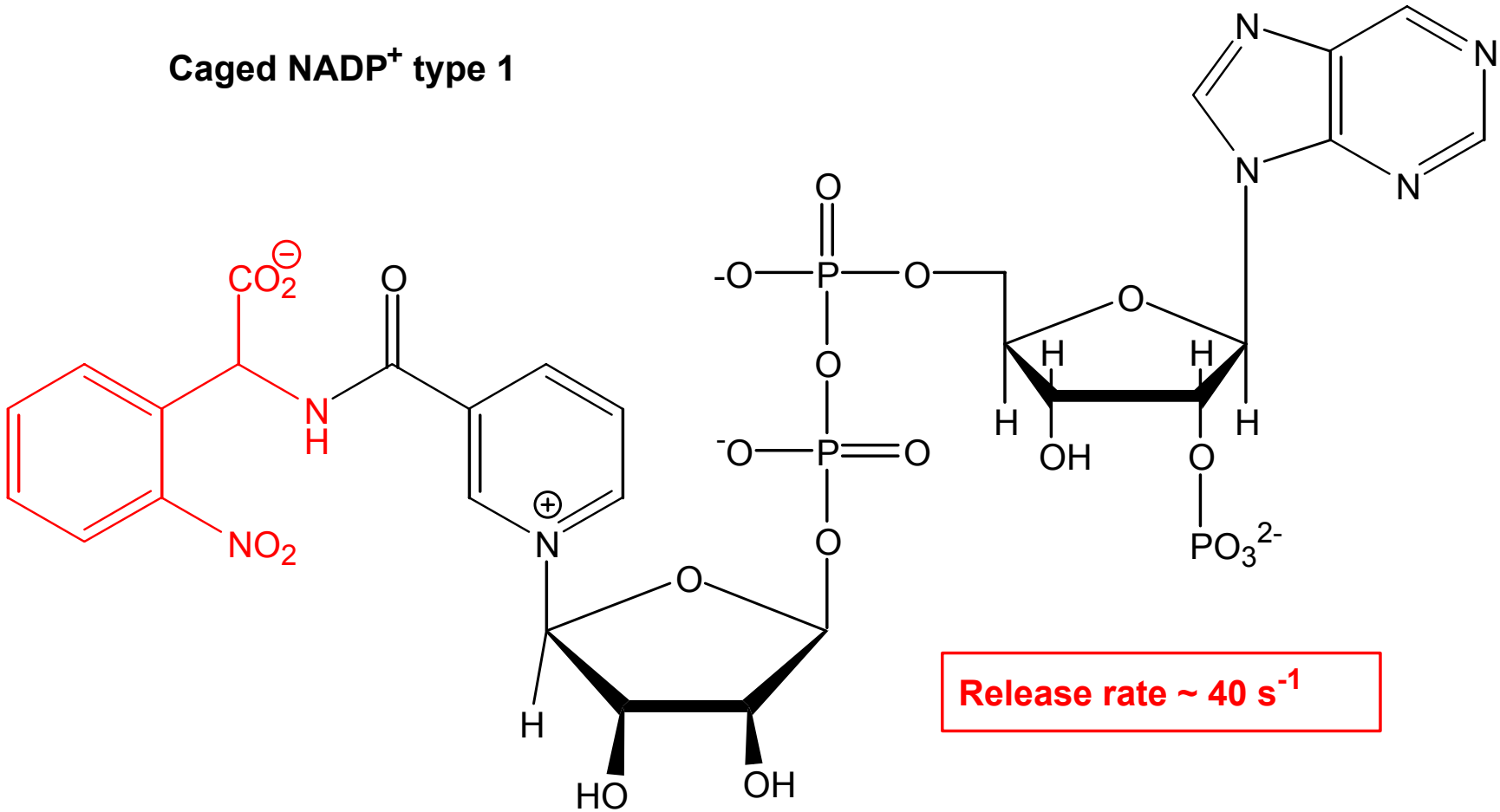
Isocitrate

caged isocitrate

Release rate = 10 s^{-1} (pH = 8)
= 200 s^{-1} (pH = 6.5)

Strategy # 2

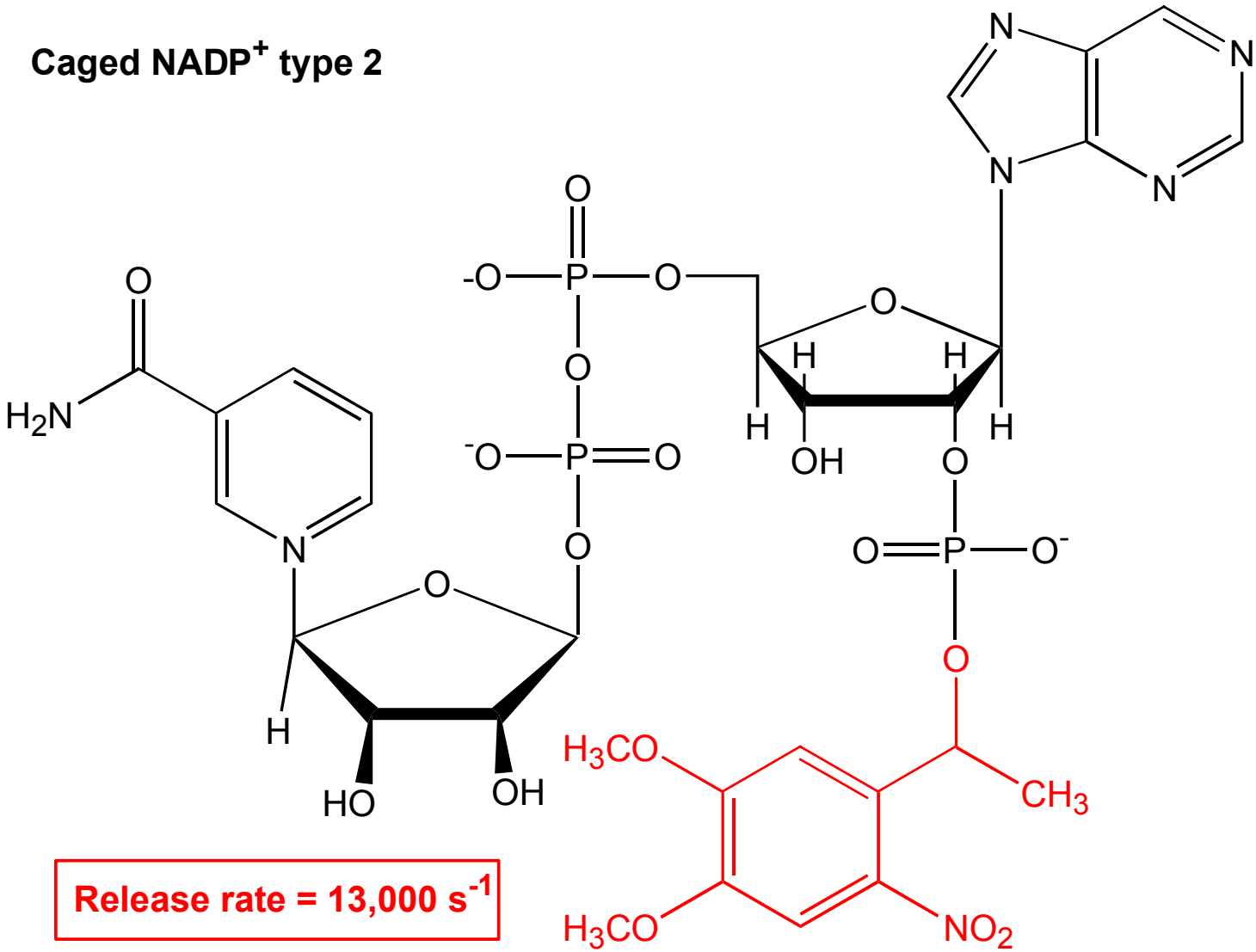
Caged NADP$^+$ type 1



Release rate ~ 40 s^{-1}

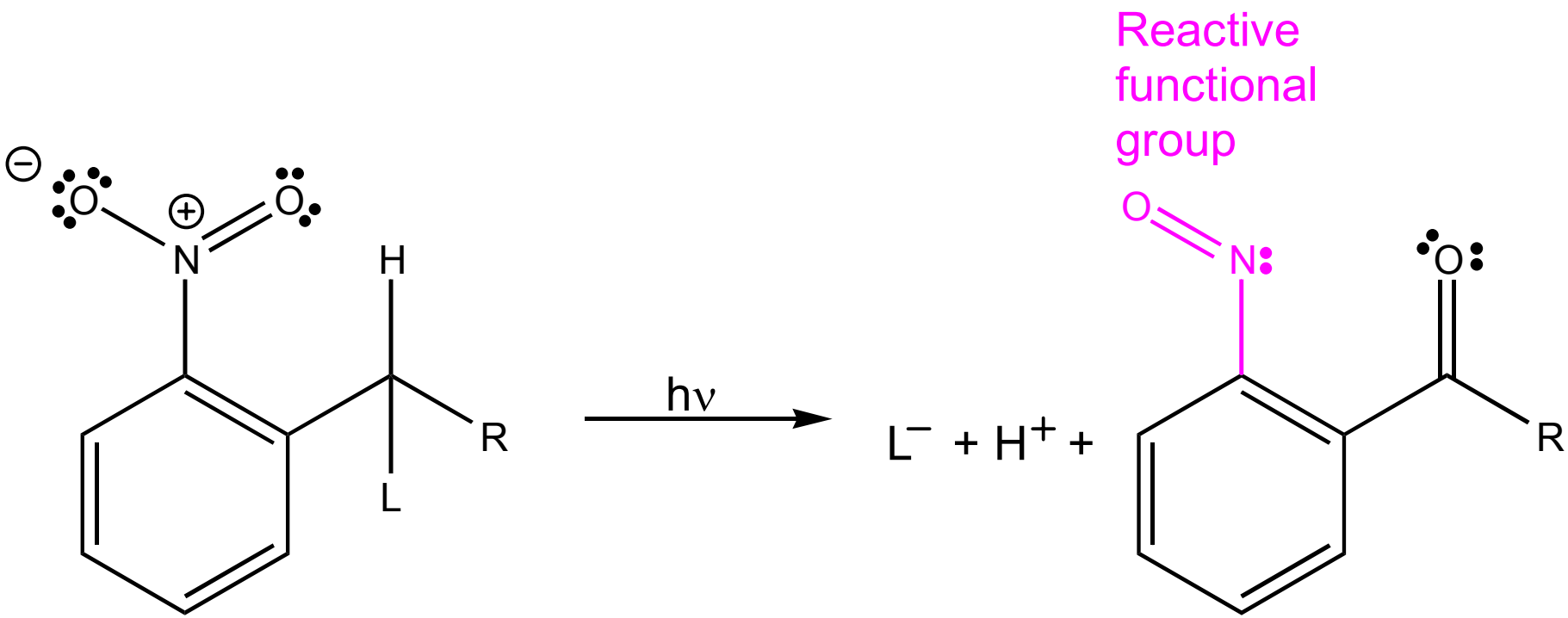
Strategy # 3

Caged NADP$^+$ type 2



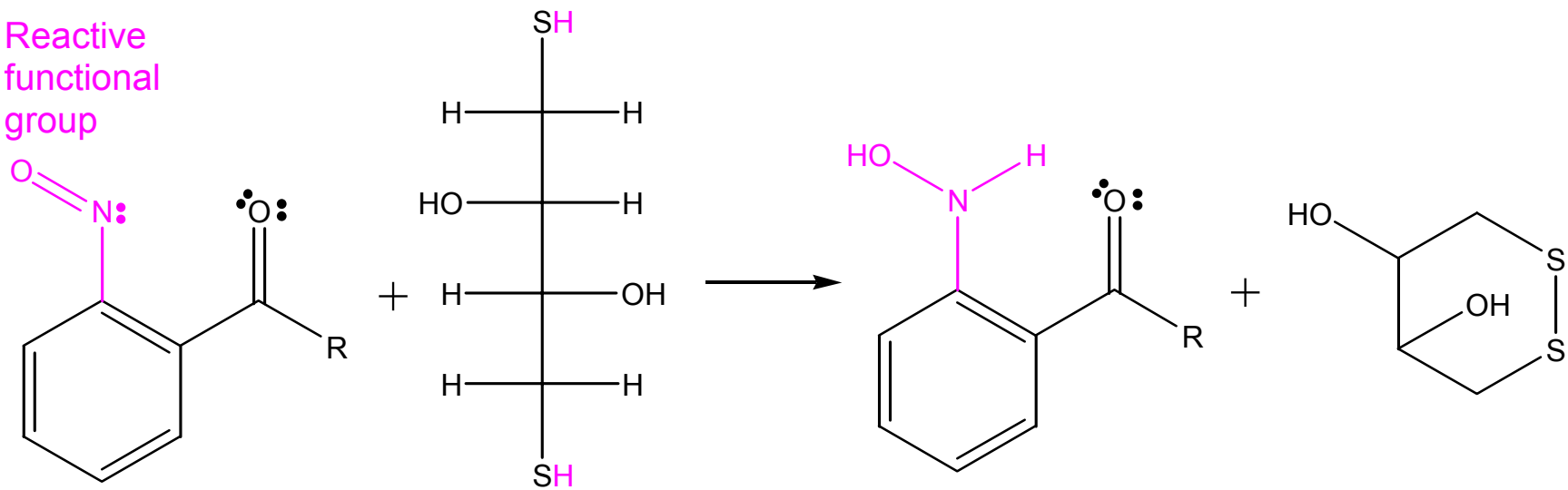
Release rate = 13,000 s^{-1}

Potential problem #1: side reactions of blocker group after uncaging



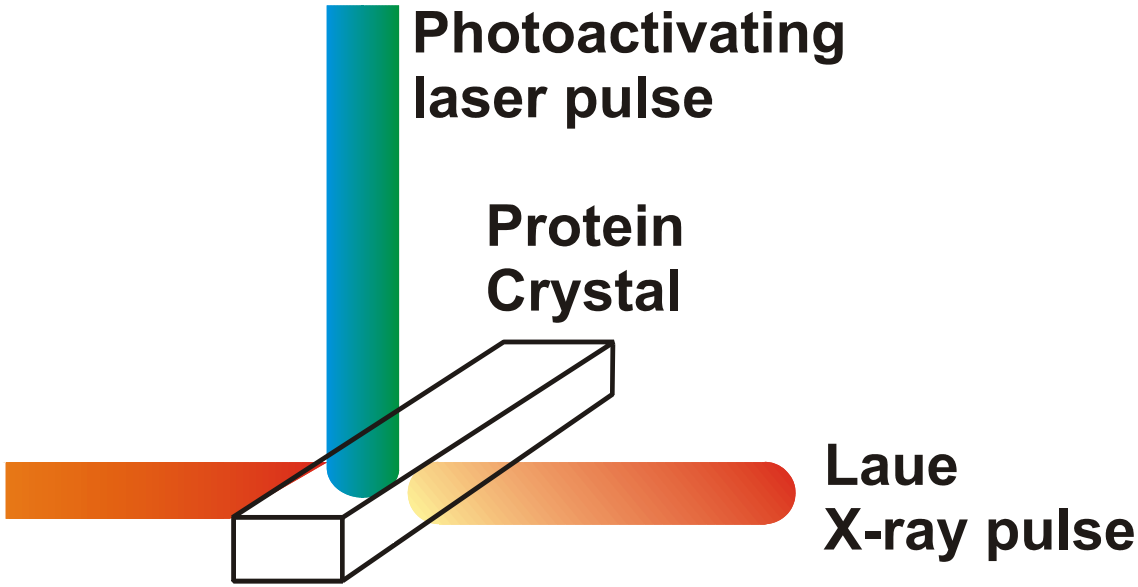
Possible Solution

Cohen et al. *Biochemistry* **1997**, *36*, 9035-9044



dithiothreitol

Potential problem #2: Self-screening prevents uniform sample photoactivation



Protein crystal absorbs most laser light near the incident surface

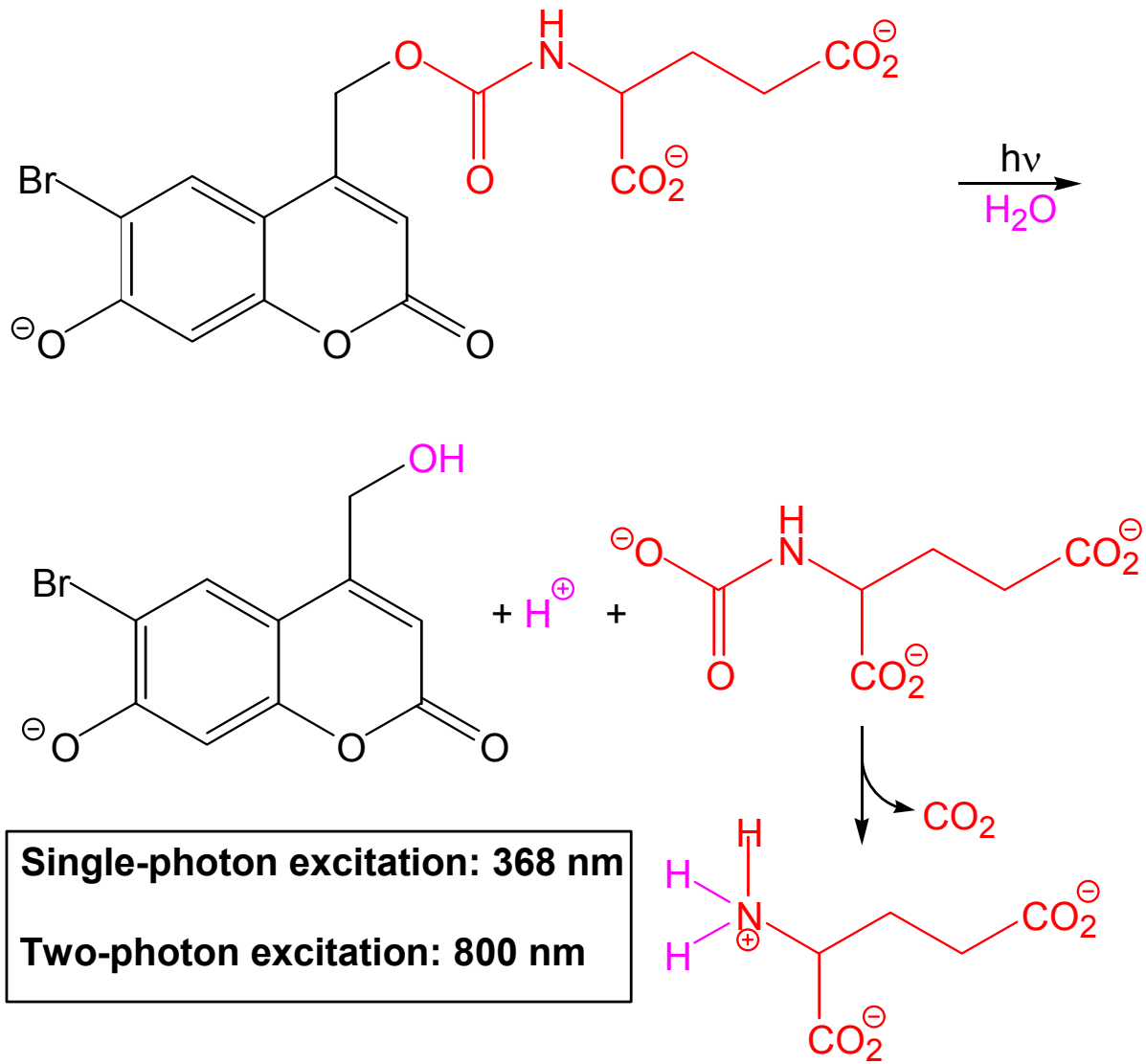
Inhomogeneous photochemistry within the crystal

Possible solution:
2-photon excitation

- Requires caged compounds with significant 2-photon excitation cross sections

Example: Coumarins

Furuta et al. *Proc. Natl. Acad. Sci. USA* **1998**,*96*, 1193-1200

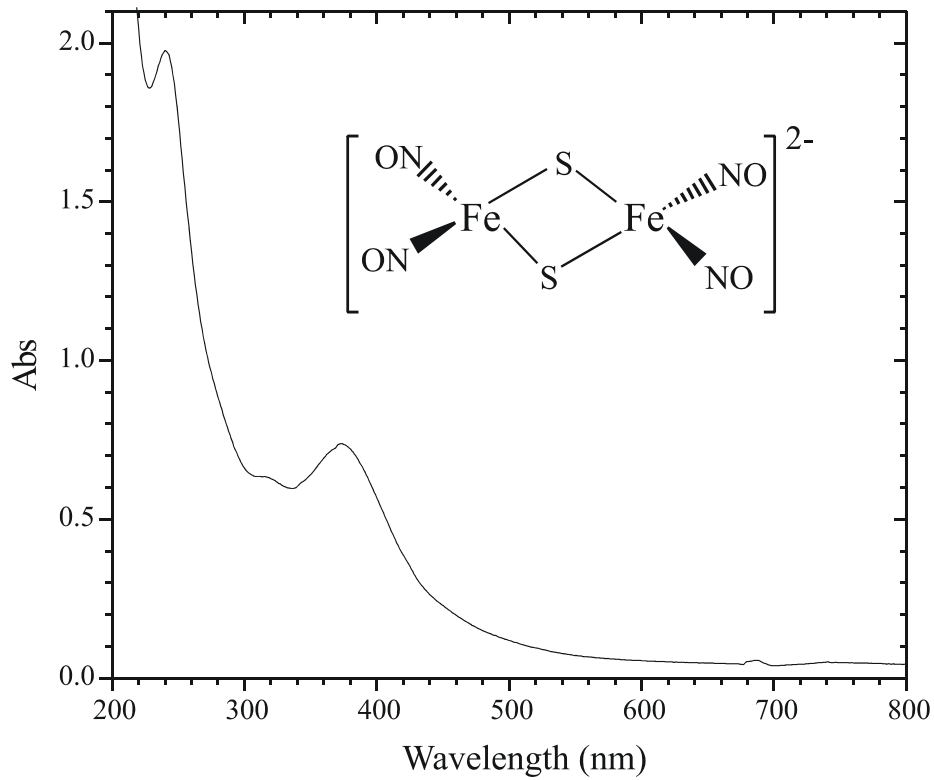


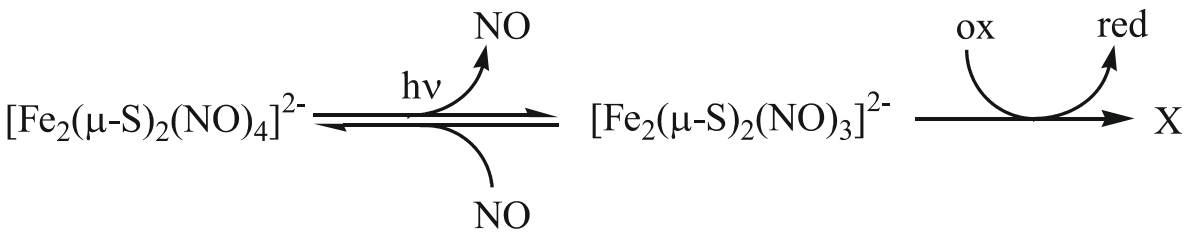
Single-photon excitation: 368 nm

Two-photon excitation: 800 nm

Caged Nitric Oxide
(Roussin's Red Salt)

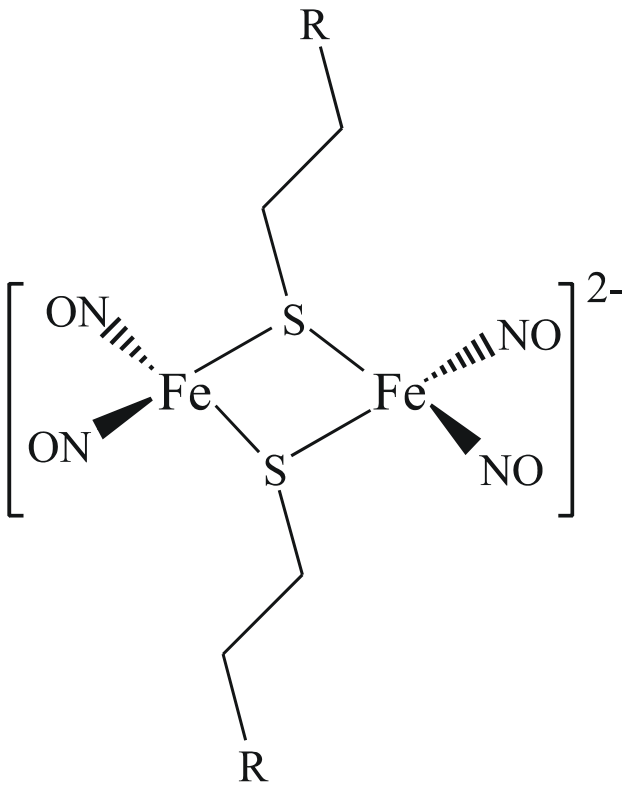
Bourassa et al. *J. Am. Chem Soc.* **1997**, *119*, *2853-2860*

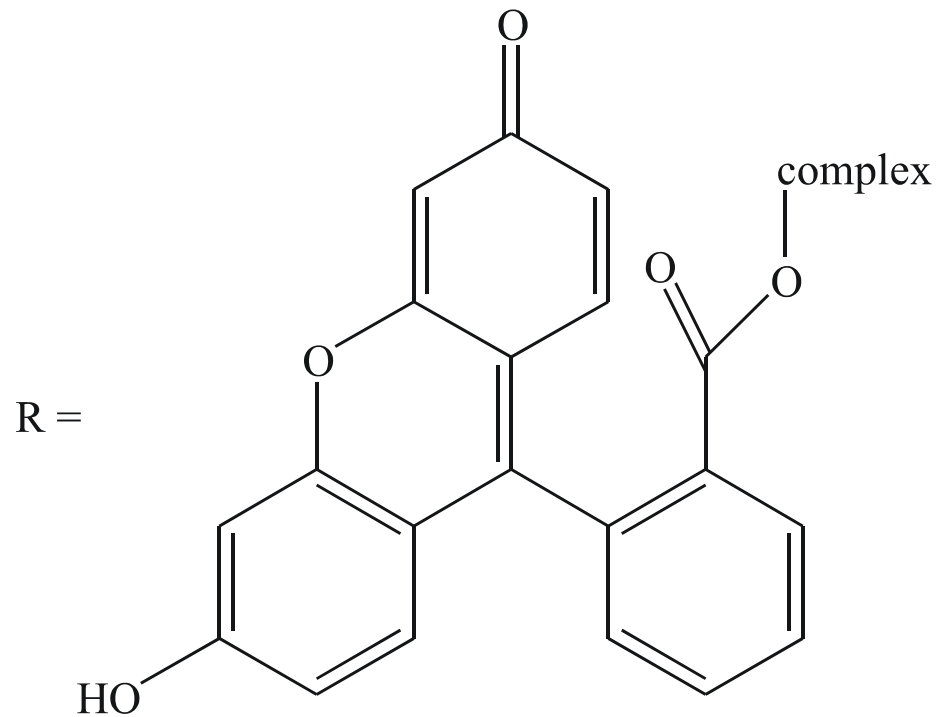




Roussin's Red Salt with attached 2-photon antenna complex (fluorescein)

Wecksler et al. *J. Am. Chem. Soc.* **2006**, *128*, 3831-3837





λ_{max} = 500 nm

1-photon excitation (436 nm)

2-photon excitation (800 nm)

Caged Complexes
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