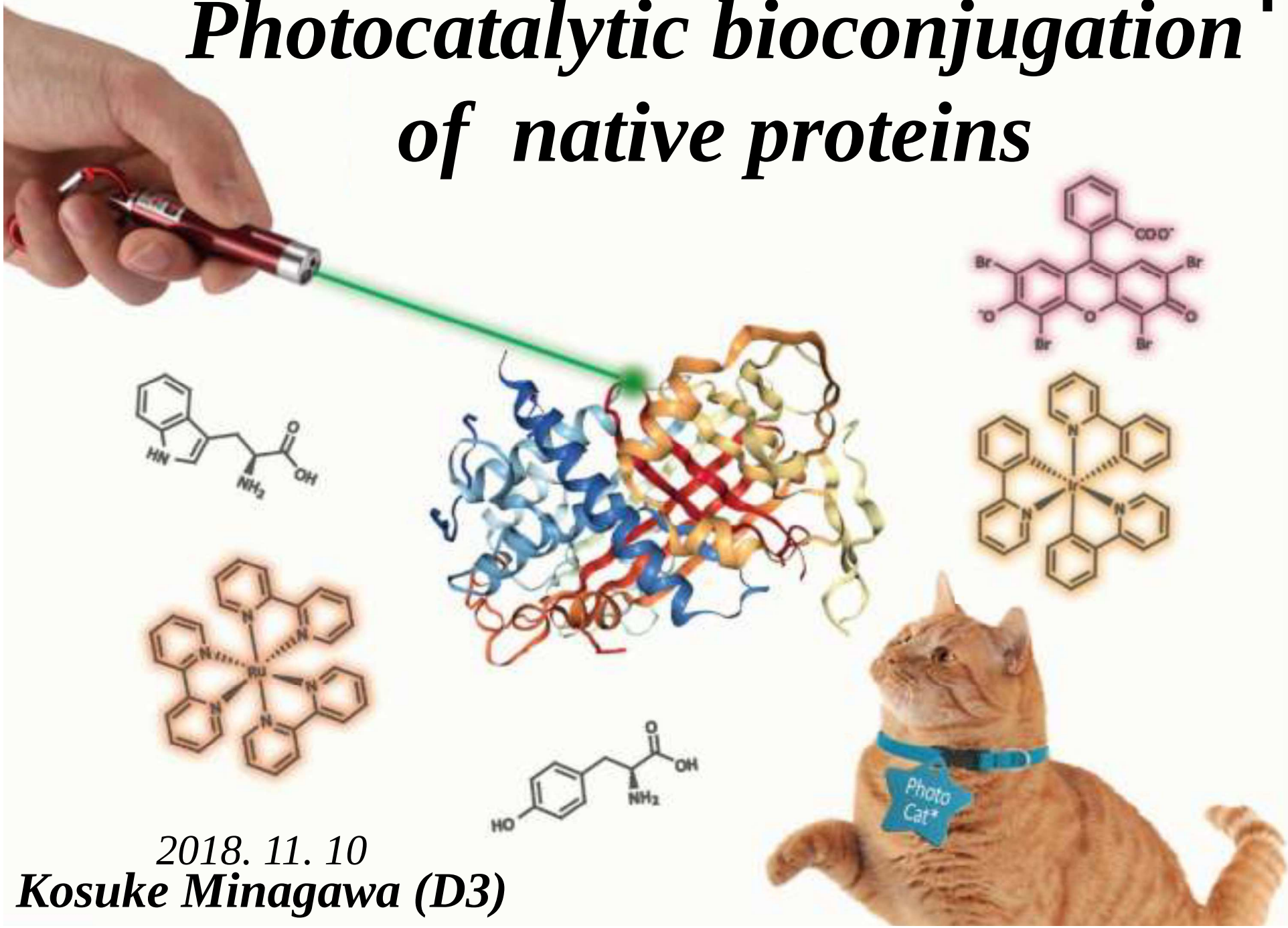
Photocatalytic bioconjugation of native proteins



2018. 11. 10

Kosuke Minagawa (D3)

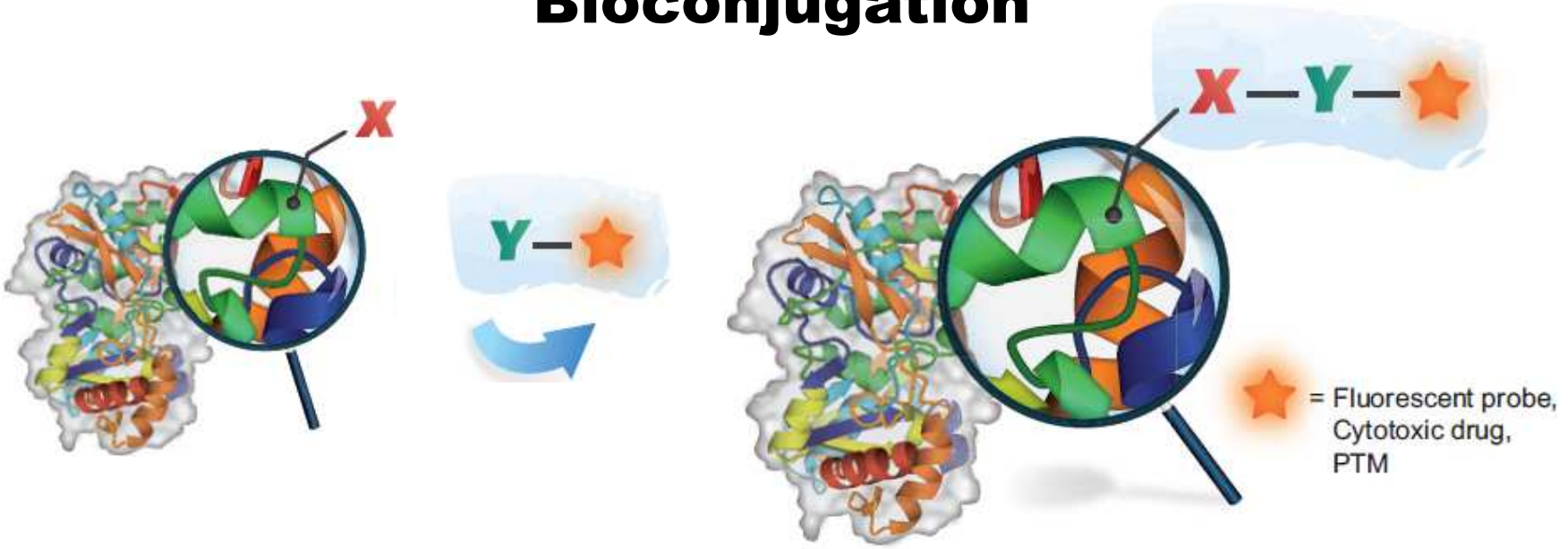
Today's contents

1. Introduction
   - -Bioconjugation
   - -Existing method for protein bioconjugation (two-electron pathway)

2. Bioconjugation using radical reaction (Tyr, Dha residues)

3. Bioconjugation for carboxyl group

Bioconjugation[1)]



"the covalent derivatization of biomolecules"

Advantage of...

- ✔ **Discovery of biological interactions**
- ✔ **Biochemical assays**
- ✔ **Conformational study**
- ✔ **Antibody-drug conjugate (ADC, 武装抗体)** →

cf.
Dr. Asaba_LS_150926_"Chemically Synthesized Antibody"
Dr. Hashimoto_LS_161115_"Antibody-drug Conjugates"

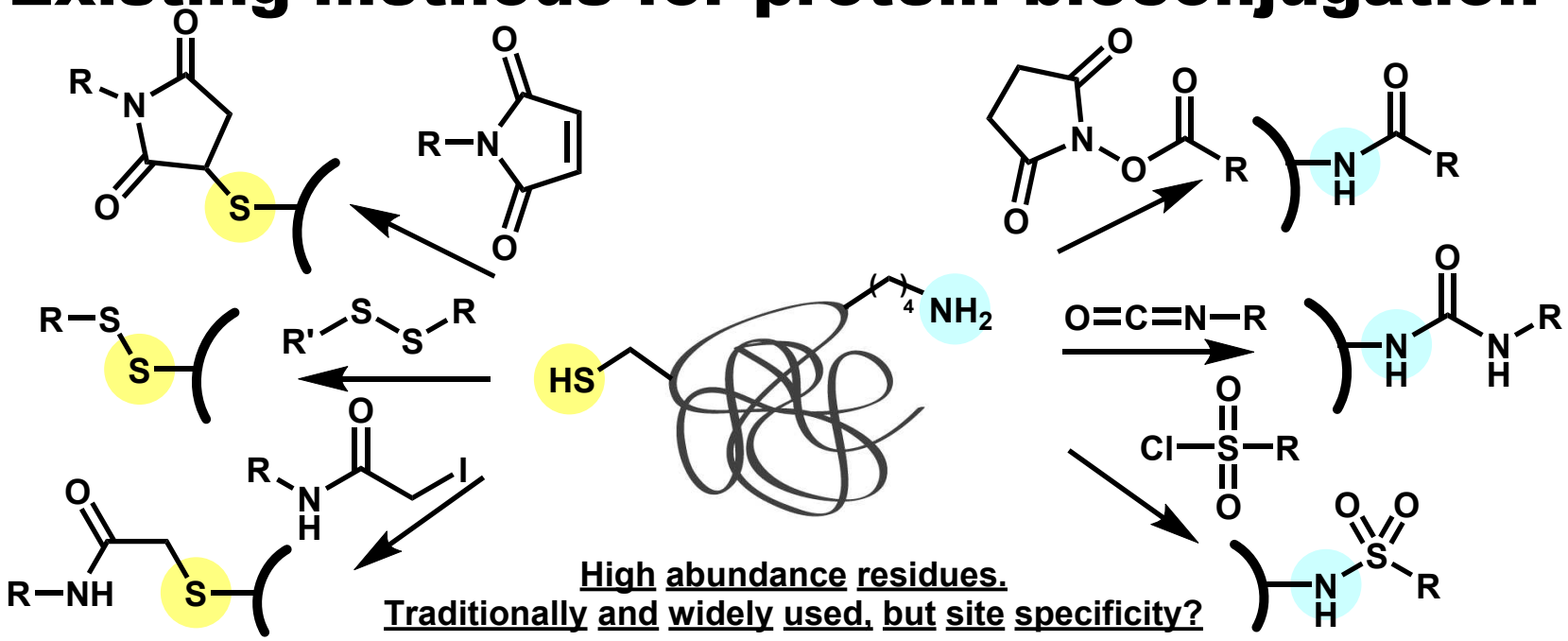
2017年 世界で最も売れた医薬品[2)]

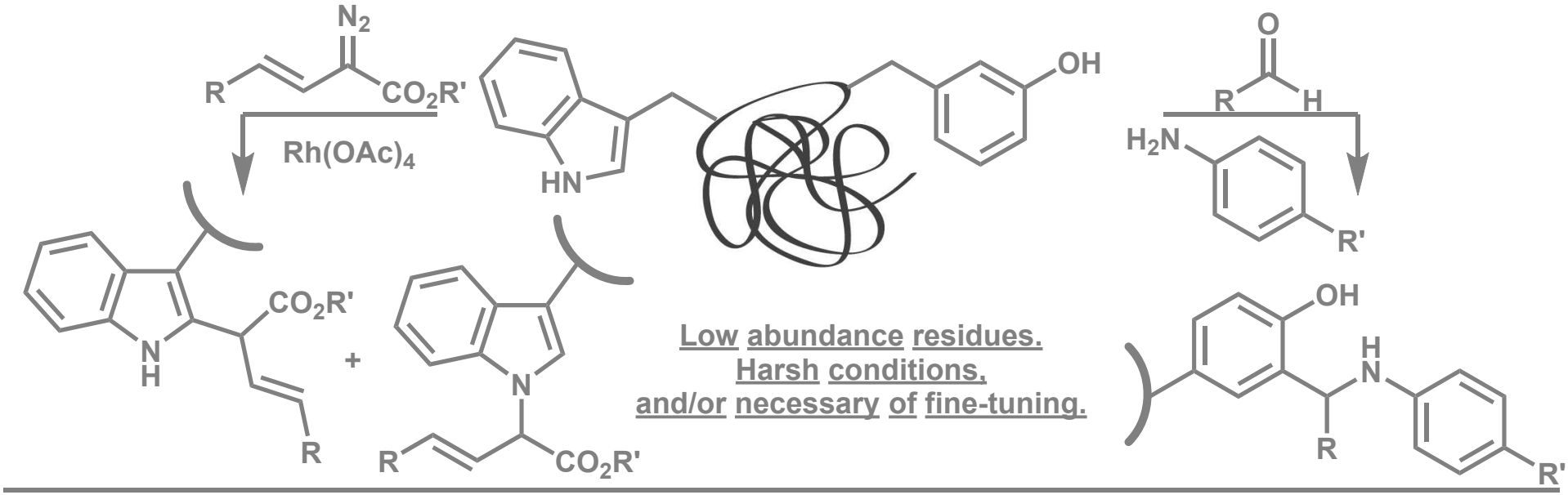
【単位：百万ドル】カッコ内は前年比（%）

ヒュミラ	22,713	(16.9)
ランタス	11,325	(▲7.1)
エンブレル	11,282	(2.6)
ハーボニー	8,432	(▲49.0)
レミケード	8,224	(▲2.6)
ザレルト	7,674	(18.7) 日本製品名・イグザレルト
エリキュース	7,010	(52.9)
マブセラ	6,937	(1.6) 日本製品名・リツキサン
ノボラビット	6,895	(15.3)
セレタイド	6,845	(▲7.0) 日本製品名・アドエア

1) Bernardes, G. J. L *et al. Nat. Chem.* **2016**, *8*, 103. 2) https://answers.ten-navi.com/pharmanews/14187/

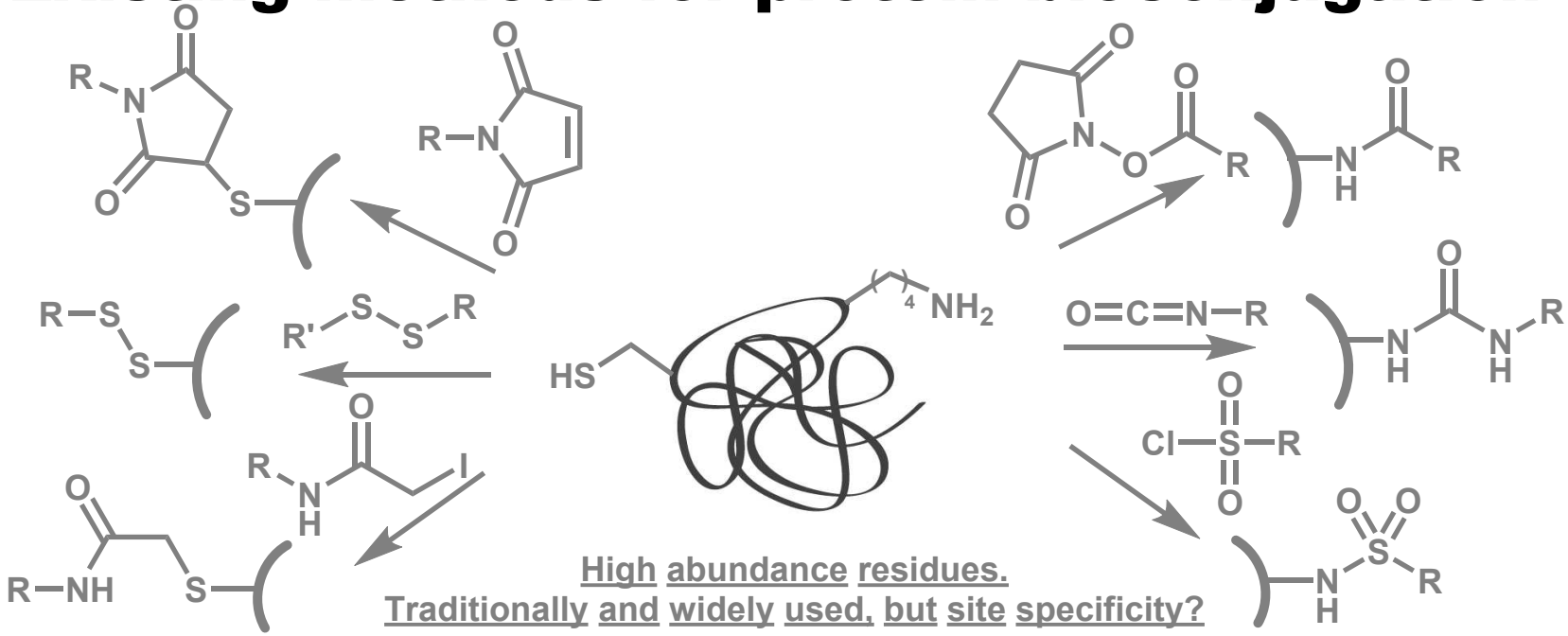
Existing methods for protein bioconjugation 4a

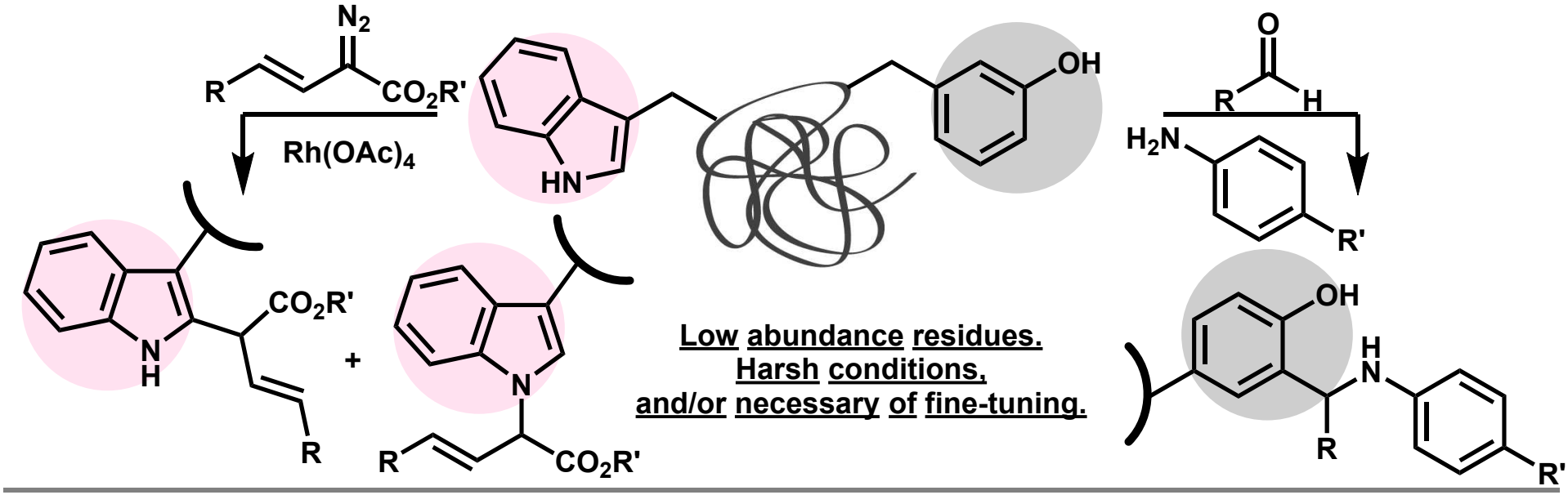




Sletten, E. M.; Bertozzi, C. R. *Angew. Chem. Int.Ed.* **2009**, *48*, 6974.

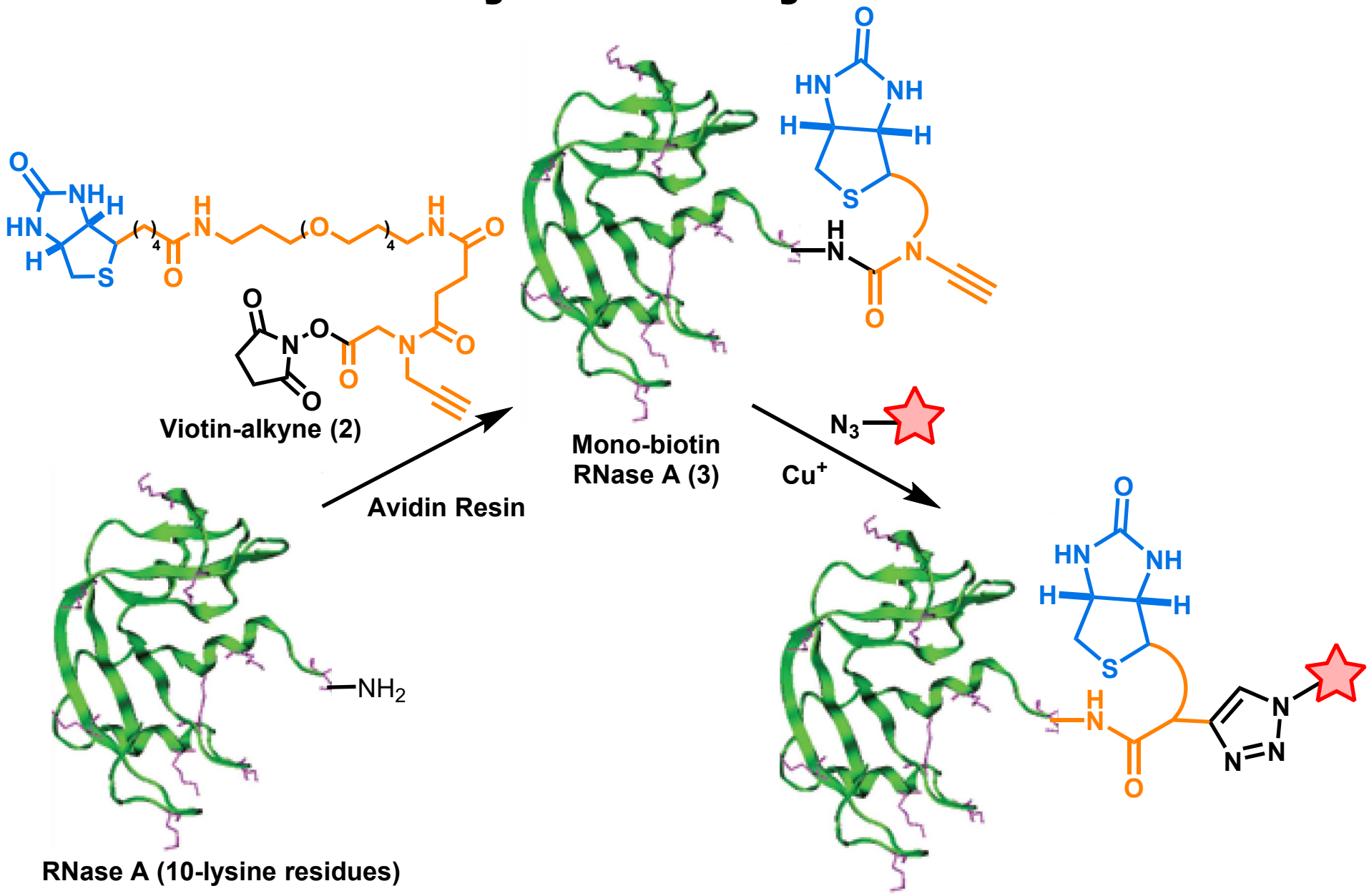
Existing methods for protein bioconjugation 4b





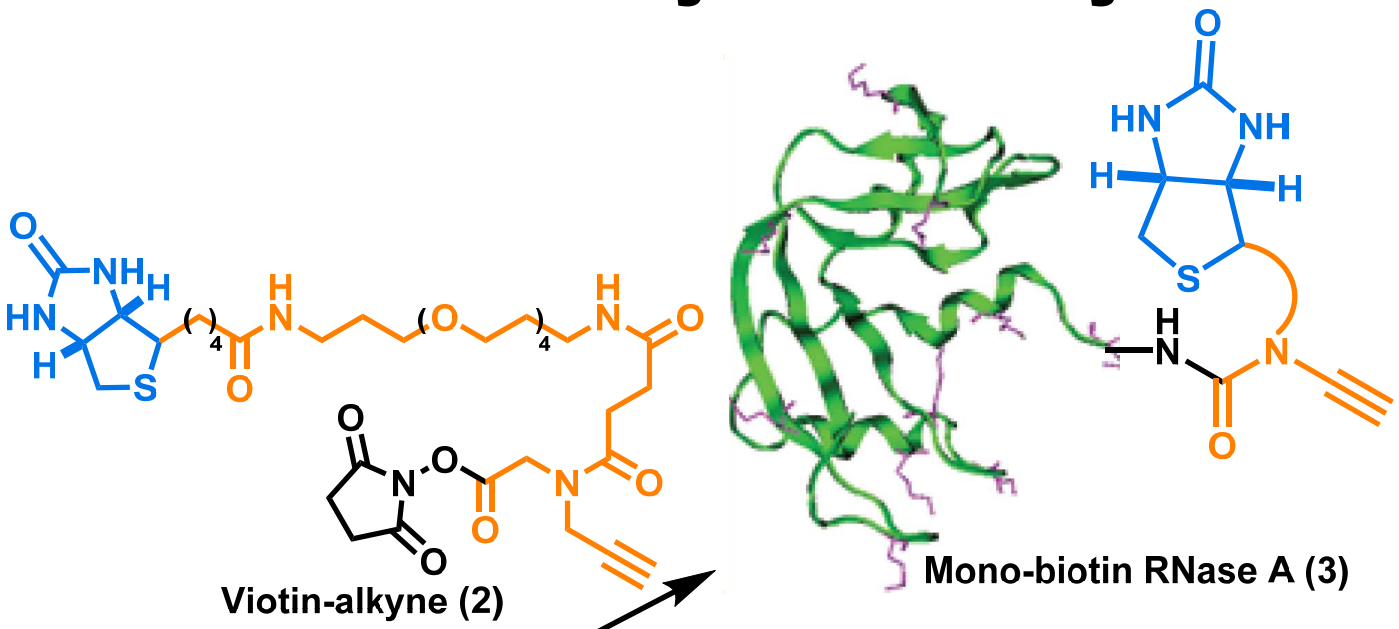
Sletten, E. M.; Bertozzi, C. R. *Angew. Chem. Int.Ed.* **2009**, *48*, 6974.

Mono-alkylation of Lysine residue



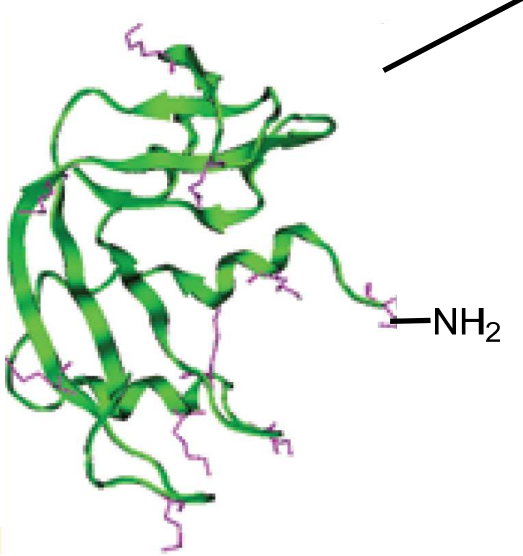
Totaro, K. A. *et. al. Bioconj. Chem.* **2016**, *27*, 994.

Mono-alkylation of Lysine residue

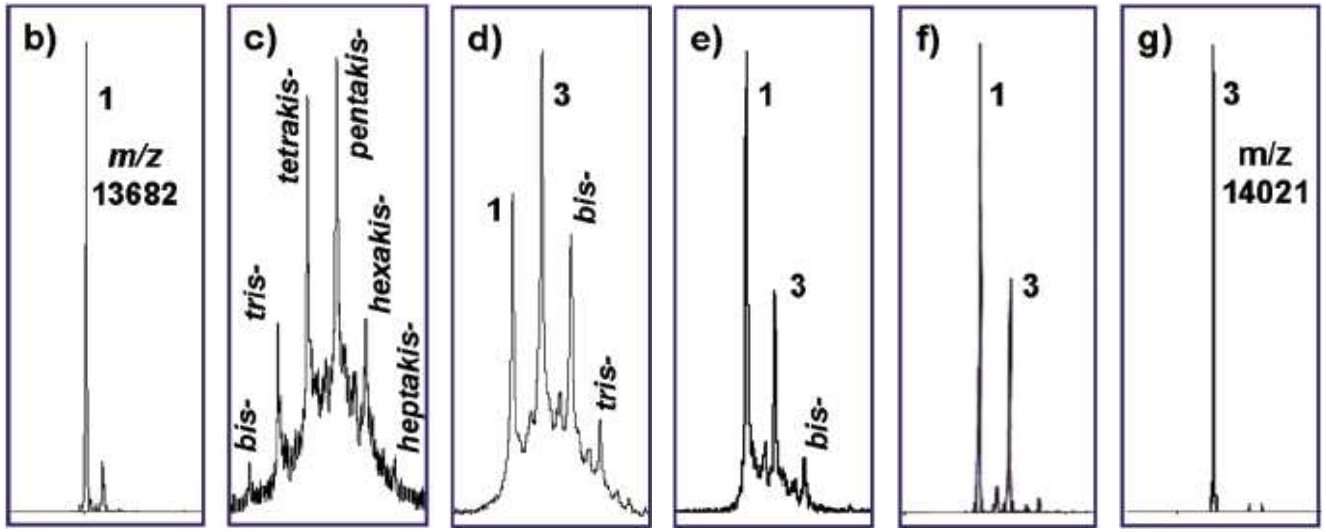


Viotin-alkyne (2)

Mono-biotin RNase A (3)



RNase A (10-lysine residues)



RNase A | 10 eq. (2) one-portion | 1 eq. (2) one-portion | 0.5 eq. (2) one-portion | 0.5 eq. (2) 100-portion | Mono-biotin RNase A (3)

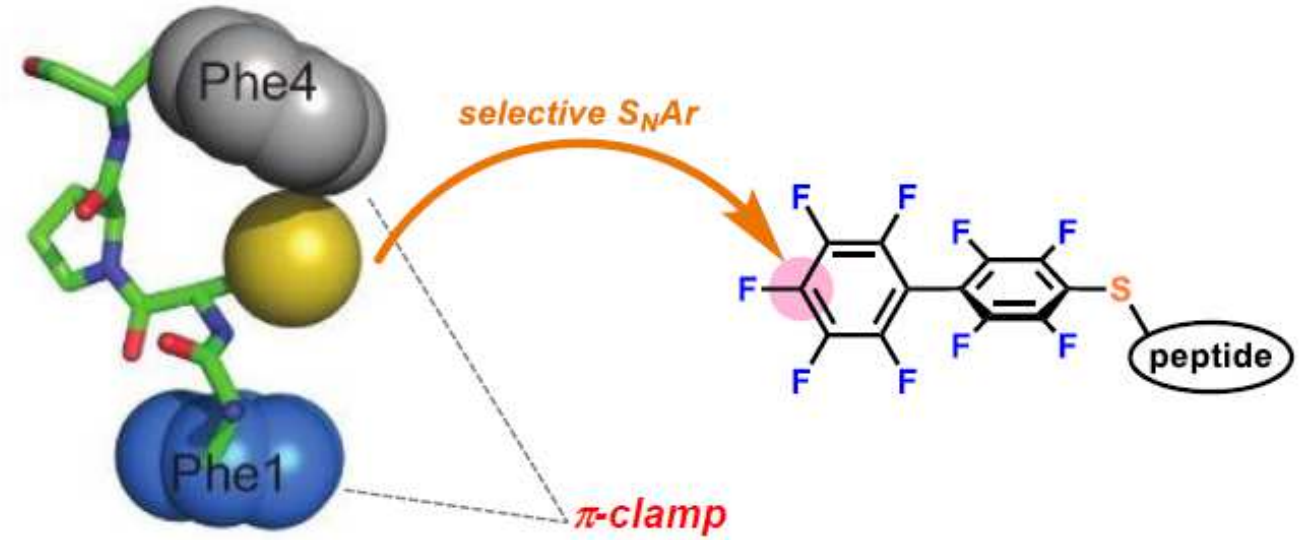
Totaro, K. A. *et. al. Bioconj. Chem.* **2016**, *27*, 994.

Mono-labelling was achieved, but generality?

Another method for bioconjugation

Protein bioconjugation using "π-clamp"

2016/6/4 Kotaro Tokumoto



Zhang, C.; Welborn, M.; Zhu, T.; Yang, N. J.; Santos, M. S.; Van Voorhis, T.; Pentelute, B. L. *Nat. Chem.* **2015**, *8*, 120.

Today's contents

1. Introduction
   - -Bioconjugation
   - -Existing method for protein (two-electron pathway)

2. **Bioconjugation using radical reaction (Tyr, Dha residues)**

3. Bioconjugation for carboxylic acid

Bioconjugation using radical reaction

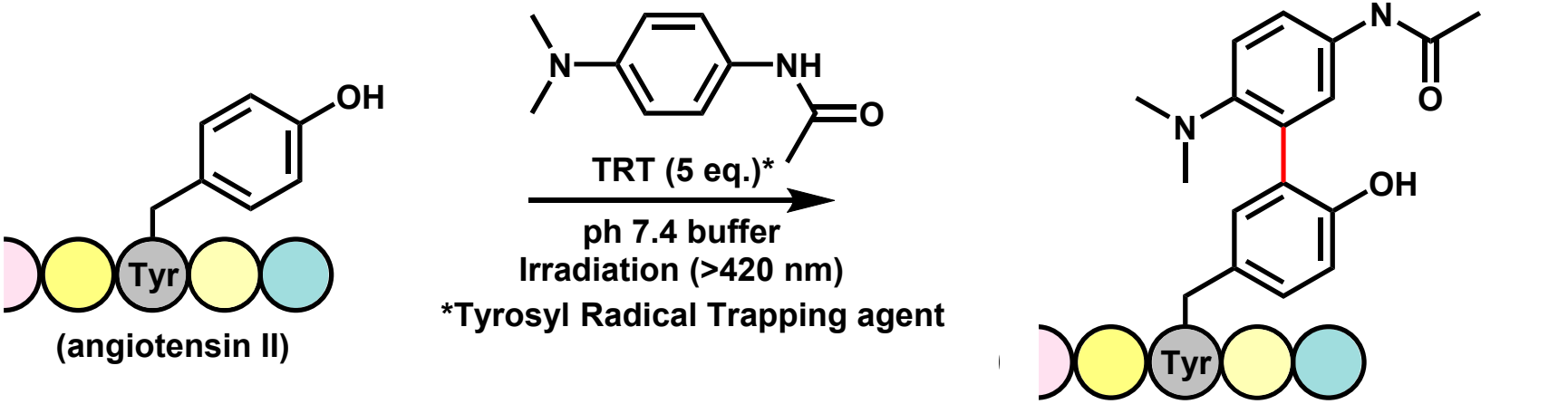
Bioconjugation
"the covalent derivatization of biomolecules"

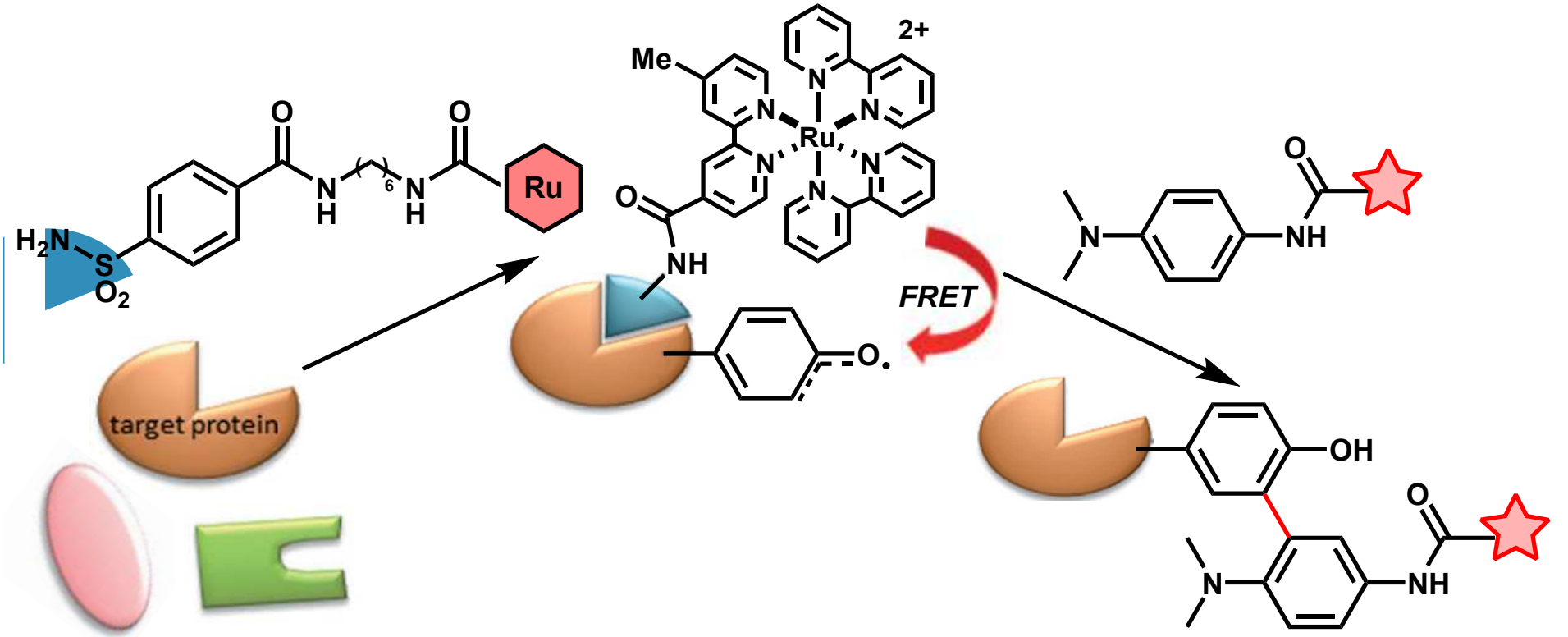
- In water
- High dilution
- Mild condition (keep tertiary structure)
- Functional group tolerance
  (containes variety of functionnal groups)

Radical reaction

- ✓ water-compatible
- ✓ High reactivity
- ✓ Mild condition
  (proceed at room temperature)
- ✓ Functional group tolerance

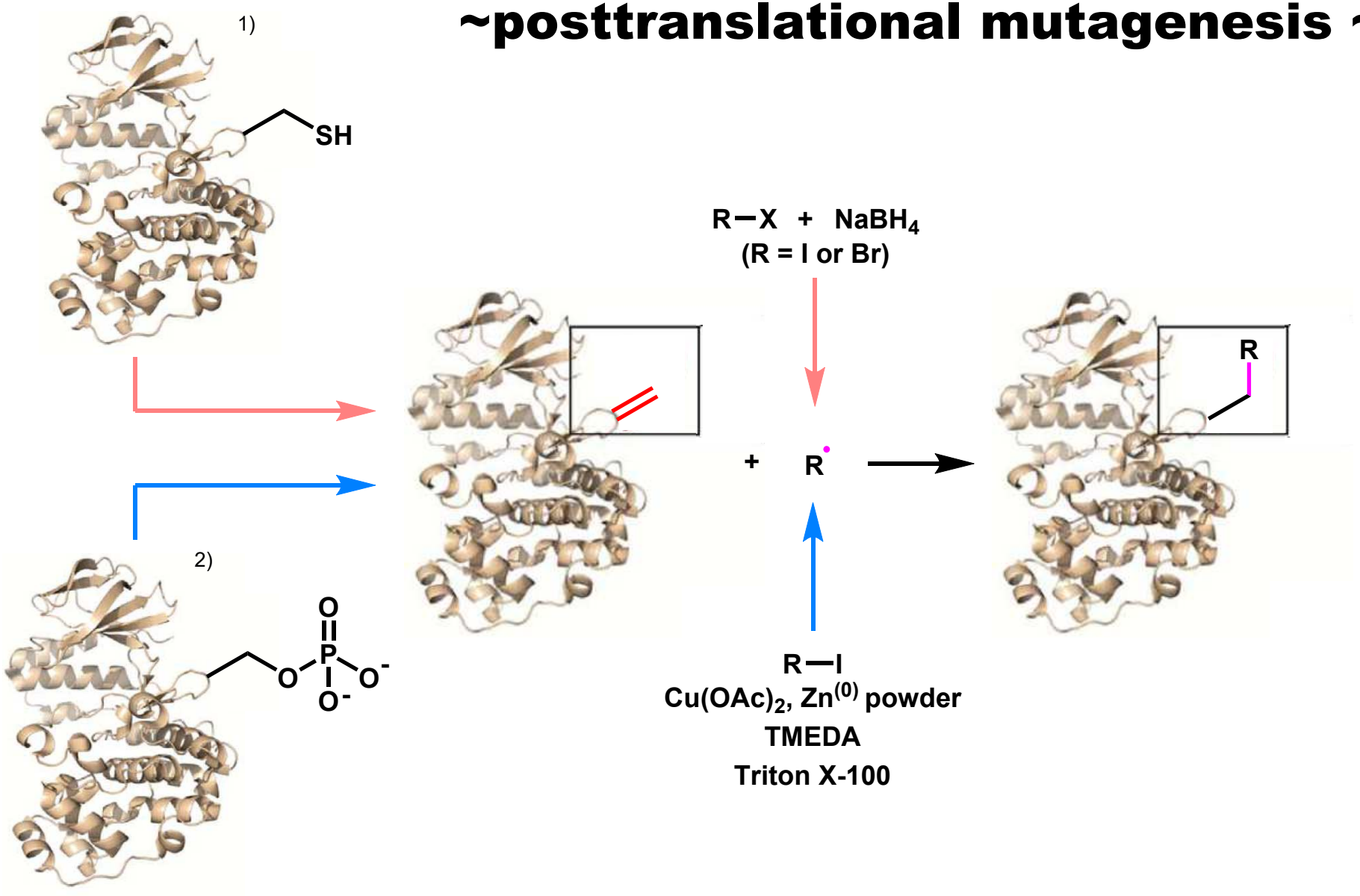
Radical approach to Tyrosine residue





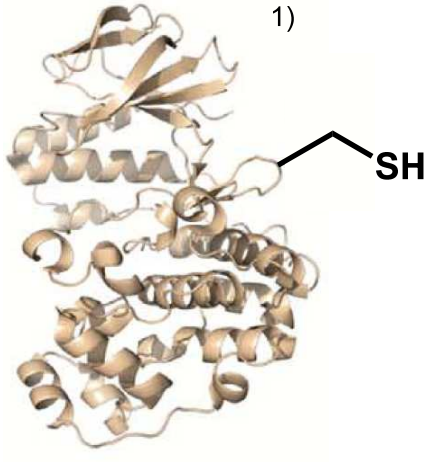
Sato, S.; Nakamura, H. *Angew. Chem. Int. Ed.* **2013**, *52*, 8681.

Radical approach for Dehydroalanine residue 11a ~posttranslational mutagenesis ~

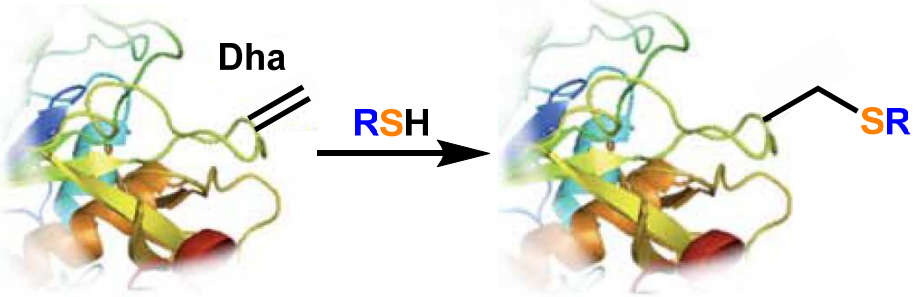


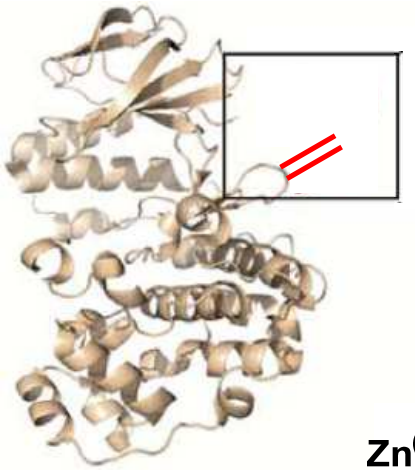
1) Wright, T. H. *et al. Science*, **2016**, *354*, 597. 2) Yang, A. *et al. Science,* **2016**, *354*, 623.

Radical approach for Dehydroalanine residue 11b



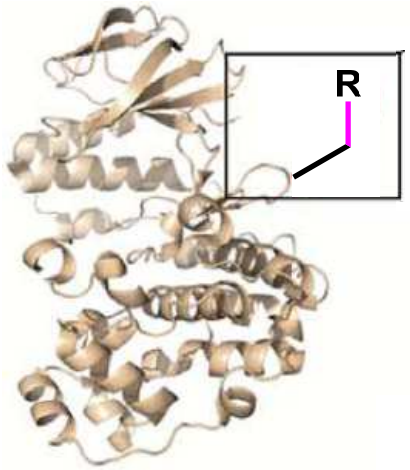
Traditionally, unnatural thioether bond was contained...



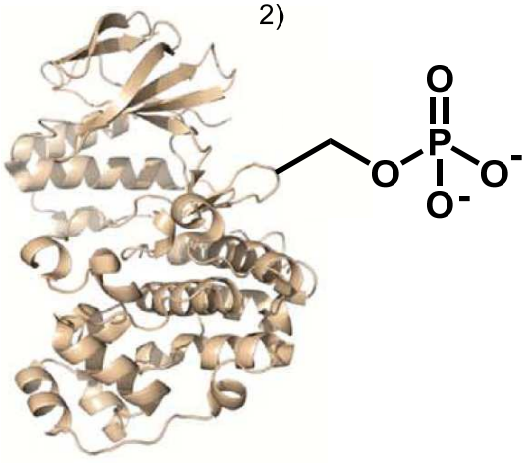


$R{-}X + NaBH_4$
(R = I or Br)

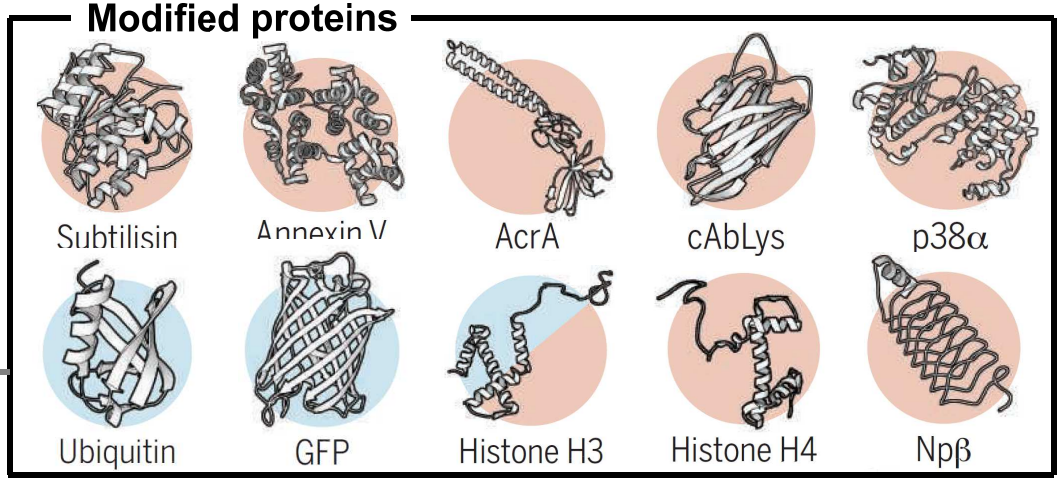
+ R•



$R{-}I + Cu(OAc)_2$
$Zn^{(0)}$ powder, TMEDA



Modified proteins



1) Wright, T. H. *et al. Science*, **2016**, *354*, 597.
2) Yang, A. *et al*. *Science,* **2016**, *354*, 623.

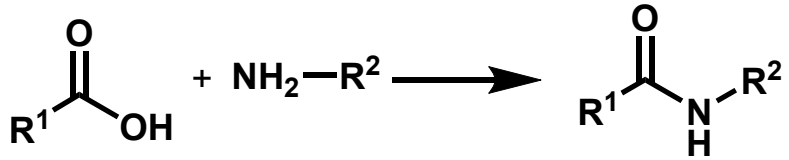
Today's contents

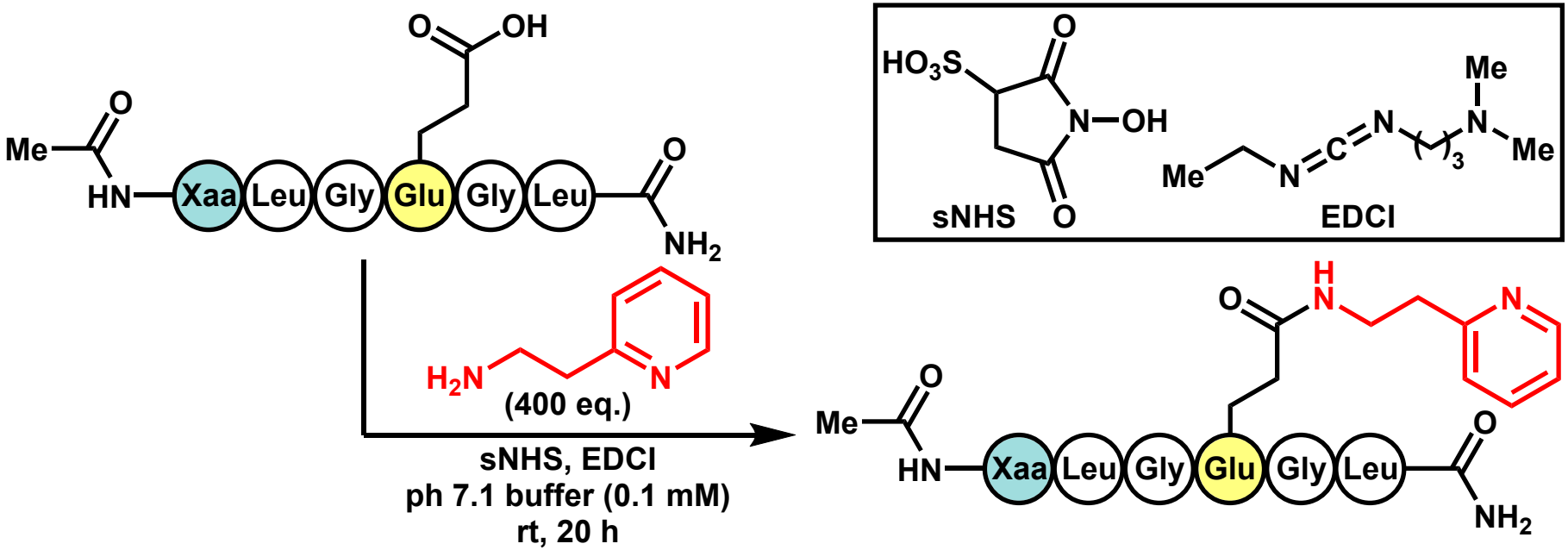
1. Introduction
   - -Bioconjugation
   - -Existing method for protein (two-electron pathway)

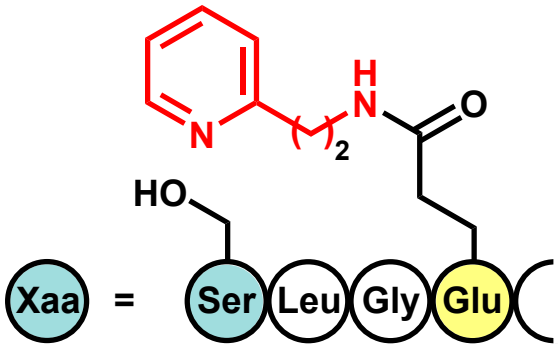
2. Bioconjugation using radical reaction (Tyr, Dha residues)

3. Bioconjugation for carboxylic acid

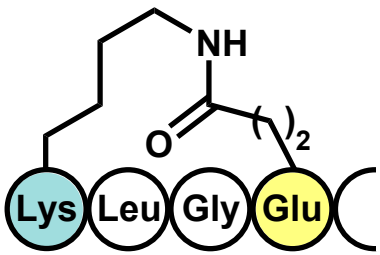
Bioconjugation for carboxyl group (1)



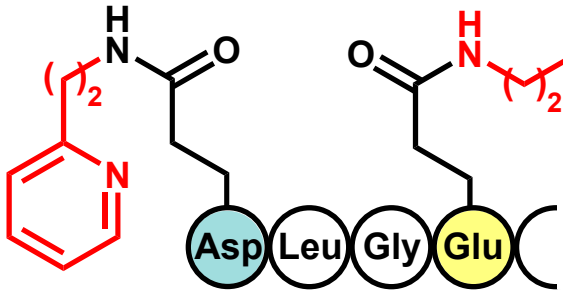




relative yield: 100% (desired)



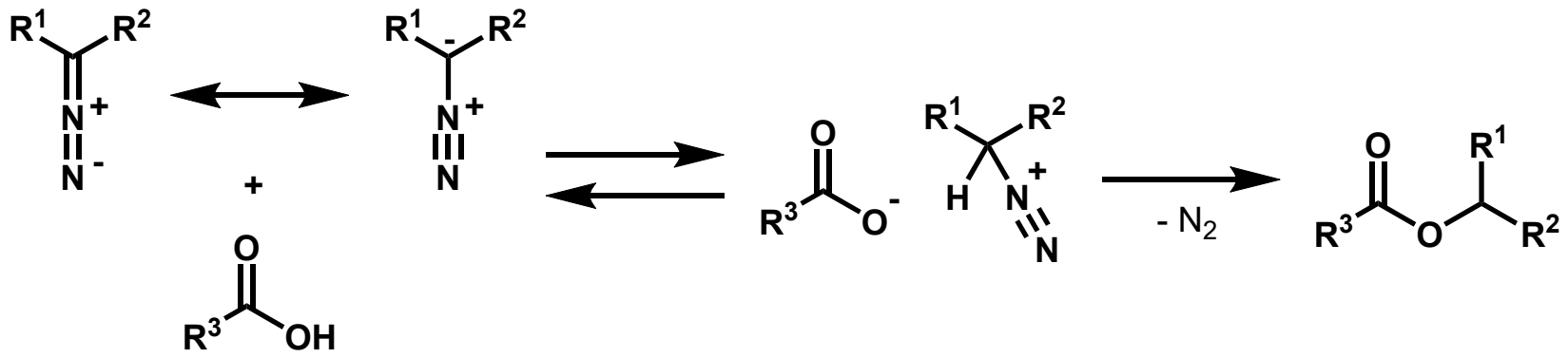
68%, 15% (bridged prod.)

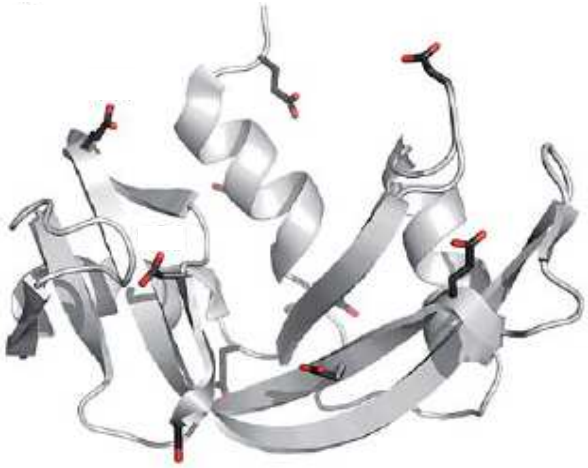


18%, 82% (second labeled prod.)

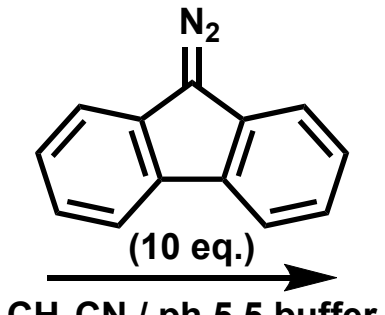
Totaro, K. A. *et. al. Bioconj. Chem.* **2016**, *27*, 994.

Bioconjugation for carboxyl group (2)





RNase A ($(CO_2H)_{11}$)



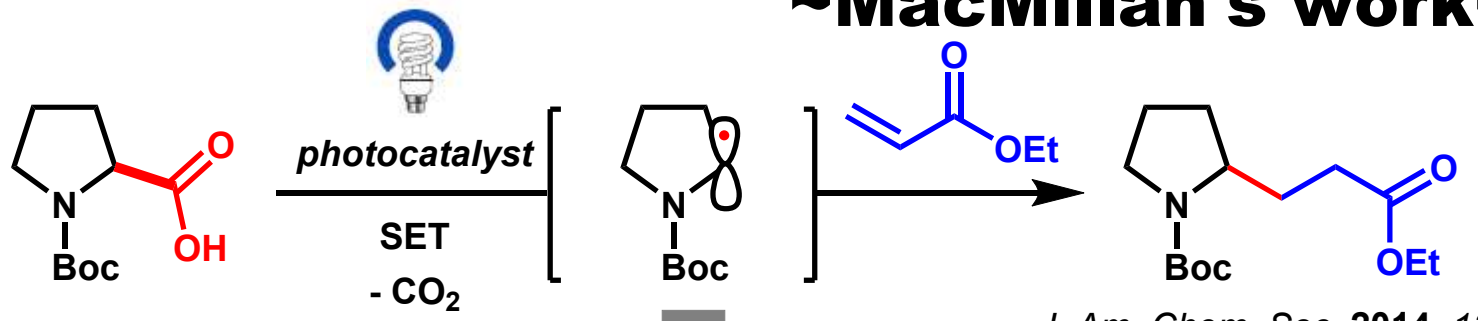
(10 eq.)

CH_3CN / ph 5.5 buffer
(10 mM)
37 °C, 4 h

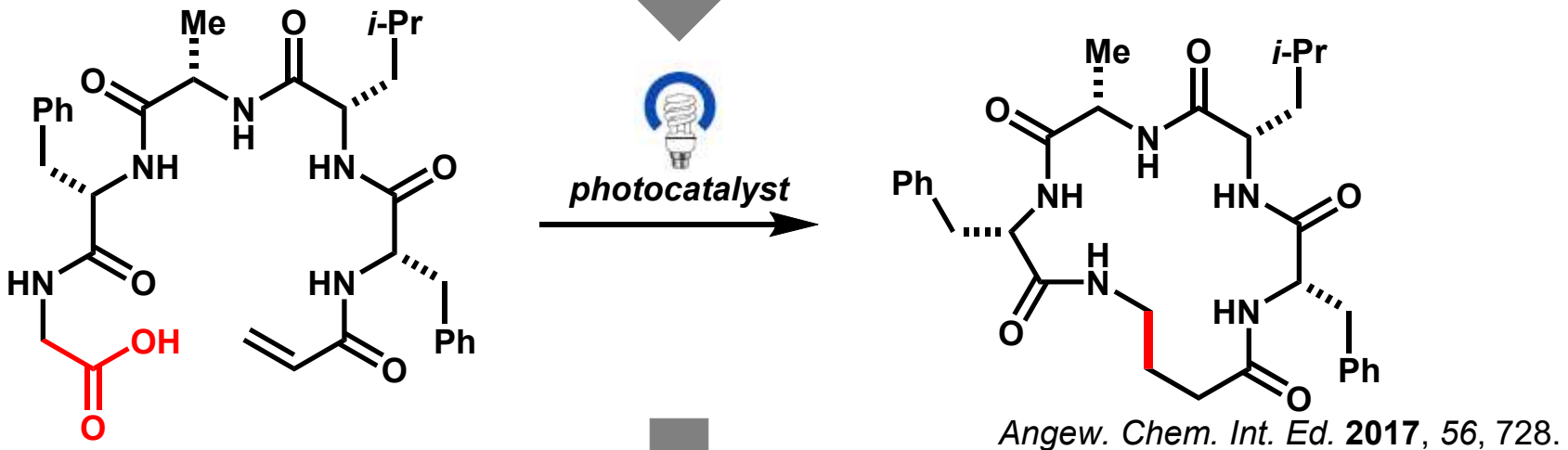
Labelled 2-Asp and 2-Glu residues

McGrath, N. A.; Andersen, K. A.; Davis, A. K. F.; Lomax, J. E.; Raines, R. T. *Chem. Sci.* **2015**, *6*, 752.

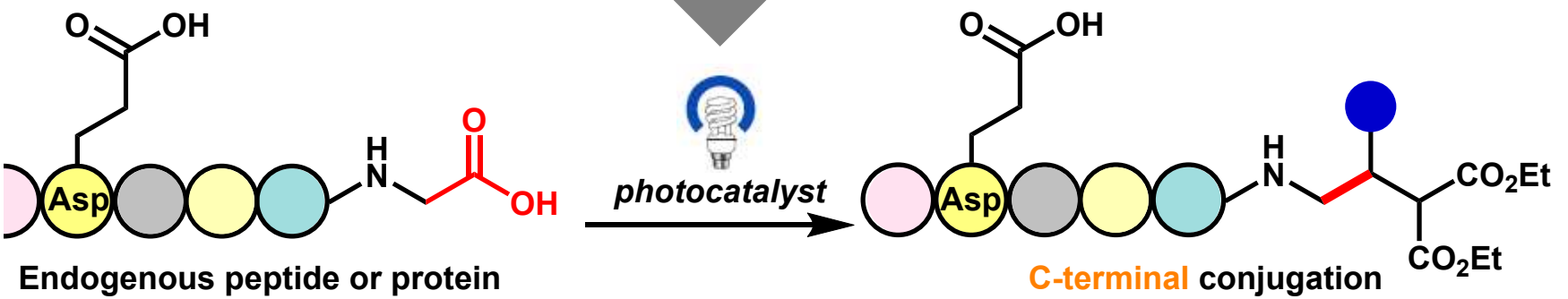
Development of photo-catalyst for bioconjugation ~MacMillan's work~ 15



J. Am. Chem. Soc. **2014**, *136*, 11602.

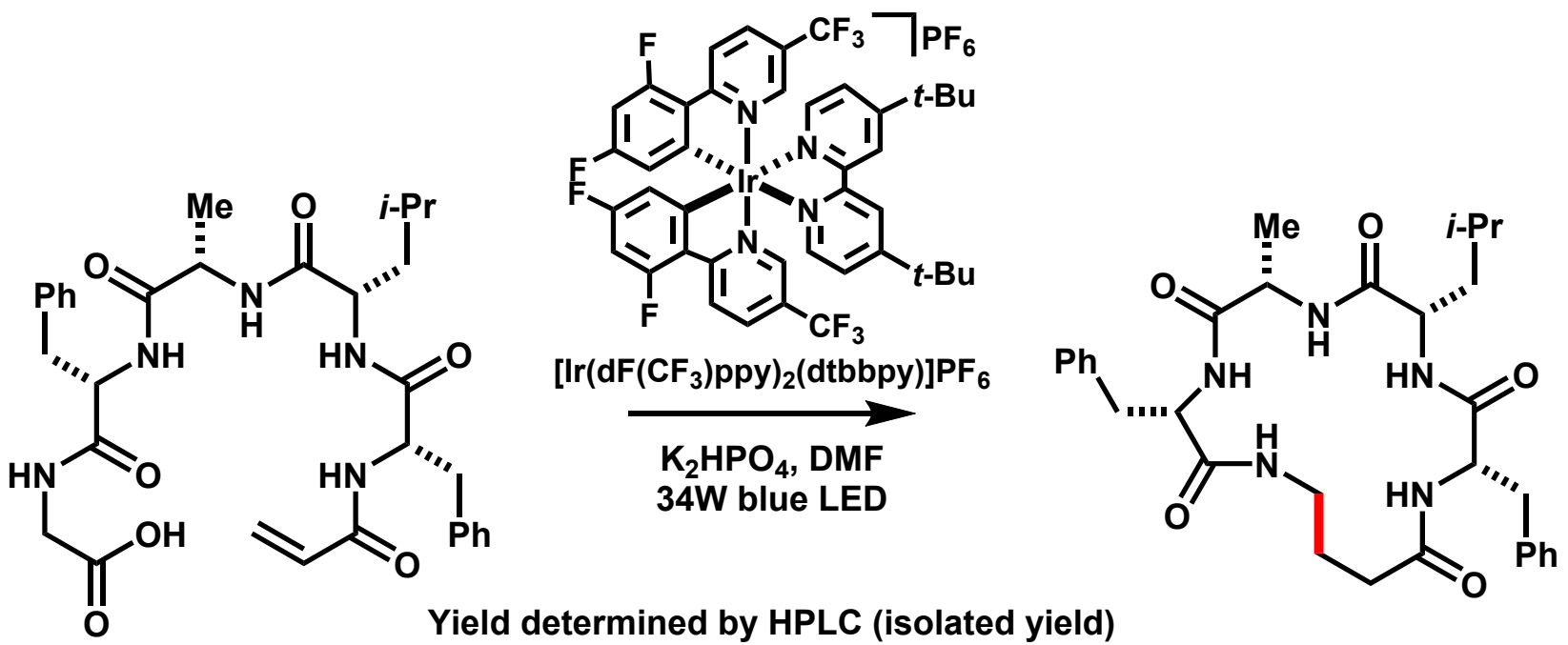


Angew. Chem. Int. Ed. **2017**, *56*, 728.



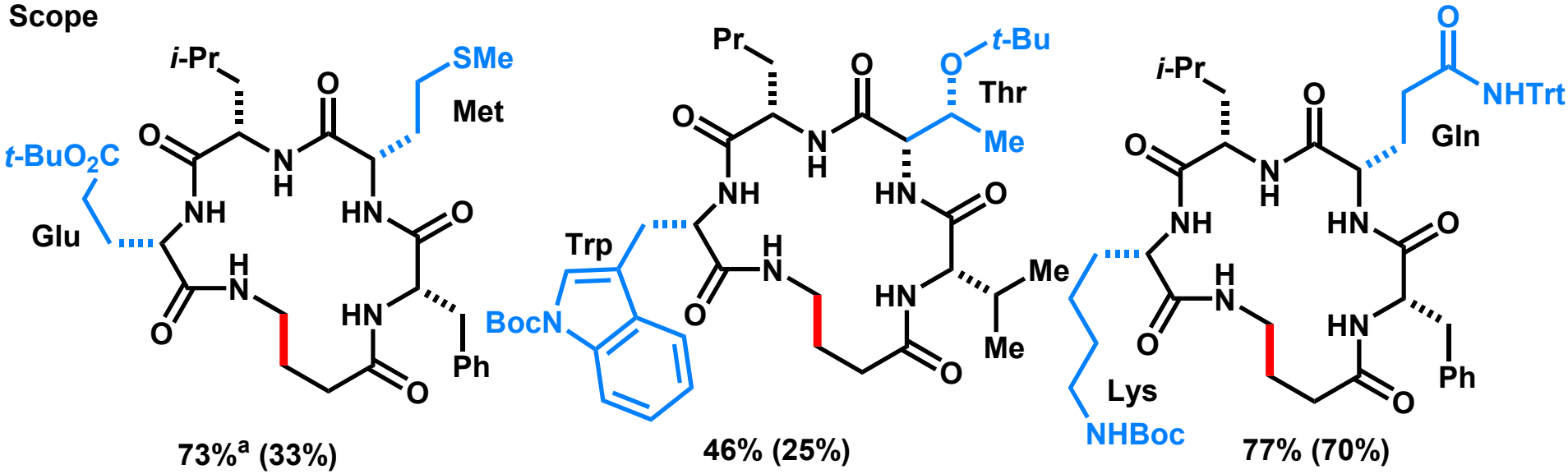
Nat. Chem. **2017**, *10*, 205.

Ir-cat. decarboxylation (intramolecular cyclization)



Yield determined by HPLC (isolated yield)

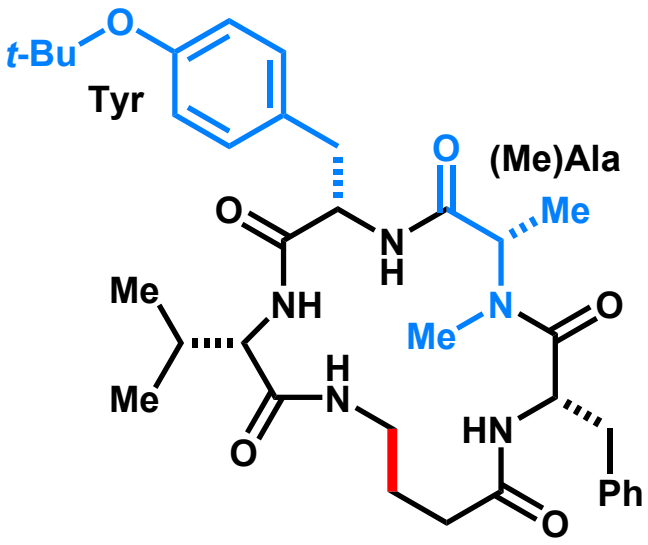
Scope



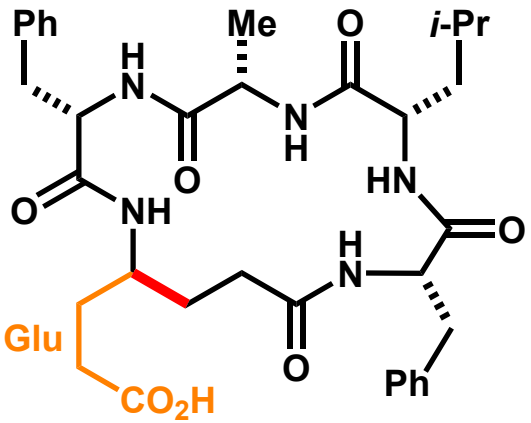
a10mol% 2,4,6-triisopropylthiophenol added.

MacMillan, D. W. C. *et. al. Angew. Chem. Int. Ed.* **2017**, *56*, 728.

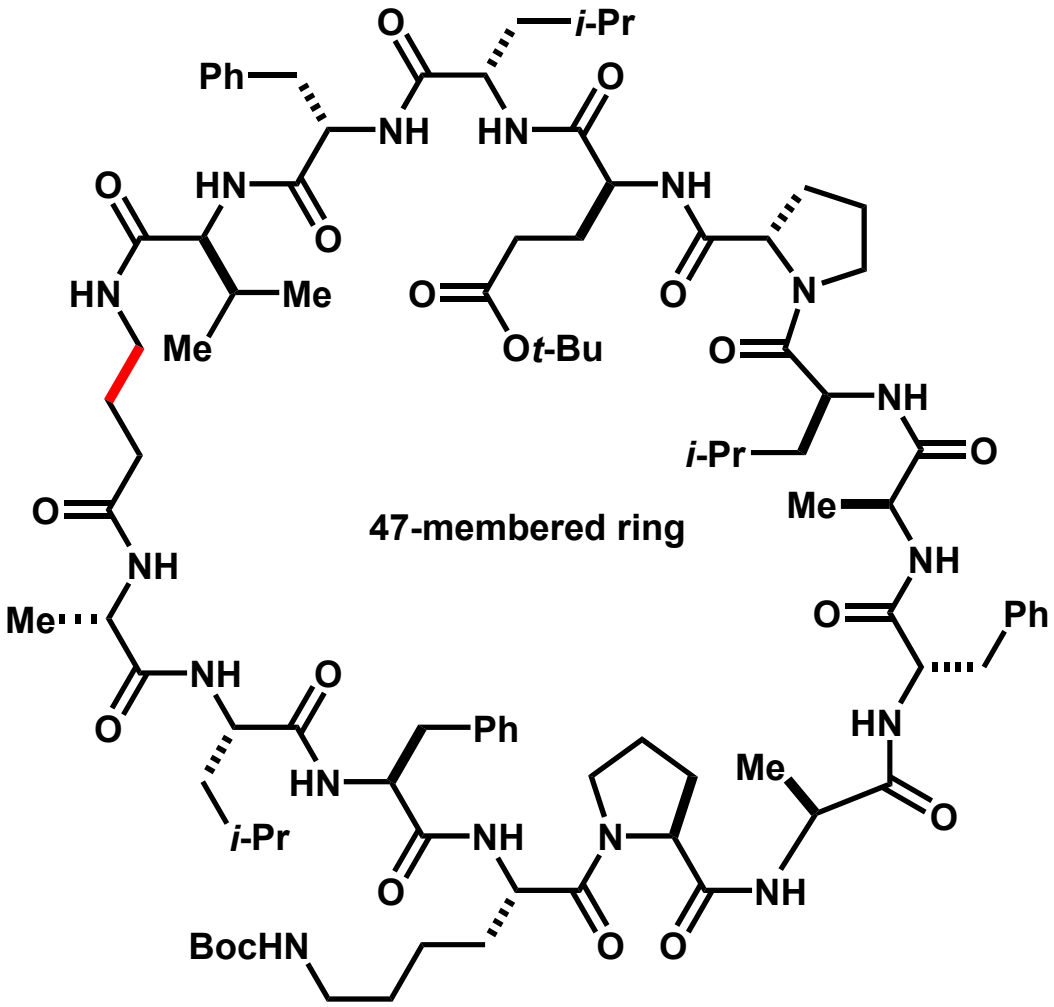
Selective decarboxylation at the C-term



82% (67%)



50%, dr = 2:1, (25%)

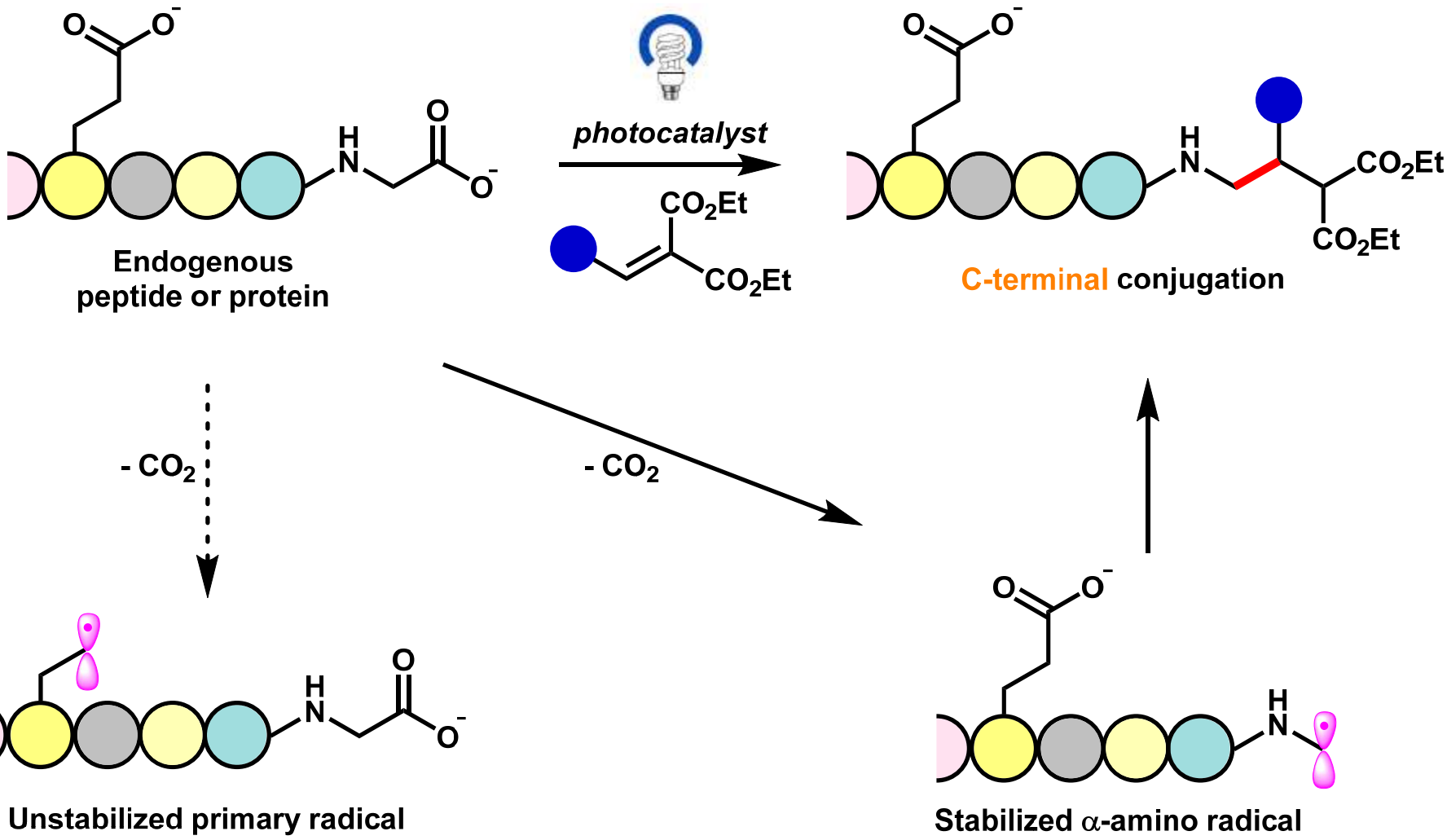


47-membered ring

53% (39%)

Yield determined by HPLC (isolated yield)

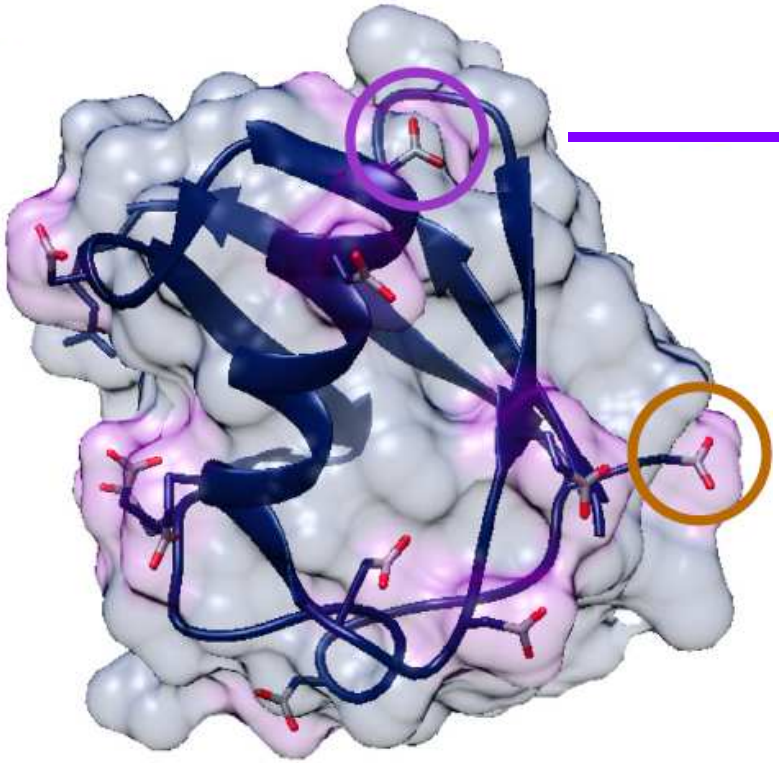
Selective oxidation of C-term carboxylate over other acid-containing side chains



Stability of the resulting radical intermediate following decarboxylation is inherently linked to the carboxylate's ground-state oxidation potential.

MacMillan, D. W. C. *et al. Nat. Chem.* **2017**, *10*, 205.

Selective oxidation of C-term carboxylate over other acid-containing side chains[1)]



Aspartic or glutamic acid carboxylates

High abundance

Difficult to oxidize

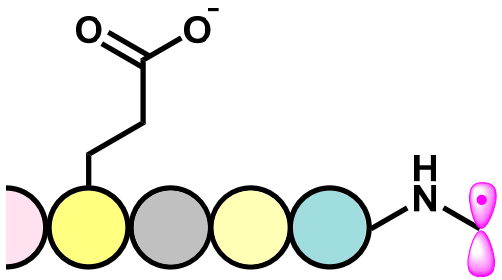
$E_{1/2}^{red}$~1.25 V (vs SCE)

C-terminal carboxylate

Single site

Readily oxidized

$E_{1/2}^{red}$~0.95 V (vs SCE)



$\Delta G = -F\Delta E$ [2)]

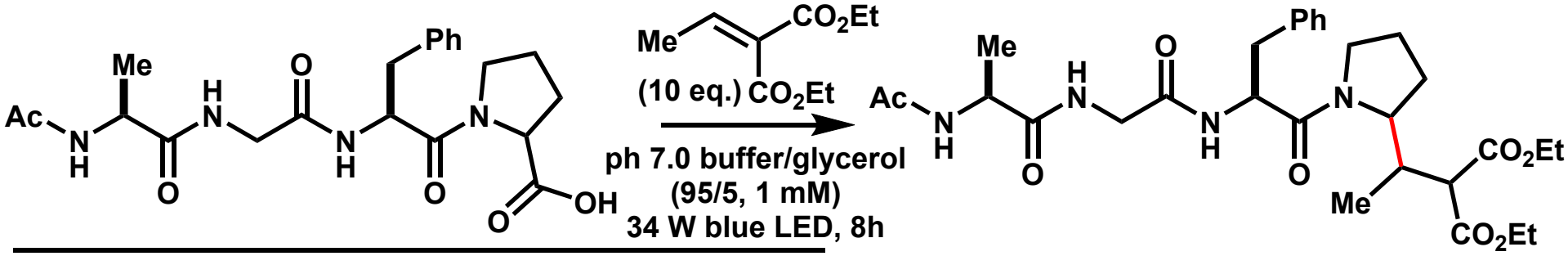
***F*: Faraday constant**

$\Delta E = 0.1$ V ≈ $\Delta G = -2.3$ kcal/mol

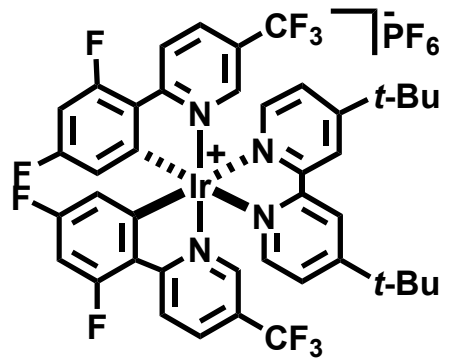
$\Delta E_{1/2}^{red} = 0.3$ V ≈ $\Delta G = -$ 6.9 kcal/mol

1) MacMillan, D. W. C. *et al. Nat. Chem.* **2017**, *10*, 205.
2) Kamakura_LS_171118_"Coupling reaction using excited state organonickel complex"

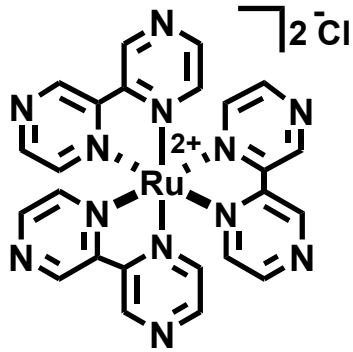
Photocatalyst evaluation



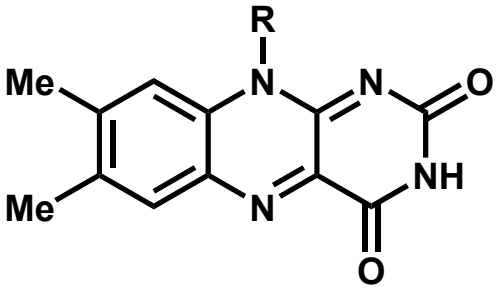
entry	photocatalyst (30 mol%)	yield
1	$[Ir(dF(CF_3)ppy)_2(dtbbpy)]PF_6$	0%
2	$Ru(bpz)_3Cl_2$	0%
3	mesityl-acridinium	21%
4	riboflavin	30%
5	riboflavin *N*-ethyl carbamate	28%
6	riboflavin tetrabutyrate (cat A)	75%
7	lumiflavin (cat B)	20%

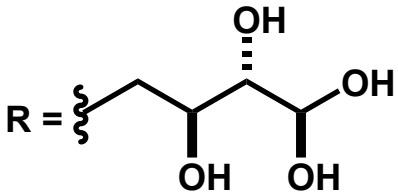


$[Ir(dF(CF_3)ppy)_2(dtbbpy)]PF_6$

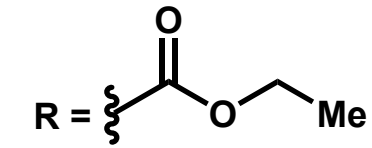


$Ru(bpz)_3Cl_2$

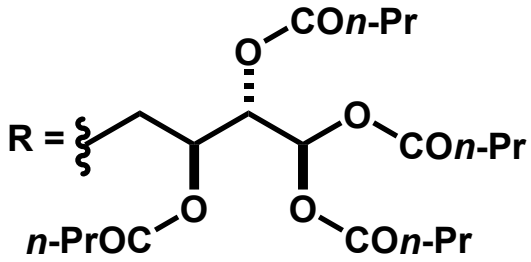




riboflavin

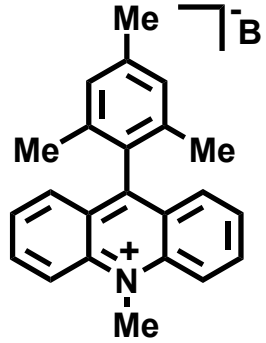


riboflavin *N*-ethyl carbamate



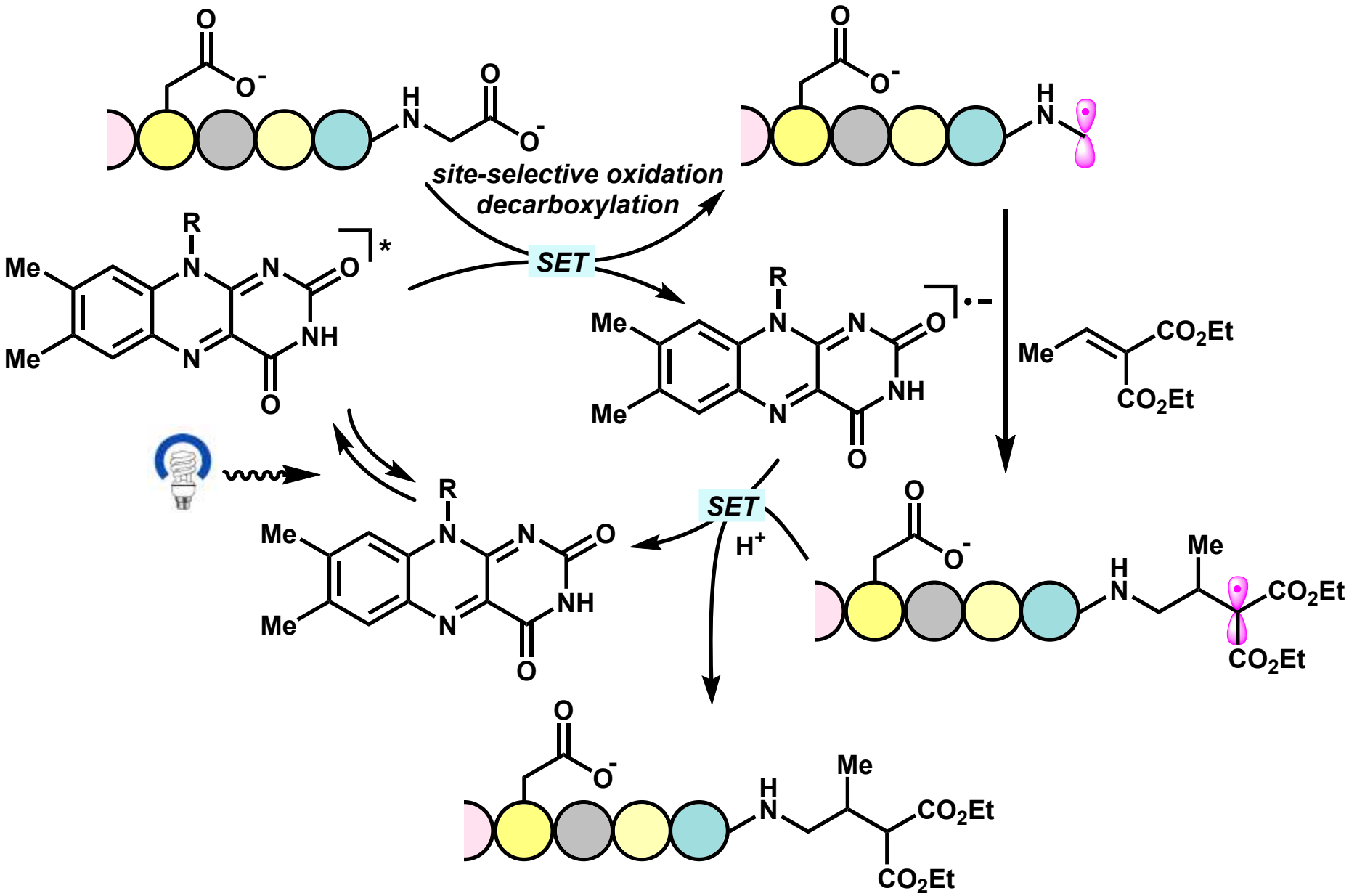
riboflavin tetrabutyrate
(cat A)

lumiflavin (cat B)



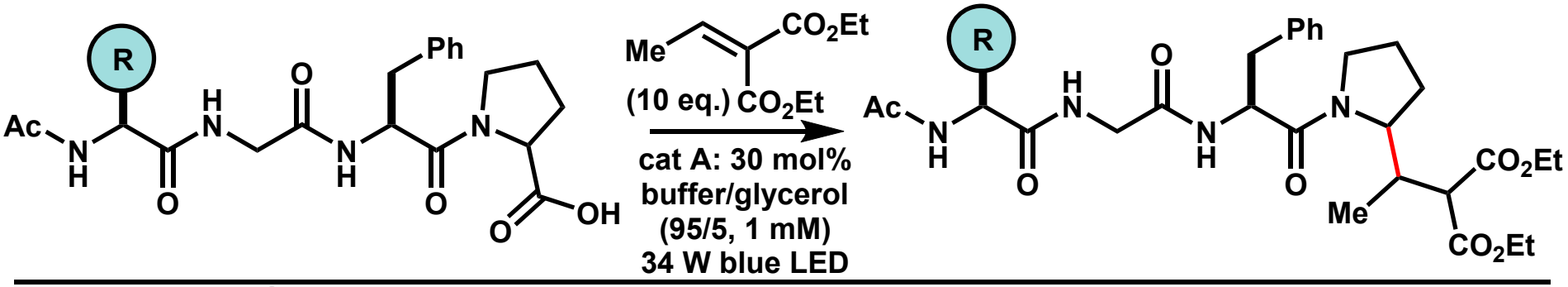
mesityl-acridinium

Proposed reaction mechanism



MacMillan, D. W. C. *et al. Nat. Chem.* **2017**, *10*, 205.

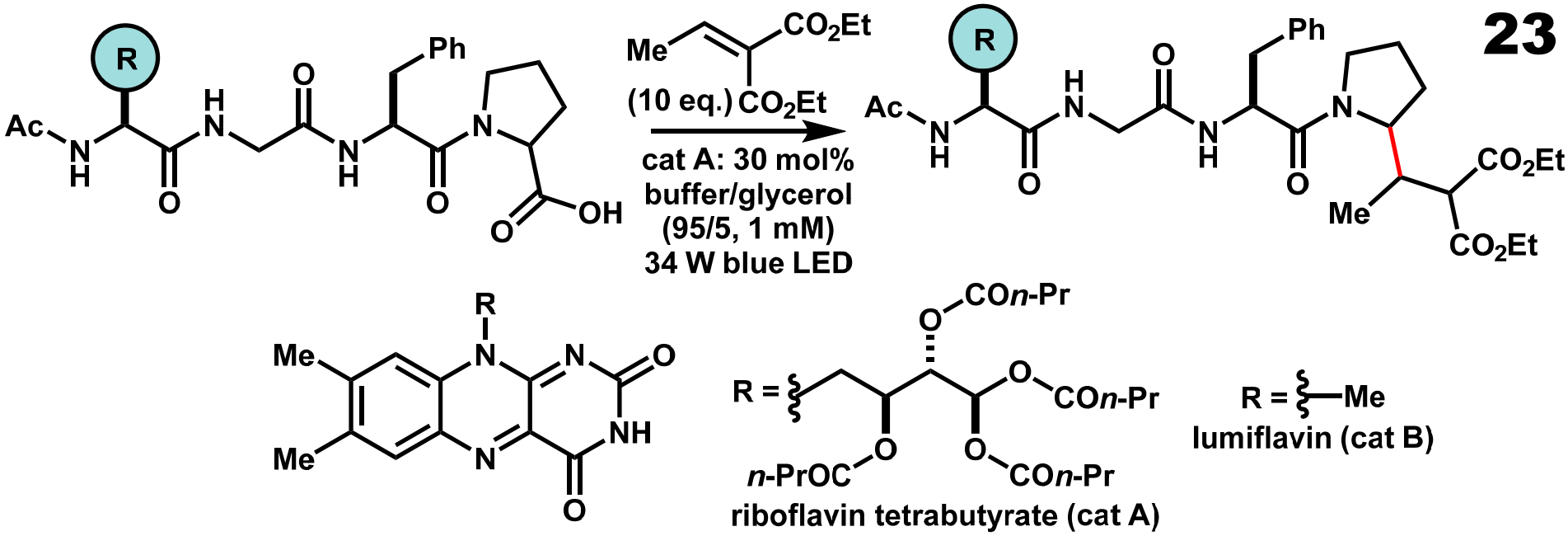
Survey of functional group tolerance

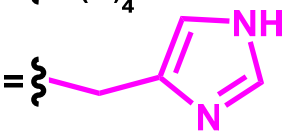
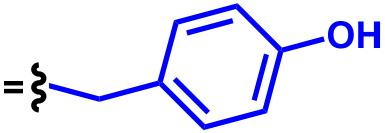


entry	R side chain	Yield (ph 7.0, 8 h)	Yield (ph 3.5, 6 h)	Surface abundance
1	Ala R = –Me	79%	92%	7.9%
2	Asp R = –CH_2CO_2H	77%	93%	7.4%
3	Ile R = –*sec*-Bu	77%	90%	3.0%
4	Asn R = –CH_2CONH_2	76%	91%	6.3%
5	Val R = –*i*-Pr	76%	95%	4.6%
6	Ser R = –CH_2OH	75%	87%	8.9%
7	Glu R = –$(CH_2)_2CO_2H$	75%	91%	6.2%
8	Leu R = –*iso*-Bu	74%	95%	4.3%
9	Thr R = –CH(Me)OH	73%	90%	7.1%
10	Gln R = –$(CH_2)_2CONH_2$	71%	94%	4.5%
11	Arg R = –$(CH_2)_3NHC(=NH)NH_2$	71%	87%	4.0%

MacMillan, D. W. C. *et al. Nat. Chem.* **2017**, *10*, 205.

Survey of functional group tolerance (Lys, His, Tyr)

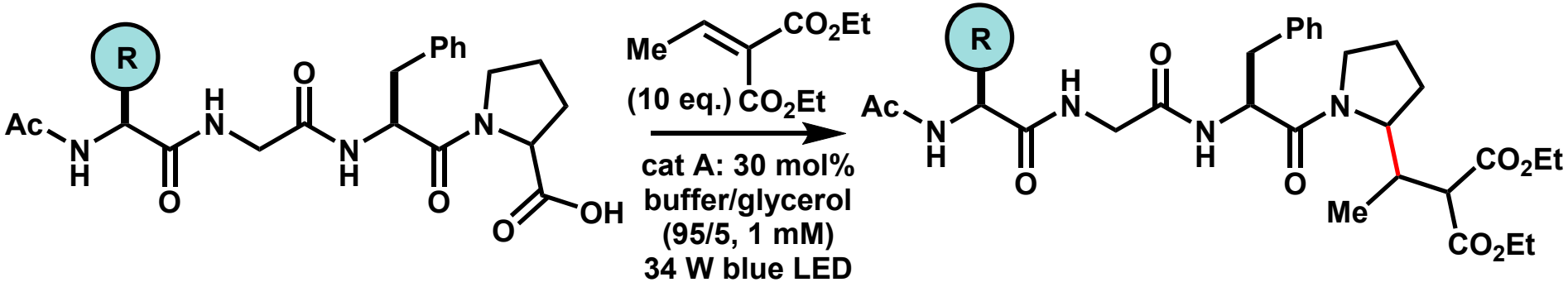


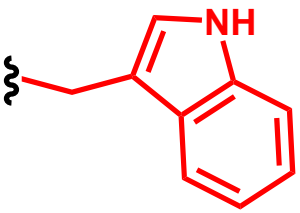
entry	R side chain	Yield (ph 7.0, 8 h)	Yield (ph 3.5, 6 h)	Surface abundance
2	Asp R = CO_2H	77%	93%	7.4%
7	Glu R = $(\ )_2CO_2H$	75%	91%	6.2%
12	Lys R = $(\ )_4NH_2$	52%	65%	8.9%
13	His R = 	11%	70%	2.2%
14	Tyr R = 	8%[a]	23%[a]	4.8%

[a]catalyst B was used instead of A

MacMillan, D. W. C. *et al. Nat. Chem.* **2017**, *10*, 205.

Survey of functional group tolerance (Trp, Cys, Met)



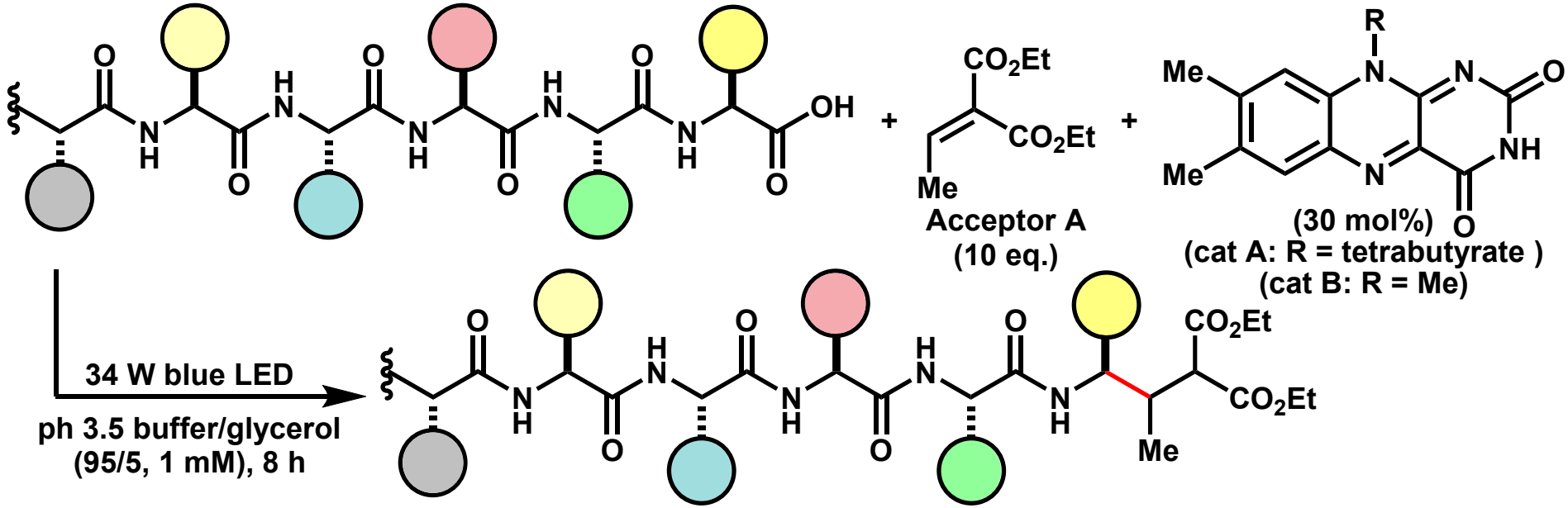
entry	R side chain	Yield (ph 7.0, 8 h)	Yield (ph 3.5, 6 h)	Surface abundance
15	Trp R = 	<10%[a]	15-20% (cat A:60mol%, 12h)	N.D.
16	Cys R = 	considerably lower[b]	26%	N.D.
17	Met R = 	N.D.	31%[c]	N.D.

[a]electron transfer occures between the indole moiety and photocatalyst.

[b]because the acidity of the thiol is pKa~8, the nucleophilicity is high at ph 7.

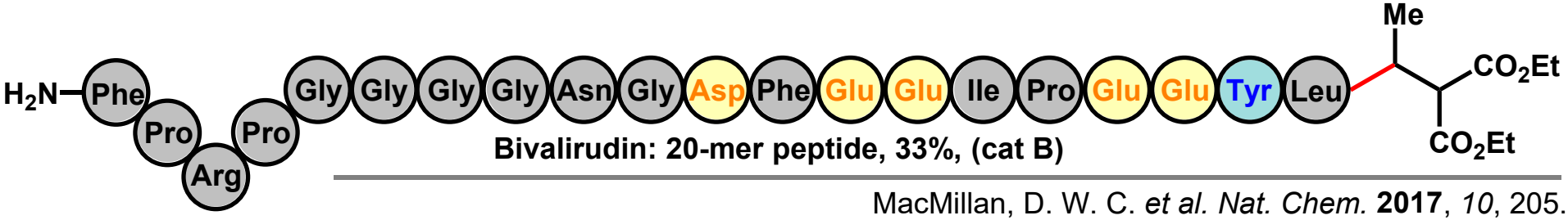
[c]combind yield of desired product and the respective sulfoxide.

Application to endogenous peptides



Fibronectin binding inhibitor: 11-mer peptide, 66%, (cat A)

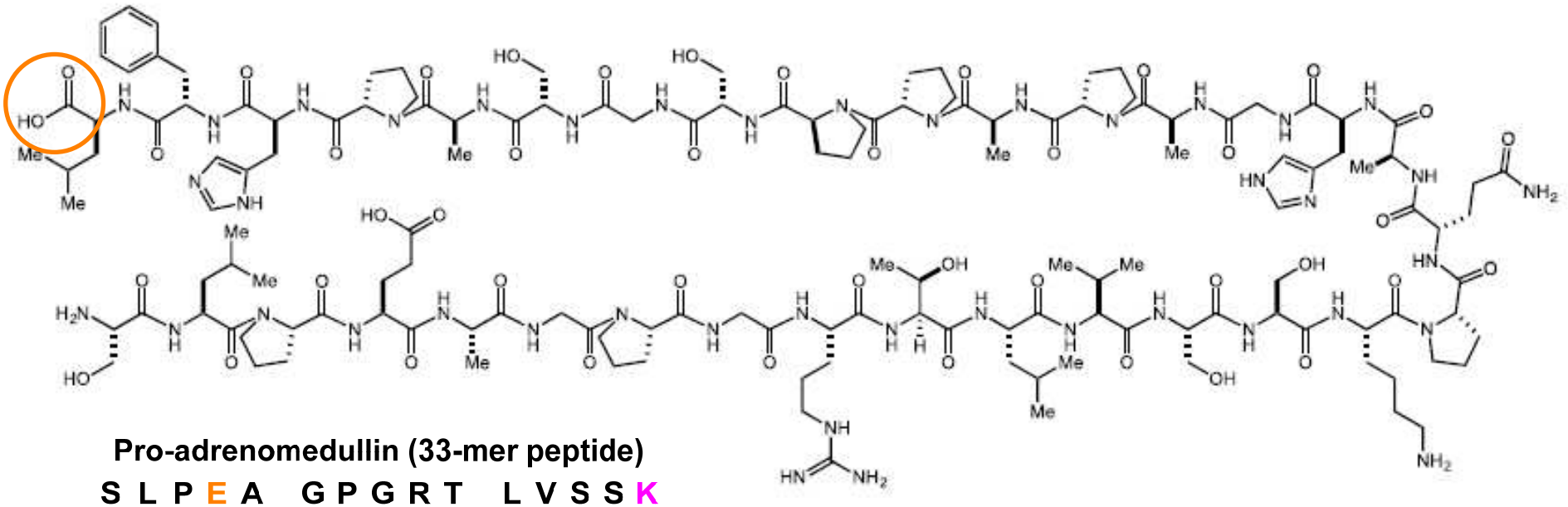
Type II collagen fragment: 15-mer peptide, 41%, (cat A)



Bivalirudin: 20-mer peptide, 33%, (cat B)

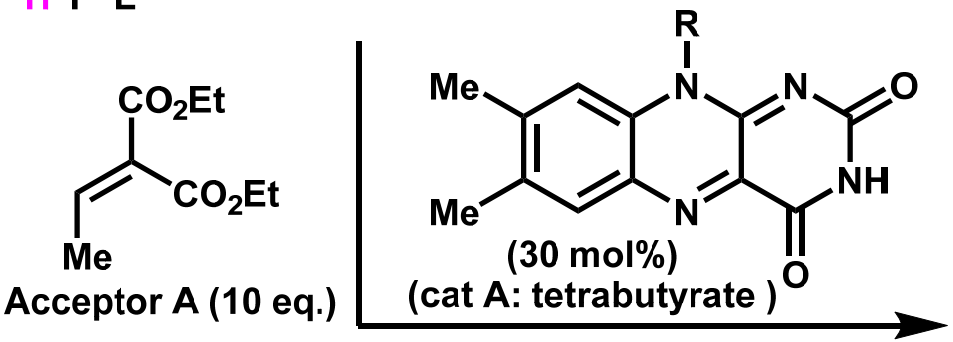
MacMillan, D. W. C. *et al. Nat. Chem.* **2017**, *10*, 205.

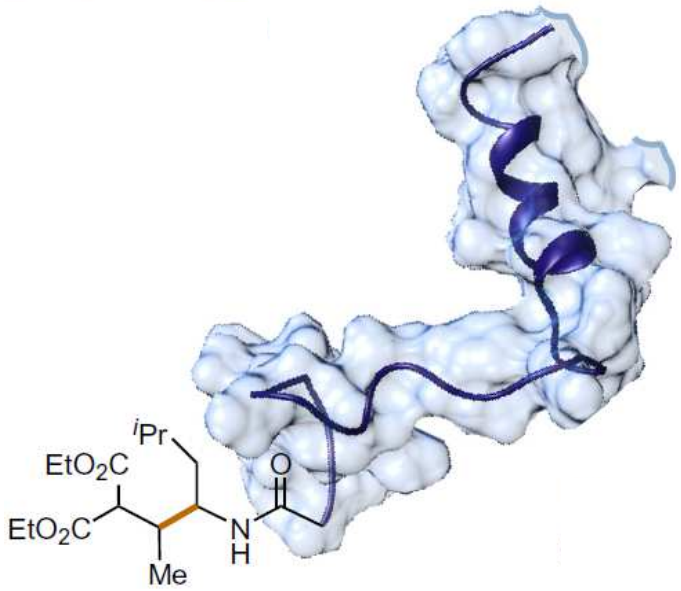
Application to seconday structure peptide 26



Pro-adrenomedullin (33-mer peptide)

S L P E A G P G R T L V S S K
P Q A H G A P A P P S G S A P
H F L



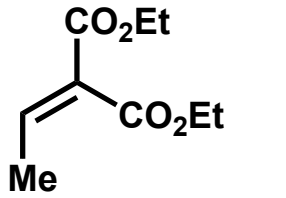


MacMillan, D. W. C. *et al. Nat. Chem.* **2017**, *10*, 205.

Application to tertiary structure peptide

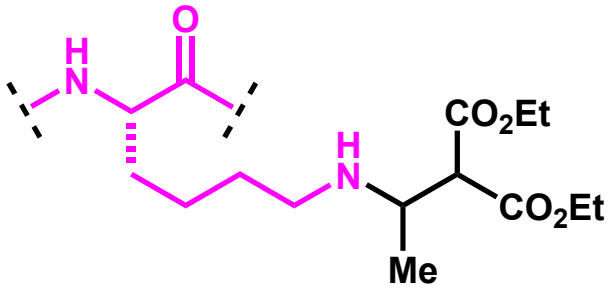
ZHER2 Affibody (58-mer peptide)

V D N K F N K E Q Q N A F Y E I L H L P
N L N E E Q R N A F I Q S L K D D P S Q
S A N L L A E A K K L N D A Q A P K

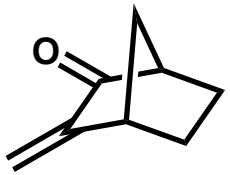


Acceptor A (10 eq.)

cat B (300 mol%)
34 W blue LED
ph 3.5 buffer/glycerol
(95/5, 1 mM), 8 h

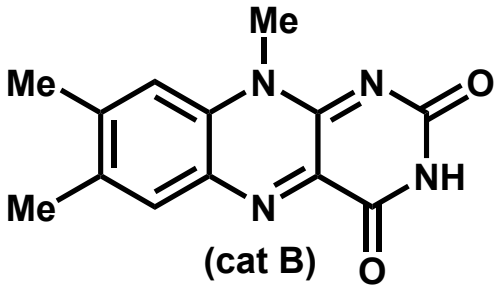


Lys residue was reacted with Acceptor A

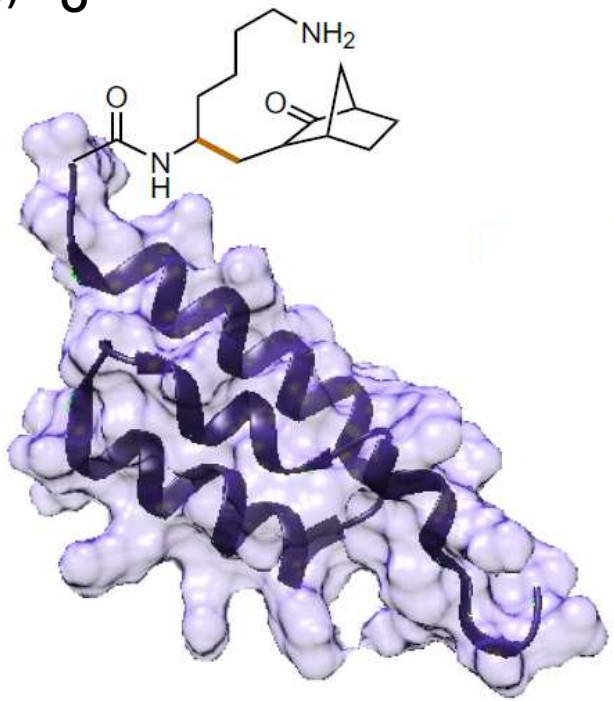


Acceptor B (10 eq.)

31%



(cat B)



MacMillan, D. W. C. *et al. Nat. Chem.* **2017**, *10*, 205.

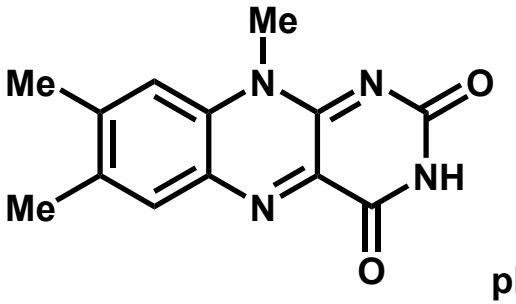
Selective functionalization of human insulin 28

Insulin: 2 parent chains, 3 disulfide bonds, 4 Glu residues, 4 Tyr residues, 2 C-terminal carboxylic acids

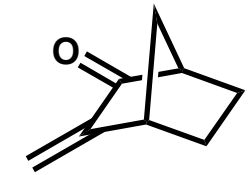
A chain G I V E Q C C T S I C S L Y Q L E N Y C N

B chain F V N Q H L C G S H L V E A L Y L V C G E R G F F Y T P K T

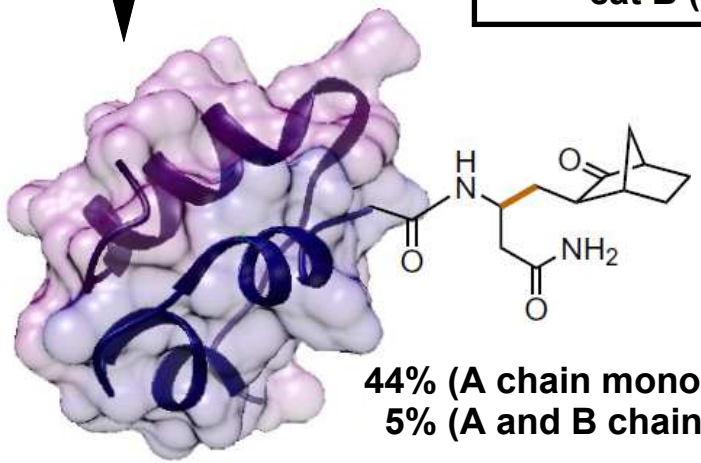
conditions:



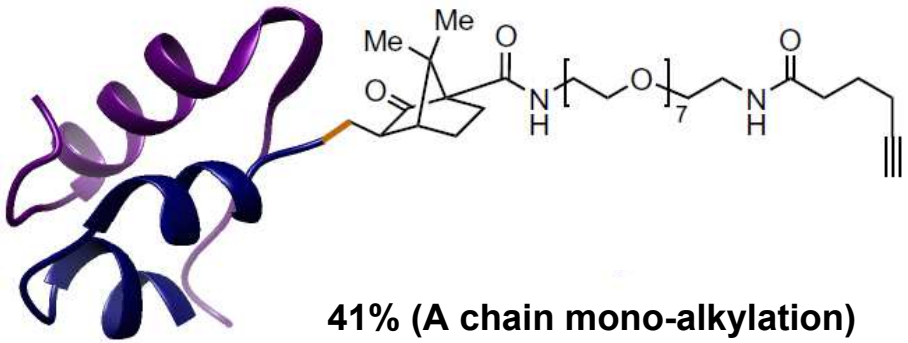
cat B (300 mol%)



Acceptor (10 eq.)
34 W blue LED
ph 3.5 buffer/glycerol
(95/5, 1 mM), 8 h



44% (A chain mono-alkylation)
5% (A and B chain alkylation)



41% (A chain mono-alkylation)

- ✓ No reduction of disulfide bonds
- ✓ No functionalization of Glu residues
- ✓ No heteroatom conjugate addition
- ✓ Selective A chain functionalization

MacMillan, D. W. C. *et al. Nat. Chem.* **2017**, *10*, 205.

conclusion

·the methods of Bioconjugation

·Traditional methods via tow-electron pathway were difficult to proceed site-selective modification

·Recently, radical methods were reported and some of the problems were solved.

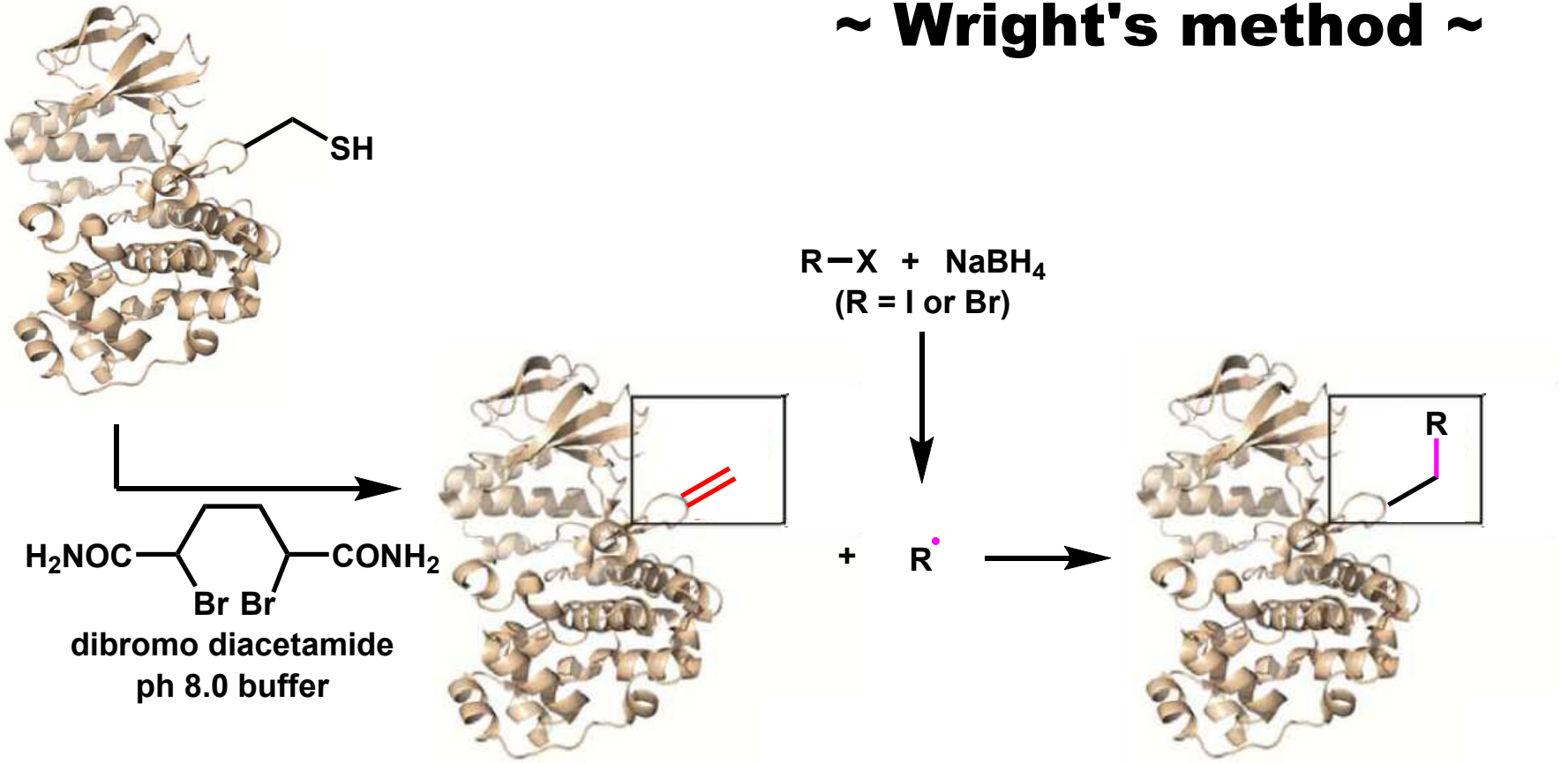
·Decarboxylative alkylation for site-selective bioconjugation of native proteins via oxidation potentials

·This strategy shows the possibility of radical reaction and/or photoredox catalyst for site-selective bioconjugation.

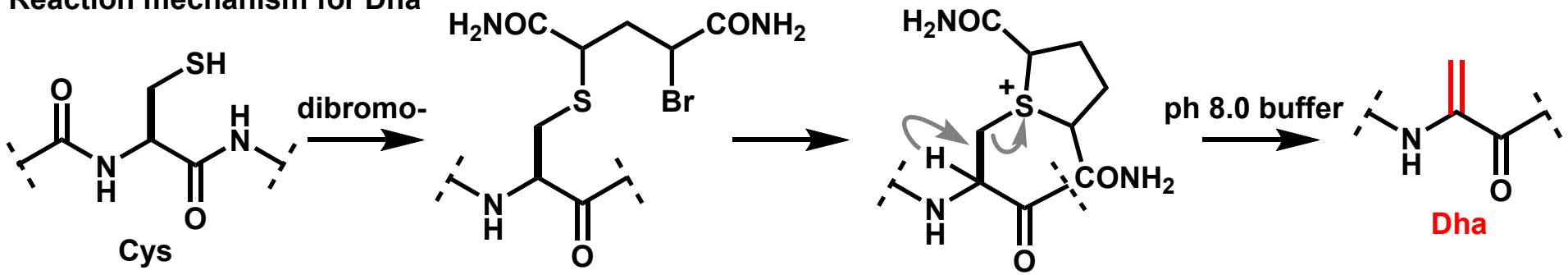
·However, the reaction condition, ph 3.5 buffer, is the problem for biological reaction.

Appendix

Radical approach for Dehydroalanine residue
~ Wright's method ~

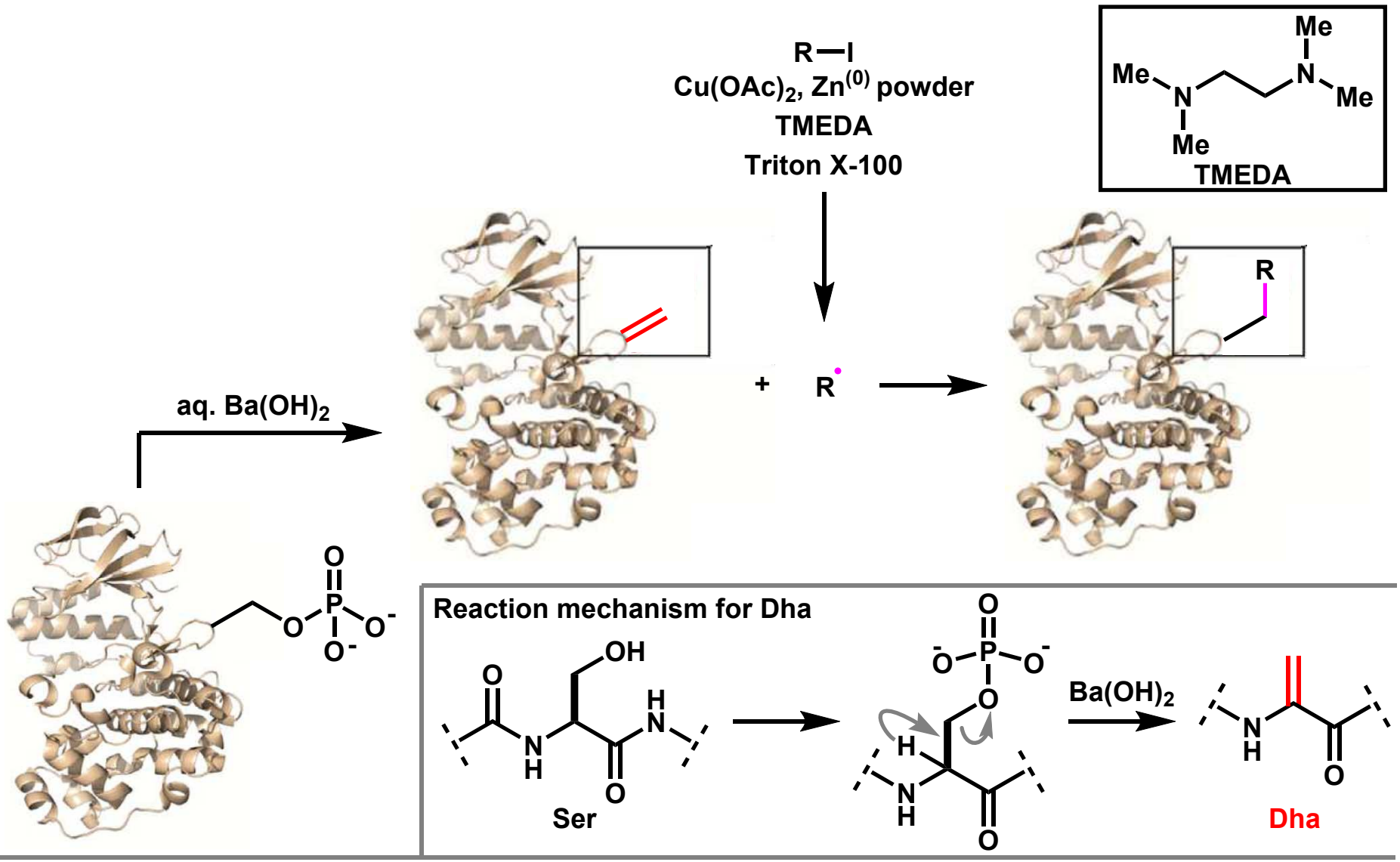


Reaction mechanism for Dha



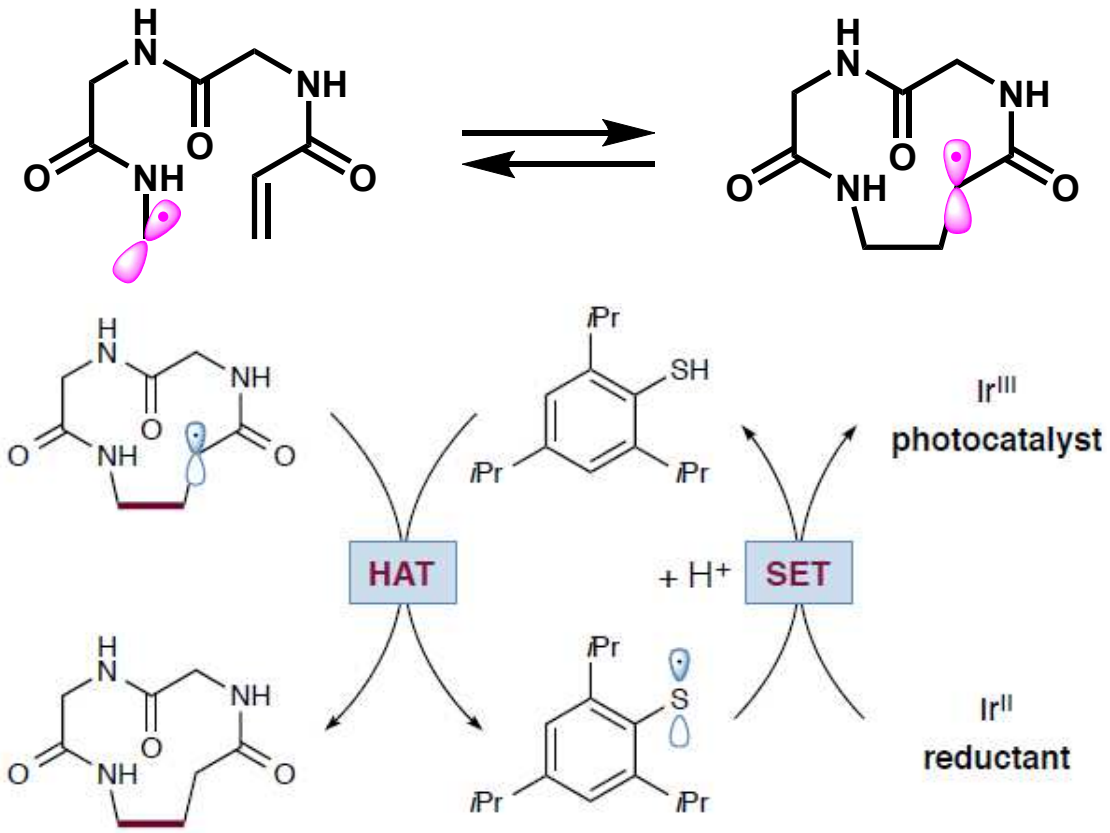
Wright, T. H. *et al. Science*, **2016**, *354*, 597.

Radical approach for Dehydroalanine residue ~ Yang's method ~



Yang, A. *et al*. *Science,* **2016**, *354*, 623.

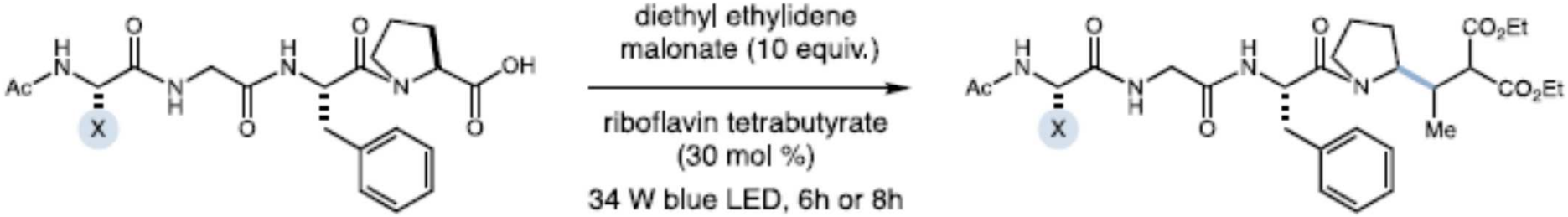
Effect of 2,4,6-triisopropylthiophenol



#	Sequence	% Yield (no thiol)	% Yield (10% thiol)
8	acryloyl-Phe-Met-Leu-Glu(O*t*Bu)-Gly	64%	73%
10	acryloyl-Phe-Arg(Boc)$_2$-Ser(*t*Bu)-Ala-Gly	32%	45%
21	acryloyl-Phe-Ala-Pro-Glu(O*t*Bu)-Leu-Phe-Ala-Gly	26%	52%

MacMillan, D. W. C. *et. al. Angew. Chem. Int. Ed.* **2017**, *56*, 728.

Supplementary Table 2 – Tetramer functional group tolerance and solvent evaluation.

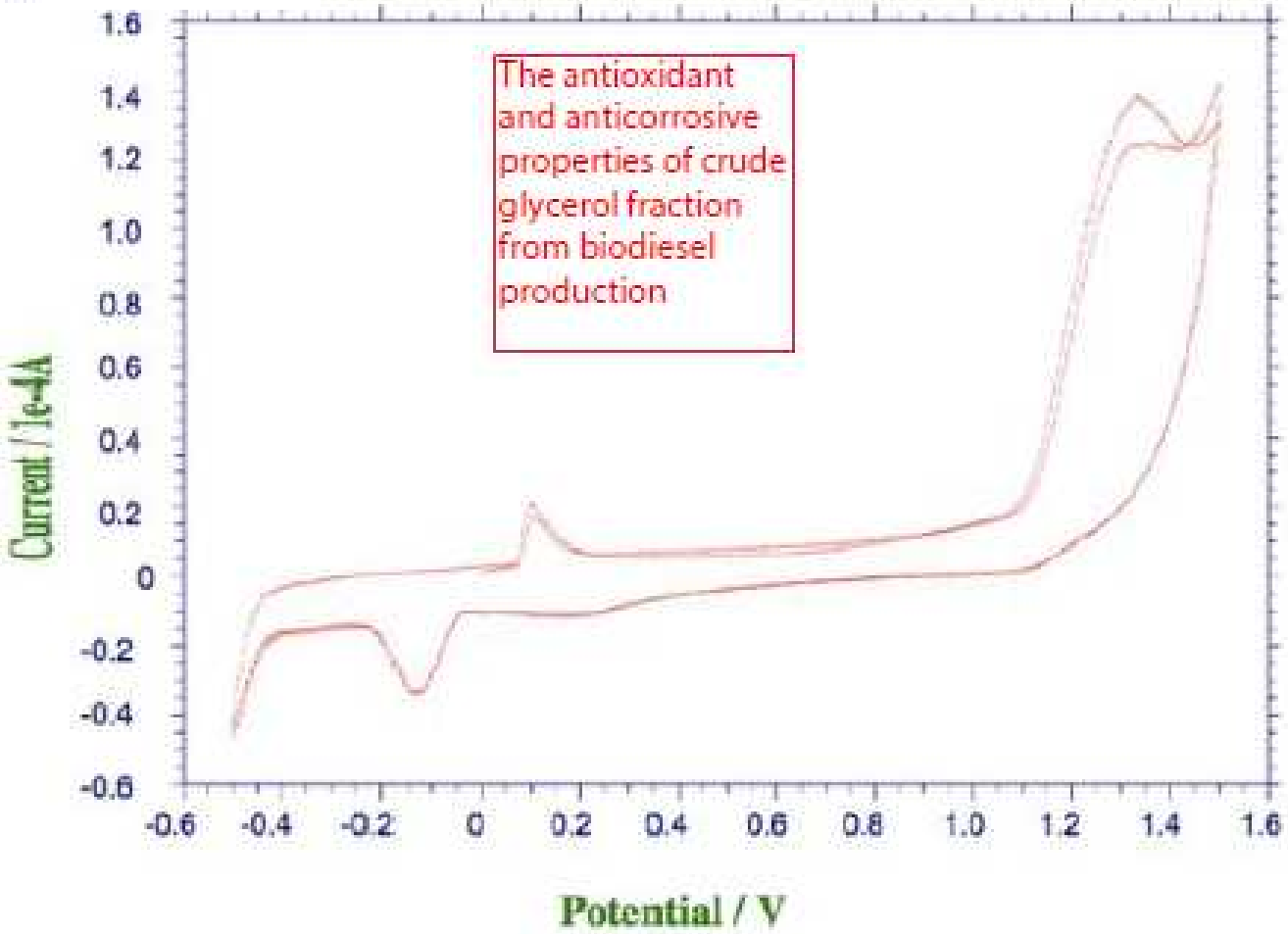


entry	tetramer	time	solvent (1 mM)	yield
1	Ac-AGFP-OH	8h	10 mM pH 7.0 potassium phosphate buffer	75%
2	Ac-AGFP-OH	8h	95:5 10 mM pH 7.0 potassium phosphate buffer: glycerol	60%
3	Ac-AGFP-OH	6h	10 mM pH 3.5 cesium formate buffer	84%
4	Ac-AGFP-OH	6h	95:5 10 mM pH 3.5 cesium formate buffer: glycerol	72%
5	Ac-NGFP-OH	8h	10 mM pH 7.0 potassium phosphate buffer	30%
6	Ac-NGFP-OH	8h	95:5 10 mM pH 7.0 potassium phosphate buffer: glycerol	51%
7	Ac-NGFP-OH	6h	10 mM pH 3.5 cesium formate buffer	37%
8	Ac-NGFP-OH	6h	95:5 10 mM pH 3.5 cesium formate buffer: glycerol	66%
9	Ac-KGFP-OH	8h	10 mM pH 7.0 potassium phosphate buffer	11%
10	Ac-KGFP-OH	8h	95:5 10 mM pH 7.0 potassium phosphate buffer: glycerol	25%
11	Ac-KGFP-OH	6h	10 mM pH 3.5 cesium formate buffer	13%
12	Ac-KGFP-OH	6h	95:5 10 mM pH 3.5 cesium formate buffer: glycerol	52%

D. Cyclic Voltammogram of Ac-AGFP-OH in water

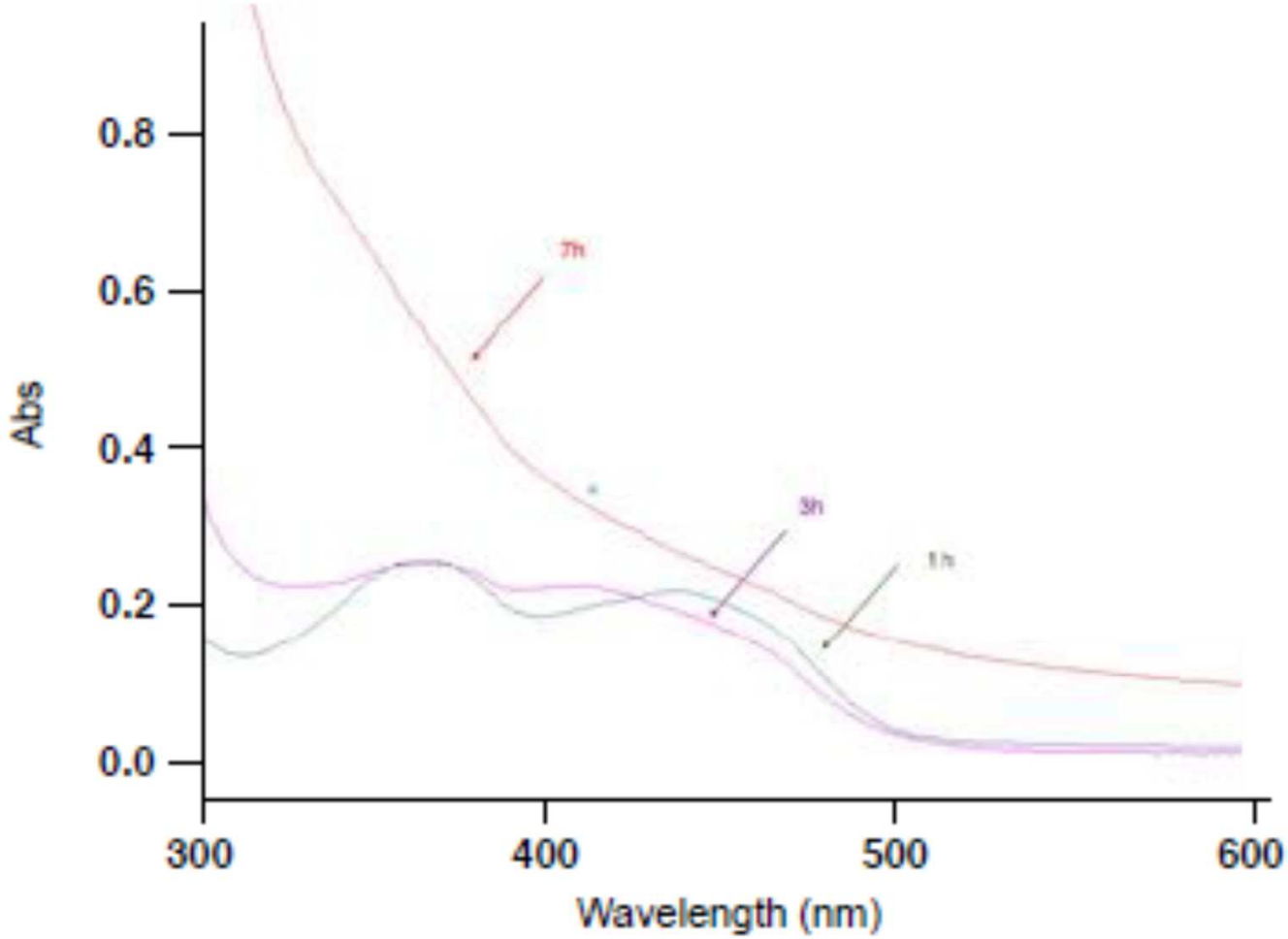
0.001 M Ac-AGFP-OH
0.1 M KCl supporting electrolyte

working electrode: platinum disk
counter electrode: platinum
reference electrode: saturated calomel
initial E (V) = 0
high E (V) = 1.5
low E (V) = -0.5
initial P/N = positive (P)
scan rate (V/s) = 0.1
segment = 4
smpl interval (V) = 0.001
quiet time (s) = 2
sensitivity (A/V) = $1e^{-4}$



E. UV-Vis Studies of Lumiflavin

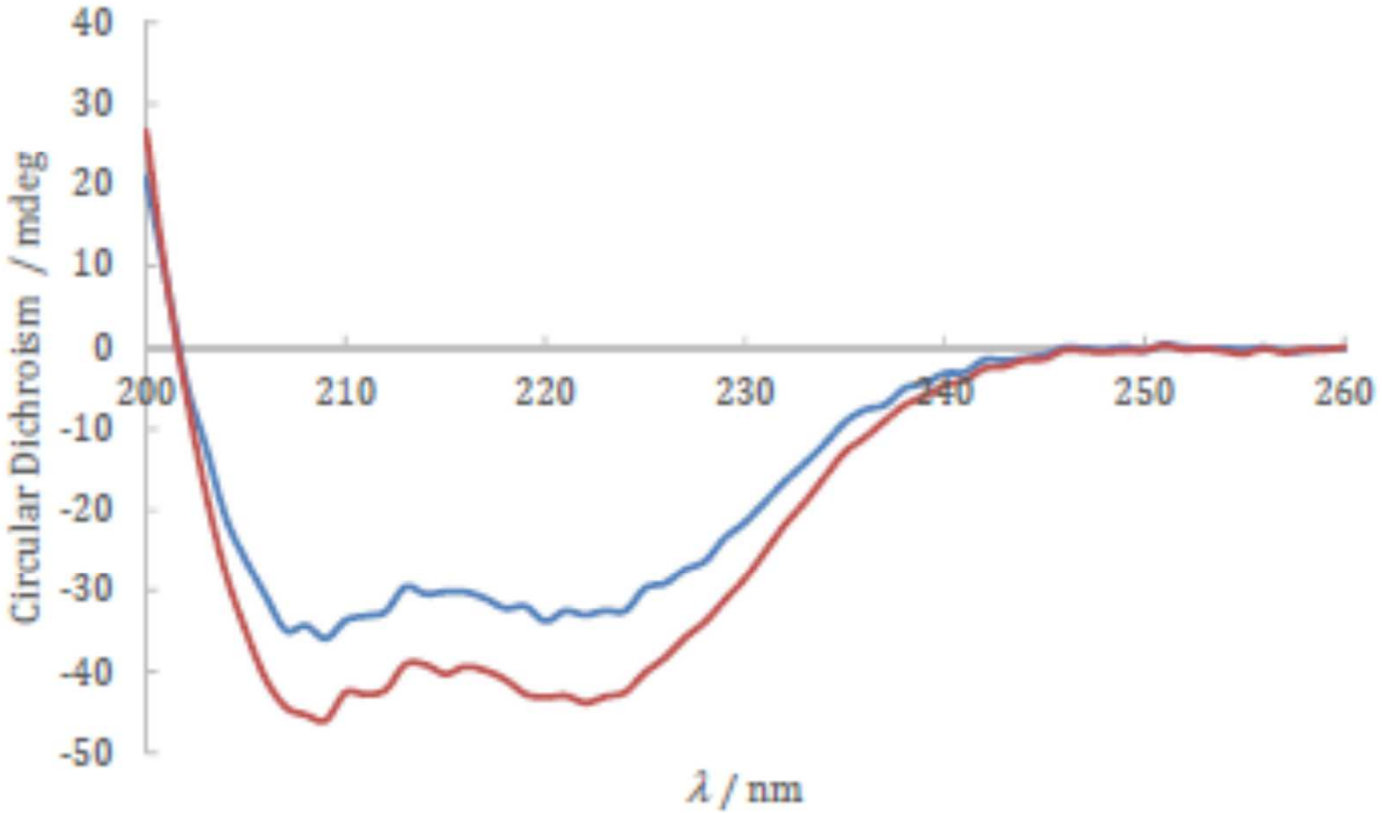
Lumiflavin spectra at 1 h, 3 h, and 7 h for standard reaction with Bivalirudin:



Analysis of lumiflavin by UV-Vis spectroscopy at various time points during the reaction reveals a considerable loss of the characteristic absorption bands of the photocatalyst.

F. Circular Dichroism Spectra of ZHER2 Affibody

CD spectra of unmodified ZHER2 Affibody (blue) and modified protein product (obtained after HPLC purification, red):



The spectra were recorded at a concentration of 20 μM in PBS and were obtained by averaging 20 individual spectra of the respective compounds.