XU LAB
Innovation through Catalysis

北京大学
PEKING UNIVERSITY

XU GROUP
Department of Chemistry, Peking University

Selected Weekly Literature Presentations

Disclaimer:
These slides contain personal interpretations and may include errors or subjective views.

Applications of Carbon-Centered Radicals in Protein Manipulation

Reporter: Tianxing Jiang

Supervisor: Yan Xu

2024.12.28

Outline

- *Introduction*
- *“Off-protein” strategy*
- *“On-protein” strategy*
- *Summary*

Outline

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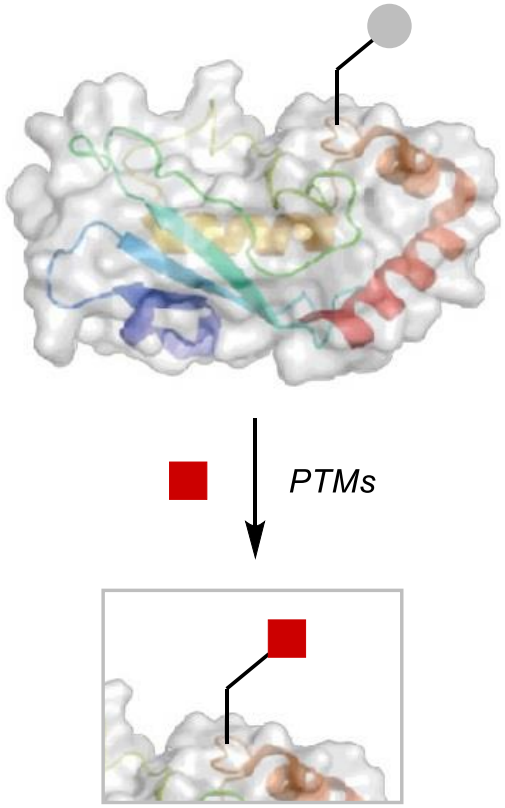
- *“On-protein” strategy*
- *Summary*

Introduction

Post-translational modifications (PTMs)



1) Chemo/regio-selectivity on AAs?

…IDSSYYTGSLNWVPVSVEGYWQI…

2) Benign reaction environment

temp: *0 ~ 37 °C*

solvent: *water*

conc.: *≤ x mM*

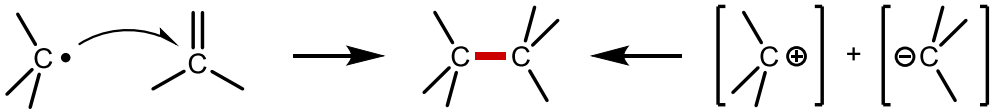
time: *≤ x h*

Davis, B. G. *et al. ACS Cent. Sci.* **2023**, *9*, 614–638.

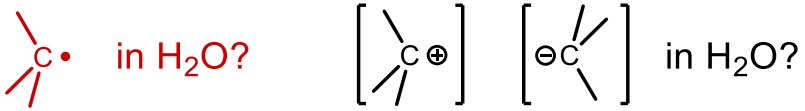
Introduction

Radical methods VS traditional two-electron chemistry

- *construction of C-C bond*



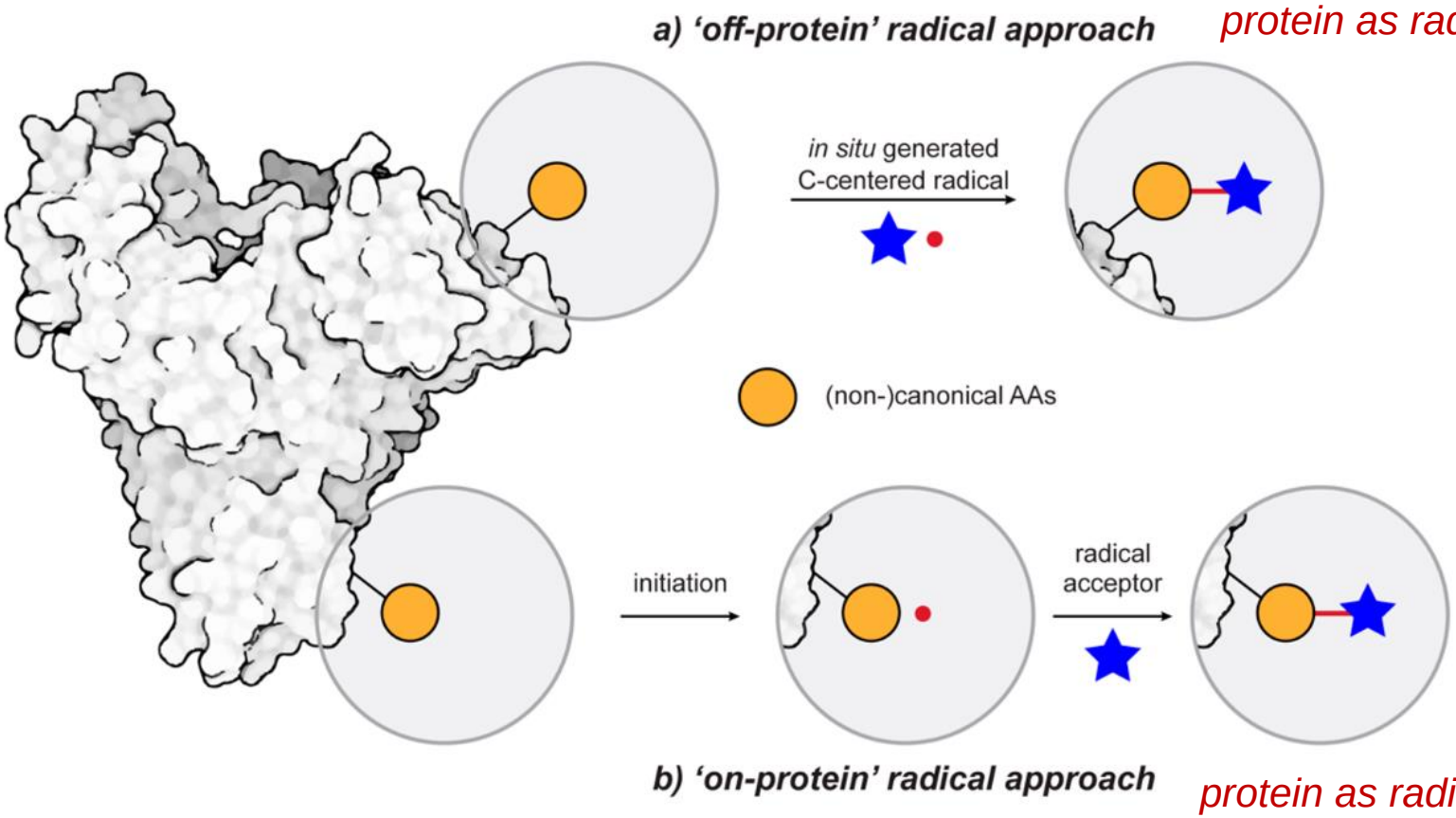
- *reliability in aqueous media*



- *chemical selectivity*

Introduction

"off-protein" & "on-protein"



Introduction

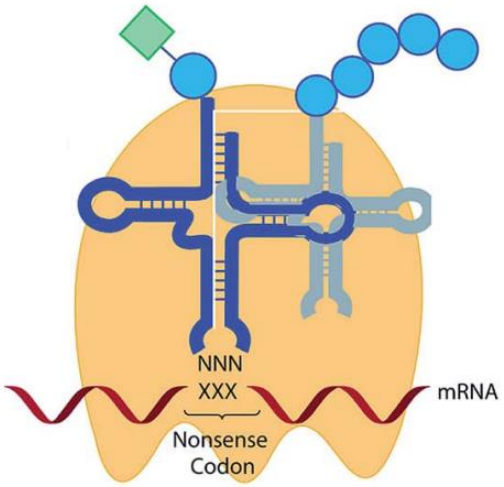
*Canonical amino acids **or** noncanonical amino acids?*

canonical AAs	***noncanonical AAs***
Advantages:	*Advantages :*
➢ *naturally abundant*	➢ *better reactivity*
➢ *simple procedure*	➢ *site-specific*
Disadvantages:	*Disadvantages :*
➢ *excess reactive sites*	➢ *difficult preparation*
➢ *limited reactivity*	➢ *difference from natural protein*

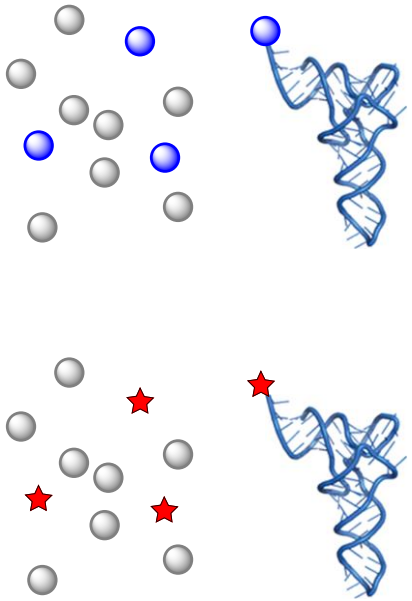
Introduction

Introducing of noncanonical amino acids (ncAAs):

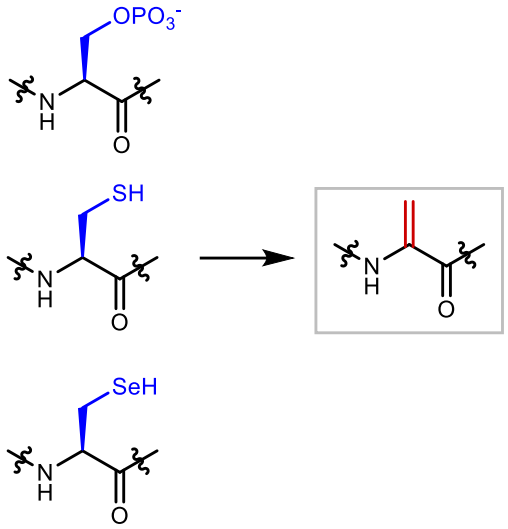
1) alternative codons



2) auxotrophic replacement



3) post-modifications



Chin, J. W. *Nature* **2017**, *550*, 53-60.
Chin, J. W. *et al. Chem. Rev.* **2014**, *114*, 4764–4806.
Davis, B. G. *et al. Curr. Opin. Chem. Biol.* **2018**, *46*, 71–81.

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- "On-protein" strategy
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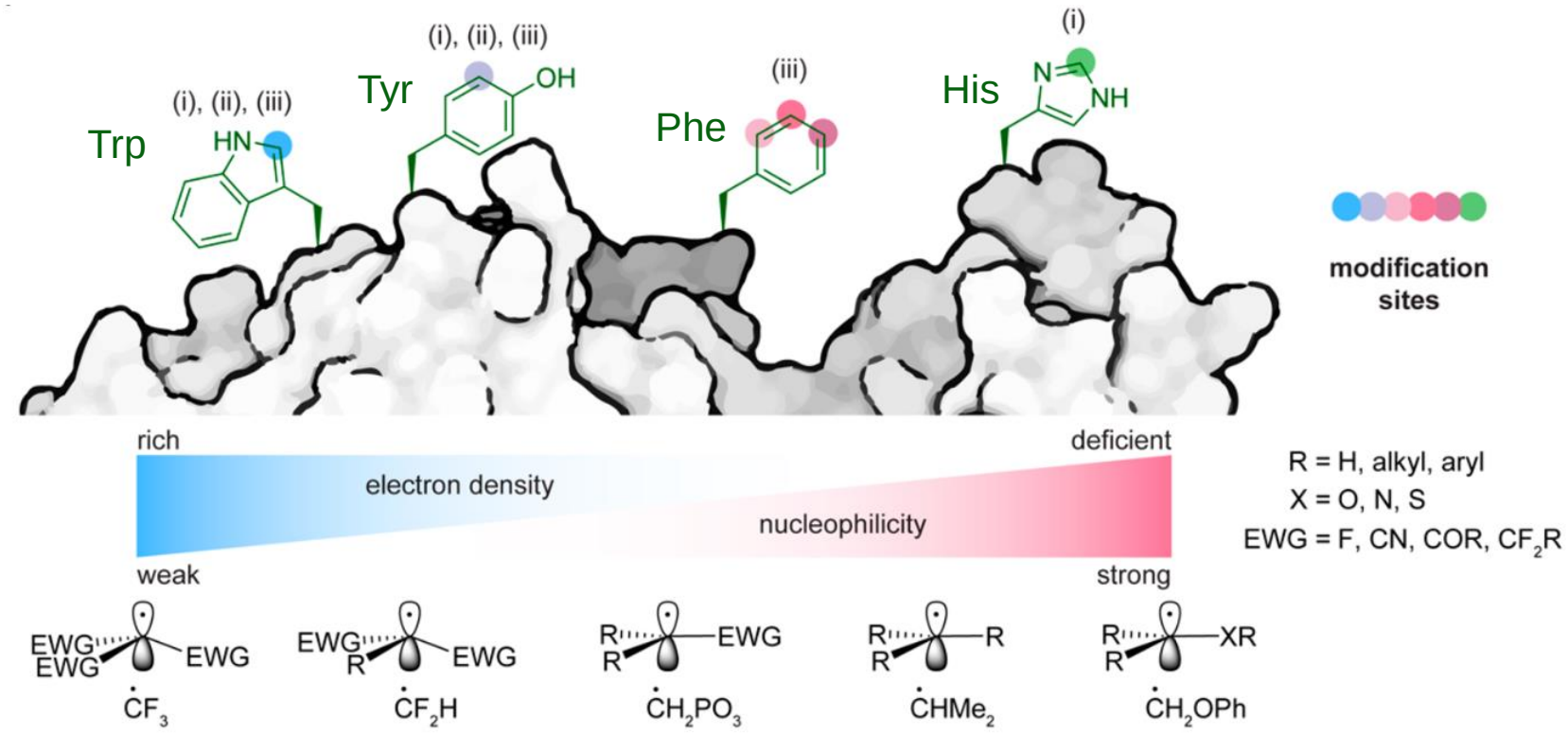
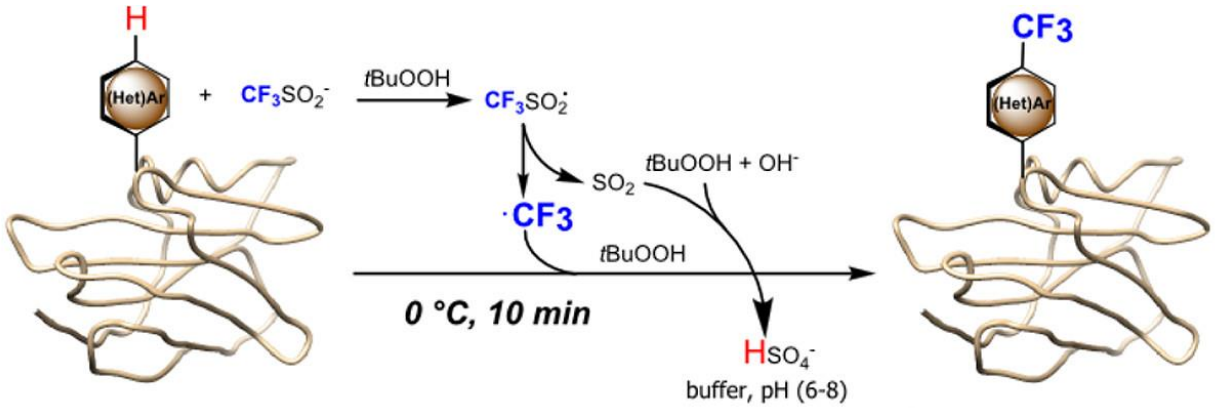
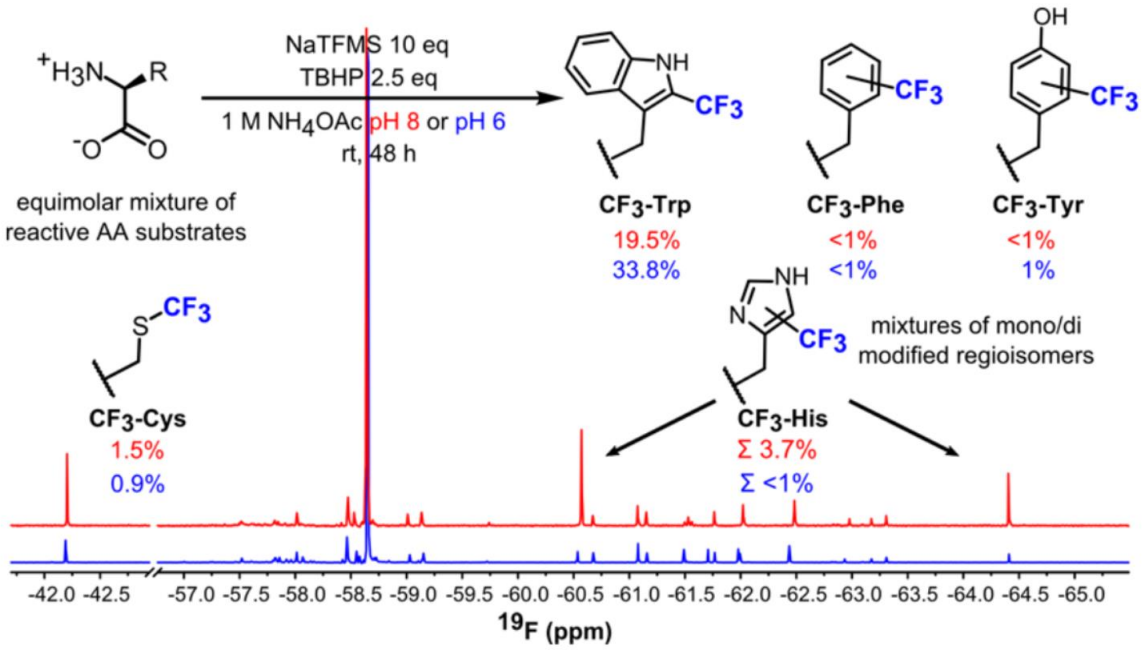
"Off-protein" Strategy

Canonical AAs: radical addition to (hetero)aromatic rings



“Off-protein” Strategy

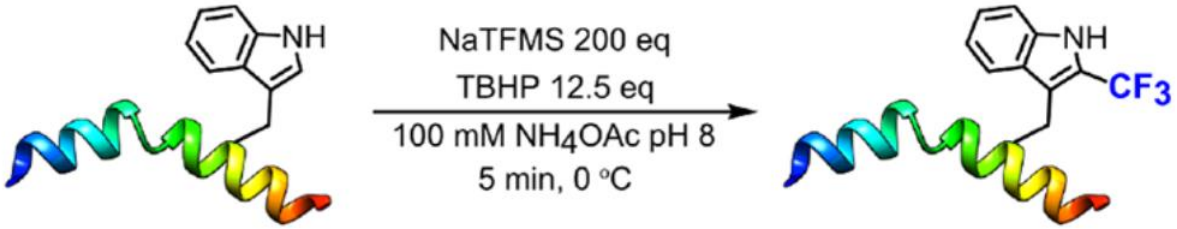


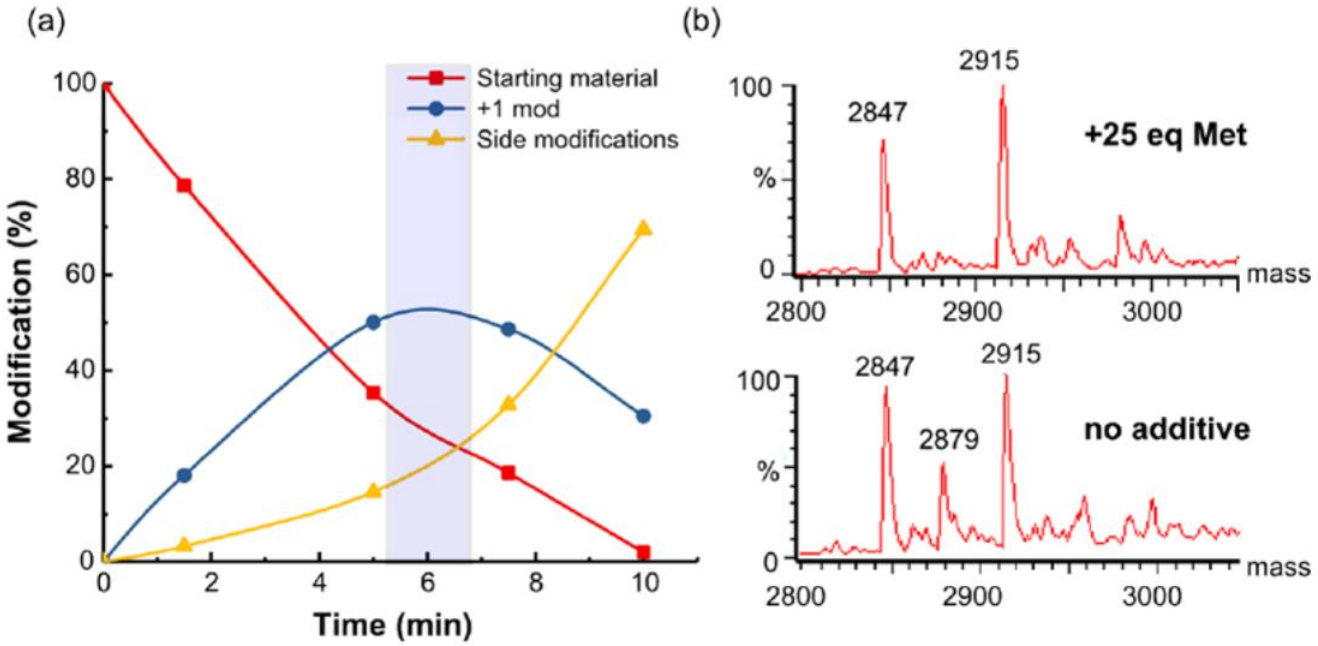


Davis, B. G. *et al. J. Am. Chem. Soc.* **2018**, *140*, 1568–1571.

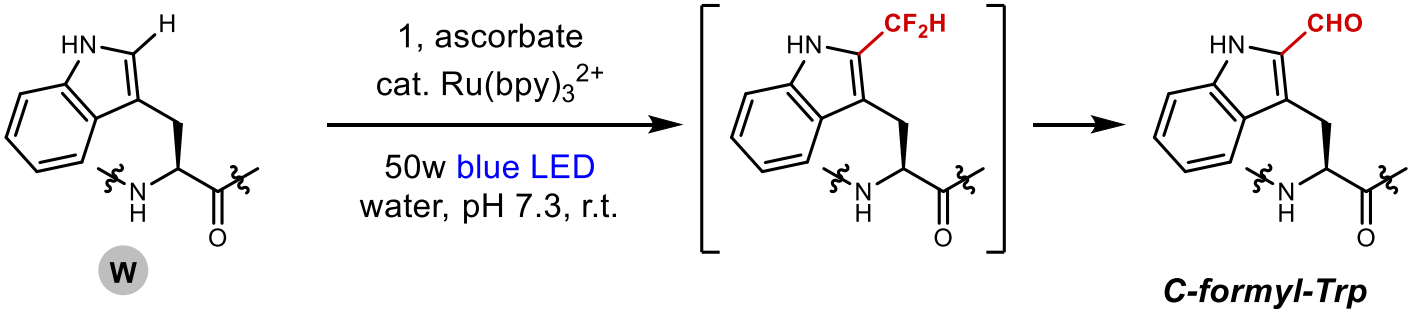
"Off-protein" Strategy

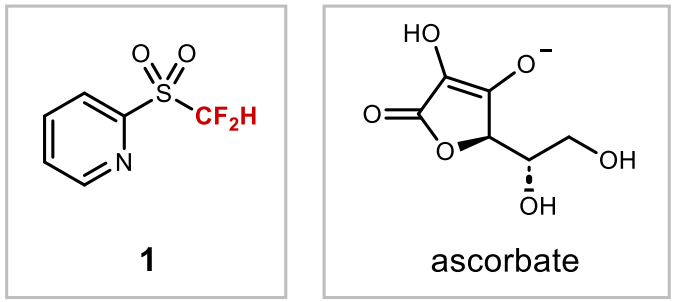
Over-oxidation caused by TBHP:

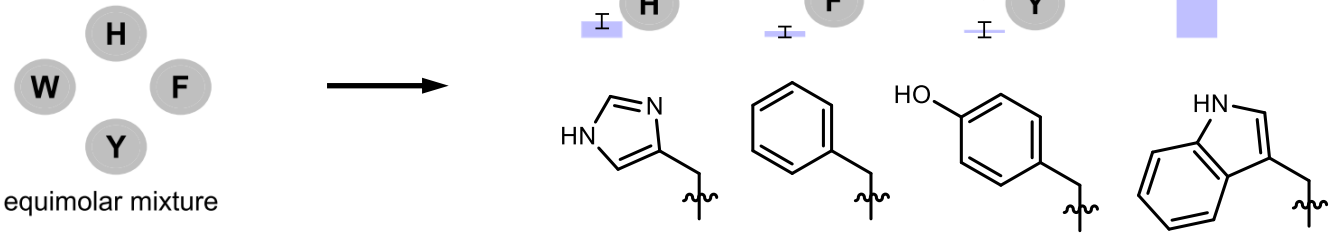




“Off-protein” Strategy

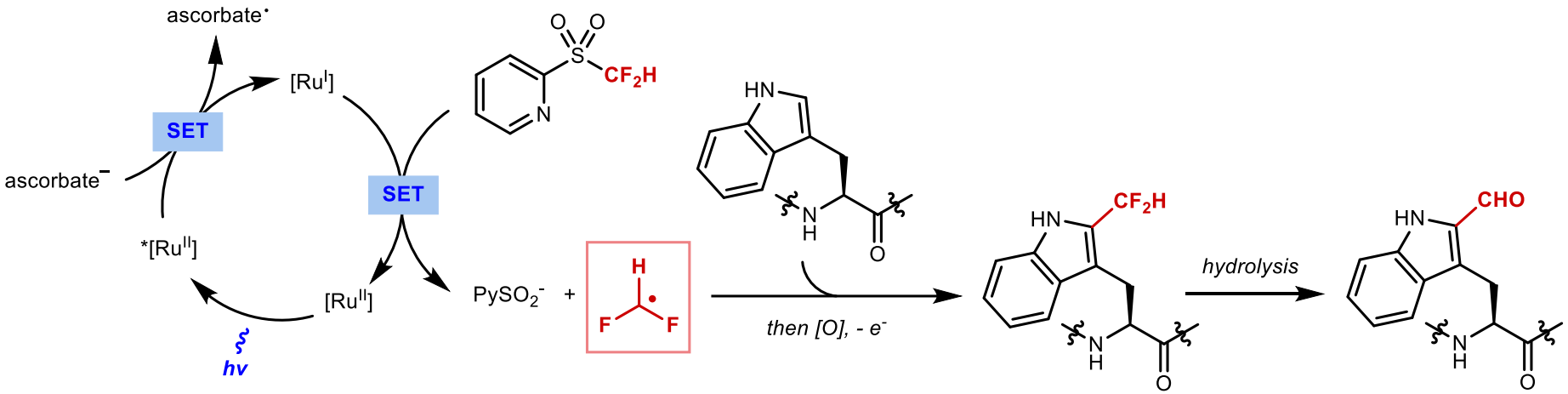


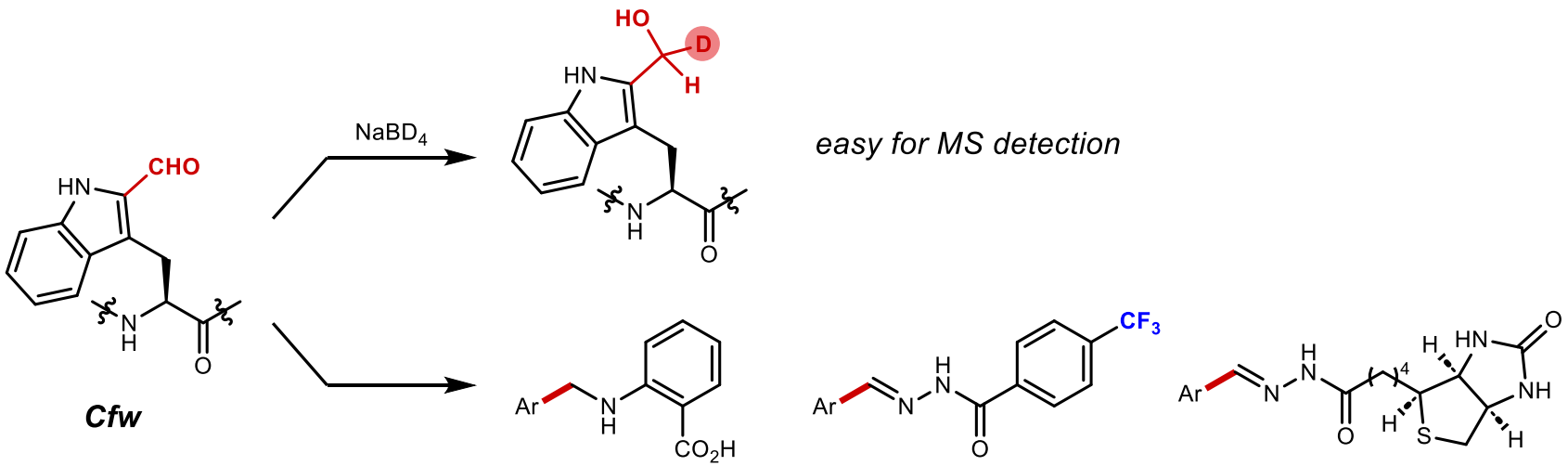




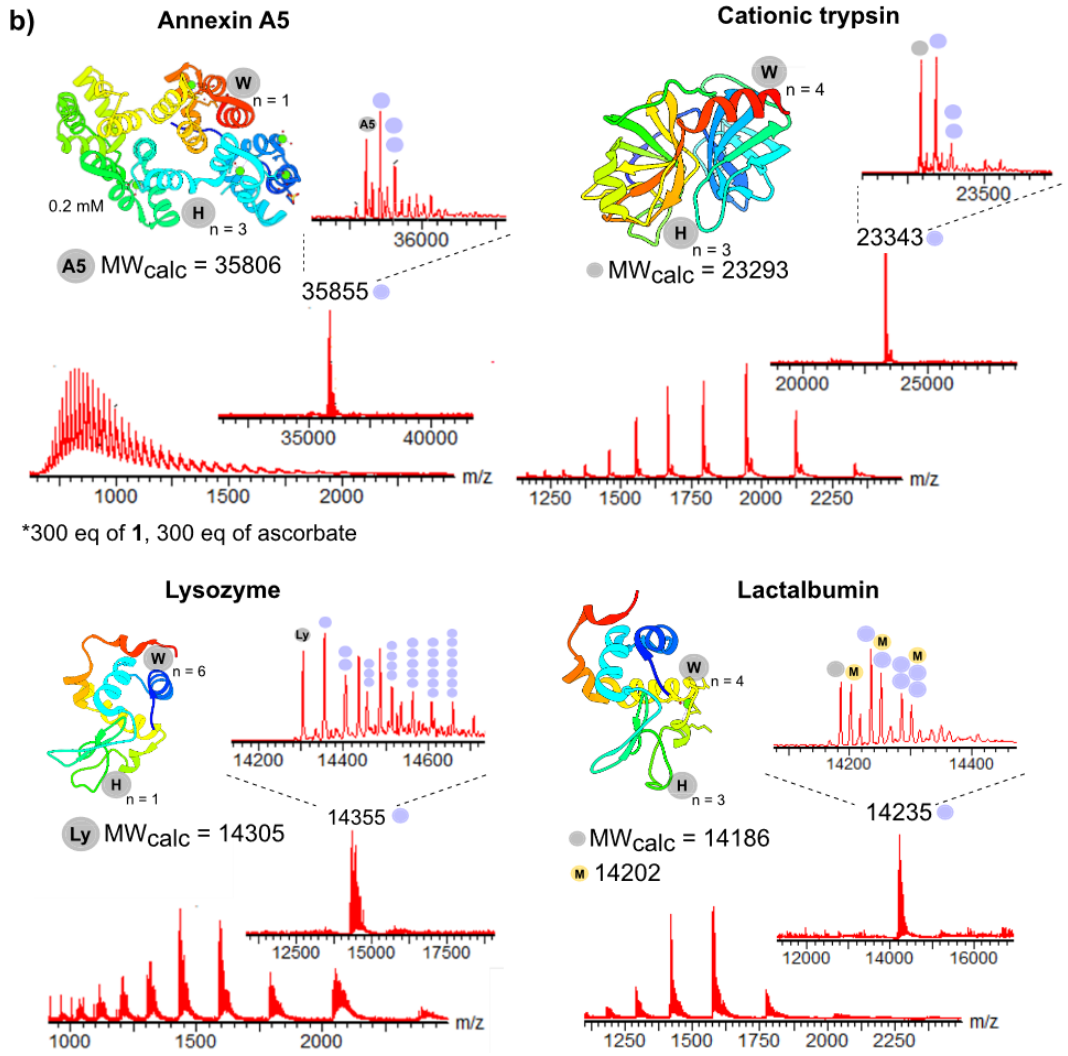
Davis, B. G. *et al. ACS Cent. Sci.* **2021**, *7*, 145–155.

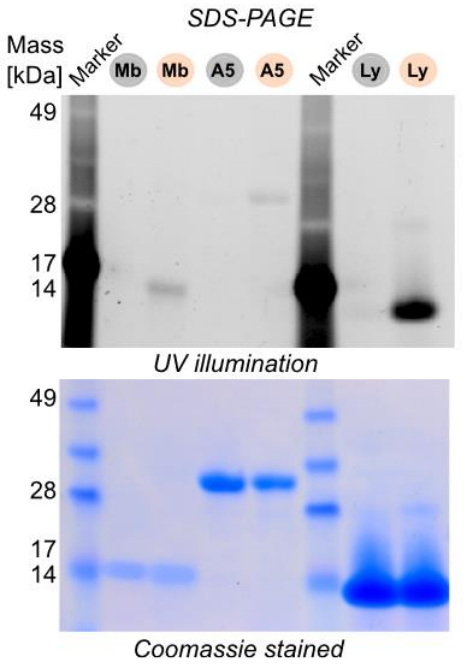
“Off-protein” Strategy

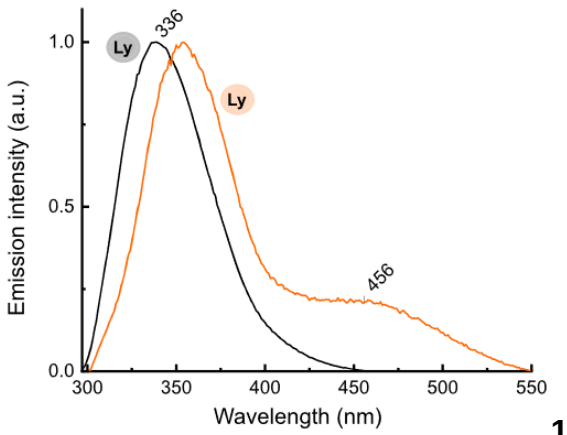




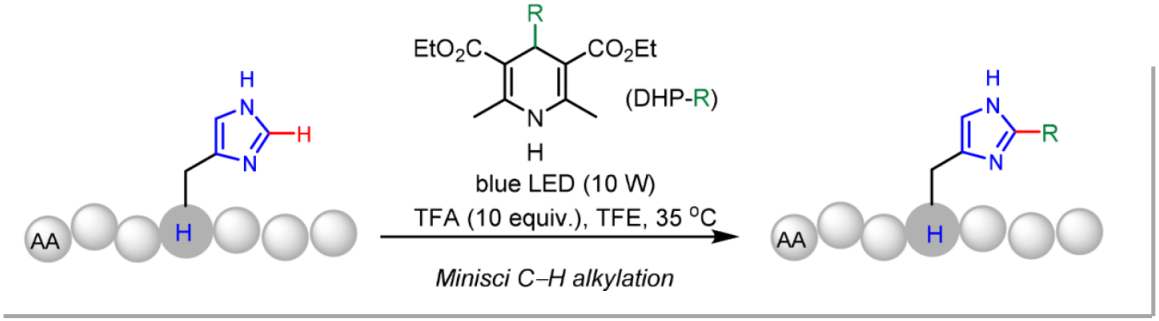
“Off-protein” Strategy







“Off-protein” Strategy



a

R = **a** = cyclohexyl

H_2N—M D W R F S Y K L—CO_2H **7-a**, 0%

H_2N—M H D W R F S Y K L—CO_2H **8-a**, 40% [34%][a]

H_2N—G R L Y H S P K E—CO_2H **9-a**, 52% [42%]

H_2N—G R M Y S P K T H—CO_2H **10-a**, 49% [39%]

H_2N—V H L T P E E K S—CO_2H **11-a**, 59% [53%]

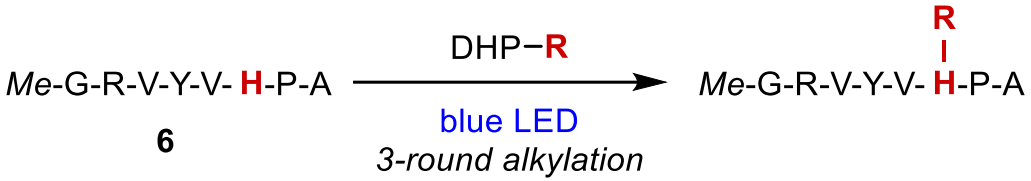
H_2N—C R I Y H P K E S—CO_2H **12-a**, complex mixture

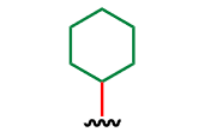
H_2N—C R I Y H P K E S—CO_2H (Acm) **13-a**, 46% [41%]

Ubiquitin
21-g, 95% [69%]

Wang, P. *et al. J. Am. Chem. Soc.* **2019**, *141*, 18230–18237.

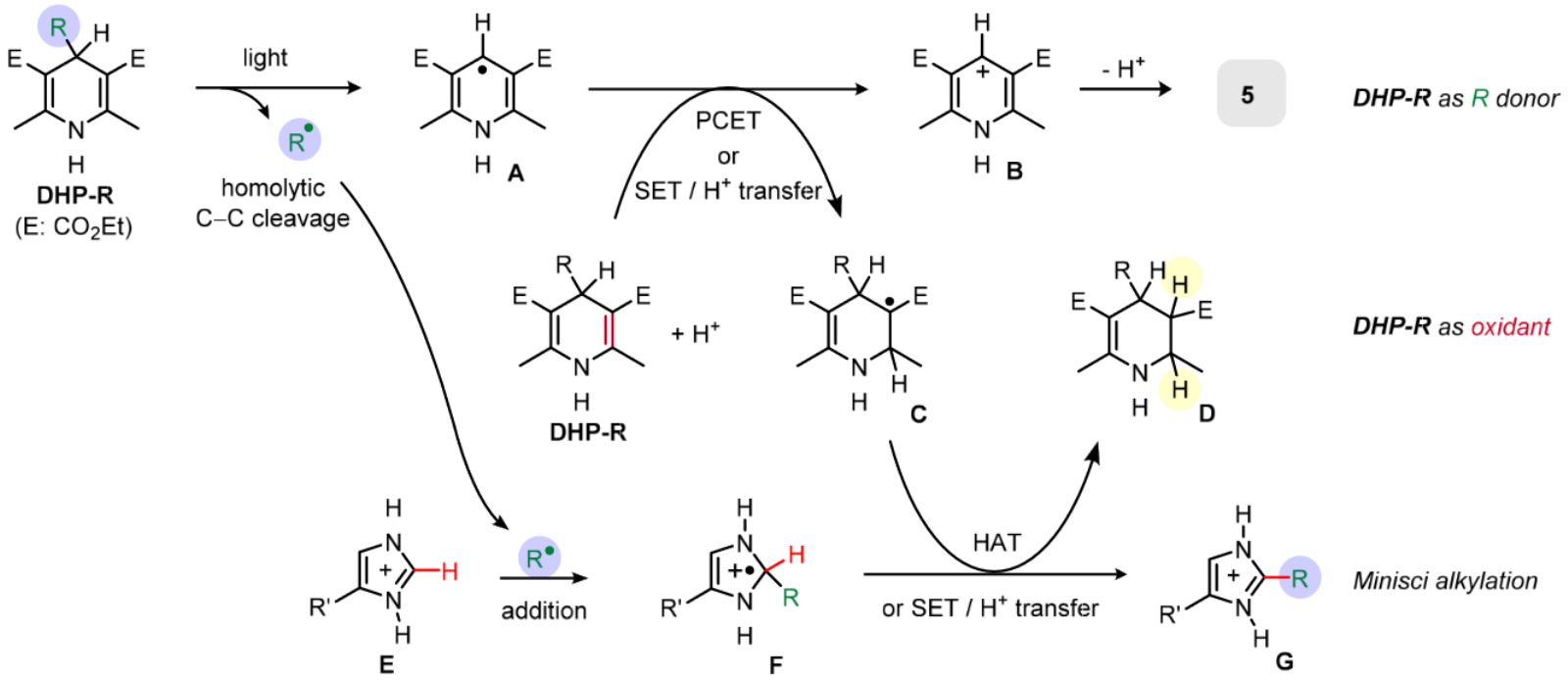
“Off-protein” Strategy





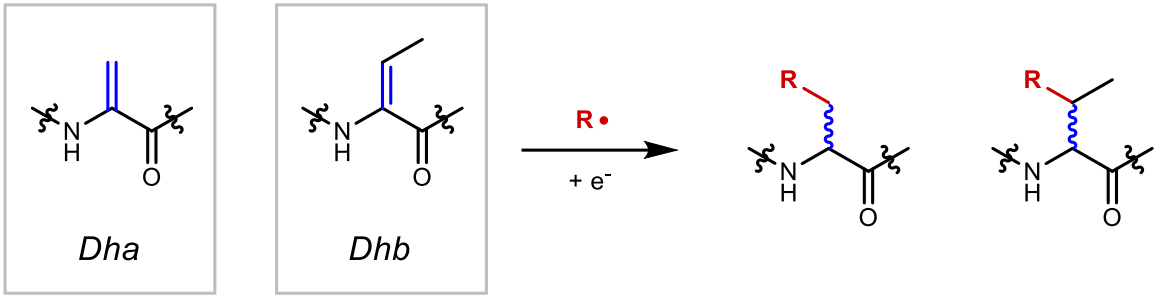
6-a 76% [57%]
6-b 61% [49%]
6-c 41% [32%]
6-d 50% [44%]
6-e 45% [41%]
6-f 0%
6-g 95% [68%]
6-h 45% [31%]
6-i 75% [57%]
6-j 65% [38%]
6-k 59% [48%]
6-l 51% [35%]
6-m 34% [23%]
6-n 60% [46%]
6-o 78% [53%]
6-p 84% [61%]
6-q 85% [60%]
6-r 95% [66%]

“Off-protein” Strategy

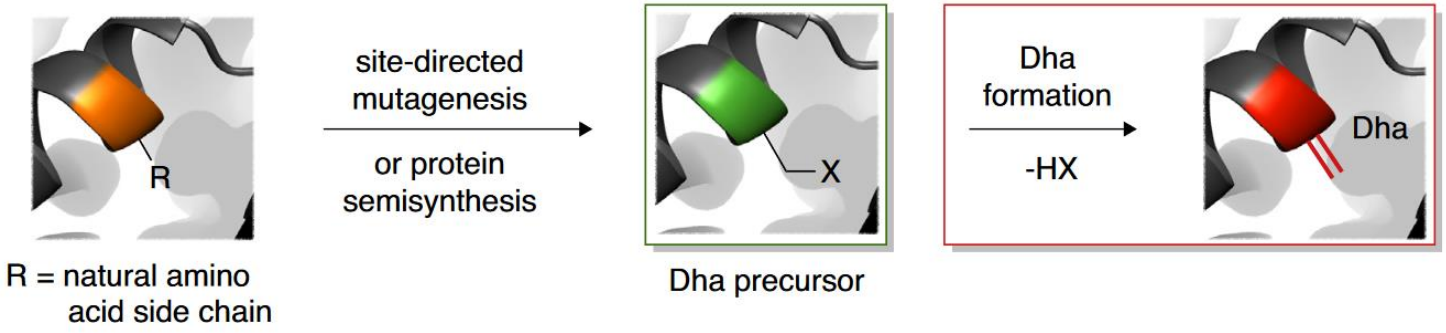


“Off-protein” Strategy

Non-canonical amino acids (ncAAs):

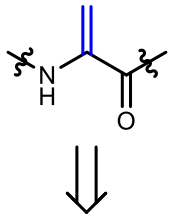


Introduction of Dha:

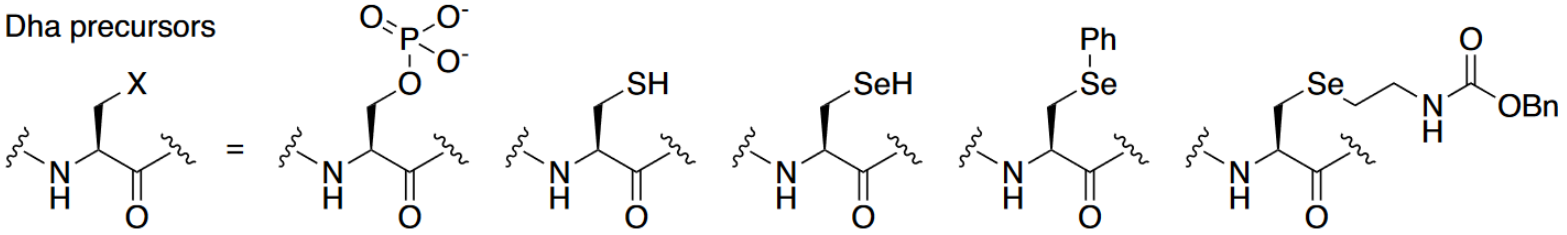


Davis, B. G. *et al. Curr. Opin. Chem. Biol.* **2018**, *46*, 71–81.

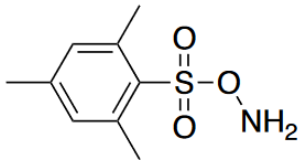
“Off-protein” Strategy



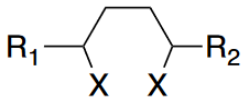
Dha precursors



Dha precursor	Conditions for Dha formation
pSer	lyases (OspF, SpvC, HopAI1), $Ba(OH)_2$
Cys	MSH (**1**), alkylating reagents (**2-4**)
SeCys	DBHDA (**3**), $NaIO_4$
PhSeCys	H_2O_2
Cbz-γSeLys	H_2O_2



MSH (**1**)

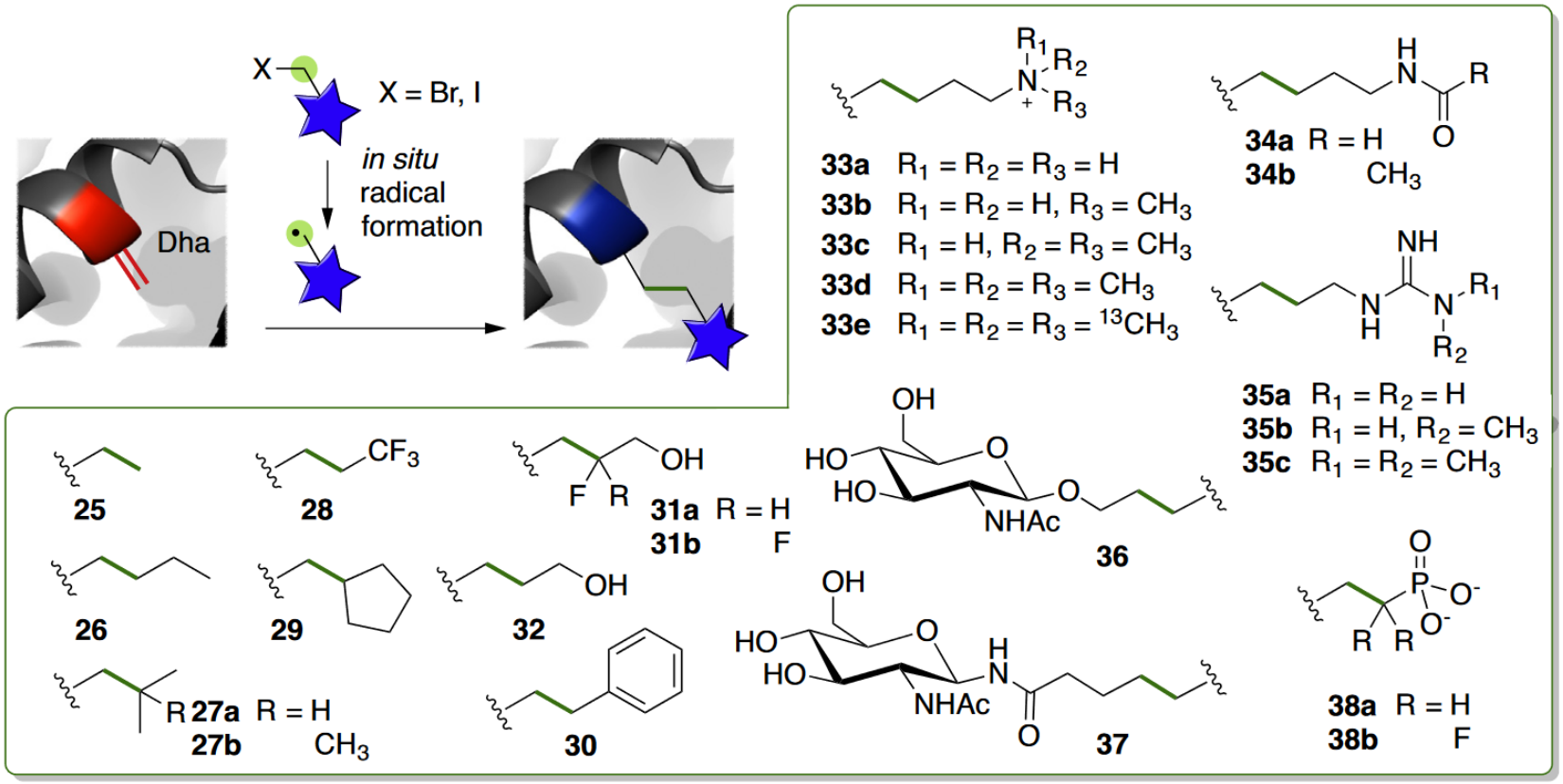


2-4

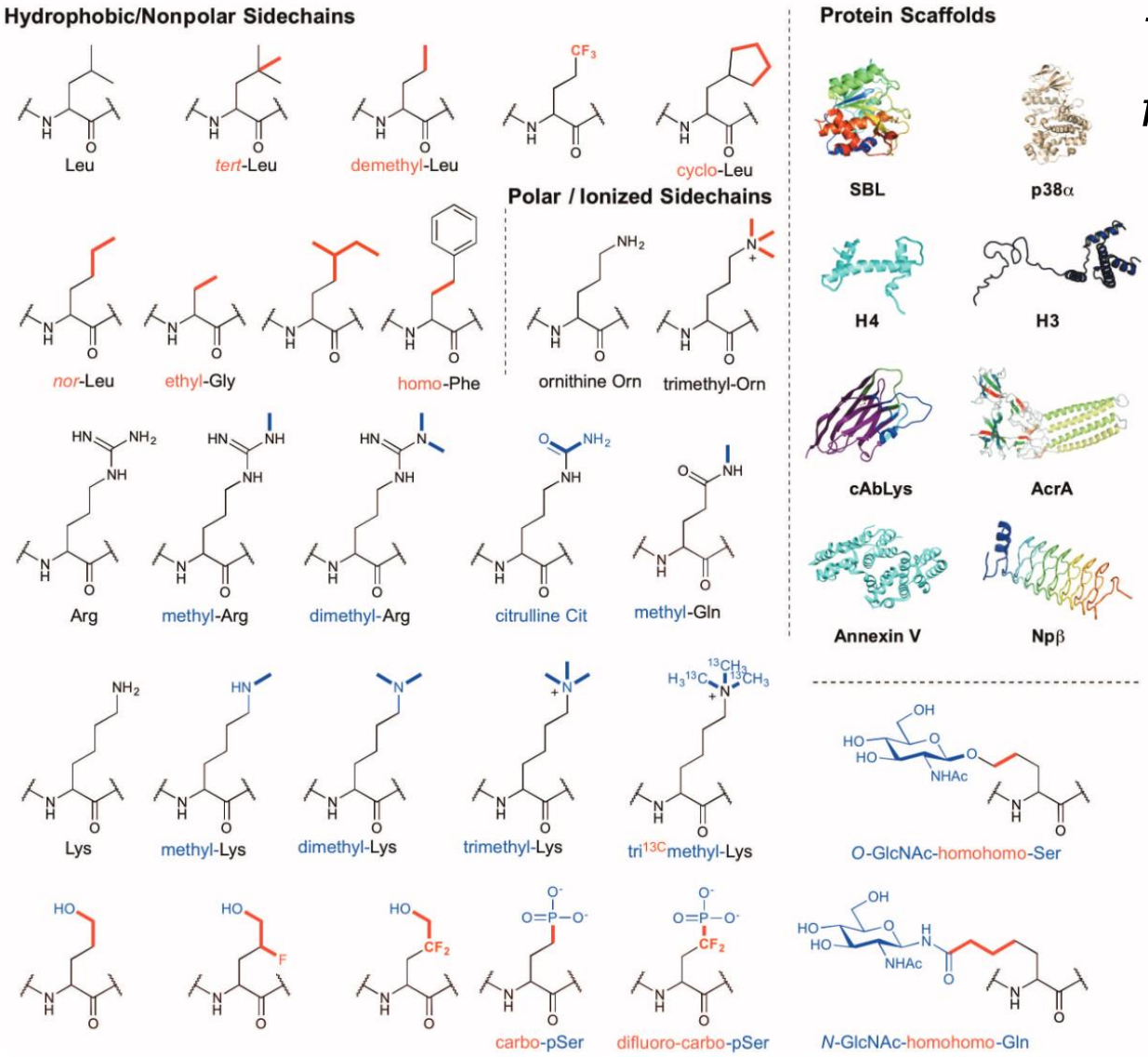
Alkylating reagent	X	R_1	R_2
DIB (**2**)	I	H	H
DBHDA (**3**)	Br	$CONH_2$	$CONH_2$
MDBP (**4**)	Br	$COOCH_3$	H

“Off-protein” Strategy

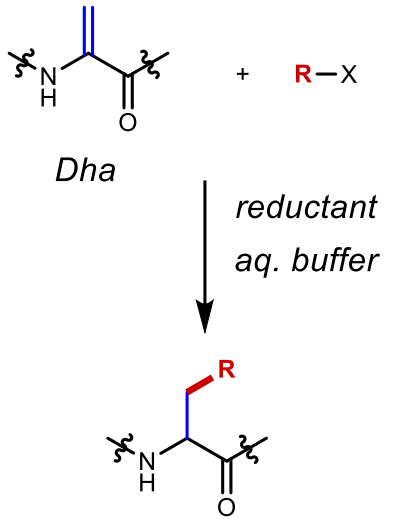
Reaction scopes



“Off-protein” Strategy



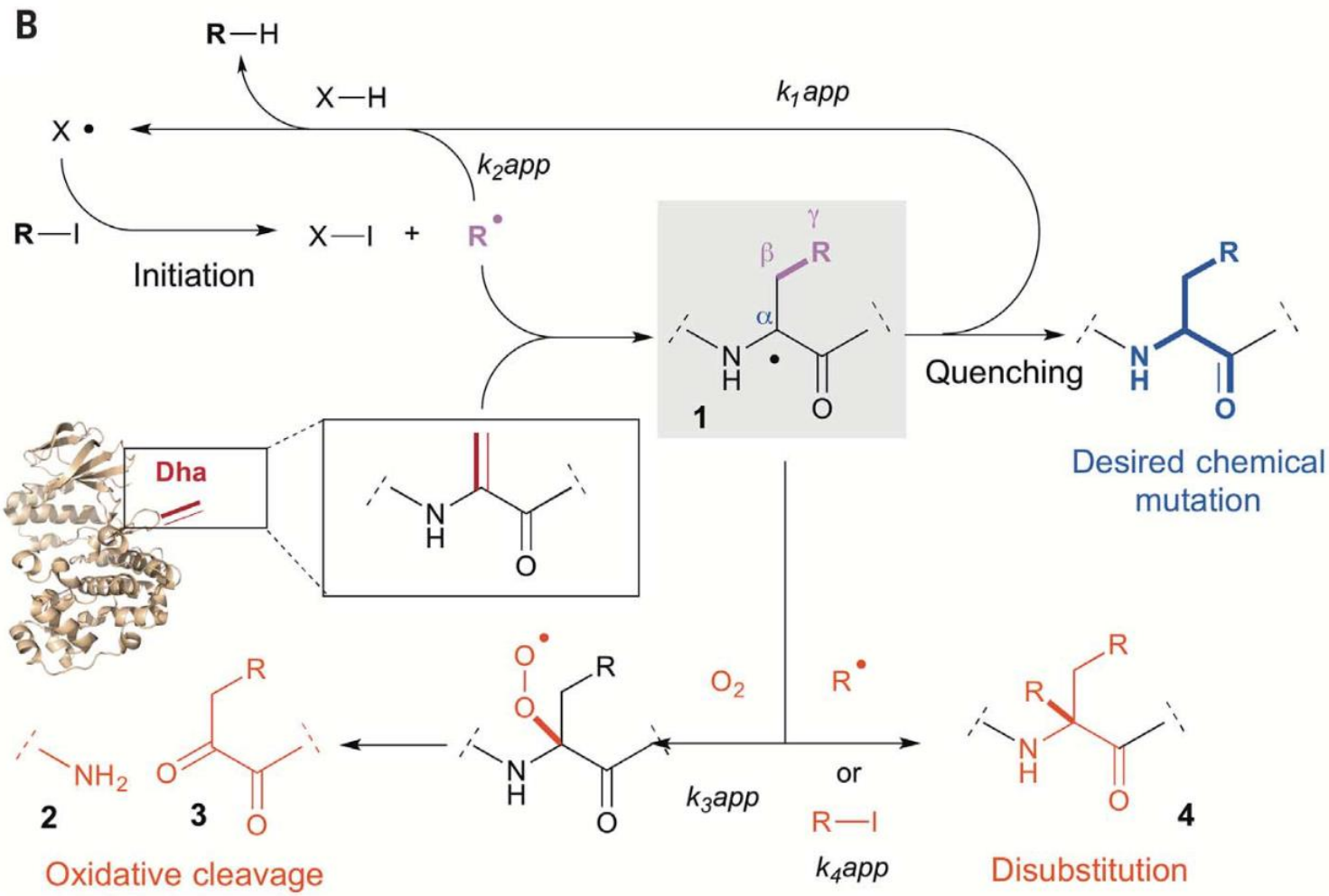
The first C(sp^3)–C(sp^3) bond formation on proteins



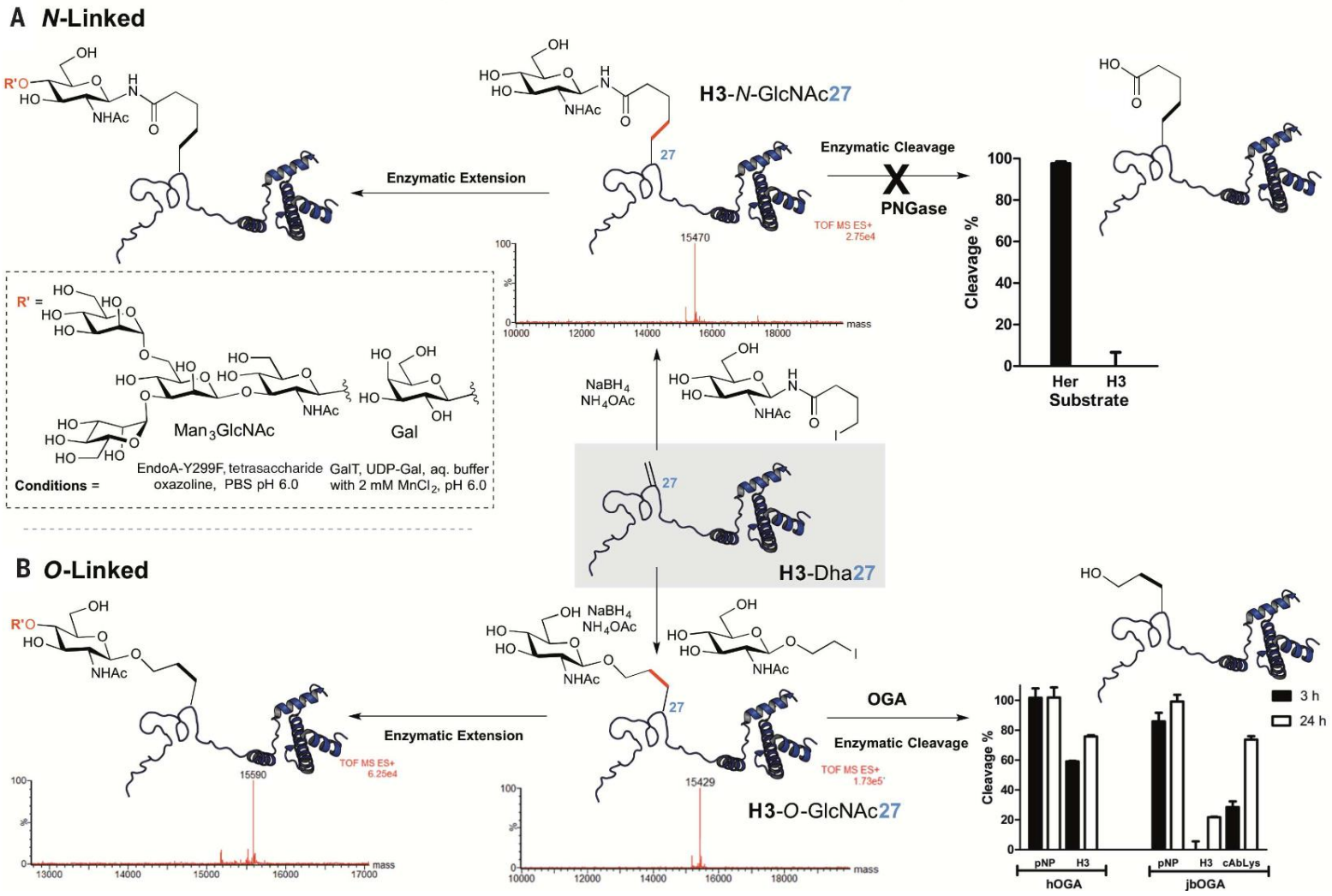
X = Br, I
reductant = $NaBH_4$, Zn, In

Davis, B. G. *et al. Science* **2016**, *354*, aag1465.

“Off-protein” Strategy

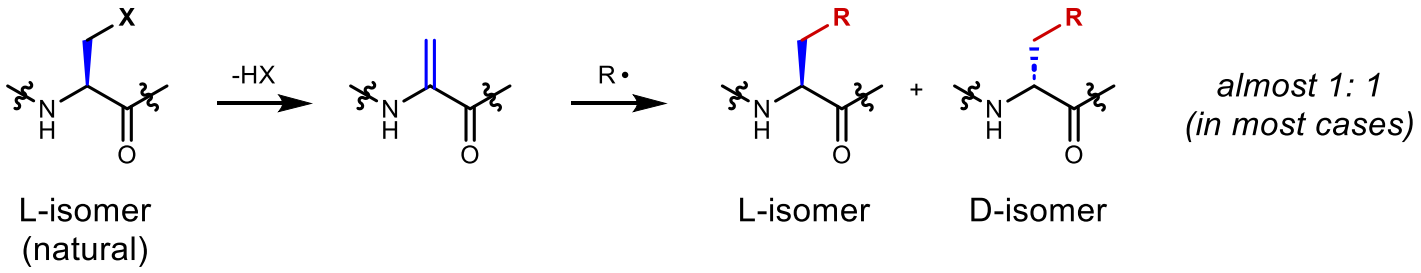


“Off-protein” Strategy

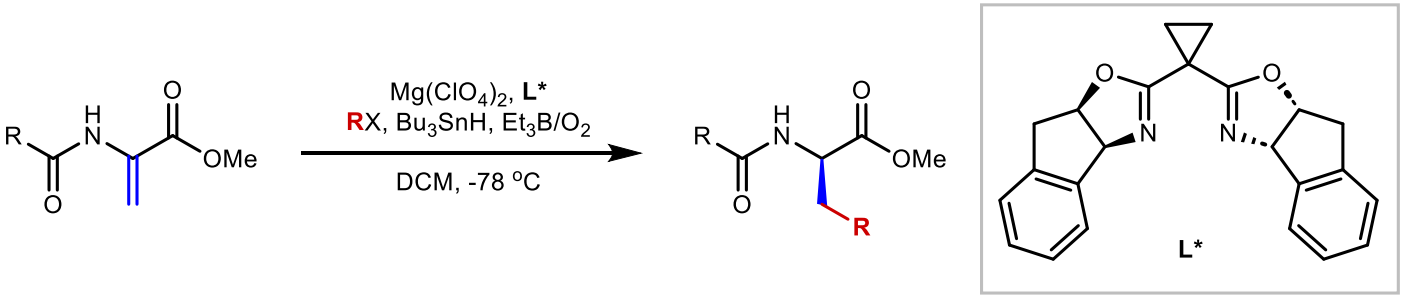


“Off-protein” Strategy

A problem: racemization



possible strategy: (diastereo- or enatio-)selective HAT



but hard to access on real protein

Sausker, J. B. *et al. Angew. Chem. Int. Ed.* **2001**, *40*, 1293–1296.

Outline

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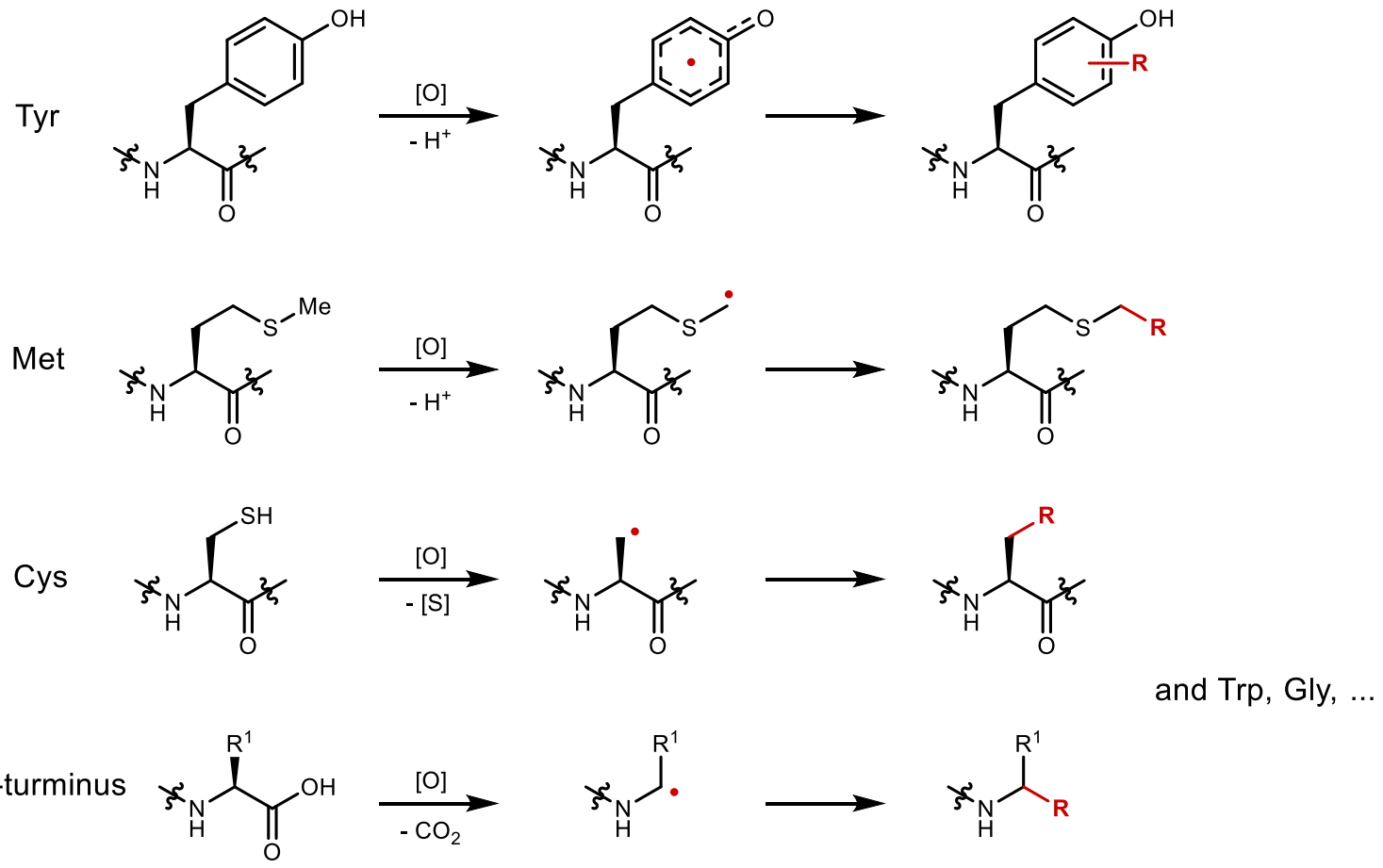
“On-protein” Strategy

Canonical AAs as radical precursors:

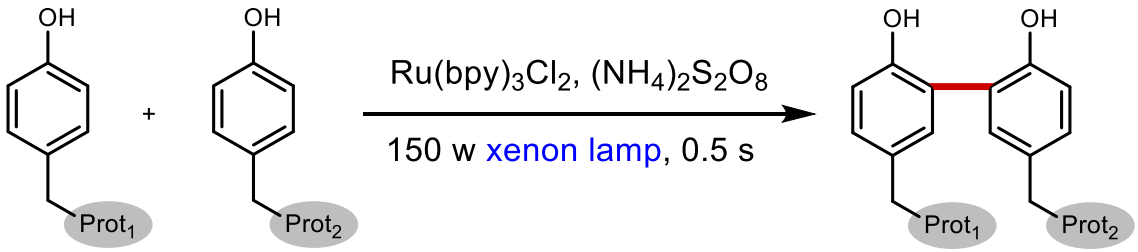


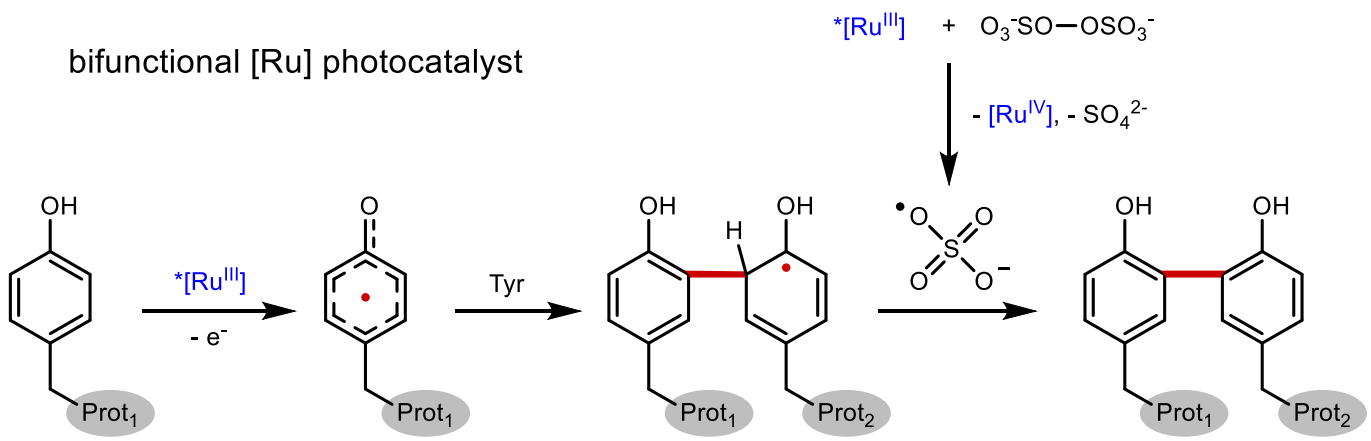
and Trp, Gly, ...

Gouin, S. G. *et al. Chem. Eur. J.* **2020**, *26*, 14257–14269.

“On-protein” Strategy

Protein cross-linking on Tyr

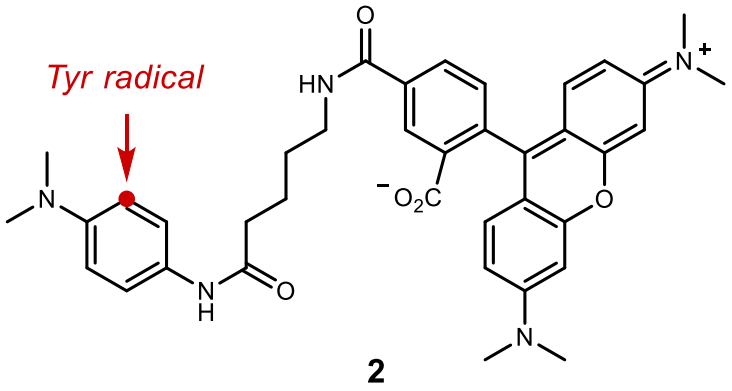




Kodadek, T. *et al. Proc. Natl. Acad. Sci. USA* **1999**, *96*, 6020–6024.

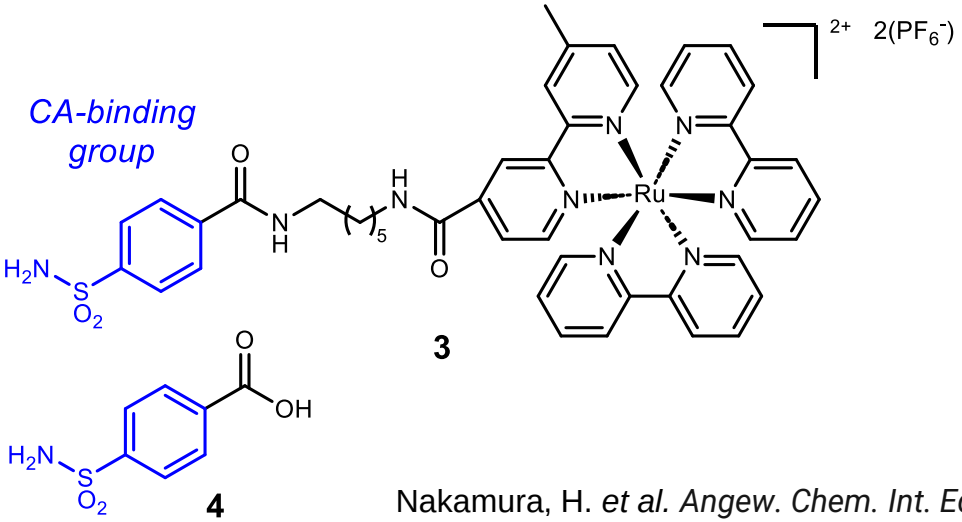
“On-protein” Strategy

“Local SET catalyst”: better site-selectivity



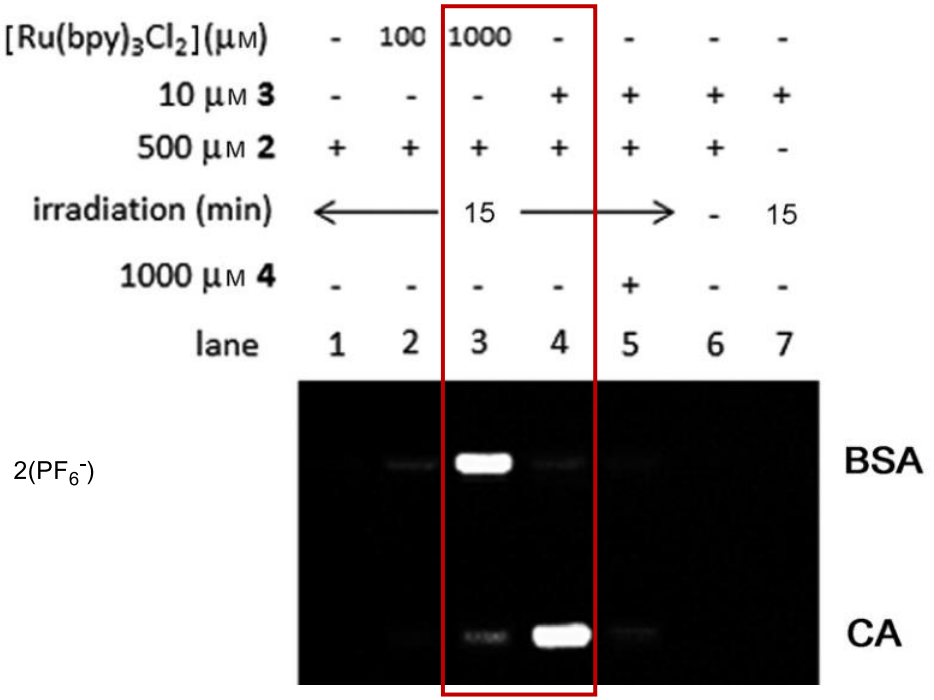
2

radical acceptor with fluorescence



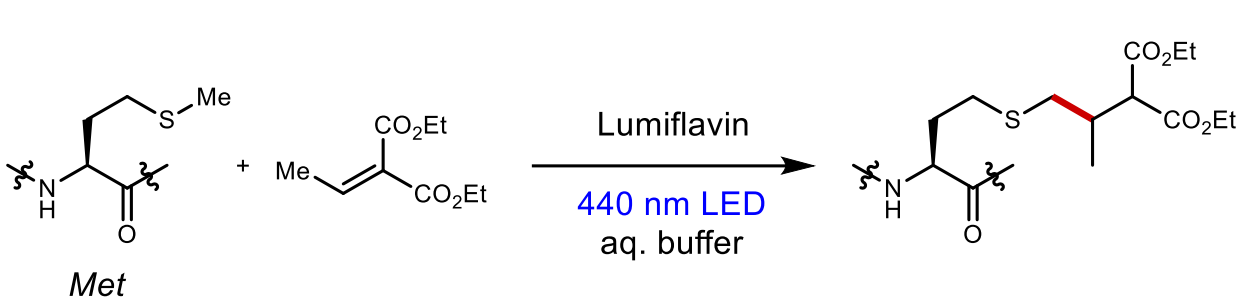
3

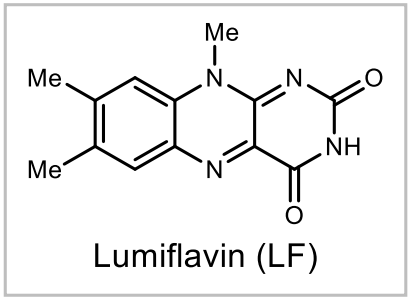
4

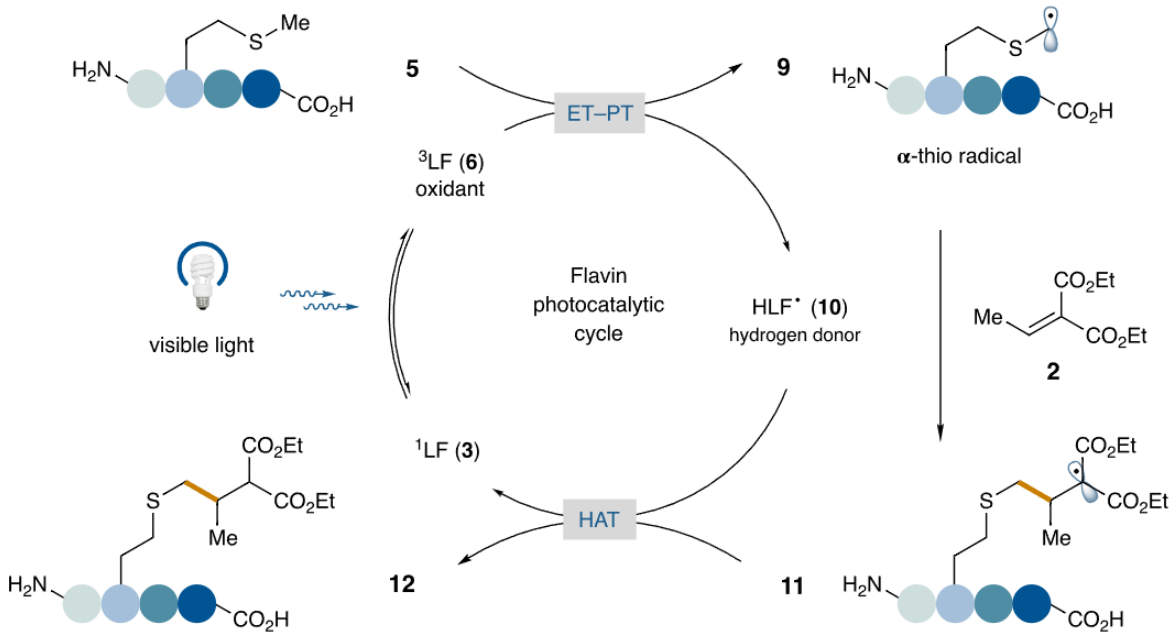


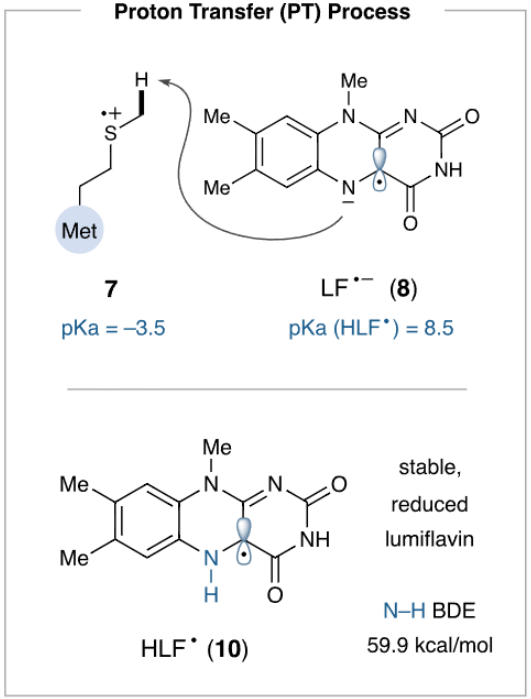
Nakamura, H. *et al. Angew. Chem. Int. Ed.* **2013**, *52*, 8681–8684.

“On-protein” Strategy

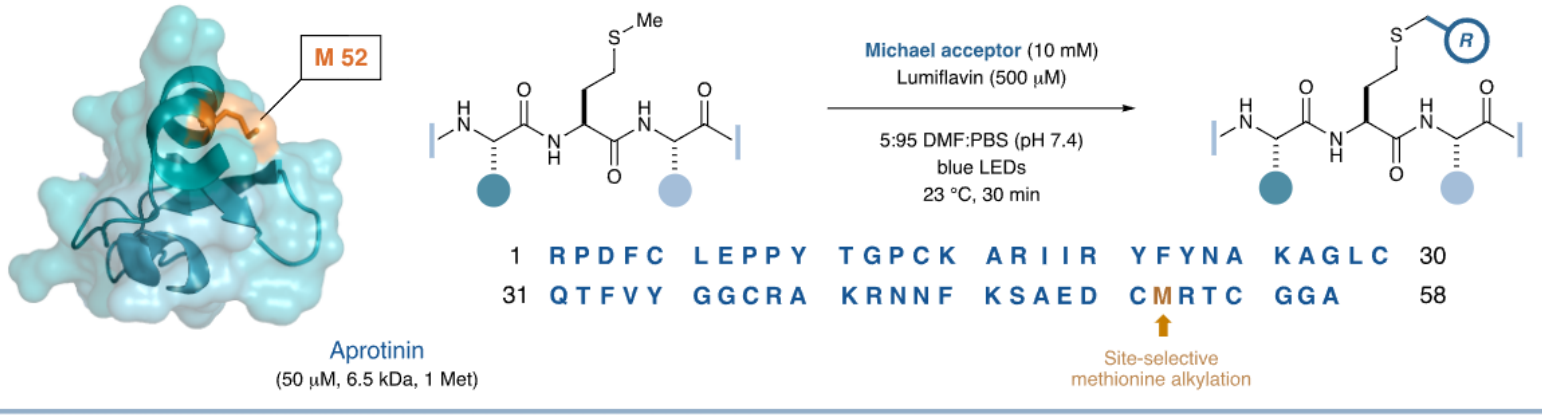


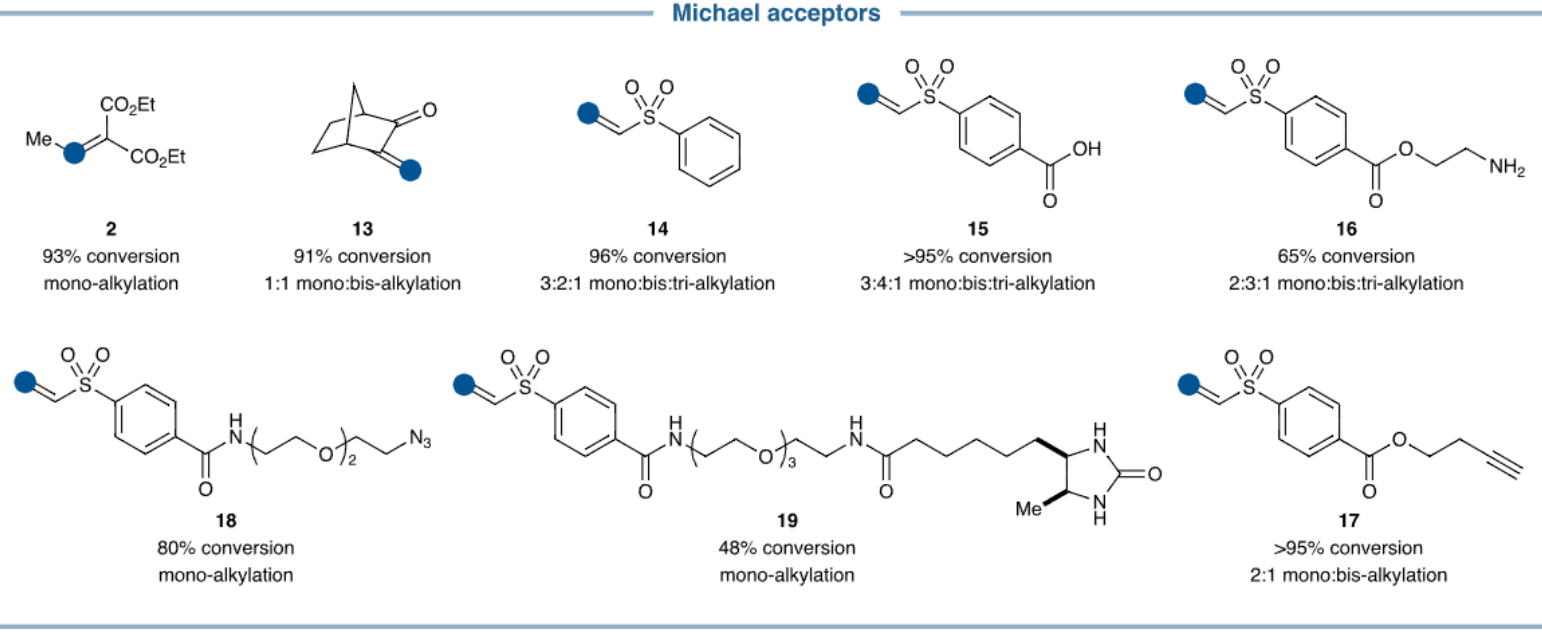




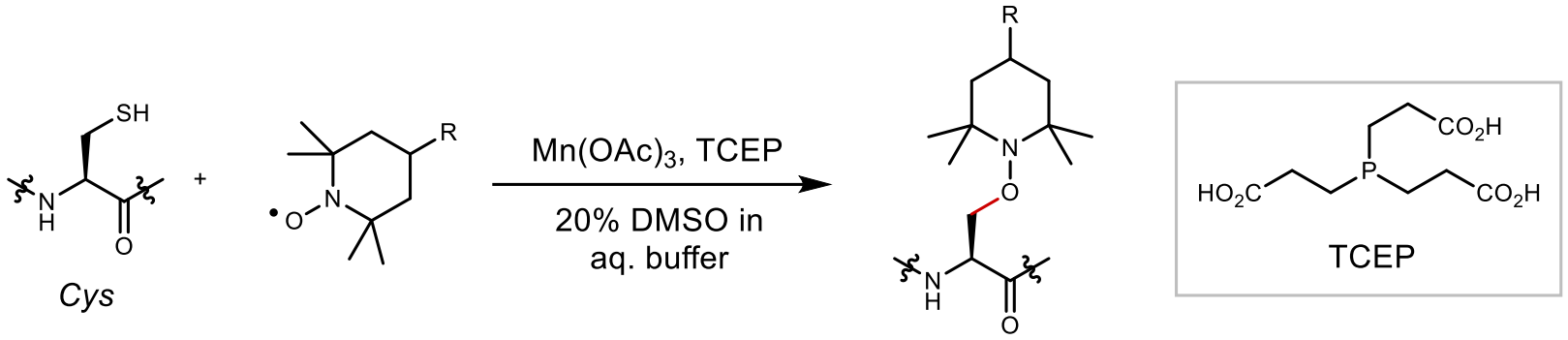


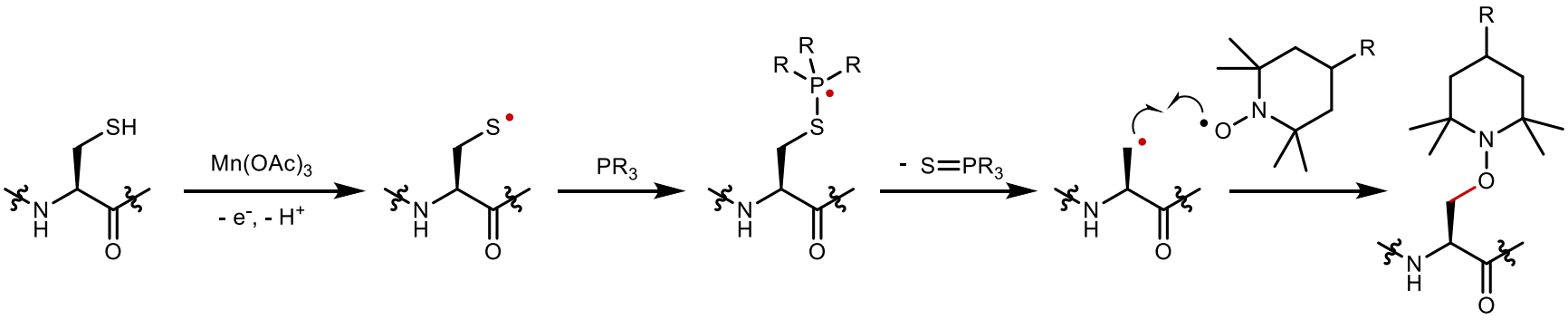
“On-protein” Strategy





"On-protein" Strategy

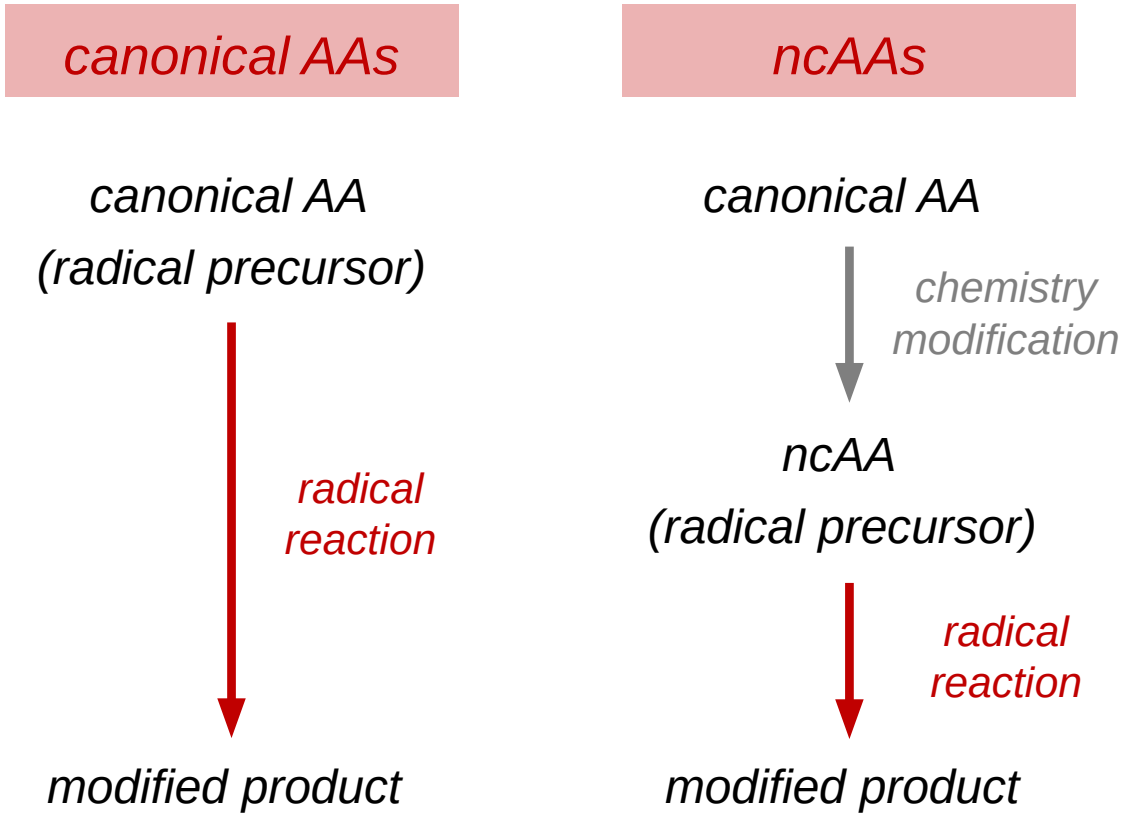




➢ *limitation for proteins: PR_3 cleaves R–S–S–R*

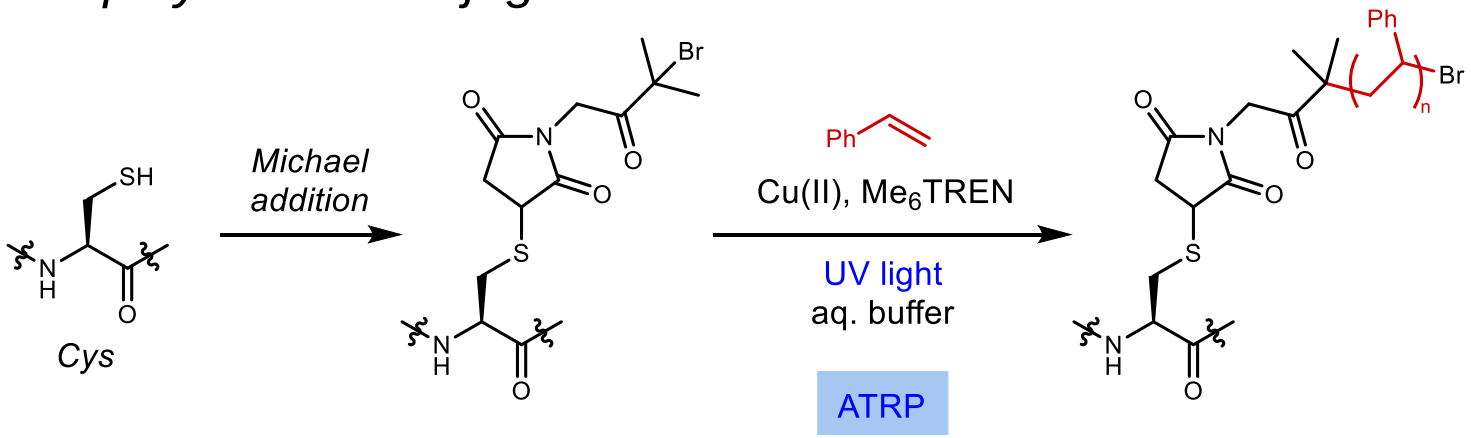
“On-protein” Strategy

“on-protein” strategy of:

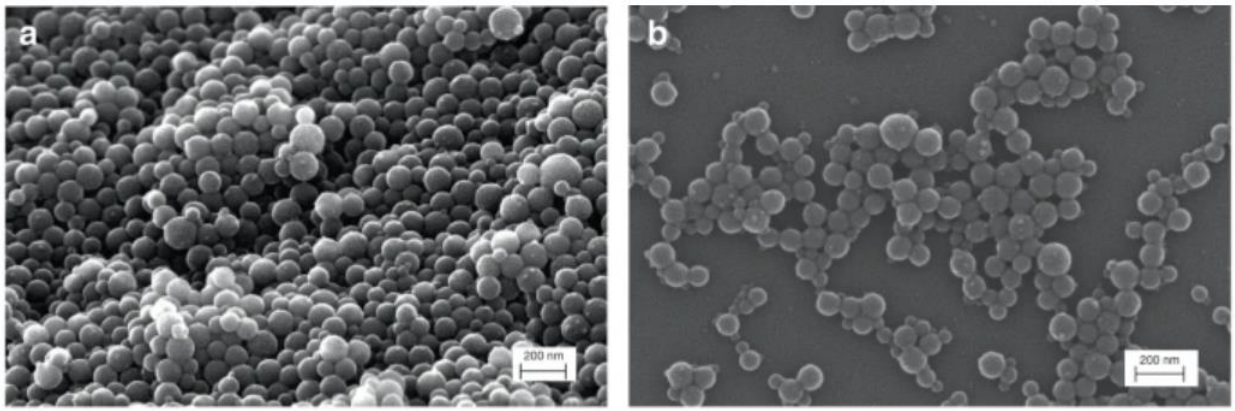


"On-protein" Strategy

Protein-polymer bioconjugates



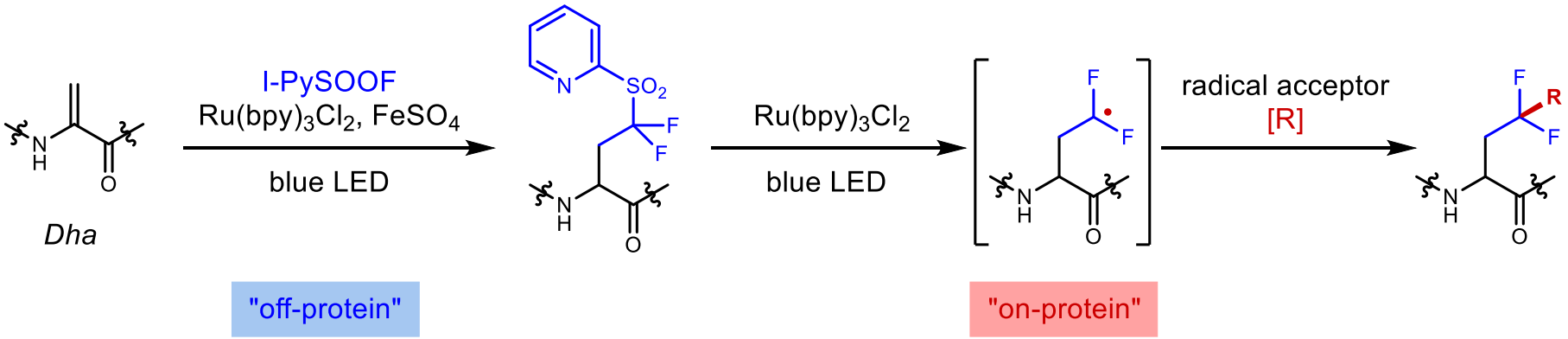
Cu(II) as low as 0.09 mM | *Oxygen tolerant* | *Capable with tap water / sea water*

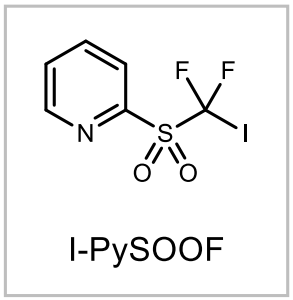


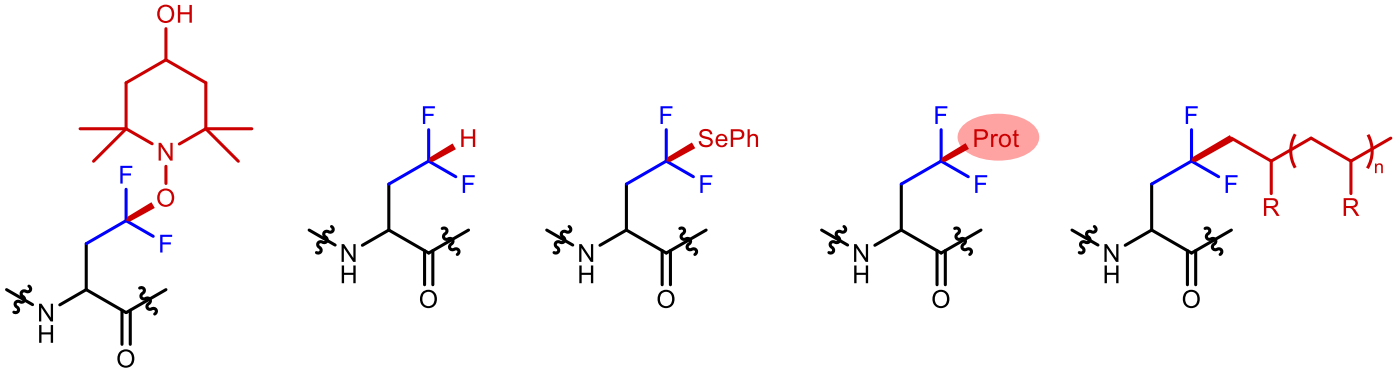
Anastasaki, A.; Velonia, K. *et al. Nat. Commun.* **2020**, *11*, 1486.

“On-protein” Strategy

Sequential PTMs via radical procedure:





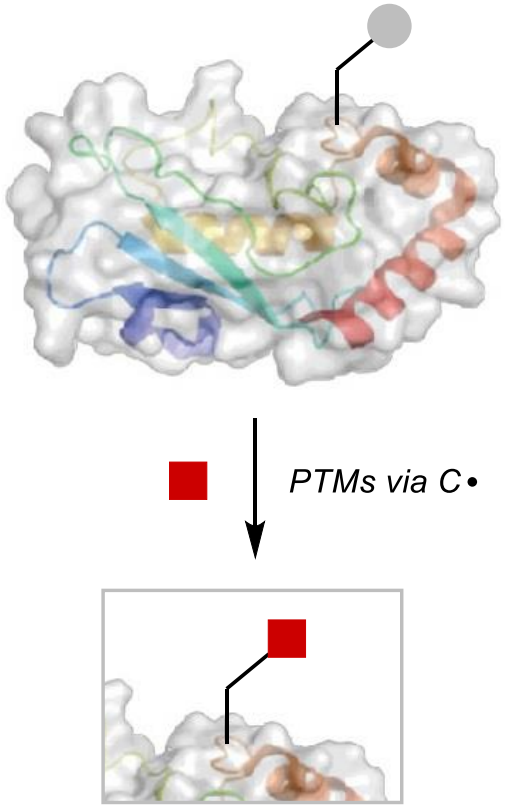


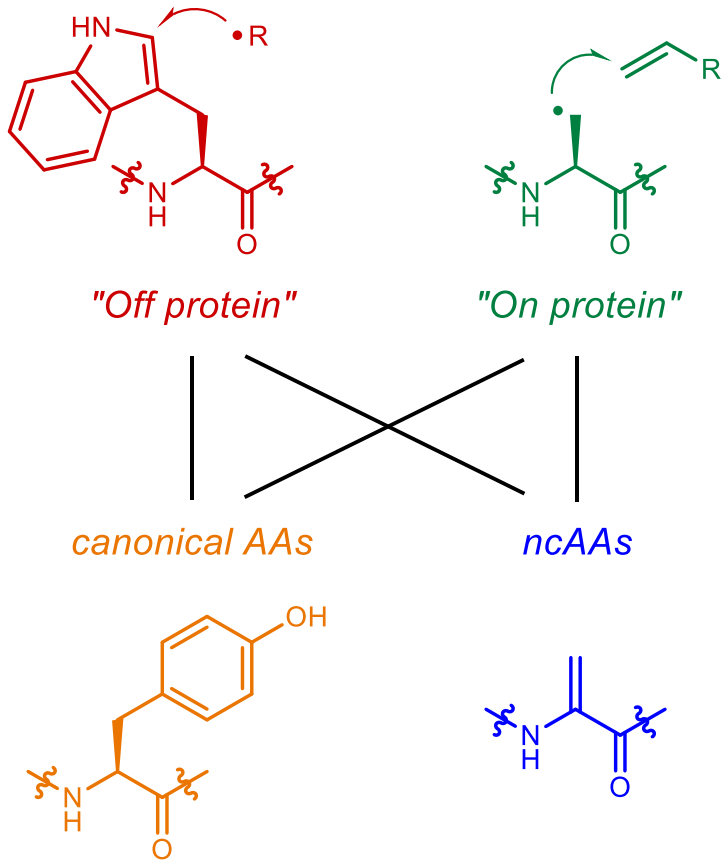
Davis, B. G. *et al. Nature* **2020**, *585*, 530–537.

Outline

- *Introduction*
- *“Off-protein” strategy*
- *“On-protein” strategy*
- *Summary*

Summary


PTMs via C•


HN
•R
N
H
O
"Off protein"
R
N
H
O
"On protein"
canonical AAs
ncAAs
OH
N
H
O
N
H
O

Summary

Think about energy input forms:

- *Stoichiometric chemical reagent*
- *Photocatalysis*
- *Electrocatalysis?*

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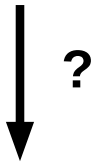
conjugation (with dye, polymer, drugs…) of proteins



?

function change & optimization of proteins