XU LAB
Innovation through Catalysis

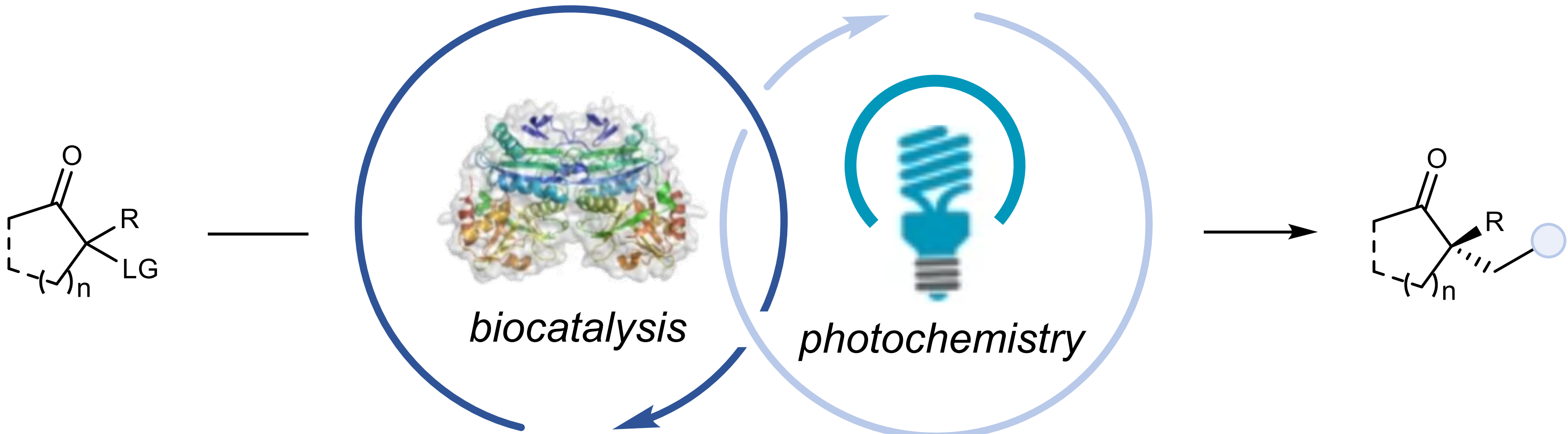
北京大学
PEKING UNIVERSITY

XU GROUP
Department of Chemistry, Peking University

Selected Weekly Literature Presentations

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

Photobiocatalysis for Abiological Transformations



Ken Chen

Group meeting

Jun. 8th, 2024

- ***Background of photobiocatalysis***
  - Brief introduction to enzymatic catalysis
  - Modification of enzyme catalysts
  - Combining photocatalysis and enzymatic catalysis
- ***Photobiocatalysis for organic synthesis***
  - Cofactor-dependent photocatalysis
  - Artificial photoenzymes
  - Combination of external photocatalysis and enzymes
- ***Summary and perspective***

- ***Background of photobiocatalysis***
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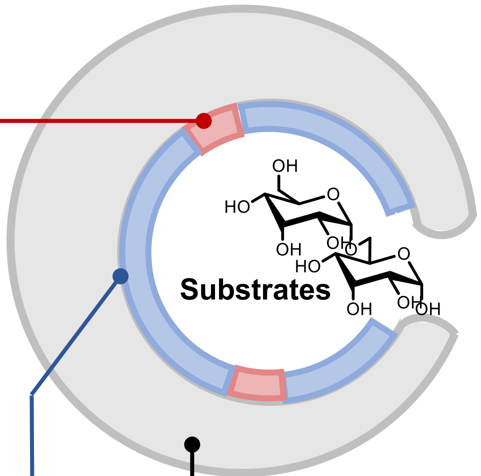
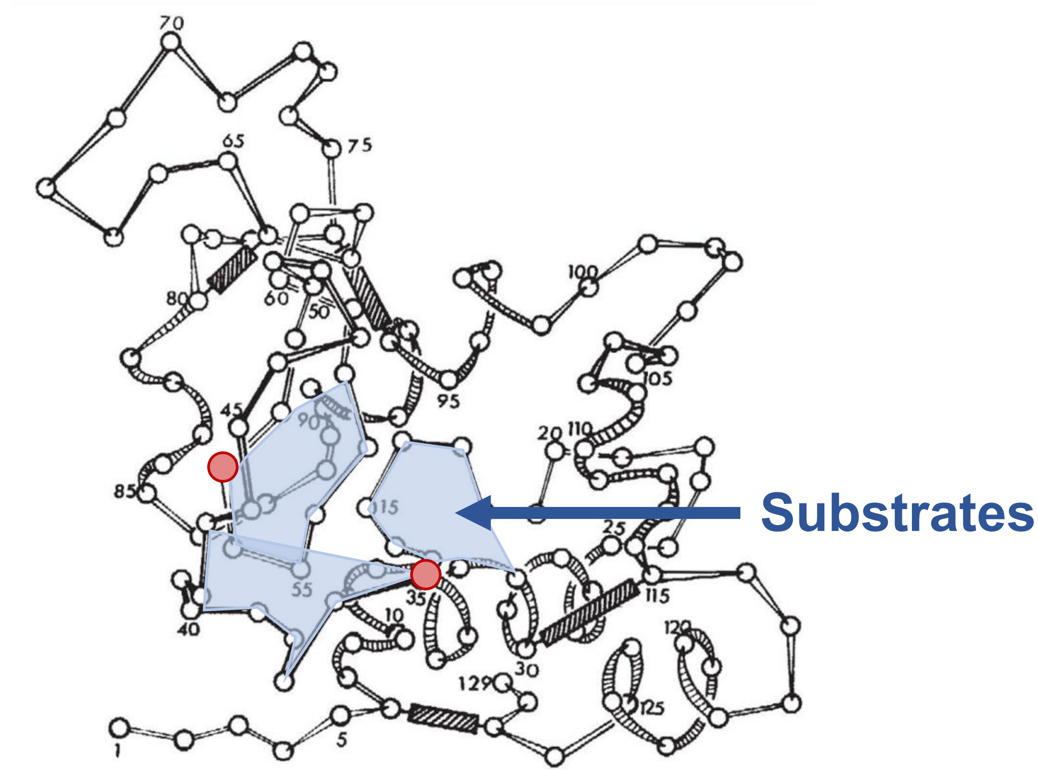
Enzymes for chemical synthesis

- Definition
  - Enzymes are macromolecular biological catalysts.
  - Catalyze more than 5,000 biochemical reaction types.
  - Most enzymes are proteins, a few are catalytic RNA molecules.
- Advantages
  - Higher enantioselectivity and regioselectivity
  - Can be effective in both aqueous and organic media
  - Typically do not require protecting groups
  - Operate under mild conditions with high efficiency

Structure and functions of enzymes





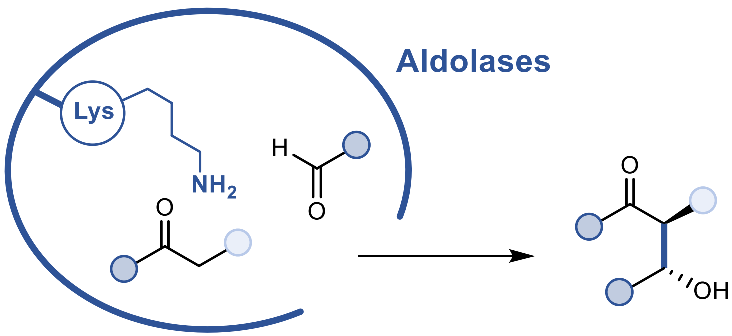
- Protein structure: Scaffold to support and position active site
- Binding sites: Bind and orient substrates
- Catalytic sites

Sarma, V. R. *et al*. *Nature*, **1965**, *206*, 757.

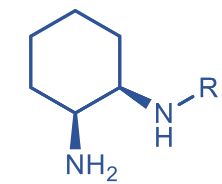
Catalytic sites of enzymes

Catalytic sites

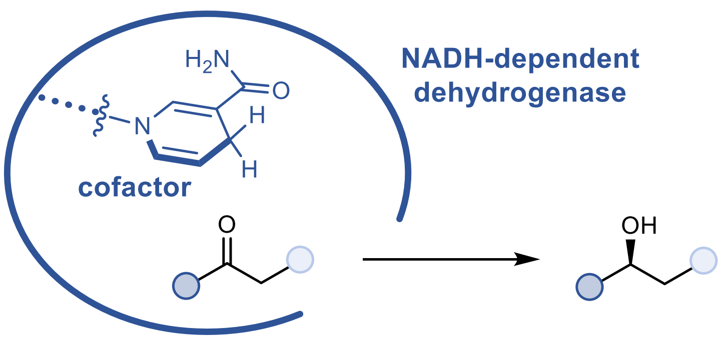
Catalyzing by residues of amino acid

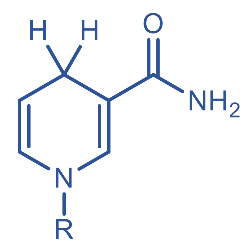


Derived from enzymes



Catalyzing by cofactor

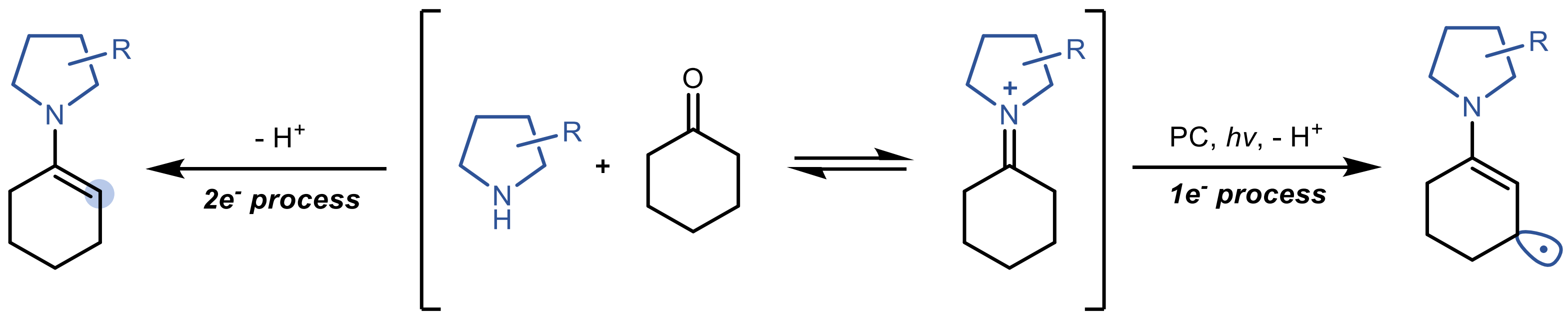


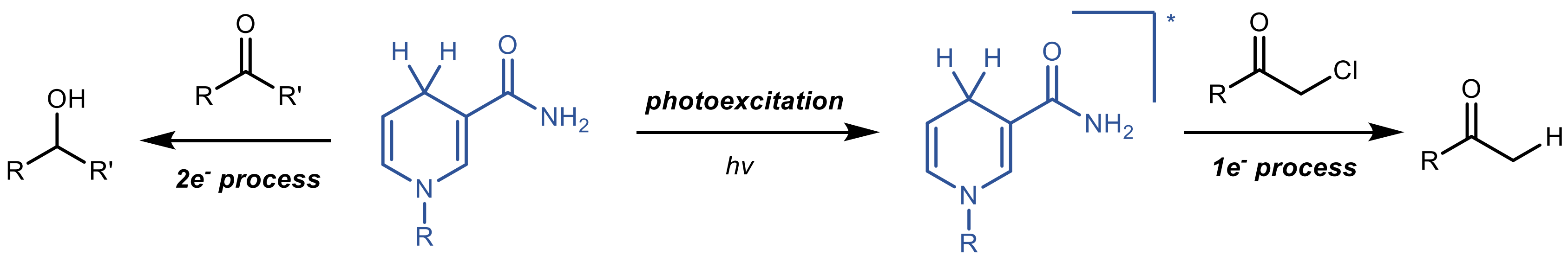


Guérard-Hélaine, C. *et al. ACS Catal.* **2022**, *12*, 733–761.
Matsudaetal, T. *et al. Tetrahedron: Asymmetry* **2009**, *20*, 513–557.

Discovering unnatural reactivity of enzymes

- The same catalyst presents different catalytic properties in different scenarios



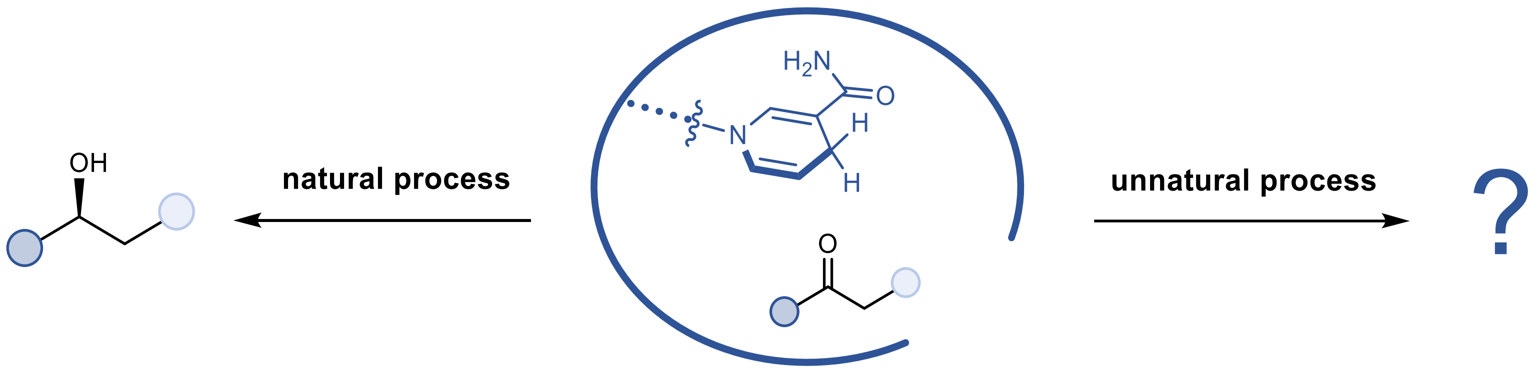


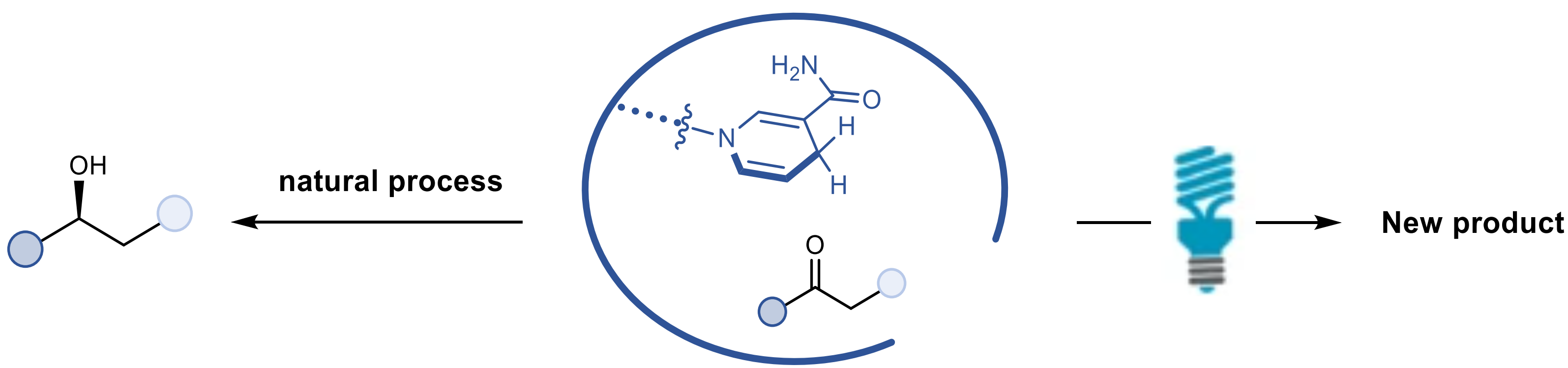
$E^{ox}_{1/2}$ **= 0.57 vs SCE**

$E^{ox}_{1/2}$ **= -2.60 vs SCE**

MacMillan, D. W. C. *et al. Science* **2013**, *339*,1593.
Suenobu, T. *et al. J. Am. Chem. Soc.* **2003**, *125*, 4808–4816.

Discovering unnatural reactivity of enzymes

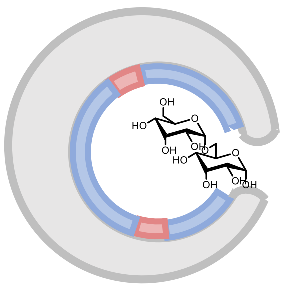




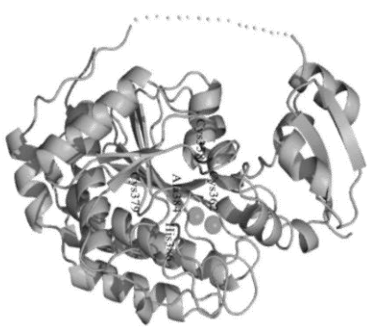
Combining photocatalysis and enzymatic catalysis

Advantages	***Challenges***
■ Mild reaction conditions	■ Compatibility of conditions
■ Diverse reactive patterns	■ Unmatched dynamics
■ Exquisite selectivity and high efficiency	■ Limited substrates

Overcoming the limitation of enzymes in organic synthesis



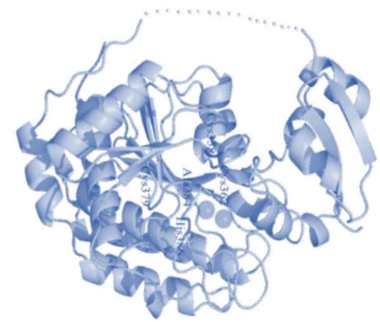
- Binding site can be modified to optimize the reaction



Naturally occurring enzyme

Activity for specific substrates

Unstable under certain conditions



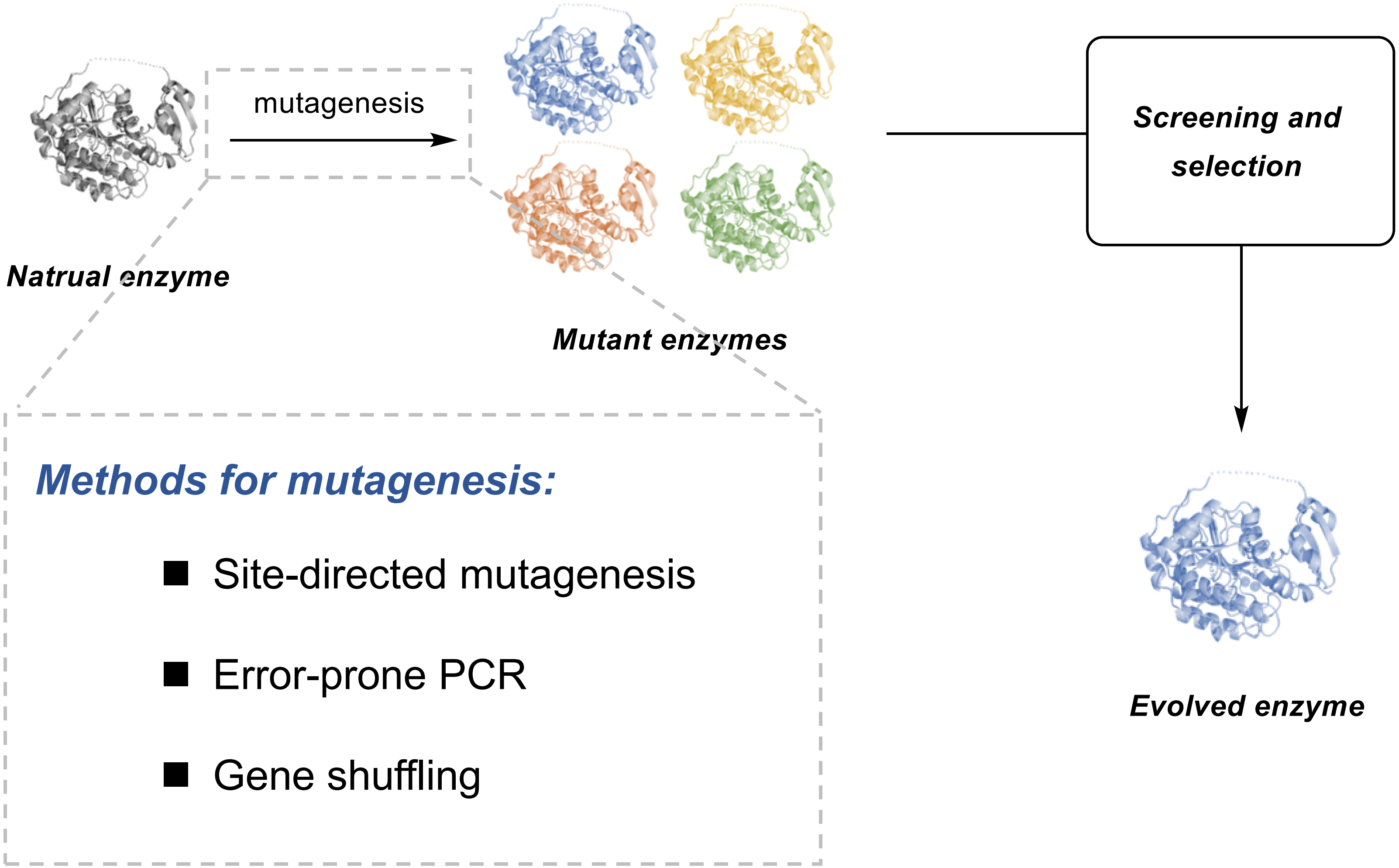
Ideal biocatalyst

Activity for desired substrates

Tolerates process conditions

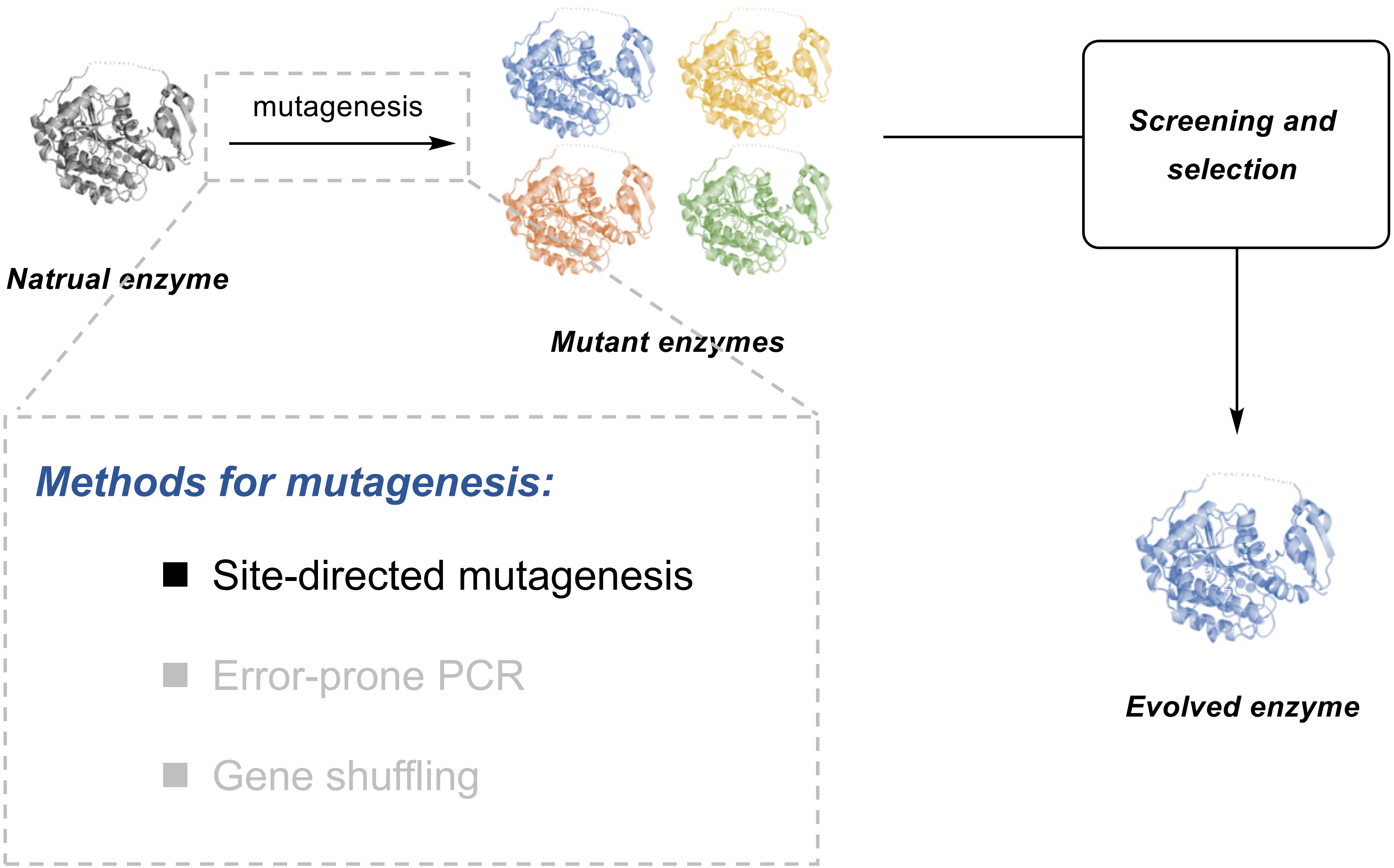
Modification of enzyme catalysts

Directed evolution



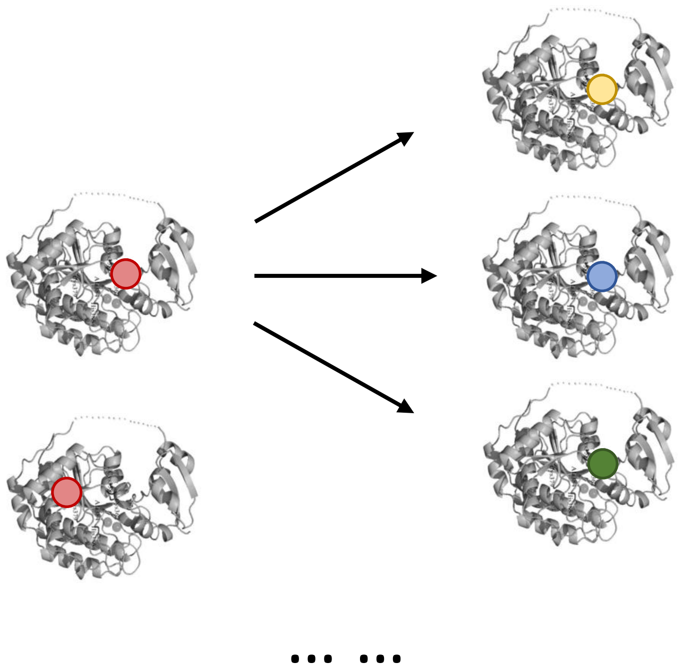
Modification of enzyme catalysts

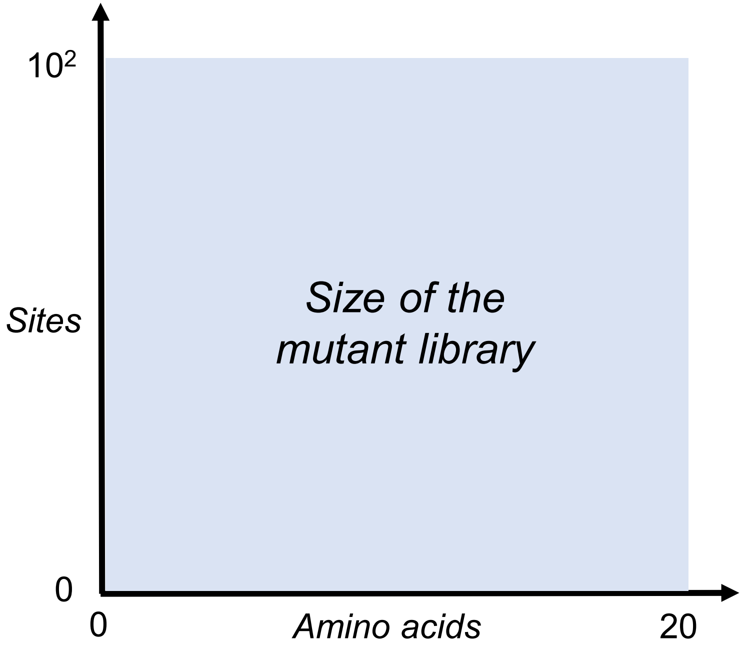
- Directed evolution



Modification of enzyme catalysts

- Site-directed mutagenesis
  - Substituting individual amino acids in a protein
  - Requires a lot of structural information

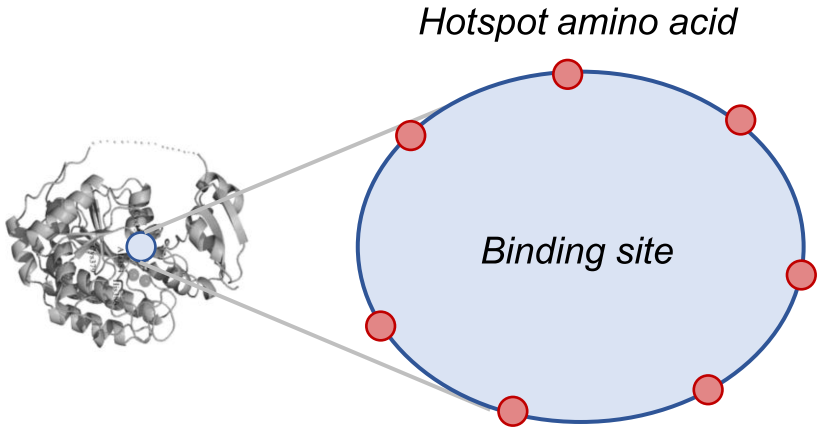


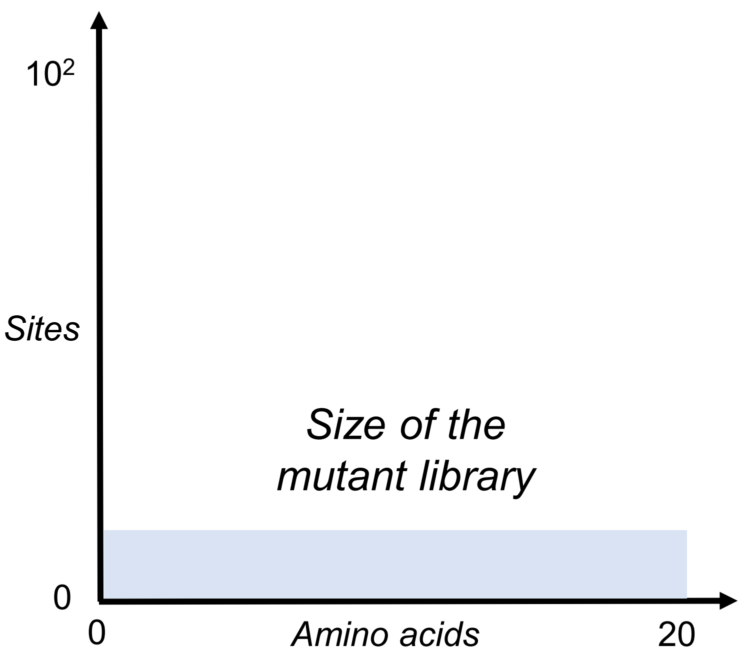


Modification of enzyme catalysts

- Site-directed mutagenesis

  - Identify the appropriate mutational residues (hotspots)





- Crystal structures
- Homologous models
- Computational means

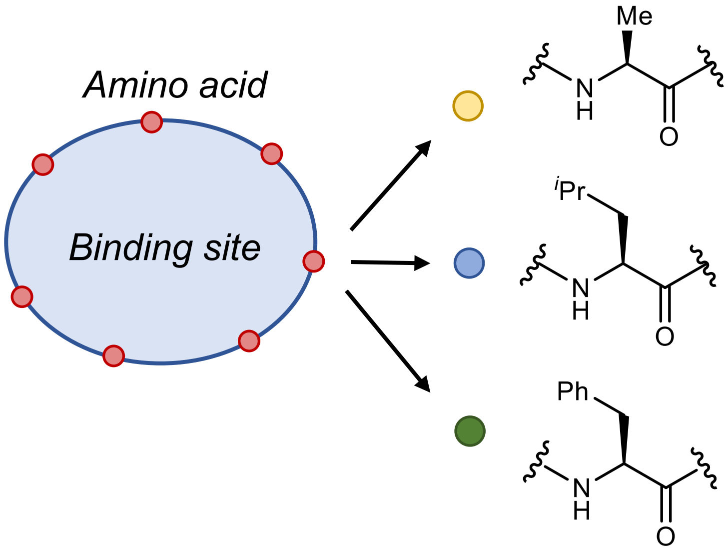
… …

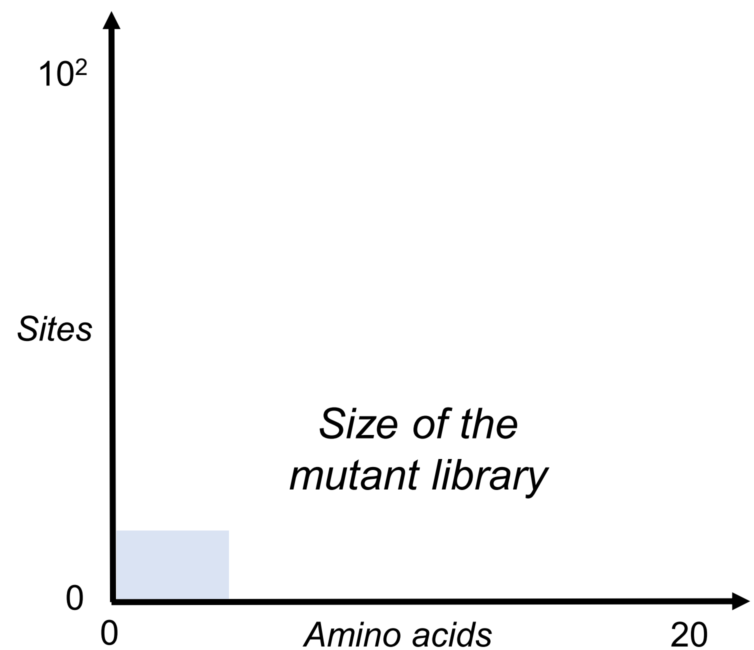
Huang, X. *et al. Mol. Catal.* **2024**, *553*, 113755.

Modification of enzyme catalysts

- Site-directed mutagenesis

  - Introduce few different amino acids per hotspot





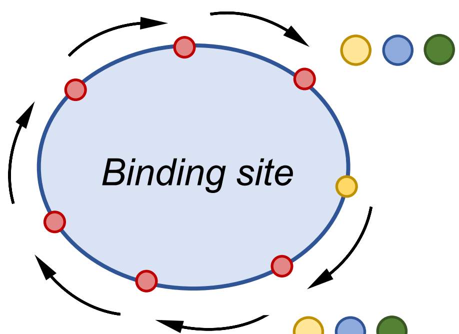
- Based on the assumption which property of residues influence the most

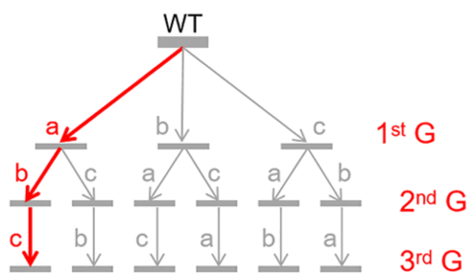
Huang, X. *et al. Mol. Catal.* **2024**, *553*, 113755.

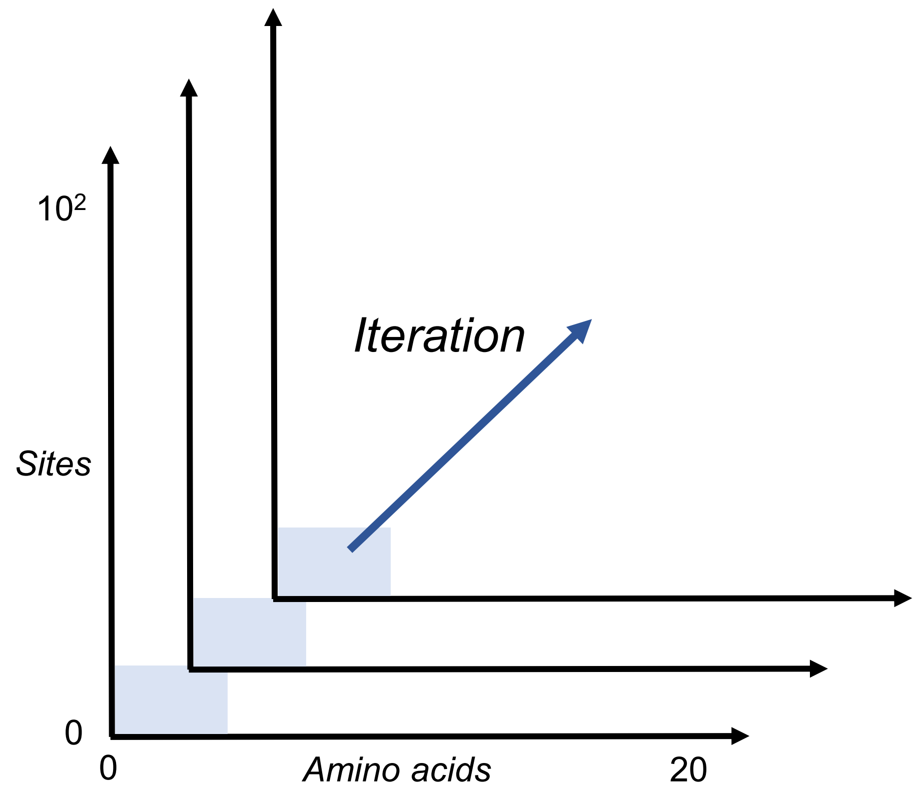
Modification of enzyme catalysts

Site-directed mutagenesis

- Iteration using the same steps







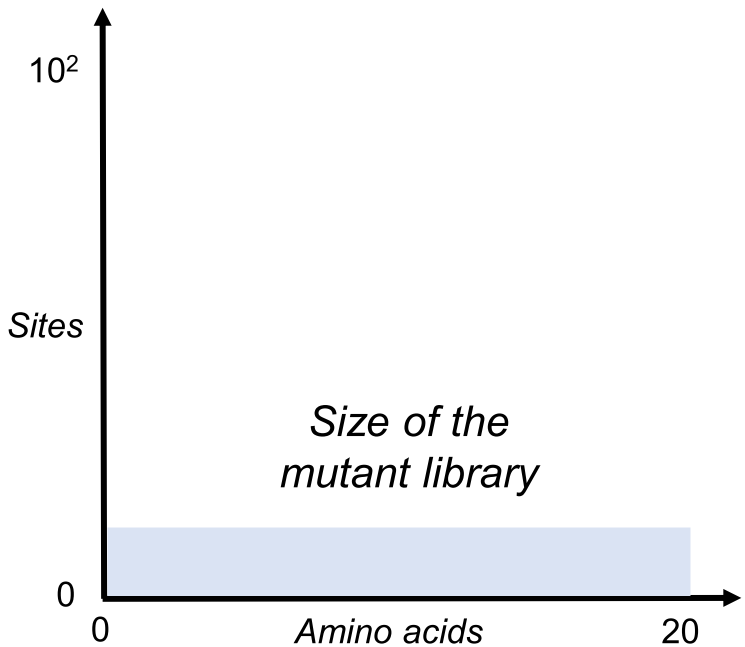
- The optimal mutant is used as the starting point for iteration

Huang, X. *et al. Mol. Catal.* **2024**, *553*, 113755.

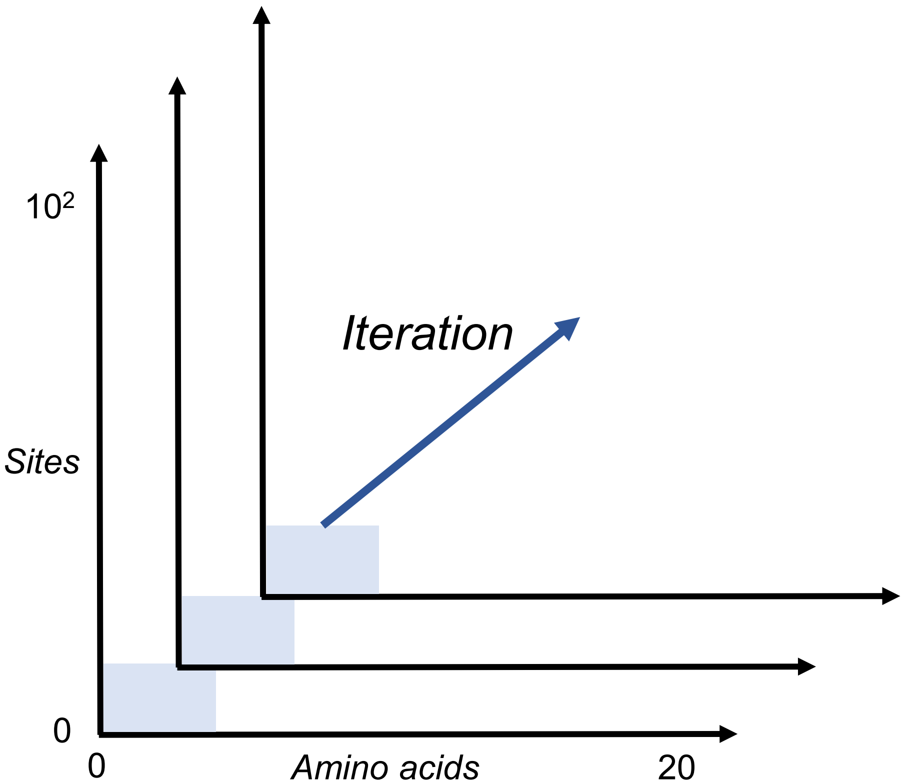
Modification of enzyme catalysts

Site-directed mutagenesis

- Site-saturation mutagenesis (SSM)



- Focused rational iterative site-specific mutagenesis (FRISM)

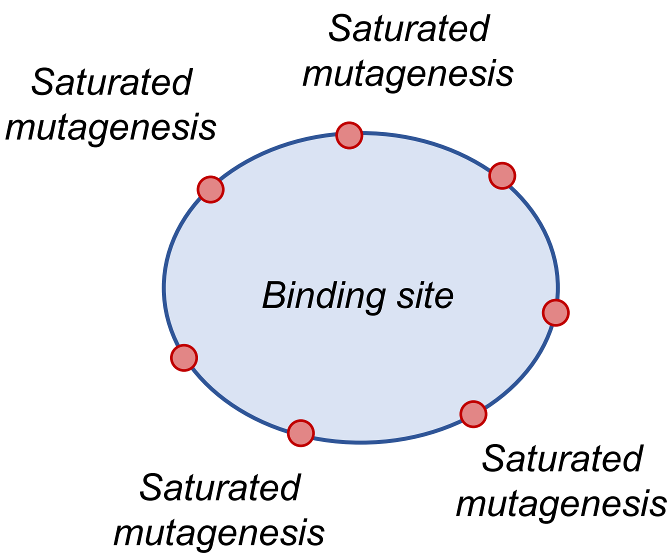


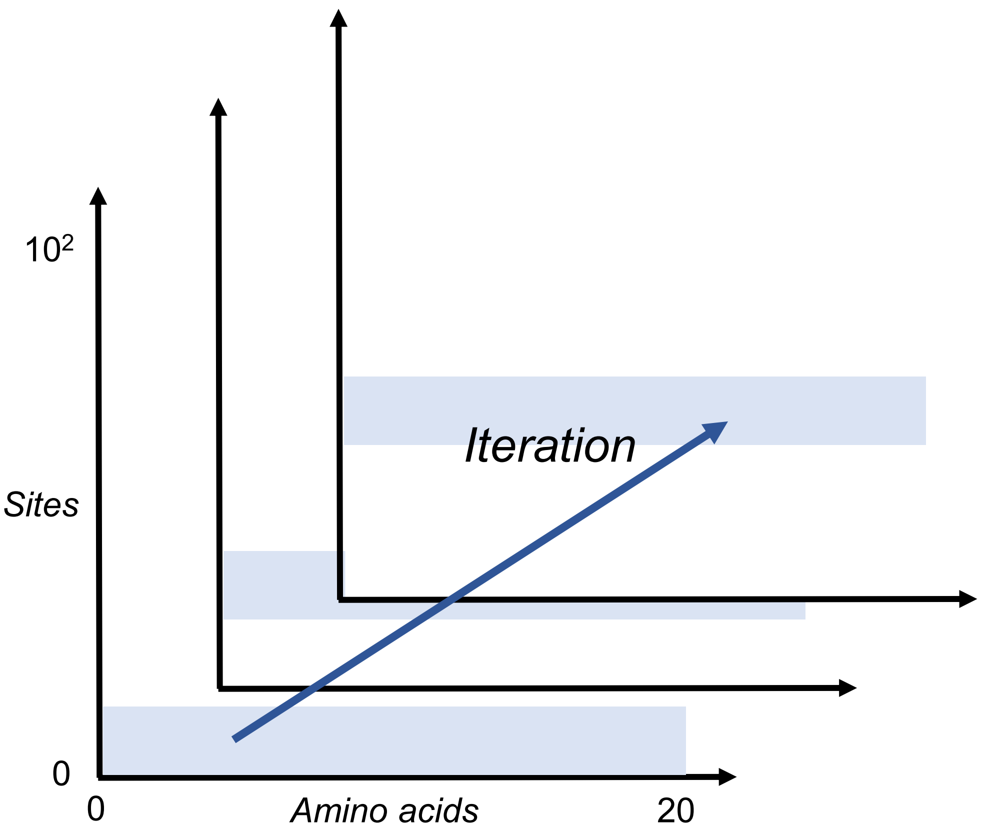
Huang, X. *et al. Mol. Catal.* **2024**, *553*, 113755.

Modification of enzyme catalysts

- Site-directed mutagenesis

  - Combination of different methods

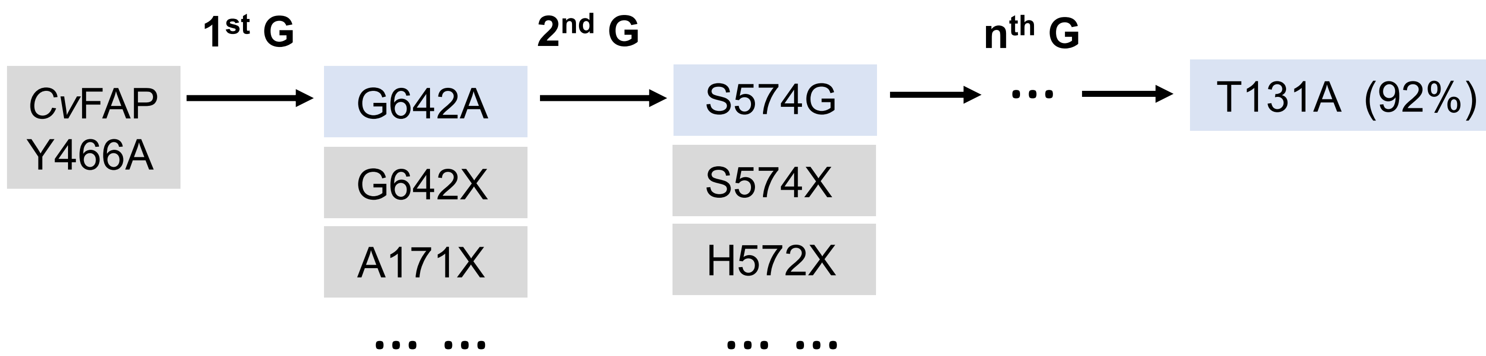




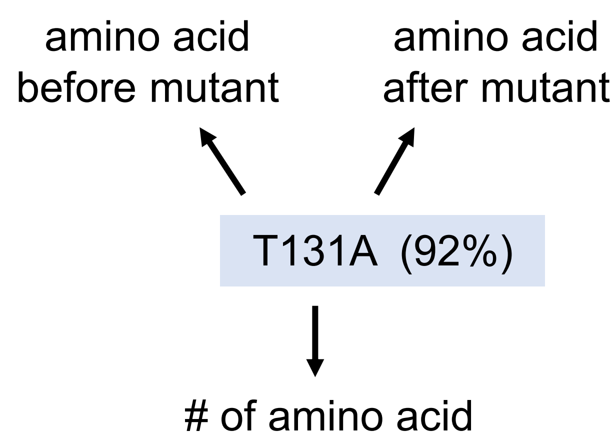
Huang, X. *et al. Mol. Catal.* **2024**, *553*, 113755.

Modification of enzyme catalysts

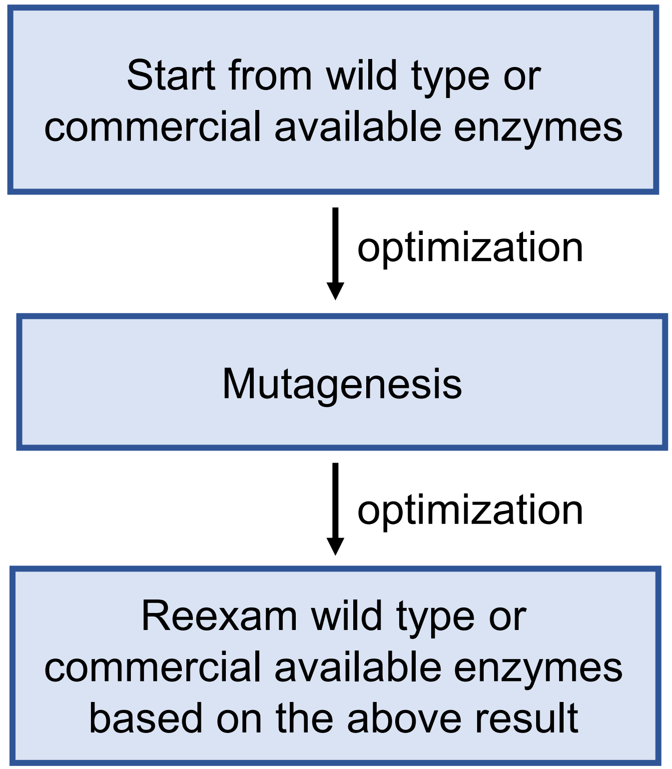
Note of mutagenesis



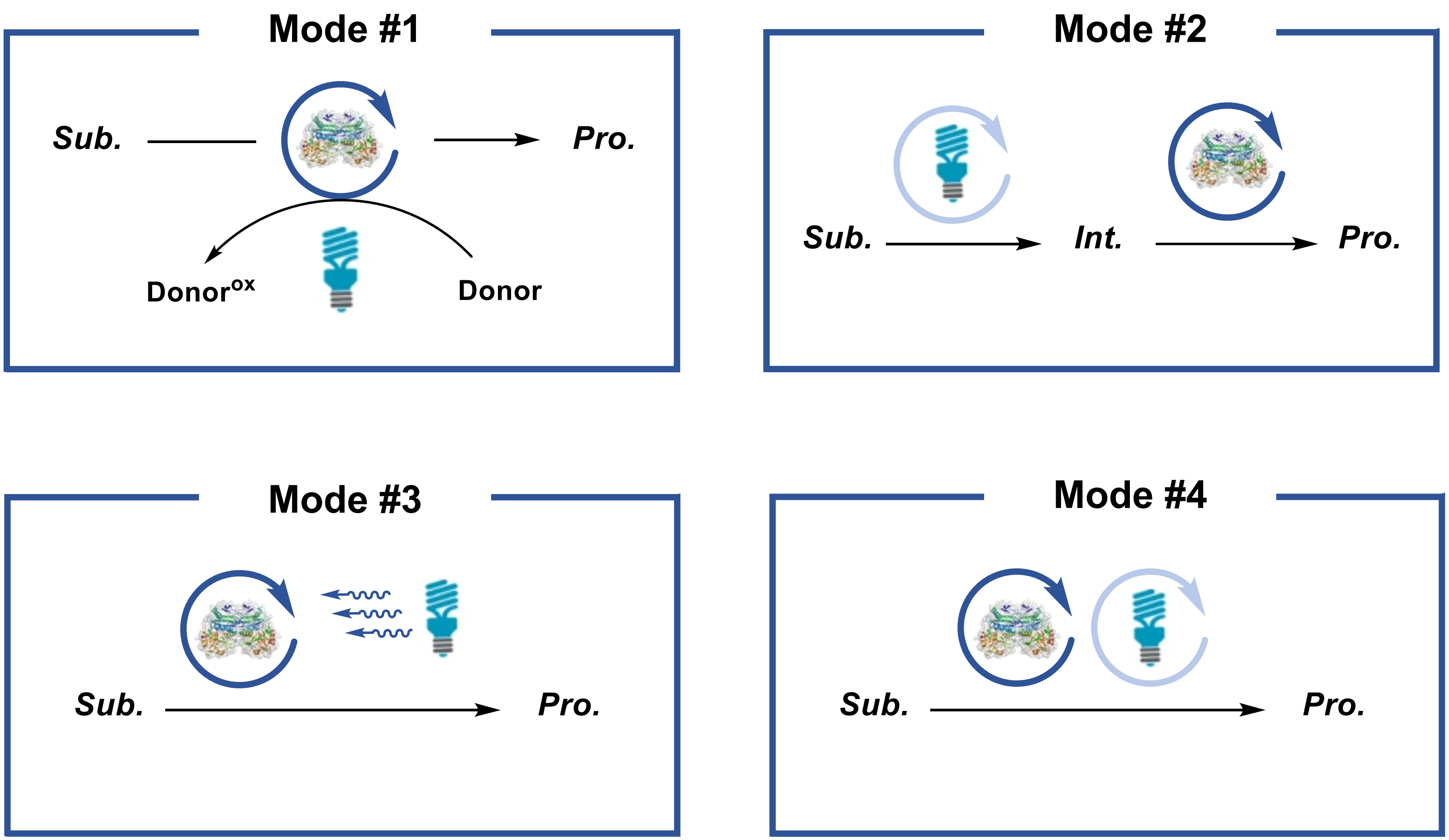
Entry	CvFAP variant	Yield of 2a
1	*Cv*FAP Y466A	13%
2	*Cv*FAP Y466A G462A	48%
3	*Cv*FAP Y466A G462A S574G	87%
4	*Cv*FAP Y466A G462A S574G S464A	89%
5	*Cv*FAP Y466A G462A S574G S464A T131A (***Cv*FAP**$^{\mathbf{AAGAA}}$ = ***Cv*RAP**$_1$)	**91%**
6	*Cv*FAP Y466A G462A S574A	89%
7	*Cv*FAP Y466A G462A S574A S464A	91%
8	*Cv*FAP Y466A G462A S574A S464A T131A (***Cv*FAP**$^{\mathbf{AAAAA}}$ = ***Cv*RAP**$_2$)	**92%**



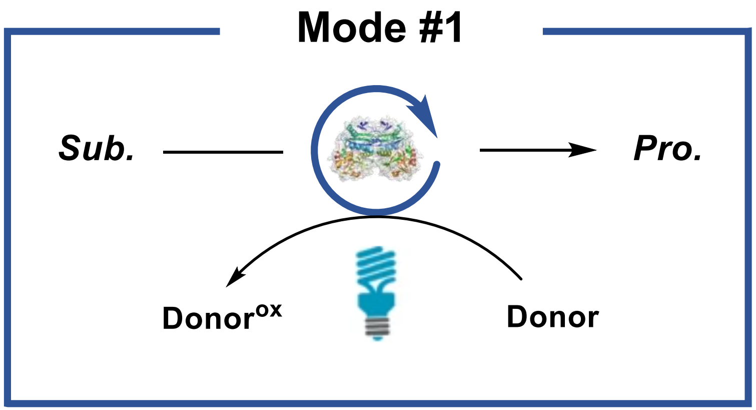
General methods for optimization



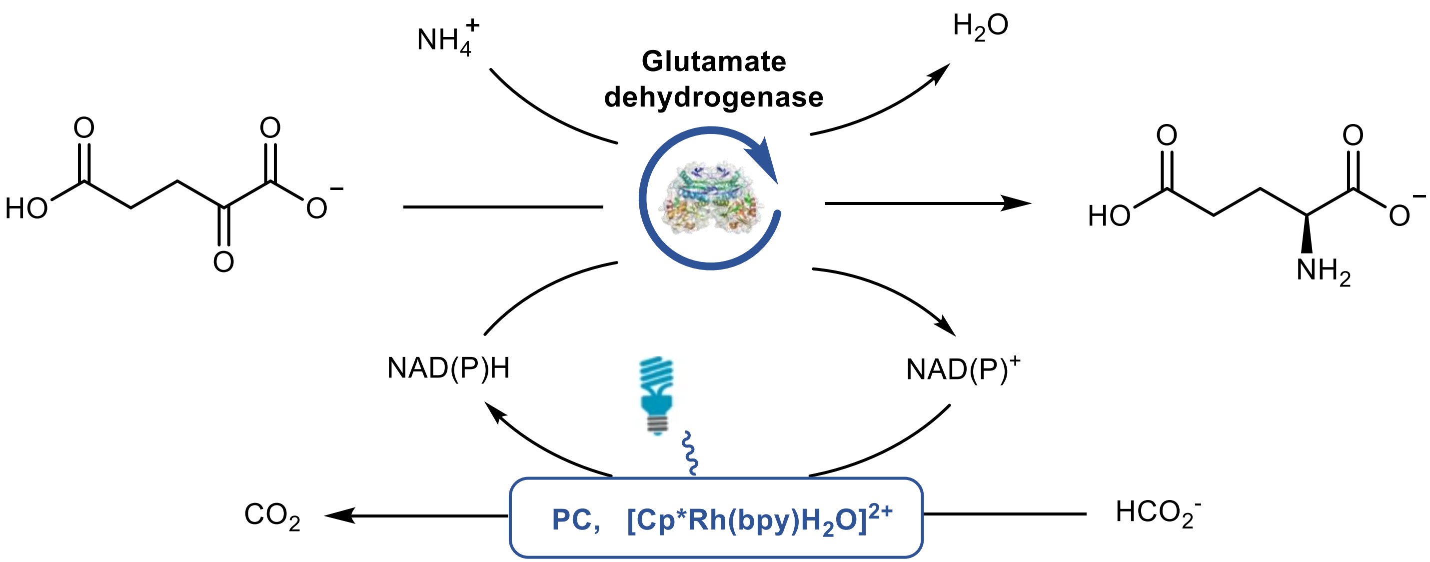
Combining photocatalysis and enzymatic catalysis



Combining photocatalysis and enzymatic catalysis

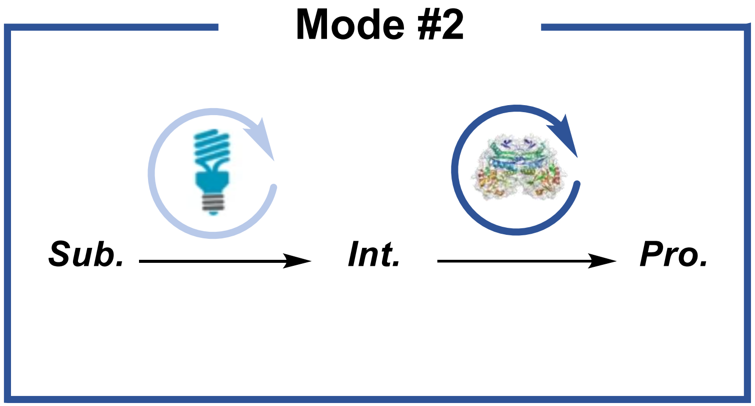


- Replaces the native cofactor regeneration system

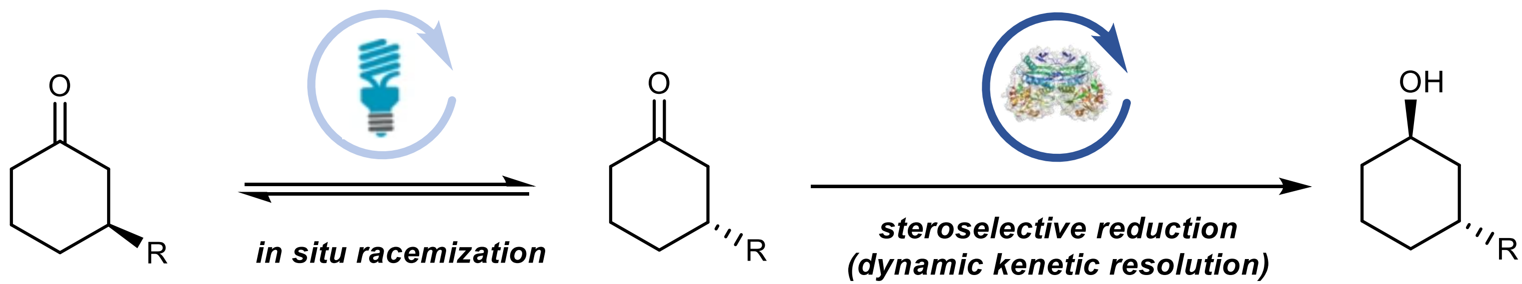


Park, C. B. *et al. Angew. Chem., Int. Ed.* **2018**, *57,* 7958−7985.

Combining photocatalysis and enzymatic catalysis



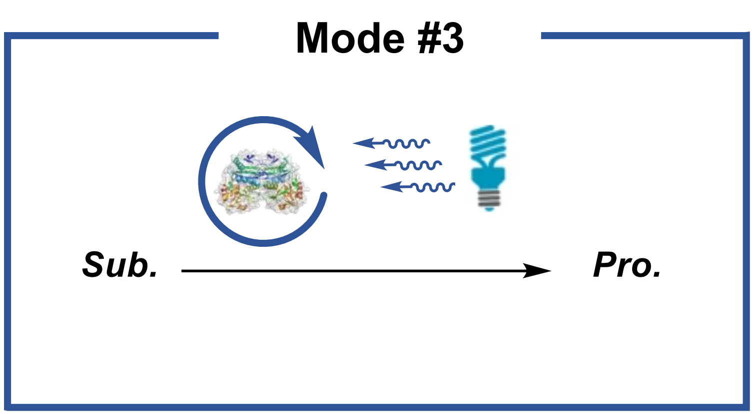
- Catalyzing the cascade respectively

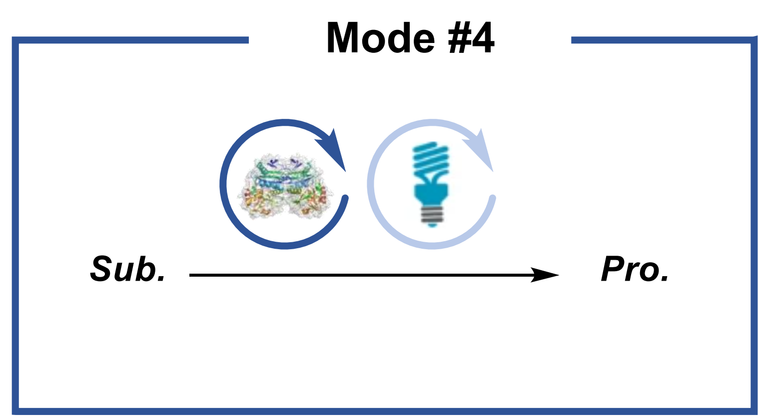


MacMillan, D. W. C. *et al. Science* **2020**, *369*, 1113−1118.

Combining photocatalysis and enzymatic catalysis

- Photocatalyst and enzymes runs separately
- Enzymes work as under thermo conditions





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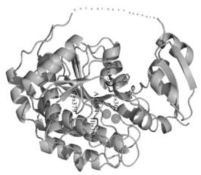
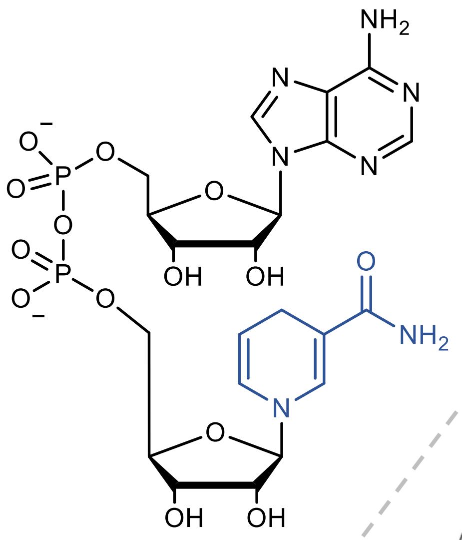
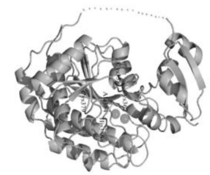
Photosensitive cofactors in nature



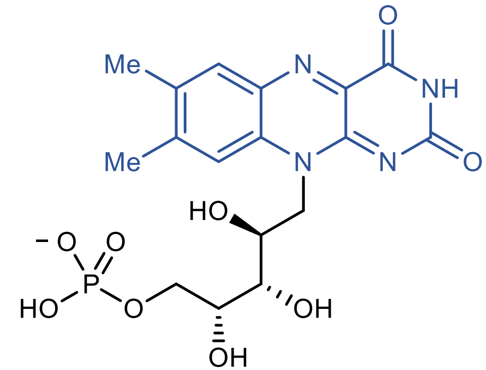
From ketoreductases (KREDs)



Nicotinamide adenine dinucleotide (NADH)

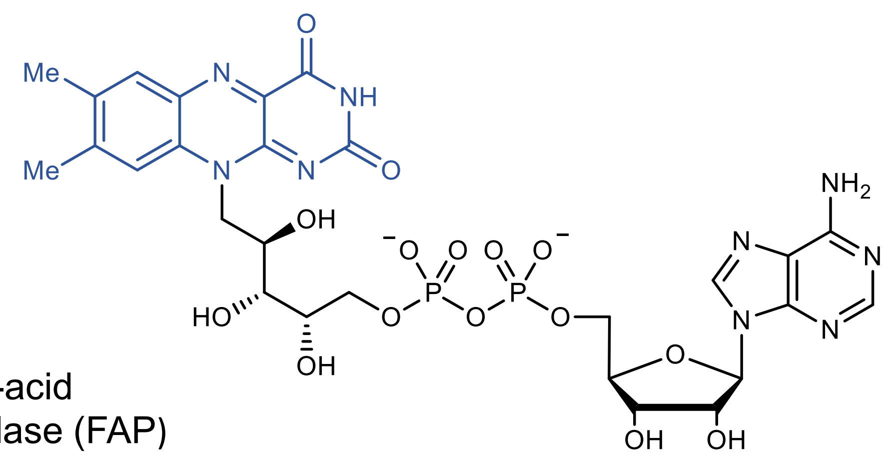


From "ene"-reductases (EREDs)



Flavin mononucleotide (FMN)

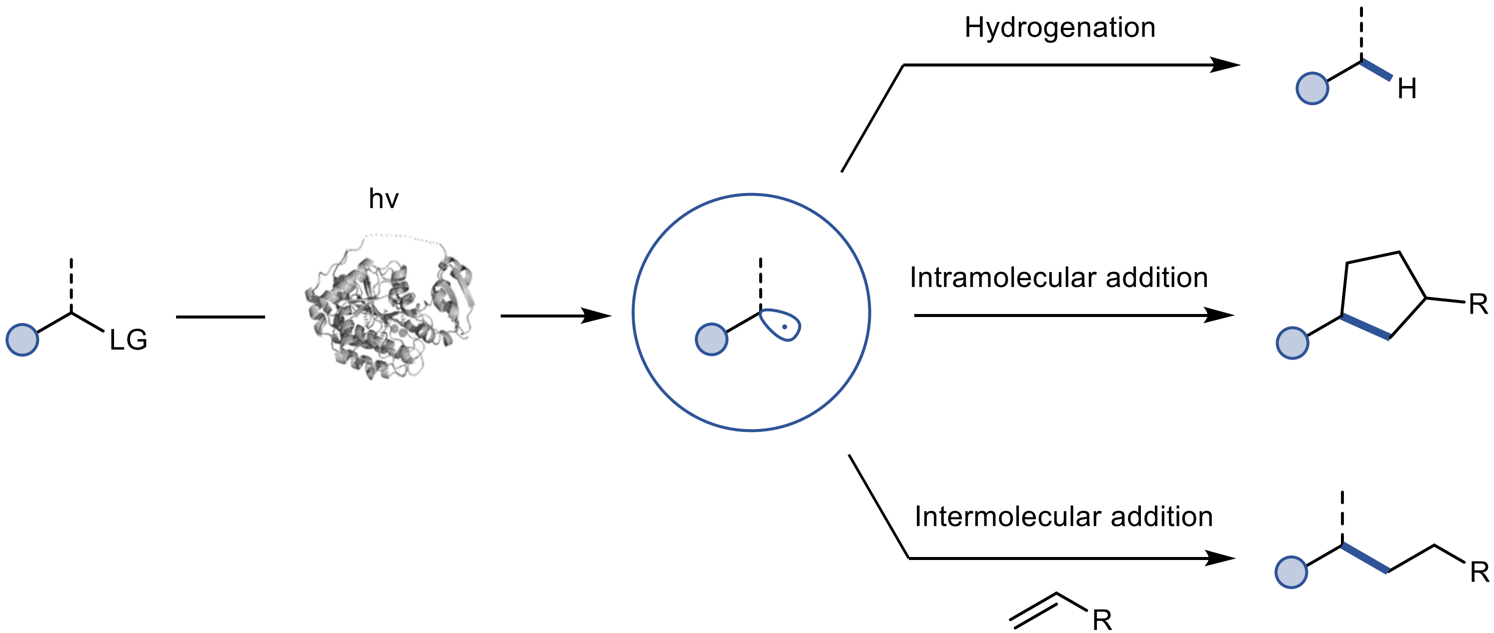
From fatty-acid photodecarboxylase (FAP)



Flavin adenine dinucleotide (FAD)

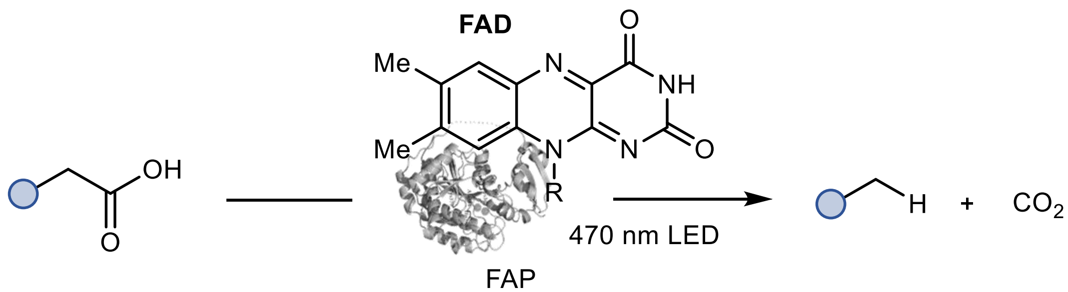
Hyster, T. K. *et al. Chem. Rev.* **2023**, *123,* 5459−5520.

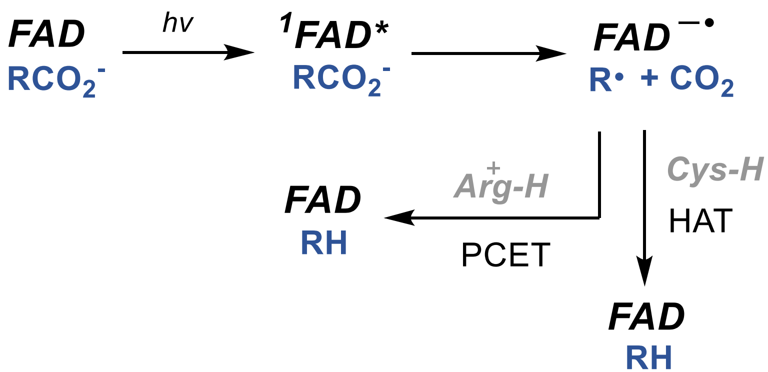
Developed reactions with photoenzyme



■ Stereoselective radical process is rare in these scenarios

Hydrogenation with fatty acid photodecarboxylase (FAP)





- Redox neutral

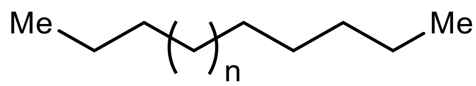
Substrate	Relative Activity (%)
C12:0	8% ± 1
C14:0	48% ± 10
C16:0	**99% ± 8**
C17:0	**96% ± 12**
C18:0	36% ± 3
C22:0	<1

Hyster, T. K. *et al. Chem. Rev.* **2023**, *123,* 5459−5520.

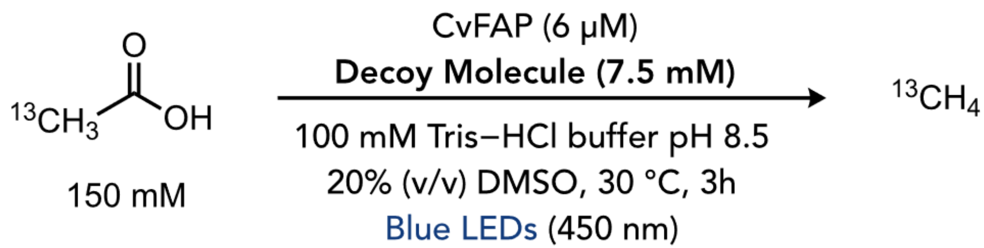
Strategies to improve reactivity of FAP to other substrates

Using decoy

Decoy



- Mimic the conformational change caused by long-chain fatty acid



CvFAP
2.7 ± 0.2 μM

CvFAP + $C_{14}H_{30}$
7.5 ± 0.8 μM

2.5 fold increase

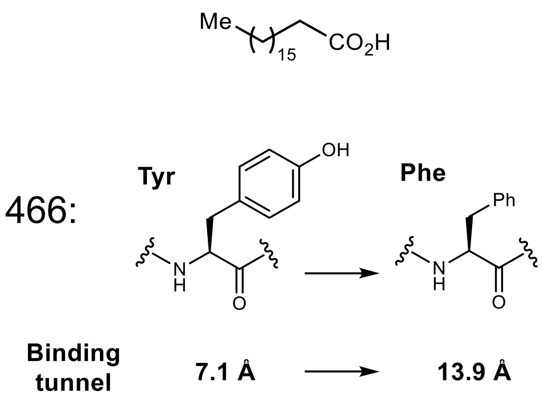
Representative Substrate Scope

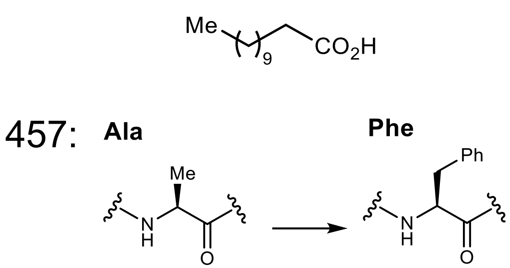
Decoy = C_9H_{20}	**Decoy = $C_{13}H_{28}$**	**Decoy = $C_{14}H_{30}$**	**Decoy = $C_{14}H_{30}$**
2440 ± 62.4 μM	2.1 ± 0.3 μM	9.3 ± 1.2 μM	291.7 ± 14.2 μM
2.8 fold improvement	no activity w/o decoy	no activity w/o decoy	6.0 fold improvement

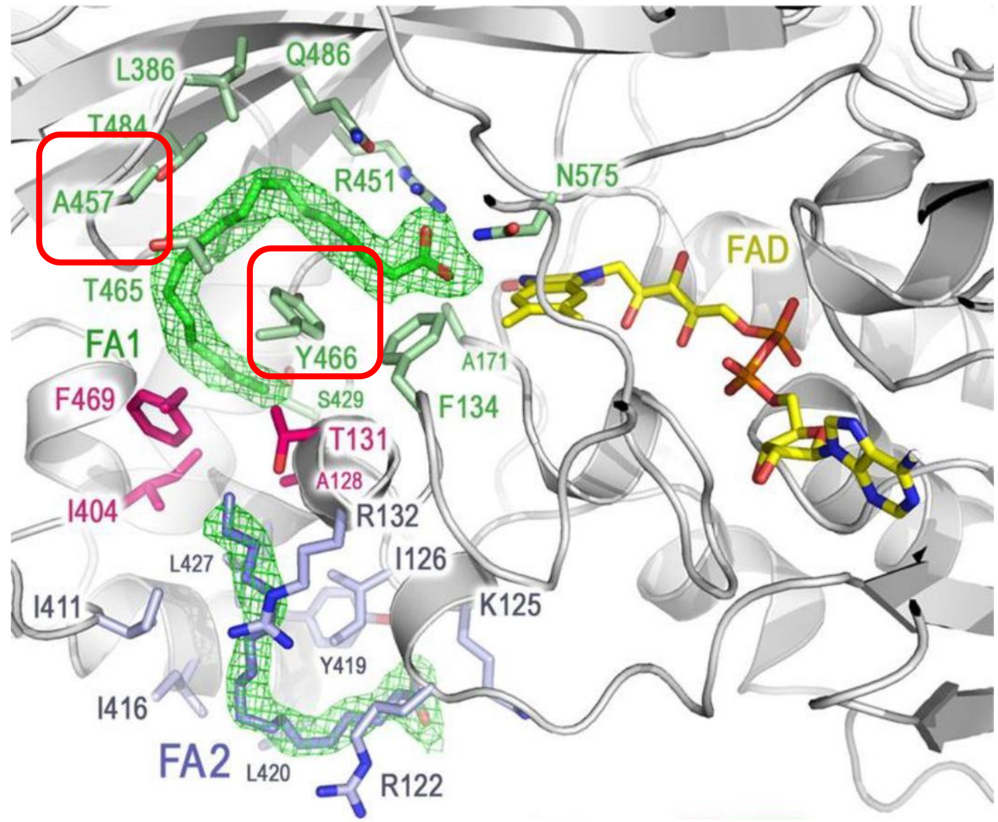
Berkel, W. J. H. *et al. J. Am. Chem. Soc.* **2019**, *141*, 3116−3120.

Strategies to improve reactivity of FAP to other substrates

- Using other type wild-type FAPs
- Using engineered FAP variants



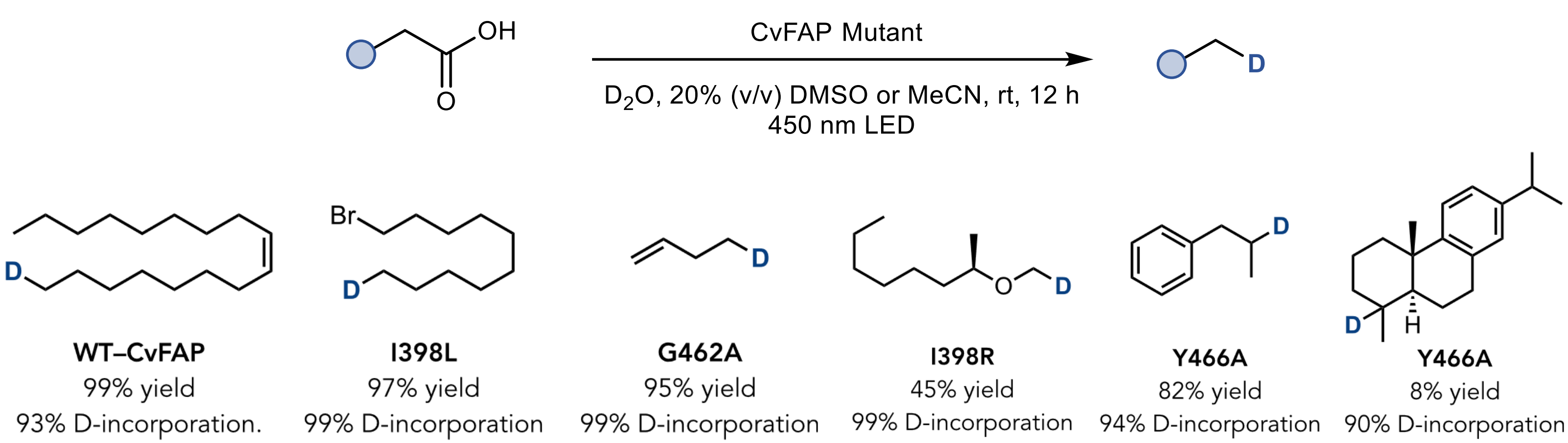




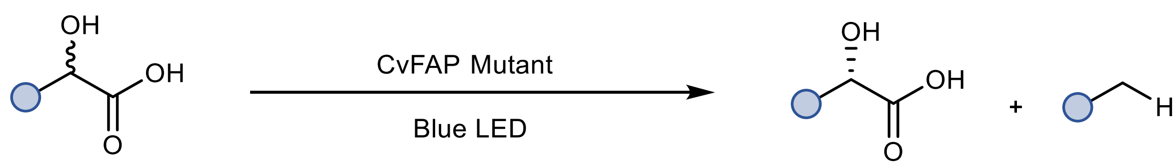
Wu, Q. *et al. Mol. Catal.* **2022**, *524*,112261.

Hydrogenation reactions with FAP

Isotope incorporation

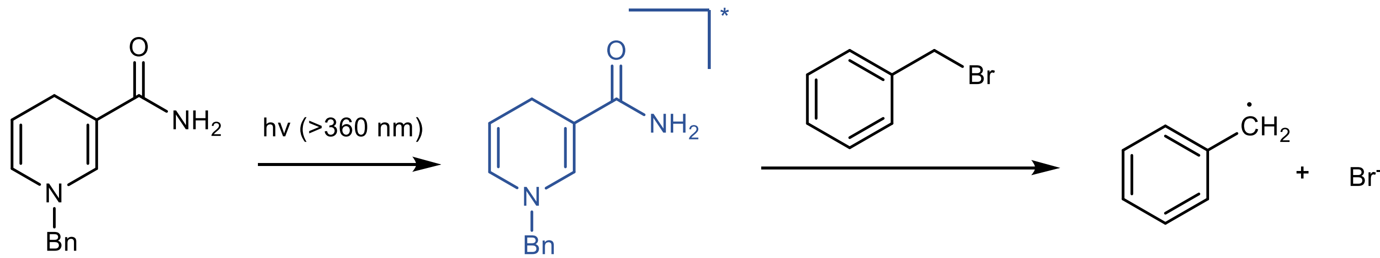


Kinetic resolution



Wu, Q. *et al. Nat. Commun.* **2021**, *12,* 3983.
Wu, Q. *et al. Angew. Chem., Int. Ed.* **2019**, *58*, 8474−8478.

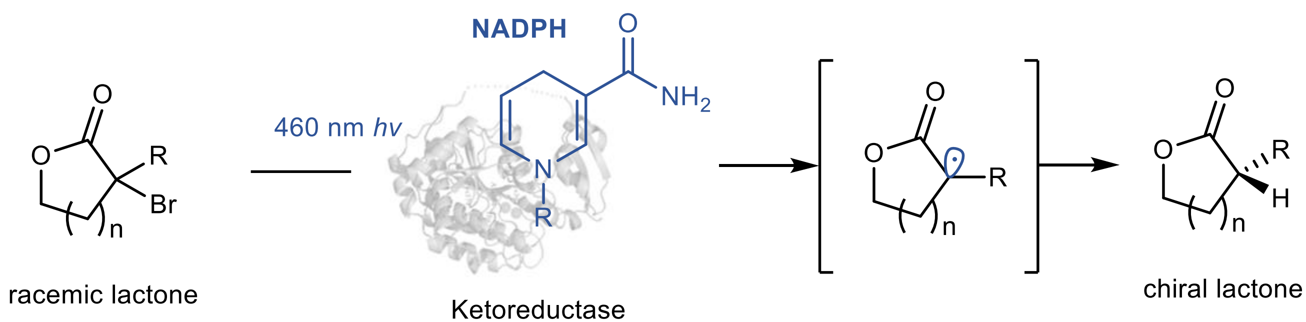
Hydrogenation with nicotinamide-dependent ketoreductase



$E^{ox}_{1/2}$ = 0.57 vs SCE

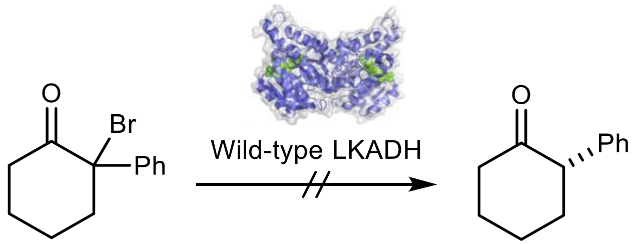
$E^{ox}_{1/2}$ = -2.60 vs SCE

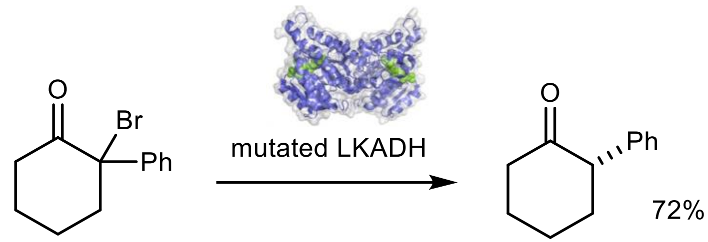
Hypothesis



Tanaka, T. *et al. J. Am. Chem. Soc.* **1983**,*105*, 4722–4727.
Hyster, T. K. *et al. Nature* **2016**, *540*, 414.

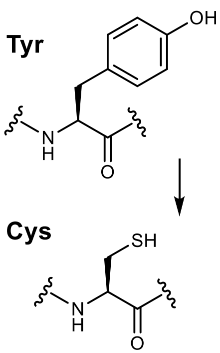
Reactivity of nicotinamide-dependent ketoreductase



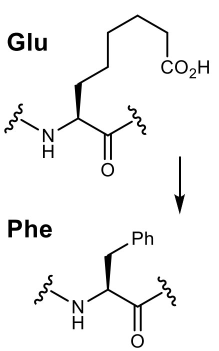


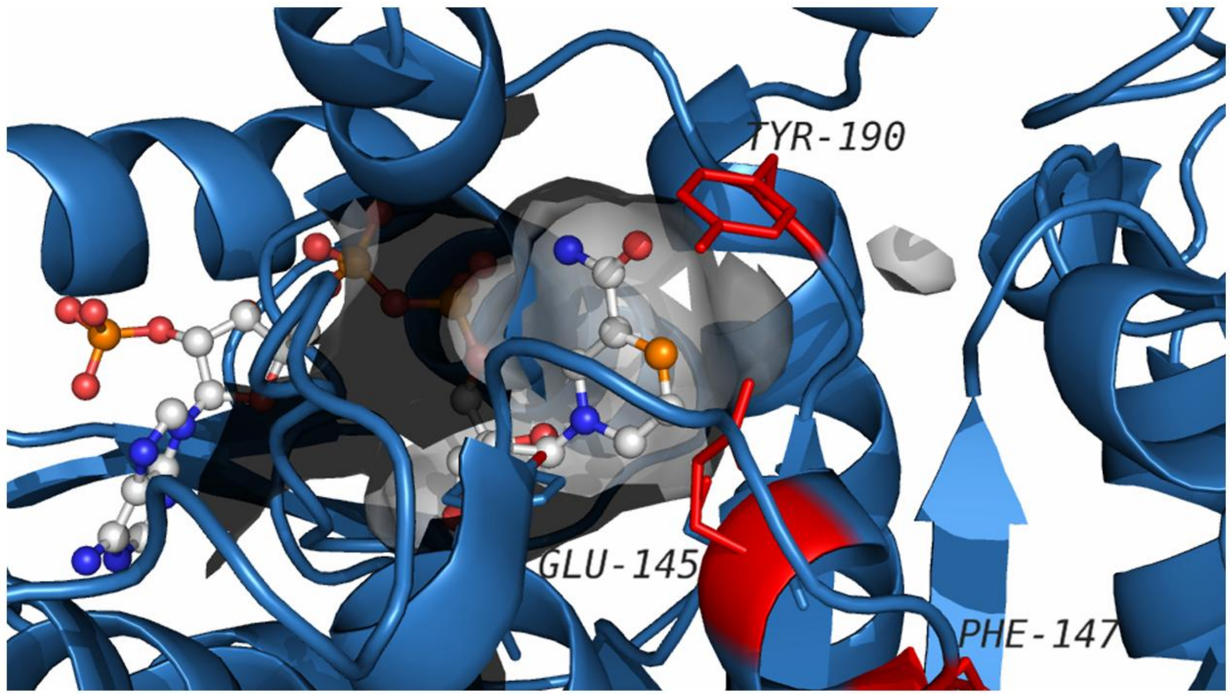
A larger active site

190:



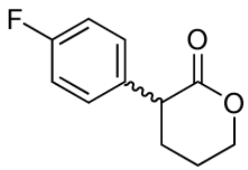
145:





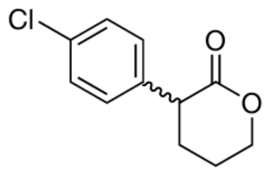
Hyster, T. K. *et al. Nature* **2016**, *540*, 414.

Reactivity of nicotinamide-dependent ketoreductase



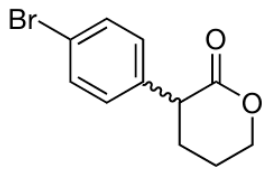
5
Ras-ADH
Yield 47%
e.r. 97/3

LKADH
Yield 91%
e.r. 2/98



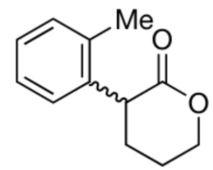
7
Ras-ADH
Yield 39%
e.r. 87/13

LKADH
Yield 86%
e.r. 5/95



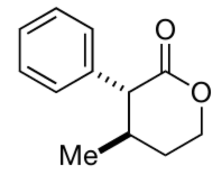
9
Ras-ADH
Yield 22%
e.r. 88/12

LKADH
Yield 35%
e.r. 6/94



11
Ras-ADH
Yield 81%
e.r. 81/19

LKADH
Yield 80%
e.r. 6/94

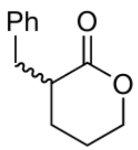


13
Ras-ADH
Yield 79%
e.r. 3/97

LKADH
Yield 56%
e.r. 4/96

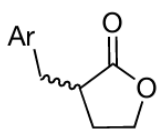
Additional stereocentres

decreased redox potentials



15
Ras-ADH
29% yield
e.r. 80/20

LKADH
80% yield
e.r. 4/96

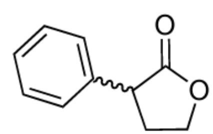


Ar = C_6H_4(3-OMe)

21
Ras-ADH
Yield 30%
e.r. 75/25

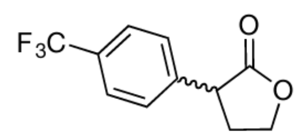
LKADH
Yield 50%
e.r. 12/88

γ-lactones (with KRED-3)



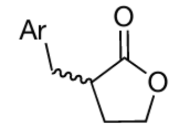
17
Ras-ADH
Yield 82%
e.r. 81/19

LKADH
Yield 74%
e.r. 9/91



19
Ras-ADH
Yield 26%
e.r. 84/16

LKADH
Yield 78%
e.r. 10/90



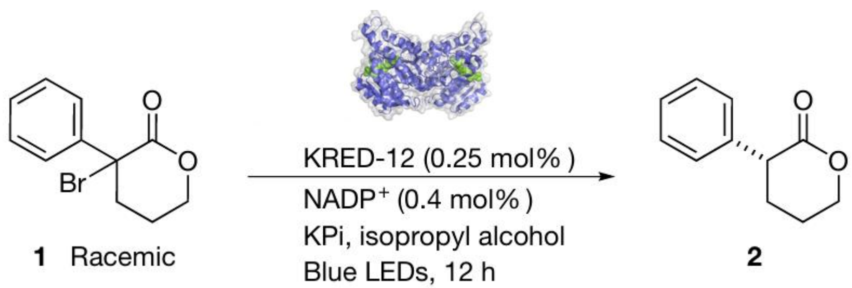
Ar = C_6H_4(3-OMe)

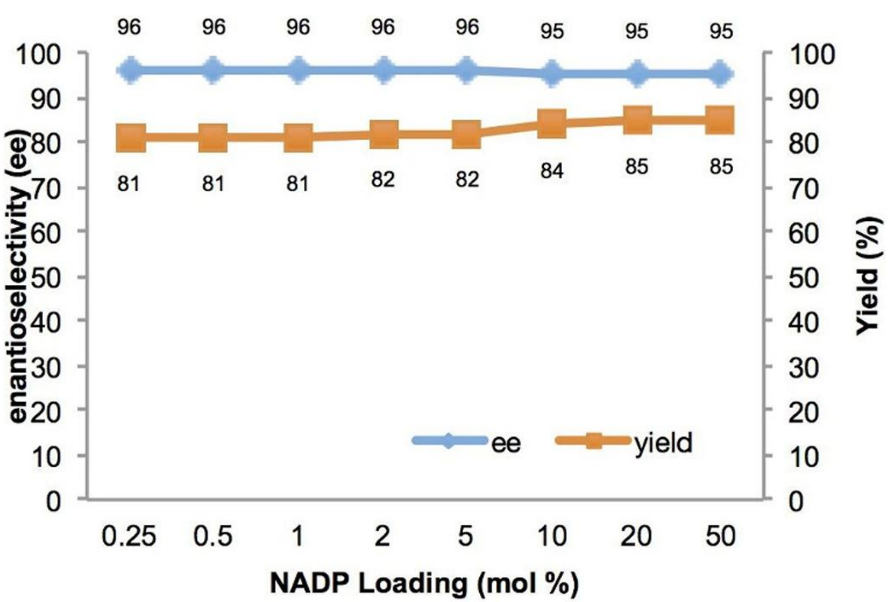
21
Ras-ADH
Yield 30%
e.r. 75/25

LKADH
Yield 50%
e.r. 12/88

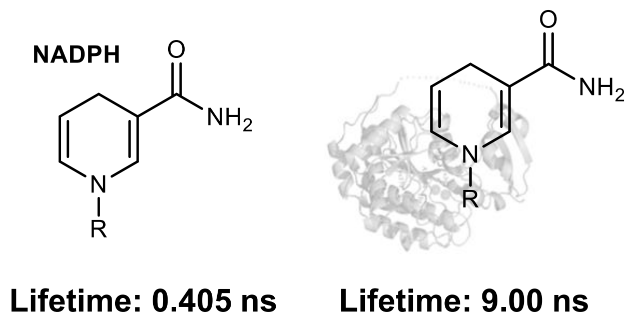
Hyster, T. K. *et al. Nature* **2016**, *540*, 414.

Nicotinamide-Dependent Ketoreductase

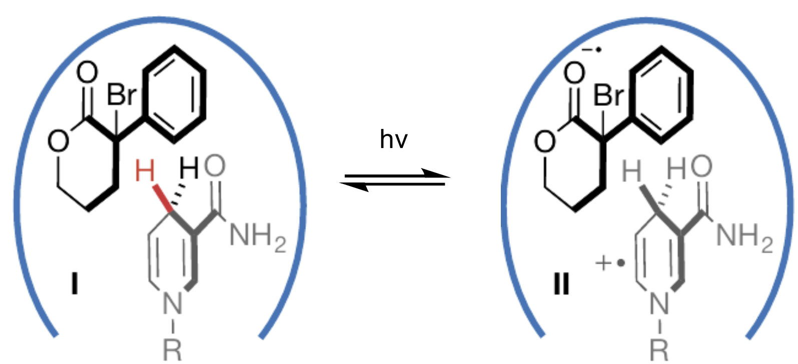




- NADPH is stabilized by the protein

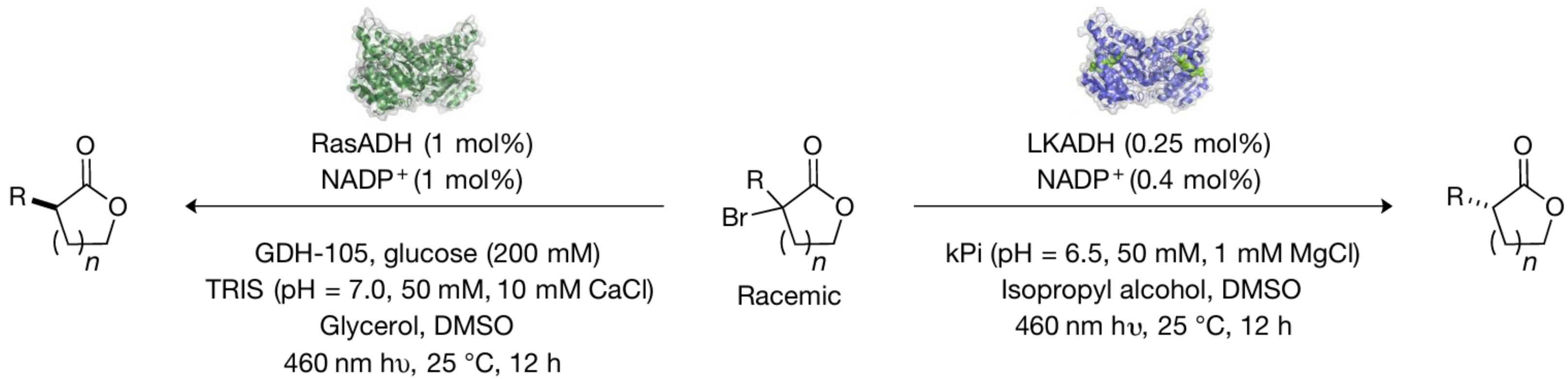


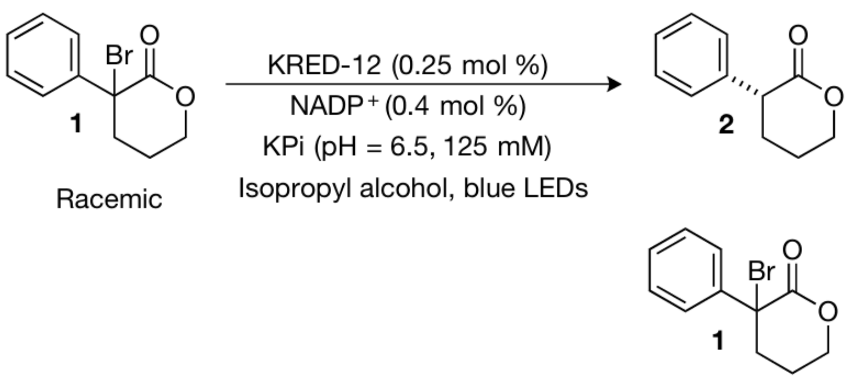
- Cofactor-substrate complex



Hyster, T. K. *et al.* *Nature* **2016**, *540*, 414.

Stereoselectivity

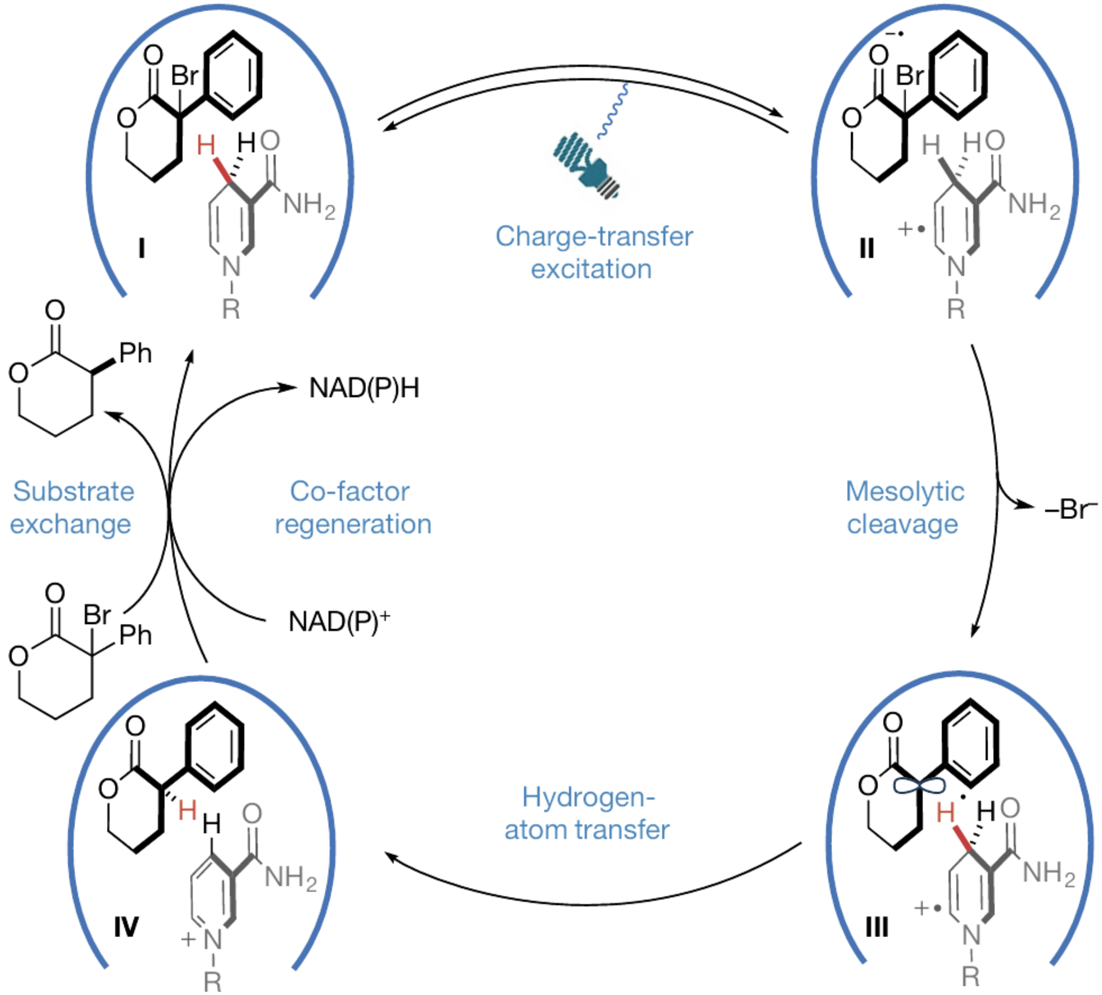




Time (min)	Product	Recovered starting material
30	Yield 17% e.r. 95/5	Yield 79% e.r. 54/46
60	Yield 34% e.r. 96/4	Yield 65% e.r. 54/46
120	Yield 50% e.r. 97/3	Yield 50% e.r. 53/47

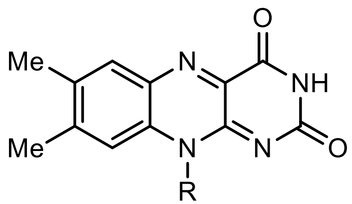
Hyster, T. K. *et al. Nature* **2016**, *540*, 414.

Mechanism

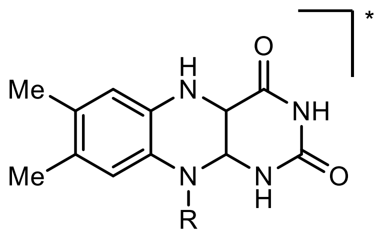


Hyster, T. K. *et al. Nature* **2016**, *540*, 414.

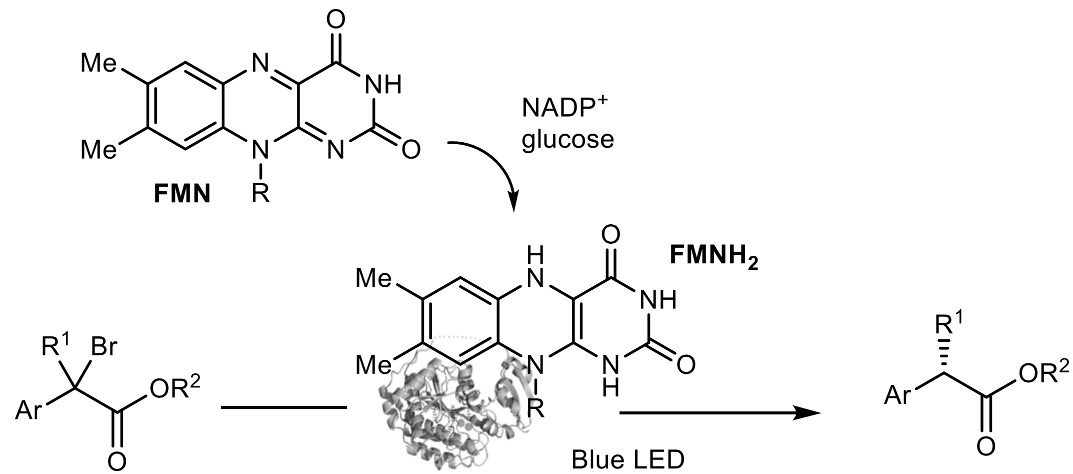
Hydrogenation with flavin-dependent "ene"-reductase



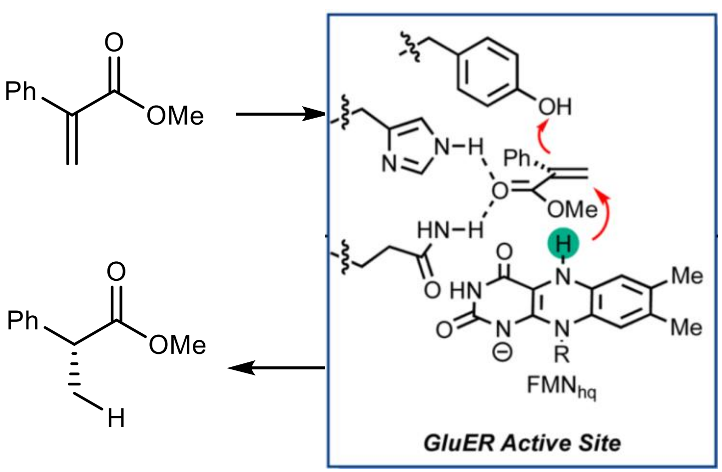
FMN $E^*_{1/2}$ = - 0.45 V (vs SCE)

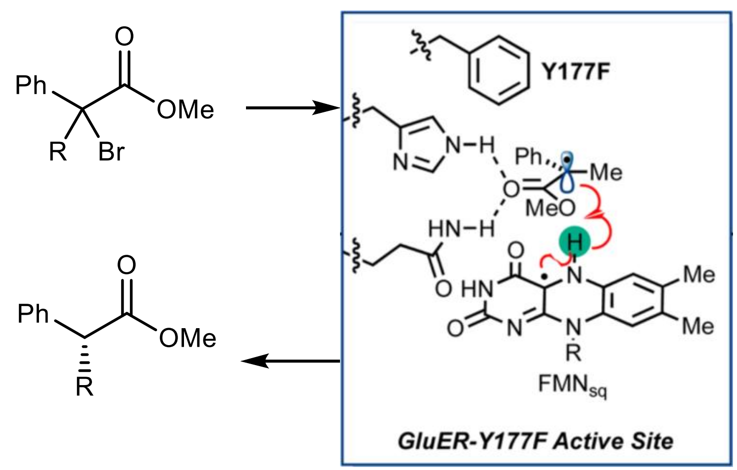


$FMNH_2$ $E^*_{1/2}$ = - 2.26 V (vs SCE)



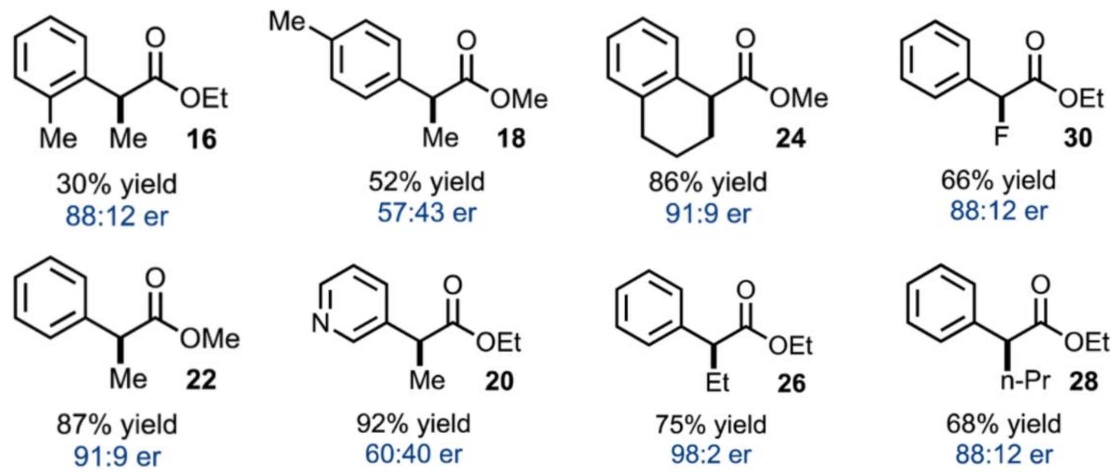
- Mutate Y177 to improve stereoselectivity



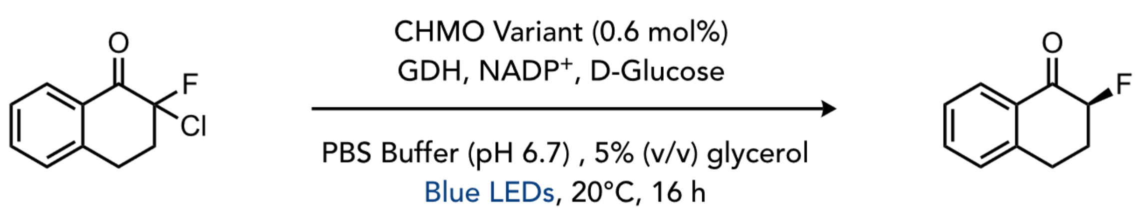


Hyster, T. K. *et al. J. Am. Chem. Soc.* **2017**, *139,* 11313−11316.

Hydrogenation with flavin-dependent “ene”-reductase



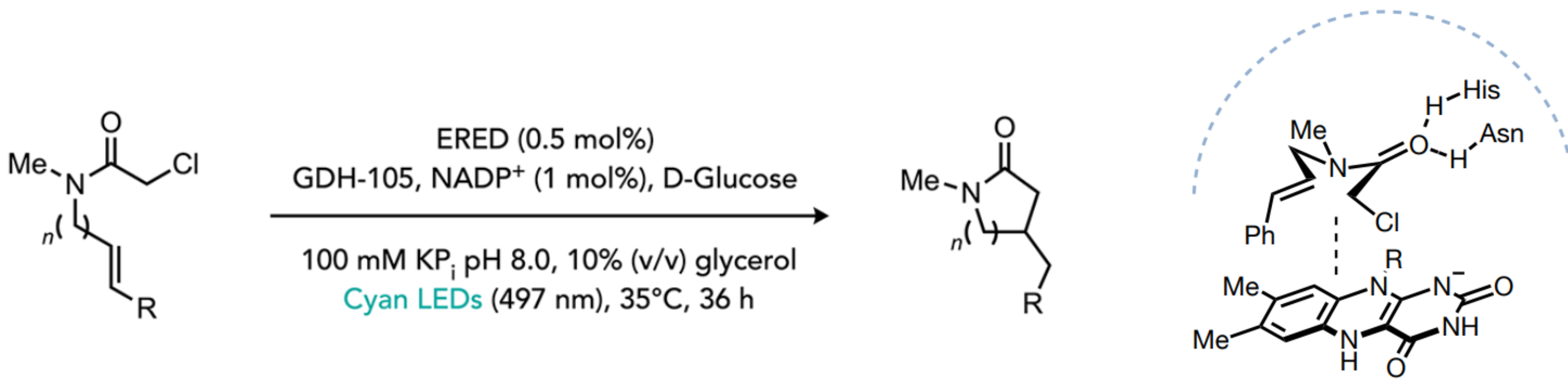
Hydrogenation of α-fluoro ketone



Hyster, T. K. *et al. J. Am. Chem. Soc.* **2017**, *139,* 11313−11316.

Cyclization with flavin-dependent "ene"-reductase

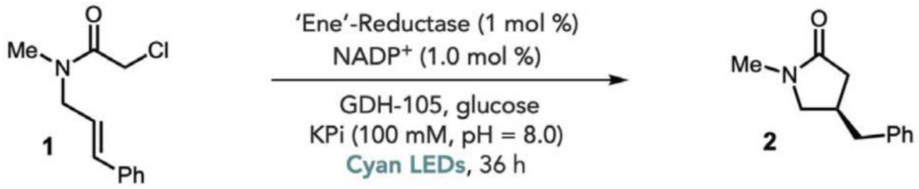
- From reduction to cyclization
  - Prevent undesired hydrogenation
  - Large, substrate-promiscuous active sites needed
  - Realizing stereoselective intramolecular radical addition



- ERED features a large active pocket

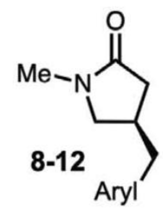
Hyster, T. K. *et al. Science*. **2019**, *364*, 1166−1169.

Cyclization with flavin-dependent "ene"-reductase



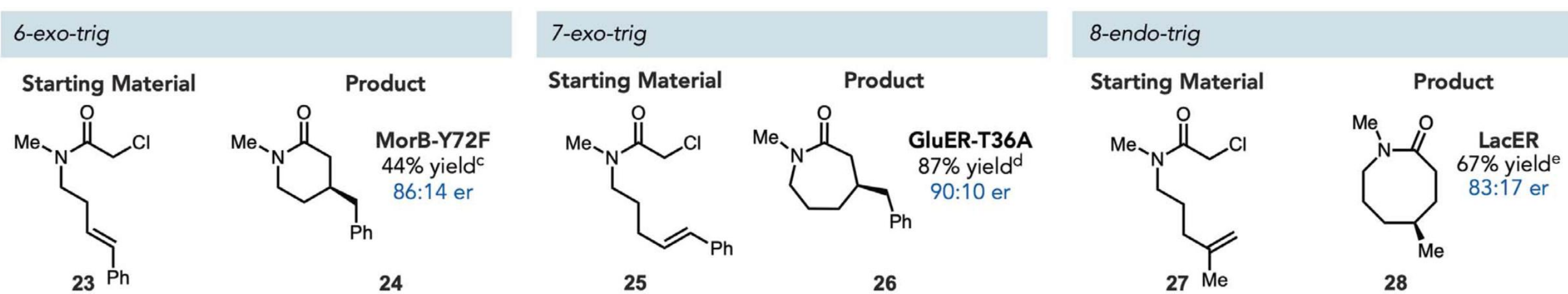
entry	'Ene'-reductase	yield (%)	er
1	GluER	68	94:6
2	OYE1	34	31:69
3	GluER-T36A	89	94:6
4[a]	GluER-T36A (0.42 mol %)	92	94:6

- Cause no structural change in active site



8-12

Aryl	GluER-T36A
4-OMeC_6H_4	91% yield, 96:4 er
3-OMeC_6H_4	73% yield, 95:5 er
2-OMeC_6H_4	84% yield, 95:5 er
4-BrC_6H_4	80% yield, 95:5 er
4-$CF_3C_6H_4$	98% yield, 96:4 er



Hyster, T. K. *et al. Science*. **2019**, *364*, 1166−1169.

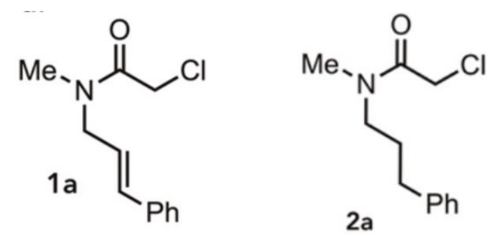
Cyclization with flavin-dependent "ene"-reductase

Enzyme	Quantum Yield
GluER-WT	0.024
GluER-T36A	0.068
GluER-G6	0.105

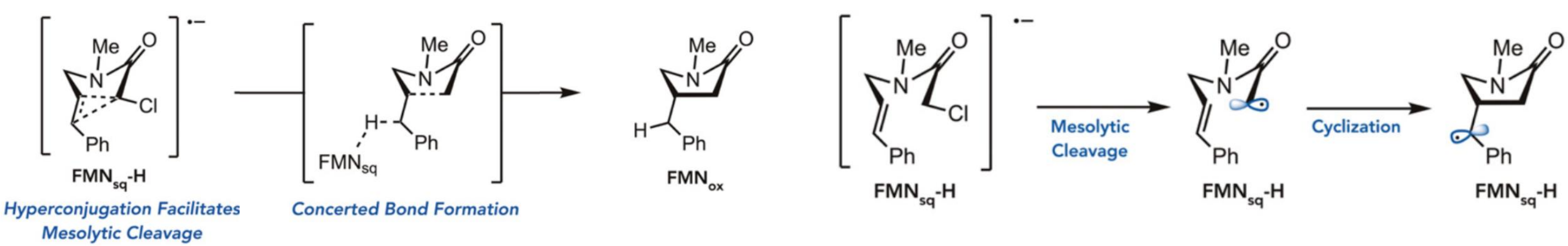
ERED Variant	Semiquinone Lifetime	Isotope Incorporation
GluER-WT	710 ps	80%
GluER-G6	20 ps	89%

ERED Variant	Substrate	Radical Pair Lifetime
GluER-WT	1a	2.8 ps
GluER-G6	1a	0.8 ps
GluER-WT	2a	4.3 ps
GluER-G6	2a	3.2 ps

- **GluER-G6 = GluER-T36A-K317M-Y343F**



- Indicating a concerted mechanism for GluER-G6
- Interaction between σ* C-Cl and π-electrons of the alkene

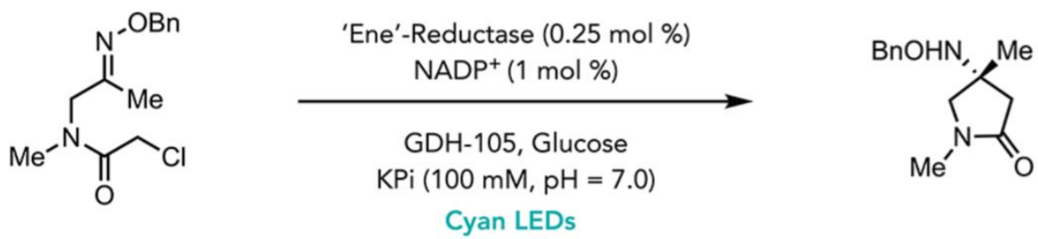


Hyster, T. K. *et al. Angew. Chem., Int. Ed.* **2021**, e202113842.

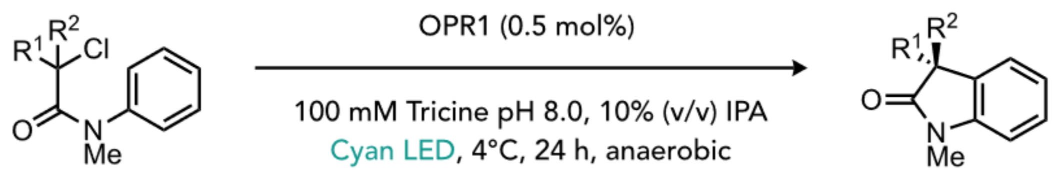
Cyclization with flavin-dependent “ene”-reductase

- Other type intramolecular addition

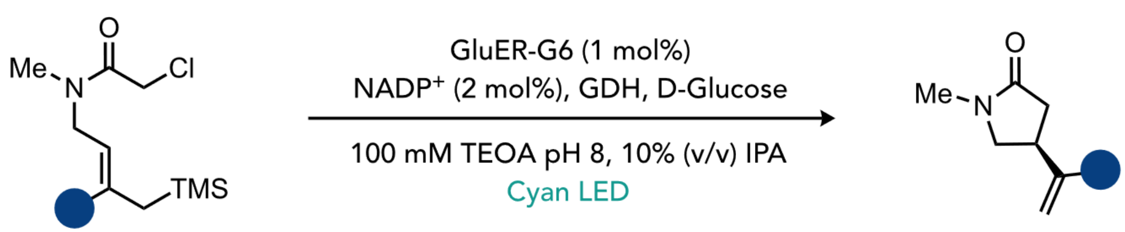
- with oxime



- with arene



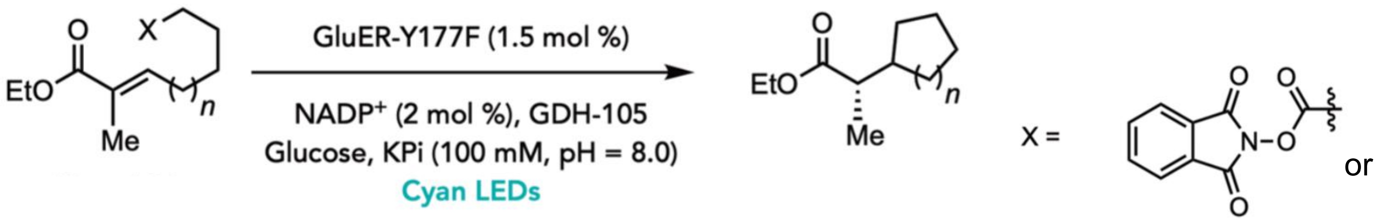
- with a tandem *β*-session



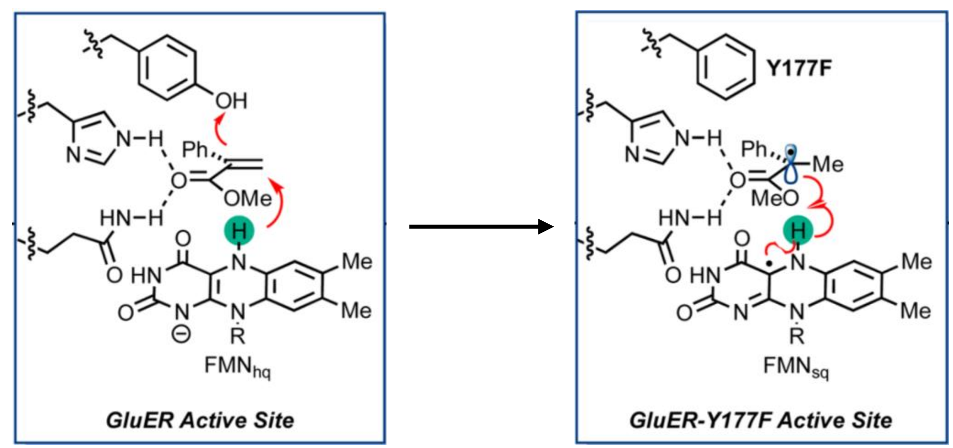
Hyster, T. K. *et al. Nat. Chem.* **2020**, *12*, 71−75.
Hyster, T. K. *et al. J. Am. Chem. Soc.* **2021**, *143*, 19643−19647.
Hyster, T. K. *et al. ACS Catal*. **2022**, *12*, 9801−9805.

Cyclization with flavin-dependent "ene"-reductase

- Other type intramolecular addition

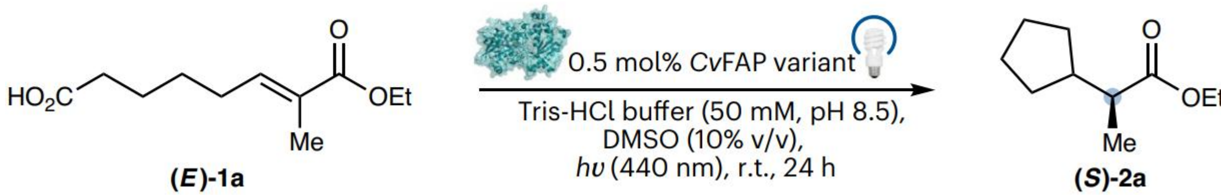


- Prevent direct reduction

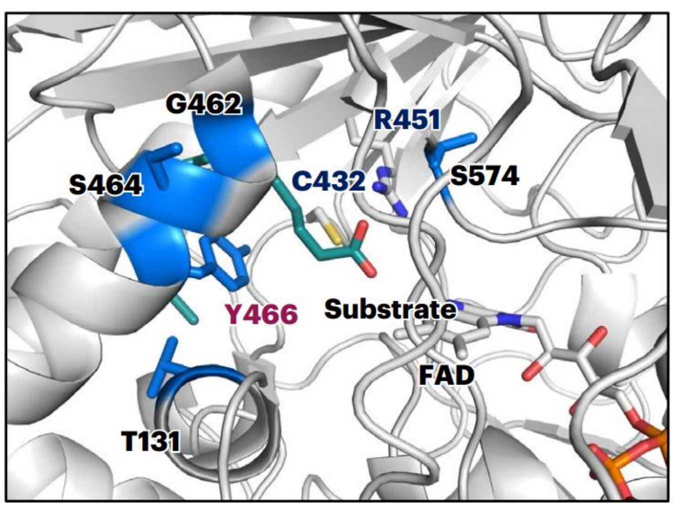


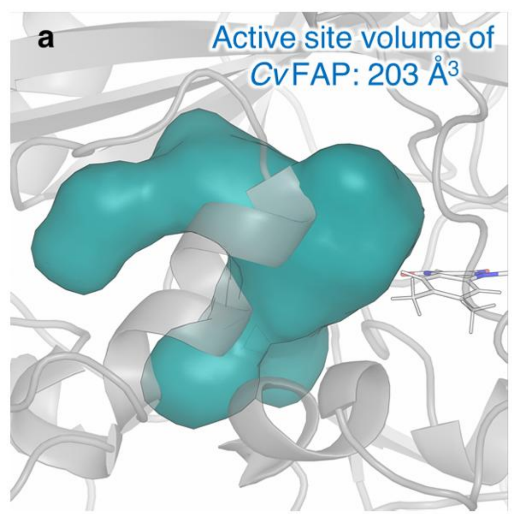
Hyster, T. K. *et al. J. Am. Chem. Soc.* **2020**, *142,* 15673−15677.

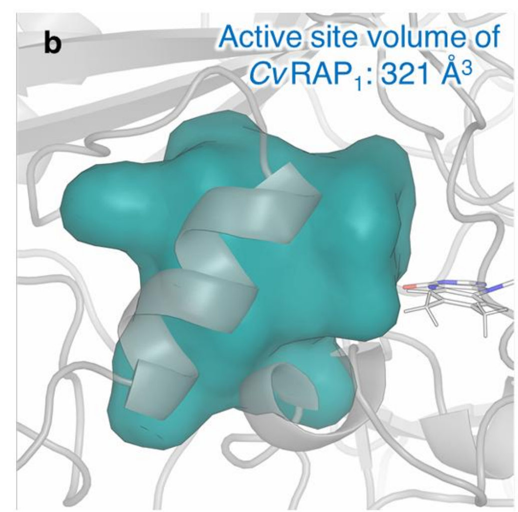
Cyclization with FAP



- Prevent the premature HAT/PCET
- Limited stability of natural FAPs
- The mechanism of HAT/PCET remains unclear

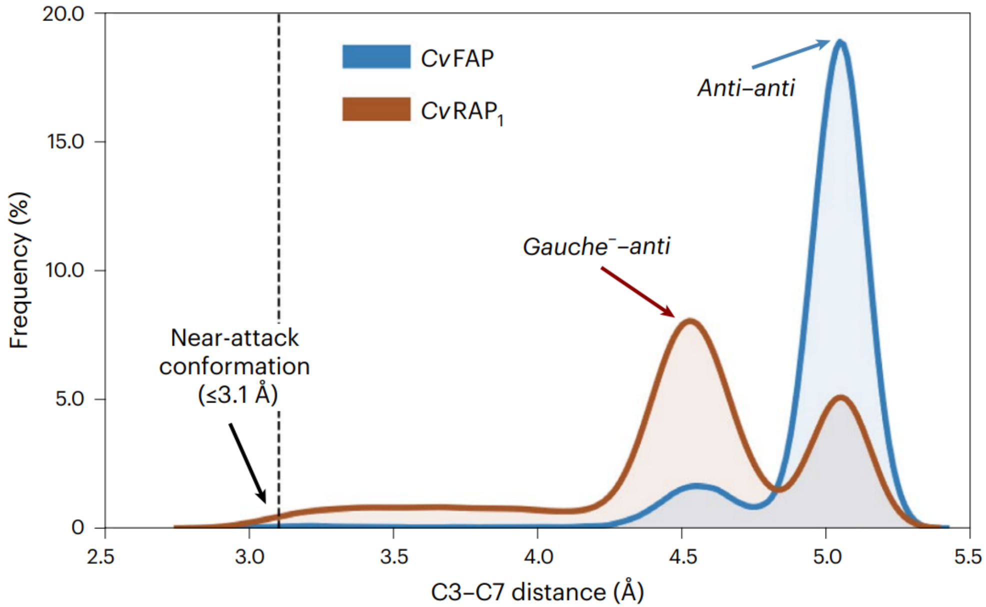


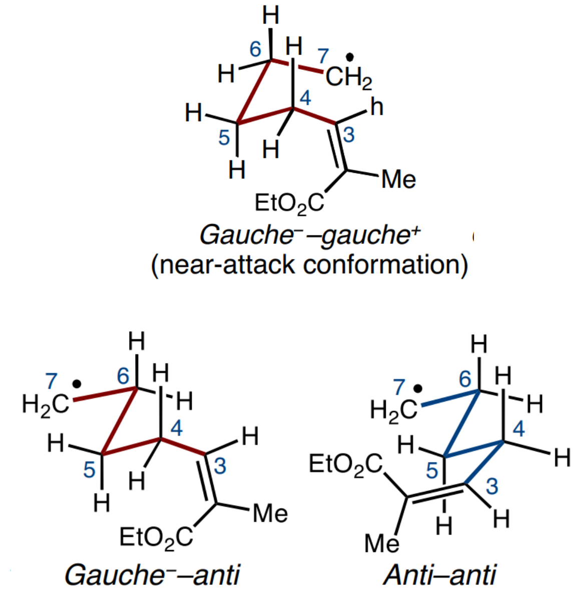


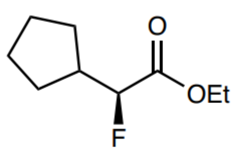


Yang, Y. *et al. Nat. Chem.* **2024**, *doi.org/10.1038/s41557-024-01494-0*

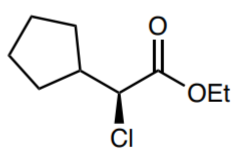
Cyclization with FAP



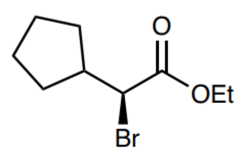




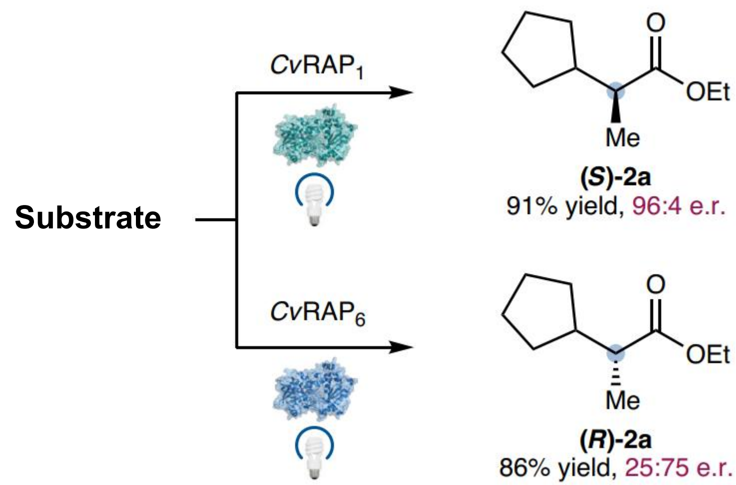
2f
42% yield[c]
94:6 e.r.



2g
71% yield[c]
94:6 e.r.



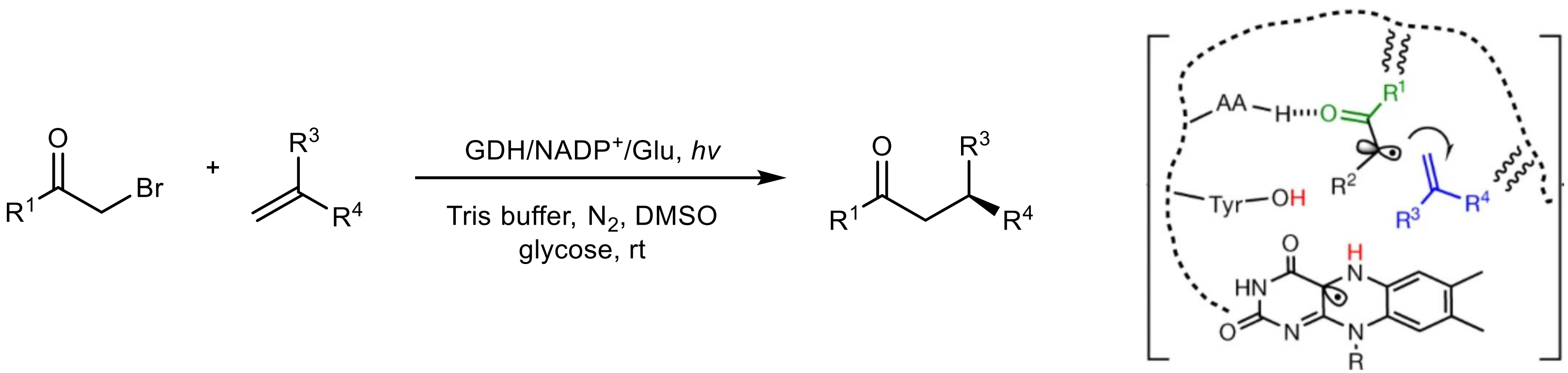
2h
66% yield[c]
92:8 e.r.



Yang, Y. *et al. Nat. Chem.* **2024**, *doi.org/10.1038/s41557-024-01494-0*

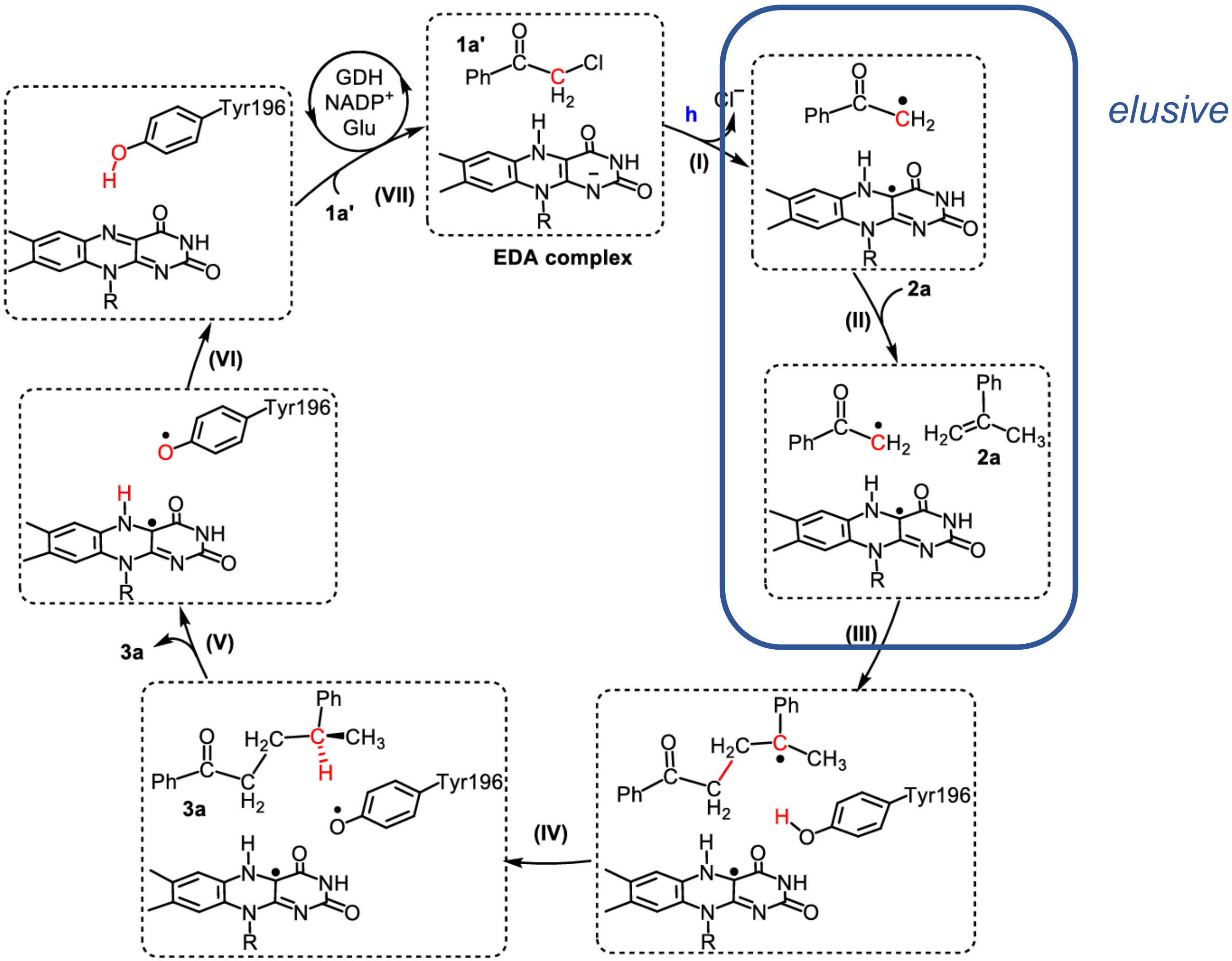
Intermolecular addition with flavin-dependent "ene"-reductase

- From intramolecular to intermolecular
  - Initially generated electrophilic radical needs to be long-lived.
  - Both substrates are accessible to the enzyme active site and appear at applicable positions.
  - Stereochemical induction is more difficult because of the flexible prochiral center.



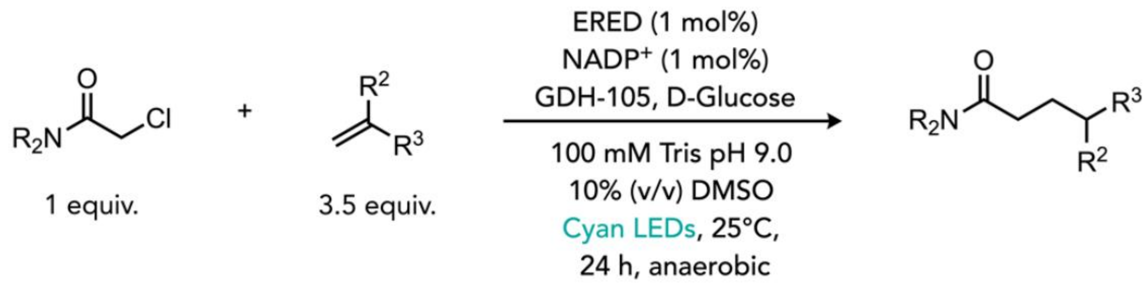
Zhao, H. *et al. Nature* **2020**, *584,* 69–74.

Intermolecular addition with flavin-dependent “ene”-reductase

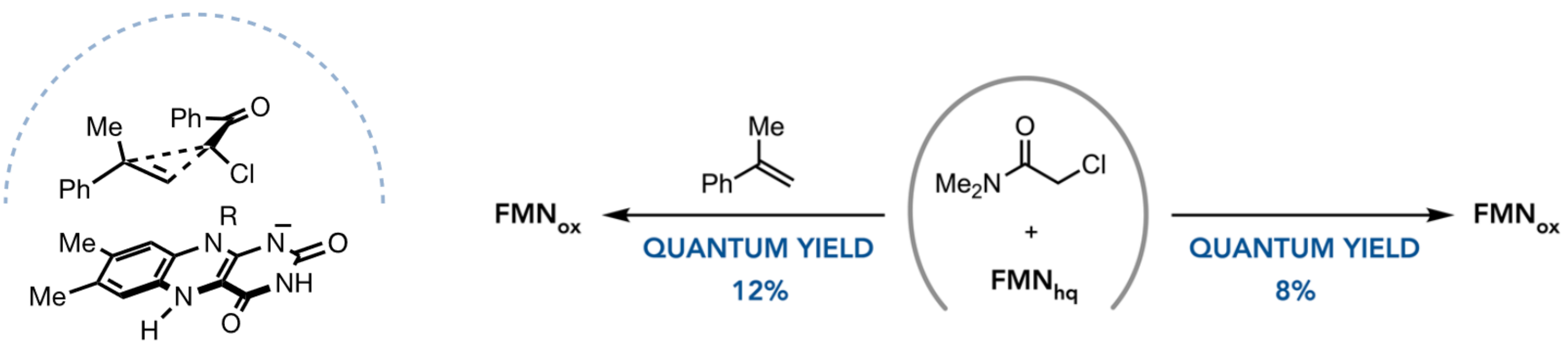


- Tyr196 account for remote stereoselectivity

Intermolecular addition with flavin-dependent "ene"-reductase



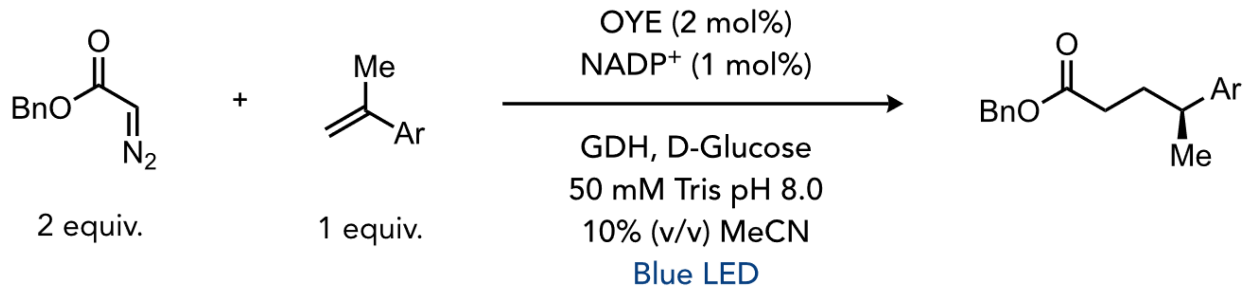
- To ensure radical formation only occurs in the presence of alkene
- GluER-T36A was used



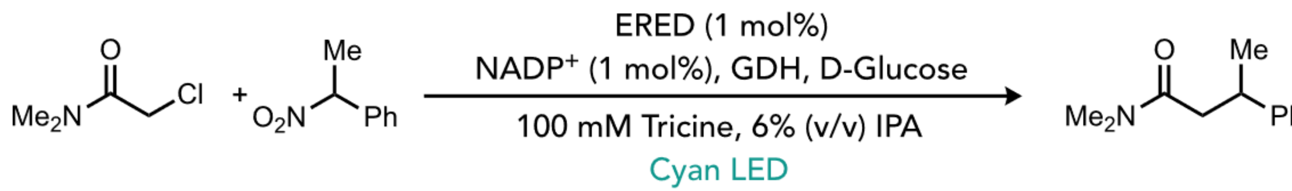
Hyster, T. K. *et al. J. Am. Chem. Soc.* **2021,** *143,* 97−102.

Intermolecular addition with flavin-dependent "ene"-reductase

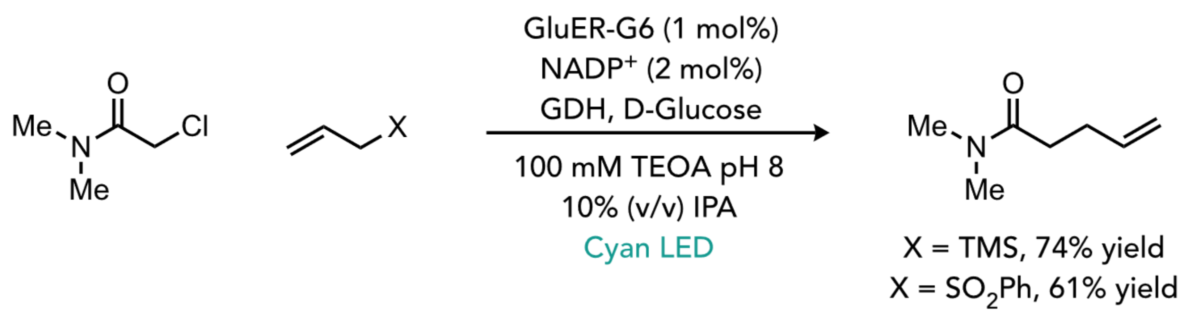
- with azine



- with sp^3-C



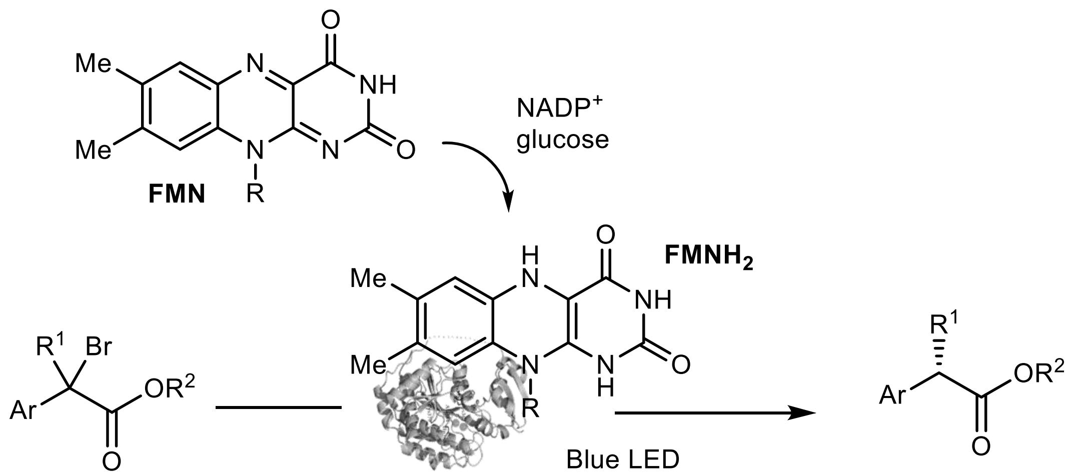
- with a tandem *β*-session

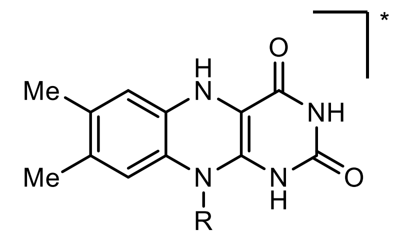


Xu, J. *et al. Angew. Chem., Int. Ed.* **2023**, *62*, e202214135.
Hyster, T. K. *et al. Nature* **2022**, *610*, 302−307.
Hyster, T. K. et al. *ACS Catal.* **2022**, *12*, 9801−9805.

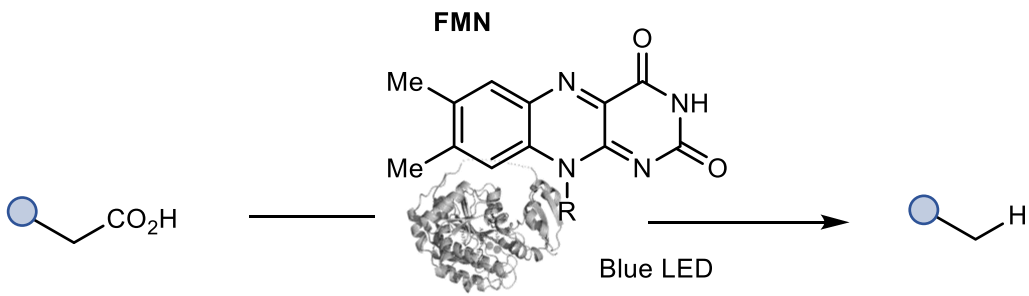
Direct excitation of FMN

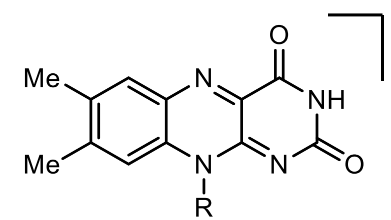
Previous work





$FMNH_2$ $E^*_{1/2}$ = - 2.26 V (vs SCE)



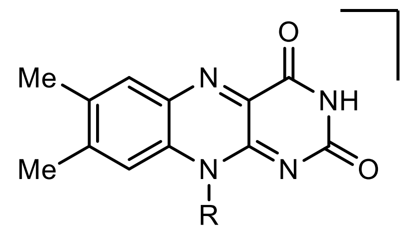


FMN $E^*_{1/2}$ = + 1.76 V (vs SCE)

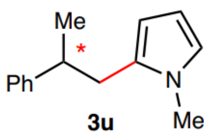
Huang, X. *et al. Nat. Catal.* **2023**, *6,* 996–1004.

Direct excitation of FMN

$E_{1/2}$ = +1.42 V

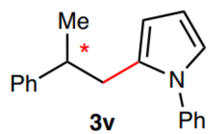


FMN $E^*_{1/2}$ = + 1.76 V (vs SCE)



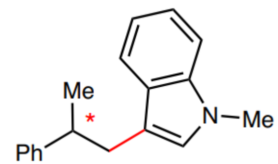
3u

ER1: 79% y, 91.5:8.5 er
ER3: 83% y, 6.5:93.5 er



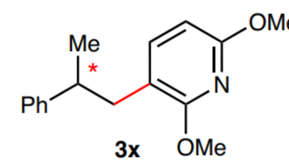
3v

ER1: 17% y, 87.5:12.5 er
ER3: 23% y, 39:61 er



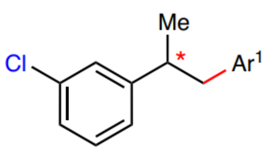
3w

ER1: 21% y, 94:6 er
ER3: 20% y, 2:98 er



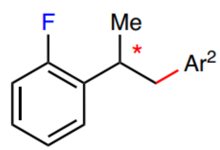
3x

ER1: 18% y, 99:1 er
ER3: 22% y, 4:96 er



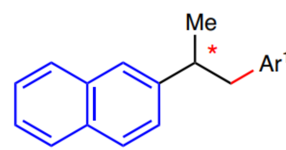
3h

ER2: 50% y, 91.5:8.5 er
ER4: 55% y, 4:96 er



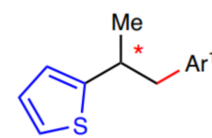
3i

ER1: 99% y, 95.5:4.5 er
ER3: 88% y, 15:85 er



3j

ER2: 46% y, 58:42 er
ER4: 51% y, 4:96 er



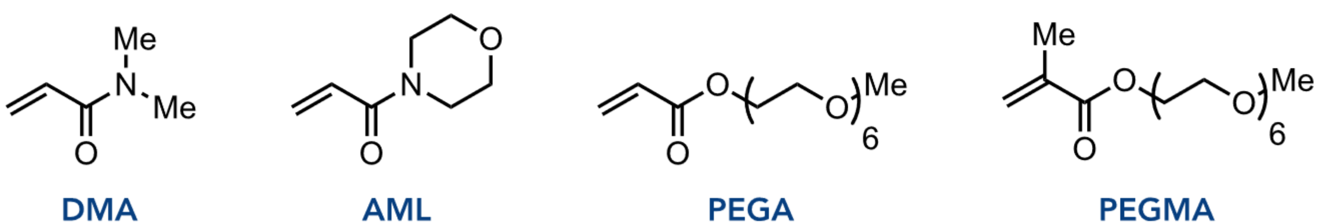
3k

ER1: 72% y, 97:3 er
ER3: 64% y, 1.5:98.5 er

Huang, X. *et al. Nat. Catal.* **2023**, *6,* 996–1004.

Flavin-dependent photoenzymes for CRP

Polymer	Conv (%)	M_{th} (kg/mol)	M_{GPC} (kg/mol)	Đ
poly-DMA_{200}	>99	20.4	22.8	1.04
poly-AML_{188}	94	27.2	23.4	1.13
poly-$PEGA_{200}$	>99	96.6	82.1	1.16
poly-$PEGMA_{200}$	>99	100.6	100.1	1.14



Huang, X. *et al. Nat. Catal.* **2023**, *6,* 996–1004.

- ***Background of photobiocatalysis***
  - Brief introduction to enzymatic catalysis
  - Modification of enzyme catalysts
  - Combining photocatalysis and enzymatic catalysis
- ***Photobiocatalysis for organic synthesis***
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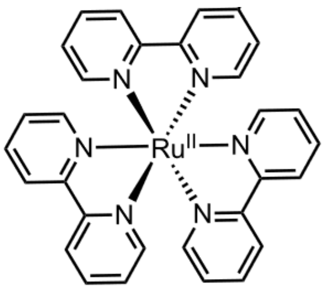
  - Artificial photoenzymes
  - Combination of external photocatalysis and enzymes
- ***Summary and perspective***

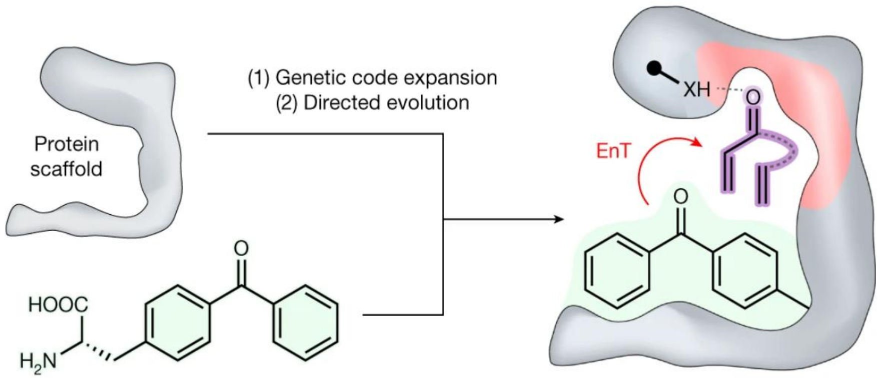
Utilizing non-natural cofactors

- Photoenzymes using non-natural cofactors and amino acids

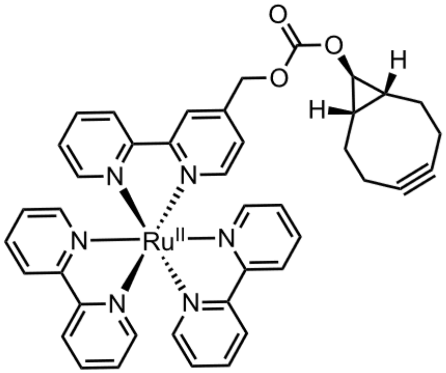


- Methods for binding non-natural cofactors into enzymes

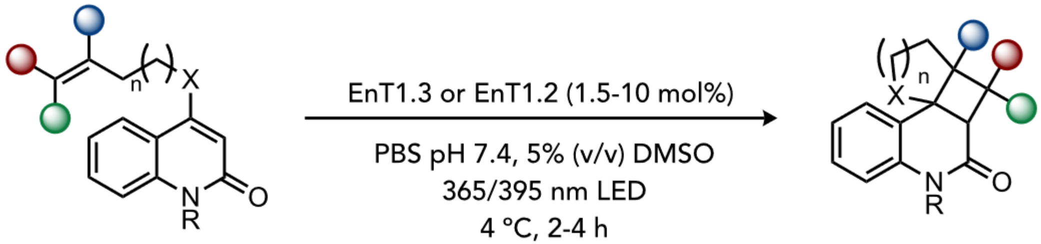
- Genetic code expansion

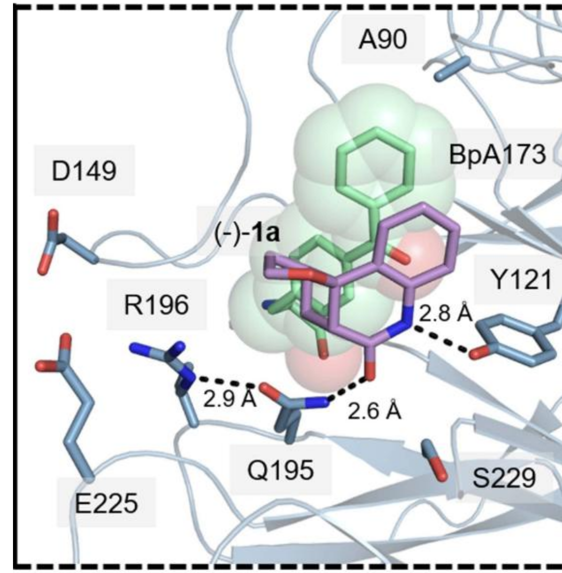


- Post-translational modification

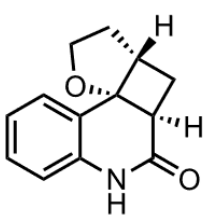


Flavin-dependent photoenzymes for CRP

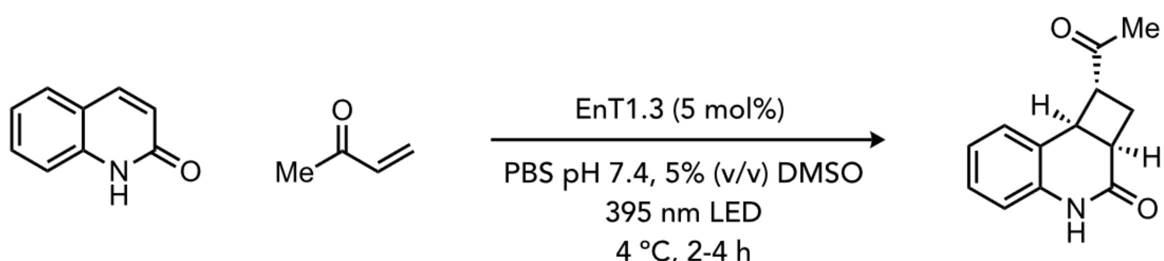




- Substrate π-stacking interactions contributed to triplet energy transfer
- Hydrogen bonding interactions between the quinolone and residues Y121 and Q195



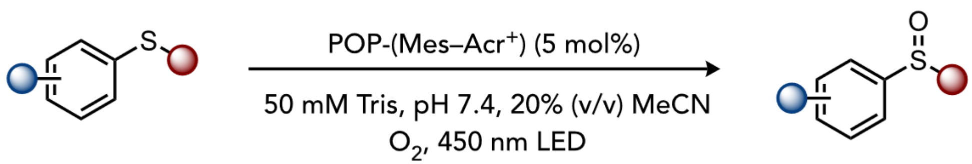
100% conversion
9:1 r.r., 99.5:0.5 e.r.

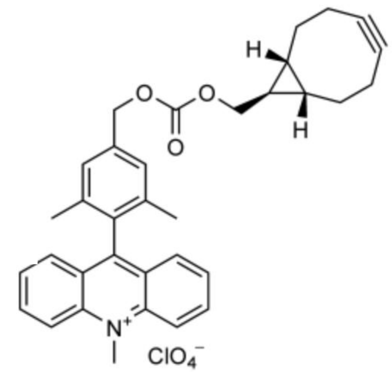


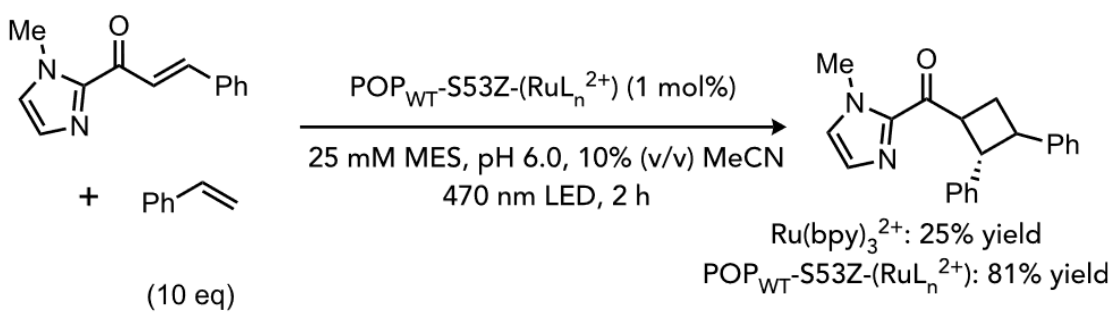
71% conversion
98.5:1.5 e.r.

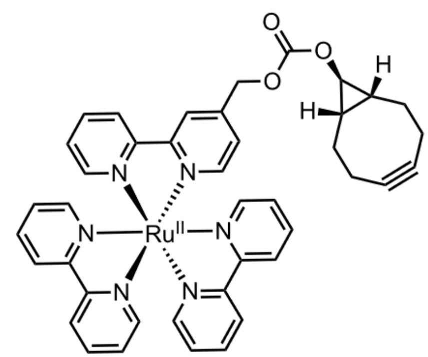
Green. A. P. *et al. Nature* **2022**, *611*, 709−714.

Reactions with artificial photoenzymes









Lewis, J. C. *et al. ChemBioChem.* **2015**, *16,* 1880−1883.
Lewis, J. C. *et al. Chem. Sci.* **2022**, *13,* 1459−1468.

- ***Background of photobiocatalysis***
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  - Combining photocatalysis and enzymatic catalysis
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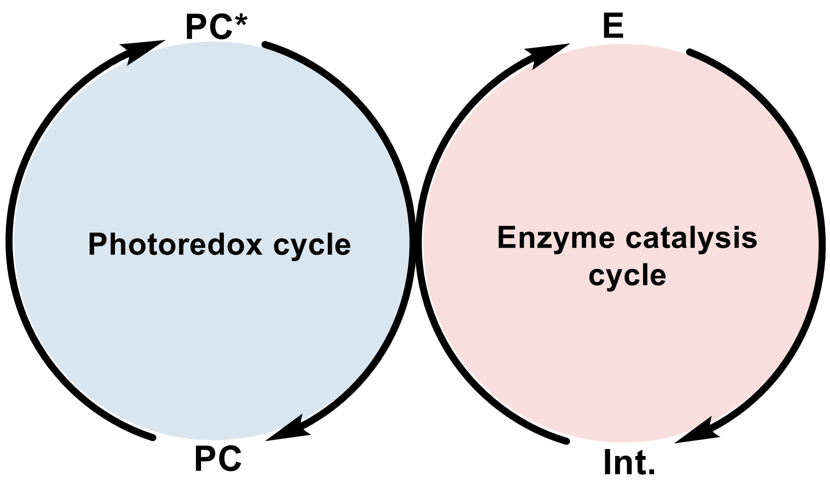
  - Combination of external photocatalysis and enzymes
- ***Summary and perspective***

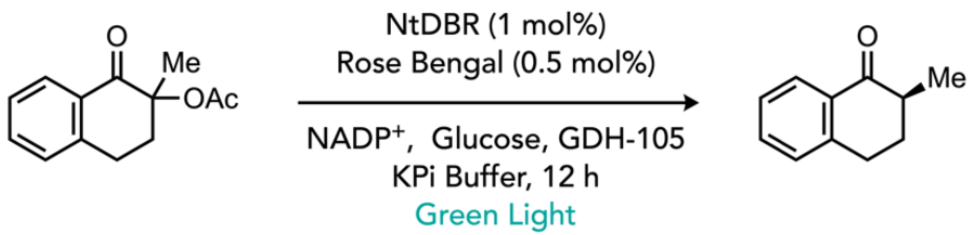
Combination of external photocatalysis and enzymes

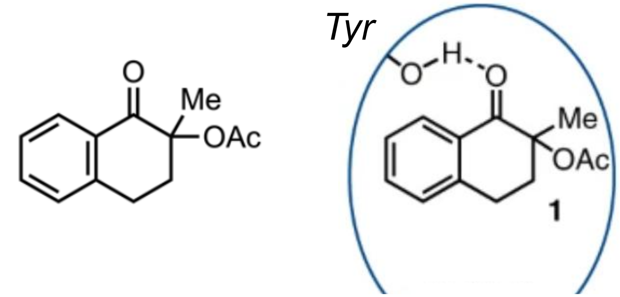


- Radical formation only occurs within the protein's active site
- Radical selectively react within the protein's active site
- Improve the compatibility of system

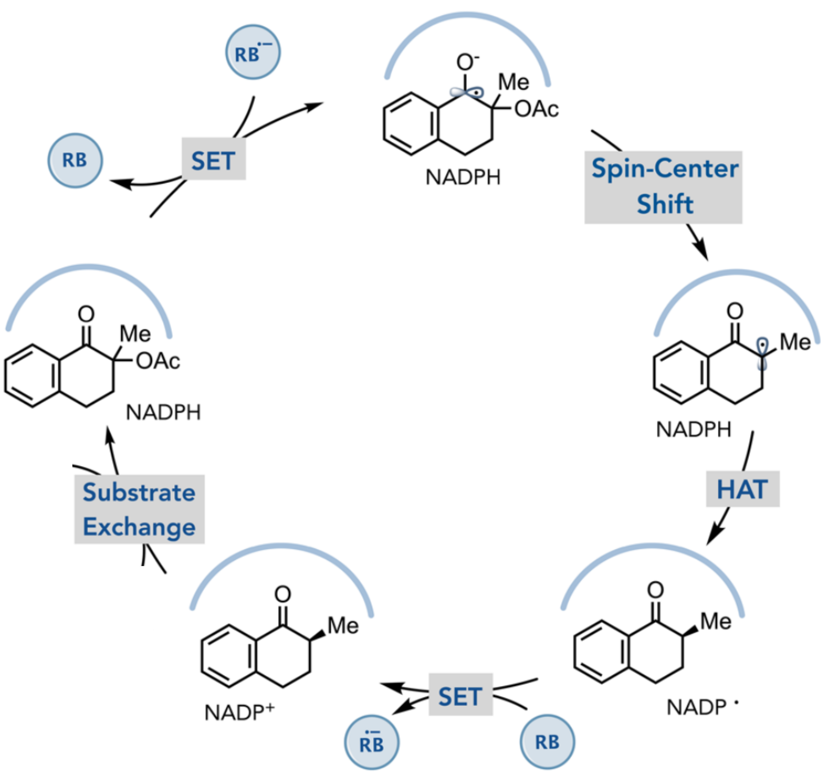
- **Long range electron transfer**
- Binding to proteins alters the redox properties of substrates or cofactors (**acceleration**)

Redox accelerated by enzymes

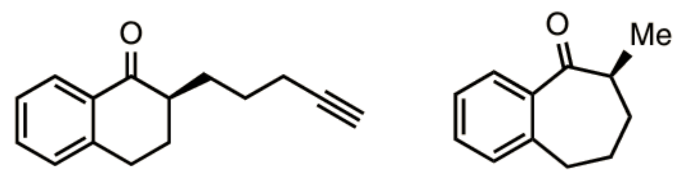




$E_{1/2}$ = - 1.30 V　　　　$E_{1/2}$ = - 1.13 V



DBR from Nicotiana tabacum
(NtDBR)

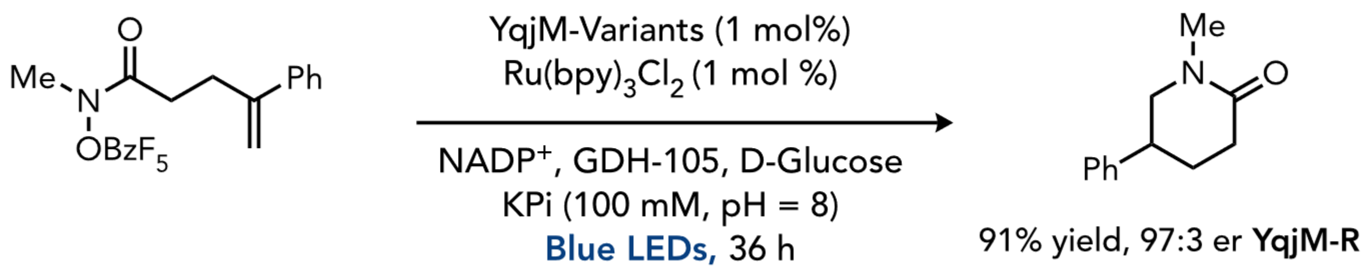


43% yield
87:13 e.r.

38% yield
88:12 e.r.

Hyster, T. K. *et al. Nat. Chem.* **2018**, *10*, 770−775.

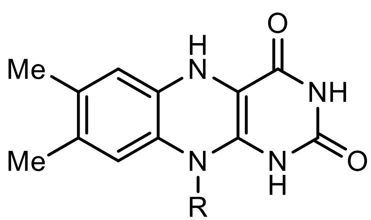
Improve the compatibility of system



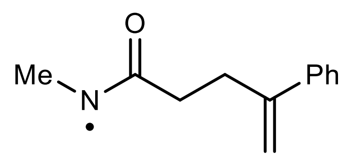
91% yield, 97:3 er **YqjM-R**

89% yield, 14:86 er **YqjM-S**

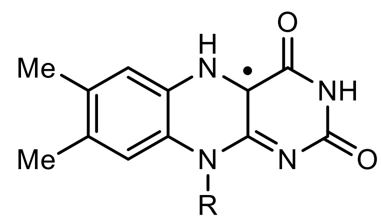
- N-centered radical is incompatible with FMNsq



FMNH$_2$



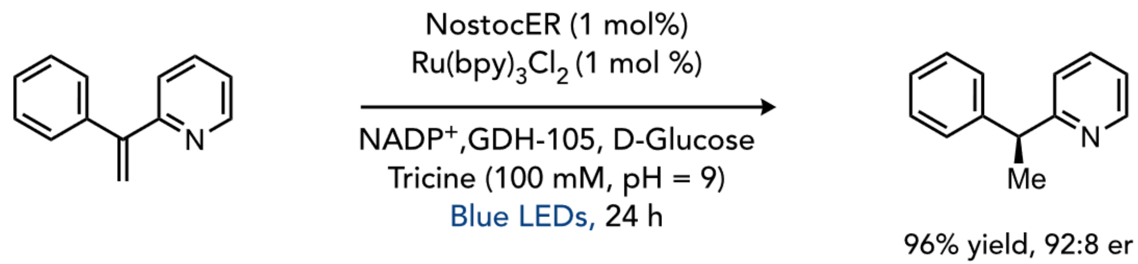
nitrogen centered radical



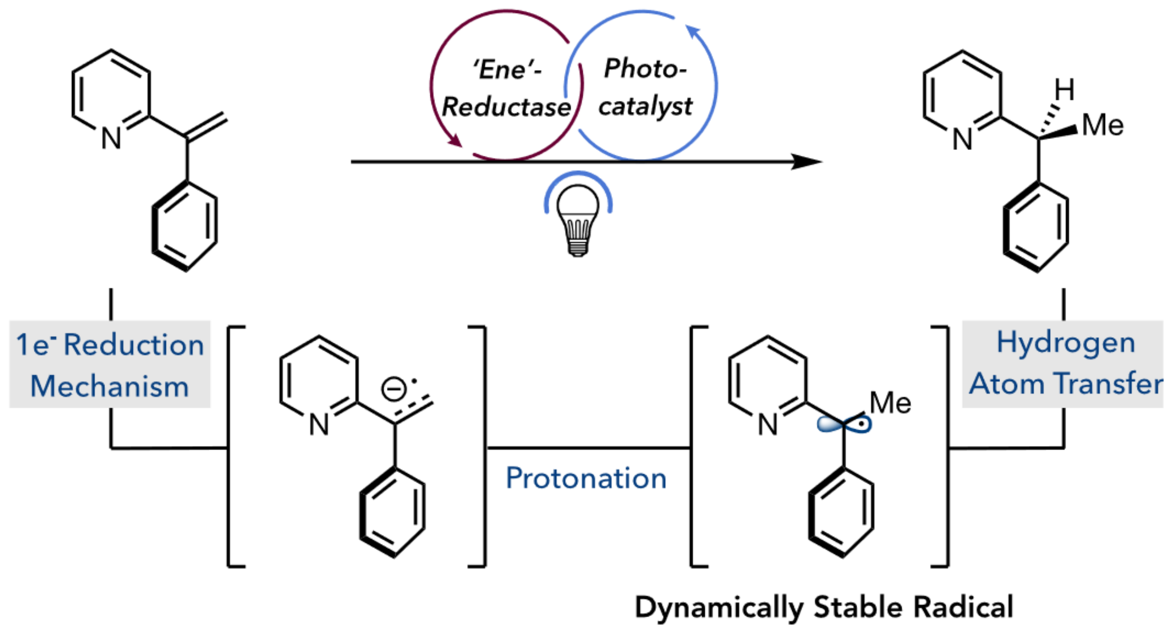
FMN$_{sq}$

Hyster, T. K. *et al. Nat. Chem.* **2023**, *15,* 206−212.

Forming a persistent radical

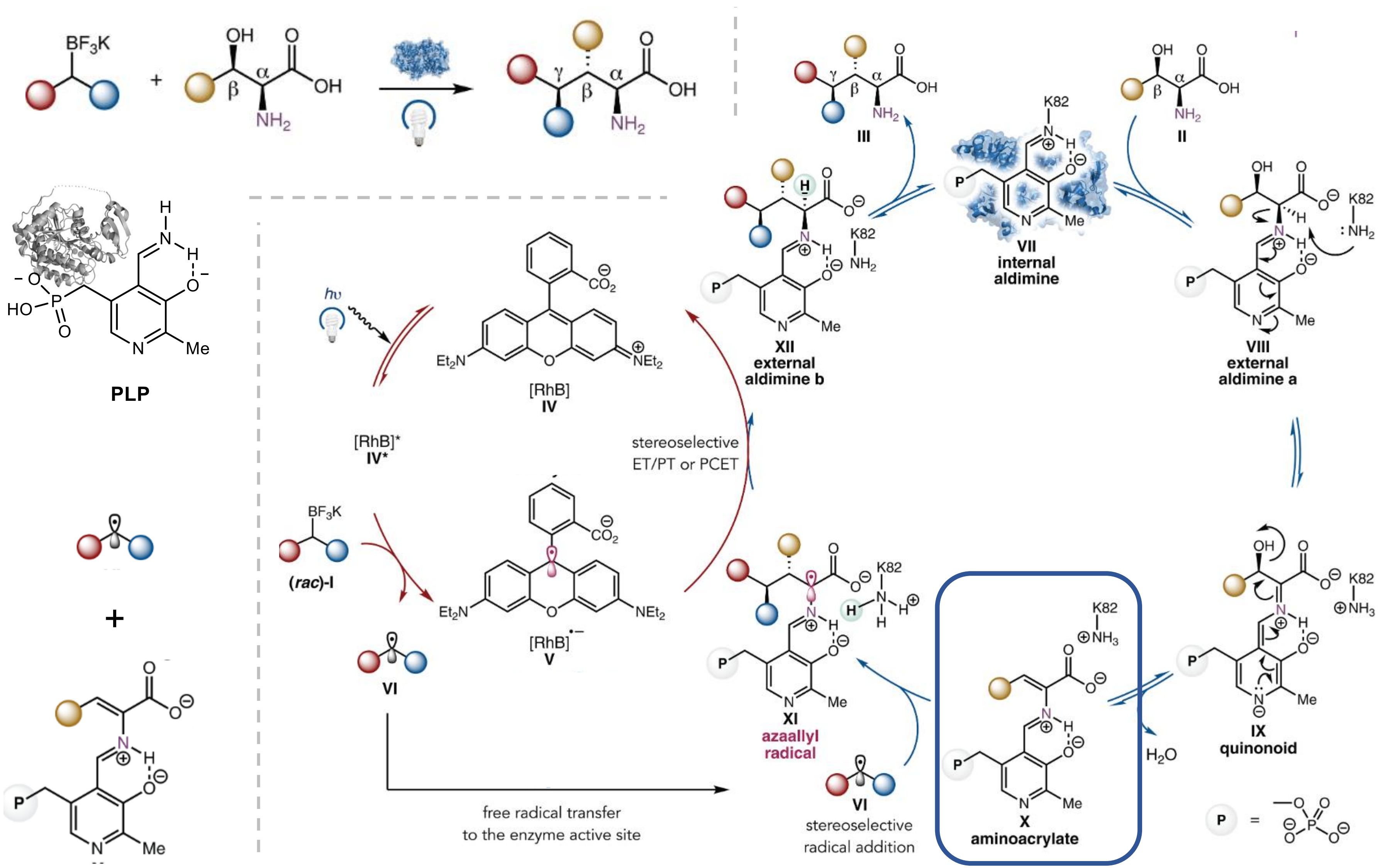


- Reduced only within enzymes



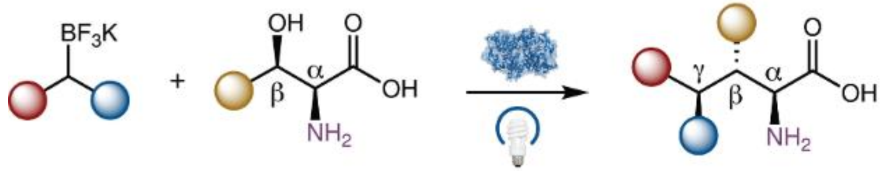
Hyster, T. K. *et al. Angew. Chem., Int. Ed.* **2020**, *59,*10484−10488.

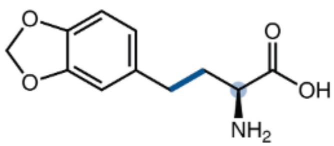
Combining persistent radical with PLP



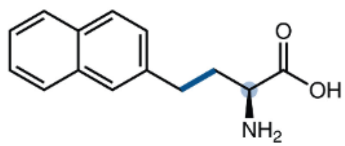
Yang, Y. *et al. Science* **2023**, *381*, 444–451.

Combining persistent radical with PLP

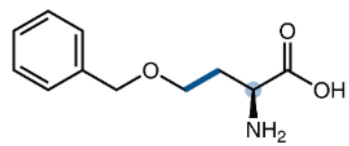




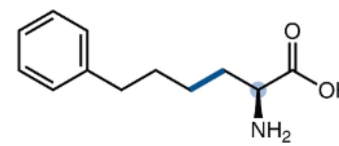
L-3k
(42 ± 2)% yield
95:5 e.r.



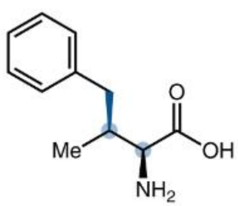
L-3l
(47 ± 5)% yield
90:10 e.r.



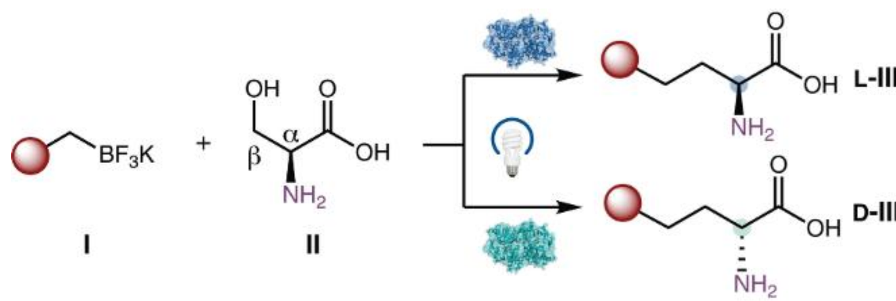
L-3m
(6 ± 1)% yield
96:4 e.r.



L-3n
(15 ± 1)% yield
71:29 e.r.

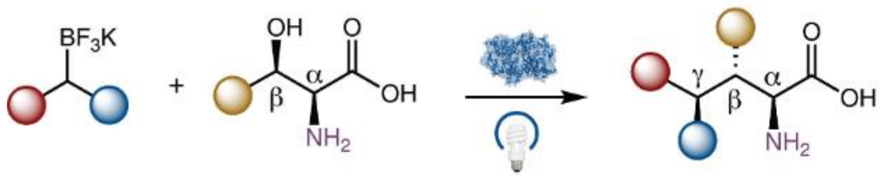


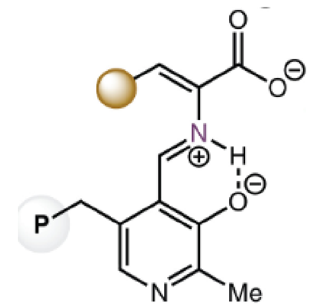
4a
(80 ± 2)% yield
d.r. = 19 : 1
e.r.(major) > 99 : 1
e.r.(minor) = 94 : 6



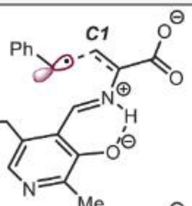
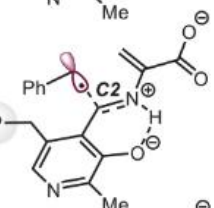
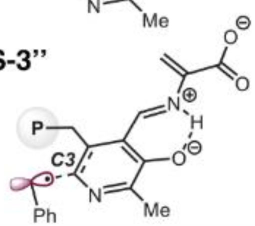
Yang, Y. *et al*. *Science* **2023**, *381*, 444–451.

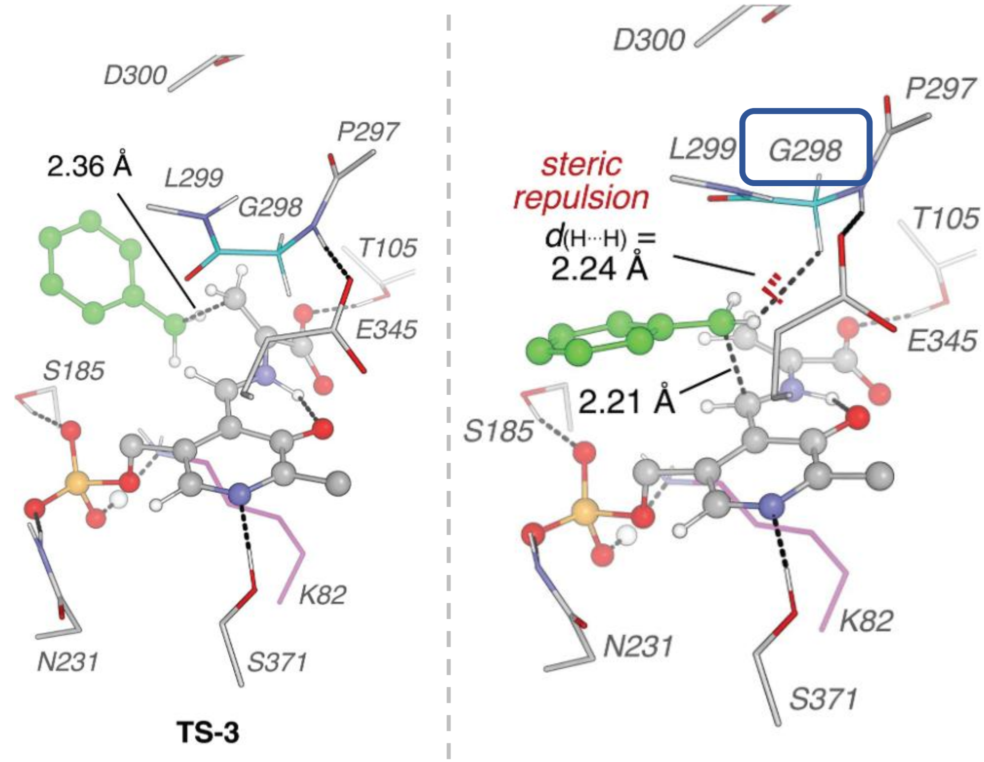
Combining persistent radical with PLP





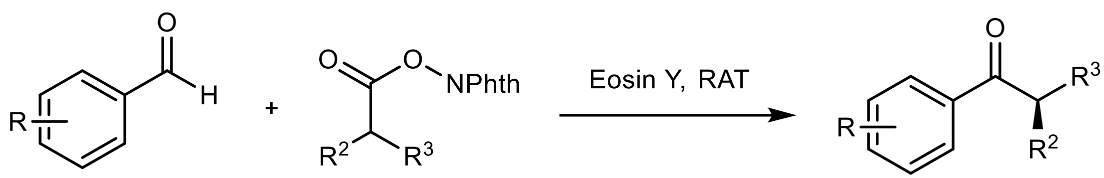
- Enzyme helps to control the regioselectivity

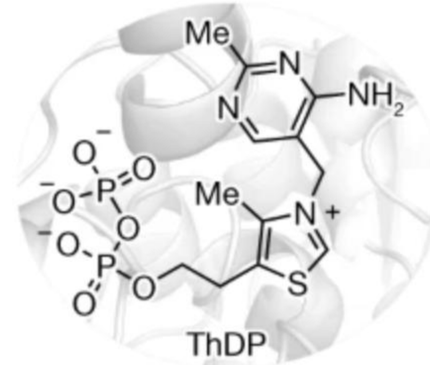
radical addition transition state		ΔH‡ (kcal/mol)	
		theozyme	free PLP cofactor
TS-3		5.6	8.3
TS-3'		8.8	8.1
TS-3''		16.1	16.1

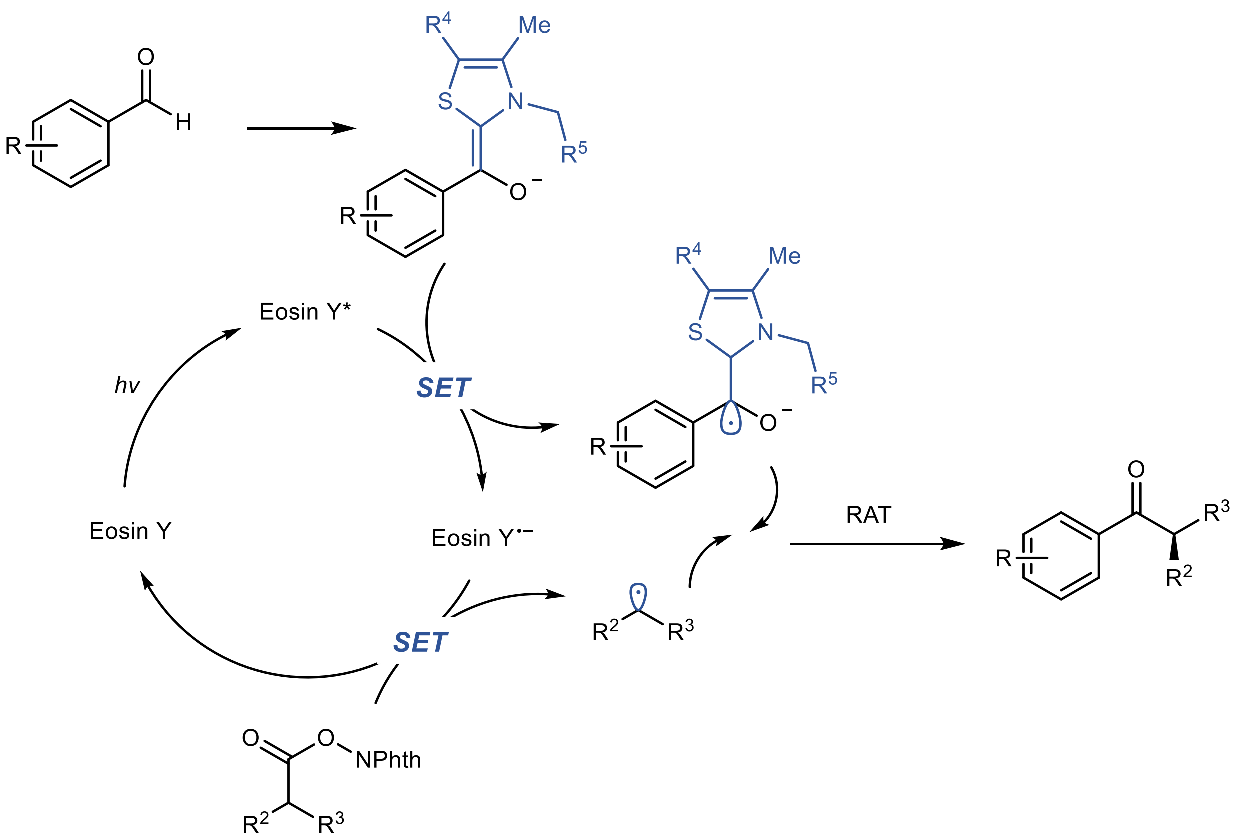


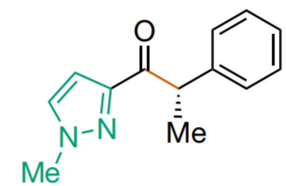
Yang, Y. *et al. Science* **2023**, *381*, 444–451.

Combining persistent radical with ThDP

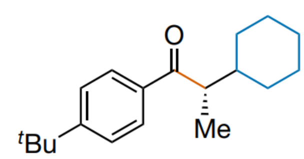








3q, RAT2
30% yield, 96% e.e.



3ad, RAT3
25% yield, 86% e.e.

Huang, X. *et al. Nature* **2024**, *625,* 74–78.

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Summary

- Combining photocatalyst with enzymic catalysis
- Derive non-natural enzymes
  - SSM
  - FRISM

- Cofactor-dependent photocatalysis
  - Hydrogenation
  - Intramolecular addition
  - Intermolecular addition
  - FAD (FAP)
  - NADPH (KRED)
  - FMN (ERED)

Summary

- Artificial photoenzymes
  - Genetic code expansion
  - Post-translational modification
- Combination of external photocatalysis and enzymes
  - Acceleration within enzymes
  - Improving compatibility
  - Utilizing persistent radicals with different enzymes

Perspective