Synergistic photobiocatalysis for enantioselective triple radical sorting

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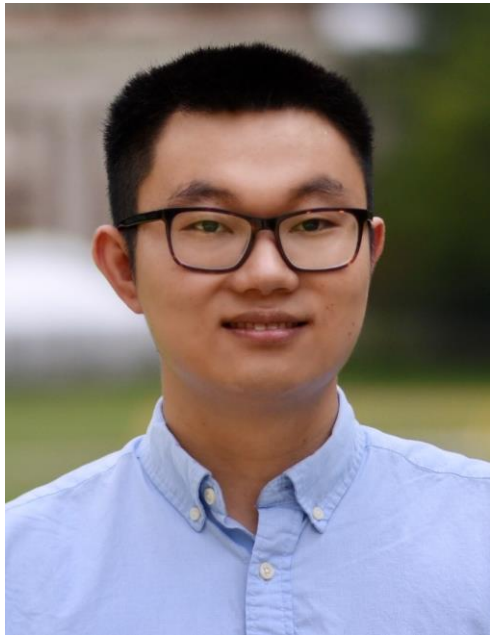
Zhongqiu Xing, Fulu Liu, Jianqiang Feng, Lu Yu, Zhouping Wu, Beibei Zhao, Bin Chen, Heng Ping, Yuanyuan Xu, Aokun Liu, Yue Zhao, Chuanyong Wang, Binju Wang & Xiaoqiang Huang

Background

Author



Dr. Xiaoqiang Huang

State Key Laboratory of Coordination Chemistry, Chemistry and Biomedicine Innovation Center (ChemBIC)，Nanjing University

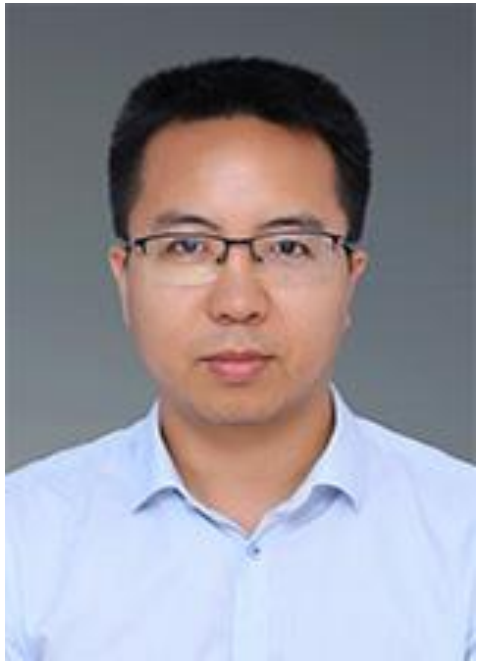
- B.E, July 2013, Peking University
- M.S, July 2015, Peking University （Supervisor: Prof. Ning Jiao)
- PhD, February 2019, Philipps-Universität Marburg (Supervisor: Prof. Eric Meggers)
- Post-Doctor training, March 2021, UIUC (Supervisor:Huimin Zhao)

- Research interest: Repurposing Natural Enzymes with Light and Engineering

C. Wang, B. Wang, X. Huang, *Nature* **2024**.

Background

Author



Dr. Binju Wang

College of Chemisty and Chemical Engineering, Xiamen University

- B.E, July 2007, Harbing Institute of Technology
- PhD, July 2012, Xiamen University
- Post-Doctor training, September 2016, The Hebrew University of Jerusalem

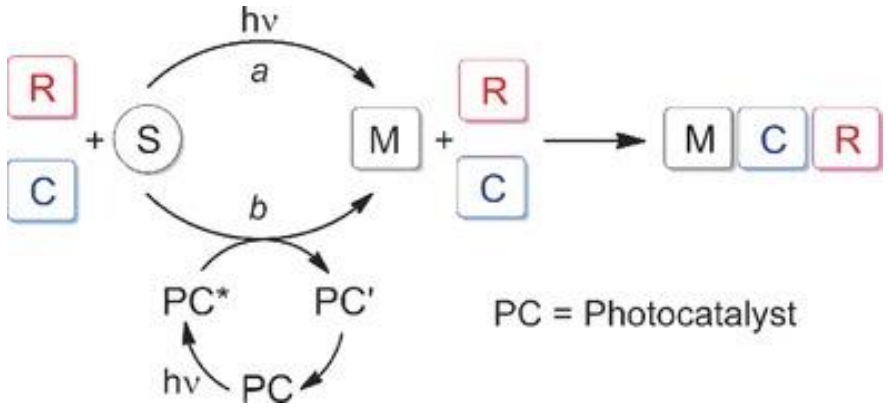
- Research interest: Multiscale theoretical simulation of chemical reactions in aqueous solutions and protein environments

C. Wang, B. Wang, X. Huang, *Nature* **2024**.

Background

Multicomponent reactions

- More than two substrates react together in a multistep, one-pot process to form a single product, with most of the atoms of the starting materials incorporated
- Rapid achievement of high levels of complexity and diversity
- Practical and time-saving operations



Atom economy

Avoidance of hazardous compounds

Waste prevention

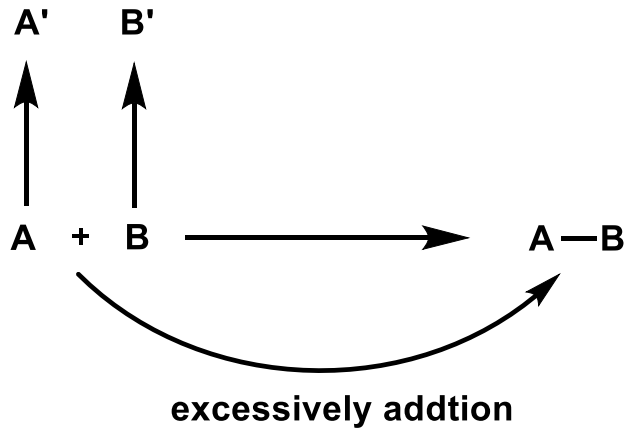
Energy efficiency

S. Protti, A. Basso et al., *Angewandte Chemie International Edition* **2016**, *55*, 15476-15484.

Background

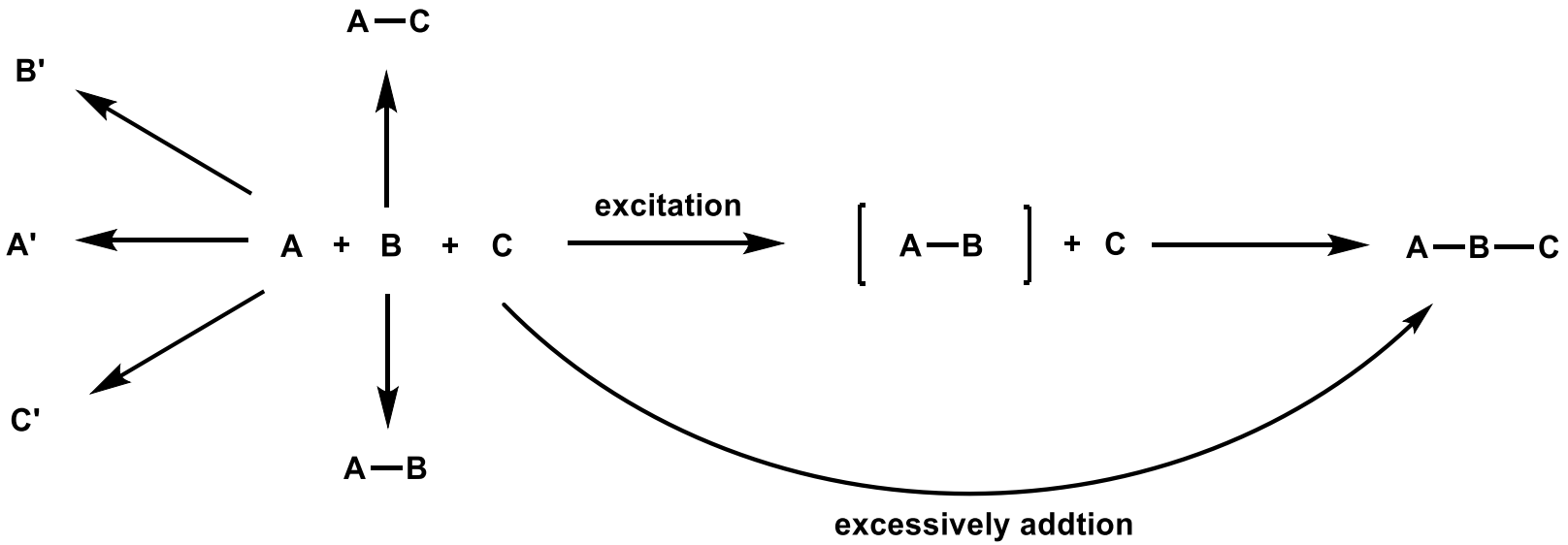
Challenges of radical multicomponent reactions: chemoselectivity

2 component



Possible side reactions
Increase exponentially!

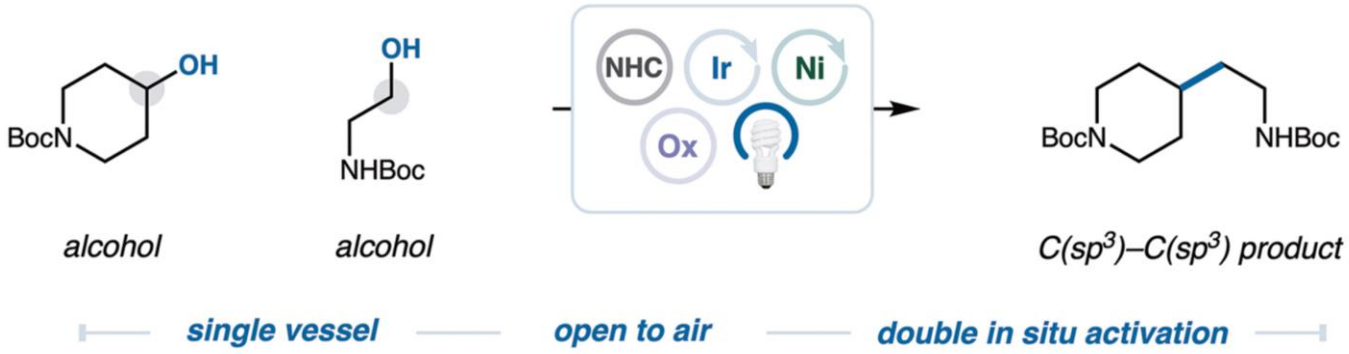
3 component



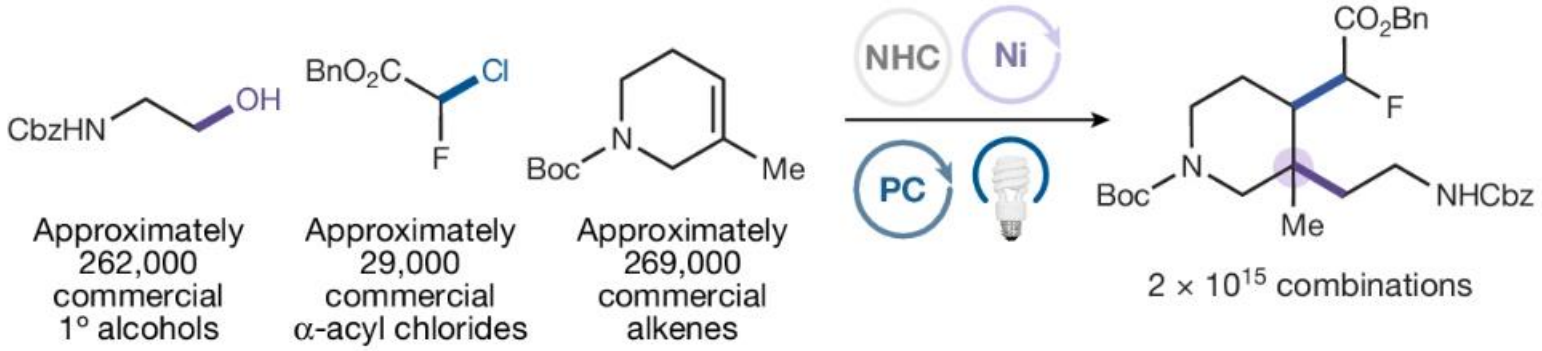
C. Wang, B. Wang, X. Huang, *Nature* **2024**.

Background

Chemical catalytic radical sorting

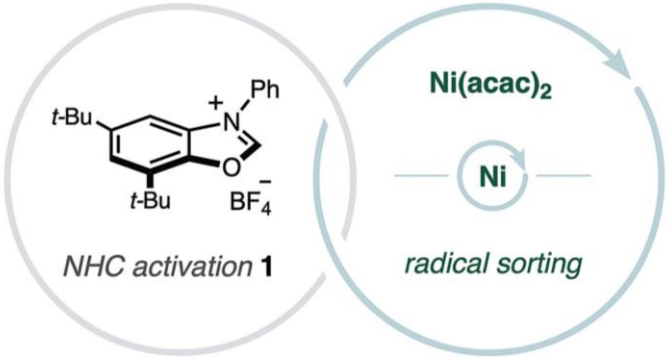


D. W. C. MacMillan, *Science* **2024**



D. W. C. MacMillan, *Nature* **2024**

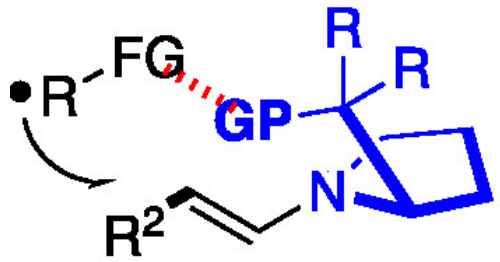
Chemical catalytic radical sorting has emerged as an enabling strategy for a variety of useful reactions



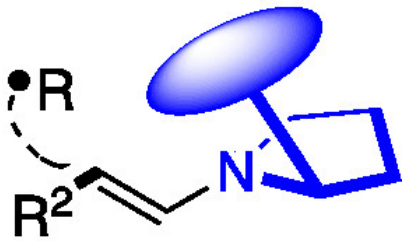
Background

Challenges yet to be solved：stereoselectivity

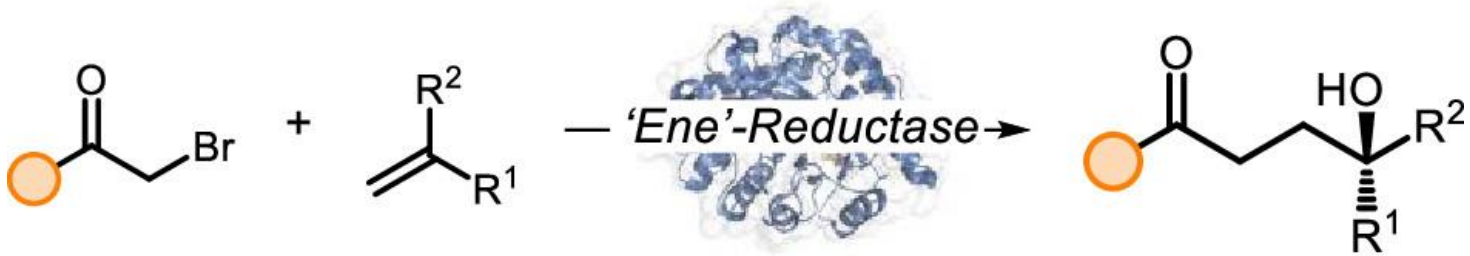
Weak-bond-directed control



Steric-directed control



M. P. Bertrand, M. Nechab, *Chemical Reviews* **2022**

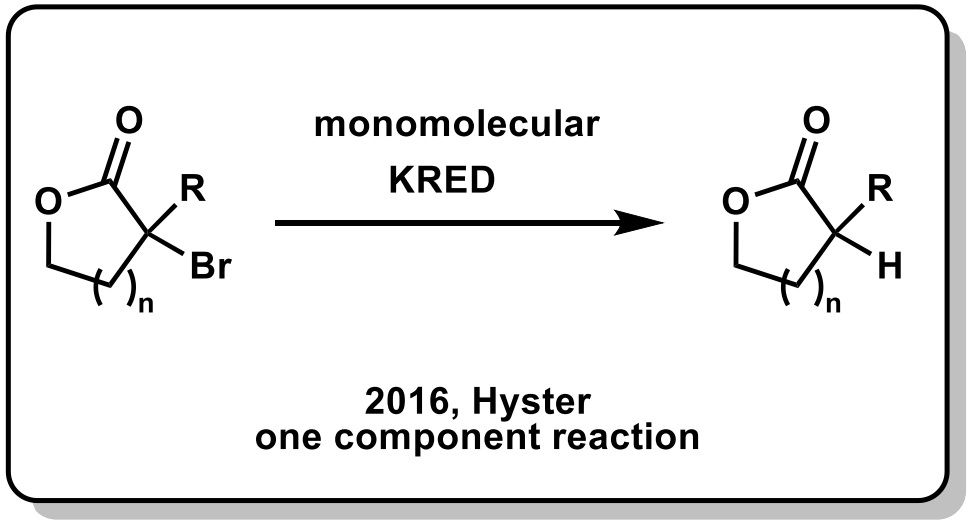


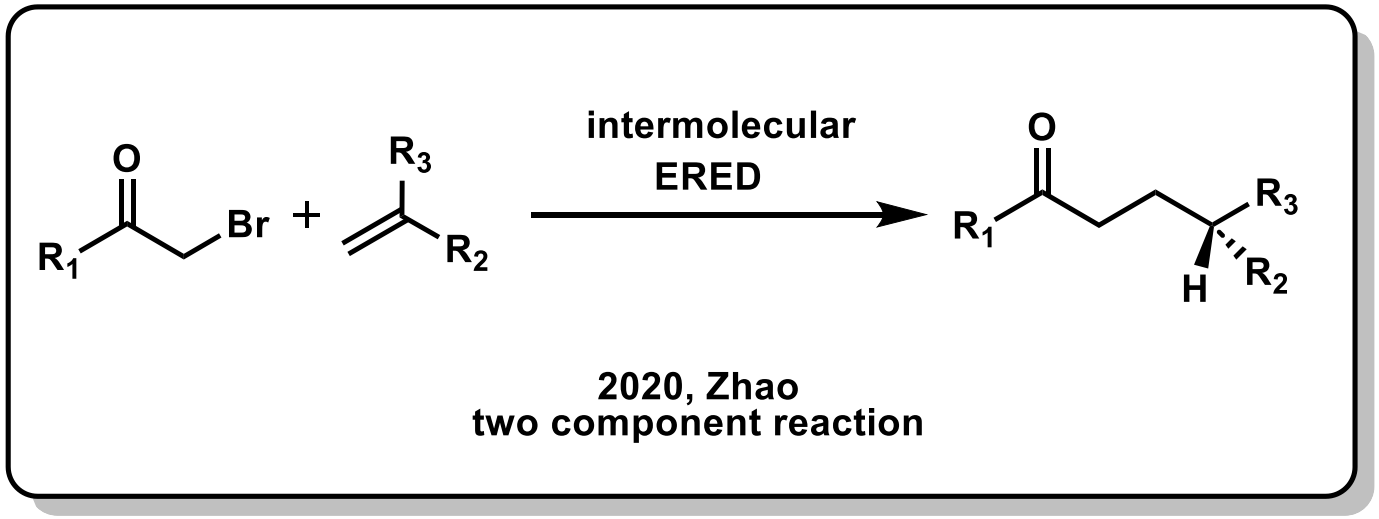
30 examples
up to 95% yield
up to >95:5 er

T. K. Hyster, *Journal of the American Chemical Society* **2023**

Background

Milestones in new-to-nature photobiocatalysis





How to use one protein to regulate the orderly transformation of three substrates?

C. Wang, B. Wang, X. Huang, *Nature* **2024**.

Background

Target

- Develop a new photobiocatalytic method

  to achieve a triple radical coupling reaction

- Enzymes evolve for specific functions

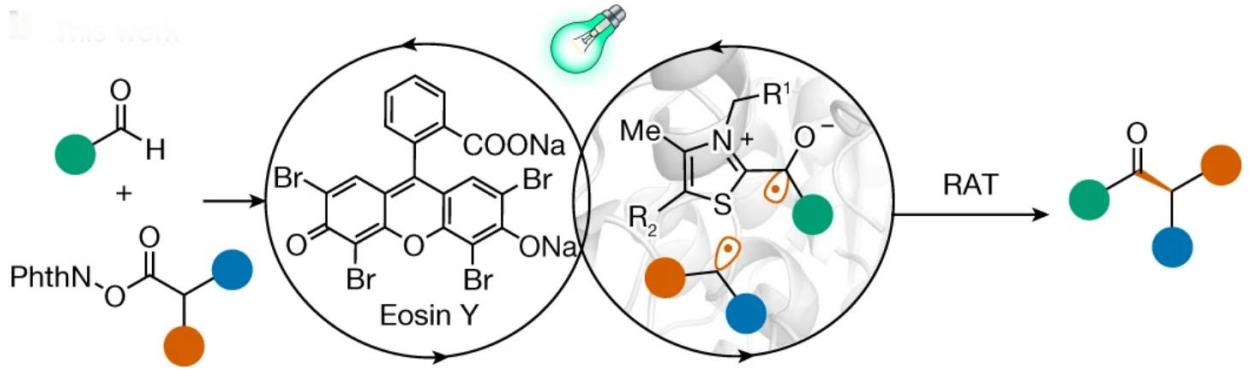
- Restricted confines of the active site

- Reactions involving multiple substrates/radicals are inherently difficult

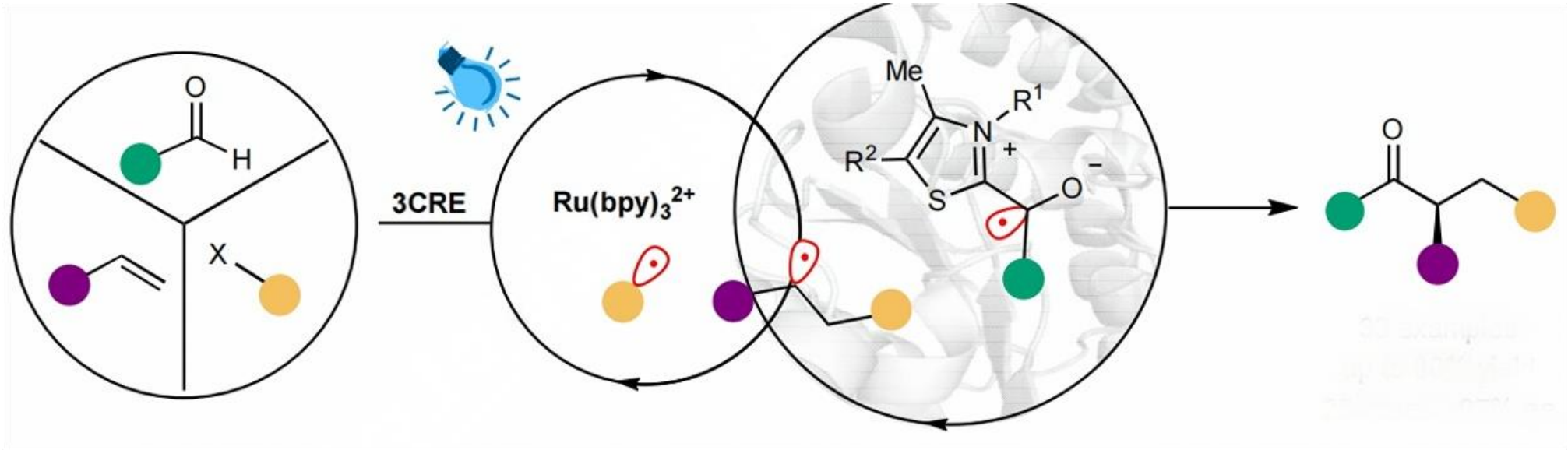
C. Wang, B. Wang, X. Huang, *Nature* **2024**.

Background

Research ideas



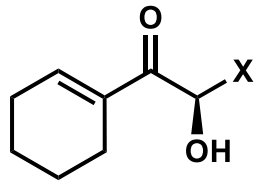
X. Huang, *Nature* **2024**, 625, 74-78.

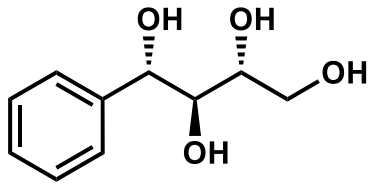


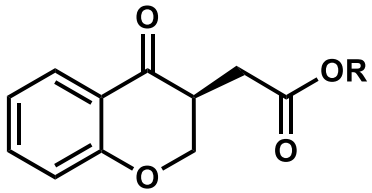
C. Wang, B. Wang, X. Huang, *Nature* **2024**.

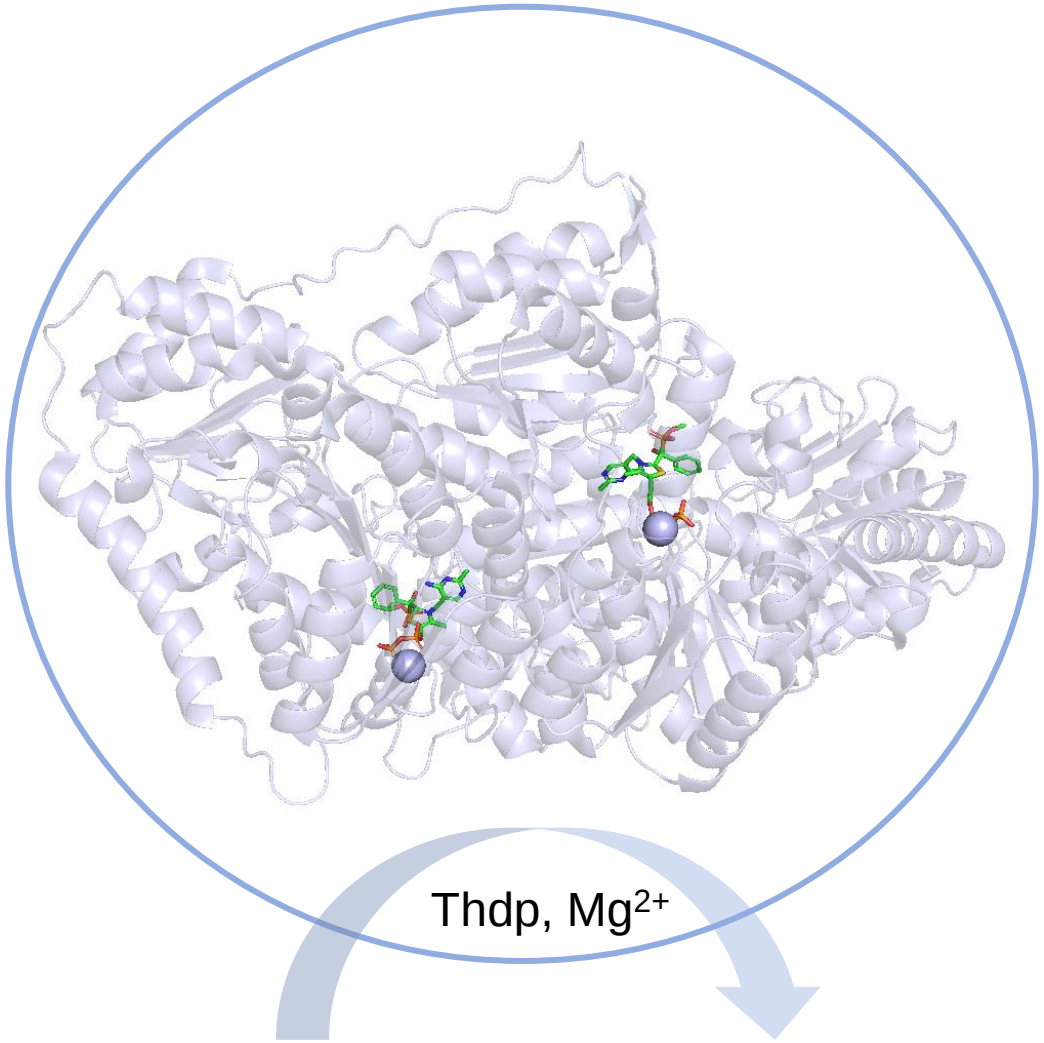
Background

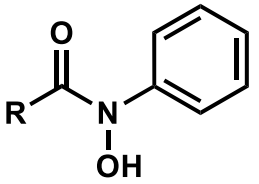
PfBAL

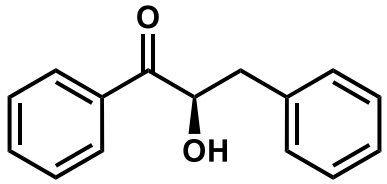


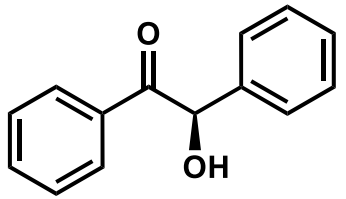








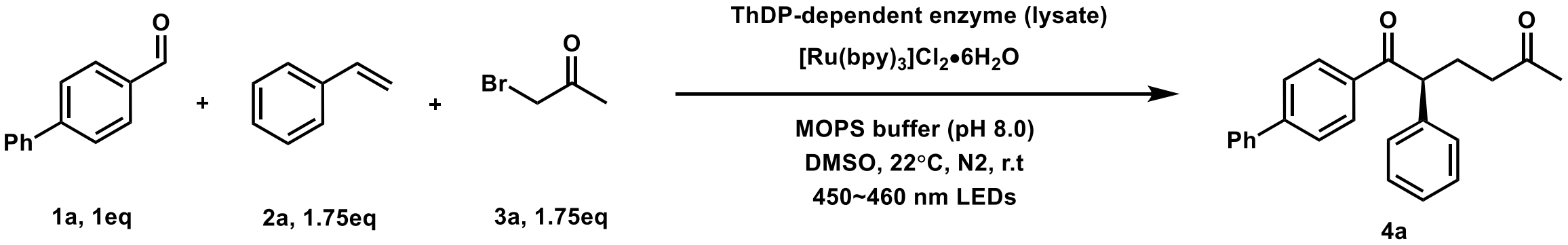




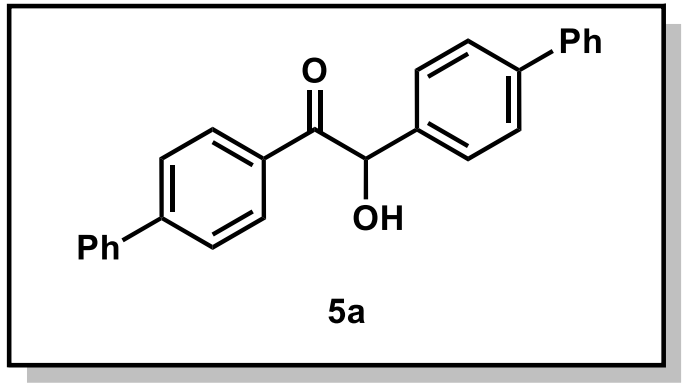
D. C. Howell, F. Chen, *Green Synthesis and Catalysis* **2022**

Results

Standard conditions and control experiment



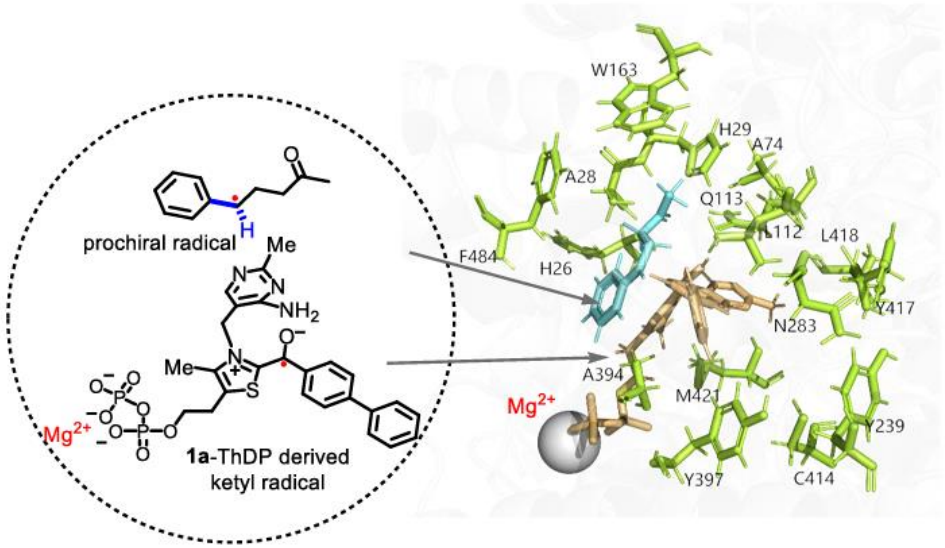
entry	enzyme	variations	yield of **4a**	yield of **5a**
1	wild-type P*f*BAL	none	3%(90%ee)	86%
2	3CRE-1	none	70%(99.5%ee)	5%
3	No enzyme	none	0%	0%
4	3CRE-1	w/o [Ru]	5%	87%
5	3CRE-1	w/o light	0%	99%
6	3CRE-1	0.2 mol% enzyme	117 tons	n.d.
7	3CRE-1	purified enzyme	53%(99.5%ee)	12%



C. Wang, B. Wang, X. Huang, *Nature* **2024**.

Results

Reaction development and engineering



Semi-rational iterative site-specific mutagenesis strategy

Wild type *Pf*BAL

↓ Site-saturation mutagenesis

*Pf*BAL_T481L

↓ MD simulation

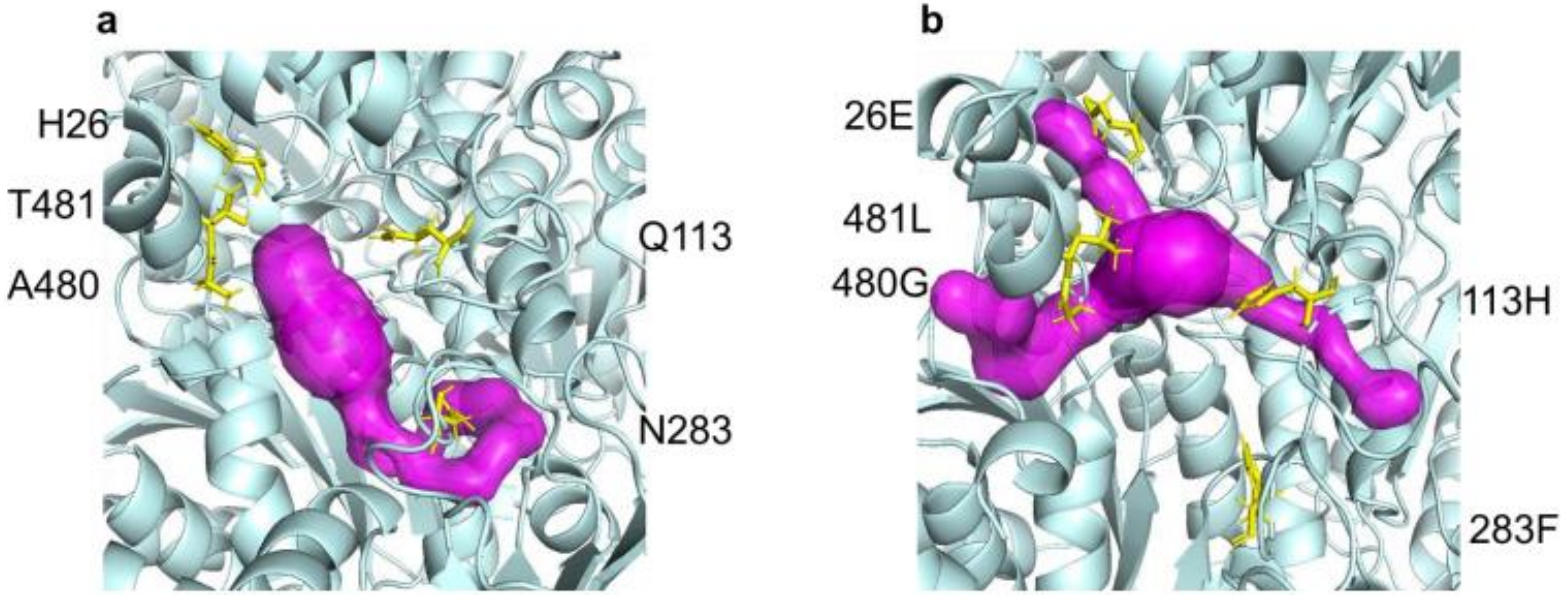
3CRE-2

entry	conditions	4a (yield, ee)	5a (yield, ee)	5b (yield)	5c (yield)
1	Wild type *Pf*BAL	3% y, 90% ee	86% y, 98% ee	9% y	1.6% y
2	*Pf*BAL_T481L	10% y, 95% ee	67% y, 98% ee	21% y	1% y
3	*Pf*BAL_T481L-A480G	18% y, 99% ee	67% y, 98% ee	14% y	1% y
4	*Pf*BAL_T481L-A480G-Q113H	48% y, 98% ee	14% y, 98% ee	38% y	0.3% y
5	*Pf*BAL_T481L-A480G-Q113H-N283F (**3CRE-2**)	66% y, 98% ee	5% y, 94% ee	29% y	1.4% y
6	*Pf*BAL_T481L-A480G-Q113H-N283F-H26E (**3CRE-1**)	70% y, 99.5% ee	5% y, 92% ee	23% y	2.3% y

C. Wang, B. Wang, X. Huang, *Nature* **2024**.

Results

MD revealed the mechanism of action of several key mutation sites



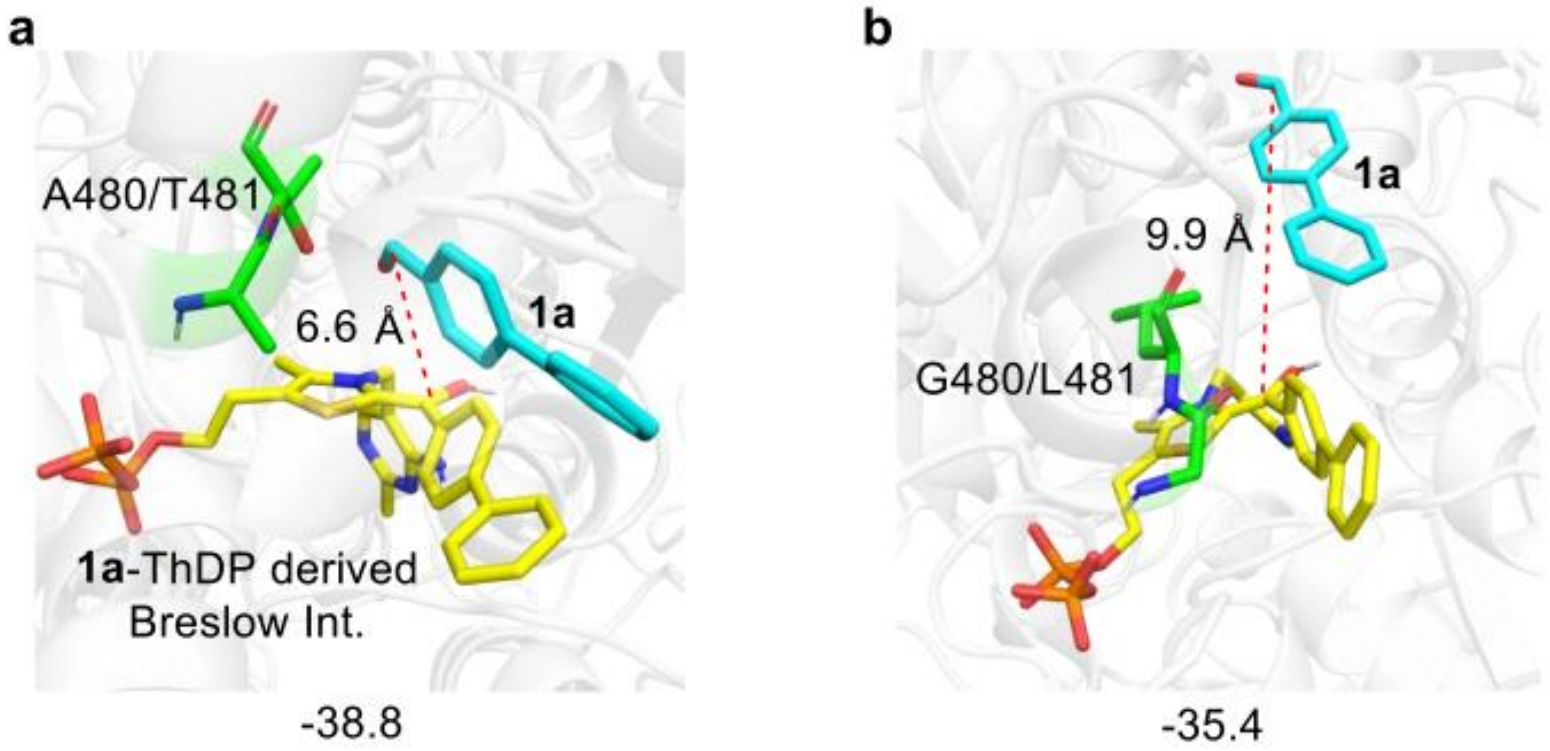
The tunnels of the wild-type P*f*BAL (a) and the five-site mutated 3CRE-1 (b)
CAVER Web 1.0[1] P*f*BAL：PDB code: 3D7K

- L481 and G480 double mutant significantly enhanced enantioselectivity
- H113 mutant greatly improves the yield and decreases the native benzoin condensation
- The volume of tunnels has expanded from 1835 Å^3 for the wild type to 2210 Å^3 for the 3CRE-1

C. Wang, B. Wang, X. Huang, *Nature* **2024**.

Results

MD simulated structures of the key intermediate for benzoin condensation enabled by (a) wild-type PfBAL, and (b) 3CRE-1



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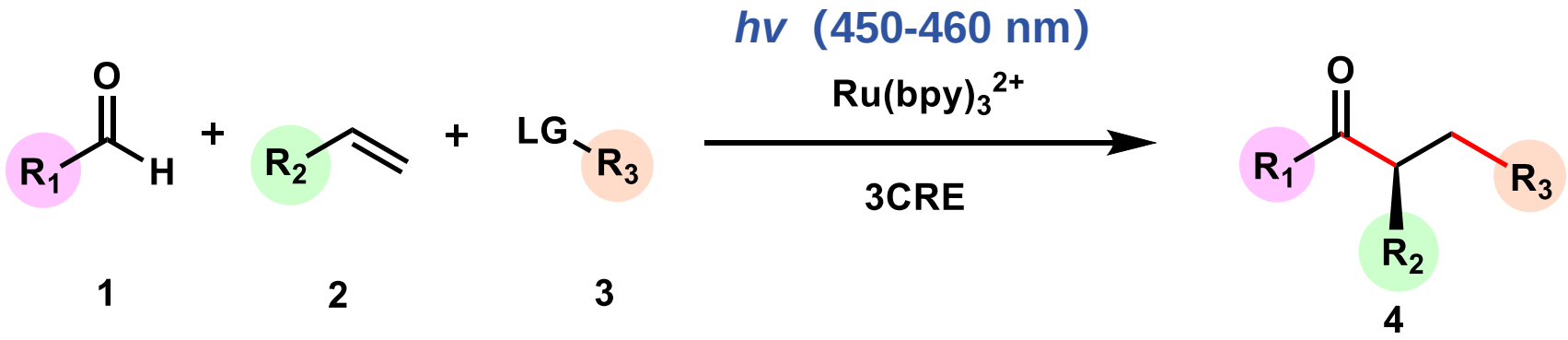
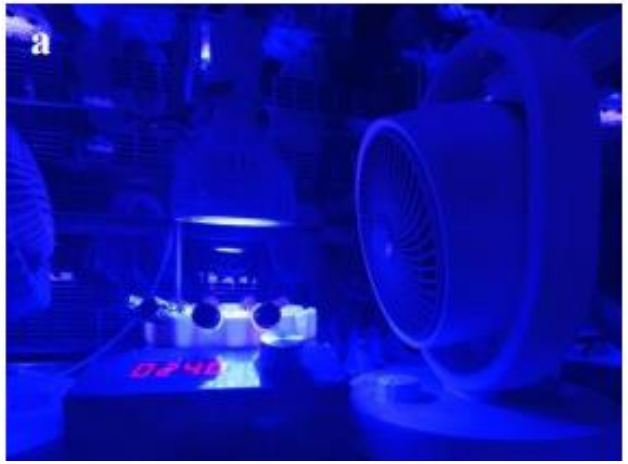
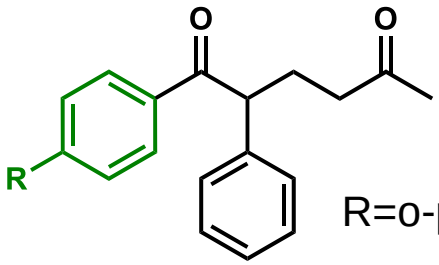
Results

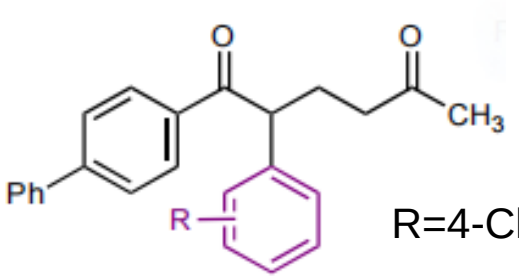
Substrate scope

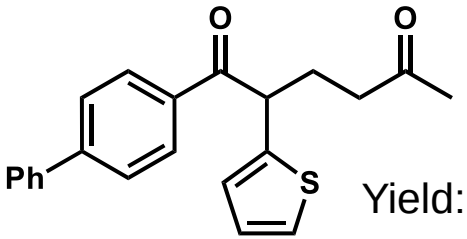






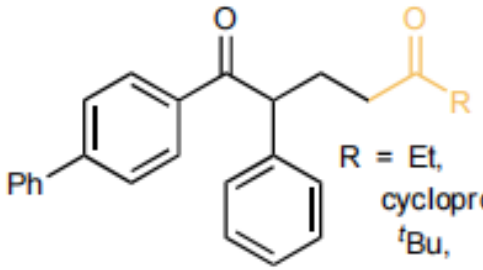
R=o-ph-CHO,ArCHO(97%-99%ee)





Yield:80%

R=4-Cl,4-F,2-F,3-F, heterocyclic ring



3CRE-1

R = Et, **4y**, 53% yield, 98% ee
cyclopropyl, **4z**, 53% yield, 97% ee
^{t}Bu, **4aa**, 35% yield, 96% ee

R=-CN,-COOR,-COR,R

33 examples
up to 80% yield
25 cases ≥ 97% ee

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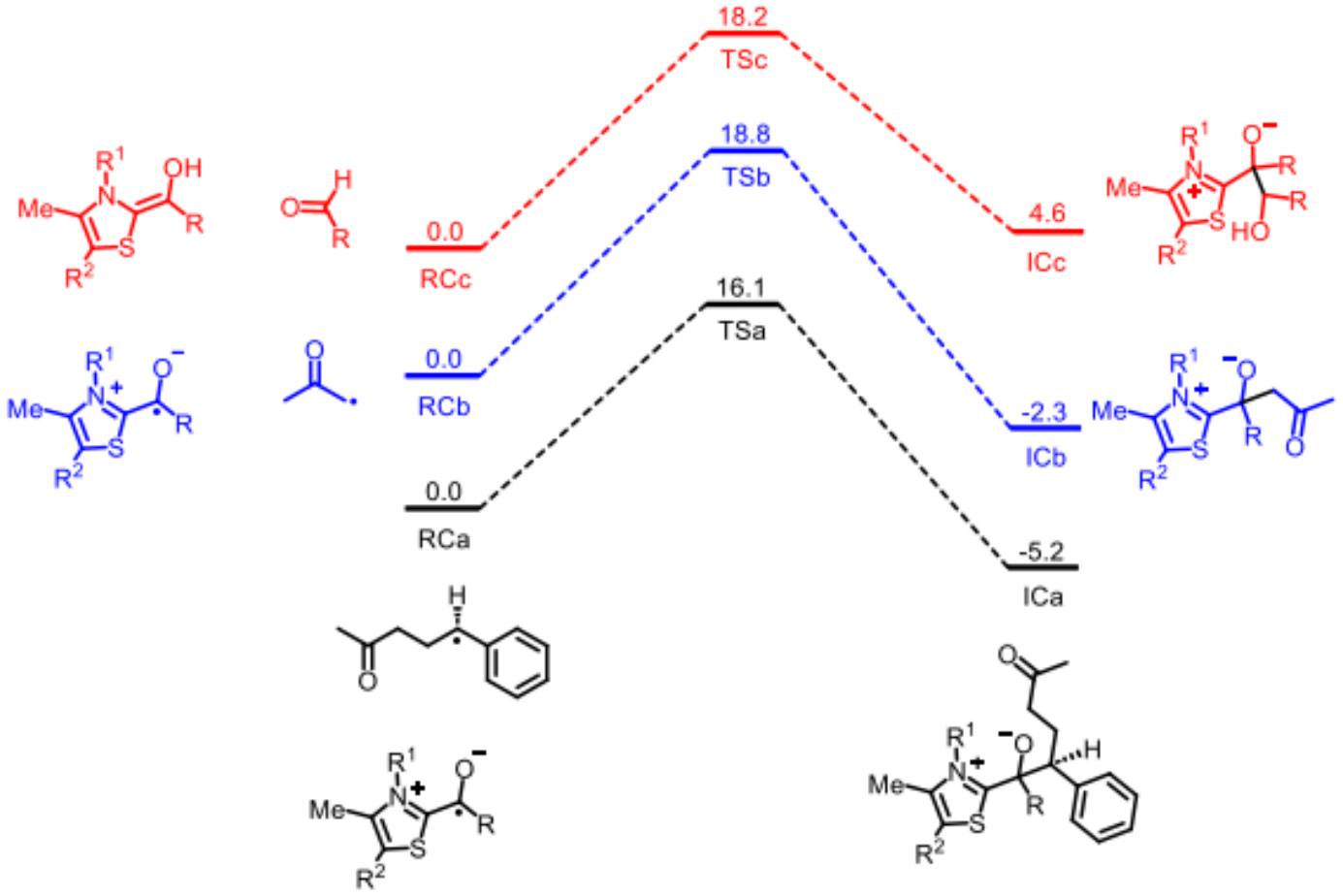
Results

Side reactions vs main reaction



Geometry optimization、IRC: B3LYP-D3/def2-SVP
Single point energy: B3LYP-D3/def2-TZVP

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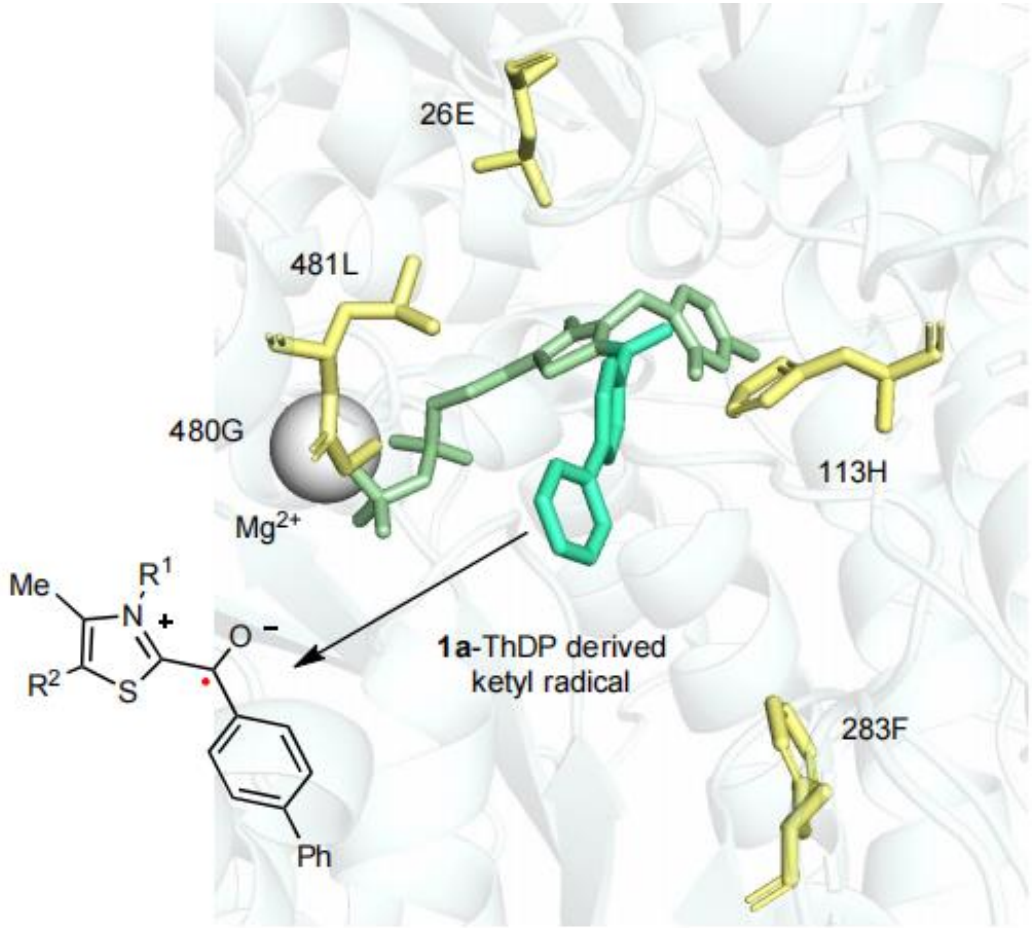
Results

Mutated sites view



QM (B3LYP-D3/B2)/MM

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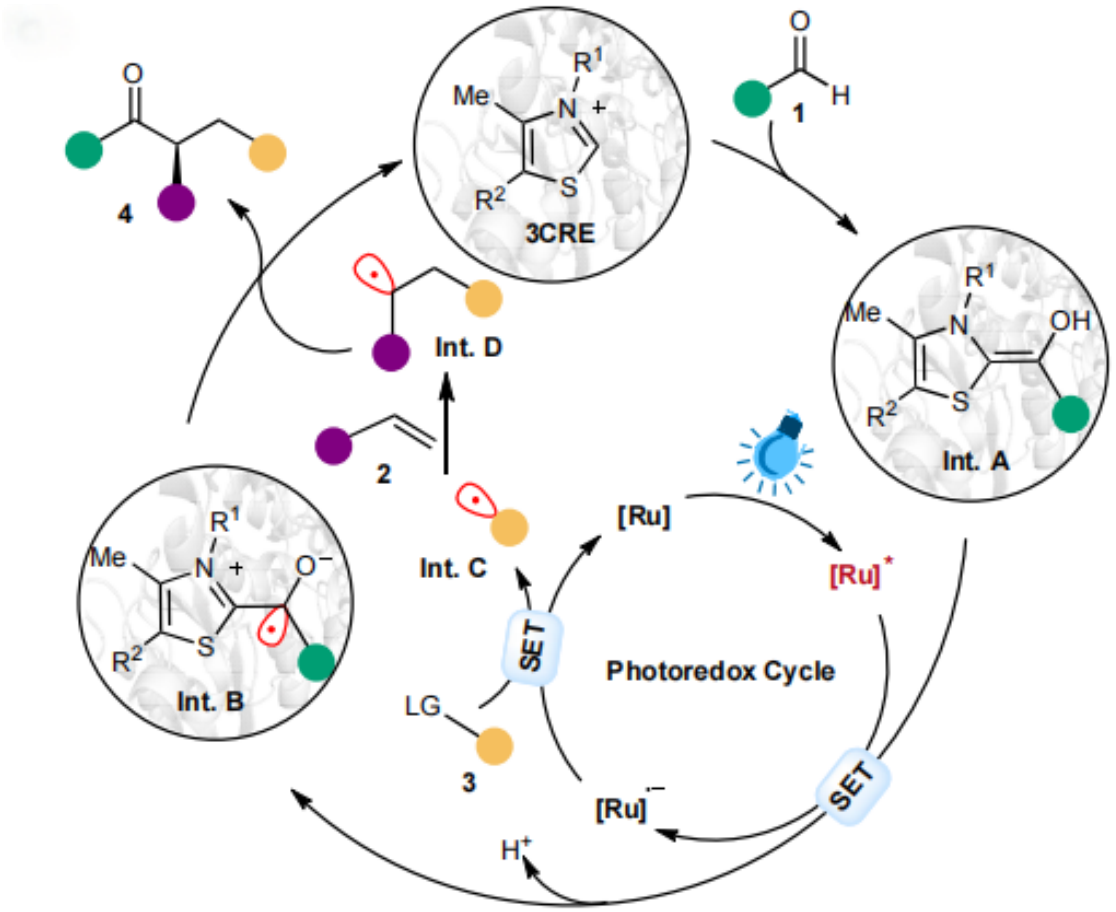
Results

Proposed mechanism



a) fomation of Breslow intermediate

b) [Ru] complex oxidate int.A and [Ru]$^{-}$reduce the bromo-substrate to generate the radical(Int.C)

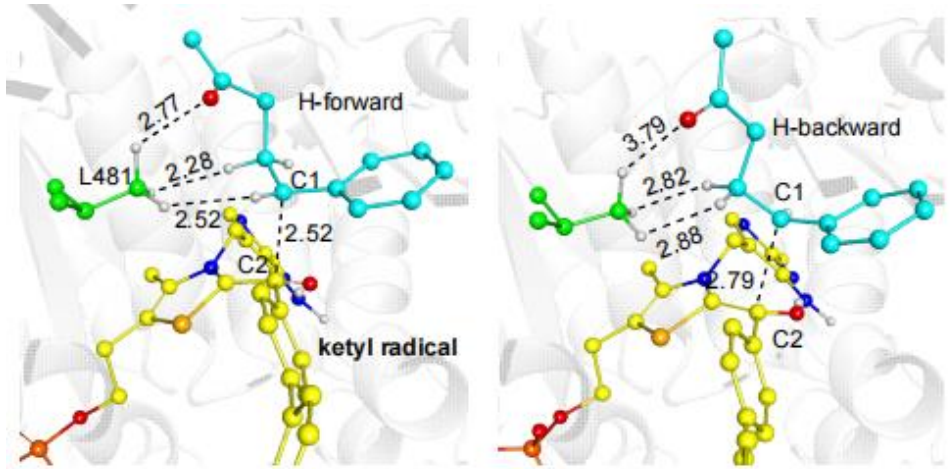
c) Int.C add to alkene to generate int.D

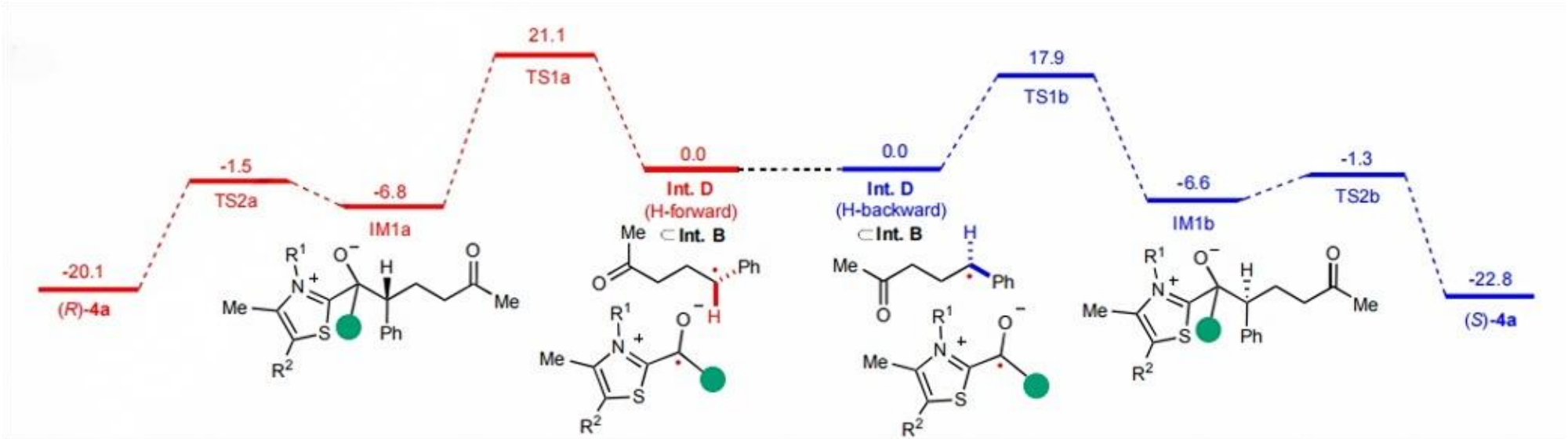
d) Int. B and Int. D undergo stereoselective radical coupling and regenerating the enzyme

C. Wang, B. Wang, X. Huang, *Nature* **2024**.

Results

The steric hindrance effect is the key to achieving excellent enantioselective control





C. Wang, B. Wang, X. Huang, *Nature* **2024**.

Discussion

Conclusion

- **Achieving chemo-/enantio-selective enzymatic triple radical sorting scaled to preparative dimensions (gram scale)**
- **System with excellent substrate tolerance, outstanding stereochemical control and mild reaction conditions, which is not easily attainable with chemo-NHC**
- **A synergistic dual photoredox and biocatalytic cycle mechanism is proposed**

Prospect

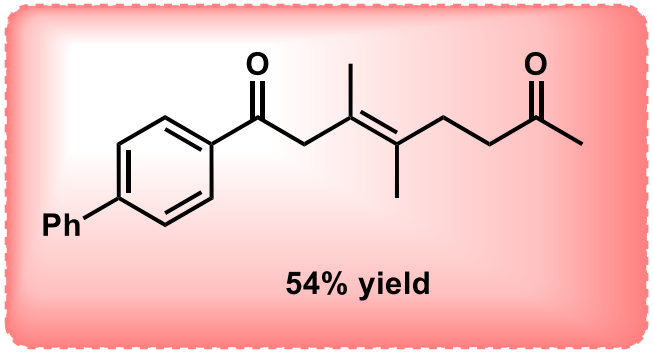
- **Provide a broad playground for exploring new-to-nature biocatalysis system**
- **Evolved enzymes containing NHC cofactors have more potential for development.**

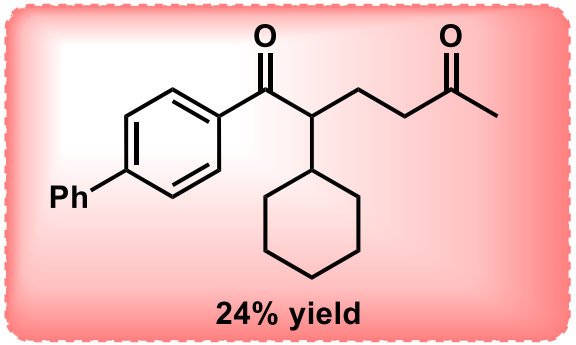
C. Wang, B. Wang, X. Huang, *Nature* **2024**.

Discussion

Limitations

- **Show low yield when involving unactivated alkene**
- **Excessive amounts of alkene and bromocarbonyl compound (1.75 equivalents) were used in the reaction (poor atom economy)**
- **Calculation：theory method（B3LYP-D3/def2-TZVP）may need optimization**
- **Lack of direct experimental evidence regarding the binding site of the [Ru] photocatalyst on the enzyme**





C. Wang, B. Wang, X. Huang, *Nature* **2024**.

Discussion

Driving innovation in chemical synthesis methods

Expanding the boundaries of biocatalytic functions

Deepening the understanding of the synergy mechanism of ThDP-dependent biocatalysis

Facilitating the development of evolved enzymes

C. Wang, B. Wang, X. Huang, *Nature* **2024**.

NIBS

清華大學 Tsinghua University

Thanks for listening!

Qi Lab NIBS