Photo-enzymatic Synthesis : From Natural to Abiological Transformations

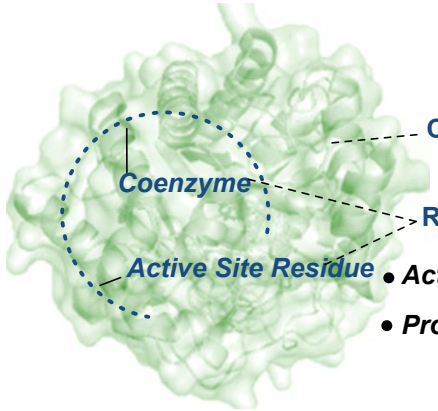
Yilun Li

Denmark Group Meeting

2024-07-16

Introduction

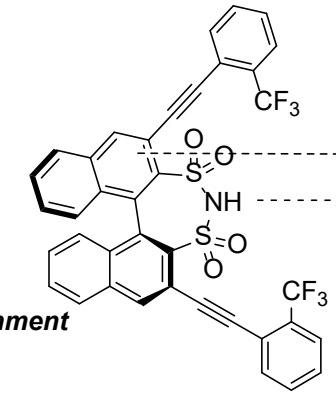
Enzyme and Chiral Catalyst



Chiral Environment

Reactivity

- *Active site ensures the reactivity*
- *Protein skeleton provides Chiral Environment*



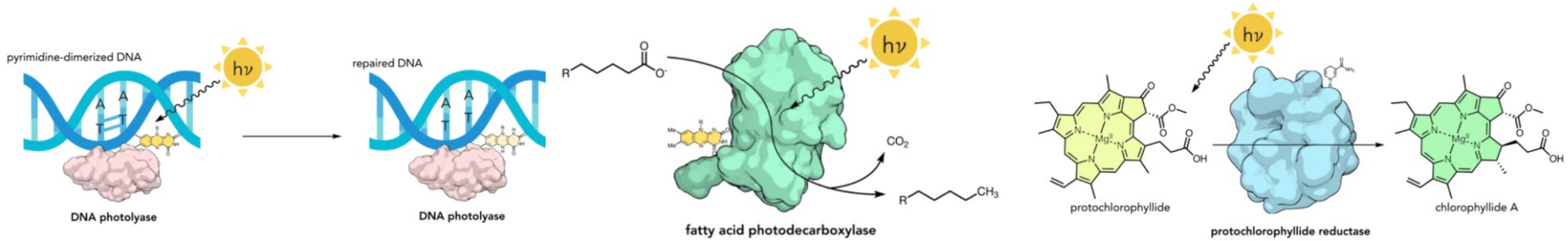
Chiral Environment

Reactivity

- *Main functional group ensures the reactivity*
- *Chemical skeleton provides Chiral Environment*

Only Three Light-dependent Enzymes in Nature

Visible Light Photocatalysis: An immensely powerful tool for synthetic transformations, using photon-absorbing dyes or metal complexes to enable electron- and energy-transfer reactions with high-energy intermediates.



Leverage photoexcitation in biocatalysis to unlock new reactivities for natural enzymes.

Harrison, W.; Huang, X.; Zhao, H. *Acc. Chem. Res*. **2022**, *55*, 1087-1096

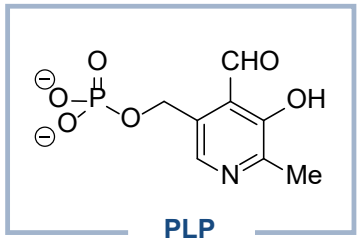
Hyster, T. K, **Hyster Group Website**, "*Photoenzymatic catalysis for selective chemical synthesis*"

Outline

- **Pyridoxal phosphate (PLP) Dependent Photo-enzymatic Synthesis**
- **Flavin Dependent Photo-enzymatic Synthesis**
- **Summary**
- **Perspective**

PLP Dependent Natural Enzyme

Pyridoxal 5'-Phosphate (PLP)

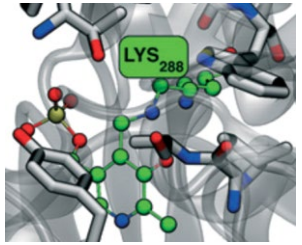


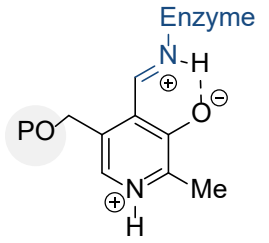
PLP

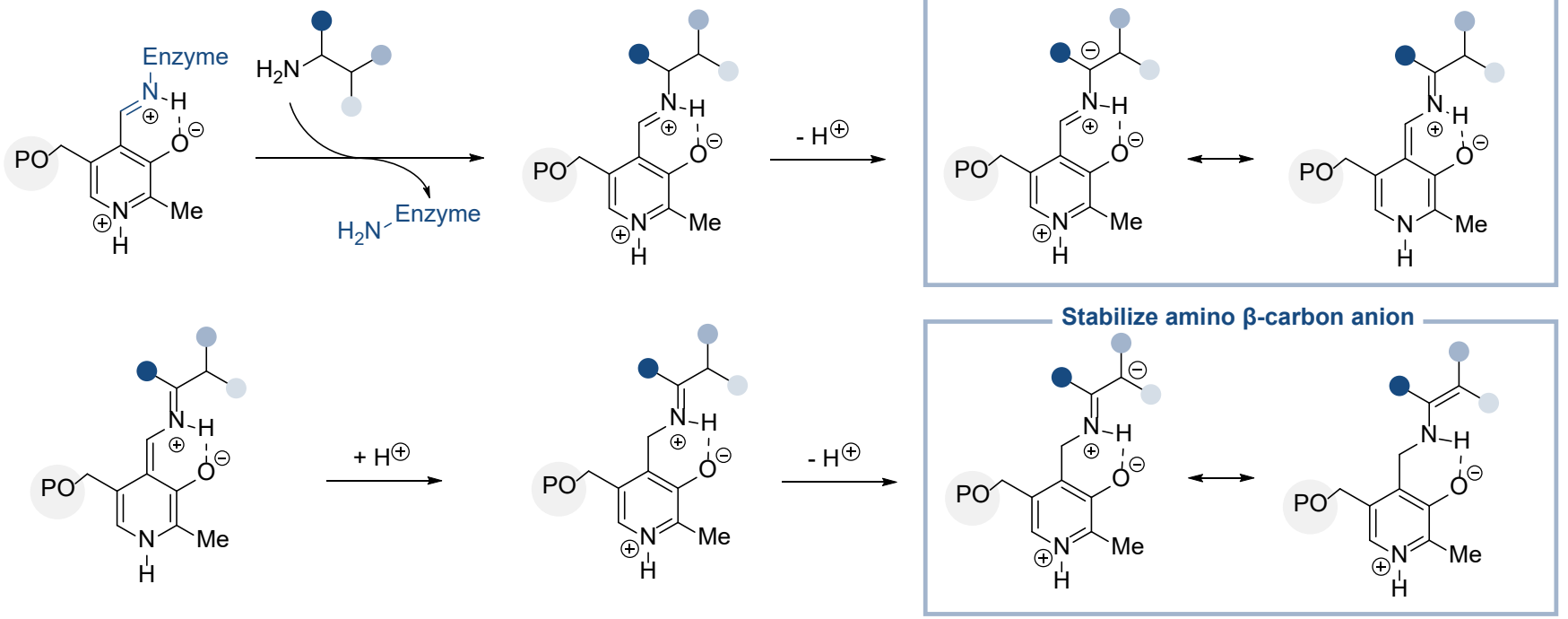
PLP Dependent Enzyme

- Transaminase
- Glutamate Decarboxylase
- Tryptophan Synthase

Coenzyme PLP



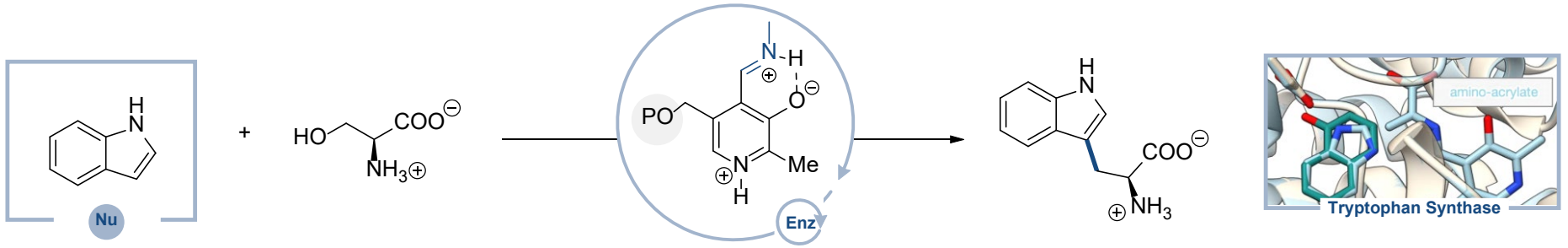


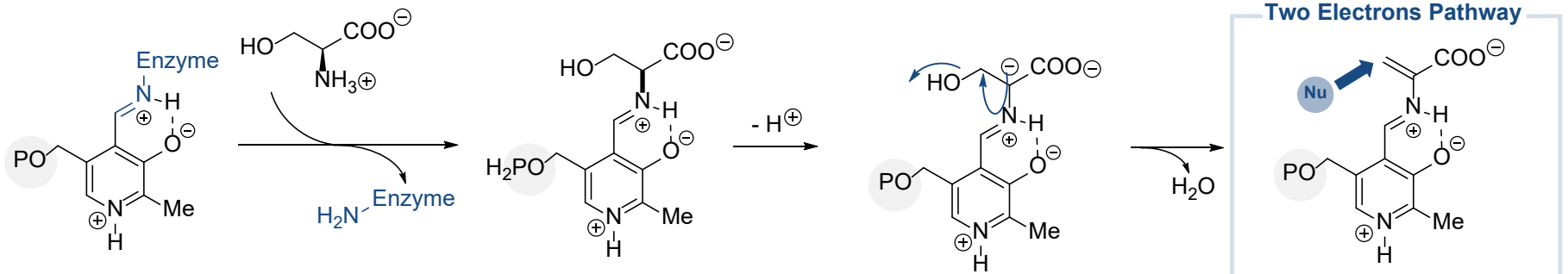


PLP: Stabilize amino α, β- carbon anion

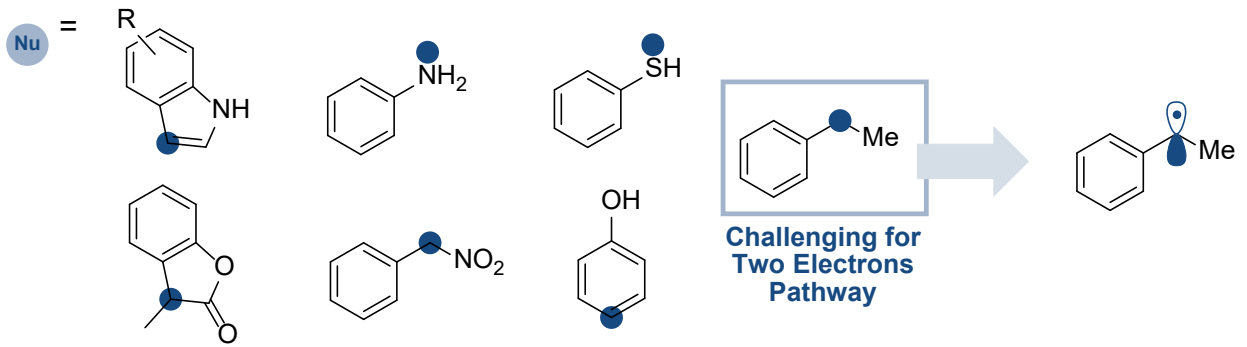
Rocha, F.; Pina, A. F.; Sousa, S. F.; Cerqueira, N. M. F. S. A. *Catal. Sci. Technol.*, **2019**, *9*, 4864–4876

Natural Enzyme: Tryptophan Synthase





Previous Two Electrons Pathway Study

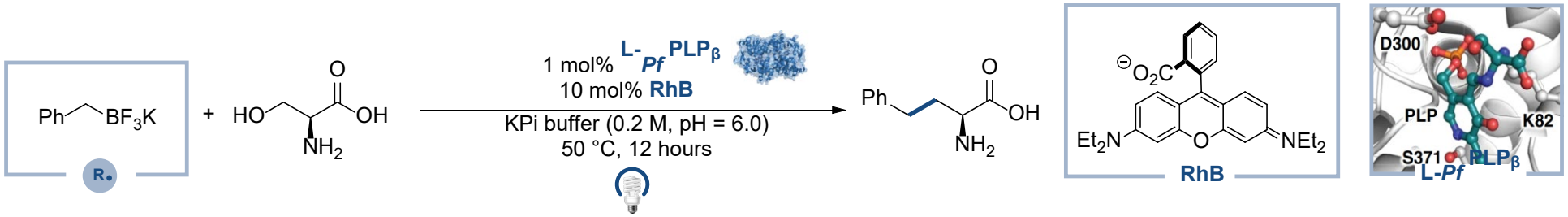


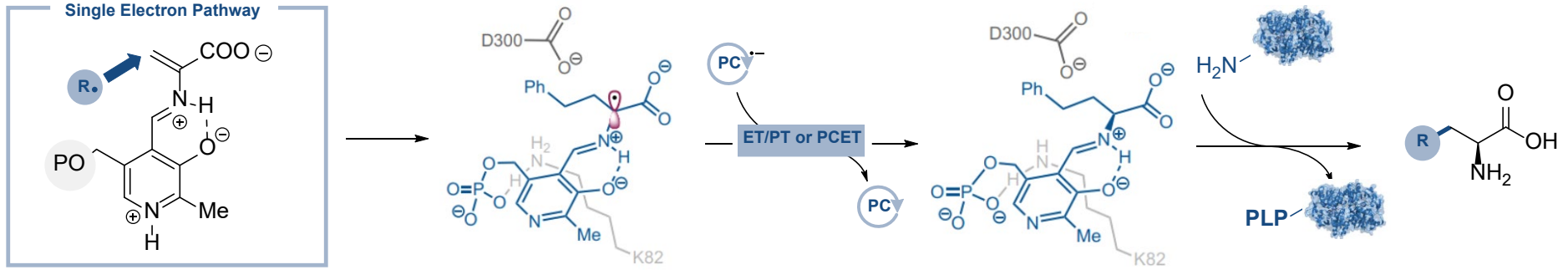
Miles, E. W. *Chem. Rec.*, **2001**, *1*, 140

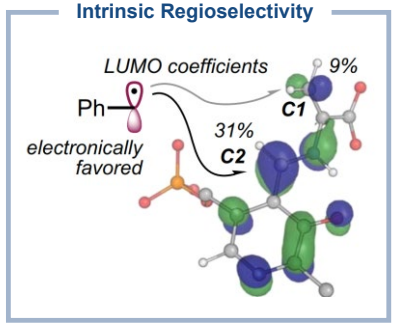
Dulaney, E. W.; Straathof, S.; Arnold, F. H. *ChemBioChem*, **2021**, *22*, 5-16

Almhjell, P. J.; Johnston, K. E.; Porter, N. J.; Kennemur, J. L.; Bhethanabotla, V. C.; Ducharme, J.; Arnold, F. H. *Nat. Chem. Bio.*, **2024**, https://doi.org/10.1038/s41589-024-01619-z

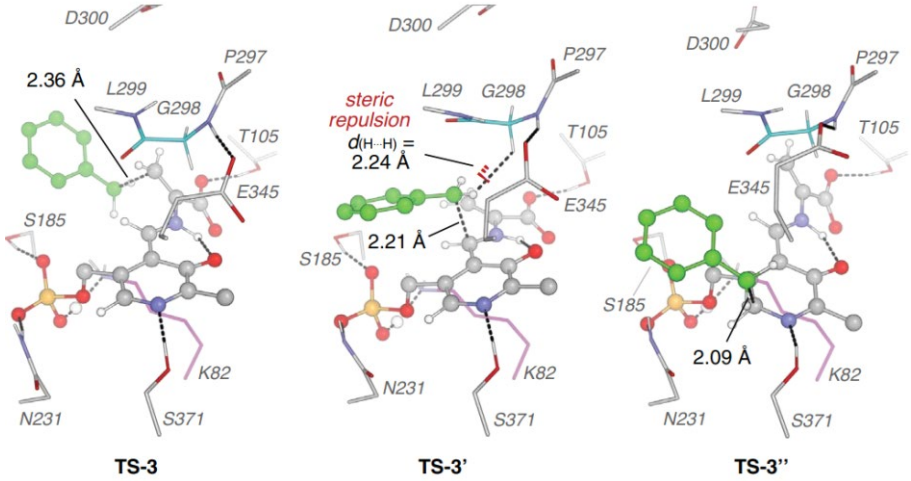
Radical Approach to Tryptophan Synthase







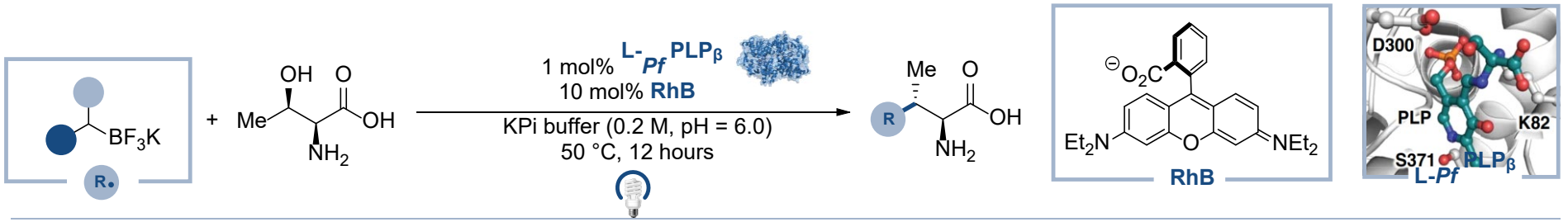
radical addition transition state	ΔH‡ (kcal/mol)	
	theozyme	free PLP cofactor
TS-3	5.6	8.3
TS-3'	8.8	8.1
TS-3''	16.1	16.1



The enzymatic skeleton will change the regioselectivity for radical addition.

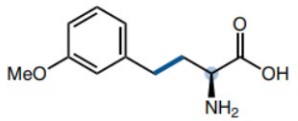
Cheng, L.; Li, D.; Mai, B. K.; Bo, Z.; Cheng, L.; Liu, P.; Yang, Y. *Science,* **2023**, *381*, 444-451

Radical Approach to Tryptophan Synthase

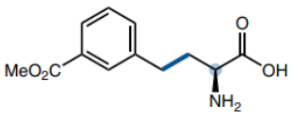


Directed Evolution

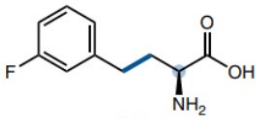
1	wt *Pf*TrpS β-subunit P12L E17G I68V F274S T292S T321A	50	91:9
2	wt *Pf*TrpS β-subunit P12L E17G I68V F274S T292S T321A L12P I16V F95L V384A (L-*Pf*PLP$^{\beta}$)	73	93:7
3	L-*Pf*PLP$^{\beta}$ V68I L91P L161A V173E S274L A321T	26	75:25
4	L-*Pf*PLP$^{\beta}$ M139L N166D L212P	46	71:29
5	L-*Pf*PLP$^{\beta}$ M139L N166D L212P E104G	54	13:87
6	L-*Pf*PLP$^{\beta}$ E104G (D-*Pf*PLP$^{\beta}$)	74	11:89
7	L-*Pf*PLP$^{\beta}$ M139L I165F N166D Y301H	13	95:5



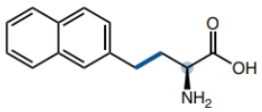
L-3e
(62 ± 4)% yield
93:7 e.r.



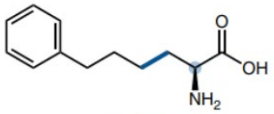
L-3h
(23 ± 3)% yield
96:4 e.r.



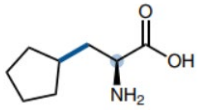
L-3f
(65 ± 5)% yield
92:8 e.r.



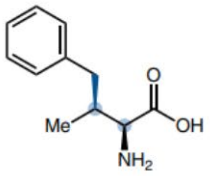
L-3l
(47 ± 5)% yield
90:10 e.r.



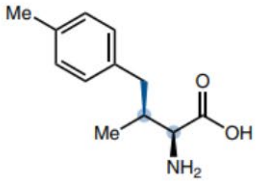
L-3n
(15 ± 1)% yield
71:29 e.r.



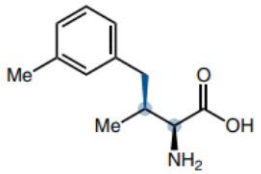
L-3o
(4 ± 1)% yield
88:12 e.r.



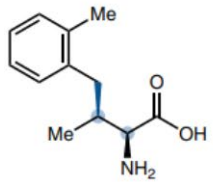
4a
(80 ± 2)% yield
d.r. = 19 : 1
e.r.(major) > 99 : 1
e.r.(minor) = 94 : 6



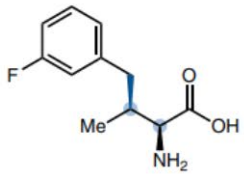
4b
(75 ± 2)% yield
d.r. = 13 : 1
e.r.(major) > 99 : 1
e.r.(minor) = 97 : 3



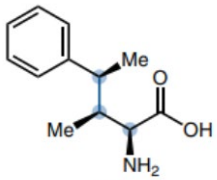
4c
(74 ± 1)% yield
d.r. = 10 : 1
e.r.(major) > 99 : 1
e.r.(minor) = 90 : 10



4d
(79 ± 1)% yield
d.r. = 14 : 1
e.r.(major) > 99 : 1
e.r.(minor) = 98 : 2



4e
(83 ± 2)% yield
d.r. = 11 : 1
e.r. (major) > 99 : 1
e.r. (minor) = 98 : 2



5a
(84 ± 3)% yield
d.r. > 20 : 1
e.r. (major) > 99 : 1

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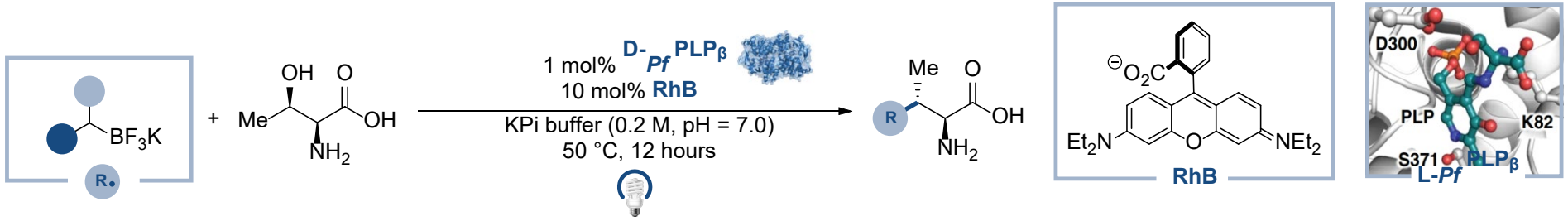
Radical Approach to Tryptophan Synthase



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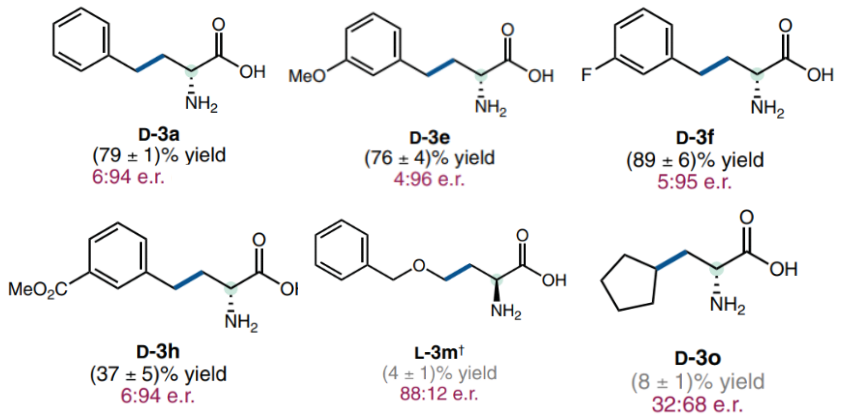

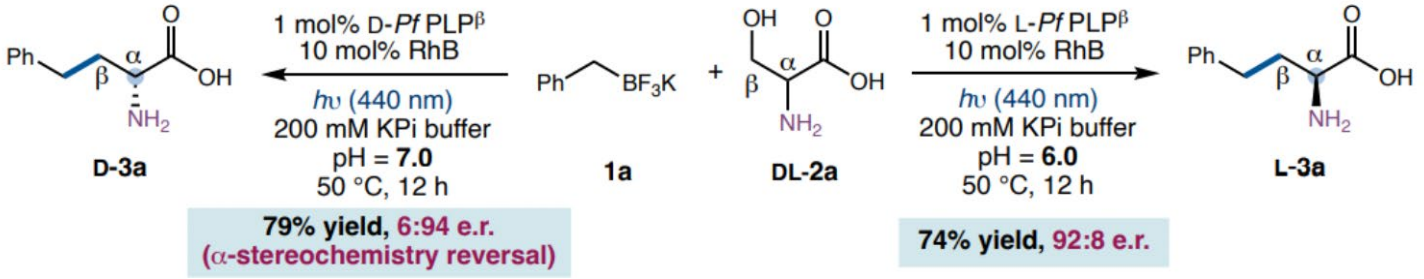

All the biocatalytic transformations described in this paper could be performed with DL-serine.

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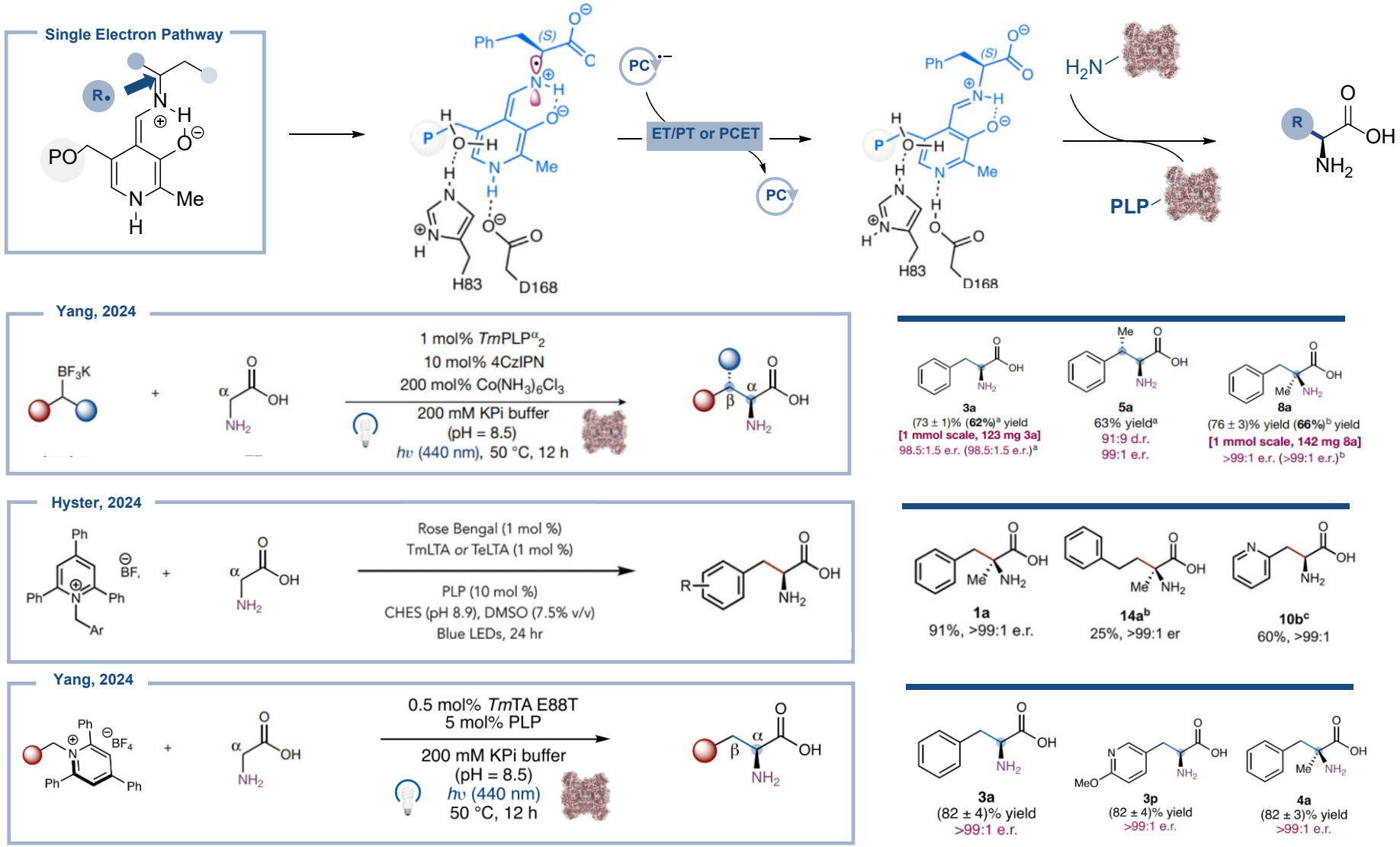

Wang, T.-C.; Mai, B. K.; Zhang, Z.; Bo, Z.; Li, J.; Liu, P.; Yang, Y. *Nature,* ***2024***, *629*, 98-104

Ouyang, Y.; Page, C. G.; Bilodeau, C.; Hyster, T. K. *J. Am. Chem. Soc.* **2024**, *146*, 13754−13759

Wang, T.-C.; Zhang, Z.; Rao, G.; Li, J.; Shirah, J.; Britt, R. D.; Zhu, Q.; Yang, Y. *J. Am. Chem. Soc.* **2024**, *ASAP*, 10.1021/jacs.4c05949

Outline

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- **Flavin Dependent Photo-enzymatic Synthesis**
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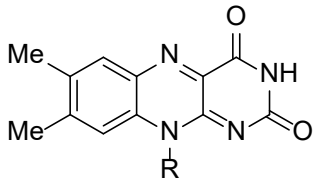
Flavin Dependent Enzymes

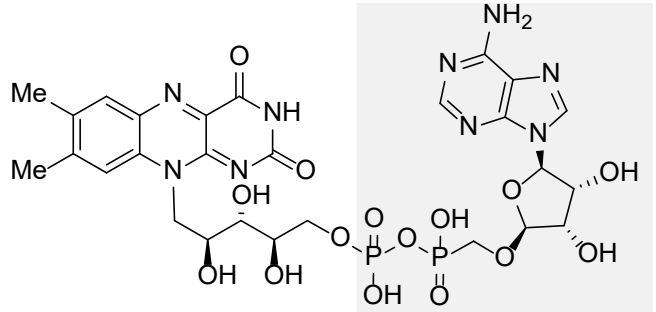
Flavin



Flavin Dependent Enzyme

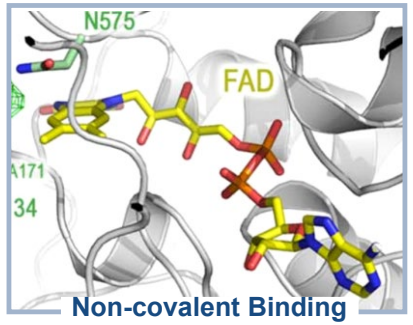
- Fatty Acid Photodecarboxylase
- Ene-reductase
- DNA Photolyase

Flavin Adenine Dinucleotide (FAD)



Riboflavin

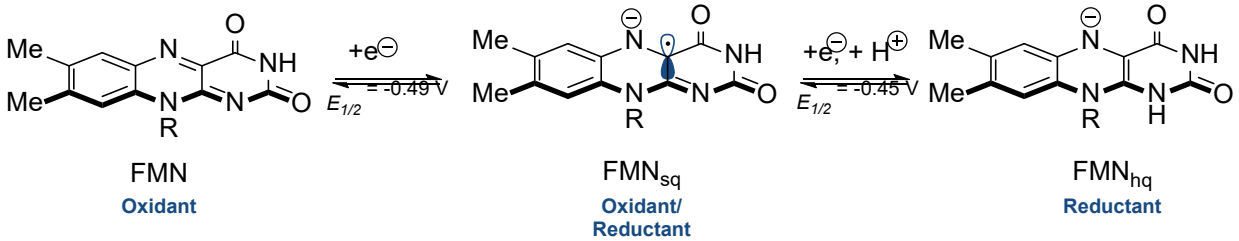
Flavin Mononucleotide (FMN)



Non-covalent Binding

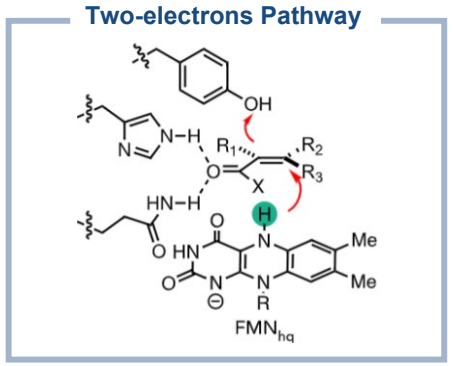
Two-electrons Pathway

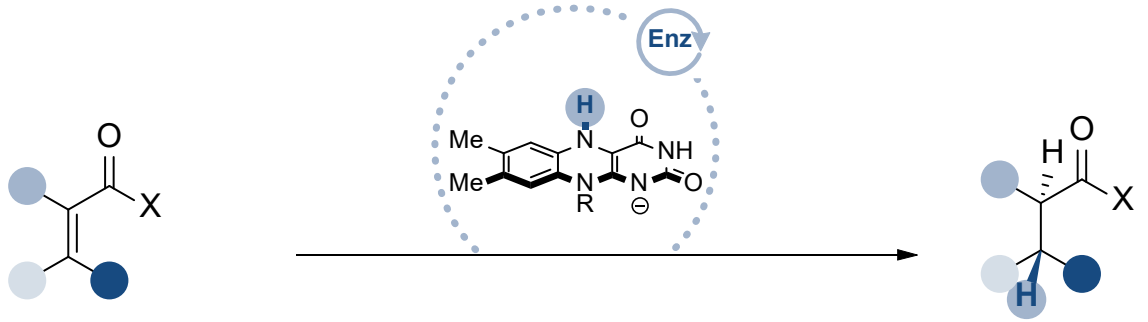
Single-electron Pathway

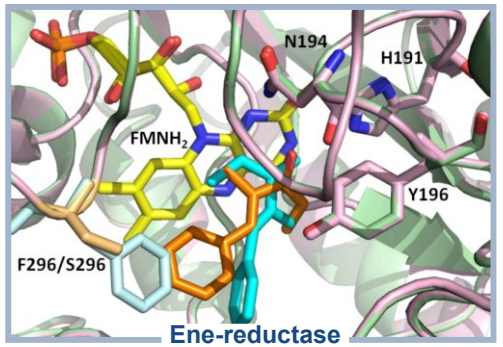


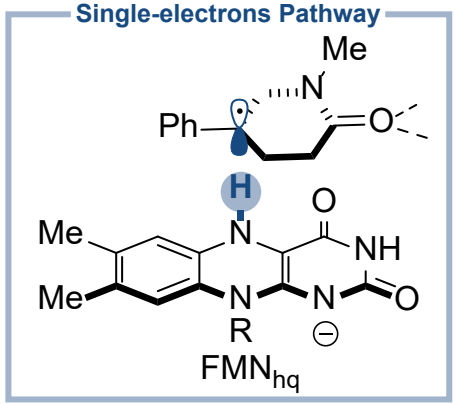
Biegasiewicz, K. F.*; Cooper, S. J.*; Gao, X.*; Oblinsky, D. J.; Kim, J. H.; Garfinkle, S. E.; Joyce, L. A.; Sandoval, B. A.; Scholes, G. D.; Hyster, T. K *Science* **2019**, *364*, 1166–1169

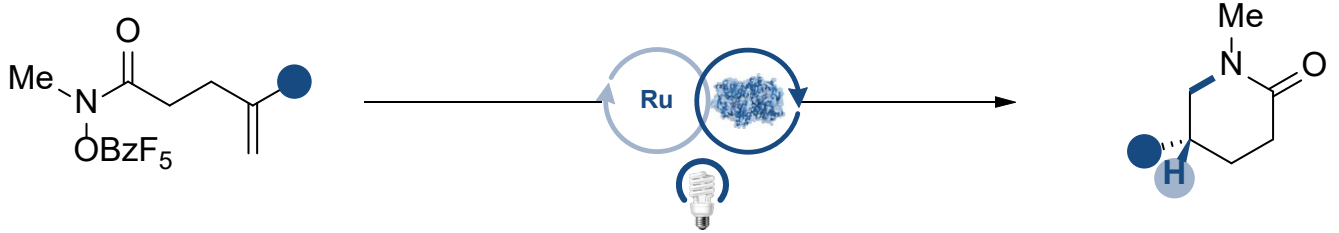
Ene-Reductase: From Hydride Transfer to Hydrogen Atom Transfer

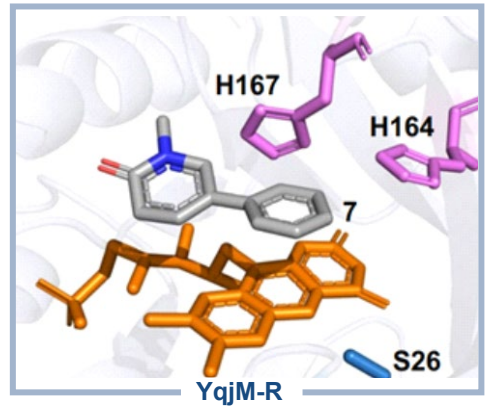








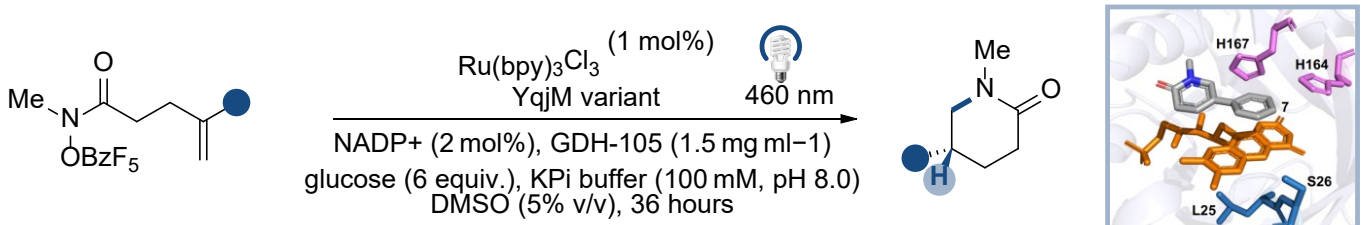




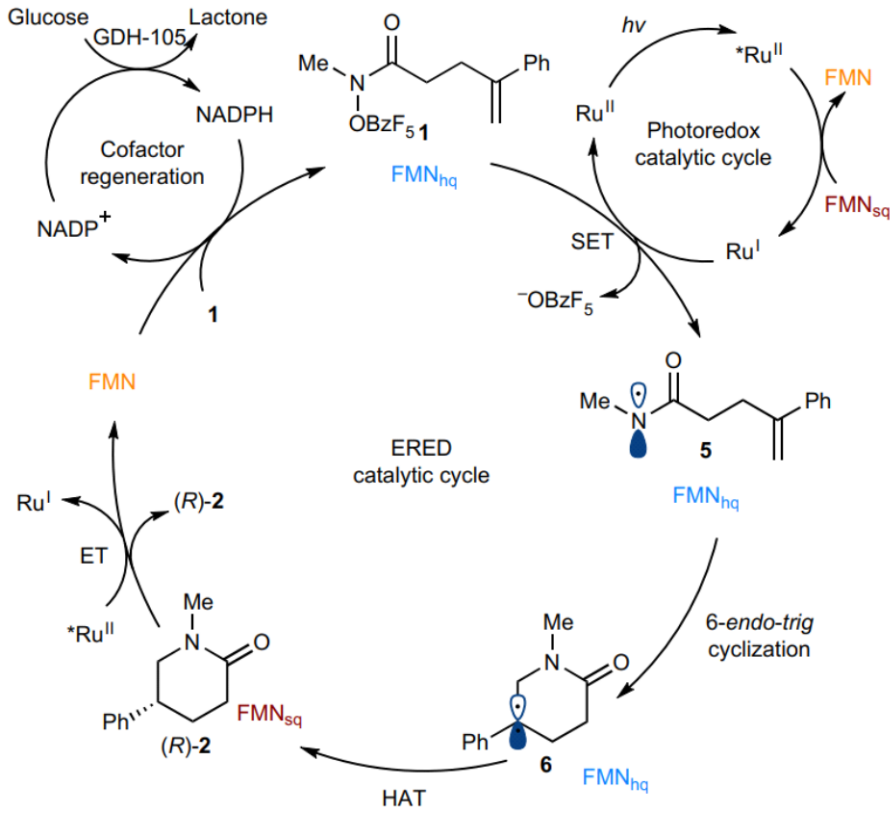
Kohli, R. M.; Massey, V. J. *Biol. Chem,* **1998**, *273*, 32763-32770

Ye, Y.; Cao, J.; Oblinsky, D. G.; Verma, D.; Prier, C. K.; Scholes, G. D.; Hyster, T. K. *Nat. Chem.* **2023**, *15*, 206-212

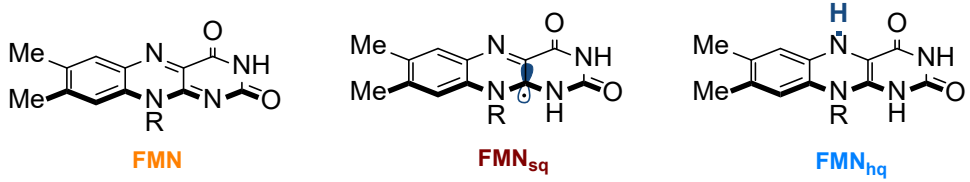
Ene-Reductase: Hydrogen Atom Transfer



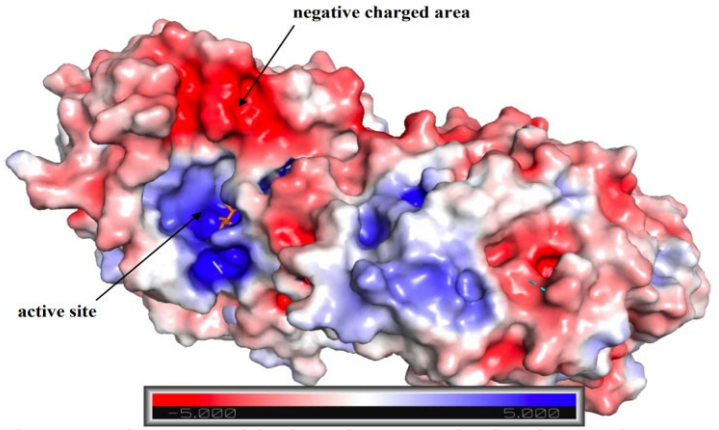
Proposed Catalysis Cycle



Flavin



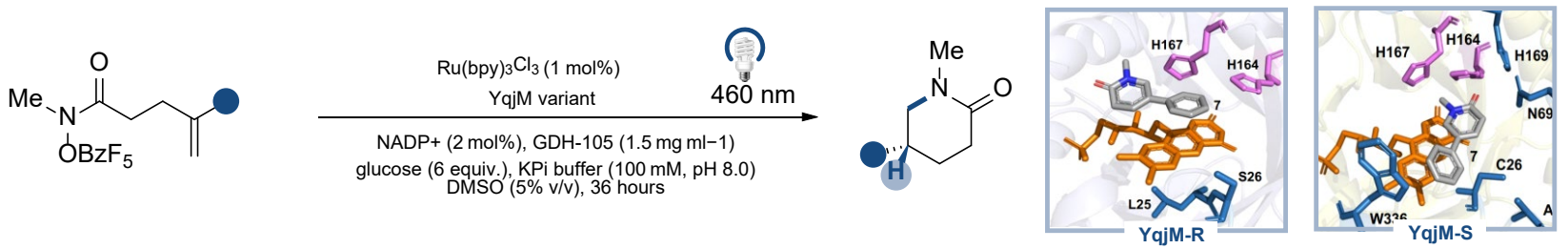
Calculated Surface Charge of YqjM

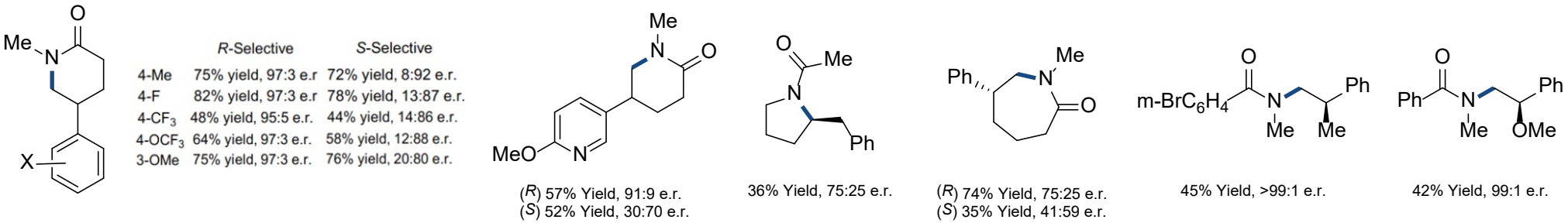


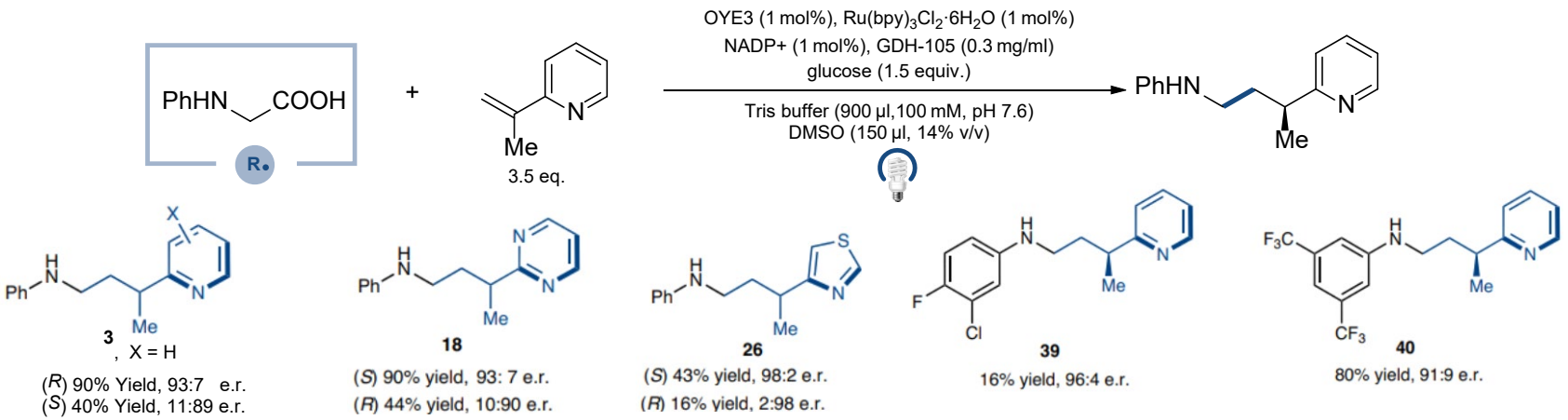
The photocatalyst may bind to the negatively charged area near the enzyme active site to facilitate the SET.

Ye, Y.; Cao, J.; Oblinsky, D. G.; Verma, D.; Prier, C. K.; Scholes, G. D.; Hyster, T. K. *Nat. Chem.* **2023**, *15*, 206-212

Ene-Reductase: Hydrogen Atom Transfer



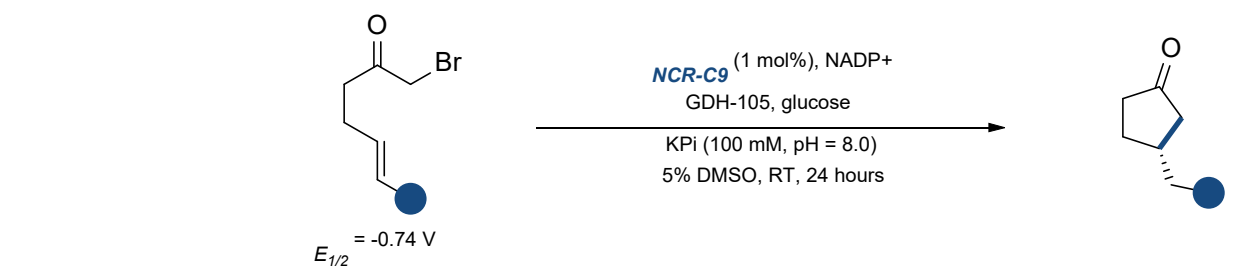


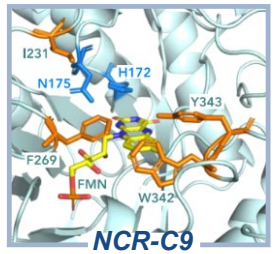


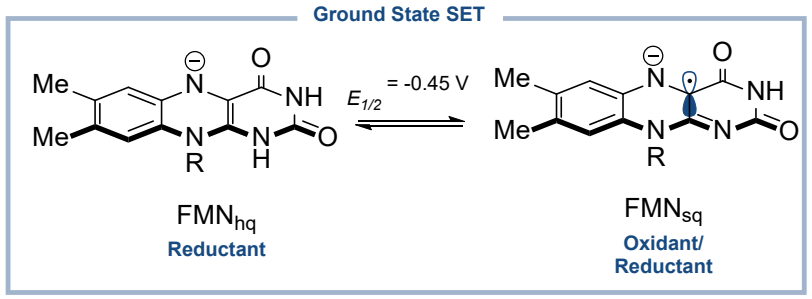
Ye, Y.; Cao, J.; Oblinsky, D. G.; Verma, D.; Prier, C. K.; Scholes, G. D.; Hyster, T. K. *Nat. Chem.*, **2023**, *15*, 206-212

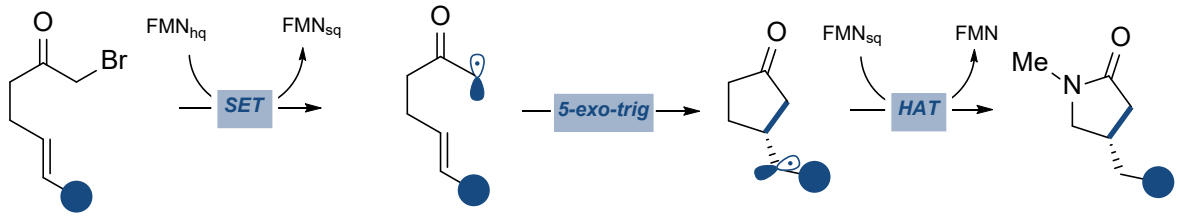
Sun, S.- J.; Nicholls, B. T.; Bain, D.; Qiao, T.; Page, C. G.; Musser, A. J.; Hyster, T. K. *Nat. Catal.*, **2024**, *7*, 35-42

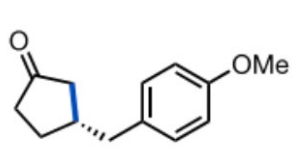
Flavin Hydroquinone: Ground State Reductant



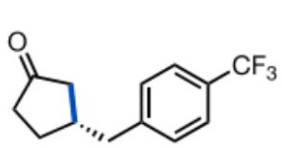




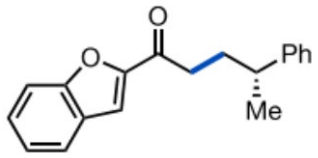




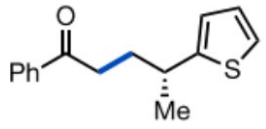
2f
86% yield
95:5 er



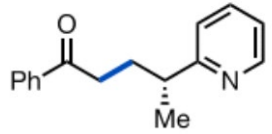
2i
77% yield
94:6 er



8m
85% yield
98:2 er



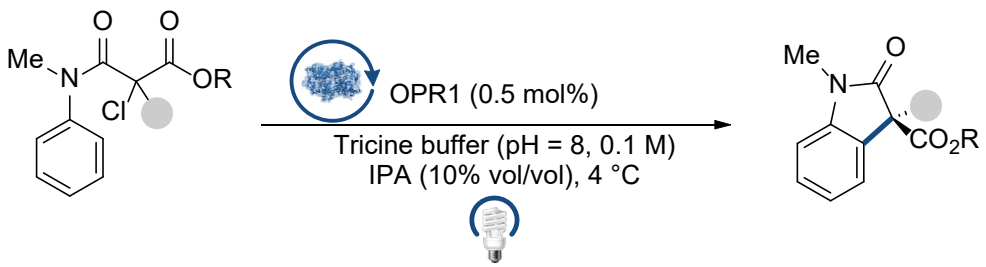
8x
74% yield
99:1 er

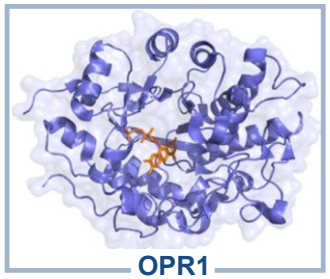


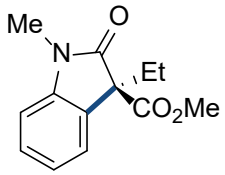
8w
61% yield
81:19 er

Fu, H.; Lam, H.; Emmanuel, M. A.; Kim, J. H.; Sandoval, BG. A .; Hyster, T. K *J. Am. Chem. Soc*., **2021** *143*, 9622-9629

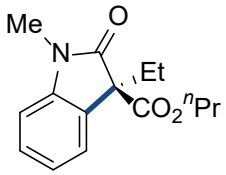
Flavin Semiquinone: Ground State Reductant



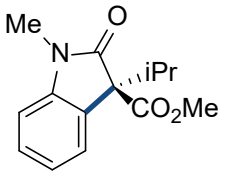




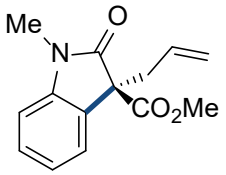
95% Yield
95:5 e.r.



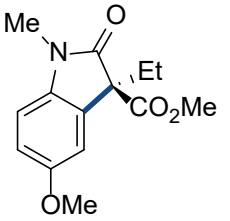
80% Yield
94:6 e.r.



39% Yield
82:18 e.r.

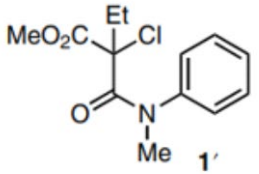


94% Yield
97.5:2.5 e.r.



41% Yield
84:16 e.r.

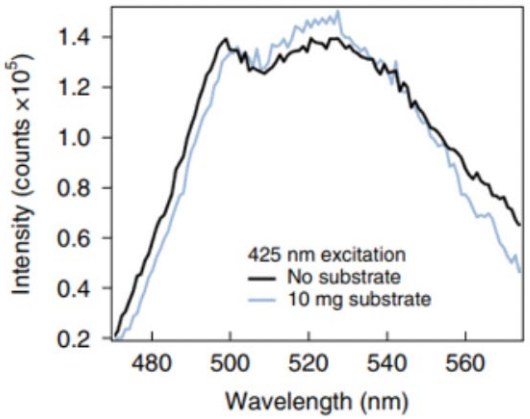
Experiments to exclude FMN_{hq}



OPR1 (0.5 mol%)
NADPH (20 mol%)
Tricine (pH 8.0, 100 mM)
*i*PrOH (10% vol/vol), 24 °C
2 h

No reaction

∴ Ground-state FMN_{hq} is not responsible for reduction

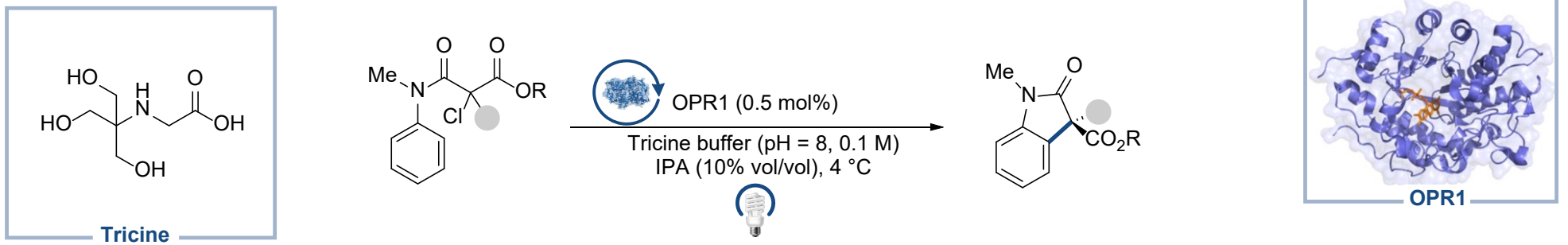


FMN_{hq} fluorescence is not quenched by the substrate

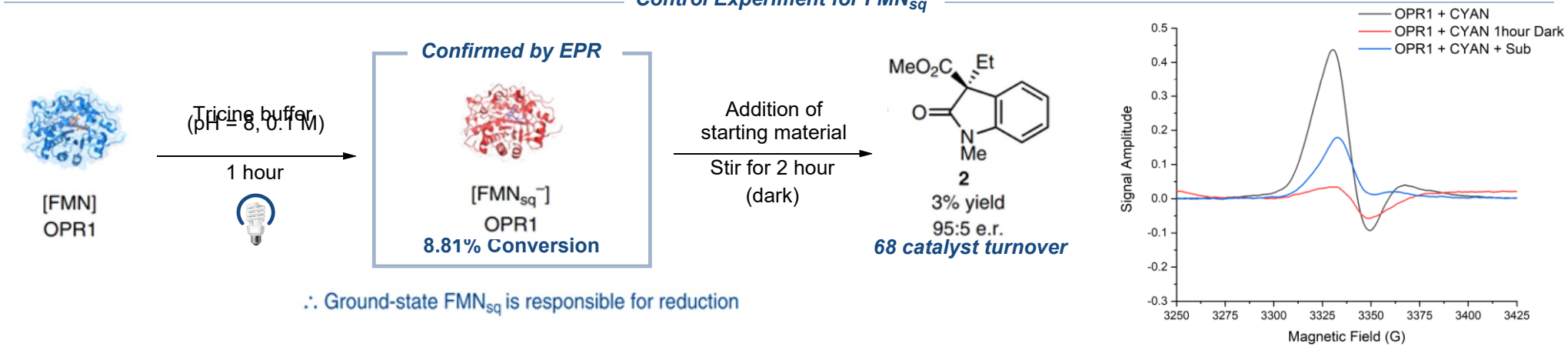
∴ Excited-state FMN_{hq} is not responsible for reduction

Black, M. J.; Biegasiewicz, K. F.; Meichan, A. J.; Oblinsky, D. G.; Kudisch, B.; Scholes, G. D.; Hyster, T. K. *Nat. Chem* **2020**, *12*, 71-75

Flavin Semiquinone: Ground State Reductant

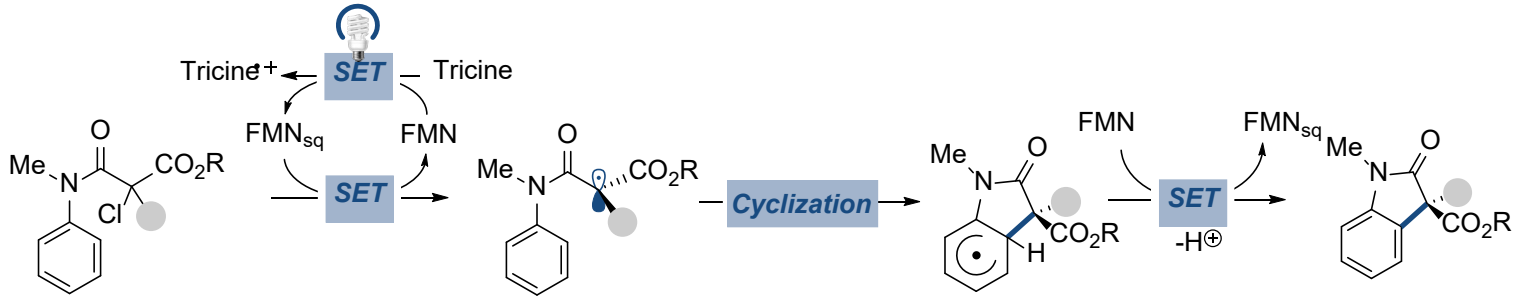


Control Experiment for FMN_{sq}



∴ Ground-state FMN_{sq} is responsible for reduction

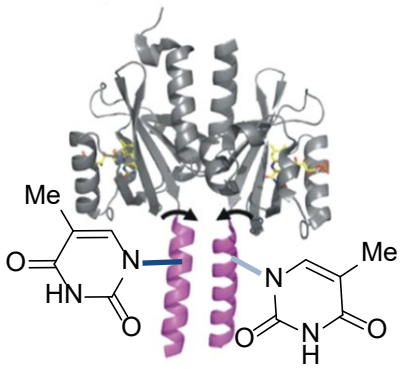
Possible Mechanism

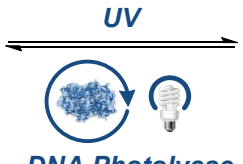


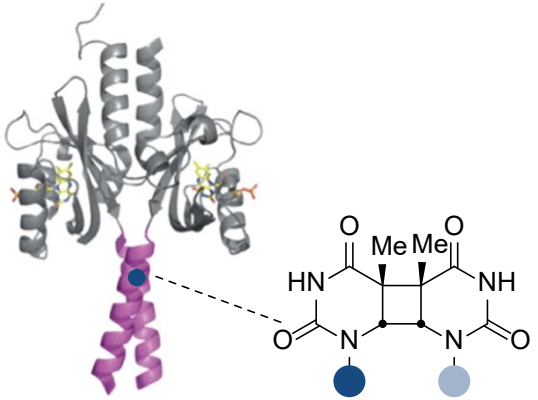
Black, M. J.; Biegasiewicz, K. F.; Meichan, A. J.; Oblinsky, D. G.; Kudisch, B.; Scholes, G. D.; Hyster, T. K. *Nat. Chem* **2020**, *12*, 71-75

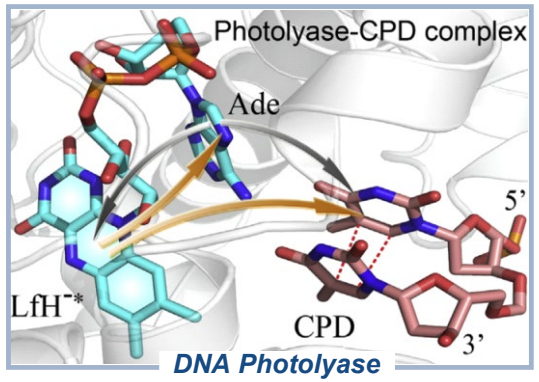
Photo-actived Natural Enzyme: DNA Photolyase

DNA Dimeric Lesion and Photoreactivation

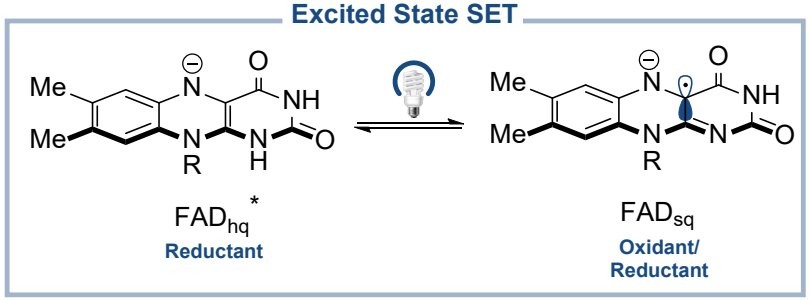


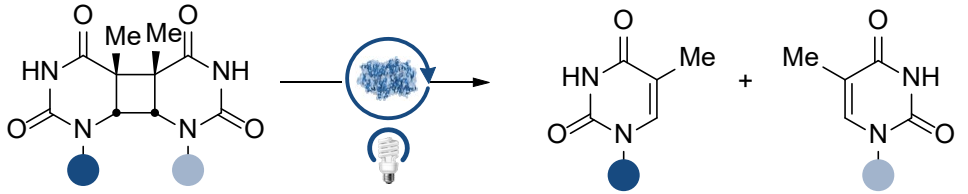


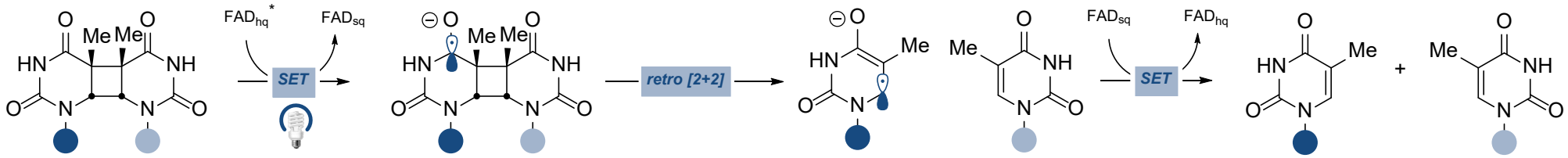




Excited State SET



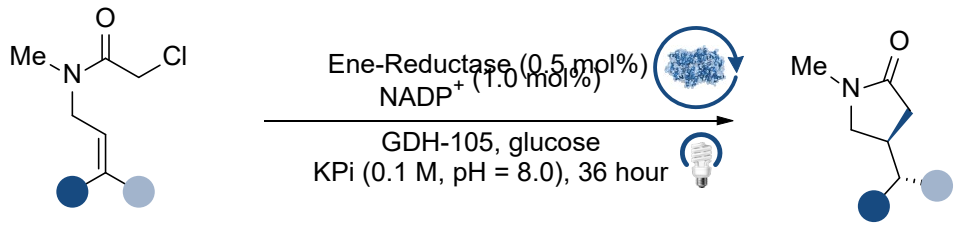




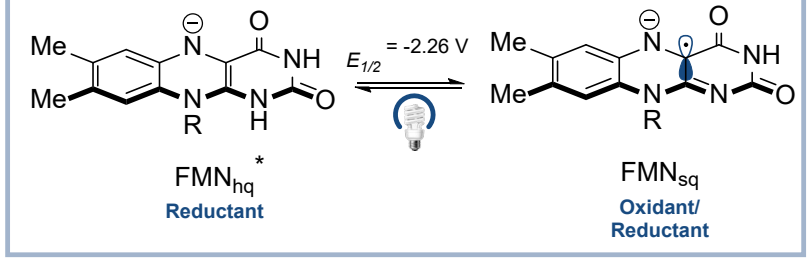
Zhang, M.; Wang, L.; Zhong, D. *Arch. Biochem.Biophys.* **2017**, *632*, 158−174

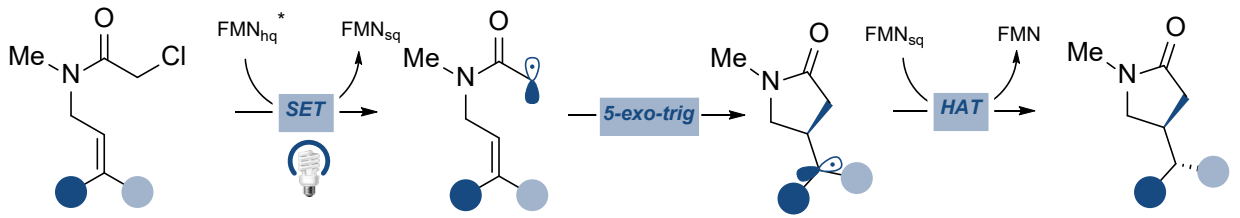
Conrad, K. S.; Manahan, C. C.; Crane, B. R. *Nat. Chem. Biol*. **2014**, *10*, 801−809.

Flavin Hydroquinone: Excited State Reductant

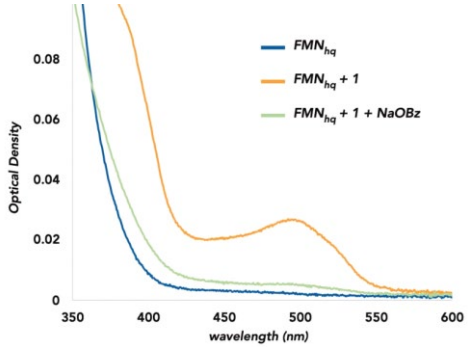


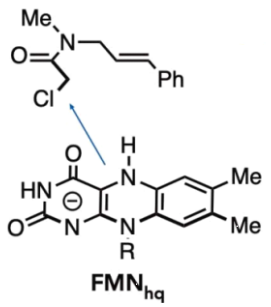
Excited State SET



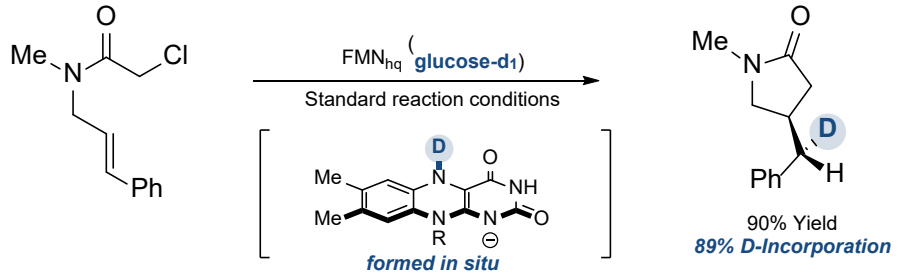


Formation of EDA Complex



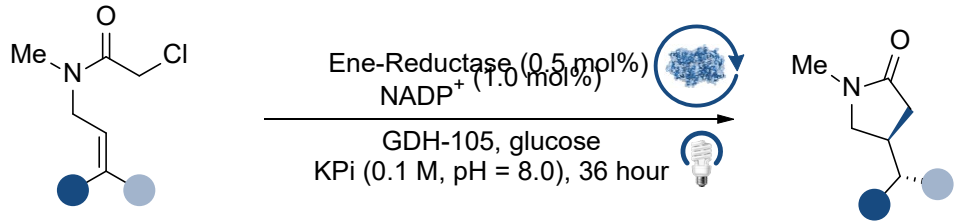


Deuterium-labelling Evidence for HAT

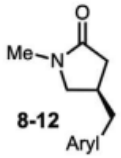


Biegasiewicz, K. F.; Cooper, S. J.; Gao, X.; Oblinsky, D. G.; Kim, J. H.; Garfinkle, S. E.; Joyce, L. A.; Sandoval, B. A.; Scholes, G. D.; Hyster, T. K. *Science* **2019**, *364*, 1166–1169

Flavin Hydroquinone: Excited State Reductant

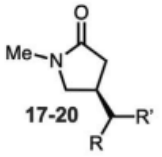


5-exo-trig (aryl substitution)



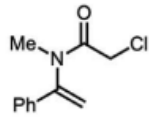
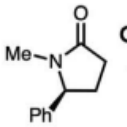
Aryl	GluER-T36A
4-OMeC$_6$H$_4$	91% yield, 96:4 er
3-OMeC$_6$H$_4$	73% yield, 95:5 er
2-OMeC$_6$H$_4$	84% yield, 95:5 er
4-BrC$_6$H$_4$	80% yield, 95:5 er
4-CF$_3$C$_6$H$_4$	98% yield, 96:4 er

5-exo-trig (alkyl substitution)

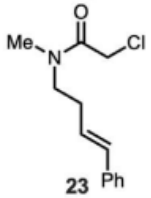
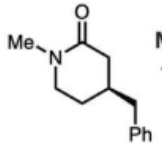


R, R'	GluER-T36A
Me, H	97% yield, 74:26 er
Et, H	99% yield, 76:24 er
	NostocER
Me, Me	81% yield, 15:85 er[a]
C_4H_8	99% yield, 10:90 er[a]

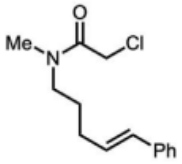
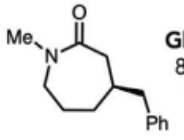
5-endo-trig

Starting Material	Product
	 GluER-T36A 51% yield[b] 93:7 er
21	22

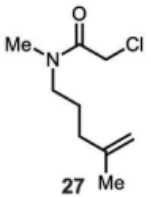
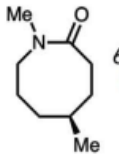
6-exo-trig

Starting Material	Product
	 MorB-Y72F 44% yield[c] 86:14 er
23	24

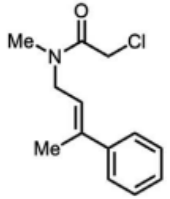
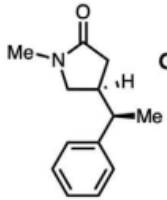
7-exo-trig

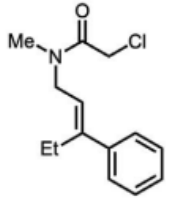
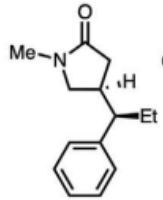
Starting Material	Product
	 GluER-T36A 87% yield[d] 90:10 er
25	26

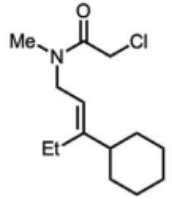
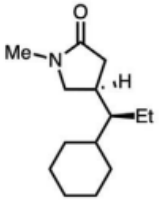
8-endo-trig

Starting Material	Product
	 LacER 67% yield[e] 83:17 er
27	28

Diastereoselective Cyclization

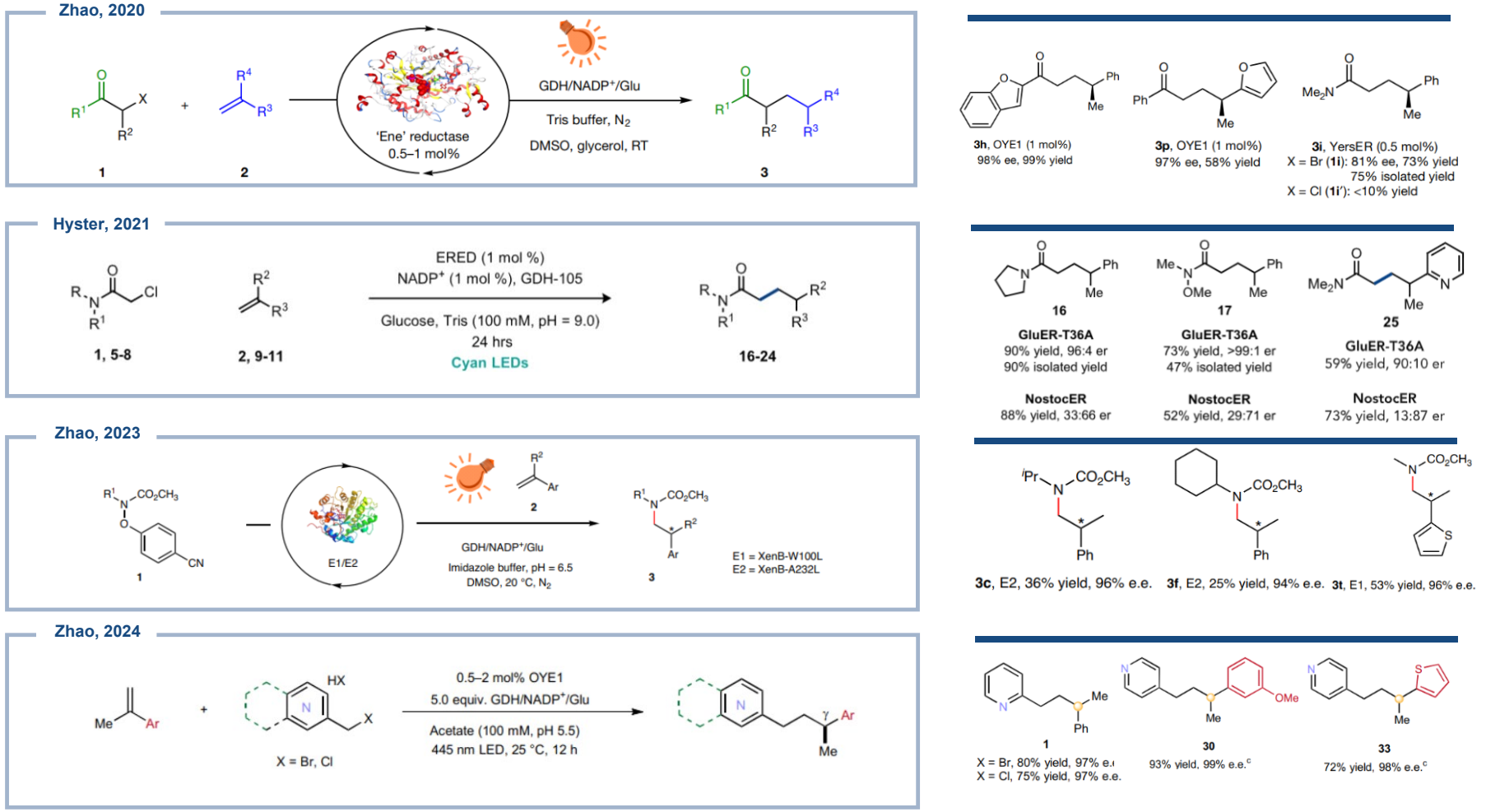
Starting Material	Product
	 GluER-T36A 75% yield >99:1 er 92:8 dr
29	30

Starting Material	Product
	 GluER-T36A 67% yield 95:5 er 87:13 dr
31	32

Starting Material	Product
	 GluER-T36A 84% yield >99:1 er 79:21 dr
33	34

Biegasiewicz, K. F.; Cooper, S. J.; Gao, X.; Oblinsky, D. G.; Kim, J. H.; Garfinkle, S. E.; Joyce, L. A.; Sandoval, B. A.; Scholes, G. D.; Hyster, T. K. *Science* **2019**, *364*, 1166–1169

Flavin Hydroquinone: Excited State Reductant



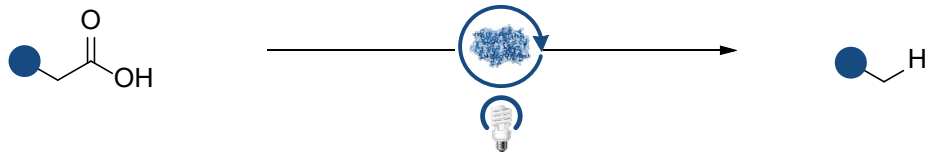
Huang, X., Wang, B., Wang, Y. et al. *Nature* **2020**, *584*, 69–74

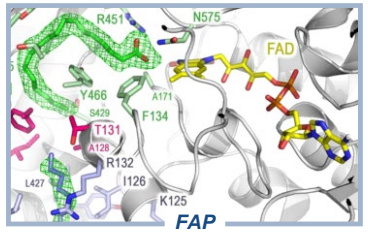
Page, C. G.; Cooper, S. J.; DeHovitz, J. S. et al. *J. Am. Chem. Soc*. **2021** *143*, 97-102

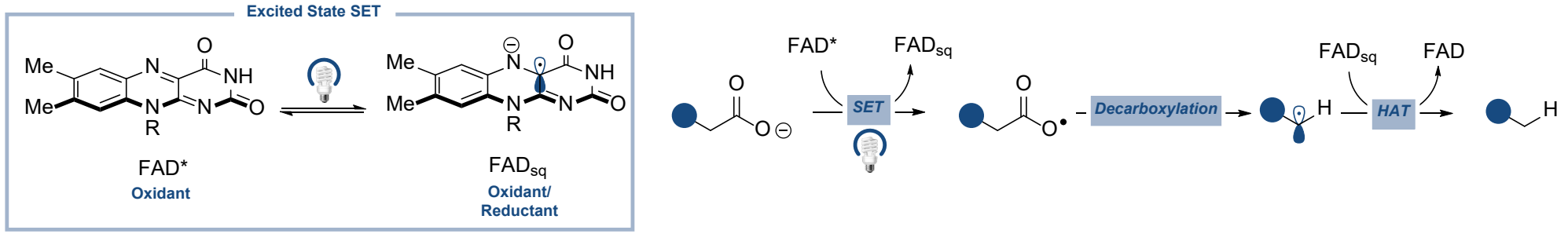
Zhang, Z., Feng, J., Yang, C. et al. *Nat Catal* **2023**, *6*, 687–694

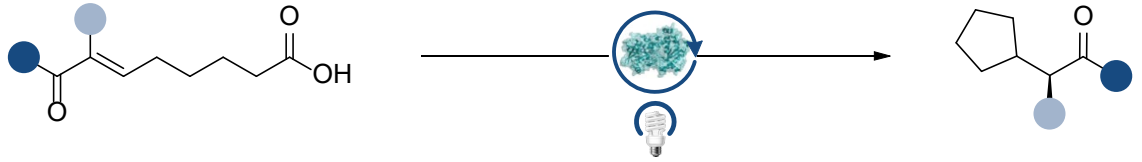
Li, M., Harrison, W., Zhang, Z. et al. *Nat. Chem.* **2024**, *16*, 277–284

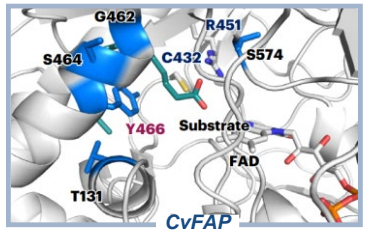
Fatty Acid Photodecarboxylase: Flavin as Excited State Oxidant

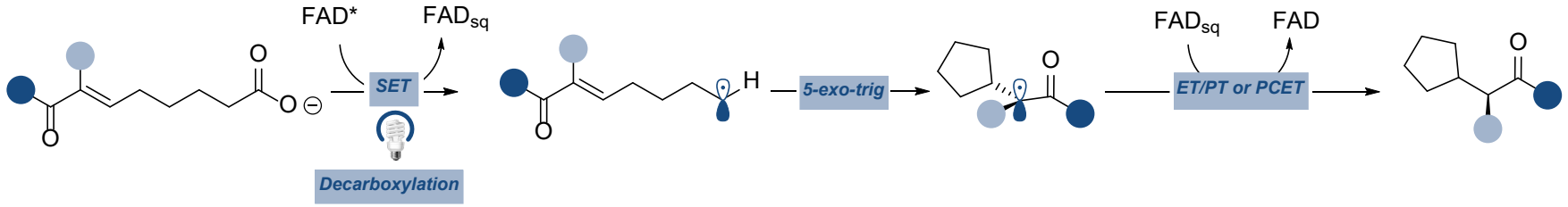








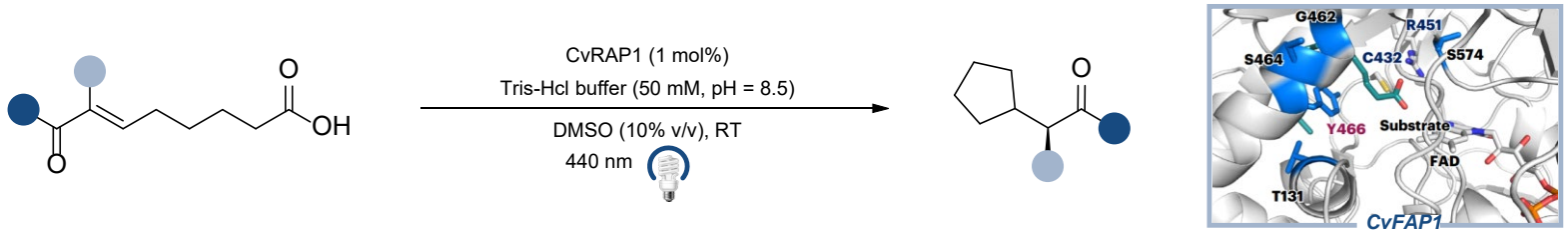


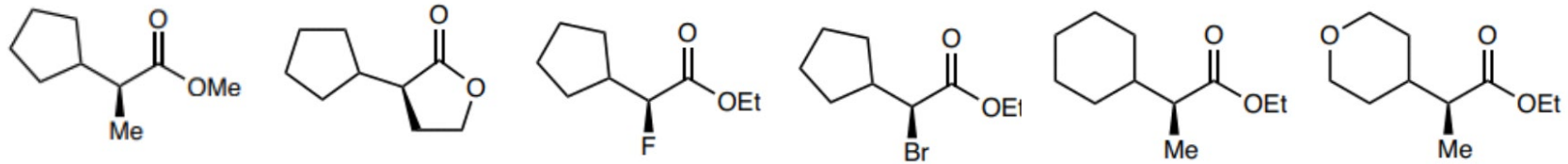


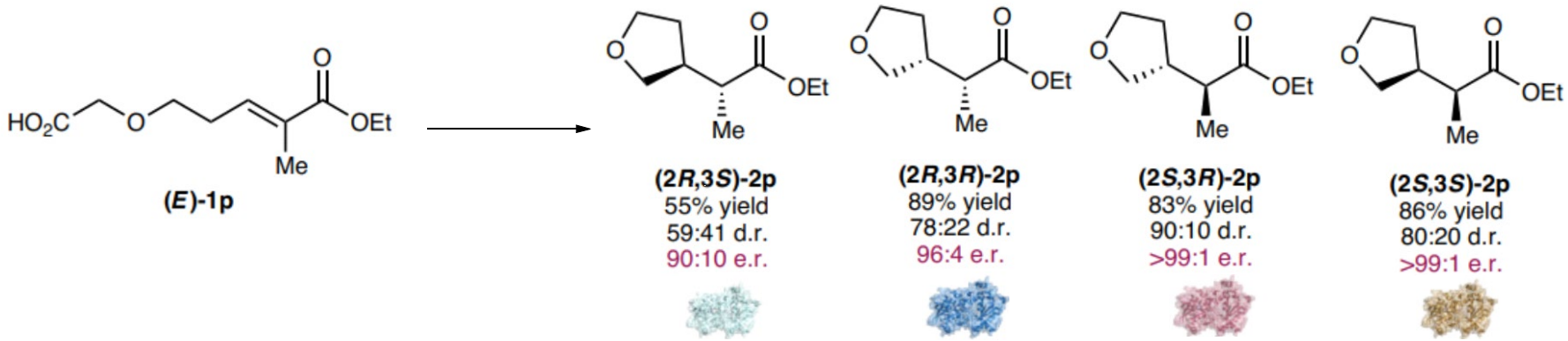
Sorigué, D. et al., *Science,* **2017**, *357*, 903-907

Ju, S., Li, D., Mai, B.K. et al. *Nat. Chem.*, **2024**. https://doi.org/10.1038/s41557-024-01494-0

Flavin: Excited State Oxidant







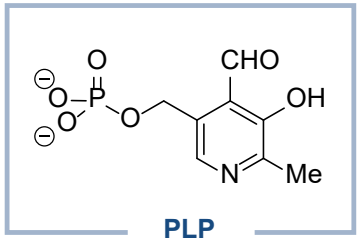
Ju, S., Li, D., Mai, B.K. et al. *Nat. Chem.*, **2024**. https://doi.org/10.1038/s41557-024-01494-0

Outline

- Pyridoxal phosphate (PLP) Dependent Photo-enzymatic Synthesis
- Flavin Dependent Photo-enzymatic Synthesis
- **Summary**
- Perspective

Summary

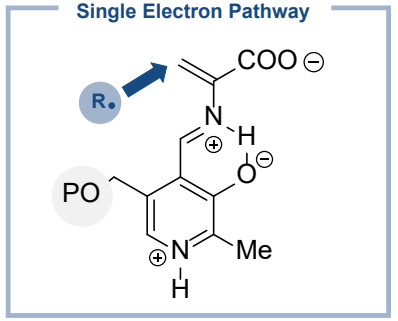
Pyridoxal 5'-Phosphate (PLP)

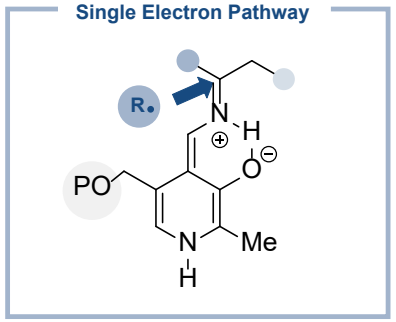


PLP

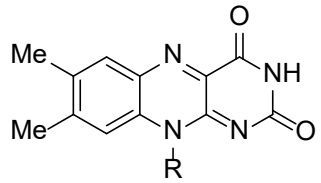
PLP Dependent Enzyme

- Transaminase
- Glutamate Decarboxylase
- Tryptophan Synthase



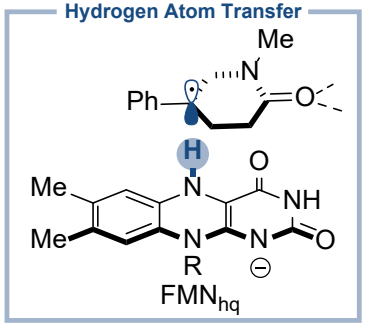


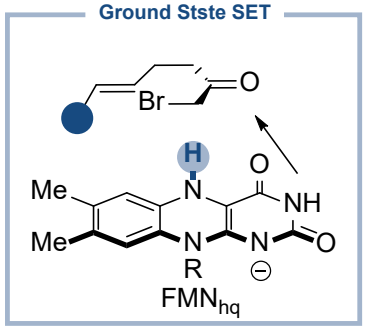
Flavin

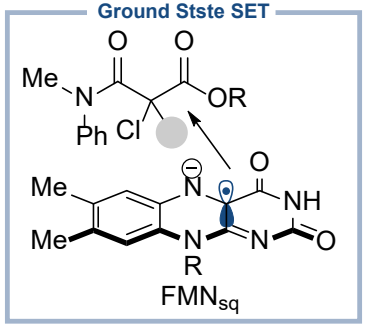


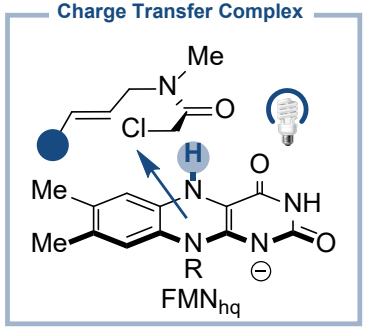
Flavin Dependent Enzyme

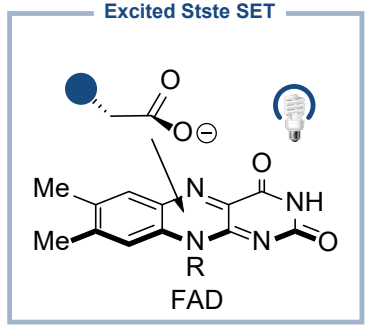
- Fatty Acid Photodecarboxylase
- Ene-reductase
- DNA Photolyase











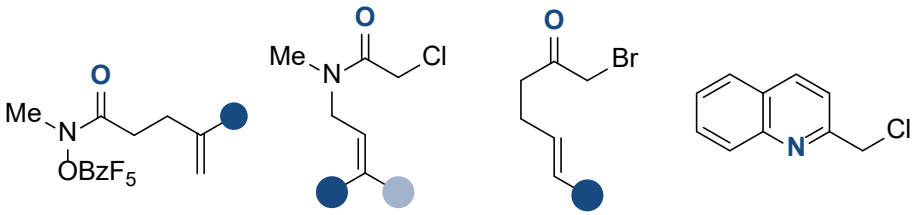
Outline

- Pyridoxal phosphate (PLP) Dependent Photo-enzymatic Synthesis
- Flavin Dependent Photo-enzymatic Synthesis
- Summary
- **Perspective**

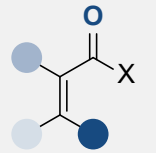
Perspective

- **Discover New enzymes to Suit different Types of Substrate.**

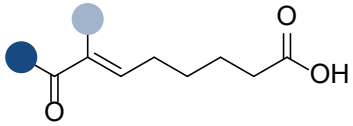
Ene-Reductase

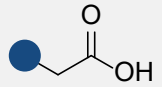


Natural Enzyme



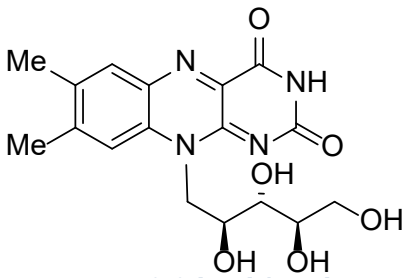
Fatty acid photodecarboxylase



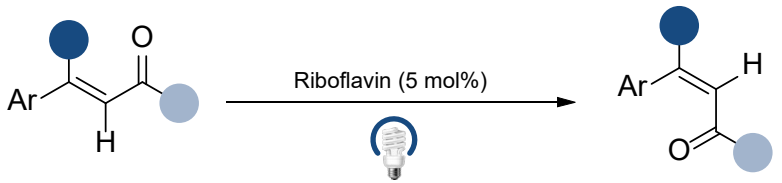


- **Explore Novel Functions of Established Coenzymes**

Riboflavin



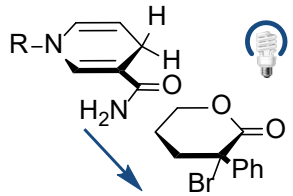
E_T 50.0 kcal / mol



Perspective

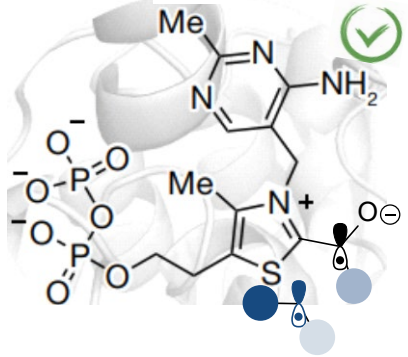
- **Develop Novel Coenzymes in Photoenzymatic Synthesis**

NAD(P)H



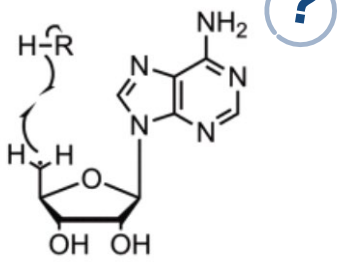
Charge Transfer Complex

ThDP

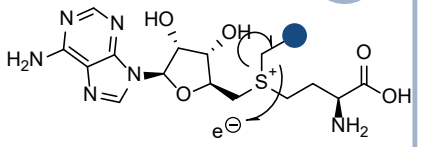


Compound 1

5'-Deoxyadenosyl Radical



SAM



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Questions?