Seminar

酶促电合成在惰性小分子转化方面的应用

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导 师：朱灿 青年研究员
日 期：2024.01.05

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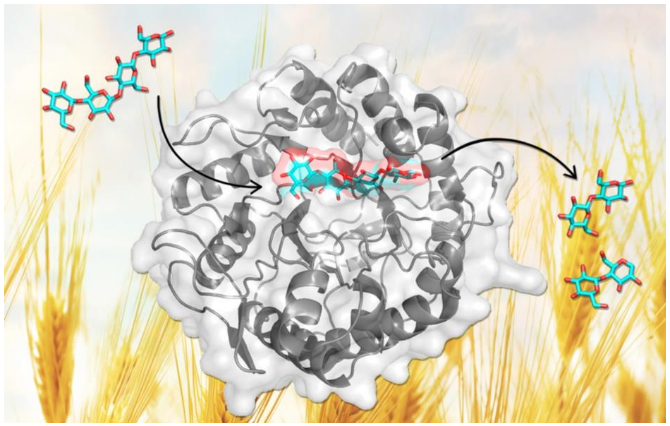
研究背景

➢ 酶促合成的应用



酶（enzyme）

由活细胞产生的、对其底物具有高度特异性和高度催化性能的蛋白质或RNA

- 单纯酶 （结构中仅含蛋白质的酶）
- 结合酶
  - 酶蛋白
  - 辅酶因子：金属离子，小分子化合物 (MoFe辅酶因子)（NADH）

✓ 高度专一性，高活性 ✓ 低毒性 ✓ 反应条件温和

✓ 种类繁多，容易获得 ✓ 可针对活性位点进行修饰改造

in vivo √

氮气	固氮微生物	氨
二氧化碳	植物光合作用	碳水化合物
碳氢化合物	体内代谢	醇，酮，酸等

in vitro ？

氮气	固氮酶	氨
二氧化碳	光系统 I，II，etc.	碳水化合物
碳氢化合物	加氧酶	醇，酮，酸等

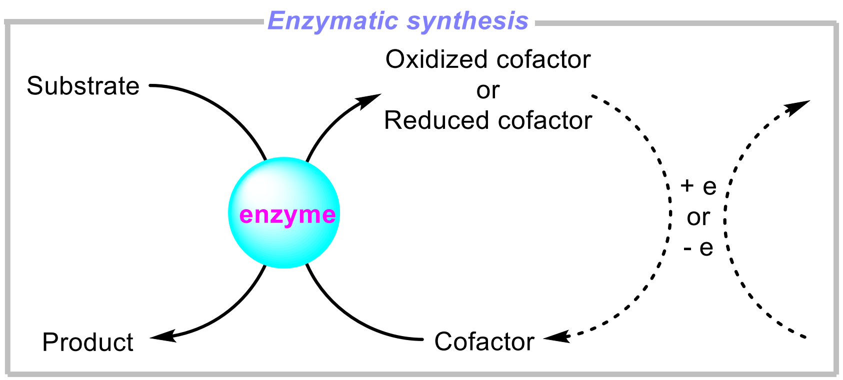
N_2	CO_2	Petroleum
946 kJ/mol	*803 kJ/mol*	*413 kJ/mol*

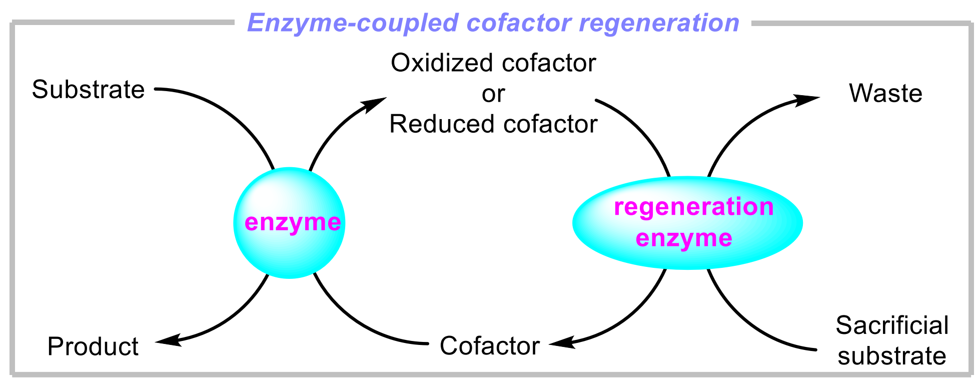
- ***Kinetic stability***
- ***Thermodynamic stability***

K. tipton; et al. *Nucleic Acids Res.* **2000**, *28*, 304–305.

研究背景

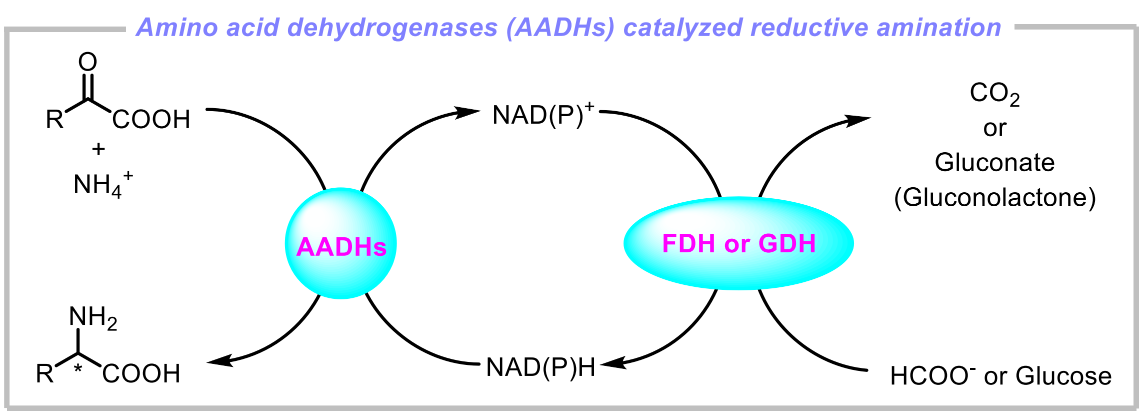
➢ 体外酶促合成

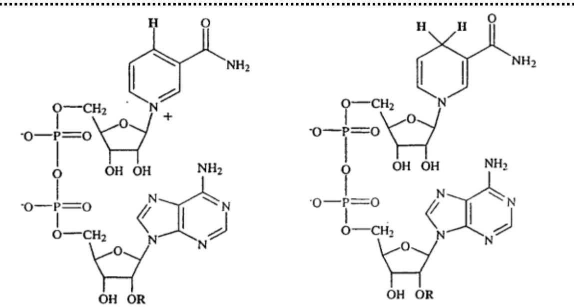




In vivo
Multienzyme cascade reactions

In vitro
Challenge





NAD$^+$: R = H
NADP$^+$: R = PO_3^{2-}

NADH: R = H
NADPH: R = PO_3^{2-}

FDH: Formate dehydrogenase　　**GDH: Glucose dehydrogenase**

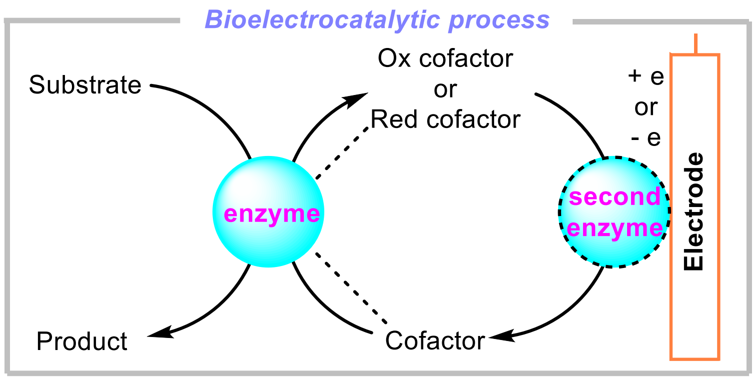
$$NAD^+ + H^+ + 2e^- \rightleftharpoons NADH$$

原子不经济！！！

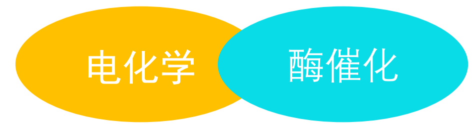
R. H. Mueller; et al. *Bioorg. Chem.* **1990**, *18*, 116–130.　S. J. Novick; et al. *J. Am. Chem. Soc.* **2006**, *128*, 10923–10929.
Y.-G. Zheng; et al. *Chem. Soc. Rev.* **2018**, *47*, 1516–1561.

研究背景

➢ 酶促电合成

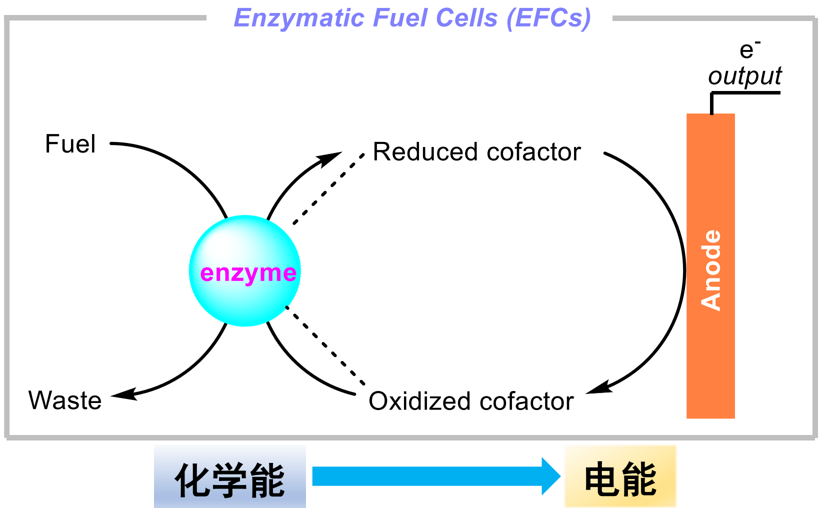


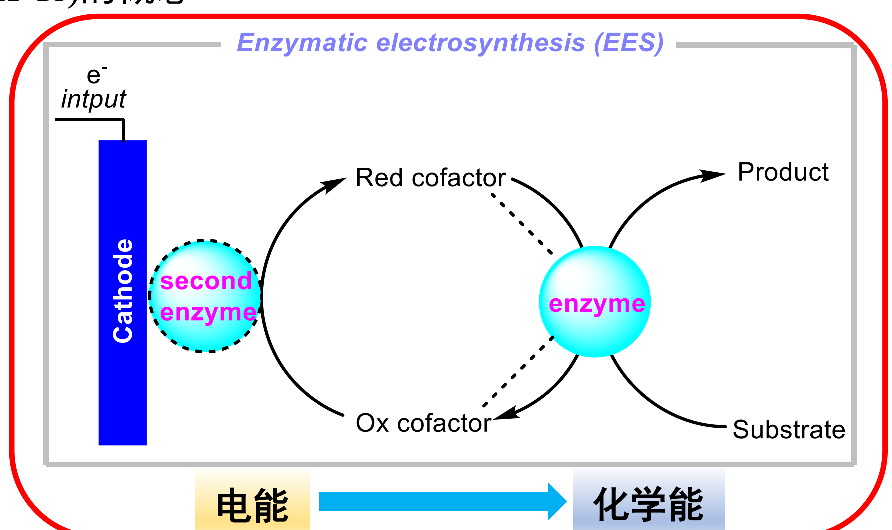
- ✓ 无需添加牺牲底物或再生酶
- ✓ 无需添加当量氧化还原试剂
- ✓ 电极电势可调控，灵活实现辅酶因子的再生
- ✓ 催化效率高



Electrons are among the cheapest redox equivalents available ！！！

1964年，Kimble提出了Enzymatic (Bio)Fuel Cells (EFCs)的概念

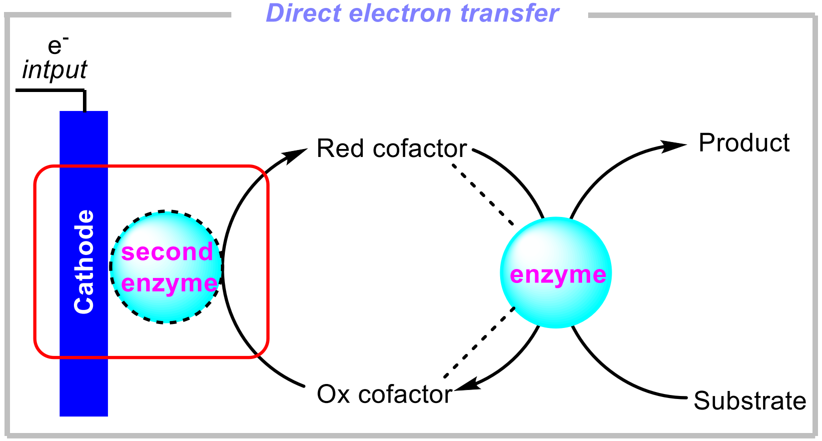


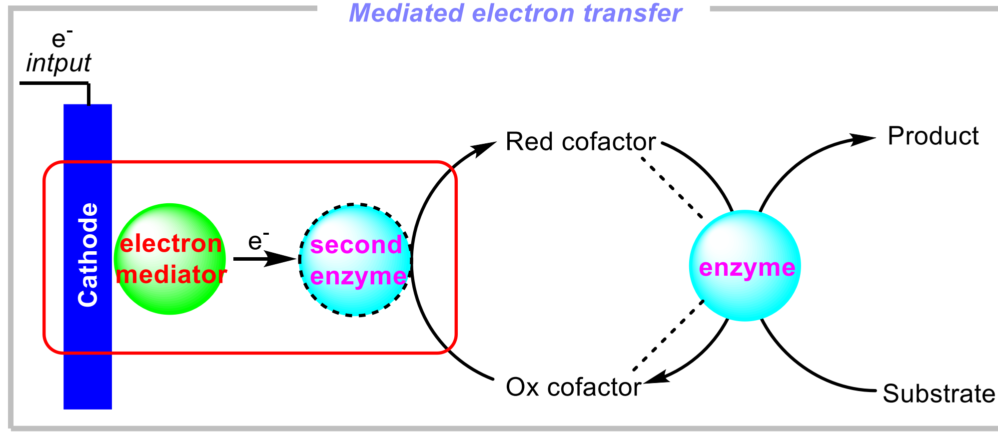


D. O. _Kimble; et al. *Biochimica et biophysica acta* **1964**, *88*, 375−383. S. Lütz; et al. *J Mol Catal B- Enzym* **2008**, *51*, 57–72.
Z.G. Zhu; et al. *Current Opinion in Electrochemistry* **2020**, *19*, 1–7. S. D. Minteer; et al. *Chem. Rev.* **2020**, *120*, 12903−12993.

研究背景

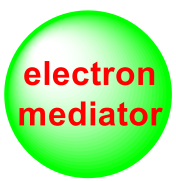
➢ 酶促电合成 (EES)





Direct electron transfer ⇒ ● *Diffusional cofactors* ● *Buried active sites* ⇒ **Mediated electron transfer**

➢ EES with mediated electron transfer



- 具有与酶活性中心相近的电势
- 具有可逆电化学性质

惰性小分子转化方面的应用

- ◆ 氮气的固定
- ◆ 二氧化碳的固定
- ◆ 惰性碳氢化合物转化

A. Heller; et al. *Acc. Chem. Res.* **1990**, *23*, 128−134. S. D. Minteer; et al. *ACS Energy Lett.* **2018**, *3*, 2736−2742.

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酶促电合成在氮气固定方面的应用

➢ **固氮酶(Nitrogenase)的结构及作用机制**

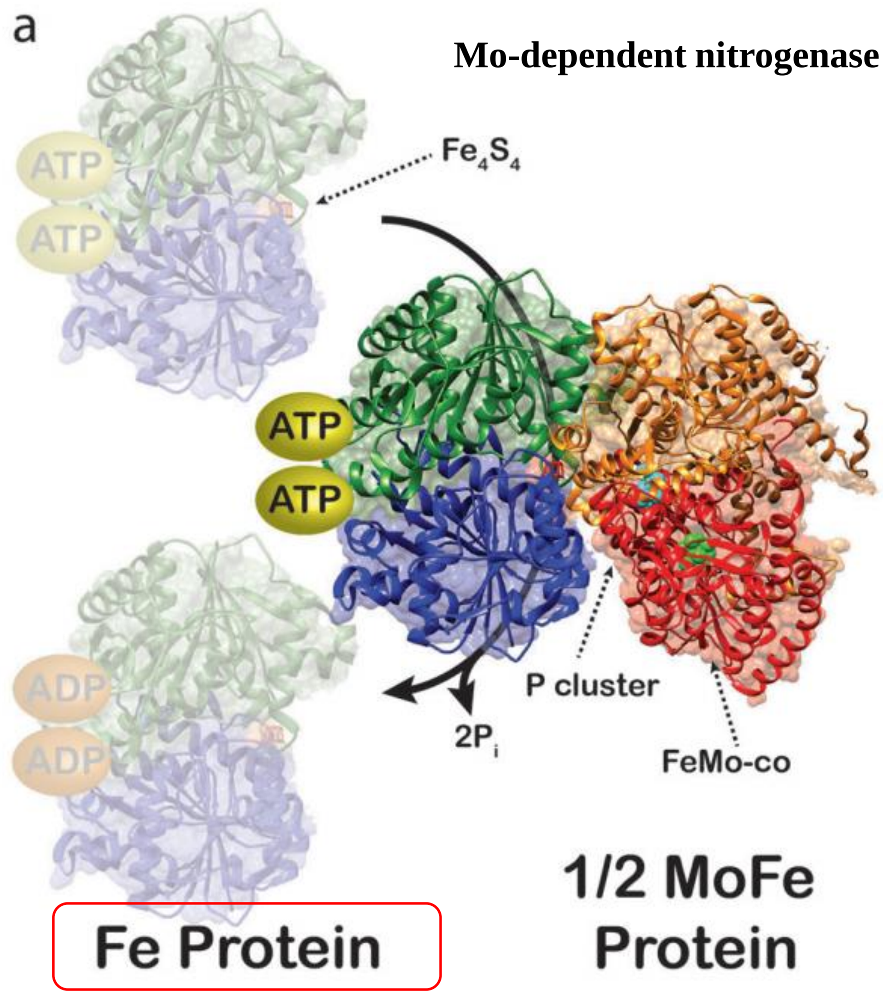
Nitrogenase
已知的唯一
催化氮气还原为氨的酶

- **Mo-dependent nitrogenase（含有MoFe-co）**
- V-dependent nitrogenase（含有VFe-co）
- Fe-dependent nitrogenase（含有FeFe-co）

$$N_2 + H^+ \xrightarrow[\textit{In vivo}]{\text{Nitrogenase, Ferredoxin or Flavodoxin, ATP}} NH_3$$

Mo-dependent nitrogenase
(highly sensitive to O_2)

- **Fe protein (还原蛋白)**
- **MoFe protein (催化蛋白)（含有FeMo-co）** 活性位点



铁氧还蛋白（Ferredoxin）
黄素氧还蛋白（Flavodoxin） ⇐ **In vivo** — *Oxidized Fe protein regeneration*

$$16MgATP + 8e^- + 8H^+ + N_2 \longrightarrow 16MgADP + 2NH_3 + H_2 + 16P_i$$

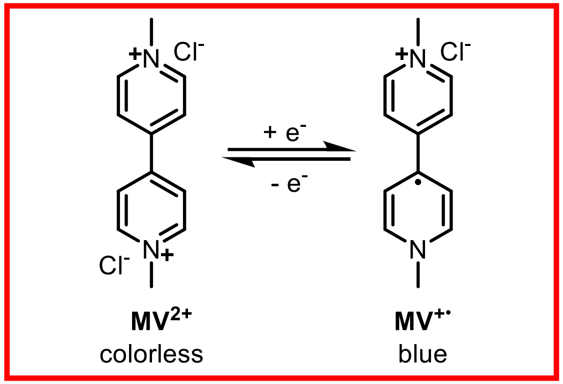
D. R. Dean; et al. *Annu. Rev. Plant. Physiol. Plant. Mol. Biol*. **2001**, *52*, 269-295.

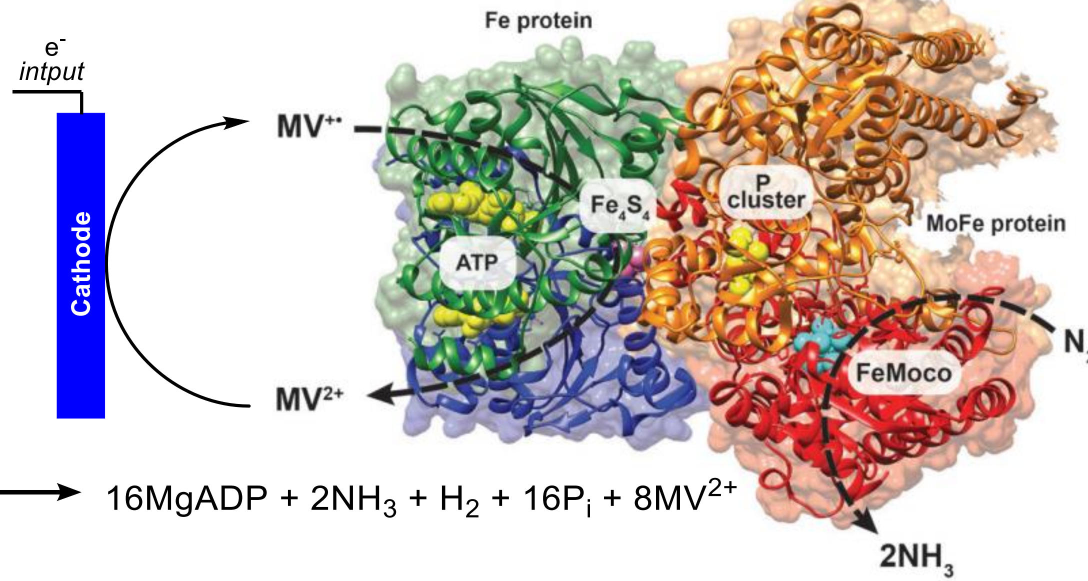
酶促电合成在氮气固定方面的应用

➢ N_2 fixation by enzymatic electrosynthesis

Minteer's group (2017)

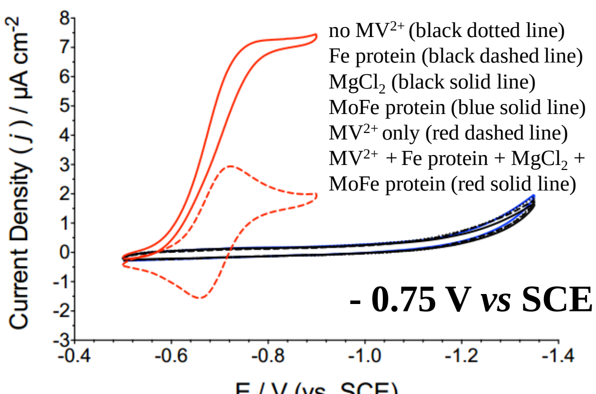
Methyl viologen



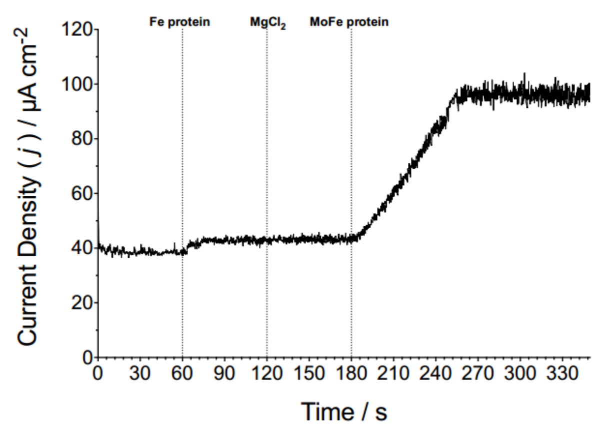


$$16MgATP + 8MV^{+\bullet} + 8H^+ + N_2 \longrightarrow 16MgADP + 2NH_3 + H_2 + 16P_i + 8MV^{2+}$$

☐ Cyclic voltammetric investigation



☐ Amperometric i-t analysis



Faradaic efficiency: 59%

$$FE = \frac{4\ Fn}{\int_0^t I\ dt} \times 100\%$$

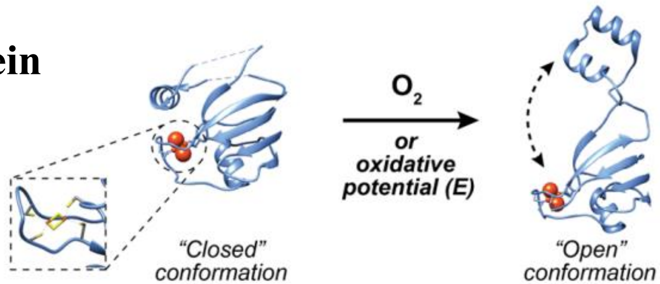
S. D. Minteer; et al. *Angew. Chem. Int. Ed.* **2017**, *56*, 2680 –2683.

酶促电合成在氮气固定方面的应用

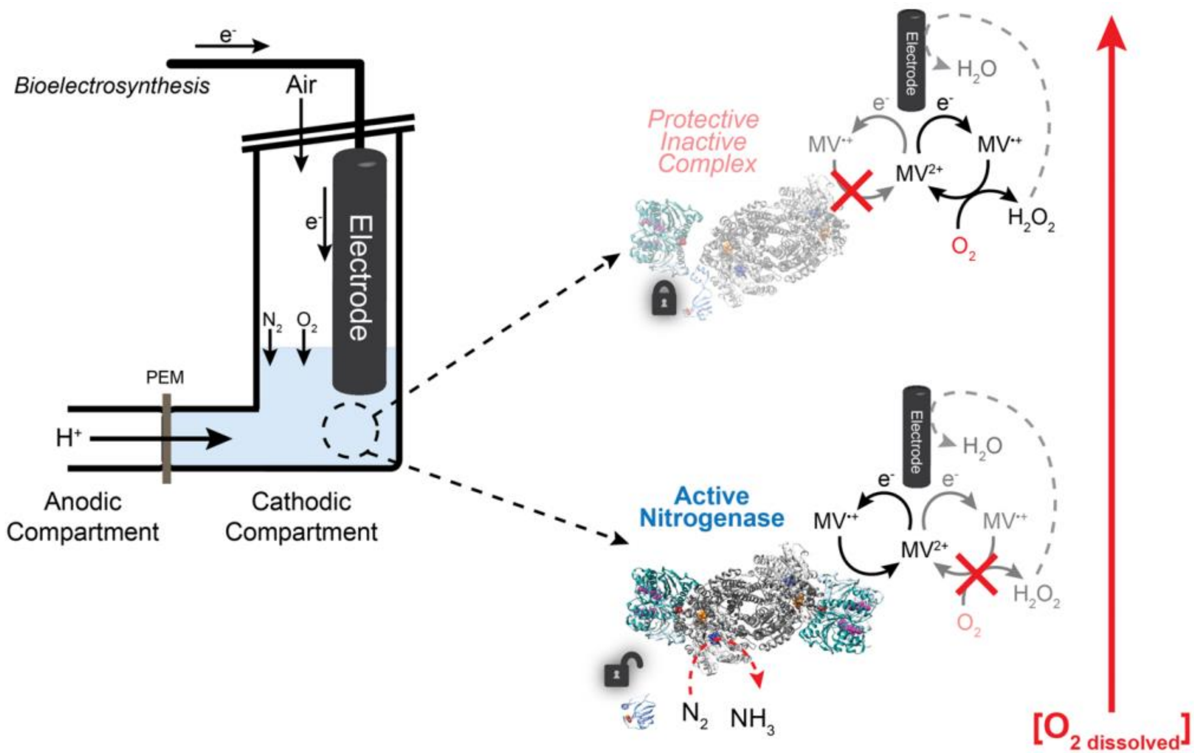
➢ **Aerobic bioelectrochemical dinitrogen reduction in vitro**

“Shethna” protein

[2Fe-2S] cluster



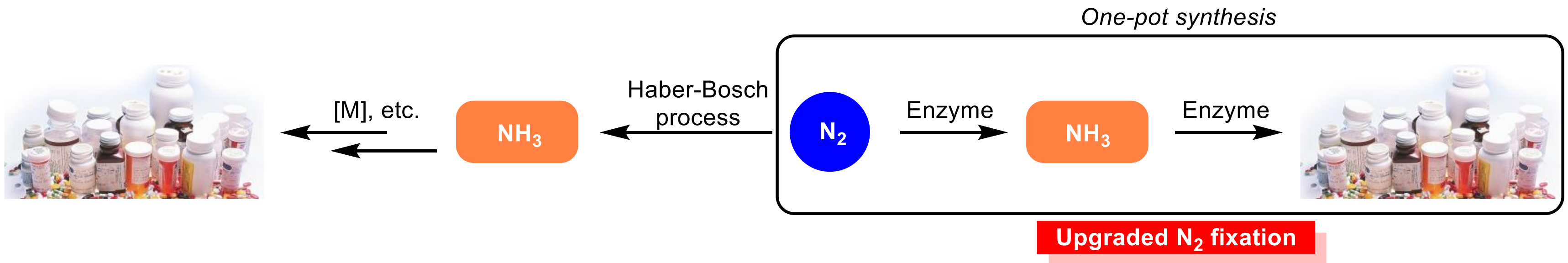
FeSII:Fe:MoFe complexes



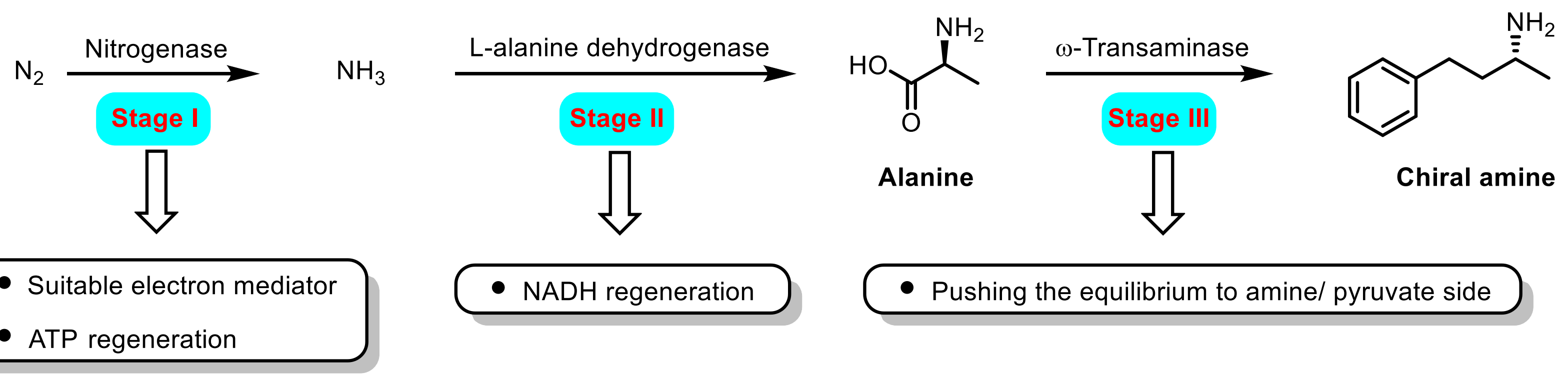
S. D. Minteer; et al. *J. Am. Chem. Soc.* **2017**, *139*, 9044−9052.

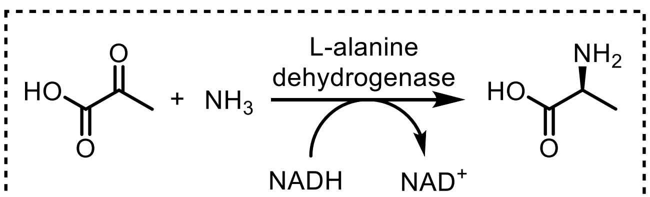
酶促电合成在氮气固定方面的应用

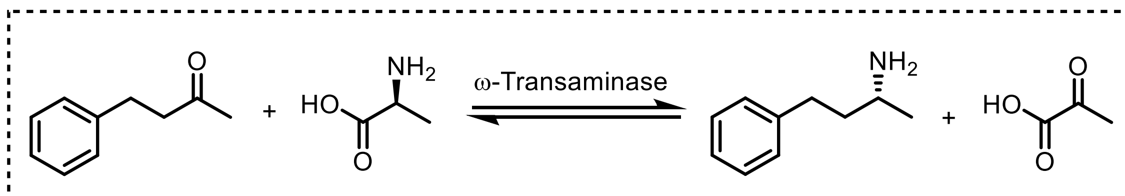
➢ Upgraded N_2 fixation



Minteer's group (2019)



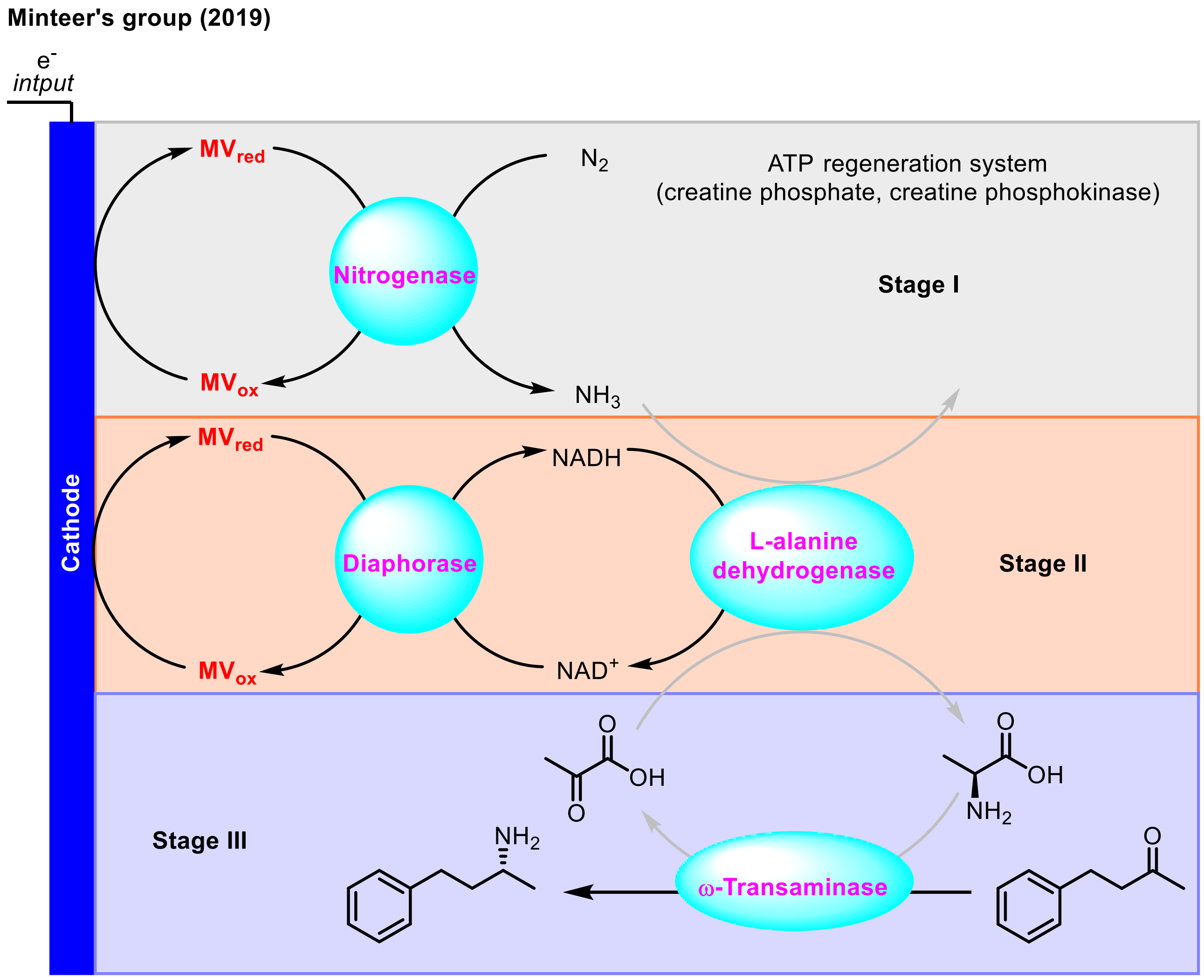




S. D. Minteer; et al. *J. Am. Chem. Soc.* **2019**, *141*, 4963−4971.

酶促电合成在氮气固定方面的应用

- **Upgraded N_2 fixation**

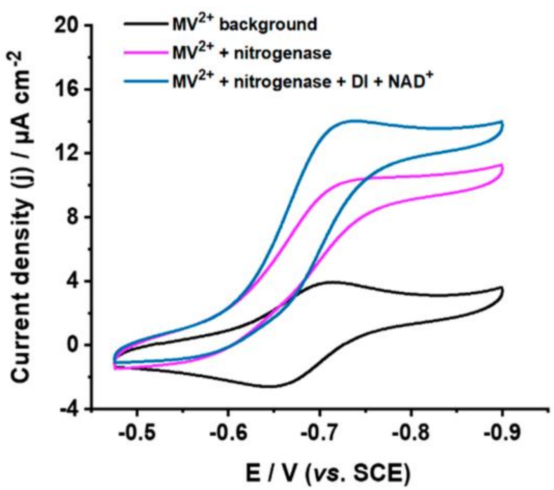


S. D. Minteer; et al. *J. Am. Chem. Soc.* **2019**, *141*, 4963−4971.

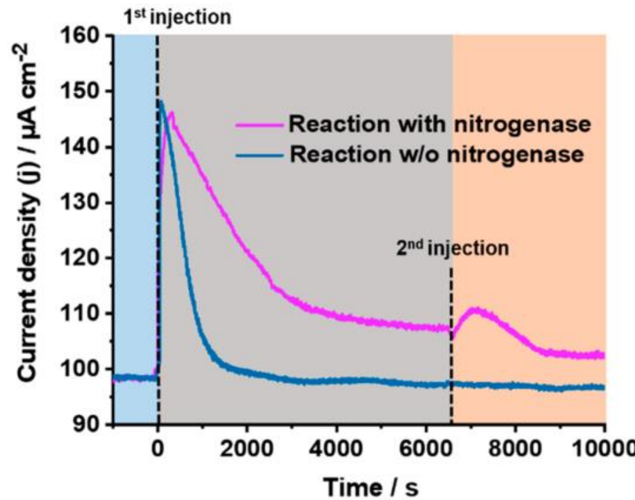
酶促电合成在氮气固定方面的应用

- **Upgraded N_2 fixation**

Cyclic voltammetric investigation



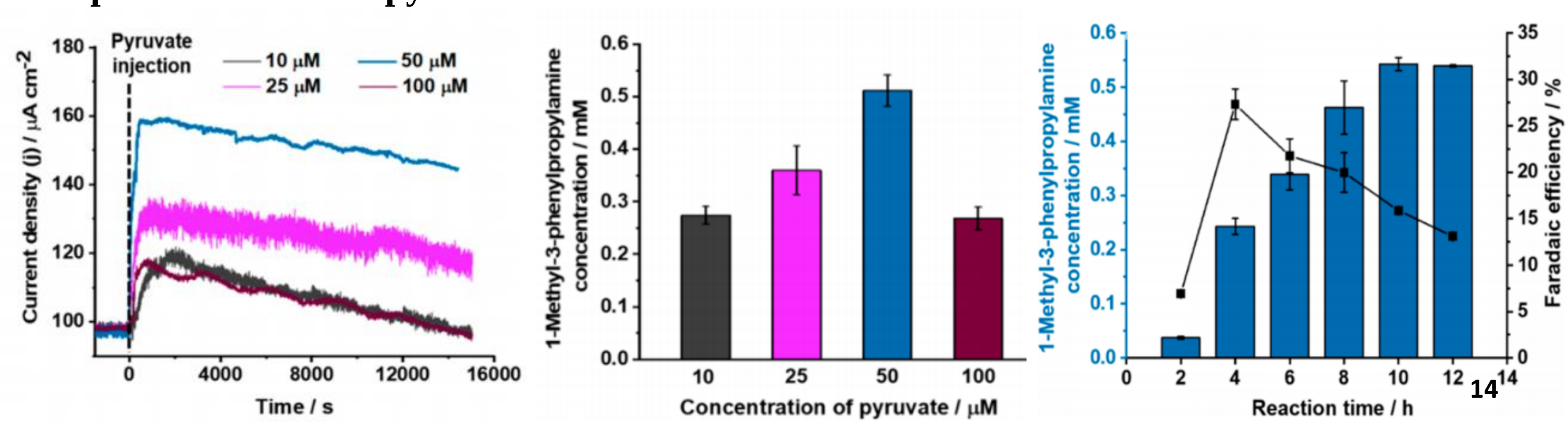
Amperometric i-t analysis



1st injection: 0.18 U/mL AlaDH, 0.16 U/mL DI, 200 μM NAD^+, and 50 μM pyruvate

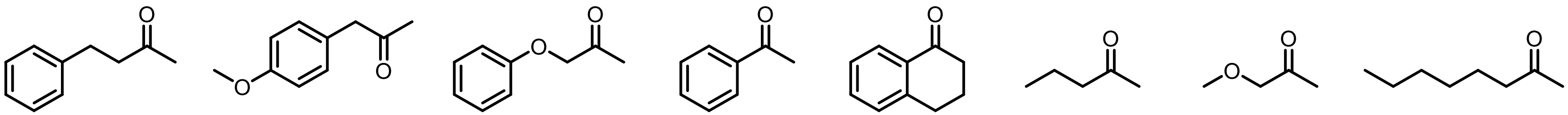
2nd injection: 2 mM 4-phenyl-2butanone

Optimization of the pyruvate concentration



酶促电合成在氮气固定方面的应用

➤ **底物扩展**



1a **2a** **3a** **4a** **5a** **6a** **7a** **8a**

Entry	Substrate	product concentration (mM)[b]	ee_p (%)[c]	yield rate (μmol/L/h)
1	**1a**	0.54	>99 (R)	54
2	**2a**	0.34	>99 (R)	34
3	**3a**	0.61	>99 (R)	61
4	**4a**	n.d.[d]	n.d.[d] (R)	n.d.
5	**5a**	n.d.[d]	n.d.[d] (R)	n.d.
6	**6a**	0.14	>99 (R)	14
7	**7a**	0.53	>99 (R)	53
8	**8a**	0.35	>99 (R)	35

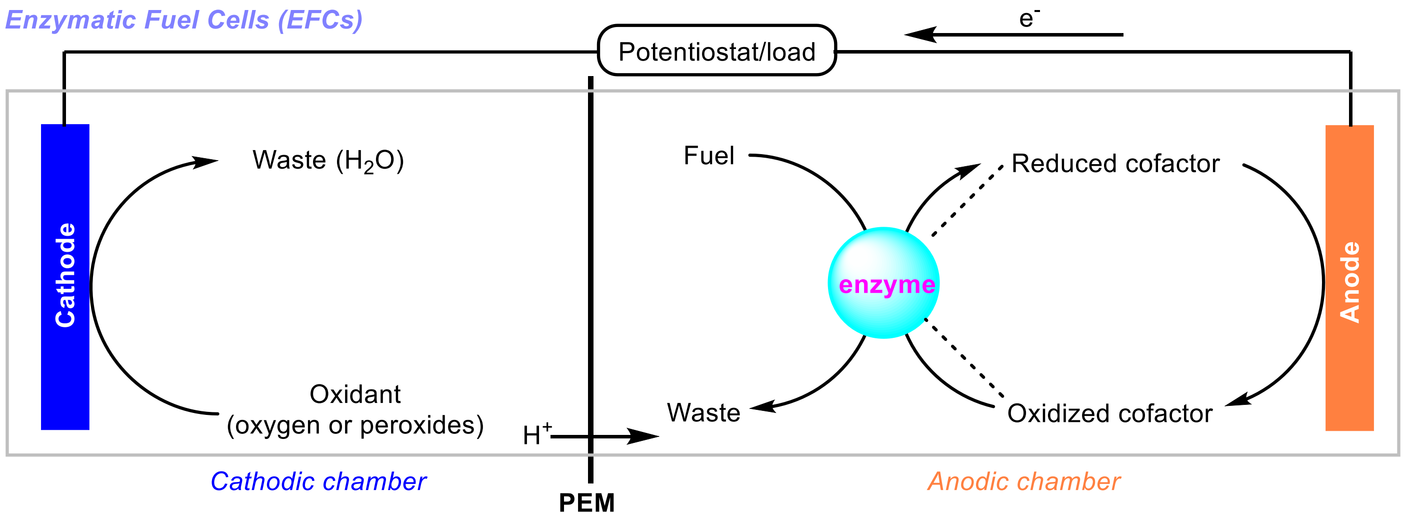
[a]Each value represents the mean from triplicate experiments. [b]The reactions contain MOPS buffer (100 mM, pH 7.0), MV^{2+} (750 μM), NAD^+ (200 μM), PLP (1 mM), substrate (2 mM), pyruvate (50 μM), nitrogenase (0.16 U/mL), AlaDH (0.18 U/mL), DI (0.16 U/mL), and dry E. coli cells containing HN-ωTA (0.1 U/mL). [c]In [%]. Determined by GC on a chiral stationary phase after derivatization to the corresponding acetamide. [d]n.d. Not determined because of too low a conversion.

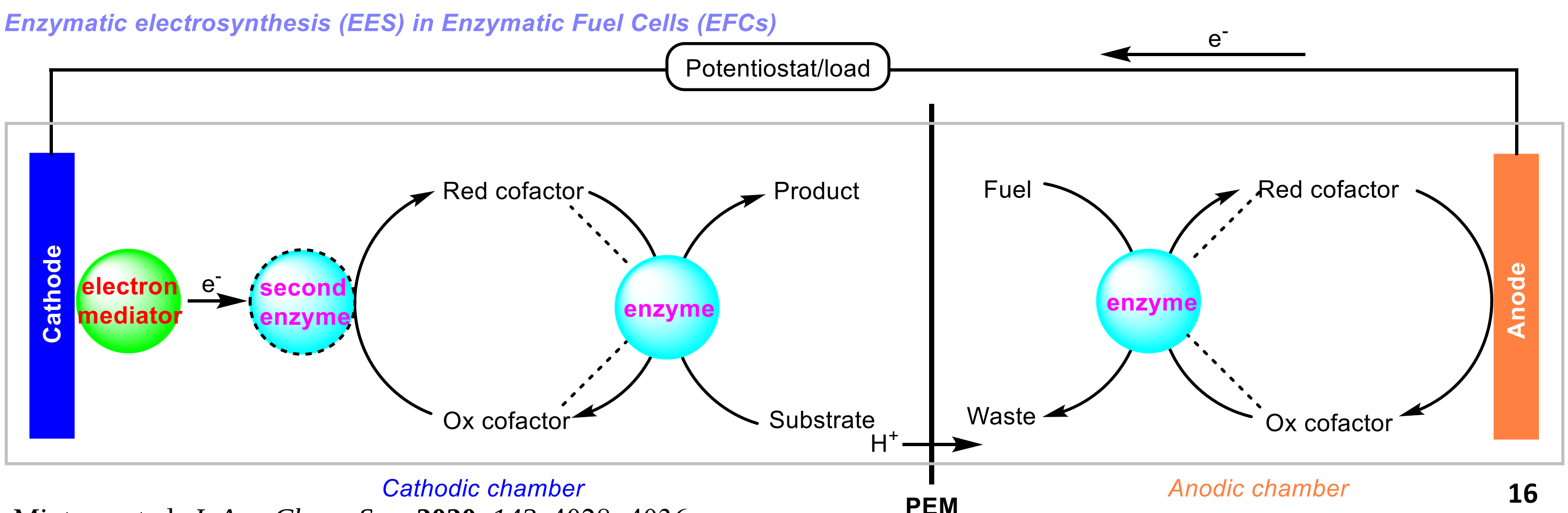
- The low utilization ratio of generated NH_3 (approximately 40%)
- The low faradaic efficiency (approximately 27.6%)
- The reliance of the system on the external electrical energy input

S. D. Minteer; et al. *J. Am. Chem. Soc.* **2019**, *141*, 4963−4971.

酶促电合成在氮气固定方面的应用

➢ **Bioelectrocatalytic conversion from N_2 to chiral amino acids in a H_2/α-keto acid enzymatic fuel cell**

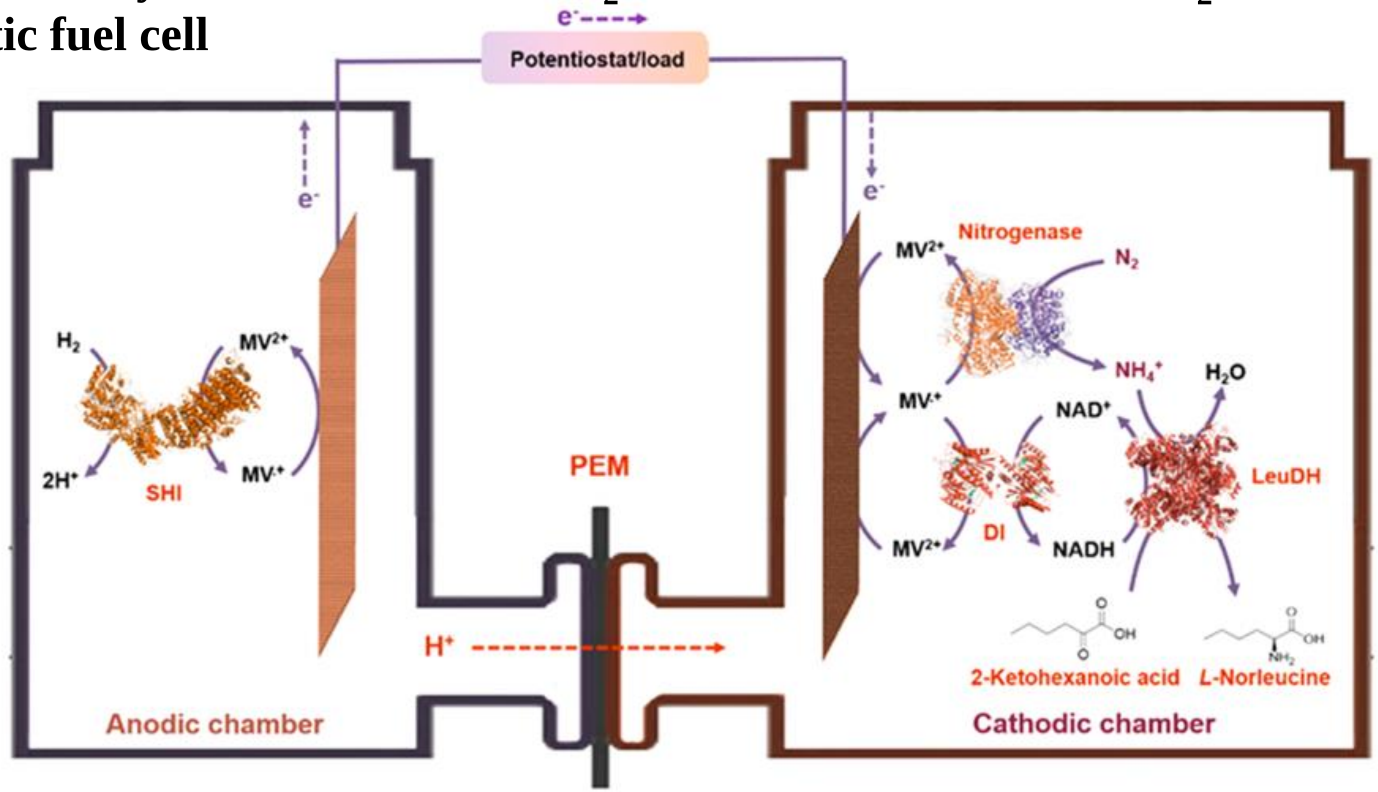


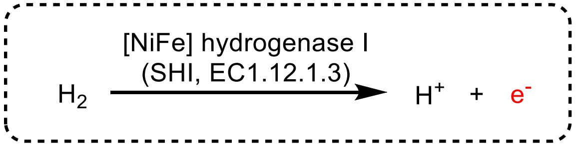


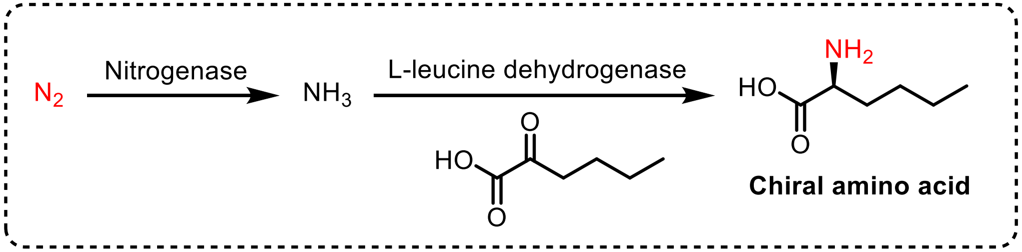
S. D. Minteer; et al. *J. Am. Chem. Soc.* **2020**, *142*, 4028−4036.

酶促电合成在氮气固定方面的应用

- **Bioelectrocatalytic conversion from N_2 to chiral amino acids in a H_2/α-keto acid enzymatic fuel cell**







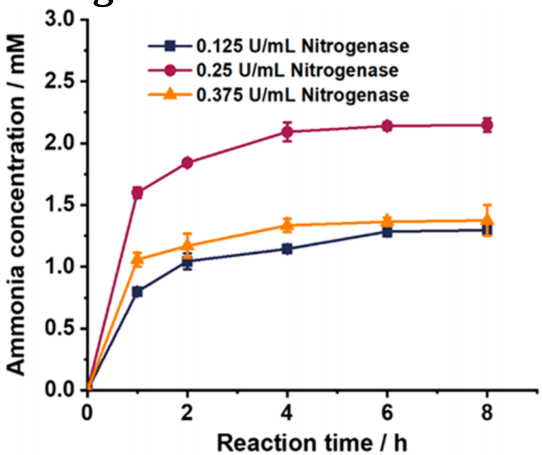
- ✓ The low utilization ratio of generated NH_3
- ✓ The low faradaic efficiency
- ✓ The reliance of the system on the external electrical energy input

S. D. Minteer; et al. *J. Am. Chem. Soc.* **2020**, *142*, 4028−4036.

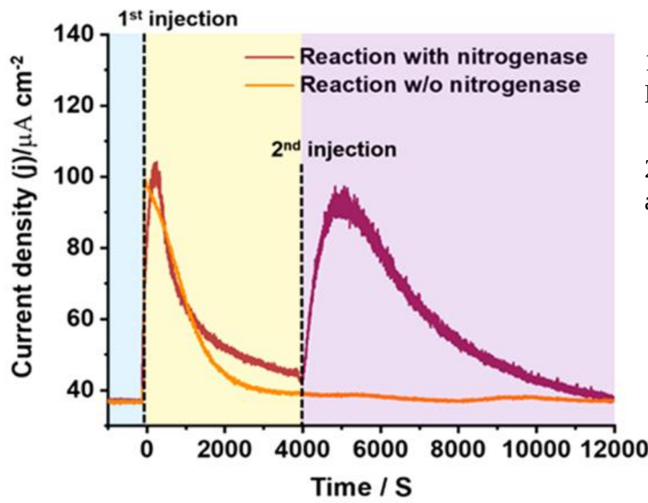
酶促电合成在氮气固定方面的应用

➢ **Bioelectrocatalytic conversion from N_2 to chiral amino acids**

☐ **Optimization of the nitrogenase concentration**



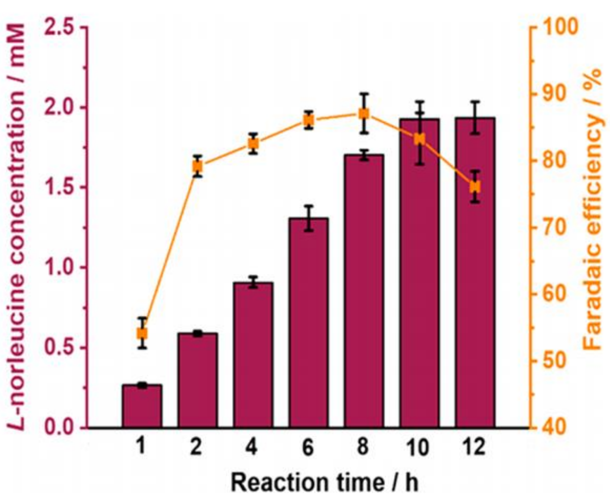
☐ **Amperometric i-t analysis**



1st injection: 0.25 U/mL DI and 200 μM NAD+

2nd injection: 2.5 mM 2-ketohexanoic acid and 0.45 U/mL

☐ **Bioelectrocatalytic L-norleucine production powered by an applied voltage**

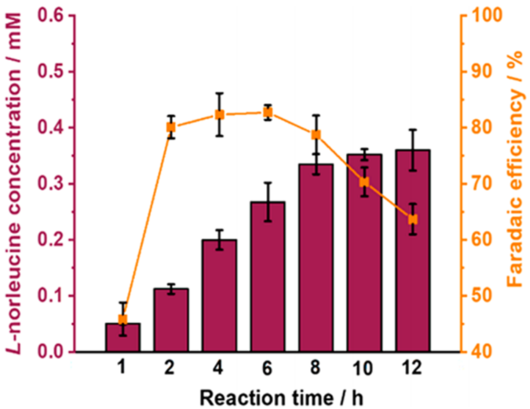


- *The Faradaic efficiency was up to 87.1% (2.3 times as many as previous research)*
- *The utilization ratio of NH_3 was up to 92% (3.4 times as many as previous research)*

S. D. Minteer; et al. *J. Am. Chem. Soc.* **2020**, *142*, 4028−4036.

酶促电合成在氮气固定方面的应用

➢ **Fuel-cell-powered bioelectrosynthetic L-norleucine production.**



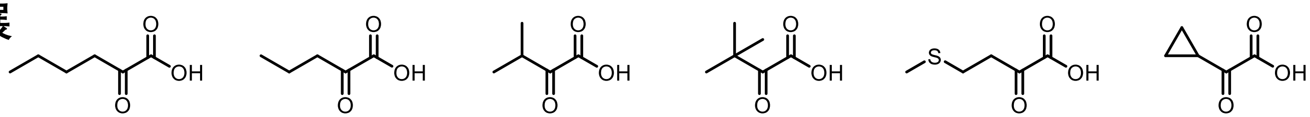
- *The highest L-norleucine concentration was 0.36 mM*
- *The Faradaic efficiency: 64%-82%*

外加电压 vs	自供电
1.97 mM	0.36 mM

L-norleucine concentration

S. D. Minteer; et al. *J. Am. Chem. Soc.* **2020**, *142*, 4028−4036.

□ 底物扩展



1a 2a 3a 4a 5a 6a

Entry	Substrate	product concentration (mM)[b]	ee_p (%)[b]	yield rate (μmol/L/h)
1	**1a**	0.36	>99 (L)	36
2	**2a**	0.38	>99 (L)	38
3	**3a**	0.40	95.2 (L)	40
4	**4a**	0.40	>99 (L)	40
5	**5a**	n.d.[c]	n.d.[c]	n.d.[c]
6	**6a**	0.28	>99 (L)	28

[a]Values represent the means from three separate individual U-shaped dual-chamber electrochemical cells. For every single cell, the values were obtained based on triplicate amino acid derivatization. [b]In %. Determined by SFC after derivatization. [c]n.d. = not determined because the conversion was too low.

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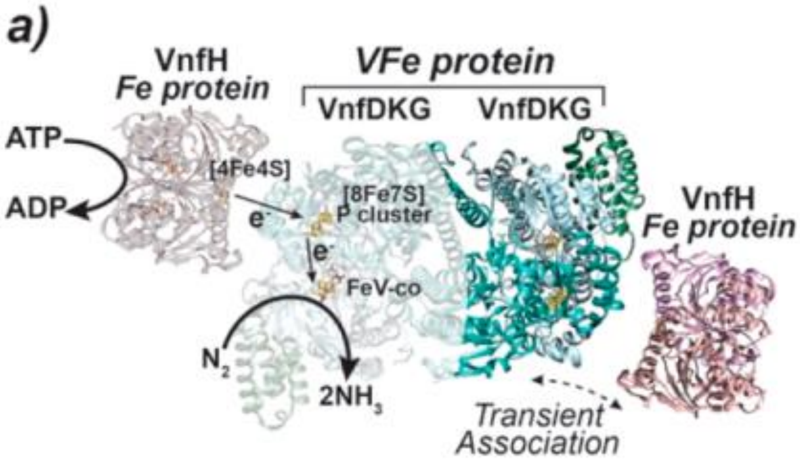
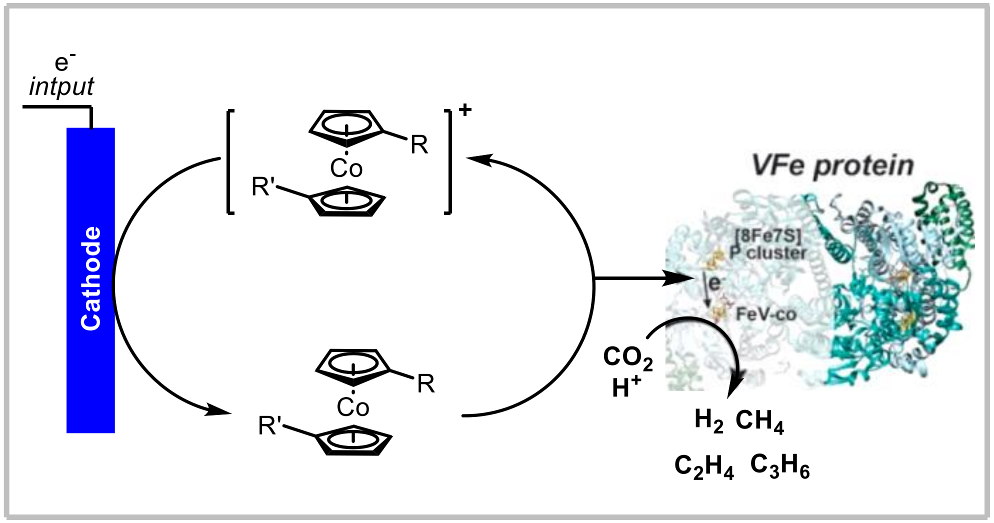
酶促电合成在二氧化碳固定方面的应用

➢ **Electroenzymatic CO_2 Reduction by VFe nitrogenase**



Minteer's group (2018)



Without Fe protein
Without ATP-hydrolysis

$$CO_2 + 8e^- + 8H^+ \longrightarrow CH_4 + 2H_2O$$

$$2CO_2 + 12e^- + 12H^+ \longrightarrow C_2H_4 + 4H_2O$$

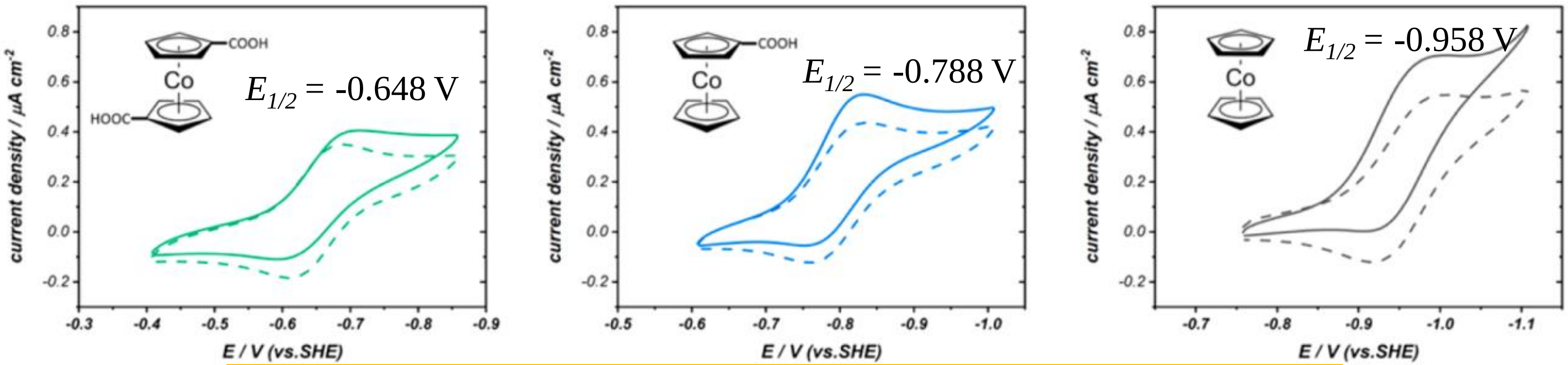
$$3CO_2 + 18e^- + 18H^+ \longrightarrow C_3H_6 + 6H_2O$$

S. D. Minteer; et al. *J. Am. Chem. Soc.* **2018**, *140*, 5041−5044.

酶促电合成在二氧化碳固定方面的应用

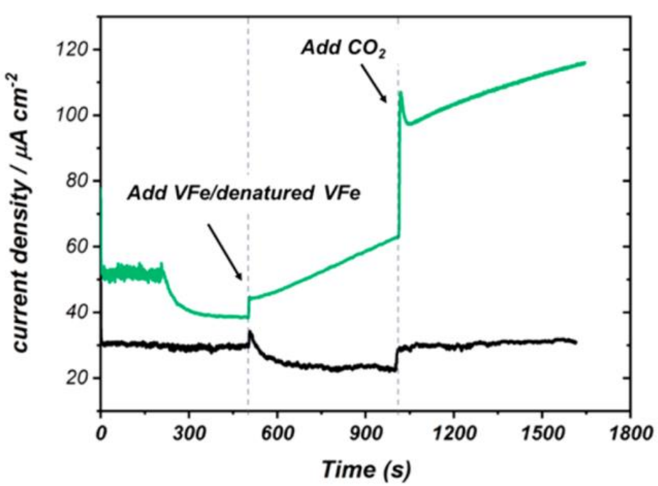
➢ Electroenzymatic CO_2 Reduction by VFe nitrogenase

☐ Cyclic voltametric investigation

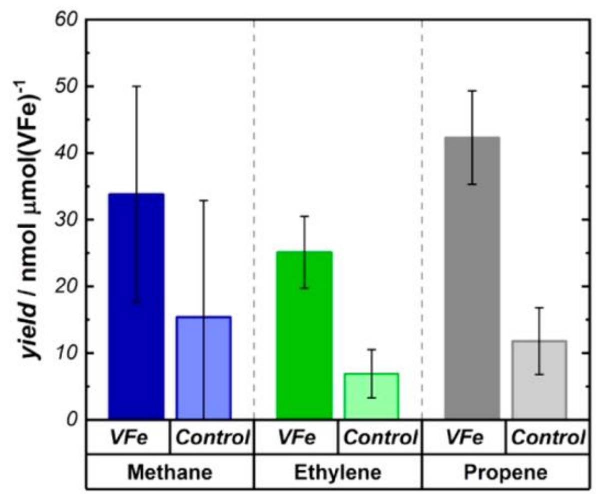


$E^0_{(CO_2/CH_4)}$ = -0.24 V, $E^0_{(2CO_2/C_2H_4)}$ = -0.34 V, $E^0_{(3CO_2/C_3H_6)}$ = -0.31 V

☐ Amperometric i-t analysis



☐ Product distribution



S. D. Minteer; et al. *J. Am. Chem. Soc.* **2018**, *140*, 5041−5044.

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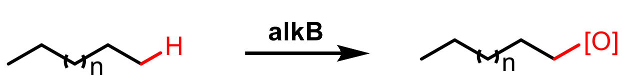
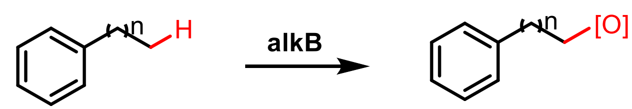
酶促电合成在惰性碳氢化合物转化方面的应用

➢ Selective Electroenzymatic Oxyfunctionalization by Alkane Monooxygenase

Alkane monooxygenase (alkB) from *Pseudomonas putida* GPO1
（恶臭假单胞菌）



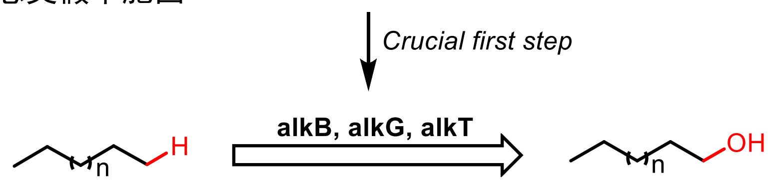
Gasoline-range alkanes (C5-C12)

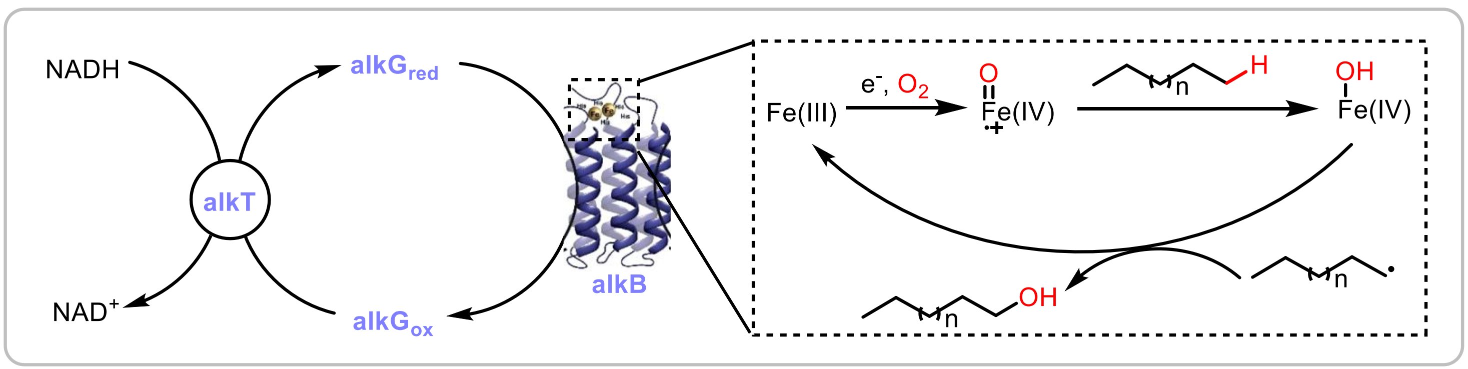


Aromatic compounds

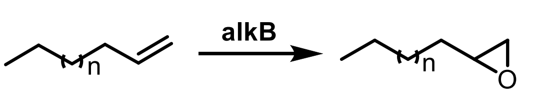
Gene cluster alkBFGHJKLST

恶臭假单胞菌 alkanes as a carbon source

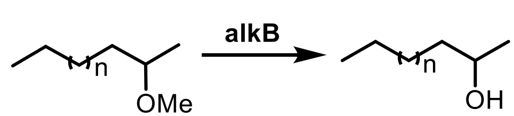




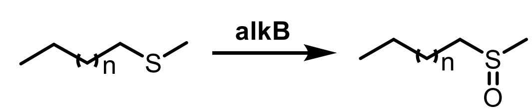
Epoxidation of olefins



Demethylation of branched methyl ethers



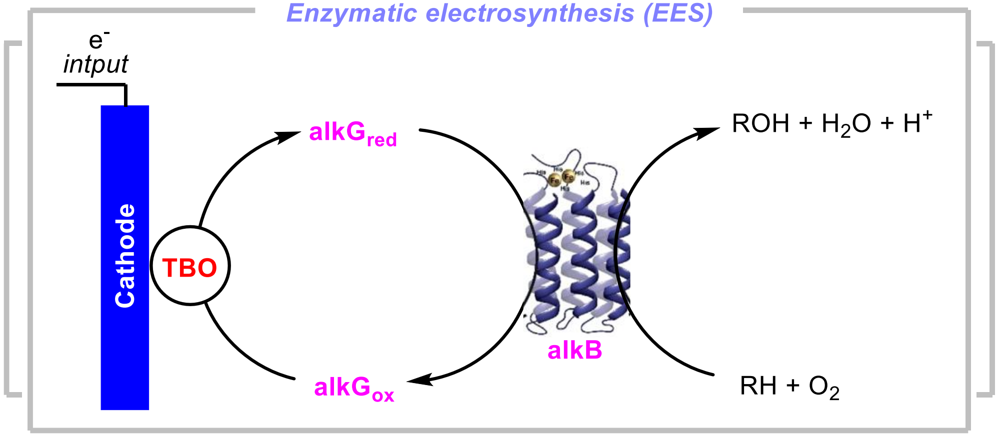
Sulfoxidation of thioethers



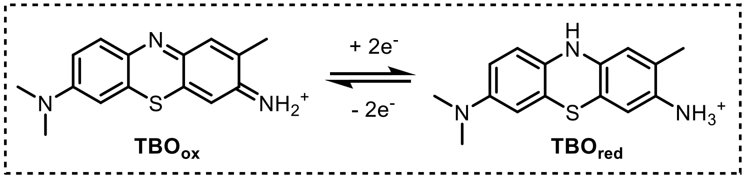
S. D. Minteer; et al. *Angew. Chem. Int. Ed*. **2020**, *59*, 8969–8973.

酶促电合成在惰性碳氢化合物转化方面的应用

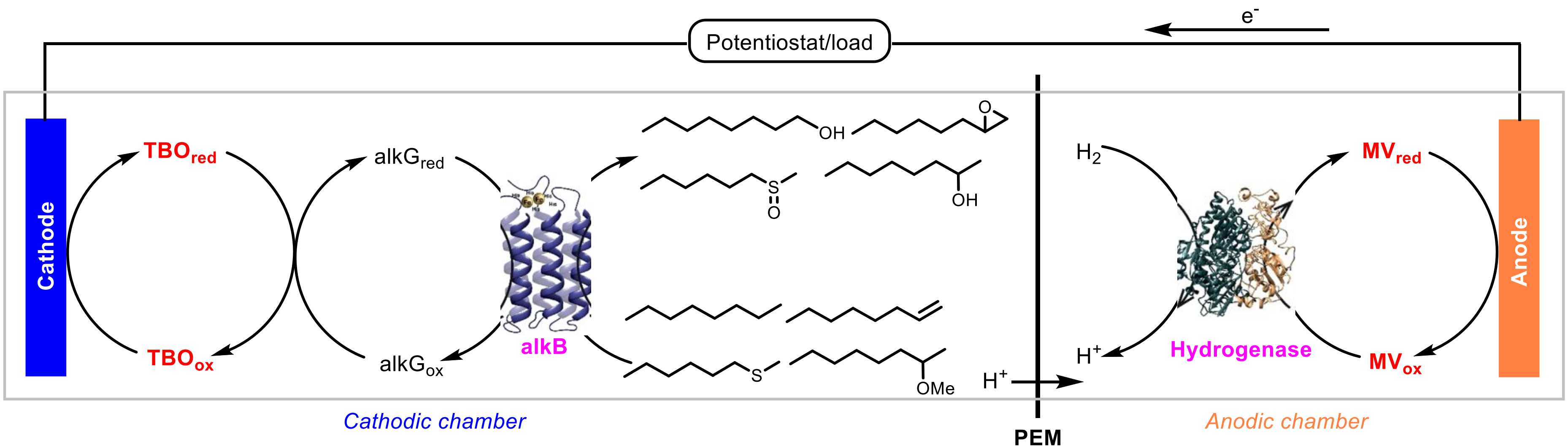
➢ **Selective Electroenzymatic Oxyfunctionalization by Alkane Monooxygenase**



Toluidine blue O (TBO)



☐ **Enzymatic electrosynthesis in a Biofuel Cell**

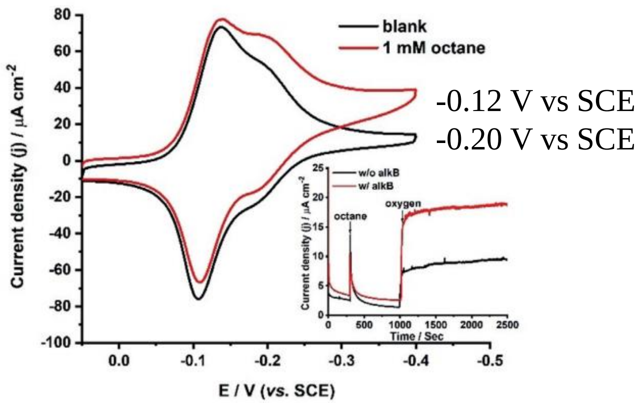


S. D. Minteer; et al. *Angew. Chem. Int. Ed*. **2020**, *59*, 8969–8973.

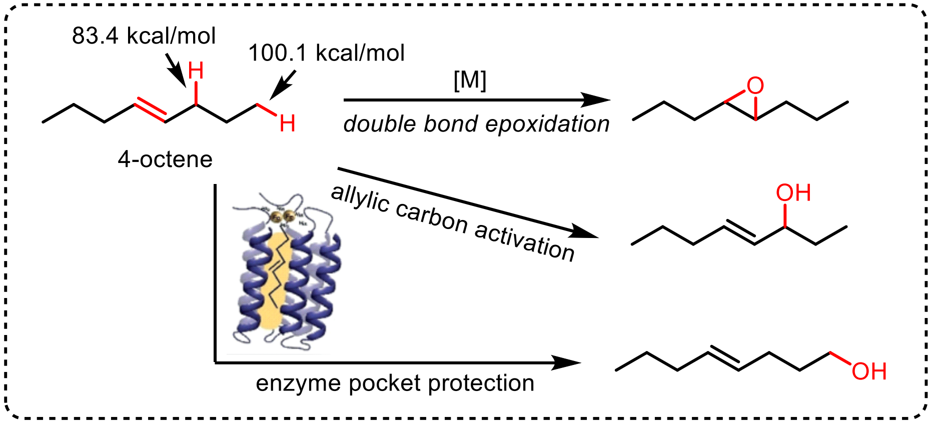
酶促电合成在惰性碳氢化合物转化方面的应用

➢ Selective Electroenzymatic Oxyfunctionalization by Alkane Monooxygenase

□ Cyclic voltammetric investigation
Amperometric i-t analysis



□ AlkB catalyzes terminal C-H activation



□ 底物扩展

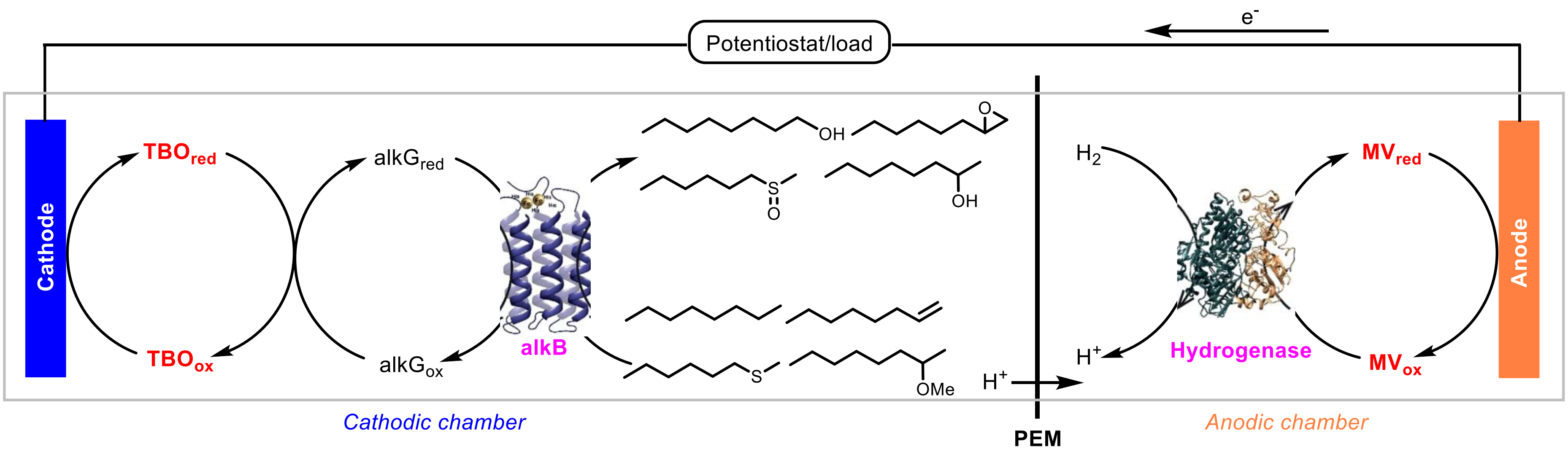
Substrate	Product	Product detected (nmol/h)	Faradaic efficiency (%)[b]	Rate (µmol/h/mg of alkB)
Octane	1-Octanol	265	25	1.77
Cyclohexane	Cyclohexanol	29	2.6	0.19
Ethylbenzene	2-Phenylethanol	71	5.1	0.47

Substrate	Product	Product detected (nmol/h)	Faradaic efficiency (%)[b]	Rate (µmol/h/mg of alkB)
1-Octene	1,2-Epoxyoctane	144	16	0.96
Methyl n-octyl sulfide	1-Methanesulfinyloctane	110	8.7	0.36
2-Methoxyoctane	2-Octanol	241	18	1.61

S. D. Minteer; et al. *Angew. Chem. Int. Ed.* **2020**, *59*, 8969–8973.

酶促电合成在惰性碳氢化合物转化方面的应用

➢ **The EFC with an alkB/alkG biocathode and hydrogenase bioanode**

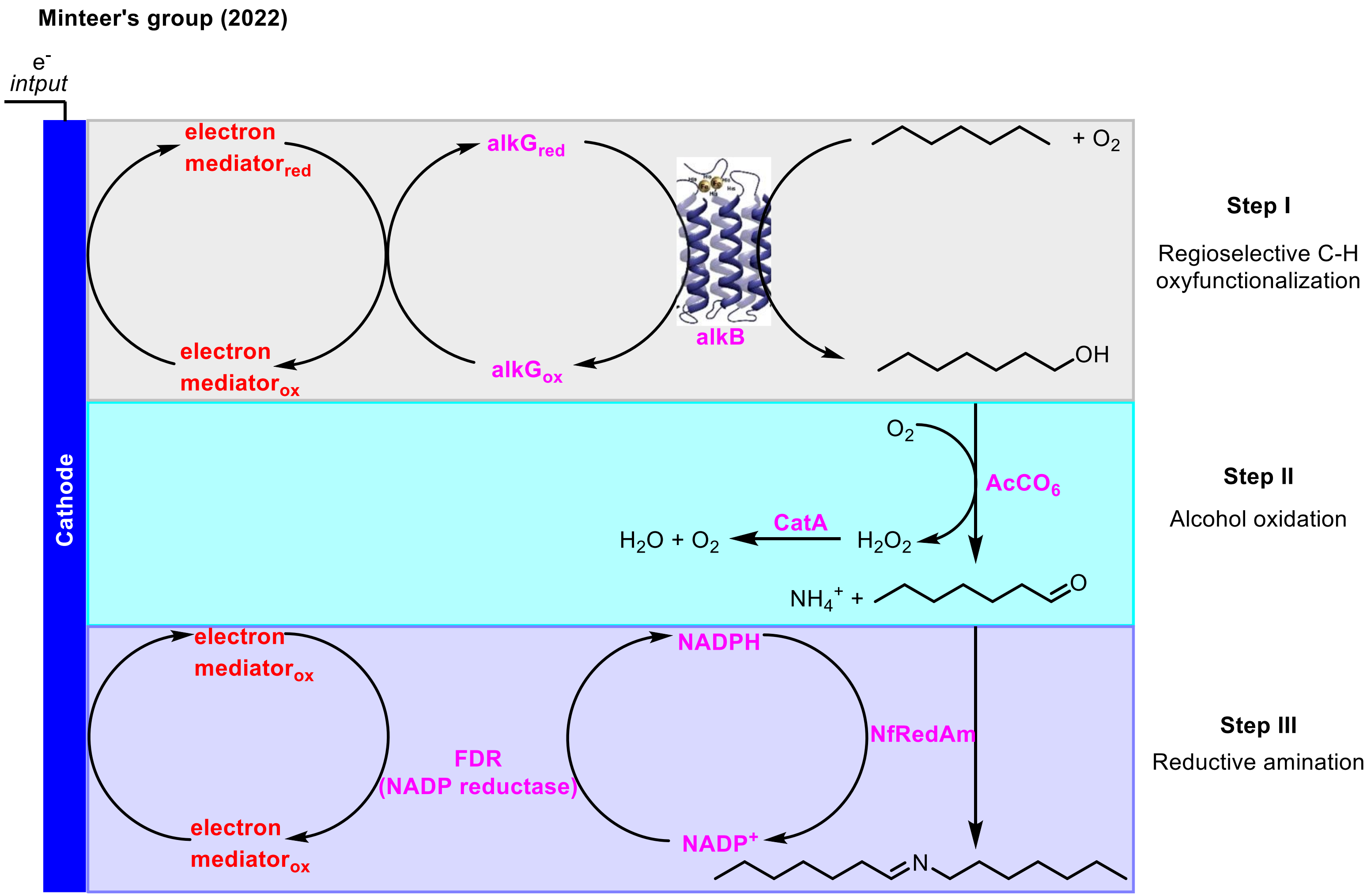


Cathode substrate	OCP [V]	Maximum current density [μA cm^{-2}]	Maximum power density [μW cm^{-2}]	Product quantification [nmol cm^{-2}]	Faradaic efficiency [%]	Rate [μmol h^{-1} mg^{-1} of alkB]
Octane	0.65 ± 0.01	318 ± 7	45 ± 1	690 ± 34	23 ± 1	1.15
1-Octene	0.657 ± 0.009	190 ± 16	36 ± 3	230 ± 12	15 ± 1	0.38
Methyl n-octyl sulfide	0.647 ± 0.002	335 ± 3	50 ± 5	220 ± 16	9.0 ± 0.4	0.18
2-Methoxyoctane	0.663 ± 0.004	280 ± 26	47 ± 4	420 ± 56	17 ± 2	0.70

S. D. Minteer; et al. *Angew. Chem. Int. Ed.* **2020**, *59*, 8969–8973.

酶促电合成在惰性碳氢化合物转化方面的应用

- **bioelectrocatalytic multienzyme cascade reaction**

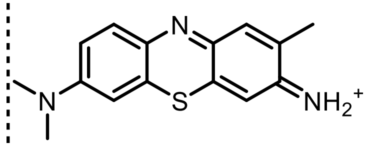


S. D. Minteer; et al. *J. Am. Chem. Soc.* **2022**, *144*, 4047−4056.

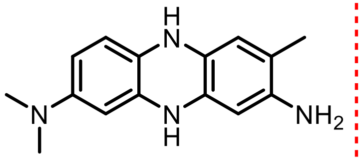
酶促电合成在惰性碳氢化合物转化方面的应用

➢ **Design of reaction system**

alkG氧化还原电势：-0.24 V *vs* SCE
FDA氧化还原电势：-0.55 V *vs* SCE

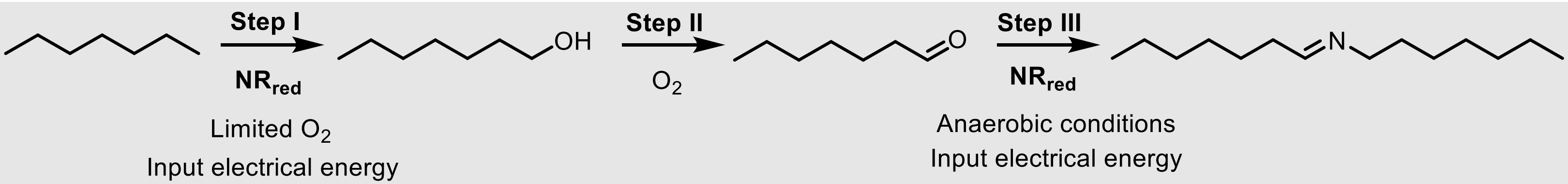


Toluidine blue O (TBO)

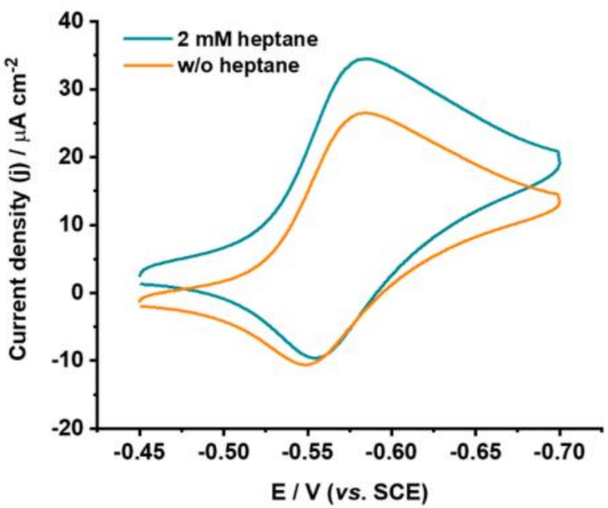


Neutral red (NR)

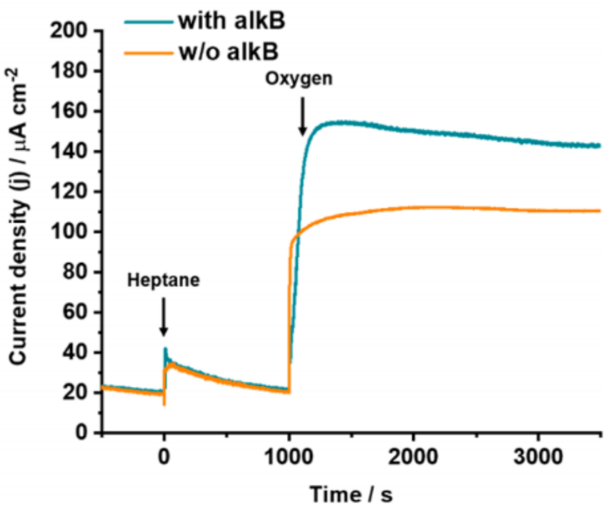
-0.59 V vs SCE



☐ **Cyclic voltammetric investigation**



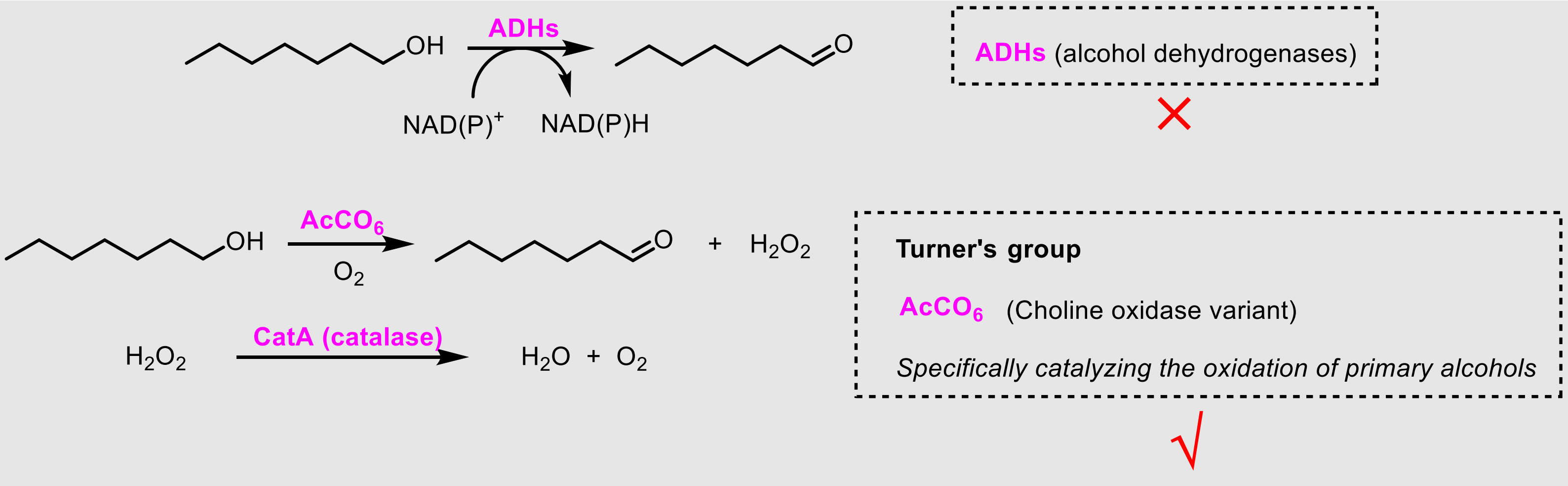
☐ **Amperometric i-t analysis**

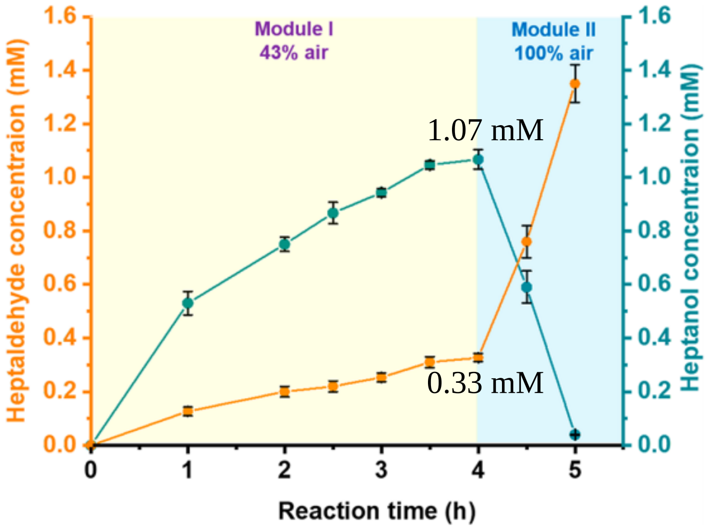


S. D. Minteer; et al. *J. Am. Chem. Soc.* **2022**, *144*, 4047−4056.

酶促电合成在惰性碳氢化合物转化方面的应用

➢ **Step II: Oxidation of 1-Heptanol**





Step II

Cutting off power input

Changing the air ratio from 43% to 100%

Injection of 0.8 mg/mL (0.14 U/mL) $AcCO_6$

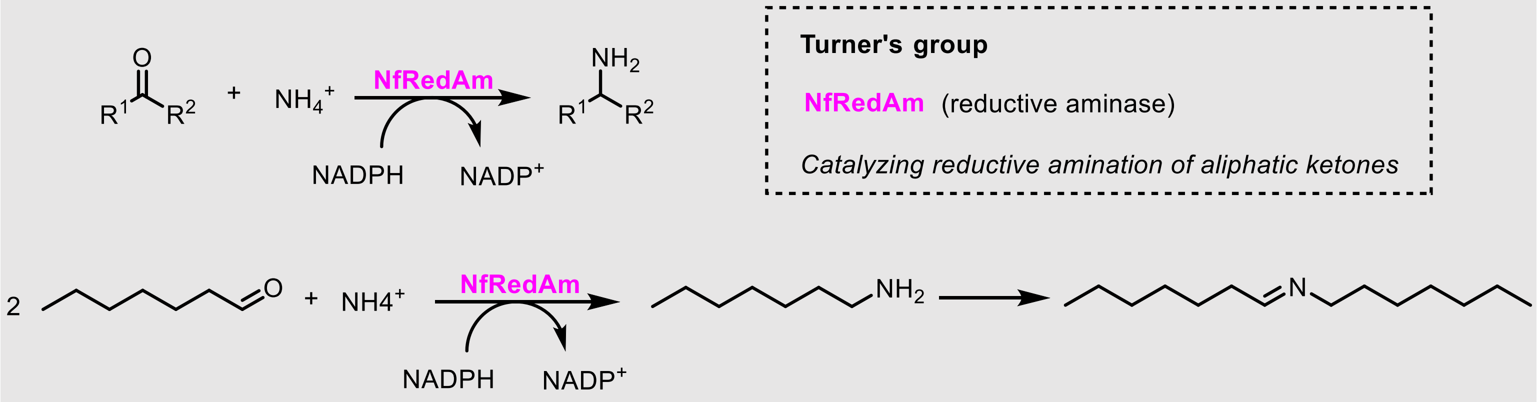
Conversion ratio of 1-heptanol 98%

The highest concentration of heptanal 1.38 mM

S. D. Minteer; et al. *J. Am. Chem. Soc*. **2022**, *144*, 4047−4056.

酶促电合成在惰性碳氢化合物转化方面的应用

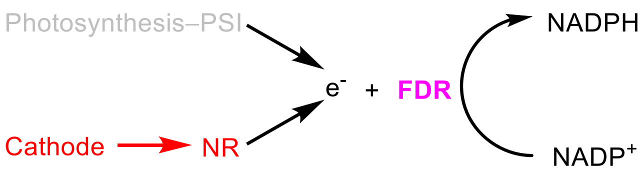
➢ **Step III: Reductive amination of heptanal**



Turner's group

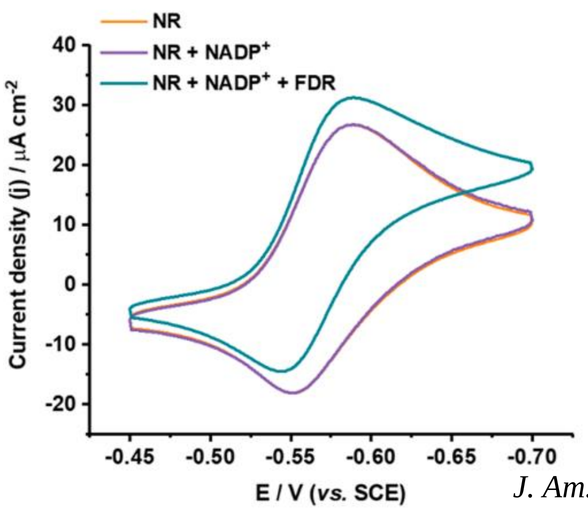
NfRedAm (reductive aminase)

Catalyzing reductive amination of aliphatic ketones

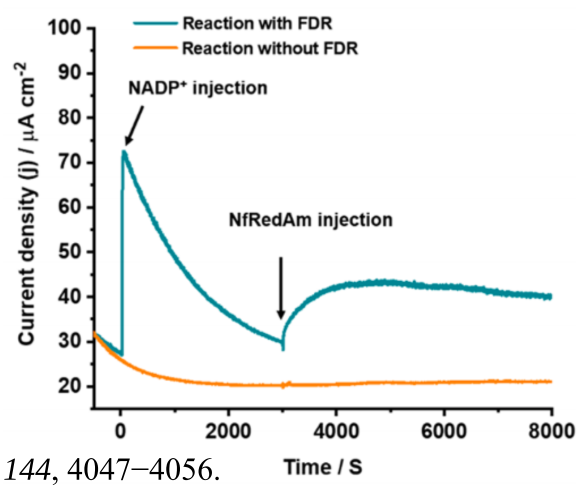


FDR (the flavoenzyme ferredoxin–NADP$^+$ reductase)

☐ **Cyclic voltammetric investigation and amperometric i-t analysis**

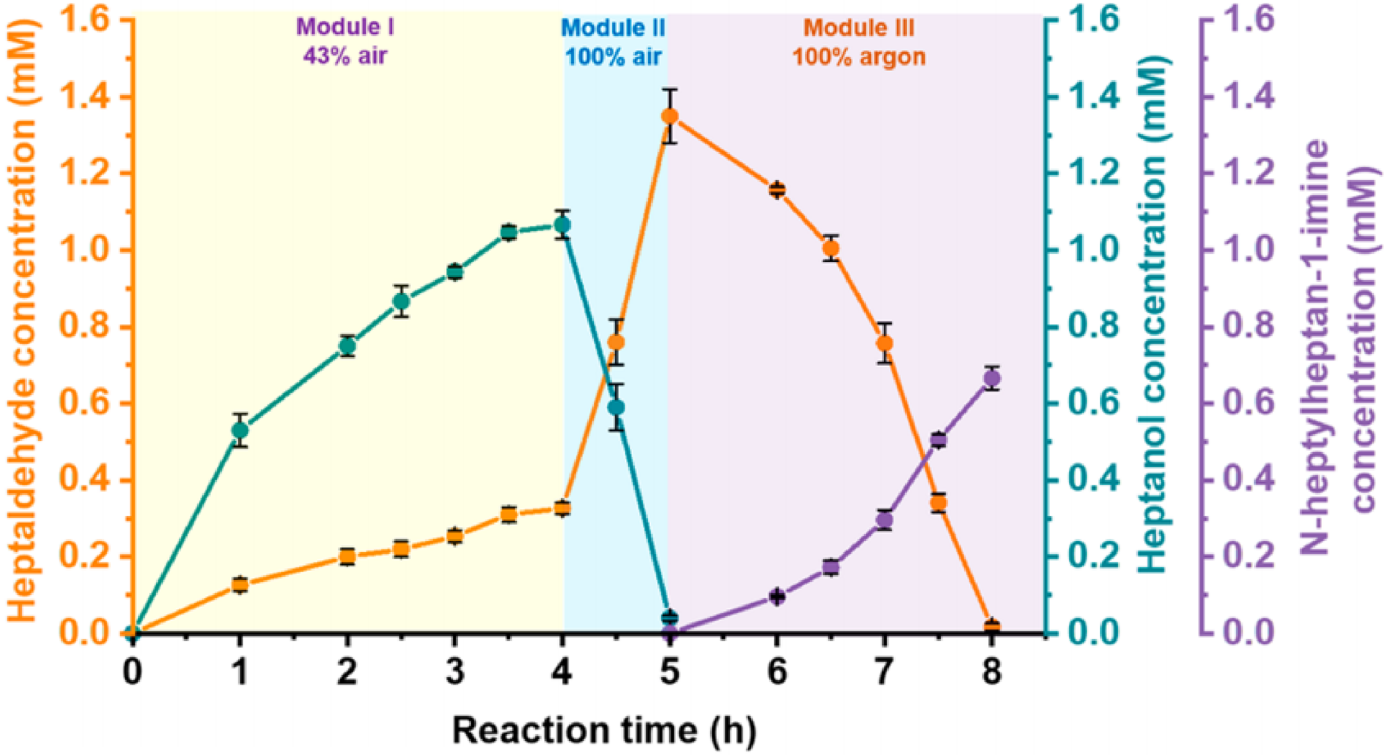


J. Am. Chem. Soc. **2022**, *144*, 4047–4056.



酶促电合成在惰性碳氢化合物转化方面的应用

➢ **Step III integrated with steps I and II**



Step III

- Resupplying power input
- Changing from 100% air to 100% argon gas
- Injection of 0.15 mM $NADP^+$, 30 mM $(NH_4)_3PO_4$ (90 mM NH_4^+), 0.6 mg/mL FDR, and 1.1 mg/mL (1.2 U/mL) NfRedAm

Conversion ratio of heptanal 97%
The highest concentration of N-heptylheptan-1-imine 0.67 mM

S. D. Minteer; et al. *J. Am. Chem. Soc*. **2022**, *144*, 4047−4056.

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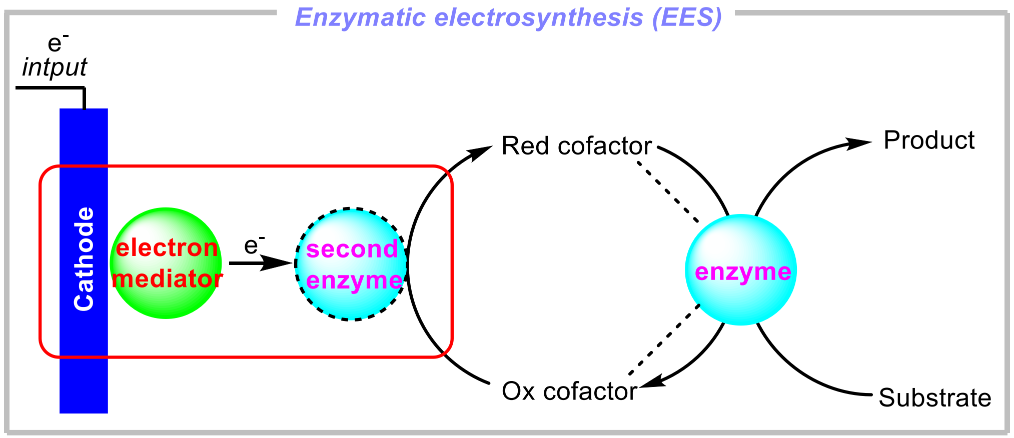
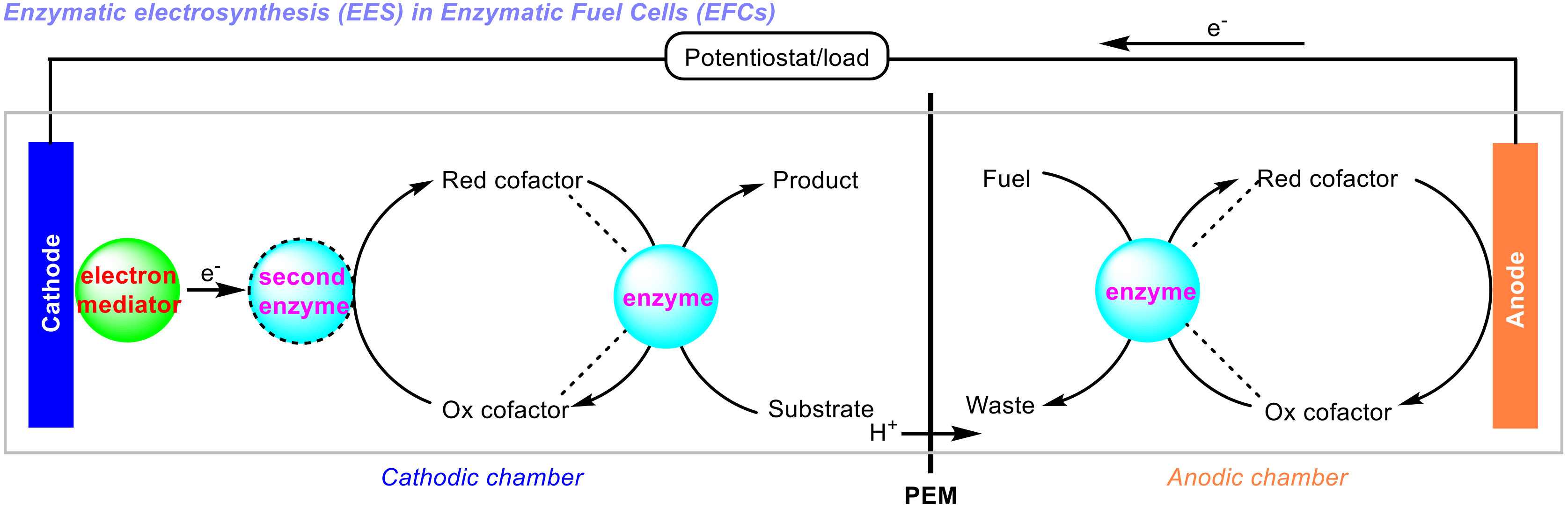
总结与展望

➢ Conclusions



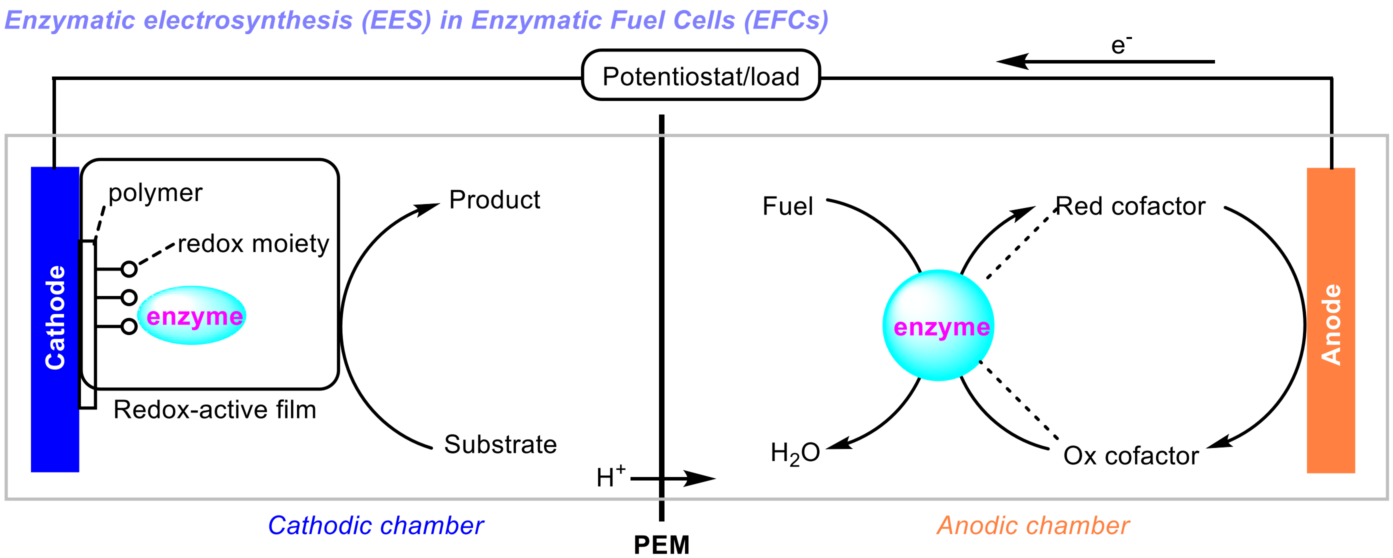
惰性小分子转化方面的应用

- ◆ 氮气的固定
- ◆ 二氧化碳的固定
- ◆ 惰性碳氢化合物转化



总结与展望

➢ Outlook



Ideal enzymatic electrosynthesis

- 无需外部能量输入，实现自供电
- 高度稳定性：空气的稳定性，电极的稳定性等
- 高的电流密度以及高的电子利用率
- 进行大规模的合成

⇨

Solution

- ✓ 开发新型的电子介质
- ✓ 酶负载于聚合物，纳米材料上
- ✓ 酶进行改造修饰
- ✓ 电子介质连接在聚合物中与酶共同固定在电极上
- ✓ 发展新型的电极材料

Thank you！