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Communication

# Semiartificial Photoelectrochemistry for $CO_2$-Mediated Enantioselective Organic Synthesis

Tessel Bouwens, Samuel J. Cobb, Celine W. S. Yeung, Yongpeng Liu, Guilherme Martins, Inês A. C. Pereira, and Erwin Reisner*

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**ABSTRACT:** Photoelectrochemical (PEC) cells are under intensive development for the synthesis of solar fuels, but $CO_2$ reduction typically only results in simple building blocks such as $HCOO^-$. Here, we demonstrate that $CO_2$-converting PEC cells can drive integrated enzymatic domino catalysis to produce chiral organic molecules by using $CO_2/HCOO^-$ as a sustainable redox couple. First, we establish a semiartificial electrode consisting of three enzymes co-immobilized on a high surface area electrode based on carbon felt covered by a mesoporous indium tin oxide (ITO) coating. When applying a mild cathodic potential (−0.25 V vs the reversible hydrogen electrode (RHE)), $CO_2$ is reduced to $HCOO^-$ using a W-formate dehydrogenase ($FDH_{NvH}$) from *Nitratidesulfovibrio vulgaris* Hildenborough, which then enables the reduction of $NAD^+$ to NADH by an $NAD^+$-cofactor-dependent formate dehydrogenase from *Candida boidinii* ($FDH_{CB}$). Subsequently, an alcohol dehydrogenase (ADH) uses NADH generated from $CO_2/HCOO^-$ cycling to reduce acetophenone to chiral 1-phenylethanol in good enantiomeric excess (93%) and conversion yields (38%). Depending on the specific ADH ($ADH_S$ or $ADH_R$), either (*S*)- or (*R*)-1-phenylethanol can be synthesized at pH 6 and 20 °C. To illustrate solar energy utilization, we integrate the three nanoconfined enzymes with a PEC platform based on an integrated organic semiconductor photocathode to allow for enantioselective synthesis (at +0.8 V vs RHE) based on a solar fuel device. This proof-of-principle demonstration shows that concepts and devices from artificial photosynthesis can be readily translated to precise and sustainable biocatalysis, including the production of chiral organic molecules using light.

Photosynthesis has long inspired scientists as it harnesses solar energy to convert simple building blocks ($CO_2$, $H_2O$) into complex organic chemicals (glucose) while releasing $O_2$.[1,2] Accordingly, artificial photosynthetic devices such as photoelectrochemical (PEC) cells aim to mimic this process, but they currently only produce relatively simple chemical fuels (e.g., $H_2$, $HCOO^-$, CO, $CH_4$, ethylene, ethanol, or propanol).[3−7] Current efforts to mimic the natural process have mainly focused on the light-dependent reactions in the thylakoid membrane, while the compartmentalized multienzyme machinery responsible for glucose synthesis from $CO_2$ occurring in the light-independent stromal reactions has received limited attention.[8] As mature PEC devices with high efficiency and stability are emerging, a growing opportunity arises to expand the reactivity space from simple fuels to more complex organic molecules by integrating catalytic cascade reactions in devices, thereby bringing us a step closer to truly mimicking the reactivity of natural photosynthesis.

State-of-the-art PEC cells provide an excellent platform to expand from classical solar fuels reactions (water splitting, $CO_2$ reduction) to enantioselective organic synthesis, in line with our ongoing mission to integrate the fields of artificial photosynthesis and organic chemistry.[9] While the recent renaissance of organic photoredox catalysis has made substantial progress in developing visible light responsive photocatalysts for many noteworthy reactions, it has not yet taken full advantage of the opportunities provided by chiral biocatalytic synthesis under benign, aqueous conditions and solid-state devices employing state-of-the-art semiconductors.

In this study, we aim to close this gap and develop a platform that uses solar energy to drive biocatalysis integrated into a PEC device for chiral organic synthesis. We employ evolutionarily optimized enzymes as biocatalysts to tackle the bottleneck of synthetic catalysts to overcome efficiency, selectivity, and reactivity issues.[10−12] While semiartificial PEC cells employing enzymes are known, only the production of simple fuels such as $H_2$,[13,14] CO,[15] $HCOO^-$,[16,17] and MeOH[18,19] has been demonstrated. However, oxidoreductases provide an opportunity to catalyze an exciting diversity of reactions, including control of chemo-, regio-, and stereoselectivities, which outcompetes the possibilities provided by synthetic catalysts.[20−22] To provide a glimpse of possibilities for the future, we demonstrate here that PEC cells producing classical solar fuels, such as $HCOO^-$ from $CO_2$, can be readily modified using cofactor-dependent enzymes for chiral synthesis using light (Figure 1).

The integrated enzyme cascade consists of three enzymes. The first enzyme is formate dehydrogenase ($FDH_{NvH}$) from *Nitratidesulfovibrio vulgaris* Hildenborough (NvH),[23,24] which is known to attach in an electroactive configuration to porous

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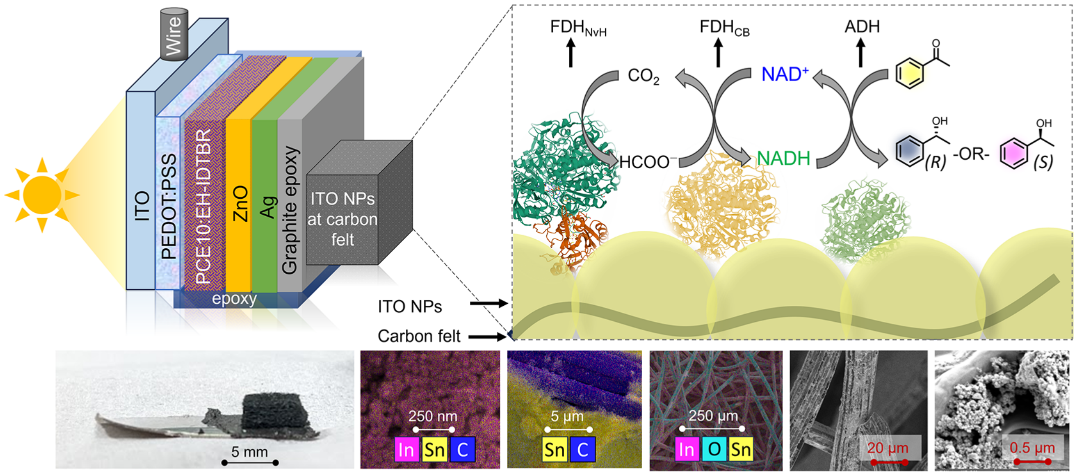

**Figure 1.** Schematic representation of the PEC-cascade system using $CO_2/HCOO^-$ as a mediator to generate chiral building blocks featuring $FDH_{NvH}$, $FDH_{CB}$, and ADH. All three enzymes are integrated into a Ti|ITO-CF scaffold (bottom left) for electrochemical characterization and an OPV-based photocathode, giving OPV|ITO-CF|$FDH_{NvH}$/$FDH_{CB}$/ADH. The SEM and EDX images of Ti|ITO-CF electrodes show the ITO NPs on CF.

ITO electrodes via noncovalent interactions, allowing for $CO_2$ reduction to $HCOO^-$ with marginal overpotential and a $K_M$ for $CO_2$ of 0.42 mM.[25−27] The next enzyme is the NAD$^+$-cofactor-dependent formate dehydrogenase from *Candida boidinii* ($FDH_{CB}$), which uses $HCOO^-$ to catalyze the reduction of NAD$^+$ to NADH. The last enzyme is an alcohol dehydrogenase (ADH), which can use the NADH produced from $CO_2$/$HCOO^-$ cycling to reduce pro-chiral acetophenone (selected as model building block for pharmaceutical applications[28,29]).

Porous metal oxide electrodes made from indium tin oxide (ITO) provide conductivity, a robust interaction with electroactive enzymes, and their porosity gives access to a high surface area to support large quantities of enzymes.[30−32] However, the brittleness and conductivity constraints of porous ITO films only allow for film thicknesses up to approximately 50 $\mu$m.[33,34] Therefore, we developed a conducting scaffold for enzyme immobilization in this study using commercially available carbon felt (CF) cuboids ($5 \times 5 \times 3.2$ mm$^3$) coated with ITO nanoparticles. These macroscopic cuboids provide millimeter thickness and can be readily connected to a titanium foil ($20 \times 10$ mm$^2$) using graphite epoxy (Figures S1−S4, further discussion in the Supporting Information). These Ti|ITO-CF electrodes can be used as a (dark) cathode or integrated into a photocathode (see below). The maximum loading of ITO NPs onto CF was determined (Figure S1), and the CF cuboids demonstrated an apparent porosity of 78%, close to the reported porosity of these CF materials (>80%). Ti|ITO-CF was characterized by SEM and EDX (Figure 1, Figures S2−S4), which show the individual ITO NPs and the presence of In, Sn, and O on the carbon fibers.

$FDH_{NvH}$ (125 pmol) was then dropcast onto the Ti|ITO-CF electrode (0.25 cm$^2$) and analyzed using protein film voltammetry (PFV) in an aqueous electrolyte solution containing MOPS (13.5 mL, 0.1 M, pH 6), DMSO (1.5 mL), NAD$^+$ (1 mM), and acetophenone (50 mM), saturated with $CO_2$ at ambient temperature (20 °C, Table S1). The PFV response shows an onset of catalytic current for $CO_2$ reduction close to 0 V vs the reversible hydrogen electrode (RHE), as expected for the reversible $CO_2$ to $HCOO^-$ biocatalyst (Figure 2).[32] During the back scan, an anodic wave is observed with a peak current at approximately +0.2 V vs RHE, which is only present upon scan reversal following the catalytic cathodic wave (Figure 2b). This observation suggests that this cathodically induced anodic wave is due to the oxidation of $HCOO^-$ produced from the reduction of $CO_2$ in the porous stationary Ti|ITO-CF|$FDH_{NvH}$ electrode. In the absence of $CO_2$, no catalytic currents are observed (Figure 2a).

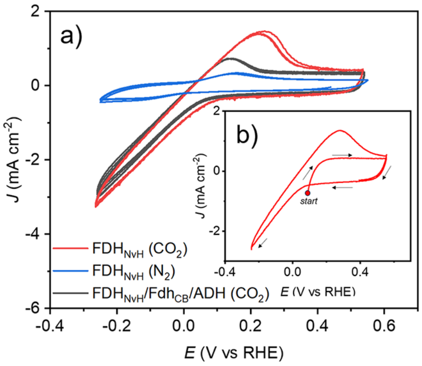

**Figure 2.** (a) Protein film voltammetry (PFV) scans of $FDH_{NvH}$ (red and blue traces) and the 3-enzyme cascade (black trace) under $CO_2$ (red and black traces) or $N_2$ (blue trace). (b) PFV analysis of $FDH_{NvH}$, starting at +50 mV to 550 mV to −250 mV and back (red trace). PFV scans recorded using 10 mV s$^{-1}$ in electrolyte comprised of MOPS (13.5 mL, 0.1 M, pH 6), NAD$^+$ (1 mM), acetophenone (50 mM), and DMSO-$d_6$ (1.5 mL) (Table S1).

Next, we studied the electrochemical response with all three enzymes, using $FDH_{NvH}$ (125 pmol), $FDH_{CB}$ (1 nmol), and ADH (5 nmol) dropcast onto Ti|ITO-CF (0.25 cm$^2$). Note that solely $FDH_{NvH}$ is an electroactive enzyme receiving electrons directly from Ti|ITO-CF, whereas $FDH_{CB}$ and ADH rely on $HCOO^-$ and NADH as an electron source. In a $CO_2$-saturated solution with NAD$^+$ and acetophenone we observe a qualitatively similar voltametric cathodic response for Ti|ITO-CF|$FDH_{NvH}$/$FDH_{CB}$/ADH compared to Ti|ITO-CF|$FDH_{NvH}$, but the current density of the anodic wave at +0.2 V vs RHE is significantly reduced (Figure 2a). This observation supports the idea that $HCOO^-$ is readily consumed by $FDH_{CB}$ and used to generate the NADH cofactor that is needed to produce the chiral alcohol product.

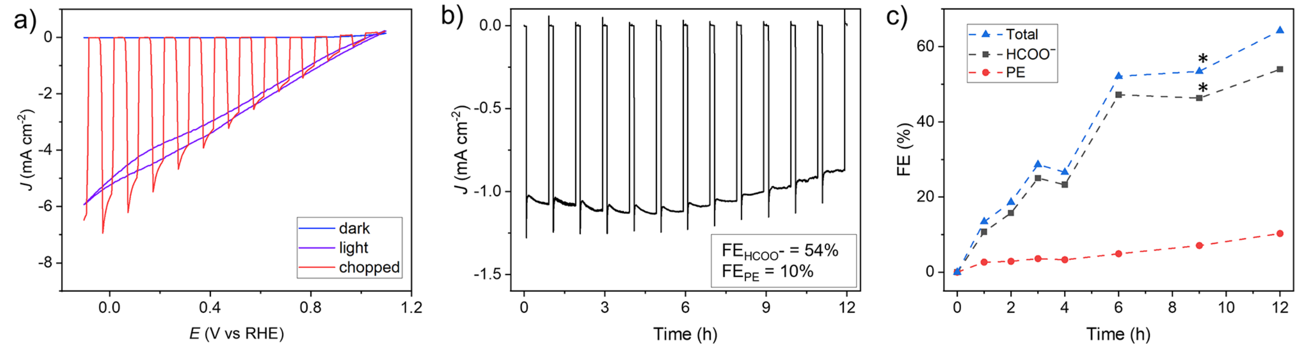

**Figure 3.** Production of chiral (*S*)-1-phenylethanol using a PEC system: OPV|ITO-CF|FDH$_{NvH}$/FDH$_{CB}$/ADH. (a) PFV scans in dark, light, and chopped with on/off cycles of 10 s. (b) Chopped light PF-CA at +0.8 V vs RHE with 50 min on- and 10 min off-cycles. NMR samples were collected after *t* = 0, 1, 2, 3, 4, 6, 9, and 12 h. (c) FE$_{PE}$, FE$_{HCOO^-}$, and FE$_{Total}$. *Calculated from deconvoluted NMR signals.

Protein film chronoamperometry (PF-CA) was performed with ITO-CF|FDH$_{NvH}$ at an applied potential of −0.25 V vs RHE (Figure S5), showing quantitative conversion of $CO_2$ into $HCOO^-$ (Faradaic efficiency, FE$_{HCOO^-}$ = 99%; determined by $^1$H NMR spectroscopy, Figures S6−S8). PF-CA with all three enzymes produces 1-phenylethanol (PE), demonstrating that the cascade is functional electrochemically (FE$_{HCOO^-}$ = 72% and FE$_{PE}$ = 27%, FE$_{total}$ = 99%).

The turnover number of ADH (TON$_{ADH}$) for 1-phenylethanol synthesis is estimated from PF-CA at approximately 5 × 10$^3$ after 12 h (Figures S5, Figures S9 and S10). The cascade generates chiral 1-phenylethanol in high conversions after 12 h (38 ± 8%, Table S2, Figure S7f). Depending on the choice of ADH (Figures S11 and S12), we were able to produce both the (*S*)-enantiomer (enantiomeric excess, *ee* = 93%, determined by chiral HPLC) or the (*R*)-enantiomer (*ee* = 59%) (Figure 2b, Figures S13 and S14).

Isotopic labeling experiments using $^{13}CO_2$ with the Ti|ITO-CF|FDH$_{NvH}$/FDH$_{CB}$/ADH electrode at an applied potential of −0.25 V vs RHE for 4 h confirm the origin of $HCOO^-$ solely from $CO_2$ ($^1$H NMR, $\delta$ = 8.44 ppm, doublet, $J_{C-H}$ = 194 Hz, Figure S15). A control experiment using ITO-CF|FDH$_{NvH}$/FDH$_{CB}$/ADH without $NAD^+$ resulted in negligible amounts of 1-phenylethanol (Figure S16).

The FE and *ee* for Ti|ITO-CF|FDH$_{NvH}$/FDH$_{CB}$/ADH are comparable to previous work employing electrocatalytic enzymatic cascades based on NAD(P)H-recycling using ferrodoxin NADP$^+$ reductase.[22,35,36] Synthetic cofactor regeneration strategies have also been applied previously, e.g., using Cp*Rh(bpy)-derived mediators, but these systems suffer from side reactions, such as $H_2$ formation (FE$_{NADH}$ < 86%).[18,37−45] Our semiartificial platform operates selectively (quantitative FE$_{NADH}$, no side-products detected) with a high $k_{cat}(NAD^+)_{FDH}$ = 8400 h$^{-1}$, whereas Cp*Rh(bpy)-derived mediators only reach $k_{cat}(NAD^+)_{Rh}$ = 36 h$^{-1}$.[46] Electrocatalytic production was also integrated with ADH-promoted synthesis of 1-propanol from 1-propanal via Cp*Rh(bpy)-mediated cofactor regeneration,[44] but this system requires a flow-through electrolytic cell with a negative potential of −1.6 V vs RHE.

The interfacial electron transfer kinetics were investigated by electrochemical impedance spectroscopy (EIS)[47] over the same potential range used for the PFV scans with 0.1 V intervals and a sinusoidal perturbation of 15 mV. Quantitative analysis with Nyquist plot fitting (Figures S17−S20) has previously been validated for electroenzymatic reactions involving H$_2$ase and FDH$_{NvH}$.[48−50] The values for the resistance $R_e$ remain similar across different systems; thus the values of time constant $\tau_e$, defined by $\tau_e = R_e \times C_e$, are driven by the distinct differences in $C_e$ values. The time constant describes all of the processes occurring within the measurement time domain. The addition of FDH$_{CB}$ and FDH$_{CB}$/ADH leads to a smaller $\tau_e$, suggesting that the $CO_2$/$HCOO^-$-cycling for Ti|ITO-CF|FDH$_{NvH}$/FDH$_{CB}$/ADH is faster than the single process at the Ti|ITO-CF|FDH$_{NvH}$ electrode (further discussion in the Supporting Information).[51]

After establishing the semiartificial electrocatalytic domino system, we integrated an organic photovoltaic (OPV)-based PEC system based on a recently established $\pi$-conjugated organic semiconductor PEC platform.[50] Specifically, we employed an OPV device based on PCE10:EH-IDTBR that is encapsulated by graphite epoxy to (i) protect the OPV from the aqueous electrolyte solution and (ii) integrate the ITO-CF electrode scaffold (Figure 1).[52] The ITO-CF cuboid is connected to the OPV using graphite-epoxy paste, and the photocathode is wired to a metal rod. The enzymes were drop-cast onto the OPV|ITO-CF using the same procedure as that for the electrochemical studies on Ti|ITO-CF.

The OPV|ITO-CF|FDH$_{NvH}$/FDH$_{CB}$/ADH photocathode displays an onset potential of 1 V vs RHE with a high photocurrent density of approximately −6 mA cm$^{-2}$ at 0 V vs RHE under standard solar spectrum air mass 1.5 global (AM1.5G) irradiation at 20 °C (pH 6, Figure 3a, Figure S21). PF-CA showed a relatively stable current density of ∼1 mA cm$^{-2}$ at +0.8 V vs RHE during 12 h AM1.5G irradiation (Figure 3b). The generation of the intermediate $HCOO^-$ and (*S*)-1-phenylethanol was followed by $^1$H NMR spectroscopy (Figure 3c, Figures S22 and S23). After 12 h, a FE$_{PE}$ of 10% with a FE$_{HCOO^-}$ of 54% was obtained. Our OPV|ITO-CF|FDH$_{NvH}$/FDH$_{CB}$/ADH photocathode therefore demonstrates solar-powered recycling of NADH using $CO_2$/$HCOO^-$ as a mediator for the enantioselective synthesis of (*S*)-1-phenylethanol (TON$_{ADH}$ of approximately 1.2 × 10$^3$, Figures S23 and S24). The longevity of the current domino PEC system is likely limited by NADH degradation during irradiation (Figure S25).

In summary, we have demonstrated the integration of three enzymes into a state-of-the-art PEC cell using $CO_2$ as a redox mediator to produce NADH to drive the synthesis of chiral organics (both (*R*)- and (*S*)-enantiomers). This strategy provides a possible platform to leverage enzyme-driven photoelectrochemistry to synthesize chiral organic chemicals from simple substrates for the chemical and pharmaceutical industry in the future.[28,29,53] This principle can be readily adopted by contemporary solar fuel devices producing (i) other fuels, e.g., $H_2$ using a cofactor-dependent hydrogenase,[13,54] and (ii) other photocathode materials, e.g., encapsulated silicon, copper oxide, and lead halide perovskite.[4,5,55] This work also intends to inspire and motivate efforts to connect the materials- and device-focused artificial photosynthesis community with organic chemists employing biocatalysis and photoredox catalysis.

## ■ ASSOCIATED CONTENT

### Data Availability Statement

Experimental data of this study can be accessed through the University of Cambridge data repository: 10.17863/CAM.117123.

### Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/jacs.5c02250.

Experimental procedures, material characterization, and additional spectroscopic data (PDF)

## ■ AUTHOR INFORMATION

### Corresponding Author

**Erwin Reisner** − *Yusuf Hamied Department of Chemistry, University of Cambridge, Cambridge CB2 1EW, U.K.;* orcid.org/0000-0002-7781-1616; Email: reisner@ch.cam.ac.uk

### Authors

**Tessel Bouwens** − *Yusuf Hamied Department of Chemistry, University of Cambridge, Cambridge CB2 1EW, U.K.;* Present Address: Department of Chemical Engineering, Delft University of Technology, Van der Maasweg 9, 2629 HZ Delft, The Netherlands

**Samuel J. Cobb** − *Yusuf Hamied Department of Chemistry, University of Cambridge, Cambridge CB2 1EW, U.K.;* Present Address: Department of Chemistry, The University of Manchester, Oxford Road, Manchester M13 9PL, UK.; orcid.org/0000-0001-5015-8090

**Celine W. S. Yeung** − *Yusuf Hamied Department of Chemistry, University of Cambridge, Cambridge CB2 1EW, U.K.*

**Yongpeng Liu** − *Yusuf Hamied Department of Chemistry, University of Cambridge, Cambridge CB2 1EW, U.K.;* orcid.org/0000-0002-4544-4217

**Guilherme Martins** − *Instituto de Tecnologia Química e Biológica António Xavier (ITQB NOVA), Universidade NOVA de Lisboa, 2780-157 Oeiras, Portugal*

**Inês A. C. Pereira** − *Instituto de Tecnologia Química e Biológica António Xavier (ITQB NOVA), Universidade NOVA de Lisboa, 2780-157 Oeiras, Portugal;* orcid.org/0000-0003-3283-4520

Complete contact information is available at: https://pubs.acs.org/10.1021/jacs.5c02250

### Author Contributions

The manuscript was written through contributions of all authors.

### Notes

The authors declare no competing financial interest.

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## ■ REFERENCES

(1) Lewis, N. S.; Nocera, D. G. Powering the Planet: Chemical Challenges in Solar Energy Utilization. *Proc. Natl. Acad. Sci. U. S. A.* **2006**, *103* (43), 15729−15735.

(2) Yoshino, S.; Takayama, T.; Yamaguchi, Y.; Iwase, A.; Kudo, A. $CO_2$ Reduction Using Water as an Electron Donor over Heterogeneous Photocatalysts Aiming at Artificial Photosynthesis. *Acc. Chem. Res.* **2022**, *55* (7), 966−977.

(3) Roh, I.; Yu, S.; Lin, C.-K.; Louisia, S.; Cestellos-Blanco, S.; Yang, P. Photoelectrochemical $CO_2$ Reduction toward Multicarbon Products with Silicon Nanowire Photocathodes Interfaced with Copper Nanoparticles. *J. Am. Chem. Soc.* **2022**, *144* (18), 8002−8006.

(4) Xia, M.; Pan, L.; Liu, Y.; Gao, J.; Li, J.; Mensi, M.; Sivula, K.; Zakeeruddin, S. M.; Ren, D.; Grätzel, M. Efficient $Cu_2O$ Photocathodes for Aqueous Photoelectrochemical $CO_2$ Reduction to Formate and Syngas. *J. Am. Chem. Soc.* **2023**, *145* (51), 27939−27949.

(5) Andrei, V.; Reuillard, B.; Reisner, E. Bias-Free Solar Syngas Production by Integrating a Molecular Cobalt Catalyst with Perovskite-$BiVO_4$ Tandems. *Nat. Mater.* **2020**, *19* (2), 189−194.

(6) Rahaman, M.; Andrei, V.; Wright, D.; Lam, E.; Pornrungroj, C.; Bhattacharjee, S.; Pichler, C. M.; Greer, H. F.; Baumberg, J. J.; Reisner, E. Solar-Driven Liquid Multi-Carbon Fuel Production Using a Standalone Perovskite-$BiVO_4$ Artificial Leaf. *Nat. Energy* **2023**, *8* (6), 629−638.

(7) Zhou, B.; Ou, P.; Pant, N.; Cheng, S.; Vanka, S.; Chu, S.; Rashid, R. T.; Botton, G.; Song, J.; Mi, Z. Highly Efficient Binary Copper-iron Catalyst for Photoelectrochemical Carbon Dioxide Reduction toward Methane. *Proc. Natl. Acad. Sci. U. S. A.* **2020**, *117* (3), 1330−1338.

(8) Miller, T. E.; Beneyton, T.; Schwander, T.; Diehl, C.; Girault, M.; McLean, R.; Chotel, T.; Claus, P.; Cortina, N. S.; Baret, J.-C.; Erb, T. J. Light-Powered $CO_2$ Fixation in a Chloroplast Mimic with Natural and Synthetic Parts. *Science* **2020**, *368* (6491), 649−654.

(9) Reisner, E. When Does Organic Photoredox Catalysis Meet Artificial Photosynthesis? *Angew. Chem., Int. Ed.* **2019**, *58* (12), 3656−3657.

(10) Goldberg, K.; Schroer, K.; Lütz, S.; Liese, A. Biocatalytic Ketone Reduction - A Powerful Tool for the Production of Chiral Alcohols - Part I: Processes with Isolated Enzymes. *Appl. Microbiol. Biotechnol.* **2007**, *76* (2), 237−248.

(11) Hollmann, F.; Opperman, D. J.; Paul, C. E. Biocatalytic Reduction Reactions from a Chemist's Perspective. *Angew. Chem., Int. Ed.* **2021**, *60* (11), 5644−5665.

(12) Chen, H.; Simoska, O.; Lim, K.; Grattieri, M.; Yuan, M.; Dong, F.; Lee, Y. S.; Beaver, K.; Weliwatte, S.; Gaffney, E. M.; Minteer, S. D. Fundamentals, Applications, and Future Directions of Bioelectrocatalysis. *Chem. Rev.* **2020**, *120* (23), 12903−12993.

(13) Edwardes Moore, E.; Andrei, V.; Zacarias, S.; Pereira, I. A. C.; Reisner, E. Integration of a Hydrogenase in a Lead Halide Perovskite Photoelectrode for Tandem Solar Water Splitting. *ACS Energy. Lett.* **2020**, *5* (1), 232−237.

(14) Sokol, K. P.; Robinson, W. E.; Warnan, J.; Kornienko, N.; Nowaczyk, M. M.; Ruff, A.; Zhang, J. Z.; Reisner, E. Bias-Free Photoelectrochemical Water Splitting with Photosystem II on a Dye-Sensitized Photoanode Wired to Hydrogenase. *Nat. Energy* **2018**, *3* (11), 944−951.

(15) Bachmeier, A.; Hall, S.; Ragsdale, S. W.; Armstrong, F. A. Selective Visible-Light-Driven $CO_2$ Reduction on a p-Type Dye-Sensitized NiO Photocathode. *J. Am. Chem. Soc.* **2014**, *136* (39), 13518−13521.

(16) Edwardes Moore, E.; Andrei, V.; Oliveira, A. R.; Coito, A. M.; Pereira, I. A. C.; Reisner, E. A Semi-artificial Photoelectrochemical Tandem Leaf with a $CO_2$-to-Formate Efficiency Approaching 1%. *Angew. Chem., Int. Ed.* **2021**, *60* (50), 26303−26307.

(17) Sokol, K. P.; Robinson, W. E.; Oliveira, A. R.; Warnan, J.; Nowaczyk, M. M.; Ruff, A.; Pereira, I. A. C.; Reisner, E. Photoreduction of $CO_2$ with a Formate Dehydrogenase Driven by Photosystem II Using a Semi-Artificial Z-Scheme Architecture. *J. Am. Chem. Soc.* **2018**, *140* (48), 16418−16422.

(18) Kuk, S. K.; Singh, R. K.; Nam, D. H.; Singh, R.; Lee, J.-K.; Park, C. B. Photoelectrochemical Reduction of Carbon Dioxide to Methanol through a Highly Efficient Enzyme Cascade. *Angew. Chem., Int. Ed.* **2017**, *56* (14), 3827−3832.

(19) Wang, P.; Wang, X.; Chandra, S.; Lielpetere, A.; Quast, T.; Conzuelo, F.; Schuhmann, W. Hybrid Enzyme-Electrocatalyst Cascade Modified Gas-Diffusion Electrodes for Methanol Formation from Carbon Dioxide. *Angew. Chem., Int. Ed.* **2025**, *64* (12), No. e202422882.

(20) Zhang, C.; Zhang, H.; Pi, J.; Zhang, L.; Kuhn, A. Bulk Electrocatalytic NADH Cofactor Regeneration with Bipolar Electrochemistry. *Angew. Chem., Int. Ed.* **2022**, *61* (3), No. e202111804.

(21) Cheng, B.; Wan, L.; Armstrong, F. A. Progress in Scaling up and Streamlining a Nanoconfined, Enzyme-Catalyzed Electrochemical Nicotinamide Recycling System for Biocatalytic Synthesis. *ChemElectroChem.* **2020**, *7* (22), 4672−4678.

(22) Morello, G.; Megarity, C. F.; Armstrong, F. A. The Power of Electrified Nanoconfinement for Energising, Controlling and Observing Long Enzyme Cascades. *Nat. Commun.* **2021**, *12* (1), 340.

(23) Oliveira, A. R.; Mota, C.; Mourato, C.; Domingos, R. M.; Santos, M. F. A.; Gesto, D.; Guigliarelli, B.; Santos-Silva, T.; Romão, M. J.; Cardoso Pereira, I. A. Toward the Mechanistic Understanding of Enzymatic $CO_2$ Reduction. *ACS Catal.* **2020**, *10* (6), 3844−3856.

(24) Note that *Nitratidesulfovibrio vulgaris* Hildenborough was formerly named *Desulfovibrio vulgaris* Hildenborough.

(25) Cobb, S. J.; Badiani, V. M.; Dharani, A. M.; Wagner, A.; Zacarias, S.; Oliveira, A. R.; Pereira, I. A. C.; Reisner, E. Fast $CO_2$ Hydration Kinetics Impair Heterogeneous but Improve Enzymatic $CO_2$ Reduction Catalysis. *Nat. Chem.* **2022**, *14* (4), 417−424.

(26) Edwardes Moore, E.; Cobb, S. J.; Coito, A. M.; Oliveira, A. R.; Pereira, I. A. C.; Reisner, E. Understanding the Local Chemical Environment of Bioelectrocatalysis. *Proc. Natl. Acad. Sci. U. S. A.* **2022**, *119* (4), No. e2114097119.

(27) Cobb, S. J.; Dharani, A. M.; Oliveira, A. R.; Pereira, I. A. C.; Reisner, E. Carboxysome-Inspired Electrocatalysis Using Enzymes for the Reduction of $CO_2$ at Low Concentrations. *Angew. Chem., Int. Ed.* **2023**, *62* (26), No. e202218782.

(28) Raynbird, M. Y.; Sampson, J. B.; Smith, D. A.; Forsyth, S. M.; Moseley, J. D.; Wells, A. S. Ketone Reductase Biocatalysis in the Synthesis of Chiral Intermediates Toward Generic Active Pharmaceutical Ingredients. *Org. Process. Res. Dev.* **2020**, *24* (6), 1131−1140.

(29) Jakoblinnert, A.; Mladenov, R.; Paul, A.; Sibilla, F.; Schwaneberg, U.; Ansorge-Schumacher, M. B.; De María, P. D. Asymmetric Reduction of Ketones with Recombinant E. Coli Whole Cells in Neat Substrates. *Chem. Commun.* **2011**, *47* (44), 12230−12232.

(30) Mersch, D.; Lee, C.-Y.; Zhang, J. Z.; Brinkert, K.; Fontecilla-Camps, J. C.; Rutherford, A. W.; Reisner, E. Wiring of Photosystem II to Hydrogenase for Photoelectrochemical Water Splitting. *J. Am. Chem. Soc.* **2015**, *137* (26), 8541−8549.

(31) Kato, M.; Cardona, T.; Rutherford, A. W.; Reisner, E. Photoelectrochemical Water Oxidation with Photosystem II Integrated in a Mesoporous Indium-Tin Oxide Electrode. *J. Am. Chem. Soc.* **2012**, *134* (20), 8332−8335.

(32) Miller, M.; Robinson, W. E.; Oliveira, A. R.; Heidary, N.; Kornienko, N.; Warnan, J.; Pereira, I. A. C.; Reisner, E. Interfacing Formate Dehydrogenase with Metal Oxides for the Reversible Electrocatalysis and Solar-Driven Reduction of Carbon Dioxide. *Angew. Chem., Int. Ed.* **2019**, *58* (14), 4601−4605.

(33) Fang, X.; Kalathil, S.; Divitini, G.; Wang, Q.; Reisner, E. A three-dimensional hybrid electrode withelectroactive microbes for efficient electrogenesisand chemical synthesis. *Proc. Natl. Acad. Sci. U. S. A.* **2020**, *117* (9), 5074−5080.

(34) Fang, X.; Sokol, K. P.; Heidary, N.; Kandiel, T. A.; Zhang, J. Z.; Reisner, E. Structure-Activity Relationships of Hierarchical Three-Dimensional Electrodes with Photosystem II for Semiartificial Photosynthesis. *Nano Lett.* **2019**, *19* (3), 1844−1850.

(35) Megarity, C. F.; Siritanaratkul, B.; Cheng, B.; Morello, G.; Wan, L.; Sills, A. J.; Heath, R. S.; Turner, N. J.; Armstrong, F. A. Electrified Nanoconfined Biocatalysis with Rapid Cofactor Recycling. *ChemCatChem.* **2019**, *11* (23), 5662−5670.

(36) Megarity, C. F.; Weald, T. R. I.; Heath, R. S.; Turner, N. J.; Armstrong, F. A. A Nanoconfined Four-Enzyme Cascade Simultaneously Driven by Electrical and Chemical Energy, with Built-in Rapid, Confocal Recycling of NADP(H) and ATP. *ACS Catal.* **2022**, *12* (15), 8811−8821.

(37) Wienkamp, R.; Steckhan, E. Indirect Electrochemical Regeneration of NADH by a Bipyridinerhodium(I) Complex as Electron-Transfer Agent. *Angew. Chem., Int. Ed. Engl.* **1982**, *21* (10), 782−783.

(38) Hildebrand, F.; Kohlmann, C.; Franz, A.; Lütz, S. Synthesis, Characterization and Application of New Rhodium Complexes for Indirect Electrochemical Cofactor Regeneration. *Adv. Synth. Catal.* **2008**, *350* (6), 909−918.

(39) Zhang, B.; Xu, S.; He, D.; Chen, R.; He, Y.; Fa, W.; Li, G.; Wang, D. Photoelectrochemical NADH Regeneration Is Highly Sensitive to the Nature of Electrode Surface. *J. Chem. Phys.* **2020**, *153* (6), 064703.

(40) Xing, F.; Xue, X.; Li, J.; Liu, J.; Wang, W.; Dong, W.; Yuan, H.; Liu, J. Sustainable Photocatalytic Biological Cofactor Regeneration Fueled by Selective Alcohol Oxidation over Polarized $ZnIn_2S_4$. *ACS Catal.* **2024**, *14* (15), 11366−11377.

(41) Chen, M.; Liu, F.; Wu, Y.; Li, Y.; Liu, C.; Zhao, Z.; Zhang, P.; Zhao, Y.; Sun, L.; Li, F. Bioinspired Photoelectrochemical NADH Regeneration Based on a Molecular Catalyst-Modified Photocathode. *Chem. Commun.* **2024**, *60* (24), 3319−3322.

(42) Castañeda-Losada, L.; Adam, D.; Paczia, N.; Buesen, D.; Steffler, F.; Sieber, V.; Erb, T. J.; Richter, M.; Plumeré, N. Bioelectrocatalytic Cofactor Regeneration Coupled to $CO_2$ Fixation in a Redox-Active Hydrogel for Stereoselective C-C Bond Formation. *Angew. Chem., Int. Ed.* **2021**, *60* (38), 21056−21061.

(43) Lee, D.; Yamauchi, K.; Sakai, K. Water-Induced Switching in Selectivity and Steric Control of Activity in Photochemical $CO_2$ Reduction Catalyzed by RhCp*(Bpy) Derivatives. *J. Am. Chem. Soc.* **2024**, *146* (46), 31597−31611.

(44) Wang, C.; Dong, W.; Zhang, P.; Ma, Y.; Han, Z.; Zou, Y.; Wang, W.; Li, H.; Hollmann, F.; Liu, J. Formate-Mediated

Electroenzymatic Synthesis via Biological Cofactor NADH. *Angew. Chem., Int. Ed.* **2024**, *63* (41), No. e202408756.

(45) Meyer, J.; Romero, M.; Thöming, J.; Baune, M.; Reimer, N.; Dringen, R.; Bösing, I. Experimental Insights into Electrocatalytic $[Cp^*Rh(Bpy)Cl]^+$ Mediated NADH Regeneration. *Sci. Rep.* **2023**, *13*, 22394.

(46) Hollmann, F.; Witholt, B.; Schmid, A. $[Cp^*Rh(Bpy)(H_2O)]^{2+}$: A Versatile Tool for Efficient and Non-Enzymatic Regeneration of Nicotinamide and Flavin Coenzymes. *J. Mol. Catal. B Enzym.* **2002**, *19*−*20*, 167−176.

(47) Pandey, K.; Islam, S. T. A.; Happe, T.; Armstrong, F. A. Frequency and Potential Dependence of Reversible Electrocatalytic Hydrogen Interconversion by [FeFe]-Hydrogenases. *Proc. Natl. Acad. Sci. U. S. A.* **2017**, *114* (15), 3843−3848.

(48) Liu, Y.; Pulignani, C.; Webb, S.; Cobb, S. J.; Rodríguez-Jiménez, S.; Kim, D.; Milton, R. D.; Reisner, E. Electrostatic [FeFe]-Hydrogenase-Carbon Nitride Assemblies for Efficient Solar Hydrogen Production. *Chem. Sci.* **2024**, *15* (16), 6088−6094.

(49) Liu, Y.; Bin Mohamad Annuar, A.; Rodríguez-Jiménez, S.; Yeung, C. W. S.; Wang, Q.; Coito, A. M.; Manuel, R. R.; Pereira, I. A. C.; Reisner, E. Solar Fuel Synthesis Using a Semiartificial Colloidal Z-Scheme. *J. Am. Chem. Soc.* **2024**, *146* (43), 29865−29876.

(50) Yeung, C. W. S.; Liu, Y.; Cobb, S.; Andrei, V.; Coito, A.; Manuel, R.; Pereira, I.; Reisner, E. Semi-Artificial Leaf Interfacing Organic Semiconductors and Enzymes for Solar Fuel Synthesis. ChemRxiv preprint, Submission: December 20, 2024. DOI: 10.26434/chemrxiv-2024-f49zl.

(51) Wieczorek, A.; Liu, Y.; Cho, H.-H.; Sivula, K. Assessing the Charge Carrier Dynamics at Hybrid Interfaces of Organic Photoanodes for Solar Fuels. *J. Phys. Chem. Lett.* **2024**, *15* (24), 6347−6354.

(52) Yeung, C. W. S.; Andrei, V.; Lee, T. H.; Durrant, J. R.; Reisner, E. Organic Semiconductor-$BiVO_4$ Tandem Devices for Solar-Driven $H_2O$ and $CO_2$ Splitting. *Adv. Mater.* **2024**, *36* (35), 2404110.

(53) Huang, C.; Xiong, P.; Lai, X.-L.; Xu, H.-C. Photoelectrochemical Asymmetric Catalysis. *Nat. Catal* **2024**, *7* (12), 1250−1254.

(54) Lauterbach, L.; Lenz, O.; Vincent, K. A. $H_2$ -driven Cofactor Regeneration with $NAD(P)^+$ -reducing Hydrogenases. *FEBS J.* **2013**, *280* (13), 3058−3068.

(55) Jia, X.; Stewart-Jones, E.; Alvarez-Hernandez, J. L.; Bein, G. P.; Dempsey, J. L.; Donley, C. L.; Hazari, N.; Houck, M. N.; Li, M.; Mayer, J. M.; Nedzbala, H. S.; Powers, R. E. Photoelectrochemical $CO_2$ Reduction to CO Enabled by a Molecular Catalyst Attached to High-Surface-Area Porous Silicon. *J. Am. Chem. Soc.* **2024**, *146* (12), 7998−8004.