

# Bioelectrocatalytic CO<sub>2</sub> Reduction by Redox Polymer-Wired Carbon Monoxide Dehydrogenase Gas Diffusion Electrodes

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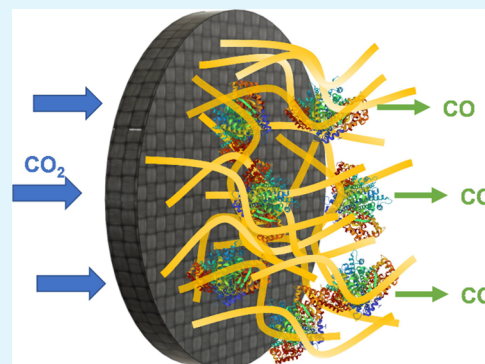
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**ABSTRACT:** The development of electrodes for efficient CO<sub>2</sub> reduction while forming valuable compounds is critical. The use of enzymes as catalysts provides the advantage of high catalytic activity in combination with highly selective transformations. We describe the electrical wiring of a carbon monoxide dehydrogenase II from *Carboxydotherrmus hydrogenoformans* (ChCODH II) using a cobaltocene-based low-potential redox polymer for the selective reduction of CO<sub>2</sub> to CO over gas diffusion electrodes. High catalytic current densities of up to  $-5.5 \text{ mA cm}^{-2}$  are achieved, exceeding the performance of previously reported bioelectrodes for CO<sub>2</sub> reduction based on either carbon monoxide dehydrogenases or formate dehydrogenases. The proposed bioelectrode reveals considerable stability with a half-life of more than 20 h of continuous operation. Product quantification using gas chromatography confirmed the selective transformation of CO<sub>2</sub> into CO without any parasitic co-reactions at the applied potentials.

**KEYWORDS:** carbon monoxide dehydrogenase, CO<sub>2</sub> reduction, enzymes, gas diffusion electrodes, redox polymers



## INTRODUCTION

As a greenhouse gas, increasing amounts of CO<sub>2</sub> in the atmosphere contribute significantly to climate change.<sup>1</sup> The reduction of CO<sub>2</sub> has become of significant importance over the course of the last few years, as it offers the possibility to decrease the amounts of CO<sub>2</sub> present in the atmosphere. Furthermore, it allows for the use of CO<sub>2</sub> as an alternative carbon feedstock to fossil fuels.<sup>2</sup> Due to the inert nature of CO<sub>2</sub>, an efficient catalyst is crucial to reducing CO<sub>2</sub> for the synthesis of valuable products. So far, substantial research has been put into using abiotic catalysts, such as metals, metal oxides, N-doped carbon materials, and molecular catalysts for electrocatalytic CO<sub>2</sub> reduction.<sup>3–7</sup> However, these catalysts are commonly operated under harsh conditions and often suffer from low product specificity due to the competing H<sub>2</sub> evolution reaction. Therefore, considerable efforts are needed for the design and operation of catalysts providing high conversion selectivities.<sup>8</sup> The ability of enzymes to effectively catalyze reactions toward specific products in aqueous electrolytes at mild pH and temperature makes them highly advantageous catalysts to be investigated.<sup>9</sup> Recently, several studies have been devoted to the characterization and potential applications of the only two enzymes capable of directly reducing CO<sub>2</sub> to CO and formate, namely, carbon monoxide dehydrogenase and formate dehydrogenase, respectively.<sup>10</sup>

Carbon monoxide dehydrogenases (CODHs) are highly active and efficient enzymes known to catalyze the conversion between CO and CO<sub>2</sub>.<sup>11</sup> Among the different enzyme classes,

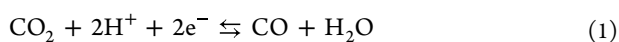
Mo/Cu-containing CODHs are air-stable but only capable of the unidirectional oxidation of CO to CO<sub>2</sub>.<sup>12</sup> In contrast, the Ni-containing CODHs are extremely oxygen sensitive<sup>13</sup> but allow for bidirectional reversible catalysis (see eq 1) and thereby are suited for CO<sub>2</sub> reduction. The active site, containing a [NiFe<sub>4</sub>S<sub>4</sub>] cluster, possesses at least three different redox states. Two of these redox states, C<sub>red1</sub> and C<sub>red2</sub>, are thought to take part in the catalytic cycle.<sup>13–16</sup> It is assumed that CO<sub>2</sub> binds to the Ni ion in the C<sub>red2</sub> state, via the carbon in a bent configuration where one of the oxygens interacts with a dangler iron ion and the other is stabilized by hydrogen bonds from the protein environment.<sup>15</sup> The [3Fe–4S–Ni] part of the C-cluster is thought to transfer two electrons to CO<sub>2</sub>, while the dangler iron facilitates C–O bond cleavage and hydroxide formation.<sup>13</sup> The redox states interconvert with an average redox potential of  $E(\text{C}_{\text{red1}}/\text{C}_{\text{red2}}) = -530 \text{ mV vs SHE}$ , very close to the thermodynamic potential for the CO<sub>2</sub>/CO couple at pH 7.<sup>13,16</sup> Additionally, an inactive oxidized state of the enzyme (C<sub>ox</sub>) can form at potentials more positive than  $-300 \text{ mV vs SHE}$ .<sup>13</sup> Thus, the enzyme can be inactivated by exposure to high potentials.<sup>17</sup>

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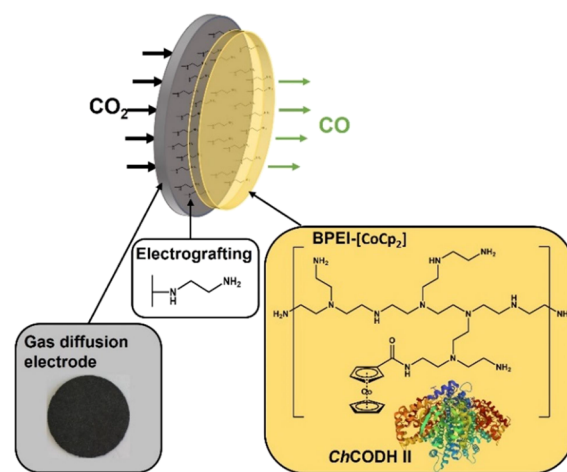
Recent reports have shown the fabrication of highly active bioelectrodes for  $\text{CO}_2$  reduction using a recombinant CODH from *Rhodospirillum rubrum* in direct electron transfer (DET) with carbon nanotube-modified electrode surfaces.<sup>16,18</sup> Although the current densities of these electrodes reached up to  $-4.9 \text{ mA cm}^{-2}$ ,<sup>18</sup> approaching the performance of metal- and molecular-based catalysts,<sup>16</sup> the use of specific linkers for adequate enzyme immobilization was crucial for optimum performance. Under DET configuration, the enzymes must be in direct contact with the electrode and in an appropriate orientation to allow electrical wiring to the conductive surface.<sup>19</sup> In contrast, the use of redox mediators to shuttle electrons between enzymes and the electrode provides effective electron transfer independent of the biomolecule orientation and with the possibility to wire more enzymes than only those in direct contact with the electrode surface. Such a mediated electron transfer configuration has been demonstrated for a CODH from *Moorella thermoacetica* in solution using methyl viologen as the redox mediator.<sup>20</sup> However, the use of freely diffusing redox mediators has the disadvantage of potential mediator leakage, limiting the long-term stability of the electrode.<sup>21</sup> Furthermore, it can lead to short-circuiting in a complete electrolyzer. In that respect, redox polymers are advantageous for the fabrication of bioelectrodes due to the covalent tethering of the redox moieties to a polymer backbone. In addition, the three-dimensional polymeric matrix is beneficial for the stable entrapment of enzymes on the electrode surface, allowing immobilization and electrical wiring of an increased number of enzyme molecules.<sup>22</sup>

Here, we present the electrical wiring of CODH II from *Carboxydotherrmus hydrogenoformans* using a low-potential redox polymer, constituting the first report on the integration of a CODH within a redox hydrogel. A cobaltocene derivative bound to branched polyethylenimine (BPEI-[CoCp<sub>2</sub>])<sup>23,24</sup> is employed for enzyme immobilization and electrical wiring with the electrode surface enabling the fabrication of efficient gas diffusion bioelectrodes for bioelectrocatalytic  $\text{CO}_2$  reduction.

## RESULTS AND DISCUSSION

For the development of the CODH-based  $\text{CO}_2$ -reducing bioelectrode, a gas diffusion layer was used as the electrode substrate. Gas diffusion electrodes circumvent mass transport limitations by decreasing the diffusional pathways of gaseous substrates and therefore increasing the flux of substrate toward the catalytic sites, which results in an increased catalytic current.<sup>25</sup> This strategy has been demonstrated for the fabrication of bioelectrodes integrating isolated enzymes within redox polymer matrices for the highly efficient conversion of gaseous substrates such as  $\text{H}_2$ <sup>26–28</sup> and  $\text{CO}_2$ .<sup>29</sup>

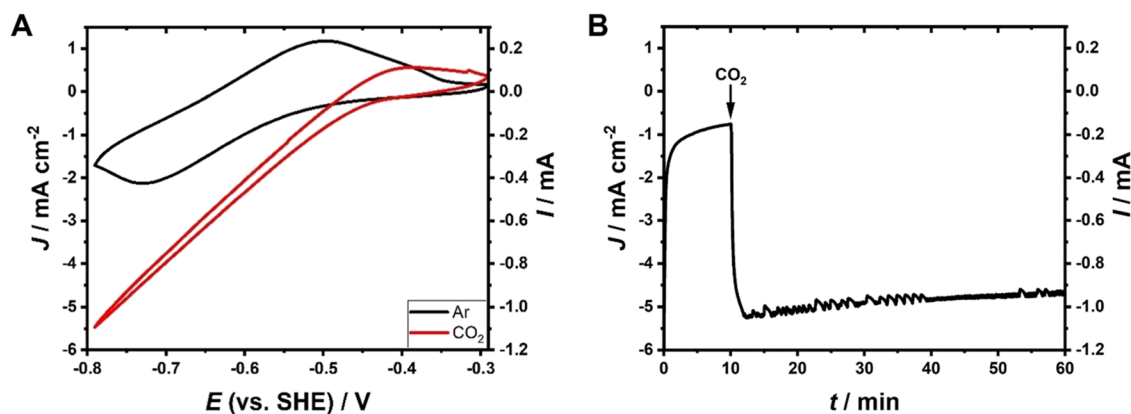
The carbon-based gas diffusion electrode was first modified by means of electrografting, leading to a monolayer of free amino groups on the electrode surface to attain an adequate stability of the modification layer. Then, the electrode was modified with a mixture of the redox polymer BPEI-[CoCp<sub>2</sub>] and the isolated enzyme ChCODH II. To attach the film covalently to the electrode surface and generate a cross-linked three-dimensional network entrapping the enzyme over the electrode, the crosslinker poly(ethylene glycol) diglycidyl ether (PEGDGE) was added. Figure 1 depicts a schematic representation of the modified electrode.



**Figure 1.** Schematic representation of the redox polymer/enzyme gas diffusion electrode for  $\text{CO}_2$  reduction. The image includes an optical micrograph of the modified gas diffusion electrode, the electrografted layer of ethylenediamine, the structures of the redox polymer BPEI-[CoCp<sub>2</sub>], and the enzyme CODH II from *C. hydrogenoformans* (ChCODH II, PDB ID: 1SUF<sup>30,31</sup>).

We first evaluated the ability of the redox polymer to wire CODH to the electrode. The cobaltocene-based redox polymer deposited on a glassy carbon electrode in the absence of enzyme (Figure S1) revealed a midpoint potential of about  $-620 \text{ mV vs SHE}$ . This midpoint potential was more negative than that of the enzyme  $E(C_{\text{red1}}/C_{\text{red2}})$ , ensuring adequate wiring for biocatalytic reduction processes. The modified electrodes integrating ChCODH II were characterized using cyclic voltammetry in the absence and presence of  $\text{CO}_2$ . The cyclic voltammograms (CVs), displayed in Figure 2a, show a pronounced catalytic current in the presence of  $\text{CO}_2$ , while under an Ar atmosphere, only the response of the redox polymer can be seen. The current under turnover conditions reached up to  $-1.1 \text{ mA}$  at an applied potential of  $-0.79 \text{ V vs SHE}$ . This current accounts for a maximum current density of up to  $-5.5 \text{ mA cm}^{-2}$  considering a geometric surface area of  $0.2 \text{ cm}^2$ . The difference between cathodic currents recorded under  $\text{CO}_2$  and Ar at an applied potential of  $-0.79 \text{ V vs SHE}$  was calculated to specifically determine the catalytic current density for  $\text{CO}_2$  reduction, which amounted to  $-4.4 \text{ mA cm}^{-2}$  (Figure 2b). The attained performance exceeds any previous reports on enzymatic  $\text{CO}_2$  reduction, making use of either CODH or formate dehydrogenase with the enzyme in the DET regime or wired by redox mediators (see Table S1). A potential of  $-0.79 \text{ V vs SHE}$  was chosen to examine the catalytic current since thereby a fully reduced redox polymer was ensured. However, more negative applied potentials were prevented to avoid interferences from reductive background processes taking place at the modified electrode. A control electrode was fabricated following the same modification approach but without integrating the CODH enzyme. Even at more negative applied potentials, no catalytic current response could be observed in the presence of  $\text{CO}_2$  (Figure S2), confirming that the conversion was catalyzed by the enzyme.

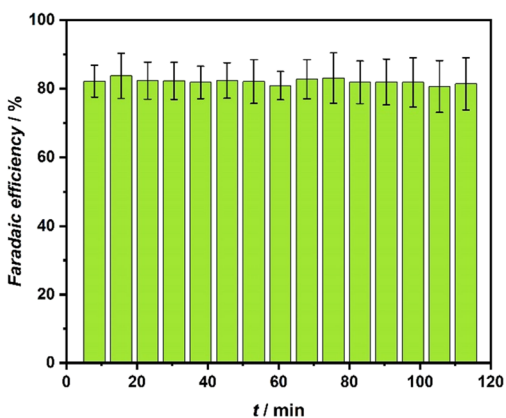
The reaction products were quantified using gas chromatography to determine the selectivity and assess if other products were formed directly at the electrode or the redox polymer at the applied negative potentials. The gas diffusion cell was coupled to a gas chromatograph for in-line analysis of gaseous



**Figure 2.** Electrochemical characterization using (A) cyclic voltammetry and (B) amperometry of a BPEI-[CoCp<sub>2</sub>]/ChCODH II-modified gas diffusion electrode using Ar or CO<sub>2</sub> applied to the backside of the electrode. Electrolyte: Ar-saturated 0.1 M phosphate buffer (pH 7.3). Scan rate in (A): 5 mV s<sup>-1</sup>. Applied potential in (B): -0.79 V vs SHE. Current densities were calculated with respect to the modified geometric surface area ( $d = 5$  mm).

product species during electrochemical measurements (Figure S3).

The analysis of a BPEI-[CoCp<sub>2</sub>]/ChCODH II-modified electrode at a constant applied potential of -0.79 V vs SHE with CO<sub>2</sub> provided to the backside of the electrode revealed the specific reduction of CO<sub>2</sub> to CO. CO, the expected product from the enzymatic conversion, was solely obtained with a Faradaic efficiency to be (82.1 ± 0.8)% (Figure 3;  $n =$



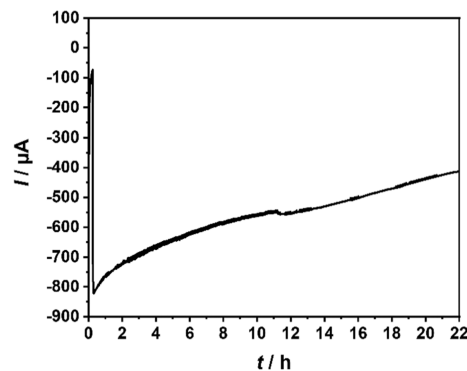
**Figure 3.** Faradaic efficiency calculated for the conversion of CO<sub>2</sub> with a BPEI-[CoCp<sub>2</sub>]/ChCODH II-modified electrode. The bio-electrode was operated under constant turnover conditions (CO<sub>2</sub>,  $E_{app} = -0.79$  V vs SHE) in Ar-saturated 0.1 M phosphate buffer (pH 7.3). Error bars represent the standard deviation ( $n = 3$ ).

3), while no other side products were observed (Figure S4). The samples for the analysis were only taken from the gaseous stream passing the backside of the electrode (see Figure S3), since CO exhibits a low solubility in aqueous solutions and will hence predominantly diffuse back to the gas phase from the three-phase reaction zone within the gas diffusion electrode.

A BPEI-[CoCp<sub>2</sub>]-modified control electrode without enzyme was analyzed at different applied potentials in the presence of CO<sub>2</sub> provided to the backside of the gas diffusion electrode. No products could be detected at any of the applied potentials (Figure S5), confirming that unspecific conversion of CO<sub>2</sub> or the formation of side products such as H<sub>2</sub> at the carbon-based gas diffusion electrode material or the redox polymer is negligible. The current observed during this

measurement indicates that the remaining faradaic efficiency could be ascribed to background processes that are not related to enzymatic CO<sub>2</sub> conversion nor hydrogen evolution. The electrode could be safely operated at applied potentials as low as -0.79 V vs SHE where high catalytic currents were observed.

The stability of the modified bioelectrode under continuous turnover conditions was evaluated by means of amperometry at constant applied potentials (Figure 4). For the first 10 min,



**Figure 4.** Stability measurement under continuous turnover conditions with a BPEI-[CoCp<sub>2</sub>]/ChCODH II-modified gas diffusion electrode.  $E_{app} = -0.79$  V vs SHE. Electrolyte: Ar-saturated 0.1 M phosphate buffer (pH 7.3).

Ar was provided to the backside of the gas diffusion electrode to determine the background current before changing the gas feed to CO<sub>2</sub>. The initial catalytic current was -0.75 mA, corresponding to a maximum turnover frequency of 2.7 s<sup>-1</sup>. The amperometric measurement was run until the catalytic current had reached 50% of the initial catalytic current after 22 h. Although promising stability under continuous turnover was observed, the decrease in activity over time was likely due to an instability of the redox polymer film, as confirmed by a comparison of CVs recorded before and after the long-term catalytic conversion experiment (Figure S6).

## CONCLUSIONS

To conclude, we report the successful wiring of the enzyme CODH II from *C. hydrogenoformans* on gas diffusion electrodes using the low potential redox polymer BPEI-[CoCp<sub>2</sub>]. Product

analysis via GC confirmed selective enzymatic CO<sub>2</sub> conversion to CO without the formation of any side products at negative applied potentials. The outstanding performance of the fabricated bioelectrode in terms of achieved current densities and operational stability highlights the potential of redox enzymes for the fabrication of functional CO<sub>2</sub>-electroreduction systems. Based on the developed bioelectrode, the synthesis of products with a higher value is envisaged by the establishment of catalytic cascade reaction systems.

## EXPERIMENTAL SECTION

**Chemicals and Materials.** All chemicals (reagent or higher grade) were purchased from Sigma Aldrich, Merck, VWR, or Fisher Scientific and were used as received. The cobaltocene precursor was purchased from MCAT. Carbon dioxide (≥99.9%) was from Air Liquide. Distilled water was obtained using a Millipore water purification system.

**Enzyme Expression and Purification.** A gene encoding CODH II (AF249899.1) from *Carboxydotherrmus hydrogenoformans* strain Z-2901 was ordered from GenScript as a codon-optimized DNA sequence in pUC57 containing KasI and PstI restriction sites at the 5' and 3' ends, respectively. The *ChCODH II* gene was subcloned into the expression plasmid pASK-IBA17+ between the KasI and PstI sites giving a construct that encodes *ChCODH II* with an amino-terminal Strep-tag II and TEV protease cleavage site. BL21(DE3)  $\Delta$ iscR cells<sup>1</sup> were transformed with the *ChCODH II* construct and grown at 37 °C in LB-phosphate containing 0.5% (w/v) glucose, 1 mM NiCl<sub>2</sub>, 2 mM ammonium iron citrate, 5 mM L-cysteine, 100  $\mu$ g mL<sup>-1</sup> ampicillin, and 50  $\mu$ g mL<sup>-1</sup> kanamycin to an optical density of 0.6 at 600 nm. The cell suspension was then transferred to sealed bottles and purged with N<sub>2</sub> before protein production was induced by addition of 200  $\mu$ g L<sup>-1</sup> anhydrotetracycline overnight at room temperature. Cells were harvested by centrifugation, resuspended in 100 mM Tris-HCl, 150 mM NaCl, pH 8, and lysed by sonication. The cell suspension was clarified by centrifugation, and the protein was purified from the supernatant using a Streptactin column (IBA). The purified protein was concentrated to 486  $\mu$ M, sealed in glass vials, and stored at -80 °C until use.

**Redox Polymer Synthesis.** BPEI (40 mg, 50 wt % in water) was dried overnight in a vacuum. Then, 20 mg of the dried polymer backbone was dissolved in dry dimethyl sulfoxide (DMSO) (1.3 mL) under an Ar atmosphere and fresh Ar-saturated Et<sub>3</sub>N (12  $\mu$ L) was added. The solution was stirred for 5 min. Three vacuum-Ar cycles were carried out to ensure completely O<sub>2</sub>-free conditions. The redox mediator 1-(2,5-dioxopyrrolidinylcarboxy)-cobaltocenium hexafluorophosphate (12 mg) was dissolved in dry DMSO (1 mL). Then, the yellow mediator containing solution was slowly added to the BPEI solution and the whole mixture was stirred overnight (20 h) under an Ar atmosphere at 28 °C. Afterward, Et<sub>2</sub>O (10 mL) was added, which leads to the precipitation of a dark yellow oil. Et<sub>2</sub>O was added additionally two times (10 mL each) until the solution was colorless. Thereafter, the crude product sample was washed first with CH<sub>2</sub>Cl<sub>2</sub> (10 mL) and second using MeOH (10 mL). The residue was left to dry under vacuum for two nights. The dried polymer was resublimized in H<sub>2</sub>O (2 mL) leading to a polymer concentration of 7.0 mg mL<sup>-1</sup>.

**Electrode Preparation.** The electrode material was first modified by means of an electrografting procedure using a  $\mu$ Autolab Type III potentiostat. A carbon-based gas diffusion layer (H23, Freudenberg Performance Materials; 210  $\mu$ m thickness,  $d = 1.8$  cm), used as an electrode substrate, was fixed in a cell exposing a defined electrode area and only one side of the electrode surface for modification. Then, cyclic voltammetry in a range from 0.0 to 1.4 V vs pseudo-Ag/AgCl reference electrode at a scan rate of 50 mV s<sup>-1</sup> for 20 cycles was performed in 15 mM *N*-Boc-ethylenediamine and 0.15 M TBABF<sub>4</sub> in MeCN. Afterward, the electrode was rinsed with EtOH. For deprotection of the *N*-Boc-protected amino groups, the electrode was treated with 4 M HCl in dioxane for at least 1 h. Then, the electrode was submerged in EtOH (2 min) and then washed with

H<sub>2</sub>O. After drying under ambient conditions, the electrode was transferred into an anaerobic glovebox for the modification with the catalytically active layer. A solution of BPEI-[CoCp<sub>2</sub>] (20  $\mu$ L, 7.0 mg mL<sup>-1</sup>), CODH (3  $\mu$ L, 486  $\mu$ M), and PEGDGE (4  $\mu$ L, 1:40 dilution in H<sub>2</sub>O) was deposited over the electrode surface by means of drop-casting, modifying an area of 0.2 cm<sup>2</sup>, and left to dry overnight in the anaerobic glovebox before being used for electrochemical measurements. For control experiments, the electrode was modified only with BPEI-[CoCp<sub>2</sub>] (15.4  $\mu$ L, 13.5 mg mL<sup>-1</sup>) and PEGDGE (4.62  $\mu$ L, 1:40 dilution in H<sub>2</sub>O). An electrode modified with BPEI-[CoCp<sub>2</sub>] (20  $\mu$ L, 7.0 mg mL<sup>-1</sup>) and PEGDGE (4  $\mu$ L, 1:40 dilution in H<sub>2</sub>O) was used for the control experiment with in-line coupled gas chromatography. The gas diffusion cell was assembled inside the glovebox. To avoid leakage of electrolyte through the hydrophilized electrode, an H23I2 gas diffusion layer (Freudenberg Performance Materials, 210  $\mu$ m thickness,  $d = 1.8$  cm) with an additional hydrophobic treatment was positioned behind the modified electrode. For the electrochemical measurements, the cell was taken out of the glovebox but kept under an Ar atmosphere.

For the characterization of the low potential redox polymer, a glassy carbon electrode was modified with BPEI-[CoCp<sub>2</sub>] (3  $\mu$ L, 7.0 mg mL<sup>-1</sup>) and let to dry under ambient conditions.

**Electrochemical Measurements.** All electrochemical measurements were performed in a standard three-electrode setup, employing a gas diffusion cell.<sup>2</sup> The enzyme-modified electrode acted as the working electrode. An Ag/AgCl/3 M KCl reference electrode and a Pt wire counter electrode were employed. A Gamry Reference 1000 potentiostat was used for all electrochemical measurements. The potentials are given vs the standard hydrogen electrode (SHE) according to  $E_{\text{SHE}} = E_{\text{Ag/AgCl/3 M KCl}} + 210$  mV. The reported current densities were calculated regarding the modified geometric surface area of the electrode ( $A = 0.2$  cm<sup>2</sup>). The experiments were conducted outside of the glovebox under anaerobic conditions by purging the buffer continuously with Ar while providing either Ar or CO<sub>2</sub> to the backside of the gas-diffusion electrode. To adjust to the different gas feeds to the backside of the electrode between measurements, the electrodes were exposed for 5 min to the corresponding gas feed before starting the measurement. As electrolyte, 0.1 M phosphate buffer with a pH value of 7.3 was used.

The modified gas diffusion electrodes were electrochemically characterized by means of cyclic voltammetry in a potential range between -0.29 and -0.79 V vs SHE at a scan rate of 5 mV s<sup>-1</sup>. The long-term stability of the fabricated bioelectrode was evaluated using amperometry at a constant applied potential of -0.79 V vs SHE. To determine the background current, the first 10 min of the chronoamperometric measurement was recorded under Ar flow before switching to CO<sub>2</sub>. For characterization of the redox polymer, the modified glassy carbon electrode was placed in a standard electrochemical cell. The measurement was conducted in Ar-purged 0.1 M phosphate buffer (pH 7.3) at a scan rate of 50 mV s<sup>-1</sup>.

**Gas Chromatography.** To analyze the products formed during conversion, a three-electrode setup was used, with a Ag/AgCl/3 M KCl reference electrode and a stainless-steel canula counter electrode. The gas diffusion cell was coupled to a multiple gas analyzer #1 gas chromatograph (GC, SRI Instruments). The GC has a 3 m HayeSep D column and two detectors. A flame ionization detector equipped with a methanizer for the detection of CO and a thermal conductivity detector for the H<sub>2</sub> analysis were used. As the carrier gas, N<sub>2</sub> was used. The electrode was exposed to a constant flow of CO<sub>2</sub>, controlled with a mass flow controller (AALBORG) to the backside of the electrode while applying a constant potential of -0.79 V vs SHE. A photograph of the cell can be seen in Figure S3. The gas phase at the backside of the electrode was directly injected into the GC. For control experiments, an electrode modified following the same fabrication procedure but in the absence of enzyme was used. A chronoamperometric measurement was performed to investigate the possible formation of side products by stepping the applied potential from -0.29 to -0.79 V vs SHE in steps of 100 mV. Each potential was applied for 15 min. An Autolab PGSTAT302N was used during electrochemical measurements coupled to GC detection. The faradaic

efficiency (FE, %) was calculated according to eq 2,<sup>3</sup> with  $F$  = Faraday constant (96,485 C mol<sup>-1</sup>),  $ne^-$  = number of electrons needed for the reduction reaction,  $nr(\text{CO})$  = concentration of CO detected by GC (vol%),  $f$  = gas flow rate (L s<sup>-1</sup>),  $V_m$  = molar volume of an ideal gas (22.4 L mol<sup>-1</sup>), and  $I$  = measured current at the time of sampling:

$$\text{FE} = \frac{F \times ne^- \frac{nr(\text{CO}) \times f}{V_m}}{I} \times 100 \quad (2)$$

## ■ ASSOCIATED CONTENT

### SI Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acsami.2c09547>.

Literature comparison; redox polymer characterization; gas chromatography setup and results; and voltammetric characterization before and after long-term measurement (PDF)

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## Notes

The authors declare no competing financial interest.

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## ■ REFERENCES

- (1) Solomon, S.; Plattner, G.-K.; Knutti, R.; Friedlingstein, P. Irreversible Climate Change due to Carbon Dioxide Emissions. *Proc. Natl. Acad. Sci. U. S. A.* **2009**, *106*, 1704–1709.
- (2) Finn, C.; Schnittger, S.; Yellowlees, L. J.; Love, J. B. Molecular Approaches to the Electrochemical Reduction of Carbon Dioxide. *Chem. Commun.* **2012**, *48*, 1392–1399.
- (3) Jin, S.; Hao, Z.; Zhang, K.; Yan, Z.; Chen, J. Advances and Challenges for the Electrochemical Reduction of CO<sub>2</sub> to CO: From Fundamentals to Industrialization. *Angew. Chem., Int. Ed.* **2021**, *60*, 20627–20648.
- (4) Ma, W.; He, X.; Wang, W.; Xie, S.; Zhang, Q.; Wang, Y. Electrocatalytic Reduction of CO<sub>2</sub> and CO to Multi-Carbon Compounds over Cu-Based Catalysts. *Chem. Soc. Rev.* **2021**, *50*, 12897–12914.
- (5) Suominen, M.; Kallio, T. What we currently know about Carbon-Supported Metal and Metal Oxide Nanomaterials in Electrochemical CO<sub>2</sub> Reduction. *ChemElectroChem* **2021**, *8*, 2397–2406.
- (6) Wang, G.; Chen, J.; Ding, Y.; Cai, P.; Yi, L.; Li, Y.; Tu, C.; Hou, Y.; Wen, Z.; Dai, L. Electrocatalysis for CO<sub>2</sub> Conversion: From Fundamentals to Value-Added Products. *Chem. Soc. Rev.* **2021**, *50*, 4993–5061.
- (7) Boutin, E.; Merakeb, L.; Ma, B.; Boudy, B.; Wang, M.; Bonin, J.; Anxolabéhère-Mallart, E.; Robert, M. Molecular Catalysis of CO<sub>2</sub> reduction: Recent Advances and Perspectives in Electrochemical and Light-Driven Processes with Selected Fe, Ni and Co Aza Macrocyclic and Polypyridine Complexes. *Chem. Soc. Rev.* **2020**, *49*, 5772–5809.
- (8) Azcarate, I.; Costentin, C.; Robert, M.; Savéant, J.-M. Through-Space Charge Interaction Substituent Effects in Molecular Catalysis Leading to the Design of the Most Efficient Catalyst of CO<sub>2</sub>-to-CO Electrochemical Conversion. *J. Am. Chem. Soc.* **2016**, *138*, 16639–16644.
- (9) Monti, D.; Ottolina, G.; Carrea, G.; Riva, S. Redox Reactions Catalyzed by Isolated Enzymes. *Chem. Rev.* **2011**, *111*, 4111–4140.
- (10) Meneghello, M.; Léger, C.; Fourmond, V. Electrochemical Studies of CO<sub>2</sub>-Reducing Metalloenzymes. *Chem. – Eur. J.* **2021**, *27*, 17542–17553.
- (11) Svetlitchnyi, V.; Peschel, C.; Acker, G.; Meyer, O. Two Membrane-Associated NiFeS-Carbon Monoxide Dehydrogenases from the Anaerobic Carbon-Monoxide-Utilizing Eubacterium Carboxydotherrus Hydrogeniformans. *J. Bacteriol.* **2001**, *183*, 5134–5144.
- (12) Hille, R.; Dingwall, S.; Wilcoxon, J. The Aerobic CO Dehydrogenase from *Oligotropha Carboxidovorans*. *J. Biol. Inorg. Chem.* **2015**, *20*, 243–251.
- (13) Can, M.; Armstrong, F. A.; Ragsdale, S. W. Structure, Function, and Mechanism of the Nickel Metalloenzymes, CO Dehydrogenase, and Acetyl-CoA Synthase. *Chem. Rev.* **2014**, *114*, 4149–4174.

- (14) Lindahl, P. A. The Ni-Containing Carbon Monoxide Dehydrogenase Family: Light at the End of the Tunnel? *Biochemistry* **2002**, *41*, 2097–2105.
- (15) Jeoung, J.-H.; Dobbek, H. Carbon Dioxide Activation at the Ni<sub>4</sub>Fe-Cluster of Anaerobic Carbon Monoxide Dehydrogenase. *Science* **2007**, *318*, 1461–1464.
- (16) Contaldo, U.; Guigliarelli, B.; Perard, J.; Rinaldi, C.; Le Goff, A.; Cavazza, C. Efficient Electrochemical CO<sub>2</sub>/CO Interconversion by an Engineered Carbon Monoxide Dehydrogenase on a Gas-Diffusion Carbon Nanotube-Based Bioelectrode. *ACS Catal.* **2021**, *11*, 5808–5817.
- (17) Dobbek, H.; Svetlitchnyi, V.; Gremer, L.; Huber, R.; Meyer, O. Crystal Structure of a Carbon Monoxide Dehydrogenase Reveals a Ni<sub>4</sub>Fe-SS Cluster. *Science* **2001**, *293*, 1281–1285.
- (18) Contaldo, U.; Curtil, M.; Pérard, J.; Cavazza, C.; Le Goff, A. A Pyrene-Triazacyclononane Anchor Affords High Operational Stability for CO<sub>2</sub> RR by a CNT-Supported Histidine-Tagged CODH. *Angew. Chem., Int. Ed.* **2022**, No. e202117212.
- (19) Habermüller, K.; Mosbach, M.; Schuhmann, W. Electron-Transfer Mechanisms in Amperometric Biosensors. *Fresenius' J. Anal. Chem.* **2000**, *366*, 560–568.
- (20) Shin, W.; Lee, S. H.; Shin, J. W.; Lee, S. P.; Kim, Y. Highly Selective Electrocatalytic Conversion of CO<sub>2</sub> to CO at –0.57 V (NHE) by Carbon Monoxide Dehydrogenase from Moorella Thermoacetica. *J. Am. Chem. Soc.* **2003**, *125*, 14688–14689.
- (21) Schuhmann, W.; Wohlschläger, H.; Lammert, R.; Schmidt, H.-L.; Löffler, U.; Wiemhöfer, H.-D.; Göpel, W. Leaching of Dimethylferrocene, a Redox Mediator in Amperometric Enzyme Electrodes. *Sens. Actuators, B* **1990**, *1*, 571–575.
- (22) Heller, A. Electron-Conducting Redox Hydrogels: Design, Characteristics and Synthesis. *Curr. Opin. Chem. Biol.* **2006**, *10*, 664–672.
- (23) Tapia, C.; Milton, R. D.; Pankratova, G.; Minteer, S. D.; Åkerlund, H.-E.; Leech, D.; De Lacey, A. L.; Pita, M.; Gorton, L. Wiring of Photosystem I and Hydrogenase on an Electrode for Photoelectrochemical H<sub>2</sub> Production by Using Redox Polymers for Relatively Positive Onset Potential. *ChemElectroChem* **2017**, *4*, 90–95.
- (24) Yuan, M.; Sahin, S.; Cai, R.; Abdellaoui, S.; Hickey, D. P.; Minteer, S. D.; Milton, R. D. Creating a Low-Potential Redox Polymer for Efficient Electroenzymatic CO<sub>2</sub> Reduction. *Angew. Chem., Int. Ed.* **2018**, *57*, 6582–6586.
- (25) So, K.; Sakai, K.; Kano, K. Gas Diffusion Bioelectrodes. *Curr. Opin. Electrochem.* **2017**, *5*, 173–182.
- (26) Szczesny, J.; Birrell, J. A.; Conzuelo, F.; Lubitz, W.; Ruff, A.; Schuhmann, W. Redox-Polymer-Based High-Current-Density Gas-Diffusion H<sub>2</sub>-Oxidation Bioanode Using FeFe Hydrogenase from *Desulfovibrio Desulfuricans* in a Membrane-Free Biofuel Cell. *Angew. Chem., Int. Ed.* **2020**, *59*, 16506–16510.
- (27) Lielpetere, A.; Becker, J. M.; Szczesny, J.; Conzuelo, F.; Ruff, A.; Birrell, J.; Lubitz, W.; Schuhmann, W. Enhancing the Catalytic Current Response of H<sub>2</sub> Oxidation Gas Diffusion Bioelectrodes Using an Optimized Viologen-Based Redox Polymer and [NiFe] Hydrogenase. *ELSA* **2022**, *2*, No. e2100100.
- (28) Ruff, A.; Szczesny, J.; Vega, M.; Zacarias, S.; Matias, P. M.; Gounel, S.; Mano, N.; Pereira, I. A. C.; Schuhmann, W. Redox-Polymer-Wired NiFeSe Hydrogenase Variants with Enhanced O<sub>2</sub> Stability for Triple-Protected High-Current-Density H<sub>2</sub>-Oxidation Bioanodes. *ChemSusChem* **2020**, *13*, 3627–3635.
- (29) Szczesny, J.; Ruff, A.; Oliveira, A. R.; Pita, M.; Pereira, I. A. C.; de Lacey, A. L.; Schuhmann, W. Electroenzymatic CO<sub>2</sub> Fixation Using Redox Polymer/Enzyme-Modified Gas Diffusion Electrodes. *ACS Energy Lett.* **2020**, *5*, 321–327.
- (30) Sehnal, D.; Bittrich, S.; Deshpande, M.; Svobodová, R.; Berka, K.; Bazgier, V.; Velankar, S.; Burley, S. K.; Koča, J.; Rose, A. S. Mol\* Viewer: Modern Web App for 3D Visualization and Analysis of Large Biomolecular Structures. *Nucleic Acids Res.* **2021**, *49*, W431–W437.
- (31) Dobbek, H.; Svetlitchnyi, V.; Liss, J.; Meyer, O. Carbon Monoxide Induced Decomposition of the Active Site Ni<sub>4</sub>Fe-SS