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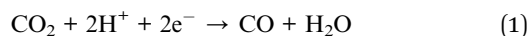
# Electrocatalytic CO<sub>2</sub> reduction by a cobalt porphyrin mini-enzyme†

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Cobalt-mimochrome VI\*a (CoMC6\*a), a synthetic mini-enzyme with a cobalt porphyrin active site, is developed as a biomolecular catalyst for electrocatalytic CO<sub>2</sub> reduction in water. The catalytic turnover number reaches ~14 000 for CO production with a selectivity of 86 : 5 over H<sub>2</sub> production under the same conditions. Varying the applied potential and the p*K*<sub>a</sub> of the proton donor was used to gain insight into the basis for selectivity. The protected active site of CoMC6\*a is proposed to enhance selectivity for CO<sub>2</sub> reduction under conditions that typically favor H<sub>2</sub> production by related catalysts. CoMC6\*a activity and selectivity change only marginally under air, indicating excellent oxygen tolerance.

## Introduction

Electrochemical carbon dioxide (CO<sub>2</sub>) reduction is an appealing route to renewable fuel production.<sup>1,2</sup> Achieving selectivity for CO<sub>2</sub> reduction over proton reduction is an omnipresent challenge, since the reduction of CO<sub>2</sub> to CO, or any stable product, requires protons (eqn (1) and (2)).<sup>3,4</sup> Achieving selectivity in a protic solvent such as water is particularly challenging. However, there is significant interest in developing catalysis in water as an abundant source of protons and a desirable environmentally-friendly solvent.<sup>5–7</sup> An additional challenge raised by use of water as a solvent is the poor solubility of CO<sub>2</sub>.<sup>8,9</sup> Developing catalysts with microenvironments that sequester and activate CO<sub>2</sub> in the presence of protons thus is of high interest.<sup>10–15</sup>



Nature's enzymes achieve high selectivity and activity for reactions such as CO<sub>2</sub> reduction by providing an active-site microenvironment to promote substrate binding and transformation and by controlling electron and proton delivery.<sup>16–19</sup> Inspired by Nature's catalysts, artificial enzymes for CO<sub>2</sub> reduction (see examples in Table S1†) have been prepared by incorporation of synthetic CO<sub>2</sub> reduction catalysts, such as [Ni(cyclam)]<sup>2+</sup>,<sup>20</sup> Ni(terpyridine),<sup>21</sup> or cobalt porphyrins,<sup>22–24</sup> into

proteins including azurin,<sup>20</sup> cytochrome *b*<sub>562</sub>,<sup>23</sup> myoglobin,<sup>24</sup> an artificial protein αRep,<sup>25</sup> or an engineered photosensitizer protein.<sup>21</sup> Some of these systems have been reported to achieve enhanced activity<sup>23</sup> and selectivity<sup>20</sup> relative to the synthetic catalyst outside of the protein environment. For example, improved selectivity for CO<sub>2</sub> over proton reduction by [Ni(cyclam)]<sup>2+</sup> bound to the protein azurin was attributed to the protein scaffold providing restricting conformational flexibility of the catalyst and an active site buried within a solvent-excluded hydrophobic patch.<sup>20</sup>

Inspired by the importance of proton transfer steps in enzymatic catalysis,<sup>17,26–29</sup> roles for endogenous<sup>4,17,30–32</sup> and exogenous<sup>7,22,30,33</sup> proton donors in determining CO<sub>2</sub> reduction selectivity and activity have been proposed. The use of relatively weak Brønsted acids as proton donors is proposed to slow metal-hydride formation and thus disfavor the competing H<sub>2</sub> evolution pathway.<sup>1,34,35</sup> Electrochemical studies on an iron-porphyrin electrocatalyst<sup>7</sup> and a cobalt macrocyclic catalyst<sup>36</sup> showed that using a higher-p*K*<sub>a</sub> buffer increases selectivity for CO over H<sub>2</sub> production. Furthermore, in photochemical studies employing cobalt porphyrin catalysts, presence of a higher-p*K*<sub>a</sub> buffer (bicarbonate, as opposed to phosphate) was shown to increase selectivity for CO over H<sub>2</sub> production.<sup>37,38</sup> Other properties of buffers have also been implicated in determining selectivity. For [Ni(cyclam)]<sup>2+</sup>, buffer steric properties and charges were found to impact selectivity for CO over H<sub>2</sub> production; cationic buffers were proposed to stabilize an activated Ni–CO<sub>2</sub> species in a second-sphere interaction, favoring CO production.<sup>33</sup>

In a previous study, we reported CO<sub>2</sub> reduction catalysis by a semisynthetic cobalt-porphyrin-containing mini-enzyme, CoMP11-Ac, consisting of a cobalt porphyrin with a covalently attached peptide donating an axial histidine ligand on the proximal side of the porphyrin (Fig. 1a). For CoMP11-Ac,

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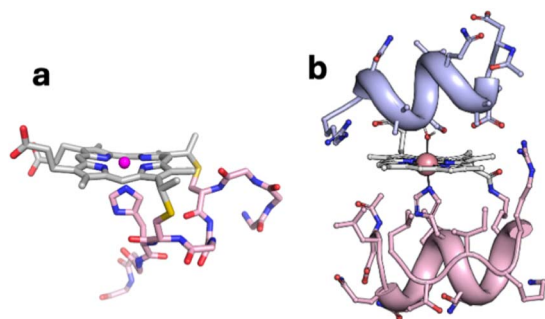


Fig. 1 Models of (a) CoMP11-Ac; (b) CoMC6\*a.

selectivity for CO over H<sub>2</sub> production in water is increased by using a higher- $pK_a$  buffer as an exogenous proton donor, which is proposed to disfavor the formation of a metal-hydride species that yields H<sub>2</sub>. Furthermore, catalysis at a more negative potential ( $-1.4$  V vs. Ag/AgCl/KCl<sub>(1M)</sub>) lowers selectivity for CO over H<sub>2</sub> production, while applying a less negative potential ( $-1.2$  V) increases selectivity.<sup>22</sup>

We now investigate effects of biocatalyst structure on selectivity for CO<sub>2</sub> vs. proton reduction. We have chosen a catalyst that, like CoMP11-Ac, has a cobalt porphyrin active site and axial His ligand, but that also has a peptide covering the distal side of the heme. This catalyst is a synthetic mini-enzyme, cobalt-mimochrome VI\*a (CoMC6\*a, Fig. 1b). Mimochromes are miniaturized porphyrin-based metalloproteins consisting of a deuteroporphyrin sandwiched between two peptide chains covalently bound to the porphyrin.<sup>39,40</sup> MC6\*a is a proven framework for catalysis, displaying peroxidase,<sup>41–43</sup> peroxygenase<sup>42–44</sup> or hydrogenase<sup>45,46</sup> activities depending on conditions and the metal ion. Its scaffold consists of a distal decapeptide and a proximal tetradecapeptide that provides the axial His ligand to the metal ion. Helical secondary structure is favored by the inclusion of two 2-aminoisobutyric acid residues in the distal peptide.<sup>47</sup>

Previously, CoMC6\*a was shown to act as an electrocatalyst for H<sub>2</sub> evolution from water with a turnover number (TON) exceeding 230 000 (ref. 45) as well as a catalyst in a system for photochemical H<sub>2</sub> evolution.<sup>46</sup> Subsequent studies of CoMC6\*a catalysis of H<sub>2</sub> evolution from water revealed that buffer acid species play a critical role in proton delivery to CoMC6\*a during catalysis, with their structures and  $pK_a$  values impacting catalytic rate, potential, and mechanism.<sup>48</sup> In particular, proton-coupled electron transfer (PCET) was shown to be required for H<sub>2</sub> production by CoMC6\*a, with the catalytic potential shifting with the  $pK_a$  of the buffer acid in a Nernstian fashion. Furthermore, catalytic rate was shown to depend on buffer sterics, an observation attributed to the impact of the distal peptide in hindering proton delivery by protonated buffer.<sup>48</sup> Interestingly, the specific effects of buffer acid on H<sub>2</sub> production catalysis differ from those observed for CoMP11-Ac, for which buffer  $pK_a$ , but not buffer structure, plays a role in determining catalytic rate, likely as a result of the solvent-exposed active site of CoMP11-Ac.<sup>49</sup>

Having observed these impacts of catalyst structure on H<sub>2</sub> evolution catalysis by CoMP11-Ac vs. CoMC6\*a, we now turn to investigating the impact of structure on CO<sub>2</sub> reduction by CoMC6\*a. We hypothesized that the more hydrophobic and enclosed active site of CoMC6\*a would favor CO<sub>2</sub> reduction. Using conditions applied to CoMP11-Ac to facilitate comparison, the roles of both applied potential and exogenous proton donor  $pK_a$  in determining CO<sub>2</sub> vs. proton reduction selectivity and activity by CoMC6\*a are investigated. Comparison to previous results on CoMP11-Ac indicates that the distal peptide plays a role in enhancing selectivity for CO<sub>2</sub> reduction. Finally, we demonstrate that this catalyst exhibits excellent tolerance for oxygen, with minimal impact on CO<sub>2</sub> reduction activity or selectivity.

## Results and discussion

CoMC6\*a was prepared and characterized as described in the ESI (Fig. S1 and S2)<sup>†</sup> as well as previous publications.<sup>45,47</sup> Cyclic voltammetry (CV) of 1  $\mu$ M CoMC6\*a was carried out using a hanging mercury drop electrode, used in previous related work.<sup>22,45,48</sup> As was observed for CoMP11-Ac,<sup>22</sup> dip-and-stir experiments<sup>50</sup> indicate that CoMC6\*a adsorbs to the electrode, acting as an immobilized catalyst (Fig. S3 and S4<sup>†</sup>).

### Effects of applied potential

CV of 1  $\mu$ M CoMC6\*a at pH 6 in 50 mM 3-morpholinopropane-1-sulfonic acid (MOPS,  $pK_a$  7.2) under N<sub>2</sub> (Fig. 2) shows faradaic current beginning at an onset potential of  $\sim -1.2$  V vs. Ag/AgCl/KCl<sub>(1M)</sub> (all potentials reported herein are reported against this reference). The rise in current forming a single peak is attributed to CoMC6\*a electrocatalytic H<sub>2</sub> evolution activity *via* protonated buffer consumption, which was previously reported under similar conditions.<sup>45,48</sup> When the solution is saturated with CO<sub>2</sub> and placed under 1 atm CO<sub>2</sub>, two peaks are observed at  $\sim -1.2$  V and  $\sim -1.5$  V (Fig. 2). The resulting increase in current at  $\sim -1.2$  V may indicate selective CO<sub>2</sub> reduction over proton reduction at this potential. Furthermore, the anodic shift of the catalytic onset potential may be due to CO<sub>2</sub> coordination and reduction or a coupled EC/CE reaction.

To characterize product formation, controlled potential electrolysis (CPE) experiments were run on 1  $\mu$ M CoMC6\*a in

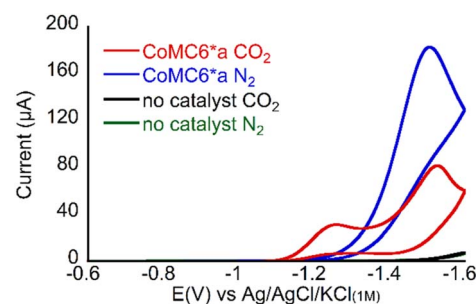


Fig. 2 Cyclic voltammograms of 1  $\mu$ M CoMC6\*a pH 5.9 in 50 mM MOPS, 0.1 M KCl, at 100 mV s<sup>-1</sup>, scan 2, under 1 atm of the indicated gas.



the presence of MOPS for two hours, after which the headspace gas was sampled and analyzed by gas chromatography (GC). Experiments were run at  $-1.2$  and  $-1.4$  V to aid comparison to published results on CoMP11-Ac at these conditions (Table S2†).<sup>22</sup> At  $-1.4$  V under  $N_2$  with no  $CO_2$  present,  $H_2$  is produced with nearly quantitative faradaic efficiency ( $FE_{H_2}$   $96 \pm 4\%$ ), consistent with previous results.<sup>45,48</sup> When a  $CO_2$ -saturated solution of CoMC6\*a under one atmosphere of  $CO_2$  is subjected to CPE, the major product is CO (Tables 1, S3 and Fig. S5†). However, selectivity for CO formation over  $H_2$  under these conditions changes with applied potential, with higher selectivity ( $85 : 6$   $FE_{CO} : FE_{H_2}$ ) at  $-1.2$  V compared to  $68 : 24$  at  $-1.4$  V (Tables 1, S3 and Fig. S5†). The turnover number (TON) for CO production also is dependent on potential, with double the value ( $2200 \pm 300$ ) at the less cathodic potential of  $-1.2$  V. In comparison with results on CoMP11-Ac under the same conditions (Table S2†),  $FE_{CO}$  ( $85 \pm 2\%$ ) and  $FE_{H_2}$  ( $8 \pm 2\%$ ) are nearly the same as the values for CoMC6\*a at  $-1.2$  V. However, at  $-1.4$  V (Table 2), CoMP11-Ac favors  $H_2$  production, with  $FE_{CO}$  of  $21 \pm 5\%$  and  $FE_{H_2}$  of  $63 \pm 13\%$ . Thus, under these conditions at  $-1.4$  V, CoMC6\*a shows significantly greater selectivity for  $CO_2$  over proton reduction compared to CoMP11-Ac, supporting the hypothesis that protection of the CoMC6\*a active site by the distal peptide enhances selectivity.

### Effects of proton donor $pK_a$

An important tool for addressing product selectivity and gaining mechanistic insights in  $CO_2$  reduction electrocatalysis is tuning proton donor properties.<sup>31,51</sup> For a number of catalysts in water, protonated buffers have been shown to be the primary proton donors in proton-requiring catalysis (except at low pH values)<sup>52</sup> for  $H_2$  production<sup>48–50</sup> and  $CO_2$  reduction,<sup>7,33,49</sup> with buffer properties impacting catalytic rate, mechanism, and selectivity.<sup>36–38</sup> For CoMC6\*a, properties of buffer acids have been shown to impact electrocatalytic  $H_2$  evolution efficiency, activity, and mechanism: lower- $pK_a$  buffers result in an anodic shift in the catalytic wave, which has been attributed to their

Table 2 FE values for CoMP11-Ac and CoMC6\*a at  $-1.4$  V<sup>a</sup>

Buffer	Catalyst	$FE_{(H_2)}$ %	$FE_{(CO)}$ %
CAPS ( $pK_a$ 10.4)	CoMP11-Ac	$29 \pm 6$	$48 \pm 10$
	CoMC6*a	$4 \pm 1$	$76 \pm 10$
CHES ( $pK_a$ 9.3)	CoMP11-Ac	$43 \pm 9$	$57 \pm 4$
	CoMC6*a	$14 \pm 1$	$67 \pm 12$
MOPS ( $pK_a$ 7.2)	CoMP11-Ac	$63 \pm 13$	$21 \pm 5$
	CoMC6*a	$24 \pm 4$	$68 \pm 8$

<sup>a</sup> Data on CoMP-11 from ref. 22. Data collected under 1 atm  $CO_2$ , 0.5 M buffer, pH 6.5. Full table of comparative results in ESI.

role in PCET,<sup>48</sup> and less bulky buffers increase catalytic current, a phenomenon attributed to distal CoMC6\*a peptide hindering proton donor access to the active site.<sup>48</sup> To determine the effect of proton donor on  $CO_2$  reduction selectivity by CoMC6\*a, we chose three structurally related buffers: MOPS, used above ( $pK_a = 7.2$ ), *N*-cyclohexyl-2-aminoethanesulfonic acid (CHES,  $pK_a = 9.3$ ) and 3-(cyclohexylamino)-1-ethanesulfonic acid (CAPS  $pK_a = 10.4$ ; structures are shown in Fig. 3).

First, we collected CVs of CoMC6\*a under  $N_2$  or  $CO_2$ , with the solution saturated with the respective gas. Under  $N_2$ , there is only one feature, which is between  $-1.4$  and  $-1.6$  V, and was previously shown to be associated with catalytic  $H_2$  evolution.<sup>45,48</sup> The peak current of this low-potential feature decreases with increasing buffer  $pK_a$ , consistent with lower  $H_2$  production activity with less acidic proton donors (Fig. 3).

Under  $CO_2$ , the CV changes dramatically but in a manner dependent on the buffer present. With all three buffers, an increase in current under  $CO_2$  relative to that under  $N_2$  is seen at  $\sim -1.2$  V, a potential at which CPE experiments show (*vide infra*) there is minimal  $H_2$  production (Fig. 3 and Table 1). This result suggests that there may be enhanced  $CO_2$  reduction  $\sim -1.2$  V.

To determine products formed, two-hour CPE experiments on CoMC6\*a in MOPS, CHES and CAPS buffers at pH 6 were performed at  $-1.2$  and  $-1.4$  V, with results in Tables 1, S3–S5 and Fig. S5–S7.† The UV-vis spectrum of the catalyst in bulk

Table 1 Results of CPE experiments on CoMC6\*a<sup>a</sup>

Gas	Buffer	$E^b$ (V)	$FE_{(H_2)}$ %	$FE_{(CO)}$ %	$TON_{(H_2)}$	$TON_{(CO)}$	$Q_T$ (C)
$CO_2$	CAPS ( $pK_a$ 10.4)	$-1.4$	$4 \pm 1$	$76 \pm 10$	$110 \pm 20$	$2100 \pm 600$	$2.6 \pm 0.4$
		$-1.2$	$4 \pm 4$	$73 \pm 5$	$11 \pm 10$	$230 \pm 10$	$0.3 \pm 0.1$
$CO_2$	CHES ( $pK_a$ 9.3)	$-1.4$	$14 \pm 1$	$67 \pm 12$	$280 \pm 10$	$1300 \pm 400$	$1.9 \pm 0.1$
		$-1.2$	$11 \pm 1$	$86 \pm 11$	$100 \pm 20$	$800 \pm 200$	$0.9 \pm 0.1$
$CO_2$	MOPS ( $pK_a$ 7.2)	$-1.4$	$24 \pm 4$	$68 \pm 8$	$390 \pm 120$	$1100 \pm 200$	$1.6 \pm 0.5$
		$-1.2$	$6 \pm 1$	$85 \pm 11$	$160 \pm 40$	$2200 \pm 300$	$2.5 \pm 0.2$
$N_2$	CAPS ( $pK_a$ 10.4)	$-1.4$	$88 \pm 10$	$\sim 0$	$1100 \pm 400$	$\sim 0$	$1.2 \pm 0.3$
		$-1.2$	No above-background activity <sup>c</sup>				
$N_2$	CHES ( $pK_a$ 9.3)	$-1.4$	$97 \pm 14$	$\sim 0$	$1800 \pm 200$	$\sim 0$	$1.8 \pm 0.1$
		$-1.2$	$78 \pm 14$	$\sim 0$	$130 \pm 30$	$\sim 0$	$0.2 \pm 0.1$
$N_2$	MOPS ( $pK_a$ 7.2)	$-1.4$	$96 \pm 4$	$1.0 \pm 0.3$	$3900 \pm 1500$	$45 \pm 12$	$3.9 \pm 1.4$
		$-1.2$	No above-background activity				

<sup>a</sup> Two-hour CPE experiments conducted on 1  $\mu$ M catalyst in 0.5 M buffer with 1 M KCl. Data shown corresponds to the average of at least three individual runs, the error corresponds to the difference between the average and the replicate with the greatest difference from the average; ESI shows detailed results. The pH of all MOPS, CHES, and CAPS solutions after purging with  $CO_2$  was  $6.5 \pm 0.2$ ; and  $7.2 \pm 0.2$  when purged with  $N_2$ . <sup>b</sup> Potentials reported vs. Ag/AgCl/KCl(1M). <sup>c</sup> Activity is not reported if it did not exceed three times background in more than one replicate.





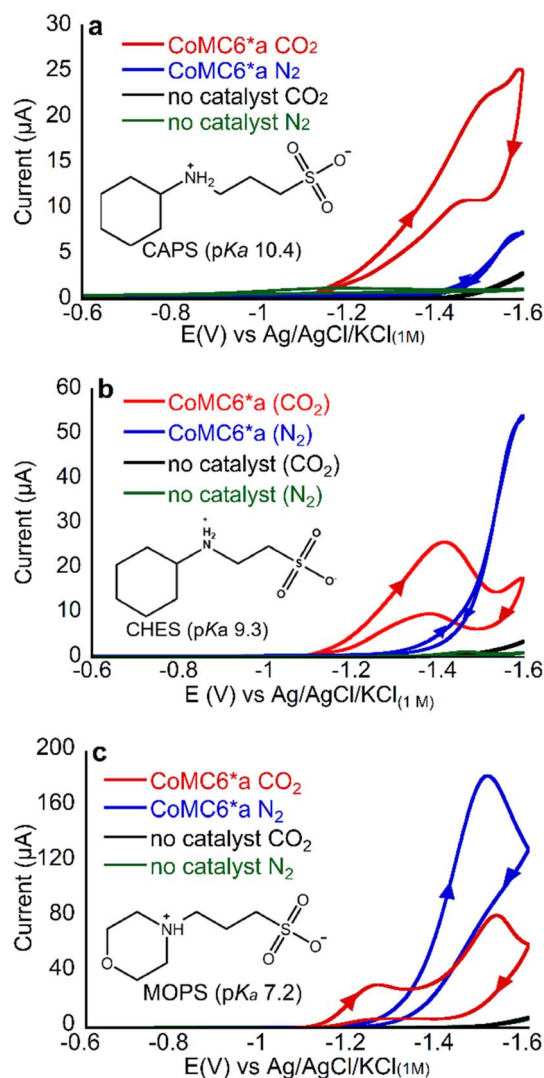


Fig. 3 CVs of 1  $\mu\text{M}$  CoMC6\*a in 50 mM (a) CAPS, (b) CHES, (c) MOPS. For all CVs, pH = 5.9,  $[\text{KCl}] = 0.1 \text{ M}$  and scan rate =  $100 \text{ mV s}^{-1}$ . Arrows in the CV traces indicate the scanning direction.

solution shows minimal change before and after CPE, indicating catalyst robustness (Fig. S8†). Under  $\text{N}_2$  at  $-1.2 \text{ V}$ , no activity above background was observed in the presence of CAPS or MOPS, and very low activity was observed in CHES, indicating that minimal  $\text{H}_2$  production occurs at  $-1.2 \text{ V}$  in the presence of all three buffer acids under these conditions, consistent with prior results on CoMC6\*a.<sup>48</sup> At  $-1.4 \text{ V}$  under  $\text{N}_2$ , the charge passed exceeds background for all three buffers, with  $\text{H}_2$  formation with  $\text{FE}_{\text{H}_2}$  values from 88 to 97%. As we lower buffer  $\text{pK}_a$ , we see an increase in  $\text{TON}_{\text{H}_2}$ , supporting the hypothesis that more acidic proton donors enhance  $\text{H}_2$  production activity, in line with prior results.<sup>48</sup>

When CPE of CoMC6\*a is performed under  $\text{CO}_2$ , CO becomes the major product under all conditions used here. At  $-1.2 \text{ V}$  under  $\text{CO}_2$ ,  $\text{FE}_{\text{CO}}$  is approximately the same for experiments run with the three different buffer acids (ranging from 73 to 85%) and the  $\text{FE}_{\text{H}_2}$  values are also similar (4–11%), indicating

that the  $\text{pK}_a$  of the buffer does not have a significant impact on selectivity at  $-1.2 \text{ V}$ . In contrast, at  $-1.4 \text{ V}$  under  $\text{CO}_2$ ,  $\text{FE}_{\text{H}_2}$  increases from  $4 \pm 1\%$  to  $14 \pm 1\%$  to  $24 \pm 4\%$  as buffer  $\text{pK}_a$  decreases, showing that increased buffer acidity enhances  $\text{H}_2$  evolution under a  $\text{CO}_2$  atmosphere, possibly by promoting formation of a metal hydride or its protonation.  $\text{FE}_{\text{CO}}$  shows minimal change with buffer  $\text{pK}_a$  at  $-1.4 \text{ V}$ , (67–76%), indicating that the effect of increased buffer  $\text{pK}_a$  on enhancing selectivity for CO production at  $-1.4 \text{ V}$  results primarily from decreasing  $\text{H}_2$  production.

Comparison to results on CoMP11-Ac (Fig. 1) provides insight into how catalyst structure impacts selectivity. Similar to CoMC6\*a, at  $-1.2 \text{ V}$ , CO :  $\text{H}_2$  selectivity of CoMP11-Ac shows no dependence on buffer acid  $\text{pK}_a$  (Table S2†). At  $-1.4 \text{ V}$ , also like CoMC6\*a, CoMP11-Ac shows an increase in selectivity for  $\text{CO}_2$  reduction over proton reduction as the  $\text{pK}_a$  of the buffer acid is increased (Table 2 and S2†).<sup>22</sup> CoMP11-Ac and CoMC6\*a thus show similar trends in CO :  $\text{H}_2$  selectivity with buffer acid  $\text{pK}_a$ , with no dependence at  $-1.2 \text{ V}$  and an increased  $\text{FE}_{\text{CO}} : \text{FE}_{\text{H}_2}$  with decreased buffer acidity at  $-1.4 \text{ V}$ , dominated by an impact on  $\text{FE}_{\text{H}_2}$ . However, CoMC6\*a has a higher CO :  $\text{H}_2$  selectivity under all conditions, always in favor of  $\text{CO}_2$  reduction. These results indicate that the CoMC6\*a structure enhances  $\text{CO}_2$  reduction selectivity over proton reduction, an effect primarily seen at the more negative potential used herein.

For CoMP11-Ac, two mechanisms were proposed at the two different potentials.<sup>22</sup> At  $-1.4 \text{ V}$ , a mechanism invoking formal Co(I) formation was proposed, consistent with an estimated Co(II/I) reduction potential of  $-1.42 \text{ V}$ .<sup>52</sup> Cobalt hydride is proposed to yield  $\text{H}_2$  upon protonation, and this process accounts for the greater  $\text{FE}_{\text{H}_2}$  at a more negative potential. This mechanism is in line with the observed selectivity dependency on the buffer acid  $\text{pK}_a$  at  $-1.4 \text{ V}$ , as a more acidic proton donor will favor Co(I) protonation,<sup>48</sup> thus biasing the system toward  $\text{H}_2$  formation. At  $-1.2 \text{ V}$ , a mechanism in which  $\text{CO}_2$  binding couples to electron transfer to form a formal Co(I)– $\text{CO}_2$  adduct was invoked, which avoids directly forming a Co(I) species and accounts for the lack of dependence of selectivity on buffer  $\text{pK}_a$  at this potential. This mechanism has a selectivity-determining step prior to any protonation step, which suggests that selectivity will not depend on proton donor  $\text{pK}_a$ , in line with the experimental results at  $-1.2 \text{ V}$ .

To consider this model for CoMC6\*a, we measured the formal Co(II/I) reduction potential. This was accomplished under  $\text{N}_2$  at high pH and with a rapid scan rate, conditions at which  $\text{H}_2$  evolution is suppressed. From quasi-reversible CVs at pH 10–12, a midpoint potential of  $\sim -1.58 \text{ V}$  was measured (Fig. S9†). Thus, under the conditions used here for catalysis, direct formation of Co(I) is not possible. For  $\text{CO}_2$  reduction, reaching this formal oxidation state will require  $\text{CO}_2$  binding before or coupled with reduction. For proton reduction, PCET is required, as was previously demonstrated.<sup>48</sup> These observations lead to the proposed mechanism in Fig. 4, which has its basis in published mechanisms for  $\text{CO}_2$  reduction and proton reduction by cobalt porphyrins.<sup>53</sup> However, the low potential of Co(II/I) MC6\*a precludes direct formation of a Co(I) species under these conditions, a process typically invoked in related systems.<sup>22,37,53</sup>



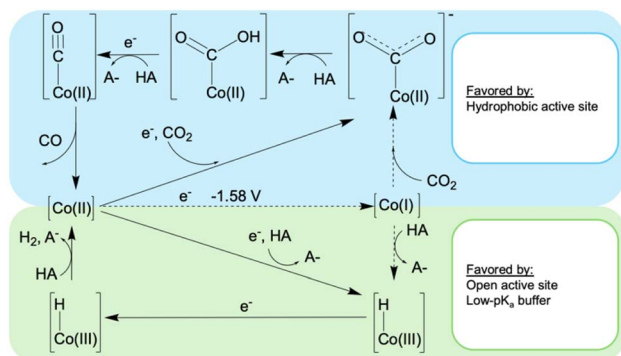


Fig. 4 Proposed mechanisms for  $\text{H}_2$  and  $\text{CO}$  formation catalyzed by CoMC6\*a. The dotted lines indicate processes not observed or expected under the conditions used herein.

To provide additional data to test this model, effects of  $\text{CO}_2$  concentration on catalysis were measured.

### Effects of $\text{CO}_2$ partial pressure

Prior experiments examined the effect of proton donor (buffer) concentration on catalysis. Next, we examined effects of  $\text{CO}_2$  by collecting voltammograms as a function of  $\text{CO}_2$  partial pressure ( $P_{\text{CO}_2}$ ).<sup>22</sup> In the presence of increasing partial pressures of  $\text{CO}_2$  (Fig. 5), a CV wave develops on the anodic side of the voltammogram, consistent with a process that is dependent on the concentration of  $\text{CO}_2$ . The proposed mechanism, invoking coupled  $\text{CO}_2$  binding and reduction, should be dependent on

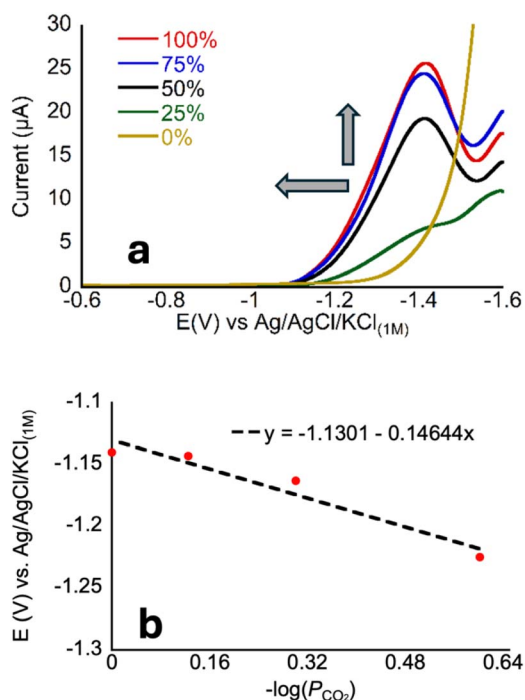


Fig. 5 (a) Linear sweep voltammograms of 1  $\mu\text{M}$  CoMC6\*a in 50 mM CHES, 0.1 M KCl, pH  $5.9 \pm 0.1$  at  $100 \text{ mV s}^{-1}$  under different  $P_{\text{CO}_2}$ , the arrows indicate the direction of increasing  $P_{\text{CO}_2}$ . (b) Plot of  $E_i$  vs.  $-\log(P_{\text{CO}_2})$  showing a slope of  $\sim 150 \text{ mV}$  per decade.  $R^2 = 0.94$ .

the following equation under equilibrium conditions. Note that  $E_h$  refers to the half-wave potential:

$$\text{M} + \text{e}^- + \text{CO}_2 \rightleftharpoons [\text{M}-\text{CO}_2]^- \quad (3)$$

$$E = E^{\circ'} + \frac{0.0592}{n} \log \left( \frac{[\text{M}-\text{CO}_2]^-}{[\text{M}]P_{\text{CO}_2}} \right) \quad (4)$$

$$E_h = E^{\circ'} - 0.0592 \log(P_{\text{CO}_2}) \quad (5)$$

To analyze these data, we chose a current near the foot of the wave ( $1.5 \mu\text{A}$ ) to reflect the  $\text{CO}_2$ -dependent process that occurs at less cathodic potentials than  $\text{H}_2$  production because a distinct peak is not always present in the voltammograms of CoMC6\*a. We then define  $E_i$  as the potential at which this current is reached; we have used this approach when  $E_h$  (eqn (5)) cannot be readily defined (Fig. 5).<sup>22</sup>

$$E_i = -0.0592 \log(P_{\text{CO}_2}) + E^{\circ'} \quad (6)$$

The negative non-zero slope seen in Fig. 5 reflects the increasing current with increasing  $P_{\text{CO}_2}$ , consistent with a relationship between  $\text{CO}_2$  concentration and electron transfer, which supports our proposed mechanism. However, because a clear peak is not present reflecting primarily  $\text{CO}_2$  reduction, defining a quantitative relationship is not possible from these data.

Examination of Fig. 5a reveals that the voltammogram is nearly the same under 75% and 100%  $\text{CO}_2$ , which contrasts with the clear changes from 0 to 75%. This change in dependence suggests that, above 75%, substrate ( $\text{CO}_2$ ) availability is no longer a limiting factor in catalysis. Notably, this observation differs from what is seen for CoMP11-Ac, for which the anodic shift continues for all  $P_{\text{CO}_2}$  values in the same range. To determine whether the proton donor becomes limiting under these conditions, we measured CVs for CoMC6\*a under a  $\text{CO}_2$  atmosphere under varied concentrations of CHES buffer (the buffer used in Fig. 5). In contrast with the increase in catalytic current seen as a function of [CHES] (and all buffers)<sup>48</sup> under  $\text{N}_2$ , the CVs under  $\text{CO}_2$  are nearly invariant as a function of [CHES] (Fig. S10 and S11†). These observations for CoMC6\*a indicate that, in the presence of  $\text{CO}_2$ , a process other than  $\text{CO}_2$  or proton delivery limits catalysis. This may be a conformational rearrangement of the catalyst, *i.e.*, of the distal peptide to facilitate substrate access, or a later step in catalysis such as C-O bond breakage.

### Effect of air on catalysis

Since practical sources of  $\text{CO}_2$  such as flue gas tend to have impurities such as oxygen ( $\text{O}_2$ ), which has been shown to negatively affect many  $\text{CO}_2$  reduction catalysts, developing catalysts that can facilitate  $\text{CO}_2$  reduction in the presence of oxygen is a priority.<sup>54</sup> To test whether  $\text{O}_2$  impacts  $\text{CO}_2$  reduction catalysis by CoMC6\*a, a CV of a CoMC6\*a solution saturated with  $\text{CO}_2$  was collected under room air (Fig. 6). The CV of CoMC6\*a was not significantly impacted by the presence of air,

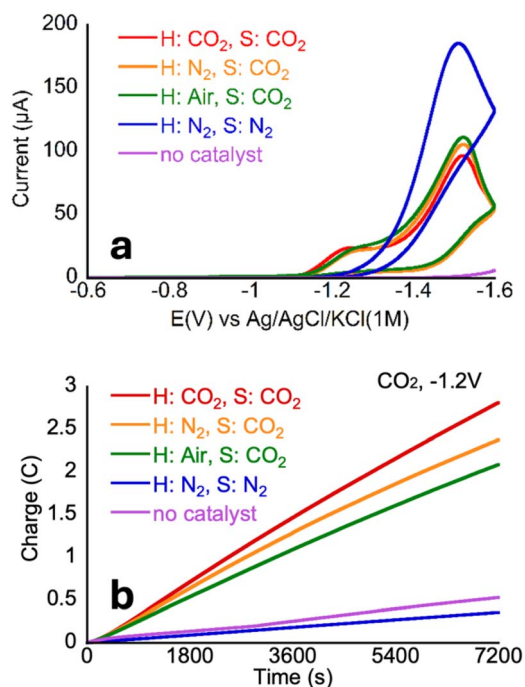


Fig. 6 (a) CVs of 1  $\mu\text{M}$  CoMC6\*a in 50 mM MOPS, pH  $5.9 \pm 0.1$ . For all CVs,  $[\text{KCl}] = 0.1 \text{ M}$  and scan rate =  $100 \text{ mV s}^{-1}$ . Arrows in the CV traces indicate the scanning direction. (b) CPE experiments run in 0.5 M MOPS, 1 M KCl, the concentration of catalyst was 1  $\mu\text{M}$  when present. The pH of all MOPS after purging with  $\text{CO}_2$  was  $6.5 \pm 0.1$ ; and  $7.2 \pm 0.2$  when purged with  $\text{N}_2$ . Potentials reported vs.  $\text{Ag}/\text{AgCl}/\text{KCl}_{(1\text{M})}$ . H: headspace S: solution.

overlying closely with CVs under  $\text{CO}_2$  and nitrogen, suggesting the possibility of air-tolerant  $\text{CO}_2$  reduction. Results were similar for CVs of CoMC6\*a solutions saturated with  $\text{CO}_2$  whether under 1 atmosphere of  $\text{CO}_2$ ,  $\text{N}_2$ , or air. Next, two-hour CPEs were run to determine the impact of air on product formation. The resulting CPEs (Fig. 6 and Table 3) showed no significant difference in selectivity. The overall charge passed and TON values decreased when  $\text{CO}_2$  was removed from the headspace. This observation is consistent with lower activity with a decrease in available substrate and demonstrates an effect of changing the headspace on the two-hour CPE experiment. These results indicate that CoMC6\*a maintains  $\text{CO}_2$  reduction activity and selectivity in the presence of  $\text{O}_2$ . Note that

air tolerance for  $\text{H}_2$  evolution by CoMC6\*a was previously demonstrated.<sup>45</sup>

While more investigations are needed to understand the basis for this air tolerance, there are a few reported examples that provide context. One is a cobalt phthalocyanine catalyst anchored to carbon nanotubes for  $\text{CO}_2$  reduction. In this system,  $\text{FE}_{\text{CO}}$  drops from 93% to 0% in the presence of 5%  $\text{O}_2$ . However, protecting the cobalt phthalocyanine with a bio-inspired polymer of intrinsic microporosity increased  $\text{FE}_{\text{CO}}$  in the presence of 5%  $\text{O}_2$  to 75.9%. At levels of  $\text{O}_2$  in air of 22%, however,  $\text{FE}_{\text{CO}}$  decreased to 49.7%.<sup>55</sup> Another oxygen-tolerant transition-metal catalyst for  $\text{CO}_2$  reduction is an iron-porphyrin catalyst with four ferrocenes in its distal site that displays a 500-fold faster rate of  $\text{CO}_2$  binding compared to  $\text{O}_2$  binding, giving the catalyst high  $\text{FE}_{\text{CO}}$  of 84% in the presence of 25%  $\text{O}_2$ .<sup>56</sup> Its  $\text{O}_2$  tolerance is also attributed to its favorable 4-electron reduction of  $\text{O}_2$  to  $\text{H}_2\text{O}$  that avoids the formation of destructive reactive oxygen species, as well as rapid  $\text{CO}_2$  binding.<sup>56</sup>

### Insights into effects of catalyst structure on activity

Nature's enzymes have enviable properties, typically rapid catalysis, high substrate and product specificity, and great efficiency (*i.e.* low overpotential). These properties are attributed to the active-site microenvironment provided by the polypeptide matrix.<sup>16,57</sup> However, Nature's metalloenzymes can be challenging to isolate in significant quantities and often are large structures with a low density of active sites. Furthermore, many enzymes that make  $\text{H}_2$  and that reduce  $\text{CO}_2$  are sensitive to oxygen. Thus, there has been interest in developing biomolecular catalysts that are relatively easy to prepare and work with, but retain the advantage of having polypeptide matrix that can be tuned to engineer the active site environment.<sup>6,58</sup> However, despite the progress made to date, there are few examples in which structure–function relationships have been demonstrated in engineered biomolecular catalysts,<sup>20,23,45,59,60</sup> especially for systems that exhibit high activity and robustness (*i.e.*, high TON values).

Prior investigation of the mechanism of electrochemical proton reduction by CoMC6\*a revealed that proton delivery to CoMC6\*a is slow relative to CoMP11-Ac and is impacted by steric hindrance of the proton donor.<sup>48</sup> The data are consistent with the requirement of a conformational rearrangement of

Table 3 Results of CPE experiments on CoMC6\*a in the presence and absence of air<sup>a</sup>

GAS <sub>Headspace</sub>	GAS <sub>Solution</sub>	$E^b$ (V)	$\text{FE}_{(\text{H}_2)}$ %	$\text{FE}_{(\text{CO})}$ %	$\text{TON}_{(\text{H}_2)}$	$\text{TON}_{(\text{CO})}$	$Q_T$ (C)
$\text{CO}_2$	$\text{CO}_2$	−1.2	$6 \pm 1$	$85 \pm 11$	$160 \pm 40$	$2200 \pm 300$	$2.5 \pm 0.2$
Air	$\text{CO}_2$	−1.2	$4 \pm 1$	$86 \pm 7$	$67 \pm 30$	$1500 \pm 500$	$1.7 \pm 0.6$
$\text{N}_2$	$\text{CO}_2$	−1.2	$5 \pm 4$	$90 \pm 10$	$80 \pm 60$	$1500 \pm 200$	$1.6 \pm 0.1$
$\text{N}_2$	$\text{N}_2$	−1.2	No above-background activity <sup>c</sup>				

<sup>a</sup> Two-hour CPE experiments conducted on 1  $\mu\text{M}$  catalyst in 0.5 M MOPS with 1 M KCl. Results correspond to the average of at least three individual runs, the error corresponds to the difference between the average and the replicate with the greatest difference from the average. The pH of all solutions was adjusted to 6 for experiments. CPEs under air were purged with  $\text{CO}_2$  before the headspace was replaced with air ~99% of the  $\text{CO}_2$  was replaced. <sup>b</sup> Potentials reported vs.  $\text{Ag}/\text{AgCl}/\text{KCl}_{(1\text{M})}$ . <sup>c</sup> Activity is not reported if it did not exceed three times background in more than one replicate.



CoMC6\*a to facilitate proton delivery, *i.e.*, to expose the distal side of the porphyrin, which is protected by a helix in the folded mini-protein (Fig. 1). In contrast, CoMP11-Ac reacts with proton donors in a diffusion-controlled manner, provided the proton donor has a  $pK_a$  below  $\sim 7.5$ .<sup>49</sup> Those results revealed the impact of the distal helix on  $H_2$  evolution reactivity of CoMC6\*a: it slows proton delivery, changes mechanism, and increases catalyst robustness, as reflected by  $TON_{H_2}$  values nearly 10-fold higher (230 000) than what is seen for CoMP11-Ac (25 000).<sup>45,61</sup>

Given the more hydrophobic nature of the CoMC6\*a active site relative to CoMP11-Ac, we hypothesized that it may display greater  $CO_2$  reduction activity and/or selectivity compared to CoMP11-Ac. This prediction is consistent with reports that hydrophobic microenvironments can improve activity and selectivity for  $CO_2$  reduction in MOF- and materials-based catalytic systems.<sup>14,62–64</sup> and also for catalysts within protein environments.<sup>20,23</sup>

For electrocatalytic  $CO_2$  reduction at  $-1.2$  V, CoMP11-Ac<sup>22</sup> and CoMC6\*a (Table 1) yield similar and high selectivities for CO production (Table S2† compares results on these catalysts). For CoMP11-Ac at  $-1.2$  V in the presence of MOPS, CHES, or CAPS buffers, values of  $FE_{CO}$  range from 81 to 88%, and  $FE_{H_2}$  ranges from 5 to 8%, similar to the respective ranges for CoMC6\*a (73–86% and 4–11%). The measure that does change when comparing these catalysts under these conditions is TON measured in 2-hour experiments; CoMP11-Ac generally has higher TON values for both  $H_2$  and CO production at  $-1.2$  V, by a factor of four- to six-fold for CO production and two- to seven-fold for  $H_2$  production, suggesting that the more solvent-accessible active site of CoMP11-Ac facilitates reaction turnover at  $-1.2$  V. However, when CPE is run at  $-1.2$  V for 24 hours (Fig. S12†), the gap in TON values for CO production between these catalysts closes, with a  $TON_{CO}$  of 14 000 for CoMC6\*a compared to 32 000 for CoMP11-Ac (Table S6†). This result is attributed to a loss of overall activity for CoMP11-Ac in this longer experiment, in which it yields  $FE_{CO}$  of 61% compared to 86% for CoMC6\*a. We propose that the more protected nature of the CoMC6\*a active site maintains catalyst integrity and activity in this longer experiment. Its total value of  $FE_{H_2} + FE_{CO}$  is 91%, but this value is only 70% for CoMP11-Ac. We propose that catalyst degradation, which is significant for CoMP11-Ac, accounts for the balance of FE, consistent with the observation that CoMP11-Ac undergoes deactivation and degradation in longer CPE experiments.<sup>61</sup> These results illustrate how supermolecular structure confers advantages for CoMC6\*a catalysis that translate to it maintaining high activity and selectivity for CO production in longer (24-hour) experiments.

These differences in selectivity between these catalysts change substantially for reactions run at more negative potential. At  $-1.4$  V in the three different buffers, CoMC6\*a has  $FE_{CO}$  values that vary little (67–76%), while  $FE_{CO}$  is lower and more variable (21–48%) for CoMP11-Ac.  $FE_{H_2}$  values differ significantly between these two catalysts at  $-1.4$  V, ranging from 4 to 24% for CoMC6\*a and 29–63% for CoMP11-Ac in the three buffers. Overall, for both catalysts, a decreased buffer acid  $pK_a$  is correlated with a higher  $FE_{H_2}$ . We also see that the  $TON_{CO}$  value for CoMC6\*a at  $-1.4$  V is highest with the least acidic proton

donor (CAPS), but for CoMP11-Ac,  $TON_{CO}$  at  $-1.4$  V with CAPS is its lowest value among the three buffers. While the basis for this difference is speculative, we propose that these observations support the proposal that the protected and hydrophobic active site of CoMC6\*a facilitates  $CO_2$  binding and inhibits proton delivery to both enhance CO production and inhibit  $H_2$  evolution, especially at lower potentials that enhance  $H_2$  evolution activity. However, in CoMP11-Ac, with its solvent-exposed distal site, the  $pK_a$  of the proton donor is the key factor determining overall catalytic activity, such that CO production activity (TON) increases with a more acidic proton donor even as  $FE_{CO}$  decreases.

## Conclusions

CoMC6\*a is a synthetic mini-enzyme that electrochemically catalyzes  $CO_2$  reduction to CO in water. We provide evidence that its selectivity for  $CO_2$  over proton reduction is enhanced relative to CoMP11-Ac, particularly at more negative potentials, which we attribute to protection of its active site and its lower Co(II/I) potential. The catalytic mechanism for CO formation requires  $CO_2$  binding before or coupled with Co(II) reduction for CO formation. CoMC6\*a displays an outstanding  $TON_{CO}$  of 14 000 over 24 hours and excellent selectivity of 86:5  $CO:H_2$  products in the same 24-hour experiment, demonstrating that a small artificial biocatalyst can be active, robust, and selective for  $CO_2$  reduction in water. Furthermore, the activity of CoMC6\*a is minimally impacted by air, an unusual and desirable property for a  $CO_2$  reduction catalyst.

## Data availability

Data supporting this article have been published as ESI.†

## Author contributions

Conceptualization: JLA-H, KLB, AL; funding acquisition: AAS, KLB, AL; investigation: AAS, JLA-H, LL, KBR; supervision: KLB, AL; writing – original draft: AAS, JLA-H; writing – review & editing: KLB, AL, LL.

## Conflicts of interest

There are no conflicts to declare.

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