Single-Atom Cobalt Catalysts Coupled with Peroxidase Biocatalysis for C–H Bond Oxidation

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ABSTRACT: This paper reports a robust strategy to catalyze in situ C–H oxidation by combining cobalt (Co) single-atom catalysts (SACs) and horseradish peroxidase (HRP). Co SACs were synthesized using the complex of Co phthalocyanine with 3-propanol pyridine at the two axial positions as the Co source to tune the coordination environment of Co by the stepwise removal of axial pyridine moieties under thermal annealing. These structural features of Co sites, as confirmed by infrared and X-ray absorption spectroscopy, were strongly correlated to their reactivity. All Co catalysts synthesized below 300 °C were inactive due to the full coordination of Co sites in octahedral geometry. Increasing the calcination temperature led to an improvement in catalytic activity for reducing O₂, although molecular Co species with square planar coordination obtained below 600 °C were less selective to reduce O₂ to H₂O₂ through the two-electron pathway. Co SACs obtained at 800 °C showed superior activity in producing H₂O₂ with a selectivity of 82−85% in a broad potential range. In situ production of H₂O₂ was further coupled with HRP to drive the selective C–H bond oxidation in 2-naphthol. Our strategy provides new insights into the design of highly effective, stable SACs for selective C–H bond activation when coupled with natural enzymes.

KEYWORDS: single-atom catalysts, biocatalysis, Co phthalocyanine, horseradish peroxidase, oxygen reduction reaction (ORR), synthesis of hydrogen peroxide, C–H bond activation

1. INTRODUCTION

Hydrogen peroxide (H₂O₂) is of great importance for chemical syntheses, paper industries (pulp bleaching), and intercellular signaling in physiological processes.¹–³ As a strong oxidant, H₂O₂ has been used in many oxidative synthetic reactions, e.g., epoxidation and hydroxylation.⁴ In nature, H₂O₂ can be produced in animals including humans as byproducts from the metabolism of fatty acids or in various fungi through the metabolism of glucose. A very well-known example is glucose oxidase, which produces H₂O₂ and D-glucono-1,5-lactone by the oxidation of glucose. H₂O₂ can activate many iron heme enzymes, including peroxidases and catalases, for biocatalytic oxidations.⁵⁻⁷ In addition to their biological pathways, the combination of H₂O₂-producing enzymes like glucose oxidases and H₂O₂-consumption enzymes like peroxidases can catalyze organic oxidations not relevant to their biofunctions.⁷⁻⁹

On an industrial scale, H₂O₂ is produced through the anthraquinone process, where the redox cycle of hydroquinone and quinone reduces molecular O₂ to H₂O₂. The regeneration of hydroquinone requires costly palladium (Pd) catalysts. On top of the typical time-consuming separation and recycling of anthraquinone, the long-term storage of relatively unstable...
H$_2$O$_2$ is problematic. There are a number of catalytic and noncatalytic processes to produce H$_2$O$_2$ in controlled quantitative yield.$^{10-14}$ For example, electrochemical synthesis of H$_2$O$_2$ from O$_2$ is effective, controllable, and, more importantly, can be done on demand.$^{12}$ In the electrochemical reduction of O$_2$, H$_2$O$_2$ can be synthesized by a two-electron transfer process: O$_2$ + 2H$^+$ + 2e$^-$ → H$_2$O$_2$, $E^0 = 0.695$ V vs the reversible hydrogen electrode (RHE). While an oxygen reduction reaction (ORR) on metal catalysts often involves a four-electron transfer, controlling the spatial distribution of noble metals like isolated Pd sites has shown to be very selective in reducing O$_2$ by a two-electron transfer.$^{15,16}$ A similar perspective is demonstrated in earth-abundant metal-based catalysts, e.g., molecular Cu catalysts.$^{13,14}$ It has been reported that H$_2$O$_2$ production can be stimulated by changing the surrounding atomic structure of the metal center.$^{17}$ By fine-tuning the environment and the local interactions of the metal...
Single-atom catalysts (SACs) have attracted a great deal of research interest due to their high catalytic activity per unit mass, excellent stability, and low cost. Heterogeneous SACs consist of metal atoms often dispersed or stabilized on a support such as conductive carbon, providing a platform for metal and surrounding environmental interactions to tune catalytic reactivity. Previous studies have suggested that nitrogen-coordinated metal sites in the form of M-N$_4$ and M-N$_3$ on nitrated carbon are very active catalysts to reduce O$_2$. These catalyst sites are structurally similar to metal atoms (or ions) surrounded by porphyrin-like ligands in heme-containing proteins. As an example, single-atom Fe sites coordinated with N$_4$ ligands are active to reduce O$_2$ through a four-electron pathway, while Co$_2$N$_4$ is active to reduce O$_2$ to H$_2$O$_2$ due to the weak interactions between Co sites with precise N-coordinated SACs for ORR.

In this paper, we report the synthesis and applications of Co$_2$N$_4$ SACs for H$_2$O$_2$ production and its use for a cascade oxidative C–H activation catalyzed by horseradish peroxidase (HRP). The chemically stable complex cobalt phthalocyanine (CoPC) was used to examine the impact of the coordination structure of Co sites on the activity of Co SACs. CoPC was first coordinated with 3-propanol pyridine at the two axial positions (CoPC-2Py, Scheme 1) and then loaded on activated carbon. Through annealing at high temperatures, the stepwise removal of axial pyridine moieties allowed to tune the number of N coordination of Co, as ascertained by infrared spectra and XAS analysis, and structural features were correlated with the reactivity of Co SACs. With Co SACs obtained at 800 °C, a selectivity of >80% for H$_2$O$_2$ was achieved in the potential window of −0.3 to −0.9 V vs the saturated calomel electrode (SCE, the same hereafter). In situ production of H$_2$O$_2$ by Co SACs was used to activate HRP to catalyze the oxidative coupling of 2-naphthol to binaphthol. The combined use of Co SACs and HRP allows the controlled synthesis of H$_2$O$_2$ and shuts down unselective radical pathways for C–C bond coupling, with a total yield of binaphthol of approximately 80% and selectivity close to 100%.

2. RESULTS AND DISCUSSION

We chose CoPC as the molecular Co source to make Co SACs due to their high chemical and thermal stability. Since it also has poor solubility in organic solvents, 3-propanol pyridine was used as the axial ligand to increase its solubility. CoPC was first mixed with 3-propanol pyridine in chloroform at 1:2 (mol). The solubilization of CoPC was evidenced by the bright green solution (Figure 1b), while it was nearly colorless in the absence of 3-propanol pyridine. The UV–vis absorption spectroscopy of CoPC was monitored by UV–vis spectroscopy. A strong absorption peak at around 665 nm was assigned to the Q-band ($\pi \rightarrow \pi^*$) excitation arising from CoPC. The peak intensity increased with time as the concentration of bound
CoPC increased. Without stirring, the absorbance increased to 1.6 in 4 h, corresponding to 0.017 mg/mL with the molar absorption coefficient of CoPC, $\sim 1 \times 10^5 \text{L} \cdot \text{mol}^{-1} \cdot \text{cm}^{-1}$. In chloroform, the bound CoPC complex was highly fluorescent. The maximum emission peak appeared at 706 nm (Figure 1c), very close to that of CoPC, as reported previously.\(^3\) The dissolving was much faster under stirring, and the solubility of CoPC could reach 0.3 mg/mL after 48 h.

The formation of the CoPC complex was confirmed by $^1$H NMR. In CDCl$_3$, 3-propanol pyridine showed well-resolved proton peaks. After coordination with paramagnetic Co, all proton peaks were significantly broadened, where the first-order coupling of adjacent protons disappeared (Figure 2a). The aromatic protons on the pyridine ring had an obvious shift, and the protons on the propyl alcohol far away from the coordination center were less impacted (Supporting Information, Figure S1a). From $^1$H--$^1$H correlation spectroscopy (COSY) (Figure 2b), the protons adjacent to N (HC$\equiv$N, H$_a$, and H$_b$) were largely shifted. H$_a$ with no spin–spin interaction of neighboring protons downshifted from 8.4 to 7.4 ppm, and H$_b$ shifted from 8.4 to 9.6 ppm. The NMR results suggest that the coordination is complete without free 3-propanol pyridine, and bound 3-propanol pyridine is likely under the same chemical environment. We also used mass spectroscopy to examine this complex of CoPC and 3-propanol pyridine. The complex has a molecular ion peak at $m/z$ 845.2455, corresponding to CoPC with two 3-propanol pyridines (CoPC-2Py, C$_{42}$H$_{26}$N$_{10}$Co, inset of Figure 1d). Therefore, the square planar Co was fully coordinated as an elongated octahedron.

Synthesis of Co SACs was done by thermal annealing. In a typical synthesis, a chloroform solution of CoPC-2Py (2 mg, 2.4 $\mu$mol) was mixed with activated carbon (200 mg, $\sim$50 nm nanospheres, soft-nitrided)\(^4\) at a mass loading of 1 wt % of CoPC-2Py in chloroform relative to carbon. After being sonicated overnight, the solvent was slowly evaporated under N$_2$ at room temperature. The loading of CoPC-2Py was confirmed by thermogravimetric analysis (TGA), where the distinct decomposition for CoPC-2Py was around 400 °C (Figure S1). The loading amount of CoPC-2Py estimated from the weight difference was 1.2 wt %. The powder was collected and calcined at different temperatures for 2 h. We denote the samples as Co-X, where X represents the calcination temperature. Taking Co-800 calcined at 800 °C as an example,

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**Figure 3.** Morphological and coordination analysis. (a) TEM and (b) HAADF-STEM images of Co-800 supported on a carbon film. (c) XAS experiment data from the Co K-edge of the sample, reference Co metal, and Co oxides. (d) Fourier transform magnitudes of $k^2$-weighted EXAFS spectra collected of the sample, reference Co metal, and Co oxides. The k-range used in the Fourier transform was from 2 to 8 Å$^{-1}$.

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\(^{33,35–39}\)
transmission electron microscopy (TEM) was first used to characterize the morphology of carbon and the dispersity of Co sites. Low-resolution TEM in Figure 3a shows the carbon nanospheres with a diameter of 50 ± 1.1 nm (Figure S2) without a morphological change after thermal annealing. High-angle annular dark-field imaging scanning TEM (HAADF-STEM) imaging provides atomic resolution of Co sites and confirms their uniform distribution on the carbon support. Under a dark field, Co sites appeared as white dots (with some representative ones encircled), indicating that Co sites are well-dispersed without any agglomeration (Figure S3). Complementary X-ray absorption near-edge structure (XANES) (Figure 3c) and extended X-ray absorption fine structure (EXAFS) (Figure 3d) results, together with reference Co metals and oxides, are consistent with the conclusion that Co sites in Co-800 are atomically dispersed. The final Co loading relative to carbon is about 0.13 wt % close to its initial content (see mapping analysis in Figure S4).

To further investigate the local structures of Co sites, we used XANES for Co-X catalysts calcined at different temperatures (Figure 3e). The change in Co coordination was evidenced from the pre-edge peak at 7716 eV in XANES, which is assigned to the 1s → 4p electronic transitions of Co.40 When the sample was treated at 400 °C, the pre-edge peak intensity had a clear decrease due to the loss of the axial pyridine moiety. The pre-edge of Co is known to decrease from octahedral to square planar coordination or, in our case, by losing the axial ligands. Linear combination analysis was conducted to estimate the fraction of the pyridine moiety. The spectrum of Co-400 was fitted to 30% of the initial sample (Co-20, as-prepared without any calcination) spectrum and 70% of CoPC, indicating that the CoPC framework with square planar coordination was retained but partial loss of the axial pyridine moiety had occurred. When the calcination temperature reached 800 °C, the pre-edge feature (1s → 4p) in the XANES spectra disappeared due to the destruction of the well-defined 4-coordinated square planar structure of CoPC and the formation of atomically dispersed Co sites with lower symmetry. This structural change was also associated with a significant increase in the white line intensity of Co-800. The XANES spectrum showed nearly identical spectral features with Co SACs (as Co–N$_2$ supported on the defective carbon) synthesized supported on C$_3$N$_4$ as reported previously (Figures S5 and S6). The EXAFS region in the k-space was too short (2–8 Å$^{-1}$) for quantitative analysis by theoretical fitting. However, qualitative conclusions can be made based on Figure 3f. The fact that features in the 2–3 Å range were different from those of Co metals or Co oxides (Figure 3b) is consistent with the formation of atomically dispersed Co catalysts.

The ORR activity of Co-800 was evaluated by cyclic voltammetry (CV) in 0.1 M Na$_2$SO$_4$ saturated with O$_2$. The working electrode was made on a pyrolytic graphite rod coated with a catalyst ink containing Co-800 and Nafion as the binder (see details in Section 4). The concentration of the Co sites was estimated to be 3.0 nmol/cm$^2$ on the electrode. Figure 4a shows typical CVs of Co-800 at a scan rate of 50 mV/s. In the N$_2$-saturated electrolyte, no reduction peak was observed in cathodic scans. In contrast, there was an obvious peak in the O$_2$-saturated electrolyte at ~0.276 V vs SCE. As our control, the bare electrode or the pyrolytic graphite has nearly no background reduction of O$_2$. Those results suggest that Co-800 is catalytically active in reducing O$_2$.

We examined the ORR activity of Co SACs obtained at different temperatures under the same conditions (Figure S7). There was a strong correlation between the ORR activity and the calcination temperature. First of all, the catalysts were inactive to reduce O$_2$ when the annealing temperature was below 250 °C, as evidenced by no reduction peak and no current increase in comparison with CVs under N$_2$. At 300 °C, the Co catalysts became active. The peak potential ($E_p$) at the maximum reduction current is in the range of ~0.27 to ~0.25 V, and the onset potential ($E_{onset}$) defined as the potential where the reduction current starts to increase sharply with the potential, was estimated at ~0.14 V. Since $E_p$ and $E_{onset}$ are essentially independent of calcination temperature (Figure 4c), we assume that the active Co sites are similar in terms of their coordination geometry and environment. The current intensity ($I_p$), however, increased with the thermal treatment temperature. For example, the $I_p$ of Co-300 was ~14.3 ± 0.2 μA and it
nearly doubled to $-25.3 \pm 2.6 \mu A$ for Co-400. This increment slowed down above 400 °C, but the current trajectory continued as the calcination temperature was increased.

To understand the structural change of Co sites with the temperature, we measured the infrared spectra of CoPC-2Py. CoPC-2Py was annealed in a diffuse reflectance infrared Fourier transform spectroscopy (DRIFT) cell under N$_2$ at a heating rate of 2 °C per min. The infrared spectra were collected at every 50 °C, as shown in Figure 4d. While the aromatic C–H stretching/bending and the ring breathing mode (1600–1400 cm$^{-1}$) cannot be distinguished between the CoPC and 3-propanol pyridine, we analyzed the change of the level of the O–H (broad peak at 3350 cm$^{-1}$) and sp$^3$C–H (sharp peaks at 2930 and 2856 cm$^{-1}$) stretching from 3-propanol pyridine. The intensities of both stretching peaks decreased along with the temperature (Figure 4e). There was a sharp decrease at 300 °C. We attributed this change to the thermal removal of the axial ligands of CoPC-2Py, where the axial position of Co became available for catalysis. This temperature correlates well with the ORR activity, as Co catalysts made at 250 °C and below were inactive. We could conclude that fully coordinated octahedral Co sites are not catalytically active in reducing O$_2$. Moreover, the change in the extent of the O–H and sp$^3$C–H stretching also shows a continuous decrease with T until ~420 °C, where the axial ligands are likely removed completely. These active Co sites are structurally close to molecular CoPC with sp$^2$C–H stretching at 3030 cm$^{-1}$ and aromatic C–N stretching at 1321 and 1290 cm$^{-1}$. At 600 °C and above, CoPC-like spectral features disappeared, indicating that phthalocyanine carbonized around 600 °C and square planar Co sites became less symmetric or as SACs (see Figure S8 for details). We note that the further increase of temperature (>600 °C) would increase the graphitization degree of the ligand environments of Co SACs, known to improve the conductivity and activity of SACs.$^{41}$

ORR kinetics were investigated using rotating disk voltammetry (RDV) in 0.1 M Na$_2$SO$_4$. Figure 5a exhibits the RVDs of Co-800 at rotation rates of 400–3600 rpm. The diffusion-limited current density increased with the rotation speed of the electrode as a typical characteristic for catalytic reduction of O$_2$. The onset potential for the reduction of the O$_2$ was approximately $-0.27$ V, close to that measured by CV. These curves were fitted using the Koutecky–Levich (K−L) method by plotting the reciprocal of current density ($J^{-1}$) as a function of the square root of the rotation rate ($\omega^{1/2}$) (Figure 5b). The linearity of the fit is considered as a standard first-order kinetics plot with respect to the concentration of dissolved O$_2$. The number of electrons transferred ($n$) obtained from the slope was 2.2, 2.0, and 1.9 at $-0.4$, $-0.6$, and $-0.8$ V, respectively. Since the number of electrons transferred was very close to 2 and independent of the potential, this suggests that Co-800 is active in reducing O$_2$ through two-electron reduction.

Figure 5. Electrochemical reduction of O$_2$. (a) RDV of Co-800 at different rotation speeds in O$_2$ saturated 0.1 M Na$_2$SO$_4$ (pH 7) at a scan rate of 50 mV/s. (b) K−L plots at different potentials and the corresponding number of electrons transferred of Co-800. (c) RRVD of Co-800 at 1600 rmps. (d) Number of electrons transferred and H$_2$O$_2$ yields of Co-800 at 1600 rmps. (e) $i$−$t$ curves at different potentials to investigate the stability.
The relative yield of $\text{H}_2\text{O}_2$ was measured by rotating-ring-disk voltammetry (RRDV). The platinum ring held at a constant potential of 20 mV to oxidize $\text{H}_2\text{O}_2$ with a collection efficiency ($N$) of 22%. The percentage yield of $\text{H}_2\text{O}_2$ formation was determined from the currents at the ring and the disk

$$\text{H}_2\text{O}_2 \text{ (%) } = \frac{2I_R/N}{I_D + I_R} \times 100\%$$

where $I_D$ is the disk current, $I_R$ is the ring current, and $N$ is the collection efficiency of the ring electrode. The yield of $\text{H}_2\text{O}_2$ was $\sim 85\%$ in the potential range of $-0.2$ to $-0.8$ V. $\text{H}_2\text{O}_2$, as the major product during the ORR, is in agreement with the average number of electrons transferred of $\sim 2$ measured from the K–L method. Additionally, we examined the stability of Co-800 by using constant-potential electrolysis ($i-t$). Figure S5e shows the $i-t$ curves obtained at $-0.4$ to $-1$ V. Generally, the steady state current of Co-800 increased with increasing negative potential. In all cases, the catalysts were stable during electrolysis for 4 h, and no obvious decrease in activity was seen under all potentials. Co-800 as distorted square planar Co sites are strongly anchored on carbon that significantly enhances catalytic durability.

We measured the concentration of $\text{H}_2\text{O}_2$ quantitatively as the product in the electrolyte. Using an H-cell that separates the anode and cathode, the amount of $\text{H}_2\text{O}_2$ was measured using a colorimetry assay with leuco crystal violet. The oxidation of leuco crystal violet gives absorbance of the product at 596 nm, and it can be calibrated in a standard linear range of known concentrations of $\text{H}_2\text{O}_2$ (Figure S9). Figure 6a shows the amount of $\text{H}_2\text{O}_2$ produced at $-0.4$ V (see more potentials in Figure S9). The concentration of $\text{H}_2\text{O}_2$ increased linearly with the electrolysis time. The maximum yield of $\text{H}_2\text{O}_2$ was 97.4 $\mu$M after 1 h. The Faradaic efficiency (FE) that defines electron utilization efficiency to specifically produce $\text{H}_2\text{O}_2$ was in the range of $>80\%$ during the electrolysis for 1 h at $-0.4$ V. This number was consistent with those measured from RRDV, as shown in Figure 5.

Using RRDVs, we further examined the efficiency of $\text{H}_2\text{O}_2$ production for all of the Co catalysts obtained at different temperatures. Since all Co samples produced below 300 °C were inactive for the reduction of the $\text{O}_2$ concentration, their efficiency was negligible. For Co catalysts calcined in the temperature range of 300–450 °C, the selectivity of $\text{H}_2\text{O}_2$ gradually increased from 52.8 to 71.3%. Those values were close to that of molecular CoPC, as reported previously. Those results suggest that Co catalysts were still in their molecular form supported on carbon, as confirmed by XANES and DRIFT measurements. All catalysts calcined above 600 °C showed very similar $\text{H}_2\text{O}_2$ selectivity of $>85\%$, although their activity was somewhat different. Co-800 has a selectivity of 89.6% at $-0.4$ V toward $\text{H}_2\text{O}_2$. By systematically manipulating the active sites and their supporting material using rational engineering approaches, the exceptional performance of these SACs can be attributed to the synergistic effects of Co single sites and the activated carbon support, which offers a large surface area, enabling facile accessibility to active sites and efficient mass transfer during catalytic reactions. We attribute the high selectivity for $\text{H}_2\text{O}_2$ production to the atomically dispersed Co sites. The distorted Co–$\text{N}_4$ sites are likely to be the active centers for two-electron ORRs.  

The ORR kinetics of Co-800 were also examined in 0.1 M NaOH at pH 13 (Figure S10). The diffusion-limited current was higher than those obtained at pH 7. While the reduction occurred at much faster kinetics, the yield of $\text{H}_2\text{O}_2$ was still $>75\%$ in the potential range of $-0.3$ to $-1$ V. Co-800 was also very stable under alkaline conditions, as shown by amperometry (Figure S10e). These results clearly illustrate that Co-800 is viable and efficient in catalyzing two-electron ORR and producing $\text{H}_2\text{O}_2$ in basic solutions.

Co SACs that reduce $\text{O}_2$ to $\text{H}_2\text{O}_2$ selectively provide more opportunities for straightforward applications, e.g., coupled with peroxidases to activate stable C–H bonds (Scheme 2). We examined the oxidative coupling of 2-naphthol to binaphthol catalyzed by HPR and Co SACs. HPR was immobilized and cross-linked on magnetic beads through layer-by-layer assembly with polylysine. The cascade reaction was done in a bicontinuous microemulsion consisting of cetyltrimethylammonium bromide, butanol, hexadecane, and $\text{H}_2\text{O}$ (5:5.4:1:88.6, wt %), that not only provides solubility for 2-naphthol in the oil phase and water for proper enzyme function but also acts as a supporting electrolyte for the electroreduction of $\text{O}_2$. Figure 7a shows the constant-potential electrolysis in the microemulsion. Co-800 films on the electrodes were very stable in the high solvating microemulsions, where the $\text{O}_2$ reduction currents remained nearly constant with time, similar to those measured in $\text{Na}_2\text{SO}_4$. The formation of $\text{H}_2\text{O}_2$ was confirmed by using $\text{H}_2\text{O}_2$ testing stripes. After mixing HPR beads and 2-naphthol in the testing stripes. After mixing HPR beads and 2-naphthol in the microemulsion, the oxidative coupling was done at $-0.4$ V for 1 h. Using gas chromatography, the formation of binaphthol was confirmed with a well-resolved elution peak at 19.8 min. The conversion of 2-naphthol was estimated to be 80% using 6-bromo-2-naphthol as an internal standard. In negative controls, no product was detected in the absence of HPR or without Co SACs. Therefore, the two catalysts, Co-800 and HRP, worked cooperatively to drive consecutive reactions, i.e.,

Figure 6. (a) $\text{H}_2\text{O}_2$ yield and FE of Co-800 toward $\text{H}_2\text{O}_2$ at $-0.4$ V in $\text{Na}_2\text{SO}_4$. (b) Selectivity of $\text{H}_2\text{O}_2$ of Co catalysts calcined at different temperatures. The selectivity was calculated from the RRDV at $-0.4$ V.
oxidation of O2 to H2O2 by Co-800 and H2O2-activation of HRP to oxidize 2-naphthol by HRP.

In situ oxidative coupling kinetics of 2-naphthol was measured by using UV−vis spectroelectrochemistry. In brief, the three-electrode systems were built in a cuvette. A Pt wire with a diameter of 0.25 mm was used as a counter electrode, and a Ag wire with a diameter of 0.5 mm was used as a reference electrode. All electrodes were placed outside of the pathway of incident light. The reaction kinetics were monitored by absorption spectra of the solution mixture at different reaction times. Figure 7b shows the UV−vis spectra of 2-naphthol and binaphthol. Their absorption peaks were fairly close to each other (e.g., 331 nm for 2-naphthol and 334 nm for binaphthol), but the molar absorbability of binaphthol was ~27 times higher than that of 2-naphthol. With the increase of the electrolysis time, there was a fast increase of UV−vis absorbance around 330 nm. Using their molar absorbability, we calculated the conversion of 2-naphthol, as
given in Figure 7e, as the change of [S] with reaction time (see details in Figures S11–S12). Interestingly, the consumption rate of 2-naphthol at different potentials nearly overlapped. This agrees well with the formation rate of binaphthol, as plotted in Figure 7f. The formation rate (or the half reactant consumption rate) was in the range of 0.16–0.19 μM/min, with small changes at different potentials. These results suggest that the production of H2O2 is unlikely to be the rate-determining step, but the overall reaction is controlled by the enzymatic dimerization of 2-naphthol by HRP. In other words, the production rate of H2O2 was much faster than its consumption rate by HRP. The turnover frequency, i.e., the transformation of substrates on each heme center per unit time, was 0.02 s−1, close to that of HRP for peroxide activation.46

3. CONCLUSIONS

In summary, we demonstrated a robust strategy to catalyze in situ C–H oxidation by combining Co SACs on electrodes and HRP on magnetic beads. Using CoPC-2Py loaded on activated carbon, we investigated the stepwise removal of axial pyridine moieties through thermal annealing to tune the coordination environment of the Co sites. Co-800 showed excellent optimized catalytic performance in producing H2O2 at a concentration of 97 μM. Co catalysts obtained below 300 °C were inactive due to the retention of the octahedral coordination. Increasing the calcination temperature led to improvement in activity for the ORR, while the molecular Co species formed at below 600 °C were less selective to reduce the level of the O2 in a two-electron pathway. DRIFT measurements confirmed that the removal of axial ligands occurred above 300 °C, while the aromatic ring frameworks of CoPC disappeared at 600 °C and above, suggesting that phthalocyanine carbonized and Co sites converted to single atoms supported on carbon. These structural features were further confirmed by XANES and correlated with the ORR activity. The FE of Co-800 toward H2O2 was 82–85% at −0.4 V, where the distorted Co–N4 sites were likely the active centers for two-electron ORRs. Co SACs were further coupled with HRP to drive the catalytic cascade of the reaction of O2 to H2O2 and the oxidation of 2-naphthol in microemulsions. The high selectivity (~100%) and yield (~80%) of binaphthol were confirmed by gas chromatography and spectroelectrochemistry. Our results provide new insights into the design of highly effective, stable SACs potentially for selective C–H activation when coupled with natural enzymes.

4. EXPERIMENTAL SECTION

4.1. Materials and Chemicals. Cobalt phthalocyanine (CoPC, 97%), 3-propanol pyridine (ACS grade, ≥99%), sodium hydroxide (NaOH, bioXtra ≥98%), sodium sulfate (Na2SO4, BioXtra ≥98%), horseradish peroxidase (HRP, MW: 40 kDa), leucocrystal violet (MW: 373.5 g/mol), hydrochloric acid, acetate buffer (pH 4.5), hydrogen peroxide (H2O2), and Nafion were obtained from Sigma-Aldrich. Printex U carbon was gifted by the Orion-engineered materials. Aldrich. All chemicals were used without further purification. HRP was cross-linked using EDC coupling chemistry at each bilayer of PLL/HRP. A 3-propanol pyridine and the CoPC complex with a molar ratio of 1:2 (mol) in chloroform. The solution was stirred under N2 to fully dissolve and disperse CoPC. 4.2. Synthesis of the CoPC-2Py Complex. CoPC-2Py was synthesized by adding 3-propanol pyridine and the CoPC complex with a molar ratio of 1:2 (mol) in chloroform. The solution was stirred under N2 to fully dissolve and disperse CoPC. 4.3. Synthesis of Co SACs. Activated carbon was first nitrided using a soft nitriding route, as reported previously.38–40 4 g of Printex U carbon (4 g) was mixed with 6 g of urea. After grounded together, the mixture was sealed in a crucible with foil and calcined at 150 °C for 2 h and then 300 °C for another 2 h.58,59 The obtained product was washed with water and ethanol several times to remove residual urea and then dried at 50 °C overnight. Co SACs were produced by a pyrolysis method. In a typical synthesis, the concentrated solution of CoPC-2Py (2 mg) in 5 mL of CHCl3 was mixed with 200 mg of activated carbon dispersed in CHCl3, and then stirred overnight. The solvent was then evaporated slowly in a fume hood under a flow of N2. The dried powder was collected and placed on a ceramic crucible tray for thermal treatment in a tube furnace under N2. The temperature was increased at a ramping rate of 5 °C/min, and the desired temperature was held for 2 h. The sample was cooled down with a decrement rate of 15 °C/min. Using the same protocol, the sample was treated at different temperatures ranging from 100 to 900 °C. 4.4. Electrochemical Measurements. An electrochemical CH 1040 workstation was used for the electrochemical characterization. An SCE was used as a reference electrode, and the graphite rod was used as a counter electrode. The working electrode was made on a pyrolytic graphite rod with the catalyst ink. The ink was prepared by adding 6 mg of catalyst in 1140 μL of water, 350 μL of isopropanol, and 10 μL of 5% Nafion solution. After the solution was sonicated for 25 min, 10 μL of the resultant solution was dropped on a pyrolytic graphite rod. The CVs were measured in 0.1 M Na2SO4 and 0.1 M NaOH at pH 7 (neutralized with trace NaOH) and 13, respectively. The pH was calibrated with a pH meter. The scan rate was 50 mV/s unless otherwise noted. The electrolyte was saturated with N2 or O2 prior to the test. For RRDE and RRDV measurements, pin wave vortex 10 was used as an electrode rotator, and the E7R8 thin gap fixed-disk electrode (ring-disk gap of 180 μm) from PINE research was used. For the RRDE measurement, the collection efficiency was calibrated using potassium ferricyanide at a 1600 rpm rotation rate in 0.1 M Na2SO4 and 0.1 M NaOH at the scan rate of 50 mV/s. The disk electrode was scanned cathodically, while the ring potential was constant at 20 mV. The production of H2O2 was also determined using the leucocrystal violet assay. The calibration curve was used with the standard concentrations of H2O2 (Figure S9).

4.5. In Situ Oxidative Coupling. For in situ conversion of 2-naphthol to binaphthol, the reaction system was built in a UV cell. The electrodes were placed in 2 mL of microemulsions without interfering with the pathway of the probe UV light. The kinetics of the reaction were determined using the change of absorbance. The concentration of 2-naphthol and binaphthol was calculated from the UV–vis spectra (see details in Figure S11). The concentration of H2O2 was 6.35 μg/mL in the reaction mixture.

4.6. Other Characterizations. Scanning electron microscopy (SEM) and energy dispersive X-ray spectroscopy (EDX) were performed using an FEI Nova NanoSEM 450. The samples were drop-cast onto a silicon wafer and dried at room temperature. The EDX mapping was measured on a large area of the sample at 10 kV, and the exposure to the beam for a long time was avoided to minimize the beam-induced effect, as both activated carbon and cobalt are sensitive to the X-ray beam. HAADF-STEM imaging was recorded on a Titan Themis probe-corrected microscope. The samples were first cleaned by 17 h of beam shower and 15 s of plasma. The UV–vis results were collected on a Cary 60 spectrometer. TGA Q500 was used to analyze the sample annealing from 100 to 700 °C with a rate of heating at 10 °C/min under N2. 1H NMR spectra were recorded on a Bruker Avance 400 MHz spectrometer. X-ray absorption spectroscopy (XAS) measurements at the Co K-edge (7709 eV) were performed at the 7-BM (QAS) beamline of National Synchrotron Light Source II (NSLS II), Brookhaven National Laboratory. The CoPC sample was mixed and ground with boron nitride, prepared as a pellet and measured in the transmission mode. Other samples were prepared as pellets and measured in fluorescence mode. The infrared spectra at different temperatures were analyzed on a Thermo Nicolet 40351.
NEXUS 670 with a DRIFT cell. The fluorescence spectra of CoPC-2Py were recorded on a Cary Eclipse fluorescence spectrophotometer.

**ASSOCIATED CONTENT**

**Supporting Information**

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsami.3c03053.

Further characterization of CoPC complexes and SACs, electrochemical analysis, and kinetic data on HRP catalysis (PDF)

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- J.H., M.L., and J.R. conceived the idea and co-wrote the first draft, M.L. carried out the synthesis of materials and electrochemical characterizations, R.N.T.K. worked on enzyme immobilization and catalysis, H.D. conducted the characterization of CoPC complexes, R.S., E.S., and A.I.F. conducted the XAS characterizations and calculations, J.S. and P.G. carried out the DRIFT measurement, and M.N. and Y.Z. did STEM characterizations. All authors discussed the results and commented on the manuscript.

**Notes**

The authors declare no competing financial interest.

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