



Article Photoelectrochemical Water Oxidation by Cobalt Cytochrome C Integrated-ATO Photoanode

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Abstract: Here, we report the immobilization of Co-protoporphyrin IX (Co-PPIX) substituted cytochrome *c* (Co-cyt *c*) on Antimony-doped Tin Oxide (ATO) as a catalyst for photoelectrochemical oxidation of water. Under visible light irradiation ($\lambda > 450$ nm), the ATO-Co-cyt *c* photoanode displays ~6-fold enhancement in photocurrent density relative to ATO-Co-PPIX at 0.25 V vs. RHE at pH 5.0. The light-induced water oxidation activity of the system was demonstrated by detecting evolved stoichiometric oxygen by gas chromatography, and incident photon to current efficiency was measured as 4.1% at 450 nm. The faradaic efficiency for the generated oxygen was 97%, with a 671 turnover number (TON) for oxygen. The current density had a slow decay over the course of 6 h of constant irradiation and applied potential, which exhibits the robustness of catalyst-ATO interaction.

Keywords: water oxidation; co-cytochrome *c*; semiartificial photosynthesis; antimony-doped tin oxide (ATO); photoelectrochemistry

1. Introduction

The efficient conversion of sunlight energy into fuels and chemicals is one of the main goals and challenges to develop a sustainable and renewable future energy system. Photoelectrochemical (PEC) water splitting using semiconductor materials is a promising technology to harvest solar energy in the form of fuel [1-5]. Water splitting comprises two half reactions: (1) water oxidation (WO), in which O_2 , $4H^+$, and $4e^-$ are generated; and (2) proton reduction, in which H_2 is the final product. The bottleneck of the full process is the first step, which occurs on the photoanode and involves multiple electron and proton transfers, as well as O-O bond formation, thus requiring a high overpotential [6-8]. Hence, the development of stable and cost-effective photoanodes with high efficiency is a crucial step in designing water splitting systems. One effective approach to overcome the high overpotential barrier of water splitting is the development and study of water oxidation catalysts (WOCs) operating at a low overpotential [7]. In nature, the photoinduced oxidation of water is accomplished at the cubane-like Mn₃CaO₄ oxygen evolving center of photosystem II (PS II) [9,10]. However, the complexity and sensitivity of PSII hinders its application in devices and has led to extensive efforts for mimicking its function using molecular catalysts [7,10]. Molecular coordination complexes based on ruthenium, iridium, iron, nickel, or cobalt have been reported as efficient WOCs [11–16]. The coordination complexes can be incorporated in heterogeneous materials, resulting in effective long-lasting WOCs under (photo) electrochemical conditions [4,17–19]. In this context, cobalt complexes bearing corrole or porphyrin ligands



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Copyright: © 2021 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). have been shown to catalyze water oxidation, either in the form of homogeneous [15,20] or heterogenized systems [21–26], due to the capacity of porphyrin ligands to stabilize high oxidation states [27]. For instance, Grove et al. reported a Co-porphyrin complex [Co^{II}(TDMImp)(OH₂)] ([Co-5,10,15,30-tetrakis-(1,3-dimethylimidazolium-2-yl)porphyrin]) active in electrochemical WO at an onset potential of 1.4 V (vs. SHE) at pH 7 (0.2M NaPi buffer), and producing 1400 s⁻¹ [21]. Likewise, Sakai and co-workers reported a Co porphyrin Co^{III}TPPS (TPPS = [meso-tetra(4-sulfonatophenyl)porphyrinato]) in combination with $Ru^{II}(bpy)_3](NO_3)_2$ (bpy = 2,2'-bipyridine) as photoredox catalyst for photocatalytic WO yielding 122 TON O_2 [20]. Porphyrins also act as cofactors in many natural metalloproteins such as cytochromes and can coordinate various transition metals such as Ni, Fe, Mn, and Co [28,29]. Cytochrome c (cyt c) is a heme binding redox-active protein in which the cofactor is covalently bound through two cysteine residues and axially coordinated by one histidine and one methionine, as shown in Figure 1, and serves as an electron transport component of the respiratory chain of anaerobic specious [30]. The affinity of cyt c to a series of transparent conducting oxides (TCOs), including indium-doped tin oxide (ITO), WO₃, SnO₂, and TiO₂, has been investigated [31–33], yet its capability to serve as a heterogeneous hybrid catalyst remains unexplored. Among all studied materials, antimony-doped tin oxide (ATO) has emerged as a favored TCO due to its low-cost and excellent optical properties [18]. In addition, ATO's high electrical conductivity combined with its unique large surface area marks it as a promising framework for integrating biomolecules [18,34,35]. We previously demonstrated that wild type (WT) cyt c could be absorbed in thin films of highly mesoporous ATO with retention of its native structure and redox properties [36]. Moreover, cobalt porphyrins have been previously interfaced with TCOs and other semiconductors [37–39]. The immobilization strategy for binding cyt c to ATO takes advantage of the electrostatic interaction between substantial negative charges on the metal oxide surface and the protein's positively charged surface, mediated by lysine residues [40,41].



Figure 1. Crystal structure of Co-cyt *c* active site (PDB 1LFM). Highlighted residues are His/Met biaxial ligands.

In this work, we used a mesoporous ATO- cyt *c* assembly to develop a catalytic system for photoelectrochemical water oxidation by swapping the iron within the heme active site for cobalt following a transmetalation strategy. We show that the system exhibits a maximum TON of 671 at pH 5.0 with a faradaic efficiency of 97% in O₂ generation. Increased photocurrent density relative to Co-protoporphyrin IX (Co-PPIX) suggests the following: (i) the protein scaffold facilitates absorption to the ATO surface; and (ii) the protein scaffold provides a protecting environment on the active site by slowing down the degradation processes.

2. Results and Discussion

Co-cyt *c* was obtained by removing iron from WT cyt c via reductive demetallation and the insertion of cobalt by treatment with cobalt acetate [42–44]. The absorption spectrum of the resulting product is consistent with six-coordinated Co (III) porphyrin. Co-cyt *c* was then deposited on highly mesoporous ATO-coated FTO by soaking the ATO electrode in 50 μ M solution of the protein in 50 mM tris pH 7.0, overnight. As shown by UV-vis spectroscopy, the position of the Soret and Q bands remains unchanged upon grafting in the mesoporous ATO (Figure 2), indicating that Co-cyt *c* retains its native structure within the pores.



Figure 2. UV-Vis absorption spectra of Co-cyt c (3.2 μ M) in 100 mM tris, pH 7.5 (red), and Co-cyt c absorbed on ATO coated slide in air (blue). The absorption spectrum of ATO Co-cyt c was multiplied by 3 to improve the visuality. The Soret band maxima at 421 nm for both Co-cyt c in solution and on ATO represents the six-coordinated Co (III)-porphyrin.

We assessed the integrity of Co-cyt *c* when absorbed within the pores of ATO by ATR-FTIR spectroscopy. The most intense bands in the IR spectra of proteins, amide I (1600–1700 cm⁻¹) and amide II (1480–1575 cm⁻¹), are sensitive to changes in secondary structure [45]. The comparison of the spectra of Co-cyt *c* in solution vs. the ATO-absorbed form reveals a similar prominent band 1640 cm⁻¹ from amide I (Figure 3). These results indicate facile binding of Co-cyt *c* to the ATO, without compromising protein folding [36]. In our conditions (pH 5–7.5), cyt c (isoelectric point of 9.6) is positively charged and thus can be adsorbed on the negatively charged surface of ATO via electrostatic attraction [41].



Figure 3. FT-IR spectra of blank ATO (gray), ATO with incorporated Co-cyt *c* (black), and Co-cyt *c* in solution (dotted blue). The spectrum of Co-cyt *c* in solution was taken in 100 mM tris pH 7.5 in liquid IR cell. The spectra for solid samples were obtained directly on the diamond ATR. The IR spectra of ATO-Co-cyt *c* and Co-cyt *c* show the characteristic band for amide I at 1640 cm⁻¹.

The electrochemical activity of ATO-incorporated Co-cyt *c* was investigated by cyclic voltammetry, revealing a quasi-reversible peak centered at -0.39 V vs. RHE, consistent with the Co ^(III/II) redox couple (Figure S1A). Peak separation of 250 mV indicates a relatively slow interfacial electron transfer kinetics. The reduction potential of Co-cyt *c* in solution was measured to be -0.48 V vs. RHE for the Co ^(III/II) redox couple (Figure S1C); the similarity between Co-cyt *c*'s redox potential in solution and immobilized on ATO suggests that the protein is in a native conformation.

The linear dependence of the peak current density vs. the scan rate, at scan rates from 40 to 400 mV·s⁻¹ (Figure S2), reflects the expected behavior for surface-immobilized species and indicates the strong binding of Co-cyt c to the ATO surface; in contrast, the absorption of cyt c on ITO surface results in diffusion-limited behavior, as previously reported [26]. Based on the area under the CV wave of Co^(III/II) redox couple, we estimated an electrochemically active surface coverage of 2.2 \pm 0.5 nmol·cm⁻², which was in a good agreement with previous reports [36]. The total amount of cobalt deposited on the surface was assessed by ICP-MS and corresponded to the average surface coverage of 5.45 ± 0.48 nmol·cm⁻², indicating that approximately 40% of Co-cyt c in the ATO-Co-cyt *c* assembly was electroactive. The high loading of Co-cyt *c* in its electroactive form is in agreement with previously reported studies on cyt c [26]. To build a photoelectrochemical cell (PEC), a three-electrode configuration system was used, consisting of ATO-Co-cyt c as the working photoelectrode, Pt wire as the counter electrode, and SCE as the reference electrode, under a visible light irradiation source ($\lambda > 450$ nm). The electrodes were placed in a glass cell, which was sealed with rubber septa and equipped with inlet and outlet syringes for degassing purge system.

First, we optimized the pH, at which the photoelectrocatalytic studies with ATO-Cocyt *c* should be performed, within the pH range, where our protein is stable (5-9) [40,46]. The ability of the ATO-Co-cyt *c* photoanode to generate a photocurrent upon irradiation with a blue light is highest at pH 5 in citrate buffer, resulting in a photocurrent of 55 μ A·cm⁻² when 0.25 V of potential vs. RHE was applied (Figure 4). The positive photocurrent is indicative of electron flow of the electrons from the ATO-Co-cyt *c* photoanode to the Pt counter electrode, followed by water oxidation by oxidized cobalt in ATO-Co-cyt c. Notably, this photocurrent remained stable during 30 s light illumination. The photocurrent observed decreased drastically to 11.6 μ A·cm⁻² upon decreasing the pH to 5.8, and values of less than 5 μ A·cm⁻² were recorded at pH values from 8.9 to 6.4. As shown in Figure 1, Co-PPIX in cyt c is axially coordinated to a histidine and a methionine. The increase in the generated photocurrent from 11.6 μ A·cm⁻² at pH 5.8 to 48 μ A·cm⁻² at pH 5.0 indicates higher activity of the catalyst, most likely due to the de-coordination of the axial histidine upon protonation, resulting in an open coordination site for the water molecule. Conversely, we can rule out leaching of the Co-PPIX prosthetic group because of its covalent linkage to the protein scaffold. Leaching of the protein is also unlikely in this pH range because the pI of cyt c is 9.6. The data suggest that protonation and de-coordination of the axial histidine are involved in the catalysis, which have been previously postulated with other systems bearing pendant bases [5,46]. A similar pendantbase assisted water oxidation mechanism has been observed with a "xanthene-hangman" corrole complex ([Co^{III}(bpfxc)], bpfxc = 5, 15-bis-(pentafluorophenyl)-10-(2,7-di-tert-butyl-5-carboxy-9,9-dimethyl-9H-xanthene-4-yl)corrole) that is active for electrocatalytic WO when immobilized in Nafion films, with a TOF close to 0.8 s^{-1} at 1.6 V vs. SHE applied potential (pH = 7) [22]. In this study, the authors argued that the hanging moiety assists in the intramolecular proton transfers by preorganizing the water molecule within the system and has a decisive role for water oxidation. Because the water oxidation activity of Co-cyt c is highest at pH 5.0, we selected citrate buffer at this pH for further studies.





Light/dark photoelectrocatalytic experiments were performed to test the longevity and stability of the system. Upon blue light illumination, the ATO-Co-cyt *c* photoanode generates an averaged photocurrent of 51 μ A·cm⁻² at 0.25 V vs. RHE applied potential (Figure 5). The photocurrent remains stable over 30 s light-on intervals and subsequently decays to zero when the light is off. Moreover, the photocurrent was maintained over 400 s of light-on/off cycles.



Figure 5. Light on/off photocurrent measurement for ATO-Co-cyt *c*. The data were collected in 100 mM citrate buffer, 1 M KNO₃, pH 5.0. The applied potential bias was 0.25 V vs. RHE. The irradiation was performed with a blue light for 30 s at each cycle.

Increasing the external bias (0.5 V vs. RHE) in the same conditions did not result in higher photocurrent (Figure S4). Bare ATO shows negligible (2 μ A·cm⁻²) photocurrent density upon blue light irradiation at 0.25 V vs. RHE, suggesting ATO-Co-cyt *c* as the active catalyst.

In order to compare the water oxidation activity of Co-cyt *c* with Co-PPIX, ATO was incubated in a 50 μ M solution of Co-PPIX in methanol, overnight. The photocurrent density of ATO-Co-PPIX in citrate buffer was measured and a maximum of 9 μ A·cm⁻² was observed, while ATO-Co-cyt *c* shows a much stronger signal at the same concentration used for the incubation (Figure 6). To garner more information on the increased activity observed for the protein, we compared the photoelectrocatalytic activity of Co-cyt *c* and Co-PPIX in solution. We measured a generated photocurrent of 8 μ A·cm⁻² for Co-cyt *c*, while only 2 μ A·cm⁻² was obtained from Co-PPIX at identical 5 μ M concentration.





To investigate whether the difference in photoelectron oxidation reactivity observed in solution could be attributed to the differences in interaction with the electrode, we assessed the diffusion coefficients of Co-PPIX and Co-cyt *c* in solution using the Randles– Sevcik equation,

$$\mathbf{i}_{\mathrm{p}} = 0.446\mathrm{nFAC}^0 \left(\frac{nF\nu D_0}{RT}\right)^{1/2} \tag{1}$$

where *n* is the number of transferred electrons, A (cm²) the electrode surface area, C⁰ (mol·cm⁻³) the analyte bulk concentration, and D_0 (cm²·s⁻¹) the diffusion coefficient of the analyte. This equation describes the peak current i_p (A) dependence on the square root of scan rate v (V·s⁻¹) for electrochemically reversible electron transfer processes [47].

The current produced at different scan rates was studied for Co-cyt *c* and Co-PPIX in solution at the same concentration (5 μ M) using a three-electrode configuration, where a 0.4 cm² glassy carbon electrode was used as a working electrode; a SEC as a reference electrode; a Pt wire was used as a counter electrode in 100 mM citrate buffer pH 5.0 and under inert atmosphere; and the current vs. square root of the scan rate was plotted (Figures S5 and S6). The calculated diffusion coefficient from these plots is 1.68×10^{-9} and 2.31×10^{-9} cm²·s⁻¹ for Co-cyt *c* and Co-PPIX, respectively. The diffusion coefficient is higher for Co-PPIX, ruling out this factor as a driver for the higher photocurrent observed for Co-cyt *c* in solution and suggesting that the protein environment has a direct effect on enhancing the porphyrin's water oxidation photoelectrocatalytic activity, in line with previous reports for protein-embedded hydrogen production catalysts [48].

Bulk electrolysis for ATO-Co-cyt *c* photoanode was conducted in a gas-tight photoelectrochemical cell with a three-electrode configuration (Figure S7) in 100 mM citrate buffer at pH 5.0 (1 M KNO₃ supporting electrolyte) with a 0.25 V vs. RHE applied bias. Stable photocurrent was obtained under blue light irradiation with blue illumination of the photoanode over 1000 s (Figure 7). The evolution of oxygen and hydrogen was confirmed and measured with gas chromatography (GC-TCD) by taking a sample of the photoelectrochemical cell's headspace at the end of the photo-electrolysis. 1.9 μ mol H₂ and 1.1 μ mol O₂ was produced, and a TON of 671 was calculated for O₂ generation.

Comparison of the charge passed through the system during the illumination with the amount of generated O_2 gives the faradaic efficiency of 97% for oxygen (Supplementary Materials, for more information). The incident photo-to-current efficiency (IPCE) of the system has also been measured using actinometry (see Supplementary Materials for further details). Using a light source with a wavelength at 450 nm, the calculated IPCE was 4.1%.



Figure 7. Bulk electrolysis measurement with a 0.25 V vs. RHE with ATO-Co-cyt *c* as the working electrode in 100 mM citrate buffer pH 5.0; illumination was performed with a blue light source.

3. Materials and Methods

3.1. Preparation of ATO Film on FTO Glass

A previously established sol–gel route for producing highly mesoporous ATO coatings was adapted for the fabrication of macroporous ATO coatings [26,38,40,45,46]. Briefly, a clear solution with 1.00 g of SnCl₄·5H₂O (Alfa Aesar, 98%) (Haverhill, MA, USA) and 0.05 g of SbCl₃ (Alfa Aesar, \geq 99.9%) in 6.50 g of n-BuOH and 1.0 g of deionized water was prepared and cooled to about 4 °C in ice bath, and then transferred into an Ultra-Turrax tube drive with a rotor stator element. A separate mixture containing 12.4 wt % carbon black, acetylene (Alfa Aesar, 50% compressed \geq 99.9%, surface area = 75 m²/g, bulk density of 0.095–0.103 g/cm³), 25.8 wt % polyethylene glycol bisphenol A, epichlorohydrin copolymer (PEG) (Sigma-Aldrich (St. Louis, MO, USA), 15–20 kDa), and 61.8 wt % \pm epichlorohydrin (Fluka (Charlotte, NC, USA), \geq 99.9%) was homogeneously blended in a separate bottle. From this mixture, 6.15 g was transferred to the metal salt solution in the tube drive. The tube drive was run on speed 3 for 10 s, followed by vigorous shaking. Subsequently, the mixing was carried out with a setting of 6, for 1 min, followed by vigorous shaking, and finally, the tube drive run on the maximum setting, for 1 min, to produce the reaction mixture.

For production of coatings, fluorine-doped tin oxide (FTO, Hartford Glass (Mishawaka, IN, USA), sheet resistance 8 Ω /sq) was washed with soapy water and sonicated in an acetone bath. Two opposing edges were masked by applying double-thick transparent tape (Scotch, St. Paul, MN, USA) to cover 1 mm of the FTO face, and a single layer of tape was applied to a third edge. About 0.25 mL of the reaction mixture was pipetted on the single tape layer and was drawn smoothly and quickly across the entire slide length with a Pasteur pipet. After drying for 3 h under ambient conditions, the tubes were placed in a tube furnace that was flushed with oxygen for 1 h, before increasing the temperature to 500 °C. The initially black coatings were calcined at 500 °C for 5 h under an oxygen flow to yield translucent, pale blue coatings.

3.2. Metal Substitution Procedure

10 mg lyophilized cyt c was dissolved in 10 mL of glacial acetic acid and 500 μ L of concentrated HCl was added to the solution (final pH = 2.2). Working in the dark, the solution was added to a Schlenk flask, and oxygen was removed via 3 freeze-pump-thaw cycles. 100 mg of FeCl₂ was added and the solution was refluxed under argon for 8 h until its color changed from red to purple, indicating a successful demetallation [43]. The acid was removed under vacuum and 2 mL of water was added and evaporated. The resulting powder was dissolved in 2.5 mL of 50 mM sodium acetate, 100 mM EDTA, and desalted with Sephadex G-25 PD10 desalting columns [44].

3.3. Cobalt Insertion

40 mg cobalt acetate was dissolved in 2 mL methanol and added to the protein. The solution was degassed with argon and refluxed for 2 h [42]. Completion of the reaction was detected with the Soret band at 421 nm. The reaction mixture was then purified with RP-HPLC C18 semi-Preparative column (Agilent Technologies, Santa Clara, CA, USA).

3.4. Incorporation of Co-cyt C into ATO Films

ATO-coated FTO slides were dried by heating to 180 °C, for 30 min, and cooled to room temperature prior to immersion in the protein solution. The binding of Co-cyt *c* to the ATO pores was accomplished by incubating the ATO-coated slides in a solution of 50 μ M Co-cyt *c*, overnight. The slides were then rinsed with water to remove the physisorbed species and were dried for further experiments.

3.5. Photocurrent Measurements and Cyclic Voltammetry

Photoelectrochemical measurements and Cyclic Voltammetry (CV) were performed on a CH-Instruments (Austin, TX, USA) model electrochemical workstation with a threeelectrode configuration containing a SCE reference electrode, a Pt counter electrode, and ATO-Co-cyt working electrode. All scans were performed at 100 mV/s unless otherwise mentioned. ATO-Co-cyt *c* slides were illuminated using a Kessil (Richmond, CA, USA) blue lamp.

3.6. ICP-MS

To prepare the ICP-MS samples, Co-cyt *c* functionalized ATO slides were immersed into 10 mL of concentrated OmniTrace sulfuric acid and heated at 60 °C, for 20 min. The solution was then sonicated for 1 h and diluted for the measurement. Three samples of Co-cyt *c* functionalized ATO were prepared, as well as a blank ATO as the control. Data were collected from a Thermo-Finnigan Neptune ICP-MS (Thermo Fischer Scientific, Waltham, MA, USA). The reported data are the average of at least 3 replicates.

3.7. Photoelectrochemical Water Splitting

All photoelectrochemical water splitting experiments were performed in a threeelectrode configuration, as mentioned before. The electrolyte was 100 mM citrate buffer, 1 M KNO₃ pH 5.0, and were purged with argon for 1 h prior to each test. The evolved hydrogen and oxygen were measured by taking 150 μ L of the cell's headspace with a gas tight syringe and injected on a GC (SRI instruments (Torrance, CA, USA), Model no. 310C) using a 5Å molecular sieve column, a TCD detector, and argon carrier gas. The GC calibration was performed using a gas standard (1% H₂ and bulk N₂). The reported data are the average of at least 3 replicates.

3.8. FTIR

FTIR spectra were recorded on a Bruker instrument (Billerica, MA, USA) by placing the ATO-Co-cyt *c* on a diamond ATR for surface measurements and putting a drop of Co-cyt *c* solution in a liquid cell for in-solution measurements.

4. Conclusions

In conclusion, we have prepared a new semiartificial photoanode for light-driven water oxidation based on a Co-cyt *c* supported on highly mesoporous ATO under photoelectrochemical conditions. We found that the incorporation of Co-cyt *c* onto ATO results in a 5-fold increase in the photoelectrochemical water oxidation to oxygen regarding the free Co-cyt *c* as well as free or ATO-supported Co-PPIX. These results suggest an increase in the stability of the Co-cyt *c* catalytic system upon immobilization, as compared with the immobilized Co-PPIX. Nevertheless, further studies may provide a more detailed picture of the mechanism of action.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10 .3390/catal11050626/s1, Figure S1: Cyclic voltammogram of (A) ATO-Co-cyt c, in 100 mM citrate buffer, 1M KNO₃, pH 5.0 at scan rate of 100 mV·S⁻¹, the redox wave at -0.34 V vs SCE (-0.39V vs RHE) is assigned to Co (III) to Co(II); (B) blank ATO in the same buffer and scan rate; and (C) Co-cyt c in solution in the same buffer and scan rate, the redox wave at -0.43 V vs SCE (-0.48 V vs RHE) is assigned to Co (III) to Co(II), Figure S2: Anodic peak current dependence on scan rate for ATO-Co-cyt c, in 100 mM citrate buffer, 1M KNO₃, pH 5.0 at the following scan rates: 40, 100, 150, 200, 250, 300, 350 and 400 mV s⁻¹, Figure S3: Linear sweep voltammograms of the working electrode ATO-Co-cyt c, in the dark (orange line) and under light illumination (blue line) in 100 mM citrate buffer at pH 5.0, Figure S4: Chronoamperometry behavior of ATO-Co-cyt c at 0.5 (blue) and 0.25 V (red) vs RHE applied bias in 100 mM citrate buffer at pH 5.0, Figure S5: (A) Cyclic voltammograms of $5 \ \mu M$ Co-PPIX in 100 mM citrate buffer, 1M KNO₃, pH 5.0, at varying scan rates: 50, 80, 100, 200, 250 and $300 \text{ mV} \cdot \text{s}^{-1}$, respectively. (B) The Ipc vs square root of scan rate obtained from the data in (A) was used to calculate the diffusion coefficient, Figure S6: (A) Cyclic voltammograms of 5 µM Co-cyt c in 100 mM citrate buffer, 1 M KNO₃, pH 5.0, at varying scan rates: 50, 80, 100, 150, 200, 250, 300, 350, 400, 450 and 500 mV s⁻¹, respectively. (B) The Ipc vs square root of scan rate obtained from A was used to calculate the diffusion coefficient, Figure S7: Evolution of gas bubbles on the ATO-CocytC working electrode and Pt counter electrode upon illumination.

Author Contributions: C.C., G.G., and D.-K.S. designed the experiments. C.C. and D.J.S. performed the synthesis and experiments. H.Z., S.C., and D.J.S. prepared the ATO Film on FTO Glass. All authors participated in data analysis. C.C. and G.G. wrote the paper with contributions from all authors. All authors have read and agreed to the published version of the manuscript.

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