Protein Secondary-shell Interactions Enhance the Photoinduced Hydrogen Production of Cobalt Protoporphyrin IX

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Supplemental Information

Materials

All chemicals were purchased from commercial suppliers and used without further purification. UV-Vis spectra were acquired on a Varian Cary 50 Bio Spectrophotometer. Gas chromatography was carried out on a SRI instruments, Model no. 310C GC using a 5Å molecular sieve column with a thermal conductivity detector and argon carrier gas.

Point mutants were prepared by the method of Gibson *et al*. and were confirmed by direct sequencing of the PUC19 vector harboring the insert.[1] CoMyoglobin was prepared following previously reported procedures.[2] Briefly, BL21 *E. coli* harboring the pMB413a vector, purchased from Addgene, were induced with 100 μM IPTG at an OD_{600} of 0.6 and grown for 5 hours. Harvested cells were lysed by continuous sonication on ice. Clarified lysate was brought to 60% saturation with Ammonium Sulfate and the precipitated proteins discarded. The solution was then brought to 95% saturation, in which myoglobin precipitated. Resuspended proteins were ran over a 1 m, Sephadex G50 Size Exclusion Column, followed by batch bound to DEAE cellulose. Unbound myoglobin was concentrated and heme was removed using the acid:acetone method.[2a] CoPP(IX), 10 molar excess, was dissolved in 90% water, 10% pyridine, and added slowly to a solution of apomyoglobin. After 1 hour of incubation, excess porphyrin was removed through desalting on a GE healthcare PD10 column. Protein concentration was calculated using the extinction coefficient of 170,000 M^{-1} cm^{-1} at the soret.

Electrochemical Methods

Electrochemical experiments were carried out using a CH-instruments 1242B potentiostat. For all electrochemical measurements, a three-electrode system was used. The electrodes used were a 3-mm

diameter glassy carbon working electrode with a surface area of 0.28 cm^2, platinum mesh counter electrode, and a saturate calomel reference electrode. All potentials were normalized to SHE by the addition of 240 mV to the SCE values. All electrolyte solutions were degassed by incubation in a Coy anaerobic chamber for 2 days prior to use. Working electrodes were polished with 1 μm alumina for 5 minutes, followed by 10 minutes of sonication, prior to use.

Photoinduced H_2 Production

Irradiation was performed using a 450W xenon lamp with a 400 nm cutoff filter, irradiating at a constant 1100 W/m^2 throughout the experiment. For each experiment, 1 mM $Ru(Bpy)_3^{2+}$, 100 mM sodium ascorbate, and the desired catalyst were added to a 1 M potassium phosphate buffer at the appropriate pH. The 400 μL total reaction volume was added to a custom made airtight 1 mm cuvette and degassed extensively with argon prior to illumination. During irradiation time course 100 μL samples of the headspace were removed with a gas-tight syringe and injected directly for analysis by GC. Calibration was achieved by injection of various volumes of a 1% H_2, 99% N_2 gas mixture onto the GC.

Photophysical Parameters

Quantum efficiency was calculated starting from the irradiance spectra of the 450W Xenon Arc lamp. Individual light wavelengths were converted to photon energy through the use of SI equation 1.

$$E = \frac{hc}{\lambda}$$

Each wavelength was then converted to photons irradiated for each experiment following SI equation 2:

$$\frac{Photons\,Irradiated}{nm} = \left(\frac{\frac{W}{m^2}\,Xenon\,Arc}{Photon\,Energy} \right) (Experiment\ Time)\,(Cuvette\ Area)$$

Integration under the curve from 400-550 nm ($Ru(Bpy)_3^{2+}$ absorbance bands) resulted in total photons available for photoexcitation during the experiment time course. Quantum efficiency was then calculated by SI Equation 3:

$$Quantum\ Efficiency = \left(\frac{2 * mol\ H_2\ Evolved}{mol\ Photon\ Irradiated} \right) * 100\%$$

Utilizing the above equations, quantum efficiency for 2.5 μM the H64/97A mutant was calculated to be 0.0035 %.

Supplemental Figures:

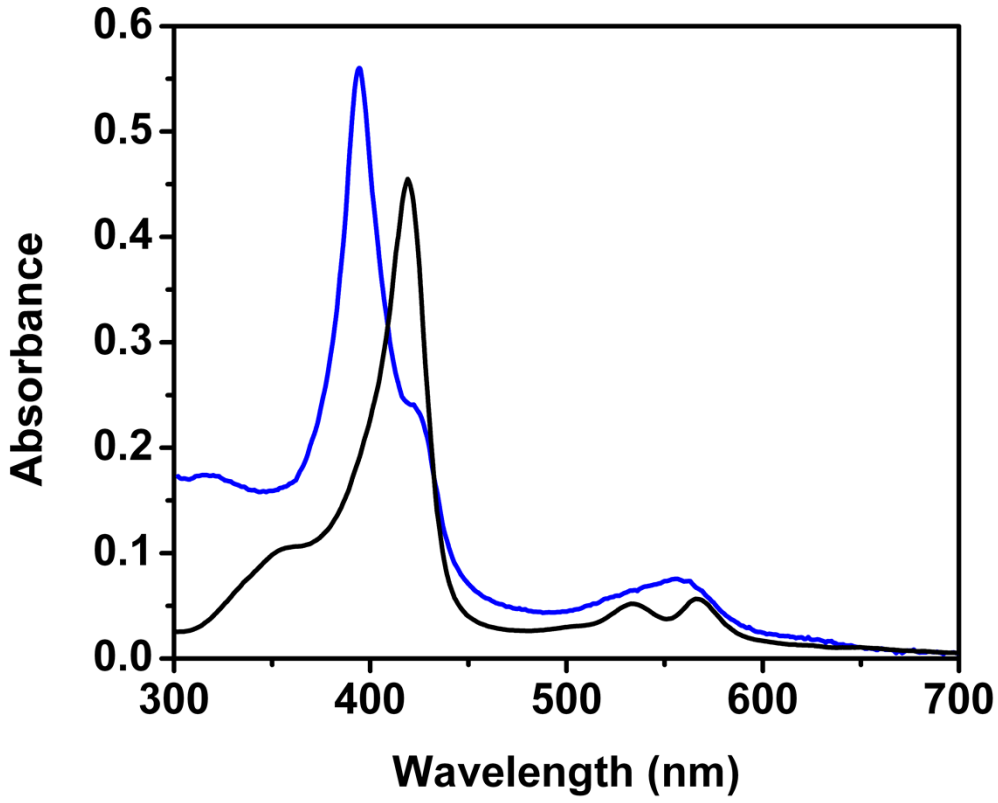

Figure S1: UV-Vis traces of 2.6 μM CoMyo in 100 mM KPi pH 7.5 in the oxidized (black) and dithionite reduced (blue) states.

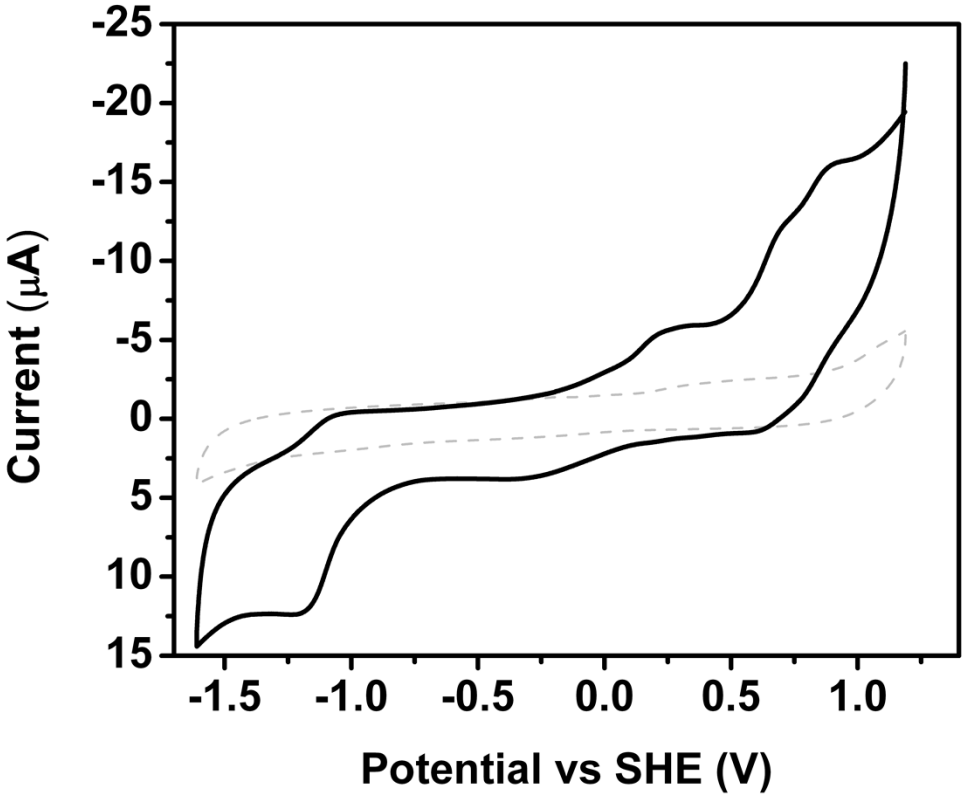

Figure S2: Cyclic Voltammagram of a 1 mM CoPP(IX) solution in MeCN with 0.1 M (n-$Bu_4N)PF_6$ (solid line), compared to a blank scan (dotted line), at 100 mV/s with a glassy carbon working electrode.

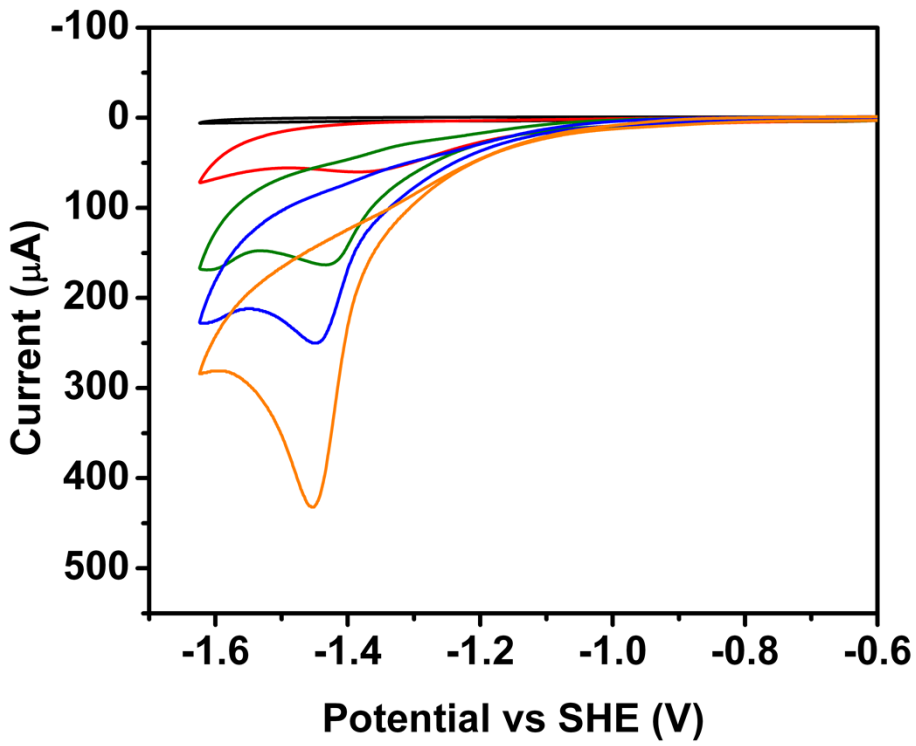

Figure S3: Cyclic voltammograms of 250 μM CoPP(IX) in MeCN with 0.1 M (n-Bu_4N)PF_6 with increasing concentrations of tosic acid. (Black: 0 mM, Red: 1.2 mM, Green: 3.6 mM, Blue: 4.8 mM, Orange: 7 mM) Scan rate was 100 mV/s with a glassy carbon electrode.

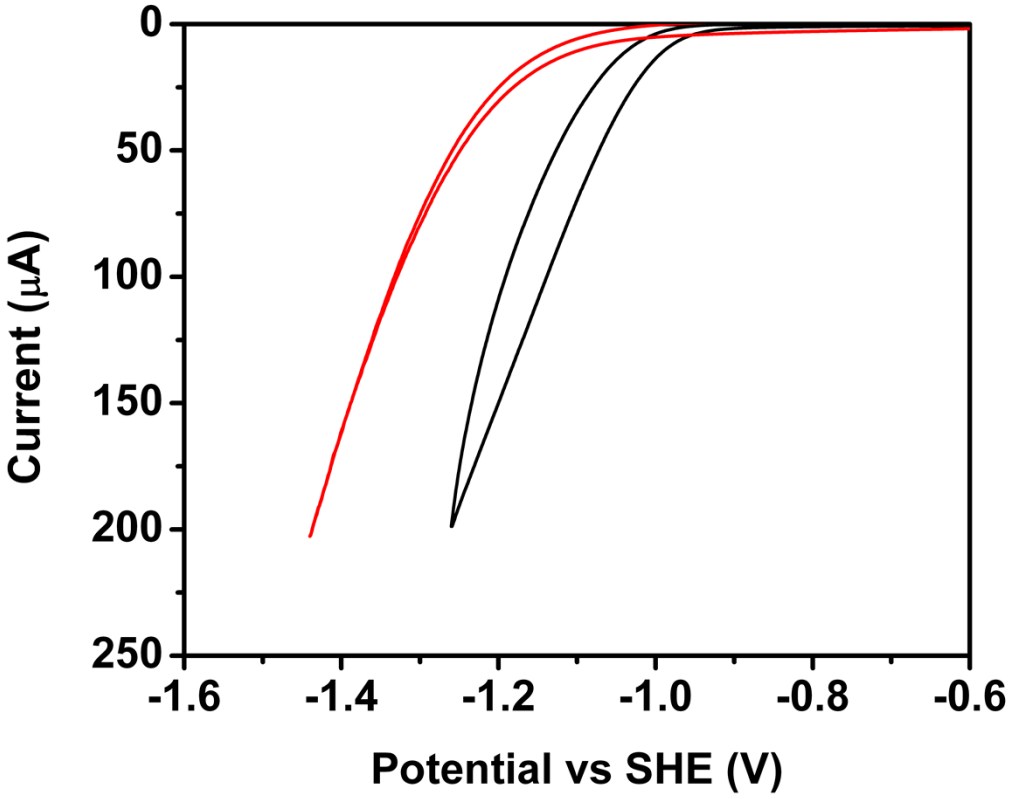

Figure S4: Cyclic Voltammograms of a blank glassy carbon electrode (red) compared to 1.5 μM H64A mutant (black) at pH 7.0 in 100 mM KPi, 200 mM KCl at a scan rate of 100 mV/s.

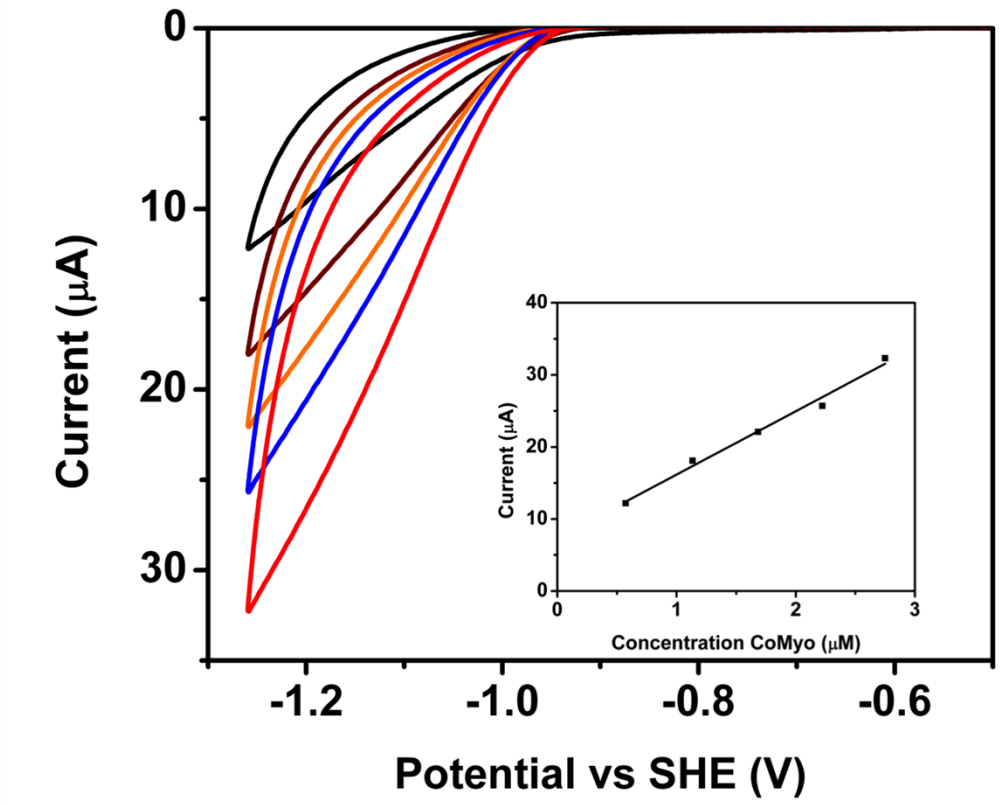

Figure S5: Cyclic voltammograms of WT CoMyo at varied concentrations in 100 mM Tris-HCl, 200 mM NaCl, pH 7.5 at 100 mV/s with a glassy carbon electrode. Inset: Linear fit of current at -1.26 V vs SHE for various concentrations of CoMyo.

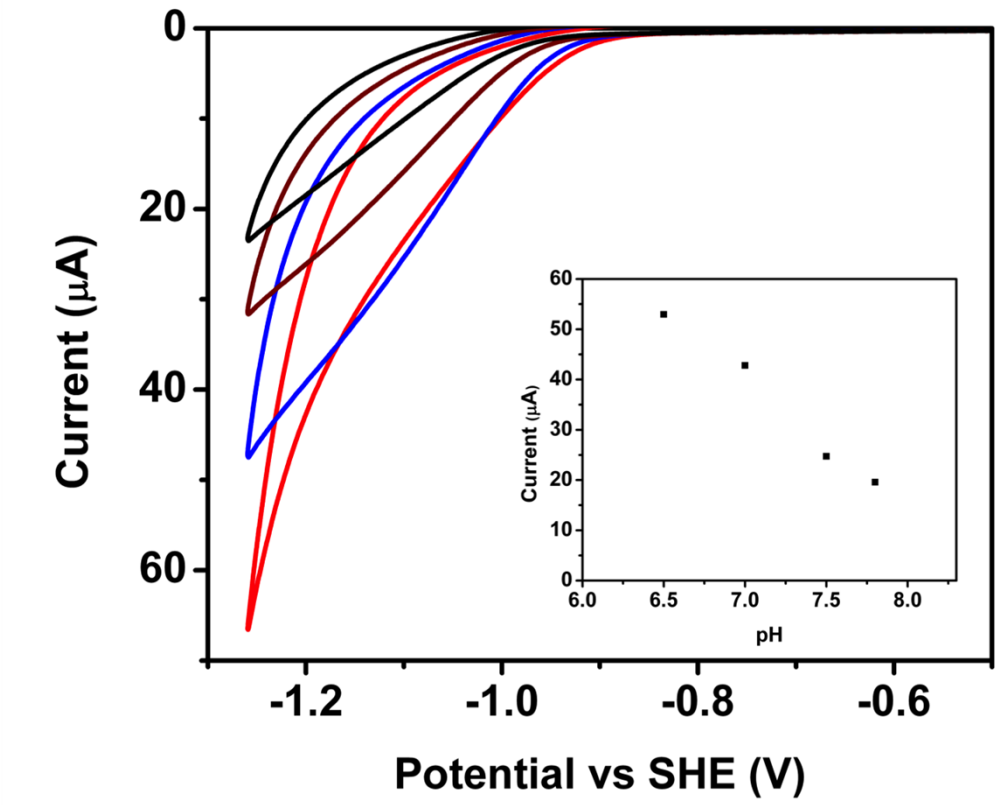

Figure S6: Cyclic voltammograms of 1.6 μM WT CoMyo at varied pH levels of 100 mM Tris-HCl, 200 mM NaCl at 100 mV/s with a glassy carbon electrode. Inset: Current at -1.26 V vs SHE for varied pH levels.

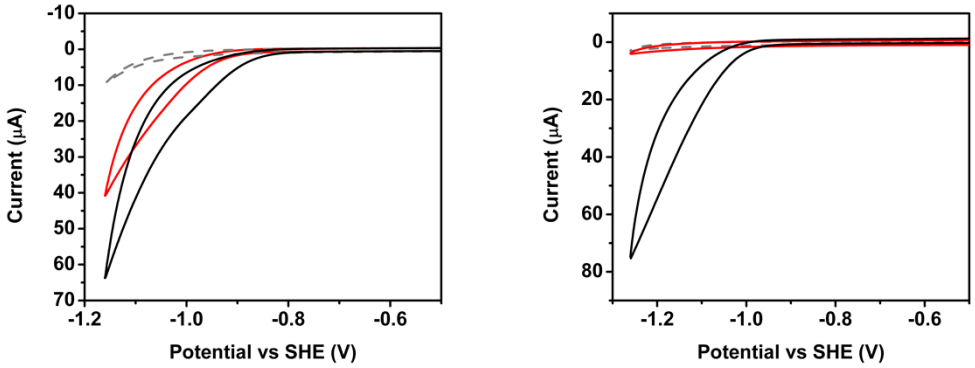

Figure S7: A) Cyclic voltammogram in pH 4.5, 100 mM Citrate, 100 mM NaCl of the blank scan (grey dashed), after addition of 2.6 μM WT CoMyo (black), and after placing rinsed electrode back into fresh buffer (red). B) Cyclic voltammogram in pH 7.5, 100 mM Tris-HCl, 100 mM NaCl of the blank scan (grey dashed), after addition of 5.5 μM WT CoMyo (black), and after placing rinsed electrode back into fresh buffer (red).

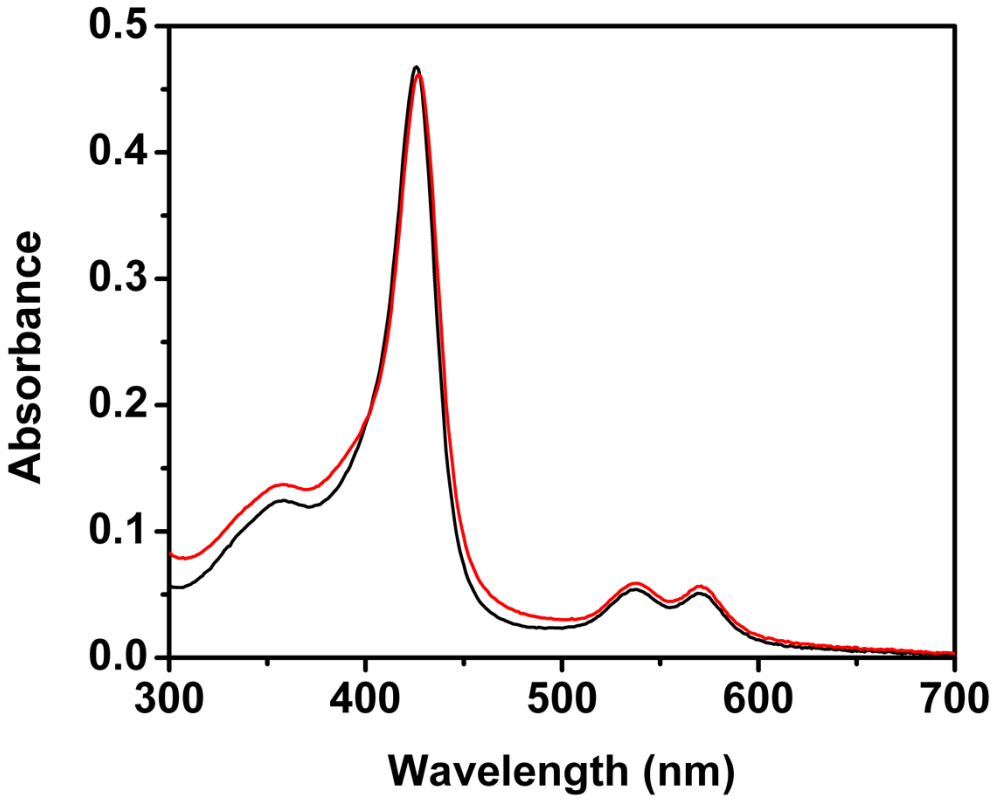

Figure S8: UV-Vis spectra of 2.6 µM WT CoMyo in 100 mM Tris, 100 mM NaCl, pH 7.5 (black trace) and 2.6 µM WT CoMyo in 100 mM Citrate, 100 mM NaCl, pH 4.5.

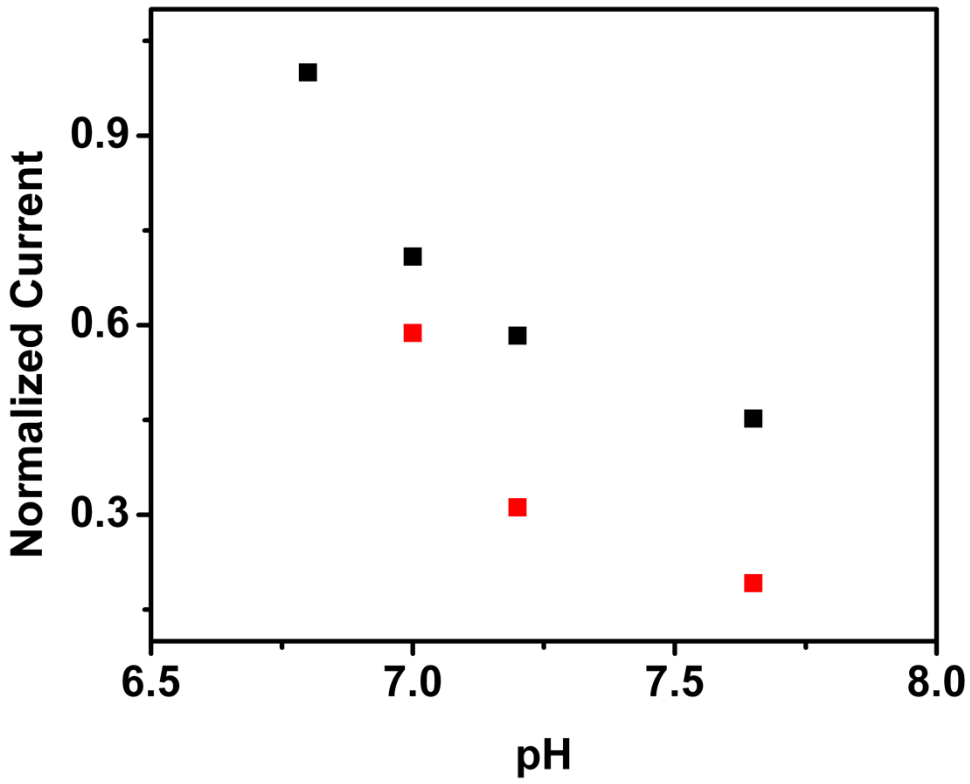

Figure S9: Catalytic current at -1.26 V for WT (black) and H64A (red) CoMyo at various pH levels in 100 mM KPi, 200 mM KCl with a glassy carbon working electrode at 100 mV/s. Data were normalized to current at pH of 6.8 to account for variation in catalytic activity of the two systems.

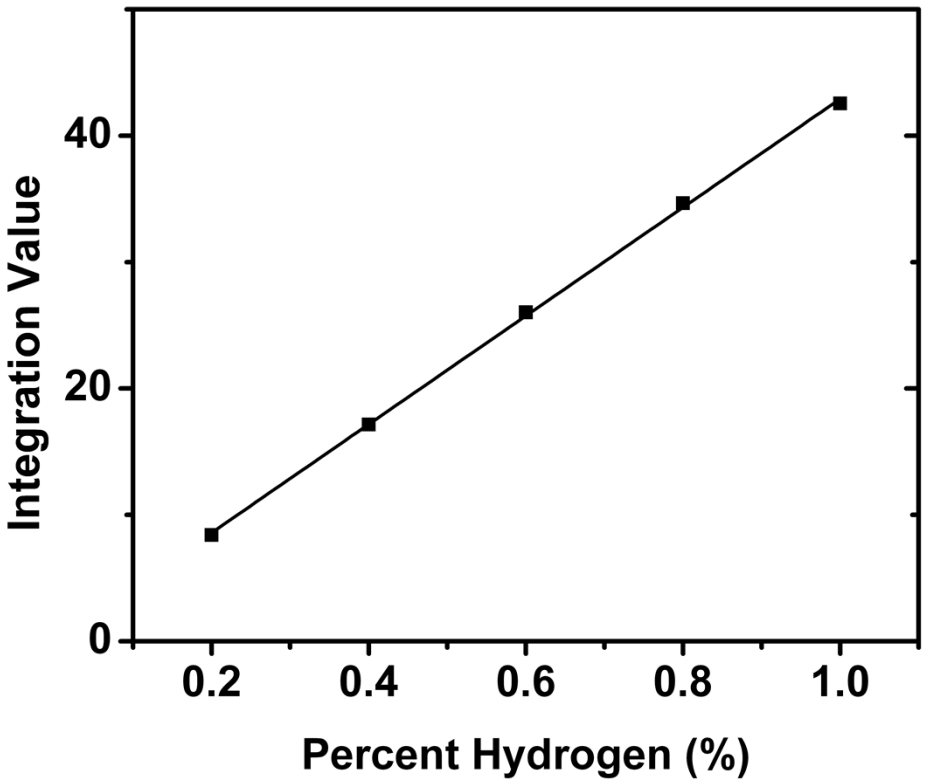

Figure S10: Typical GC standardization curve obtained for photoinduced hydrogen production assays. Data were fit to the line y = 42.90 x + .003

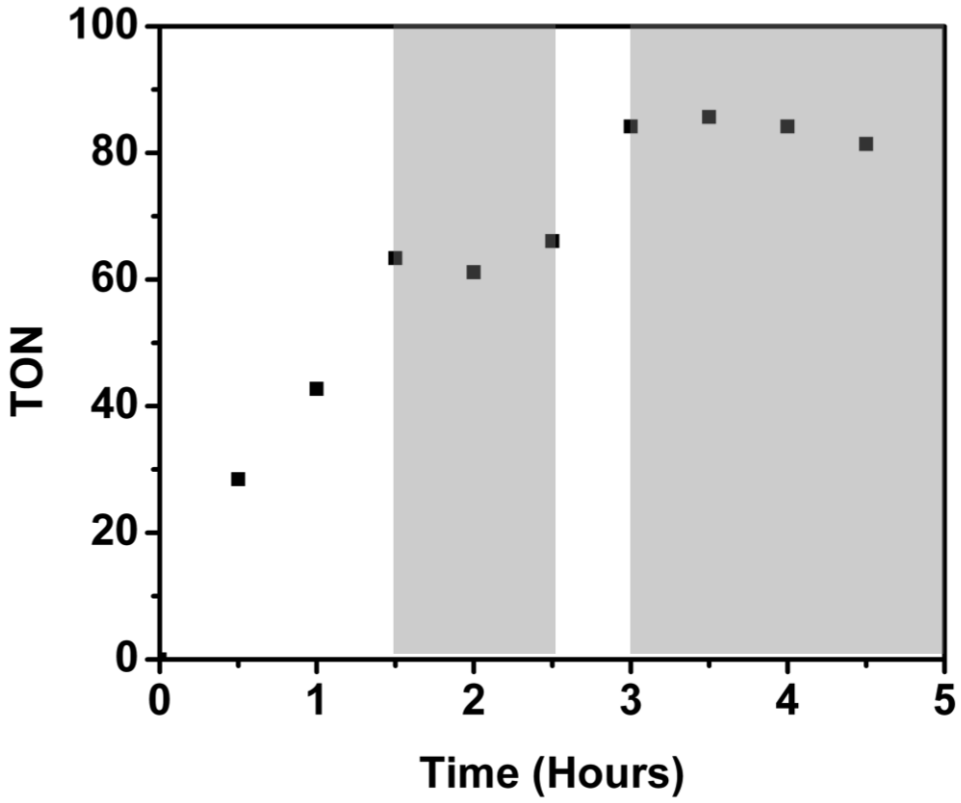

Figure S11: Photoinduced hydrogen production of 2.5 μM WT CoMyo under varied light conditions. White area corresponds to irradiation with 1100 W/m^2, grey corresponds to no irradiation of the sample.

References:

1 D. G. Gibson, H. O. Smith, C. A. Hutchison, 3rd, J. C. Venter and C. Merryman, *Nat. Methods*, 2010, **7**, 901.

2(a) E. A. Brucker, J. S. Olson, G. N. Phillips, Y. Dou and M. Ikeda-Saito, *J. Biol. Chem.*, 1996, **271**, 25419; (b) B. A. Springer and S. G. Sligar, *P. Natl. Acad. Sci. USA*, 1987, **84**, 8961.