Single-Walled Carbon Nanotube/Protein Integrated Systems for Light Harvesting

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Supporting Information

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Part I: Experimental Methods

Preparation of Zn-cyt *c*.: Synthesis of Zn-cyt *c* was performed as described in Ref. [1] with modifications, as follows. Lyophilized horse heart cytochrome *c* (Sigma) (30 mg) was added to a 250-mL bomb flask and dissolved in 4 mL of glacial acetic acid and 0.5 mL of hydrochloric acid. The sample was then degassed and the chamber was backfilled with argon. Under argon flow, approximately 5 mg of $FeCl_2$ were added to a bomb flask wrapped in aluminum foil to minimize light exposure. The flask was then sealed and stirred at ambient temperature for 30 minutes. The solution changed from a deep red to magenta, characteristic of heme demetallation. The acid solution was removed under vacuum, after which 5 mL of water were added to the flask and then removed under vacuum. The dried demetallated cyt *c* (P-cyt *c*) was dissolved in 50 mM sodium acetate pH 4.5 and 100 mM EDTA in water. The solution was exchanged into 5 mL of 50 mM sodium acetate buffer, pH 4.5 using a PD-10 desalting column (GE Life Sciences). A 100-fold molar excess $Zn(O_2CCH_3)_2$ was then added to the solution, which was heated on a heating block at 70 ºC for 15 min, inverting to mix gently once during the reaction. The UV-vis absorption spectra of wild-type cyt *c*, P-cyt *c* and Zn-cyt *c* are shown in Figure S6.

Preparation of SWNT/Zn-cyt *c* suspensions: 25 mg CoMoCAT SWNTs (SouthWest Nanotechnologies, Inc.), 0.25 g sodium cholate and 25 mL of 50 mM HEPES (pH 7.5) were sonicated using a Branson 450 Sonifier with a 1/8" tip at ~15 W for 1 hour. The resulting solution was centrifuged for 1 hour at ~16,000 *g* using a Heraeus Instruments Biofuge Pico tabletop centrifuge. In order to perform optical spectroscopic and spectrophotometric measurements, the top ~75% of the supernatant was removed and used. In order to prepare films for photocurrent measurements, the top 75% of the supernatant was removed and then centrifuged again in a Beckman Coulter Optima ultracentrifuge using an SW-41 Ti rotor at 35,000 RPM for 4 hours at 20°C. The top ~80% of the supernatant was then removed and used in preparation of films.

In order to create aqueous solutions of SWNT:Zn-cyt *c*, small volumes (between 1 and 50 μL) of Zn-cyt *c* stock solutions were combined with SC-suspended SWNTs to spontaneously form SWNT:Zn-cyt *c*.

The concentration of SWNTs was determined by fitting the absorption spectra of the E_{11} peaks for the three major species ((*6*,*5*), (*8*,*3*) and (*7*,*5*)) with Gaussian functions, and determining their relative carbon concentration using published absorption coefficients. The concentration of SWNTs was then calculated by assuming an average length of 400 nm, and determining the number of carbon atoms composing a 400 nm long SWNT of each species.

There were some bundles of SWNTs still visible after centrifugation leading to the scattering seen in the UV-Vis spectra. Control samples of Zn-cyt *c* were also sonicated under the same conditions in the absence of SWNTs and centrifuged in the same way. These solutions showed negligible Zn-cyt *c* concentration changes, confirming that SWNTs will remove Zn-cyt c from solution during centrifugation.

Preparation of Zn-cyt *c*/SWNT films: ITO-coated quartz (50±5nm ITO deposited on 1/16" thick quartz) was cut into ~3 mm x ~3 cm strips using a diamond scribe. Strips were then coated using a layer-by-layer method. Each substrate underwent an initial 10 minute soak in a 1% wt. poly(diallyldimethylammonium chloride) solution to increase adhesion of the subsequent layers to the substrate. 2 mM HEPES buffer (pH 7.5) was used to wash the films between depositions of each layer. 20 layers of SWNT and 20 layers of Zn-cyt *c* solutions were then deposited by alternating 5-minute soaks (1 mg/mL SWNT solution and 5 mg/mL Zn-cyt *c* solution both in 50 mM HEPES and 1 mg/mL sodium cholate (SC), pH 7.5). The backside of the film (non-ITO side) was then removed by gently wiping it away. Films were dried overnight in ambient conditions. Controls were done for both removals of the Zn-cyt *c* as well as of the SWNTs. For removal of the Zn-cyt *c*, a 5% wt. solution of poly-L-lysine in 50 mM HEPES and 1 mg/mL SC, pH 7.5 was used in its place and films were fashioned as above. For removal of the SWNTs, a 5%

wt. solution of poly(acrylic acid) in 50 mM HEPES and 1 mg/mL SC, pH 7.5 was used in its place. Films were fashioned as previously described.

Photocurrent Measurements: Photocurrent and photoaction spectra were measured on films in solutions, pH 7.5, of 50 mM HEPES buffer, 100 mM NaCl, and 10% v/v triethanolamine (TEOA) with TEOA used as a sacrificial electron donor. Measurements were performed in an electrochemical cell with the ITO substrate of the film as the working electrode, platinum wire as the counter electrode, and silver/silver chloride as the reference electrode. To measure photocurrents, 0.9 mW of 421-nm light generated by a Xe lamp passing through a SpectraPro 150 monochromator was used, matching the Soret band λ_{max} of Zn-cyt *c*. Currents were measured using a potentiostat (CH Instruments Electrochemical Analyzer).

Spectroscopic Measurements: Photoluminescence excitation spectroscopy (PLE) measurements were performed on a home-built fluorometer system. A SpectraPro 150 excitation monochromator and a SpectraPro 300i emission monochromator (Acton Research Corp.) were in a right-angle geometry with respect to the sample chamber. Excitation wavelengths from 400-750 nm were scanned in 5-nm steps with a Xe lamp as the light source. Emission wavelengths from 900-1200 nm were scanned in 5-nm steps. SWNT fluorescence was detected using a L-N_2 cooled Ge detector (North Coast Scientific). Absorption spectra were collected on both Lambda 19 and Lambda 950 UV/vis/NIR spectrophotometers (Perkin-Elmer).

Part II: Percent Internal Quantum Efficiency Calculations

The percent internal quantum efficiency (ϕ_{int}) can be determined by the ratio of charge carriers produced per second (n) to the photon flux (q_p).

$$\phi_{int} = \frac{number\ of\ charge\ carriers\ per\ second}{number\ of\ photons\ absorbed\ per\ second} \times 100\% = \frac{n}{q_p} \times 100\%$$

The photon flux (the number photons absorbed per second) was calculated by the following equation with P_{abs} (W) being the power of the light absorbed by the sample, λ being the wavelength of the Xe lamp light source in meters, h being Planck's constant (J-s), and c being the speed of light (m/s).

$$q_p = \frac{P_{abs} \times \lambda}{hc}$$

The power of light absorbed by the sample (P_{abs}) was determined by the relationship between the absorbance of the sample at the excitation wavelength (A_λ), the radiant power of the light entering the sample (P_0), and the radiant power of the light leaving the sample (P_f). Subtracting P_f from P_0 yields P_{abs}.

$$A_\lambda = log_{10}\frac{P_0}{P_f} \qquad P_0 - P_f = P_{abs}$$

The number of charge carriers per second (n) was determined by the relationship between I, being the average photocurrent measured from the film (A), and Q_e, being the magnitude of the charge of an electron (C).

$$n = \frac{average\ photocurrent\ produced\ by\ film}{elementary\ charge} = \frac{I}{Q_e}$$

Sample calculation:

For the film producing the highest amount of photocurrent:
λ = 421 nm
P_0 = 900 μW
A_{421} = 0.127
I = 5.627 nA

$$0.127 = log_{10}\frac{9 \times 10^{-7} W}{P_f}$$

$$P_f = \frac{9 \times 10^{-7} W}{10^{0.127}} = 6.718 \times 10^{-7}\ \text{W} \qquad 9 \times 10^{-7}\ \text{W} - 6.718 \times 10^{-7}\ \text{W} = 2.282 \times 10^{-7}\ \text{W} = P_{abs}$$

Assume 95% transmission through photocurrent cell

$$q_p = \frac{2.282 \times 10^{-7} W \times 421 \times 10^{-9} m}{6.626 \times 10^{-34} J - s \times 3.00 \times 10^{8}\ m/s} = 4.591 \times 10^{11}\ \text{photons/s}$$

$$n = \frac{5.627 \times 10^{-9} A}{1.602 \times 10^{-19} C} = 3.512 \times 10^{10} \text{ electrons/s}$$

$$\phi_{\text{int}} = \frac{3.512 \times 10^{10}\ electrons/s}{4.591 \times 10^{11}\ photons/s} \times 100\% = \mathbf{7.65\%}$$

Part III: Supporting Figures

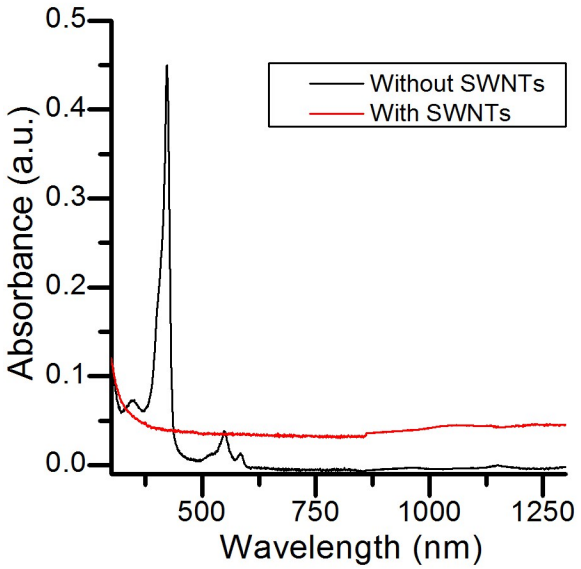

Figure S1: UV-Vis-NIR absorption spectra of Zn-cyt *c* after sonication and centrifugation with (red) and without (black) SWNTs. When SWNTs are present, Zn-cyt *c* is not detected in the supernatant. The background absorbance seen for this sample is due to scattering from protein-SWNT aggregates. Without SWNTs, the Zn-cyt *c* remains in solution after centrifugation.

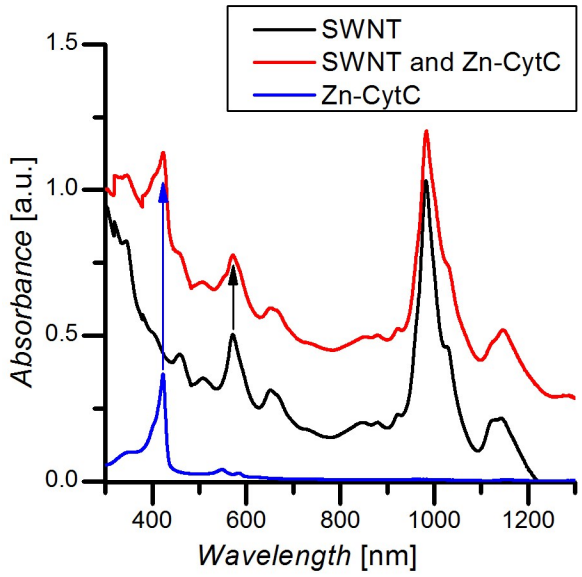

Figure S2: UV-Vis-NIR spectra of Zn-cyt *c* (blue), and SWNT solutions with (red) and without (black) the addition of Zn-cyt *c* to a final concentration of 0.06 mg/mL. The SWNT E_{11} transitions for different SWNT structures can be seen between 900 and 1200 nm while the E_{22} transitions can be seen between 450 and 700 nm. The Zn-cyt *c* Soret band, centered at 422 nm, is seen in the spectrum of the Zn-cyt *c*-SWNT complex. Arrows indicate the relative positions of the E_{22} and Soret transitions upon forming the complex.

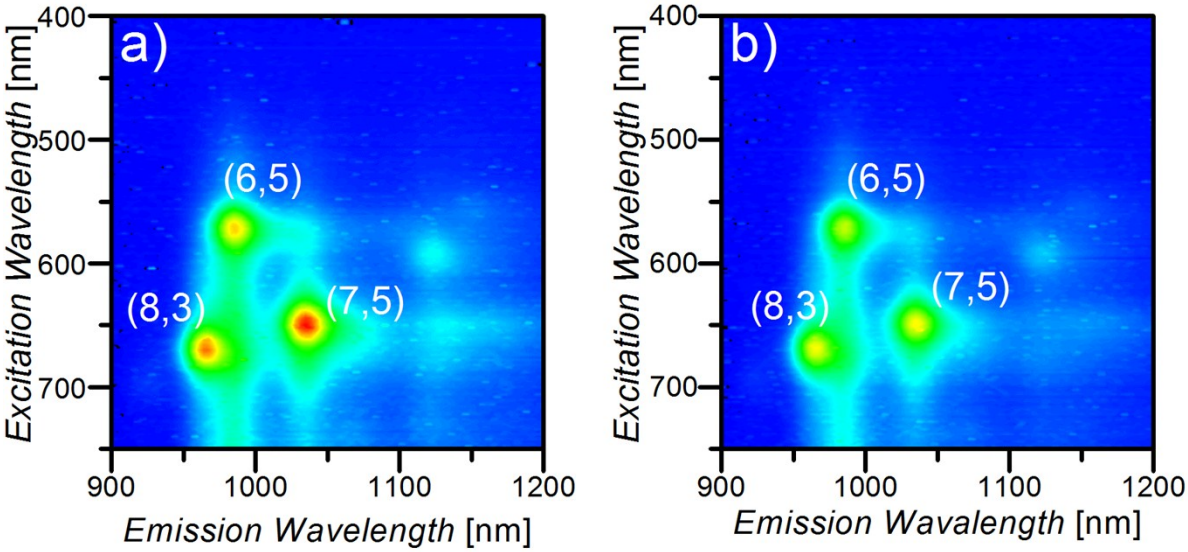

Figure S3: PLE maps of SWNT fluorescence without (a) and with (b) Zn-cyt *c*. PL intensity scales are identical in both maps. The lack of a SWNT fluorescence peak upon excitation of the Zn-cyt *c* (~420 nm) in (b) demonstrates a lack of energy transfer from ZnP to the SWNTs.

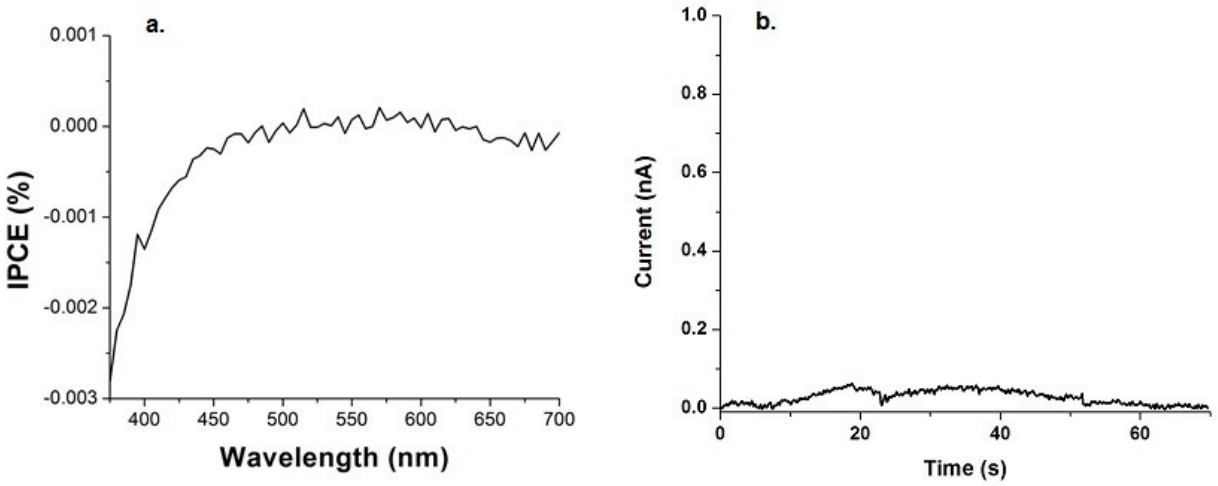

Figure S4: (a) Photoaction spectrum of poly-L-lysine/SWNT films. Small negative IPCE values for the blue wavelengths arise from electrons flowing into the ITO. (b) Photocurrent over time of Zn-cyt *c*/poly (acrylic acid) films. Light is blocked and unblocked every 15 seconds. No appreciable photocurrent can be seen.

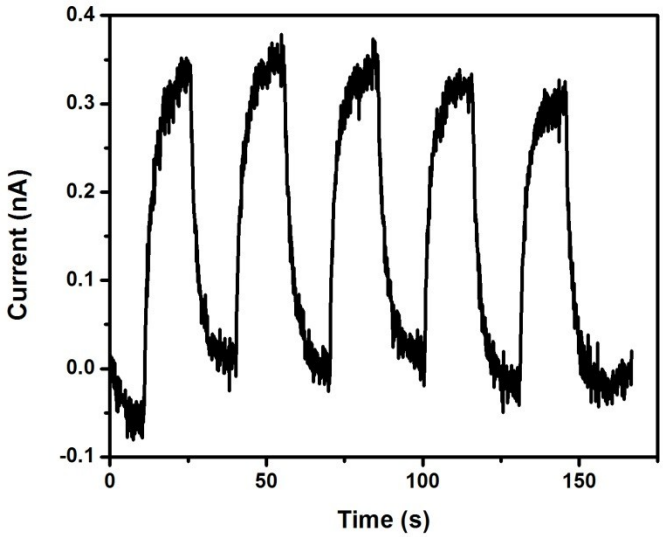

Figure S5: Repeatable photocurrent over time of 10 layer SWNT/Zn-cyt *c* film in 50 mM HEPES buffer, 100 mM NaCl and 10% TEOA with a 0 mV bias generated with 421-nm monochromatic light by manually blocking and unblocking the light source roughly every 15 seconds.

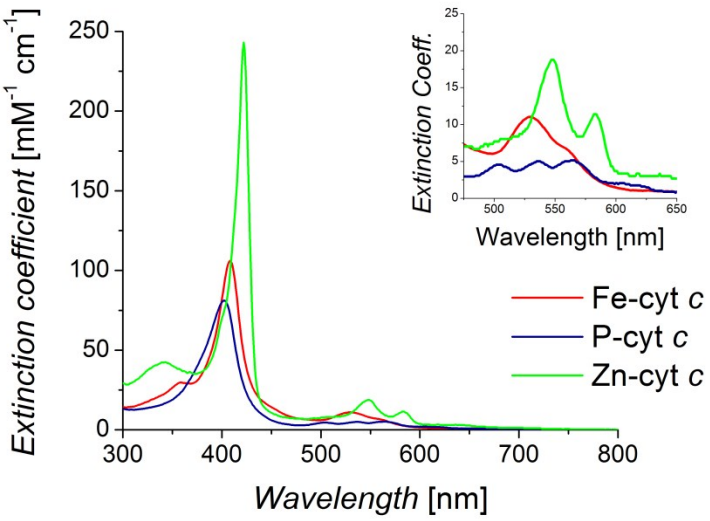

Figure S6: UV-Vis absorption spectrum showing spectral changes of native horse heart cytochrome *c* (green) after reductive demetallation (red) and subsequent zinc insertion (blue). The native protein is present as a mixture of oxidized and reduced forms.

References

[1] Primus, J. L., Boersma, M. G., Mandon, D., Boeren, S., Veeger, C., Weiss, R., and Rietjens, I., "The effect of iron to manganese substitution on microperoxidase-8 catalysed peroxidase and cytochrome P450 type of catalysis," *J. Biol. Inorg. Chem.* **4**, 274-283 (1999).