

Electrostatically arranged cytochrome *c*–fullerene photoelectrodes†Israel Zilbermann,^a Andrew Lin,^a Maria Hatzimarinaki,^b Andreas Hirsch*^b and Dirk M. Guldi*^a^a Radiation Laboratory, University of Notre Dame, Notre Dame, IN 46556, USA. E-mail: guldi.1@nd.edu; Fax: 1 574 631 8068; Tel: 1 574 631 7441^b Institut für Organische Chemie, Universität Erlangen-Nürnberg, Henkestr. 42, 91054 Erlangen, Germany. E-mail: hirsch@organik.uni-erlangen.de; Fax: 0049 9131 85 26864; Tel: 0049 9131 85 22537

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We have developed a molecular-level switch—a C₆₀/cytochrome *c* modified ITO electrode—that reversibly transmits and processes solar energy.

Bioengineering of photovoltaic entities is a rapidly progressing field of interdisciplinary interest, providing important information about the integration of biomolecules into functional systems.¹ The *layer-by-layer* technique emerged as a powerful technique towards functional systems and photoactive electrodes due to a fine-tuned interplay of interactions at the molecular level.² In this work we wish to describe a simple molecular-level switch—a C₆₀/cytochrome *c* modified ITO electrode—that reversibly transmits/processes solar energy.

The strategy involves the use of mitochondrial cytochrome *c* (FeCyt_c), which is a polycationic redox protein. It plays a dominating role as a charge carrier and/or charge mediator complex in numerous biological³ and synthetic systems.⁴ FeCyt_c is then electrostatically linked to a negatively-charged dendritic fullerene (C₆₀DF)—see Scheme 1.⁵ The function of the construct is given by the redox activity of the protein's heme group, that is, the Fe^{III}Cyt_c/Fe^{II}Cyt_c transformation.

Once Fe^{II}Cyt_c/C₆₀DF is deposited onto an ITO electrode, the fullerene constitutes the primary electron acceptor (A₁; C₆₀DF/C₆₀DF^{•-}: -0.70 ± 0.05 V vs. SCE; ¹*(C₆₀DF)/C₆₀DF^{•-}: +1.07 ± 0.05 V vs. SCE); it accepts the electron from the electron donating Fe^{II}Cyt_c redox protein (D; Fe^{II}Cyt_c/Fe^{III}Cyt_c: +0.25 ± 0.05 V vs. SCE) and relays it to the electrode. In the current context the ITO electrode serves as a secondary electron acceptor (A₂: -0.25 V vs. SCE)⁶ that ultimately accumulates and stores the electrons. Scheme 1 underlines the aspect of a fine-tuned redox gradient in the integrated donor–acceptor–acceptor (*i.e.*, D–A₁–A₂) construct.

The stepwise assembly of substrate/C₆₀DF/Fe^{II}Cyt_c includes the following sequence. First, deposition of the negatively-charged dendritic fullerene necessitates a base layer of poly(diallyldimethylammonium) chloride (PDDA), adsorbed onto the hydrophilic substrate.‡ Mainly Coulombic forces, which are pH and ionic strength sensitive, govern the physisorption process between the opposite charged ions of PDDA and C₆₀DF.⁵ Similar principles apply then for implanting a layer of Fe^{III}Cyt_c on top of the C₆₀DF surface. The distinct absorption features of C₆₀DF (*i.e.*, ~260 and

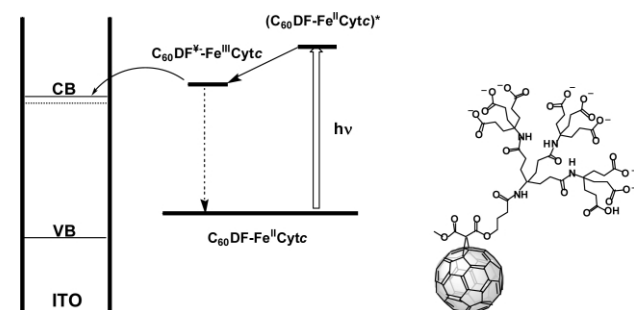
330 nm) and Fe^{III}Cyt_c (*i.e.*, Soret- and Q-bands around 410 nm and 525 nm, respectively) facilitate their convenient detection—see Supporting Information Fig. S1.

After completing each deposition step—PDDA, C₆₀DF, Fe^{III}Cyt_c—the surface structure was analyzed by AFM. While the image of the PDDA base layer can be best described by a fairly flat surface with occasional pores—see Fig. 1,⁷ the visualization of the dendritic C₆₀DF layer and subsequently the Fe^{III}Cyt_c layer led to different representations—see Fig. 1. Now, fine-grained structures of C₆₀DF and Fe^{III}Cyt_c layers are clearly discernible. Fig. 1 shows that in the case of the dendritic C₆₀DF, the surface coverage, that is, characteristic 20–50 nm 2D nanoaggregates, amalgamates on a macroscopic picture into a continuous film. Layers of Fe^{III}Cyt_c reveal, despite the amorphous nature of the protein matrix, a similar texture.

Repetition of the C₆₀DF/Fe^{III}Cyt_c sequencing leads to substrate/(C₆₀DF/Fe^{III}Cyt_c)_n, where *n* could be as large as 10. To confirm the regularities of the sequential sandwich packing we followed the *layer-by-layer* build-up *via* absorption spectrophotometric and ellipsometric means on quartz and silicon wafers, respectively. Figures S2 and S3 illustrate that both variables, namely, C₆₀DF/Fe^{III}Cyt_c visible transitions and film thickness, gave rise to linear dependences.⁸ We conclude we have achieved success in fabricating layered, closely-packed ITO-surfaces with tailorable absorption-cross sections throughout the visible region and, thus, enhancing an efficient use of the solar spectrum. For example, fairly high absorbances of up to 0.2 (*i.e.*, Soret-band region) were accomplished for the sandwich structures.

Linear sweep wave voltammetry performed with ITO/C₆₀DF/Fe^{III}Cyt_c electrodes revealed, on the cathodic side, two distinct and well-separated reduction steps.§ Linear scan rate dependences substantiated surface-based, thin-layer electrochemical processes rather than diffusion controlled events. The Fe^{III}Cyt_c/Fe^{II}Cyt_c redox process appeared first, at -0.25 ± 0.05 V—Fig. S4—followed by the one-electron reduction of C₆₀DF/C₆₀DF^{•-} at -0.70 ± 0.05 V—both potentials are given *versus* SCE. These values are in excellent agreement with measurements conducted with the individual constituents and confirm that both chromophores sustain their redox characteristics in the layered films. From these redox potentials we estimate the energy of the C₆₀DF^{•-}/Fe^{III}Cyt_c charge-separated state as ~ 0.7 eV, sufficiently less energetic than the photoexcited chromophores; > 1.80 eV.

Owing to the good conductivity that layered films of C₆₀DF/Fe^{III}Cyt_c exhibit, we probed the electrochemical Fe^{III}Cyt_c/Fe^{II}Cyt_c⁹ processes as a function of ITO/(C₆₀DF/Fe^{III}Cyt_c)_n (*n* = 0–6). What is most interesting is that we found a satisfying linear dependence of



Scheme 1 Electron transfer processes occurring in photoexcited ITO/C₆₀DF/Fe^{II}Cyt_c and structure of C₆₀DF.

† Electronic supplementary information (ESI) available: experimental details. See <http://www.rsc.org/suppdata/cc/b3/b310289k/>

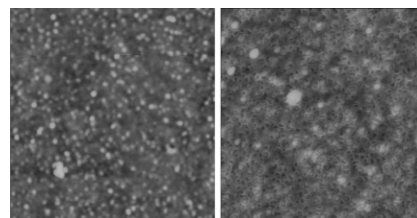


Fig. 1 AFM images of (left) silicon/PDDA/C₆₀DF and (right) silicon/PDDA/C₆₀DF/Fe^{III}Cyt_c (1 μm × 1 μm).

the currents, which relate to the Fe^{III}Cytc reduction, in films of up to 4 sandwich layers. Beyond this point, however, the currents level off and any further current increase is suppressed. The latter observation is consistent with a strongly impacted connection between the ITO electrode, on one end, and (Fe^{III}Cytc)₅ or (Fe^{III}Cytc)₆, on the opposite end, in ITO/(C₆₀DF/Fe^{III}Cytc)_n.

The photocurrent generation of an ITO/C₆₀DF/Fe^{III}Cytc cell is quite small—about 0.1 μA (Fig. 2)—a deficit that finds many causes. Most importantly, the electron donating ability of Fe^{III}Cytc, that is, its one-electron oxidation to form presumably Fe^{IV}Cytc, renders it an inappropriate choice for reducing C₆₀DF upon illumination. This picture should change dramatically, once the electrochemical reduction of the trivalent iron center is carried out. In fact, after electrolyzing the ITO/C₆₀DF/Fe^{III}Cytc cell at −0.25 V, a potential that leaves C₆₀DF as a primary electron acceptor intact, a nearly 10-fold amplification of the photocurrent was registered—see Fig. 2. The photocurrent was measured after the completion of preparative electrolysis—5 minutes—without any active bias potential on during the photocurrent measurements. The designed gradient in the integrated ITO/C₆₀DF/Fe^{II}Cytc construct ensures the efficient formation of C₆₀DF^{•−}/Fe^{III}Cytc in the excited device (*i.e.*, singlet excited state) plus a thermodynamically supported electron injection from C₆₀DF^{•−} into the secondary electron acceptor, ITO.^{7a} The photoaction spectrum—Fig. S5—reveals broadening relative to the ITO/C₆₀DF/Fe^{II}Cytc ground state features, indicating electronic interactions between the moieties. To confirm the photocurrent results ¹C₆₀DF fluorescence experiments were carried out, which indicate the presence and absence of electron transfer quenching in ¹C₆₀DF/Fe^{II}Cytc and ¹C₆₀DF/Fe^{III}Cytc, respectively were conducted. Reversing the electrode's bias and to reoxidize the redox protein at +0.2 V led again to the termination of the photocurrent, that is, reverting back to about 0.1 μA. Again, the photocurrents were recorded without any bias, but after 5 minutes of preparative electrolysis. We would like to emphasise that these switching features are completely reproducible: the photoelectrochemical cell performance depends solely on either a negative (*i.e.*, switching ON *via* reducing Fe^{III}Cytc) or a positive (*i.e.*, switching OFF *via* oxidizing Fe^{II}Cytc) bias, applied to the electrode.

The above-mentioned electrochemical results point to fairly good contacts between the ITO electrode, the ultimate electron acceptor, and Fe^{II}Cytc, the electron donor, situated in one of the outermost layers for *n* up to 4. Interestingly, the photocurrents in the corresponding ITO/(C₆₀DF/Fe^{III}Cytc)_n (*n* = 0–4) give rise to a similar trend, that is, they are linearly dependent on the number of layers (*n*) and, hereby, confirm the electrochemistry. Also, intermolecular interactions within the individual layers, as evidenced by the broadening of the spectra, ensure good charge migration/delocalization. The reversible ON/OFF switching remains reproducible even in ITO/(C₆₀DF/Fe^{III}Cytc)₄.

In conclusion, specific integration of ITO, C₆₀DF and Fe^{II}Cytc building blocks yields an environmentally very stable molecular

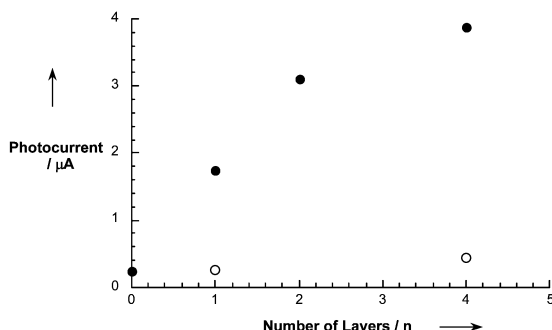


Fig. 2 Photocurrent of (C₆₀DF/Fe^{III}Cytc)—filled circle—and (C₆₀DF/Fe^{II}Cytc)—open circle—asssembled on ITO electrode. Experimental conditions: deoxygenated aqueous solutions containing 0.1 M NaCl as supporting electrolyte; either −0.25 V or +0.2 V bias voltage is applied.

switch. The function of the redox protein-based system, that is, reversible switching between two defined states, is attained by altering the redox state of the iron center: applying different electrochemical potentials switches the photoperformance ON (Fe^{II}Cytc) or OFF (Fe^{III}Cytc). A promising asset of our work is that, for the first time, stacking donor–acceptor sandwich layers (*i.e.*, C₆₀DF/Fe^{III}Cytc) helps to enhance the photosensitivity of the switch nearly linearly with the absorption cross section of the modified ITO electrodes.

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Notes and references

‡ The sequence of operations resulting in production of layered films was the following: (i) dipping of the substrate into a solution of 0.5% PDDA at pH 3 for 10 minutes; (ii) rinsing with water for 1 minute; (iii) dipping of the substrate into a solution of C₆₀DF at pH 7.6 for 40 minutes; (iv) rinsing with water again for 1 minute; (v) dipping of the substrate with the C₆₀DF layer into a solution of Fe^{III}Cytc for 120 minutes; (vi) final washing for 1 minute with water.

§ Platinum auxiliary electrode; ITO working electrode; 1 cm × 1 cm film area; Xe-lamp – 500 mW output; 350 nm cut off filter.

- (a) E. Braun, Y. Eichen, U. Sivan and G. Ben-Yoseph, *Nature*, 1998, **391**, 775; (b) I. Willner and E. Katz, *Angew. Chem., Int. Ed.*, 2000, **39**, 1180; (c) J. Fritz, M. K. Baller, H. P. Lang, H. Rothuizen, P. Vettiger, E. Meyer, H. J. Guntherodt, C. Gerber and J. K. Gimzewski, *Science*, 2000, **288**, 316; (d) A. N. Shipway and I. Willner, *Acc. Chem. Res.*, 2001, **34**, 421; (e) T. Chen, S. C. Barton, G. Binyamin, Z. Q. Gao, Y. C. Zhang, H. H. Kim and A. Heller, *J. Am. Chem. Soc.*, 2001, **123**, 8630; (f) J. J. Wei, H. Y. Liu, D. E. Khoshdariya, H. Yamamoto, A. Dick and D. H. Waldeck, *Angew. Chem., Int. Ed.*, 2002, **41**, 4700; (g) I. Willner, *Science*, 2002, **298**, 2407; (h) N. Mano, F. Mao and A. Heller, *J. Am. Chem. Soc.*, 2002, **124**, 12962; (i) S.-J. Park, T. A. Taton and C. A. Mirkin, *Science*, 2002, **295**, 1503.
- (a) M. Ferreira, J. H. Cheung and M. F. Rubner, *Thin Solid Films*, 1994, **244**, 806; (b) J. H. Fendler and F. C. Meldrum, *Adv. Mater.*, 1995, **7**, 607; (c) S. W. Keller, S. A. Johnson, E. S. Brigham, E. H. Yonemoto and T. E. Mallouk, *J. Am. Chem. Soc.*, 1995, **117**, 12879; (d) G. Decher, *Science*, 1997, **277**, 1232; (e) N. A. Kotov, *Nanostruct. Mater.*, 1999, **12**, 789.
- (a) G. W. Pettigrew and G. R. Moore, *Cytochromes c: Biological Aspects*, Springer, Berlin, 1987; (b) N. M. Kostic, *Met. Ions Biol. Syst.*, 1991, **27**, 129; (c) G. McLendon and R. Hake, *Chem. Rev.*, 1992, **92**, 481; (d) J. R. Winkler and H. B. Gray, *Chem. Rev.*, 1992, **92**, 369; (e) J. M. Nocek, J. S. Zhou, S. D. Forest, S. Priyadarshy, D. N. Beratan, J. N. Onuchic and B. M. Hoffman, *Chem. Rev.*, 1996, **96**, 2459; (f) R. H. Holm and E. I. Solomon, *Chem. Rev.*, 1996, **96**, 2237.
- (a) S. S. Isied, G. Worosila and S. J. Artherton, *J. Am. Chem. Soc.*, 1982, **104**, 7659; (b) S. S. Isied, *Adv. Chem. Ser.*, 1997, **253**, 331; (c) J. V. McArdle, H. B. Gray, C. Creutz and N. Sutin, *J. Am. Chem. Soc.*, 1974, **96**, 5737; (d) J. V. McArdle, K. Yocoon and H. B. Gray, *J. Am. Chem. Soc.*, 1977, **12**, 4141; (e) M. Meier and R. van Eldik, *Inorg. Chim. Acta*, 1994, **225**, 95; (f) M. Meier, R. van Eldik, I. J. Chang, G. A. Mines, D. S. Wuttke, J. R. Winkler and H. B. Gray, *J. Am. Chem. Soc.*, 1994, **116**, 1577; (g) M. Meier, J. Sun, R. van Eldik and J. F. Wishart, *Inorg. Chem.*, 1996, **35**, 1564; (h) M. Meier and R. van Eldik, *Inorg. Chim. Acta*, 1996, **242**, 185; (i) M. Meier and R. van Eldik, *Chem. Eur. J.*, 1997, **3**, 39.
- (a) A. Hirsch and O. Vostrowsky, *Eur. J. Org. Chem.*, 2001, 829; (b) M. Braun, S. Atalick, D. M. Guldi, H. Lanig, M. Brettreich, S. Burghardt, M. Hatzimarinaki, E. Ravanelli, M. Prato, R. van Eldik and A. Hirsch, *Chem. Eur. J.*, 2003, **9**, 3867.
- F. Fromherz and W. Arden, *J. Am. Chem. Soc.*, 1980, **102**, 6211.
- (a) C. Luo, D. M. Guldi, M. Maggini, E. Menna, S. Mondini, N. A. Kotov and M. Prato, *Angew. Chem., Int. Ed.*, 2000, **39**, 3905; (b) D. M. Guldi, C. Luo, D. Koktysh, N. A. Kotov, T. Da Ros, S. Bosi and M. Prato, *Nano Lett.*, 2002, **2**, 775.
- Reproducible images were recorded for all the following C₆₀DF and Fe^{III}Cytc layers.
- (a) Z. Zhang, A. E. F. Nassar, Z. Lu, J. B. Schenkman and J. F. Rusling, *J. Chem. Soc., Faraday Trans.*, 1997, **93**, 1769; (b) Y. M. Lvov, Z. Lu, J. B. Schenkman, X. Zu and J. F. Rusling, *J. Am. Chem. Soc.*, 1998, **120**, 4073.