CHEMISTRY
A EUROPEAN JOURNAL

A Low-Cost Biofuel Cell with pH-Dependent Power Output Based on Porous Carbon as Matrix

Ying Liu, Mingkui Wang, Feng Zhao, Baifeng Liu, and Shaojun Dong*[a]

Abstract: A glucose/O_2 biofuel cell (BFC) possessing a pH-dependent power output was fabricated by taking porous carbon (PC) as the matrix to load glucose oxidase or fungi laccase as the catalysts. The electrolytes in the anode and cathode compartments contain ferrocene monocarboxylic acid and 2,2′-azino-bis-(3-ethylbenzthiazoline-6-sulfonic acid) diammonium salt as the mediators, respectively. The power of the BFC was enhanced significantly by using PC as the matrix, rather than glassy carbon electrode. Additionally, the power output of the BFC decreases as the pH of the solution increases from 4.0 to 7.0, which provides a simple and efficient method to achieve the required power output. More importantly, the BFC can operate at pH 6.0, and even at pH 7.0, which overcomes the requirement for cathode solutions of pH < 5.0 when using fungi laccase as a catalyst. Operation of the BFC at neutral pH may provide a means to power medical devices implanted in physiological systems. The facile and low-cost fabrication of this BFC may enable its development for other applications.

Keywords: biofuel cell · carbon · enzyme catalysis · glucose oxidase · laccase

Introduction

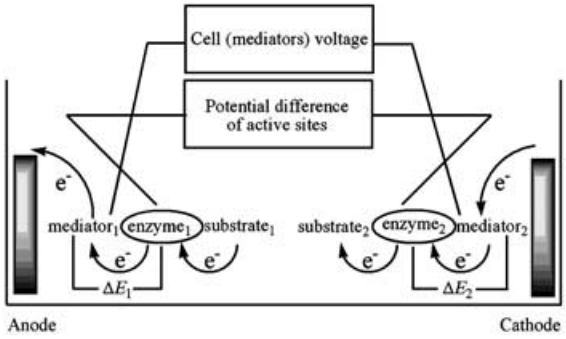
The biofuel cells (BFCs) that use biocatalysts, such as microbes[1–3] and enzymes,[4–9] can generate a power source by making use of animal or plant body fluids, without the production of poisonous materials. Such BFCs provide a green power source that protects the environment. The low and stable output of power that is generated can supply devices that require relatively little power, such as small sensor-transmitter systems in animals and plants,[10] microdevices, nanorobots, and pacemakers. Abundant biomass products, such as methanol or glucose, can be used as substrates (fuels) at the anode, whereas H_2O_2 or O_2 serve as the reducible products at the cathode (oxidizer).[4] Because they have the potential to act as an alternative power source to, for example, rechargeable batteries, these BFCs have become the subject of extensive research.[8–15] To satisfy the needs of increasingly diverse power sources, many novel and "smart" BFCs have been developed.[8,10,15] In particular, the miniature BFC made of two carbon fibers[11,14] is expected to act as an implantable medical device. Recently, the Willner group demonstrated how the adoption of a constant magnetic field parallel to the electrode surface could affect the rate of electrochemical reactions and enhance the performance of BFCs.[16] The performance of BFCs can be evaluated mainly by measuring the power output (W), which is determined by the cell voltage (V_{cell}) and cell current (I_{cell}) ($W = V_{cell} \times I_{cell}$). The communication of electrons between the biomolecules and electrodes is the key factor influencing the performance of BFCs in bioelectronic systems.[6] However, because the active centers of most redox enzymes are buried deep within the protein matrices, there is no direct communication between the electrons and the electrodes. Many attempts have been adopted to enhance the electron transfer rate between enzyme and electrode, for example, the reconstitution of apoprotein by using gold nanoparticles as an electron relay,[17] carbon nanotubes as the electrical contacting connector,[18] or boronic acid as the relay.[19] Another useful method is the orientation and covalent binding of an enzyme onto a monolayer-modified electrode.[4,20] Incorporation of the enzyme into redox-active polymers to facilitate the electrical connection of the enzyme with electrodes[11,21] has also been adopted. The use of a variety of electron mediators to facilitate the electrical communication between enzyme and electrodes is often involved in the fab-

[a] Y. Liu, Dr. M. Wang, Dr. F. Zhao, Prof. B. Liu, Prof. S. Dong
State Key Laboratory of Electroanalytical Chemistry
Changchun Institute of Applied Chemistry
Changchun Jilin, 130022
Graduate School of the Chinese Academy of Sciences
Beijing 100039 (China)
Fax: (+86) 431-568-9711
E-mail: dongsj@ns.ciac.jl.cn

Supporting information for this article is available on the WWW under http://www.chemeurj.org/ or from the author.

DOI: 10.1002/chem.200500308

rication of BFCs,[7,22,23] and has greatly promoted their development. The cell voltage can be controlled by the formal potential of the two mediators used in the cathode and anode, as shown in Scheme 1. The performance of the cell



Scheme 1. Mechanism of a mediated BFC.

can be improved by minimizing the potential difference (ΔE) between the enzyme and the mediator. This is achieved by choosing mediators with a formal potential close to that of the enzyme.

The catalysts in the anode of BFCs usually contain enzymes, such as glucose oxidase (GOD), glucose deghydrogenase, lactate dehydrogenase, and alcohol dehydrogenase. The microperioxidase-11, cytochrome oxidase, bilirubin oxidase (BOD), and laccase are often adopted as the cathodic catalysts of BFCs.[6,12,24–26] Due to their plentiful sources and good catalytic properties, GOD and laccase have been cooperatively assembled into BFCs.[9,11–13] Mediators often adopted for GOD include polymer-Os,[8,9,13,14] pyrroloquinoline quinone (PQQ), ferrocene (Fc) and its derivatives, ferricyanide, methylene blue, benzoquinone, and *N*-methyl phenazine.[25] So far, the best mediator of GOD that has been adopted in the anode of BFCs is PQQ, due to its formal potential being relatively low; however, the high costs involved limit its application. In comparison, the stable and low-cost Fc has been used as mediator in the anode of BFCs.[22] Common mediators of laccase include 2,2′-azinobis(3-ethylbenzthiazoline-6-sulfonic acid) diammonium salt (ABTS), syringal dazine, Fc, $K_3Fe(CN)_6/K_4Fe(CN)_6$, I_2/I_3^-, and polymer-Os.[7–9,13,14] ABTS was found to be the best electron mediator for use in the cathode of BFCs, due to it having a relatively high formal potential and being easily obtainable.

The material and microstructure of the electrode surface are also important factors for the improvement of the performance of BFCs.[27] A variety of materials, such as glassy carbon (GC),[7] Au,[25] carbon felt sheet,[28] carbon cloth,[26,29] carbon fiber,[26] acetylene carbon black, and colloid graphite[30] have been adopted as electrode materials to fabricate BFCs. Porous materials with three-dimentional structure have also attracted interest in a variety of fields;[21,31,32] however, to our knowledge, the use of porous carbon (PC) as the matrix for BFCs has not yet been reported. The three-dimensional structure and higher surface area of PC relative to planar materials makes it suitable for preparing cells required to improve power output. The one-dimensional, porous electrode model transporting oxygen has been evaluated,[27] and indicates that electrode materials can increase the diffusion of gas or ions and generate higher current density. In fact, the performance of conventional fuel cells has been improved by using PC as the matrix for the catalyst.[32] The use of carbon cloth, with a similarly porous structure, as the matrix to load laccase resulted in increased catalytic ability and the improvement of laccase stability.[21] In addition, PC has much greater mechanical strength and better conductivity than the previously used carbon felt and carbon cloth.

The present work involved the fabrication of a facile and economic BFC with a pH-dependent power output based on a PC matrix. GOD and laccase were entrapped in separate suspensions of carbon nanotubes–chitosan (CNTs–CS), which were then cast onto the PC matrix to form the catalyst of the anode and cathode, respectively. The mediators ferrocene monocarboxylic acid (FMCA) and ABTS were dissolved in the electrolytes of the anode and cathode, respectively. Glucose was used as the fuel at the anode, and oxygen acted as the oxidant at the cathode. The power of this BFC was shown to be higher than that of the cell prepared with the GC matrix. Furthermore, a BFC that can operate at pH 6.0–7.0 overcomes the restriction of using fungi laccase, which normally requires a solution of $pH < 5.0$.[9,14,21] The system described is substantially less expensive than that involving the mediators PQQ and polymer-Os,[4–6,21,25–27] which is advantageous for the practical and extensive application of such BFCs.

Results and Discussion

The electro-catalytic behavior of oxygen and glucose at the GC electrode: The catalytic activity of laccase towards O_2 decreases as the pH of the solution increases from 4.0 to 7.0 (data not shown). Cyclic voltammograms of ABTS in the N_2-saturated and O_2-saturated solution (pH 6.0) at the GC electrode modified with laccase were studied, respectively (see Supporting Information). A pair of peaks at 0.44 and 0.58 V can be ascribed to the redox reaction of $ABTS^{\bullet-}/ABTS^{2-}$ (N_2-saturated). Upon saturation of the solution with oxygen, the cathodic catalytic current increases dramatically, whereas the anodic current drops. This effect is similar to that observed upon using another multicopper oxidase, BOD, to catalyze the reduction of O_2 in the presence of ABTS as the mediator.[28] This is due to laccase catalyzing the oxidation of $ABTS^{2-}$, while the reduction of $ABTS^{\bullet-}$ occurs on the cathode. This result shows that the fungi laccase can still catalyze the reduction of O_2 under conditions of pH 6.0, which overcomes the difficulty in using most fungi laccase in solutions of $pH < 5.0$.[21]

The catalytic activity of GOD towards glucose increases as the pH is increased from 4.0 to 7.0 (data not shown). Cyclic voltammograms of FMCA at a GOD-modified GC electrode in the absence and presence, respectively, of 7 mM

glucose in the anode compartment (pH 6.0) were investigated (data not shown). In the absence of glucose, a distinct pair of FMCA peaks at 0.31 and 0.37 V were obtained. After addition of 7 mM glucose, the anodic current of FMCA increases, accompanied by the dramatic decrease in the cathodic current. This is consistent with results reported previously by our group,[33] and means that GOD, reduced by glucose, catalyzes the reduction of FMCA, which is oxidized at the anode. The phenomena described illustrate that ABTS and FMCA are effective mediators of laccase and GOD, respectively. The ΔE, based on the formal potential of ABTS and FMCA, is about 170 mV. In principle, these mediators can be used to create a BFC. In fact, for the BFC, the open-circuit voltage (V_{oc}) is approximately 410 mV and the short-circuit current (I_{sc}) is approximately 1 μA (corresponding to 15 μA cm^{-2}, according to the geometrical area of the GC electrode), which are similar to previously reported results.[4]

Performance of the BFC based on PC as matrix: The electro-catalytic behavior of oxygen and glucose with laccase and GOD, respectively, at the PC electrode is similar to that observed at the GC electrode. The performance of the BFC with PC as the matrix was investigated in solutions of pH 4.0–7.0 at ambient temperature. The values of cell voltage and current were recorded immediately after the circuit was closed. The BFC generated V_{oc} of approximately 0.66 (pH 4.0), 0.55 (pH 5.0), 0.42 (pH 6.0), and 0.16 V (pH 7.0), and I_{sc} of around 1.52 (pH 4.0), 1.18 (pH 5.0), 0.51 (pH 6.0), and 0.23 mA (pH 7.0). The current density of I_{sc} corresponds to 950 (pH 4.0), 738 (pH 5.0), 319 (pH 6.0), and 144 μA cm^{-2} (pH 7.0), according to the geometric area of the PC electrode, and is much higher than that of the planar GC electrode. These results suggest that PC is a more suitable material for the preparation of cells, as its three-dimensional, porous structure facilitates the increase in active monolayer enzyme, which in turn leads to a higher loading and degree of dispersion of the enzyme. Figure 1, top and bottom, shows the cell voltage and current, respectively, operating at variable external loads and in solutions of different pH. As the external load increases, the voltage of the BFC increases, reaching a plateau at a load of around 20 kΩ (Figure 1 top). By contrast, the cell current decreases, and reaches almost zero at an external load of around 30 kΩ (Figure 1 bottom). Figure 2, top and bottom, shows plots of the current versus voltage and the power output versus voltage, respectively, at various external loads of the BFC and at different pH. The maximum power output is 159.6 (0.27 V, pH 4.0), 23.6 (0.15 V, pH 5.0), 12.7 (0.26 V, pH 6.0), and 3.2 μW (0.11 V, pH 7.0), which corresponds to a power density of 99.8 (pH 4.0), 14.75 (pH 5.0), 7.94 (pH 6.0), and 2.0 μW cm^{-2} (pH 7.0), according to the geometric area of the PC electrode. The dependence of power output on pH is consistent with that of laccase activity on pH; the reverse relationship applies in the case of GOD. This implies that the laccase activity at the cathode limits the power of the BFC. Thus, the activity of the enzyme is usually the key factor in

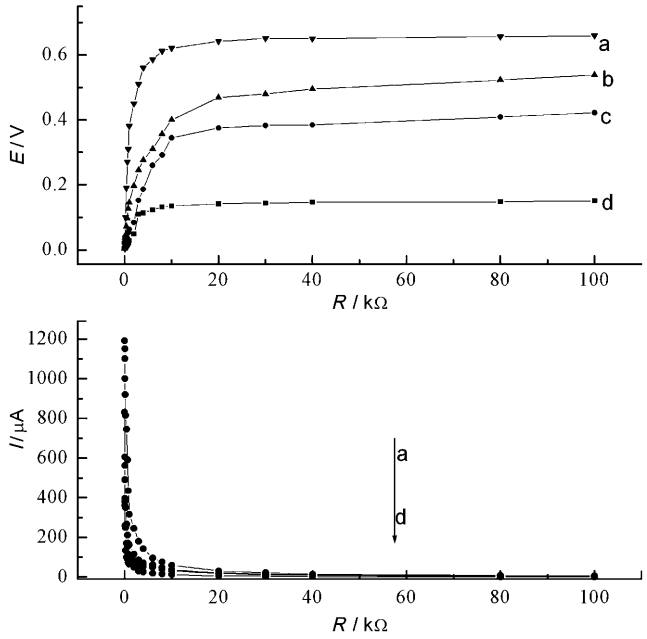


Figure 1. The voltage (top) and current (bottom) for the BFC at different external loads. a) pH 4.0, b) pH 5.0, c) pH 6.0, and d) pH 7.0.

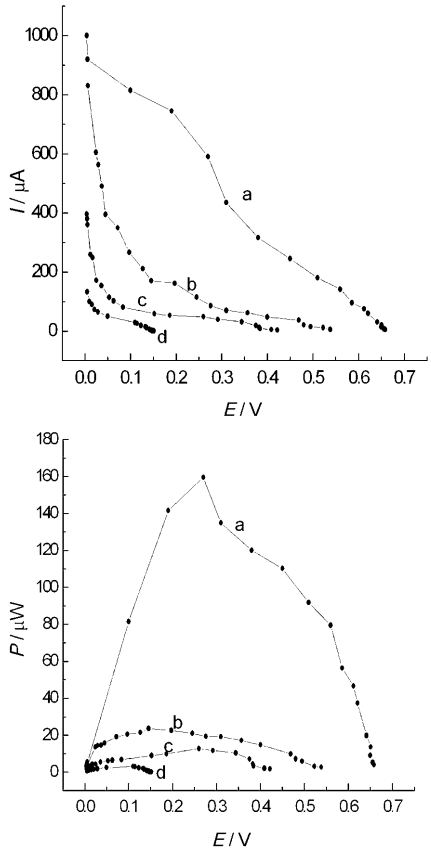


Figure 2. Current-voltage behavior of the BFC at different external loads (top), and the power output of the BFC at different cell voltages (bottom). a) pH 4.0, b) pH 5.0, c) pH 6.0, and d) pH 7.0.

influencing the performance of the cells. The relationship between pH and power output may provide an alternative means of adjusting the output as required, and eliminate the possible side-effects caused by tuning the external load.[15] The greater the initial external load, the greater the decrease in the BFC current. This may be attributed to the limitation of electron-transfer kinetics at the mediator/electrode interface or the ohmic cell resistance.[5] Thus, it could result in the linear and non-rectangular relationship of the *I* versus *E* plot (Figure 2 top), which is similar to previously reported results.[4,5] Although the operating voltage of the cell described here is lower than the highest reported voltage of 0.88 V,[13] the current and voltage of the BFC would be enhanced greatly by optimizing the mediator of GOD, for example, by substituting FMCA by PQQ[4] or polymer-Os.[11] Because PQQ and polymer-Os exhibit more negative formal potential (−0.155 V vs saturated calomel electrode (SCE)[4] and +0.10 V vs Ag/AgCl[11]) than FMCA (+0.34 V), the power output of the BFC incorporating mediators other than FMCA would be enhanced greatly. More importantly, we emphasize that the BFC incorporating laccase can operate at pH 6.0–7.0, which may provide a microenvironment with better biocompatibility for immobilizing laccase. This enzyme is often inactive at neutral pH and usually requires an environment of pH 5.0.[9,14,21] A unique characteristic of BOD is that this copper enzyme is not inhibited at neutral pH. No BFCs with fungi laccase as the catalyst operating at pH 6.0–7.0 have yet been reported. Operation at neutral pH facilitates the use of BFCs as a power supply for implantable physiological devices. There is no change in the magnitude of cell voltage and current during continuous operation of the BFC for 3 h. To maintain the long-term working stability of the BFCs, the electrolyte in the anodic and cathodic compartments needs to be refreshed every 3 h, or a flow system should be applied to eliminate possible depletion of fuels (glucose) and oxidants (oxygen), or degradation of the mediators.

Conclusion

We describe a BFC with a pH-dependent power output based on PC as matrix. The power output was enhanced greatly by using PC as the matrix, relative to that achieved with the GC electrode. The maximum power density was around 99.8 (pH 4.0), 14.75 (pH 5.0), 7.94 (pH 6.0), and 2.0 $\mu W cm^{-2}$ (pH 7.0), according to the geometric area of the PC electrode. The BFC with laccase as the cathodic catalyst can operate at neutral or near neutral pH, which overcomes the major impediment of using laccase below pH 5.0. This makes the application of the BFC to implantable medical devices possible. The facile and low-cost fabrication is also advantageous for the extension of BFC applications.

Experimental Section

Materials: Laccase (EC 1.10.3.2, *p*-diphenol:dioxygen oxidoreductases, from *Coriolus versicolor*) was purchased from Fluka (Switzerland). 2,2′-Azino-bis-(3-ethylbenzthiazoline-6-sulfonic acid) diammonium salt (ABTS), glucose oxidase (GOD) (EC 1.1.3.4 from *Aspergillus niger*), ferrocene monocarboxylic acid (FMCA), and chitosan (CS) (from crab shells, minimum 85% deacetylation) were obtained from Sigma (Canada). All reagents were used as received. Multiwall carbon nanotubes (CNTs) (95%, 20–50 nm), purchased from Shenzhen Nanotech Port. Co. (Shenzhen, China), were purified by using nitric acid. All other chemicals were of analytical grade. Phosphate buffer solutions (0.1 M PBS, pH 5.0–7.0) were made from Na_2HPO_4 and NaH_2PO_4. The pH 4.0 buffer solution was made from 0.1 M Na_2HPO_4 and citric acid. Pure water was used throughout, and was obtained by using a Millipore Q water purification apparatus with resistivity greater than 18 MΩ cm.

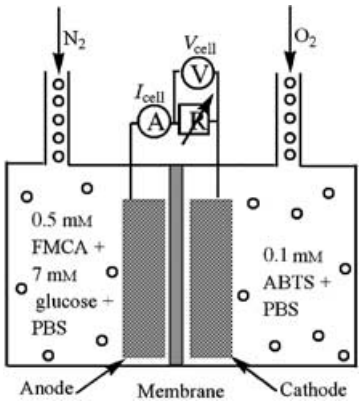
Porous carbon (PC) (T204), obtained from Harbin Electric Carbon Factory (Harbin, China), had a thickness of 3–4 mm, average pore size of 5.1 μm, and porosity of 39.4%.

Apparatus: Cyclic voltammetry was performed at a CHI 660B electrochemical workstation (CH Instruments, USA) with a conventional three-electrode cell. The modified electrodes were used as the working electrode. Coiled platinum wire and Ag/AgCl (saturated KCl) electrodes were used as the counter electrode and reference electrode, respectively. The PBS was purged with high-purity nitrogen or oxygen for at least 30 min prior to use, and the solutions in the cell were maintained under a nitrogen or oxygen environment.

Preparation of CNTs–CS and enzyme–CNTs–CS suspensions: CNTs (0.3 mg) were added to a solution of CS in acetic acid (1 mL, 1%) with ultrasonic agitation for 15 min. A uniformly black, viscous suspension was obtained, which displayed good film-forming ability, conductivity, and biocompatibility.[33,34] The enzymes GOD (12.5 mg) and laccase (7.5 mg) were dissolved separately in PBS (1 mL), and the enzyme suspension was obtained by mixing the CNTs–CS suspension with the GOD or laccase solution in a ratio of 2:1 (*v*/*v*), respectively.

Glassy carbon (GC) electrode as the matrix of the loading enzyme: GC electrode (3 mm in diameter) was used as a matrix to load either GOD or laccase. The enzyme suspension (5 μL) was spread evenly onto the surface of the GC electrode and dried at 4 °C for 24 h. The electro-catalytic characteristics of the relevant substrates on the electrodes modified with the enzymes were investigated.

PC as the matrix of the loading enzyme: The suspension of GOD or laccase (30 μL) was spread onto the mounted PC (8×8×1 mm) in a stepwise manner, so that the PC electrode became thoroughly wetted and evenly coated by the suspension. The modified PC electrodes were then dried at 4 °C.



Scheme 2. Schematic configuration of the BFC employing glucose and oxygen as substrate and oxidant, respectively.

Assembly of biofuel cells (BFCs): The perfluorinated membrane (Nafion® 115), with thickness 0.125 mm, was used to separate the anodic and cathodic compartments, which each had a volume of 5 mL. The anolyte with nitrogen-saturated solutions of different pH contained FMCA (0.5 mM) and glucose (7 mM). The oxygen-saturated solutions of different pH acted as the catholyte containing ABTS (0.1 mM). The variable external load, ranging from 0–100 kΩ, was connected in series between anode and cathode. The construction of the BFC is illustrated schematically in Scheme 2.

Acknowledgements

This research was supported by the National Natural Science Foundation of China (Nos. 20275036 and 2021130506).

[1] M. J. Cooney, E. Roschi, I. W. Marison, C. Comniellis, U. von Stocker, *Enzyme Microb. Technol.* **1996**, *18*, 358–365.
[2] W. Habermann, E. H. Pommer, *Appl. Microbiol. Biotechnol.* **1991**, *35*, 128–133.
[3] T. Ikeda, K. Kano, *Biochim. Biophys. Acta* **2003**, *1647*, 121–126.
[4] I. Willner, G. Arad, E. Katz, *Bioelectrochem. Bioenerg.* **1998**, *44*, 209–214.
[5] E. Katz, B. Filanovsky, I. Willner, *New J. Chem.* **1999**, *23*, 481–487.
[6] I. Willner, *Science* **2002**, *298*, 2407–2408.
[7] G. T. R. Palmore, H.-H. Kim, *J. Electroanal. Chem.* **1999**, *464*, 110–117.
[8] N. Mano, F. Mao, A. Heller, *J. Am. Chem. Soc.* **2003**, *125*, 6588–6594.
[9] N. Mano, F. Mao, W. Shin, T. Chen, A. Heller, *Chem. Commun.* **2003**, *4*, 518–519.
[10] E. Katz, A. F. Bückmann, I. Willner, *J. Am. Chem. Soc.* **2001**, *123*, 10752–10753.
[11] T. Chen, S. C. Barton, G. Binyamin, Z. Gao, Y. Zhang, H.-H. Kim, A. Heller, *J. Am. Chem. Soc.* **2001**, *123*, 8630–8631.
[12] R. F. Service, *Science* **2002**, *296*, 1223.
[13] V. Soukharey, N. Mano, A. Heller, *J. Am. Chem. Soc.* **2004**, *126*, 8368–8369.
[14] N. Mano, F. Mao, A. Heller, *J. Am. Chem. Soc.* **2002**, *124*, 12962–12963.
[15] E. Katz, I. Willner, *J. Am. Chem. Soc.* **2003**, *125*, 6803–6813.
[16] E. Katz, O. Lioubashevski, I. Willner, *J. Am. Chem. Soc.* **2005**, *127*, 3979–3988.
[17] Y. Xiao, F. Patolsky, E. Katz, J. F. Hainfeld, I. Willner, *Science* **2003**, *299*, 1877–1881.
[18] F. Patolsky, Y. Weizmann, I. Willner, *Angew. Chem.* **2004**, *116*, 2165–2169; *Angew. Chem. Int. Ed.* **2004**, *43*, 2113–2117.
[19] M. Zayats, E. Katz, I. Willner, *J. Am. Chem. Soc.* **2002**, *124*, 2120–2121.
[20] T. Lotzbeyer, W. Schuhmann, E. Katz, J. Falter, H. L. Schmidt, *J. Electroanal. Chem.* 1994, 377, 291–294.
[21] S. C. Barton, H.-H. Kim, G. Binyamin, Y. C. Zhang, A. Heller, *J. Phys. Chem. B* **2001**, *105*, 11917–11921.
[22] A. Pizzariello, M. Stred′ansky, S. Miertuš, *Bioelectrochemistry* **2002**, *56*, 99–105.
[23] F. Barrière, Y. Ferry, D. Rochefort, D. Leech, *Electrochem. Commun.* **2004**, *6*, 237–241.
[24] N. Mano, F. Mao, A. Heller, *J. Am. Chem. Soc.* **2003**, *125*, 6588–6594.
[25] E. Katz, I. Willner, A. B. Kotlyar, *J. Electroanal. Chem.* **1999**, *479*, 64–68.
[26] N. Mano, H.-H. Kim, A. Heller, *J. Phys. Chem. B* **2002**, *106*, 8842–8848.
[27] N. Mano, H.-H. Kim, Y. C. Zhang, A. Heller, *J. Am. Chem. Soc.* **2002**, *124*, 6480–6486.
[28] S. Tsujimura, M. Fujita, H. Tatsumi, K. Kano, T. Ikeda, *Phys. Chem. Chem. Phys.* **2001**, *3*, 1331–1335.
[29] S. C. Barton, H.-H. Kim, G. Binyamin, Y. C. Zhang, A. Heller, *J. Am. Chem. Soc.* **2001**, *123*, 5802–5803.
[30] M. R. Tarasevich, V. A. Bogdanovskaya, A. V. Kapustin, *Electrochem. Commun.* **2003**, *5*, 491–496.
[31] Y. Y. Song, D. Zhang, W. Gao, X. H. Xia, *Chem. Eur. J.* **2005**, *11*, 2177–2182.
[32] G. S. Chai, S. B. Yoon, J.-S. Yu, J.-H. Choi, Y.-E. Sung, *J. Phys. Chem. B* **2004**, *108*, 7074–7079.
[33] Y. Liu, M. Wang, F. Zhao, Z. Xu, S. Dong, *Biosens. Bioelectron.* **2005**, in press.
[34] M. Zhang, A. Smith, W. Gorski, *Anal. Chem.* **2004**, *76*, 5045–5050.

Received: March 18, 2005
Published online: June 21, 2005