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Photoswitching of Enzyme Activity of Horseradish Peroxidase Intercalated into Semiconducting Layers

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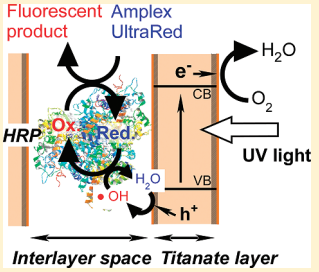
Supporting Information

ABSTRACT: The present study demonstrates that enzymatic reaction of horseradish peroxidase (HRP) inserted into semiconducting iron-doped titanate (FT) layers can be controlled by ultraviolet (UV) light irradiation. The resting HRP in the interlayer space is transformed into activated state (Compound I) as a result of oxidation with holes generated in the valence band of the irradiated FT layers. Subsequently, Compound I leads to the enzymatic conversion of organic substrates accompanied by two-electron reduction to regenerate the resting state. The FT layers play significant roles not only as sources of photo-oxidants (holes) for the HRP but also as UV light screeners to inhibit photodenaturation of HRP. In contrast,the titanate layer not including Fe is inappropriate as a host because of the lower absorbance of UV light. The intermittent UV light irradiation causes a clear photoswitching behavior of peroxidase activity. That is, the proposed photoinduced enzymatic reaction requires no additives such as peroxides (typically H_2O_2) and acidic stop solutions, which are frequently employed for batch-type operations. In the present study, advantages of the proposed technique over conventional enzymatic reaction systems are discussed in detail.



KEYWORDS: peroxidase, intercalation, photoelectrochemistry, semiconducting layer, enzymatic reaction cycle

INTRODUCTION

Enzyme biocatalysts are utilized in numerous industrial applications including chemical manufacturing and pollutant purifications because of their excellent efficiencies and substrate specificities. Among them, one of the most useful enzymes is peroxidase, which catalyzes the oxidation of electron donors (e.g., phenolic derivatives) in the presence of peroxide (typically hydrogen peroxide (H_2O_2)). When the enzymatic reaction is performed in a batch-type reactor, in general, the operation is initiated and terminated by the addition of H_2O_2 and acidic solution, respectively. Needless to say about the acidic solution, the exposure to H_2O_2 for long duration induces irreversible denaturation of the peroxidase.[1] Hence, the continuous utilization of the valuable enzyme without denaturation remains an ongoing challenge especially in the industrial fields exploiting peroxidases.

It is believed that one of the solutions for the above problem is a photochemical control of peroxidase activity.[2−8] Since the photon energy has no positive effect on the enzymatic reaction of natural peroxidase, hybridization with a photosensitizer is demanded to construct the photochemical system. Soares et al. have reported that incident light accelerates the peroxidase cycle under the coexistence of photoreductants.[9] Niemeyer et al. have claimed that ultraviolet (UV) light illumination to peroxidase-adsorbed semiconducting quantum dots (QDs) brings about the formation of reactive oxygen species (ROS; superoxide anion radical, hydroxyl radical) on the QD surface, and then the ROS trigger catalytic oxidation by the peroxidase.[10,11] Similarly, UV light illumination on the QDs covered with peroxidases on electrodes has enhanced the detection current of electrochemical biosensors.[12] In these approaches, since the enzyme molecules are exposed to the outside environment, the excitation light (especially UV region) may result in serious photo- and/or thermal denaturation of the enzymes.[13,14] Furthermore, the UV light irradiation largely exceeding band gap energy brings about photodissolution of the chalcogenide QDs.[15,16]

In the past decade, several groups, ours included, have studied that intercalation into the interlayer space of the physicochemically stable inorganic layer is useful for the protection of enzymes from denaturation at high temperature and nonphysiological pH, or in organic solvent.[17−22] Moreover, it is considered that the enzymes sandwiched between semiconducting layers having a band gap energy equivalent to the UV region possesses outstanding durability for photodenaturation.[23]

In the present study, to achieve the photochemical control of peroxidase activity without serious denaturation, the peroxidase-intercalated semiconducting layers are synthesized using horseradish peroxidase (HRP) and layered titanate as a guest and a host, respectively. We investigated whether the peroxidase activity of HRP inserted into the titanate layers can be switched by turning on or off the UV light in the absence of the aggressive reagents. In addition, the effect of the optical absorption property of the host layer on the photoreaction rate is also studied.

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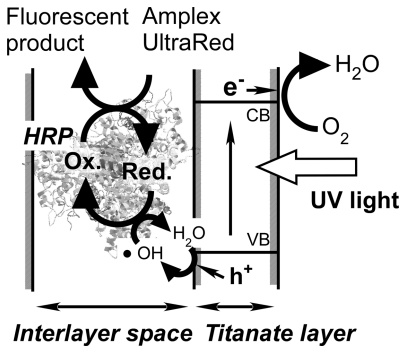

Figure 1. Schematic model of UV light-induced enzymatic reaction of HRP intercalated into titanate layers.

EXPERIMENTAL SECTION

Predicted Photochemical Control Mechanism of Enzyme Activity Using Peroxidase-Intercalated Semiconducting Titanate Layers. Figure 1 shows a schematic model for controlling the peroxidase reaction that is triggered by the photoirradiation of peroxidase-intercalated semiconducting oxide layers. The UV light irradiation generates photocarriers (electron and hole) in the semiconducting layer, and then the hole with an oxidation activity initiates the reaction cycle. The incorporation into the interlayer space guarantees the accessibility of substrate molecules to the peroxidase through semiopen structures along the two-dimensional layers. When compared with the others, a significant advantage of the present system is that the semiconducting oxide layers with a prominent photostability shield the peroxidase in the interlayer from the excitation light. As a result, the photodenaturation of peroxidase is inhibited for long durations.

Synthesis and Delamination of Layered Titanate. Pristine $K_{0.8}Fe_{0.8}Ti_{1.2}O_4$ (Fe-doped, FT) and $Cs_{0.7}Ti_{1.825}O_4$ (undoped, UT) were prepared by a conventional solid-state reaction method as described in our previous papers.[17] The alkali metal ions in the FT and the UT were substituted with H^+ during stirring in 1 M HCl over 24 h. The protonated powder was filtered and washed with copious water followed by drying at room temperature. The resultant powder was exfoliated to single titanate nanolayers by vigorous stirring in tetrabutylammonium hydroxide (TBA^+OH^-) solution for more than 1 week, where the amount of TBAOH was equivalent to 2-fold molar excess of the ion exchange capacity of protonated titanate.

Intercalation of HRP into Titanate Layers. Titanate layers intercalated with HRP were formed by the mixing of HRP solution with the exfoliated titanate suspension. The stock solution of titanate (pH 6.0, 0.72−0.93 mg/mL) was prepared by dilution and addition of acetic acid to the basic titanate suspension, followed by the removal of undelaminated particles by centrifugation. The contents of titanate layers in the obtained suspention were estimated by measuring the maximum absorbance at 255 and 267 nm for the FT and the UT, respectively. The lyophilized HRP powder (Wako Pure Chemical Ind., Osaka, Japan, MW: 40,200) was dissolved into 0.02 M potassium acetate buffer solution at pH 4.0 (0.44−2.00 mg/mL). The equivalent volumes of each stock solution were blended and stirred for 3 h at 298 K. After that, the precipitate (HRP-FT and HRP-UT) was collected by centrifugation and washed with copious water several times. The amount of HRP intercalated was calculated from a comparison of absorbances at the Soret band of HRP (403 nm) for the stock solution and the supernatant including unbound HRP after the centrifugation. The HRP/titanate ratio was controlled by adjusting the concentration of HRP stock solution. The incorporation of HRP molecules into the interlayer space of titanates was confirmed by the powder X-ray diffraction (XRD) technique.

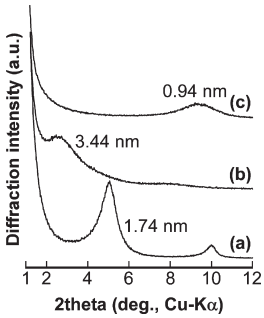

Figure 2. X-ray diffraction patterns of Fe-doped titantate (FT) intercalated with (a) tetrabutyl ammonium ions and (b) HRP (HRP/FT = 0.26 (w/w)). The pattern of HRP-FT after deintercalation of HRP in phosphate buffer solution is also displayed as in c. *d*-Spacings of the (020) plane in lepidocrocite-type titanate are written around each peak.

Peroxidase Activity Evaluation. Effect of the intercalation into the titanate layers on peroxidase activity of HRP was evaluated by measuring the oxidation rate of guaiacol (2-methoxyphenol) as a substrate. The reaction solution was prepared by the addition of the free HRP or the HRP-titanate and guaiacol (0.1−30 mM) to buffer solution (pH 4 (acetate) or 7.4 (phosphate)) in a disposable cuvette. The formation of oxidation products was monitored by absorbance change at 470 nm and 298 K immediately after the addition of H_2O_2 (0.4 mM) to the mixed solution. The extinction coefficient of the product ($\varepsilon_{470\ nm}$ = 26.6 $mM^{-1}\ cm^{-1}$) was used to calculate initial oxidation velocities (V_0).

Photochemical Control of Peroxidase Activity. Photochemical control (photoswitching) of peroxidase activity was performed in the citrate buffer solution (pH 6.0) containing Amplex UltraRed (AUR, purchased from Invitrogen, Carlsbad, CA) as a fluorogenic substrate. The solution (100 μL) consisting of AUR (14−50 μM) and the free HRP or the HRP-titanate (w_{HRP} = 1−25 μg/mL) was added to a 96-well black microplate and then was exposed to UV light from a UV-LED light source (Asahi Spectra, Tokyo, Japan, POT-365, 10 mW/cm^2 at 365 nm, fwhm: 10 nm) at ambient temperature for various durations. The formation of the fluorescent product was monitored by a microplate reader using fluorescence detection mode (excitation, 530 nm; emission, 590 nm).

RESULTS AND DISCUSSION

The mixing of stock solutions of HRP and the FT layer in acetate buffer solution (pH 4.0) achieved the intercalation of HRP into the FT layers. According to zeta potential measurements, the FT layer possessed a negative surface charge at pH 4.0 in contrast to that of HRP, which was positively charged. These facts imply that they are assembled via electrostatic interaction to form the periodical structure of the FT layers intercalated with the HRP. The absorbance at the Soret band of HRP (403 nm) in the supernatant after centrifuging the reaction mixture was smaller than that of the HRP stock solution (Figure 1S, Supporting Information), indicating the existence of HRP in the solid product. As shown in Figure 2a and b, the peak attributed to the (020) plane of the HRP-FT (d = 3.44 nm) appeared at a lower diffraction angle than that of TBA^+-intercalated FT (d = 1.74 nm).[24] This demonstrated that the HRP-FT layered structure was spontaneously formed by the intercalation of positively charged HRP having a large molecular size.[25−27]

First of all, to clarify the influence of intercalation into the FT layers on the peroxidase activity of HRP, the oxidation rate of the guaiacol substrate using the free-HRP or the HRP-FT was measured in the presence of H_2O_2 at pH 4.0. Figure 3a depicts

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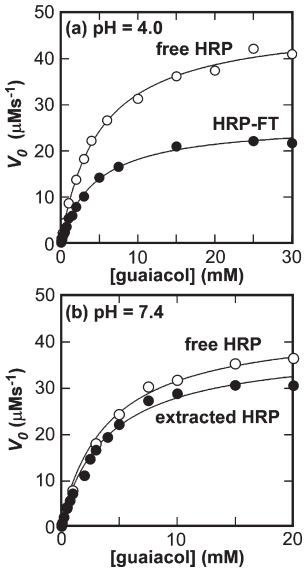

Figure 3. (a) Peroxidase activities of free HRP and HRP bound to FT (HRP/FT = 0.94 (w/w)). Initial reaction velocities (V_0) of guaiacol and H_2O_2 (0.4 mM) catalyzed by 17 μg/mL HRP in acetate buffer solution (pH 4.0) are plotted as a function of guaiacol concentration. (b) Peroxidase activities of two kinds of unbound HRP (12 μg/mL) at pH 7.4; free and extracted HRP from the HRP-FT in phosphate buffer solution. All solid lines indicate nonlinear regression curves to determine kinetic parameters (K_m and V_{max}).

Table 1. Catalytic Properties of Free HRP, HRP-FT, and HRP Extracted from the Interlayer

pH	sample	K_m (mM)	V_{max} (mM s^{-1})	k_{cat} (s^{-1})	k_{cat}/K_m (mM^{-1} s^{-1})
4.0	free HRP[a]	4.95	0.048	115	23
	HRP-FT[a]	4.40	0.026	61	14
7.4	free HRP[b]	4.02	0.044	147	37
	extracted HRP[b]	4.04	0.039	131	32

[a] 17 μg/mL. [b] 12 μg/mL.

the variation of initial oxidation velocity of the polymerized product[28] (V_0) as a function of the guaiacol concentration. The kinetic constants of the Michaelis−Menten equation (K_m and V_{max}) were determined by curve fitting of the relationship between the V_0 and the substrate concentration (Table 1). The V_{max} value of the HRP-FT is 0.0259 mM s^{-1} while that of the free HRP is 0.0484 mM s^{-1}, suggesting that the activity of HRP was reduced to about 54% after the intercalation. However, the HRP molecules existing in the interlayer could be released in phosphate buffer solution (PBS, pH 7.4) due to electrostatic repulsion between negatively charged HRP and FT at the neutral pH. In fact, 93% of HRP in the HRP-FT was extracted after stirring in the PBS at 298 K for 3 h and subsequent centrifugation to remove the FT layers deintercalated. The release of HRP was also confirmed by reduction of the interlayer distance as shown in Figure 2c. Figure 3b illustrates the guaiacol oxidation velocities of extracted and free HRP in the PBS. It was revealed that the peroxidase activity of the extracted HRP was comparable with that of the free HRP. Therefore, the decline in the V_{max} after the intercalation (Figure 3a) is not indicative of essential denaturation of HRP during the intercalation, and in other words, the intercalation process only reduces an apparent activity. Considering the nearly invariable K_m which corresponds to the relative affinity of the substrate for the enzyme compared with solvent molecules,[29] it is believed that the decreased V_{max} is mainly reflected by a decrease of the formation rate of the product from the substrate−peroxidase complex (k_{cat}) originated from slow product diffusion in the narrow interlayer space. Consequently, the synthesis of HRP-FT via the electrostatic self-assembly realized the fixation of HRP molecules in the interlayer space without serious denaturation of the peroxidase activity.

The photodenaturation of HRP under UV light illumination is inhibited by interstratification with the semiconducting titanate layers having a band gap energy corresponded to UV region. As illustrated in Figure 1, the catalytic performance of HRP bound to the FT layers will be controlled if charge transfer from the titanate to the HRP occurs under the UV light irradiation. The UV light absorption by the titanate layers causes generation of photocarriers (hole and electron) by the band gap excitation. On account of a high oxidizability, the holes produced in the valence band (ca. + 2.8 V *vs.* Ag|AgCl) encourage oxidation of the resting HRP (Red.) to Compound I (Ox.; production of oxy-ferryl (Fe^{IV}=O) and porphyrin radical cation)[30,31] as similar to a conventional peroxidase reaction using H_2O_2 as an initiator. The hydroxyl radicals, which are formed as a result of interaction between the hole and water molecule, may be concerned with the oxidation of the resting HRP. The Compound I oxidizes other substrates and then is regenerated to the resting form accompanied by two-electron reduction. The presence of electron in the conduction band may inhibit the enzymatic photocycle by recombination with the holes or reduction of the Compound I. Thus, the photochemical control of enzyme reaction is realized on the basis of the photoirradiation to the layered titanate with the interlayer HRP molecules.

The proposed photochemical technique was tested using Amplex UltraRed (AUR) as a fluorogenic substrate under the presence of free HRP or HRP-FT in citrate buffer solution (pH 6.0). According to the manufacturer's protocol, the non-fluorescent AUR is transformed into a bright fluorescent product in the presence of H_2O_2 and HRP. Figure 4a shows the variations of relative fluorescence unit (RFU) during weak UV light irradiation (10 mW/cm^2 at 365 nm) to the solution containing the AUR and the free HRP, the HRP-FT, or the FT. The solution temperature rose by several degrees after the irradiation for 21 min. As shown in Figure 4a, no rise of RFU in the AUR solution containing only the FT was confirmed, indicating that the photocatalytic oxidation of the AUR by the FT layers did not occur under the UV light irradiation. The RFUs in the HRP-FT solution were linearly increased with the irradiation time, and their slopes are significantly larger than those for the free HRP. The linearities in Figure 4a suggest that thermal activation of substrate conversion and photo- or thermal deactivation of HRP including photocatalytic decomposition can be neglected within the present experimental conditions. Figure 4b displays the dependence of HRP concentration on the slopes of RFU changes in Figure 4a which were calculated through a linear regression analysis. In the cases of free HRP, the slopes were almost constant and independent of the HRP concentration. This fact revealed that the slight enhancement of RFU in the free HRP solution during the irradiation was caused not by the enzymatic reaction but by direct photodecomposition of the AUR with a poor photostability. Actually, a minor increment of RFU under

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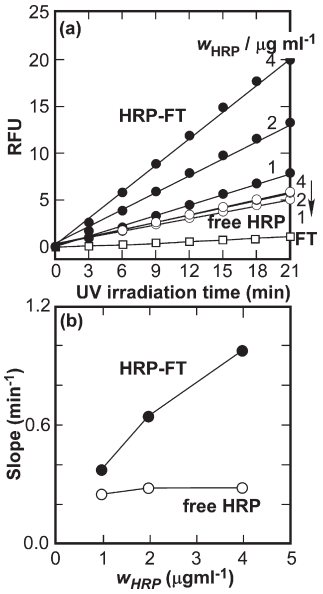

Figure 4. (a) Enhancements of the relative fluorescence unit (RFU) of 50 μM Amplex UltraRed including various amounts of free HRP, HRP-FT (HRP/FT = 0.54 (w/w)), or FT (0.05 mg/mL) during UV light irradiation at room temperature. (b) Plot of slopes in panel a versus weight concentration of HRP.

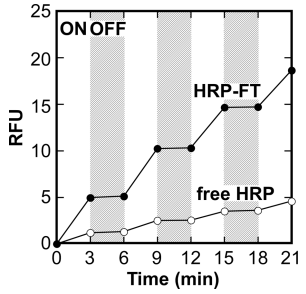

Figure 5. Photoswitching behaviors of catalytic oxidation of 15 μM Amplex UltraRed by HRP-FT (w_{HRP} = 0.025 mg/mL; HRP/FT = 0.54 (w/w)) or free HRP (0.25 mg/mL) under intermittent UV light irradiation.

the irradiation was observed even in the absence of HRP. In contrast, the positive relationship between the slope and the HRP-FT concentration is clearly discerned. Taking into account no change in the RFU under the dark state, it was suggested that the oxidation of AUR in the HRP-FT solution was triggered by the photoexcitation of the FT layers exposed to the UV light. Hence, the FT layers intercalated with HRP were effective for the photochemical promotion of peroxidase activity without addition of any other reagents.

In the previous studies describing the photoinduced reaction on the HRP-adsorbed QDs, the ROS ($O_2^{-}\cdot$ and OH$\cdot$) generated by band gap excitation of the QDs had activated the enzymatic reaction.[10] Because of the long lifetime of $O_2^{-}\cdot$ in neutral ~basic solution, however, the enzymatic reaction continued even after turning off the irradiation. The phenomenon appears to be inappropriate to attain a precise time and/or spatial resolution of the enzyme reaction. Therefore, the photoswitching ability of the peroxidase activity was evaluated using HRP-FT.

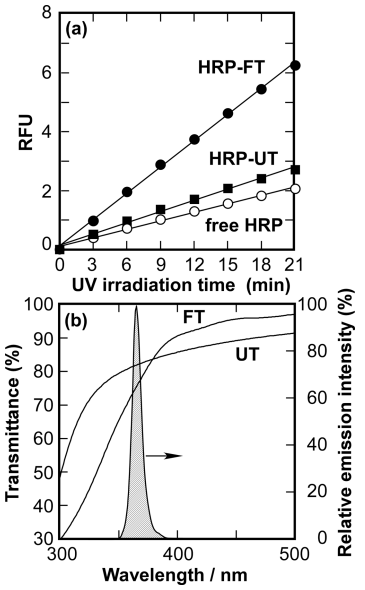

Figure 6. (a) Effect of the host layer on the photochemical reaction of intercalated HRP (4 μg/mL) in 15 μM Amplex UltraRed. Weight ratio of HRP/FT and HRP/UT employed is 0.54 and 0.50, respectively. (b) UV−vis transmission spectra of a colloidal solution of FT (12 μg/mL) and UT (13 μg/mL) at pH 6.0, where the emission spectrum of the UV-LED light source is also displayed.

Figure 5 shows the RFU changes in the solution including the free HRP or the HRP-FT under intermittent irradiation of UV light (every 3 min). In the free HRP solution, only a slight rise of the RFU assigned to the nonenzymatic photo-oxidation of AUR was observed with advance of the reaction. However, although the HRP content in the HRP-FT solution was one-tenth compared with that of the free HRP, the RFU was largely increased under UV light irradiation in contrast to little change under the dark state. Judging from these facts, it is concluded that the peroxidase activity of HRP inserted into the FT layers can be easily and precisely switched by turning on or off the UV light. The concentration of dissolved oxygen gas (under N_2 or O_2 bubbling) did not affect the reaction rate (Figure 2S, Supporting Information), indicating that the ROS such as stable $O_2^{-}\cdot$ are not concerned with the present photoenzymatic reaction cycle. Consequently, the unstable and highly reactive OH$\cdot$ radicals produced by the reaction of H_2O with hole could lead to the enzymatic reaction as shown in Figure 1, and hence, the clear photoswitching succeeded using HRP-FT.

Since the photoinduced reaction rate of HRP in the interlayer would be influenced by the optical absorption property of the semiconducting layers, the photochemical reaction was also carried out using a layered titanate without Fe (undoped; UT). The intercalation of HRP into the UT layers was easily accomplished through an identical procedure with FT. The RFU changes of the AUR solution with three types of HRP under continuous UV light irradiation are compared in Figure 6a. The photoassisted peroxidase activities of the interlayer HRP were largely different depending on the host layer. Only a slight influence of the UT layer on the RFU enhancement was confirmed. The optical absorption spectra of colloidal solution of the exfoliated titanate layers are depicted in Figure 6b. The absorption edge of the UT is observed around 320 nm, whereas that of the FT is largely shifted to the red side (ca. 380 nm) as a

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result of Fe doping. Judging from the emission spectrum of the light source (shaded peak in Figure 6b), it is considered that the FT layer can absorb the UV light more effectively than the UT. That is, the difference between the FT and the UT in Figure 6a may be explained by the discrepancy of photocarrier density in the titanate layer under irradiation. Thus, the FT layer plays important roles both as a shielding layer against the UV light and as a source of photocarriers that stimulate the peroxidase activity.

CONCLUSIONS

The present study demonstrated that UV light irradiation of the semiconducting layered titanate intercalated with the HRP molecules could trigger the enzymatic conversion of organic substrates. The reaction mechanism was explained as follows: (1) generation of holes in the valence band of the layered titanate under UV light irradiation, (2) direct or indirect hole oxidation of the resting HRP to Compound I at the HRP/titanate interface, and (3) oxidation of the organic substrate accompanied by two-electron reduction of Compound I to the resting state. The photoswitching of enzymatic reaction in the solution containing the HRP-titanate could be easily executed by turning on or off the UV light. Furthermore, the photochemical reaction rate depended on the band gap energy of the layered titanate. Since the titanate layers prevent the HRP molecules from UV-light-induced denaturing, the photoreaction cycle will continue for a longer period as compared with the conventional HRP-adsorbed QD systems. The photoswitching of peroxidase activity proposed here is considered to be a prominent technique from the viewpoint that no addition of peroxide initiator or acidic stop solution is required and would be effective for other redox enzymes and/or organic substrates. In addition, the utilizing of inorganic layers with a narrow band gap may realize the visible-light-driven activity control, and hence, the present photochemical system would be applied to various combinations of enzymes and organic substrates in the near future.

ASSOCIATED CONTENT

Supporting Information. UV−vis absorption spectra of HRP solution before and after intercalation into FT layers and effect of gas babbling on photochemical enzymatic reaction rate of HRP-FT (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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REFERENCES

(1) Ogushi, Y.; Sakai, S.; Kawakami, K. *J. Biosci. Bioeng.* **2007**, *104*, 30.
(2) Bretschneider, J. C.; Reismann, M.; von Plessen, G.; Simon, U. *Small* **2009**, *5*, 2549.
(3) Kurayama, F.; Matsuyama, T.; Yamamoto, H. *Adv. Powder Technol.* **2005**, *16*, 517.
(4) Kurayama, F.; Matsuyama, T.; Yamamoto, H. *Adv. Powder Technol.* **2004**, *15*, 51.
(5) Falguera, V.; Pagán, J.; Ibarz, A. *LWT Food Sci. Technol.* **2011**, *44*, 115.
(6) Song, Y. Y.; Hildebrand, H.; Schmuki, P. *Electrochem. Commun.* **2009**, *11*, 1429.
(7) Kuwabata, S.; Nishida, K.; Tsuda, R.; Inoue, H.; Yoneyama, H. *J. Electrochem. Soc.* **1994**, *141*, 1498.
(8) Kumar, S.; Nahar, P. *Talanta* **2007**, *71*, 1438.
(9) Soares, V. A.; Severino, D.; Junqueira, H. C.; Tersariol, I. L. S.; Shida, C. S.; Baptista, M. S.; Nascimento, O. R.; Nantes, I. L. *Photochem. Photobiol.* **2007**, *83*, 1254.
(10) Fruk, L.; Rajendran, V.; Spengler, M.; Niemeyer, C. M. *ChemBioChem* **2007**, *8*, 2195.
(11) Ipe, B. I.; Lehnig, M.; Niemeyer, C. M. *Small* **2005**, *1*, 706.
(12) Yang, X.; Wang, P.; Zhu,Y.; Li, C. *J. Solid State Electrochem.* **2011**, *15*, 731.
(13) Neves-Petersen, M. T.; Klitgaard, S.; Carvalho, A. S. L.; Petersen, S. B.; Aires de Barros, M. R.; Pinho e Melo, E. *Biophys. J.* **2007**, *92*, 2016.
(14) Maccarrone, M.; D'Andrea, G.; Salucci, M. L.; Avigriano, L.; Finazzi-Agrò, A. *Phytochemistry* **1993**, *32*, 795.
(15) Torimoto, T.; Paz Reyes, J.; Iwasaki, K.; Pal, B.; Shibayama, T.; Sugawara, K.; Takahashi, H.; Ohtani, B. *J. Am. Chem. Soc.* **2003**, *125*, 316.
(16) Torimoto, T.; Kontani, H.; Shibutani, Y.; Kuwabata, S.; Sakata, T.; Mori, H.; Yoneyama, H. *J. Phys. Chem. B* **2001**, *105*, 6838.
(17) Kamada, K.; Tsukahara, S.; Soh, N. *J. Mater. Chem.* **2010**, *20*, 5646.
(18) Chen, G. J.; Yen, M. C.; Wang, J. M.; Lin, J. J.; Chiu, H. C. *Bioconjugate Chem.* **2008**, *19*, 138.
(19) Mudhivarthi, V. K.; Bhambhani, A.; Kumar, C. V. *Dalton Trans.* **2007**, 5483.
(20) Gao, L.; Gao, Q.; Wang, Q.; Peng, S.; Shi, J. *Biomaterials* **2005**, *26*, 5267.
(21) Wang, Q.; Gao, Q.; Shi, J. *J. Am. Chem. Soc.* **2004**, *126*, 14346.
(22) Kumar, C. V.; Chaudhari, A. *Chem. Commun.* **2002**, 2382.
(23) Wei, M.; Yuan, Q.; Evans, D. G.; Wang, Z.; Duan, X. *J. Mater. Chem.* **2005**, *15*, 1197.
(24) Sasaki, T.; Watanabe, M.; Hashizume, H.; Yamada, H.; Nakazawa, H. *J. Am. Chem. Soc.* **1996**, *118*, 8329.
(25) Cai, W. Y.; Xu, Q.; Zhao, X. N.; Zhu, J. J.; Chen, H. Y. *Chem. Mater.* **2006**, *18*, 279.
(26) Chen, X.; Fu, C.; Wang, Y.; Yang, W.; Evans, D. G. *Biosens. Bioelectron.* **2008**, *24*, 356.
(27) Zhang, L.; Zhang, Q.; Lu, X.; Li, J. *Biosens. Boioelectron.* **2007**, *23*, 102.
(28) Tonami, H.; Uyama, H.; Nagahata, R.; Kobayashi, S. *Chem. Lett.* **2004**, *33*, 796.
(29) Zhou, Y.; Hu, N.; Zeng, Y.; Rusling, J. F. *Langmuir* **2002**, *18*, 211.
(30) Savenkova, M. I.; Newmyer, S. L.; Ortiz de Montellano, P. R. *J. Biol. Chem.* **1996**, *271*, 24598.
(31) Mahmoudi, A.; Nazari, K.; Moosavi-Movahedi, A. A.; Saboury, A. A. *Thermochim. Acta* **2002**, *385*, 33.