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Short communication

Glucose biosensor based on the room-temperature phosphorescence of TiO_2/SiO_2 nanocomposite

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ABSTRACT

The direct immobilization of glucose oxidase (GOD) on TiO_2/SiO_2 nanocomposite and its application as glucose biosensor were investigated. The room-temperature phosphorescence of TiO_2/SiO_2 nanocomposite can be quenched by hydrogen peroxide (H_2O_2). The detection of glucose may be accomplished by monitoring the formation of hydrogen peroxide which generated in the oxidation process of glucose with the catalysis of GOD. To our surprise, by using a 96-hole polyporous plate accessory of fluorescence spectrophotometer, the biosensor exhibits excellent linear response to glucose concentrations ranging from 1.0×10^{-9} to 1.0×10^{-2} M with a detection limit of 1.2×10^{-10} M. The TiO_2/SiO_2 nanocomposite can be used as both supporting material and signal transducer. The phosphorescence intensity and color of the biosensor change obviously and even could be observed with naked eyes by continuous addition of glucose. Based on the room-temperature phosphorescence of TiO_2/SiO_2 nanocomposite, a new method of solid substrate-room-temperature phosphorimetry (SS-RTP) for glucose determination is proposed. A glucose biosensor was fabricated with wide determination concentration range, low detection limit, high sensitivity, and fast response time. And the biosensor has been successfully applied to the determination of glucose in human blood serum. The coacervation of GOD enzyme and its interaction with TiO_2/SiO_2 nanocomposite enlarge the surface area and enhance the chemical stability of GOD. The nice biocompatibility, large surface area, good chemical stability and nontoxicity of the TiO_2/SiO_2 nanocomposite have made this material suitable for functioning as biosensor.

1. Introduction

Glucose is a very important metabolite for living organisms. The characteristic and the amount of glucose play very important roles in living organisms. The blood glucose concentration higher or lower than the normal range of 4.4–6.6 mM mostly indicates the inability to produce or utilize insulin in human body (Wang, 2008).

From the first report about enzyme electrode for the determination of glucose (Clark and Lyons, 1962), numerous methods have been reported for glucose analysis, such as fluorescence (Pickup et al., 2005), electrochemistry (Sugawara et al., 2000), flow injection (Yu et al., 2003), and biosensors (Han et al., 2008). Biosensors have attracted special interest for researchers owing to their own advantages, such as simplicity, low cost, high selectivity, fast response time and suitability for real time detection (Dai et al., 2009; Hart et al., 2004). In order to fabricate excellent biosensors, various materials have been used for the immobilization of enzymes in the preparation of biosensors. New materials with nice biocompatibility, good stability and large surface area for immobilization of enzymes are the focus of studies.

Silica-based materials have large surface areas and three-dimensional netlike structures. This kind of inorganic solid material has property that can be used as supporting material for enzymes and proteins (Lev et al., 1995; Lin et al., 2007; Wang et al., 2009).

In our previous work, TiO_2/SiO_2 nanocomposite has been prepared by a sol–gel scheme. And the phosphorescence intensity of the material exhibits good linear response to H_2O_2 solution concentrations ranging from 7.0×10^{-6} to 7.0×10^{-2} M (Shu et al., 2007). The phosphorescence properties of the TiO_2/SiO_2 nanocomposite will not be affected by some common ions (including phosphate and sulfate) and organic acids (Shu et al., 2007). In this work, TiO_2/SiO_2 nanocomposite was synthesized and used for the immobilization of GOD by physical adsorption. The phosphorescence intensity of TiO_2/SiO_2 nanocomposite decreased with the addition of glucose in the presence of GOD. The proposed biosensor exhibits excellent linear response to glucose concentrations ranging from 1.0×10^{-9} to 1.0×10^{-2} M with a detection limit of 1.2×10^{-10} M. And it has been successfully applied to the determination of glucose in human blood serum. Based on the room-temperature phosphorescence of TiO_2/SiO_2 nanocomposite, a new method of solid substrate-room-temperature phosphorimetry (SS-RTP) for glucose determination

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is proposed. The results demonstrate that the TiO_2/SiO_2 nanocomposite can provide good microenvironment for the immobilized enzyme. The TiO_2/SiO_2 nanocomposite is a potential matrix for immobilization of enzymes and fabrication of biosensors, due to the nice biocompatibility, good chemical stability, large surface area and nontoxicity of the material.

2. Experiments

2.1. Reagents and instrumentation

Tetraethoxysilane, isopropyl alcohol, and tetrabutyl titanium were obtained from Chengdu Chemicals (Sichuan, China). Glucose oxidases (GOD) were obtained from sigma (264 Units/mg). All chemicals were of analytical reagent grade and were used without further purification. Redistilled water was used throughout all experiments. Human blood serum samples were obtained from local hospital. Phosphorescence measurements were performed with fluorescence spectrophotometer F-7000 (Hitachi) with a polyporous plate accessory. The 96-hole (8 × 12, A1 to H12) polyporous plate accessory allows continuous automatic detection. The capacity of single hole of it is 300 μl (see Fig. S1). Atomic force microscopic (AFM) experiments were performed with SPM-9600 (Shimadzu, Japan).

2.2. The fabrication of biosensor

The nanometer particles of TiO_2/SiO_2 composite were prepared by the sol–gel method (Yu et al., 2001; Samantaray and Parida, 2001). Ti and Si were obtained from tetrabutyltitanium solution and tetraethoxysilane solution, respectively. The TiO_2/SiO_2 nanocomposite was prepared following a recipe similar to that reported by our precious work (Shu et al., 2007). The nanometer particles were kept in the desiccators and triturated before use. The immobilization of GOD on TiO_2/SiO_2 nanocomposite consists of two steps: (1) 0.010 g GOD was dissolved in redistilled water (10.0 ml) and stored in fridge at 4 °C before use; (2) Drop the GOD solution into TiO_2/SiO_2 nanocomposite powder until the material becomes mushy. Put the biosensor in refrigerator at 4 °C before use.

2.3. Measurement procedure

Put the biosensor into each hole of a polyporous plate with the same weight (0.020 g). The biosensor must be well-proportioned. Then 50 μl analyte solution was added into the hole one by one orderly (from A1 to H12). For example, 50 μl of 1.0×10^{-9} M H_2O_2 solution was added into A1 hole, 50 μl of 1.0×10^{-8} M H_2O_2 solution was added into A2 hole, and then 50 μl of 1.0×10^{-7} M H_2O_2 solution was added into the next hole that would be A3. Glucose solutions and human blood serum samples were added in the same way. Any change in phosphorescence intensity of the biosensor was recorded for each addition of analyte solution. The serum samples are measured with photometry mode provided by fluorescence spectrophotometer F-7000 (Hitachi). The phosphorescence spectral scans were recorded from 450 to 650 nm with an excitation wavelength of 403 nm. The excitation and emission bandwidth slits were set at 10 and 20 nm, respectively. The time scan phosphorescence spectra were obtained by sequential additions of glucose into the same hole. The time scan phosphorescence spectra were obtained at the maximum excitation wavelength of 403 nm and the emission wavelength of 550 nm. The bandwidth slits were set at 5 and 5 nm, respectively. The limit of detection for the method was determined as that giving phosphorescence intensity higher than the mean + 3 S.D. of the blank phosphorescence intensity of the biosensor.

3. Results and discussion

3.1. Performance of TiO_2/SiO_2 nanocomposite for H_2O_2

There have been reported that the phosphorescence intensity of TiO_2/SiO_2 nanocomposite exhibited good linear response to different concentrations of H_2O_2 solutions (7.0×10^{-6} to 7.0×10^{-2} M) in our laboratory (Shu et al., 2007). In order to enable comparison with previous work, a 96-hole polyporous plate accessory of fluorescence spectrophotometer F-7000 (Hitachi) was used. The response of the biosensor at increasing H_2O_2 concentration was shown in Fig. S2. Upon the addition of different concentrations of H_2O_2, the phosphorescence intensity of the TiO_2/SiO_2 nanocomposite decreased. The phosphorescence intensity of TiO_2/SiO_2 nanocomposite and the concentrations of H_2O_2 accord with the logarithmic quantitative equation, $\log[(P_0 - P)/P] = a + b \log C$, in which P_0 is the original phosphorescence intensity of TiO_2/SiO_2 nanocomposite, P is the phosphorescence intensity of TiO_2/SiO_2 nanocomposite in different concentrations of H_2O_2 solution, and C is the concentration of H_2O_2 solution (M). The calibration curve of the biosensor was shown as an inset in Fig. S2. The linear equation is $y = -0.2740x + 1.3685$, in which y is for $\log[(P_0 - P)/P]$, and x is for $-\log C$. The linear response range of the material to H_2O_2 concentrations was from 1.0×10^{-9} to 1.0×10^{-2} M (correlation coefficient $R^2 = 0.9949$). The average relative standard deviation (R.S.D.) of the plots was 5.2%.

3.2. Performance of the biosensor for glucose

3.2.1. Wavelength scan phosphorescence spectrum of the biosensor

The wavelength scan spectrum of the biosensor to glucose was shown in Fig. 1. The top line (a) is the original phosphorescence intensity of the biosensor without any glucose. With the addition of increasing glucose concentration, the phosphorescence intensity of the biosensor decreased. The corresponding calibration curve was shown in inset of Fig. 1. The linear equation is $y = -0.2489x + 1.5662$, in which y is for $\log[(P_0 - P)/P]$, and x is for $-\log C$. The resulting calibration plot was linear from 1.0×10^{-9} to 1.0×10^{-2} M with a linear correlation coefficient of $R^2 = 0.9939$. The detection limit of this biosensor to glucose was estimated to be 1.2×10^{-10} M. The average relative standard deviation of the plots was 5.9%. The linear

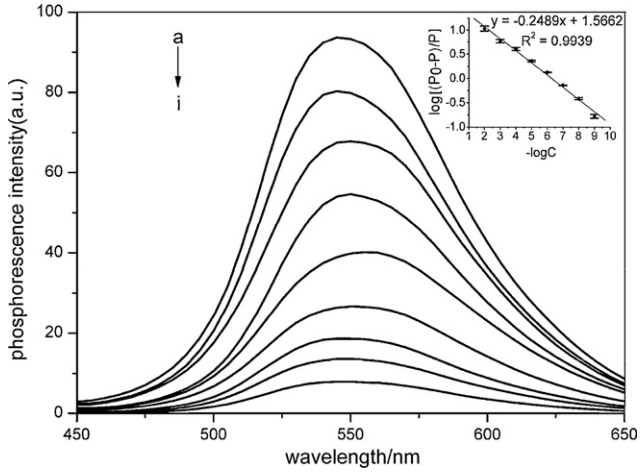

Fig. 1. Phosphorescence intensity of TiO_2/SiO_2 nanocomposite with the addition of different concentrations of glucose ranged from 1.0×10^{-9} to 1.0×10^{-2}. (a) 0, (b) 1.0×10^{-9}, (c) 1.0×10^{-8}, (d) 1.0×10^{-7}, (e) 1.0×10^{-6}, (f) 1.0×10^{-5}, (g) 1.0×10^{-4}, (h) 1.0×10^{-3}, and (i) 1.0×10^{-2} M.

range of the biosensor was much wider and suitable for diagnosing diabetics.

3.2.2. Time scan phosphorescence spectrum of the biosensor

Time scan phosphorescence spectrum of the biosensor to different concentrations of glucose was shown in Figs. S3 and S4. They display the phosphorescence intensity of the biosensor upon the successive injection of glucose. The phosphorescence intensity of the biosensor decreased steeply with the addition of different concentrations of glucose. The result shows that the response time of the biosensor is very fast (ca. 6 s in all, see supplementary material Fig. S4). The decay curves of the biosensor before and after the addition of glucose are shown in Fig. S5. Line (a) is the original phosphorescence lifetime of the biosensor with the addition of H_2O, and Line (b) is the phosphorescence lifetime with the addition of 1.0×10^{-2} M glucose solution. The phosphorescence lifetime of the biosensor decreased considerably with the addition of 1.0×10^{-2} M glucose. As an analytical method, time-resolved, room-temperature phosphorescence can minimize the light source noise or quench the background signal, and this is desirable for routine analytical chemistry (Shu et al., 2007). According to the equation $\ln I_t = \ln I_0 - t/\tau$, a plot of $\ln I_t$ versus t gives a straight line, with a slope equal to $1/\tau$, which is used for calculations. I_0 was the maximum intensity at time $t = 0$, I_t was the phosphorescence intensity at decay time t after excitation, and τ was the apparent lifetime of decay (Wei et al., 2005). The phosphorescence lifetime of the biosensor in the absence of glucose is 1.61 and 1.06 s for addition of 1.0×10^{-2} M glucose. The effect of glucose on the phosphorescence lifetimes of the biosensor provides further evidence for quenching by glucose.

3.2.3. Optical performance of the biosensor

The decreasing phosphorescence intensity of the biosensor could be recorded by fluorescence phosphorescence spectrophotometer. Moreover, the change of phosphorescence intensity and color of the biosensor could be observed with naked eyes. Fig. S6 (a) showed the room-temperature phosphorescence photograph of the biosensor in glucose concentrations ranging from 1.0×10^{-9} to 1.0×10^{-2} M, captured with a commercial digital camera, right after turning off a UV lamp. It can be seen that the phosphorescence intensity of the biosensor decreased by addition of different concentrations of glucose. In Fig. S6 (b), we can see that the color of the biosensor deepens gradually with the addition of high concentrations of glucose under daylight. The color of the biosensor changed from white (0 M glucose) to distinct yellow (1.0×10^{-3} M). The result indicates that the proposed biosensor provides a simple colorimetric method for the glucose analysis.

3.3. Immobilization of GOD on TiO_2/SiO_2 nanocomposite

There have been reported that the porous materials can be immobilized with protein simply by immersing the porous material in the protein solution (Diaz and Balkus, 1996). The AFM images of TiO_2/SiO_2 nanocomposite before (A and C) and after (B and D) GOD loading were shown in Fig. 2. It can be observed that the diameter of TiO_2/SiO_2 nanocomposite was about 10–15 nm. After GOD

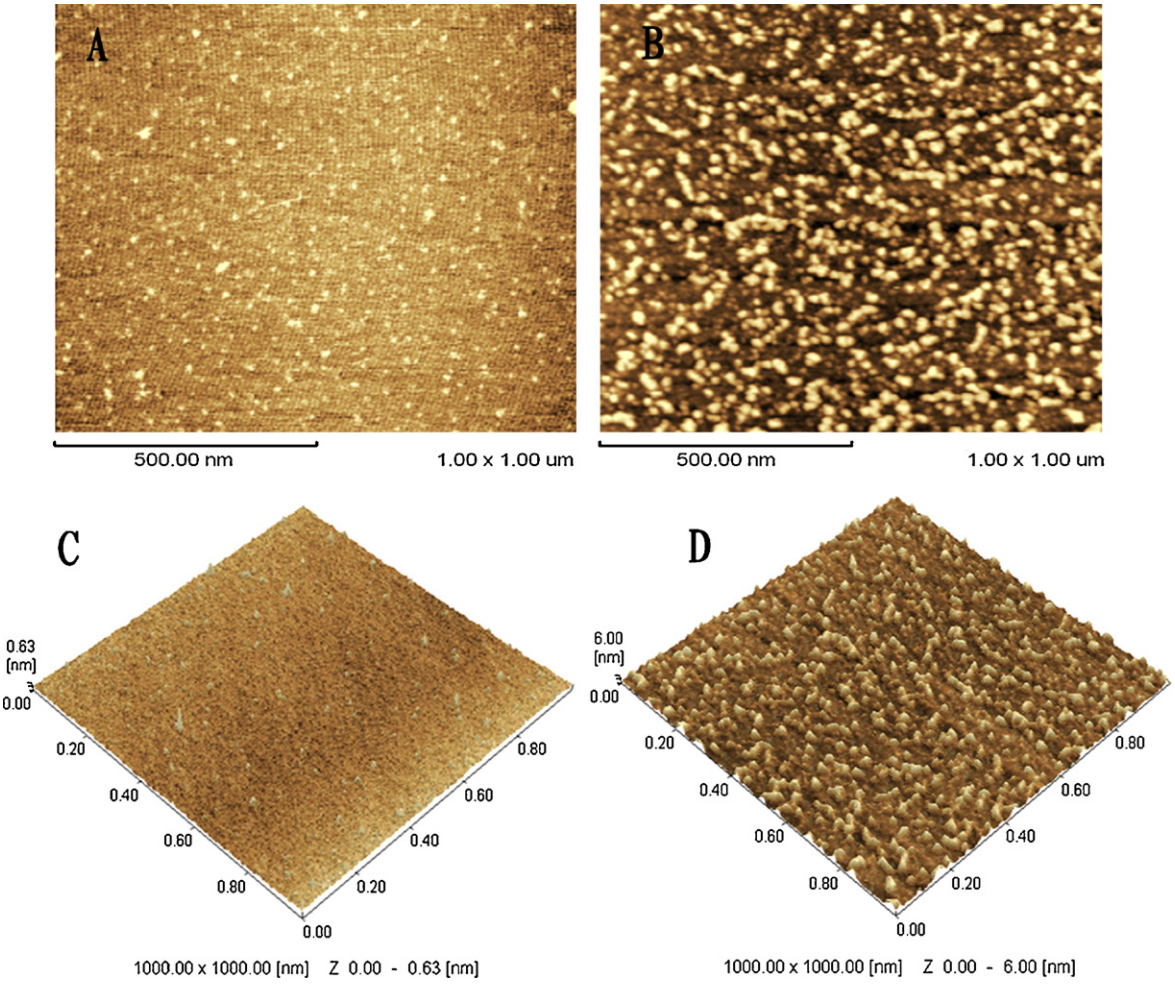

Fig. 2. AFM images of TiO_2/SiO_2 nanocomposite before (A and C) and after (B and D) GOD loading.

was loaded, the diameter of the material was increased to about 40–50 nm. The original TiO_2/SiO_2 nanoparticles became larger and their profiles became smooth. The result indicates that the GOD was adsorbed on the surface of the TiO_2/SiO_2 nanocomposite, and intercalated into the interspaces of Ti and Si. The GOD has an enlarged surface area due to the interaction between GOD and TiO_2/SiO_2 nanocomposite. It can be concluded that the TiO_2/SiO_2 nanocomposite might provide GOD with a favorable microenvironment.

3.4. Effect of pH

The effect of pH on the performance of this glucose biosensor was examined over a range of pH values (from pH 4.0 to pH 10.0) at 25 °C. At each different PBS solution, the biosensor was used to detect the glucose concentrations ranging from 1.0×10^{-9} to 1.0×10^{-2} M. As pH increased from 4.0 to 10.0, the phosphorescence intensity of the biosensor did not increase or decrease obviously. And the linear related coefficients of the biosensor to glucose were all above 0.99. The results indicate that the biosensor is pH independent. The optimum pH value for the activity of GOD is 5.5, while it has a broad activity pH range of 4.0–7.0 (Bright and Appleby, 1969). In this work, the GOD immobilized on TiO_2/SiO_2 nanocomposite could keep activity over a large range of pH values. The interaction between GOD and TiO_2/SiO_2 nanocomposite has enhanced the chemical stability of the GOD. This demonstrates that the TiO_2/SiO_2 nanocomposite is a good matrix for GOD immobilization.

3.5. Interference studies

The interferences of the common compounds in serum were investigated. The tolerable concentration ratios for interference at the 5% level were higher than 100 for lactose, uric acid, sucrose aminophenol, Na^+, K^+, Ca^{2+}, NO_3^-, PO_4^{3-} and SO_4^{2-} in glucose solutions of 1.0×10^{-2} M. The results show that the proposed biosensor has good selectivity to glucose and potential application in the detection of glucose in blood samples.

3.6. Lifetime of the biosensor

To demonstrate the stability of the biosensor, three months later, the proposed biosensor was used to detect the glucose concentrations according to the process mentioned above. The wavelength spectrum of the biosensor was shown in Fig. S7. The original phosphorescence intensity of the biosensor decreased from 93.70 to 89.76 (decreased ca. 4.5%). Upon the addition of different concentrations of glucose (from 1.0×10^{-9} to 1.0×10^{-2} M), the phosphorescence intensity of the biosensor became lower and lower. The linear equation of it is $y = -0.2350x + 1.3138$, in which y is for $\log[(P_0 - P)/P]$, and x is for $-\log C$. And the linear related coefficient of the biosensor is $R^2 = 0.9930$ compared with the $R^2 = 0.9939$ three months before. The result shows that the biosensor exhibits satisfactory stability.

3.7. Detection of glucose in blood serum samples

The proposed glucose biosensor could be applied to assay the real blood glucose. To demonstrate the reliability of this biosensor, it was applied to detect the glucose in blood serum samples. The natural value of glucose in human blood serum is from 4.4 mM to 6.6 mM. There were 241 human blood serum samples in our experiment, including 161 samples with unnatural glucose values. Error Grid analysis was used to show the clinical accuracy of this biosensor compared with the instrumental method. The maximum concentration of the samples was 24.84 mM and the CV was 3.30. The minimum concentration of the samples was 2.43 mM and the CV was 0.64. As shown in Fig. 3, the clinical performances of the two methods were observed to be identical based on the Error Grid analysis. The *F*-test and *T*-test also show no significant difference at the 90% confidence level between our proposed biosensor and the automatic glucose analyzer. The similar electrochemical performance between ascorbic acid and glucose will not affect the phosphorescence of our proposed biosensor. As to acetaminophen, the interaction between acetaminophen and glucuronic acid does not affect the process of glucose determination at all. Therefore, the presents of ascorbic acid and acetaminophen will not affect the human serum glucose determination with our proposed phosphorescence biosensor. The results indicate that the biosensor supply an alternative method for the determination of glucose in human blood serum samples, with the advantage of high stability, wide determination concentration range, low detection limit, high sensitivity, and fast response time.

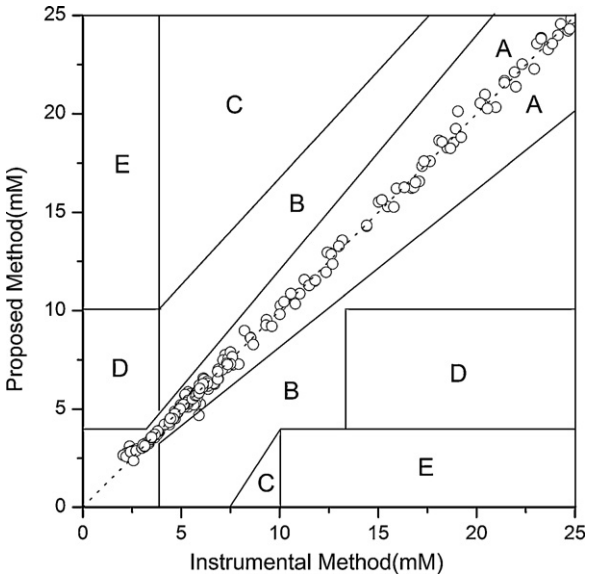

Fig. 3. Error grid analysis of two methods on blood serum samples ($n = 241$). (A) Clinically accurate; (B) deviating from the reference method by 20% but would lead to benign or no treatment error; (C) deviating from the reference method by 20% and would lead to unnecessary corrective treatment errors; (D) potentially dangerous failure to detect and treat blood glucose concentrations outside of desired target range; (E) would result in erroneous treatment.

4. Conclusions

A glucose biosensor based on TiO_2/SiO_2 nanocomposite has been fabricated and investigated for the determination of glucose in human blood serum. The room-temperature phosphorescence of TiO_2/SiO_2 nanocomposite can be quenched by H_2O_2. The detection of glucose may be accomplished by monitoring the formation of H_2O_2 which generated in the oxidation process of glucose with the catalysis of GOD. In this work, a new method of solid substrate-room-temperature phosphorimetry (SS-RTP) for glucose determination is proposed. The TiO_2/SiO_2 nanocomposite was used as both supporting material and signal transducer. The proposed biosensor exhibits excellent linear response to glucose concentrations ranging from 1.0×10^{-9} to 1.0×10^{-2} M with a linear related coefficient of $R^2 = 0.9939$. And the detection limit of the biosensor is estimated to be 1.2×10^{-10} M. This biosensor has been successfully applied to detect the glucose in human blood serum. The results indicate that the TiO_2/SiO_2 nanocomposite is an excellent immobilizing matrix and activity holder for enzyme. This study provides a new strategy for the direct immobilization of enzyme on the TiO_2/SiO_2 nanocomposite and the further development of biosensors.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bios.2009.05.033.

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