

Journal of Materials Chemistry B

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An electrochemical sensor for detecting triglyceride based on biomimetic polydopamine and gold nanocomposite

Wei Zhang ^a, Yan Tang ^a, Jia Liu ^a, Yujie Ma ^b, Ling Jiang ^c, Wei Huang ^d, Feng-wei Huo ^e, Danbi Tian ^{a*}

We developed a novel electrochemical sensing strategy for tributyrin detection based on polydopamine and gold nanoparticles hybrid (GNPs@PDA) to significantly improve the performance of the sensor. The adhesive hybrid has been easily synthesized in situ via oxidative polymerization of dopamine in the presence of H₂O₂ and the lipase was then conjugated with the GNPs@PDA easily, remaining enzymatically active and catalyzing hydrolyzation of tributyrin. The biomimetic material PDA here performs well both as an ideal binding agent for attaching to sensor surface and as a good platform for grafting enzyme lipase. A linear responses ranging from 50 to 300 mg·dL⁻¹ and the detection limit of tributyrin as low as 0.84 mg·dL⁻¹ with fast response time (20 s) are achieved on the GNPs@PDA platform. The value of the apparent Michaelis–Menten constant (K_m^{app}) was obtained as 7.94 mg·dL⁻¹, which indicated high affinity of lipase with tributyrin. Further, the sensor has strong anti-interference ability as well as a long shelf-life. We also use this sensor to detect triglycerides concentration in human serum samples.

1. Introduction

Estimation of triglyceride (TG) concentration is extremely important since it is an important diagnostic marker for coronary heart diseases,¹ lipoprotein disorder.² Elevation of normal triglyceride levels in the serum along with other serum parameters, is considered unhealthy.^{3,4} Among various methods available for TGs detection, electrochemical biosensors are comparatively more simple, sensitive, specific and rapid, and method for chemical modifying the electrode surface is a crucial step for the construction of an electrochemical biosensor. Although widely implemented in research, many available methods have limitations for widespread

practical use including the requirement for chemical specificity between interfacial modifiers and surfaces (e.g., alkanethiols on noble metals and silanes on oxides) and the need for multistep procedures for implementation (layer-by-layer assembly) and sophisticated and expensive synthesis procedures.

In recent years, the uses of gold nanoparticles (GNPs) for the biological components immobilization and enzyme electrode construction have attracted great attention due to its biocompatibility, high specific surface area, and reaction activity, etc.⁵⁻⁸

Dopamine, a biomolecule that was found in mussel adhesive proteins could polymerize at alkaline pHs to form adherent polydopamine (PDA) that is able to firmly adhere to almost all surfaces.⁹⁻¹² The PDA also enables a wide variety of chemical reactions for functional modifications due to its reactive catechol/quinone groups and is capable of reacting with thiols and amines, thus enabling protein conjugation/attachment and surface modification without need of any activation process.¹³

Inspired by the intriguing properties of PDA and GNPs, we developed a novel electrochemical sensing strategy based on PDA and GNPs hybrid (GNPs@PDA) to significantly improve the performance of electrochemical biosensor for tributyrin, which is a form of TG, being composed of butyric acid and glycerol. On the surface of sensor, GNPs@PDA hybrid was in situ synthesized via oxidative polymerization of dopamine in the presence of H₂AuCl₄. The PDA serves both as a conformal coating for the purposes of lipase immobilization onto GNPs and as highly adhesive to electrode surface. The lipase was secondarily conjugated with the GNPs@PDA easily, remaining enzymatically active and catalyzing the hydrolyzation of tributyrin. A detection limit of tributyrin as low as 0.84 mg·dL⁻¹ with fast response time (20 s) are achieved on a GNPs@PDA platform, which was demonstrated to be economical, facile and efficient.

2. Materials and methods

2.1 Chemicals

Lipase (Solarbio L3126 from porcine pancreas) and tributyrin (Purity 98.5%) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Tris (hydroxyl methyl)amino methane, Ascorbic acid, Uric acid were purchased from Sinopharm Chemical Reagent Co., Ltd. Human serum was obtained from Solarbio (Beijing, China). All other chemicals used are of analytical grade. Lipase ($1\text{-}5\text{ mg}\cdot\text{mL}^{-1}$) is freshly prepared in phosphate buffer (50 mM, pH 7.4) prior to being used. The tributyrin solution ($100\text{ g}\cdot\text{dL}^{-1}$) prepared in absolute ethanol was stored at $4\text{ }^{\circ}\text{C}$ refrigerator. This standard solution was further diluted to make different tributyrin concentrations with 50 mM phosphate buffer solution (PBS, pH 7.0).

2.2 Fabrication of the model of the amperometric tributyrin biosensor

The strategy for the preparation of the GNPs@PDA hybrid was as follow: The 1 mL dopamine solution ($2\text{ mg}\cdot\text{mL}^{-1}$) was added to 0.5 mL Tris-HCl buffer solution (0.1M, pH 8.5). Different volumes of chloroauric acid solution containing 0.1M $\text{HAuCl}_4\cdot 3\text{H}_2\text{O}$ were added in the above solution, and the total volume comes 2 mL . The as pretreated ITO electrodes were immersed in the dopamine solution for 4 h and then washed with distilled water, dried by nitrogen. For lipase immobilization, 20 μL solution of lipase prepared in PBS (pH 7.4, 50 mM, 0.9% NaCl) is dropped onto 0.5 cm^2 area of ITO surface and kept overnight. The bioelectrode was then washed with PBS buffer solution three times, dried under nitrogen flow and then stored in a refrigerator (4°C) before in use.

2.3 Apparatus and Electrochemical measurement

The GNPs@ PDA /ITO electrode and lipase/ GNPs@ PDA /ITO electrode have been characterized using electrochemical impedance spectroscopy (EIS) and scanning electron microscopy (SEM, QUANTA 2000). All Electrochemical analysis was performed in a conventional three-electrode cell at room temperature. Cyclic voltammograms (CVs) were measured using a three-electrode conventional cell with a CHI660C electrochemical workstation. The modified ITO as working electrode, a Pt wire as the counter electrode and a saturated calomelelectrode (SCE) as reference

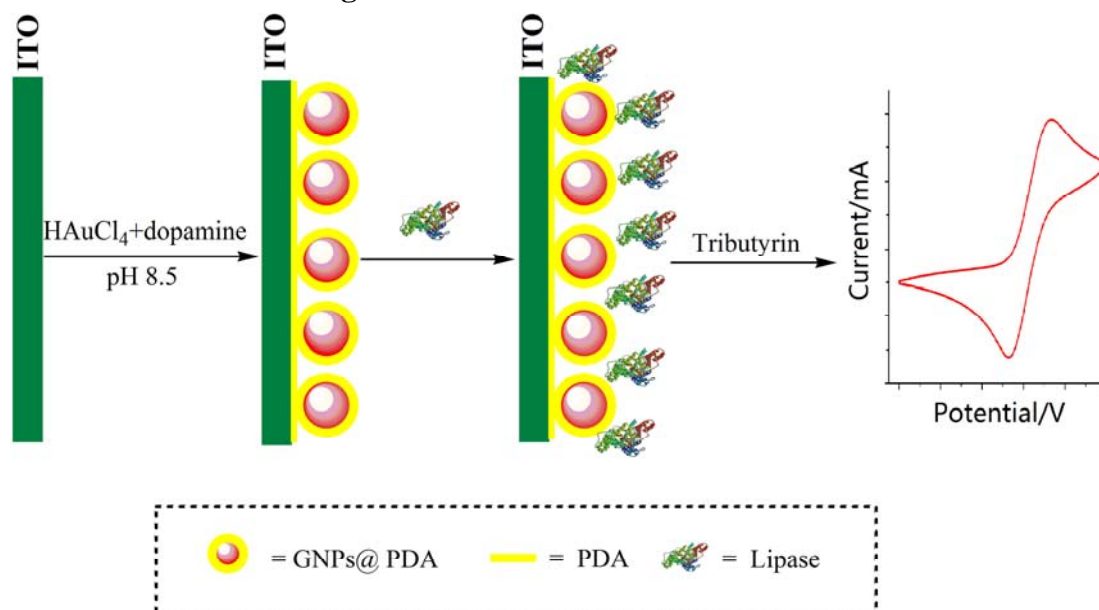
electrodes in phosphate buffer saline (PBS, 0.1M, pH 7.5, 0.9 % NaCl) containing 5 mM $[\text{Fe}(\text{CN})_6]^{3-}$ as redox probe. All potentials were referred to the SCE. The electrolyte solutions were deaerated by N_2 bubbling for 20 min prior to electrochemical measurements.

2.4 Detection of TGs concentration in serum

1 mL serum samples and 2 mg lipase were mixed and incubated for overnight at 35 °C to hydrolyze the inherent triglyceride in the serum. The sample was mixed with equal volume absolute ethanol, and then centrifuged at 12, 000 rpm for 5 min to remove protein. The treated sample was diluted by 50 times with PBS (50 mM, pH 7.5). Different concentrations of tributyrin were added to the diluted serum samples, in a three-electrode system with the lipase/ GNP@ PDA /ITO as working electrode.

3. Results and discussion

3.1 Mechanism of TG sensing



Scheme 1. Schematic illustration of the electrochemical strategy of measuring tributyrins.

Scheme 1 outlines the sensing mechanism employed in this study. When tributyrin in solution is hydrolyzed in the presence of lipase, the products formed are glycerol and butyric acid, which produced the changes of solution pH value. The change of pH is proportional to the concentration of tributyrin and could be detected by electrochemical biosensor.^{1, 14-16} The present designed electrochemical biosensor for measurement of TG was prepared by mounting GNP@PDA membrane bound to

lipase onto ITO electrode to use it as working electrode.

The polymerization of dopamine occurs under mild alkaline conditions and the sequent product PDA also allows spontaneous deposition of Au via reduction of the metal ion without any reductant assistance.⁹ So GNPs@PDA hybrid film was synthesized in situ by the simultaneous self-polymerization of dopamine and the reduction of HAuCl₄. The GNPs@PDA coating is able to firmly adhere to the surface of ITO electrode and also could be used as an versatile platform for secondary reactions, polydopamine-assisted grafting of lipase was accomplished through simple dropping the solution of lipase on the surface of GNPs@PDA, it is believed to involve reaction between terminal amino or thiol groups of lipase and the catechol/quinone groups of the GNPs@PDA coating.¹¹

3.2 Characterization of the fabricated lipase/ GNPs@PDA /ITO electrode

A control experiment was carried out to investigate whether GNPs@PDA hybrid film is better one for fabricating the biosensor. Compared to simultaneously synthesized GNPs@PDA film, another film was synthesized in situ on ITO electrode following the method reported by Guo et al,¹⁷ where a PDA film was synthesized by buffering dopamine solution to pH 8.5 firstly and then used the polydopamine coating as a reducing agent to reduce HAuCl₄ to form the PDA/GNPs film.

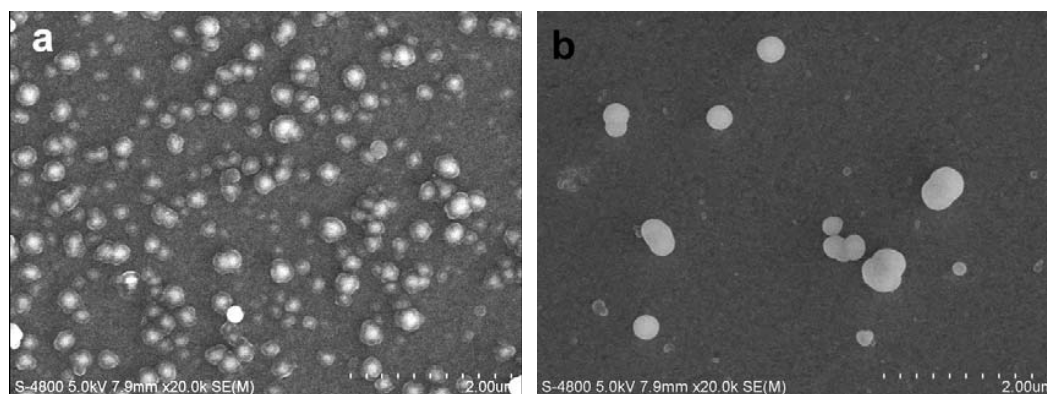


Fig. 1 SEM images of a) the GNPs@PDA hybrid film b) the PDA/GNPs film

The surface morphology of the GNPs@PDA and the PDA/GNPs film on the ITO electrode was checked by scanning electron microscopy (SEM) (Fig. 1). Image Fig.1a showed that at GNPs@PDA/ITO the GNPs were well capsulated by PDA and uniformly distributed on the surface of the ITO electrode, but image Fig.1b can't

prove the GNPs be capsulated by PDA at PDA/GNPs/ITO.

Electrochemical impedance spectroscopy (EIS) was used to monitor the features of these modified electrodes, the semicircle diameter at higher frequencies corresponds to the electron-transfer resistance (R_{et}), and the linear part at lower frequencies corresponds to the diffusion process.¹⁸ As shown in Fig. 2 A, the R_{et} of the $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ probe at the GNPs@PDA/ITO electrode was much lower than that at the PDA/GNPs/ITO electrode, indicating that the GNPs@PDA was an excellent conducting material to accelerate electron transfer.

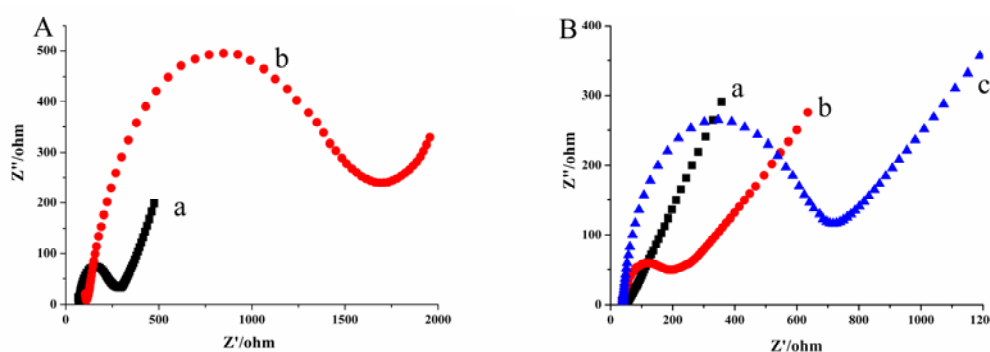


Fig. 2 Electrochemical impedance characterization: A) EIS of two modified electrode a) GNPs@PDA/ITO; b) PDA/GNPs/ITO; (B) (a) Bare ITO electrode; (b) GNPs @ PDA /ITO electrode; (c) Lipase/ GNPs @ PDA /ITO bioelectrode.

Fig.2B shows the results of EIS at different modification stages. The EIS of the bare ITO electrode was almost straight line (curve a). After the deposition of GNPs@PDA on the ITO electrode, the R_{et} slightly increased (curve b). When the lipase was grafted at the GNPs@PDA /ITO electrode (curve c), the R_{et} increased obviously, suggesting the presence of lipase, which could have hindered electron transfer due to the insulating characters.¹

3.3 Optimal detection conditions

The effects of pH, temperature and time in hydrolysis of TGs catalysed by lipase have been investigated, and the effects of $HAuCl_4$ and the lipase concentrate in fabrication of the tributyrin biosensor were also be investigated. As a result, lipase concentration of $2 \text{ mg}\cdot\text{mL}^{-1}$, incubation temperature of 40°C , pH 7.5 and chloroauric acid concentration of $6 \text{ }\mu\text{mol}\cdot\text{L}^{-1}$ were selected as the optimal detection conditions (Fig.S1).

To determine the type of the controlled process of TG at the lipase/GNPs@PDA /ITO electrode, the effects of the scan rate ($10 \text{ mV}\cdot\text{s}^{-1}$ to $150 \text{ mV}\cdot\text{s}^{-1}$) on the peak current were studied separately. Fig. 3 showed the changes of the anodic and cathodic peak currents of TG ($200 \text{ mg}\cdot\text{dL}^{-1}$) with the square root of the scan rate respectively. The anodic peak currents of TG increased linearly with the square root of scan rate with a correlation coefficient R of 0.985 and that of cathodic peak currents was 0.984, indicating that the electrode reaction of TG was a diffusion-controlled process.^{19, 20}

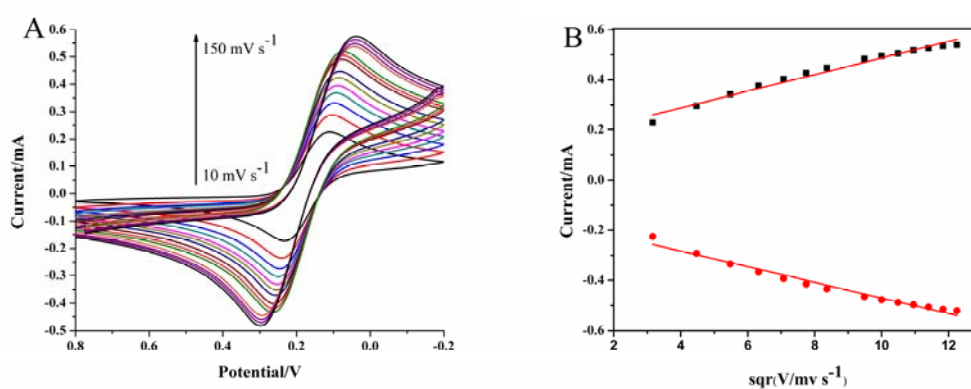


Fig. 3 The lipase/ AuNPs@ PDA /ITO bioelectrode with $200 \text{ mg}\cdot\text{dL}^{-1}$ TG (A) CVs of the modified electrode at different scan rates (range from 10 to $150 \text{ mV}\cdot\text{s}^{-1}$); (B) Plot of the anodic and cathodic peak currents with respect to the $v^{1/2}$.

3.4 Electrochemical behavior of lipase on GNPs@PDA /ITO electrode

For such a surface process, the average surface coverage (Γ^*) can be calculated according to the Laviron equation²¹

$$i_p = \frac{nFQv}{4RT} = \frac{n^2 F^2 v A \Gamma^*}{4RT} \quad (1)$$

the average surface coverage (Γ^*) of the electroactive lipase immobilized on GNPs@PDA was estimated to be $3.17 \times 10^{-8} \text{ mol}\cdot\text{cm}^{-2}$ ($n = 1$). This value was larger than previously reported values for immobilization of lipase on silicon nitride and nanoporous gold ($1.79 \times 10^{-9} \text{ mol}\cdot\text{cm}^{-2}$, $1.41 \times 10^{-8} \text{ mol}\cdot\text{cm}^{-2}$, respectively).^{16, 22} In addition to more loaded lipase on GNPs@PDA/ITO, a sound microenvironment

provided by PDA (electroactive catechol/quinone groups) should be responsible for the effective direct electron transfer.

3.5 Storage stability of lipase/ GNPs@ PDA /ITO bioelectrode

Stability is necessary for the fabrication of a biosensor. The lipase activity on the surface of the GNPs@ PDA /ITO electrode has been investigated by measuring electrochemical current response with regular interval of one week. The average amperometric current of the electrode retained 90 % of the initial signal after they were stored at 4 °C for two months, indicating that both the GNPs and the PDA might have provided a favorable microenvironment for lipase retain its bioactivity.

3.6 Determination of TG and samples in Human serum sample

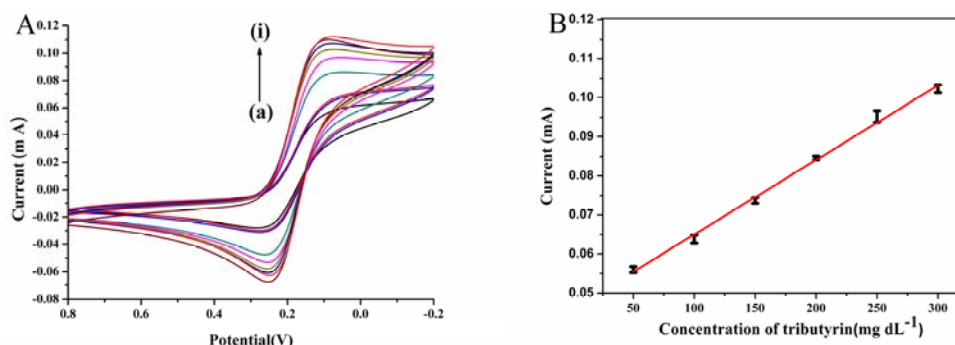


Fig. 4 Different concentrations of tributyrins (A) cyclic voltammogram; (B) calibration curves.

Under the optimized conditions, the electrochemical response of the lipase/ GNPs@PDA /ITO electrode was investigated as a function of tributyrin concentrations using CV technique at $50 \text{ mV}\cdot\text{s}^{-1}$ scan rate in PBS (50 mM, pH 7.5, 0.9% NaCl). The cathodic peak currents were all proportional to the concentration of tributyrin in the range of $50\text{--}300 \text{ mg}\cdot\text{dL}^{-1}$, and the correlation coefficient was 0.995. The detection limit was $0.84 \text{ mg}\cdot\text{dL}^{-1}$ and the sensitivity of $0.38 \mu\text{A}\cdot\text{mg}^{-1}\cdot\text{dL}^{-1}\cdot\text{cm}^{-2}$. This value indicated that the lipase/ GNPs@PDA /ITO electrode had much lower detection limit and higher sensitivity than most currently available lipase-based biosensors as shown in Table S1(supporting information).

The apparent Michaelis-Menten constant (K_m^{app}) can reveal affinity of lipase for the substrate (tributyrin). From the Hanes plot (Fig.5A), we can found that this value is $7.94 \text{ mg}\cdot\text{dL}^{-1}$, which is much smaller than that prepared by sol-gel derived

nanostuctured cerium oxide($22.27 \text{ mg}\cdot\text{dL}^{-1}$)¹ and nanoporous gold material($10.67 \text{ mg}\cdot\text{dL}^{-1}$)¹⁶ The low value of K_m^{app} indicates immobilized lipase possessed high catalytic activity and has a much better affinity to tributyrin.

Finally, this TG biosensor was tested in human serum using the optimum operation procedures and reaction conditions. Different concentrations of TGs were added to the diluted serum samples to prepare the spiked samples. Current values are observed using CV technique at $50 \text{ mV}\cdot\text{s}^{-1}$ scan rate. The concentration of TGs in the human serum was calculated from the calibration curve and compared with spiked samples. The obtained results were shown in Table 1.

Table 1 Results of the tributyrin assay and the recovery test for serum samples.

No	Tributyrin added ($\text{mg}\cdot\text{dL}^{-1}$)	Tributyrin found ($\text{mg}\cdot\text{dL}^{-1}$)	Recovery (%)
1	50	49.2	98.4
2	100	102.3	102.3
3	200	204.8	102.4

3.7 Specificity and interference of lipase/ GNPs@ PDA /ITO electrode

The potential interference of urea, uric acid, cholesterol and glucose for the detection of the TG concentration in human serum was assessed experimentally. 1 mM urea, 0.2 mM uric acid, 5 mM cholesterol, and 5 mM glucose were added into the tributyrin solution ($200 \text{ mg}\cdot\text{dL}^{-1}$), respectively, based on the normal concentration ranges of these components in blood. The detecting current difference showed changes 2.4%, 2.1%, -2.0% and -0.3% , respectively (Fig. 5B), indicating that the interfering components just have a negligible effects with respect to the detection of TG using this method.

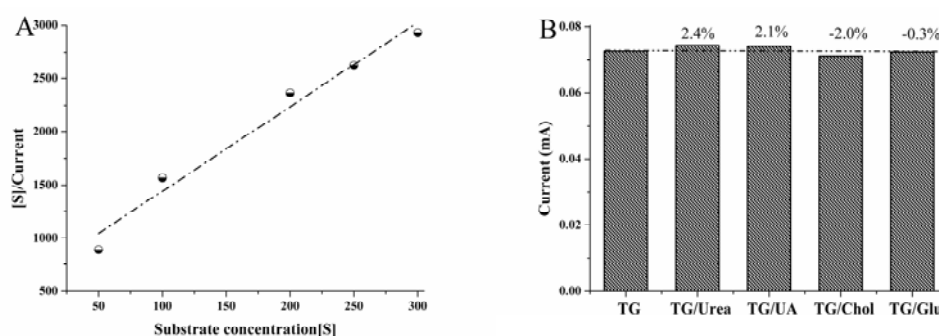


Fig. 5 (A) Hanes plot between the substrate concentration and [S]/current value; (B) Effects of interferents on lipase/ GNPs@ PDA /ITO electrode

4. Conclusion

We introduced a facile approach to fabricate lipase/ GNPs@ PDA biosensor for TG detection. The resulting lipase/ GNPs@ PDA biosensor presented excellent sensing performance with high sensitivity, anti-interference ability, and good stability for TGs detection in human serum. This surface fabrication strategy is distinctive in its ease of application, use of simple ingredients and mild reaction conditions, showing that the GNPs@PDA hybrid can provide a new opportunity for the design of high-performance TG biosensors in practical clinical analysis. Moreover, the preparation of the GNPs@PDA nanomaterial in which the inherent functionality of an enzyme is controlled on the molecular level by a nonbiological component is an interesting concept that may lead to more efficient usage of biomolecules in applications at the interface of biology and technology.^{23,24} It might be provide a promising potential in clinical applications, relative research are currently in progress.

Acknowledgements

This work was supported by the Natural Science Foundation of Jiangsu Province, China (No. BK2012822), the National Science Foundation for Distinguished Young Scholars of China (No. 21225626), the National High Technology Research and Development Program of China (No. 2012AA021700), the National Natural Science

Foundation of China for Young Scholars (No.21106064).

Notes and References

a. College of science, Nanjing Tech University Nanjing, Nanjing 211816, China

E-mail: danbi@njtech.edu.cn

b. Institute of Materials Engineering, Nanjing University, Nantong 226019, China

c. College of Food Science and Light Industry, Nanjing Tech University, Nanjing, 211816, China

d. Singapore-Jiangsu Joint Research Center for Organic/Bio-Electronics and Information Displays & Institute of Advanced Materials (IAM), Nanjing Tech University, Nanjing, 211816, China

e. School of Materials Science and Engineering, Nan yang Technological University, 50 Nan yang Avenue, Singapore 639798, Singapore

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