Molecularly Imprinted TiO_2 Thin Film by Liquid Phase Deposition for the Determination of L-Glutamic Acid

Liang Feng, Yongjun Liu, and Jiming Hu*

College of Chemistry and Molecular Sciences, Wuhan University, Wuhan 430072, People's Republic of China, and State Key Laboratory of Chemo/Biosensing and Chemometrics, Hunan University, Changsha 410082, People's Republic of China

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For the first time, the feasibility of a molecularly imprinted liquid phase deposition (LPD) thin film has been demonstrated. Thin films of titanium oxide imprinted with L-glutamic acid were prepared by the LPD method on a gold-coated quartz crystal microbalance. The imprinted molecule could be removed upon treatment with immersion in deionized water. A sensor was developed on the basis of this method and showed good sensitivity, selectivity, and reproducibility to the template molecule. An equation was deduced to characterize the interaction between molecularly imprinted films and the template by virtue of Scatchard analysis. X-ray photoelectron spectroscopy was introduced to show the evidence for the molecular imprinting phenomenon. The linear relationship between the frequency shifts and the concentration of analyte in the range of 10−200 μM was obtained. LPD proves to be a powerful method for imprinting titanium oxide thin films.

Introduction

Molecularly imprinted polymers (MIPs) are synthetic polymers that are obtained by polymerizing a monomer with a cross-linker around a template (the analyte) molecule. After polymerization, the template is removed by washing and the sites capable of selectively rebinding the target analyte are left.[1,2] Since the paper about theophylline MIPs was reported by Mosbach et al. in *Nature* in 1993,[3] interest in the molecular imprinting technique has surged because of its predetermination, selectivity, and practicability.[4−6] The usefulness of MIPs was shown in an array of analytical techniques for the analysis of many classes of molecules including herbicides,[7,8] drugs,[9,10] pesticides,[11] proteins and peptides,[12,13] cholesterol,[14] dyes,[15] and carbohydrates.[16]

Currently, two basic approaches to molecular imprinting may be distinguished: One is the pre-organized approach, mainly developed by Wulff,[17] where the aggregates in solution prior to polymerization are maintained by (reversible) covalent bonds. The other is the self-assembly approach, mainly developed by Mosbach and co-workers,[18,19] where the pre-arrangement between the printing molecule and the functional monomers is formed by noncovalent or metal coordination interactions. Compared with the pre-organized approach, the second method avoids complicated synthesis and chemical cleavage steps. Furthermore, the noncovalent interactions of different functionalities can be utilized simultaneously, and the imprinting system can be readily optimized to give optimal binding characteristics.[20] In noncovalent molecular imprinting, ionic interaction, hydrogen bonding interaction, and hydrophobic effects can be employed to great effect.[21]

Recently, molecular imprinting of recognition sites of carboxylic acid-functionalized substrates in TiO_2 sol−gel deposited thin films has also been developed as a means to tailor specific binding sites.[22] This novel surface sol−gel process can be used to design individual metal oxide layers with molecular precision and is broadly applicable to varied metal alkoxides. But the gel formation, pulverization, and extraction are intricate and time-consuming procedures. Therefore, it is necessary to develop a fast and convenient method to molecularly imprinted deposited thin films.

More recently, a promising technique, called liquid phase deposition (LPD), can provide an alternative approach to imprinting. LPD, also known as chemical bath deposition, of oxide films was first realized by Nagayama et al.,[23] who used the technique to prepare SiO_2 coatings

* Corresponding author. E-mail: ghu@whu.edu.cn. Address: College of Chemistry and Molecular Sciences, Wuhan University, Wuhan 430072, People's Republic of China. Tel.: +86-27-87682439, ext. 8304.

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on silicon wafers. The process has since been extended to the formation of other oxides, including those of Ti, Sn, Zr, V, Fe, Ni, Zn, and Cd.[24] Compared to other membrane techniques, LPD is a low-temperature, low-cost, and reliable method. It offers lower capital equipment costs (based on aqueous precursors), energy efficiency, and flexibility.

In this work, analytical performances of the sensor-based on L-glutamic acid (GA)-imprinted TiO_2 thin film will be reported, together with those of the nonimprinted TiO_2 thin film. Quartz crystal microbalance (QCM), microgravity detecting equipment, is used as a transducer of the sensors for measuring mass loading on the surface of a crystal on the basis of the famous Sauerbrey equation.[25] Ultimately, the sensor exhibits good sensitivity, selectivity, and reproducibility to the template molecule.

Experimental Section

Materials. GA, L-3,4-dihydroxyphenylalanine, and glycine were purchased from Sigma Co. Tryosine, leucine, and tryptophan were supplied by Shanghai Chemical Reagent Co. D-Glutamic acid was obtained from GL Biochem (Shanghai), Ltd. All the other chemicals were of analytical grade and used as received without further purification. Deionized water was used throughout.

Apparatus. The QCMs were gold-deposited AT-cut piezoelectric crystals with a 9-MHz basic resonance frequency from Beijing Chengjing Co. (Beijing, China). The crystal consisted of a 12.5 × 0.2 mm (diameter × thickness) quartz wafer, placed between 6-mm gold electrodes. The quartz crystal was mounted by concentric rubber seals (O-rings) into a holder to provide contact with one side of the quartz crystal to the liquid. The frequency counting was performed by a self-made frequency collecting card plugged into a personal computer. The high-resolution frequency counter (CN3165, Sampo Co., Taiwan, China) was used for calibration. The determination of the pH was performed using a digital pH counter (PHS-2F, Shanghai, China). An X-ray photoelectron spectrometer (XSAM800, KRATOS Co., U.K.) was used for the X-ray photoelectron spectroscopy (XPS) analysis.

Preparation of the Molecularly Imprinted TiO_2 Deposited Film. GA was dissolved in 10 mL of deionized water at a concentration of 80 mmol dm^{-3}. $(NH_4)_2TiF_6$ and H_3BO_3 were dissolved in this solution at concentrations of 0.1 and 0.3 mol dm^{-3}, respectively. The mixture was stirred quickly until all the compounds were mixed uniformly and used as the solution for the deposition.

The quartz crystal was washed by pirannha etch solution (1:3 30% (v/v) H_2O_2/concentrated H_2SO_4), deionized water, and absolute ethanol and dried in nitrogen gas prior to use. Then, the crystals were placed into a beaker for deposition. The resonant frequency was monitored by personal computer for 1 h at intervals of 1 s. The temperature was controlled at 35 ± 1 °C using a thermostat water bath. The nonimprinted LPD film was prepared under exactly the same conditions without the template molecule GA.

Procedure of Measurement. The imprinted quartz crystal was dipped in a solution containing of 10 mL of a 0.05 M phosphate buffer solution (pH = 6.8). A steady resonant frequency (F_0) was recorded. Then, small aliquots of stock GA solution were successively added by a microsyringe to the solution to allow the reaction to occur between the analyte and the imprinted sensor. The frequency was recorded until the steady resonant frequency (F_i) was obtained again. A shift in the frequency was calculated by the difference between F_0 and F_i. The same procedure was performed to the nonimprinted sensor under exactly the same conditions without the template molecule. The environmental temperature was always controlled at ~25 °C using an air conditioner.

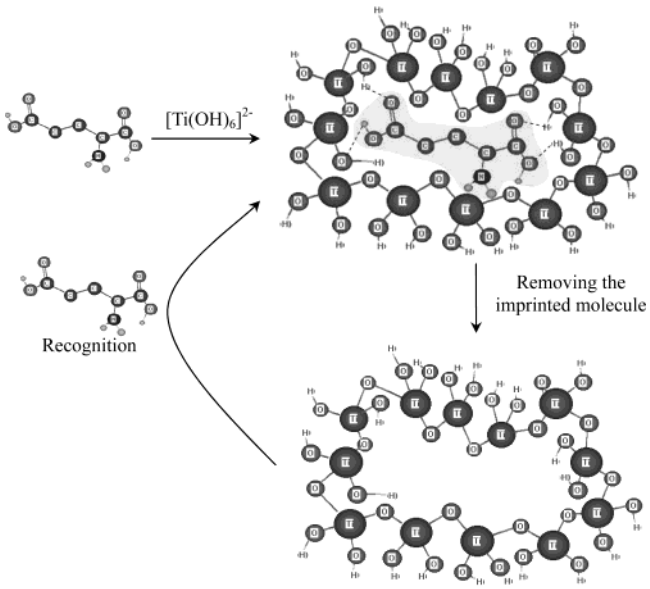

Figure 1. Mechanism of the molecularly imprinted LPD film.

Results and Discussion

Mechanism of the Molecularly Imprinted LPD Film. Figure 1 shows the preparation of the imprinted sensor for GA. $[TiF_6]^{2-}$ is hydrolyzed with water following the ligand-exchange equilibrium reaction.[26]

$$[TiF_6]^{2-} + nH_2O \rightleftharpoons [TiF_{6-n}(OH)]^{2-n} + nHF$$

This reaction can be shifted to the right-hand side by adding boric acid, which reacts with the F^- ions to form more stable complex ions:

$$H_3BO_3 + 4HF = BF^{4-} + H_3O^+ + 2H_2O$$

The addition of H_3BO_3 to the $(NH_4)_2TiF_6$ solution accelerates the ligand-exchange (hydrolysis) reaction. Simultaneously, several functional groups of the analyte react with the $[Ti(OH)_6]^{2-}$ species generated by the hydrolysis reaction of $[TiF_6]^{2-}$. The hydrogen bonds are formed between the carboxyl groups on GA and the hydroxyl groups of $[Ti(OH)_6]^{2-}$ on the LPD film surface during the deposition. Consequently, a titanium oxide molecularly imprinted thin film forms on the substrates upon dehydration of the $[Ti(OH)_6]^{2-}$ species generated by the hydrolysis reaction of $[TiF_6]^{2-}$.

The frequency shifts of the quartz crystal during the deposition are recorded. A remarkable decrease in resonant frequency (~1470 Hz) is observed. A similar reduction in resonant frequency (~1405 Hz) is obtained during the deposition of the molecularly nonimprinted film, suggesting the parallel masses deposited on the sensors. The control experiment is also performed for 1 h using a blank QCM in phosphate buffer (pH = 6.8). The response of the resonant frequency of less than 6 Hz proves that the impact of environmental drift is so small that it can even be omitted. The decreasing of frequency is partly due to a mass increase on the surface of the QCM; however, the variation of the density and the viscosity also play an important role during the deposition.

The imprinted QCM is washed by a small quantity of distilled water, and then immersed in the 10 mL 0.05 M

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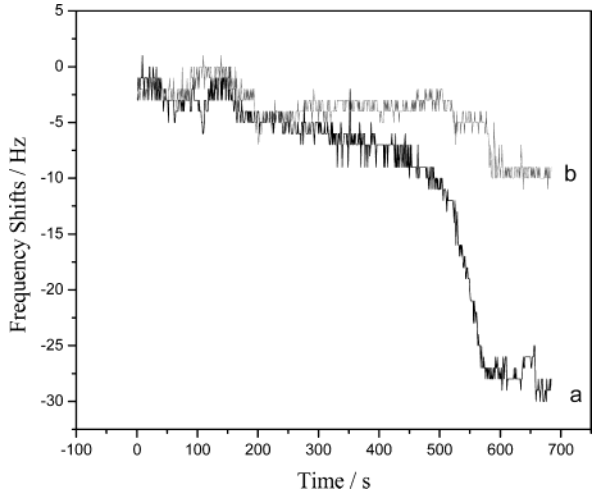

Figure 2. Response to the 10 μM GA of the imprinted sensor (a) and nonimprinted sensor (b).

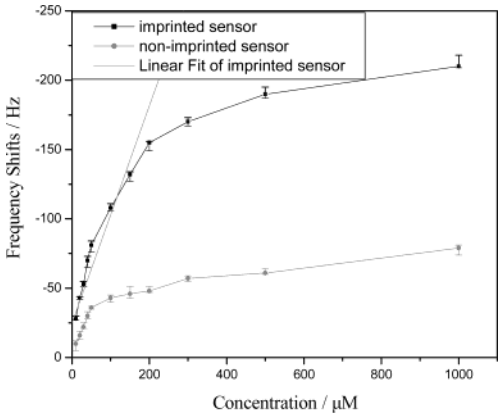

Figure 3. Corresponding frequency shifts of the imprinted and nonimprinted sensors for the different concentrations of GA.

phosphate buffer solution (pH = 6.8) to remove the imprinted molecules. The nonimprinted sensor is performed under exactly the same conditions.

Evaluation for the Effect of the Binding Interaction. The responses to 10 μM GA of the imprinted sensor and nonimprinted sensor are shown separately in Figure 2a,b. A notable decrease of the resonant frequency is obtained for the imprinted sensor, and then a steady value is reached in ~12 min. The frequency decrease approaches a limiting value of ~30 Hz or so. However, few frequency shifts are observed for the nonimprinted sensor under the same conditions. The similar experiment is also performed in a blank QCM, and the frequency decrease is approximately 5 Hz. It indicates that the injection of the solution containing analyte does not nearly induce the variation of the frequency. Therefore, the sensor based on imprinted GA has a sensitive response to the template relative to the nonimprinted sensor.

Figure 3 shows the corresponding frequency shifts of the sensor for the different concentrations of GA. There is a linear relationship between the frequency shifts and the concentration of analyte in the range of 10−200 μM. It can be described by the linear fit equation $\Delta f = -782\,540C - 24.97$ ($r = -0.9792$). The response time increases with the increasing of concentration of analyte. Response saturation is obtained at ~1 mM. The nonimprinted sensor without an imprinted molecule also shows a small quantity of frequency shifts. This can be explained by a mass of liquid loading.

Selectivity of the Sensor. The specificity of the binding mechanism in the imprinted sensor has been also taken into account by comparing its response to analyte with some similar molecular structures. In this experiment, L-3,4-dihydroxyphenylalanine, glycine, tryosine, leucine, and D-glutamic acid are considered. As is shown in Figure 4a, glycine shows similar results as GA, and it can be tentatively explained that glycine has an analogous structure but smaller molecule size. Consequently, the glycine molecule can easily enter the recognition sites applicable for GA. Only a small quantity of frequency shifts can be observed for other analogues in a lower concentration range relative to GA, and the nonimprinted sensor exhibits few frequency shifts for the same concentration under exactly the same condition. This confirms that the sensor based on the imprinted LPD film has a good selectivity of recognition to GA. Furthermore, the sensors are capable of discrimination between the stereoisomers. There is an initial linear decrease in the frequency corresponding to an increase in the D-glutamic acid concentration in the range of 10−200 μM. It can be also described by the linear fit equation $\Delta f = -166\,970C - 11.98$ ($r = -0.9592$). The enantioselectivity of the MIP coating can be assessed by evaluating the slopes of the initial linear equation of GA to D-Glutamic acid. Hence, the enantiomeric selectivity coefficent of the MIP used in this work is 4.69. It is in good agreement with the values reported by Haupt et al.[5,27] ranging between 3.6 and 5. However, all these compounds have an obvious response to the sensors for a large concentration range (Figure 4b). It can be interpreted that a mass of liquid loading can induce the notable changes in the density and viscosity of the solution and interfere with the observed results of the sensors.

Regeneration of the Sensor. The regeneration of the imprinted sensor binding 50 μM analyte in 10 mL of 0.05 M phosphate buffer solution is reported in Figure 5. An increase of the resonant frequency is obtained. The frequency of the sensor can be partly recovered. It can be tentatively explained by the washing of analyte from the binding sites. Every sensor can be used repeatedly more than 25 times.

Reproducibility of the Sensor. Because of using the LPD method, the amount of polymeric films can be controlled. This gives the possibility of reproducibility for the sensor, consequently. Three similar new QCMs are used to fabricate the sensor under the same conditions. The frequency shift of the sensor is about 1410 ± 120 Hz in the LPD experiment for 1 h. For the 50 μM GA solution, the average shift of the resonant frequency of the three sensors is 78 ± 13 Hz.

GA and Imprinted Titanium Oxide Film (i-TOF) Interactions. Information on the equilibrium is extracted by Scatchard analysis of the calibration curve, a tool already applied in MIP work.[3,28]

$$\mathrm{GA} + \text{i-TOF} \underset{k_2}{\overset{k_1}{\leftrightarrows}} \mathrm{GA{-}TOF}$$

It has been confirmed that the Sauerbrey eqaution is still valid if the viscosity and density of the liquid do not change

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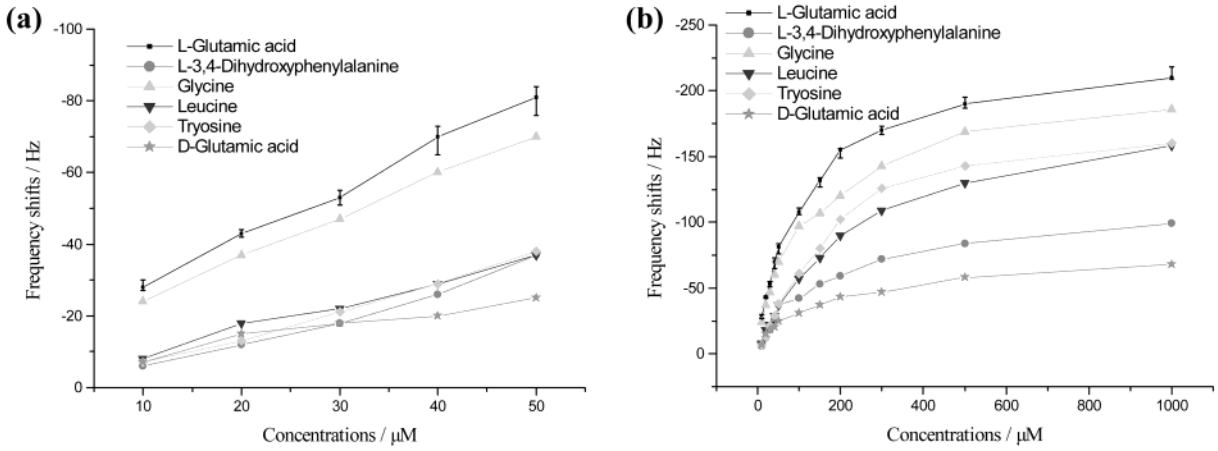

Figure 4. Corresponding frequency shifts of the sensors for the different concentrations GA, L-3,4-dihydroxyphenylalanine, glycine, tryosine, leucine, and D-glutamic acid, respectively. (a) The range of concentrations is 10−50 μM. (b) The range of concentrations is 10 μM−1 mM.

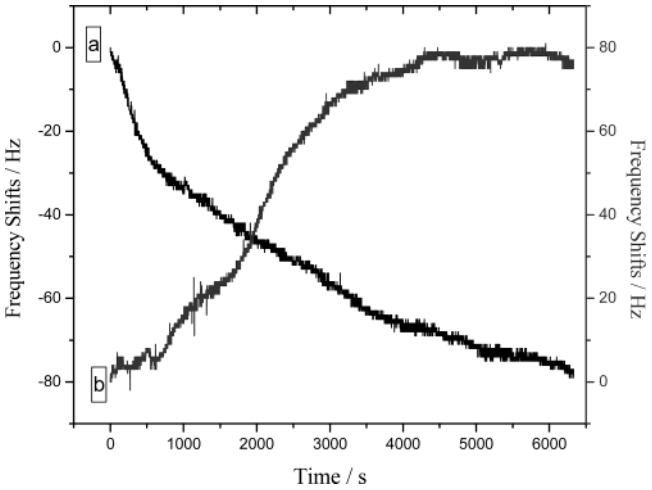

Figure 5. Regeneration of the imprinted sensor. (a) The frequency decrease during the binding of 50 μM GA. (b) The frequency recovery of the imprinted sensor after the binding of 50 μM analyte in a phosphate buffer solution.

during the QCM experiment in liquid.[29] That is,

$$\Delta f = -2\Delta m f_0^2/A(\eta_Q \rho_Q)^{1/2}$$

where A is the surface area of the electrode on the QCM. η_Q and ρ_Q are the elastic modulus and density of the quartz.

In this experiment, the changes in the densities and viscosities of the resultant solution by the injection of GA stock solution were infinitesimal for the lower concentration range (10−200 μM). It was, therefore, valid to apply the Sauerbrey equation in calculating the Δm on the electrodes. Ultimately, the Scatchard equation is converted to

$$-\frac{\Delta f}{[\mathrm{GA}]} = \frac{1}{K_D}\Delta f + a\frac{1}{K_D}[\text{i-TOF}]_0$$

where a is a constant, K_D is the dissociation constant of the complex GA−TOF, and [GA] is the concentration of free GA (approximated by the analytical concentration of GA).

A plot of the data in Figure 3 is reported in Figure 6. The linear fitting equation between 10 and 200 μM is described by $-\Delta f/[\mathrm{GA}] = 14\,585\Delta f + (2.82 \times 10^6)$. So,

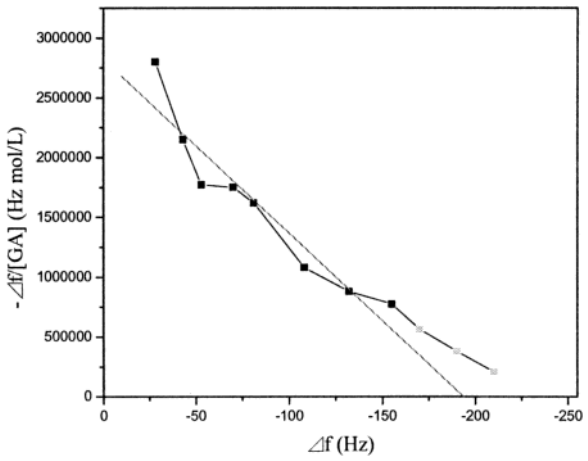

Figure 6. Scatchard analysis of the calibration curve in Figure 3.

the dissociation constant K_D of the GA−TOF is 68.6 μmol/L. It can be partly deduced that there exists a strong interaction between molecularly imprinted binding sites and the analyte.

Spectroscopic Characterization of i-TOF. XPS was employed to compare the chemical structure of the i-TOF with that of the nonimprinted TOF.

A remarkable N(1s) XPS spectrum appears after depositing the molecularly imprinted film. This finding agrees with the idea that GA can be integrated to the liquid phase deposited film. The relative height of the N(1s) peak decreases after washing the sensor to remove the imprinted molecule.

A further comparison has been performed after exposure of the film just described to 50 μM GA. Figure 7 shows the N(1s) XPS spectrum for an i-TOF after interaction with 50 μM GA. Because the content of titanium was kept invariable in all the experiments, the amount of imprinting recognition is evaluated by comparing the N/Ti values for the washed i-TOF sample and i-TOF−GA sample. The obtained difference is about 0.008. For the same GA concentration, the N/Ti value evaluated by the response for the imprinted sensor and by the deposited mass of the TiO_2 thin film (∼0.009) is in good agreement with the value estimated by the XPS method. A similar behavior is not obtained in the experiments involving nonimprinted TOF. It indicates that molecular recognition occurs during the exposure of the washed i-TOF to the template molecule.

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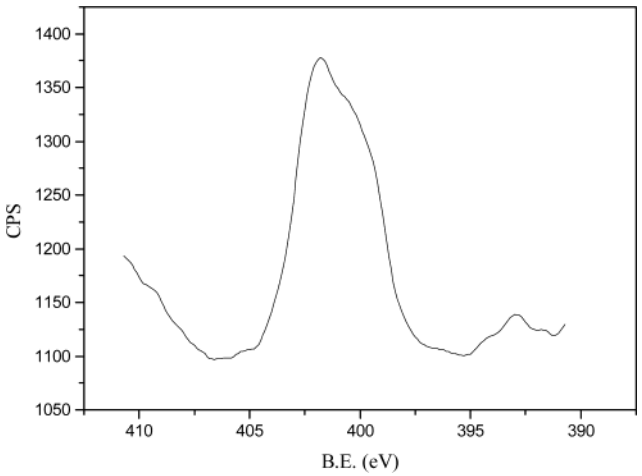

Figure 7. N(1s) XPS spectrum for an i-TOF after interaction with 50 μM GA.

Conclusions

In this work, we have developed a novel method for a GA sensor based on the molecularly imprinted TiO_2 LPD film. The assembled sensor exhibits good sensitivity, selectivity, and reproducibility for analyte by virtue of the interaction between the molecularly imprinted binding sites and the template. XPS characterization of the imprinted TiO_2 LPD film supports the feasibility of this method. Schatchard analysis shows the strong interaction between the imprinted sensor and the template molecule. The simplicity and efficacy of this method has profound application for the construction of an applied sensor and has enormous potential.

On this basis, we suggest that the proposed method will provide a significant easy procedure for the preparation of a sensor system with a desired selectivity and sensitivity.

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