Efficient One-Pot Synthesis of Mussel-Inspired Molecularly Imprinted Polymer Coated Graphene for Protein-Specific Recognition and Fast Separation

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Supporting Information

ABSTRACT: Molecular imprinting at nanomaterial surfaces has shown good prospects to extract templates easily and to achieve excellent performances such as large binding capacity and fast adsorption. In this work, we describe a one-step approach to synthesize a novel surface protein-imprinted nanomaterial employing graphene as the supporting substrate and dopamine as the polymerizing monomer. By simply immersing graphene oxide (GO) in a weak alkaline solution of dopamine (DA) containing bovine hemoglobin (BHb), GO nanosheet was readily converted to reduced GO (RGO) by dopamine with simultaneous capping by a thin polydopamine film imprinted with BHb leading to the BHb imprinted PDA@RGO nanomaterials. Fourier transform infrared (FT-IR), ultraviolet−visible (UV−vis), Raman spectra, X-ray diffraction (XRD), scanning electron microscopy (SEM), X-ray photoelectron spectroscopy (XPS), and nitrogen adsorption experiments have been used to characterize the resulting imprinted PDA@RGO. The whole reaction process was conducted in aqueous solution at ambient temperature, which is easy to scale up at a low cost without pollution. In addition, because of the unique properties of graphene (large surface area, high surface-to-volume ratio) and polydopamine (high biocompatibility and controllable thickness), the prepared imprinted PDA@RGO not only possessed high binding capacity (198 mg/g) but also exhibited a fast adsorption kinetics (adsorb 89% of the maximum amount within 5 min) and good selectivity toward template protein (the imprinting factor α is 4.95). The outstanding recognizing behavior coupled to the low production cost and facile, quick, green preparation procedure makes the imprinted PDA@RGO attractive in specific protein recognition and separation, biosensors, and biochips.

INTRODUCTION

Molecularly imprinted technique has become a powerful tool for the preparation of polymeric materials with special recognition capacity. The good mechanical/chemical stability, easy and cheap preparation, flexibility in choosing copolymerizing monomers, and low cost of molecular imprinting polymers (MIPs) render them promising alternatives to enzymes, antibodies, and natural receptors for use in areas including biosensors, bioseparation, medical diagnostics, catalysis, and drug delivery. Although the bulk MIPs prepared by the conventional method exhibit high selectivity, some disadvantages such as time-consuming and complicated preparation process, low-affinity binding, high diffusion barrier, low-rate mass transfer, and poor site accessibility still exist. Recently, combining surface imprinting with nanomaterials became an effective solution to solve the above-mentioned problems and to achieve excellent performances. Since nanostructured imprinted materials have a small dimension with extremely high surface-to-volume ratio, most template molecules are situated at or approximate to the materials surface, which are expected to possess several remarkable advantages over normal imprinted materials (e.g., more complete removal of templates, higher binding capacity, and faster binding kinetics). Some literature has reported molecular imprinting strategy at the surface of some nanosized substrates, including monodispersed silica nanoparticles, Fe₃O₄ nanoparticles, polystyrene core colloids, carbon nanotubes, and silicon nanowires.

Graphene, with extraordinary properties and two-dimensional structure, has enjoyed wide attention since Novoselov et al. first isolated single-layer samples from graphite in 2004. This unique nanostructure with extremely large specific surface area (theoretical value 2630 m² g⁻¹) and small dimension makes graphene an excellent candidate as supported material for preparing molecularly imprinted materials. The prepared MIPs over the surface of graphene would possess large surface area with most template molecules situated at the surface, which provides a high loading capacity, improves the

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accessibility to target species, and also reduces the binding time. In addition, the large delocalized electron system also provides graphene a strong affinity for carbon-based ring materials, which are widely present in drugs, pollutants, and biomolecules. Furthermore, graphene has unique electronic properties with low noise, which is beneficial for the fabrication of MIP electrochemical sensor with high sensitivity. In fact, MIP nanofilms have been successfully grafted on the two-dimensional structure of graphene or graphene oxide. At a weak alkaline pH solution, in which the substrates were immersed, dopamine underwent self-polymerization to produce an adherent PDA coating on the substrates with the accompanied oxidation of catechol groups to the quinone form. Recently, Kang et al. employed this procedure to immobilize a wide variety of molecules in PDA coating on many diverse surfaces by codissolving the molecules of interest with dopamine. Meanwhile, several successful examples of using PDA in molecular imprinting were reported by Zhou et al. to prepare novel protein-imprinted Fe3O4@PDA nanoparticles, but the binding capacity of this promising application of graphene as supporting material in the preparation of MIPs. However, because of the strong tendency of graphene sheets to agglomerate, surface modification of graphene should be performed to maintain its large surface area before MIP is synthesized on its surface. It means the preparation process of graphene−MIP composite needs at least two steps. In addition, the creation of MIP took place in organic solvent, which is quite disadvantageous for imprinting biological molecules such as protein.

Previously, Lee and co-workers proposed and developed a novel and important protocol for multifunctional coatings of thin, surface-adherent polydopamine (PDA) films onto a wide range of substrates on the basis of dopamine (DA) self-polymerization. At a weak alkaline pH solution, in which the substrates were immersed, dopamine underwent self-polymerization to produce an adherent PDA coating on the substrates with the accompanied oxidation of catechol groups to the quinone form. Recently, Kang et al. employed this procedure to immobilize a wide variety of molecules in PDA coating on many diverse surfaces by codissolving the molecules of interest with dopamine. Meanwhile, several successful examples of using PDA in molecular imprinting were published. PDA film has a cross-linked structure which could generate stable three-dimensional imprinting sites. In addition, the thickness of the PDA film, which decides the depth of the imprinted cavities, is in nanoscale range and could be adjusted by changing the polymerization time. Furthermore, its multifunctional groups (amino and catechol groups) and its properties of hydrophilicity and biocompatibility make it appropriate for imprinting bimacromolecules. Ouyang et al. reported a clever method for creating protein-imprinted PDA nanowires by using porous alumina as a sacrificial nanomold. However, this elegant method is limited by the multistep treatment of the alumina and the difficult control of the polymerization. Fe3O4 nanoparticles were used as the supporting material by Zhou et al. to prepare novel protein-imprinted Fe3O4@PDA nanoparticles, but the binding capacity of this valuable strategy was low. More recently, molecular imprinting at the surface of silicon nanowires (SiNWs) using DA as the monomer was also reported by Chen et al. The resulting imprinted silicon nanowires showed good performance such as fast adsorption kinetics, high selectivity, and large binding capacity. However, the synthesis of silicon nanowires is complicated and is of high cost which limits its large-scale application.

Considering the above-mentioned attractive properties of graphene and PDA in the preparation of MIP, it is quite expected that novel molecular imprinted materials with good performance could be obtained using graphene as supporting material and dopamine as polymerizing monomer. Herein, a new protein imprinted nanomaterial was fabricated combining the advantages of graphene and DA. By simply immersing graphene oxide in a weak alkaline solution of DA containing bovine hemoglobin (BHB), a thin adherent polypolydopamine (PDA) film imprinted with BHB was spontaneously obtained on the surface of graphene sheets to produce the BHB imprinted PDA@RGO nanomaterial. In this one-pot preparation procedure, DA not only acts as the polymerizing monomer but also as the reducing agent for graphene oxide (GO); thus, three reactions are involved: the reduction of graphene oxide to graphene sheets by dopamine, the simultaneous self-polymerization of dopamine to form PDA film on the in situ formed RGO sheets, and molecular imprinting of BHB in PDA film. This one-pot fabrication eliminates the use of toxic reducing agent and avoids the surface modification of graphene surface representing a rapid, efficient, and green approach to fabricate protein-imprinted nanomaterials. In addition, the polymerization and adsorption conditions were investigated in detail in order to obtain the highest selectivity and binding capacity. The prepared imprinted PDA@RGO exhibited fast binding kinetics, large binding capacity, and excellent selective recognition behavior toward template protein in aqueous solution. All these features make this simple procedure a potential route for the fabrication of low-cost and high-performance imprinted materials for protein-specific recognition and fast separation.

### EXPERIMENTAL SECTION

**Reagents.** Graphite powder, dopamine hydrochloride (DA), BHB, bovine serum albumin (BSA), lysozyme (Lyz), and egg albumin were purchased from Alladin. All other chemicals were of analytical grade and were used as received without further purification unless stated otherwise. Doubly distilled water was used throughout the work.

**Characterization.** UV−vis spectra were recorded on a TU-1901 spectro-photometer (Beijing Purkinje General Instrument Co., Ltd.). Fourier transform infrared (FTIR) spectra were recorded on an FTLA 2000-104 FTIR spectrophotometer (ABB Bomem, Canada). Raman was recorded with a Renishaw in Via Raman Microscope operating at 514 nm with a charge-coupled device detector. X-ray diffraction (XRD) profiles of Cu-modified graphene were obtained (XD-3A, Shimadzu) with high-intensity Cu Kα radiation (λ = 1.5406 nm). X-ray photoelectron spectroscopy (XPS) measurement was made on a VG ESCALAB Mkll spectrometer with a Mg Kα X-ray source (1253.6 eV photos). The X-ray source was operated at 14 kV and 20 mA. The morphologies of samples were determined using scanning electron microscopy (SEM) (Hitachi S-3700N, Tokyo, Japan). Thermal gravimetric analysis (TGA) was conducted on an SDT 2960 instrument from room temperature to 850 °C with a heating rate of 20 °C min−1 in the nitrogen flow (10 mL min−1). Nitrogen adsorption−desorption isotherms were measured using a Micromeritics ASAP 2050 system. Atomic force microscopy (AFM) images were taken in tapping mode with the SPM Dimension 3100 from Veeco.

**Synthesis of BHB Imprinted PDA@RGO.** Graphene oxide was synthesized from natural graphite following a method reported by Hummers and Oﬀeman. In a typical BHB imprinted PDA@RGO synthesis, GO (5 mg) was dispersed in 5 mL Tris buffer (10 mM, pH 8.0) by ultrasonication. BHB (5 mg) was then added. The mixture was mechanically stirred for 0.5 h at room temperature. Subsequently, DA (10 mg) was added, and the reaction was continued for another 5 h at room temperature. After reaction, the product was washed with water.
to remove the unreacted monomer, then was washed with a mixture of hydrochloric acid solution (1 mol/L) and methanol (1:4, v/v) to extract the template protein, and then was rewash
thoroughly with distilled water. For comparison, nonimprinted
PDA@RGO was prepared by the same procedure only without
the addition of the template protein. In addition, protein
extraction (template removal) process was also applied to
nonimprinted PDA/RGO composite to make it a good control
for specific protein absorption experiments.

Binding Experiments. All the binding experiments were
carried out in glass vials by using a batch technique. Before
binding experiments, a calibration curve was obtained from the
UV−vis absorption spectra of the BHb solutions with different
concentrations. For the kinetics experiments, 10.0 mg
imprinted PDA@RGO or control nonimprinted PDA@RGO
was ultrasonically dispersed in 20 mL of BHb solution for 1
min, and then the mixture was shaken for half an hour. Samples
were withdrawn at appropriate time intervals, and supernatant
liquid was separated by centrifugation at 10 000 rpm for 5 min.
For the adsorption isotherm experiments, an amount of 5 mg of
imprinted PDA@RGO or control nonimprinted PDA@RGO
was added to 20 mL BHb solutions of different concentrations.
After shaking at room temperature for 1 h, the solid and liquid
phases were separated by centrifugation at 10 000 rpm for 5
min. Then, the concentration of BHb in the supernatant was
measured by UV−vis absorption spectroscopy using the
characteristic peak of BHb (405 nm). The amount of BHb
adsorbed by the imprinted PDA@RGO or nonimprinted
PDA@RGO (Q, mg/g) was calculated from the differences in the
protein concentration before and after adsorption using the
following formula: \[ Q = \frac{(C_0 - C)V}{W} \]
where \( C_0 \) and \( C \) were the template concentrations (mg mL\(^{-1}\)) in the solutions which
were measured initially and after sorption, respectively, \( V \) (mL)
was the volume of the bulk solution, and \( W \) (g) was the weight
of the imprinted or nonimprinted PDA@RGO.

The selectivity of the imprinted PDA@RGO was investigated
using BSA, egg albumin, and Lyz as comparative proteins with
initial concentrations of 0.4 mg mL\(^{-1}\). The concentration of
comparative proteins in the supernatant was measured by UV−vis
absorption spectroscopy at peaks of 280 nm for BSA, egg
albumin, and Lyz.

\[ Q = \frac{(C_0 - C)V}{W} \]

## RESULTS AND DISCUSSION

Preparation and Characterization of BHb Imprinted PDA@RGO. Our fabrication procedure for the BHb imprinted
PDA@RGO is illustrated in Scheme 1. GO was first mixed with
BHb in Tris buffer (10 mM, pH 8.0), and DA was then added.
It was recently found that dopamine could be used simultaneously as a reducing agent for GO and as a capping
agent to stabilize and decorate the resulting reduced GO (RGO) because of its fascinating (reduction, self-polymerization,
and adhesion) properties.\(^{29−33}\) Thus, when DA was
mixed with GO in a weak alkaline solution containing BHbs, the
GO nanosheets could be readily reduced by dopamine
accompanied by the spontaneous deposition of a thin adherent
PDA film (from self-polymerization of dopamine) around the
resulting graphene sheet surface. Meanwhile, the template
proteins were embedded in the PDA film. The BHb imprinted
PDA@RGO material was eventually obtained after the removal
of the embedded template proteins. This one-pot synthesis
avoids the tediousness in the multiple procedures for the
previously reported preparation of graphene molecularly
imprinted polymer.\(^{16,17}\) In addition, the whole synthesis is
carried out in aqueous solution at ambient temperature which is
environmentally friendly.

During the preparation process, the dark brown graphene
oxide suspension turned into a black solution, the color
typically observed for a dispersion of graphene sheets,
indicating the reduction of GO to graphene by dopamine. In
our research, the simultaneous surface functionalization and
reduction of GO by PDA coating were evaluated by UV−vis,
FTIR, Raman, AFM, XRD, TGA, and XPS spectroscopy. For
comparison, the spectrum and image of GO was also shown. As
shown in Figure 1A, GO displays a maximum absorption peak
centered at 230 nm and a shoulder peak at about 300 nm. After
PDA modification, the characteristic absorption peaks of GO
disappeared indicating the reduction of GO. Meanwhile, two
new peaks at 280 and 208 nm because of the presence of PDA
were observed. The presence of PDA layer on the graphene
surface was further evaluated by the alkaline etching method (1 M NaOH).\(^{34}\) After the removal of PDA, the above two peaks
could not be detected. For the FTIR spectrum of BHb
imprinted PDA@RGO (Figure 1B), the appearance of two
typical absorption peaks of phenyl group at 1627 and 1543
\( \text{cm}^{-1}\) and the stretching vibration of \( -\text{NH}_2 \) at 3240 \( \text{cm}^{-1}\)
provide evidence of the formation of PDA layer. In addition,
compared to GO, the disappearance of the C\(=\text{O} \) peak at 1727
\( \text{cm}^{-1}\) provided a solid indication of GO reduction. The results
provide evidence for the conversion of GO to RGO and the
formation of PDA film on its surface.

The successful reduction of GO to graphene was also verified by Raman (Figure S1A of the Supporting
Information) and X-ray diffraction (XRD) (Figure S1B of the
Supporting Information) spectroscopies. The intensity ratio of
the D to G band \( (I_D/I_G) \), which reflects the graphitization
degree of carboxenous materials and the defect density,\(^{35}\)
increased from 1.1 for GO to 1.31 for PDA@RGO. This
change implied the creation of more small size of the in-plane
sp\(^2\) domains upon the reduction of GO to RGO by PDA
modification.\(^{36}\) The sharp XRD peak in GO (d-spacing of 0.96
nm at 2\(\theta\) = 9.2) decreased dramatically after reduction and a
new broad diffraction peak (d-spacing of 0.36 nm at 2\(\theta\) = 24.5),
which was closer to the typical diffraction peak of graphite (d-
spacing of 0.33 nm at 2\(\theta\) = 26.6),\(^{37}\) appeared in PDA/RGO
(Figure S1B of the Supporting Information).

As a sensitive surface analytical tool, X-ray photoelectron
spectroscopy (XPS) was used to analyze the surface chemical
composition of the PDA@RGO. As shown in Figure 2, the
survey spectrum of GO showed the C 1s peak at 285 eV and
the O 1s peak at 527 eV because of the existence of hydroxyl
groups and carboxylic groups on its surface, which is in
accordance with the literature (Figure 2, curve a). After the

Scheme 1. Preparation of BHb Imprinted PDA@RGO”

\( ^{”}(1) \) Self-polymerization of DA, (2) reduction of GO, (3) remove
protein molecules.
surface modification by PDA, a new peak of N 1s at 401 eV was clearly observed in the survey spectrum of PDA@RGO (Figure 2, curve b) demonstrating successful formation of PDA polymer layer on the surface of graphene. In addition, compared to GO, a significant decrease of XPS signals at 286.4 eV, which corresponds to C−O groups, was observed for PDA@RGO (Figure S2 of the Supporting Information) indicating the reduction of GO by dopamine. Moreover, the ratio of nitrogen to carbon (N/C) peak areas was 0.11, which was near the theoretical value of 0.125 of DA, indicating that graphene had been completely coated by the PDA polymer shell. The BHb imprinted PDA@RGO composite and GO were also characterized by thermogravimetric analysis. From the TGA results (Figure S2 of the Supporting Information), the weight loss of GO at 200 °C was about 30 wt %, which is due to the evaporation of adsorbed water and the decomposition of labile oxygen. In comparison, the weight loss of the PDA@RGO at 200 °C was much lower (about 10 wt %), which is likely due to the reduction of GO by the PDA coating. In addition, PDA@RGO composite showed more weight loss than GO upon heating to 850 °C further confirming the formation of PDA layer on graphene surface.

The morphological structures of GO and imprinted PDA/RGO were examined by SEM. GO shows a typically curved, layerlike structure with a fairly smooth surface (Figure 3a). It was quite different from Figure 3b where the BHb incorporated PDA/RGO had a rough surface indicating the deposition of PDA layer over the surface of graphene sheets. Furthermore, the removal of template molecules (Figure 3c) did not obviously change the surface morphology of PDA@RGO as compared with that of BHb incorporated PDA@RGO (Figure 3b) demonstrating the robustness of the PDA layer. These results also showed that the different recognition behaviors of BHb imprinted PDA@RGO toward the template proteins resulted from the efficient footprints and not from their morphological differences. AFM images were used to characterize the 2D surface morphology and the thickness of the pristine GO and PDA@RGO. As shown in Figure S4A of the Supporting Information, the representative AFM image and cross section analysis of GO clearly shows flat sheets with some wrinkles and an average thickness of about 1.02 nm. Compared with pristine GO, the PDA@RGO presents an apparently increased thickness of about 9.2 nm (Figure S4B of the Supporting Information), which demonstrates that the PDA layer of about 8 nm was attached to the GO surface successfully.

To further check the effect of template imprinting, nitrogen adsorption experiments of the imprinted PDA@RGO and control nonimprinted PDA@RGO were carried out. As shown
in Figure 4 and Table 1, although both samples showed the adsorption of nitrogen, a characteristic of a porous structure, because of the presence of imprinted cavities on the surface of BHB-imprinted PDA layer.

**Optimization of Preparation Conditions.** To obtain efficient protein recognition, the preparation conditions of BHB imprinted PDA@RGO, including the concentrations of monomer dopamine and the template molecule BHB, the polymerization time, and the washing time, were optimized as discussed below in detail.

To evaluate the influence of dopamine, 20 mg GO, 8 mg BHB, and different amounts of DA (ranging from 0 to 100 mg) were selected in the preparation of BHB imprinted PDA@RGO. As shown in Figure 5A, when the amount of DA was below 60 mg, the amount of BHB adsorbed \( (Q) \) increased with increasing monomer DA content. We presume that the increasing DA amount could increase the thickness of PDA film on the surface of RGO which can accommodate more BHB molecule and thus lead to the increase in the number of recognition cavities. However, a little decrease in the adsorption amount was observed with DA amounts above 60 mg, which may be because overthickness of the PDA film blocked the site accessibility and led to the decrease in the binding amount of BHB. Thus, an optimized DA content of 60 mg was selected.

Similarly, the effect of the amount of BHB was also investigated in the range from 8 to 18 mg, and the results are given in Figure 5B. The adsorption BHB amount increased with increasing template molecule content because of the increase in the number of recognition cavities. A maximum binding amount was achieved at the BHB content of 12 mg. When the BHB concentration was more than 12 mg, the imprinting efficiency of BHB was apparently weakened, which is attributed to the interaction and impact between template molecules at a higher concentration of BHB in the process of BHB-MIPs fabrication and may cause a decrease of valid recognition.

<table>
<thead>
<tr>
<th>sample</th>
<th>surface area (m²/g)</th>
<th>total pore volume (cm³/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>imprinted PDA@RGO</td>
<td>40.35</td>
<td>0.306</td>
</tr>
<tr>
<td>nonimprinted PDA@RGO</td>
<td>16.87</td>
<td>0.069</td>
</tr>
</tbody>
</table>

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Figure 4. Nitrogen adsorption isotherms of BHB on imprinted PDA@RGO (■) and control nonimprinted PDA@RGO (●).

Table 1. Surface Area and Total Pore Volumes Calculated from Nitrogen Adsorption–Desorption Isotherm

![Figure 5](image-url)

Figure 5. Effect of the amount of (A) DA, (B) BHB, (C) reaction time, and (D) washing time on the recognition behavior of the imprinted PDA@RGO toward BHB. The points represent mean values of three measurements.
cavities. Thus, the optimized template protein content was selected to be 12 mg.

To create more imprinted sites and to get rapid response, the PDA film thickness on graphene surface was adjusted via controlling reaction time. As shown in Figure 5C, the binding amount of BHb significantly increased with the increasing polymerization time and reached maximum at 8 h and then tended to decrease with the polymerization time extended, which may be because longer time would increase the thickness of the PDA film on the surface of graphene, and protein molecules are perhaps buried deep within overthick PDA layer and are difficult to extract to form effective recognition sites.

Extraction is a fairly important step for MIPs formation because of its direct influence on the rebinding capacity and selectivity to the template recognition. In our work, a mixture of hydrochloric acid solution (1 mol/L) and methanol (1:4, v/v) was used to extract the template molecules from the PDA layer. With the extraction going on, more and more template molecules were extracted from the PDA layer leading to an increasing number of imprinted cavities and thus to an increase in the rebinding amount of template protein. As shown in Figure 5D, at the extracting time of 3 h, the rebinding amount reached the maximum value. With prolonging extraction time, the imprinted cavities may be partly destroyed.

**Binding Analysis of Imprinted PDA@RGO.** The BHb recognition ability of the imprinted PDA@RGO was investigated by the static absorption experiments. The binding isotherms of BHb onto imprinted and nonimprinted PDA@RGO are shown in Figure 6. It can be seen that the imprinted PDA@RGO displayed much higher binding capacity toward BHb (curve a), and only small amounts of BHb were bound to control PDA@RGO (curve b) because of surface nonspecific adsorption confirming highly selective recognition ability toward template protein and the good preservation of the sterically complementary imprinted cavity structure of the template protein. In addition, the BHb bound by both imprinted and control nonimprinted PDA@RGO increased with the increase of initial concentration and achieved a saturation value when the BHb concentration reached 0.4 mg/mL. The maximum adsorption capacities are 198 mg/g for imprinted PDA@RGO, about 5 times higher than that of control nonimprinted PDA@RGO (40 mg/g). The adsorption capacities of the imprinted PDA@RGO in our work were superior to those of most imprinted polymers using BHb as the template as reported in the literature.39-45 This remarkably high binding capacity may lie in a combination of the thin PDA layers on the surface of graphene and the large surface area of graphene. Graphene has a small dimension with high surface-to-volume ratio, and thus, more recognition sites could be created at its surface, whereas the thin PDA layer ensures the complete removal of the template molecules. Furthermore, compared with the previously reported imprinted materials using DA as the monomer, the binding capacity of the imprinted PDA@RGO is much higher than those of protein-imprinted PDA nanowires9 and Fe₃O₄@PDA nanoparticles confirming the advantages of graphene as supporting material in the preparation of MIPs. Very recently, Chen et al. reported protein molecular imprinting on the surface of silicon nanowires, and the obtained imprinted nanowires had a large binding capacity of 213.7 mg/g.13 However, silicon nanowires need a complicated synthesis and complex equipment and, thus, are of high cost. In addition, graphene has high electrical conductivity and is an extremely low noise material electronically, which is beneficial for the fabrication of MIP electrochemical sensor with high sensitivity.16,17,46 Considering low production cost and good electronic property of graphene, our imprinted PDA@RGO material should be more applicable. To further study the adsorption mechanism, Langmuir isotherm equation is used to fit the experimental data. The Langmuir model is expressed as

\[
\frac{C_e}{q} = \frac{1}{Q_m k_L} + \frac{C_e}{Q_m}
\]

where \(C_e\) is the equilibrium concentration of BHb in the supernatant (mg/L), \(Q_m\) represents the maximum adsorption capacity of BHb, \(k_L\) is the Langmuir constant related to the energy of adsorption, and \(Q_e\) is the adsorption capacity at adsorption equilibrium. In the BHb concentration range studied, the Langmuir plot is linear indicating the presence of independent noninteracting sites conforming to the Langmuir model (Langmuir constant \(k_L\) 0.26 L/mg; the maximum adsorption \(q_m\) 212 mg/g; capacity correlation coefficients \(R^2\) 0.989), which should be attributed to homogeneous distribution of imprinting sites on the imprinted PDA@RGO surface.

The adsorption kinetics process of BHb was investigated by varying the adsorption time from 1 to 20 min, and the initial concentration of BHb was kept at 0.4 mg/mL. As shown in Figure 7, the BHb imprinted PDA@RGO took up 87% of the equilibrium amount of the template molecules from solution within a period of 5 min, and the adsorption equilibrium was reached within 10 min. The results revealed a remarkable rapid adsorption dynamics of BHb molecules to imprinted PDA@RGO considering that the imprinted materials using PDA reported previously often required more than 1 h to achieve the adsorption equilibrium. This fast dynamics adsorption might originate from the high ratio of surface-imprinted sites, large surface-to-volume ratio, and complete removal of the BHb templates. That is to say, a great number of effectively imprinted sites are at the surface or in the proximity of the imprinted PDA@RGO surface. Therefore, the templates can be easily removed or combined from the PDA layer in such a short time.

**Selectivity of the Imprinted PDA@RGO.** The selectivity experiments were carried out using BSA (\(M_w\) 68 kDa), egg albumin (\(M_w\) 45 kDa), and Lyz (\(M_w\) 14.4 kDa) as similarities.
These proteins have different molecular mass and volume. Figure 8 shows the rebinding capacities of the imprinted and nonimprinted PDA@RGO for these proteins with a feed concentration of 0.4 mg/mL. It is obvious that BHb imprinted PDA@RGO exhibits a much higher adsorption amount of BHb than other analogues suggesting a better adsorption and binding capacity toward the template molecules. In addition, the imprinting factor $\alpha$, taken as the binding ratio of imprinted PDA@RGO/control nonimprinted PDA@RGO, was determined as 4.95 for binding the template BHb, whereas the values of $\alpha$ for binding BSA, egg albumin, and Lyz were 1.58, 1.39, and 1.67, respectively, showing that the adsorbed amounts of the nontemplate protein did not have great differences between the imprinted PDA@RGO and the nonimprinted PDA@RGO. It is quite clear that whether from the point of the imprinting factor or the specific adsorption capacity, the imprinted PDA@RGO materials showed significant selectivity for the template BHb against other proteins.

The high adsorption selectivity and specificity of the imprinted PDA@RGO toward the template protein could be attributed to specific binding sites in the imprinted PDA layer on graphene surface imparted by the imprinting procedure. The specific binding sites involve two roles including multiple weak interactions (such as amino-containing, hydroxy-containing groups, $\pi-\pi$ bonds, and van der Waals forces) within the imprinted cavities for interaction with the template protein and the sterically complementary imprinted structure just fitting for the unique molecular structure of BHb. They have not only stronger binding force for template proteins but also steric effects hindering the adsorption of other nontemplate proteins, which led to the outstanding recognition behavior toward the target molecules. The imprinted sites formed in the polydopamine films were spatially oriented to BHb and were almost complementary to the BHb in terms of size and shape. Although the competitor proteins (BSA, egg albumin, and Lyz) have similar or even smaller weight with BHb, the microenvironment and the steric complementarity of imprinted cavity are not suitable for these proteins.

To prove the potential use of imprinted PDA@RGO in purification of target protein from the mixture, competitive binding experiments were performed. Three proteins including BSA, egg albumin, and Lyz were used as competing proteins in the coexistence of equivalent template (Figure 9). In the presence of competing proteins, the relative rebinding of template proteins to the imprinted PDA@RGO could still reach above 82% demonstrating that the imprinted PDA@RGO could be used for affinity material for purification of proteins.

**Regeneration and Stability of the Imprinted PDA@RGO.** Desorption and regeneration is one of the most important properties for the application of an MIP platform. The imprinted PDA@RGO could be regenerated after washing with a mixture of hydrochloric acid solution and methanol. To investigate the stability and regeneration of the imprinted PDA@RGO, the adsorption–desorption cycle was repeated four times using the same imprinted PDA@RGO sample. The result showed that the imprinted PDA@RGO was stable up to four adsorption cycles with less than 18% decrease in the binding capacity. The possible reason for the decrease in absorption is that some recognition cavities in the PDA layer might be deformed during the regeneration process, and thus, they no longer match the template molecules. The loss of imprinted PDA@RGO from the centrifugation process may also contribute to the decrease in adsorption capacity.

**CONCLUSIONS**

In summary, using graphene as the supporting material and dopamine as the monomer, a novel protein-imprinted nanomaterial has been successfully fabricated. The one-step preparation procedure is simple, efficient, environmentally friendly, and cost-effective.
friendly, and low cost, which involves the reduction of GO to RGO by dopamine, the self-polymerization of dopamine to form PDA layer on the surface of the RGO sheets, and molecular imprinting of BHB in PDA film simultaneously. The as-prepared BHb imprinted PDA@RGO, combining the advantages of graphene nanosheets and dopamine, showed high binding capacity and fast adsorption kinetics as well as good selectivity toward the template protein. The outstanding recognizing behavior coupled with the low cost and facile single-step preparation makes the imprinted PDA@RGO attractive for separation and specific protein recognition.

**ASSOCIATED CONTENT**

Supporting Information
Raman, XRD, XPS spectra, TGA curves, and AFM images of GO and the imprinted PDA@RGO. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes
The authors declare no competing financial interest.

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