Molecular Imprinting Based Hybrid Ratiometric Fluorescence Sensor for the Visual Determination of Bovine Hemoglobin

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S Supporting Information

ABSTRACT: We describe a simple and effective strategy to construct a molecular imprinting ratiometric fluorescence sensor (MIR sensor) for the visual detection of bovine hemoglobin (BHb) used as a model protein. The sensor was prepared by simply mixing the solution of green and red CdTe quantum dots (QDs), which were embedded in core-shell structured molecularly imprinted polymers and silica nanoparticles, respectively. The resultant hybrid MIR sensor can selectively bind with BHb and thus quench the fluorescence of the green QDs, while the red QDs wrapped with silica are insensitive to BHb with the fluorescence intensity unchanged. As a result, a continuous obvious fluorescence color change from green to red can be observed by naked eyes, with the detection limit of 9.6 nM. Moreover, the MIR sensor was successfully applied to determine BHb in bovine urine samples with satisfactory recoveries at three spiking levels ranging from 95.7 to 101.5%, indicating great potential application for detecting BHb in real samples. This strategy of using different fluorescence emission materials incorporated to construct a ratiometric fluorescence sensor is reasonable and convenient, which can be extended to the preparation of other ratiometric fluorescence systems for targeted analytes.

KEYWORDS: molecular imprinting, protein imprinting, ratiometric fluorescence, bovine hemoglobin, quantum dots

Hemoglobin (Hb) plays an important role in a variety of physiological activities. It is a conjugated iron-protein compound in red blood cells and participates in the transportation of oxygen, carbon dioxide, and nitric oxide.¹ A deficiency of hemoglobin can result in various diseases, such as anemia, thalassemia, and so on.² Thus, to analyze Hb is of important significance for clinical diagnoses, and biomedical and proteomics research. Because the similarity between bovine hemoglobin (BHb) and Hb is as high as 90%,^{2,3} BHb can be used as the target protein instead of Hb in the analysis of Hb. At present, protein detection from complex matrices is still a challenging issue. So, it is essential to ameliorate the detection selectivity toward target proteins.

Molecular imprinting technology is famous for its ability in providing and improving selectivity, which has been demonstrated by the creation of molecularly imprinting polymers (MIPs) with tailor-made binding sites complementary to the template molecules in shape, size, and functional groups.⁴ Owing to high selectivity, good physical stability, easy preparation, and low cost, MIPs have gained popularity in many fields, including sample pretreatment/chromatographic

separation and chemical/biological sensing.⁴ So far, the imprinting of small molecules has attained great success; however, the preparation of MIPs for biomacromolecules, like proteins, still remains challengeable due to the large molecular size, complexity, flexible conformation, and solubility.^{5,6} To solve the above problems, various strategies have been developed, such as surface imprinting,^{7,8} boronate affinity molecular imprinting,^{9,10} and epitope imprinting.^{11,12} Among them, surface imprinting is promising, because the imprinting cavities on or near the support surfaces can facilitate removal and adsorption of the template. Moreover, when combined with support nanomaterials, e.g., SiO₂ and quantum dots (QDs), the obtained MIPs not only get enhanced density of surface binding sites, but also have regular morphologies in favor of chemical modifications, rendering MIPs tailored for sensors.

Received: October 30, 2017

Accepted: January 16, 2018

Published: January 16, 2018

Scheme 1. Schematic Illustration of the Proposed Synthetic and Construction Strategy for MIPs Ratiometric Sensor

Development of MIPs based fluorescence sensors is of great concern by virtue of the combination high selectivity of MIPs with high sensitivity of fluorescence detection. Among fluorescent label materials, semiconductor QDs enjoy great popularity, thanks to their bright photoluminescence, narrow and symmetric emission with tunable colors, and high photostability.^{13–15} Some groups have reported QDs@MIPs fluorescence sensors for proteins, such as bovine hemoglobin,^{16–18} lysozyme,^{19,20} cytochrome *c*,^{12,21} and α -fetoprotein.^{22,23} Compared to conventional sole responsive fluorescence detection, ratiometric fluorescence technique using dual fluorescence signals has recently attracted increasing attention, since it can eliminate environmental effects and give improved sensitivity for trace level analytes.^{24–26} Excitingly, novel MIPs based ratiometric fluorescence sensors have been reported and displayed the potential in practical application. For example, Xu's and Chen's groups have respectively carried out related research on MIPs ratiometric fluorescence sensors for TNT,²⁷ melamine,²⁸ and bisphenol A,²⁹ and for phycocyanin³⁰ and 2,4-dichlorophenoxyacetic acid.³¹ These studies have confirmed that MIPs ratiometric fluorescence sensors can obtain high sensitivity and visual recognition by the naked eye.

In general, building ratiometric fluorescence sensors needs two individual materials with different fluorescence emission wavelengths. A simple approach is to use one emission peak as response signal to the target analyte and the other as reference signal, leading to the color change of the two emission fluorescent materials obviously and easily observed by the naked eye. Since the ratiometric fluorescence response relies on the fluorescence intensity of the two individual fluorescence materials, which is related to their amounts, it is imperative to optimize their proportions. However, optimization during the preparation process of core-shell MIPs ratiometric fluorescence sensors is usually hard to control for ideal color change, relatively time-consuming, and laborious.^{27,28,31}

Herein, we developed a simple and easy way to construct MIPs ratiometric fluorescence sensor (MIR sensor) for the visual detection of BHb, through simply mixing two solutions of green and red CdTe QDs, which were embedded in core-shell structured MIPs (CdTe@MIPs) and silica nanoparticles (CdTe@SiO_2), respectively. BHb as imprinting model protein demonstrated the proof-of-principle of our strategy. Two different sizes of CdTe QDs emitting green and red fluorescence were used as response signal and reference signal, respectively. Green CdTe@MIPs and red CdTe@SiO_2 were synthesized separately, and then they were mixed according to an appropriate proportion for obtaining the ratiometric fluorescent system. Upon the addition of BHb, CdTe@MIPs could bind BHb, resulting in the fluorescence intensity decreasing gradually with the increase of BHb concentration. While the fluorescence of CdTe@SiO_2 slightly fluctuated in the presence of BHb, which was negligible when compared with that of CdTe@MIPs . The variation of fluorescence intensity ratios resulted in a continuous fluorescence color change from green to red, allowing the visual detection of BHb. Such mixed-type construction effectively avoided time-consuming synthesis of composite nanoparticles, providing a simple, convenient and alternative construction approach to ratiometric fluorescence sensors.

■ EXPERIMENTAL SECTION

Reagents and Materials. Tellurium powder, cadmium nitrate ($\text{Cd}(\text{NO}_3)_2$), sodium hydroxide, ethanol, ammoniumhydroxide (25%), acetonitrile (ACN), tetraethoxysilane (TEOS), 3-aminopropyltriethoxysilane (APTES), 3-mercaptopropyltrimethoxysilane (MPS), and phosphate buffered saline (PBS) were supplied by Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China). Sodium borohydride (NaBH_4), bovine hemoglobin (BHb), bovine serum albumin (BSA), lysozyme (Lyz), protamine, and thioglycolic acid (TGA) were purchased from Sigma-Aldrich (Shanghai, China).

Instrumentation. Fluorescence spectra were recorded by using a Fluoromax-4 spectrofluorometer (Horiba Scientific). The morphological evaluation was taken on a transmission electron microscope (TEM, JEM-1230, operating at 100 kV) and scanning electron microscope (SEM, Hitachi S-4800 FE-SEM, operating at 5 kV). FT-IR analyses were recorded by using a FT-IR spectrometer (Thermo Nicolet Corporation, USA). Zeta potential and dynamic light scattering (DLS) tests were performed on a Malvern Zetasizer Nano-ZS90 (ZEN3590, UK). Elemental analysis was measured using a Vario Microcube element analyzer (Elementar Company, Germany).

Preparation of Red CdTe QDs Embedded Silica Nanoparticles (CdTe@SiO₂). TGA-modified red CdTe QDs were obtained according to our previous synthesis procedure for QDs,¹⁵ and changing the refluxing time from 2 to 48 h. Then, CdTe@SiO₂ was synthesized by sol–gel process according to the reported method with some modifications²⁶ as below. Typically, 40 mL of ethanol and 5 mL of red CdTe QDs solution were mixed and stirred for 10 min. Then 20 μ L of MPS was added into the above solution. After stirring for 12 h, 200 μ L of NH₃·H₂O and 200 μ L of TEOS were added and stirring continued for 5 h. Finally, the products were centrifuged and washed with ethanol and ultrapure water.

Preparation of BHB Imprinted Nanoparticles (CdTe@MIPs). TGA-modified green CdTe QDs were synthesized according to our previous procedure,¹⁵ and SiO₂ nanoparticles were synthesized according to our report.³⁰ Then, 2 mL of the green CdTe QDs was added to 18 mL of ultrapure water containing 800 μ L of 12.5 mg/mL SiO₂. After stirring for 30 min, 10 mg of BHB and 20 μ L APTES were added into the above solution; after stirring for 1 h, 40 μ L of NH₃·H₂O and 40 μ L of TEOS were added and stirring continued overnight in the dark. Finally, the resultant CdTe@MIPs were centrifuged and washed with ethanol/acetonitrile (8:2, v/v) to remove BHB, and then washed with ultrapure water three times. As a control, its corresponding nonimprinted polymers (CdTe@NIPs) were synthesized using the same procedure without the template BHB.

Construction of the Molecular Imprinting Ratiometric Fluorescence Sensor (MIR Sensor). To obtain the MIR sensor for BHB determination, 100 μ L of CdTe@MIPs solution (2 mg/mL) and 20 μ L of the CdTe@SiO₂ solution were mixed thoroughly in 1 mL of PBS solution. The fluorescence intensity of the MIR sensor was evaluated under a single wavelength excitation at 420 nm with a recording emission range of 440–700 nm.

Analysis of Real Samples. Urine samples were randomly collected from a healthy bovine from a rural household (Local in Yantai City, China). The real urine samples were filtered with a 0.45 μ m microfiltration membrane to remove any possible particulate suspension, and then they were diluted 100-fold for spiking. Recovery experiments were performed by using the spiked BHB at three concentration levels of 0.3, 1.0, and 2.0 μ mol L^{−1}, and then these samples were analyzed using the MIR sensor. All the tests were performed in triplicate.

RESULTS AND DISCUSSION

Construction and Characterization of Ratiometric Fluorescent Sensor. The synthesis of CdTe@MIPs, CdTe@SiO₂ and the construction of the dual emission fluorescence nanohybrid for visual detection of BHB were illustrated in Scheme 1; CdTe@MIPs and CdTe@SiO₂ were synthesized separately (Scheme 1A) and then mixed in an appropriate proportion (Scheme 1B). By using silica nanoparticles as support for surface imprinting, BHB as imprinted template, APTES as functional monomer, TGA-modified green CdTe QDs as assistant functional monomer, and TEOS as cross-linker, the core–shell structured CdTe@MIPs were prepared. The as-prepared CdTe@MIPs exhibited an emission maximum at 541 nm with a strong green fluorescence (Figure 1a). CdTe@SiO₂ prepared by sol–gel process displayed an emission maximum at 647 nm with a strong red fluorescence (Figure 1b). When CdTe@MIPs were mixed with CdTe@SiO₂

Figure 1. Fluorescence emission spectra ($\lambda_{\text{ex}} = 420$ nm) of (a) CdTe@MIPs, (b) CdTe@SiO₂, and (c) MIPs ratiometric sensor, respectively. The inset shows the corresponding fluorescence images under a 365 nm UV lamp.

in a certain proportion, producing an appropriate fluorescence color ratio, a new MIR sensor was obtained, which displayed dual fluorescence emission peaks at 541 and 647 nm under a single excitation at 420 nm (Figure 1c). Since there were recognition sites on the surface of CdTe@MIPs, BHB could enter the recognition sites, quenching the fluorescence of the green QDs. The fluorescence emission spectrum of CdTe@MIPs had no spectral overlap with the absorption spectrum of BHB (Figure S1), so the fluorescence quenching of QDs could be ascribed to the electron transfer between QDs and BHB. With the presence and increase of BHB, the green fluorescence of QDs was quenched gradually. However, the red QDs wrapped with silica would prevent the direct contact with the external BHB. There are no carboxyl and amino groups on the surface of CdTe@SiO₂, so red fluorescence of QDs was insensitive to BHB. As a result, a variation of fluorescence intensity ratios was shown and a distinct fluorescence color change from green to red could be used for the ratiometric detection of BHB.

The morphological structures of SiO₂, CdTe@MIPs, CdTe@NIPs and CdTe@SiO₂ were investigated by TEM and SEM. As shown in Figure 2, SiO₂ nanoparticles had good dispersion with an average diameter of 50–60 nm and their surface was very smooth (Figure 2a, d). The CdTe@MIPs and CdTe@NIPs had similar morphologies and displayed rough polymeric surface (Figure 2b, c, e, and f). The MIPs layer could be estimated to <10 nm in thickness calculated from the TEM image. Besides, CdTe@SiO₂ also had good dispersion with an average diameter of 30–40 nm. The size distributions of SiO₂, CdTe@MIPs, and CdTe@NIPs were obtained by DLS measurement as shown in Figure S2. As seen, the intensity contribution versus diameters of the above nanoparticles showed good size-distribution and the main distribution peak of SiO₂ was about 54 nm, and the dominant distribution peak of CdTe@MIPs and CdTe@NIPs was around 60 nm, which were consistent with TEM results. These indicated that they could well disperse in aqueous solution.

Furthermore, elemental analysis was performed to examine the nitrogen, carbon, hydrogen and sulfur content (Table S1). As seen, there was no sulfur element in SiO₂; however, it existed in CdTe@MIPs and CdTe@NIPs, which indicated that TGA-QDs were successfully introduced. The marked enlarge-

Figure 2. TEM and SEM images of the synthesized (a, d) SiO_2 , (b, e) CdTe@MIPs , (c, f) CdTe@NIP , and (g) CdTe@SiO_2 .

ment in the nitrogen atomic composition from 0.411% for SiO_2 to 2.7785%, 2.486% for CdTe@MIPs and CdTe@NIPs , respectively, which revealed that amine groups were successfully introduced onto the surface of SiO_2 . Besides, the carbon and hydrogen atomic compositions for CdTe@MIPs and CdTe@NIPs also increased significantly compared with SiO_2 and the results indicated that the existence of carboxyl groups in the CdTe@MIPs and CdTe@NIPs . Overall, the CdTe@MIPs and CdTe@NIPs were successfully prepared.

The successful preparation of CdTe@MIPs and CdTe@NIPs was also proved through the existence of carboxyl groups and amino groups as shown in the FT-IR spectra (Figure S3). As seen, the peak at 1469 cm^{-1} was the characteristic peak of the propylamino groups. The peaks at 1545 and 1388 cm^{-1} quite possibly corresponded with the antisymmetrical and symmetric vibrations of COO^- , respectively.³² All these characteristic bands in the spectra suggested the existence of TGA-QDs and APTES in the CdTe@MIPs and CdTe@NIPs .

The surface charge of the synthesized SiO_2 , CdTe@MIPs and CdTe@NIPs particles was measured by a zeta potentiometer. The surface charge of SiO_2 particles is -35 mV , and the surface charge of CdTe@MIPs and CdTe@NIPs was 27.8 mV and 32.5 mV , respectively. Because the surface of SiO_2 is mainly hydroxyl, which makes its surface with a strong negative power. After the modification of SiO_2 with amino groups, the shell is strong positive due to amino groups with positive. All above results indicated CdTe@MIPs and CdTe@NIPs was successfully synthesized.

Ratio Optimal for CdTe@MIPs and CdTe@SiO_2 in the MIR Sensor. The ratiometric fluorescence response of the MIR sensor relied on the fluorescence intensity at 541 and 647 nm , which was related to the proportion of CdTe@MIPs and CdTe@SiO_2 , respectively. When the ratio of CdTe@MIP and CdTe@SiO_2 was $10:1$, it could only lead to a fluorescent color change from green to orange upon addition of BHb (Figure S4A). A wide color change from green to red was obtained for the MIR sensor with a ratio of $5:1$ (Figure 4A). Continuous increase of the ratio to $10:3$ only resulted in color change from yellow to red (Figure S4B). Thus, the hybrid consisting of $5:1$ of CdTe@MIPs and CdTe@SiO_2 was selected to build the MIR sensor for BHb detection.

Condition Optimization for the MIR Sensor. Fluorescence stability, pH and response time were three major

parameters, which could highly influence the fluorescence properties of the MIR sensor, and optimized as below. The stability of the MIR sensor was tested by fluorescence spectra. The fluorescence intensity ratios (I_{541}/I_{647}) of the ratiometric sensor remained almost unchanged over 2 h (Figure S5), displaying its good fluorescence stability. The influence of pH was examined by $(F_0 - F)/F$ in the range of 5.0 – 9.0 , as shown in Figure S6; herein, F_0 and F stand for the fluorescence intensity of green QDs in the absence and presence of the BHb, respectively. It was seen from the figure that the quenching efficiency was affected at different pH values. As pH increased, the quenching efficiency first increased and then decreased. Under pH 8.0 , the quenching efficiency was the maximum, possibly owing to the same condition as CdTe@MIPs synthesis. At the pH of 8 , BHb with negative charges most readily bound with amino groups of APTES, just as Turner et al. reported,³³ “optimum binding occurs when polymer is exposed to the same conditions as those used for polymerization”. Consequently, the most remarkable quenching occurred. Besides, under high or low pH, BHb may be denatured. So, the optimum pH of 8 was used for the following work.

Furthermore, to investigate the kinetics of the MIR sensor, the fluorescence intensity at about 541 nm was recorded at different reaction time after adding BHb (Figure S7). With the addition of BHb, the fluorescence intensity at about 541 nm was decreased rapidly and then kept unchanged in less than 10 min , demonstrating that BHb could quickly reach the imprinted cavities, which could well attribute to the ultrathin imprinting layer. Hence, 10 min was chosen as the reaction/incubation time.

Sensitivity and Selectivity of the MIR Sensor. The sensitivity of the MIR sensor was evaluated upon addition of different amounts of BHb. As seen from Figure 3A, the fluorescence intensity of the green QDs gradually decreased along with increasing the concentration of BHb, while the fluorescence intensity of the red QDs kept almost unchanged. Owing to the red QDs as reference, with the decrease of green QDs intensity, the ratios of the two fluorescence intensities varied, leading to a distinguishable fluorescence color change from light green to orange red and to red, which could be clearly observed by the naked eye. As can be seen in Figure 3a, a good linear relationship was obtained by plotting the ratio of

Figure 3. Fluorescence spectra and corresponding fluorescence intensity ratio of (A,a) MIPs ratiometric sensor, (B,b) only CdTe@MIPs, and (C,c) NIPs ratiometric sensor upon the addition of different amounts of BHb; The ratio of CdTe@MIPs or CdTe@NIPs and CdTe@SiO₂ is 5:1. The images in the bottom are their corresponding fluorescence images taken under a UV lamp (365 nm). The concentrations of BHb from left to right are 0, 0.05, 0.1, 0.3, 0.5, 0.75, 1, 1.5, 2, 2.5, and 3 μM , respectively.

the green and red QDs fluorescence intensity $(I_{541}/I_{647})_0/(I_{541}/I_{647})$ versus the concentrations of BHb within 0.050–3.0 μM . The limit of detection (LOD) was down to 9.6 nM, on the basis of $3\sigma/s$, in which σ means the standard deviation of the

blank measurements, and s means the slope of calibration curve.³¹

In order to demonstrate the advantages of the MIR sensor, single-green emission of CdTe@MIPs was determined similarly. As seen in Figure 3B, with the addition and increase

Figure 4. Selectivity of MIPs ratiometric sensor and NIPs ratiometric sensor for BHB over other analogues including BSA, Lyz, and Protamine at concentration of 1 μ M.

Table 1. Results of Spiked Recoveries and RSDs (%) for Determination of BHB in Bovine Urine Samples Using the Constructed MIR Sensor

sample	added (μ M)	found (μ M)	recovery $\pm$ RSD (%)
bovine urine	0	0	
	0.3	0.287	95.7 $\pm$ 3.6
	1.0	0.981	98.1 $\pm$ 3.1
	2.0	2.03	101.5 $\pm$ 4.2

of BHB, the fluorescence intensity of green QDs decreased, resulting in the color change of the green QDs fluorescence quenching being hard to distinguish by the naked eye compared with that of the MIR sensor. Moreover, as displayed in Figure 3b, the single fluorescence quenching of CdTe@MIPs sensor was less sensitive than that of the MIR sensor for the visual detection of BHB.

The sensitivity of the NIPs ratiometric sensor was also examined as shown in Figure 3C. The green fluorescence intensity of NIPs ratiometric sensor could also be quenched with the addition of BHB; however, the decrease of fluorescence intensity was not obvious compared with the MIR sensor under the same concentration of BHB, which could be attributed to that there no specific recognition site was in the NIPs. As a result, the decrease of green fluorescence intensity was not large enough to change colors and it was hard to be distinguished by the naked eye. Accordingly, a high imprinting factor was obtained of 6.8, calculated as the ratio of the slopes of linear relationship of ratiometric MIPs and NIPs.

In the meanwhile, the selectivity of the MIR sensor for BHB was also assessed by recording the fluorescence intensity ratios (I_{541}/I_{647}) of the sensor at the same concentration with other proteins (BSA, Lyz, Protamine). As shown from Figure 4, the

fluorescence intensity at 541 nm of the MIR sensor was highly quenched by BHB and orange red fluorescence color was present. Conversely, the value of I_{541}/I_{647} and the fluorescence color have no obvious change with other proteins presence at 1 μ M. The reason is mainly because that the MIR sensor has specific recognition sites in shape, size, and functional groups with the template BHB, and can easily bind with imprinted sites, quenching the fluorescence of green QDs. Whereas, other proteins were not complementary to the specific recognition sites and thereby could not effectively influence the fluorescence intensity of green QDs. The results suggested the MIR sensor had good selectivity and specificity for BHB.

Practical Applicability and Method Performance Comparison. The practical applicability of the MIR sensor was further studied in urine sample spiked with different concentration of BHB. It was clearly shown in Table 1 that the recoveries ranged from 95.7–101.5% with relative standard deviations (RSDs) of 3.1–4.2% by the MIR sensor. So, the MIR sensor was demonstrated practically applicable and reliable for sensitive and accurate determination of low BHB contents in real samples.

The strategy and performance of our developed MIR sensor for detection of BHB was compared with other different MIPs-based fluorescence detection approaches, as listed in Table 2. As seen, most fluorescence sensors for BHB based on molecular imprinting have been designed with only one fluorescence emission wavelength^{6,16–18,34,35} and the detection limits are slightly higher compared with ratiometric fluorescence systems. Besides, by ratiometric fluorescence technique, visual detection and analysis of BHB can be achieved.³⁶ However, the synthesis process of core–shell composite MIPs ratiometric fluorescence sensor is tedious and time-consuming, since the amount of two different fluorescent materials needs to be optimized. Interestingly, our presented strategy avoided complicated synthesis process, just needed to mix the as-prepared MIPs and CdTe@SiO₂ within 10 min for forming dual-emission fluorescence sensor.

CONCLUSIONS

In summary, we have successfully developed a novel MIR sensor by using CdTe QDs with two different fluorescence colors for the detection of BHB. The green CdTe QDs embedded in MIPs was used as responsive signal while the red QDs wrapped in SiO₂ was used as reference signal. The sensor relied on the mixture of the two kinds of QDs in a certain proportion. In the presence of BHB, the green MIPs could bind with BHB, leading to fluorescence quenching whereas the red QDs fluorescence remained constant. Thus, the sensor exhibited high selectivity and reliability of visual detection and provided a practical solution for selective recognition and

Table 2. Comparison of Analytical Performances with Other Reported MIPs Fluorescence Sensors for BHB Detection

system	fluorescence mode	linear range	detection limit	ref
UCNPs/MOFs/MIP	single	0.1–0.6 mg/mL	0.062 mg/mL	34
Mn-doped ZnS QDs/MIP	single	0.1–5 μ M	0.038 μ M	16
Mn-doped ZnS QDs/MIP	single	0.1–10 μ M	0.032 μ M	17
CdTe/thermosensitive MIPs	single	0.28–4 μ M	0.16 μ M	18
Si-NP/CdTe/MIP	single	0.02–2.1 μ M	9.4 nM	6
BHB CdTe/MIPs	single	0.3–5.0 μ M	0.069 μ M	35
CdTe@SiO ₂ /CdTe/MIPs ratiometric fluorescence sensor	dual (ratiometric)	0.02–2.0 μ M	6.3 nM	36
CdTe@MIPs + CdTe@SiO ₂ ratiometric fluorescence sensor	dual (ratiometric)	0.05–3 μ M	9.6 nM	this work

detection of target protein in biological samples. By virtue of the reasonable mixing of different fluorescence emission materials and fully utilizing the technical superiority of molecular imprinting and ratiometric fluorescence, such type of simple, convenient, and universal strategy would promote the development of MIR sensors for wide applications in separation science, proteomics, and chemo/biosensing.

■ ASSOCIATED CONTENT

■ Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acssensors.7b00804.

Fluorescence emission spectra of CdTe@MIPs (blue) and absorption spectra of BHb (red); statistic particle size distribution of SiO₂, CdTe@MIPs, and CdTe@NIPs; FT-IR of SiO₂, CdTe@MIPs, and CdTe@NIP; ratiometric fluorescence response of the MIR sensor upon addition of different amounts of BHb; ratios of CdTe@MIPs and CdTe@SiO₂; stability of the fluorescence intensity of MIR sensor in 2 h; effect of pH value on quenching efficiency of MIR sensor in the presence of BHb at concentration of 1 μM; fluorescence response time of MIR sensor for BHb at concentration of 1 μM; fluorescence intensity recorded at the wavelength of 541 nm; atomic composition ratios (%) of SiO₂, CdTe@MIPs, and CdTe@NIPs from elemental analysis (PDF)

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The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This work was financially supported by the National Natural Science Foundation of China (21477160, 21575159, 81573393), the Science and Technology Development Plan of Shandong Province of China (2014GGF01074), the Youth Innovation Promotion Association CAS (2017256), the Defense Science and Technology Innovation Fund of the Chinese Academy of Sciences (CXJJ-17-Q148), and the Instrument Developing Project of the Chinese Academy of Sciences.

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