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A biosensing platform for sensitive detection of concanavalin A based on fluorescence resonance energy transfer from CdTe quantum dots to graphene oxide

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In this paper, we constructed a novel fluorescence resonance energy transfer (FRET) system between CdTe quantum dots (QDs) and graphene oxide (GO) for sensitive detection of concanavalin A (Con A). In this system, CdTe QDs as an energy donor and GO as an energy acceptor were both covalently functionalized by glucosamine via an amide linkage. The specific combination of Con A with glucosamine could bring CdTe QDs and GO into appropriate proximity and therefore induce efficient energy transfer. The concentration of Con A caused the modulation of the energy transfer efficiency between GO and QDs, thus enabling the Con A detection. Under the optimized experimental conditions, the decrease of fluorescence intensity ratio I/I_0 (I_0 and I were the fluorescence intensity of the sensing system in the absence and presence of Con A, respectively) is proportional to the concentration of Con A in the range of 9.8-196.1 nM. This method has high sensitivity with the detection limit as low as 3.3 nM. The results suggested that the graphene FRET platform could be used for sensitive and selective sensing of Con A and could be applied for protein-carbohydrate studies.

Key words: Graphene oxide; CdTe quantum dots; Fluorescence; Concanavalin A.

Introduction

Graphene with fascinating thermal, electronic and mechanical properties has attracted persistent attention in nanoelectronic devices, electron transportation and supercapacitor since its discovery. [1-3] Graphene oxide (GO), an intermediate obtained during the graphene preparation by modified Hummer's method, has numerous kinds of hydrophilic functional groups on its surfaces and edges, such as epoxyl, carboxyl and hydroxyl groups to enable its high water dispersibility and facile surface modification. [4-7] Semiconductor quantum dots (QDs), a type of promising fluorescent materials, have been widely used as effective energy transfer donor due to their unique optical properties such as high quantum yield, narrow emission peaks and tunable size-dependent emission. [8, 9] In the previous studies, scientists have devoted their attentions to develop fluorescence assays based on the high efficiency energy transfer between GO and fluorescent dyes, such as DNA assays, [10-12] proteins assays, [13, 14] and other molecules. [15-17] Researchers discovered that GO also had an extraordinary quenching efficiency for various kinds of quantum dots, such as CdSe, CdTe and CdSe–ZnS. [18-21] For exemple, Li *et al.* reported a fluorescence method for sensitive detection of matrix metalloproteinase and thrombin activity using a protease substrate peptide as the linker between the donor (CdSe–ZnS QDs) and the acceptor (GO). [18] The high efficiency of GO in quenching the donor emission make it a perfect selection to develop new fluorescence sensors in analytical chemistry.

Recently, a class of proteins that could interact with many carbohydrates by means of specific molecular recognition came into the forefront of biological research, such as concanavalin A (Con A) and peanutagglutinin (PNA). [22-27] It is well known that concanavalin A is a legume lectin from Jack beans containing four identical binding sites. Each monomer subunit of Con A is approximately 4×4×4 nm with a single small binding site capable of binding to α-D-glucopyranose with unmodified

hydroxyls at positions C3, C4 and C6. [28-30] In addition, Con A has been shown to interact with many mannosyl and glucosyl residues of polysaccharides, such as dextran, β-cyclodextrin, chitosan and glycodendrimer. [25, 31-34] Carbohydrate recognition is predominant in many important functions, such as cell-surface recognition, host pathogen interactions, cell-cell interactions and immune response. Studying on the carbohydrate-protein interactions have been developed because it is the foundation of the pathogen detection and prevention of bacterial infection. Although various methods have been widely used for Con A detection, some of them are time-consuming, technical expertise and low sensitivity. [35-39] Thus, fluorescence methods have been devoted to overcome such shortcomings due to their simplicity, convenience and high sensitivity. The fluorometric assays for lectin detection were generally based on the agglutination of sugar-QDs, [27, 40] for example, Badu and co-workers reported a assay for lectin detection through the agglutination of carbohydrates stabilized CdSe-ZnS QDs. [40] Few methods had been exploited with the combination of CdTe QDs and GO to develop for fluorescence Con A determination.

The previously reported FRET models based on graphene oxide as a fluorescence quencher most rely on the π-π stacking interaction. In this work, we constructed a different model in which donor and acceptor were brought into FRET proximity through specific molecular recognition. The present fluorescence sandwich assay for Con A detection was based on the FRET between CdTe quantum dots and graphene oxide (GO). In this system, Con A was firstly interacted with glucosamine functionalized GO (GO-G) to form GO-G/Con A hybrid, and subsequently the free identical binding sites of Con A specifically combined with glucosamine modified QDs (QDs-G) to form a sandwich configuration (GO-G/Con A/QDs-G), which allowed QDs-G and GO-G to be in appropriate proximity. Then the fluorescence of QDs-G could be quenched by GO-G, and therefore assembled an

efficient FRET biosensing system for Con A detection. Compared with the previous reports, the proposed sandwich method is simpler and more sensitive and can possibly provide a versatile tool for the analysis of different kinds of lectins containing several binding sites with high sensitivity just by exchanging the carbohydrates functionalized on the QDs and GO.

2. Experimental

2.1 Reagents and chemicals

All chemicals used were of analytical reagent grade and used without further purification. Graphite powder and Tris(hydroxymethyl)aminomethane (Tris) were attained from Sinopharm Chemical Reagent Co. Ltd. (China). Tellurium powder (200 mesh, 99.8%), cadmium chloride ($CdCl_2$, 99+%), Mercaptopropionic acid (MPA) (99%), glucosamine and Con A were obtained from Sigma-Aldrich Chemical Co. 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) and *N*-Hydroxy-sulfosuccinimide (NHS) were purchased from Aldrich Chemical Co. Ltd. Bovine serum albumin (BSA), trypsin, pepsin, human serum albumin (ALH) and lysozyme (Lys) were attained from Sino-American Biotechnology Co. Ltd. The water used in all experiments had a resistivity higher than 18 MΩ cm^{-1}.

2.2 Instrumentation

The FT-IR spectra were recorded on a Nicolet 400 Fourier transform infrared spectrometer. Fluorescence measurements were performed on a Shimadzu RF-5301 PC spectrafluorophotometer with a 1 cm path-length quartz cuvette. The fluorescence spectra were recorded with the excitation wavelength of 400 nm. The slit widths of the excitation and emission were 5, 10 nm, respectively.

2.3 Synthesis of graphene oxide

Graphene oxide was prepared by a modified Hummer's method and characterized in our previous

work [41]. Briefly, graphite powder (2 g) was added to 98% H_2SO_4 (12 mL) consisting of $K_2S_2O_8$ (2.5 g) and P_2O_5 (2.5 g) and reacted for 4.5 h at 80 ℃. The mixture was then diluted with 0.5 L water. Then, the mixture was filtered and washed with water to remove the residual acid. The product was dried under ambient conditions overnight. This pre-oxidized graphite was stirred in 98% H_2SO_4 (120 mL), $KMnO_4$ (15 g) was gradually added while keeping the temperature < 20 ℃. This mixture was stirred at 35 ℃ for 30 min and 90 ℃ for 90 min. Afterwards, the mixture was diluted with water (250 mL) and kept at 105 ℃ for 25 min. After the resulting mixture was stirred for 2 h, the reaction was terminated by addition of 0.7 L water and 20 mL of 30% H_2O_2. For purification, the mixture was filtered and washed with 1:10 HCl aqueous solution and water many times. Finally, the product was further purified by dialysis for 1 week to remove the remaining metal species. The obtained dispersion was centrifuged at 10000 rpm to remove any unexfoliated GO. The supernatant solution was collected and freezed dried. The GO powder was redispersed in water to obtain 0.5 mg mL^{-1} GO stock solution.

2.4 Synthesis of MPA-capped CdTe quantum dots

CdTe QDs were synthesized by refluxing routes as described in detail in our previous paper. [42] Briefly, the precursor solution of CdTe QDs was formed in water by adding fresh NaHTe solution to $CdCl_2$ solution at pH 11 in the presence of MPA as stabilizing agent in N_2 atmosphere. Cd^{2+}/MPA/HTe^- ratio was set at 1:2.4:0.5. The CdTe precursor solution was subjected to reflux at 100℃ under open-air conditions with condenser attached, and CdTe QDs with different sizes would be obtained at different refluxing time. The fluorescence emission wavelength of CdTe QDs used in this study was 630 nm.

2.5 Preparation of glucosamine functionalized CdTe QDs and GO

The synthesis of glucosamine functionalized CdTe QDs (QDs-G) and GO (GO-G) were both carried

out using the EDC and NHS linkage reaction for covalently attaching the glucosamine to the carboxyl groups of the QDs and GO via amide bond formation. In a typical experiment, appropriate amount of EDC and NHS were respectively added to the CdTe QDs and GO solution with stirring for 30 min. Next, glucosamine was added to the above solutions and incubated 3 h with continuous gentle mixing. After the reaction was complete, the solution of GO-G was filtered and washed with water to move free glucosamine as well as other molecules. In order to block the adsorption site of GO-G surface, the obtained GO-G was dissolved in water consisting of 0.5％ Tween 20. The QDs-G solution was precipitated by centrifugation at 8000 rpm for 10 min after the addition of ethanol , then redissolved in water and stored at 4 ℃ in the darkroom.

2.6 Fluorescence experiments

In our experiment, the detection of Con A was carried out in Tris-HCl buffer solution (20 mM, pH 7.0) at room temperature. Con A was dissolved in Tris-HCl buffer solution (20 mM, pH 7.0) augmented with $CaCl_2$ (0.5 mM) and $MnCl_2$ (0.5 mM) to obtain a stock solution (1.0 mg mL^{-1}) that was stored at 4℃. 15 μg mL^{-1} GO-G was mixed with different concentrations of Con A and incubated for 1 h， and then 20 μL QDs-G was added into above solution and allowed to react for 30 min before measurement. The fluorescence spectra were recorded in the 500–780 nm emission wavelength range with the excitation of 400 nm.

3. Results and discussion

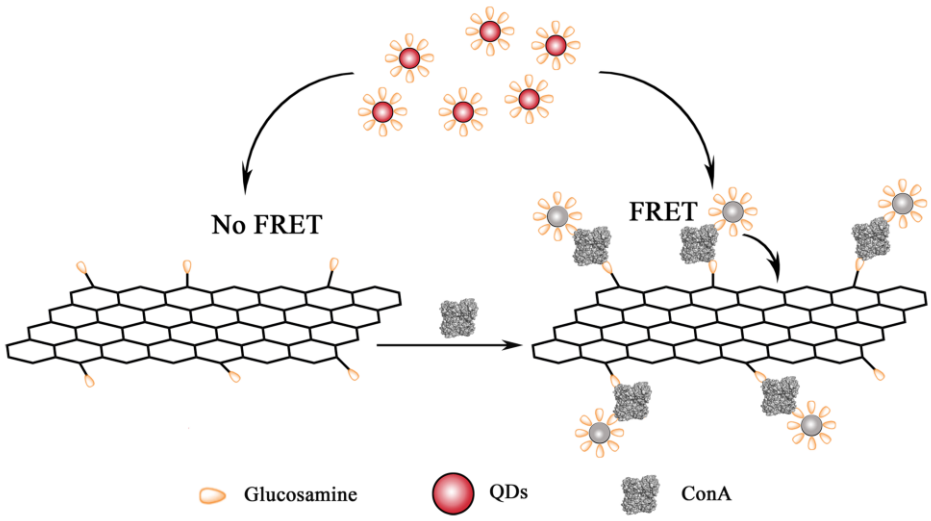
3.1 FT-IR spectra of CdTe QDs, QDs-G, GO and GO-G.

Glucosamine functionalized CdTe quantum dots (QDs-G) and graphene oxide (GO-G) complex were obtained by covalently linking glucosamine with the carboxyl group of CdTe quantum dots (QDs) and graphene oxide (GO) in our study. Fig. S1 and Fig. S2 showed the FT-IR spectra of the CdTe QDs,

QDs-G, GO and GO-G. For the QDs-G and GO-G complex, the peak at ~1035 cm^{-1} was the signature of C-O bond in glucosamine. As seen in Fig.S1 the amide I band (1656 cm^{-1}) for amide-carbonyl stretching vibration appeared which was due to the conjugation of glucosamine to the MPA-capped CdTe QDs. It can be seen from Fig. S2 that the amide II band (1560 cm^{-1}) and the amide III band (1384 cm^{-1}) for -CONH group were appeared, suggesting that the GO was modified with glucosamine via amide bound.

3.2 Design of the florescence sensing system

A previous study reported that GO could quench the fluorescence of QDs when brought GO and QDs into appropriate proximity. [18] The biosensing platform for concanavalin A (Con A) detection is based on the modulation of the fluorescence resonance energy transfer (FRET) efficiency between the glucosamine functionalized CdTe QDs (QDs-G) and GO (GO-G) complex. The mechanism of GO-based sensing system is outlined in scheme 1. The specific combination of Con A with glucosamine allows GO-G and QDs-G to be in proximity, resulting in the efficient fluorescence quenching. As shown in Fig. 1A, the fluorescence of QDs-G was greatly quenched by GO-G hybrid in the presence of Con A, while the fluorescence quenching of QDs-G in the absence of Con A was only 80.2％of its original intensity. Considering that GO-G complex could influence the fluorescence intensity of QDs-G, the effect of incubation times of GO-G on the fluorescence of QDs-G without Con A was investigated firstly. As demonstrated in Fig. 1B, a slow decrease in the fluorescence intensity of QDs-G/GO-G system was observed and the fluorescence intensity gradually decreased before 10 min and was relatively stable after 10 min. It implied that GO-G was not responsible for further fluorescence quenching in the following experiment.



Scheme 1 Schematic illustration of the GO-based fluorescence resonance energy transfer platform for concanavalin A detection.

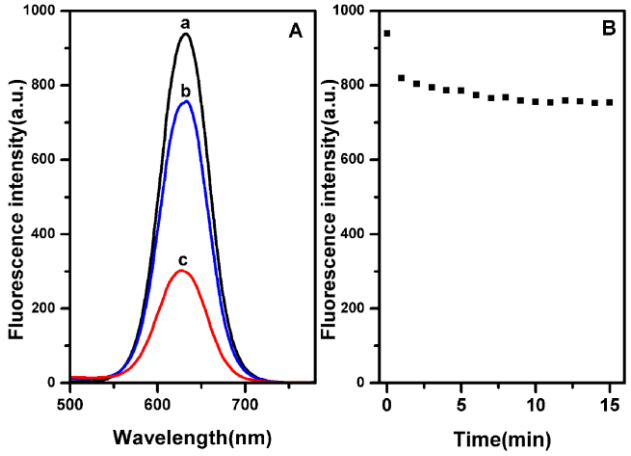


Fig.1 (A) The fluorescence spectra of QDs-G (a) and GO-based sensing system in the absence (b) or presence (c) of Con A. (B) The effect of incubation times of GO-G on the fluorescence of QDs-G without Con A.

3.3 The influence of incubating time

In order to achieve the maximum sensitivity for Con A detection, we studied the effect of incubation times on the fluorescence of sensing system. Fig. 2 showed the fluorescence intensity changes as a function of incubating time in the absence and presence of GO-G. In the presence of GO-G, the

fluorescence intensity ratio I/I_0 (I_0 and I referred to the fluorescence intensity of QDs-G/GO-G system in the absence or presence of Con A, respectively) decreased rapidly with the increasing of incubation time and reached a plateau within 30 min at room temperature. So we chose 30 min as the optimal reaction time in the following experiments. To demonstrate that the fluorescence quenching of QDs-G was resulted from the existence of GO-G in the presence of Con A, the effect of Con A on the fluorescence of QDs-G was performed in the absence of GO-G. From Fig. 2, it could be seen that the fluorescence intensity ratio I/I_0 (I_0 and I referred to the fluorescence intensity of QDs-G in the absence or presence of Con A, respectively) decreased gradually with the increasing of incubation time in the absence of GO-G. The fluorescence quenching extent of QDs-G/GO-G system was obviously greater than that of QDs-G system, which indicated that the fluorescence quenching of QDs-G was more efficient by using GO-G as a fluorescence quencher.

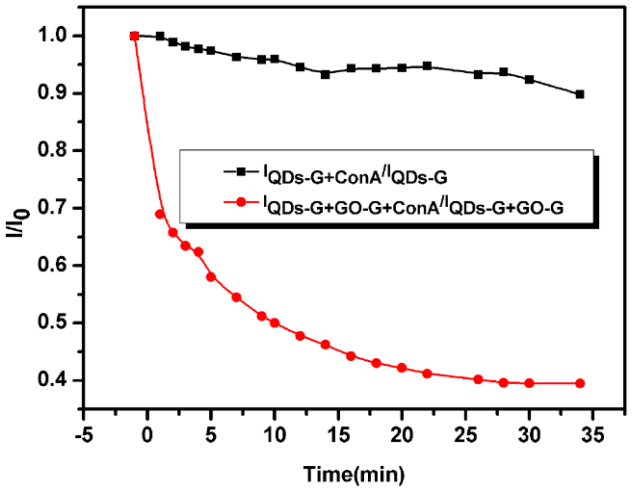


Fig. 2 The effect of incubating time on the fluorescence intensity of QDs-G in the absence (Square) and presence (Circle) of GO-G.

3.4 The optimized concentration of GO-G

The concentration of GO-G is also a key parameter in the sensing system for sensitive Con A detection. A series of different concentrations of GO-G hybrid were incubated in the fixed Con A and

subsequently incubated in the solution of QDs-G to form a sandwich configuration (GO-G/Con A/QDs-G). As shown in Fig. 3A, the fluorescence of QDs-G was quenched with the increasing amount of GO-G without the existence of Con A. However, in the presence of Con A, the fluorescence intensity decreased obviously when the concentration of GO-G complex below 15 mg L^{-1}. Con A could specifically combine with the GO-G to form more GO-G/Con A hybrid, the free binding sits of Con A on the formed GO-G/Con A hybrid could capture QDs-G and bring QDs-G and GO-G into appropriate proximity, resulting in the remarkable fluorescence quenching. When the concentration of GO-G was higher than 15 mg L^{-1}, the free binding sits of Con A on the formed GO-G/Con A hybrid would continually bind to the excess GO-G before the addition of QDs-G, leading to the less fluorescence quenching. Fig. 3B showed the relationship between the fluorescence intensity ratio I/I_0 (I_0 and I referred to the fluorescence intensity of QDs-G with different concentrations of GO-G hybrid in the absence or the presence of Con A, respectively) and the concentrations of GO-G hybrid. It could be seen that the fluorescence intensity ratio I/I_0 decreased with the increasing concentrations of GO-G up to 15 mg L^{-1} and then increased. Therefore, 15 mg L^{-1} of GO concentration was chosen as the optimal concentration in the following experiments.

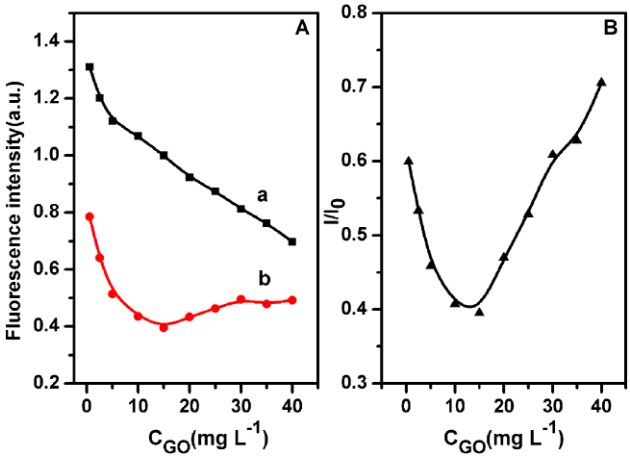


Fig. 3 The influence of the different concentrations of GO-G on the concanavalin A detection system. (A) The

fluorescence intensity of the GO-based sensing system in the absence (a) or presence (b) of Con A. (B) The fluorescence intensity ratio I/I_0 of the sensing system (I_0 and I referred to the fluorescence intensity of QDs-G with different concentration of GO-G hybrid in the absence or presence of Con A, respectively).

3.5 The effect of pH

Con A appears to be dimeric below pH 5.5 and exists as a tetramer when the pH was higher than 7.0. And there is a smooth transition between the dimer and tetramer as a function of the pH value. [43] Therefore, we systematically investigated the effect of pH on the sandwich assay for Con A. From Fig. 4A, it could be observed that the fluorescence intensity ratio I/I_0 increased with the increase of pH in the range of pH 5-10. When pH value was lower than 7.0, the fluorescence intensity ratio I/I_0 increased slowly, which might be ascribed to the isoforms of Con A in different pH values. However, high pH value caused the denaturation of Con A, resulting in low binding amount of Con A and the rapid increasing of the fluorescence intensity ratio I/I_0. The fluorescence intensity of the sandwich system with and without of Con A at different pH were also shown in Fig. 4B. To ensure higher sensitivity of the sensing system, a pH 7.0 Tris-HCl buffer solution was selected for the determination of Con A.

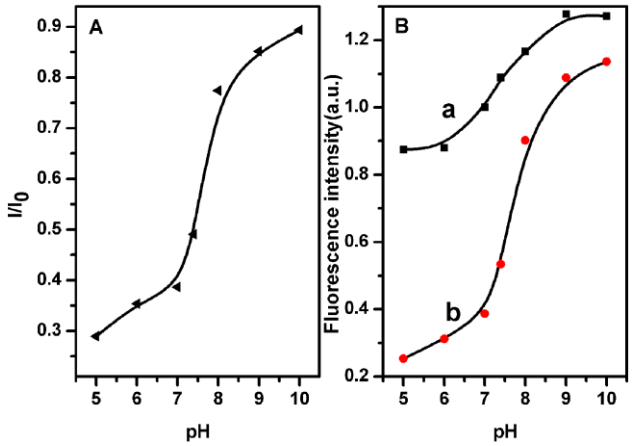


Fig. 4 The effect of the pH on the sensing system. (A) The fluorescence intensity ratio I/I_0 of the sensing system (I_0 and

I referred to the fluorescence intensity of sensing system at different pH value in the absence or the presence of Con A, respectively). (B) The fluorescence intensity of the GO-based sensing system in the absence (a) or presence (b) of Con A.

3.6 Con A detection

To further study the performance of the GO-based sandwich assay for Con A detection, we investigated the fluorescence emission spectra of this system with different concentrations of Con A under the optimized conditions. As shown in Fig. 5, the fluorescence intensity of QDs-G decreased upon the increasing concentration of Con A from 0 to 392.2 nM. The inset of Fig. 5 showed a good linear relationship between the fluorescence intensity ratio I/I_0 (I_0 and I were the fluorescence intensity of the sensing system in the absence and presence of Con A, respectively) and the concentrations of Con A in the range of 9.8 to196.1 nM with a correlation coefficient $R^2 = 0.994$. The linear regression equation was $I/I_0=0.982– 0.003C_{Con\ A}$ (nM) and the detection limit for Con A was found to be 3.3 nM based on 3σ rule, the standard deviation for six replicate measurements of 24.5 nM Con A was 1.0%. These results indicated that the sensing system could be applied to quantitative determination of Con A with high sensitivity. Compared with the previous reports for Con A determination in linear range and detection limit (Table 1), our method obtained the similar or superior detection limit and linear range.

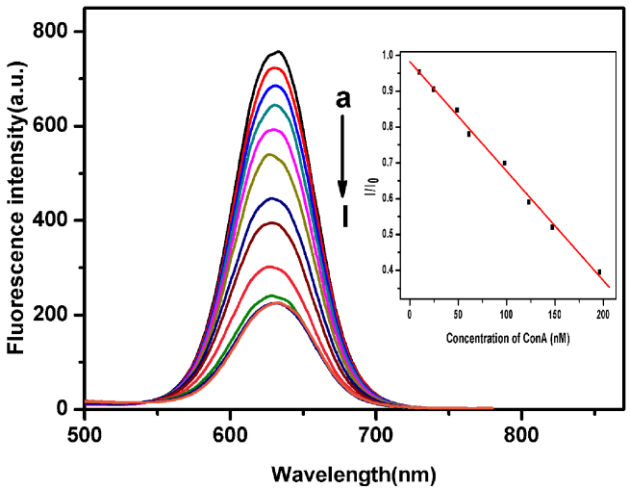


Fig.5 Fluorescence emission spectra of the sensing system with different concentrations of Con A, a-l represented the concentrations of Con A of 0, 9.8, 24.5, 49.0, 61.3, 98.0, 122.5, 147.1, 196.1, 245.1, 294.1 and 392.2 nM. The inset showed the calibration curve between the fluorescence intensity ratio I/I_0 and the concentrations of Con A in the range of 9.8 to 196.1 nM (B). I_0 and I referred to the fluorescence intensity of the proposed system in the absence or the presence of Con A, respectively.

Table 1 Comparison of different methods for the detection of Con A.

Methods	Linear range (nM)	Detection limit (nM)	Ref.
Electrochemistry	2-80	0.8	[37]
	1.96×10^{-3}-98	1.5×10^{-3}	[38]
Colorimetry	40-100	40	[39]
	80-260	100	[39]
Surface plasmon resonance	9.8-196.1	3.8	[31]
	98.0-588.2	11.2	[40]
Fluorometry	20-1000	0.8	[14]
	1.96-196	0.73	[24]
	0-150	1.5	[26]
	9.8-196.1	3.3	This work

3.7 Interference study

The selectivity of the GO-based sensing system was studied in the paper. Potential interference

substances such as bovine serum albumin (BSA), human serum albumin (ALH) and lysozyme (Lys), trypsin and pepsin were examined to assess the selectivity of the presented method for Con A detection. The interference study was carried out at a Con A concentration of 196.1 nM by monitoring the fluorescence response of the sensing system to other coexistence substances. Blank represented the fluorescence intensity ratio I/I_0 of the sensing system without Con A. The concentration of BSA and ALH were 5-folds of Con A, and the concentrations of Lys, trypsin and pepsin were about 10-folds higher than Con A. Fig. 6 showed the changes in the fluorescence intensity ratio I/I_0 of the sensing system with the addition of various substances. The results showed that no obvious influence was observed in coexistence with high concentration of the tested substances and the established fluorescence strategy exhibited good selectivity in the determination of Con A.

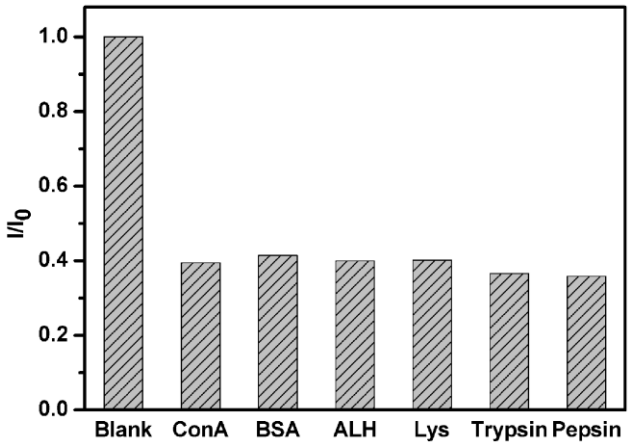


Fig. 6 The selectivity of the established sensing system to other coexistence substances, including bovine serum albumin (BSA), human serum albumin (ALH) and lysozyme (Lys), trypsin and pepsin. The concentration of Con A was 196.1 nM.

4. Conclusion

In summary, we have developed a new fluorescence method for Con A determination by using CdTe QDs as fluorescence donor and GO as fluorescence quencher. Glucosamine functionalized QDs and

GO could be used to construct a fluorescence sandwich biosensor for Con A based on the special carbohydrate-protein interactions. GO-G and QDs-G were brought into FRET proximity through the specific molecular recognition of Con A, resulting in the fluorescence decreasing. Under the optimized conditions, a linear correlation was established between the fluorescence intensity ratio I/I_0 (I_0 and I were the fluorescence intensity of the sensing system in the absence and presence of Con A, respectively) and the concentration of Con A in the range of 9.8-196.1 nM with the detection limit of 3.3 nM. The proposed sandwich method is simpler and more sensitive than other methods and can be conveniently extended to the quantitative analysis of various lectins with high sensitivity just by exchanging the carbohydrates functionalized on the QDs and GO.

Acknowledgements

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