CdTe/CdSe quantum dot-based fluorescent aptasensor with hemin/G-quadruplex DNzyme for sensitive detection of lysozyme using rolling circle amplification and strand hybridization

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ABSTRACT

Lysozyme with a small monomeric globular enzymatic protein is part of the innate immune system, and its deficiency can cause the increased incidence of disease. Herein, we devise a new signal-enhanced fluorescence aptasensing platform for quantitative screening of lysozyme by coupling with rolling circle amplification (RCA) and strand hybridization reaction, accompanying the assembly of CdTe/CdSe quantum dots (QDs) and hemin/G-quadruplex DNzyme. Initially, target-triggered release of the primer was carried out from DNA duplex *via* the reaction of the aptamer with the analyte, and the released primer could be then utilized as the template to produce numerous repeated oligonucleotide sequences by the RCA reaction. Following that, the formed long-stranded DNA simultaneously hybridized with the CdTe/CdSe QD-labeled probe and hemin/G-quadruplex DNzyme strand in the system, thereby resulting in the quenching of QD fluorescent signal through the proximity hemin/G-quadruplex DNzyme on the basis of transferring photoexcited conduction band electrons of quantum dots to Fe(III)/Fe(II)-protoporphyrin IX (hemin) complex. Under optimal conditions, the fluorescent signal decreased with the increasing target lysozyme within the dynamic range from 5.0 to 500 nM with a detection limit (LOD) of 2.6 nM at the $3s_{\text{blank}}$ criterion. Intra-assay and interassay coefficients of variation (CVs) were below 8.5% and 11.5%, respectively. Finally, the system was applied to analyze spiked human serum samples, and the recoveries in all cases were 85 – 111.9%.

KEYWORDS: fluorescent aptasensor; rolling circle amplification; hemin/G-quadruplex DNzyme; CdTe/CdSe quantum dots; lysozyme

1. Introduction

Nucleic acid-based fluorescence quantitative analysis for microRNA (Hong et al., 2016; Liao et al., 2016; Cheng et al., 2016), proteins/small molecules (Chen et al., 2016; Xue et al., 2012), and metal ions (Freage et al., 2014; Tan et al., 2016; He et al., 2014; Zhou et al., 2016) has emerged as a very useful technique in the past years, thus stimulating the fast development of the fluorescent aptasensors (Nakano et al., 2013; Ruckh, et al., 2016; Liu et al., 2016; Kong et al., 2015) owing to their inherent advantages, *e.g.*, sensitivity, specificity, speed and economy. Despite some advances in this field, these approaches usually suffer from some unfavorable limitations, such as poor photostability and solubility in water, high cost, a complicated synthesis process and surface modification. In contrast, semiconductor nanocrystals or quantum dots (QDs) (Fernando et al., 2015; Zhou, et al., 2015; Martín-Yerga et al., 2016; Shahmuradyan et al., 2016) are chosen as the valuable supplements relative to conventional fluorescent proteins (Duwé et al., 2015; Jablonski, et al., 2015) and organic dyes (Venkateswararao et al., 2014), since they have the broad absorption spectra, size-tunable emission spectra, easy functionalization and strong photostability (Morales-Narváez et al., 2015; Pan, et al., 2016; Samanta et al., 2012). Highly fluorescent quantum dots have shown promising applications in optical device (Bakulin et al., 2013), biological imaging (Allen et al., 2010), bioconjugate (Vinayaka et al., 2011), and optical biosensor (Huang et al., 2016). However, single-component quantum dots are easy to reunite because of surface defect. Core-shell quantum dot composites depending on selecting the core-shell materials can improve this phenomenon and endow some new properties.

Aqueous type-II core-shell CdTe/CdSe QDs have potential as important near-infrared fluorescent probes for drug screenings, biological imagings, and medical diagnostics due to low fluorescence background and high penetration of near infrared emission (Xia et al., 2008; Antanovich, et al., 2015). Especially, the insoluble fluorescent probes are of the interests in the biological application because of low cost and low toxicity relative to the organic synthesis (Zhao et al., 2016). Moreover, the optical readout signals of the size-controlled quantum dots usually depend on the different photophysical mechanisms, such as fluorescence electron transfer quenching (Zhang et al., 2013), fluorescence resonance energy transfer (FRET) (Wu et al., 2016), and chemiluminescence resonance energy transfer (Lee et al., 2012). Freeman et al. reported a new detection system for small molecule and metal ion based on the self-assembly of hemin/G-quadruplex DNzyme, aptamer and nucleic acid-modified quantum dots (Freeman et al., 2011). Shahmuradyan and Krull

devised intrinsically labeled fluorescent oligonucleotide probes on quantum dots for transduction of nucleic acid hybridization (Shahmuradyan et al., 2012). Sharon et al. utilized the interaction of CdSe/ZnS quantum dots with hemin/G-quadruplex for construction of luminescence aptasensor (Sharon et al., 2010). Further signal-amplified strategies have been developed coupling with quantum dots (Zeng et al., 2014; Zhao, et al., 2015). Unfavorably, the detectable signals were always limited, especially using the single quantum dot. Rolling circle replication, a process of unidirectional nucleic acid replication, can rapidly synthesize multiple copies of circular molecules of DNA/RNA (Zhuang et al., 2010). In this case, a short DNA or RNA primer can form a long single-stranded DNA or RNA containing tens to hundreds of tandem repeats by using a circular DNA template and special polymerases (Liu et al., 2014). Compared with other signal-amplified strategies, rolling circle replication is capable of generating 10^{9-15} -fold signal amplification (Qian et al., 2003). To this end, our motivation in this work is to design a signal-amplified fluorescence detection platform based on the core-shell quantum dots and rolling circle amplification strategy.

Lysozyme with a small monomeric globular enzymatic protein (glycoside hydrolases) is part of the innate immune system, and its deficiency can cause the increased incidence of disease. Elevated concentrations of lysozyme in serum/urine are related to meningitis, leukemia, and renal diseases (Huang et al., 2010). Herein, we report the proof-of-concept of feasible and *in-situ* amplified fluorescent aptasensor for the detection of lysozyme using CdTe/CdSe quantum dots and hemin/G-quadruplex DNzyme with rolling circle amplification (RCA)-based strand hybridization reaction (Scheme 1). Introduction of rolling circle replication is expected to enhance the conjugated amount of QD-based probes through the strand hybridization reaction, whilst hemin/G-quadruplex DNzyme is utilized to readily quench the fluorescent signal of quantum dots in the close proximity moieties. The released primer after the formation of the aptamer-target complex is used as the template for the RCA reaction with the help of ligase and polymerase. The quenching efficiency of free CdTe/CdSe quantum dots relies on the concentration of target lysozyme in the sample. During this process, the photoexcited conduction band electrons of quantum dots are transferred to the Fe(III)-protoporphyrin IX (hemin) complex (Shahmuradyan et al., 2016). Moreover, the lowest energy conduction band electrons of quantum dots are largely localized in the shell while the lowest energy valence band hole is localized in the core. This spatial distribution enables ultrafast electron transfer to the surface-adsorbed electron acceptors owing to the enhanced electron density on the

shell, while simultaneously retarding the charge recombination process because the shell acts as a tunneling barrier for the core localized hole (Antanovich et al., 2015; Zhu et al., 2011; Chuang et al., 2010).

Scheme 1. (A) Schematic illustration of CdTe/CdSe quantum dots (QDs)-based fluorescent aptasensing platform with hemin/G-quadruplex DNzyme for the ultrasensitive detection of lysozyme using rolling circle amplification (RCA) and strand hybridization reaction, and (B) the appropriate energy levels diagram of hemin/G-quadruplex DNzyme and CdTe/CdSe ODs associated with the electron transfer quenching mechanism.

2.1. Materials and reagents

oligonucleotides were synthesized and purified by Sangon Biotechnol. Co., Ltd. (Shanghai, China).

The sequences of the oligonucleotide used in this work are as follows:

DNA₁ (aptamer): 5'-ATCAGGGCTAAAGAGTGCAGAGTACTTAG-3'

DNA₂: 5'-CTAAGTAACTCTGCACTCTTTAGCCCTGAT-3'

DNA₃: 5'-phosphate-TGCAGAGTACTTAGCGTATCGTATGTTTCGATCGATTTCGTTTATCAGGGCTAAAGAG-3'

DNA₄: 5'-NH₂-ATCGATTTCGTTT-3'

DNA₅: 5'-CGTATCGTATGTTTCGTTGGGTAGGGCGGGTTGGG-3'

2.2. Synthesis of Core-Shell CdTe/CdSe Quantum Dots

Core-shell CdTe/CdSe quantum dots were synthesized at two steps. At the first step, CdTe nanocrystals were prepared through typical one-pot synthesis method (Huang et al., 2015). 52 μ L of 3-mercaptopropionic acid was initially injected to 50 mL of 0.5 mmol Cd(NO₃)₂ aqueous solution containing 200-mg trisodium citrate dehydrate, and pH of the resulting mixture was then adjusted to 10.5. Following that, 0.1 mmol of Na₂TeO₃ and 50 mg of NaBH₄ were added into the mixture in sequence. During this process, the solution changed from colorless and yellow. Finally, the resultant solution was refluxed for 60 min at room temperature, and the obtained CdTe nanocrystals were centrifuged and washed thoroughly with *n*-propanol (1:1, v/v) for use.

Next, the as-prepared CdTe nanocrystals were used as the cores for the synthesis of CdTe/CdSe quantum dots. Firstly, the Se precursor solution (NaHSe) was prepared by dissolving 20 mg of Se powder (0.25 mmol) in 10-mL NaBH₄ aqueous solution (1.0 mmol) under the protection of nitrogen. After that, the purified CdTe nanocrystals with a known concentration were suspended into 25-mL degassed water, and the pH was adjusted to 10.5. Meanwhile, the Cd²⁺ solution was prepared by dissolving 0.3-mmol Cd(NO₃)₂ and 32- μ L 3-mercaptopropionic acid in 10-mL degassed water and adjusting the pH to 10.5. Following that, the deliberate amounts of Cd²⁺ and NaHSe solutions were added into the CdTe solution in discrete steps, and the temperature was held at 90 °C for 20 min. Finally, the core-shell CdTe/CdSe quantum dots were precipitated by adding *n*-propanol (1:1, v/v), followed by centrifugation at 10000 rpm for 10 min.

2.3. Preparation of CdTe/CdSe QD-Conjugated Nucleic Acid (DNA₄)

All samples were prepared in 10 mM HEPES [2-4-(2-Hydroxyethyl)-1-piperazinylethanesulfonic acid, pH 7.4] buffer. The DNA₄ (10 nmol) was added to CdTe/CdSe quantum dots on a gentle shaker for 5 min. Following that, the suspension was injected into the mixture containing EDC and NHS (Molar ratio: QD : DNA₄ : EDC : NHS = 3: 1 : 30 : 30), and reacted for 120 min at 37 °C with gently shaking. After that, the suspension was deposited overnight at room temperature. Finally, the unconjugated DNA₄ was removed with a millipore tube, and DNA₄-modified CdTe/CdSe QDs were dissolved to 10 mM HEPES (pH 7.4) buffer. The conjugates (designated as DNA₄-QD) were detected by 3% agarose gel electrophoresis.

2.4. Fluorescence measurement toward target lysozyme

5 μ L of 2.0 μ M DNA₁ and 5 μ L of 2.0 μ M DNA₂ were initially mixed into 90 μ L HEPES buffer (25 mM, pH 7.4) containing 20 mM KCl, 200 mM NaCl, 0.05 wt % Triton X-100 and 1.0 wt % dimethyl sulfoxide. And then, the resulting mixture was denatured for 4 min at 94 °C, annealed for 5 min at 50 °C, and finally cooled to room temperature. A 5- μ L volume of lysozyme standard with a certain concentration was added into the above-prepared mixture, and incubated for 80 min at 37 °C. Following that, 400 U of T₄ DNA ligase, 1.0- μ M DNA₃ 200 μ M dNTP and 10 U phi29 DNA polymerase were added and incubated for 70 min at 37 °C to implement the RCA reaction (note: The RCA reaction was terminated by incubation at 65 °C for 10 min). Subsequently, 10- μ L DNA₄-QDs and 10- μ L DNzyme-DNA₅ (note: Hemin/G-quadruplex DNzyme-based DNA₅, designated DNzyme-DNA₅, was prepared by mixing 1.0-nmol DNA₅ and 70-nM hemin into 100 μ L pH 7.4 HEPES buffer containing 20 mM KCl for 60 min at 37 °C) was added into the resulting mixture, and incubated for 70 min at 37 °C. Finally, the fluorescent spectra of the suspension were recorded on a fluorescence spectrophotometer (F-4600, Hitachi, Japan) in a quartz cuvette (λ_{ex} = 365 nm, λ_{em} = 645 nm). The fluorescent intensity was collected and registered as the sensing signal relative to target PPase activity. All measurements were performed at room temperature (25 $\pm$ 1.0 °C). Analyses were always made in triplicate.

3. Results and discussion

3.1. Design and characterization of RCA-based fluorescence aptasensing platform

Scheme 1A represents the schematic illustration of CdTe/CdSe QD-based fluorescent aptasensing platform with hemin/G-quadruplex DNzyme for detection of lysozyme by using rolling circle amplification and strand hybridization reaction. On a whole, this system mainly involves in a short double-stranded DNA including the lysozyme aptamer, a circular template, QD-labeled DNA₄, hemin/G-quadruplex- based DNA₅ and the corresponding enzymes. The detection consists of analyte-induced release of the primer from DNA duplex, template-triggered RCA reaction, product-based strand hybridization reaction and fluorescence measurement. The signal is amplified by fluorescence resonance energy transfer between QDs and hemin/G-quadruplex DNzymes on the RCA products. In the presence of lysozyme, the analyte initially reacts with the aptamer (DNA₁) to release the DNA₁ (primer) from the DNA duplex, which initiates the RCA reaction to produce a long-stranded DNA with the help of circular template and enzymes. Thereafter, QD-DNA₄ and DNzyme-DNA₅ hybridize with RCA product. In this case, the fluorescence of quantum dots are quenched by the proximity DNzymes, therefore resulting in the decreasing fluorescent intensity. The redox state of hemin/G-quadruplex DNzyme as compared to the energy levels of the QDs is depicted in Scheme 1B, implying that the conduction band electrons of the QDs is transferred to hemin complex.

Fig. 1A-B gives UV-vis absorption and fluorescence emission spectra of CdTe nanocrystals and core-shell CdTe/CdSe quantum dots, respectively. Compared with CdTe nanocrystals alone, the absorption/emission spectrum of CdTe/CdSe hybrids exhibited a gentle red shift **due to the quantum confinement effect**. Further, these two nanocrystals were investigated using X-ray diffraction (XRD) (Fig. 1C). The diffraction pattern of the as-synthesize CdTe nanocrystals is consistent with cubic phase referring to the standard spectra (JCPDS 15-0770). The growth of CdSe shell on CdTe core caused the shift in the diffraction peak, and the scattering peaks located between bulk cubic CdTe and the bulk CdSe. Additionally, transmission electron microscopy (TEM) and X-ray photoelectron spectroscopy (XPS) were employed for the characterization of CdTe/CdSe QDs. The average size of the as-prepared CdTe/CdSe QDs was 5 nm (Fig. 1D-E). The elements of the nanocrystals were determined through energy dispersive spectroscopy (EDS) (Fig. 1D, inset). The peak at 1.48 KeV corresponded to the Se element. According to the XPS analysis (Fig. 1F), a Se 3d peak at 53.14 eV of the binding energy was obviously observed and further confirmed the successful synthesis of CdTe/CdSe quantum dots.

Fig. 1. (A) UV-vis adsorption spectroscopy, (B) fluorescence emission spectra and (C) XRD patterns of (a) CdTe and (b) CdTe/CdSe quantum dots, respectively (insets: the corresponding photographs); (D) TEM image and (E) HRTEM image of CdTe/CdSe quantum dots (inset: EDS patterns); and (F) XPS patterns of CdTe/CdSe quantum dots (inset: Se 3d).

3.2. Elucidation of target-triggered RCA fluorescence aptasensing platform

To further verify the feasibility of target-induced release of the primer for RCA reaction, the resulting products of the DNA duplex after RCA reaction were monitored in the presence and absence of lysozyme by using gel electrophoresis, respectively (Fig. 2A). As seen from lane 'a', the strong spots were observed at the beginning of the gel electrophoresis, and the base number was largely more than 1500 bp. In contrast, almost no RCA product was appeared in the absence of target analyte (lane 'b'). The results indicated that analyte-triggered release of DNA₂ from the duplex could be readily carried out, and its product could be used the primer for RCA reaction. Further, we used agarose gel electrophoresis to investigate CdTe/CdSe QDs before and after conjugation with DNA₄ (Fig. 2B). As shown in lane 'b', QD-DNA₄ exhibited a slightly slower electrophoretic mobility than that of CdTe/CdSe QDs alone (lane 'a'). The reason was attributed to the fact that introduction of single-stranded DNA₄ on the quantum dots caused the increase in the surface charge of nanocrystals because of the negatively charged phosphate backbone. Meanwhile, the bigger-sized QD-DNA₄ relative to pure quantum dots was unfavorable for the migration in the

gel pores (Noor et al., 2013). The results in Fig. 2B revealed that the aminated DNA₄ could be covalently bond onto the CdTe/CdSe quantum dots.

Logically, another puzzling question arises to whether our design based on RCA product could amplify the fluorescence signal of the aptasensing platform using QD-DNA₄ and DNzyme-DNA₅. To demonstrate this issue, this system was used for detection of 5.0 nM lysozyme (as an example). As seen from curve 'a' in Fig. 2C, the as-prepared CdTe/CdSe QDs displayed strong fluorescence emission intensity. Compared with curve 'd' and curve 'b' in Fig. 2C, the presence of the lysozyme in the system resulted in the decreasing of the fluorescence intensity. Maybe, we also asked whether the decrease in the intensity derived from the interaction between QD-DNA₄ and DNzyme-DNA₅. As the control test, the resultant product in the absence of ligase, dNTP and phi29 DNA polymerase (*i.e.*, without the RCA reaction) was detected. As shown from curve 'c' in Fig. 2C, the fluorescent intensity was almost the same as that in the absence of target analyte (curve 'b'), suggesting that the DNzyme on the DNA₅ could not quench the fluorescence signal of quantum dots on the QD-DNA₄ without the RCA product. These results further indicated the feasibility of RCA-based fluorescence aptasensing platform for the determination of target lysozyme.

Fig. 2. (A) Agarose gel electrophoresis images for the resulting RCA products in the (a) presence and (b) absence of target lysozyme (M: DNA marker); (B) agarose gel electrophoresis images of (a) CdTe/CdSe QDs and (b) QD-DNA₄; and (C) fluorescence spectra of (a) CdTe/CdSe QDs; (b) DNA₁-DNA₂ + DNA₃ + QD-DNA₄ + DNzyme-DNA₅, (c) DNA₁-DNA₂ + 5.0 nM lysozyme + QD-DNA₄ + DNzyme-DNA₅, and (d) DNA₁-DNA₂ + 5.0 nM lysozyme + DNA₃ + QD-DNA₄ + DNzyme-DNA₅, respectively (note: DNA₃ for RCA reaction).

3.3. Optimization of experimental condition

Typically, the sensitivity of this system relies on the following factors, *e.g.*, the conjugated ratio between DNA₄ and QD, incubation time between DNA duplex and lysozyme, RCA reaction time and hybridization time of QD-DNA₄ and DNzyme-DNA₅ with RCA product. To investigate the effects of the factors on the fluorescent signal of our strategy, 100 nM of lysozyme was utilized as an example in all cases. As described above, the fluorescence signal originated from the conjugated quantum dots on QD-DNA₄. Too many DNA₄ probes labeled on the QD would affect the quenching efficiency of quantum dots, while too few DNA₄ would be unfavorable for hybridization reaction. As shown in Fig. 3A, an optimal fluorescence change in the signal (relative to the background signal) was obtained at the molar ratio of 1:3 (DNA₄ : QD). Therefore, 1:3 of DNA₄ and CdTe/CdSe QD with the molar ratio was used for the preparation of QD-DNA₄ in this study.

Fig. 3. Effects of (A) the molar ratio of DNA₄ and CdTe/CdSe QD, (B) incubation time between DNA duplex and lysozyme, (C) RCA reaction time and (D) hybridization time of QD-DNA₄ and DNzyme-DNA₅ with RCA product on the fluorescence intensity of RCA-based aptasensing platform (100 nM lysozyme used in this case).

Generally, it takes some time to execute the target-aptamer reaction, rolling cycling replicates and strand hybridization reaction. As shown from Fig. 3B-D, the change in the fluorescence initially increased with the increasing incubation/reaction time, and then tended to level off. A longer time did not cause the significant change of the fluorescence intensity. To save the assay time, 80 min, 70 min and 70 min were employed for target-induced release of the primer from DNA duplex (*i.e.*, the reaction of target with the aptamer) (Fig. 3B), DNA₂-induced RCA reaction (Fig. 3C) and the assembly of QD-DNA₄ and DNzyme-DNA₅ on the RCA product (Fig. 3D), respectively.

3.4. Analytical performance

Under the optimal experimental conditions, RCA-based fluorescence aptasensing platform was utilized for the quantitative monitoring of target lysozyme coupling with DNzyme-QD-based quenching mechanism. The inset in Fig. 4A shows the fluorescence emission spectra of this system (at 645 nm) toward the different-concentration lysozyme standards, and the fluorescence intensity decreased with the increasing lysozyme. A good linear relationship between the relative fluorescence intensity $[(F_0 - F)/F_0]$, where F_0 and F represent the fluorescence intensities in the absence and presence of target analyte, respectively] and lysozyme concentration in the sample was acquired within the dynamic range from 5.0 nM and 500 nM, as indicated from Fig. 4A. The linear regression equation was fitted to $y = 0.266 \times \log C_{[\text{target}]} - 0.144 (R^2 = 0.9843, n = 6)$. The limit of detection (LOD) was estimated to 2.6 nM at a signal-to-noise ratio of 3. Obviously, the LOD of our strategy was comparable with those of previous reports, *e.g.*, **voltammetric detection (0.2 $\mu\text{g mL}^{-1}$) (Cheng et al., 2007), enzyme-linked immunosorbent assay (0.264 $\mu\text{g mL}^{-1}$) (Chuang et al., 2010), magnetic relaxation switch detection (0.5 nM) (Bamrungsap et al., 2011), naked-eye detection (0.36 $\mu\text{g mL}^{-1}$) (Wang et al., 2010), electrochemical biosensor ($1.5 \times 10^{-8} \text{ mg mL}^{-1}$) (Zhang et al., 2015), and fluorescence assay ($2.86 \times 10^{-6} \text{ mg mL}^{-1}$) (Tang et al., 2011).** The results further revealed that use of RCA-based fluorescence aptasensing strategy enabled a high sensitivity and a low detection limit for the detection of target lysozyme. Such a high sensitivity was ascribed to the high fluorescence quenching efficiency of the electron transfer based on the target-induced rolling circle amplification between QD-DNA₄ and DNzyme-DNA₅.

The reproducibility of RCA-based fluorescence aptasensing system was investigated by assaying

the high-middle-low three lysozyme concentrations including 10 nM, 100 nM and 300 nM based on the different-batch QD-DNA₄ and DNzyme-DNA₅, respectively. Experimental results indicated that the coefficients of variation of using the same-batch QD-DNA₄ and DNzyme-DNA₅ were 7.3%, 4.2% and 8.1% ($n = 3$) toward the above-mentioned analytes, respectively, while those with the different batches were 11.2%, 9.1% and 10.7% ($n = 3$), respectively, thus suggesting a good reproducibility.

The specificity of RCA-based fluorescence aptasensing platform was studied by challenging our system against other biomolecules or proteins, *e.g.*, bovine serum albumin (BSA), streptavidin, and thrombin. As seen in Fig. 4B, a strong change in the fluorescence intensity was obtained toward target lysozyme, while those of other non-target analytes were almost the same as the background signal. Importantly, the existence of the interfering materials with target analyte did not cause the significant change in the fluorescence signal of the aptasensing platform. Hence, the selectivity of our scheme is satisfactory.

Fig. 4. (A) Calibration curve of RCA-based fluorescence aptasensing platform toward lysozyme standards with different concentrations (inset: the corresponding fluorescence emission spectrum), and (B) the specificity of this system against BSA (50 nM), thrombin (50 nM), streptavidin (50 nM), and target lysozyme (5 nM) (note: the mixture was prepared by mixing the above-mentioned analytes with the same concentration).

3.5. Recovery Evaluation for Real Samples

To investigate the feasibility and method accuracy of RCA-based fluorescence aptasensing platform, several spiked human serum specimens with various concentrations of target lysozyme were detected by our strategy. **Initially, human serum samples (gifted from our local hospital of our**

University) were diluted to different concentrations with 10 mM HEPES (pH 7.4) buffer, and then lysozyme standards with various levels were spiked in these samples. To evaluate the recovery, the initial diluted samples were first measured by using commercial human lysozyme enzyme-linked immunosorbent assay (ELISA) kit, respectively, and the spiked samples were then assayed by the fluorescence aptasensor, respectively. The method accuracy was performed using a t -test for comparison of means proceeded by the application of an F -test between the fluorescence aptasensor and ELISA. As shown from Table 1, the recoveries in all cases were 85 – 111.9%, and all t_{exp} values were below t_{crit} ($t_{\text{crit}[4, 0.05]} = 2.77$). So, the RCA-based fluorescence aptasensing scheme could be applied for quantitative monitoring of lysozyme in the complex system.

Table 1. Analysis of the spiked human serum samples toward target lysozyme by using RCA-based fluorescence aptasensor and commercial human lysozyme ELISA kit

Sample no	Concentrations for diluted samples by ELISA (nM)	Added (nM) for spiked sample	Concentration (nM, $n = 3$) ^a			Recovery (%) ^b
			By aptasensor	By ELISA	t_{exp}	
1	43.2	10	51.7	53.8	2.11	85
2	12.8	50	65.7	61.4	1.43	105.8
3	24.5	100	132.4	126.3	2.45	107.9
4	8.9	150	155.3	161.2	0.98	97.6
5	32.4	200	256.2	228.7	1.94	111.9
6	19.3	300	302.7	323.8	1.63	94.5

^aAnalyses were made in triplicate, and the data were obtained as mean values of three assays ($n = 3$). ^bThe recovery was calculated on the basis of the obtained results from the fluorescence aptasensor.

4. Conclusions

In summary, this work designs a new fluorescence aptasensing protocol with signal amplification based on analyte-induced rolling cycling amplification, accompanying the assembly of CdTd/CdSe QD-labeled oligonucleotides and hemin/G-quadruplex DNzyme-conjugated DNA. Experimental results revealed that our strategy could exhibit a high sensitivity and low detection limit. Compared with classical lysozyme immunoassays, the target analyte can be determined without the needs of sample multiple separation and multiple washing steps. Easily, this methodology can be extended for detection of other small molecules/mycotoxins by changing the sequence of the corresponding

aptamer. Moreover, other fluorescence donors (*e.g.*, carbon dots and g-C₃N₄ QDs) can also be used for the labeling of DNA probes. By combining with high-efficient fluorescence nanocrystals and the quenchers, a series of fluorescence aptasensing schemes can be constructed, thereby representing a versatile sensing platform. Nevertheless, one disadvantages of this method lie in the long incubation time and the signal-off detection mode. Therefore, future works should focus on their improvement in the practical application.

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