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ORIGINAL PAPER

A fluorescent probe composed of quantum dot labeled aptamer and graphene oxide for the determination of the lipopolysaccharide endotoxin

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Abstract

Endotoxins are complex lipopolysaccharides (LPS) and key components of the outer cell membrane of Gram-negative bacteria. The authors report on a fluorescent aptamer-based probe for the determination of LPS of Gram-negative bacteria. An aptamer against LPS was fluorescently labeled with CdSe/ZnS quantum dots. Its emission is quenched on addition of graphene oxide (GO). On addition of LPS, the aptamer binds LPS and GO is released. This results in the recovery of fluorescence, typically measured at excitation/emission wavelengths of 495/543 nm. The probe responds to LPS in the 10–500 ng·mL^{-1} concentration range, and the detection limit is 8.7 ng·mL^{-1}. It can be used for selective detection of LPS from different Gram-negative bacteria, in the presence of biological interferents.

Keywords Biosensor · Gram-negative bacteria · DNA · Fluorescence quenching · Resonance energy transfer · CdSe/ZnS quantum dots · Fluorescence turn-on · Receptor

Introduction

Lipopolysaccharide (LPS) is a significant contaminant in food and pharmaceutical products, and its level is a strict control index for Food and Drug Administrations all over the world. Hence, the detection of LPS is of great concern in pharmaceutical quality control, health care and food industry [1, 2]. Enzymatic Limulus amebocytes lysates (LAL) [3–5] assay is the currently employed detection method for LPS. However, the low reproducibility due to its dependence on pH and temperature, and massive horseshoe crabs death due to the excess collection of blood are major limitations of LAL assay [6, 7].

Many enzyme-free methods have been developed to detect LPS, of which the biosensors based LPS analysis attract more

Highlights
- A fluorescence probe was constructed in combination of QDs labeled aptamers with GO.
- The GO-aptamer-QD probe can recognize and bind with LPS, releasing GO and recovering fluorescence.
- The constructed probe can detect LPS in injections sensitively with low LOD 8.7 ng mL^{-1}.

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attention due to their high sensitivity and simple experimental procedures [7]. According to the transduction form, they can be categorized to two main classes: electrochemical detection methods and optical detection methods. Electrochemical sensing takes advantages of very high sensitivity [8–11], if combined with aptamer probe and gold atomic clusters modified Au electrodes, the detection limit of LPS can reach as low as 7.94×10^{-21} M [9].

Optical LPS biosensors often are based on measurement of fluorescence and surface plasmon resonance (SPR). Based on SPR, silver nanoparticles (AgNPs)-Polymyxin B (PMB) colloidal system [12, 13] were designed to detect LPS in urine and traditional Chinese medicine injections. Fluorescence based LPS sensors focus on synthesis of fluorescently labeled ligands those discriminate and conjugate LPS. Fluorescence resonance energy transfer (FRET) is a sensitive readout for the probe, in which binding of LPS induces a conformational change and resulting in fluorescence recovery. Based on ion pair interactions, peptide functionalized polydiacetylene liposomes [14], and tetraphenylethylene [15] were synthesized as fluorescence turn-on sensor for LPS. The ligand-binding aptamers and peptides based LPS fluorescence sensors are more robust and specific. FRET sensor with detection limit of 20 μg mL^{-1} was constructed by peptides derived from the LPS-binding domain of CD14 [16]. Graphene oxide (GO) displayed high fluorescence quenching efficiency [17]. When it is used for FRET probes of biomolecules detection, a dye labeled ligand is assembled on the surface through π-π stacking interactions, and the conjugation of targets with ligand enables the release of GO to induce fluorescence recovery. A tetramethylrhodamine labeled peptide-assembled graphene oxide (GO) fluorescent turn-on sensor [18] enabled the LPS detection limit down to 1.3 ng mL^{-1}. Zhang et al. [19] synthesized the 6-carboxy-X-rhodamine labeled LPS aptamer combining GO to detect LPS in drink samples with a detection limit of 15.7 ng mL^{-1}.

Compared with organic dyes, quantum dots (QDs) take advantages of high photo bleaching threshold, good chemical stability, and readily tunable spectral properties [20]. QD-GO fluorescence quenching system displays high sensitivity and reproducibility, which are applied widely in biomolecules sensing, such as proteins [21], nucleic acid [22], bacteria [23], and aflatoxin B1 [24]. However, there is no reports on QD labeled aptamer combined with GO for LPS detection.

Thus, we present the first QD-aptamer-GO fluorescence turn-on probe for the detection of LPS. 5'-NH_2 modified aptamer that has LPS binding domain was synthesized and labeled by QDs. Combining with GO as fluorescence quencher, a sensitive fluorometric turn-on system was constructed for LPS assay. Under the optimized conditions, LPS in real samples were quantitatively detected. This work provides an alternative method for optical LPS detection.

Experiment

Materials and reagents

The sequences of LPS aptamer (NH_2–5′-CTTCTGCC CGCCTCCTTCCTAG CCGGATCGCGCTGGCCAGAT GATATAAAGGGTCAGCCCCCCAGGAGACGA GATAGGCGGACACT-3′) [25] were synthesized by Shanghai Sangon Biotechnology Co. Ltd. (Shanghai, China) (https://www.sangon.com) and purified by high performance liquid chromatography (HPLC). 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC), N-hydroxysuccinimide (NHS), phosphate buffered saline (0.01 M, pH 7.4), graphene oxide and LPS from *Escherichia coli* 055:B5, *Pseudomonas aeruginosa*, and *Salmonella typhosa* were purchased from Sigma–Aldrich (St. Louis, MO, USA) (https://www.sigmaaldrich.com). Water soluble carboxyl-functionalized CdSe/ZnS quantum dots with maximum emission wavelength at 545 ± 5 nm were purchased from Wuhan Jiayuan Quantum Dots co. Ltd. (Wuhan, China) (http://www.qds.net.cn). Vitamin B1 and 0.9% NaCl injections were purchased from Southwest Pharmaceutical Co. Ltd. (Chongqing, China) (http://www.swp.cn). All solutions were prepared with ultrapure water with a resistivity of 18.0 MΩ from a Millipore Milli-Q water purification system. All the other chemicals are of analytical grade and used as received.

Fluorescence spectra were measured by RF-5301 fluorescence spectrophotometer (Shimadzu, Japan) (https://www.shimadzu.com) with 150 W xenon light source, excitation light and emission light slit width of 5.0 nm.

Synthesis of QDs labeled aptamers

Solutions of 1-ethyl-3-(3-dimethylamino propyl)-carbodiimide (EDC) (60 mM), QDs (1 μM), N-hydroxysulfosuccinimide (NHS) (30 mM), and amine modified aptamers (25 μM) were prepared by PBS.

80 μL EDC and 80 μL QDs solutions were mixed and stirred at 25 °C on a shaker. After 15 min, 80 μL NHS were added and stirred for 20 min. After that, 18 μL aptamer solution was added and reacted for 1 h in dark at 25 °C on the shaker. Then, 80 μL 10 mM Tris-HCl buffer was added and stirred for 2 h to terminate the unreacted carboxyl groups on the surface of QDs.

The QDs aptamer conjugates were collected and washed with PBS for three times by centrifugation at 12000 rpm for 15 min to completely remove free aptamer. The QD-aptamer was suspended at 8 mL PBS (pH 7.4) with a concentration of 0.8 μM.

Fluorescence recovery of LPS interaction with QD-aptamer-GO probe

LPS was dissolved in ultrapure water with concentrations of 8.5, 10, 50, 100, 200, 300, 400, 500, and 1000 ng mL^{-1}. The LPS stock solutions were vortexed and warmed to 42 °C before using to avoid micelles/aggregates formation.

500 μL QD-Apt solutions (0.8 μM) were added to 200 μL GO (100 μg mL^{-1}) solutions and incubated for 30 min. Then, 200 μL LPS solutions (200 ng mL^{-1}) were added and incubated. Then, the fluorescence intensities at excitation/emission wavelength $\lambda_{ex}/\lambda_{em}$ = 495/543 nm at 25 °C were recorded before and after the LPS addition with different incubation time.

Detection of LPS in PBS and vitamin B1 injections

500 μL QD-Apt solutions were mixed with 200 μL GO (100 μg/mL) solutions, and the mixture was incubated for 30 min. Then 200 μL LPS solutions with different concentrations 500–8.5 ng mL^{-1} were added and incubated for another 30 min. The calibration was plotted by fluorescence intensity ratio F/F_0 versus LPS concentrations, where F and F_0 refers to the fluorescence intensity at $\lambda_{ex}/\lambda_{em}$ = 495/543 nm with and without LPS, respectively.

The commercially available Vitamin B1 injections ($C_{12}H_{17}ClN_4OSHCl$ 50 mg mL^{-1}) are taken for real sample analysis. It is sterile solution of Vitamin B1 with pH 2.5–4.0. Vitamin B1 injections were diluted ten times with PBS (0.01 M, pH 7.4), which were then added to 400 μL LPS standard solutions with concentrations of 50, 150, 300, 500 and 1000 ng mL^{-1} to a constant volume of 2 mL to get the spiked solutions with LPS concentrations of 10, 30, 60, 100 and 200 ng mL^{-1}, respectively. 200 μL spiked solutions were taken for LPS detection and the procedures were the same as those described for LPS in PBS. The concentrations of LPS, spike recovery and relative standard deviation were calculated by the calibration plot.

Results and discussion

Choice of materials

Our group has focused on pathogenic bacteria detection for a long time. We designed the electrochemical impedance biosensors PDDA/AuNP@Ag [26] and Man/MUA-MH/Au [27] for *E. coli* detection, and fluorescence biosensors CdSe/ZnS QD–IgG-antibody [28] and carbon dots (CDs)-aptamer [29] for *Salmonella typhimurium* detection. The results suggest that the aptamers based fluorescence sensors have similar specificity as antibody sensor, but its fabrication procedures are much simpler. We therefore now want to design a LPS fluorescence probe based on QD labeled aptamers, which is different from our former method CD-aptamer. We also want to introduce a fluorescence acceptor to construct a more sensitive system based on FRET. GO displays very high fluorescence quenching efficiency and can strongly adsorb single-stranded DNA via its hydrophobic and π-π stacking interactions with nucleobases but less affinity towards double-stranded DNA or secondary and tertiary-structured DNA. Thus, we designed the QD-Apt-GO fluorescent LPS probe, and the components CdSe/ZnS QDs, aptamers, and GO were the fluorescence donor, ligands for LPS recognition, and fluorescence quencher, respectively.

Mechanism of the QD-Apt-GO fluorescent probe

The principle of LPS QD-Apt-GO fluorescence probe is shown in Fig. 1. The amino-modified single-stranded DNA with LPS-binding domain was synthesized and labeled at *N*-terminal by QDs. The QD-aptamer complex with single-stranded conformation randomly adsorb to GO physically via π-π stacking in solution, and the distance between QD-Apt and GO is less than 10 nm, resulting in fluorescence quenching via FRET (Fig. 1). On addition of LPS, the single-stranded nucleic acid aptamer is identified and bound by LPS via van der Waals forces, base stacking interaction, hydrogen chemical bond, or electrostatic interaction, to form the well folded QD-Apt-LPS complex. The occupations of the purine and pyrimidine bases of aptamer enable the interactions between QD-Apt and GO much weaker. This result in releasing of GO and fluorescence recovery (Fig. 1). On addition of 200 ng mL^{-1} LPS, the fluorescence intensity at 543 nm was increased 20 times after incubation for 30 min (Fig. 2). It is suggested that the QD-Apt-GO probe is sensitive to the change of LPS concentration. This fluorescence quenching system displays fluorescence spectrum in the range of 500–600 nm upon excitation wavelength 495 nm, and the maximum emission wavelength is 543 nm (Fig. 2).

Optimization of the LPS detection methods

The following parameters were optimized: (a) GO amount; (b) Incubation time of QD-Apt with GO; (c) Incubation time of QD-Apt-GO with LPS; (d) Sample pH value. Respective data and figures are given in the Electronic Supporting Material (Fig. S1-Fig. S4). The following experimental conditions are found to give best results: (a) 100 μg mL^{-1} GO; (b) incubation time of QD-Apt with GO is 30 min; (c) incubation time of QD-Apt-GO with LPS is 30 min; (d) Best sample pH 7.0–8.0. The Stern–Volmer plot (Fig. S2) displays an upward curve shape in the range of 0 to 100 μg·mL^{-1}, which is the characteristic feature of the combination of dynamic and static quenching.

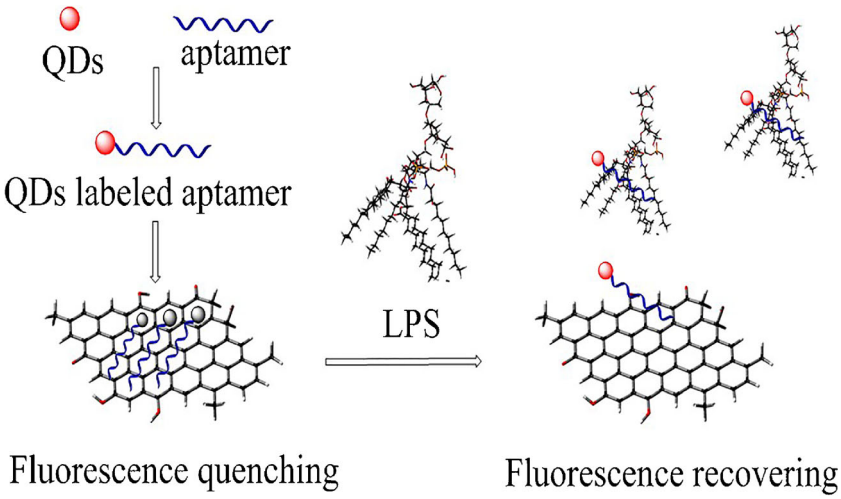

Fig. 1 Mechanism of the QD-Apt-GO fluorescent probe

Selectivity and specificity of QD-Apt-GO for LPS

Though LAL is the major method of LPS detection, its operations are harsh and can be interfered by β-D-glucose or ions such as Zn^{2+}, Mg^{2+} and EDTA, resulting in false negative results. In addition, for pharmaceutical injections, the active ingredients usually exist as salts containing ions such as Cl^-, PO_4^{3-}, and Na^+ etc.. In order to investigate the selectivity of QD-Apt-GO fluorescent probe, the effects of EDTA (known to effect LAL), Zn^{2+}, Mg^{2+}, H_3PO_4, BSA, DNA, and β-D-glucose (function groups of LPS) (Fig. 3) were investigated. It is noted that all these interferents have no significant influence on the fluorescence intensity ratio F/F_0, even at concentrations 100 times of LPS (Fig. 3 black columns). In contrast, on addition of 500 ng mL^{-1} LPS solution to these interferents, the fluorescence recovery occurred and the F/F_0 changed by at least 10 times (Fig. 3 gray columns).

The LPS detection in real sample 0.9% NaCl injection without any pretreatments was carried out. It is shown in Fig. 3 that there is little change in F/F_0 for 0.9% NaCl injection. Thus, this QD-Apt-GO LPS probe is highly selective to LPS, even in the presence of interferents and in real pharmaceutical injection samples.

To investigate whether this QD-Apt-GO probe is capable of detecting LPS from other Gram-negative bacteria, 200 ng mL^{-1} purified LPS from *E. coli*, *P. aeruginosa*, and *S. typhosa* were added to the probe solutions. The results (Fig. 4) show that this probe is more sensitive to LPS from *P. aeruginosa*, and the fluorescence recovery abilities of LPS from *E. coli* and *S. typhosa* are similar. This suggests that this probe displays higher binding affinity to *P. aeruginosa* LPS. The results also imply that this QD-Apt-GO probe is capable of detecting LPS of wide range of Gram-negative bacteria, and this is very important for its applications on endotoxin detection.

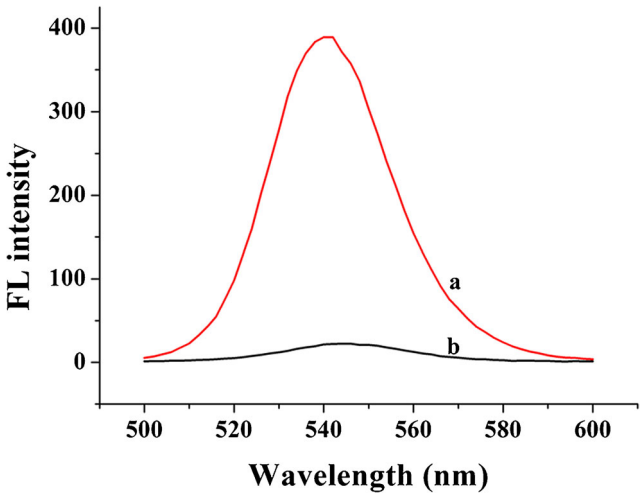

Fig. 2 Fluorescence spectra of the QD-Apt-GO with or without LPS (200 ng mL^{-1}) at excitation wavelength 495 nm

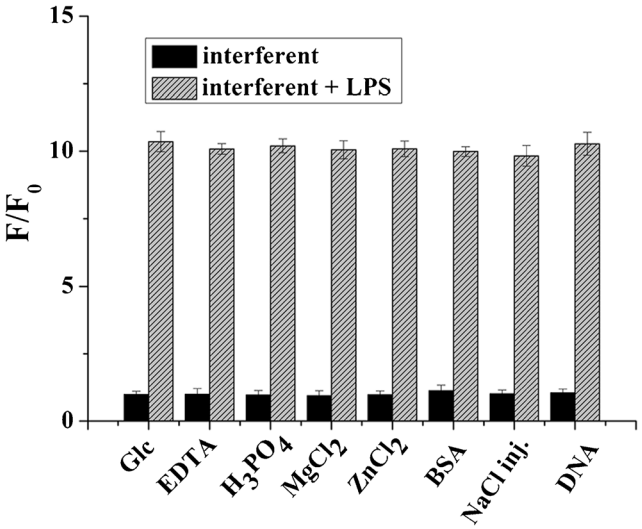

Fig. 3 Fluorescence intensity ratio at $\lambda_{ex}/\lambda_{em}$ = 495/543 nm of the QD-Apt-GO probe in the presence of various interferents. Gray and black columns represent the F/F_0 ratio of these interferents (50 μg mL^{-1} for interferents and 0.9% NaCl injection) with and without 500 ng mL^{-1} LPS solution. Results are average of 6 experiments

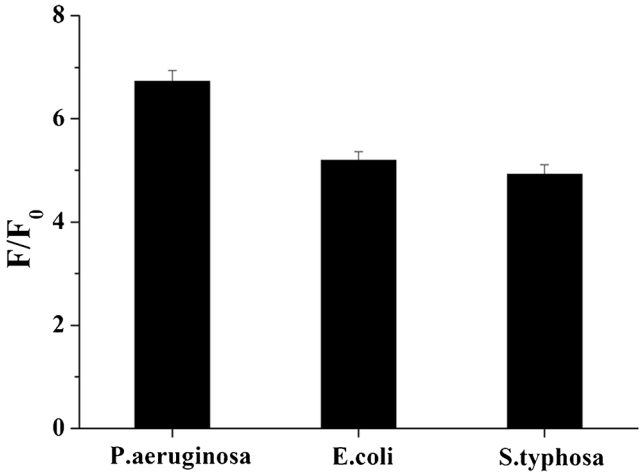

Fig. 4 Fluorescence intensity ratio at $\lambda_{ex}/\lambda_{em}$ = 495/543 nm of the QD-Apt-GO probe in the presence of 200 ng mL^{-1} LPS from different Gram-negative bacteria. Results are average of 3 experiments

Detection of LPS based on QD-Apt-GO fluorescent probe

Under the optimized experimental conditions, fluorescent intensities of LPS in PBS were detected using the QD-Apt-GO fluorescent probe. Fig. 5a shows the fluorescence emission spectra of QD-Apt-GO incubating with LPS with different concentrations. The fluorescence emissions increase gradually with LPS concentration (0–1000 ng mL^{-1}). Plotting the fluorescence intensity ratio F/F_0 versus LPS concentration, a good linear relationship between F/F_0 and LPS concentration is obtained (Fig. 5b). It can be expressed as $F/F_0 = 0.02091C + 0.97763$, with $R^2 = 0.983$ (Fig. 5b). The limit of detection (LOD) of this probe was estimated as 8.7 ng mL^{-1}.

In comparison with the other LPS assay methods reported previously (Table 1), the LOD of QD-Apt-GO LPS probe is at average level. Although the sensitivity is lower than that of electrochemical biosensors [10, 30] and FRET methods based on 5′-FAM- aptamers/3′-DABCYL-complement sequence (qCS) [18] and tetramethylrhodamine-peptides/GO [19], this probe can be operated directly by mixing QD-aptamer, GO, and LPS. It is an alternative for LPS detection.

Detection of LPS in injections based on QD-Apt-GO fluorescent probe

The QD-Apt-GO fluorescence probe was utilized to detect the LPS in Vitamin B1 injection. Synthetic samples were prepared by adding LPS to Vitamin B1 injections. It is shown in Table S1 that the recoveries of LPS were 92.2% ~ 102.1%, RSD less than 5% (n = 6). Thus, this QD-Apt-GO fluorescent probe can be used for LPS detection in real samples.

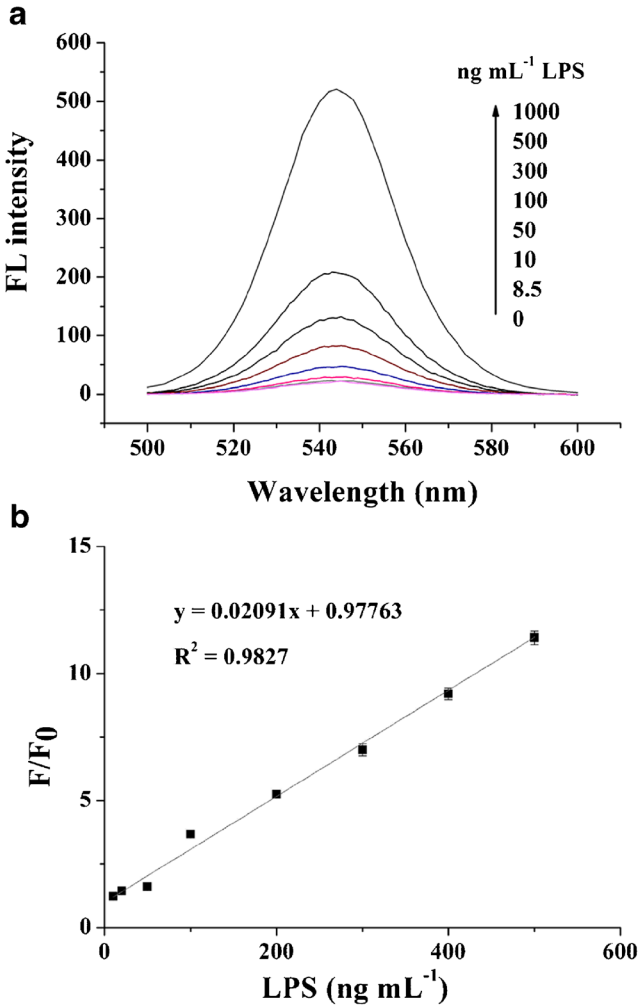

Fig. 5 **a** Fluorescent spectra of QD-Apt-GO in the presence of 8.7, 10, 50, 100, 300, 500, 1000 ng mL^{-1} LPS; **b**: Calibration plot of fluorescence intensity F/F_0 ($\lambda_{ex}/\lambda_{em}$ = 495/543 nm) versus LPS concentration (10–500 ng mL^{-1}), and results are average of 6 experiments

Conclusions

In summary, a QD-Apt-GO fluorescent probe based on FRET was developed for the detection of LPS. LPS is able to be recognized and conjugated by the QD labeled aptamer, resulting in release of GO and fluorescence recovery. This probe is specific to LPS compared with other biological interferents. It is also sensitive and selective to LPS from a wide range of Gram-negative bacteria. This provides an alternative for LPS detection. However, there are still some limitations to be improved. This method is not sensitive enough, especially for quality control of pharmaceutical injections. The irreversible probe increases the cost of the assay, because the nanoparticles QDs and GO are not low cost materials even today. Besides, as ultraviolet excitation (UV) probe, it is limited when some biosamples are analyzed, such as blood, cells, and marine water, because the UV absorbers in these complicated samples are likely to weaken the fluorescence signal.

Table 1 Comparison to known methods

Methods	Materials	Analytical range	LOD	Ref.
Electrochemical biosensor	Au@Fe_3O_4-LBA-DNA1/ AuNPs–Tb–Gra-DNA2	10 fg mL^{-1} -50 ng mL^{-1}	8.7 fg·mL^{-1}	[30]
	Aptamer/AuAC/Au	0.1 ag·mL-10 pg mL^{-1}	7.94×10^{-5} fg·mL^{-1}	[10]
Colorimetric probe	Polymyxin B-AgNPs	25–175 ng·mL^{-1}	20 ng·mL^{-1}	[12]
SPR	grating Au substrates- peptides	50 ng·mL^{-1}-180 μg·mL^{-1}	32.5 ng·mL^{-1}	[19]
FRET	5'-FAM-aptamer/3'-DABCYL- qCS	5–250 ng·mL^{-1}	3.0 ng·mL^{-1}	[31]
	tetramethylrhodamine-peptides/GO	4–200 ng·mL^{-1}	1.3 ng·mL^{-1}	[18]
	6-carboxy-X-rhodamine-aptamer/GO	25–1600 ng·mL^{-1}	15.7 ng·mL^{-1}	[19]
	QD-Aptamer/GO	10–500 ng·mL^{-1}	8.7 ng·mL^{-1}	This work

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Compliance with ethical standards

The author(s) declare that they have no competing interests.

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