

Ultrasensitive Biosensor for Detection of Mercury(II) Ions Based on DNA-Cu Nanoclusters and Exonuclease III-assisted Signal Amplification

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This paper describes a novel method for label-free mercury(II) ion detection based on exonuclease III-induced target signal recycling amplification using double-stranded DNA templated copper nanoclusters. The synthesized DNA-Cu nanoclusters were used with exonuclease III loop amplification technology for ultra-high sensitivity detection of mercury(II) ions, which were detected by significantly decreased fluorescence intensity. Under the optimal experimental conditions, there was a clear linear relationship between Hg^{2+} concentration in the range of 0.04 to 8 nM and fluorescence intensity. The detection limit for Hg^{2+} was 4 pM. In addition, the interference of other metal ions on the mercury(II) ion detection was also studied. To confirm the application of the fluorescent sensor, it was applied to determine the concentrations of mercury(II) ions in tap water, and the results showed that the method can be used to detect mercury(II) ions in water samples successfully.

Keywords Mercury(II) ion detection, copper nanoclusters, signal amplification, fluorescent biosensor

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Introduction

Mercury is a highly toxic environmental pollutant. It is released from many human activities, including solid waste incineration, coal and oil combustion, pyrometallurgical processes, and production of mercury and gold, so it is widely distributed in soil, water, and ambient air.¹ Mercury is non-biodegradable and usually forms methyl mercuric compounds by microbial biomethylation of solvated Hg^{2+} .² In aquatic systems, methylmercury ions, CH_3Hg^+ , which are the most toxic form of mercury, bioaccumulate in fish from very low concentrations of methylmercury (MeHg) to significant concentrations in humans who eat the fish.^{1,3} MeHg has been reported to be neurotoxic, particularly in the central and peripheral nervous systems of children.⁴ Therefore, a highly sensitive and selective assay method for determination of solvated Hg^{2+} in aquatic systems is highly desirable.

In the past years, numerous techniques for the detection of mercury(II) ions have been reported, including inductively coupled plasma mass spectrometry,⁵ atomic absorption spectrometry,⁶ fluorometric methods,^{7,8} electrochemical methods,^{9,10} colorimetric methods,^{11,12} and chromatography techniques.¹³ Fluorescent methods have attracted wide attention because of their simplicity, convenient signal transfer, quick detection, and easy operation.^{14,15} Recently, the method for

detecting Hg^{2+} has been gaining attention to developing highly selective oligonucleotide-based sensors. Hg^{2+} could selectively bind for the thymine (T) nucleobase of DNA, and the interaction force among the thymine- Hg^{2+} -thymine link is strong.¹⁶ It has been demonstrated that the thymine- Hg^{2+} -thymine link is stronger than the adenine-thymine attraction, and the Hg^{2+} attraction to thymine is more specific than for other metal ions, such as Ag^+ , Cu^{2+} , Ni^{2+} , Pd^{2+} , Co^{2+} , Mn^{2+} , Zn^{2+} , Pb^{2+} , Cd^{2+} , Mg^{2+} , Ca^{2+} , Fe^{2+} , Fe^{3+} , and Ru^{3+} .^{17,18} Based on this specificity, various sensors for detecting Hg^{2+} have been developed. For example, a sensor of MoS_2 nanosheet/DNA/carbon dot nanoassembly was developed for the detection of mercury(II) ions in environmental samples.¹⁹ This shows some promise, but the labeled DNA fluorescent probes required complex modifications to experiments or were expensive,^{20,21} and most of the systems have relatively low sensitivity.

Recently, metallic nanoclusters with diameters smaller than 2 nm for new fluorescent probes have attracted great interest in biological sensing and imaging.²² Ultrasmall metallic nanoclusters, including gold/silver/copper nanoclusters, are usually synthesized by using templates, such as amino acids, DNA, proteins, and dendrimers.²³ Compared to traditional fluorescent nanoparticles, the nanoclusters have outstanding fluorescence, low cost, and good biocompatibility. Among the various reported templates, DNA has specific recognition abilities for metal ions and biomolecules, so it has been used widely. DNA templated gold nanoclusters with thrombin as a fluorescent indicator were utilized for fluorescent sensing of target DNA and molecular recognition.²⁴ Single-stranded DNA (ssDNA) templated Cu/Ag nanoclusters were employed to

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detect adenosine by using S-adenosylhomocysteine hydrolase.²⁵ The template of 24 adenine-thymine pairs in dsDNA with six-base loops was used to synthesize fluorescent Cu nanoclusters (CuNCs) for S1 nuclease detection.²⁶

Signal recycling amplification is an important technique for highly sensitive detection. Nuclease cleavage is an efficient strategy for target recycling. Nicking endonucleases and quantum dots have been employed for enzyme-catalyzed signal recycling amplification for Hg²⁺ detection.²⁷ However, sequence-dependent activation limits the extended application of nicking enzymes. To overcome that weakness, exonuclease III (Exo III) that contains 3'-5' exonuclease activity could be used. It does not require a specific recognition site like other nicking endonucleases. It has high exodeoxyribonuclease activity for removing mononucleotides gradually from blunt or recessed 3' termini of double-stranded DNA (dsDNA), but it is inactive for ssDNA and the protruding 3' end of dsDNA.²⁸ For Hg²⁺ detection, target signal recycling amplification leads to benefits from letting a target Hg²⁺ ion interact with DNA signal probes based on the T-Hg²⁺-T structure. The nuclease cleavage will release the target to a new recycling route, so one Hg²⁺ ion can repeatedly produce a signal response.

In this paper, we describe a novel approach for label-free detection of Hg²⁺ based on exonuclease III-induced target signal recycling amplification using dsDNA templated CuNCs. In a hairpin-structure ssDNA of the ds-DNA template, the middle stem of the ssDNA is hybridized and the 3' and 5' termini end of the ssDNA have three bases of thymine. Hence, the hairpin-structure ssDNA has a protruding 3' end and shows inactivity against the exonuclease III. In the presence of Hg²⁺, T-Hg²⁺-T mismatch sequence structures would be formed and the protruding 3' end of the hairpin-structure ssDNA would be changed to a blunt 3' terminus. The exonuclease III could digest the hairpin-structure ssDNA and release the Hg²⁺, allowing it to take part in the next cycling route. The decrease of ssDNA would lead to no hybridization of dsDNA, which would result in an outstanding decrease of fluorescence intensity. The CuNCs fluorescent sensor is simple, low-cost, and easy to operate.

Experimental

Apparatus

Fluorescence measurements were carried out on a Shimadzu RF-5301 PC spectra fluorophotometer. Transmission electron microscope (TEM) images were obtained with a Tecnai G2 F20 S-Twin. Fluorescence spectra were obtained at 570 nm excitation wavelength. All pH measurements were made with a Sartorius PB-10 pH meter (Sartorius Instruments Co. Ltd., Beijing, China). Ultrapure water was purified using a Milli-Q Reagent Water System (Millipore, Bedford, MA, USA).

Reagents

All DNA oligonucleotides used for synthesis of copper nanoclusters were purchased from Sangon Biotech Co., Ltd. (Shanghai, China). The sequences of the DNA oligonucleotides were as follows:

Oligo A: TTTCGTCATGGGTTGACGTTT

Oligo B: CGTCAACCCATGACG

Exonuclease III and sodium ascorbate were purchased from Sangon Biotech Co., Ltd. (Shanghai). CuSO₄·5H₂O, Hg(NO₃)₂, and MgCl₂ were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Disodium hydrogen phosphate dodecahydrate (Na₂HPO₄·12H₂O), sodium dihydrogen phosphate (NaH₂PO₄·2H₂O), and sodium nitrate (NaNO₃) were purchased

from Aldrich Chemical Co. We prepared phosphate-buffered saline (PBS) (20 mM, pH 7.4) from 3.58 g Na₂HPO₄·12H₂O, 1.56 g NaH₂PO₄·2H₂O, and 1.7 g NaNO₃ in one liter ultrapure water.

Fluorescence assay

Typically, according to our previous work,²⁹ 9 μL 25 μmol L⁻¹ ssDNA strands (Oligo A) and 10 μL 50 mmol L⁻¹ Mg²⁺ were first mixed together in 40 μL of 20 mmol L⁻¹ PBS buffer (pH 7.4) with different concentrations of 5 μL Hg²⁺ and reacted for 5 min at 37°C. Next, the 10 μL 1 U L⁻¹ exonuclease III was added to the mixed solution, incubated for 1 h at 37°C, and then put in an 80°C water bath for 5 min. Finally, 9 μL 25 μmol L⁻¹ ssDNA strands (Oligo B) were added to the mixed solution and reacted for 20 min at 37°C. In order to synthesize the DNA-Cu nanoclusters, a mixed solution containing 140 μL 20 mmol L⁻¹ PBS buffer (pH 7.4), 50 μL 1 mol L⁻¹ NaCl, and 10 μL 45 mmol L⁻¹ sodium ascorbate were added to the dsDNA solution. After shaking for 1 min, 10 μL 3.6 mmol L⁻¹ Cu²⁺ was added to the mixed solution with the final concentrations of 750 nmol L⁻¹ DNA, 120 μmol L⁻¹ Cu²⁺ and 1.5 mmol L⁻¹ sodium ascorbate. The mixed solution was 300 μL and kept for 10 min at 37°C, and then the fluorescent DNA-Cu nanoclusters were formed. All experiments were repeated three times.

Results and Discussion

Sensor design

We constructed a fluorescent biosensor with specific dsDNA as a template to synthesize DNA-Cu nanoclusters, which was used for label-free detection of mercury(II) ions based on exonuclease III-induced target signal recycling amplification. Figure 1 shows the design process for the detection of mercury ions by this fluorescent biosensor. DNA hybridization strictly follows the principle of base pairing, so Oligo A and Oligo B were formed to a rigid dsDNA. The hybridized dsDNA serves as a template for the formation of Cu nanoclusters, which have remarkable fluorescence intensity in comparison to the ssDNA.³⁰

For ultrasensitive detection of Hg²⁺, exonuclease III plays an important role in the sensor. In the physiological process, it can repair damaged dsDNA,³¹ make DNA hybridization more accurate,³² promote genetic recombination,³³ and prevent genomic disorders.³⁴ Exonuclease III acts to digest the dsDNA by the stepwise removal of mononucleotides in the direction of 3' to 5' with blunt or recessed 3' termini of dsDNA. However, it does not digest the ssDNA and the dsDNA with protruding 3' end. In our fluorescence sensor, exonuclease III cannot degrade the hairpin-structure ssDNA of Oligo A in the absence of mercury(II) ion, because in the Oligo A there is a protruding 3' end of three thymine bases. Hence, Oligo A and Oligo B, the two partly complementary nucleic acid strands, hybridize to form the template of stable DNA duplexes. After addition of sodium ascorbate and copper(II) ions, copper(II) ions are reduced to copper(I) ions, and the Cu⁺ disproportionates to form Cu²⁺ and Cu, resulting in stable Cu nanoclusters.³⁰ Cu nanoclusters accumulate in the dsDNA and the fluorescence intensity of the system is enhanced. However, when mercury(II) ions are present, they form a "T-Hg²⁺-T" mismatch structure at the end of the hairpin-structure ssDNA, changing the protruding 3' end of three thymine bases to a blunt 3' terminus. With the addition of exonuclease III, the Oligo A was cleaved to fragments in the 3' to 5' direction due to high exodeoxyribonuclease activity. The decrease of Oligo A would result in a smaller volume of dsDNA templates hybridized by

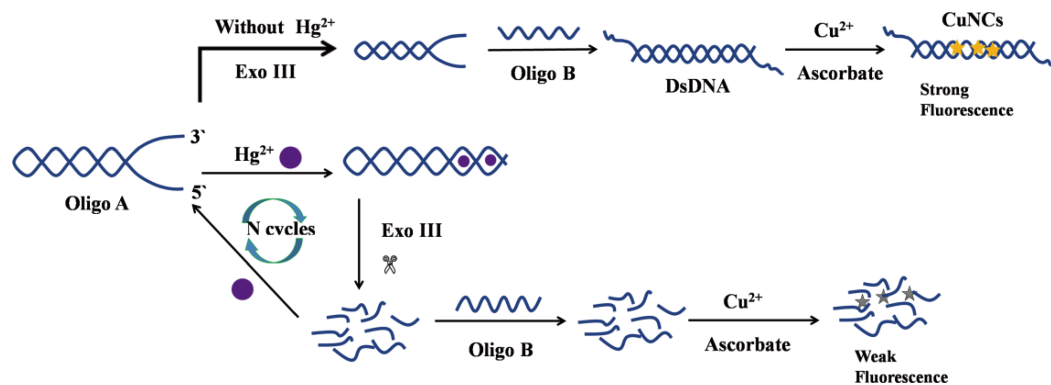


Fig. 1 Schematic illustration of mercury(II) ion detection using dsDNA-templated fluorescent Cu nanoclusters.

Oligo A and Oligo B. After the addition of sodium ascorbate and copper(II) ions, the DNA-Cu nanoclusters would not be formed for the lack of dsDNA templates. Therefore, the fluorescence intensity of the system is decreased.

In order to verify the feasibility of this new method to detect the mercury(II) ions, the fluorescence spectra of the following four conditions were measured: (a) no Hg^{2+} ions and no exonuclease III, (b) Hg^{2+} ions and no exonuclease III, (c) exonuclease III and no Hg^{2+} ions, and (d) Hg^{2+} ions and with exonuclease III. Figure 2A shows the TEM image for the formation of DNA-Cu nanoclusters with no Hg^{2+} ions and no exonuclease III. The diameter of nanoclusters is in the range of 2–3 nm. Figure 2B shows the relationship between wavelength and fluorescent intensity for these four conditions. A comparison of (a) and (b) shows that when exonuclease III is absent, adding Hg^{2+} ions leads to an 11.6% signal decrease in fluorescence intensity. Unfortunately, a comparison of (c) and (a) shows that when there are no Hg^{2+} ions, employed exonuclease III leads to a certain decrease in background fluorescence. This is because exonuclease III shows limited activity on the ssDNA.²⁸ Nevertheless, the comparison of (d) and (c) shows that the fluorescence intensity decreases rapidly when both mercury(II) ions and exonuclease III are added. We can observe a 75.2% signal decrease when exonuclease III amplification was employed. In contrast, a signal decrease of only 11.6% was observed without the exonuclease III. The result demonstrates that the method is feasible for ultrasensitive detection of mercury(II) ions.

Optimization of experimental conditions

The concentrations of Cu^{2+} ions and sodium ascorbate have a significant impact on the fluorescence intensity of the synthesized dsDNA-Cu nanoclusters. In order to obtain the best experimental conditions for fluorescent biosensors to detect mercury(II) ions, we optimized the experimental conditions.

Because dsDNA plays a role as a template in the formation of fluorescent copper nanoclusters, we first investigated the effect of dsDNA concentration on the fluorescence intensity of copper nanoclusters. As shown in Fig. 3(a), the fluorescence intensity of dsDNA-Cu nanoclusters increased as the dsDNA concentration gradually increased from 100 to 1000 nmol L^{-1} . Moreover, Fig. 3(b) shows that the fluorescence intensity of the dsDNA-Cu nanoclusters has a clear linear relationship with the concentration of dsDNA in the range of 100 to 1000 nmol L^{-1} . This indicated that the formation of Cu nanoclusters varies depending on the concentration of dsDNA when the other reaction conditions are fixed. Considering the fluorescence

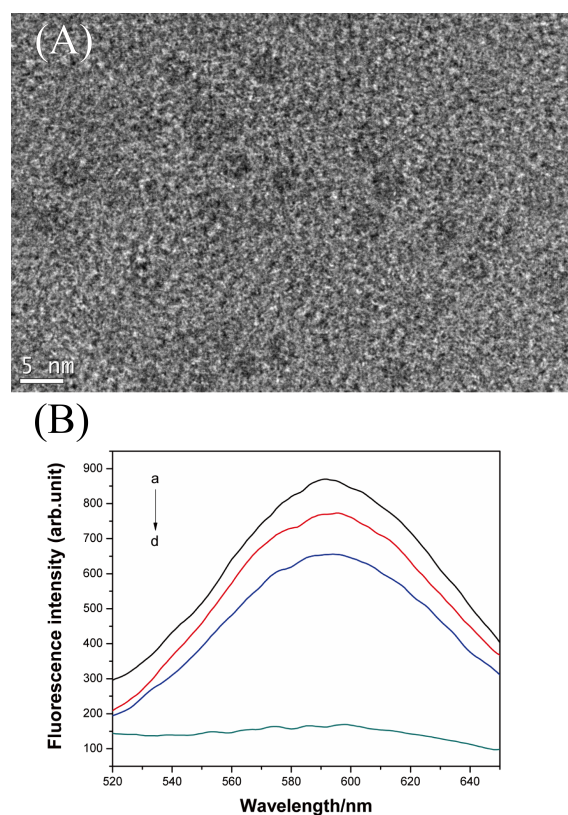


Fig. 2 (A) The TEM images of the dsDNA-Cu nanoclusters. (B) Fluorescence spectra of dsDNA-Cu nanoclusters obtained under different conditions: (a) without exonuclease III and Hg^{2+} , (b) with Hg^{2+} , without exonuclease III, (c) with exonuclease III, without Hg^{2+} , (d) with exonuclease III and Hg^{2+} . Conditions: 56 nmol L^{-1} Hg^{2+} ; 750 nmol L^{-1} dsDNA; 120 $\mu\text{mol L}^{-1}$ Cu^{2+} ; 1.5 mmol L^{-1} sodium ascorbate; 33 U mL^{-1} Exo III; 20 mmol L^{-1} PBS, pH 7.4.

intensity of the Cu nanoparticles satisfying the detection requirement, we chose to perform the following experiment using the concentration of dsDNA of 750 nmol L^{-1} .

Because Cu^{2+} ions have a significant influence on the formation of Cu nanoparticles, we next investigated the effect of Cu^{2+} ion concentration on the fluorescence intensity of Cu nanoparticles. Figure 3(c) shows that when the other reaction conditions are unchanged, the fluorescence intensity of the system increases with the increase of Cu^{2+} ion concentration.

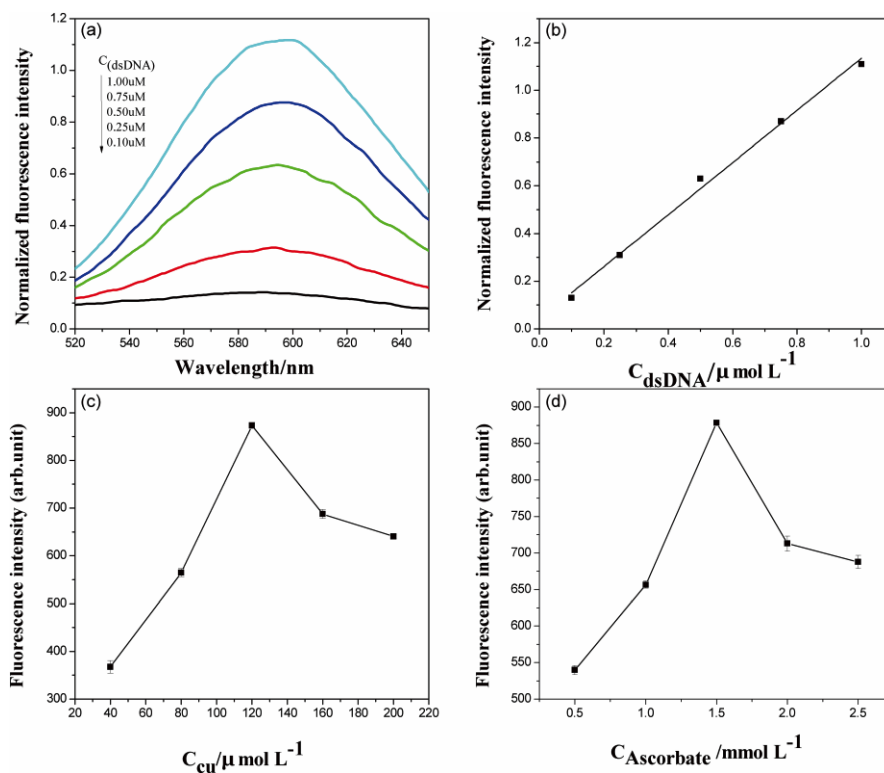


Fig. 3 (a) Effect of dsDNA concentration on the fluorescence spectra of dsDNA-Cu nanoclusters without the addition of exonuclease III and Hg^{2+} . The dsDNA concentrations were (from top to bottom) 1, 0.75, 0.5, 0.25 and 0.1 $\mu\text{mol L}^{-1}$. (b) The linear relationship between dsDNA concentration and fluorescence intensity. (c) Effect of Cu^{2+} concentration on the fluorescence spectra of dsDNA-Cu nanoclusters without the addition of exonuclease III and Hg^{2+} . The Cu^{2+} concentrations were (from left to right) 0.10, 0.25, 0.50, 0.75 and 1.00 $\mu\text{mol L}^{-1}$. (d) Effect of sodium ascorbate concentration on the fluorescence spectra of dsDNA-Cu nanoclusters without the addition of exonuclease III and Hg^{2+} .

Because copper(II) ions are at low concentrations, copper(II) ions can first bind to phosphate-negative groups on DNA molecules *via* electrostatic adsorption.³⁵ When the concentration of copper(II) ions is slightly increased, there is interaction between the copper(II) ions and the DNA bases and their interaction is greater.³⁶ At the same time, sodium ascorbate can also reduce the copper(II) ions to form stable fluorescent Cu nanoclusters. Therefore, it is clear that as the concentration of copper(II) ions increases, the fluorescence intensity also increases. However, the fluorescence intensity of the system decreases after the concentration of copper(II) ions reaches 120 $\mu\text{mol L}^{-1}$. This might be due to the decreasing dsDNA templates with the destruction of the DNA double helix by the hydroxyl radicals generated at the higher Cu^{2+} concentration.³⁷ Therefore, we chose the Cu^{2+} ion concentration of 120 $\mu\text{mol L}^{-1}$ for the following experiment.

In our experiment, sodium ascorbate reduces Cu^{2+} , which leads to the formation of stable Cu nanoclusters. Therefore, we further studied the effect of sodium ascorbate on the fluorescence intensity of copper nanoclusters. Figure 3(d) shows that as the sodium ascorbate concentration increases, the fluorescence intensity of the system also increases, indicating that sodium ascorbate is an effective reducing agent in the formation of Cu nanoclusters.³⁸ However, when the concentration of sodium ascorbate reached 1.5 mmol L^{-1} , the fluorescence intensity of the system gradually decreased, so we chose the sodium ascorbate concentration of 1.5 mmol L^{-1} for the later experiments.

As shown in Fig. 4, we studied the effect of the concentrations (a) and incubation time (b) of the Exo III on the fluorescence

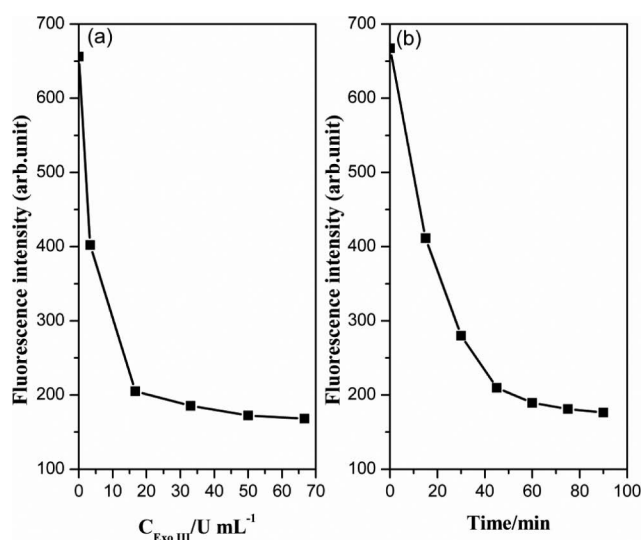


Fig. 4 Fluorescence spectra of different concentrations (a) and incubation time (b) of exonuclease III. Conditions: 56 nmol L^{-1} Hg^{2+} ; 750 nmol L^{-1} dsDNA; 120 $\mu\text{mol L}^{-1}$ Cu^{2+} ; 1.5 mmol L^{-1} sodium ascorbate; 33 U mL^{-1} Exo III; 60 min incubation time; 20 mmol L^{-1} PBS, pH 7.4.

intensity of the sensor. It can be seen that the fluorescence intensity decreased with the change of concentrations from 0 to 66 U mL^{-1} , or incubation time from 0 to 90 min. The

fluorescence intensity became almost constant at concentrations greater than 33 U mL^{-1} , or incubation time greater than 60 min. On the basis of the enzymatic reaction kinetics, the concentration of 33 U mL^{-1} and incubation time of 60 min were chosen for the Exo III enzyme in this sensing system.

Mercury(II) ion detection

Exonuclease III played a key role in our experiments. After the addition of exonuclease III, the sensor shows obvious fluorescence responses of exonuclease III-aided amplification assay to Hg^{2+} of varying concentrations. Figure 5(a) shows that with an increase of Hg^{2+} ion concentration from 0.004 to 80 nmol L^{-1} , the fluorescence intensity decreased gradually. This is because when Hg^{2+} is present, it is tightly bound to thymine (T) in the ssDNA of Oligo A, producing a stable T- Hg^{2+} -T complex.³⁹ After addition of exonuclease III, it can cleave the Oligo A into fragments along the 3'→5' direction, resulting in the lack of dsDNA templates, which leads to decreased fluorescence intensity of the system. Figure 5(b) shows that the fluorescence intensity of CuNCs has a clear linear relationship with the concentration of Hg^{2+} ions in the

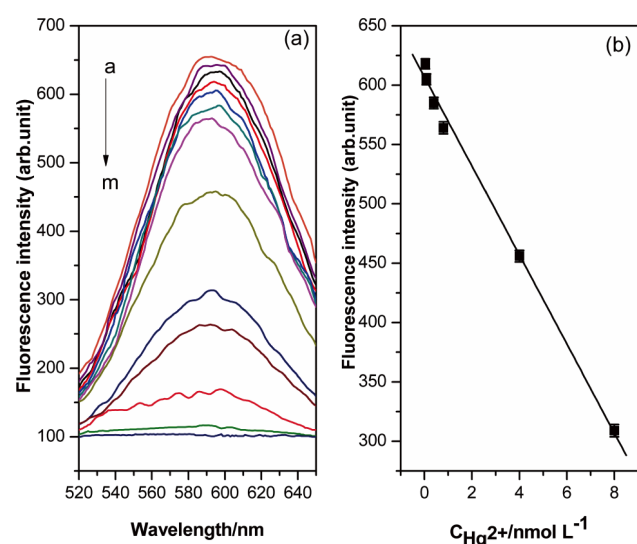


Fig. 5 (a) Fluorescence spectra of dsDNA-Cu nanoclusters detection of mercury(II) ions. Hg^{2+} concentrations were (from a to m) 0, 0.004, 0.008, 0.04, 0.08, 0.4, 0.8, 4, 8, 24, 40, 56, and 80 nmol L^{-1} . (b) The linear relationship between mercury(II) ion concentration and fluorescence intensity. Conditions: 750 nmol L^{-1} dsDNA; $120 \text{ }\mu\text{mol L}^{-1}$ Cu^{2+} ; 1.5 mmol L^{-1} sodium ascorbate; 33 U mL^{-1} Exo III; 20 mmol L^{-1} PBS, pH 7.4.

range of 0.004 to 8 nmol L^{-1} . The linear regression equation was $F = 606.99 - 37.51C_{\text{Hg}^{2+}}$, the linear correlation coefficient was $R^2 = 0.992$, and the limit of detection (LOD) of mercury(II) ions was 4 pmol L^{-1} . Importantly, our method could satisfy the requirement of the US Environment Protection Agency guideline for Hg^{2+} in tap drinking water ($\leq 10 \text{ nM}$). The reproducibility of the fluorescent sensors was determined by repeatedly detecting 2 nmol L^{-1} Hg^{2+} and the relative standard deviation (RSD) was 3.7% ($n = 8$). The results indicated satisfactory reproducibility of the fluorescent sensor. Table 1 shows that the detection limit was significantly lower than that of previous reported methods.

Selectivity and interference of the method for Hg^{2+} detection

In order to prove the selectivity of Hg^{2+} ion detection, we replaced Hg^{2+} ions with other metal ions (such as Fe^{2+} , Fe^{3+} , Ca^{2+} , Mg^{2+} , Zn^{2+} , Al^{3+} , Mn^{2+} , K^{+} , Ag^{+} , Ni^{2+} and Cd^{2+}) of 1 mmol L^{-1} in control experiments. The experimental process and the detection method were the same as for the mercury(II) ion detection. Figure 6(a) shows that compared with other metal ions, only Hg^{2+} ions have a significant influence on the fluorescence intensity of the system under the same conditions. The results show that this experiment has good selectivity for the detection of Hg^{2+} ions.

To verify the ability of fluorescent biosensors to specifically recognize Hg^{2+} ions, we examined the effects of a series of other metal ions that may interfere, such as Fe^{2+} , Fe^{3+} , Ca^{2+} , Mg^{2+} , Zn^{2+} , Al^{3+} , Mn^{2+} , K^{+} , Ag^{+} , Ni^{2+} and Cd^{2+} . Figure 6(b) shows the results of combining 40 nmol L^{-1} Hg^{2+} ions and 1 mmol L^{-1} of the other metal ions to the DNA-Cu nanoparticle system. The results show that when Hg^{2+} ions are present and different metal ions are added to the fluorescent sensor, there is no significant effect on the fluorescence intensity of Cu nanoclusters, indicating that other metal ions do not interfere with the detection of Hg^{2+} ions by the fluorescent biosensor.

Actual sample test

To confirm that our process can detect mercury(II) ions in real samples, we tested the presence of Hg^{2+} in tap water. Tap water containing Hg^{2+} can be harmful to the environment and human health. The results of this experiment displayed in Table 2 show that there is no Hg^{2+} in the tap water, and the addition of Hg^{2+} concentrations of 0.01, 2, and 6 nM was applied for the recovery test using the procedure described above. The average recoveries of Hg^{2+} ions were in the range from 97.5 to 102.17%. According to the results, our method can be successfully used to detect Hg^{2+} in tap water samples.

Table 1 Different methods for the determination of Hg^{2+}

Serial number	Usage method	Materials used	Detection range	Detection limit	Reference
1	Electrochemical method	Novel EDTA modified PANI/SWCNTs nanocomposite electrode	$2 \text{ mM} - 2 \text{ }\mu\text{M}$	$0.68 \text{ }\mu\text{M}$	40
2	Fluorometric method	enzyme-free DNA molecular machine	$0.1 \text{ nM} - 100 \text{ nM}$	65 pM	41
3	Fluorometric method	9AnPD and polymer	$50 \text{ nM} - 100 \text{ }\mu\text{M}$	$0.5 \text{ }\mu\text{M}$	42
4	Fluorometric method	Au nanoparticles	$0.067 \text{ nM} - 23.3 \text{ nM}$	26 pM	43
5	Colorimetric assays	Silver nanoparticles/carboxymethyl cellulose	$2.5 \text{ nM} - 250 \text{ nM}$	5 nM	44
6	Fluorometric method	G-Quadruplex complex	$0.3 \text{ }\mu\text{M} - 4 \text{ }\mu\text{M}$	10 nM	45
7	Fluorometric method	Graphene oxide (GO) catalytic hairpins self-assembly	$50 \text{ pM} - 1200 \text{ pM}$	25 pM	46
8	Fluorometric method	Cu nanoparticles, Exonuclease III	$0.04 \text{ nM} - 8 \text{ nM}$	4 pM	This work

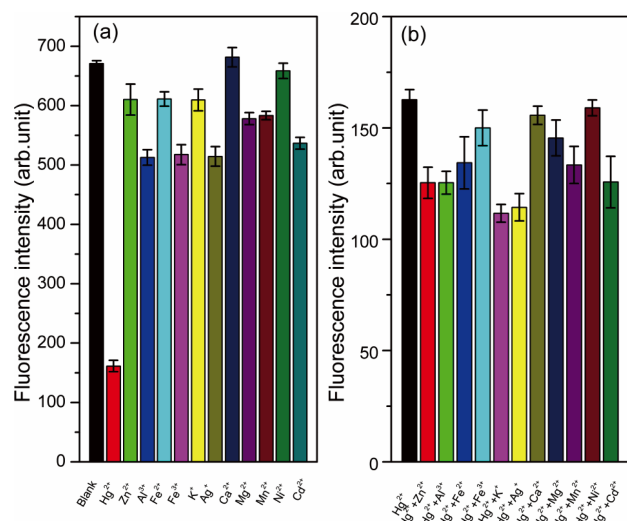


Fig. 6 (a) Selectivity of the dsDNA-Cu nanoclusters sensing system for Hg²⁺ detection. The concentration of Hg²⁺ was 40 nmol L⁻¹, and the concentrations of the other metal ions were 1 mmol L⁻¹. (b) Anti-interference for the dsDNA-Cu nanoclusters sensing system for Hg²⁺ detection. The concentration of Hg²⁺ was 40 nmol L⁻¹, and the concentrations of all other ions that might interfere were 1 mmol L⁻¹. Conditions: 750 nmol L⁻¹ dsDNA; 120 μmol L⁻¹ Cu²⁺; 1.5 mmol L⁻¹ sodium ascorbate; 33 U mL⁻¹ Exo III; 20 mmol L⁻¹ PBS, pH 7.4.

Table 2 Detection of Hg²⁺ in tap water using the fluorescence sensing platform

Sample	Found amount/ nmol L ⁻¹	Added amount/ nmol L ⁻¹	Detection amount/ nmol L ⁻¹	Recovery, %
Tap water	—	0.1	0.098	9.8
	—	2.0	1.95	97.5
	—	6.0	6.13	102.17

Conclusions

In summary, we describe a novel approach for label-free detection of Hg²⁺ based on exonuclease III-induced target signal recycling amplification using dsDNA templated CuNCs. In the presence of Hg²⁺, T-Hg²⁺-T mismatch sequence structures would be formed and the protruding 3' end of hairpin-structure ssDNA would be changed to a blunt 3' terminus. The exonuclease III could digest the hairpin-structure ssDNA and release the Hg²⁺, allowing it to take part in the next cycling route. The decrease of ssDNA would lead to no hybridization of dsDNA, which would result in an outstanding decrease of fluorescence intensity. Experimental results showed that the addition of exonuclease III could obviously enhance the fluorescent intensity of the hairpin DNA-based fluorescence sensor without exonuclease III. Compared with previous methods, the CuNCs fluorescent sensor is simple, low cost, easy to operate and offers sensitivity. At the same time, this method exhibits high sensitivity and selectivity for the detection of Hg²⁺ and the fluorescent sensor has been successfully applied for the assay of Hg²⁺ in tap water.

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