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Fluorescence Regulation of Copper Nanoclusters via DNA Template Manipulation toward Design of a High Signal-to-Noise Ratio Biosensor

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ABSTRACT

Due to bioaccumulation of food chain and disability of biodegradation, concentration of toxic mercury ions (Hg^{2+}) in the environment dramatically varies from picomolar to micromolar, indicating the importance of well-performed Hg^{2+} analytical methods. Herein, reticular DNA is constructed by introducing thymine (T)-Hg^{2+}-T nodes in poly(T) DNA, and copper nanoclusters (CuNCs) with aggregate morphology are prepared using this reticular DNA as templates. Intriguingly, the prepared CuNCs exhibit enhanced fluorescence. Meanwhile, the reticular DNA reveals evident resistance to enzyme digestion, further clarifying the fluorescence enhancement of CuNCs. Relying on the dual-function of DNA manipulation, a high signal-to-noise ratio biosensor is designed. This analytical approach can quantify Hg^{2+} in a very wide range (50 pM -

500 μM) with an ultra-low detection limit (16 pM). Besides, depending on the specific interaction between Hg^{2+} and reduced L-glutathione (GSH), this biosensor is able to evaluate the inhibition of GSH toward Hg^{2+}. In addition, pollution of Hg^{2+} in three lakes are tested using this method, and the obtained results are in accord with that from inductively coupled plasma mass spectrometry. In general, this work provides an alternative way to regulate properties of DNA-templated nanomaterials, and indicates the applicability of this way by fabricating an advanced biosensor.

KEYWORDS

copper nanoclusters, DNA template manipulation, fluorescence regulation, Hg^{2+} quantification, biosensor

INTRODUCTION

Mercury ions (Hg^{2+}), widespread cytotoxic ions in river, food, and cosmetics, may result in hydrargyrism by damaging DNA and inhibiting DNA repair.[1] Meanwhile, according to bioaccumulation of food chain and disability of biodegradation, concentration of Hg^{2+} in the environment dramatically varies in a range of picomolar to micromolar.[2-4] Currently, various methods including electrochemistry,[5,6] electrochemiluminescence,[7] photoelectrochemistry,[8] room-temperature phosphorescence,[9] colorimetry,[10] and surface enhanced Raman spectroscopy,[11] have been created to determine Hg^{2+}. Although these methods reveal their superiority in sensitivity, they are normally lack of the capability of sensing Hg^{2+} in a broad

range. Therefore, fabrication of Hg^{2+} biosensors with a wide detection range and an ultra-low detection limit is still urgent to us.

DNA-templated nanomaterials have been widely used to design analytical approaches for their low toxicity, ease of preparation, and in particular tunability of fluorescence.[12,13] It is commonly known that the fluorescence of DNA-templated nanomaterials can be tuned by altering the DNA sequence, length, and structure.[14] In detail, DNA with a specific sequence and length is an essential prerequisite to prepare DNA-templated nanomaterials.[15-18] Besides, specific DNA sequence can induce fluorescence enhancement by setting them in close proximity to nanomaterials.[19,20] DNA structure is another key factor to affect the property of DNA-templated metal nanoclusters. For instance, both single stranded DNA (ssDNA) and double stranded DNA (dsDNA) can act as templates for the preparation of copper nanoparticles, but result in different fluorescence emission.[21] Meanwhile, it has been reported that rigid structure of DNA may minimize the quenching effect caused by environmental stimuli.[22] Therefore, taking melamine-bound dsDNA as templates, florescence of copper nanoclusters (CuNCs) is greatly enhanced. The aforementioned studies demonstrated that fluorescence of DNA-templated nanoclusters can be significantly affected by DNA sequence, length, and structure. In comparison with alternation of DNA sequence and length, manipulation of DNA structure is the most convenient choice. Accordingly, exploring novel principles that can improve the rigidity of DNA structure offers exciting opportunities to enhance the fluorescence of DNA-templated nanomaterials.

Integrating enzyme catalysis into analytical methods is an alternative way to improve the performances of DNA-based biosensors.[23,24] Especially, relying on their interactions with nucleosides, metal ions can regulate enzyme catalysis. To date, Hg^{2+}, silver ions (Ag^{+}), and copper ions (Cu^{2+}) have been employed to induce or restrain the activity of enzyme by forming

metal ions-mediated base pairs. For example, Hg^{2+} and Ag^{+} can trigger "illusionary" polymerase activity by constructing thymine (T)-Hg^{2+}-T, cytosine (C)-Ag^{+}-C, and C-Ag^{+}-adenine (A) base pairs.[25,26] Similarly, Cu^{2+} can mediate the formation of artificial base pairs to enable the activity of DNA polymerase.[27] Apart from DNA polymerase, exonuclease III can also be regulated by metal ions.[28] These studies indicate that metal ions can alter the structure of DNA, thus influencing the behaviors of enzyme catalysis and the following applications.

Taking multi-functional manipulation of DNA in regulating properties of nanomaterials into account, we believe poly(T) DNA is promising to design a fluorescence Hg^{2+} biosensor with a high signal-to-noise ratio. First, poly(T) DNA is a preferential template in preparing CuNCs that can emit intense fluorescence. Second, it has been demonstrated by a number of studies that Hg^{2+} can specifically bind to T to form T-Hg^{2+}-T base pairs.[29-33] Using T-Hg^{2+}-T base pairs as nodes, single stranded poly(T) DNA tends to become a reticular structure. Reticular structure is more rigid than single stranded structure, which is propitious to enhance the fluorescence of CuNCs. Third, single stranded structure is an ideal substrate of exonuclease I (exo I), while Hg-induced reticular structure may restrain the digestion of exo I. Therefore, background noise from redundant poly(T) DNA can be decreased by exo I, and enhanced signal from Hg-induced reticular DNA is maintained. Overall, because of fluorescence regulation via DNA template manipulation, the signal-to-noise of a Hg^{2+} biosensor is remarkably improved. As a result, this biosensor is able to quantify Hg^{2+} in a wide range with an ultra-low detection limit. Besides, the analytical approach is applied to evaluate the inhibition of L-glutathione (GSH) toward Hg^{2+} and test Hg^{2+} pollution in lakes.

EXPERIMENTAL SECTION

Chemicals and materials. Mercury acetate ($Hg(Ac)_2$), copper chloride ($CuCl_2$), ascorbic acid (Vc), reduced L-glutathione (GSH), sodium chloride (NaCl), potassium chloride (KCl), lithium chloride monohydrate ($LiCl \cdot H_2O$), magnesium chloride hexahydrate ($MgCl_2 \cdot 6H_2O$), calcium chloride dihydrate ($CaCl_2 \cdot 2H_2O$), cobalt acetate tetrahydrate ($Co(Ac)_2 \cdot 4H_2O$), barium nitrate ($Ba(NO_3)_2$), nickel acetate tetrahydrate ($Ni(Ac)_2 \cdot 4H_2O$), tris(hydroxymethyl)aminomethane hydrochloride (Tris-HCl), boric acid (H_3BO_3), ethylene diamine tetraacetic acid (EDTA), and 3-(N-Morpholino)propanesulfonic acid (MOPS) were purchased from Sigma-Aldrich Co., Ltd. (Shanghai, China). Exo I was obtained from Takara Biotechnology Co., Ltd. (Dalian, China).

Oligonucleotides (poly(30T): 5'-TTTTTTTTTTTTTTTTTTTTTTTTTTTTTT-3') were obtained and purified with HPLC by Shanghai Sangon Biological Engineering Technology & Services Co., Ltd (Shanghai, China).

Poly(30T) was dissolved in ultrapure water. $CuCl_2$ was dissolved in MOPS (10 mM, pH 7.5) as a stock solution. Other solutions were prepared with ultrapure water (18.2 MΩ cm) obtained from a Milli-Q purification system (Bedford, MA).

Instrumentation. Fluorescence measurements were carried out on a Fluoromax-4 spectrometer (Horiba, France). The fluorescence emission spectra of CuNCs were recorded from 500 nm to 800 nm with a 340 nm excitation wavelength by a 400 nm optical filter. The transmission electron microscopy (TEM) images and high-resolution transmission electron microscopy (HRTEM) images were recorded on an instrumentation (JEOL-2100F) at an accelerating voltage of 200 kV. Circular dichroism (CD) measurements were obtained on a Chirascan (Applied Photophysics, Britain), using quartz cells and a 1 nm bandwidth, 1 nm step size, 1 mm path length. The scan range was from 350 nm to 190 nm. Inductively coupled plasma

mass spectrometry (ICP-MS) measurements were accomplished with NexION 300X (PerkinElmer, USA).

Preparation of CuNCs. In a typical procedure, DNA template solution was first mixed with adequate MOPS solution to the final volume of 275 μL. Then, 10 μL of 1 mM $CuCl_2$ and 15 μL of 20 mM Vc were added into the mixture. After keeping the solution in dark for 15 min, DNA-templated CuNCs were obtained and ready for fluorescence characterization.

Construction of a Novel Fluorescence Hg^{2+} Biosensor. First, Hg^{2+}-treated DNA templates were prepared. Specifically, 15 μL of 10 μM poly(30T) and 15 μL of Hg^{2+} at different concentrations were incubated at 37 °C for 30 min. Afterwards, exo I (8 U) was added into the solution, and the mixture was kept at 37 °C for another 30 min. Then, MOPS, $CuCl_2$, and Vc were added into the mixture to form CuNCs, and fluorescence spectra were recorded 15 min later.

Selectivity Investigation of the Biosensor. To testify the selectivity of this biosensor, 15 μL of 1 mM Na^+, K^+, Li^+, Mg^{2+}, Ca^{2+}, Co^{2+}, Ba^{2+}, and Ni^{2+} were incubated with 15 μL of 10 μM poly(30T) at 37 °C for 30 min, respectively. Then, exo I (8 U) was added into the solutions that were incubated at 37 °C for another 30 min. After that, the above solutions were used to prepare CuNCs.

Inhibition Assay of GSH toward Hg^{2+}. To accomplish inhibition assay, 15 μL of GSH at various concentrations and 15 μL of 1 mM Hg^{2+} were first mixed and incubated for 30 min. After that, 15 μL of 10 μM poly(30T) was added into the mixture and kept at 37 °C for 30 min. The following procedure was similar to that of biosensor construction.

Analysis of Hg^{2+} in Real Water Samples. Real water samples were collected from three lakes (Xianlin Lake, Yangshan Lake, and Lanyue Lake) in Nanjing (China). These lake water samples were filtrated by filtering membrane with 0.22 μm inner diameter to remove some impurity. The amount of Hg^{2+} in pretreated samples was analyzed by the proposed biosensor and ICP-MS, respectively.

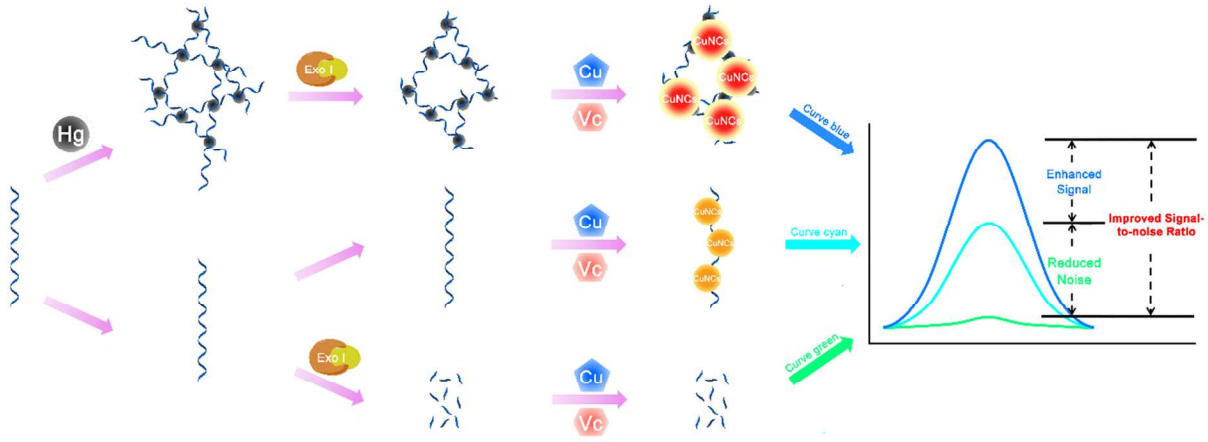
TEM Characterization of CuNCs. To characterize poly(30T)-templated CuNCs and Hg^{2+}-treated poly(30T)-templated CuNCs with TEM, the prepared CuNCs were first diluted into proper concentration. Then, 10 μL of the as-diluted CuNCs were dropped onto carbon-coated copper grid substrates, which were dried naturally for 3 hours in a dryer.

Circular Dichroism (CD) Assay. Sample preparation for CD assay was the same with that for fluorescence detection, while higher concentrations of DNA and Hg^{2+} were needed. The final concentrations of poly(30T) and Hg^{2+} were 10 μM and 1 mM, respectively.

Polyacrylamide Gel Electrophoresis (PAGE) and Staining. The construction of Hg^{2+} biosensor was confirmed by 12% non-denaturing polyacrylamide gel electrophoresis, which was carried out at room temperature in 1×TBE buffer (9 mM Tris-HCl, 9 mM boric acid, 0.2 mM EDTA, pH 8.0) for 1.5 h with a constant voltage of 100 V. After electrophoresis, the gel was stained by fluorescent CuNCs as described previously.[34] Briefly, the gel was first immersed in a 50 mL of MOPS buffer solution (10 mM MOPS, 50 mM $MgCl_2$, pH 7.5) containing 100 μM $CuCl_2$ for 15 min at room temperature. Then, 1 mM Vc was added to the above solution and incubated for another 15 min to synthesize CuNCs. The gel was re-stained by repeating the above procedure to enhance the fluorescence intensity.

RESULTS AND DISCUSSION

Mechanism of Hg^{2+} Sensing Based on Fluorescence Regulation of CuNCs via DNA Template Manipulation. It is generally accepted that some metal ions can specifically interact with certain nucleotides to build special DNA structure, such as T-Hg^{2+}-T base pairs. In poly(T) DNA, T-Hg^{2+}-T base pairs can be considered as nodes. As a result, poly(T) DNA becomes interlaced, leading to the formation of reticular DNA. In comparison with single stranded poly(T) DNA, Hg^{2+}-treated poly(T) DNA is speculated to be more rigid, which is apt to form CuNCs with enhanced fluorescence. Relying on the fluorescence enhancement, Hg^{2+} can be conveniently detected. However, given that poly(T) DNA is excessive in the procedure of Hg^{2+} detection, this method suffers from high background noise. To reduce the background noise, exo I is employed to digest redundant poly(T) DNA. Due to substrate specificity of enzyme catalysis, exo I can only remove nucleotides from ssDNA instead of reticular DNA. Therefore, background noise is reduced, while enhanced signal is protected. This sensing mechanism has been illustrated in Scheme 1. Notably, because of Hg^{2+}-induced fluorescence enhancement and enzyme restraint, signal-to-noise ratio of this biosensor is remarkably enlarged, which lays groundwork for sensitive quantification of Hg^{2+} in a wide range.



Scheme 1. Schematic illustration of Hg^{2+} quantification based on fluorescence regulation of CuNCs via DNA template manipulation.

Hg^{2+}-Induced Fluorescence Enhancement and Enzyme Restraint. Effect of Hg^{2+} on the morphology of poly(30T)-templated CuNCs is first investigated. TEM image in Figure 1A shows that poly(30T)-templated CuNCs are monodisperse. Crystal planes of the CuNCs with a lattice spacing of 2.05 Å are clearly observed in HRTEM (Inset of Figure 1A), which is corresponding to the (111) plane of the fcc phase of metal copper.[35] If poly(30T) is treated with Hg^{2+} in advance, morphology of prepared CuNCs becomes aggregate (Figure 1B). The phenomenon should be attributed to the structural change of DNA templates. According to CD spectra in Figure 1C, it can be found that there is an evident difference in the structures of poly(30T) and Hg^{2+}-treated poly(30T). In detail, the interaction between Hg^{2+} and T may introduce a large number of T-Hg^{2+}-T nodes in poly(30T), resulting in interleaving of ssDNA into reticular DNA. It is noteworthy that a negative peak emerges at 265 nm, indicating the reticular DNA is more rigid.[36] Using reticular DNA as templates, the prepared CuNCs is naturally in aggregate morphology.

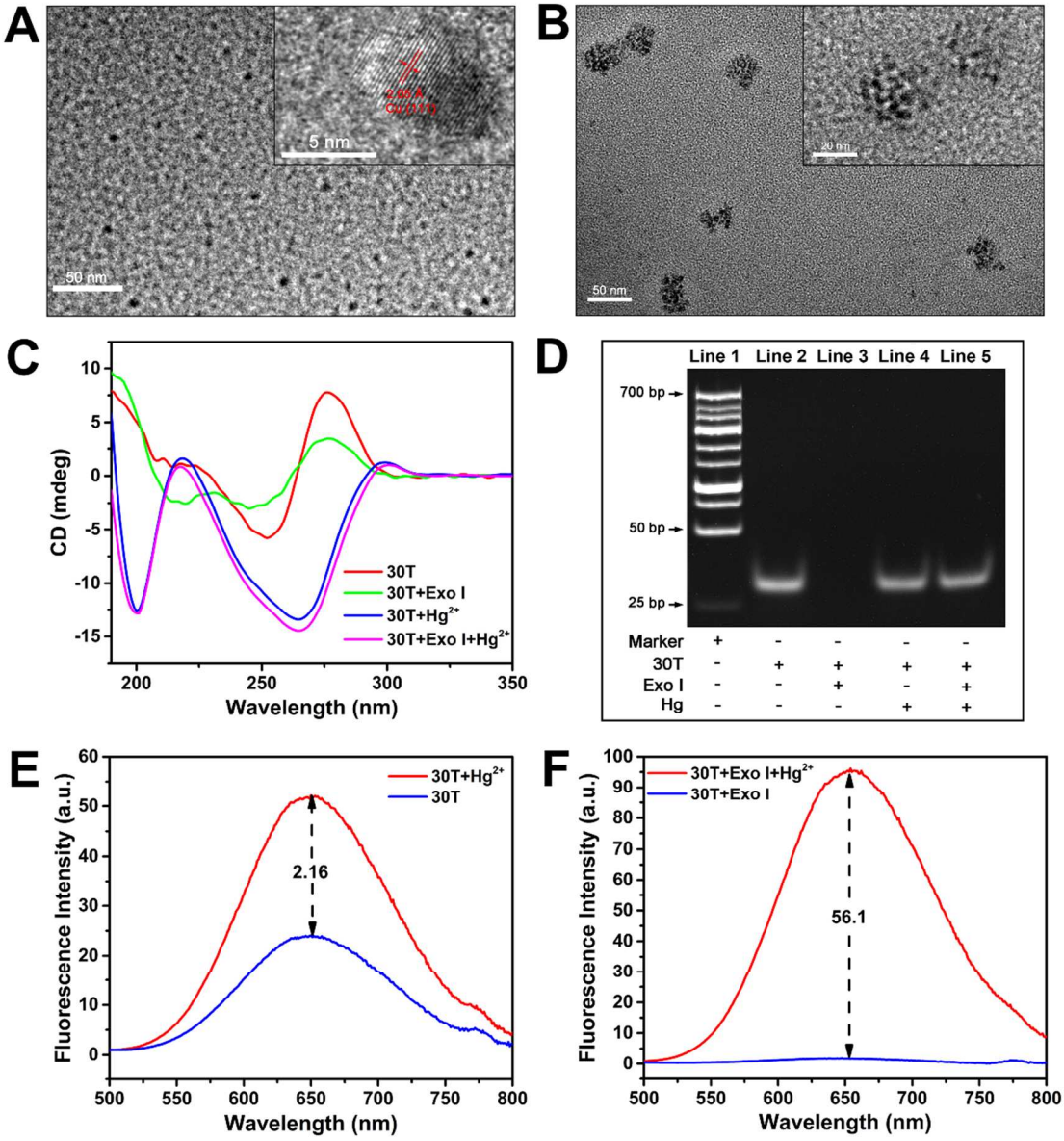


Figure 1. (A) TEM image of CuNCs using poly(30T) as templates. Inset: HRTEM image of CuNCs with a lattice spacing of 2.05 Å. (B) TEM image of CuNCs using Hg^{2+}-treated poly(30T) as templates. Inset: Enlargement of one aggregate of CuNCs. (C) CD spectra of poly(30T), poly(30T) with exo I, poly(30T) with Hg^{2+}, and poly(30T) with Hg^{2+} and exo I. (D) PAGE image of different samples stained by CuNCs. The symbol "+" means the presence of each reagent, whereas the symbol "–" means the absence of each reagent. (E) Fluorescence spectra of CuNCs

using poly(30T) and Hg^{2+}-treated poly(30T) as templates. (F) Fluorescence spectra of CuNCs using poly(30T) and Hg^{2+}-treated poly(30T) as templates in the presence of exo I.

Meanwhile, it is found that Hg^{2+} can restrain the digestion of exo I to its substrate. For ssDNA, intensities of CD peaks become weak in the presence of exo I, since a large number of poly(30T) is digested by exo I. In contrast, for reticular DNA, only slight change is observed after the addition of exo I, reflecting great resistance to exo I digestion. The results are in good consistent with the observation from PAGE. To our knowledge, normal dyes, such as ethidium bromide and SYBR Green I, are incapable in staining poly(T) DNA. Fortunately, Zhu and coworkers establish a novel protocol for poly(T) staining by in situ synthesis of fluorescent CuNCs in the gel,[34] which is adopted in this assay. In comparison with line 2 in Figure 1D, there is no band in line 3 as a consequence of exo I digestion. However, in the presence of Hg^{2+}, evident bands of poly(30T) can be observed in line 4 and line 5, meaning that exo I cannot work on Hg^{2+}-treated poly(30T). From the results of CD spectra and PAGE image, it can be concluded that the interaction between Hg^{2+} and T results in the structural change of poly(30T), inducing restraint of exo I digestion.

Furthermore, we survey the fluorescence property of reticular DNA-templated CuNCs. In comparison with poly(30T)-templated CuNCs, the absorption wavelength, excitation wavelength and maximum emission wavelength of Hg^{2+}-treated poly(30T)-templated CuNCs are not obviously changed, but the fluorescence intensity is remarkably enlarged (Figure 1E, Figure S1). As reported, DNA with rigid structure is of great importance in enhancing fluorescence of DNA-templated nanomaterials.[37,38] Since reticular DNA is much more rigid than ssDNA, fluorescence of Hg^{2+}-treated poly(30T)-templated CuNCs is thus greatly enhanced. However, in this case, an obvious background noise is unavoidable. To further highlight the fluorescence enhancement,

exo I is introduced to digest redundant poly(30T). As identified above, exo I only work on ssDNA rather than reticular DNA. As a result, background noise is greatly reduced, while enhanced fluorescence is maintained to the most extent (Figure 1F). According to the participation of exo I, signal-to-noise ratio of Hg^{2+}-induced fluorescence enhancement enlarges from 2.16 to 56.1.

Determination of Hg^{2+} with a High Signal-to-Noise Ratio Method. Since fluorescence of CuNCs is enhanced with the increase of Hg^{2+}, Hg^{2+} can be simply detected by taking fluorescence intensity at 650 nm ($F_{650\,nm}$) as the signal (Figure 2A). In a range of 10 to 500 μM, a linear dependency between $F_{650\,nm}$ and concentration of Hg^{2+} ($[Hg^{2+}]$) is obtained. The regression equation is $F_{650\,nm} = 34.2\lg[Hg^{2+}] - 100$, $R = 0.998$. The calculated detection limit of this biosensor is 6.9 μM (S/N = 3, the detailed description of the calculation is given in the supporting information). However, due to the high background noise, Hg^{2+} under the concentration of 10 μM cannot be sensed in this way. Therefore, exo I is employed to pursue better analytical performances. As can be seen in Figure S2, the amount of exo I used in this assay is firstly investigated. Under the optimized condition, background noise is greatly reduced, clarifying Hg^{2+}-induced fluorescence enhancement (Figure 2B). After carefully analyzing the dependence of $F_{650\,nm}$ and $[Hg^{2+}]$, it is found that quantification of Hg^{2+} can be accomplished in two linear ranges. In the range of 50 pM to 2.5 μM, the regression equation is $F_{650\,nm} = 3.1\lg[Hg^{2+}] + 5.9$, $R = 0.998$. In the range of 2.5 μM to 500 μM, the regression equation is $F_{650\,nm} = 13.3\lg[Hg^{2+}] - 25.6$, $R = 0.994$. The calculated detection limit of this method is 16 pM (S/N = 3) (Figure 2C). As the increase of signal-to-noise ratio, linear range of Hg^{2+} biosensor is enlarged approximately 5 orders of magnitude in accompany with the decrease of detection limit from 6.9 μM to 16 pM. Since Hg^{2+} can be measured with different strategies, analytical performances of

several Hg^{2+} biosensors have been summarized in Table S1. It can be found that detection limit of our biosensor is better than or comparable to the listed methods. More importantly, linear range of our biosensor is at least 2 orders of magnitude wider than them, which is desirable in current Hg^{2+} approaches. To evaluate the selectivity of this analytical approach, the proposed Hg^{2+} biosensor is challenged with several other metal ions. As depicted in Figure 2D, by replacing Hg^{2+} with other metal ions, $F_{650\ nm}$ is not significantly enhanced, suggesting the satisfactory selectivity of this fluorescence biosensor, which should be ascribed to the specificity of T-Hg^{2+} interaction.

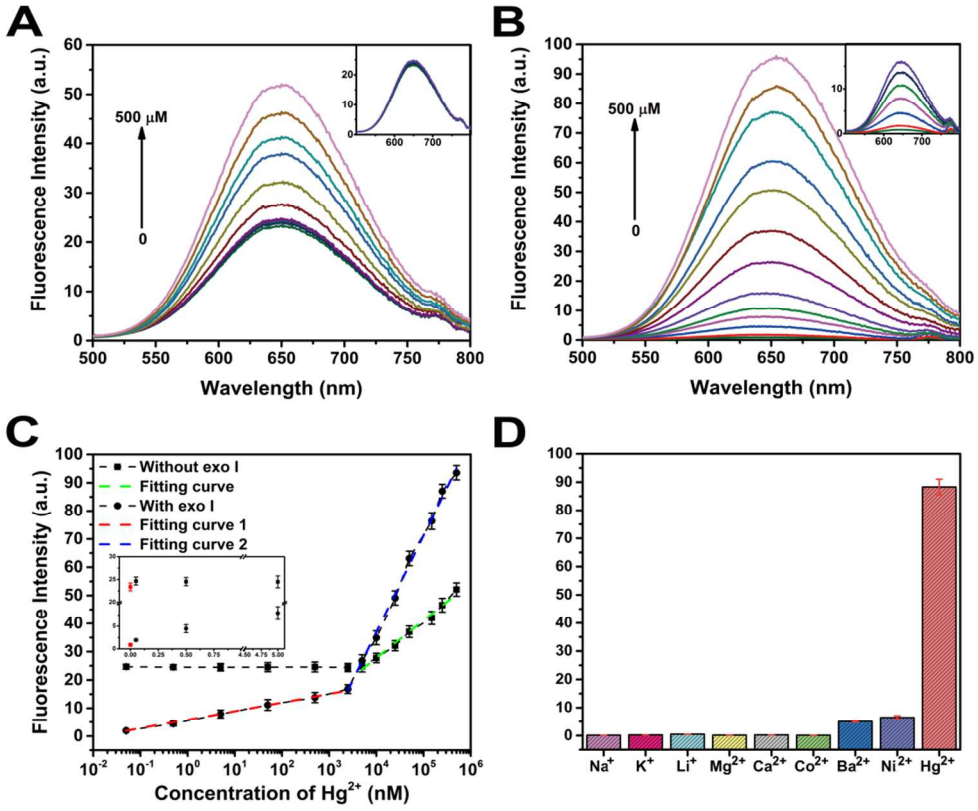


Figure 2. (A) Fluorescence spectra of CuNCs using Hg^{2+}-treated poly(30T) as templates. The concentration of Hg^{2+} varies from 0 to 500 μM. Inset: Regionally enlarged drawing with the concentration of Hg^{2+} varies from 0 to 2.5 μM. (B) Fluorescence spectra of CuNCs using Hg^{2+}-

treated poly(30T) as templates in the presence of exo I. The concentration of Hg^{2+} varies from 0 to 500 μM. Inset: Regionally enlarged drawing with the concentration of Hg^{2+} varies from 0 to 2.5 μM. (C) The dependence of fluorescence intensity on the concentration of Hg^{2+} in the absence and presence of exo I. Inset: Regionally enlarged drawing with the concentration of Hg^{2+} varies from 0 to 5 nM. The fluorescence intensities of blank samples are marked in red. (D) Fluorescence responses of CuNCs using different metal ions-treated poly(30T) as templates in the presence of exo I.

Evaluation of the Inhibition of GSH toward Hg^{2+}. GSH plays an essential role in regulating the redox balance of biological processes,[39] thus methods that can quantify GSH are valuable in studying oxidative stress reaction and related diseases. Previous works have confirmed that Hg^{2+} can directly interact with the thiol group of GSH, leading to the formation of an GSH-Hg complex.[40] Thus, GSH can serve as an inhibitor of Hg^{2+} in our body. Since the fluorescence intensity is highly related to the concentration of Hg^{2+}, the proposed biosensor is further used to evaluate the inhibition of GSH toward Hg^{2+}. With the increase of GSH, concentration of Hg^{2+} decreases by forming GSH-Hg complex, and thus the fluorescence of CuNCs is gradually reduced (Figure 3). A linear dependency between $F_{650\,nm}$ and the concentration of GSH ([GSH]) is obtained in a range of 0.5 to 300 μM with a detection limit of 0.2 μM. The regression equation was $F_{650\,nm}$=97.5-0.18[GSH], R=0.997. As the concentration of GSH is about micromolar level in human serum.[41] this biosensor is promising in analyzing GSH in real biological samples.

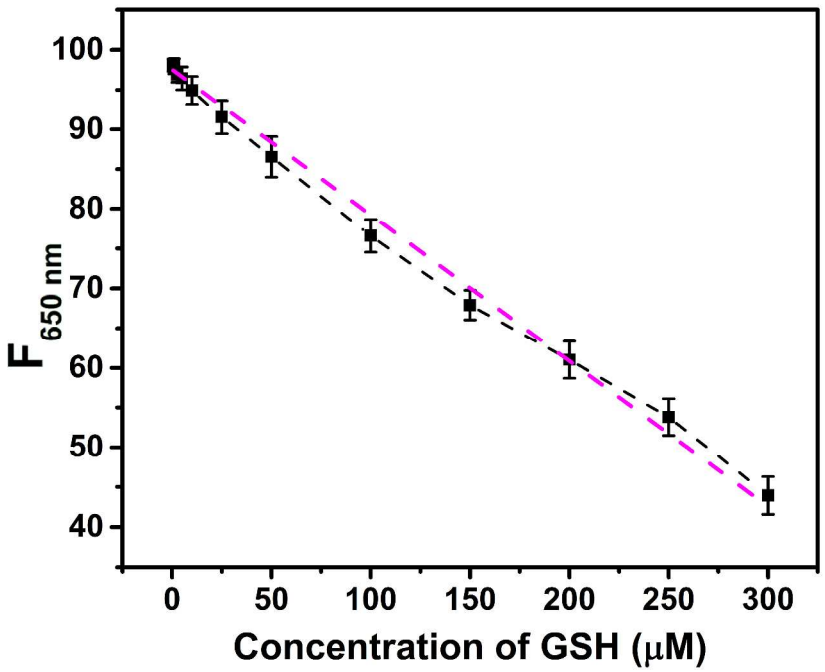


Figure 3. The dependence of fluorescence intensity of Hg^{2+}-responsive system on the concentration of GSH.

Quantification of Hg^{2+} in Lake Water Samples. Thanks to the improved signal-to-noise ratio, the proposed biosensor is very suitable to quantify Hg^{2+} in real samples. Despite the fact that there are several contaminants in lake water samples, high recovery percentage and low relative standard deviation (RSD) reveal the applicability of this biosensor in real sample detection (Table 1). More importantly, the results obtained from this biosensor get a fine match with the data from the ICP-MS, which is desirable in related biosensors. It is reported that guideline values for Hg^{2+} in drinking-water are 6 μg/L (Guidelines for Drinking-water Quality, World Health Organization),[42] so the quality of drinking-water can be assessed by this analytical method.

Table 1. Quantification of Hg^{2+} in lake water samples using ICP-MS and our proposed biosensor.

Sample	Hg^{2+} spiked (μg/L)	ICP-MS (μg/L)	Recovery (%)	RSD (%, n=3)	This biosensor (μg/L)	Recovery (%)	RSD (%, n=3)
Yangshan Lake	0.0	12.3	N/A[a)]	5.8	12.8	N/A	3.5
	5.0	17.7	108	8.2	18.0	104	4.1
	100.0	118.3	106	2.9	117.8	105	3.7
Xianlin Lake	0.0	6.7	N/A	5.0	5.8	N/A	2.8
	5.0	11.9	104	5.6	10.7	98	4.6
	100.0	111.7	105	6.2	109.8	104	1.6
Lanyue Lake	0.0	5.7	N/A	3.4	5.2	N/A	3.3
	5.0	10.8	102	4.2	10.1	98	2.9
	100.0	110.7	105	5.2	107.2	102	4.5

a) N/A represents Not Applicable

CONCLUSIONS

In this work, reticular DNA is constructed relying on the interaction between Hg^{2+} and poly(30T). Taking the reticular DNA as templates, novel CuNCs with aggregate morphology are prepared. It is found that the reticular DNA-templated CuNCs exhibit enhanced fluorescence emission than ssDNA-templated CuNCs. Meanwhile, this reticular DNA can resist the digestion of exo I. Therefore, signal is greatly improved by Hg^{2+}-induced fluorescence enhancement, while background noise is substantially reduced by Hg^{2+}-induced enzyme restraint. On a basis of this principle, a novel Hg^{2+} biosensor with high signal-to-noise ratio is designed, and reveal the capability of sensing Hg^{2+} in a wider linear range with outstanding sensitivity and selectivity.

Due to the specific interaction between Hg^{2+} and GSH, this biosensor can also be used to evaluate the inhibition of GSH toward Hg^{2+} and test lake water samples. More importantly, the results obtained using this method are in accord with related data from ICP-MS, suggesting accuracy and reliability of this biosensor. Overall, this work provides new perspectives of preparing DNA-templated nanomaterials with enhanced property and fabricating prominent biosensors.

ASSOCIATED CONTENT

Supporting Information

Absorption and excitation spectra of CuNCs, effect of Exo I on CuNCs, calculation of detection limit, and comparison of different Hg^{2+} biosensors.

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