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# Colorimetric detection of mercury ion based on unmodified gold nanoparticles and target-triggered hybridization chain reaction amplification

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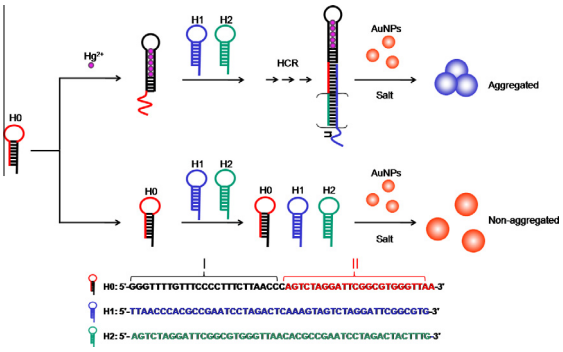
## HIGHLIGHTS

- A colorimetric method for $Hg^{2+}$ detection was proposed based on AuNPs.
- A detection limit of around 30 nM was achieved due to HCR amplification.
- It did not require enzymatic reactions, modifications or expensive instruments.

## GRAPHICAL ABSTRACT

A novel unmodified gold nanoparticles-based colorimetric strategy for label-free, sensitive and specific mercury ion detection was demonstrated by using thymine–mercury–thymine recognition mechanism and hybridization chain reaction amplification strategy.

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## ABSTRACT

A novel unmodified gold nanoparticles (AuNPs)-based colorimetric strategy for label-free, specific and sensitive mercury ion ($Hg^{2+}$) detection was demonstrated by using thymine–$Hg^{2+}$–thymine (T–$Hg^{2+}$–T) recognition mechanism and hybridization chain reaction (HCR) amplification strategy. In this protocol, a structure-switching probe (H0) was designed to recognize $Hg^{2+}$ and then propagated a chain reaction of hybridization events between two other hairpin probes (H1 and H2). In the absence of $Hg^{2+}$, all hairpin probes could stably coexist in solution, the exposed sticky ends of hairpin probes were capable of stabilizing AuNPs. As a result, salt-induced AuNPs aggregation could be effectively prevented. In the presence of $Hg^{2+}$, thymine bases of H0 could specifically interact with $Hg^{2+}$ to form stable T–$Hg^{2+}$–T complex. Consequently, the hairpin structure of H0 probe was changed. As H1/H2 probes were added, the HCR process could be triggered and nicked double-helixes were formed. Since it was difficult for the formed nicked double-helixes to inhibit salt-induced AuNPs aggregation, a red-to-blue color change was observed in the colloid solution as the salt concentration increased. With the elegant amplification effect of HCR, a detection limit of around 30 nM was achieved (S/N = 3), which was about 1–2 orders of magnitudes lower

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than that of previous unmodified AuNPs-based colorimetric methods. By using the T–$Hg^{2+}$–T recognition mechanism, high selectivity was also obtained. As an unmodified AuNPs-based colorimetric strategy, the system was simple in design, convenient in operation, and eliminated the requirements of separation processes, chemical modifications, and sophisticated instrumentations.



## Introduction

Mercury is a highly toxic and widespread pollutant in the environment, which can cause serious and permanent damage to the human body [1,2]. Therefore, sensitive and selective mercury detection is very important for environment monitoring and food analysis. Up to now, several conventional methods have been developed, such as cold vapor atomic fluorescence spectrometry [3], inductively coupled plasma mass spectrometry [4,5], surface enhanced Raman scattering [1,6], electrochemical methods [7,8], fluorescent methods [9–11] and colorimetric methods [12–21]. Among these methods, gold nanoparticles (AuNPs)-based colorimetric assay has drawn great attention [12–23], since it is a simple, fast and efficient method for determining targets with the aid of obvious color change. Since Ono and co-workers described the stabilization of DNA duplexes via the formation of thymine–$Hg^{2+}$–thymine (T–$Hg^{2+}$–T) [24], a variety of colorimetric assays based on DNA and AuNPs for the determination of $Hg^{2+}$ have been developed [12–19]. For colorimetric detection of $Hg^{2+}$, there are two major classes of AuNP-based assays. One strategy is based on DNA-functionalized AuNPs [12–14]. In this strategy, DNA-functionalized AuNPs were linked by the formation of T–$Hg^{2+}$–T complex, and the linked AuNPs would aggregate with a red-to-blue color variation. The other one relied on the difference in binding properties of single-stranded DNA and double-stranded DNA toward unmodified AuNPs [15–19]. Single-stranded DNA could stabilize AuNPs, and salt-induced AuNPs aggregation could be effectively prevented. While double-stranded DNA could not stabilize AuNPs, and AuNPs underwent aggregation as the salt concentration increased. Although colorimetric assays based on DNA and AuNPs for $Hg^{2+}$ detection have many advantages, a number of constraints still exist in conventional prototype designs [19,25,26]. The strategy based on DNA-functionalized AuNPs needs complicated steps, such as modifying oligonucleotides onto AuNPs and separating the modified AuNPs from the unmodified AuNPs or surplus oligonucleotides [17,26]. The strategy based on unmodified AuNPs suffers from the constraint of limited sensitivity. For example, the detection limit of most unmodified AuNPs-based colorimetric assays for $Hg^{2+}$ is above 200 nM [15–19]. Thus, constructing a simple colorimetric method based on unmodified AuNPs for high sensitive analysis of $Hg^{2+}$ is particularly intriguing.

Recently, our group has developed a DNA detection system that combined unmodified AuNPs colorimetric detection and HCR amplification [25]. This method not only avoided modification steps, but also offered a high sensitivity which was comparable to that of the enzyme-mediated AuNPs-based colorimetric assays. Herein, a novel unmodified AuNPs-based colorimetric detection system for $Hg^{2+}$ using T–$Hg^{2+}$–T recognition mechanism and HCR amplification strategy was developed. As shown in Fig. 1, three hairpin probes, H0, H1 and H2 were designed. The hairpin probe H0 contained two fragments termed as I and II according to their different functions. The fragment I (red line) which was partially caged in the duplex structure of the stem by hybridization with fragment II (black line) could initiate a chain reaction of hybridization events with H1 and H2. The fragment II contained a designed thymine-rich sequence which could recognize $Hg^{2+}$ specifically. In the absence of $Hg^{2+}$, all the hairpin probes could stably coexist in solution because of the low hybridization efficiency and their exposed sticky end could protect AuNPs against the salt-induced aggregation. Thus the color of the colloid solution retained red. Upon adding $Hg^{2+}$, fragment II of probe H0 could interact with $Hg^{2+}$ to form the T–$Hg^{2+}$–T base pairs, the structure of hairpin probe H0 was opened up and the fragment I of H0 was exposed. Thereby, fragment I of the H0 probe could trigger a chain reaction of hybridization events with H1 and H2 to form a nicked double-helix. The formed nicked double-helix was stiff and had its negatively charged phosphate backbone exposed [27,28]. The strong repulsion between double-stranded DNA and negatively charged AuNPs made their binding effects negligible. Consequently, double-stranded DNA provided little stabilization, and could not prevent the AuNPs aggregation at elevated salt concentrations. The aggregation of AuNPs is accompanied by a red-to-blue color change and red shift of the UV–vis absorption spectra. Hence, $Hg^{2+}$ detection results could be read out by the naked eye or a UV–vis spectrometer.

## Experimental section

### *Reagents*

Oligonucleotides listed in Fig. 1 were synthesized by Sangon Biotech Co. Ltd. (Shanghai, China). Chloroauric acid ($HAuCl_4{\cdot}4H_2O$) and trisodium citrate were purchased from Shanghai Reagent Co. (Shanghai, China). $Hg^{2+}$ was prepared by dissolving some Hg $(NO_3)_2$ with 0.5% $HNO_3$ as the stock solution, and $HNO_3$ was added to adjust the solution to pH 6.0 to prevent the formation of HgO particles. A sodium phosphate-sodium nitrate buffer solution (50 mM $Na_2HPO_4$/0.4 M $NaNO_3$, pH 7.5) was used in all the assays. All of the other chemical reagents were of analytical grade or higher. Ultrapure water (18.2 MΩ cm) was used throughout the experiment.

### *Preparation of AuNPs*

AuNPs (~13 nm) were synthesized by the citrate reduction of $HAuCl_4$ [29]. Absorption spectra of AuNPs were recorded on a Shimadzu UV-1601 UV–vis spectrophotometer (Japan) at room temperature.

### *Agarose gel electrophoresis demonstration of the $Hg^{2+}$-initiated HCR*

First, H0, H1 and H2 were heated to 95 °C for 2 min respectively and then cooled to room temperature. $Hg^{2+}$ were incubated with H0 at 25 °C. Then H1 and H2 were added to this mixture. The final concentration of H0, H1, H2 and $Hg^{2+}$ were 50 nM, 1 μM, 1 μM and 20 μM, respectively. Subsequently, the mixture was incubated at 25 °C for 2 h. A 1% agarose gel was prepared using 1 × TAE buffer (40 mM Tris AcOH, 2.0 mM $Na_2EDTA$, pH 8.5). The SYBR Gold was used as DNA stain and mixed with the samples. The gel was run at 42 V for 150 min in 1 × TAE buffer with loading of 10 μL of sample into each lane at room temperature. Then it was photographed using the gel image analysis system (Tanon 2500R, Tianneng Ltd., Shanghai, China).