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Naked-eye colorimetric sensor for Hg^{2+} monitoring with cascade signal amplification based on target-induced conjunction of split DNAzyme

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A colorimetric sensor for the visual detection of Hg^{2+} with cascade signal amplification has been successfully constructed using the split DNAzyme subunits as the sensing elements and gold nanoparticles as the signal reporter. The biosensor is ultrasensitive, enabling the visual detection of trace levels of Hg^{2+} as low as 10 pM without instrumentation.

Routine detection of mercury ion (Hg^{2+}) with high sensitivity and selectivity is significant important for field monitoring and disease diagnosis because it is not degradable in the environment and has adverse effects on human health even at low concentrations.[1] In recent years, many efforts have been paid to develop elegant sensing platform for Hg^{2+} detection employing electrochemical,[2a-g] fluorescent,[2h-m] or colorimetric[2n-s] signal as the output on the basis of thymine-Hg^{2+}-thymine (T-Hg^{2+}-T) interaction. In these developed methods, colorimetric sensors have received considerable interest due to their instrument-free format, naked-eye readout, short assay time, and ease of use.[3] These advantages make the colorimetric approach to be an ideal candidate for the development of a rapid Hg^{2+} detection kit for point-of-care (POC) diagnostics and on-site quick screening. However, the colorimetric assay often suffers from low sensitivity, which cannot meet the requirement of the maximum allowable level of Hg^{2+} in drinking water to be 10 nM,[4] a standard set by the U.S. Environmental Protection Agency (EPA). Thus, it is still highly desirable to develop a new biosensor with simplified operation and cost-effectiveness for Hg^{2+} detection with amplified colorimetric signal.

Signal amplification is an efficient way to construct sensing systems with high sensitivity.[5] Employing protein enzymes as biocatalysts, various amplification strategies have been proposed for targets detection with enhanced signal outputs.[6] Despite their high sensitivities, the requirement of protein enzymes encounters the disadvantages of irreverersible denaturation, exorbitant price, poor stability, and complicated operation, which greatly limit the practical applications of these sensing platforms. As an alternative, DNAzymes have found growing interest as amplifying labels for biosensing events due to their good stability, easy preparation, and low nonspecific adsorption.[7] A DNAzyme is a synthetic nucleic acid that can catalyze the cleavage of a ribonucleobase (rA)-containing DNA substrate. Because the catalytic cleavage reaction is carried out with multiple turnovers, the DNAzyme affords an amplified signal.[8] For example, the Mg^{2+}-dependent DNAzyme was used as an ideal biocatalyst to design enzyme-free assay platforms for amplified detection of DNA and other analytes.[9] It was reported that the Mg^{2+}-dependent DNAzyme can be split into two fragments that lack catalytic activity, but upon target-induced cooperative association of the subunits, the cleavage activity is reactivated.[10] Herein, we developed a naked-eye colorimetric sensor for Hg^{2+} monitoring with cascade signal amplification using split DNAzyme fragments as the sensing elements and gold nanoparticles as the signal indicators. The integration of DNAzyme with gold nanoparticles to give a simple on-site screening technique can be used in remote settings.

The principle of our proposed naked-eye colorimetric sensing strategy for Hg^{2+} detection is illustrated in Scheme 1. The binding DNA, the split Mg^{2+}-dependent DNAzyme subunits (A and B), the hairpin DNA, and the caged substrate (substrate DNA-blocking DNA) are used as the sensing elements to construct the biosensor. In the presence of Hg^{2+}, the parts A and B of the DNAzyme subunits co-recognizes and binds with the binding DNA *via* T-Hg^{2+}-T coordination chemistry to form an active DNAzyme. The Hg^{2+}-guided assembly of the active DNAzyme results in the cleavage of the caged substrate and releases the protected segments E and C-D. The formed active DNAzyme can undergo many cycles to trigger the continuous cleavage of the caged substrate for signal amplification (Cycle I). The single-stranded sequence C-D can cross-link AuNPs-1 and AuNPs-2 through DNA hybridization, forming an aggregate network of gold nanoparticles, which is blue in color due to the

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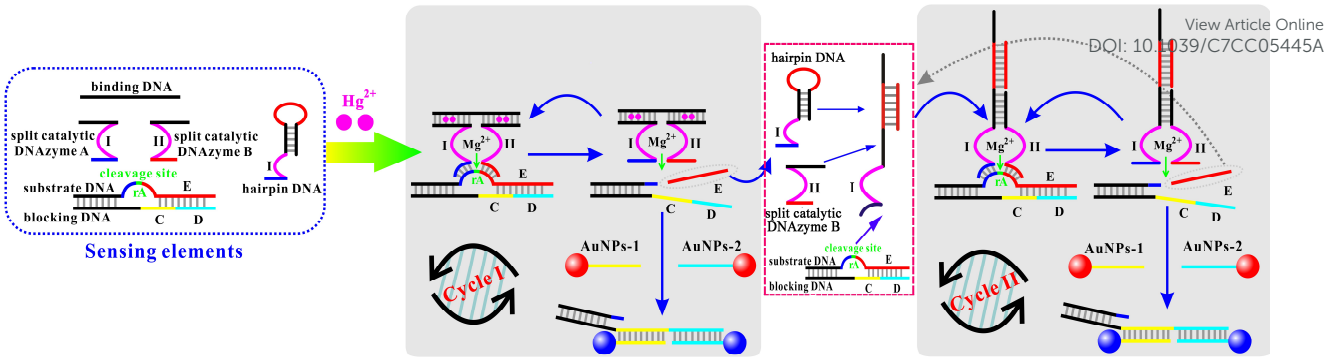

Scheme 1 Schematic representation of the naked-eye colorimetric sensor for Hg^{2+} monitoring based on target-induced conjunction of split DNAzyme. The sensing system consists of the binding DNA, the split catalytic DNAzymes A and B, the hairpin DNA, and the caged substrate. The Hg^{2+}-induced activation of the Mg^{2+}-dependent DNAzyme leads to the cleavage of the caged substrate and to the release of the protected C-D sequence for the formation of the colorimetric signal. AuNPs-1 and AuNPs-2 are the gold nanoparticles modified with probes 1 and 2, respectively.

red-shifting and dampening of the nanoparticle plasmon resonance.[11] The red-to-blue color change can be visualized by the naked eye.

In addition, the released domain E can initiate another signal amplification process (Cycle II). E can open the hairpin DNA, which contains the split catalytic DNAzyme A sequence. The synergistic binding of the opened hairpin to the split catalytic DNAzyme B results in the formation of another active DNAzyme stucture, leading to the cleavage of the caged substrate and to the generation of the unblocked DNA of E and C-D. The liberated E sequence can be recycled to open the hairpin DNA. This process leads to the enhanced formation of the active DNAzyme and the increased scission of the caged substrate, concomitantly generating numerous available C-D. In the detection step, a mix of AuNPs-1 and AuNPs-2 is added to the sample solution to get the colorimetric readout. In the absence of Hg^{2+}, the formation of an active DNAzyme and the release of the occupied C-D are inhibited due to the T-T mismatches. Thus, AuNPs-1 and AuNPs-2 do not get cross-linked and the solution remains red.

The feasibility of our developed colorimetric sensor for Hg^{2+} detection was verified by UV-vis absorption spectra, transmission electron microscopy (TEM) and dynamic light scattering (DLS). As shown in Fig. 1A, in the absence of a target, an obvious characteristic adsorption peak at ~520 nm was obtained (corresponding to the AuNPs specific surface plasmon resonance absorption), and the solution color was red (inset in Fig. 1A). When target Hg^{2+} was present in the sensing system, an absorption band at 650 nm appeared, and the absorbance at 520 nm decreased. Correspondingly, the color of the solution turned to blue (inset in Fig. 1A). TEM micrographs showed that AuNPs were well-dispersed without target Hg^{2+} (Fig. 1B), indicating that no active DNAzyme was formed. While in the presence of Hg^{2+}, AuNPs aggregated heavily (Fig. 1C), revealing that target-induced activation of catalytic DNAzyme structure had taken place. Meanwhile, we also used DLS to investigate the size of AuNPs before and after the reaction with Hg^{2+}. As shown from the insets in Fig. 1B and C, the average hydroynamic diameter of AuNPs increased from 26.4 to 840.9 nm. The reason for the size increase originated from the aggregation of AuNPs, which was in good agreement with the TEM images. These results indicated that Hg^{2+}can trigger the formation of active DNAzymes to cleave the caged substrates for generating numerous free sequence C-D, which interacts with AuNPs-1 and AuNPs-1 to form a cross-linked network of nanoparticles.

To investigate the dynamic range and sensitivity of the colorimetric assay, a series of samples containing different concentrations of target Hg^{2+} were tested under optimal experimental conditions (Fig. S1-S3, ESI†). As shown in Fig.2A, the color of AuNPs was gradually changed from red to blue with the increase of Hg^{2+} concentration, indicating an increase in the aggregation of AuNPs. We could easily distinguish the color change for a target Hg^{2+} concentration as low as 10 pM by the naked eye. Meanwhile, the UV-vis spectra presented in Fig. 2B shows that the peak absorbance at 520 nm gradually shifted and decreased along with the increasing of the target concentration, while the absorbance at 650 nm increased. Such red shift is attributed to the surface plasmon resonance of the gold nanoparticle, which has been widely used in biosensor design for organizing nanoparticles into aggregate structures.[12] Fig. 2C presents the A650/A520 ratio as a function of Hg^{2+} concentration. The resulting calibration curve exhibited a good linear relationship between the absorbance ratio and the logarithm of Hg^{2+} concentration within the dynamic range from 10 pM to 100 nM. The limit of detection (LOD) was calculated to be 5 pM (S/N=3), which is approximately three orders of magnitude higher than the previously reported AuNPs colorimetric sensors for Hg^{2+} detection without signal amplification (the LOD is 100 nM and 190 nM, respectively).[2o, 3b] Such excellent sensitivity is comparable to the reported Hg^{2+} biosensor by integrating bifunctional strand displacement amplification and multiplexed short linear quencher-fluorophore probes[13a] (LOD is 2 pM) and superior to the method by the utilization of fluorescent covalent organic frameworks[13b] for Hg^{2+} sensing (LOD is 125 nM). This high sensitivity could be attributed to the continuous turnover capability of the catalytic DNAzyme for cascade signal amplification. As the maximum permissible level of Hg^{2+} in

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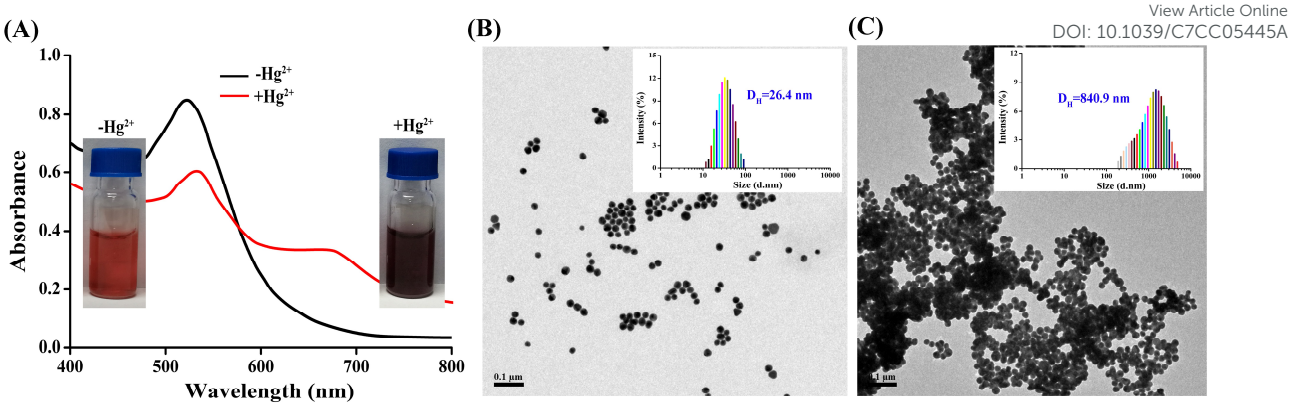

Fig. 1 (A) UV-vis absorption spectra of the colorimetric sensor in the presence and absence of 1 nM Hg^{2+} (Insets: the corresponding photographs). (B,C) TEM images of AuNPs mixed with the test solution without (B) or with (C) 1 nM Hg^{2+} (Insets: DLS analyses of the corresponding samples). Scale bar: 0.1 μm.

drinking water is set by the United States Environmental Protection Agency (EPA) to be 10 nM, this colorimetric sensor holds great potential in POC testing with its naked-eye readout, simplified operation, large dynamic range, and superior detection sensitivity.

The selectivity of the sensing protocol for Hg^{2+} was also evaluated by a visual assay to other metal ions, including Pb^{2+}, Cu^{2+}, Ag^{+}, Cd^{2+}, Sn^{2+}, Cr^{3+}, Co^{2+}, and Mn^{2+}. As shown in Fig. 3, the presence of the control metal ions (100 nM) did not cause any obvious color change compared with the blank sample

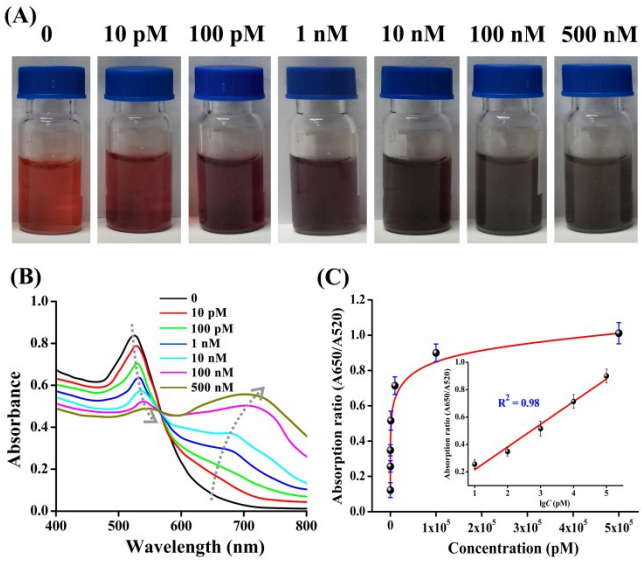

Fig. 2 (A) Photograph showing visual changes of the detection system in the presence of various concentrations of Hg^{2+}. (B) Their corresponding UV-vis absorption spectra. Two dotted gray arrows were used to precisely define the absorbance ratio (A650/A520) with the consideration of the red shift. (C) Plot of Hg^{2+} concentration *vs* absorbance ratio (A650/A520) for the target assay. Inset is the calibration curve for concentrations ranging from 10 pM to 100 nM. Error bars represent the standard deviation of three independent measurements.

(red color), while the addition of the target Hg^{2+} (1 nM) resulted in a blue color change of the solution. When Hg^{2+} (1 nM) was coexisted with the control metal ions (100 nM), no significant changes were observed compared to the Hg^{2+} existed alone. The results indicated that our proposed biosensor is good enough to selectively detect Hg^{2+} from other metal ions which may coexist in environmental sample. Such a high specificity of this assay can be attributed to the specific T-Hg^{2+}-T base pairing.

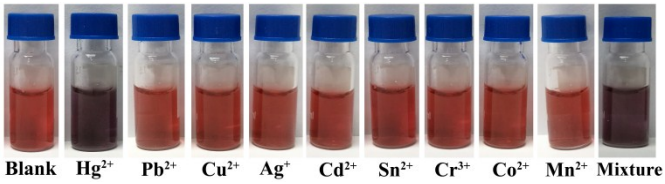

Fig. 3 Selectivity of the colorimetric sensor for Hg^{2+} against other metal ions. Concentrations of Hg^{2+} and other metal ions were 1 nM and 100 nM, respectively. The mixture contained 1 nM Hg^{2+} and 100 nM of other interfering metal ions.

To validate the applicability of this sensing system in practical sample analysis, recovery experiments for river water samples spiked with different concentrations of Hg^{2+} were carried out. The results are summarized in Table S1 (ESI†). Satisfactory values between 88% and 106% were obtained for the recovery experiments, which indicated that the possible interference from practical water samples was negligible in the present system. Moreover, the proposed method was further tested with several other water samples including tap water, lake water, and pond water. Those water samples were analyzed separately using the colorimetric sensor and the standard AFS (atomic fluorescence spectrometer). The results of the Hg^{2+} determination in real water samples were summarized in Table S2 (ESI†). The concentrations of the three real samples measured by AFS were 82.5 pM, 145.6 pM, and 963.8 pM, respectively. The concentrations of the three real samples measured by our proposed colorimetric sensor were 86.5 pM, 137.4 pM, and 985.6 pM, respectively. The data revealed that no significant difference existed between the values measured

using the proposed method and AFS, confirming the accuracy of the developed sensor for detecting Hg^{2+}. These results demonstrated that our established colorimetric sensor can be successfully applied to Hg^{2+} analysis in real environmental samples.

In conclusion, we have successfully developed a colorimetric assay for Hg^{2+} detection with cascade signal amplification on the basis of target-guided recycling assembly of the active DNAzyme. Using gold nanoparticles as the signal indicators, the target solution with a concomitant red-to-blue color change can be recognized readily by the naked eye. Because the catalytic cleavage reaction can be carried out with multiple turnovers, the DNAzyme exhibits significant signal amplification efficiency, enabling the visual detection of Hg^{2+} down to 10 pM without instrumentation. Meanwhile, the colorimetric sensor shows excellent selectivity for Hg^{2+} against other possible competing metal ions. This sensor is robust and can be applied to the determination of spiked Hg^{2+} in river water samples with satisfactory recovery. Importantly, the naked-eye colorimetric Hg^{2+} sensor with simple operation, excellent sensitivity and selectivity, and instrument-free feature holds great potential for routine on-site monitoring and POC testing. In addition, the proposed sensing system is versatile. By conjugating with other metal ion-base pairs, it can be easily employed to detect a wide range of metal ions.

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