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Chen Ma

machenfighting@126.com

Gansu University of Political Science and Law

Yida Zhang

Lanzhou University

Shuai Mu

The First Hospital of Lanzhou University, The First Clinical Medical College of Lanzhou University

Yuan Zhang

Gansu University of Political Science and Law

Shuoyun Wei

Gansu University of Political Science and Law

Quanfang Li

Gansu University of Political Science and Law

Shufang Ren

Gansu University of Political Science and Law

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An aptamer-controlled Mn-Au oxidase-mimicking nanozyme for smartphone-assisted colorimetric detection of mercury ions

Chen Ma^{1,*}, Yida Zhang², Shuai Mu³, Yuan Zhang¹, Shuoyun Wei¹, Quanfang Li¹, Shufang Ren¹.

¹Gansu Province Key Laboratory of Evidence Science Techniques Research and Application, Gansu University of Political Science and Law, Lanzhou, China

²College of Chemistry and Chemical Engineering, Lanzhou University, Lanzhou, China

³Department of Cardiology, Gansu Clinical Medical Research Center for Cardiovascular Diseases, The First Hospital of Lanzhou University, The First Clinical Medical College of Lanzhou University, Lanzhou, China

* Corresponding Author. E-mail address: machenfighting@126.com.

Abstract

Growing concerns over environmental sustainability have motivated the development of rapid analytical strategies for detecting mercury ions (Hg^{2+}). In this study, we developed a novel Hg^{2+} sensing strategy based on a Mn-Au hybrid nanozyme with enhanced oxidase-mimicking activity and an aptamer-controlled catalytic switching mechanism. First, a Mn-Au hybrid nanozyme (MnAuNPs) with a distinctive “fried-egg-like” core-shell morphology was synthesized, which exhibited prominent oxidase-like activity and rapidly oxidized 3,3',5,5'-tetramethylbenzidine (TMB) into the characteristic blue product (OxTMB) without H_2O_2 . The Hg^{2+} -specific aptamer can adsorb onto the surface of MnAuNPs through electrostatic attraction and Au-N interactions, which blocks the catalytic sites and markedly suppresses the oxidase-mimicking activity. In the presence of Hg^{2+} , the specific T- Hg^{2+} -T coordination triggers the desorption of the aptamer from the MnAuNPs surface, thereby restoring oxidase-like activity and inducing distinct color and absorbance change. Accordingly, an effective colorimetric platform for Hg^{2+} detection was

developed, exhibiting a linear response over the range of 1.0-50 μM with a limit of detection (LOD) of 0.32 μM . In parallel, a paper-based smartphone-assisted sensing platform was developed, enabling on-site Hg^{2+} detection in the range of 5.0-50 μM with LOD of 5.0 μM . Through the colorimetric and smartphone-assisted method, accurate quantification of Hg^{2+} in Yellow River water and wastewater samples were achieved with the results in good agreement with those obtained by ICP-OES. Overall, this work provides a facile strategy for constructing Mn-Au oxidase-mimicking nanozymes and reveals an aptamer-controlled catalytic switching mechanism through specific molecular recognition, thereby establishing an effective platform for environmental Hg^{2+} monitoring.

Keywords: Mn-Au nanozymes; Oxidase-mimicking activity; Aptamer-controlled catalytic switch; Mercury ions; Colorimetric sensing; Smartphone-assisted detection

1. Introduction

Nanozymes, as emerging artificial enzyme mimics, have attracted considerable attention due to their high catalytic efficiency, excellent stability, low cost, and suitability for large-scale production [1-3]. Recently, hybrid nanozymes constructed from multiple components or heterostructured architectures have emerged as a promising class of catalytic nanomaterials.[4-7]. Through the rational integration of distinct functional components, hybrid nanozymes can generate synergistic effects that increase the exposure of catalytic active sites and facilitate electron transfer, thereby enhancing their enzyme-mimicking activity [8,9]. Owing to these advantages, hybrid nanozyme-based sensing platforms have shown great potential for the sensitive detection of toxic environmental pollutants, particularly heavy metal ions [10-12].

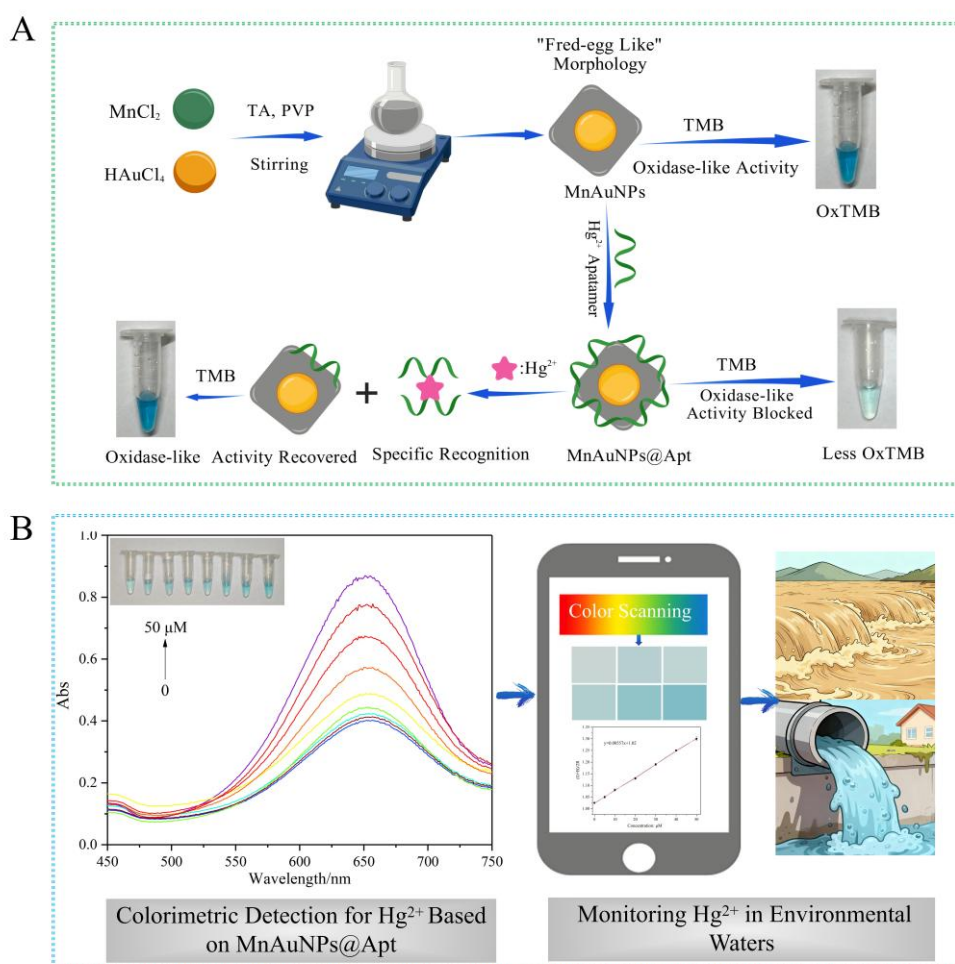
Mercury contamination represents a severe environmental and public health concern worldwide. Mercury ions (Hg^{2+}) are extremely toxic and readily bioaccumulate through food chains, causing irreversible damage to the brain, kidneys, cardiovascular, nervous, and endocrine systems [13-15]. Therefore, the development of rapid, sensitive, and reliable methods for Hg^{2+} monitoring is of great importance.

Conventional analytical techniques, such as atomic absorption spectroscopy (AAS) [16], inductively coupled plasma mass spectrometry or optical emission spectrometry (ICP-MS or ICP-OES) [17], and high-performance liquid chromatography (HPLC) [18], provide high sensitivity and accuracy but generally require sophisticated instrumentation and tedious sample pretreatment, limiting their applicability for on-site analysis. In recent years, nanozyme-based colorimetric assays have emerged as promising alternatives for Hg^{2+} detection [19-21]. However, many reported nanozymes still suffer from low catalytic activity and mainly rely on peroxidase-like catalysis requiring H_2O_2 as a substrate, which complicates the detection process and compromises environmental sustainability. Therefore, the development of highly efficient oxidase-mimicking nanozymes capable of catalyzing substrate oxidation without exogenous H_2O_2 has become an attractive strategy for simple, green, and sensitive Hg^{2+} detection.

To endow nanozyme-based sensing systems with high target specificity, aptamers have emerged as attractive biorecognition elements owing to their excellent chemical stability, low-cost synthesis, and high affinity toward specific targets [22-24]. Recent studies have demonstrated that some aptamers can adsorb onto the surface of Au-based peroxidase-mimicking nanozymes through Au-N coordination interactions between nucleobases and gold atoms, thereby suppressing the intrinsic catalytic activity of nanozymes [25-27]. Upon target binding, the aptamer dissociates from the nanozyme surface, resulting in the recovery of catalytic activity, which provides an effective strategy for constructing colorimetric biosensors for the target. Nevertheless, the engineering of aptamer-modified oxidase-mimicking hybrid nanozymes remains limited, and the corresponding sensing platforms are still in their infancy.

Herein, as illustrated in Scheme 1, we report a facile one-pot synthesis of a “fried-egg-like” core-shell Mn-Au hybrid nanozyme (MnAuNPs) with enhanced oxidase-like activity. The negatively charged Hg^{2+} -specific aptamer is functionally immobilized on the surface of MnAuNPs via electrostatic interactions and Au-N coordination, effectively blocking catalytic active sites and suppressing oxidase-like activity. Upon the addition of Hg^{2+} , specific T- Hg^{2+} -T coordination induces aptamer

desorption, restoring catalytic activity and producing distinct changes in absorbance and solution color. Based on this mechanism, a selective colorimetric assay was fabricated and integrated within a smartphone-assisted paper-based platform to achieve on-site Hg^{2+} detection. The method was validated by accurate determination of Hg^{2+} in Yellow River water collected from different regions of Gansu Province and domestic wastewater samples. In brief, this work offers a facile and versatile strategy for designing aptamer-controlled Mn-Au hybrid nanozyme systems through specific molecular recognition for practical and highly sensitive Hg^{2+} monitoring in environmental samples.



Scheme 1. Schematic illustration for the preparation of MnAuNPs and MnAuNPs@Apt (A), and construction of the MnAuNPs@Apt colorimetric sensing platform combined with smartphone readout for Hg^{2+} assay (B).

2. Experimental section

2.1 Material and instrumentation

MnCl₂·4H₂O, Polyvinylpyrrolidone (PVP, Mw=10000), NaOH, CaCl₂, FeCl₃, 3,3',5,5'-tetramethylbenzidine (TMB), and tannin acid (TA) were purchased from J&K Scientific (Beijing, China). HAuCl₄·3H₂O, Hg(NO₃)₂·H₂O, Co(NO₃)₂·6H₂O and Al(NO₃)₃·9H₂O were brought from Macklin Biochemical Technology Co., Ltd. (Shanghai, China). Cd(NO₃)₂·4H₂O, CuSO₄·5H₂O and ZnCl₂ were supplied by Aladdin Biochemical Technology Co., Ltd (Shanghai, China). All chemicals were of analytical grade and used as received. Specific recognition aptamer for Hg²⁺ was obtained from Sangon Biotech (Shanghai, China), and the sequence is 5'-TTT TTT TTT T-3' [28]. Ultrapure water (18.2 MΩ·cm, 25 °C) was obtained from a Thermo Fisher Scientific water purification system and used throughout all experiments.

Transmission electron microscopy (TEM) images and energy dispersive spectroscopy (EDS) mappings were collected using FEI Talos F200X microscope (FEI, USA). X-ray photoelectron spectroscopy (XPS) measurements were performed on a K-Alpha instrument (Thermo Scientific, USA). The ultraviolet-visible (UV-Vis) spectrophotometry was performed by UV-Vis spectrophotometer Evolution 210 (Thermo Scientific, USA). The particle size distribution of MnAuNPs and zeta potentials of MnAuNPs, Hg²⁺-aptamer and MnAuNPs@Apt were tested by ZEN 3600 (Malvern, UK). Fourier-transform infrared (FT-IR) spectra were recorded using a Spectrum Two spectrometer (PerkinElmer, USA). The photos of solution color and paper were taken through a smartphone and the RGB value were collected through Color Assist App downloaded from Apple App Store. The concentrations of Hg²⁺ in real water samples were measured using Agilent 5110 ICP-OES (Agilent, USA).

2.2 Synthesis of the MnAuNPs.

MnAuNPs were synthesized based on an improved one-step fast redox strategy according to previous work [29]. In brief, 200 μL of HAuCl₄·3H₂O (1%, w/v) were dispersed with 20 mL of deionized water, then 200 μL of MnCl₂·4H₂O (0.1 M) and 50 μL of tannin acid (0.60 mM) were added under vigorous stirring for 2 min. After that,

2 mL of PVP (1 mM) were added and stirred for 5 min. Subsequently, 1 mL of NaOH (0.1 M) were introduced into the above mixture solution rapidly. The color of the mixture solution immediately changed from light yellow to brownishblack which indicated the fabrication of MnAuNPs. The solution mixture was continually stirred for 20 min. Finally, the MnAuNPs were separated by centrifuging and resuspended with ultrapure water for further use.

2.3 Evaluation of oxidase-like activity and steady-state kinetics assay

The oxidase-like activity of MnAuNPs was evaluated using TMB as the chromogenic substrate. Steady-state kinetic assays were performed to obtain the kinetic parameters according to the Michaelis-Menten model. To achieve optimal catalytic performance, the effects of key experimental parameters on the oxidase-like activity were systematically investigated, including solution pH (3.0-9.0), temperature (25 °C-50 °C), TMB concentration (0-100 μ M), and MnAuNP concentration (0-400 ng·mL⁻¹).

2.4 Construction of MnAuNPs@Apt

First, various concentrations of the Hg²⁺-specific aptamer (0, 2 μ M, 4 μ M, 6 μ M, 8 μ M, 10 μ M, 15 μ M, 20 μ M) were mixed with a fixed concentration of MnAuNPs (200 ng/mL) in citric acid buffer (0.1 M, pH=4) and incubated at 37°C for 20 min with vigorous vortexing. The successful fabrication of MnAuNPs@Apt was characterized by zeta potential measurements and FT-IR spectroscopy. The oxidase-like activity of MnAuNPs@Apt was evaluated by measuring the absorbance of OxTMB at 652 nm. A lower absorbance indicated a stronger inhibitory effect of the aptamer on the oxidase-mimicking activity.

2.5 Colorimetric detection of Hg²⁺

In a total reaction volume of 300 μ L, 50 μ L of MnAuNPs (200 ng mL⁻¹), 50 μ L Hg²⁺-specific aptamer and 50 μ L of Hg²⁺ solutions at various concentrations (or other potential interfering analytes) were sequentially added into tubes. Subsequently, 150 μ L of TMB (0.1 mM) prepared in pH=4.0 buffer was introduced and the mixture was incubated for 20 min. All reactions were strictly maintained at pH 4.0 to ensure consistent catalytic conditions. After incubation, the UV-Vis absorption spectra of the

resulting solutions were recorded, and the characteristic absorbance at 652 nm was specifically employed for quantitative analysis. To achieve optimal sensing performance for Hg^{2+} detection, several experimental parameters were systematically optimized: (a) the pH was regulated from 3 to 9; (b) the reaction temperature was adjusted from 25 °C to 50 °C; (c) the incubation time was examined over a range of 1 to 30 min; and (d) the concentration of TMB ranged from 0 to 300 μM .

2.6 Smartphone-assisted paper-based platform for Hg^{2+} analysis

For on-site Hg^{2+} analysis, a paper-based colorimetric sensing platform coupled with smartphone-assisted RGB readout was integrated. Circular pads (1 cm diameter) were punched from Whatman No. 1 filter paper and uniformly coated with MnAuNPs@Apt solution (pH 4.0, 200 ng/mL MnAuNPs and 6 μM Hg^{2+} -specific aptamer were incubated for 20 min), followed by drying at 30°C for 30 min to obtain the paper-based sensor. Subsequently, 10 μL of Hg^{2+} solutions at different concentrations or real samples spiking with Hg^{2+} were applied to the paper-based sensor. After sample absorption, 10 μL of TMB solution (0.1 mM) was added, and the pads were incubated for 5 min to generate a measurable colorimetric response. The color changes were recorded using a smartphone camera, and the RGB values were extracted via the Color Assist application. Through converting the RGB parameters into concentration values with the standard curve, on-site quantitative of Hg^{2+} was achieved. All images were captured using an iPhone 14 Pro Max under controlled illumination to ensure reproducible RGB readout.

2.7 Determination of Hg^{2+} in real water samples

To evaluate the performance of the developed colorimetric assay and the practicality of smartphone-assisted on-site detection method, water samples from Yellow River in Gansu Province, as well as from domestic wastewater were collected. To minimize interference, each sample was first filtered five times using a Buchner funnel, followed by filtration through a 0.22 μm microporous membrane. The resulting filtrate was adjusted to pH 4.0 with 0.1 M citric acid buffer. For recovery experiments, 10 μM and 40 μM Hg^{2+} standard solutions were spiked into the pretreated samples. The samples were then analyzed according to the previously

described in the Section 2.5 and 2.6. The obtained results were compared with those determined through ICP-OES.

3. Results and discussion

3.1 Structural and optical characterization of MnAuNPs.

The Mn-Au hybrid nanozyme (MnAuNPs) was fabricated via a one-pot Mn(II)-mediated redox route, which affords a highly stable brownish-black colloidal dispersion with broad and featureless UV-Vis absorption (Fig. 1A). TEM observations revealed that the MnAuNPs exhibited a uniform “fried-egg-like” core-shell structure with an average diameter of 42 nm (Fig. 1C and Figure S1). As shown in Fig. 1B, high-resolution TEM clearly resolved the distinct core-shell architecture, which was further confirmed by EDS elemental mapping, demonstrating strong Au signals localized in the inner domain and Mn signals distributed along the outer shell. To further investigate the surface elemental composition and chemical states of MnAuNPs, XPS analysis was performed. As shown in Fig. 1D, the survey spectrum revealed the presence of C, N, O, Au, and Mn elements. The C, N, and O signals are mainly attributed to the TA and PVA used during the synthesis process, which contribute to the surface functionalization and stabilization. As shown in Fig. 1E, the high-resolution Au 4f spectrum exhibited two characteristic peaks at approximately 84.0 and 87.7 eV, corresponding to Au 4f_{7/2} and Au 4f_{5/2}, respectively, indicating the presence of metallic Au⁰. As shown in Fig. 1F, the high-resolution Mn 2p spectrum displayed the characteristic Mn 2p_{3/2} and Mn 2p_{1/2} peaks. Further peak deconvolution revealed the coexistence of Mn²⁺, Mn³⁺, and Mn⁴⁺ species, suggesting the formation of mixed-valence Mn on the surface of MnAuNPs. The coexistence of metallic Au and mixed-valence Mn further confirms the successful formation of MnAuNPs. Such a unique structure combining the Mn-rich catalytic shell with the electron-transfer-promoting Au core, endows the MnAuNPs with remarkable oxidase-mimicking activity.

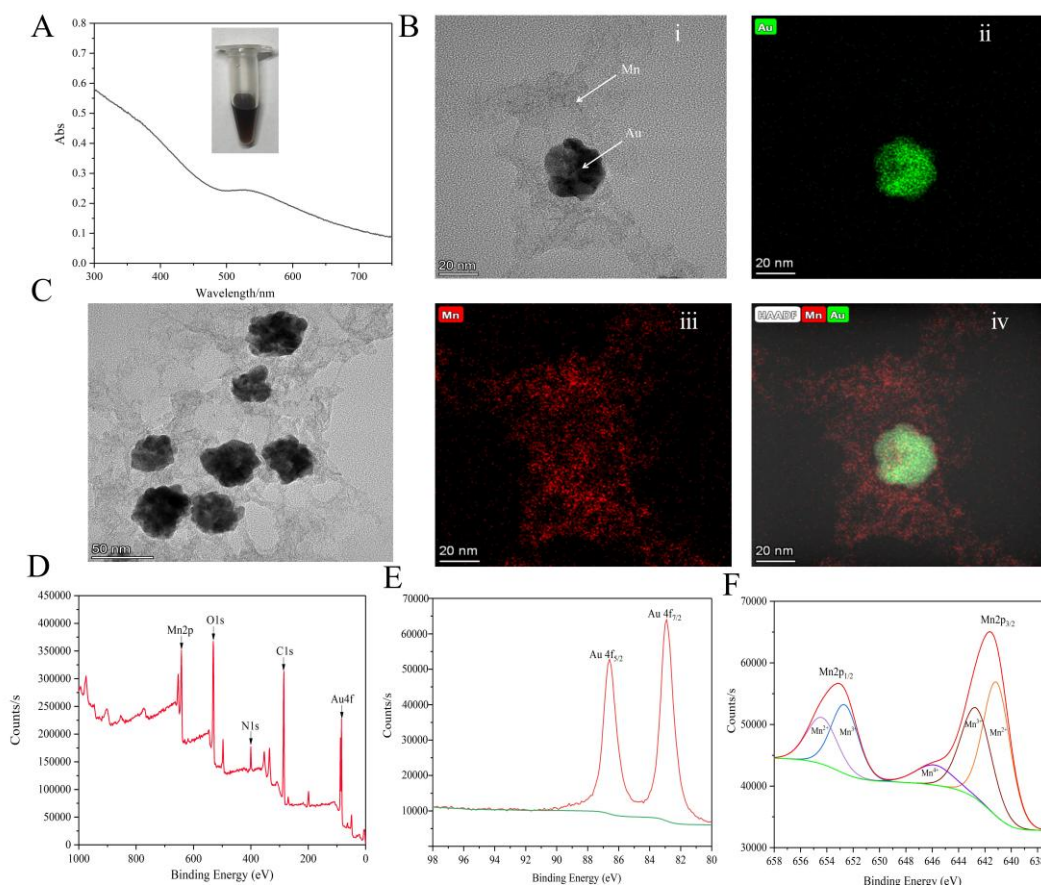


Fig. 1. Structural and optical characterization of MnAuNPs. (A) UV-Vis absorption spectra of MnAuNPs and the inset represents the photograph of MnAuNPs solution; (B) High-resolution TEM image of an individual MnAuNP along with the corresponding EDS elemental mapping of Au, Mn and merge; (C) TEM image of MnAuNPs. (D) XPS spectra of MnAuNPs; (E) High-resolution XPS spectrum of the Au 4f; (F) High-resolution XPS spectrum of the Mn 2p.

3.2 The oxidase-like catalytic performance of MnAuNPs

As shown in Fig. 2A and 2B, under acidic conditions (pH=4.0), MnAuNPs immediately (in 2 s) catalyzed the oxidation of TMB without the addition of H₂O₂, producing an immediate blue color and a characteristic absorption peak at 652 nm that remained stable for up to 120 min. The rapid chromogenic response demonstrates the excellent oxidase-like catalytic activity of MnAuNPs. Using TMB as the chromogenic

substrate, the catalytic kinetics were further evaluated using the Michaelis-Menten model:

$$v = \frac{V_{max}[S]}{K_m + [S]} \quad (1)$$

where v is the initial reaction rate, $[S]$ is the substrate concentration, V_{max} is the maximum reaction velocity, and K_m (Michaelis-Menten constant) represents the substrate concentration at which the reaction rate reaches half of V_{max} . Additionally, Lineweaver-Burk plots, the double-reciprocal form of the Michaelis-Menten equation, were constructed to accurately determine kinetic parameters:

$$\frac{1}{v} = \frac{K_m}{V_{max}} \cdot \frac{1}{[S]} + \frac{1}{V_{max}} \quad (2)$$

From these plots, the apparent K_m and V_{max} were determined to be 0.21 mM and $38.8 \times 10^{-6} \text{ M} \cdot \text{s}^{-1}$, respectively (Fig. 2C and 2D). The relatively low K_m suggests a strong affinity between MnAuNPs and TMB, whereas the high V_{max} demonstrates its efficient catalytic activity. The absorbance at 652 nm increased with increasing MnAuNPs concentration, reaching a plateau at 200 ng/mL (Figure S2); this concentration was subsequently employed in all further experiments. Moreover, MnAuNPs retained stable oxidase-like activity after 30 days of storage (Figure S3), demonstrating excellent long-term stability. Remarkably, the catalytic performance of MnAuNPs surpassed that of many reported oxidase-like nanozymes (Table S1), not only highlighting their excellent catalytic efficiency but also demonstrating their significant potential as sensing platform.

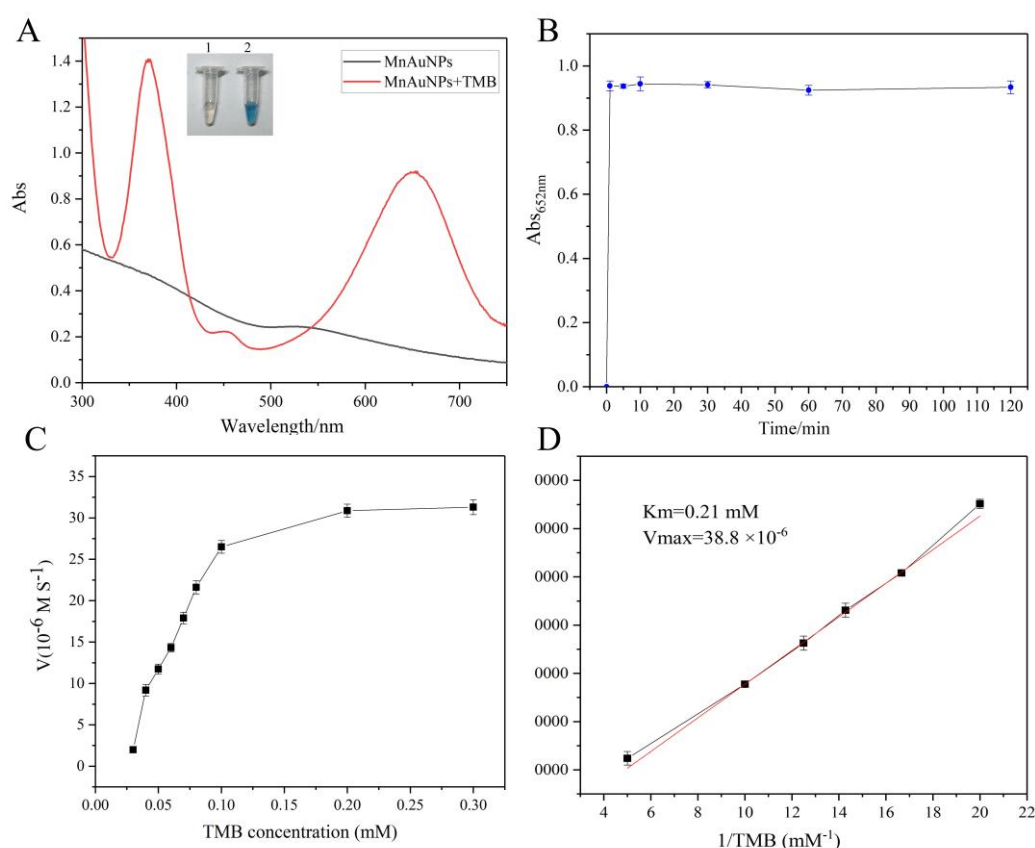


Fig. 2. Oxidase-like characterization of MnAuNPs. (A) UV-Vis absorption spectra of MnAuNPs before and after the addition of TMB. Inset: Photographs of solutions of MnAuNPs (ten-fold diluted, 1) and MnAuNPs with TMB (2); (B) Time-dependent oxidation of TMB; (C) Michaelis-Menten kinetic curves obtained at varying TMB concentrations; (D) Corresponding Lineweaver-Burk plots for kinetic parameter determination.

3.3 Study on the catalytic mechanism of MnAuNPs

Considering that nanozyme-catalyzed oxidation processes are commonly dependent on dissolved oxygen (DO), it is important to further discriminate whether the catalytic process follows a true oxidase-mimicking pathway or involves a peroxidase-like mechanism [30]. As shown in Fig. 3A, MnAuNPs efficiently catalyzed TMB oxidation even after DO removal by N₂ purging, and no significant enhancement was observed upon the addition of H₂O₂. These results suggest that the

catalytic reaction is predominantly governed by an oxidase-mimicking pathway and is not strictly dependent on DO concentration. To further investigate the catalytic mechanism, reactive oxygen species (ROS) scavenging experiments were performed. NaN₃, IPA, BQ, and EDTA-2Na were used to quench ¹O₂, ·OH, O₂^{·-}, and holes, respectively [31, 32]. As shown in Fig. 3B, IPA and BQ showed negligible effects, whereas NaN₃ and EDTA-2Na exerted a certain inhibitory effect on catalytic activity at higher concentrations (5 mM), indicating that ¹O₂ and holes are involved in the oxidation process, which in agreement with previous work [33, 34].

As the crucial role of electron transfer in regulating the catalytic activity of nanozymes, cytochrome c (Cyt c), a typical electron mediator [35], was employed as a model electron acceptor to investigate the electron transfer behavior of MnAuNPs. As shown in Fig. 3C, the characteristic absorption peak of oxidized Cyt c at 530 nm was markedly enhanced in the presence of MnAuNPs, confirming their efficient electron-transfer capability. This enhanced electron transfer facilitates the generation of reactive intermediates such as ¹O₂ thereby promoting TMB oxidation [35,36]. Overall, the superior catalytic performance of MnAuNPs can be attributed to their efficient electron-transfer properties and the involvement of reactive species (¹O₂ and holes).

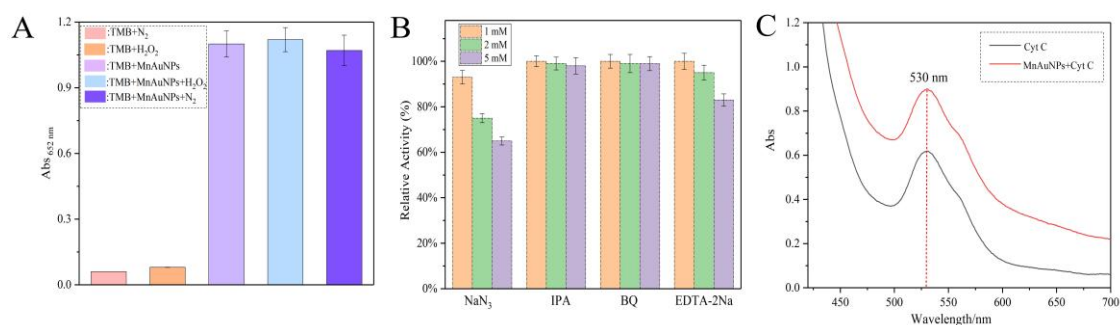


Fig. 3. Investigation of the catalytic mechanism of MnAuNPs. (A) The absorption intensities at 652 nm of different solutions; (B) Effects of ROS scavengers at different concentrations on catalytic activity; (C) Electron-transfer capability evaluated using Cyt c.

3.4 Aptamer-regulated oxidase-like activity enabling selective Hg²⁺ recognition

Aptamer-functionalized MnAuNPs (MnAuNPs@Apt) were prepared through electrostatic adsorption between the Mn-rich surface and Hg²⁺-specific aptamer, accompanied by Au-N interactions between the nucleobases and the Au element [22-24]. As depicted in Fig. 4B, the successful formation of MnAuNPs@Apt was confirmed by zeta potential and FT-IR measurements. MnAuNPs exhibited a positive surface potential due to exposed Mnⁿ⁺ species, whereas the Hg²⁺-specific aptamer carried a negative charge. After incubation, the zeta potential shifted to an intermediate value, indicating the formation of MnAuNPs@Apt. As shown in the FT-IR spectra (Fig. 4C), the appearance of characteristic DNA absorption bands at 1085 cm⁻¹ (asymmetric P-O stretching) and around 3000 cm⁻¹ (C-H vibration) after aptamer immobilization also confirms the successful formation of MnAuNPs@Apt.

Following, the effect of Hg²⁺-aptamer modification on MnAuNPs activity was investigated. As shown in Fig. 4D, MnAuNPs@Apt exhibited a markedly reduced absorbance at 652 nm upon addition of TMB compared with that of MnAuNPs, along with only a slight color change in the reaction solution. These phenomena indicate that the modification of Hg²⁺-specific aptamer markedly suppresses the oxidase-like activity of MnAuNPs. This inhibition is mainly attributed to the adsorption of Hg²⁺-aptamer blocks the catalytic active sites, which restricts substrate accessibility and impedes interfacial electron transfer [37,38]. This mechanism was further supported by cytochrome c assays (Figure S4), MnAuNPs@Apt displayed a reduced absorption peak of oxidized Cyt c at 530 nm, confirming the suppression of electron-transfer processes. As demonstrated in Fig. 4E and 4F, when the Hg²⁺-aptamer concentration is 6 μM and the incubation time was 20 min, the absorption ratio of MnAuNPs to MnAuNPs@Apt at 652 nm reached a maximum of 3.5, which further verified the remarkable inhibitory effect of Hg²⁺-aptamer toward MnAuNPs nanozyme.

Interestingly, upon the introduction of Hg²⁺, a significant absorption enhancement at 652 nm and an obvious color change were observed. This phenomenon is attributed to the specific formation of a rigid T-Hg²⁺-T complex [39], which induces aptamer conformational rearrangement, weakens its interaction with

the MnAuNPs surface, and consequently restores the oxidase-like activity. Accordingly, a remarkable enhancement at 652 nm and a distinct color change were occurred (Fig. 4A and 4D). Through the catalytic switching mechanism regulated by aptamer-target specific recognition, a simple, sensitive, and highly selective colorimetric sensing platform for Hg^{2+} detection is established.

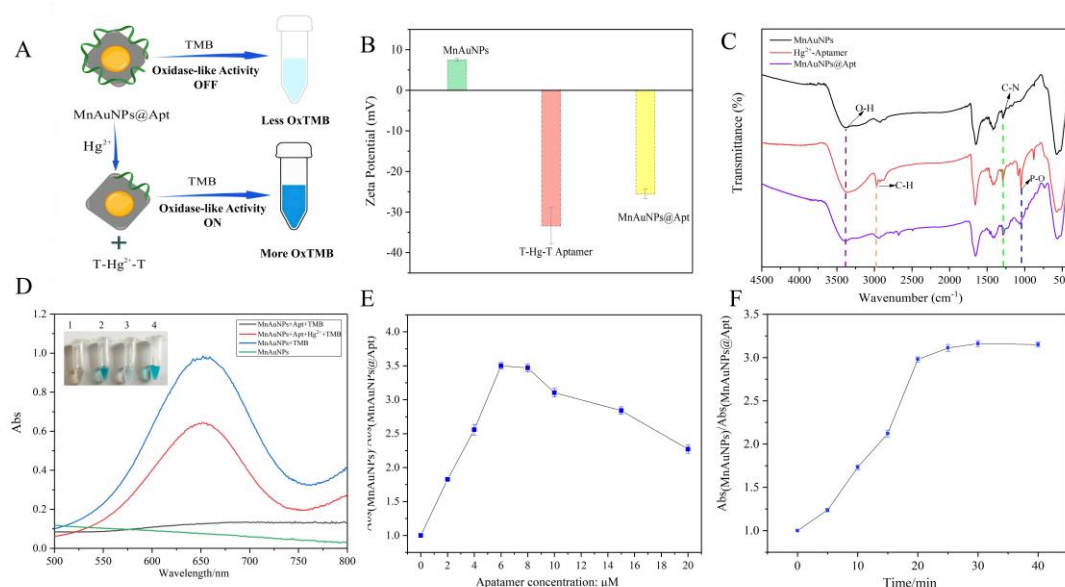


Fig. 4. Formation of MnAuNPs@Apt and its feasibility for Hg^{2+} detection. (A) Schematic illustration of the MnAuNPs@Apt sensing platform for Hg^{2+} detection; (B) Zeta potentials of MnAuNPs, Hg^{2+} -aptamer, and MnAuNPs@Apt; (C) FT-IR spectra of MnAuNPs, Hg^{2+} -aptamer, and MnAuNPs@Apt; (D) UV-Vis absorption spectra of different solutions. Insets 1-4 correspond to MnAuNPs, MnAuNPs+TMB, MnAuNPs@Apt+TMB, and MnAuNPs@Apt+ Hg^{2+} +TMB, respectively; (E) Optimization of Hg^{2+} -aptamer concentration; (F) Optimization of incubation time between MnAuNPs and Hg^{2+} -aptamer.

3.5 Optimization of parameters for the colorimetric detection system

To establish a reliable MnAuNPs@Apt-based colorimetric sensing platform, the key experimental parameters were systematically optimized. As shown in Fig. 5A, MnAuNPs exhibited the highest absorbance at 652 nm at pH=4, where the signal difference between MnAuNPs and MnAuNPs@Apt was also maximized; therefore, pH=4 was selected for the sensing platform. Temperature-dependent studies (Fig. 5B)

indicated that the sensing response was insensitive to temperature, and all measurements were performed at 25 °C. In addition, the absorbance at 652 nm reached a stable plateau within 20 min in the presence of Hg^{2+} (Fig. 5C), which was chosen as the optimal reaction time. The absorbance of $\text{MnAuNPs}@Apt+\text{Hg}^{2+}$ solution at 652 nm enhanced as TMB concentration increasing and reached a plateau at 0.1 mM, accordingly, 0.1 mM TMB was used in all subsequent detection experiments.

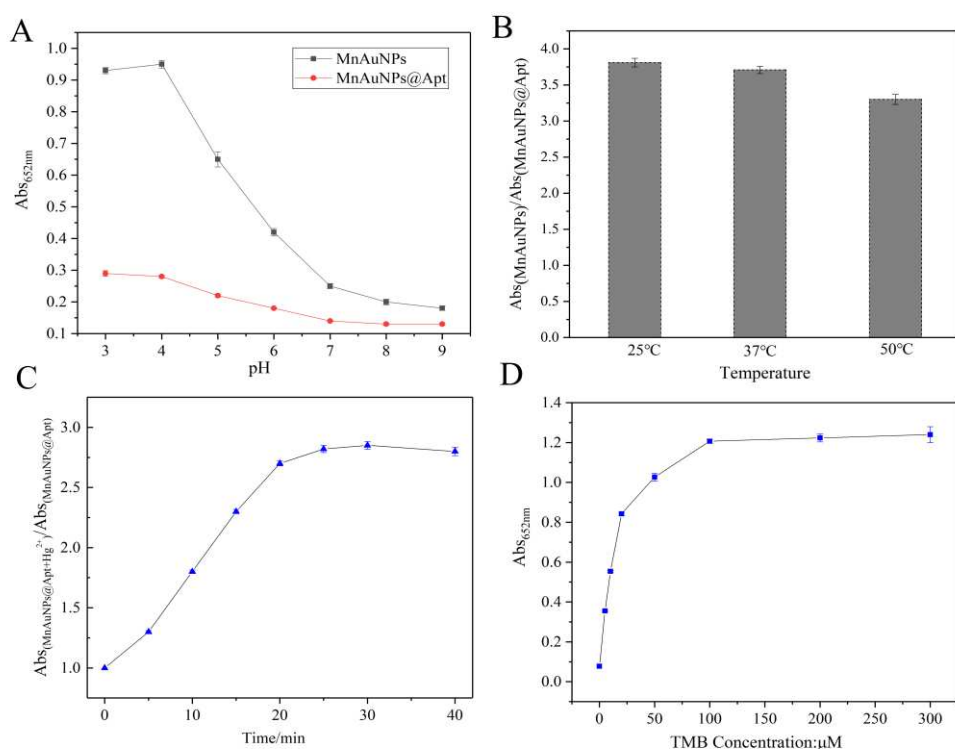


Fig. 5. Optimization of pH (A), temperature (B), reaction time (C), and TMB concentration (D) for the $\text{MnAuNPs}@Apt$ -based colorimetric detection for Hg^{2+} .

3.6 Colorimetric determination of Hg^{2+}

Based on the above findings, the colorimetric detection performance of the $\text{MnAuNPs}@Apt$ system toward Hg^{2+} was systematically evaluated. As shown in Fig. 6A, upon the introduction of Hg^{2+} , the absorbance at 652 nm increased proportionally with Hg^{2+} concentration over the range of 1.0-50 μM , yielding a linear calibration curve ($y=0.00935x+0.396$, $R^2=0.999$), where x represents the Hg^{2+} concentration (μM)

and y denotes the absorbance at 652 nm. The limit of detection (LOD), calculated as $3\sigma/k$, was 0.32 μM , indicating the high sensitivity of this sensing platform. Also, the solution color gradually deepened as Hg^{2+} concentration increased (the inset in Fig. 6B). These results demonstrate that the $\text{MnAuNPs}@Apt$ system enables sensitive, robust, and quantitative colorimetric detection of Hg^{2+} .

3.7 Selectivity and anti-interference performance of the $\text{MnAuNPs}@Apt$ system

The selectivity of the $\text{MnAuNPs}@Apt$ colorimetric platform was assessed by exposing the system to various potentially interfering metal ions at concentrations four times higher (200 μM) than that of Hg^{2+} (50 μM), including Co^{2+} , Ca^{2+} , Cd^{2+} , Al^{3+} , Fe^{3+} , Mg^{2+} , K^+ , Na^+ , Zn^{2+} , Ag^+ , Pb^{2+} , Cu^{2+} and Mn^{2+} . As shown in Fig. 6C, none of these ions produced a noticeable increase in absorbance at 652 nm, whereas Hg^{2+} induced a prominent response. Such excellent selectivity can be attributed to the specific T- Hg^{2+} -T coordination with high affinity and structural selectivity. To further verify its anti-interference performance, Hg^{2+} detection was conducted in the presence of the above coexisting ions. As depicted in Fig. 6D, the absorbance at 652 nm variations caused by these ions were negligible. These findings confirm that the $\text{MnAuNPs}@Apt$ platform not only discriminates Hg^{2+} from common metal ions but also maintains stable analytical performance in complex environments, demonstrating its strong potential for practical applications.

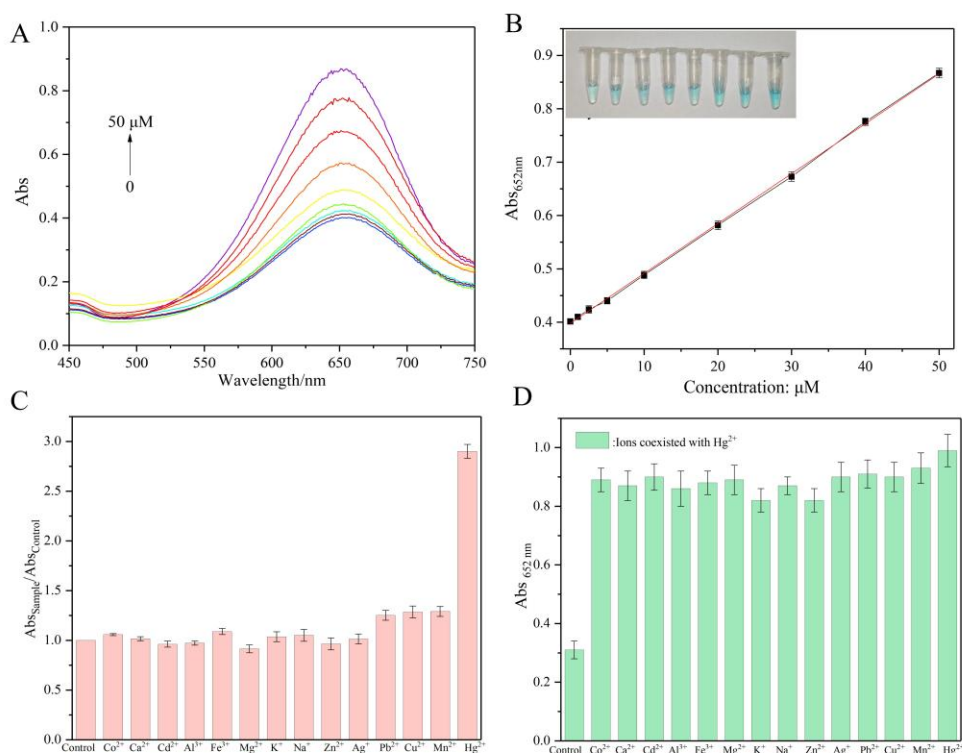


Fig. 6. Analytical performance of the MnAuNPs@Apt colorimetric sensor for Hg²⁺. (A) UV-Vis absorption spectra upon addition of varying Hg²⁺ concentrations (0, 1, 2.5, 5, 10, 20, 30, 40, 50 μM from bottom to top); (B) Linear calibration curve between absorbance at 652 nm and Hg²⁺ concentration; the inset shows corresponding color changes (0, 2.5, 5, 10, 20, 30, 40, 50 μM from left to right); (C) Selectivity toward Hg²⁺ over other metal ions; (D) Anti-interference test. n=3.

3.8 Development of a smartphone-integrated MnAuNPs@Apt sensing platform

To establish a convenient platform for Hg²⁺ detection, as illustrated in Fig. 7, a smartphone-integrated MnAuNPs@Apt paper sensor was fabricated. A color-recognition application (Color Assist, downloaded from the Apple App Store) was employed to convert the colorimetric response into quantitative RGB values (Fig. 7B). The paper-based sensor (1 cm diameter, punched from Whatman No. 1 filter paper and uniformly coated with MnAuNPs@Apt solution) exhibited a distinct color transition from light blue to dark blue upon exposure to increasing concentrations of Hg²⁺, enabling visual screening (Figure S5). The corresponding RGB signals were

simultaneously extracted by the smartphone application, allowing real-time quantification of the colorimetric reaction (Fig. 7A). As shown in Fig. 7C, the parameter $(G+B)/2R$ displayed an excellent linear correlation with Hg^{2+} concentration in the range of 5.0-50 μM , following the calibration equation $(G+B)/2R = 0.00557C + 1.02$, $R^2 = 0.997$, where C is the Hg^{2+} concentration (μM) and the LOD is 5.0 μM . These results demonstrate that the MnAuNPs@Apt-based portable paper sensor enables rapid on-site quantification of Hg^{2+} , highlighting its promising applicability for field environmental monitoring. As shown in Table 1, compared with existing colorimetric Hg^{2+} assays [40-44], the MnAuNPs@Apt platform combines a wide linear range, competitive LOD and smartphone-assisted portability, making it particularly suitable for environmental Hg^{2+} analysis in field settings.

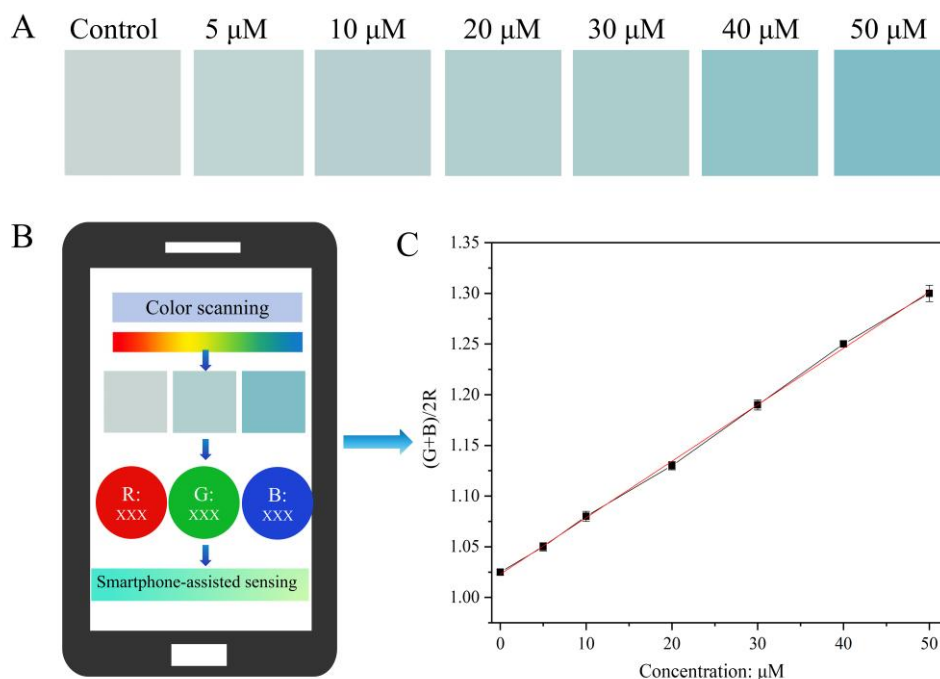


Fig. 7. Smartphone-integrated MnAuNPs@Apt sensing platform for Hg^{2+} detection. (A) Representative RGB images of the paper-based MnAuNPs@Apt sensor exposed to Hg^{2+} solutions ranging from 5 to 50 μM ; (B) Schematic illustration of the smartphone-assisted colorimetric readout to obtain RGB values; (C) Calibration plot of the parameter $(R+B)/2G$ versus Hg^{2+} concentration.

Table 1. Performance comparison of various colorimetric Hg²⁺ detection methods.

Sensor Name	Linear Range	LOD	References	Paper-based Device
BPP	0-30 μ M	1.2 μ M	40	No
Probe L	10-70 μ M	7.8 μ M	41	No
TMB@CMs	1-100 μ M	1.0 μ M	42	No
Pt/POP	0.2-50 μ M	0.04 μ M	43	No
Au@Ag NPs	0-25 μ M	0.53 μ M	44	No
MnAuNPs@Apt	1-50 μ M	0.32 μ M	This work	Yes

3.9 Quantification of Hg²⁺ in real water samples

To verify the practical applicability of the MnAuNPs@Apt sensing platform, Hg²⁺ spike-recovery experiments were conducted using Yellow River water samples collected from different regions of Gansu Province and domestic wastewater samples. All samples were filtered, passed through a 0.22 μ m membrane, adjusted to pH 4.0, and spiked with Hg²⁺ at two concentration levels (10 and 40 μ M) for recovery evaluation. The pretreated samples were analyzed using both the developed colorimetric assay and the smartphone-assisted paper-based sensor. As summarized in Table 2, the colorimetric method achieved recoveries ranging from 95.3% to 108%, with relative standard deviations (RSDs) below 3.24%. In parallel, the smartphone-based sensing platform yielded recoveries of 92.2% to 110%, with RSDs below 5.78%, showing good agreement with the colorimetric results. Moreover, the analytical results obtained by both methods were in good agreement with those measured by ICP-OES, confirming the outstanding accuracy of the proposed strategy. These results confirm that the MnAuNPs@Apt colorimetric platform permits reliable Hg²⁺ quantification in complex matrices, and the smartphone-integrated system offers a portable, cost-effective tool for on-site monitoring of Hg²⁺ in water samples.

Table 2. Comparison of colorimetric, smartphone-integrated and ICP-OES methods for Hg^{2+} determination in real water samples

Sample	Spiked (μM)	Colorimetric detection			Smartphone sensing platform			ICP-OES		
		Found (μM)	Recovery (%)	RSD (%)	Found (μM)	Recovery (%)	RSD (%)	Found (μM)	Recovery (%)	RSD (%)
Yellow River	10.0	9.53	95.3	1.98	9.22	92.2	2.55	9.72	97.2	2.67
(Hekou)	40.0	38.3	95.7	1.61	38.6	96.5	2.77	39.2	98.0	2.57
Yellow River	10.0	10.7	107	2.88	10.5	105	2.34	10.5	105	0.66
(Xisha Bridge)	40.0	42.3	106	2.47	41.4	104	3.35	41.3	103	0.90
Yellow River	10.0	10.6	106	4.01	11.6	116	3.09	11.2	112	3.76
(Malasong Park)	40.0	41.2	103	2.44	42.0	105	2.88	44.6	111	1.47
Yellow River	10.0	10.3	103	3.24	11.2	112	5.78	10.7	107	3.57
(Donggang)	40.0	42.1	105	1.79	43.2	108	4.92	42.6	106	1.70
Waste Water	10.0	10.6	106	1.26	10.9	109	4.54	10.5	105	3.49
	40.0	43.2	108	1.74	44.2	110	4.88	43.5	108	0.79

4. Conclusion

In summary, a novel Mn-Au hybrid nanozyme with intrinsic oxidase-like activity was successfully synthesized via a facile one-pot strategy and combined with a Hg^{2+} -specific aptamer to construct an aptamer-controlled catalytic switch for sensitive and selective colorimetric Hg^{2+} detection. The aptamer-regulated catalytic switching mechanism through aptamer-target specific recognition enabled efficient signal transduction, resulting in a wide linear range, low detection limit, and excellent selectivity toward Hg^{2+} . Furthermore, the sensing strategy was extended to a paper-based, smartphone-assisted platform for portable Hg^{2+} detection in environmental water samples. Benefiting from its simple operation, H_2O_2 -free condition, good practical applicability, and flexible design, the proposed aptamer-controlled Mn-Au nanozyme strategy provides a promising analytical method for environmental monitoring of Hg^{2+} . Moreover, the sensing strategy can be readily extended to the detection of other targets by simply replacing the corresponding aptamers.

Author contributions

Chen Ma: Investigation, Data curation, Writing-original draft, Formal analysis, Resources; Yida Zhang: Software, Supervision, Writing-review&editing; Shuai Mu: Project administration, Resources; Yuan Zhang: Supervision; Shuoyun Wei: Formal analysis; Quanfang Li: Data curation; Shufang Ren: Methodology.

Data availability

Data will be made available on request.

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Declarations

Competing interests The authors declare no competing interests.

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