Electronic Supplementary Information

Experimental Section

Materials and Instruments

Gelatin, 3,3,5,5-tetramethylbenzidine (TMB), Silver nitrate (AgNO₃), Sodium hydroxide, Mercuric nitrate, hydrogen peroxide (H₂O₂) were purchased from Sigma-Aldrich (Beijing, China). All of the chemicals were of analytical grade, and all glass containers were cleaned by aqua regia and ultrapure water.

UV-3600 spectrophotometer (Shimadzu, Japan) and Transmission electron microscopy (TEM, Tecnai G20, FEI, USA) imaging operated at 100 kV were employed to characterize the prepared catalytic Gel-AgNPs in the absence and presence of Hg²⁺ ions. Moreover, colorimetric measurements were performed using Infinite M 200 PRO (TECAN, Switzerland).

Synthesis and characterization of small Gelatin-Ag nanoparticles (Gel-AgNPs)

Typically, the synthesis of small Gelatin-AgNPs was conducted by adding 0.20 mL AgNO₃ (20 mM) into 0.5 mL gelatin solution with different concentrations (1.0 – 5.0 % wt) at 37 °C by magnetic stirring. Then, each of the mixtures was incubated under opaque condition at 37 °C to be vigorously stirred for 8 h. Subsequently, the dialysis and purification were performed for the mixture to be stored at 4 °C in the fridge. TEM imaging and UV-vis measurements were conducted for the resulting Gel-AgNPs in the absence and presence of Hg²⁺, including the ones that were pre-treated under UV lamp (365 nm, 6 w) for 6 h. The hydrodynamic diameters of Gel-AgNPs before and after Hg²⁺ treatment were investigated comparably by using dynamic light scattering (DLS), with a Zetasizer Nano ZS (Malvern Instruments, UK) setup equipped with a helium−neon laser (λ = 632.8 nm, 4 mW).

Colorimetric analysis

Colorimetric investigations of peroxidase-like catalysis activities of Gel-AgNPs in the absence and presence of Hg²⁺ were conducted using commercially-available TMB-H₂O₂ substrate and 96-well plates or plastic tubes. An aliquot of Gel-AgNPs was pipetted into the TMB-H₂O₂ reaction substrates at pH 7.1. An appropriate amount of Hg (NO₃)₂ was added to be mixed thoroughly by vortex to be further incubated for 20 minutes. Then, colorimetric measurements were performed, with absorbance values recorded for the reaction products of

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TMB-H$_2$O$_2$ reactions.

The optimization of catalytic reaction conditions was carried out with 96-well plates by following the same procedure above separately under different reaction conditions of ionic strengths of KNO$_3$ (2.5, 5.0, 10.0, 15.0, and 20.0 mM), temperatures (4 °C, 10 °C, 25 °C, 37 °C, 45 °C, 60 °C), pH values (2.0, 4.1, 6.2, 7.1, 8.0, 10.0, 12.1), and reaction time (2.0, 5.0, 10, 15, 20, 25, 30, and 35 min). Moreover, comparable studies on the peroxidase-like activities of Gel-AgNPs were performed among different ions including Na$^+$, Fe$^{3+}$, Co$^{2+}$, Pb$^{2+}$, Cu$^{2+}$, Mg$^{2+}$, K$^+$, Ca$^{2+}$, Cr$^{3+}$, Mn$^{2+}$, Al$^{3+}$, S$_2$O$_3^{2-}$, SO$_3^{2-}$, NO$_2^-$, Cl$^-$, SCN$^-$, PO$_4^{3-}$, S$^2$ (each 5.0 x 10$^{-5}$ M), and Hg$^{2+}$ (4.5 x 10$^{-7}$ M). Each of ions was mixed with 7.2 x 10$^{-6}$ M Gel-AgNPs with TMB-H$_2$O$_2$ substrate.

Colorimetric measurements were conducted with for TMB and H$_2$O$_2$ reaction substrates with different concentrations using Gel-AgNPs in the absence and presence of 4.5×10$^{-7}$ M Hg$^{2+}$ to obtain Michaelis-Menten curves. Typically, 10 μL Gel-AgNPs with desired concentrations were added to the wells of 96-well plates to be further incubated at 4°C overnight. Colorimetric measurements were conducted alternatively at a fixed concentration of one substrate (10 mM H$_2$O$_2$ or 2.5×10$^{-4}$ M TMB) versus varying concentrations of the second substrate of TMB (10, 50, 100, 150, 250, 300 μM) or H$_2$O$_2$ (1.0, 2.0, 4.0, 6.0, 8.0, 10, 12, 15 mM). Dynamics parameters of catalysis activities of Gel-AgNPs and Hg$^{2+}$-stimulated Gel-AgNPs were thereby calculated by double-reciprocal plotting, including the Michaelis constant ($K_m$) and the maximal reaction velocity ($V_{max}$).

Moreover, colorimetric analysis for different Hg$^{2+}$ concentrations in water, blood, and wastewater samples were carried out using the TMB-H$_2$O$_2$ reaction substrates and 96-well plates pre-incubated at 4°C with Gel-AgNPs of 7.2 x 10$^{-6}$ M. Hg$^{2+}$ samples, which concentrations range separately from 0.25 nM to 800 nM in water, 1.0 nM to 700 nM in blood, and 0.5 nM to 800 nM in wastewater, were analyzed to obtain calibration detection curves for different Hg$^{2+}$ concentrations.
Fig. S1 Investigation of catalytic activities of Gel-AgNPs in TMB-H$_2$O$_2$ reactions stimulated by various anions of $S_2O_3^{2-}$, $SO_3^{2-}$, NO$_2^-$, Cl$^-$, SCN$^-$, PO$_4^{3-}$, S$^2$ (each $5.0\times10^{-5}$ M), compared with Hg$^{2+}$ ($4.5\times10^{-7}$ M).

Fig. S2 DLS data of Gel-AgNPs in the (A) absence and (B) presence of Hg$^{2+}$ ions.
Fig. S3 (A) TEM images for the UV-treated Gel-AgNPs alone (left) and UV-treated Gel-AgNPs with Hg$^{2+}$ (right), each with amplified view of one particle (Insert); (B) UV-vis spectra of (a) Gel, (b) UV-treated Gel-AgNPs alone, and (c) UV-treated Gel-AgNPs with Hg$^{2+}$, corresponding to the photographs (Insert).
Fig. S4 Colorimetric dynamic measurements for (A) different TMB concentrations and (C) different H$_2$O$_2$ concentrations in the TMB-H$_2$O$_2$ reactions catalyzed by Gel-AgNPs in the (a) absence and (b) presence of 4.5 × 10$^{-7}$ M Hg$^{2+}$, with corresponding K$_m$ and V$_{max}$ values obtained by double-reciprocal plotting for (B) TMB and (D) H$_2$O$_2$. Colorimetric measurements were conducted alternatively at a fixed concentration of one substrate (10 mM H$_2$O$_2$ or 2.5 × 10$^{-4}$ M TMB) versus varying concentrations of the second substrate, with details shown in the Experimental.
Fig. S5 The time-dependent responses of Gel-AgNPs to Hg²⁺ ions with stimulated catalysis activities.

Fig. S6 (A) Gel concentration-dependent UV-vis spectra of Gel-AgNPs synthesized using different Gel amounts of 1.0 %–5.0 %, corresponding to catalysis activities in TMB-H₂O₂ reactions (Insert); (B) Gel-AgNPs concentration-dependent catalysis for TMB-H₂O₂ reactions in the (a) absence and (b) presence of 4.5 ×10⁻⁷ M Hg²⁺ ions.
Fig. S7 Optimization of the reaction conditions of TMB-\(\text{H}_2\text{O}_2\) reactions catalyzed by Gel-AgNPs in the (a) absence and (b) presence of \(\text{Hg}^{2+}\) \((4.5 \times 10^{-7}\) M\)) under different (A) ionic strengths, (B) temperatures, (C) pH values, and (D) color reaction time.
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