Supporting Information

In situ generated nanozyme-initiated cascade reaction for amplified surface plasmon resonance sensing

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EXPERIMENTAL SECTION

Reagents and Materials

All DNA fragments were prepared and purified through HPLC technique by Shanghai Sangon Biotechnology Co., Ltd. (Shanghai, China). $HgCl_2$, $HAuCl_4 \cdot 3H_2O$, $NH_2OH \cdot HCl$, tween 80, 6-mercapto-1-hexanol (MCH), 3,3′,5,5′-tetramethylbenzidine (TMB), H_2O_2, CH_3COOH, CH_3COONa, NaH_2PO_4, Na_2HPO_4, NaCl, KCl, and other chemicals were received from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). 10 mM Tris-HCl buffer (10 mM NaCl, 10 mM KCl, pH 7.4) was used to dissolve DNA for preparing the stock solution, and dissolve $HAuCl_4$, NH_2OH, and tween 80 for fabricating AuNPs. 10 mM CH_3COOH/CH_3COONa buffer (pH 4.4) was used to dissolve TMB and H_2O_2 for catalyzed oxidation reaction.

Apparatus

Morphology characterizations were conducted on a HT7700 transmission electron microscope (Hitachi, Japan) and a JEOL7500F scanning electron microscope (Tokyo, Japan), respectively. Electrochemical measurements including cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS) were carried out on an Autolab PGSTAT 302N electrochemical analyzer (Metrohm Autolab B. V., Utrecht, the Netherlands) based on a three-electrode system (working/reference/counter electrode). UV-vis information was obtained on an ultraviolet spectrophotometer TU-1902 (Persee, China). SPR characterizations were conducted on a SPR spectrometer (SPR Navi 200, BioNavis, Tampere, Finland) through using a 650 nm light source.

Preparation of AuNPs In the Absence/Presence of Hg^{2+}

The preparation of AuNPs was performed in a 1.0 mL solution containing 0.5 mM $HAuCl_4$, 20 mM NH_2OH, 0.04% tween 80, and 100 μM Hg^{2+}. Then the solution reacted at 25 ℃ for different time. UV-vis and TEM techniques were employed to study the formation of AuNPs.

When Hg^{2+} was absent in the reaction solution, the contrast experiment was also carried out.

Fabrication of the Modified SPR Disk MCH/T30/Au.

SPR disk used in this work was treated according to the method reported in our previous literatures. After that SPR disk was placed in 1.0 μM T30 solution and reacted for 12.0 h to obtain T30 modified SPR disk (denoted as T30/Au). Through washing with Tris-HCl buffer, T30/Au was incubated with 1.0 mM MCH solution for 1.0 h to get the resulting disk MCH/T30/Au, which was cleaned with Tris-HCl buffer and saved for SPR characterizations.

Development of Nanozyme-Based SPR Sensor for Hg^{2+}.

MCH/T30/Au disk was immobilized on a cuvette flow cell and rinsed with water until the baseline reached the equilibration. Then, 500 μL Hg^{2+} solution with different concentration was poured into and kept running for sufficiently capturing Hg^{2+} to form Hg^{2+}/MCH/T30/Au, closely followed with water washing. Further, 1.0 mL solution containing 20 mM NH_2OH, 0.5 mM $HAuCl_4$, and 0.04% tween 80 was injected into the flow cell and reacted for 14 min to produce AuNPs on Hg^{2+}/MCH/T30/Au surface, denoted as AuNPs/Hg^{2+}/MCH/T30/Au, closely followed with water washing. Finally, 1.0 mL solution containing 0.2 mM TMB and 0.7 M

H_2O_2 was pumped into the instrument and reacted with AuNPs/Hg^{2+}/MCH/T30/Au for 30 min to induce the TMB oxidation reaction and oxTMB deposition on disk. The resulting modified disk was denoted as oxTMB/AuNPs/Hg^{2+}/MCH/T30/Au. Meanwhile, SPR angle verse reaction time at different stages was recorded.

For Hg^{2+} detection in real samples, tap water and lake water were treated through 220 nm membrane-mediated filtering. Then Hg^{2+} with different amount was added into the water and was determined according to the procedures mentioned above.

Table S1. Sequences of the oligonucleotides used in the experiments

Name	Primer sequence (5' to 3')
T30	SH-TTTTTTTTTTTTTTTTTTTTTTTTTTTTTT
A30	SH- AGAAGACAAGAGGAAGAAGAGACCGGAGCA
B30	SH- CCAGCAGCAGCACGACGACGCCGCGCAGCG

Table S2. Comparison of Hg^{2+} assay using our strategy and other biosensors.

Typical methods for Hg^{2+} detection	Linear Range	LOD	Ref.
PEC method	0.5–50000 nM	0.13 nM	1
PEC method	10–2×10^8 fM	3.3 fM	2
PEC method	5–500 pM	1 pM	3
PEC method	0.01–10 nM	2.5 pM	4
Electrochemical method	10–100 nM	1.7 nM	5
Electrochemical method	0.5– 80 nM	0.2 nM	6
Electrochemical method	10–50000 pM	2.7 pM	7
Fluorescence method	1–10 μM	0.173 μM	8
Fluorescence method	-	9.7 nM	9
Fluorescence method	0.3–1 nM	0.3 nM	10
colorimetric method	0–5 nM	1.45 nM	11
colorimetric method	1–10000 pM	1 pM	12
colorimetric method	80–50000 nM	25 nM	13
SPR method	1–2000 pM	0.46 pM	This work

Table S3. Determination results of spiking Hg^{2+} with different concentrations into tap water and lake water.

Sample No.		Added (pM)	Detected (pM)	Recovery[a] (%)	RSDs (%)
Tap water	1	10	10.30	103.00	3.61
	2	50	48.60	97.20	3.42
	3	500	498.2	99.24	2.40
lake water	1	10	10.90	109.00	6.86
	2	50	52.80	105.60	5.26
	3	500	503.40	100.68	2.96

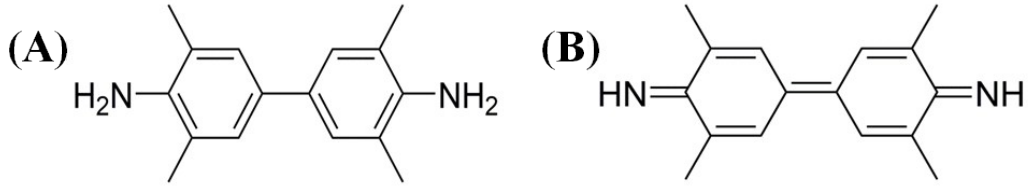

Fig. S1 The molecular structures of MB (A) and oxTMB (B).

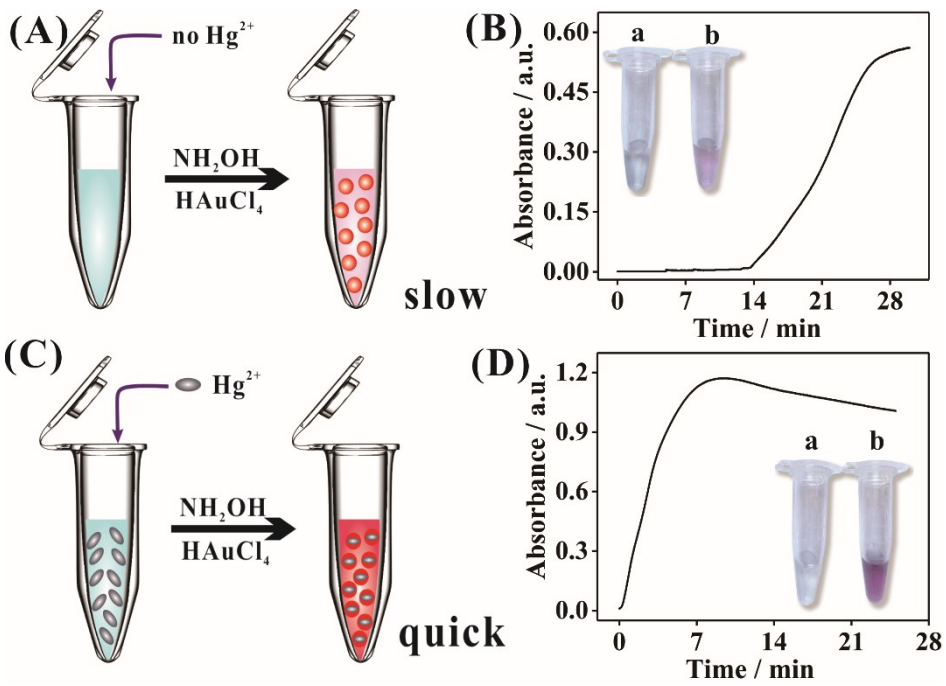

Fig. S2 (A) Diagram illustration of AuNPs' generation in the absence of Hg^{2+}. (B) Absorbance in the absence of Hg^{2+} versus reaction time. Inset indicates the images of solution at 14 min (a) and 28 min (b), respectively. (C) Diagram illustration of AuNPs' generation in the presence of Hg^{2+}. (D) Absorbance of solution in the presence of Hg^{2+} versus reaction time. Inset indicates the images of solution at 0 min (a) and 7 min (b), respectively.

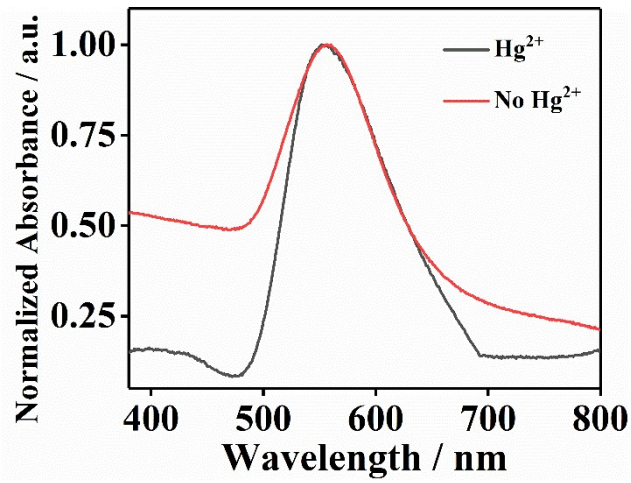

Fig. S3 Normalized UV-vis spectra of AuNPs in the absence/presence of Hg^{2+}.

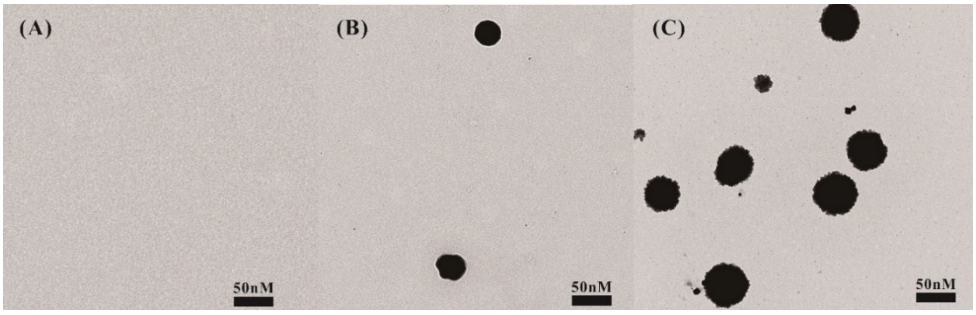

Fig. S4 TEM images of the solution in the absence of Hg^{2+} at the time of 14 min (A) and 28 min (B), and in the presence of Hg^{2+} at the time of 14 min (C).

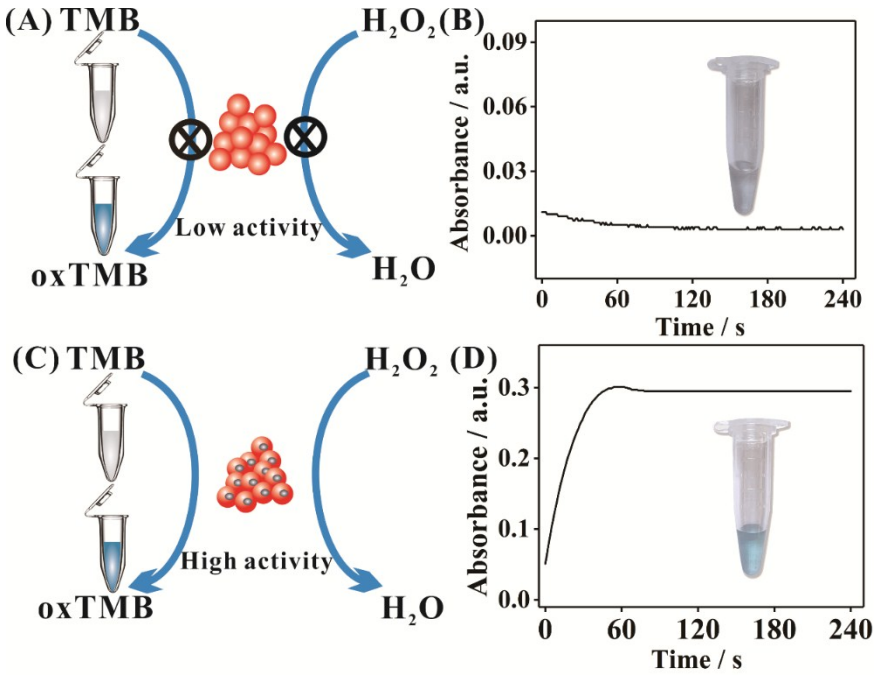

Fig. S5 (A) Diagram illustration of interaction of AuNPs (obtained in the absence of Hg^{2+}) and TMB with the aid of H_2O_2. (B) Absorbance at 650 nm versus reaction time. Inset indicates the image of solution at the time of 240 s. (C) Diagram illustration of interaction of AuNPs (obtained in the presence of Hg^{2+}) and TMB with the aid of H_2O_2. (D) Absorbance at 650 nm versus reaction time. Inset indicates the image of solution at the time of 60 s. [AuNPs] = 0.5 mM, [TMB] = 0.2 mM, [H_2O_2] = 0.7 M.

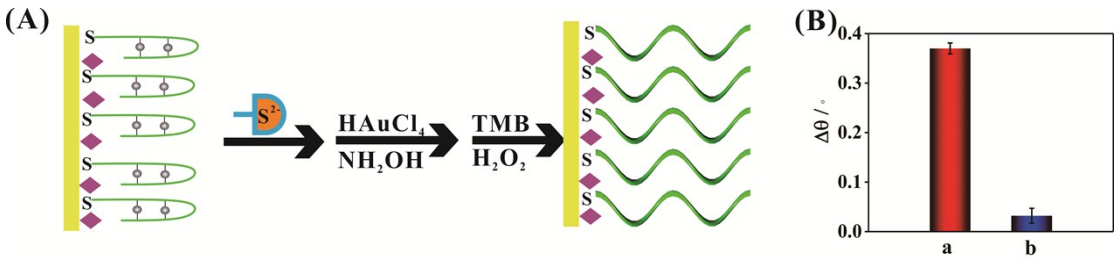

Fig. S6 (A) Diagram illustration of the influence of S^{2-} on SPR sensor for Hg^{2+} diagnosis. (B) Δθ of the proposed SPR sensor in the absence (a) and presence (b) of S^{2-}.

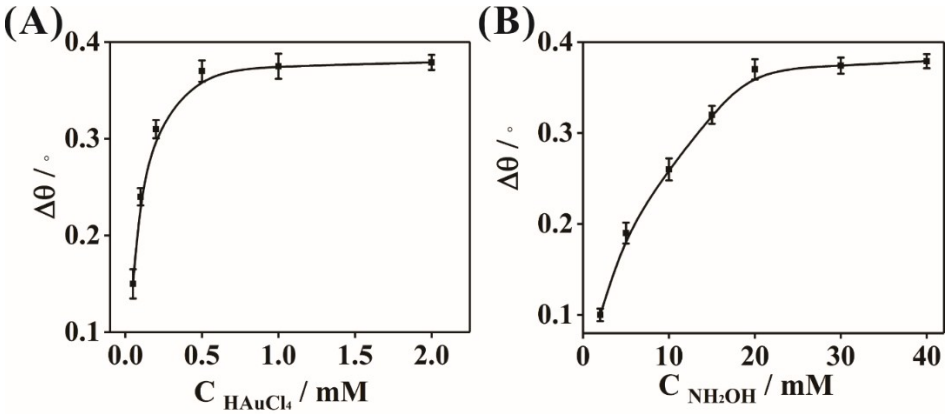

Fig. S7 (A) Δθ versus $HAuCl_4$ concentrations. (B) Δθ versus NH_2OH concentrations.

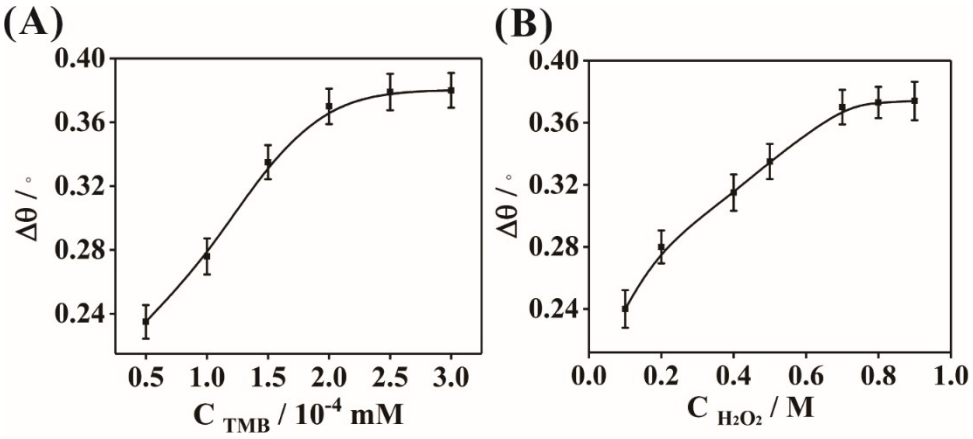

Fig. S8 (A) Δθ versus TMB concentrations. (B) Δθ versus H_2O_2 concentrations.

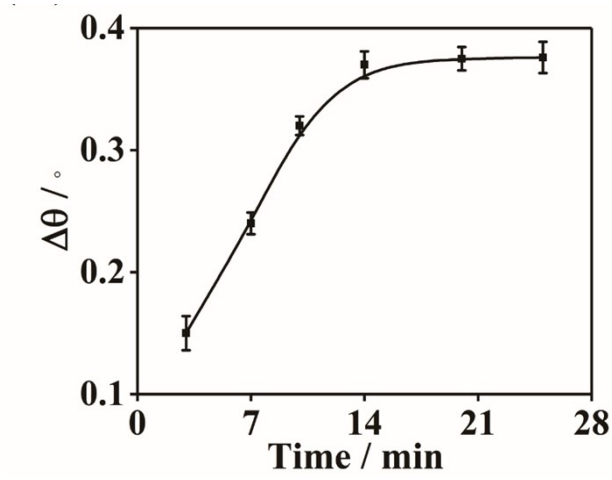

Fig. S9 Δθ versus AuNPs generation time.

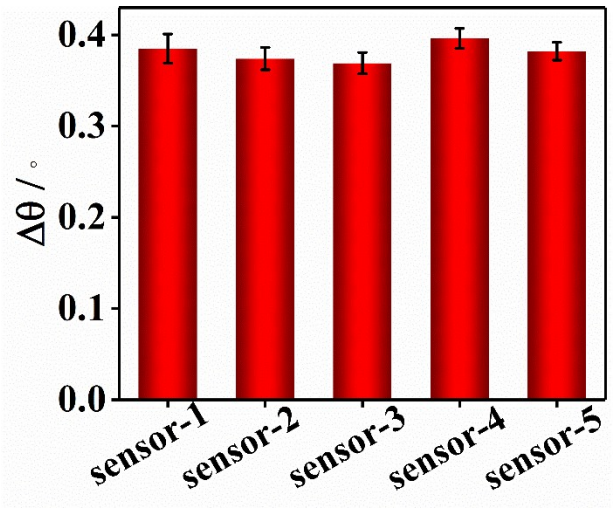

Fig. S10 Δθ of the five freshly fabricated SPR sensors in the presence of 2000 pM Hg^{2+}.

Reference:

1. Y. Hao, Y. Cui, P. Qu, W. Sun, S. Liu, Y. Zhang, D. Li, F. Zhang and M. Xu, *Electrochimica Acta.*, 2018, 259, 179–187.
2. M. Zhao, G. C. Fan, J. J. Chen, J. J. Shi and J. J. Zhu, *Anal. Chem.*, 2015, 87, 12340–12347.
3. Z. Y. Ma, J. B. Pan, C. Y. Lu, W. W. Zhao, J. J. Xu and H. Y. Chen, *Chem. Commun.* , 2014, 50, 12088–12090.
4. Y. Zhang, A. Shoaib, J. Li, M. Ji, J. Liu, M. Xu, B. Tong, J. Zhang and Q. Wei, *Biosens. Bioelectron.*, 2016, 79, 866–873.
5. P. Miao, Y. Tang and L. Wang, *ACS Appl. Mater. Interfaces*, 2017, 9, 3940-3947.
6. F. Xuan, X. Luo and I. M. Hsing, *Anal Chem*, 2013, 85, 4586–4593.
7. H. Xu, X. Zhu, H. Ye, L. Yu, G. Chen, Y. Chi and X. Liu, *Chem. Commun.* , 2015, 51, 15031–15034.
8. J. Ru, X. Chen, L. Guan, X. Tang, C. Wang, Y. Meng, G. Zhang and W. Liu, *Anal. Chem.*, 2015, 87, 3255–3262.
9. Q. Liu, J. J. Peng, L. N. Sun, F. Y. Li, *ACS Nano*, 2011, **5**, 8040–8048
10. J. Huang, X. Gao, J. Jia, J. K. Kim and Z. Li, *Anal. Chem.*, 2014, 86, 3209–3215.
11. Z. Chen, C. Zhang, Q. Gao, G. Wang, L. Tan and Q. Liao, *Anal. Chem.*, 2015, 87, 10963–10968.
12. J. Chen, S. Zhou and J. Wen, *Anal. Chem.*, 2014, 86, 3108–3114.
13. G. L. Wang, L. Y. Jin, X. M. Wu, Y. M. Dong and Z. J. Li, *Anal. Chim. Acta.*, 2015, 871, 1–8.