Supporting Information

Complementary atomic flame/molecular colorimetry dual-mode assay for sensitive and wide-range detection of cancer cells

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Materials and reagents. Copper chloride dihydrate ($CuCl_2 \cdot 2H_2O$), strontium

chloride hexahydrate ($SrCl_2 \cdot 6H_2O$) and selenium dioxide (SeO_2) were purchased from Shanghai Titan Scientific Co.Ltd. Disodium rhodizonate was obtained from Shanghai Yuanye Bio-Technology Co., Ltd (Shanghai, China). Chloroplatinic acid hexahydrate ($H_2PtCl_6 \cdot 6H_2O$) was acquired from Aladdin (Shanghai, China). L-ascorbic acid, sodium hydroxide (NaOH) and hydrogen peroxide (H_2O_2) were obtained from XiLong Scientific Co., Ltd. (Guangzhou, China). 3,3′,5,5′-tetramethylbenzidine dihydrochloride (TMB) was purchased from TCI (Shanghai, China). Thiolated AS14111 aptamer with a sequence of HS- TTT TTT TTT GGT GGT GGT GGT TGT GGT GGT GGT GG (from 5′ to 3′) was synthesized by Sangon Biotechnology Co., Ltd (Shanghai, China). Phosphate buffered saline (PBS, pH 7.4, 10 mM), Dulbecco's modified essential medium (DMEM) cell culture medium and fetal bovine serum (FBS) were purchased from Thermo Fisher Scientific Inc. (Waltham, MA, USA). Methyl thiazolyl tetrazolium (MTT) and dimethyl sulfoxide (DMSO) were acquired from Sigma-Aldrich (St. Louis, MO). Human HeLa cervical cancer cells, human breast carcinoma MCF-7 cells, and mouse fibroblast L929 cells were provided by Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences. The human serum sample was provided from a healthy donor and informed consent was obtained for any experimentation with human subjects. All related experiments were performed in compliance with the relevant laws and institutional guidelines of China and approved by the Institutional Ethical Committee (IEC) of Guangxi Normal University. All solutions were prepared with ultrapure water produced by Millipore purified water system (MILli-Q, USA).

Instrumentation. Transmission electron microscopy (TEM) images were obtained with a Talos F200S microscope (Thermo Fisher Scientific, USA). Zeta potential characterization was performed using a Malvern Zetasizer (Nano ZS-90, Malvern, UK). Cell viability was measured on a Synergy H1 multi-mode microplate spectrophotometer (Bio-Tek, USA).

Flame reaction. Typically, 10 μL of Sr^{2+} solution (50 mM) was firstly dropped onto a man-made twisted iron wire, and then placed on the burned flame of an alcohol lamp. Then, the flame photograph was taken with a smartphone. In some experiments,

the metal type and concentration, and the used volume were changed.

Volumetric analysis of Sr^{2+} solution using the detection modes of atomic flame and molecular colorimetry. Volumetric analysis of Sr^{2+} solution was performed using the flame/precipitation reactions, respectively. For the detection mode of atomic flame, the flame reaction of 50 mM Sr^{2+} solution with different volumes was carried out, respectively. Meanwhile, different volumes of Sr^{2+} solution (50 mM) were added and reacted with 1 mL of NaRho (3 mM) for 15 s. Finally, the photographs of the resultant flame and the resultant solution of NaRho were taken using a smartphone, respectively.

Synthesis of AS1411-PtNPs. PtNPs were firstly synthesized according to a previous work.[1] Firstly, 20 mL of H_2O containing 1.89 mM $H_2PtCl_6 \cdot 6H_2O$, 7.5 mM NaOH, and 10.0 mM L-ascorbic acid was stirred in a beaker. Then, the mixture was heated at 60 ℃ for 10 min, followed by cooling down to room temperature. The formed PtNPs were stored at 4 ℃ for further use.

Then, the synthesis of AS1411-PtNPs was carried out using the salt aging method.[2] In short, thiolated AS14111 aptamer and PtNPs were mixed and incubated overnight at room temperature. Then, 100 μL of NaCl (100 mM) was added slowly for several times within 12 h, followed by stirring for 24 h. After the removal of free DNA by centrifugation, AS1411-PtNPs were obtained.

MTT assay. Cytotoxicity of AS1411-PtNPs was measured by MTT assay. Briefly, 10^5 cells were seeded into a well of 96-well plate and cultured for 24 h. Then, AS1411-PtNPs with different concentrations (0, 0.05, 0.25, 0.49, 0.74 mg/mL) were added and incubated for another 4 h. After incubation with 10 μL of MTT (5 mg/mL) for 4 h, 150 μL of DMSO was added. Finally, the absorbance at 490 nm was measured.

Visual detection of cancer cells. Different concentrations of HeLa cells were firstly plated in 96-well plates and cultured in DMEM medium overnight. Then, the cell media containing 100 μL of AS1411-PtNPs (0.49 mg/mL) were added and incubated for 4 h. After washing with PBS (10 mM, pH 7.4), the cells were collected and transferred into the gas-generation tube containing 110 μL of H_2O_2 (30 wt%).

After 10 min, the volume of overflown Sr^{2+} solution (50 mM) was fixed to 860 μL with H_2O. Then, 10 μL of diluted solution was directly used and reacted with 1 mL of NaRho (3 mM) for the flame/precipitation reactions, respectively. Finally, the photographs of the resultant flame and the resultant solution of NaRho were taken using a smartphone, respectively. In some experiments, the types of cell and nanozyme were changed.

Analysis of real samples. To investigate the application of the proposed sensing platform, HeLa cells with different concentrations (50, 10^2, 10^4, 10^5 cells/mL) were spiked into 1000-fold diluted human blood, respectively. Then, the culture media containing 100 μL of AS1411-PtNPs (0.49 mg/mL) were added and incubated for 4 h. After washing, the cell suspension was mixed with 110 μL of H_2O_2 (30 wt %), followed by visual analysis using the proposed sensing platform.

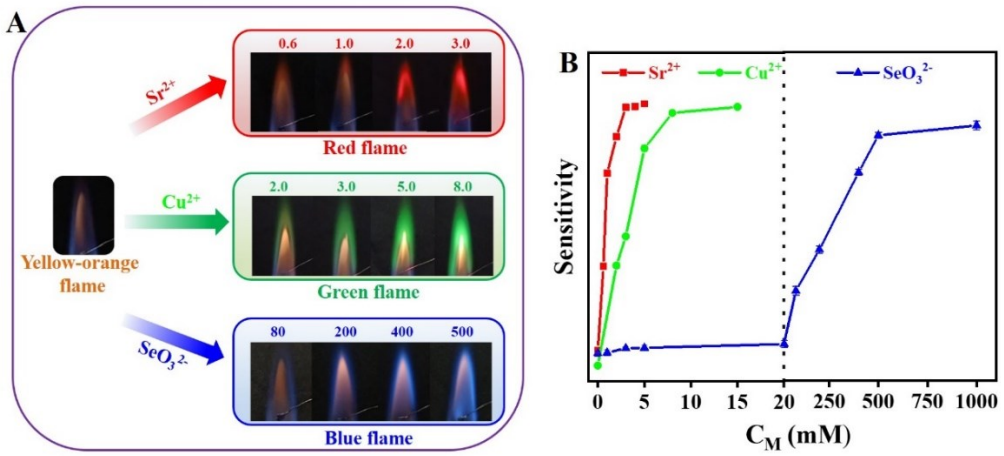

Fig. S1 (A) The photographs and (B) the corresponding sensitivity of different ions with different concentrations. The sensitivity was acquired according to the normalized R/G/B value.

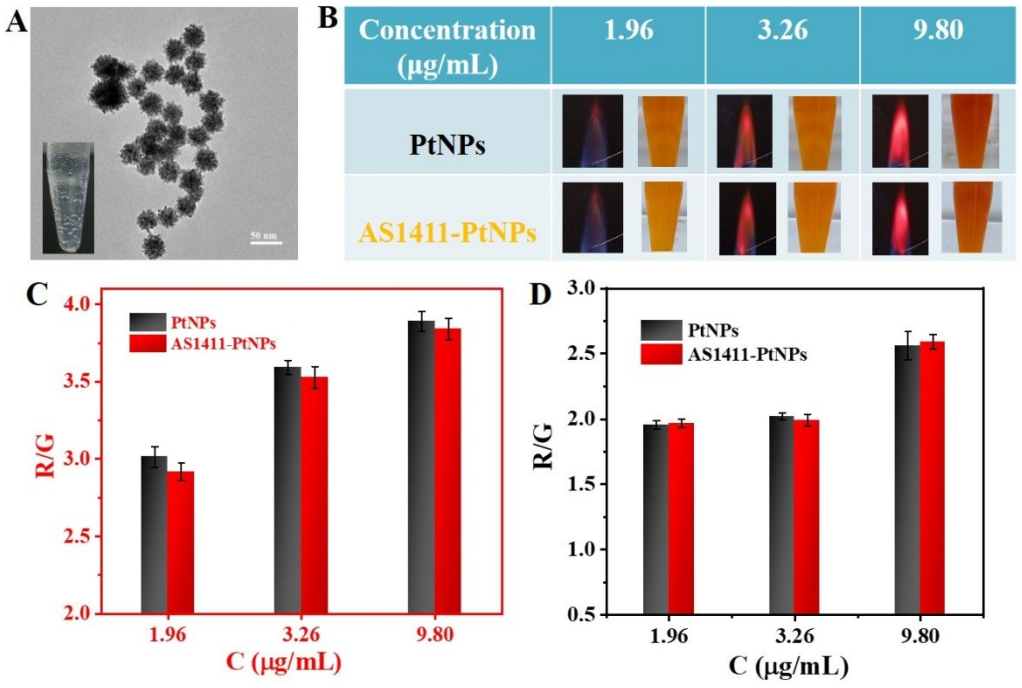

Fig. S2 (A) TEM image of PtNPs. The inset showed the catalytic reaction of H_2O_2 by PtNPs. (B) The effect of aptamer modification on the catalytic activity of PtNPs. Comparison of the catalytic activity between PtNPs and AS1411-PtNPs using the detection mode of (C) atomic flame and (D) molecular colorimetry, respectively.

Almost the same R/G value demonstrated the negligible effect of AS1411 modification on the catalytic activity of PtNPs.

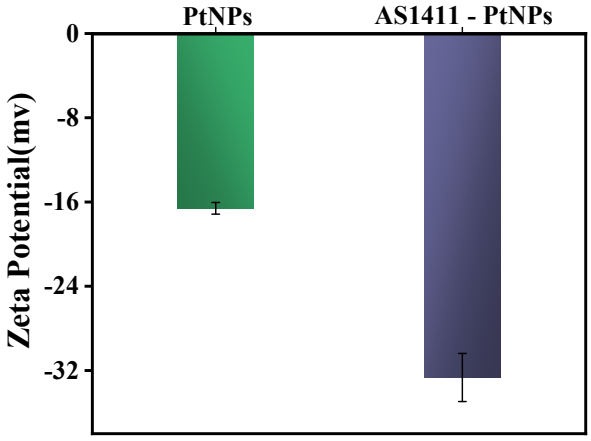

Fig. S3 Potential characterization of AS1411 functionalization of PtNPs. Almost twice the potential increase indicated effective modification of thiolated AS1411 aptamer on the surface of PtNPs.

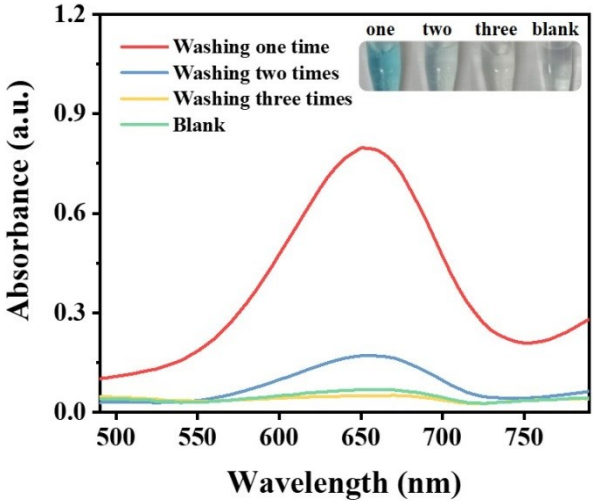

Fig. S4 UV-vis absorption spectra of the TMB-H_2O_2 solution in the presence of the supernatant of the media after washing different times. The inset shows the corresponding photographs of the TMB-H_2O_2 solution.

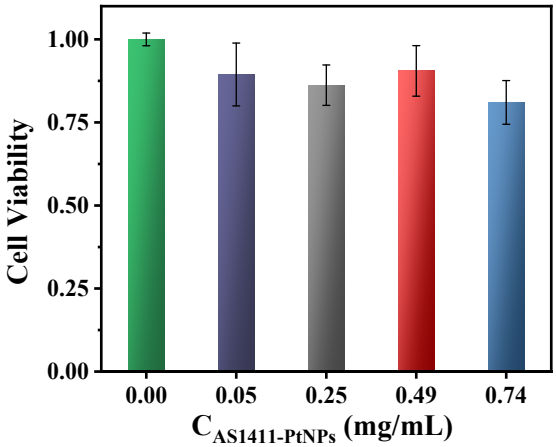

Fig. S5 Viability of the HeLa cells in the presence of different concentrations of AS1411-PtNPs. Over 80% of cell viability for all the samples demonstrated excellent biocompatibility of AS1411-PtNPs.

Table S1. Comparison of different methods for cancer cell assay

Detection mode	**Cell type**	**Response range (cells/mL)**	**LOD (cells/mL)**	**Ref.**
ICP-MS	MCF-7	$2 \times 10^2 - 1.2 \times 10^4$	81	[3]
Electrochemistry	MCF-7	$50 - 1 \times 10^6$	20	[4]
pH	HeLa	$20 - 1 \times 10^4$	20	[5]
Molecular Fluorescence	HeLa	$50 - 2 \times 10^3$	23	[6]
Pressure	HeLa	$50 - 4 \times 10^2$	50	[7]
Molecular colorimetry	CCRF-CEM	$50 - 5 \times 10^3$	50	[8]
Molecular colorimetry	MCF-7	$125 - 8 \times 10^3$	30	[9]
Pressure and molecular fluorescence	HeLa	$50 - 1 \times 10^3$	50	[10]
Atomic flame and molecular colorimetry	HeLa	$25 - 1 \times 10^4$	10	our work

Additional reference

1. S. Hu, L. Tong, J. Wang, X. Yi and J. Liu, *Anal. Chem.*, 2019, **91**, 15418-15424.
2. P. Peng, Q. Wang, Y. Du, H. Wang, L. Shi and T. Li, *Anal. Chem.*, 2020, **92**, 9273-9280.
3. B. Yang, B. Chen, M. He, X. Yin, C. Xu and B. Hu, *Anal. Chem.*, 2018, **90**, 2355-2361.
4. N. Liu, J. Song, Y. Lu, J. J. Davis, F. Gao and X. Luo, *Anal. Chem.*, 2019, **91**, 8334-8340.
5. L. Wang, C. Chen, H. Huang, D. Huang, F. Luo, B. Qiu, L. Guo, Z. Lin and H. Yang, *Biosens. Bioelectron.*, 2018, **121**, 153-158.
6. M. Xu, J. Zhuang, X. Jiang, X. Liu and D. Tang, *Chem. Commun.*, 2019, **55**, 9857-9860.
7. E. Ding, J. Hai, T. Li, J. Wu, F. Chen, Y. Wen, B. Wang and X. Lu, *Anal. Chem.*, 2017, **89**, 8140-8147.
8. X. Ye, H. Shi, X. He, K. Wang, D. He, L. a. Yan, F. Xu, Y. Lei, J. Tang and Y. Yu, *Anal. Chem.*, 2015, **87**, 7141-7147.
9. L.-N. Zhang, H.-H. Deng, F.-L. Lin, X.-W. Xu, S.-H. Weng, A.-L. Liu, X.-H. Lin, X.-H. Xia and W. Chen, *Anal. Chem.*, 2014, **86**, 2711-2718.
10. Z. Wang, J. Hai, T. Li, E. Ding, J. He and B. Wang, *ACS Sustainable Chem. Eng.*, 2018, **6**, 9921-9929.