# Dual-aptamer-based colorimetric assay for the accurate identification of circulating tumor cells via $Fe_3O_4$@Pt NP nanozymes and G-quadruplex/hemin for signal amplification

Ye He,‡[*ab] Panlin Wang,‡[ab] Zhuzheng Wu,[ab] Yating Chen,[ab] Xiaohao Gan,[ab] Fangjie Li,[ab] Wenxiang Wang*[ab]

[a]Department of Health Inspection and Quarantine, School of Public Health, Fujian Medical University, Fuzhou, Fujian, China

[b]Fujian Province Key Laboratory of Environment and Health, School of Public Health, Fujian Medical University, Fuzhou, Fujian, China

* Corresponding author. Tel.: +86-59122862023; Fax: +86-59122862023.

E-mail address: wangwenxiang@fjmu.edu.cn (W. Wang). heye@fjmu.edu.cn (Y. He).

‡The first two authors contributed equally to this work.

**The DNA sequence, buffer solutions and apparatus involved in this work were as follows:**

All DNA was HPLC-purified and provided by Sangon Biotechnology Co., Ltd. (Shanghai, China). Their sequences were as follows: MUC1 aptamer ($Apt_{MUC1}$, 5′-biotin- TTT TTG CAG TTG ATC CTT TGG ATA CCC TGG -3′), AS1411 aptamer ($Apt_{AS1411}$, 5′-biotin- TTT TTG GTG GTG GTG GTT GTG GTG GTG GTG G-3′), signal probe ($S_p$, 5′-biotin- TTT TTT GGG TAG GGC GGG TTG GGA AA-3′). PBS buffer (8 mM $Na_2HPO_4$, 137 mM NaCl, 2 mM $KH_2PO_4$, 2.7 mM KCl, pH 7.4), PBST buffer (8 mM $Na_2HPO_4$, 137 mM NaCl, 2 mM $KH_2PO_4$, 2.7 mM KCl, 0.01% Tween 20, pH 7.4), acetate buffer (0.2 M HAc, 0.2 M NaAc, pH 4.0), TES buffer (10 mM Tris, 1 mM EDTA, 2 M NaCl, pH 7.5) and TEST buffer (5 mM Tris, 0.5 mM EDTA, 1 M NaCl, 0.01% Tween 20, pH 7.5).

Transmission electron microscopy (TEM) analysis was performed on an FEI Tecnai G2 F20 (FEI Co. Ltd., USA). The UV–vis spectra were collected on an Infinite 200 Pro spectrophotometer (Tecan Ltd., Austria).

**Peroxidase-like activity of $Fe_3O_4$@Pt NPs-$Apt_{AS1411}$/$S_p$ conjugates**

50 μg/mL $Fe_3O_4$@Pt NPs-$Apt_{AS1411}$/$S_p$ conjugates were resuspended to the 100 μL HAC-NaAC buffer (pH 4.0) in the presence of different concentrations of TMB or $H_2O_2$. The catalytic parameters were determined by fitting the absorbance data to Michaelis-Menten equation:

$$\frac{1}{V} = \frac{K_m}{V_{max}}\left(\frac{1}{[S]} + \frac{1}{K_m}\right)$$

The Michaelis–Menten equation describes the relationship between the rates of substrate conversion by an enzyme and the concentration of the substrate. In this equation, $V$ is the initial velocity, $V_{max}$ is the maximal reaction velocity, $[S]$ is the substrate concentration, and $K_m$ is the Michaelis constant.

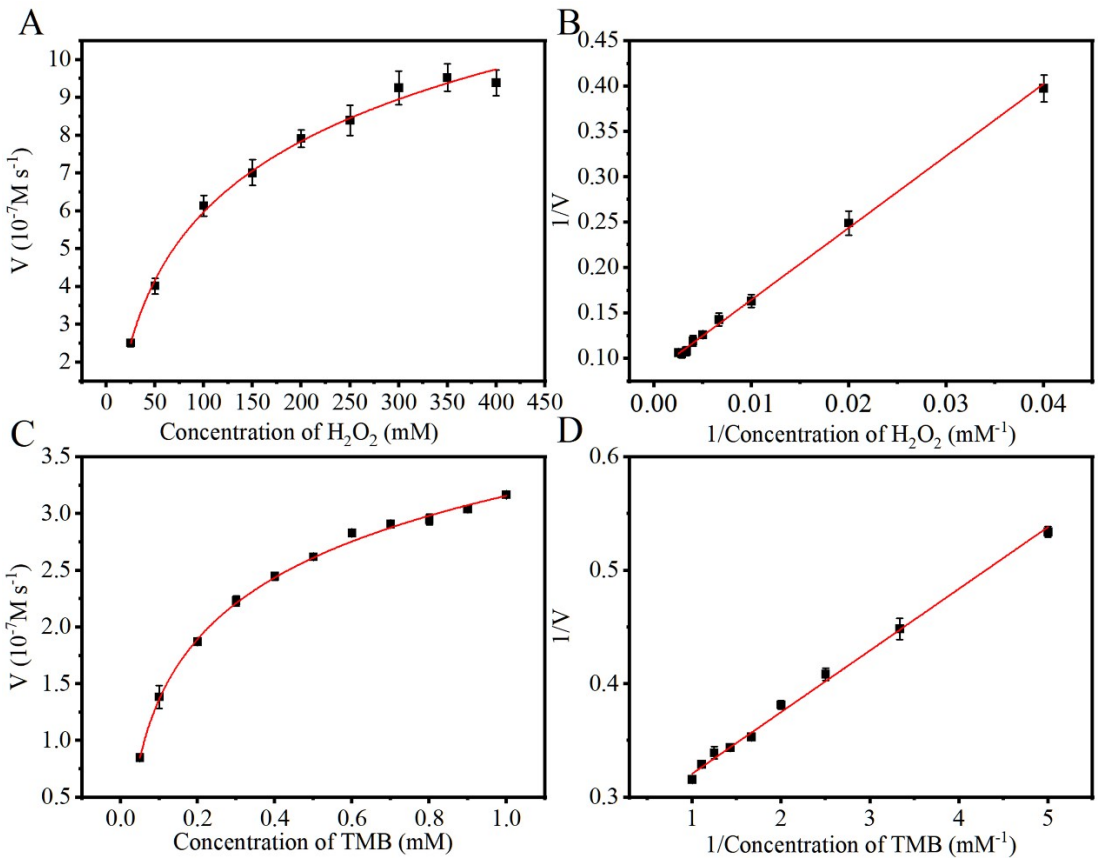


**Figure S1** Steady-state kinetic assays and the corresponding double reciprocal (Lineweaver-Burk) plots of $Fe_3O_4$@Pt NPs for $H_2O_2$ (A, B) and TMB (C, D).

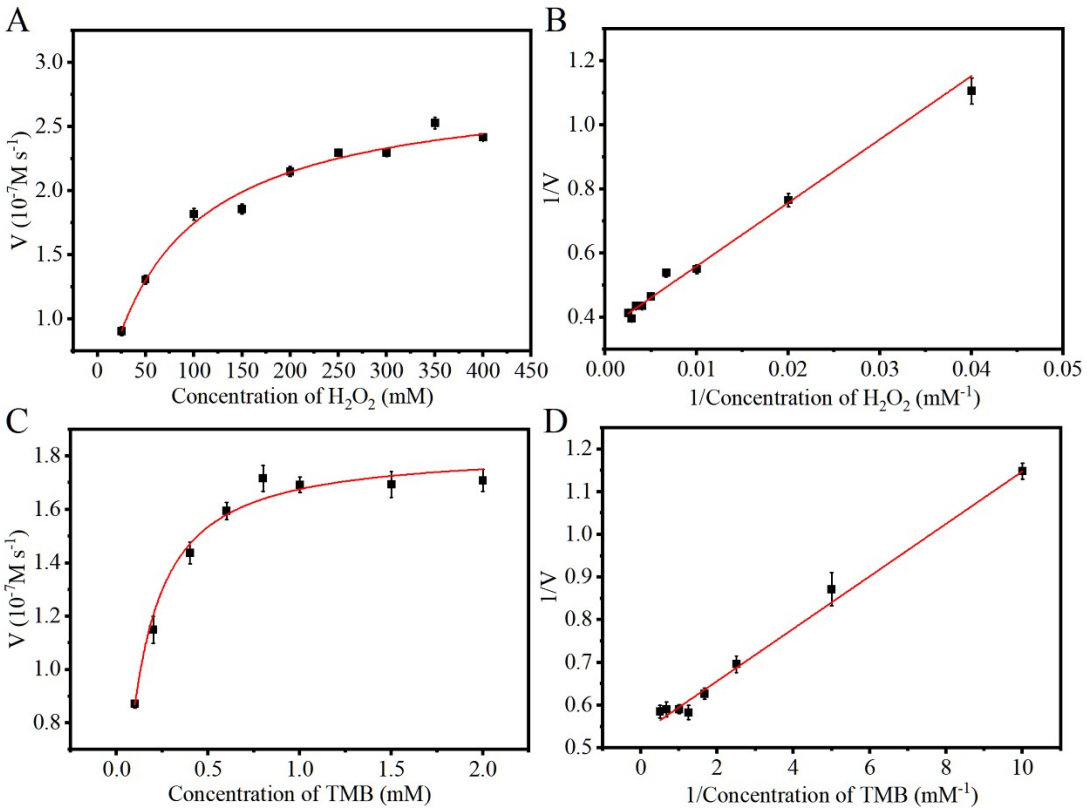


**Figure S2** Steady-state kinetic assays and the corresponding double reciprocal (Lineweaver-Burk) plots of $Fe_3O_4$@Pt NPs-$Apt_{AS1411}$/$S_p$ conjugates for $H_2O_2$ (A, B) and TMB (C, D).

**Table S1** Apparent kinetic parameters of $Fe_3O_4$@Pt NPs and $Fe_3O_4$@Pt NPs-$Apt_{AS1411}/S_p$ conjugates as peroxidase mimetics.

| Catalyst | Substrate | $K_m$ (mM) | $V_{max}$ ($10^{-7}$M $s^{-1}$) |
|---|---|---|---|
| $Fe_3O_4$@Pt NPs-$Apt_{AS1411}/S_p$ conjugates | $H_2O_2$ | 54.7 | 2.8 |
| | TMB | 0.1 | 1.9 |
| $Fe_3O_4$@Pt NPs | $H_2O_2$ | 92.9 | 11.7 |
| | TMB | 0.2 | 3.8 |
| HRP[1] | $H_2O_2$ | 3.7 | 8.7 |
| | TMB | 0.4 | 10.0 |

$K_m$: Michaelis constant. $V_{max}$: maximal reaction velocity. HRP: horseradish peroxidase.

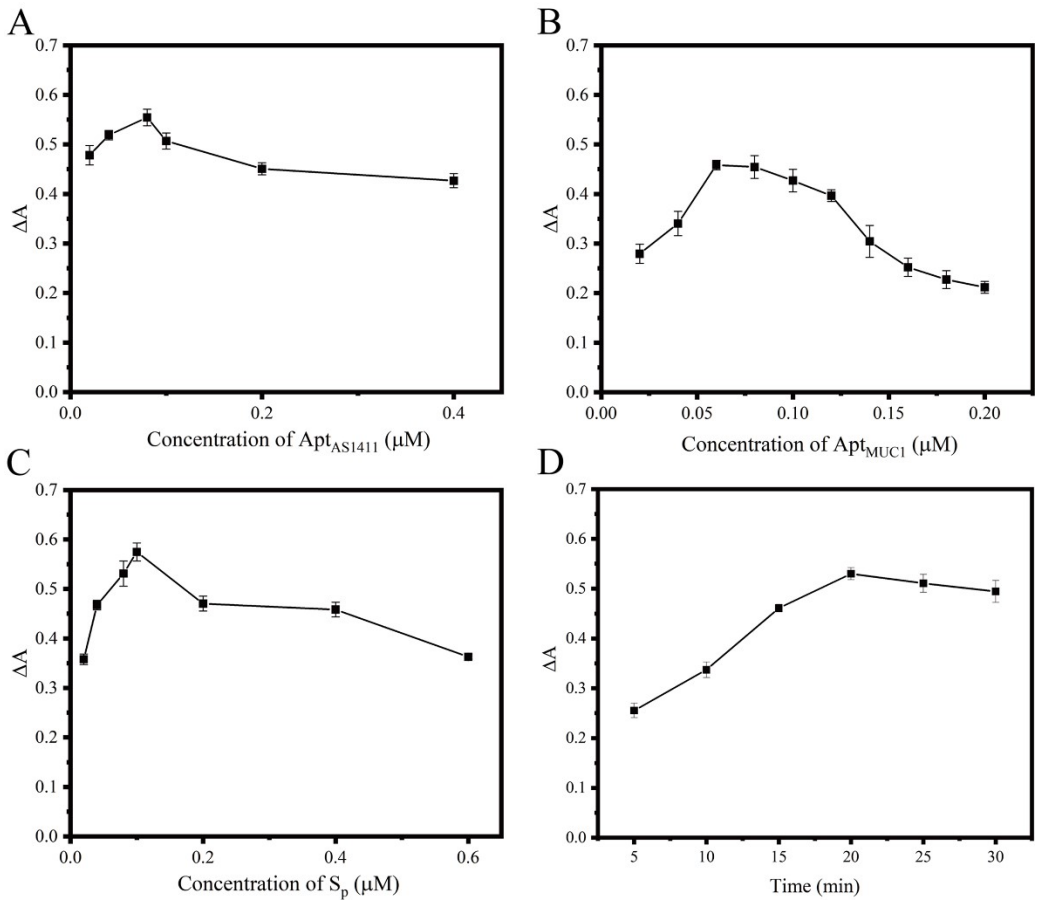


**Figure S3** Effect of the concentration of $Apt_{AS1411}$(A), the concentration of $Apt_{MUC1}$(B), the concentration of $S_p$ (C) and the incubation time of $Fe_3O_4$@Pt NPs-$Apt_{AS1411}$/$S_p$ conjugates and target MCF-7 cells (D) on the sensitivity of the method. The number of MCF-7 cells was 3000.

**Table S2** Analytical performances of various methods for CTC detection.

| Method | Cell type | Signal reporter | Linear range (cells) | Detection limit (cells) | Ref. |
|---|---|---|---|---|---|
| Electrochemistry | HepG2 | methylene blue | 10-50000 | 10 | [2] |
| ICP-MS | MCF-7 | TB | 250-1×10000 | 87 | [3] |
| Photoelectrochemistry | MCF-7 | HCNT | 100-100000 | 17 | [4] |
| Electrochemistry | MCF-7 | Ncomp | 1×10-1000000 | 4 | [5] |
| Colorimetry | HeLa | $A_{30}$AS1411-AuNFs | 10-3000000 | 10 | [6] |
| Fluorescence | MCF-7 | rGO | 100-20000 | 22 | [7] |
| Electrochemistry | 4T1 | AuNPs | 80-10000000 | 50 | [8] |
| Colorimetry | MCF-7 and HT-29 | GNCs | 50-20,000 | 221 | [9] |
| Colorimetry | MDA-MB-231 | $Fe_3O_4$@$MnO_2$ NPs | 250-5×1000 | 186 | [10] |
| Fluorescence and Colorimetry | HeLa | Pd NPs/CMC-COF-LZU1 | 0-1×1000000 | 100 | [11] |
| Colorimetry | MCF-7 | $Fe_3O_4$@Pt NPs-$Apt_{AS1411}$/$S_p$ conjugates | 50-4000 | 4 | This work |

# References


1. L. Gao, J. Zhuang, L. Nie, J. Zhang, Y. Zhang, N. Gu, T. Wang, J. Feng, D. Yang, S. Perrett and X. Yan, *Nature Nanotechnology*, 2007, **2**, 577-583.
2. H. Lu, D. Jin, L. Zhu, T. Guo, X. Li, X. X. Peng, G. Mo, L. Tang, G. J. Zhang and F. Yang, *Biosensors & Bioelectronics*, 2020, **170**, 112662.
3. X. Yin, B. Chen, M. He and B. Hu, *Analytical Chemistry*, 2020, **92**, 10308-10315.
4. J. Luo, D. Liang, X. Li, L. Deng, Z. Wang and M. Yang, *Mikrochimica acta*, 2020, **187**, 257.
5. F. Shafiei, R. S. Saberi and M. A. Mehrgardi, *Bioelectrochemistry (Amsterdam, Netherlands)*, 2021, **140**, 107807.
6. Z. Wei, Y. Yu, S. Hu, X. Yi and J. Wang, *ACS Applied Materials & Interfaces*, 2021, **13**, 16801-16811.
7. W. Qian, Z. Miao, X. J. Zhang, X. T. Yang, Y. Y. Tang, Y. Y. Tang, L. Y. Hu, S. Li, D. Zhu and H. Cheng, *Mikrochimica Acta*, 2020, **187**, 407.
8. R. Zhang, Q. You, M. Cheng, M. Ge, Q. Mei, L. Yang, W. F. Dong and Z. Chang, *Frontiers in Bioengineering and Biotechnology*, 2021, **9**, 783661.
9. A. Sanati, Y. Esmaeili, M. Khavani, E. Bidram, A. Rahimi, A. Dabiri, M. Rafienia, N. Arbab Jolfaie, M. R. K. Mofrad, S. Haghjooy Javanmard, L. Shariati and A. Zarrabi, *Analytica Chimica Acta*, 2023, **1252**, 341017.
10. X. Chen, H. Tao, Y. Guo, Z. Wang, R. Li, Y. Zhao, C. Liu, X. Zhao, X. Wang and S. Duan, *Analytica Chimica Acta*, 2023, **1249**, 340947.
11. P. Sun, J. Hai, S. Sun, S. Lu, S. Liu, H. Liu, F. Chen and B. Wang, *Nanoscale*, 2020, **12**, 825-831.