Supporting information

Cu-MOFs@AuPtNPs nanozyme-based immunosorbent assay for colorimetric detection of alpha-fetoprotein.

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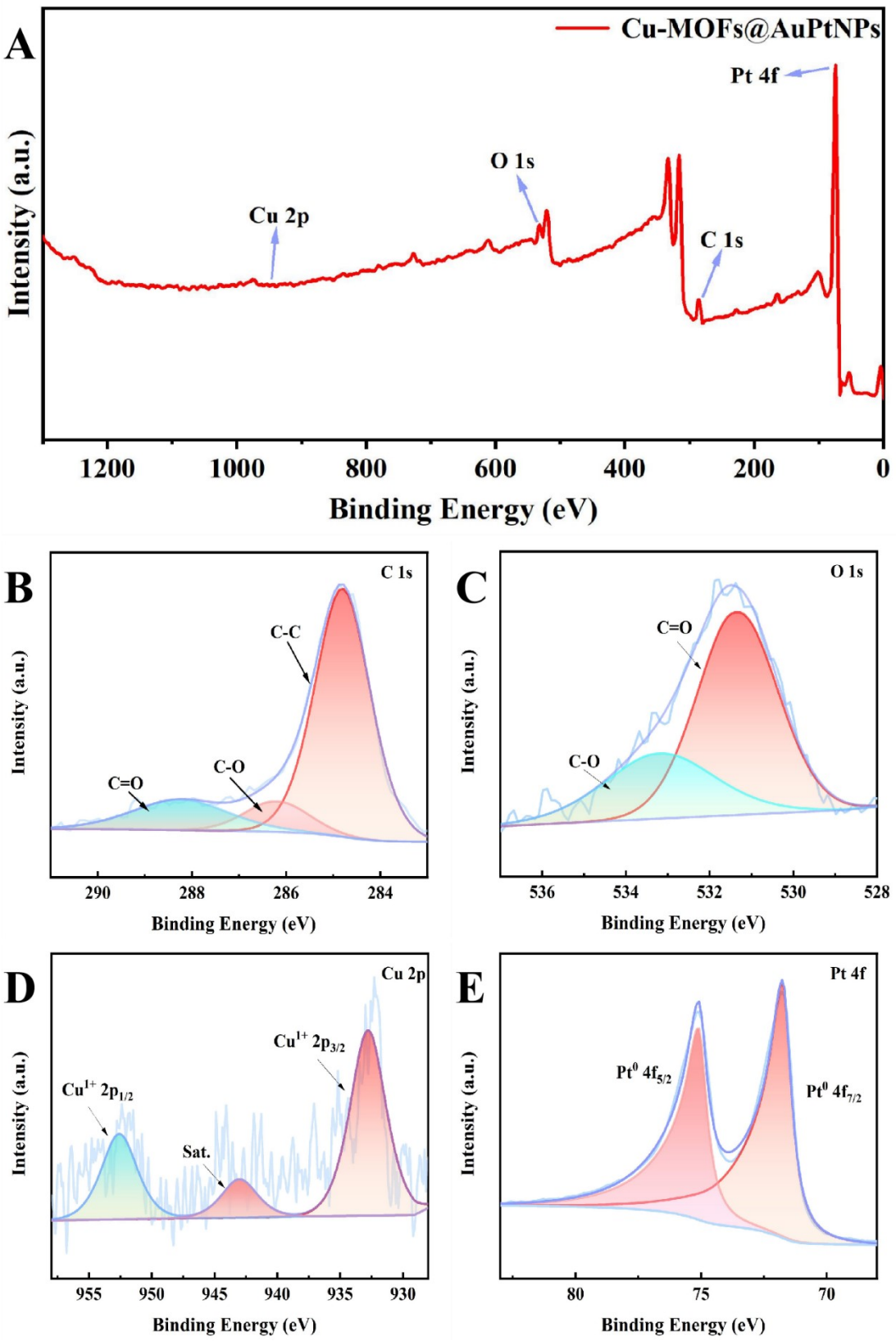

Fig. S1 *XPS results for (A) Cu-MOFs@AuPtNPs-Ab, (B) C1s, (C) O1s, (D)Cu 2p, and (E) Pt 4f*

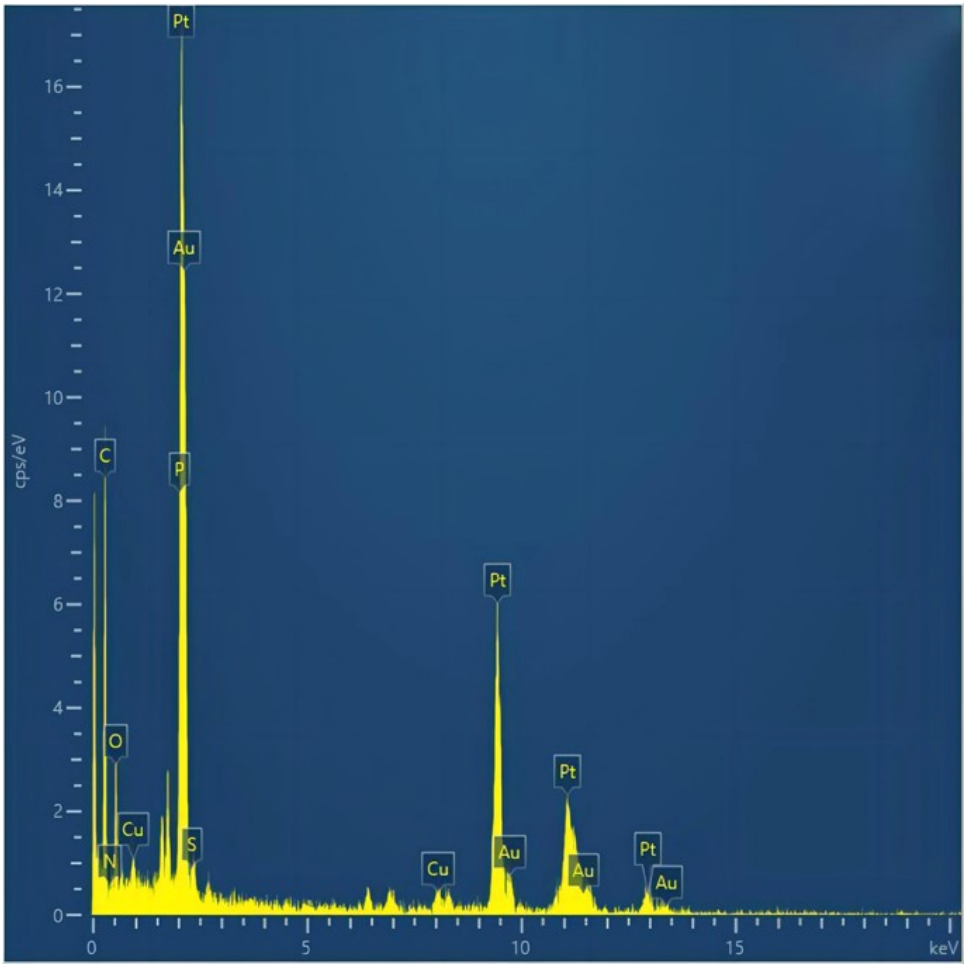

Fig. S2 *EDS pattern of Cu-MOFs@AuPtNPs-Ab*

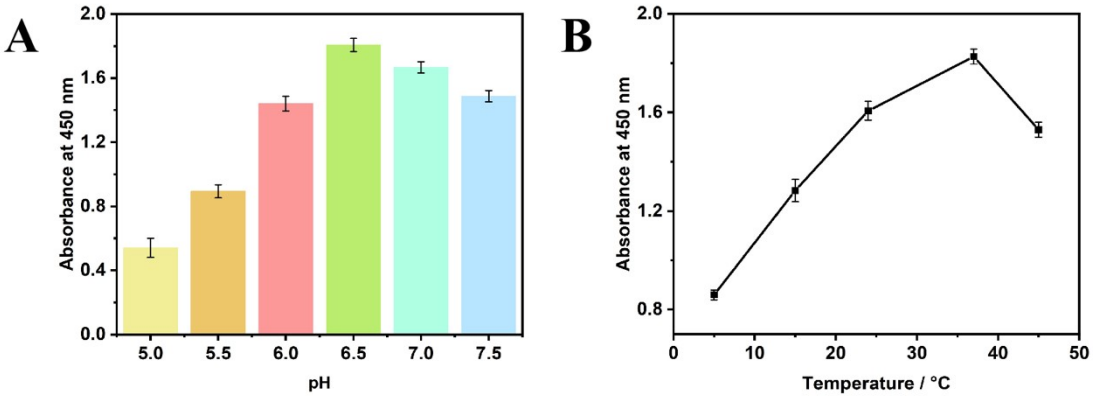

Fig. S3 *Effect of the (A) The pH values of the reaction solution, (B) the reaction temperature of the AFP and signal probes*

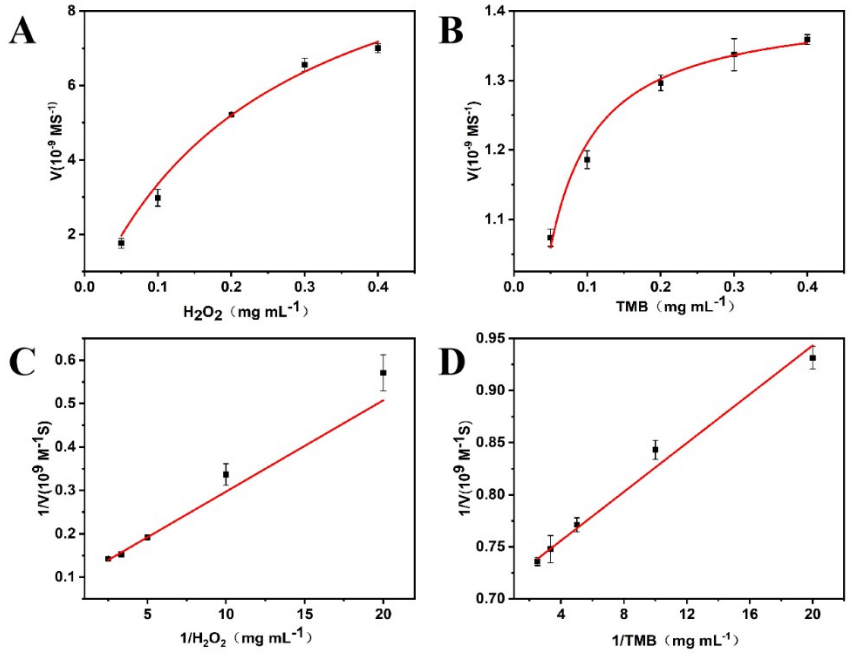

Fig. S4. Determination of the steady-state kinetics of Cu-MOFs@AuPtNPs. (A) The concentration of H_2O_2 varied, while keeping the concentration of TMB constant (0.2 mg mL-1). (B) The concentration of TMB varied, while keeping the concentration of H_2O_2 constant (0.3 mg mL-1). (C) and (D) were the double reciprocal plots corresponding to (A) and (B) respectively.

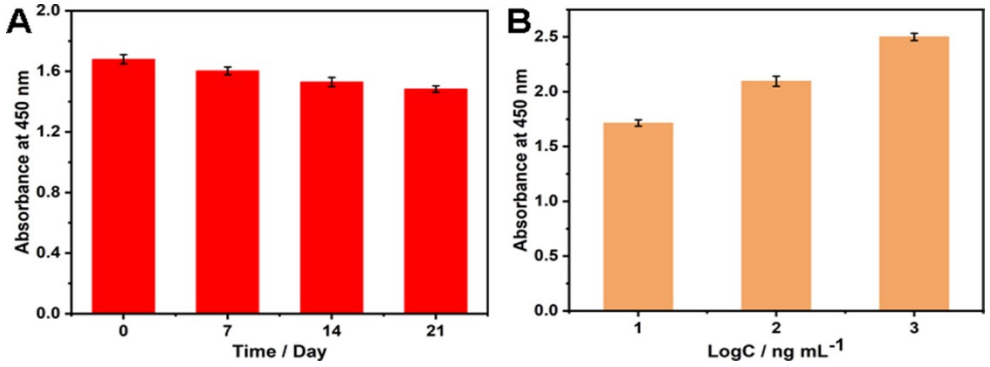

Fig. S5 *(A) Stability of the MeLISA after stored at 4 °C for 7, 14, and 21 days. (10ng mL^{-1}of AFP) Error bars = RSD (n = 5). (B) Reproducibility of the MeLISA: the absorbance at 450 nm of 1, 10, and 100 ng mL^{-1} were recorded. Error bars = RSD (n = 5).*

Table S1. *Comparison of steady-state kinetic parameters (Km and Vmax) of Cu-MOFs@AuPtNPs and HRP.*

Catalyst	*K*m/mM		*V*max/M s^{-1}	
	H_2O_2	TMB	H_2O_2	TMB
Cu-MOFs@AuPtNPs	0.246	0.017	1.16×10^{-8}	1.41×10^{-8}
HRP	3.7	0.434	8.71×10^{-8}	1.0×10^{-7}

Table S2*. Comparison of the detection limit with other AFP detection systems*

Detection method	Linear range (ng mL^{-1})	Detection limit (pg mL^{-1})	Reference
Fluorescence	0.5–10^4	170	[1]
photoelectrochemical immunosensor	0.1-300	82	[2]
plasmonic ELISA	2 - 200	230	[3]
potentiometric immunoassays	0.01 - 100	7.9	[4]
MeLISA	0.001-1000	0.86	Present work

References:

1. Y. Liu, Y. Chen, Y. Zhang, Q. Zhong, X. Zhu and Q. Wu, *Analyst*, 2022, **147**, 3186-3192.
2. X. Zhong, M. Zhang, L. Guo, Y. Xie, R. Luo, W. Chen, F. Cheng and L. Wang, *Biosens Bioelectron*, 2021, **189**, 113389.
3. Z. Xuan, M. Li, P. Rong, W. Wang, Y. Li and D. Liu, *Nanoscale*, 2016, **8**, 17271-17277.
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Table S3 *AFP detected by the proposed MeLISA in the real blood (indicated by relative standard deviation, RSD, n=5).*

Spiked AFP ng mL^{-1}	Detected AFP ng mL^{-1}	Recovery (%)	Spiked AFP ng mL^{-1}	Detected AFP ng mL^{-1}	Recovery (%)	Spiked AFP ng mL^{-1}	Detected AFP ng mL^{-1}	Recovery (%)
0.1	0.0985	98.5	1	1.018	101.8	10	9.44	94.4
0.1	0.107	107.0	1	1.054	105.4	10	10.35	103.5
0.1	0.104	104.0	1	0.965	96.5	10	10.84	108.4
0.1	0.957	95.7	1	0.950	95.0	10	9.86	98.6
0.1	0.101	101.0	1	0.940	94.0	10	10.58	105.8
	RSD%	4.39		RSD%	4.94		RSD%	5.51