A novel chemiluminescence sensor for alpha-fetoprotein detection based on aptamer-luminol modified magnetic graphene oxide and copper-based MOFs composite

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Reagents and apparatus

Reagents

Luminol, trimesic acid (H$_3$BTC), N,N-dimethylformamide (DMF), ethylene glycol, HAuCl$_4$, and zinc acetate were purchased from Shanghai Macleans Biochemical Technology Co., Ltd. Glutaraldehyde, methanol, disodium hydrogen phosphate, and sodium dihydrogen phosphate were purchased from Sinopharm Chemical Reagent Co., Ltd. Polyvinyl pyrrolidone (PVP), Hydrogen peroxide (H$_2$O$_2$) and sodium acetate were purchased from Tianjin Damao Chemical Reagent Factory. 6-Mercapto-1-hexanol (MCH), 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide (EDC, 95%) and N-hydroxysuccinimide (NHS, 99%) were purchased from Shanghai Civic Chemical Technology Co., Ltd. Tris(hydroxymethyl) aminomethane (Tris) was purchased from Shanghai Aladdin Biochemical Technology Co., Ltd. Alpha fetoprotein (AFP) and carcinoembryonic antigen (CEA) were purchased from Shanghai TargetMol Biological Technology Co., Ltd. Thrombin (THR), bovine serum albumin (BSA), tryptophan (Try) and Cysteine (Cys) were purchased from Beijing Suo Laibao Technology Co., Ltd. The aptamer of alpha fetoprotein (Apt) and the complementary strand of aptamer (AFP-cDNA) were synthesized and purchased by Shanghai Sangon Biotech Co., Ltd. The oligonucleotide sequences are follows: 5'-NH$_2$-(CH$_2$)$_6$-ATCAGGTGCAGTTCTCGACTCGGTCTTGATGTGGG-3' (Apt), 5'-NH$_2$-(CH$_2$)$_6$-CCCACATCAAGACCGCCCACATCAAGACCG-3'(AFP-Apt). The Common reagents of NaCl NaOH, KOH and other chemicals were all analytical reagent grade. Ultra-pure water was used thoroughly in this work. Phosphate buffer

solution (PBS, 0. 1 mol/L, pH = 7.2) was used to prepare all sample tube flow fluid.

Apparatus

The morphology of the prepared materials was characterized by a scanning electron microscope (SEM, Germain SEM 300, Carl Zeiss, Germany). The internal crystal structure of the materials was characterized by an X-ray diffractometer (XRD, Model D8FOCUS, Bruker AXS, Germany). The functional groups of the synthetic materials were characterized by an infrared analyzer (FTIR, Model FT-1760X, PerkinElmer, USA). The specific surface area and pore size distribution of prepared materials were characterized by a specific surface area and pore size distribution meter (BET, AMD 2460, USA). The optical properties of the prepared synthetic were verified by an ultraviolet-visible spectrophotometer (UV-Vis, Spectrometer Co., Ltd. UV-2550) and a flow injection CL analyzer (Model IFFM-E, Xi'an Ruimai, China). The CL analyzer with IFFM-E system was used for AFP detection in the whole work.
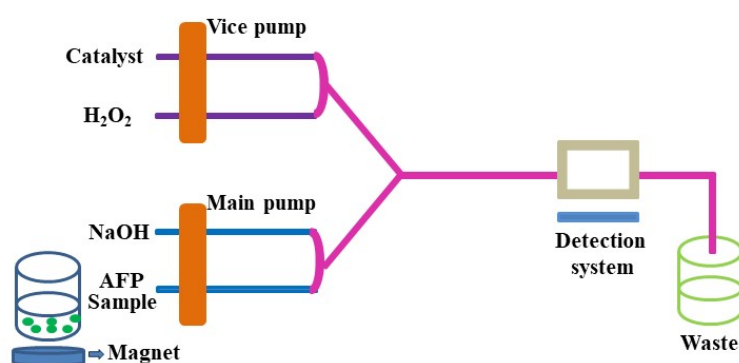
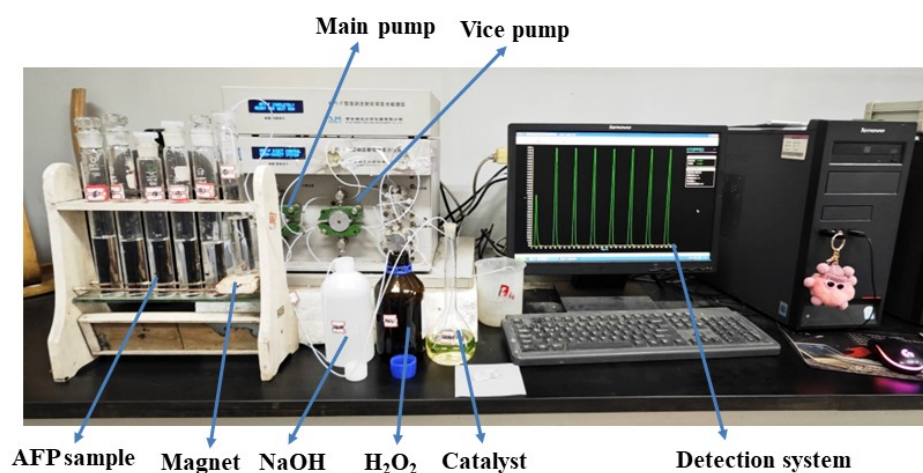**Fig. S1.** The diagram of the IFFM-E CL analyzer for AFP detction.

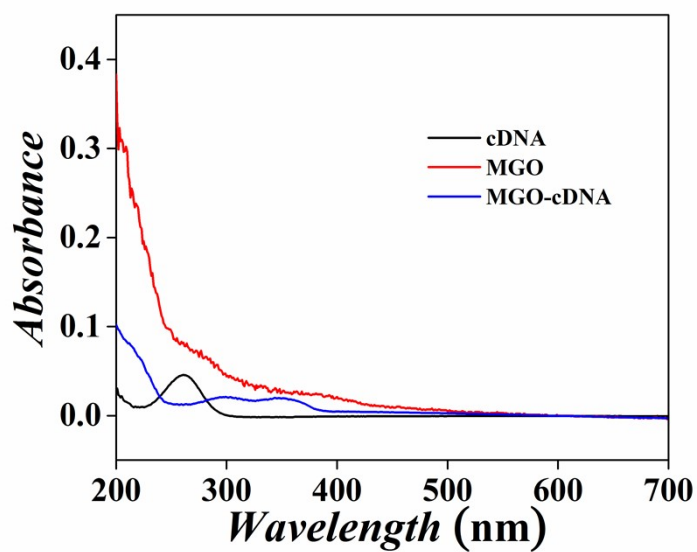**Fig. S2.** The sensor photo diagram for AFP detection.
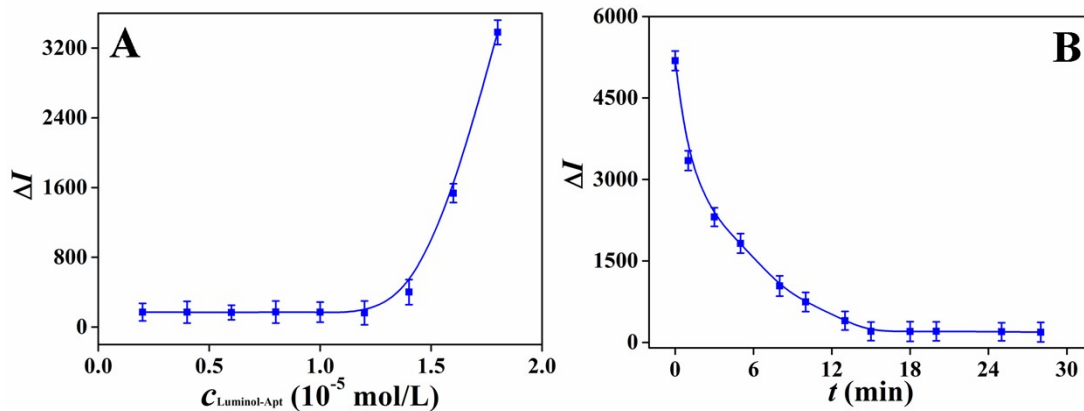

Fig. S3. The UV-vis curves of cDNA, MGO and MGO-cDNA.

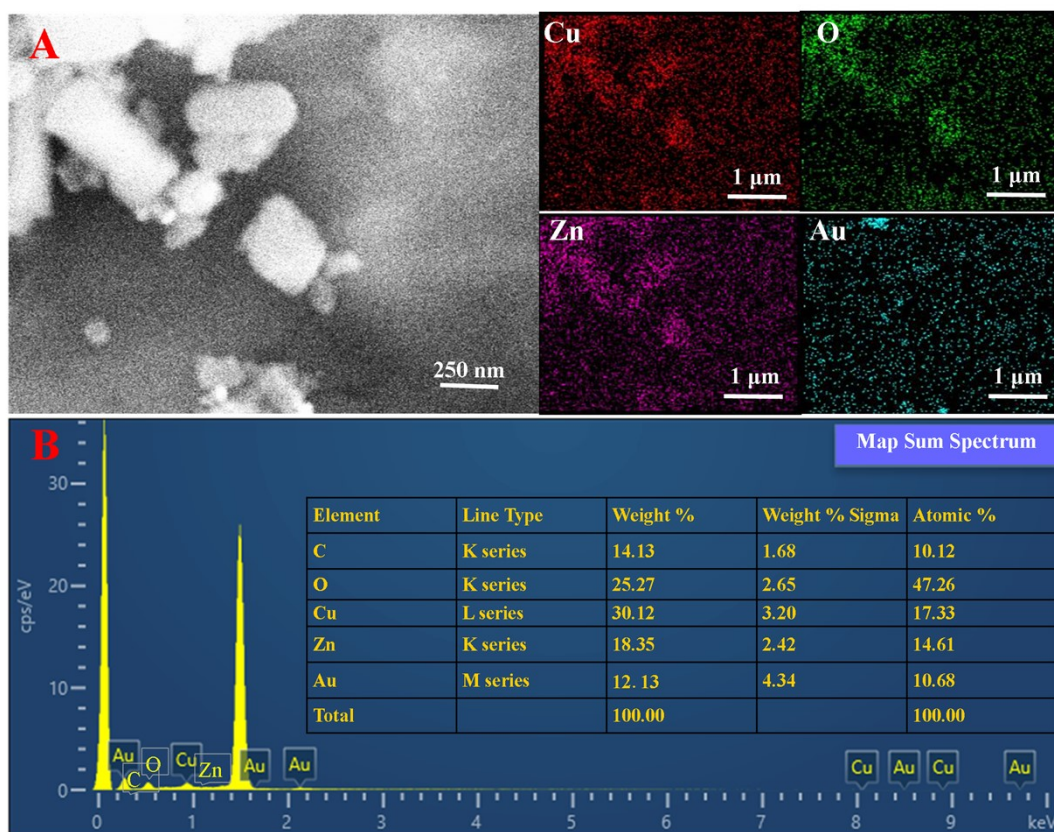**Fig. S4**. The saturated fixation amount (A) and equilibration time (B) of Apt-luminol on MGO-cDNA.

Element	Line Type	Weight %	Weight % Sigma	Atomic %
C	K series	14.13	1.68	10.12
O	K series	25.27	2.65	47.26
Cu	L series	30.12	3.20	17.33
Zn	K series	18.35	2.42	14.61
Au	M series	12. 13	4.34	10.68
Total		100.00		100.00

Fig. S5. The mapping and EDS spectrum of ZnONPs-Au-CuMOFs.
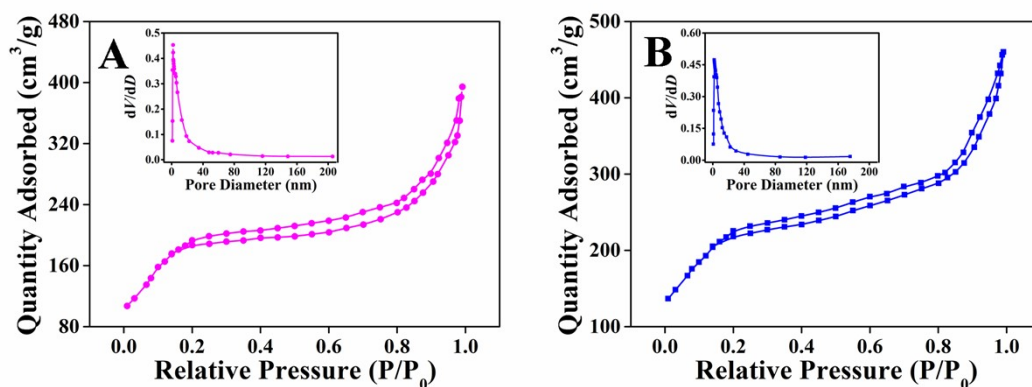
Fig. S6. The BET characterization of Au@CuMOFs (A) and ZnONPs-Au@CuMOFs (B), internal diagrams are pore size distribution diagram.
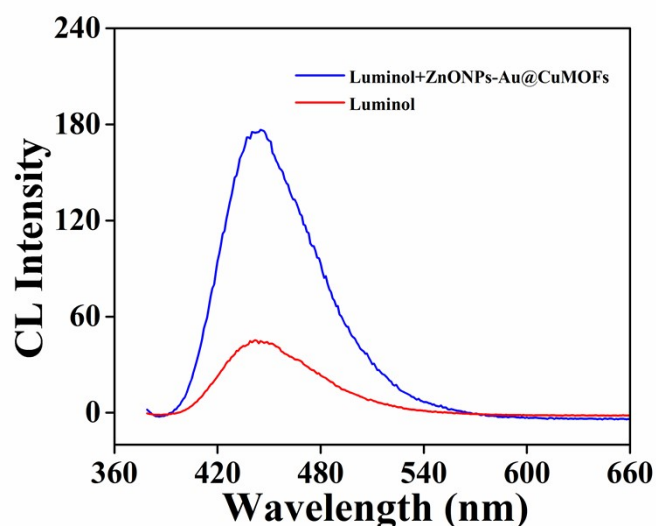

Fig. S7. The CL curves of luminol and luminol+ZnONPs-Au@CuMOFs, conditions: 1.0×10^{-6} mol/L luminol and 0.05 mg ZnONPs-Au@CuMOFs.

The Lineweaver-Burk formula

Michaelis-Menten constant (K_m) is known as an important parameter to evaluate the peroxidase activity. The Lineweaver-Burk formula is as followed: $1/v = (K_m/v_{max})(1/[S]) + 1/v_{max}$, where v is initial velocity, v_{max} is the maximum velocity, and $[S]$ is concentration of substrate.
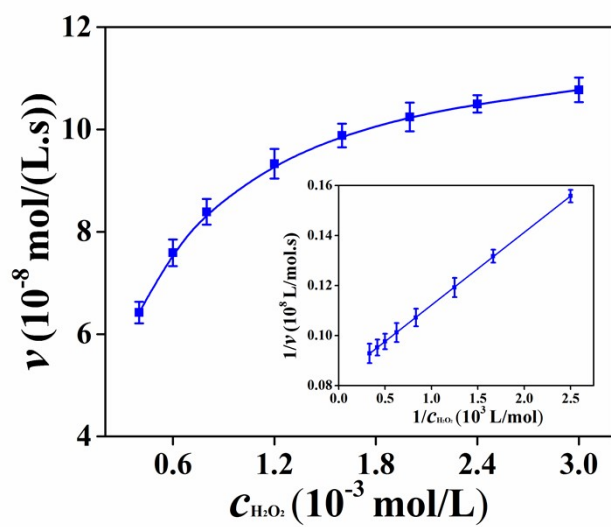
Fig. S8. The steady-state kinetic assay of Au@CuMOFs, error bars ($n = 5$).
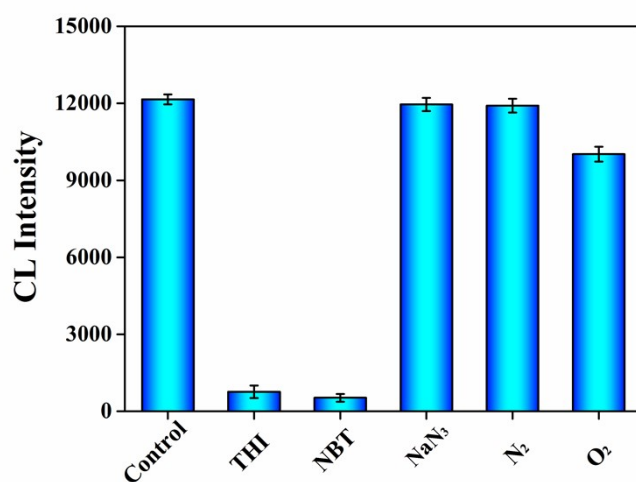
Fig. S9. The CL intensity of ZnONPs-Au@CuMOFs with 1.5×10^{-6} mol/L NaN$_3$, 2.5×10^{-7} mol/L THI and NBT, N$_2$-enriched and O$_2$-enriched for 30 min, respectively, error bars ($n = 5$).
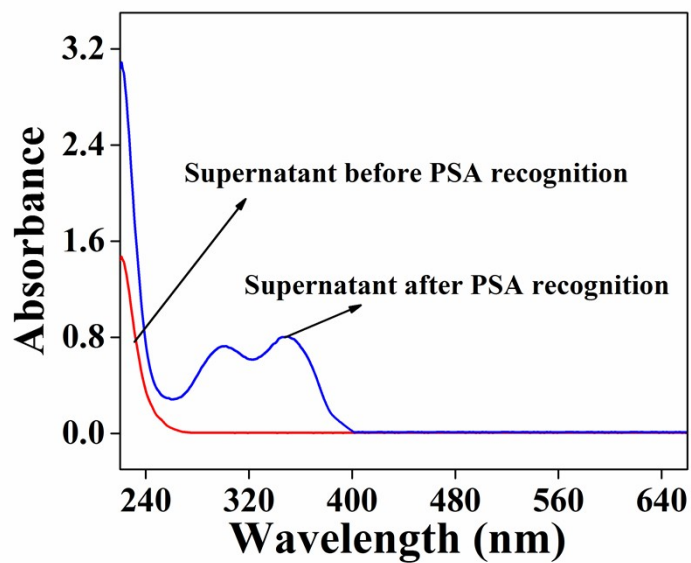
Fig. S10. The UV-vis curve of supernatant before and after PSA recognition
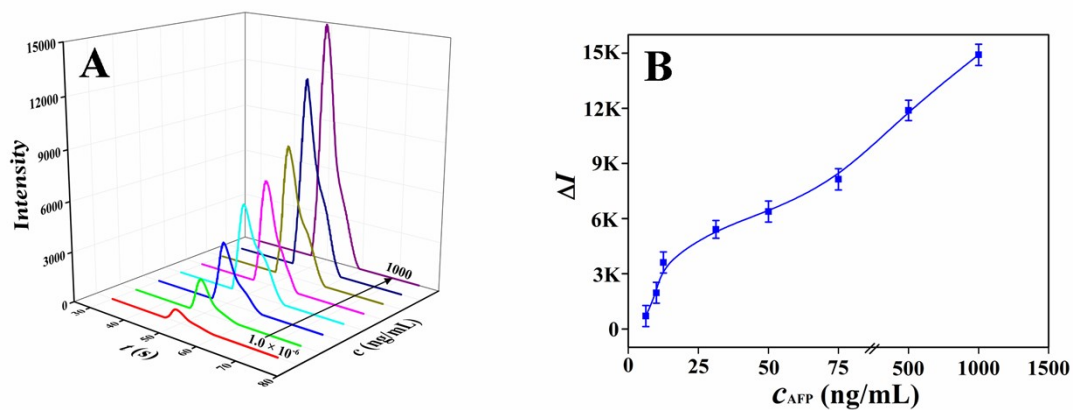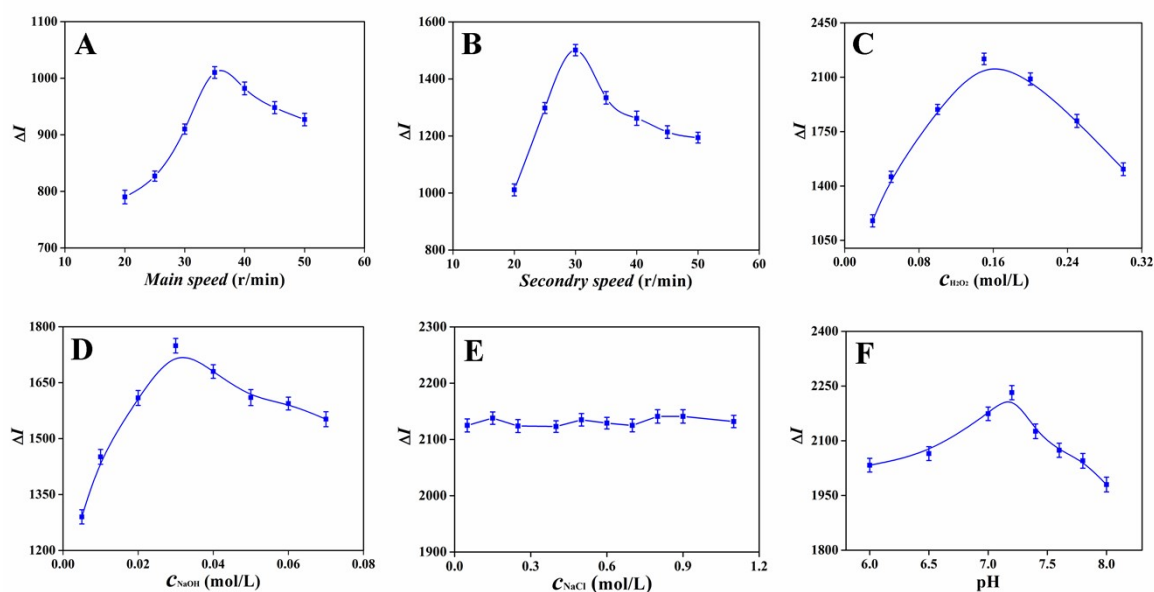

Fig. S11. The CL curves of AFP at different concentration (A) and the CL intensity of AFP at different concentration, error bars (n = 5) (B).

Fig. S12. Optimizations of main pump speed, conditions: 20 rmp of vice pump speed, 0.10 mol/L NaOH, 0.10 mol/L H_2O_2, 1.0×10^{-6} mol/L MGO-cDNA/Apt-luminol, 10 μg/mL ZnONPs-Au@CuMOFs, 1.0 ng/mL AFP (A); vice pump speed, conditions: 35 rmp of main pump speed, 0.10 mol/L NaOH, 0.10 mol/L H_2O_2, 1.0×10^{-6} mol/L MGO-cDNA/Apt-luminol, 10 μg/mL ZnONPs-Au@CuMOFs, 1.0 ng/mL AFP (B); the concentration of H_2O_2, conditions: 35 rmp of main pump speed, 30 rmp of vice pump speed, 0.10 mol/L NaOH, 1.0×10^{-6} mol/L MGO-cDNA/Apt-luminol, 10 μg/mL ZnONPs-Au@CuMOFs, 1.0 ng/mL AFP (C); the concentration of NaOH, conditions: 35 rmp of main pump speed, 30 rmp of vice pump speed, 0.15 mol/L H_2O_2, 1.0×10^{-6} mol/L MGO-cDNA/Apt-luminol, 10 μg/mL ZnONPs-Au@CuMOFs, 1.0 ng/mL AFP (D); ion strength, conditions: 35 rmp of main pump speed, 30 rmp of vice pump speed, 0.03 mol/L NaOH, 0.15 mol/L H_2O_2, 1.0×10^{-6} mol/L MGO-cDNA/Apt-luminol, 10 μg/mL ZnONPs-Au@CuMOFs, 1.0 ng/mL AFP (E); pH, conditions: conditions: 35 rmp of main pump speed, 30 rmp of vice pump speed, 0.03 mol/L NaOH, 0.15 mol/L H_2O_2, 1.0×10^{-6} mol/L MGO-cDNA/Apt-luminol, 10 μg/mL ZnONPs-Au@CuMOFs, 1.0 ng/mL AFP (F).