

A ratiometric photoelectrochemical immunosensor based on g-C₃N₄@TiO₂ NTs
amplified by signal antibodies-Co₃O₄ nanoparticles conjugates

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1. Preparation of carboxylated g-C₃N₄

The carboxylated g-C₃N₄ was prepared according to the method in ref. [S1]. Briefly, 5 g melamine were heated at 550 °C in air for 4 h to obtain g-C₃N₄ yellow powder. Then 0.5 g of bulk g-C₃N₄ powder were dispersed in 50 mL of ultrapure water and sonicated for 1 h in a round bottom flask. After adding of 50 mL concentrated HNO₃, the mixture were refluxed in slight boiling for 24 h. Cooled to room temperature, the supernatant was collected by centrifugation and washed with ultrapure water until pH neutral. Finally, the resulting product was dried by vacuum and ground carefully to obtain the carboxylated g-C₃N₄.

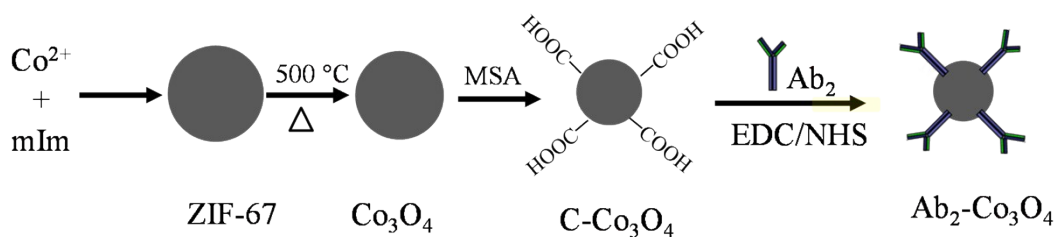
1. Preparation of TiO₂ NTs array

TiO₂ NTs were fabricated by the electrochemically anodizing Ti foil in the fluorine-containing electrolyte according to the method according to a previous paper [S2]. Briefly, Ti foils (2.0×1.0 cm²) were pretreated by ultrasonic cleaning in acetone, ethanol and ultra-pure water and then dried in nitrogen stream. Then the Ti foil was immersed in an electrolyte solution containing 98 mL ethylene glycol, 0.33 g NH₄F and 2 mL of ultra-pure water. The electrochemical anodizing was performed in a two-electrode cell at a constant 30 V anodic potential for 2 h at room temperature, with a graphite plate as the counter electrode. Finally, the Ti foils were directly heated to 550 C at a rate of 5 °C min⁻¹ and then kept at this temperature for another 3 h in a muffle furnace to prepare TiO₂ NTs photoanodes.

3 Preparation of Ab₂-Co₃O₄ conjugates

The preparation process of Ab₂-Co₃O₄ conjugates is shown in Scheme S1.

Scheme S1. Construction process of the Ab₂-Co₃O₄ NPs.



Firstly, ZIF-67 crystals were synthesized by a simple room-temperature precipitation reaction [S3]. In a typical synthesis, 4 mmol of Co(NO₃)₂ and 16 mmol of mIm were respectively dissolved in 100 mL of methanol. The methanol solution of Co(NO₃)₂ was slowly added to the methanol solution of mIm. The resultant mixture was stirred for 6 h and the solids were collected by centrifugation. Washed with methanol for several times to remove excess mIm present on the surface and pores, the ZIF-67 crystals were dried overnight in the ventilation oven. Secondly, a combustion boat loaded the ZIF-67 powders was directly placed in a tube furnace. The temperature inside the furnace was gradually increased from room temperature to 500 °C with a heating rate of 2 °C min⁻¹. After annealed at 500 °C for 3 h under air, the porous Co₃O₄ powder were formed by the decomposition and oxidation of ZIF-67. Thirdly, the 50 mg of Co₃O₄ powder were ground carefully in an agate mortar and dispersed in 20 mL 0.1% MSA aqueous solution. After ultrasonication for 20 min, the carboxylated Co₃O₄ NPs were acquired by centrifugation and washed several times with ultrapure water. Finally, the carboxylated Co₃O₄ NPs were re-dispersed in 5 mL phosphate buffer and stored at 4 °C for use.

The synthetic procedure of Ab₂-Co₃O₄ conjugates were prepared according to the method in ref. [S4] with slight modification. Firstly, 200 μL of newly prepared EDC (10 mg mL⁻¹) and NHS (10 mg mL⁻¹) solutions were mildly mingled with 1 mL of carboxylated Co₃O₄ NPs

suspension (0.2 mg mL^{-1}) for 30 min at room temperature. The supernatant was removed by centrifugation. Then 1 mL of Ab_2 solution ($200 \text{ } \mu\text{g mL}^{-1}$) was added and incubated for 12 h under shaking at 4°C . After washing with phosphate buffer solution (PBS, pH 7.4, $0.081 \text{ M Na}_2\text{HPO}_4 + 0.019 \text{ M NaH}_2\text{PO}_4 + 0.1 \text{ M NaCl}$) several times, the resultant $\text{Ab}_2\text{-Co}_3\text{O}_4$ NPs conjugates were acquired by centrifugation, re-dispersed to 1 mL PBS and stored at 4°C for the further use.

4 Construction of PEC Immunosensor

Firstly, $30 \text{ } \mu\text{L}$ of newly prepared EDC (10 mg mL^{-1}) and NHS (10 mg mL^{-1}) solutions was scattered onto the electrode to activate the carboxyl groups on $\text{C-g-C}_3\text{N}_4@\text{TiO}_2$ NTs for 30 min at room temperature. The electrode was covered with $30 \text{ } \mu\text{L}$ 2% (v/v) ethylenediamine (En) for 30 min to form $\text{En-g-C}_3\text{N}_4@\text{TiO}_2$ NTs. Then, $\text{En-g-C}_3\text{N}_4@\text{TiO}_2$ NTs. was rinsed with water and dried with N_2 . Subsequently, $30 \text{ } \mu\text{L}$ of newly prepared EDC (10 mg mL^{-1}) and NHS (10 mg mL^{-1}) solutions was scattered onto the electrode again to activate the amino groups on $\text{En-g-C}_3\text{N}_4@\text{TiO}_2$ NTs for 30 min at room temperature. Afterward, $30 \text{ } \mu\text{L}$ of $100 \text{ } \mu\text{g mL}^{-1}$ Ab_1 and allowed to incubate at 4°C for at least 12 h. After being rinsed with the washing buffer solution (PBS, pH 7.4, 10 mM), the electrode was incubated with $20 \text{ } \mu\text{L}$ of 10 mM PBS (pH 7.4) containing 1% (w/v) BSA at 37°C for 30 min to block nonspecific binding sites and then rinsed with the washing buffer solution thoroughly. The sensing photoelectrode of $\text{Ab}_1\text{-En-g-C}_3\text{N}_4@\text{TiO}_2$ NTs was obtained. For the purpose of concision, the term of $\text{Ab}_1\text{-En-g-C}_3\text{N}_4@\text{TiO}_2$ NTs is simplified as $\text{Ab}_1\text{-g-C}_3\text{N}_4@\text{TiO}_2$ NTs. Next, $30 \text{ } \mu\text{L}$ of different concentrations of target Ag was dropped on the BSA blocked electrode for an incubation of 1 h at 37°C followed by washing with washing buffer solution. After specific

immunoreaction between Ab_1 and Ag, the electrode was allowed for labeling by additional incubation with 20 μL of $Ab_2\text{-Co}_3\text{O}_4$ conjugates solution for 1 h at 37 $^\circ\text{C}$. Eventually, the resulting electrode was washed carefully with washing buffer solution and introduced into the photocurrent test. Similarly, the reference photoelectrode of $\text{BSA-g-C}_3\text{N}_4@\text{TiO}_2$ NTs was prepared by immobilizing BSA using EDC/NHS reaction.

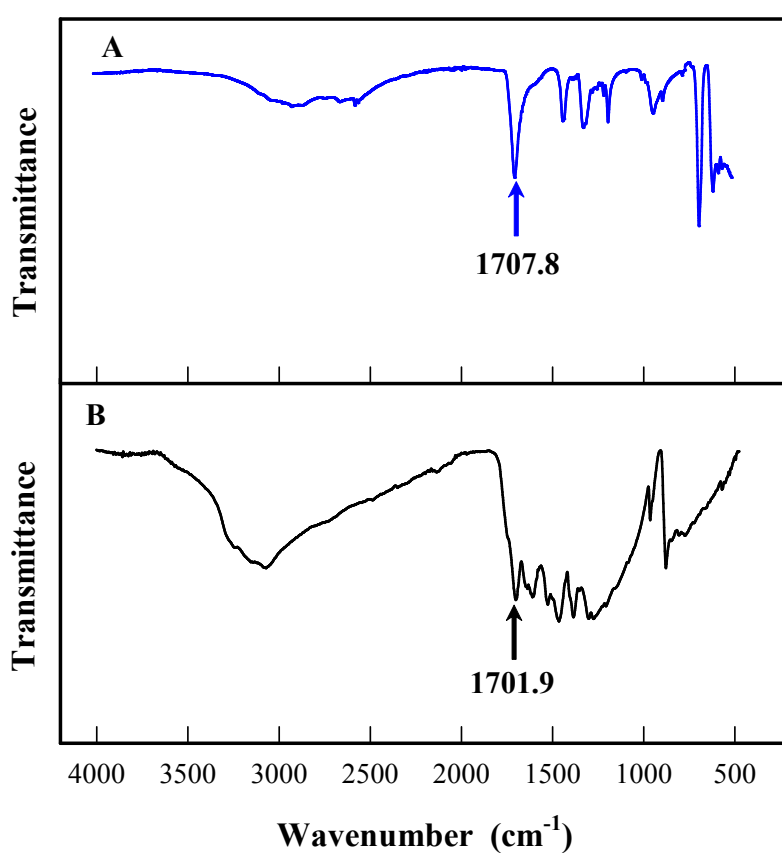


Fig. S1 FTIR of carboxylated Co_3O_4 NPs (A) and $\text{g-C}_3\text{N}_4$ (B).

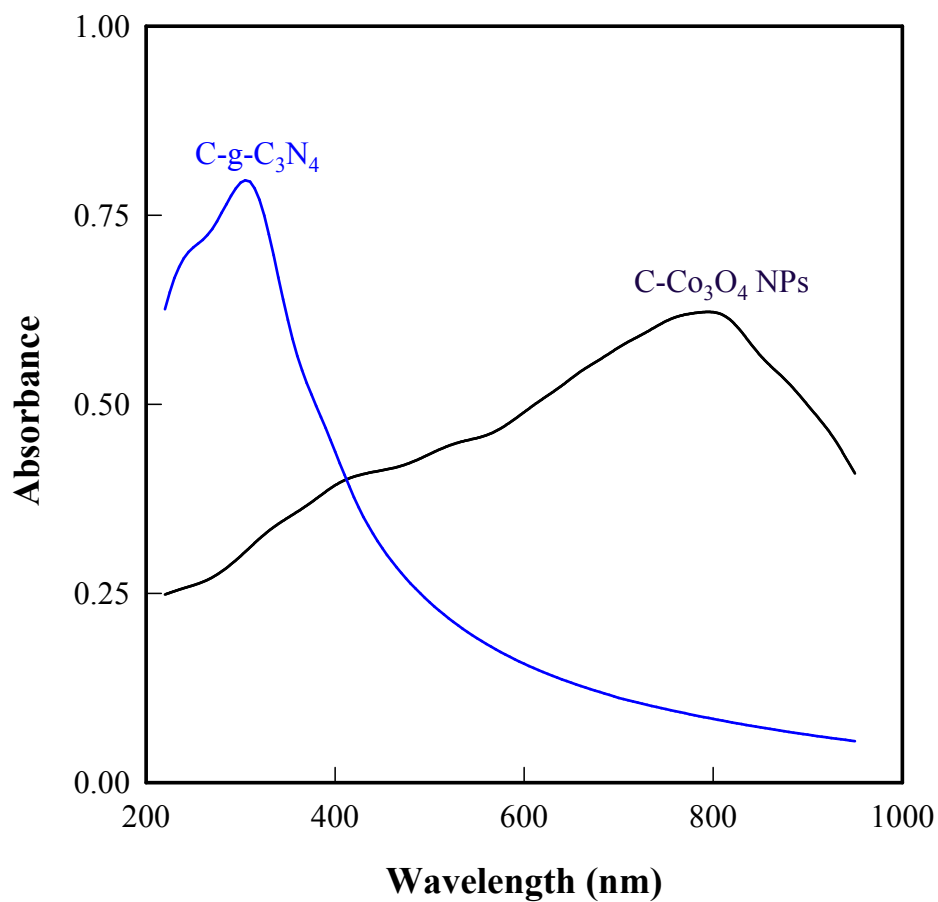


Fig. S2 UV-vis absorbance spectra of carboxylated g-C₃N₄ and Co₃O₄ NPs.

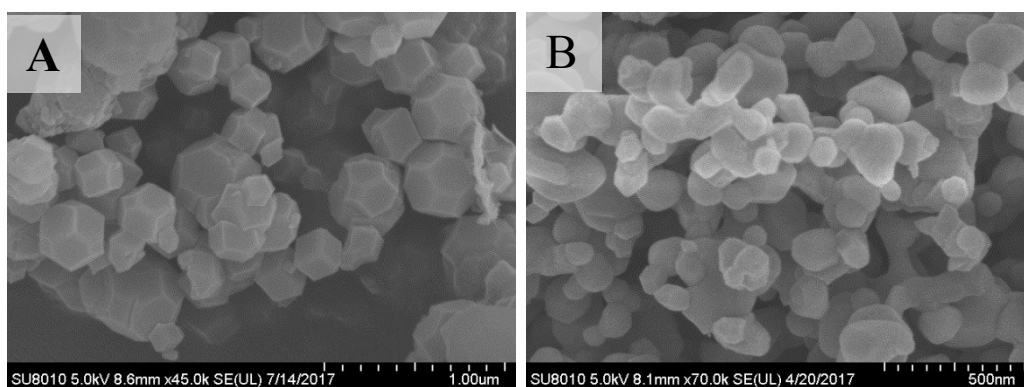


Fig. S3 SEM images of ZIF-67 (A) and Co₃O₄ (B) powders.

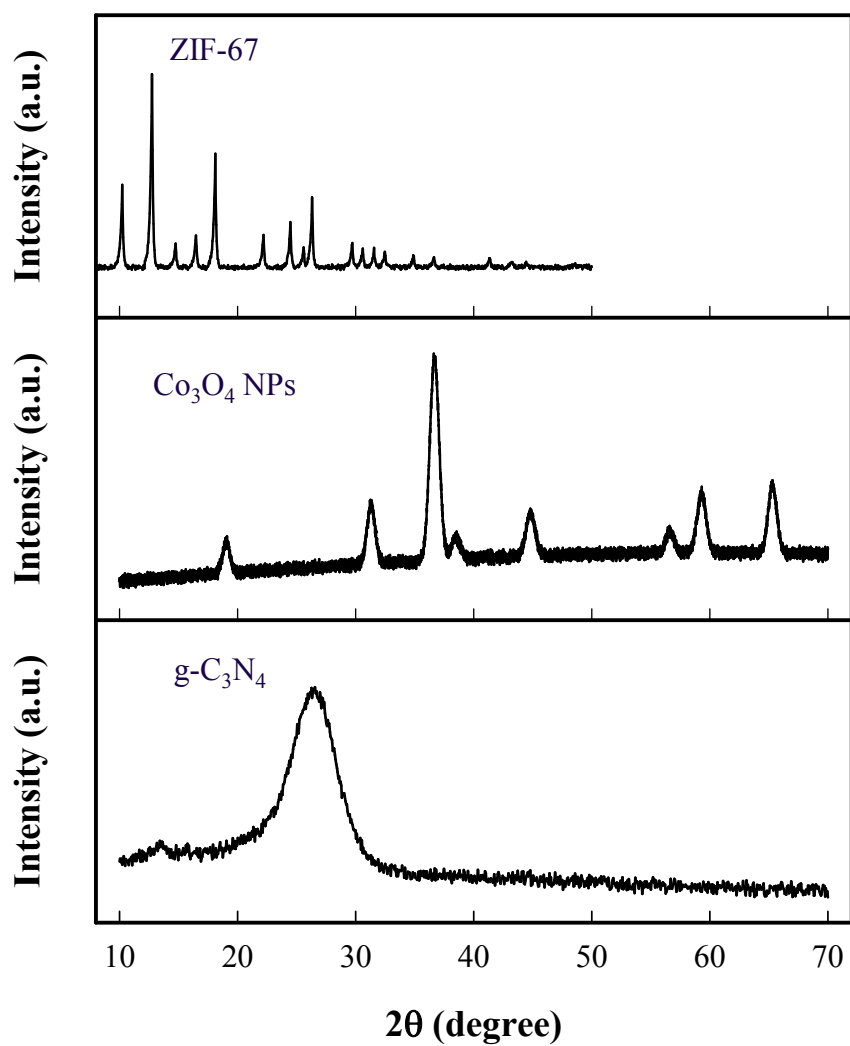


Fig. S4 X-ray powder diffraction (XRD) patterns of ZIF-67, Co_3O_4 NPs and carboxylated $\text{g-C}_3\text{N}_4$.

Table S1. Comparison of analytical performance of some immunoanalysis methods for AFP detection

Immunoprobe *	Methods **	Linear range (ng mL ⁻¹)	LoD (ng mL ⁻¹)	Refs.
Au NPs-Ab ₂ -AFP-Ab ₁	ICP-MS	0.005–2.0	0.002	S5
MnFe ₂ O ₄ -Ab ₁ -AFP-Ab ₁ - MnFe ₂ O ₄	Paramagnetic	1–500	0.3	S6
AFP-Ab ₁ -SiNB	FETs	3–100	2	S7
	CEC-IA-LIF	0.1–1×10 ³	0.05	S8
CuO-Ab ₂ -AFP-Ab ₁	Fluorescence	1–80	0.45	S9
CuO-Ab ₂ -AFP-Ab ₁	Fluorescence	0.025–5	0.012	S10
Hemin-Au@MOF-Ab ₂ -AFP-Ab ₁	Colorimetric	0.08–43	0.02	S11
Ab ₁ -AFP-Ab ₂ -PbS NPs	RLS	3×10 ⁻⁴ – 1	1×10 ⁻⁴	S4
PAADs@CNDs@Ab ₂ -AFP-Ab ₁ -GO@C ₆₀	ECL	1×10 ⁻⁶ –80	3.3×10 ⁻⁷	S12
AFP-Ab ₁ -AuNPs/Ru(bpy) ₃ ²⁺	ECL	0.05–50	0.04	S13
AFP-Ab ₁ -HAP@GO	EC	0.01–10	0.005	S14
Pt@CuO-MWCNTs/Ab ₂ -AFP -Ab ₁ -CD-GS	EC	0.001- 20	3.3×10 ⁻⁴	S15
Co ₃ O ₄ @MnO ₂ -thionine	EC	0.001–100	3.3×10 ⁻⁴	S16
AFP-Ab ₁ -Au-ZnO	PEC	0.005–50	5.6×10 ⁻⁴	S17
HRP-Ab ₂ -AFP- Ab ₁ -CdS/WS ₂	PEC	0.001 –20	4.3×10 ⁻⁴	S18
Co ₃ O ₄ -Ab ₂ -AFPC-g-C ₃ N ₄ @TiO ₂	PEC	4×10 ⁻⁴ –40	2×10 ⁻⁴	This work

*Au@MPTES-GS:3-mercaptopropyltriethoxysilane functionalized graphene sheets, CD-GS: β-cyclodextrin functionalized graphene, GO: graphene oxide, HRP:horseradish peroxidase, PAADs@CNDs poly(amidoamine) dendrimers functionalized carbon nanodots, SiNB: silicon nanobelt.

** CEC: capillary electrochromatography, EC: electrochemistry, ECL, electrochemiluminescence, FETs: field-effect transistors, IA: non-competitive immunoassay, ICP-MS: inductively coupled plasma-mass spectrometry, LIF: laser-induced fluorescence.

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