

Supplementary Information

A label-free ratiometric fluorescence nanoprobe for ascorbic acid based on redox-modulated dual-emission signals

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Experiment section

Materials and instruments

The polymer poly(9,9-dioctylfluorenyl-2,7-diyl) (PFO, $M_w=10000\sim 100000$ by GPC) was purchased from Xi'an Polymer Light Technology Corp. Amphiphilic functional polymer poly(styrene-co-maleic anhydride) (PSMA, terminated by cumene, content of 68% styrene, average molecular weight about 1700), L-glutathione-reduced (GSH), gold (III) chloride trihydrate ($\text{HAuCl}_4\cdot 3\text{H}_2\text{O}$) and 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Tetrahydrofuran (THF, anhydrous, 99.9%) and sodium hypochlorite pentahydrate ($\text{NaClO}\cdot 5\text{H}_2\text{O}$) were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Ascorbic acid (AA), metal salt compounds, amino acid, bovine serum albumin (BSA), trypsin, and glucose were purchased from Aladdin (Shanghai, China). All of the chemicals were of analytical grade without further treatment. The glassware used in the experiments were all rinsed by aqua regia ($\text{HCl}/\text{HNO}_3 = 3:1$, v/v). Ultrapure water was obtained through

Merck Millipore water purification system with an electric resistance 18.25 M Ω . Human serum was provided by Zi Jing Hospital (Wuhan, Hubei).

The fluorescence measurements were performed by RF-5301 PC spectrometer (Shimadzu, Japan). The UV-vis absorption spectra were measured on a UV-2550 UV-vis spectrometer (Shimadzu, Japan). TEM images were obtained on JEM-2100 transmission electron microscope with an acceleration voltage of 200 kV (Hitachi, Japan). Zetasizer Nano (Malvern) was used to measure zeta potential of the nanomaterials. FT-IR spectrum was conducted on a Magan-IR Spectrometer 500 (Nicolet, Madison, WI, USA) with the KBr pellet technique. X-ray photoelectron spectroscopy (XPS) spectra were obtained from a Kratos Axis Ultra LD spectrometer using a monochromatic Al K α source (PerkinElmer, USA). The time-resolved photoluminescence were measured by a FLS980 Lifetime Spectrometer (Edinburgh, England).

Synthesis of poly(9,9-dioctyluorenyl-2,7-diyl) dots (PFO dots)

The PFO dots were synthesized according to the literature.¹ Briefly, the polymer PFO and amphiphilic copolymer PSMA were dissolved in tetrahydrofuran (THF) to reach a stock solution of 1 mg/mL, respectively. 250 μ L of PFO and 100 μ L of PSMA were mixed and diluted with 4.65 mL THF to produce a solution mixture which contains 50 μ g/mL PFO and 20 μ g/mL PSMA. The mixture was sonicated to reach a homogeneous solution. Subsequently, 5 mL of mixture solution was added into 10 mL of ultrapure water quickly in a bath sonicator. THF was removed by nitrogen stripping overnight, followed by filtration through a 0.22 μ m filter to remove the fraction of aggregates. The resulting PFO dots dispersions were clear and stable for several months without signs of aggregation.

Preparation of CoOOH nanoflakes

The fabrication of CoOOH nanoflakes was performed according to the reported study with minor revision.² Typically, NaClO (0.2 M, 10 mL) and NaOH (0.8 M, 10 mL) were added into CoCl₂ solution (10 mM, 20 mL) at room temperature, and then

the mixture was sonicated for 10 min. After that, the CoOOH nanoflakes were collected from the suspension by centrifugation at 8000 rpm for 10 min and the products were washed several times with ultrapure water to remove the unreacted substances. The as-prepared CoOOH nanoflakes were redissolved with ultrapure water and further treated by sonication to obtain homogeneous CoOOH nanoflakes solution.

Preparation and purification of GSH-AuNCs

GSH-AuNCs were synthesized by chemical reduction of HAuCl_4 with glutathione.³ A solution of glutathione (5 mL, 15 mM) was added to an equal volume of 10 mM HAuCl_4 . The mixture was vigorously stirred till the solution slowly turn to the faint yellow, followed by the addition of 1 mL of 1 M NaOH. After the solution reaching the colorless with the stirring, the solution was incubated at 37 °C under continuous stirring for 24 h. Finally, the solution was extensively dialyzed against ultrapure water using a dialysis tube (MWCO = 3500 Da) overnight.

Figures

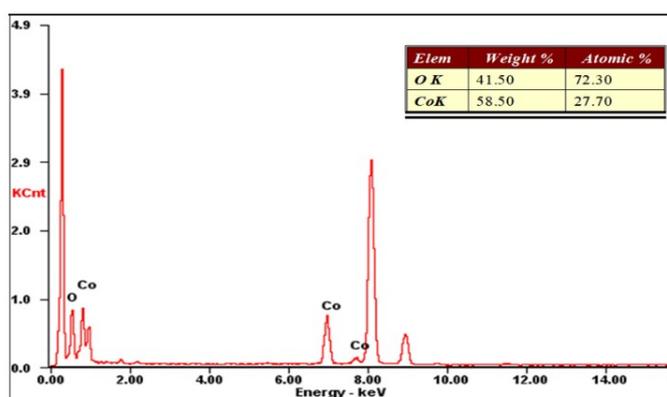


Figure S1. The EDS spectrum of the CoOOH nanoflakes.

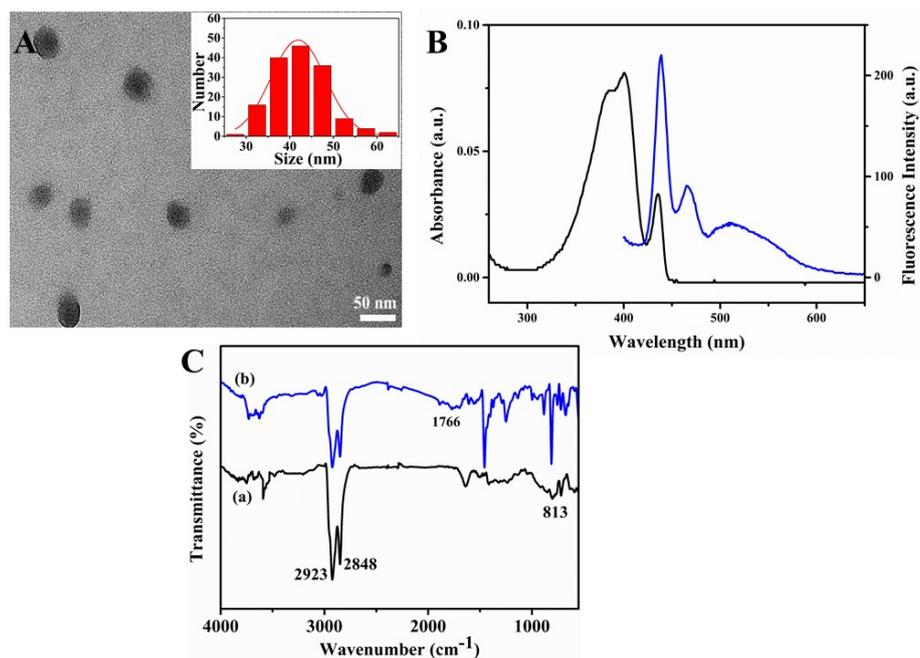


Figure S2. (A) TEM image of PFO dots, the inset image is the size distributions. (B) UV-vis absorption spectrum (black line) and fluorescence emission spectrum (blue line) of PFO dots. (C) FT-IR spectra of (a) PFO monomer and (b) PFO dots.

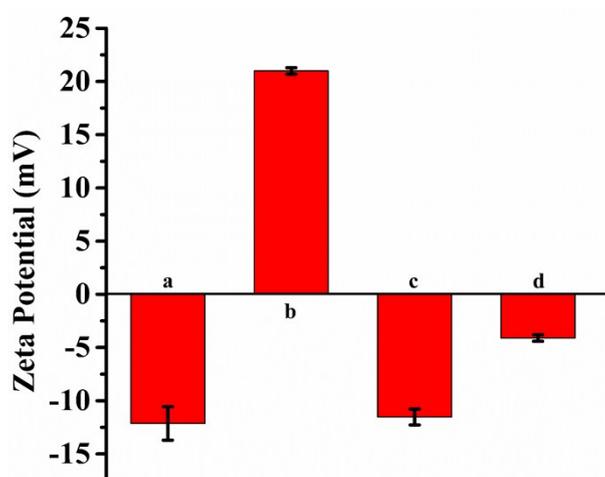


Figure S3. Zeta Potential of (a) PFO dots, (b) CoOOH, (c) GSH-AuNCs, (d) PFO dots/CoOOH/GSH-AuNCs nanoprobe.

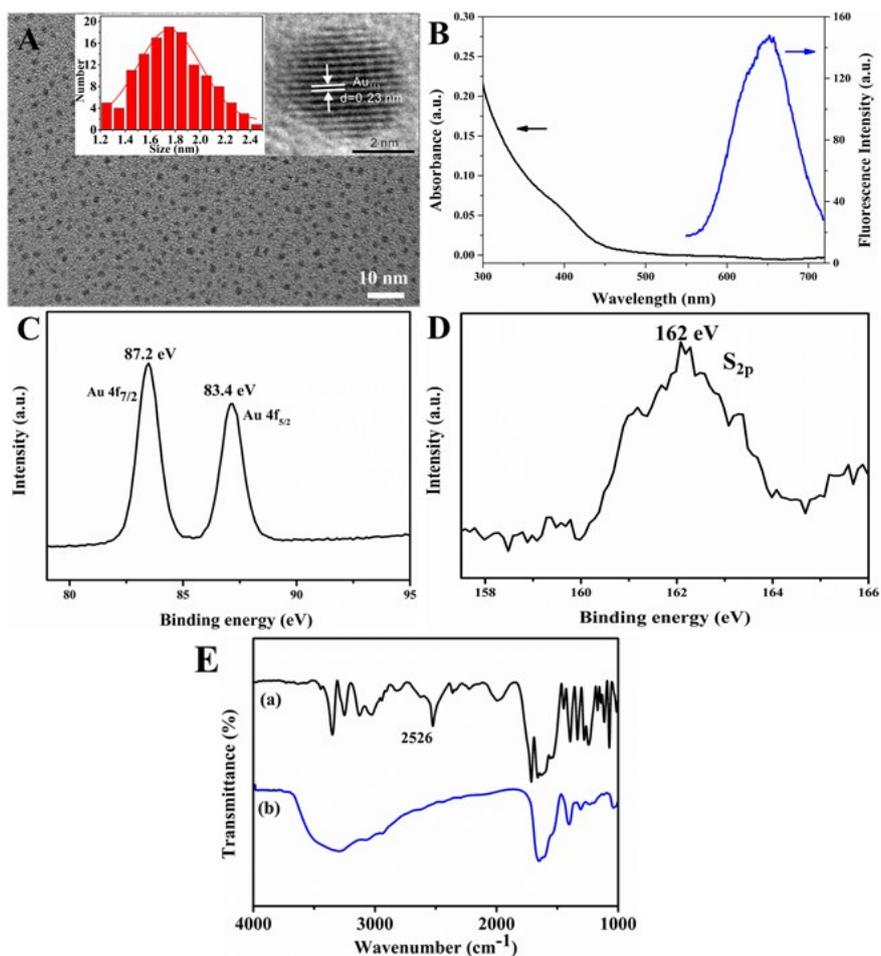


Figure S4. (A) TEM image of GSH-AuNCs. Inset image: the size distributions and the lattice fringes of GSH-AuNCs (HRTEM). (B) The UV-vis absorption (black line) and fluorescence emission (blue line) spectra of GSH-AuNCs. (C-D) The XPS spectra of Au 4f (C) and S 2p (D) for GSH-AuNCs. (E) FT-IR spectra of (a) GSH and (b) GSH-AuNCs.

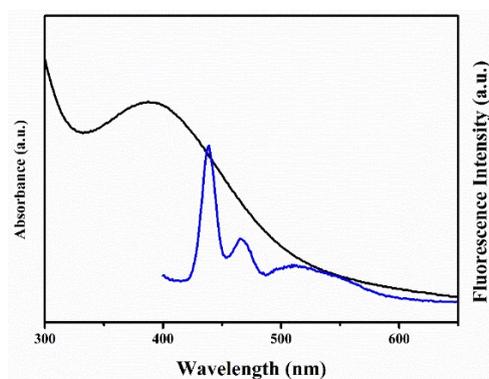


Figure S5. UV-vis absorption spectrum of CoOOH nanoflakes (black line) and fluorescence spectrum of PFO dots (blue line).

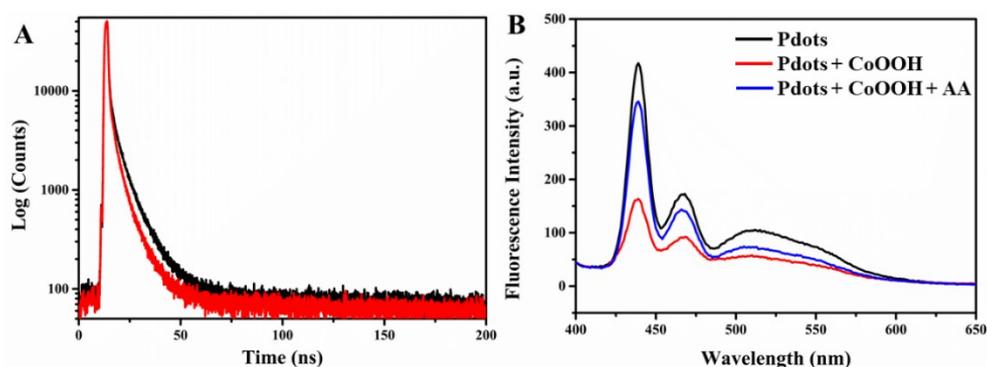


Figure S6. (A) Time-resolved fluorescence spectra of PFO dots in the absence (black line) and presence (red line) of CoOOH nanoflakes. Excitation wavelength was 380 nm. (B) The fluorescence spectra of PFO dots (black line), PFO dots incubated with 20 $\mu\text{g}/\text{mL}$ CoOOH (red line) and PFO dots incubated with CoOOH following the addition of 100 μM AA (blue line).

Table S1. Decay Parameters for PFO dots without and with CoOOH nanoflakes

Names	τ_1/ns	τ_2/ns	τ_3/ns
PFO dots	0.39	2.98	9.36
	17.32%	36.57%	46.10%
PFO dots + CoOOH	0.39	2.71	8.54
	22.75%	42.77%	34.48%

All data were fitted by a cubic exponential decay function.

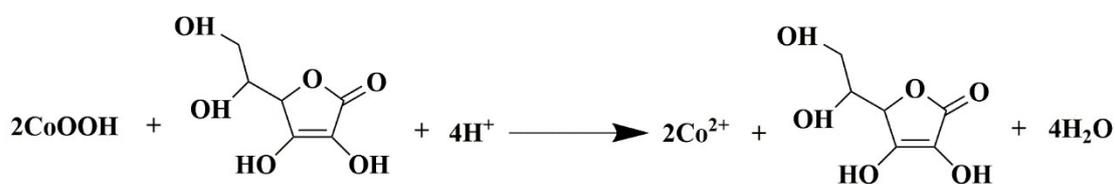


Figure S7. The redox reaction between CoOOH and AA.

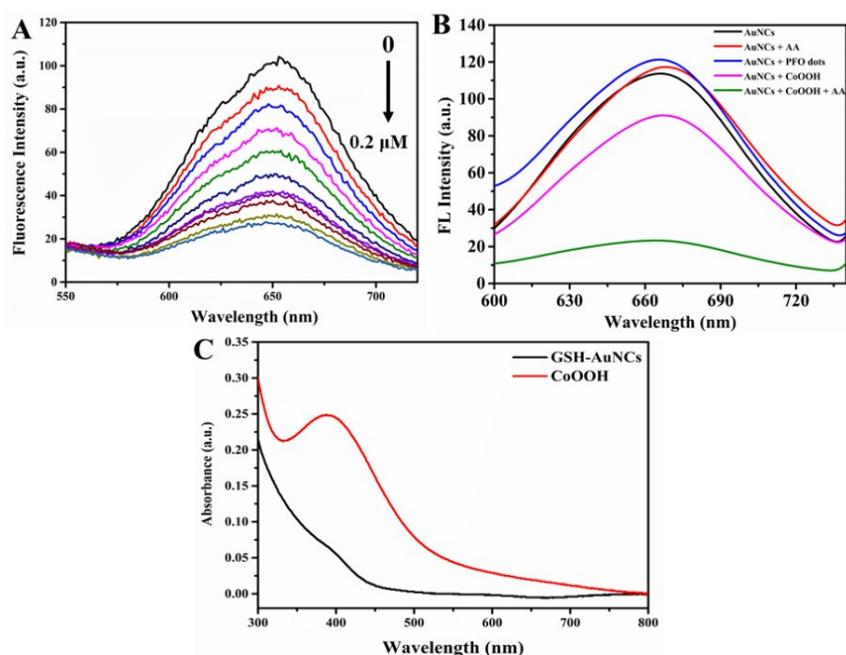


Figure S8. (A) The fluorescence spectra of AuNCs with different concentration of Co^{2+} (0-0.2 μM). (B) The fluorescence spectra of AuNCs (black line), AuNCs + AA (red line), AuNCs + PFO dots (blue line), AuNCs + CoOOH (pink line) and AuNCs + CoOOH + AA (green line). (C) UV-vis absorption spectra of AuNCs (black line) and CoOOH nanoflakes (red line).

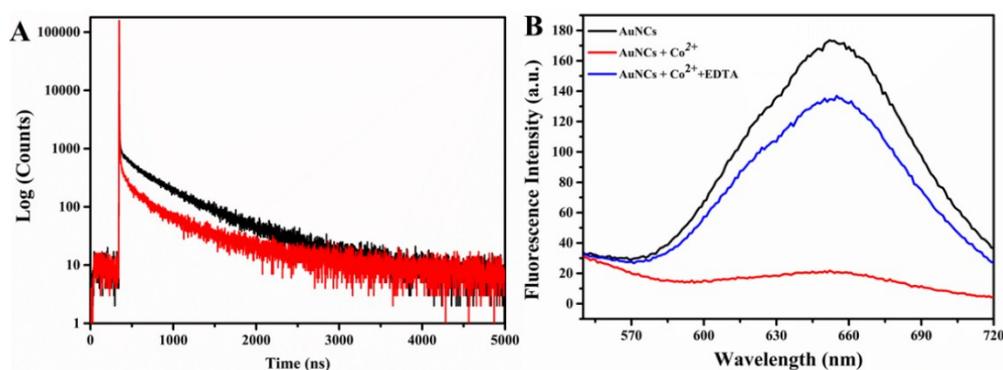


Figure S9. (A) Time-resolved fluorescence spectra of the AuNCs in the absence (black line) and presence (red line) of Co^{2+} . Excitation wavelength was 380 nm. (B) Emission spectra of the AuNCs in the absence (black line) and presence (red line) of 0.2 μM Co^{2+} , and in the presence of 0.2 μM Co^{2+} with 0.2 μM EDTA (blue line).

Table S2. Decay Parameters for AuNCs without and with Co²⁺

Names	τ_1 /ns	τ_2 /ns
AuNCs	49.91	587.68
	7.83%	92.17%
AuNCs + Co ²⁺	41.45	546.56
	17.79%	82.21%

All data were fitted by a bi-exponential decay function.

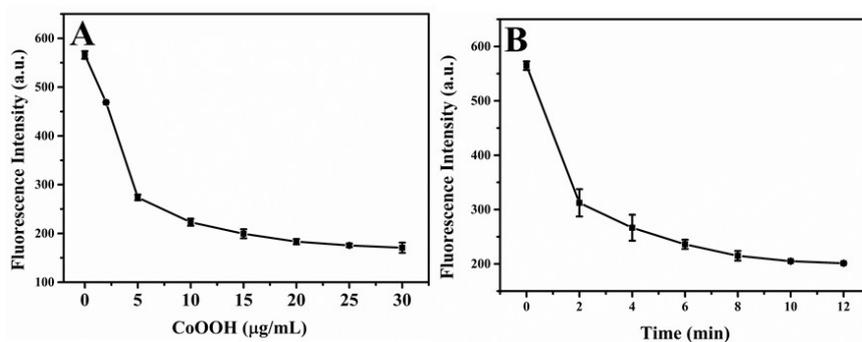


Figure S10. (A) Fluorescence quenching effect of PFO dots by varying amounts (0-30 µg/mL) of CoOOH nanoflakes. (B) Fluorescence intensity of PFO dots in response to different incubating time with CoOOH nanoflakes.

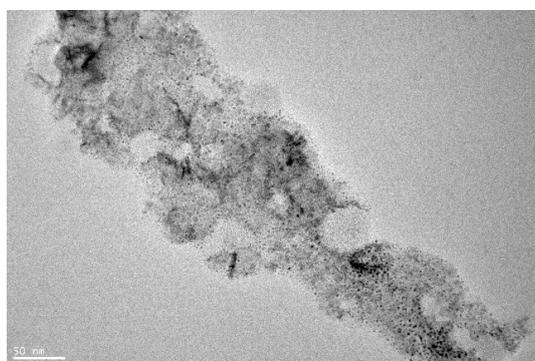


Figure S11. TEM image of the as-constructed nanoprobe.

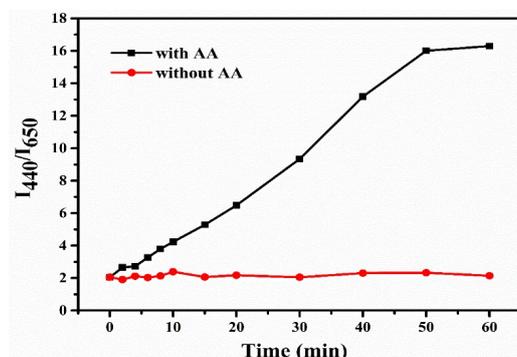


Figure S12. Fluorescence response of the nanoprobe in the absence (red line) and presence (black line) of 100 µM AA as a function of time.

Table S3. Comparison with other reported methods for AA detection

Method	Probe	Linear range (μM)	Detection limit (μM)	References
Amperometry	RGO-ZnO ^a	50-2350	3.71	4
Amperometry	CTAB/rGO/ZnS ^b	50-1000	30	5
Voltammetry	PCN-333 (Al) MOFs ^c	14.1-5500	4.60	6
Voltammetry	CL-TiN ^d	50-1500	1.52	7
DPV	Pd@Au/RGO ^e	50-2856.6	2.88	8
Fluorescence	Hydroxycoumarin/MnO ₂	10-50	1.6	9
Fluorescence	Fe ³⁺ /Fe ²⁺ -CdTe QDs@SiO ₂	3.33-400	1.25	10
Fluorescence	PFO dots/CoOOH/AuNCs	10-300	1.9	This work

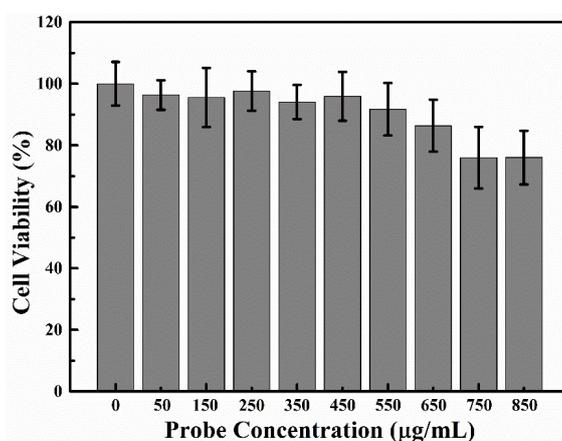
a RGO-ZnO-reduced graphene oxide-zinc oxide

b CTAB-hexadecyl trimethyl ammonium bromide, ZnS-zinc sulfide nanocomposite

c Porous coordination network, Metal-organic frameworks

d Chrysanthemum-like titanium nitride

e Pd@Au-Pd-Au core-shell, RGO-reduced graphene oxide, GCE-glassy carbon electrode.

**Figure S13.** Viability of Hela cells treated with PFO dots/CoOOH/AuNCs nanoprobe.

References

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