Supplementary Information

Single-atom copper-activated carbon dots@silica nanozyme with neutral-pH peroxidase-like activity and room-temperature phosphorescence for dual-mode glyphosate detection

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Materials and instruments

Ethylenediamine (EDA), phosphoric acid (H_3PO_4), 3-aminopropyltrimethoxysilane (APTMS), and 3,3',5,5'-tetramethylbenzidine (TMB) were purchased from Aladdin Reagents Co., Ltd. (Shanghai, China). Tetraethyl orthosilicate (TEOS), sodium carbonate (Na_2CO_3), hydrogen peroxide (30% H_2O_2), ammonia ($NH_3 \cdot H_2O$), sodium acetate (CH_3COONa), copper chloride dihydrate ($CuCl_2 \cdot 2H_2O$) were purchased from Sinopharm Group Chemical Reagent Co., Ltd. (Shanghai, China). Glyphosate, phoxim, malathion, glufosinate, and acetamiprid were purchased from McLean Biochemical Technology Co., Ltd. (Shanghai, China). Dursban and parathion were purchased from Yuanye Biotechnology Co., Ltd. (Shanghai, China). Sodium chloride (NaCl), magnesium chloride ($MgCl_2$), potassium chloride (KCl), calcium chloride ($CaCl_2$), zinc chloride ($ZnCl_2$), manganese chloride ($MnCl_2$), sodium sulfate (Na_2SO_4), sodium phosphate (Na_3PO4), sodium carbonate (Na_2CO_3) and sodium nitrate ($NaNO_3$) were purchased from Damao Technology Group Co., Ltd. (Tianjin, China).

CDs were synthesized by Midea M1-201B microwave oven (Midea, China), RTP measurements were performed by F-7000 fluorescence spectrometer (Hitachi, Japan), and UV-visible (UV-vis) absorption spectra were measured on U-3900 UV-vis spectrophotometer (Hitachi, Japan). Fourier transform infrared spectra were collected by a Nicolet-6700 Fourier transform infrared spectrometer (Thermo Fisher, USA). The morphology of CDs@SiO_2@Cu was characterized by a JEM-2011 transmission electron microscope (JEOL, Japan) and a JEM-ARM200F ACCELARM atomic resolution analytical electron microscope (JEOL, Japan). X-ray diffraction patterns were collected from an X-ray diffractometer (PANalytical, Netherlands). Reactive oxygen species (ROS) measurements were carried out on an electron spin resonance (ESR) 5000 spectrometer (Bruker, Germany).

Synthesis of CDs@SiO_2@Cu

CDs and CDs@SiO_2 were synthesized according to previously reported literature with minor modifications.[1]

Synthesis of CDs: 1.2 mL of EDA and 2 mL of H_3PO_4 were added to 14.8 mL of deionized water and mixed thoroughly. The mixture was then transferred to a microwave oven and heated at 750 W for 130 s to obtain the dark brown crude product. Then centrifuged to remove the large particles and dialyzed (MWCO: 500 Da) for 7 days. After freeze-drying, a pale yellow solid CDs powder was obtained.

Synthesis of CDs@SiO_2: 37.5 mg of CDs and 4.5 mL of TEOS were added to 9 mL of water and mixed thoroughly. Then 0.1 mL $NH_3 \cdot H_2O$ was added dropwise while stirring, and the reaction was carried out at room temperature for 72 h. After that, centrifuged at 12,000 rpm for 15 min to remove large particles and filtered through a 0.22 μm filter to obtain a clear CDs@SiO_2 solution.

Synthesis of CDs@SiO_2@Cu: 2 mL above CDs@SiO_2 solution and 0.035 mL of APTMS were added to 7.495 mL of deionized water, then ultrasonically mixed and reacted at room temperature for 6 h while stirring, Next, introduced 0.5 mL 20 mg mL^{-1} $CuCl_2 \cdot 2H_2O$ solution and continue stirring at room temperature for another 8 h. The precipitate was then collected by centrifugation (12,000 rpm, 15 min) and washed three times with deionized water to remove unreacted $CuCl_2$ and APTMS. Finally, white CDs@SiO_2@Cu powder was obtained after freeze-drying.

Peroxidase-like activity analysis and steady-state kinetic analysis

Peroxidase (POD) -like activity analysis: Prepared a series of PBS buffer solutions (pH 7.0) containing 0.6 mM TMB, 0.6 mM TMB and 0.6 mM H_2O_2, 1.2 mg mL^{-1} CDs@SiO_2@Cu and 0.6 mM TMB, or 1.2 mg mL^{-1} CDs@SiO_2@Cu, 0.6 mM TMB, and 0.6 mM H_2O_2. After thorough mixing, incubated the solutions at 40°C for 30 min, and collected the absorbance at 652 nm.

Steady-state kinetic analysis: For the H_2O_2-depend kinetic assay, a series of PBS buffer solutions (pH 7.0) containing 1.2 mg mL^{-1} CD@SiO_2@Cu, 0.6 mM TMB, and varying concentrations of H_2O_2 ranging from 0.05 to 1.0 mM were prepared. For the TMB-depend kinetic assay, solutions were prepared with the same concentration of CD@SiO_2@Cu and 0.6 mM H_2O_2, but with varying TMB concentrations from 0.05 to 1.0 mM. The solutions were incubated at 40°C and the absorbance at 652 nm was recorded every 15 s. The kinetic parameters (K_m and V_{max}) were calculated based on the relationship between reaction rate and substrate concentration.

Detection of ROS by ESR assay

The trapping agent of TEMP (25 mM) was used to detect the generation of $^1O_2$ and DMPO (100 mM) was employed to trap $O_2^{\cdot-}$ and ·OH. PBS buffer solutions (7.0) containing 1.2 mg mL^{-1} CD@SiO_2@Cu and 0.6 mM H_2O_2 were incubated at 40°C for 30 min. Then 200 μL of the above solution was mixed thoroughly with 50 μL ROS trapping agents and collect the ESR spectra.

Detection of glyphosate

50 μL different concentrations of glyphosate were incubated with 20 μL CDs@SiO_2@Cu (30 mg mL^{-1}) for 5 min. Then 20 μL TMB (20 mM) solution, 20 μL H_2O_2 (20 mM) solution, and 390 μL of PBS (pH=7) were added and thoroughly mixed, then solutions were incubated at 40°C for 30 min. The RTP intensities at 510 nm and the absorbance at 652 nm were recorded for RTP and colorimetric dual-mode analysis.

Detection of glyphosate in real samples

Drinking water was obtained from a local supermarket. After filtered through a 0.22 μm filter, 200 μL of drinking water, 20 μL of 30 mg·mL^{-1} CDs@SiO_2@Cu solution, and 20 μL of glyphosate solutions at different concentrations (5, 12.5, and 17.5 μg·mL^{-1}) were incubated for 5 min. Then, 20 μL of 20 mM TMB solution, 20 μL of 20 mM H_2O_2 solution, and 220 μL of PBS

(pH=7) were added to the mixture and incubated at 40°C for 30 min. Finally, the RTP intensity at 510 nm and the absorbance at 652 nm were recorded for the calculation of the glyphosate content.

Soybeans were purchased from a local supermarket. According to previously reported studies,[2, 3] 5 g of soybeans were thoroughly cleaned using ultrasound and dried. Then, 0.02 mg, 0.05 mg, and 0.07 mg glyphosate were sprayed and then mixed with 10 mL of water and ultrasonicated for 15 min. The mixture was then centrifuged for 10 min at 4500 rpm and filtered through a 0.22 μm membrane. Then 50 μL of the above solution was mixed with 20 μL of 30 $mg \cdot mL^{-1}$ CDs@SiO_2@Cu solution and incubated for 5 min. 20 μL of 20 mM TMB solution, 20 μL of 20 mM H_2O_2 solution, and 390 μL of PBS (pH=7) were then added, mixed thoroughly, and incubated at 40°C for 30 min. Finally, the RTP intensity at 510 nm and the absorbance at 652 nm were recorded for the calculation of the glyphosate content.

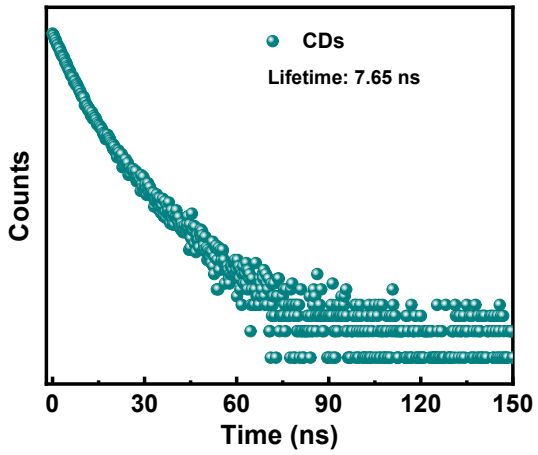

Fig. S1 FL lifetime decay scatterplot of CDs.

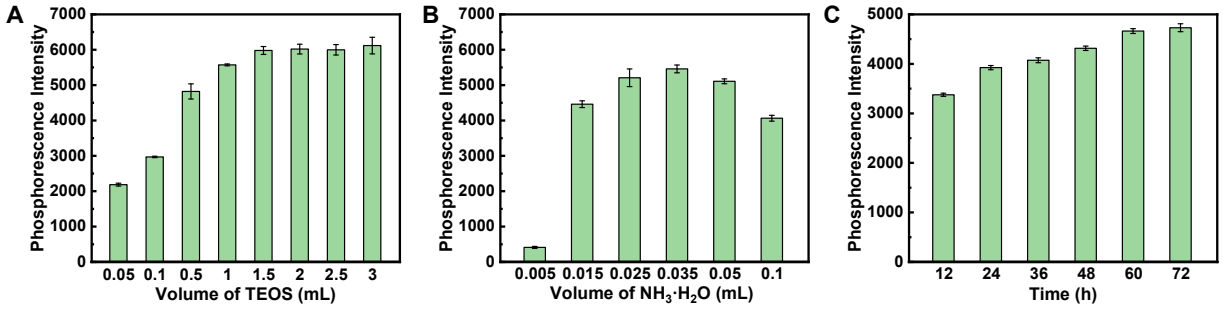

Fig. S2 Effects of (A) TEOS volume, (B) $NH_3 \cdot H_2O$ volume, and (C) reaction time on the RTP intensity of CDs@SiO_2 at 510 nm.

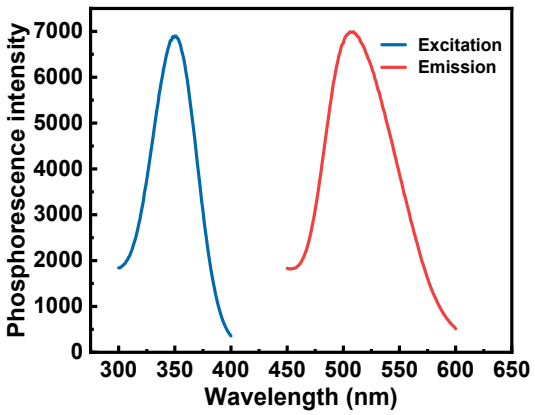

Fig. S3 RTP excitation and emission spectra of CDs@SiO_2.

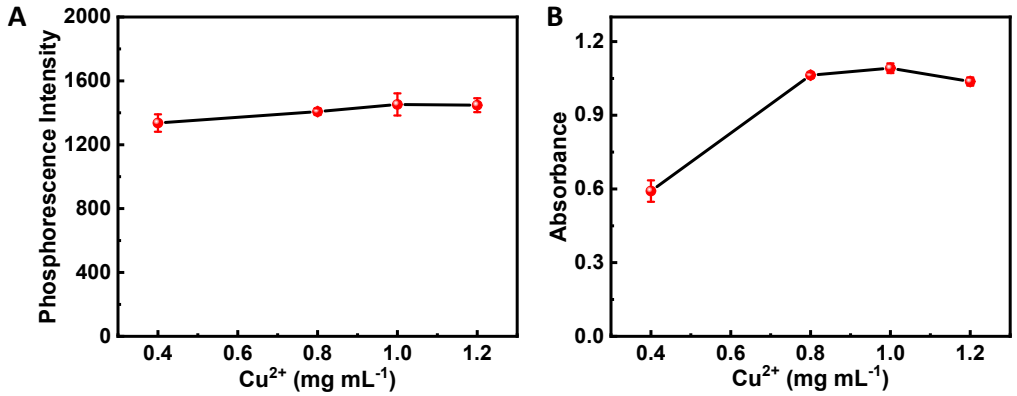

Fig. S4 (A) RTP intensity of CDs@SiO_2@Cu obtained at different Cu^{2+} concentrations and (B) the corresponding absorbance at 652 nm after reacting with TMB and H_2O_2.

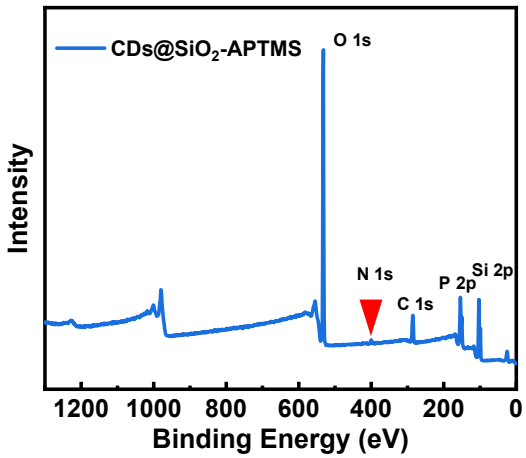

Fig. S5 XPS spectrum of CDs@SiO_2-APTMS.

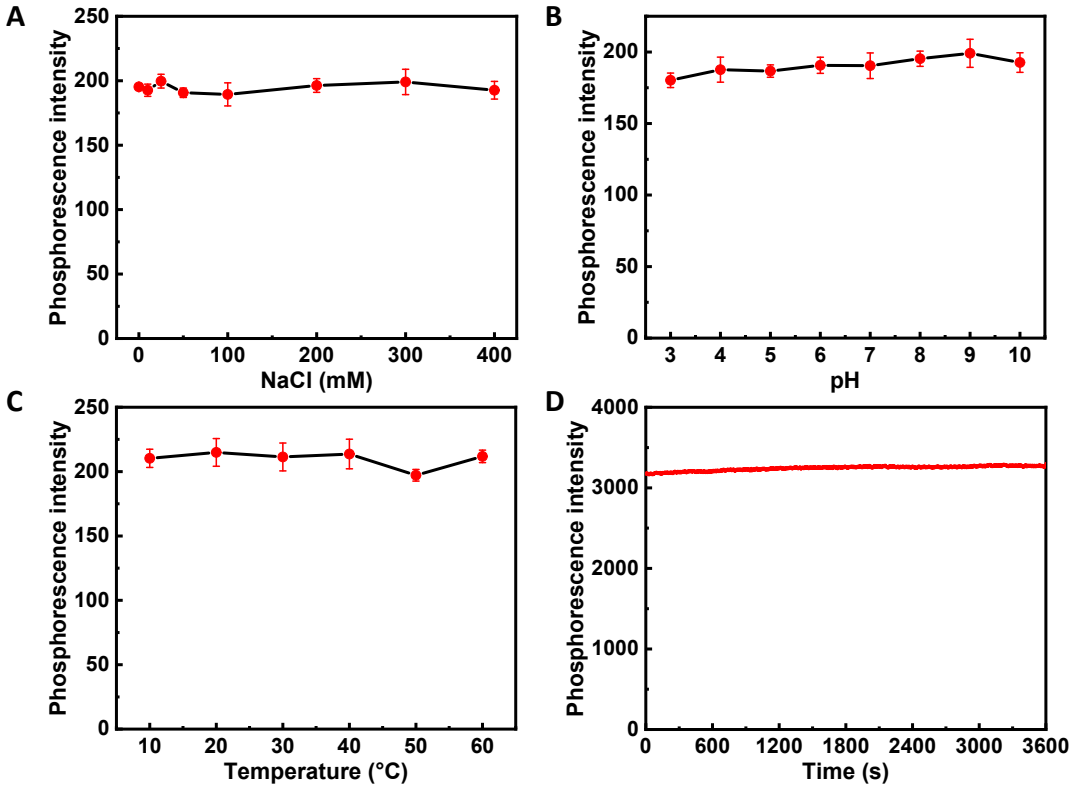

Fig. S6 (A) Ionic strength, (B) pH, (C) temperature, and (D) photobleaching resistence of RTP CDs@SiO_2@Cu. (Photobleaching resistence: RTP intensity at 510 nm under continuous 365 nm excitation light radiation)

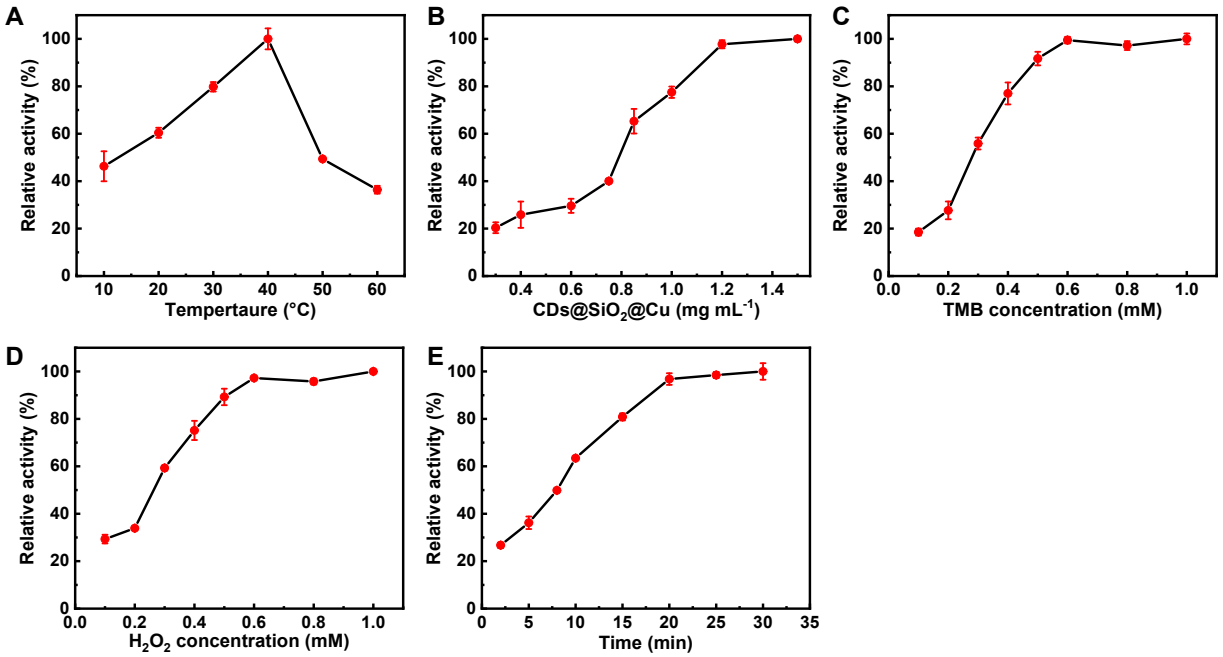

Fig. S7 Influence of various parameters on the POD-like activity of CDs@SiO_2@Cu: (A) temperature, (B) concentration of CDs@SiO_2@Cu, (C) concentration of TMB, (D) concentration of H_2O_2, and (E) incubation time.

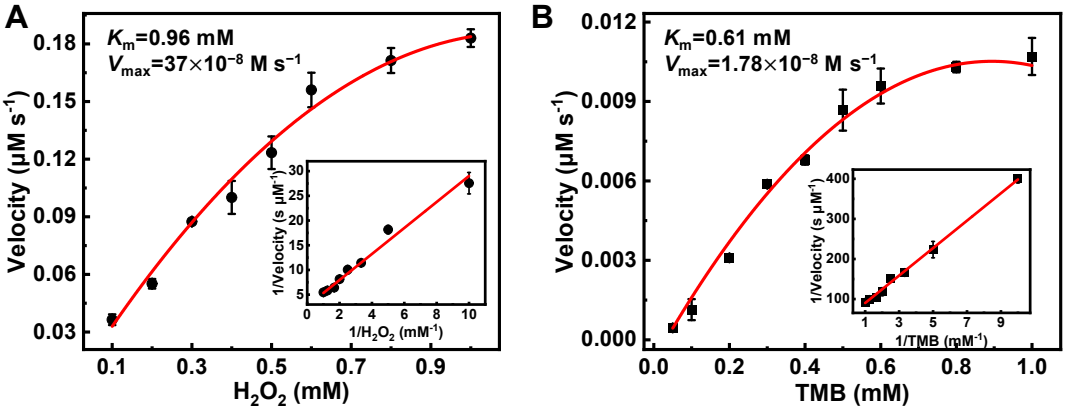

Fig. S8 Steady-state kinetic assay of CDs@SiO_2@Cu with (A) H_2O_2 and (B) TMB.

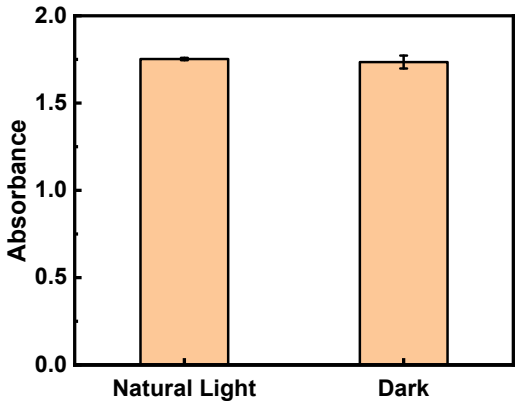

Fig. S9 POD-like activity of CDs@SiO_2@Cu under dark and natural light conditions.

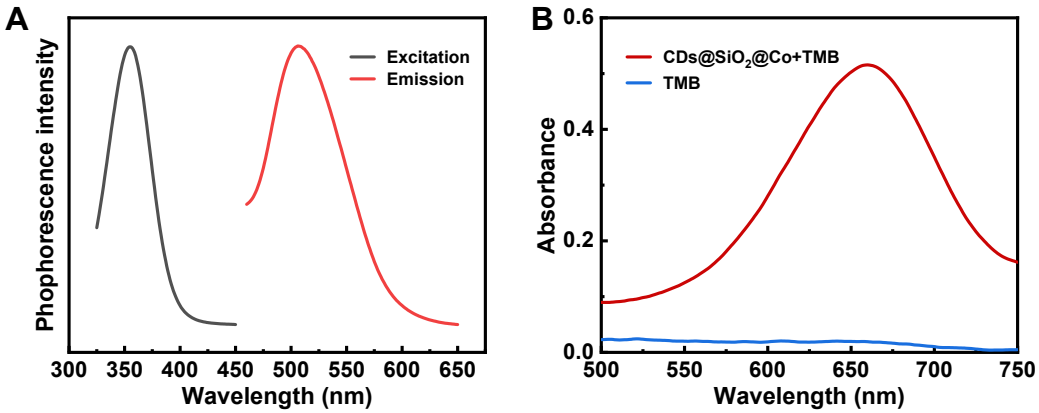

Fig. S10 (A) RTP excitation and emission spectra of CDs@SiO_2@Co. (B) oxidase-like activity of CDs@SiO_2@Co.

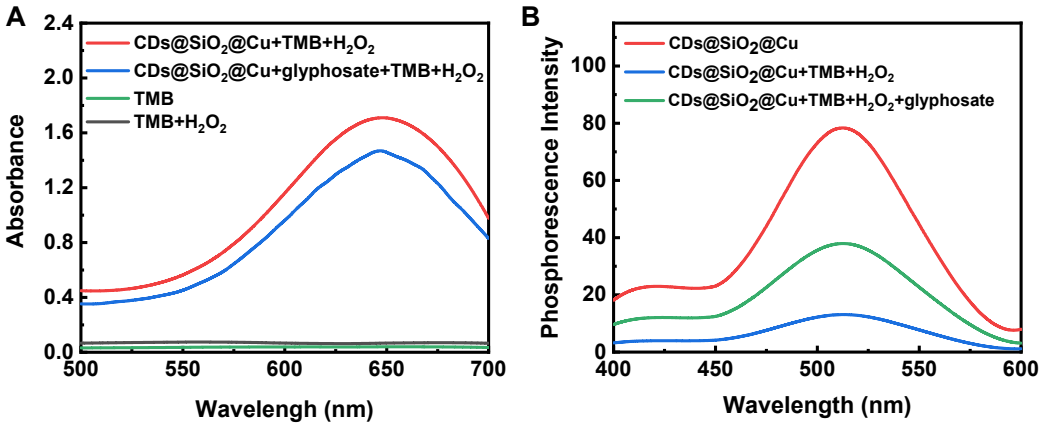

Fig. S11 Feasibility of glyphosate detection. (A) colorimetric mode and (B) phosphorescent mode. (CDs@SiO_2@Cu: 1.2 mg mL^{-1}, H_2O_2: 0.5mM, TMB: 0.5mM, glyphosate: 8 μM).

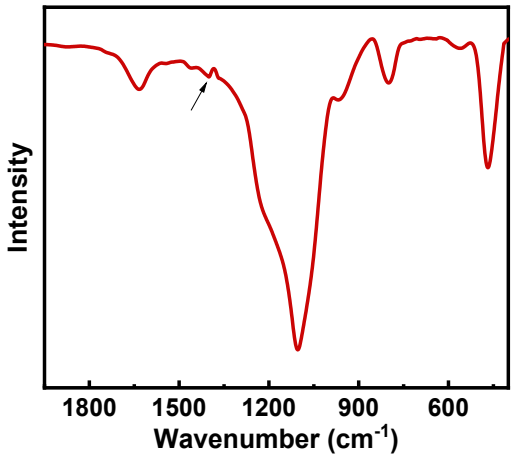

Fig. S12 FTIR spectrum CDs@SiO_2@Cu after mixed with glyphosate.

The FTIR spectrum of the glyphosate-mixed CDs@SiO_2@Cu shows a distinct peak near 1400 cm^{-1}, associated with the symmetric stretching of carboxylate groups from glyoxylate-a known degradation product of glyphosate.[4] This evidence confirms that glyphosate binds to the CDs@SiO_2@Cu's surface, blocking its catalytic sites and inhibiting its POD-like activity.[4, 5]

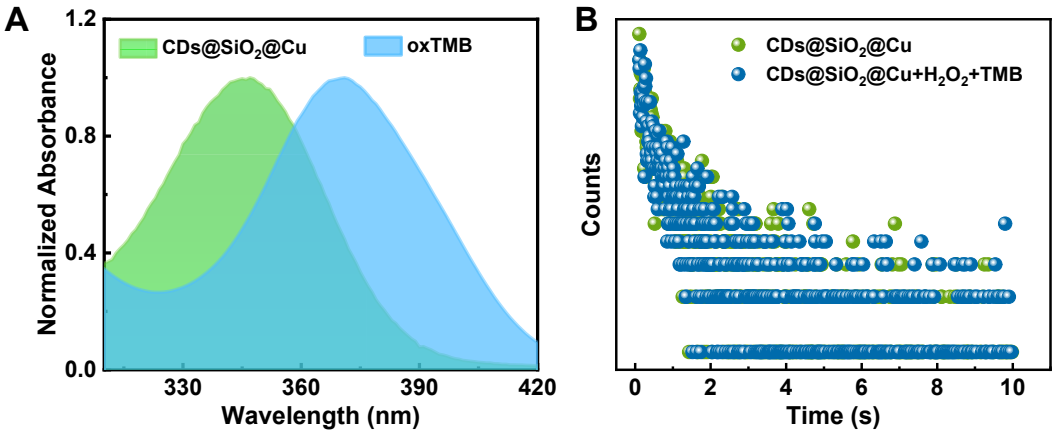

Fig. S13 (A) RTP excitation spectrum of CDs@SiO2@Cu and UV-vis spectrum of oxTMB. (B) RTP lifetime decay scatter plot of CDs@SiO_2@Cu before and after mixing with oxTMB.

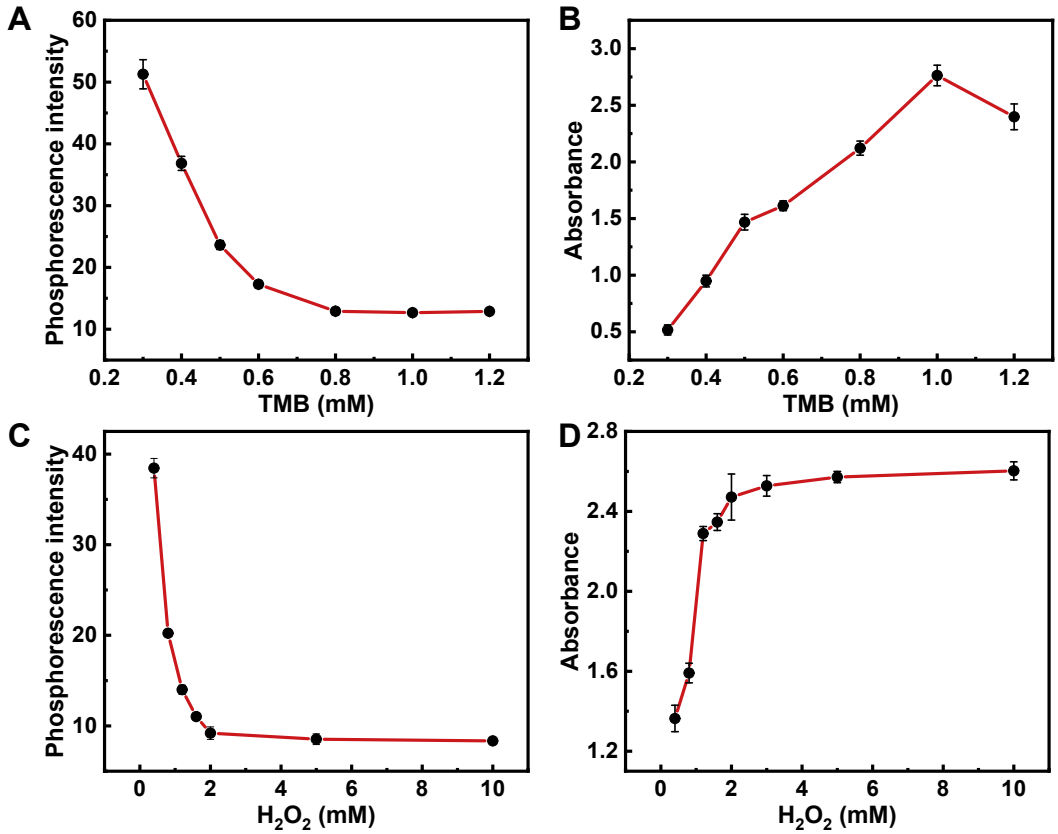

Fig. S14 Effect of different concentrations of (A and B) TMB and (C and D) H_2O_2 on the detection performance of glyphosate.

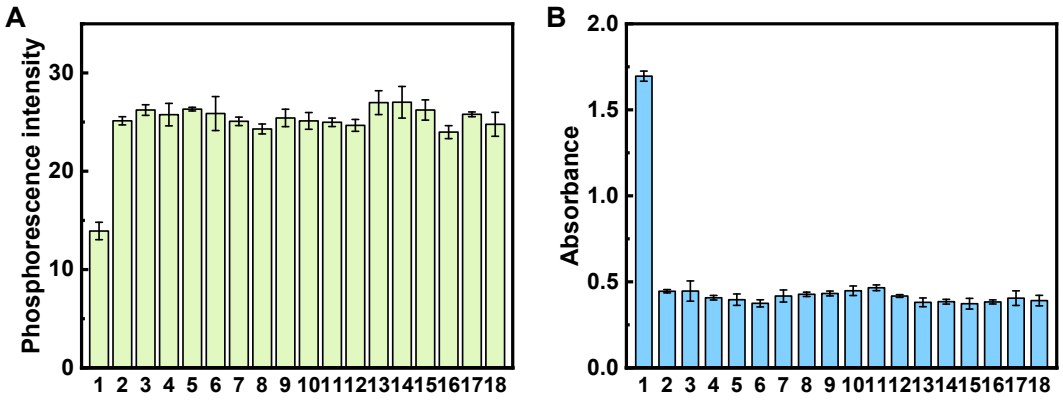

Fig. S15 Influence of various interferents on glyphosate detection. (1: Blank, 2: glyphosate (8 μM), 3: glufosinate (50 μM), 4: phoxim (50 μM), 5: dursban (50 μM), 6: parathion (50 μM), 7: malathion (50 μM), 8: acetamiprid (50 μM), 9: K^+ (10 mM), 10: Na^+ (10 mM), 11: Ca^{2+} (10 mM), 12: Mg^{2+} (10 mM), 13: Mn^{2+} (10 mM), 14: Zn^{2+} (10 mM), 15: SO_4^{2-} (10 mM), 16: PO_4^{3-} (10 mM), 17: CO_3^{2-} (10 mM), 18: NO_3^- (10 mM)).

Table S1. Comparison of reaction kinetics parameters between natural enzymes and other enzymes

Materials	pH	K_m (mM)		V_{max} (10^{-8} M s^{-1})		Ref.
		TMB	H_2O_2	TMB	H_2O_2	
HRP	7.0	0.20	0.16	/	/	[6]
HRP	6.0	1.73	8.84	71.6	500	[7]
GO-AuNCs	7.0	0.16	142.39	/	/	[6]
Co-m-ceria	6.0	1.46	3.00	823	93.4	[7]
Fe_3O_4 + ATP	7.4	0.374	54.6	2.6	1.8	[8]
Au-NCs	7.0	3.59	16.71	0.86	1.30	[9]
Au-NCs+heparin	7.0	1.97	37.81	7.39	3.03	[9]
Fe-phosphotungstates	7.0	0.38	18	5.28	1.32	[10]
Bare CuS	7.0	0.11	101.8	0.038	0.043	[11]
CuS-$Asp_{0.05}$	7.0	0.09	103.2	33.1	38.2	[11]
Fe_1@CN-S	7.0	7.70	2.12	56.11	101.95	[12]
CDs@SiO_2@Cu	7.0	0.61	0.96	1.78	37	This work

Table S2. Comparison of analytical performance of glyphosate detection methods

Materials	Method	Line range (μM)	LOD (μM)	Reference
PHQCA-Cu^{2+}	Fluorescent	2.0-3.7	0.01	[13]
PDHN-Cu^{2+}	Fluorescent	0.2-2.0	0.07	[14]
B-CDs and R-AuNCs	Fluorescent	1.77-71.0 (0.3-12 μg/mL)	1.29 (0.218 μg/mL)	[15]
CDs	Fluorescent	1.0–110.0	0.60	[16]
Ponceau 4 R	Colorimetric	1.0-90.0 (0.17-15.21 μg/mL)	0.14 (0.023 μg/mL)	[17]
Oxidized MXene quantum dots@CuNi bimetal	Colorimetric	0-100	1.13	[18]
Fe_3O_4@Cu nanozyme	Colorimetric	1.66-4.14 (0.28-0.70 μg/mL)	0.51 (0.086 μg/mL)	[5]
	Chemiluminescent	0.30-1.00 (0.05-0.17 μg/mL)	0.11 (0.019 μg/mL)	
Carbryl	Colorimetric	5-600	0.63	[19]
	Fluorescent	1.0–40.0	0.084	
CDs@SiO_2@Cu	Colorimetric	0.5-10	0.16	This work
	Phosphorescence	0.1-10	0.02	

Table S3. Detection of glyphosate in drinking water samples

Sample	Spiked ($\mu g \cdot mL^{-1}$)	Mode	Found ($\mu g \cdot mL^{-1}$)	Recovery (%)	RSD (%)
1	0.2	Phosphorescence	0.192	96.0	4.9
		Colorimetric	0.189	94.5	8.3
2	0.5	Phosphorescence	0.512	102.4	5.2
		Colorimetric	0.491	98.2	7.8
3	0.7	Phosphorescence	0.694	99.1	4.6
		Colorimetric	0.712	101.7	6.6

Table S4. Detection of glyphosate in soybean samples

Sample	Spiked ($mg \cdot Kg^{-1}$)	Mode	Found ($mg \cdot Kg^{-1}$)	Recovery (%)	RSD (%)
1	4	Phosphorescence	3.74	93.5	7.3
		Colorimetric	3.80	95.0	7.8
2	10	Phosphorescence	9.87	97.8	9.1
		Colorimetric	10.16	101.6	8.7
3	14	Phosphorescence	13.94	99.5	2.9
		Colorimetric	13.78	98.4	3.1

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