Electronic Supplementary Information

V_2O_5 nanowires as robust and efficient peroxidase mimic at high temperature in aqueous media

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Materials and methods

Reagents and materials

All chemicals and reagents were of analytical grade and used without further purification, and ultra pure water was used throughout. TMB, o-phenylenediamine (OPD), 2,2'-azino-bis(3-ethylbenzo-thiazoline-6-sulfonic acid) diammonium salt (ABTS) and glucose oxidase (GOx, EC 1.1.3.4. 47, 200 U mg^{-1}) were purchased from Sigma-Aldrich (Shanghai, China). A 0.01 M luminol was prepared by dissolving luminol (Merck, Germany) in 0.01 M NaOH solution, and the diluted solutions of luminol were prepared as needed with 0.2 M Na_2CO_3. H_2O_2, glucose, NH_4VO_3, P123 ($EO_{20}PO_{70}EO_{20}$), H_2O_2, NaOH, HCl, Na_2CO_3 and $NaHCO_3$ were obtained from Chongqing Chemical Reagents Company (Chongqing, China). All glassware was soaked in 10% nitric acid and thoroughly rinsed before use.

Apparatus

UV−visible spectroscopy measurements were performed on a Shimadzu UV−2450 spectrophotometer (Suzhou, China). The CL spectra were recorded under the fluorescence scanning model by turning off the excitation source with a Hitachi F-7000 spectrofluorimeter (Tokyo, Japan). CL measurements were performed on an IFFL-D MCFL-A flow injection CL Analyzer (Ruike Electronic Equipment Company Ltd., Xi'an, China). The powder X-ray diffraction (XRD) patterns of the as-prepared products were performed on a XD-3 X-ray diffractometer (PuXi, Beijing, China) under the conditions of nickel filtered CuKα radiation (λ=0.15406 nm) at current of 20 mA and a voltage of 36 KV. The scanning rate was 4° min^{-1} in the angular range of 5°–55° (2θ). FT-IR spectra were recorded on a Nicolet 170SX instrument (Madison, WI, USA) in the transmission mode using KBr pellets to prepare the sample. The ESR spectra were obtained on a Bruker ESR 300E with Microwave Bridge (receiver gain, 1×10^5; modulation amplitude, 2 Gauss; microwave power, 10 mW; modulation frequency, 100 kHz). The scanning transmission electron microscopy (SEM) images were taken on Hitachi model S-4800 field emission scanning electron microscope (Hitachi, Japan), with an accelerating voltage of 20 kV. The size of the prepared V_2O_5 was characterized by a model Tecnai G2 20 TEM operating at 200 kV accelerated voltage.

Synthesis of V_2O_5 Nanowires, V_2O_5 Nanorods, V_2O_5 Nanoparticles, V_2O_5 Nanosheets, V_2O_5 Nanobelts and V_2O_5 Spheres

V_2O_5 nanowires were prepared by the hydrothermal treatment of NH_4VO_3 as described elsewhere[1] with a slight modification. Briefly, 0.75 g NH_4VO_3 and 1.25 g of P123 were dissolved in 75 mL of water containing 3.75 mL of 2 M HCl. The mixture was stirred at room temperature for 7 h, and then transferred into 100 mL Teflon-lined autoclave. After the autoclave was sealed and maintained in an electric oven at 120 °C for 24 h, it was cooled down to room temperature. The product was rinsed with water and acetone several times and dried at 80 °C for 12 h. The as-prepared nanowires could be well-dispersed in aqueous buffer.

V_2O_5 nanorods were synthesized by a facile thermal-decomposition of vanadyl oxalate by reacting micro-sized V_2O_5 with oxalic acid.[2] Briefly, 1.2 g V_2O_5 and 1.83 g $H_2C_2O_4$ were added to 30 mL of the distilled water under stirring at room temperature until the color of the solution changed from yellow to blue. The obtained solution was dried at 80 °C and then calcined at 400 °C for 2 h.

V_2O_5 nanoparticles were prepared by using a surfactant-mediated method.[3] The first micelle solution was constituted of 6 g CTAB, 10.8 g 1-hexanol and 3 g water which contained 0.2 g ammonium metavanadate, while the second micelle solution contained 6 g CTAB, 10.8 g 1-hexanol 3.1 g water and 0.1 g sulfuric acid. After stirring and getting clear solutions, the second micelle solution was added to the first micelle solution. The mixed micelle solution was stirred at 50 °C for 3 h and then left for two days at room temperature to allow precipitation. The precipitates were washed with deionized water and ethanol for several times and then heated at 400 °C for 2 h in air.

V_2O_5 nanosheets were prepared via a simple and direct exfoliation of bulk V2O5 crystals in formamide solvent.[4] Briefly, 50 mg V_2O_5 powder was added into 50 mL of formamide solution, then the mixture was shaken overnight to suspend the powder. After the resulting suspension was then sonicated at room temperature for 3 days, the exfoliated V_2O_5 nanosheets were isolated from the upper solution via centrifugation and washed with ethanol for several times and then dried overnight at 70 °C.

V_2O_5 nanobelts were synthesized based on method by Liu et al.[5] Briefly, 0.234 g ammonium metavanadate was dissolved in 20 mL of the deionized water to form a light yellow clear solution. Then, the concentrated nitric acid was added dropwisely to adjust the pH value of the solution to about 2.0 to 3.0 to obtain a clear orange solution. Then the resulting solution was transferred to autoclave with a Teflon liner and filled with deionized water up to 80% of the total volume. The autoclave was heated to 180 °C and kept for 24 h. After cooling down to room temperature naturally, the final product was washed with deionzed water and alcohol for several times and then dried at 60 °C under vacuum for 6 h.

References

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2. Pan, A.; Zhang, J.; Nie, Z.; Cao, G.; Arey, B. W.; Li, G.; Liang, S.; Liu, J. *J. Mater. Chem.* **2010**, *20*, 9193–9199.
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4. Rui, X.; Lu, Z.; Yu, H.; Yang, D.; Hng, H. H.; Lim T. M.; Yan, Q. *Nanoscale* **2013**, *5*, 556–560.
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Electron Spin Resonance

175 μL samples were prepared at room temperature by adding 5 μL of 3% H_2O_2, 20 μL of 0.2 M DMPO, 5 μL of 1×10^{-4} M luminol and 95 μL of water into a 1 mL plastic tube in the presence of 50 μL of 200 μg mL^{-1} V_2O_5 nanowires. Then, the prepared sample solution was transferred to a quartz capillary tube and placed in the ESR cavity to obtain the ESR spectra. In this experiment, DMPO was used to trap the •OH radicals to form the DMPO/•OH spin adduct.

Procedures for CL detection and catalytic property assay

The CL intensity was measured by a flow injection CL system, in which two peristaltic pumps (30 r min^{-1}, Longfang Instrument Factory, Wenzhou, China) were used to deliver all solutions at a flow rate of 3 mL min^{-1} (per tube). One was used for delivering V_2O_5 nanowires solution as catalyst and water carrier stream, and the other for delivering luminol solution and H_2O_2 solution (or sample solution). PTFE tubing (0.8 mm i. d.) was used to connect all components in the flow system. For CL measurement, flow

lines were inserted into the luminol solution, water, and H_2O_2 solution or sample solution, respectively. Then the pumps were started until a stable baseline was recorded. Injection of V_2O_5 nanowires solution was made by using an eight-way injection valve equipped with a 120 μL sample loop. The CL signal produced in the flow cell was monitored by a photomultiplier tube (PMT, operated at -600 V) of the Type IFFL-D MCFL-A flow injection CL Analyzer and was recorded by a computer equipped with a data acquisition interface. Data acquisition and treatment were performed with REMAX software running under Windows XP. The net CL intensity $\Delta I = I - I_0$ was used for the quantitative determination, where I and I_0 were the CL intensities of sample and blank solutions, respectively. At each target concentration, the injection was repeated for three times, and the average CL signal was obtained.

The performance of catalytic properties was evaluated by varying the concentrations of hydrogen peroxide at fixed concentration of V_2O_5 nanowires (18.2 mg/L) and luminol (8 μM, pH 11.3). Catalytic properties can be evaluated by CL emission intensities versus concentration of hydrogen peroxide.

Procedure for temperature effect on catalytic activity of V_2O_5 nanowires

The effect of temperature on catalytic activity of V_2O_5 nanowires was evaluated by incubating the V_2O_5 nanowires solution (18.2 mg/L) in a water bath at a range of temperature from 5 to 70 ^oC (5, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, and 70 ^oC) for 10 min, then their activities were measured under the following conditions: 8 μM luminol in 0.2 M buffer solution of Na_2CO_3-$NaHCO_3$ (pH 11.3), 1.0 μM hydrogen peroxide. The catalytic activity of V_2O_5 nanowires at 65 ^oC was set to 100%.

Procedure for glucose detection in serum samples

For glucose analysis, first, the calibration curve for glucose was made by: (1) 0.1 mL of 1 mg mL^{-1} GOx and 0.1 mL of glucose of different concentrations in 0.2 mL of 0.2 M NaAc buffer (pH 7.0) were incubated at 37 °C for 30 min (with final glucose concentration of 0.05–10 μM); (2) the obtained mixture was diluted to 10 mL with ultra pure water, and used for standard curve measurement.

For glucose determination in serum, the samples were first treated by ultra filtration with 5 kDa Amicon cell at 3000 rpm for 10 min. The filtrate was diluted properly with 0.2 M NaAc buffer (pH 7.0). Then 0.1 mL of the diluted filtrate was

added into 0.1 mL of 1 mg mL^{-1} GOx. The obtained mixture was incubated at 37 °C for 30 min and then used for the glucose measurement by the same way as glucose standard. In control experiments, buffer solution, 10 mM lactose and 10 mM fructose were used instead of 1.0 μM glucose. The comparison study was carried out by an OneTouch Ultra glucose meter using glucose paper (Johnson and Johnson Medical Ltd., Shanghai, China).

Procedure for hydrogen peroxide analysis in real water samples

In this study, the rain water and lake water samples were selected for investigation. Rain water samples were collected from the roof of the located building near our lab. Lake water sample was taken from the ChongDe Lake located in Southwest University. Before experiment, all the water samples were filtered through 0.45 μm micropore membrane prior to the preparation of samples by spiking the original samples with known amounts of H_2O_2 at three different concentrations (0.5, 1.0, and 5.0 μM). EDTA as masking agent was added to water sample with final concentration of 1.0 μM for eliminating the potential interference of metal ions. Each measurement was done in triplicate and the average was presented with standard deviation. The results summarized in Table S4 shows that the recoveries of H_2O_2 in the spiked lake water sample ranged from 90 to 108%, indicating the utility of the proposed method for the detection of H_2O_2.

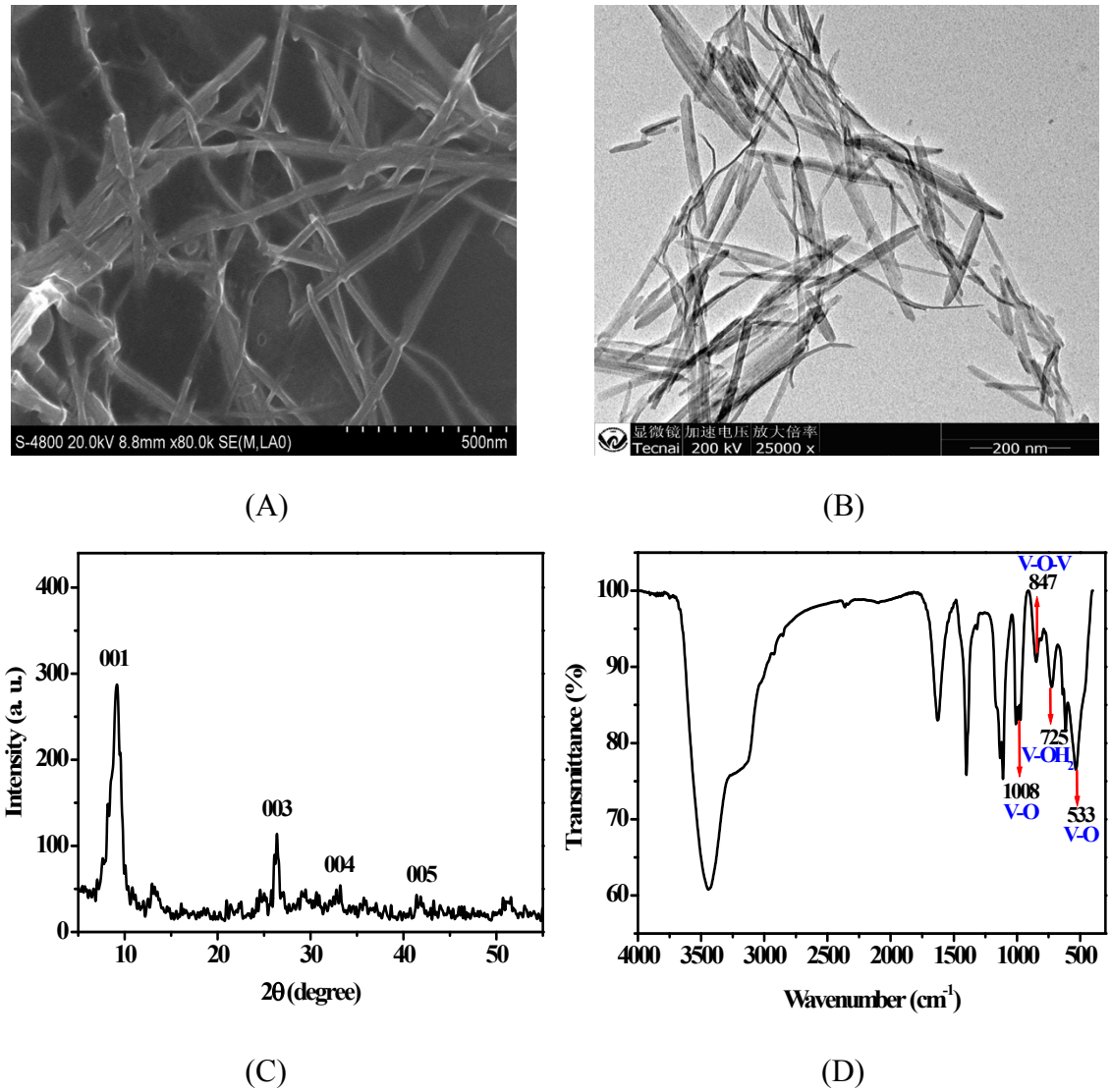


Figure S1. (A) SEM image of V_2O_5 nanowires. (B) TEM image of V_2O_5 nanowires. (C) XRD pattern of V_2O_5 nanowires. (D) FT-IR spectra of V_2O_5 nanowires.

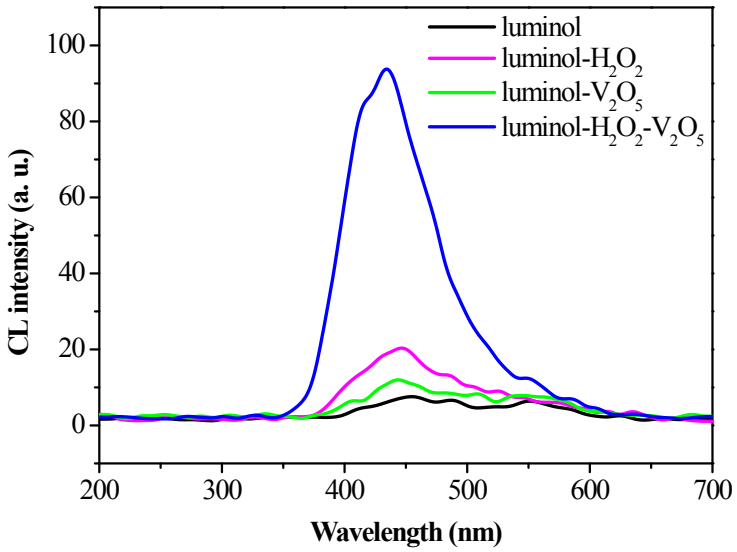
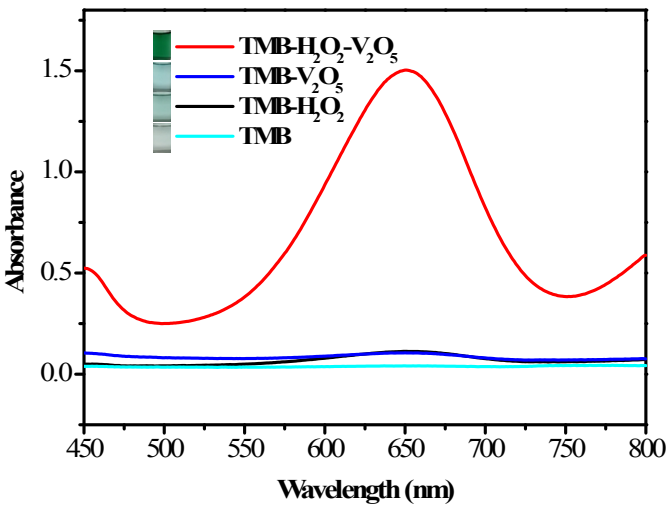
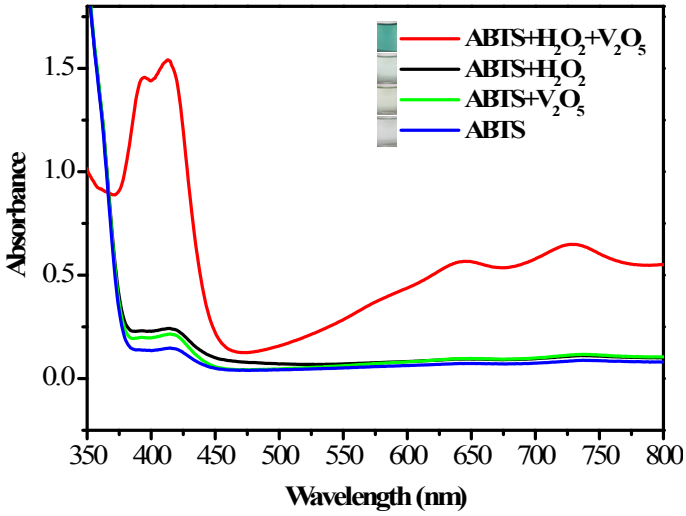


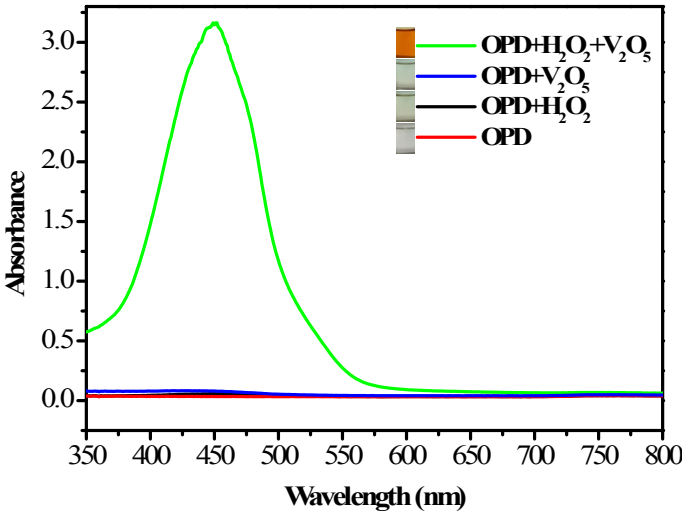
Figure S2. CL spectra for the luminol-H_2O_2-V_2O_5 nanowires system. Reaction conditions: 10 μM of luminol in 0.2 M Na_2CO_3 (pH 11.3), 10 μM of H_2O_2, 20 mg L^{-1} of V_2O_5 nanowires.



(A)



(B)



(C)

Figure S3. (A) The UV-visible absorption spectra of 0.1 mM TMB with and without 5 mg L^{-1} of V_2O_5 nanowires in the absence and presence of 0.1 mM H_2O_2 for 30 min reaction at pH 4.0 and 35 °C. (B) The UV-visible absorption spectra of 0.1 mM ABTS with and without 5 mg L^{-1} of V_2O_5 nanowires in the absence and presence of 0.1 mM H_2O_2 for 30 min reaction at pH 4.0 and 35 °C. (C) The UV-visible absorption spectra of 1 mM OPD with and without 5 mg L^{-1} of V_2O_5 nanowires in the absence and presence of 1 mM H_2O_2 for 30 min reaction at pH 4.0 and 35 °C. Insets: Images of color reaction of TMB/ABTS/OPD with and without V_2O_5 nanowires in the absence and presence of H_2O_2.

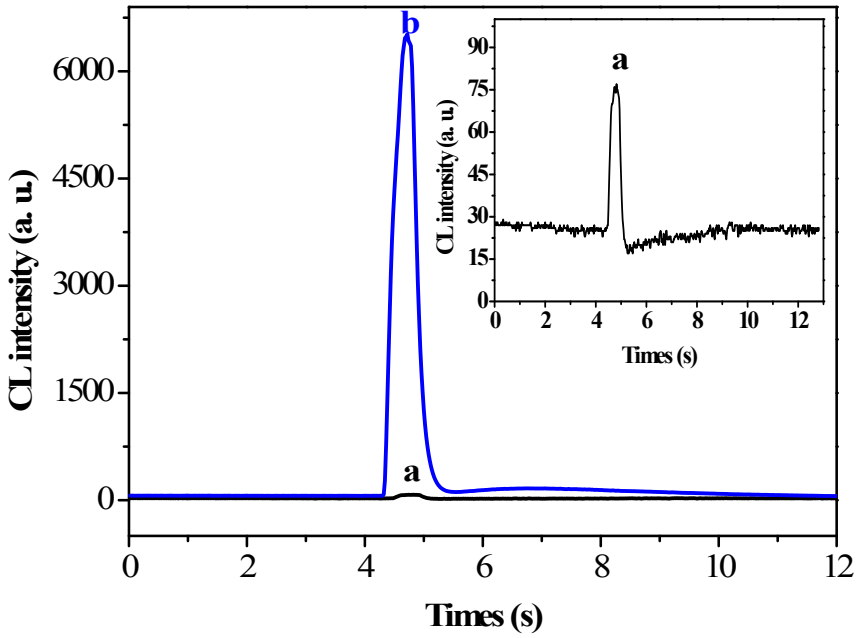


Figure S4. Kinetic characteristics of the luminol-H_2O_2 CL system in the absence (a) and presence (b) of V_2O_5 nanowires. Inset: Amplified kinetic response of the luminol-H_2O_2 CL system. Reaction conditions: 8.0×10^{-6} M of luminol in 0.2 M Na_2CO_3 (pH 11.3), 1.0×10^{-6} M of H_2O_2, 20 mg L^{-1} of V_2O_5 nanowires.

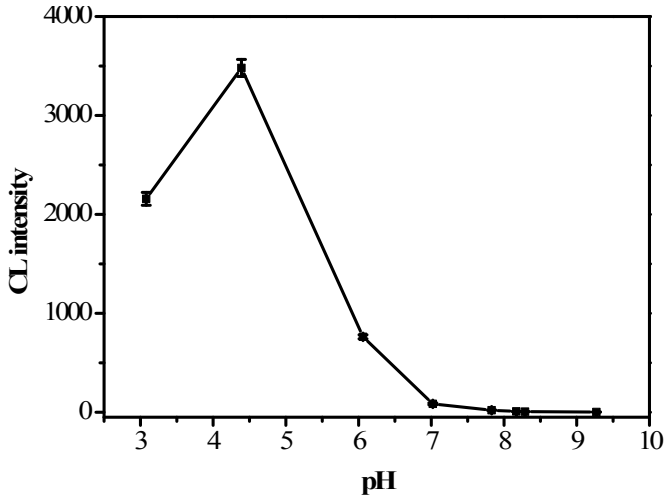


Figure S5. Effect of solution pH on catalytic activity of V_2O_5 nanowires. Experimental conditions: 8 μM luminol in 0.2 M Na_2CO_3 buffer (pH 11.3), 1.0 μM hydrogen peroxide, 18.2 mg L^{-1} V_2O_5. Error bars represent one standard deviation for three measurements.

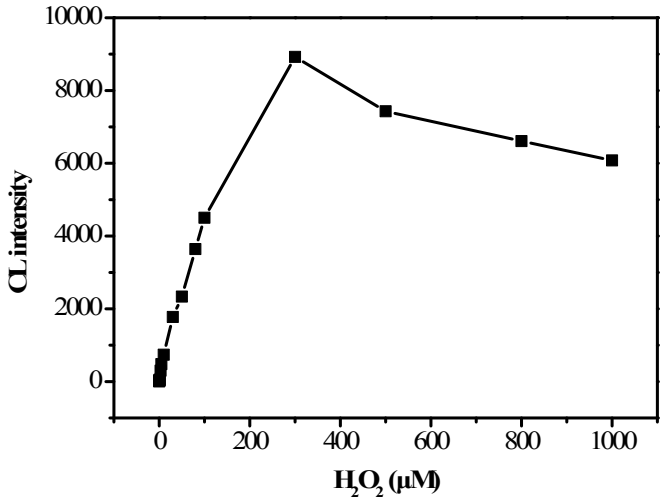


Figure S6. Effect of the hydrogen peroxide concentration on catalytic activity of V_2O_5 nanowires. Experimental conditions: 8 μM luminol in 0.2 M Na_2CO_3 buffer (pH 11.3), 18.2 mg L^{-1} V_2O_5. Error bars represent one standard deviation for three measurements.

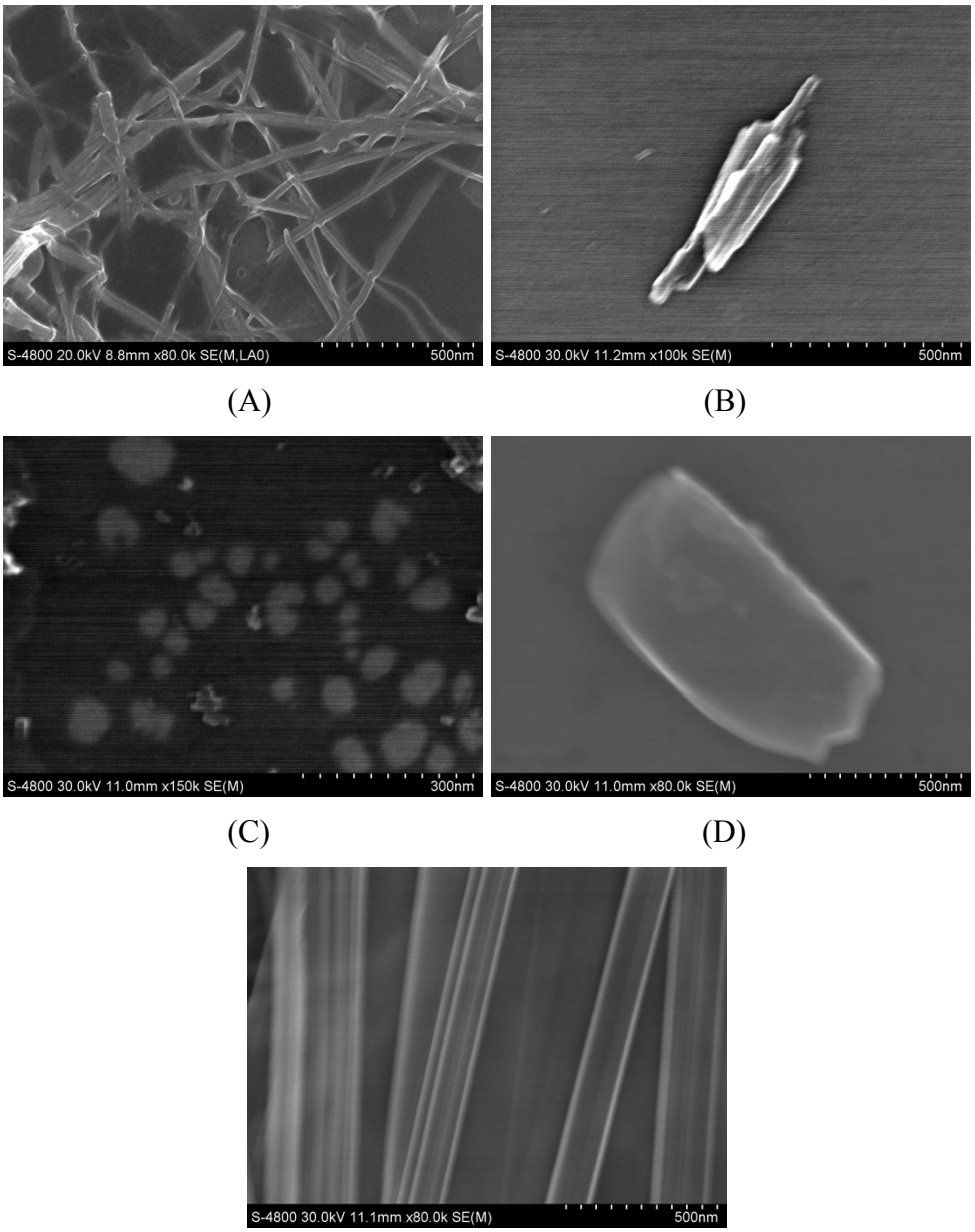


Figure S7. SEM image of (A) V_2O_5 nanowires; (B) V_2O_5 nanorod; (C) V_2O_5 nanoparticle; (D) V_2O_5 nanosheet and (E) V_2O_5 nanobelt.

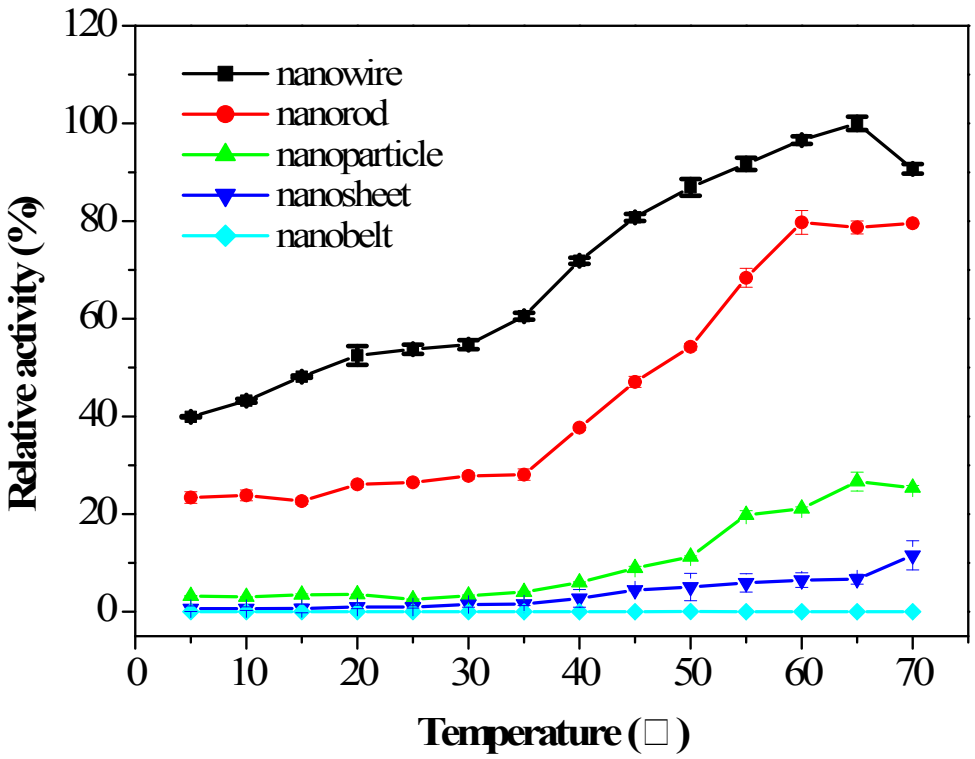


Figure S8. Effect of different V_2O_5 structures on the catalytic activities. Experimental conditions: 8 μM luminol in 0.2 M Na_2CO_3 buffer (pH 11.3), 1.0 μM hydrogen peroxide, 18.2 mg L^{-1} V_2O_5. Error bars represent one standard deviation for three measurements.

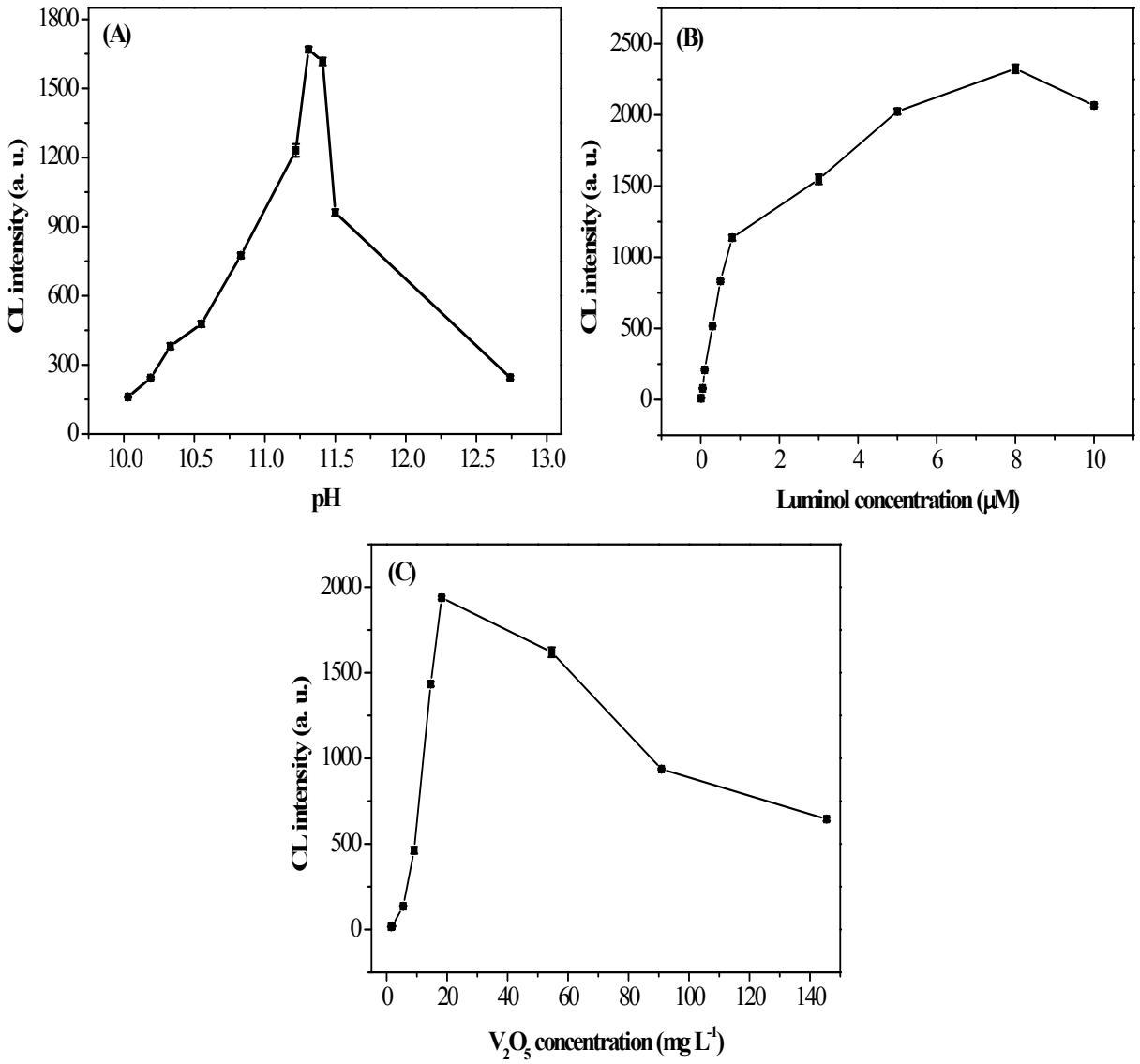


Figure S9. (A) Effect of pH of luminol solution. Reaction conditions: 1.0 μM of luminol, 10 mg L^{-1} of V_2O_5 nanowires, 1.0 μM of H_2O_2; (B) Effect of luminol concentration. Reaction condition: pH 11.3, 10 mg L^{-1} of V_2O_5 nanowires, 1.0 μM of H_2O_2 (C) Effect of V_2O_5 nanowires concentration. Reaction conditions: 8.0 μM of luminol in 0.2 M Na_2CO_3 (pH 11.3); 1.0 μM of H_2O_2.

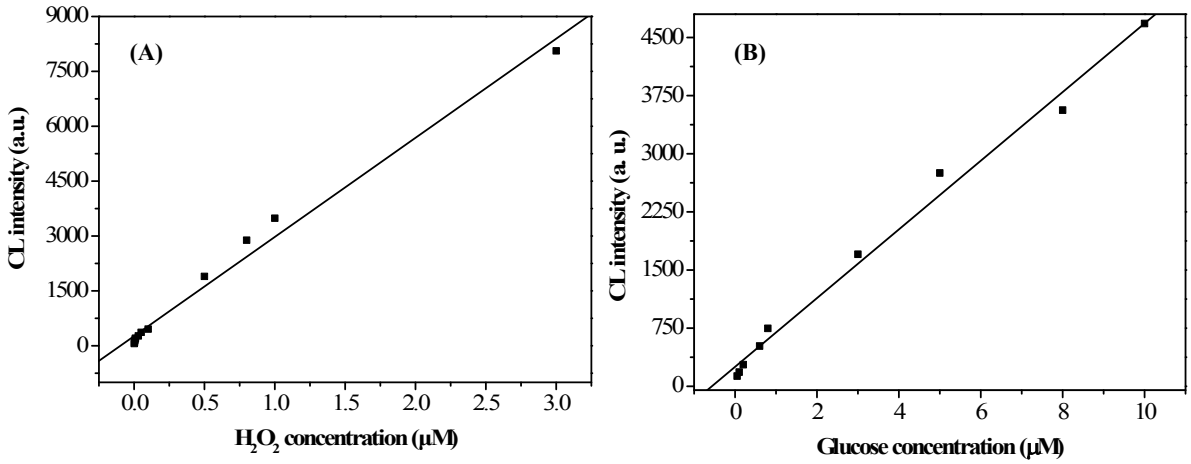


Figure S10. The response curves of H_2O_2 (A) and glucose (B).

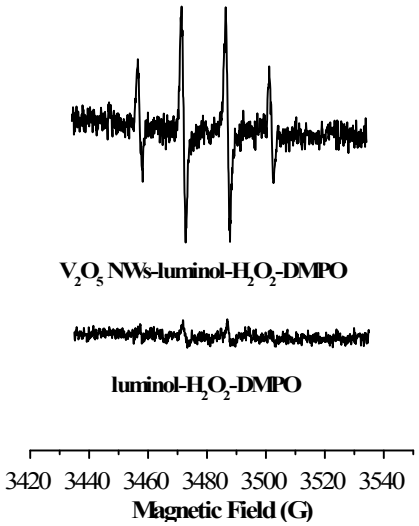


Figure S11. Spin-trapping ESR spectra of ·OH free radicals in the systems of H_2O_2–luminol–DMPO and H_2O_2–luminol–DMPO–V_2O_5 nanowires. Conditions: 23 mM DMPO, 3 μM luminol, 30 mM H_2O_2, and 57 mg L^{-1} of V_2O_5 nanowires.

Table S1. Comparison of the apparent Michaelis–Menten constant (K_m) of V_2O_5 nanowires and $CoFe_2O_4$ nanoparticles (NPs) using H_2O_2 as substrate

	K_m/μM (n=3)
Catalyst	H_2O_2
V_2O_5 nanowires	58.9±2.9
$CoFe_2O_4$ NPs	75.2±2.6

Table S2. Recoveries of H_2O_2 in the presence of foreign species (H_2O_2: 1.0 μM)

Coexisting species	At concentration	Recovery (%)[a]	Coexisting species	At concentration	Recovery (%)[a]
Na^+	23 mg L^{-1}	99.75±0.43	*Ni^{2+}	0.29 mg L^{-1}	103.35±0.58
K^+	39 mg L^{-1}	100.12±0.78	*Pb^{2+}	1.04 mg L^{-1}	99.62±0.78
Ca^{2+}	40 mg L^{-1}	104.73±0.94	*Co^{2+}	58.9 μg L^{-1}	95.63±0.42
NH_4^+	18 mg L^{-1}	95.34±0.15	Cl^-	36 mg L^{-1}	99.75±0.43
Mg^{2+}	24 mg L^{-1}	100.76±0.76	NO_3^-	62 mg L^{-1}	100.90±1.46
Zn^{2+}	0.65 mg L^{-1}	99.40±2.49	SO_4^{2-}	96 mg L^{-1}	102.74±0.78
Al^{3+}	0.27mg L^{-1}	100.42±0.80	PO_4^{3-}	9.5 mg L^{-1}	101.56±1.70
*Cu^{2+}	64 μg L^{-1}	101.13±0.87	I^-	2.54 mg L^{-1}	95.78±0.52
*Fe^{3+}	112 μg L^{-1}	99.31±0.45			

[a] Average of three measurements (mean±SD, n=3). * with 1 μM EDTA.

Table S3. Results of determination of glucose in serum samples

sample	proposed method[a] (mM)	Glucose paper method[b] (mM)
serum 1	4.27±0.01	4.5
serum 2	5.19±0.02	5.2
serum 3	9.92±0.01	10.4

[a] Average of three measurements (mean±SD, n=3); [b]The glucose determination was performed in the laboratory for clinical analysis, the Ninth People's Hospital of Chongqing.

Table S4. Application of the proposed method to the Determination of H_2O_2 in rain Water and Local Lake Water Samples[a]

Sample	Found (μmol L^{-1})	Added (μmol L^{-1})	Total found (μmol L^{-1})	Recovery (%)
Rain water	0.23±0.004	0.5	0.77±0.02	108.0±3.42
		1.0	1.15±0.06	92.1±5.93
		5.0	5.39±0.08	103.2±1.64
Lake water	n.d [b]	0.5	0.45±0.02	90.0±3.39
		1.0	0.95±0.05	95.5±4.58
		5.0	5.00±0.05	100.0±1.02

[a] Values shown were the calculated mean H_2O_2 concentration for each sample and were determined from three replicates. [b] n.d = not detected.

Table S5. Effects of different radical scavengers on the CL of luminol-H_2O_2- V_2O_5 system[a]

Scavengers	Intermediates	Concentration	Percent inhibition (%)[b]
H_2O	/	/	0
NaN_3	$^1O_2$	5 mM	76.1
ascorbic acid	OH•, $O_2^{\bullet-}$	1 μM	98.6
thiourea	OH•	5 mM	99.4
SOD	$O_2^{\bullet-}$	0.07 mg mL^{-1}	91.1

[a] Solution conditions were 1 μM H_2O_2, 8 μM luminol in 0.2 M Na_2CO_3 (pH 11.3), 18.2 mg L^{-1} V_2O_5. [b]Mean value of three measurements.