Supporting Information

In Situ Hg^{2+} Improved Peroxidase-like Activity and Triggered "on" Oxidase-like Activity of Yolk-shell Co_3S_4 Microspheres for Detection of Hg^{2+}

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Associated Content

Experimental Section (Materials, Instruments, Preparation of Co_3S_4 yolk-shell microspheres, Assay of the peroxidase-like activity of Co_3S_4, Steady-state kinetics, Assay of the oxidase-like activity, Assay of detection of Hg^{2+});

XRD pattern of Co_3S_4 microspheres (a),SEM image of precursor (b), SEM image of Co_3S_4 (c), and TEM image of Co_3S_4 (d) (Fig. S1);

BET data of Co_3S_4 yolk-shell spheres (Fig. S2);

Assay of catalytic activity of Co_3S_4 (Fig. S3);

(a) Assay of the peroxidase-like activity of Co_3S_4, (b) Fluorescence intensity, (c) and (d) ESR data of Co_3S_4 (Fig. S4);

Assay of the oxidase-like activity of Co_3S_4 (Fig. S5);

Steady-state kinetic (Fig. S6);

Assay of the oxidase-like activity (Fig. S7);

TEM image of HgS/Co_3S_4 (a), XRD data of Co_3S_4 and HgS/Co_3S_4 (b), and TEM-EDX mapping images of HgS/Co_3S_4 (c) (Fig. S8);

XPS spectra of Co_3S_4 and HgS/Co_3S_4 (Fig. S9);

(a) and (b) Assay of the oxidase-like activity, (c) ESR data of HgS/Co_3S_4 (Fig. S10);

Selectivity of sensor (Fig. S11);

Comparison of K_m (Table S1);

Comparison of Hg^{2+} detection (Table S2);

Color changes of the reaction systems in the presence of Hg^{2+} or in the absence of Hg^{2+} (Video S1).

Experimental Section

Materials

$HgCl_2$, thioacetamide (TAA) and 3,3',5,5'-tetramethylbenzidine dihydrochloride (TMB) were commercially purchased from Macklin (Shanghai, China). $Fe(NO_3)_3•9H_2O$, $Co(NO_3)_2•6H_2O$, NaCl, KCl, $MgSO_4$, H_2O_2 (30 wt%), Terephthalic acid, disodium ethylenediaminetetraacetic acid (EDTA-2Na), Lactose (Lac), D-histidine (Hi), isopropanol (IPA), p-benzoquinone (PBQ), Dserine (Ser), L-arginine (Arg), and Sucrose (Suc) were obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China).

Instruments

The surface state of elements and the phase of Co_3S_4 yolk-shell microspheres were systematically characterized by X-ray photoelectron spectroscopy (XPS, Thermo ESCALAB 250Xi, USA) and X-ray diffractometer (Cu-Kα radiation, 2θ = 10-80°, 5°/min), respectively. The morphology was investigated by the transmission electron microscope with the energy dispersive X-ray spectroscopy (TEM, FEI, APREO, USA). Fluorescent spectra and UV–vis absorption spectra were recorded on a fluorescence spectrophotometer (FL, Hitachi F-4600, Japan) and a UV-8000PC spectrophotometer (Puxi, TU 1810, China), respectively. The Bru-nauer-Emmett-Teller surface were analyzed on a Micromeritics ASAP 2460 at 77.3 K.

Preparation of Co_3S_4 yolk-shell microspheres

In a typical synthesis process, 0.2734 g $Co(NO_3)_2•6H_2O$ were dissolved in the mixed solvents glycerol (7.5 mL) and isopropanol (52.5 mL) resulting a clear pink

solution. The formed mixture was then transferred into Teflon-sealed autoclave and heated at 150 °C for 24 h. Natural cooling to room temperature, the purple precipitate was obtained via centrifugation, rinsed with absolute ethanol six times and dried at 60 ^{o}C.

For the synthesis of hollow Co_3S_4 yolk-shell microspheres, 0.0300 g purple precipitate was dispersed into 20.0 mL ethanol solution including 0.0500 g thioacetamide (TAA). Then, the resulting solution was sealed in Teflon-sealed autoclave and performed at 180 ^{o}C for 12 h. After centrifugation and rinsed with distilled water and ethanol several times, the hollow Co_3S_4 coreshell nanospheres were finally synthesized.[1]

Assay of the peroxidase-like activity of Co_3S_4

Typically, 200 μL of Co_3S_4 aqueous suspension, 200 μL of H_2O_2 and 200 μL of TMB were added into 1400 μL buffer solution in turn, and the absorbance at 652 nm was recorded after reaction for 2 min. The optimal catalytic conditions of the Co_3S_4 nanozyme were evaluated by tuning pH values of buffer solutions as well as the various temperatures.

Steady-state kinetics

The catalytic kinetics of Co_3S_4 dual nanozymes was studied by changing the concentration of the substrates TMB or H_2O_2, and keep the other unchanged, respectively. Herein, the kinetic study was carried out using 200 μL of Co_3S_4 microspheres (0.3 mg/mL) in a reaction volume of 200 μL with 0.1 mM TMB or 25 mM H_2O_2. K_m and V_{max} were calculated by the double reciprocal graph of the

Michaelis-Menten equation, that is, $1/v = (K_m/V_{max}) \times (1/[S]) + 1/V_{max}$, where V_{max} and v represent the maximum reaction velocity and initial speed, as well as [S] and K_m represent the substrate concentration and Michaelis constant, respectively.

Assay of the oxidase-like activity

Briefly, 200 μL of Co_3S_4, 200 μL of Hg^{2+} and 200 μL of TMB were added into 1400 μL buffer solution reacted for 2 min, and the absorbance at 652 nm was recorded. Similar to the study of peroxidase-like activity of Co_3S_4, it was necessary to study the influences of the experimental temperatures and pH values on the oxidase-like activity.

Assay of detection of Hg^{2+}

Colorimetric Hg^{2+} detection based on the peroxidase-like activity of Co_3S_4

Firstly, different concentrations of Hg^{2+} (200 μL) and peroxidase-like nanozyme Co_3S_4 (200 μL, 0.3 mg/mL) were added to HAc-NaAc buffer (pH = 4) and incubated at room temperature for 2 min. Then, add H_2O_2 (1 mM, 200 μL) together with TMB (0.1 mM, 200 μL) into the above mixture reacted for 2 min and report the absorbance at 652 nm.

Colorimetric Hg^{2+} detection based on the oxidase-like activity of Co_3S_4

Different concentrations of Hg^{2+} (200 μL), Co_3S_4 (200 μL, 0.3 mg/mL) and TMB (0.1 mM, 200 μL) were added into HAC-NaAC buffer (pH = 5), which was incubated at room temperature for 2 min. Then, the absorbance at 652 nm was reported.

XPS spectra of HgS/Co_3S_4

From Fig. S9a, the elements Hg, Co, and S are detected in the final production

111 HgS/Co_3S_4. The Co 2p peak in HgS/Co_3S_4 shows a positive shift of 1.0 eV due to the
112 influence of the introduced Hg^{2+} (Fig. S9b). The typical peaks centered at 161.4 and
113 162.2 eV are S 2p1/2 and S 2p3/2, respectively, indicating that the oxidation state of
114 S^{2-} exists in Co_3S_4 (Fig. S9c). [2] For HgS/Co_3S_4, a decrease in the main peak at 165.9
115 eV is detected (Fig. S9c), indicating the influence of the produced HgS. In addition, as
116 seen from Fig. S9d, two peaks at 100.22 and 104.22 eV in HgS/Co_3S_4 indicate that Hg
117 mainly exists in the 2+ oxidation state. [3] Thereof, it can be suggested that the
118 produced HgS is attached to Co_3S_4 subsequently.

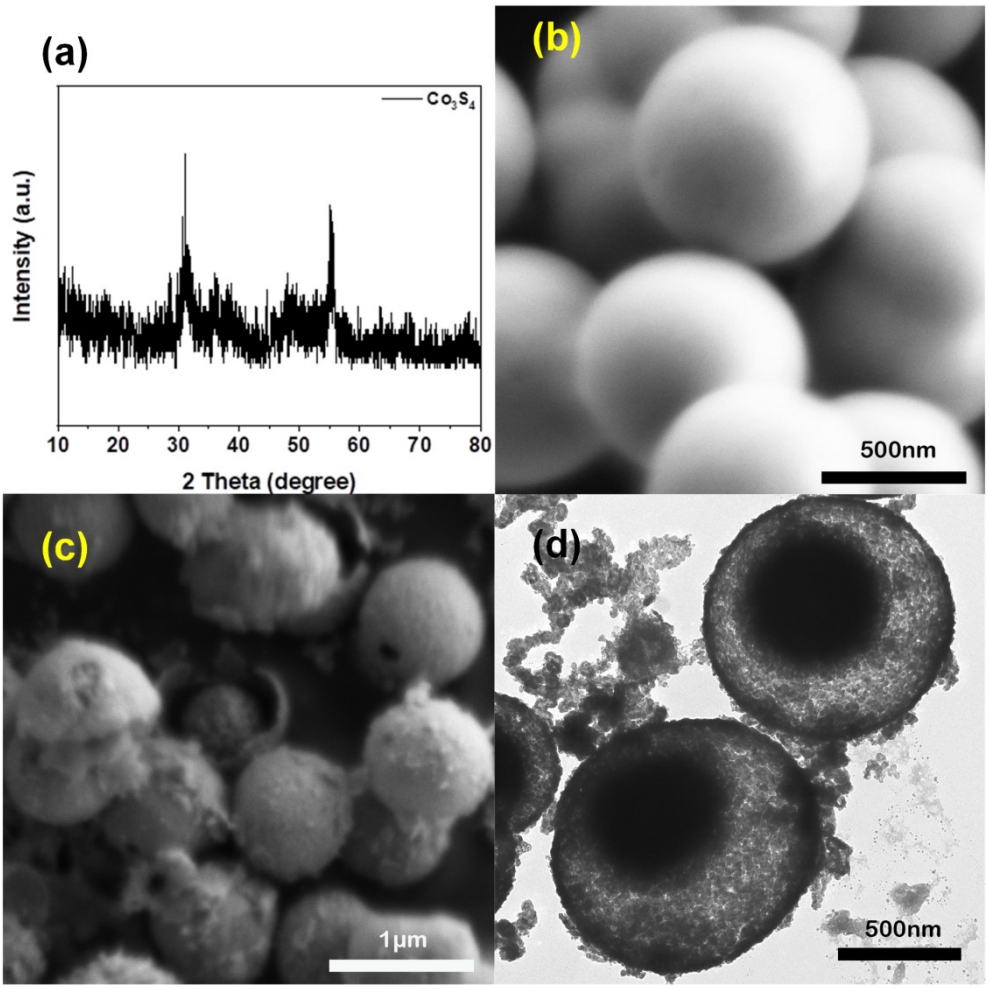


Fig. S1 XRD pattern of Co_3S_4 microspheres(a); SEM image of precursor (b); Images of SEM (c) and TEM (d) of yolk-shell Co_3S_4

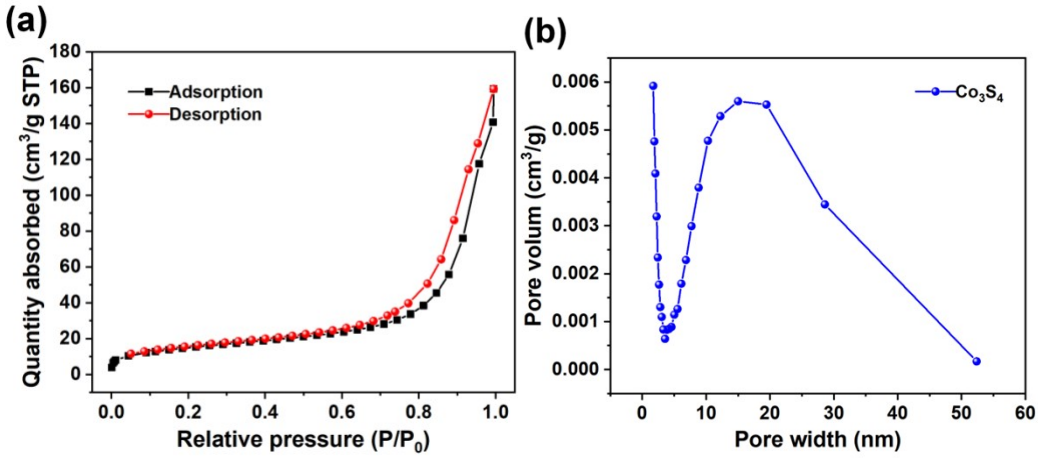


Fig. S2 N_2 adsorption/desorption isotherms (a) and the pore volume (b) of Co_3S_4 yolk-shell spheres.

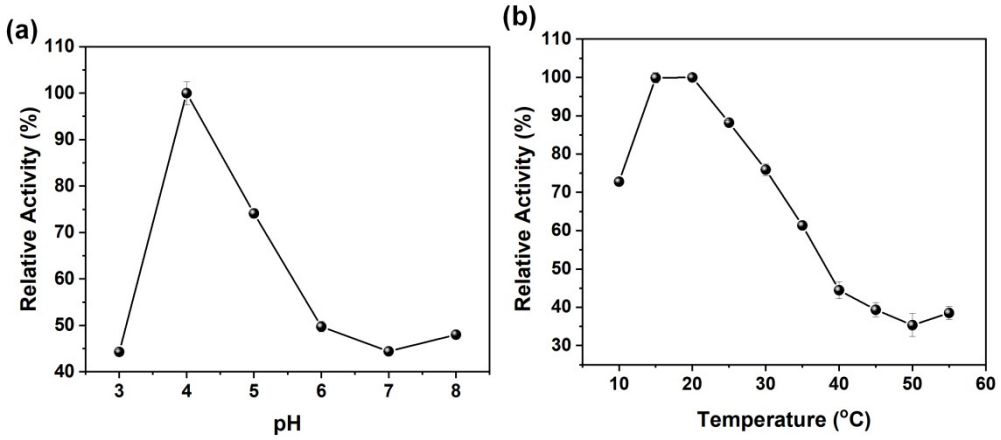


Fig. S3 Influences of the catalytic activity of Co_3S_4 on pH (a) and temperature (b), respectively.

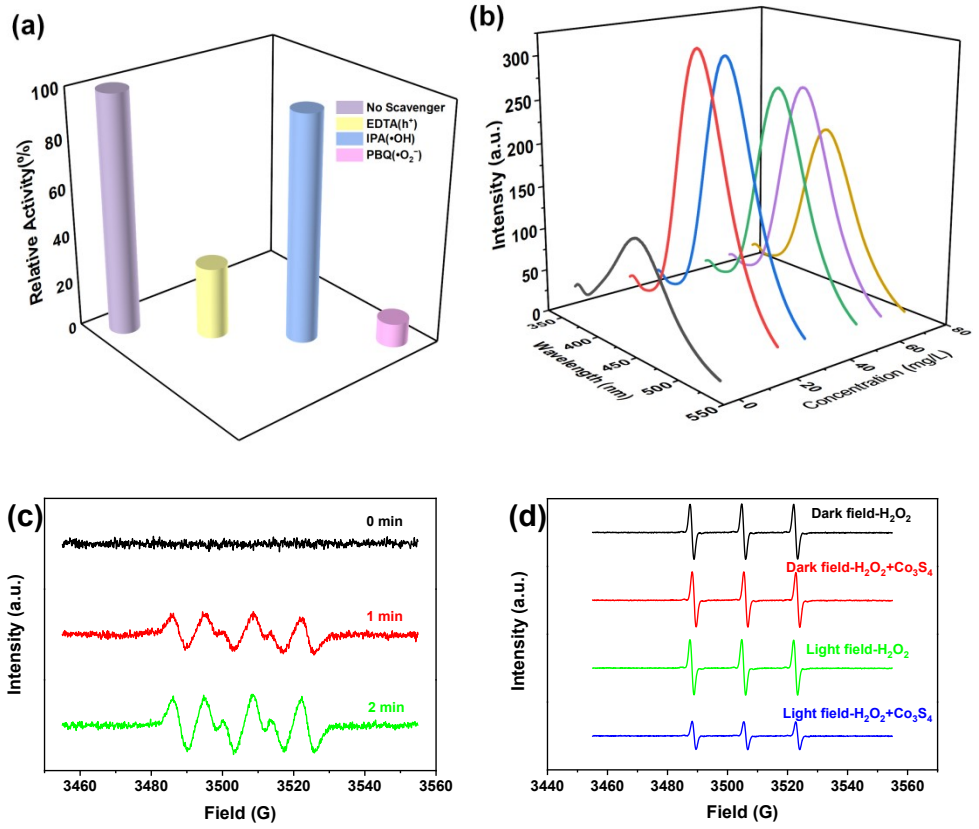


Fig. S4 (a) Effects of various active scavengers during the catalytic oxidation of TMB by Co_3S_4 under natural light. (b) Fluorescence intensity varies with the amount of Co_3S_4. Fluorescent tests: Co_3S_4 as catalyst, H_2O_2 24 mM, TA 0.5 mM, pH of 4, 20 °C, 30 min. (c) Generation of $•O_2^-$ by Co_3S_4 nanozymes in the presence of 1mM H_2O_2 (methanol system), and (d) photogenerated hole

in water system.

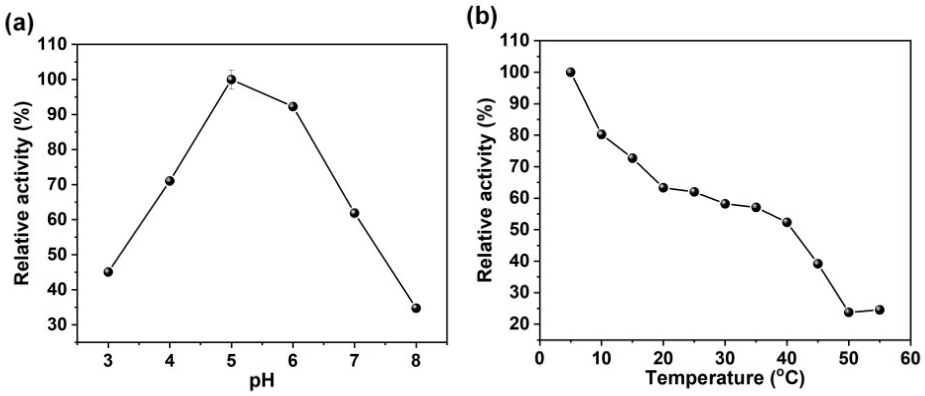


Fig. S5 Influences of pH (a) and temperature (b) on the oxidase-like activity of Co_3S_4, respectively.

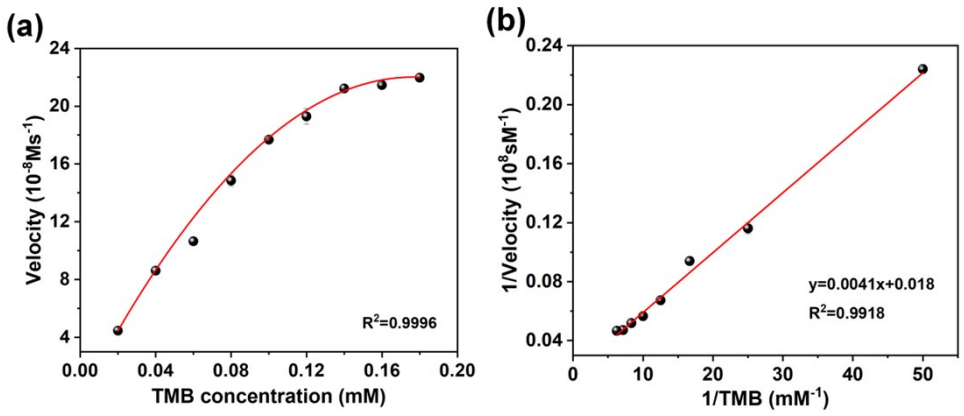
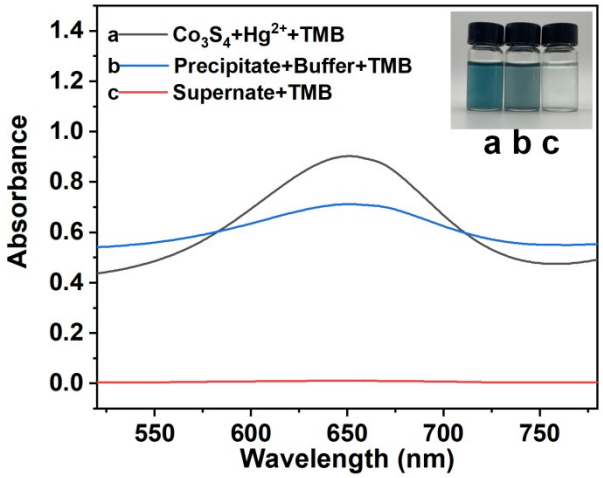


Fig. S6 Steady-state kinetic assay of Hg^{2+} triggered "ON" Co_3S_4 oxidase-like activity by (a) varying the concentrations of TMB from 20 to 200 μM with a fixed amount of Hg^{2+} (1 mM) (b) is corresponding double reciprocal curve towards TMB.



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148 **Fig. S7** Comparison of the oxidase-like activity whether precipitate (HgS/Co_3S_4) or supernate

149 taken from the reaction system after Hg^{2+} mixed with Co_3S_4 under natural light.

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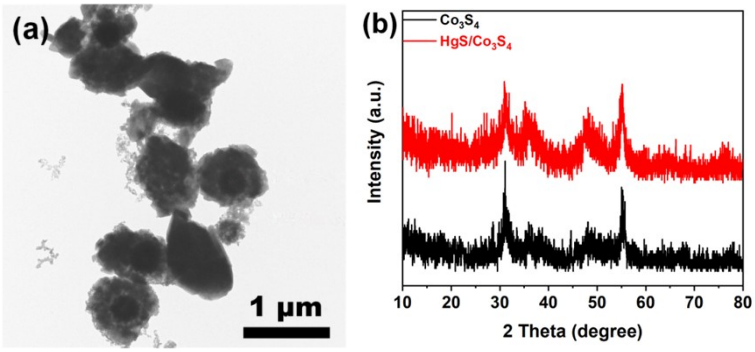

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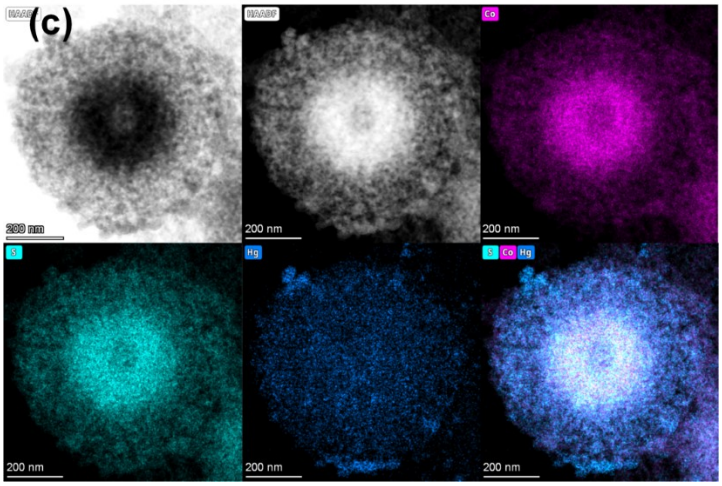

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153 **Fig. S8** TEM image of HgS/Co_3S_4 (a), XRD data of Co_3S_4 and HgS/Co_3S_4 (b), and TEM-EDX

154 mapping images of HgS/Co_3S_4 (c), respectively.

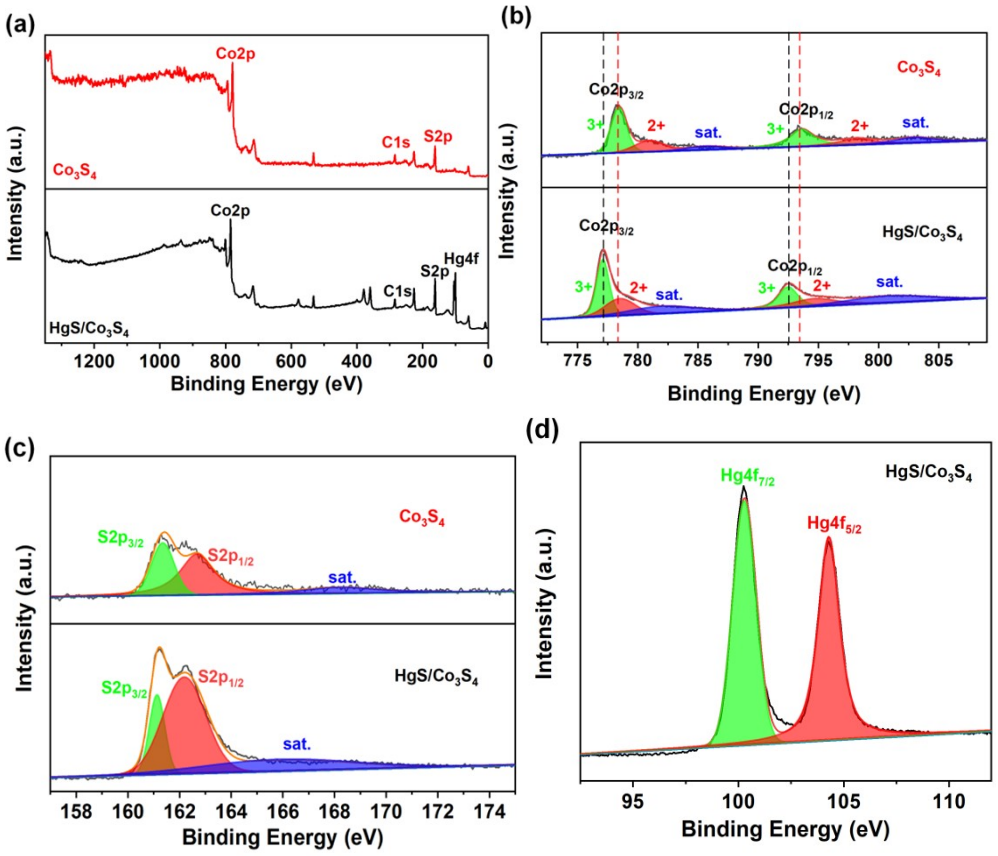

Fig. S9 High-resolution XPS spectra of Co_3S_4 and HgS/Co_3S_4: survey (a), Co 2p (b), S 2p (c) and Hg 4f (d), respectively.

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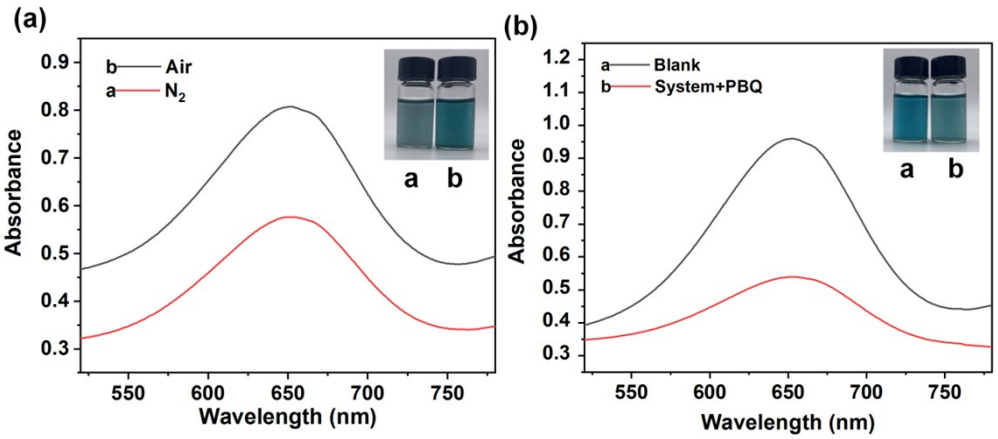

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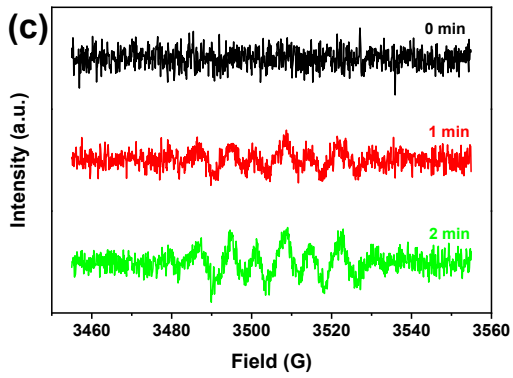

162 **Fig. S10 (**a) The influences on the oxidase-like activity of Hg-Co_3S_4 under air-saturated or N_2-

163 saturated conditions (inset: the corresponding photographs); **(**b**)** In the presence or absence of PBQ.

164 (c) Generation of $\bullet O_2^-$ by HgS/Co_3S_4 without the presence of H_2O_2 (methanol system).

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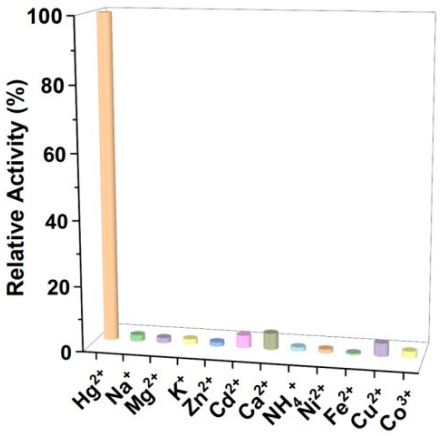

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167 **Fig. S11** The selectivity of the Co_3S_4-based sensor towards Hg^{2+} (1.0 mM) in the presence of

168 interferents (100 mM).

Table S1 Comparison of K_m of nanozymes towards TMB substrate.

Nanozymes	K_m/mM	Ref.
Co-V MMO (oxidase)	0.44	4
CeO_2 (oxidase)	0.80	5
Selenium (oxidase)	8.30	6
Co_3S_4 (peroxidase)	0.82	This work
Co_3S_4-Hg^{2+} (oxidase)	0.22	This work

Table S2 Comparison of Hg^{2+} detection based on Hg^{2+}-triggered Co_3S_4 oxidase-like activity with the previous studies.

Nanozymes	LOD (nM)	Ref.
GO-Ag (fluorimetric)	590	7
Ag/GO (colorimetric)	338	8
p-PDA/Ag(colorimetric)	800	9
Ficuscaricastem-Ag(colorimetric)	1060	10
Co_3S_4 (peroxidase)	170	This work
Co_3S_4-Hg^{2+}(oxidase)	55	This work

video.mp4

Video S1 Color changes of the reaction systems in the presence of Hg^{2+} or in the absence of Hg^{2+}.

Left system: Buffer solution (pH = 4), Co_3S_4 (0.3 mg/L), TMB (1 mM) and Hg^{2+} (1 mM).

Right system: Buffer solution (pH = 4), Co_3S_4 (0.3 mg/L) and TMB (1 mM).

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