Electronic Supplementary Information (ESI) for:

Glutathione-driven Cu (I)-O_2 chemistry: a new light-up fluorescent assay of intracellular glutathione

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Experimental section.

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Supplementary experimental section.

Materials and Reagents.

All chemicals are of analytical grade and used without further treatment. All chemicals including copper chloride ($CuCl_2$•$2H_2O$), selenium dioxide (SeO_2), sodium bicarbonate ($NaHCO_3$), polyvinyl-pyrrolidone (PVP, M_W 55000), terephthalic acid (TPA), glutathione and the oxidized glutathione (GSSG) were obtained from Aladdin (China). The CuO nanoparticels used for imaging assay was from Sigma-Aldrich (America). All solutions were prepared using ultra-pure water (Millipore, 18.2 MΩ).

Apparatus.

Powder X-ray diffraction (PXRD) pattern was obtained with a XRD-7000 X-ray diffractometer (Shimadzu, Kyoto, Japan). X-ray photoelectron spectra (XPS) of the as-prepared sample were characterized using an ESCALAB 250 X-ray photoelectron spectrometer (Thermo Fisher Scientific Inc., Waltham, MA, USA). Fluorescence spectra were performed with an F-2500 fluorescence spectrophotometer (Hitachi, Tokyo, Japan). Ultraviolet-visible light (UV-vis) absorption spectra were recorded with a UV-3600 spectrophotometer (Hitachi, Tokyo, Japan). Scanning electron microscopy (SEM) was performed on S-4800 scanning electron field emission microscope (Hitachi, Tokyo, Japan). The high-resolution transmission electron microscopy (HRTEM) and were conducted with a JEM-2010F field emission TEM (JEOL Ltd., Tokyo, Japan). Nitrogen (N_2) adsorption/desorption isotherms were measured by using an Rristar II 3020M Analyzer. Fluorescent imaging was performed on an IX81 confocal laser scanning microscope (Olympus, Japan). The fluorescent images were acquired with Image-Pro Plus 6.0 Software.

Preparation of Hollow Porous CuO MSs.

0.6 mmol $CuCl_2 \cdot 2H_2O$ (102.3 mg), 0.3mmol SeO_2 (33.3 mg), and 0.25 g PVP (M_W 55000) were dissolved in 8 mL distilled water, then, 2 mL $NaHCO_3$ solution (75 mg/mL) was added dropwise. Subsequently, the reaction mixture solution was transferred into a 25 mL-capacity Teflon-lined stainless steel autoclave and maintained at 160^{o}C for 12 h, and cooled to room temperature naturally. Finally, the precipitates were separated by centrifugation, washed with anhydrous ethanol and distilled water, and then dried at 80^{o}C in vacuum.

Procedure of GSH Detection.

The GSH detection sensing solutions was made by mixing 80 μL of 100 mM TPA, 40 μL of the hollow porous CuO MSs stock solution (10 mg/mL) and 50 μL GSH of different concentrations in 0.33 mL of 0.2 M phosphate buffer (pH 6.5). The mixed solutions were then stirring incubated in a 50^{o}C water bath for 30 min. The reaction solutions were centrifuged afterwards to separate unreacted CuO MPs and measured by F-2500 fluorescence spectrophotometer under the excitation wavelength of 315 nm.

Fluorescent imaging assay of the GSH in cells.

300 μL HeLa cells (about 1×10^5 cells per mL) were planted onto the bottom of an imaging dish in RPMI 1640 medium which is supplemented with 2% fetal bovine serum. Then the cells were cultured in an incubator (37^{o}C, 5% CO_2) for 24 h. The cells were cultured with 900 μL RPMI 1640 medium and 100 μL of different samples (Control; 50 μM of TPA only, 50 μM of TPA and 40 μg/mL of CuO nanoparticles; 50 μM of TPA and 40 μg/mL of CuO nanoparticles pretreated with 500 μM NEM for 30 min) for 2 h, and then were rinsed three times with the RPMI 1640 medium. After the treatment with T1 (0, 500 nM), cells were then used for imaging by an IX81 confocal microscope.

Supplementary figures.

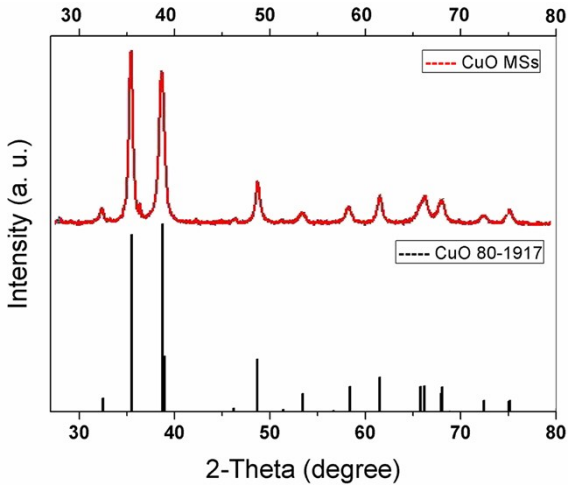

Fig. S1 PXRD pattern of the hollow porous CuO MSs and the CuO (JCPDS card no. 80-1917).

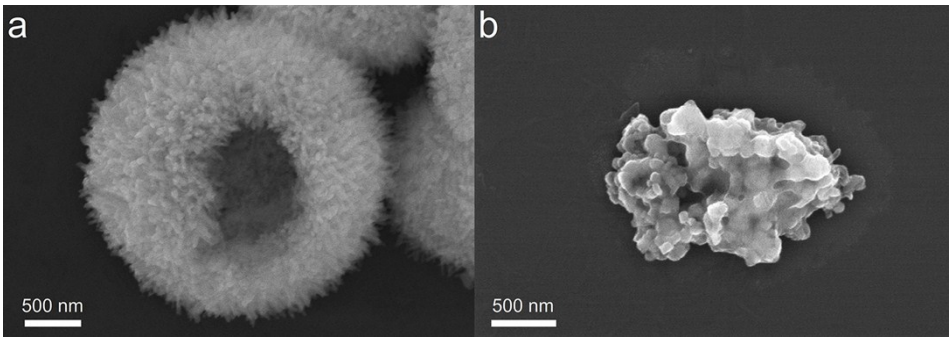

Fig. S2 SEM characterization of the CuO MSs (a) before and (b) after excessive GSH was added.

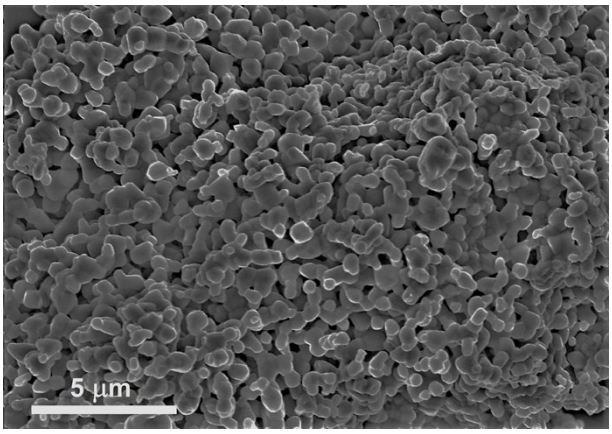

Fig. S3 SEM of the commercial available solid CuO MSs.

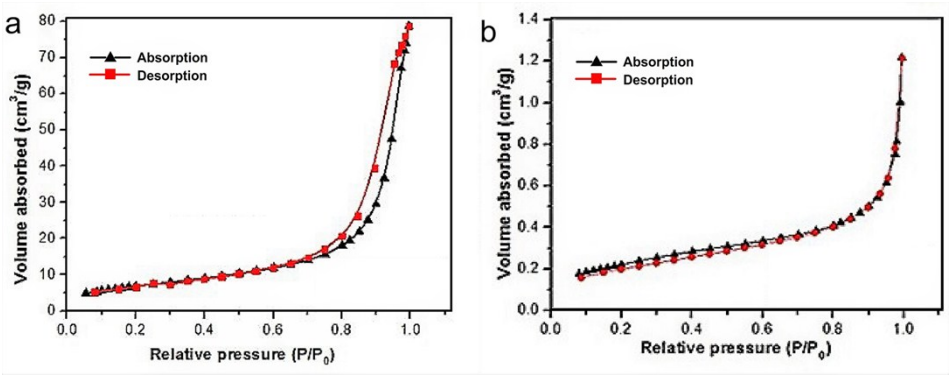

Fig. S4 Nitrogen adsorption–desorption isotherms of the (a) hollow porous CuO MSs and (b) commercial solid CuO MSs.

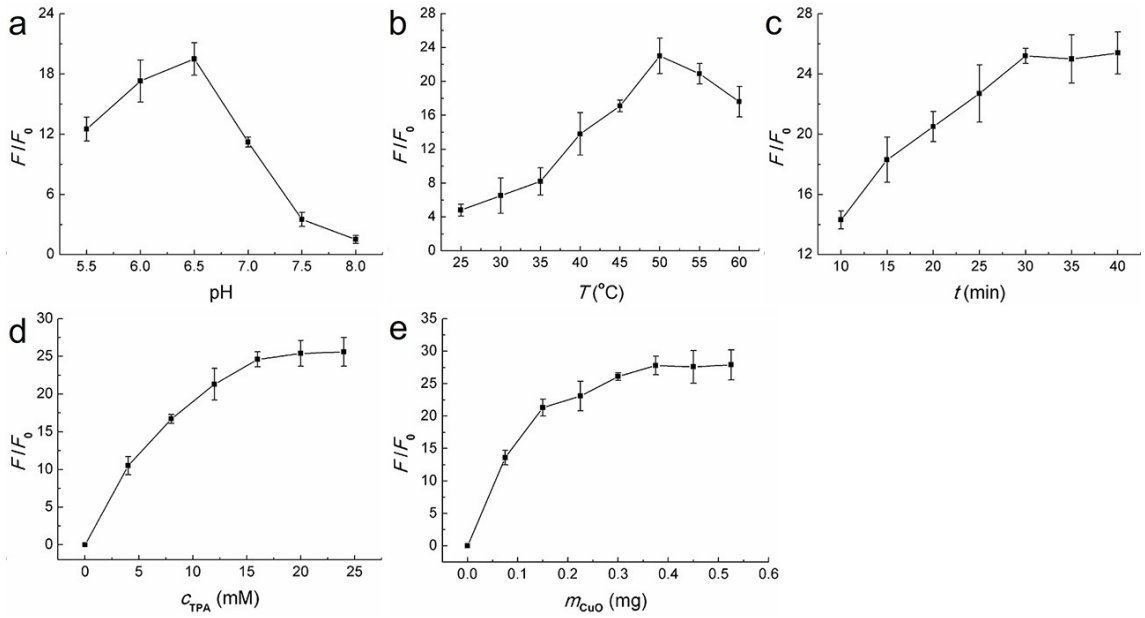

Fig. S5 Effects of (a) pH, (b) reaction temperature (*T*), (c) reaction time (*t*), (d) TPA concentration, (b) CuO dosage on the fluorescence response of CuO-O_2-TPA system. Experimental condition: For (a), 50 °C, 30 min; c_{GSH}, 5.0 × 10^{-5} M; c_{TPA}, 1.6 × 10^{-2} M; c_{CuO}, 0.4 mg/mL. For (b), pH 6.5, 30 min; c_{GSH}, 5.0 × 10^{-5} M; c_{TPA}, 1.6 × 10^{-2} M; c_{CuO}, 0.4 mg/mL. For (c), pH 6.5, 50 °C, 30 min; c_{GSH}, 5.0 × 10^{-5} M; c_{TPA}, 1.6 × 10^{-2} M; c_{CuO}, 0.4 mg/mL. For (d), pH 6.5, 50 °C, 30 min; c_{GSH}, 5.0 × 10^{-5} M; c_{CuO}, 0.4 mg/mL. For (e), pH 6.5, 50 °C, 30 min; c_{GSH}, 5.0 × 10^{-5} M; c_{TPA}, 1.6 × 10^{-2} M. Error bars represent the standard deviations (n = 3).

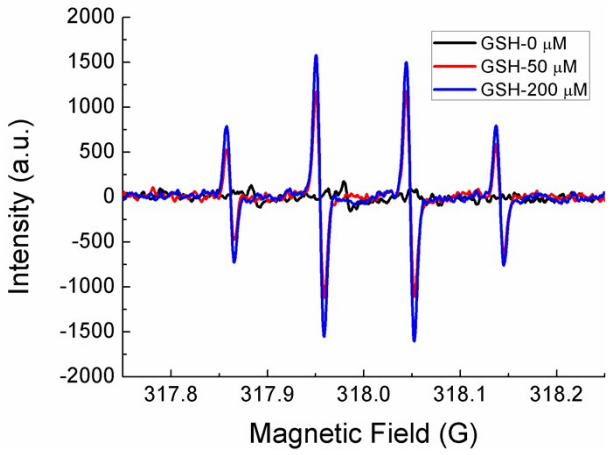

Fig. S6 ESR determination of the GSH-dose dependent formation of •OH by CuO MSs (pH 6.5 PBS).

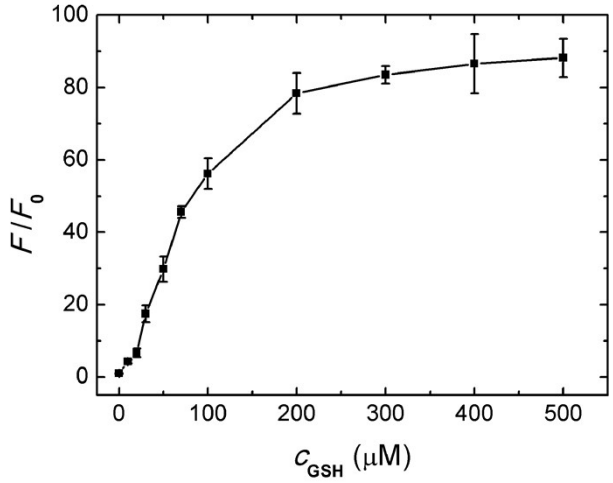

Fig. S7 The trend of fluorescence intensity (F/F_0, at 430 nm) of the CuO MSs-O_2-TPA system at large range of GSH concentrations from 0 to 500 μM. Experimental condition: pH 6.5; 50°C, 30 min; c_{TPA}, 1.6 × 10^{-2} M; c_{CuO}, 0.4 mg/mL.

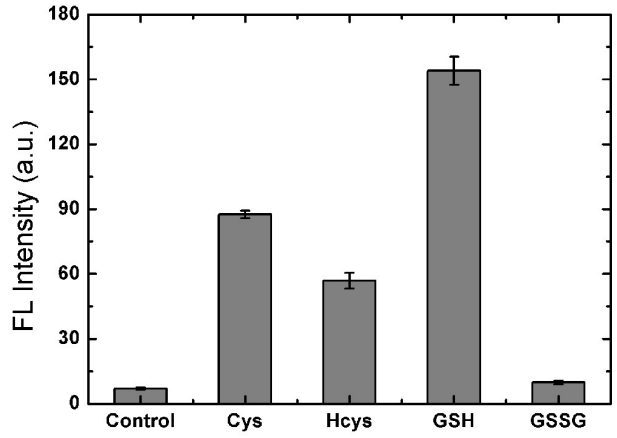

Fig. S8 Fluorescence intensity of the CuO MSs-O_2-TPA system with different biothiols. Experimental condition: pH 7.4, 50°C, 30 min; c_{thoils}, 5.0 × 10^{-5} M; c_{TPA}, 1.6 × 10^{-2} M; c_{CuO}, 0.4 mg/mL.

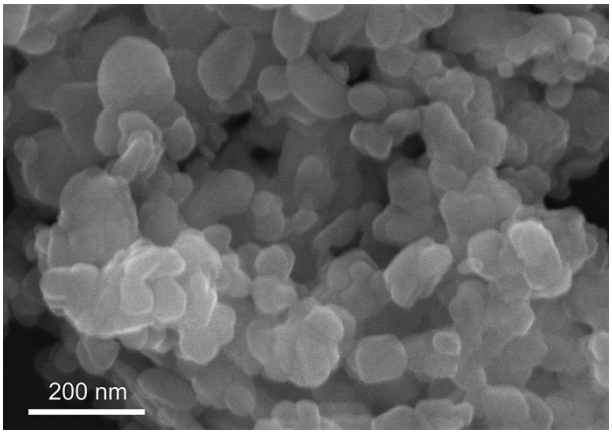

Fig. S9 SEM of the CuO nanoparticles used for the intracellular GSH fluorescent assay.

Supplementary table.

Table S1 | Concentrations of the investigated interferants.

Interferent	Concentration
Histidine (His)	1 mM
Aspartic (Asp)	1 mM
Proline (Pro)	1 mM
Valine (Val)	1 mM
Isoleucine (Ile)	1 mM
Lysine (Lys)	1 mM
Alanine (Ala)	1 mM
Arginine (Arg)	1 mM
Serine (Ser)	1 mM
Threonine (Thr)	1 mM
Na^+	0.2 mM
K^+	0.25 mM
Zn^{2+}	7μM
Mg^{2+}	40 μM
Fe^{3+}	0.25 μM
Ca^{2+}	2 mM
Glucose (Glu)	1 mM
Vitamine C (Vc)	7 μM
Homocysteine (HCy)	2 μM
Cysteine (Cys)	32 μM