A sensitive turn-on fluorescence probe for intracellular imaging of glutathione using single-layer MnO$_2$ nanosheets-quenched fluorescent carbon quantum dots

Dinggeng He, Xiaoxiao Yang, Xiaoxiao He,* Kemin Wang,* Xue Yang, Xing He and Zhen Zou

State Key Laboratory of Chemo/Biosensing and Chemometrics, College of Chemistry and Chemical Engineering, College of Biology, Hunan University, Key Laboratory for Bio-Nanotechnology and Molecular Engineering of Hunan Province, Changsha 410082, China.

*E-mail: kmwang@hnu.edu.cn; xiaoxiaohe@hnu.edu.cn.
EXPERIMENTAL SECTION

Materials. Manganese chloride tetrahydrate (MnCl$_2$•4H$_2$O), hydrogen peroxide (H$_2$O$_2$, 30 wt%), tetramethylammonium hydroxide pentahydrate (TMA•OH), L-cysteine (Cys) and L-glutathione reduced (GSH) were purchased from Alfa Aesar (China). 3-[4,5-dimethylthialzol-2-yl]-2,5-diphenyltetrazolium bromid (MTT) and DL-homocysteine (HCys) were purchased from Sigma-Aldrich (USA). Dimethyl sulfoxide (DMSO) was obtained from Xilong reagent company (Guangdong, China). Citric acid monohydrate was obtained from Shanghai Shiyi Chemicals Reagent Co., Ltd. (China) and urea was obtained from Sinopharm Chemicals Reagent Co., Ltd. (China). All chemicals were of analytical grade and were used as received without further purification. All aqueous solutions were prepared using ultrapure water, which was prepared through a Millipore Milli-Q water purification system, with an electrical resistance >18.2 MΩ.

Apparatus and characterization. Ultraviolet-visible light (UV-vis) absorption spectra were recorded on a UV-2600 UV-vis spectrometer (Shimadzu, Japan) in the range of 200-700 nm. Fluorescence spectra were collected using a Hitachi Model F-7000 Fluorometer (Hitachi Co., Ltd., Japan). The samples were excited at 350 nm and the fluorescence emission ranged from 365 nm to 600 nm, in steps of 1 nm and fluorescence measurements were performed at room temperature under ambient conditions. Atomic force microscopy (AFM) images of samples were analyzed on a Multimode 8 (Bruker, USA). Transmission electron microscope image was obtained on a F20 field-emission transmission electron microscope and an accelerating voltage of 200 KV. Zeta potential and DLS measurements were performed at 25°C using a Nano ZS90 laser particle analyzer (Malvern Instruments, UK), equipped with a He-Ne laser (633 nm) at a fixed scattering angle of 90°. Fourier transform infrared (FT-IR) spectra were obtained from a TENSOR 27 spectrometer (Bruker Instruments Inc., Germany) in the range of 4000-400 cm$^{-1}$. The MTT assay was obtained in a Benchmark Plus, Biorad Instruments Inc, Japan. The confocal laser scanning
microscopy (CLSM) images were obtained on a Fluoview FV500, Olympus.

**Synthesis of MnO$_2$ nanosheets.** Single-layer MnO$_2$ nanosheets were synthesized according to a literature procedure reported previously.$^1$ In a typical reaction, 20 mL of mixture containing 0.6 M TMA•OH and 3.0 wt% H$_2$O$_2$ were added to 10 mL aqueous solution of 0.3 M MnCl$_2$•4H$_2$O within 15 s. The resulting mixture a dark brown suspension was formed, then the product was stirred vigorously overnight in the open air at room temperature, which was accompanied by the generation of oxygen. Subsequently, the crude product was collected by centrifugation at 10000 rpm for 10 min, washed with alcohol and water several times and finally the solid was then placed under high vacuum at -60 °C for 12 h to remove the residual solvent. To acquire 1 mg mL$^{-1}$ MnO$_2$ solution, 30 mg MnO$_2$ solid was dispersed in 30 mL ultrapure water and was degraded by ultrasonic cleaning machine and ultrasonic cell crasher.

**Preparation of nitrogen-doped fluorescent carbon quantum dots (CQDs).** Here, the CQDs were compounded utilizing microwave synthesis methods.$^2$ 10 mL mixture containing 3 g citric acid monohydrate and 3 g urea were then heated for about 4 min in a domestic 800 W microwave oven, after the solution changed from a colorless liquid to a dark-brown clustered solid, suggesting that the crude product was formed. Afterwards, the solid was then heated at 60 °C vacuum oven for 1 h to remove the remaining small molecules. Then the solid was redispersed in 20 mL of ultrapure water. After that the aqueous solution of the CQDs was purified by centrifugation at 3000 rpm for 20 min to remove large or agglomerated particles. The concentration of CQDs solution was acquired by dried 1 mL CQDs solution in 80 °C revolve vacuum oven for 4 h and the solution was diluted according to the demand.

**Fluorescence quenching of the single-layer MnO$_2$ nanosheets toward CQDs.** On the basis of the procedure above, various concentrations MnO$_2$ nanosheets (0 to 100 μg mL$^{-1}$) were added into CQDs (2.4 μg mL$^{-1}$) reaching a total volume of 200 μL.
for each sample. The fluorescence detections were performed.

**Analysis of GSH in aqueous solutions.** For fluorescent sensing detection of GSH solution, the turn-on fluorescence probe based on MnO$_2$-CQDs nanocomposite was first prepared by hybridized MnO$_2$ nanosheets (30 μg mL$^{-1}$) with CQDs (2.4 μg mL$^{-1}$), and then various concentrations of GSH (0 to 200 μM) were added into MnO$_2$-CQDs nanocomposite, respectively, reaching a total volume of 200 μL for each sample. The mixtures were incubated at room temperature for 5 min and then the fluorescence detections were performed.

**Selectivity of the fluorescence nanocomposite probe toward GSH.** To explore the selectivity of the fluorescence probe toward GSH. Different electrolytes or biomolecules (KCl, NaCl, Na$_2$SO$_4$, MgSO$_4$, MnCl$_2$, Gly, Glu, Glucose, Sucrose, BSA, Cys, HCys and GSH) were added into MnO$_2$-CQDs nanocomposite solutions, respectively, reaching a total volume of 200 μL for each sample. After being incubation with GSH (200 and 500 μM), and different electrolytes and biomolecules (1 mM for each; 1 mg mL$^{-1}$ for BSA, 500 μM for Cys, HCys) for 5 min at room temperature, fluorescence intensities of MnO$_2$-CQDs nanocomposite solutions were detected.

**Cytotoxicity experiment.** The cytotoxicity of MnO$_2$-CQDs nanocomposite and CQDs were evaluated by an MTT assay using HeLa cells. HeLa cells were cultured in 96-well plates at a density of $7 \times 10^3$ cells per well in 200 μL of medium containing 10% fetal bovine serum (FBS) and incubated at 37 °C and 5% CO$_2$. After grown for 24 h, various concentrations of MnO$_2$-CQDs nanocomposite (0-100 μg mL$^{-1}$) and CQDs (0-120 μg mL$^{-1}$) in culture medium were added into each well and then incubated for 24 h. MTT was used to assess cell viability. The MTT solution (0.5 mg mL$^{-1}$) was further incubated with cells in fresh medium for another 4 h. Then the medium was withdrawn and 150 μL of DMSO was added into each well to dissolve the precipitated formazan violet crystals at 37 °C for 10 min. The absorbance was
measured at 490 nm by a multidetection microplate reader.

**Cell imaging.** HeLa cells were maintained at 37 °C in 5% CO₂ in RPMI media. The cells were plated at around 60-70% confluency 24 h before imaging experiments in 35-mm culture dishes. Prior to imaging experiments, the HeLa cells were treated with ALA (500 μM) for 24 h to enhance the GSH concentration. To decrease GSH concentration in living cells, HeLa cells were treated with NEM (500 μM) for 20 min. Both cell lines were washed for three times with cell culture media and incubated with MnO₂-CQDs nanocomposites (20 μg mL⁻¹) for 3 h. The cell lines were further washed with cell culture media and subsequently imaged at ambient temperature.

To further investigate the capacity of the probe to distinguish the normal cells and cancer cells, HepG2, HeLa and L02 cells were incubated with MnO₂-CQDs nanocomposites (20 μg mL⁻¹) for 3 h. The cell lines were then washed with cell culture media and imaged at ambient temperature.
**Fig. S1** AFM images of CQDs (a, b) and single-layer MnO$_2$ nanosheets (c, d) deposited on mica substrates.

**Fig. S2** DLS results of fluorescent CQDs (a) and single-layer MnO$_2$ nanosheets (b).
Fig. S3 FT-IR of CQDs, MnO\textsubscript{2} nanosheets and MnO\textsubscript{2}-CQDs nanocomposite.

Fig. S4 TEM (a) and high-resolution TEM (b) images of MnO\textsubscript{2}-CQDs nanocomposite.
Fig. S5 The fluorescence quenching efficiency of the different concentrations of MnO$_2$ nanosheets toward CQDs in H$_2$O (black) and RPMI 1640 medium (red). \((F_0 - F)/F_0\) as a function of MnO$_2$ concentrations. F and F$_0$ are fluorescence intensities of CQDs solutions with and without MnO$_2$ nanosheets, respectively.

Fig. S6 Fluorescence emission spectra (a) and the white light/UV irradiation digital photographs (b) of fluorescent CQDs, quenching CQDs and dequenching CQDs solution.
**Fig. S7** Time dependence of fluorescence intensity at 447 nm for the MnO$_2$-CQDs nanocomposites in the presence of GSH (1 mM).

**Fig. S8** Optimization of MnO$_2$ concentration for GSH sensing. $F_0$ is the fluorescence emission intensity of CQDs (2.4 µg mL$^{-1}$) in the presence of MnO$_2$ nanosheets; F is that in the presence of MnO$_2$ nanosheets and GSH (100 µM).
Fig. S9 (a) Fluorescence emission spectra of probe with use of 60 μg mL⁻¹ MnO₂ nanosheets in the presence of different GSH concentrations. (b) The plot of the fluorescence intensities versus GSH concentrations. The approach using 60 μg mL⁻¹ MnO₂ nanosheets showed a lower fluorescence background than that using 30 μg mL⁻¹ MnO₂ nanosheets. By the addition of GSH, the fluorescence of CQDs quenched by MnO₂ nanosheets was restored. The fitted curve could be used for the quantification of GSH with a correlation coefficient of 0.9956. And the detection limit as low as 1.71 μM could be reached based on the definition of three times the deviation of the blank signal (3σ).

Fig. S10 Viability of HeLa cells after being incubated with different concentrations of CQDs for 24 h.
Fig. S11 CLSM images of HepG2, HeLa and L02 cells after being incubated with MnO$_2$-CQDs nanocomposite (20 μg mL$^{-1}$) for 3 h. Top row of images show fluorescence microscopy images; bottom row of images show the overlap of fluorescence and bright-field images. The scale bar is 20 μm.

Fig. S12 Photoluminescence intensities of HepG2, HeLa and L02 cells measured after treatment with different chemicals and MnO$_2$-CQDs nanocomposites.
Table S1. Comparison of Different Fluorescence Methods for GSH Sensing.

<table>
<thead>
<tr>
<th>probe</th>
<th>principle</th>
<th>linear range</th>
<th>LOD</th>
<th>note</th>
<th>ref.</th>
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<tbody>
<tr>
<td>Coumarin-malonitrile conjugate</td>
<td>Michael addition</td>
<td>no give</td>
<td>no give</td>
<td>complicated operation, special equipment, poor photostability</td>
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<tr>
<td>organoselenium fluorescent probe</td>
<td>selenium-nitrogen bond</td>
<td>0.015-1.0 μM</td>
<td>144 pM</td>
<td>complicated operation, time-consuming, require purification</td>
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<tr>
<td>naphthalimide-based colorimetric fluorescent probe</td>
<td>disulfide bonds</td>
<td>0.5-10 mM</td>
<td>28 μM</td>
<td>low sensitivity, complicated operation, time-consuming</td>
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<td>CdSe-ZnS QDs-MV²⁺</td>
<td>metal complexes-displace coordination</td>
<td>5-250 μM</td>
<td>0.6 μM</td>
<td>complicated operation, time-consuming, inherent toxicity</td>
<td>6</td>
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<tr>
<td>CdTe-Hg(II)</td>
<td>metal complexes-displace coordination</td>
<td>0.6-20 μM</td>
<td>0.1 μM</td>
<td>inherent toxicity, chemical instability</td>
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<tr>
<td>g-CNQDs-Hg²⁺</td>
<td>metal complexes-displace coordination</td>
<td>0.16-16 μM</td>
<td>37 nM</td>
<td>rigorous pH conditions, toxic heavy metal, disable for cellular assay</td>
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<tr>
<td>Fe₃O₄@PFR@Au nanocomposites</td>
<td>metal complexes-oxidation-reduction</td>
<td>0.8-10 μM</td>
<td>0.5 μM</td>
<td>costly material, low sensitivity, rigorous pH conditions</td>
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<td>MnO₂-modified UCNPs</td>
<td>GSH-decomposable MnO₂ nanosheets</td>
<td>no given</td>
<td>0.9 μM</td>
<td>low sensitivity, special equipment, complicated operation</td>
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<td>g-C₃N₄-MnO₂</td>
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<td>200-500 μM</td>
<td>0.2 μM</td>
<td>sophisticated technique, complicated operation, high temperature</td>
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<tr>
<td>MnO₂-CQDs nanocomposites</td>
<td>GSH-decomposable MnO₂ nanosheets</td>
<td>1-200 μM</td>
<td>10 nM</td>
<td>high sensitivity, good biocompatibility, no label, low cost</td>
<td>this work</td>
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</table>
References