

Electronic Supplementary Information

A FRET-based carbon dots-MnO₂ nanosheets architecture for glutathione sensing in human whole blood samples

Yuhui Wang,^a Kai Jiang,^{a,b} Jiali Zhu,^{a,c} Ling Zhang^a and Hengwei Lin^{a*}

^a Ningbo Institute of Materials Technology & Engineering (NIMTE), Chinese Academy of Sciences, Ningbo 315201, P. R. China.

^b Department of Applied Physics, Chongqing University, Chongqing 401331, P. R. China.

^c School of Materials Science and Engineering, Shanghai University, Shanghai 200444, P. R. China.

*E-mail: linhengwei@nimte.ac.cn

Experimental Section

1. Reagents and instruments. All chemicals are of at least analytical-pure reagent and used directly without further purification. Citric acid and potassium permanganate were purchased from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). C-dots were purified by a dialysis membrane with a molecular weight cutoff (MWCO) of 500 (Spectra, America). The other reagents were obtained from Aladdin Reagent Co., Ltd (Shanghai, China). Human whole blood samples were collected from healthy volunteers by Zhongnan Hospital of Wuhan University. Deionized (DI) water was used to prepare aqueous buffer.

The morphology of the fluorescent C-dots and MnO₂ nanosheets were characterized by a Tecnai F20 transmission electron microscope (TEM). The FT-IR spectrum of the C-dots was performed on a Nicolet 6700 spectrometer (Thermo Fisher, America) with KBr pellet technique. The UV-vis absorption spectrum of MnO₂ nanosheets was recorded on a PERSEE T10CS spectrophotometer (Beijing, China). Fluorescence spectra were measured with a Hitachi F-4600 spectrophotometer (Hitachi, Japan). The fluorescence lifetimes of the C-dots and the C-dots-MnO₂ composite were measured using Fluorolog 3-11 (HORIBA, Jobin Yvon Inc). The absolute fluorescence quantum yield of the C-dots was determined by a Fluoromax-4 quantum yield measurement system (HORIBA, Jobin Yvon Inc).

2. Synthesis of the C-dots. An aqueous mixture (15 mL) comprising citric acid (1.05 g), 6-aminocaproic acid (0.65 g) and NaOH (0.2 g) was transferred into a 25 mL Teflon autoclave and heated to 200 °C for 5 h. Thereafter, the Teflon autoclave was cooled to room temperature naturally, and a brown fluid was obtained. The fluid was extensively dialyzed against DI water through a dialysis membrane (500 MWCO) for three days. After that, the solution was concentrated and dried under vacuum. Finally, a tawny powder (*ca.* 180 mg) was obtained.

3. Preparation of the C-dots-MnO₂ composite. The composite of the C-dots and MnO₂ nanosheets was prepared following the literatures.^{S1,S2} Briefly, 30 μL KMnO₄ solution (100 mM) was added into a 970 μL 2-(N-morpholino)ethanesulfonic acid (MES) aqueous

buffer (0.1 M, pH 6.0) containing 10 μg of the C-dots. Subsequently, the mixture was sonicated for 0.5 h until a brown solution formed. Then, the brown mixture was centrifuged with 12000 rpm for 15 min to obtain a precipitate. The precipitate was then washed twice with DI water, and finally diluted with 500 μL HEPES buffer (20 mM, pH 7.2). As a comparison, MnO_2 nanosheets were as well as prepared with the same procedure in the absence of C-dots.

4. GSH detection based on the FRET sensing system of C-dots- MnO_2 nanosheets.

First of all, various amounts of KMnO_4 (100 mM) were added into the C-dots (10 $\mu\text{g}/\text{mL}$) MES buffer and sonicated for 0.5 h. After purification and treatment of the mixture by the above described procedure, the samples were subjected to the fluorescence measurements. In the process of GSH detection, different amounts of GSH were added into the dispersion of C-dots- MnO_2 composite that was prepared at the optimized condition (i.e. 10 $\mu\text{g}/\text{mL}$ C-dots and 0.3 mM MnO_2 nanosheet). After 10-min equilibrium, the fluorescence spectra of the mixture solutions were recorded. To investigate the selectivity of the sensor, interferents were added into the C-dots- MnO_2 dispersion in place of GSH under the same experimental conditions. All fluorescence measurements were performed in HEPES buffer (20 mM, pH 7.2). All the fluorescence was recorded with the excitation at 325 nm (based on their fluorescence excitation spectrum, Fig. S1B), and the emission intensity at 430 nm was taken for quantitative analysis.

5. Procedure for determining GSH concentration in human whole blood.

Human whole blood sample was collected in a heparin anticoagulated tube. The blood sample was pretreated according to the procedure reported in the literatures.^{S3-S5} In short, 20 μL serine-borate complex (100 mM) was added into 1 mL whole blood to inhibit gamma-glutamyltranspeptidase (GGT) activity. Then, the sample was centrifuged at 12000 rpm for 15 min at room temperature to remove haemocytes. The resulting supernatant fluid was diluted 100-fold and added into the FRET-based sensing system immediately. The subsequent operations and fluorescence measurements were the same as above. The concentration of GSH in blood was calculated using the calibration curve that obtained in plasma matrix.

Characterizations

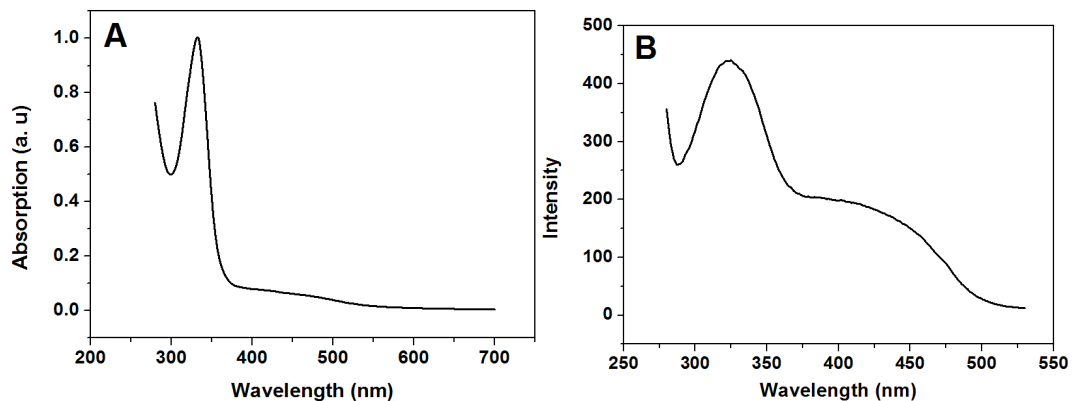


Fig. S1 (A) UV-Vis absorption spectrum of the C-dots. (B) Fluorescence excitation spectrum of the as-prepared C-dots.

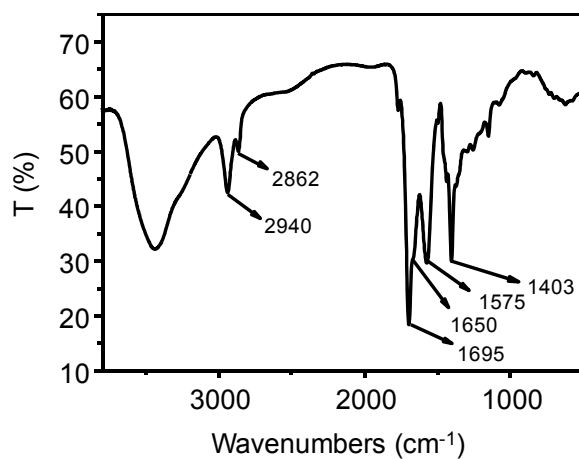


Fig. S2 FT-IR spectrum of the carboxyl functionalized C-dots.

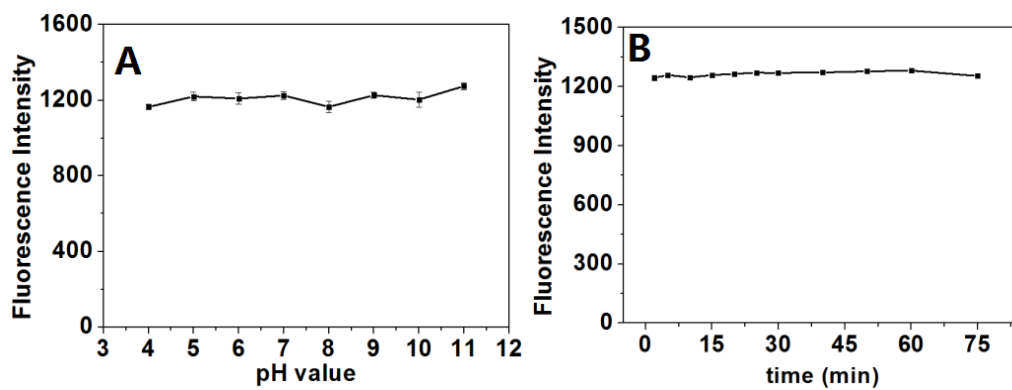


Fig. S3 (A) The fluorescence emission intensity of the C-dots at 430 nm versus pH. (B) The relationship between fluorescence emission intensity of the C-dots at 430 nm and the period of time excitation ($\lambda_{ex}=325$ nm).

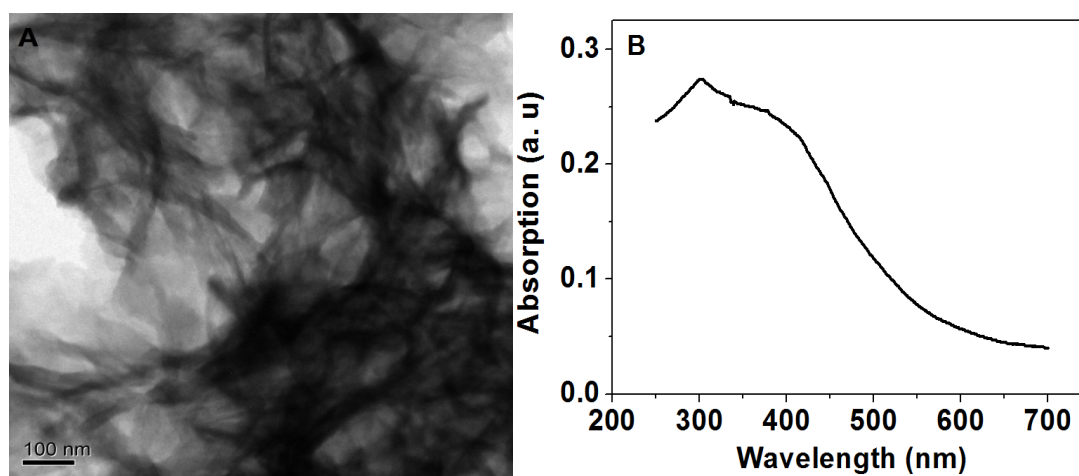


Fig. S4 (A) TEM image of MnO₂ nanosheets. (B) UV-vis absorption spectrum of MnO₂ nanosheets (0.3 mM).

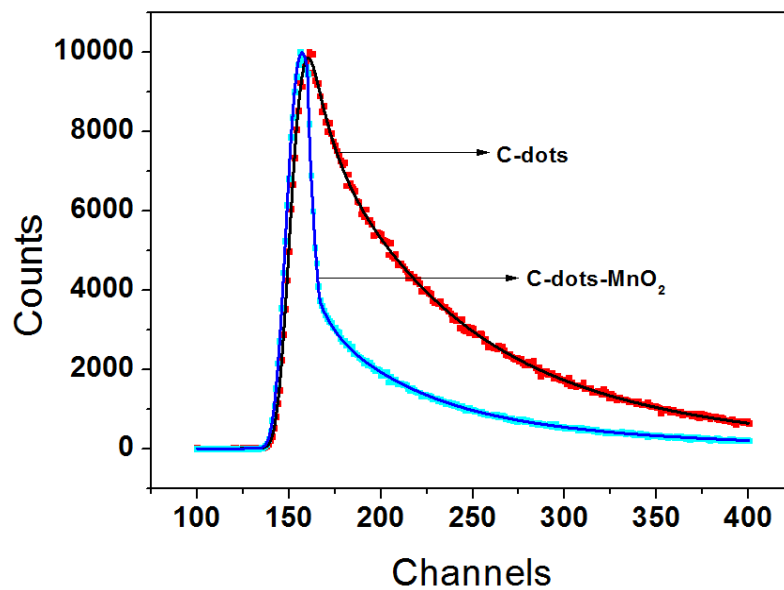


Fig. S5 Fluorescence decays of the C-dots and the C-dots-MnO₂ nanosheets composite (10 $\mu\text{g/mL}$ C-dots and 0.3 mM MnO₂).

Table S1. Fitting results of the fluorescence lifetimes for the C-dots and the C-dots-MnO₂ hybrid.

Sample	τ_1 (ns)	A ₁ (%)	τ_2 (ns)	A ₂ (%)	τ_3 (ns)	A ₃ (%)	τ_{average} (ns)
C-dots	4.4	31.8	8.9	56.0	17.8	12.2	10.4
C-dots-MnO₂	1.3	69.9	2.3	25.1	4.6	6.0	2.1

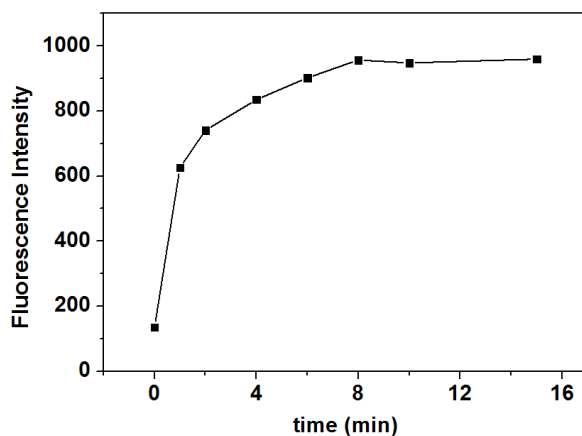


Fig. S6 Kinetics of fluorescence restoration (intensity at 430 nm) of the C-dots-MnO₂ composite in the presence of 0.6 mM GSH.

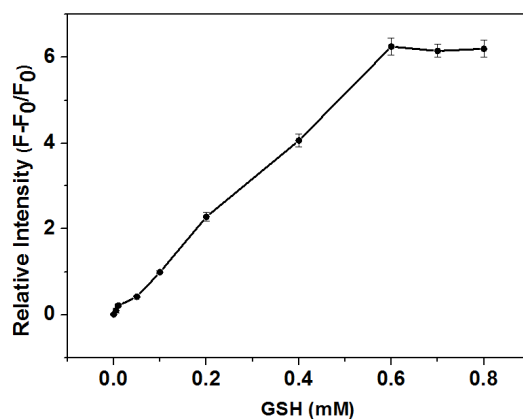


Fig. S7 Fluorescence restoration of the C-dots-MnO₂ nanosheet composite in the presence of various concentrations of GSH (F and F₀ represent the fluorescence intensity in the presence and absence of GSH, respectively).

Table S2. Comparison of the current work with non-fluorescence approaches for the detection of GSH.

Method	Linear range	LOD	Reference
AuNPs-based colorimetry	0.025 ~ 2.28 μ M	17 nM	S6
AuNPs-based colorimetry	8 ~ 250 nM	8 nM	S7
AuNPs-based colorimetry	0 ~ 6 μ M	29 nM	S8
electrochemistry	5 ~ 1250 nM	5 nM	S9
spectrophotometry	0.182 ~ 45.5 μ M	34.9 nM	S10
HPLC	2.5 ~ 1250 nM	2.5 nM	S11
electrophoresis	2.5 ~ 30 μ M	2.5 μ M	S12
current work	0.2 ~ 600 μ M	22 nM	--

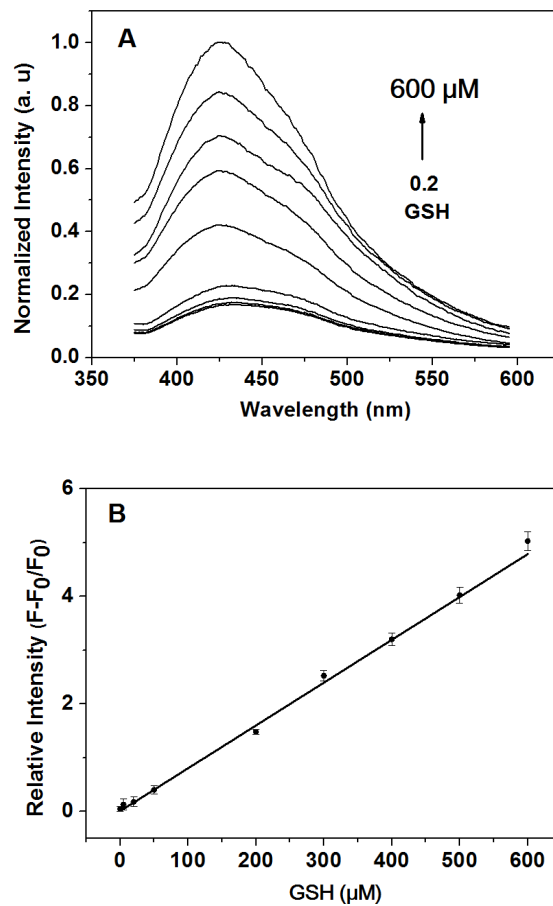


Fig. S8 (A) Fluorescence restoration of the C-dots-MnO₂ nanosheet composite towards increased concentrations of GSH (i.e 0.2, 5, 20, 50, 200, 300, 400, 500 and 600 μM) in plasma matrix. (B) Relationship and linear fitting between the fluorescence recoveries and the concentrations of GSH.

Table S3. Concentrations of the investigated interferants.

Interferent	Concentration
Na ⁺	1.6 mM
K ⁺	2.0 mM
Mg ²⁺	0.29 mM
Ca ²⁺	14 mM
Zn ²⁺	50 μM
Fe ³⁺	2 μM
Glucose (Glu)	6 mM
Alanine (Ala)	50 μM
Glutamine (Gly)	50 μM
Histidine (His)	50 μM
Glutamic acid (Gln)	50 μM
Vitamine C (Vc)	50 μM
Homocysteine (HCy)	15 μM
Cysteine (Cys)	250 μM

Table S4. Detection of GSH in human whole blood samples by the FRET-based C-dots-MnO₂ sensor.

Sample no	Measured (μM)	RSD (n=3)	Added (μM)	Found (μM)	RSD (n=3)	Recovery
1	8.1	4.5%	100	96.6	2.3	88.5%
2	9.0	2.9%	200	199.0	5.1	95%
3	8.4	1.4%	400	416.1	3.9	101.9%

References

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