Electronic Supplementary Information

$Ti_3C_2T_x$ MXene derived TiO_2/C-QDs as an oxidase mimic for efficient diagnosis of glutathione in human serum

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Experimental Section

Materials: Ti_3AlC_2 bulk materials (90~95 wt % purity) were obtained from Shanghai Yuehuan New Material Co., Ltd. (Shanghai, China). Potassium bromide (KBr, SP) received from Tianjin Fuyu Fine Chemical Co., Ltd. (Tianjin, China). Disodium hydrogen phosphate dodecahydrate ($Na_2HPO_4 \cdot 12H_2O$, AR), monobasic sodium phosphate dihydrate ($NaH_2PO_4 \cdot 2H_2O$, AR), glucose (Glu, AR), sodium chloride (NaCl, AR), potassium chloride (KCl, AR), magnesium chloride($MgCl_2$, AR), L-glutathione (GSH), L-cysteine (Cys, 99 %) and ethanol absolute (AR) were from Sinopharm Chemical Reagent Co., Ltd. China. 3, 3′, 5, 5′-Tetramethylbenzidine (TMB) was purchased from the Shanghai Yuanye Bio-Technology Co., Ltd. Lithium fluoride (LiF, AR), hydrochloric acid (HCl), terephthalic acid (TA), dopamine hydrochloride (DA, 99 %), L-arginine (Arg, 99%), L-histidine (His, 99 %), L-serine (Ser) and glycine (Gly) were obtained from Shanghai Macklin Biochemical Technology Co., Ltd. L-ascorbic acid (AA, AR) was from Tianjin Heowns Biochemical Technology Co., Ltd. Zinc chloride ($ZnCl_2$, AR) received from Shanghai Aladdin Bio-Chem Technology Co., Ltd. And all reagents were analytical grade and used without further purification. Deionized water 18.2 MΩ/cm) was gotten from a Milli-Qultrapure system (Qingdao, China).

Apparatus: The PL spectra and life time were collected on an Edinburgh Instruments Spectrofluorometer FS5 (United Kingdom). The UV-vis absorption spectra were collected using a Mapada UV-6500 spectrophotometer (Shanghai, China). The XRD was carried out on a Rigaku D-MAX 2500/PC with the Cu Kα radiation (λ =1.54056 Å) (Tokyo, Japan). Morphologys of the MXenes were characterized on a HITACHI UHR FE-SEM SU8010 (Tokyo, Japan) and JEM-2010 transmission electron microscope (JEOL Ltd., Japan) respectively. The XPS were performed on an ESCALab 220 i-XL electron spectrometer (VGScientific, West Sussex, UK) using 300 W Al K α radiation. The FTIR were collected on a Nicolet 5700 FTIR spectrometer (Thermo Electron Scientific Instruments Corp., USA). ECL signals were measured using a

chemiluminescence system (Xi'an Remex Electronics Co. Ltd. Xi'an, China) combined with a CHI 760D electrochemical workstation (CH Instruments, Shanghai, China). The voltage of the photomultiplier tube was set at 1000 V in the process of detection, the Pt electrode and Ag/AgCl electrode used are from Tianjin Hengyi Group Co., Ltd. (Tianjin, China).

The TiO_2/C-QDs preparation: According to the classic MXene preparation method reported by Ghidiu, et al[S1]., Ti_3AlC_2 MAX is first immersed in a mixed solution of 2 M LiF and 9 M HCl, and stirred at 40 ℃ temperature to remove the aluminum (Al) layer. The obtained $Ti_3C_2T_x$ MXene powder was washed by repeated centrifugation until pH>6 to remove residual acid and salt, and dried under vacuum at 80 °C for 12 h. Then, the $Ti_3C_2T_x$ MXene powder was separately dispersed in a solvent of deionized water, and it was treated under 100 W sonicate for 3 h. Next, centrifugation was carried out for 10 min at 10,000 rpm, and the supernatant suspension was taken. Then, the solvent heat treatment in a vacuum oven at 120 °C for 10 h, followed by natural cooling to room temperature. Finally, centrifuge 12000 rpm for 30 min, and take the supernatant as the TiO_2/C-QDs suspension.

Oxidase-like activity of TiO_2/C-QDs studies: The Oxidase-like activity of TiO_2/C-QDs was investigated by oxidizing TMB without H_2O_2. The amounts of TiO_2/C-QDs suspension, 0.1 M PBS (pH = 5) and 20 mM TMB added in a 1 mL reaction system were 200 μL, 700 μL and 100 μL, respectively. The final concentration of TMB in this system was 2 mM. After reacting for 3 min at room temperature (25 °C), observe the color change and measure the UV-vis absorbance of the mixed solution at 652 nm.

Colorimetric detection of GSH: The measurement of GSH was performed at room temperature (25 °C). 200 μL of TiO_2/C-QDs suspension, 650 μL of PBS (0.1 M, pH=5), and 100 μL of TMB (20 mM) solution were sequentially added in the order of PBS, TiO_2/C-QDs suspension, and TMB solution. Then, 50 μl of different concentrations of GSH solution were added to the above mixed solution to form a 1 mL system, and the reaction was carried out for 3 min. The final concentration of GSH in this system was 0 μM, 0.5 μM, 1 μM, 2 μM, 4 μM, 6 μM, 8 μM, 10 μM, 15 μM, 20 μM, 25 μM, 30 μM,

respectively. Observe the color change and measure the UV-vis absorbance of the mixed solution at 652 nm.

The calibration curve of GSH was obtained by plotting A calibration curve for detecting GSH is obtained by plotting: $\Delta A = aX + b$ ($\Delta A = A_0 - A$), where A_0 is the UV-vis absorbance value (652 nm) of the system with 0 μM GSH solution added, A is the UV-vis absorbance value (652 nm) after adding different concentrations of GSH solution, and X is the final concentration of GSH in the system. Measured 3 times and the error bar is standard deviation (SD).

Cytotoxicity test: HuVec (Human umbilical Vein epithelial cell, ATCC number: CRL-1730) were cultured in RPMI 1640 (containing 10% fetal bovine serum and 1 % penicillin-streptomycin (Gibco)) at 37 °C in 5% CO_2. All the experiments were carried out at 60-70 % confluency of cells.

HUVEC cells were cultured in 96-well plates with a density of 5×103 per well for 12 h. TiO_2/C-QDs suspension with different concentrations were added into the medium. After incubation for 24 h, cells were treated with MTT to allow the formation of formazan crystal. The purple formazan product was dissolved with DMSO, and measured on a microplate reader.

Detection of GSH in human serum samples by standard addition methods: The detection of GSH in human serum samples was performed at room temperature (25 °C). Human serum samples were centrifuged several times at 10000 rpm for 15 minutes and the supernatant was taken. The human serum was diluted 100-fold, and different amounts of GSH were added to increase the GSH concentration in the serum by 0, 5, and 10 μM, respectively, and then these serum samples were tested by GSH colorimetry. First add 200 μL of TiO_2/C-QDs suspension, 250 μl of 0.2 M PBS (pH=5), and 50 μL TMB solution (40 mM), then serum samples with GSH were added to the system. And after 3 minutes of reaction, the UV-vis absorbance was measured, at the same time, the recovery rate were calculated by equation (1)[S2,S3]:

$$Recovery = \frac{C_{GSH} - C_0}{C_{SC}} \times 100\% \qquad (1)$$

Wherein C_0 is a measured value of GSH concentration in serum diluted 100 times; a standard concentration of GSH is added to serum diluted 100 times, and C_{SC} is a theoretical value of added GSH concentration; C_{GSH} is the measured value of the total concentration of GSH in serum after the addition of GSH. The recovery rate of each sample was measured 3 times, and the average was taken as the sample recovery rate. In addition, their relative standard deviation (RSD) is calculated by equation (2)[S2,S3]:

$$RSD = \frac{\sqrt{\frac{1}{N-1}\sum_{i=1}^{N}(x_i - x_0)^2}}{x_0} \times 100\% \qquad (2)$$

Wherein N is the number of measurements (N=3), X_i (i=1, 2, and 3) is the recovery calculated by equation 1, and X_0 is the average of X_i.

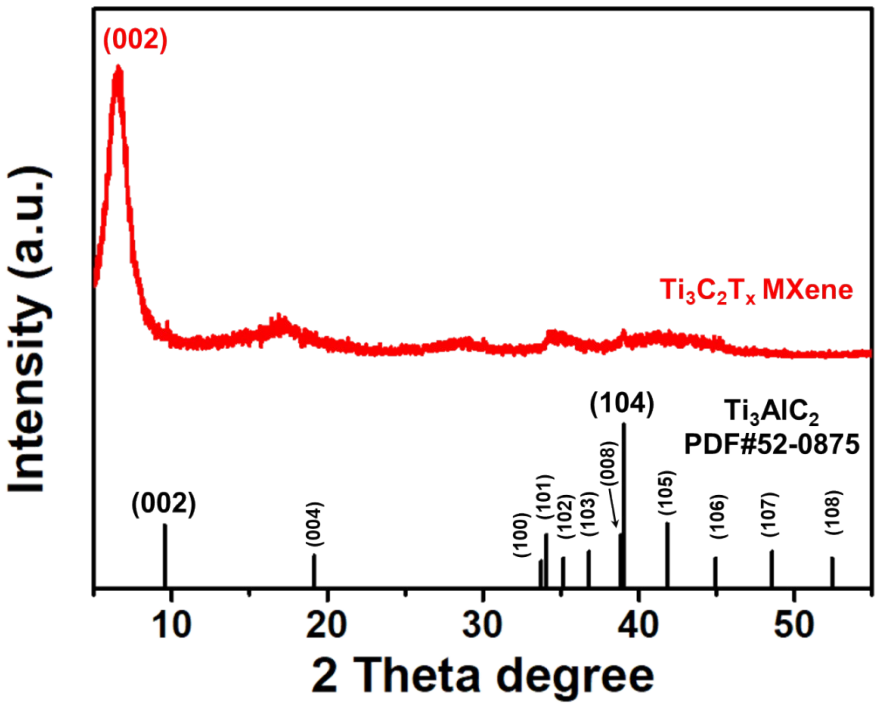

Fig. S1 The XRD patterns of $Ti_3C_2T_x$ MXene.

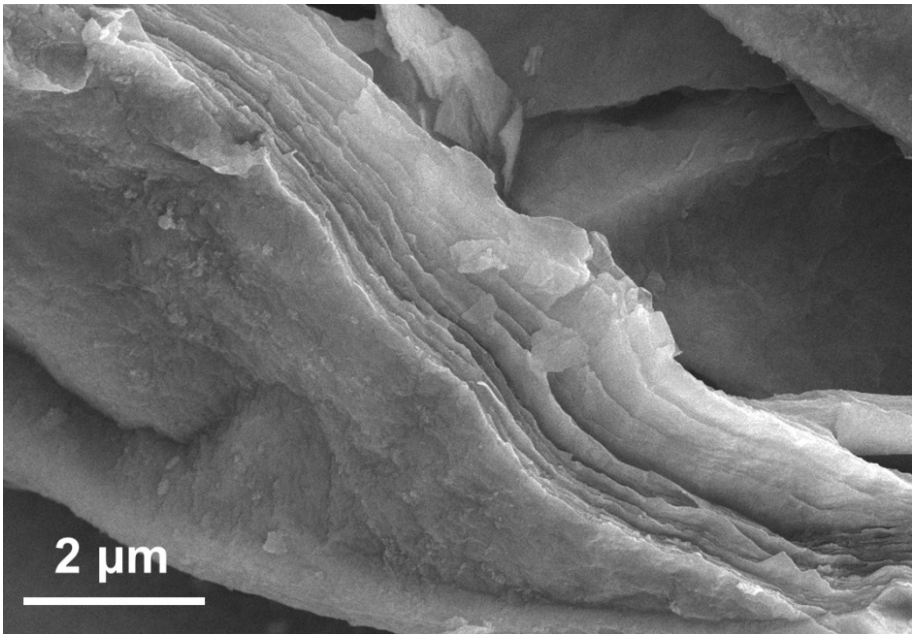

Fig. S2 SEM image of $Ti_3C_2T_x$ MXene after stripping with LiF-HCl mixed solution.

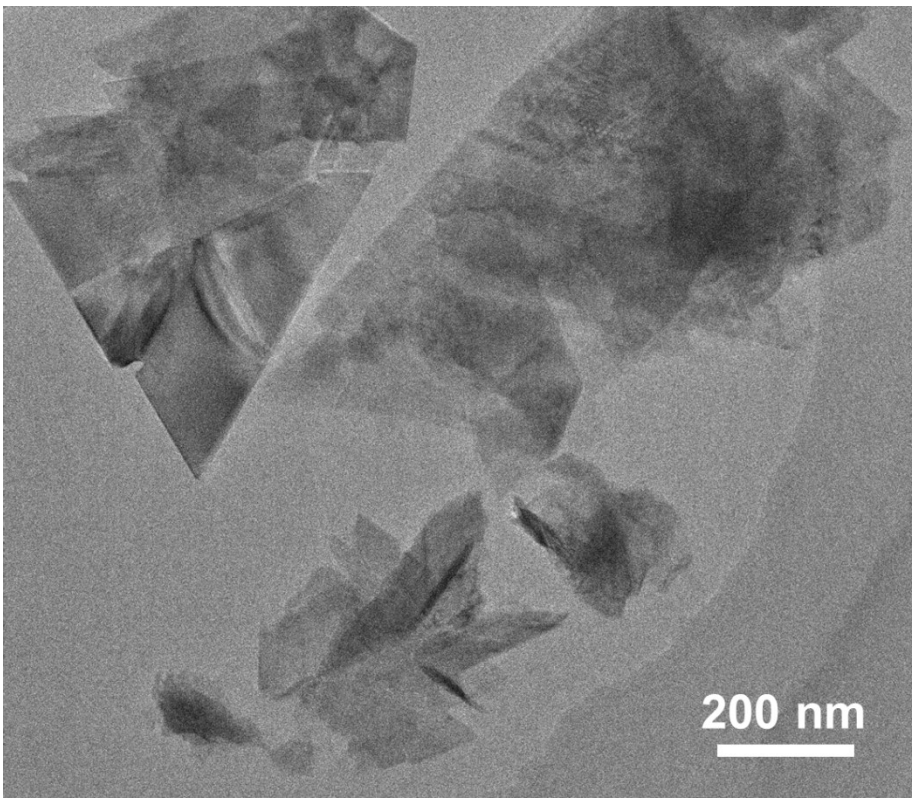

Fig. S3 TEM image of fewe-layer $Ti_3C_2T_x$ MXene nanosheet suspension.

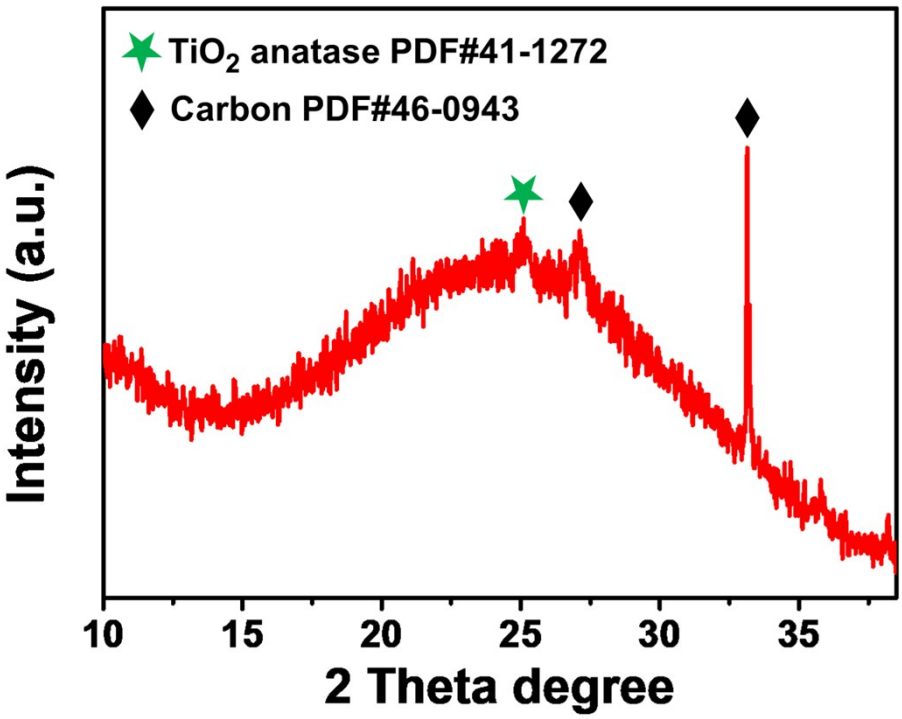

Fig. S4 XRD patterns of TiO_2/C-QDs.

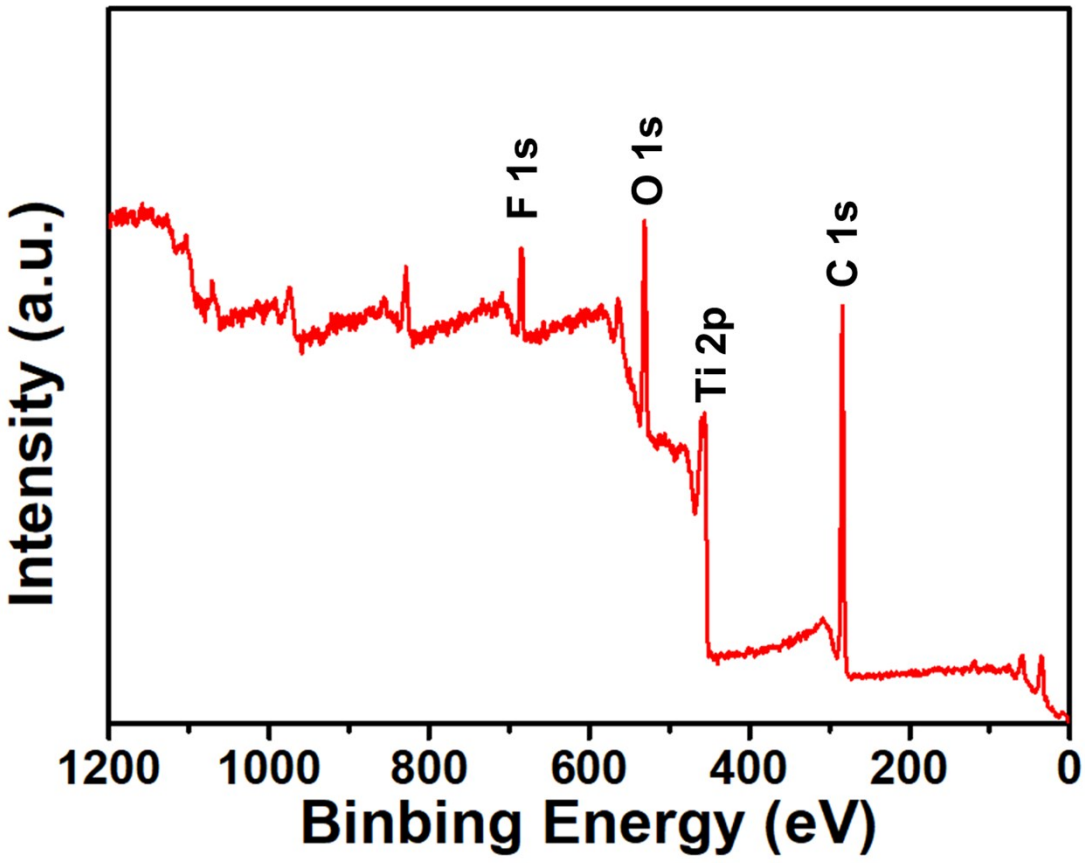

Fig. S5 The survey XPS spectra of TiO_2/C-QDs.

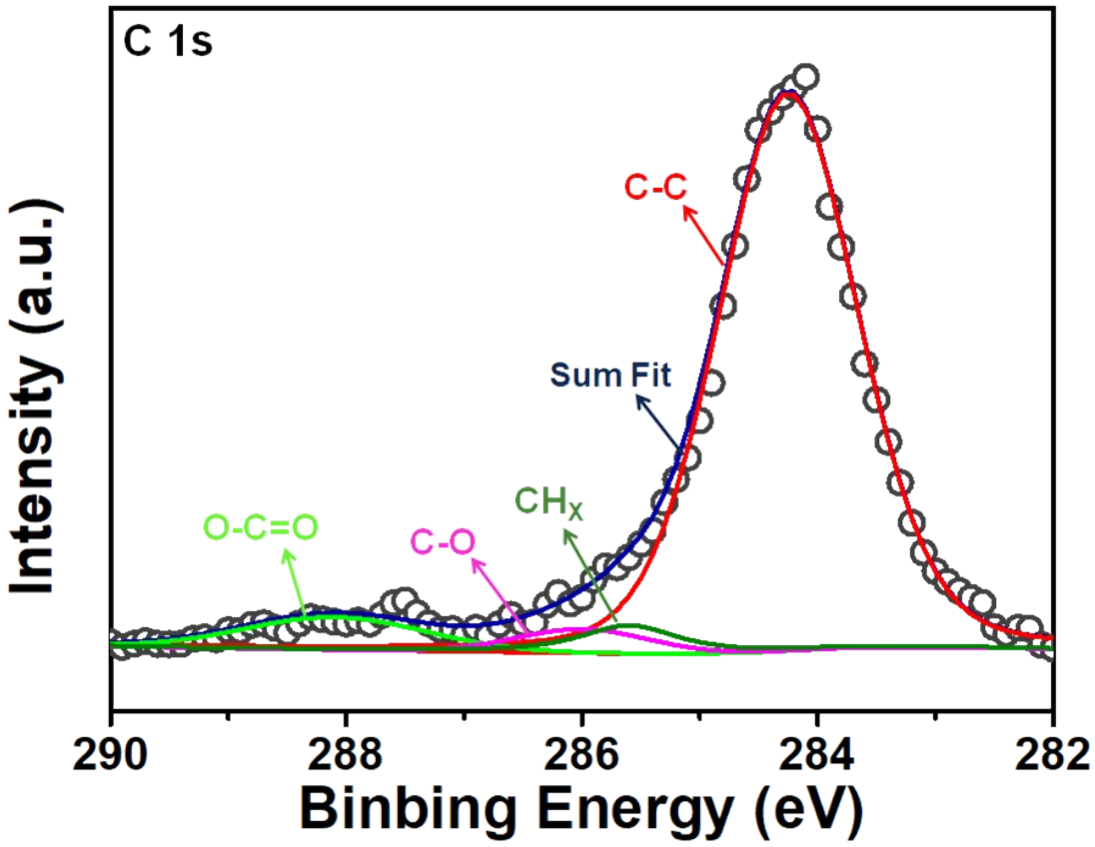

Fig. S6 The XPS narrow scan spectra of C 1s.

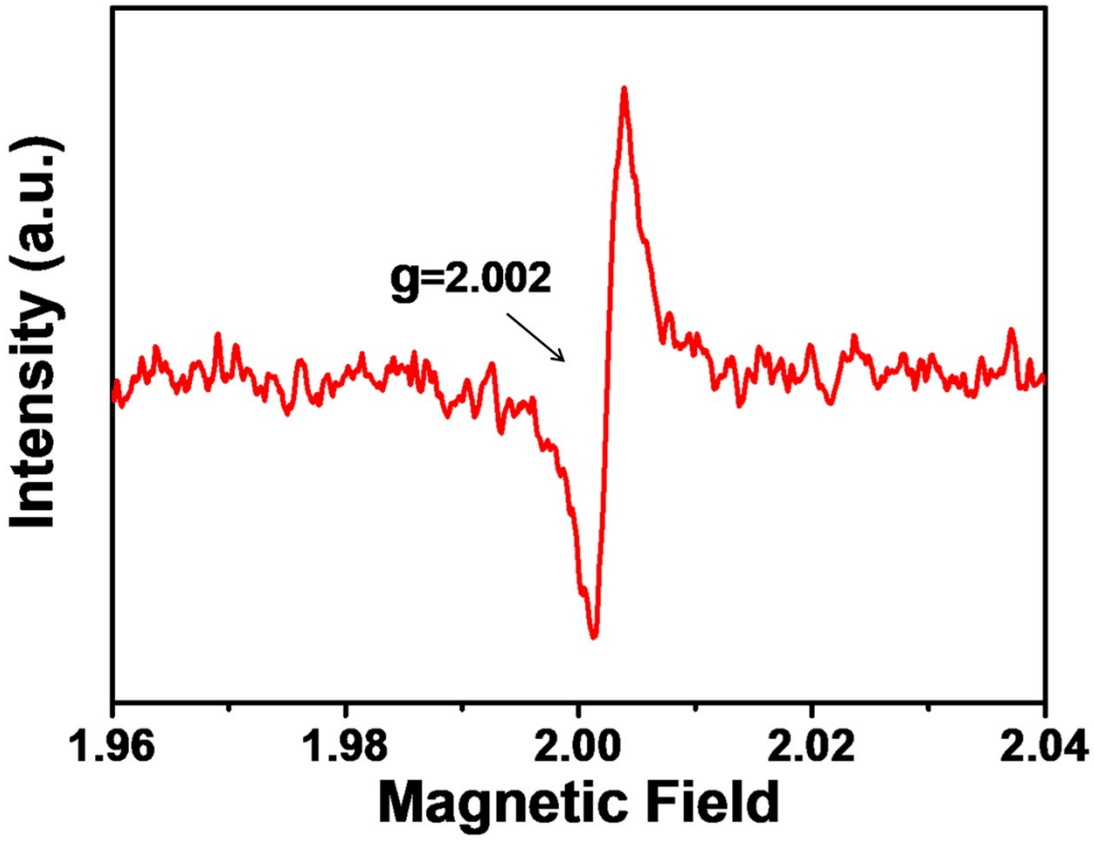

Fig. S7 The EPR spectra of TiO_2/C-QDs suspension.

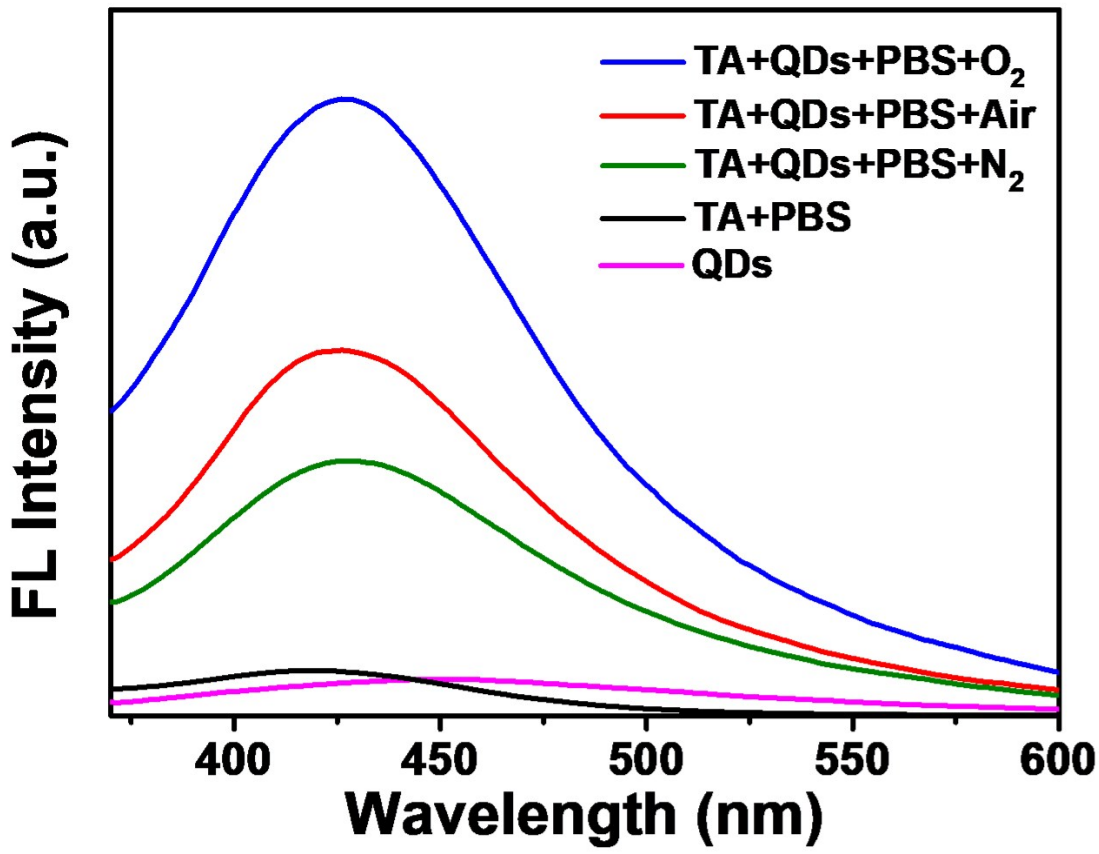

Fig. S8 The Fluorescence spectra in different reaction systems. (TA, 0.5 mM, pH = 5, λ_{ex}: 315 nm, λ_{em}: 435 nm.)

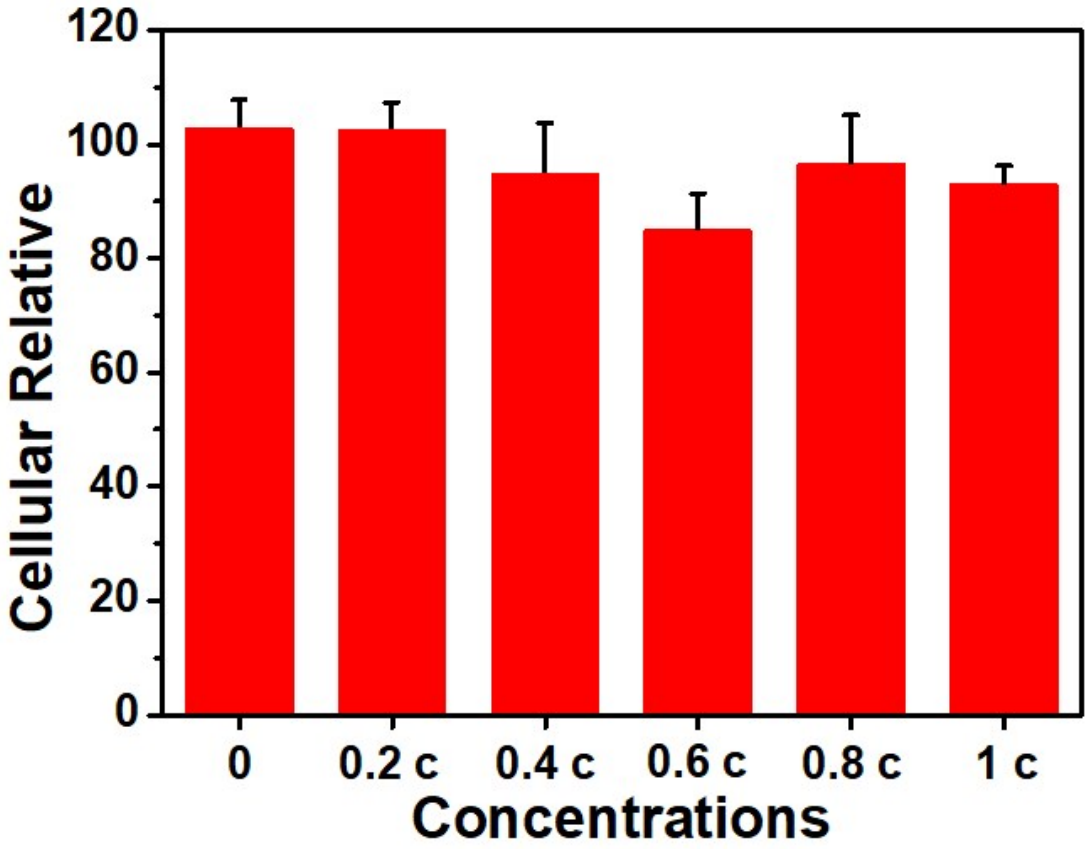

Fig. S9 Cell viability of HeLa cells (n = 3, error bar = SD.) after being treated with TiO_2/C-QDs suspensions of various final concentrations (where c is the concentration of the TiO_2/C-QDs original suspension). Untreated cells were served as the control, whose viability was set as 100%.

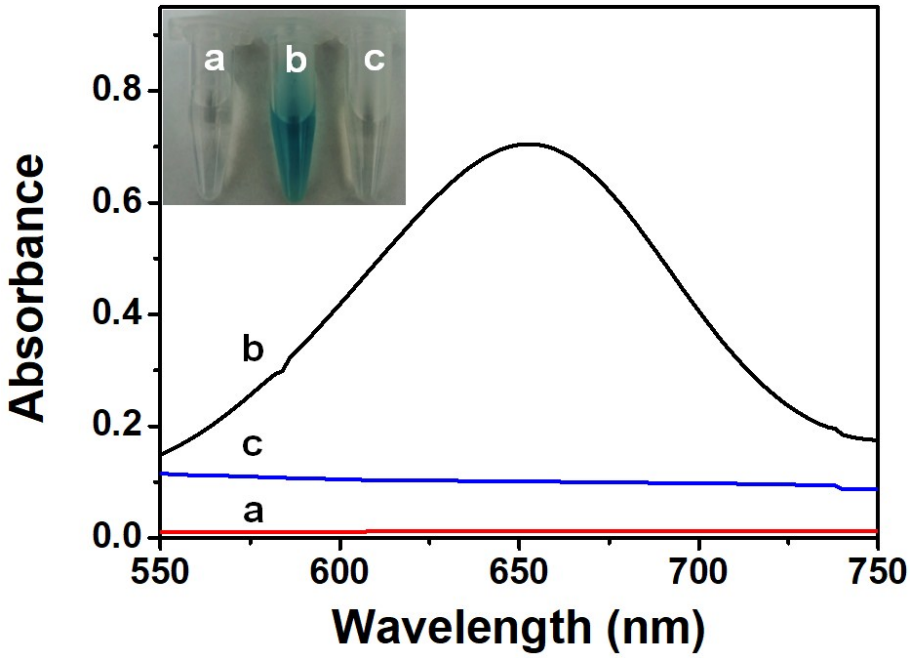

Fig. S10 (A) The visual color changes and absorbance curve in different reaction systems (a: PBS + TMB, b: PBS + TMB + TiO_2/C-QDs and c: PBS + TMB + TiO_2/C-QDs system reacts for 3 minutes, then GSH is added).

Table S1. Comparison of kinetic parameters of published reports.

Materials	K_m (mM)	V_{max} (10^{-8} M s^{-1})	Kcat (s^{-1})	Ref.
MoO_3-TPP nanoparticles	0.59	3.52	2.78	[S4]
Mutant of sulfite oxidase	0.332	-	4.8	[S5]
Au@Pt-based	0.069	0.226	1.33	[S6]
N-PCNSs	0.0549	12.6	-	[S7]
TiO_2/C-QDs	0.16	2.5	6.04	This work

References

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