Visual detection of biological thiols based on lightening quantum dots/TiO_2 composite

Qin Mu, Yan Li*, Yunfei Ma and Xinhua Zhong*

1 Experimental Section

1.1 Chemicals

Cadimim chloride hemi(pentahydrate) ($CdCl_2 \cdot 2.5H_2O$，99.995%), Te powder (-200 mesh, 99.8%), Sodium borohydride ($NaBH_4$，96%), Thiourea (99%), 3-Mercaptopropionic acid (MPA, 99%), L-Glutathione reduced (GSH, 99+%), L-Cysteine (Cys, 99%), DL-Homocysteine (Hcy, 95%), and other amino acids were purchased from Sigma-Aldrich. Titanium(IV) isopropoxide (98+%) was supplied by Acros. Citric acid, Sodium citrate, triphenyl phosphine, hydrochloric acid (HCl), methanol, acetone, and acetonitrile were purchased from Sinopharm Chemcial Reagent Co., Ltd. All reagents were of analytical grade and used as received without further purification. Deionized (DI) water and ultrapure water were used throughout.

1.2 Characterization

Fluorescence spectra were monitored on a Cary Eclipse (Varian) fluorescence spectrophotometer. Transmission electron microscopy (TEM) images of the QDs were obtained from a JEOL JEM-1400 transmission electron microscope at an acceleration voltage of 100 kV. The fluorescence lifetime study was recorded on an Edinburgh Instruments LifeSpec-ps spectrometer equipped with a Hamamatsu C8898 picosecond light pulser. The excitation light (440 nm) was obtained from a 441 nm laser with frequency of 1.0 MHz.

1.3 Synthesis of CdTe/CdS quantum dots

CdTe/CdS QDs were synthesized according to the reported method with minor modifications.[1] The detailed procedures were as follows:

1.3.1 Preparation of Te source NaHTe

25.5 mg (0.2 mmol) of Te powder and 40 mg (1.06 mmol) of $NaBH_4$ were mixed in a 25 mL three-necked flask, into which 10 mL of DI water was added. The flask was first pumped down at room temperature repeated three times to get rid of the atmosphere in the reaction

system. The system was then heated to 90 ^{o}C by a heating jacket under the protection of high purity nitrogen. The reaction proceeded at this temperature for 40 min. Accordingly, the color of the mixed solution was changed from colorless to petunia, indicating the generation of NaHTe. After that, the heating jacket was removed, and the reaction system was cooled down to room temperature. The obtained NaHTe (0.02 M) solution was stored under the protection of high purity nitrogen for further use.

1.3.2 Synthesis of CdTe cores

0.183g (0.8 mmol) of $CdCl_2$ and 1.6 mmol (135 μL) of MPA were mixed with 40 mL of DI water in a three-necked flask. The pH of the above mixed solution was adjusted to 10.0 by dropwise addition of NaOH solution (2.0 M) under stirring. After repeated pumped down at room temperature for three times, the reaction system was heated with a heating jacket under the protection of high purity nitrogen. When the temperature of the system reached to 100 ^{o}C, 8 mL of the as-prepared NaHTe solution was injected into the flask through a syringe. Thus, the molar ratio of Cd : MPA : Te in the reaction solution was 1:2:0.2. Nitrogen was continued pumped in and the temperature was maintained to complete this reaction. Aliquots of sample were taken at different time intervals and their PL spectra were recorded. When the PL spectrum reached to 520 nm, the heating source was removed to stop the reaction. The prepared CdTe core solution was cooled down under the protection of nitrogen and would be used in the next step.

1.3.3 Synthesis of CdTe/CdS core/shell quantum dots

0.152 g of thiourea (2.0 mmol) was dissolved in 2 mL of DI water, which was then added into the cooled CdTe core solution prepared in the previous step. The pH of the mixed solution was adjusted to 10.0 by dropwise addition of NaOH solution (2.0 M) under stirring. The flask was pumped down at room temperature for three times again, and was heated to 100 ^{o}C under nitrogen protection. Timing started when the temperature reached to 100 ^{o}C. Aliquots of the sample were taken at different time intervals and their UV-vis and PL spectra were record to monitor the reaction. When the PL emission spectrum reached to 580 nm, the heating jacket was removed to stop the reaction and cooled down. The obtained CdTe/CdS QDs solution was stored for further use. The synthesized CdTe/CdS QDs can be stored for several months in dark without aggregation or weakness of the fluorescence intensity.

1.4 Synthesis of TiO_2 nanoparticles

The TiO_2 NPs was synthesized according to the reported method.[2] Briefly, 7.18 mL of methanol, 1.24 mL of DI water, and 1.55 mL of HCl were mixed in a three-necked flask. 3 g (3.16 mL) of titanium(IV) isopropoxide was dropwise added into the flask. The mixture was first stirred for 1 h at room temperature, then heated to 70 ^{o}C and maintained for 4 hours. After cooled down to room temperature, the solution was transferred into a plastic centrifuge tube. Acetone was added to precipitate TiO_2 NPs. The mixture was centrifuged. The supernate was discarded and the obtained white sediment was redissolved in 4 mL DI water to get a TiO_2 NPs solution. When used for the detection of biothiols, the obtained TiO_2 NPs solution was first diluted 10 times. The prepared TiO_2 NPs can be stored for months without aggregation.

1.5 Detection of biothiols

Before used to detect biothiols, the prepared CdTe/CdS QDs need to be purified. Acetone was added to CdTe/CdS QDs solution to make QDs aggregation. The mixture was centrifuged and the obtained sediment was redissolved in DI water to get an aqueous CdTe/CdS solution. For the detection of biothiols, a small aliquot of CdTe/CdS solution was dispersed in a clear quartz cuvette which loaded with 2 mL of citrate buffer (1.0 mM). Then, 25 μL of diluted TiO_2 NPs solution was added, and the PL spectra of the CdTe/CdS QDs solution were recorded by the excitation wavelength at 400 nm. After the fluorescence of CdTe/CdS QDs were quenched, different amounts of biothiols solution of different concentrations were added. After stirring for 15 min, the fluorescence spectra were recorded again.

1.6 Detection of biothiols in real sample

The human urine sample was collected from two healthy male volunteers. Biothiols analysis in biological fluids generally requires the reduction of disulfide to free thiols. The reduction can be accomplished according to the literature method.[3] Briefly, 500 μL of human urine sample was first centrifuged at 5000 rpm for 10 min, and the supernate was collected. Then the collected supernate was mixed with 40 μL of HCl (0.2 M) and 20 μL of triphenyl phosphine solution (0.4 M). After incubated for 30 min at room temperature, the disulfide was reduced and the free thiols were dissolved in the biological sample followed by the addition

of 500 μL acetonitrile. The mixture was centrifuged at 8000 rpm for 15 min. The supernatant which contained free thiols was used for further analysis. Before measurement, the urine sample was appropriately diluted with 1.0 mM citrate buffer (pH = 6.0) to ensure the concentration of biothiols was within our linear range. For recovery studies, GSH solutions with accurate concentration were added to the urine samples. The total biothiol content in human urine samples was then determined by the standard addition method.

2. Fluorescence quenching of the CdTe/CdS QDs by TiO_2 nanoparticles

The fluorescence quenching of CdTe/CdS QDs by TiO_2 NPs was examined. As shown in Fig. S1, upon the addition of TiO_2, the fluorescence intensity of MPA capped CdTe/CdS QDs decreased remarkably in 20 min and then was constant in 4 h.

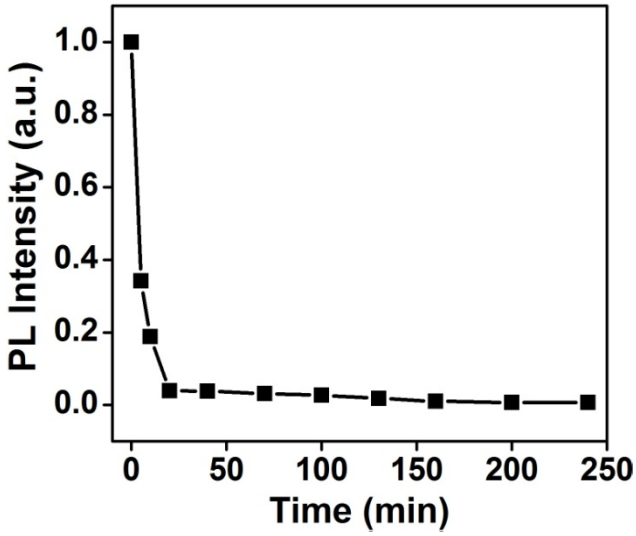

Fig. S1 Evolution of relative PL intensities of MPA capped CdTe/CdS QDs after the addition of TiO_2 ($0.37 g \cdot L^{-1}$).

3. The effect of pH value on the recovery of fluorescent QDs

The fluorescence recovery of the QD-TiO_2 system for biothoils under different pH condition adjusted by using citrate buffer was examined. As shown in Fig. S1, the efficiency of recovery processes increased significantly with the increase of pH value from 5.6 to 6.0, while the recovery efficiency decreased rapidly when pH value continually increased. Therefore, the pH of 6.0 was the optimal pH value for the detection of biothiols.

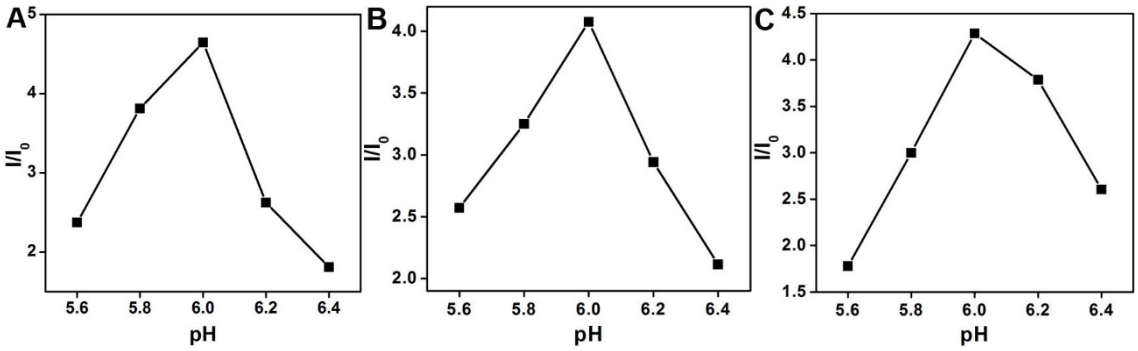

Fig. S2 The effect of the pH value on PL enhancement of QDs for GSH (A), Cys (B) and Hcy (C). I_0 and I are the PL intensities in the absence and presence of 10 μM biothoils in QD-TiO_2 solution.

4. Time dependence

The fluorescence spectra were monitored at different time to investigate the recovery kinetics of the QD-TiO_2 probe for the detection of biothoils. As can be seen from Fig. S2, the fluorescence intensities were enhanced as the reaction time is prolonged, and it leveled off to a saturation value after ca. 15 min. Thus, the fluorescence spectra were recorded after the addition of biothiols into the probe system for 15 min for the detection of biothiols.

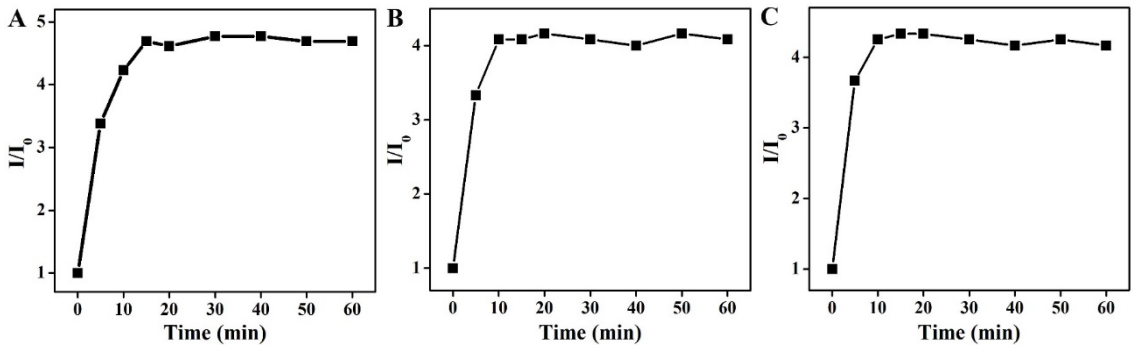

Fig. S3 The time-dependent fluorescence intensity change upon the reaction of the QD-TiO_2 probe with GSH (A), Cys (B) and Hcy (C) at pH value of 6.0. I_0 and I are the PL intensities in the absence and presence of 10 μM biothoils in QD-TiO_2 solution with pH value of 6.0.

5. The detection of Cys

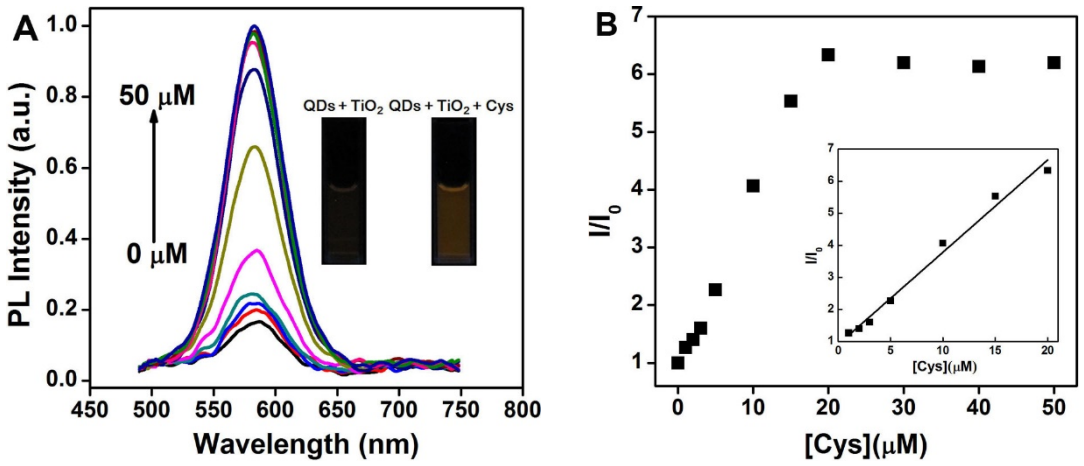

Fig. S4 (A) Fluorescence enhancement of the QD-TiO_2 probe solution with the addition of Cys in optimal condition (Incubation time 15 min, 1.0 mM citrate buffer with pH 6.0). Inset shows colorful images under a UV lamp before and after the addition of 50 μM Cys. (B) The relationship between I/I_0 and Cys concentration (where I_0 and I are the fluorescence intensity of QDs in the absence and presence of Cys, respectively). The inset is the linear restoration of the PL in the Cys concentration range from 1.0 to 20.0 μM with standard deviation R=0.985. The limit of detection of the proposed method for Cys was 0.28 μM.

6. The detection of Hcy

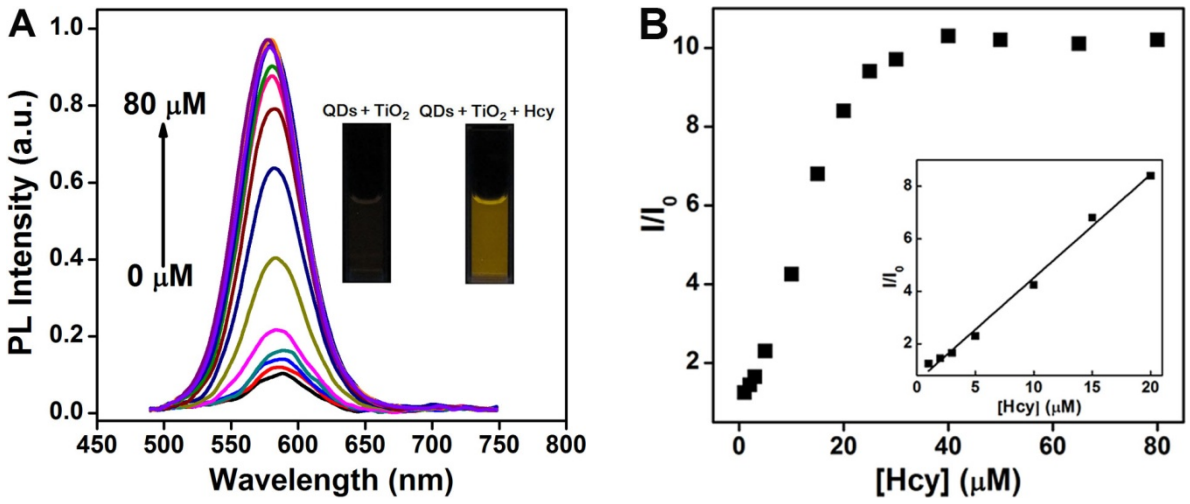

Fig. S5 (A) Fluorescence enhancement of the QD-TiO_2 probe solution with the addition of Hcy in optimal condition (Incubation time 15 min, 1.0 mM citrate buffer with pH 6.0). Inset shows colorful images under a UV lamp before and after the addition of 80 μM Hcy. (B) The relationship between I/I_0 and Hcys concentration (where I_0 and I are the fluorescence intensity of QDs in the absence and presence of Hcy, respectively). The inset is the linear restoration of the PL in the Hcy concentration range from 1.0 to 20.0 μM with standard deviation R=0.992. The limit of detection of the proposed method for Hcy was 0.15 μM.

7. Fluorescence lifetime

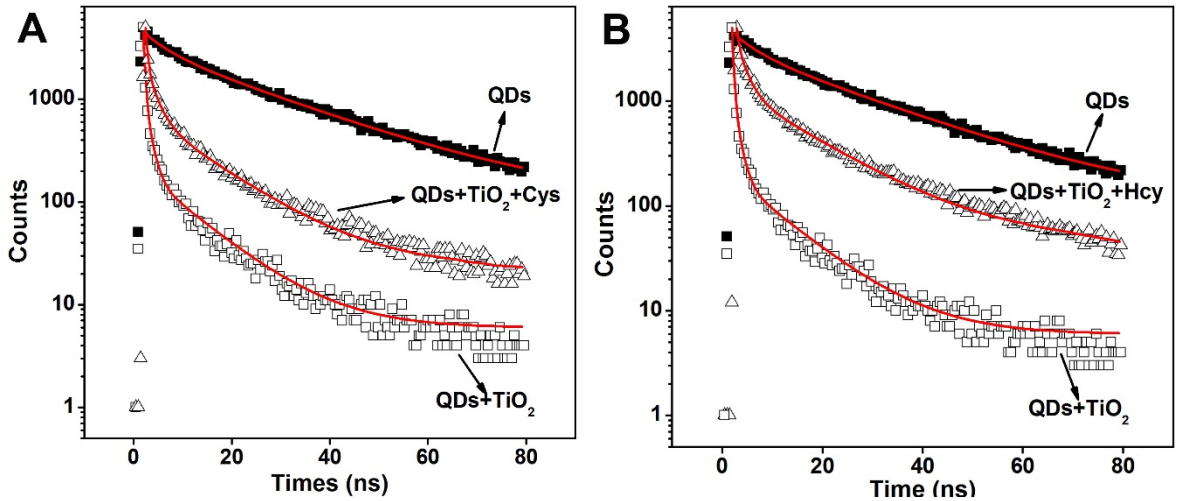

Fig. S6 Normalized PL decay curves of native QDs, and QD-TiO_2 system in the absence and presence of Cys (A) and Hcy (B). The red lines represent the corresponding fitting curves.

Table S1 Fluorescence lifetime data for the QDs, QDs-TiO_2 composites in the absence and presence of biothiols using the fitting function: $F(t) = A + a_1\exp(-t/\tau_1) + a_2\exp(-t/\tau_2) + a_3\exp(-t/\tau_3)$.

Substrate	a_1	τ_1/ns	a_2	τ_2/ns	a_3	τ_3/ns	χ^2	τ_{av}/ns
QDs	811.04	4.64	2143.23	20.48	664.57	50.75	1.16	32.29
QDs+TiO_2	2052.86	0.62	335.96	4.34	57.73	21.83	1.04	8.67
QDs+TiO_2+GSH	2426.25	1.74	1107.16	11.18	242.78	42.61	1.04	21.76
QDs+TiO_2+Cys	500.75	2.05	508.58	9.25	136.50	34.71	1.15	20.07
QDs+TiO_2+Hcy	1334.11	1.92	663.49	10.47	180.57	38.62	1.18	21.05

The average lifetime values were calculated using the expression $\tau_{av} = \sum a_i\tau_i^2 / \sum a_i\tau_i$.

8. Fluorescence response of CdTe/CdS QDs after the addition of GSH

In order to further illustrate the fluorescence recovery mechanism, the evolution of PL intensities of MPA-QDs with the addition of GSH was examined. As shown in Fig. S7, The fluorescent intensity of pure MPA-QDs exhibit negligible change (less than 5%) in 3 hours after the addition of 20 μM GSH, indicating that GSH can hardly affect the electronic properties of MPA-QDs.

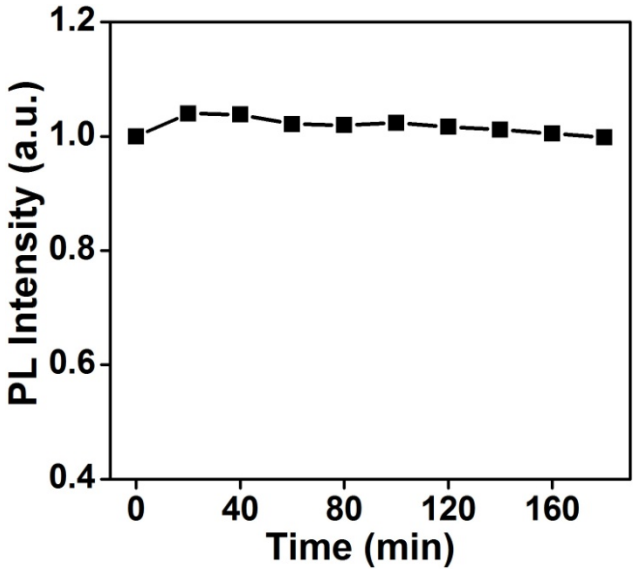

Fig. S7 Evolution of relative PL intensities of pure MPA-QDs after the addition of GSH.

9. Selectivity

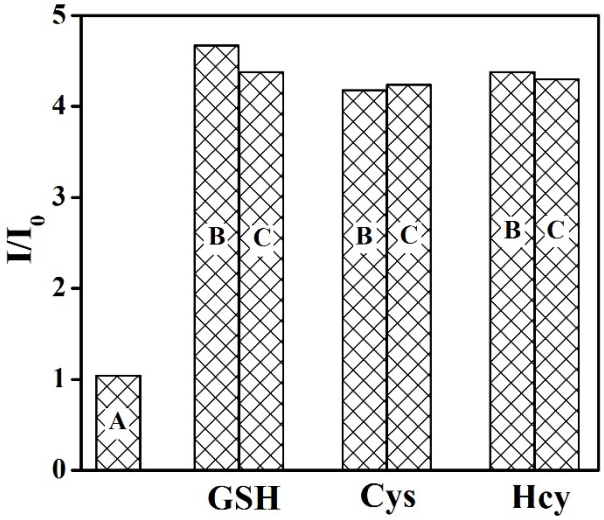

Fig. S8 Relative PL response (I/I_0) of QDs in the presence of (A) mixed 19 nature amino acids (each at 100 μM) except Cys, (B) mixed 19 nature amino acids with 10 μM of GSH, Cys and Hcy, respectively, (C) only 10 μM of GSH, Cys and Hcy, respectively.

10. TEM images of MPA capped QDs and TiO_2 NPs

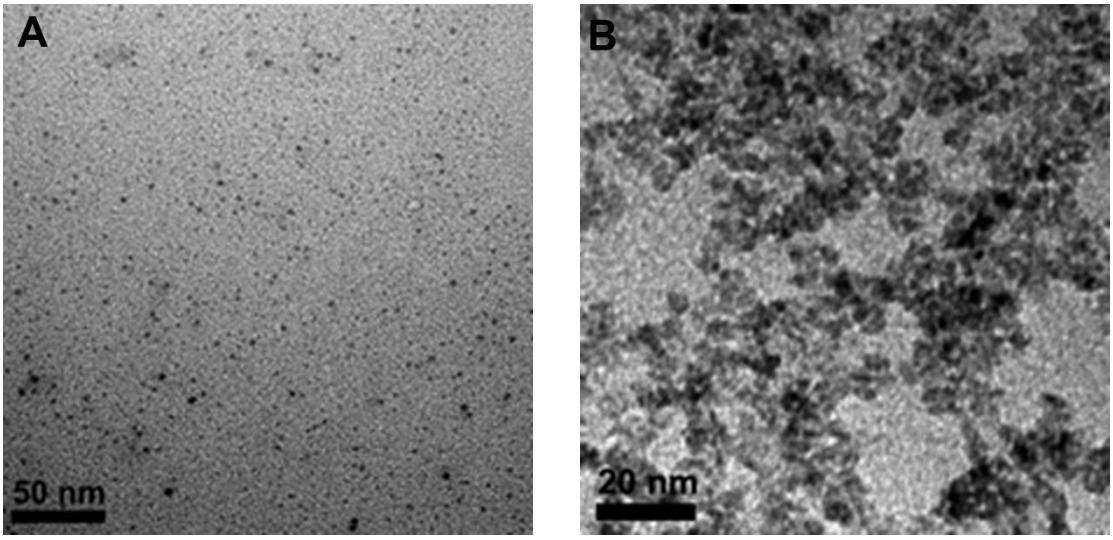

Fig. S9 TEM images of the MPA-capped QDs (A) and the as-prepared TiO_2 NPs (B).

11. The detection of biothiols in biological fluids

To demonstrate the applicability of the CdTe/CdS QDs probe for the detection of biothiols, the analysis performance in human urine was tested. In urine, most of the thiol-containing amino acids such as Cys and Hcy are bound to proteins or other thiols in the disulfide form. Thus, before used for analysis, the urine samples were first treated by using triphenyl phosphine reagent to reduce the disulfides to free thiols. The treated urine samples was appropriately diluted with 1 mM citrate buffer (pH = 6.0) to ensure the concentration of biothiols was within our linear range and to obtain quantitative recovery of the spiked thiols. The total biothiols content in human urine samples were determined by the standard addition method using GSH as the standard, and the results are summarized in Table S2. Recovery of added known amount of GSH to the urine samples was found to be in the range of 97.41–101.57%. As we known, many inorganic cations and anions, uric acid, glucose, amino acids and other organic components are present in human urine. The recovery results indicate that the presence of these co-existence components has no significant interference with the determination of biothiols in urine samples after appropriate dilution.

Table S2 Application of the designed probe for the detection of GSH in the simulated biological fluids[a].

Urine samples	Determined Biothiols (μM)	Add GSH (μM)	Measured (μM)	Recovery (%)	RSD (n = 3, %)
1	3.47	5.00	8.32	98.23	2.8
		10.00	13.12	97.41	3.4
2	2.71	5.00	7.82	101.42	3.1
		10.00	12.91	101.57	4.2

a All values are reported as average of three determination ± standard deviation.

12. Comparison of our QDs/TiO_2 sensor with recently reported instrumental and chemical technologies

Compared to the literatures related to instrumental method that were recently reported, the linear range of our sensor is narrower than electrochemistry and capillary electrophoresis,[4] and the LOD is higher than electrochemistry and HPLE,[4a,5] but lower than capillary electrophoresis.[4b] The present sensor exhibits better detection limit and linear range than that of the recently reported MS methods.[6] Compared to fluorescent method based on QDs, the LOD of the present method is a little higher than the probe that was based on Rh 6G and MPA-CdTe QDs,[7] but the LOD is lower to the probe based on CdSe/ZnS QDs or CdTe QDs.[8]

Table S3 Comparison of the linear range and detection limit of the instrumental and chemical technologies for the determination of GSH.

Methods	Linear range (μM)	LOD (μM)	References
Electrochemsitry	0.08–100	0.006	4a
Capillary electrophoresis	1.58–200	0.62	4b
HPLC	0.07–10	0.07	5
LC-MS/MS	0.5–8	0.5	6
Ratiometric fluorescence	0.05–80	0.015	7
Fluorescence method	5–250	0.6	8a
Fluorescence method	0.6–20	0.1	8b
This work	1–25	0.17	–

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