

Supporting Information

K⁺-mediated G-quadruplex formation enhancement fluorescence polarization

system based on quantum dot for detection of Hg²⁺ and biothiols

Experimental section

Reagents

Mercaptoacetic acid (TGA, 98%), NaBH₄ (98%) were purchased from J&K Scientific Ltd. Tellurium powder (99.999%), CdCl₂·2.5H₂O(99%) and all the metal salts were obtained from Shanghai Chemical Reagents Company and used without purified. Reduced L-glutathione (GSH), L-cysteine (Cys), Homocysteine (Hcy), 1-Ethyl-3(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC), N-hydroxysuccinimide (NHS) and other amino acids were purchased from Sigma-Aldrich. Streptavidin (SA) was obtained from Biosynthesis Biotechnology Co. Beijing, China. G-Riched DNA (5'-GGGTTAGGGTTAGGGTTAGGGT₂₅-3'), T-Riched DNA (5'-Biotin-T₂₅-3') and random DNA (5'-C₂₁-T₂₅3') in the present study were synthesized and purified with HPLC by Sangon Corp. Shanghai, China. The Hg²⁺ stock solution (1.0×10⁻³ M), Cys, GSH, Hcy and other amino acids stock solution (1.0×10⁻³ M) were prepared in ultrapure water and stored in refrigerator at 4°C within one week. All aqueous solutions were prepared with deionized water

(specific resistance > 18.2 MΩ/cm) obtained with a Millipore purification system.

PBS buffer (10mM, pH=7.4), HEPES buffer (10mM, pH=7.4), Tris-HCl (10mM, pH=7.4, 3M KCl) were referred to the whole experiment.

Apparatus

The fluorescence polarization value and photoluminescence (PL) intensities were measured and recorded with a LS-55 luminescence spectrometer (Perkin Elmer, USA).

Circular dichroism (CD) spectroscopy was monitored by a J-810 circular dichroism spectrometer (JASCO, Japan). UV-vis absorption spectroscopy was recorded with a TU-1901 UV-visible spectrophotometer (Beijing Purkinje General Instrument Co., Ltd., China).

Methods

Synthesis of thioglycolic acid-capped CdTe/CdS quantum dots

The high fluorescence CdTe quantum dots were prepared according to the literature¹. The pre-prepared CdTe QDs solution were concentrated and purified with ethanol precipitation and collected by centrifugal separation, the precipitation was redissolved with ultrapure water by ultrasonic dissolving method, the purified CdTe QDs was used as core QDs in the following step. Cd²⁺-TGA precursor solution ([Cd²⁺]=1.25 mM, [TGA]=6.0 mM, pH=9.0) was added into the purified CdTe core QDs solution and the mixture solution was stirred strongly for 10 minutes. Then, the

final solution was added into a Teflon-lined stainless steel autoclave and kept it at a reaction temperature of 160 °C for different time. The synthesized CdTe/CdS core-shell QDs nanocrystals were purified by ethanol precipitation and then stocked in Tris-HCl buffer solution. The concentration and sized of CdTe/CdS core-shell QDs was estimated by UV-vis absorption spectroscopy according to a previously published protocol.² The final concentration and size of QDs was 1μM and 3.5nm, respectively.

Preparation of DNA-functionalized CdTe/CdS core-shell QDs

50μL of QDs (1 μM) was mixed with EDC and NHS in 10mM phosphate buffer saline (PBS, pH 7.4) with the molar ratio of 1:200:100. After incubated at 37°C for 20 min, 20μL of 1mg·mL⁻¹ streptavidin solution were added into the centrifuge tube and incubated at 37°C for 3h with gentle shaking, the excess streptavidin were separated by ultrafiltration with a Millipore Microcon 50 kDa molecular weight cut-off centrifugal filter. The streptavidin coated QDs were redissolved in 300μL 10mM phosphate buffer saline, the 20μL of 100μM T-riched DNA solution were then added into the QDs solution and kept for 1h at 37°C, after that, the mixed solution were moved into the refrigerator and keep at 4°C overnight before centrifugalization using centrifugal filter devices (Millipore Microcon 50 kDa molecular weight cut-off). Finally, the T-riched DNA functionalized QDs were dispersed into 500μL 10mM HEPES-HCl buffer by vortex and stored at 4°C for future usage. The number of the oligonucleotides loaded on the QDs was also estimated by measuring the absorbance difference at 260nm before and after modification with oligonucleotides according to the literature.³ The numbers of oligonucleotides immobilized on each QDs were about 30.

Circular dichroism spectroscopy

The G-rich DNA were dissolved into Tris-HCl (10mM, pH=7.4, 3M KCl) and the concentration was confirmed as 10 μ M. The solution were heated at 95 $^{\circ}$ C for 5min and gradually cooled to room temperature. Then, the DNA solution was kept in the refrigerator at 4 $^{\circ}$ C overnight. The formation of G-quadruplexes was confirmed by the Circular dichroism spectroscopy. The CD spectroscopy measurement parameters were set as follows: three scans with a scanning speed of 200nm \cdot min $^{-1}$, the optical chamber with a 1cm path length and a band width of 2 nm. The spectra from 360 to 200nm were accumulated and averaged at room temperature. The background of the buffer solution was subtracted from the CD data.

Fluorescence polarization measurement for Hg $^{2+}$

To detect Hg $^{2+}$ in buffer or real environmental water samples, the 15 μ L of 100nM T-riched DNA functional QDs was mixed well with prepared G-quadruplexes by vortex, then, various concentrations of Hg $^{2+}$ was added into the mixture and incubated at 25 $^{\circ}$ C for 1h in 500 μ L of 10mM HEPES buffer to form the T-Hg $^{2+}$ -T complex. The fluorescence polarization assay was carried out by a LS-55 luminescence spectrometer (Perkin Elmer, USA). The excitation wavelength was 355 nm and the emission wavelength was 617 nm, the slit width was 10 nm. Each point was the mean of five

experiments with the standard deviation as the error bar.

Fluorescence polarization measurement for Cys

Competition reaction was used for the detection of Cys. Firstly, the 15 μL of 100 nM T-riched DNA functional QDs mixed well with prepared G-quadruplexes by vortex, then, 800 nM Hg^{2+} and various concentrations of Cys were mixed simultaneously with the mixture in 500 μL of 10 mM HEPES buffer. Before fluorescence polarization measurement, the final solution was incubated at 25 $^{\circ}\text{C}$ for 1 h. Each point was the mean of five experiments with the standard deviation as the error bar. The excitation wavelength was 355 nm and the emission wavelength was 617 nm, the slit width was 10 nm.

Pretreatment of real sample

The real environmental water samples was obtained from Jiang Jun Lake (Guilin, China) and filtered by 0.22 μm filter membrane. The treated sample was spiked with Hg^{2+} for Hg^{2+} detection by the standard additions method.

Human urine samples were collected from three healthy young men. Before carrying out fluorescence polarization experiments, the samples were separated by ultrafiltration with a Millipore Microcon 10 kDa molecular weight cut-off centrifugal filter, and the filtrate was collected and diluted with ultrapure water according to the ratio of 1:10. The diluted samples were spiked with different concentration of Cys

(5 μ M, 20 μ M, 50 μ M) for biothiol detection by the standard additions method. The volume of 15 μ L diluted sample or spiked diluted sample were further used in the detection process (see the experiment section- Fluorescence polarization measurement for Cys).

Characterization of the As-Prepared QDs-T

A typical UV-vis absorption spectra of functional QDs is shown in Figure S1A. Compare to the free QDs (Figure S1A, red curve), an obviously characteristic absorption peak of the DNA was observed at approximately 260nm after functional with T-riched DNA(Figure S1A, black curve). The result displayed that the T-riched DNA were successfully loaded on the surface of QDs by the affinity of biotin-streptavidin. Furthermore, the insert of Fig.S1A showed that the characteristic absorption peak of QDs had a slight blue shift from 556nm to 544nm after functional by T-riched DNA. Meanwhile, Figure S1B revealed that the shape and width of the normal fluorescence spectra of T-riched DNA modified QDs was very similar with free QDs. However, the emission peaks of the fluorescence spectra of DNA-functionalized QDs had a red shift about 5 nm compared with that of QDs alone. These results may be attributed to the electronic effects of the ligand on surface of QDs.⁴

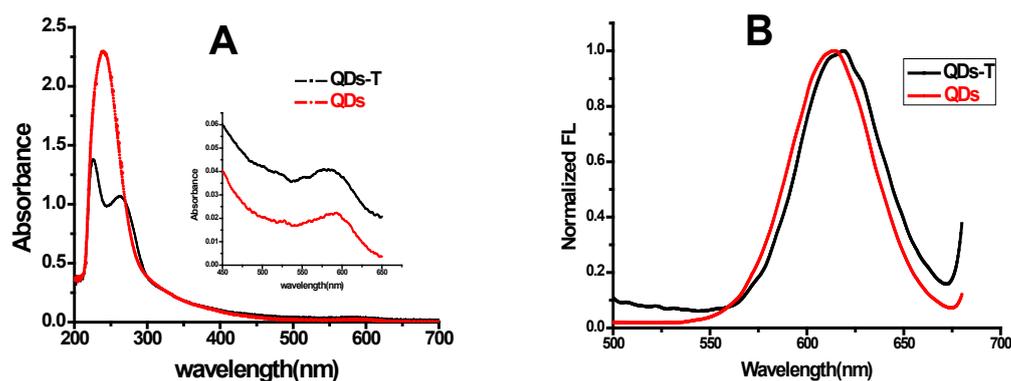


Figure S1 UV-Vis (A) and normalized Fluorescence spectra (B) of ODs functionalized before (red curve) and after (black curve). Insert: the characteristic absorbance spectra of QDs functionalized before and after.

CD study for G-quadruplex formation

CD has become a very useful technique for the characterization of G-quadruplex-forming oligonucleotides. The secondary structure formation of G-rich DNA is a key factor to the sensitivity of present method. Thus, CD spectra of G-rich DNA were recorded upon addition or not addition of K^+ . As shown in the Figure S2, in the absence of K^+ , the CD spectra exhibited a positive peak around 280 nm and weak negative peak around 245nm, which means a part of G-quadruplex formation exist in the buffer. However, after the addition of K^+ , the spectrum revealed a positive band at 285nm, a shoulder peak at 268 nm, and a smaller negative peak at 245nm, which indicated the formation of a mixture of the hybrid, parallel/anti-parallel G-quadruplex⁵ and is nearly consistent with the literatrue.⁶ These results confirm that G-

riched DNA could be efficiently formed G-quadruplex with K^+ .

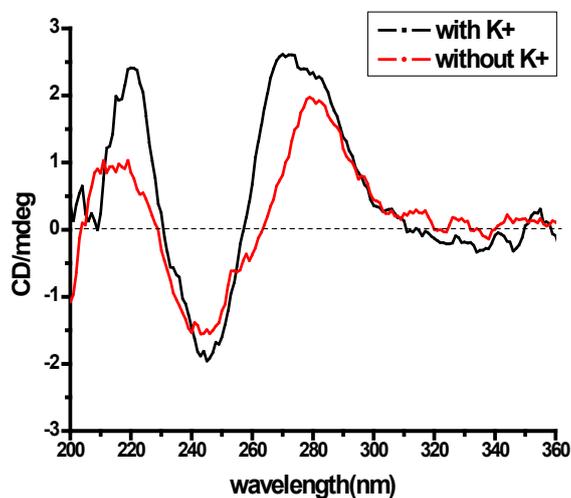


Figure S2 Circular dichroism spectra of G-Riched DNA with and without K^+ in the Tris-HCl buffer.

Optimization experiment

In the present strategy, the concentration of G-quadruplex (GQ- DNA) and hybridization times played crucial roles in the detection sensitivity. In order to obtain the optimal analytical parameters, experimental conditions were investigated in the presence and absence of Hg^{2+} respectively.

To evaluate the effect of the G-quadruplex (GQ-DNA), the FP values were recorded at the different concentrations of G-quadruplex (GQ-DNA). As shown in Figure S3A, the fluorescence polarization (FP) response elevated and reached a

maximum when the concentration of G-quadruplex (GQ-DNA) was 500nM. Then the FP response decreased gradually with higher concentration G-quadruplex (GQ-DNA) due to the excessive G-quadruplex which could inhibit the mismatched hybridization between QDs-T probe and G-quadruplex. The results indicated that the optimum G-quadruplex(GQ-DNA) concentration in this assay was 500nM .

After the introduction of Hg^{2+} to prompt the T-Hg -T formation, the incubation time was also investigated, and the results were shown in Figure S3B. The fluorescence polarization (FP) value of QDs increased when Hg^{2+} was introduced and then tended to stabilize after more than 55min, while the FP value of background solution remained unchanged. The signal to noise ratio reached maximum after incubation for 55min. Thus, the optimum incubation time was chosen as 55 min in next experiment. Herein, 400nM was used as the Hg^{2+} concentration to optimize the experimental conditions.

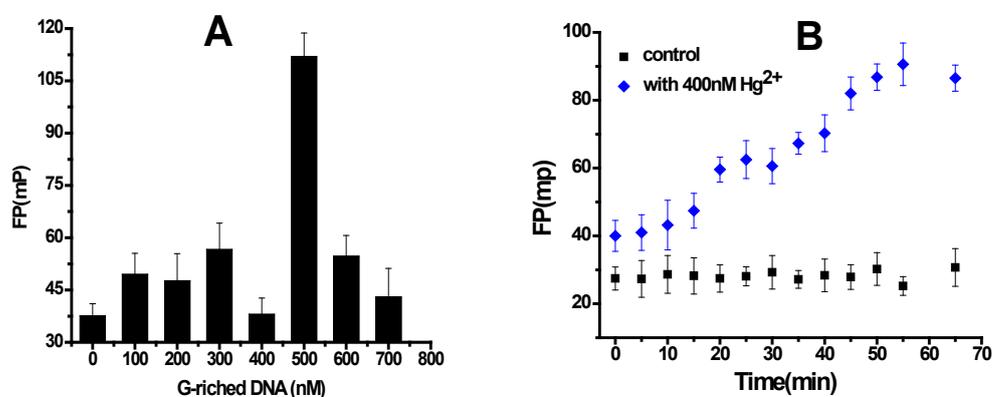


Figure S3 (A) The effect of the G-quadruplex concentrations on the FP value of QDs;

(B) Time-dependent curves of QDs Fluorescence polarization value with and without

Hg^{2+} . The Concentration of Hg^{2+} was 400nM.

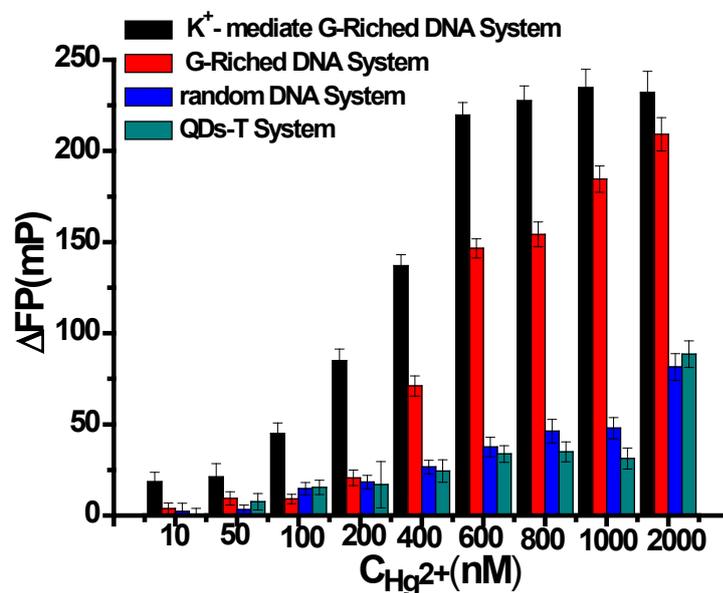


Figure S4 Plot of fluorescence polarization changes of QDs solution as a function of the Hg^{2+} concentration under different conditions

Table S1 determination of Hg^{2+} in environmental water sample using the proposed method

Sample	Found in Sample	Added (nM)	Total Found (nM)	Recovery(%, n=3)	RSD(%, n=3)
lake water	No Found	50	$48.3^a \pm 4.7^b$	96.5	9.8
		400	$398.1^a \pm 5.2^b$	99.5	1.3

^a Mean values of tree determinations. ^b Standard deviation.

Table 2 Determination of biothiols in complex biological samples using the proposed

method	Found in	RSD	Added	Total	Recovery	RSD	Found in
Sample	diluted	(%, n=3)	(μM)	Found(μM)	(%, n=3)	(%, n=3)	original
	(μM)						(μM)
Human urine sample 1	$21.55^{\text{a}} \pm 1.15^{\text{b}}$	5.3	5	$26.72^{\text{a}} \pm 0.35^{\text{b}}$	103.5	0.4	215.5
Human urine sample 2	$22.43^{\text{a}} \pm 0.84^{\text{b}}$	3.8	20	$40.61^{\text{a}} \pm 1.10^{\text{b}}$	91.0	2.8	224.3
Human urine sample 3	$29.04^{\text{a}} \pm 0.09^{\text{b}}$	0.3	50	$77.71^{\text{a}} \pm 3.14^{\text{b}}$	97.3	4.07	290.4

^a Mean values of three determinations. ^b Standard deviation. ^{*}Mean the urine sample was diluted for 10 times.

Annotations: The original Cys found in urine (215.5 -290.4 μM) were within the reported normal concentration range.⁷

References

1. J. N. Tian, R. J. Liu, Y. C. Zhao, Y. Peng, X. Hong and S. L. Zhao, *Nanotechnology*, 2010, 21(30), 5101–5108.
2. W.W. Yu, L. H. Qu, W. Z. Guo and X. Peng, *Chem. Mater.*, 2003, 15, 2854–2860.
3. R. Gill, I. Willner, I. Shweky and U. Banin, *J. Phys. Chem. B*, 2005, 109(49), 23715–23719.
4. C. Bullen and P. Mulvaney, *Langmuir*, 2006, 22, 3007–3013.
5. I. M. Pedroso, L. F. Duarte, G. Yanez, K. Burkewitz and T. M. Fletcher,

Biopolymers, 2007, 87(1), 74-84.

6. (a) Y. Xu, T. Ishizuka, K. Kurabayashi and M. Komiyama, *Angew. Chem. Int. Ed.*

2009, 48, 7833-7836; (b) A. Ambrus, D. Chen, J. X. Dai , T. Bialis , R. A. Jones

and D. Z. Yang, *Nucleic Acids Research*, 2006, 34(9), 2723–2735.

7. I. Fermo, C. Arcelloni and R. Paroni, *Anal. Biochem.*, 2002, 307, 181-183.