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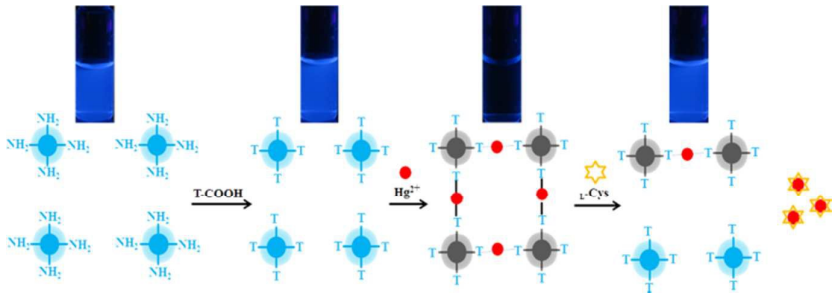
Graphic Abstract



The as-prepared thymine-modified carbon dots were applied to as a sensor for detecting Hg^{2+} and $_{L}$-Cysteine with high sensitivity and selectivity.

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ARTICLE

Highly Selective and Sensitive Fluorescence Probe Based on Thymine-modified Carbon Dots for Hg^{2+} and $_L$-Cysteine Detection

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Hui Xu [a], Shanshan Huang [a], Caiyun Liao [a], Yang Li [a], Baozhan Zheng [a], Juan Du *[a] and Dan Xiao *[a,b]

Herein, an "on-off-on" fluorescence probe for mercury ions and L-Cysteine was designed and fabricated with thymine (T) covalently modified on the surface of carbon dots (CDs) by a simple, economical and effective strategy. The prepared thymine-coated CDs (CDs-T) could detect Hg^{2+} ions with good selectivity and sensitivity by forming the specific thymine-Hg^{2+}-thymine (T-Hg^{2+}-T) structure, which induced aggregation of CDs-T and resulted in significant decrease of CDs's fluorescence intensity (on-off). In the presence of $_L$-Cys, the fluorescence was obviously recovered as the result of the disaggregation of CDs-T (off-on). In this process, Hg^{2+} ions were removed from the comparatively weaker connected complex of T-Hg^{2+}-T by the formation of stronger Hg^{2+}-S bond between Hg^{2+} and $_L$-Cys, thus the aggregates were broken and the fluorescence could be turned on. Under the optimal conditions, the concentration linear range for detecting Hg^{2+} and $_L$-Cys were 0.03-8 μM (R^2 = 0.9978) and 0.003-7 μM (R^2 = 0.9957), respectively. The limits of detection (LOD) were as low as 0.93 nM and 0.88 nM, respectively. This method was successfully used to analyze Hg^{2+} ions in real water samples and determine $_L$-Cys in human urine and blood serum samples.

Introduction

Heavy metal pollution is widespread and arises from a variety of industrial sources.[1, 2] Mercury ions (Hg^{2+}) is one of the most potently toxic heavy metals, which can cause serious problems to human health and the environment.[3-5] Therefore, monitoring Hg^{2+} in the aqueous environment has received an increasing interest among researchers. Recently, a number of highly sensitive and selective methods for sensing Hg^{2+} have been developed, based on quantum dots,[6, 7] fluorochrome,[8, 9] DNAzymes,[10-13] polymer materials,[14] proteins[15] and so on. It is well-known that thymine has a simple chemical structure and good photoresponsiveness.[16, 17] On the other hand, thymine has proven to be one of the most specific ligands that binds to Hg^{2+} in the form of T-Hg^{2+}-T complexes through the nitrogen atoms of it without any interference from other heavy metal ions.[12, 13] Based on this interaction, many highly selective and sensitive Hg^{2+} ions sensors have been developed by combining thymine or thymine derivatives into the detection systems.[18, 19]

$_L$-Cysteine ($_L$-Cys) is an important amino acid, which is the only one that containing a free thiol moiety in living systems. A deficiency of $_L$-Cys can result in many diseases, such as slowed growth in children, liver damage, muscle and fat loss, skin lesions, and weakness.[20] However, high levels of it in living systems also cause many human diseases including developmental retardation, cardiovascular and osteoporosis, Alzheimer's disease, *etc*.[21] Increasingly, $_L$-Cys has got considerable concern in recent years, and there have been many reports about the detection of $_L$-Cys.[22-25]

Carbon dots (CDs), as a promising new class of fluorescent carbon nanoparticles, have recently received growing research interest since they were discovered in 2004.[26] Compared with conventional semiconductor quantum dots (QDs), CDs exhibit many unique merits such as chemical inertness and stability, high photostability against photobleaching, tunable excitation and emission spectra, excellent biocompatibility and low toxicity.[27-32] With these features, the potential applications of CDs in the biological field are keenly anticipated.[33-36] However, only a little explorations about the modification on the surface of CDs have been reported at present,[37-39] and many scientific issues with CDs still await further investigation.

In this work, we presented a very simple and sensitive method to prepare an "on-off-on" fluorescence probe for the detection of Hg^{2+} and $_L$-Cys by using thymine molecule covalently modified CDs (CDs-T). Specifically, the CDs-T probe could detect Hg^{2+} by forming T-Hg^{2+}-T complexes. In this process, Hg^{2+} acted as a bridge to link the neighboring thymine groups (T), and therefore induced the

[a] College of Chemistry, Sichuan University, 29 Wangjiang Road, Chengdu, 610064, China. E-mail: xiaodan@scu.edu.cn; Fax: +86-28-85416029; Tel: +86-28-85415029

[b] College of Chemical Engineering, Sichuan University, 29 Wangjiang Road, Chengdu, 610064, China.

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aggregation of CDs-T, which would facilitate the non-radiative electron/hole recombination annihilation through an effective electron/energy transfer process.[40, 41] As a consequence, the fluorescence of CDs-T got quenched. Then, the fluorescence could gradually recover with the addition of $_L$-Cys as the result of the strong binding preference of $_L$-Cys towards Hg^{2+} by forming the Hg^{2+}-S bond, which could release free fluorescent CDs-T from the aggregates. Therefore, the CDs-T probe made it possible to effectively detect Hg^{2+} and $_L$-Cys.

Experimental

Reagents and materials

Citric acid was purchased from Tianjin Chemical Reagent Company (Tianjin, China). Urea was purchased from Chengdu Chemical Reagent Company (Chengdu, China). N-(3-dimethylaminopropyl)-N-ethylcarbodiimide hydrochloride (EDC, 98.5%) and N-hydroxysuccinimide (NHS, 98.5%) were purchased from Sigma-Aldrich. Thymine-1-acetic acid was purchased from Alfa Aesar (China) Chemical Co. Ltd.. Quinine sulfate in 0.1 M H_2SO_4 was chosen as a standard to measure the quantum yield (QY) of the resulting CDs-T. Phosphate buffered solutions (PBS, 25 mM) was prepared by mixing NaH_2PO_4 and Na_2HPO_4. The human serum samples were obtained from Wangjiang Hospital of Sichuan University. Human urine samples were collected from two healthy volunteers. All of reagents were of analytical grade and used without further purification. The purified water used in this study was double-distilled.

Apparatus

Transmission electron microscope (TEM) images were obtained by an H-800 electron microscope and H-8010 scanning system (Hitachi, Japan). X-Ray photoelectron spectra (XPS) were acquired on a Kratos XSAM 800 spectrometer (Manchester, U.K.) with an Al-Kα X-Ray (1486.6eV) excitation source running at 15 KV. Fourier transform infrared spectra (FT-IR) were recorded on a Thermo Scientific Nicolet 6700 FT-IR spectrometer (Sugar Land, TX, USA) with the KBr pellet technique in the range of 400-4000 cm^{-1}. UV-visible spectra were measured on Hitachi U-2900 spectrophotometer equipped with a 1 cm quartz cell. Fluorescence spectra were measured on Hitachi F-7000 spectrophotometer equipped with a 1 cm quartz cell.

Preparation of CDs

Amino-coated CDs were synthesized according to the literature with slight modification.[42] 0.5 g citric acid, 0.5 g urea and 10 mL H_2O were added into a 25 mL Teflon-lined autoclave and then heated at 200 ^oC for 5 h. After being cooled down to room temperature, the purification of the CDs was conducted through a dialysis tube (1000 Da, molecular weight cut-off) for about 24 h in the dark.

Preparation of CDs-T (Scheme 1)

The T moieties were conjugated onto the surface of CDs via EDC/NHS coupling chemistry.[39, 43] Thymine-1-acetic acid (76.4 mg) was dissolved in DMF (6 mL), EDC (38.4 mg) and NHS (43.4 mg) were added into the solution and stirred at room temperature for 1 h. Afterwards the CDs solution (4 mL) was added into the above solution. The reaction solution was stirred at room temperature for 72 h. Finally, the solution was purified by distilled water using a dialysis membrane (1000 Da, molecular weight cut-off) for 24 h.

Quantum yield measurement

The quantum yield (QY) of CDs-T was measured according to an established method. Quinine sulfate in 0.1 M H_2SO_4 aqueous solution (Φ = 0.54 at 360 nm) was selected as standard. The QY was determined by comparing the integrated fluorescence intensity (excited at 360 nm for CDs-T) and the absorbance value (less than 0.1 at excitation wavelength) of CDs-T samples with that of the references.[44] The slope method was used to calculate the QY using the following equation:

$$\phi_x = \phi_{std} \times \frac{I_x}{I_{std}} \times \frac{A_{std}}{A_x} \times \frac{\eta_x^2}{\eta_{std}^2}$$

Where ϕ is the quantum yield, I is the integrated area of the corrected emission spectrum, A is the absorbance at the excitation wavelength, η is the refractive index of the solvent (1.33 for water and a 0.1 M H_2SO_4 aqueous solution). The subscript "*std*" refers to standard with known QY and "*x*" for the sample.

Detection of Hg^{2+} and $_L$-Cys

The detection of Hg^{2+} was performed at room temperature in PBS buffer solution (25 mM, pH = 7.4). 2.00 mL of CDs-T (10 μg·mL^{-1}) was added to the cell and incubated for 30 min at room temperature before the spectral measurements. The final concentration of Hg^{2+} ranged from 0 to 15 μM. For comparison, different metal ions with the final concentration of 20 μM were added to 2.00 mL of CDs-T (10 μg·mL^{-1}), and incubated for 30 min at room temperature. All the fluorescence spectra were recorded under excitation at 360 nm.

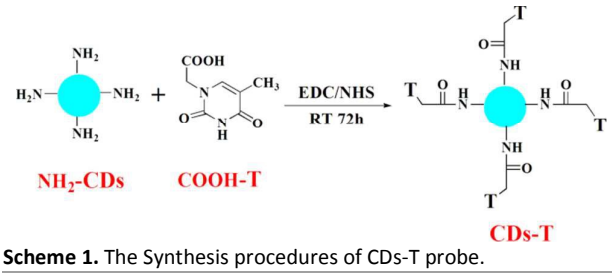
For the detection of $_L$-Cys, Hg^{2+} with a final concentration of 5 μM was added to 2.00 mL of CDs-T (10 μg·mL^{-1}) to form a mixture solution. The final concentration of $_L$-Cys ranged from 0 to 15 μM, and incubated for 30 min at room temperature. All the fluorescence spectra were recorded under excitation at 360 nm.

Results and discussion

Preparation and characterization of CDs and CDs-T probe

The probe was formed by the reaction between the -COOH of thymine-1-acrtid acid and the $-NH_2$ on the surface of CDs. In Scheme 1, the synthesis procedure of CDs-T was exhibited. The as-prepared CDs and CDs-T were fully characterized by TEM, XPS, FT-IR spectra. Fig. 1A and 1B showed the transmission electron microscope (TEM) images of the as-prepared CDs and CDs-T. These nanoparticles were uniformly dispersed without apparent aggregation, and the corresponding particles size did not change too much. The distribution histogram of the CDs and CDs-T size indicated that these nanoparticles had diameter ranging from 2-5 nm with the average diameter of 3.2 nm and 3.7 nm, respectively

(Fig. S1), which was quite similar to the CDs obtained from citric acid.[45, 46]



Scheme 1. The Synthesis procedures of CDs-T probe.

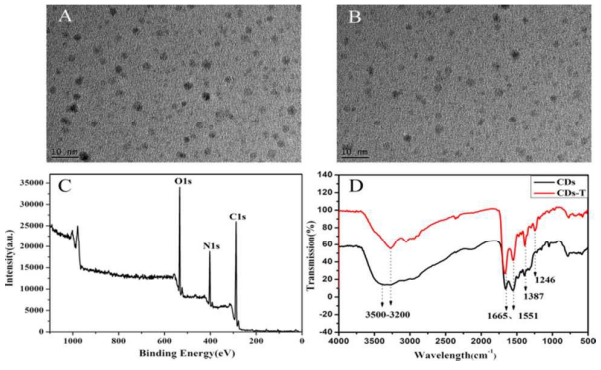


Fig. 1 TEM images of the resultant CDs (A) and CDs-T (B); XPS spectrum of CDs-T (C), FT-IR spectra of CDs and CDs-T (D).

The surface composition and element analysis for the overall composition of the resultant nanoparticles were characterized by X-ray photoelectron spectroscopy (XPS). The XPS spectrum of CDs-T (Fig. 1C) showed three peaks at 284.75, 400.67 and 531.29 eV, which were attributed to C_{1s}, N_{1s}, and O_{1s}, respectively.[47] This result indicated that CDs-T were mainly composed of three elements of C, O, and N. Similar to the previous studies,[48-50] the C_{1s} spectrum (Fig. S2A) revealed four peaks at 284.8, 285.8, 287.6 and 288.1 eV, which could be attributed to C-C, C-N, C-O, C=N/C=O bonds respectively. The N_{1s} spectrum (Fig. S2B) showed three peaks at 399.6, 400.8, 401.3 eV, which can be assigned to the C-N-C, N-$(C)_3$ and N-H bonds, respectively. As we can see from the O_{1s} spectrum (Fig. S2C), there was one peak shown at 531.3 eV, which was attributed to C=O groups. The Fourier-transform infrared spectroscopy (FT-IR) was revealed in Fig. 1D, these absorption peaks can be observed in CDs and CDs-T: the band at 3500-3200 cm^{-1} was assigned to N-H groups; the band located at 1665 cm^{-1} and 1551 cm^{-1} corresponded to the stretching vibration of C=O and the bending vibrations of C-N-H; the 1387 cm^{-1} peak was due to the stretching vibration of HN-C=O. Compared with the black line, these peaks at 1665 cm^{-1} and 1387 cm^{-1} in the red line became obviously more pointed and stronger. The new peak at 1246 cm^{-1} in the red line represented the stretching vibration of C-N.[49] All results displayed that thymine (T) group was successfully modified on the surface of CDs.

Spectral characterization of CDs and CDs-T

The UV-vis absorption spectra and fluorescence spectra of CDs-T were initially recorded. As is shown in Fig. 2A, the aqueous solution of CDs-T can be excited with handheld UV light at 365 nm and emitted blue fluorescence (Quantum yield 54% using quinine sulfate as the standard) while appearing as completely colorless transparent solution under daylight (Fig. 2A-inset). The UV-vis absorption spectra showed one absorbance band at around 350 nm and a feature peak at 235 nm, which were ascribed to the π–π* transition of C=C and n–π* transition of C=O, respectively.[51] The optical absorption at 350 nm matched with the maximum fluorescence excitation (Ex), and the corresponding emission wavelength (Em) was at 450 nm (Fig. 2A). Excitation-dependent PL behavior can be observed in Fig. 2B. The emission spectra of the as-synthesized CDs-T from 450 nm to 495 nm showed changes in the fluorescence intensity as the excitation wavelength varied from 300 nm to 420 nm, and revealed the maximum emission intensity at 450 nm when CDs-T were excited at 360 nm. After overall consideration, we chose 360nm as the optimum excitation conditions the following detection system.

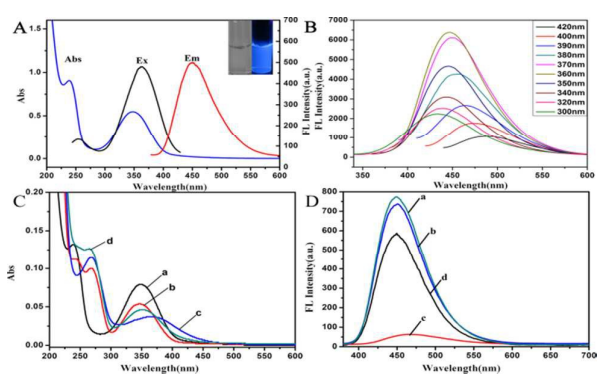


Fig. 2 (A) UV-vis absorption (Abs), fluorescence excitation (Ex) and emission spectra (Em) of CDs-T in PBS buffer solution (25 mM, pH = 7.4). (λ_{ex} = 360 nm, λ_{em} = 450 nm). The inset shows the photograph of CDs-T in aqueous solution under illumination of sunlight (left) and UV light (365 nm, right). (B) Fluorescence emission spectra of CDs-T at different excitation wavelength. (C) The UV-vis absorption spectra of CDs (a), CDs-T (b), CDs-T + Hg^{2+} (c), CDs-T/Hg^{2+} + $_L$-Cys (d). (D) Fluorescence emission spectra of CDs (a), CDs-T (b), CDs-T+Hg^{2+} (c), CDs-T/Hg^{2+} + $_L$-Cys (d).

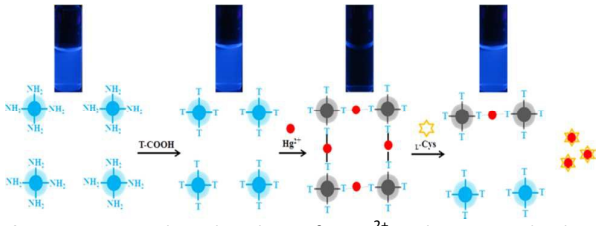
We compared with the UV-vis absorption spectra of CDs and CDs-T (Fig. 2C), the figure revealed that CDs-T had a new peak at 275 nm, which was obviously different from the UV-vis absorption spectra of CDs (Fig. 2C-curve a and b). While Hg^{2+} was added into the CDs-T solution, it can be observed that the peak at 235 nm disappeared, and the other peak at 275 nm still remained (Fig. 2C-curve c). When $_L$-Cys was added in the system of CDs-T/Hg^{2+}, the obtained spectra (Fig. 2C-curve d) was similar to curve b.

The fluorescence emission spectra of CDs and CDs-T were exhibited in Fig. 2D-curve a and b. It can be observed that the fluorescence intensity of CDs was slightly decreased after the

modification of thymine. It can be noted that the strong peak at 450 nm dramatically decreased as soon as 20 μM Hg^{2+} was added, which indicated that Hg^{2+} can effectively quench the fluorescence of CDs-T (Fig. 2D-curve c). Interestingly, this peak markedly increased when 10 μM $_L$-Cys was added (Fig. 2D-curve d) and the fluorescence intensity recovered about 80%.

Mechanism for the analysis of Hg^{2+} and $_L$-Cys

The detection mechanism of the fluorescence probe was shown in Scheme 2, the surface of CDs were coated with abundant amino groups, and exhibited a strong blue fluorescence under the UV light (365 nm). Usually, the fluorescence of CDs was quenched through the charge- or energy- transfer process with the addition of metal ions (Hg^{2+}, Cu^{2+}, Fe^{3+} and so on).[45-47] In this study, the prepared CDs exhibited poor selectivity towards various metal ions (Fig. S3). Then we modified CDs with thymine through the chemical bonds combination (-NH_2 and -COOH). In the presence of Hg^{2+} ions, thymine (T) could interact with it and form specific T-Hg^{2+}-T complex, inducing the CDs-T aggregated[41] and finally leading to the fluorescence quenching of CDs. On the other hand, the aggregated CDs-T could be disassociated in the presence of $_L$-Cys, since it can bind with Hg^{2+} ions to form the more stable Hg^{2+}-S bond, therefore, the free CDs-T can be released and "turn-on" fluorescence can be observed.



Scheme 2. An analytical pathway for Hg^{2+} and $_L$-Cys involved with fluorescence switching of CDs-T.

Effect of pH value

The pH value of the solution was another key factor that affected the sensing system, because the initial FL intensity (in the absence of Hg^{2+}) and the quenched FL intensity (in the presence of Hg^{2+}) of CDs-T are both pH-dependent (Fig. S4). CDs-T alone (Fig. S4-black line) had strong FL activities in the range of pH 4 ~ 8, and comparatively weak FL activities in the pH range from 9 to 11. In strongly acidic media (pH ≤ 4), the addition of Hg^{2+} had nearly no effect on the CDs-T's FL intensity (Fig. S4-red line), which may be attributed to the fact that the nitrogen atoms of thymine at the surface of CDs-T were well protonated and thus unable to complex with Hg^{2+} to form FL-quenching T-Hg^{2+}-T structure. However, the most obvious and stable fluorescence quenching of CDs-T occurred in the pH range of 5 ~ 11. Especially, in the weakly acidic and neutral media (pH 5 ~ 8), the quenching efficiencies had similar high values, suggesting that these weakly acid and neutral media can be chosen for the sensitive detection of Hg^{2+}. Therefore, a weak alkaline condition of pH = 7.4 (PBS buffer solution, 25 mM) was chosen for this sensing system.

Detection of Hg^{2+}

Fluorescence titration experiments were performed to investigate the selectivity of CDs or CDs-T towards various metal ions. The results were shown in Fig. S3 and Fig. 3A. For the unmodified CDs, Fig. S3 manifested that besides Hg^{2+}, some other metal ions such as Cr^{3+}, Ag^{+} and Pb^{2+} could induce minor fluorescence quenching of CDs. However, in the CDs-T detecting system (Fig. 3A), none of other metal ions had effect on the fluorescence quenching of CDs-T except Hg^{2+}, indicating the excellent selectivity of CDs-T for Hg^{2+} detection. In addition, the potential interfering effects from some other metal ions frequently encountered in biological or environmental systems were investigated by using the present sensor system, and the results were shown in Fig. 3B. It was observed that no obvious interference for Hg^{2+} detection with the coexistence of other metal ions can be detected. These results indicated that CDs-T can act as a sensitive Hg^{2+}-specific fluorescence sensor and were expected to find potential application in selectively and efficiently detection of Hg^{2+} in real water samples and biological environments.

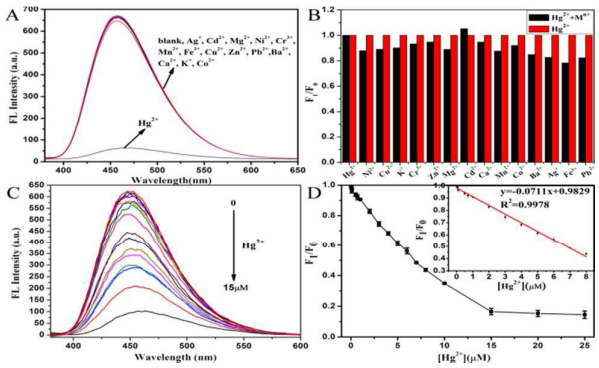


Fig. 3 (A) Fluorescence responses of CDs-T (10 μg·mL^{-1}) upon the addition of various metal ions (20 μM) in PBS buffer solution (25 mM, pH = 7.4) (λ_{ex} = 360 nm). (B) Selective fluorescence responses of CDs-T (10 μg·mL^{-1}) towards various metal ions. The black bars represent the addition of an excess of metal ions (200 μM for other metal ions). The red bars represent the subsequent addition of 20 μM Hg^{2+} to the CDs-T. (C) Fluorescence intensity changes of CDs-T (10 μg·mL^{-1}) in the presence of different concentration of Hg^{2+}. (D) The plot of the fluorescence intensity ratio of CDs-T (10 μg·mL^{-1}) at 450 nm versus the concentration of Hg^{2+} (performed in pH 7.4, 25 mM PBS buffer solution; F_0 and F_1 correspond to the fluorescence intensity of the CDs-T at 450 nm in the absence and presence of Hg^{2+}, respectively. λ_{ex} = 360 nm), the inset shows the linear range of the curve.

The fluorescence responses of CDs-T toward different concentrations of Hg^{2+} were shown in Fig. 3C, the fluorescence intensity of CDs-T at around 450 nm decreased gradually and the emission peaks had a little red shift with the Hg^{2+} concentration increasing. Furthermore, the quenching ratio (F_1/F_0) *vs.* Hg^{2+} concentration was shown in Fig. 3D, the probe exhibited a good linear response toward Hg^{2+} at a wide concentration range from

0.03 μM to 8 μM (R^2 = 0.9978) (Fig. 3D-inset). The detection limit was estimated to be 0.93 nM at a signal-to-noise ratio of 3, which also has been compared with other previously reported values (Table S1). Overall, the results demonstrated that our CDs-T sensing system can serve as an excellent sensor for the detection of Hg^{2+} and exhibited superior sensitivity to the previously reported sensing systems based on nanoparticales.

The excellent specificity combined with high sensitivity and selectivity of CDs-T to Hg^{2+} ions suggest that the present probe might be directly applied for detecting Hg^{2+} in real water samples (pond water and tap water). The samples collected were simply pretreated by filtration before further determination. The results summarized in Table 1 were satisfying and reasonable, and the recovery of Hg^{2+} was between 103.2 % and 103.7 % for tap water and 97.2 % and 98.3 % for pond water, which suggested that the proposed sensor CDs-T was suitable for detecting Hg^{2+} in practical samples.

Table 1. The recovery study of Hg^{2+} in tap water and pond water with proposed sensing system.

Sample	Added(μM)	Found(μM)	Recovery (%)	RSD (%)
	5.00	5.17	103.5	
Tap water	5.00	5.18	103.7	0.23
	5.00	5.16	103.2	
	5.00	4.89	97.9	
Pond water	5.00	4.86	97.2	0.54
	5.00	4.91	98.3	

Detection of $_L$-Cys

As we all known that biothiols possess strong affinities toward Hg^{2+} by forming the stable Hg^{2+}-S bond. One key feature of these biothiols lies in its thiol group, which may remove Hg^{2+} on the surface of CDs-T, and finally leading to the fluorescence recovery of CDs-T. To further evaluate the detection selectivity of CDs-T/Hg^{2+} toward $_L$-Cys, we studied the influence of different amino acids on the detection system. We tested the selectivity to various amino acids in CDs-T/Hg^{2+} solution. It can be clearly observed from Fig. S5 that only the addition of $_L$-Cys can lead to the fluorescence recovery, manifesting the satisfactory selectivity of CDs-T/Hg^{2+} to $_L$-Cys over other amino acids. Moreover, we investigated the fluorescence response in the presence of different biothiols, $_L$-cysteine ($_L$-Cys), glutathione (GSH), cysteamine and mercaptoacetic acid (TGA). As illustrated in Fig. S6, only $_L$-Cys can recover the fluorescence of CDs-T efficiently, while others led to only a little fluorescence recovery. We further evaluated the fluorescence enhancement efficiency of CDs-T/Hg^{2+} in the presence of different concentration of $_L$-Cys, and the results were suggested in Fig. 4A. It indicated that the fluorescence intensity of CDs-T gradually recovered with increasing concentrations of $_L$-Cys. A working curve was established by plotting the fluorescence intensity ratio (F_2/F_1) *vs.* the concentration of $_L$-Cys (Fig. 4B). As shown in Fig. 4B-inset, the best linear response concentration range of $_L$-Cys was from 0.003 to 7 μM (R^2 = 0.9957) and the detection limit was estimated to be 0.88 nM based on three times the standard deviation rule (LOD=3δ/s), which was also compared with other previously reported values (Table S2). The results in Table S2 revealed the superior detecting ability of our CDs-T probe over other reported nanoparticales-based $_L$-Cys probes.

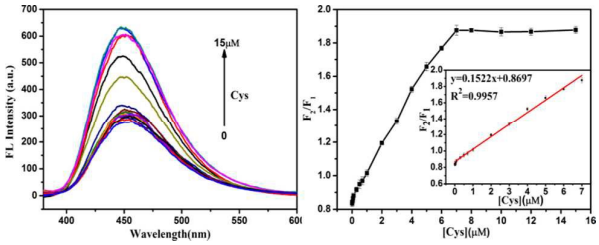


Fig. 4 (A) Fluorescence intensity changes of CDs-T (10 μg·mL^{-1}) containing Hg^{2+} (5 μM) in the presence of different concentrations of $_L$-Cys (0-15 μM). (B) The plot of the fluorescence intensity ratio of CDs-T/Hg^{2+} at 450 nm versus the concentrations of $_L$-Cys (performed in pH = 7.4, 25 mM PBS buffer solution; F_1 and F_2 correspond to the fluorescence intensity of the CDs-T/Hg^{2+} at 450 nm in the absence and presence of $_L$-Cys, respectively. λ_{ex}= 360 nm), the inset shows the linear region of the curve.

Table 2. Determination of $_L$-Cys in human urine and blood serum samples.

Sample	Present measured (μM)	Added (μM)	Found(μM)	Recovery(%)
	183.6 ± 2.4	150	160.5 ± 3.4	107± 2.27
Urine		200	204.1 ± 2.5	102.05 ± 1.25
	168.6 ± 3.0	150	148.3 ± 2.1	98.87± 1.39
		200	194.6 ± 1.8	97.3 ± 0.9
Blood	169.5 ± 2.4	150	156.8 ± 0.7	104.53 ± 0.47
serum		200	203.6 ± 2.3	101.8 ± 1.15
	171.9 ± 3.1	150	148.8 ± 1.6	99.2 ± 1.07
		200	210.5 ± 3.8	105.25 ± 1.9

[a] Mean ± SD of three measurements.

To illustrate the reliability and accuracy of our sensing system, the practical applications were evaluated by determining the $_L$-Cys in human urine and blood serum samples. Both samples were pretreated according to the procedures outlined in the section of sample pretreatment. It was necessary to do appropriate dilution for the samples, so as to ensure the signals fall into the linear range. The calibration curve was obtained by standard addition method. From the analytical results shown in Table 2, the results were in good agreement with those obtained by the previous methods (pre- and post- column derivatization with chromophores / fluorophores).[52, 53] The good recoveries (from 97.3 % to 107 %) of the known amount $_L$-Cys in real samples definitely indicated the reliability and practicability of our method in biological liquids.

Reversibility and reproducibility of CDs–T

Reversibility is another important aspect for a chemosensor. Fig. S7 showed the fluorescence intensity ratio(F/F_0) for the probe when Hg^{2+} (20 μM) or $_L$-Cys (10 μM) was added into the solution of CDs-T (10 μg·mL^{-1}) for five times. The ratio increased after adding $_L$-Cys into the CDs-T/Hg^{2+} system due to the decomplexation of Hg^{2+} by S^{2-} of $_L$-Cys by forming the stable Hg^{2+}-S bond. After five on-off cycles, the ratio(F/F_0) remained relatively stable. The reproducibility of the ratio of the emission intensity clearly demonstrated the excellent reversibility and the reliability of CDs-T.

Conclusions

In summary, we prepared a novel fluorescence probe (CDs-T) based on thymine (T) modified CDs. The as-prepared CDs-T have been successfully used for "on-off-on" detection of Hg^{2+} and $_L$-Cys respectively with high selectivity and sensitivity. In addition, the limits of detection were as low as 0.93 nM for Hg^{2+} and 0.88 nM for $_L$-Cys, which were much lower than conventional organic probes. This method has been directly applied to the determination of Hg^{2+} in pond water and tap water with recovery in the range of 95.8 %-104.2 %. Meanwhile, this probe could be used to indirectly detect $_L$-Cys in human urine and blood serum samples with determination in general from 97.3 % to 107 %. The excellent performance of the proposed fluorescence probe showed the potential value to detect Hg^{2+} and $_L$-Cys in environmental and biological applications.

Acknowledgements

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Notes and references

1 A. Renzoni, F. Zino and E. Franchi, *Environmental Research*, 1998, **77**, 68-72.
2 J. Benoit, W. Fitzgerald and A. Damman, *Environmental Research*, 1998, **78**, 118-133.
3 J. R. Miller, J. Rowland, P. J. Lechler, M. Desilets and L. C. Hsu, *Water, air, and soil pollution*, 1996, **86**, 373-388.
4 E. M. Nolan and S. J. Lippard, *Journal of the American Chemical Society*, 2003, **125**, 14270-14271.
5 E. M. Nolan and S. J. Lippard, *Chemical Reviews*, 2008, **108**, 3443-3480.
6 B. Han, J. Yuan and E. Wang, *Analytical Chemistry*, 2009, **81**, 5569-5573.
7 B. Cao, C. Yuan, B. Liu, C. Jiang, G. Guan and M. Y. Han, *Analytica Chimica Acta*, 2013, **786**, 146-152.
8 C. Wang, D. Zhang, X. Huang, P. Ding, Z. Wang, Y. Zhao and Y. Ye, *Sensors and Actuators B: Chemical*, 2014, **198**, 33-40.
9 D. H. Kim, J. Seong, H. Lee and K. H. Lee, *Sensors and Actuators B: Chemical*, 2014, **196**, 421-428.
10 M. Li, Q. Wang, X. Shi, L. A. Hornak and N. Wu, *Analytical Chemistry*, 2011, **83**, 7061-7065.
11 R. M. Kong, X. B. Zhang, L. L. Zhang, X. Y. Jin, S. Y. Huan, G. L. Shen and R. Q. Yu, *Chemical Communications*, 2009, 5633-5635.
12 M. Hollenstein, C. Hipolito, C. Lam, D. Dietrich and D. M. Perrin, *Angewandte Chemie International Edition*, 2008, **47**, 4346-4350.
13 H. Urata, E. Yamaguchi, T. Funai, Y. Matsumura and S. I. Wada, *Angewandte Chemie*, 2010, **122**, 6666-6669.
14 Y. Zhao and Z. Zhong, *Journal of the American Chemical Society*, 2006, **128**, 9988-9989.
15 P. Chen and C. He, *Journal of the American Chemical Society*, 2004, **126**, 728-729.
16 N. Lake, J. Ralston and G. Reynolds, *Langmuir*, 2005, **21**, 11922-11931.
17 H. Itoh, A. Tahara, K. Naka and Y. Chujo, *Langmuir*, 2004, **20**, 1972-1976.
18 J. S. Lee, M. S. Han and C. A. Mirkin, *Angewandte Chemie International Edition*, 2007, **46**, 4093-4096.
19 D. Li, A. Wieckowska and I. Willner, *Angewandte Chemie International Edition*, 2008, **47**, 3927-3931.
20 S. Shahrokhian, *Analytical Chemistry*, 2001, **73**, 5972-5978.
21 C. Jacob, G. I. Giles, N. M. Giles and H. Sies, *Angewandte Chemie International Edition*, 2003, **42**, 4742-4758.
22 M. JungáKim, *Chemical Communications*, 2010, **46**, 2751-2753.
23 Y. Zhang, A. Yang, X. Zhang, H. Zhao, X. Li and Z. Yuan, *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, 2013, **436**, 815-822.
24 S. Chen, H. Gao, W. Shen, C. Lu and Q. Yuan, *Sensors and Actuators B: Chemical*, 2014, **190**, 673-678.
25 M. Zhang and B.-C. Ye, *Analytical Chemistry*, 2011, **83**, 1504-1509.
26 X. Xu, R. Ray, Y. Gu, H. J. Ploehn, L. Gearheart, K. Raker and W. A. Scrivens, *Journal of the American Chemical Society*, 2004, **126**, 12736-12737.
27 S. N. Baker and G. A. Baker, *Angewandte Chemie International Edition*, 2010, **49**, 6726-6744.
28 H. Li, Z. Kang, Y. Liu and S. T. Lee, *Journal of Materials Chemistry*, 2012, **22**, 24230-24253.
29 P. G. Luo, S. Sahu, S. T. Yang, S. K. Sonkar, J. Wang, H. Wang, G. E. LeCroy, L. Cao and Y. P. Sun, *Journal of Materials Chemistry B*, 2013, **1**, 2116-2127.
30 X. Zhai, P. Zhang, C. Liu, T. Bai, W. Li, L. Dai and W. Liu, *Chemical Communications*, 2012, **48**, 7955-7957.
31 F. Wang, M. Kreiter, B. He, S. Pang and C. Y. Liu, *Chemical Communications*, 2010, **46**, 3309-3311.
32 L. Bao, Z. L. Zhang, Z. Q. Tian, L. Zhang, C. Liu, Y. Lin, B. Qi and D. W. Pang, *Advanced Materials*, 2011, **23**, 5801-5806.
33 D. Qu, M. Zheng, P. Du, Y. Zhou, L. Zhang, D. Li, H. Tan, Z. Zhao, Z. Xie and Z. Sun, *Nanoscale*, 2013, **5**, 12272-12277.
34 L. Shen, L. Zhang, M. Chen, X. Chen and J. Wang, *Carbon*, 2013, **55**, 343-349.
35 K. Qu, J. Wang, J. Ren and X. Qu, *Chemistry – A European Journal*, 2013, **19**, 7243-7249.
36 H. Wu, C. Mi, H. Huang, B. Han, J. Li and S. Xu, *Journal of Luminescence*, 2012, **132**, 1603-1607.
37 J. M. Liu, L.P. Lin, X. X. Wang, S. Q. Lin, W. L. Cai, L. H. Zhang and Z. Y. Zheng, *Analyst*, 2012, **137**, 2637-2642.
38 X. Hou, F. Zeng, F. Du and S. Wu, *Nanotechnology*, 2013, **24**, 335502.
39 C. Yu, X. Li, F. Zeng, F. Zheng and S. Wu, *Chemical Communications*, 2013, **49**, 403-405.
40 L. Zhou, Y. Lin, Z. Huang, J. Ren and X. Qu, *Chemical Communications*, 2012, **48**, 1147-1149.
41 H. X. Zhao, L. Q. Liu, Z. De Liu, Y. Wang, X. J. Zhao and C. Z. Huang, *Chemical Communications*, 2011, **47**, 2604-2606.
42 Q. Niu, K. Gao, Z. Lin and W. Wu, *Analytical Methods*, 2013, **5**, 6228-6233.
43 Y. Huang, D. Hu, S. Wen, M. Shen, M. Zhu and X. Shi, *New Journal of Chemistry*, 2014, **38**, 1533-1539.
44 B. De and N. Karak, *RSC Advances*, 2013, **3**, 8286-8290.

45 Y. Guo, Z. Wang, H. Shao and X. Jiang, *Carbon*, 2013, **52**, 583-589.
46 S. Zhu, Q. Meng, L. Wang, J. Zhang, Y. Song, H. Jin, K. Zhang, H. Sun, H. Wang and B. Yang, *Angewandte Chemie International Edition*, 2013, **52**, 3953-3957.
47 S. Liu, J. Tian, L. Wang, Y. Zhang, X. Qin, Y. Luo, A. M. Asiri, A. O. Al‐Youbi and X. Sun, *Advanced Materials*, 2012, **24**, 2037-2041.
48 F. Yan, Y. Zou, M. Wang, X. Mu, N. Yang and L. Chen, *Sensors and Actuators B: Chemical*, 2014, **192**, 488-495.
49 X. Yang, Y. Zhuo, S. Zhu, Y. Luo, Y. Feng and Y. Dou, *Biosensors and Bioelectronics*, 2014, **60**, 292-298.
50 X. Zhai, P. Zhang, C. Liu, T. Bai, W. Li, L. Dai and W. Liu, *Chemical Communications*, 2012, **48**, 7955-7957.
51 M. Chen, W. Wang and X. Wu, *Journal of Materials Chemistry B*, 2014, **2**, 3937-3945.
52 T. Toyo'oka, *Journal of Chromatography B*, 2009, **877**, 3318-3330.
53 F. Carlucci and A. Tabucchi, *Journal of Chromatography B*, 2009, **877**, 3347-3357.