Supporting Information

Nitrogen Doped Thiol Functionalized Carbon Dots for Ultrasensitive Hg (II) Detection

Abhishek Gupta, Abhishek Chaudhary, Pooja Mehta, Charu Dwivedi, Syamantak Khan, Navneet Chandra Verma, Chayan Kanti Nandi*

School of Basic Sciences, Indian Institute of Technology, Mandi, Himachal Pradesh, 175001 India

Corresponding author

*Chayan K. Nandi

School of Basic Sciences,

Indian Institute of Technology Mandi,

Himachal Pradesh-175001, India

Email: chayan@iitmandi.ac.in

Table of Contents:

Experimental Section:

Materials: All Glass wares were washed with aqua regia (3 HCl: 1 HNO_3), followed by rinsing several times with double distilled water. Chitosan, polyethylene glycol (PEG), dithiothreitol (DTT), Glutathione and amino acids were purchased from Sigma Aldrich. All metal ions including chloride of lead, mercury, chromium, cadmium, magnesium, copper, cobalt, nickel, manganese, zinc and arsenic oxide with purity >99% and EDTA were purchased from Merck India chemicals. Sodium Iodide (NaI, 99%) was purchased from Fisher Scientific. Double distilled 18.3 mΩ deionized (DI) water (Elga Purelab Ultra) was used throughout for the preparation of solutions.

UV-Vis Absorption spectroscopy

The UV-Vis absorption spectra were recorded using Shimadzu UV-Vis 2450 spectrophotometer. The spectra were collected using a quartz cuvette of 10 mm path length and volume 1 ml. All the measurements were repeated at least three times.

Transmission Electron Microscope (TEM)

The particle size and dispersity of the synthesized C-dots were checked using a TECNAI G2 200 kV TEM (FEI, Electron Optics) electron microscope with 200 kV input voltage. TEM grids were prepared by placing 5 μL diluted and well sonicated sample solutions on a carbon coated copper grid and evaporated the solution at room temperature completely. Precautions were taken to avaoid contamination from various sources like dust particles and glasswares.

Fourier Transform Infrared Spectroscopy (FTIR)

FTIR spectra of C-dots and DTT modified C-dots were measured using a Perkin-Elmer FTIR spectrophotometer equipped with a horizontal attenuated total reflectance (ATR) accessory containing a zinc selenide crystal and operating at 4 cm^{-1} resolution. The use of the spectral subtraction provided reliable and reproducible results.

Zeta Potential measurement

Zeta potential measurements were carried out on a Malvern Zetasizer Nano system equipped with 633 nm He-Ne laser. 1 ml of the sample was used in a cuvette (Malvern-DTS1061) and measured for 10 runs with 10 second time interval. The average of atleast five measurements were reported in the data.

Atomic Force Microscopy (AFM)

AFM analysis of the synthesized C-dots for particle size determination was carried out using a Digital Instruments Bruker AFM. Standard Veeco tapping mode silicon probes were used for scanning the samples. Typically, aqueous suspensions of C-dots samples were dried on silicon substrate for 3 hours. Once dried, samples were placed on the AFM stage and scanned. Pertinent scanning parameters were as follows: Resonant frequency (probe): 60-80 kHz; Tip velocity for all measurements are: (4 μm/s for 2 μm), (15 μm/s for 5 μm), (30 μm/s for 10 μm). Aspect ratio: 1:1; Resolution: 512 samples/line, 256 lines.

X-ray photoelectron spectroscopy (XPS)

X-ray Photo-Electron Spectroscopy (XPS) with Auger Electron Spectroscopy (AES) module PHI 5000 Versa Prob II, FEI Inc. and C60 sputter gun have been used for characterization and scanning the spectra from C1s, N1s, O1s and S2p region. Al Kα X-ray radiation was used as the

source for excitation (1486.8 eV, 500 mm). Samples were loaded on copper strips, and surface adherence done by double sided adhesive tape. C-dots samples were prepared by concentrating the solution by centrifugation at 23000 rpm, followed by drying. The interference of DTT was excluded by dialyzing the DTT modified C-dots in 3-5 kDa membrane (D-Tube™ Dialyzers, Merck Millipore) for 24 hours. The resulting sample was concentrated by centrifugation at 23000 rpm and the pellet was dried to obtain DTT modified C-dots in powder form. Thus obtained C-dots samples were used for recording XPS spectra.

Steady state and Time Resolved Fluorescence Spectroscopy

Steady state fluorescence was measured using Horiba Fluorolog-3 Spectrofluorometer. All the experiments including Hg^{2+}detection were performed at room temperature after equilibrating the samples for 15 minutes. The fluorescence was measured in 1 ml quartz cuvette. For titration experiment, to calculate the detection limit, different concentration of Hg^{2+} ions were added to a fixed concentration of C-dots and the final volume is maintained as 1 ml. For determining the selectivity for Hg^{2+} ion, 50 μM of each metal ion were added into a fixed concentration of DTT/C-dots. The kinetics of the detection was investigated by recording the fluorescence spectra of DTT/C-dots, immediately after the addition of Hg^{2+} ion upto 5 minutes (as most of the quenching processes were over within 5 minutes of the contact time). The fluorescence lifetime was measured using Horiba scientific Delta Flex TCSPC system with Pulsed LED Sources. Ludox has been used as an IRF for de-convolution of the spectral value. 3 ml C-dots solution has been used for all the lifetime measurements.

Calculation of quantum yield:

In the comparative method, the quantum yield (Φ) is calculated using the slope of the line determined from the plot of the absorbance against the integrated fluorescence intensities. 2-amino pyridine in 0.1 M H_2SO_4 is used as standard for determining the quantum yield. Equation 1 was used for calculating the quantum yield.

$$\Phi = \Phi_R \left[\frac{m}{m_R}\right]\left[\frac{n^2}{n_R^2}\right] \qquad \text{(Equation 1)}$$

Where, m is the slope of the line obtained from the plot of the integrated fluorescence intensity vs. absorbance, n is the refractive index of solvent (water n=1 in both cases) and the subscript R refers to the reference (2-Amino pyridine).

Synthesis of C-dots and its Surface functionalization with DTT

Chitosan (CHS) gel (2%) was prepared by dissolving 2 gm CHS in 99 ml of water in the presence of 1 ml of acetic acid, under vigorous stirring condition. 25 ml of PEG was dissolved in 75 ml water (25 % PEG solution). For synthesizing C-dots, in a beaker 4.5 ml of the prepared CHS gel was mixed with 4.5 ml of the 25% of PEG. In the resulting solution 1 ml of 5 M NaOH was added. The mixture was heated inside a domestic microwave at 600 W powers for 3 minutes. The resulting material was dissolved in 15 ml of distilled water. The solution was filtered by Whatman filter paper. 1.5 ml of the filtered solution was centrifuged twice (Sorvall Lynx 6000, thermo scientific) at 23000 rpm for 30 minutes each. The resulting pellets were removed each time and the final supernatant was collected and used for experiments. For surface functionalization with DTT, the above C-dots solutions were incubated for 2 hour with 10 μM DTT. Excess of DTT was removed as a supernatant by centrifugation at 25000 rpm for 5 minutes.

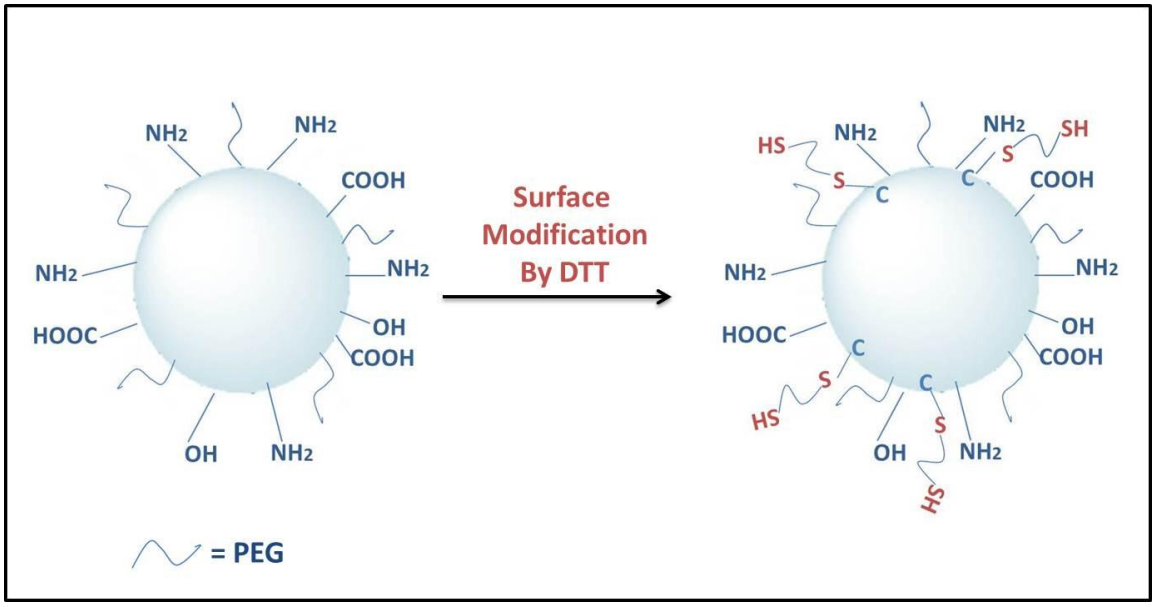

Figure S1. Schematic representation of DTT modified C-dots surface.

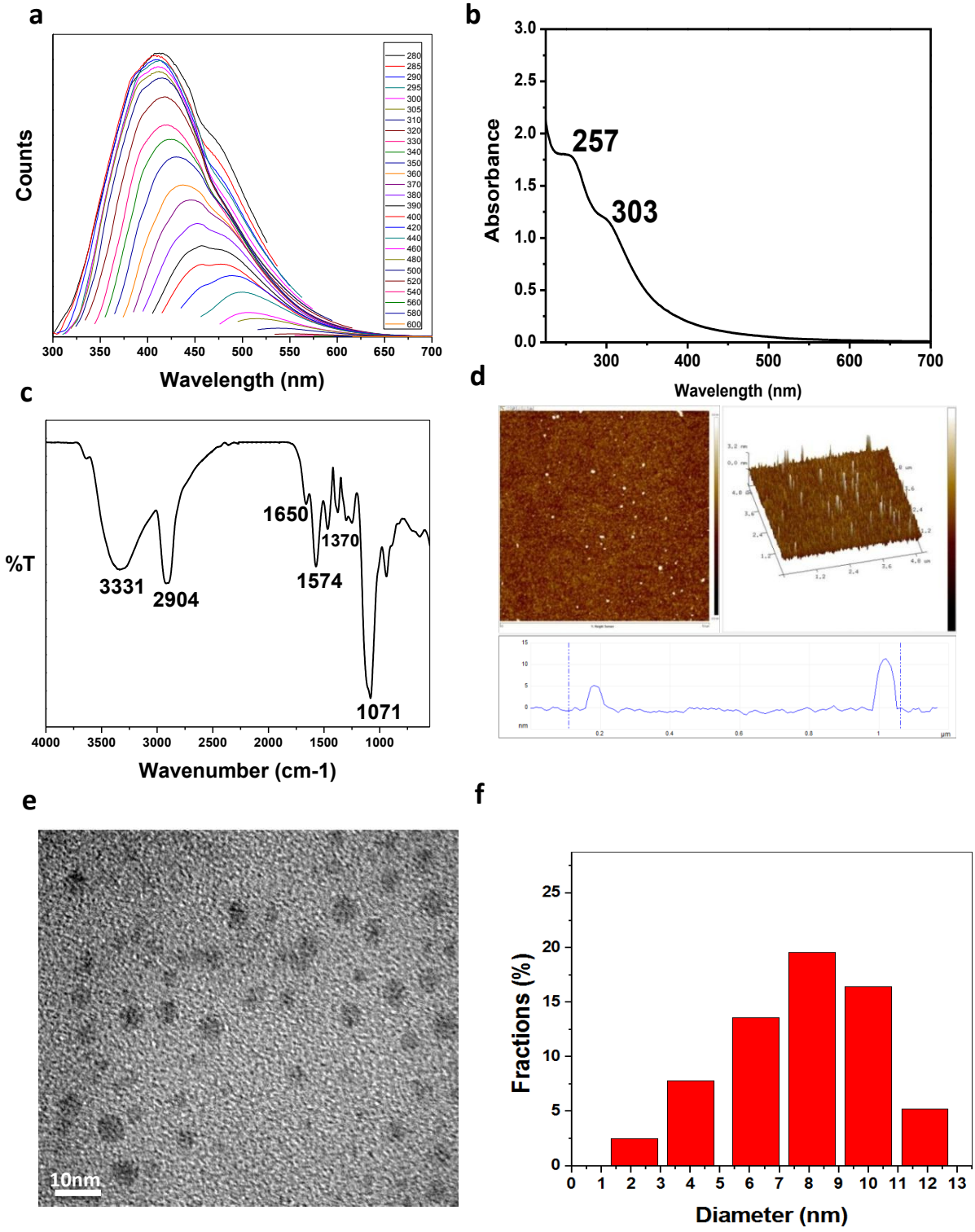

Figure S2. Characterization of synthesized C-dots (a) Multicolor fluorescence spectra recorded from 280 to 600 nm with variable excitation with 20 nm increment (b) UV-Vis absorbance spectrum (c) FTIR spectrum (d) AFM images and the height profile (e) TEM image and (f) TEM size distribution of the C-dots.

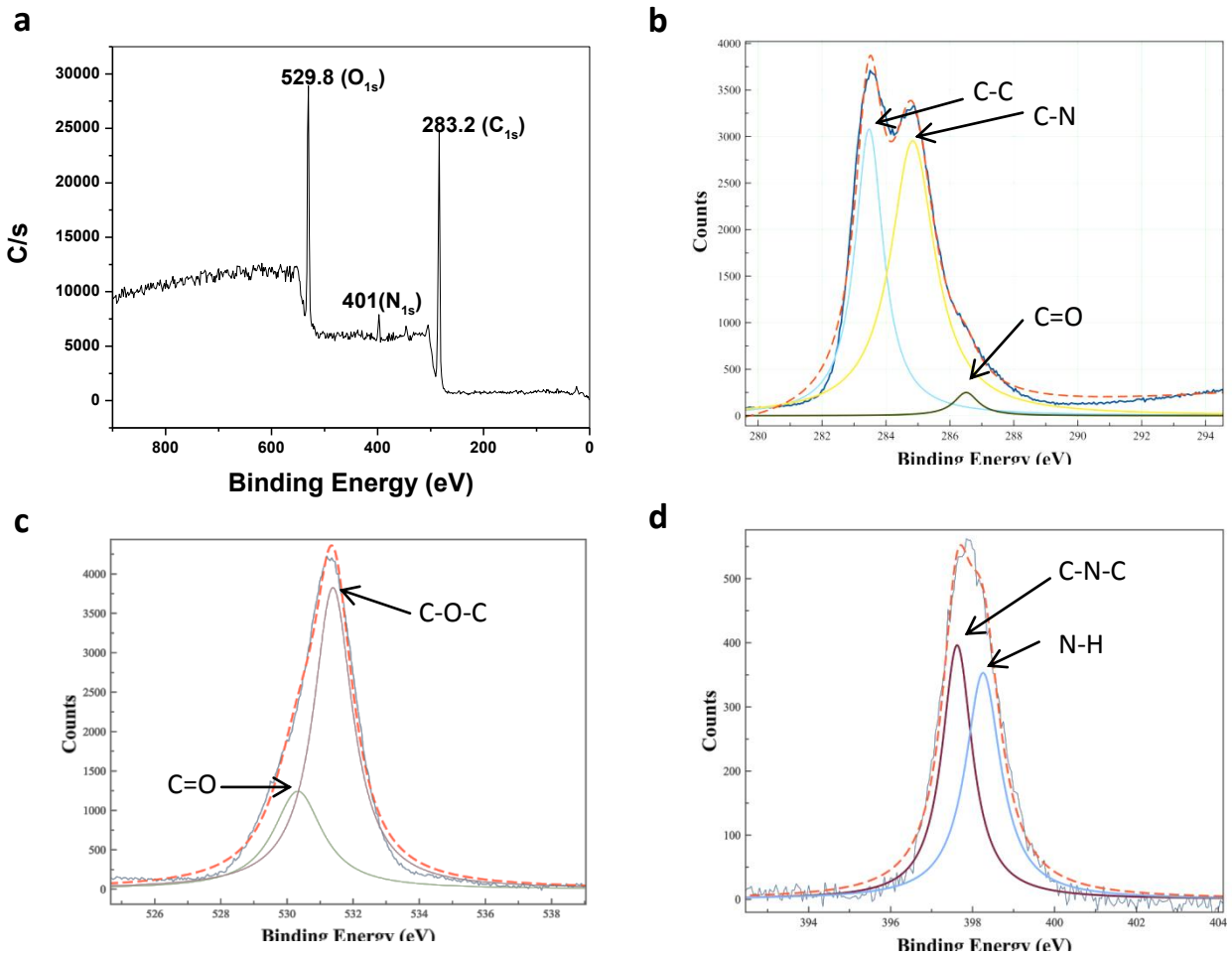

Figure S3. (a) XPS spectrum of the as synthesized C-dots shows three major peaks of C1s, O1s and N1s respectively. (b), (c) and (d) showed the high resolution C1s, O1s and N1s spectra of the as synthesized C-dots for characterizing the different functional groups.

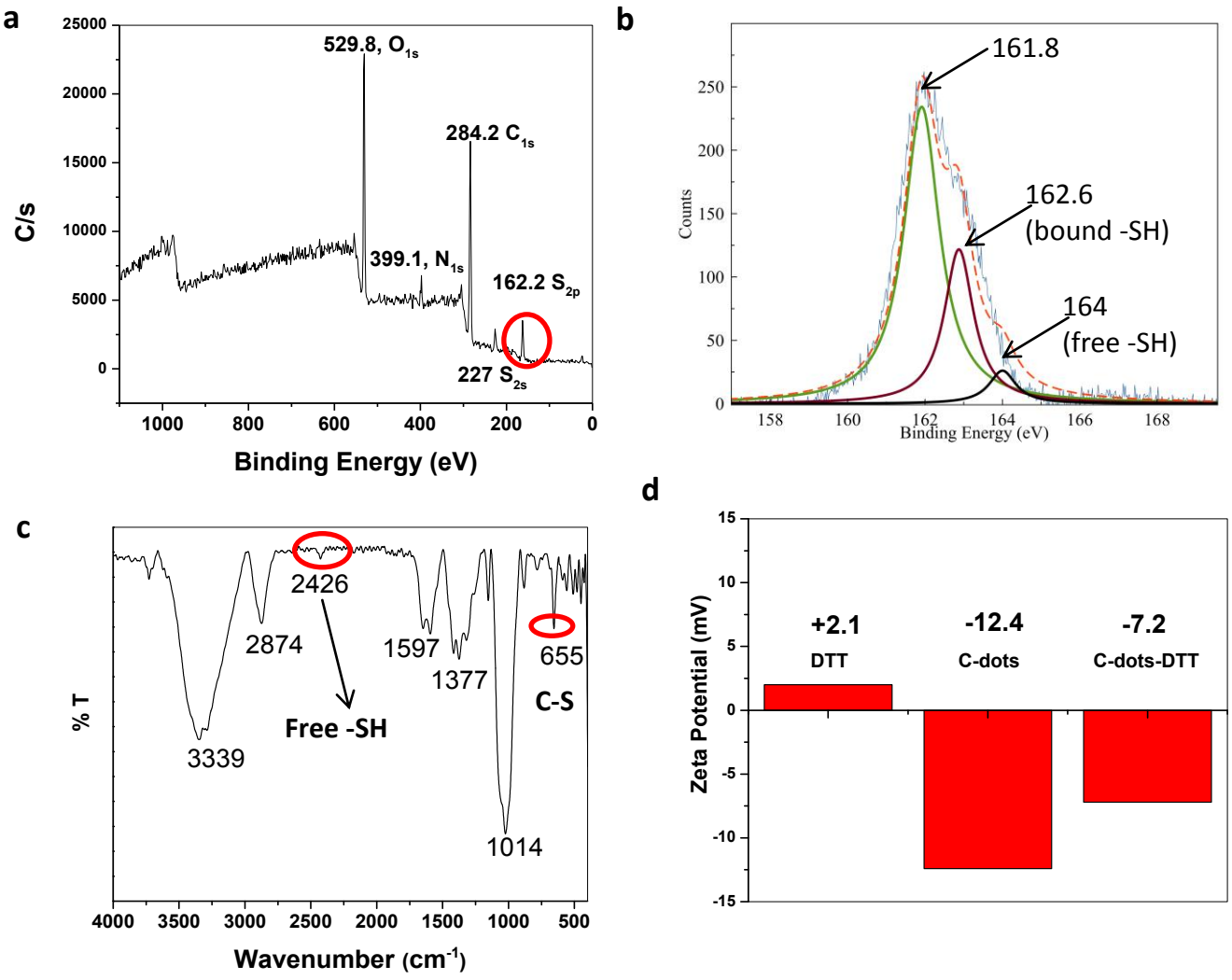

Figure S4. (a) XPS spectrum of DTT/C-dots shows a new peak at 162.2 eV corresponding to S2p. (b) the high resolution scan of S2p confirms the presence of bound and free thiol group in DTT functionalized C-dots (DTT/C-dots).[1,2] (c) FTIR spectra of DTT/C-dots: a new absorbance bands at 655 and 2426 cm^{-1} appear in DTT/C-dots corresponding to C-S and free –SH stretch[3] and (d) change in the zeta-potential value of C-dots after DTT functionalization.

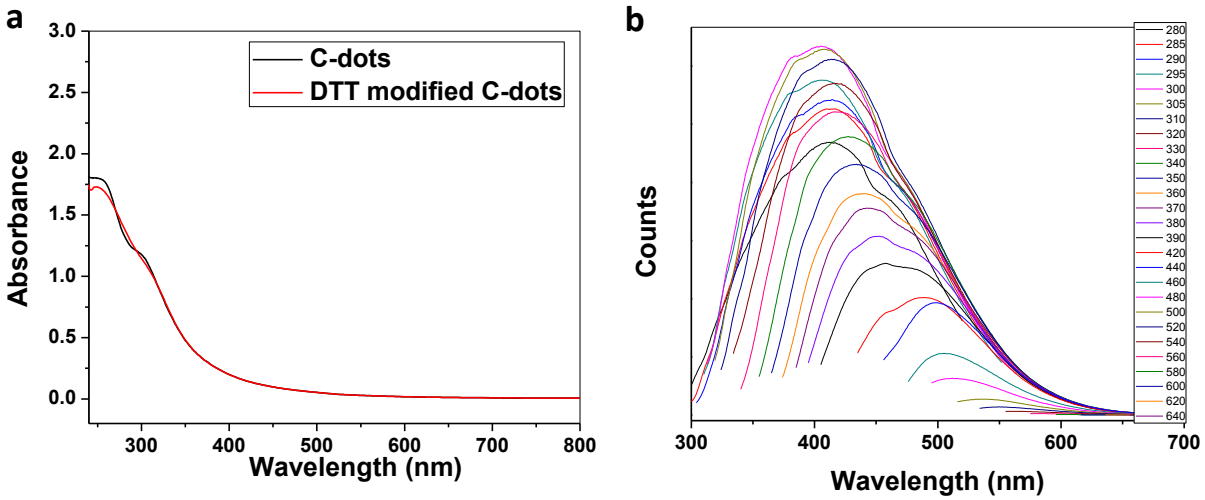

Figure S5. (a) Comparison of UV-Vis absorbance spectra of only C-dot and DTT/C-dots shows no significant change in the spectra after the modification with DTT and (b) multicolor fluorescence emission spectra of DTT/C-dots recorded from 280 to 640 nm excitation with 20 nm increment.

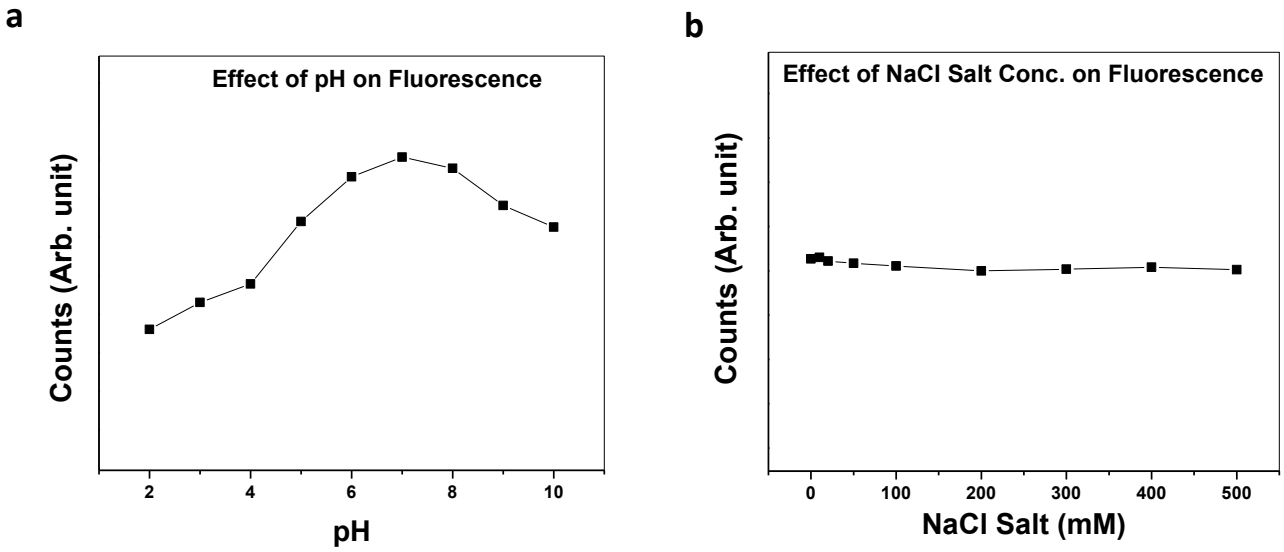

Figure S6. Effect of (a) pH and (b) NaCl concentration (0, 10, 50, 100, 200, 300, 400 and 500mM) on the fluorescence of C-dots. These results suggest that C-dots shows maximum fluorescence intensity at physiological pH (pH 7.4) and also its fluorescence is not affected even at high salt concentration.

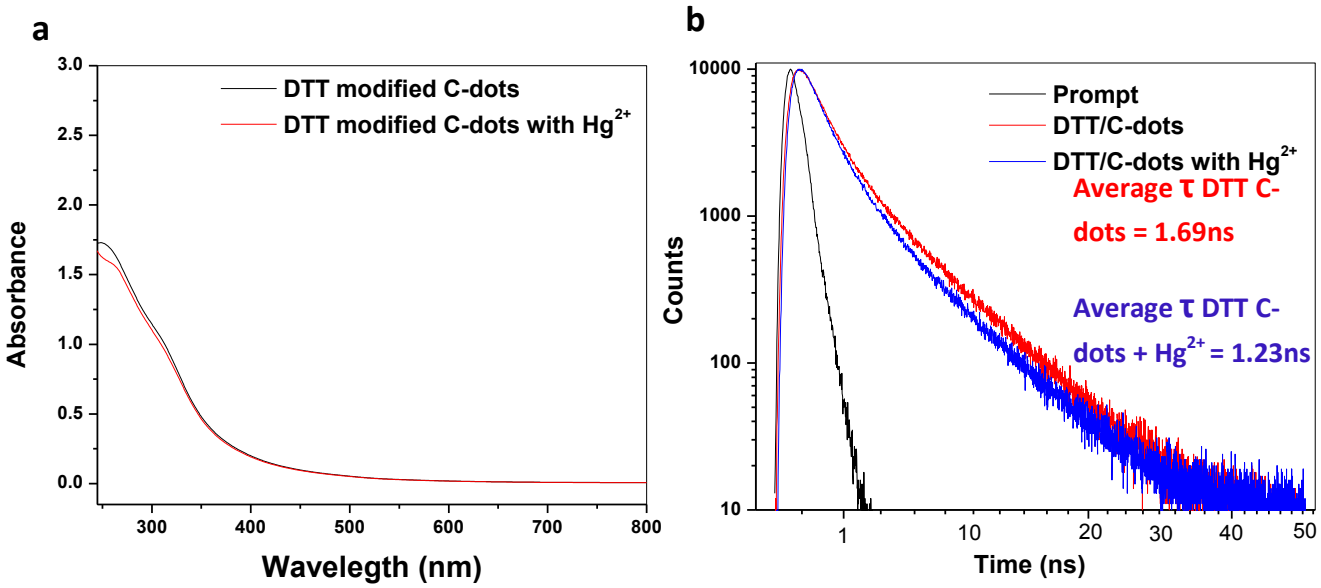

Figure S7. (a) UV-Vis absorbance spectra of DTT/C-dots and conjugated with Hg^{2+} (50 μM) and (b) life time data of only DTT/C-dots and with Hg^{2+} (50 μM).No significant changes were observed in the UV-Vis spectrum. However, a decrease in excited state lifetime after the Hg^{2+} complex formation confirms that the quenching process is dynamic in nature.

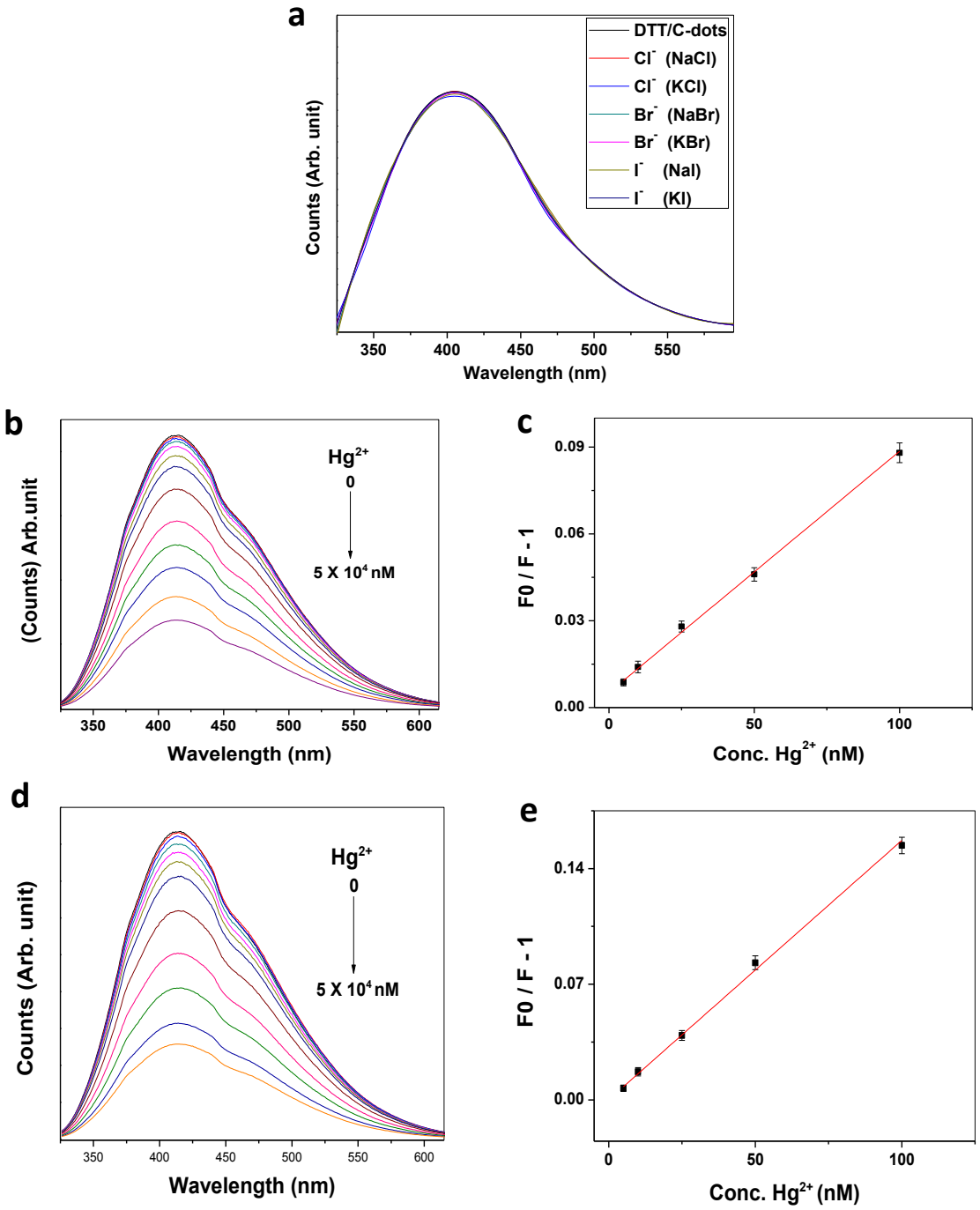

Figure S8. (a) The fluorescence spectra of DTT/C-dots in the presence of different halide ions (50 μM). No interference of halide ions (chloride, bromide and iodide) was observed on the fluorescence intensity of DTT/C-dots (b) Fluorescence quenching of DTT/C-dots with increasing concentration of Hg^{2+} in presence of 1 mM NaCl (c) the DL is determined to be as 19 pM in presence of 1 mM NaCl when the data of (b) is fitted using Stern-Volmer equation (d) fluorescence quenching of DTT/C-dots with increasing concentration of Hg^{2+} in presence of 1 mM KI (e) the DL is determined to be 23 pM when the data of (d) fitted using Stern-Volmer equation. Each data point is presented as standard deviation from three replicate assays.

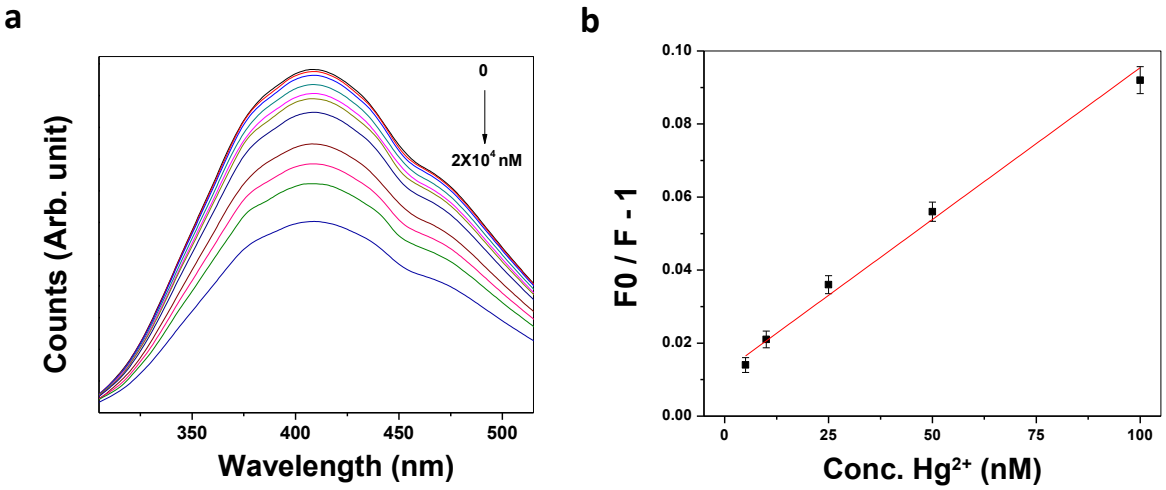

Figure S9. (a) Fluorescence quenching of the as synthesized C-dots in the presence of Hg^{2+}(b) the detection limit of 6.8 nM was observed when the data of (a) fitted using Stern-Volmer equation. Each data point is presented as standard deviation from three replicate assays.

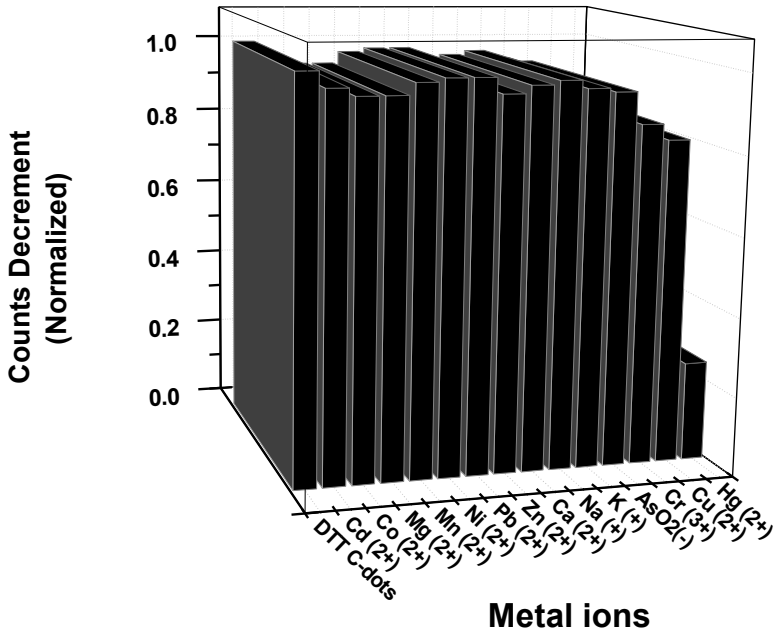

Figure S10. Normalized fluorescence intensity changes in DTT/C-dots in presence of various other metal ions (50 μM).

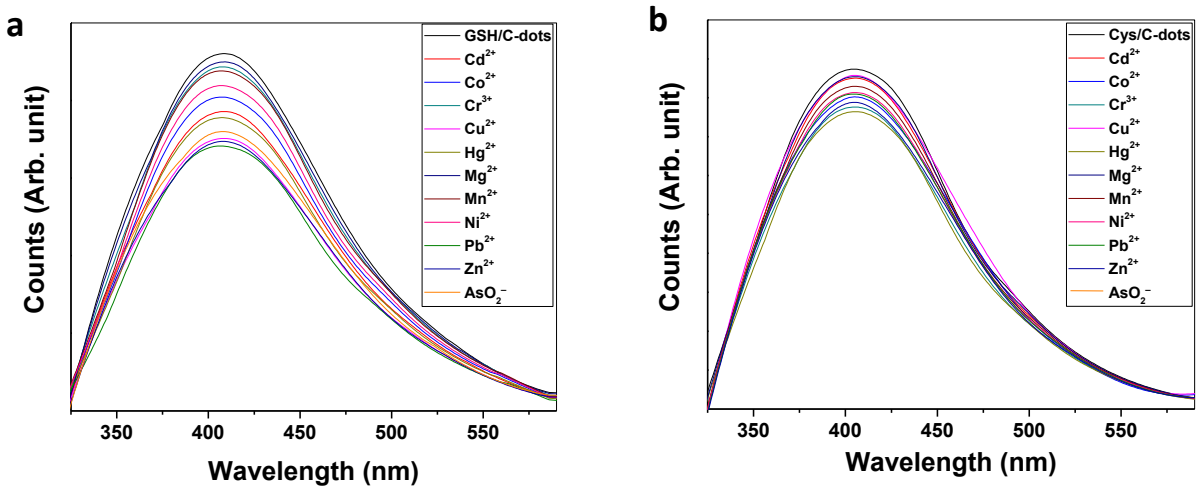

Figure S11. Selectivity of different metal ions (50 μM) in presence of C-dots surface functionalized with (a) GSH and (b) Cysteine

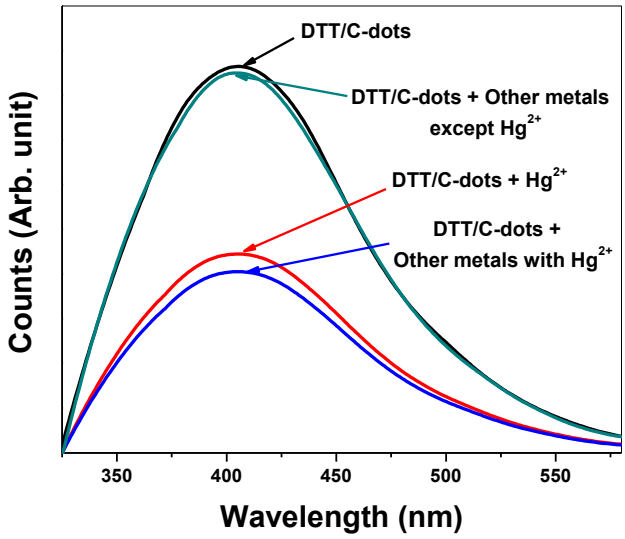

Figure S12. The data showed the change in the fluorescence intensity of DTT/C-dots in presence of the mixture of various metal ions with and without adding Hg^{2+}. No change in the fluorescence intensity of the DTT/C-dots was observed when all the metal ions were added excluding Hg^{2+}, however, extensive quenching was observed when Hg^{2+} was added to the system.

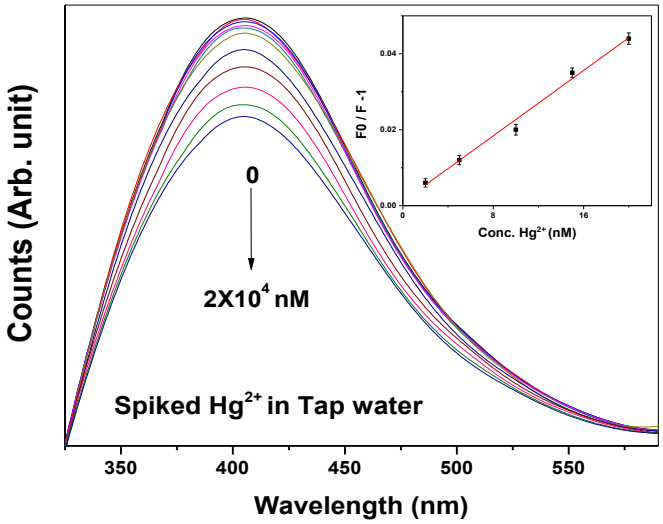

Figure S13. Fluorescence quenching pattern of DTT/C-dots in presence of Hg^{2+} ion spiked in tap water. Inset is showing the Stern-Volmer fitting to calculate the detection limit as 50 pM. Each data point is presented as standard deviation from three replicate assays.

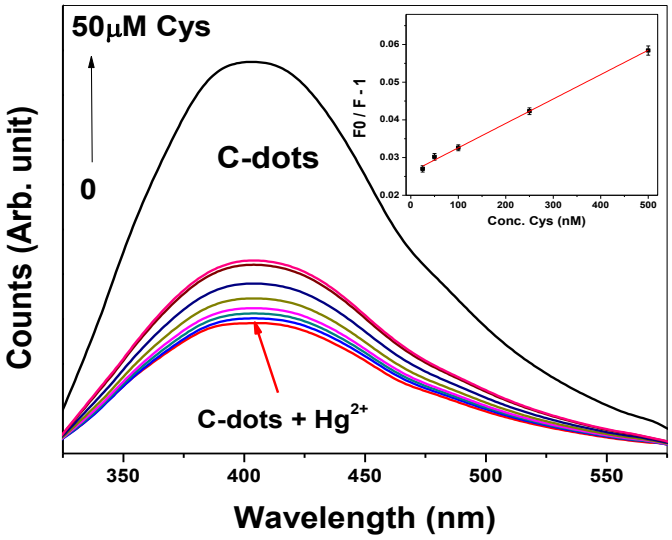

Figure S14. Fluorescence recovery of quenched C-dots/Hg^{2+} assay after the addition of Cysteine, The Stern-Volmer fitting (inset) showed detection limit as 72 nM. Each data point is presented as standard deviation from three replicate assays.

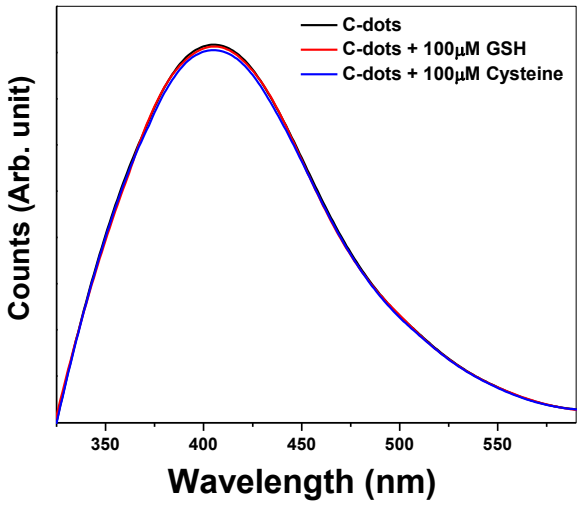

Figure S15. Control experiment (without addition of Hg^{2+}) revealed that the fluorescence intensity of the as synthesized C-dots (without DTT modification) had a negligible change in presence of biothiols (GSH and Cys).

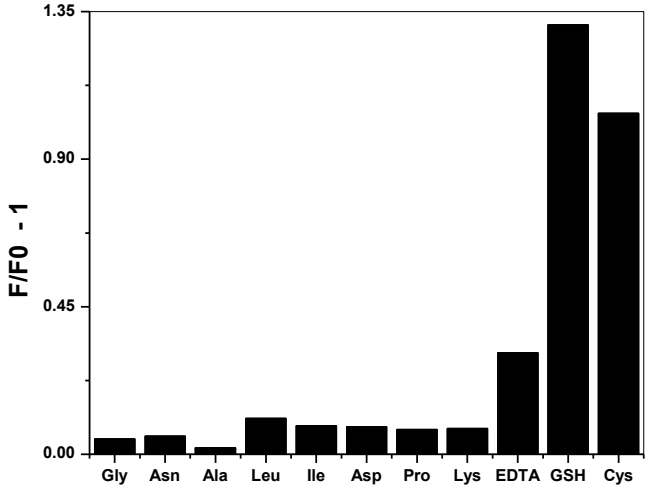

Figure S16. Relative change in the fluorescence recovery of quenched C-dots/Hg^{2+}after addition of EDTA, GSH, cysteine and several other amino acids (50μM). Apart from GSH and Cys, no other amino acids showed any significant effect on fluorescence recovery of quenched C-dots/Hg^{2+}.

Table S1: Comparison of the detection limit using different nanoparticle-based probes for Hg^{2+} detection.

S. No.	Methods	Detection limit	Supporting References
1	Surface-modified CdTe quantum dots	4 nM	4
2	Fluorescent gold nanoparticle	5 nM	5
3	Au@Ag core-shell Nanoparticles	9 nM	6
4	DNA-functionalized gold nanoparticles	25 nM	7
5	Mononucleotides-stabilized gold nanoparticles	50 nM	8
6	Cysteine functionalized Ag nanoparticles	65 nM	9
7	Carbon dots	226 and 845 nM	10
8	Carbon quantum dots	60 nM	11
9	Fluorescent carbon dots	5.9 nM	12
10	Carbon nano dots	4.2 nM	13
11	Carbon dots-labeled oligodeoxyribonucleotide	2.6 nM	14
12	Fluorescent carbon nanoparticles	0.23 nM	15
13	Carbon-dot	0.05 nM	16
14	Disposable strip biosensor	1 pM	17
15	Gold nanorod	2.4 pM	18
16	Lysozyme type VI stabilized gold nanoclusters	3.0 pM	19
17	**DTT/ C-dots and C-dots**	**18 pM and 6.8 nM**	**(our method)**

Table S2: Physicochemical properties of the Uhl River water, Kamand Village of Mandi, Himachal Pradesh 175001, India

Physicochemical property	**Experimental value**
Turbidity (NTU)	4.9 NTU
Conductivity (μS)	95.6μS
pH	7.56
Alkalinity	40.00 mg/L
Total Hardness as $CaCO_3$	80.00 mg/L
Ca^{2+}	16.00 mg/L
Mg^{2+}	9.76 mg/L
Na^{+}	2.70 mg/L
K^{+}	1.80 mg/L
Cl^{-}	25.56 mg/L
SO_4^{2-}	2.90 mg/L
HCO_3^{-}	40.00 mg/L
F^{-}	0.00 mg/L

Table S3: Physicochemical properties of the tap water of Mandi, Himachal Pradesh 175001, India.

Physicochemical property	Experimental value
Turbidity (NTU)	0.00 NTU
Conductivity (μS)	34.8 0μS
pH	7.79
Alkalinity	64.00 mg/L
Total Hardness as $CaCO_3$	240.00 mg/L
Ca^{2+}	64.00 mg/L
Mg^{2+}	19.50 mg/L
Na^{+}	11.30 mg/L
K^{+}	5.30 mg/L
Cl^{-}	19.88 mg/L
SO_4^{2-}	2.80 mg/L
HCO_3^{-}	64.00 mg/L
F^{-}	0.35 mg/L

Supporting References

1- L. Tortech, Z. Mekhalif, J. Delhalle, F. Guittard and S. Geribaldi, *Thin Solid Films*, 2005, **491**, 253.

2- T. Laihoa, J. A. Leiro, M. H. Heinonena, S. S. Mattila and J. Lukkari, *J. Electron Spectrosc. Relat. Phenom.*, 2005, **142,** 105.

3- M. F. Brigatti, C. Lugli, S. Montorsi and L. Poppi, *Clays Clay Miner.*, 1999, **47**, 664.

4- Y. Xia and C. Zhu, *Talanta*, 2008, **75**, 215–221.

5- C. C. Huang, Z. Yang, K. H. Lee and H. T. Chang, *Angew. Chem. Int. Ed.*, 2007, **46**, 6824–6828.

6- S. Guha, S. Roy and A. Banerjee, *Langmuir*, 2011, **27**, 13198–13205.

7- C. W. Liu, C. C. Huang and H. T. Chang, *Langmuir*, 2008, **24**, 8346–8350.

8- Y. Xu, L. Deng, H. Wang, X. Ouyang, J. Zheng, J. Li and R. Yang, *Chem. Commun.*, 2011, **47**, 6039–6041.

9- L. Shang, J. Yin, J. Li, L. Jin and S. Dong, *Biosens. Bioelectron.*, 2009, **25**, 269–274.

10- F. Yan, Y. Zou, M. Wang, X. Mu, N. Yang and L. Chen, *Sens. Actuators, B*, 2014, **192**, 488.

11- J. Hou, F. Zhang, X. Yan, L. Wang, J. Yan, H. Ding and L. Ding, *Anal. Chim. Acta*, 2012, **859**, 72–78.

12- I. C. Mora, V. Romero, I. Lavilla, and C. Bendicho, *Anal. Chem.*, 2014, **86**, 4536.

13- L. Zhou, Y. Lin, Z. Huang, J. Ren and X. Qu, *Chem. Commun.*, 2012, **48**, 1147.

14- X. Cui, L. Zhu, J. Wu, Y. Hou, P. Wang, Z. Wang and M. Yang, *Biosens. Bioelectron.*, 2015, **63**, 506.

15- W. Lu, X. Qin, S. Liu, G. Chang, Y. Zhang, Y. Luo, A. M. Asiri, A. O. Al-Youbi and X. Sun, *Anal. Chem.*, 2012, **84**, 5351.

16- S. Mohapatra, S. Sahu, N. Sinhab and S. K. Bhutia, *Analyst*, 2015, **140**, 1221.

17- J. Chen, S. Zhou and J. Wen, *Anal. Chem.* 2014, **86**, 3108.

18- G. Chen, Y. Jin, L. Wang, J. Dengab and C. Zhang, *Chem. Commun.*, 2011, **47**, 12500.

19- Y. H. Lin and W. L. Tseng, *Anal. Chem*. 2010, **82**, 9194.