Electronic Supplementary Information (ESI) †

Facile and green synthesis of fluorescent carbon dots from onion waste and their potential applications as sensors and multicolour imaging agents

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Experimental section:

Materials:

Onions were purchased from a local supermarket. All the reagents used were of analytical grade and used without further purification. All the solutions were prepared in double distilled water.

Synthesis of Carbon dots (CDs): Here we report a novel green approach for the synthesis of carbon dots using a simple autoclave and onion waste as the source material. To 30 g of finely crushed Onion waste (top & bottom parts and two external layers), 100 mL of double distilled water was added and boiled for 1 h. The resultant solution was filtered after cooling down to room temperature. To 20 mL of the filtrate taken in a boiling tube, 200 µL of ethylene diamine (EDA) was added. The tube was closed with aluminium foil and kept in the autoclave under 15 lbs pressure and 120 ^{o}C temperature for 2 h. After cooling down to room temperature naturally, the obtained dark brown solution was centrifuged to remove insoluble black particles. It was then filtered through a 0.22 µm microporous membrane and dialyzed for 24 h against double distilled water by using a dialysis membrane (MWCO=3500) to remove the unreacted molecules and ions. Double distilled water was replaced several times during the dialysis

process. Finally the product was dried using a freeze dryer and stored in the refrigerator for further usage. As and when required, certain amount of dried product was dissolved in ultrapure water to prepare desired solutions (1 mg/mL).

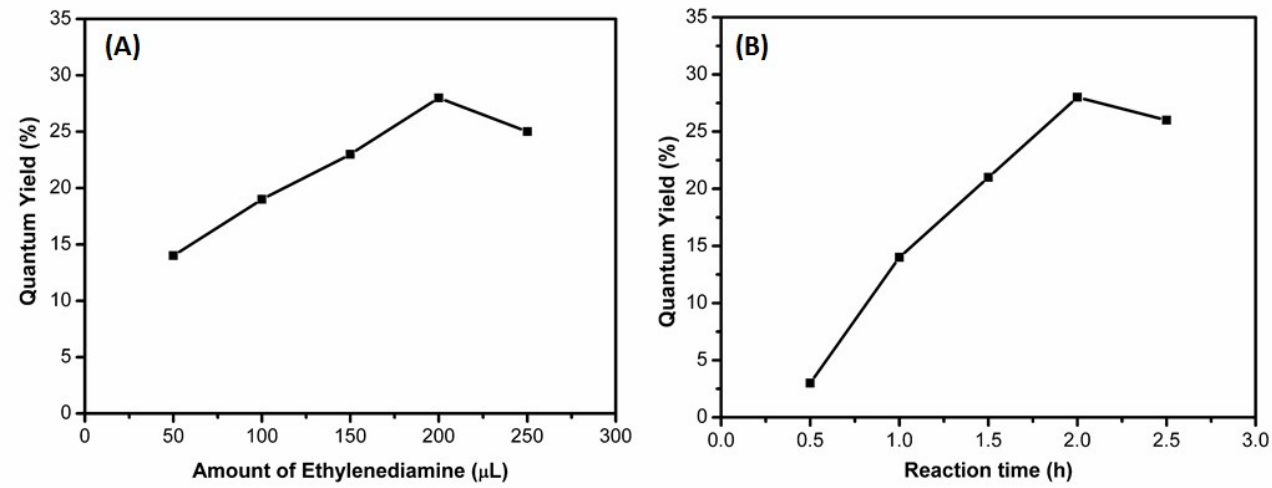

Figure. S1. Optimization of synthetic conditions: Quantum yield as a function of A) amount of ethylene diamine B) reaction time.

Characterizations: Fluorescence spectral measurements were carried out using a RF-5301 PC Spectrofluorophotometer (Shimadzu, Kyoto, Japan) set with excitation and emission slit widths at 3nm. Absorbance spectra were recorded using a Dual Beam UV-visible Spectrophotometer (Shimadzu-3600, Kyoto, Japan). High resolution Transmission electron microscopy (HRTEM) images were acquired on a JEOL 3010 microscope operating at 200 kV by drop casting an appropriate dilution of CDs aqueous solution onto the carbon-coated copper grids. X-ray photoelectron spectroscopy (XPS) measurements were carried out by Kratos AXIS Ultra spectrometer with a Al Kα X-ray as excitation source (1486.71eV). Zeta potential and DLS measurements were conducted using a Malvern, Nano ZS 90 zetasizer. Elemental composition was determined using a Flash 2000 CHN Analyser (Thermo Scientific). Raman spectra were measured with a Horiba Jobin-Yvon LABRAM HR with a focal length of 800 mm and equipped with a He-Ne 633 nm Laser. Thermogravimetric analysis (TGA) was performed using METTLER TA3000 thermal analyser by heating the sample under the flow of N_2 gas to 800 ^{o}C at the rate of 10 °C min^{-1}. X-ray diffraction (XRD) patterns of CDs were obtained using a X' pert Pro powder X-ray diffractometer (Netherland) with Cu-Kα radiation,

λ = 1.5406 Å. Identification of functional groups was done using an IR Prestige-21 spectrometer Shimadzu using KBR pellets. Fluorescence decay analysis was done using a Horiba Jobin Yvon "Fluoro Cube Fluorescence Lifetime System" equipped with a 370nm Nano LED source.

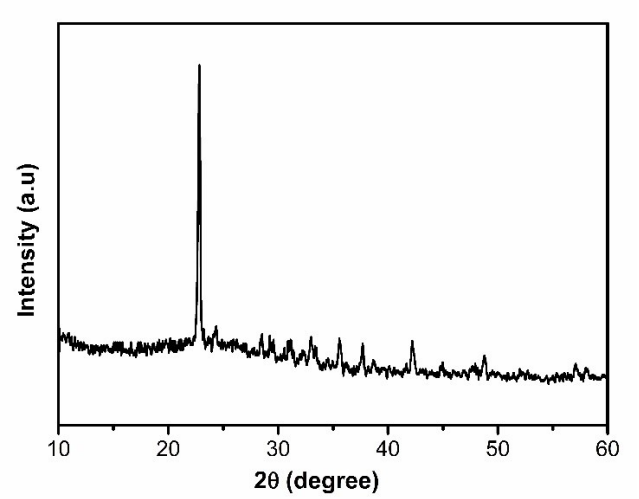

Figure. S2. Powder XRD pattern of CDs.

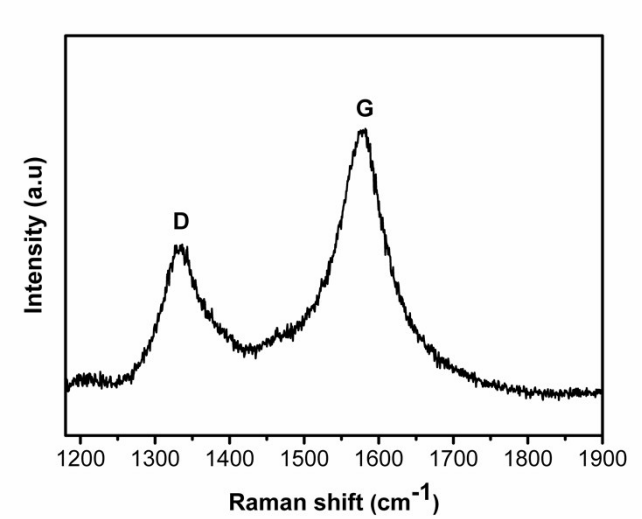

Figure. S3. Raman spectrum of CDs.

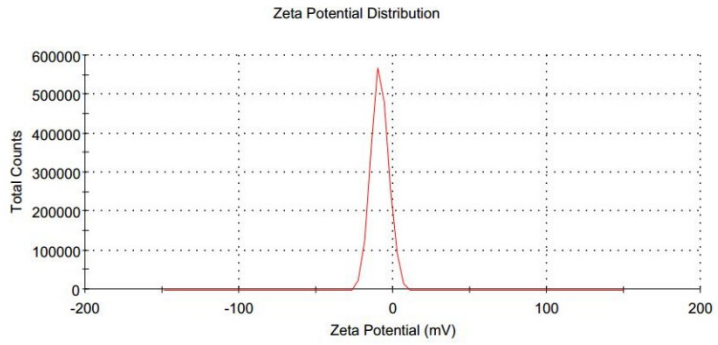

Figure. S4. Zeta potential of CDs aqueous solution.

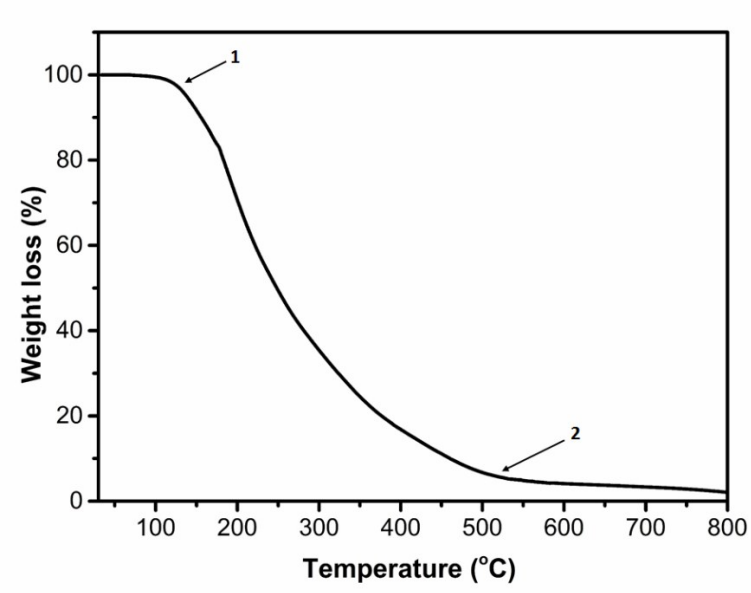

Figure. S5. Thermogravimetric analysis (TGA) graph of CDs.

Quantum yield measurement: Quantum yields of the as synthesized carbon dots (CDs) were calculated by comparing the integrated photoluminescence intensities and the absorbance value using Quinine sulphate (QS) as standard (Φ = 54%). In this method, first several samples of CDs aqueous solution and quinine sulphate in 0.1M H_2SO_4 solution was prepared by keeping the absorbance values less than 0.1 at their excitation wavelengths. Then the integrated photoluminescence intensities of all the samples excited at their optimal excitation wavelengths (360 nm for QS & 380 nm for CDs) was measured. The integrated photoluminescence intensity was plotted against absorbance and the slope values of the obtained linear plots were measured. Then by using the following equation quantum yield values were calculated.

$$\Phi_x = \Phi_{st}\left(\frac{Grad_x}{Grad_{st}}\right)\left(\frac{\eta_x^2}{\eta_{st}^2}\right)$$

Where Φ is the quantum yield, *Grad* the gradient from the plot of integrated fluorescence intensity *vs* absorbance and η is the refractive index of the solvent (both 1.33). The subscript *st* and *x* are denoted to standard (QS) & CDs respectively.

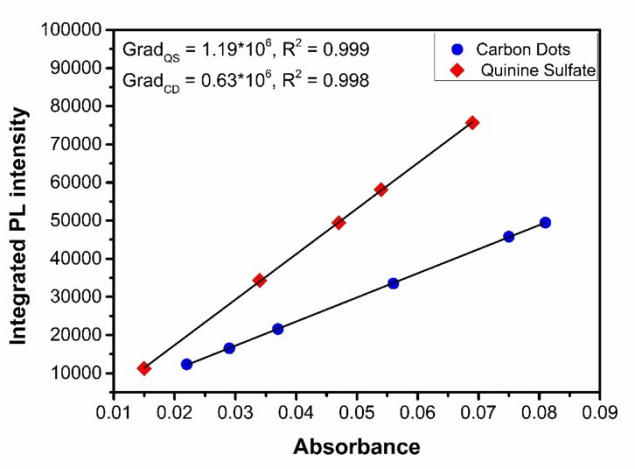

Figure. S6. Plot of integrated fluorescence intensity *vs* absorbance of CDs and QS

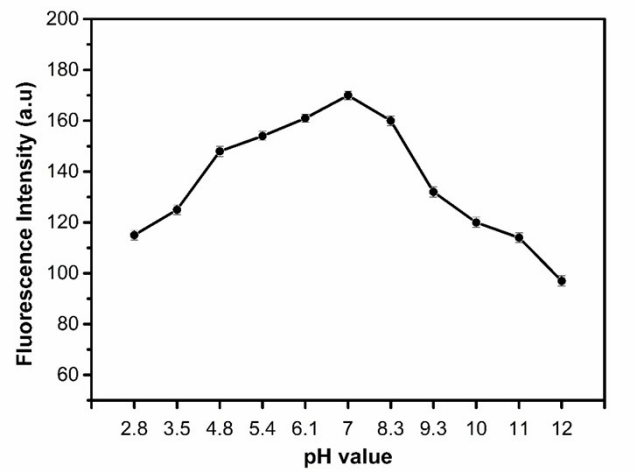

Figure. S7. Dependence of fluorescence emission of CDs on pH (λ_{ex} = 380 nm; λ_{em} = 464 nm)

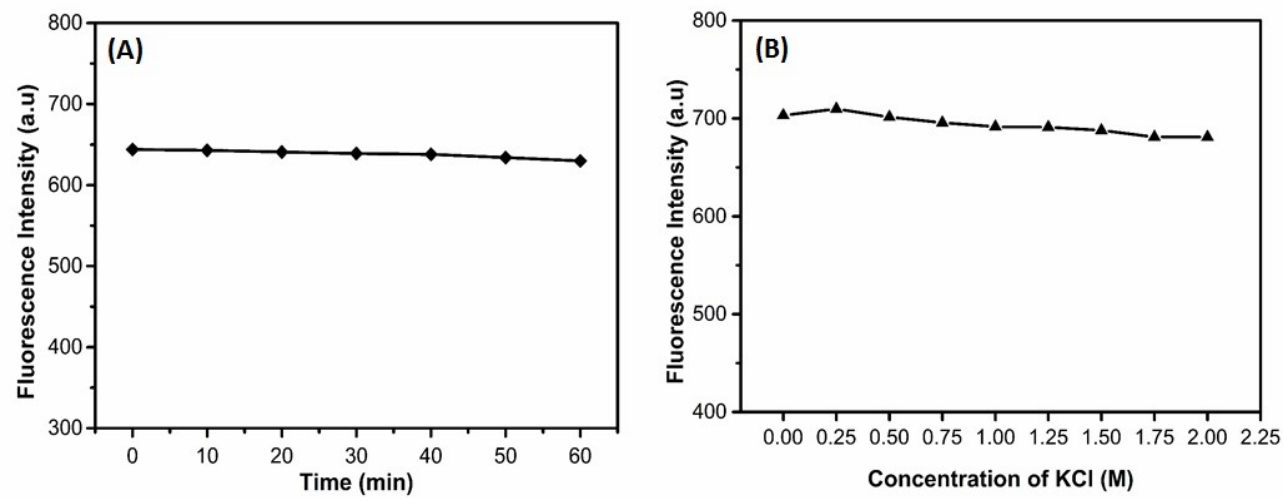

Figure. S8. Dependence of fluorescence emission intensity of CDs on (A) irradiation time (photostability) (B) ionic strength at λ_{ex} = 380 nm; λ_{em} = 464 nm.

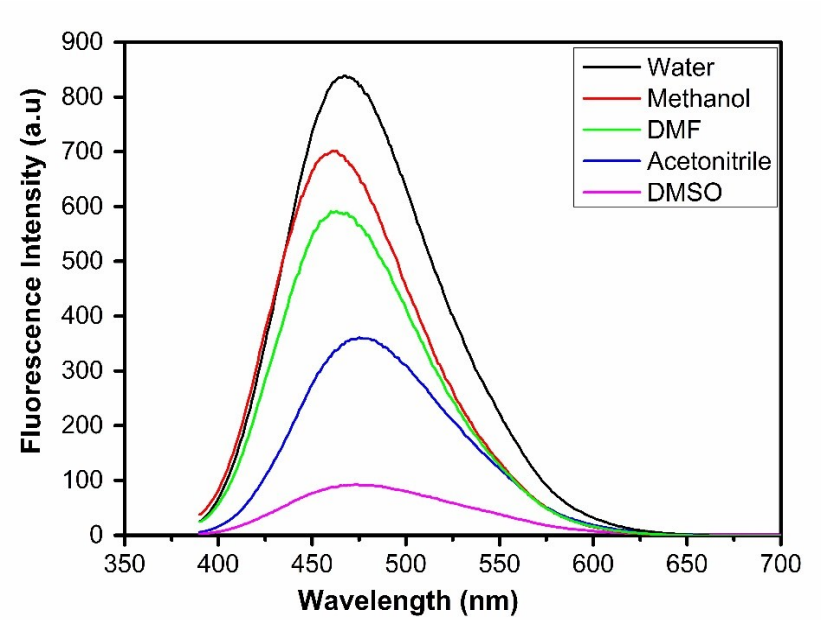

Figure. S9. Effect of various solvents on fluorescence intensity of CDs (λ_{ex} = 380 nm)

Procedure of Fe^{+3} sensing: All metal ion solutions were prepared from their respective salts using deionized water. 50 µL of 0.1 mg/mL CDs solution was added to 2 mL of deionized water and the fluorescence intensity of this sample at λ_{em} = 464nm (λ_{ex} = 380nm) was taken as F_0. In the same way 50 µL of 0.1 mg/mL CDs solution was added to 2 mL of different metal ion solutions having 100 µM

concentration. The samples were shaken well and incubated for 3 min and then the fluorescence intensity was measured and taken as F. For quantitative determination of Fe^{+3} similar procedure was followed. In a typical assay, 50 µL of CDs (0.1mg/mL) solution was added to 2 mL of Fe^{+3} salt solution having varying concentrations.

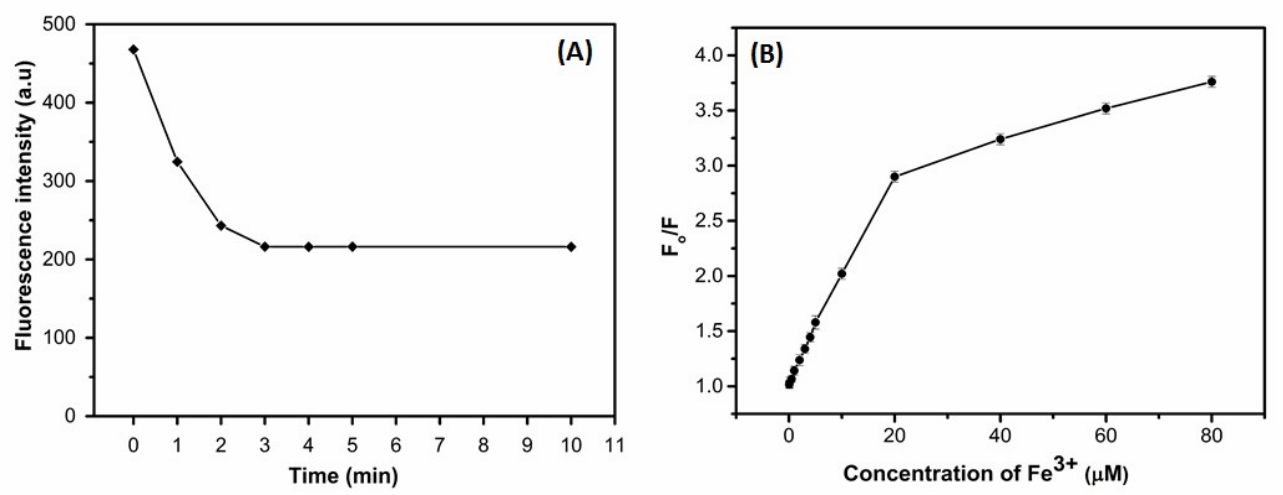

Figure. S10. (A) Fluorescence intensity of (CDs + Fe^{3+}) system as a function of time (B) Relative fluorescence intensity of CDs as a function of Fe^{3+} concentration (λ_{ex} = 380 nm; λ_{em} = 464 nm).

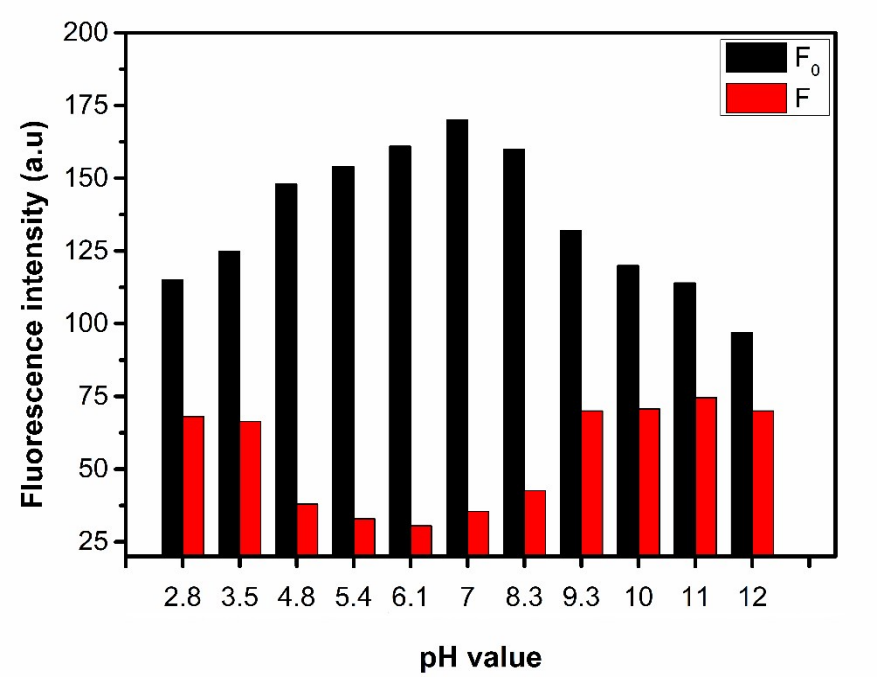

Figure. S11. Fluorescence response of CDs in the absence (black) and presence (red) of 100 µM Fe^{3+} at different pH values. (λ_{ex} = 380 nm; λ_{em} = 464 nm)

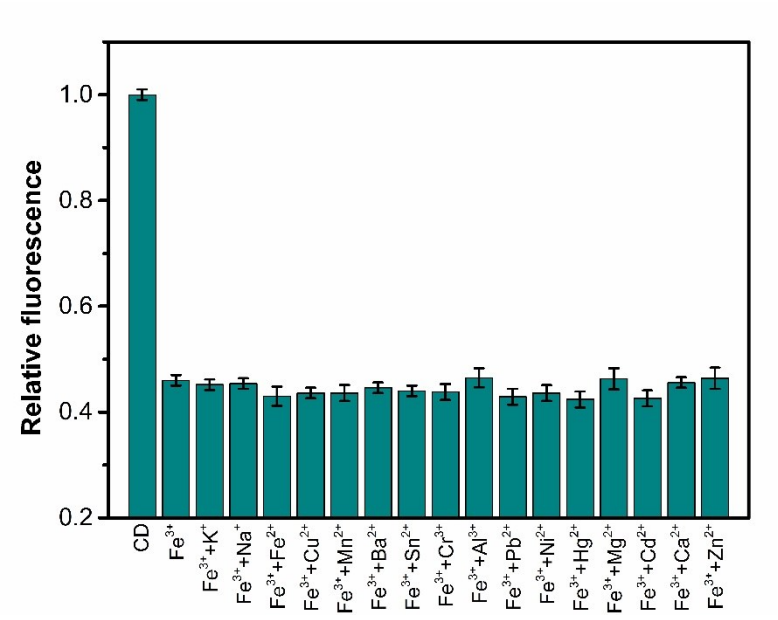

Figure. S12. Relative fluorescence response (F_o/F) of CDs towards Fe^{+3} ions in the presence of other metal ions (Anti-interference ability).

Detection of Fe^{+3} in real water samples: Detection capability of CDs in real water samples was evaluated by analysing the spiked samples of tap and lake water. For this purpose water samples collected from nearby lake in Osmania University campus (centrifuged and filtered through 0.45 μm membrane filter) and tap water samples were spiked with different concentrations of Fe^{+3} and the obtained recoveries were given in the following table.

Table S1: Results of Fe^{+3} in real water samples

Samples	Added (μM)	Found (μM)	RSD (n = 3, %)	Recovery (%)
Tap water	15	15.2, 15.1, 14.9	0.9	99.3-101.3
	10	10.3, 10.4, 10.1	1.5	101-104
Lake water	15	15.1, 14.9, 14.8	1	98.6-100.6
	10	9.7, 10.1, 9.8	2	97-101

Cytotoxicity test:

In vitro cytotoxicity of CDs was evaluated on both cancerous (HeLa) and normal (HEK293) cell lines by using MTT Assay. Cellular toxicity was determined by measuring the activity of mitochondrial enzymes in live cells to transform the soluble yellow MTT solution to an insoluble purple formazan product. The cells were cultured in 96-well tissue culture plates at a density of 1×10^4 cells per well. After attachment, the cells were incubated with a medium containing different doses of CDs for 24 hours. Subsequently, after incubation the medium from each well was removed and the cells were washed in phosphate buffered solution (PBS). A fresh medium containing 10 µL of 5 mg mL^{-1} solution of MTT was added to each well and incubated for 4 h and then the medium was replaced with 150 µL of DMSO followed by shaking for 15 min to dissolve the formazan crystals. The absorbance of each well was recorded at 570 nm using a multimode microplate reader (Biotek, Cytation3). The untreated cells were used as controls for calculating the relative percentage cell viability from the following equation:

% Cell viability = (A_{570} in treated sample / A_{570} in control sample) × 100%

Multicolour imaging:

Bio imaging potential of CDs was tested using HeLa Cells. HeLa cells were seeded in 6-well culture plates at a density of 10^5 cells per well in DMEM containing 10 % Fetal bovine serum (FBS) and incubated for 24 h at 37^{o}C and 5 % CO_2. Then the medium was replaced with a fresh medium containing 0.2 mg/mL CDs and further incubated for 6 h. After that the cells were washed thrice with PBS to remove extracellular CDs, fixed with 4% paraformaldehyde and mounted using 50 % glycerol. Fluorescent images were recorded using Zeiss LSM 510 Meta confocal Microscopy at laser excitations of 405, 488 and 561 nm.

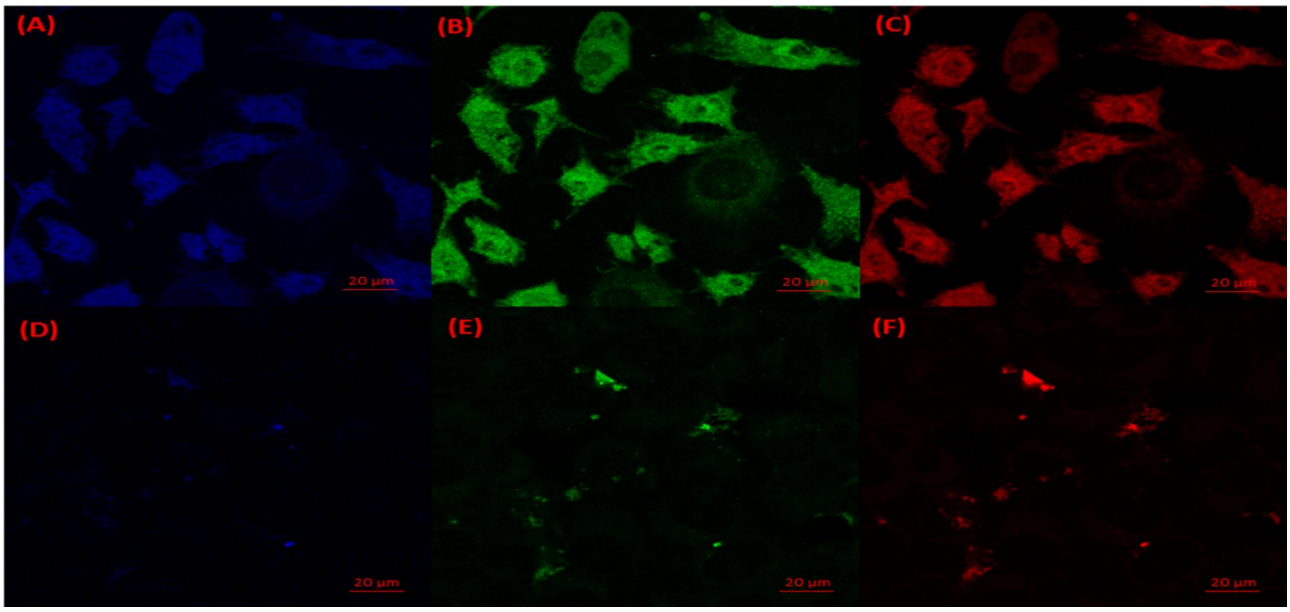

Figure. S13. Confocal laser scanning microscopic images of HeLa cells treated with CDs + Fe^{+2} (A, B & C) and CDs + Fe^{+3} (D, E, & F) at excitation wavelengths of (A & D) 405 (blue), (B&E) 488 (green) and (C&F) 561 (red) nm. Scale bar indicates 20 µm. Concentrations of CDs and metal ions are 0.2 mg/mL and 0.1 mM respectively.