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Supporting Information

A cucurbit[6]uril-carbon dot system: a potentially new bioimaging agent

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Contents

S1. Experimental Section

Chemicals and Reagents: Q[6] was synthesized according to the literature[1]. The reaction solution was cooled to room temperature, filtered, and added with appropriate amount of methanol added to the filtrate. The solid was filtered out, washed with a small amount of distilled water, recrystallized with distilled water, and characterized by NMR to obtain pure Q[6]. *m*-Phenylenediamine was purchased from Aladdin (Shanghai, China). All solvents used below were purchased from Aladdin (Shanghai, China). All metal ion reagents (nitrate salt and perchlorate salt) are analytically pure without further purification. Deionized water was used throughout. All metal ions are configured with deionized water in a solution of 0.02M concentration. PC-3 cells purchased from Kunming Cell Bank, China Academy of Sciences.

Apparatus and Instruments: The UV-Vis spectra were recorded at room temperature using an 8453 UV-Vis spectrophotometer purchased from Agilent (Agilent Technologies, Santa Clara, California, USA). The fluorescent spectra were recorded at room temperature using a Varian Cary Eclipse fluorescence emission spectrophotometer (Varian, Inc., Palo Alto, California, USA). Model 818 pH meter (Orilon of America) was used to adjust the pH of the solution. Centrifuge the solution using an H1850 centrifuge. The IR spectrum was tested on a Bruker infrared spectrometer. TEM images were obtained using Tecnai G2 F20 Field Emission Transmission Electron Microscope (FEI Corporation of America). The particle size was determined by a Malvern laser particle size analyzer (Zetasizer Nano ZS90). XRD patterns were tested with X-ray diffractometer (X 'pert Powder). PC-3 cell imaging images are derived from Ti inverted fluorescence phase contrast microscope and imaging system (Nikon, Japan). x-ray photoelectron spectrometer (Thermo Fisher, USA), freeze dryer

Synthesis of *m*-Q[6]-CQDs: Q[6] (160 mg, 0.161 mmol) and *m*-phenylenediamine (23 mg, 0.213 mmol) were dissolved in 60 ml ultrapure water, and the mixture was mixed uniformly by ultrasonic shock. The mixture was transferred to a 100 mL reactor and reacted at 180 °C for 12 h. After the reaction was completed, the yellow liquid was

obtained. The liquid product was centrifuged at 10000 r/min for 20 min. to obtain the supernatant. Then the ultrafiltration tube with molecular cut-off flow of 5 W was centrifuged at 6500 r/min. for 10 min., and the obtained liquid was moved into the dialysis bag with molecular cut-off mass of 1000 u, and placed in a large beaker filled with deionized water for 24 h. Then the dialysis solution was freeze-dried to obtain CQDs, weighing 38 mg, which equates to a yield of 23 %.

Transmission electron microscopy (TEM) sample processing: A certain concentration of *m*-Q[6]-CQDs was diluted and dropped on the dark side of the copper net (the concentration should not be too large, otherwise the test results would be affected). Then *m*-Q[6]-CQDs was dried with an infrared lamp. The samples were placed under transmission electron microscopy and irradiated under 200KV acceleration voltage to observe and analyze the morphology and structure of the samples.

X-ray diffraction (XRD) sample treatment: The *m*-Q[6]-CQD obtained after freeze-drying was placed in a special glass mold for X-ray diffractometer, pressing and spreading, and detected by X-ray diffractometer. The conditions were set as follows: Wavelength 0.15406 nm, voltage 45 KV, current 40 mA (variable), scanning range 10-90 °, scanning speed 5/min. The test results were analyzed and processed by software Jade.

X-ray photoelectron spectroscopy (XPS) sample processing: the solid powder obtained after drying *m*-Q[6]-CQDs was tested by X-ray photoelectron spectroscopy, using non-monochromatic al-k laser (1486.6eV) with a power of 150W;Semi-quantitative analysis was performed on the relative content of elements, which was calculated by Vision software and Shirley baseline. Finally, Advantage software is used for data processing and peak fitting.

Fourier Transform infrared spectroscopy (FTIR) sample processing: The above *m*-Q[6]-CQDs and spectrally pure potassium bromide were dried in a vacuum drying oven and mixed at a ratio of about 1 : 200. The mixture was thoroughly ground and mixed in an agate mortar, and then the tablets were pressed on a pressure machine for sample preparation using the matching mold of an infrared spectrometer. The

absorption/transmission spectrum was tested by an infrared spectrometer with a scanning range of 400-4000 cm^{-1}.

Preparation of UV-VIS solution: the 38 mg *m*-Q[6]-CQDs solid was dissolved in 10 mL ultrapure water to obtain a concentration of 3.8 mg/mL reserve solution. Then 200 μL of the reserve solution was diluted to 2 mL with ultrapure water to obtain 0.38 mg/mL *m*-Q[6]-CQDs solution. The solution was transferred into a UV cuvette, and the UV-visible absorption spectrum of *m*-Q[6]-CQDs was measured by UV-visible spectrophotometer (scanning speed was medium, scanning range was 200-800 nm).

Preparation of fluorescence spectrum (FL) solution: 200 μL of the above *m*-Q[6]-CQDs reserve solution with a concentration of 3.8 mg/mL was diluted to 2mL with ultrapure water to obtain a concentration of 0.38 mg/mL *m*-Q[6]-CQDs solution. The solution was transferred into a fluorescence colorimetric dish, and the fluorescence emission spectrum of *m*-Q[6]-CQDs was measured by fluorescence spectrophotometer.

Detection of metal ions by *m*-Q[6]-CQDs: 200 μL *m*-Q[6]-CQD (3.8 mg/mL) stock solution was fully mixed with 1.8 mL ultrapure water to obtain *m*-Q[6]-CQD solution with a concentration of 0.38 mg/mL and transferred into a 5 mL centrifuge tube. Then, 20 μL of different metal cations (Ag^{+}, Cu^{2+}, Fe^{3+}, Hg^{2+}, K^{+}, Mg^{2+}, Ni^{2+}, Al^{3+}, Cd^{2+}, Pb^{2+}, Zn^{2+}) with a concentration of 0.02 M were added to the centrifuge tube, respectively. The fluorescence emission spectra of the carbon quantum dot solution in each centrifuge tube excited at 385 nm were measured, and the change of fluorescence intensity was compared with that of blank solution without metal ions.

Determination of detection limits for Hg^{2+} and Al^{3+} by *m*-Q[6]-CQDs: After optimizing the test conditions, 100 μL *m*-Q[6]-CQD (3.8 mg/mL) was added into 1.9 mL ultrapure water, and 0.19 mg/mL *m*-Q[6]-CQD solution was obtained, and then moved into 5 mL centrifuge tube. After adding different concentrations of mercury ion solution, the fluorescence spectrum was measured. The final concentration range of mercury ion was 0-160 μM. Aluminum ion was detected by the same method as the mercury ion, and its concentration range was 0-60 μM.

Particle size distribution experiment of *m*-Q[6]-CQDs: 3 mL aqueous solution of *m*-Q[6]-CQDs (0.38 mg/mL) was filtered by 0.45 μM filter membrane and injected into a

dynamic light bottle by syringe. The particle size distribution in the solution was tested by dynamic light dispersive spectrometer.

MTT cytotoxicity test: In this study, human prostate PC-3 cancer cells were selected to investigate the toxicity of *m*-Q[6]-CQDs. PC-3 cells were digested with trypsin, then prepared with DMEM culture medium into cell suspension with a certain concentration, and inoculated into 96 well cell culture plates with a capacity of 100 μL per well. The culture plate was placed in the cell incubator for culture at 37 ℃ and 5% CO_2. After 24 h, the culture plate was removed to remove the supernatant from each well, and the cells were washed with PBS buffer once before adding the culture medium. In the experimental group, 90 μL was added with 10 μL *m*-Q[6]-CQDs solution of different concentrations (0, 0.1, 0.2, 0.4, 0.6, 0.8, 1 $mg \cdot mL^{-1}$) in each well, and 100μL in the control group was placed in an incubator (37℃, 5% CO_2) for 24 h. Then, the cell culture plate was taken out, the supernatant of each well was discarded and the cells were washed twice with PBS buffer solution, then 90 μL PBS buffer solution and 10 μL CCK-8 solution were added to each well, and incubated in the cell culture box for 1 h. Finally, the absorbance value (wavelength 405 nm) of each well was measured with a microplate reader, and the toxicity was obtained after data recording and processing.

Cell imaging experiment: Human prostate cancer cell PC-3 was also selected as the model to observe the labeling effect of carbon quantum dots on cells through inverted fluorescence microscope. The specific experimental process was as follows: firstly, PC-3 cells were digested with 0.05% Trypsin, then the cells were counted by the cell counting plate, then the cells were inoculated on the 24-well plate containing DMEM in a sterile state at a density of 1×10^4 cells per well, and cultured at 37 ℃ with 5% CO_2 for 24 h. Then remove the old medium, add 100 μL *m*-Q[6]-CQDs(1mg/mL) into the well, static culture at 37℃, 5% CO_2 for 15 min, then wash with PBS buffer for two or three times, finally use fluorescence inverted microscope for observation. Then, 50 μM Hg^{2+} solution was injected into the well and incubated in a constant temperature incubator for 60 min. The obtained cells were washed twice with PBS buffer and placed under an inverted fluorescence microscope for fluorescence imaging.

S2. Determination of fluorescence quantum yield of quantum dots

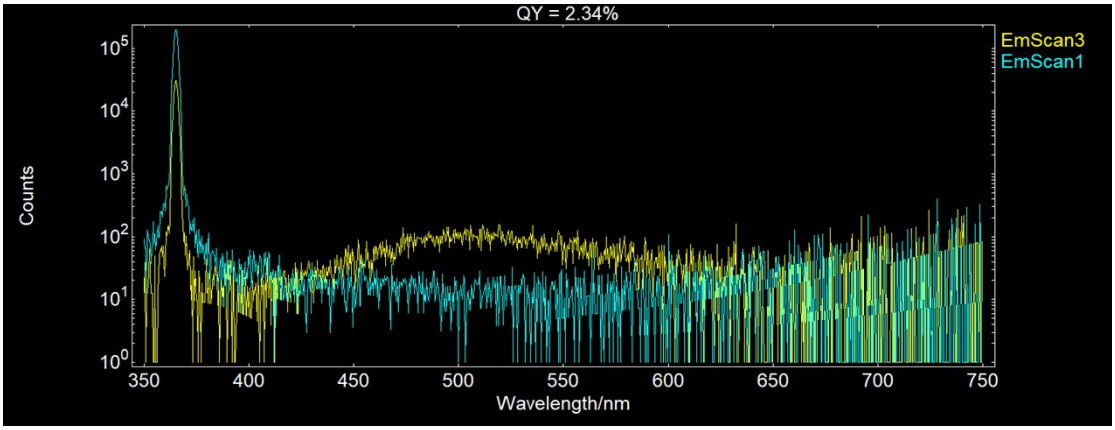

Figure S1. Fluorescence quantum yields of *m*-Q[6]-CQDs. the solution is ultrapure water, [*m*-Q[6]-CQDs] = 0.38 mg/mL.

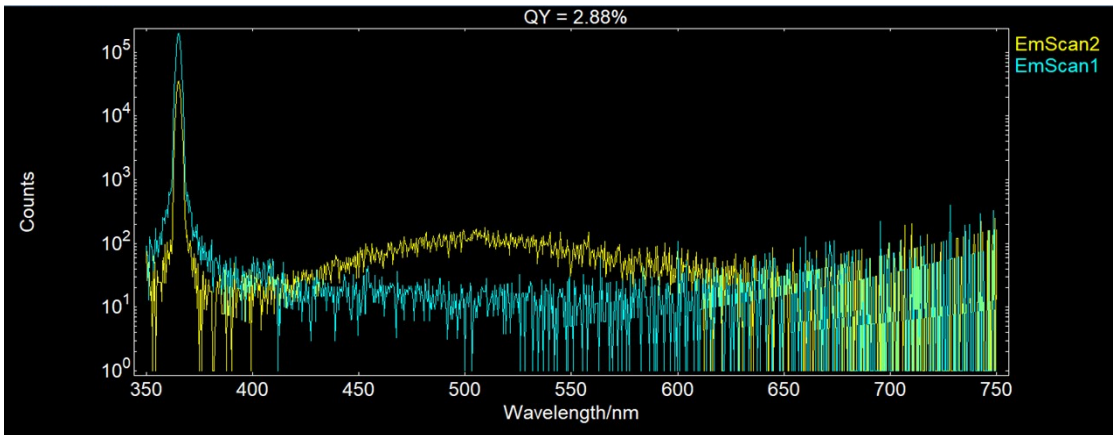

Figure S2. Fluorescence quantum yield of *m*-Q[6]-CQDs after interaction with Al^{3+}. the solution is ultrapure water, [*m*-Q[6]-CQDs] = 0.38 mg/mL, [Al^{3+}] = 0.2 mM.

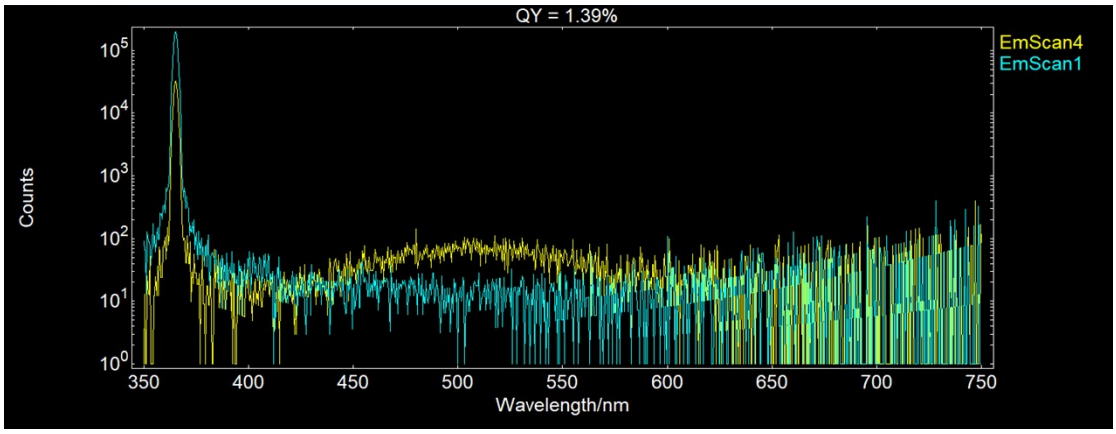

Figure S3. Fluorescence quantum yield of *m*-Q[6]-CQDs after interaction with Hg^{2+}. the solution is ultrapure water, [*m*-Q[6]-CQDs] = 0.38 mg/mL, [Hg^{2+}] = 0.2 mM.

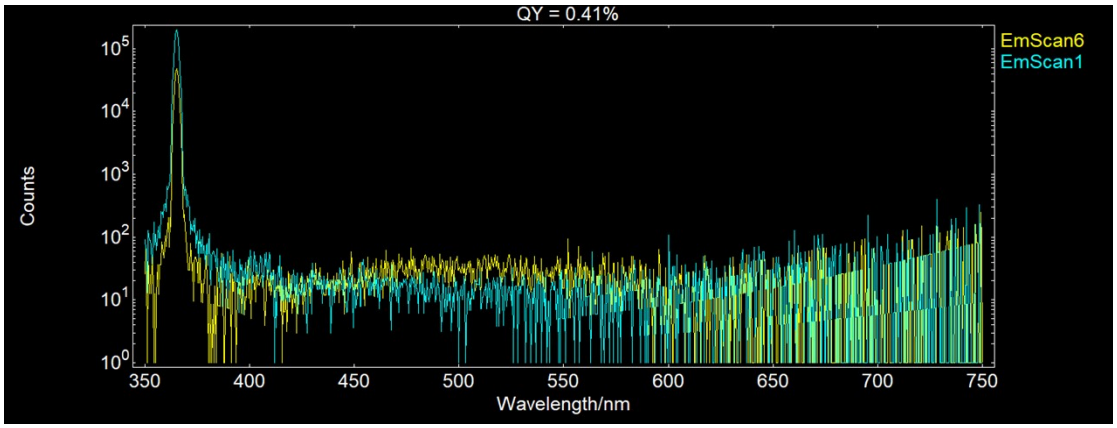

Figure S4. Fluorescence quantum yields of *m*-CQDs. the solution is ultrapure water, [*m*-Q[6]-CQDs] = 0.38 mg/mL.

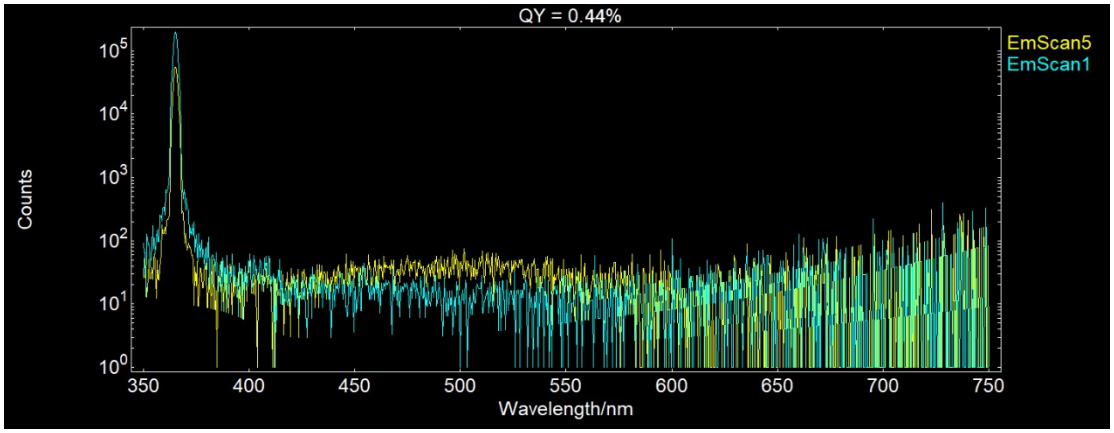

Figure S5. Fluorescence quantum yield of *m*-CQDs after interaction with Al^{3+}. the solution is ultrapure water, [*m*-Q[6]-CQDs] = 0.38 mg/mL, [Al^{3+}] = 0.2 mM.

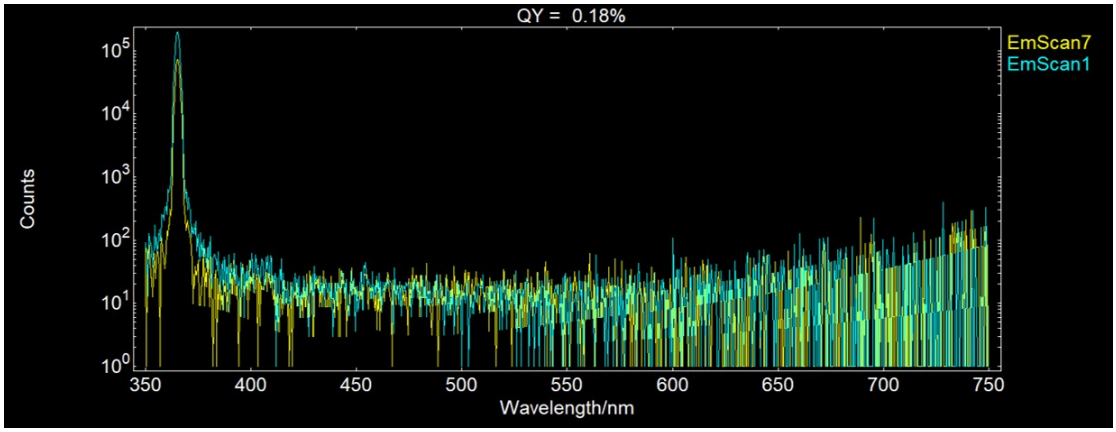

Figure S6. Fluorescence quantum yield of *m*-CQDs after interaction with Hg^{2+}. the solution is ultrapure water, [*m*-Q[6]-CQDs] = 0.38 mg/mL, [Hg^{2+}] = 0.2 mM.

S3. UV and fluorescence spectra of *p*-phenylenediamine formed quantum dots with Q[6](*p*-Q[6]-CQDs) and *o*-phenylenediamine formed quantum dots with Q[6](*o*-Q[6]-CQDs).

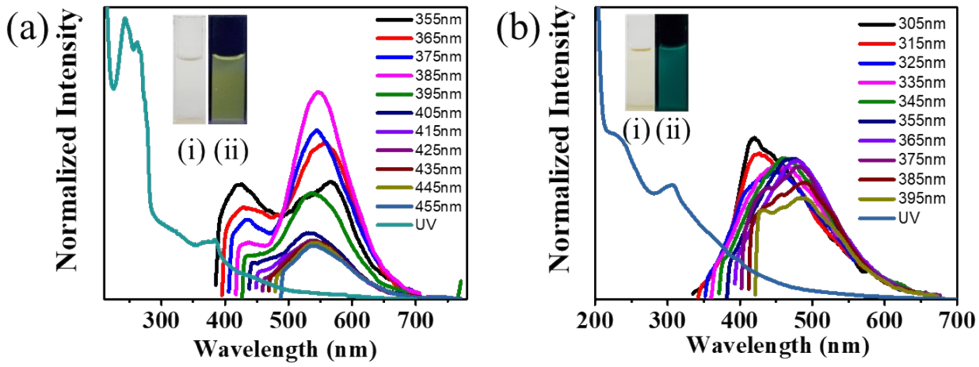

Figure S7. (a) UV absorption spectrum of the *o*-Q[6]-CQDs and Fluorescence emission spectra of *o*-Q[6]-CQDs at excitation wavelengths of 355–445 nm. (i) under natural light irradiation and (ii) ultraviolet lamp irradiation. (b) UV absorption spectrum of the *p*-Q[6]-CQDs and Fluorescence emission spectra of *p*-Q[6]-CQDs at excitation wavelengths of 355–445 nm. (i) under natural light irradiation and (ii) ultraviolet lamp irradiation. The concentration is 0.38 mg/mL, the solution is ultrapure water.

S4. TEM images and particle size distribution of *m*-Q[6]-CQDs

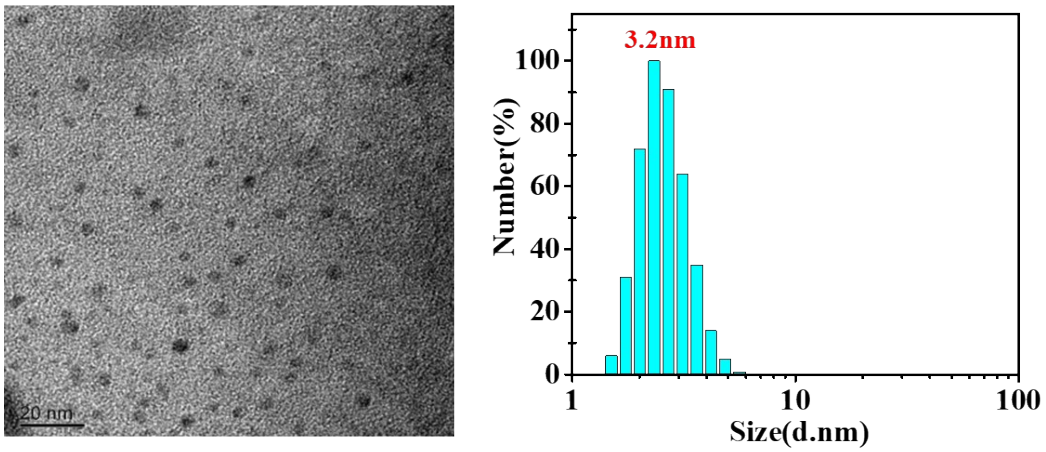

Figure S8. The left image is the image of *m*-Q[6]-CQDs projection under an electron microscope, and the right is the number distribution of particle size.

S5. *m*-CQDs (*m*-phenylenediamine) and Q[6]-CQDs(Q[6]) obtained by hydrothermal reaction of *m*-phenylenediamine and Q[6] alone.

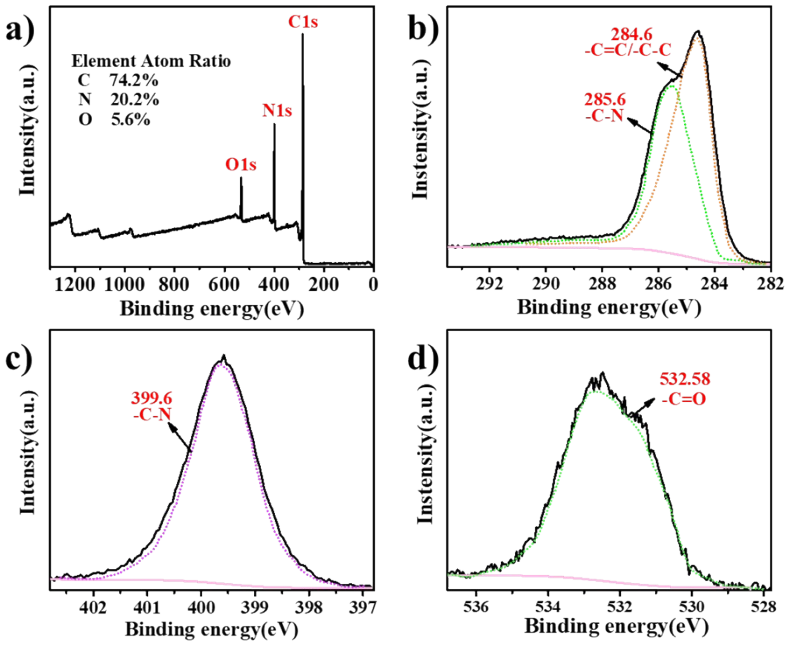

Figure S9. a) XPS spectra of *m*-CQDs; b), c) and d) high resolution spectra of C1s, N1s and O1s, respectively.

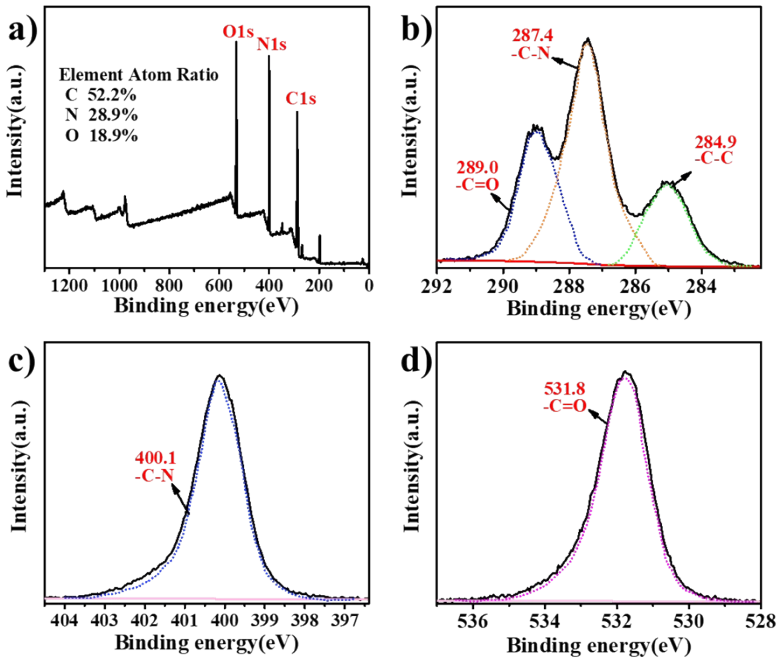

Figure S10. a) XPS spectra of Q[6]-CQDs; b), c) and d) high resolution spectra of C1s, N1s and O1s, respectively.

S6. Interaction of *m*-CQDs with metal ions

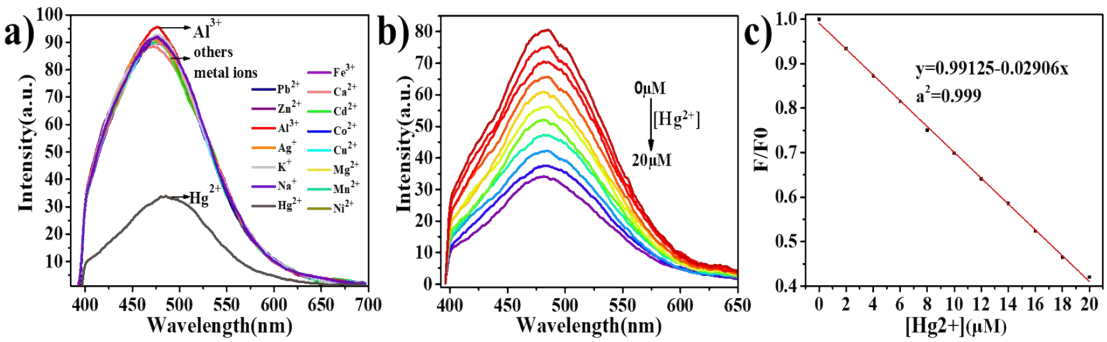

Figure S11. a) Interaction of the *m*-CQDs with different metal ions under neutral conditions, the *m*-CQDs concentration is 0.38 mg/mL, the concentration of all metal ions is 0.2mM, λex / λem = 385 / 485 nm; b) Spectral changes of the *m*-CQDs solution containing different concentrations of mercury ions; c) The linear curve between the fluorescence quenching efficiency of the *m*-CQDs and the concentration of mercury ions. The illustration shows that the fluorescence quenching efficiency is proportional to the concentration of mercury ions over the range 0-20 μM. The excitation wavelength is 385 nm, the concentration of *m*- CQDs is 0.19 mg/mL, and the solvent used is ultrapure water.

Reference

[1] A. Day, A. P. Arnold, R. J. Blanch and B. Snushall, Controlling Factors in the Synthesis of Cucurbituril and Its Homologues, *J. Org. Chem*. 2001, 66, 8094-8100.