

Supporting Information

Biocompatible Fluorescent Carbon Quantum Dots from Beetroot Extract for in vivo Live Imaging in *C. elegans* and BALB/c Mice

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Movie clip: download in Google Chrome from

<https://mega.nz/#!kCwiybBZ!f9NQqXrmMInt8uhzj0aJDcvcyFuuP7XL9jySO0rC-vg>

Table of Content

Instrumentations and synthesis of CQDs	S2
High resolution XPS spectra of B-CQDs and G-CQDs	S4
Normalized fluorescence spectra of both CQDs in different excitation wavelength	S5
Photostability study of both CQDs under UV light exposure	S6
pH dependent study of both CQDs	S7
Materials and Methods for live animal imaging	S8—S10
Control experiment and Images of <i>C. elegans</i>	S11
References	S12

Instrumentation:

UV-visible electronic absorption spectra measurements were carried out in Agilent Cary 100-UV-visible Spectrophotometer. Fluorescence emission spectra of the samples were measured using Varian Cary Eclipse spectrofluorometer. Fluorescence lifetime experiments were performed using a Horiba Jobin Yvon instrument in a Time-Correlated Single Photon Counting (TCSPC). A nano-LED of 370 nm was used as source of excitation for CQDs. LUDOX AS40 colloidal silica, scattering medium was used for instrument response function. The pulse repetition rate of TCSPC was fixed at 1 MHz. Average size of CQDs was carried out by using FEI Titan G2 300 kV high resolution transmission electron microscope (HR-TEM). Surface functionality characterization was carried out by X-ray photoelectron spectroscopy (XPS) in using PHI 5000 Versaprobe II, FEI. Zeta potential measurement of water dispersed CQDs was carried out by using Malvern Zetasizer Nano ZS-90 dynamic light scattering (DLS) instrument.

Synthesis of carbon quantum dots in aqueous medium

(a) Hydrothermal treatment

The preparation of CQDs from beetroot aqueous extract was carried out by hydrothermal treatment as follows. The aqueous beetroot extract (20 mL) was filtered (11 μm pore sizes) and transferred into a 50 mL Teflon-Lined autoclave heated at 150 $^{\circ}\text{C}$ for 16 h. After the reaction is over, the autoclave was cooled down naturally and brownish aqueous solution was obtained. Subsequently, the resultant CQDs mixture was purified by centrifugation at 1500 rpm for 10 min. The supernatant solution was again centrifuged for 10 min at 2500 rpm to remove heavy organic and inorganic

residues. Finally, the large particles were further removed from aqueous solution by centrifugation at 4000 rpm for 10 min. A clear brownish solution was obtained with a concentration of 40 mg/mL.

(b) Ortho-Phosphoric acid treatment

The synthesis of CQDs from acidic treatment from aqueous beetroot extract was described below. Concentrated *ortho*-phosphoric acid (10 mL) was added into 20 mL of beetroot extract and placed in a 50 mL round bottom flask and kept at 100 °C for 2 hr. The reaction mixture was naturally cool down to room temperature, neutralized with 1 M sodium hydroxide solution. The resultant solution was purified by repeated centrifugation (1500, 2500, and 4000 rpm) for 10 min to remove unreacted natural plant ingredients and heavy residual impurities. A clear yellowish solution was obtained with a concentration of 328 mg/mL.

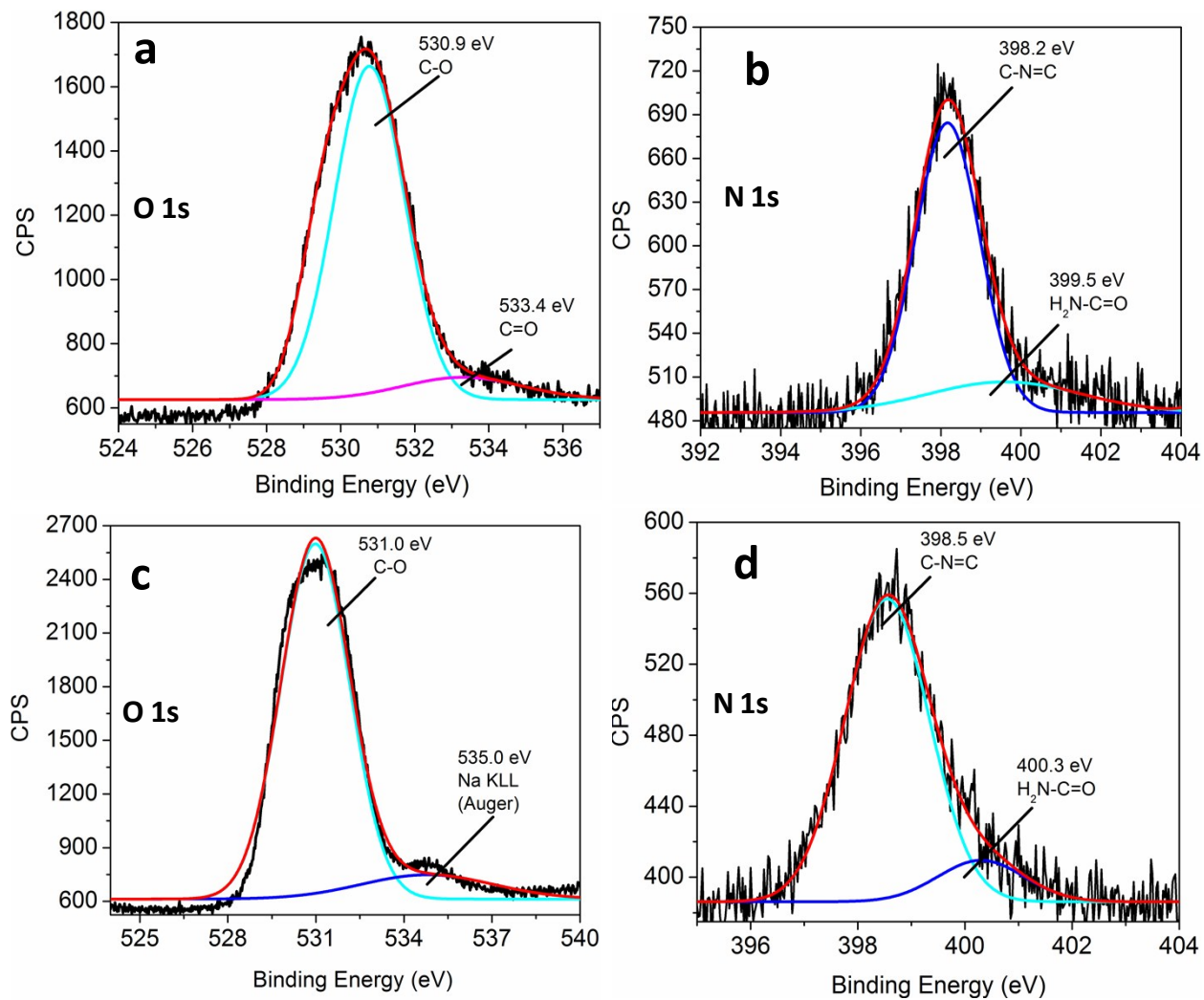


Figure S1. High resolution XPS spectra of the B-CQDs in (a) O1s region, (b) N1s region and G-CQDs in (c) O1s region (d) N1s region

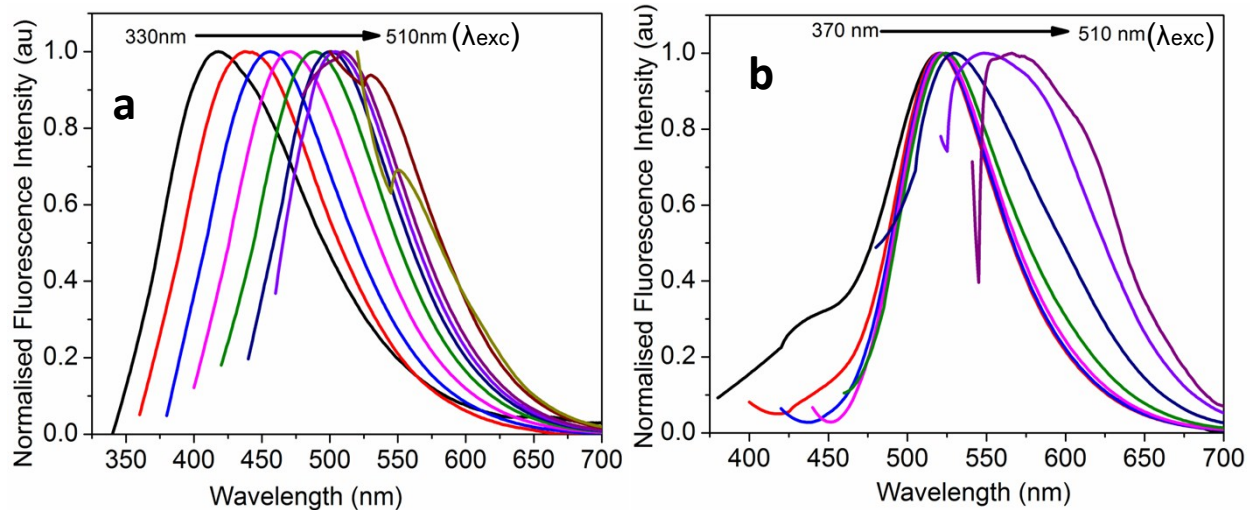


Figure S2. Normalised fluorescence spectra of (a) B-CQDs and (b) G-CQDs in different excitation wavelength in aqueous medium

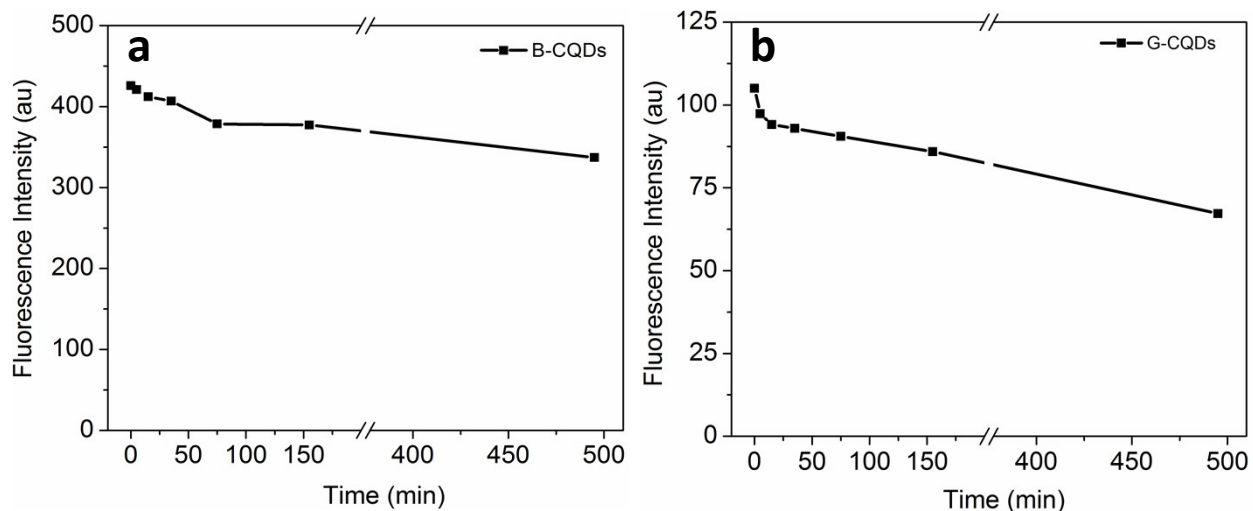


Figure S3. Photostability test of CQDs under continuous irradiation of the 365 nm light with different time intervals. (a) Plot of the fluorescence intensity of B-CQDs at 450 nm emission and (b) Plot of the fluorescence intensity of G-CQDs at 522 nm emission (performed in aqueous medium with excitation wavelength is 370 nm)

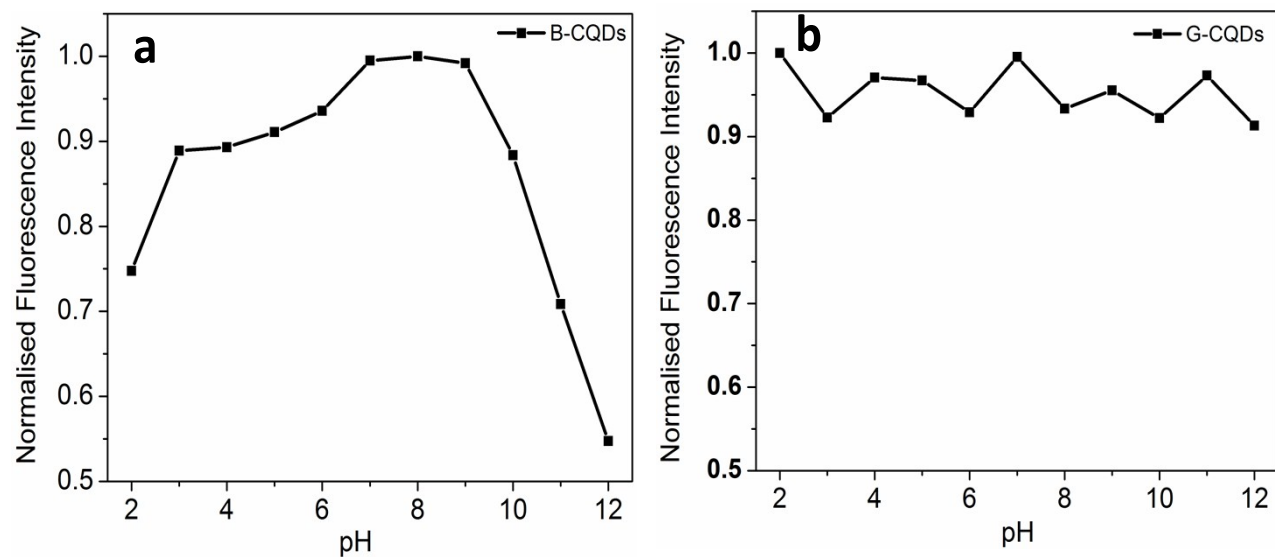


Figure S4. pH dependence of fluorescence response of (a) B-CQDs and (b) G-CQDs in various pH ranges

Materials and Methods

Animal Studies: All of the animal studies were performed in compliance with guidelines set by the CDRI Institutional Animal Ethics Committee (CDRI-IAEC) with approval number IAEC/2012/71N/Renew-05(214/16) dated 04.11.2016.

Culture and maintenance of *C. elegans*:

The culturing and propagation of *C. elegans* was carried out under standard conditions¹. Wild type bristol N2 worm strain was fed on a diet of *Escherichia coli* OP50 seeded Nutrient Growth Medium (NGM), prepared by adding 3g of sodium chloride, 2.5g of peptone and 17g of agar to 975mL double distilled water which was then autoclaved for about 40-50 minutes at 15 psi. The media cooled gradually to 55-60°C and then 1mL of cholesterol (5mg/ml of absolute alcohol); 1mL of 1.0 M calcium chloride; 1.0 mL of 1.0 M magnesium sulphate; and 25mL of 1M potassium dihydrogen phosphate (pH adjusted to 6.0) was added to it in sterile condition. Both CQDs were thoroughly mixed and then poured into petri dishes that were used for culturing of the nematodes.

Age synchronization and feeding of worms with the compounds:

Adult gravid worms that were washed of OP50 seeded NGM plates and subjected to axenization towards obtaining of embryos. A solution of 1.0 M sodium hydroxide and household bleach (freshly prepared) was used to axenize the cuticle of the worms. This is done for obtaining uniformly aged population of worms in order to avoid differences arising due to subtle changes in age of worms^{2,3}. The embryos were placed onto the compound seed NGM plates. A suspension of both synthesised CQDs at a concentration of 1.5mg/mL was used mixed with bacteria OP50 towards feeding the nematodes.

Confocal Microscopy:

The nematodes of various treatment groups that were continuously fed on carbon quantum dots (B-CQDs and G-CQDs), post embryo isolation were assayed for the absorption and localization of CQDs within various parts of the body. Standard microscopy analysis procedures were followed; briefly, worms at pre-adult stage (48 hours post embryonic age) were washed thrice with M9 buffer to eliminate adhering bacteria and then immobilized in a final concentration of 100 mM sodium azide and 50% (v/v) glycerol. After mounting onto 2 % agar padded slides and sealing the coverslips with nail polish, confocal microscopy was performed using confocal microscope LSM 510 META (Carl Zeiss, Jena, Germany). Different worms were analysed and images were captured for multiple segments with an excitation of 405 nm for both B-CQDs and G-CQDs while the emissions were collected within band pass 460-510 nm and 500-550 nm respectively. Bright-field images were also captured for identification of specific fields of examination of the respective worms.

Animal model and in vivo imaging:

All of the animal studies were performed in compliance with guidelines set by the CDRI Institutional Animal Ethics Committee. The mice were intravenously injected with 50 μ L of the G-CQDs solution at a concentration of 20 mg per 50 μ L. The mice were anesthetized using isoflurane and placed on an animal plate (2% flow in the anesthesia chamber and 2.5% flow in the *in vivo* imaging system). The time-dependent biodistribution in the mice was imaged in *in vivo* fluorescence-imaging system using IVIS[®]spectrum (Perkin Elmer, USA). Light with a central wavelength of 430 nm was selected as the excitation source. In vivo spectral imaging was conducted at 520 nm,

with an exposure time of 150 ms for each image frame. Autofluorescence was removed using spectral unmixing software. Scans were carried out at 5 min, 10 min, 15 min and 24h post-injection. Moreover, the mice were also sacrificed at 24 h post-intravenous injection of the C-Dots. The organs, including the heart, lungs, liver, spleen, ovaries, kidneys, stomach and the intestines were excised after 24h and imaged using the IVIS system for ex vivo fluorescence imaging. Three mice were used at each time point after injection.

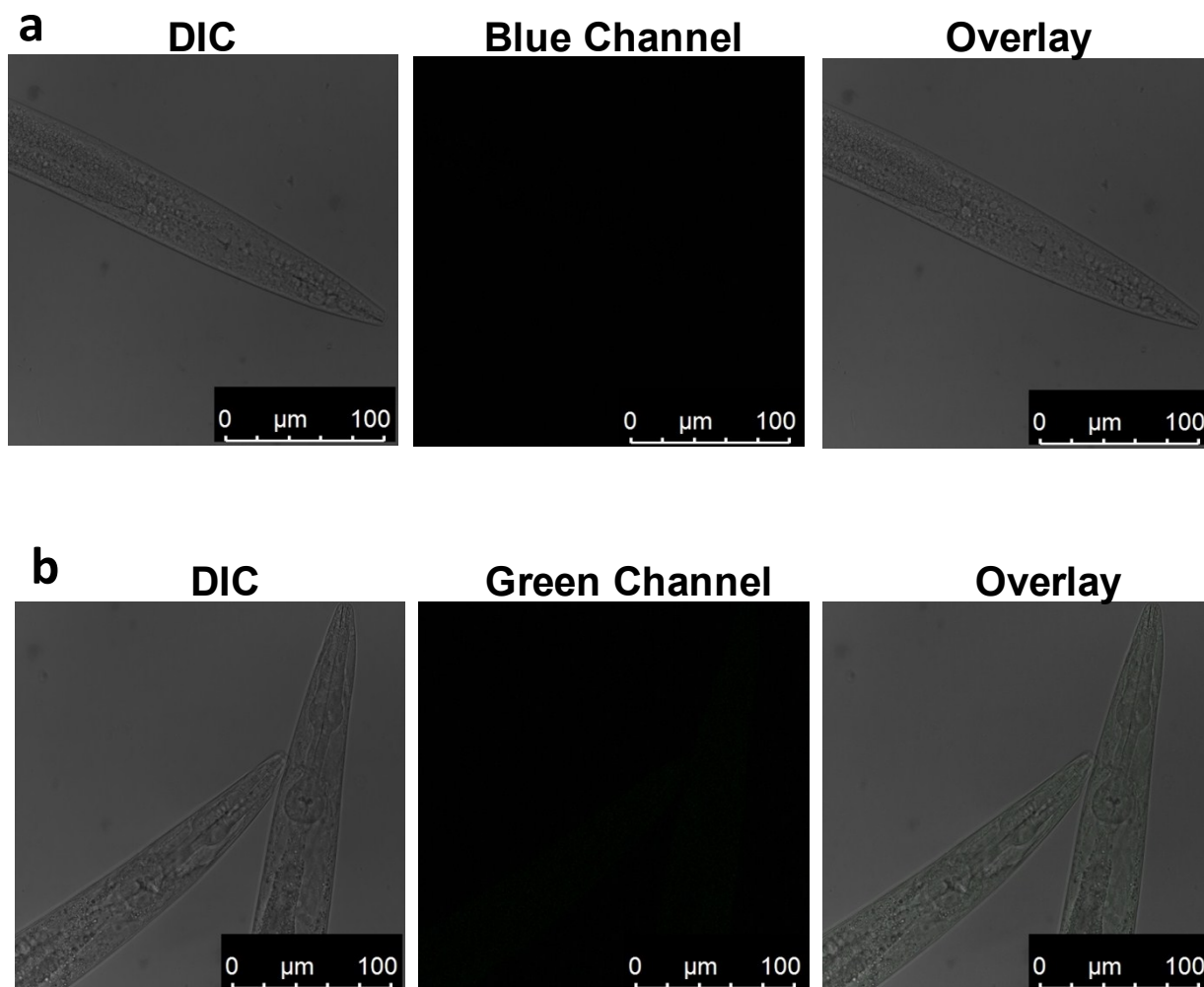


Figure S5. Confocal fluorescence microscope images of *C. elegans* in the absence (control) of (a) B-CQDs and (b) G-CQDs: Blue channel: $\lambda_{\text{ex}} = 405 \text{ nm}$, $\lambda_{\text{em}} = 460\text{-}510 \text{ nm}$. Green channel: $\lambda_{\text{ex}} = 405 \text{ nm}$, $\lambda_{\text{em}} = 500\text{-}550 \text{ nm}$ band pass.

References

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