Absorption and Emission of Light in Red Emissive Carbon NanoDots

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Experimental

Materials

All glasswares were washed with aqua regia, followed by rinsing several times with double distilled water. o-Phenylenediamine, Dopamine and Catechol were purchased from Merck Chemicals. HCl was purchased from a local vendor. All chemicals were used without further purification. Double-distilled (18.3 mΩ) deionized water (ELGA PURELAB Ultra) was used throughout the entire process.

Characterization

The UV-Vis absorption spectra were recorded using Shimadzu UV-Vis 2450 spectrophotometer. The spectra were collected using a quartz cuvette having a 10 mm path length and 1 ml volume. Steady state fluorescence spectra were recorded on Horiba spectrophotometer from excitation wavelength range of 280 to 600 nm. Thermal properties were measured by Perkin Elmer Pyris Thermogravimetric (TGA) analyser under nitrogen atmosphere from room temperature to 1000 °C with the heating rate of 10 °C min^{-1}. The particle morphology was observed by transmission electron microscopy (TEM) using FEI Tecnai TEM equipped with a LaB6 source operating at 200 kV. Fourier transform infrared (FTIR) spectra 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. The Raman spectrum was measured by the confocal microscope Raman spectrometer (Horiba Scientific, XploRA ONE). The morphology and energy dispersive x-ray analysis (EDS) were observed using a field emission scanning electron microscopy FESEM (NOVA NanoSEM 450) at an accelerating voltage of 10 kV. Powder X-ray diffraction (PXRD) pattern was recorded on a Rigaku Smart Lab diffractometer, using CuKα radiation from 5° to 80° with a scanning rate of 2°/min. ^{1}H NMR spectra were recorded in DMSO-d6 on a Jeol-ECX-500 MHz spectrometer using tetramethylsilane (TMS) as an internal standard and HRMS spectra were recorded on a Bruker impact-HD spectrometer.

Fluorescence Correlation Spectroscopy (FCS)

Approx. 50μl sample solutions (~nanomolar concentrations) were drop-casted on a cleaned glass coverslip and placed on an inverted confocal microscope (A1R+, Nikon, Japan) using a 1.4 NA oil-immersion 60x objective for measuring the FCS data. An appropriate laser was used to excite the sample at room temperature (25 °C) using appropriate dichroic and filters in the optical path. The emission beam was directed through a side-port of the microscope delivering it to a Hybrid Photomultiplier Detector Assembly single photon counting module (PicoQuant GmBh. Berlin, Germany). The fluorescence fluctuations were analyzed within a point region of interest (ROI) of the sample. The following general autocorrelation function (for n number of diffusion species) was used for fitting and calculating diffusion coefficients.

$$G(t) = \left[1 + T\left[e^{-\left(\frac{t}{\tau_{Trip}}\right)} - 1\right]\right]\sum_{i=0}^{n-1} \frac{\rho(i)}{\left[1 + \frac{t}{\tau_{Diff}[i]}\right]\left[1 + \frac{t}{\tau_{Diff}[i]\kappa^2}\right]^{0.5}}$$

Where τ_{Trip} is the time of triplet blinking and diffusion through the confocal volume, T is triplet contribution and ρ[i] is the contribution of ith species. Our study used the single species model with triplet contribution. The instrument parameter κ was calibrated with a standard Atto 647 with a known diffusion coefficient. All data were analyzed using the SymPhoTime 64 software (PicoQuant GmBh).

Confocal Imaging of HeLa cells

Coverslip preparation: The glass slides and coverslips were cleaned by incubating in freshly prepared Piranha solution for 30 min and finally washing by MiliQ water in bath sonication then dried under nitrogen.

Cell Culture, fixation and staining: HeLa cells were grown in Dulbecco's Modified Eagle Medium (DMEM) with 10% fetal bovine serum. The cells were grown in 6-well plate on the coverslips with density 10^4 cells per 100 µl. Each well was filled with 2 ml of cell suspension in growth medium and the cells were allowed to grow overnight for the proper adherence and growth. The growth and the attachment of the cells to the coverslips were examined by an optical microscope. Once the cells reached at proper attachment and growth stage, they were stained synthesized material to achieve enough labelling density for confocal microscopy. Finally, the cells were fixed by incubating with 4% paraformaldehyde solution in 1x PBS buffer for 15 min. The fixed cells were then washed 4-6 times by PBS buffer to remove extra agents. The coverslips were fixed on a glass slide before imaging.

Confocal microscopy: Nikon Eclipse Ti inverted microscope was used for the confocal microscopy and images were acquired using Nikon Nis-Element software. The cell samples were excited by the three lasers 401, 488 and 639 nm. Finally, the images were collected by choosing a proper filter set.

Lifetime Measurement

The fluorescence lifetime measured using Horiba Scientific Delta Flex TCSPC system with Pulsed LED Sources. Ludox has been used to calculate IRF for de-convolution of the spectral value.

Fluorescence Lifetime Imaging Microscopy (FLIM)

Nikon Eclipse Ti inverted Confocal microscope along with Nikon Nis-Element software was used for image acquisition before the FLIM measurement. The FLIM was performed using a time-correlated single photon counting (TCSPC) system PicoHarp 300 and a hybrid PMT System (both from Picoquant Germany) attached to the same confocal microscope. A pulsed diode laser, LDH-P-C-405B, PicoQuant, 405 nm (Pulse FWHM <50ps and Max Repetition rate 40Mhz) was used for the sample excitation and fluorescence signals were collected by choosing a proper dichroric-filter set. The acquired data were fitted using n-exponential

reconvolution function and analysis has been done using Symphotime software provided with the setup.

Computational Details

Density functional theory (DFT) calculations were employed to compute ground state geometries and vibrational frequencies, whereas, for the excited states, time-dependent DFT (TD-DFT) calculations were used. The performance of three different density functionals, hybrid B3LYP (Becke's three-parameter hybrid with Lee-Yang-Parr correlation),[1,2] long-range-corrected version of B3LYP using the Coulomb-attenuating method, CAM-B3LYP,[3] and hybrid meta-GGA Minnesota functional M06[4] was compared. All DFT and TDDFT calculations were performed in conjunction with double-ξ 6-31G(d,p) basis set. The superfine integration grid was employed throughout all calculations. Grimme's D3 dispersion with Becke-Johnson damping (D3BJ)[5] was used during all calculations to account for the electron correlation effects due to dispersion interactions. In order to mimic the solvation effects on the ground and excited states, we employed the polarizable continuum model (PCM) with ethanol solvent ($\varepsilon = 24.852$). All calculations were performed using Gaussian 09 (Revision D.01) suite of quantum chemistry program.[6] The structural depiction and molecular orbitals were generated by using the Chimera visualization program.[7]
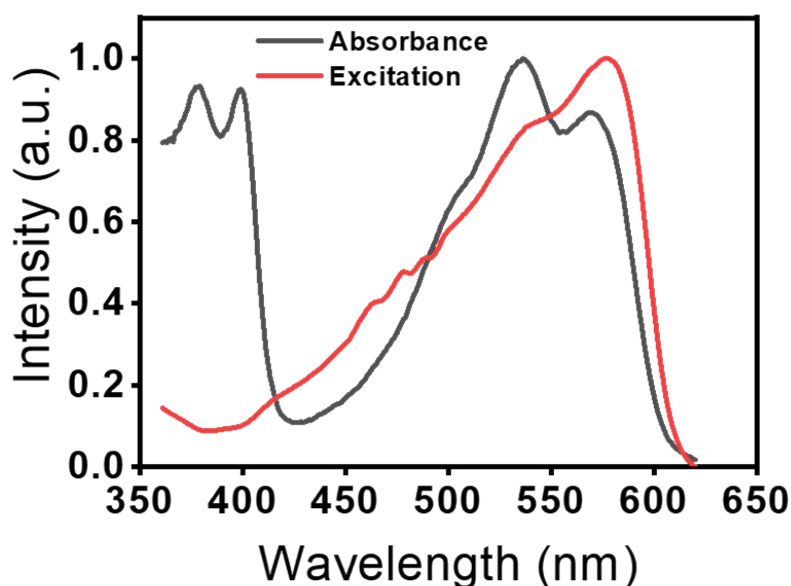

Figure S1: Comparison of absorption and excitation spectra of red CNDs. The data show the similarity in excitation and absorption spectra.
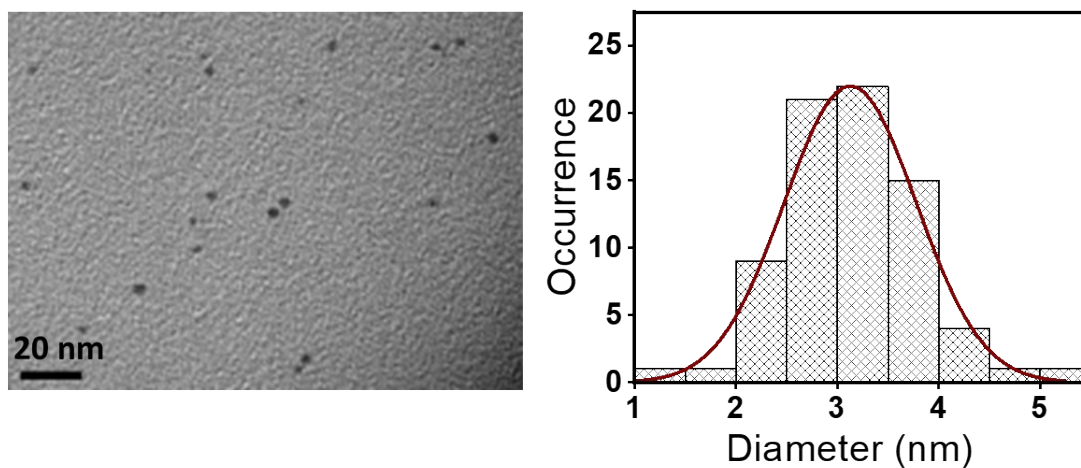
Figure S2: TEM image and the size distribution of the blue component.
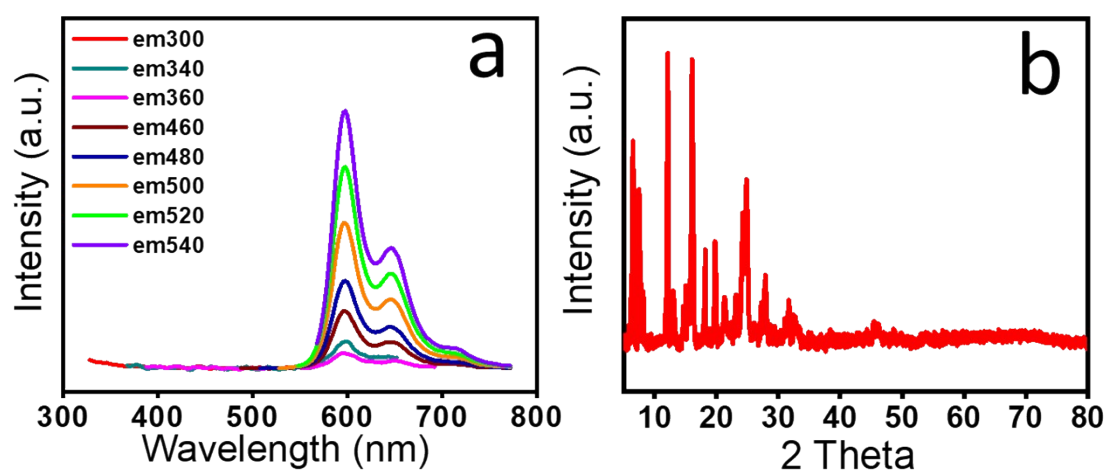

Figure S3: (a) Emission spectra of the red component after performing the dialysis with a 1 kDa membrane. It shows extreme similarity with the original red component. This suggests the small size of the red component. (b) Powder XRD data of the red component.
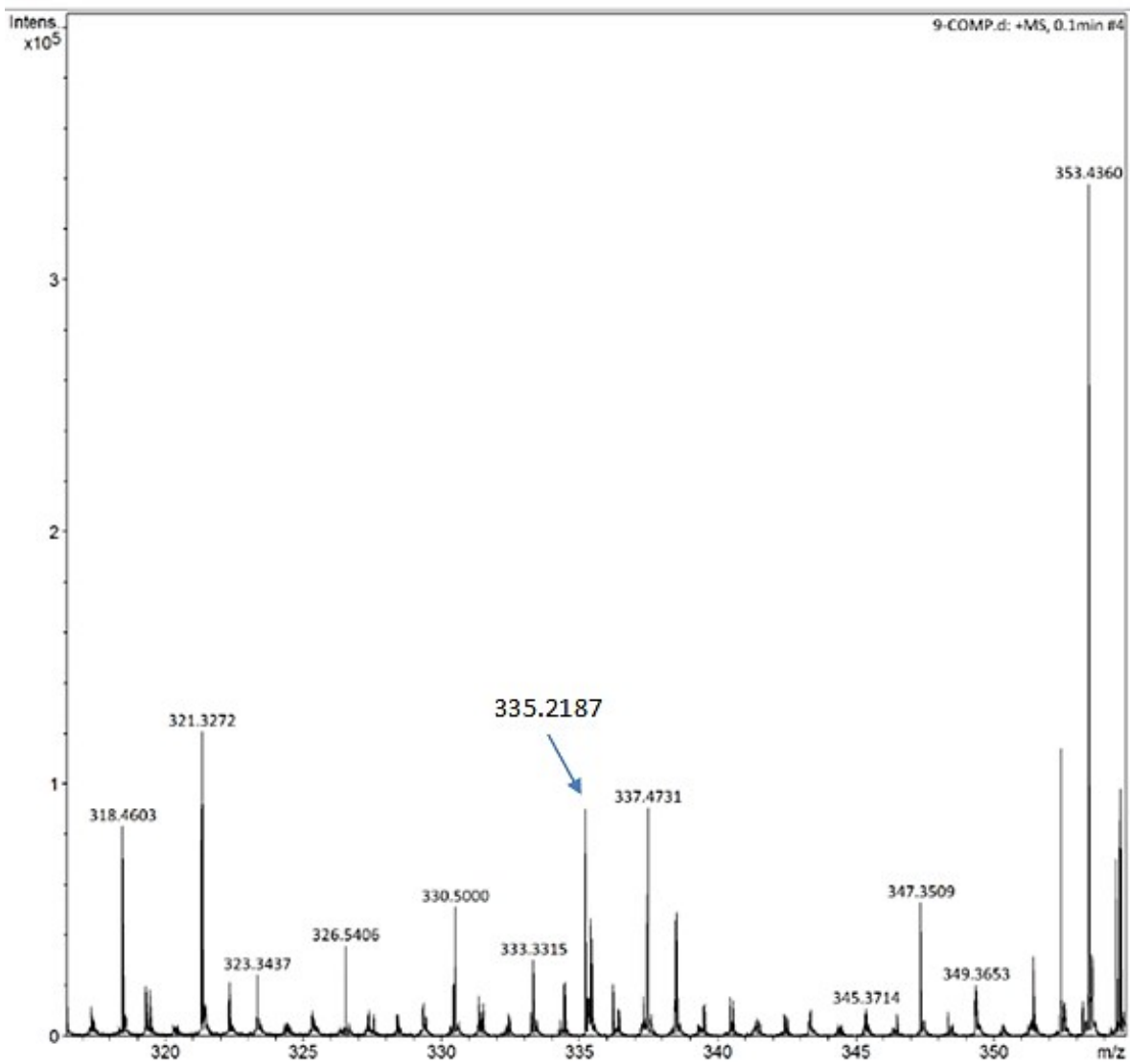
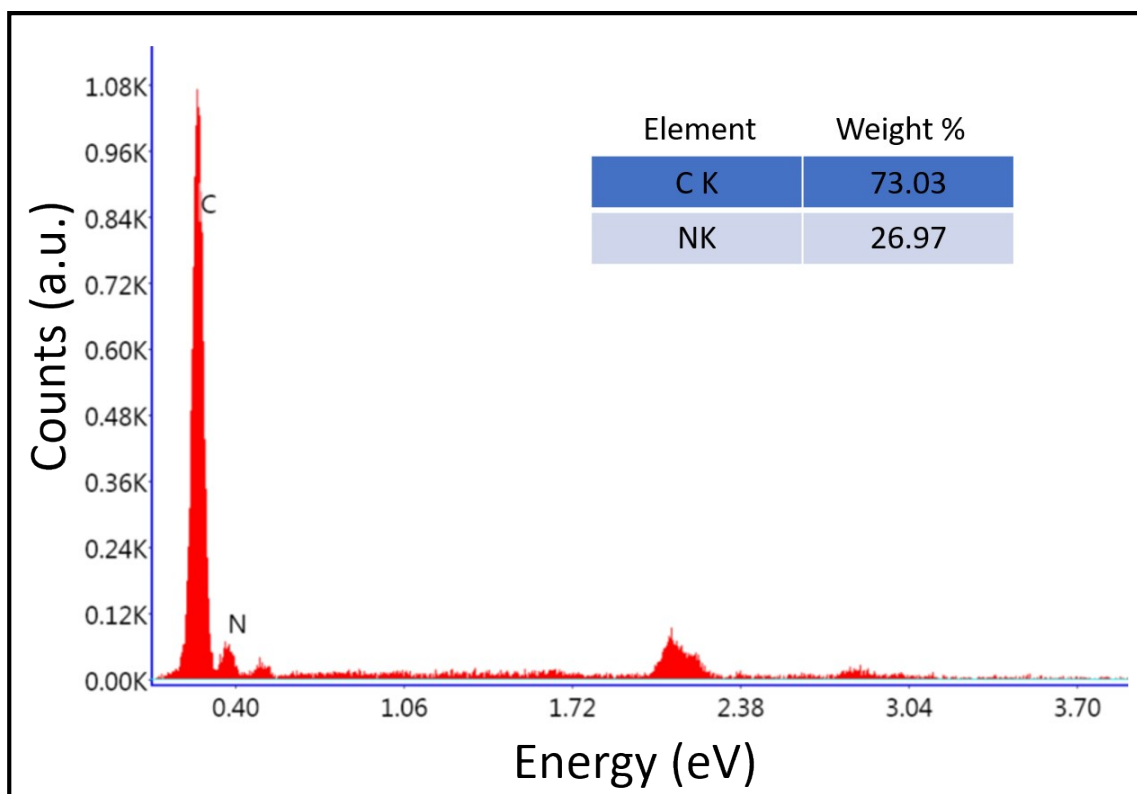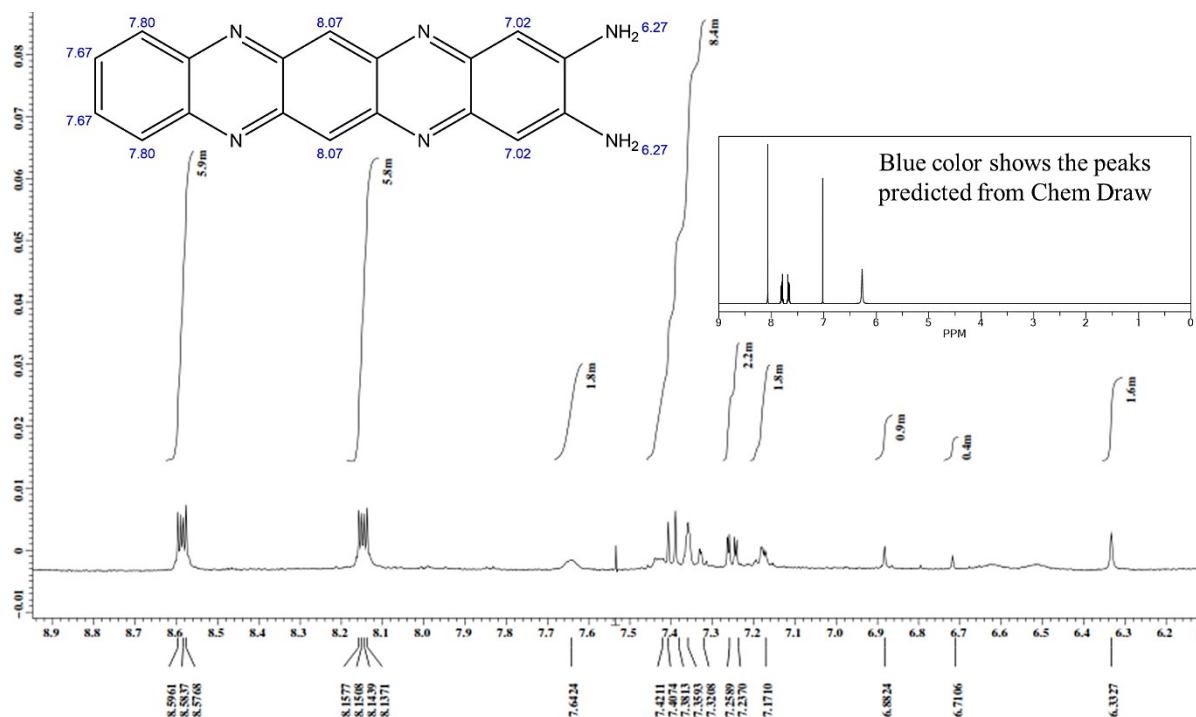**Figure S4**: Mass spectrum of the red emissive component shows the tentative structure as QXPDA.

Element	Weight %
C K	73.03
NK	26.97

Figure S5: EDAX analysis of the QXPDA.

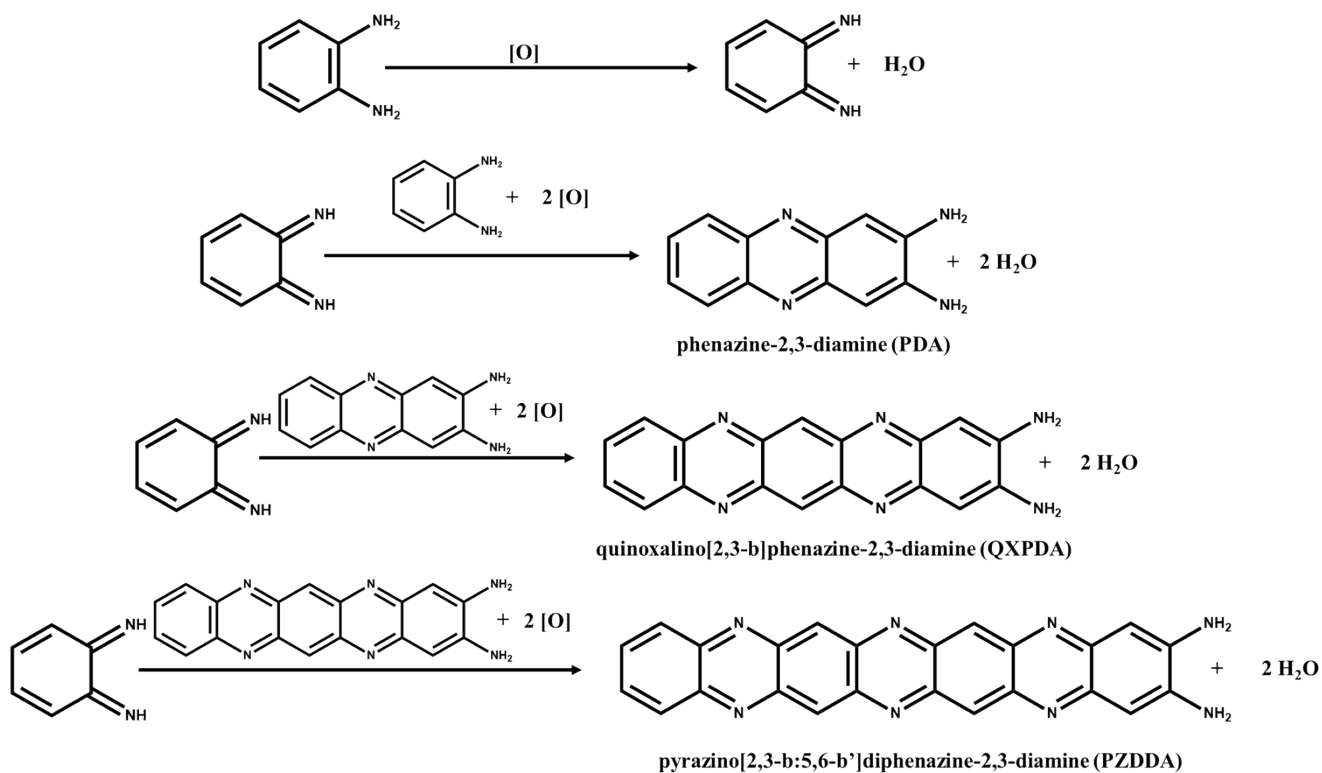**Figure S6**: NMR spectrum of the red emissive component shows the tentative structure as QXPDA.

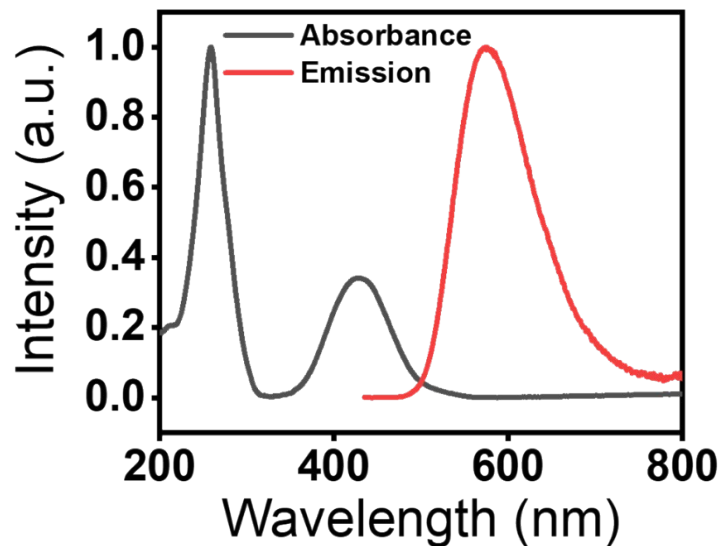**Figure S7**: Synthesis scheme and the possible formation of the products

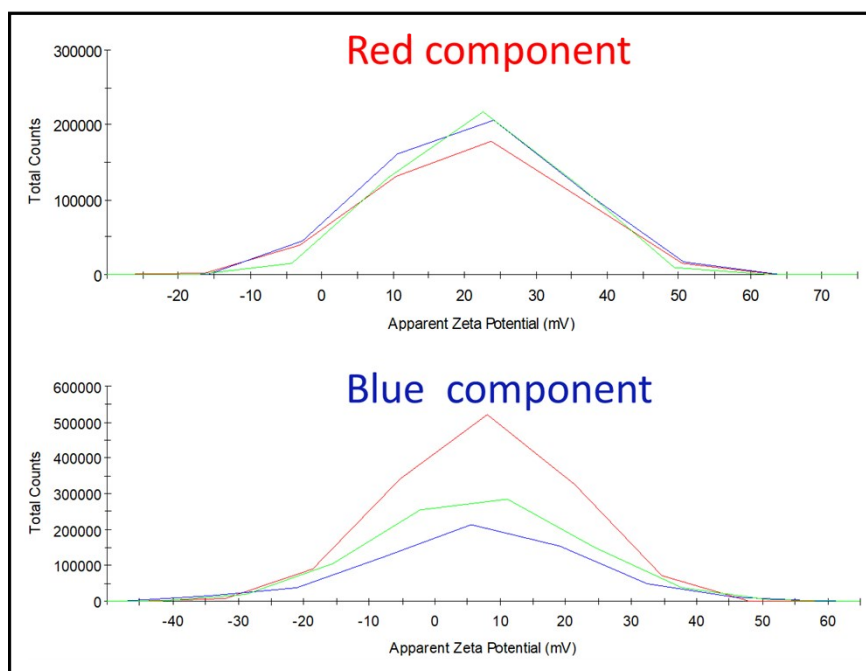**Figure S8**: Absorption and emission spectra of PDA.

Figure S9: Zeta potential intensity distributions of red and blue component.
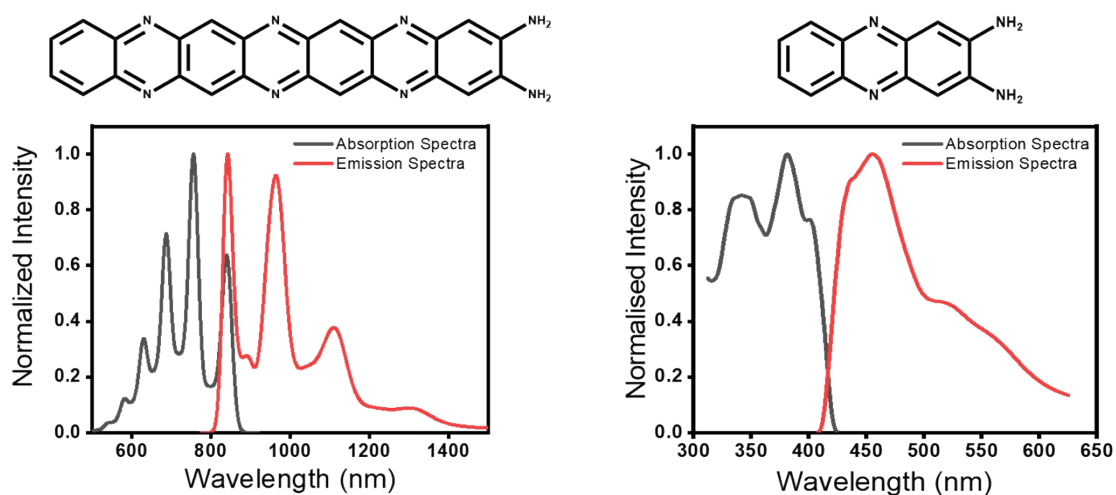
Figure S10: Theoretically simulated absorption and emission spectra of PZDDA and PDA at the TDDFT-CAM-B3LYP level.
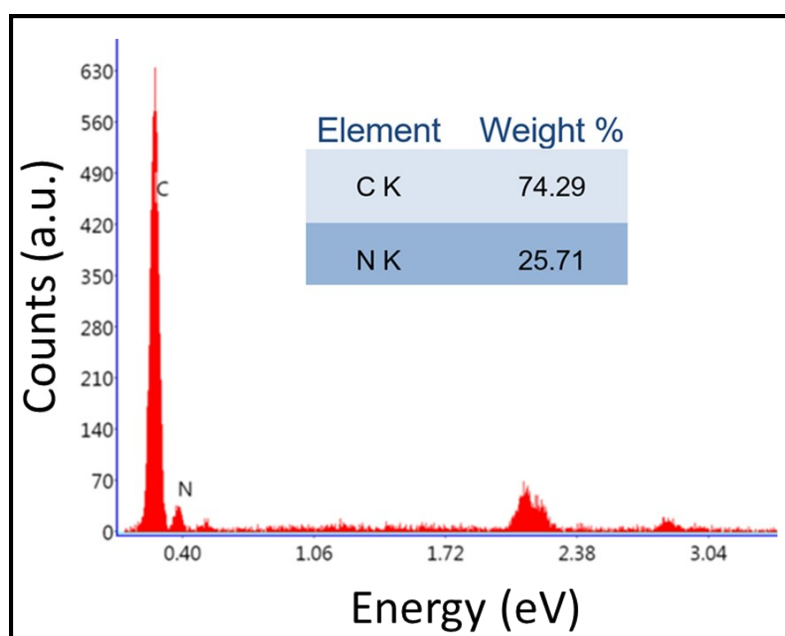

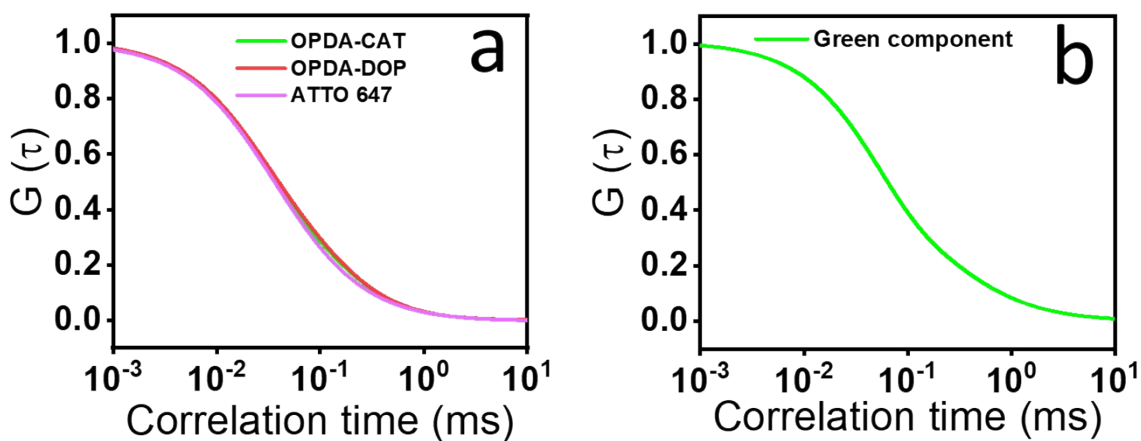**Figure S11:** EDAX analysis of the red CNDs.

Figure S12: (a) Fluorescence correlation spectroscopic data of the column purified red component obtained from OPDA-DOP and OPDA-CAT. (b) Fluorescence correlation spectroscopic data of the green component.
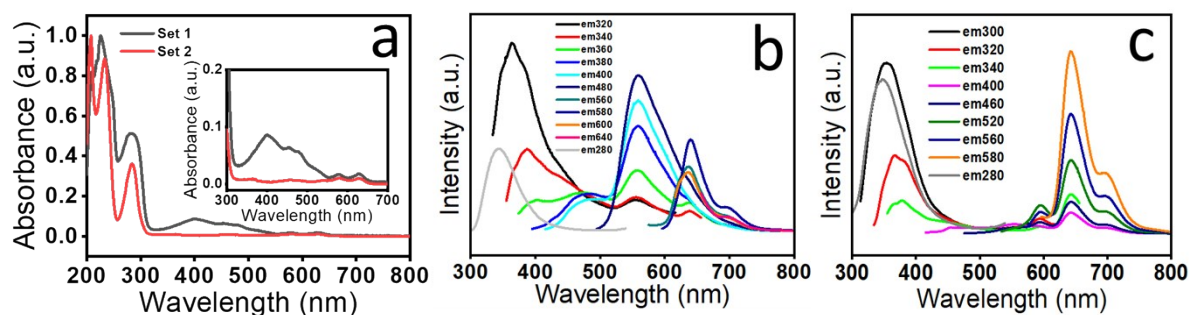

Figure S13: Temperature dependent synthesis of red CNDs (a) absorption spectra and (b-c) emission spectra. It shows the different extent of core structured green and blue components along with red component.
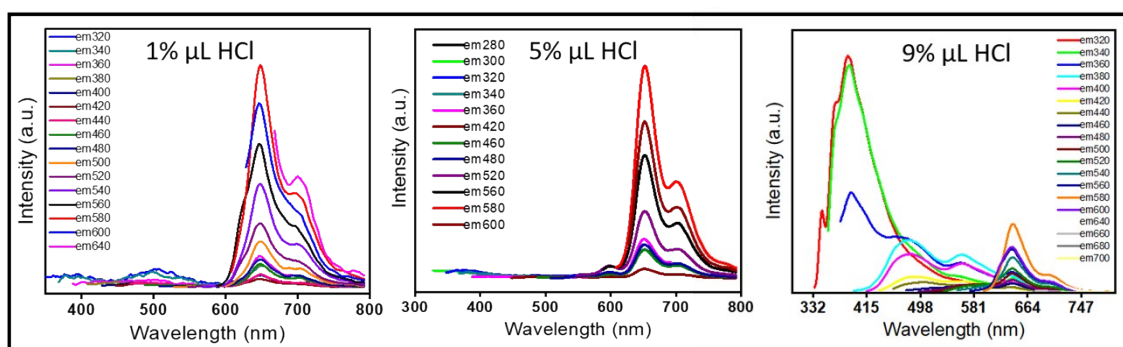
Figure S14: Effect of HCl percentage during the synthesis.

Table S1: CNDs synthesized by employing o-Phenylenediamine as precursors in various conditions.

S.N.	Precursors	Solvent	Emission	Method	References
1.	o-Phenylenediamine	Ethanol	535 nm	Solvothermal 180 °C, 12 h	[8]
2.	o-Phenylenediamine	Water	573 nm	Microwave	[9]
3.	o-Phenylenediamine	Water	567 nm	Solvothermal 300 °C, 2 h	[10]
4.	o-Phenylenediamine & HCl	Water	650 nm	Solvothermal 180 °C, 10 h	This Work
5.	o-Phenylenediamine & Dopamine	Water	710 nm	Solvothermal 200 °C, 8 h	[11]
6.	o-Phenylenediamine & Phosphoric acid	Water	622 nm	Solvothermal 160 °C, 5 h	[12]
	o-Phenylenediamine	Water	568 nm		
7.	Citric acid & o-Phenylenediamine	Water	562 nm	Ultra-sonication	[13]
8.	L-Glutamic acid & o-Phenylenediamin	Ethanol	594 nm	Solvothermal	[14]

	e			210 °C, 10 h	

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