Supplementary information for

Excitation Wavelength Dependent Quantum Yield in Water Soluble CdTe Quantum Dots

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1.1. Materials

Cadmium chloride hemipentahydrate, $CdCl_2.2.5H_2O$, Heavy water (D_2O) and Mercaptosuccinic acid (MSA), were purchased from Sigma. Sodium tellurite was purchased from Alfa-Aesar. Sodium borohydride was purchased from Merck. Hydrazine hydrate 80% was purchased from Loba Chemie private limited. Sulphuric acid and 30% hydrogen peroxide were purchased from fisher scientific. Double-distilled (18.3 MΩ) deionized water was used throughout the entire process.

1.2. Synthesis of CdTe quantum dot solution

CdTe Quantum dots (CQDs) with emission maxima at 604 nm were synthesized following the slightly modified protocol of J. Tan et al., 0.3425 g (1.5 mmol) $CdCl_2.2.5H2O$ was dissolved in deionized water with 0.2477 g (1.65 mmol) MSA, which was then deaerated for 25–30 minutes. The pH of this solution was adjusted to 9–10 using 5 M NaOH. The obtained solution was called solution A. 0.0554 g (0.25 mmol) Sodium tellurite was dissolved separately in 10 mL deionized water and called as solution B. Then, solutions A and B were mixed with 0.0662 g (1.75 mmol) Sodium borohydride. This solution was kept on stirring, and a yellow color solution was obtained after 3–4 minutes, which turned deep orange after fifteen minutes. This solution was then divided into five fractions of 10 mL each, and 400 µL of Hydrazine hydrate 80% was added to each of these fractions for CQDs with ~600 nm emission maxima. These test tubes were then kept on water bath at 90–95 °C for 30 minutes and obtained quantum dots were then allowed to cool under room temperature.

1.3. D_2O-H_2O solvent exchange

Two vials of ~1 mL as synthesised CQDs were freeze-dried/lyophilized for ~14 hours. 0.5 mL D_2O and H_2O were used to disperse the lyophilised CQDs. These CQDs were then used for further studies.

1.4. Cell line maintenance, slide preparation and CQDs internalization:

HEK-293t cells were maintained in DMEM media (Gibco) containing 10% fetal bovine serum (Gibco), 1% Penstrap (Gibco), 1% anti-anti and 1% NEA (Gibco) at 37 ºC in humidified CO_2 (5%) incubator. For CQDs internalization inside the cells, healthy HEK cells were seeded on coverslips (prior coated with poly-L-lysin: Sigma) in 2 $X10^5$ confluency. After proper adherence cells were washed with 1X PBS buffer and incubated with CQDs for 12 hrs at 37 ºC in humidified CO_2 (5%) incubator. Further cells were

properly washed after CQDs incubation and fixed with 4% p-formaldehyde, followed by mounting on a glass slide prior to confocal imaging.

1.5. Steady-state ensemble experiments

1.5.1. 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 having a 10 mm path length and 1 ml volume.

1.5.2. Fluorescence spectroscopy

Steady-state fluorescence spectra were recorded on a Horiba fluorolog spectrophotometer and using Agilent Technologies Cary eclipse fluorescence spectrophotometer. Spectra was collected using quartz cuvette of path length 10 mm and 1 mL volume.

1.5.3. Fluorescence lifetime (Time-resolved fluorescence decay) spectroscopy

Fluorescence lifetime measurements were performed using Horiba Scientific Delta Flex TCSPC system with interchangeable pulsed LED sources. For this experiment, pulsed LEDs of wavelength 390 nm, 454 nm, 574 nm were used as they lie in the excitation region of CQDs. Ludox has been used to calculate IRF for de-convolution of obtained lifetime spectrum. Horiba DAS6, data analysis software, was used for the fitting of data. Data was fitted using tri-exponential decay function.

$$I(t) = \sum_{i}^{n} A_i e^{-\left(\frac{t}{\tau_i}\right)}$$

Where I(t) is the counts at time t, τ_i is the lifetime value of i^{th} component. A_i is the amplitude of i^{th} component, and n are the number of components

$$\tau_{avg} = \frac{\sum_i^n A_i \tau_i^2}{\sum_i^n A_i \tau_i}$$

Where τ_{avg} is the average lifetime value obtained using relation.

1.5.4. Absolute QY determination using integrated sphere method

We performed the absolute quantum yield measurements using integrating sphere approach on a QuantaMaster 8450-22 Spectrofluorometer (Horiba) instruments situated at Sophisticated Analytical and Technical Help Institutes (SATHI), IIT Delhi. CQDs were ensured to have absorbance < 0.1 at all excitation wavelengths to reduce any artifacts. For a particular wavelength e.g. for 390 nm excitation, an emission spectrum was taken from 380 nm to 700 nm (scattering and emission part combined) firstly for solvent (water) and then with CQDs. Same settings were employed for both measurements. Then the excitation part (excitation λ ± 10 nm) and emission part (500-700 nm) of the graphs were integrated separately for solvent (ref) and sample (CQDs).

Absolute quantum yield is represented as the following equation

$$Absolute\ quantum\ yield = \frac{Total\ number\ of\ photons\ emitted}{Total\ number\ of\ photons\ absorbed}$$

Therefore, the equation can be reformulated to

$$Absolute\ quantum\ yield = \frac{\int(Emission\ curve\ for\ sample) - \int(Emission\ curve\ for\ solvent)}{\int(Excitation\ curve\ for\ solvent) - \int(Excitation\ curve\ for\ sample)}$$

Using this equation, "Felix GX 4.9.0.10329" software calculated the reported absolute quantum yield values.

1.5.5. Radiative and non-radiative decay measurements

From the fluorescence lifetime and QY values, radiative and non-radiative rates were calculated using the following relations.

$$QY = \frac{k_r}{k_r + k_{nr}}$$

$$Since, \tau = \frac{1}{k_r + k_{nr}}$$

$$QY = \tau \times k_r$$

$$on\ rearranging\ k_r = \frac{QY}{\tau}$$

$$also\ k_{nr}\ can\ be\ calculated\ as,$$

$$k_{nr} = \frac{1}{\tau} - k_r$$

where, k_r = radiative rate, k_{nr} = non-radiative rate, τ = fluorescence lifetime

1.6. Single particle level experiments

1.6.1. Fluorescence correlation spectroscopy (FCS)

Nikon Eclipse Ti inverted microscope with 60x water immersion objective was used to excite a drop of very diluted (nM) solution of CQDs. The drop was placed over a glass coverslip with thickness ~0.1 mm. The emission from sample was directed to a combination of hybrid photomultiplier detector assembly (Picoquant, GmBh Berlin, Germany) placed at 90 degrees from each other. A 600/50 nm bandpass filter was used to filter the emission from CQDs. The filtered light is then directed to both detectors through a 50:50 beam splitter. Then the signal received on one detector is cross correlated to other detector. The signal was analysed and fitted in the Symphotime 64 software supplied with the instrument. Triplet fitting model was used to fit the data obtained from all the excitations.

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

Where, τ_{trip} is the triplet state lifetime, τ_{Diff} is the diffusion time of fluorophore from the confocal volume, T is the triplet fraction, n is the number of fluorescing species, $\rho(i)$ is the contribution of i^{th} species. This instrument is equipped with 405, 488, and 561 nm continuous wave lasers, which were used to excite the samples. The laser power of all lasers was calculated over the objective before starting the experiment to take equal power of all lasers with the help of ThorLabs PM160 optical power meter.

Then, FCS measurements were conducted using a pulsed laser source with a wavelength of 532 nm and a repetition rate of 2 MHz. To obtain information at the single-particle level, the average number of colloidal quantum dots (CQDs) within the confocal volume was maintained below 1, specifically around 0.85 to 0.95. The intensity-time traces of the quantum dots (QDs) as they diffused through the confocal

volume were analyzed further for lifetime characterization at different intensity levels, utilizing the SymphoTime 64 software.

1.6.2. Total correlation in FCS and fluorescence antibunching measurements

Total correlation FCS (from lifetime of fluorophore i.e. nanoseconds to seconds) and antibunching experiments were performed at SATHI Facility, IIT Delhi using Picoquant MicroTime 200. This instrument consists of PDL 828 Sepia II, MultiHarp 150, an Olympus inverted microscope with 60x water immersion objective and a laser combining unit with 405, 485, 532 nm pulsed diode laser. Microtime 200 system was used in T2 mode with sync killed. A drop of very diluted CQDs sample (average number of particles < 0.6) was kept over glass coverslip. Then the excitation laser in CW mode was focused at the sample, emitted light was observed with two detector setups (both Excelitas single photon counting module SPCM AQRH single photon avalanche photo diode). For observing correlation below microseconds range, we need to use the two-detector system and can only be done in T2 mode with sync killed. separate 582/64 BP filters were used in front of both detectors. Data acquisition was done for nearly one hour. The observed data was then analysed using total correlation and antibunching analysis scripts in Symphotime 64 software.

1.6.3. Cleaning of glass coverslips

Since the single particle experiments are very sensitive to the contamination, utmost care was taken to ensure that the glass coverslips were clean. Glass coverslips were treated with Piranha solution (3:1 solution of Sulphuric acid and Hydrogen peroxide) for half an hour, then the mixture was discarded, and glass coverslips were washed with ultrapure deionized water for 4 times and then were ultrasonicated. This process was repeated 4 times. Then the coverslips were kept in water till they were required for the experiment. Freshly cleaned glass coverslips were used for all the experiments. Two empty glass coverslips were analysed for testing impurities to ensure the cleanliness of glass coverslips.

1.6.4. Single particle fluorescence experiments

1.6.4.1. Single particle fluorescence spectroscopy data acquisition

CQDs solution was first diluted at nM to pM concentration, and the sample was spin-coated over a glass coverslip at ~5000 RPM (with 500 RPM/s acceleration). The

coverslip was then mounted over a home-built inverted Nikon Ti epifluorescence microscope objective 100x, 1.49 NA, TIRF objective. Laser beams of wavelengths 488 nm, and 532 nm were aligned for simultaneous measurements. The laser beam reaches the glass coverslip using a 590 nm high pass dichroic mirror (AHF analysentechnik) for both 488 nm and 532 nm lasers. After the sample is excited by these lasers, the emission is collected using the same objective. Then, the excitation beam and emission is separated by the same dichroic mirror, i.e., 590 nm high pass filter. Emission is then further filtered using a band pass filter of 580 ± 35 nm. Emission is finally collected at Andor EMCCD iXon Ultra 897. Andor Solis 64-bit software was used to record the data. EMCCD was used in photon counting mode with an EM gain 300, exposure time 50 ms (~20 fps), and pixel readout rate 17 MHz. A movie with above mentioned settings, with 5000 frames (~250 s) was recorded and saved in. FITS format. In the recorded video, one pixel corresponds to 160 nm x 160 nm area and an area of 20.48 µm x 20.48 µm (128 x 128 pixels) was recorded.

1.6.4.2. Single particle fluorescence spectroscopy data analysis

The area of the recorded video is 20.48 µm x 20.48 µm, and this huge area contains 30-70 number of CQDs particles, and they show intensity fluctuations across the recorded video duration. We custom-built a script using ImageJ macro language to analyse the total photon counts and ON-OFF dwell times for 300-400 individual CQDs. This script first identifies the bright QDs by using maximum intensity projection (Z-project of ImageJ), and NanoJ-core's peak localization (Nearest neighbour analysis). Then a ROI box of 7 x 7 pixels is created around all of these identified localizations, and then the intensity vs. time graph is extracted for individual QDs. These obtained curves are single-particle raw time traces. Now a threshold is set up on all QDs to separate the ON and OFF states. Above the threshold, all emissions are considered ON and below a threshold value, all intensity fluctuations are considered to be OFF. All of these ON and OFF times are noted and photon counts are also extracted from all of such single-particle time traces. A fit line shows the ON states; for OFF states, the fit line reaches zero. It was ensured that no two ROI box overlap each other, in such cases, the ROIs are discarded from the study. An ON time is considered as the time a CQD particle spent in ON state without turning OFF. An OFF time is the time between two subsequent ON-states. If a CQD turns dark and do not turn ON to the end of acquisition, that time is excluded from the OFF state.

All photon counts, and ON-OFF dwell times are then recorded in a separate Excel file. Total photon counts are histogrammed and fitted using exponential decay in data analysis and plotting software Origin. ON-OFF dwell times are then histogrammed with 50 ms bins and plotted in log-log scales, and fitted using power law and truncated power law equations.

$$\text{Inverse power law: } P_{event} = a \times t_{event}^{-m}$$

$$\text{Truncated power law: } P_{event} = a \times t_{event}^{-m} \times e^{-kt_{event}}$$

Where P_{event} is the probability of event (ON or OFF) time t and k is the inverse of truncation time t_c

1.6.4.3. Power density equalization for 488 nm and 532 nm lasers

To study the effect of excitation wavelength over single particle blinking and photon counting statistics, it was ensured that the power density of lasers (488 nm and 532 nm) were same. This was done to avoid any effect of laser power/intensity on fluorescence blinking, which is already known and reported for several QDs.

At first, the laser power was monitored over the objective using a power meter (Thorlabs PM160) for both lasers. Then the output power (obtained from power meter) and input power (given from software) were plotted and fitted. Now we can determine the output power for any input. Since power density is given as

$$Power\ density = \frac{Output\ laser\ power}{Area\ of\ laser\ incidence}$$

Now to get the area of incidence of the laser on the glass coverslip, we took images of glass coverslip, keeping the output power same for both lasers. Then we measured the area of incidence using ImageJ. We back-calculated the output laser power needed to equate the power density of two lasers using the fitted equation of output power vs input given by software.

For this experiment, power density was kept at 0.0107 kW/cm^2 (or 10.68 W/cm^2).

1.6.4.4. Number calculation study

Maximum-intensity projection images were made with the help of ImageJ. Then with the help of a custom-built ImageJ macro language script, all signals were localized, and

there locations were marked. Then an ROI (rectangular selection) of 7x7 pixels is made around all identified signals. The non-overlapping and aggregated CQDs were removed from the counting of bright CQD particles. Then the number of CQDs were counted.

1.7. Relationship between QY (ensemble) and no of bright particles (single particle)

$$QY^{Bulk} = \frac{Photons\ emitted\ by\ Bright\ CQDs + Photon\ emitted\ by\ dark\ CQDs}{Photons\ absorbed\ by\ Bright\ CQDs + Photon\ absorbed\ by\ dark\ CQDs}$$

$$Photons\ emitted\ by\ dark\ CQDs = 0$$

$$QY^{Bulk} = \frac{Photons\ emitted\ by\ Bright\ CQDs}{Photons\ absorbed\ by\ Bright\ CQDs + Photon\ absorbed\ by\ dark\ CQDs}$$

$$Dividing\ whole\ equation\ by\ 'Photons\ absorbed\ by\ bright\ CQDs'$$

$$QY^{Bulk} = \frac{\frac{Photons\ emitted\ by\ Bright\ CQDs}{Photons\ absorbed\ by\ Bright\ CQDs}}{\frac{Photons\ absorbed\ by\ Bright\ CQDs}{Photons\ absorbed\ by\ Bright\ CQDs} + \frac{Photon\ absorbed\ by\ dark\ CQDs}{Photons\ absorbed\ by\ Bright\ CQDs}} \cdots eqn\ 1$$

Quantum yield of bright fraction can be written as

$$QY^{Bright\ fraction} = \frac{Photons\ emitted\ by\ Bright\ CQDs}{Photons\ absorbed\ by\ Bright\ CQDs}$$

$$therefore, equation\ 1\ can\ be\ rewritten\ as$$

$$QY^{Bulk} = \frac{QY^{Bright\ fraction}}{1 + \frac{Photon\ absorbed\ by\ dark\ CQDs}{Photons\ absorbed\ by\ Bright\ CQDs}}$$

Based on the assumption that both dark and bright CQDs absorb in similar fashion

$$QY^{Bulk} = \frac{QY^{Bright\ fraction}}{1 + \frac{Dark\ fraction}{Bright\ fraction}}$$

$$QY^{Bulk} = \frac{QY^{Bright\ fraction}}{\frac{Bright\ fraction}{Bright\ fraction} + \frac{Dark\ fraction}{Bright\ fraction}} = \frac{QY^{Bright\ fraction}}{\frac{Bright\ fraction + Dark\ fraction}{Bright\ fraction}}$$

$$\because Bright\ fraction + Dark\ fraction = 1$$

$$QY^{Bulk} = \frac{QY^{Bright\ fraction}}{\frac{1}{Bright\ fraction}}$$

$$\therefore QY^{Bulk} = Bright\ fraction \times QY^{Bright\ fraction} \cdots eqn\ 2$$

$$\gg QY^{Bulk} \propto Bright\ fraction \propto no\ of\ bright\ particles$$

1.8. Relationship between apparent rates and intrinsic rates of fluorescent bright fractions

$$From\ eqn\ 2,$$

$$QY = Bright\ fraction \times QY^{Bright\ fraction}$$

$$QY = (1 - D) \times QY^{B} \cdots eqn\ 3$$

$$Where\ B = Bright\ fraction, \quad D = Dark\ fraction,$$
$$QY = observed\ quantum\ yield, \quad QY^{B} = QY\ of\ bright\ fraction$$

$$QY = k_r^{App} \times \tau^{App}\ \ and\ \ QY^{B} = k_r^{B} \times \tau^{B}$$

Where, $k_r^{App} = Apparant\ radiative\ rate$, $k_{nr}^{App} = apparant\ non\ radiative\ rate$,
$k_r^{B}, k_{nr}^{B} = Intrinsic\ radiative, and\ non\ radiative\ rate\ of\ bright\ fraction$

$$But\ since\ the\ lifetime\ is\ independent\ of\ the\ dark\ fraction\ for\ CdTe\ QDs$$

$$\tau^{App} = \tau^{B} = \tau$$

$$k_r^{App} = B \times k_r^{B}\ or = (1 - D) \times k_r^{B} \cdots eqn\ 4$$

$$\tau = \frac{1}{k_r^{App} + k_{nr}^{App}} = \frac{1}{k_r^{B} + k_{nr}^{B}}$$

$$k_{nr}^{App} = \frac{1}{\tau} - k_r^{App} = \frac{1}{\tau} - (1 - D) \times k_r^{B}$$

$$k_{nr}^{App} = \frac{1}{\tau} - (1 - D) \times \left(\frac{1}{\tau} - k_{nr}^{B}\right)$$

$$on\ rearranging, k_{nr}^{App} = \frac{D}{\tau} + (1 - D) \times k_{nr}^{B} \cdots eqn\ 5$$

Equation 4 and 5 gives the relationship between apparent rates and the intrinsic rates of bright fraction. We have also added a discussion in 'Results and discussion' section of the manuscript.

Dependency of dark fraction on apparent rates can be seen from the special cases when there is no dark fraction (Dark fraction = 0), and when there is no bright fraction (Dark fraction = 1).

When dark fraction is absent, equation 4 yields

$$k_r^{App} = (1 - D) \times k_r^B = (1 - 0) \times k_r^B$$

$$k_r^{App} = k_r^B \cdots eqn\ 6$$

Also, when D=0, equation 5 becomes,

$$k_{nr}^{App} = \frac{D}{\tau} + (1 - D) \times k_{nr}^B = \frac{0}{\tau} + (1 - 0) \times k_{nr}^B$$

$$k_{nr}^{App} = k_{nr}^B \cdots eqn\ 7$$

Equation 6, and 7 shows that in the absence of dark fraction, both radiative and non-radiative apparent rates are the actual rates of bright fraction only.

Now, when the whole solution comprises of dark fraction only (no bright fraction), D = 1

Equation 4 becomes

$$k_r^{App} = (1 - D) \times k_r^B = (1 - 1) \times k_r^B$$

$$k_r^{App} = 0 \cdots eqn\ 8$$

Equation 5 becomes

$$k_{nr}^{App} = \frac{D}{\tau} + (1 - D) \times k_{nr}^B = \frac{1}{\tau} + (1 - 1) \times k_{nr}^B$$

$$k_{nr}^{App} = \frac{1}{\tau} \cdots eqn\ 9$$

Equation 8 shows that when there is no bright fraction, the apparent rate of complete solution is independent of the radiative rate of bright fraction and equals to zero. Equation 9 shows that k_{nr}^{App} is inversely proportional to the fluorescence lifetime of solution only. But this must be

considered that for a non-fluorescent solution i.e., D=1 and B=0, the fluorescence lifetime cannot be defined and hence the k_{nr}^{App} is undefined and independent to k_{nr}^{B}.

1.9. Extrapolation of fluorescence lifetime values at 488 nm, and 532 nm excitations

For performing the fluorescence lifetime experiment, our setup has 390 nm, 454 nm, 574 nm pulsed excitation sources. We extrapolated the value of fluorescence lifetime at 488 nm, and 532 nm excitation. Since, there exist very less difference in the fluorescence lifetime spectra at all excitation, the values at 488 nm, and 532 nm should not be much different then the other excitation values. For obtaining the extrapolated values, experimental lifetime values were plotted with excitation wavelengths. Then the plotted points were linear fitted. Then the lifetime values at 488 nm, and 532 nm were obtained from the fitted curve.

Supplementary figures

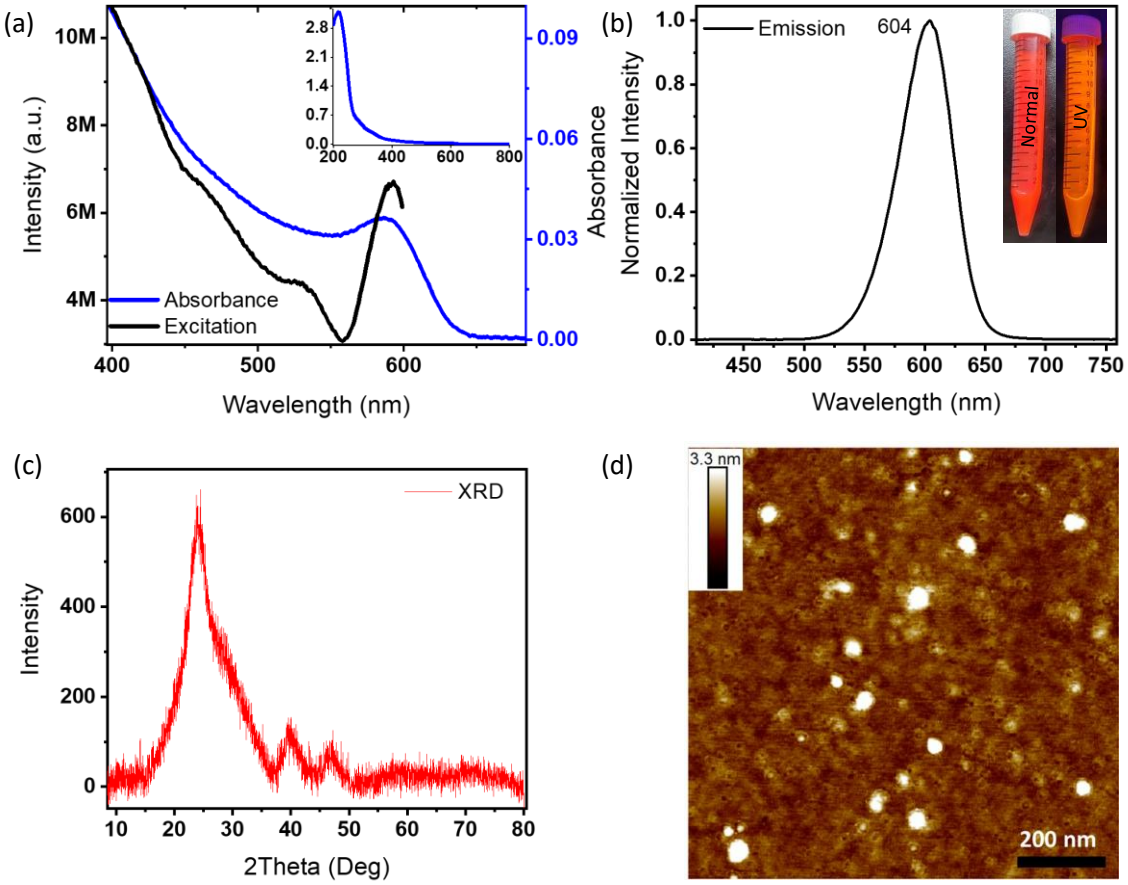


Fig. S1: Photophysical characterization of synthesized water-soluble CQDs: (a) UV-Vis absorption spectrum (Blue) and fluorescence excitation spectrum (Black) of CQDs with band edge peak at 587 nm, inset shows the full wavelength absorption spectrum, (b) fluorescence emission spectrum obtained by 390 nm excitation wavelength with peak maxima at 604 nm. Inset shows photographs of CQDs under normal white light and UV light excitation. (c) shows the powder XRD analysis of freeze-dried CQDs. (d) AFM height profile data of CQDs spin-coated over Si wafer.

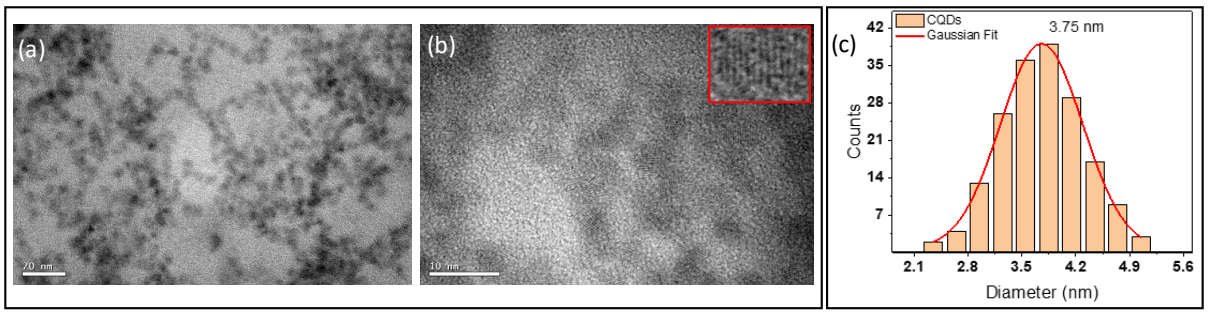


Fig. S2: (a, b) TEM micrograph images showing the uniform distribution of sizes of CQDs. (b) inset shows lattice fringe with size ~0.3 nm for CdTe. (c) showing particle size distribution obtained from ~175 individual CQDs with standard deviation of 0.52 nm. Scale bar: 20 nm and 10 nm in (a) and (b) respectively.

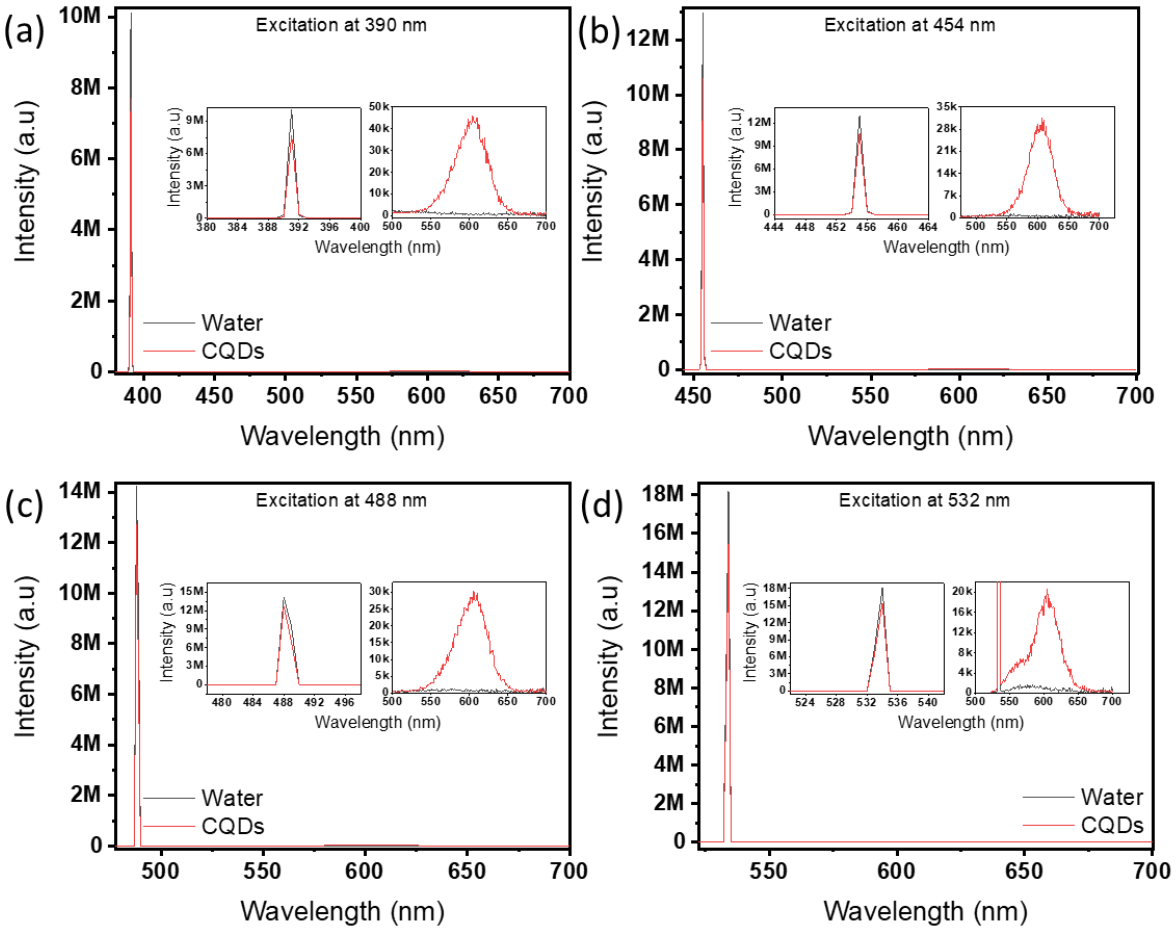
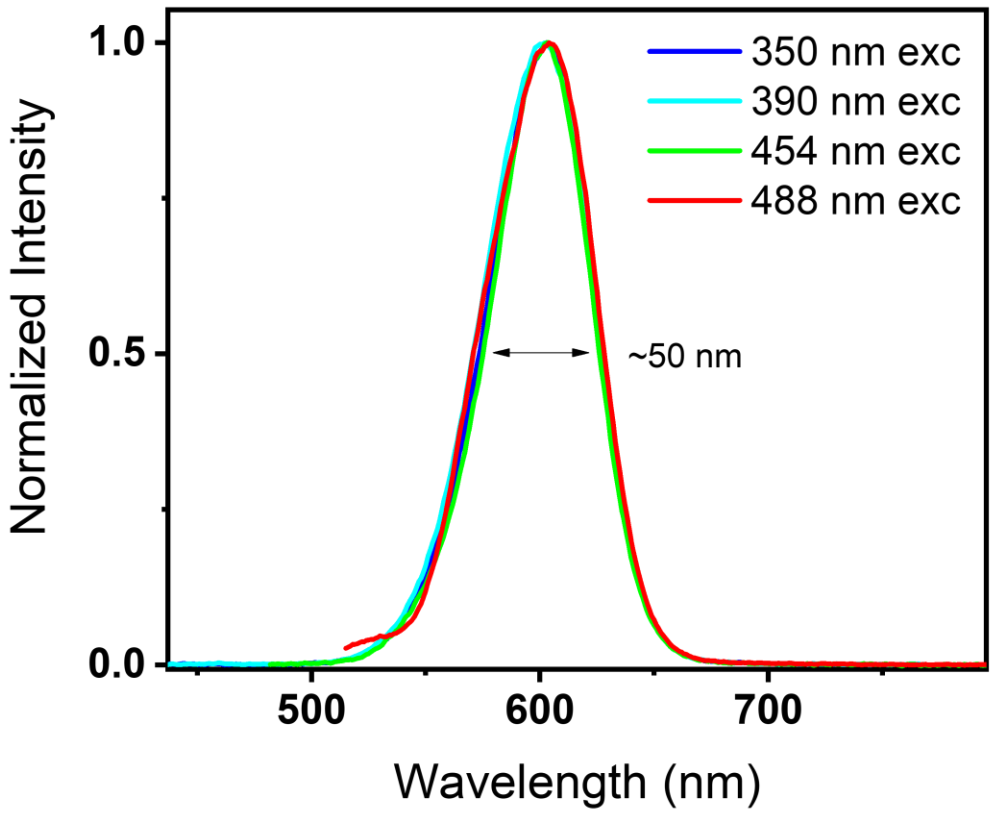


Fig. S3: Complete range (excitation and emission) spectrum taken keeping the CQDs solution inside integrating sphere and exciting the sample with (a) 390 nm, (b) 454 nm, (c) 488 nm, and (d) 532 nm. Black curve is for solvent i.e. water and red curve is for CQDs. inset shows zoomed curves at the excitation range (excitation $\lambda \pm 10$ nm) and emission range (500-700 nm).

307

308 **Table S1:** Absolute quantum yield graph integration values for solvent (ref) and CQDs (sample) at excitation
309 wavelength range (excitation λ ± 10 nm) and emission wavelength range (500-700) at various excitations

Exc (nm)	$\int I_{em}$[CQDs]	$\int I_{em}$[ref]	$\int I_{ex}$[CQDs]	$\int I_{ex}$[ref]
390	2363313	143882	9115139	11861320
454	1509863	88115.23	12527510	15324570
488	1532335	105343.1	22067910	26582710
532	958763.6	95440.09	22346560	26214710



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311 **Fig. S4:** Normalized excitation wavelength dependent emission spectra showing homogeneous emission profile
312 with narrow emission spectrum of full width half maximum of ~50 nm.

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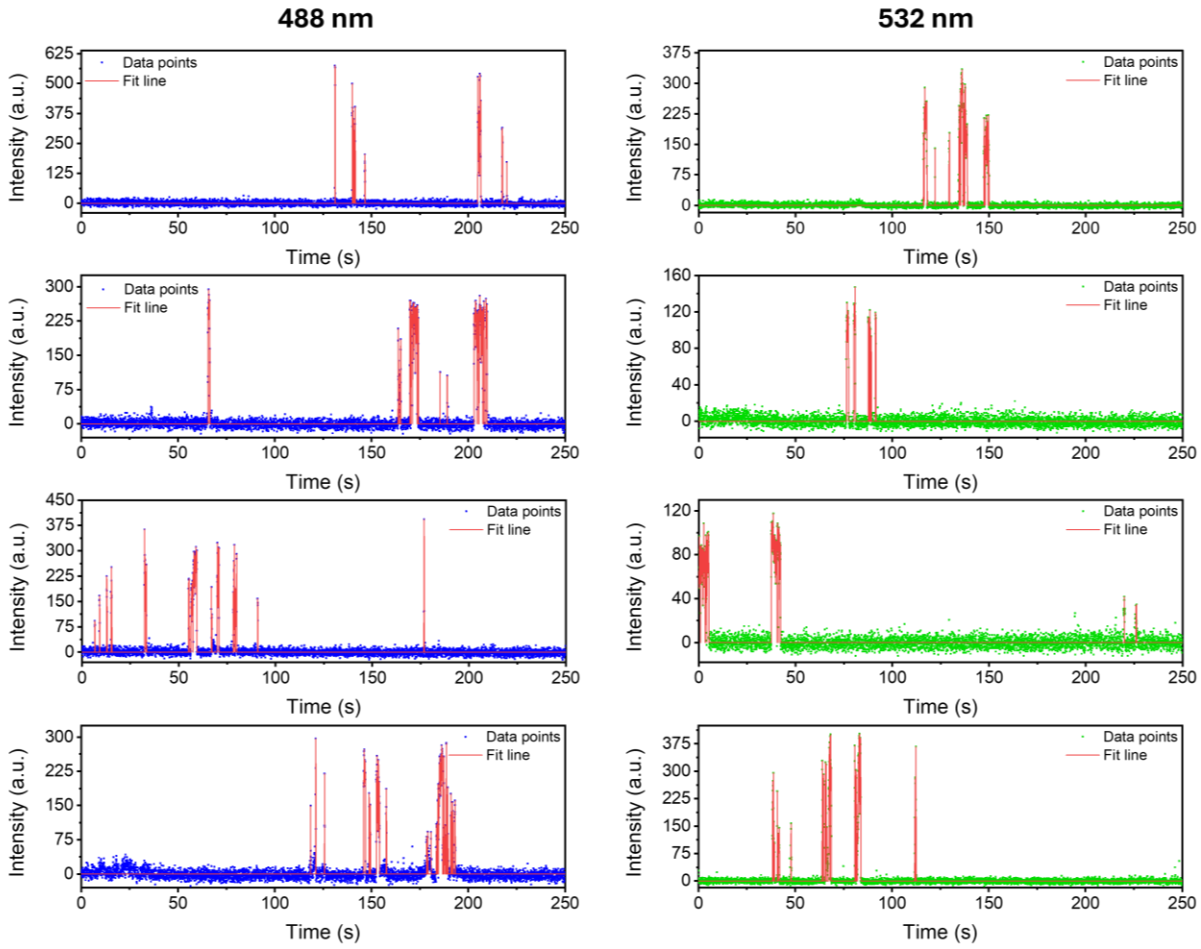

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315 **Fig. S5:** Some representative intensity time traces obtained from individual CQDs excited with 488 nm (left panel)
316 and 532 nm excitation (right panel)

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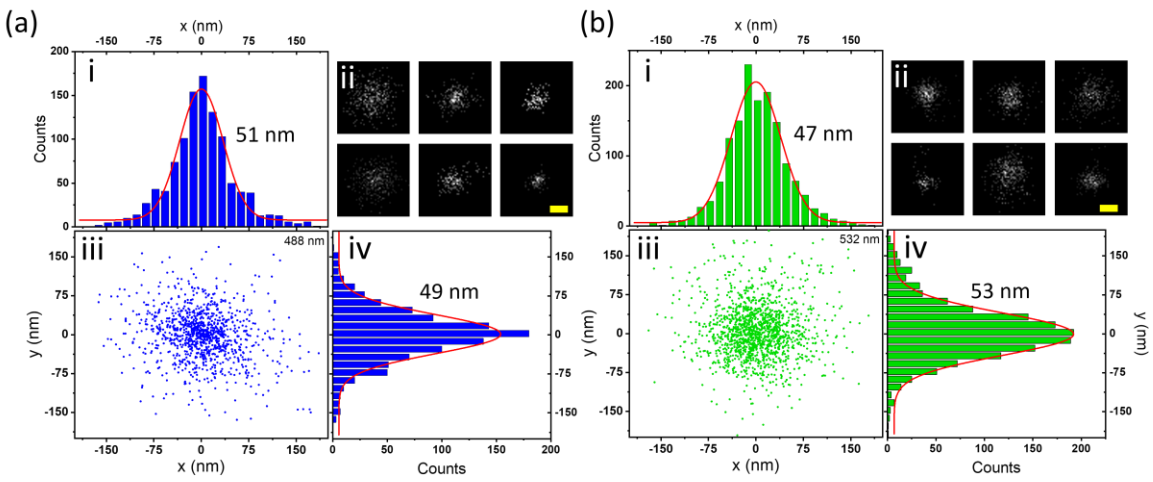


Fig. S6: (a, b) shows the experimental localization precision determination at both 488 nm & 532 nm excitations. The scatter plot (a, b) (iii) is obtained by the localization of ~8 QDs keeping the center of mass of localizations at origin. (a, b) (i & iv) shows distributions of these localizations along x and y-direction. (iv) shows distributions of these localizations in y direction. (a, b) (ii) shows representative individual localizations obtained from Thunder-STORM used for localization precision study. Fit line in (i & iv) shows Gaussian distribution with standard deviation values ~50 nm. Scale bar in single localizations (ii) is 100 nm.

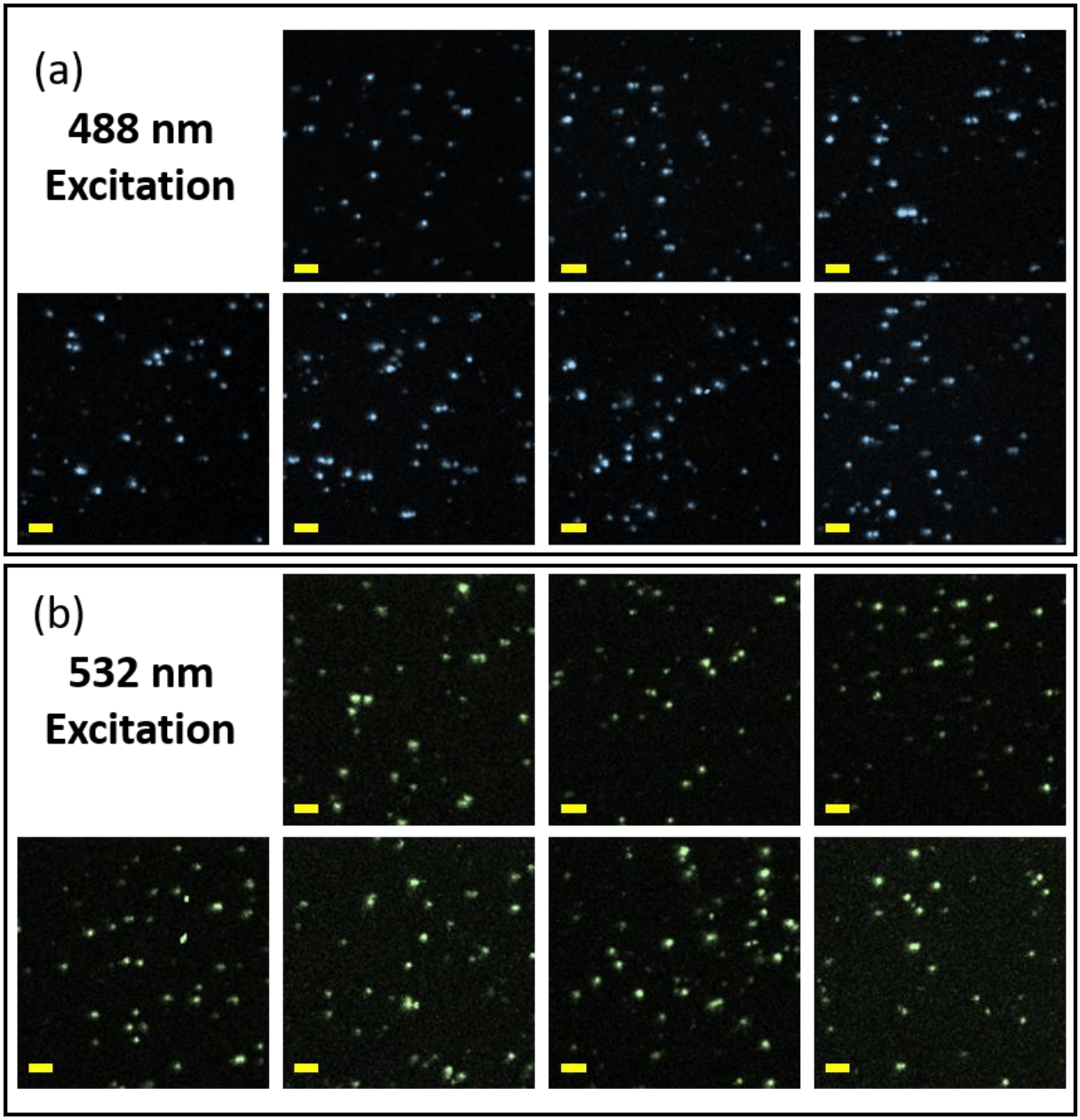
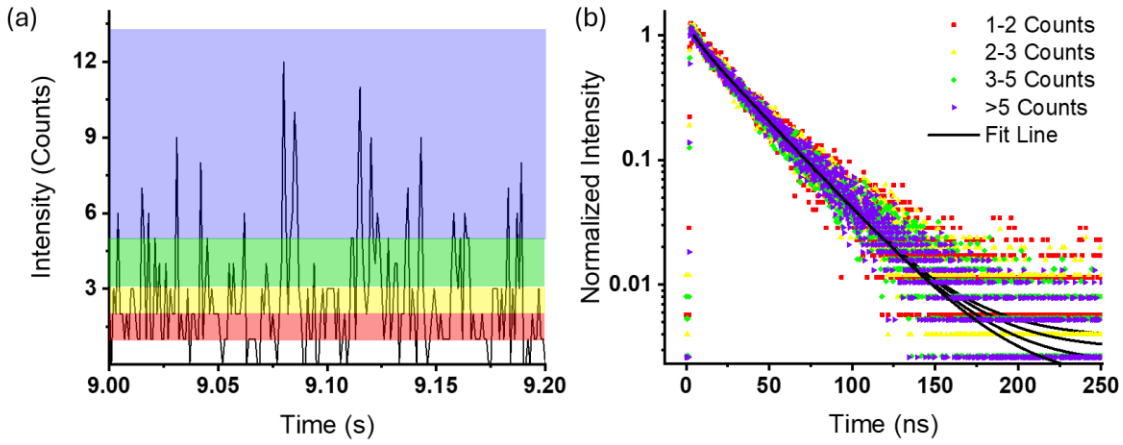
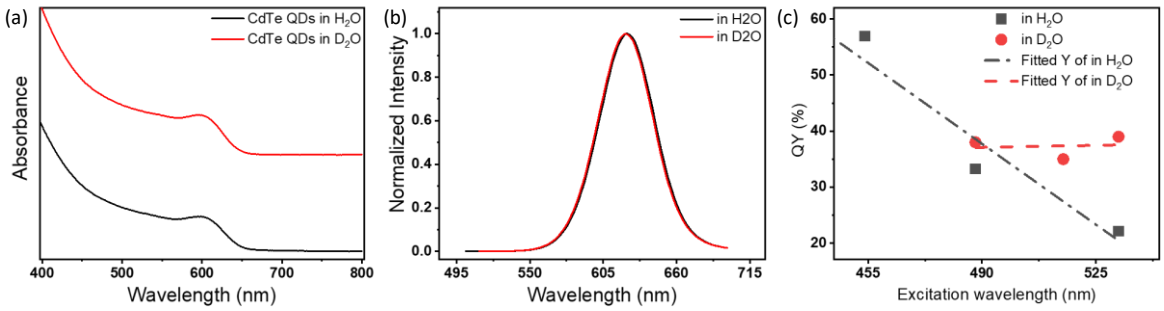


Fig. S7: Max intensity projection of videos recorded for single particle studies under 488 nm and 532 nm excitations with the same power density. Image area is 20.48 µm x 20.48 µm, Scale bar: 2 µm.



329

330 **Fig. S8:** (a) Fluorescence intensity–time traces of quantum dots (QDs) diffusing through a confocal volume under
331 pulsed excitation at 532 nm recorded with an average particle count of closely ~1 in the confocal volume to isolate
332 single-particle intensity fluctuations. The complete measurement time was ~60 s. Shaded regions indicate
333 different intensity states analyzed for fluorescence lifetime measurements. (b) Fluorescence lifetimes
334 corresponding to the intensity states highlighted in (a). The data reveal that all intensity states exhibit the same
335 fluorescence lifetime, thus suggesting emission from single emitter species.



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337 **Fig. S9:** CdTe QDs were redispersed in H_2O (Black) and D_2O (Red) after freeze drying. (a) UV-Vis absorption
338 spectra (offset for better view), (b) overlapping normalized emission spectrum. (c) Excitation wavelength
339 dependent QY for QDs in water and excitation independent QY for QDs dispersed in D_2O.

340

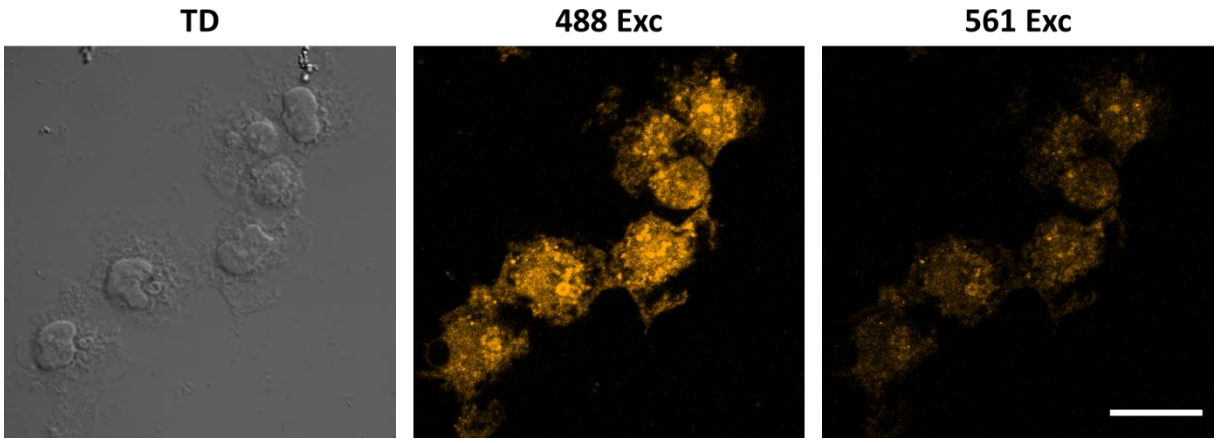

Fig. S10: Confocal laser scanning microscope image taken of CdTe QDs stained HEK cells. Samples were excited with 488 nm and 561 nm lasers, and emission was collected in the TRITC channel (595/50 bandpass) with the same detector setting, keeping the excitation laser power at 3.5 µW. High intensity can be observed under 488 nm excitation in comparison to 561 nm excitation. Scale bar: 20 µm.