Electronic Supplemental Information for

Fluorescent Vesicles Formed by Single Surfactant Induced by Oppositely-Charged Carbon Quantum Dots

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containing 0.5 mg·mL^{-1} CQDs and 1.0 mmol·L^{-1} SO.

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Table S1. Fluorescence lifetime (τ) and quantum yield (Φ) of CQDs in water and micellar phases

formed by SO and SDS.

1. Experimental Section

1.1 Materials

1-aminopropyl-3-methyl-imidazolium bromide ([APMIm][Br]) was obtained from Lanzhou Institute of Chemical Physics, Chinese Academy of Sciences (China). Citric acid monohydrate, sodium oleate (SO), sodium dodecyl sulphate (SDS), bis (2-ethylhexyl) sulfosuccinate sodium salt (Aerosol OT, abbreviated to AOT), cetyltrimethylammonium bromide (CTAB) and Triton X-100 (TX-100) were purchased from Aladdin Reagent Co. and used as received. Ultrapure water with a resistivity of 18.25 MΩ cm prepared from a UPH-IV ultrapure water purifier (China) was used throughout the experimental process.

1.2 Synthesis of CQDs

Ionic liquid modified CQDs were prepared with modifications on our recently-developped procedures.[1] In brief, citric acid monohydrate (1.0 g) and [APMIm][Br] (3.44 g) were added to a round-bottom flask containing 25 mL water with vigorous stirring until the mixture was dissolved totally. The mixture was then heated to ~180 ºC by an oil bath to remove water until a uniform yellow gel was obtained, which was then pyrolyzed at 240ºC for 2 h. After cooling to room temperature, the mixture was re-dispersed in 25 mL water and dialyzed with a Snake Skin Pleated Dialysis Tubing membrane (1000 Da cutoff) against water for several days. Finally, the as-prepared CQDs aqueous solution was lyophilized to get the powder, which can be easily re-dispersed in water wherever necessary.

1.3 Characterizations

Zeta potential was measured with a Zeta PALS potential analyzer instrument (Brookhaven, USA) with parallel-plate platinum black electrodes spaced 5 mm apart and a 10 mm path length rectangular organic glass cell. All samples were measured using a sinusoidal voltage of 80 V with a

frequency of 3 Hz. For each sample, totally ten values were automatically given by the instrument, which were then averaged to give the final value and the standard deviations. X-Ray photoelectron spectroscopy (XPS) data were collected by an X-ray photoelectron spectrometer (ESCALAB 250) with a monochromatized Al Kα X-ray source (1486.71 eV). Dynamic light scattering (DLS) measurements were performed on a Brookhaven BI-200SM instrument at a constant scattering angle of 90°. The intensity of the function ΓG(Γ) was analyzed by the method of CONTIN. The samples were passed through 0.45 μm filters to remove the dusts.

For negative-staining transmission electron microscopy (TEM) observations, ~5 μL of the CQDs solution was placed on a carbon-coated copper grid which was then stained by adding 5 μL of 1.6 wt% uranyl acetate in ethanol. The excess solution was wicked away with a piece of filter paper and the copper grids were dried at room temperature for 24 h before observation on a JEOL JEM-100 CXII (Japan) at an accelerating voltage of 120 kV. High-resolution transmission electron microscopy (HRTEM) images were recorded on a HRTEM JEOL 2100 system operating at 200 kV. For atomic force microscopy (AFM) observations, ~5 μL of CQDs solution was placed on a silica wafer. The excess solution was removed with a piece of filter paper and the silica wafer were dried at room temperature for 24 h before observation on a Tapping Mode operating with a Nanoscope IIIA at a scan frequency of 1.5 Hz and a resolution of 512×512 pixels. Confocal laser scan microscopy (CLSM) observations were performed using an inverted microscope (model IX81, Olympus, Tokyo, Japan) equipped with a high-numerical-aperture 60 oil-immersed objective lens, a UV-mercury lamp (OSRAM, HBO, 103w/2, Germany), a mirror unit consisting of a 330-385 nm excitation filter, a 535-565 nm emission filter, and a 16 bit thermoelectrically cooled EMCCD (Cascade512B, Tucson, AZ, USA). The EMCCD was used for collecting the fluorescent images. Details for FF-TEM and cryo-TEM observations can be found elsewhere.[2,3]

Fluorescent measurements were conducted on a Hitachi F-4500 fluorescence spectrophotometer.

The fluorescence lifetimes and time-resolved emission fluorescence spectra were measured on an Edinburgh Instruments FLS920 with a time correlated single photon containing (TCSPC) method. The data were deconvoluted with the instrument response function, recorded using dispersed light, and fitted to a multi-exponential function. The absolute fluorescence quantum yields were measured with a spectrofluorometer (FLSP920, Edinburgh Instruments LTD) equipped with an integrating sphere, which consists of a 120 mm inside diameter spherical cavity. 3 mL of sample solution was sealed in a quartz cell (1 cm × 1 cm) with a plug. The same volume of solvent was used as the blank sample.

1.4 Cytotoxicity assay

In vitro cytotoxicity of the CQDs against Hek293 cells was assessed by the standard MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. Typically, 100 μL of Hek293 cells were seeded in a 96-well plate with a density of 1×10^4 cells per mL and allowed to adhere overnight. After incubation for 24 h at 37 ºC, the culture medium was discarded and then cells were treated with Dulbecco's Modified Eagle's Medium (DMEM) and 10% Fetal Bovine Serum (FBS), containing various concentrations of CQDs (0-200 μg /mL) for another 24 h. At the end of the incubation, the culture medium was removed, and 10 μL of MTT (5.0 mg/mL in PBS) was added into each well. After additional 4 h incubation, the growth medium was removed and the formazan crystals formed were dissolved in 150 mL of dimethyl sulfoxide (DMSO) and shaken. Finally, the absorbance values of formazan were measured with PE EnSpire instrument at 490 nm.

1.5 Calculation of surface charge density of CQDs

The mean diameter of the CQDs was determined by HRTEM to be 2.6 nm (Fig. S2), thus the volume of each CQD (V) can be obtained according to equation 1:

$$V = \frac{4}{3}\pi R^3 = \frac{4}{3}\times 3.14\times 1.3^3 = 9.20\ nm^3 \qquad (1)$$

Assuming that the density of the CQDs (ρ) equals 1.8 g·cm^{-3}, the quality of each CQD (m) can be

calculated according to equation 2:

$$m=\rho V=1.8\times 9.20\times 10^{-21}=1.66\times 10^{-20}\ g \quad (2)$$

For 1 mL CQDs aqueous solution with a concentration of 0.5 mg·mL^{-1}, the number of CQDs (N_1) can be obtained by equation 3:

$$N_1=\frac{0.5\times 10^{-3}}{1.66\times 10^{-20}}=3.01\times 10^{16} \quad (3)$$

For 1 mL surfactant aqueous solution with a concentration of 0.5 mmol·L^{-1}, the number of surfactant molecules (N_2) can be calculated by equation 4:

$$N_2=n_c\times N_A=0.5\times 10^{-3}\times 10^{-3}\times 6.02\times 10^{23}=3.01\times 10^{17} \quad (4)$$

Then, the number of positive charges on each CQD (n) can be obtained by equation 5:

$$n=\frac{N_2}{N_1}=\frac{3.01\times 10^{17}}{3.01\times 10^{16}}=10 \quad (5)$$

The surface charge density (σ) can be obtained according to equation 6:

$$\sigma=\frac{n}{4\pi R^2}=\frac{10}{4\times 3.14\times\left(1.3\times 10^{-7}\right)^2}=4.7\times 10^{13}\ per\ cm^2 \quad (6)$$

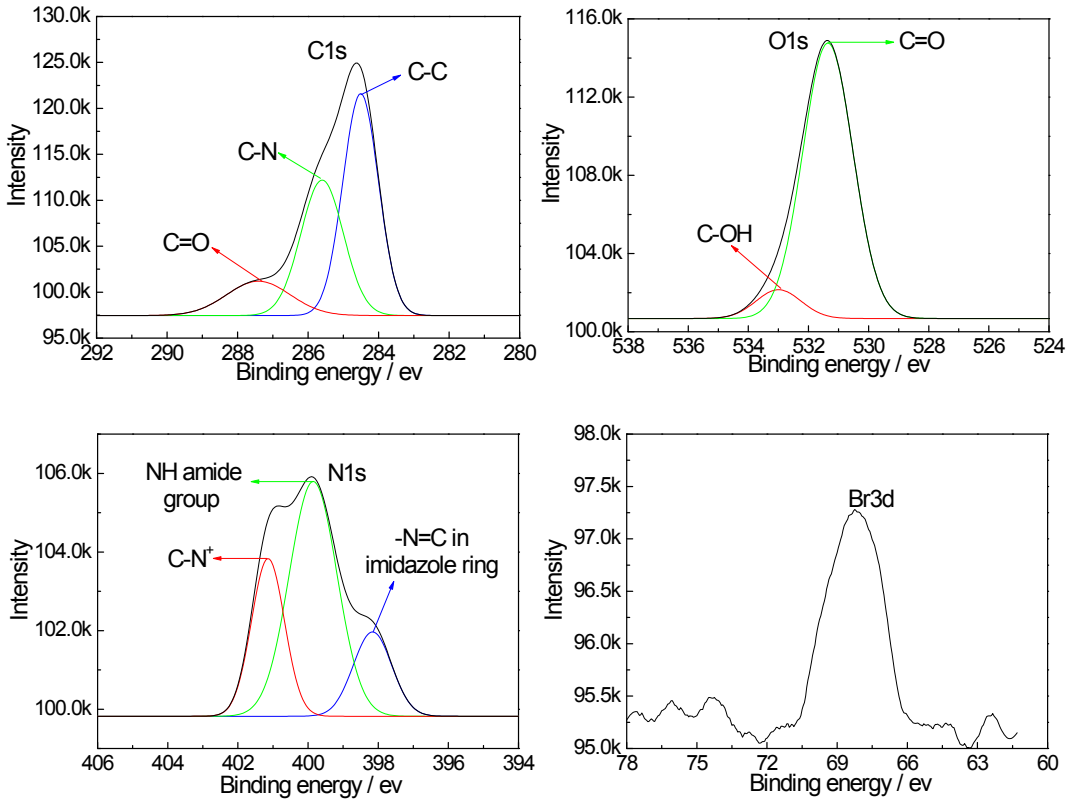

Fig. S1. XPS fine structure spectra of C_{1s}, O_{1s}, N_{1s}, and Br_{3d} of CQDs.

Peaks of C1s, O1s, N1s could be well attributed to the respective structures. Specifically, the presence of nitrogen and bromine confirms the successful attachment of [APMIm][Br] onto the surfaces of CQD.

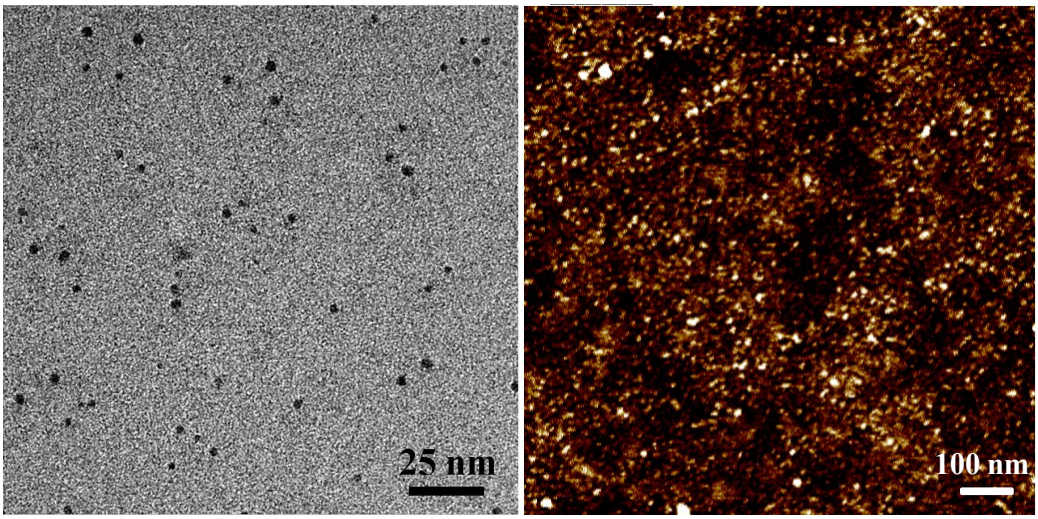

Fig. S2 A typical micrograph of CQDs aqueous solution: HRTEM images (left) and AFM images (right) denoting the existence of spherical CQDs with a mean diameter of ~2.6 nm.

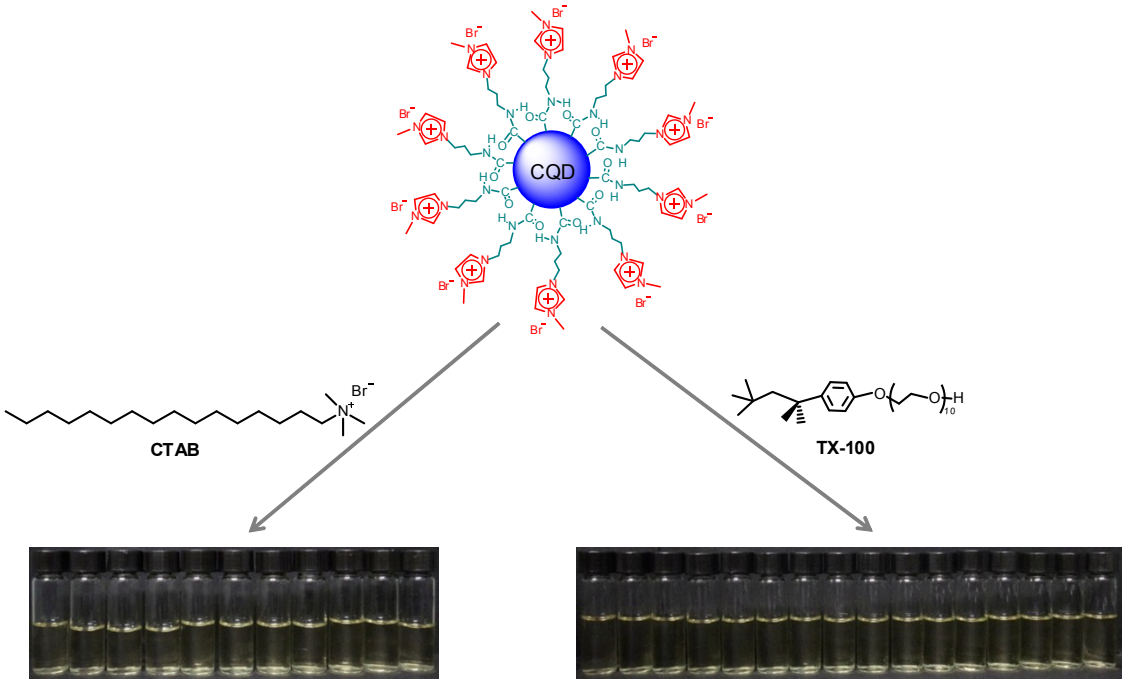

Fig. S3 Photographs of samples of 0.5 mg·mL^{-1} CQDs mixed with varying amount of CTAB (left) and TX-100 (right). The concentration of CTAB (from left to right) is 0, 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4, 1.6, 1.8, 2.0 mmol·L^{-1} while that of TX-100 (from left to right) is 0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.1, 1.2, 1.3, 1.4, 1.5 mmol·L^{-1}, respectively.

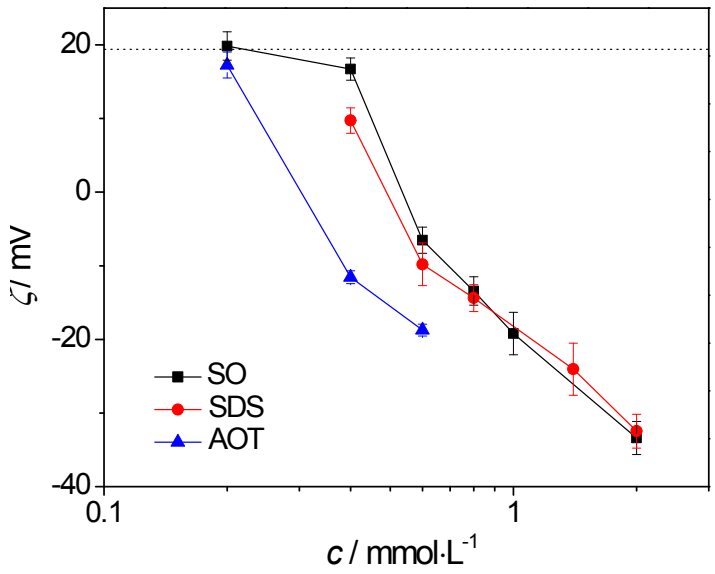

Fig. S4 Zeta potential (ξ) of the turbid samples in CQDs/anionic surfactant mixed solutions. The dotted line denotes the value of CQDs before surfactant addition.

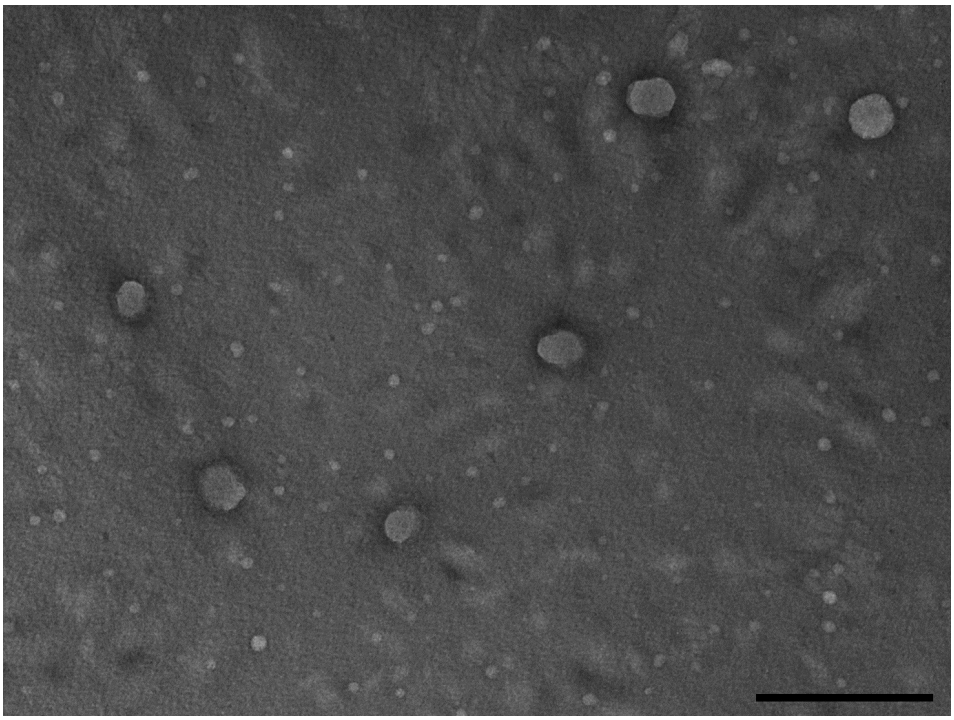

Fig. S5 A typical NS-TEM image denoting the coexistence of the large and small vesicles for the sample containing 0.5 mg·mL^{-1} CQDs and 1.0 mmol·L^{-1} SO. The scale bar corresponds to 500 nm.

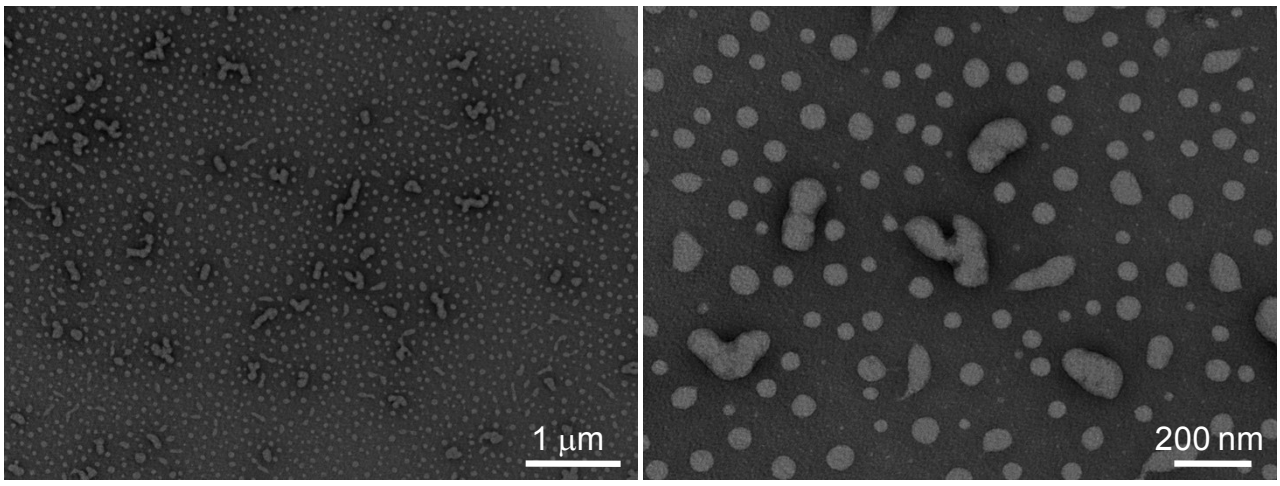

Fig. S6 Typical NS-TEM images denoting the fusion and/or clustering of the vesicles for the sample containing 0.5 mg·mL^{-1} CQDs and 1.2 mmol·L^{-1} SDS.

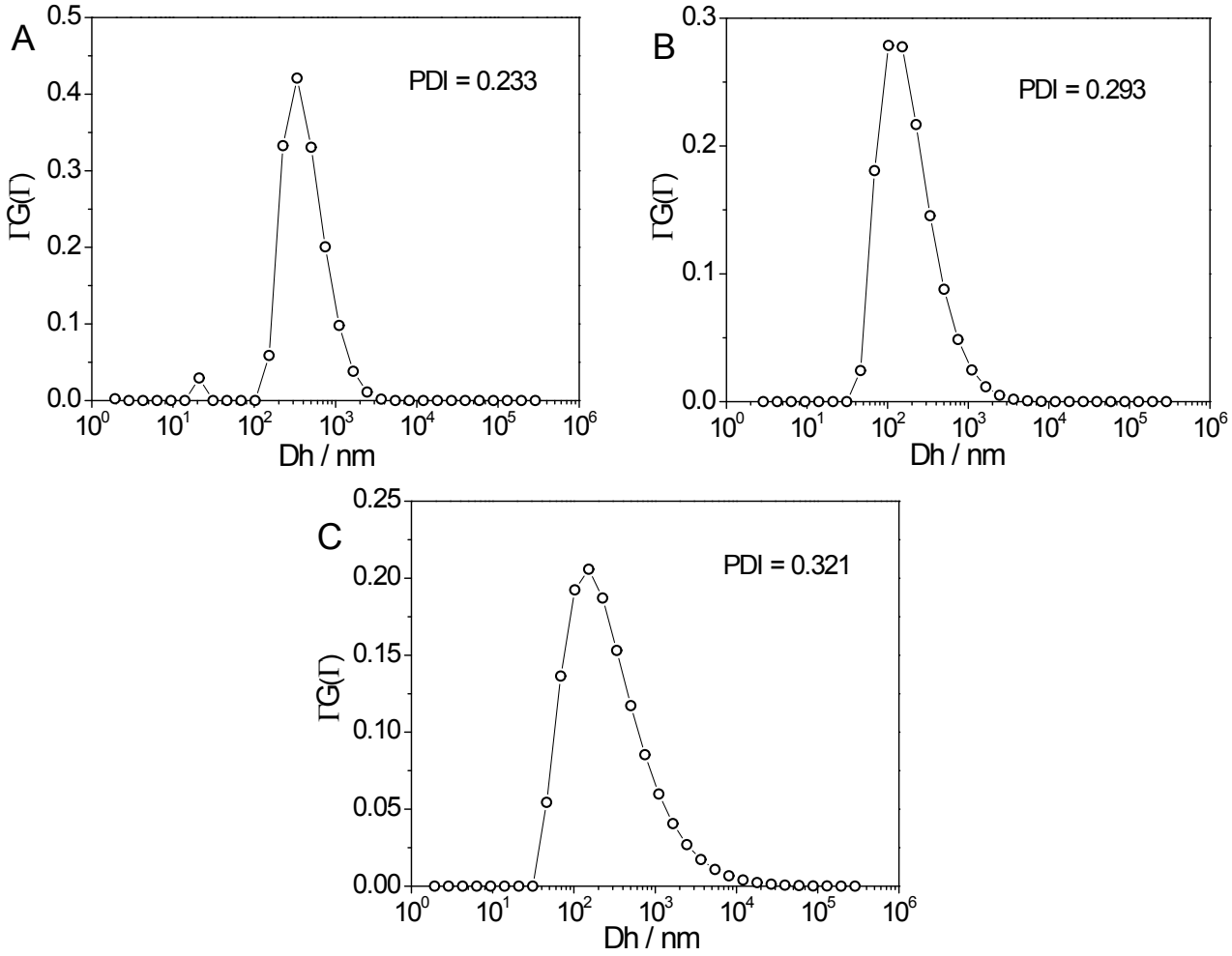

Fig. S7 Hydrodynamic diameter distributions and polydispersity index of the vesicles formed at 1.0 mmol·L^{-1} SO (A), 1.2 mmol·L^{-1} SDS (B) and 0.6 mmol·L^{-1} AOT (C), respectively. The concentration of CQDs is fixed at 0.5 mg·mL^{-1}.

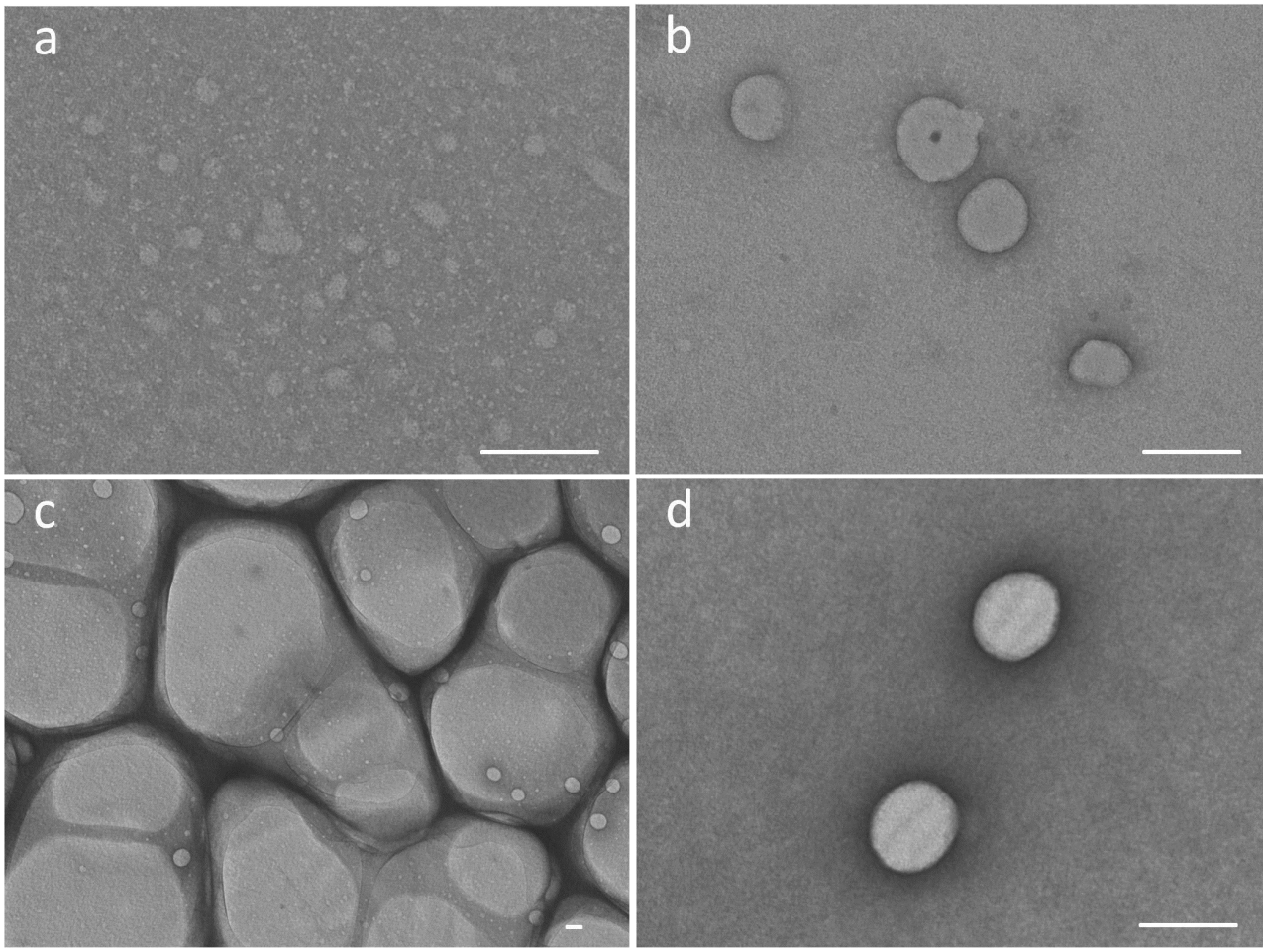

Fig. S8 Typical NS-TEM images of the turbid samples containing 0.4 mmol·L^{-1} SO (a), SDS (b) and AOT (c, d), respectively. The scale bar corresponds to 200 nm.

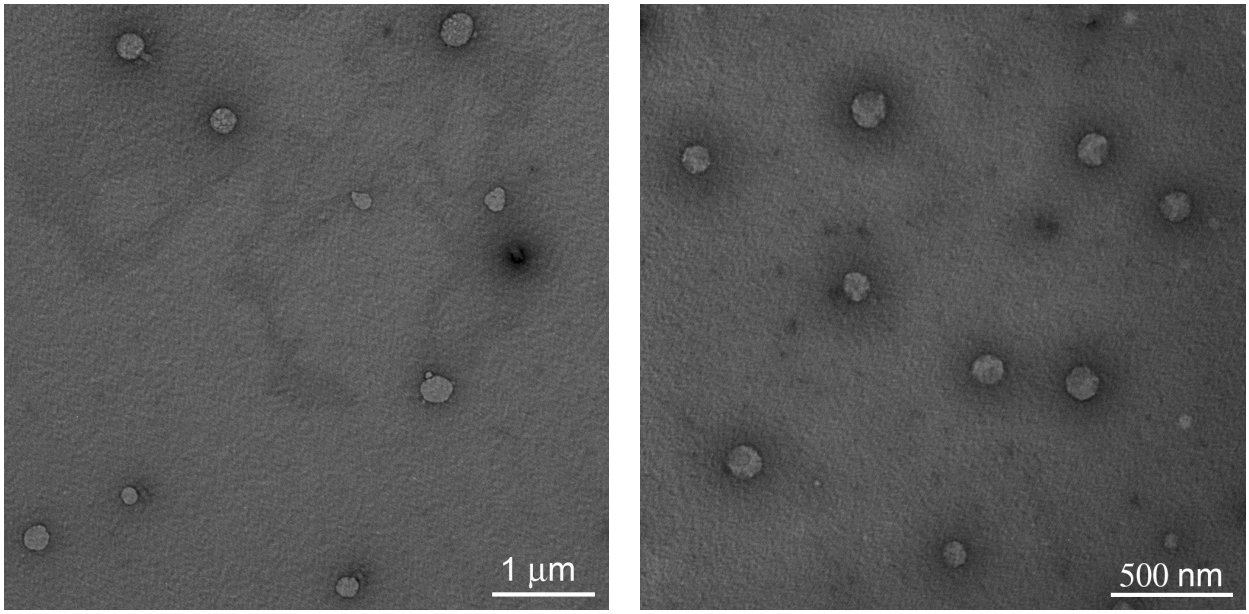

Fig. S9 NS-TEM micrographs of the turbid samples formed in 0.5 mg·mL^{-1} negatively-charged CQDs mixed with 2.0 mmol·L^{-1} CTAB in water. Taken one week (left) and one month (right) after sample preparation, respectively.

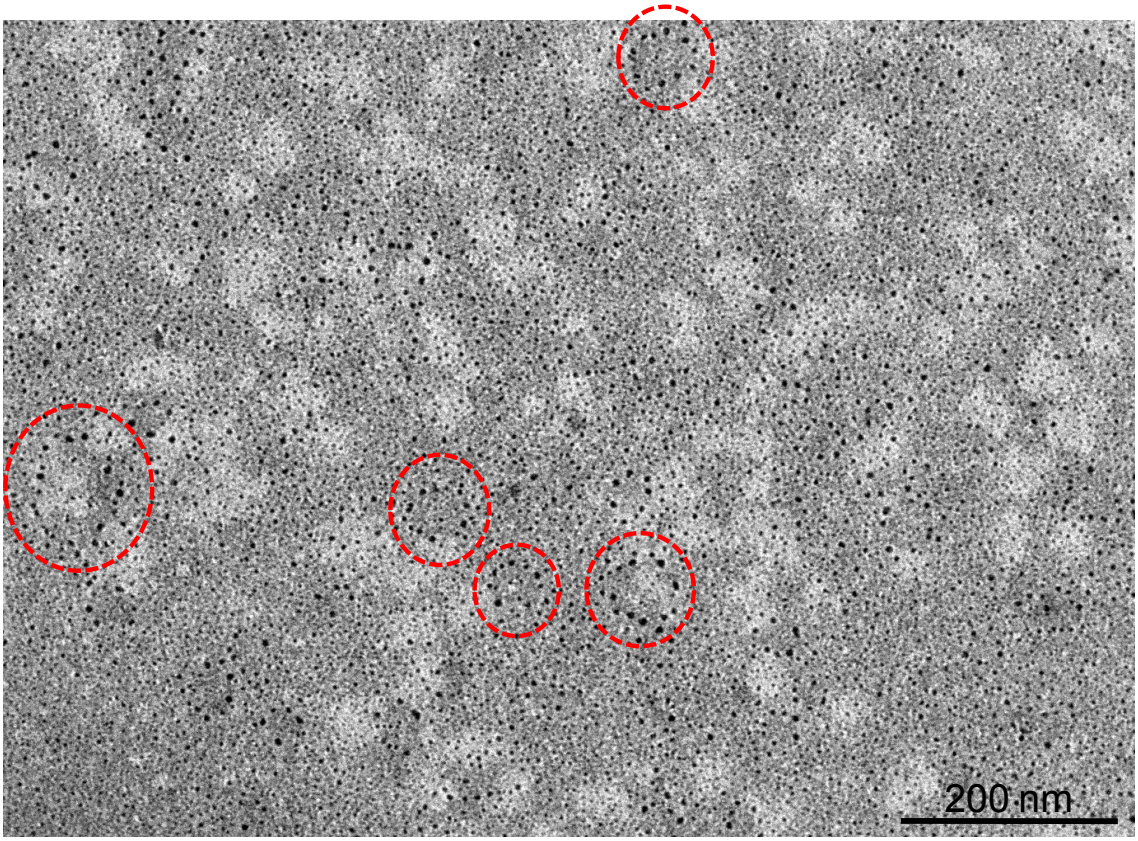

Fig. S10 HRTEM image showing the existence of the CQDs-based vesicles (denoted by the red circles) and giant micells with CQDs as the cores.

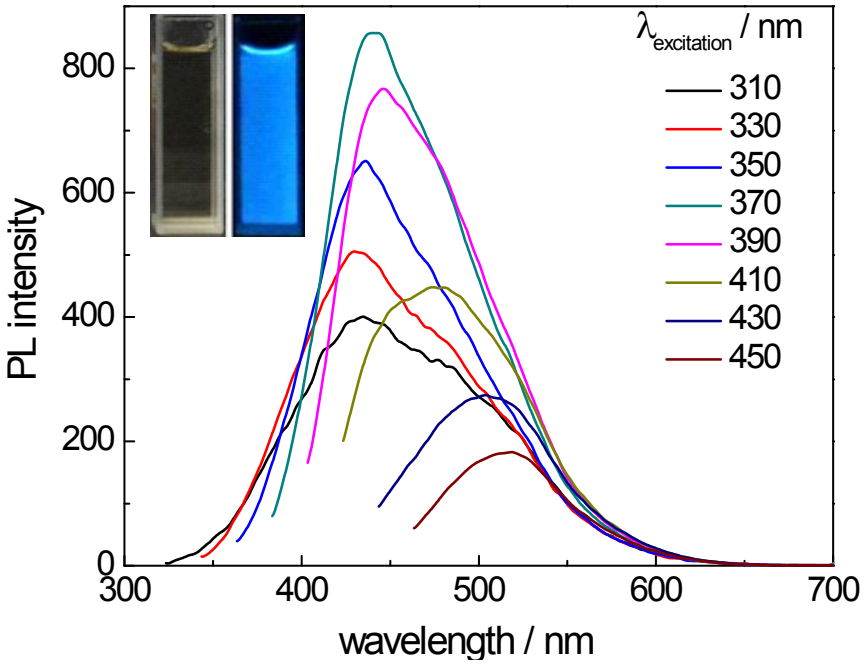

Fig. S11 Emission spectra at varying excitation wavelengths of the CQDs aqueous solution. Insets are photos of the sample solution under room light (left) and 365 nm UV lamp (right).

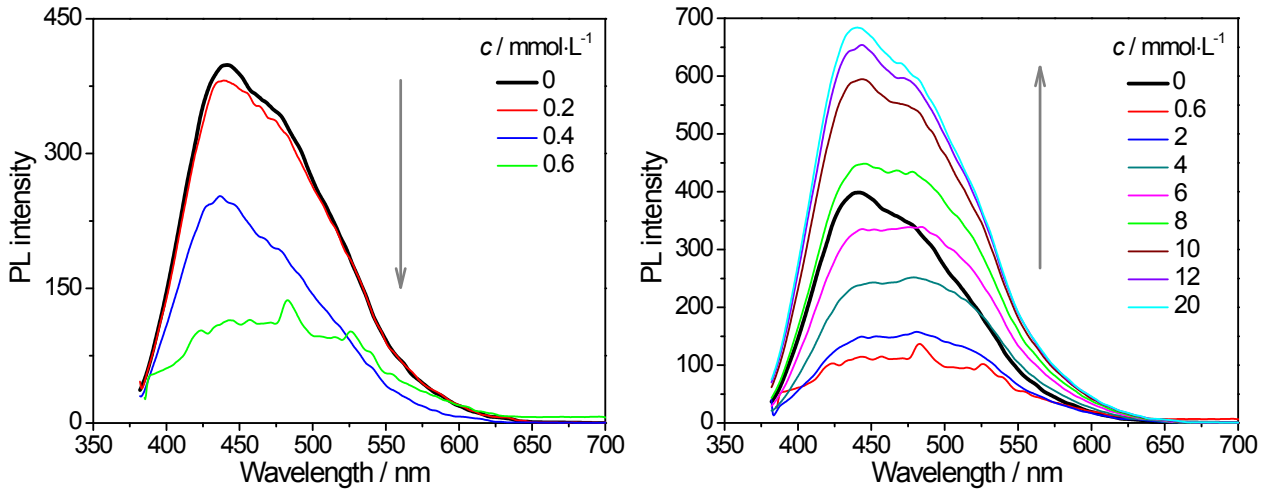

Fig. S12 Emission spectra at 370 nm of samples containing increasing amount of SDS

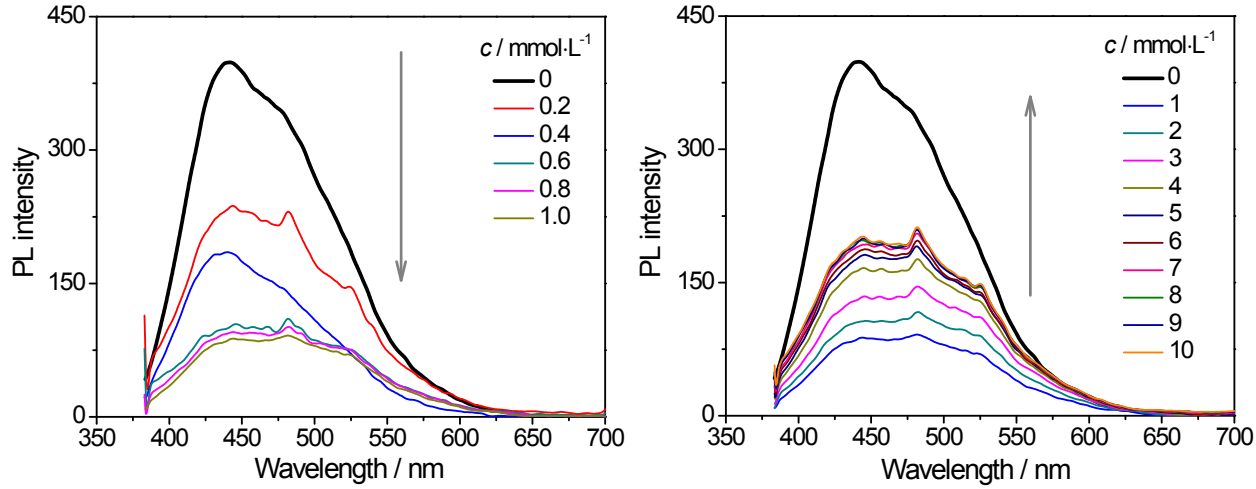

Fig. S13 Emission spectra at 370 nm of samples containing increasing amount of AOT.

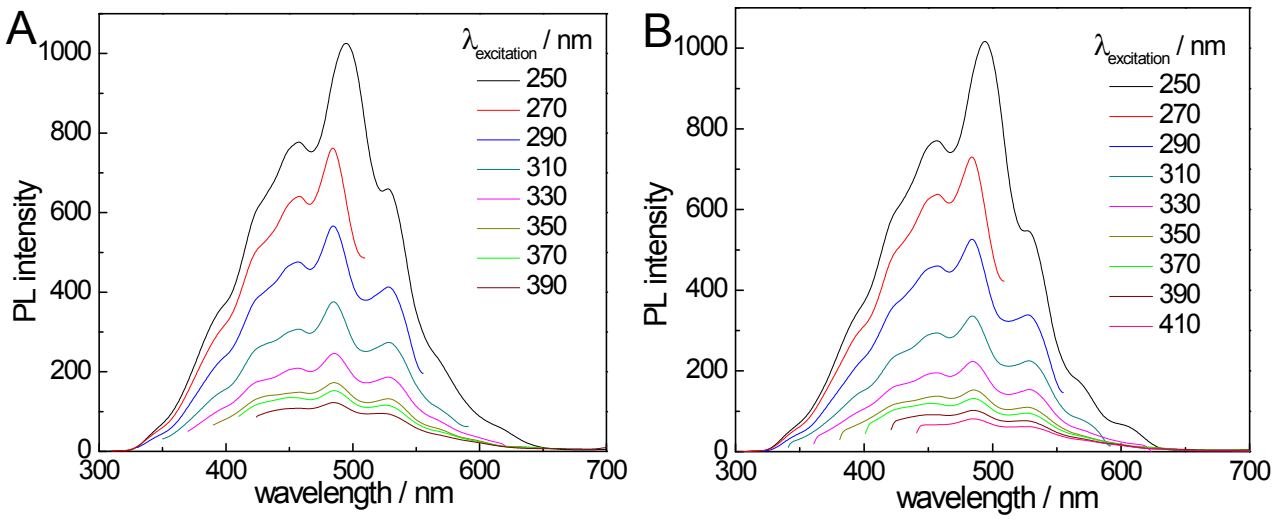

Fig. S14 Emission spectra at varying excitation wavelengths of two typical turbid samples containing 1.0 mmol·L^{-1} SO and 1.2 mmol·L^{-1} SDS, respectively.

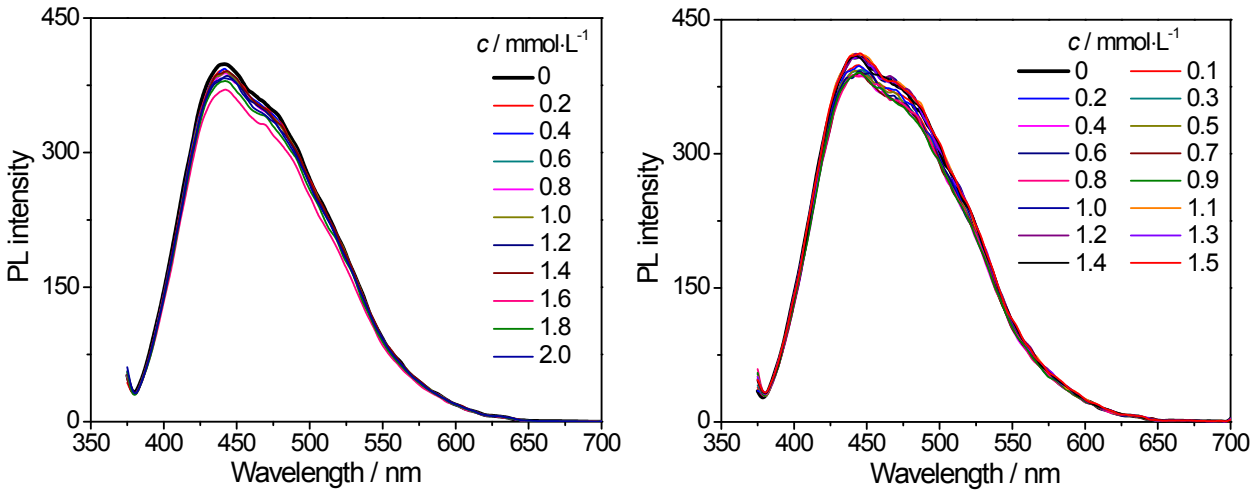

Fig. S15 Emission spectra at 370 nm of samples containing increasing amount of CTAB (left) and TX-100 (right).

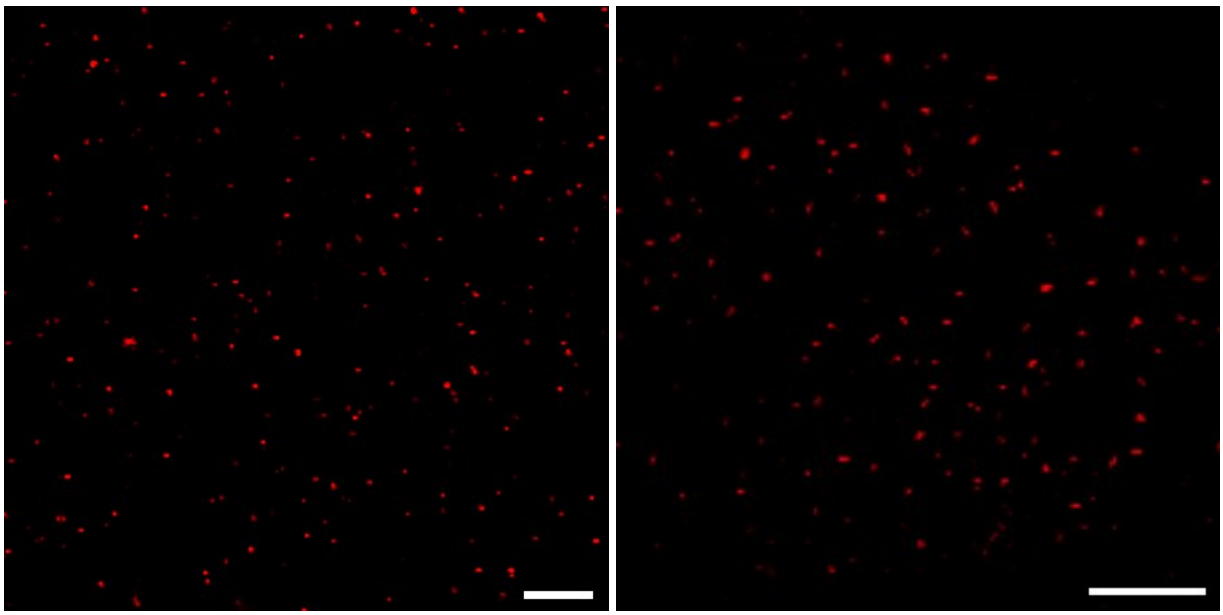

Fig. S16 Confocal fluorescence micrographs of the samples containing 1.2 mmol·L^{-1} SDS (left) and 0.6 mmol·L^{-1} AOT (right). The scale bar corresponds to 10 μm.

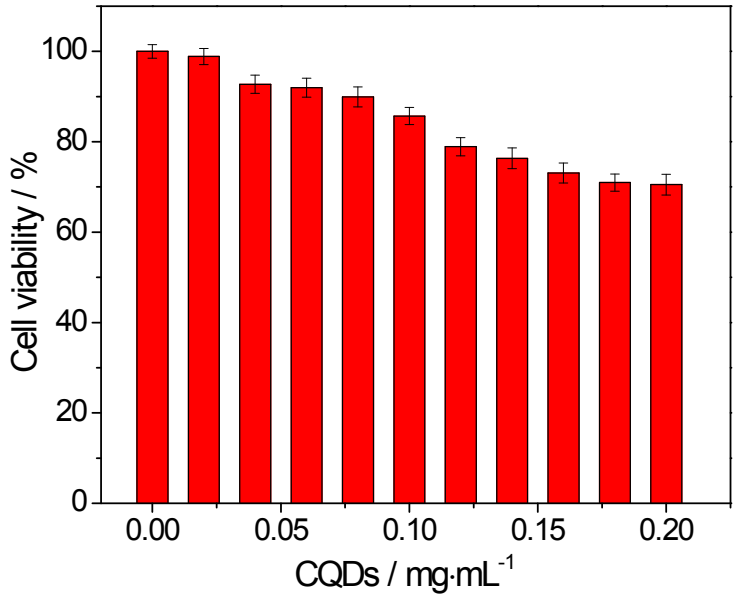

Fig. S17 Cellular cytotoxicity assessment of CQDs.

Table S1. Fluorescence lifetime (τ) and quantum yield (Φ) of CQDs in water and micellar phases formed by SO and SDS.

	τ_1 / ns	τ_2 / ns	$\langle\tau\rangle^a$ / ns	χ^2	Φ
CQDs in water	2.01 (44.61%)	9.29 (55.39%)	6.04	1.19	11.37
CQDs in 10 mmol·L^{-1} SO	2.04 (49.47%)	8.57 (50.53%)	5.34	1.15	12.92
CQDs in 20 mmol·L^{-1} SDS	2.18 (46.49%)	9.48 (53.51%)	6.08	1.16	13.24

[a] mean fluorescence lifetime $\langle\tau\rangle = \tau_1 \times A_1 + \tau_2 \times A_2$

References

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2. Y. Li, H. Li, J. Chai, M. Chen, Q. Yang and J. Hao, *Langmuir*, **2015**, *31*, 11209-11219.
3. Y. Li, H. Li, J. Chai, M. Chen, Q. Yang and J. Hao, *J. Colloid Interface Sci.*, **2016**, *472*, 157-166.