**SUPPORTING INFORMATION**

*Highly Luminescent Biocompatible Carbon Quantum Dots by Encapsulation with an Amphiphilic Polymer.*

Colin Fowley¹, Bridgeen McCaughan¹, Andrea Devlin¹, Ibrahim Yildiz², Francisco M Raymo² and John F Callan¹*.

¹. Department of Pharmacy and Pharmaceutical Sciences, School of Biomedical Sciences, The University of Ulster, Northern Ireland, BT52 1SA. 2. Laboratory for Molecular Photonics, Department of Chemistry, University of Miami, 1301 Memorial Drive, Coral Gables, Florida 33146-0431, United States.

* To whom correspondence should be addressed: email: j.callan@ulster.ac.uk; fraymo@miami.edu

**Materials and Methods.**

**Reagents and Equipment:** Citric acid anhydrate, 1-octadecene (ODE), 1-hexadecylamine, acetone and chloroform were purchased form Sigma-Aldrich at the highest purity available and used as received without further purification. Amphiphilic polymer was prepared according to the procedure documented by Yildiz et al.¹ Absorbance measurements were recorded on a Varian Cary 50 Spectrometer using 10 mm quartz cuvettes. The hydrodynamic diameter of the nanoparticles was determined on a Malvern Nano-ZS zetasizer.
Preparation of Carbon Quantum Dots (CQDs): ODE (15 mL) and HDA (1.5 g) were placed into a 100 mL three-neck flask and degassed with Argon for 10 minutes. The temperature was raised to 300ºC, and citric acid (1 g, 47.5 mmol) which had been dissolved in the minimum volume of ODE was quickly added into the solution under vigorous stirring. The contents were stirred at 300ºC for 2 h and then allowed to cool to room temperature. The resulting product was diluted to twenty times its volume with acetone and centrifuged at 5000 rpm for 5 min. The resulting pellet was re-suspended in CHCl₃ and centrifuged. This process was repeated twice more. The CQDs obtained were dispersed in chloroform or toluene.

Preparation of Aqueous Carbon Quantum Dots (aqCQDs): 1 (10 mg) was dissolved in CHCl₃ (4 mL) in an open vial and CQDs (280 µg) were added. The solution was heated at 40ºC until all of the solvent had evaporated. The residue was then dispersed in 4 ml of PBS at pH 7.4. The vial was shaken vigorously and the resulting solution was sterile filtered through a 0.1 µM filter. The aqCQDs were used directly without further purification.

Quantum Yield Determination: The quantum yield values for the CQDs and aqCQDs were determined by reference to Rhodamine 6G using a literature procedure.² Both samples were fixed at the same optical density and the quantum yield was determined by Equation 1:

\[
QY_X = QY_S \times \frac{A_X}{A_S} \times \left(\frac{n_X}{n_S}\right)^2
\]

where \(QY_X\) is the quantum yield of the sample, \(QY_S\) is the quantum yield of the standard (Rhodamine 6G; 0.95 in EtOH), \(A_X\) and \(A_S\) are the integrated area of the emission peaks for the sample and standard, respectively, and \(n_X\) and \(n_S\) are the refractive indices of the solvents used to dissolve the sample and standard, respectively.
**Intracellular Imaging Experiments**: supplemented with fetal bovine serum (10%, v/v), penicillin (200 U mL⁻¹), streptomycin (200 μg/mL⁻¹), and glutamine (2 mM), and incubated overnight at 37 °C in O₂/CO₂/air (20:5:75, v/v/v). The cultured cells were incubated further with media/PBS dispersion (90:10, v/v) of the aqCQDs (70μg/ml) for 24 h on glass coverslips. After being washed three times with PBS (1 mL), the coverslips were imaged with an inverted Leica SP5 confocal/multiphoton microscope. The samples were excited at 490 nm, and the emission was recorded from 500 to 560 nm.

**Cytotoxicity Experiments**: CHO cells were seeded in 96 well plates at a density of 50,000 cells per well, incubated for 24 h at 37°C in O₂/CO₂/air (20:5:75, v/v/v), then the cells were spiked with aqCQDs at concentrations of 0, 175 and 350 μg/mL. The cells were incubated in the dark for a 6 h, then all of the media was removed and replaced with fresh media. The cells were incubated in the dark for a further 24 h (20:5:75, v/v/v), then 20 μL 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) solution (5 mg mL⁻¹ in PBS) was added to each well, the plates were incubated at 37°C for 4 h, 185 μL media was removed from each well, 75 μL DMSO was added and the formazan crystals were dissolved by incubation at room temperature for 30 min on an orbital shaker. The absorbance of each well at 595 nm was measured using a Flex Station plate reader. The mean absorbance value of the blank control wells (media containing no cells) was subtracted from the absorbance value of the test wells. Cell viability was calculated by expressing the adjusted absorbance values of cells treated with 175 and 350 μg/mL aqCQDs as a percentage of the mean ± SD adjusted absorbance value of cells treated with 0 μg/mL aqCQDs from triplicate experiments (n = 3 per experiment).
**Figure S1:** Photograph of CQDs (left vial) and aqCQDs (right vial) both at the same concentration (0.7mg/mL), the former in toluene and the latter in PBS buffer. ($\lambda_{EX} = 365$ nm).
Figure S2: Emission Spectra for aqCQDs recorded at pH 1, 3, 7 and 12. ($\lambda_{\text{EX}} = 350$ nm).
**Figure S3:** Emission Spectra for aqCQDs recorded over a one month period. ($\lambda_{EX} = 350$ nm).
Figure S4: Dynamic light scattering plots for CQDs (top, recorded in toluene) and aqCQDs (bottom, recorded in water).
**Figure S5:** Line graphs illustrating the evolution of emission intensity for a line drawn across CHO cells. The plots reveal high intensity at the periphery of the cell with reduced intensity in the central region indicative of cytosolic localisation.
Figure S6: Confocal image of CHO cells with no aqCQDs added.

References