

## **Homogenous, Far-reaching Tuning and Highly Emissive QDs-Silica Core-Shell Nanocomposite Synthesized via a Delay Photoactive Procedure; Their Applications in Two-Photon Imaging of Human Mesenchymal Stem Cells**

### **Supporting Information**

*Chih-Wei Lai, Yu-Hsiu Wang, Yu-Chun Chen, Cheng-Chih Hsieh, Borade Prajakta Uttam, Jong-Kai Hsiao,\* Cheng-Chih Hsu and Pi-Tai Chou\**

### **Experimental Section:**

#### ***Synthesis of QDs (CdSe/ZnS, CdTe/CdSe/ZnS):***

According to our previous synthetic protocol, a series of different sizes of CdSe quantum dots with diameters of range from 3.5 to 5.1 nm were prepared.<sup>[1]</sup> Briefly, a Se injection solution containing Se (1 mmol) was prepared in a glovebox by dissolving Se powder in 0.3 mL of tri-*n*-butylphosphine (TBP) and then diluted with 1.681 g di-*n*-octylamine. The Se injection solution was then brought out of the glovebox in a vial sealed with a rubber subseal. A mixture of CdO (0.1 mmol) and stearic acid (0.40 mmol) was heated in a three-neck flask to 140 °C under Ar flow. After CdO was completely dissolved, the mixture was allowed to cool to room temperature. 1.94 g of the tri-*n*-octylphosphine oxide (TOPO) and hexadecylamine was then added to the flask, and the mixture was heated to 320 °C. At this temperature, the Se injection solution was quickly injected into the hot solution. The reaction mixture was allowed to cool to 290 °C for the growth of the CdSe nanocrystals. Various sizes of CdSe QDs were obtained by the time required to terminate the reaction upon cooling. CdSe QDs were precipitated out from the growth solution by adding methanol. To prepare the ZnS shell, zinc stearate and sulfur powder were used as precursors.<sup>[1]</sup> In a typical protocol to obtain the CdSe/ZnS core-shell nanoparticles, the precipitated CdSe QDs (0.020 g) were dispersed in TOPO (2.20 g) and HDA (1.26 g) before being heated to 200 °C. In addition, zinc stearate (0.316 g) was dissolved in TBP (3 mL) by

gentle heating (ca. 80°C). After cooling to room temperature, the resulting 0.2 M solution was mixed with sulfur (3 mL, 0.2M) in TBP. This mixture was then injected with a syringe pump within 1.5 hr into the reaction flask containing the core nanocrystals at 190 °C. After the addition was completed, the crystals were annealed at 190°C for an additional 1-1.5 hr. Core-shell nanoparticles of various sizes were obtained by adjusting the concentrations of zinc stearate and sulfur in TBP as well as the corresponding injection periods. The prepared CdSe/ZnS QDs were further purified by centrifugation and were reprecipitated twice from methanol. The CdTe core from CdO was prepared according to a method previously reported by Chen *et. al.*<sup>2</sup> To obtain the CdTe/CdSe core/shell nanoparticles, the precipitated CdTe QDs (0.020 g) were dispersed in TOPO (2.20 g) and HDA (1.26 g) before being heated to 190 °C. In addition, CdCl<sub>2</sub> (0.092 g) was dissolved in 2.5 mL of TBP upon gentle heating (ca. 80 °C). After being cooled to room temperature, the resulting 0.2 M solution was mixed with 2.5 mL of a 0.2 M solution of Se in TBP. With a syringe pump, this mixture was injected within 1hr into the reaction flask containing the core nanocrystals at 200 °C. After the addition was completed, the crystals were annealed at 200 °C for an additional 1–1.5 hr. Core/shell nanoparticles of various sizes were obtained by adjusting the concentrations of CdCl<sub>2</sub> and Se in TBP as well as the corresponding injection periods. The prepared CdTe/CdSe QDs were further purified by centrifugation and twice re-precipitation from methanol. The ZnS shell coating on CdTe/CdSe was similar to the method described earlier for ZnS shell onto CdSe. Finally, QDs included CdSe/ZnS and CdTe/CdSe/ZnS was precipitated and prepared for phase transfer.

### ***Instrument information:***

An integrating sphere (Laboratory sphere) was applied to measure the quantum yield in the QDs-Cy and QDs-Cy/SiO<sub>2</sub> DI water solution, respectively, for which the solution was measured after extraction and centrifugation procedures and excited by an argon ion laser at

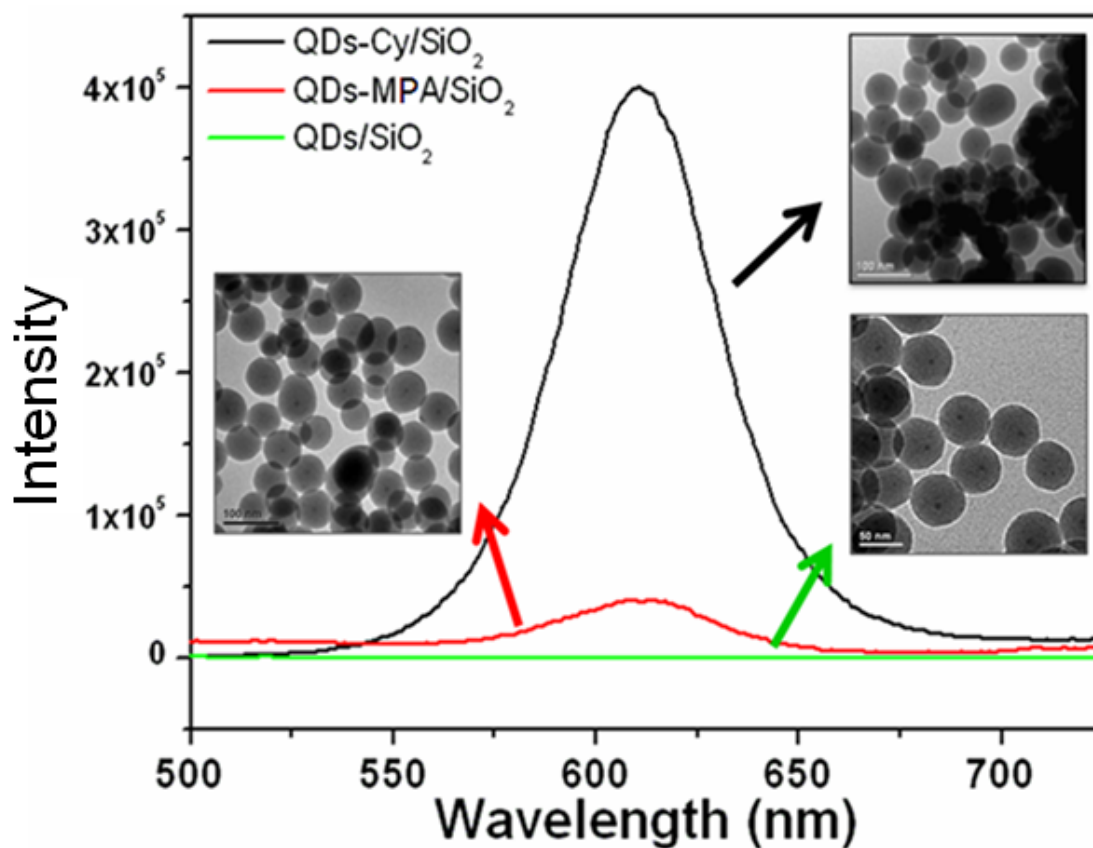
363 nm. The resulting luminescence was acquired with an intensified charge-coupled detector (ICCD) for subsequent quantum yield analyses according to a reported method.<sup>3</sup> The analysis of QDs-Cy/SiO<sub>2</sub> concentration was conducted using ICP-MS (Agilent 7500ce). The as-prepared nanoparticles were characterized with a Hitachi H-7100 transmission electron microscope, powder X-ray diffractometer (model PANalytical X'Pert PRO), and high-resolution transmission electron microscope (FEI Tecnai G2) including EDX spectroscopy and a CCD camera with Diff pack program. Absorption and emission spectra were measured by Hitachi (U-3310) spectrophotometer and Edinburgh (FS920) fluorimeter. A traveling-wave optical parametric amplifier of white-light continuum (TOPAS-C, Spectra Physics) was used to produce the NIR femtosecond laser source. TOPAS-C was driven by a mode-locked Ti:sapphire laser (Tsunami, Spectra Physics) coupled to a regenerative amplifier that generated a ~120 fs, 1 mJ pulse (800 nm, 1 kHz, Spitfire Pro, Spectra Physics). The output of TOPAS-C produced NIR signal beam (1150~1560 nm, 1kHz, 80 μJ) was used as the pumping source for two photon emission measurement. An NIR interference filter (1200 nm, FWHM 10 nm, T: 40%) was used to eliminate the idler pulse and some weak non-phase matched sum-frequency and second harmonic of the fundamental pulse. The IR images were captured by a handheld infrared camera from Eletrophysics (ElectroViewer 7215) and recorded by a black and white CCD camera from Sampo (VK-B120). For *in vitro* two photon imaging, the cell samples examined by Leica TCS SP2 Confocal Spectral Microscope & MP Two-Photon Imaging System with a X40 water immersion objective, using 800 nm two-photon diode laser and 543 nm He-Ne laser as excitation source.

***Calculation of QD/cell:***

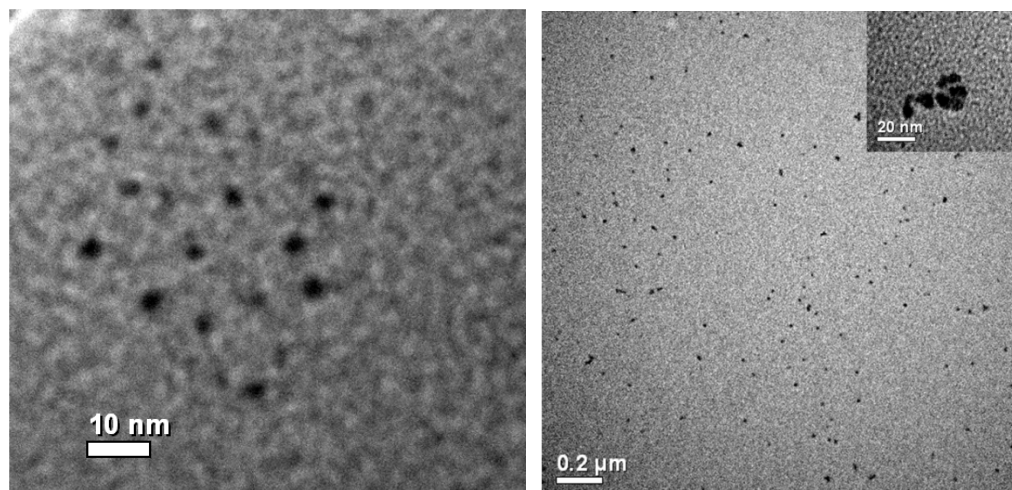
The number of QD in samples was calculated using the equation

$$D = M / V \dots \text{eq(1)}$$

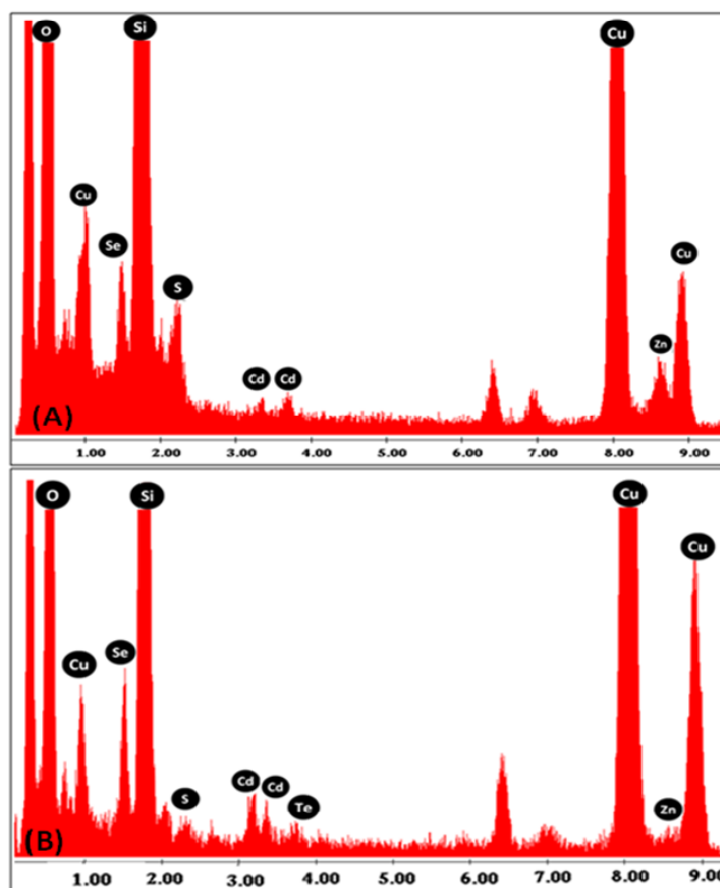
Where  $D$  is the density, which is  $5.816 \text{ g/cm}^3$  for CdSe with core diameter of  $3.5 \text{ nm}$ <sup>[4]</sup>;  $M$  is the mass in gram; and  $V$  is the volume of spherical QDs.



**Figure S1.** The emission spectra of silica coated QDs-Cy, QDs-MPA and QDs, without phase-transfer, under identical treatment of 6 hr illumination under oxygen atmosphere. TEM images are provided to illustrate the shape of each nanoparticles.

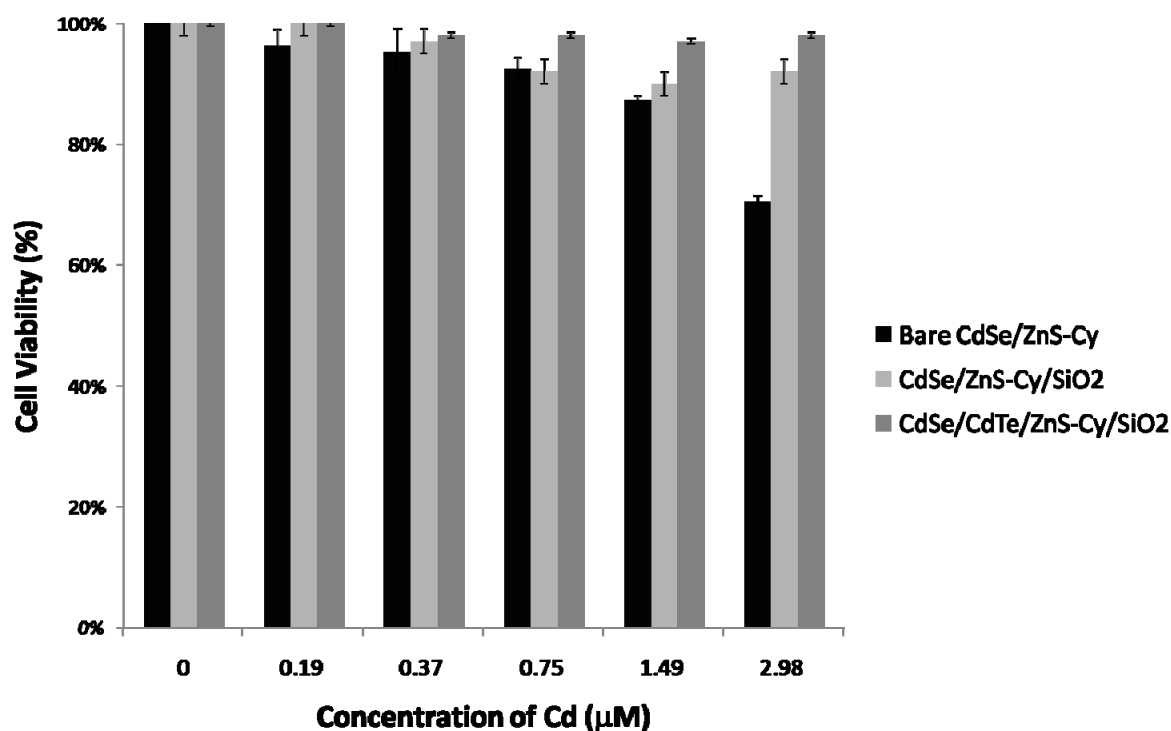


**Figure S2.** Left: TEM image of CdSe/ZnS-Cy with 3.5nm in diameter. Right: TEM image of CdTe/CdSe/ZnS-Cy with 7.1nm in diameter.



**Figure S3.** The QDs-Cy/SiO<sub>2</sub> EDX spectra of (A) CdSe/ZnS-Cy/SiO<sub>2</sub> and (B) CdTe/CdSe/ZnS-Cy/SiO<sub>2</sub> nanocomposite, respectively. Atomic percentage ratios (in parentheses) of O(K $\alpha$ : 0.523 eV), Cu(K $\alpha$ : 8.040 eV, L $\alpha$ : 0.930 eV, copper mesh), Cd(L $\alpha$ : 3.133 eV), Se(L $\alpha$ : 1.379 eV), Zn(K $\alpha$ : 8.637), S(K $\alpha$ : 2.307 eV), Te(L $\alpha$ : 3.759 eV in S3B), and Si(K $\alpha$ : 1.740 eV)

## Viability of hMSC treating with QDs



**Figure S4.** Viabilities of human mesenchymal stem cell (hMSC) treated with different QDs: Bare CdSe/ZnS-Cy ( $E_m$ :530nm, black bar), CdSe/ZnS-Cy/SiO<sub>2</sub> ( $E_m$ :530nm, light gery), and CdSe/ZnS-Cy/SiO<sub>2</sub> ( $E_m$ :780nm, dark gery). Error bar represents the standard error of 3 replicas.

### References:

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