Electronic Supplementary Information (ESI)

Highly Fluorescent Quantum Dot@Silica Nanoparticles by a Novel Post-treatment for Live Cell Imaging

Xiebing Wang,[a] Wanwan Li,*[a] Bingxia Zhao,[a] Delong Zhang,[a] Kang Sun,*[a] Xiao An,[b] Zhaofeng Zhang,[b] Zunli Shen[b]

[a] *State Key Lab of Metal Matrix Composites, Shanghai Jiao Tong University, 800 Dongchuan Road, Shanghai, 200240, P. R. China.*
[b] *The First People's Hospital Affiliated to Shanghai Jiao Tong University School of Medicine, 100 Haining Road, Shanghai 200080, P. R. China*
** Email: wwli@sjtu.edu.cn (Wanwan Li) ; ksun@sjtu.edu.cn (Kang Sun)*

Experimental Section

Materials:

Tetraethylorthosilicate (TEOS, 99%), poly(5)oxyethylene-4-nonylphenyl-ether (NP-5), mono-methylamine (40 wt.% in H_2O), ultra-pure TEOS (99.999 %), Phosphate buffered saline (PBS, pH~7.2 at 25 °C) were purchased from Sigma Aldrich. Ammonium hydroxide (NH_4OH, 28-30 %), dimethylamine (40 wt.% in H_2O, analytical reagent), cyclohexane (analytical reagent), *n*-hexane (analytical reagent), acetone (analytical reagent), *n*-butanol (analytical reagent) and ethanol (analytical reagent) were purchased from Sinopharm Chemical Reagent Co., Ltd. All chemicals were used as received without further purification.

Synthesis of QD@SiO_2:

The CdSe/CdS/ZnS core/multi-shell QDs (CSS QDs) were prepared according to the reference.[1] SiO_2 coating was performed through the formation of a water-in-cyclohexane reverse microemulsion. Typically, 0.1 mL TEOS and 10 mL QDs (1 mM) cyclohexane solution were mixed and stirred for 10 min. Then surfactant (1 mL) was consecutively added into above solution under stirring and stirred for thirty minutes until the microemulsion system was formed. Then bases (0.1 mL) were introduced to initiate the polymerization. After stirring for 24 h at room temperature, the silica growth completed. Then the nanoparticles were collected by centrifugation, washed three times by ethanol and water and finally redispersed in ethanol. The fluorescence data were acquired by RF–5301PC Shimadzu spectrofluorophotometer respectively at room temperature.

Post-treatment on QD@SiO_2

Firstly,annealing treatment was taken by loading the purified QD@SiO_2 solution (redispersed in *n*-butanol) in Teflon-lined stainless autoclave and heated at designated temperature for a certain period of time under stirring. Then the products were further purified and redispersed in ethanol. And then, the photoactivation process was carried out by irradiating the QD@SiO_2 NPs suspension under an 8W 365nm UV light source for a designated period of time. The fluorescence data were acquired by RF–5301PC Shimadzu spectrofluorophotometer respectively at different time intervals.

Characterization:

The quantum yield was determined as follows: QD@SiO_2 was dispersed in ethanol/toluene mixture to match refractive index, thus minimizing effect from light scattering by silica spheres. An appropriate reference dye in methanol was chosen with closely matched optical densities (<0.05) of QD@SiO_2 at excitation wavelength. Both photoluminescence spectra were acquired by a RF–5301PC Shimadzu spectrofluorophotometer. The quantum yield of the sample was determined by comparison of their corresponding integrated emission. Transmission electron microscopy (TEM) images were recorded on a JEOL-JEM 2100 electron microscope operating at an accelerating voltage of 200 kV. High-resolution TEM (HRTEM) images were obtained on a JEM-2100F transmission electron microscope. X-Ray diffraction spectra were recorded with a BRUKER-AXS X-ray diffractometer. Fluorescent decay curves were obtained by an FLS-920 Combined Steady State and Phosphorescence Lifetime Fluorimeter (Edinburgh Instruments). The NPs after post-treatment was subjected to a stability test in physiological environment by immersing the NPs in PBS (pH ~7.2) for different periods of time ranging from 0 to 14 days. The aforementioned pH values were chosen with respect to cytosol environment, respectively. Additionally, another NPs was immersed in DI water for the same period of time.

Cell Viability Assay

Colorimetric 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) assays were performed to assess the metabolic activity of cells in the presence of NPs. The assay was carried out in triplicate in the following manner. For MTT assay, NIH/3T3 or Hela cells were seeded into 96-well plates at the density of 3000~10000 cells per well in 200 μL of media and grown overnight. The cells were then incubated with various concentrations of CSS QD@SiO_2 NPs for 24h at 37°C under the humidified atmosphere (5% CO_2) in dark. Then the medium containing QDs were replaced by fresh culture medium. The cells were incubated for another 24h time. Afterwards, cells were incubated in media containing 0.5 mg mL^{-1} MTT for 4 h at 37 °C. The medium were removed and 150 μL DMSO was added to each well. The cells were then incubated for 0.5 h at 37 °C. Absorbance was measured at 450 nm by POLARstar OPTIMA Multidetection Microplate Reader (BMG LABTECH).

Hela Cell Labeling and Fluorescent Imaging

Hela cells were propagated in Roswell Park Memorial Institute's medium (RMPI1640) supplemented with FBS (10%) and penicillin/streptomycin (1%). Then the cultured cells were trypsinized and resuspended in the RMPI1640 medium at a concentration of about 7.5 × 105 mL^{-1}. The cell suspension (200 μL) was transferred to each Petri dish (35 mm). After 24 h of incubation, the cells were carefully rinsed with PBS (0.01 mol L^{-1}, pH7.4). For each Petri dish, RMPI1640 medium (without fetal blood serum and FAs, 200 μL) and the CSS QD@SiO_2 (20 μL, 0.1 mg mL^{-1}) were added and incubated for 24 h. The labeled cells were carefully rinsed with PBS to remove the unbonded NPs, and the fresh serum-free medium (200 μL) was added to the Petri dishes. The fluorescent imaging of the Hela cells was performed on an IX71 inverted fluorescence microscope (Olympus).

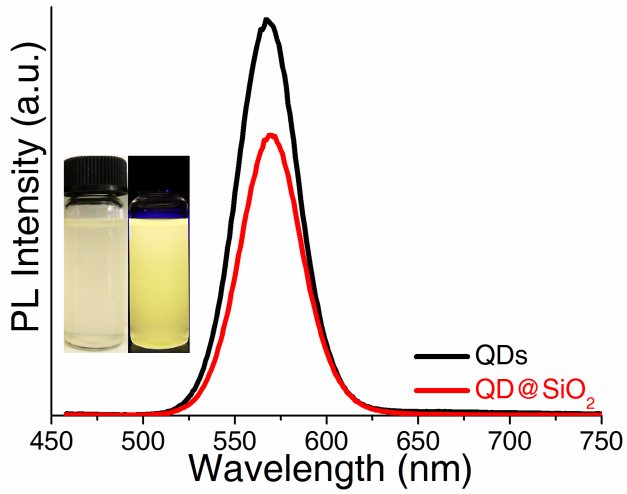


Figure. S1 Representative PL spectra of CSS QDs and corresponding QD@SiO_2 NPs. The inset shows the photographs of QD@SiO_2 NPs suspension under natural light (left) and excited with 365 nm UV light (right).

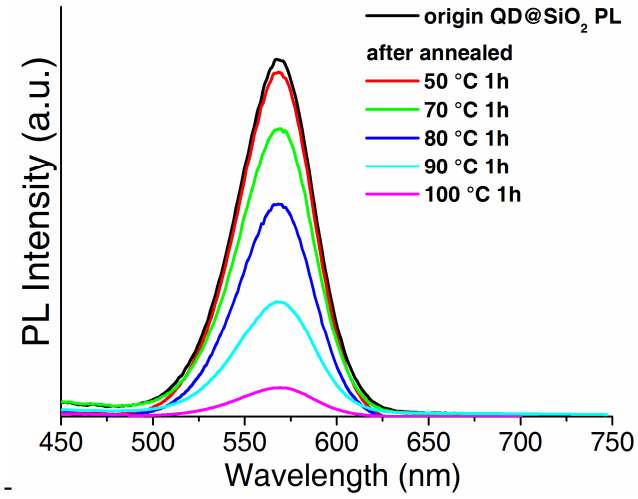


Figure. S2 Evolution of PL spectra of CSS QD@SiO_2 NPs before and after different annealing temperatures.

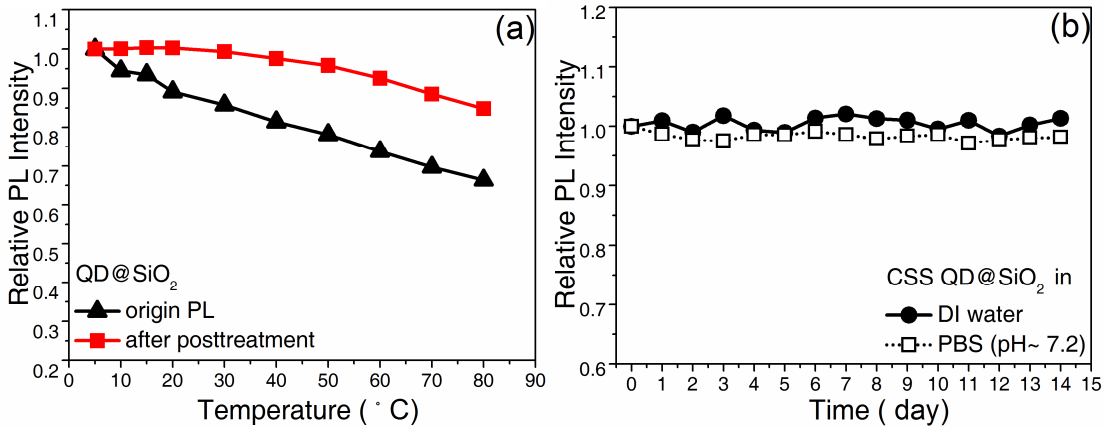


Figure. S3 (a)The temperature stability of the QD@SiO_2 NPs (dispersed in PBS solution) before and after post-treatment. (b) the stability of the QD@SiO_2 NPs after post-treatment when dispersed in DI water (solid circles with solid line) and PBS solution (open square with dash line) versus time.

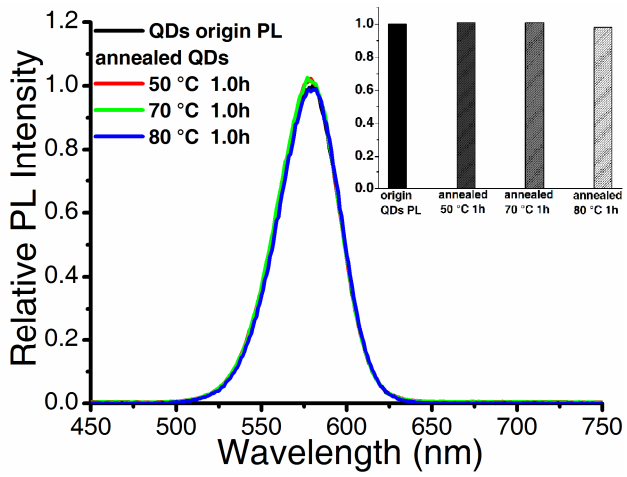


Figure. S4 Evolution of the PL spectra of CSS QDs NPs before and after different annealing temperatures. The inset is the corresponding relative intensity.

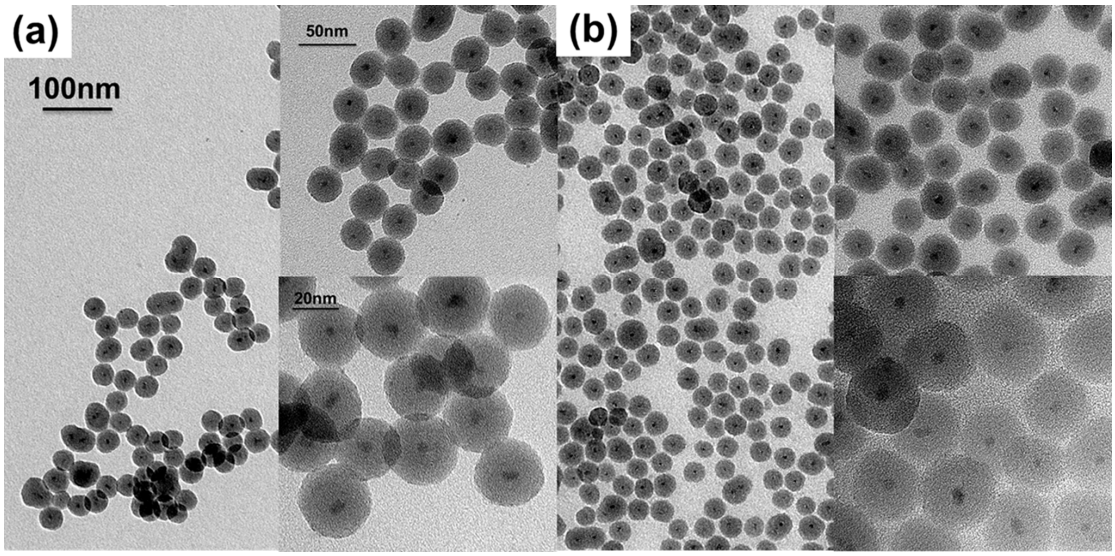


Figure. S5 TEM and HRTEM images of QD@SiO_2 NPs before (a) and after post-treatment (b).

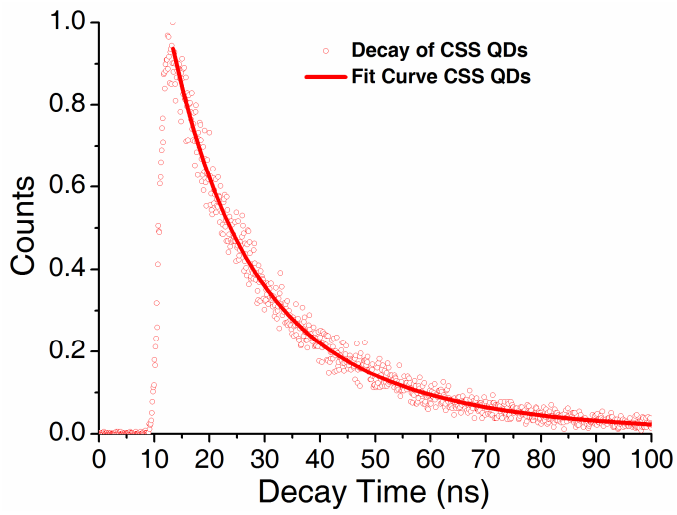
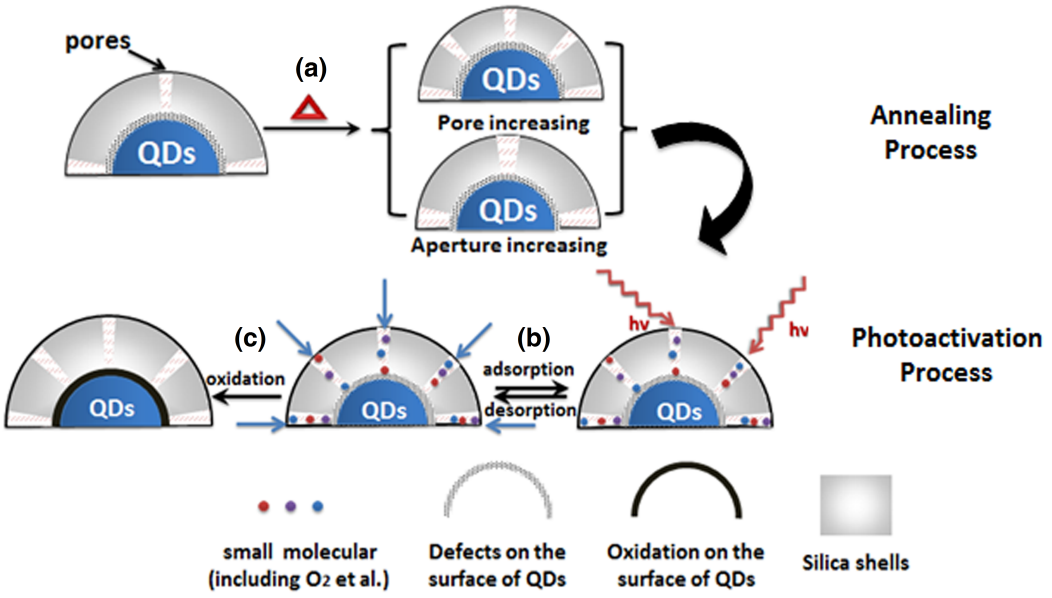


Figure. S6 Fluorescence decay curve of CdSe/CdS/ZnS QDs($\bar{\tau}$ = 18ns).

Scheme. S1 Schematic representation of possible mechanism proposed for PL enhancement of CSS QD@SiO_2 NPs. There is a reversible dynamic process in the solution that small molecules (including O_2) adsorbed onto the surface of QDs through the silica matrix under UV irradiation and desorbed from surface of QDs if the irradiation was terminated[2]. (a) During annealing process, the porosity of the silica shells of CSS QD@SiO_2 NPs increased, resulted in more pores or larger aperture inside the silica. (b) Thus, more small molecules in solvent could connect onto the surfaces of QDs. (c) By the followed continuous UV photoactivation, the surfaces of QDs could be smoothed by forming a very thin oxidation layer and a subsequent transformation of the surface. As a consequence, the surface defects of the QDs were steadily shielded and the PL intensity of the CSS QD@SiO_2 NPs was irreversibly enhanced.



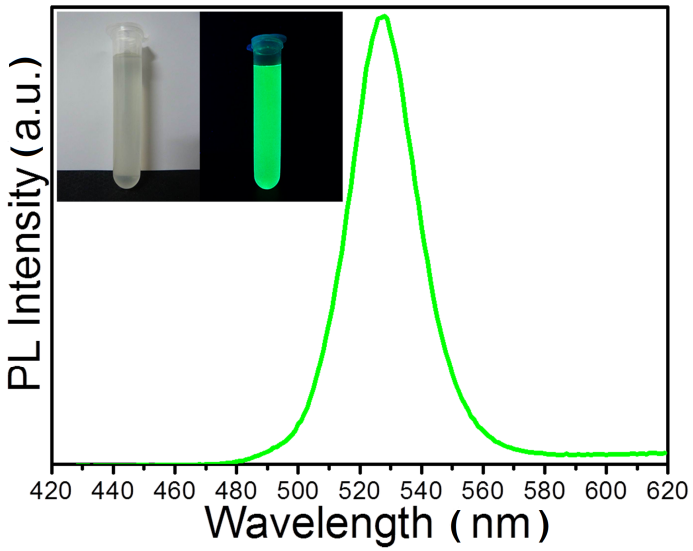


Figure. S7 Representative PL sprectra of a 527 nm green-emitting CSS QD@silica NPs used for Hela cell uptaken. The inset showed the photographs of QD@SiO_2 NPs suspension under natural light (left) and excited with 365 nm UV light (right).

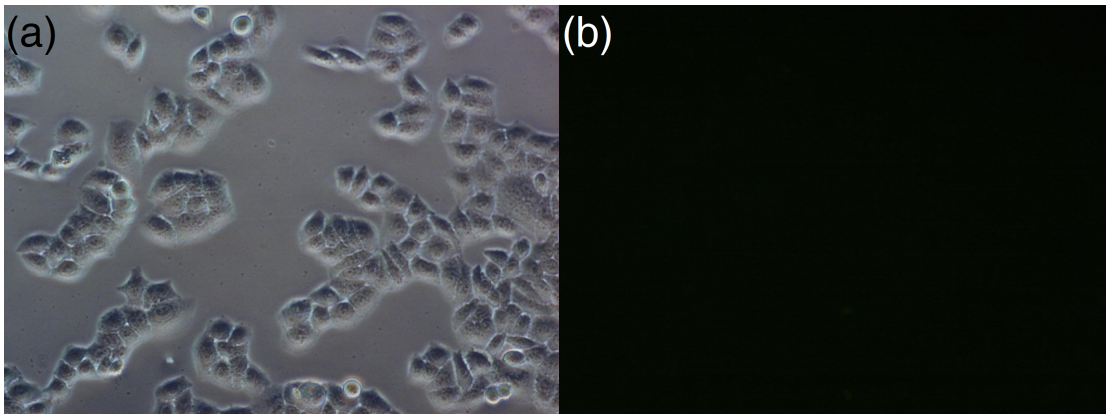


Figure. S8 (a) Bright field and (b) fluorescent images of Hela cells incubated for 24 h without adding CSS QD@SiO_2 NPs (control).

References

[1] Xiebing Wang; Wanwan Li; Kang Sun. *J. Mater. Chem.* 2011, **21,** 8558.

[2] Sofia Dembski, Christina Graf, Tim Krüger, Uwe Gbureck, Andrea Ewald, Anne Bock, Eckart Rühl. *Small* 2008, **4**, 1516.