Supporting Information

Bright, Non-blinking, and Less-cytotoxic SiO_2 Beads with Multiple CdSe/ZnS Nanocrystals

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1. Experimental section

As explained using Scheme S1, the preparation of the beads consists of three steps: silanization of the nanocrystals (NCs) (Step 1), phase transfer and assembly of the silanized NCs to form seeds (Step 2), and the growth of a SiO_2 shell to form beads (Step 3). The beads, unless indicated otherwise, were prepared at room temperature.

In Step 1, tetraethyl orthosilicate (TEOS) was added to toluene solution of CdSe/ZnS NCs, which was then stirred for 3–5 h to obtain precursor solution A. Precursor solution B was obtained by mixing 3-mercaptopropyltrimethoxysilane (MPS) with ethanol, H_2O, and ammonia with stirring. Typical molar ratios of MPS, ethanol, H_2O, and NH_3 were 1, 1.59×10^5, 7.23×10^4, and 2.05×10^3.

In Step 2, precursor solutions A and B were mixed, stirred for 3–4 h, and centrifuged to separate the seeds. The seeds thus prepared were re-dispersed in pure water.

In Step 3, the re-dispersed seeds were condensed by means of a 3000–MWCO filter to adjust the NC concentration. TEOS was then added drop by drop. Typically, the molar ratios of NCs, TEOS, ethanol, H_2O, and NH_3 were 1, 2.39×10^5, 7.33×10^8, 1.49×10^8, and 7.86×10^6. After a reaction time of 3–4 h, SiO_2 beads with encapsulated CdSe/ZnS NCs were separated from the solution by centrifuging. The beads were re-dispersed in H_2O for further characterization.

Carboxyl surface modification of the beads was performed by using carboxyethylsilanetriol sodium (CES). Because CES quickly nucleates in ethanol with ammonia and H_2O compared with TEOS, we investigated three approaches to identify the effect of CES on bead formation: (1) CES was directly added after Step 3, (2) CES was mixed initially with TEOS and then gradually added during Step 3, and (3) CES was first mixed with TEOS and hydrolyzed. Typically, the molar ratios of H_2O, TEOS, and CES were 1.0, 3.8, and 0.2. The pre-hydrolyzed TEOS and CES were added during Step 3. The preparation conditions for Beads A1 to A7 are listed in Table S1. The results are shown in Fig. S7. Elemental analysis of the luminescent SiO_2 beads was performed using an inductively coupled plasma atomic emission spectrometer (IR1S Advantage, Nippon Jarrell-Ash Co. Ltd.). The absorption and PL spectra were measured using conventional spectrometers (Hitachi U-4000 and F-4500). The PL efficiencies of the emitting beads and QDs in solution were estimated using the method described by us.[1] Briefly, the PL and absorption spectra of the standard quinine solution (quinine in 0.1-N H_2SO_4 solution, PL efficiency η_0 of 55%) were measured in a 1-cm quartz cell as a function of its concentration. Emission intensity P_0 (in units of the number of photons) is expressed using efficiency η_0: $P_0 \approx K \eta_0 a_0 10^{-0.5 a_0}$, where a_0 is absorbance at the excitation wavelength (365 nm), and K is the apparatus function. After the measurement of absorbance a and PL intensity P of the sample using the same apparatus parameters, the PL efficiency η of the sample was derived by comparing with PL intensity P_0 of the quinine solution. The wavelength dependence of the sensitivity of spectrometer was corrected according to the procedure indicated by the producer. The error in the PL efficiency is estimated to be within 10% by comparing the results using two standards, namely quinine and R6G.

The ability to confine Cd^{2+} in the surrounding material (SiO_2 or polymer) was investigated for our beads, commercial Q-dots (CdSe/ZnS QDs with a polymer coating, ~20 nm in diameter), and Q-tracker (for bio-labeling). The beads, Q-dots, and Q-tracker were re-dispersed in HEPES buffer (30 mM N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid, 30 mM KCl, 1 mM EGTA, 2 mM $MgCl_2$, and 10 mM dithiothreitol, pH ~7.4) and in HEPES buffer with 137 mM of NaCl with an NC

concentration of 20 nM. After re-dispersion of 15 h, the solutions were filtered by means of a 3000-MWCO filter to remove the beads, Q-dots, or Q-tracker. The amount of free Cd^{2+} in the solution was then determined by means of an inductively coupled plasma mass spectrometer (Finnigan Element2, Thermo Fisher Scientific).

Transmission electron microscopic (TEM) observation (Figs. 2, 3, S3, S5, S6, S7 and S9) was carried out using Hitachi H-9000NA (300 kV) and Topcon 002B (200 kV) electron microscopes. To obtain 3D images by electron tomography (*Video S1*), serial tilt images (from -64° to +64° with 1° increments) were recorded with a high-angle annular dark-field scanning transmission electron microscope (HADDF-STEM) FEI Tecnai G2 F20 (200 kV). Tomograms were aligned and reconstructed using FEI Inspect3D and visualized using Mercury Computer Systems Avizo 5.

The NCs and beads were imaged using a fluorescence microscope based on an inverted microscope (IX71, Olympus) via a 100×1.4 NA oil immersion objective lens. Light from a mercury lamp was used for excitation after band pass filtering (WIG, BP 530-550 nm, Olympus). The fluorescence signal was collected by the same objective and imaged by an electron-multiplying charge coupled device (EM-CCD) camera with 512 × 512 pixels (PhotonMAX, Princeton Instruments Inc.) after passing through a dichroic mirror and filters removing the excitation light. Movies (*Video S2*) were recorded at a rate of 5 frames per second over 300 frames and analyzed using a home-made program.

2. Discussion on cytotoxicity

In the case of Cd-based NCs, cytotoxicity can be a problem due to the release of highly toxic free Cd^{2+} ions. Therefore, the cytotoxicity of CdSe and CdSe/ZnS NCs has been investigated for different surface modifications such as coating with various ligands, silanization, and polymer coating.

The free Cd^{2+} concentration in a 0.25 mg/mL (at a Cd concentration of ~1300 μM) solution of mercaptoacetic acid (MAA)-capped CdSe NCs was investigated. The authors indicated that a free Cd^{2+} concentration of 6 ppm (~ 50 μM) released from non-oxidized MAA-capped CdSe NCs (which were kept in inert atmosphere before ligand exchange) is safe for hepatocyte while another free Cd^{2+}

concentration of 126 ppm (~1.05×10^3 μM) released from oxidized MAA-capped CdSe NCs (which were kept in air for 30 min before ligand exchange) resulted in hepatocyte death.[2,3] This indicates that the release of free Cd^{2+} ions from the NCs depended strongly on their surface modification.

Quarta and co-workers reported an intracellular Cd concentration of 300 nM (at a Cd concentration of 25 μM for CdSe/ZnS quantum rods in surrounding solution) resulted in cell viability of around 90% after an incubation of 24 h.[4] The SiO_2 shell on NCs is known to reduce the cytotoxicity of Cd-based NCs compared with a polymer coating because of the dense network structure. In current experiments, a NCs concentration of 20 nM (Cd concentration of ~2.5 μM in the NCs solution) was used to measure the concentration of released Cd^{2+} ions because this concentration was usually used for cell labeling.[5] The concentration of free Cd^{2+} ions released from carboxyl-coated SiO_2 beads in HEPES buffer with 137 mM of NaCl was 0.03 ppb (0.27 nM). Since this value is three orders of magnitude smaller than the value (300 nM) of Quarta *et al.,* the beads are substantially nontoxic for bioapplications.

References

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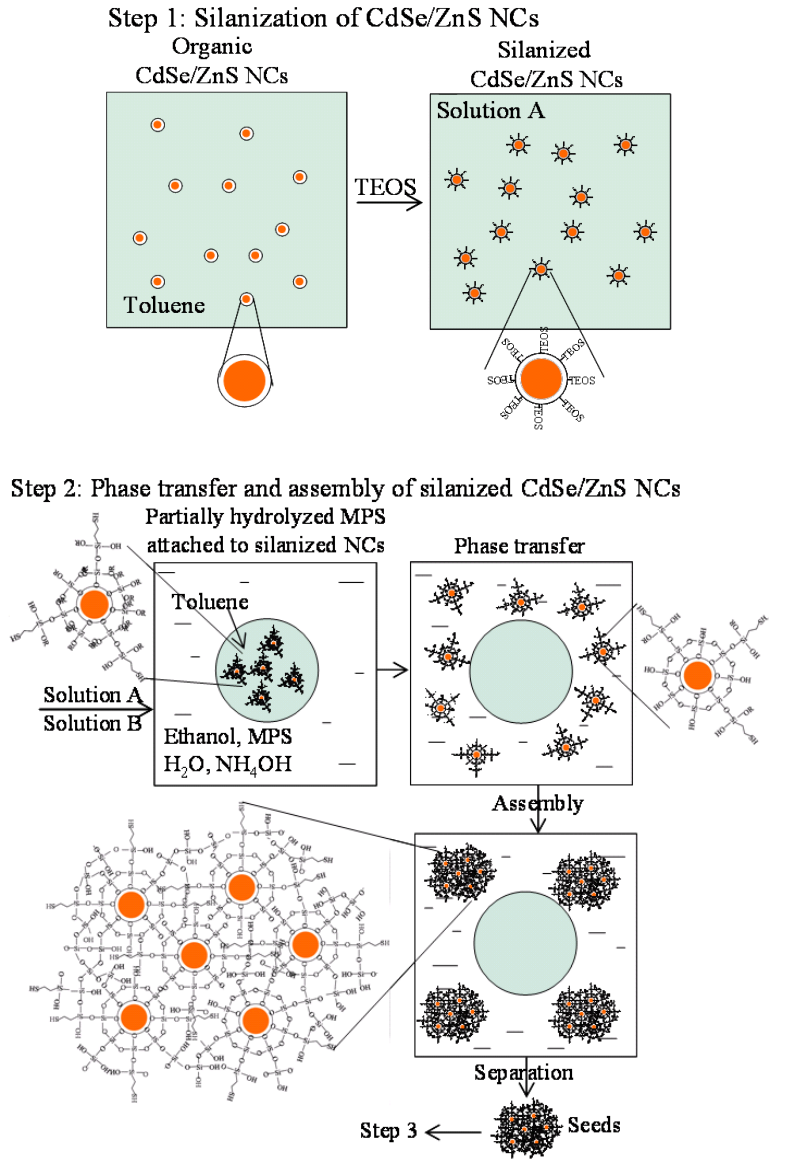
Table S1. Preparation conditions for Beads A1 to A7 in Step 3.

Bead	Molar ratio of TEOS/NCs	Molar ratio of CES/NCs	Type of seeds	Reaction time / h
A1	2.39×10^5	N/A	1	3.5
A2	2.39×10^5	N/A	4	3.5
A3	2.28×10^5	1.20×10^4	3	3.5
A4	2.28×10^5	1.20×10^4	3	3.5
A5	2.28×10^5	1.20×10^4	3	3.5
A6	2.39×10^5	N/A	4	3.5
A7	2.28×10^5	1.20×10^4	4	3.5



Scheme S1. Steps in assembling multiple CdSe/ZnS NCs into SiO_2 beads by phase transfer and seed growth. Solution A: silanized CdSe/ZnS NCs in toluene; Solution B: partially hydrolyzed MPS in solution of ethanol, H_2O, and NH_4OH.

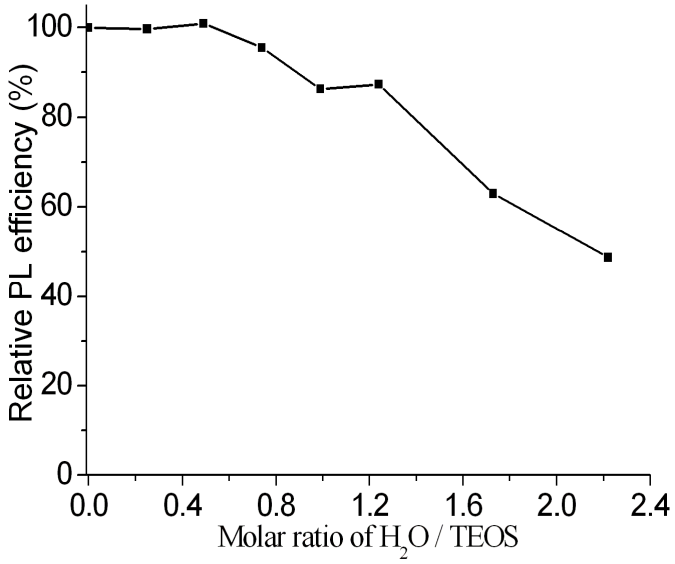


Fig. S1 Relative PL efficiency of CdSe/ZnS NCs in toluene with TEOS versus molar ratio of H_2O/TEOS. PL efficiencies were measured after mixing H_2O in toluene with TEOS and NCs for 3 h. Concentration of CdSe/ZnS NCs in solution was 25 nM, and molar ratio of TEOS/NCs was 4.5×10^5. When amount of H_2O was large, quick hydrolysis of TEOS resulted in irregular arrangement on surface of NCs. This led to a decrease in PL efficiency due to increase in surface defects.

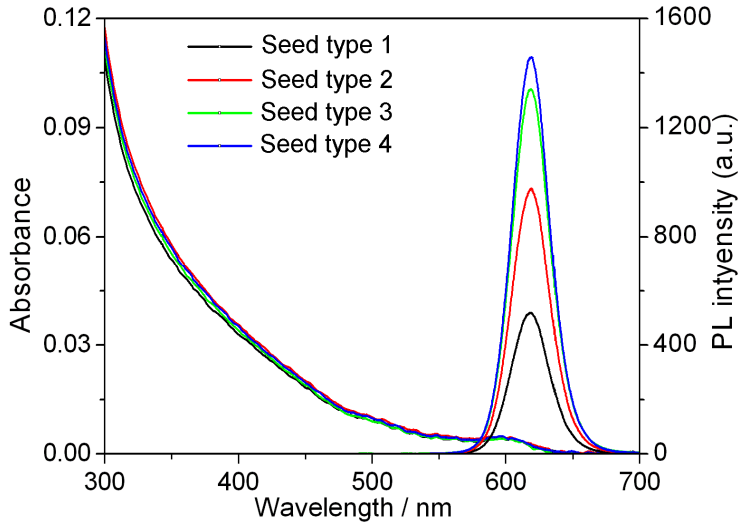


Fig. S2 Absorption and PL spectra of seeds for different MPS concentrations. Concentrations of MPS for seed types 1, 2, 3, and 4 during Step 2 were 0, 4.7×10^{-5}, 9.4×10^{-5}, and 1.9×10^{-4} M, respectively. PL efficiencies were 11, 25, 34, and 35%, respectively. PL spectra of seeds did not reveal any difference in FWHM or PL peak wavelength compared with those of initial CdSe/ZnS NCs.

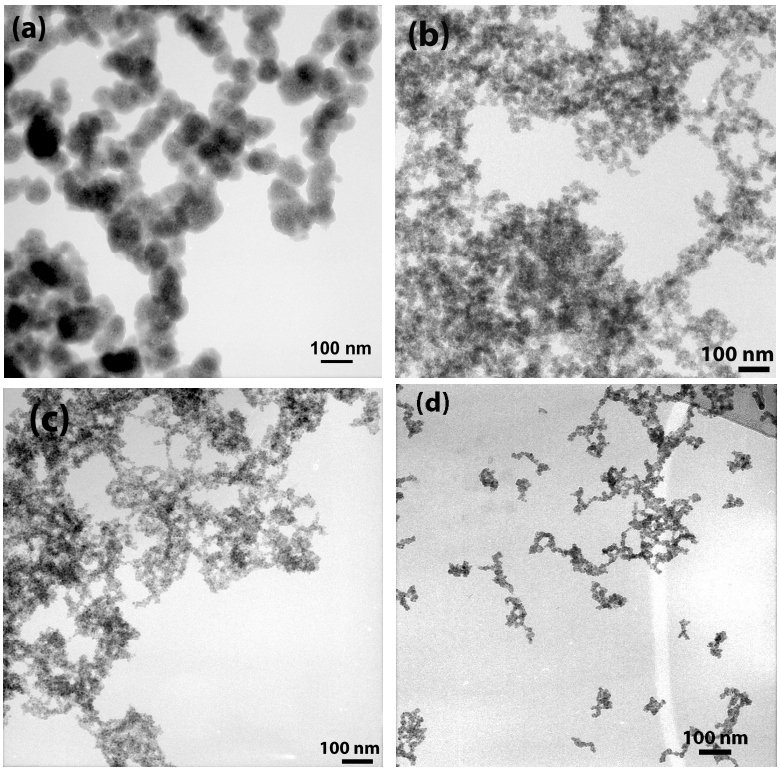


Fig. S3 TEM images of seeds prepared with different concentrations of MPS during Step 2: (a) seed type 1, (b) 2, (c) 3, and (d) 4. Preparation conditions were listed in Table S1. Results indicate that seed size can be controlled by changing concentration of MPS.

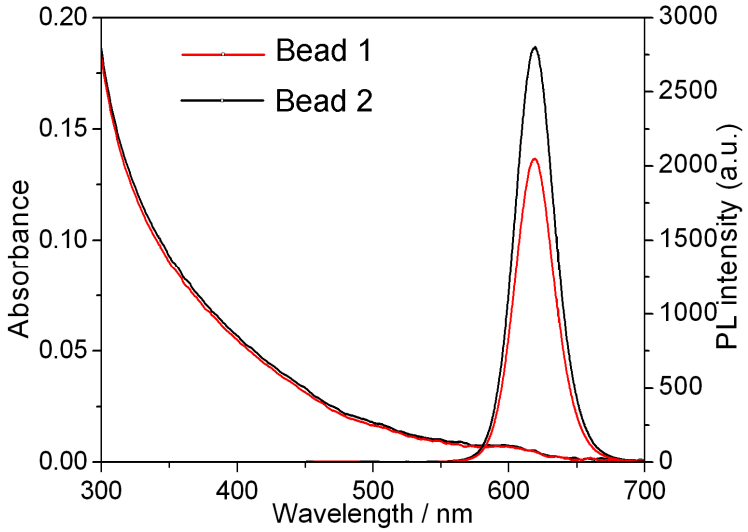


Fig. S4 Absorption and PL spectra of luminescent SiO_2 beads. No change was observed for PL peak wavelength and FWHM of PL spectra compared with those of initial CdSe/ZnS NCs.

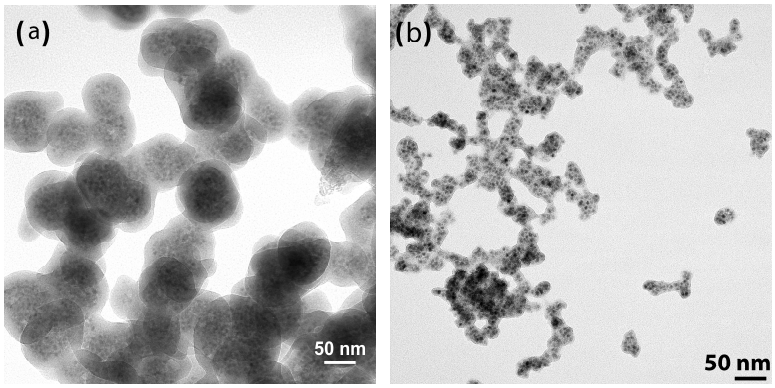


Fig. S5 TEM images of luminescent SiO_2 beads: (a) Bead A1 prepared using seed type 1; (b) Bead A2 prepared using seed type 4. High concentration of MPS for type 4 resulted in more mercapto groups on seed surfaces, making it difficult to deposit SiO_2 monomers on surface of Bead A2 during Step 3.

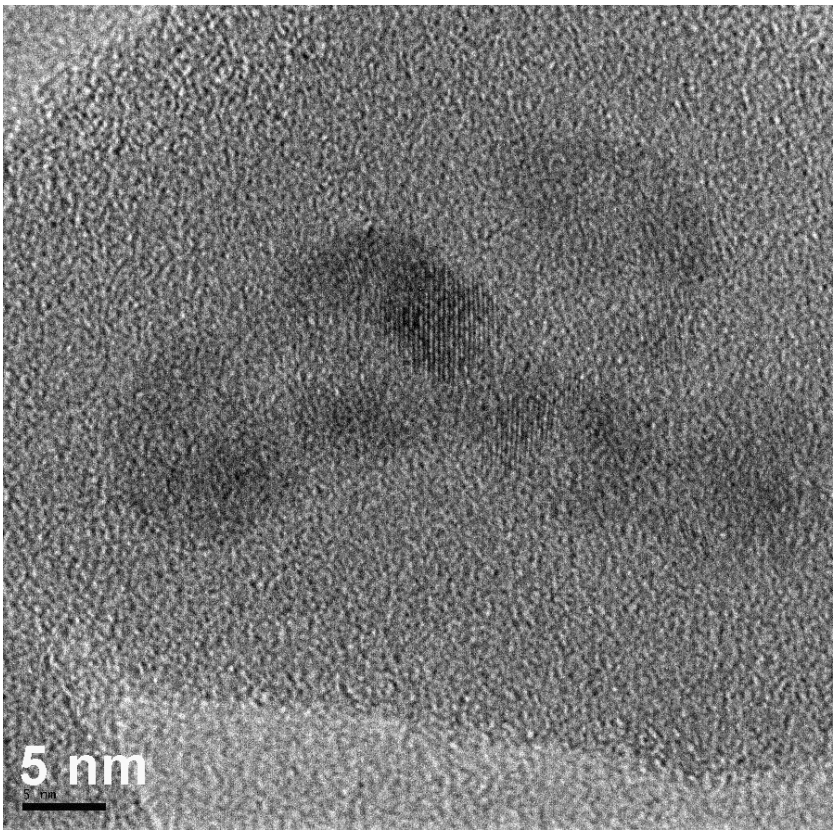


Fig. S6 TEM image of luminescent SiO_2 bead (Beads 4). Well-developed lattice fringes in the NCs were observed.

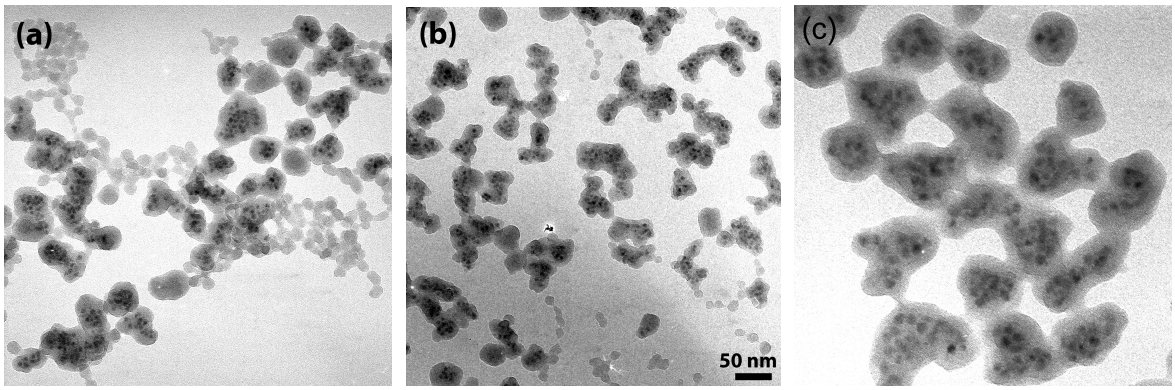


Fig. S7 Effect of CES on formation of luminescent SiO_2 beads prepared using seed type 3: (a) Bead A3: CES added directly after Step 3; (b) Bead A4: CES as-mixed with TEOS and gradually added during Step 3; (c) Bead A5: CES pre-hydrolyzed with TEOS for two days and gradually added during Step 3. Molar ratio of CES to TEOS was 5%. Except for the added orders of CES, other preparation parameters remain unchanged. Results indicate that pre-hydrolysis of CES and TEOS is necessary to obtain beads with narrow size distribution and to prevent formation of pure SiO_2 beads without NCs.

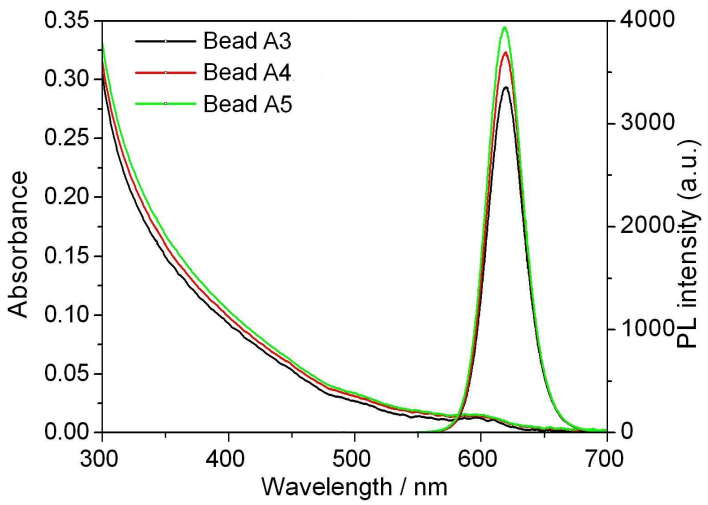


Fig. S8 Absorption and PL spectra of luminescent SiO_2 beads. PL efficiencies of Beads A3, A4, and A5 were 29, 27, and 29%, respectively, while initial value for NCs was 35%.

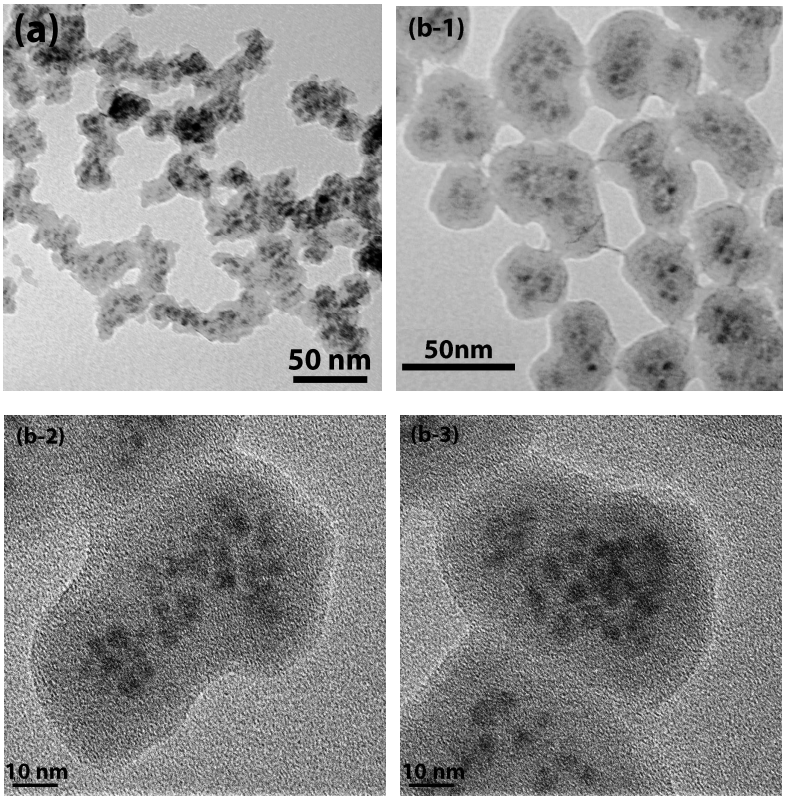


Fig. S9 TEM images of luminescent SiO_2 beads: (a), Bead A6: pure TEOS used during Step 3; (b-1) Bead A7: pre-hydrolyzed TEOS and CES used during Step 3. Molar ratio of CES to TEOS was 5%. CES affected condensation kinetics of SiO_2 monomers. Figs. (b-2) and (b-3) with higher resolution clearly show distribution of NCs in beads. Both Beads A6 and A7 were prepared from seed type 4. Results indicate that use of CES resulted in deposition of SiO_2 monomers even though there were many more mercapto groups on the surface.

3D_image-2.avi

Video S1. A representative 3D Image of a luminescent SiO_2 bead (Bead 4 shown in Fig. 3). Distribution of CdSe/ZnS NCs in the bead is clearly discerned as white dots. The average size of the dots is 5.5 nm. The SiO_2 matrix of the bead is shown as a slightly white part.

a.mpg

b.mpg

Video S2. a: Blinking by individual CdSe/ZnS NCs in initial toluene solution. b: Non-blinking by individual luminescent carboxyl-coated SiO_2 beads in water (Bead A7 shown in Fig. S7).