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Supporting Information

Synthesis of Gd doped CdSe nanoparticles for potential optical and MR imaging applications

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Experimental Section

Materials

We obtained trioctylphosphine oxide (TOPO, Technical grade, 90%), trioctylphosphine (TOP, Technical grade, 90%), selenium (Se, powder, -100 mesh, 99.5+%), gadolinium(III) Chloride, anhydrous ($GdCl_3$, 99.99%), mercaptopropionic acid (MPA, 99%), and *N*-(3-dimethylaminopropy)-*N*-ethyl-carbodiimide (EDC, commercial grade, >97%) from Aldrich, n-tetradecylphosphonic acid (TDPA, 98%) from Alfa Aescar, cadmium oxide (CdO, 99.999%-Cd) from Strem Chemicals, and DL-lysine (>95%) from Fluka.

Synthesis of CdSe:Gd nanoparticles

For a typically synthesis for Gd doped CdSe nanoparticles, a mixture with $GdCl_3$ (0.05 mmol), 0.57 g CdO (0.44 mmol), 0.1 g n-tetradecylphosphonic acid (TDPA) and 3.5 g of trioctylphosphine oxide (TOPO) was introduced to a 50 mL three-necked flask at 100 °C and degassed by Argon gas for one hour. We heated the mixture to 340°C under nitrogen, and the solution gradually turned from a dark red to a clear, colorless solution. The mixture then cooled to the desired injection temperature: 280°C. The chalcogen precursor (Se-TOP) was prepared by dissolving 0.5 mmol of Se powder in 1 mL of trioctylphosphine (TOP) and heating to 120°C. Then we quickly injected 0.8 mL of Se-TOP mixture into the Cd-mixture. Upon injection of

the Se-TOP at 280°C, the colorless mixture turned various colors with different growth times. Next, the solution was immediately cooled to room temperature. We purified the nanocrystals by precipitating the particles with excess methanol and discarding the supernatant after centrifugation. The synthetic procedure for pure CdSe nanoparticles was the same as that of Gd doped CdSe nanoparticles except for the $GdCl_3$ addition.

Preparation of the water soluble CdSe:Gd nanoparticles

The CdSe:Gd nanoparticles dispersed in 10 mL of chloroform premixed with MPA (60 μL) stirred for 24 hr at room temperature. Afterward 3 mL of ammonia solution (28%) was added, the cloudy solution was vigorous stirred for 1 h. After ceasing the stirring, the cloudy solution separated into two layers spontaneously. We extracted the aqueous phase of the upper layer containing MPA-capped QDs, and removed the excess MPA by precipitating the particles with 3 mL methanol and discarding the supernatant after centrifugation. A MPA-capped nanoparticles precipitate dispersed in 18 mL of deionic water, then 60 mg of lysine and 1mg of EDC (as a cross-linking reagent) were added, and the solution was vigorous stirred for 1 h. We dialyzed a solution of lysine-modified nanoparticles (pore size MW 1000) for 1 h three times by deionic water to remove excess lysine and residual EDC.

Calculation of number of Gd atoms in a single CdSe:Gd nanoparticles

Although we have conducted EDS measurements using HR-TEM to determine Gd element amount per quantum dot, the HR-TEM (Hitachi HF-2000) available in our campus has detection limit of 0.1 wt% on element in sample matrix for EDS determination, where Gd amount per QD (wt%) = Gd/(Gd+Cd+Se)×100 %. Based on the ICP-AES (detection limit (ppb)) analysis, the Gd amount in a CdSe was 0.061 wt%, which was lower than HR-TEM detection limit.

Alternatively, the ICP-AES measurements were used to derive the amount of Gd per CdSe particle. The total number of Gd atoms of the as-prepared CdSe:Gd nanoparticles was obtained by dissolution of as-synthesized CdSe:Gd nanoparticles using concentrated HNO_3, followed by the measurements of ICP-AES spectrometer to determine the concentration of total Gd atoms. To determine the number of Gd atoms in a single CdSe:Gd nanoparticle, we considered CdSe:Gd nanoparticles with *x* nm in diameter (measured by TEM). In a *x* nm nanoparticle we would find *y* Gd atoms, which was calculated based on CdSe unit cell. CdSe is a wurtzite-type structure.

Characterization

The morphologies were characterized by TEM (100KV, Hitachi) and high-resolution TEM (200KV, JEOL 2100).We characterized the crystalline structure of the products by XRD (Rigaku D-Max IIIV diffractometer using Cu Ka radiation,

λ=1.5418 Å at 30 kV and 30 mA). The extinction characteristics of the nanomaterials were determined using a UV-vis spectrophotometer (Hewlett-Packard Model 8453). Fluorescence spectra were recorded on a Hitachi F-4500 fluorescence spectrophotometer. We obtained the Quantum yield of the CdSe:Gd nanoparticles by comparison with a standard Rhodamine 6G dye (Rh6G in ethanol, QY = 95%). Purified nanoparticles were dissolved in chloroform or water and measured in a standard 1 cm quartz cell at the excitation wavelength (450 nm).

Cell viability of CdSe:Gd nanoparticles in the Vero cell line

MTT assays were employed on the Vero cell line. The MTT assay method is based on the formation of dark-red formazan by the metabolically active cells after their exposure to MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide). We added the serial medium dilutions of the CdSe:Gd nanoparticles with particle concentrations of 200, 100, 10, 1, 0.1, and 0.01 μg/mL to the culture wells to replace the original culture medium with a final volume of 100 μL. The cells were incubated with the particles for 24 h. After incubation, the culture medium was removed and replaced by 100 mL of the new culture medium containing 10% MTT reagent. The cells were then incubated for 4 h to allow formation of the formazan dye at 37^{o}C. Next, the culture medium in each well was removed, followed by the addition of dimethylsulfoxide (200 μL per well) for an additional 10 min incubation.

After centrifugation, We then transferred the resulting formazan in each well to an ELISA plate. The quantification determining cell viability was done using optical absorbance (540/650 nm) and an ELISA plate reader.

In vitro MR assay

The experiments were performed using a spectroscope (3T MRI Biospec; Bruker, Ettlingen, Germany). A gradient system mounted on the table of the 3 T magnet with an inner diameter of 6 cm and a maximal gradient strength of 1000 mT m^{-1} was used to yield high-resolution images. A quadrature coil with an inner diameter of 3.5 cm was used for RF transmission and reception. For in vitro MR images and both T_1 and T_2 measurements, all CdSe:Gd nanoparticles were dispersed in water with various gadolinium atoms concentrations (0, 0.01, 0.025, 0.05, 0.1, and 0.15mM). The relaxivity measurements were performed at 20 ^{o}C. The array was embedded in a phantom to allow the appropriate image acquisition. Acquired images had a matrix size of 256T192, a field of view of 60T60 mm, and a slice thickness of 3 mm, yielding an in-plane resolution of 234 *m*m after image smoothing. Both T_1- and T_2-weighted images were acquired using a multi-slice multi-echo (T_1-weighted) and fast spin-echo (T2-weighted) sequence with a repetition time/echo time (TR/TE) of 472/9.4 ms with a number of averages (NEX) of 8 and TR/TE of 4500/62.7 ms with a NEX of 6, respectively. T_1 values were measured using a multi-slice multi-echo

sequence with a TR of 6000 ms, a TE of 8.7 ms, and 45 inversion recovery points (TI from 13.3 to 6000 ms). The field of view was 60T60 mm, the slice thickness was 6 mm, and the image matrix was 128T128. This allowed for simultaneous imaging of 26 vials with 0.3 mL of contrast agent for each vial. An average signal of 50 voxels was evaluated for all TI values. T_2 values were performed with a spin-echo sequence of TR/TE¼4000/ 10.1 ms, 60 echo points of 60, and a NEX of 5. The field of view was 60T60 mm, the slice thickness was 6 mm, and the imaging plane was 256T192.

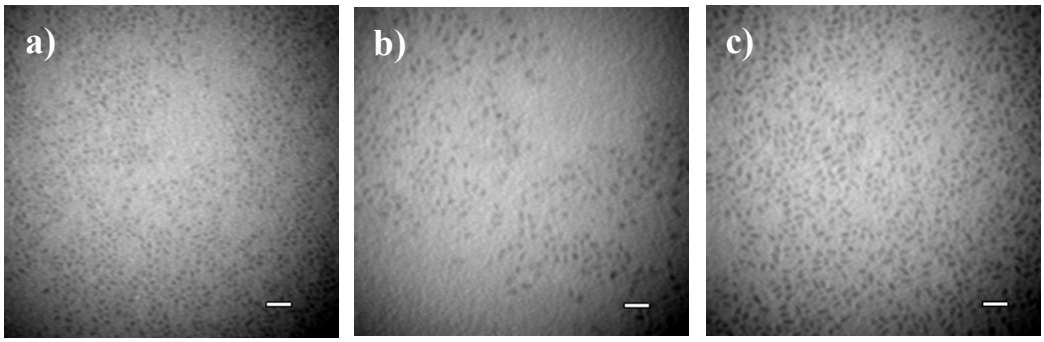

Figure S1. TEM images of CdSe NPs growing under the different reaction times of (a) 30 s, (b) 1 min, and (c) 5 min. Scale bar = 20 nm

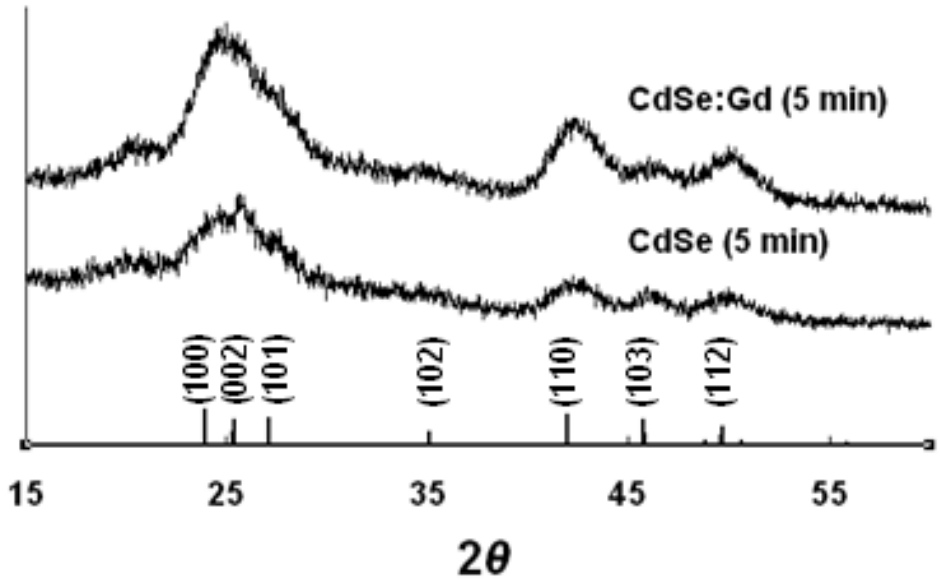

Figure S2. XRD patterns of CdSe NPs (reaction time: 5 min) and CdSe:Gd NPs (reaction time: 5 min). The vertical lines indicate the positions of the diffraction patterns for the wurtzite structure of bulk CdSe.

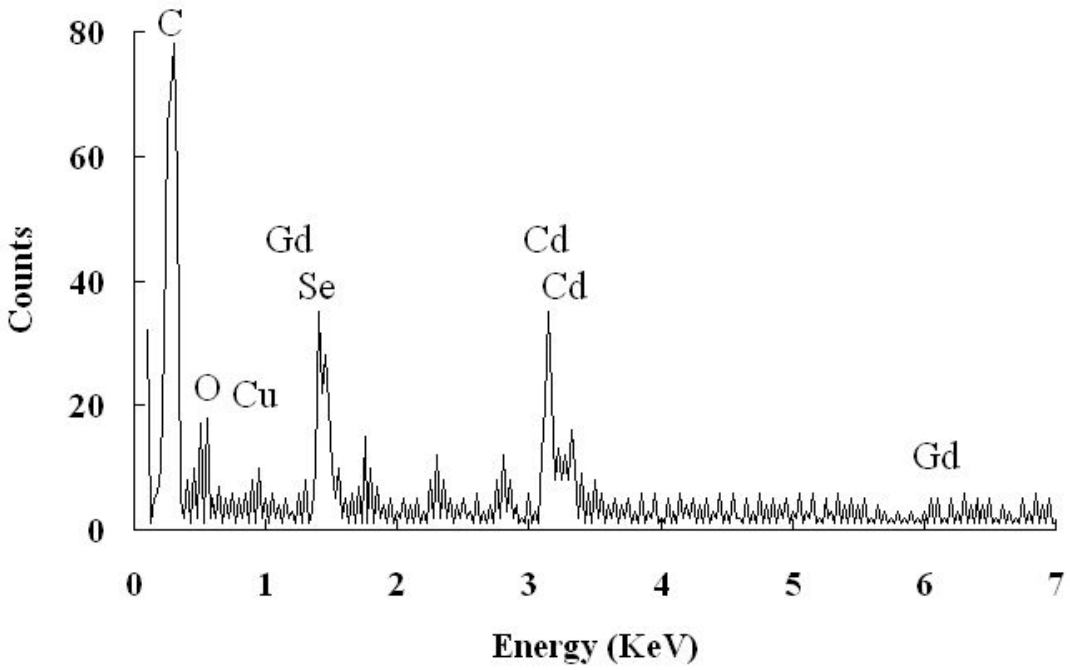

Figure S3. EDX spectrum of CdSe:Gd NPs (reaction time: 5 min)

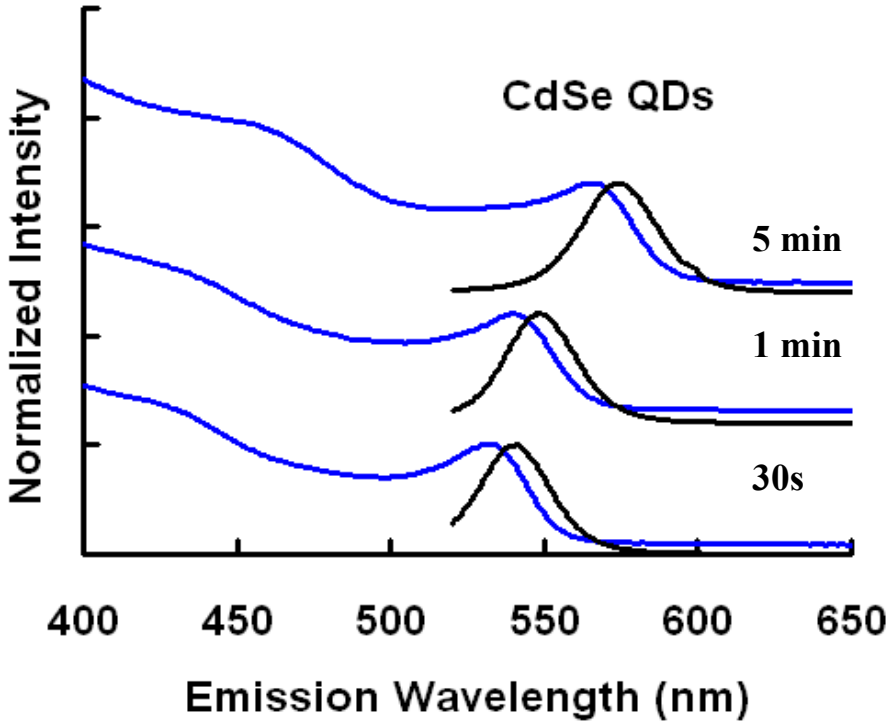

Figure S4. Absorption and photoluminescence (PL) for TOPO-capped CdSe NPs at different reaction times (absorption: blue; emission: black; emission spectra excited by 450 nm)

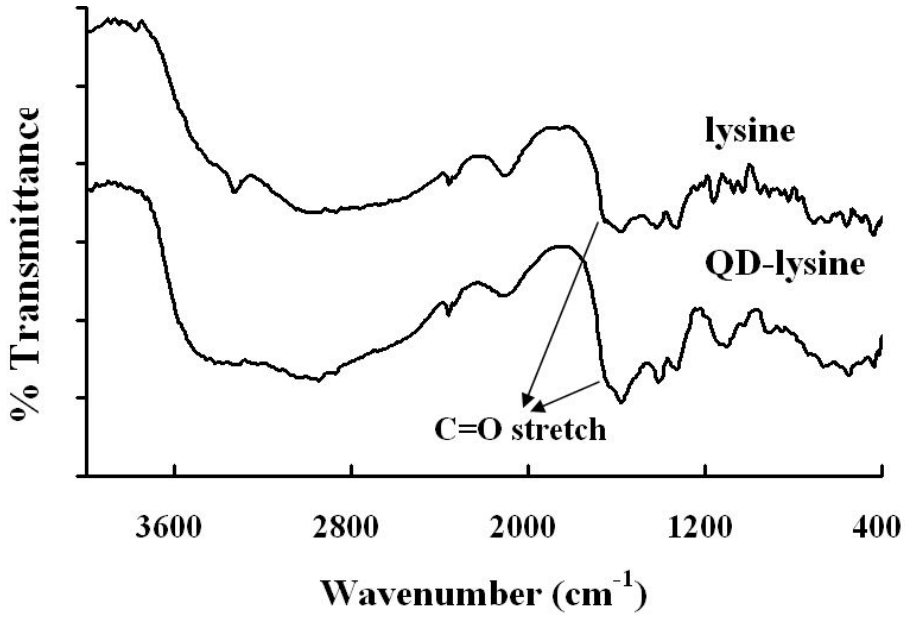

Figure S5. FTIR spectra of lysine and lysine-capped CdSe:Gd NPs. The absorption bands at 1660 cm^{-1} is related to the C=O stretching from the lysine

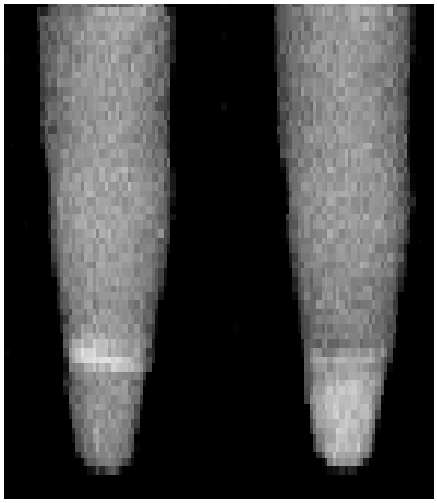

Figure S6. T_1-weighted images of vero cells alone (left) and vero cells incubated with CdSe:Gd NPs (right) at 0.013 mM of Gd ions measured at 3T

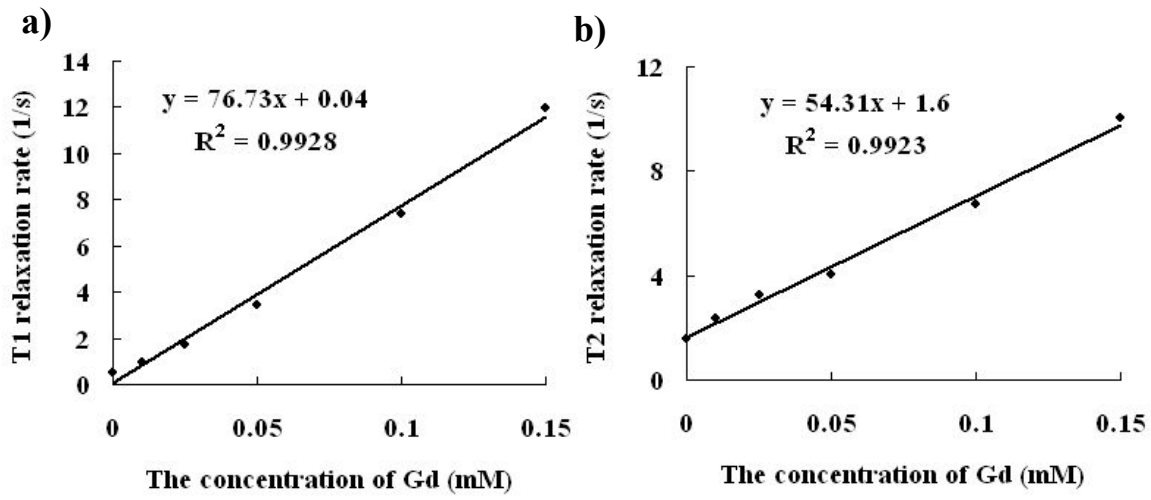

Figure S7. T_1 relaxation (s^{-1}) and T_2 relaxation (s^{-1}) rates of CdSe:Gd NPs as a function of Gd concentrations (mM)

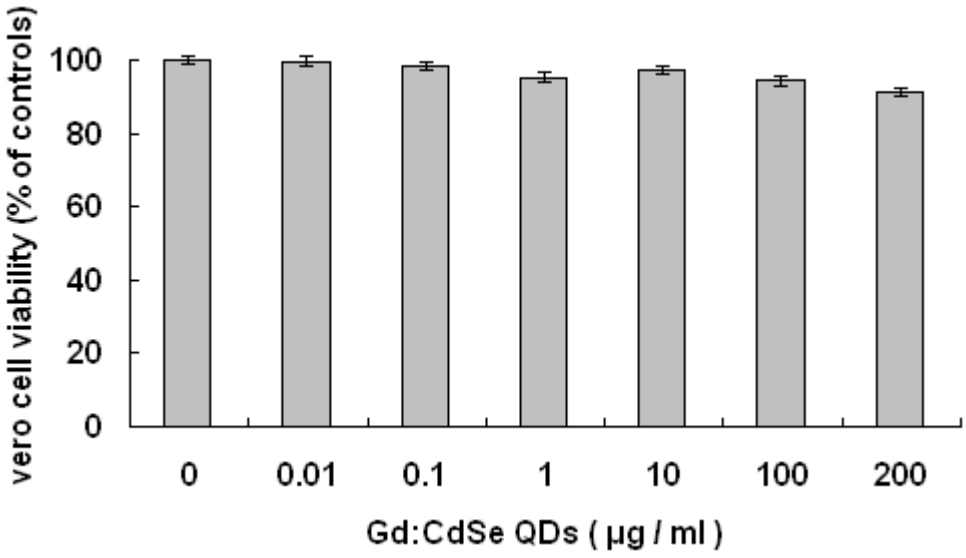

Figure S8. The biocompatibility of the CdSe:Gd NPs were analyzed using MTT assays. Vero cells were incubated with particles for 24 h