# SUPPORTING INFORMATION

## A general approach to silicate nanoshells: gadolinium silicate and gadolinium silicate:europium nanoshells for dual-modality optical and MR imaging

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## Experimental

*Chemicals:* All reagents were of analytical purity and used without further purification. Tetraethyl orthosilicate (TEOS, 98%) and polyvinylpyrrolidone (PVP, *Mw*. 4,0000) were purchased from Acros Organics (Geel, Belgium) and Sigma-Aldrich Co. (St Louis, MO), respectively. The gadolinium(III) acetylacetonate [$Gd(acac)_3$] hydrate (99.9%), iron(III) acetylacetonate [$Fe(acac)_3$] (99.9%), Gallium(III) acetylacetonate [$Ga(acac)_3$] (99.99%), and terbium(III) acetylacetonate [$Tb(acac)_3$] were purchased from Sigma-Aldrich. Erbium(III) acetylacetonate [$Er(acac)_3$] was purchased from Alfa Aesar (Taipei, Taiwan). Europium (Eu)(III) nitrate hexahydrate (Strem Chemicals, Inc., Boston, MA 99.9%) was used as doping material. The isopropyl alcohol (2-proponol, $C_3H_5OH$) solvent was purchased from Mallinckrodt, Inc. (Raleigh, NC).

*Synthesizing Gd silicate nanoshells.* In a typical synthesis, 0.22 mL of ammonia solution (28-30%), 1.25 mL of PVP aqueous solution (0.6 mM), and 0.048 mL of TEOS were dissolved in 10 mL of 2-propanol with vigorous stirring for 8 h. The resulting solution was added with 0.4 mL of $Gd(acac)_3$ solution (0.1 M in 2-propanol) to form a milky solution for an 18-h reaction. The process was repeated by adding 0.045 mL of TEOS and mixing for 2 h, and then 0.55 mL of 0.1M $Gd(acac)_3$ was added for a 6-h reaction. The solution was then transferred into a Teflon-lined stainless steel autoclave (23 mL) for 18 h at 120 °C. Finally, the white products were collected using centrifugation (10000 rpm) and rinsed several times with 2-propanol. We used the same experimental parameters, except for the metal(III) acetylacetonate, to prepare Tb silicate, Er silicate, Fe silicate, and Ga silicate nanoshells. The annealed silicate nanoshells were prepared by heating as-prepared silicate nanoshells in air for 1h.

*Synthesizing Gd silicate:Eu nanoshells.* We used the same procedure as for Gd silicate to synthesize Gd silicate:Eu nanoshells: we prepared a mixture of 0.22 mL of ammonia solution (28-30%), 1.25 mL of PVP aqueous solution (0.6 mM), 0.048 of mL TEOS, 0.4 mL of

$Gd(acac)_3$ in a 10 mL of 2-propanol solution, to which we added 0.045 mL of TEOS with magnetic stirring for a total reaction time of 26 h. Next, 0.55 mL of lanthanide solutions with a series of $EuNO_3 \cdot 6H_2O$ solutions (0.1 M) in 0.015, 0.03, 0.0582, 0.11, 0.198, and 0.527 mL were used, respectively, to dope Eu ions in nanoshells by maintaining the 0.1 M concentration of lanthanide ions ($Gd^{3+}$ + $Eu^{3+}$). Finally, the resulting solution was solvothermally treated at 120 °C for 18 h to obtain Gd silicate:Eu nanoshells. Ga silicate:Eu nanoshells were synthesized using the same procedures, except for the acetylacetonate. The annealed silicate:Eu nanoshells were prepared by heating as-prepared silicate:Eu nanoshells in air for 1h.

*In vitro MRI.* The experiments were done using a spectroscope (3T MRI Biospec; Bruker Optik GmbH, Ettlingen, Germany). A gradient system mounted on the table of a 3T magnet (inner diameter: 6 cm; maximal gradient strength: 1000 mT $m^{-1}$) was used to yield high-resolution images. A quadrature coil (inner diameter: 3.5 cm) was used for RF transmission and reception. For $T_1$ and $T_2$ measurements, all Gd silicate nanoshells with various $Gd^{3+}$ concentrations (0-2.75 mM) were dispersed in 0.5% agarose gel. The acquired MRIs (matrix size: 256 × 192; field of view: 60 × 60 $mm^2$; slice thickness: 3 mm) had an in-plane resolution of 234 μm 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 ($T_2$-weighted) sequence with a repetition-time/echo-time (TR/TE) of 427/9.4 ms and a NEX (number of averages) of 10 ($T_1$) and with a TR/TE of 4500/62.7 ms and a NEX of 5 ($T_2$). $T_1$ value measurements were taken 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: 13.3-6000 ms; field of view: 60 × 60 $mm^2$; slice thickness: 6 mm; image matrix: 128 × 128). This allowed for simultaneous imaging of 26 vials with 0.3 mL of contrast agent per vial. An average signal of 50 voxels was evaluated for all TI values. $T_2$ value measurements were taken with a spin-echo sequence of TR/TE of 4000/10.1 ms, 60

echo points of different 60 echo time, and a NEX of 5 (field of view: 60 × 60 $mm^2$; slice thickness: 6 mm; imaging plane: 256 × 192).

*The biocompatibility of Gd silicate and Gd silicate:Eu (48%) nanoshells*. To evaluate the biocompatibility of the Gd-containing nanoshells, a Vero (monkey kidney) cell line was treated with nanoshells (0-500 μg/mL) using an MTT assay. The Vero cells (4 × 103 cells/well) were cultured in a 96-well microplate with α-modified minimal essential medium (MEM) (containing 20% FBS+ 1% PSN+ basic FGF 4 μg/mL) at 37 °C supplied with 5% $CO_2$/95% air. After 24 h, serial diluents of loaded nanoshells samples (at concentrations of 500, 200, 100, 10, 1, 0.1, 0.01, and 0.001 μg/mL) were added to the culture wells to replace the original culture medium. Subsequently, the cells were incubated with the hollow samples for 24 h. The culture medium was then removed and replaced with 100 μL of fresh culture medium (α-modified MEM) containing 10% MTT reagent. The resulting cells were incubated at 37 °C for 4 h to allow the formation of formazan dye. The cultural medium in each well was centrifuged and collected, and then transferred to an ELISA plate. The quantification of cell viability was done using an ELISA plate reader at an optical absorbance of 540/650 nm.

*Measuring annealed Gd silicate and annealed Gd silicate:Eu (48%) nanoshells using confocal laser scanning microscopy*. Cells were cultured in α-modified MEM at 37 °C supplied with 5% $CO_2$/95% air. Cells were trypsinized and seeded in 8-well chamber slides with $1.2 \times 10^4$ cells of each well. After 24 h of incubation, each well was washed twice with phosphate-buffered saline (PBS), and then 0.3 mL of annealed nanoshells (40 μg/mL in α-modified MEM) were added. After 1 h and 6 h of incubation, the treated cells were washed with PBS and then fixed using 4% paraformaldehyde/PBS for 30 min at 37 °C. The cytoskeleton was stained using Alexa 594 phalloidin (red) for annealed Gd silicate nanoshells and Alexa 488 phalloidin (green) for annealed Gd silicate:Eu nanoshells (48%) for confocal

laser scanning microscopy (Digital Eclipse C1si; Nippon Instruments Corporation, Tokyo, Japan) analysis.

*Characterization:* The crystalline structures were identified using an X-ray diffractometer (XRD-7000S; Shimadzu Corporation, Tokyo, Japan) with copper-potassium alpha (CuK*a*) radiation ($\lambda$ = 1.54060 Å) at 30 kV and 30 mA. Electron micrographs of the samples were taken using transmission electron microscopes (TEM) at 200 kV (JEOL 2010; Jeol USA, Inc., Peabody, MA, and CM-200; Philips Research Europe, Eindhoven, The Netherlands). A drop of the sample was placed on a Cu mesh coated with an amorphous carbon film and then the solvent was evaporated in a vacuum desiccator. Field emission scanning electron microscope (FE-SEM) images of the nanoshells on the Cu plate substrates were taken using an FE-SEM at 10 kV (XL-40 FEG; Philips). Infrared (IR) spectra were measured using a Fourier transformation (FT)-IR spectrometer (200E; Jasco International Co., Ltd., Tokyo, Japan). Nitrogen ($N_2$) adsorption measurements were taken at 77 K using an accelerated surface area and porosimetry analyzer (ASAP 2010; Micrometrics Instrument Corporation, Norcross, GA) with Brunauer-Emmett-Teller (BET) calculations for the surface area. A thermogravimetric analysis (TGA) was done using ~10 mg of sample on a thermogravimetric analyzer (Netzsch TG209; Trendtop Scientific Corp., Chung-Ho City, Taipei Hsien, Taiwan) at a heating rate of 10 °C/min under dried air. A fluorescence spectrophotometer (F-2500; Hitachi Koki Co., Ltd., Tokyo, Japan) and a UV-Vis spectrophotometer (8452A; Hewlett-Packard Company, Palo Alto, CA) were used to record photoluminescence (PL) characteristics. The quantum yields were determined by comparing the integrated emission of the colloidal solutions with the emission from a DCM (4-dicyanomethylene-2-methyl-6-p-dimethylaminostyryl-4H-pyran) dye solution. $^{29}$Si MAS NMR spectra were recorded on a spectrometer (9.395 Tesla) (AV-400; Bruker) with resonance frequencies of 59.63 MHz. The spin rate of the sample was 4.0 kHz, and the number of scans was 19000-20000. The chemical shifts were referenced to tetramethylsilane (TMS) for $^{29}$Si.

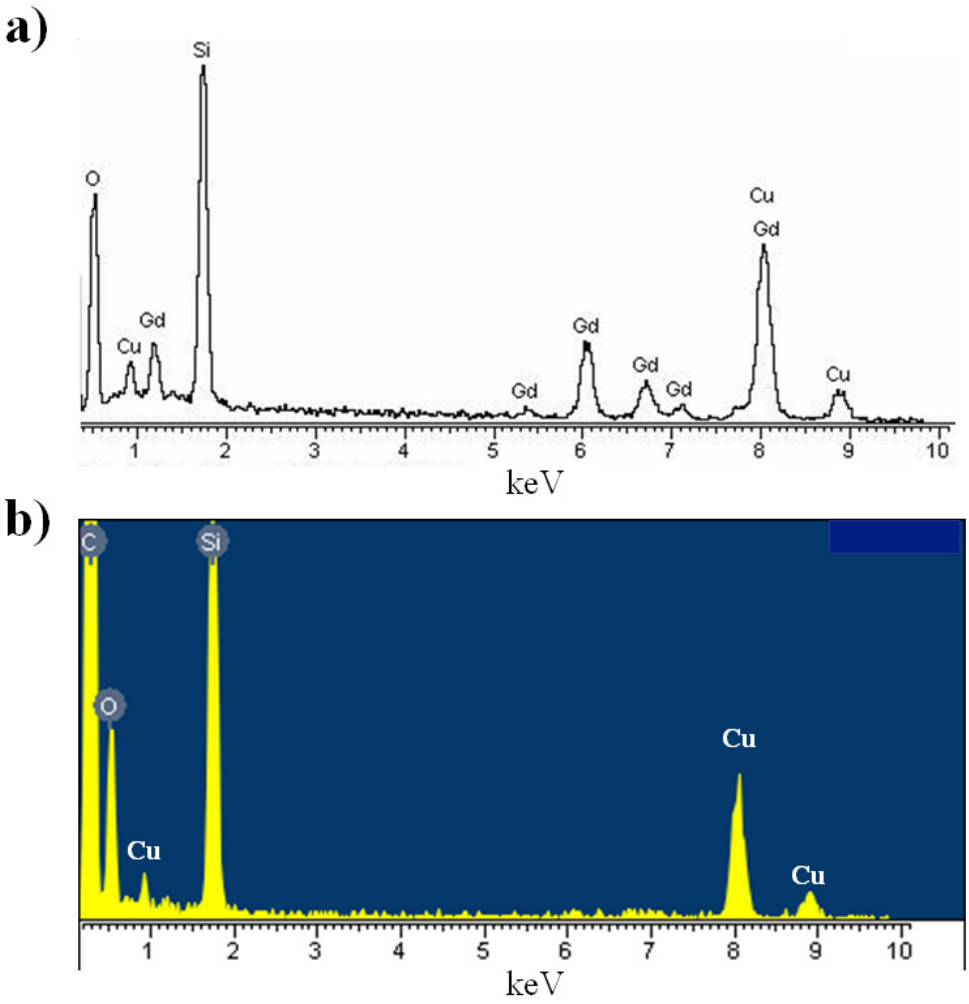


**Figure S1.** EDS analyses of as-prepared gadolinium silicate nanoshells a) before and b) after HCl etching treatment. The Cu signals are attributed to the copper substrate.

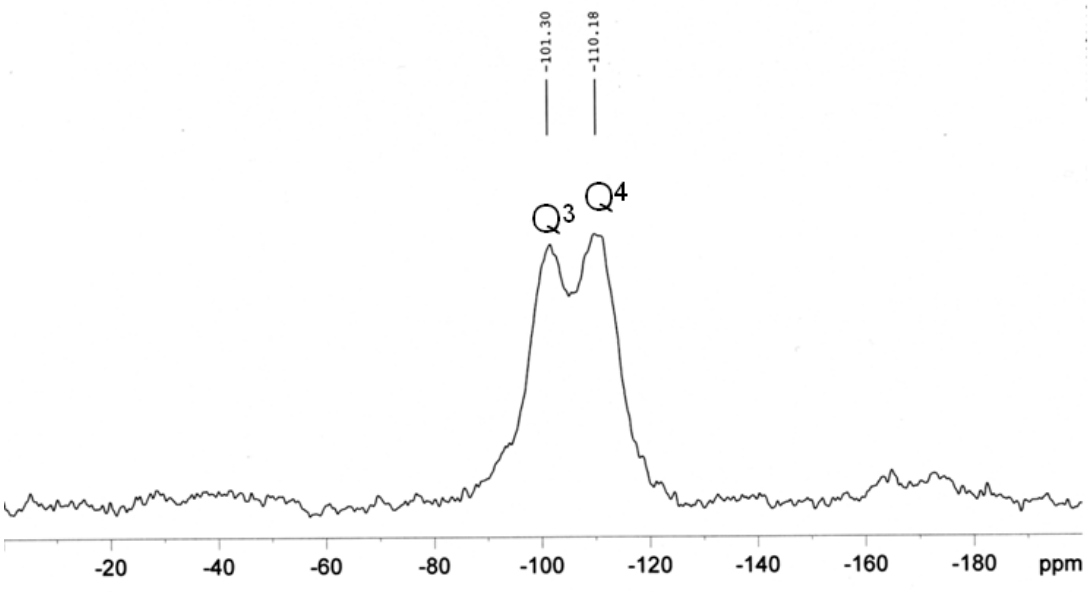


**Figure S2.** $^{29}$Si MAS NMR spectrum obtained after HCl etching treatment with gadolinium silicate nanoshells.

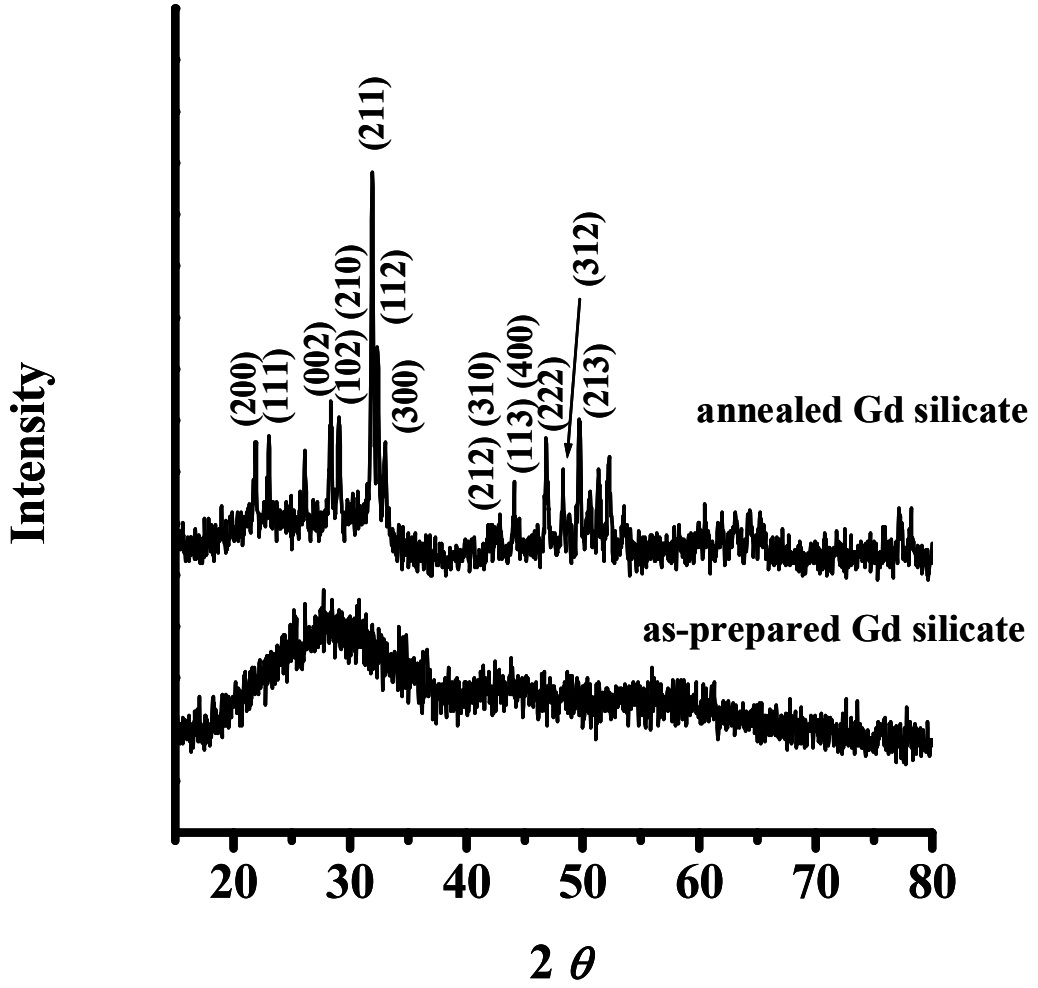


**Figure S3.** Evolution of XRD patterns for as-prepared and annealed (1100 $^{o}$C) gadolinium silicate nanoshells.

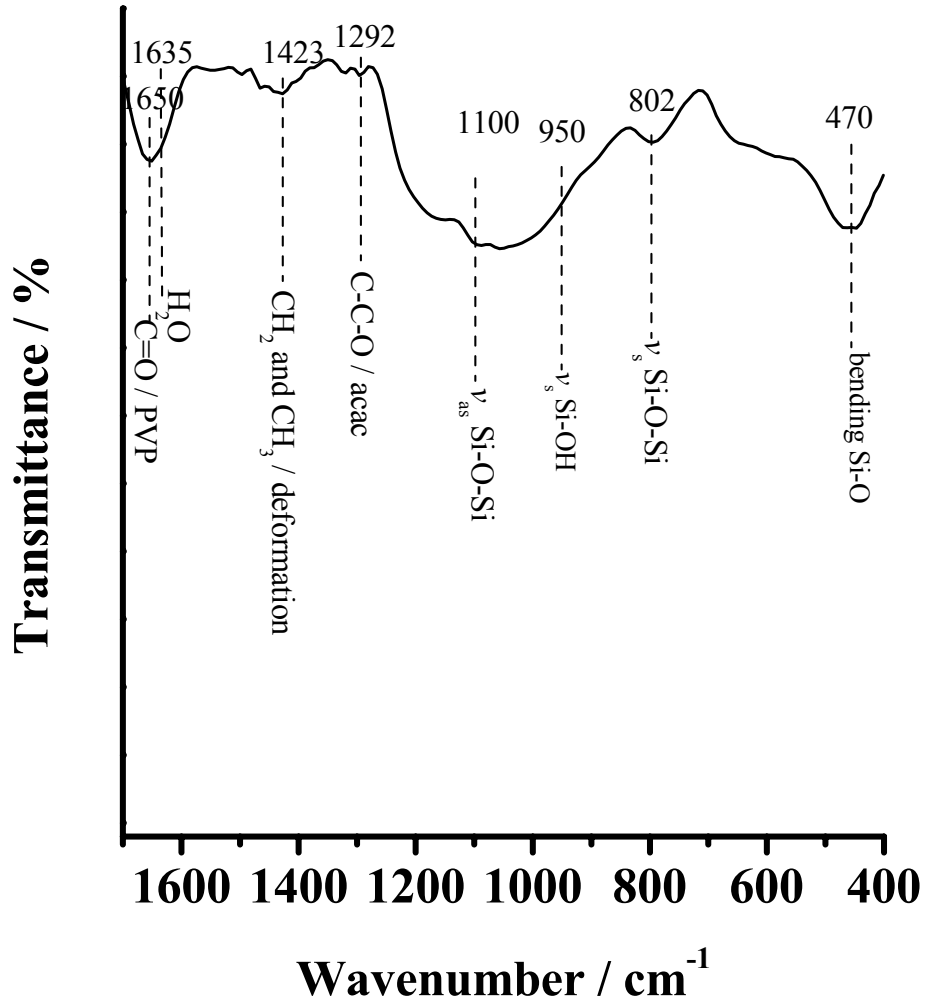


**Figure S4.** FT-IR spectrum (in the range of 400-1700 $cm^{-1}$) for as-prepared gadolinium silicate nanoshells. The absorption bands in the range of 400-1200 $cm^{-1}$ correspond to silicate vibrations.[1,2] A C-C-O peak of acetylacetone ligand molecule was found at 1292 $cm^{-1}$.[3] A peak center at 1423 $cm^{-1}$ was originated from the coupling of pyrollidone ring and $CH_3/CH_2$ deformation.[3] The C=O stretching band of PVP polymer located at 1650 $cm^{-1}$.[4] The O-H stretching in molecular $H_2O$ was marked at 1635 $cm^{-1}$.[1,2]

[1] M. Yu, J. Lin, Y. H. Zhou, S. B. Wang, H. J. Zhang, *J. Mater. Chem* **2002**, *12*, 86.

[2] X. M. Han, J. Lin, R. B. Xing, J. Fu, S. B. Wang, Y. C. Han, *J. Phys.: Condens. Matter* 2003, **15**, 2115.

[3] J. R. Sohn, S. I. Lee, *Langmuir* **2000,** *16,* 5024.

[4] T. Saegusa, *Pure Appl. Chem.* **1995**, *67*, 1965.

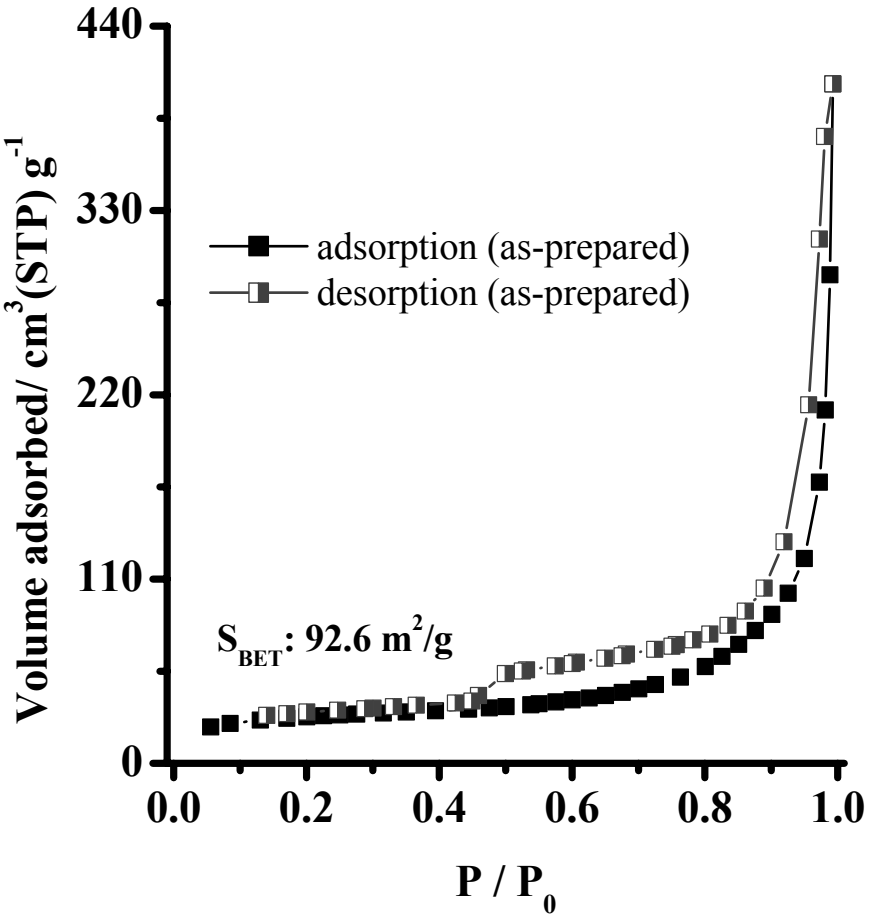


**Figure S5.** $N_2$ adsorption-desorption isotherms of as-prepared gadolinium silicate nanoshells.

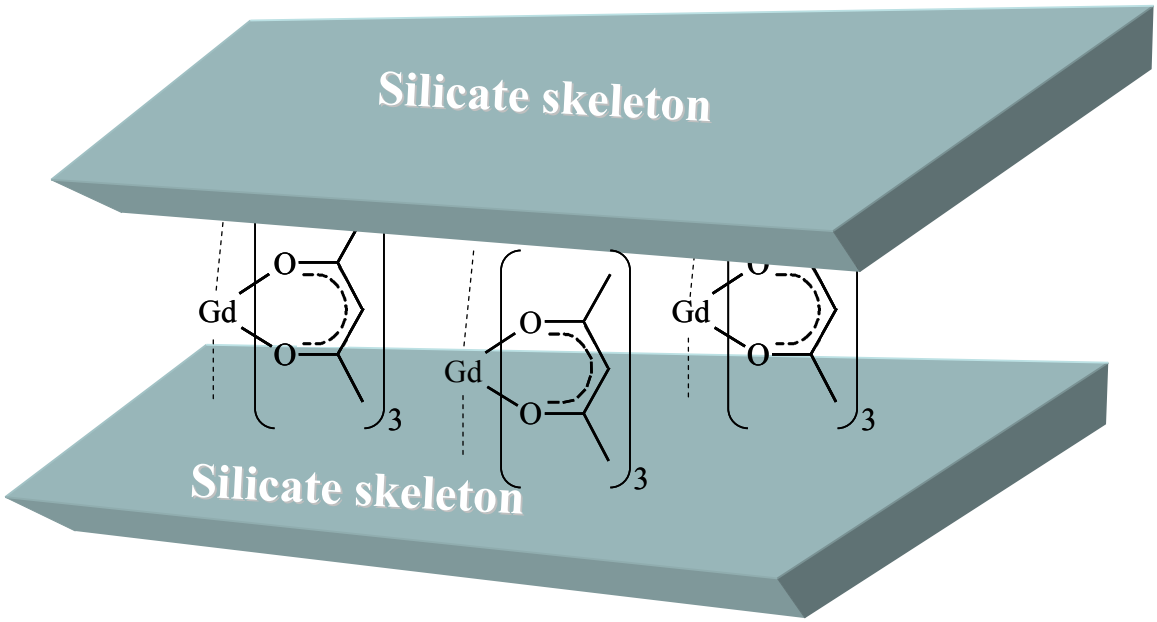


**Figure S6.** Schematic illustration depicts the proposed complex formation of the acetylacetone molecules coordinating gadolinium cation between the silicate layers.[1]

[1] J. R. Sohn, S. I. Lee, *Langmuir* **2000,** *16,* 5024

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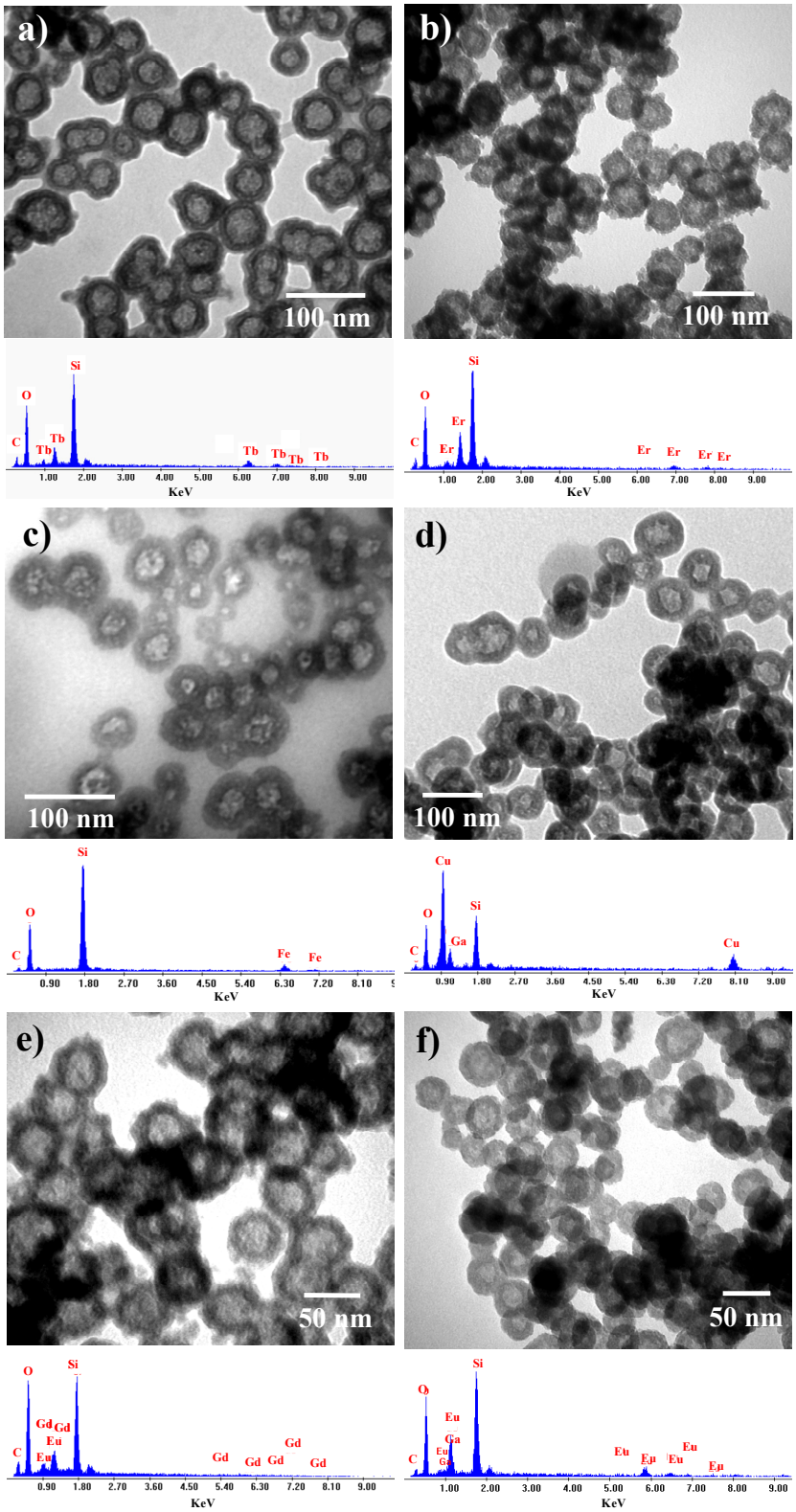



**Figure S7.** TEM images of a) terbium silicate nanoshells (~49.9 nm; shell thickness: ~10.1 nm), b) erbium silicate nanoshells (~52.2 nm nm; shell thickness: ~13.4 nm), and c) iron silicate nanoshells (~45.5 nm; shell thickness: ~15.3 nm), d) gallium silicate nanoshells (~52.0 nm; shell thickness: ~15.0 nm), e) gadolinium silicate:Eu (48%) nanoshells (~48.1 nm; shell thickness: ~9.2 nm), f) gallium silicate:Eu (48%) nanoshells (~48.0 nm; shell thickness: ~10.0 nm), and corresponding EDS spectra. The Cu element is attributable to the background of copper substrate.

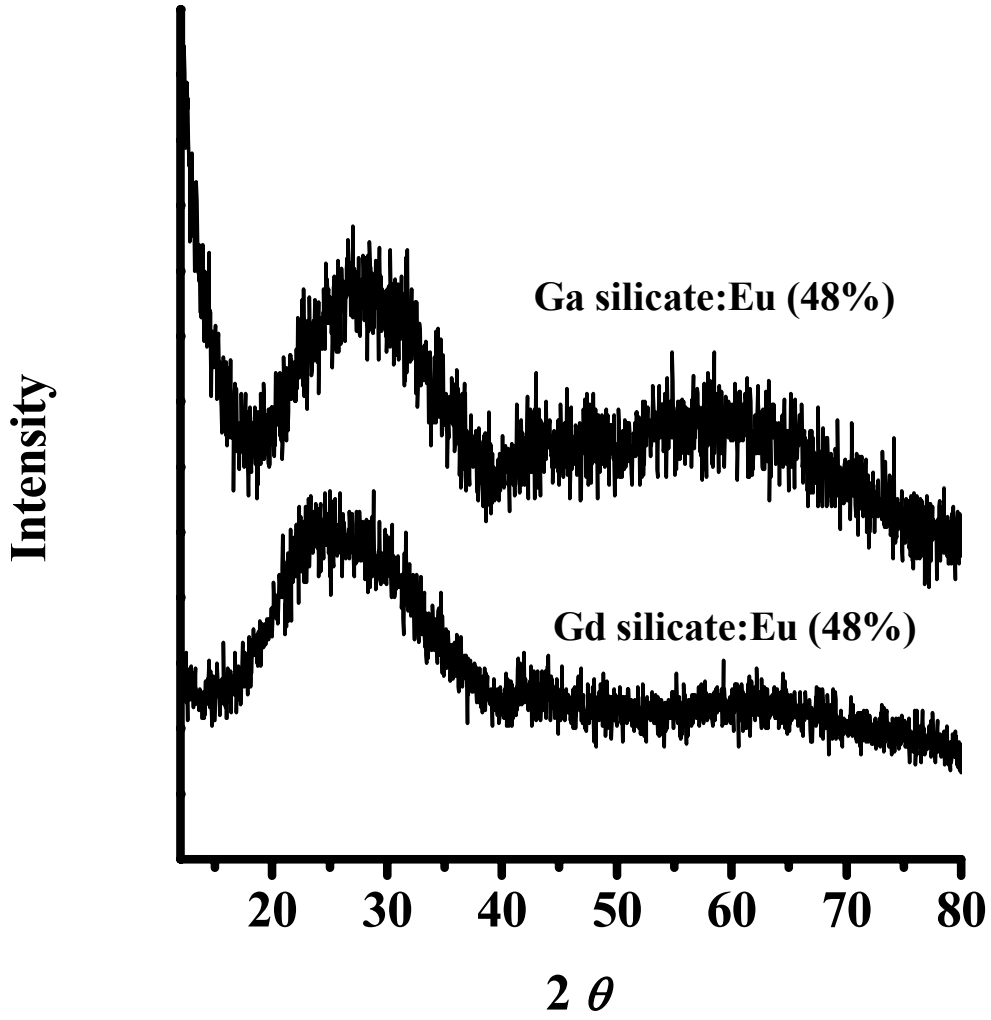
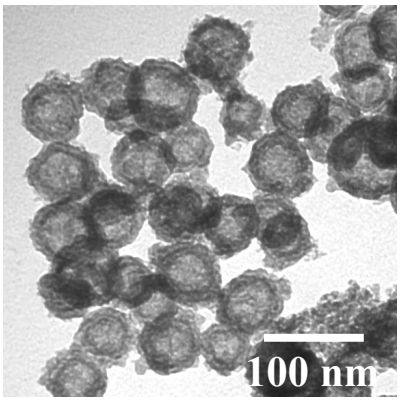
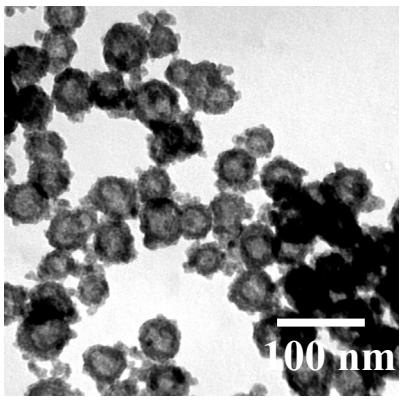



**Figure S8.** XRD patterns for as-prepared Gd silicate:Eu (48%) and Ga silicate:Eu (48%) nanoshells.

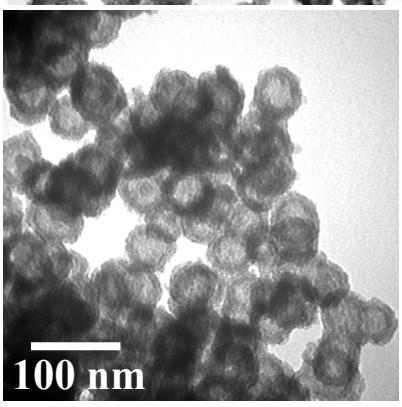
Gd silicate nanoshells




Gd silicate:Eu nanoshells




Ga silicate nanoshells




Ga silicate:Eu nanoshells

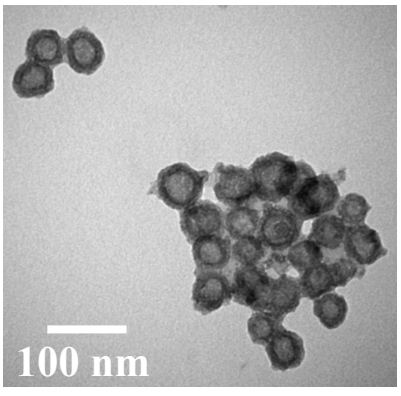



**Figure S9.** TEM images of Gd silicate, Gd silicate:Eu, Ga silicate, and Ga silicate:Eu nanoshells after annealing at 860 $^{o}$C for 1 h.

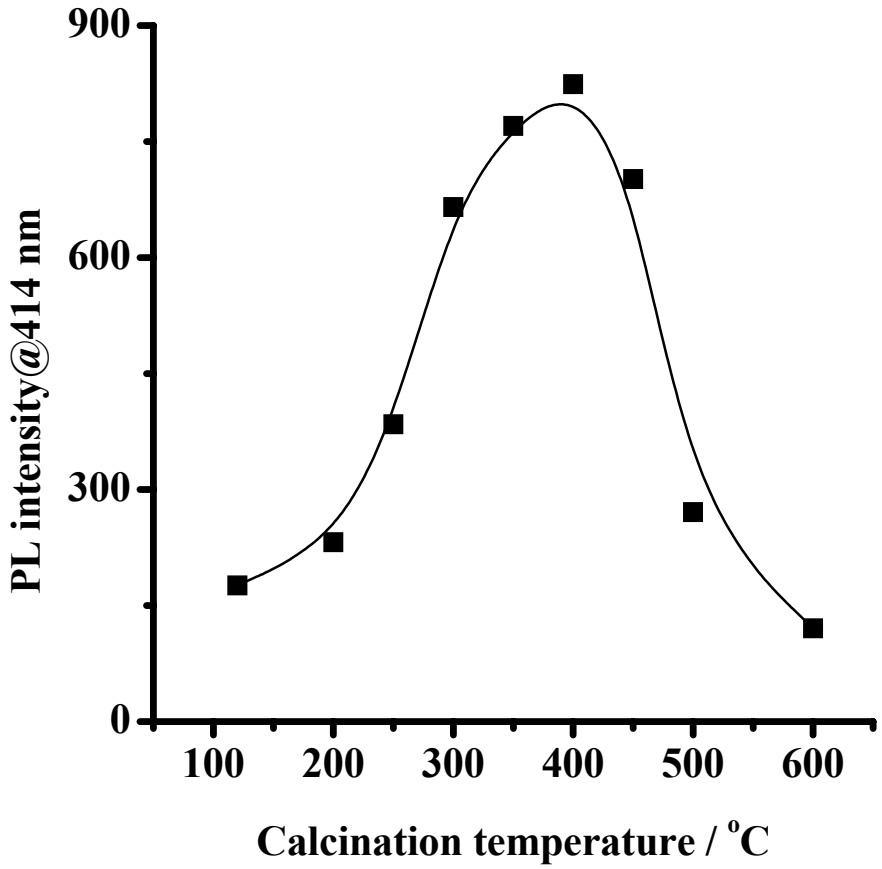



**Figure S10.** The photoluminescence intensity of as-prepared gadolinium silicate nanoshells as a function of annealing temperatures (120-600 $^{o}$C) recorded at 414 nm emission peak upon excitation at 330 nm. The sample concentrations were all fixed at 1.8 of O.D. value measured at 200 nm wavelength.

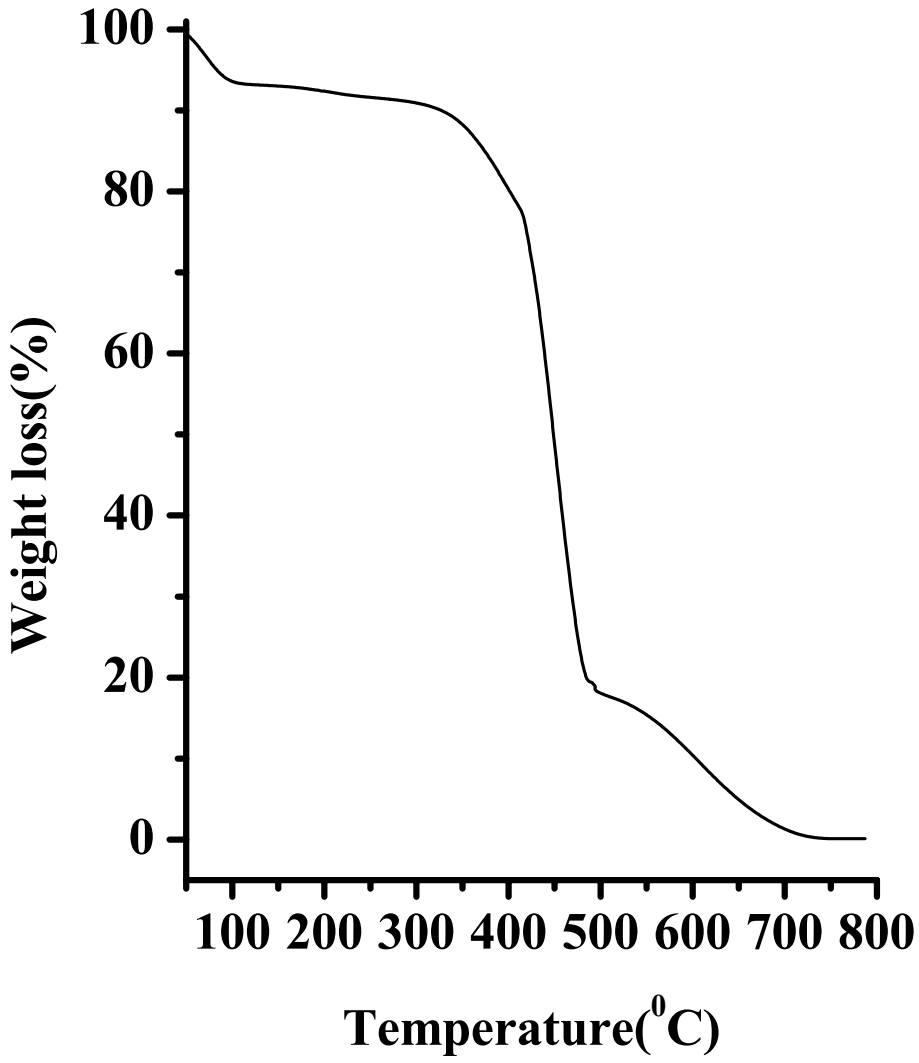



**Figure S11.** TGA traces of pure PVP polymer.

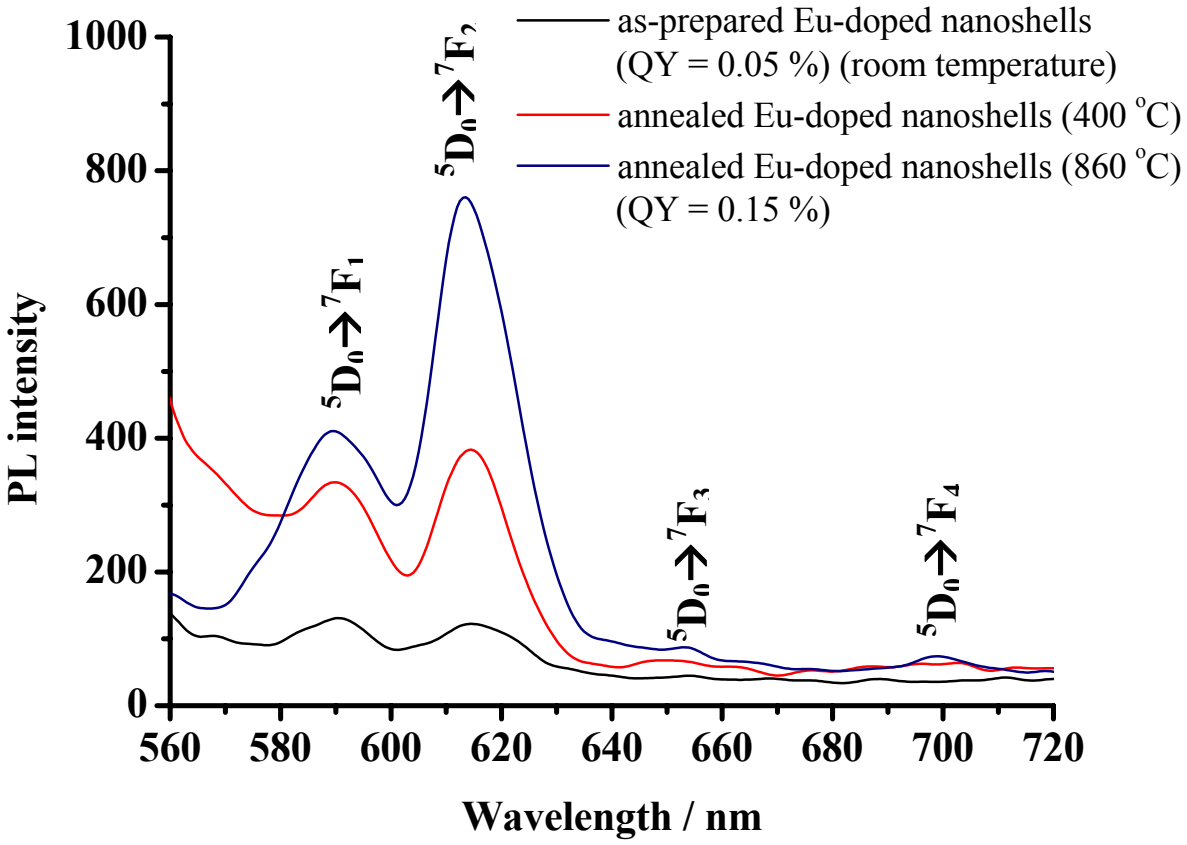



**Figure S12.** The evolution of PL spectra for gadolinium silicate:Eu (48%) nanoshells as a function of annealing temperature upon excitation at 250 nm.

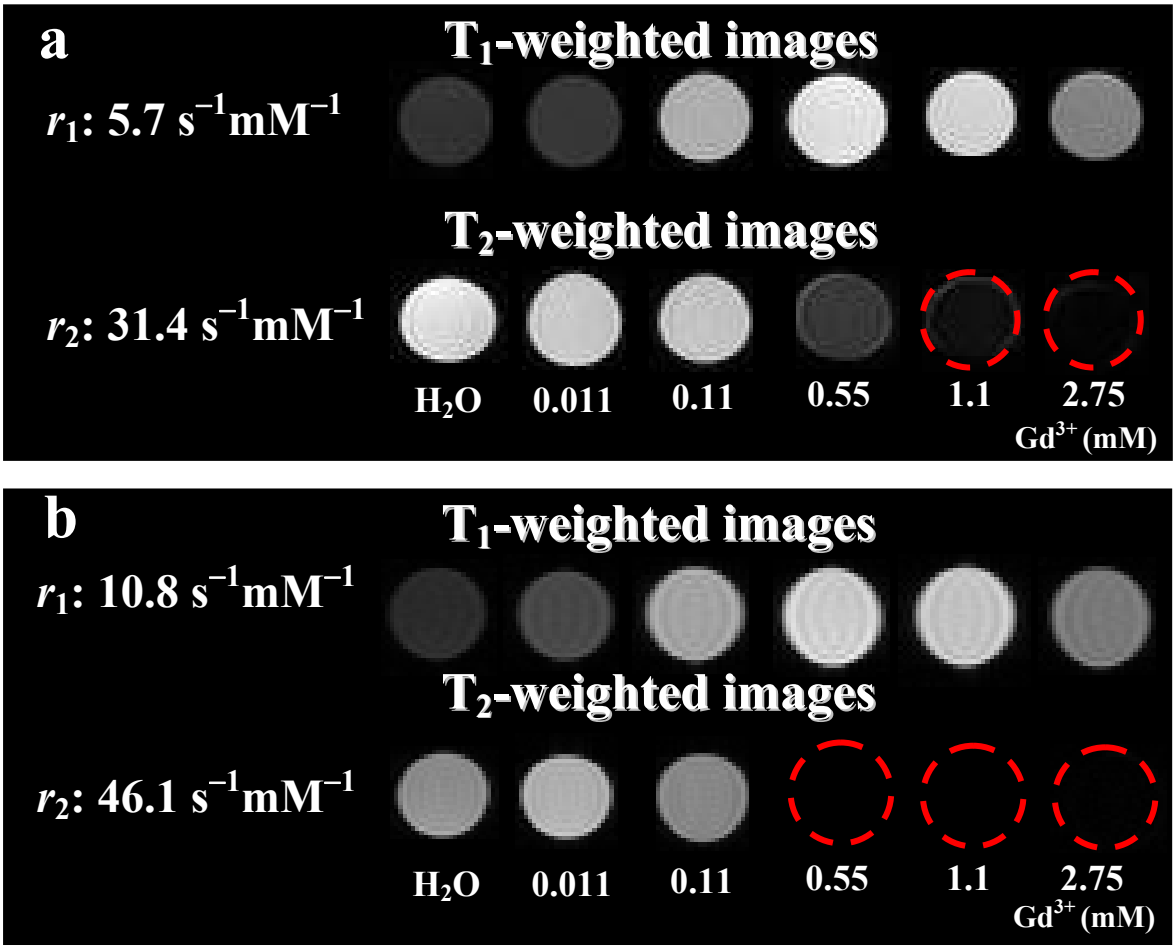



**Figure S13.** MR *in vitro* assays of $T_1$-weighted and $T_2$-weighted images of a) as-prepared gadolinium silicate nanoshells and b) as-prepared gadolinium silicate:Eu (48%) nanoshells in 0.5% agarose gel.

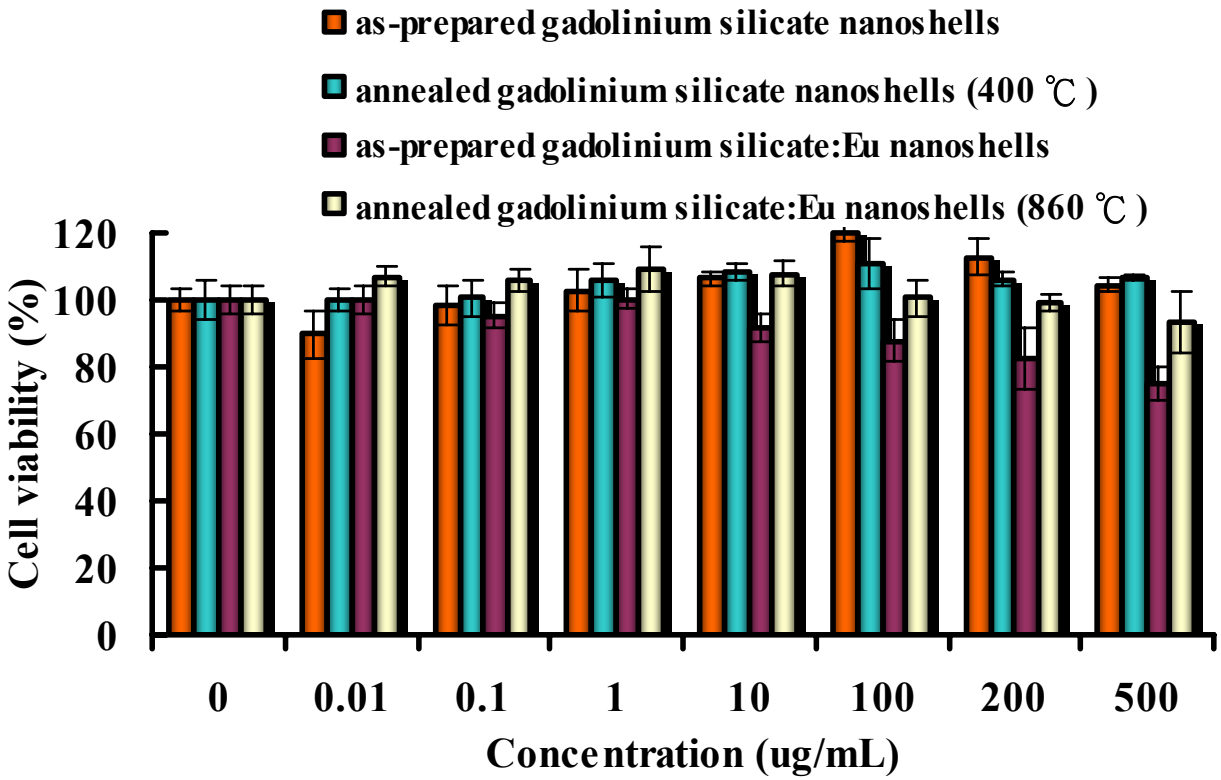



**Figure S14.** The biocompatibility of gadolinium silicate and gadolinium silicate:Eu (48%) nanoshells treated with Vero cells for 1 day cell culture using MTT assay.