Electronic Supplementary Information (ESI) for

$CePO_4$:Tb,Gd Hollow Nanospheres as Peroxidase Mimic and Magnetic-Fluorescent Imaging Agent

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1. Materials and Methods

(1) Materials

Cerium (IV) oxide (CeO_2, 99.99%), terbium oxide (Tb_4O_7, 99.99%) and gadolinium oxide (Gd_2O_3, 99.99%) were obtained from Shanghai Sanpu Chemical Corporation (Shanghai, China), and are of SpecPure grade. NaCl, $(NH_4)_2HPO_4$, glycerol, nitric acid and hydrogen peroxide (H_2O_2) were obtained from Shanghai Chemical Corporation (Shanghai, China). 3-(4,5-Dimethylthiozol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT), 2-[4-(2-hydroxyethyl)-1-pipera-zinyl]ethanesulfonic acid (HEPES) and 3,3',5,5'-tetramethylbenzidine dihydrochloride hydrate ($TMB•2HCl•H_2O$) supplied by Sigma were used without further treatment.

(2) Methods

Synthesis of $CePO_4$:Tb,Gd hollow spheres

$CePO_4$:Tb,Gd hollow nanospheres were prepared by a solvothermal technique as follows: stoichiometric amounts of high purity CeO_2, Tb_4O_7 and Gd_2O_3 (Ce:Tb:Gd=95:2.5:2.5, mol ratio) were dissolved in concentrated nitric acid and H_2O_2 by heating to form rare earth solutions. Appropriate volume of $(NH_4)_2HPO_4$ solution was dripped into the former solution. After that glycerol (40 mL) was added under vigorous stirring. And then the mixture was transferred into a Teflon-lined stainless steel autoclave with a filling capacity of 40%. The solvothermal reaction lasted for 18 h at 180 °C. The resulting products were washed with ethanol and de-ionized water for several times, and centrifuged at 8000 rpm. The obtained precipitates were redispersed in PBS (10 mL) to form $CePO_4$:Tb,Gd colloid.

Peroxidase mimetic activity study of $CePO_4$:Tb,Gd hollow nanospheres:

300 μL of TMB solution (5 mM) was mixed and sonicated with 100 μL of $CePO_4$:Tb,Gd colloid (100 μg/mL). Different volume of 30 wt.% H_2O_2 were then added into the mixture. The color change with reaction time was recorded by a digital camera and read on a spectrophotometer at 370 and 653 nm.

In order to investigate the effect of redox state on the catalytic properties, $CePO_4$:Tb,Gd samples were dipped in 0.5 M H_2O_2 or L-ascorbic acid for 24 h to obtain oxidized $CePO_4$:Tb,Gd (o-$CePO_4$) and reduced $CePO_4$:Tb,Gd (r-$CePO_4$), respectively.

Biocompatibility assessment of $CePO_4$:Tb,Gd hollow nanospheres

MTT viability assay, a colorimetric measure of mitochondrial activity, is used to assess the biocompatibility of the nanospheres. HeLa cells were cultured in a 96-well plate (Costar, approximately 4×10^3 cells per well) with DMEM Medium containing 10% FBS and different concentrations of the $CePO_4$:Tb,Gd nanospheres for 24 h. Then, 20 μL of MTT solution (5 mg/mL MTT in phosphate buffer solution, pH 7.4) was added to each well and incubated for 4 h at 37 °C. After removing the medium, intracellular formazan crystals were extracted into 150 μL of DMSO and quantified by measuring the absorbance of the cell lysate at 490 nm. Cell viability was expressed as a percentage of the control. All results are averages ± SD of five samples.

In Vitro Cellular Uptake of $CePO_4$:Tb,Gd hollow nanospheres

HeLa cells were used in this study. The DMEM Medium containing 10% fetal bovine serum (FBS) was utilized as cell culture medium. Cells were precultivated in 6

well plates (Costar) with 5% CO_2 in the medium at 37 °C until confluence has reached. $CePO_4$:Tb,Gd colloid diluted in the medium were added into the chamber, respectively. The cells were then incubated at 37 °C for 96 h to allow the cells to internalize the spheres. Then the cells were rinsed three times with PBS in order to remove the spheres remained in the chambers. Further, 80% acetone was used to fix the cells before fluorescent imaging.

TEM characterization of cells

HeLa cells incubated with $CePO_4$:Tb,Gd nanospheres (100 μg/mL) for 24 h were used in this study. The HeLa cells were thoroughly washed with PBS to remove the nanospheres that not taken up by the cells. Cells were then scraped from the culture flask (Corning) and centrifuged at 1000 rpm for 10 min. The cell pellets were fixed in a 0.1 M PBS solution containing 2.5% gluteraldehyde for 1 h. After postfixed in 4% osmium tetroxide solution, dehydrated in a graded series of acetone and embedded in epoxy resin, ultrathin sections of HeLa cells were obtained by an ultramicrotome and imaged under a 80 kV TEM.

Biodegradability investigation of $CePO_4$:Tb,Gd hollow nanospheres

The biodegradability of the $CePO_4$:Tb,Gd nanospheres was investigated by soaking them in saline solution (HBS) made from 150 mM NaCl buffered with 20 mM 2-[4-(2-hydroxyethyl)-1-pipera-zinyl]ethanesulfonic acid (HEPES) at pH=7. Typically, 0.05 g of $CePO_4$:Tb,Gd nanospheres was dispersed in 5 mL of HBS and placed in a dialysis membrane bag (8~12 kDa cut off) sanked into 100 mL of HBS. The entire system was kept at 37 °C with continuously shaking at a rate of 120 strokes

min^{-1} using a stroke length of 3 cm. After a predetermined period, 5 mL of the HBS medium was drawn out from release system for analysis, and 5 mL of fresh medium was added into the release system. The released Ce, Gd, Tb and P element amounts were determined by ICP. The results demonstrate that after 10 days of immersion in HBS solution (37 °C), the $CePO_4$:Tb,Gd nanospheres did not show detectable Ce/Gd/Tb leakage (Table S1). Moreover the weight and the morphology of the $CePO_4$:Tb,Gd samples before and after soaking in HBS are almost the same (data not shown), indicating the negligible biodegradability of the products within 10 days.

BET Analysis

BET analysis was performed to detect the cavities of the $CePO_4$:Tb,Gd nanospheres on a BET surface area analyzer. Surface area calculations were carried out using the BET method, whereas the pore size distribution was calculated according to the BJH algorithm. The results show that BET surface and total pore volume are 96.414 $m^2 g^{-1}$ and 0.248 $cm^3 g^{-1}$, respectively. The diameter of the internal cavities has a mean value of ca. 11.3 nm (inset of Fig. S9) which is derived from the nitrogen adsorption-desorption isotherm (Fig. S9) and calculated by BJH method, demonstrating the porous inner structure of the prepared $CePO_4$:Tb,Gd nanospheres.

Characterization

XRD studies were conducted on a Rigaku D/max-2500 X-ray powder diffractometer using Cu Kα radiation (λ=1.5406 Å). Inductively coupled plasma-optic emission spectrometry (ICP-OES, Perkin-Elmer Optima 2000) was used to analyze the element contents of the samples dispersed in ultrapure water. The morphological

investigations were carried out with field-emission scanning electron microscopy (FESEM, JEOL JSM-6700F), and transmission electron microscopy (TEM, JEOL JEM-2010). Fluorescent properties were performed at room temperature by a fluorescence spectrophotometer (F-4600, Hitachi). The magnetic relaxation properties of the $CePO_4$:Tb,Gd hollow nanospheres were examined with its aqueous suspension using a 1.5 T MRI system (Bruker Minispec mq60 NMR analyzer) at 300 K. Biocompatibility evaluated by MTT viability assay was performed on a Bio-Rad model 680 microplate reader. An inverted microscope (Eclipse TE2000S, Nikon) was used to image the cellular uptake of the products by HeLa cells. UV-Vis-NIR spectrophotometer (Cary500, Varian) was utilized to monitor the reaction between TMB and H_2O_2 catalyzed by the $CePO_4$:Tb,Gd nanospheres. Liquid nitrogen adsorption isotherms were measured by a BET surface area analyzer (NOVA1000, Quantachrome).

2. Figure S1-S11

Figure S1. XRD pattern $CePO_4$:Tb,Gd hollow nanospheres.

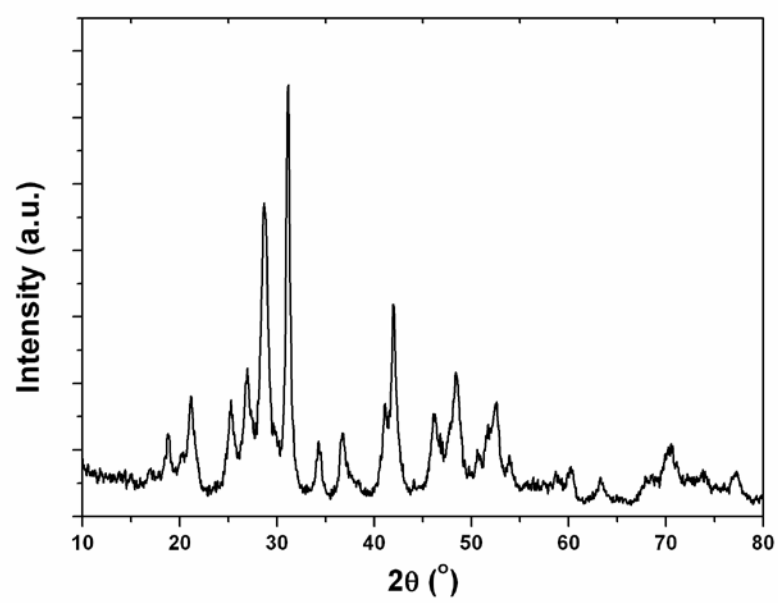

Figure S2. EDS spectrum of $CePO_4$:Tb,Gd hollow nanospheres.

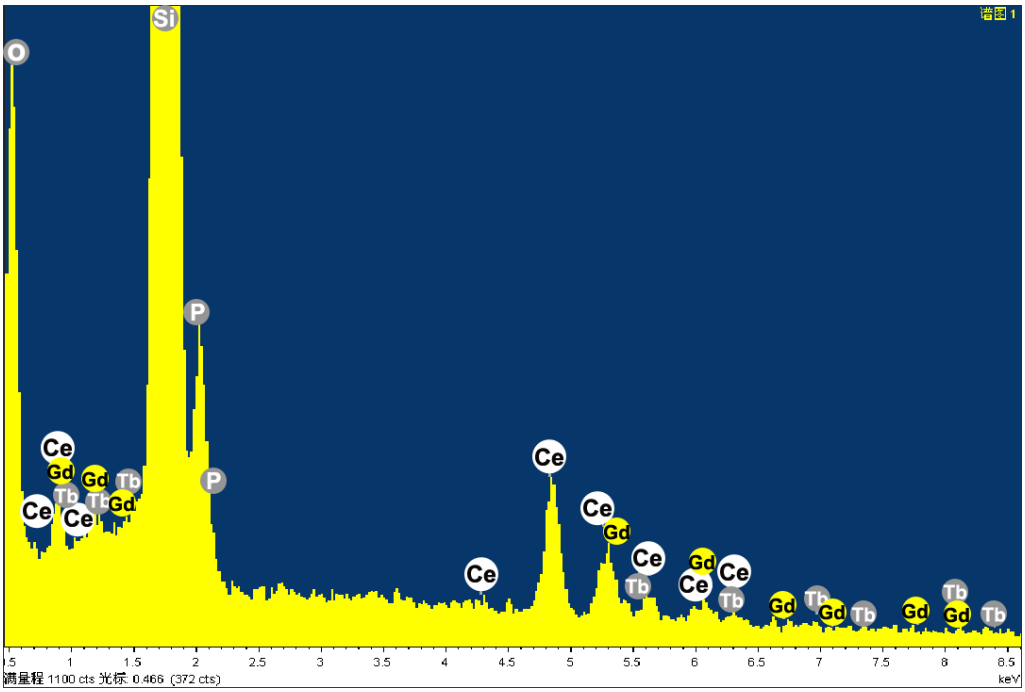

Figure S3. SEM images of $CePO_4$:Tb,Gd spheres obtained with different amount of H_2O_2: (A) 5 mL, (B) 2 mL and (C) 1 mL. The scale bars are 200 nm.

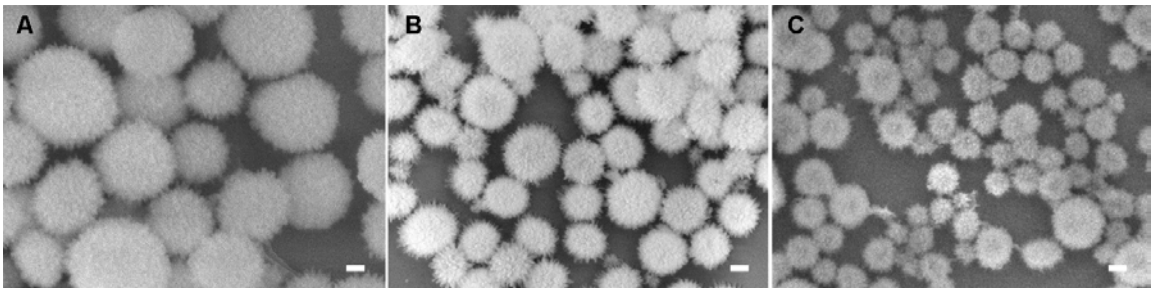

Figure S4. SEM images of $CePO_4$:Tb,Gd obtained at different reaction time: (A) 6 h, (B) and (C) 12 h.

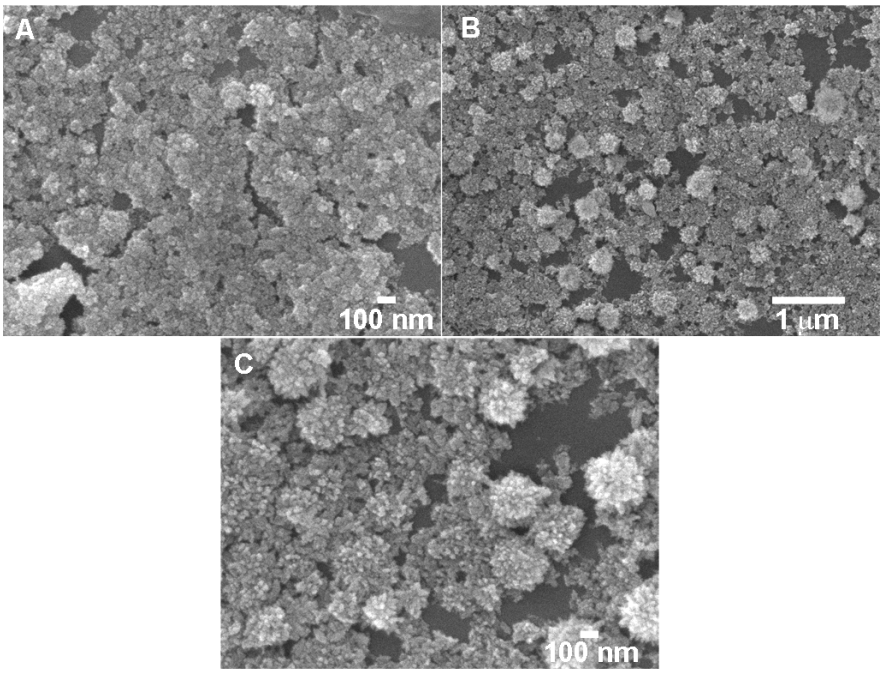

Figure S5. UV-Vis spectra of $CePO_4$:Tb,Gd samples oxidized by H_2O_2 (o-$CePO_4$), reduced by L-ascorbic acid (r-$CePO_4$) and $CePO_4$:Tb,Gd sample without any special treatment.

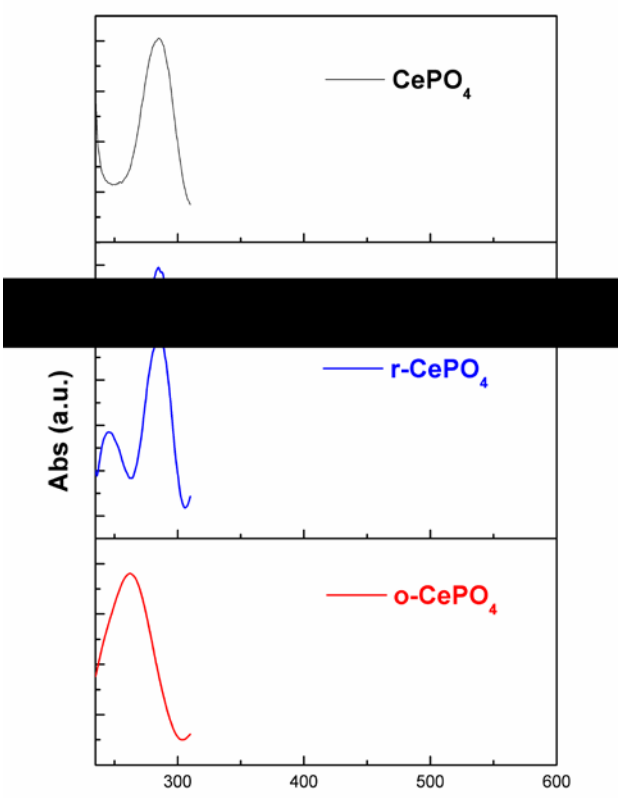

Figure S6. Time-dependent absorbance changes at 653 nm of TMB-H_2O_2 reaction systems catalyzed by different $CePO_4$:Tb,Gd samples.

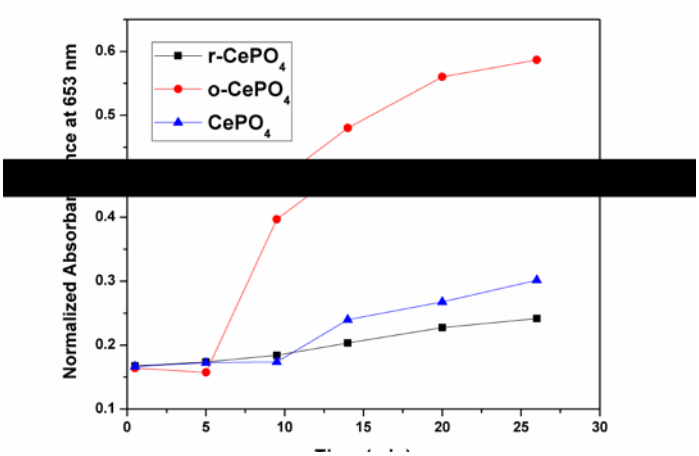

Figure S7. T_2 (left) and T_1 (right) relaxivity plots of aqueous suspensions of $CePO_4$:Tb,Gd hollow nanospheres, measured at 25 °C and 1.5 T.. Insets of (A) and (B) are T_2-weighted and T_1-weight images, respectively, at different Gd concentrations, measured at 25 °C and 3.0 T.

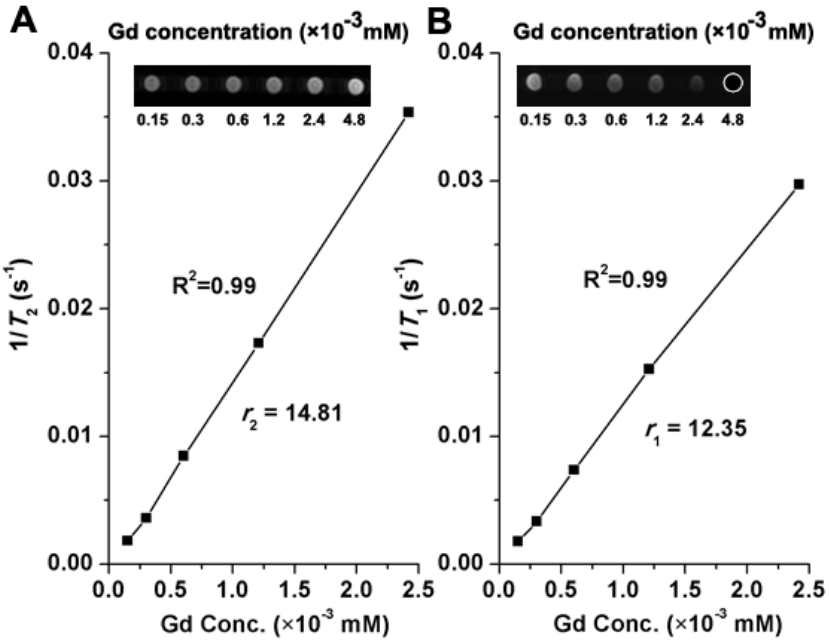

Figure S8. The biocompatibility of $CePO_4$:Tb,Gd hollow nanospheres analyzed using MTT assays. The HeLa cells were incubated with (A) $CePO_4$:Tb,Gd with varied concentrations for 24 h, (B) $CePO_4$:Tb,Gd with a fixed concentration of 100 μg/mL for 24~144 h before evaluation.

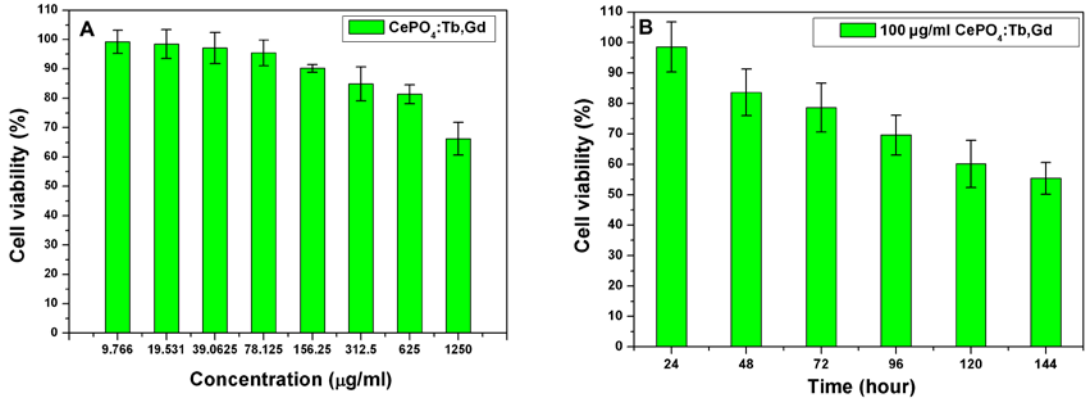

Figure S9. Nitrogen adsorption-desorption isotherm of $CePO_4$:Tb,Gd hollow nanospheres. The inset is its BJH average pore size distribution curve.

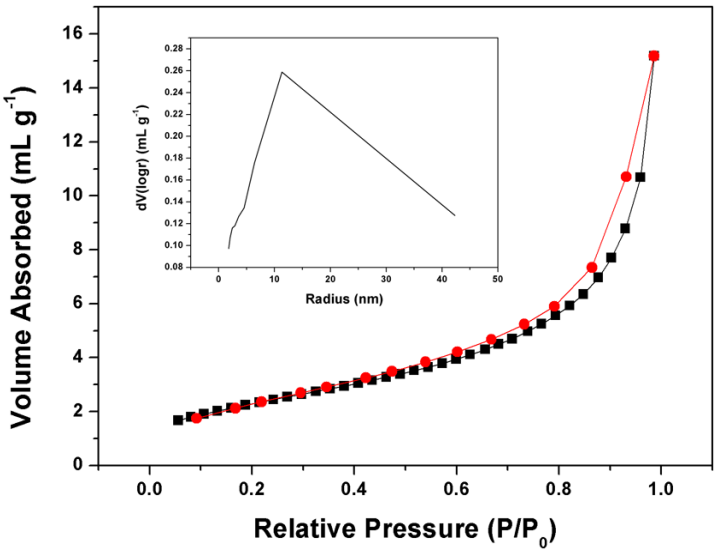

Figure S10. (A) Optical and (B) fluorescent images of HeLa cells without $CePO_4$:Tb,Gd hollow nanospheres. The scale bars are 10 μm.

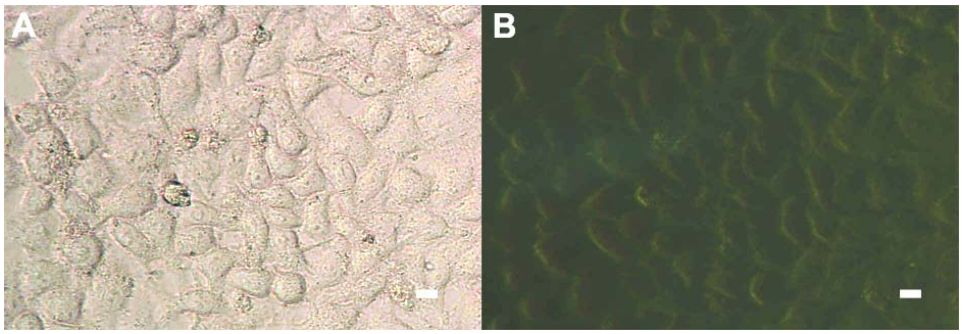

Figure S11. TEM image of $CePO_4$:Tb,Gd hollow nanospheres.

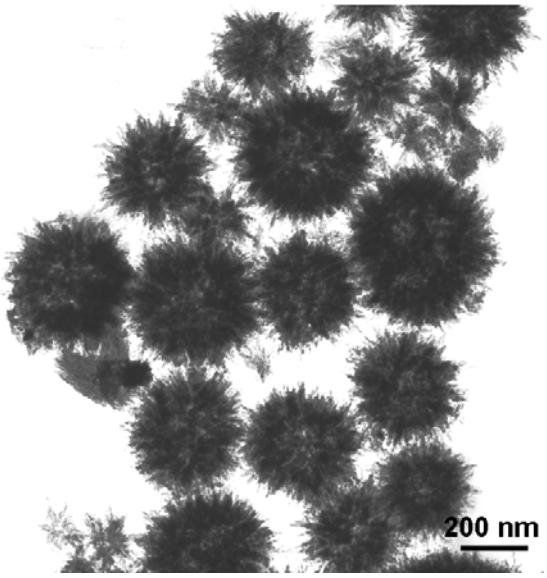

Table S1. Element contents of HBS solution after 10 days soaking of $CePO_4$:Tb,Gd hollow nanospheres measured by ICP*.

Element	Content ($\times 10^{-4}$ wt%)			
	3 days	5 days	7 days	10 days
Ce	0.0014	0.0021	0.0035	0.0034
P	2.5396	2.9867	3.1154	3.2597
Tb	0.0004	0.0005	0.0004	0.0009
Gd	0.0003	0.0002	0.0004	0.0004

*Samples were dispersed in ultra pure water with the concentration of 0.01 g/mL