

Supporting information

Tb-doped core-shell-shell nanophosphors for enhanced X-ray induced luminescence and sensitization of radiodynamic therapy

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Experimental

Synthesis of NaGdF₄: 15Tb @NaYF₄ core/shell nanoparticles. In a typical synthesis of NaGdF₄: 15Tb core nanoparticles, Gd(CH₃CO₂)₃·H₂O (0.85 mmol) and Tb(CH₃CO₂)₃·H₂O (0.15 mmol) were dissolved in mixture of 6 ml oleic acid and 15 ml octadecene. The mixture was heated to 130 °C under vacuum for 60 min to remove residual water and then cooled down naturally to room temperature. Subsequently, 10 ml methanol solution containing 2.5 mmol of NaOH and 4 mmol of NH₄F were injected to the mixture and stirred overnight. Thereafter, the reaction mixture was initially heated to 110 °C for 30 min to remove methanol and then heated to 300 °C for 2 hours under the nitrogen flow. The products were cooled down to room temperature and precipitated with ethanol. Finally, the obtained core/shell nanoparticles were washed with ethanol for three times and dispersed in 5 ml cyclohexane. To conduct the epitaxial shelling, the 1 mmol of Y(CH₃CO₂)₃·H₂O (99.9%) were dissolved in mixture of 6 ml oleic acid and 15 ml octadecene. The mixture was then heated to 130 °C under vacuum for 60 min to remove residual water and form the transparent oleate precursor. After that, the solution was cooled down to 80 °C and 2 ml of above prepared NaGdF₄: 15Tb core nanoparticles were injected to the solution. The resulting mixture was kept at 80 °C for 30 min under vacuum to remove excess cyclohexane and cooled to room temperature later. Subsequently, 10 ml methanol solution containing 2.5 mmol of NaOH and 4 mmol of NH₄F were injected to the mixture and stirred overnight. Thereafter, the reaction mixture was initially heated to 110 °C for 30 min to remove methanol and then heated to 300 °C for 2 hours under the nitrogen flow. The products were cooled down to room temperature and precipitated with ethanol. Finally, the obtained core/shell nanoparticles were washed with ethanol for three times and redispersed in 2 ml cyclohexane.

Synthesis of ligand-free (LF) CSS nanoparticles. The as-prepared core/shell/shell (CSS) nanoparticles (20 mg) were firstly dispersed in ethanol (750 μ l). Next, 750 μ l of HCl solution (1N) was added to the nanoparticle suspension and resulting mixture was then sonicated for 5 min to remove the oleate ligand. The LF nanoparticles were obtained by centrifugation at 14000 rpm for 20 min, washed three times with Milli-Q (MQ) water and ethanol and finally dispersed in MQ water.

Phantom X-ray Luminescence Experiments. Various concentrations of ligand-free nanoparticles (core only, core/shell and core/shell/shell) were prepared in black-bottom 96-well plates (200 μ l per well). The plate was placed on the treatment bed of a small-animal conformal irradiator (X-RAD SmART, Precision X-ray Irradiation). The gantry was rotated 90° to allow for the sample to be imaged optically from the top. In that configuration, the X-ray tube is on the side. An X-ray CT scan was performed to ensure that the plate is within the irradiated field. For X-ray luminescence imaging, the X-ray tube was turned to full power (225 kVp, 13 mA); at this power, the dose rate is approximately 3 Gy/min for open field (uncollimated). After \sim 10 s, a sequence of optical images ($n = 100$) were acquired with 0.1 s exposure, 2×2 pixel binning, and $50\times$ electron-multiplication gain. For background correction, the process was then repeated but with the samples removed from the imaging chamber. We also acquired dark images with the X-ray turned off. The images were processed to remove hot pixels due to X-ray photons hitting the charge-coupled device sensor. The basic procedure relies on the fact that X-ray noise is randomly distributed in the image, therefore there always exists at least one image (out of 100) for which a given pixel is free of X-ray noise. Images taken for the same experimental conditions were averaged (while excluding hot pixels), then filtered with a 2D Gaussian filter ($\sigma = 0.5$ pixels). Background images were subtracted. ROI analysis was performed by placing circular regions of interest (diameter =

10 pixels) on the wells and on the background to calculate the mean signal within the ROI. Error bars are ± 1 standard error of the mean. For cell imaging, the cell pellets were placed in 15 mL conical tubes, placed on the treatment bed, and irradiated using the same procedure.

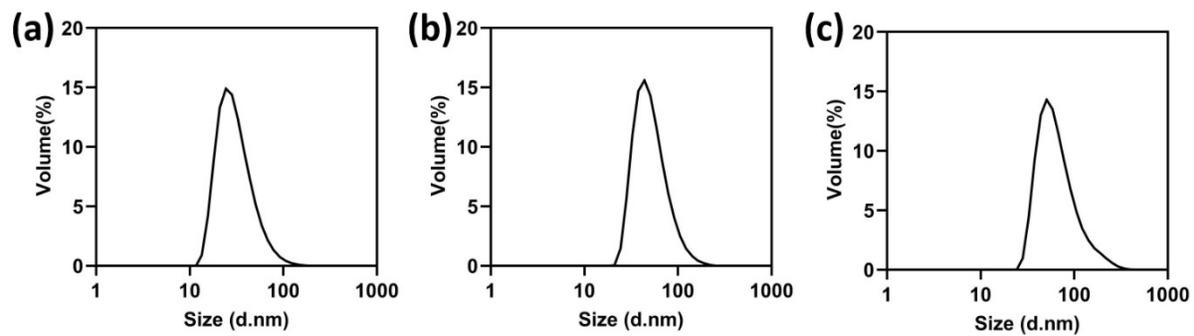


Figure S1. Size distribution of (a)LF-CSS, (b)CSS@Cit and (c)CSS@Cit@PLL nanoparticles in MQ water analyzed by DLS.

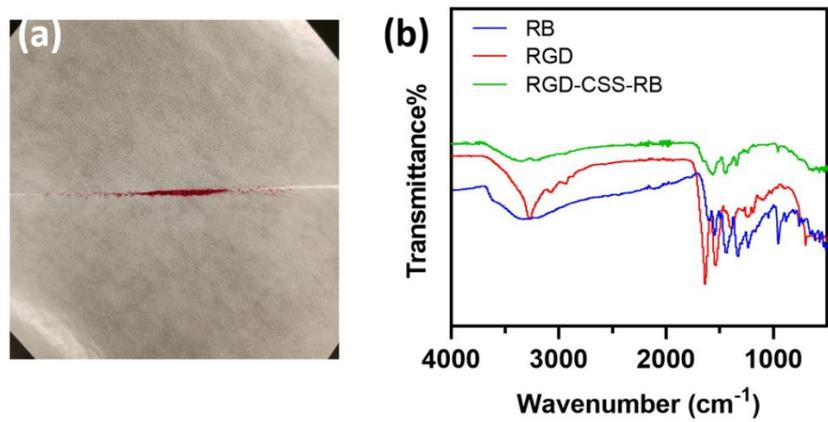


Figure S2. (a) Photo of lyophilized RGD-CSS-RB nanoparticles and (b) FTIR spectra of RB, RGD and RGD-CSS-RB.

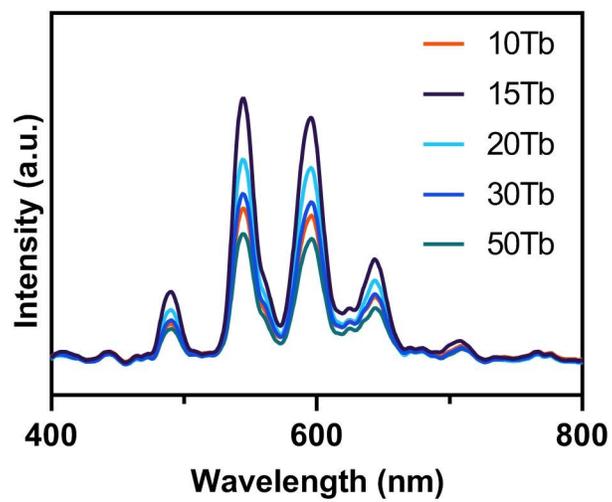


Figure S3. X-ray luminescence spectra of CSS nanoparticle with varied mol% Tb dopants.

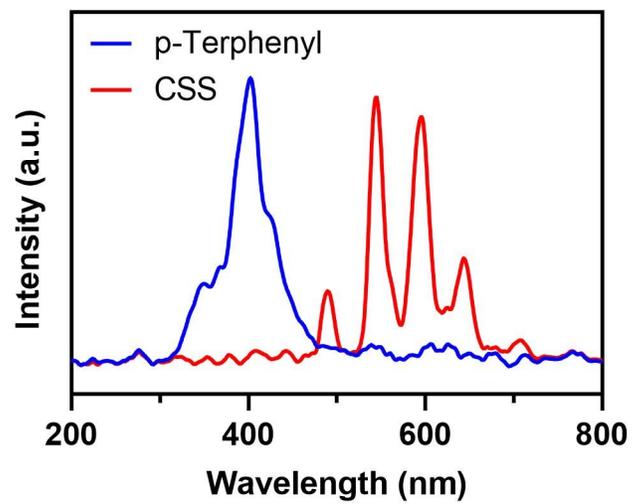


Figure S4. X-ray luminescence spectra of as-prepared CSS nanoparticle and organic liquid scintillator p-terphenyl toluene solution at 5 g/l.

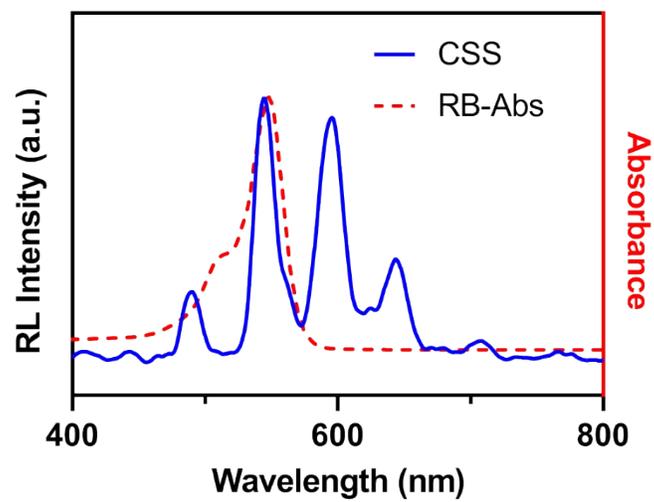


Figure S5. X-ray luminescence spectrum of CSS nanoparticle (blue line) and absorption spectrum of rose bengal (red line).

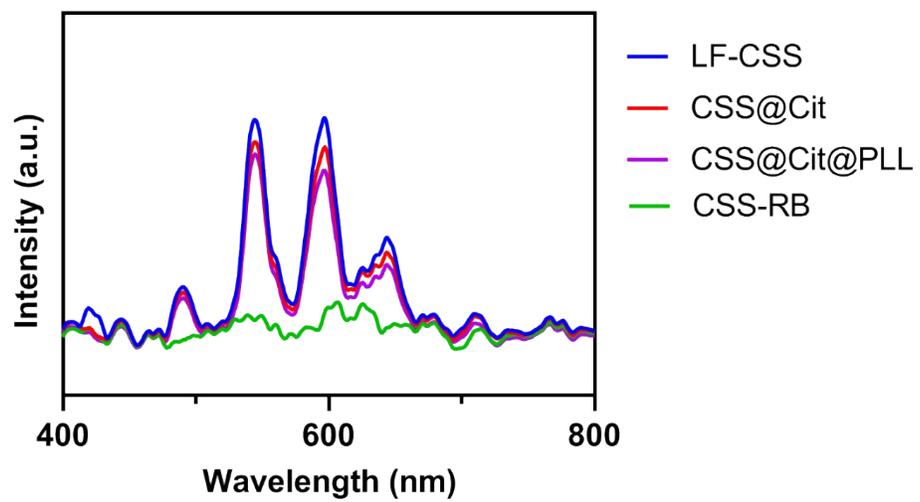


Figure S6. Luminescence spectra of CSS, CSS@Cit, CSS@Cit@PLL and CSS-RB nanoconjugate upon X-ray irradiation.

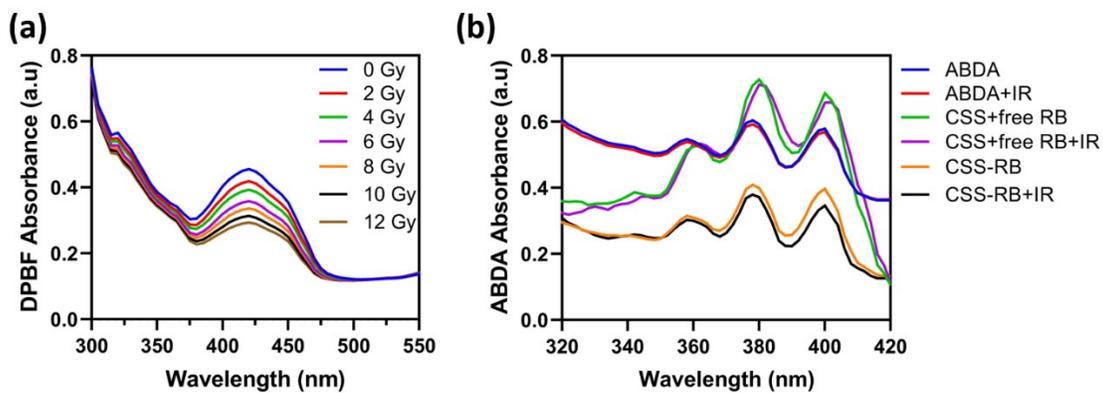


Figure S7. (a) The absorption spectra of DPBF in presence of CSS-RB nanoconjugate with varied X-ray Doses and (b) The absorption spectra of ABDA in different solutions with or without X-ray irradiation (4 Gy).

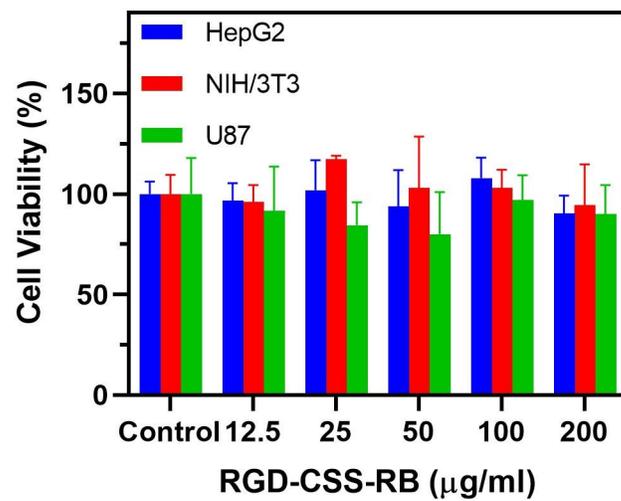


Figure S8. Cell viability of HepG2, NIH/3T3 and U87 cells incubated with RGD-CSS-RB nanoconjugates.

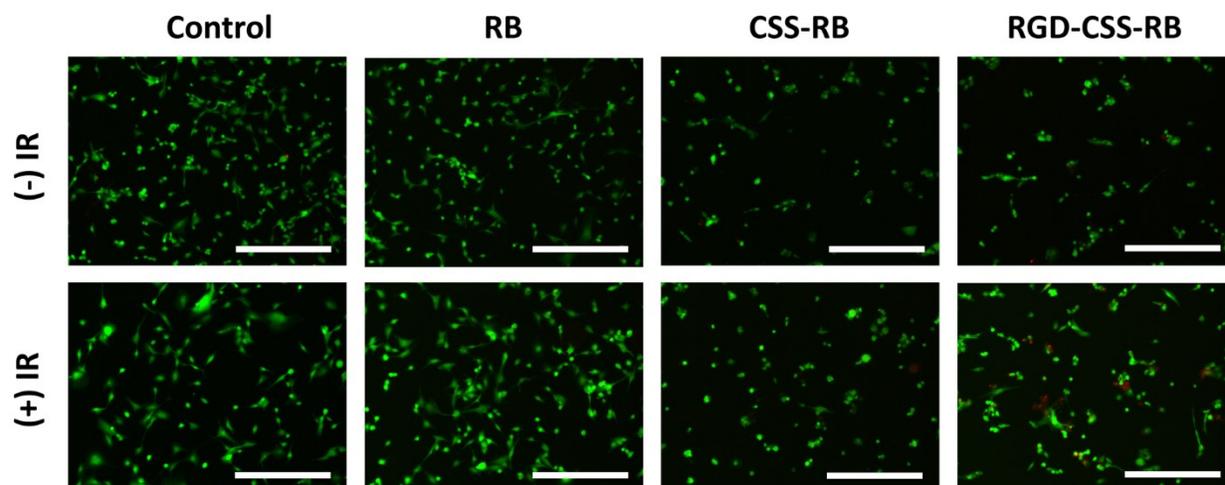


Figure S9. Live-dead staining of U87 cells with different treatments, Calcein AM (green fluorescence) for live cells and BI (red fluorescence) for dead cells. Scale bar represents 200 μm .