Supporting Information

High-Efficiency X-Ray Luminescence in Eu^{3+}-Activated Tungstate Nanoprobes for Optical Imaging through Energy Transfer Sensitization

Tao Guo,[a] Yan Lin,[a] Wei-Jian Zhang,[b] Jin-Sheng Hong,[b] Ru-Hui Lin,[c] Xiao-Ping Wu,[a] Juan Li,[a] Chun-Hua Lu,[*a] and Huang-Hao Yang,[*a]

[a]Key laboratory for analytical science of food safety and biology of the MOE, State Key Laboratory of Photocatalysis on Energy and Environment, College of Chemistry, Fuzhou University, Fuzhou 350116, P. R. China.

[b]Department of Radiation Oncology, The First Affiliation Hospital of Fujian Medical University, Key Laboratory of Radiation Biology (Fujian Medical University), Fujian Province University, Fuzhou 350005, P. R. China

[c]Academy of Integrative Medicine, Biomedical Research Center, Fujian University of Traditional Chinese Medicine, Fuzhou 350122, P. R. China

E-mail: chunhualu@fzu.edu.cn; hhyang@fzu.edu.cn

Experimental Section

Chemicals and apparatus. Gadolinium nitrate hexahydrate ($Gd(NO_3)_3 \cdot 6H_2O$) and Sodium tungstate dihydrate ($Na_2WO_4 \cdot 2H_2O$) were purchased from Sinopharm Chemical Reagent Co. Ltd (China). Europium(III) nitrate hexahydrate ($Eu(NO_3)_3 \cdot 6H_2O$) was purchased from Sigma-Aldrich. All other chemicals were of analytical grade and were used as received from manufacturer. Ultrapure water obtained from a Millipore water purification system (18.2 MΩ resistivity) was used in all runs.

Apparatus. Transmission electron microscopy (TEM) images were taken on the FEI Tecnai G20 transmission electron microscope with an accelerating voltage of 200 kV. X-ray diffraction (XRD) pattern was obtained by using an X-ray powder diffractometer (D/MAX-3C, Rigaku Co., Japan). The metal concentration of the ions in samples was determined by an Agilent 7500ce inductively coupled plasma mass spectrometry (ICP-MS). Photoluminescence (PL) measurements were performed using an Edinburgh FS5 fluorescence spectrophotometer (Edinburgh Instruments Ltd., UK). X-rays generated from a mini-X X-ray tube (Amptek, Inc.). UV-excited luminescence and X-ray-excited luminescence imaging were performed using a SI Imaging Amix small animal imaging system (Spectral Instruments Imaging Co., USA). The thermogravimetric analysis was performed on a Q5000 thermogravimetric analyzer (TA Instruments, USA).

Synthesis of PEG-NGW:xEu nanorods with different Eu^{3+} doped concentration: In a typical synthesis, the 4 mmol $Gd(NO_3)_3 \cdot 6H_2O$ and x mmol $Eu(NO_3)_3 \cdot 6H_2O$ (x = the mole ratio of $Eu^{3+}/(Gd^{3+} + Eu^{3+})$ is 0%, 5%, 10%, 15%, 20%) were dissolved in 5 mL deionzed water, respectively, and then transferred into a 25 mL flask. After vigorous stirring for 30 mins, 5 mmol of $Na_2WO_4 \cdot 2H_2O$ was slowly added dropwise into the above solution. After that, these

mixed solution were transferred into a 50 mL Teflon-lined autoclave with a stainless steel shell and then heated in a oven at 240 ^{o}C for 10 h. The resulting white precipitate was centrifuged (10000 rpm, 15 mins) and then washed with ethanol (10 mL) and water (10 mL) for three times. After this procedure, the products was redispersed in 2 mL of deionized water. To obtain water-soluble mPEG-SH modified NGW:Eu nanorods, the 2 mL of the NGW:Eu nanorods solution (2 mg mL^{-1}) were mixed with 2 mL of 30 mg methoxy-poly(ethylene glycol)-thiol (mPEG-SH, MW: 1000) and then stirred in water for 8 h. The excess mPEG-SH was removed by centrifugation (20000 rpm, 15 mins) and then washed with ethanol (10 mL) and water (10 mL) for three times. Finally, the products was redispersed in water and stored at room temperature for further use. To synthesis the PEG-NGW:10%Eu of big size with length for 2400 nm and width for 120 nm, the only change is that using 16 mmol $Gd(NO_3)_3{\cdot}6H_2O$ and 1.7 mmol $Eu(NO_3)_3{\cdot}6H_2O$ in the above reaction.

Synthesis of $NaGdF_4$:15%Eu nanorods. The $NaGdF_4$:15%Eu nanorods were synthesized according to the previous report with slight modification.[1] In the first step, 0.85 mmoL of $Gd(NO_3)_3{\cdot}6H_2O$ and 0.15 mmoL of $Eu(NO_3)_3{\cdot}6H_2O$ were dissolved in 10 mL of aqueous solution, and then kept stirring for 10 min. Then 25 wt% of ammonia solution was introduced dropwise to the above vigorously stirred solution until pH = 9. After kept stirring for 1 h, the as-obtained white colloidal precipitate was heated to 200 °C for 12 h in a 25 mL stainless Teflon-lined autoclave. After this procedure, the precursors were collected by centrifugation (10000 rpm, 20 mins), washed with water for three times, and then redispersed in 5 mL water. In the second step, a 12 mL of aqueous solution containing 0.12 g NaF and 0.5 mL HF (40%) were dropwise added into the above precursors solution and then kept stiring for 1 h. After the reaction, the above solution was heated to 200 °C for 12 h in a

25 mL stainless Teflon-lined autoclave. Finally, the resulting precipitates were collected by centrifugation (12000 rpm, 10 mins), washed with water for three times, and then redispersed in water for further use.

Synthesis of Gd_2O_3:18%Eu nanorods. Gd_2O_3:18%Eu nanorods were prepared using a well-established method.[2] Briefly, a 5 mL of aqueous solution containing $GdCl_3 \cdot 6H_2O$ (0.82 mmol) were added into 5 mL of aqueous solution of $EuCl_3 \cdot 6H_2O$ (0.18 mmol) under the stirring condition. Then 25 wt% of ammonia solution was introduced dropwise to the above vigorously stirred solution until pH = 7-9. After kept stirring for 1 h, the as-obtained white colloidal precipitate was heated to 200 °C for 24 h in a 50 mL stainless Teflon-lined autoclave. When the reaction was completed, the reaction mixture was cooled to room temperature. After this procedure, the precipitates were collected by centrifugation (10000 rpm, 20 mins), washed with water for three times, and then dried under vacuum at 60 °C. Finally, the products was placed in a tube furnace and heated to 900 °C for 3 h.

Quantification of Gd, W and Eu ions in PEG-NGW:Eu nanorods by using ICP-MS: Typically, 2.5 mL HNO_3 were added to a beaker which contained 50 μL PEG-NGW:Eu nanorods, and then sealed for predigestion overnight. After that, 1.5 mL of 30% H_2O_2 were added to the above beaker and subsequently heated to 150 °C. After the digestion was complete, the solution was cooled to room temperature. Finally, this solution was diluted to 10 mL with 3% HNO_3 for ICP-MS measurement.

Cell culture and cytotoxicity assay: HeLa cells were cultured in RPMI-1640 medium (Gibco) with 10% fetal bovine serum (FBS, Gibco) at 37 ^oC in a humidified atmosphere with 5% CO_2.

Cell viabilities was measured with CCK-8 according to the manufacturer's protocol. In a typical experiment, HeLa cells (1×10^4) were seeded in 96-well plates and then incubated with 20 μL of varying concentration of PEG-NGW:Eu nanorods for 24 h at 37 ^oC in a humidified 5% CO_2 atmosphere. Cell viabilities were determined by CCK-8 according to the manufacturer's protocol.

MRI phantom and relaxivity studies at 0.5 T: The MRI phantom and relaxivity studies were performed on a 0.5 T NMI20-Analyst NMR system (Niumag Corporation, Shanghai, China). A series of PEG-NGW:Eu nanorods or Gd-DTPA aqueous solution with the same Gd ions concentration (0.05, 0.1, 0.2, and 0.4 mM) were prepared. T_1-weighted phantom images were acquired using a 2D multi-slice spin-echo (MSE) sequence with the following parameters: TR/TE = 100/2 ms, 512 × 512 matrices, slices = 1, thickness = 1 mm, NS = 4, temperature = 32 ^oC. The T_1 times were measured by an inversion recovery (IR) sequence. The r_1 values were determined from the slope of the plot of $1/T_1$ against Gd ions concentration.

In vivo MRI: The BALB/c nude mice (weight ~20 g) were obtained from Shanghai SLAC laboratory Animal Co., Ltd. All animal experiments were performed in accordance with the Guide for the Care and Use of Laboratory Animals (Ministry of Science and Technology of China, 2006) and approved by the Animal Ethics Committee of Fujian Medical University. Tumor-bearing mice were prepared by subcutaneously injecting a suspension of 2×10^6 Hela cells in PBS (100 μL) into the mice.

In vivo MRI was performed on a 7 T MRI scanner (Bruker Biospec 70/30 USR). Axial and coronal two-dimensional (2D) fast spin echo sequence images were first acquired to ensure

the imaging position of the implanted tumor. T_1-weighted multislice spinecho images were collected before and after intratumor injection of PEG-NGW:Eu nanorods (40 μL, 50 μg mL^{-1}) using the following parameters: TR/TE = 800/4 ms, matrix = 256 × 256, thickness = 2 mm, FOV = 60 × 60.

CT imaging: *CT imaging was carried out by using a Micro-CT system for small animal imaging.* For in vitro iamging, the PEG-NGW:Eu nanorods and Iohexol with the same concentrtion of 0, 0.29 mM, 0.58 mM, 1.15 mM, 2.3 mM, 4.6 mM for W and I elements were used for assessing the CT imaging effect. For in vivo imaging, 45 μL of 75 μg mL^{-1} PEG-NGW:Eu nanorods were intratumor injection into the Tumor-Bearing nude mice for CT imaging (Imaging parameter: 60 mm FOV, 90 kV tube voltage, 160 μA tube current).

In vivo X-ray luminescence imaging of PEG-NGW:Eu nanorods: All animal experiments were performed in accordance with the Guide for the Care and Use of Laboratory Animals (Ministry of Science and Technology of China, 2006) and approved by the Animal Ethics Committee of Fujian Medical University. BALB/c nude mice (weight ~20 g) were obtained from Shanghai SLAC laboratory Animal Co., Ltd. Tumor-bearing mice were prepared by subcutaneously injecting a suspension of 2×10^6 HeLa cells in PBS (100 μL) into the mice.

In vivo X-ray luminescence imaging of PEG-NGW:Eu nanorods were performed with SI Imaging Amix small animal imaging system. PEG-NGW:Eu nanorods (50 μL, 100 μg mL^{-1}) and InP/ZnS QDs (50 μL, 45 μg mL^{-1}) aqueous solution were injected through intramuscular into two mice, respectively. In addition, the PEG-NGW:Eu nanorods (200 μL, 300 μg mL^{-1}) and InP/ZnS QDs (200 μL, 135 μg mL^{-1}) aqueous solution were injected into another two mice via intravenous injection. These mice were imaging under X-ray (40 kV, 70 μA) or UV excitation

(Ex 430 nm), and acquired the luminescence signal by small animal imaging system (SI Imaging Amix). A scientific CCD (size: 25.9×17.3 mm, operating temperature: -90 ℃, pixel: ≥ 880000) provided by Cold Spring Harbor Laboratory Press was used throughout the study.

1. Z. Xu, C. Li, D. Yang, W. Wang, X. Kang, M. Shang and J. Lin, *Physical chemistry chemical physics : PCCP*, 2010, **12**, 11315-11324.

2. J. Yang, C. X. Li, Z. Y. Cheng, X. M. Zhang, Z. W. Quan, C. M. Zhang and J. Lin, *J. Phys. Chem. C*, 2007, **111**, 18148–18154.

Supporting Figures

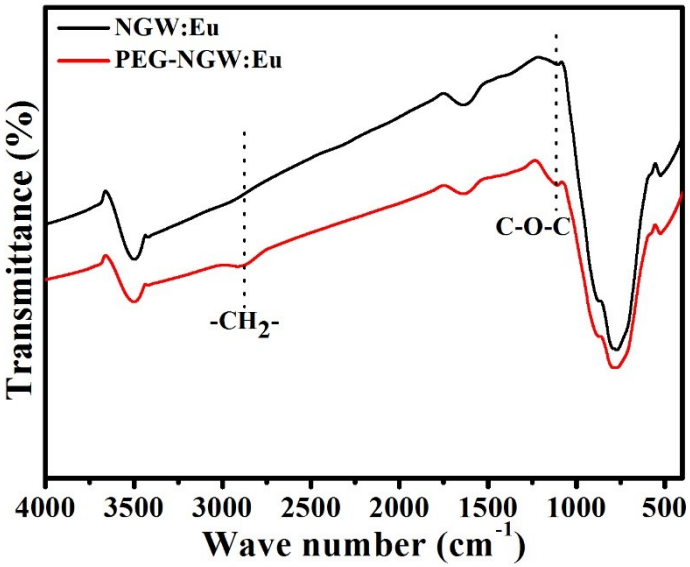

Figure S1. Fourier transform infrared (FT-IR) spectrum of PEG-NGW:Eu nanorods. The IR absorption bands located at 2870 cm^{-1} and 1110 cm^{-1} were ascribed to the symmetric (v_s) stretching vibrations of methylene (CH_2) groups and stretching vibrations of C-O-C in PEG chain, respectively. This results indicated that the successfully formation of PEGylated NGW:Eu nanorods.

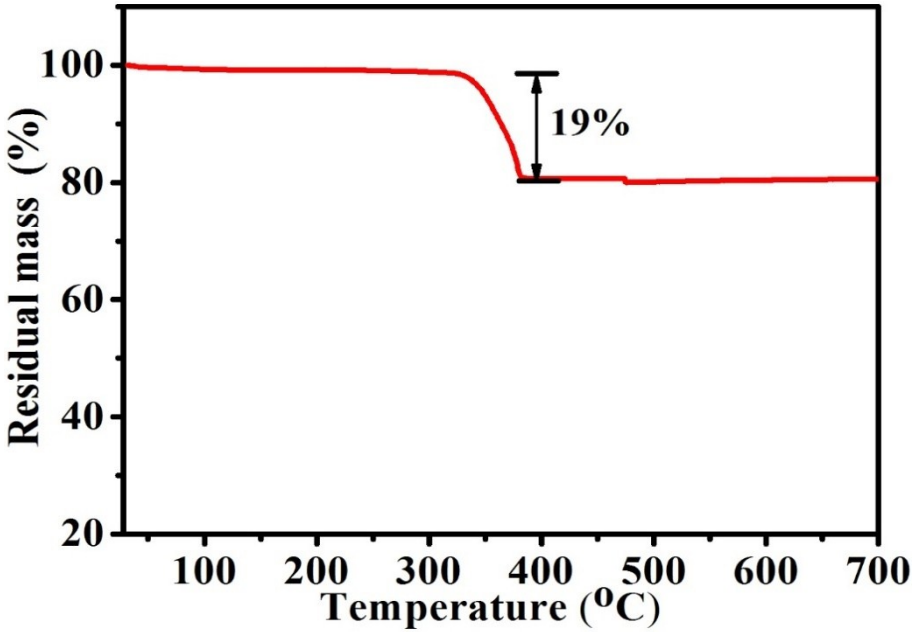

Figure S2. Residual mass (%) versus temperature thermogram of PEG-NGW:Eu nanorods evaluated using TGA measurement.

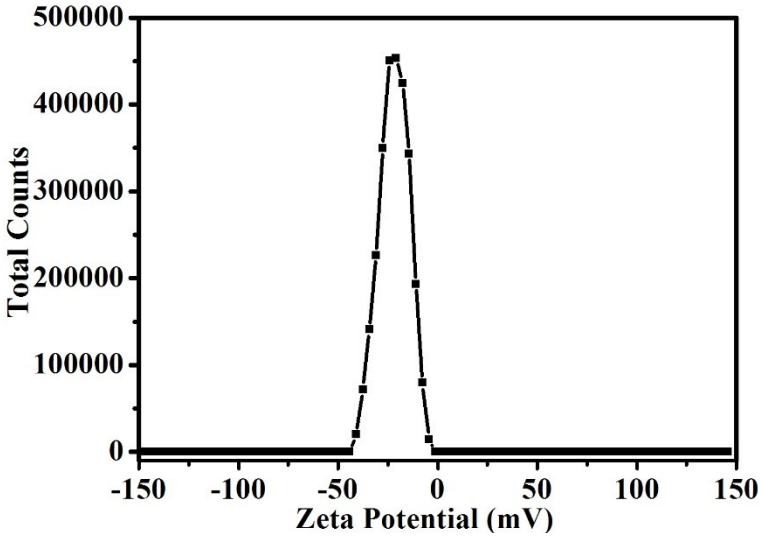

Figure S3. Zeta-potential of PEG-NGW:Eu nanorods dispersed in water.

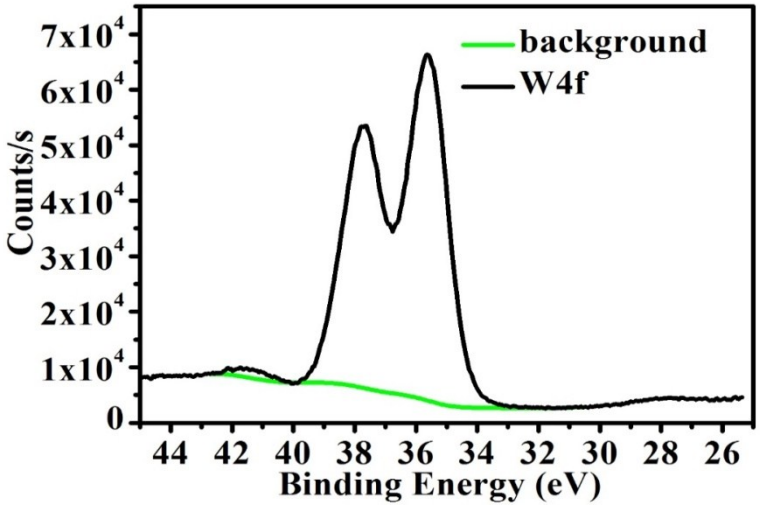

Figure S4. XPS spectrum of the W ions in PEG-NGW:Eu nanorods.

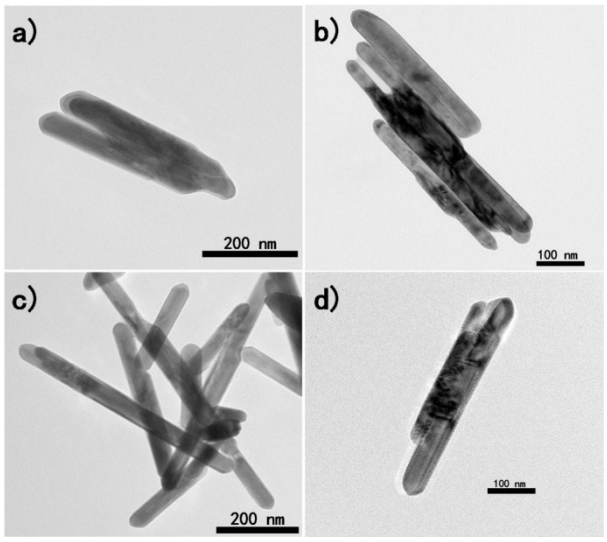

Figure S5. TEM images of PEG-NGW:xEu nanorods. a) x = 0%. b) x = 5%. c) x = 15%. d) x = 20%.

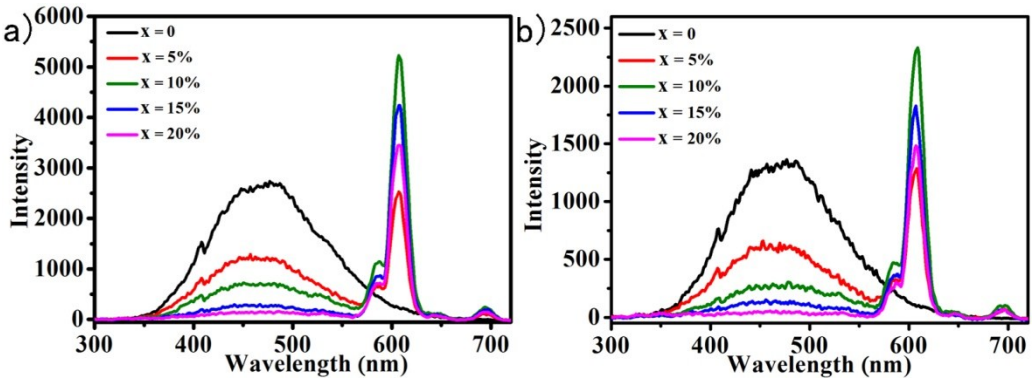

Figure S6. PL spectra of PEG-NGW:xEu nanorods with various Eu^{3+} doping concentration (0, 5, 10, 15, 20%) under a) X-ray excitation (50 KV, 35 μA) and b) X-ray excitation (50 KV, 20 μA). The concentrations of PEG-NGW:xEu nanorods in aqueous solution were 200 μg mL^{-1}.

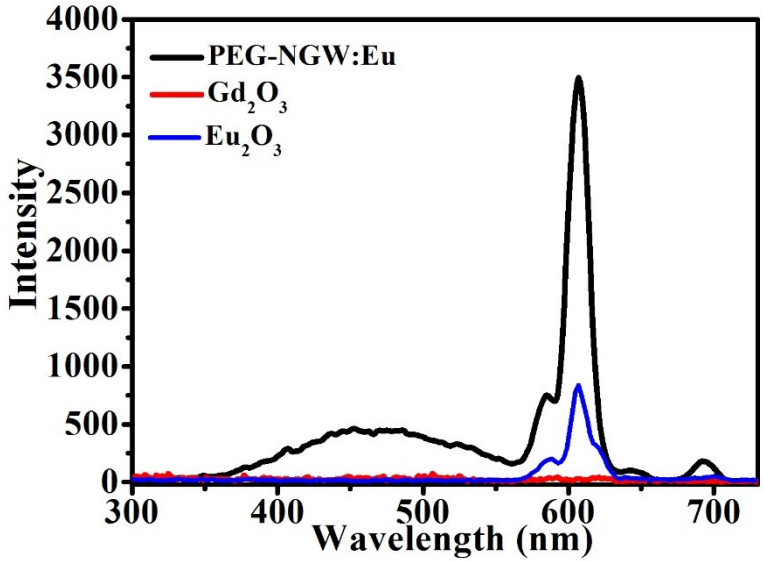

Figure S7. PLE spectrum of PEG-NGW:Eu, Gd_2O_3 and Eu_2O_3 nanoparticles under X-ray excitation. The luminescence intensities were measured at the concentration of 65 μg mL^{-1} for all particles in aqueous solution and under the same X-ray excitation condition (40 KV, 70 μA). Compared to the PEG-NGW:Eu, there was no luminescence peak (λ = 470 nm) in both spectra of Gd_2O_3 and Eu_2O_3 nanoparticles under X-ray excitation condition. These results further supported that the luminescence peak of PEG-NGW:Eu at λ = 470 nm (5d-2p) was ascribed to the WO_4^{2-} group.

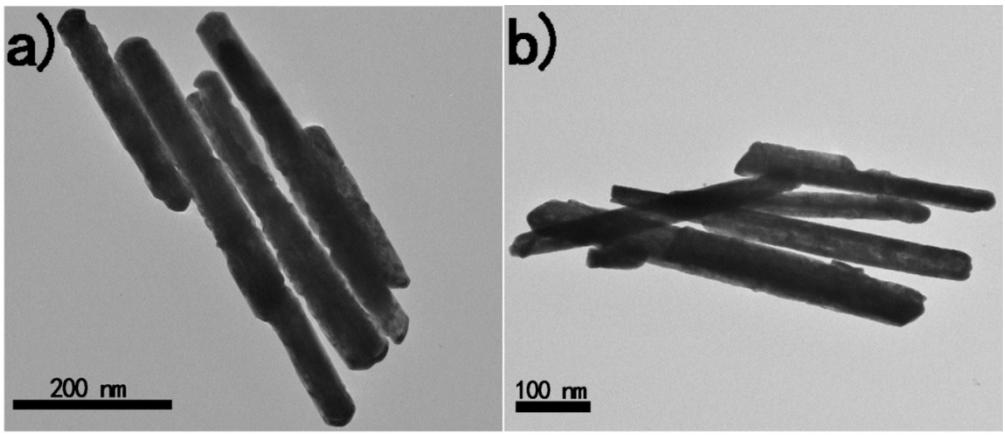

Figure S8. TEM images of a) $NaGdF_4$:15%Eu and b) Gd_2O_3:18%Eu nanorods.

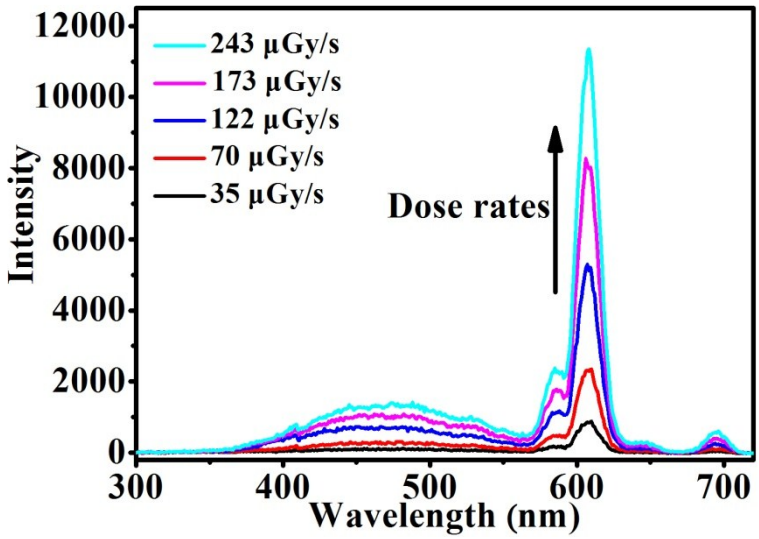

Figure S9. PL spectra of PEG-NGW:Eu nanorods under X-ray excitation with the dose rates range from 35 to 243 µGy/s.

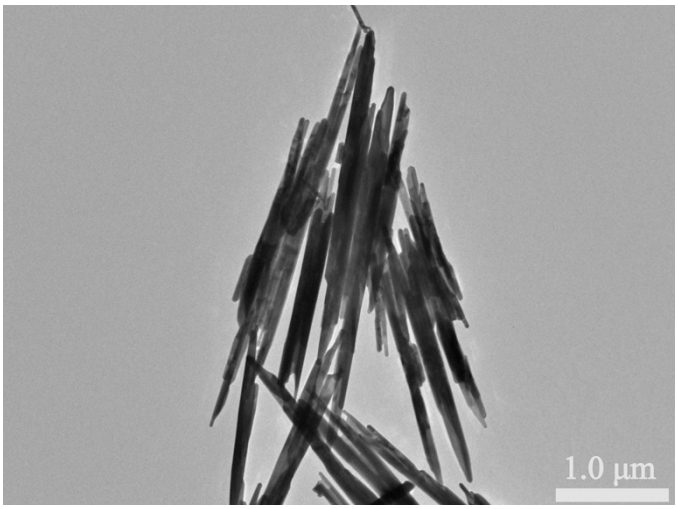

Figure S10. TEM image of the PEG-NGW:10%Eu nanorods of big size with the average length for 2400 nm and width for 120 nm.

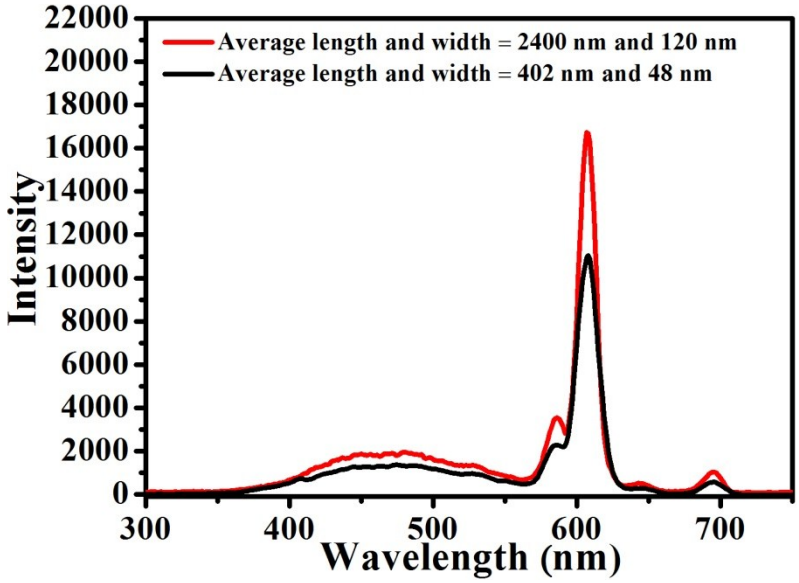

Figure S11. PL spectra of PEG-NGW:10%Eu with two different sizes (average length and width = 2400 nm and 120 nm; average length and width = 402 nm and 48 nm) under X-ray excitation. The luminescence intensities were measured at the concentration of 200 μg mL^{-1} for all particles in aqueous solution and under the same X-ray excitation condition (40 KV, 70 μA).

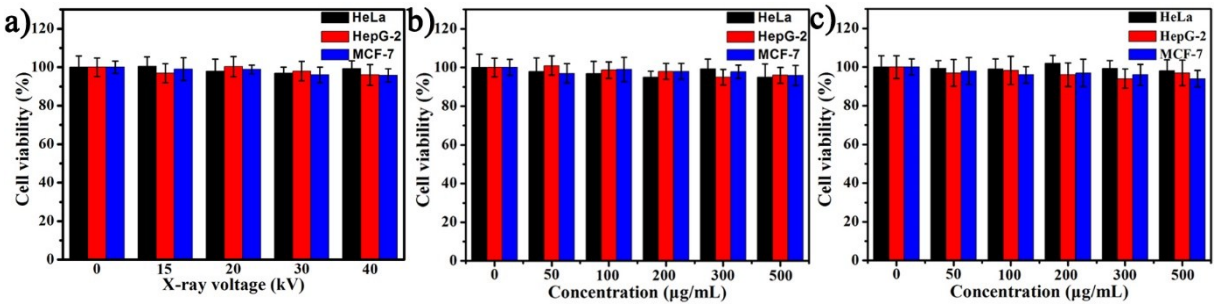

Figure S12. The biological safety of X-ray light source and the biocompatibility of the PEG-NGW:Eu nanorods. (a) Cell viabilities of HeLa, HepG-2, MCF-7 cells under X-ray irradiation for 5 mins with different X-ray tube voltage (40 KV, 70 μA). (b) Cell viabilities of the HeLa, HepG-2, MCF-7 cells incubated with the PEG-NGW:Eu nanorods for diffierent concentration. (c) Cell viabilities of the HeLa, HepG-2, MCF-7 cells incubated with the PEG-NGW:Eu nanorods for diffierent concentration under X-ray irradiation (5 mins, 40 kV, 70 μA).

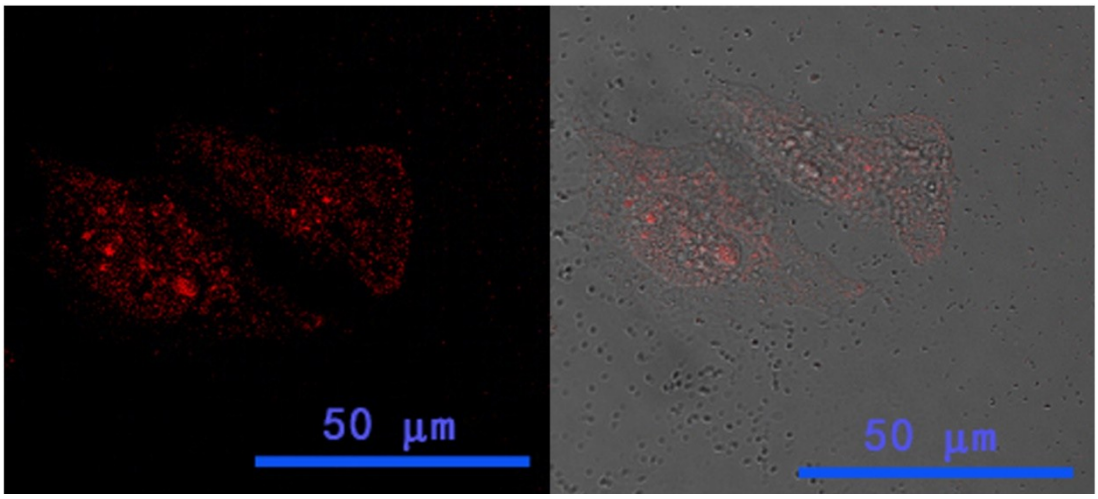

Figure S13. (a) Confocal fluorescence image and (b) overlay image of bright field and confocal fluorescence image of the HeLa cells incubated with PEG-NGW:Eu nanorods for about 4 h (excitation wavelength: 488 nm).

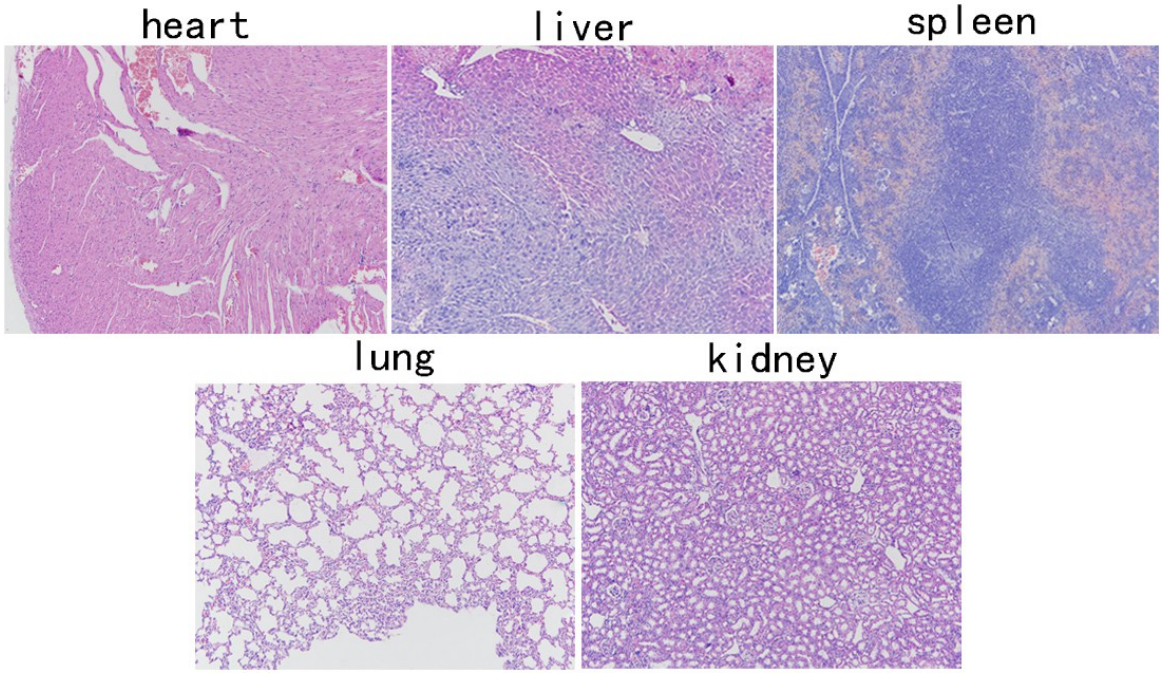

Figure S14. H&E stained images of major organs of mice at the 7 days after intravenous injection with PEG-NGW:Eu nanoprobes.

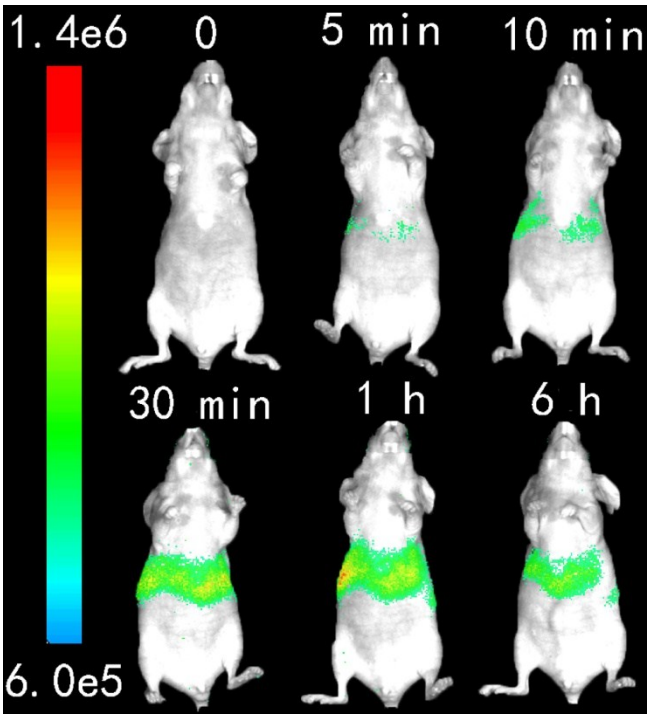

Figure S15. The time-dependent *in vivo* X-ray excited luminescence bioimaging by intravenous injection with PEG-NGW:Eu nanorods (200 µL, 300 µg mL^{-1}).

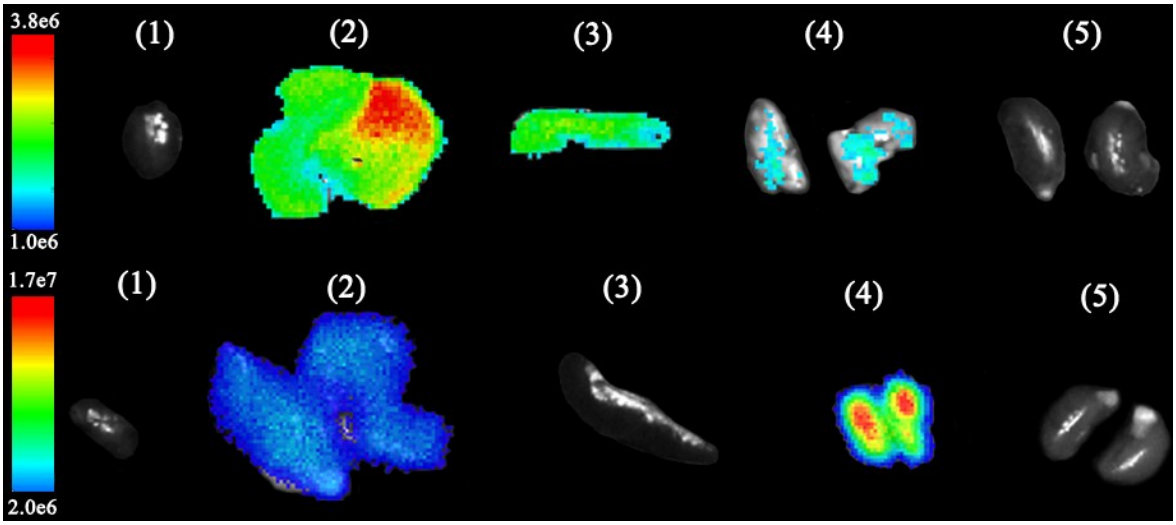

Figure S16. Representative ex vivo X-ray excited (upper row) and UV excited (lower row) luminescence images of isolated organs from two mice at thirty minutes post-intravenous injection of PEG-NGW:Eu and InP/ZnS QDs respectively: (1) heart, (2) liver, (3) spleen, (4) lung, (5) kidney.

Injection Method / signal-to-background / Imaging Model	Intramuscular Injection			Intravenous Injection		
	Signal	Background	signal-to-background ratio	Signal	Background	signal-to-background ratio
UV-excited luminescence	9.67E+7	3.02E+7	3.2	6.07E+7	4.56E+7	1.3
X-ray excited luminescence	3.11E+6	1.35E+5	23.1	1.04E+6	5.07E+4	20.5

Figure S17. Luminescence signal intensities of target and background for imaging.

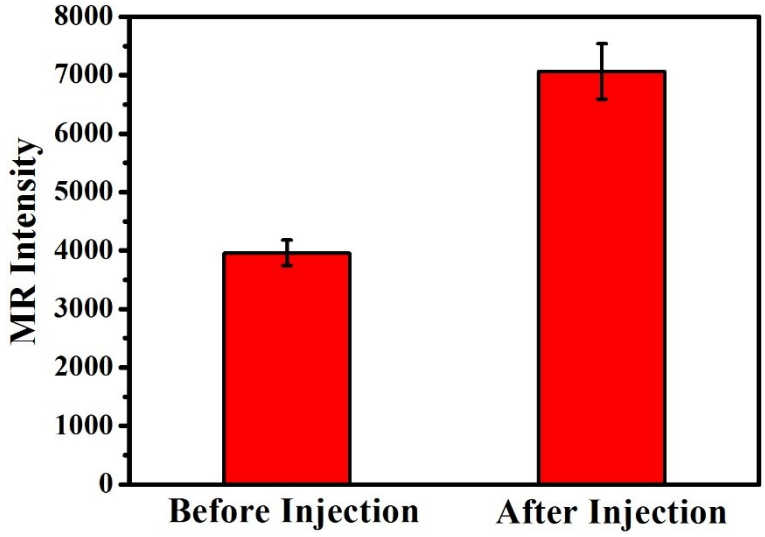

Figure S18. Quantification of MRI images intensities for mice tumor sites before and after the injection of PEG-NGW:Eu nanorods.

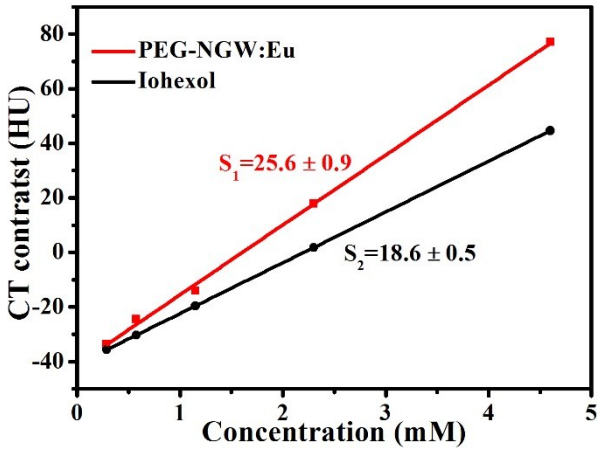

Figure S19. HU values of PEG-NGW:Eu nanorods and commercial Iohexol as a function of the concentration of tungsten (red) and Iohexol (black), respectively. The slope values were determined by three independent experiments.

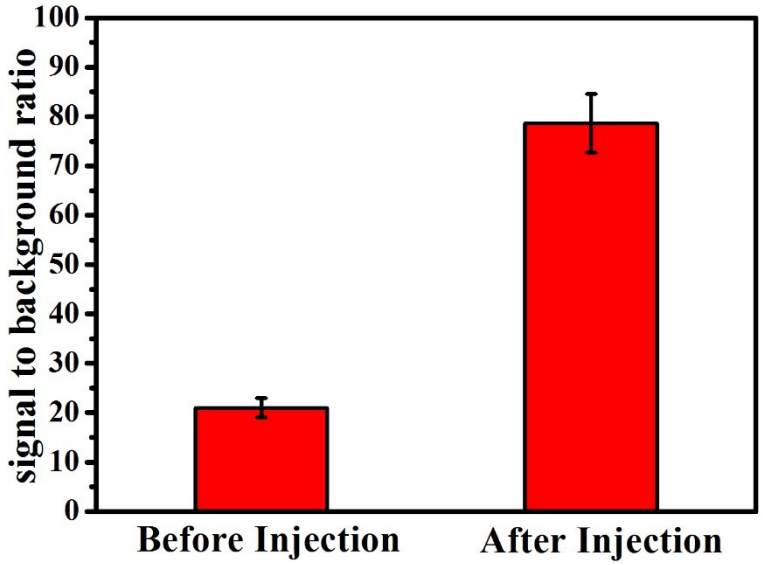

Figure S20. Quantification of CT images signal-to-background ratio for mice tumor sites before and after the intratumoral injection of PEG-NGW:Eu nanorods.