

Supporting information

Multi-functional mesoporous β -Ga₂O₃:Cr³⁺nanorod with long lasting near infrared luminescence for *in vivo* imaging and drug delivery

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1. Detailed Experimental Section

Materials: Gallium Oxide (Ga₂O₃, 99.99%), hydrogen peroxide (H₂O₂, 30%), chromic nitrate (Cr(NO₃)₃•9H₂O, 99.95%) were purchased from Aladdin (Shanghai, China). Doxorubicin hydrochloride (DOX) was purchased from Dalian Mei lun Biology Technology Co. Ltd (Dalian, China). Griess reagent was purchased from Beyotime institute of Biotechnology (Jiangsu, China). Cell Counting Kit-8 (CCK-8) was purchased from DOJINDO(Japan). All the materials were used without purification.

L-929, HeLa, MCF-7, Raw 264.7 cells were obtained from the Institute of Biochemistry and Cell Biology, SIBS, CAS (Shanghai, China). Fetal bovine serum (FBS) was purchased from Zhejiang Tian hang Biological Technology Co. Ltd (Hu zhou, China). RPMI 1640 and DMEM culture medium with penicillin (100 unit/ml) and streptomycin (100 unit/ml) were purchased from Ji nuo Biomedical Technology Co. Ltd (Hangzhou, China). Cells were cultivated in RPMI 1640 with 10% FBS, at 37 °C with 5% CO₂.

BALB/C nude mice were purchased from shanghai laboratory animal center. The animal studies were approved by the Ethical Committee of Zhejiang University, Hangzhou, China.

Synthesis and characterization of GaOOH:Cr³⁺ and β -Ga₂O₃:Cr³⁺nanorod: The β -Ga₂O₃:Cr³⁺nanorod was synthesized by the hydrothermal process followed by calcination. Firstly, Ga₂O₃ (0.1874 g) was dissolved in 40 ml HCl (1 M) through stirring and heating. Then 5 ml PEG-400 and 100 μ l Cr(NO₃)₃ (0.1 M) were mixed and diluted with H₂O to the final volume of 50 ml. The above solution was mixed homogeneous by magnetic stirring at room temperature for 0.5 h, and then adjusted pH to 7.0 by NaOH (2 M) for the formation of white floccules. The floccules were put into a teflon-lined autoclave with 80 ml capacity and maintained at 120°C for 1 h. After the autoclave was cooled to room temperature, the white precipitate was collected by centrifugation (4000rpm, 10min). After being maintained at 110°C for 24h, the precipitate was pre-fired at 600°C in air for 3h followed by another calcination at 1000°C for 3h.

GaOOH:Cr³⁺ was characterized by Zeta sizer Nano (S90 Malvern), Thermal Analysis (DSCQ1000, AT America) with the heating rate of 10.00 °C/min from room temperature to 800 °C in air. GaOOH:Cr³⁺ and β -Ga₂O₃:Cr³⁺ were characterized by X-ray diffraction (X'Pert PRO, Panalytical), transmission electron microscopy with an acceleration voltage of 80kV (TEM; JEM-1200EX, Japan). Pore structure of the β -Ga₂O₃:Cr³⁺ was characterized by automated surface area and pore size analyzer (TRISTAR 3020, micromeritics). The excitation and emission spectra of nanoparticle were acquired by fluorescence spectrophotometer (FLS920, Edinburgh Instruments, England).

In vitro characterization of afterglow: solid β -Ga₂O₃:Cr³⁺ was exposed to UV for 3min, and then coated by tinfoil for protection from light. After appropriate time, the afterglow image of the β -Ga₂O₃:Cr³⁺ was acquired by in-vivo imaging system (NIGHTOWL LB-981 BERTHOLD) with 2 min exposure time. At the end of 72 h in dark, β -Ga₂O₃:Cr³⁺ was re-excited with white light for 3min and the afterglow image of the β -Ga₂O₃:Cr³⁺ was acquired.

In vivo characterization of afterglow: β -Ga₂O₃:Cr³⁺ suspension (5mg/ml) was subcutaneous injected with 300 μ g, 200 μ g, 100 μ g from head to tail at the HeLa tumor-bearing mouse. For the intravenous injection, 5mg (15mg/ml suspension) β -Ga₂O₃:Cr³⁺ was injected at HeLa tumor-bearing mouse. Before injection, β -Ga₂O₃:Cr³⁺ suspension was

exposed to UV for 3min, and then afterglow images in body was acquired by in-vivo imaging system(NIGHTOWLLB-981 BERTHOLD) with 2 min exposure time. At 48 h-post *i. v.* injection, nude mouse was sacrificed. The isolated organs and tumour from the mouse bearing Hela was re-excited by white light for the afterglow image by in-vivo imaging system(NIGHTOWLLB-981 BERTHOLD) with 2 min exposure time.

In vivo fluorescence imaging: $\beta\text{-Ga}_2\text{O}_3\text{:Cr}^{3+}$ suspension(5mg/ml) was subcutaneous injected with 200 μg at bottom of nude mouse. And then fluorescence image was acquired by in-vivo fluorescence imaging system (MK50101-EX, CRI, America)e quipped with 150 W xenon lamp and 675nm filter. Excitation wavelength is 635 nm.

Cytotoxicity assay: The cytotoxicity assay was performed by using Cell Counting Kit-8(CCK-8) method. MCF-7 and L-929 cells were seeded in 96-well plates for 24 h to adherence. After treated with various concentrations of the $\beta\text{-Ga}_2\text{O}_3\text{:Cr}^{3+}$ nanorod for 24 h, 10 μl of CCK-8 were added to each well for another 1.5 h, and then the 96-well plates was measured the optical density (OD) at 450 nm by microplate reader(RT-6000, Rayto). The blank cell was add corresponding concentrations of $\beta\text{-Ga}_2\text{O}_3\text{:Cr}^{3+}$ nanorod as the background. So, the cell viability was calculated by the following formula:

$$\text{Cell viability} = \frac{\text{OD}_{\text{treated}} - \text{OD}_{\text{background}}}{\text{OD}_{\text{control}} - \text{OD}_{\text{background}}} \times 100\%$$

NO measurement: Raw 264.7 macrophages were seeded in 96-well plates. After treated with various concentrations of the $\beta\text{-Ga}_2\text{O}_3\text{:Cr}^{3+}$ nanorod for 24 h, the supernatant was collected to measure NO concentration by Griess reagent. Briefly, 50 μl of supernatant from each well were mixed with 100 μl of Griess reagent in a 96-well plate. After 15 min incubation at room temperature, OD was determined at 540 nm with a microplate reader. The nitrite production of each sample was calculated from a standard curve prepared with sodium nitrite.

Drug loading and release: 25 mg of $\beta\text{-Ga}_2\text{O}_3\text{:Cr}^{3+}$ nanorod were mixed with 25 ml DOX solution (PBS, pH 6.8, 0.2 mg/ml) followed by ultrasonication for 0.5 h to disperse the nanorod. After stirring for 12 h under dark condition, the DOX-loaded nanorod was centrifuged and washed with PBS (pH 6.8) for 3 times followed by lyophilization. The centrifugate and washing solution were collected to measure unloaded drug by using UV-vis absorption spectroscopy at 480nm. The drug-loading rate was determined by the following formula:

$$\text{Loading rate} = \frac{m_{\text{loaded}}}{m_{\text{loaded}} + m_{\text{nanorod}}} \times 100\%$$

m_{loaded} was the amount of DOX loaded in $\beta\text{-Ga}_2\text{O}_3\text{:Cr}^{3+}$ nanorod; m_{nanorod} was the amount of $\beta\text{-Ga}_2\text{O}_3\text{:Cr}^{3+}$ nanorod.

For the *in vitro* DOX releasing, 5 mg of DOX/ $\beta\text{-Ga}_2\text{O}_3\text{:Cr}^{3+}$ nanorod (DOX/ Ga_2O_3) were dispersed in 3ml PBS solution (pH 5.5 or 7.4) at 37 °C with 100 rpm shaking. At suitable intervals, absorbance of centrifugate solution (4000rpm, 10min) was measured at 480 nm. The sediment was dispersed in another 3ml PBS for further releasing test. All the drug release tests were performed three times.

2. Supplementary Results:

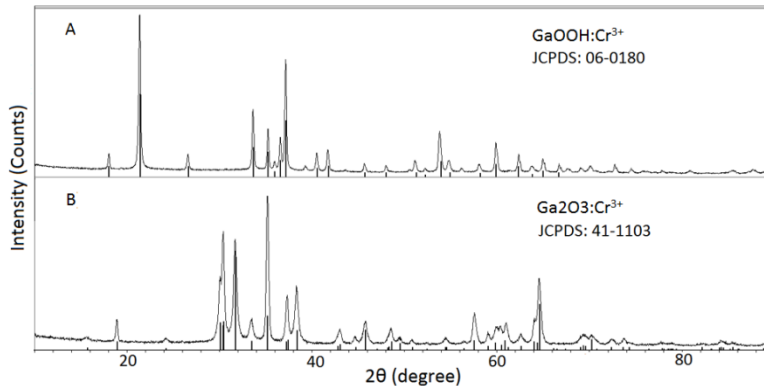


Fig. S1 XRD of GaOOH:Cr³⁺nanorod(A) and β-Ga₂O₃:Cr³⁺nanorod(B)

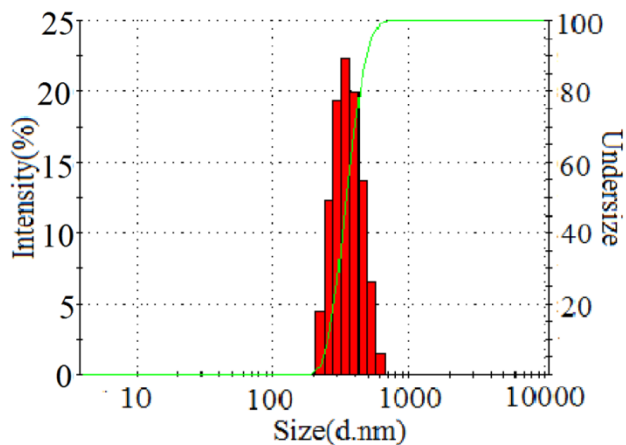


Fig. S2 Hydrodynamic size distribution of GaOOH:Cr³⁺nanorod

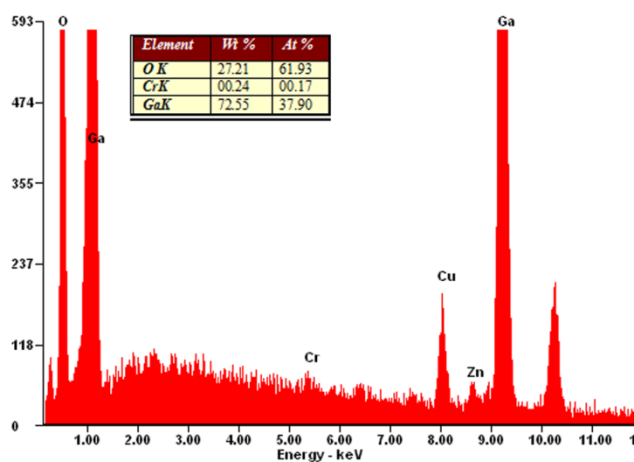


Fig. S3 Energy-dispersive spectrometry (EDS) of β-Ga₂O₃:Cr³⁺crystallattice.

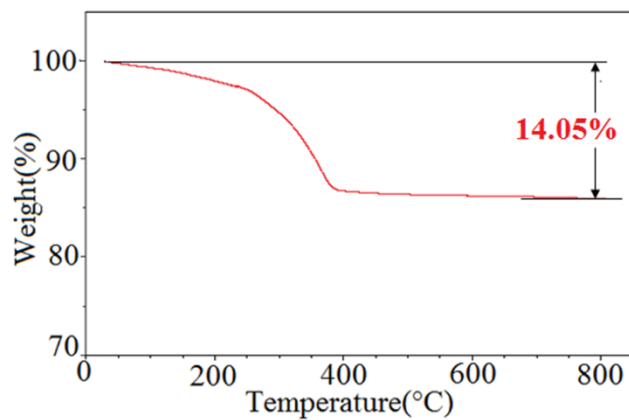


Fig. S4 TGA of GaOOH:Cr³⁺nanorod

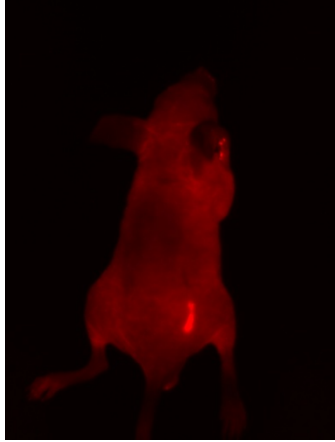


Fig. S5 *In vivo* fluorescent image acquired by in-vivo fluorescence imaging system with 150 W xenon lamp. Excitation wavelength was 635 nm. The light spot near right hind leg was the fluorescence signal of $\beta\text{-Ga}_2\text{O}_3\text{:Cr}^{3+}$ nanorod.