Electronic Supplementary Information for

One-pot synthesis of multifunctional radiolabeled upconversion nanorods for enhanced multimodal imaging performance

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Materials. $GdCl_3•6H_2O$ (99.99%), $YCl_3•6H_2O$ (99.99%), $YbCl_3•6H_2O$ (99.99%), $ErCl_3•6H_2O$ (99.99%), NaOH, RGD peptide, triethylamine, chloramine-T and oleic acid (OA) came from Aladdin, NH_4F came from Macklin. All drugs were used as starting materials without further purification. Ethanol, cyclohexane, chloroform, chloramine-T and dimethyl sulfoxide (DMSO) were purchased from Aladdin. $Na^{125}I$ was purchased from Chengdu Gaotong Isotope Co. Ltd. High Q water was used throughout the research.

Characterization

X-ray diffraction (XRD) was carried out using a X'Pert-Pro MPD X-ray

diffractometer at 40 KV and 40Ma with Cu Kα radiation. The morphology of the nanrods was measured using a transmission electron microscope (TEM, TecnaiG220).

Synthesis of $NaYF_4$: Yb/Er/Gd (18/2/60 mol%) nanorods. The typical procedure to the synthesis of lanthanide-doped $NaYF_4$ nanorods were followed as reported previously. Typically, 0.3 g NaOH was dispersed in 1.5 ml H_2O. Then added 5 ml ethanol and 5 ml OA under stirring. 2 ml of $RECl_3$ (0.2 M, RE= Yb, Er, Gd) and 1 ml of NH_4F (2 M) were added. Then the solution was transferred into a 20 ml of Teflon-lined autoclave and heated to 200 ^oC for 2 h. The obtained nanrods were washed with etheanol three times and dispersed in cyclohexane.

Synthesis of poly(maleic anhydride-alt-1-octadecene)-mPEG (5000(C18PMH-PEG)). 5000 (C18PMH-PEG) polymers were synthesized following a previous protocol[1]. One molar equivalent(eq.) of 5kmPEG-NH_2 (PEG Bio, China) were reacted with poly(maleic anhydride -alt-1-octadecene)(PMHC18, Sigma-Aldrich) at 2:1 in 2 ml dichloromethane for one day in the presence of 2 eq. of N-(3-Dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDC, Sigma-Aldrich) and 4 eq. triethylamine. The reaction solution was blown-dry by nitrogen, yielding a solid product which was then dissolved in water. The amphiphilic polymer solutions were dialyzed against water using a 14 kDa cut-off membrane and then lyophilized. The final products were 100%-5kPEG-C18PMH.

Surface modification of $NaYF_4$: Yb/Er/Gd (18/2/60 mol%) nanorods. 0.5 ml stock solution of $NaYF_4$: Yb/Er/Gd (18/2/60 mol%) nanorods were precipitated by centrifugation and redispersed in chloroform. Another solution of 10 mg C18PMH-PEG polymer in 2 ml chloroform was added. The mixture was then stirred for 2 h. The chloroform solvent was blown-dried by N_2 and the residue was readily dissolved in water. The resultant solution was filtered through a 0.22-μm syringe filter to remove large aggregates. $NaYF_4$: Yb/Er/Gd (18/2/60 mol%)-PEG was centrifuged to remove coating polymers, re-dispersed in water, and stored under 4 ℃ for further use.

Radiolabeling of UCNRs. The RGD peptide (Fig. S1) located on the surface of UCNRs, which can be readily labeled by $Na^{125}I$ through the standard chloramine-T

method to afford the radioactive nanoprobe ^{125}I-UCNRs. The probes were separated from free iodine by PD-10 column. Briefly, 5 mg of UCNRs was dissolved in 500 μl water and labeled with Na^{125}I (500 μCi), and then 100 μg chloramine-T in 10 μl water was added. The reaction mixture was incubated at room temperature for 10 min with shaking. Then ^{125}I-UCNRs were washed with water for at least three times.

In vitro **cytotoxicity assay by methylthiazoletetrazolium (MTT).** The effect of ^{125}I-UCNRs on the cell viability was carried out by the MTT assay. The Hela cells seeded in 96-well plates (1×10^4 cells per well) for 24 h, respectively. Afterwards a series of concentrations of ^{125}I-UCNRs were added into the two cell cultures. After 24 h incubation, 20 ml of MTT solution was mixed in each well for another 4 h. After that, DMSO was added into the well. Finally, the cell viability was measured by a microplate reader (Model 680 Bio-RAD).

In vitro MRI phantom study. The MR imaging of ^{125}I-UCNRs in all samples were scanned using a 1.5 T clinical MRI scanner (GE Medical systems, Signa HDX) at room temperature. The T_1 values at different iron concentrations were measured by a manually drawn region-of-interest (ROI) after obtaining the T_1-weighted MR imaging.

In vivo **SPECT imaging.** All animal experiments were approved by the Animal Care and Use Committee and all animal protocols conformed to the Guide for the Care and Use of Laboratory Animals. In the experimental group, Balb/c mice were intravenously injected with 4 mg/ml free ^{125}I and ^{125}I-UCNRs. The whole-body SPECT imaging was performed under general anesthesia induced by inhalation of 3% isoflurane. The representative SPECT images of free ^{125}I and ^{125}I-UCNRs were acquired at different time points (5 h) post injection.

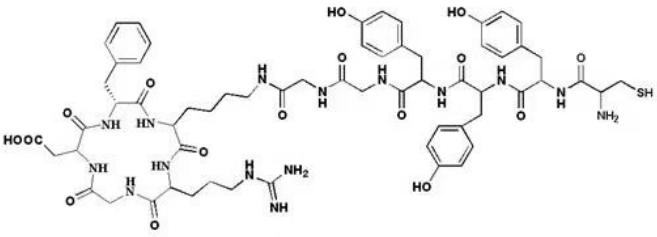

Fig. S1 The structure of RGD peptide.

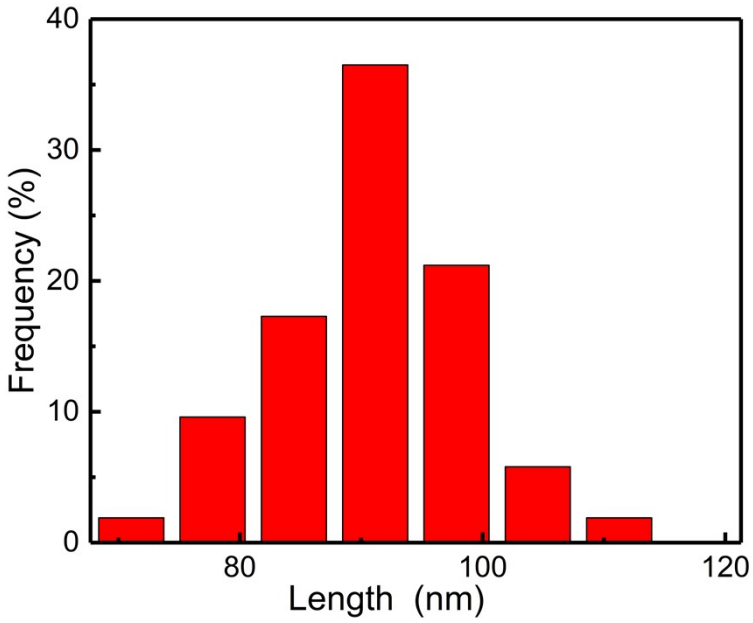

Fig. S2 The size distribution of upconversion nanoparticles.

1 X. Liu, H. Tao, K. Yang, S. Zhang, S. Lee, Z. Liu, *biomaterials*, 2011, **32**, 144.