Electronic Supplementary Material (ESI) for

Rational Synthesis of Three-Dimensional Core-double Shell Upconversion Nanodendrites with Ultrabright Luminescence for Bioimaging Application

Murad M.A. Abualrejal,[a,b] Kamel Eid,[c] Rongrong Tian,[a,b] Lin Liu,[a,d] Hongda Chen,[a] Aboubakr M. Abdullah,[c] and Zhenxin Wang[a*]

[a.] *State Key Laboratory of Electroanalytical Chemistry, Changchun Institute of Applied Chemistry, Chinese Academy of Sciences, Changchun, 130022, P. R. China. *E-mail: wangzx@ciac.ac.cn*

[b.] *University of Science and Technology of China, Road Baohe District, Hefei, Anhui, 230026, P. R. China*

[c.] *Center for advanced materials, Qatar University, Doha 2713, Qatar*

[d.] *College of Chemistry, Jilin University, Changchun 130012, P. R. China.*

Content

1. Additional Experimental Section

Chemicals and Materials

Rare-earth oxides, including Y_2O_3 (99.99%), Er_2O_3 (99.99%), Nd_2O_3 (99.99%), and Yb_2O_3 (99.99%) were obtained from Alfa Aesar (Ward Hill, USA). $Ca(CH_3COOH)_2$ H_2O (98%) was obtained from Tianjin Guangfu Fine Chemical Research Institute (Tianjin, China). The Y_2O_3, Yb_2O_3, Nd_2O_3, and Er_2O_3 were reacted with excess hydrochloric acid to form the rare-earth chloride compounds, respectively. Then, the rare earth chloride compounds were dried by solvent evaporation, and redispersed in water to yield the YCl_3 (1.6 mol L^{-1}), $YbCl_3$ (0.6 mol L^{-1}), $NdCl_3$ (0.6 mol L^{-1}) and $ErCl_3$ (0.1 mol L^{-1}) aqueous stocking solutions, respectively. 1-octadecene (1-ODE, ≥90%), oleic acid (OA, ≥90%), Triton X-100 and tetraethyl orthosilicate (TEOS, 99.999%) were purchased from Sigma-Aldrich Co. (St Louis, USA). Dulbecco's modified Eagle's medium (DMEM) and fetal bovine serum (FBS), ammonium fluoride (NH_4F, 98%), and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were purchased from Beijing Dingguo Biotechnology Ltd. (Beijing, China) The HeLa cell line was purchased from Shanghai Cell Bank, CAS (Shanghai, China). The 980 nm laser was purchased from Changchun Laser Optoelectronics Technology Co. Ltd. (Changchun, China). And the short pass filter was provided by Shenzhen Gengxu Photonics Technology Co. Ltd. (Shenzhen, China). Other reagents (analytical grade) were purchased from Beijing Chemical Reagents Company (Beijing, China).

All reagents were used as received without further purification. Milli-Q water (18.2 MΩ cm) was used in all experiments.The Balb/c mice (six week old, 20 ± 0.2 g, male) were purchased from Beijing HFK Biotechnology Ltd. (Beijing, China). Animal experiments were conformed to the guidelines of the Regional Ethics Committee for Animal Experiments established by the Jilin University Institutional Animal Care and Use.

Synthesis of UCND@SiO_2-COOH

To synthesis UCND@SiO_2-COOH, 7.5 mL cyclohexane containing UCNDs (6 mg mL^{-1}), 1.8 mL TritonX-100, 1.8 mL *n*-hexanol, and 0.48 mL H_2O were mixed and stirred for 30 min.

Then 90 μL NH_4OH (28 wt%) and 120 μL TEOS were added into the mixture. After stirring for 5.75 h, 140 μL CTES was added and kept stirring at room temperature for 24 h. The final product was precipitated by addition of 5 mL acetone, collected by centrifugation (10,000 rpm), and washed with ethanol for three times. Finally, the UCND@SiO_2-COOH was dispersed in water.

Cytotoxicity Study of UCND@SiO_2-COOH

The HeLa cells were cultured in fresh DMEM medium supplemented with 10% fetal bovine serum (FBS) and 100 U mL^{-1} penicillin-streptomycin under a humidified 5% CO_2 at 37 °C. To evaluate the cytotoxicity of UCND@SiO_2-COOH, the HeLa cells were cultured in 96-well cell-culturing plate (1×10^4 cells per well in 100 μL culture medium) for 24 h. After removed the culture medium and washed with PBS for three times, 100 μL fresh culture medium containing the UCND@SiO_2-COOH with desired concentrations (6.25, 12.5, 25, 50, 100 and 200 μg mL^{-1}) were introduced into the wells and incubated for another 24 h, respectively. The cell viabilities were determined by traditional 3-(4,5-dimethylthiazol-2-yl)- 2,5-diphenyltetrazolium bromide (MTT) assay [1, 2].

UCL Imaging of Cells

For UCL imaging, HeLa cells (5×10^4 cells per well in 0.5 mL culture medium) were seeded in a 48-well culturing plate for 24 h. After discharged the culture medium and washed with PBS for three times, 0.5 mL fresh culture medium containing UCND@SiO_2-COOH with varying concentrations (25, 50, 100 and 200 μg mL^{-1}) were added into the corresponding wells and incubated for 24 h. After washed with PBS (3 times), the NP-stained cells were fixed with 4% paraformaldehyde for 20 min and subjected to UCL imaging under an external 980 nm laser (0.5 W cm^{-2}).

Besides, fresh culture medium containing 100 μg mL^{-1} UCND@SiO_2-COOH were added to the wells and incubated at the same conditions for different time (0.5, 1, 2, 4,

6, 8, 12 and 24 h), respectively.

In vivo UCL Imaging of UCND@SiO_2-COOH.

200 μL 0.9 wt% NaCl solutions containing UCND@SiO_2-COOH (1.5 mg mL^{-1}) were intravenously injected into a healthy Balb/c mouse. Then the UCL images of livers were recorded at the appropriate time points (0, 0.5, 1, 2, 4, 6, 8, 10, 12 and 24 h post-injection) under the excitation of 980 nm NIR laser (1.5 W cm^{-2}), respectively. In addition, the mouse injected with 200 μL 0.9 wt% NaCl solutions containing UCND@SiO_2-COOH (1.5 mg mL^{-1}) was sacrificed at 8 h post-injection, and the organs (heart, liver, spleen, lung, and kidneys) were collected for ex vivo UCL imaging.

All of the UCL images were recorded by M2590 (Genie^{TM} Nano Cameras) with a 700 nm short pass filter (SP700, FWHM 150 nm).

In Vivo Toxicology Analysis of UCND@SiO_2-COOH.

6 healthy Balb/c mice were randomly divided into two groups, which were received intravenous injections of 200 μL 0.9 wt% NaCl solution only (control group), 200 μL 0.9 wt% NaCl solution containing UCND@SiO_2-COOH at a dose of 10 mg kg^{-1} through tail veins, respectively. The body weight of each mouse was monitored every five days. After 30 days, blood samples and organs (heart, liver, spleen, lung, and kidneys) were harvested from both the control and the experiment mice. The organs were fixed in 4% (w/v) paraformaldehyde solution, embedded in paraffin, sectioned, and finally stained with hematoxylin-eosin (H&E) for histological examinations. The blood samples were analyzed by the blood biochemistry assay.

2. Additional Figures S1-S8

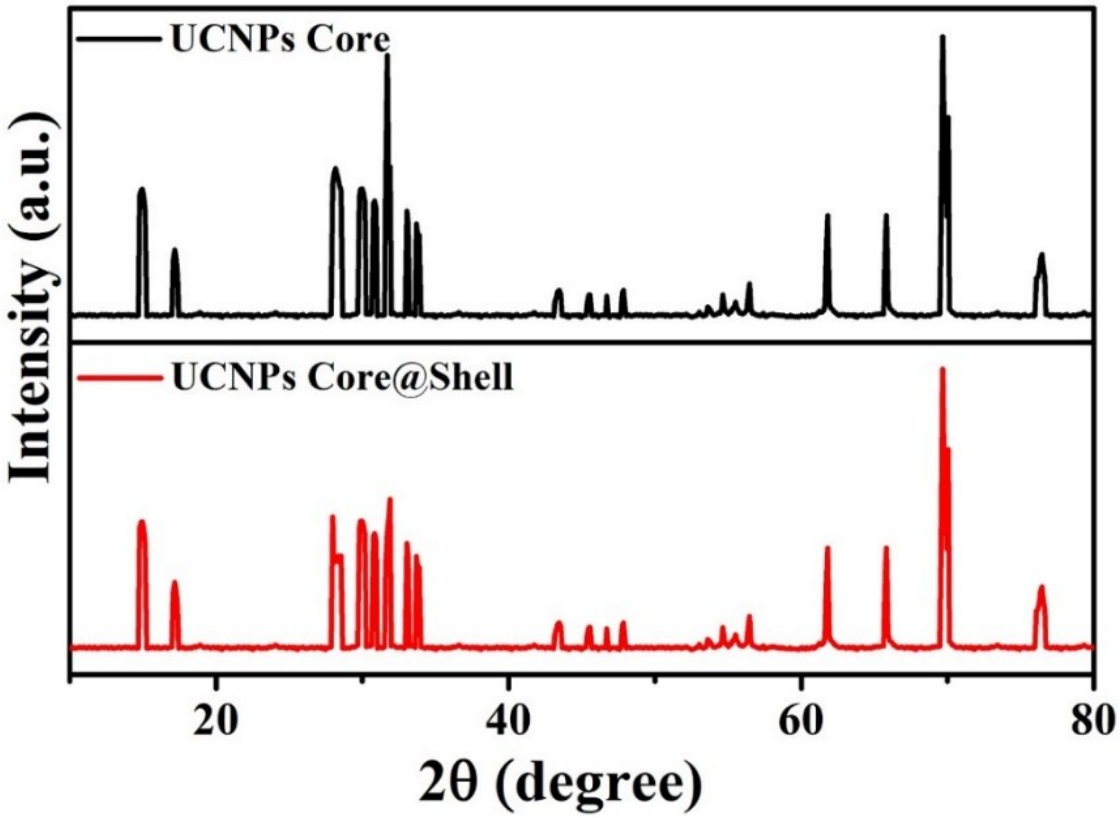

Fig. S1. XRD patterns of UCNPs core and UCNPs core-shell.

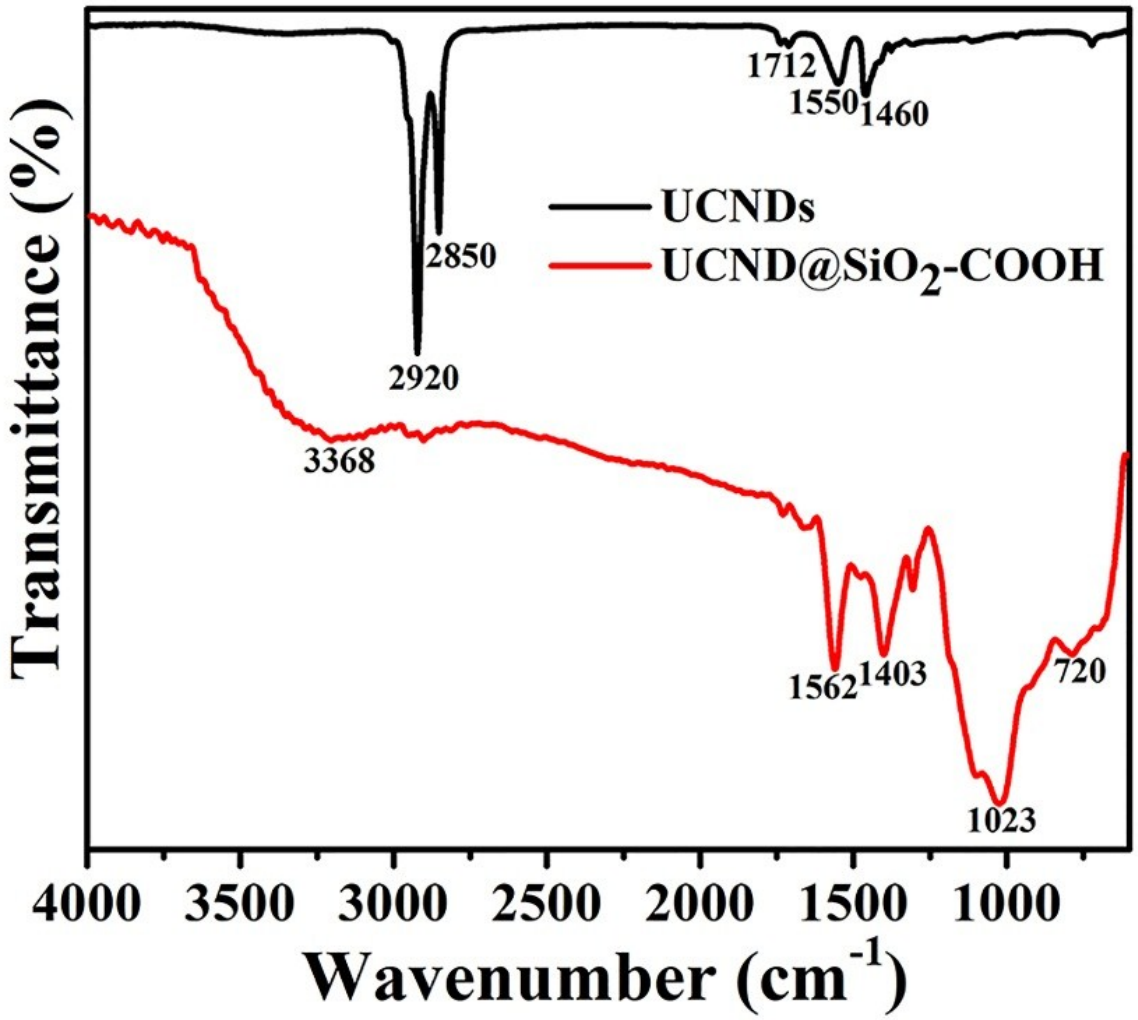

Fig. S2. FT-IR spectra of UCNDs and UCND@SiO_2-COOH.

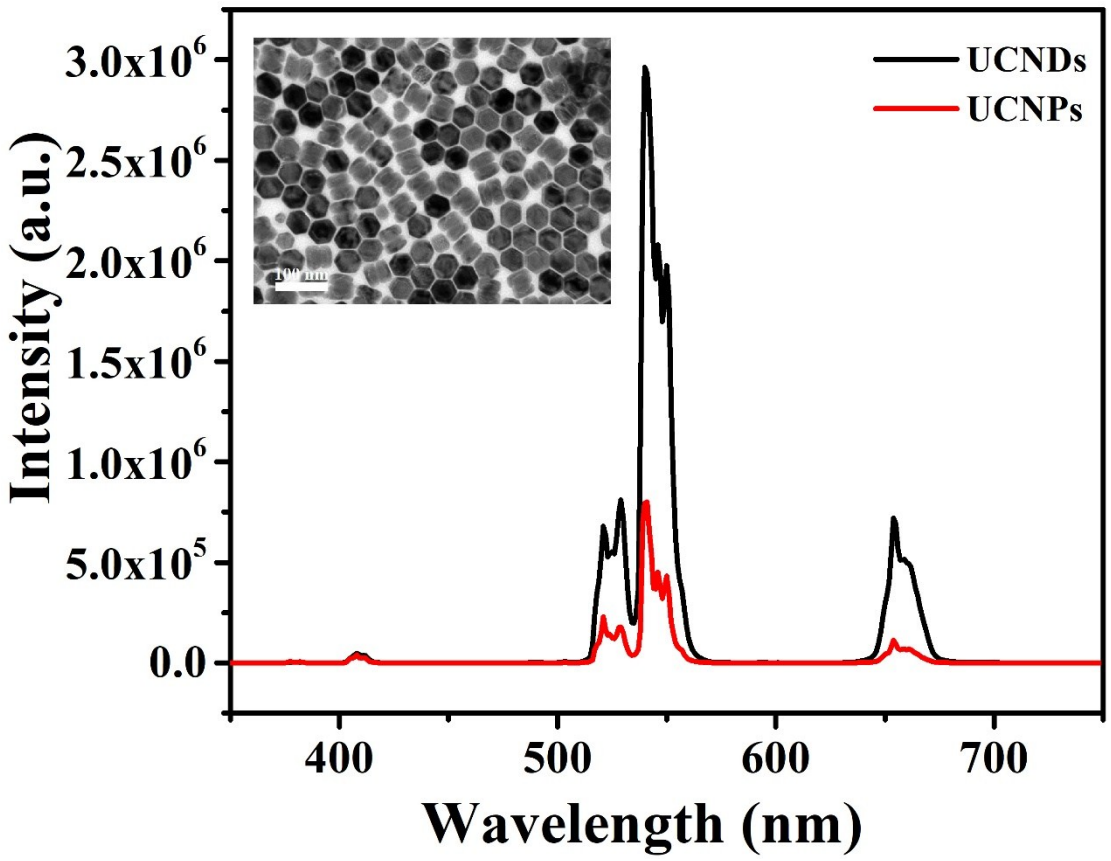

Fig. S3. The UCL spectra of UCNDs (black line) and $NaYF_4$: Yb^{3+}, Er^{3+}@$NaGdF_4$ UCNPs (red line). The UCL spectra were recorded under same experimental conditions. The concentrations of nanoparticles are 6 mg mL^{-1} in cyclohexane. The inset is the TEM image of $NaYF_4$: Yb^{3+}, Er^{3+}@$NaGdF_4$ UCNPs. The $NaYF_4$: Yb^{3+}, Er^{3+}@$NaGdF_4$ UCNPs were synthesized by the literature reported method. [S2-7]

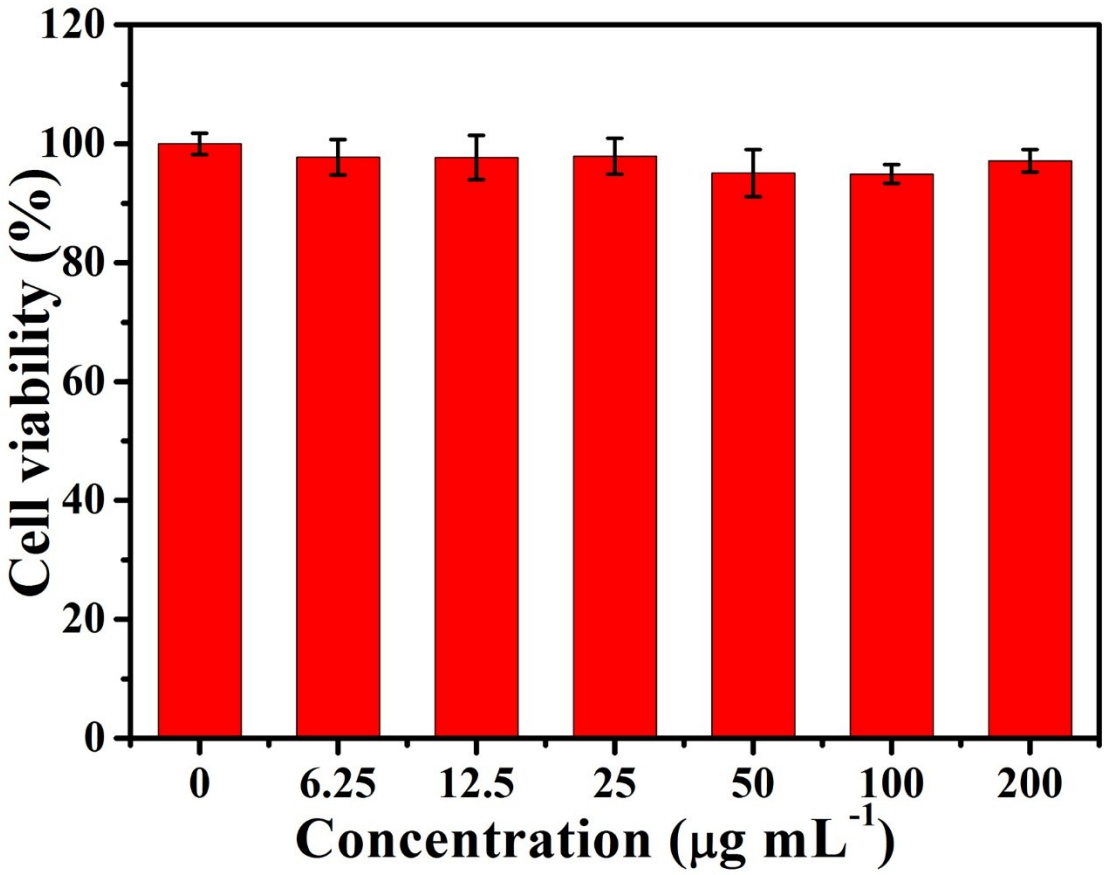

Fig. S4. In vitro cell viabilities of HeLa cells incubated with various concentrations (0, 6.25, 12.5, 25, 50, 100 and 200 μg mL^{-1}) of UCND@SiO_2-COOH.

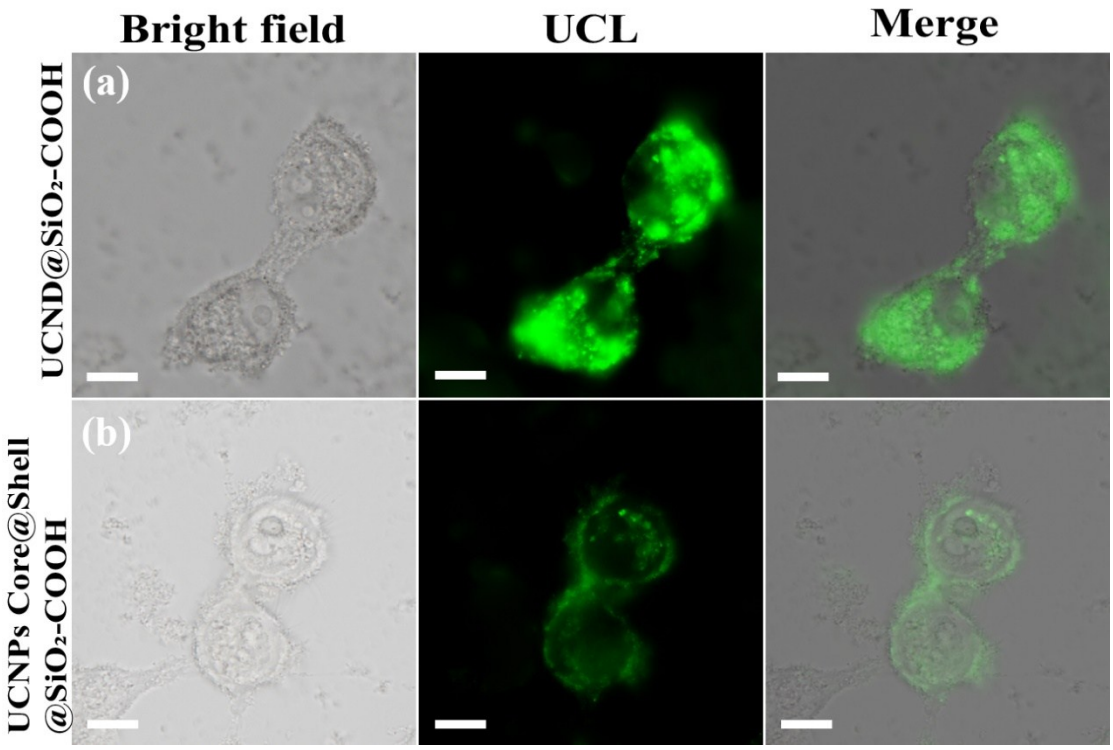

Fig. S5. High-resolution confocal fluorescence microscopy images of HeLa cells treated with 100 μg mL^{-1} of (a) UCND@SiO_2-COOH and (b) UCNPs core@shell@SiO_2-COOH for 24 h. The nanoparticle stained HeLa cells were excited by a 980 nm NIR laser. The indicated scale bars are 10 μm.

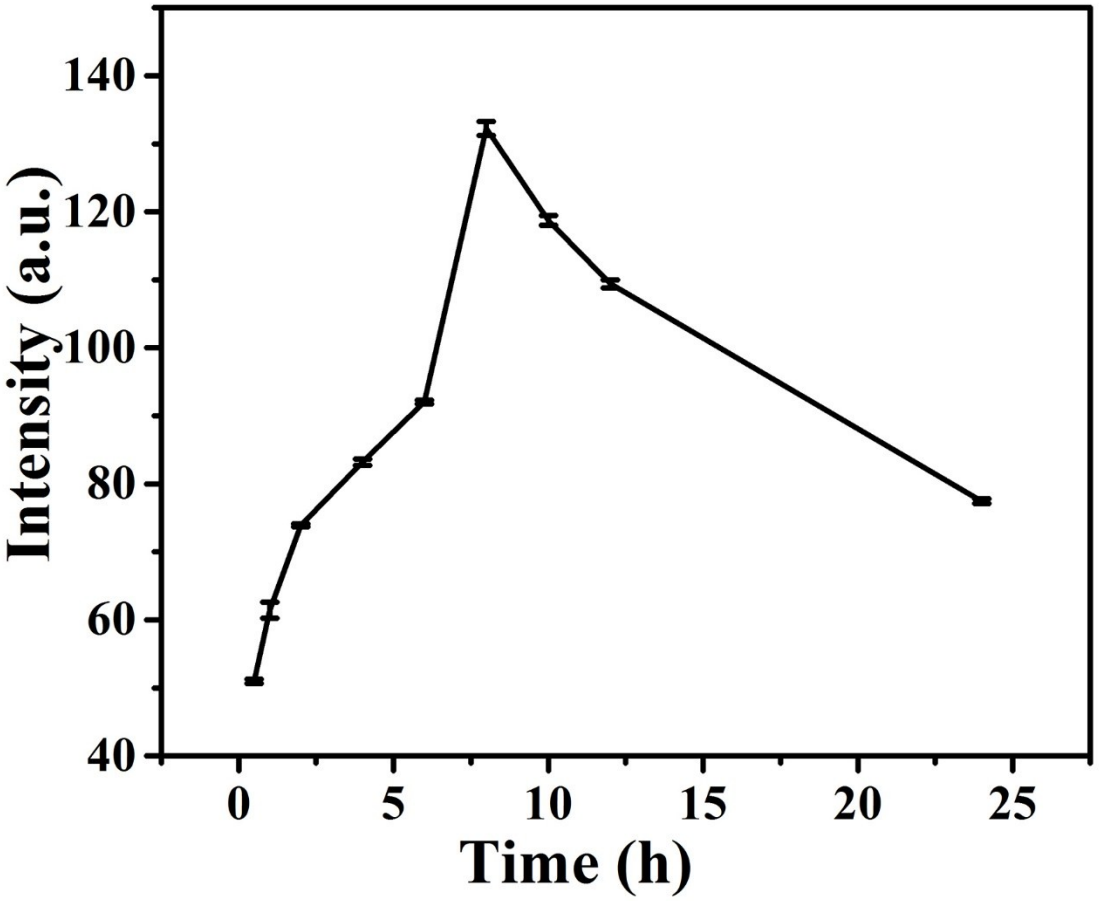

Fig. S6. The UCL intensity of liver as a function of post-injection time. The mice were injected with UCND@SiO_2-COOH through the tail vein.

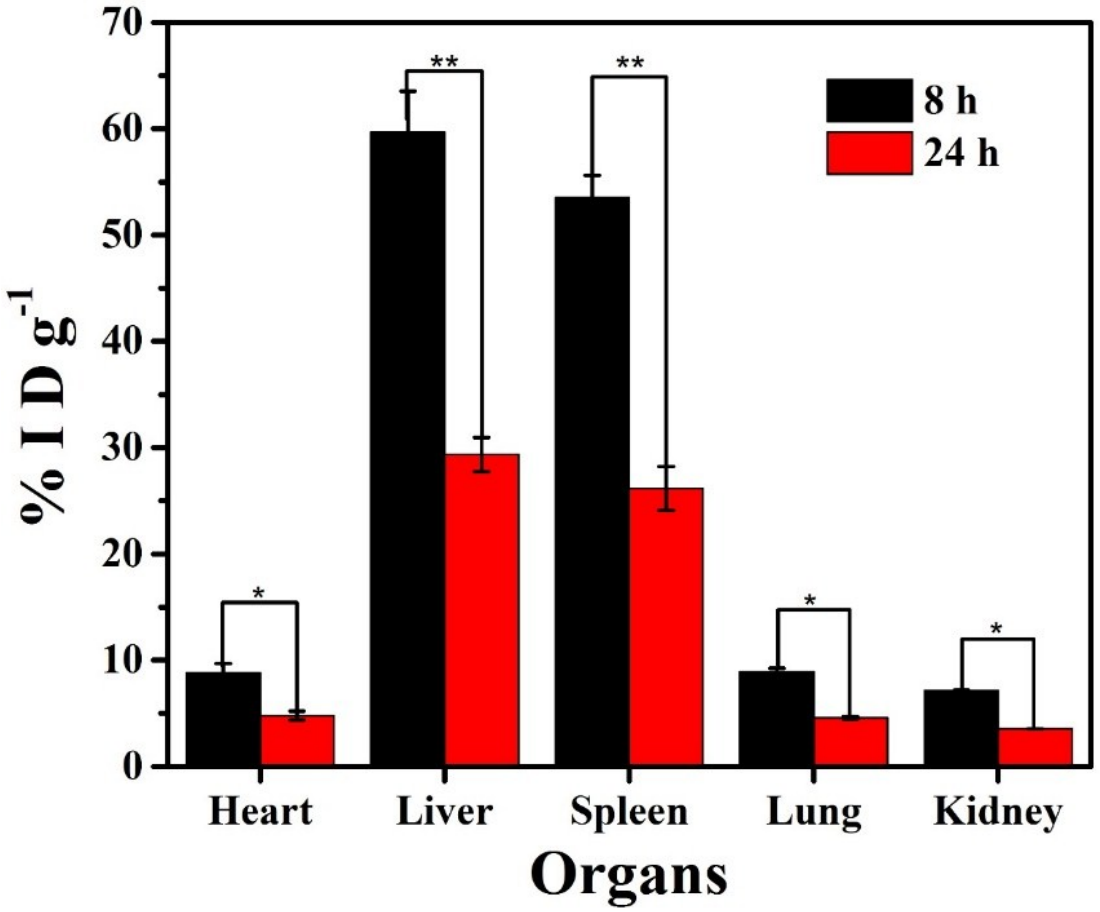

Fig. S7. Biodistributions of Nd element in main organs of mice at 8 and 24 h of post-intravenous injection of UCND@SiO_2-COOH, respectively. The Error bars mean standard deviations, n = 5, *P< 0.05 or **P < 0.01 from an analysis of variance with Tukey's post-test.

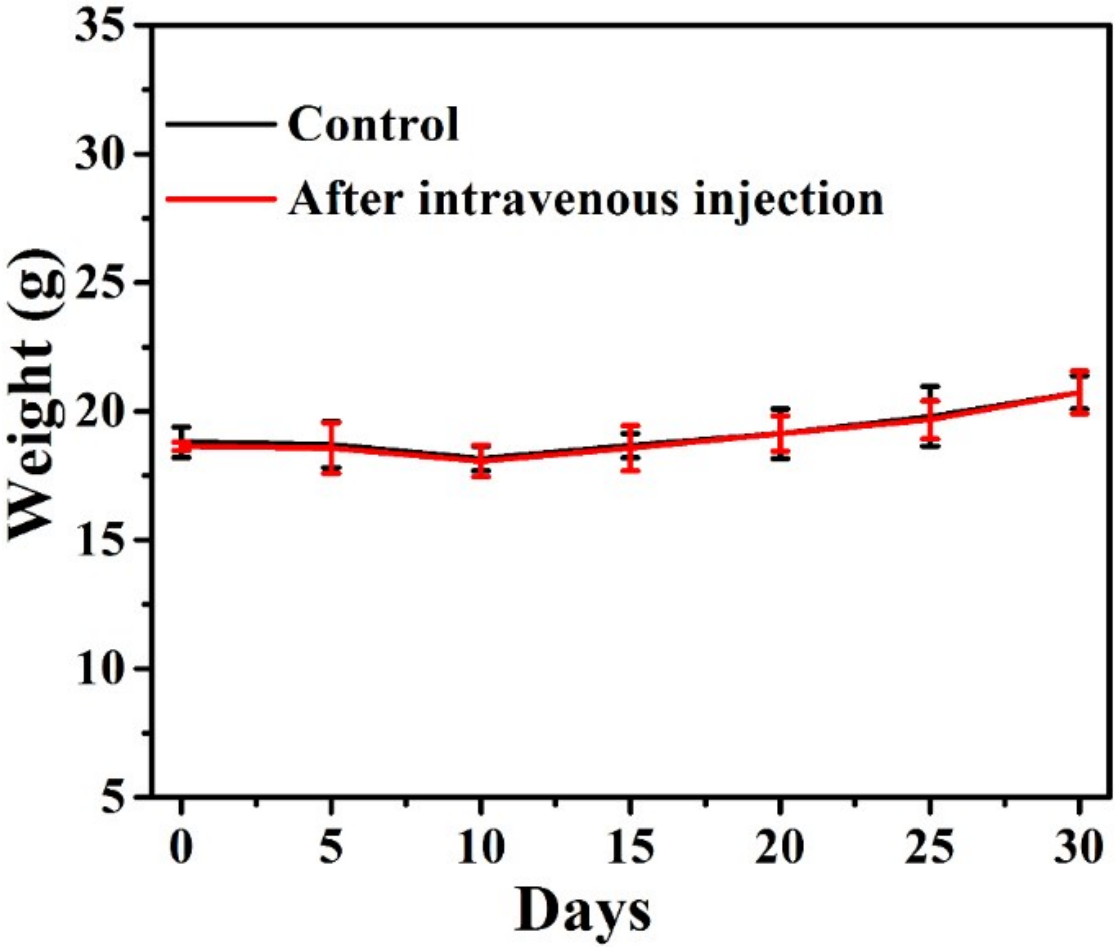

Fig. S8. Change in body weight obtained from mice injected with UCND@SiO_2-COOH through tail vein (n = 3, dose =10 mg/kg, Test) and without injection (n = 3, Control).

3. Additional Table S1-S5

Table S1. Atomic contents of UCND@SiO_2-COOH

Elements	Ca3933	Er3372	Nd4303	Si2124	Y_3242	Yb3289
Unit	ppm	ppm	ppm	ppm	ppm	ppm
Average value	2.435	0.3726	82.29	48.42	24.31	22.71

Table S2 Ratio of the UCL intensity at 654 nm to the UCL intensity at 542 nm (I_{654}/I_{542}).

UCNPs	I_{654}/I_{542}
$NaYF_4$: Yb, Er, Ca	0.0046
$NaYF_4$: Yb, Er, Ca@$NaYF_4$: Yb, Ca	0.051
$NaYF_4$: Yb, Er, Ca@$NaYF_4$: Yb, Ca@$NaNdF_4$: Yb, Ca	0.241
UCND@SiO_2-COOH	0.053

Table S3. The elemental; distribution in the organs determined by the ICP-MS after 8 h of post-injection with (10 mg kg^{-1}).

Organs Elements	Heart	Liver	spleen	lung	Kidneys
Unit	ppm	ppm	ppm	ppm	ppm
Nd	1.833	59.680	53.56	9.715	2.34
Yb	0.3054	18.72	14.792	2.033	0.7692
Y	0.3514	19.185	15.468	2.037	0.9354
Er	0.07982	0.621	0.468	0.1198	0.09342

Table S4. The elemental; distribution in the organs determined by the ICP-MS after 24 h of post-injection with (10 mg kg^{-1}).

Organs Elements	Heart	Liver	spleen	lung	Kidneys
Unit	ppm	ppm	ppm	ppm	ppm
Nd	0.99403	29.370	26.19	5.01289	1.16013
Yb	0.16554	9.21259	7.23325	1.04901	0.38135
Y	0.19048	9.44143	7.56381	1.05108	0.46375
Er	0.04326	0.30561	0.22885	0.06181	0.04631

Test	*Units*	*Control (mean ± sd)*	*Treatment (mean ± sd)*
WBC	$\times 10^9$/L	2.10±0.06	1.85±0.12
RBC	$\times 10^{12}$/L	10.26±0.90	9.30±0.80
HGB	g/L	166.00±4.60	165.80±2.10
HCT	%	51.50±5.10	45.10±3.20
MCV	fL	50.20±2.30	50.10±1.70
MCH	Pg	15.50±0.30	15.90±0.74
MCHC	g/L	324.00±4.60	329.60±6.60
PLT	$\times 10^9$/L	663.70±6.70	652.00±2.60
LYMPH	%	76.64±7.50	71.22±6.20

Table S5. Results of hematology analysis and blood biochemical assays.

References

S1. R. Tian, H. Zhang, H. Chen, G. Liu and Z. Wang, *Adv. Sci.* 2018, 1800214.

S2. L. Liu, H. Zhang, D. Song and Z. Wang, *Analyst*, 2018, **143**, 761-767.

S3. F. Liu, X. He, L. Liu, H. You, H. Zhang and Z. Wang, *Biomaterials*, 2013, **34**, 5218-5225.

S4. F. Liu, X. He, J. Zhang, H. Zhang and Z. Wang, *Small*, 2015, **11**, 3618-3618.

S5. Y. Liu, Q. Jia, X. Zhai, F. Mao, A. Jiang and J. Zhou, *Chem. sci.*, 2019, **10**, 1193-1200.

S6. D. Zhang, L. Wei, M. Zhong, L. Xiao, H.-W. Li and J. Wang, *Chem. sci.*, 2018, **9**, 5260-5269.

S7. L. Liu, H. Zhang, Z. Wang and D. Song, *Biosens. Bioelectron.*, 2019, **141**, 111403.