Supporting Information

Simultaneously High Sensitive Fluoride Ions Detection and Fluorosis Therapy *via* Rational Designed Upconversion Nanoprobe

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The supporting information contains a description of the further characterization and condition investigation data in support of the manuscript.

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1. Experiment section

1.1. Materials

Rare-earth oxide Lu_2O_3 (99.999%), Yb_2O_3 (99.99%), and Tm_2O_3 (99.99%) were purchased from STREM Chemicals, Inc. USA. Na_2SO_4, KCl, $MgSO_4$, $CaCl_2$, $FeSO_4$, $ZnCl_2$, NaCl, $NaNO_3$, KBr, KI, $Ti(SO_4)_2$, NaOH, ethanol, cyclohexane, and dichloromethane (CH_2Cl_2) were purchased from Beijing Chemical Reagent Company. Oleic acid (OA), NaF, KF, nitrosonium tetrafluoroborate ($NOBF_4$), glycine (Gly), cysteine (Cys), lysine (Lys), serine (Ser), aspartic acid (Asp), dopamine hydrocloride (DA), glucose (Glu), reduced glutathione (GSH), methyl cyanide, and ascorbic acid (AA) were purchased from Sigma Aldrich. 1-Octadecene (ODE) and NH_4F were purchased from Alfa Aesar Chemical Co. Ltd. Bovine serum albumin (BSA), human serum albumin (HSA), and pyrogallic acid (PA) were purchased from Energy Chemical Co. Ltd. Rare earth chlorides ($LnCl_3$, Ln: Lu, Yb, Tm) were prepared by dissolving the corresponding metal oxide in HCl solution at elevated temperature and then evaporating the water completely under reduced pressure. All other chemical reagents were of analytical grade and were used directly without further purification. Deionized (DI) water was used throughout.

1.2. Synthesis of $NaLuF_4$:Yb,Tm@$NaLuF_4$ nanoparticles (UCNP)

In a typical experiment, a mixture of 1 mM $LnCl_3$ (Ln: 83%Lu, 16.9%Yb, and 0.1% Tm), 15 mL OA, and 15 mL ODE were added into a 100 mL three-necked flask. Under the vacuum, the mixture was heated to 160 ^oC to form a clear solution, and then cooled to room temperature. After the solution cooling down, 0.025 mmol NaOH

and 0.04 mmol NH_4F were added into the flask directly and stirred for 30 min. The solution was slowly heated with gently stirred, degassed at 100 ^oC, and then heated to 300 ^oC and maintained for 1 h under the argon atmosphere. After the solution was cooled naturally, the $NaLuF_4$:Yb,Tm nanoparticles were separated *via* centrifugation (10000 rpm) and washed with ethanol/cyclohexane (1:1 v/v) three times. The hydrophobic $NaLuF_4$:Yb,Tm was stored under room temperature in cyclohexane. The UCNP with core-shell structure were obtained with the same method.

1.3. Synthesis of UCNP-PA-Ti

In a typical experiment, 5 mL of dichloromethane solution of $NOBF_4$ (0.01 M) was dropped into 5 mL of hydrophobic UCNP dispersion in cyclohexane (~5 mg mL^{-1}) at room temperature. Then, the bare UCNP were washed with ethanol several times and dispersed in DI water. PA-Ti complex was obtained *via* a simple complexation reaction by mixing 5 mL 5 mg mL^{-1} $Ti(SO_4)_2$ with 5 mL 10 mg mL^{-1} PA at room temperature and ultrasonically treating for 5 min. Bare UCNP were then dispersed in the solution of PA-Ti (pH = 7.4), being vigorous stirring for another 20 min at room temperature. The resultant mixture was separated to obtain UCNP-PA-Ti *via* centrifugation and washed with DI water several times to remove the extra PA-Ti complex. The UCNP-PA-Ti was stored under 4 ^oC in DI water.

1.4. Characterization

The sizes and morphologies of $NaLuF_4$:Yb,Tm and UCNP were determined using a Tecnai G^2F30 high-resolution transmission electron microscope (HR-TEM). Samples of the UCNP were dispersed in cyclohexane and dropped on the surface of a copper

grid. The size distribution was counted and calculated from TEM images (α=0.90, 500 particles were measured) UV-vis-NIR absorption spectra were obtained on a Shimadzu UV-3600 UV-vis-NIR spectrophotometer. Powder X-ray diffraction (XRD) pattern was measured with a Brucker D8 advance X-ray diffractometer from 10^{o} to 70^{o} (Cu Kα radiation, λ = 1.54 Å). Dynamic light scattering (DLS) and zeta potential experiments were carried out on an ALV-5000 spectrometer goniometry equipped with an ALV/LSE-5004 light scattering electronic and multiple tau digital correlator and a JDS Uniphase He–Ne laser (632.8 nm) with an output power of 22 mW. The hydrodiameter distribution was measured at 25 ^{o}C with a detection angle of 90^{o}. The upcoversion luminescence (UCL) spectra were taken on a Maya LIFS-980 fluorescence spectrometer (Shanghai Oceanhood Opto-electronics tech Co. LTD) equipped with an external 0-8 W 980 nm adjustable laser as the excitation source. Inductively coupled mass spectroscopy (ICP-MS) analysis was performed on Agilent 7500ce ICP-MS.

1.5. Cell culture

CCC-HEL-1 cells were grown in DMEM (Dulbecco's modified Eagle's medium) supplemented with 10% FBS (fetal bovine serum) and 1% penicillin-streptomycin at 37 ^{o}C with 5% CO_2. For use in the MTT and oxidative stress experiments, 1×10^5 cells well^{-1} were seeded in 96-well plates and allowed to attach for 24 h prior to the assay. For use in the *in vitro* experiments, 1×10^7 cells well^{-1} were seeded in 6-well plates and allowed to attach for 24 h prior to the assay.

1.6. *In vitro* UCL imaging

CCC-HEL-1 cells with F^- pretreated were incubated with UCNP-PA-Ti solution (500 μg mL^{-1}) for 5 h, washed with PBS several times, and were resupplied with fresh DMEM. The UCL imaging was performed by optical microscopic study excited by CW 980 nm. Signals were collected at 400-600 nm and 650 ± 15 nm, respectively.

1.7. *In vivo* UCL imaging

In vivo UCL imaging was performed with a modified *in vivo* luminescence imaging system using an external 0–8 W adjustable CW infrared laser (980 nm, Hide-Wave Co., China) as the excited source. Signals were collected at 400-600 nm and 800 ± 12 nm by Andor iXon Ultra EMCCD, respectively. Images of signals were analyzed with corresponding software. Nude mice (m = 19 ± 2 g) were anesthetized with 10% chloral hydrate (150 μL) and were intravenously injected with UCNP-PA-Ti solution (10 mg kg^{-1}). Undoped $NaLuF_4$@$NaLuF_4$-PA-Ti was used for regular administration. At the 4 h post-injection, whole-body imaging was performed.

1.8. MTT assay

In vitro cytotoxicity was measured by performing methyl thiazolyl tetrazolium (MTT) assays on the CCC-HEL-1 cells. Different concentrations of UCNP-PA-Ti (0, 2, 4, 6, 8 and 10 mg mL^{-1}) were then added to the wells. The cells were subsequently incubated for 24 or 48 h at 37 ^oC under 5% CO_2. Thereafter, MTT (10 μL, 5 mg mL^{-1}) was added to each well and the plate was incubated for an additional 4 h at 37 ^oC under 5% CO_2. The optical density value (*Abs.*) of each well, with background subtraction at 690 nm, was measured by means of a Tecan Infinite M200 monochromator-based multifunction microplate reader. The following formula was

used to calculate the inhibition of cell growth.

Cell viability (%) = (mean of Abs. value of treatment group/mean of Abs. value of control)×100%

1.9. Hematology studies

All the animal procedures were in agreement with institutional animal use and care committee and carried out ethically and humanely. The blood were harvested from mice intravenously injected with UCNP-PA-Ti (n = 3, dose = 20 mg kg^{-1}, Test) and from mice receiving no injection for 1 day, 7 days, and 30 days post-injection (n = 3, dose = 0 mg kg^{-1}, Control), respectively. Blood was collected from the orbital sinus by quickly removing the eye ball from the socket with a pair of tissue forceps. Five important hepatic indicators (ALT, alanine aminotransferase; AST, aspartate amino transferase; TBIL, total bilirubin; ALB, albumin; TP, Total protein) and one indicator for kidney functions (CREA, Creatinine) were measured. Blood smears were prepared by placing a drop of blood on one end of a slide, and using another slide to disperse the blood along the length of the slide. Upon completion of the blood collection, mice were sacrificed.

1.10. Hematoxylin and eosin (H&E) stained tissues

The heart, liver, spleen, lung, and kidney were harvested from mice intravenously injected with UCNP-PA-Ti (n = 3, dose= 20 mg kg^{-1}, Test) and from mice receiving no injection (n = 3, dose = 0 mg kg^{-1}, Control), 1 day, 7 days, and 30 days post-injection, respectively. The tissues were fixed in paraformaldehyde, embedded in paraffin, sectioned, and stained with H&E. The histological sections were observed

under an optical microscope.

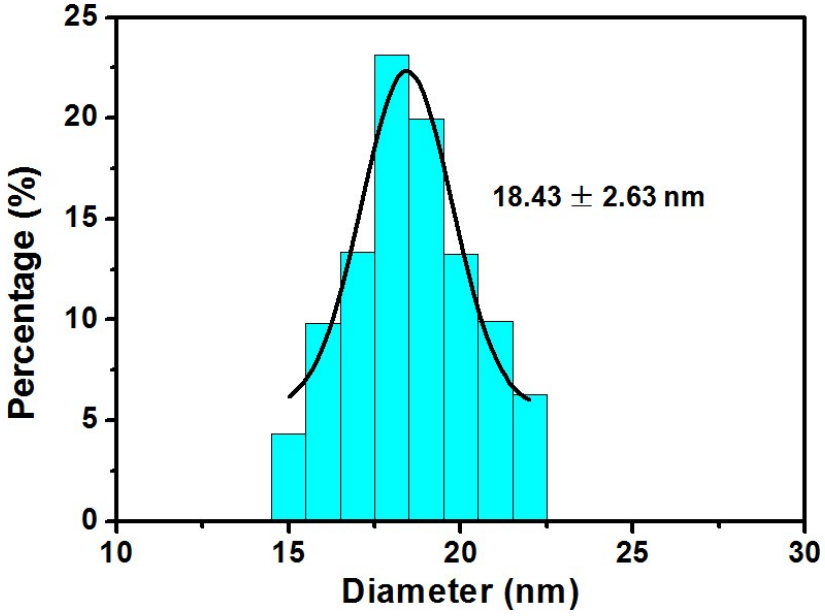

Figure S1. Size distribution of $NaLuF_4$:Yb,Tm.

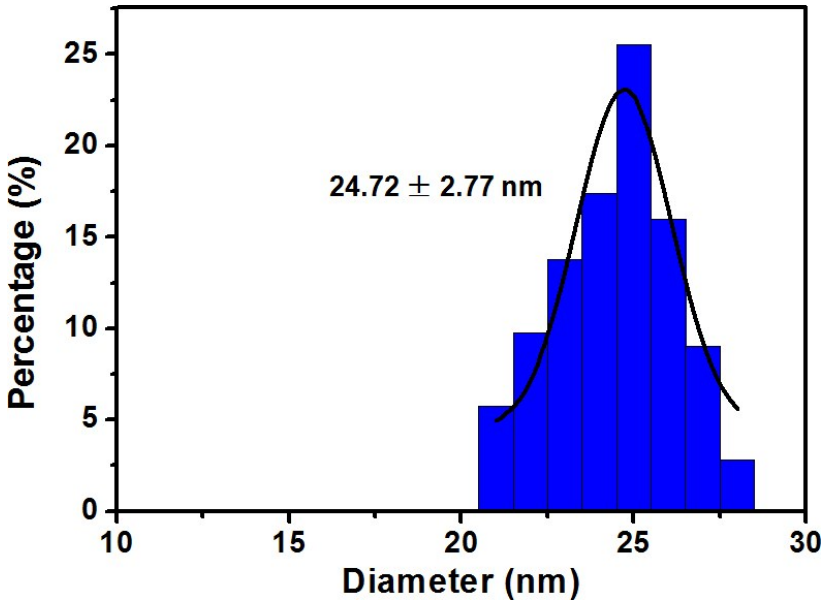

Figure S2. Size distribution of UCNP.

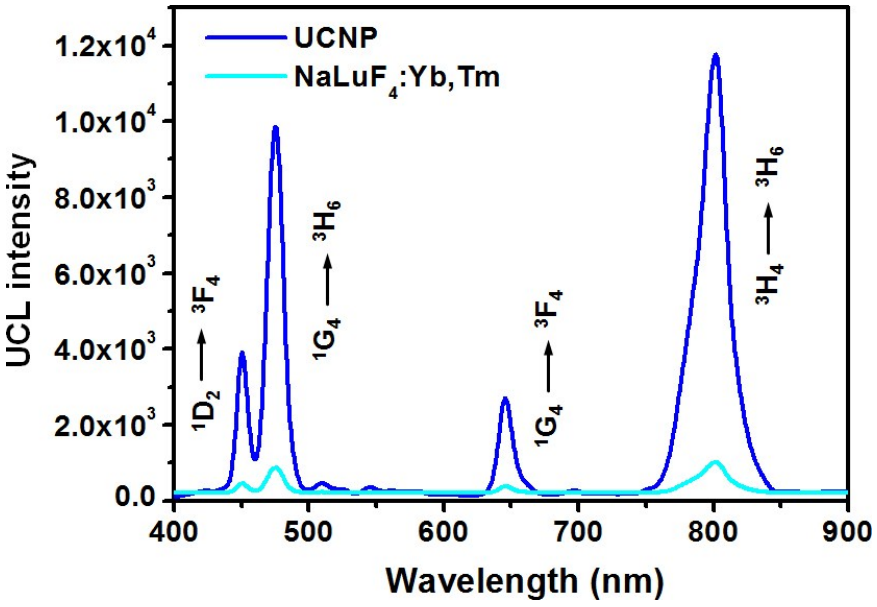

Figure S3. UCL spectra of $NaLuF_4$:Yb,Tm and UCNP.

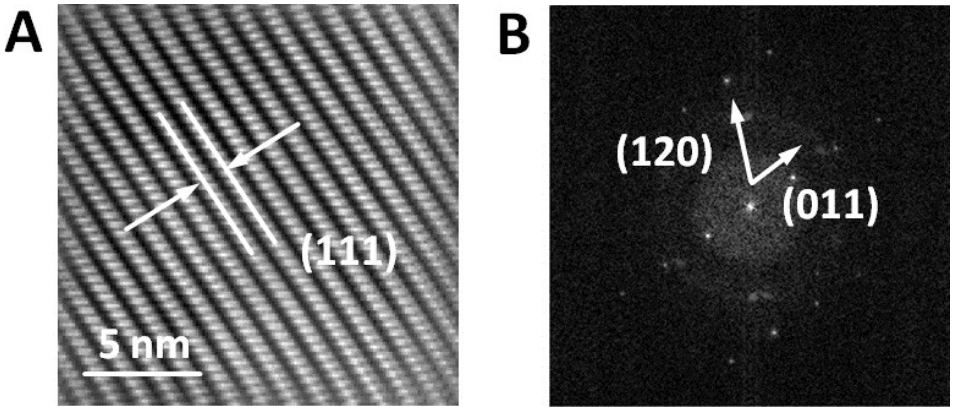

Figure S4. High-resolution TEM (HR-TEM) image A) and SAED patterns B) of UCNP. The SAED patterns were obtained from HR-TEM image *via* FFT.

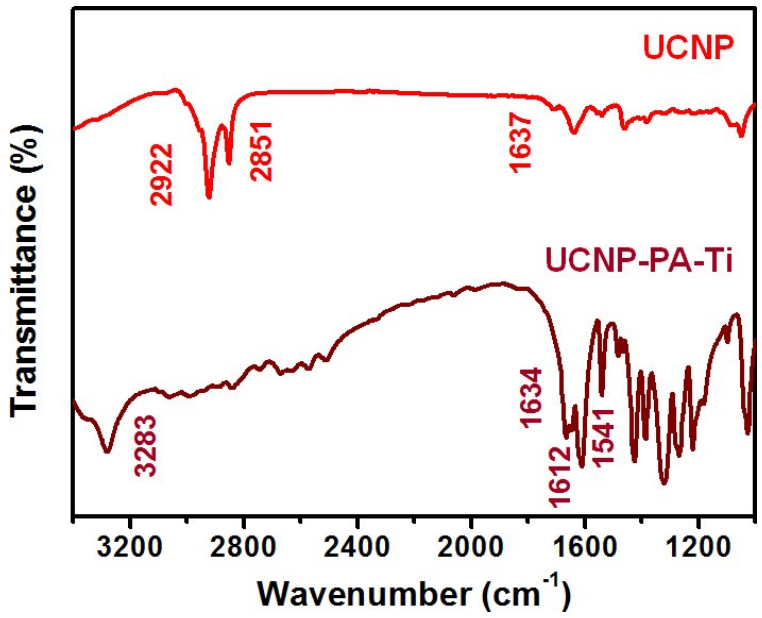

Figure S5. FTIR spectra of UCNP and UCNP-PA-Ti. The band of UCNP at 1637 cm^{-1} corresponds to stretching vibration of carbonyl. The bands of UCNP at 2851 cm^{-1} and 2922 cm^{-1} correspond to stretching vibration of C-H. New bands of UCNP-PA-Ti at 1541 cm^{-1}, 1612 cm^{-1}, and 1634 cm^{-1} correspond to stretching vibration of C=C in phenyl. The band of UCNP-PA-Ti at 3283 cm^{-1} corresponds to stretching vibration of O-H.

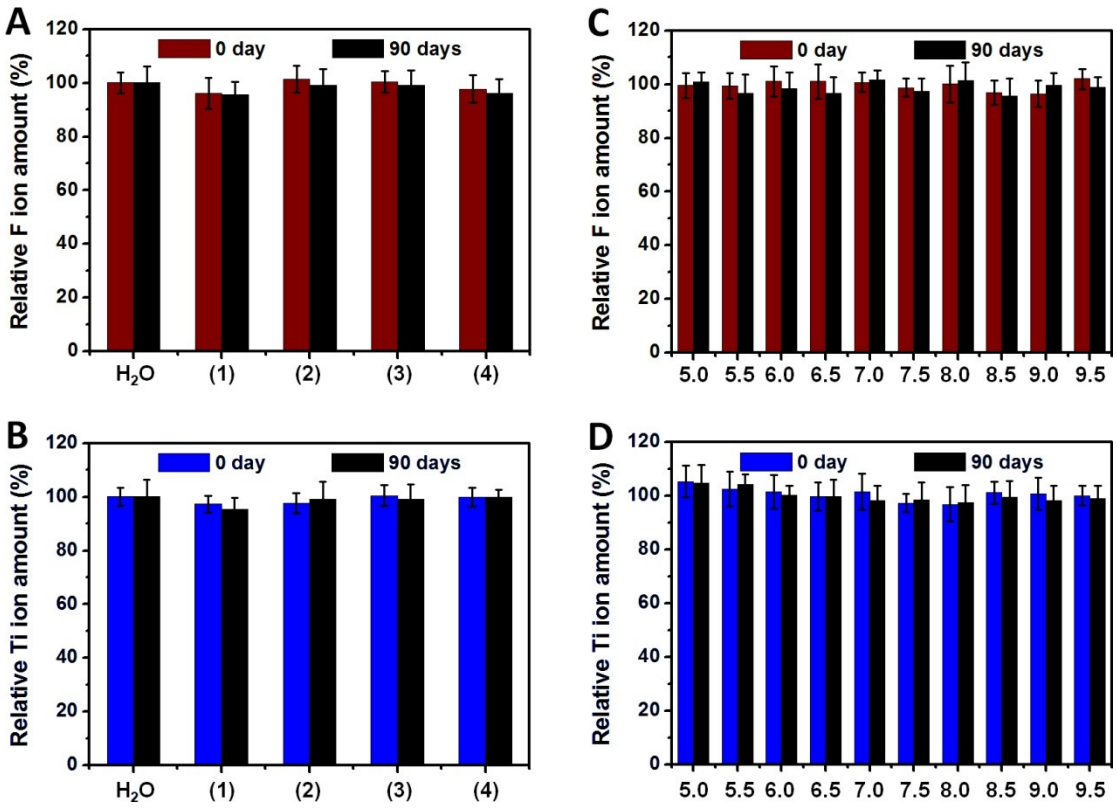

Figure S6. F A) and Ti B) amount in the supernatant of UCNP-PA-Ti-contained serum (1), 0.9% NaCl solution (2), 5% glucose solution (3), and artificial cerebrospinal fluid (4) within 90 days standing. F C) and Ti D) amount in the supernatant of UCNP-PA-Ti-contained PBS solutions with various pH within 90 days standing.

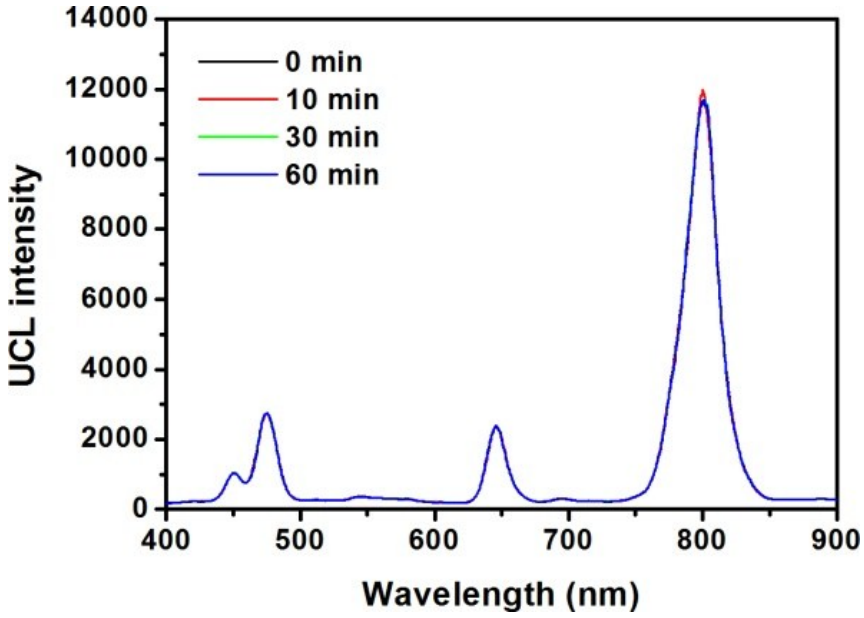

Figure S7. UCL spectra of UCNP-PA-Ti after receiving 10, 30, and 60 min irradiation.

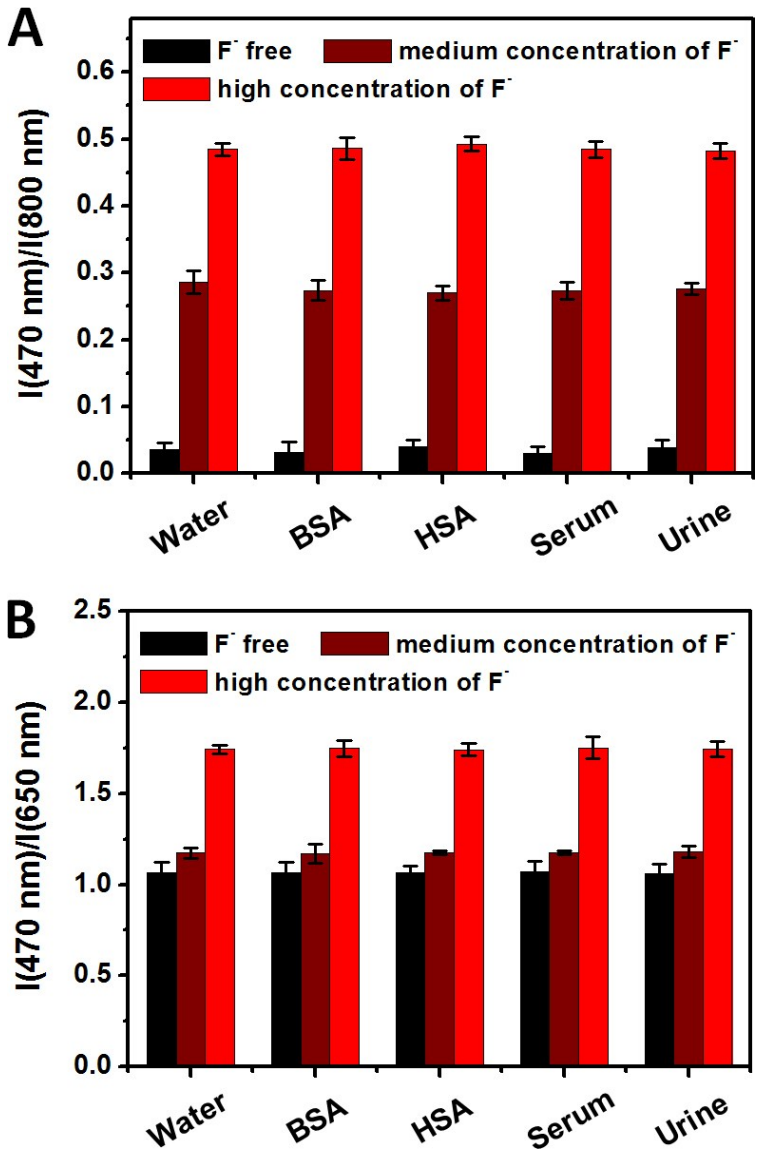

Figure S8. I(470 nm)/I(800 nm) A) and I(470 nm)/I(650 nm) B) of pretreated UCNP-PA-Ti solution with various concentration F^- (medium concentration: 10 μM; high concentration: 20 μM) in BSA solution, HSA solution, serum, and urine.

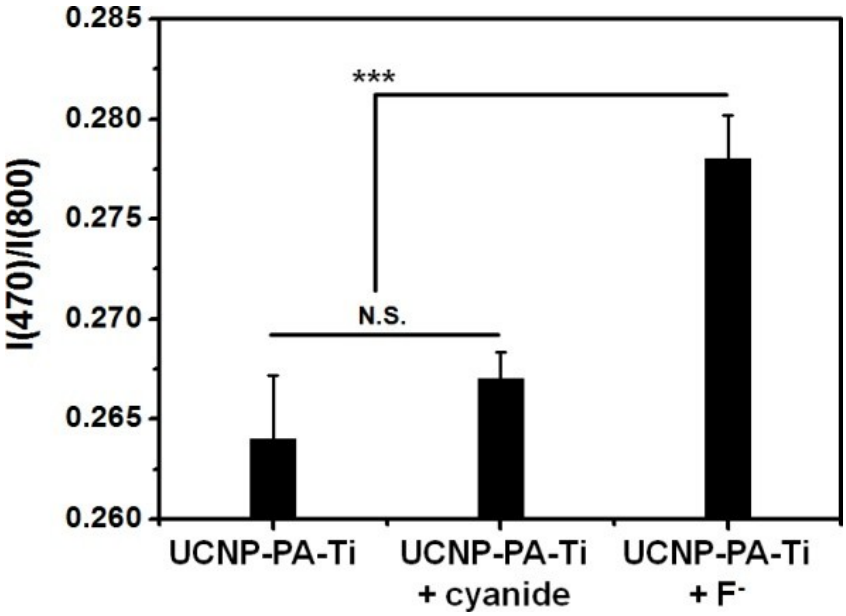

Figure S9. I(470)/I(800) of UCNP-PA-Ti with cyanide (UCNP-PA-Ti + cyanide) and F- (UCNP-PA-Ti + F^-) addition, respectively.

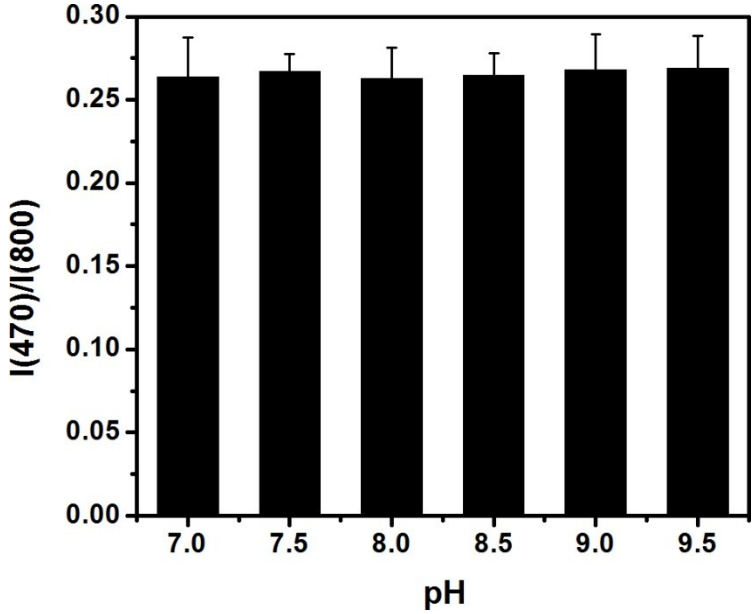

Figure S10. I(470)/I(800) of UCNP-PA-Ti in various basic conditions (pH = 7.0, 7.5, 8.0, 8.5, 9.0, and 9.5), respectively.

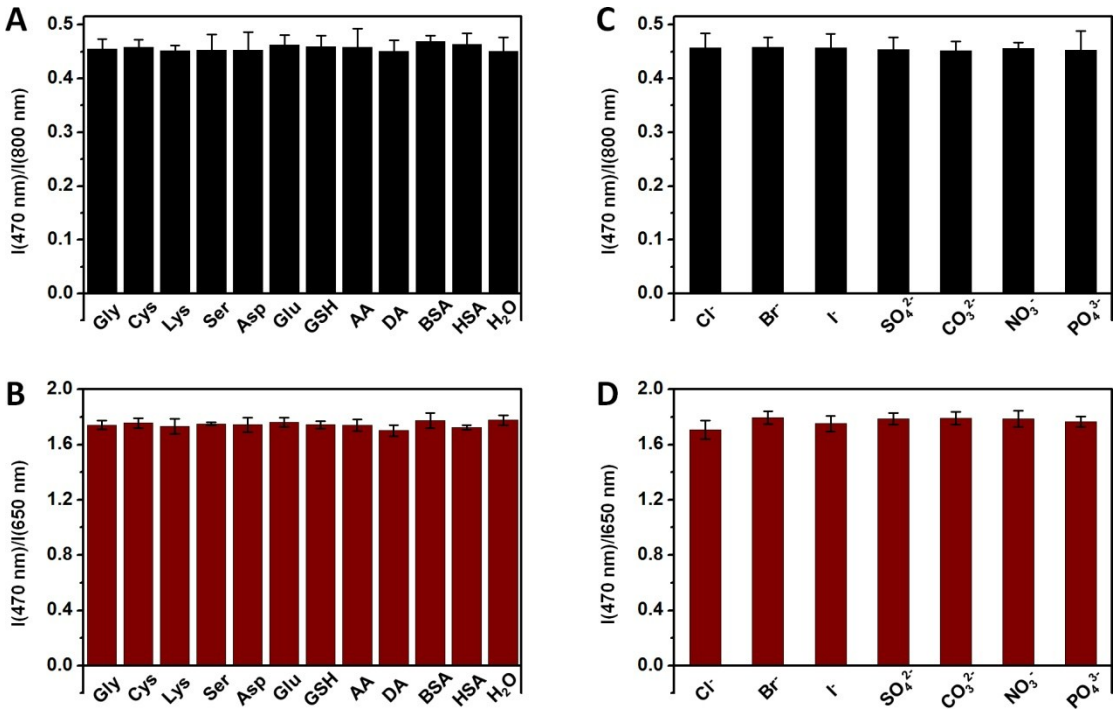

Figure S11. I(470 nm)/I(800 nm) A) and I(470 nm)/I(650 nm) B) of pretreated UCNP-PA-Ti solution with F^- in aqueous condition containing various biomolecules. I(470 nm)/I(800 nm) C) and I(470 nm)/I(650 nm) D) of pretreated UCNP-PA-Ti solution with F^- in aqueous condition containing various anions.

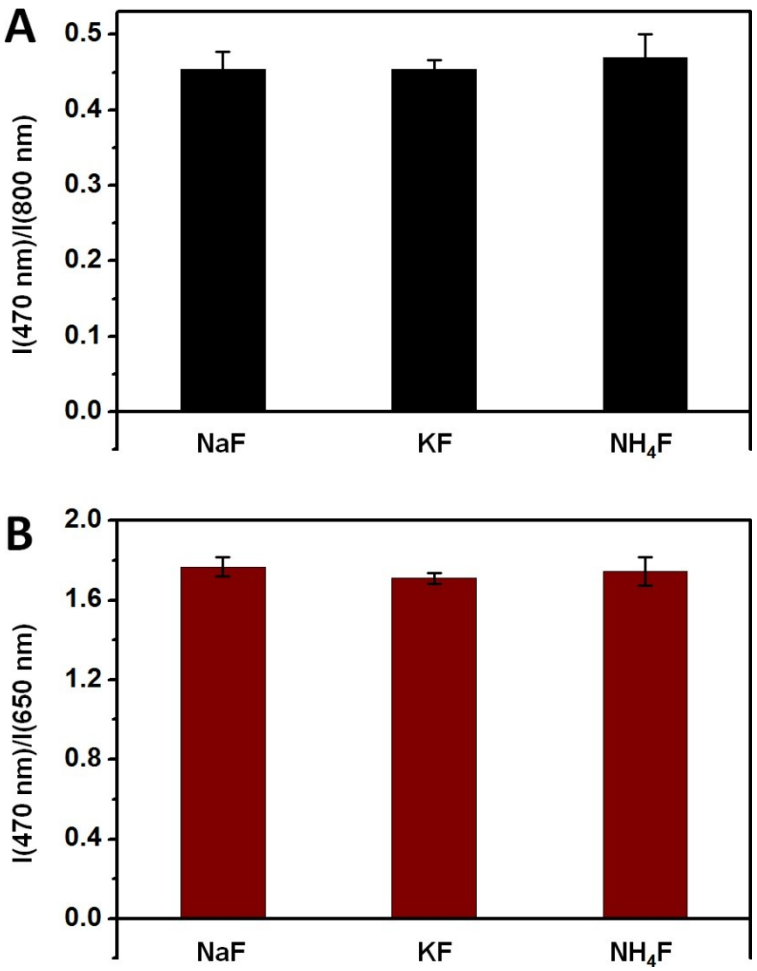

Figure S12. I(470 nm)/I(800 nm) A) and I(470 nm)/I(650 nm) B) of UCNP-PA-Ti solution with various soluble fluorides.

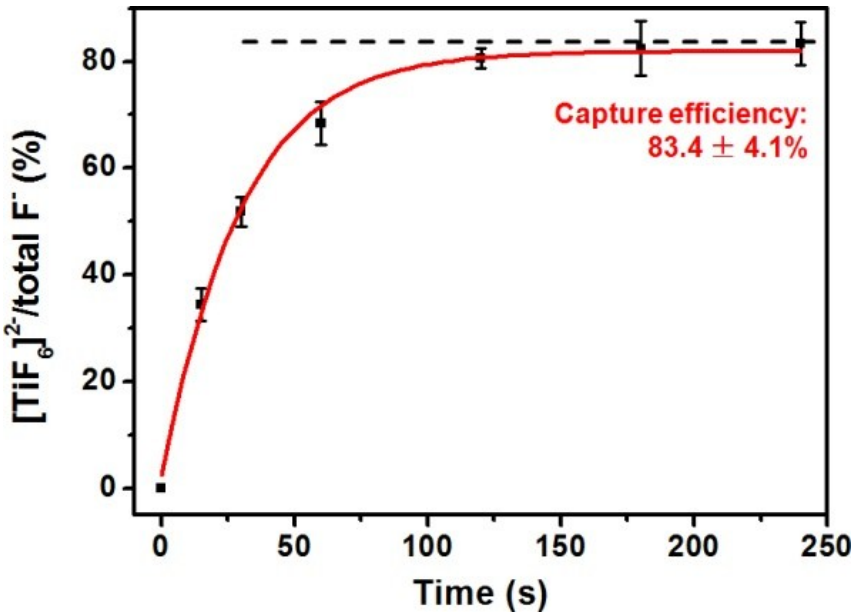

Figure S13. $[TiF_6]^{2-}$/total F^- ratio of albumin-conjugated F^- solution treated with UCNP-PA-Ti within 4 min.

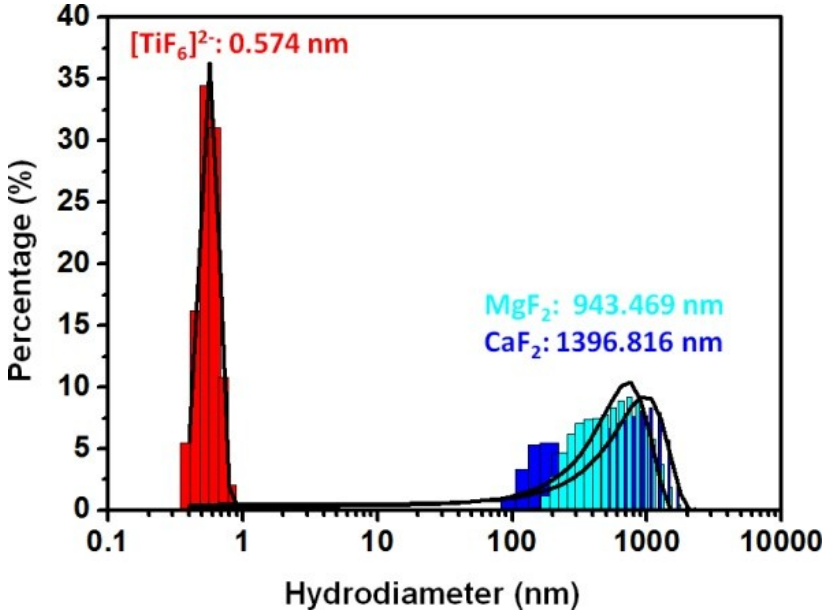

Figure S14. Hydrodiameter of produced $[TiF_6]^{2-}$, CaF_2, and MgF_2 *in vitro*.

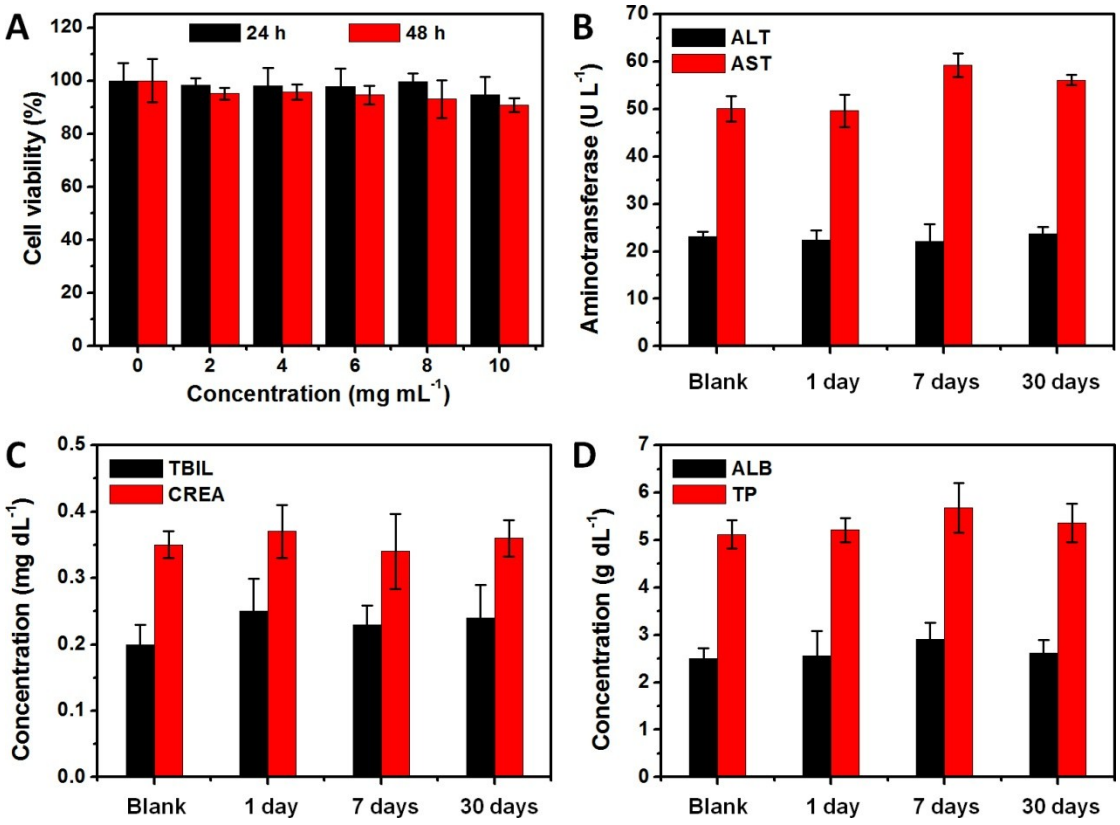

Figure S15. A) Cytotoxicity of UCNP-PA-Ti determined by MTT assay. B-D) Serum biochemistry tests including indices of liver and kidney function. Alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin (TBIL), creatinine (CRE), albumin (ALB), and total protein (TP) were tested.

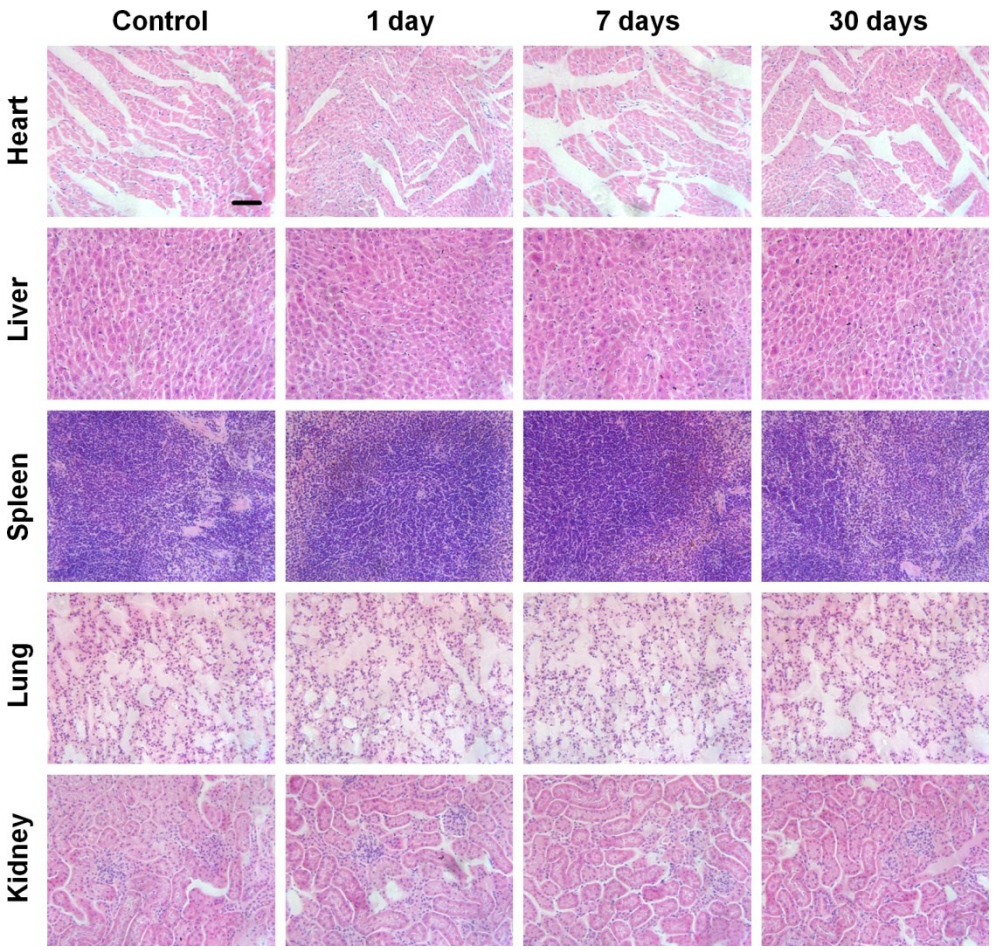

Figure S16. H&E-stained tissue sections of major organs, including heart, liver, spleen, lung, and kidney. Scale bar: 50 μm.

Table S1. Hydrodiameter of UCNP-PA-Ti in various biological fluids, including (1) serum, (2) 0.9% NaCl solution, (3) 5% glucose solution, and (4) artificial cerebrospinal fluid, and pH conditions (pH = 5.0 - 9.0).

	H_2O	(1)	(2)	(3)	(4)
HD (nm)	28.0	27.6	28.2	28.4	27.9
RSD (%)	1.346	1.667	2.614	2.639	1.271

	5.0	**5.5**	**6.0**	**6.5**	**7.0**	**7.5**	**8.0**	**8.5**	**9.0**	**9.5**
HD (nm)	27.3	28.3	28.9	27.5	27.9	28.9	29.0	27.5	28.6	27.0
RSD (%)	1.789	2.674	1.528	1.468	2.389	2.128	2.400	1.349	1.863	2.505

Table S2. F^- capture efficiency of UCNP-PA-Ti in various anions presence.

	Cl^-	Br^-	I^-	SO_4^{2-}	CO_3^{2-}	NO_3^-
F^- Capture Efficiency (%)	84.3 ± 1.7	83.8 ± 2.2	85.4 ± 0.9	84.7 ± 2.3	83.9 ± 3.4	84.0 ± 2.7

Table S3. Comparison of the UCNP-PA-Ti and previous reported nanoprobes for F^- detection.

	Strategy	LOD (M)	Linear Range (M)	Reference
UCNP-PA-Ti	ratiometric	4.2×10^{-9}	5.0×10^{-8} – 2.6×10^{-5}	this work
Eu-MOF	ratiometric	2×10^{-6}	4×10^{-6} – 8×10^{-5}	[1]
CdS QDs-Ca	turn-off	6×10^{-6}	1×10^{-5} – 3×10^{-4}	[2]
Eu(L–S15)$_2$TTA	turn-off	1.6×10^{-9}	1.0×10^{-7} – 1.0×10^{-6}	[3]

Table S4. UCL intensity of tubes presented in Figure 3E and F.

	F^- free	5 μM	10 μM	20 μM
I(470 nm)	3970.63	5786.52	6898.03	9083.40
I(650 nm)	4308.30	4619.54	4504.47	4417.51
I(800 nm)	15796.50	15837.48	15821.63	15132.10
I(470 nm)/I(650 nm)	0.9216	1.2526	1.5314	2.0562
I(470 nm)/I(800 nm)	0.2514	0.3654	0.4360	0.6003