Supporting Information

Rationally Designed Pure-inorganic Upconversion Nanoprobe for Ultra-highly Selective Hydrogen Sulfide Quantification and Elimination *In Vivo*

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Experimental section

Materials

Rare-earth oxide Lu_2O_3 (99.999%), Yb_2O_3 (99.99%), Er_2O_3 (99.99%), and Tm_2O_3 (99.99%) were purchased from STREM Chemicals, Inc. USA. Citric acid hydrate, HCl, KCl, NaOH, ethanol, cyclohexane, and dichloromethane (CH_2Cl_2) were purchased from Beijing Chemical Reagent Company. 1-Octadecene (ODE), $K_3[Fe(CN)_6]$, $NOBF_4$, and NH_4F were purchased from Alfa Aesar Chemical Co. Ltd. Bovine serum albumin (BSA) and human serum albumin (HSA) were purchased from Energy Chemical Co. Ltd. Other chemicals were purchased from Sigma Aldrich. Rare earth chlorides ($LnCl_3$, Ln: Lu, Yb, Er, 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.

Synthesis of core-shell $NaLuF_4$:Yb,Er,Tm@$NaLuF_4$ nanoparticles with 20% Er dopant (UCNPs) and 2% Er dopant (UCNPs-L)

In a typical experiment, a mixture of 1 mM $LnCl_3$ (Ln: 64.9% Lu, 15% Yb, 20% Er, 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 °C and maintained for 1 h under the argon atmosphere. After the solution was cooled naturally, the 20% Er-doped $NaLuF_4$:Yb,Er,Tm nanoparticles were separated *via* centrifugation (10000 rpm, 5 min) and washed with the mixture of ethanol and cyclohexane (1:1 v/v) for three times.[1] 2% Er-doped nanoparticles were obtained with the same method with 20% Er-doped nanoparticles using the rare-earth ratio of 82.9% Lu, 15% Yb, 2% Er and 0.1% Tm.

The core-shell UCNPs were obtained with the same solvothermal method using 20% Er-doped $NaLuF_4$:Yb,Er,Tm nanoparticles as core and $LuCl_3$ as only rare-earth source of inner shell. The core-shell UCNPs-L were obtained with the same method with 2% Er-doped $NaLuF_4$:Yb,Er,Tm nanoparticles as core. The hydrophobic core-shell UCNPs and UCNPs-L were stored under room temperature in cyclohexane.

Synthesis of PB-functionalized upconversion nanoprobes (UC-PB)

In a typical experiment, 5 mL of dichloromethane solution of $NOBF_4$ (10 mM) was dropped into 5 mL of hydrophobic UCNPs dispersion in cyclohexane (~5 mg mL^{-1}) at room temperature. Then, the bare UCNPs were washed with ethanol several times and dispersed in DI water. Further citric acid modification was performed by dispersing bare UCNPs in a saturated solution of citric acid and ultrasonic treatment.[2] Bare UCNPs-L were obtained with the same method.

The synthesis of PB-functionalized upconversion nanoprobes (UC-PB) were in accordance with a previously reported protocol by our group.[3] In a typical experiment for UC-PB_5 nanoprobes preparation, 5 mL HCl solution (25 mM) containing $FeCl_3$ (1 mM) and 5 mL citric acid solution (30 mM) containing $K_3[Fe(CN)_6]$ (1 mM), ascorbic

acid (30 mM), and KCl (0.1 mM) were slowly mixed with 10 mL citric acid-modified UCNPs suspension (5 mM) at room temperature. After vigorous stirring for another 20 min, the resulting colloidal solution was centrifuged and washed with deionized water three times. The reaction for PB shell coating can be described as the following two equations:

$$2[Fe(CN)_6]^{3-} + C_6H_8O_6 = 2[Fe(CN)_6]^{4-} + C_6H_6O_6 + 2H^+ \quad \text{(Eq.S1)}$$

$$3[Fe(CN)_6]^{4-} + 4Fe^{3+} = Fe_4[Fe(CN)_6]_3 \text{ (PB)} \quad \text{(Eq.S2)}$$

The synthetic procedure of UC-PB_8 and UC-PB_{11} nanoprobes was same to that of UC-PB_5 nanoprobes except that the concentration of UCNPs was 3 mM and 2 mM, respectively. UCNPs-L with 5 nm PB shell were obtained with the same method using UCNPs-L instead of UCNPs. The UC-PB nanoprobes were stored under 4 ^oC in DI water, respectively.

Characterization

The sizes and morphologies of the nanoprobes were determined at 200 kV by a JEOL JEM-2010 transmission electron microscope (TEM). The high-resolution lattice image, scanning transmission electron microscope (STEM) image, energy dispersive X-ray analysis (EDXA) spectra, and EDXA line scan were performed using a Tecnai G^2F30 high-resolution transmission electron microscope (HR-TEM). Samples were dispersed in cyclohexane or DI water 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 absorbance 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° to 70° (Cu Kα radiation, λ = 1.54 Å). Fourier transform infrared (FTIR) spectra were measured using a Shimadzu Fourier Transform Infrared Spectrophotometer IRPRESTIGE-21 from samples in KBr pellets. X-ray photoelectron spectroscopy (XPS) spectra were performed on Thermo escalab 250Xi. The sample for XRD, FTIR, and XPS determination was previously dried in nitrogen atmosphere at 120 °C. 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 °C with a detection angle of 90°. The upconversion luminescence (UCL) spectra were taken on a Edinburgh FLS980 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. Square-wave voltammetry (SWV) curves were obtained by electrochemical station (Chenhua Instruments Co. CHI600). A three-electrode system was made up of a glassy carbon electrode (GCE, 3 mm in diameter) as the working electrode, an Ag/AgCl electrode (saturated KCl) as reference electrode, and a Pt wire as counter electrode.

Detection of H_2S *in vitro*

The H_2S standard solution was prepared by adjusting the pH of freshly formulated NaHS solution to neutral (pH = 7.0) with diluted hydrochloric acid solution. All H_2S

standard solutions and UC-PB_5 nanoprobes suspension were used immediately after formulation. To set up the standard curve for H_2S detection, 1 mL UC-PB_5 nanoprobes suspension (0.7 mg mL^{-1}) was added into 1 mL H_2S standard solutions with concentrations from 2 nM to 300 μM, respectively. After incubated for 10 min, the UV-vis-NIR absorbance spectra and UCL spectra of all samples were determined, respectively.

Other biomolecules and ions was also studied by the same method to demonstrate the selectivity of UC-PB_5 nanoprobes, including common biosulfurs (reduced glutathione (GSH), cysteine (Cys), homocysteine (Hcy), SO_3^{2-}, and $S_2O_3^{2-}$), essential/non-essential amino acids, reactive oxygen species (HClO, H_2O_2, and $ONOO^-$), cations (K^+, Na^+, Ca^{2+}, Mg^{2+}, Mn^{2+}, Zn^{2+}, and NH_4^+), anions (Cl^-, CO_3^{2-}, NO_3^-, SO_4^{2-}, SiO_4^{4-}, and PO_4^{3-}), glucose, dopamine, dihydroxy-phenyl acetic acid (DOPAC), bovine serum albumin (BSA), and human serum albumin (HSA). The concentration of UC-PB_5 nanoprobes suspension for selectivity study was (0.2 mg mL^{-1}). The concentration of studied biomolecules and ions was 30 mM.

Dynamic study of H_2S detection

The dynamic free S^{2-} amount in solution was monitored by Ag/S ion selective electrode (Shanghai Leici Pag/S-1). In a typical experiment, 10 mL H_2S standard solution (300 μM) was placed in flask with generously magnetic stirring. After 10 min continuous monitoring, 100 μL UC-PB_5 nanoprobes suspension (30 mg mL^{-1}) was added rapidly. The residual free S^{2-} amount was calculated using the initial S^{2-} amount as the standard of normalization. The dynamic absorbance spectra were performed in

dark room by UV-vis-NIR spectra. After mixing 1 mL UC-PB_5 nanoprobes suspension (0.7 mg mL^{-1}) with 1 mL H_2S standard solution (200 nM, 20 μM, 40 μM, 100 μM, 200 μM, and 300 μM), the dynamic absorbance spectra was taken within 350 s. The video of UC-PB_5 nanoprobes suspension was recorded after H_2S standard solution addition within 20 s.

Stability of UC-PB_5 nanoprobes

The absorbance at 720 nm and UCL signal of UC-PB_5 nanoprobes in various biological solutions (DI water, PBS, and saline, 0.35 mg mL^{-1}) were recorded by UV-vis-NIR absorbance spectra and UCL spectra within 720 min. The DLS of UC-PB_5 nanoprobes after various storage time points were determined after voticity for 1 min.

Mechanism study of H_2S detection

To obtain the products in reaction, including reacted UC-PB_5 nanoprobes and produced sulfides, 20 mL UC-PB_5 nanoprobes suspension (0.7 mg mL^{-1}) was mixed with 20 mL H_2S standard solution (300 μM) under generous magnetic stirring for 60 min. The reacted UC-PB_5 nanoprobes were collected *via* centrifugation (10000 rpm, 5 min). The supernatant was collected to obtain produced sulfides by high-speed centrifugation (20000 rpm, 15 min). All the above products were washed with DI water for nine times, dried under nitrogen protection, and stored in individual sealed container before further determination, respectively. Further, the products from the reaction between ferricyanide (70 μM) or ferric isothiocyanate (70 μM) and H_2S standard solution (300 μM). The TEM images and XPS spectra of studied products were determined within 24 h post-preparation.

For electrochemical study, 100 μL UC-PB_5 nanoprobes suspension (15 mg mL^{-1}) was dropped on the surface of a clean electrode and dried under 37 ^oC. The SWV curve of as-modified electrode was tested in the presence of 20 mL KCl solution (0.1 M). After the first SWV determination, same electrode was soaked into 20 mL H_2S standard solution (150 μM) for 5 min. Then, another SWV determination was performed in the same condition after gentle washing.

In neutral condition, the H_2S was existed in HS^- state. To describe the reaction between PB and HS^- in neutral condition, the following chemical equation was proposed:

$$Fe_4[Fe(CN)_6]_3 + 6HS^- = 3[Fe(CN)6]^{4-} + 2S + 4FeS + 6H^+ \quad \text{(Eq.S3)}$$

Cell culture

Adenocarcinomic human alveolar basal epithelial cells (A549 cell line provided by provided by the Institute of Basic Medical Sciences Chinese Academy of Medical Sciences) were grown in DMEM (Dulbecco's modified Eagle's medium) supplemented with 10% FBS (fetal bovine serum) and 1% penicillin-streptomycin at 37 ^oC with 5% CO_2. For use in the cytotoxicity 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 cell imaging experiments, 1×10^7 cells $well^{-1}$ were seeded in 6-well plates and allowed to attach for 24 h prior to the assay.

MTT assay

In vitro cytotoxicity was measured by performing methyl thiazolyl tetrazolium (MTT) assays on the A549 cells. Different concentrations of UC-PB_5 nanoprobes (0-

4.5 mg mL^{-1}) were then added to the wells. The cells were subsequently incubated for 24 or 48 h at 37 ^{o}C 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 ^{o}C under 5% CO_2. The optical density value (*Abs.*) of each well, with background subtraction at 570 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%

Hematology and histology 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 C57BL/6 mice intravenously injected with UC-PB_5 nanoprobes (n = 3, dose = 20 mg kg^{-1}, Test) and from mice receiving no injection for 1 day, 7 days, 15 days, 30 days, and 60 days post-injection (n = 3, dose = 0 mg kg^{-1}, Control), respectively. The body weight of mice were also recorded. Blood was collected from the femoral vein. 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 after anesthetic and their liver, spleen, and kidney were harvested. The

tissues were fixed in paraformaldehyde, embedded in paraffin, sectioned, and stained with H&E. The histological sections were observed under an optical microscope (Olympus IX71).

In vitro UCL ratiometric imaging

A549 cells were pretreated with PBS, DL-PAG (4.5 mM), L-Cys (4 mM), L-Gly (4 mM), and NaHS (100 μM) for 24 h, respectively. The DL-PAG/L-Cys co-pretreated cells were treated with DL-PAG (4.5 mM) and L-Cys (4 mM) in sequence for 24 h, respectively. The cells with various pretreatments were then incubated with UC-PB_5 nanoprobes suspension (1.5 mg mL^{-1}) for 12 h, washed with PBS several times, and were resupplied with fresh DMEM and DAPI solution for nucleus staining. The dynamic cell imaging was taken by optical microscopy every 60 s using UC-PB_5 nanoprobes-stained cells without any pretreatment. After 240 s continuous monitoring, 1 mL NaHS solution (100 μM) was separately covered on cells. The UC-PB_5 nanoprobes and DAPI were excited by CW 980 nm and 360 nm, respectively. Signals were collected at 450 ± 20 nm (DAPI), 550 ± 20 nm (G), and 650 ± 15 nm (R), respectively. The signals were analyzed and UCL ratio was calculated by attached software of Andor iXon Ultra EMCCD.

Acute pancreatitis (AP) modal establishment

All animal procedures were performed in accordance with the Guidelines for Care and Use of Laboratory Animals of Beijing Vital River Laboratory Animal Technology Co., Ltd. and approved by the Animal Ethics Committee of the Vital River Institutional Animal Care and Use Committee (VR IACUC). The establishment

of AP mice was performed by Beijing Vital River Laboratory Animal Technology Co., Ltd. To establish the AP mice modal, C57BL/6 mice were intraperitoneally injected with L-arginine solution (18 mmol kg^{-1}) twice and were continuously observed within the following 72 h.[4] The successful establishment of AP mice modal was confirmed by the increasing serum amylase and IL-6 level, as well as corresponding H&E-stained pancreas sections from randomly selected mice.

Biobehavior and excretion study

All the animal procedures were in agreement with institutional animal use and care committee and carried out ethically and humanely. C57BL/6 mice (n = 9) were randomly divided into 3 groups for blood, tissue, and excretion collection, respectively. The blood were harvested from the femoral vein of mice receiving intravenous injection of UC-PB_5 nanoprobes (n = 3, dose = 20 mg kg^{-1}) within 8 h post-injection. The tissues were harvested from mice receiving intravenous injection of UC-PB_5 nanoprobes (n = 3, dose = 20 mg kg^{-1}) at 1, 4, and 24 h post-injection. The excretion were collected from mice receiving intravenous injection of UC-PB_5 nanoprobes (n = 3, dose = 20 mg kg^{-1}) within 168 h post-injection. All blood, tissues, and excretion samples were broke up and centrifuged to obtain clear diluted resultant for ICP-MS determination. The blood circulation curve of UC-PB_5 nanoprobes was fitted by the typical formulation of $C_t = C_0 e^{-ket} + A$.

In vivo UCL ratiometric 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. Images of signals were analyzed with corresponding software. AP mice, normal mice, and AP mice receiving DL-PAG injection (450 μmol kg^{-1}) were anesthetized with 10% chloral hydrate (200 μL) and were intravenously injected with UC-PB_5 nanoprobes suspension (10 mg kg^{-1}). At the 24 h post-injection, whole-body imaging was performed. Upon completion of whole-body imaging, the mice were sacrificed after anesthetic and their organs were harvested. The organs were simply washed by ethanol and PBS once respectively and then imaged under same conditions. Signals were collected at 550 ± 20 nm (G), and 800 ± 12 nm (N), respectively. The signals were analyzed and UCL ratio was calculated by attached software of Andor iXon Ultra EMCCD.

AP-associated lung injury alleviation

AP mice were randomly divided into 4 groups (n = 6, m = 18 ± 3 g) for further study. 6 AP mice in test group (AP + DL-PAG + UC-PB_5) were intraperitoneally injected with DL-PAG (450 μmol kg^{-1}) and intravenously injected with UC-PB_5 nanoprobes suspension (10 mg kg^{-1}) per 3 h for 4 times. The other three groups of AP mice, including PBS-injected mice (AP + PBS), DL-PAG-injected mice (AP + DL-PAG), and UC-PB_5 nanoprobes-injected mice (AP + UC-PB_5) were used as the controls. Another 12 normal mice were randomly divided into 2 groups and were injected with PBS (normal + PBS) or UC-PB_5 nanoprobes (normal + UC-PB_5) respectively as blank groups. The serum H_2S concentration was determined per hour within 5 hours post-injection of drugs by typical colorimetric assay. The relative MPO in lung tissues was tested at 72 h post-injection by commercial ELISA kit (Thermo

Fisher™ EMMPO Mouse Myeloperoxidase ELISA Kit). The H&E-stained tissue sections of pancreases and lungs harvested from mice with various treatments at various time points were also performed.

Supporting Information

Supporting Information is available, including detailed Experiment section; TEM images, size distribution, XRD patterns, EDXA spectrum, FTIR spectrum, UCL spectrum, UV-vis-NIR spectra of as prepared $NaLuF_4$:Yb,Er,Tm, UCNPs, and UC-PB nanoprobes (Figure S1-5); dynamic absorbance spectra of UC-PB_5 nanoprobes suspension in the absence and presence of H_2S (Figure S6,7); real-time video of UC-PB_5 nanoprobes suspension in the addition of H_2S (Movie S1); selectivity of UC-PB_5 nanoprobes among amino acids, ions, and biomolecules (Figure S8-11); comparison of H_2S detection capacities with previous reported upconversion nanoprobes (Table S1); cytotoxicity and biocompatibility studies of UC-PB_5 nanoprobes (Figure S12-14); luminescence images of A549 cells receiving various pre-treatments and UC-PB_5 nanoprobes incubation (Figure S15); serum biochemical data and H&E-stained sections for establishing AP modal (Figure S16); UCL images of mice receiving various treatments (Figure S17); biobehavior of UC-PB_5 nanoprobes in mice (Figure S18); H&E-stained sections of lungs harvested from mice receiving various treatments at various time points (Figure S19).

Reference

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2. A. G. Dong, X. C. Ye, J. Chen, Y. J. Kang, T. Gordon, J. M. Kikkawa and C. B. Murray, *J. Am. Chem. Soc.*, 2010, **133**, 998-1006.
3. Y. Liu, Q. Guo, X. Zhu, W. Feng, L. Wang, L. Ma, G. Zhang, J. Zhou and F. Li, *Adv. Funct. Mater.*, 2016, **26**, 5120-5130.
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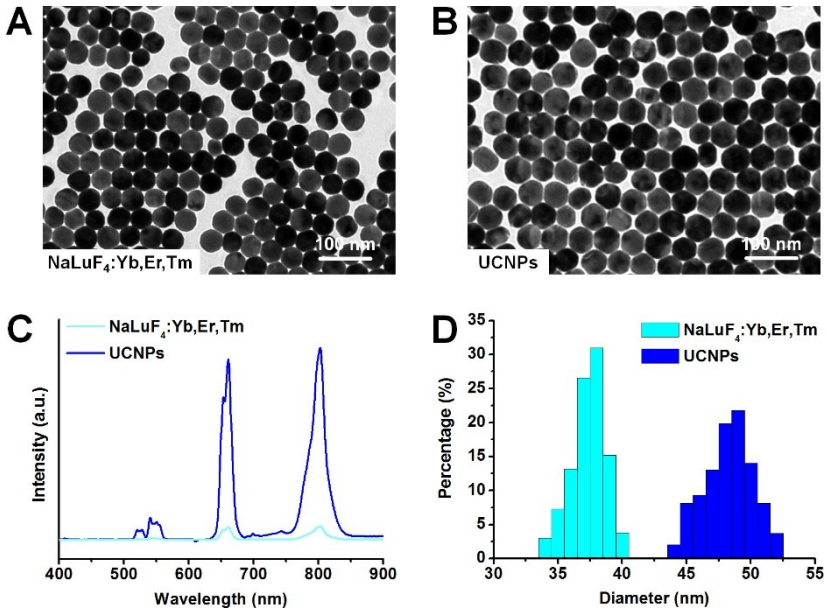


Figure S1. TEM image of 20% Er-doped $NaLuF_4$:Yb,Er,Tm core A) and core-shell UCNPs B). The UCL spectra C) and size distribution D) of 20% Er-doped $NaLuF_4$:Yb,Er,Tm core and core-shell UCNPs.

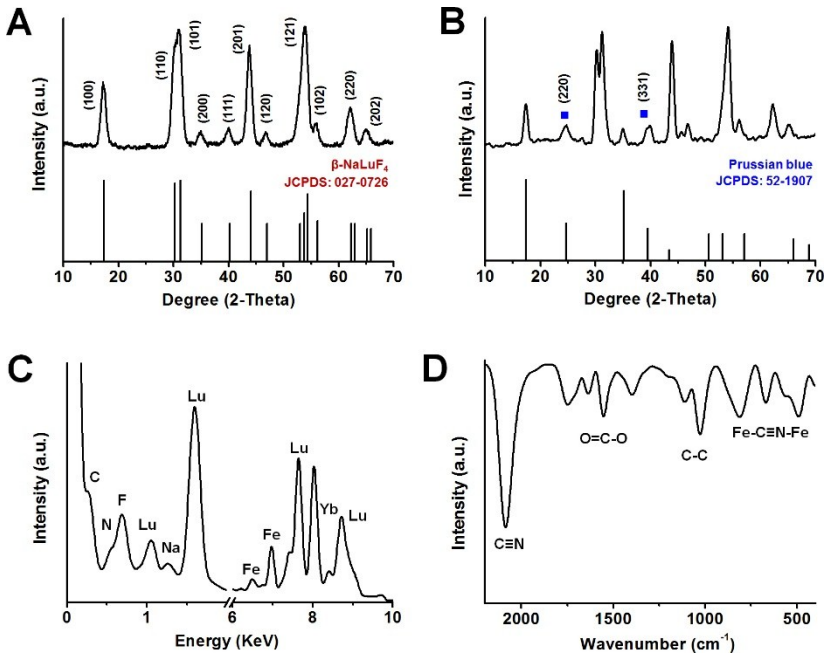


Figure S2. XRD patterns of core-shell UCNPs A) and UC-PB nanoprobes B). The EDXA spectrum C) and FTIR spectrum D) of UC-PB nanoprobes.

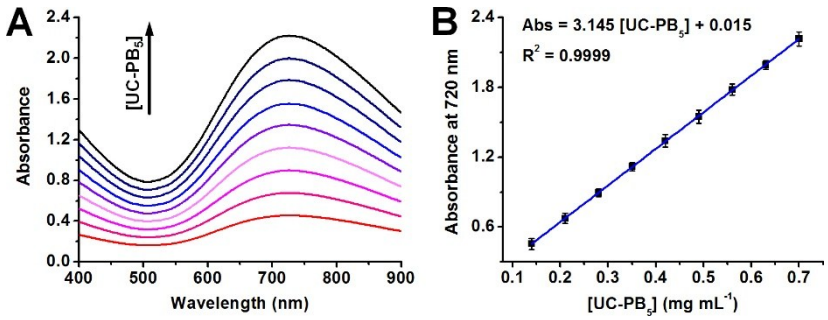


Figure S3. A) UV-vis-NIR spectra of UC-PB nanoprobes suspension in various concentrations. B) Linear curve of absorbance at 785 nm *vs* concentration of UC-PB nanoprobes suspension. Data are represented as mean ± SD.

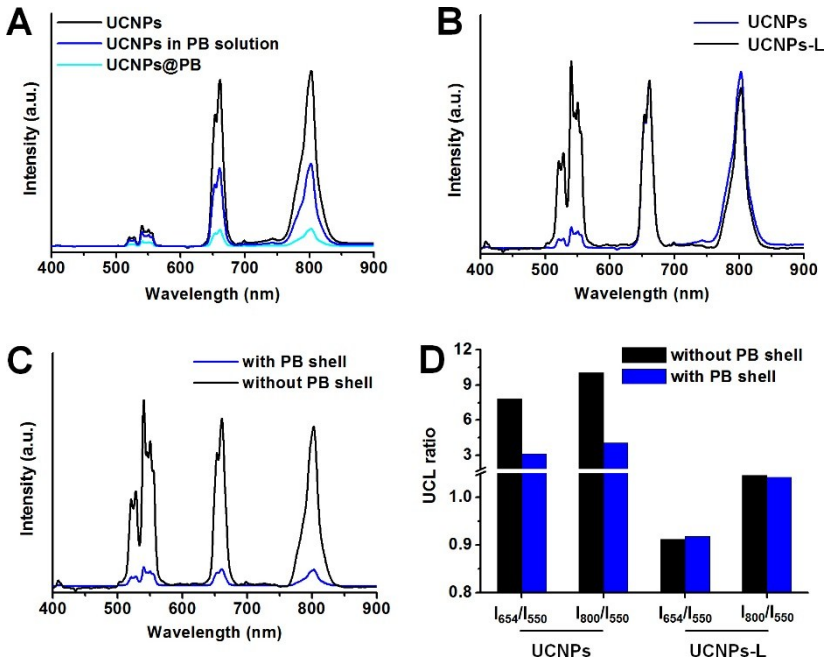


Figure S4. UCL spectra of UCNPs, UCNPs in PB solution, and UC-PB A), UCNPs and UCNPs-L B), and UCNPs-L with and without PB shell C). D) I_{654}/I_{550} and I_{800}/I_{550} UCL ratio of UCNPs and UCNPs-L with and without PB shell.

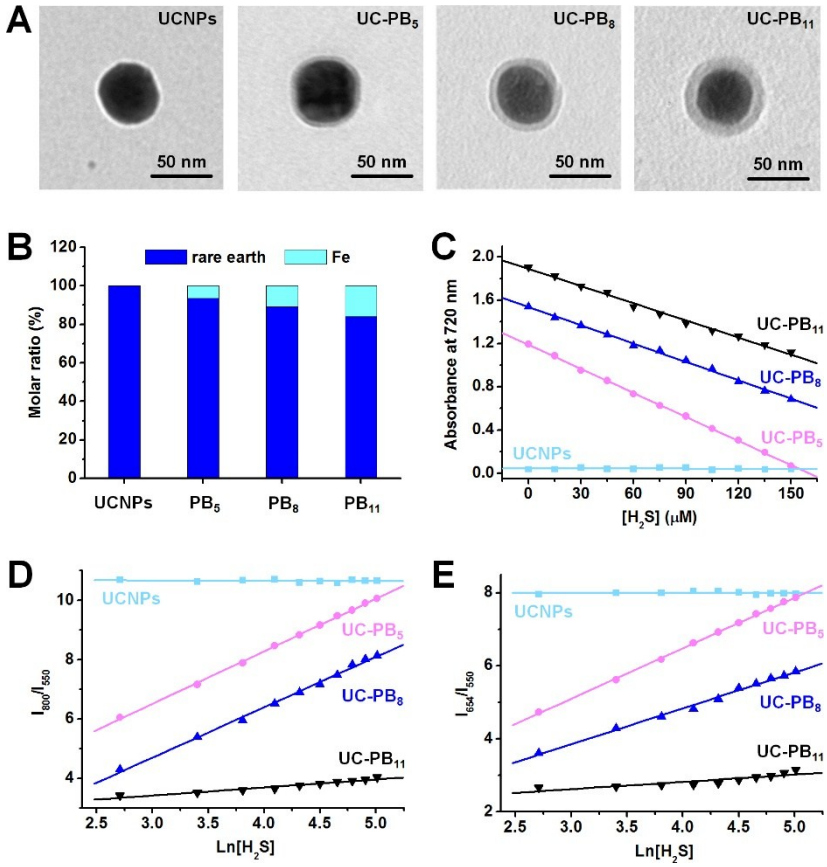


Figure S5. TEM image A) and element ratio B) of UCNPs, UC-PB_5, UC-PB_8, and UC-PB_{11}. Linear relationship between absorbance at 720 nm C), between I_{800}/I_{550} UCL ratio D), or between I_{654}/I_{550} UCL ratio E) of various nanoprobes and concentration of H_2S.

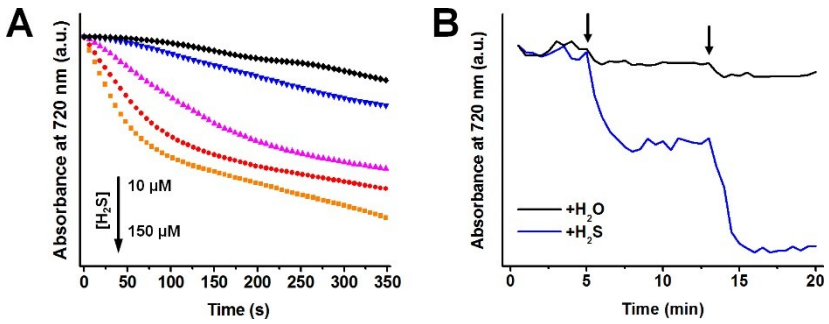


Figure S6. Dynamic absorbance spectra of UC-PB_5 nanoprobes suspension at 720 nm in response to various concentrations of H_2S A) and UC-PB_5 nanoprobes suspension at 720 nm with and without multiple H_2S addition B).

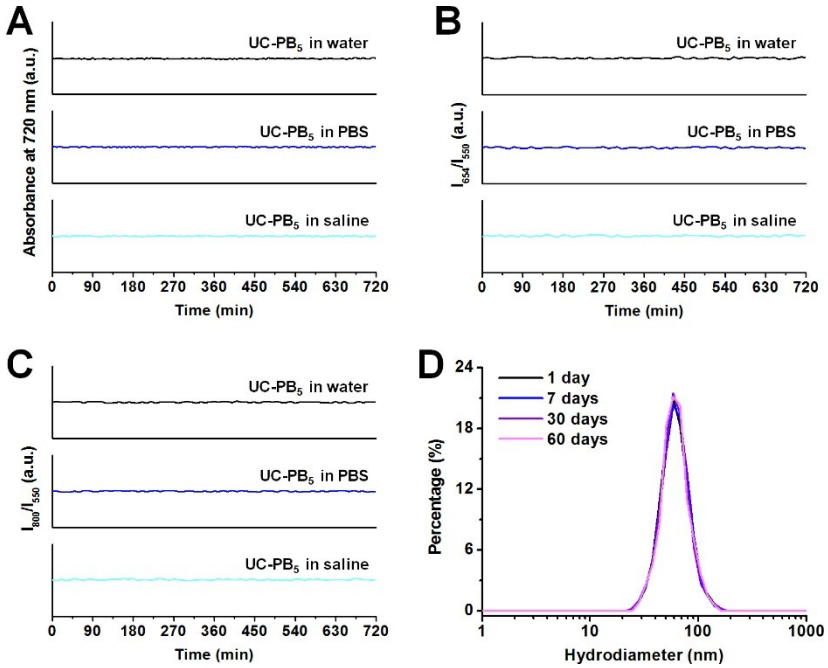


Figure S7. A) Dynamic absorbance spectra of UC-PB_5 nanoprobes at 720 nm in various biofluids. Dynamic UCL ratio of I_{654}/I_{550} B) and I_{800}/I_{550} C) calculated from dynamic luminescence spectra of UC-PB_5 nanoprobes at 720 nm in various biofluids. D) Hydrodiameter of UC-PB_5 nanoprobes with various storage periods.

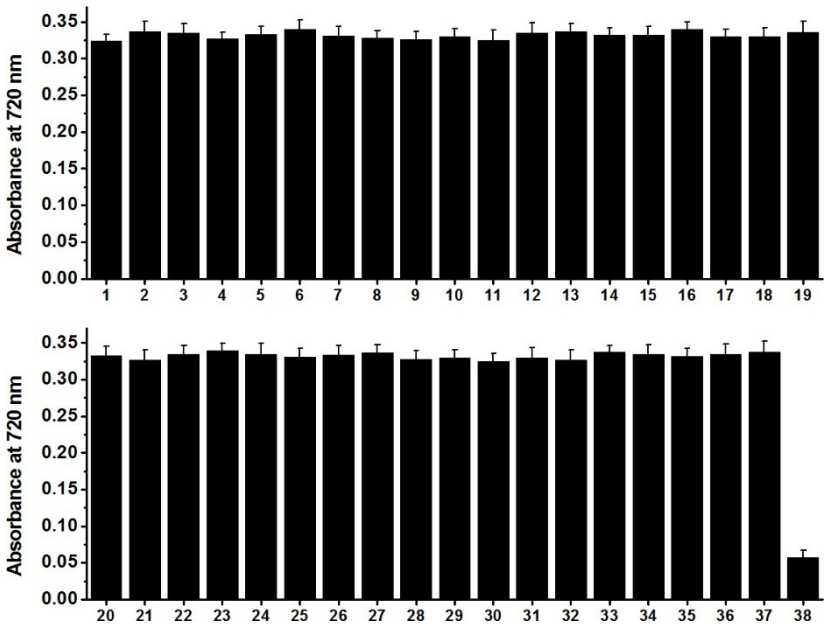


Figure S8. Absorbance of UC-PB_5 nanoprobes at 720 nm in response to various ions and biomolecules. 1) glycine; 2) alanine; 3) valine; 4) leucine; 5) isoleucine; 6) phenylalanine; 7) tryptophan; 8) tyrosine; 9) aspartic acid; 10) asparagine; 11) glutamic acid; 12) lysine; 13) glutamine; 14) methionine; 15) serine; 16) threonine; 17) proline; 18) histidine; 19) arginine; 20) K^+; 21) Na^+; 22) Ca^{2+}; 23) Mg^{2+}; 24) Mn^{2+}; 25) Zn^{2+}; 26) NH_4^+; 27) Cl^-; 28) CO_3^{2-}; 29) NO_3^-; 30) SO_4^{2-}; 31) SiO_4^{4-}; 32) PO_4^{3-}; 33) glucose; 34) dopamine; 35) dihydroxy-phenyl acetic acid (DOPAC); 36) bovine serum albumin (BSA); 37) human serum albumin (HSA); 38) H_2S. Data are represented as mean ± SD.

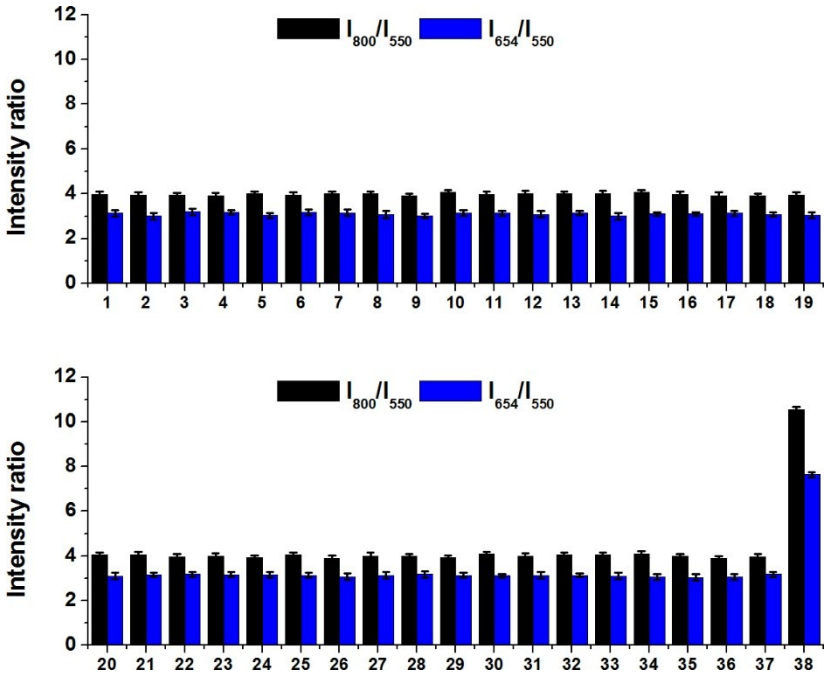


Figure S9. UCL ratio of UC-PB_5 nanoprobes in response to various ions and biomolecules. 1) glycine; 2) alanine; 3) valine; 4) leucine; 5) isoleucine; 6) phenylalanine; 7) tryptophan; 8) tyrosine; 9) aspartic acid; 10) asparagine; 11) glutamic acid; 12) lysine; 13) glutamine; 14) methionine; 15) serine; 16) threonine; 17) proline; 18) histidine; 19) arginine; 20) K^+; 21) Na^+; 22) Ca^{2+}; 23) Mg^{2+}; 24) Mn^{2+}; 25) Zn^{2+}; 26) NH_4^+; 27) Cl^-; 28) CO_3^{2-}; 29) NO_3^-; 30) SO_4^{2-}; 31) SiO_4^{4-}; 32) PO_4^{3-}; 33) glucose; 34) dopamine; 35) dihydroxy-phenyl acetic acid (DOPAC); 36) bovine serum albumin (BSA); 37) human serum albumin (HSA); 38) H_2S. Data are represented as mean ± SD.

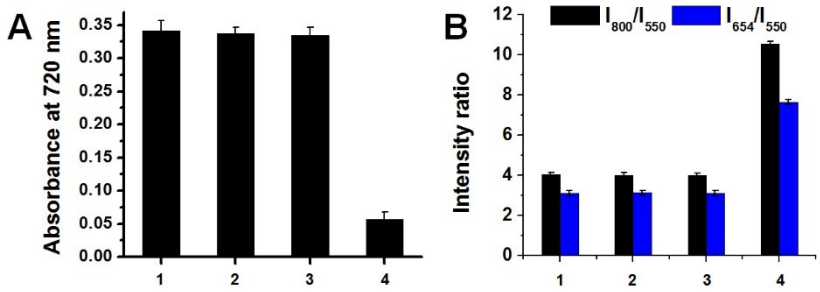


Figure S10. Absorbance of UC-PB_5 nanoprobes at 720 nm A) and UCL ratio of UC-PB_5 nanoprobes B) in response to various ROS. 1) HClO; 2) H_2O_2; 3) $ONOO^-$; 4) H_2S. Data are represented as mean ± SD.

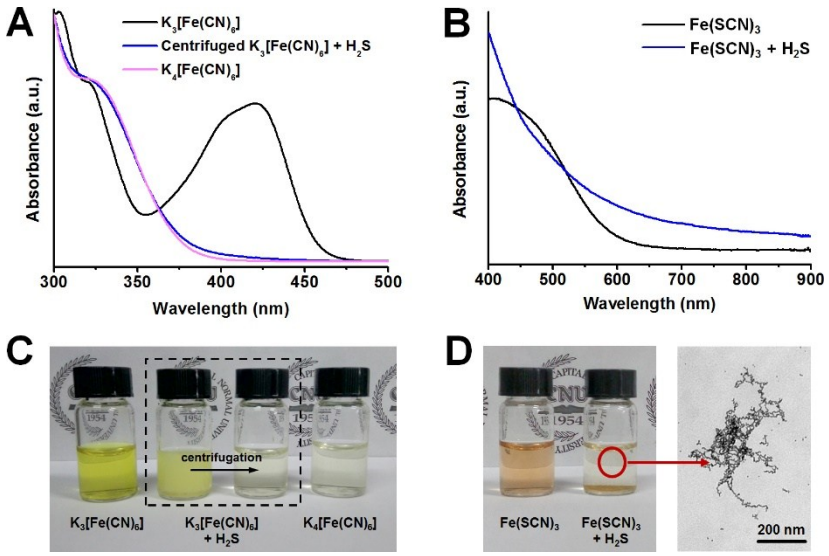


Figure S11. UV-vis absorbance spectra A) and photographs C) of $K_3[Fe(CN)_6]$, centrifuged $K_3[Fe(CN)_6]$ with H_2S addition, and $K_4[Fe(CN)_6]$. UV-vis-NIR absorbance spectra B) and photographs D) of $Fe(SCN)_3$ and $Fe(SCN)_3$ with H_2S addition. TEM images of $Fe(SCN)_3$ with H_2S addition was also provided in the right side of Figure 4D.

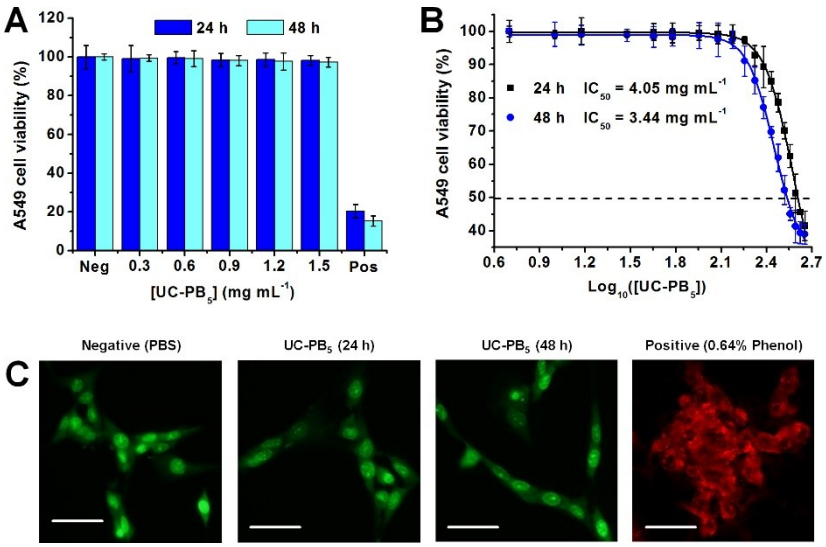


Figure S12. Cell viability A) and IC50 determination B) of UC-PB_5 nanoprobes within 24 and 48 h by MTT assay. C) Luminescence images of cells incubated with UC-PB_5 nanoprobes with SYTO-9/PI staining. The scale bar was 20 μm. Data are represented as mean ± SD.

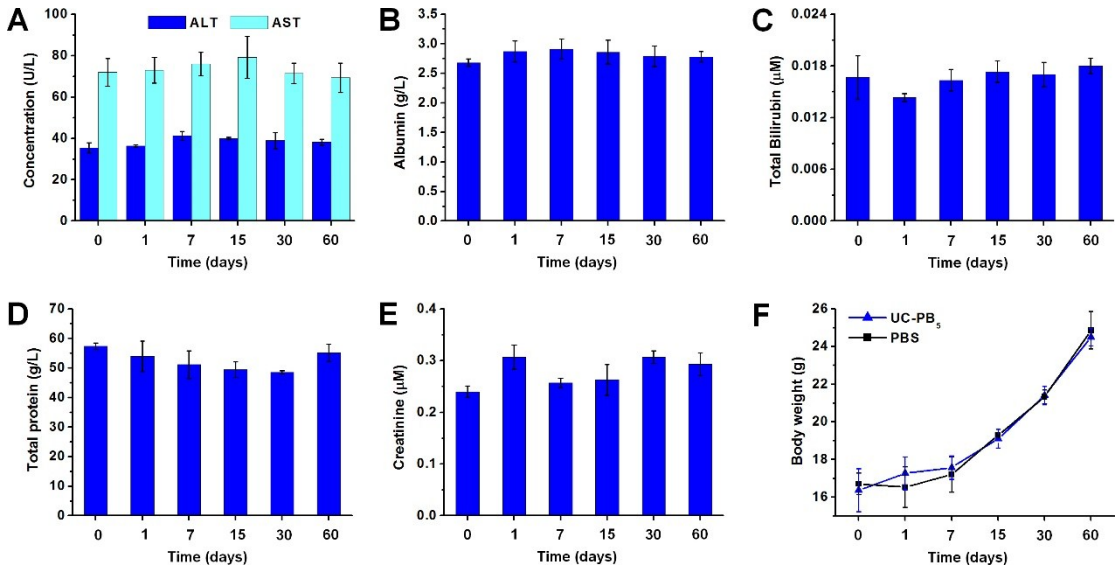
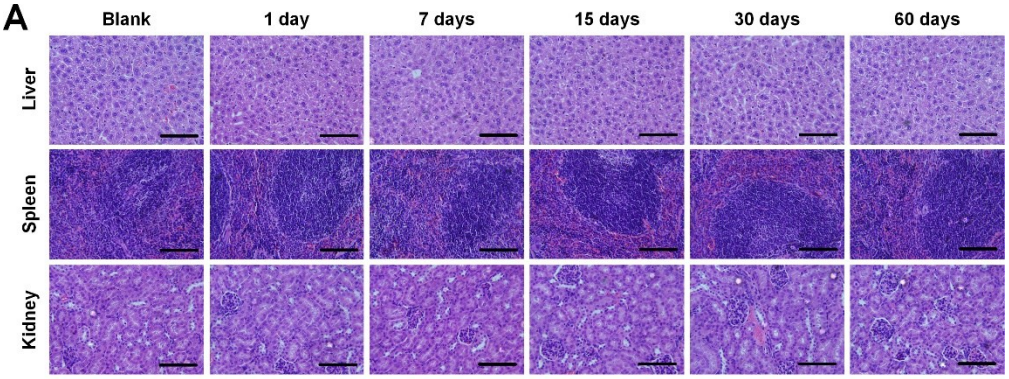


Figure S13. Serum biochemistry results A-E) and body weight F) obtained from mice receiving UC-PB_5 nanoprobes injection (20 mg kg^{-1}) at various time points post-injection (1 day, 7 days, 15 days, 30 days, and 60 days) and mice receiving PBS injection (0 day). A) ALT (alanine aminotransferase) and AST (aspartate aminotransferase); B) ALB, albumin; C) TBIL, total bilirubin; D) TP, total protein; E) CREA, creatinine. Data are represented as mean ± SD.

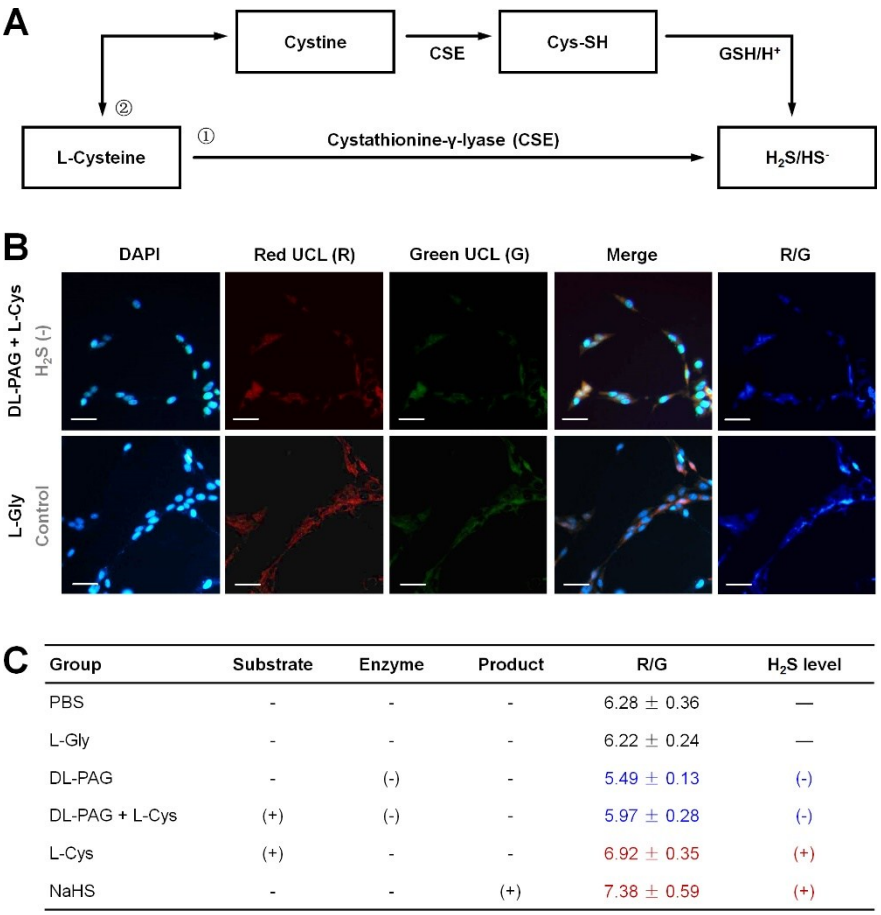
A



B

		blank	1 day	7 days	15 days	30 days	60 days
Liver		1343.8	1404.7	1365.9	1397.4	1353.2	1383.5
Spleen		87.3	84.2	90.0	92.5	79.8	96.5
Kidney	R	197.9	218.4	186.5	196.6	184.8	176.8
	L	171.1	198.4	185.1	192.3	175.2	175.6

Figure S14. H&E-stained tissue sections A) and organs weight B) from mice receiving UC-PB_5 nanoprobes injection (20 mg kg^{-1}) at various time points post-injection (1 day, 7 days, 15 days, 30 days, and 60 days) and mice receiving PBS injection (0 day). Tissues were harvested from liver, spleen, and kidney. The scale bar was 100 μm.



C

Group	Substrate	Enzyme	Product	R/G	H_2S level
PBS	-	-	-	6.28 ± 0.36	—
L-Gly	-	-	-	6.22 ± 0.24	—
DL-PAG	-	(-)	-	5.49 ± 0.13	(-)
DL-PAG + L-Cys	(+)	(-)	-	5.97 ± 0.28	(-)
L-Cys	(+)	-	-	6.92 ± 0.35	(+)
NaHS	-	-	(+)	7.38 ± 0.59	(+)

Figure S15. A) Schematic illustrations of the production process of H_2S. Luminescence images B) and signal statistic C) of A549 cells receiving various pre-treatments and UC-PB_5 nanoprobes incubation. The nucleus was stained with commercial DAPI staining solution. The scale bar was 20 μm. GSH: reduced glutathione; Substrate: L-Cys; Enzyme: CSE activity; Product: H_2S/HS^-; (-): down-regulating; (+): up-regulating.

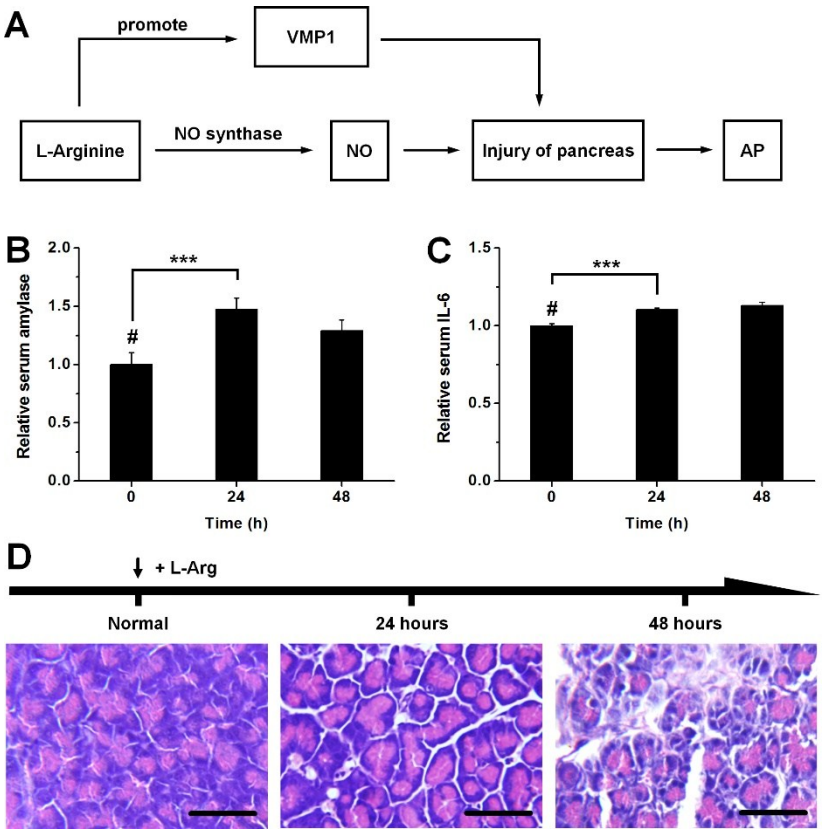


Figure S16. A) Schematic illustrations of the mechanism of L-arginine-induced AP. Relative serum amylase B), serum IL-6 C), and H&E-stained pancreas sections D) of mice receiving L-arginine injection at various time points post-injection. Data are represented as mean ± SD. Statistical significance was determined from one-way t tests. *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$. The scale bar was 50 μm. VMP1: vacuole membrane protein 1; #: the group as the standard of normalization.

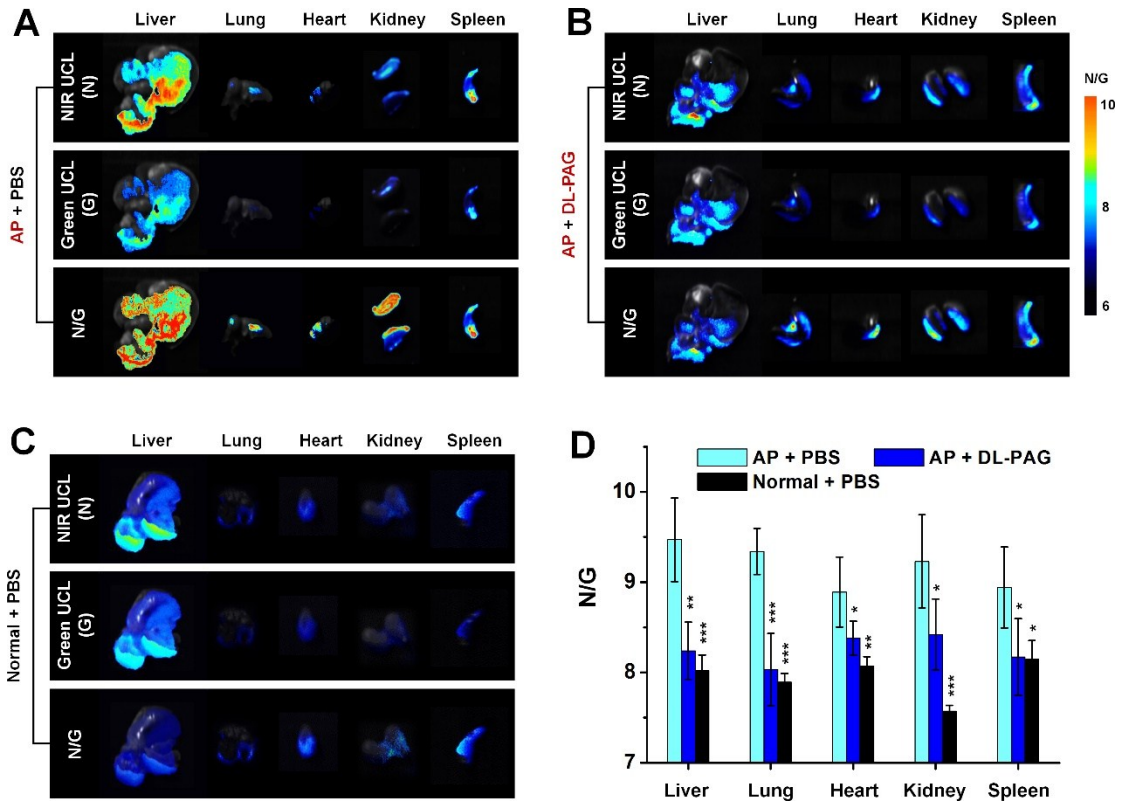


Figure S17. Representative UCL images of dissected organs of AP mice A), AP mice receiving DL-PAG injection B), and normal mice C) sacrificed 24 hours after intravenous injection with UC-PB_5 nanoprobes. D) Quantitative analysis of N/G obtained from UCL images of dissected organs. Data are represented as mean ± SD. Statistical significance was determined from one-way t tests. *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$.

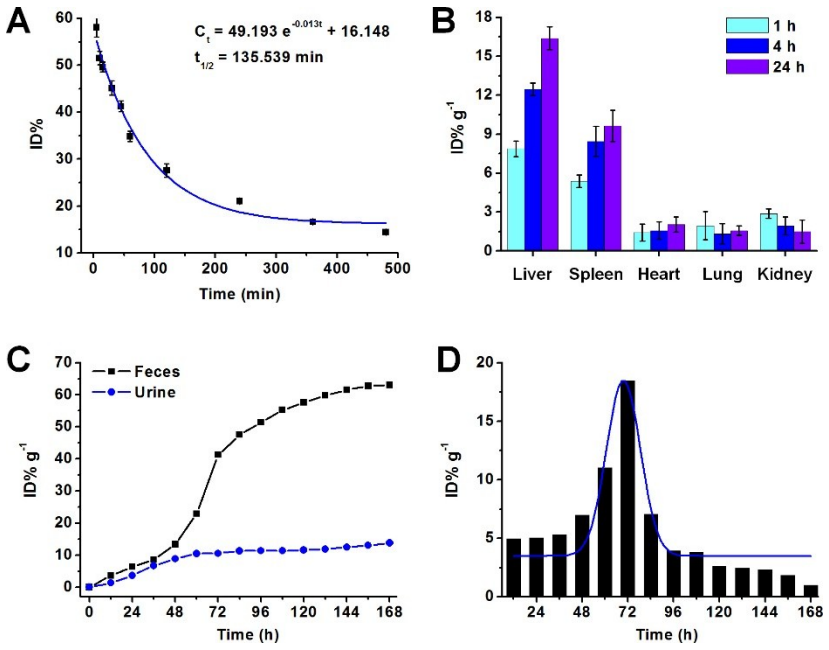


Figure S18. A) Blood circulation curve of UC-PB_5 nanoprobes at various time points post-injection. B) Retention amount of UC-PB_5 nanoprobes in various organs at 1, 4, and 24 h post-injection. C) Accumulated amount of UC-PB_5 nanoprobes in feces and urine at various time points post-injection. D) UC-PB_5 nanoprobes distribution in excretion at various time points post-injection. The unit is percentage of injected dose per gram of tissue (% ID g^{-1}). Data are represented as mean ± SD.

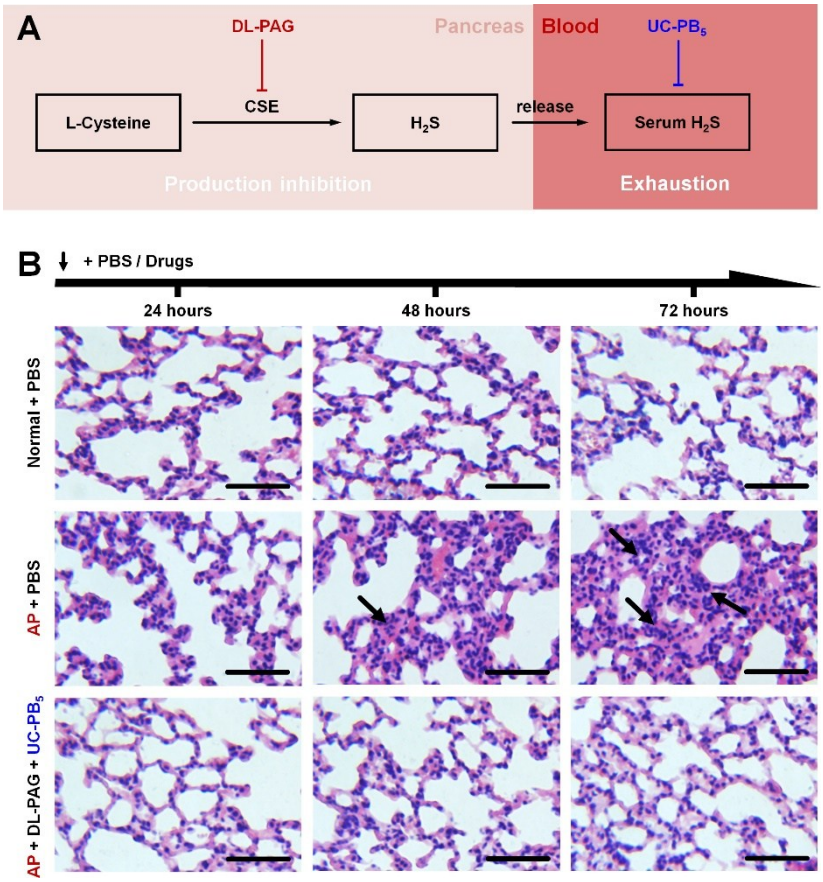


Figure S19. A) Schematic illustrations of the mechanism of DL-PAG and UC-PB_5 nanoprobes for alleviation of AP-associated lung injury. B) H&E-stained sections of lungs harvested from mice receiving various treatments at various time points post-injection. The scale bar was 50 μm.

Table S1. Comparison of H2S detection capacities with previous reported upconversion nanoprobes. LOD[1]: limit of detection (3 times higher than background); O: Organic acceptors; Io: Inorganic acceptors.

Donor		Acceptor	LOD[1]	Linear range	Strategy	Ref.
$NaYF_4$:Yb,Er,Tm	O[2]	Chromophores 1	—	—	Ratiometric	20
$NaYF_4$:Yb,Er,Tm	O	Chromophores 2	—	0 – 100 μM	Ratiometric	
$NaYF_4$:Yb,Er,Tm	O	Chromophores 3	—	0 – 115 μM	Ratiometric	
$NaYF_4$:Yb,Er,Mn	O	Chromophores 3	—	—	Turn-on	
$NaYF_4$:Yb,Er,Tm	O	CHC1 dye	0.13 μM	0 – 50 μM	Ratiometric	21
$NaYF_4$:Yb,Er,Tm	O	MC dye	0.58 μM	0 – 115 μM	Ratiometric	24
$NaYF_4$:Yb,Er	Io[3]	Cu^{2+}	0.022 μM	0.026 – 225 μM	Turn-on	25
$NaLuF_4$:Yb,Er,Tm	Io	Prussian Blue	**0.01 μM**	0 – 150 μM	Ratiometric	This work

Movie S1. Real-time color change in response of UC-PB_5 nanoprobes suspension upon addition of H_2S solution.