Supporting Information

Short-wave Infrared *in vivo* Imaging with Gold Nanoclusters

Yue Chen[†], Daniel M. Montana[†], He Wei[†], Jose M. Cordero[†], Marc Schneider[‡], Xavier Le Guével[§], Ou Chen[‖], Oliver T. Bruns[†], Moungi G. Bawendi*[†]*

[†]Department of Chemistry, Massachusetts Institute of Technology, Cambridge, MA 02139, United States

[‡]Biopharmaceutics and Pharmaceutical Technology, Saarland University, D-66123 Saarbrücken, Germany

[§]Cancer Targets & Experimental Therapeutics, Institute for Advanced Biosciences (IAB), University of Grenoble Alpes (UGA), INSERM-U1209/ CNRS-UMR 5309- 38000 Grenoble, France

[‖]Department of Chemistry, Brown University, Providence, RI 02912, United States

*To whom the correspondence should be addressed: mgb@mit.edu, obruns@mit.edu

General

Materials. Lipoic acid, trimethylamine, methanesulfonyl chloride, organic solvents (acetone nitrile, methanol, chloroform, etc.), PBS buffer, Fetal bovine serum (FBS), and other reagents were purchased from Sigma Aldrich (St. Louis, MO, USA) unless otherwise noted. N,N-dimethyl-1,3-propanediamine, 1,3-propanesultone, and hydrogen tetrachloroaurate (III) hydrate, 99.9% (metals basis) were purchased from Alfa Aesar (Haverhill, MA, USA). All chemicals were used as received.

Instrumentation. Transmission electron microscopy (TEM) images of LA-sulfobetaine Au NCs were taken on JEOL 2010 advanced high performance TEM. Absorption spectra were taken using a Cary 5000 UV-Vis-NIR spectrophotometer. Photoluminescence measurements were conducted at room temperature using an Edinburgh FS5 fluorometer with NIR extension. Matrix-assisted laser desorption/ionization (MALDI) spectra were taken on a Bruker Daltonics UltrafleXtreme MALDI TOF/TOF using α-cyano-4-hydroxycinnamic acid as the matrix in positive mode. Thermogravimetric analysis (TGA) was performed using Discovery TGA thermogravimetric analyzer. All samples were equilibrated at 100 ºC for one hour before increasing the temperature to remove water. Experiments were conducted under nitrogen flow. The gel filtration chromatography (GFC) setup was an AKTAprime Plus system equipped with a Superose 6 10/300 GL column. The mobile phase was 1x PBS, and measurements were carried out at a flow rate of 0.5 mL/min. 250 μL of each sample was injected, and absorbance at 280 nm was recorded. Liquid chromatography-mass spectrometry (LC-MS) analyses were conducted on the Agilent 1290 Infinity LC System equipped with an Agilent 6140 Quadruple mass spectrometer, using acetonitrile and water containing 0.1% formic acid as the mobile phases at a rate of 0.6 mL/min. Gold concentrations for the Au NCs dissolved by potassium cyanide were

measured using an Agilent 5100 ICP-OES. Mouse tissues and blood were digested using aqua regia using a Milestone UltraWave microwave sample digestion system, and the gold content in each tissue or blood sample was measured using an Agilent 7900 ICP-MS. Quantum yields were measured using an integration sphere (Labsphere RTC-060-SF) under 785 nm diode laser excitation. An 850 nm longpass filter was used to block the excitation, and signal from the sample was collected using a calibrated germanium detector (Newport: 818-IR) through a lock-in amplifying system. The measured quantum yields were then corrected to account for leakage of the excitation light and the transmittance of the filter. *In vivo* imaging data were recorded using an InGaAs-based SWIR camera (900-1,600 nm) (Nirvana, Princeton Instruments) and a silicon based camera (PIXIS, Princeton Instruments) under 808 nm excitation and different longpass filters (Thorlabs and Edmund Optics) as indicated.

Animals. Eight-week-old NCRNU-M mice were purchased from Taconic (Hudson, NY). Fifteen-week-old FVB mice were purchased from Charles River Laboratories. A C57BL/6 mouse (male, 8 weeks) was purchased from The Jackson Laboratory. The animal protocol was reviewed and approved by the Institutional Animal Care and Use Committee at the Massachusetts Institute of Technology.

Synthesis of lipoic acid based sulfobetaine (LA-sulfobetaine)

DHLA-amide was synthesized according to a previously reported protocol [1] except that the solvent was changed to acetone nitrile and the buffer used for extraction was changed to 1 M sodium bicarbonate aqueous solution. Next, 1.5 g of DHLA-amide was dissolved in 50 mL of methanol and stirred for 10 min, followed by the successive addition of ammonium hydroxide (0.362 mL, 28% aqueous solution) and 1,3-propanesultone (0.510 mL). The reaction mixture

was stirred at room temperature for 4 days before it was completely rotary-evaporated, resulting an oily crude product. Ethanol (50 mL) and hexanes (50 mL) were then added and the mixture was allowed to stand overnight. LA-sulfobetaine as the precipitate was collected and dissolved in 3 mL of water for storage.

Synthesis of SWIR-emitting Au NC

SWIR Au NCs were synthesized by modifying a literature method.[2] For Au NCs (1:1), briefly, 1 mg of LA-sulfobetaine was dissolved in 5 mL of DI water. Upon stirring, sodium hydroxide aqueous solution (100 μL, 0.2 M) and hydrogen tetrachloroaurate (III) hydrate (1 mg) in DI water (50 μL) were added. The mixture was stirred at room temperature for 5 min, followed by the dropwise addition of sodium borohydride aqueous solution (100 μL, 50 mM). The mixture was stirred overnight, and Au NCs were purified via dialysis using a 10K cutoff dialysis filter for 3 times. The Au NCs were dissolved in DI water (400 μL) and stored at 4 ºC. The reaction yield was about 97%.

Treatment of Au NCs with extra LA-sulfobetaine ligands

10 mg LA-sulfobetaine in 100 μL DI water was added to the stock 400 μL Au NC (1:1) solution under stirring. The reaction was allowed to stand overnight. Au NCs were purified using a 10K cutoff dialysis filter for 3 times and a NAP-10 column before characterizations. The final product was dissolved in 0.5 mL aqueous solvent (DI water, 1x PBS or isotonic saline) and stored at 4 ºC. The reaction yield was about 96%.

Serum stability test

150 μL of Au NCs (1:1) treated with LA-sulfobetaine in isotonic saline was mixed with an equal volume of FBS in an Eppendorf tube. As controls, 150 μL of Au NCs (1:1) treated with LA-sulfobetaine ligands solution was mixed with 150 μL of isotonic saline, and 150 μL of FBS was diluted with 150 μL of isotonic saline in separate tubes. The three tubes were placed in a shaker, warmed to 37 ºC, and shaken for 4 h. Each solution was then filtered through a 0.2 μm syringe filter prior to GFC measurements. An Agilent 1260 Series Bio-inert HPLC system equipped with an absorbance diode array detector and a Superose 6 column was used for analysis. The mobile phase was composed of Tris/Tris base (pH 7.5), isotonic NaCl, and 0.02% NaN_3. Analysis was carried out at a flow rate of 0.5 mL/min for all samples: 100 μL of each sample was injected into the instrument, and absorbance at 280 and 750 nm was recorded.

Cell toxicity test

4T1 breast cancer cells were seeded into a 96-well plate with a cell density of 4k/well and incubated at 37 ºC for 12 h for cell attachment. Au NCs with different concentrations were added, and cells were incubated for 3 days. Cell medium was removed, and cells were washed with PBS twice. MTT reagent (ATCC, 10 μL) was added to each well and incubate at 37 ºC for 3 h. Purple precipitates were observed. DMSO was then added to dissolve the purple precipitates for 20 min upon shaking. Absorbance at 570 nm was recorded on a plate reader

In vivo SWIR imaging

NCRNU-M mice were anesthetized by intraperitoneal injection of ketamine (200 μL) and xylazine (100 μL) in isotonic saline (700 μL) (150 μL/30g mouse). A tail vein catheter was inserted after mice were unresponsive to toe pinch. Mice were placed on the imaging stage to collect background images for the assessment of animal autofluorescence. Mice were then intravenously injected with Au NCs (1:1) treated with LA-sulfobetaine ligands (200 μL/mouse, 0.125 mg of Au) to observe the injection and initial distribution of Au NCs in the blood. First dose of Au NCs (0.125 mg of Au) was injected, and video was taken from the front side. About 20 min later, a second dose of Au NCs (0.125 mg of Au) was injected via the tail vein, and video was taken from the back side of the mice.

A C57BL/6 mouse was anesthetized using the same method as mentioned above, and the hair on the left leg was shaved. Au NCs (0.5 mg of Au) was injected via tail vein, and images of the left leg were taken using a silicon camera with an 850 nm longpass filter applied. Another dose of Au NCs (0.5 mg Au) was injected via the tail vein, and images of the left leg were taken using an InGaAs camera equipped with 1,000 nm, 1,150 nm, and 1,250 nm longpass filters, respectively.

Biodistribution

200 μL of Au NCs (0.089 mg Au determined by ICP-MS) was injected via tail vein into six FVB mice. Three control FVB mice were injected with 200 uL1X PBS each. Mouse urine was collected at 0.5 h, 1.5 h and 3.0 h post injection, respectively. Organs and blood were harvested from both the experiment and control group of mice at 3 h post injection and stored in pre-weighed petri dishes and Eppendorf tubes. The petri dishes and Eppendorf tubes were weighed

again to get the weight of each organ or blood sample. The samples were then digested using aqua regia by the microwave digester. The resulting clear solutions were diluted for ICP-MS analysis.

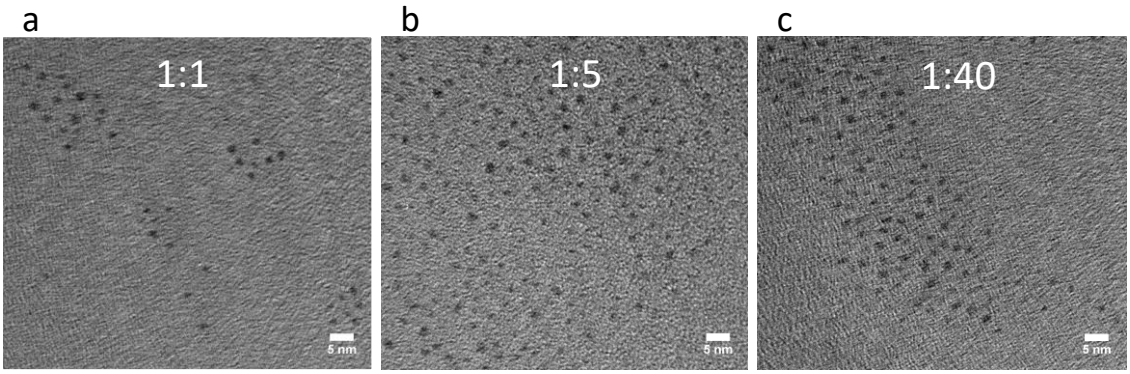

Figure S1. TEM images of (a) Au NCs (1:1), (b) Au NCs (1:5), and (c) Au NCs (1:40). Scale bar represents 5 nm.

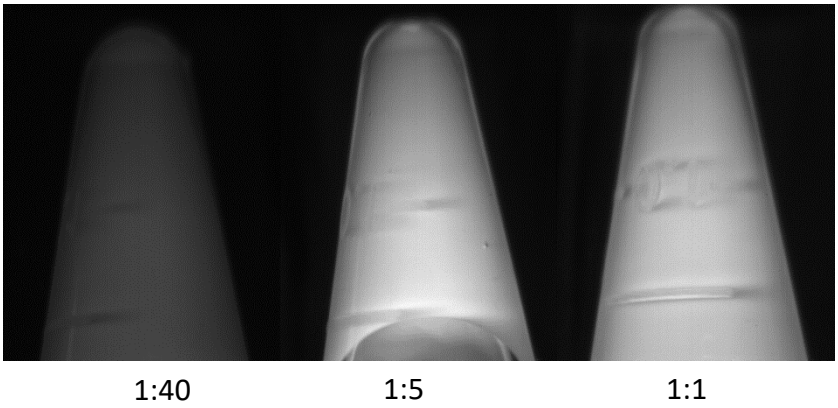

Figure S2. Images of Au NCs (1:40, 1:5, and 1:1) in Eppendorf tubes taken with an InGaAs camera and 808 nm excitation.

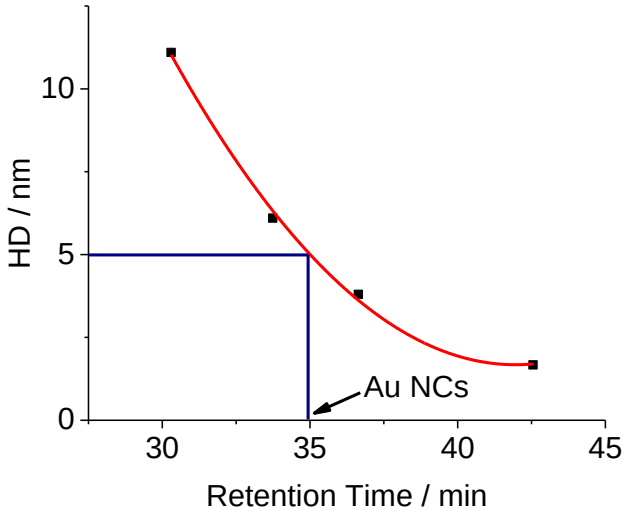

Figure S3. Superose 6 size-exclusion column was calibrated with protein standards containing γ-globulin, ovalbumin, myoglobin, and vitamin B12 from Bio-Rad (black squares). The red curve is a calibration curve based on the protein standards. Our Au NCs had a retention time of about 35 min (arrow), which corresponds to a HD of 5 nm.

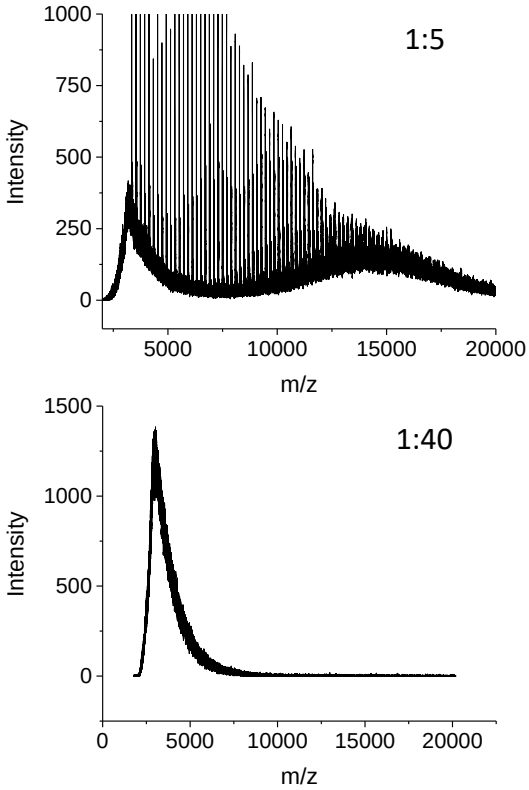

Figure S4. MALDI spectra of Au NCs (1:5) and Au NCs (1:40).

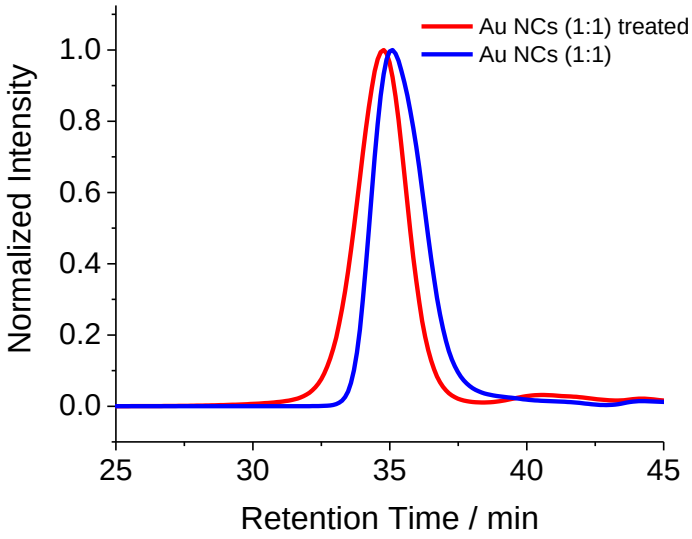

Figure S5. GFC traces of Au NCs (1:1) before and after treated with extra LA-sulfobetaine ligands.

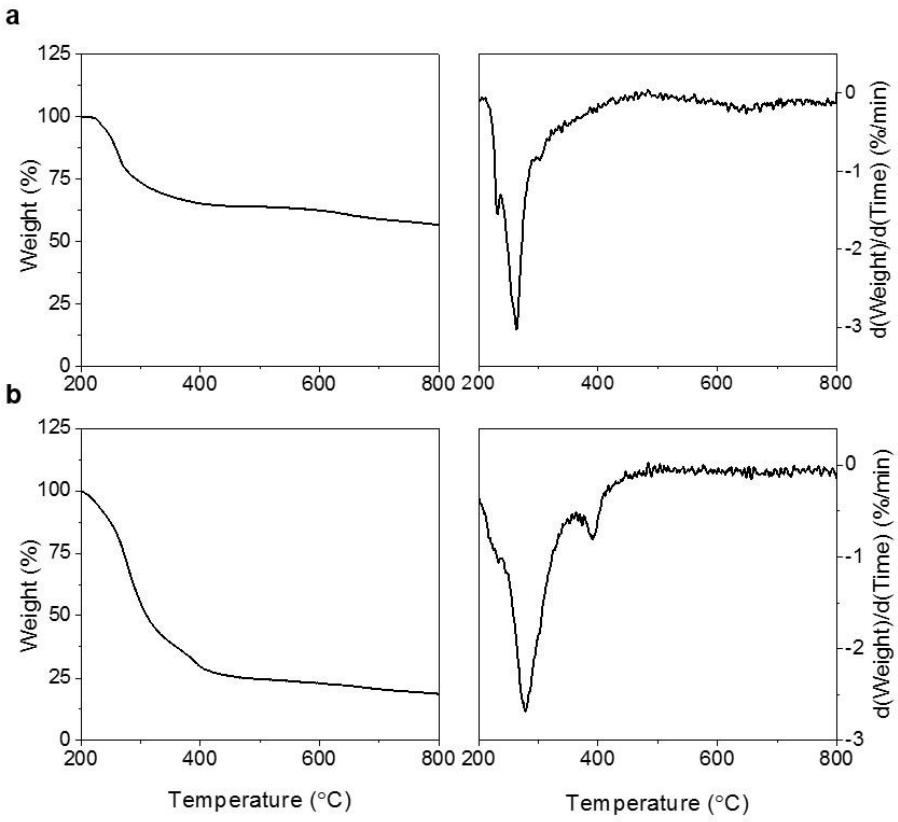

Figure S6. TGA spectra of (a) Au NCs (1:1), and (b) Au NCs (1:1) treated with extra LA-sulfobetaine ligands.

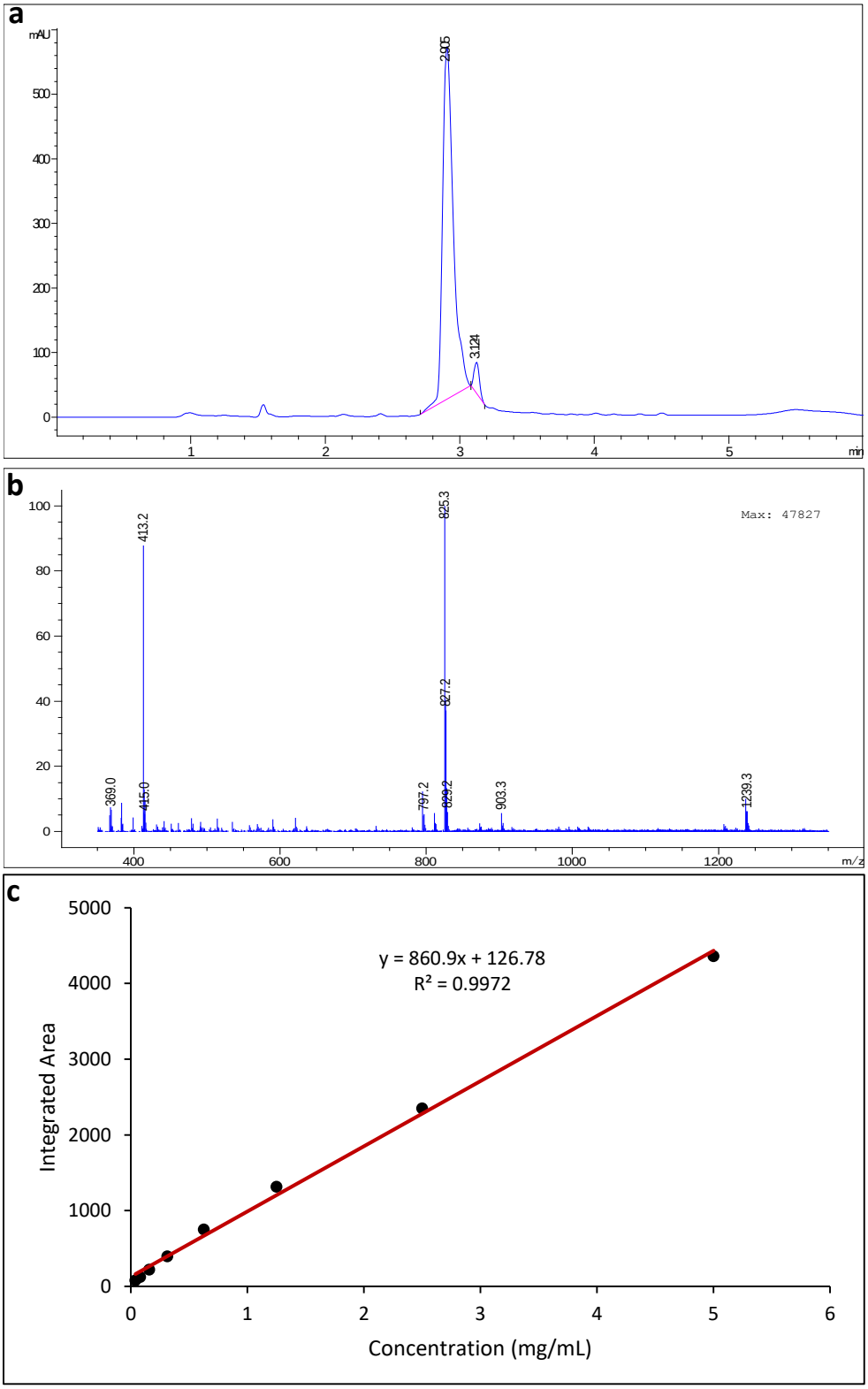

Figure S7. (a) HPLC trace of LA-sulfobetaine with 254 nm absorption and (b) Mass spectrum of the largest peak in (a) using LC-MS. (c) Fitting curve of HPLC integrated area versus concentration of LA-sulfobetaine.

Table S1. Quantitative analysis of ligand to gold ratio of Au NCs. Au NCs were dissolved using potassium cyanide, followed by LC-MS analysis. [LA-sulfobetaine] was calculated using the standard curve in Figure S7(c), and [Au] was measured using ICP-OES.

Au NCs	Volume (mL)	Integrated Area	[LA-sulfobetaine] (mg/mL)	[Au] (mg/mL)	LA-sulfobetaine/Au (mg/mg)
Nontreated	0.51	477.7	0.40762	0.47225	0.86631
Treated	0.66	1328.57	1.39597	0.35350	3.94899

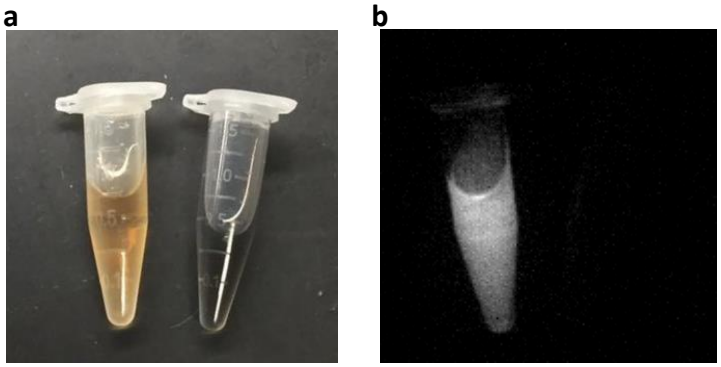

Figure S8. Images of Au NCs (left) and SWNTs (right) at the same concentration (0.25 mg/ml) (a) under room light, and (b) using a SWIR camera with 808 nm excitation.

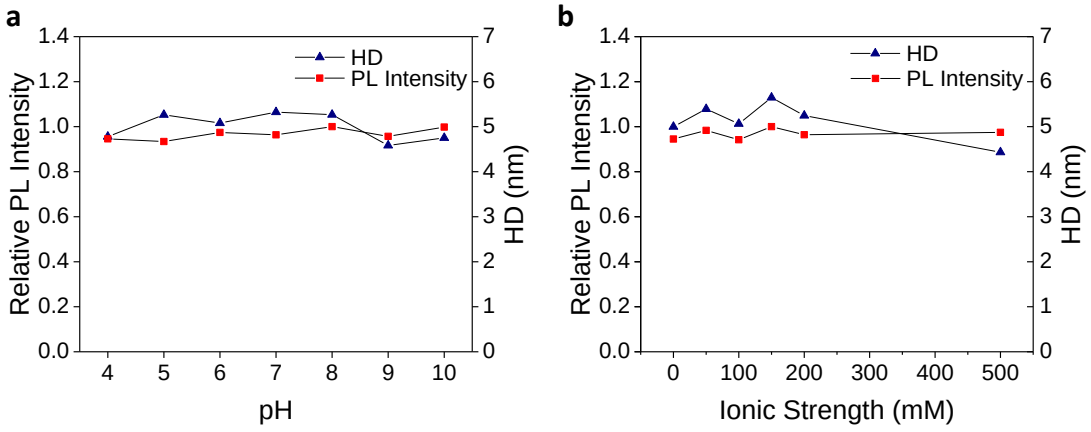

Figure S9. Relative photoluminescence intensity and hydrodynamic diameter of Au NCs at (a) different pH, and (b) different ionic strength.

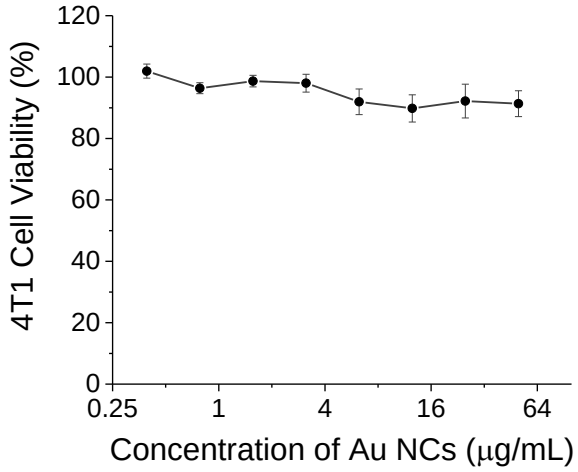

Figure S10. 4T1 cell viability after treatment with Au NCs at different concentrations for 3 days.

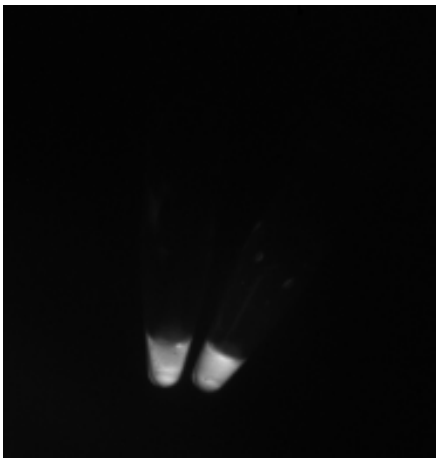

Figure S11. Images of Au NCs (1:1) treated with extra LA-sulfobetaine ligands after 4-h incubation in 1X PBS (left) and FBS (right) at 37 °C, respectively.

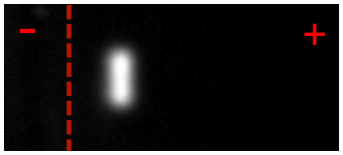

Figure S12. Agarose gel image of Au NCs (1:1) treated with extra ligands. Au NCs were loaded on 0.5% agarose gel and run under 70 V in 1x TAE buffer for 30 min.

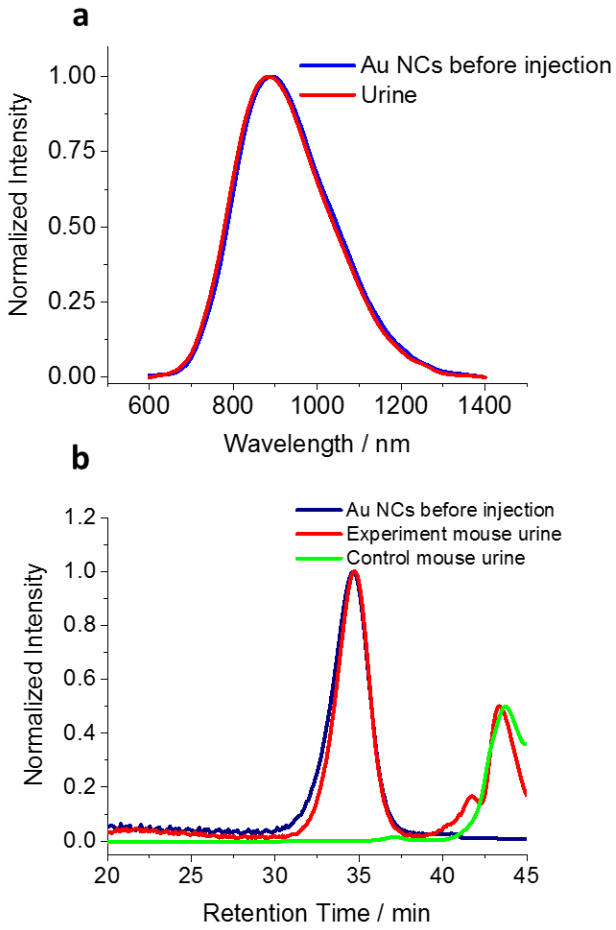

Figure S13. (a) Emission spectra and, (b) GFC traces of Au NCs before injection into the mouse (blue curve) and the urine collected from both the experimental mouse bladder (red curve) and the control mouse bladder (black curve).

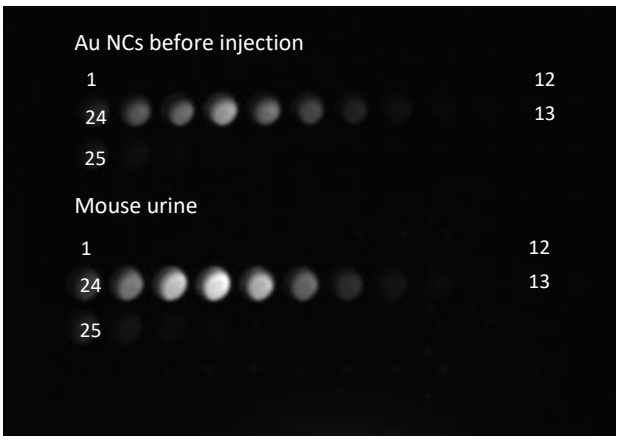

Figure S14. Images of GFC fractions of original Au NCs solution (top three rows) and urine collected from mice that were treated with the Au NCs (bottom three rows), respectively. Fractions were collected starting from 20 min with 0.6 min per fraction.

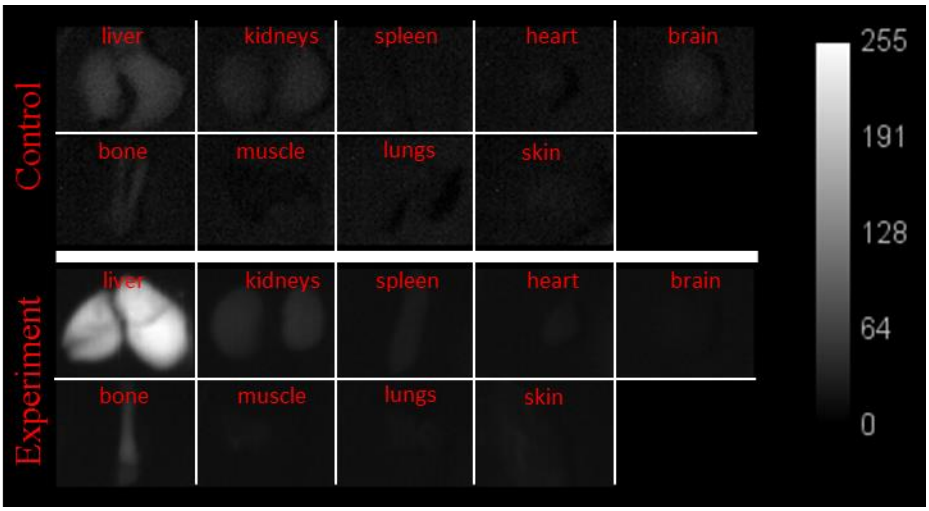

Figure S15. Ex vivo fluorescence imaging of tissues harvested from mice treated with 1X PBS (n=3) or Au NCs (n=6) at 3 h post injection (top two rows: control mouse; lower two rows: experiment mouse; exposure time: 25 sec).

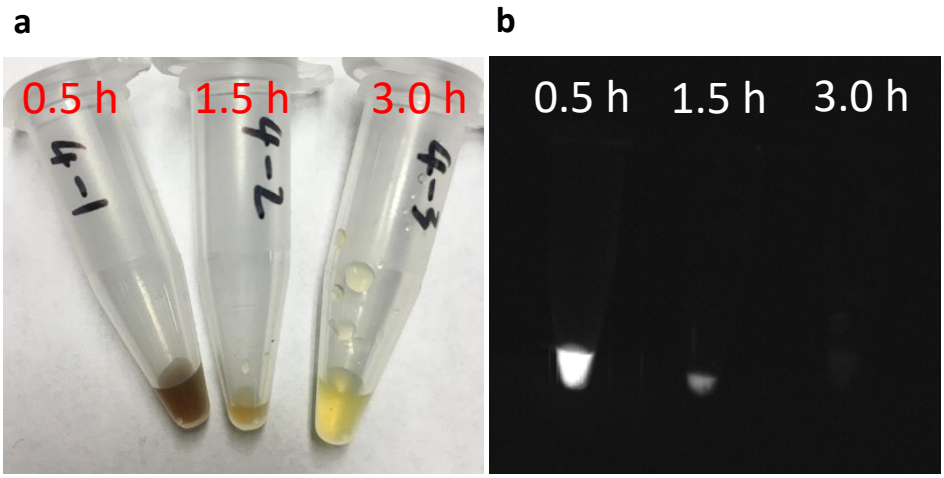

Figure S16. Image of mouse urines collected at 0.5 h, 1.5 h and 3.0 h post injection (a) under room light and (b) under SWIR camera (exposure time 2.5 sec, 10x shorter than in Figure S11).

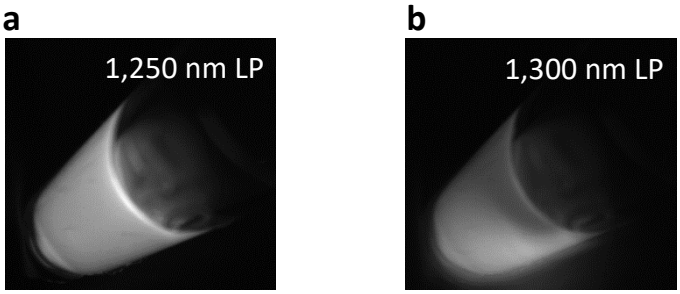

Figure S17. Images of Au NCs (1:1) treated with extra LA-sulfobetaine ligands taken using an InGaAs camera with (a) 1250 nm LP filter and (b) 1300 nm LP filter applied.

Reference:

[1] N. Q. Zhan, G. Palui, H. Grise, H. L. Tang, I. Alabugin, H. Mattoussi, *Acs Appl Mater Inter* **2013**, *5*, 2861-2869.

[2] X. Le Guevel, O. Tagit, C. E. Rodriguez, V. Trouillet, M. P. Leal, N. Hildebrandt, *Nanoscale* **2014**, *6*, 8091-8099.