Supporting Information:

Multicolor Lanthanide-doped CaS and SrS Near-Infrared Stimulated Luminescence Nanoparticles with Bright Emission: Application to Broad-spectrum Lighting, Information Coding, and Bio-imaging

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Materials

All starting materials were obtained from commercial supplies and used directly. Europium (III) nitrate ($Eu(NO_3)_3$, 99.99%), Samarium (III) nitrate ($Sm(NO_3)_3$, 99.99%), Cerium (III) nitrate ($Ce(NO_3)_3$, 99.95%), Hexadecyltrimethyl ammonium bromide (CTAB, 99%), 3-Mercaptopropionic Acid (3-MPA, 98%), 1-Pentanol (98%), 1-Dodecanethiol (95%), Tergitol (NP-10), 3-Mercaptopropionic Acid (99%), N-(3-Dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (EDC·HCl,98%), N-Hydroxysuccinimide (NHS, 98%), Diaminopolyethylene glycol (H_2N-PEG-NH_2, M.W 2000), D-Mannosamine hydrochloride (D-Man, 98%) were purchased from Aladdin Reagent, Co.,Ltd. (Shanghai, China). Cyclohexane (99.5%), Calcium nitrate tetrahydrate ($Ca(NO_3)_2$•$4H_2O$, 99%), Strontium nitrate tetrahydrate ($Sr(NO_3)_2$•$4H_2O$, 99%), Manganese acetate tetrahydrate ($Mn(CH_3COO)_2$•$4H_2O$, 99%), Ammonium sulfate ($(NH_4)_2SO_4$, 99%), Succinic anhydride (99%), Triethylamine (99%), Sodium hydroxide (NaOH, 96%) and Nitric acid (HNO_3, 70%) were purchased from Sinopharm Chemical Reagent Co.,Ltd. (Shanghai, China). 11-Mercaptoundecanoic acid (3-MUA, 98%) was purchased from Energy Chemical Reagent Co.,Ltd. (Shanghai, China). Ethanol (anhydrous, 99.7%) and Acetone (99%) were obtained from Beijing Chemical Regent Co.,Ltd. N,N-dimethyl formamide (DMF, 99.5%) was obtained from Tianjin Kermel Chemical Co.,Ltd. All aqueous solutions were prepared using ultrapure water (Mill-Q, Millipore, 18.2 MΩ resistivity).

Characterization

TEM images of the nanomaterials were collected by JEOL JEM-2010 transmission electron microscope at 200 kV. Powder X-ray diffraction patterns were recorded by D8 Advance Bruker powder X-ray diffractometer (Cu Kα radiation, λ=1.5406 Å) from 10° to 80° at scanning rate of 4°/min. The fluorescence emission spectrum were acquired on a Hitachi F-4600 spectrofluorometer with an external 980 nm laser device (Changchun New Industries Optoelectronics Technology Co.,Ltd.). The Fourier transformation infrared spectroscopy was measured in a Nicolet FTIR 5700. The size distribution and Zeta-potential were measured using Malven Nano Zetasizer system by Malvern Instruments. The Nuclear Magnetic Resonance spectrum was obtained using a Bruker Ascend 400 spectrometer. Thermoluminescencecurves were measured with a FJ-427A TL meter (Beijing Nuclear Instrument) by heating the irradiated samples from room temperature to 600 K. The samples were pre-irradiated by using a UV lamp for 2 min and then heated at a linear rate of 5 K/s. Fluorescence images of cell and tissue slides were acquired in Nikon confocal microscopy A1. *In vivo* and *ex vivo* fluorescence imaging was measured with a modified Lumina XR system, two external CW 980 nm lasers (Connet Fiber Optics Co.,Ltd.) were used as the excitation sources. Elements distribution in organs were measured by Inductively coupled plasma mass spectrometry (Agilent 7900). Fluorescence images of the QR code and Morse code were irradiated by the mini-980 nm laser pointer purchased from Shenzhen Fuzhe Technology Co., Ltd. NIR emitting chips were purchased from Shenzhen Looking Long Technology Co., Ltd. All the fluorescence photographs were collected by smartphone (Beijing MIUI Scientice and Technology Co.,Ltd.) equipped with a NIR filter to eliminate 980 nm excitation light.

Synthesis of DT/MUA@CaS:Eu,Sm,Mn nanoparticles (CS-ESM NPs)

The CS-ESM NPs were prepared by mixing 20 mL cyclohexane containing 2.75 mmol hexadecyltrimethyl ammonium bromide (CTAB) and 1.5 mL 1-pentanol with 0.5 mL $Ca(NO_3)_2$ (0.1 M) aqueous solution containing 0.3 mM $Eu(NO_3)_3$, 0.3 mM $Sm(NO_3)_3$ and 0.8 mM $Mn(CH_3COO)_2$. The mixture was stirred vigorously for 30 min to form a water-oil emulsion, followed by a stand for 1 h. Then 0.055 mmol $(NH_4)_2SO_4$ in water-oil emulsion ($V_{cyclohexane}/V_{water}$=10:1) was added into the above water-oil emulsion，and followed by slow agitation for 3 min. After aging for 10 min, 10 mL acetone was added to the mixture. The resultant particles were isolated by centrifuge at 10000 rpm for 10 min, and alternately washed with acetone and ethanol. The precipitate was dried in vacuum and annealed at 850 °C for 60 min under CO flow to acquire the CaS: Eu,Sm,Mn NPs.[1]

Under a dry nitrogen atmosphere the as-prepared CaS: Eu,Sm,Mn NPs were then modified with 1-dodecanethiol (DT) by dispersing them in 10 mL absolute ethanol containing 20 μL Tergitol NP-10. After sonication for 30 min, the mixture was added into 5 mL absolute ethanol containing 0.5 mL DT at pH 8.0, obtained by drop-wise addition of 0.25 M NaOH solution in ethanol. The solution was stirred at 50 °C for 24 h, then centrifuged and washed with ethanol, and finally dispersed in 2 mL cyclohexane. The DT-modified CaS: Eu,Sm,Mn NPs were then encapsulated by 3-mercaptoundecanoic acid (MUA). For this purpose, the DT-modified CaS: Eu,Sm,Mn NPs in cyclohexane were added into 8 mL absolute ethanol, and sonicated for 10 min. Then 150 mg MUA and 10 μL 3-mercaptopropionic acid (MPA) were dissolved in 5 mL absolute ethanol adjusted to pH 8.5 with a 0.25 M NaOH ethanol solution. After stirring for 30 min the solution was added to the cyclohexane solution containing the DT-modified CaS: Eu,Sm,Mn NPs, and stirred vigorously for 48 h. The DT/MUA@CaS:Eu,Sm,Mn NPs were collected by centrifuge at 10000 rpm and washed with ethanol. The product were dispersed in water or ethanol, and could be illuminated by ultraviolet lamp for 3min to get fully activated.

Synthesis of DT/MUA@SrS:Eu,Sm,Mn nanoparticles (SS-ESM NPs)

The synthetic procedure of SS-ESM NPs were similar as that used to synthesize CS-ESM NPs, except 0.5 mL aqueous solution of $Ca(NO_3)_2$ (0.1 M) was replaced by 0.5 mL aqueous solution of $Sr(NO_3)_2$ (0.1 M), and changes in reaction condition: aging time (10 min) was adjusted to 5 min, and annealing condition was adjusted at 1000 °C for 60 min.

Synthesis of DT/MUA@CaS:Ce,Sm,Mn nanoparticles (CS-CSM NPs)

The synthetic procedure of CS-CSM NPs were similar as that used to synthesize CS-ESM NPs, except 0.3 mM $Eu(NO_3)_3$ solution was replaced by 0.075 mM $Ce(NO_3)_3$ solution, and concentration of $Mn(CH_3COO)_2$ (1.5 mM) was adjusted from 0.8 mM to 0.6 mM.

Synthesis of DT/MUA@CaS:Ce,Mn nanoparticles (CS-CM NPs)

The synthetic procedure of CS-CM NPs were similar as that used to synthesize CS-ESM NPs, except 0.3 mM $Eu(NO_3)_3$ solution was replaced by 0.15 mM $Ce(NO_3)_3$ solution, 0.3 mM $Sm(NO_3)_3$ solution was removed, and concentration of $Mn(CH_3COO)_2$ (1.5 mM) was adjusted from 0.8 mM to 0.6 mM.

Synthesis of DT/MUA@SrS:Ce,Sm,Mn nanoparticles (SS-CSM NPs)

The synthetic procedure of SS-CSM NPs were similar as that used to synthesize CS-ESM NPs, except 0.5 mL aqueous solution of $Ca(NO_3)_2$ (0.1 M) was replaced by 0.5 mL aqueous solution of $Sr(NO_3)_2$ (0.1 M), 0.3 mM $Eu(NO_3)_3$ solution was replaced by 0.075 mM $Ce(NO_3)_3$ solution, and changes in reaction condition: aging time (10 min) was adjusted to 5 min, and annealing condition was adjusted at 1000 °C for 60 min.

Measurement of NIR Stimulated Fluorescence Efficiency

The NIR (980 nm) stimulated fluorescence efficiency was measured according to the reported method.[2] It is carried out on a Steady-State&Time-Resolved Fluorescence Spectrofluorometer (PTI Corporation QM/TM/IM) equipped with an integrating sphere (80 mm in diameter) from EVERFINE Corporation. The integrating sphere was mounted on the fluorescence spectrofluorometer with the entry and output ports of the sphere located in 90 geometry from each other in the plane of the spectrometer. The sample was thermally excited with a UV lamp for 3 min,, then stimulated with 980 nm illumination using a 980 nm laser device (Shanghai Dream Laser Technology Co.,Ltd) which was coupled to a fiber at an excitation density of $0.5 W \cdot cm^{-2}$ to excite the trapped electrons producing a fluorescence emission. The spectrum (970 ~ 1000 nm) of excitation radiation which was not absorbed by the sample was measured through neutral density filters. The spectrum (400 ~ 700 nm) of emission of each sample was measured without any neutral density filter. AES NPs samples dispersed in ethanol, and pure ethanol was used as the reference to record blank background. The fluorescence efficiency (FE) of each sample was calculated according to the following equation:

$$\mathrm{FE}\ (\%) = \frac{N_{\mathrm{emi}}}{N_{\mathrm{abs}}} \times 100\%$$

where N_{emi} is emissive photon number, N_{abs} is absorbent excitation photon number corrected with background subtraction.

Synthesis of D-Mannosamine modified SS-CSM NPs

The as-prepared SS-CSM NPs were dispersed in 5 mL DMF and sonicated for 10 min to obtain a transparent solution. Then EDC•HCl (10 mg) was added to the solution and stirred for 15 min. Afterward, NHS (5 mg) was added and stirred at 37℃ for 2 h. The nanoparticles were isolated via centrifugation at 8000 rpm and re-dispersed in DMF (2.5 mL), in which 2.5 mL DMF containing 25 mg PEG-NH_2 was then added, and stirred for 24 h. The resulting SS-CSM NPs-PEG were collected by centrifugation at 10000 rpm and washed with ethanol three times. In a flask D-mannosamine (Man, 50 mg) was dissolved in DMF (1 mL), and succinic anhydride (150 mg) was added then reacted at 60

℃ for 48 h. The reaction mixture was poured in ether and waiting for crystallization, then filtered and washed with ether to obtain succinic anhydride linked D-mannosamine (SA-Man). The SS-CSM NPs-PEG (~5 mg) were dispersed in DMF (1 mL), and triethylamine (10 μL) was dropped slowly, then synthesized SA-Man (5 mg), EDC (15 mg), and NHS (8 mg) was added into solution step by step, vigorously stirring at room temperature for 24 h. The mixture was centrifuged at 10000 rpm and washed with ethanol, and the product was dialyzed against deionized water (5 kDa)..

Fabrication of the AES NPs-based light emitting devices

The CS-ESM, CS-CM and SS-CSM NPs were dispersed with silicone and stirred thoroughly, and the AES NPs-silicone mixture was deposited on the surface of the NIR chips, and after a baking process the red, green, blue, and white light LED devices could be fabricated.

Cytotoxicity of the AES NPs

In vitro cytotoxicity was measured by performing the CCK-8 assay on Hela cells. Cells were seeded into a 96-well cell culture plate at 5×10^3/well, under 100% humidity, and were cultured at 37 °C in 5 % CO_2 for 24 h; different concentrations of the AES NPs (0, 100, 200, 400 and 800 μg/mL diluted in DMEM) were then added to the wells. The cells were subsequently incubated for 5 h at 37 °C in 5% CO_2. Thereafter, the CCK-8 solution (10 μL) was added to each well and incubated with the cells for another 1 h. After thorough mixing, the absorbance was measured at 450 nm by means of a Tecan Infinite M200 monochromator-based multifunction microplate reader. The given result was the average of five wells. The cytotoxicity was expressed as the percentage of the cell viability compared to that of untreated control cells. The following formula was used to calculate the inhibition of cell growth: Cell viability (%) = (Mean of Abs. value of treated group- Mean of Abs. value of blank group) / (Mean of Abs. value of untreated group - Mean of Abs. value of blank group)×100%.

Hemolysis test of the AES NPs

In vitro hemolysis test was measured by colorimetry on mice whole blood. Mice whole blood was preserved in tubes containing 10 mL normal saline and 0.6 mL sodium citrate (4 %). Then blood was centrifuged three times to collect haemocytes, and resuspended in normal saline solution. Then, different concentrations of AES NPs solution were added to 2.5 mL haemocytes solution to give final AES NPs concentrations of 0, 5, 10, 20, 50, and 100 μg/mL, respectively. The mixture was incubated at 37 °C for 0.5 h, followed by centrifugation at 4000 rpm for 5 min. Haemocytes solution incubated

with normal saline and ultrapure water were set as negative control and positive control, respectively. The percentage of supernatant was calculated using the following equation: Hemolysis (%) = (Mean of Abs. value of treatment group- Mean of Abs. value of negative group) / (Mean of Abs. value of positive group- Mean of Abs. value of negative group)×100%.

In vivo and *ex vivo* fluorescence imaging

Balb/c mice (weight 20~30g) were obtained from Shanghai SLAC laboratory Animal Co., Ltd. All the experiments were carried out in strict accordance with the guidelines established by the Committee on the Use and Care of Animals at the Hunan Province, P. R. China, and the protocol was approved by the Ethics Committee of the University of Hunan Normal University. Balb/c mice were intravenously injected with the AES NPs saline solution. Mice were anesthetized by chloral hydrate solution for *in vivo* and *ex vivo* fluorescence imaging under 980 nm light irradiation. The major organs such as heart, liver, spleen, lung, stomach and kidneys were collected, and washed for *ex vivo* fluorescence imaging. Corresponding emissive filters were used to collect different wavelengths under 980 nm laser irradiation respectively, and analyzed with Lumina XR Living Image Software. Corresponding organs were embedded in Tissue-Tek optimal cutting temperature (O.C.T.) and slides were carried out in Leica CM 1950 freezing microtome, and imaged by fluorescence microscopy analyzed with Image J Software. To determinate CS-ESM NPs and SS-CSM NPs distribution (Eu and Ce content), organ samples were ground and dissolved in deionized water (4.5 mL) and HNO_3 (0.5 mL), then undergoing a digestion process and centrifuged at 8000 rpm. The supernatant was analyzed by ICP-MS.

Supplemental Data

Table S1. Experimental parameters of the AES NPs preparation.

Compositions	Eu (%)	Sm (%)	Mn (%)	Annealing temperature/time
CS-ESM NPs	0.30	0.30	0.80	850 °C/ 60min
SS-ESM NPs	0.30	0.30	0.80	1000 °C/ 60min
CS-CSM NPs	0.075	0.30	0.60	850 °C/ 60min
CS-CM NPs	0.15	0	0.60	850 °C/ 60min
SS-CSM NPs	0.075	0.30	0.80	1000 °C/ 60min

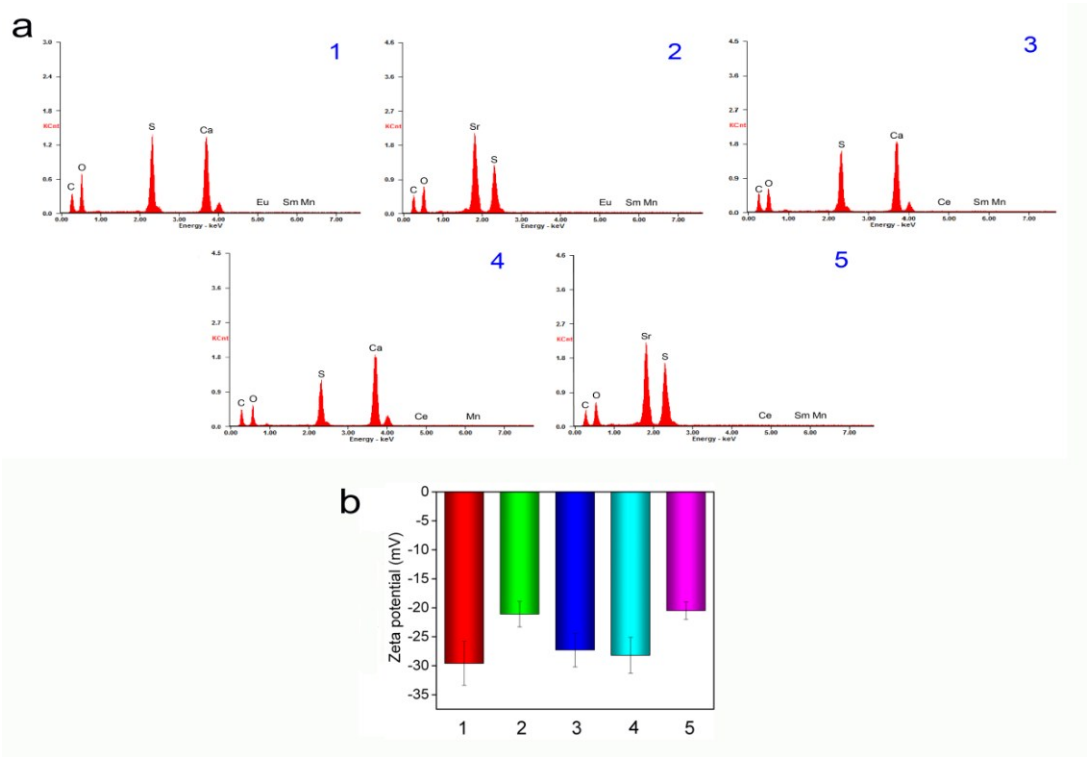

Figure S1. (a) EDX and (b) Zeta potential of the five AES NPs, (1)~ (5) represent CS-ESM, SS-ESM, CS-CSM, CS-CM, and SS-CSM NPs, respectively.

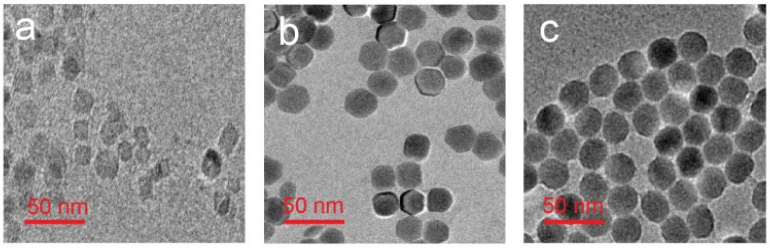

Figure S2. TEM images of the synthesized (a) r-$NaYF_4$ NPs, (b) g-$NaYF_4$ NPs, and (c) b-$NaYF_4$ NPs, respectively.

Table S2. Experimental NIR stimulated fluorescence efficiency of the AES NPs. The value specially refers to conversion efficiency from NIR excitation light to emission light.

Compositions	Emissive photon number	Absorbent photon number	Fluorescence efficiency [%]
CS-ESM NPs	274315	462935	59.3
SS-ESM NPs	291233	575560	50.6
CS-CSM NPs	85563	361026	23.7
CS-CM NPs	15788	130967	12.1
SS-CSM NPs	103428	287298	36.0

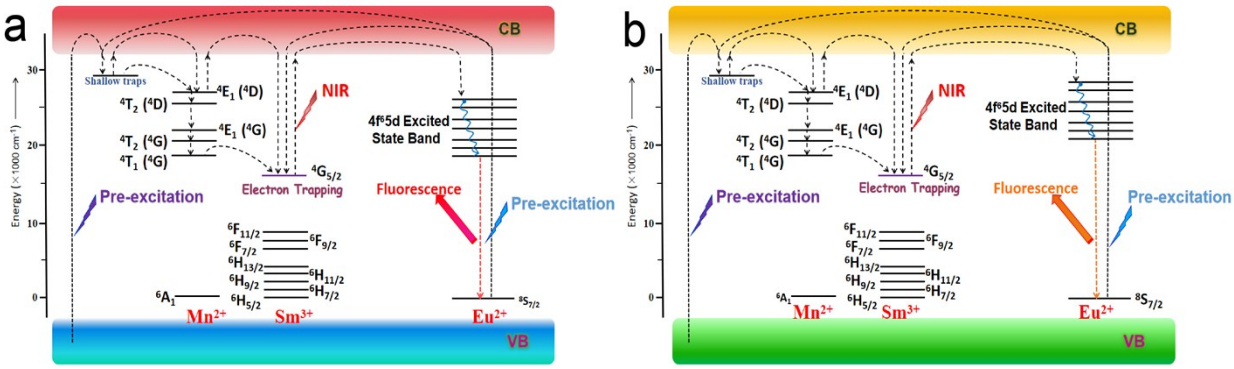

Figure S3. Proposed fluorescence mechanism of (a) CS-ESM NPs and (b) and SS-ESM NPs.

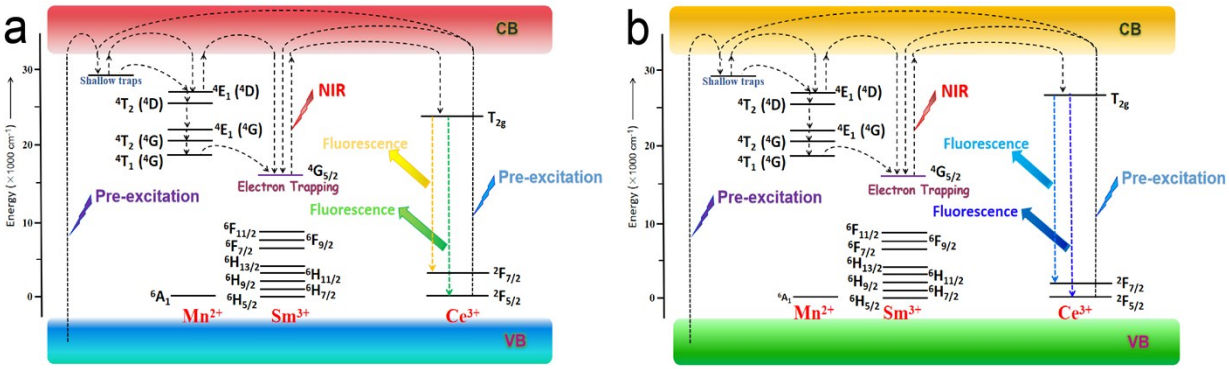

Figure S4. Proposed fluorescence mechanism of (a) CS-CSM NPs and (b) and SS-CSM NPs.

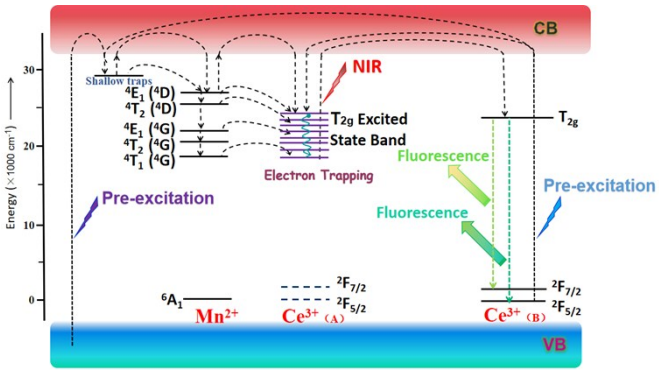

Figure S5. Proposed fluorescence mechanism of CS-CM NPs. Ce^{3+} ions were affected by symmetry environment, which not only acted as luminescence center ($Ce^{3+}_{(B)}$), but also acted as trapping center ($Ce^{3+}_{(A)}$).

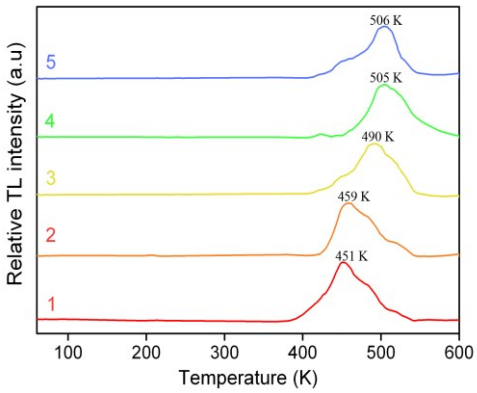

Figure S6. Thermoluminescence (TL) curves of the five AES NPs samples. (1)~ (5) represent CS-ESM, SS-ESM, CS-CSM, CS-CM, and SS-CSM NPs, respectively. The trap depth of the CS-ESM, SS-ESM, CS-CSM, CS-CM, and SS-CSM NPs were calculated to be 0.90 eV, 0.92 eV, 0.98 eV, 1.01 eV, and 1.01 eV, respectively.

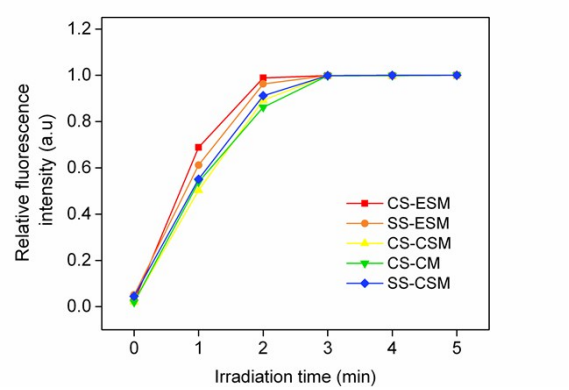

Figure S7. NIR stimulated fluorescnece intensity of the five AES NPs under different UV light irradiation time.

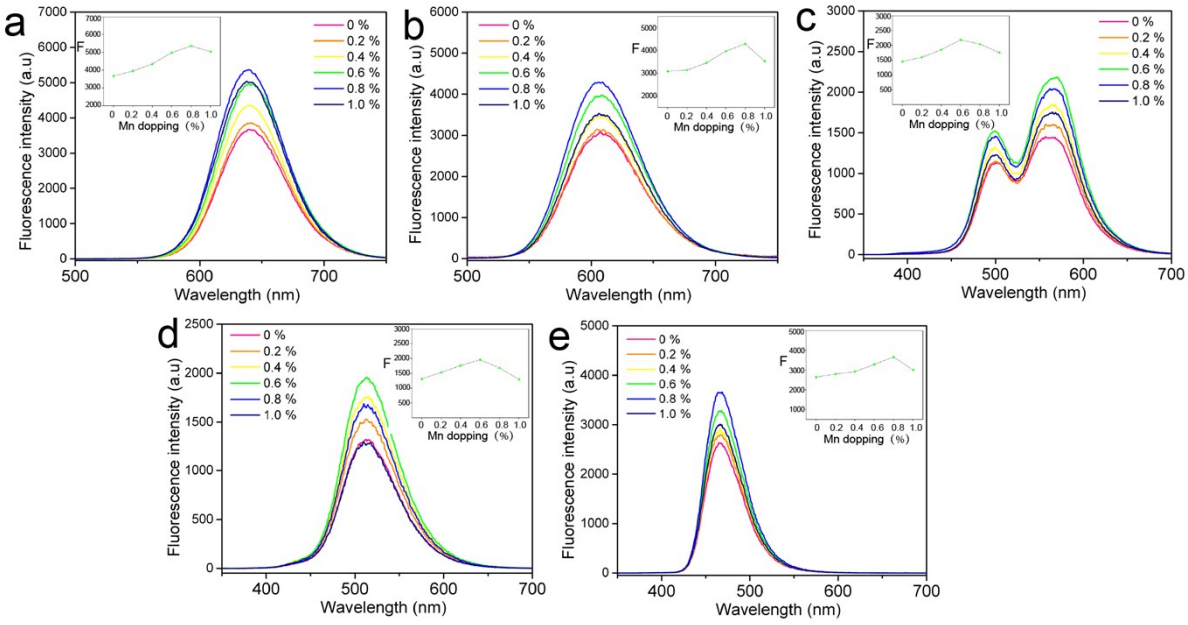

Figure S8. Fluorescence spectrum and (inset) the corresponding maximum peak value of (a) CS-ESM, (a) SS-ESM, (a) CS-CSM, (a) CS-CM, and (a) SS-CSM NPs with different Mn ions doping concentration.

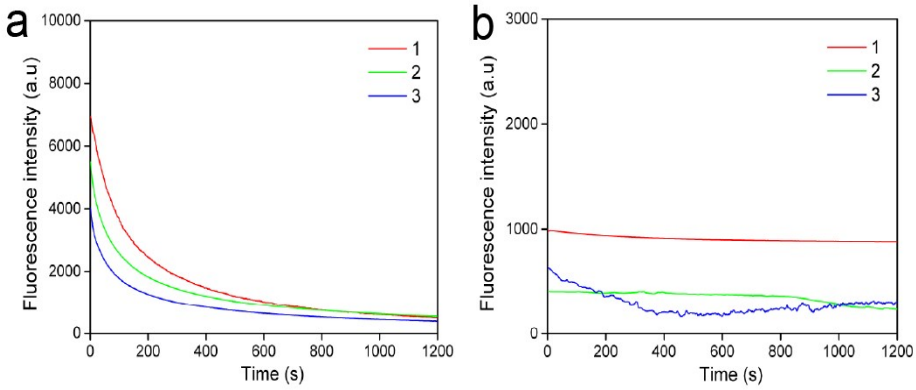

Figure S9. (a) Fluorescence decay curves of CS-ESM NPs (1), CS-CM NPs (2), and SS-CSM NPs (3), and (b) fluorescence decay curves of r-$NaYF_4$ NPs (1), g-$NaYF_4$ NPs (2), and b-$NaYF_4$ NPs (3) under continuous 980 nm laser irradiation.

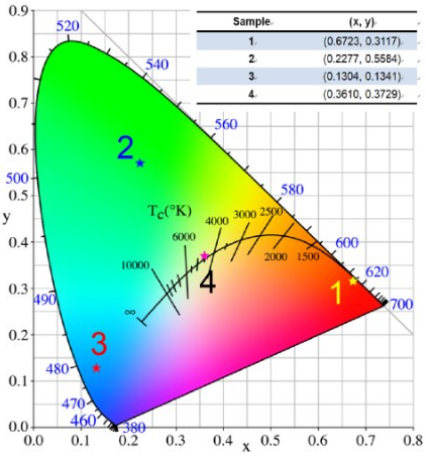

Figure S10. Corresponding CIE color coordinate of the red, green, blue, and white light emitting devices.

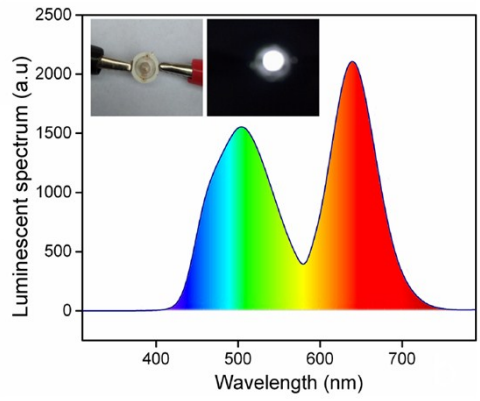

Figure S11. Luminescence spectrum and photographs of white light emitting device.

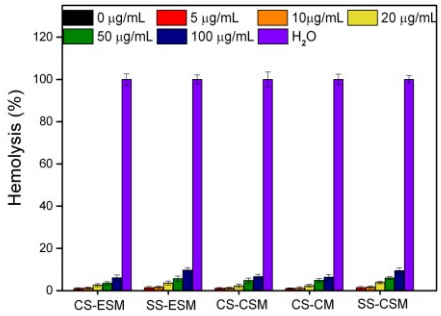

Figure S12. Hemolysis results of the five AES NPs at different concentrations.

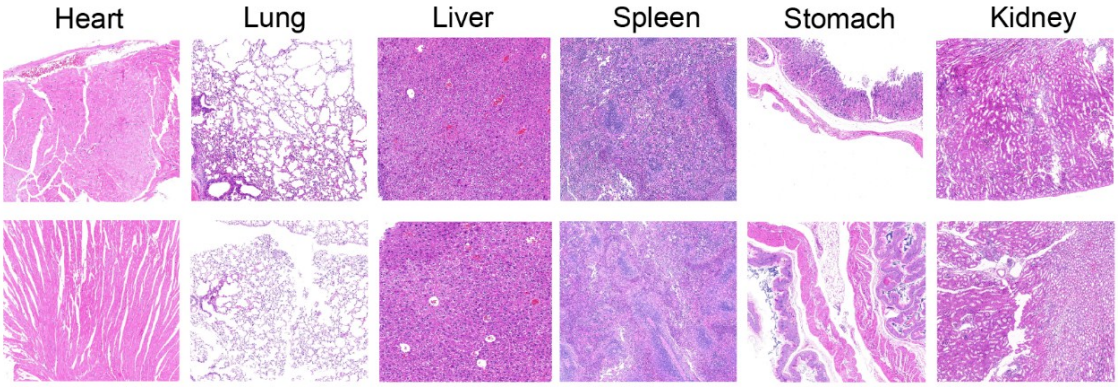

Figure S13. Typical H&E stained images of main organs from mice intravenously injected with mixed AES solution (CS-ESM NPs/SS-CSM NPs) and normal saline solution.

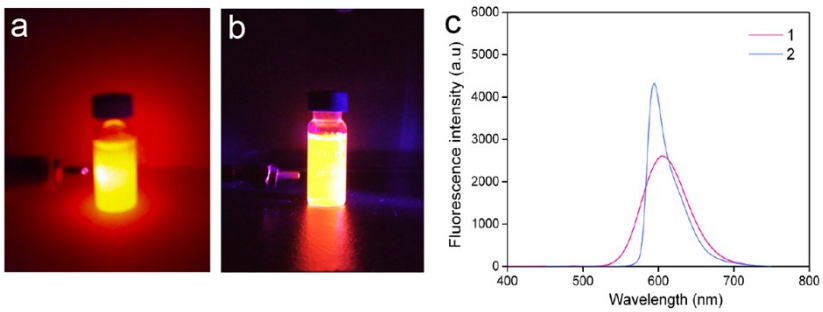

Figure S14. Fluorescence images of orange fluorescent emissive (a) SS-ESM NPs and (b) Rhodamine 6G solution with identical concentration. (c) Corresponding fluorescence emission spectrum of SS-ESM NPs (1) and Rhodamine 6G (2) . Rhodamine 6G was excited by 405 nm laser device, and SS-ESM NPs was excited by 980 nm laser device.

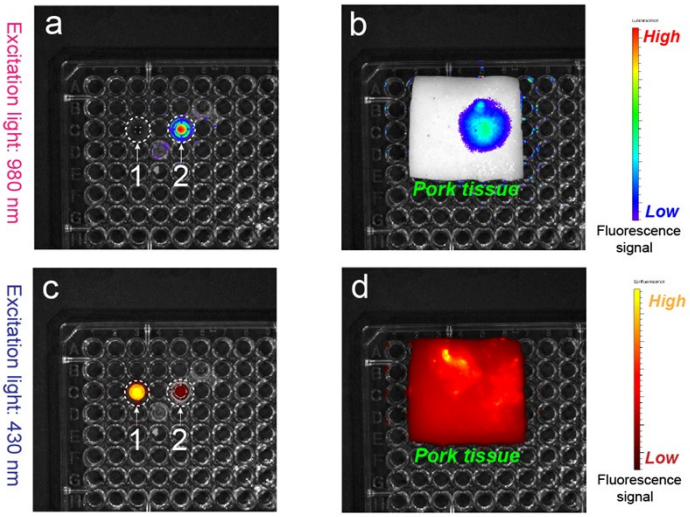

Figure S15. Fluorescence images of equal amount of Rhodamine (1) and SS-ESM NPs (2) solutions seed in a 96-well plate under (a, b) 980 nm and (c, d) 430 nm irradiation, respectively. A layer of 4 mm thick pork tissue (b, d) was applied on the plate to investigate fluorescence imaging through organisms.

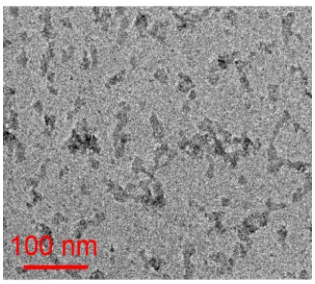

Figure S16. TEM image of PEG-NH_2 and D-Mannosamine modified SS-CSM NPs (SS-CSM NPs-PEG-Man).

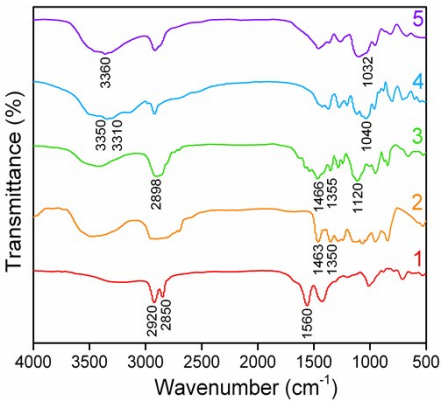

Figure S17. FT-IR of SS-CSM NPs (1), PEG-NH_2 (2). SS-CSM NPs-PEG (3), D-Mannosamine (Man; 4), and SS-CSM NPs-PEG-Man (5).

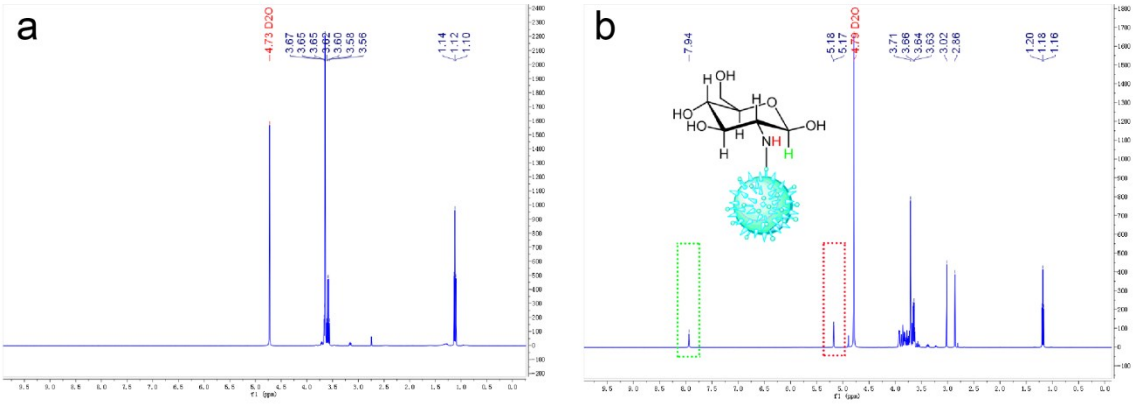

Figure S18. [1]HNMR of SS-CSM NPs-PEG (a) and SS-CSM NPs-PEG-Man (b). D_2O was used as solvent (4.79 ppm).

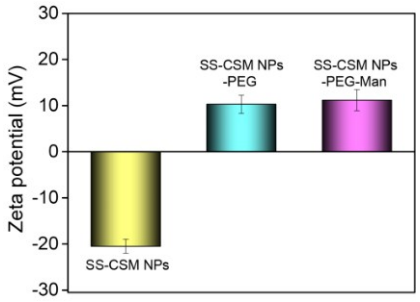

Figure S19. Zeta potential of SS-CSM NPs, SS-CSM NPs-PEG and SS-CSM NPs-PEG-Man, respectively.

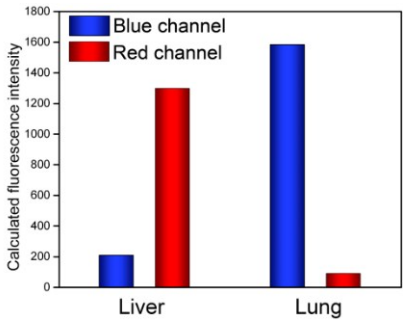

Figure S20. Corresponding quantitative analysis for tissue slides fluorescence intensity.

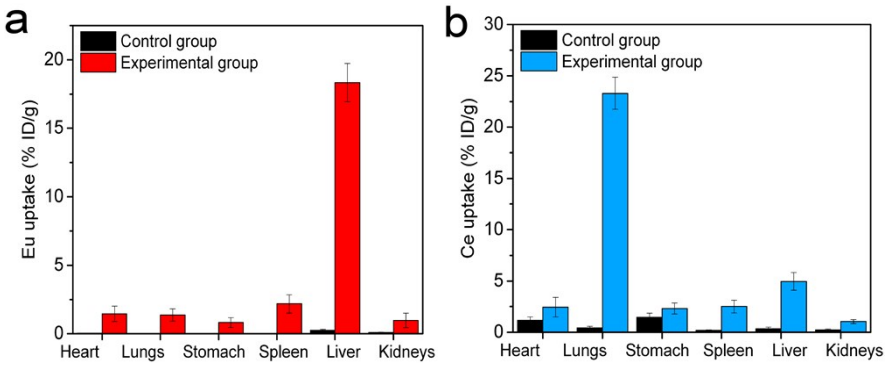

Figure S21. ICP-MS analysis of bio-distribution of the AES NPs in various organs.

REFERENCES

(1) J. Wang, N. He, Y. Zhu, Z. An, P. Chen, C. A. Grimes, Z. Nie and Q. Cai, *Chemical Communications,* 2018, **54,** 591-594.

(2) J Boyer , F Van Veggel, Nanoscale, 2010, **2(8),** 1417.