Highly-luminescent Eu,Sm,Mn-doped CaS Up/Down conversion Nanoparticles: Application to Ultra-Sensitive Latent Fingerprint Detection and *in vivo* Bioimaging

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Materials

All starting materials were obtained from commercial supplies and used directly. Europium (III) oxide (Eu_2O_3, 99.9%), Samarium (III) oxide (Sm_2O_3, 99.9%), Hexadecyl trimethyl ammonium bromide (CTAB, 99%), 3-Mercaptopropionic Acid (3-MPA, 98%), N-(3-Dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDC·HCl,98%), N-Hydroxysuccinimide (NHS, 98%), 1-Pentanol (98%), 1-Dodecanethiol (95%), Tergitol (NP-10), L-Arginine (98%), Diaminopolyethylene glycol (H_2N-PEG-NH_2, M.W 2000) were purchased from Aladdin Reagent, Co.,Ltd. (Shanghai, China). Cyclohexane (99.5%), Calcium nitrate tetrahydrate ($Ca(NO_3)_2 \bullet 4H_2O$, 99%), Manganese acetate tetrahydrate ($Mn(CH_3COO)_2 \bullet 4H_2O$, 99%), Ammonium sulfate ($(NH_4)_2SO_4$, 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). N,N-dimethyl formamide (DMF, 99.5%) was obtained from Tianjin Kermel Chemical Co.,Ltd. Ethanol (anhydrous, 99.7%) and Acetone (99%) were obtained from Beijing Chemical Regent Co.,Ltd. All aqueous solutions were prepared using ultrapure water (Mill-Q, Millipore, 18.2 MΩ resistivity). $RE(NO_3)_3$ was prepared by adding RE_2O_3 into an excessive nitric acid solution (0.2M) and stirred at 50°C for 30min and then evaporated to obtain nitrate powder.

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 K_α radiation, λ=1.5406 Å) from 10° to 80° at scanning rate of 4°/min. The Up-conversion luminescence (UCL) emission spectrum were acquired on a Hitachi F-4600spectrofluorometer with an external 980 nm NIR laser (Changchun New Industries Optoelectronics Technology Co.,Ltd.). The excitation spectrum and the Down-conversion luminescence (DCL) emission spectrum were recorded on a Hitachi F-4600 spectrofluorometer with a Xe lamp as the excitation source. The measurement of UCL absolute quantum were acquired in the Steady-State&Time-Resolved Fluorescence Spectrofluorometer (PTI Co.,Ltd. QM/TM/IM) equipped with integrating sphere (EVERFINE Co.,Ltd. 80 mm in diameter). The size distribution and Zeta-potential were measured using Malven Nano Zetasizer system by Malvern Instruments. The Flourier transformation infrared spectroscopy was measured in a Nicolet FTIR 5700. ^1H Nuclear Magnetic Resonance spectra were obtained using a Bruker Ascend 400 spectrometer. The thermogravimetric analysis was performed on a NETZSCH STA 449c simultaneous differential scanning calorimetry and thermogravimetry. *In vivo* and *ex vivo* UCL 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.

Synthesis of CaS:Eu,Sm,Mn nanoparticles (ESM-CaS NPs)

2.75 mmol hexadecyl trimethyl ammonium bromide(CTAB) and 1.5 mL 1-pentanol were added to 20 mL cyclohexane, then mixed with aqueous solution of $Ca(NO_3)_2$ (0.5mL, 0.1M), $Eu(NO_3)_3$ (0.1mL, 1.5mM), $Sm(NO_3)_3$ (0.1mL, 1.5mM) and $Mn(CH_3COO)_2$ (0.26 mL, 1.5 mM). The mixture was stirred vigorously for 30min forming a water-in-oil emulsion, and then left to stand for 1 h. Meanwhile, a water-in-oil emulsion containing $(NH_4)_2SO_4$ (0.55mL, 0.1M) was prepared, and mixed with it, 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 via centrifugation 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.

DT Modification of ESM-CaS NPs

First, 0.5mL 1-Dodecanethiol (DT) was mixed with 5 mL absolute ethanol in a three-neck flask, in which ethanol solution containing 0.1 M NaOH was slowly added to adjust pH to 8.5 and then stirred for 30 min in dry N_2 atmosphere. During this process, the as-prepared ESM-CaS NPs were dispersed in 10 mL absolute ethanol containing 20 μLTergitol NP-10, sonicated for 30 min, and added into the DT solution. The solution was stirred at 50 C for 24h under dry N_2 atmosphere. The

DT@ESM-CaS NPs were collected by centrifugation at 10000 rpm, washed with ethanol, and finally dispersed in 2 mL cyclohexane.

Synthesis of DT/MUA@ESM-CaS NPs

The as-prepared DT@ESM-CaS NP solution in cyclohexane was added into 8 mL absolute ethanol, and sonicated for 10 min. Then 150 mg 11-mercaptoundecanoic acid and 10 μL 3-mercaptopropionic acid were dissolved in 5 mL absolute ethanol. The ethanol solution was adjusted to pH 9.0 with 0.1 M NaOH ethanol solution. After stirring for 30 min under dry N_2 atmosphere, the solution was added into the DT@ESM-CaS NP solution, and stirred vigorously for 48 h. The DT/MUA@ESM-CaS NPs were collected by centrifugation at 10000 rpm and washed with ethanol.

Synthesis of Arg-DT/MUA@ESM-CaS NPs

The as-prepared DT/MUA@ESM-CaS NPs were dispersed in 20 mL DMF and sonicated for 10 min to obtain a transparent solution. Then EDC·HCl (10mg) was added to the solution and stirred for 15 min. Afterward, NHS (5mg) was added and stirred at 37C for 2 h. The nanoparticles were isolated via centrifugation at 8000 rpm and re-dispersed in DMF (10 mL), in which 10 mL DMF containing 15 mg L-Arginine was then added, and stirred for 24 h in dark. The resulting Arg-DT/MUA@ESM-CaS NPs were collected by centrifugation and washed with ethanol.

Measurement of UCL Absolute Quantum

The as-prepared ESM-CaS NPs in anhydrous ethanol (1mg/mL) were placed in quartz cuvette, which was inserted into an integrating sphere (80 mm in diameter) from EVERFINE Corporation. The integrating sphere was mounted on the Steady-State&Time-Resolved Fluorescence Spectrofluorometer (PTI Corporation QM/TM/IM) with the entry and output ports of the sphere located in 90 geometry from each other in the plane of the spectrometer. Samples were excited with a 980 nm laser device (Shanghai Dream Laser Technology Co.,Ltd) coupled to fibre, the excitation density is 0.5W•cm^{-2}. The spectrum of excitation radiation not absorbed by the sample was measured at the wavelength from 970 to 1000 nm through neutral density filters. The spectrum of emission of each sample was measured from 400 to 900 nm without any neutral density filter. Pure anhydrous ethanol was used as the reference sample to record blank background. The absolute quantum yield of each sample was then determined according to the equation:

$$QY = \frac{L_{\text{sample}}}{E_{\text{reference}} - E_{\text{sample}}}$$

where QY is the absolute quantum yield, L_{sample} is the emission intensity, $E_{reference}$ and E_{sample} are the intensities of the excitation light not absorbed by the sample and the reference sample, respectively.

Latent Fingerprint Detection

Latent fingerprints were imprinted onto the surface of a substrate. After aging for 12 h, 1 mL Arg-DT/MUA@ESM-CaS NP aqueous solution (2.0 mg/mL) was dropped upon the general area of the fingerprints and allowed to incubate for 15 min at room temperature. Subsequently, the surface was rinsed (wash bottle) to remove excess nanoparticles and allowed to dry for UCL imaging. All UCL images were collected under 980 nm excitation (power ≈ 200 mW), by a commonly used smart-phone equipped with a12-megapixel camera.

Cytotoxicity of PEG-DT/MUA@ESM-CaS NPs

In vitro cytotoxicity was measured by performing the CCK-8 assay on A549 cells. Cells were seeded into a 96-well cell culture plate at 5×10^3 /well, under 100% humidity, and were cultured at 37Cin 5% CO_2 for 24 h; different concentrations of PEG-DT/MUA@ESM-CaS NPs (0, 100, 200, 400 and 600 μg/mL diluted in DMEM) were then added to the wells. The cells were subsequently incubated for 5 h and 24 h at 37C in 5% CO_2. Thereafter, the CCK-8 solution (10μL) 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 treatment group/mean Abs. value of control)×100%.

In vivo and ex vivo UCL imaging

Female 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 PEG-DT/MUA@ESM-CaS NPs saline solution (2 mg/mL, 200 μL). Mice were anesthetized by chloral hydrate solution for *in vivo* UCL imaging and sacrificed. The major organs such as heart, liver, spleen, lung, stomach and kidneys were collected, and washed for *ex vivo* UCL imaging. UCL signals were collected at 625-675 nm, and analyzed with Lumina XR Living Image Software.

Tab. S1. The $L_9(4^5)$ Orthogonal Table and corresponding UCL intensity under orthogonal experimental conditions

Serial number	Eu (mol%)	Sm (mol%)	Mn (mol%)	T (C)	Time (min)	UCL intensity (a.u)
Exp.1	0.1	0.1	0.2	850	30	2788
Exp.2	0.1	0.2	0.4	950	60	5382
Exp.3	0.1	0.3	0.6	1050	90	2635
Exp.4	0.1	0.4	0.8	1150	120	3223
Exp.5	0.2	0.1	0.4	1050	120	3203
Exp.6	0.2	0.2	0.2	1150	90	4572
Exp.7	**0.2**	**0.3**	**0.8**	**850**	**60**	**9298**
Exp.8	0.2	0.4	0.6	950	30	7259
Exp.9	0.3	0.1	0.6	1150	60	5761
Exp.10	0.3	0.2	0.8	1050	30	5923
Exp.11	0.3	0.3	0.2	950	120	6075
Exp.12	0.3	0.4	0.4	850	90	7430
Exp.13	0.4	0.1	0.8	950	90	3473
Exp.14	0.4	0.2	0.6	850	120	3773
Exp.15	0.4	0.3	0.4	1150	30	5644
Exp.16	0.4	0.4	0.2	1050	60	4990
Mean-1	3507	3806	4606	**5822**	5404	
Mean-2	6083	4912	5415	5547	**6358**	
Mean-3	**6297**	**5913**	4857	4188	4528	
Mean-4	4470	5726	**5479**	4800	4068	
Extremum	2790	2107	873	1634	2289	

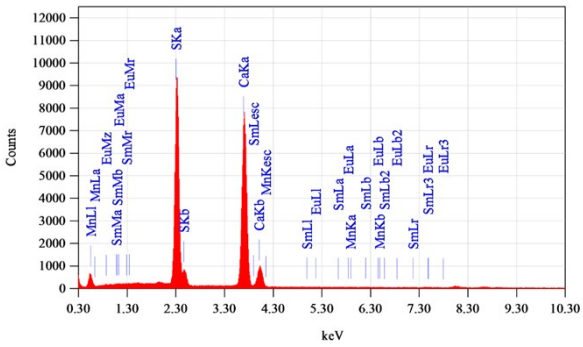

Fig. S1. Energy dispersive X-ray analysis of ESM-CaS NPs.

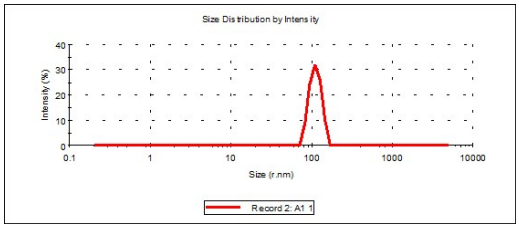

Fig. S2a. Dynamic light scattering (DLS) of ESM-CaS NPs

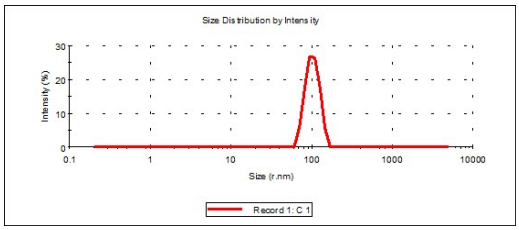

Fig. S2b. Dynamic light scattering (DLS) of DT@ESM-CaS NPs

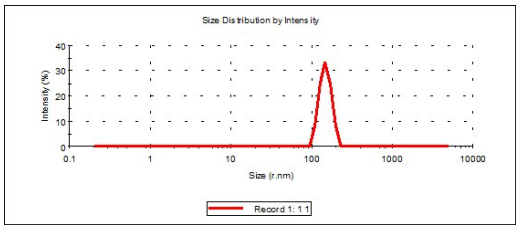

Fig. S2c. Dynamic light scattering (DLS) of DT/MUA@ESM-CaS NPs.

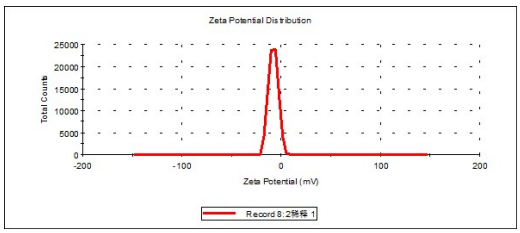

Fig. S3a. Zeta-potential of ESM-CaS NPs.

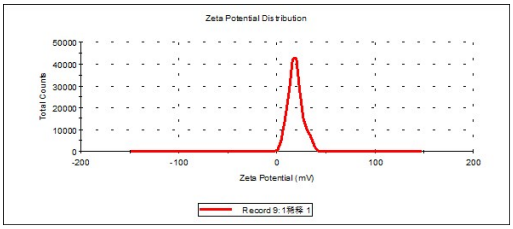

Fig. S3b. Zeta-potential of DT@ESM-CaS NPs.

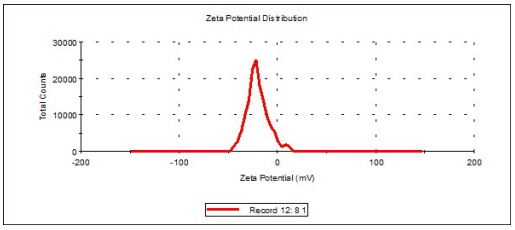

Fig. S3c. Zeta-potential of DT/MUA@ESM-CaS NPs.

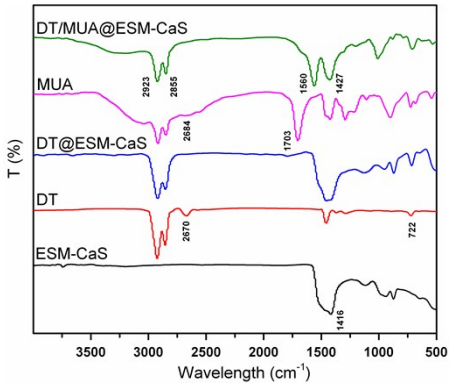

Fig. S4. Fourier transform infrared spectroscopy of ESM-CaS, DT, DT@ESM-CaS, MUA and DT/MUA@ESM-CaS NPs.

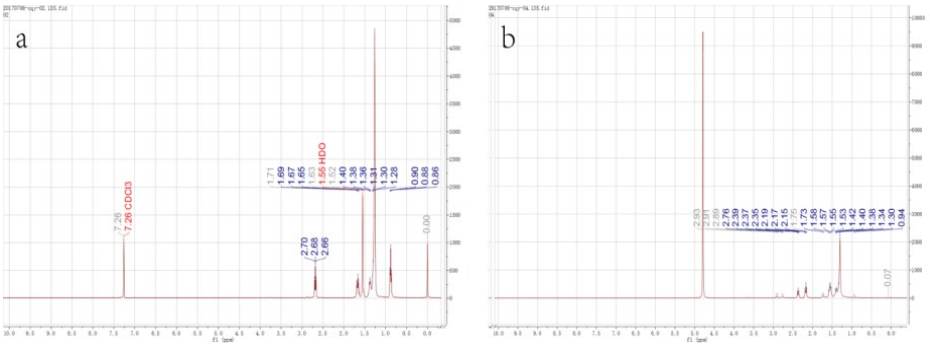

Fig. S5. Nuclear magnetic resonance spectroscopy (^{1}HNMR) of (a) DT@ESM-CaS NPs dispersed in $CDCl_3$, (b) DT/MUA@ESM-CaS NPs dispersed in D_2O.

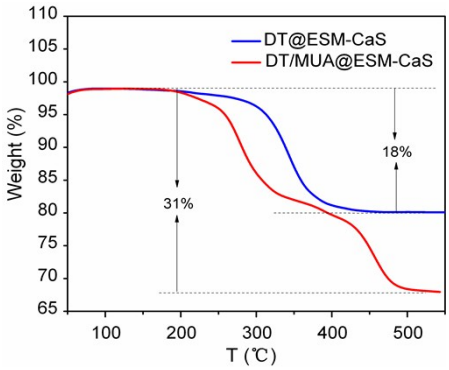

Fig. S6. Thermogravimetric analysis of DT@ESM-CaS NPs and DT/MUA@ESM-CaS NPs.

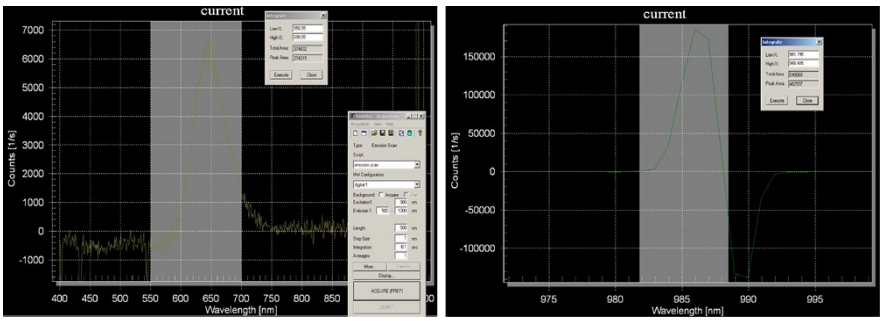

Fig. S7. The absolute quantum yield measurement of the ESM-CaS NPs.

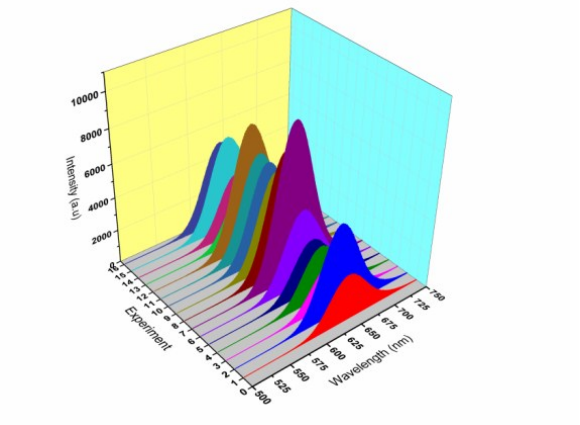

Fig. S8. UCL spectrum under different orthogonal experiment conditions.

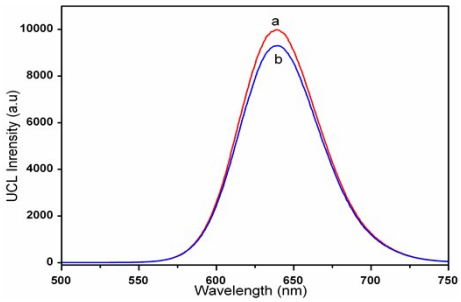

Fig. S9. The UCL spectrum: (a) 0.3% Eu, 0.3% Sm and 0.8% Mn, annealing at 850 C for 60 min. (b) 0.2% Eu, 0.3% Sm and 0.8% Mn, annealing at 850 C for 60 min (Exp.7 in Orthogonal table)

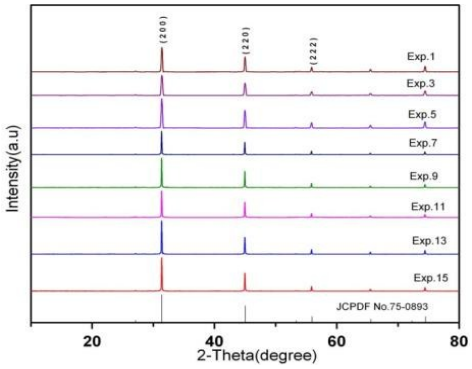

Fig. S10. X-ray diffraction spectra of CaS:Eu,Sm,Mn samples under different orthogonal experiment conditions.

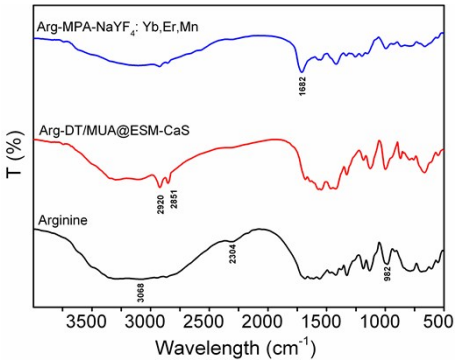

Fig. S11. FT-IR of Arginine, Arg-DT/MUA@ESM-CaS NPs and Arg-MPA-$NaYF_4$: Yb,Er,Mn NPs, respectively.

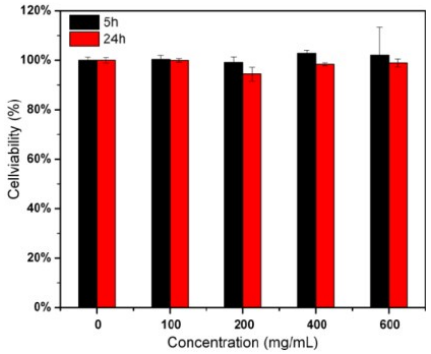

Fig. S12. Cell viability of A549 cells incubated with PEG-DT/MUA@ESM-CaS NPs at different concentrations for 5 and 24 hours.