Supporting Information

In vivo 808nm Image-guided Photodynamic Therapy Based on Upconversion Theranostic Nanoplatform

Xiaomin Liu,[a] Ivo Que,[b] Xianggui Kong,[a]* Youlin Zhang,[a] Langping Tu,[a] Yulei Chang,[a] Tong Tong Wang,[a] Alan Chan,[b,d] Clemens W. G. M. Löwik,[b] Hong Zhang[a,c]*

[a]State Key Laboratory of Luminescence and Applications. Changchun Institute of Optics, Fine Mechanics and Physics, Chinese Academy of Sciences. 130033, Changchun, Jilin, China.

[b]Experimental Molecular Imaging, Department of Radiology, Leiden University Medical Center, 2333 ZA Leiden, The Netherlands.

[c]Van't Hoff Institute for Molecular Sciences. University of Amsterdam, Science Park 904, 1098 XH Amsterdam, The Netherlands.

[d]Percuros B.V., Building Zuidhorst, Drienerlolaan 5, 7522 NB Enschede, The Netherlands.

*Corresponding author
E-mail address: xgkong14@ciomp.ac.cn; h.zhang@uva.nl

Keywords: Upconversion · Fullerene · Ligand exchange · Photodynamic therapy.

Characterization

The structure and morphology of the nanoparticles were characterized by using a Brucker D8-advance X-ray diffractometer (XRD) with Cu Ka radiation (λ= 1.5418 Å). The transmission electron microscopy (TEM) was performed on a Tecnai G2 F20 S-TWIN D573 electron microscope operated at 300 kV TEM. Ultraviolet-visible (UV-VIS) absorption was measured at room temperature by a UV-3101 spectrophotometer. The fluorescent emission spectra were measured at room temperature by a Hitachi F-4500 fluorescence spectrofluorimeter. The luminescence kinetics was recorded with a 500 MHz Tektronix digital oscilloscope and the excitation was realized by a nanosecond pulse train at 980 nm from an optical parametric oscillator. Cellular imaging was done using a Motic AE31 microscope equipped with the Andor GNIR CCD camera (iXon3 888-BV), which is capable of imaging in the range of 500-850 nm. A fiber coupled laser diode (*n*light, NL-PPS50) emitting at 980 nm was used as the light source, and the fiber was introduced through the entrance port of the microscope. The emitted light was passed through a 900nm short-wave pass and 740 nm long-wave pass filters and recorded by CCD camera.

Table S1. The energy transfer efficiencies based on the change of integral areas calculated from the steady-state upconversion luminescence spectra (Φ: integral area; η: energy transfer efficiency).

Peak (nm)	Φ(UCNPs)	Φ(Covalent)	Φ(ligand exchange)	η (covalent)	η(ligand exchange)
360	1.255	0.157	0.004	87.5%	99.7%
407	0.638	0.158	0.011	75.3%	98.3%
450	1.674	0.524	0.022	68.7%	98.7%
475	10.431	4.355	0.761	57.3%	92.7%
540	10.051	4.502	1.188	55.2%	88.2%
650	6.975	3.832	1.728	45.1%	75.2%
696	2.406	1.642	1.148	31.7%	52.3%

Table S2. The energy transfer efficiencies based on the change of integral areas calculated from luminescence decay spectra (Φ: integral area; η: energy transfer efficiency).

Peak (nm)	Φ (UCNPs)	Φ(ligand exchange)	η (ligand exchange)
450	0.00253	0.000687	72.8%
475	0.0325	0.01199	63.1%
540	0.0247	0.0108	56.3%
650	0.0568	0.0279	50.8%

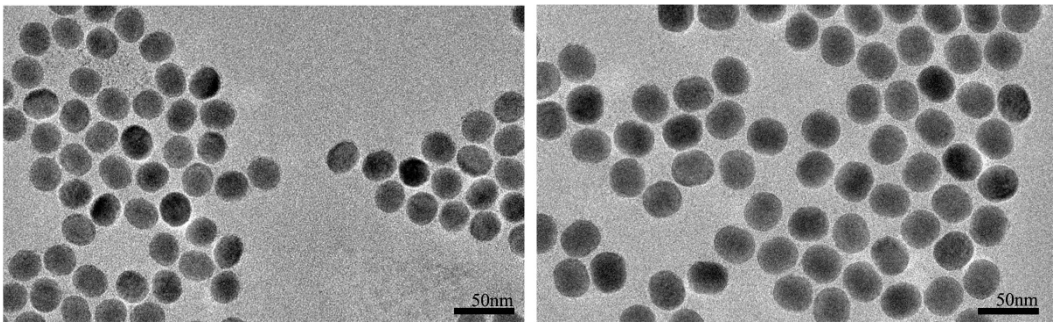

Figure S1. TEM images of core $NaYF_4$: Yb, Er (left) and core-shell $NaYF_4$: Yb, Er/ $NaYF_4$: Yb, Tm UCNPs (right).

The $NaYF_4$: Yb, Er nanoparticles had a mean particle diameter of 24 nm. Core/shell $NaYF_4$: Yb, Er/ $NaYF_4$: Yb, Tm UCNPs increased the size to 34 nm, corresponding to about 5 nm shell thickness.

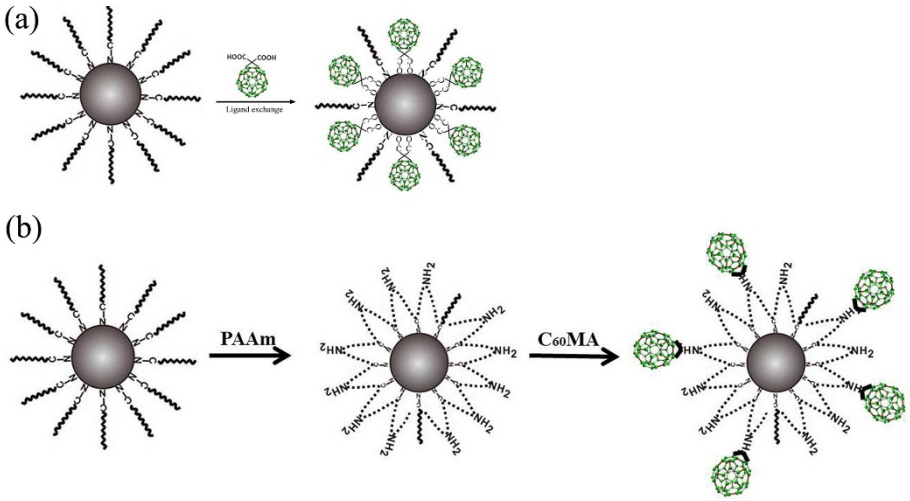

Figure S2. (a)The scheme of the ligand exchange reaction. The coordination ability of Ln^{3+} - O is stronger than that of Ln^{3+} - N, the carboxyl groups of C_{60}MA could easily replace oleylamine and coordinate to Ln^{3+}. (b) The scheme of the covalent assembled UCNPs-C_{60}MA. The oleylamine-coated NPs were firstly encapsulated by Poly(allylamine) (PAAm) giving the amino groups on the surface, followed by crosslinking reaction between the amino group of the UCNPs and the carboxyl group of C_{60}MA.

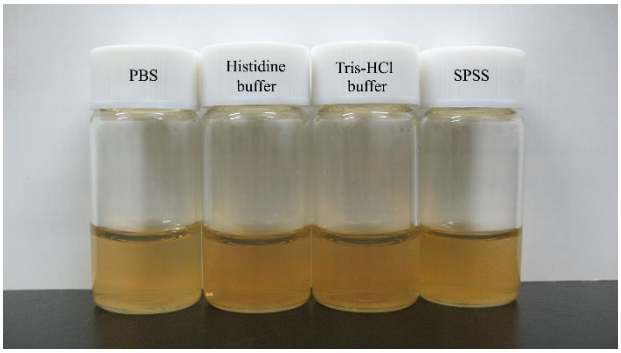

Figure S3. Photos of the ligand exchange UCNPs-C_{60}MA nanophotosensitizers dispersed in various biological media (SPSS: stroke-physiological saline solution).

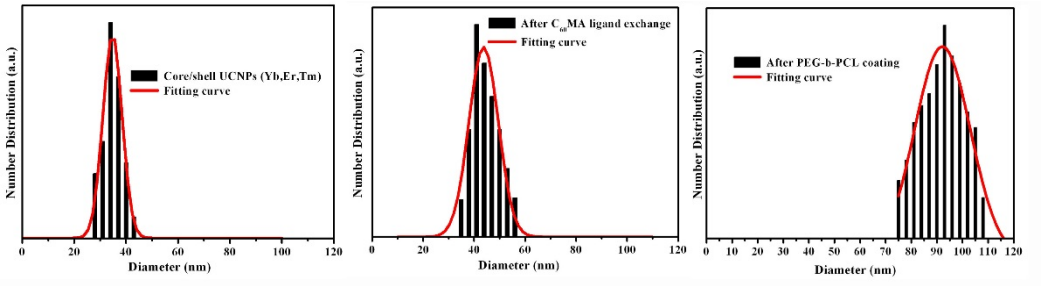

Figure S4. The hydrodynamic diameter distributions of the UCNPs before (~34 nm) and after liagand exchange (~43 nm), and after further polymer coating (~92 nm). Best fitting curves are also shown as a red solid line.

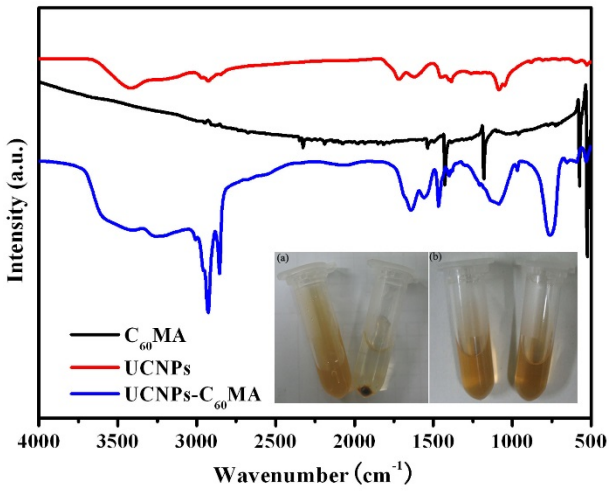

Figure S5. FTIR absorption spectra of C_{60}MA (black line), oleylamine-coated nanoparticles (red line), and ligand exchange assembled UCNPs-C_{60}MA nanoconjugate (blue line). The insert in the figure shows photos of the UCNPs-C_{60}MA nanoconjugate dissolved in THF (a) and free C_{60}MA dissolved in THF (b) before and after centrifugation (10,000 rpm for 10min).

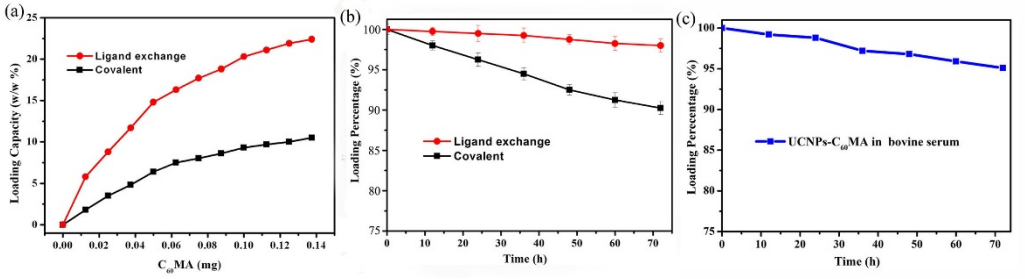

Figure S6. (a) Quantification of C_{60}MA loadings at different C_{60}MA amounts. The C_{60}MA loading capacity increased as the rise of C_{60}MA amounts and saturated at 22.5% (w/w). (b) The release of C_{60}MA from the ligand exchange constructed UCNPs-C_{60}MA nanophotosensitizer in PBS. (c)The release of C_{60}MA from UCNPs-C_{60}MA nanophotosensitizer in bovine serum.

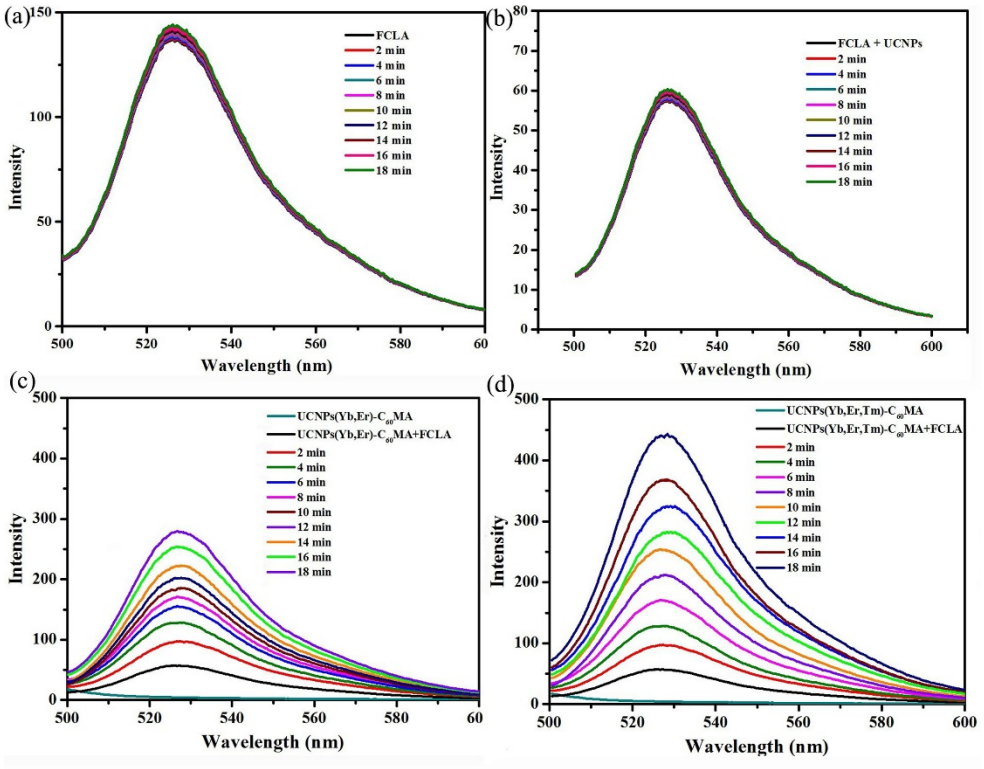

Figure S7. The spectra of FCLA luminescence intensity without UCNPs-C_{60}MA just under the illumination of 980 nm (a), and the spectra of FCLA luminescence intensity with UCNPs-C_{60}MA without 980 nm illumination (b), showing negligible change over time. FCLA assay of $^1O_2$ generation by covalent conjugated UCNPs-C_{60}MA nanophotosensitizer (c), and ligand exchange assembled UCNPs- C_{60}MA nanophotosensitizer (d).

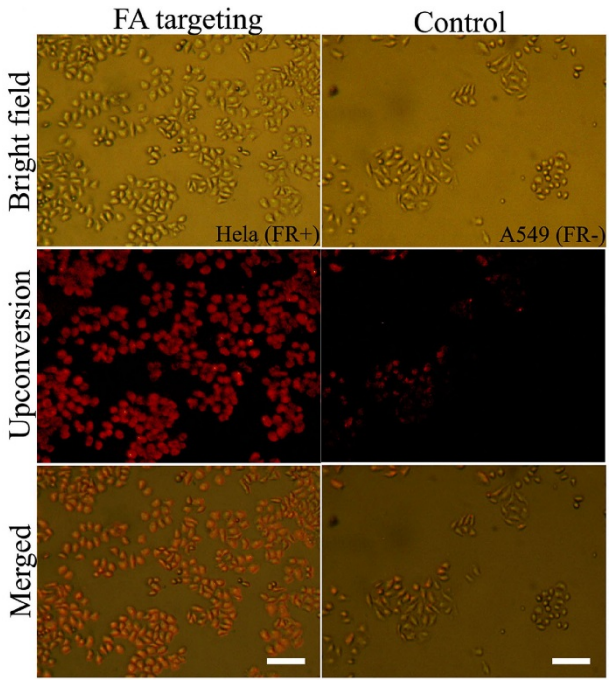

Figure S8. Specificity of the ligand exchange assembled UCNPs-C_{60}MA nanophotosensitizer. Hela cells cultured in folate-free medium (left, positive) and in A549 cells (right, negative control). Scale bar, 50 μm.

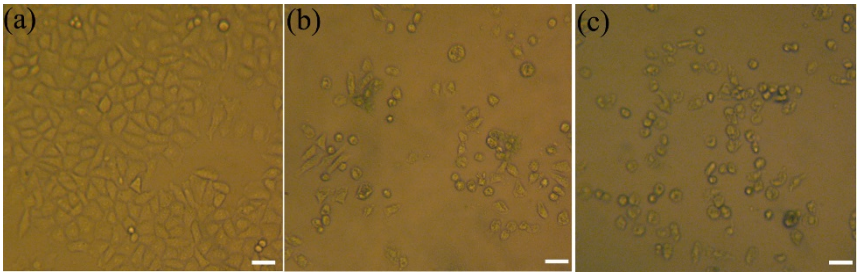

Figure S9. The cell morphology treated with different dosage of ligand exchange assembled UCNPs - C_{60}MA nanophotosensitizer after light exposure, (a) 100 μg/mL (100μL), (b) 300 μg/mL (100μL) and (c) 500 μg/mL (100μL), Scale bar, 20 μm.

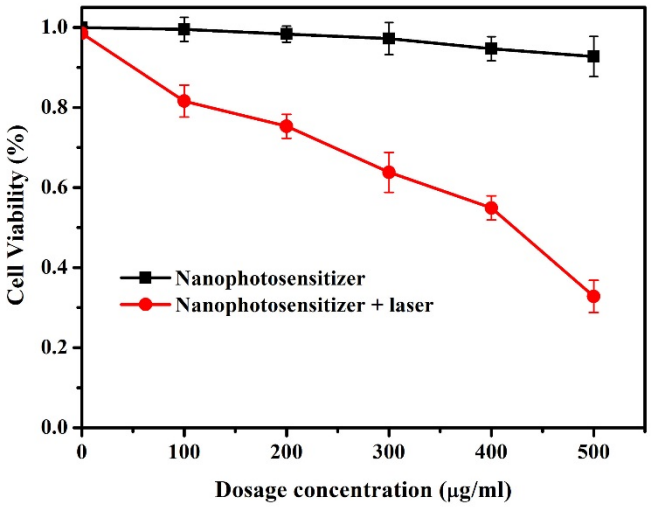

Figure S10. The photodynamic effect of ligand exchange assembled UCNPs-C_{60}MA NPS on mouse Hepa1-6 cell line (980 light dosage: 0.39 W/cm^2 for 10min).

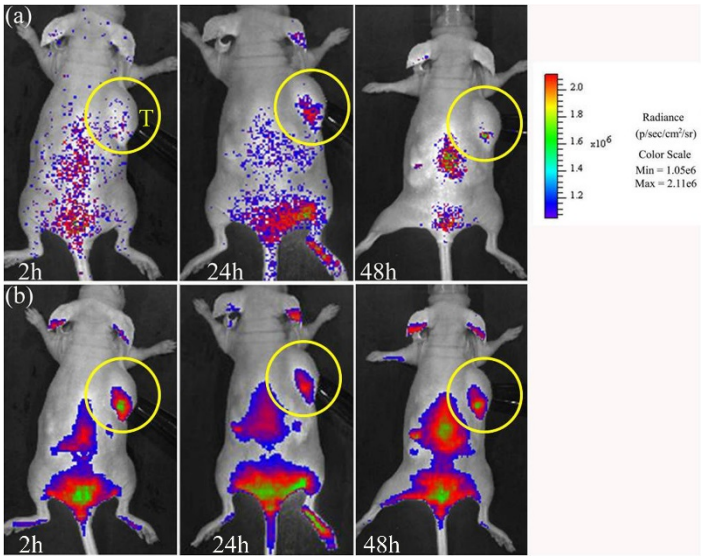

Figure S11. Mice bearing tumors which were administrated with UCNPs-C_{60}MA (a) or UCNPs-C_{60}MA/FA (b) imaged at different time points.

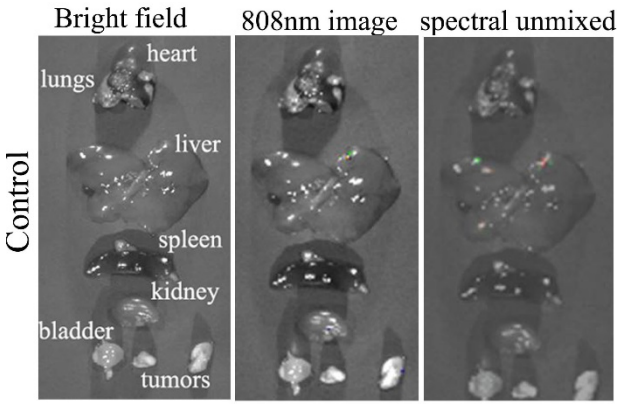

Figure S12. Fluorescence images of isolated organs separated from tumor-bearing mice without injection of NPS.