Lanthanide-Doped Upconversion Nanoparticles Electrostatically Coupled with Photosensitizers for Near-Infrared-Triggered Photodynamic Therapy

- Supporting Information

Meng Wang, Zhuo Chen, Wei Zheng, Haomiao Zhu, Shan Lu, En Ma, Datao Tu, Shanyong Zhou, Mingdong Huang, and Xueyuan Chen*

[*] M. Wang, Dr. W. Zheng, Dr. H. M. Zhu, Dr. S. Lu, E. Ma, Dr. D. T. Tu, Prof. X. Y. Chen

Key Laboratory of Optoelectronic Materials Chemistry and Physics, and Key Laboratory of Design and Assembly of Functional Nanostructures, Fujian Institute of Research on the Structure of Matter, Chinese Academy of Sciences, Fuzhou, Fujian 350002, China.

Fax: (+86)591-8764-2575

E-mail: xchen@fjirsm.ac.cn

Dr. Z. Chen, S. Y. Zhou, Prof. M. D. Huang

State Key Laboratory of Structural Chemistry and Danish-Chinese Centre for Proteases and Cancer, Fujian Institute of Research on the Structure of Matter, Chinese Academy of Sciences, Fuzhou, Fujian 350002, China.

M. Wang

University of Chinese Academy of Sciences, Beijing 100049, China.

Average particle size (nm)	OA/OM (mL/mL)	Time (min)	Temperature (°C)
47	8:2	60	310
22	8:4	60	310
8.1	8:6	90	320

 Table S1. Synthetic conditions for LiYF4:Yb/Er UCNPs with different sizes.

LiYF ₄ (this work)			NaYF ₄ (Ref.6) ¹		
Average particle size (nm)	Power density (W/cm ²)	QY (%)	Average particle size (nm)	Power density (W/cm ²)	QY (%)
47		0.6	100		0.3
22	60	0.08	30	150	0.1
8.1		0.03	10		0.005

Table S2. Comparison of the absolute upconversion quantum yield (QY) in $LiYF_4$:Yb/Er andNaYF4:Yb/Er UCNPs.

References

1. J.-C. Boyer and F. C. J. M. van Veggel, *Nanoscale*, 2010, **2**, 1417-1419.



Figure S1. XRD patterns of LiYF₄:Yb/Er UCNPs synthesized with an OA/OM ratio of (a) 8/6, (b) 8/4, (c) 8/2. All diffraction peaks match well with the standard pattern of tetragonal-phase LiYF₄ (JCPDS No. 77-861), indicating the formation of highly crystalline LiYF₄ UCNPs. The average diameters estimated by Debye–Scherrer's formula are 10, 36, and 53 nm for UCNPs synthesized with an OA/OM ratio of 8/6, 8/4, 8/2, respectively, in good agreement with the mean sizes obtained from TEM images.



Figure S2. Upconversion luminescence (UCL) decays from ${}^{4}F_{9/2}$ of Er^{3+} in LiYF₄:Yb/Er UCNPs of different sizes upon NIR excitation at 980 nm. By fitting the decay curves exponentially, the UCL lifetime of ${}^{4}F_{9/2}$ of Er^{3+} was determined to be 50, 117, 531 µs for LiYF₄:Yb/Er UCNPs with a mean size of 8.1, 22, 47 nm, respectively. The observed UCL lifetime of the UCNPs increased with the increasing UCNPs size, which is in line with the improved UCL intensity.



Figure S3. Thermogravimetric analyses (TGA) curves of oleate-capped UCNP, ligand-free UCNPs, and UCNPs-ZnPc-COOH with and without PVP capping under N_2 atmosphere in the temperature range of 35-800 °C at a rate of 10 °C min⁻¹. Different decomposition temperatures and weight losses were observed, due to different ligands on the surface of UCNPs, which indicate the successful removal of surface ligands from the oleate-capped UCNPs for the ligand-free UCNPs as well as the capping of ZnPc-COOH and PVP on the surface of ligand-free UCNPs.



Figure S4. Fourier transform infrared (FTIR) spectra of (a) oleate-capped UCNPs, (b) ligand-free UCNPs, and UCNPs-ZnPc-COOH (c) without and (d) with PVP capping. The intensity of the original asymmetric and symmetric stretching vibrations of methylene ($-CH_2-$) in the long alkyl chain peaking at 2924 and 2854 cm⁻¹ in the ligand-free UCNPs were markedly weakened, reconfirming the successful removal of oleate ligands from the surface of oleate-capped UCNPs. Strong IR band centered at ~1665 cm⁻¹ was observed for UCNPs-ZnPc-COOH, which is assigned to the C=O stretching mode of PVP. Besides, two weak bands were also observed at 1098 and 734 cm⁻¹, which are attributed to the stretching vibrations of the phthalocyanine aromatic ring and the C-H group on the phthalocyanine aromatic ring of ZnPc-COOH, respectively.



Figure S5. Hydrodynamic diameter distribution of the ligand-free UCNPs, UCNPs-ZnPc-COOH with and without the capping of PVP. The hydrodynamic diameter of the ligand-free UCNPs (50.7 nm) is in agreement with their original oleate-capped counterparts (47 ± 2.5 nm), indicating that the removal of surface ligands has no significant influence on the sizes of the UCNPs. The hydrodynamic diameter of UCNPs-ZnPc-COOH without the capping of PVP increased to 191.3 nm and exhibited a broad distribution, due to the aggregation of the UCNPs inducing by the π - π stacking interaction of the phthalocyanine aromatic ring of ZnPc-COOH on their surface. By contrast, the hydrodynamic diameter of PVP-capped UCNPs-ZnPc-COOH was 63.5 nm, indicating a recovery of hydrophilicity. The larger hydrodynamic diameter of the PVP-capped UCNPs-ZnPc-COOH than that of the ligand-free UCNPs can be attributed to the formation of PVP layer on the surface of the UCNPs.



Figure S6. In vitro cytotoxicity of ligand-free UCNPs and UCNPs-ZnPc-COOH against HELF cells after incubation for 24 h by using a CCK-8 assay. The cell viability was determined to be larger than 98 % even at a concentration as high as 1 mg/mL for all the UCNPs. Such low cytotoxicity indicates that these UCNPs are biocompatible and nearly nontoxic to live cells.



Figure S7. ζ -potentials of the ligand-free UCNPs, UCNPs-ZnPc-COOH without and with PVP capping obtained from the DLS measurement. After the loading of ZnPc-COOH, the ζ -potential of the UCNPs changed from +49.5 to +2.9 mV (pH 6.5), as a result of reduced positively-charged Ln³⁺ ions exposed on the surface of the UCNPs, thus verifying the successful conjugation of ZnPc-COOH to the surface of UCNPs. As compared to that of UCNPs-ZnPc-COOH without PVP capping, the ζ -potential of UCNPs-ZnPc-COOH with PVP capping changed from +2.9 to +15.4 mV (pH 6.5), owing to the N atom on the pyrrolidone ring.



Figure S8. Quantitative analysis of ZnPc-COOH capped on the surface of LiYF₄:Yb/Er UCNPs. (a) Absorption spectra of DMF solutions of ZnPc-COOH and UCNPs-ZnPc-COOH. (b) The standard curve of absorption for ZnPc-COOH. The loading amount of ZnPc-COOH was determined to be 1.1 % (w/w) in UCNPs-ZnPc-COOH.



Figure S9. Absorption spectra of pure ZnPc-COOH (0.5 μ g/mL) and the centrifugating supernatant of UCNPs-ZnPc-COOH after soaking for 7 days in aqueous solution of different pH ranging from 3 to 11. As compared to that of pure ZnPc-COOH, the absorbance of the photosensitizers in the supernatant of UCNPs-ZnPc-COOH solution can be neglected, indicating that a negligibly low content of ZnPc-COOH was released from UCNPs-ZnPc-COOH even for 7 days soaked in aqueous solution with a wide pH range. The inset shows the photographs of UCNPs-ZnPc-COOH soaked in aqueous solution of different pH, which reveals the excellent water-dispersibility of UCNPs-ZnPc-COOH. These results verify the high stability of the proposed electrostatic coupling of photosensitizers on the surface of UCNPs.



Figure S10. Absorption spectra of pure ZnPc-COOH (0.5 μ g/mL) and the centrifugating supernatant of UCNPs-ZnPc-COOH soaked in PBS. As compared to that of pure ZnPc-COOH, the absorbance of photosensitizers in the supernatant of UCNPs-ZnPc-COOH PBS solution can be neglected, indicating that a negligibly low content of ZnPc-COOH was released from UCNPs-ZnPc-COOH even for 7 days soaked in PBS. This result reconfirmed the high stability of the proposed electrostatic coupling of photosensitizers on the surface of UCNPs.



Figure S11. (a) TEM image of β -NaYF₄:Yb/Er UCNPs. (b) UCL spectra of β -NaYF₄:Yb/Er UCNPs before and after electrostatic coupling with ZnPc-COOH. TEM image shows that β -NaYF₄:Yb/Er UCNPs are roughly spherical with an average diameter of 30 nm. UCL spectra show that the red UCL intensity of UCNPs-ZnPc-COOH at 669 nm was obviously decreased as compared to that of the ligand-free UCNPs, which was remarkably different from their nearly unchanged green UCL intensity at 540 nm. From the UCL spectra, the energy transfer efficiency was determined to be 88.7 %.



Figure S12. (a) TEM image of β -NaLuF₄:Gd/Yb/Er UCNPs. (b) UCL spectra of β -NaLuF₄:Gd/Yb/Er UCNPs before and after electrostatic coupling with ZnPc-COOH. TEM image shows that the β -NaLuF₄:Gd/Yb/Er UCNPs are roughly spherical with an average diameter of 18 nm. UCL spectra show that the red UCL intensity of UCNPs-ZnPc-COOH at 669 nm was obviously decreased as compared to that of the ligand-free UCNPs, which was remarkably different from their nearly unchanged green UCL intensity at 540 nm. From the UCL spectra, the efficiency of energy transfer from UCNPs to ZnPc-COOH was determined to be 92.4 %. These results reconfirm the high energy transfer efficiency from UCNPs to ZnPc-COOH.



Figure S13. a) Schematic illustration of the setup layout for in vitro PDT in the presence of a tissue phantom. b) Phototoxicity of MDA-MB-231 cells incubated with different concentration of UCNPs-ZnPc-COOH after the 980-nm NIR laser and the 660-nm red laser (commonly used for the excitation of ZnPc in conventional PDT) irradiation for 20 min, respectively, in the presence of a tissue phantom. The viability of the cells exposed to the 980-nm NIR laser decreased more significantly than that exposed to the 660-nm red laser, as the concentration of the co-incubated UCNPs-ZnPc-COOH increased, which is ascribed to the deeper tissue penetration with the 980-nm NIR light. Particularly, the cells incubated with UCNPs-ZnPc-COOH at a concentration of 0.25 mg/mL almost died out upon 980-nm NIR laser irradiation for 20 min. These results reveal the great potential of our PDT agent for deep cancer therapy.



Figure S14. Phototoxicity of MDA-MB-231 cells after incubation with different size of UCNPs-ZnPc-COOH upon 980-nm NIR laser irradiation for 20 min in the presence of a tissue phantom. Each data point represents the average of quadruplicate measurements. The cell viability decreased gradually with the concentration and particle size of the UCNPs-ZnPc-COOH co-incubated upon 980-nm NIR laser irradiation, which indicates that the PDT system with larger size of UCNPs has a higher PDT efficacy against cancer cells. This can be ascribed to the higher UCL quantum yield of the larger UCNPs (47 nm) than that of the smaller ones (8.1 and 22 nm).