Versatile Photosensitizers for Photodynamic Therapy at Infrared Excitation

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Supporting Information

SiO_2 Coating of PUNPs

The PUNPs used in this study emit green color (peak at ~537 nm) when excited by a diode laser of 974 nm. The photon upconverting nanoparticles are home-made, based on the procedures in the literature [S1] with some modifications of our own. The particle size ranges from 60 nm to 120 nm from batch to batch. A TEM image of such nanoparticles is shown in Figure 1S. The nanoparticles are coated with a thin layer of SiO_2 through a variation of the well-known Stöber process. In brief, 5 mg of nanoparticles was dispersed in 5 ml of n-propanol under sonication. Then, 1.5 ml of 28% ammonia, 1.5 ml of de-ionized water, 4 µl of Merocyanine 540 solution (10 mM) and 2 µl of tetraethyl orthosilicate (TEOS) were added. The mixture was placed in a constant-temperature bath at 40˚C under vigorous stirring for over 2 hours. The resulting nanoparticles were centrifuged and collected. A thin SiO_2 layer was thus deposited on the PUNPs surface.

Surface modification of SiO_2-coated PUNPs

We adopted the procedures reported in the literature of binding the $–NH_2$ group of the antibody and the –OH group on the SiO_2 surface.[S2] To covalently attach the antibody to the SiO_2 surface, 5 mg of SiO_2-coated PUNPs were washed twice in PBS buffer (pH = 8), and dispersed in 2 ml of 2 M sodium carbonate buffer. Subsequently, 50 mg of Cyanogen Bromide (CNBr) dissolved in 0.2 ml of acetonitrile was added under stirring. After 2 minutes, the nanoparticles were washed with ice-cold water and PBS buffer, and re-dispersed in 2 ml of PBS buffer. 50 μl of antibody solution (1mg/ml) was added, and the mixture was kept at 4˚C overnight under constant stirring. Finally, the nanoparticles were collected, washed with and dispersed in PBS buffer before use. ADPA and M-540 were from Invitrogen, and used as received. All other chemicals were from Sigma-Aldrich, and used as received.

Cell culture

MCF-7/AZ cells are variants of the human mammary carcinoma cell family MCF-7. The cells are maintained on tissue culture plastic substrate (Nunc) in a mixture of Dulbecco's modified eagle's medium (DMEM) and HAMF12 (50/50) (Invitrogen, Carlsbad, CA) supplemented with 250 IU/ml penicillin, 100 μg/ml streptomycin (Invitrogen) and 10% fetal bovine serum (FBS) (Invitrogen), at 37°C in a humidified atmosphere containing 10% CO_2.[S3]

In vitro PDT study with cells of Trypan Blue staining

Single cell suspensions were prepared with trypsin/EDTA and 0.5 ml of cell suspension was brought in a covered Petri dish with glass bottom for viewing. 0.1 ml of 0.4% Trypan Blue Stain and 0.1 ml of antibody-modified PUNPs PBS buffer solution were added, mixed thoroughly and

incubated stand for 15 minutes at room temperature. The Petri dish was imaged using an inverted microscope with a 10× objective, before and after a certain period of IR irradiation. The power of the Halogen bulb used for optical imaging was maintained constant throughout the experiment. The illumination from the Halogen bulb was blocked during the IR irradiation.

In vitro PDT study with cells of PI staining

Single cell suspensions were prepared with trypsin/EDTA and 0.5 ml of cell suspension was brought in a covered Petri dish with glass bottom for viewing. 1 µl of PI solution (1 mg/ml) and 0.1 ml of antibody-modified PUNPs PBS buffer solution were added, mixed thoroughly and incubated stand for 15 minutes at room temperature. The Petri dish was imaged using an inverted microscope with a 60× oil immersion objective and a filter set (Chroma Technology, Set 31002), before and after a certain period of IR irradiation. The power of the mercury lamp used for fluorescence imaging was maintained constant throughout the experiment. The illumination from the mercury lamp was blocked during the IR irradiation.

Microscopy/Spectroscopy

The microscopic experiments were performed using an inverted microscope (Olympus IX-50), equipped with a CCD camera (Apogee Instruments, Alta U2000). Images were analysed by ImageJ software. Figures 3a and 3b have the same maximum and minimum values of intensity. Figures 3d and 3e have the same maximum and minimum values of intensity. False colour of fluorescence images was also added by ImageJ software. The spectroscopic measurements were carried out in a quartz cuvette with a PTI spectrofluorometer. A 0.5 ml M-540-coated nanoparticles suspension in buffer (pH=7.4) was mixed with 5 µl ADPA (0.2 mM). The solution was first irradiated at 540 nm using a xenon lamp for several minutes, while the 402 nm emission

peak of ADPA (excited at 374 nm) being measured at different time intervals. The decrease of the ADPA fluorescence intensity over time confirmed the singlet oxygen generation from the M-540-coated nanoparticles under visible light irradiation. The solution was then illuminated by an infrared laser. The illumination from the xenon lamp was blocked during the IR irradiation. A diode laser at 974 nm (Power Technology) of approximately 60 mW was used as the irradiation source in all experiments.

Figure 1S. TEM image of the $NaYF_4:Yb^{3+},Er^{3+}$ nanoparticles used in this study.

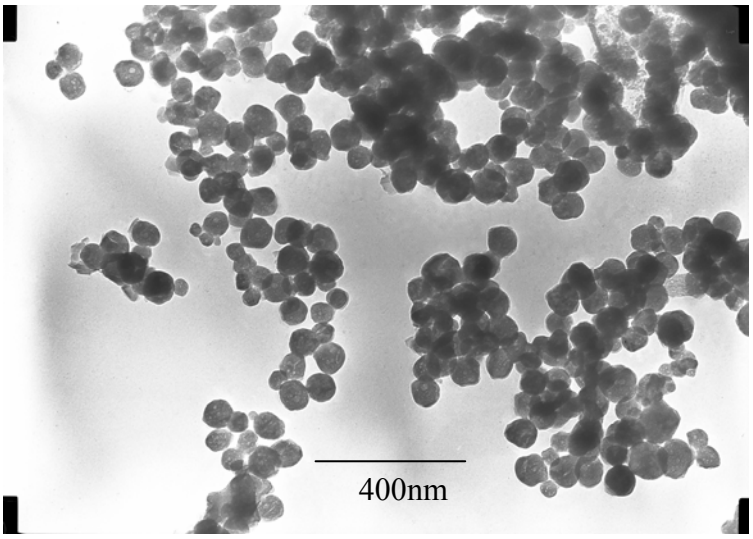
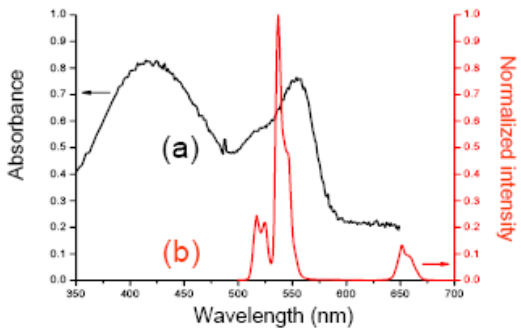


Figure 2S. (a) Absorbance spectrum of M-540. (b) Emission spectrum of PUNPs used in this study, excited by a 974 nm laser.



References

[S1] Yi, G.; Lu, H.; Zhao, S.; Ge, Y.; Yang, W.; Chen, D.; Guo, L.-H. *Nano Lett.* **2004**, *4*, 2191.

[S2] Weetall, H.; Detar, C. *Biotechnol. Bioeng.* **1975**, *17*, 295.

[S3] Bracke, M. E.; Van Larebeke, N.; Vyncke, B.; Mareel, M. M. *Br. J. Cancer.* **1991**, *63*, 867.