Supporting Information

One-Step Co-Assembly Method to Fabricate Photosensitive Peptide Nanoparticles for Two-Photon Photodynamic Therapy

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Materials and experimental section

Materials

Fluorenylmethoxycarbonyl-Leucine-Leucine-Leucine-OMe (Fmoc-L_3-OMe) was purchased from BACHEM and Meso-Tetra(p-hydroxyphenyl) porphine (m-THPP) was purchased from J&K Chemical. DMSO were purchased from Beijing Chemical Works. 9,10-anthracenediylbis (methylene) dimalonic acid (ABDA) was obtained from Sigma-Aldrich (Shanghai, China). 2', 7'-dichlorofluorescein diacetate (DCFH-DA) was purchased from R&D-SYSTEMS. Propidium Iodide (PI), Dulbecco's phosphate buffered saline (PBS), RMPI 1640, Alexa 488, Hoechst 33342, fetal bovine serum (FBS) were obtained from Invitrogen Corp..

MCF-7 cell line was obtained from the Cell Culture Center of the Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences (Beijing, China).

Millipore water was used throughout the study, with a resistivity of 18.2 MΩ cm^{-1}

Experimental section

1. Fabrication of m-THPP doped peptide nanoparticles

Fmoc-L_3-OMe and m-THPP were first dissolved in DMSO with ultrasonic treatment, where the concentration of Fmoc-L_3-OMe in DMSO kept unchanged at 0.8 wt%, while the concentration m-THPP in DMSO was changed from 0.2 wt% to 0.6 wt%. Then the mixture was quickly added to water with gentle shaking (1 volume of mixture and 20 volume of water). After aging overnight, the solution was centrifuged (13500 rpm, 20 min), washed and resuspended in water or cell culture medium for further use.

2. Morphology characterization of the nanoparticles

The scanning electron microscopy (SEM) image of the nanoparticles was acquired with S-4800 (HITACHI, Japan, 10 kV voltage) instrument measurement. For sample preparation, an aliquot of the nanoparticle suspension was dropped on a silica wafer and dried in vacuum. Before image acquisition, platinum was sputtered onto the sample to increase conductivity. Transmission electron microscopy (TEM) image was obtained by a JEOL JEM-1011 instrument at 100 kV and the sample was prepared on the carbon-coated copper grids. CLSM image of the nanoparticles was obtained using an Olympus FV500 microscope. The size distribution of the nanoparticles was determined using a Zetasizer (Malvern Instruments).

3. Two-photon CLSM image and fluorescence spectra of the nanoparticles

For the acquisition of CLSM images of nanoparticles excited by the two-photon laser, an aliquot of the nanoparticle suspension was dropped onto a piece of glass slide and dried in vacuum. The two-photon CLSM image of the nanoparticles was obtained using an Olympus FV500 microscope. The two-photon fluorescence spectra of the nanoparticles were determined under an 850 nm femtosecond pulse laser irradiation with an omni-λ monochromator and a photomultiplier.

4. Endocytosis experiment

MCF-7 cells were seeded onto confocal dish (35 mm) and incubated for 12 h (37 °C, 5% CO_2). After washed with PBS twice, the dish was refreshed with 1 mL of nanoparticles (10 μg ml^{-1}) contained RMPI 1640 cell culture medium and incubated for 4 h. The dish was again washed twice with PBS to remove the un-uptake nanoparticles. Before observation, cell membrane and nuclei were stained with Alexa 488 and Hoechst 33342, respectively. The intracellular localization of the nanoparticles was observed using an Olympus FV500 microscope.

5. $^1O_2$ detection in cells

DCFH-DA was used for the detection of $^1O_2$ in cells. The MCF-7 cancer cells were first co-cultured with m-THPP doped peptide nanoparticles (10 μg ml^{-1}) in confocal dish for 4 h. Then the cells were washed with PBS twice and refreshed with 1 mL of the fresh culture medium and 10 μL of the DCFH-DA suspension (10^{-5} M in culture medium without FBS). Twenty minutes later, the cells were again washed with PBS twice, refreshed with 1 mL of fresh culture medium and then irradiated with two-photon laser of 850 nm wavelength at 10% intensity for 20 min before CLSM observation.

6. Two-photon cytotoxicity of the nanoparticles

The MCF-7 cells were first seeded on a confocal dish (35 mm) and incubated (5% CO_2, 37 °C) for 12 h, then the cells were co- incubated with nanoparticles (10 μg ml^{-1}) for 4 h in darkness. After that, the dish was washed with PBS twice to remove un-uptake nanoparticles. For easy observation, PI (1 mg mL^{-1}, 10 μL, in PBS) was added to the dish to stain the dead cells before irradiation. Then, the cells were irradiated with two-photon laser of 850 nm wavelength at 2% intensity for 20 min and continued to culture for 10 h. The CLSM images were taken before and after irradiation. Cells with no nanoparticle uptake were also treated with the two-photon laser of 850 nm wavelength at 10% intensity for 20 min and continued to culture for 10 h before observation. Dark toxicity was tested under the same conditions but in the absence of light.

Additional data

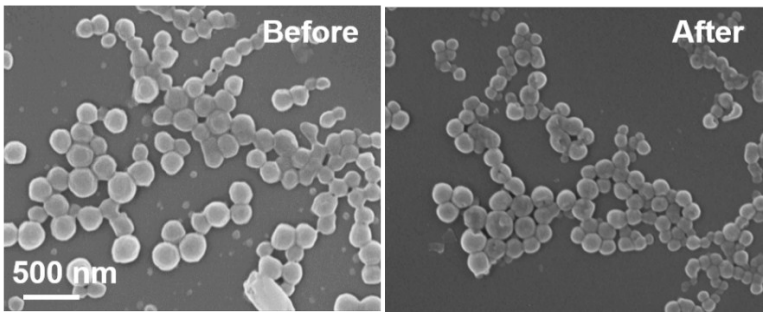

Figure S1 SEM images of nanoparticles before and after light irradiation

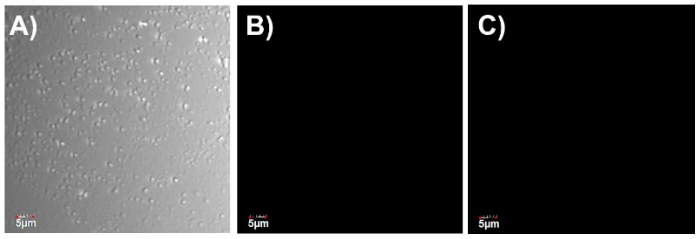

Figure S2 CLSM images of pure m-THPP nanoparticles excited by the two-photon laser of 850 nm: A) light field; B) and C) fluorescence images

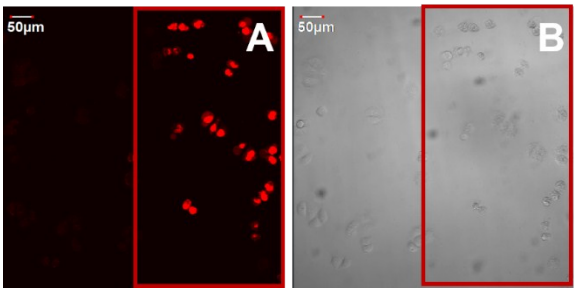

Figure S3 MCF-7 cell with PI staining and nanoparticles: area in the red frame was treated with two-photon laser.