Supporting Information

Aggregation-induced emission nanoparticles as photosensitizer for two-photon photodynamic therapy

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Calculation of TPE-red molecules in each TPE-red-PSMA nanoparticle

0.2 mg of TPE-red and 0.1 mg of PSMA were used when preparing the TPE-red-PSMA nanoparticles with the weight ratio of TPE-red to PSMA of 2:1. Assuming there was no chemicals loss during the preparation, the total concentration of TPE-red-PSMA nanoparticles in 1 mL water dispersion should be 0.3 mg/mL. As the TPE-red-PSMA nanoparticles dispersed uniformly and stably in the water, without floating and sinking, the density of them could be equivalent to the density of the water (1 g/cm^3). The total volume of the TPE-red-PSMA nanoparticles in water dispersion (1 mL) was therefore 0.3×10^{-3} cm^3. According to the DLS result (Fig. 2a, page 2), the average diameter of TPE-red-PSMA nanoparticle was 64.1 nm, so the average volume of the nanoparticles was 1.38×10^5 nm^3. Thus, the number of the TPE-red-PSMA nanoparticles in 1 mL water was 0.3×10^{-3} cm^3/1.38×10^5 nm^3=2.17×10^{12}, or 0.36×10^{-11} mol (Avogadro constant = 6.022×10^{23} mol^{-1}). In addition, 0.2 mg TPE-red (molecular weight = 520) contained 3.846×10^{-7} mol molecules. Finally, the number of TPE-red molecules in each TPE-red-PSMA nanoparticle can be calculated as 3.846×10^{-7} mol/0.36×10^{-11} mol =1.068×10^5.

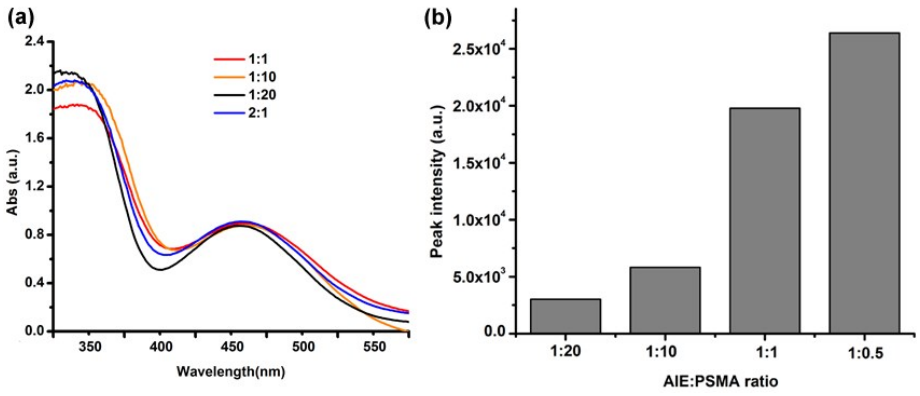

Fig. S1 (a) Absorbance spectra of TPE-red-PSMA nanoparticles with various weight ratios. (b) The peak fluorescence intensities of TPE-red-PSMA nanoparticles in different weight ratios.

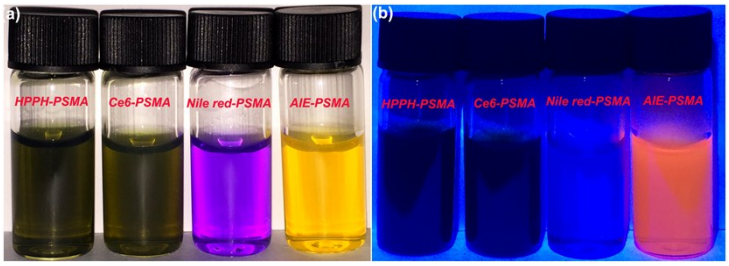

Fig. S2 (a) Photographs of the aqueous dispersion of HPPH-PSMA, Ce6-PSMA, Nile-red-PSMA and TPE-red-PSMA nanoparticles with the same concentration of encapsulated dyes/photosensitizers and the same weight ratio of dyes/photosensitizers to PSMA, under the irradiation of daylight (a) and ultraviolet lamp (b).

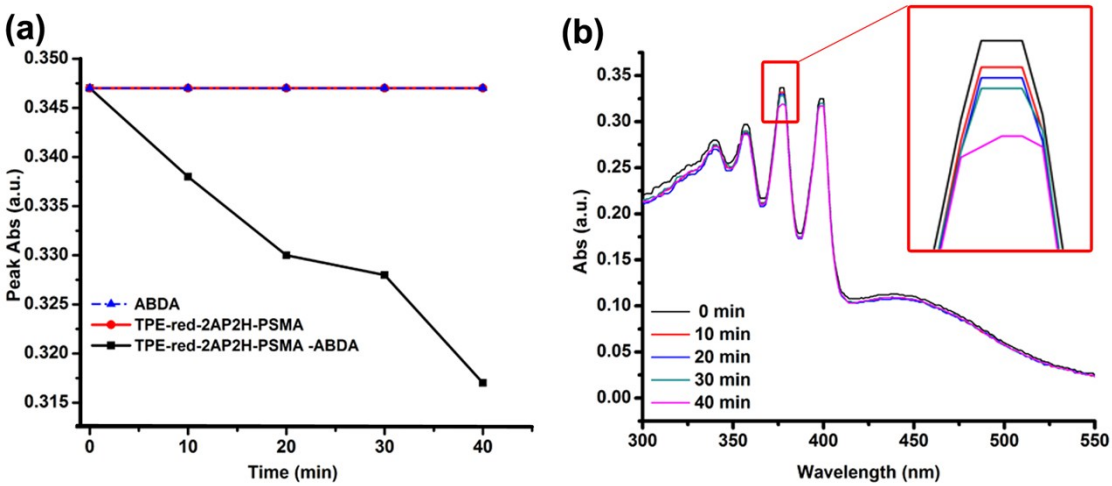

Fig. S3 (a) Decay curves of the absorbance (at 375 nm) upon 1040 nm-fs irradiation for 40 min in the case of ABDA (in blue), TPE-red-PSMA nanoparticles (in red) and ABDA mixed with TPE-red-PSMA nanoparticles (in black). (b) Absorption spectra of the mixture containing ABDA and TPE-red-PSMA nanoparticles irradiated by 1040 nm-fs for different time. Insert: The enlarged changes of the absorbance at 375 nm.

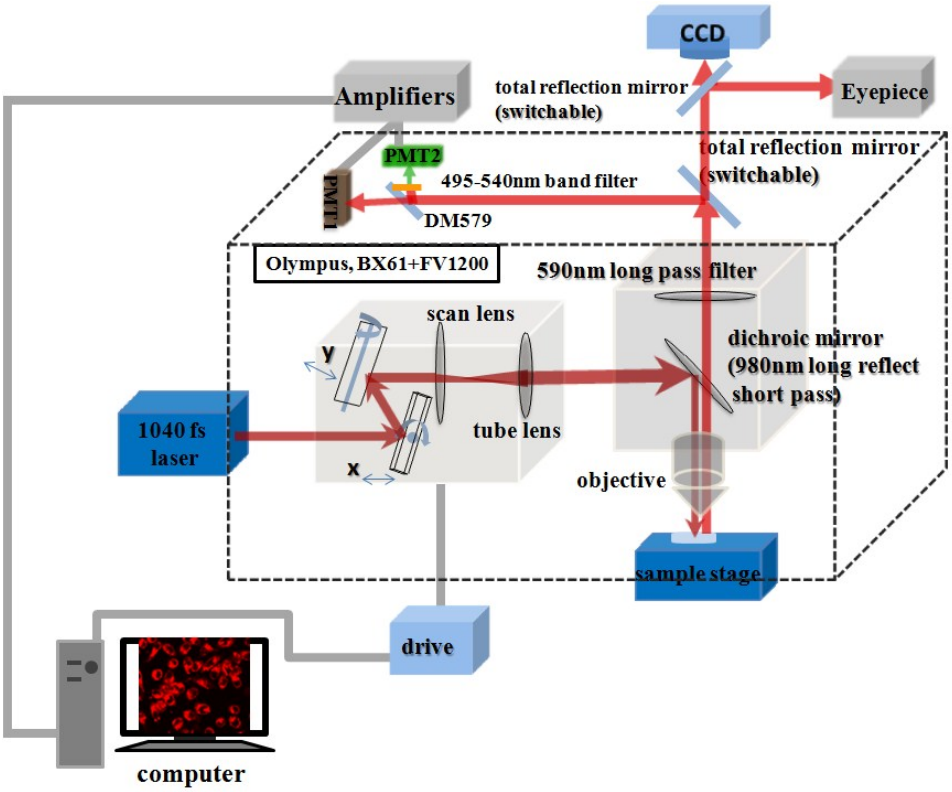

Fig. S4 Schematic illustration of the setup for two-photon fluorescence imaging and *in vitro* two–photon excited PDT.

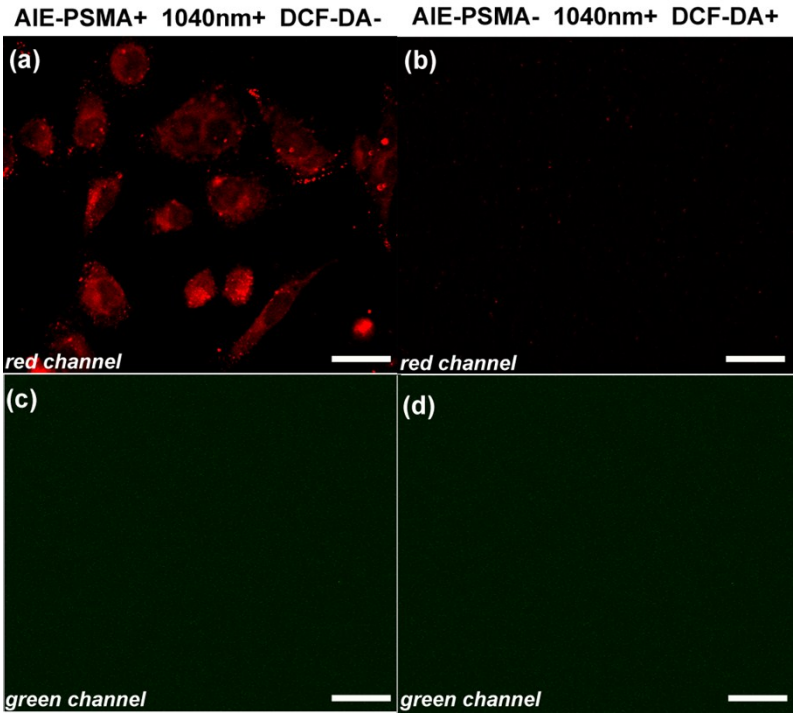

Fig. S5 HeLa cells treated with TPE-red-PSMA nanoparticles (but without DCF-DA) under1040 nm-fs excitation (60 mW) in the (a) red channel and (c) green channel. HeLa cells treated without TPE-red-PSMA nanoparticles (but with DCF-DA) under 1040 nm-fs excitation (60 mW) in the (b) red channel and (d) green channel. Scale bar is 50 μm.

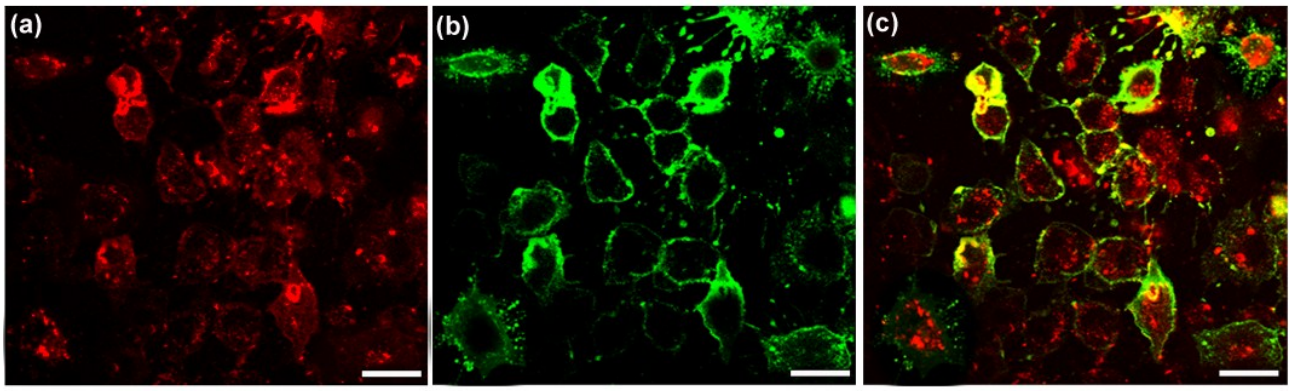

Fig. S6 Two-photon excited apoptosis rate measurement of HeLa cells. Cells were treated with both TPE-red-PSMA nanoparticles and Annexin V-FITC, followed by 1040 nm-fs excitation (60 mW) for 120 s. (a) Two-photon fluorescence from TPE-red-PSMA nanoparticles in the red channel. (b) Two-photon fluorescence from Annexin V-FITC in the green channel. (c) Overlap of the red and green channels. Scale bar is 50 μm.