

Supplementary information

# **Targeted and Image-guided Photodynamic Cancer Therapy Based on Organic Nanoparticles with Aggregation-induced Emission Characteristics**

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## Experimental Section

*Materials.* 1,2-Distearoyl-*sn*-glycero-3-phosphoethanolamine-*N*-[maleimide(polyethylene glycol)-2000] (DSPE-PEG<sub>2000</sub>-Mal) was purchased from Avanti Polar Lipids, Inc. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and other chemicals were all purchased from Sigma-Aldrich and used as received without further purification. 2-(2,6-Bis((*E*)-4-(phenyl(4'-(1,2,2-triphenylvinyl)-[1,1'-biphenyl]-4-yl)amino)styryl)-4*H*-pyran-4-ylidene) malononitrile (TTD) was synthesized according to our previous report.<sup>1</sup> Thiolated cyclic (Arg-Gly-Asp-D-Phe-Lys(mpa)) peptide (c(RGDfK)) was customized from GL Biochem Ltd (Shanghai).

Dulbecco's Modified Essential Medium (DMEM) is a commercial product of National University Medical Institutes (Singapore). Milli-Q water was supplied by Milli-Q Plus System (Millipore Corporation, Bedford, USA). 10× phosphate-buffer saline (PBS) buffer with pH = 7.4 (ultrapure grade) is a commercial product of 1st BASE (Singapore). Milli-Q water (18.2 MΩ) was used to prepare the buffer solutions from the 10× PBS stock buffer. 1× PBS contains NaCl (137 mM), KCl (2.7 mM), Na<sub>2</sub>HPO<sub>4</sub> (10 mM), and KH<sub>2</sub>PO<sub>4</sub> (1.8 mM). Fetal bovine serum (FBS) and trypsin-EDTA solution were purchased from Gibco (Life Technologies, AG, Switzerland). Annexin V-FITC apoptosis detection kit was purchased from Life Technologies.

*Instrumentation.* Particle size and size distribution were determined by laser light scattering (LLS) with a particle size analyzer (90 Plus, Brookhaven Instruments Co., USA) at a fixed angle of 90° at room temperature. TEM images were obtained from a JEOL JEM-2010 transmission electron microscope with an accelerating voltage of 200 kV. UV-vis absorption spectra were taken on a Milton Ray Spectronic 3000 array spectrophotometer. Photoluminescence (PL) spectra were

measured on a Perkin-Elmer LS 55 spectrofluorometer. All UV and PL spectra were collected at  $24 \pm 1$  °C.

*Preparation of TTD loaded NPs (TTD NPs).* A THF solution (1 mL) containing DSPE-PEG-Mal (1.0 mg) and TTD (0.5 mg) was poured into water (10 mL). This was followed by sonicating the mixture for 2 minutes at 12 W output using a microtip probe sonicator (XL2000, Misonix Incorporated, NY). The mixture was then stirred at room temperature overnight to evaporate the organic solvent. The NP suspension was further filtered with a 0.2  $\mu\text{m}$  syringe filter to obtain TTD NPs. The TTD encapsulated in the nanoparticles was calculated by measuring the absorbance at 500 nm and comparing with a standard curve. Encapsulation efficiency (%) was calculated as follows: encapsulation efficiency = [amount of TTD in the NPs]/[total amount of TTD used]  $\times$  100.

*Conjugation of cRGD to TTD NPs (T-TTD NPs).* Thiolated cRGD was conjugated to the surface of TTD NPs (denoted as T-TTD NPs) as the following procedure. The TTD NPs were suspended in HEPES buffer (0.2 mg mL<sup>-1</sup>) and incubated with excess thiolated cRGD at room temperature for 6 h. Then the cRGD functionalized nanoparticles were washed with Milli-Q water (3 mL  $\times$  3 times) by ultrafiltration (20,000 MWCO, Amicon, Millipore Corporation, Bedford, USA), resuspended in Milli-Q water and stored at 4 °C for further use.

*Detection of ROS in solution:* A ROS-sensitive probe, 1,3-diphenylisobenzofuran (DPBF), was used in our experiment to detect the generation of ROS from NPs under light irradiation.<sup>2</sup> DPBF solid was dissolved in solvent (50  $\mu\text{M}$ ) with T-TTD NPs (1  $\mu\text{g mL}^{-1}$ ) and was exposed to light irradiation for different time intervals at the power density of 0.25 W cm<sup>-2</sup>. The decomposition of DPBF was monitored by the absorbance at 418 nm.

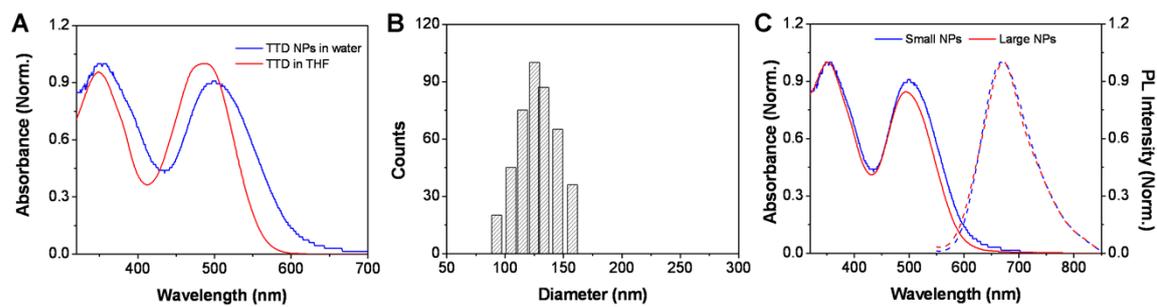
*Cell culture.* The breast cancer cell line MDA-MB-231, MCF-7 and normal cell line NIH 3T3 were provided by American Type Culture Collection (ATCC). The cells were cultured in DMEM (Invitrogen, Carlsbad, CA) containing 10% heat-inactivated FBS (Invitrogen), 100 U mL<sup>-1</sup> penicillin, and 100 µg mL<sup>-1</sup> streptomycin (Thermo Scientific) and maintained in a humidified incubator at 37 °C with 5% CO<sub>2</sub>. Before experiment, the cells were precultured until confluence was reached.

*Intracellular ROS detection.* ROS generation inside cells under light irradiation was detected using dichlorofluorescein diacetate (DCF-DA) cellular reactive oxygen species assay kit (Abcam). MDA-MB-231, MCF-7 and NIH 3T3 cells were cultured in the chambers (LAB-TEK, Chambered Coverglass System) at 37 °C. After 80% confluence, the culture medium was removed and washed twice with PBS buffer. Following incubation with T-TTD NPs (1 µg mL<sup>-1</sup>) for 1 h in the dark, DCF-DA was loaded into the cells. After 10 min incubation, cells were washed twice with PBS and then exposed to light irradiation for 10 s at the power density of 0.25 W cm<sup>-2</sup>. After irradiation, the medium was replaced with PBS and fluorescence images of treated cells were acquired using a laser confocal microscope.

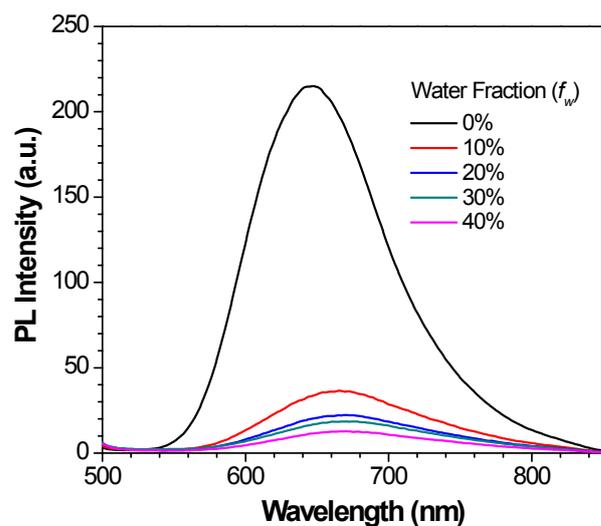
*Confocal imaging.* MDA-MB-231, MCF-7 and NIH 3T3 cells were cultured in the chambers at 37 °C. After 80% confluence, the culture medium was removed and washed twice with PBS buffer. Following incubation with T-TTD NPs (1 µg mL<sup>-1</sup>) for 1 h, the cell nucleus were living stained with Hoechst 33342 (Life Technologies), following the standard protocol of the manufacturer. The cellular apoptosis imaging measurement was carried out based on standard detection kit (Annexin V-FITC Apoptosis Detection Kit) according to manufacturer's protocol. The cells were then imaged immediately by confocal laser scanning microscope (CLSM, Zeiss

LSM 410, Jena, Germany). The excitation was 488 nm, and the emission was collected above 560 nm. The images were analyzed by Image J 1.43 × program (developed by NIH, <http://rsbweb.nih.gov/ij/>).

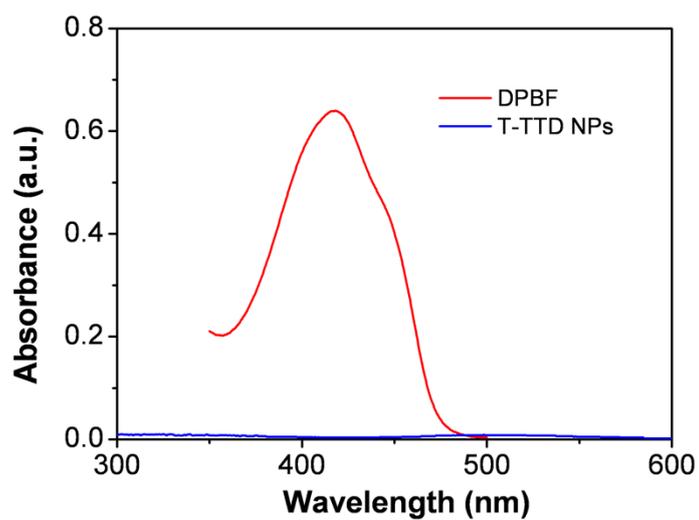
*Cytotoxicity studies:* 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assays were used to assess the metabolic activity of MDA-MB-231, MCF-7 and NIH 3T3 cells. The cells were seeded in 96-well plates (Costar, IL, USA) at an intensity of  $4 \times 10^4$  cells mL<sup>-1</sup>. After 24 h incubation, the medium was replaced with the T-TTD NPs at different concentrations and incubated at 37 °C. Following incubation with T-TTD NPs for 1 h, cells were washed twice with PBS and then exposed to light irradiation for 2 min at the power density of 0.25 W cm<sup>-2</sup>. The cells were further incubated for 24 h and then washed twice with 1× PBS buffer, and 100 μL of freshly prepared MTT (0.5 mg mL<sup>-1</sup>) solution in culture medium was added into each well. The MTT medium solution was carefully removed after 3 h incubation in the incubator at 37 °C. DMSO (100 μL) was then added into each well and the plate was gently shaken to dissolve all the precipitates formed. The absorbance of MTT at 570 nm was monitored by the microplate reader (Genios Tecan). Cell viability was expressed by the ratio of absorbance of the cells incubated with NPs to that of the cells incubated with culture medium only. The relative cell survival percentages compared to the NPs-free control were plotted against the drug concentration in logarithmic scale. The data reported represent an average of three measurements from different batches. The dose-effect profiles were obtained by sigmoidal logistic fitting by use of Origin 8.0 (OriginLab, Northampton, MA) and the half-maximal inhibitory concentration (IC<sub>50</sub>) values were determined on the basis of the fitted data.



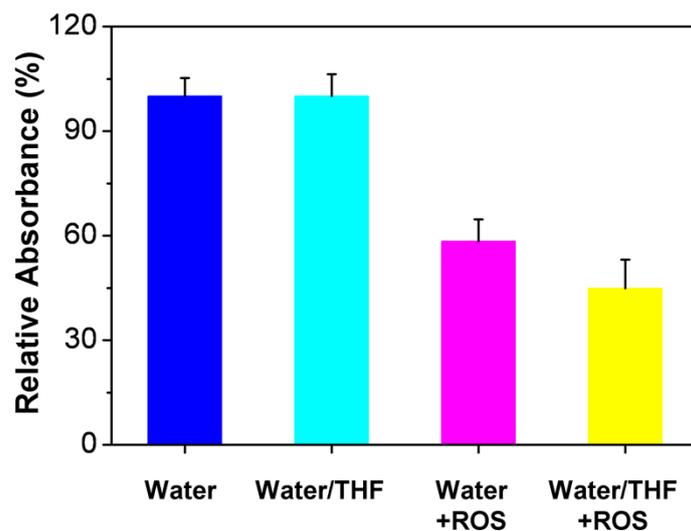
**Figure S1.** UV-vis absorption spectra of TTD NPs in water and TTD in THF. (B) Size distribution of TTD NPs prepared without sonication. (C) UV-vis absorption and emission ( $\lambda_{\text{ex}} = 502 \text{ nm}$ ) spectra of TTD NPs with different sizes (30 nm, small NPs vs 120 nm, large NPs).



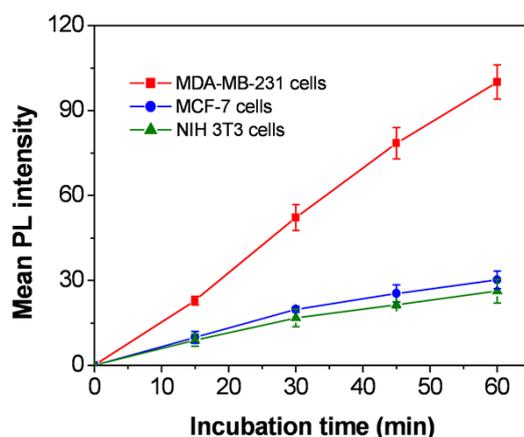
**Figure S2.** PL spectra of TTD in THF/water mixtures at the same total volume with different water fractions from 0 to 40%.



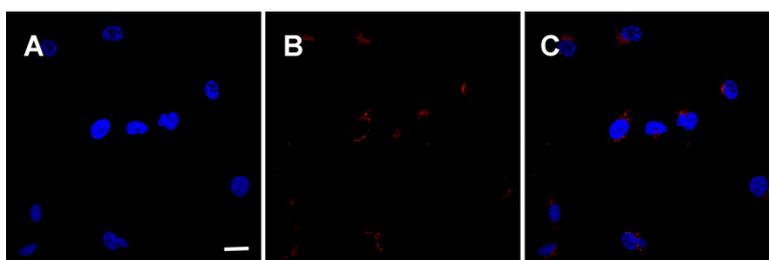
**Figure S3.** Absorption spectra of 50  $\mu\text{M}$  DPBF and 1  $\mu\text{g}/\text{mL}^{-1}$  of T-TTD NPs in water. Both have the same concentrations as those used for monitoring of ROS generation.



**Figure S4.** Plots of change in absorbance of DPBF at 418 nm with T-TTD NPs in water or in the mixed solution (THF/water = 60/40, v/v) with and without ROS produced by reacting H<sub>2</sub>O<sub>2</sub> with Fe<sup>2+</sup>.

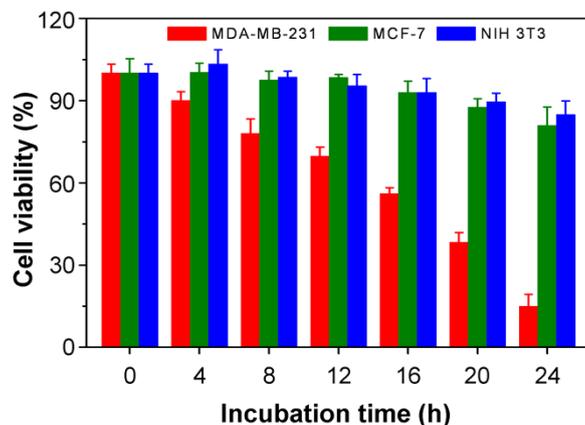


**Figure S5.** Evaluation of the targeting effect of T-TTD NPs to different cancer cells. Integrated PL intensities of cells (MDA-MB-231, MCF-7 and NIH 3T3 cells) upon incubation with T-TTD NPs (1  $\mu\text{g mL}^{-1}$ ) for different time. The error is the standard deviation from the mean ( $n = 3$ ).

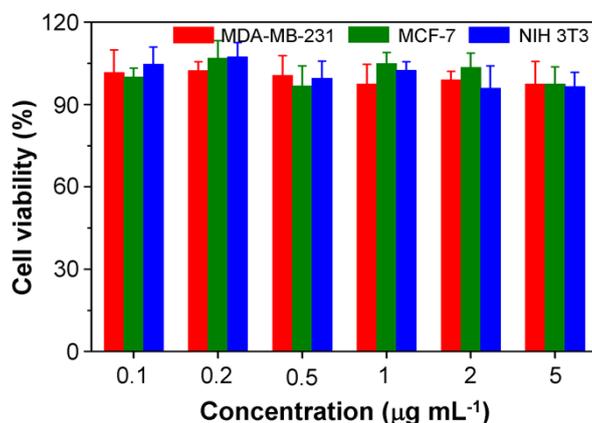


**Figure S6.** CLSM images of free cRGD (50  $\mu\text{M}$ ) pretreated MDA-MB-231 cells upon

incubation with T-TTD NPs ( $1 \mu\text{g mL}^{-1}$ ) for 1 h. The blue fluorescence from the nuclei of cells is dyed by Hoechst 33342 (A), the red fluorescence is from T-TTD NPs (B), and the overlay image is shown in (C). All images share the same scale bar ( $20 \mu\text{m}$ ).



**Figure S7.** Relative viabilities of MDA-MB-231, MCF-7 and NIH 3T3 cells incubated with T-TTD NPs ( $2 \mu\text{g mL}^{-1}$ ) after light irradiation ( $0.25 \text{ W cm}^{-2}$ , 2 min), followed by further incubation for different time.



**Figure S8.** Relative viabilities of MDA-MB-231, MCF-7 and NIH 3T3 cells after being incubated with various concentrations of T-TTD NPs in dark for 24 h. Data represent mean values  $\pm$  standard deviation,  $n = 3$ .

1. W. Qin, D. Ding, J. Liu, W. Z. Yuan, Y. Hu, B. Liu, B. Z. Tang, *Adv. Funct. Mater.* 2012, **22**, 771-779.
2. X. F. Zhang, X. Li, *J. Lumin.* 2011, **131**, 2263-2266.