Supporting information for

Study on the Transient Absorption Spectroscopy of a D-π-A Structure Aggregation-Induced Emission Luminogen and Its Photodynamic Therapy Application

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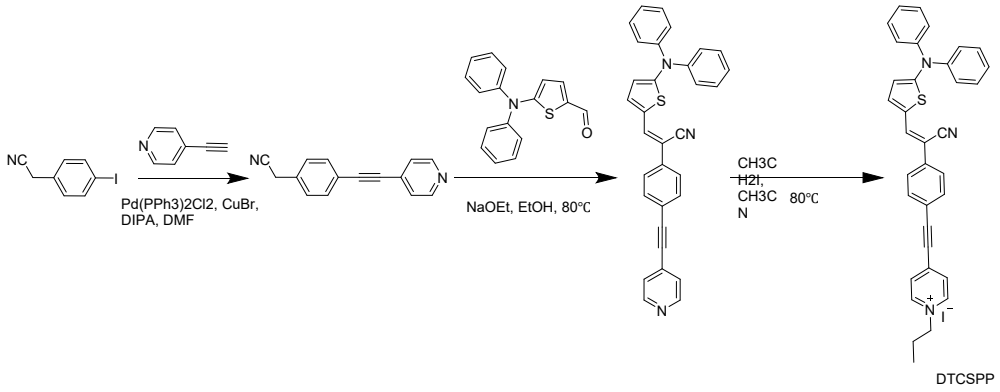
Materials and methods

Material

DTCSPP was synthesized as the method our group previously reported (as shown in Scheme S1). The solvents were purchased from Macklin. The 2', 7'-dichlorodihydrofluorescein diacetate (DCF-DA) and MitoTracker Deep Red FM were purchased from Invitrogen. Cell culture medium, phosphate-buffered saline (PBS), fetal bovine serum (FBS), and penicillin-streptomycin solution (PS) were purchased from Gibco. Anti-LC3B antibody, anti-GPX4 antibody, anti-P62 antibody, and anti-β-actin antibody were purchased from Abcam. All materials and reagents were used as received.



Scheme S1. Synthetic route of DTCSPP

Measurement

The UV-Vis absorption and photoluminescence spectra were recorded on a UV-1900I spectrophotometer (Shimadzu, Japan) and FLS1000 spectrofluorimeter (Edinburgh Instruments, U.K.) at room temperature. The particle size was measured by ZEN3700. (Zetasizer, UK). The electron paramagnetic resonance (EPR) measurements were carried out with a Bruker Model A300 spectrometer (Bruker, America) with 1 G field modulation, 20 mW and 100 G-scan range. The TEMP and DMPO (100 mM) were used as spin-trapping adducts to detect the $^1O_2$ and $\cdot O_2^-/\cdot OH$ produced by DTCSPP (0.1 mg/ml) under dark and irradiation, respectively. The TEMP/$^1O_2$ and DMPO/$\cdot O_2^-$ DMPO/$\cdot$ O.H. signals were detected in water and dimethyl sulfoxide, respectively. The femtosecond transient absorption measurements were performed on

a Helios (Ultrafast systems) spectrometer using a regeneratively amplified femtosecond Ti: sapphire laser system (Spitfire Pro-F1KXP, Spectra-Physics; frequency, 1 kHz; max pulse energy, 8 mJ; pulse width, 120 fs) at room temperature. Finally, analyze the data through commercial software (Surface Xplorer, Ultrafast Systems).

Theoretical calculation

DTCSPP was fully optimized by the density functional theory (DFT) method by using CAM-B3LYP density functional and 6-31G (d,p) basis set with Gaussian 16 program. Time-dependent density functional theory (TD-DFT) was carried out at the same level of theory to calculate energy levels of singlet-triplet states and their gap (ΔE_{st}).

Cell culture

MDA-MB-231 cells are human adherent breast cancer cells, which were obtained by the Chinese Academy of Sciences Cell Banks (Shanghai, China) and were cultured in Leibovitz's L-15 Medium with 10% fetal bovine serum (FBS) in the humidified atmosphere at 37°C without CO_2. The cellular uptakes of DTCSPP were observed in MDA-MB-231 cells by CLSM. About 1×10^5 MDA-MB-231 cells were seeded in a confocal dish and cultured in Leibovitz's L-15 Medium for 24 h. After fully attached, the cells were incubated for 1 h with 5 μM DTCSPP and then imaged by a confocal laser scanning microscope.

***In vitro* cell viability**

Cell viability was measured using human breast cancer cell line MDA-MB-231 by CCK-8 assay. Each group of cancer cells was seeded at cell density of 1×10^4 per well in a 96-well cell culture plate with Leibovitz's L-15 Medium (200 μL). After 24 h, the culture medium need to be replaced by the medium with a final concentration of DTCSPP at 0 to 10 μM. After a 48-hour incubation, the cells were washed with PBS, and then CCK8 (10 μL in 90 μL medium) was addedl. Then, cells were incubated for 2 h and can be measured by the microplate reader at a wavelength of 450 nm.

Intracellular ROS level

In intracellular ROS generation was evaluated by employing DCFH-DA as the fluorescent indicator. MDA-MB-231 cells were planted onto 35-mm confocal dishes at

a density of 1×10^5 cells. After incubation for 24 h, all the medium can be removed and washed with PBS three times. Then, MDA-MB-231 cells were incubated with 10 μM DTCSPP for 1 h and then washed with PBS three times. Then, the cells were incubated with the fluorescent probe DCFH-DA (20 μM) for 30 min at the temperature of 37 °C in the dark and washed three times with PBS. The cells were subsequently treated with a white LED irradiation (50 mW/cm^2) for 2 h and were successively examined by the CLSM (excited at 488 nm).

Western blots analysis

Briefly, MDA-MB-231 cells were collected and lysed using lysis buffer at 6 h post different treatments. Then, the extracted proteins were separated through gel electrophoresis (according to their sizes) using 10% sodium dodecyl sulfatepolyacrylamide gel electrophoresis (SDS-PAGE). Next, these proteins were transferred from the gel to PVDF membranes (Millipore, Billerica, MA, USA). After that, the membrane containing protein bands was incubated with a blocking buffer containing 5% BSA for 0.5 h. After that, membrane was incubated with primary antibody for 2 h, then incubated with HRP-conjugated secondary antibody for 1 h post three-times washing by 1×PBST. Finally, the related protein bands were visualized using Tanon-5200 Chemiluminescent Imaging System (Tanon Science and Technology) after washing.

***In vivo* anticancer studies**

The BALB/c nude mice (female, 4 weeks) were obtained from Guangdong Medical Laboratory Animal Center (Guangdong, China) and were raised to 6 weeks old to construct xenografts. All animal experiments were conducted strictly according to the guidelines and approval of the Institutional Animal Care and Use Committee of Shenzhen International Graduate School, Tsinghua University, and the Medical Laboratory Animal Center of Guangdong. China (License No.35 (year 2022). Mice were subcutaneously injected with MDA-MB-231 (1×10^7 cells) and were randomly divided into four groups with 3 mice in each group. Group I: PBS injection without irradiation; Group II: PBS injection with irradiation; Group III: DTCSPP injection without irradiation; Group V: DTCSPP injection with irradiation. After intratumoral

injection, a white LED light (150 mW/cm^2) treatment was performed on Group II and Group V by irradiating the tumor region for 20 min every other day. The effect of the different treatment groups was monitored by measuring tumor size and mice body weight for 15 days after PDT treatment. The tumor volume is calculated using the formula as follows:

$$V(mm^3) = \frac{\pi}{6} \times length(mm) \times width(mm) \times height(mm)$$

After 15 days, the tumors of the mice were dissected and weighed.

Statistical Analysis

Mean ± standard deviation (S.D.) was used for quantitative data analysis. Statistical comparisons were analyzed by one-way ANOVA with a Tukey post-hoc test and unpaired Student's t-test (two-tailed). *P < 0.05 means a statistically significant difference. **P < 0.01 and ***P < 0.001 mean highly significant.

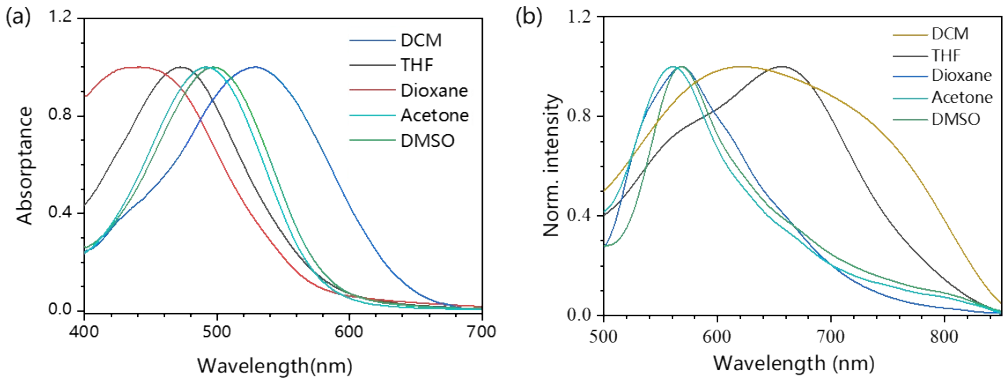


Figure S1. Normalized (a) absorption and (b) fluorescence spectra of the DTCSPP in different solvents (Concentration = 10^{-5} M). Tetrahydrofuran (THF), Dichloromethane (DCM), Dimethyl sulfoxide (DMSO).

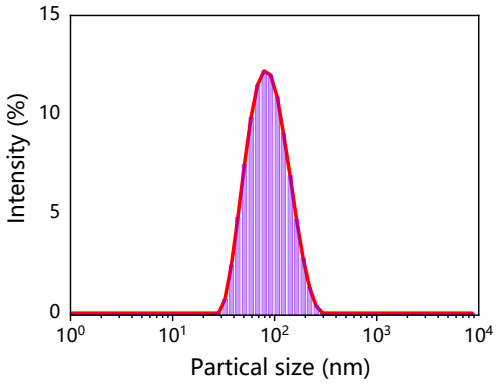


Figure S2. The DLS profile of DTCSPP in the mixture of DMSO: water =1:9 (by vol %). Concentration = 10^{-5} M.

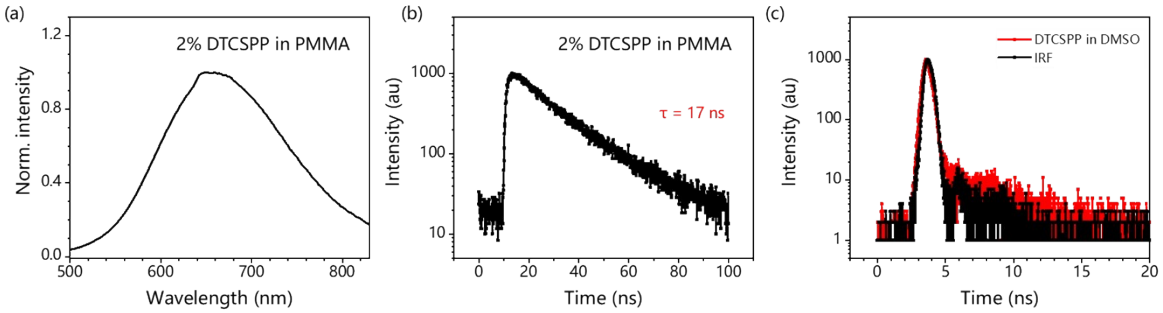


Figure S3. The (a) PL and (b) time-resolved photoluminescence of 2% DTCSPP in PMMA (by mass fraction) film. λ_{ex} = 485 nm. (c) the time-resolved photoluminescence of DTCSPP in DMSO (concentration = 10^{-5} M) and instrument response function (IRF).

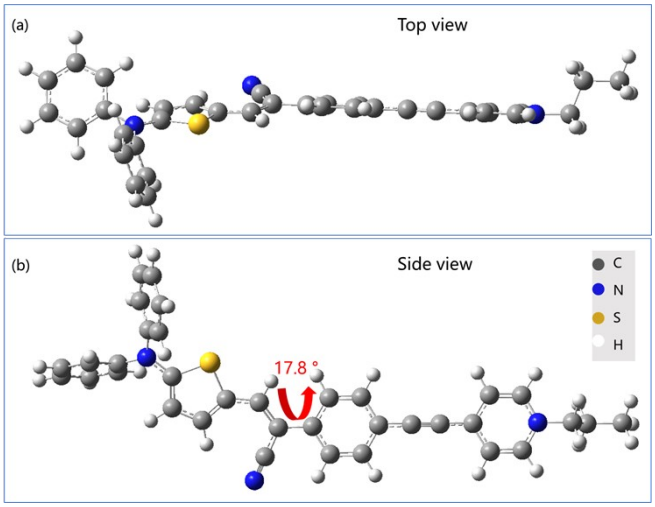


Figure S4. The DFT optimized geometries of DTCSPP at CAM-B3LYP density functional and 6-31G(d,p) basis set.

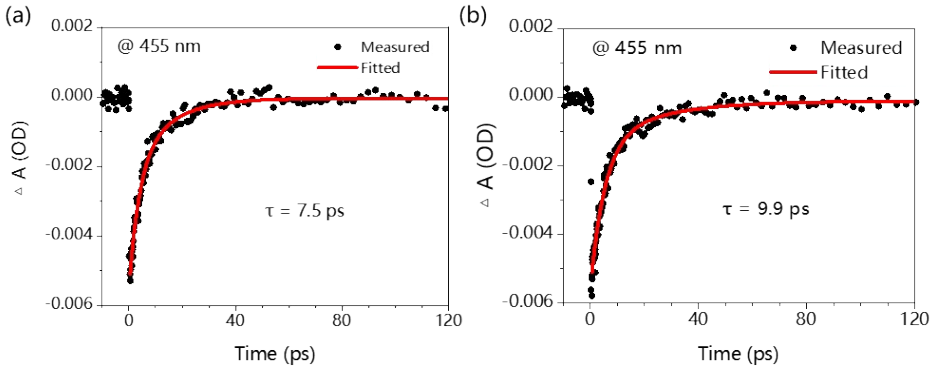


Figure S5. The kinetic fitting of the peak at 455 nm (a) in DMSO solution and (b) in the mixture of DMSO:water = 1:9

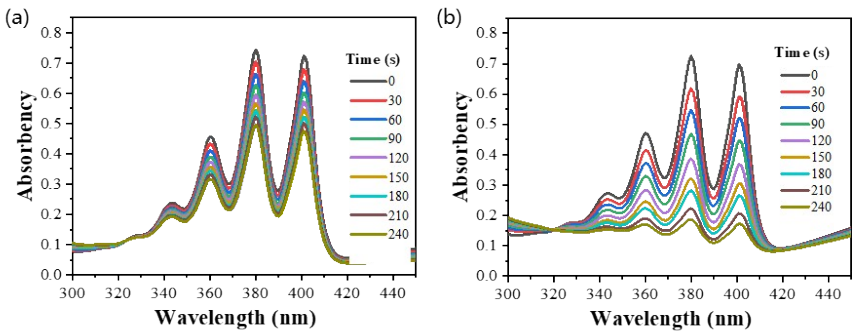


Figure S6. Chemical trapping of the $^1O_2$ generation. Photodegradation of ABDA with (a) Rose Bengal (RB), and (b) DTCSPP. The measurements were carried out under white light irradiation in PBS buffer.

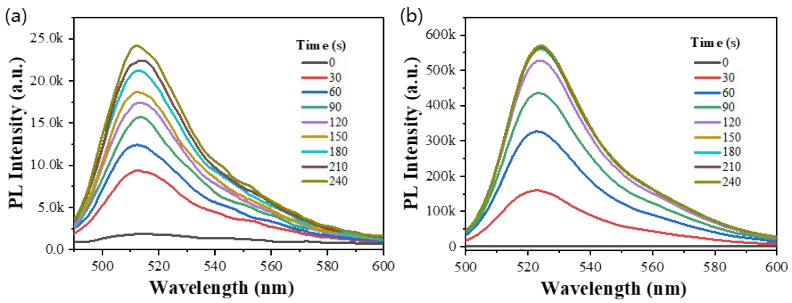


Figure S7. (a) Chemical trapping of the ·OH generation by photoactivation of HPF and (b) Chemical trapping of the ROS generation. Photoactivation of DCFH with DTCSPP.

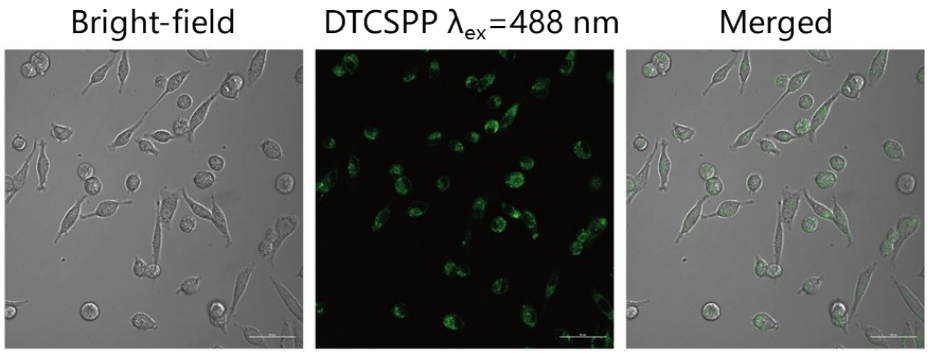


Figure S8. Bright-field, fluorescence, and their merged images for living MDDA-MB-231 cells with 10 μM DTCSPP in DMSO Scale bar: 50μm.

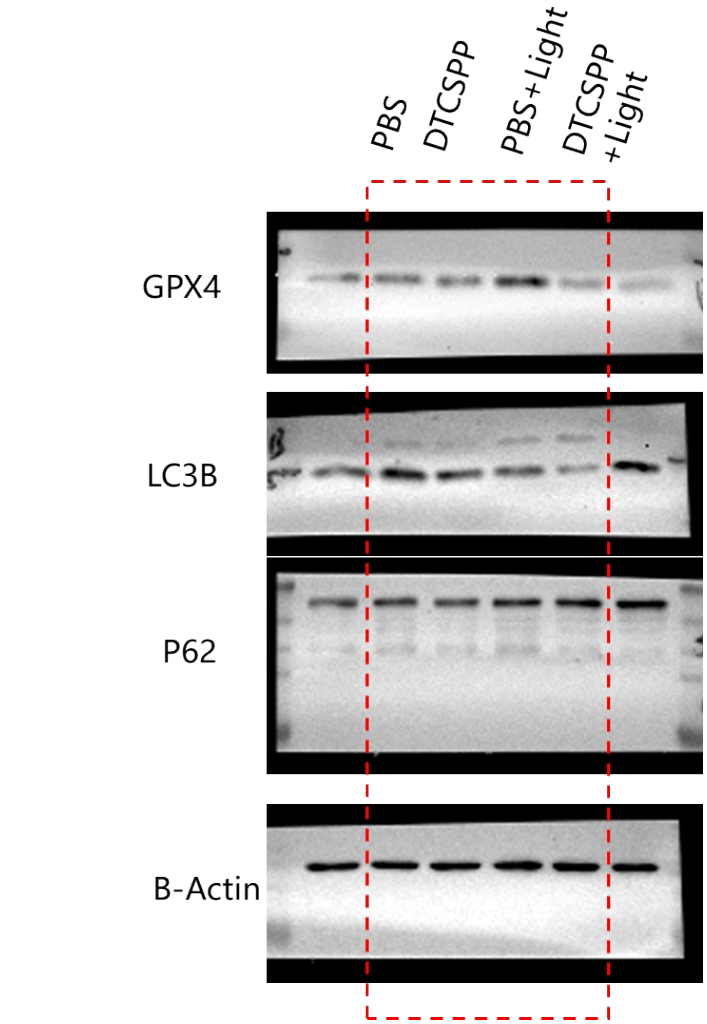


Figure S9. The uncropped original gels/blots of Figure 5h.

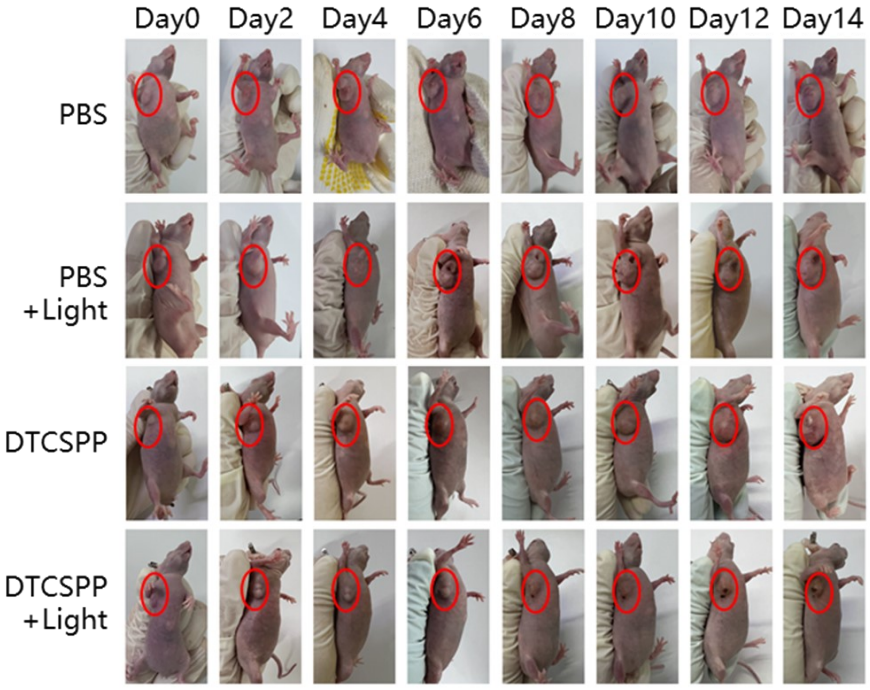


Figure S10. Photo images of MDA-MB-231 tumor-bearing mice during treatment.

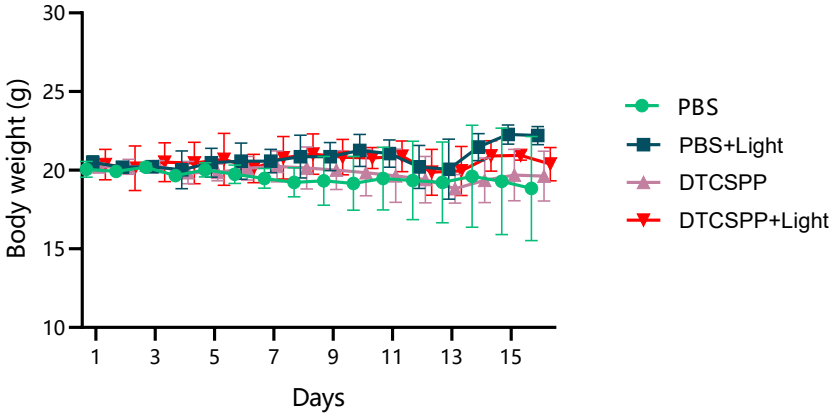


Figure S11. The body weight changes with the treatment.