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Electronic Supporting Information for

Boosting photodynamic therapy of near-infrared AIE-active photosensitizers by precise manipulation of molecular structure and aggregate-state packing

Guanyu Ding,[a,b] Jialin Tong,[b] Yingchen Duan,[c] Shuang Wang,[b] Zhongmin Su,[*c] Kuizhan Shao,[b] Lingyu Zhang,[b] Daoming Zhu,[d] Li-Li Wen,[*c] Yuanyuan Li[*e] and Guo-Gang Shan[*b]

[a]*School of Materials Science and Engineering, Changchun University of Science and Technology, Changchun, Jilin, 130022, PR China*

[b]*Institute of Functional Material Chemistry and National & Local United Engineering Lab for Power Battery, Faculty of Chemistry, Northeast Normal University, Changchun, 130024, PR China*

E-mail: shangg187@nenu.edu.cn (G. Shan)

[c]*School of Chemistry and Environmental Engineering, Changchun University of Science and Technology, Changchun, 130022, PR China*

E-mail: wll@cust.edu.cn (L. Wen), zmsu@nenu.edu.cn (Z. Su)

[d]*Department of Radiation and Medical Oncology, Hubei Key Laboratory of Tumor Biological Behaviors, Hubei Cancer Clinical Study Center, Zhongnan Hospital of Wuhan University Wuhan 430071, PR China*

[e]*College of Veterinary Medicine, Jilin University, Changchun 130062, PR China*

E-mail: li_yuanyuan@jlu.edu.cn (Y.Y. Li)

Experimental Section

Materials and Methods

Unless stated otherwise, all analytical grade chemicals used in this paper were purchased from Energy Chemical, Macklin, or Sigma as received without further. Superdry dichloromethane (DCM) and methanol (MeOH) with molecular sieves were purchased from Macklin. DCFH, DHR123, ABDA, and Rose Bengal were purchased from Bidepharm and used as received. Cell culture medium (DMEM/HIHG GLUCOSE) and fetal bovine serum (FBS) were purchased from Cytiva and Every Green. Mitotracker Green and Cell Counting Kit-8 were purchased from Beyotime.

^{1}H NMR (500 MHz), and ^{13}C NMR (500 MHz) spectra were recorded on a 500 MHz Bruker Avance spectrometer with Chloroform-d or Dimethyl sulfoxide-d_6 as the solution. High resolution mass spectra (HRMS-ESI) were measured on the matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectrometer. The photophysical characteristics of the **TCP-PF$_6$** and **TTCP-PF$_6$** in DMSO were determined with FL-4600 fluorescent spectrophotometer and Cary 500 UV-vis-NIR spectrophotometer for photoluminescence (PL) spectra (5.0×10^{-5} M) and absorption spectra (1.0×10^{-5} M), respectively. The photoluminescence quantum yields (PLQYs) of their solid and solution were determined in an integrating sphere. Excited-state lifetimes of these solid and solution were determined with the Edinburgh FLSP920 spectrofluorimeter. Cellular fluorescence images were taken using a Zeiss laser scanning confocal microscope and analyzed using ZEN 2.6 software. Cytotoxicity Assay were received by Infinite 200 Pro (Tecan). White light source for ROS generation experiment comes from a commercial LED light with 23.4 mW/cm^2 neutral white light. The model number is NVC-EXTT9029.

Theoretical calculations

All calculations were performed using the Gaussian 09 program package. The ground state (S_0) geometries of **TCP-PF$_6$** and **TTCP-PF$_6$** were optimized at the theoretical level of B3LYP/6-31g(d), energies of the first singlet and triplet excited states were calculated based on the optimized S_0 in DMSO environment at TD-B3LYP/6-31g(d).

Molecular Dynamics Simulations

All the molecular dynamics (MD) simulations were carried out by the GROMACS (version 2018.4) package. The initial confirmation was generated by randomly placing 30 **TCP-PF_6** and **TTCP-PF_6** molecules in a cubic simulation box with a length 4 nm, respectively. Here, the total number of water molecules was 1701. For each system in aqueous solution, energy minimization by conjugate gradient algorithm was first performed. Then we run 200 ps molecular dynamics annealing simulations to relax the system. The temperature increased from 0 to 298.15 in 100 ps and then systems underwent 100 ps NVT process at that temperature. The temperature was controlled by the velocity rescaling thermostat. After that, 2 ns NPT (T = 298.15 K and P = 1 atm) simulation was carried out to further equilibrate the system. Here the temperature and pressure were controlled by the velocity rescaling thermostat and parrinello-rahman barostat. The time constants of couplings for temperature and pressure were 0.1 ps and 1.0 ps, respectively. For the electrostatic interactions, the reciprocal space summation was evaluated by the particle mesh Ewald (PME) method. The direct space summation was computed at a cutoff distance of 1.0 nm. The cutoff distance of Van de Waals interactions was 1.0 nm. All bond lengths were constrained via the hbonds algorithm. Periodic boundary condition was applied in all three directions to minimize the edge effects in a finite system. Finally, in both system 1 and system 2, independent 10 ns production molecular dynamics simulations were conducted to obtain enough configurations for data collection. Here, the time step is 2 fs.

DLS and TEM

The concentrated DMSO solution of **TCP-PF_6** and **TTCP-PF_6** (500 μM) was diluted with water 100 times. Then the size and morphology of the **TCP-PF_6** and **TTCP-PF_6** in DMSO/water were determined with the dynamic light scattering and transmission electron microscope.

ROS generation measurement

A conventional ROS indicator DCFH-DA was employed to detect the ROS generation of **TCP-PF$_6$** and **TTCP-PF$_6$** in solution. The indicator was first activated through the general method. 2 mL NaOH (10 mM) and 0.5 mL DCFH-DA in ethanol (1 mM) were mixed and allowed to stir under dark at room temperature for 30 min. Then the solution was added to 10 mL of PBS and stored in dark and cool until use. The fluorescence of each sample (2 μM) was firstly set as blank. Then, 40 μM of DCFH was mixed with each sample (DMSO/PBS (v:v) = 1/99) in a dark room, and the fluorescence of the sample was measured at once. The sample mixture was then irradiated under white light (23.4 mW/cm^2) at intervals of 5 s until 120 s. DCFH aqueous solution without AIEgens was subjected to irradiation. The fluorescence of DCF was excited at 480 nm and collected within 500−650 nm.

Type I (free radical-dominated) ROS measurement

Dihydrorhodamine 123 (DHR123) was employed to evaluate type I (free radical-dominated) ROS of **TCP-PF$_6$** and **TTCP-PF$_6$** upon light irradiation. The fluorescence of the sample (2 μM) was firstly set as blank. Then, 3 μM of DHR123 was mixed with the sample (DMSO/water (v:v) = 1/99) in a dark room, and the fluorescence of the sample was measured at once. The sample mixture was then irradiated under white light (23.4 mW/cm^2) at intervals of 1 min until 10 min. DHR123 aqueous solution without AIEgens was subjected to irradiation. The fluorescence of DHR123 was excited at 480 nm and collected within 500−650 nm.

Singlet oxygen generation measurement

ABDA (9,10-Anthracenediyl-bis(methylene)dimalonic acid) was employed to evaluate $^1O_2$ generation of **TCP-PF$_6$** and **TTCP-PF$_6$** light irradiation. The absorbance of each sample (1 μM) was firstly set as blank. Then, 100 μM of ABDA was mixed with each sample (DMSO/water (v:v) = 1/99) in a dark room, and the absorbance of the sample was measured at once. The sample mixture was then irradiated under white light (23.4 mW/cm^2) at intervals of 1 min until 10 min. The absorption of ABDA at 378 nm was recorded at various irradiation times to obtain the decay rate of

the photosensitizing process. The absorbance change of ABDA alone in 10 min light irradiation time was also measured as a control.

Cell culture

HeLa cells and A549 cells were cultured in Dulbecco's minimal essential medium (DMEM) containing 10% heat-inactivated FBS at 37 ℃ in a humidity incubator with 5% CO_2.

Intracellular ROS detection

DCFH-DA was employed to detect the ROS generation inside cells under light irradiation. Hela cells were cultured in the plates at 37 ℃ with DMEM without FBS. Following incubation with **TCP-PF_6** (2 μM) and **TTCP-PF_6** (2 μM) for 30 min in the darkroom, DCFH-DA (30 μM) was added to the cells. After 20 min incubation, cells were washed thrice with a stroke-physiological saline solution. The fluorescence images of DCFH-DA were acquired using CLMS. The 488 nm laser of CLMS was utilized as an illuminant for ROS excited and the emission was collected at 500-530 nm.

Hela Cell imaging

Hela cells were seeded and cultured at 37℃ in a 35 mm glass-bottomed dish. After incubation with **TCP-PF_6**, **TTCP-PF_6**, and Mito - Tracker Green (100 nm) for 30 min, the cells were washed with Stroke-physiological saline solution three times and subjected to imaging analysis using a Zeiss Laser Scanning Confocal Microscope. Two gens can be excited with 561 nm filter and the emission filter was 600 - 750 nm. And the Mito-Tracker Green was excited with 488 nm and the emission filter was 490-590 nm. Afterward, the cells were washed with Stroke-physiological saline solution three times and then observed with CLSM. The co-localization efficiency was analyzed with ZEN 2.6 software, The **TTCP-PF_6** was calculated 0.97 of Pearson's coefficient.

Cytotoxicity Assay

Cell Counting Kit-8 (CCK-8) assay was utilized to quantitatively measure the cytotoxicity of **TCP-PF_6** and **TTCP-PF_6** based on Hela cell line and A549 cell line.

All cancer cell lines were seeded in DMEM and 10% FBS media in a 96-well round-bottom microplate with a density of 1×10^4 cells per well. The gens (**TCP-PF_6** and **TTCP-PF_6**) were added at different concentrations of 0, 0.2, 0.5, 1, 2, and 5 μM after replacing the medium, and was incubated with different cell lines respectively for 30 min, followed by irradiation with white light for 10 min (30 mW/cm^2), and another array of plates with cells were kept in the dark as the control. After 24 h incubation, 10 μL CCK-8 was added to each well. 1 hour later, the absorption of each well at 450 nm was recorded via a plate reader. Each trial was performed with 4 wells in parallel.

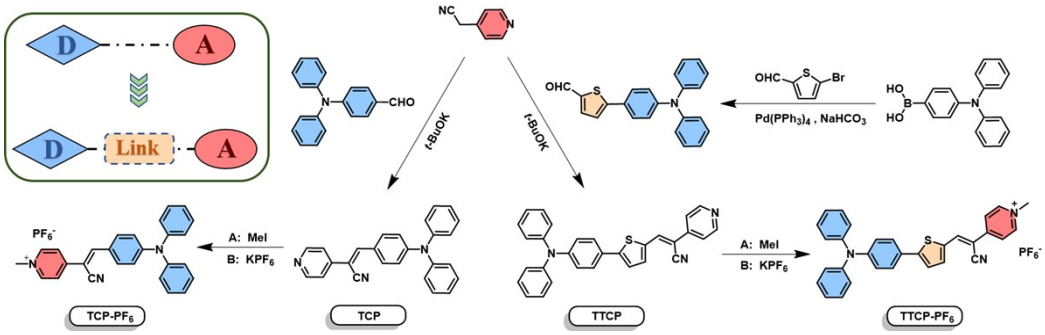
Mouse Modal

Female Balb/c nude mice aged 4-5 weeks are purchased from Vital River Company (Beijing, China). 100 μL of MCF-7 cell suspension (5×10^6 cells) were subcutaneous injected into each mouse to establish the tumor models. The animal experiments were carried out according to the protocol approved by the Ministry of Health in People's Republic of PR China and were approved by the Administrative Committee on Animal Research of the second clinical Medicine College of Wuhan University.

In vivo PDT assay

The tumor-bearing mice were randomly divided into three groups with the same number (5 mice) after tumor inoculation. A stock solution of **TCP-PF_6** and **TTCP-PF_6** was first prepared at the concentration of 10 mM in DMSO. The stock solution was further diluted with PBS to 1 mM before injection. Thereafter, the mice were treated by intratumor injection of 100 μL PBS (control group), **TCP-PF_6** and **TTCP-PF_6**. After that, each array of the tumor-bearing mice with the half number was kept in the dark. Four hours later, another number of three groups of mice were irradiated with wight light (0.1 W/cm^2) for 20 min. The irradiation was conducted every two days. Tumor volumes and weights of mice were recorded every two days. The tumor volume was calculated as: (tumor length × (tumor width)^2)/2. The relative tumor growth ratio was reflected by the relative volume V/V_0 (V_0 as the initial tumor volume before treatment). All mice were euthanized on the 14th day after the first injection. The tumors were then dissected and weighed for analysis.

Synthesis and characterization



Scheme S1. Synthesis route of the **TCP-PF$_6$** and **TTCP-PF$_6$**.

Synthesis of compound 5-(4-(diphenylamino)phenyl)thiophene-2-carbaldehyde

A mixture of (4-(diphenylamino)phenyl)boronic acid (722 mg, 2.5 mmol) and 5-bromothiophene-2-carbaldehyde (363 mg, 1.9 mmol) were dissolved in mixed solvent (DME : Ethanol : H_2O = 30 : 2 : 2 mL). Then $Pd(PPh_3)_4$ (121 mg, 0.1 mmol) was added and the reaction was heated to 80 ºC for 12 h under nitrogen. After cooling to room temperature, the mixture was extracted with dichloromethane (DCM) for three times and dried over anhydrous Na_2SO_4. The filtrate was concentrated under reduced pressure and the concentrate was purified by silica gel column chromatography with petroleum ether (PE) and DCM (1:1, v/v) to afford the desired product as a yellow solid. Yield: 37%. ^{1}H NMR (500 MHz, Chloroform-*d*) δ 9.82 (s, 1H), 7.66 (d, J = 4.0 Hz, 1H), 7.49 (d, J = 9.0 Hz, 2H), 7.28 - 7.25 (m, 5H), 7.11 (d, J = 8.0 Hz, 4H), 7.08 – 7.02 (m, 4H).

Synthesis of compound TCP

A mixture of 4-(diphenylamino)benzaldehyde (273 mg, 1.0 mmol), 2-(pyridin-4-yl)acetonitrile (118 mg 1.0 mmol) and t-BuOK (168 mg 1.5 mmol) were dissolved in 2 mL superdry MeOH. The reaction was stirred at room temperature for 5 h. Then the mixture was extracted with DCM three times and dried over anhydrous Na_2SO_4. The filtrate was concentrated under reduced pressure and the concentrate was purified by silica gel column chromatography with DCM and EAC (1:1, v/v) to afford the desired product as an orange solid. Yield: 43%.1H NMR (500 MHz, Chloroform-*d*) δ 8.68 – 8.61 (m, 2H), 7.85 – 7.80 (m, 2H), 7.61 (s, 1H), 7.57 – 7.53 (m, 2H), 7.37 – 7.31 (m,

4H), 7.21 – 7.13 (m, 6H), 7.06 – 7.01 (m, 2H).

Synthesis of compound TTCP

A mixture of 5-(4-(diphenylamino)phenyl)thiophene-2-carbaldehyde (355 mg, 1.0 mmol), 2-(pyridin-4-yl)acetonitrile (118 mg 1.0 mmol) and t-BuOK (168 mg 1.5 mmol) were dissolved in 2 mL superdry MeOH and 3 mL superdry DCM. The reaction was stirred at room temperature for 5 h. Then the mixture was extracted with DCM three times and dried over anhydrous Na_2SO_4. The filtrate was concentrated under reduced pressure and the concentrate was purified by silica gel column chromatography with DCM and ethyl acetate (1:1, v/v) to afford the desired product as orange solid. Yield: 40%. ^{1}H NMR (500 MHz, Chloroform-*d*) δ 8.66 (s, 2H), 7.81 (s, 1H), 7.65 (d, J = 4.0 Hz, 1H), 7.55 – 7.53 (m, 4H), 7.32 – 7.28 (m, 5H), 7.14 (d, J = 8.0 Hz, 4H), 7.11 – 7.06 (m, 4H).

Synthesis of compound TCP-PF_6 /TTCP-PF_6

A mixture of 198 mg TCP / 241 mg TTCP (0.5 mmol) was dissolved in 20 mL acetonitrile. After this 470 μL iodomethane (7.6 mmol) was added to the solution and heated to 80 ºC for 12 h under nitrogen. After cooling the room temperature, the mixture was decanted into diethyl ether. The sediments were filtered by suction and re-dissolved in 20 mL methanol, followed by adding saturated KPF_6 solution (15 mL). After stirring for 1 h, the solution was evaporated and the residue was filtered again, washed with diethyl ether, and dried under reduced pressure to give the target compound.

The product of **TCP-PF_6** was a red solid. Yield: 38%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.93 (d, J = 7.0 Hz, 2H), 8.56 (s, 1H), 8.31 (d, J = 7.0 Hz, 2H), 8.02 (d, J = 9.0 Hz, 2H), 7.46 (t, J = 8.0 Hz, 4H), 7.33 – 7.19 (m, 6H), 6.93 (d, J = 8.5 Hz, 2H), 4.29 (s, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 152.60, 150.94, 150.29, 145.83, 145.48, 133.66, 130.63, 127.14, 126.51, 124.23, 122.48, 118.49, 117.56, 99.80, 47.46. ESI HRMS: calcd. for $C_{27}H_{22}N_3^+$ $[M - PF_6]^-$: 388.1808, fond: 388.1808.

The product of **TTCP-PF_6** was a deep red solid. Yield: 36%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.94 (d, J = 6.5 Hz, 3H), 8.27 (d, J = 6.5 Hz, 2H), 7.97 (d, J = 4.0 Hz,

1H), 7.74 – 7.69 (m, 3H), 7.38 (t, J = 7.5 Hz, 4H), 7.17 – 7.69 (m, 6H), 6.99 (d, J = 8.5 Hz, 2H), 4.29 (s, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 154.78, 149.57, 149.48, 146.70, 145.88, 144.13, 142.38, 135.10, 130.31, 128.10, 125.71, 125.44, 124.88, 122.47, 121.84, 117.13, 99.41, 47.50. ESI HRMS: calcd. for $C_{31}H_{24}N_3S^+$ [M – PF_6]$^-$: 470.1685, fond: 470.1685.

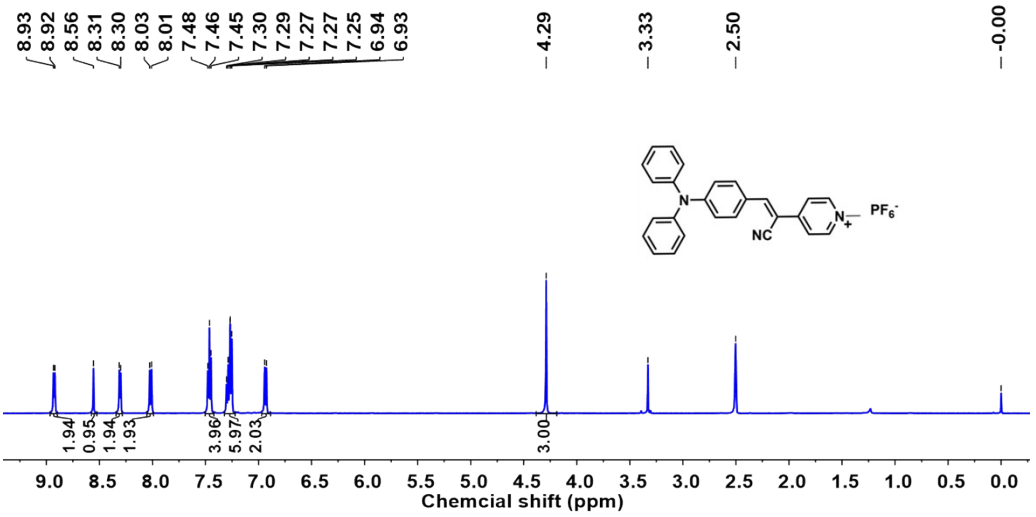


Fig. S1 ^{1}H NMR spectrum of compound **TCP-PF$_6$**.

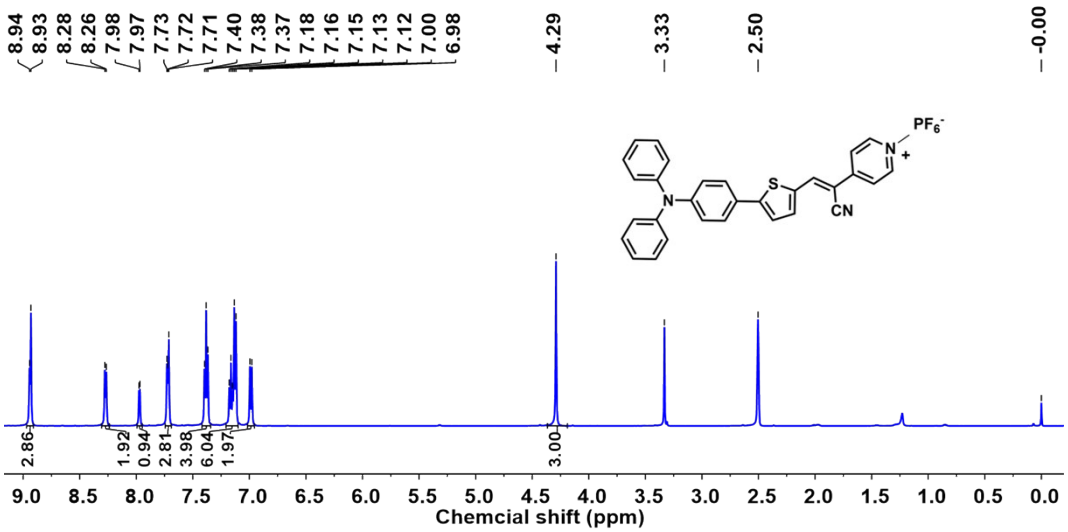


Fig. S2 ^{1}H NMR spectrum of **TTCP-PF$_6$**.

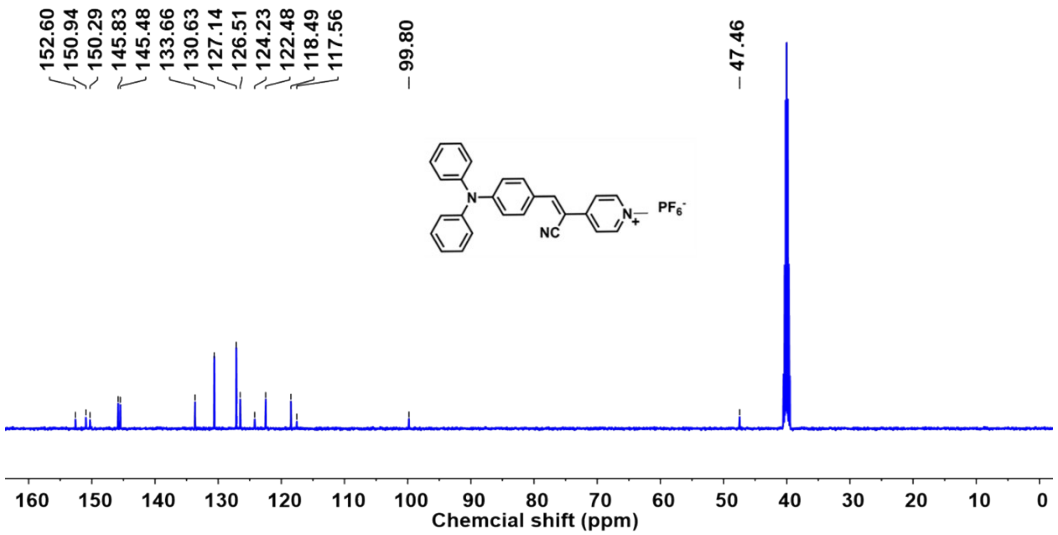


Fig. S3 ^{13}C NMR spectrum of **TCP-PF$_6$**.

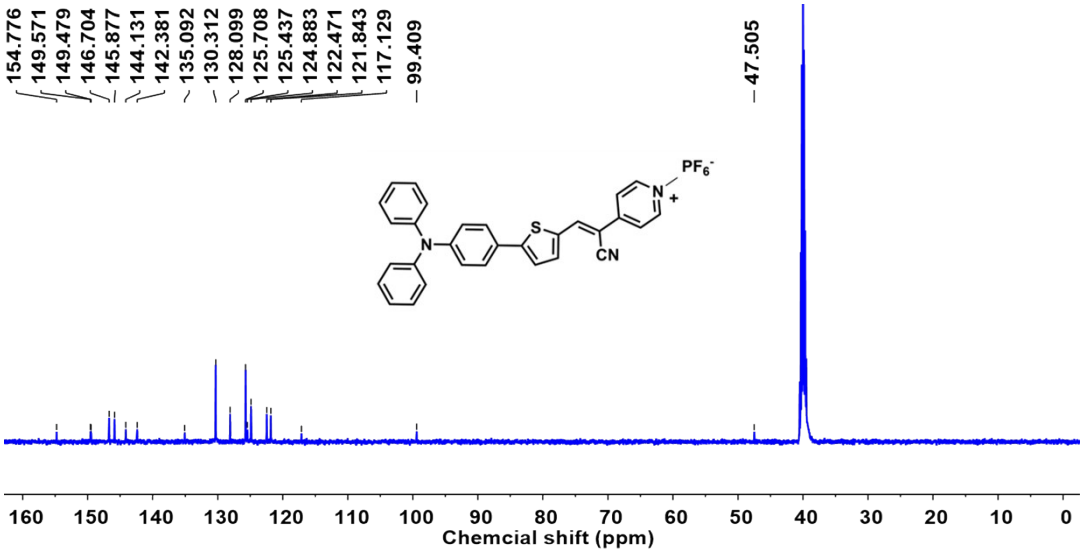


Fig. S4 ^{13}C NMR spectrum of **TTCP-PF$_6$**.

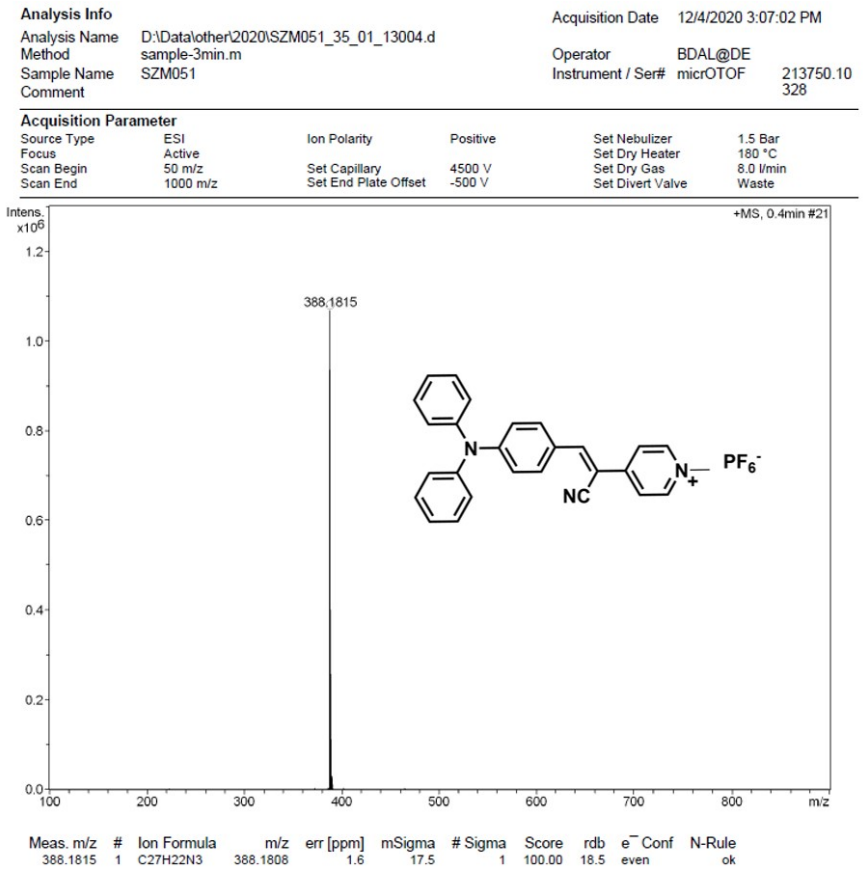


Fig. S5 HRMS spectrum of compound **TCP-PF$_6$**.

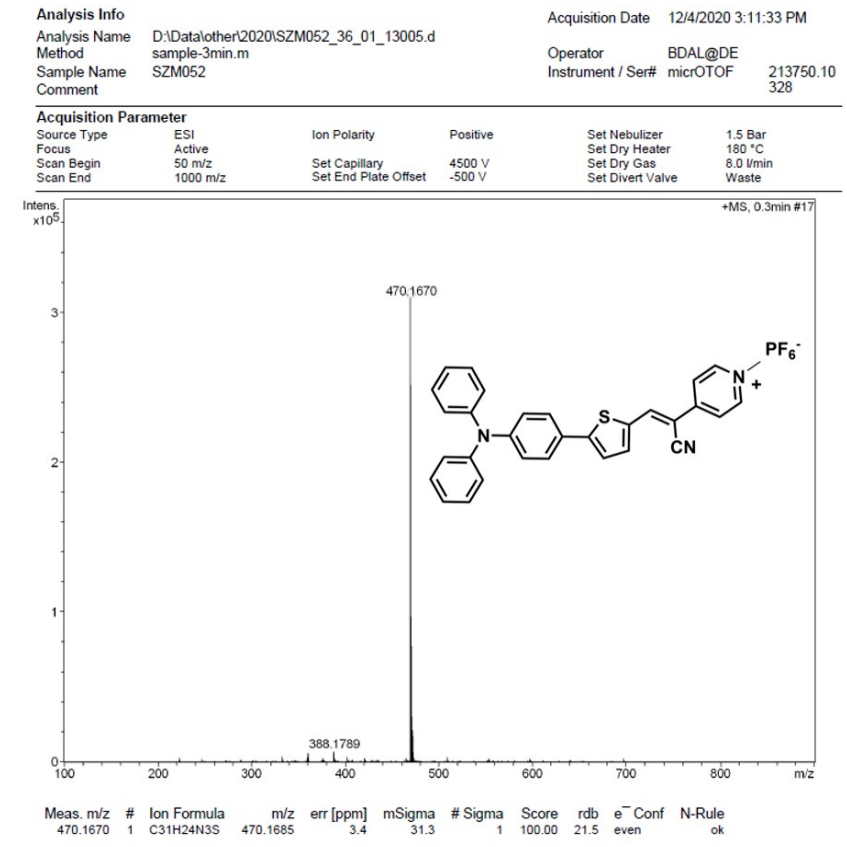


Fig. S6 HRMS spectrum of compound **TTCP-PF$_6$**.

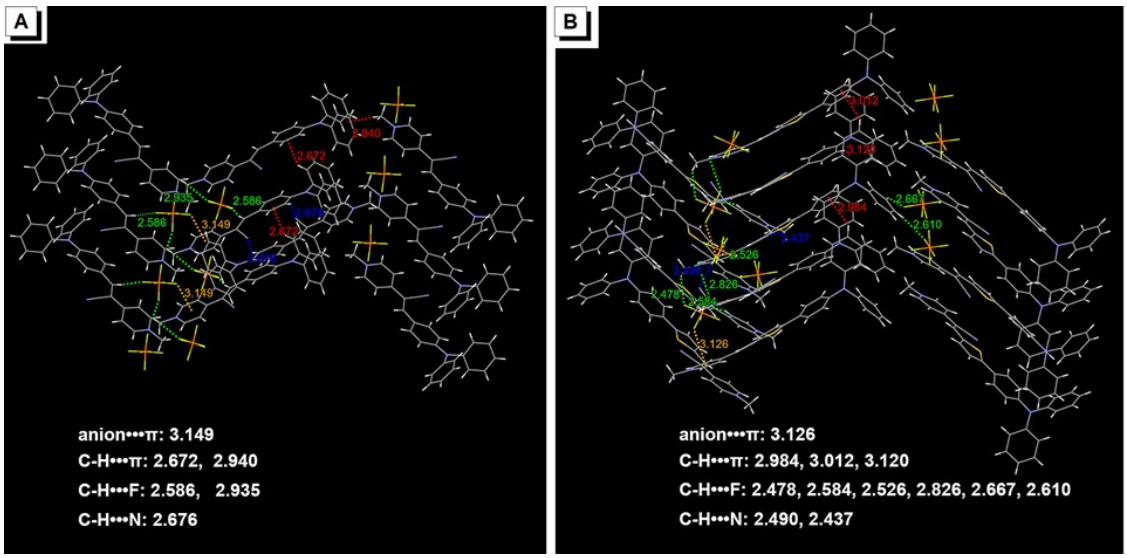


Fig. S7 The molecular stacking structures of **TCP-PF$_6$** and **TTCP-PF$_6$.**

Table S1. Crystal data and structure refinement for **TCP-PF$_6$** and **TTCP-PF$_6$**.

Empirical formula	$C_{27}H_{22}F_6N_3P$	$C_{31}H_{24}F_6N_3PS$
	TCP-PF$_6$	**TTCP-PF$_6$**
Formula weight	533.44	615.56
Temperature (K)	273.58	173.01
Crystal system	Monoclinic	Orthorhombic
Space group	$P2_1/n$	Pbca
a (Å)	5.9009(4)	19.1907(5)
b (Å)	29.1051(18)	8.6600(2)
c (Å)	14.6172(10)	33.8602(10)
α (deg)	90	90
β (deg)	94.585(4)	90
γ (deg)	90	90
V (Å^3)	2502.4(3)	5627.3(3)
Z	4	8
ρ_{calc} (g/cm^3)	1.416	1.453
μ (mm^{-1})	1.571	2.155

F (000)	1096.0	2528.0
Radiation	CuKα (λ = 1.54178)	CuKα (λ = 1.54178)
2Θ range for data collection	6.074 to 127.83	6.962 to 127.14
Index ranges	-6 ≤ h ≤ 6, -33 ≤ k ≤ 33, -16 ≤ l ≤ 16	-14 ≤ h ≤ 22, -9 ≤ k ≤ 10, -39 ≤ l ≤ 38
Reflections collected	32290	29550
Independent reflections	4114 [R_{int} = 0.0641, R_{sigma} = 0.0377]	4565 [R_{int} = 0.0949, R_{sigma} = 0.0581]
Data/restraints/parameters	4114/0/335	4565/0/380
Goodness-of-fit on F^2	1.041	1.016
Final *R* indexes [I>=2σ (I)]	R_1 = 0.0846, wR_2 = 0.2160	R_1 = 0.0613, wR_2 = 0.1357
Final *R* indexes [all data]	R_1 = 0.1025, wR_2 = 0.2288	R_1 = 0.1037, wR_2 = 0.1563
Largest diff. peak/hole (e Å^{-3})	0.63/-0.57	0.61/-0.40
CCDC NO	2131046	2131047

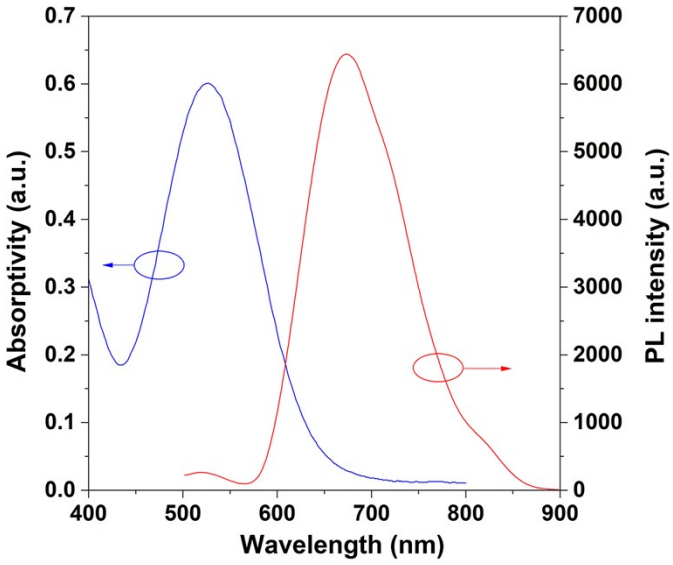


Fig. S8 UV-Vis absorption and emission spectra of **TTCP-PF_6** in DMSO/DMEM (1/99 in volume).

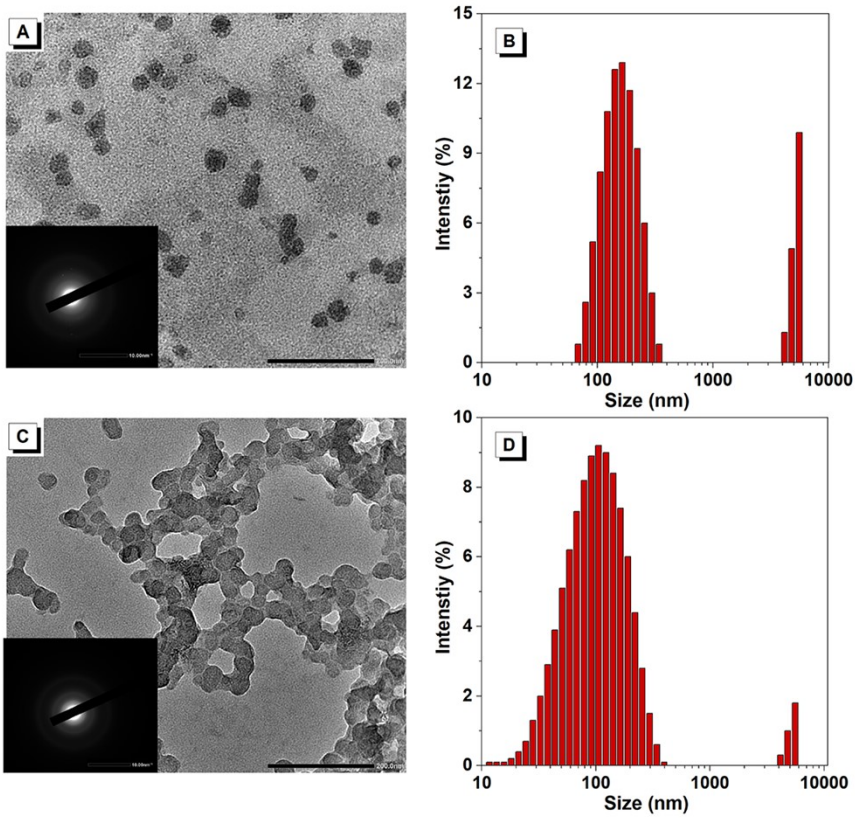


Fig. S9 TEM imaging and DLS imaging of compound **TCP-PF_6** (A, B) and **TTCP-PF_6** (C, D). Illustrations were electron diffraction pattern of the amorphous nanoaggregates.

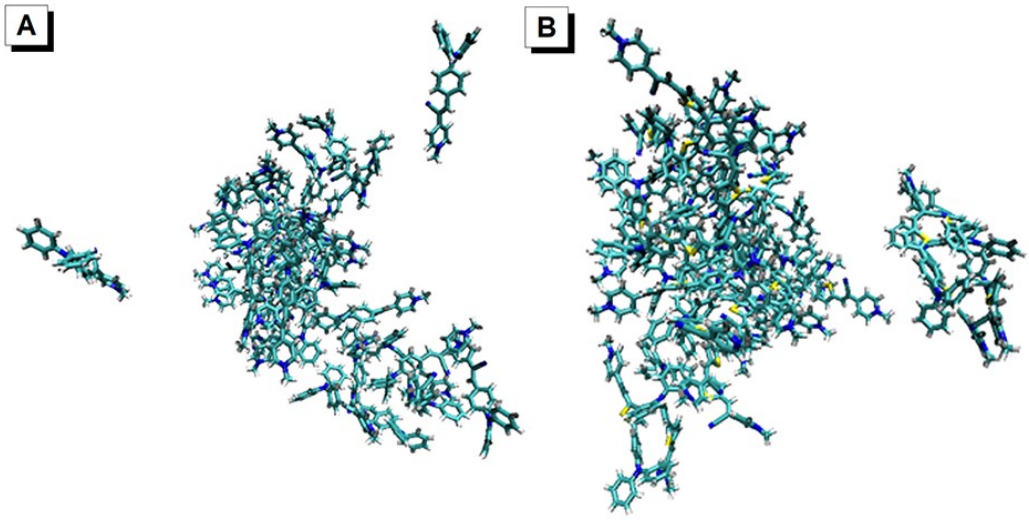


Fig. S10 TCP-PF_6 (A) and **TTCP-PF_6** (B) in aggregates obtained via MD simulations.

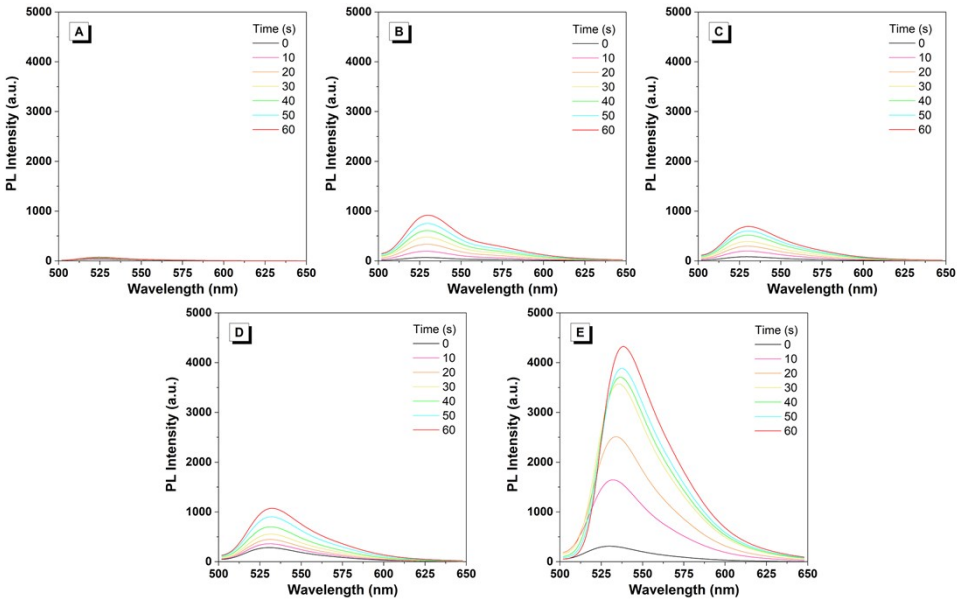


Fig. S11 Fluorescence spectra for ROS of Ctrl (A), Rose Bengal (B), Chlorin e6 (C), **TCP-PF$_6$** (D), and **TTCP-PF$_6$** (E) with white light irradiation using DCFH as fluorescence probe. Gens concentration (2×10^{-6} M), DCFH concentration (4×10^{-5} M), white light irradiation (400−700 nm, 23.4 mW/cm^2).

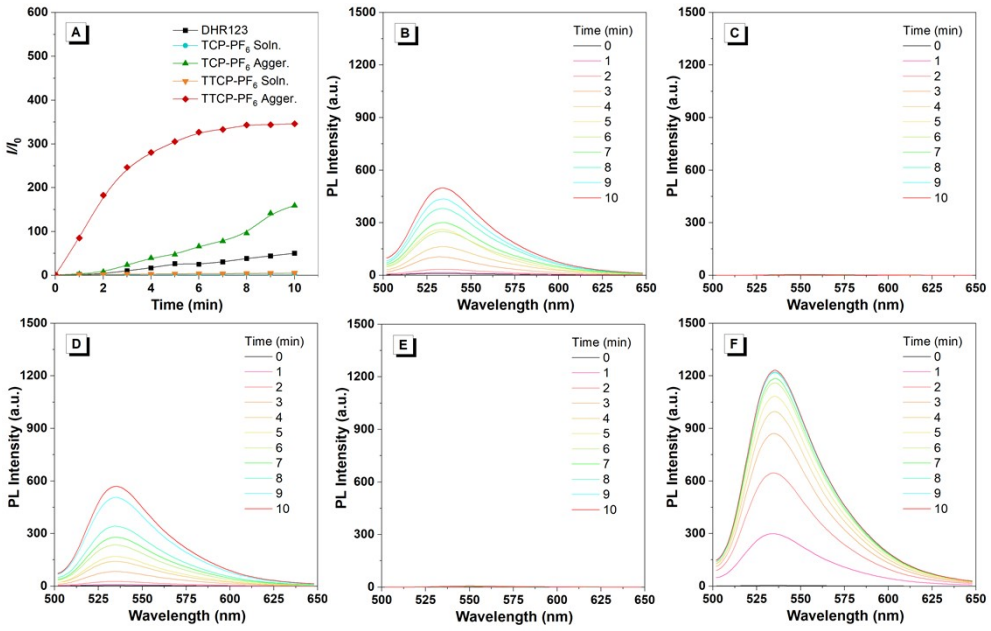


Fig. S12 Fluorescence spectra for type I (free radical-dominated) ROS of I/I_0 (A), Ctrl (B), **TCP-PF$_6$** solution (C), **TCP-PF$_6$** aggregation (D), **TTCP-PF$_6$** solution (E) or **TTCP-PF$_6$** aggregation (F) with white light irradiation using DHR123 as fluorescence probe. Gens concentration (2×10^{-6} M), DHR123 concentration (3×10^{-6} M), white light irradiation (400−700 nm, 23.4 mW/cm^2).

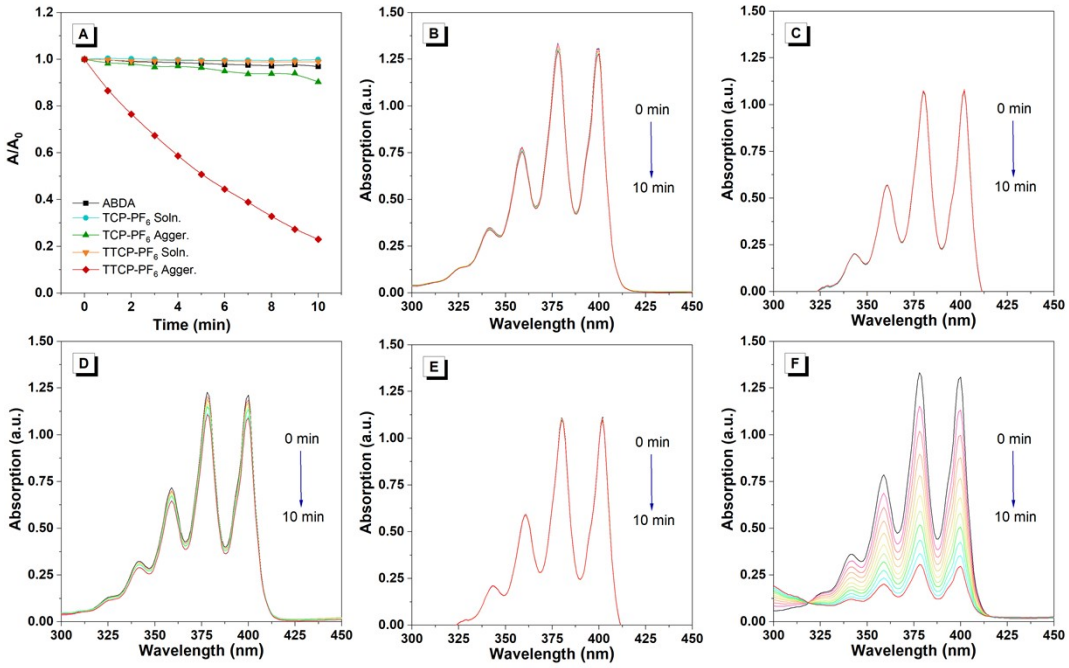


Fig. S13 Absorbance spectra for $^1O_2$ of A/A_0 (A), Ctrl (B), **TCP-PF$_6$** solution (C), **TCP-PF$_6$** aggregation (D), **TTCP-PF$_6$** solution (E) or **TTCP-PF$_6$** aggregation (F) with white light irradiation using ABDA as absorbance probe. Gens concentration (1×10^{-6} M), ABDA concentration (1×10^{-4} M), white light irradiation (400−700 nm, 23.4 mW/cm^2).