Electronic Supplementary Material (ESI) for Journal of Materials Chemistry B.

Pyridinium-substituted Tetraphenylethylene Salts-based Photosensitizers by Varying Counter Anions: Highly Efficient Photodynamic Therapy for Cancer Cell Ablation and Bacteria Inactivation

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ROS generation detection by H2DCF-DA in cells: The HeLa cells were seeded in a 96-well plate at a density of 10000 cells per well and incubated for 24 h. After being incubated with or without **TPEPy-I** and **TPEPy-PF6** at 5 μM for 30 min, the cells were washed with PBS and then incubated with 100 μL of 10 μM H2DCF-DA for 30 min. The cells were carefully washed with PBS twice and 100 μL of PBS was added. Then the plate was subjected to white light irradiation (30 $mW.cm^{-2}$). The fluorescence signal was determined by a fluorescence plate reader (excitation/emission: 488 nm/500–530 nm). Each trial was performed with 3 times in parallel.

The measurement for the determination of quantum yields

We used Hamamatsu absolute PL quantum yield spectrometer C11347 to measure absolute fluorescence quantum yields of **TPEPy-I** and **TPEPy-PF6** in PMMA film, this instrument consists of an excitation light source based on a xenon arc lamp and a high-sensitivity multichannel detector. The emitted light is collected by the integrating spheres. The use of integrating spheres has usually required a laser as the excitation source in combination with a fibre coupled CCD camera or a calibrated photodiode as the luminescence detectors.

Computational details

Since $\mathbf{PF_6^-}$ in $\mathbf{TPEPy\text{-}PF_6}$ plays a minor role in ISC, we used it cation part, i.e. $\mathbf{TPEPy^+}$ for simplicity in calculations. $\mathbf{TPEPy^+}$ was fully optimized at the level of ωB97XD/6-31G* of theory using Gaussian 09 program. For the optimization of **TPEPy-I**, the only difference from $\mathbf{TPEPy^+}$ is that we used Stuttgart/Dresden ECPs (also known as SDD) and corresponding basis set for I because 6-31G* is not applicable for I. Based on relaxed ground state structures, we conducted time-dependent density functional theory (TD-DFT) calculations and optimized their structures at lowest singlet state (S_1) at the

same level of theory as ground state calculations. Then, we carried out vertical excitation (both singlet and triplet excitations) calculations on the basis of relaxed S_1 structures in conjunction with ωB97XD functional and a larger basis set TZVP for all elements except for I (SDD is used), which allows us to obtain singlet-triplet energy gaps. Finally, to compute SOCMEs of relevant ISC channels (originated from S_1) in both **TPEPy$^+$** and **TPEPy-I**, we conducted scalar relativistic TD-DFT calculations using ZORA method at the level of ωB97XD/def2-TZVP as implemented in ORCA 4.2.1 program.

$^1O_2$ detection via chemical method.

ABDA was used as the $^1O_2$ indicator, and Rose Bengal (RB) was employed as the standard photosensitizer. To eliminate the inner-filter effect, the absorption maxima were adjusted to ~ 0.2 OD. The measurements were carried out under white light irradiation in DMSO/water mixtures with f_w = 99%. [ABDA] = 10 × [PSs], $^1O_2$ quantum of sample were calculated by the equation: $\Phi_{sample}=\Phi_{RB}(K_{sample}/K_{RB})(A_{RB}/A_{sample})$ where K_{sample} and K_{RB} are the decomposition rate constants of ABDA with sample and RB, respectively. A_{sample} and A_{RB} represent the light absorbed by sample and RB, respectively, which are determined by integration of the areas under the absorption bands in the wavelength range of 400–800 nm. Φ_{RB} is the $^1O_2$ quantum yield of RB, which is 0.75 in water. Each trial was performed with 3 times in parallel.

Cytotoxicity study

MTT assays were used to evaluate the cytotoxicity of the presented AIEgens. Cells were seeded in 96-well plates (Costar, IL, USA) at a density of 6000–8000 cells per

well. After overnight culture, the medium in each well was replaced with 100 mL fresh medium containing different concentrations of **TPEPy-I** or **TPEPy-PF6**. 24 hours later, 10 mL MTT solution (5 $mg.mL^{-1}$ in PBS) was added into each well. After 4 hours of incubation, 100 mL SDS–HCl aqueous solution (10% SDS and 0.01 M HCl) was added to each well. After incubation for 4 hours, the absorption of each well at 595 nm was recorded via a plate reader (Perkin-Elmer Victor3™). Each trial was performed with 3 wells in parallel.

Cell imaging

Cells were grown in a 35 mm Petri dish with a coverslip at 37 ^{o}C. The live cells were incubated with a certain dye at a certain concentration for a certain time. Afer adding TTVP (500 nM), the Petri dish was shaken for a few seconds at room temperature, and then the coverslip was taken out. The TTVP-labelled cells were mounted and imaged using a laser scanning confocal microscope (LSM7 DUO) at 488 nm with 5% laser power (the scanning rate was 22.4 s per frame). The emission signal in the range of 600–744 nm was collected for cell imaging.

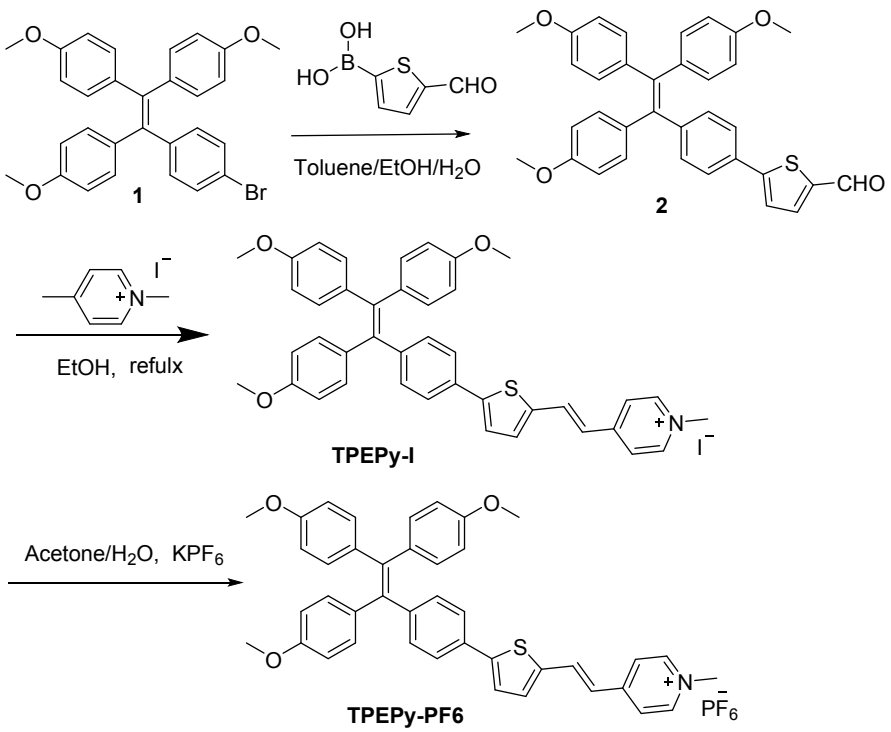
Cytotoxicity to cancer cells under light irradiation

HeLa cells were seeded in 96-well plates (Costar, IL, USA) at a density of 6000–8000 cells per well. After overnight culturing, the medium in each well was replaced with 100 mL fresh medium containing different concentrations of TTVP. After incubation for 3 s, the plates containing HeLa cells were exposed to white light (around 10 $mW.cm^{-2}$) for 10 min, and another array of plates with cells were kept in the dark as the control. Then the plates were subjected to the same treatment as the biocompatibility

test.

Photodynamic bacterial killing study

10^8 CFU bacteria were dispersed in 500 μL of PBS. After incubation with certain concentration of **TPEPy-I** or **TPEPy-PF6** for 10 min. After that, the bacteria were dispersed in PBS and exposed to white light lamp for desired time, while the control groups were put in the dark. The viability of bacteria was quantified by plate-count method. The bacteria were diluted with a dilution factor of 10^5. Then 100 μL of the bacteria solution was evenly spread on a LB agar plate, which was then subjected to culturing at 37 °C for 18 h before quantification and photo taking.



Scheme S1 The synthetic routes of **TPEPy-I** and **TPEPy-PF6**

Synthesis of Compound 2: Compound **1** (1000mg, 2mmol), 5-formyl-2-thiopheneboronic acid (468 mg, 3 mmol), $Pd(Pph_3)_4$ (100 mg, 0.1mmol), 3 mL K_2CO_3 aqueous solution (2 mol/L) , 40 mL mixed solvent (ethanol: toluene = 3:1) were stirred and refluxed for 8 h under nitrogen. After reaction finished, the mixture was cooled to room temperature, and then poured into water, and extracted with dichloromethane by above three times. The organic layers were washed with brine and dried by anhydrous magnesium sulfate. After filtration and solvent evaporation, the residue was purified by silica-gel column chromatography using a mixture of petroleum ether and ethyl acetate (10:1, v/v) as eluent to afford yellow solids in 65.7% yield. ^{1}H-NMR (400 MHz, $CDCl_3$): δ 9.86 (s, 1H), 7.70 (d, J = 3.9 Hz, 1H), 7.42 (d, J = 8.1 Hz, 2H), 7.34 (s, 1H), 7.08 (d, J = 8.0 Hz, 2H), 6.99 – 6.91 (m, 6H), 6.66 (dd, J = 8.4, 4.0 Hz, 7H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 182.71, 158.30, 158.16, 158.08, 154.40, 146.27, 141.98, 140.42,

137.82, 137.50, 136.34, 136.25, 132.66, 132.60, 132.25, 130.45, 125.64, 123.75, 113.31, 113.28, 113.25, 113.14, 55.14. HRMS (*ESI*): m/z $[M+Na]^+$ *calcd* for $C_{34}H_{28}O_4S$: 555.1700, found: 555.1605. m.p.: 193.5-194.0 ^oC.

Synthesis of TPE-PY-I: Compound **2** (0.212 mg, 0.398 mmol) and 1,4-dimethylpyridine-1-iodide (0.103 mg, 0.438mmol) were placed in a 2-neck reaction flask under argon atmosphere, and 30 ml of absolute ethanol was added. After heating to raise the temperature to 80 ° C, a few drops of piperidine were added to the reaction solution to catalyze overnight. After completion of the reaction, water was added and extracted with dichloromethane. The crude product was purified by column chromatography (dichloromethane: methanol = 98:2) to afford 0.25 g of dark red solid. m.p.: 188-189 ℃. ^1H NMR (400 MHz, DMSO-d_6) δ 8.83 – 8.76 (m, 2H), 8.20 – 8.12 (m, 3H), 7.02 (d, J = 7.4 Hz, 2H), 6.89 (s, 6H), 6.73 (s, 6H), 4.22 (s, 3H), 3.69 (s, 9H).^{13}C NMR (100 MHz, DMSO-d_6) δ 158.17, 158.10, 152.57, 147.20, 145.35, 145.22, 140.16, 139.92, 138.07, 136.29, 136.25, 136.19, 134.11, 133.91, 132.59, 132.54, 132.50, 132.23, 125.65, 125.42, 123.53, 122.18, 113.81, 55.40, 47.23. HRMS (*ESI*): m/z $[M-I]^+$ calcd for $C_{41}H_{36}NO_3S$, 622.2410, found: 622.2448.

Synthesis of TPE-PY-PF_6 : The saturated KPF_6 solution (5 mL) was added to **TPEPy-I** (50 mg) dissolved in acetone. The mixture was stirred for 2h at room temperature. After completion of the reaction the acetone was evaporated under reduced pressure and the precipitates were filtered, then washed with water and diethyl ether. The product was dried under reduced pressure with 99% yield. m.p.: 165-166 ℃. ^1H NMR (400 MHz, DMSO-d_6) δ 8.81 (d, J = 5.9 Hz, 2H), 8.22 – 8.15 (m, 3H), 7.58

(d, J = 3.8 Hz, 1H), 7.50 (t, J = 6.8 Hz, 3H), 7.15 (d, J = 16.0 Hz, 1H), 7.02 (d, J = 8.2 Hz, 2H), 6.91 – 6.84 (m, 6H), 6.75 – 6.69 (m, 6H), 4.22 (s, 3H), 3.69 (s, 9H).^{13}C NMR (100 MHz, DMSO-d_6) δ 158.29, 158.18, 158.11, 152.57, 147.24, 145.34, 145.24, 140.17, 139.89, 138.07, 136.29, 136.25, 136.19, 134.08, 133.89, 132.60, 132.54, 132.51, 132.24, 130.95, 125.65, 125.43, 123.52, 122.18, 113.88, 113.82, 113.72, 55.39, 47.25, 30.89. HRMS (ESI) (m/z): $[M\text{-}PF_6]^+$ calcd for $C_{41}H_{36}NO_3S$, 622.2410; found: 622.2415.

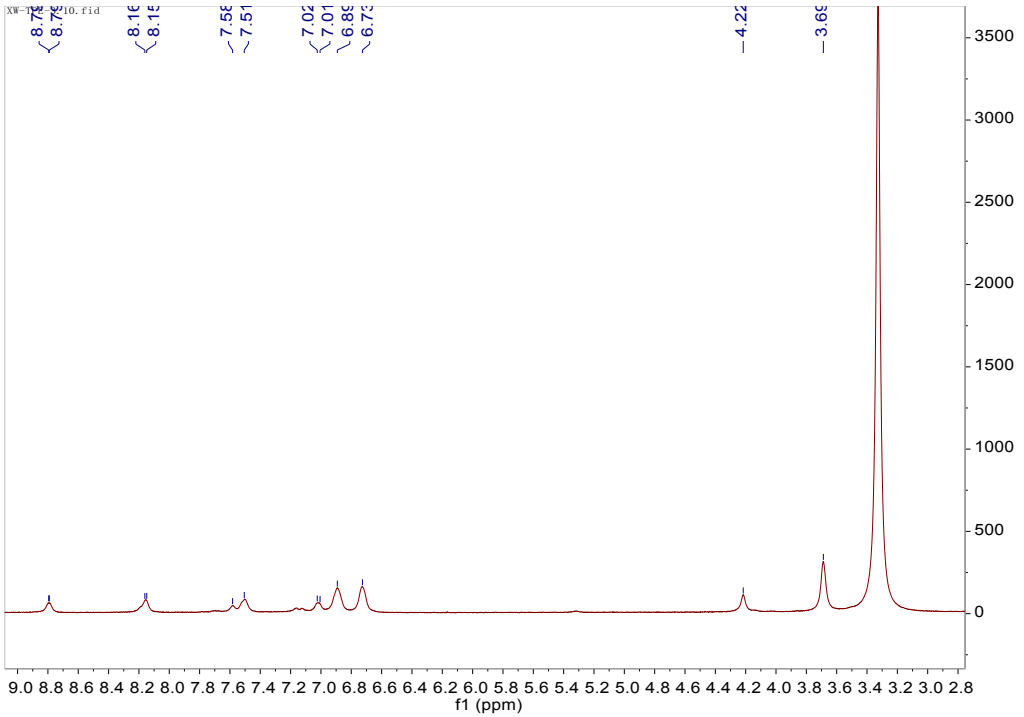


Figure S1 ^{1}H NMR spectrum of **TPEPy-I** in DMSO-d_6.

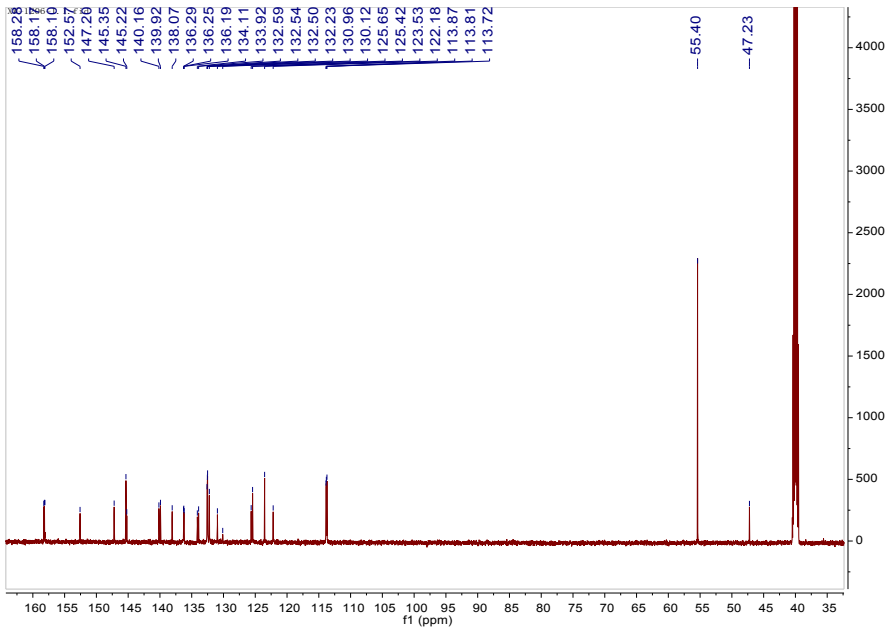


Figure S2 ^{13}C NMR spectrum of **TPEPy-I** in DMSO-d_6.

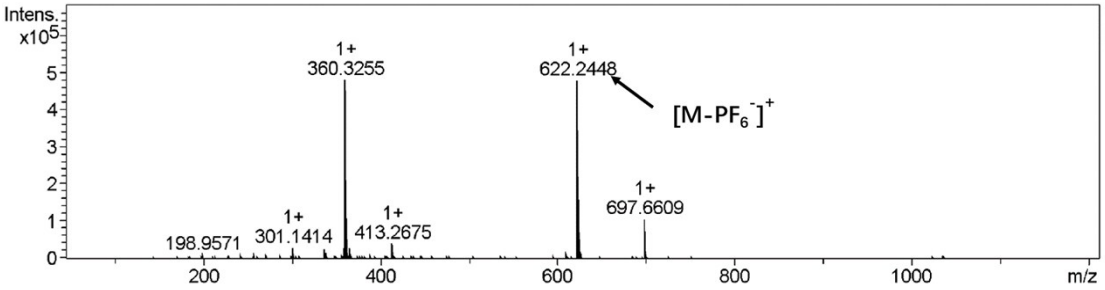


Figure S3 HRMS spectrum of **TPEPy-I**.

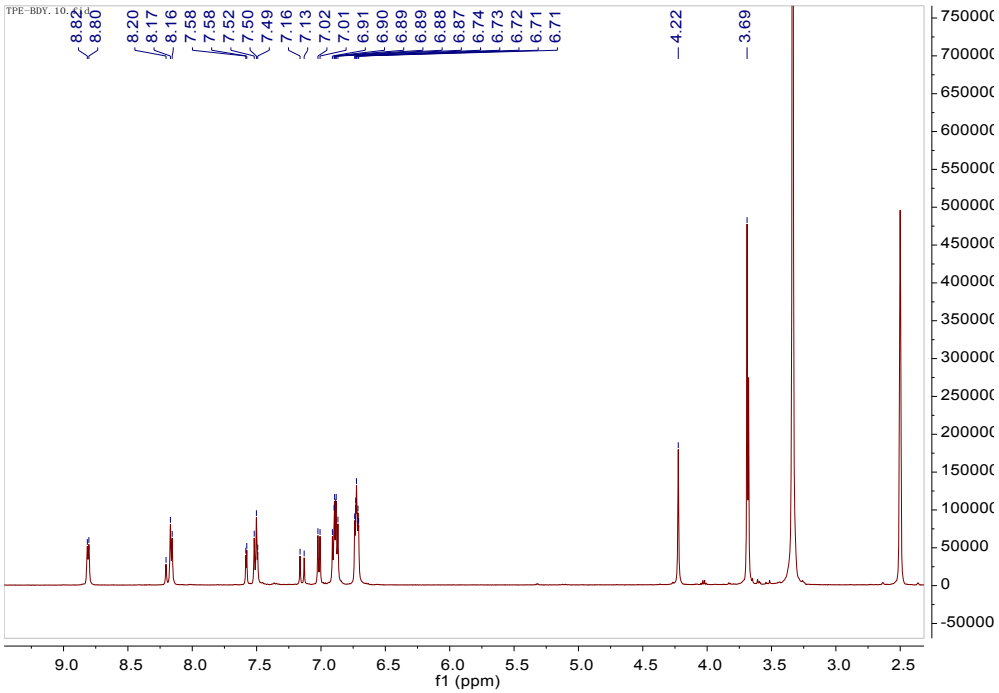


Figure S4 ^{1}H NMR spectrum of **TPEPy-PF6** in DMSO-d_6.

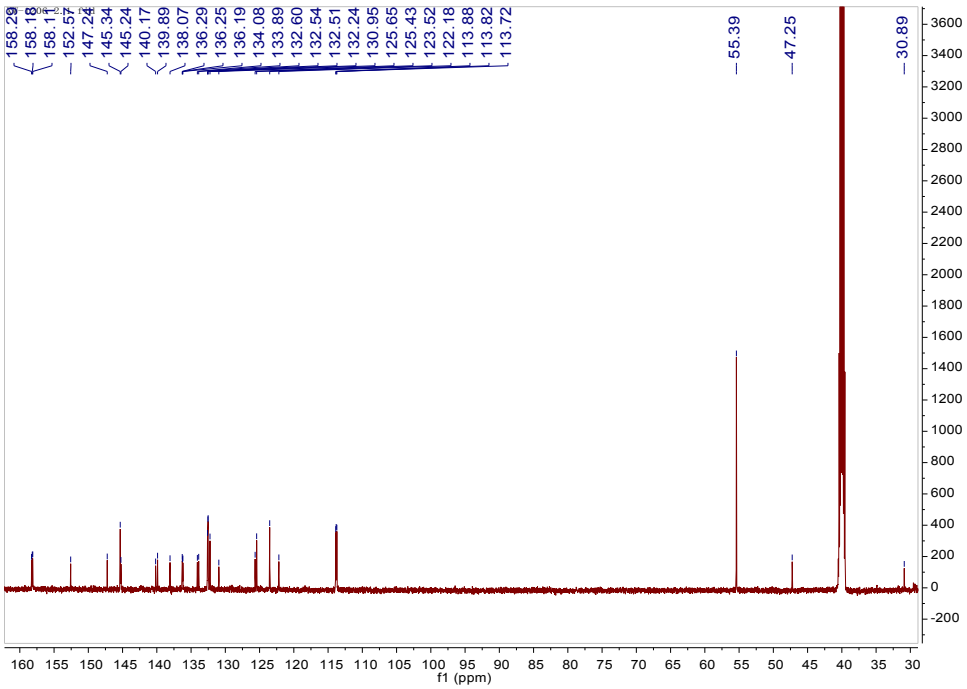


Figure S5 ^{13}C NMR spectrum of **TPEPy-PF6** in DMSO-d_6.

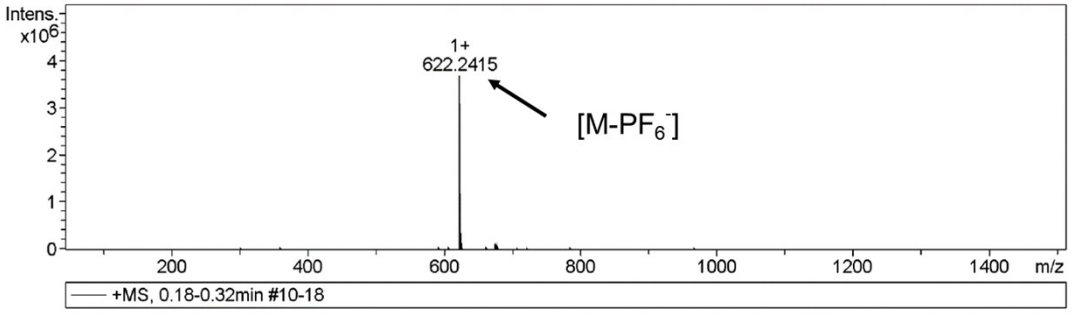


Figure S6 HRMS spectrum of **TPEPy-PF6**.

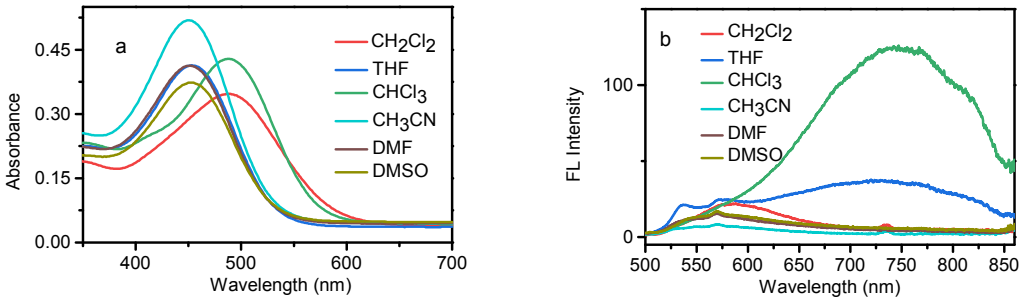


Figure S7. The (a) absorption and (b) emission spectra of **TPEPy-I** (10 μM) in different solvents.

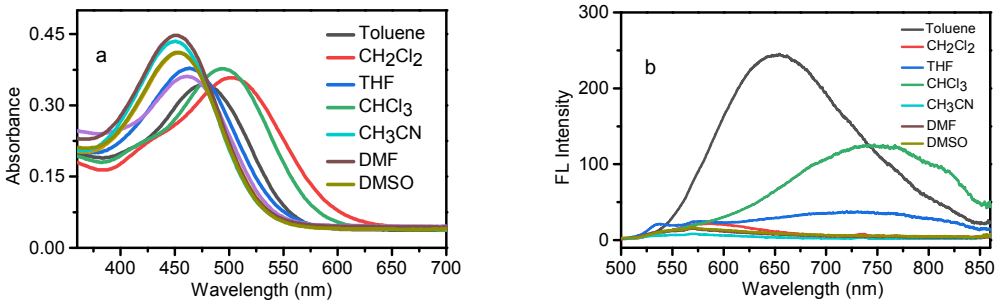


Figure S8. The (a) absorption and (b) emission spectra of **TPEPy-PF6** (10 μM) in different solvents.

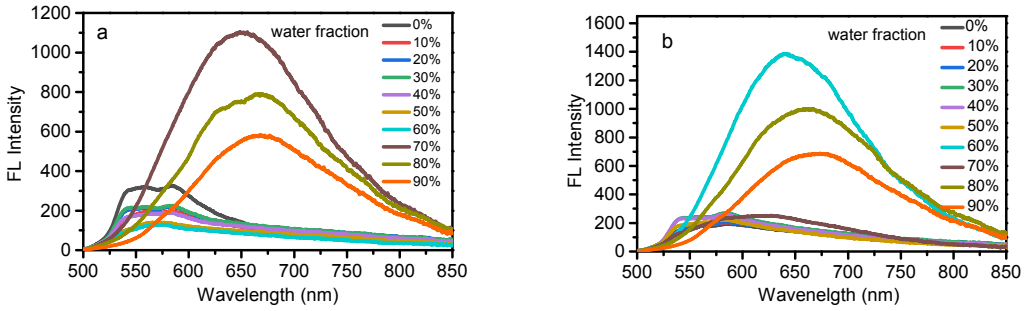


Figure S9. The emission spectra of (a) **TPEPy-I** and (b) **TPEPy-PF6** in DMSO-H_2O with different water content.

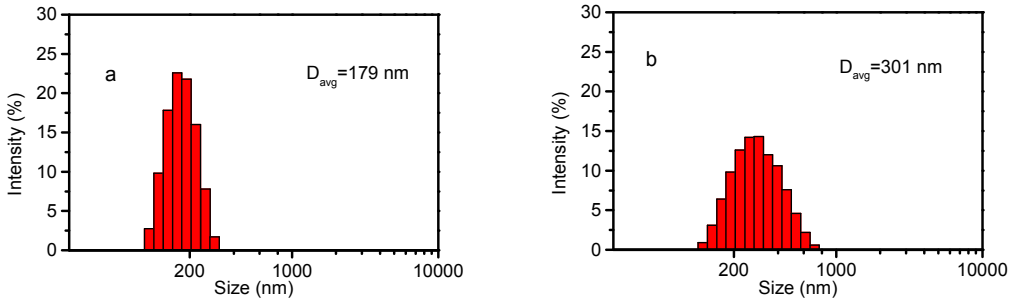


Figure S10. Particle size distributions of (a) **TPEPy-I** (10 μM) and (b) **TPEPy-PF6** (10 μM) aggregates in DMSO-H_2O (1/9, v/v).

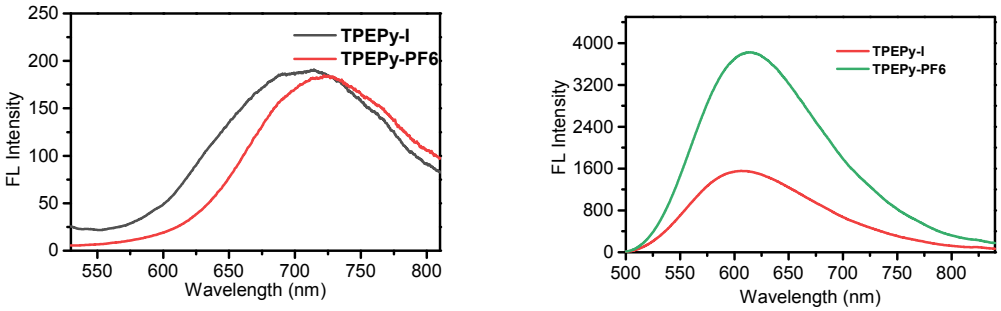
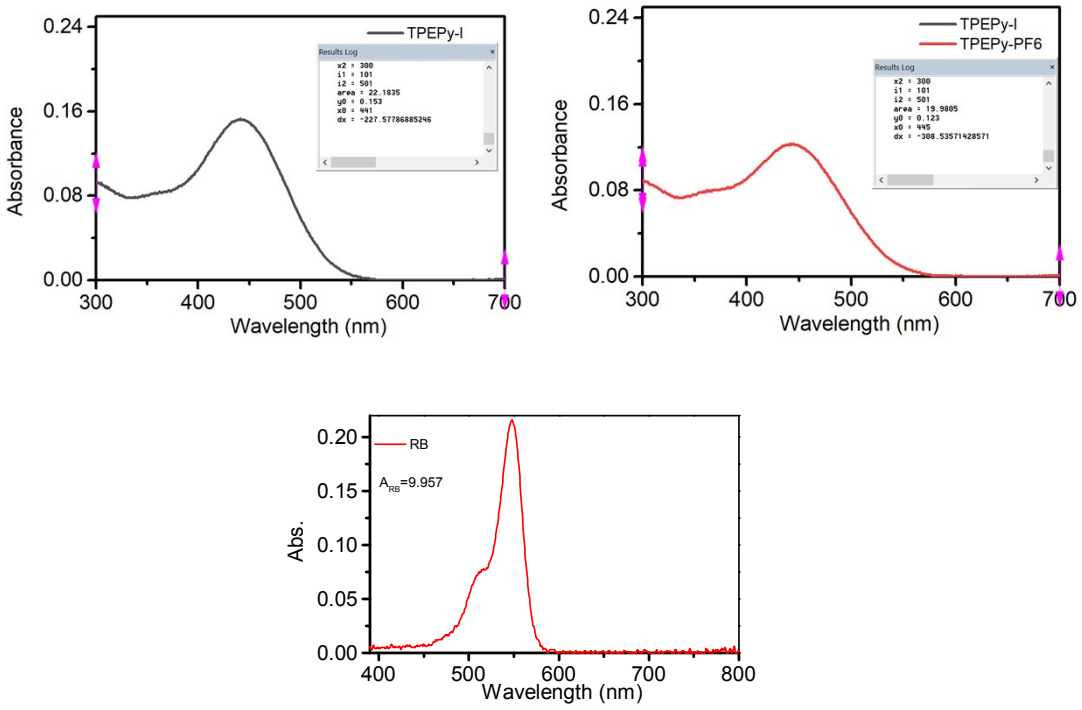


Figure S11. (Left) the emission spectra of **TPEPy-I** and **TPEPy-PF6** powder and (right) the emission spectra of 1% **TPEPy-I** and **TPEPy-PF6** PMMA film.



$$\Phi_{sample}=\Phi_{RB}(K_{sample}/K_{RB})(A_{RB}/A_{sample})$$

$$\Phi_{TPEPy\text{-}I}=0.75*(0.01201/0.00446)*(9.95/22.1835)=0.89$$

$$\Phi_{TPEPy\text{-}PF6}=0.75*(0.01111/0.00446)*(9.95/19.9805)=0.93$$

Figure S12. The integral area of **TPEPy-I**, **TPEPy-PF6** and **RB**.

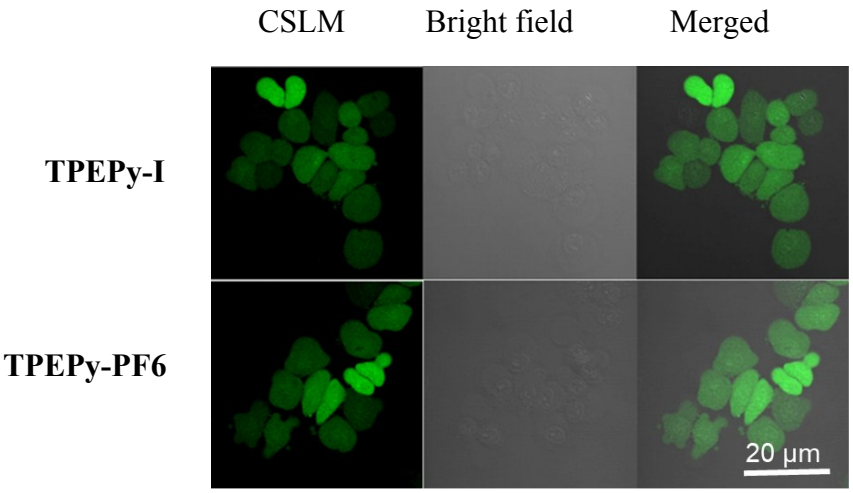


Figure S13. Detection of intracellular ROS generation using H2DCF-DA in HeLa cells incubated with **TPEPy-I** and **TPEPy-PF6** followed by irradiation with white light irradiation for 5 min.

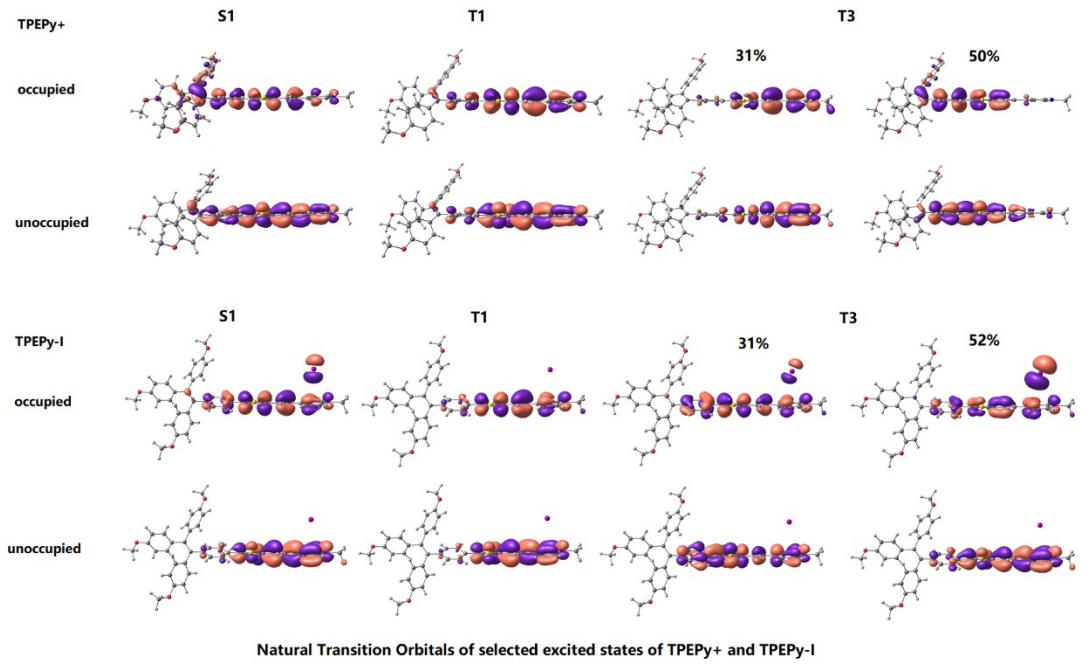


Figure S14. Natural Transition Orbitals (NTOs) analysis of S1, T1 and T3 for **TPEPy-PF_6** (represented by **$TPEPy^+$** in upper panel) and **TPEPy-I** (lower panels) in water. Isovalue of 0.03 is adopted for visualization of all NTOs.

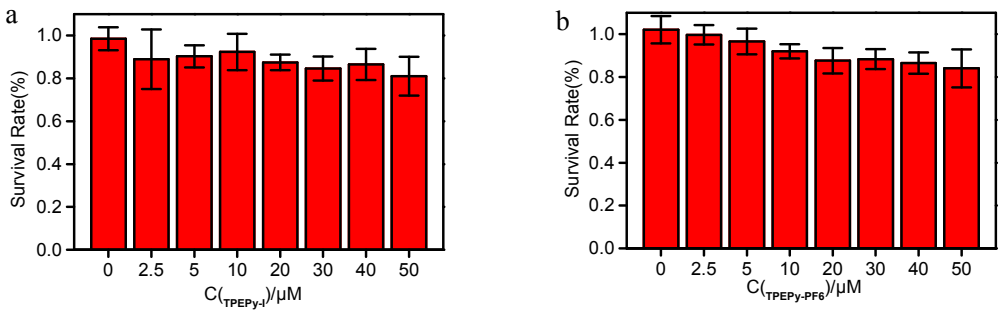


Figure S15. Viability of HeLa cells pretreated with a series of doses of (a) **TPEPy-I** and (b) **TPEPy-PF6** without light irradiation.

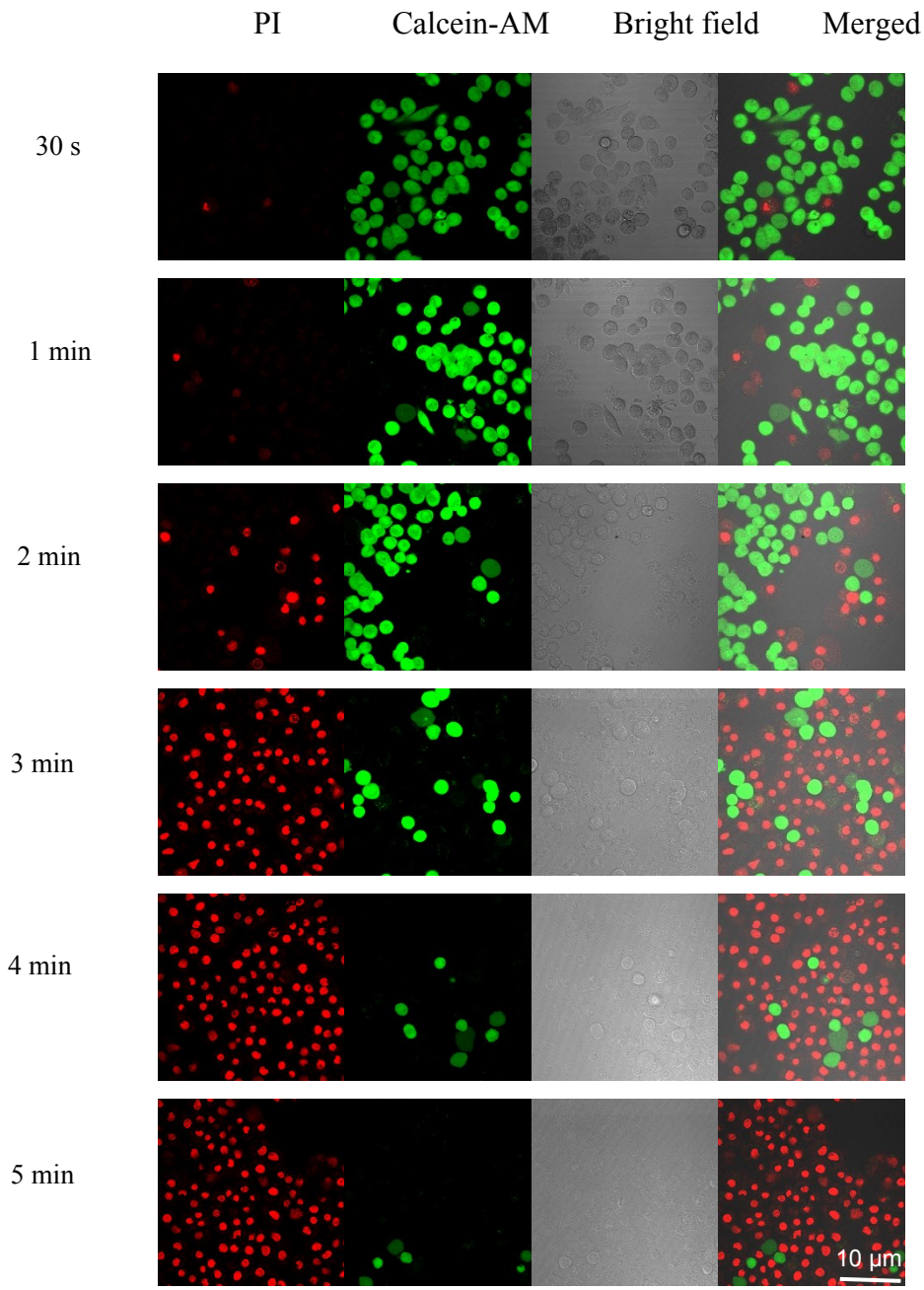


Figure S16. Live/dead staining of **TPEPy-PF6** (10 μM) treated HeLa cells with light irradiation for 30 s, 1 min, 2 min, 3 min, 4 min and 5 min. The live cells were stained by Calcein-AM (green), whereas dead cells were stained by PI (red).

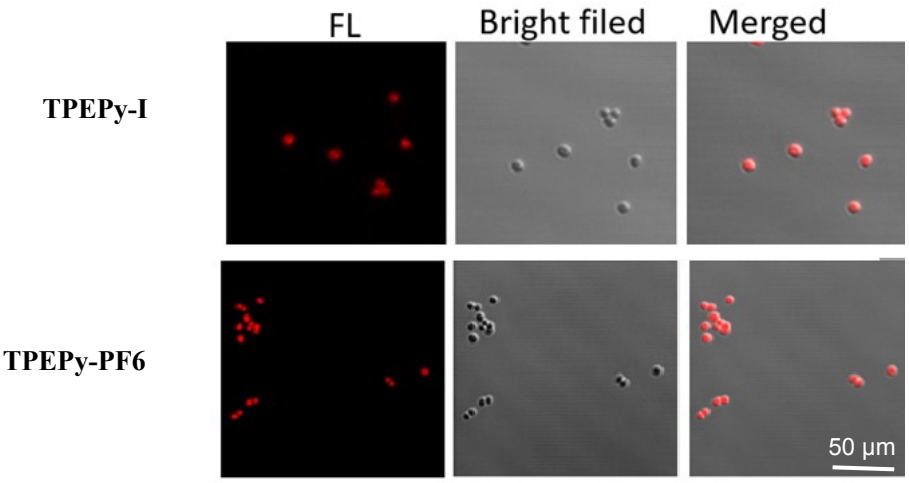


Figure 17 CLSM imaging of *S. Aureu*s stained with **TPEPy-I** and **TPEPy-PF6**.

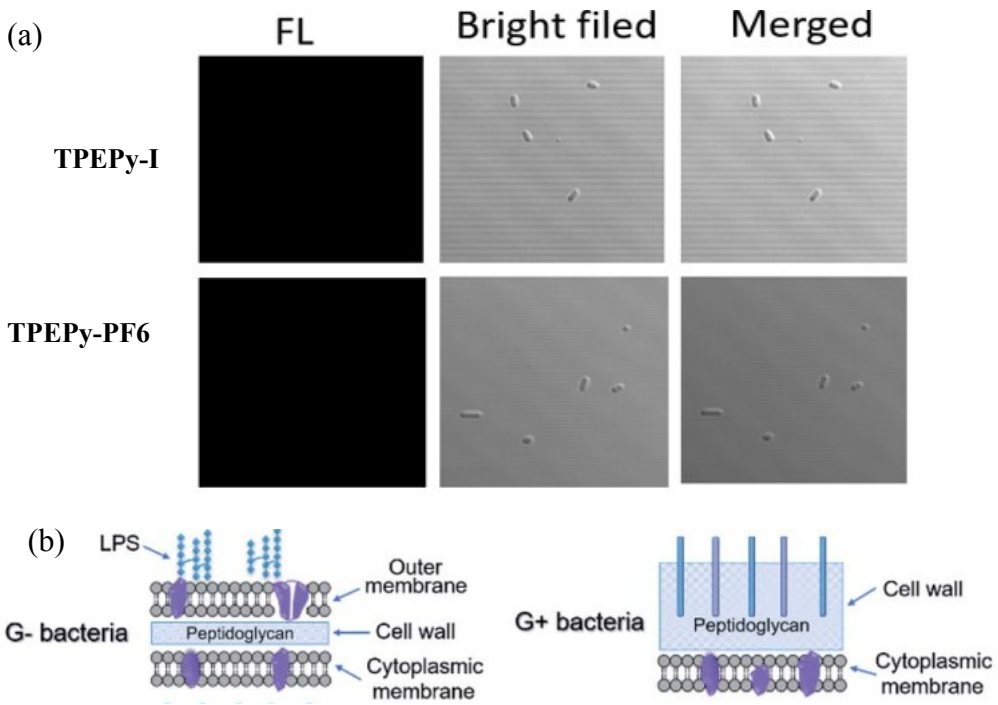


Figure S18. (a) CLSM imaging of *E. coli* stained with **TPEPy-I** and **TPEPy-PF6**. (b) Schematic of cell envelope structures of Gram-negative (G^-) bacteria, Gram-positive (G^+) bacteria.

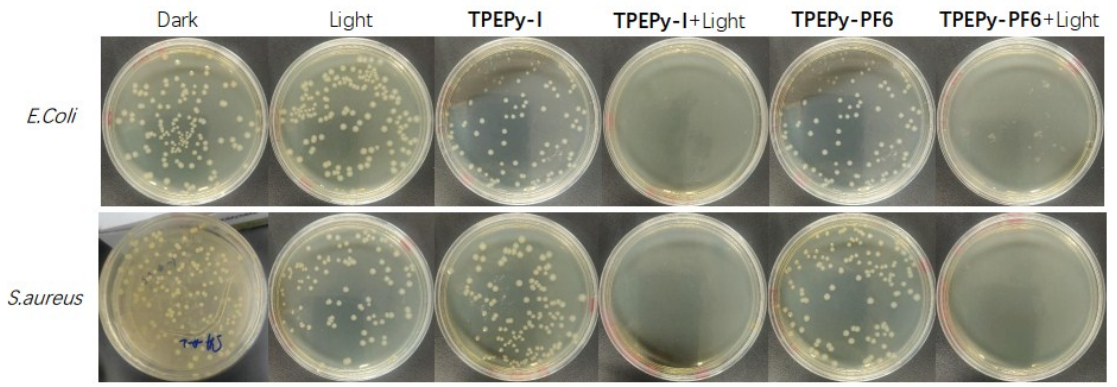


Figure S19 Photographs of *E. coli* and *S. Aureu*s cultured on agar plate supplemented with **TPEPy-I** and **TPEPy-PF6** (5 μM) under white light for 5 min along with the controls of the bacteria in dark or upon white light activation alone.

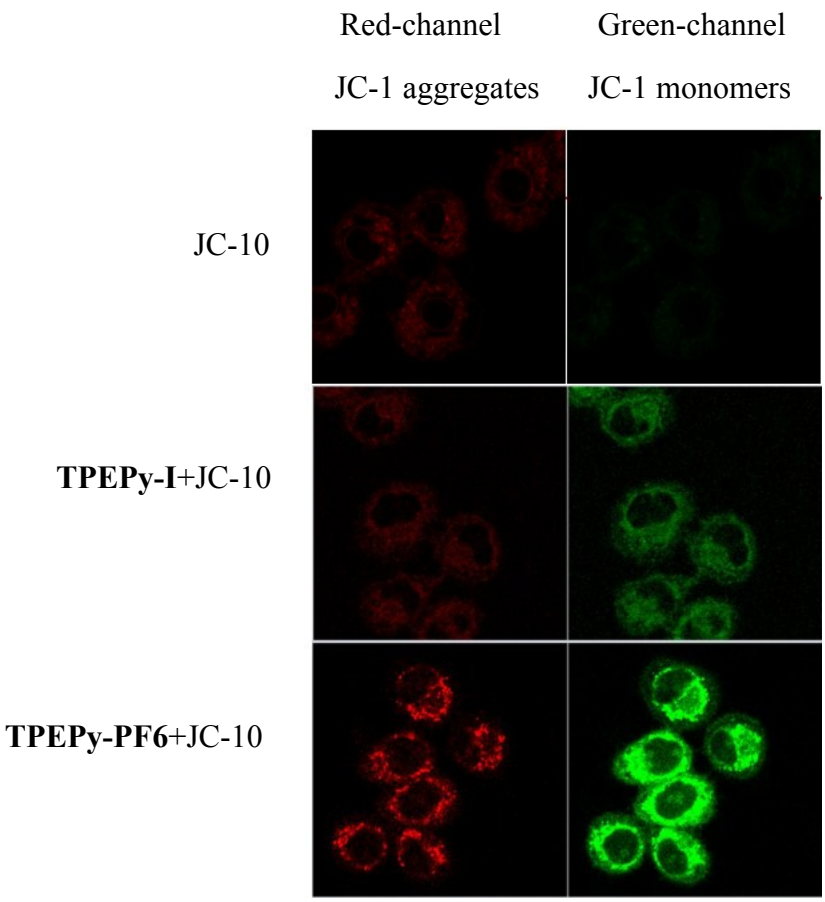
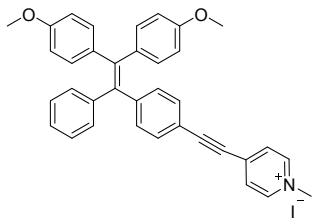
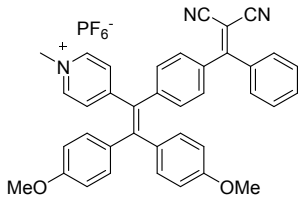
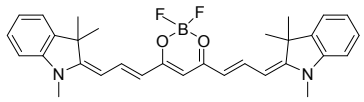
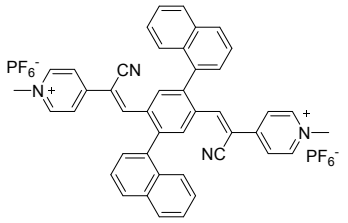
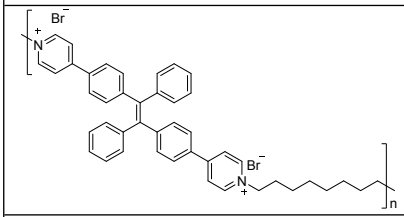
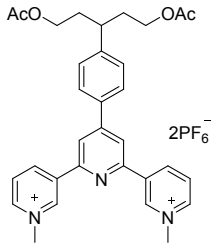
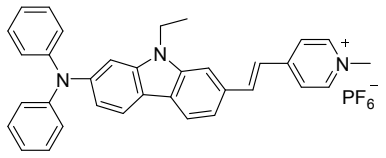
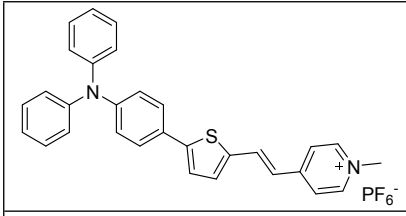
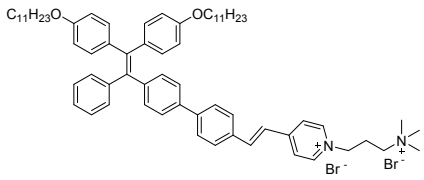
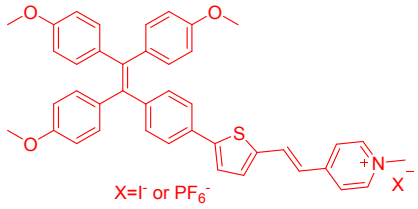


Figure S20 CLSM images of intracellular mitochondrial membrane potential stained with JC-10 dye and **TPEPy-I** (or **TPEPy-PF6**) under white light. The red channel was recorded at 580−640 nm under excitation at 561 nm to indicate the aggregated JC-10 dye. The green channel was recorded at 500−550 nm under excitation at 488 nm to indicate the monomeric JC-10 dye.

Table S1 The comparison of AIE-PS for cancer cell ablation and bacterial elimination

Chemical structure	Remarks	Ref.
	High dark toxicity, *E. coli*: 30 μM PS, white light for 7 min, almost 100% killing rate *B. subtilis*: 5 μM PS, white light for 7 min, almost 100% killing rate	Chem. - Asian J. 2017, 12, 1013-1020
	HeLa cell: 12.5 μM PS, 80% killing rate *S. epidermidis*: 5 μM PS, white light for 20 min, almost 100% killing rate *E. coli*: no killing effect	J. Mater. Chem. B, 2018, 6, 3894-3903
	little dark toxicity MCF-7 cells: 5 μM PS, 660 nm LED red light (135 J/cm^2), 95% killing rate,	Chem. Commun. 2019, DOI: 10.1039/c9cc06157f
	HeLa cell: 50 μM PS, white light for 30 min, 80% killing rate *S. aureus* and *E. coli*: 20 μM PS, white light for 30 min, almost 100% killing rate	ACS Appl. Mater. Interfaces 2019, 11, 11227−11237
	*S. epidermidis* and *E. coli*: 150 μg/mL PS, white light for 180 min, almost 100% killing rate	ACS Sustainable Chem. Eng. 2018, 6, 15064−15071
	HeLa cell: 12 μM PS, 808 nm laser for 10 min, 80% killing rate	Chem. Commun., 2019, 55, 1450−1453
	HeLa cell: 10 μM PS, white light for 10 min, 90% killing rate	ACS Nano 2018, 12, 8145−8159

	*S. aureus*: 2 μM PS, white light for 20 min, almost 100% killing rate *E. coli*: no killing effect	J. Am. Chem. Soc, 2019, 141, 16781-16789
	*S. epidermidis* and *E. coli*: 10 μM PS, room light for 60 min, 99% killing rate	ACS Appl. Mater. Interfaces 2015, 7, 7180
	HeLa cell: 1 μM PS, white light for 5 min, 95% killing rate, *B. subtilis*: 0.5 μM PS, white light for 5 min, up to 97% killing rate, *E. coli*: 5 μM PS, white light for 5 min, almost 97% killing rate, More efficient cancer ablation, Excellent antibacterial activity.	This work