Electronic Supplementary Information

# Synthesis of AIEgen Functionalized Cucurbit[7]uril for Subcellular Bioimaging and Synergistic Photodynamic Therapy and Supramolecular Chemotherapy

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## 1. *Reagents and chemicals*

The intact cucurbituril was prepared in our lab in grams scale according to a well-developed method.[1, 2] Fetal bovine serum (FBS) was purchased from Gibco (Life Technologies). 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), penicillin-streptomycin solution, 9,10-Anthracenediyl-bis(methylene)dimalonic acid (ABDA) and 2',7'-Dichlorofluorescin diacetate (DCFH_DA) were purchased from Sigma-Aldrich. Dulbecco's modified essential medium (DMEM) and PBS were commercial products from Beyotime Biotechnology Inc. All other chemicals and reagents were purchased from Sinopharm Chemical Reagent Co., Ltd without further purification. All reactions were performed in an inert gas atmosphere.

## *2. Instrumentation and methods*

The NMR spectra are obtained on Bruker 600 MHz ASCENDTM. ESI-MS (high-resolution) characterizations were executed on Thermo LTQ Orbitrap XL hybrid FTMS. We record UV-Vis absorption signals on HACH DR6000 spectrophotometer in 10 mm path quartz cell. Besides, the photoluminescence spectra of samples are measured on Horiba fluoroMax-4 spectrofluorometer. The characterization of fourier transform infrared spectra (FTIR) are executed on an IR Spectrometer (Spectrum Two, Perkin Elmer) by using potassium bromide and the scanned ranges were set from 4000 to 500 $cm^{-1}$ wavenumbers. Transmission electron microscopy (TEM) characterization is performed on Hitachi HT7700 microscope operating at 100 kV accelerating voltage. We acquire the hydrodynamic sizes of nanoparticles on Zetasizer Nano ZS, Malvern Instruments Inc. The pH values were obtained from pH meter (SevenCompact™ pH/Ion meter s220, Mettler Toledo). The light source applied in photodynamic therapy is generated from a 450 nm laser (model: OX-4502P) of OXlasers Inc. Laser confocal scanning microscope (CLSM) images are obtained from Lecia TCS SP8.

### *3. Cell culture and MTT assays*

A549 cells and LO2 cells were cultured in DMEM containing 10% FBS and 1% penicillin streptomycin at 37 °C in a humidified environment containing 5% CO2. Before experiment, the cells were pre-cultured until confluence was reached about 80%.

MTT assays were performed to assess the cytotoxicity of AIECB7 and AIECB[7]+oxaliplatin on A549 and LO2 cells. A549 and LO2 cells were seeded in 96-well plates at an intensity of $2\times10^4$ cells/mL. After 48 h incubation, the medium was replaced by AIECB[7] and AIECB[7]+oxaliplatin solutions at the concentration from 2.5 μM to 40 μM, and the cells were then incubated for another 24 h. After the designated time interval, the wells were washed once with 1×PBS buffer and freshly prepared MTT solution was added into each well. The absorbances of MTT at 570 nm were monitored by SpectraMax® M5 Microplate Reader. Cell viabilities were expressed by the ratio of the absorbances of the cells incubated with AIECB[7] and AIECB[7]+oxaliplatin solutions to that of the cells incubated with culture medium only.

### *4. Establishment of multicellular tumor spheroids (MSC)*

A549 monolayer cells were plated on Corning® ultra-low attachment surface plates, and the culture medium was replaced every 3 days. About 7 days later, the multicellular tumor spheroid models (MCTS) had formed with a homogeneous diameter around 300 μm. These MCTS were incubated with 40 μM AIECB[7] +AQ4N, AIECB[7] and AQ4N, respectively for 24 and 48 hours. The images of MCTS were acquired on Inverted Microscope Solution DMi8 S Platform, and the 3D version images were obtained by using the Z-Stack scanning from top to bottom of tumor spheroid at 1 μm interval. The PDT assays on MCTS were executed under 450 nm light irradiation at 150 $mW/cm^2$ for 4 mins. The cell viabilities of MCTS were distinguished by staining with calcein AM (indicating live cells) and propidium iodide (indicating dead cells).

## *5. Synthesis details*

### Synthesis of Monopropargyloxy CB[7] (CB[7]-alkyne)

The CB[7]-OH was prepared by following reported literatures[3] with slightly modification (characterized in Figure S1, $^1$H NMR, 600 MHz, $D_2O$). The synthesis of Monopropargyloxy CB[7] referred to the approach of our previous synthesized Monoallyloxy CB[7] ( $AO_1$-CB[7]).[4] Briefly, CB[7]-OH (300 mg, 0.254 mmol, 1 eq) was added into 25 mL anhydrous DMSO with sonication to dissolve all the CB[7]-OH, then NaH (100 mg, 2.5 mmol, 10 eq, 60% in mineral oil) was added into the solution under inert gas atmosphere at 0$^o$C and stirred at room temperature (R.T.) for 2 hours. Propargyl bromide (121 mg, 1.0 mmol, 4 eq) was slowly added via syringe at 0$^o$C, then the mixture was stirred at R.T. for 12 hours. The mixture was poured into methanol, and the precipitate was collected and washed with methanol 4 times to remove the impurities thoroughly. At last the residue was dried affording the desired product as fluffy light brown solid 253.5 mg, yield: 82%. $^1$H NMR (600 MHz; $D_2O$+NaCl puris, Figure S2): $\delta_H$ 5.82-5.80 (d, 2H), 5.75-5.71 (m, 12H), 5.65-5.57 (m, 14H), 4.52-4.64 (m, 2H), 4.35-4.25 (m, 13 H), 2.65 (m, 1H). $^{13}$C NMR (150 MHz; $D_2O$, Figure S3): 156.88, 155.46, 97.41, 77.57, 71.52, 52.61, 49.34, 47.51. ESI-MS (high-resolution, Figure S4) m/z: [Monopropargyloxy CB[7]+diaminohexane]$^{2+}$, calcd for $C_{51}H_{62}N_{30}O_{15}$, 667.2505; found, 667.2523. FTIR (KBr, Figure S16) cm$^{-1}$, 3437 (CO-N), 3280 (C≡C-H), 2118 (-C≡C-), 1736 (C=O).

### Synthesis of TPE-Br

4, 4'-dimethoxybenzophenone (1.274 g, 5.15 mmol), 4-Bromobenzophenone (1.344 g, 5.15 mmol), zinc dust (1.346 g, 20.6 mmol) and 80 mL anhydrous THF were added into a 250 mL Schlenk flask, then the mixture was stirred at -78$^o$C. 2.26 mL titanium tetrachloride (20.6 mmol) was dropwise injected into the reaction in 20 mins. After the flask was warmed to room temperature, it was heated at 80 $^o$C for 5 hours. The reaction

was quenched with $K_2CO_3$ aqueous solution and extracted with ethyl acetate. The organic phase was dried with anhydrous $MgSO_4$ and the volatile was removed by rotary evaporator. The final residue was refined with silica gel column chromatography (PE: DCM = 4: 1) affording the TPE-Br as a yellowish solid in 29% yield (704.0 mg). $^1$H NMR (600 MHz; $CDCl_3$, Figure S5): $\delta_H$ 7.21-7.20 (d, 2H), 7.10-7.08 (m, 2H), 7.00-6.99 (m, 2H), 6.93-6.87 (m, 5H), 6.66-6.64 (d, 2H), 6.62-6.61 (d, 2H), 3.74 (s, 3H), 3.71 (s, 3H). $^{13}$C NMR (150 MHz; $CDCl_3$, Figure S6): 158.32, 143.84, 143.35, 140.83, 137.94, 136.06, 133.10, 132.60, 131.39, 130.90, 127.86, 126.35, 120.07, 113.25, 55.15.

**Synthesis of TPEPy**

To a 250 mL Schlenk flask, TPE-Br (839.57 mg, 1.786 mmol, 1eq) and 4-pyridinylboronic acid (241.53 mg, 1.965 mmol, 1.1 eq), tetrabutylammonium bromide (TBAB, 57.5 mg, 0.178 mmol, 0.1 eq) and $Pd(PPh_3)_4$ (60 mg, 0.052 mmol) were added, dissolved with 50 mL anhydrous THF and stirred under $N_2$ atmosphere. 3 mL $K_2CO_3$ aqueous solution (2M) was injected, then the reaction was set at 80 $^o$C for 5 hours. After cooling to room temperature, the reaction was quenched with water and extracted with ethyl acetate. The organic phase was collected and underwent a desiccation with anhydrous $MgSO_4$, then the volatile was removed under reduced pressure. The final residue was purified with silica gel column chromatography (EA: DCM=1:1) affording the TPEPy as a yellow solid in 87% yield (729.6 mg). $^1$H NMR (600 MHz; $CDCl_3$, Figure S7): $\delta_H$ 8.61-8.60 (d, 2H), 7.47-7.46 (d, 2H), 7.41-7.40 (d, 2H), 7.14-7.12 (m, 2H), 7.06-7.05 (m, 2H), 6.99-6.94 (m, 4H), 3.74 (d, 2H). $^{13}$C NMR (150 MHz; $CDCl_3$, Figure S8): 158.31, 150.13, 147.89, 145.54, 144.02, 141.03,138.32, 136.12, 135.22, 132.65, 132.16, 131.43, 127.85, 126.18, 121.28, 113.19, 55.11.

**Synthesis of TPEPy-$N_3$ (AIEgen)**

The TPEPy-$N_3$ was synthesized by covalently connecting the TPEPy part and 1-azido-3-bromopropane part according a reported literature.[5] TPEPy (568.2 mg, 1.21 mmol, 1 eq) and 1-azido-3-bromopropane (992.26 mg, 6.05 mmol, 5 eq) were dissolved in 8 mL

anhydrous DMF and heated at 100 $^{o}$C under $N_2$ atmosphere for 24 hours. After cooling to room temperature, the mixture was directly transferred to silica gel column for separating the desired product (DCM: MeOH= 10:1). The obtained bromide salt of TPEPy-$N_3$(Br) was then dissolved in $H_2O$ and subsequently treated with $KPF_6$ resulting in precipitation of the $PF_6^-$ salt. The desired compound was then separated from the aqueous solution by centrifugation and dried under air, yielding in TPEPy-$N_3$ as an orange powder 682.3 mg, yield in 89%. $^1$H NMR (600 MHz; $CDCl_3$, Figure S9): $\delta_H$ 9.41-9.40 (d, 2H), 8.12-8.11 (d, 2H),7.55-7.54 (m, 2H), 7.23-7.22 (d, 2H), 7.15-7.14 (m, 3H), 7.03-7.02 (m, 2H), 6.96-6.93 (m, 4H), 5.13-5.06 (t, 2H), 3.75 (s, 6H), 3.60-3.47 (m, 2H), 2.74-2.39 (m, 2H). $^{13}$C NMR (150 MHz; $CDCl_3$, Figure S10): 160.80, 158.74, 158.53, 156.21, 149.98, 144.85, 143.37, 142.88, 137.37, 135.51, 133.06, 132.71, 132.63, 131.39, 130.34, 128.10, 127.20, 126.70, 124.28, 113.41, 113.10, 58.26, 55.20, 48.01, 30.83. ESI-MS (high-resolution, Figure S11) m/z: $[AIEgen]^+$, calcd for $C_{91}H_{92}N_{33}O_{17}$, 553.2598; found, 553.2541.

**Synthesis of AIECB[7]**

Monopropargyloxy CB[7] (230 mg, 0.189 mmol), TPEPy-$N_3$ (120 mg, 0.172 mmol), $CuSO_4$-$5H_2O$ (51.6 mg, 0.206 mmol) and DMSO (25 mL) were added to a 125 mL Schlenk flask. The system was degassed by freeze-pump-thaw cycles for three times, then sodium ascorbate (68.2 mg, 0.344 mmol) aqueous solution was added via syringe and stirred at 80 $^{o}$C for 24 h. After the mixture was cooled down, it was presented to centrifugation for removing the precipitates of Cu compounds. The mixture in DMSO was dispersed in 50 mL water and dialyzed against Milli-Q water with 2k Da molecular weight cutoff dialysis membrane for 5 times in 72 h. During the 5 times in 72 hours, the pH values of the dialysis solvent were adjusted from acid to neutral gradually in order to further removing Cu-based catalyst, respectively. The substances in dialysis tube were lyophilized, and the obtained fluffy solid was triturated with methanol for 3 times to remove the hydrophobic small organic molecules. At last, the obtained solid was dried in oven affording the final product as yellowish brown solid in 75% yield

(247.1 mg). $^{1}$H NMR (600 MHz; DMSO-d$_6$, 20% DCOOD added, Figure S13): $\delta_H$ 8.93 (d, 2H), 8.33 (d, 2H), 8.23 (s, 1H), 7.87 (m, 2H), 7.28 (m, 2H), 7.19-7.04 (m, 4H), 7.00-6.79 (m, 5H), 6.74-6.55 (m, 4H), 5.78-5.60 (m, 12H), 5.57-5.49 (m, 2H), 5.49-5.20 (m, 14H), 4.35 (m, 2H), 4.25-4.00 (m, 14H), 3.74-3.63 (m, 6H), 2.62 (m, 2H), 2.38 (m, 2H). Due to the complex structure and relative lower solubility in solvents, a precise characterization of $^{13}$C NMR was not possible. 2D HSQC ($^{1}$H-$^{13}$C, DMSO-d6, 10% DCOOD added) Figure S14. ESI-MS (high-resolution, Figure S15) m/z: [AIECB[7]+1-Adamantanamine hydrochloride]$^{2+}$, calcd for ($C_{91}H_{92}N_{33}O_{17}$), 960.8792; found, 960.8704.

## *6. Related Figures*

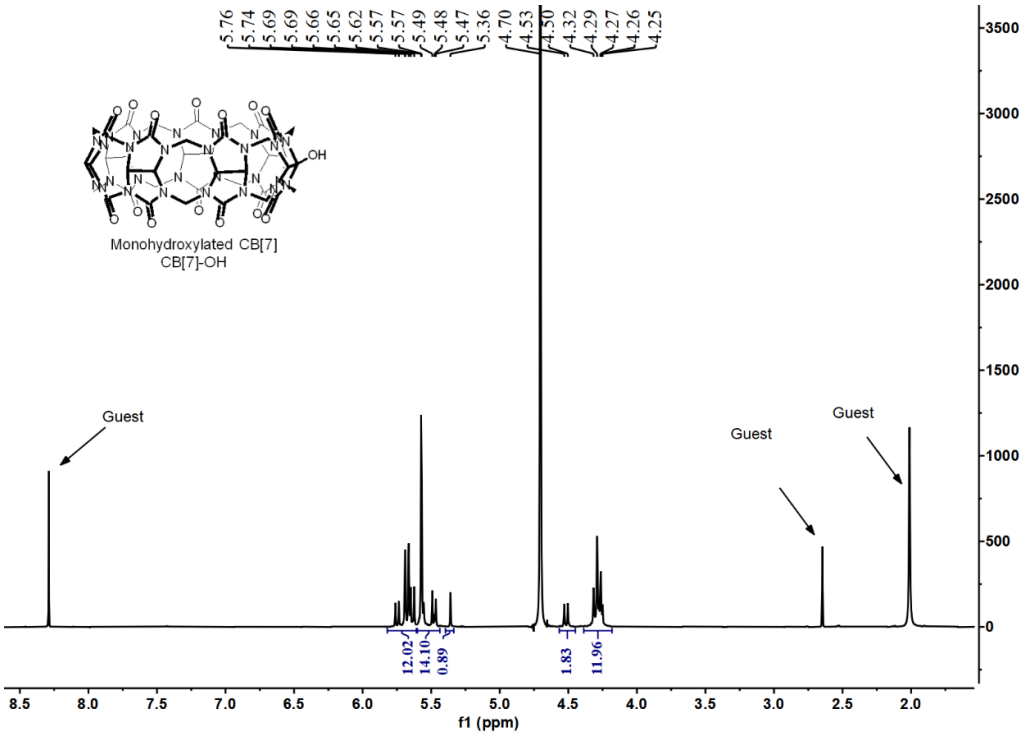


**Figure S1**. The $^{1}$H NMR spectrum of CB[7]-OH, guest: cystamine dihydrochloride.

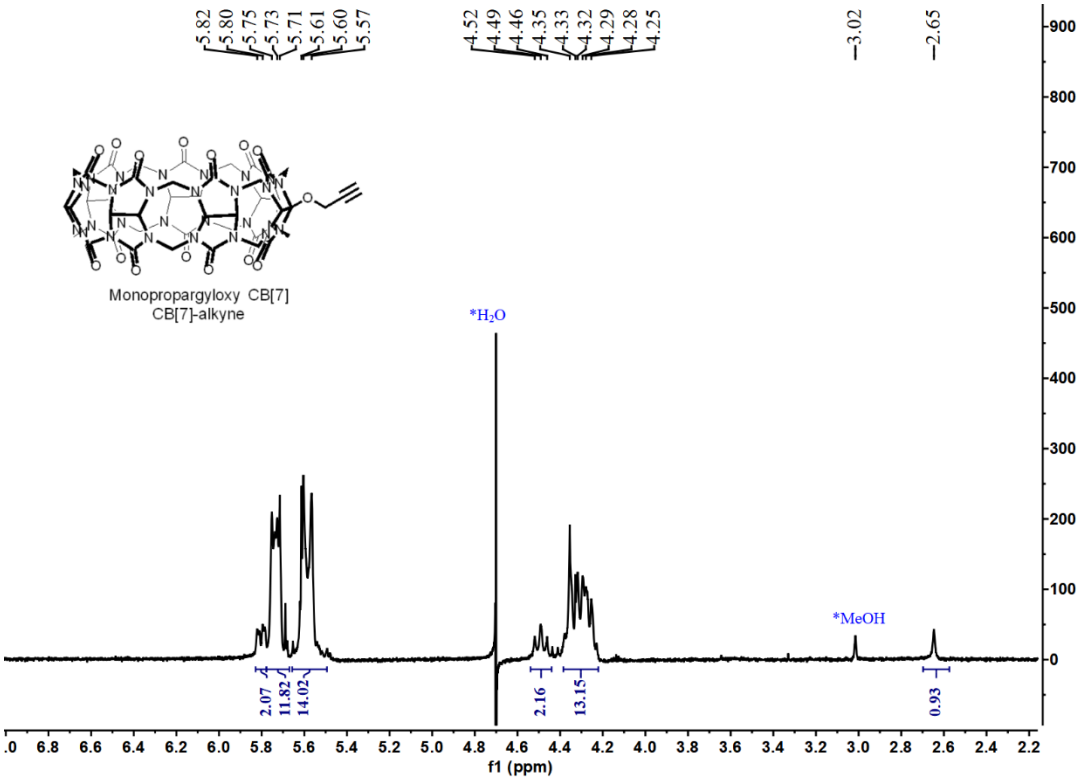


**Figure S2**. The $^{1}$H NMR spectrum of CB[7]-alkyne.

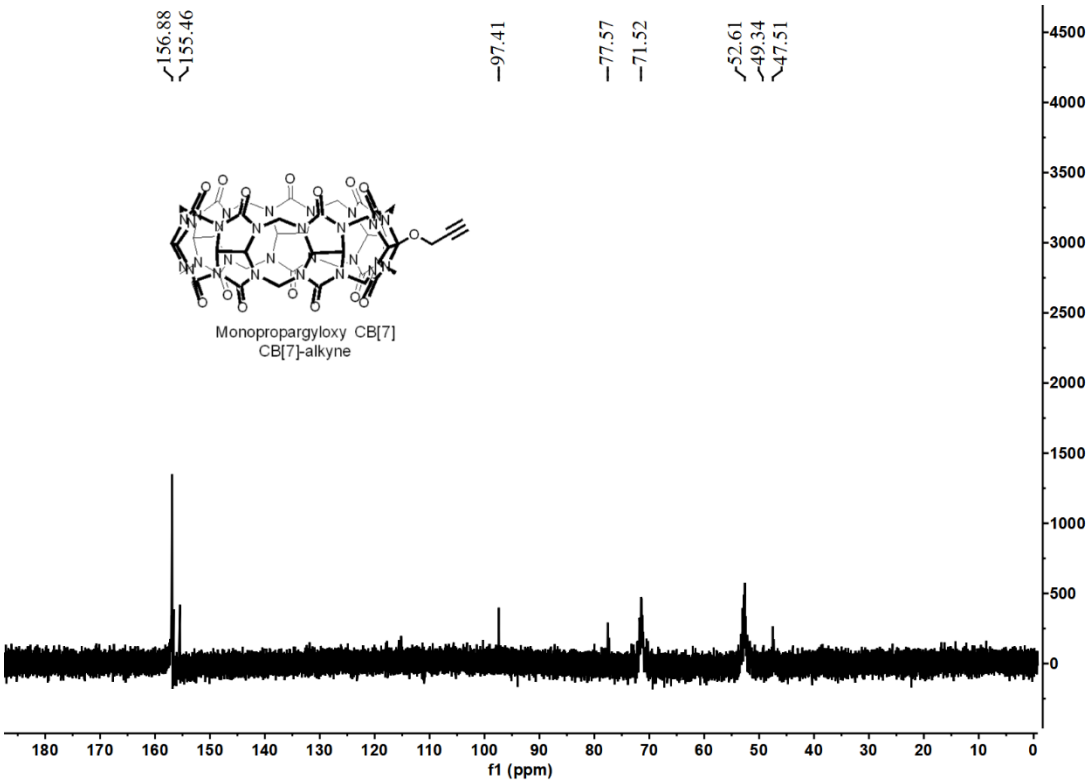


**Figure S3**. The $^{13}$C NMR spectrum of CB[7]-alkyne.

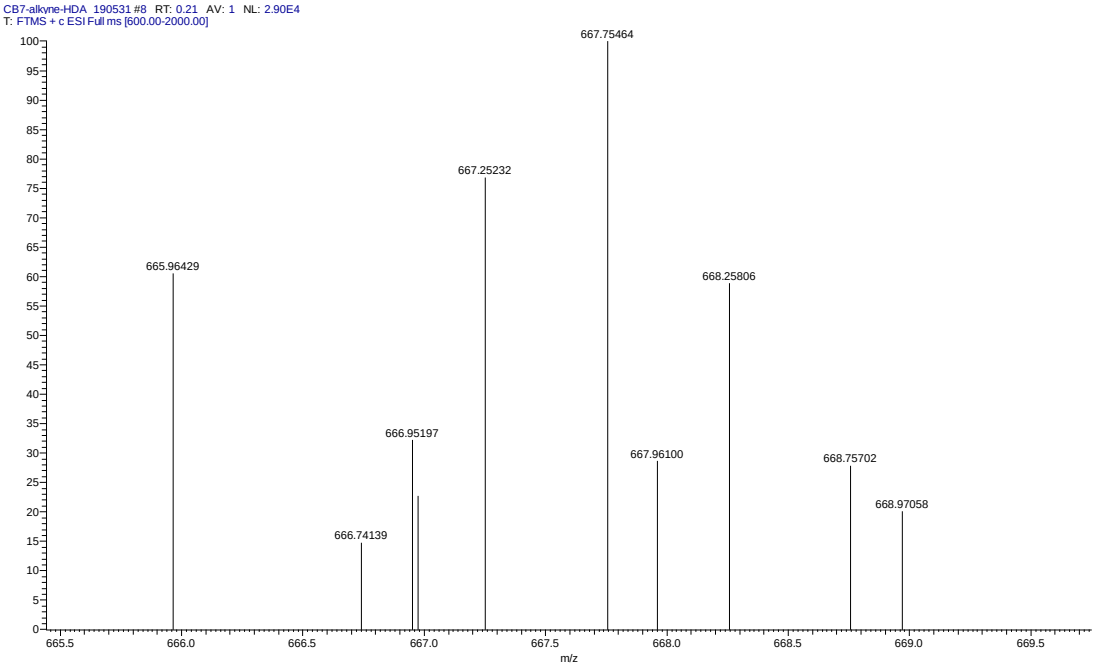


**Figure S4**. ESI-MS (high-resolution) spectrum of CB[7]-alkyne.

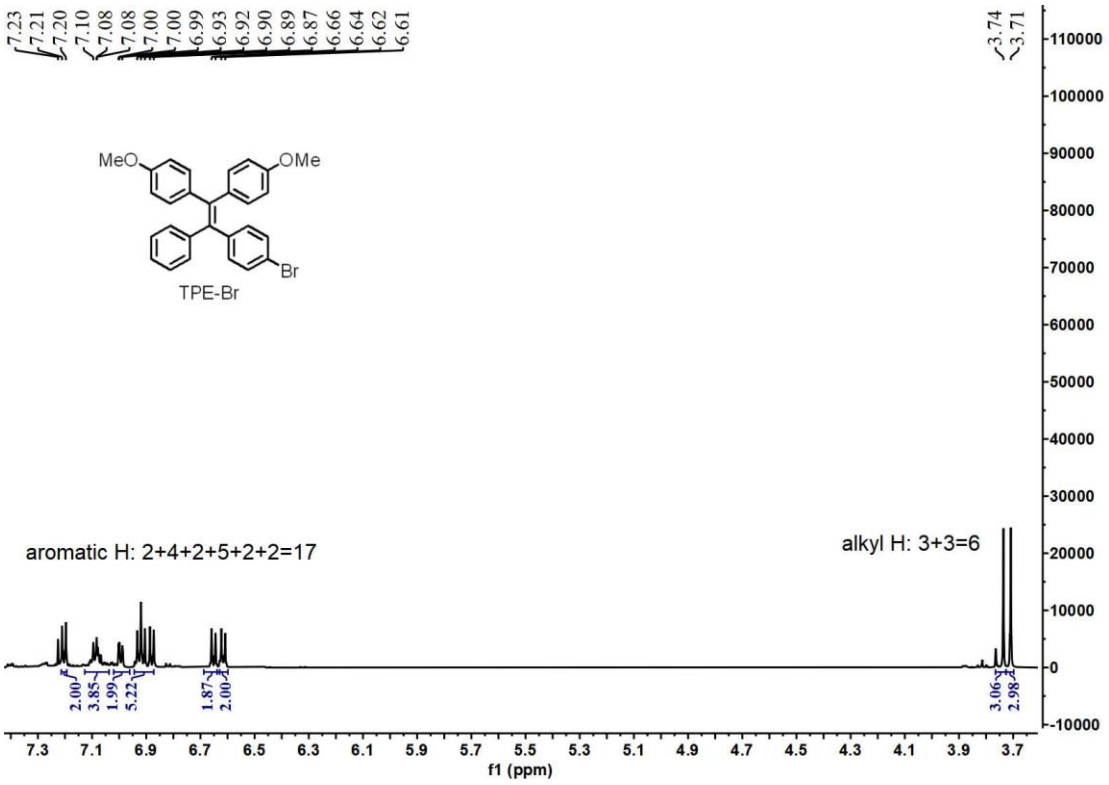


**Figure S5**. The $^{1}$H NMR spectrum of TPE-Br.

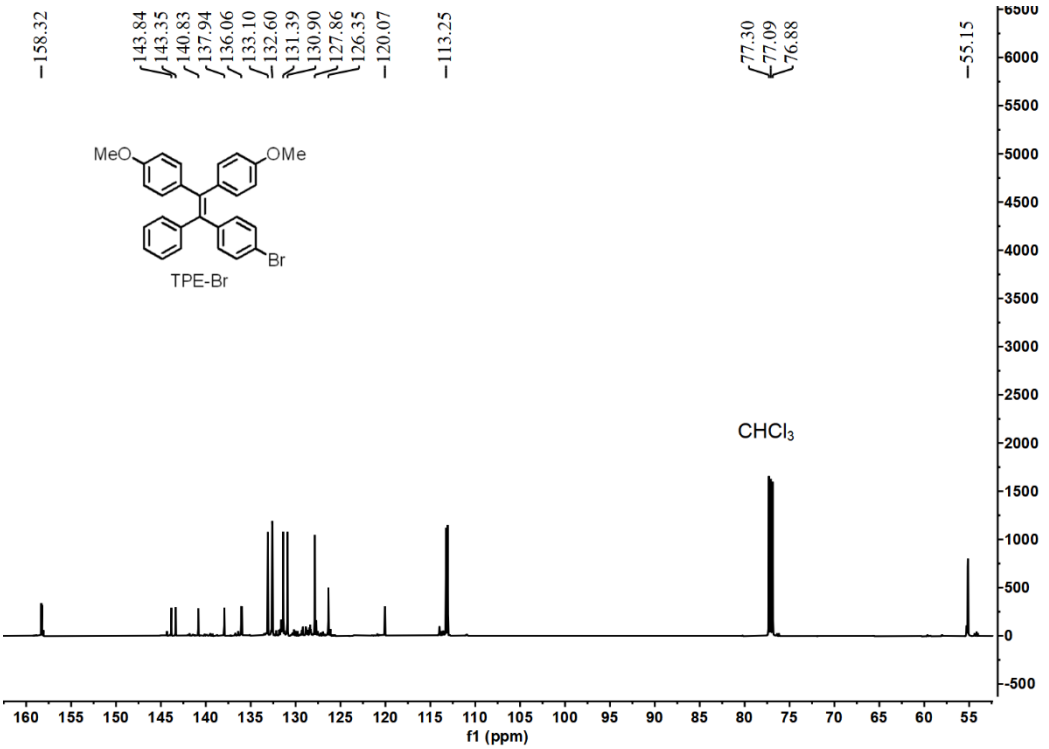


**Figure S6**. The $^{13}C$ NMR spectrum of TPE-Br.

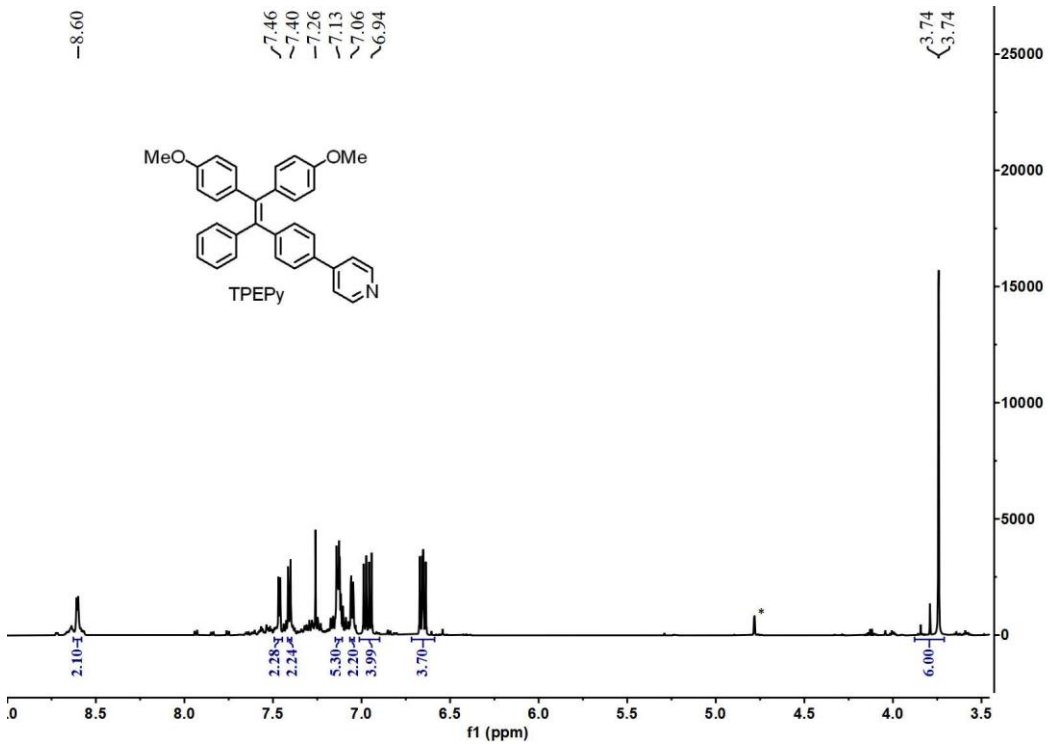


**Figure S7**. The $^{1}H$ NMR spectrum of TPEPy.

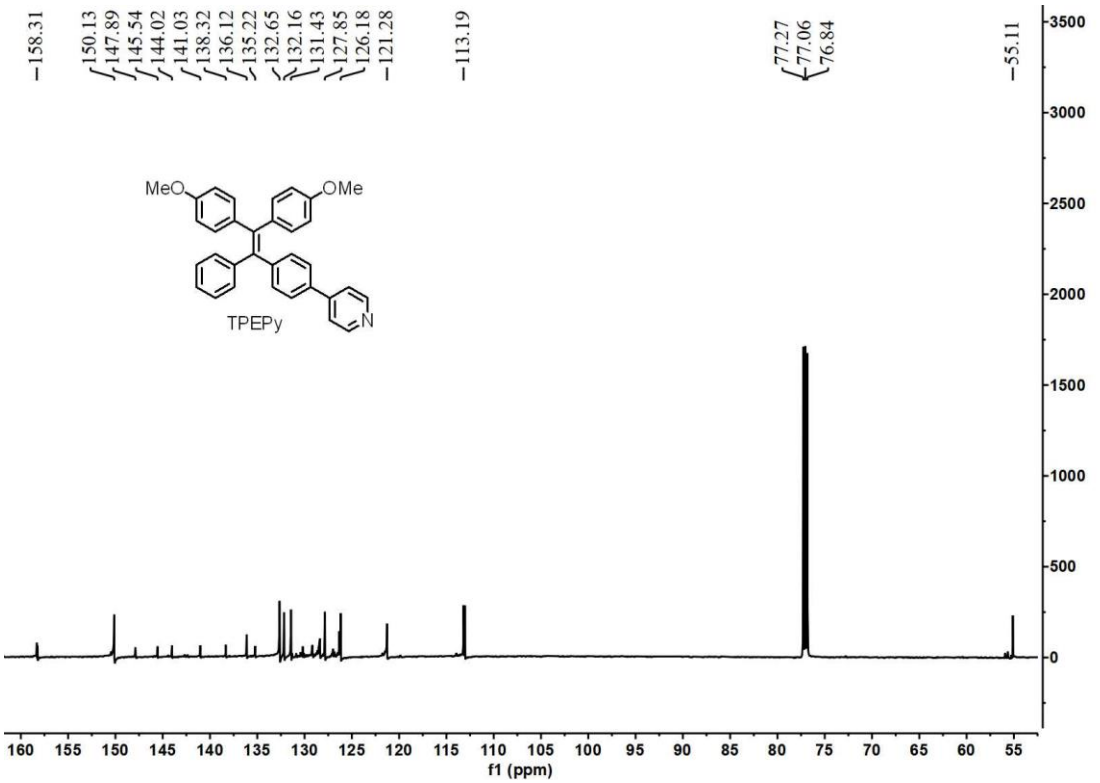


**Figure S8**. The $^{13}C$ NMR spectrum of TPEPy.

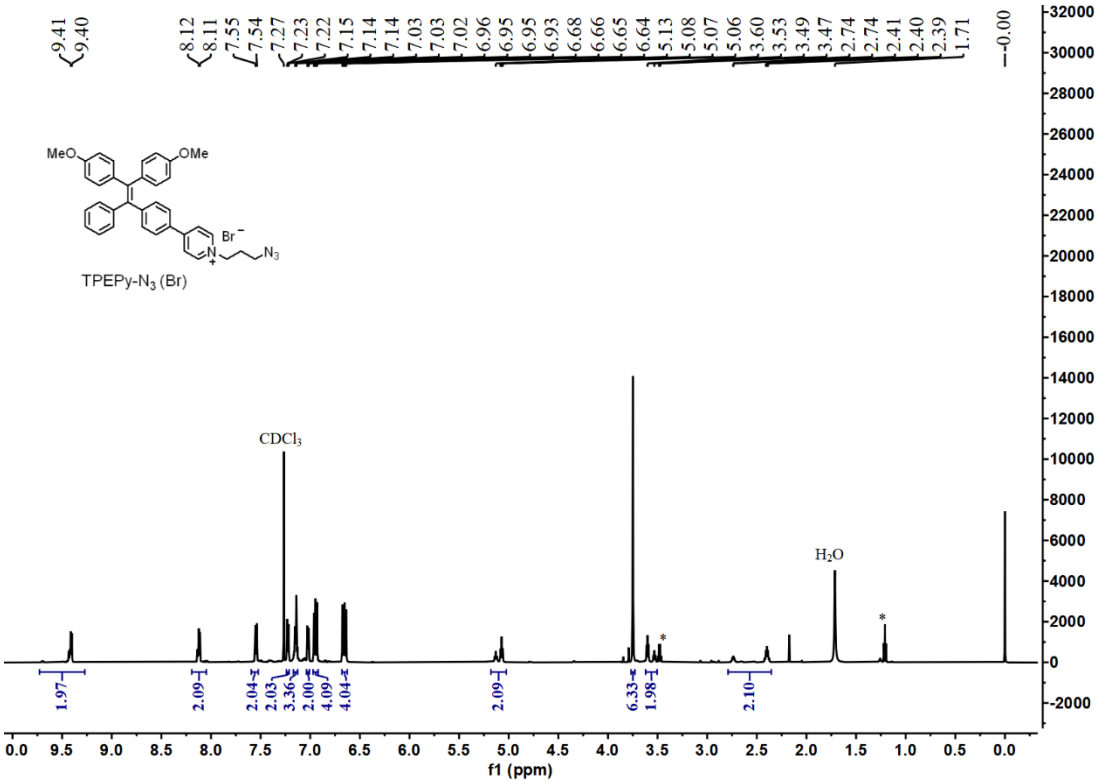


**Figure S9**. The $^{1}H$ NMR spectrum of TPEPy-$N_3$ (AIEgen). * represent trace diethyl ether.

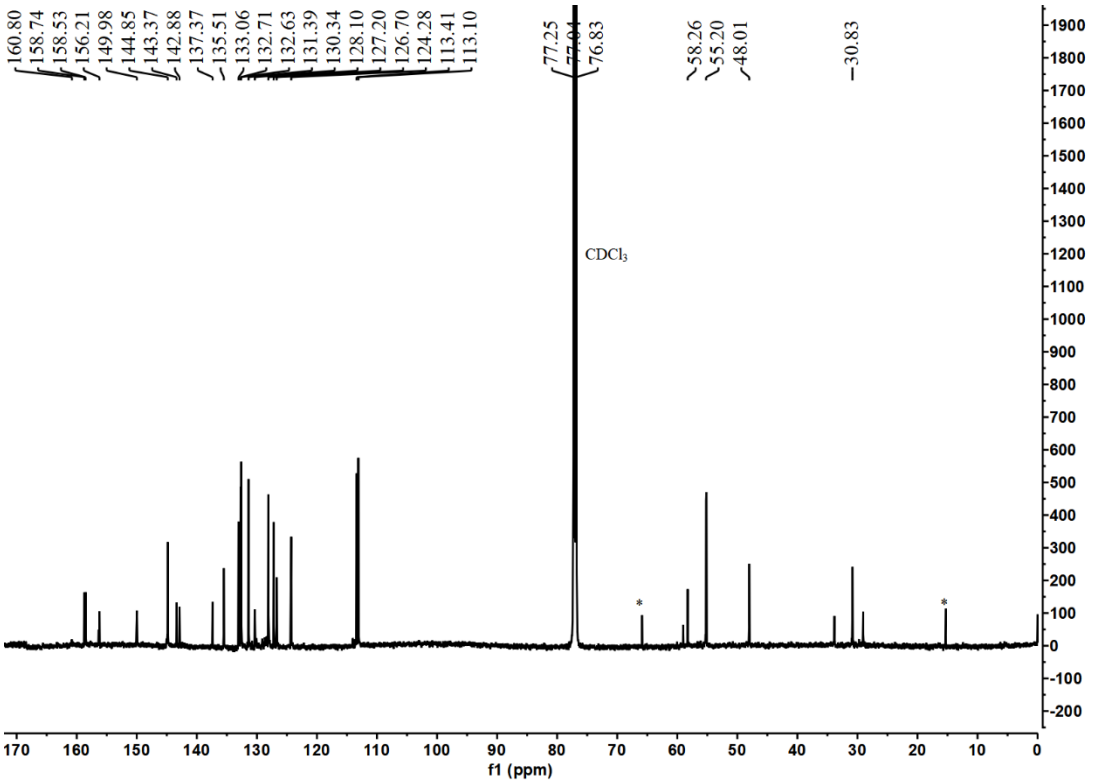


**Figure S10**. The $^{13}C$ NMR spectrum of TPEPy-$N_3$ (AIEgen). * represent trace diethyl ether.

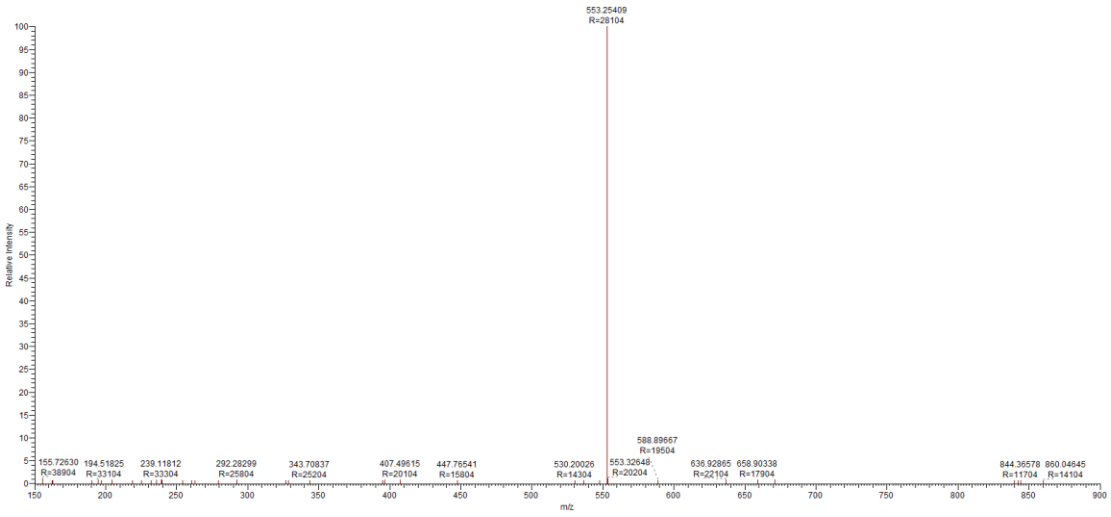


**Figure S11**. ESI-MS (high-resolution) spectrum of TPEPy-$N_3$ (AIEgen).

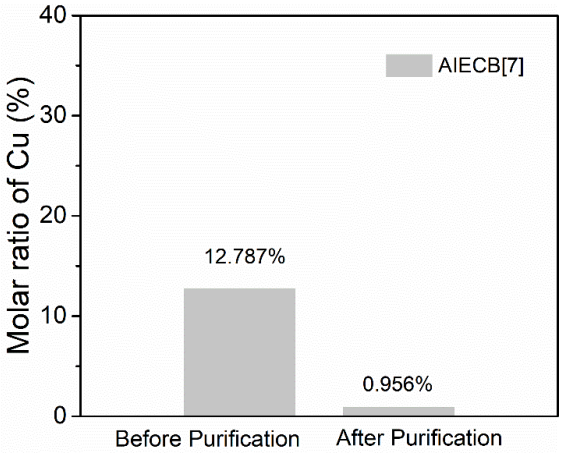


**Figure S12**. The molar ratio percentages of Cu elements in the samples of AIECB[7] indicated by ICP-MS.

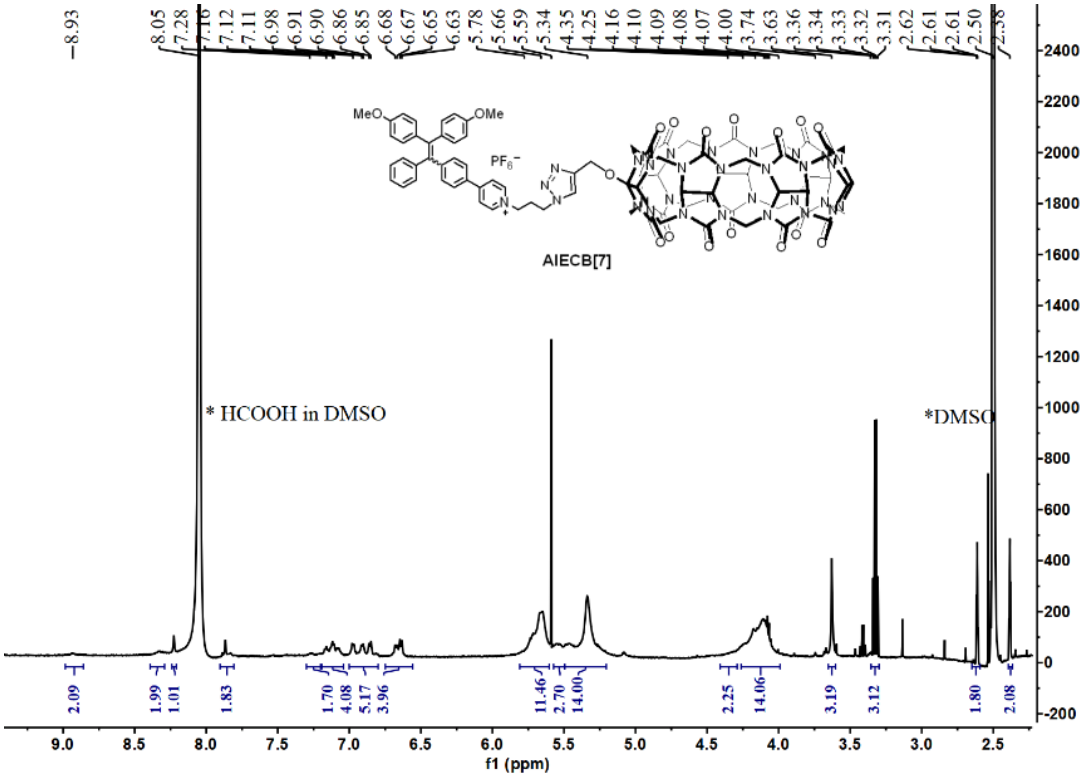


**Figure S13.** The $^{1}$H NMR spectrum of AIECB[7].

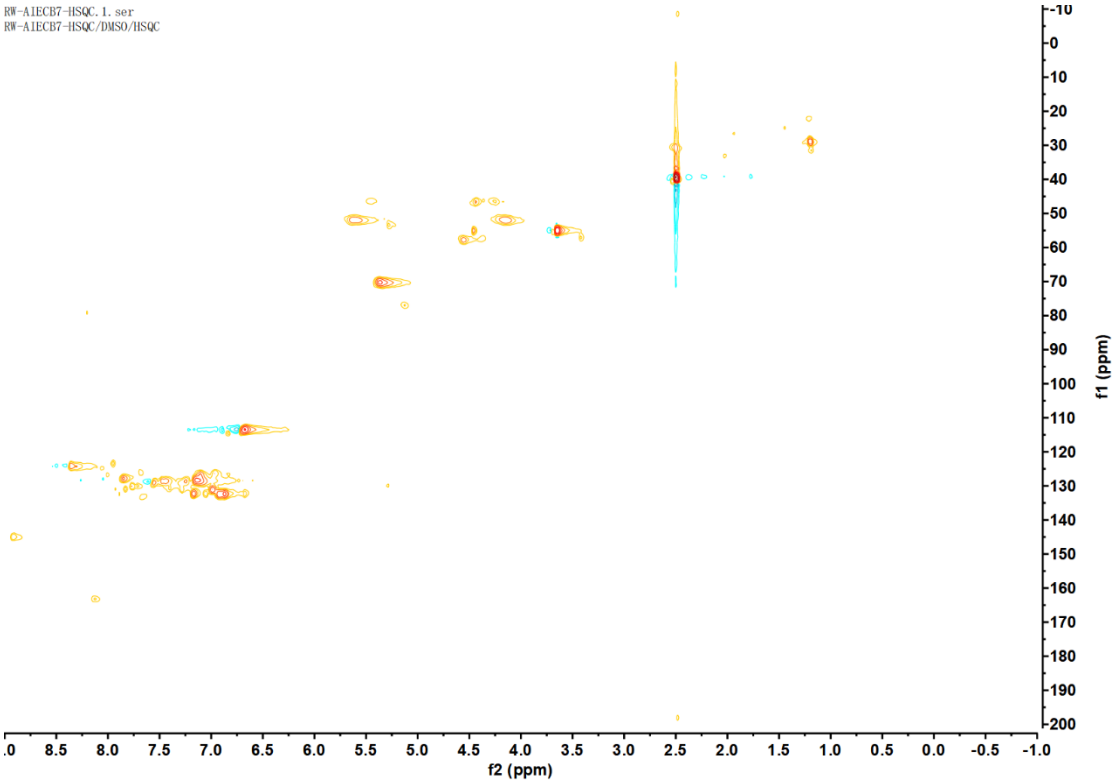


**Figure S14.** The $^{1}H$-$^{13}C$ 2D HSQC NMR spectrum of AIECB[7].

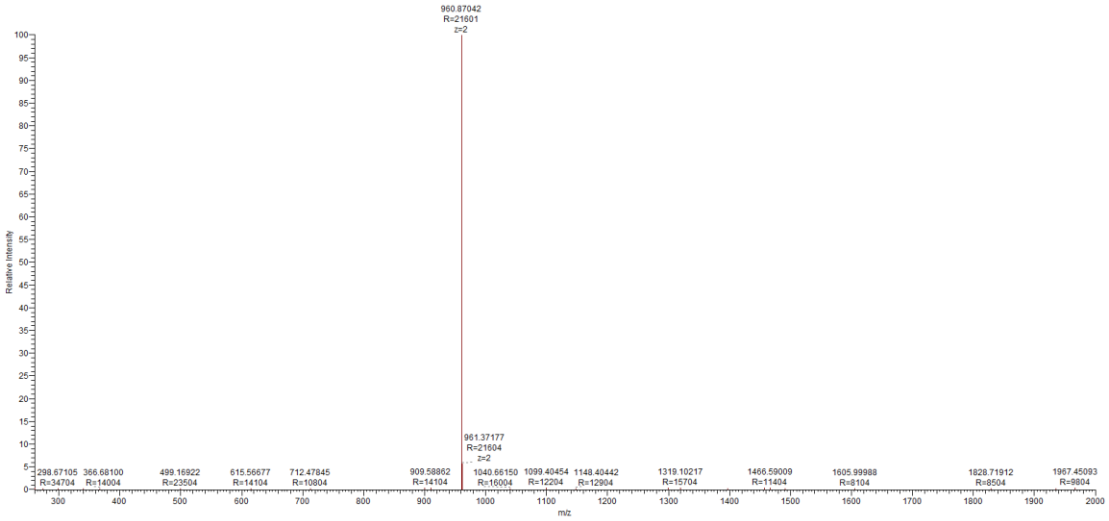


**Figure S15.** ESI-MS (high-resolution) spectrum of AIECB[7].

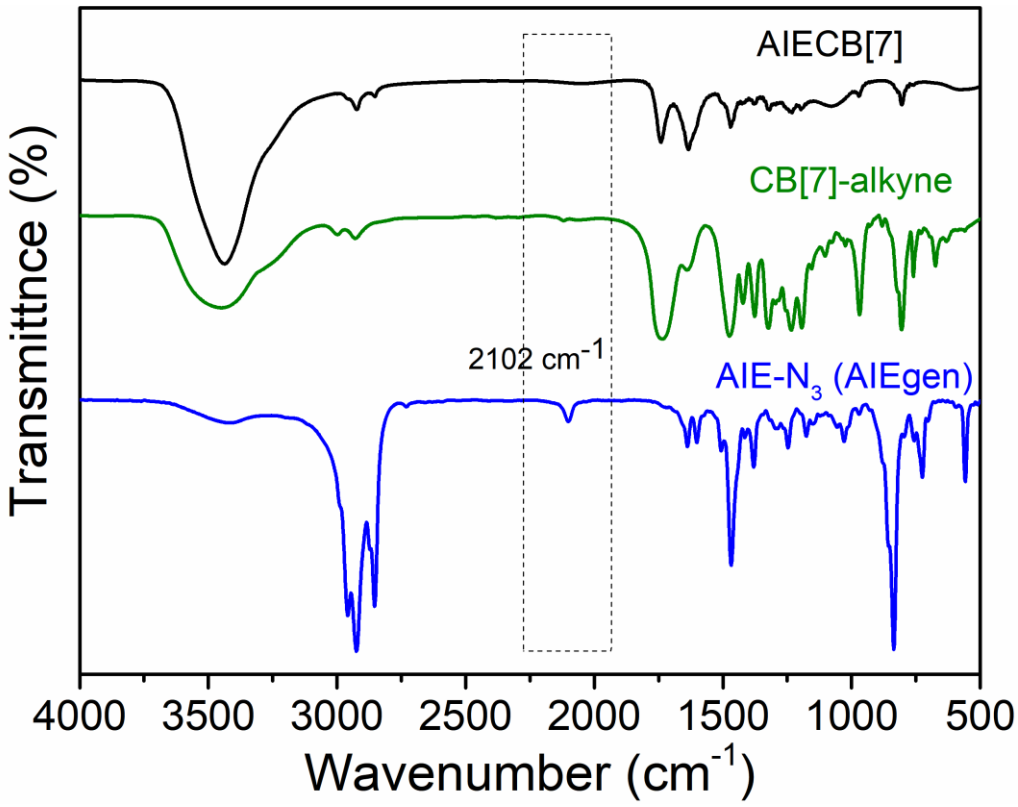


**Figure S16.** The FTIR spectrum of AIECB[7], CB[7]-alkyne and AIEgen.

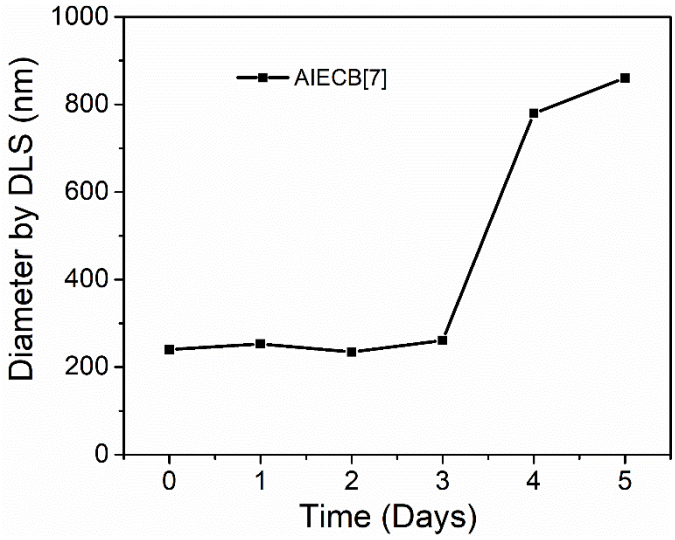


**Figure S17**. The water stability of AIECB[7] nanoaggregates in aqueous solution determined by DLS.

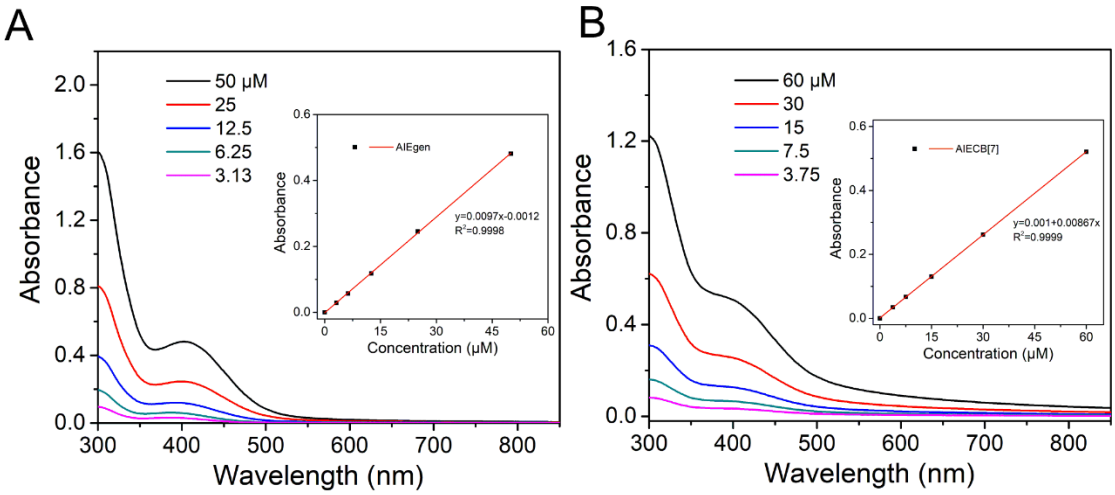


**Figure S18.** The UV-vis absorbances and standard curves based on the absorbances of AIEgen (1% THF contained, A) and AIECB[7] (B).

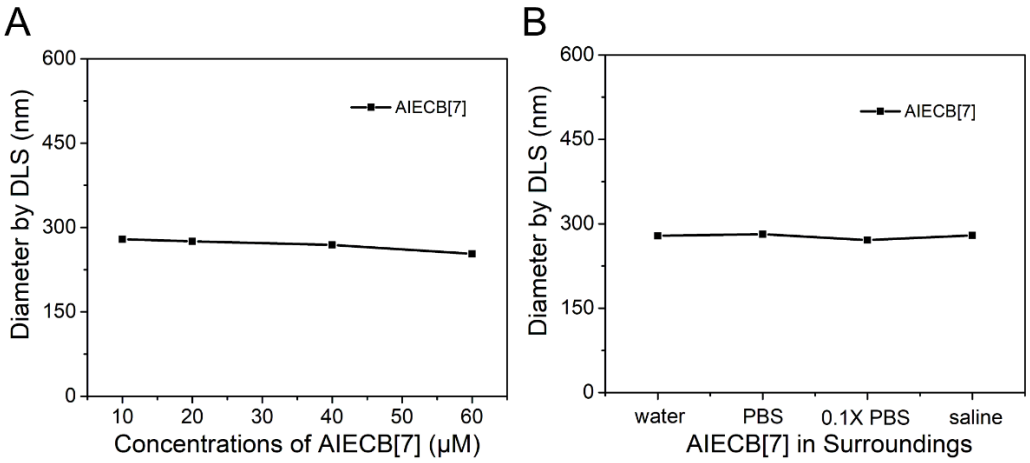


**Figure S19**. The particles sizes of AIECB[7] nano-assemblies at different concentrations (A) and in biologically relevant media (PBS, 0.1X PBS, saline) and water (B).

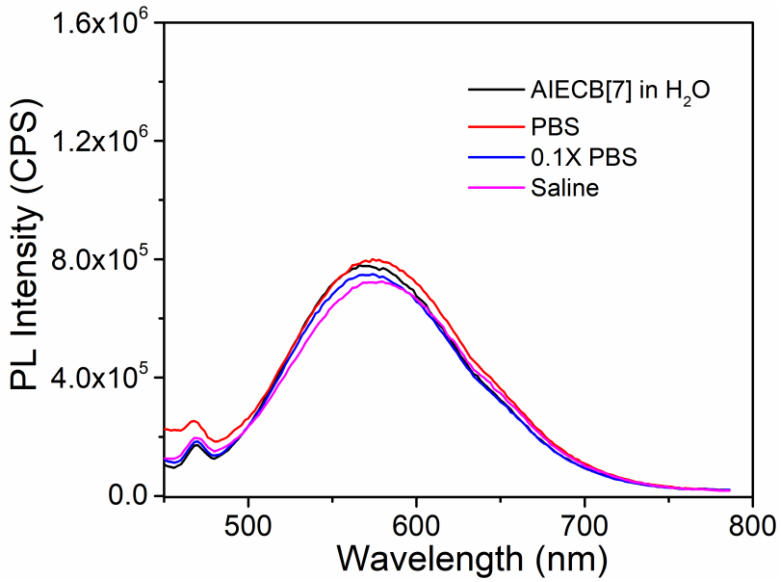


**Figure S20**. The PL intensities of AIECB[7] in $H_2O$, PBS, 0.1×PBS and saline.

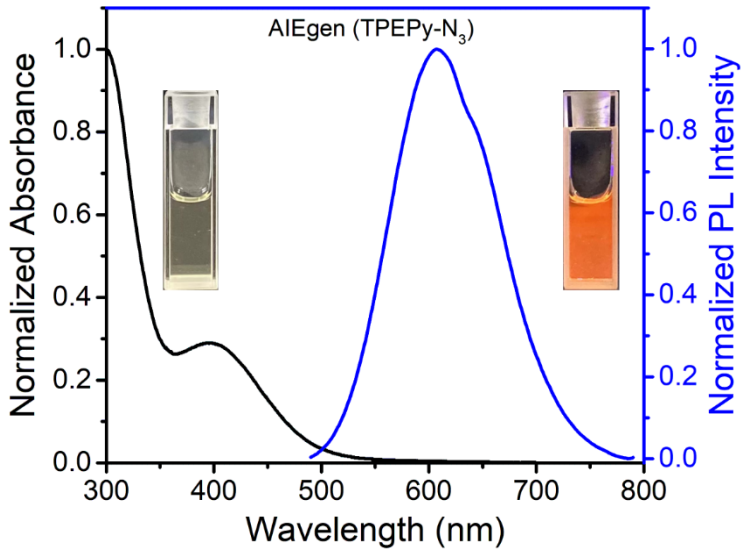


**Figure S21.** Normalized UV-vis absorbance and fluorescence spectrum of AIEgen (1% THF contained) in aqueous solution. The inset images are AIEgen aqueous solutions that were taken under white light (left) and 365 nm UV light irradiation (right).

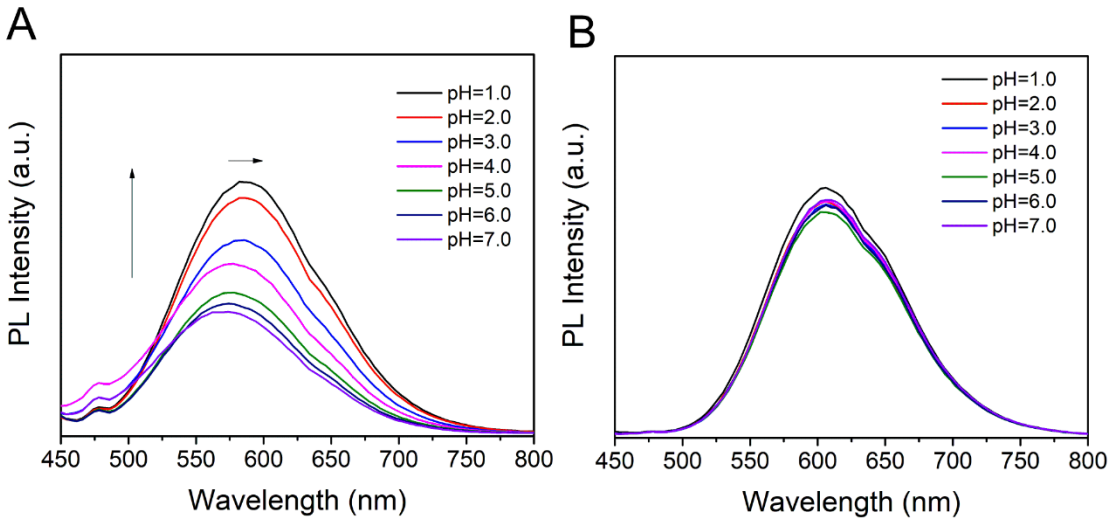


**Figure S22**. The PL intensities (a.u.) of AIECB[7] (A) and AIEgen (B) at different pH conditions (pH from 7.0 to 1.0).

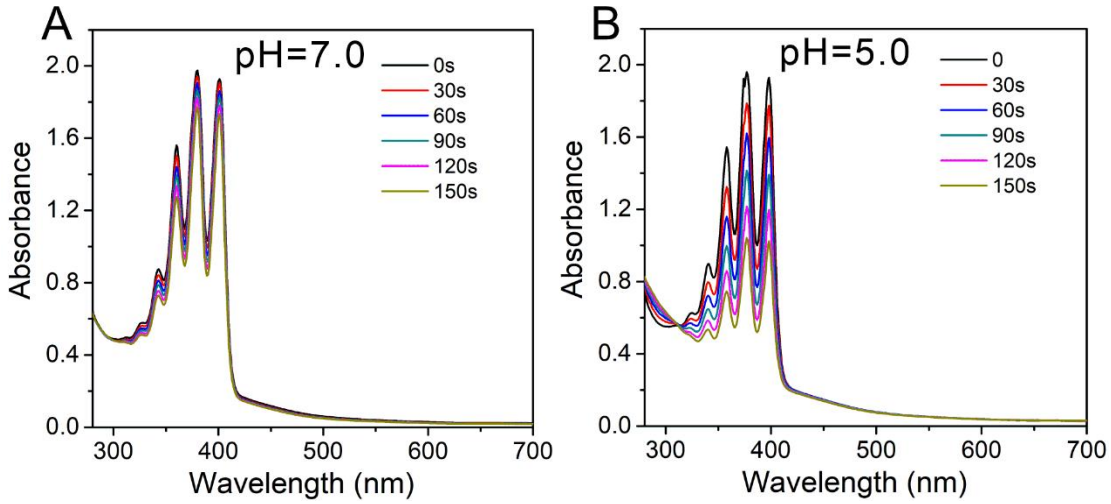


**Figure S23**. (A) The absorbance changes of ABDA in aqueous solutions of AIECB[7] (pH=7.0 (A) and pH=5.0 (B)) with extended light irradiation time.

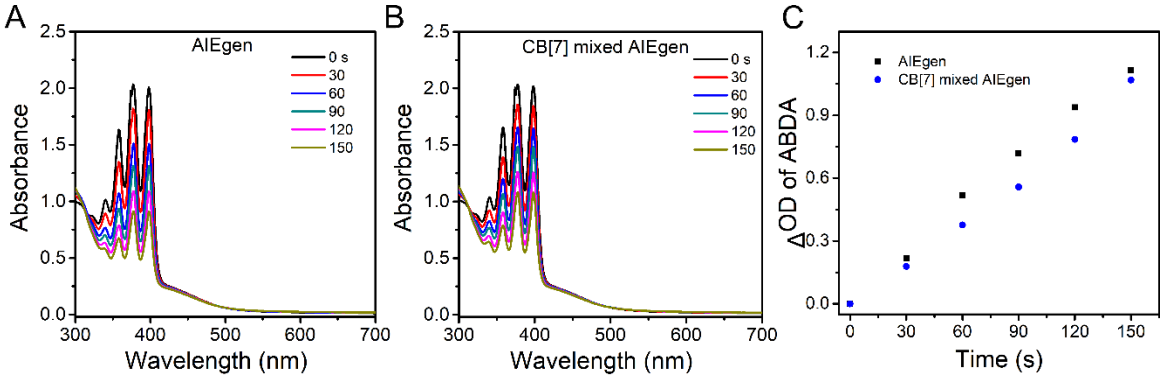

**Figure S24**. The absorbance changes of ABDA in aqueous solutions of AIEgen (A) and CB[7] mixed AIEgen (B) with extended light irradiation. (C) Singlet oxygen ($^1O_2$) generation of AIEgen and CB[7] mixed AIEgen by measuring the absorbance changes of ABDA at 378 nm.

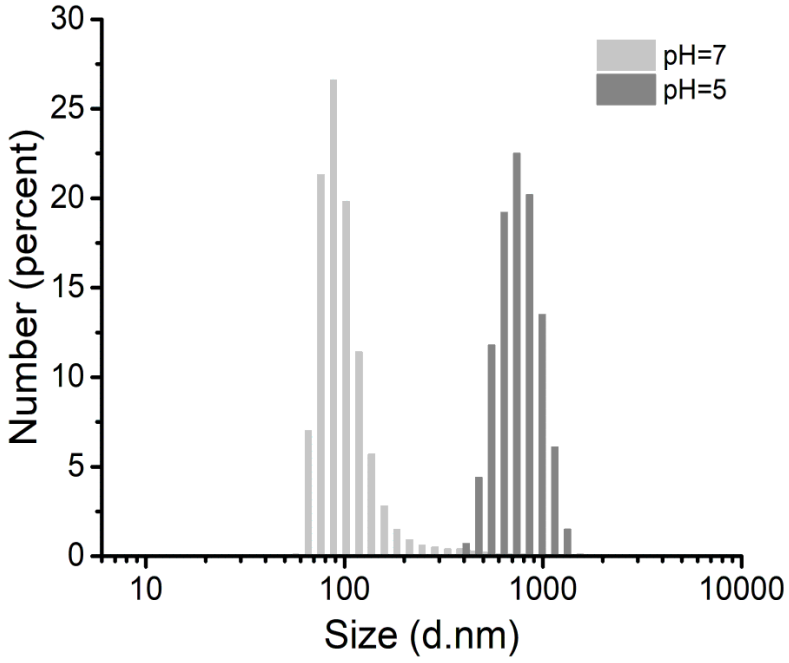


**Figure S25**. The DLS results of AIECB[7] nanoaggregates.

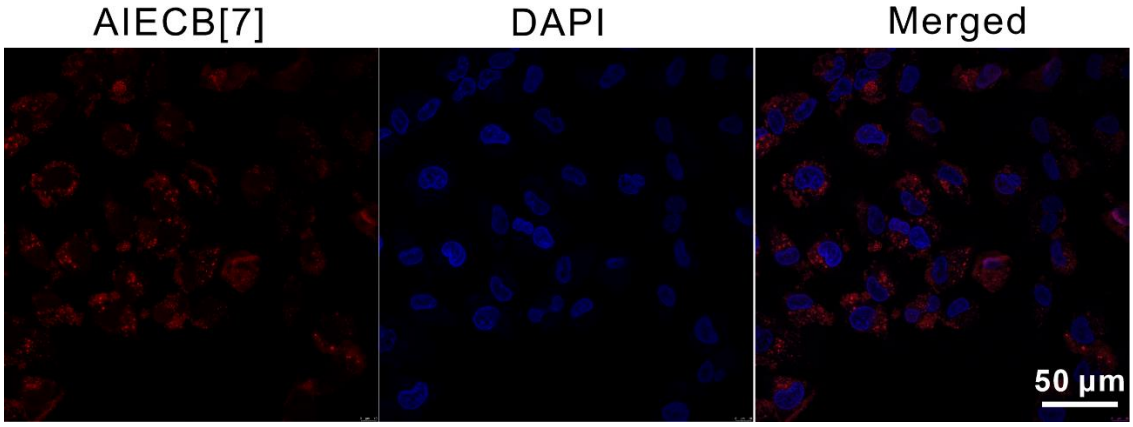


**Figure S26**. Confocal images of A549 cells after incubation with AIECB7 for 8 hours. The regions cell nuclei were stained with DAPI. Scale bars: 50 μm.

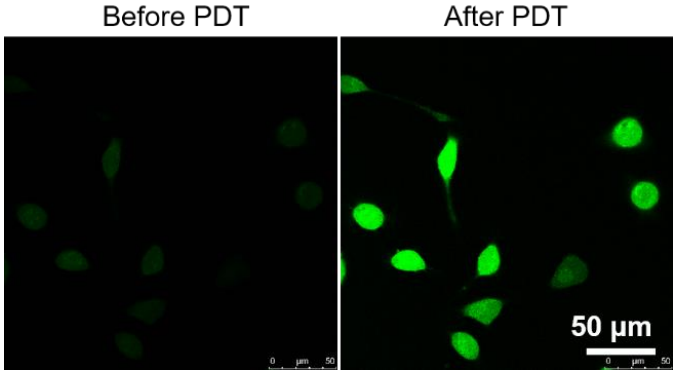


**Figure S27**. Intracellular singlet oxygen ($^1O_2$) level in A549 cells indicated by DCFH-DA staining before PDT and after PDT. Scale bars: 50 μm.

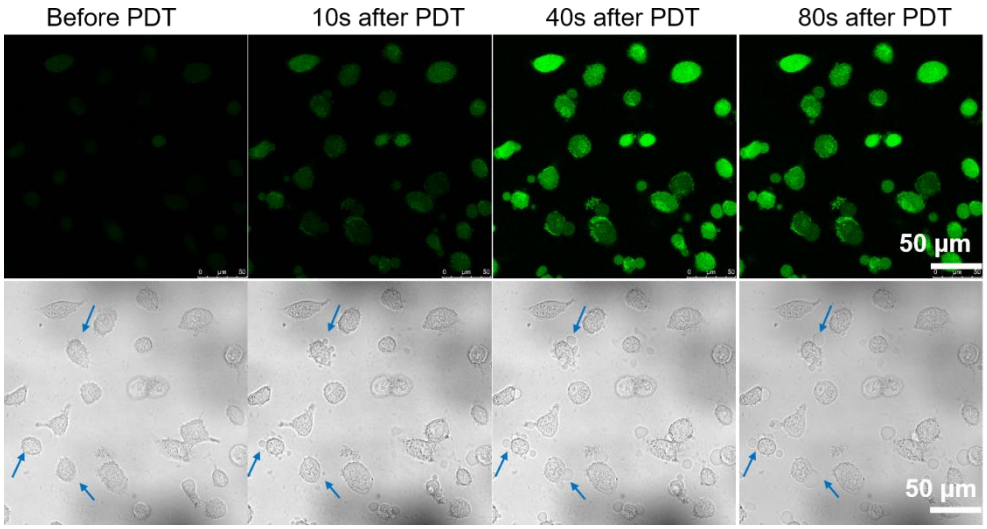


**Figure S28**. Confocal images of A549 cells involution after they were irradiated by 450 nm light at 150 mW/cm$^2$ for 3 min. Scale bars: 50 μm.

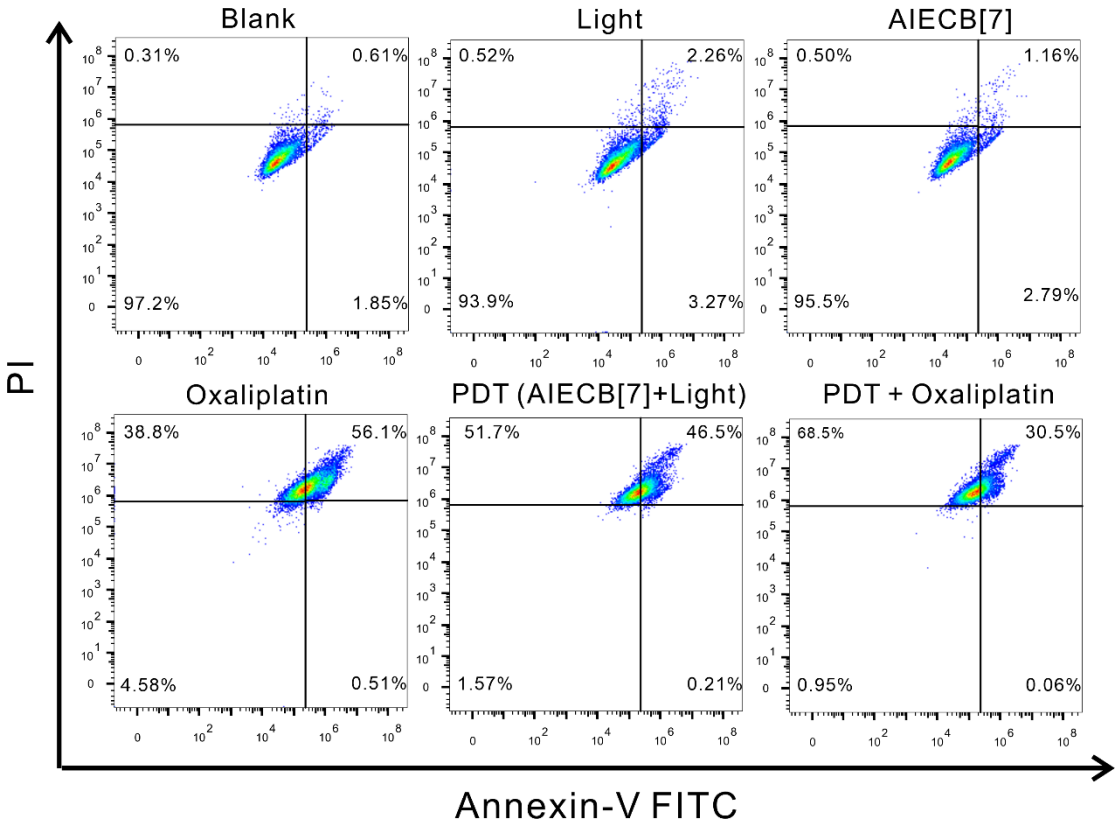


**Figure S29.** Flow cytometry analysis of A549 cells treated with light alone, AIECB[7] alone, Oxaliplatin alone, AIECB[7] PDT and AIECB[7] plus oxaliplatin upon 450 nm light irradiation at 150 mW/cm$^2$ for 4 min, respectively. All the cells were stained with Annexin V-FITC/PI kit.

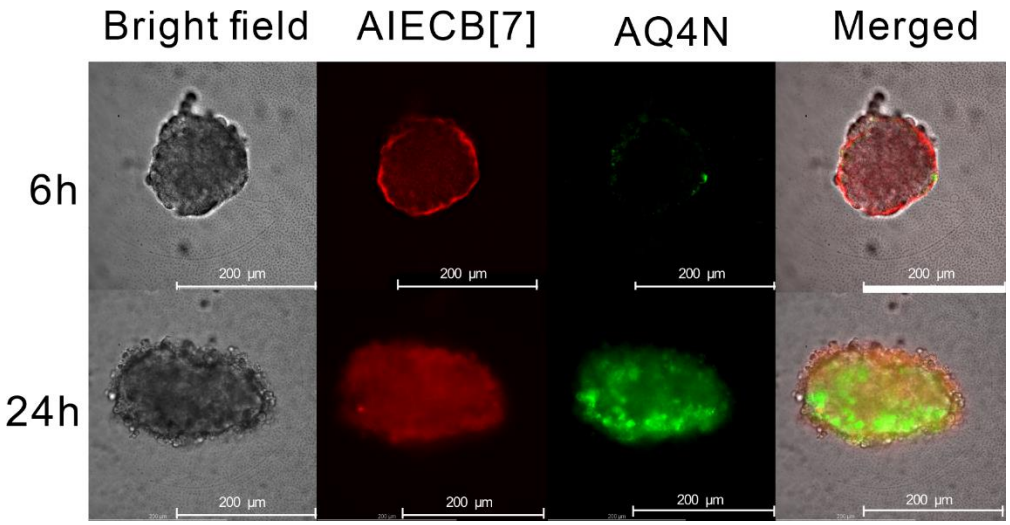

**Figure S30.** Penetration of AIECB[7] +AQ4N on A549 multicellular tumor spheroids at 6 hours (above) and 24 hours (below). AIECB[7] (Exi: 405 nm, Emi: 570-640 nm), AQ4N (Exi: 488 nm, Emi:650-700 nm). Scale bars: 200 μm.

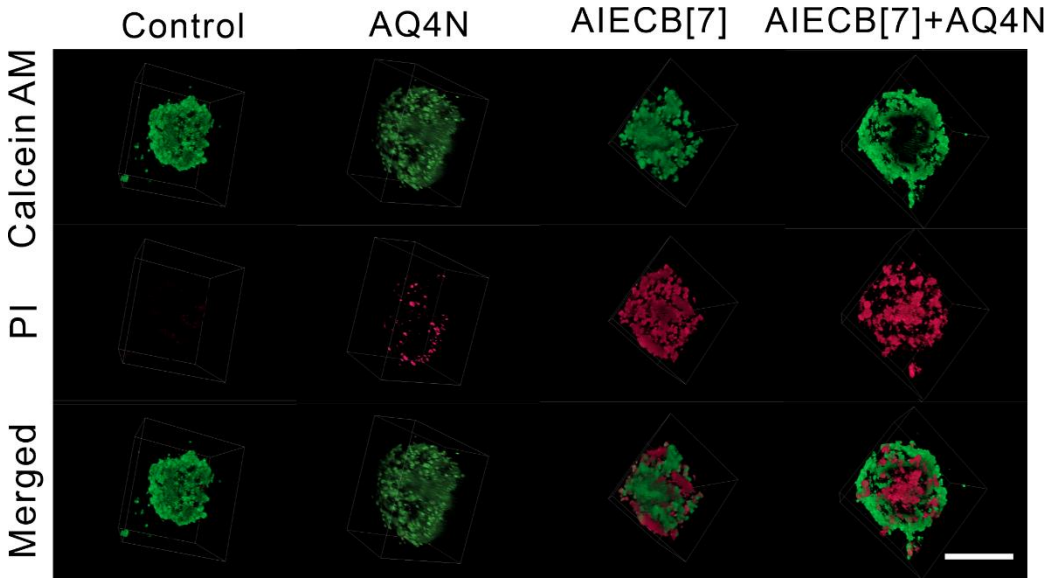


**Figure S31.** Z-stack scanning images (3D version) of A549 multicellular tumor spheroids treated with AQ4N, AIECB[7] and AIECB[7] + AQ4N, respectively. The cell viabilities of these tumor spheroids were indicated by Calcein AM (CM) and PI.

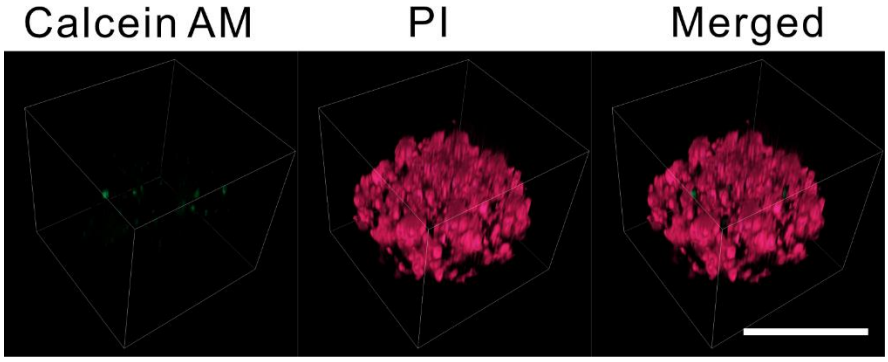


**Figure S32.** Z-stack scanning images (3D version) of the A549 multicellular tumor spheroids treated with 40 μM AIECB[7] + AQ4N upon light irradiation after 48 h. Scale bars: 200 μm.

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