Mitochondrial-targeted chemiluminescent ternary supramolecular assembly for in situ photodynamic therapy

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Experimental section

Instrumentation and methods

All solvents and reagents were commercially available and used without further purification unless noted otherwise. 4,4'-(dibenzo[a,c]phenazine-9,14-diyl) benzaldehyde (DPAC-CHO) was prepared according to the previous literature procedures.[1] NMR spectra were recorded on an Ascend 400 MHz instrument. High-resolution mass (HR-MS) spectra were performed on a Q-TOF LC-MS with an ESI mode. Absorption spectra were record on a Thermo Fisher Scientific EVO300 PC spectrophotometer in a conventional rectangular quartz cell (10×10 ×45 mm) at 25 °C. Fluorescence spectra were measured in a conventional rectangular quartz cell (10 × 10 × 45 mm) on a JASCO FP-750 spectrometer equipped with a constant temperature water bath. Transmission Electron Microscope (TEM) measurements were recorded on a high-resolution TEM (Tecnai G2 F20 microscope, FEI) equipped with a CCD camera (Orius 832, Gatan) operating at an accelerating voltage of 200 kV. Scanning Electron Microscope (SEM) image were recorded on a JEOL JSM-7500F SEM operating at an accelerating voltage of 30 keV. The light irradiation experiment was carried out employing a CEL−HXUV300 xenon lamp at a power density of 0.22 W/cm^2. Chemiluminescence is measure by IVIS Lumina in Vivo Imaging System (Caliper) (exposure time, 3 min).

Measurements

Preparation of assembled nanoparticles

DPAC-S and CB[7] were dissolved in Milli-Q water with concentration of 1mM under vigorous sonication, respectively. CPPO was dissolved in THF with concentrations of 10mg/ml. The solution of DPAC-S and CB[7] were mixed equivalently, DPAC-S@ CB[7] was obtained by fully mixing the solution with ultrasound. Then 30μl of 10mg/ml CPPO tetrahydrofuran solution was added in above mixture solution, DPAC-S@CB[7]@CPPO was obtained by ultrasound mixed solution.

Chemiluminescence measurement of supramolecular assemblies

1.5ml of 1mM DAPC-S, 1.5ml of 1mM CB[7] and 30μl of 10mg/ml CPPO were full mixed by ultrasound. Then the mixture was transferred to the quartz cell. 3μL of 35wt% H_2O_2 was added to initiate the chemiluminescence reaction. The chemiluminescence spectra and intensity were recorded in the range of 200-800 nm immediately.

Assessment of the ability of supramolecular assemblies to produce $^1O_2$ under irradiation

Evaluation of the ability of supermolecular assembly to produce singlet oxygen ($^1O_2$) and calculation of the

$^1O_2$ quantum yield was carried out using the chemical method.[2] 9, 10-Anthracenediyl-bis (methylene) dimalonic acid (ABDA) was used as a $^1O_2$ probe and rose bengal (RB) was used as a control. 20μL of ABDA (10mM) solution was added to 3ml of sample solution (5×10^{-5} M). Xenon lamp with power 0.22 W/cm^2 was used as the light source (400-700nm). The quantum yield of $^1O_2$ is calculated by the following formula:

$$\Phi_{sam} = \Phi_{RB} \times \frac{K_{sam} \times A_{RB}}{K_{RB} \times A_{sam}} \quad (1)$$

Where K_{sam} and K_{RB} are the decomposition rate constants of ABDA determined by sample and RB, A_{sam} and A_{RB} represent the light absorbed by the sample and RB, determined by integrating the optical absorption bands in the wavelength range of 400–700 nm. Φ_{sam} is represents the $^1O_2$ quantum yield of RB (Φ_{RB}=0.76).

Assessment of the ability of supramolecular assemblies to produce $^1O_2$ by Chemiluminescence

The $^1O_2$ generation ability was studied using 9, 10-Anthracenediyl-bis (methylene) dimalonic acid (ABDA) as an indicator. Under the action of $^1O_2$, Ultraviolet absorption of ABDA decreases at 300-400nm. The mixture of CPPO and ABDA was used as blank sample, the solution of DPAC-S@CB[7]@CPPO and ABDA were mixed in dark. Absorption of ABDA was measured by ultraviolet spectroscopy immediately after adding hydrogen peroxide (H_2O_2) in dark environment. Scan the UV absorption of ABDA every two minutes.

Cell culture

KYSE-150 cell were cultured in RPMI-1640 supplemented with 10% FBS and 1% penicillin-streptomycin at 37 °C under a humidified atmosphere containing 5% CO_2.

293T cell were cultured in RPMI-1640 supplemented with 10% FBS and 1% penicillin-streptomycin at 37 °C under a humidified atmosphere containing 5% CO_2.

Colocalization assay

KYSE-150 cells were seeded in 35 mm dishes for 24 h and then incubated with DPAC-S@CB[7]@CPPO at 37 °C for 24 h. The cells were further co-incubated with Mitotracker red (10 μg/ml) at 37 °C for 0.5 h. Cells were washed three times with PBS and visualized by a confocal microscope (OLYMPUS FV3000) immediately. The DPAC-S@CB[7]@CPPO was excited at 450 nm, collected at 580 nm, Mitotracker red was excited at 580nm and emission was 600 nm respectively.

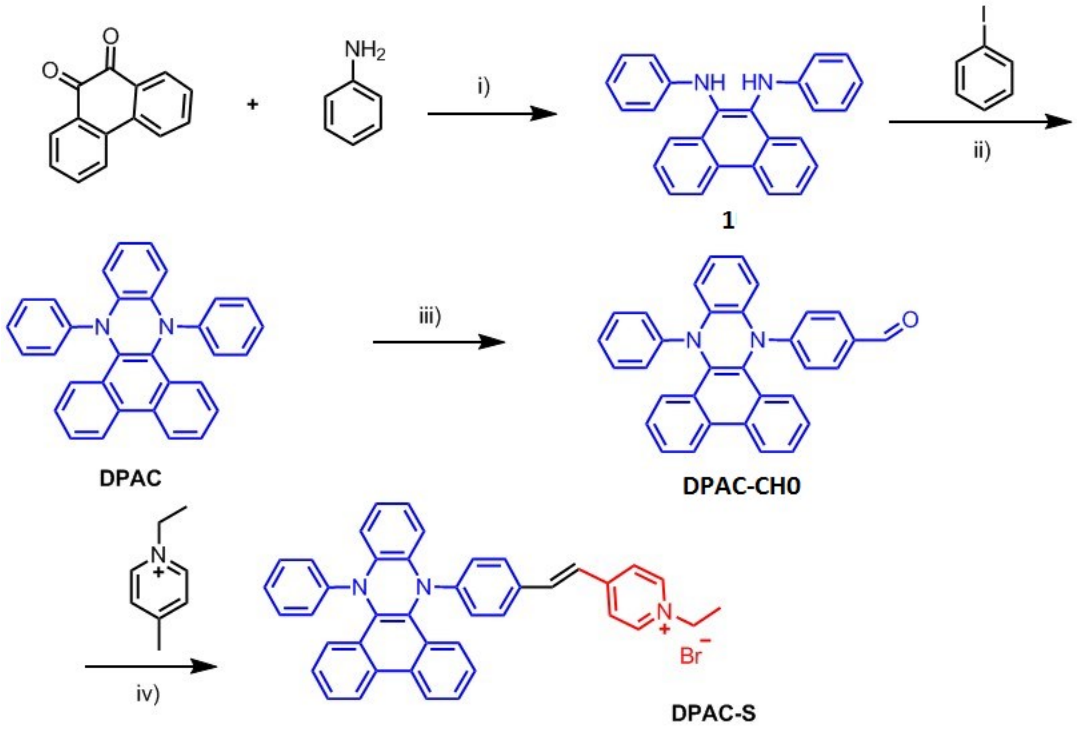
In vitro photodynamic toxicity assessment

Cytotoxicity in the KYSE-150 was assessed by a standard CCK8 assay. KYSE-150 cells were cultured in 96-well plate at a density of 6×10^3 cells/well and incubated at 37 °C overnight. Then the cells were treated with DPAC-S@CB[7](2-12$\times10^{-5}$M), DPAC-S@CB[7]@CPPO(2-12$\times10^{-5}$M) respectively. After 12 h incubation, H_2O_2 was added to interact with the cells at room temperature. After 24 h incubation, Standard CCK8 solution (100 μL/well) was added to the wells and incubated at 37 °C for 4 h. Ultraviolet absorption of formazan at 450 nm was measured by enzyme-labelled instrument. The cell viability rate (VR) was calculated according to the following equation:

$$VR = \frac{(A-A0)}{(A*-A0)} \times 100 \quad \text{.....(2)}$$

Where A represents the mean absorbance value of treatment group, and A0 represents the absorbance of CCK8 solution and DPAC-S@CB[7]@CPPO without cells. A* represents the absorbance of CCK8 solution and cells after incubation.

Synthesis and characterization of DPAC-S



Scheme S1. Synthesis route of DPAC-S. Reagents and conditions: i) $TiCl_4$, toluene, pyridine,N_2; ii) $Cu(OTf)_2$, K_2CO_3, TCB, 180℃; iii) $POCl_3$, DMF; iv) ethanol, 80℃.

Synthesis of DPAC

Compound 1 was obtained by the reaction of phenanthrene-9,10-dione and aniline, Compound 1 (3.58g, 10 mmol), iodobenzene (2.0 g,10 mmol), K_2CO_3 (2.0 g, 15 mmol), $Cu(CF_3SO_3)_2$ (0.9 g, 2.5 mmol) and 1,3,5-trichlorobenzene (TCB) (20 g) were added in a 100 mL flask. The mixture was heated to 60°C， after the TCB has fully dissolved the reactant, the mixture was heated to 180°C and continues react for 18 hours. Then the solvent was completely removed by vacuum distillation. The crude product was purifed by column chromatography on silica (petroleum ether: ethyl acetate = 30:1) to afford white powder solid (2.5 g, 50%).^{1}H NMR (400 MHz, $CDCl_3$, δ):8.75(d, J=8.74, 2H), 8.13(d, J=8.12, 2H),7.75 (m,2H),7.65(t, J=7.65, 2H), 7.55(t, J=7.54,2H),7.34(m,2H),7.02-6.98(m,8H),6.78(t, J=6.80, 2H). HRMS: m/z 434.036, ([DPAC], calcd for $C_{32}H_{22}N_2$, 434.53)

Synthesis of DPAC-CHO

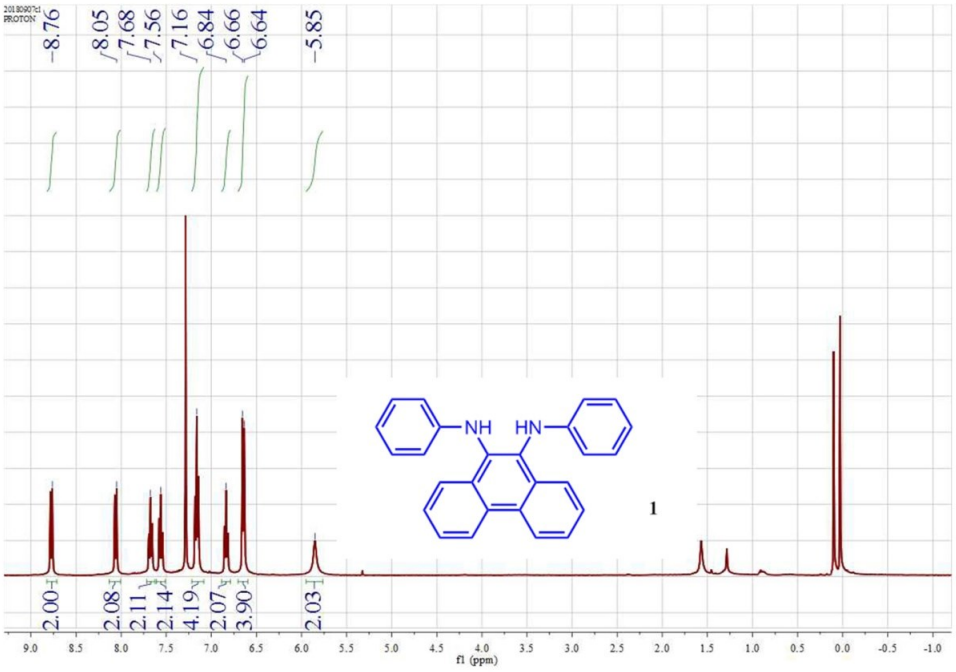
Injection of N,N-dimethylformamide (DMF)(10ml) into phosphorus oxychloride (3.45g, 23mmol) in 0℃ under the protection of nitrogen, the mixture was vigorously stirred for 2h to obtain the Vilsmeier reagent. Then the solution of DPAC (1g, 2.3mmol) in DMF (5ml) was added by dropwise. The mixture was heated to 80°C and stirred for 10h. After cooling to room temperature, the mixture poured into ice water, followed by adjustment of pH to 7.The yellow solid was filtered and purified by column

chromatography on silica(petroleum ether: dichloromethane = 1:5).Then, the yellow powder was obtained (0.85g, 78%).

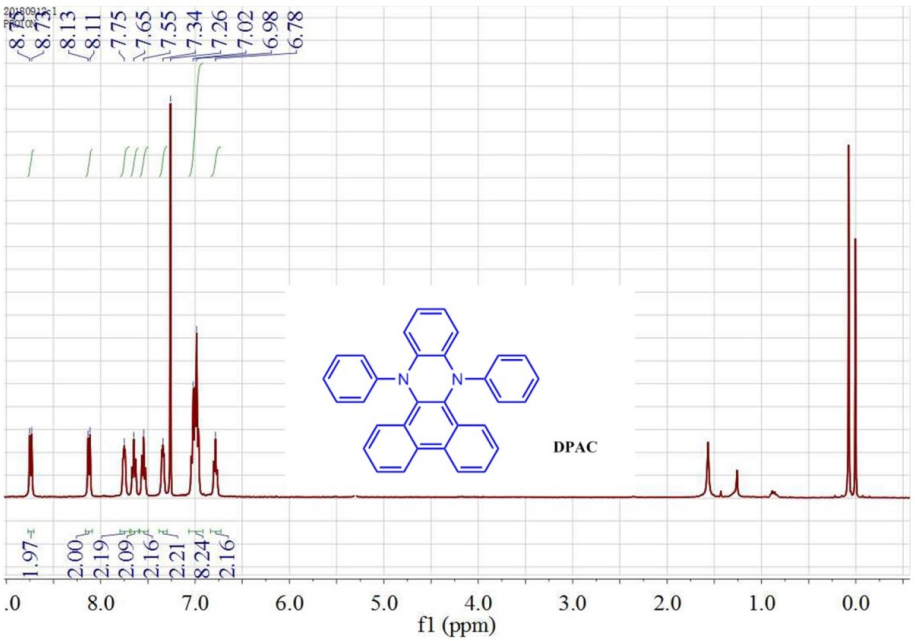
Synthesis of DPAC-S

DPAC-CHO(0.500g, 1.01mmol) and 1-ethyl-4-methylpyridin-1-ium bromide (0.617g, 3.03 mmol) were dissolved in 40 mL ethanol, and the solution was heated to reflux for 12 hours. Then the solution was concentrated to about 20 mL, and cooled by ice water. Then formed precipitate was filtered and washed by acetone. After that, the precipitate was collected, and dissolved in 20 mL ethanol for recrystallization. Formed precipitate was washed by acetone, dried in vacuo, to yield DPAC-S (0.460g, 65.3%) as orange powder. ^{1}H NMR (400 MHz, DMSO-d6, δ) 8.97 (t, J = 8.96Hz, 2H), 8.84 (t, J = 8.92Hz, 2H), 8.10(t, J= 8.09 Hz, 2H),7.95 (t, J = 7.96Hz, 4H), 7.87-7.60 (m, 6H), 7.49-7.44 (m, 3H), 7.22-7.19 (m, 1H),7.09-6.98 (m, 6H), 6.85-6.80 (m, 1H), 4.45(t, J=4.45Hz 2H),1.49(m, 3H) HRMS: m/z 566.2595, ([DPAC-S]+, calcd for $C_{41}H_{32}N_3$+,566.344)

(a)



(b)



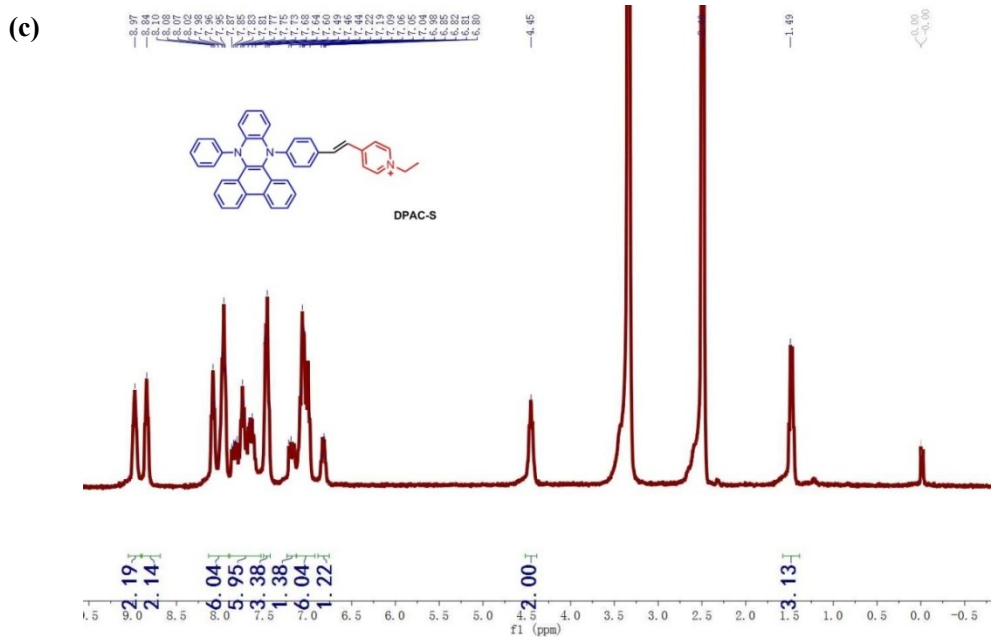


Figure S1. ^{1}H NMR (400 MHz, DMSO, 25℃) spectrum of (a) 1; (b) DPAC ; (c)DPAC-S.

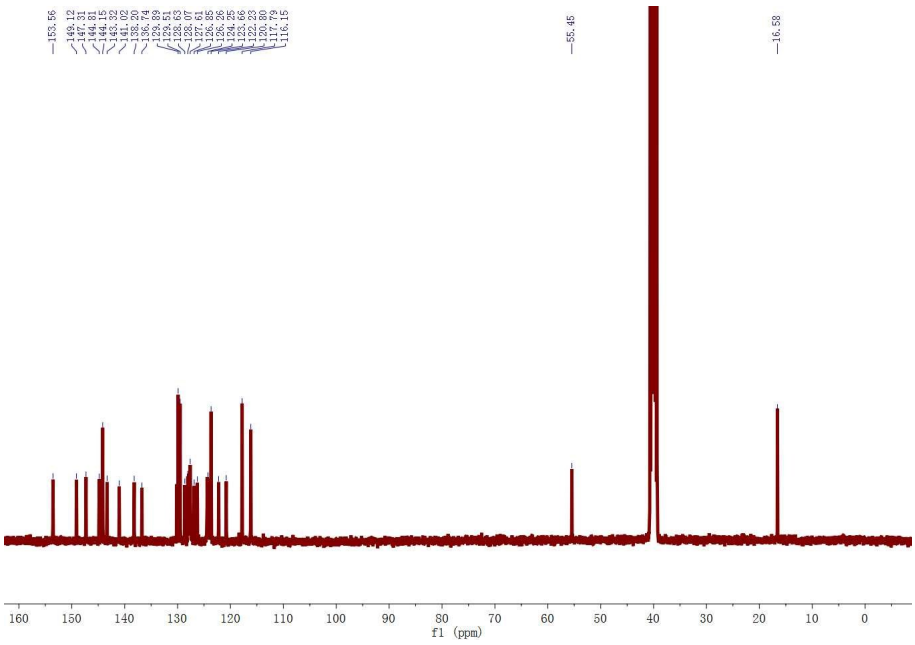


Figure S2. ^{13}C NMR (101 MHz, DMSO, 25℃) spectrum of compound DPAC-S.

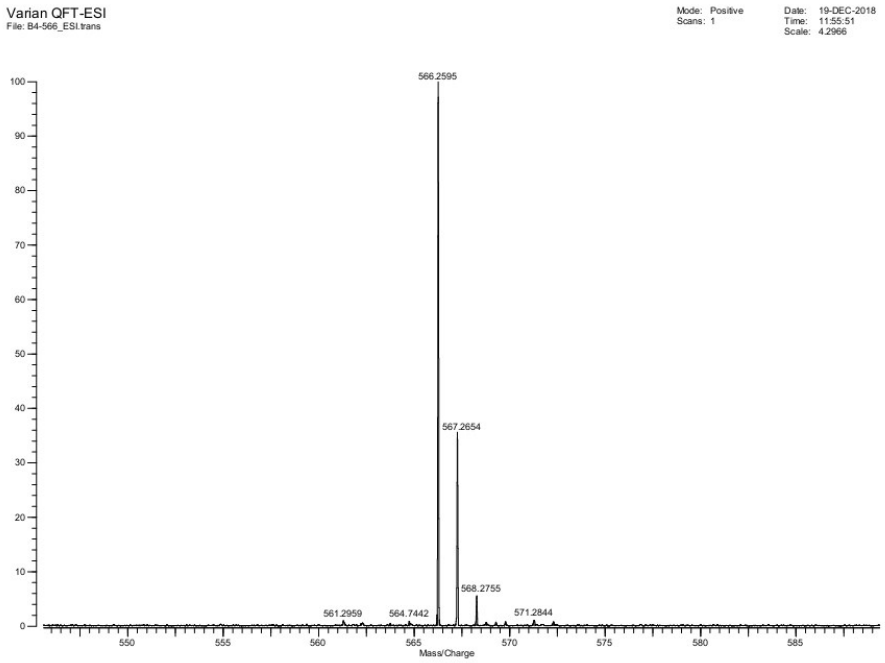


Figure S3 High Resolution Mass Spectrometry of DPAC-S

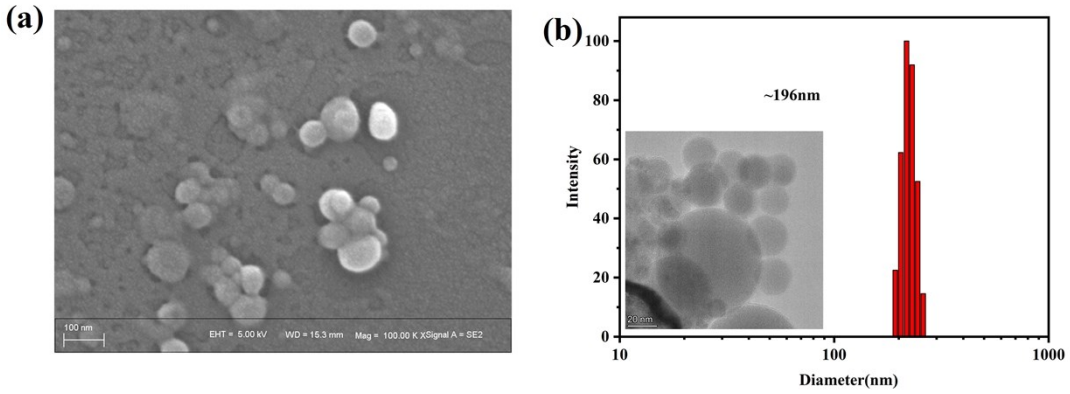


Figure S4. SEM and TEM images of DPAC-S and CB[7] and DLS image of DPAC-S@CB[7].

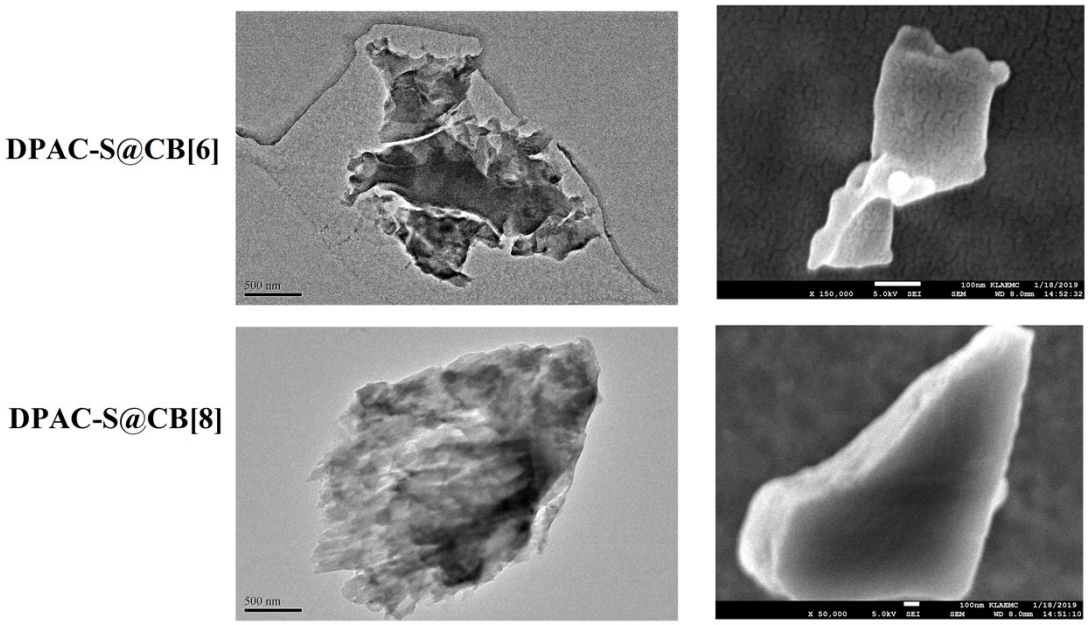


Figure S5. SEM and TEM images of DPAC-S and different cucurbituril.

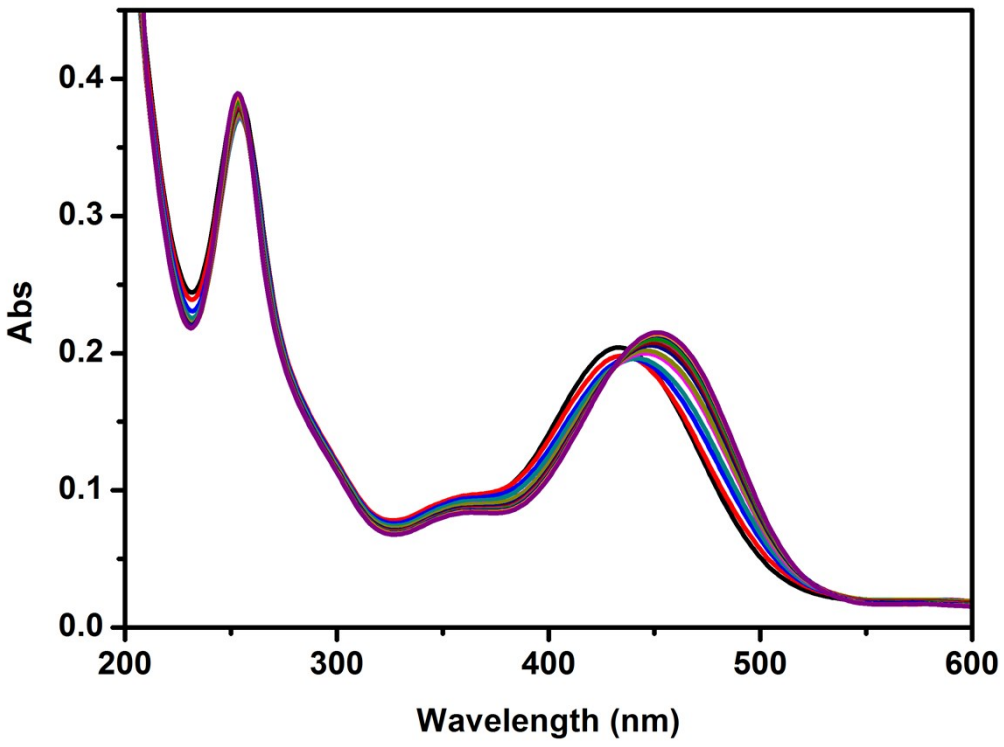


Figure S6. UV/Vis spectra of DPAC-S (0.01 mM) with varying concentrations of CB[7] from 0 to 0.013 mM in aqueous solution at 25°C.

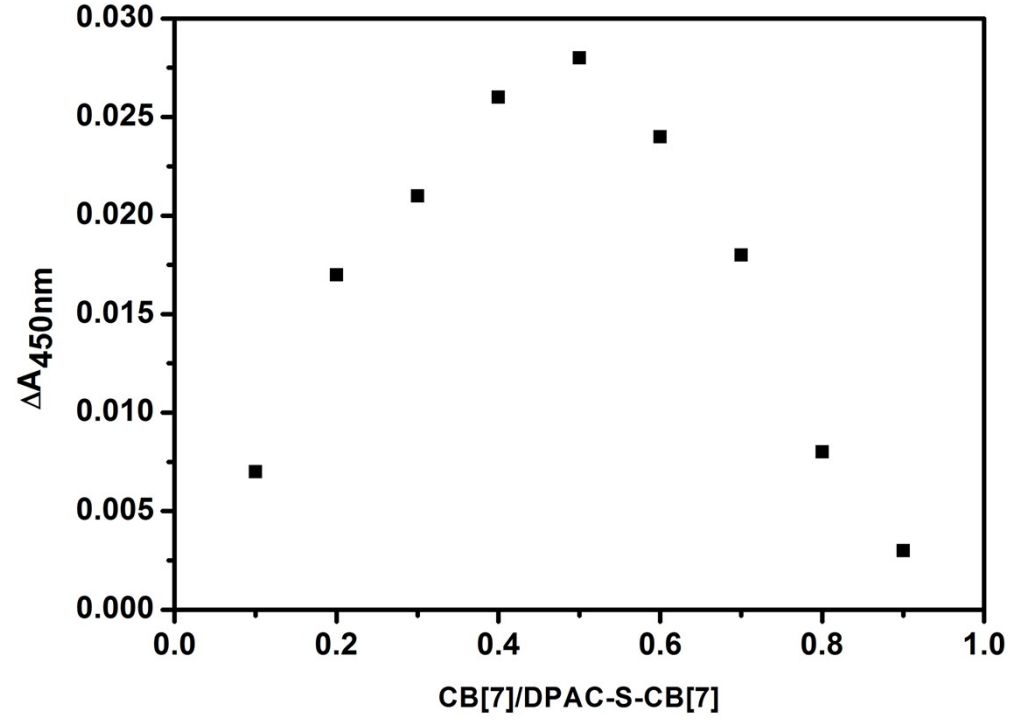


Figure S7. Job plot of DPAC-S and CB[7] obtained by recording the absorbance at 450nm at 25°C.

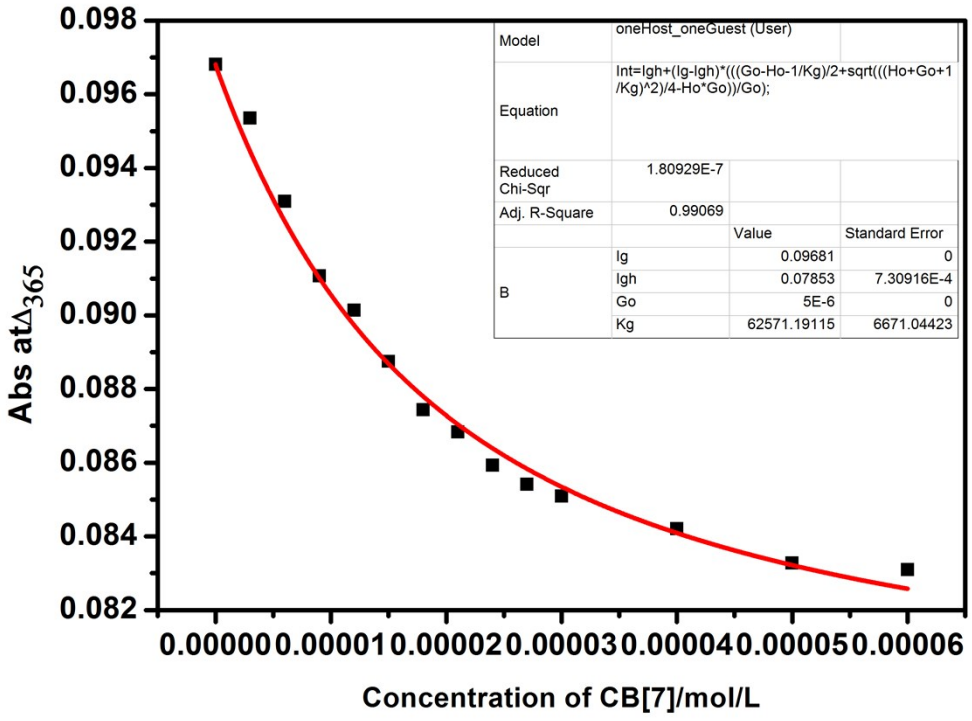


Figure S8. The nonlinear least-squares analysis of the variation of absorbance with the concentration of CB[7] to calculate the binding constant from the corresponding absorbance

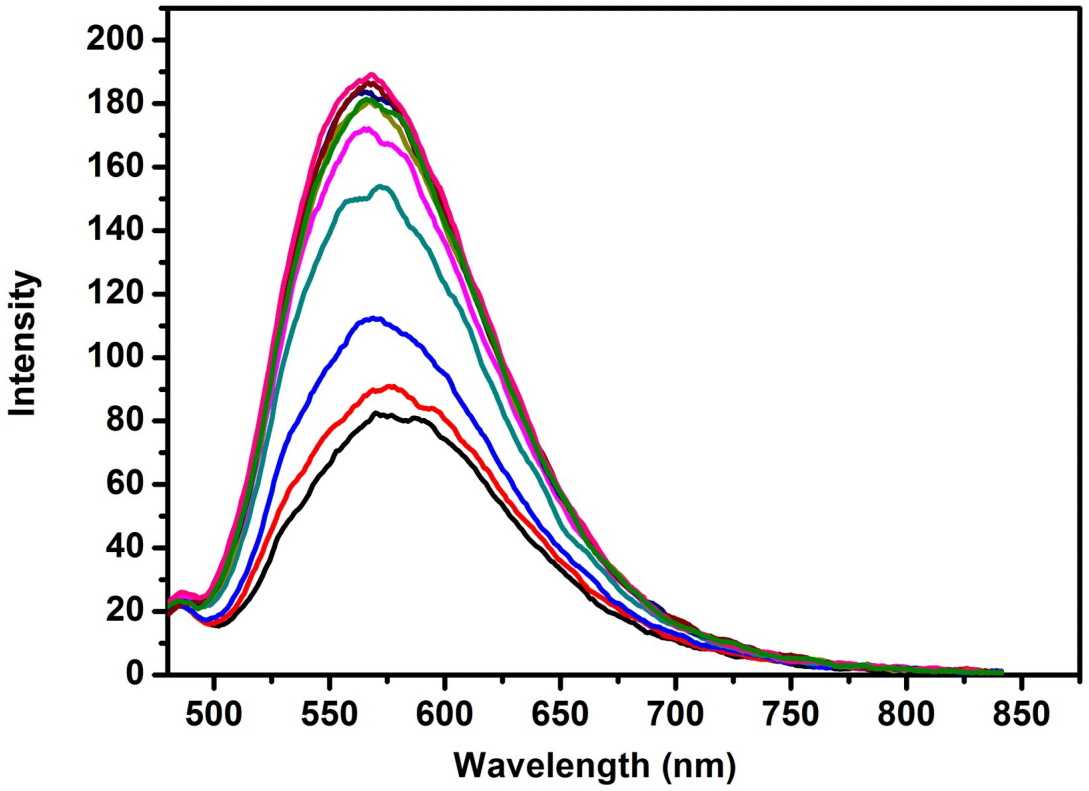


Figure S9. Emission spectra of DPAC-S in varying concentration of CB[7]. ([DPAC-S] = 0.01 mM, [CB[7]] = 0 mM to 0.013mM, respectively

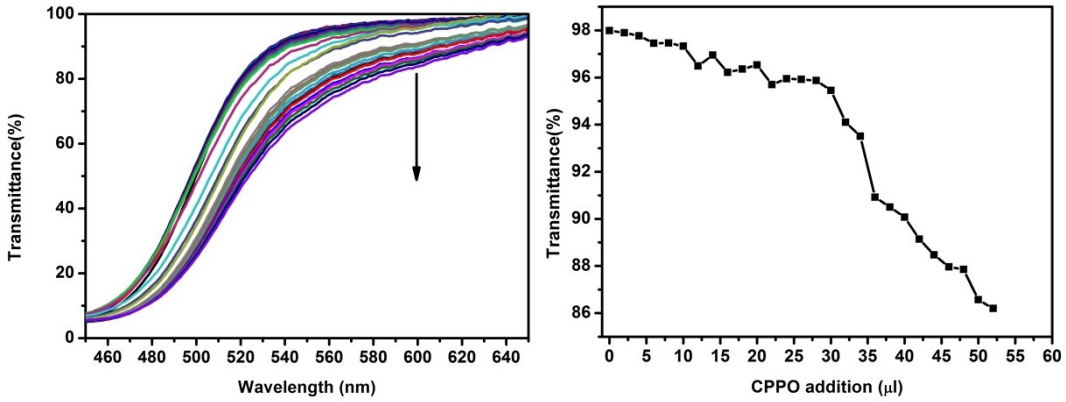


Figure S10. The transmittance of DPAC-S/CB[7]/CPPO in aqueous solution varies with the addition of CPPO.([DPAC-S] = 2×10^{-4} M 1.5ml in aqueous, [CB[7]] = 2×10^{-4} M 1.5ml in aqueous, [CPPO] = 1.48×10^{-2} M 0-54ul in THF)

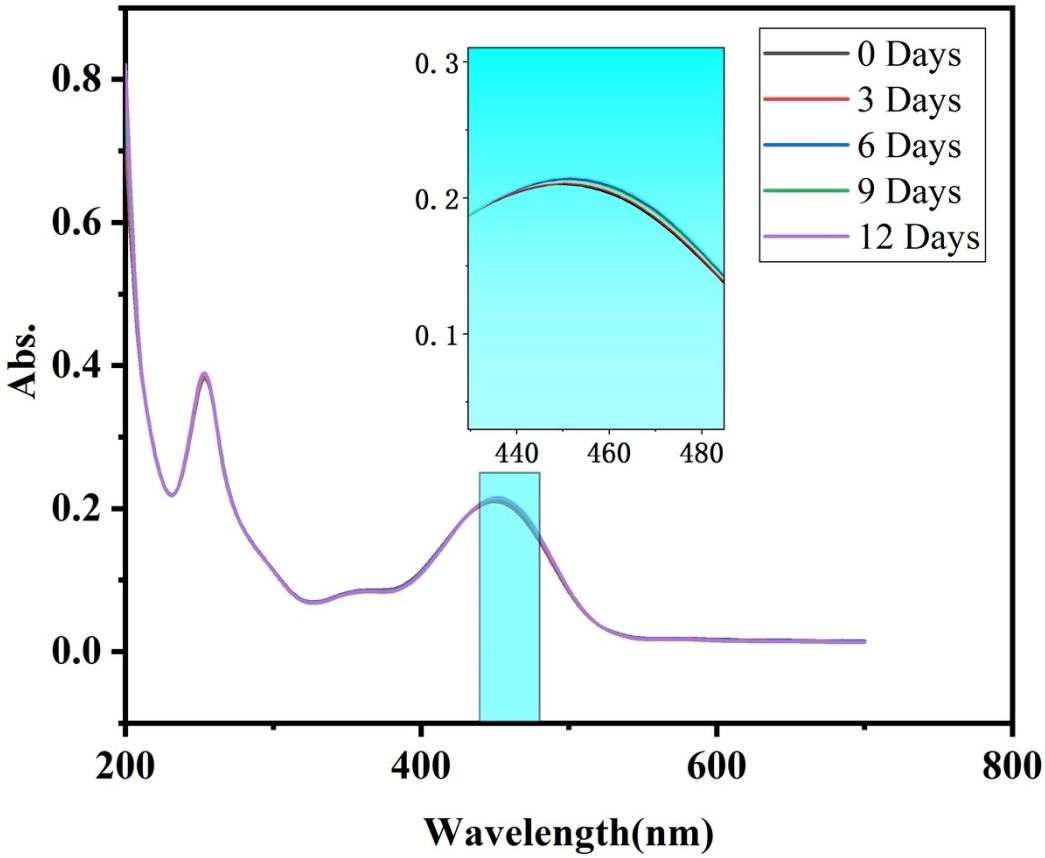


Figure S11. Stability of DPAC-S@CB[7]@CPPO in RPMI 1640 medium

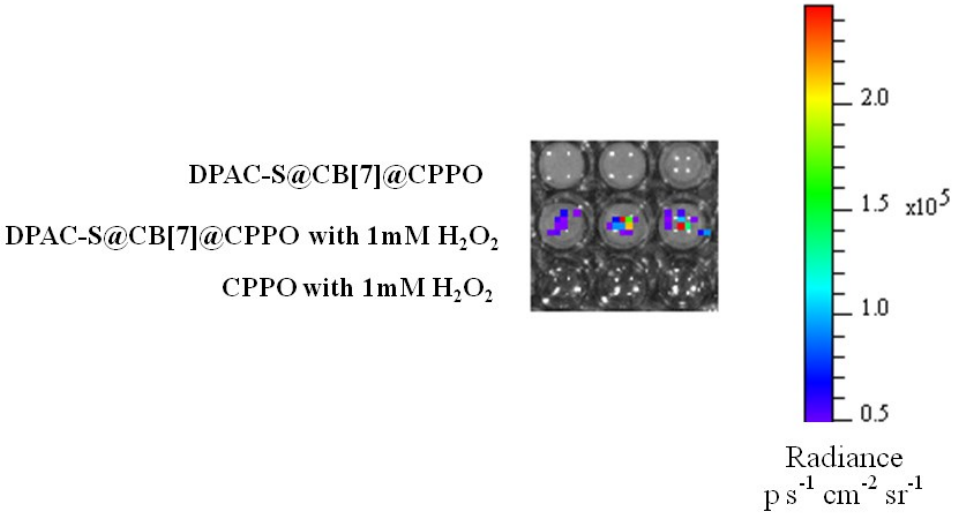


Figure S12. Chemiluminescence under different conditions

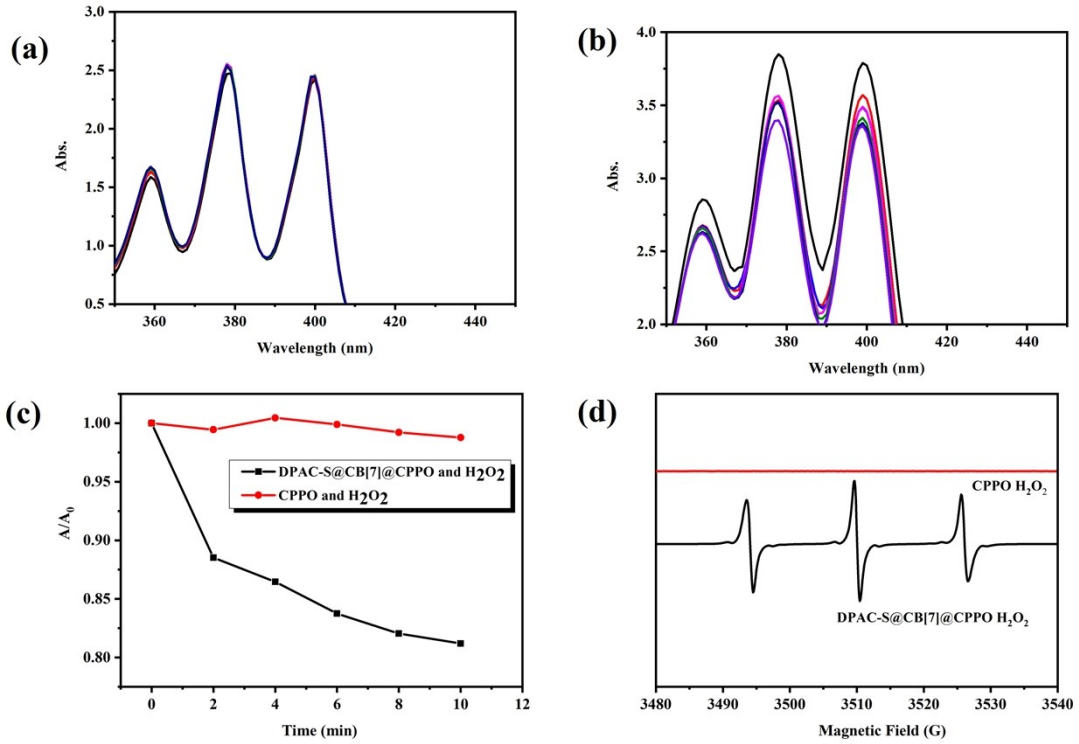


Figure S13. (a) Evaluation of CPPO $^1O_2$ generation activated by hydrogen peroxide. (b) Evaluation of DPAC-S@CB[7]@CPPO $^1O_2$ generation activated by hydrogen peroxide. (c) Evaluation of DPAC-S@CB[7]@CPPO $^1O_2$ generation activated by H_2O_2; (d) ESR signal of reactive oxygen species.

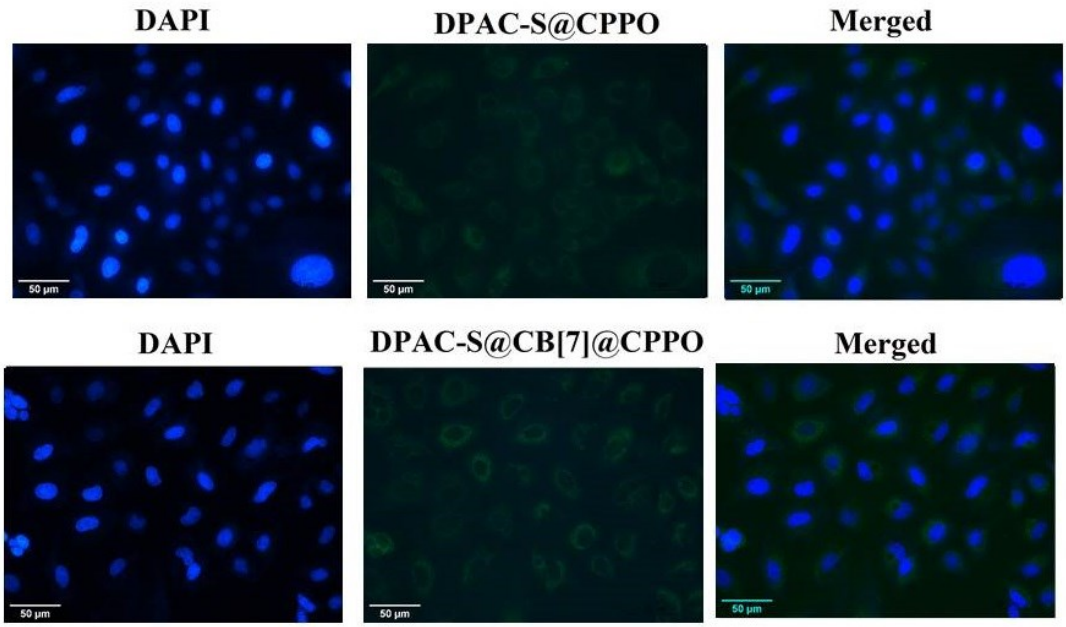


Figure S14. Confocal fluorescence images of KYSE-150 cells treated with DPAC-S@CB[7]@CPPO or DPAC-S@CPPO after 24 h. The fluorescence of DPAC-S@CB[7]@CPPO is shown in green.

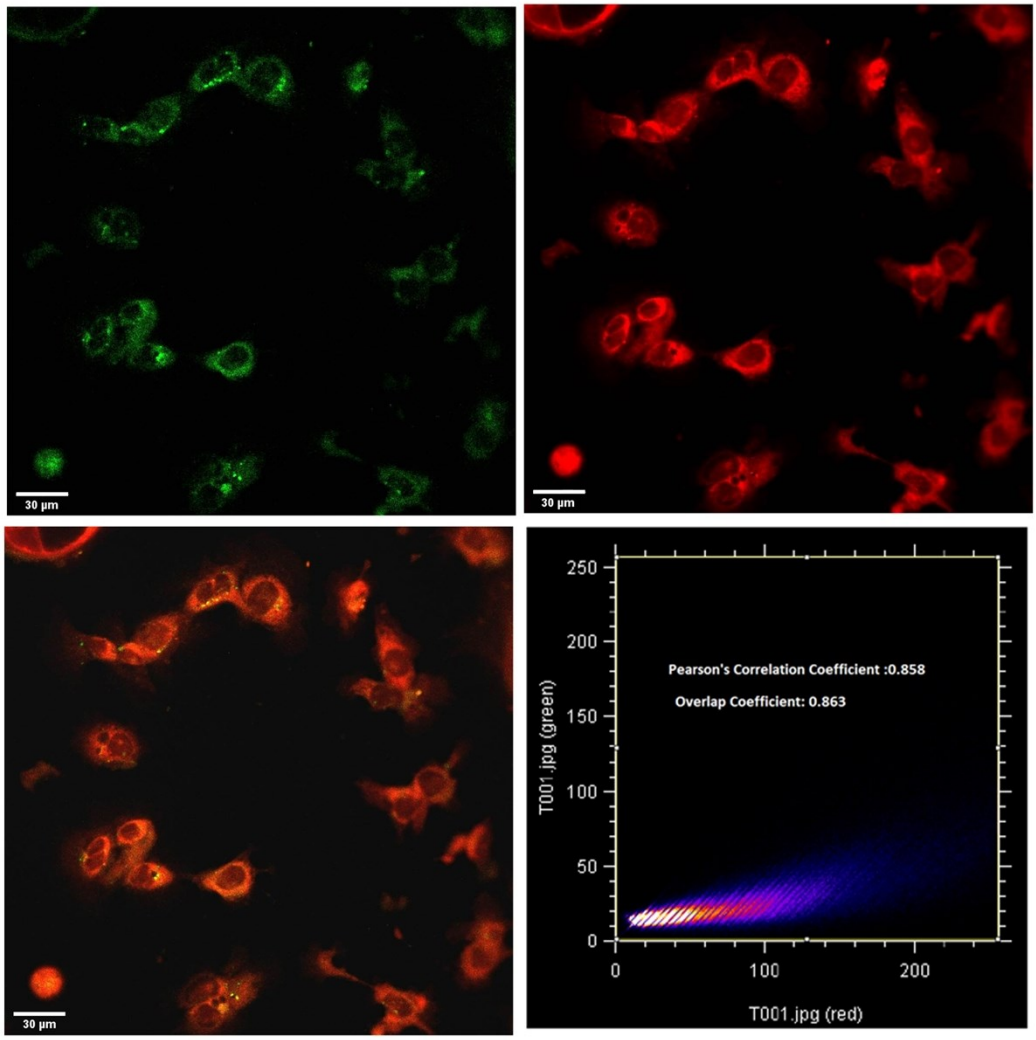


Figure S15. Confocal fluorescence images of KYSE-150 cells after incubation with DPAC-S@CB[7]@CPPO for 24 h. The cells are stained with Mitotracker red. The fluorescence of DPAC-S@CB[7]@CPPO is shown in green and Mitotracker is shown in red; Pearson's coefficient and overlap coefficient are calculated by the colorization finder plug-in of ImageJ .

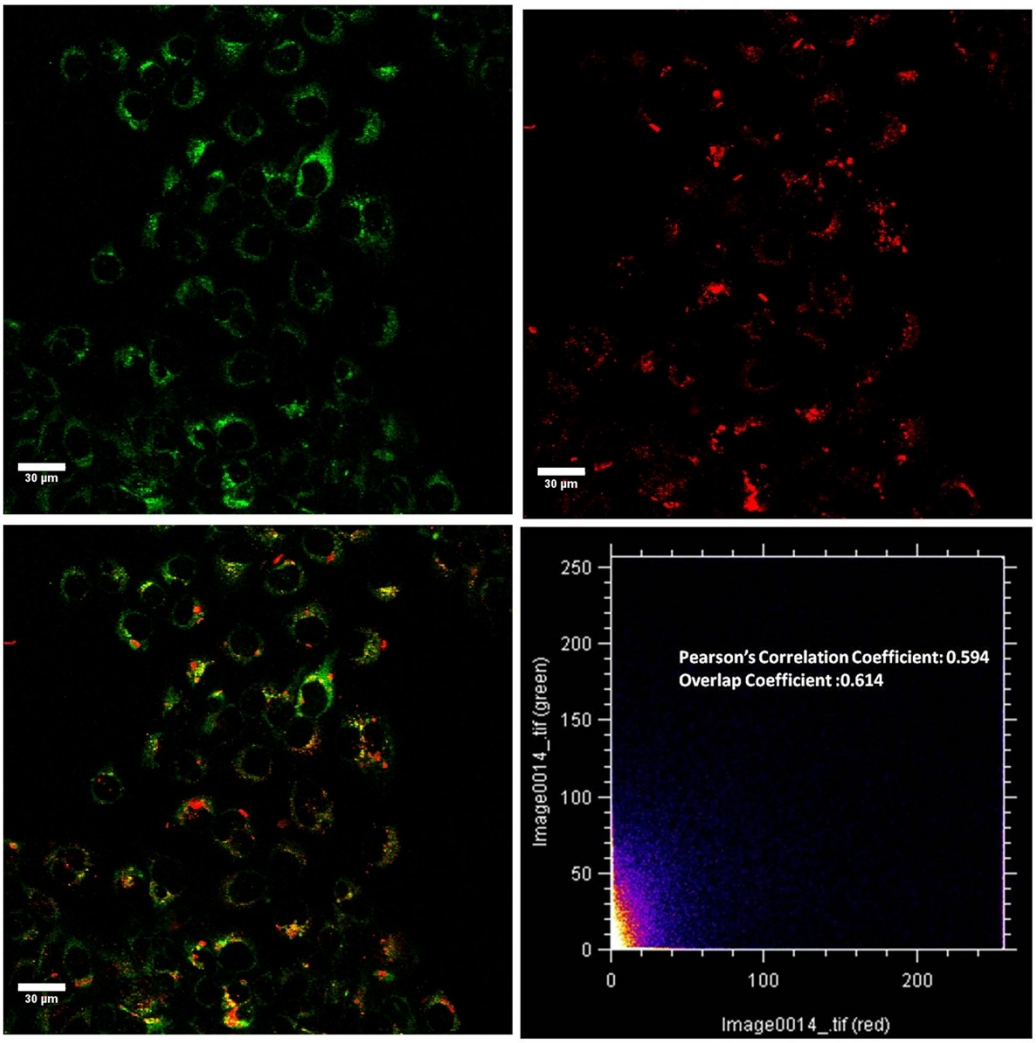


Figure S16. Confocal fluorescence images of KYSE-150 cells after incubation with DPAC-S@CB[7]@CPPO for 24 h. The cells are stained with LysoTracker red. The fluorescence of DPAC-S@CB[7]@CPPO is shown in green and LysoTracker is shown in red; Pearson's coefficient and overlap coefficient are calculated by the colorization finder plug-in of ImageJ.

References

[1] a) J. Wang, X. Yao, Y. Liu, H. Zhou, W. Chen, G. Sun, J. Su, X. Ma and H. Tian, *Adv. Opt. Mater*. 2018, **6**, 1800074; b) Z. Zhang, C. L. Chen, Y. A. Chen, Y. C. Wei, J. Su, H. Tian and P. T. Chou, *Angew. Chem., Int. Ed*. 2018, **57**, 9880.(c) W. Chen, C. L. Chen, Z. Zhang, *J. Am. Chem. Soc*. 2017, **4**, 1636.

[2] Z. Li, D. Wang, M. Xu, *J. Mater. Chem. B*, 2020, **8**, 2598.