Supporting Information for

The BSA mediating the proton coupled electron transfer to unlock the photo-generation of hydroxyl in cancer cells

Guiyan Xu,[a] Yingyong Ni,[*a] Xiaojiao Zhu,[*a] Xuan Zhao,[a] Ting Wang,[a] Shengyu Shi,[b] Jianhua Yu,[a] Qiong Zhang,[a] Xingxing Chen[a] and Hongping Zhou[*ab]

[a] School of Chemistry and Chemical Engineering, School of Materials Science and Engineering, School of Chemistry and Chemical Engineering, Institutes of Physical Science and Information Technology, Center of Free Electron Laser & High Magnetic Field, Key Laboratory of Structure and Functional Regulation of Hybrid Materials Ministry of Education, Key Laboratory of Functional Inorganic Materials Chemistry of Anhui Province, and Key Laboratory of Chemistry for Inorganic/Organic Hybrid Functionalized Materials of Anhui Province, Anhui University, Hefei 230601 P. R. China.

[b] School of Chemical and Environmental Engineering, Anhui Polytechnic University, Wuhu, 241000, P.R. China.

*To whom correspondence should be addressed

E-mail: 18895636492@163.com (Y. Y. Ni); xiaojzhu@ahu.edu.cn (X. J. Zhu); zhpzhp@263.net (H. P. Zhou)

1. Experimental Section

1.1 Materials

All reagents were purchased from commercial resources (Aladdin, Macklin, SigmaAldrich or Bioquest Co., Ltd.), including 1-(4-BroMophenyl)-1,2,2-triphenylethylene, 5-(5-Formyl-2-thienyl)-2-thiopheneboronic Acid, 4- Bis(di-tert-butyl(4-dimethylaminophenyl)phosphine) dichloropalladium(II), Methylpyridine, Iodoethane, Potassium carbonate, 1,5-Diiodopentane, etrahydrofuran, piperidine, dimethyl sulfoxide, methanol, ethanol and dichloromethane, piperidine, methanol, ethanol, triethylamine, bovine serum albumin (BSA), 9,10-anthracenediyl-bis(methylene)dimalonic acid (ABDA), dihydrorhodamine 123 (DHR123), 3'-p-(hydroxyphenyl) fluorescein (HPF), 5,5-dimethyl-1-pyrroline N-oxide (DMPO), 2,2,6,6-tetramethylpiperidine (TEMP), 3,3'-dioctadecyloxacarbocyanine perchlorate (DiO), singlet oxygen sensor green (SOSG), dihydroethidium (DHE), calcein acetoxymethyl ester/propidium iodide (Calcein AM/PI), fluorescein isothiocyanate labeled phospholipid binding protein/propidium iodide (Annexin V-FITC/PI) 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) and so on. Fluo-4 acetoxymethyl ester (Fluo-4 AM) and dulbecco's modified eagle medium (DMEM) were purchased from commercial sources (Aladdin, Energy-Chemical, Macklin, Sigma-Aldrich, Bestbio and Thermo). The HepG2 (human hepatocellular carcinoma cell) was purchased from BeNa Culture Collection.

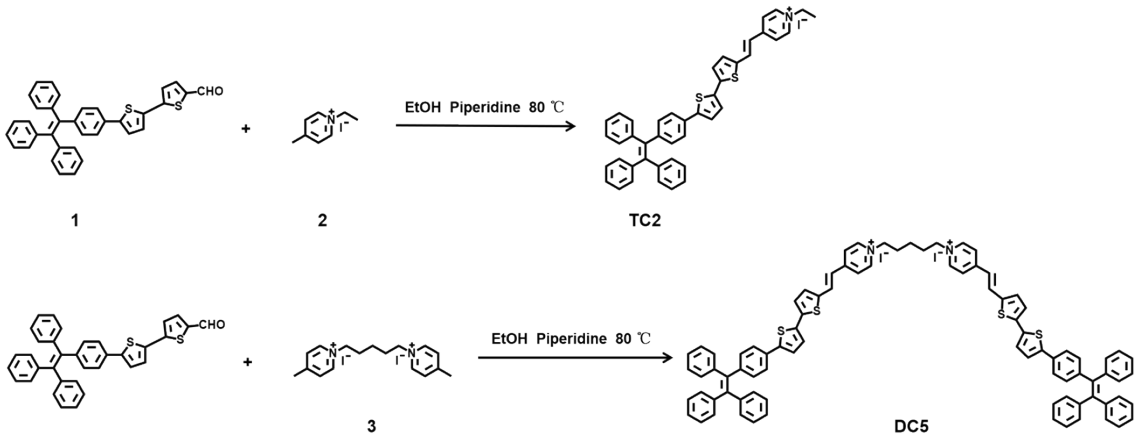
1.2 Instrumentation

^{1}H and ^{13}C NMR spectra were recorded with a JNM-ECZ400S spectrometer using tetramethyl silane (TMS) as a reference at room temperature. Electrospray ionization mass spectrometric (ESI-MS) data were performed on Thermo Fisher Scientific LTQOrbitrap XL mass spectrometer. The morphology was taken from transmission electron microscopy (TEM) (JEM-2100). The size of particles was measured by a dynamic light scattering (DLS) particle size analyzer (Nano-zs90). The UV-vis absorption and emission spectra were measured with UV-3900 spectrophotometer and

Hitachi F-7100 fluorescence spectrophotometer, respectively. ESR experiment was examined on a Bruker Nano x-band spectrometer (Magnettech ESR500). Mott-Schottky experiments were carried out by CHI-760E electrochemical workstation. Cell viability test was obtained on a Thermo Scientific Multiskan. Flow cytometry test of apoptosis was carried out with a CytoFLEX (BECKMAN) instrument. Confocal fluorescence imaging was shown on Leica confocal laser scanning microscope (CLSM) and the pictures were processed by ImageJ software.

Light sources: LED light (white light (400 nm-700 nm); 1 W cm^{-2}) was used for photostability test, ROS test and intracellular test. Xenon lamp (white light; 0.40 W cm^{-2}) was used in photocurrent test.

1.3 Synthesis and Characterization



Scheme S1. Synthesis route of **TC2** and **DC5**

Compounds 1, 2 and 3 were synthesized according to the previous literature reports.[1-3]

Synthesis of TC2

Compound 1 (0.70 g, 1.34 mmol) and Compound 2 (0.28 g, 1.11 mmol) were dissolved in 30 mL of ethanol, then 8 drops of piperidine were added to the reaction mixture. Heat the mixture to 80 ℃ and reflux for 8 hours. Thin-layer chromatography (TLC) was used to trace the reaction to the end. Afterwards, the crude product was obtained through vacuum distillation. The crude product was then separated and

purified using silica gel column chromatography with an eluent ratio of CH_2Cl_2/CH_3OH (v/v) = 65:1, and **TC2** (0.39 g, 0.52 mmol) was obtained. Yield: 47%. ^{1}H NMR (400 MHz, DMSO-d_6), δ (ppm): 8.92 (d, J = 6.70 Hz, 2H), 8.19 (d, J = 6.80 Hz, 2H), 7.55 - 7.44 (m, 6H), 7.21 - 7.10 (m, 10H), 7.06 - 6.95 (m, 9H), 4.50 (q, J = 7.40 Hz, 2H), 1.52 (t, J = 7.30 Hz, 3H); ^{13}C NMR (101 MHz, DMSO-d_6), δ (ppm): 147.18, 144.57, 144.26, 143.57, 143.41, 134.37, 134.00, 132.17, 131.44, 131.28, 131.23, 131.17, 128.56, 128.50, 128.38, 127.38, 127.28, 125.91, 125.54, 123.96, 122.34, 59.92, 33.00, 19.32, 13.88; HRMS: $C_{43}H_{34}NS_2^+$ [M/z]$^+$ calcd for: 628.2127, found: 628.2124.

Synthesis of DC5

Compound 1 (1.57 g, 3.00 mmol) and Compound 3 (0.51 g, 1.00 mmol) were dissolved in 10 mL of ethanol, then 6 drops of piperidine were added to the reaction mixture. Heat the mixture to 80°C, allow it to react in the dark, and reflux overnight. Monitor the reaction using TLC. After the reaction was complete, wash the product with petroleum ether and ethanol to obtain **DC5** (0.78 g, 0.42 mmol) as a red solid, with a yield of 42%. ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 1.11 - 1.21 (m, 2H), 1.91 - 2.02 (m, 4H), 4.44 - 4.56 (d, J = 7.60 Hz, 4H), 6.96 - 7.04 (m, 16H), 7.10 - 7.18 (p, J = 7.50 Hz, 20H), 7.36 - 7.46 (m, 12H), 8.13 - 8.23 (s, 5H), 8.23 - 8.28 (s, 1H), 8.87 - 8.95 (d, J = 6.40 Hz, 4H); ^{13}C NMR (101 MHz, DMSO-d_6) δ (ppm): 147.18, 144.57, 144.26, 143.57, 143.41, 134.37, 134.00, 132.17, 131.44, 131.28, 131.23, 131.17, 128.56, 128.50, 128.38, 127.38, 127.28, 125.91, 125.54, 123.96, 122.34, 59.92, 33.00, 19.32, 13.88; HRMS: $C_{87}H_{68}N_2S_4^{2+}$ [M/z]$^+$ calcd for: 634.2127, found: 634.2130.

1.4 Preparation of Nanoparticles

Preparation of TC2@BSA, DC5@BSA: Weigh 5 mg **TC2** or **DC5** into small glass bottles and dissolve in 10 mL of methanol, respectively. 100 mg BSA and 10 μL of triethylamine were dissolved 10 mL of deionized water, which was slowly added into the methanol solution of the **TC2/DC5**, and stir overnight at room temperature. Fill the mixed solution into a dialysis bag, dialyze with deionized water for one day, and freeze dry. Dissolve the flocculent substance obtained after freeze-drying in 5 mL of deionized water and sonicate to dissolve it. After centrifugation, take the clear

solution and filter it to obtain the assembly solution. Measure the absorbance concentration standard curve of compounds and the absorbance of the assembly solution, and calculate the concentration of compounds in the assembly solution.

1.5 UV-vis absorption and fluorescence test

A 1mM stock DMSO solution of the **TC2/DC5** was prepared, subsequently, 20 μL of this concentrated solution was diluted in deionized water. The UV absorption and fluorescence emission spectra were then measured using a 10 μM diluted solution.

The concentrations of **TC2@BSA/DC5@BSA** were obtained according to Lambert Beer's law, and then were diluted with deionized water to 10 μM to measure the UV absorption and fluorescence emission spectra.

1.6 Detection of Singlet Oxygen ($^1O_2$) by ABDA

Probe ABDA was employed to explore the $^1O_2$ generation. The as-prepared photosensitizers was first diluted with deionized water to 10 μM (2 mL) in dark conditions, then the ABDA stock solution (15 μL, 7.5 mmol L^{-1}) were added. Then, the samples were irradiated with 1 W LED light for 0 ~ 30 s, and the absorbance at ~ 380 nm were recorded.

1.7 Detection of Superoxide Anion Radical ($O_2^{-\bullet}$) by DHR123

Probe DHR123 was employed to explore the $O_2^{-\bullet}$ generation. DHR 123 (20 μL, 20 μg mL^{-1}) were added to the as-prepared photosensitizers (10 μM, 2mL) in dark conditions. Then, the samples were irradiated with 1 W LED light for 0 ~ 30 s, and the FL. intensities at ~ 525 nm were recorded (excitation wavelength: 488 nm).

1.8 Detection of Hydroxyl Radical (•OH) by HPF

HPF (20 μL, 5 mM) were added to the as-prepared photosensitizers (10 μM, 2mL) in dark conditions. Then, the samples were irradiated with 1 W LED light for 0 ~ 30 s, and the FL intensities at ~ 515 nm were recorded by the FL spectrofluorometer (excitation wavelength: 490 nm).

1.9 Stability test

Dark stability: In the dark, absorbance curves of **TC2** and **TC2@BSA**, (concentration of 10 μM) were measured, respectively, and the absorbance curves were measured every 2 min.

Photostability: The absorption curves of **TC2** and **TC2@BSA** were measured respectively, and the absorption curves were measured after each 2 min of light exposure.

1.10 ESR experiments to detect ROS

ESR trapping experiments were performed to identify the types of ROS. Detection of $^1O_2$ and $O_2^{-\bullet}$: dilute the photosensitizer stock solution with DMSO to 100 μM, than add 10 μL of 20 mM TEMP (for $^1O_2$ detection) or DMPO (for $O_2^{-\bullet}$ detection), mix thoroughly and transfer the sample into a glass capillary for ESR testing under both dark and light conditions, recording the data; Detection of •OH: dilute the photosensitizer with H_2O to 100 μM, than add 10 μL of 20 mM DMPO, mix thoroughly and transfer the sample into a glass capillary for ESR testing under both dark and light conditions, recording the data.

1.11 Molecular dynamics research

Molecular dynamics simulations were conducted using GROMACS 2023.4. The topology parameters of the **TC2** were constructed using antechamber in AmberTools24 to build the initial molecular dynamics model parameters. The parameters were based on GAFF2, and the RESP2 charges were obtained using ORCA 5.0 with the B3LYP/6-311G** method. The TIP3P water solvent model was used. Subsequently, the packmol program was used to construct the water environment system, with 20 small molecules and 4000 water molecules in each system, and the simulation box size was 70.0 × 70.0 × 70.0 nm. After the construction of the above systems, the substrate was simulated at 300 K. Energy minimization was performed for 50,000 steps using the steepest descent method and the conjugate gradient method. Then, two stages of pre-equilibration simulations were carried out for the system, namely 500 ps of NVT ensemble equilibration (V-rescale temperature coupling with constant particle number, volume,

and temperature) and 1000 ps of NPT ensemble equilibration (Berendsen pressure coupling with constant particle number, pressure, and temperature). All bond lengths and bond angles were constrained using LINCS, and the PME method was used to handle long-range electrostatic interactions. The cutoff value for van der Waals interactions was 1.0 nm. Finally, the entire system was maintained at 300 K for a 100 ns dynamics simulation with a step size of 2 fs and coordinate files were saved every 10 ps. After obtaining the all-atom simulation trajectory of this system, further analysis was conducted using the built-in analysis programs of GROMACS 2023.4 and DulvyTools.

1.12 Molecular docking studies

The IIA region of BSA was used as the binding site for **TC2** polymer to form a complex. The pocket parameters of the IIA region are: center x: 0.055, center y: 25.259, center z: 105.565, size x: 44.6, size y: 51.6, size z: 48.5. The input files for the small molecule and the receptor were constructed respectively through ADT, and the required files were generated. Molecular docking was performed using the autodock vina 1.2.7 program, with spacing set at 0.375 and the rest of the program parameters set to default. A total of 100 compound conformations were generated. After the docking was completed, the conformation with the optimal binding mode was selected. It was saved as a complex and then the binding position of the small molecule-receptor was analyzed using Pymol and Discovery studio 2024 client.

1.13 The measurement of photocurrent responses and charge transfer resistance

The photocurrent responses experiment and charge transfer resistance were carried out using a three-electrode configuration. The working electrode consisted of a carbon paper electrode with the PSs attached to it. A platinum electrode served as the counter electrode, while an Ag/AgCl electrode was used as the reference electrode. The measurements were conducted in a solution of 0.01 M phosphate buffer.

1.14 Cyclic voltammetry measurement

A cyclic voltammetry (CV) experiment was performed using a three-electrode setup. The working electrode was a platinum-carbon composite electrode, while a

platinum wire electrode and an Ag/Ag^+ electrode were employed as the auxiliary and reference electrodes, respectively. The measurement was carried out in a solution of acetonitrile containing 0.1 M tetrabutylammonium hexafluorophosphate ($(nBu)4N^+PF_6$) as the supporting electrolyte. The scan rate was optimized to 100 mV s^{-1}. Fc/Fc^+ was used as an external reference.

1.15 Cell culture

HepG2 cells were cultured in DMEM containing 10% FBS and antibiotics (100 units per mL penicillin and 100 μg mL^{-1} streptomycin) in a 5% CO_2 humidity incubator at 37 °C.

1.16 Cell Membrane Colocalization Experiments

Under dark conditions, HepG2 cells pretreated with **TC2** or **TC2@BSA** (10 μL, 1 mM) were incubated for 30 min, respectively. Then, 3 μL of commercial dye DiO was added to the above cell culture dishes for 15 min. Finally, fluorescent images of HepG2 cells were collected by CLSM (λ_{ex} = 484 nm, λ_{em} = 490 ~ 530 nm).

1.17 Detection of intracellular ROS

The intracellular ROS were examined by using SOSG/DHE/HPF as fluorescence probes. HepG2 cells were incubated with 10 μM **TC2** or **TC2@BSA** for 20 min, respectively, followed by incubation with 3 μL SOSG/DHE/HPF for 10 min under normoxic (21% O_2), respectively. Subsequently, the cells were washed with PBS for three times. Then, cells were irradiated with a LED lamp (0.5 W cm^{-2}) (5 min). Then, the fluorescence was immediately observed using CLSM.

1.18 Live/Dead Cell Staining Assay by Calcein-AM/PI

HepG2 cells were pre-cultured into 15 mm × 15 mm confocal dishes and incubated for 24 h. After incubated with 10 μM **TC2** or **TC2@BSA** for 30 min at 37 ℃ under normoxic (21% O_2), respectively. The cells were further stained by 5 μL Calcein-AM and PI for 15 min. Usually, Calcein-AM would stain live cell (green fluorescence), and PI would stain dead cell (red fluorescence). The green channel: $\lambda_{ex}/\lambda_{em}$ = 488/500–550 nm. The red channel: $\lambda_{ex}/\lambda_{em}$ = 488/580–630 nm.

1.19 Apoptosis and Necrosis Assay by AnnexinV-FITC / PI

HepG2 cells were cultured in 6-well plates for 48 h, and 15 μM of **TC2** or **TC2@BSA** were added to each well, which was irradiated by a LED Light for 30 min. Subsequently, PBS buffer (400 μL), AnnexinV-FITC (5 μL) and PI (5 μL) were added to wells (the control group was set at the same time). Finally, the cells were transferred into the flow tube and detected on the flow cytometer (AnnexinV-FITC: λ_{ex} = 495 nm, λ_{em} = 520 ~ 540 nm, PI: λ_{ex} = 530 nm, λ_{em} = 600 ~ 700 nm).

1.20 Cell Viability Investigation by Calcein-AM Assay

HepG2 cells were seeded in 96-well plates at a density of 5.0×10^3 cells per well. Subsequently, the cells were treated with **TC2@BSA**, **TC2**, or BSA at different concentrations (0~25 μM) and incubated for 4 hours. After incubation, the cells were irradiated with white light for 15 min or kept in the dark. After light irradiation treatment, the samples were incubated for 12 h, followed by replacing the medium with Calcein-AM working solution (2 μM in PBS) and incubated at 37 °C for 30 min. Fluorescence intensity was measured using a microplate reader at an excitation wavelength of 494 nm and an emission wavelength of 517 nm.

To calculate HepG2 cell viability, a background group (only Calcein-AM) and a control group (cell + Calcein-AM) should be set. The relative cell viability was calculated according to the following formula:

$$\text{Cell viability (\%)} = (OD_{sample} - OD_{background}) / (OD_{control} - OD_{background}) \times 100\%.$$

In which OD_{sample}, $OD_{background}$ and $OD_{control}$ represent the optical density of the sample group, background group and control group, respectively.

1.21 Statistical analysis

Statistical analysis was performed using GraphPad Prism 8.0 software. All quantitative data were expressed as the mean ± standard deviation (SD) (n = 3). Unpaired student's t-test was used between two-group comparison, and one-way analysis of variance for multigroup analysis. Asterisk represented statistically

significant differences (*P < 0.05; **P < 0.01; ***P < 0.001; ****P < 0.0001; n.s., not significant).

2. Supplementary Figures

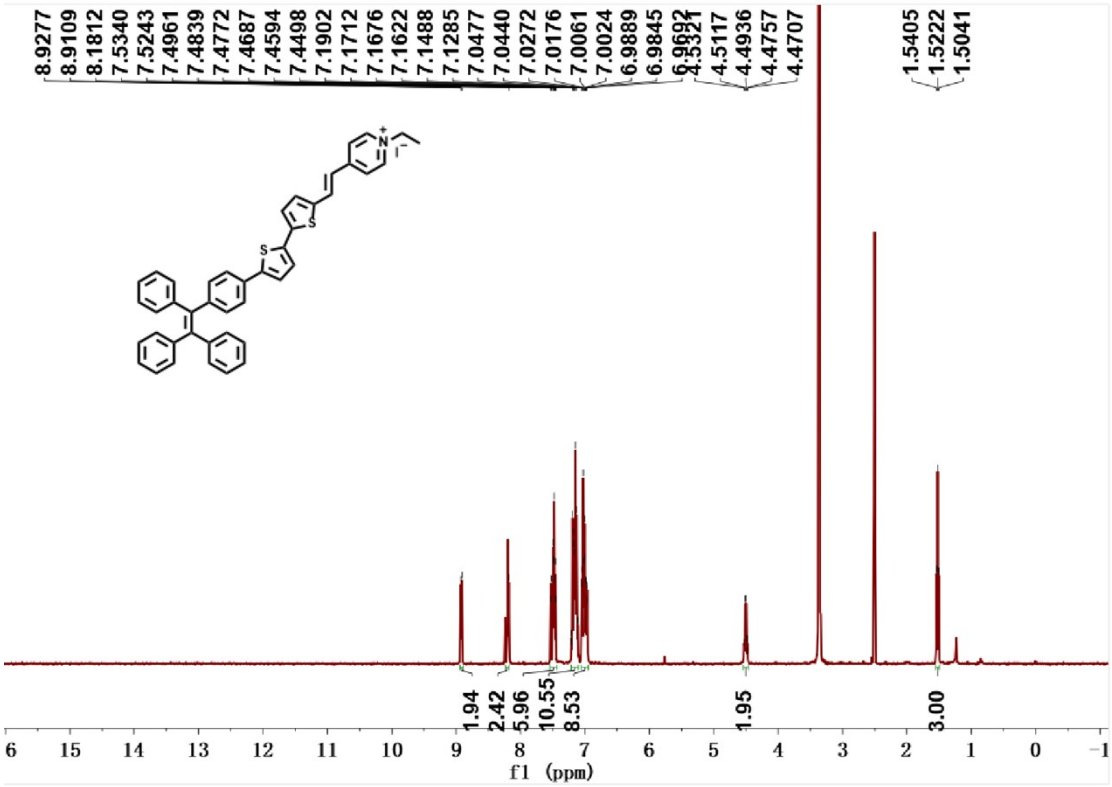


Figure S1. ^{1}H NMR spectrum of **TC2** in *d6*-DMSO at the room temperature.

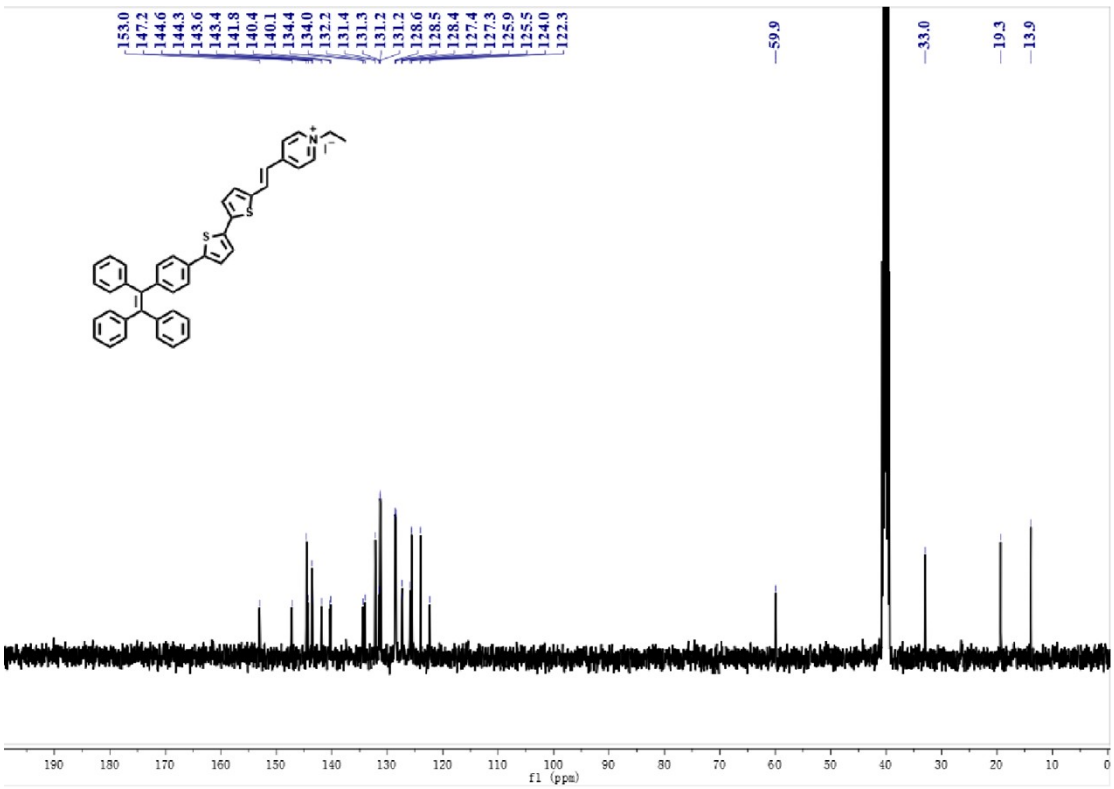


Figure S2. ^{13}C NMR spectrum of **TC2** in *d6*-DMSO at the room temperature.

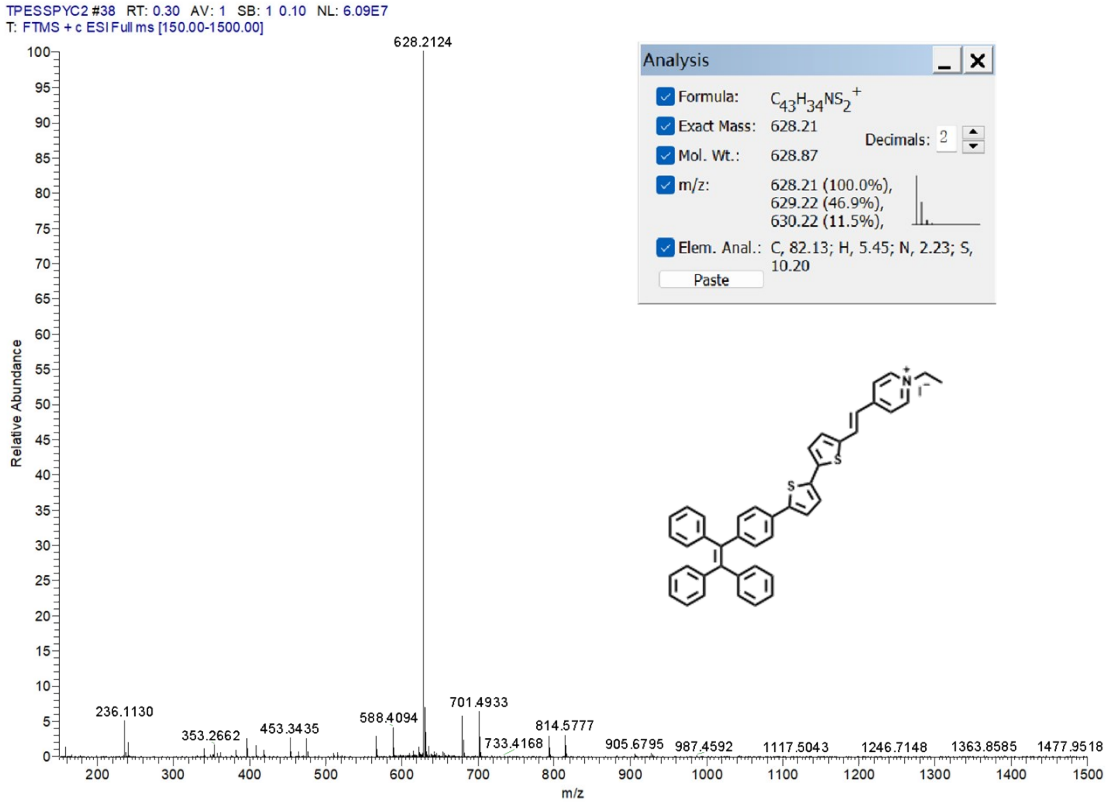


Figure S3. ESI- FTMS spectrum of **TC2**.

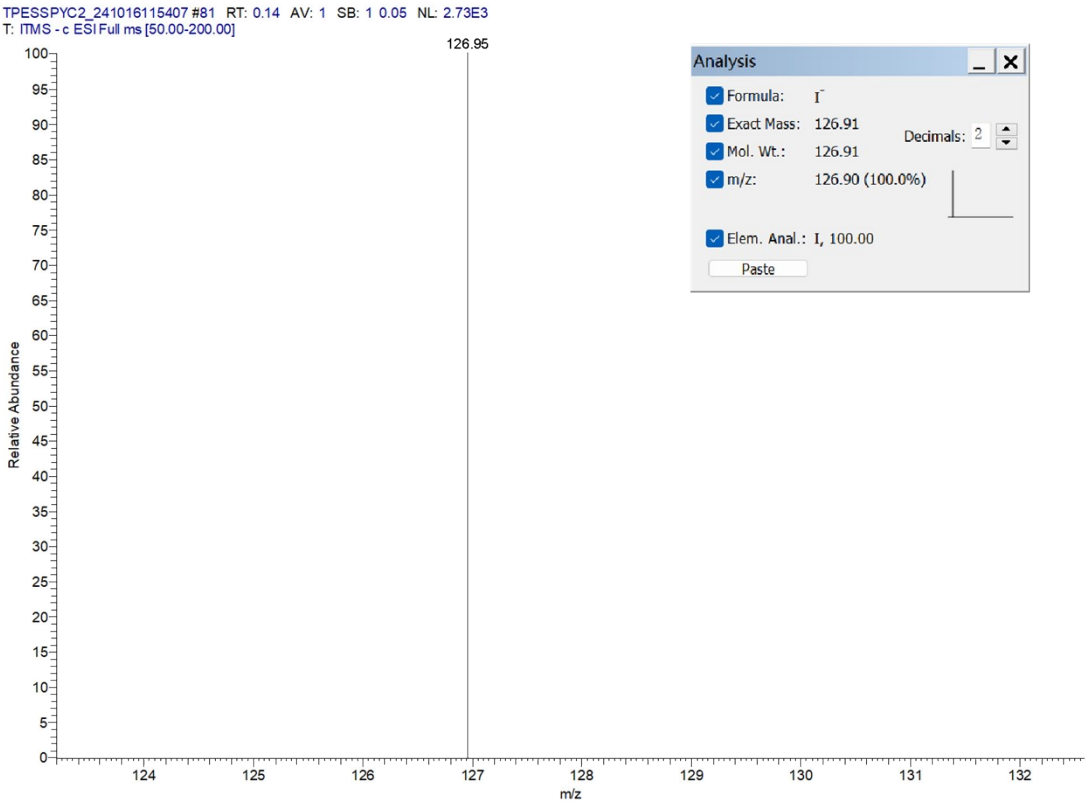


Figure S4. ESI- ITMS spectrum of **TC2**.

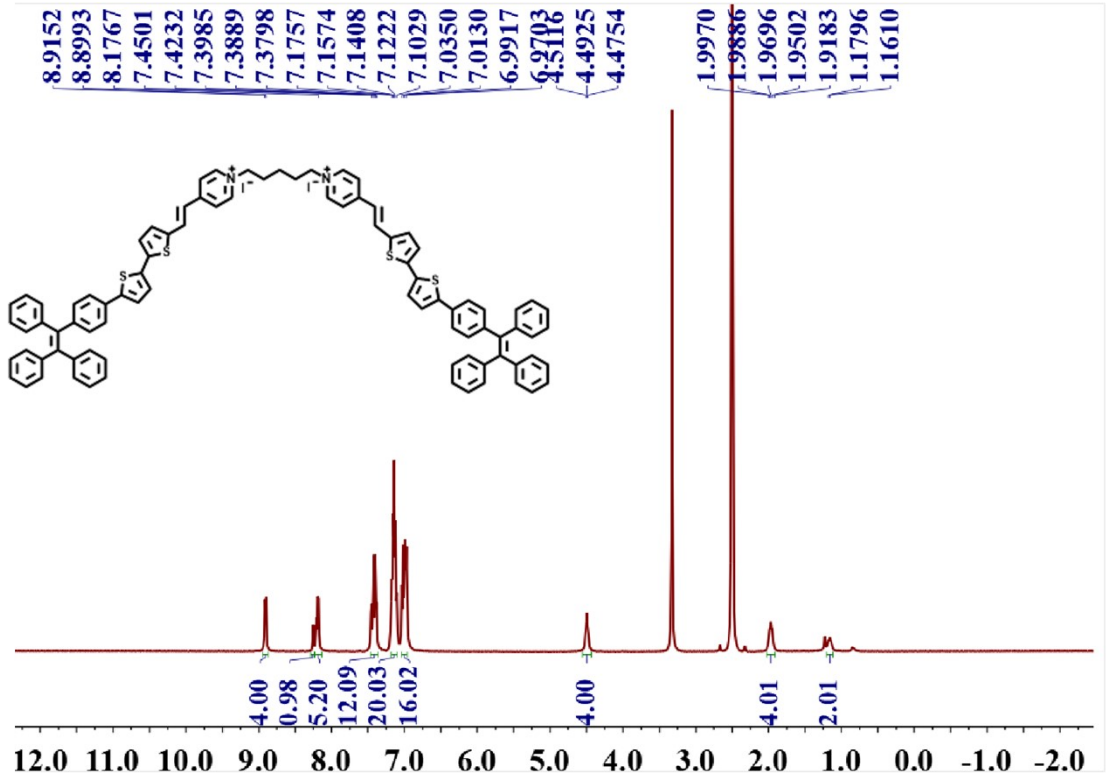


Figure S5. ^{1}H NMR spectrum of **DC5** in *d6*-DMSO at the room temperature.

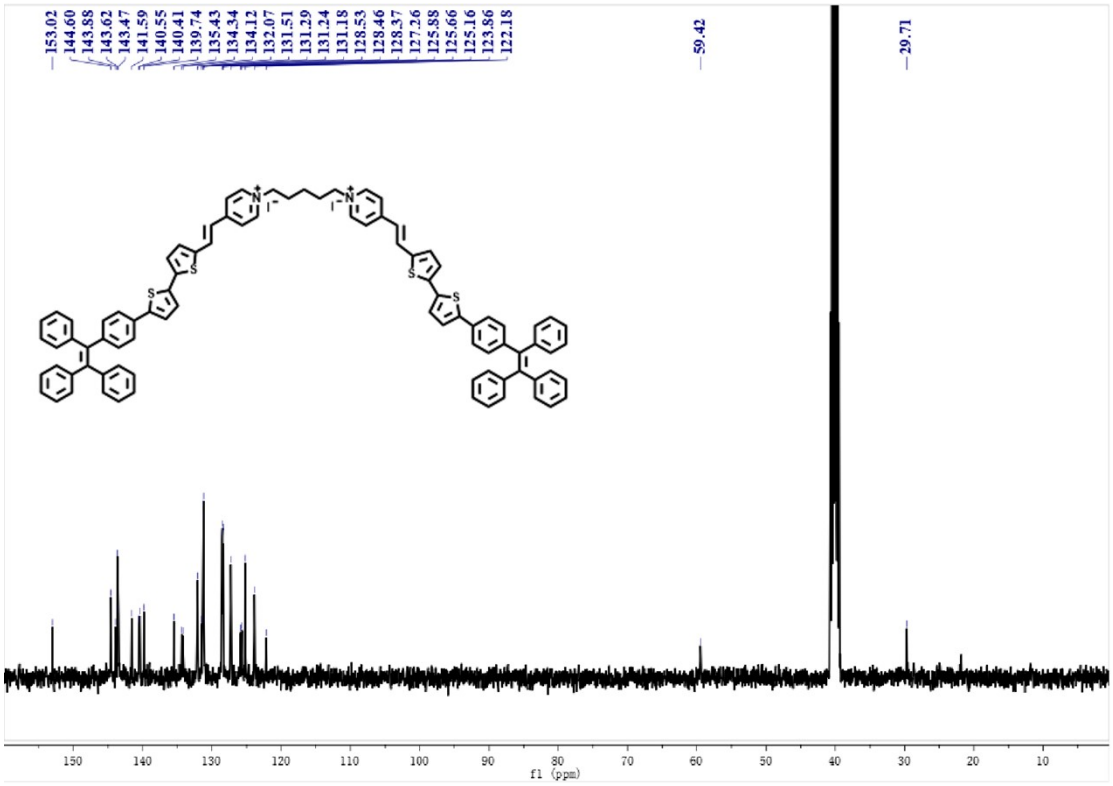


Figure S6. ^{13}C NMR spectrum of **DC5** in *d6*-DMSO at the room temperature.

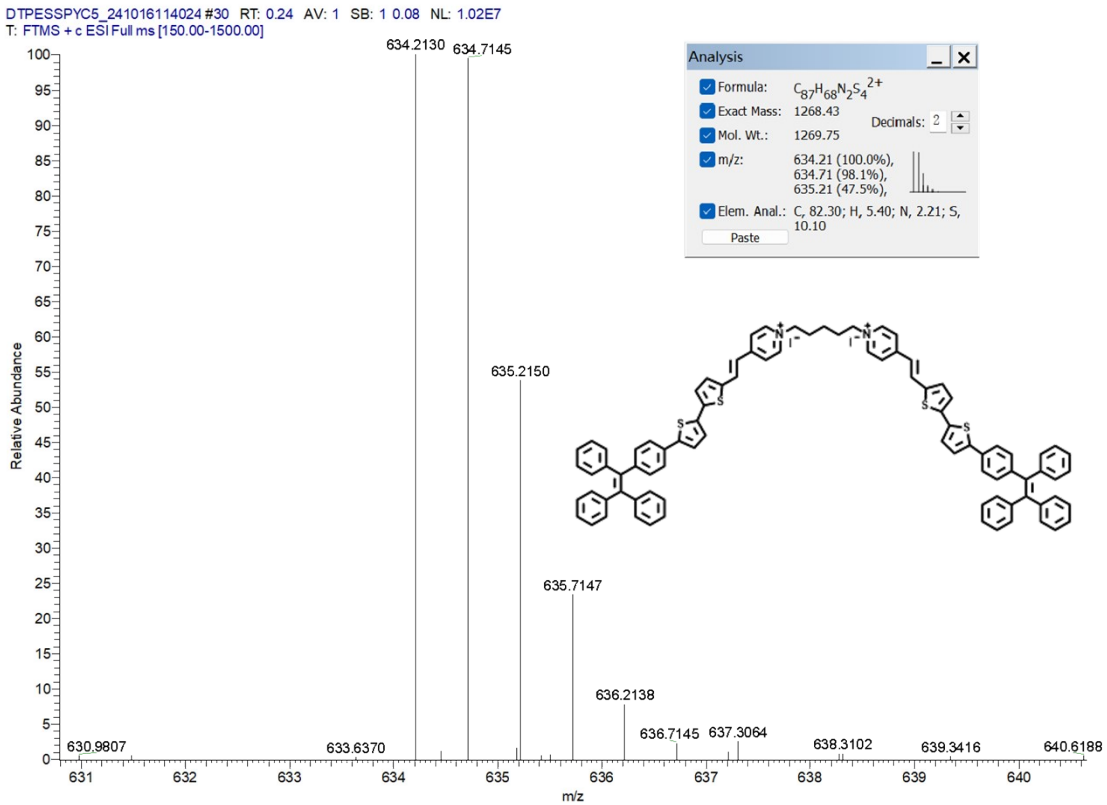


Figure S7. ESI- FTMS spectrum of **DC5**.

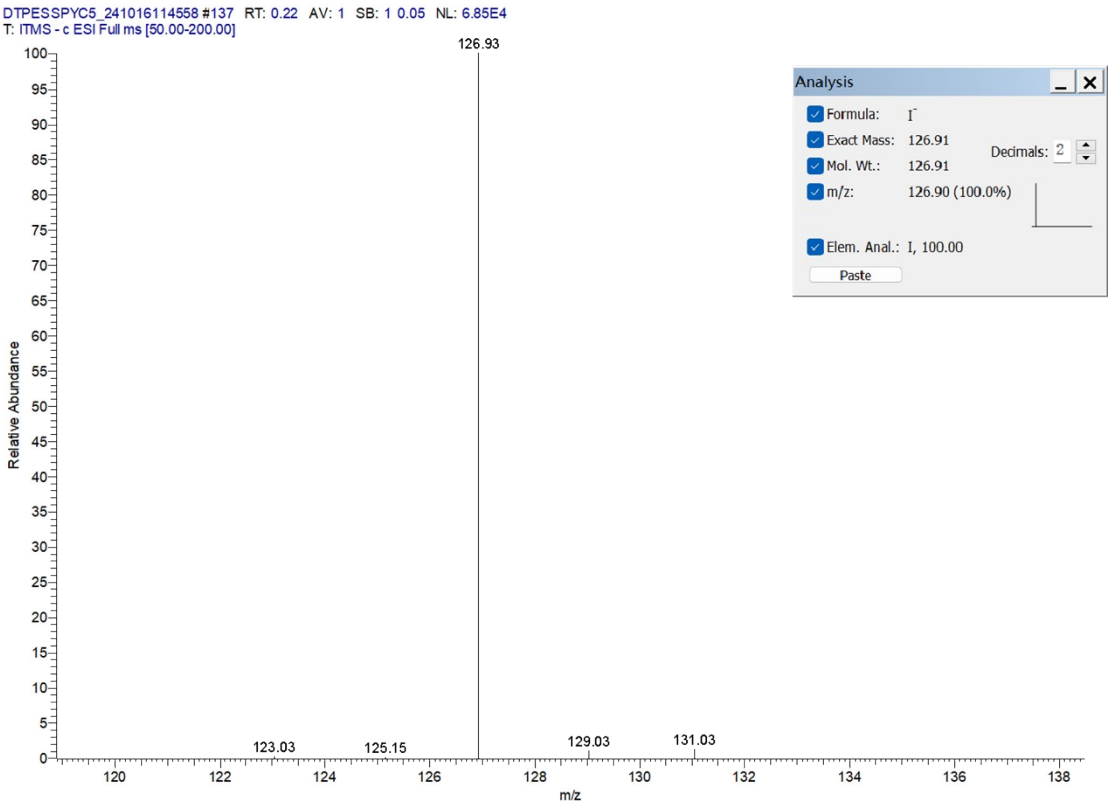


Figure S8. ESI- ITMS spectrum of **DC5**.

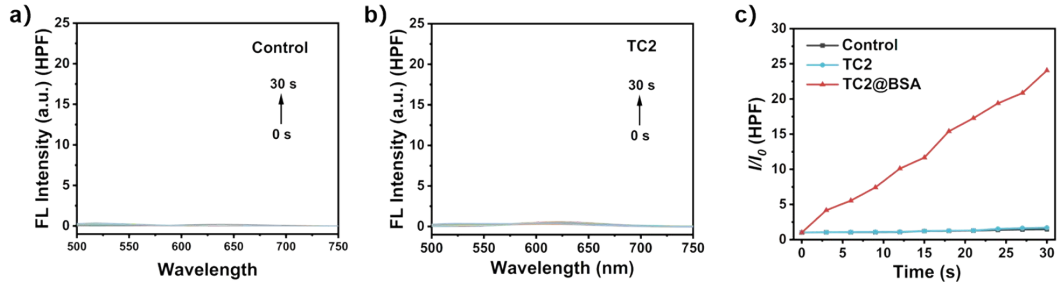


Figure S9. The detection of •OH generated upon irradiation of LED lamp using HPF, (a) Control, (b) **TC2** and (c) The relative fluorescence intensity (I/I_0) of HPF.

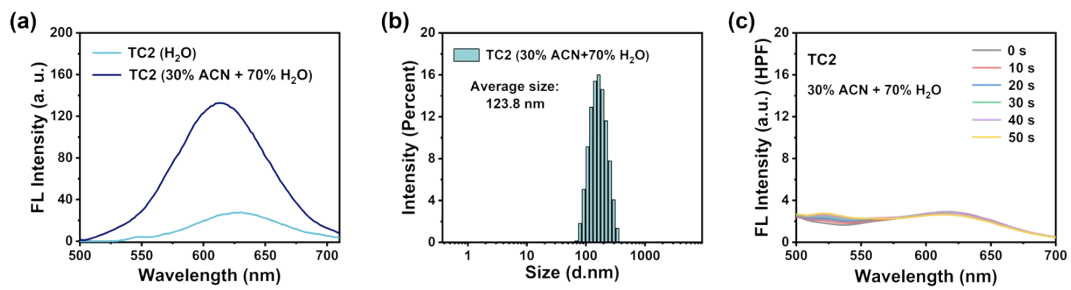


Figure S10. (a) Comparison of fluorescence intensity of **TC2** in H_2O and 30% ACN+70% H_2O; (b) DLS data and (c) •OH generation capability of **TC2** in 30% ACN+70% H_2O.

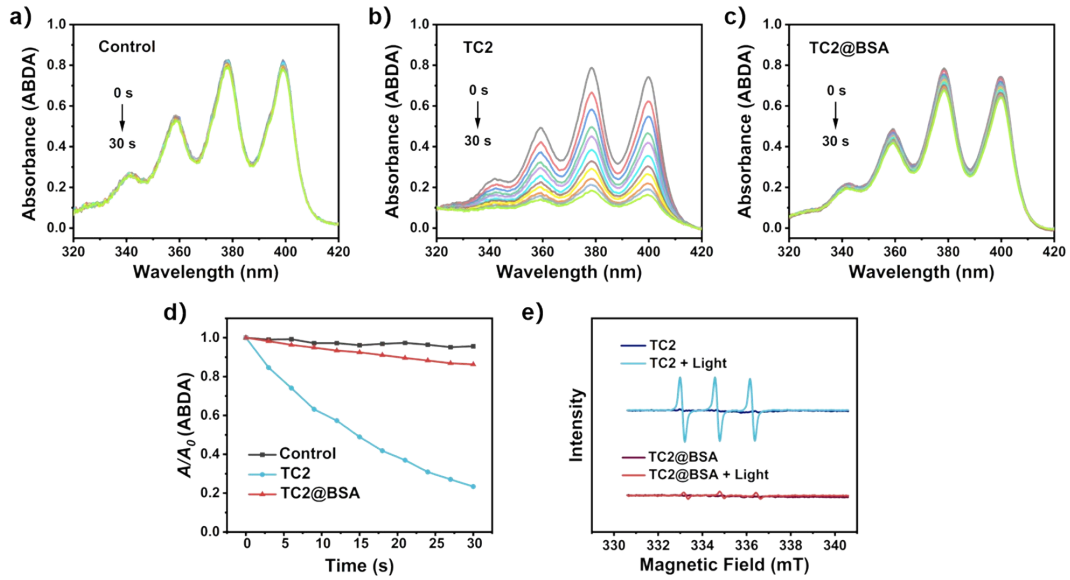


Figure S11. The detection of $^1O_2$ generated upon irradiation of LED lamp using ABDA, (a) Control, (b) **TC2**, (c) **TC2@BSA** and (d) The relative absorption spectra changes (A/A_0) of ABDA. (e) The ESR results of **TC2** and **TC2@BSA**, respectively. TEMP was used as $^1O_2$ trapping agent (white light for 3 min; 1 W cm^{-2}).

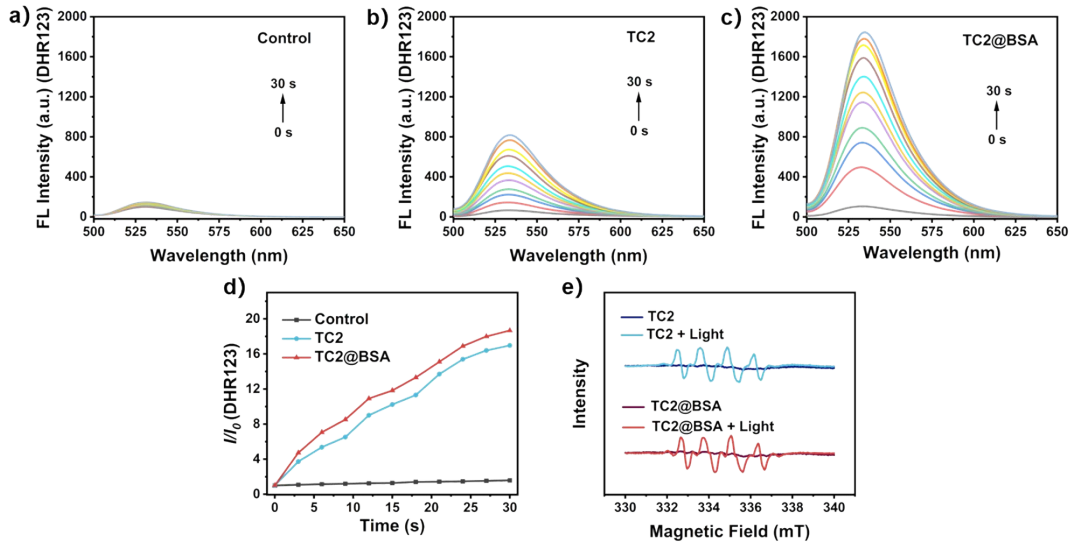


Figure S12. The detection of $O_2^{-\bullet}$ generated upon irradiation of LED lamp using DHR123, (a) Control, (b) **TC2**, (c) **TC2@BSA** and (d) The relative fluorescence intensity (I/I_0) of DHR123. (e) The ESR result of **TC2** and **TC2@BSA**, respectively. DMPO was used as $O_2^{-\bullet}$ trapping agent (white light for 3 min; 1 W cm^{-2}).

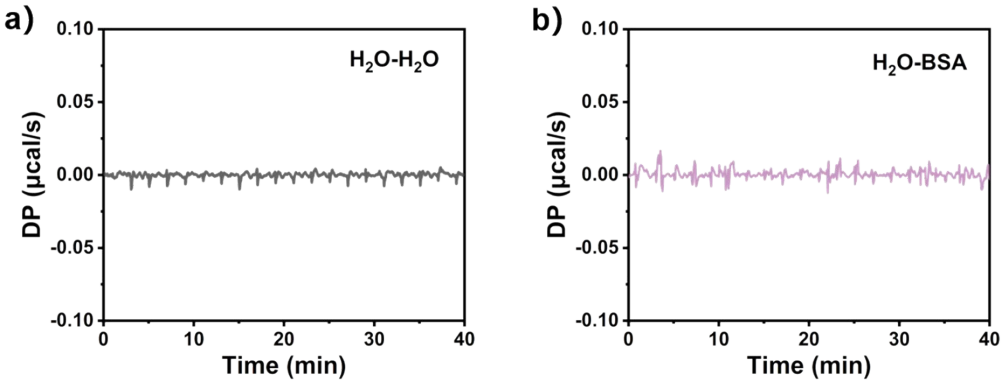


Figure S13. Calorimetric curves for titration of H_2O with serial injections of (a) H_2O and (b) BSA (10 μM) at 25 ℃.

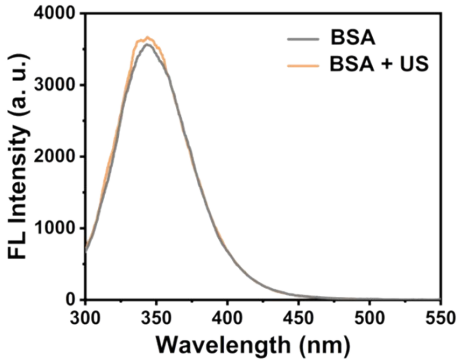


Figure S14. Changes in endogenous fluorescence of BSA solution (10 mg mL^{-1}) without $CHCl_3$ before and after sonication.

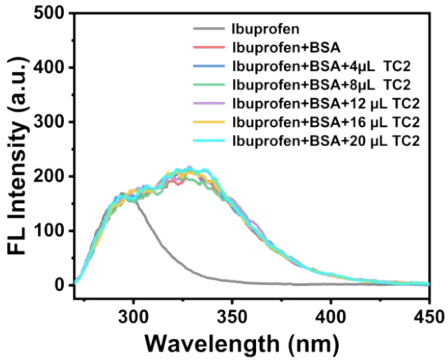


Figure S15. Ibuprofen was selected as the IIIA region marker of BSA, and the site marker replacement experiment was carried out by increasing the concentration of **TC2** (the excitation of Ibuprofen was 220 nm).

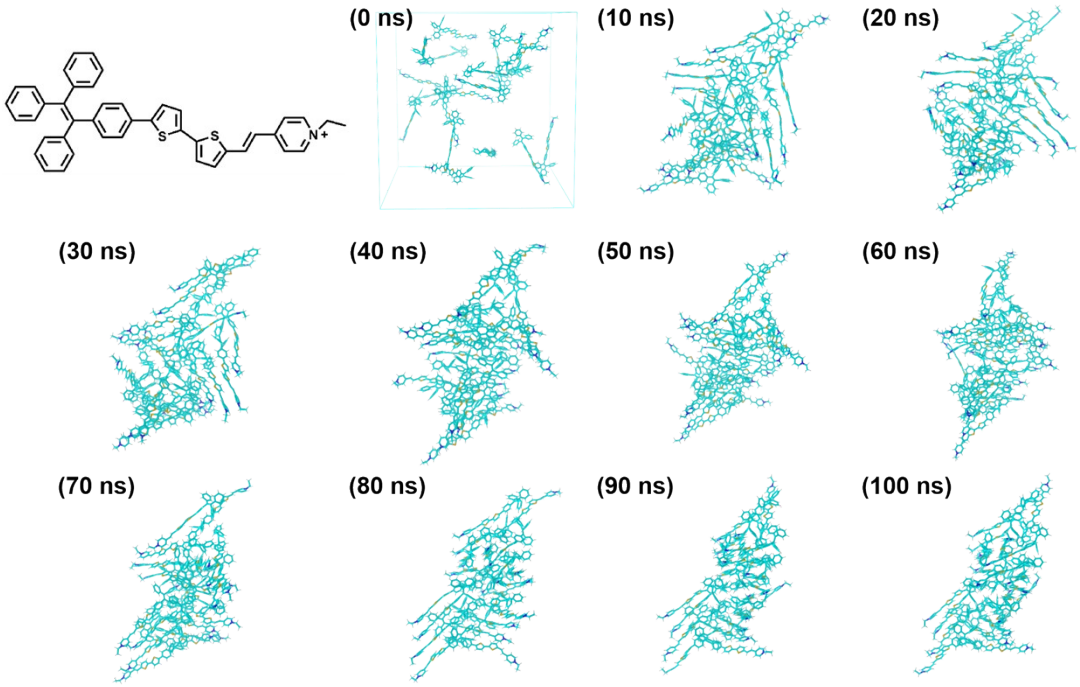


Figure S16. Molecular dynamics simulations of **TC2** (in an aqueous medium) aggregates across different time scales have yielded snapshots of amorphous aggregate structures.

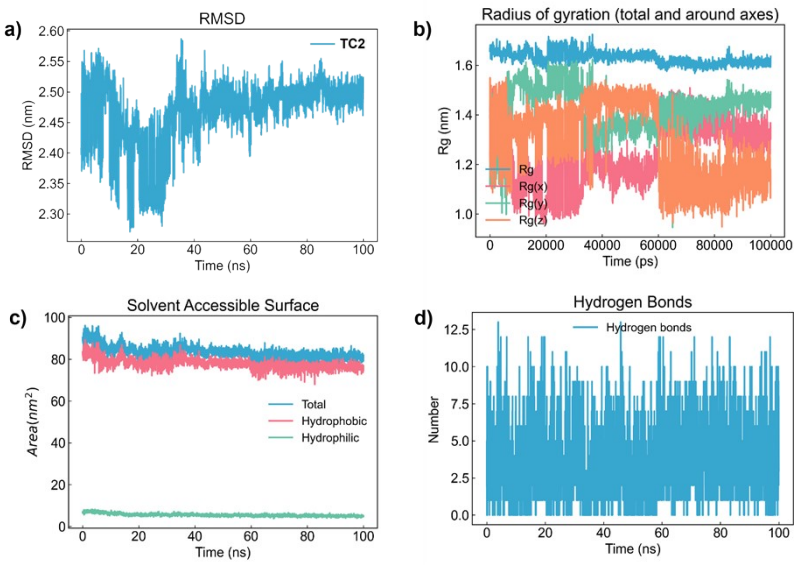


Figure S17. (a) The RMSD of **TC2**. (b) The Gyrate of **TC2**. (c) The solvent accessible surface of **TC2**. (d) The hydrogen bonds of **TC2** in water**.**

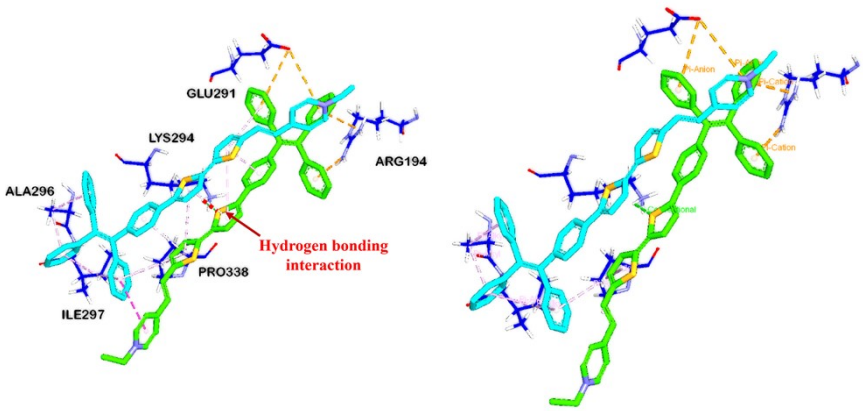


Figure S18. The interactions between BSA and **TC2**.

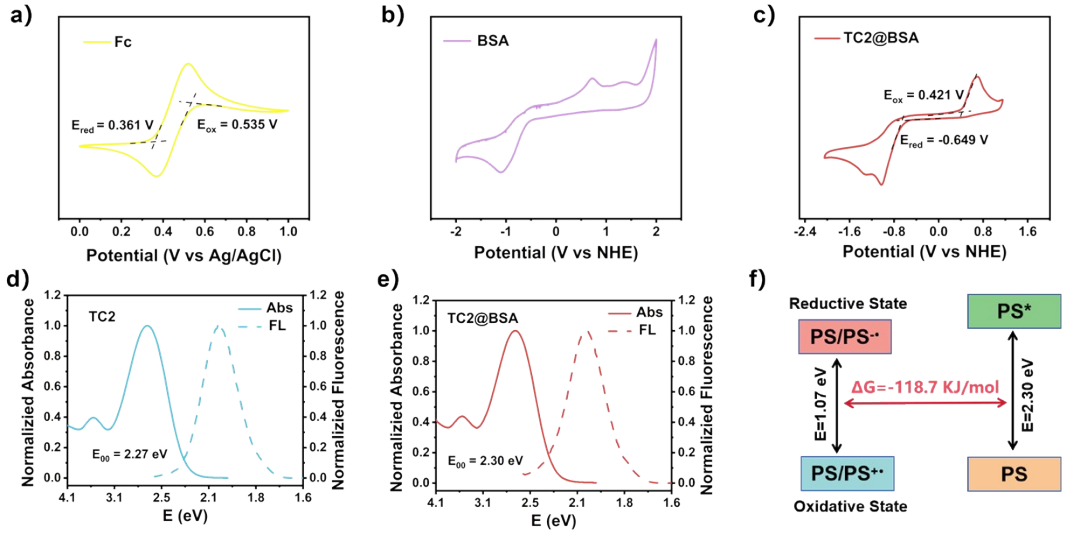


Figure S19. Cyclic voltammogram of (a) Ferrocene (Fc, 1.0 mM), (b) BSA (1.0 mM) and (c) **TC2@BSA** (1.0 mM) in CH_3CN, with $(n\text{-}Bu)_4N^+PF_6^-$ (0.1 M) as a supporting electrolyte, Ag/Ag^+ as a reference electrode, glassy carbon electrode as a working electrode and Pt wire as a counter electrode; scan rate, 100 mVs^{-1}. Normalized absorption and fluorescence spectra of (d) **TC2** and (e) **TC2@BSA**. (f) The Gibbs free energy of electron transfer between excited **TC2@BSA** and ground state **TC2@BSA** was calculated according to the Rehm-Weller equation.

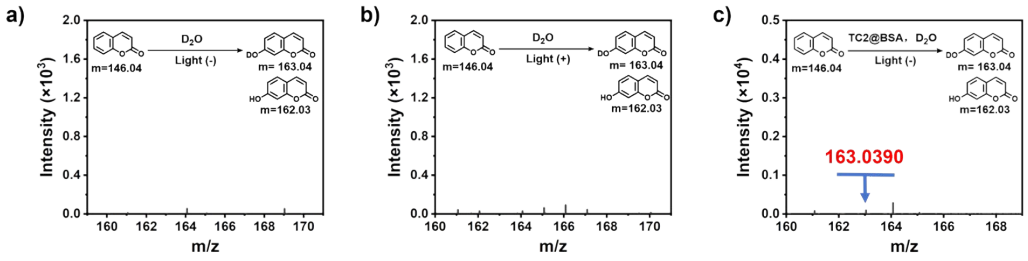


Figure S20. The recorded mass spectrometry for detection of •OH by coumarin in D_2O when **TC2@BSA** was not added under dark condition (a), **TC2@BSA** was not added under light condition (b), and **TC2@BSA** was added under dark condition(c).

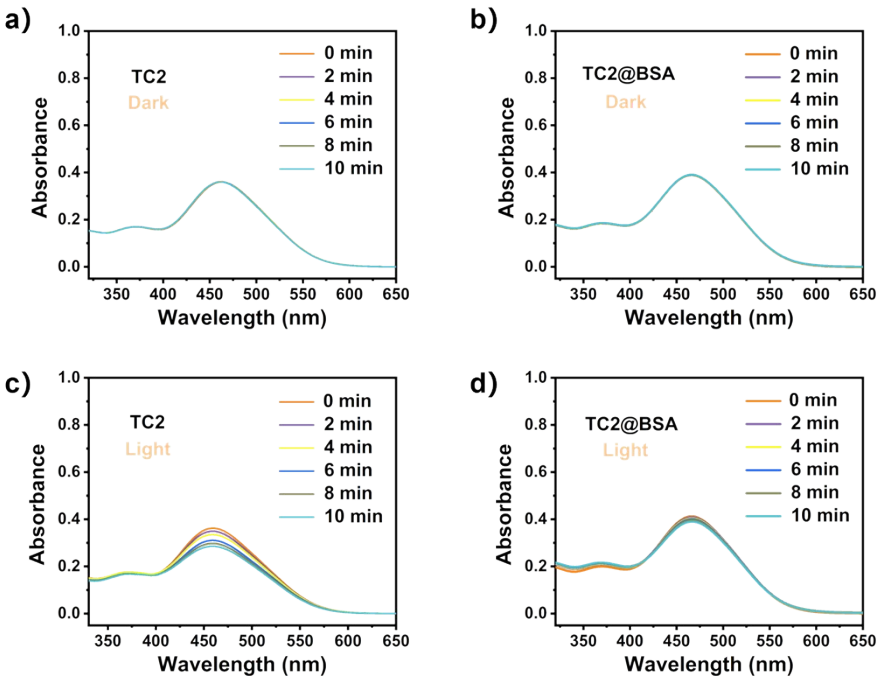


Figure S21. The stability test of **TC2** and **TC2@BSA** under dark and light conditions. Absorbance of (a) **TC2** and (b) **TC2@BSA** was measured every 2 min in the dark for a total of 10 min; Absorbance of (c) **TC2** and (d) **TC2@BSA** was measured every 2 min under light (white light; 1 W cm^{-2}) for a total of 10 min.

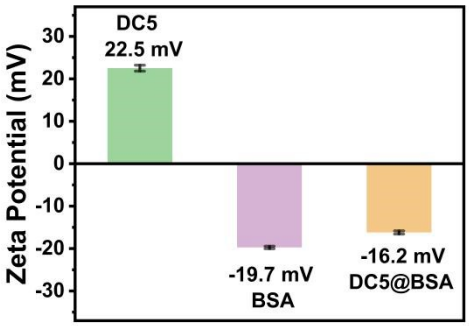


Figure S22. Zeta potentials of BSA, **DC5** and **DC5@BSA**, respectively. Error bars, mean ± SD, n = 3.

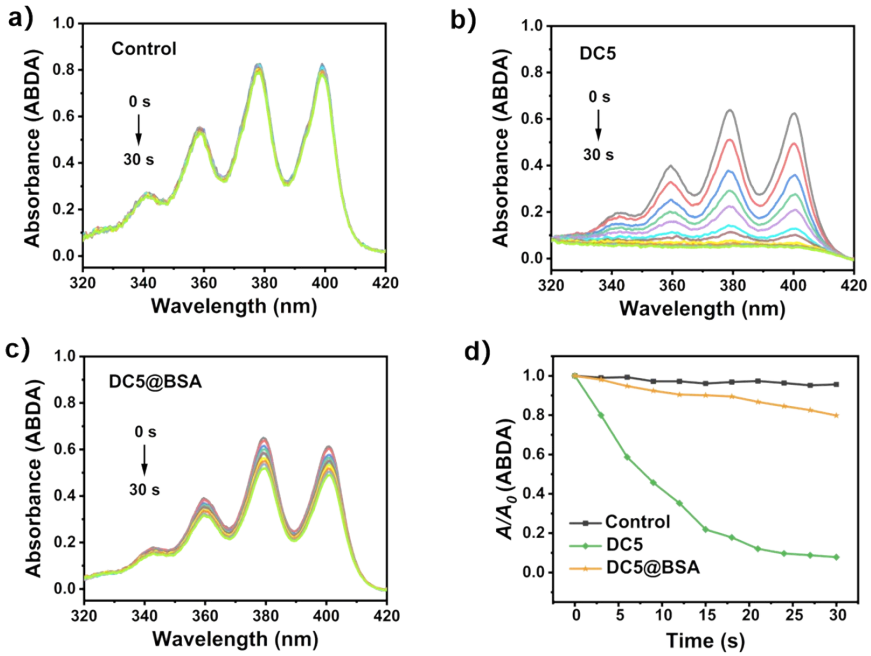


Figure S23. The detection of $^1O_2$ generated upon irradiation of LED lamp using ABDA, (a) Control, (b) **DC5**, (c) **DC5@BSA** and (d) The relative absorption spectra changes (A/A_0) of ABDA.

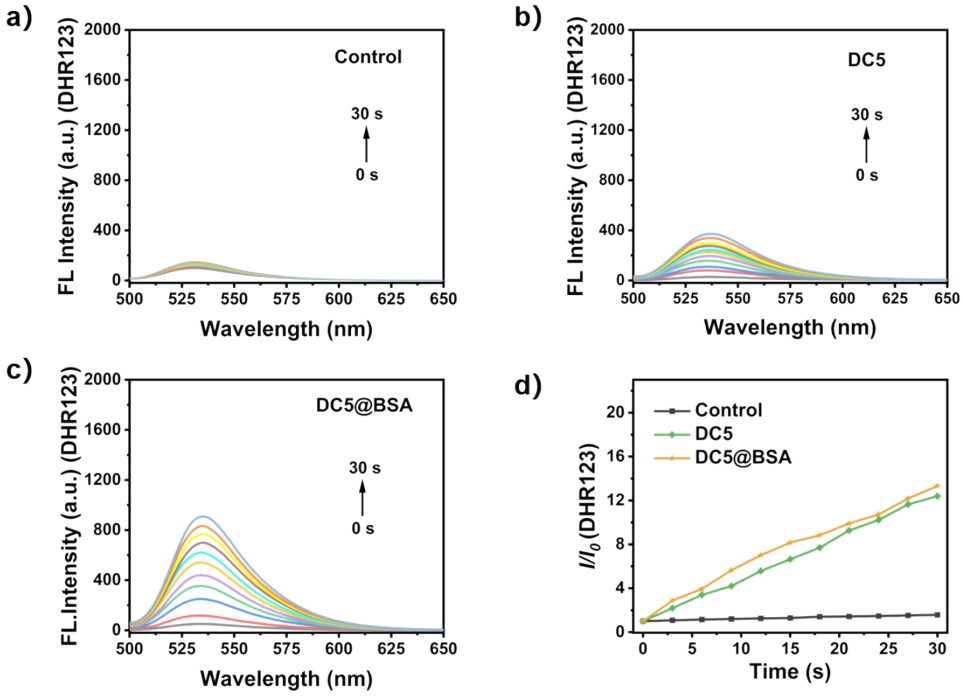


Figure S24. The detection of $O_2^{-\bullet}$ generated upon irradiation of LED lamp using DHR123, (a) Control, (b) **DC5**, (c) **DC5@BSA** and (d) The relative fluorescence

intensity (I/I_0) of DHR123.

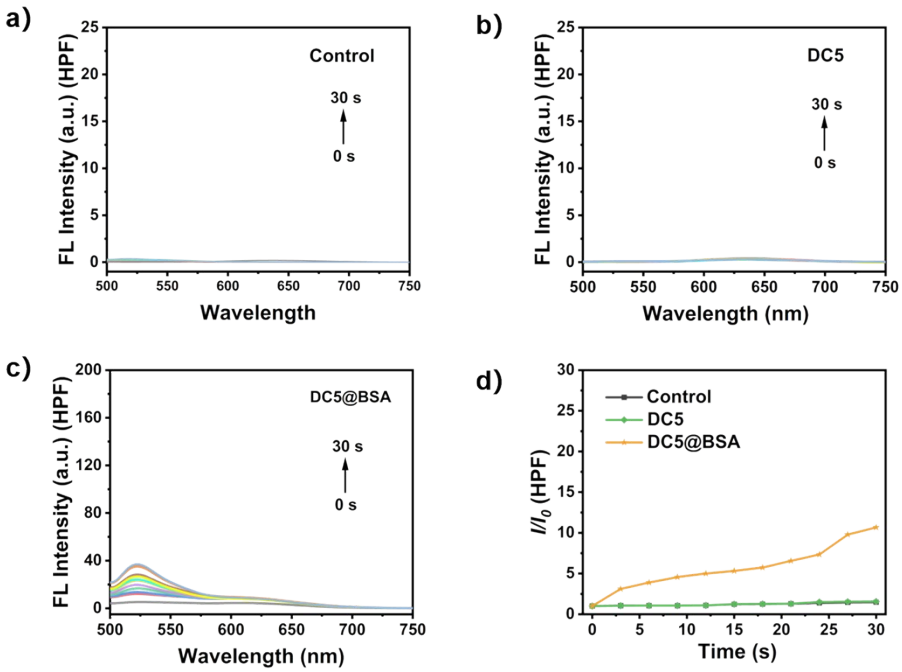


Figure S25. The detection of •OH generated upon irradiation of LED lamp using HPF, (a) Control, (b) **DC5**, (c) **DC5@BSA** and (d) The relative fluorescence intensity (I/I_0) of HPF.

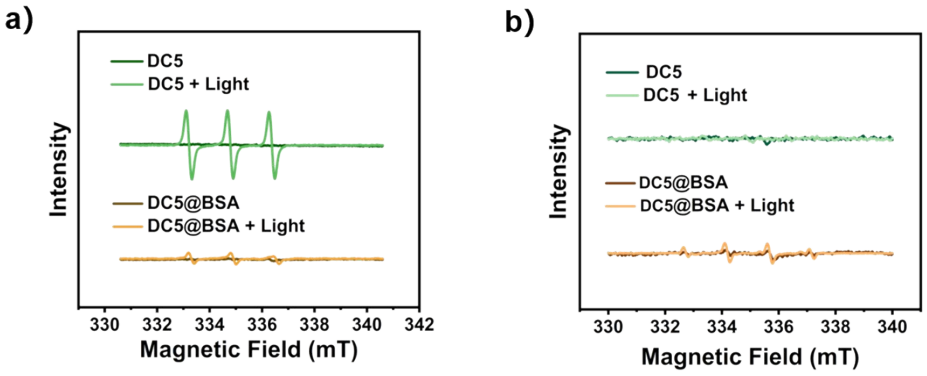


Figure S26. (a) The ESR results of **DC5** and **DC5@BSA**, respectively. TEMP was used as $^1O_2$ trapping agent (white light for 3 min; 1 W cm^{-2}). (b) ESR results of **DC5** and **DC5@BSA**, respectively. DMPO was used as $O_2^{-\bullet}$ trapping agent (white light for 3 min; 1 W cm^{-2}).

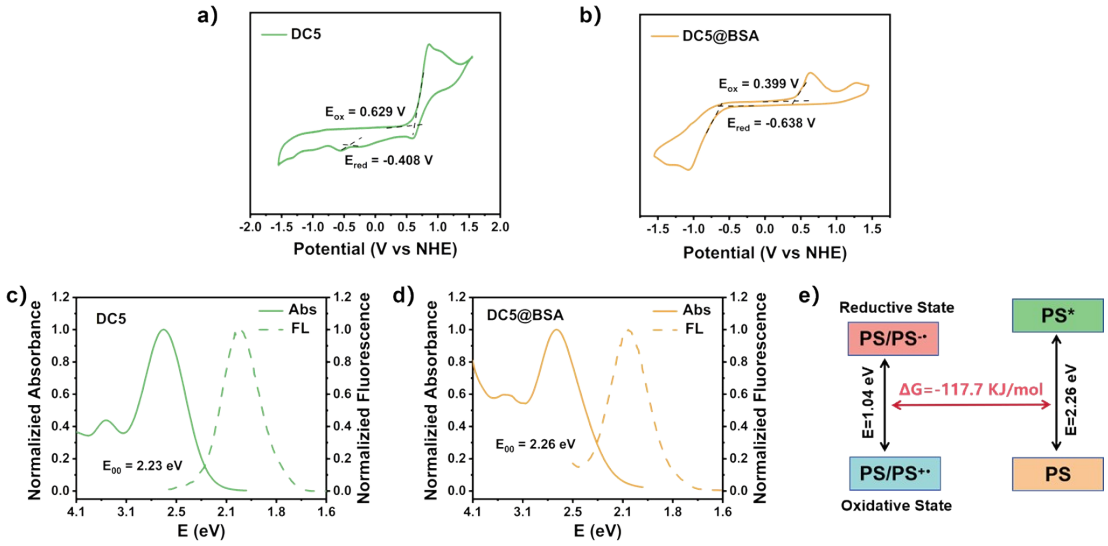


Figure S27. Cyclic voltammogram of (a) **DC5** and (b) **DC5@BSA** (1.0 mM) in CH_3CN. Normalized absorption and fluorescence spectra of (c) **DC5** and (d) **DC5@BSA**. (e) The Gibbs free energy of electron transfer between excited **DC5@BSA** and ground state **DC5@BSA** was calculated according to the Rehm-Weller equation.

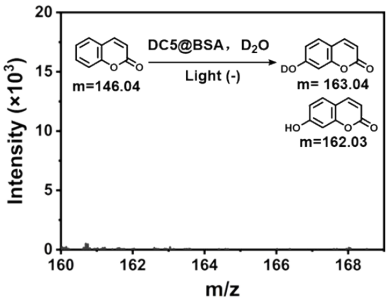


Figure S28. The recorded mass spectrometry for detection of •OH in D_2O using coumarin when **DC5@BSA** was added under dark condition.

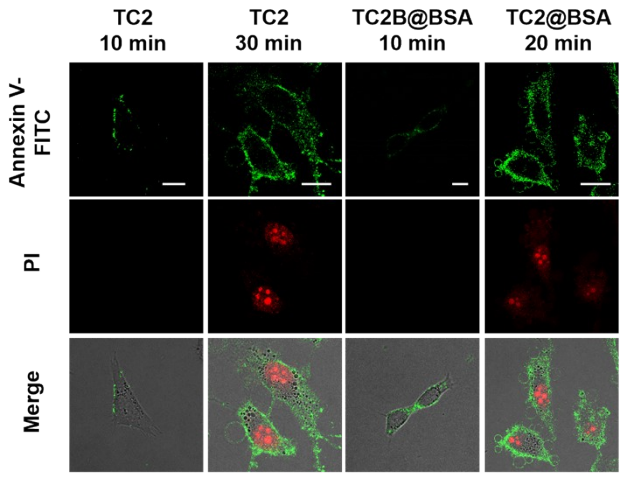


Figure S29. HepG2 cells images after they were stained with **TC2/TC2@BSA** for 15 min and then exposed to light for 30 min/20 min before staining with Annexin V-FITC and PI. The green channel: $\lambda_{ex}/\lambda_{em}$ = 488/500–550 nm, for Annexin V-FITC. The red channel: $\lambda_{ex}/\lambda_{em}$ = 488/580–630 nm, for PI. Scale bar: 20 μm.

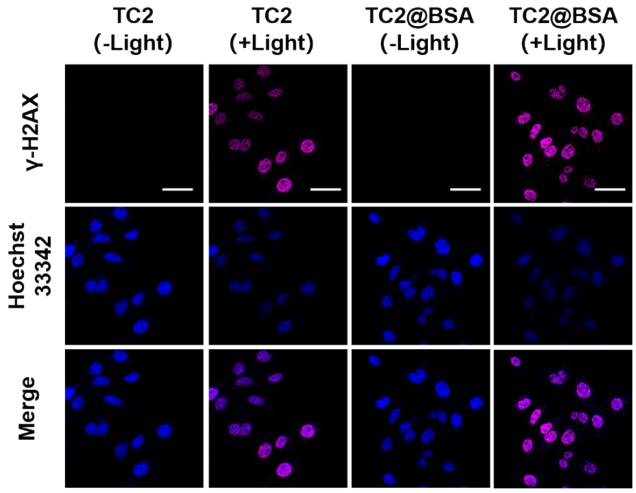


Figure S30. γ-H2AX was used as a probe to detect nuclear damage. The magenta channel: $\lambda_{ex}/\lambda_{em}$ = 495/500–550 nm, for γ-H2AX. The blue channel: $\lambda_{ex}/\lambda_{em}$ = 405/430–480 nm, for Hoechst 33342. Scale bar: 50 μm.

References

1 M.-Y. Wu, L.-J. Wang, C.-M. Qin, Y. Wang, J.-X. Song, Y. Zhao, Y. Wan, S. Feng, *Sensors and Actuators B: Chemical* **2023**, *381*, 133471.

2 Y. Ni, K. Li, X. Xu, X. Zhu, Z. Lu, X. Zhao, T. Wang, J. Wang, X. Chen, H. Zhou, *Science China Materials* **2025**, 1-9.

3 J. Wang, Y. Zhu, J. Li, A. Wang, Z. Yu, S. Wang, H. Zhou, *Science China Materials* **2023**, *66*, 2483-2491.