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Supporting Information

Molecular Engineering of Metal-based Photosensitizers with Narrow Band Gap for Efficient Photodynamic Therapy

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General

The reagents and solvents utilized in this study were sourced from chemical suppliers, including Sinopharm Chemical Reagent (SCRC), Bide Pharmatech, Macklin and KeyGEN. NMR spectra were captured on a Bruker spectrometer operating at 600 MHz for ^{1}H and 151 MHz for ^{13}C spectra. MS analysis was conducted using an BRUKER UltrafleXtreme MALDI-TOF/TOF mass spectrometer. Absorption spectra were generated using a UV-2600 Shimadzu spectrometer. Confocal fluorescence imaging was carried out using an Olympus Corporation FV3000 laser scanning confocal microscopy (LSCM), utilizing the Buccaneer-SHG laser manufactured by Changchun Femtosecond Technology Co., Ltd. [Ir_2(2-phenylpyridine)$_4Cl_2$], 2,3-di(2-pyridyl)quinoxaline, and dipyrido[2,3-a:3',2'-c]phenazine were prepared according to previous literatures.[1-3]

Synthesis and characterization

Synthesis of IrPQ1. [Ir_2(2-phenylpyridine)$_4Cl_2$] (188 mg, 0.18 mmol) in a 100 mL round-bottomed flask and dissolve it with 20 mL methanol, weigh 2,3-di(pyridin-2-yl)quinoxaline (50 mg, 0.18 mmol) in 20 mL methanol and inject it into the round-bottomed flask. Under nitrogen protection, reflux at 80 ℃ for 12 h, the obtained filtrate was spun dry, and the product was purified with silica gel column chromatography (eluent was dichloromethane/methanol = 10/1, v/v) to obtain red solid product IrPQ1 (103 mg, 70.0%). Anal. Calcd for $C_{40}H_{28}IrN_6Cl$: C, 58.56; H, 3.44; N, 10.24. Found: C, 58.48; H, 3.45; N, 10.26. ^{1}H NMR (600 MHz, d_6-DMSO) δ 8.66 (s, 1H), 8.44 (d, J = 5.3 Hz, 1H), 8.24 (dd, J = 48.3, 22.9 Hz, 4H), 8.05 (d, J = 31.1 Hz, 5H), 7.94 – 7.75 (m, 5H), 7.69 (s, 1H), 7.58 (s, 1H), 7.41 (s, 2H), 7.25 (s, 1H), 7.16 (s, 1H), 7.02 (d, J = 40.1 Hz, 3H), 6.89 (s, 1H), 6.35 (s, 1H), 6.08 (s, 1H). ^{13}C NMR (151 MHz, d_6-DMSO) δ 167.09, 166.29, 157.66, 156.24, 153.69, 153.50, 151.73, 150.29, 150.11, 149.34, 148.94, 146.95, 144.19, 143.57, 142.16, 139.90, 139.61, 139.08, 138.38, 137.34, 133.60, 132.46, 131.84, 130.95, 130.85, 130.81, 130.74, 129.90, 128.68, 127.91, 126.03, 125.90, 125.64, 124.80, 124.66, 124.03, 123.42, 122.87, 121.13, 120.27. MS (MALDI-TOF) m/z $[M-Cl]^+$ = 785.2.

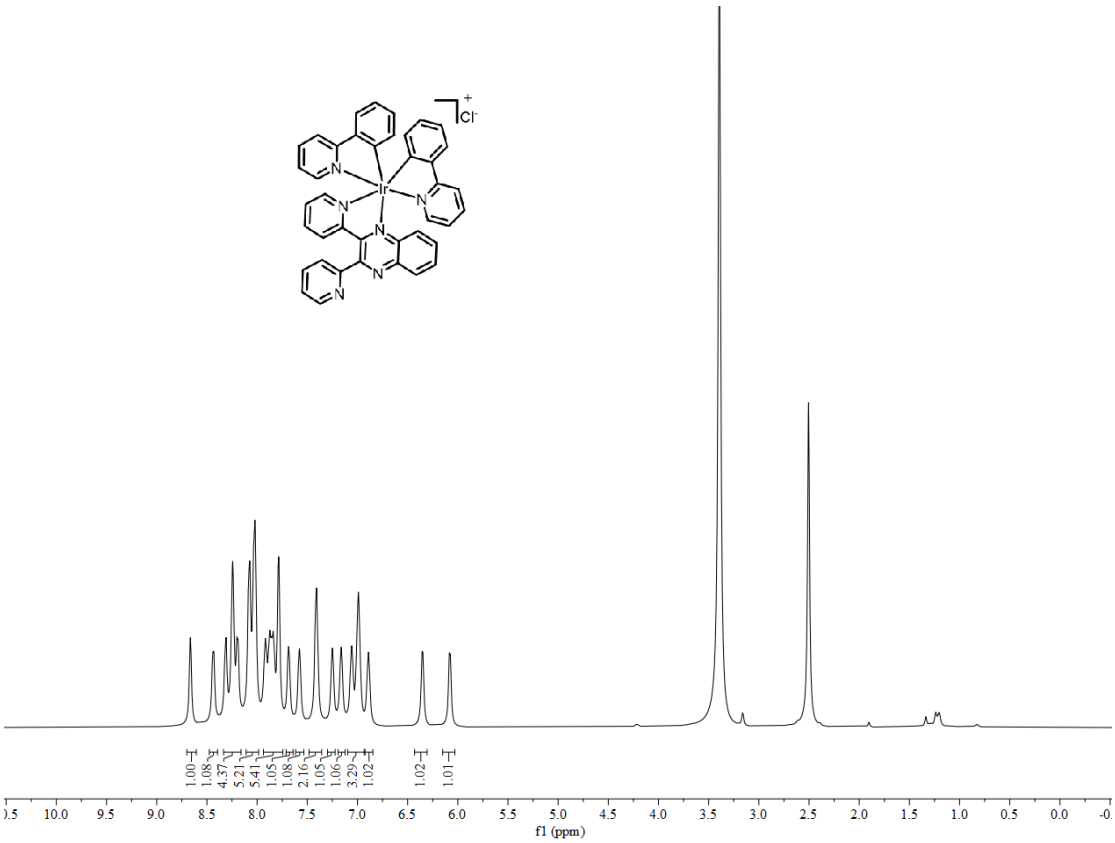

Figure S1. ^{1}H NMR spectrum of IrPQ1 (DMSO-d_6)

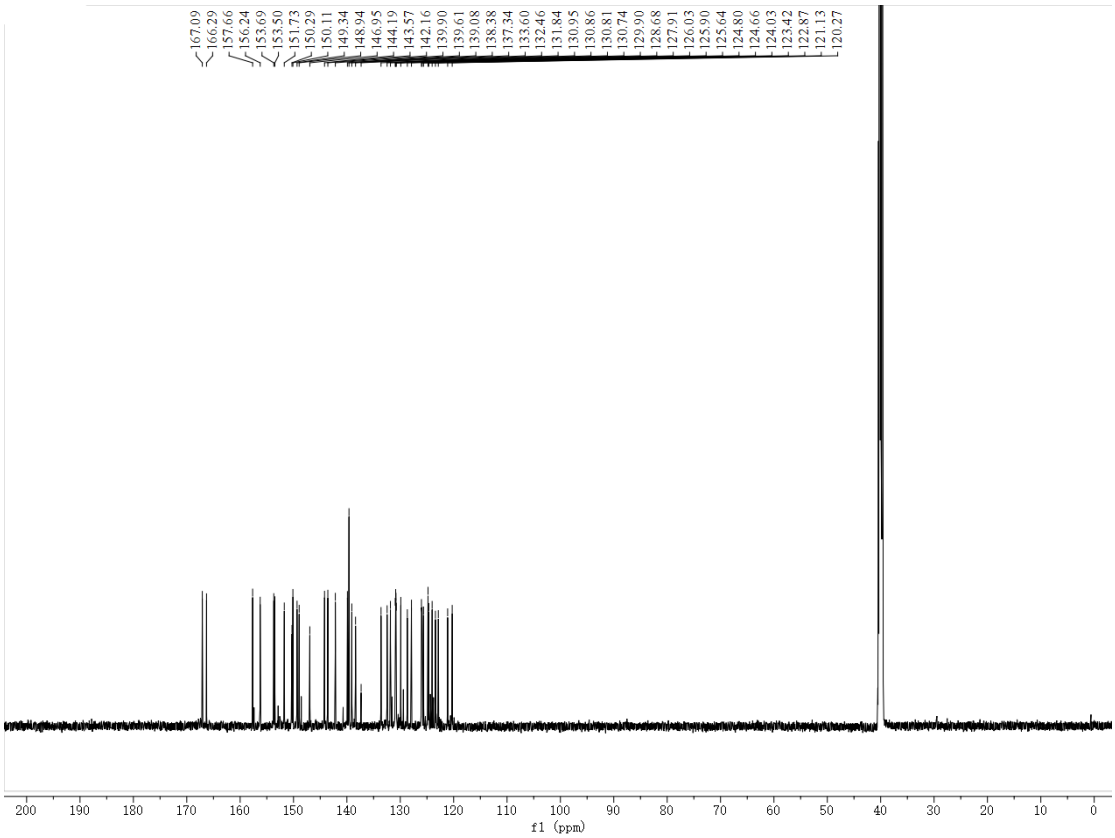

Figure S2. ^{13}C NMR spectrum of IrPQ1 (DMSO -d_6)

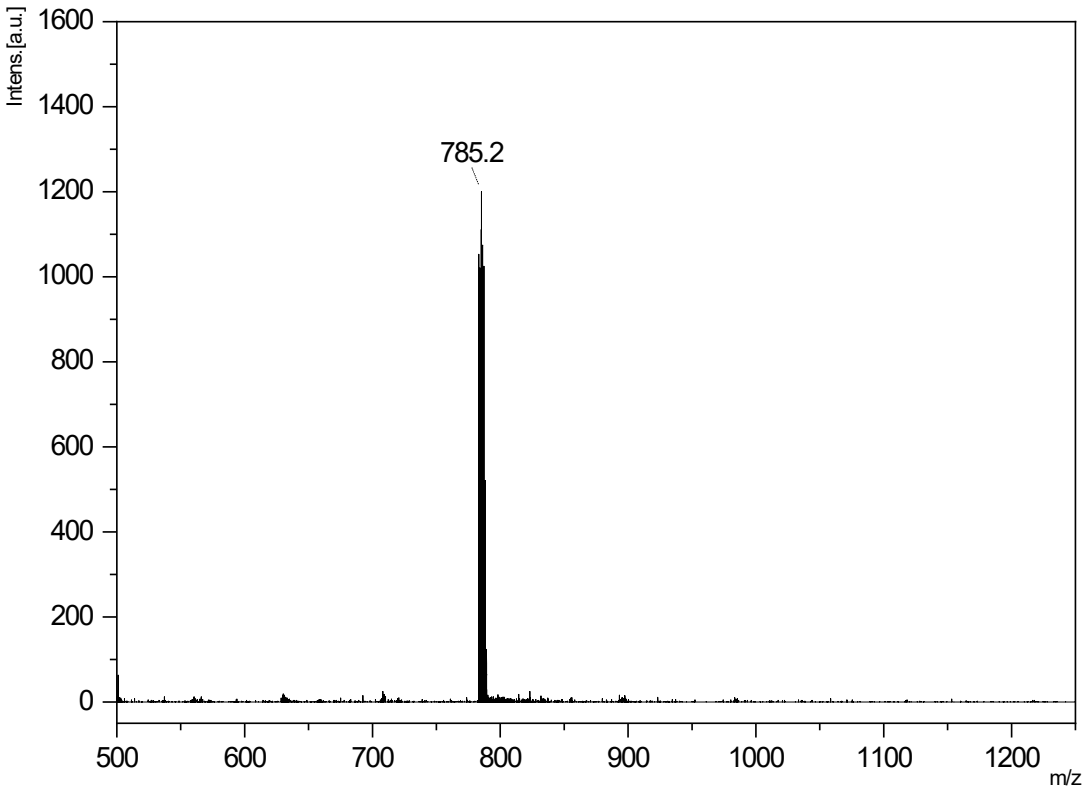

Figure S3. MALDI-TOF-MS spectrum of IrPQ1

Synthesis of IrPQ2. IrPQ2 was prepared similar to that of IrPQ1 with dipyrido[2,3-a:3',2'-c]phenazine as starting material. Green solid (56 mg, 38.2%). Anal. Calcd for $C_{40}H_{26}IrN_6Cl$: C, 58.71; H, 3.20; N, 10.27. Found: C, 58.59; H, 3.19; N, 10.29. ^{1}H NMR (600 MHz, d_6-DMSO) δ 9.65 (d, J = 8.3 Hz, 1H), 9.50 (d, J = 7.7 Hz, 1H), 9.31 (d, J = 24.8 Hz, 1H), 8.55 (dd, J = 12.8, 8.6 Hz, 1H), 8.33 (d, J = 8.1 Hz, 1H), 8.23 (d, J = 8.1 Hz, 1H), 8.21 – 8.16 (m, 2H), 8.16 – 8.12 (m, 2H), 8.05 – 7.96 (m, 3H), 7.91 (t, J = 7.9 Hz, 1H), 7.87 – 7.79 (m, 2H), 7.56 – 7.48 (m, 1H), 7.43 (d, J = 4.4 Hz, 1H), 7.13 – 7.08 (m, 2H), 7.04 (t, J = 7.4 Hz, 1H), 6.98 (td, J = 7.5, 1.2 Hz, 1H), 6.94 – 6.89 (m, 2H), 6.33 (d, J = 7.5 Hz, 1H), 6.07 (d, J = 7.6 Hz, 1H). ^{13}C NMR (151 MHz, d_6-DMSO) δ 167.21, 166.27, 152.56, 152.01, 150.95, 150.52, 150.31, 148.71, 147.91, 146.07, 145.93, 145.72, 145.20, 144.39, 144.39, 143.76, 141.44, 139.45, 139.25, 135.24, 133.73, 133.56, 133.39, 131.70, 131.58, 131.18, 130.89, 130.35, 129.21, 127.78, 126.44, 125.91, 125.88, 125.78, 124.63, 124.13, 123.57, 122.97, 120.75, 120.41. MS (MALDI-TOF) m/z $[M\text{-}Cl]^+$ = 783.2.

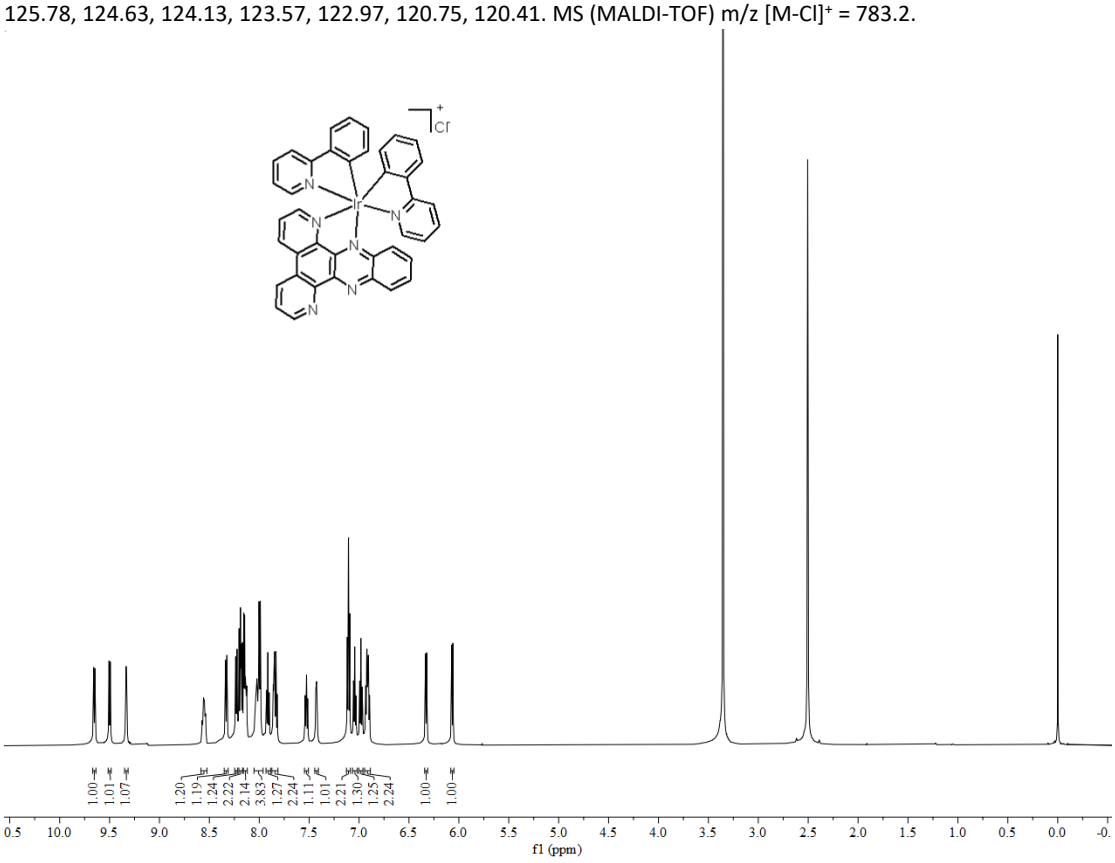

Figure S4. ^{1}H NMR spectrum of IrPQ2 (DMSO-d_6)

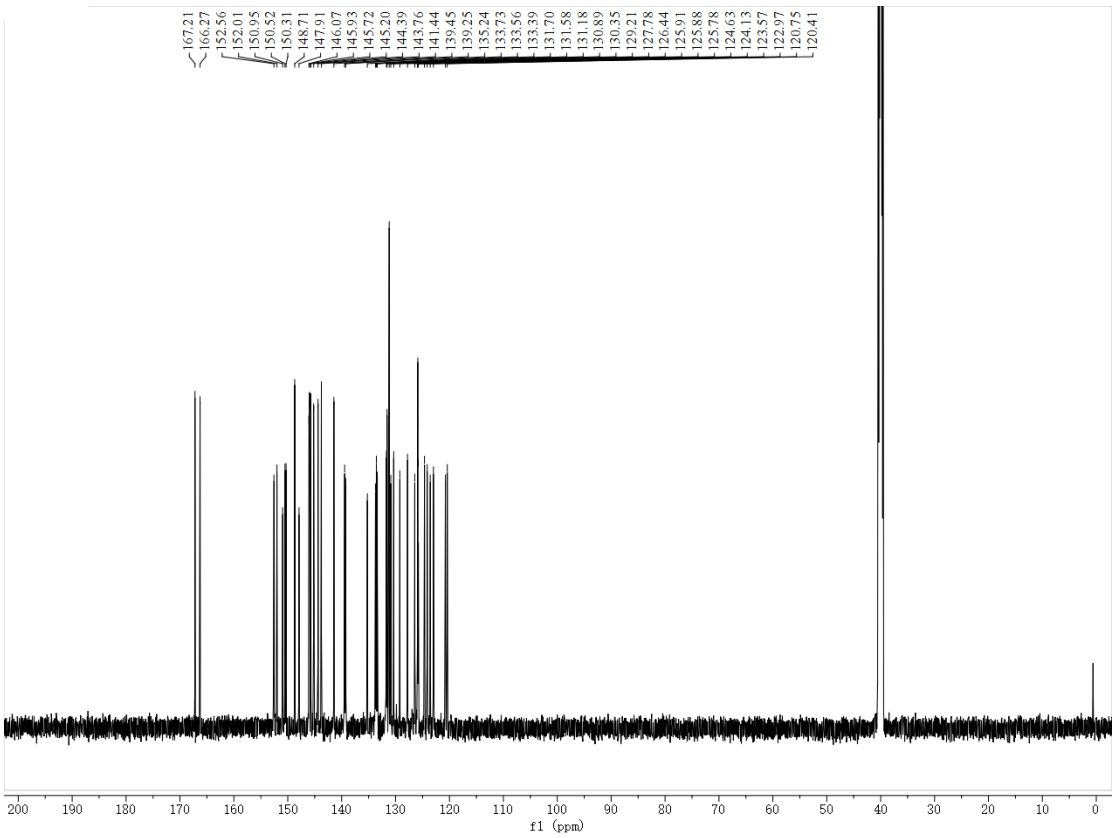

Figure S5. ^{13}C NMR spectrum of IrPQ2 (DMSO -d_6)

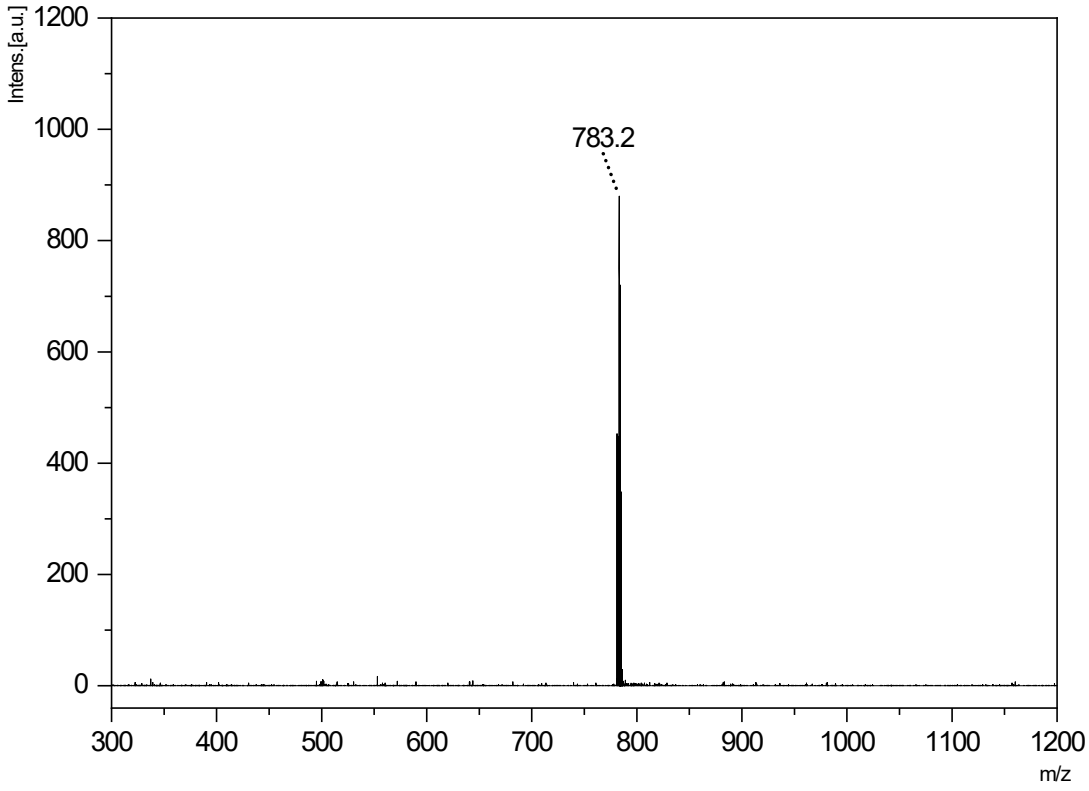

Figure S6. MALDI-TOF-MS spectrum of IrPQ2

Synthesis of IrPQ3. [Ir_2(2-phenylpyridine)$_4Cl_2$] (188 mg, 0.18 mmol) in 100 mL round-bottomed flask and dissolve it with 20 mL methanol, weigh IrPQ2 (147 mg, 0.18 mmol) in 20 mL methanol and inject it into the round-bottomed flask. Under nitrogen protection at 80 ℃ reflux for 12 h, the obtained filtrate was spun dry and purified by silica gel column chromatography (eluent was dichloromethane/methanol = 10/1, v/v) to obtain red solid product IrPQ3 (104 mg, 43.0%). Anal. Calcd for $C_{62}H_{42}Ir_2N_8Cl_2$: C, 54.98; H, 3.13; N, 8.27. Found: C, 54.89; H, 3.14; N, 8.29. ^{1}H NMR (600 MHz, d_6-DMSO) δ 8.39 (d, J = 8.1 Hz, 3H), 8.14 (d, J = 8.0 Hz, 3H), 8.08 (s, 3H), 7.99 (dd, J = 17.2, 9.4 Hz, 3H), 7.92 (d, J = 6.6 Hz, 3H), 7.82 (t, J = 7.7 Hz, 3H), 7.33 (s, 3H), 7.13 (t, J = 7.4 Hz, 5H), 7.08 – 7.03 (m, 5H), 7.02 – 6.96 (m, 5H), 6.32 (d, J = 4.0 Hz, 3H), 6.02 (s, 3H). ^{13}C NMR (151 MHz, d_6-DMSO) δ 167.12, 167.12, 167.12, 167.12, 166.87, 166.87, 166.13, 166.13, 166.12, 166.08, 166.08, 166.08, 166.07, 166.07, 152.87, 152.87, 152.84, 152.84, 150.65, 150.61, 150.58, 150.58, 149.60, 149.60, 149.60, 149.60, 144.46, 144.31, 144.31, 144.27, 143.70, 143.70, 143.63, 143.63, 139.71, 139.63, 139.42, 139.42, 131.65, 131.57, 131.25, 131.25, 130.92, 130.92, 130.92, 130.92, 125.95, 125.95, 125.90, 125.90, 124.78, 124.41, 124.28, 124.28, 123.97, 123.97, 123.91, 123.32, 120.93, 120.93, 120.82, 120.55. MS (MALDI-TOF) m/z [M-Cl]$^+$ = 1282.3.

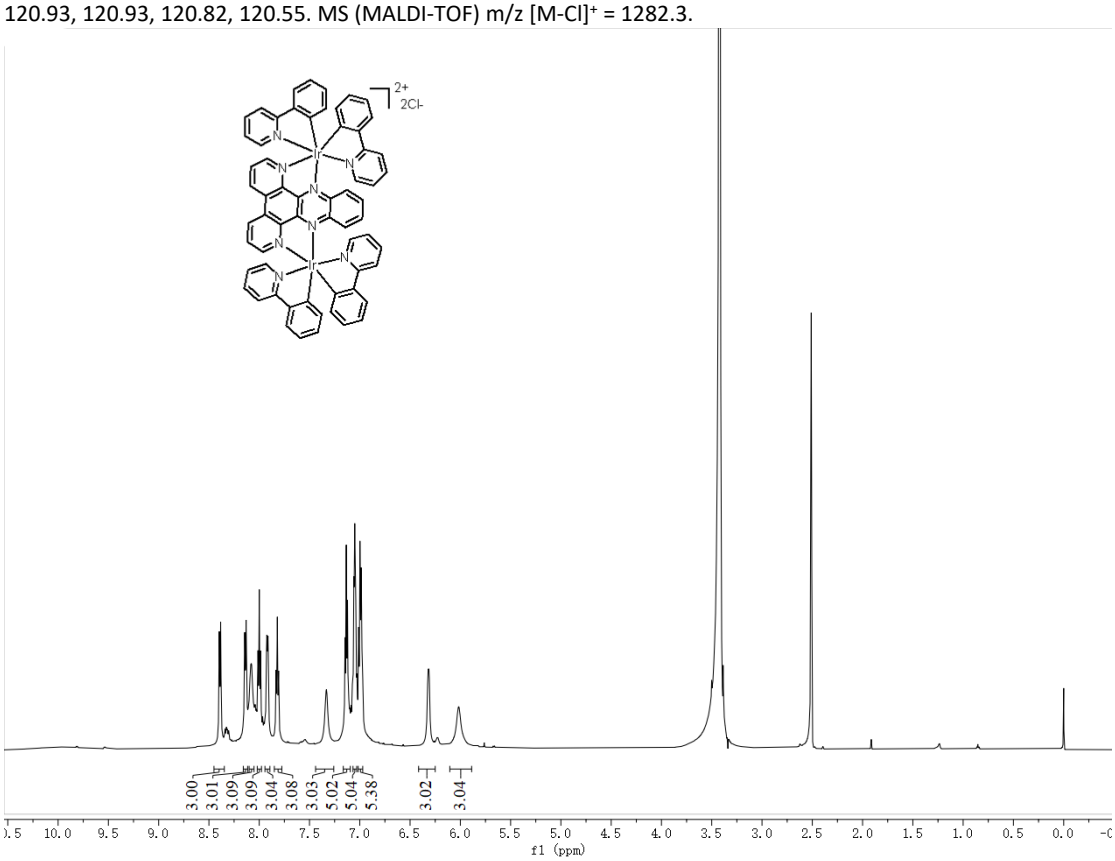

Figure S7. ^{1}H NMR spectrum of IrPQ3 (DMSO-d_6)

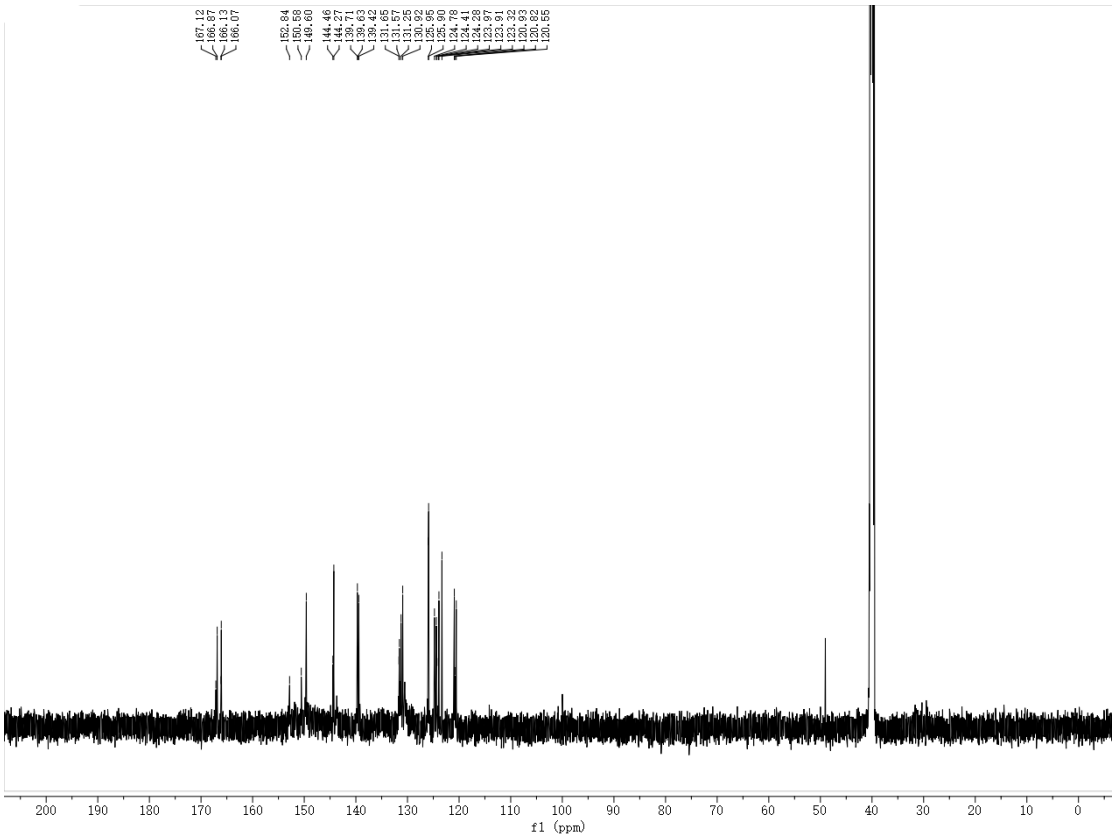

Figure S8. ^{13}C NMR spectrum of IrPQ3 (DMSO -d_6)

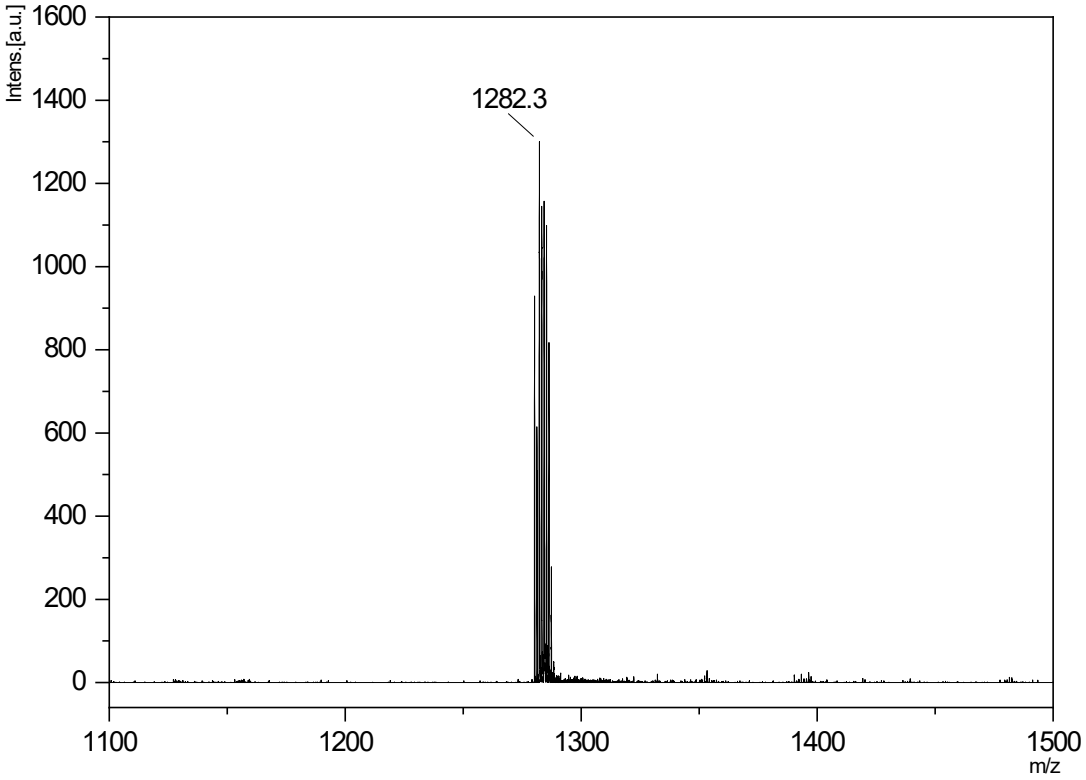

Figure S9. MALDI-TOF-MS spectrum of IrPQ3

Computational Details

DFT calculations of $[Ir(ppy)_2(phen)]Cl$ and IrPQ1-IrPQ3 were carried out at the PBE1PBE/6-31G*//LanL2TZf level with SMD solvent model by using the Gaussian 16 package.[4-6] The reorganization energies were calculated with a normal-mode analysis approach using the DUSHIN program.[7]

Photostability investigation

Acetonitrile solutions of IrPQ1-IrPQ3 (1 mg/mL) were prepared and subjected to laser irradiation at 560nm, 635nm, or 730nm for different time points(0 min, 15 min, 30 min). The absorption of the iridium complexes at a wavelength of 254 nm was measured using Agilent 1260 II HPLC system with a mobile phase consisting of 50% methanol and 50% acetonitrile. The relative content of each compound was analyzed using the instrument's software.

ROS Generation Studies

9,10-anthracenediyl-bis(methylene)dimalonic acid (ABDA) was used as an active oxygen indicator to detect the $^1O_2$ production performance of IrPQ1-IrPQ3 in methanol or mixture of methanol and water (5 : 95, v/v). A volume of 20 μL of ABDA solution was mixed with 3 mL compound solution. Thereafter the solution were irradiated with 560, 635 or 730 nm laser light (0.3 W/cm^2), and the absorption spectra or fluorescence intensity of ABDA were recorded using a UV-2600 Shimadzu spectrometer or Shimadzu RF6000 spectrophotometer.

The $^1O_2$ quantum yields of IrPQ1 and IrPQ2 were determined using the following equation: $\Phi_\Delta(PS) = \Phi_\Delta(Std)\ S_{PS} \times F_{Std} / (S_{std} \times F_{PS})$, where PS is IrPQ1 or IrPQ2 and Std designate methylene blue (MB); S is the photodecomposition rate of ABDA at 378 nm; F is given by $F = 1\text{-}10^{-OD}$ (OD means the optical density of IrPQ1 and IrPQ2 at 560 and 635 nm, respectively). As for IrPQ3, the $^1O_2$ quantum yield was calculated according to the following equation: $\Phi_\Delta(PS) = \Phi_\Delta\ (ICG) \times (r_{PS}/A_{PS}) / (r_{ICG}/A_{ICG})$, where r_{PS} and r_{ICG} were the photodecomposition rates of ABDA. A_{PS} and A_{ICG} are the absorbance of IrPQ3 and indocyanine green (ICG) at 730 nm. ($\Phi_\Delta(MB) = 0.22$ and $\Phi_\Delta(ICG) = 0.002$ in water).

ESR measurement

A mixture of DMSO and water (1 : 99, v/v)water solution containing 2,2,6,6-tetramethylpiperidine (50 mM) and IrPQ3 (100 μM) was prepared and irradiated with 730 nm laser for 0, 2 and 4 min. The signals were recorded with the ESR spectrometer.

Cell Culture

4T1 cells were used as cancer cell models to evaluate the photocytotoxicity of IrPQ1-IrPQ3. RPMI-1640 medium was used for 4T1 cell culture (37 ℃, 5% CO_2). 1% penicillin and streptomycin for playing anti-bacteria role, and 10% FBS for providing nutrition were added to the medium. In order to avoid the influence of DMSO on the state of cells and experimental results, the ratio of DMSO to buffer used in the experiment was 1:1000.

Cytotoxicity Assay

To conduct the experiment, 4T1 cells, which exhibit robust growth, were seeded into 96-well plates at a density of 10^4 cells per well. The cells were then cultured overnight at a temperature of 37 ℃. After that, the test samples were diluted in PBS buffer and further diluted into the required concentration using the culture medium. Then, the diluted samples were incubated with the cells for a period of 12 hours. Following the incubation, the cells were washed once more with PBS and exposed to laser radiation at wavelengths of 560, 635, or 730 nm for duration of 5 minutes, with an intensity of 0.3 W/cm^2. After the laser treatment, the cells were allowed to continue incubating for an additional 24 hours.

Subsequently, 10 μL of MTT solution (5 mg/ml) was added to each well, and the cells were incubated for an additional 4 hours. After ensuring that all the culture medium was carefully aspirated from the wells, 150 μL of DMSO was added to each well to solubilize the formazan. The dissolution of formazan in DMSO allows for the subsequent quantification of cell viability, as the absorbance of the resulting solution at 490 nm is directly proportional to the number of live cells in the culture. The viability of the cells was determined by measuring the absorbance of each well at a wavelength of 490 nm. Finally, after performing three parallel experiments, the half-inhibition concentration (IC_{50}) was calculated using SPSS software to analyze the data obtained from the experiments.

Cellular ROS Generation Assay

DCFH-DA fluorescent probe was employed to determine the generation of reactive oxygen species (ROS) within the cells in the presence of IrPQ1-IrPQ3. This probe is known to yield a bright green fluorescence when ROS are generated. 4T1 cells were carefully seeded into glass culture dishes (30 mm) at a density of 4×10^5 cells per well and allowed to incubate for 24 hours. Following this, the cells were treated with IrPQ1-IrPQ3 (40 μM) for a duration of 12 hours. Subsequently, the culture medium was discarded, and the cells were subjected to two washes with PBS. DCFH-DA at a concentration of 10 μM was then introduced to the cells, and they were incubated for an additional 30 minutes.

After this incubation period, the cells were once again washed with PBS buffer. They were then exposed to laser irradiation at wavelengths of 560, 635, or 730 nm, with an intensity of 0.3 W/cm^2, for a period of 5 minutes. To visualize and quantify the fluorescence emitted by the DCFH-DA probe, cell images were captured using a laser scanning confocal microscopy. The fluorescence intensity observed in the treated cells could indicate the levels of intracellular ROS production induced by IrPQ1-IrPQ3, thereby confirming their potential to generate ROS within the 4T1 cells.

Calcein AM/ PI co-staining assay

The 4T1 cells were seeded in 30 mm glass culture dishes at a density of 4×10^5 cells per well and allowed to incubate for 24 hours at 37 °C. Following this, the cells were treated with the samples containing IrPQ1-IrPQ3 at a concentration of 40 μM. After 12 hours incubation, the cells were washed with PBS to remove the residual samples. Next, the cells were exposed to laser light at wavelengths of 560, 635, or 730 nm, with an intensity of 0.3 W/cm^2, for a duration of 5 minutes.

To assess cell viability and integrity, the cells were stained using the Calcein AM/PI Double Stain Kit according to the provided instructions. This staining kit allows for the differentiation of live cells (stained with Calcein AM) and dead cells (stained with propidium iodide, PI). Finally, the stained cells were imaged using laser scanning confocal microscopy to record the fluorescent images.

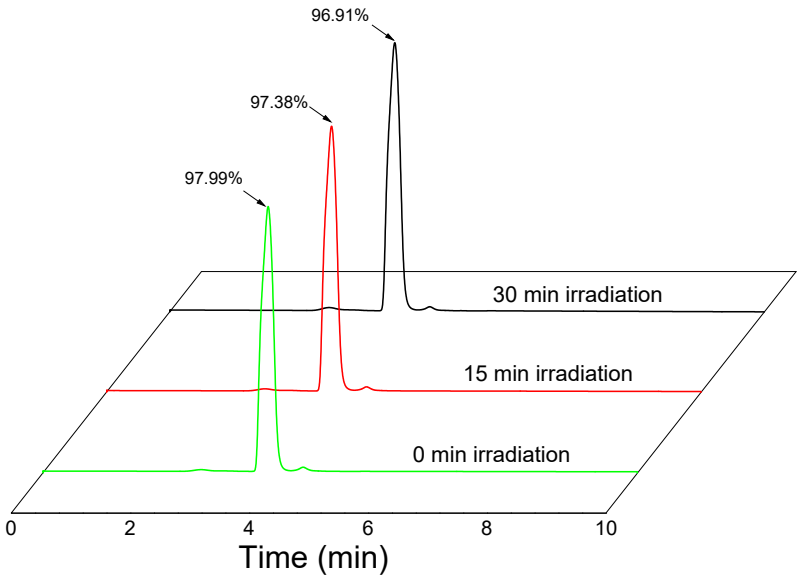

Figure S10. HPLC chromatograms of IrPQ1 upon 560 nm laser irradiation (0.3 W/cm^2) for different irradiation durations.

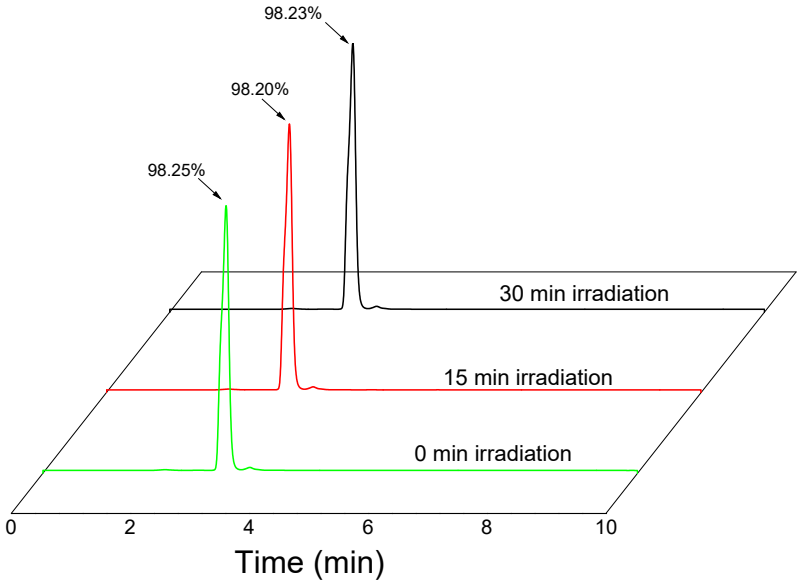

Figure S11. HPLC chromatograms of IrPQ2 upon 635 nm laser irradiation (0.3 W/cm^2) for different irradiation durations.

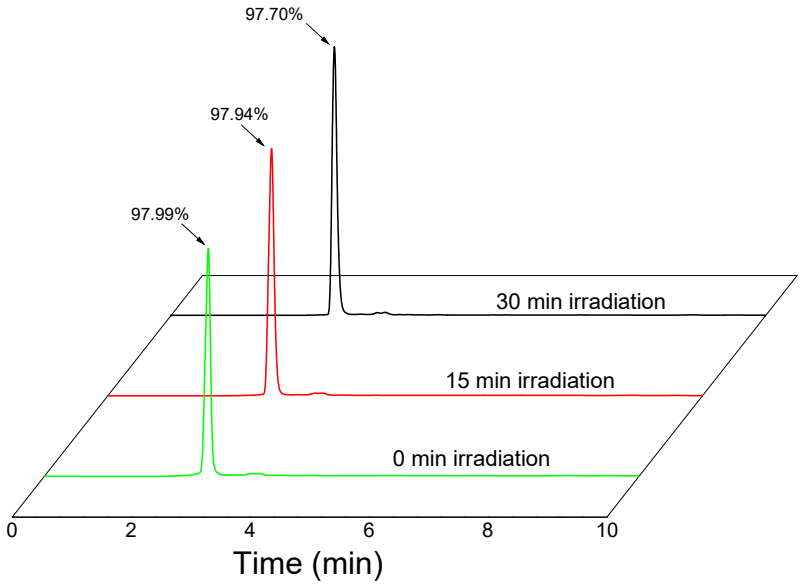

Figure S12. HPLC chromatograms of IrPQ3 upon 730 nm laser irradiation (0.3 W/cm^2) for different irradiation durations.

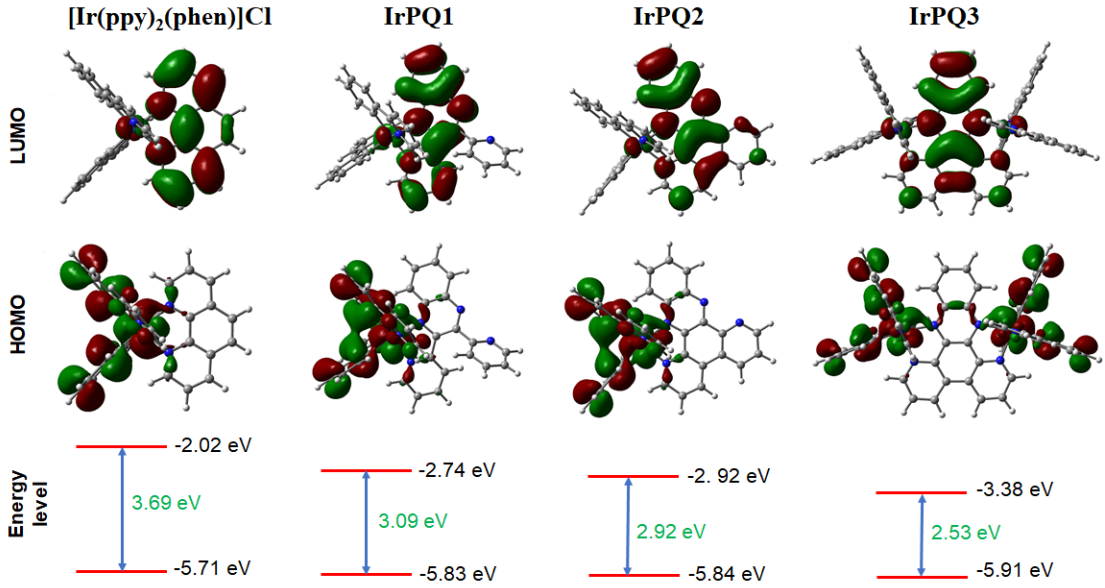

Figure S13. Frontier molecular orbitals and energy levels of [Ir(ppy)$_2$(phen)]Cl and IrPQ1-IrPQ3.

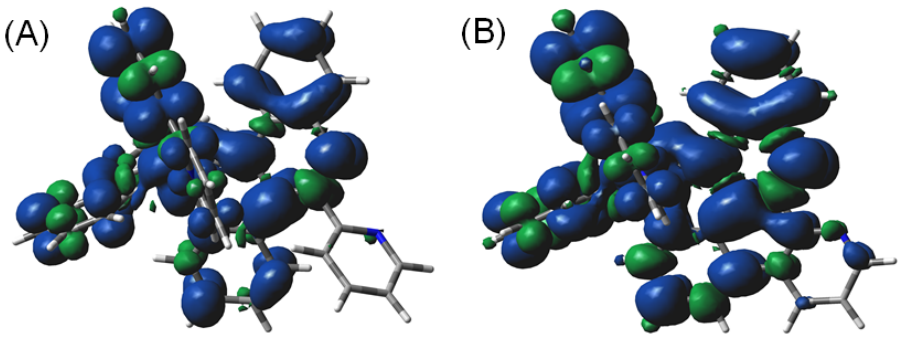

Figure S14. Isosurfaces for the spin density of (A) IrPQ1 and (B) IrPQ2 at the optimized triplet-state geometries.

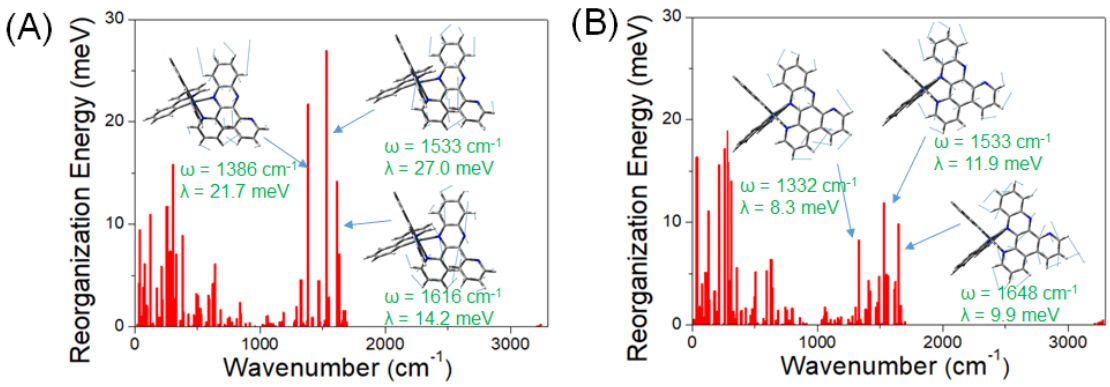

Figure S15. Mode-specific reorganization energies of (A) IrPQ1 and (B) IrPQ2 between T_1 and S_0 states.

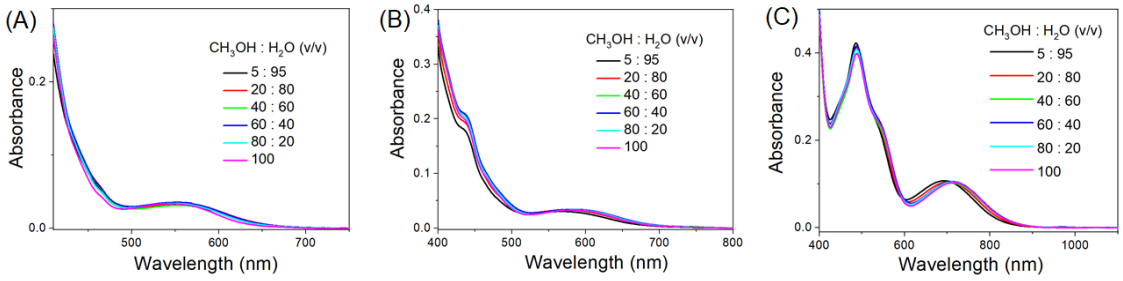

Figure S16. The absorption spectra of (A) IrPQ1, (B) IrPQ2, and (C) IrPQ3 in different ratios of methanol and water.

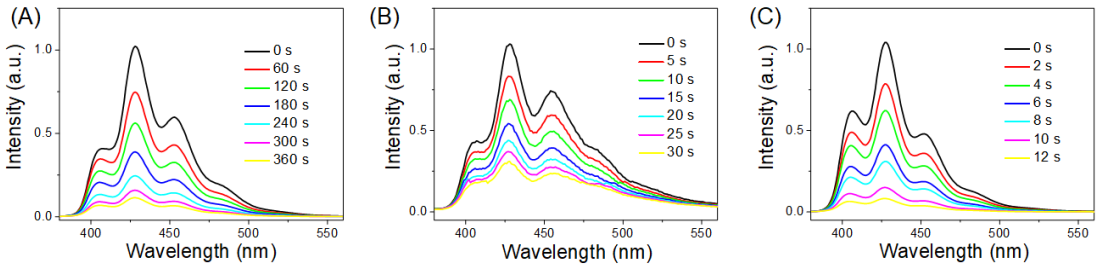

Figure S17. Photocatalytic degradation of ABDA triggered by (A) IrPQ1, (B) IrPQ2, and (C) IrPQ3 in a mixture of methanol and water (5 : 95, v/v) with the excitation of 560, 635 and 730 nm laser, respectively.

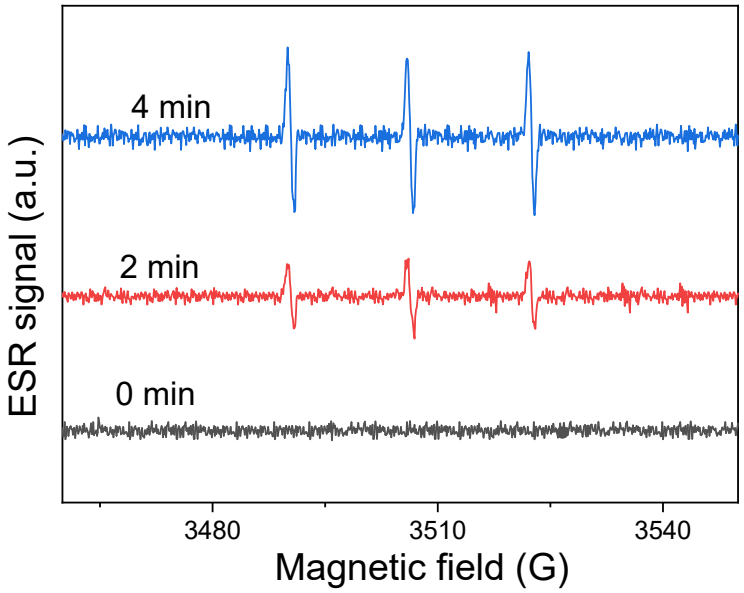

Figure S18. EPR spectra of TEMP–$^1O_2$ adduct induced by IrPQ3 upon 730 nm laser treatment (0.3 W/cm^2).

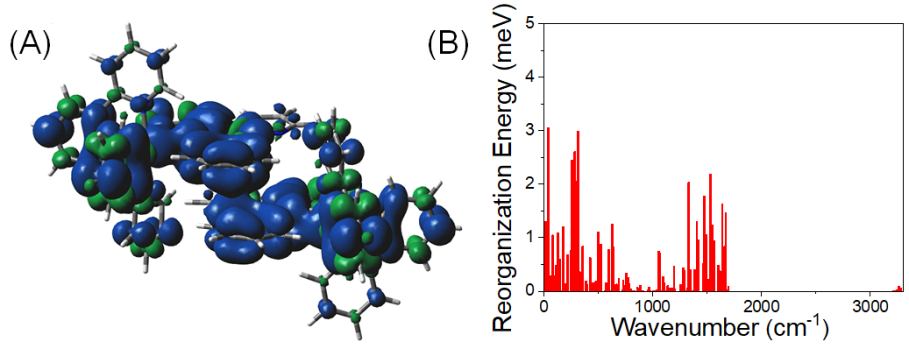

Figure S19. (A) Isosurfaces of the spin density of dimeric IrPQ2 at the optimized triplet-state geometry. (B) Mode-specific reorganization energy of IrPQ2 in dimeric forms between T_1 and S_0 states.

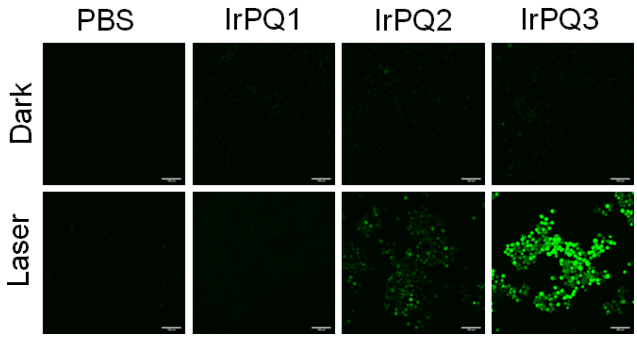

Figure S20. Confocal fluorescence images of 4T1 cells stained by DCFH-DA after treatment with PBS and IrPQ1-IrPQ3 in the presence or absence of laser irradiation (scale bar: 100 μm). Irradiation used for IrPQ1 (560 nm), IrPQ2 (635 nm), and IrPQ3 (730 nm) at 0.3 W/cm^2 for 5 min (90 J/cm^2).

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