Electronic Supporting Information

Water-soluble cyclometalated Ir(III) complexes as carrier-free and pure nanoparticle photosensitizers for photodynamic therapy and cell imaging

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1. General information and methods

Materials

Materials obtained from commercial suppliers were used without further purification unless otherwise stated. All glassware, syringes, magnetic stirring bars and needles were thoroughly dried in a convection oven. All other materials for organic synthesis were purchased from Energy Chemical Company. Indocyanine green (ICG) was purchased from Fisher Scientific. Dulbecco's Modified Eagle Medium (DMEM), RPMI-1640 Medium, and fetal bovine serum (FBS) were purchased from Sigma-Aldrich. 3-(4, 5-Dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) was obtained from Shanghai Beyotime Biotechnology Co., Ltd. (China). The cell viability (live dead cell staining) assay kit was purchased from KeyGen Biotech Co., Ltd. (China). 2', 7'-Dichlorofluorescence diacetate (DCFH-DA) were purchased from Shanghai Beyotime Biotechnology Co., Ltd..

Measurements

Reactions were monitored using thin layer chromatography (TLC). Commercial TLC plates were used and the spots were visualized under UV light at 254 and 365 nm. ^{1}H NMR spectra were recorded at 25 °C on a Varian 600 MHz spectrometer. The chemical shifts (δ) are given in parts per million relative to internal standard TMS. The ^{1}H NMR spectra were referenced internally to the residual proton resonance in DMSO-d_6 (δ 2.49 ppm) or MeOD-d_4 (δ 3.31 ppm). UV-vis absorption spectra were recorded on a Shimadzu UV-3100 spectrophotometer. The photoluminescence spectra, excited state lifetimes (τ) and photoluminescence quantum yields (Φ_{PL}) were recorded on an Edinburgh FLS920 spectrofluorimeter under air at room temperature. Transmission electron microscopy (TEM) images of the samples were taken by a TECNAI F20 microscope. Diameter and diameter distribution of the nanoparticles were determined by a Malvern Zetasizer Nano instrument for dynamic light scattering (DLS). Confocal laser scanning microscopy (CLSM) images were taken using a Zeiss LSM 700 (Zurich, Switzerland).

Cell culture

HeLa cells (the human cervical cancer cell line) was purchased from Jilin University and cultured in Dulbecco's modified Eagle's medium (DMEM, GIBCO) supplemented with 10% heat-inactivated fetal bovine serum (FBS), and 1% antibiotic (penicillin-streptomycin). The cells were cultured in a humidified incubator at 37 °C with 5% CO_2, and the culture medium was replaced once every day.

Cytotoxicity assay

The cytotoxicity of **1-H**/**2-H**/**1-Na**/**2-Na** with or without irradiation was examined via MTT protocol. HeLa cells harvested in a logarithmic growth phase were seeded in 96-well plates at a density of 10^4 cells per well and incubated in 100 μL DMEM at 37 °C in 5% CO_2 atmosphere for overnight. After removing incubation medium, the different concentrations of **1-H**/**2-H**/**1-Na**/**2-Na** of the increasing concentration (0-100 μg mL^{-1}) were added to cell wells separately. After incubation for 6 h, cells were illuminated with light (450 nm, 20 mW cm^{-2}, 40 min). And the cells treated with nothing were used as dark control group. After continued incubation 24 h, 20 μL of MTT in PBS solution with the concentration of 5 mg mL^{-1} was added to the medium and the plates were incubated at 37 °C for another 4 h. Subsequently, the incubation medium was removed and the formazan crystals were dissolved in 150 μL of dimethyl sulfoxide (DMSO). The cell viability was evaluated by measurement of the absorbance at 490 nm, using a Bio-Rad 680 microplate reader.

Cell uptake

Cell uptake by confocal laser scanning microscopy (CLSM): The cellular uptake behaviors of **1-H** and **1-Na** were examined by CLSM towards HeLa cells. The cells were seeded in 6-well plates with a clean cover slip in each well at density of 5×10^4 cells per well in 2 mL of DMEM medium and allowed to adhere for 24 h. And then the medium was replaced with **1-H** and **1-Na** diluted with fresh culture medium to a final concentration of 60 μg mL^{-1}. Thereafter cells were incubated at 37 °C for 0.5 h, 2 h and 6 h. Subsequently, the supernatant was removed and the cells were washed gently three times with PBS (pH 7.4), fixed with 4% paraformaldehyde (1 mL/each well) for 15 min at ambient temperature and washed thrice with cold PBS. The cellular uptake was obtained by CLSM with excitation at 405 nm.

Intercellular ROS assays

The intracellular $^1O_2$ generation was measured by using 2′, 7′-dichlorofluorescin diacetate (DCFH-DA) as a probe. HeLa cells were seeded in 6-well culture plates at a density of 5×10^4 cells per well for 24 h. They were incubated with **1-H** and **1-Na** with the concentration of 60 μg mL^{-1} for 6 h, and then illuminated with blue light (450 nm, 20 mW cm^{-2}, 15 min). Hela cells without illumination treatment were used as negative control. After the irradiation, the medium was replaced with culture medium. Then DCFH-DA (10 μM) was added and the cells were incubated for 20 min. After removing the DCFH-DA-containing medium and washing three times, the cells was subjected to observation fluorescence by CLSM.

Live/dead cell staining assays

To further investigate the photodynamic therapy efficacy of **1-H** and **1-Na**, HeLa cells were stained with the calcein-AM/propidium iodide (PI) to identify dead (red fluorescence) and live (green fluorescence) cells. Hela cells were seeded in 96-well plates at a density of 10^4 cells per well and incubated in 100 μL DMEM for overnight. **1-H** and **1-Na** were added into cell culture medium separately with the concentration of 60 μg mL^{-1} for 6 h, and then illuminated with blue light (450 nm, 20 mW cm^{-2} 40 min). The dark control group received nothing. After additional incubation for 24 h, phosphate-buffered saline (PBS) was used to wash the cells. Then cells were incubated with Calcein-AM/PI for 40 min at room temperature, subsequently imaged by CLSM.

2. General information and methods

Synthesis of 1-H

A yellow suspension of the dichloro-bridged diiridium complex $[Ir(ppy)_2Cl]_2$ (0.100 g, 0.1 mmol, 1.00 eq.) and 2,2′-Bipyridine-4,4′-dicarboxylic acid **L1** (0.049 g, 0.2 mmol, 2.00 eq.) in MeOH (15 mL) and CH_2Cl_2 (15 mL) was refluxed under an inert atmosphere of N_2 in the dark for 4 h. The red solution was then cooled to room temperature, and excess solid potassium hexafluorophosphate (0.184 g, 1 mmol, 10.00 eq.) was added to the solution. The mixture was stirred for 45 min at ambient temperature and the suspension was then filtered and the precipitate was washed with petroleum ether and dried. The crude product was purified by column chromatography with acetone/methyl alcohol (10/1, v/v) as eluent. A red solid was

obtained (0.151 g, 85% yield). ^{1}H NMR (600 MHz, DMSO-*d6*, δ [ppm]): 13.98 (s, 1H, OH), 9.28 (s, 1H, ArH), 8.28 (d, J =6.0 Hz, 1H, ArH), 8.11 (d, J =6.0 Hz, 1H, ArH), 8.06 (d, J =6.0 Hz, 1H, ArH), 7.96-7.93 (m, 2H, ArH), 7.68 (d, J =6.0 Hz, 1H, ArH), 7.13 (t, J =6.0 Hz, 1H, ArH), 7.05-7.02 (m, 1H, ArH), 6.92 (d, J =8.0 Hz, 1H, ArH), 6.17 (d, J =6.0 Hz, 1H, ArH). ^{13}C NMR (151 MHz, DMSO-*d6*) δ 167.06, 165.31, 156.45, 151.45, 150.31, 149.81, 144.19, 141.59, 139.39, 131.46, 130.82, 128.69, 125.56, 125.21, 124.47, 123.03, 120.55. MS: (ESI-TOF) [m/z]: 745.1423 (calcd: 745.14).

Synthesis of 2-H

The synthesis of **2-H** was similar to that of **1-H** except that the ancillary ligand 2, 2′-Bipyridine-4, 4′-dicarboxylic acid **L1** was replaced by 2, 2′-Bipyridine-5, 5′-dicarboxylic acid **L2**. **2-H** was obtained as a dark red solid (0.144 g, 81% yield). ^{1}H NMR (600 MHz, DMSO-d_6, δ [ppm]): 13.91 (s, 1H, OH), 9.05 (d, J=6.0 Hz, 1H, ArH), 8.63 (t, J =6.0 Hz, 1H, ArH), 8.38 (s, 1H, ArH), 8.28 (d, J =6.0 Hz, 1H, ArH), 7.94 (t, J =6.0 Hz, 2H, ArH), 7.74 (d, J =6.0 Hz, 1H, ArH), 7.13 (t, J =6.0 Hz, 1H, ArH), 7.04 (t, J =6.0 Hz, 1H, ArH), 6.93 (t, J =6.0 Hz, 1H, ArH), 6.19 (d, J =6.0 Hz, 1H, ArH). ^{13}C NMR (151 MHz, DMSO-*d6*) δ 167.03, 164.47, 157.77, 151.32, 150.01, 149.92, 144.23, 140.57, 139.34, 131.59, 131.51, 130.79, 126.25, 125.51, 124.45, 123.01, 120.48. MS: (ESI-TOF) [m/z]: 745.1423 (calcd: 745.14).

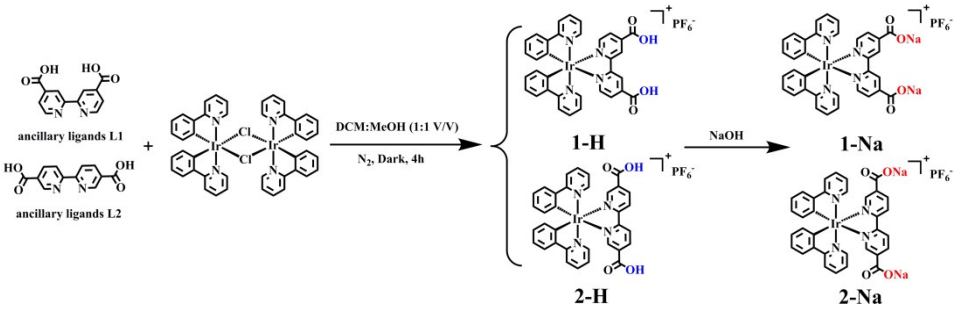
Synthesis of 1-Na

The iridium complex **1-H** (0.089 g, 0.1 mmol, 1.00 eq.) and NaOH (0.008 g, 0.2 mmol, 2.00 eq.) was dissolved in H_2O (5 mL), and the reaction mixture was stirred at room temperature for 2 h. After solvent evaporation, an orange-red solid was obtained (0.090 g, 96% yield). ^{1}H NMR (600 MHz, MeOD-d_4, δ [ppm]): 8.96 (s, 1H, ArH), 8.01 (d, J =8.0 Hz, 1H, ArH), 7.92 (d, J =5.4 Hz, 1H, ArH), 7.76-7.72 (m, 3H, ArH), 7.55 (d, J =6.0 Hz, 1H, ArH), 6.96-6.92 (m, 2H, ArH), 6.80 (t, J =6.0 Hz, 1H, ArH), 6.21 (d, J =6.0 Hz, 1H, ArH). ^{13}C NMR (126 MHz, MeOD-d_4) δ 168.51, 167.78, 156.21, 150.30, 150.18, 148.62, 143.82, 138.20, 131.38, 130.07, 127.04, 124.57, 123.72, 123.09, 122.24, 119.56. MS: (ESI-TOF) [m/z]: 789.1062 (calcd: 789.11).

Synthesis of 2-Na

The synthesis of **2-Na** was similar to that of **1-Na** except that the iridium complex **1-H** was replaced by **2-H**. **2-Na** was obtained as an orange-red solid (0.089 g, 95% yield). ^{1}H NMR (600 MHz, DMSO-d_6, δ [ppm]): 8.76 (d, J =6.0 Hz, 1H, ArH), 8.43 (d, J =6.0 Hz, 1H, ArH), 8.34 (s, 1H, ArH), 8.25 (d, J =8.0 Hz, 1H, ArH), 7.93-7.88 (m, 2H, ArH), 7.58 (d, J =6.0 Hz, 1H, ArH), 7.15 (t, J =6.0 Hz, 1H, ArH), 6.99 (t, J =6.0 Hz, 1H, ArH), 6.87 (t, J =6.0 Hz, 1H, ArH), 6.17 (d, J =6.0 Hz, 1H, ArH). ^{13}C NMR (151 MHz, MeOD-d_4) δ 168.52, 167.81, 156.25, 150.33, 150.21, 148.65, 143.86, 138.24, 131.41, 130.11, 127.08, 124.61, 123.75, 123.12, 122.28, 119.60. MS: (ESI-TOF) [m/z]: 789.1062 (calcd: 789.11).

3. Supplementary Figures and tables



Scheme S1 Synthetic routes of **1-H**/**2-H**/**1-Na**/**2-Na**.

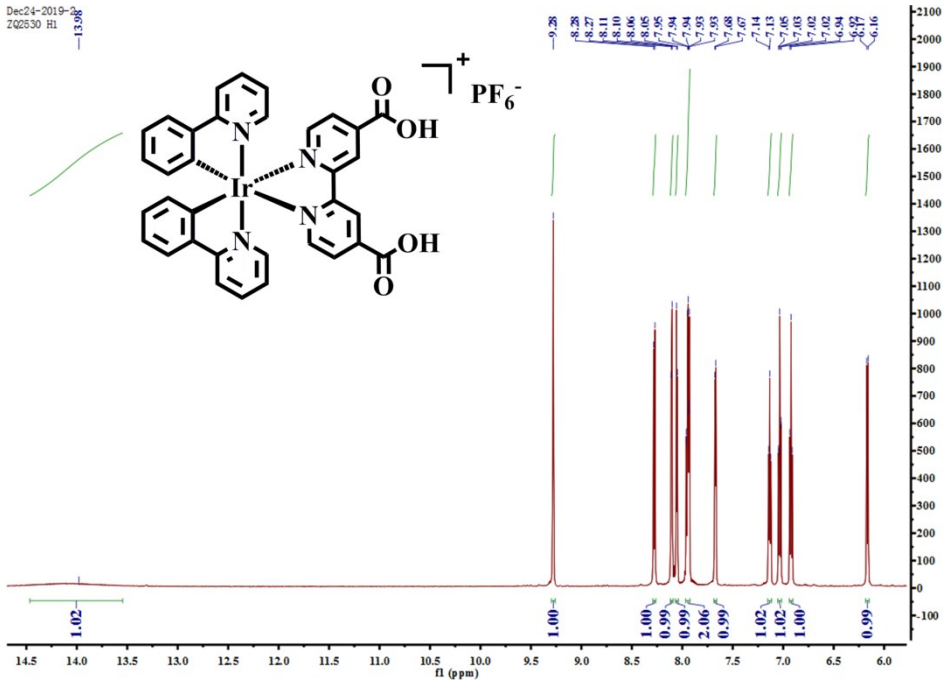


Fig. S1 ^{1}H NMR spectrum of **1-H** in DMSO-d_6.

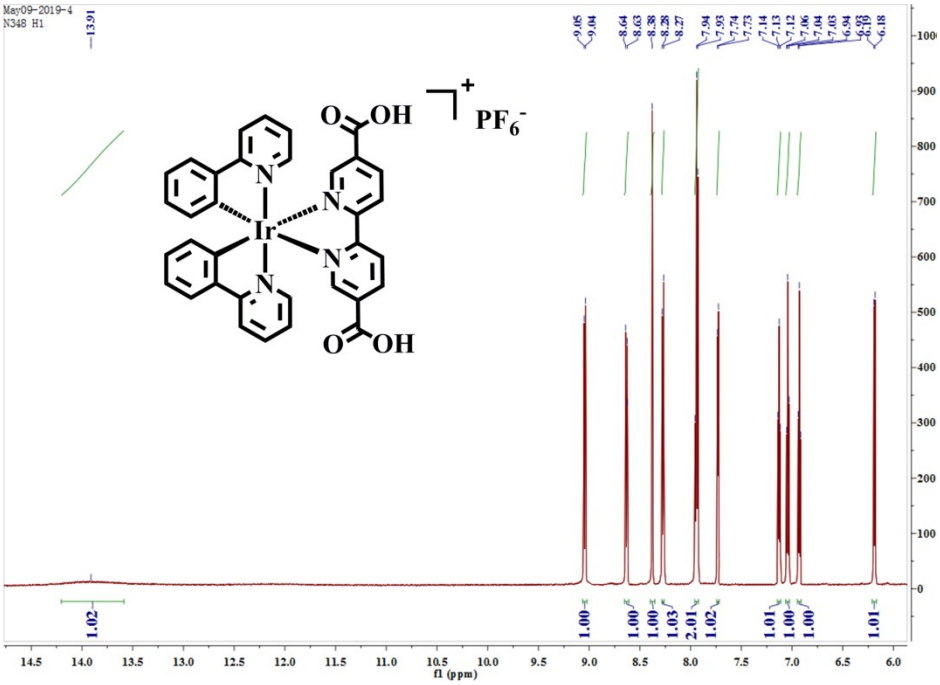


Fig. S2 ^{1}H NMR spectrum of **2-H** in DMSO-d_6.

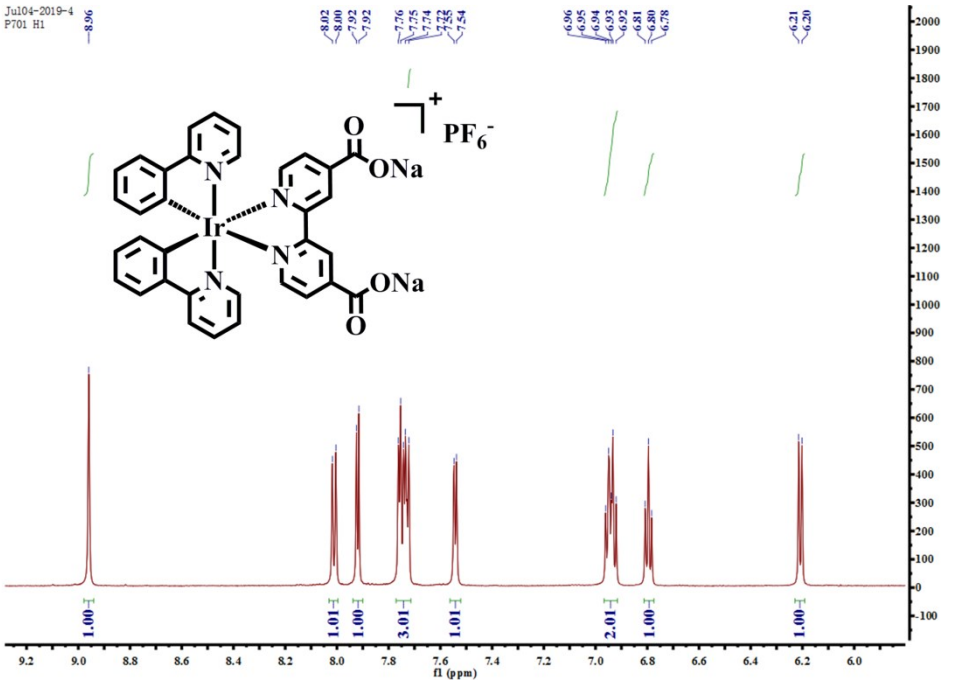


Fig. S3 ^{1}H NMR spectrum of **1-Na** in MeOD-d_4.

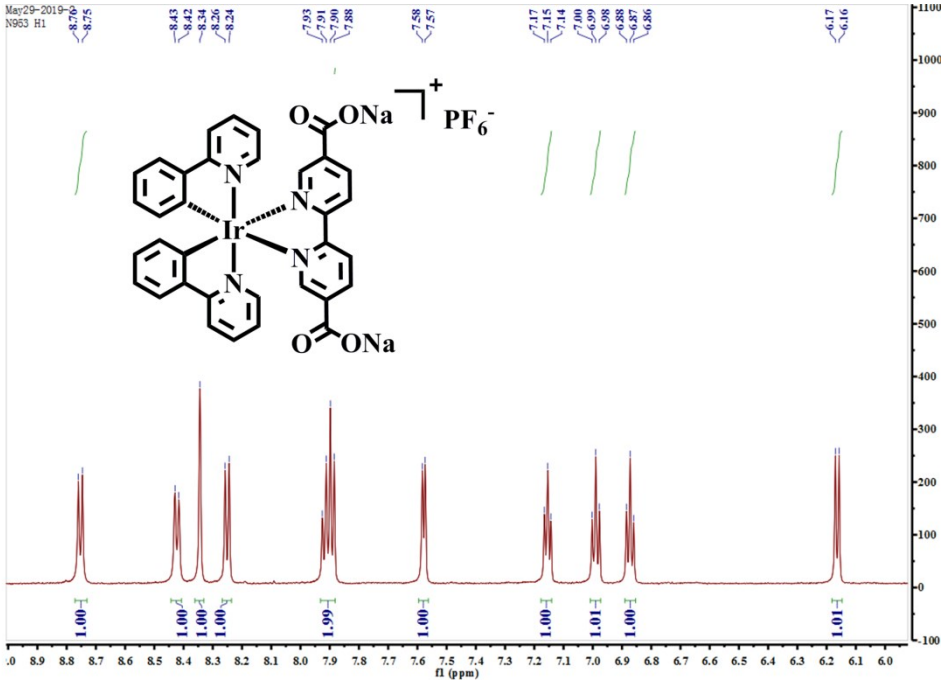


Fig. S4 ^{1}H NMR spectrum of **2-Na** in DMSO-d_6.

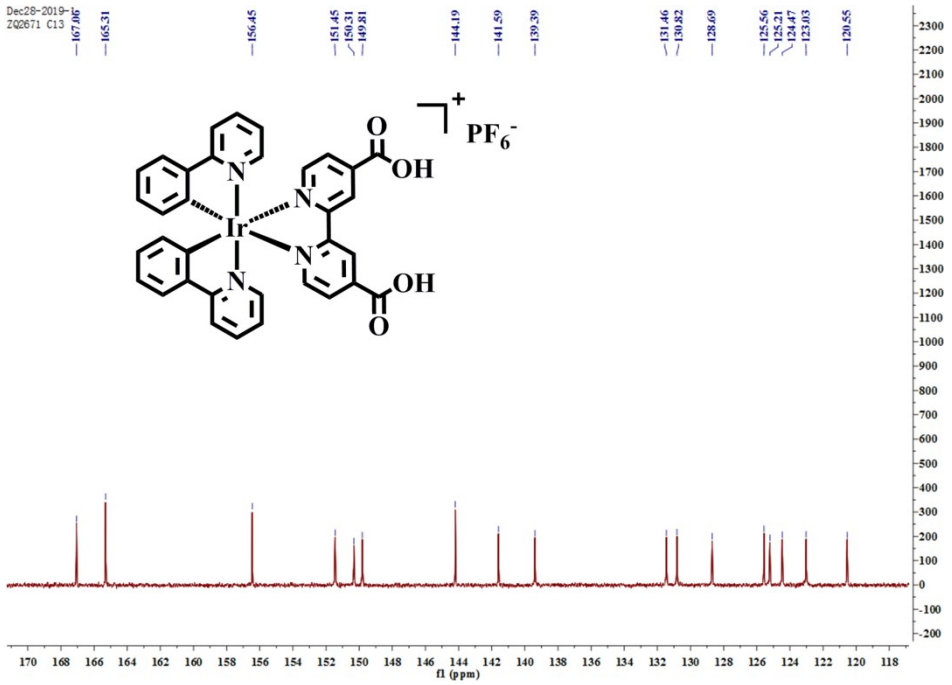


Fig. S5 ^{13}C NMR spectrum of **1-H** in DMSO-d_6.

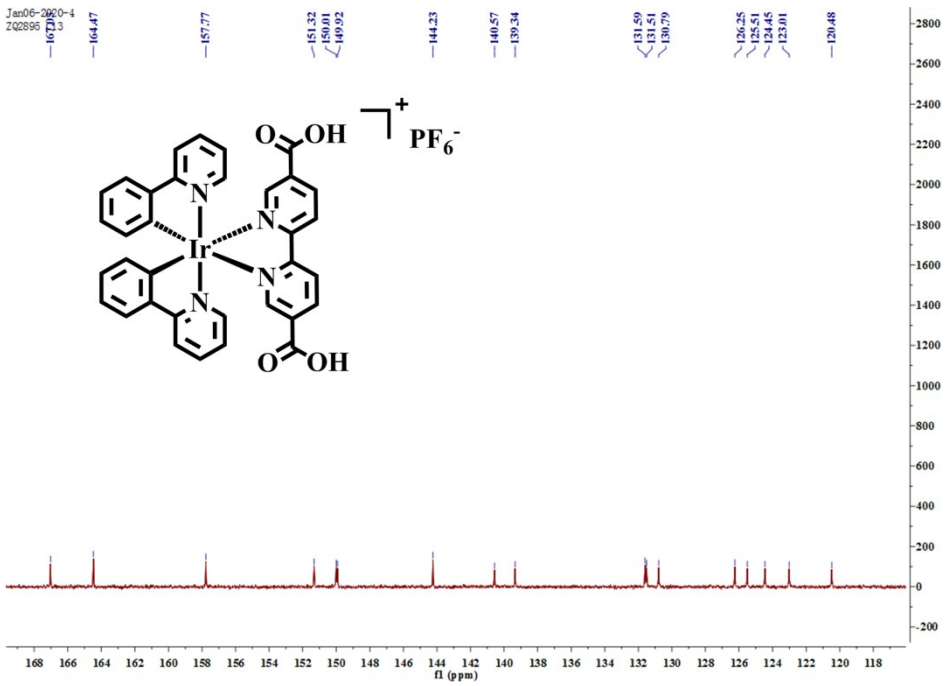


Fig. S6 ^{13}C NMR spectrum of **2-H** in DMSO-d_6.

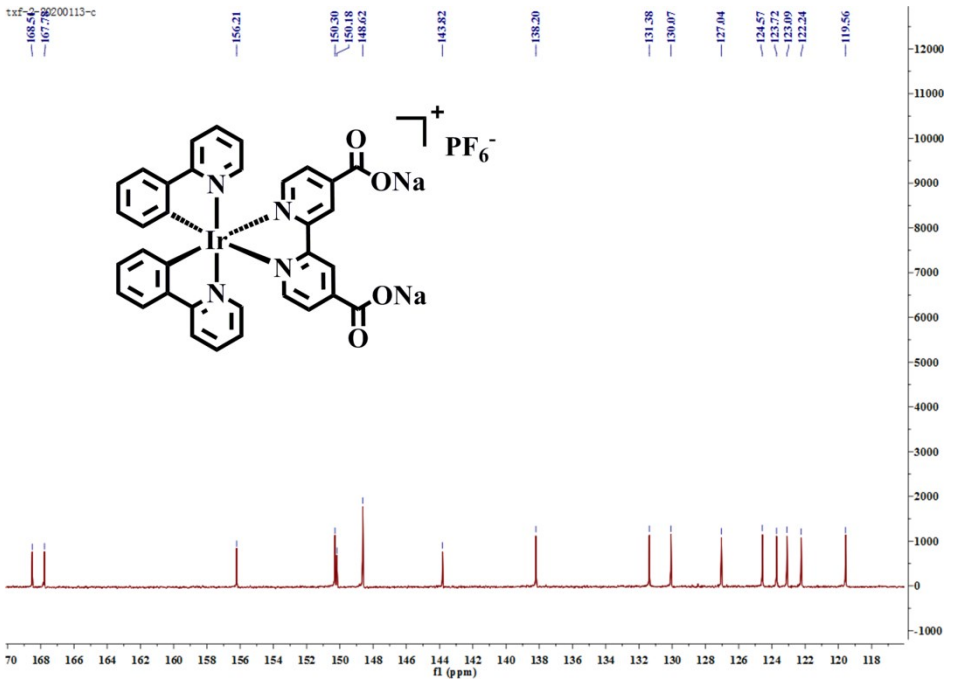


Fig. S7 ^{13}C NMR spectrum of **1-Na** in MeOD-d_4.

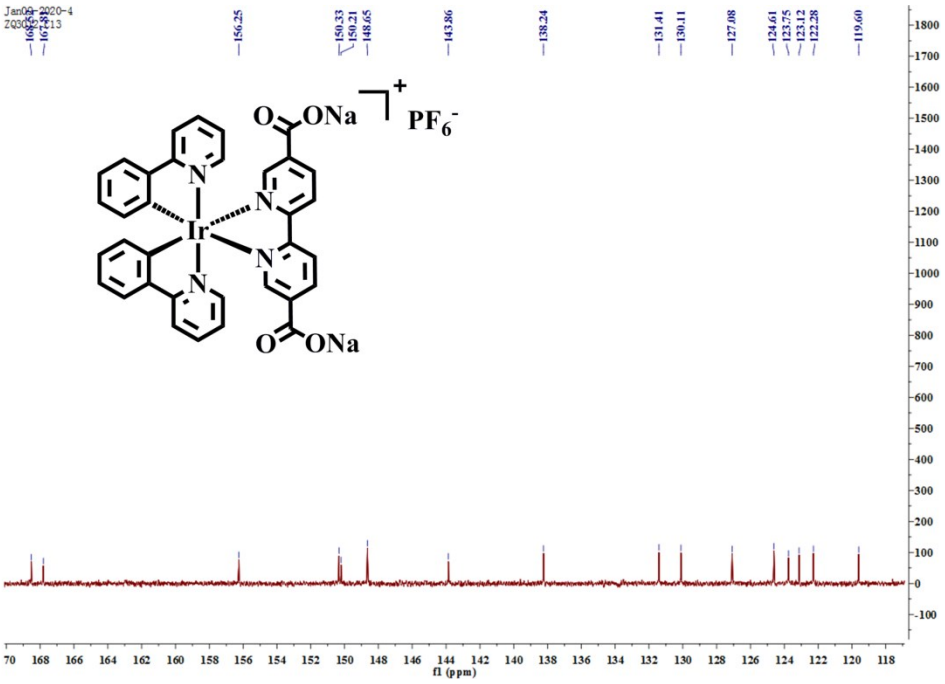


Fig. S8 ^{13}C NMR spectrum of **2-Na** in MeOD-d_4.

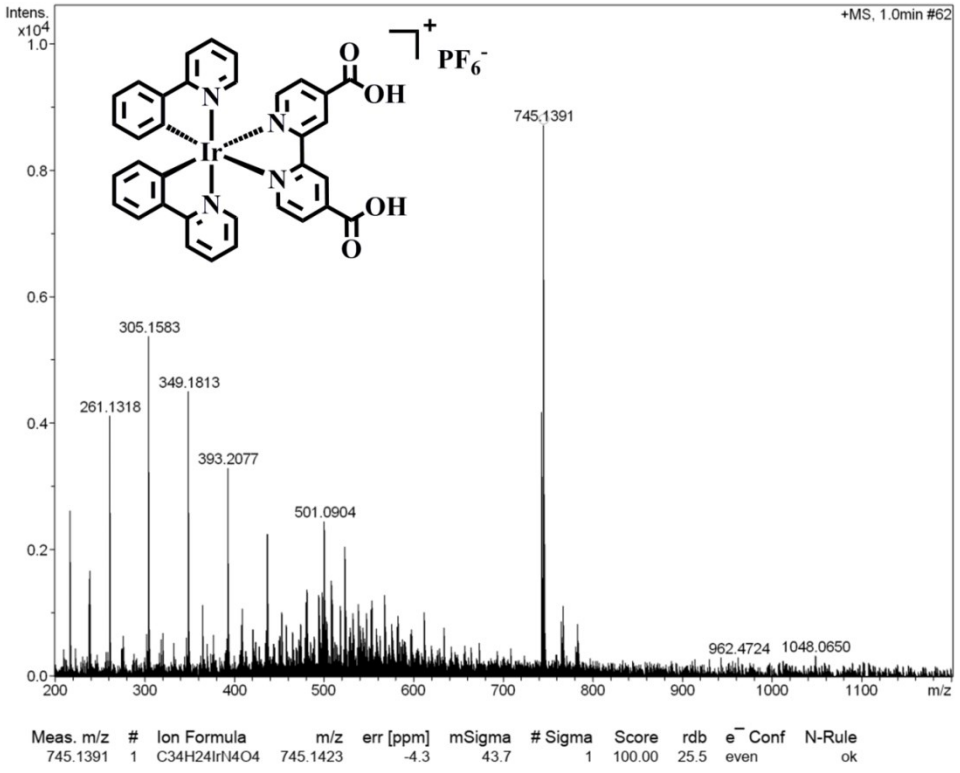


Fig. S9 ESI mass spectrum of **1-H**.

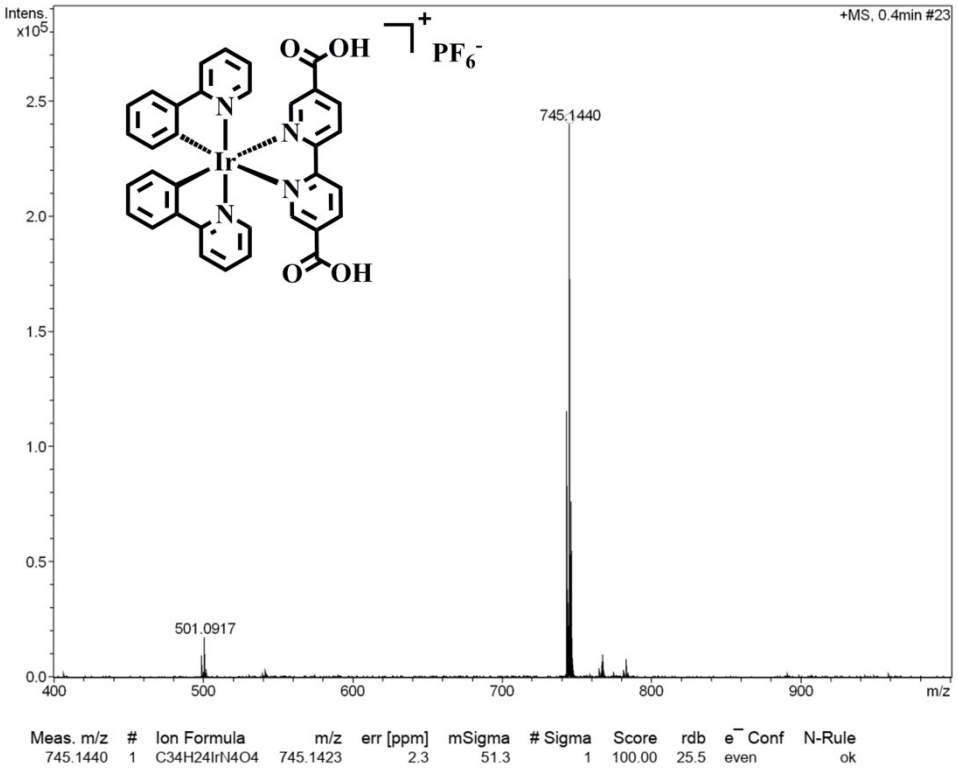


Fig. S10 ESI mass spectrum of **2-H**.

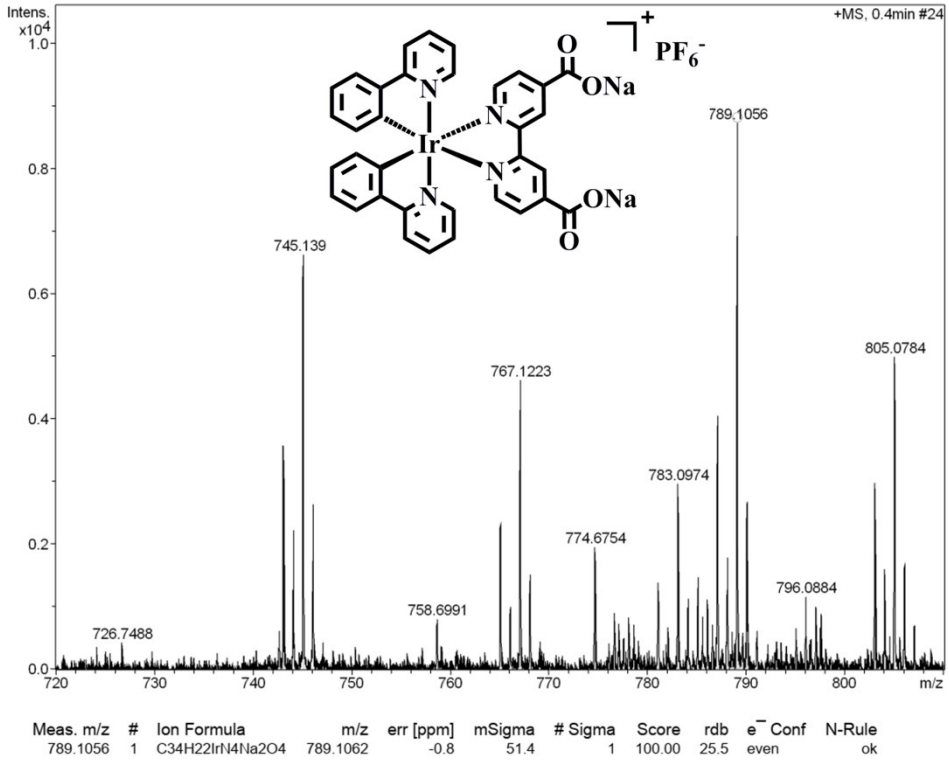


Fig. S11 ESI mass spectrum of **1-Na**.

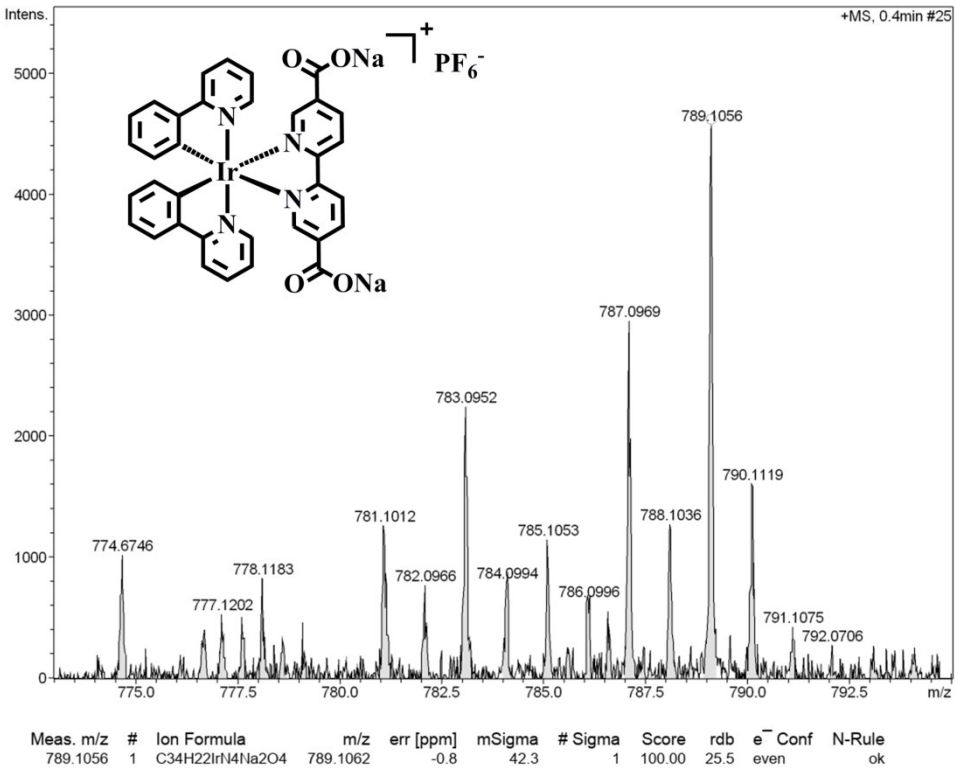


Fig. S12 ESI mass spectrum of **2-Na**.

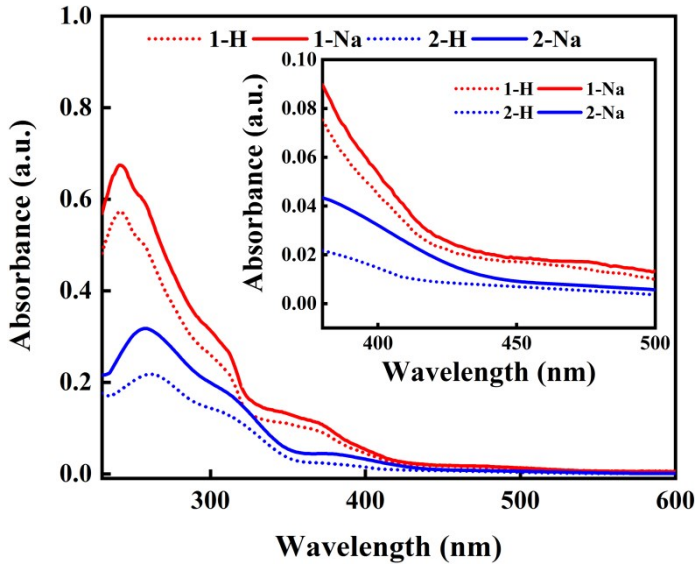


Fig. S13 UV–vis absorption spectra of **1-H**/**2-H**/**1-Na**/**2-Na** (10^{-5} M) in water, the inset is the absorption spectra at around 450 nm.

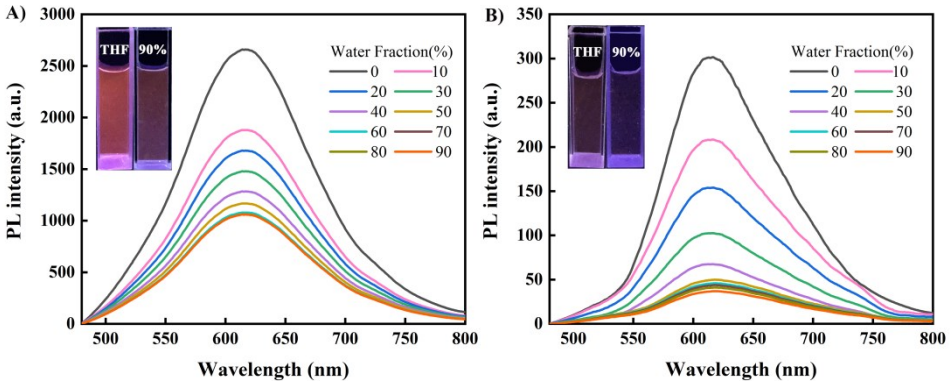


Fig. S14 Emission spectra of A) **1-H**, B) **2-H** (10^{-5} M) in THF–water mixtures with different water fractions (0–90% v/v) at room temperature.

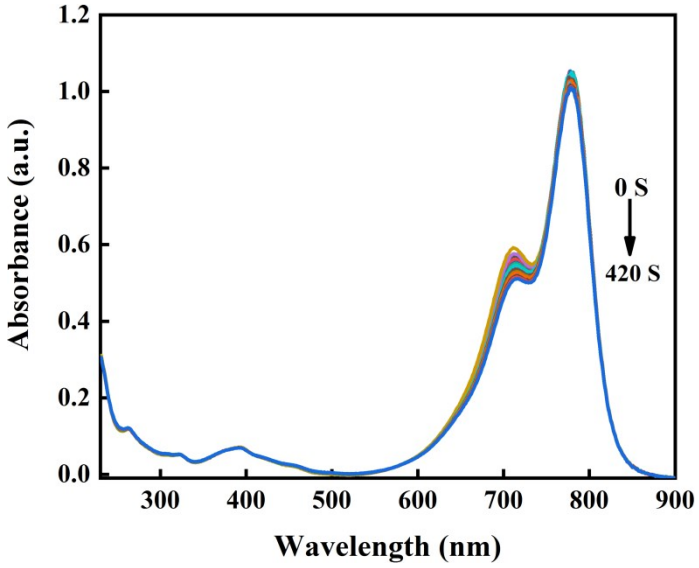


Fig. S15 UV–vis absorption spectra of ICG (5 μg mL^{-1}, 790 nm) for different times under irradiation (450 nm, 20 mW cm^{-2}).

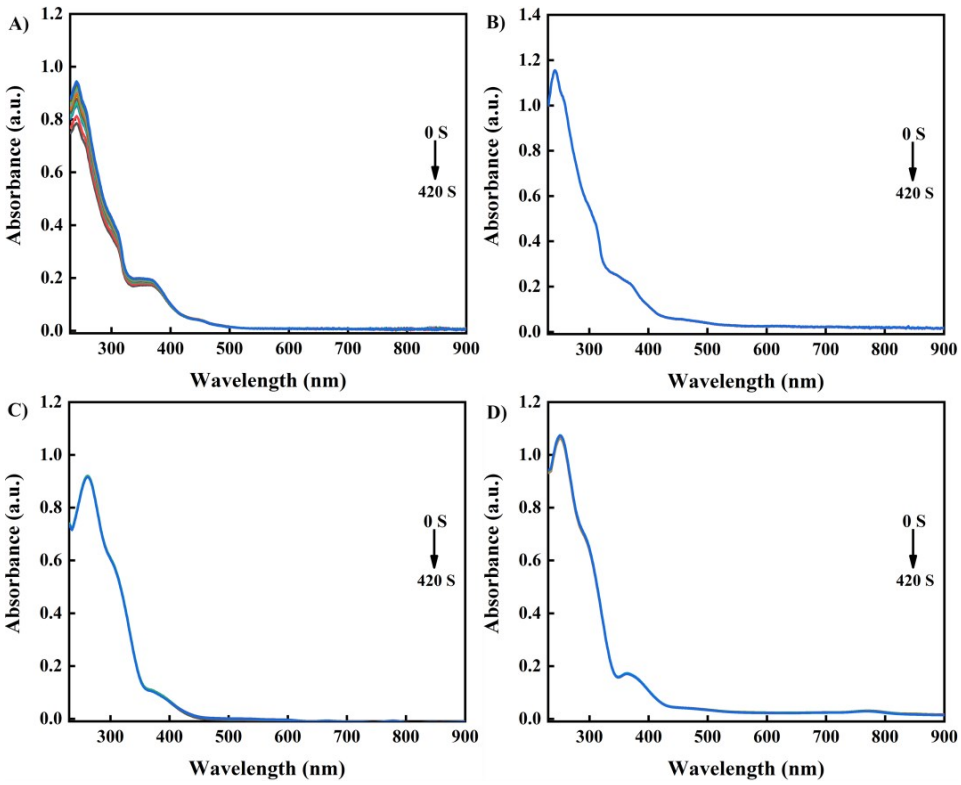


Fig. S16 UV–vis absorption spectra changes of A) **1-H**, B) **1-Na**, C) **2-H**, D) **2-Na** (50 μg mL^{-1}) for different times under irradiation (450 nm, 20 mW cm^{-2}).

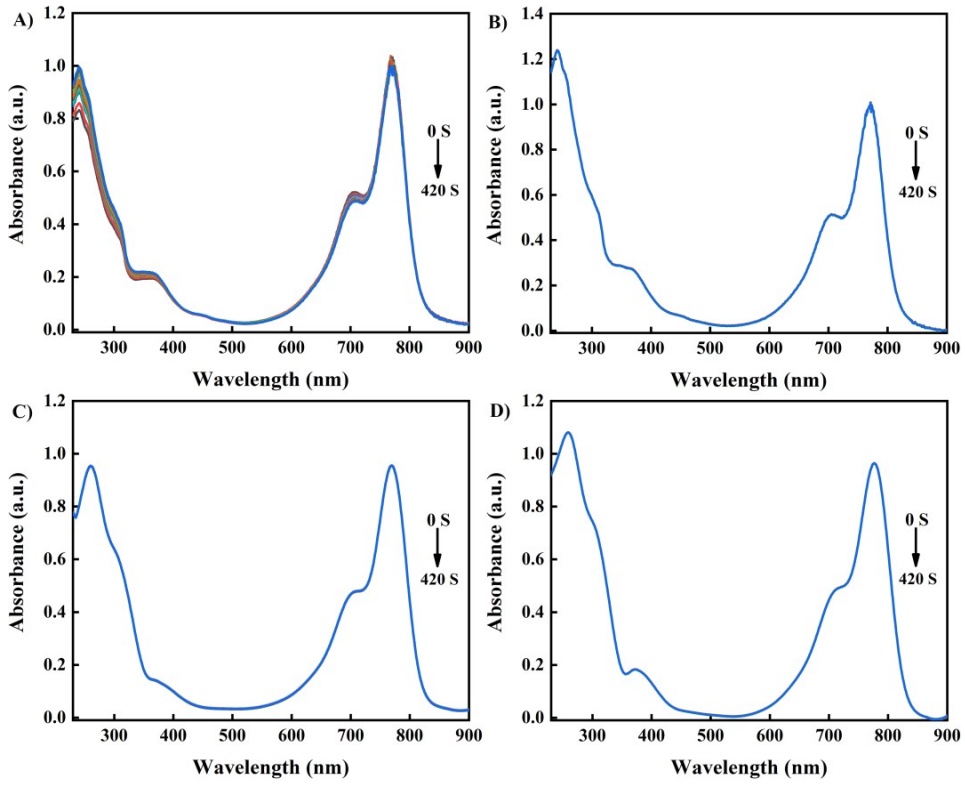


Fig. S17 UV–vis absorption spectra changes of ICG (5 μg mL^{-1}, 790 nm) in the presence of A) **1-H**, B) **1-Na**, C) **2-H**, D) **2-Na** (50 μg mL^{-1}) for different times.

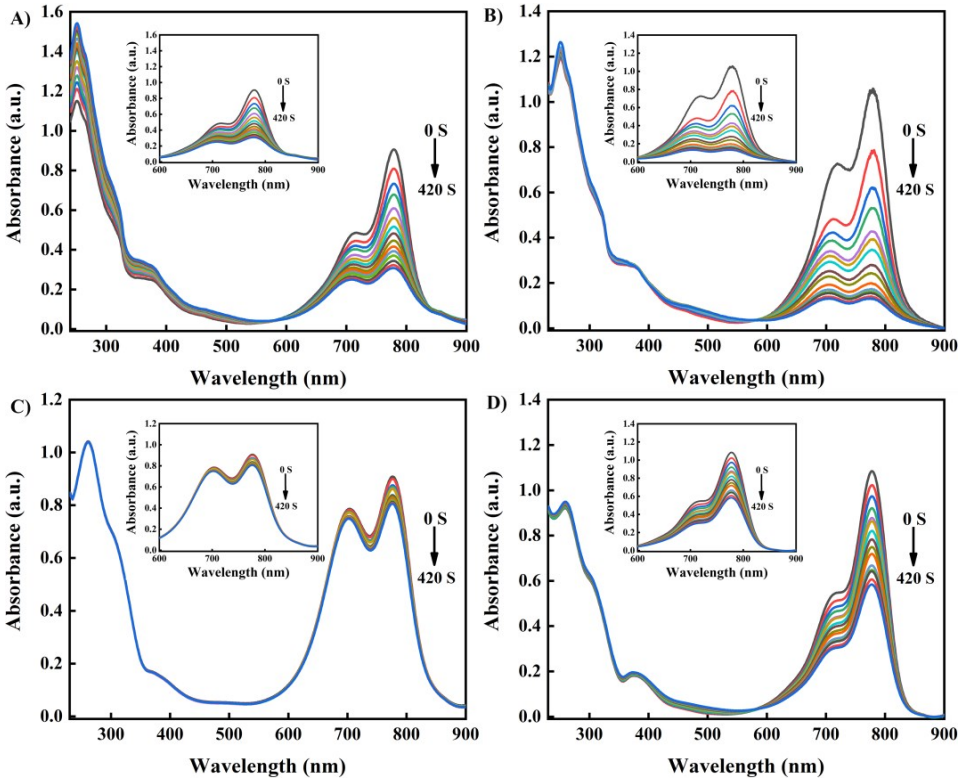


Fig. S18 UV–vis absorption spectra of ICG (5 μg mL^{-1}, 790 nm) in the presence of A) **1-H**, B) **1-Na**, C) **2-H**, D) **2-Na** (50 μg mL^{-1}) for different times under irradiation (450 nm, 20 mW cm^{-2}).

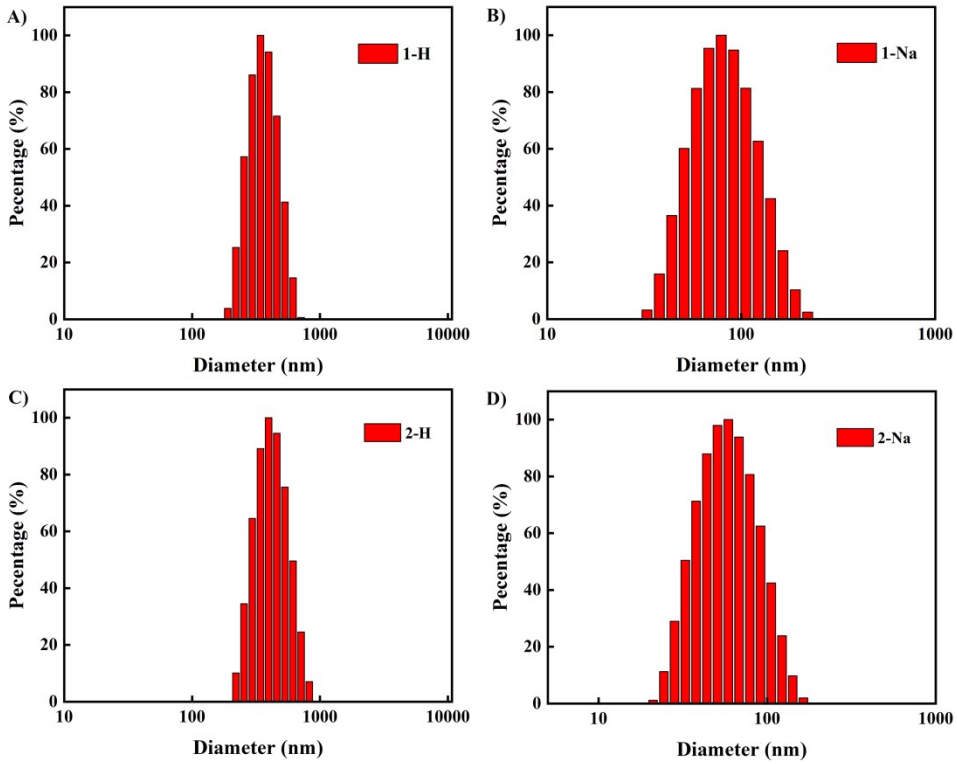


Fig. S19 Dynamic laser scattering results of A) **1-H**, B) **1-Na**, C) **2-H**, D) **2-Na** in water.

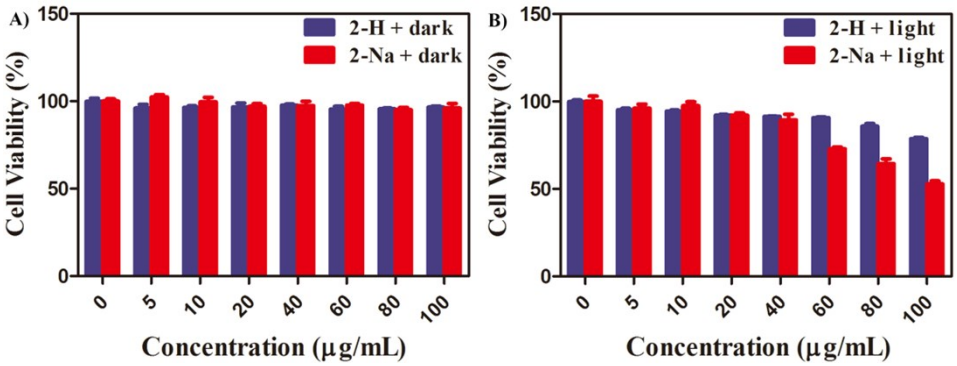


Fig. S20 Cell viability of **2-H** and **2-Na** against HeLa cells A) under dark and B) under light (450 nm, 20 mW cm^{-2}, 40 min).

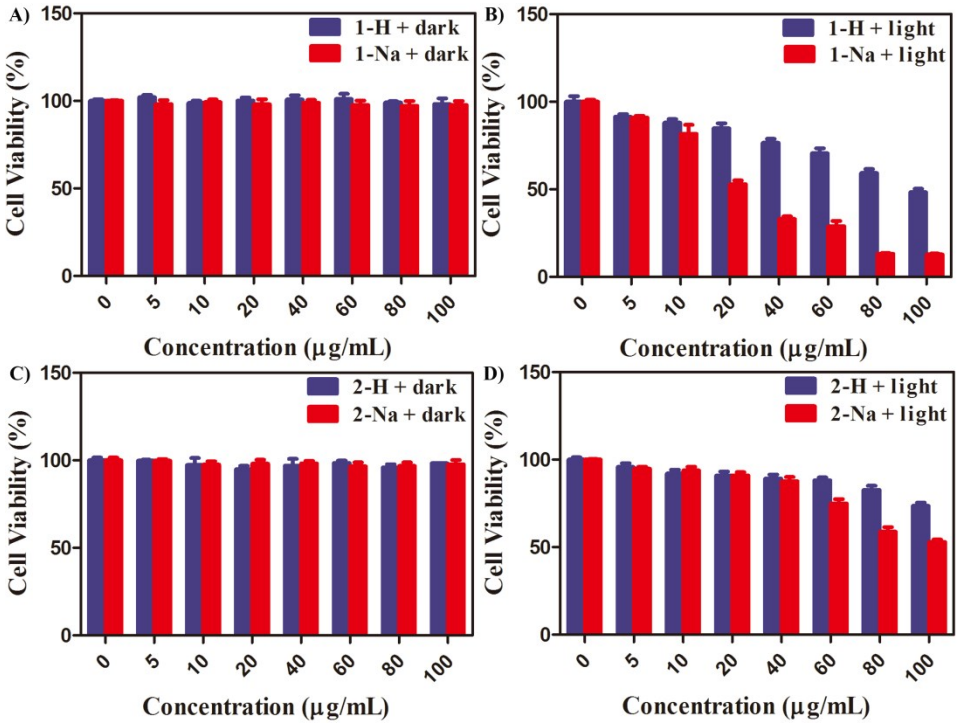


Fig. S21 Cell viability of MDA-MB-231 cells treated with **1-H** and **1-Na** A) under dark and B) under light (450 nm, 20 mW cm^{-2}, 40 min). Cell viability of MDA-MB-231 cells treated with **2-H** and **2-Na** A) under dark and B) under light (450 nm, 20 mW cm^{-2}, 40 min) .

Table S1 The photophysical data of **1-H**/**2-H**/**1-Na**/**2-Na**.

	λ_{abs} (nm)	λ_{em} (nm)	Φ_p (%)	τ_p (ns)	K_r[b] ($\times 10^5$ s^{-1})	K_{nr}[b] ($\times 10^6$ s^{-1})
1-H[a]	241,348	617	13	38.37	33.88	22.67
2-H[a]	258,355	613	1	45.47	2.20	2.17
1-Na[a]	242,349	660	34	64.04	53.09	10.30
2-Na[a]	260,358	643	7	11.44	61.19	81.29

[a] Measured in water at 298 K (1.0×10^{-5} M, λ_{ex} = 380 nm). [b] The radiative k_r and non-radiative k_{nr} values in neat film were calculated according to the equations: $k_r = \Phi/\tau$ and $k_{nr} = (1-\Phi)/\tau$, from the quantum yields Φ and the lifetime τ values.

Table S2 The average diameter and polydispersity index (PDI) results of **1-H**/**2-H**/**1-Na**/**2-Na** measured by DLS.

Sample	**1-H**	**2-H**	**1-Na**	**2-Na**
Average diameter (nm)	359	392	76	81
PDI	0.243	0.233	0.139	0.157