Electronic Supplementary Material (ESI) for New Journal of Chemistry.
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Supporting Information

Autofluorescence-free *in vivo* Imaging Using Cyclometalated Iridium Complex with Afterglow Luminescnece

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1. General information and methods

All reagents and solvents were purchased from commercial sources and were of the highest grade. Solvents were dried according to standard procedures. All reactions were magnetically stirred and monitored by thin-layer chromatography (TLC). Flash chromatography (FC) was performed using silica gel (100–200 mesh). The ^{1}H NMR and ^{13}C NMR spectra were recorded at 400 and 100 MHz, respectively. The following abbreviations were used to explain the multiplicities: s = singlet; d = doublet; t = triplet; q = quartet; m = multiplet; br = broad. High-resolution mass spectra were obtained on a Varian QFT-ESI mass spectrometer. X-ray intensity data of the compounds were collected on a Bruker D8 Venture system. The UV-visible absorption spectra were obtained with Shimadzu UV-2550 UV−vis-NIR spectrometer. Fluorescence spectra were measured using an Edinburgh FS5 fluorescence spectrometer. The afterglow emission spectrums were

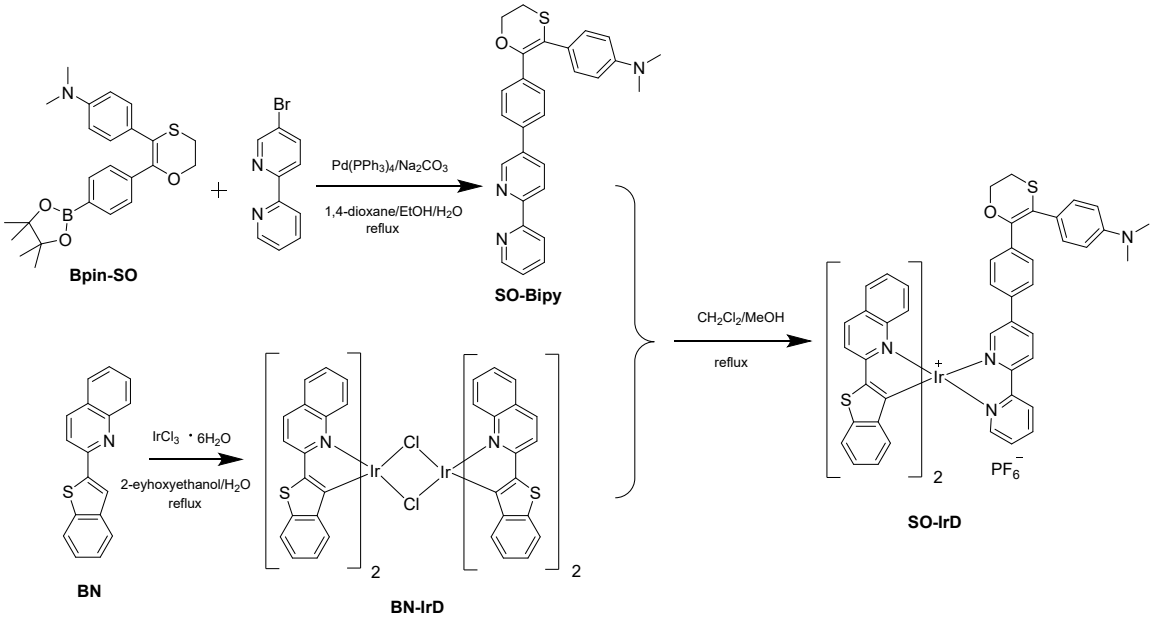
obtained by a time-resolved optical fiber spectrometer (Hamamatsu C14631).

Preparation of afterglow luminescence nanoparticles

Synthesis of nanoparticlesAf-NPs: F-127 (1 mg) was dissolved in 1 mL CH_2Cl_2, the samples SO-IrD (100 μL, 2 mM) were added the aboved solution. Then organic solvent was evaporated by a rotary evaporator to afford a thin film, and distilled deionized water (2 mL) was added under vigorous sonication to prepare Nps1. The nanomicelle solution was stored in the 4 °C refrigerator for the next use.

2. Synthesis Procedures

2.1 Synthesis of the compound SO-IrD

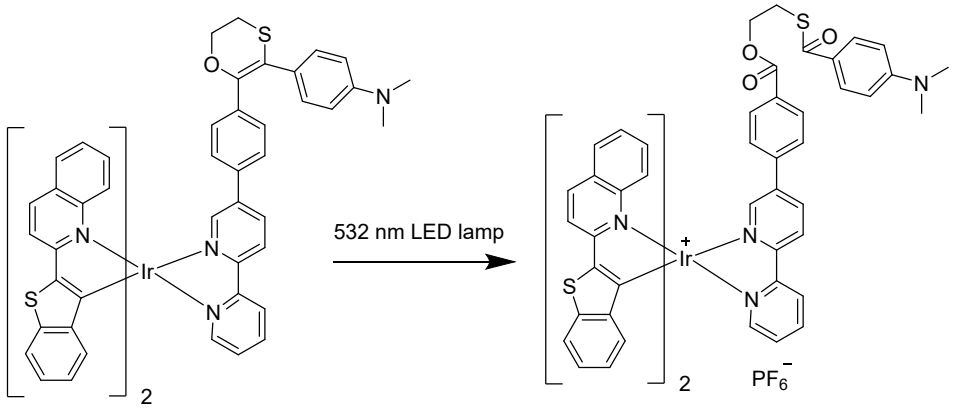


Synthesis of the compound SO-Bipy: Add 5-bromo-2,2'-bipyridine (131.6 mg, 0.86 mmol), Bpin-SO (364 mg, 0.86 mmol), $Pd(PPh_3)_4$ (50 mg, 0.043 mmol) and sodium carbonate (432 mg, 4.08 mmol) into a 50 mL two-necked flask quipped with a reflux condenser, then 1,4-dioxane/EtOH/water (10 mL, 2:1:1) was added to the reaction mixture and refluxed for 4 h under a N_2-atmosphere. The mixture solution was cooled to room temperature, quenched by 30 mL water and extracted with dichloromethane. The organic layer was dried over anhydrous Na_2SO_4 and the organic solvent was evaporated to

dryness. The crude product was purified with column chromatography over silica to give SO-Bipy, white solid.

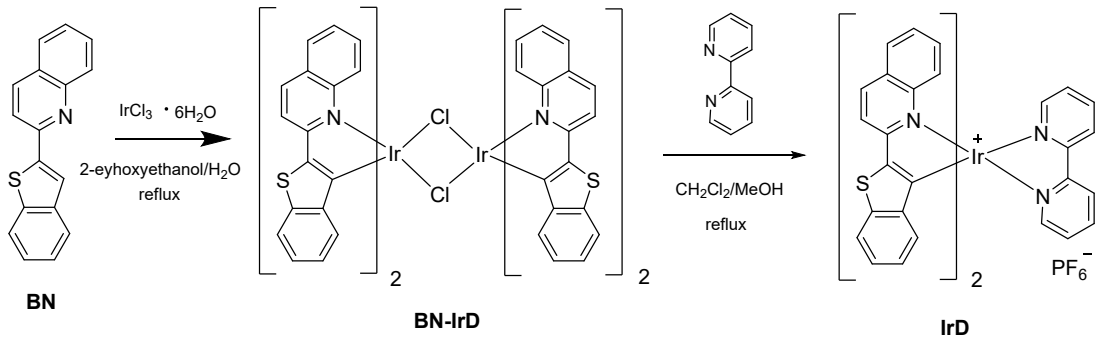
Synthesis of the compound SO-IrD: The solution of BN-IrD (750 mg, 0.5 mmol) and SO-Bipy (451 mg, 1.0 mmol) in CH_2Cl_2/MeOH (20 mL, 1:1, v/v) was heated to reflux. After 12 hours, the red solution was cooled to room temperature and then added 10-fold excess of potassium hexafluorophosphate. The suspension was stirred for 2 h and then was filtered to remove insoluble inorganic salts. The solution was evaporated to dryness under reduced pressure. The crude product was purified with column chromatography over silica to give the compound SO-IrD, red solid, 73.2% yield. ^{1}H NMR (400 MHz, CD_2Cl_2): δ 8.61 (s, 1H), 8.43 (d, J = 8.0 Hz, 1H), 8.31 (m, 2H), 8.08 (m, 6H), 7.92 (m, 2H), 7.77 (m, 2H), 7.54 (m, 1H), 7.41 (d, J = 8.0 Hz, 2H), 7.34 (m, 4H), 7.21 (m, 5H), 7.03 (m, 1H), 6.95 (m, 2H), 6.72 (m, 4H), 6.41 (m, 2H), 4.57 (t, J = 4.0 Hz, 2H), 3.31 (t, J = 4.0 Hz, 2H), 2.99 (s, 6H); ^{13}C NMR (101 MHz, CD_2Cl_2) δ 166.56, 166.41, 155.51, 153.49, 152.79, 151.73, 150.16, 148.69, 148.61, 147.74, 146.38, 145.40, 143.46, 143.13, 143.08, 141.25, 141.07, 140.22, 140.00, 136.90, 132.10, 131.37, 131.29, 130.84, 129.92, 129.35, 129.29, 127.73, 126.81, 126.74, 126.32, 126.29, 126.21, 126.14, 125.89, 124.67, 124.58, 123.76, 123.73, 123.48, 123.20, 123.08, 118.31, 117.72, 112.22, 110.95, 65.65, 40.27, 28.70. HRMS for $[M-PF_6]^+$: calcd 1164.2415, found 1164.2423。

2.2 Synthesis of the compound SE-IrD



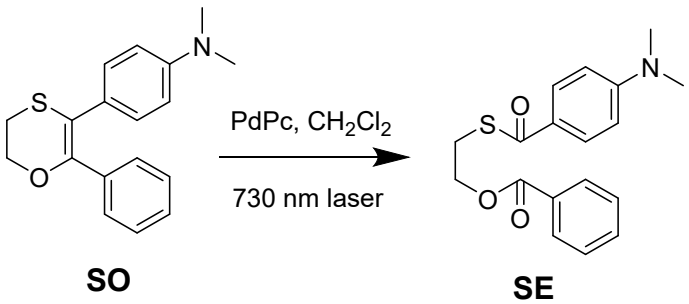
Synthesis of the compound SE-IrD: Add SO-IrD (50 mg) into a 500 mL reaction flask，then dichloromethane (200 mL) was added to the reaction mixture. The mixture solution was irradiated by a 532 nm LED lamp until the reactants are consumed, then quenched by 200 mL water. The organic layer was dried over anhydrous Na_2SO_4 and the organic solvent was evaporated to dryness. The crude product was purified with column chromatography over silica to give the SE-IrD, red solid, 92.7% yield. ^{1}H NMR (400 MHz, CD_2Cl_2): δ 8.70 (s, 1H), 8.46 (d, J = 4.0 Hz, 1H), 8.33 (m, 5H), 8.15 (m, 5H), 7.95 (m, 4H), 7.78 (m, 2H), 7.63 (m, 3H), 7.36 (m, 2H), 7.22 (m, 3H), 7.04 (d, J = 8.0 Hz, 1H), 6.96 (m, 2H), 6.74 (m, 4H), 6.43 (m, 2H), 4.59 (t, J = 8.0 Hz, 2H), 3.52 (t, J = 8.0 Hz, 2H), 3.01 (s, 6H); ^{13}C NMR (101 MHz, CD_2Cl_2) δ 188.00, 166.55, 166.39, 165.32, 155.32, 154.61, 154.02, 152.67, 151.44, 148.69, 148.59, 147.86, 146.35, 145.81, 143.12, 143.08, 141.36, 141.09, 140.19, 140.14, 139.34, 138.50, 137.84, 131.43, 131.36, 130.82, 129.38, 129.34, 129.29, 128.04, 126.98, 126.86, 126.78, 126.39, 126.28, 126.24, 126.13, 124.71, 124.61, 124.14, 123.99, 123.45, 123.26, 123.09, 118.32, 117.68, 110.62, 64.21, 39.85, 27.20. HRMS for $[M\text{-}PF_6]^+$: calcd 1196.2314, found 1196.2307。

2.3 Synthesis of the control compound IrD



Synthesis of the compound IrD: The solution of BN-IrD (750 mg, 0.5 mmol) and Bipy (156 mg, 1.0 mmol) in CH_2Cl_2/MeOH (20 mL, 1:1, v/v) was heated to reflux. After 12 hours, the red solution was cooled to room temperature and then added 10-fold excess of potassium hexafluorophosphate. The suspension was stirred for 2 h and then was filtered to remove insoluble inorganic salts. The solution was evaporated to dryness under reduced pressure. The crude product was purified with column chromatography over silica to give the compound IrD, red solid, 65.5% yield. 65.5%。^{1}H NMR (400 MHz, CD_2Cl_2): δ 8.43 (d, J = 8.0 Hz, 2H), 8.29 (d, J = 8.0 Hz, 2H), 8.06 (m, 6H), 7.91 (m, 2H), 7.77 (d, J = 8.0 Hz, 2H), 7.57 (m, 2H), 7.35 (m, 2H), 7.21 (m, 2H), 7.03 (d, J = 8.0 Hz, 2H), 6.94 (m, 2H), 6.71 (m, 2H), 6.36 (d, J = 8.0 Hz, 2H); ^{13}C NMR (101 MHz, CD_2Cl_2) δ 166.59, 155.61, 151.90, 148.60, 147.93, 146.30, 143.13, 140.97, 140.19, 140.02, 131.16, 129.30, 128.05, 126.74, 126.26, 126.20, 126.09, 124.57, 123.83, 123.39, 123.05, 118.25. HRMS for $[M-PF_6]^+$: calcd 869.1385, found 869.1385。

2.4 Synthesis of the control compound SE



Add SO (59.4 mg, 0.2 mmol, 1 eq), PdPc (2.3 mg, 0.002 mmol, 0.01 eq) into a 500 mL reaction flask, then dichloromethane (400 mL) was added to the reaction mixture. The mixture solution was irradiated by a 730 nm laser for 1 h, then quenched by 200 mL water. The organic layer was dried over anhydrous Na_2SO_4 and the organic solvent was evaporated to dryness. The crude product was purified with column chromatography over silica to give SE, white solid, 93% yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.96 (d, J= 8.0 Hz, 2H), 7.78 (d, J= 8.0 Hz, 2H), 7.44 (m, 1H), 7.33 (m, 2H), 6.53 (d, J= 8.0 Hz, 2H), 4.42 (t, J = 8.0 Hz, 2H), 3.35 (t, J = 8.0 Hz, 2H), 2.94 (s, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 188.38, 166.25, 153.74, 132.92, 129.97, 129.63, 129.37, 128.28, 124.19, 110.56, 63.73, 39.95, 27.23.

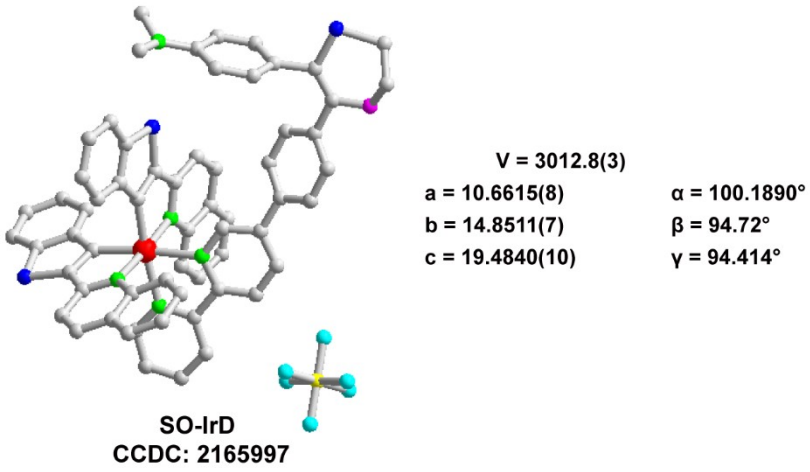


Figure S1. Single-crystal X-ray structures of afterglow molecule SO-IrD (CCDC: 2165997). Hydrogen atoms are omitted for clarity; Ir red; S blue; C gray; N green; O pink; P Turquiose; F yellow.

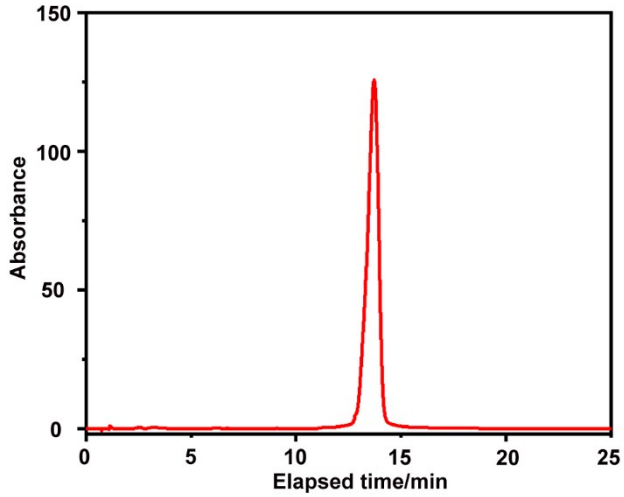


Figure S2. HPLC spectra of afterglow molecule SO-IrD monitored at 340 nm with acetonitrile/water as eluent in ratios of 80:20 to 50:50 (v/v).

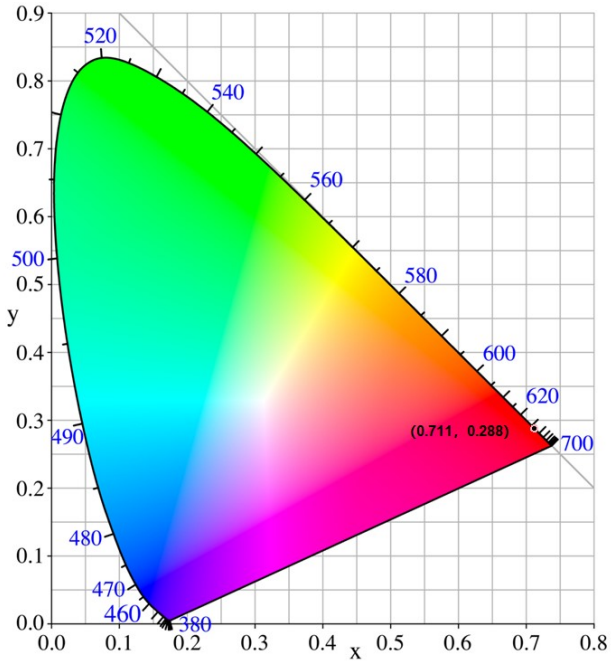


Figure S3. Corresponding CIE chromaticity coordinate diagram of afterglow luminescence of SO-IrD in dichloromethane solution.

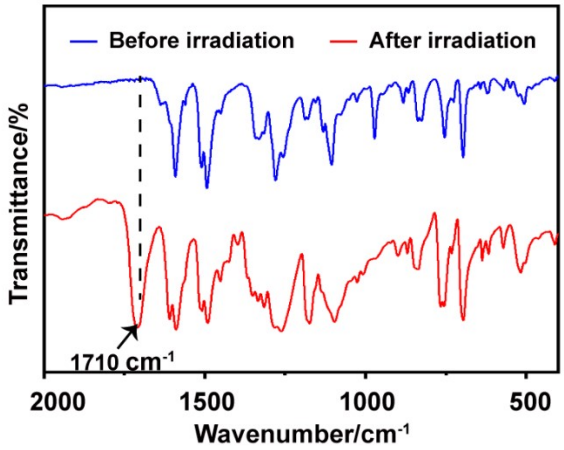


Figure S4. FTIR spectra of afterglow molecule SO-IrD before and after 532 nm LED light irradiation in dichloromethane solution.

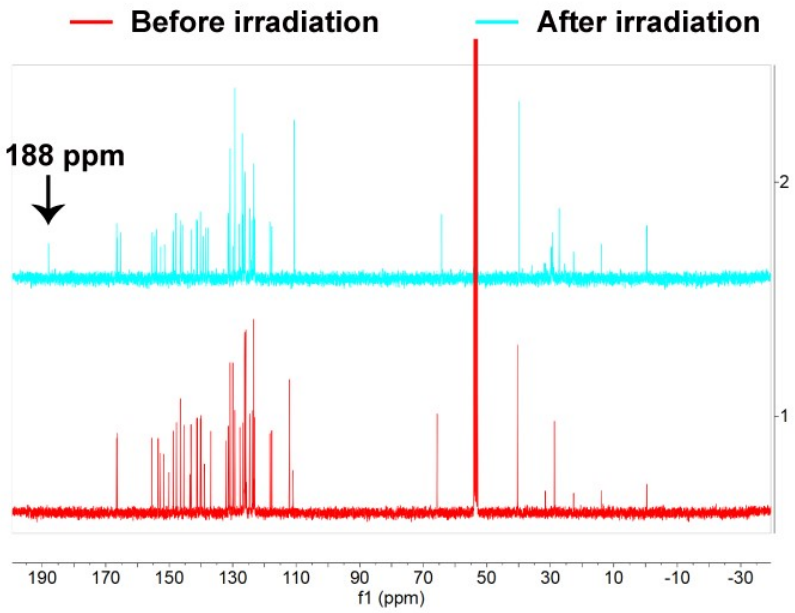


Figure S5. (a) A comparison of ^{13}C NMR spectra of SO-IrD (top) and the corresponding photochemical reaction product after 532 nm LED light irradiation (bottom) in CD_2Cl_2.

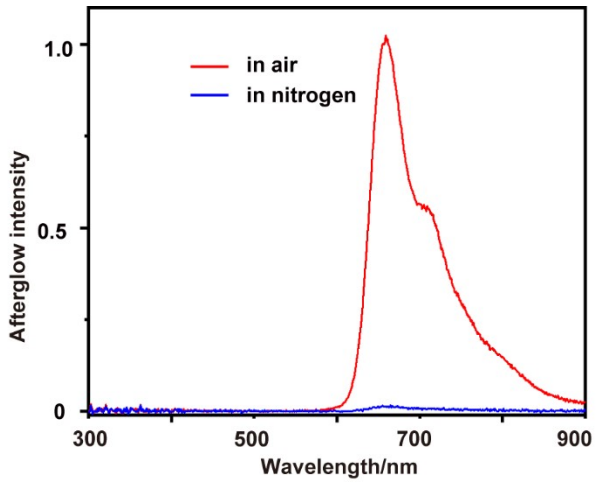


Figure S6. Afterglow emission spectra of SO-IrD in dichloromethane solution (50 μM) with an air or nitrogen atmosphere at 25 °C under irradiation with a 532 nm LED lamp irradiation.

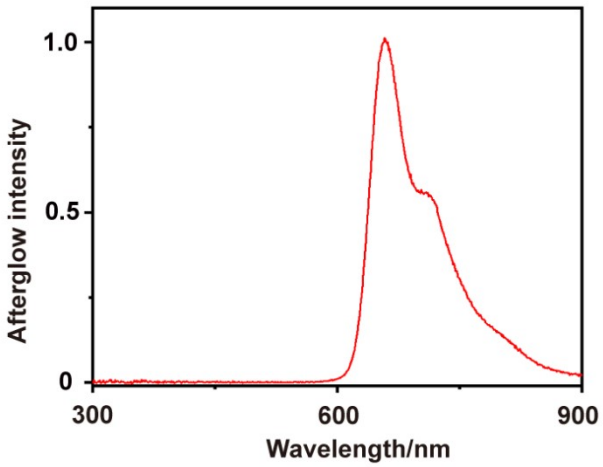


Figure S7. Afterglow emission spectra of SO-IrD in dichloromethane solution (50 μM) with the palladium(II) 1,4,8,11,15,18,22,25-octabutoxypthalacyanine (PdPc) (0.05 μM) at 25 °C under irradiation with a 730 nm LED lamp irradiation.

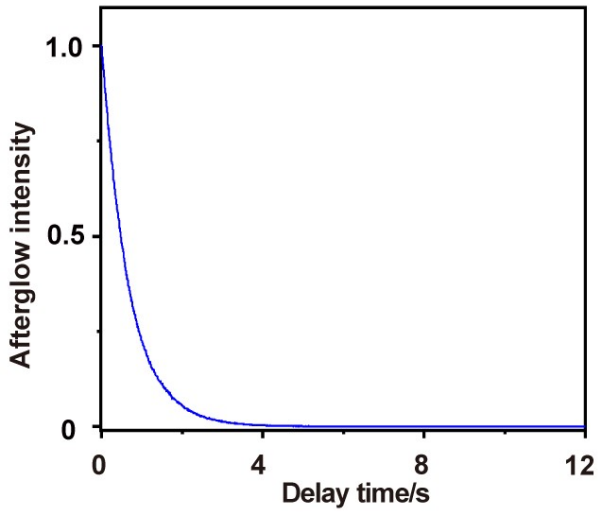


Figure S8. Time-resolved afterglow luminescence decay curves of SO-IrD in dichloromethane solution (50 μM) with the palladium(II) 1,4,8,11,15,18,22,25-octabutoxypthalacyanine (PdPc) (0.05 μM) at 25 °C under irradiation with a 730 nm LED lamp irradiation.

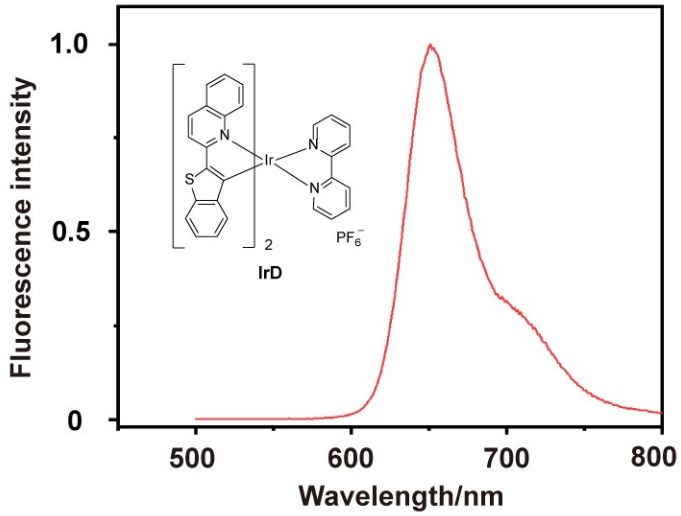


Figure S9. Fluorescence spectra of the control compound IrD in dichloromethane solution (50 μM).

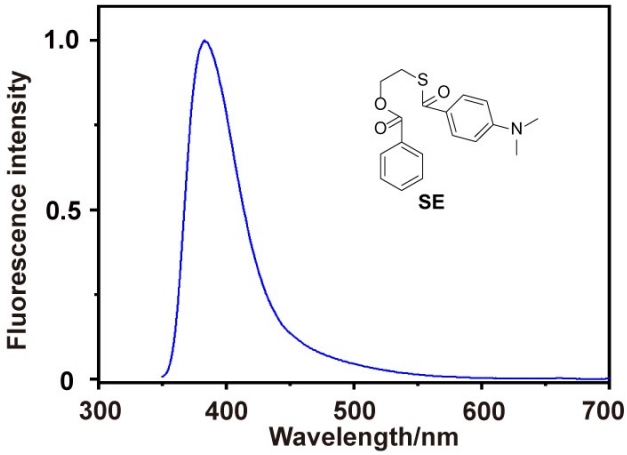


Figure S10. Fluorescence spectra of the control compound SE in dichloromethane solution (50 μM).

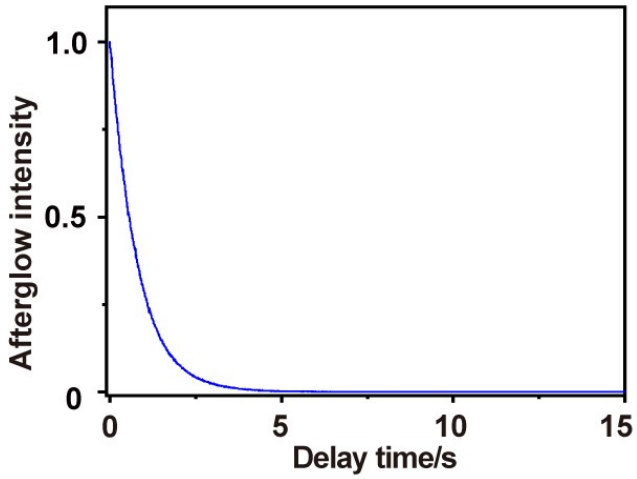


Figure S11. Time-resolved afterglow luminescence decay curves of Af-NPs in distilled water.

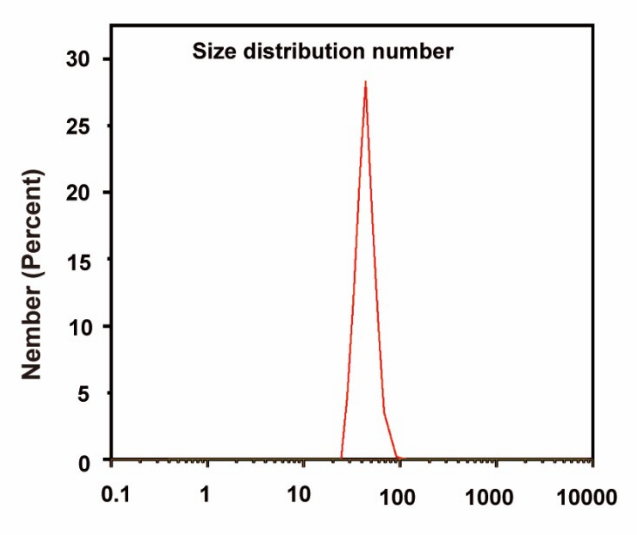


Figure S12. DLS analysis of number size of Af-NPs in distilled water.

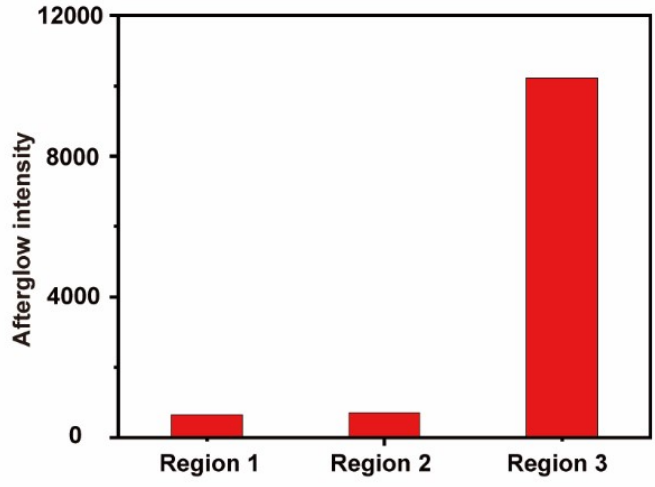


Figure S13. Analysis of the afterglow luminescence signal in regions of interest in vivo imaging.

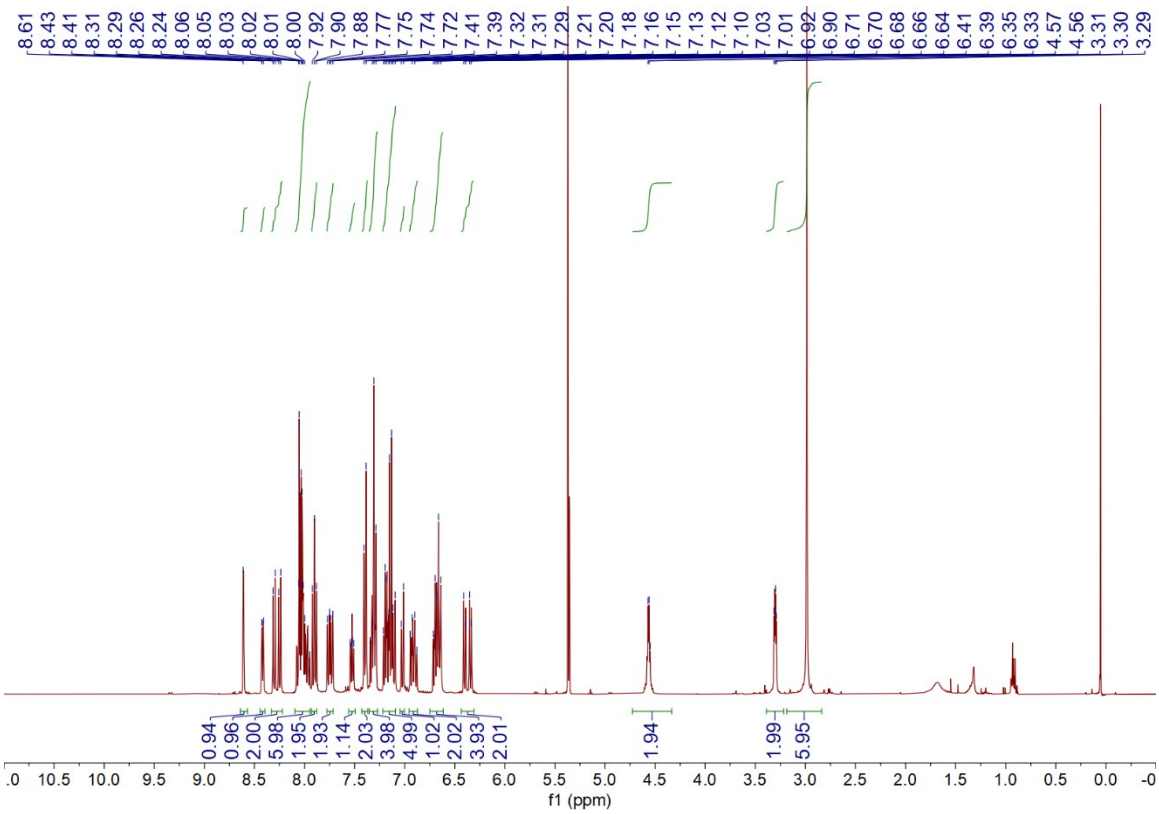


Figure S14. ^{1}H NMR chart of the compound **SO-IrD** (CD_2Cl_2, 400 MHz).

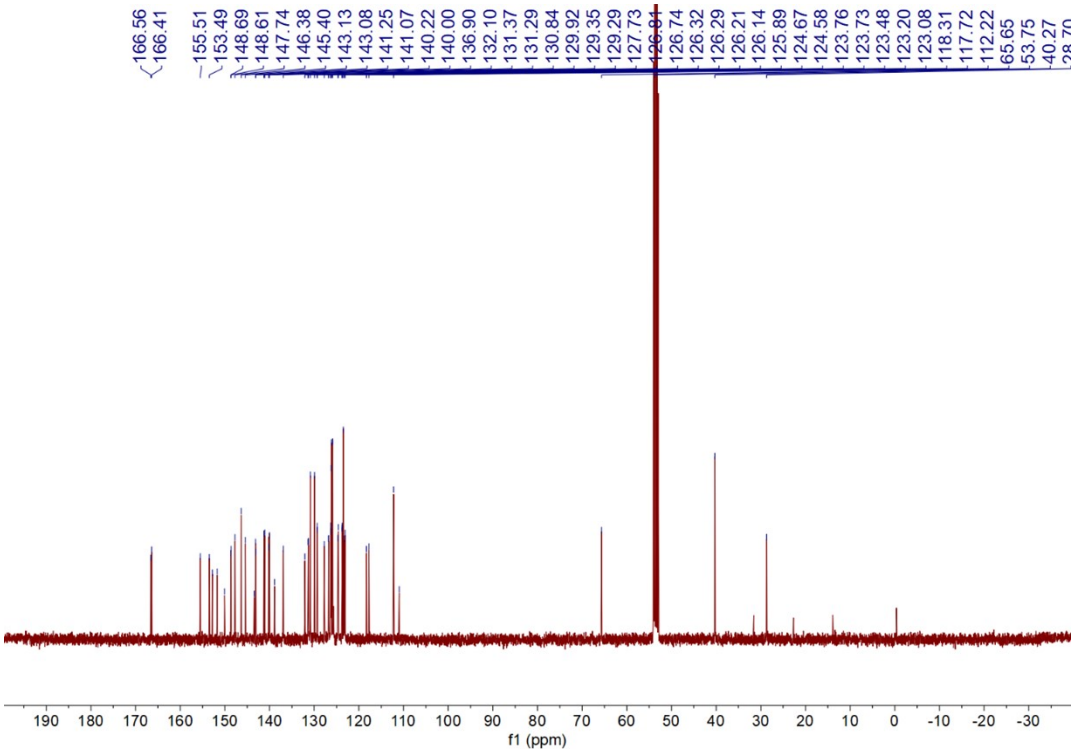


Figure S15. ^{13}C NMR chart of the compound **SO-IrD** (CD_2Cl_2, 400 MHz).

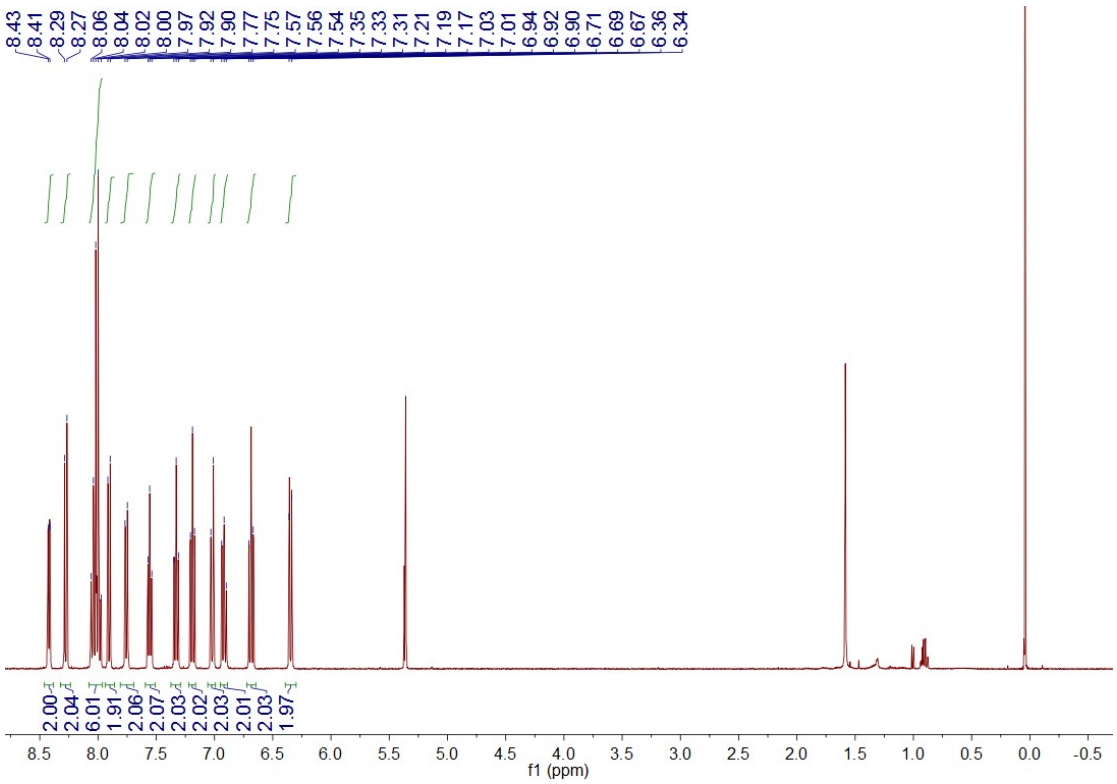


Figure S16. ^{1}H NMR chart of the compound **IrD** (CD_2Cl_2, 400 MHz).

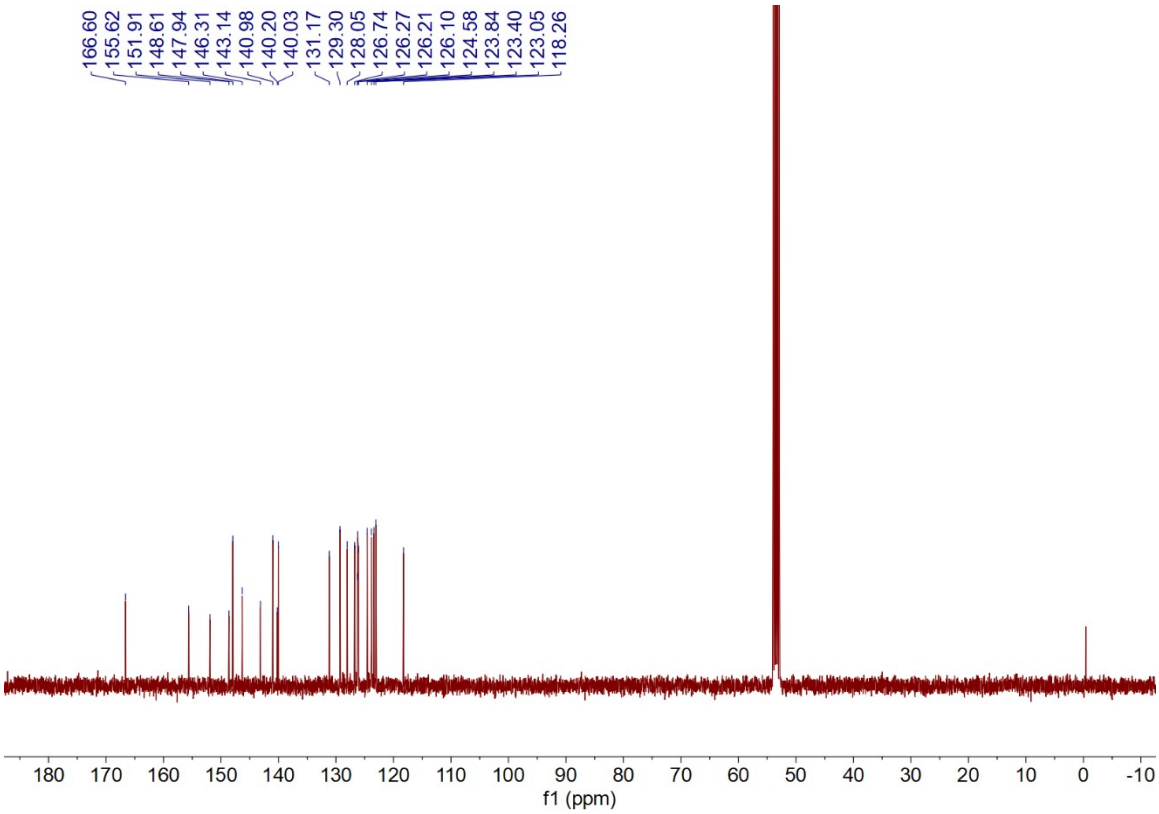


Figure S17. ^{13}C NMR chart of the compound **IrD** (CD_2Cl_2, 400 MHz).

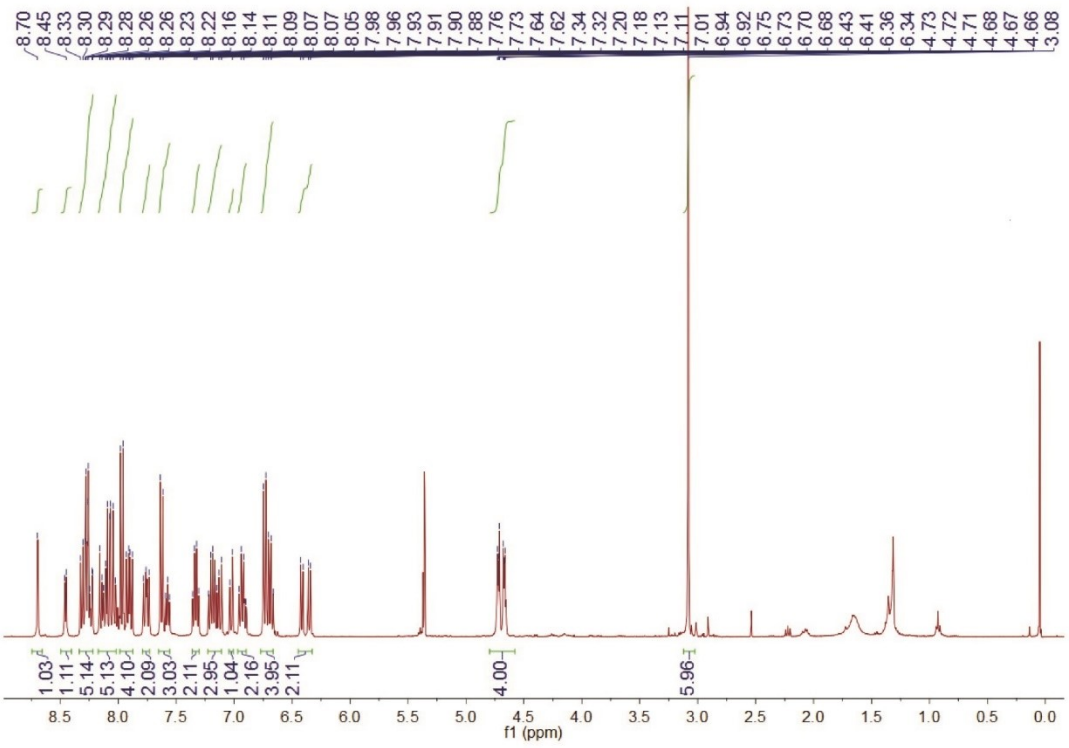


Figure S18. ^{1}H NMR chart of the compound **SE-IrD** (CD_2Cl_2, 400 MHz).

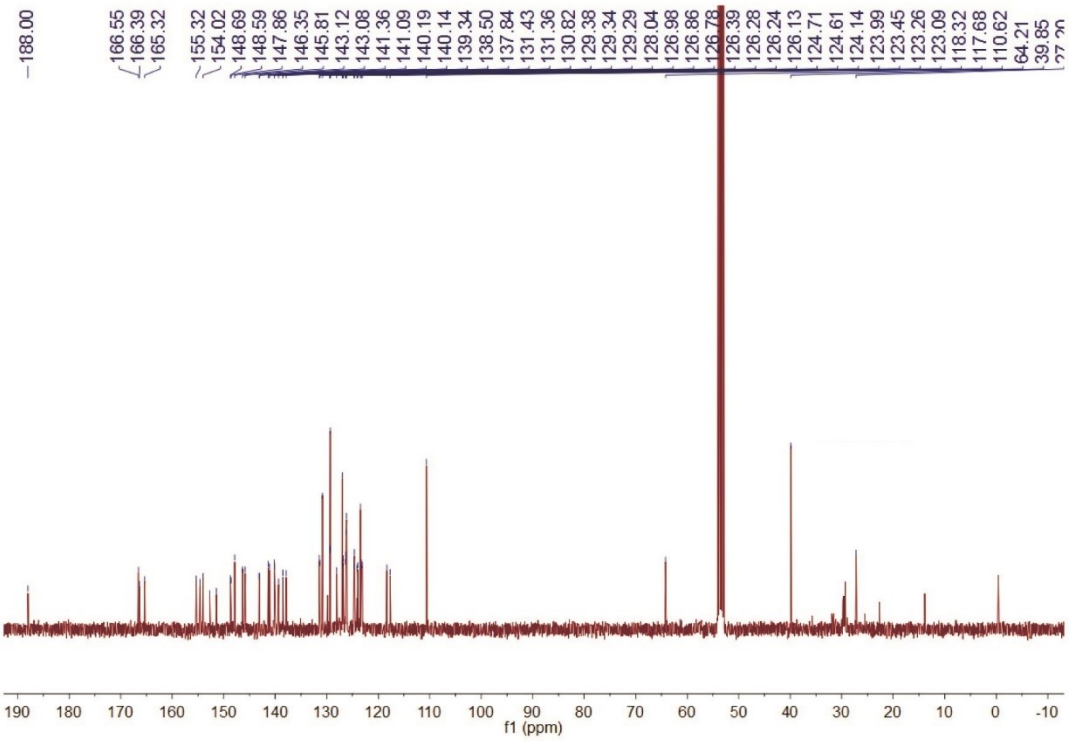


Figure S19. ^{13}C NMR chart of the compound **SE-IrD** (CD_2Cl_2, 400 MHz).

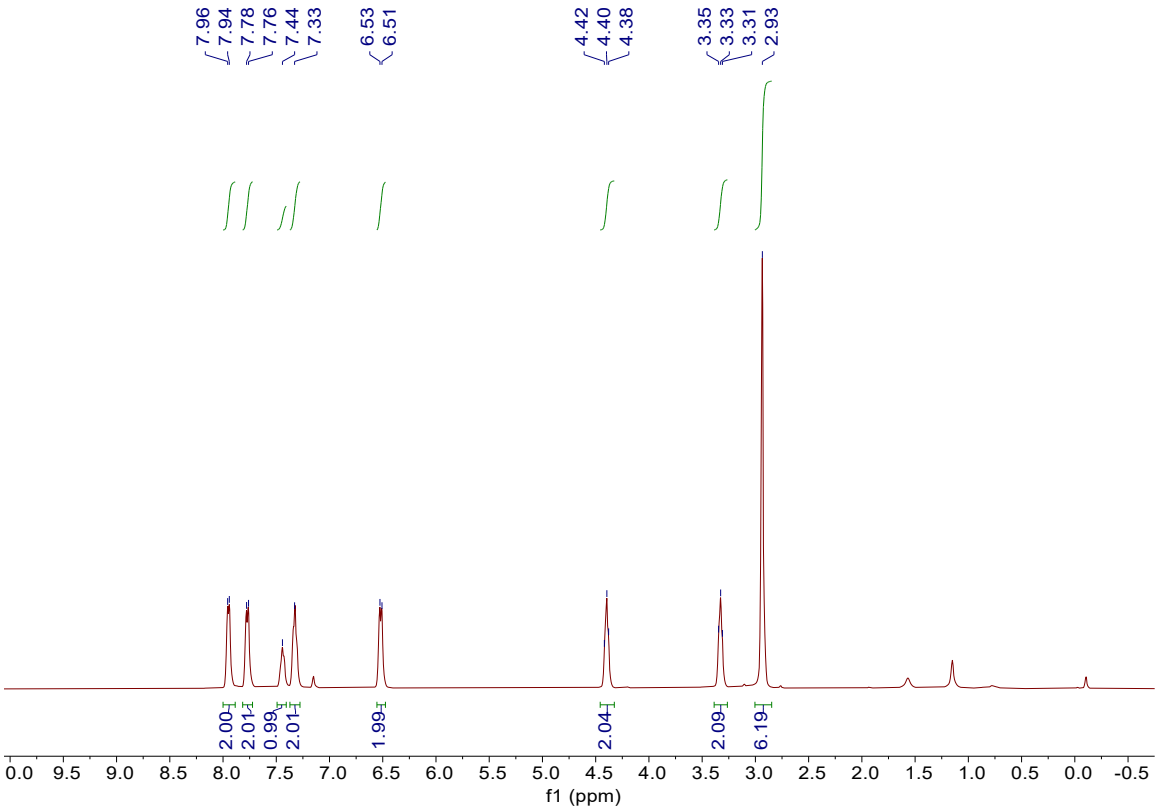


Figure S20. ^{1}H NMR chart of the compound **SE** (CD_2Cl_2, 400 MHz).

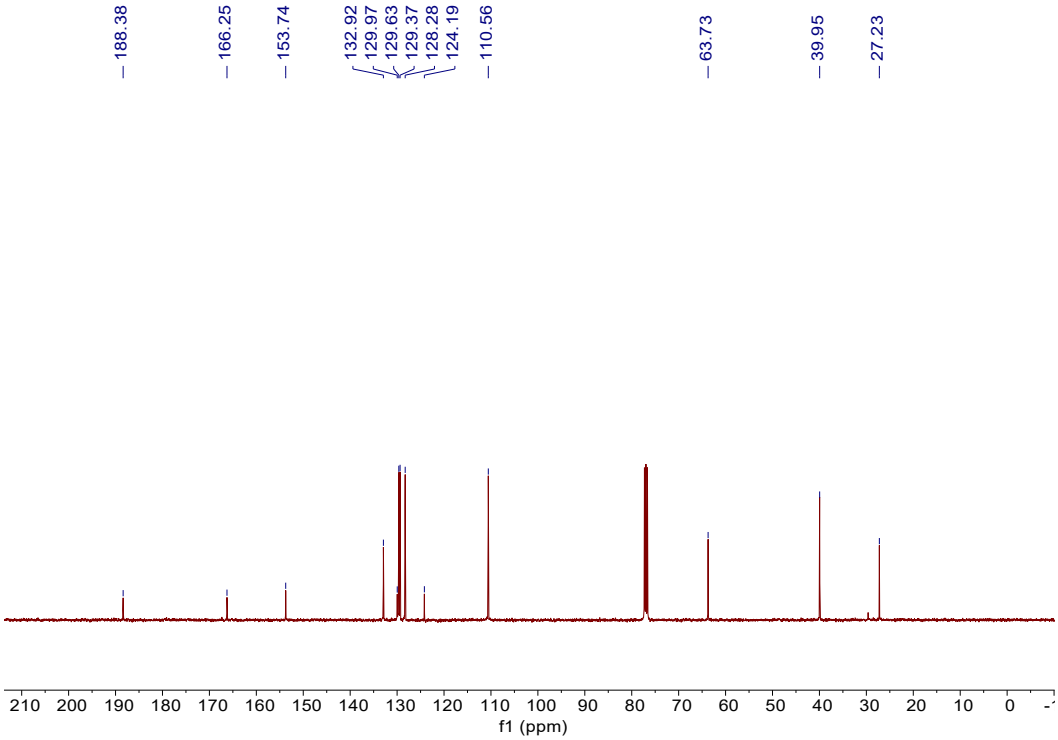


Figure S21. ^{13}C NMR chart of the compound **SE** (CD_2Cl_2, 100 MHz).

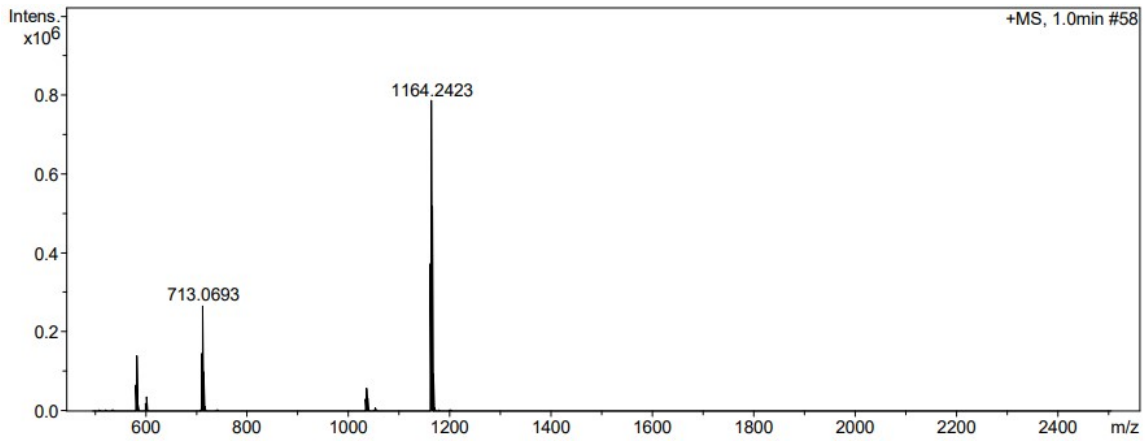


Figure S22. HRMS chart of the compound of SO-IrD.

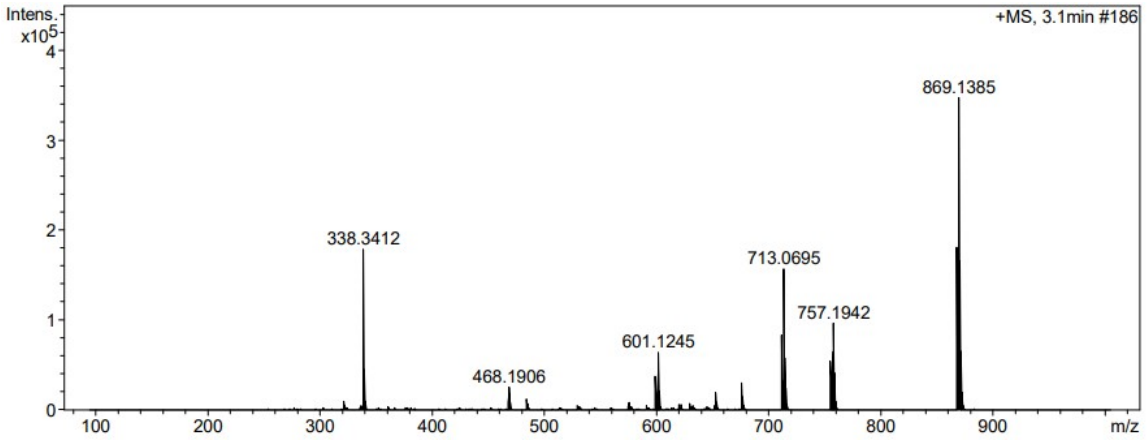


Figure S23. HRMS chart of the compound of IrD.

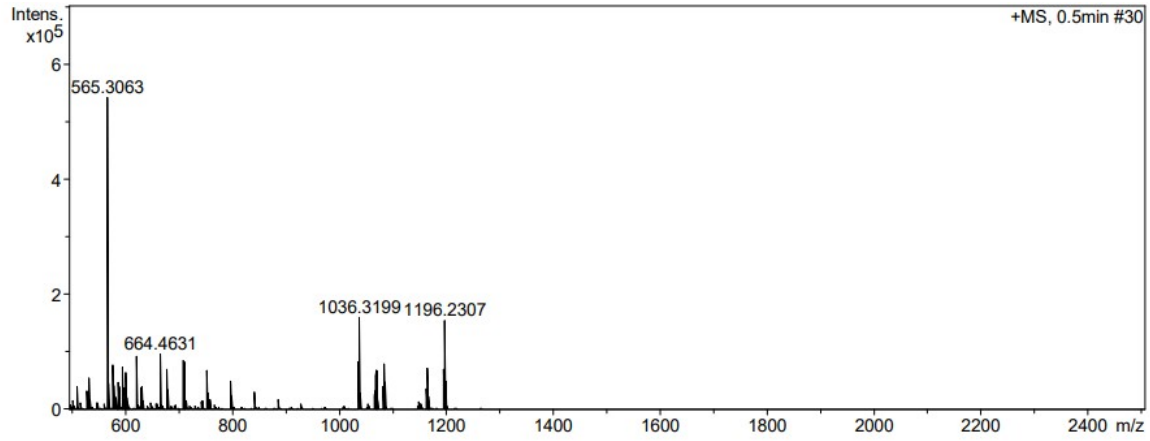


Figure S24. HRMS chart of the compound of SE-IrD.

Table 1. Crystal data and structure refinement for SO-IrD.

Identification code	ga_91126e_a
Empirical formula	C64H49Cl4F6 Ir N5OPS3
Formula weight	1479.23
Temperature	173(2) K
Wavelength	1.34138 Å
Crystal system	Triclinic
Space group	P-1
Unit cell dimensions	a = 10.6615(5) Å α= 100.1890(10)°. b = 14.8511(7) Å β= 94.720(2)°. c = 19.4840(10) Å γ= 94.4140(10)°.
Volume	3012.8(3) $Å^3$
Z	2
Density (calculated)	1.631 Mg/m^3
Absorption coefficient	5.103 mm^{-1}
F(000)	1476
Crystal size	0.250 x 0.200 x 0.170 mm^3
Theta range for data collection	3.981 to 54.966°.
Index ranges	-12<=h<=13, -18<=k<=18, -23<=l<=23
Reflections collected	97843
Independent reflections	11402 [R(int) = 0.0428]
Completeness to theta = 53.594°	99.7 %
Absorption correction	Semi-empirical from equivalents
Max. and min. transmission	0.751 and 0.352
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	11402 / 44 / 798
Goodness-of-fit on F^2	1.069
Final R indices [I>2sigma(I)]	R1 = 0.0245, wR2 = 0.0605
R indices (all data)	R1 = 0.0249, wR2 = 0.0608
Extinction coefficient	n/a
Largest diff. peak and hole	0.862 and -1.058 $e.Å^{-3}$

Table 2. Crystal data and structure refinement for SE-IrD.

Identification code	platon_sq	
Empirical formula	C62 H45 F6 Ir N5 O3 P S3	
Formula weight	1341.38	
Temperature	173(2) K	
Wavelength	1.34138 Å	
Crystal system	Triclinic	
Space group	P-1	
Unit cell dimensions	a = 14.1612(10) Å	α= 98.990(3)°.
	b = 14.3583(10) Å	β= 91.174(3)°.
	c = 15.9369(11) Å	γ = 110.964(3)°.
Volume	2978.5(4) $Å^3$	
Z	2	
Density (calculated)	1.496 Mg/m^3	
Absorption coefficient	4.062 mm^{-1}	
F(000)	1340	
Crystal size	0.160 x 0.120 x 0.090 mm^3	
Theta range for data collection	2.912 to 57.499°.	
Index ranges	-17<=h<=17, -17<=k<=18, -20<=l<=20	
Reflections collected	84349	
Independent reflections	12385 [R(int) = 0.0653]	
Completeness to theta = 53.594°	99.9 %	
Absorption correction	Semi-empirical from equivalents	
Max. and min. transmission	0.751 and 0.564	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	12385 / 188 / 860	
Goodness-of-fit on F^2	1.066	
Final R indices [I>2sigma(I)]	R1 = 0.0415, wR2 = 0.1104	
R indices (all data)	R1 = 0.0486, wR2 = 0.1148	
Extinction coefficient	n/a	
Largest diff. peak and hole	4.119 and -0.646 $e.Å^{-3}$	