Supporting Information

Two-photon photodynamic ablation of tumor cells by mitochondria-targeted iridium(III) complexes in aggregate states

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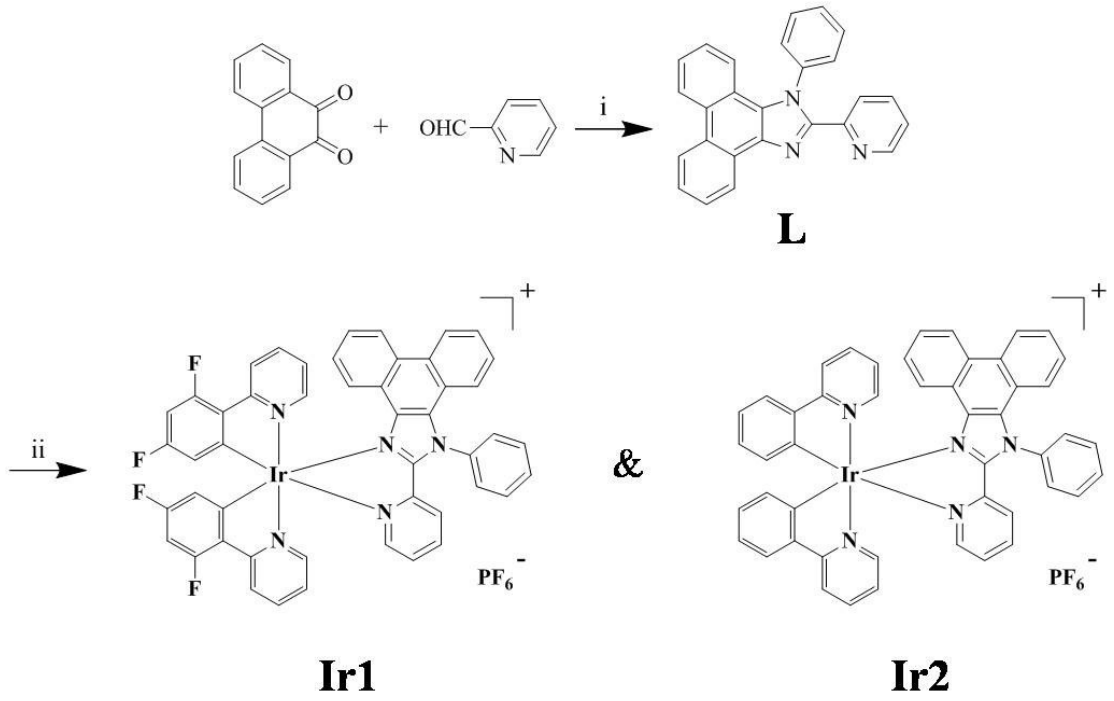

Scheme S1. Synthetic pathway of **Ir1-Ir2**. (i) CH_3COONH_4, aniline, CH_3COOH, reflux, 12 h, 45%; (ii) DMF, reflux, 12 h, 71-73%.

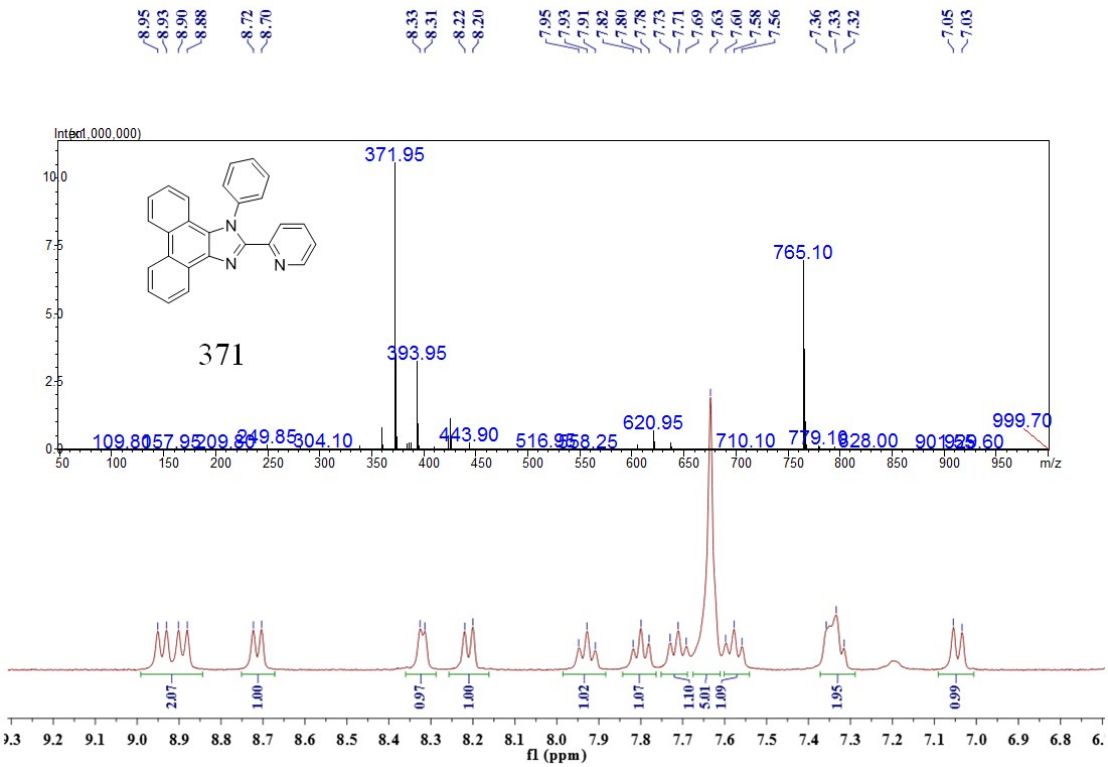

Fig. S1 ESI-MS spectrum and ^{1}H NMR spectrum of ligand **L**.

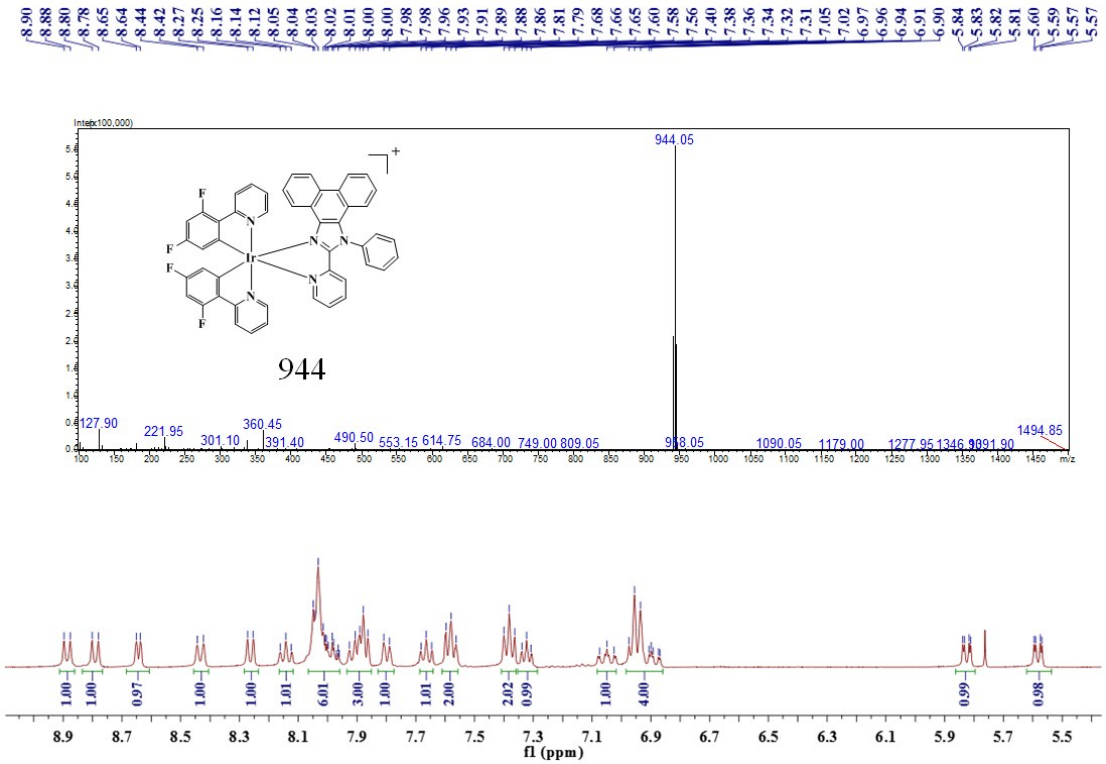

Fig. S2 ESI-MS spectrum and ^{1}H NMR spectrum of **Ir1**.

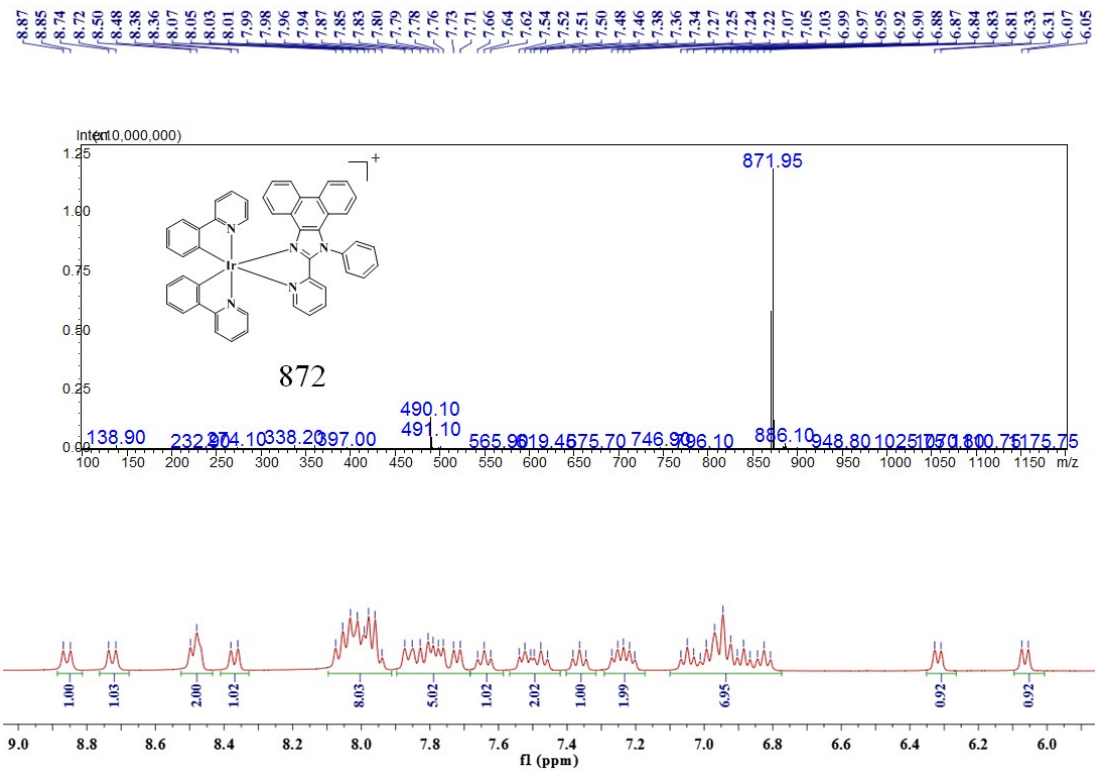

Fig. S3 ESI-MS spectrum and ^{1}H NMR spectrum of **Ir2**.

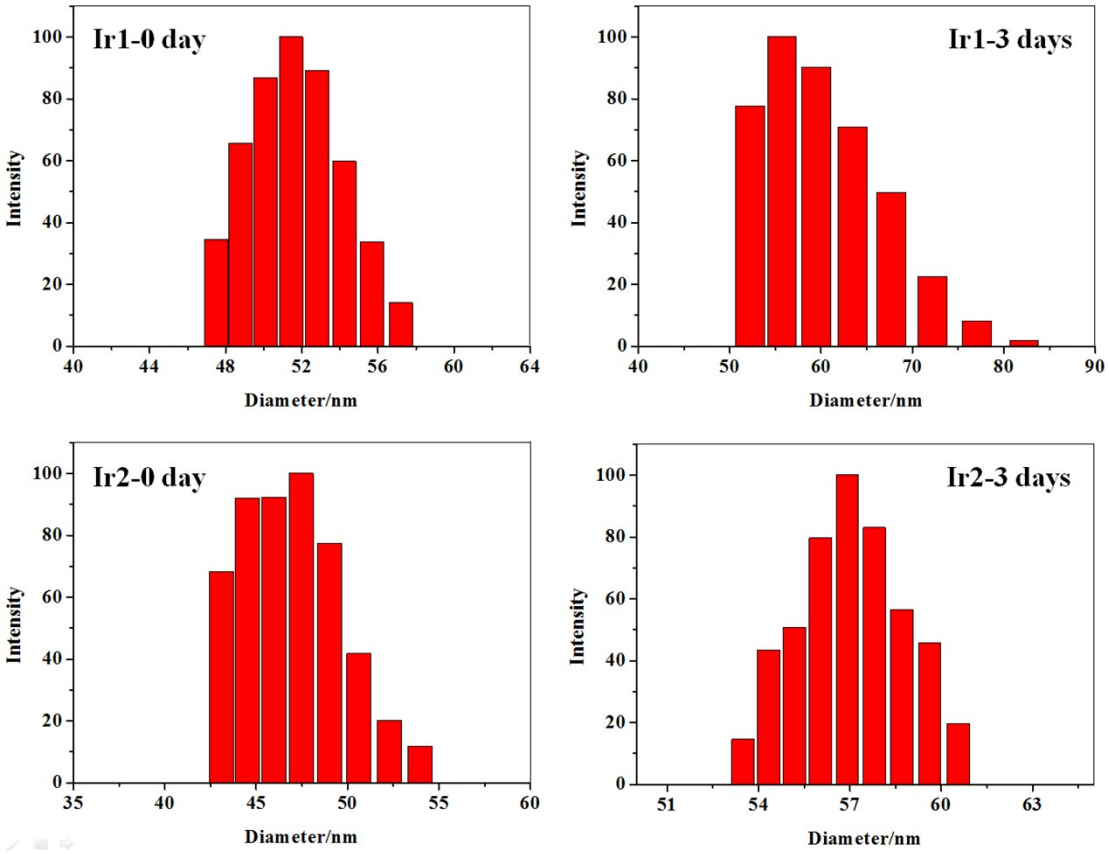

Fig. S4 The sizes of the complexes in aggregate states in water and DMSO (v/v = 95:5) at 500 μM before and after 3 days.

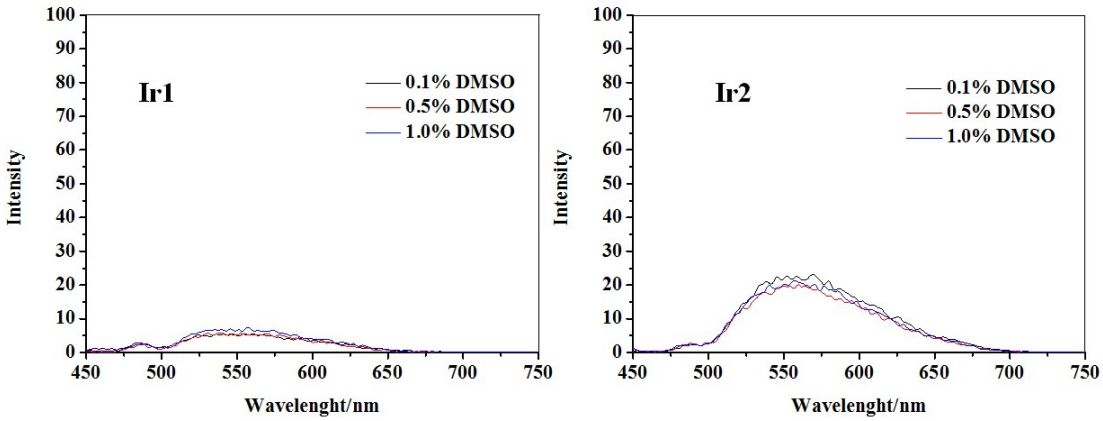

Fig. S5 The emission spectra of the complexes in water with different fractions of DMSO at 1 μM.

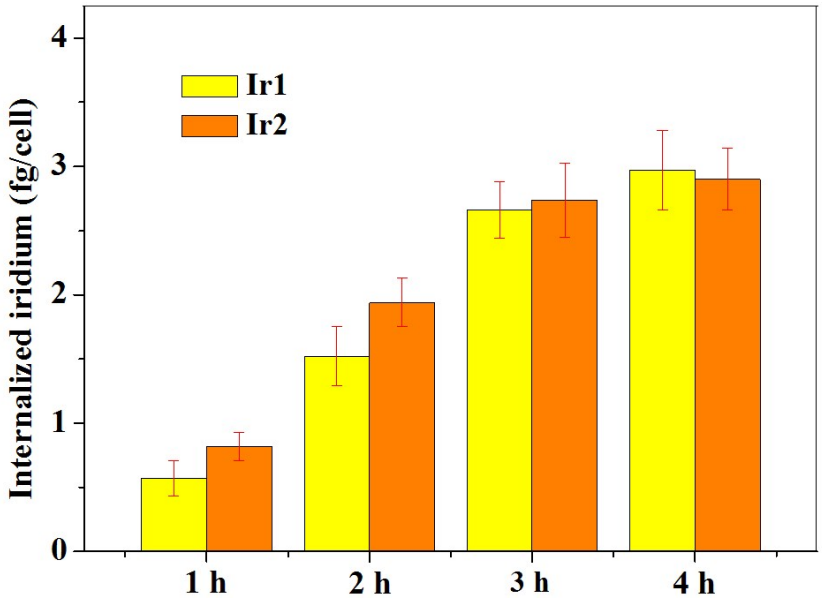

Fig. S6 The internalized iridium of the LO2 cells was quantified by ICP-MS with different incubation time.

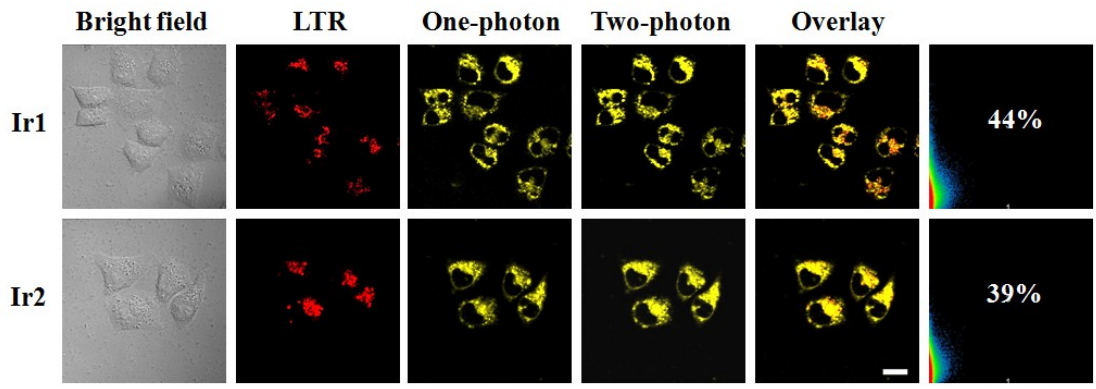

Fig. S7 OPM and TPM images of HeLa cells co-labeled with **Ir1-Ir2** (1 μM, 4 h, OPM: λ_{ex} = 405 nm, TPM: λ_{ex} = 740 nm, λ_{em} = 550 ± 20 nm) and LTR (50 nM, 0.5 h, λ_{ex} = 543 nm, λ_{em} = 610 ± 20 nm). Overlay: Overlay of the 2nd and 4th columns. Scale bar: 20 μm.

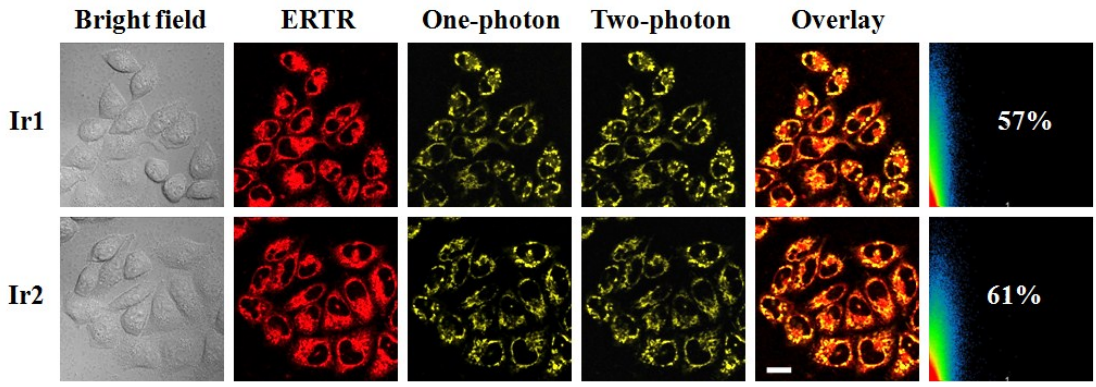

Fig. S8 OPM and TPM images of HeLa cells co-labeled with **Ir1-Ir2** (1 μM, 4 h, OPM: λ_{ex} = 405 nm, TPM: λ_{ex} = 740 nm, λ_{em} = 550 ± 20 nm) and ERTR (1 μM, 0.5 h, λ_{ex} = 543 nm, λ_{em} = 610 ± 20 nm). Overlay: Overlay of the 2nd and 4th columns. Scale bar: 20 μm.

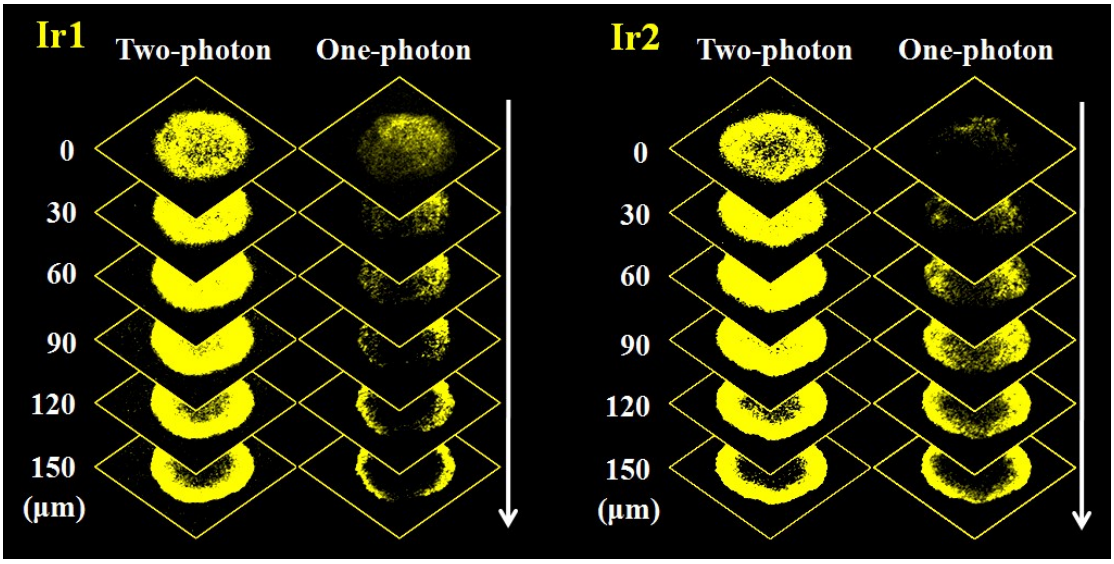

Fig. S9 OPM and TPM images of **Ir1-Ir2** in HeLa MCTSs (1 μM, 12 h, OPM: λ_{ex} = 405 nm, TPM: λ_{ex} = 740 nm, λ_{em} = 550 ± 20 nm).

Table S1. Crystallographic Data for the Ir(III) complexes

Complexes	**Ir1**	**Ir2**
Empirical formula	$C_{48}H_{29}F_{10}IrN_5P$	$C_{48}H_{33}F_6IrN_5P$
Formula weight	1088.93	1016.96
Temperature /K	120(2)	120(2)
Wavelength/Å	0.71073	0.71073
Crystal system	Triclinic	Triclinic
Space group	*P-1*	*P-1*
a / Å	9.6327(4)	9.6803(5)
b / Å	9.8230(4)	10.0240(5)
c / Å	23.0384(9)	22.9480(13)
β/°	79.9150(10)	100.758(2)
V / Å^3	2101.08(15)	2141.9(2)
Z	2	2
ρ_{cald}/g.cm^{-3}	1.721	1.577
Absorption coefficient /mm^{-1}	3.302	3.22
F (000)	1068	1004
Crystal size (mm^3)	0.202 × 0.182 × 0.176	0.188 × 0.146 × 0.052
θ range for data collection /°	2.657 to 27.510	2.521 to 27.521
Reflections collected / unique	22030 / 9514 [$R_{(int)}$ = 0.0258]	44598 / 9812 [$R_{(int)}$ = 0.0355]
Completeness to *θ* = 26.000°	99.30%	99.80%
Refinement method	Full-matrix least-squares on F^2	Full-matrix least-squares on F^2
Data / restraints / parameters	9514 / 0 / 586	9812 / 0 / 550
Goodness-of-fit on F^2	1.06	1.053
R_1, wR_2 indices [$I>2\sigma(I)$]	0.0301, 0.0515	0.0238, 0.0480
R_1, wR_2 indices (all data)	0.0382, 0.0533	0.0292, 0.0493
Largest diff. peak and hole /*e*. Å^{-3}	1.364 and -1.348	0.874 and -1.021

Table S2. Selected bond lengths (Å) and bond angles (°) for **Ir1**

Bond	Dist. (Å)	Bond	Dist. (Å)
Ir(1)-C(1)	2.002(3)	Ir(1)-C(12)	2.014(3)
Ir(1)-N(1)	2.038(3)	Ir(1)-N(2)	2.045(3)
Ir(1)-N(3)	2.141(2)	Ir(1)-N(4)	2.211(2)
Angle	(°)	Angle	(°)
C(1)-Ir(1)-C(12)	84.15(11)	C(1)-Ir(1)-N(1)	80.52(12)
C(12)-Ir(1)-N(1)	95.81(11)	C(1)-Ir(1)-N(2)	98.30(12)
C(12)-Ir(1)-N(2)	80.18(12)	N(1)-Ir(1)-N(2)	175.93(10)
C(1)-Ir(1)-N(3)	94.83(10)	C(12)-Ir(1)-N(3)	175.35(12)
N(1)-Ir(1)-N(3)	88.48(9)	N(2)-Ir(1)-N(3)	95.51(9)
C(1)-Ir(1)-N(4)	170.30(10)	C(12)-Ir(1)-N(4)	105.47(10)
N(1)-Ir(1)-N(4)	96.97(9)	N(2)-Ir(1)-N(4)	84.83(9)
N(3)-Ir(1)-N(4)	75.68(8)		

Table S3. Selected bond lengths (Å) and bond angles (°) for **Ir2**

Bond	Dist. (Å)	Bond	Dist. (Å)
Ir(1)-C(12)	2.007(2)	Ir(1)-C(1)	2.017(2)
Ir(1)-N(2)	2.041(2)	Ir(1)-N(1)	2.053(2)
Ir(1)-N(3)	2.143(2)	Ir(1)-N(4)	2.2142(19)
Angle	(°)	Angle	(°)
C(12)-Ir(1)-C(1)	84.56(10)	C(12)-Ir(1)-N(2)	80.59(10)
C(1)-Ir(1)-N(2)	95.96(9)	C(12)-Ir(1)-N(1)	98.11(10)
C(1)-Ir(1)-N(1)	80.19(10)	N(2)-Ir(1)-N(1)	176.05(8)
C(12)-Ir(1)-N(3)	95.72(9)	C(1)-Ir(1)-N(3)	175.20(9)
N(2)-Ir(1)-N(3)	88.82(8)	N(1)-Ir(1)-N(3)	95.03(8)
C(12)-Ir(1)-N(4)	171.10(9)	C(1)-Ir(1)-N(4)	104.30(8)
N(2)-Ir(1)-N(4)	97.35(8)	N(1)-Ir(1)-N(4)	84.49(7)
N(3)-Ir(1)-N(4)	75.53(7)		

Table S4. Photophysical data for **Ir1-Ir2** at 298 K

Complexes	The monomer states (CH_3CN)				The aggregate states (H_2O)			
	λ_{abs}[a] (ε[b])	λ_{em}[c] (φ_{em}[d])	τ/ns[e] ($\varphi/^1O_2$[f])	δ/GM[g]	λ_{abs}[a] (ε[b])	λ_{em}[c] (φ_{em}[d])	τ/ns[e] ($\varphi/^1O_2$[f])	δ/GM[g]
Ir1	374 (2.2)	538 (0.3%)	613 (0.29)	n.d.[h]	378 (2.4)	544 (6.8%)	4242 (0.65)	114.4
Ir2	363 (1.1)	544 (0.8%)	159 (0.51)	n.d.	378 (1.4)	556 (15.1%)	1654 (0.50)	97.1

[a] λ_{abs} maximum values of the one-photon absorption in nm. [b] Extinction coefficient in 1×10^4 $M^{-1}\times cm^{-1}$. [c] λ_{em} maximum values of the one-photon emission spectra in nm. [d] Phosphorescent quantum yields. [e] Phosphorescence lifetime. [f] $^1O_2$ quantum yields. The $^1O_2$ quantum yield of the reference $[Ru(bpy)_3]^{2+}$ was in 0.56 in aerated CH_3CN and 0.18 in aerated water. [g] Two-photon absorption cross section at 740 nm. [h] Not determined.