Supporting Information

Mitochondria-specific phosphorescent imaging and tracking in living cells with an AIPE-active iridium(III) complex

Yu Chen,‡[a] Liping Qiao,‡[a] Bole Yu,[a] Guanying Li,[a] Chunyuan Liu,[b] Liangnian Ji[a] and Hui Chao*[a]

[a] *MOE Key Laboratory of Bioinorganic and Synthetic Chemistry, State Key Laboratory of Optoelectronic Materials and Technologies, School of Chemistry and Chemical Engineering, Sun Yat-Sen University, Guangzhou 510275, P. R. China. E-mail: ceschh@mail.sysu.edu.cn; Fax: 86-20-84112245; Tel: 86-20-84110613*

[b] *Department of Chemistry, Jinan University, Guangzhou 510632, P. R. China*

Experimental Procedures

Instruments

Microanalyses (C, H, and N) were performed using a Vario EL elemental analyzer. Electrospray mass spectra (ESI-MS) were recorded on a LCQ system (Finnigan MAT, USA). Expected and measured isotope distributions were compared. The ^{1}H NMR spectra were recorded on a Varian Mercury-Plus 300 spectrometer (300 MHz). All chemical shifts are reported relative to tetramethylsilane (TMS). UV-Vis spectra were recorded on a Varian Cary 300 spectrophotometer. Steady-state emission experiments at room temperature were measured on an Edinburgh instrument FLSP-920 spectrometer with Xe lamp as excitation source. Luminescence lifetime studies were performed with an Edinburgh FLSP-920 photo-counting system with a hydrogen-filled lamp as the excitation source.

Materials

All reagents were purchased from commercial sources. All buffer components were of biological grade and used as received. $IrCl_3 \cdot 3H_2O$, 2-(2-pyridyl)benzothiophene (btp), 2-ethoxyethanol, 9,10-phenanthrenequinone, selenium dioxide and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were purchased from Sigma company and used without further purification. 1,10-phenanthroline-5,6-dione were prepared according to previously reported methods.[1] The complexes were dissolved in DMSO preceding the experiments; the calculated quantities of the drug solutions were then added to the appropriate medium to yield a final DMSO concentration of less than 1% (v/v).

Synthesis of 1,10-phenanthrolineselenazole (PhenSe)

A mixture of 1,10-phenanthroline-5,6-dione (2.1 g, 10 mmol), selenium dioxide (2.22 g, 20 mmol), NH_4Ac (7.7 g, 100 mmol) and anhydrous acetic acid (100 mL) was heated at 140 °C for 6 h. The mixture was allowed to cool to room temperature and then filtered. Subsequently, 600 mL of iced water was added to the filtrate, and the mixture was stirred for another 0.5 h. The precipitate which formed was collected by filtration and washed with water and

ether, and then dried under vacuum. Yield 2.31 g, 81%. The product was used directly in the next step without further purification.

Synthesis of iridium(III) complex Ir1

The complexes **Ir1** was synthesized similarly to previously reported methods.[2] Briefly, a mixture of 2-ethoxyethanol and water (3:1, v/v) was added to a flask containing $IrCl_3 \cdot 3H_2O$ (0.353 g, 1.0 mmol) and the btp ligand (0.528 g, 2.5 mmol). The mixture was refluxed for 24 h. After cooling, the yellow solid precipitate was filtered to give crude cyclometalated Ir(III) chloro-bridged dimmer.[3] The chloro-bridged dimer (0.259 g, 0.20 mmol) and PhenSe (0.114 g, 0.4 mmol) were placed in the 100 mL round bottomed flask with 48 mL methanol and dichloromethane (2:1, v/v). The mixture was heated at 65 °C for 6 h under Ar. The solution was filtered and the precipitate was washed three times (2 mL) with methanol. The filtrate and washings were combined and reduced by evaporation to a volume of 1 mL. After being cooled to room temperature, a bright yellow precipitate was obtained by the addition of a methanol NH_4PF_6 solution. The product was purified by column chromatography on alumina using dichloromethane-acetone (5:1, v/v) as the eluent. Yield: 0.275 g, 66%. Anal. Calcd. for $C_{38}H_{22}N_6S_2IrSePF_6$: C, 43.76; H, 2.13; N, 8.06. Found: C, 43.62; H, 2.24; N, 8.19. ES-MS [CH_3OH, m/z]: 899.2 ([M-PF_6^-]$^+$). ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.24 (d, J = 3 Hz, 2H), 8.20 (d, J = 3 Hz, 2H), 8.08 (t, J = 3 Hz, 2H), 8.02-7.93 (m, 6H), 7.70 (d, J = 3 Hz, 2H), 7.26 (t, J = 3 Hz, 2H), 6.98 (t, J = 3 Hz, 2H), 6.93 (t, J = 3 Hz, 2H), 6.00 (d, J = 6 Hz, 2H).

X-ray Crystallography

X-ray diffraction measurements were performed on Rigaku R-AXIS SPIDER Image Plate diffractometer with graphite-monochromated Mo Kα radiation (λ = 0.71073 Å). An absorption correction was applied with the SADABS program.[4] The structure solution and full-matrix least-squares refinement based on F2 for **Ir1** were performed with the SHELXS 97 and SHELXL 97 program packages, respectively.[5] Anisotropic thermal parameters were applied to all non-hydrogen atoms. All hydrogen atoms were included in calculated positions and refined with isotropic thermal parameters riding on those of the parent atoms. Crystal parameters and details of the data collection and refinement are given in Table S1. Selected bond lengths (Å) and bond angles (°) are given in Table S2. Detailed crystallographic data for the crystal structural analysis have been deposited with the Cambridge Crystallographic Data Centre, CCDC 954487 (**Ir1**) contains the supplementary crystallographic data for the present paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

Cell culture conditions and *in vitro* cytotoxicity

HeLa cells, LO2 cells and FLS cells were maintained as monolayer cultures in DMEM supplemented with 10% Fetal Bovine Serum (FBS). All cells culture was at 37 °C under 5% CO_2.

Exponentially growing HeLa and LO2 cells were seeded in triplicate into 96-well plates at 1×10^4 cells/well. After incubation for 24 h, the cells were treated with increasing concentrations of the tested complexes for various hours. To stain the viable cells, 20 μL of MTT (5 mg/mL) was added to each well. The cells were then incubated for 4 h at

37 °C. After the media had been carefully aspirated without disturbing the formed formazan crystals, the dye was dissolved in 200 μL DMSO. The absorbance of the samples was measured at 570 nm in an ELISA reader (BioTek Instruments, Winooski, VT). The following formula was used to calculate the viability of cell growth:[6]

Viability (%) = (mean of Absorbance value of treatment group / mean Absorbance value of control) × 100

Inductively coupled plasma mass spectrometry (ICP-MS)

Exponentially growing HeLa cells were harvested, and the resulting single-cell suspension was plated in 100 mm tissue culture plates (Costar). After 24 h at 37 °C, the cells were incubated with 5 μM **Ir1** for 1 h at 37 °C in either media with serum or media without serum. The cells were rinsed with PBS, detached with trypsin, counted and divided into three portions. In the first portion, the nuclei were extracted using a nucleus extraction kit (Pierce, Thermo) following the manufacturer's protocol; in the second portion, the cytoplasm was extracted using a cytoplasm extraction kit (Pierce, Thermo); and in the third portion, the mitochondrial was extracted using a mitochondrial extraction kit (Pierce, Thermo). The samples were digested with 60% HNO_3 at RT for one day. Each sample was diluted with MilliQ H_2O to obtain 2% HNO_3 sample solutions.[7] The iridium content was measured using an Agilent inductively coupled plasma mass spectrometry (ICP-MS) 7700x. Data were reported as the means ± standard deviation (n = 3).

Confocal luminescence imaging

For living cell imaging, cells were plated on 35 mm glass-bottom dishes (Corning) and allowed to adhere for 24 h. The cells were incubated with 5 μM **Ir1** for 1 h at 37 °C, followed by 50 nm MTG for another 20 min. When necessary, 10 μM CCCP was applied 1 h before **Ir1** treatment and kept in the medium during **Ir1** treatment until the cells were analyzed. Cell imaging was then carried out after washing the cells with PBS.

For fixed cell imaging, cells were detached from the culture and were fixed with 4% para-formaldehyde at room temperature for 20 min. After washing with PBS, the fixed cells were incubated with 5 μm **Ir1** in DMSO/PBS (pH 7.4, 1:99, v/v) for 1 h at 37 °C. Cell imaging was then carried out after washing the cells with PBS.

Reference

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6. P. Y. Zhang, J. Q. Wang, H. Y. Huang, L. P. Qiao, L. N. Ji and H. Chao, *Dalton Trans.*, 2013, **42**, 8907.
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Table S1 Crystal data and details of the structure determination for complex **Ir1**

Crystal data	**Ir1**
Formula	$C_{39}H_{24}Cl_2F_6IrN_6S_2PSe$
Fw	1127.79
T/ K	153(2)
Crystal system	Triclinic
Space group	$P\ \bar{1}$
a/Å	9.1798(3)
b/Å	12.2585(3)
c/Å	17.5678(6)
α/°	74.243(3)
β/°	81.025(3)
γ/°	85.881(2)
V/Å^3	1878.43(10)
Z	2
F(000)	1092
D_{calcd} /g cm^{-3}	1.994
μ /mm^{-1}	11.366
Reflns collected	5582
Unique reflns, R_{int}	5273, 0.0821
S on F^2	1.072
$R_1{}^a(I>2\sigma(I))$	0.0535
$wR_2{}^b$(all data)	0.1480

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

Ir1-C19	2.021(11)	Ir1-N4	2.103(9)
Ir1-C32	2.025(11)	Ir1-N5	2.070(9)
Ir1-N3	2.124(8)	Ir1-N6	2.066(9)
C19-Ir1-C32	88.5(4)	N3-Ir1-N4	78.3(3)
C19-Ir1-N6	98.6(4)	N3-Ir1-N5	93.9(3)
C32-Ir1-N6	79.8(4)	C19-Ir1-N3	171.7(4)
C19-Ir1-N4	95.5(4)	C32-Ir1-N3	98.3(4)
C32-Ir1-N4	172.4(4)	N4-Ir1-N6	93.1(3)
N6-Ir1-N5	178.4(3)	N3-Ir1-N6	87.3(3)
C19-Ir1-N5	80.3(4)	N4-Ir1-N5	88.1(3)
C32-Ir1-N5	98.9(4)		

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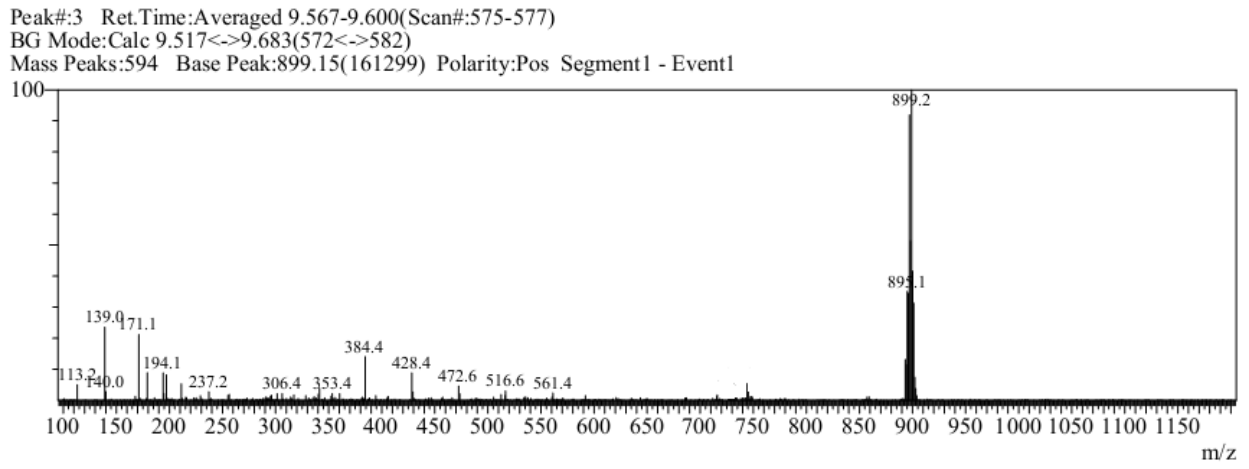

Fig. S1. ESI-MS spectrum of **Ir1** in CH_2Cl_2 solution.

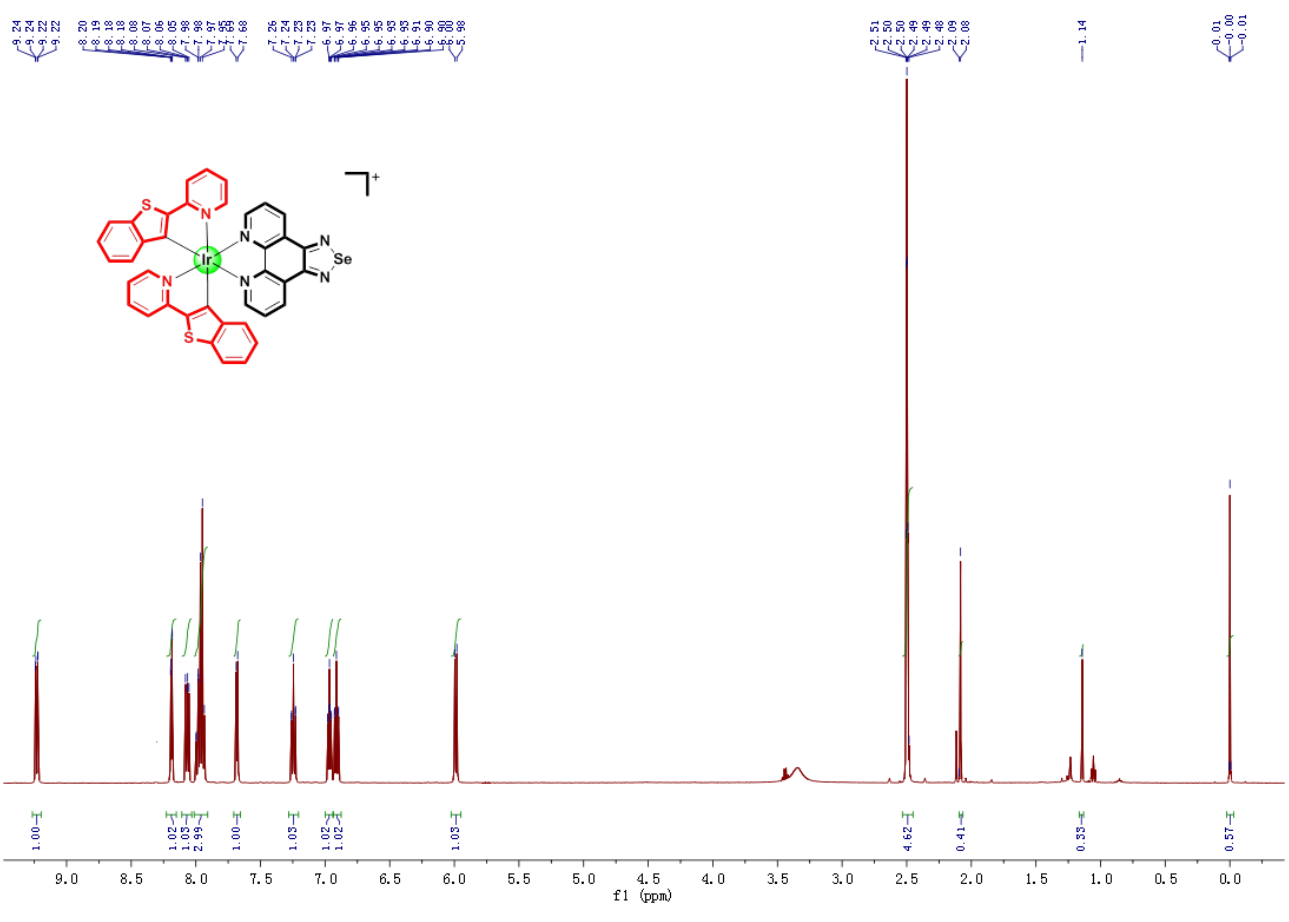

Fig. S2. ^{1}H NMR spectrum of **Ir1** in DMSO-d_6.

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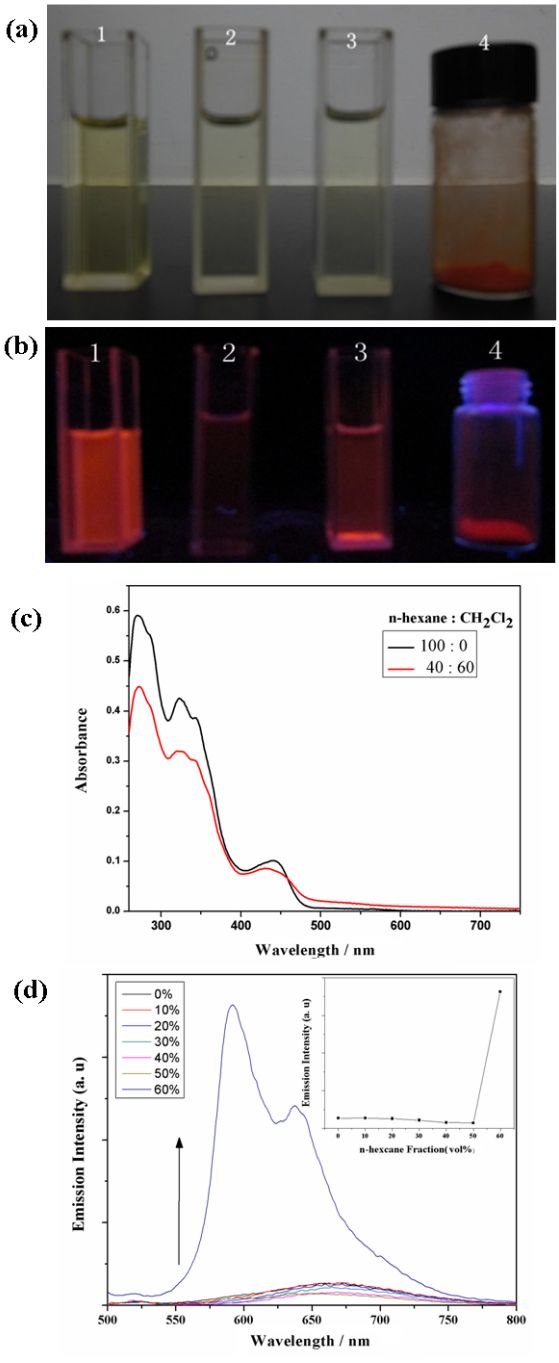

Fig. S3. (*a, b*) Images of the brightfield and room temperature luminescent emissions; lane 1, $[Ru(bpy)_3]^{2+}$ (10 μM in CH_2Cl_2); lane 2, **Ir1** (10 μM in CH_2Cl_2); lane 3, **Ir1** (10 μM in hexane/CH_2Cl_2 mixture (3/2, v/v)); lane 4, **Ir1** in solid state (λ_{ex} = 365 nm); (*c*) UV–Vis absorption spectra of **Ir1** in pure CH_2Cl_2 (black line) and hexane/CH_2Cl_2 mixture (3/2, v/v) (red line), the final concentration was kept unchanged at 10 μM; (*d*) Emission spectra of **Ir1** in the different hexane/CH_2Cl_2 fraction solutions. The inset shows the change in the emission intensity of complex versus hexane fractions in CH_2Cl_2. The final concentration was kept unchanged at 10 μM.

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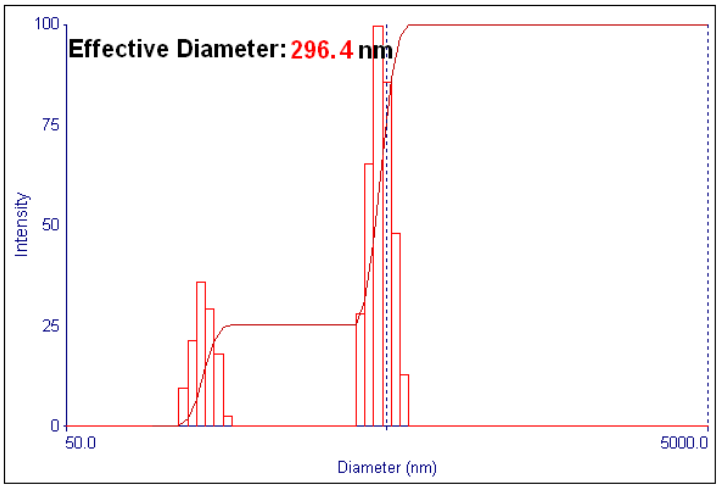

Fig. S4. Hydrodynamic diameter distributions f (D_h) of particles of **Ir1** in hexane/CH_2Cl_2 mixture (3/2, v/v) at a scattering angle of 90° at 25 °C.

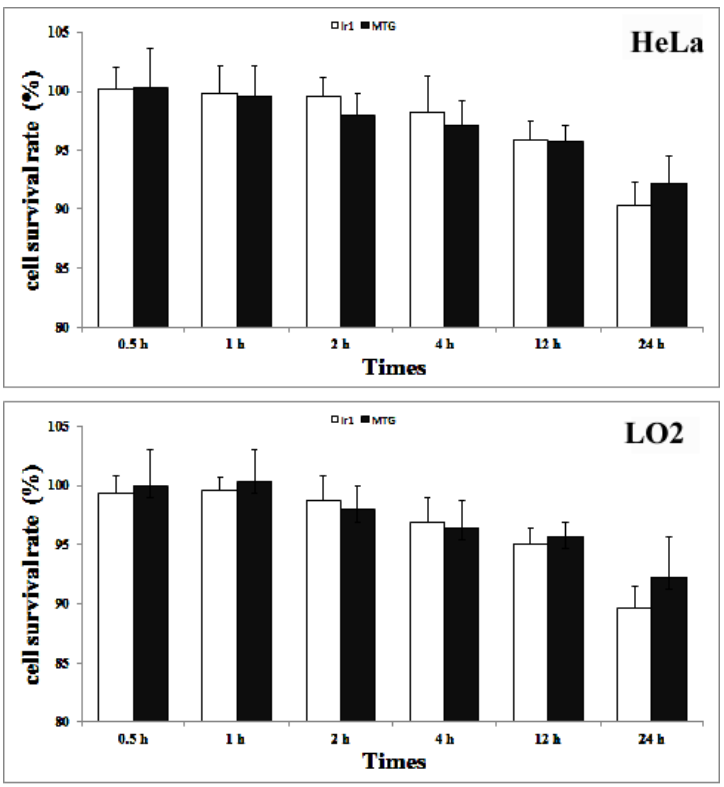

Fig. S5. *In vitro* cell viability of HeLa (top) and LO2 (bottom) incubated with 5 μM **Ir1** or 50 nM MTG at 37 °C for different time, respectively.

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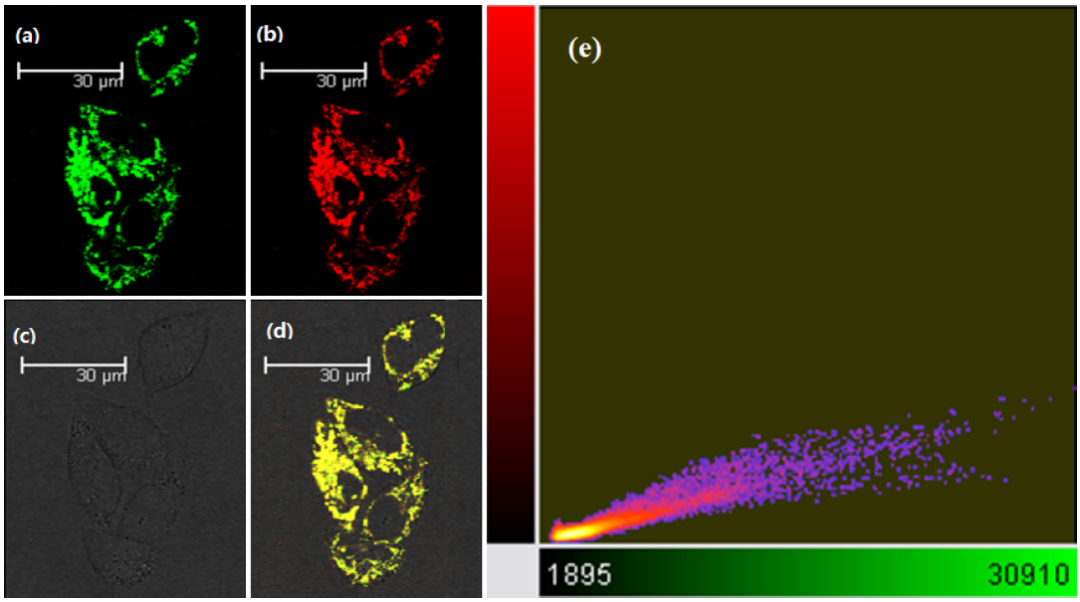

Fig. S6. Confocal fluorescence images, brightfield imagings and their overlay of living HeLa cells incubated with 5 μM **Ir1** for 1 h at 37 °C, followed by 50 nM MTG. (a) Fluorescence image of MTG, λ_{ex} = 490 nm, λ_{em} = 520 ± 20 nm. (b) Confocal phosphorescence images of **Ir1**, λ_{ex} = 590 nm, λ_{em} = 600 ± 20 nm. (c) Bright-field image of cells. (d) Overlay image of (a), (b) and (c). (e) Colocalization coefficient of **Ir1** and MTG is 0.94.

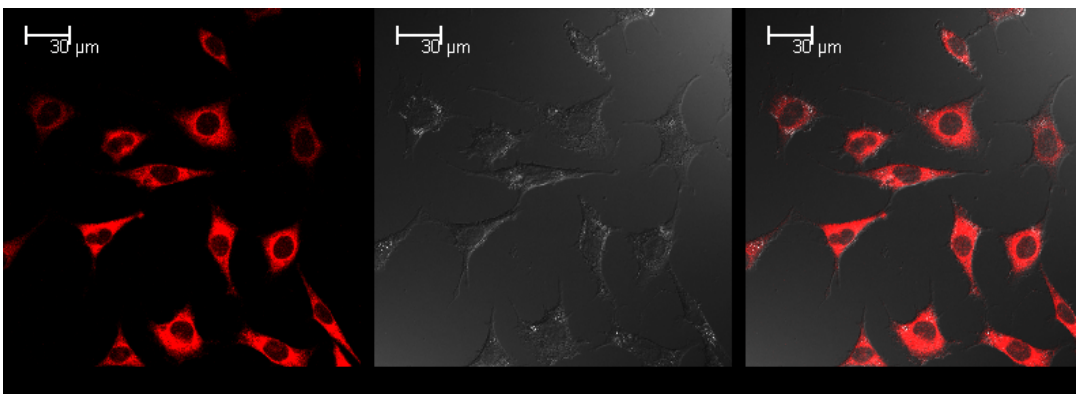

Fig. S7. Confocal luminescence images, brightfield images and their overlay of living FLS cells incubated with 5 μM **Ir1** in DMSO/PBS (pH 7.4, 1:99, v/v) for 1 h at 37 °C.

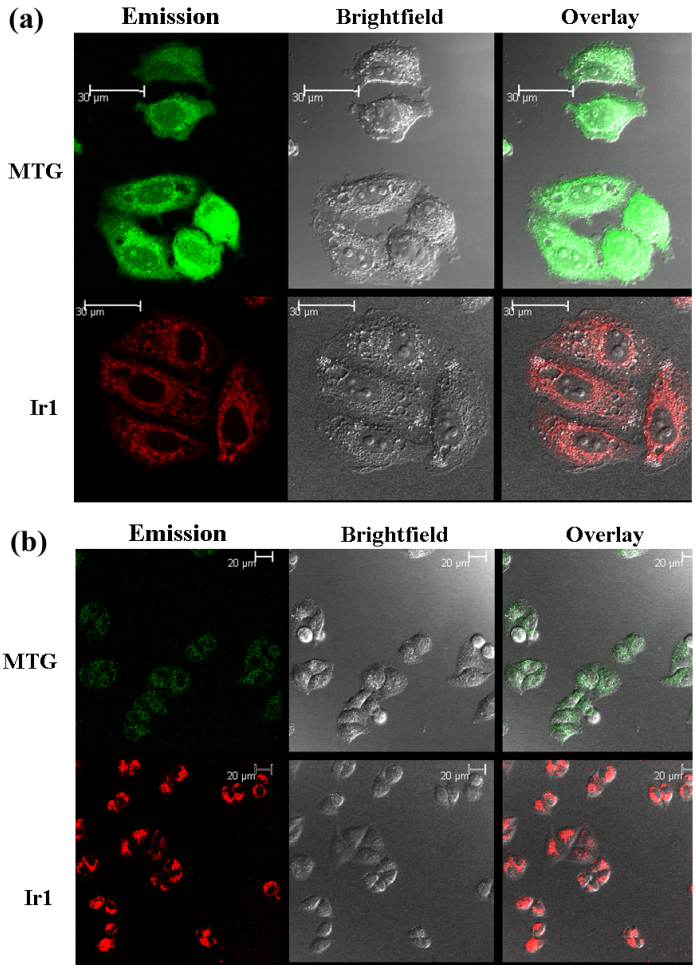

Fig. S8. Confocal luminescence images, brightfield images and their overlay of (*a*) fixed HeLa cells incubated with 50 nM MTG for 20 min or 5 μM **Ir1** for 1 h at 37 °C; (*b*) CCCP (10 μM) treated HeLa cells stained with 50 nM MTG for 20 min or 5 μM **Ir1** for 1 h at 37 °C. Excitation wavelength: 458 nm for **Ir1** and 488 nm for MTG; emission filter: 600 ± 20 nm for **Ir1** and 520 ± 20 nm for MTG.

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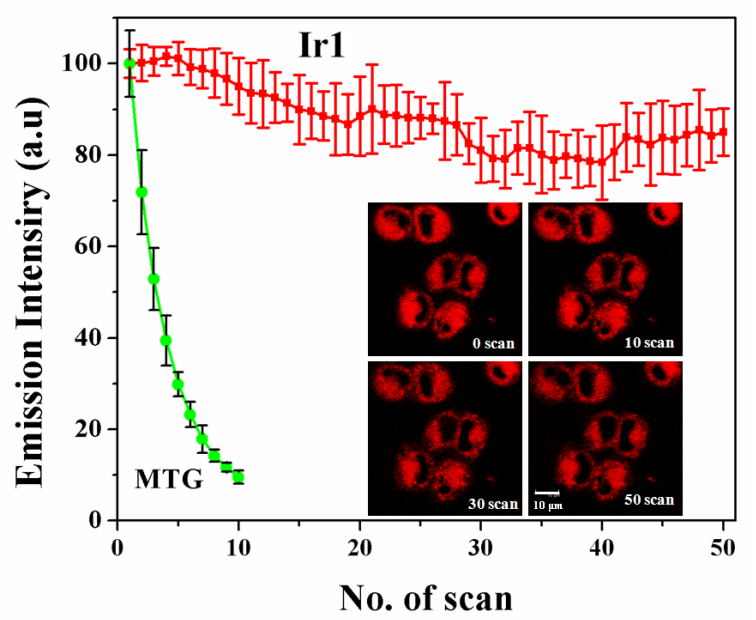

Fig. S9. Emission intensity (a.u.) of MTG (green dot) and **Ir1** (red dot) with increasing number of scans. Inset: phosphorescent images of living HeLa cells stained with **Ir1** (5 μM) with increasing number of scans (1−50 scans; the number of scans shown in lower right corner). Excitation wavelength: 458 nm for **Ir1** and 488 nm for MTG; emission filter: 520 ± 20 nm (for MTG) and 600 ± 20 nm (for **Ir1**); irradiation time: 7.75 s/scan.