Supporting Information

# Biscylometalated iridium(III) complexes target mitochondria or lysosomes by regulating the lipophilicity of main ligands

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# Materials and methods

#### *Materials and general instruments*

All reagents were purchased from commercial sources and used without further purification unless otherwise specified. The compounds 1,2-diphenyl-1*H*-imidazo[4,5-*f*][1,10]phenanthroline (**L1**),<sup>1</sup> 2-phenyl-1*H*-imidazo[4,5-*f*][1,10]phenanthroline (**L2**),<sup>2</sup> 1*H*-imidazo[4,5-*f*][1,10]phenanthroline (**L3**),<sup>3</sup> and 4-(4-(pyridin-2-yl)benzyl)morpholine<sup>4</sup> were prepared according to literature methods. IrCl<sub>3</sub>, 1,10-phenanthroline (**L4**), 2,2'-bipyridine (**L5**), and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were purchased from sigma and were used without further purification. The commercially available mitochondrial imaging agents MitoTracker<sup>®</sup> Red (MTR) and LysoTracker<sup>®</sup> Red (LTR) were purchased from Invitrogen. The complexes were dissolved in DMSO preceding the experiments, the calculated quantities of the complex solutions were then added to the appropriate medium to yield a final DMSO concentration of less than 1% (v/v).

Microanalysis (C, H and N) was carried out with a Perkin-Elmer 240Q elemental analyzer. Electrospray ionization mass spectra (ESI-MS) were recorded on an LCQ system (Finnigan MAT, USA). <sup>1</sup>H NMR spectra were recorded on a nuclear magnetic resonance spectrometer (Mercury-Plus 300, Varian, USA) at 25 °C. All chemical shifts are given relative to tetramethylsilane (TMS). The UV-vis spectra were recorded on a Perkin-Elmer Lambda 850 spectrophotometer. Emission spectra were recorded on a Perkin-Elmer LS 55 spectrofluorophotometer at room temperature (25 °C). Time-resolved emission measurements were conducted on an FLS 920 combined fluorescence-lifetime and steady-state spectrometer in aerated CH<sub>3</sub>CN. Quantum yields of luminescence at room temperature (25 °C) were calculated according to literature procedures, by using an aerated aqueous solution of [Ru(bpy)<sub>3</sub>]<sup>2+</sup> ( $\varphi = 0.028$ ) as the reference emitter.<sup>5</sup> All date were processed by using the Origin 8 software package.

### Synthesis of iridium complexes (Ir1-Ir5)

The complexes **Ir1-Ir5** were synthesized in a similar manner. Briefly, a mixture of 2ethoxyethanol and water (3:1, v/v) was added to a flask containing IrCl<sub>3</sub> (1.0 mmol) and 4(4-(pyridin-2-yl)benzyl)morpholine (pbm, 2.1 mmol). The mixture was refluxed for 24 h. After cooling, the solvent was evaporated under reduced pressure to give crude cyclometalated Ir(III) chloro-bridged dimer, which can be utilized for the next step without further purification. The chloro-bridged dimer (0.1 mmol) and N^N ligand (L1-L5, 0.2 mmol) were placed in a 50 mL round bottomed flask with 20 mL of methanol and  $CH_2Cl_2$  (1:1, v/v). The mixture was refluxed for 12 h under argon. After 12 h reflux, the solvent was evaporated under reduced pressure, and the crude product was purified by column chromatography on alumina with  $CH_2Cl_2$ /ethanol to get iridium complexes (yields = 50-60%). The complexes were further characterized by elemental analysis, <sup>1</sup>H NMR and ESI-MS measurements.

 $[Ir(pbm)_2L1]Cl (Ir1)$ : Anal. Calcd. for C<sub>57</sub>H<sub>50</sub>ClIrN<sub>8</sub>O<sub>2</sub>: C, 61.86; H, 4.55; N, 10.12. Found: C, 62.19; H, 4.87; N, 10.28. <sup>1</sup>H NMR (300 MHz, d<sub>6</sub>-DMSO)  $\delta$  9.28 (d, *J* = 9 Hz, 1H), 8.13-8.24 (m, 4H), 8.07 (s, 1H), 7.87 (d, *J* = 6 Hz, 4H), 7.73 (d, *J* = 15 Hz, 6H), 7.59 (d, *J* = 6 Hz, 2H), 7.42 (d, *J* = 9 Hz, 6H), 6.98-6.87 (m, 4H), 6.24 (d, *J* = 9 Hz, 2H), 3.42 (s, 8H), 3.18 (t, *J* = 15 Hz, 4H), 2.15 (s, 8H). ESI-MS: m/z = 536.4 [M-Cl+H]<sup>2+</sup>, 1071.5 [M-Cl]<sup>+</sup>.

 $[Ir(pbm)_2L2]Cl (Ir2)$ : Anal. Calcd. for C<sub>51</sub>H<sub>46</sub>ClIrN<sub>8</sub>O<sub>2</sub>: C, 59.43; H, 4.50; N, 10.87. Found: C, 59.68; H, 4.71; N, 11.03. <sup>1</sup>H NMR (300 MHz, d<sub>6</sub>-DMSO)  $\delta$  9.01 (d, *J* = 9 Hz, 2H), 8.34 (d, *J* = 6 Hz, 2H), 8.18 (d, *J* = 9 Hz, 2H), 7.96 (s, 2H), 7.83 (t, *J* = 6 Hz, 6H), 7.42 (t, *J* = 6 Hz, 4H), 7.32 (s, 1H), 6.95 (t, *J* = 6 Hz, 2H), 6.89 (d, *J* = 9 Hz, 2H), 6.28 (s, 2H), 3.43 (s, 8H), 3.20 (d, *J* = 12 Hz, 4H), 2.17 (s, 8H). ESI-MS: m/z = 498.4 [M-Cl+H]<sup>2+</sup>, 995.6 [M-Cl]<sup>+</sup>.

 $[Ir(pbm)_2L3]Cl (Ir3)$ : Anal. Calcd. for C<sub>45</sub>H<sub>42</sub>ClIrN<sub>8</sub>O<sub>2</sub>: C, 56.62; H, 4.43; N, 11.74. Found: C, 56.94; H, 4.75; N, 11.91. <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$  9.39 (d, *J* = 9 Hz, 2H), 8.50 (s, 1H), 8.09 (d, *J* = 3 Hz, 2H), 7.90 (d, *J* = 9 Hz, 2H), 7.66 (q, *J* = 9 Hz, 6H), 7.35 (d, *J* = 6 Hz, 2H), 7.04 (d, *J* = 9 Hz, 2H), 6.80 (t, *J* = 6 Hz, 2H), 6.35 (s, 2H), 3.63 (s, 8H), 3.36 (d, *J* = 9 Hz, 4H), 2.31 (s, 8H). ESI-MS: m/z = 460.3 [M-Cl+H]<sup>2+</sup>, 919.5 [M-Cl]<sup>+</sup>.

**[Ir(pbm)<sub>2</sub>L4]Cl (Ir4)**: Anal. Calcd. for C<sub>44</sub>H<sub>42</sub>ClIrN<sub>6</sub>O<sub>2</sub>: C, 57.79; H, 4.63; N, 9.19. Found: C, 57.93; H, 4.85; N, 9.31. <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ 8.88 (s, 2H), 8.38 (s, 2H), 8.30 (s, 2H), 7.91 (s, 4H), 7.73 (s, 2H), 7.66 (s, 2H), 7.32 (s, 2H), 7.09 (s, 2H), 6.91 (s, 2H), 6.33 (s, 2H), 3.64 (s, 8H), 3.43 (s, 4H), 2.40 (s, 8H). ESI-MS: m/z = 440.2 [M-Cl+H]<sup>2+</sup>, 879.5 [M-Cl]<sup>+</sup>.

[**Ir(pbm)**<sub>2</sub>**L5**]**Cl (Ir5)**: Anal. Calcd. for C<sub>42</sub>H<sub>42</sub>ClIrN<sub>6</sub>O<sub>2</sub>: C, 56.65; H, 4.75; N, 9.44. Found: C, 56.81; H, 5.01; N, 9.53. <sup>1</sup>H NMR (300 MHz, d<sub>6</sub>-DMSO)  $\delta$  8.86 (d, *J* = 9 Hz, 2H), 8.19 (dd, *J* = 9 Hz, 4H), 7.79-7.95 (m, 6H), 7.65 (t, *J* = 6 Hz, 2H), 7.56 (d, *J* = 6 Hz, 2H), 7.10 (t, *J* = 6 Hz, 2H), 6.85 (d, *J* = 9 Hz, 2H), 6.16 (s, 2H), 3.41 (s, 8H), 3.15 (t, *J* = 15 Hz, 4H), 2.13 (s, 8H). ESI-MS: m/z = 428.1 [M-Cl+H]<sup>2+</sup>, 855.5 [M-Cl]<sup>+</sup>.

# Hydrophilicity

A shake-flask ultraviolet spectrophotometry method was used to determine the *n*-octanol/water partition coefficients of **Ir1-Ir5**. First, the mixed solution of 50 mL of *n*-octanol and 50 mL of water was left shaking at 37 °C for 48 h. The **Ir1-Ir5** standard solutions ( $C = 10 \mu$ M) were prepared using the water phase and organic phase. Second, the **Ir1-Ir5** detection solutions ( $C = 10 \mu$ M) were prepared with mixed solvent containing 25 mL of *n*-octanol and 25 mL of water and shaking for 48 h. Third, the concentration of **Ir1-Ir5** in the water phase ( $C_w$ ) and organic phase ( $C_o$ ) of the detecting solution were determined separately using ultraviolet spectrophotometry. The *n*-octanol/water partition coefficient, namely, log *P*, is calculated by the following equation (1):

$$\log P = \log \left( C_o / C_w \right) \tag{1}$$

## Cell culture conditions and cell viability assay

HeLa was maintained as monolayer cultures in DMEM supplemented with 10 % Fetal Bovine Serum (FBS) and was cultured at 37 °C under 5 % CO<sub>2</sub>.

Exponentially grown HeLa cells were seeded in triplicate into 96-well plates at  $1 \times 10^4$  cells/well. After incubation for 24 h, the cells were treated with the tested complexes of 5  $\mu$ M for 45 min, the media was replaced and the cells were further incubated of 6 h or 12 h. To stain the viable cells, 20  $\mu$ L 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 formazan crystals that had formed, the dye was dissolved in 200  $\mu$ L of DMSO. A Tecan Infinite M200 monochromator-based multifunction microplate reader

was used to measure the optical density of each well with background subtraction at 595 nm. The cell survival rate in the control wells without complexes was considered as 100 % cell survival.

### *Cellular imaging*

The cells were trypsinized, counted, and adjusted to  $1 \times 10^5$  cells mL<sup>-1</sup> and 1 mL was added to 35 mm<sup>2</sup> Petri dish (MatTek, USA). After 24 h at 37 °C, the cells were incubated with 5 µM complexes for 45 min at 37 °C. After removing the media and washing with PBS buffer three times to remove the remaining probes, the cells were further incubated with MTR (or LTR) for another 30 min. After washing with fresh PBS three times, the cells were imaged on a Zeiss LSM 710 NLO confocal microscope (63×/NA 1.4 oil immersion objective). The incubated cells were excited at 405 nm (for Ir1-Ir5) or 543 nm (for MTR or LTR), and the emission signal was collected at 570 ± 20 nm (for Ir1-Ir5) or  $640 \pm 20$  nm (for MTR or LTR).

### Cellular uptake mechanism

After pre-treating with inhibitors and **Ir1-Ir5**, cells were then image by CLSM. The excitation wavelength for **Ir1-Ir5** was 405 nm and emission filter: 550-590 nm. For normalized incubation, HeLa cells were treated with 5  $\mu$ M complexes at 37 °C for 45 min. For low temperature inhibition, the cells were incubated with 5  $\mu$ M complexes at 4 °C for 45 min.

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Scheme S1 Synthetic route to Ir1-Ir5. (i)  $HOCH_2CH_2OCH_3-H_2O$  (3:1, v/v), IrCl<sub>3</sub>, reflux, 24 h; (ii)  $CH_3OH-CH_2Cl_2$  (1:1, v/v), reflux, 12 h.



Fig. S1 ESI-MS spectrum and <sup>1</sup>H NMR spectrum of Ir1.



Fig. S2 ESI-MS spectrum and <sup>1</sup>H NMR spectrum of Ir2.



Fig. S3 ESI-MS spectrum and <sup>1</sup>H NMR spectrum of Ir3.



Fig. S4 ESI-MS spectrum and <sup>1</sup>H NMR spectrum of Ir4.



Fig. S5 ESI-MS spectrum and <sup>1</sup>H NMR spectrum of Ir5.



**Fig. S6** Absorption spectra of **Ir1-Ir5** (10  $\mu$ M) in Britton-Robison buffer solution at pH 5.0.



Fig. S7 Emission spectra of Ir1-Ir5 (10  $\mu$ M) in Britton-Robison buffer solution at pH 5.0.



**Fig. S8** pH-sensitive emission spectra of **Ir1** (10  $\mu$ M) in Britton-Robison buffer solution. Inset: A plot of emission intensity of **Ir1** at 583 nm versus different pH values.



**Fig. S9** pH-sensitive emission spectra of **Ir2** (10  $\mu$ M) in Britton-Robison buffer solution. Inset: A plot of emission intensity of **Ir2** at 593 nm versus different pH values.



Fig. S10 pH-sensitive emission spectra of Ir3 (10  $\mu$ M) in Britton-Robison buffer solution. Inset: A plot of emission intensity of Ir3 at 581 nm versus different pH values.



**Fig. S11** pH-sensitive emission spectra of **Ir4** (10  $\mu$ M) in Britton-Robison buffer solution. Inset: A plot of emission intensity of **Ir4** at 574 nm versus different pH values.



Fig. S12 pH-sensitive emission spectra of Ir5 (10  $\mu$ M) in Britton-Robison buffer solution. Inset: A plot of emission intensity of Ir5 at 570 nm versus different pH values.



**Fig. S13** Flourescence (or phosphorescence) intensity decay of LTR, MTR, and **Ir1-Ir5** in water with irradiation by a 300 W iodine-tungsten lamp.  $I_0$  is the fluorescence (or phosphorescence) intensity before the irradiation. *I* is the fluorescence (or phosphorescence) intensity of the above compounds after a fixed time of irradiation.



Fig. S14 Viability of HeLa cells incubated with Ir1-Ir5 (5  $\mu$ M) for 6 h and 12 h.

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Complex	$\lambda_{ m abs}^{a}$	ε ε	$\lambda_{em}^{c}$	$\phi^d$	$\tau/ns^e$		
Ir1	378	8.91	566	0.166	47.6		
Ir2	380	9.32	559	0.254	126		
Ir3	381	7.86	567	0.183	43.1		
Ir4	408	3.52	556	0.458	112		
Ir5	408	3.51	556	0.291	104		
$^a$ $\lambda_{abs}$ values of the absorption in nm. $^b$ Extinction coefficient in 1×10^3 M^-1 cm^-1. $^c$ $\lambda_{em}$							
values of the emission spectra in nm. <sup>d</sup> Quantum yield . <sup>e</sup> Lifetime in CH <sub>3</sub> CN.							

**Table S1**Photophysical data for Ir1-Ir5 in Britton-Robison buffer solution at pH 5.0