Supporting Information

Tracking mitochondrial dynamics during apoptosis with phosphorescent fluorinated iridium(III) complexes

Chen Zhang,‡a Kangqiang Qiu,‡ab Chaofeng Liu,a Huaiyi Huang,a Thomas W. Rees,a Liangnian Ji,a Qianling Zhang,b and Hui Chao*ab

†MOE Key Laboratory of Bioinorganic and Synthetic Chemistry, School of Chemistry, Sun Yat-Sen University, Guangzhou, 510275, P. R. China
‡College of Chemistry and Environmental Engineering, Shenzhen University, Shenzhen, 518055, P. R. China.

‡ These authors contributed equally to this work.

Email: cescbh@mail.sysu.edu.cn (H. Chao); zhql@szu.edu.cn (Q. Zhang).
Table of Contents

Scheme S1 Synthetic route of Ir1-Ir4 .................................................................................. S2
Fig. S1 ES-MS spectrum of Ir1 ......................................................................................... S3
Fig. S2 ES-MS spectrum of Ir2 ......................................................................................... S4
Fig. S3 ES-MS spectrum of Ir3 ......................................................................................... S5
Fig. S4 ES-MS spectrum of Ir4 ......................................................................................... S6
Fig. S5 1H NMR spectrum of Ir1 .................................................................................... S7
Fig. S6 1H NMR spectrum of Ir2 .................................................................................... S8
Fig. S7 1H NMR spectrum of Ir3 .................................................................................... S9
Fig. S8 1H NMR spectrum of Ir4 ................................................................................... S10
Fig. S9 19F NMR spectrum of Ir1 .................................................................................. S11
Fig. S10 19F NMR spectrum of Ir2 ................................................................................. S12
Fig. S11 19F NMR spectrum of Ir3 ............................................................................... S13
Fig. S12 19F NMR spectrum of Ir4 ............................................................................... S14
Fig. S13 Photostability in buffer of Ir1-Ir4 ..................................................................... S15
Fig. S14 Real-time uptake monitoring of Ir1-Ir4 ............................................................ S16
Fig. S15 Colocalization imaging of LTR and Ir1-Ir4 ........................................................ S17
Fig. S16 Colocalization imaging of ERTR and Ir1-Ir4 ...................................................... S18
Fig. S17 Distribution analysis of Ir1-Ir4 in HeLa cells by ICP-MS ................................. S19
Fig. S18 Lipophilicity of Ir1-Ir4 ..................................................................................... S20
Fig. S19 Cellular uptake mechanisms of Ir1-Ir4 ............................................................ S21
Fig. S20 Photobleaching in HeLa cells ......................................................................... S22
Fig. S21 Real-time imaging of HeLa cells stained with Ir2 .............................................. S23
Fig. S22 Real-time imaging of HeLa cells stained with Ir3 .............................................. S24
Fig. S23 Real-time imaging of HeLa cells stained with Ir4 .............................................. S25
Table S1 Photophysical data for the complexes at 298 K ............................................. S26
Scheme S1. Synthetic route to Ir1-Ir4.
Fig. S1 ES-MS spectrum of complex Ir1.
**Fig. S2** ES-MS spectrum of complex Ir2.
**Fig. S3** ES-MS spectrum of complex Ir3.
**Fig. S4** ES-MS spectrum of complex Ir4.
Fig. S5 $^1$H NMR spectrum of complex Ir1 in $d_6$-DMSO.
Fig. S6 $^1$H NMR spectrum of complex Ir2 in $d_6$-DMSO.
Fig. S7 $^1$H NMR spectrum of complex $\text{Ir3}$ in $d_6$-DMSO.
Fig. S8 $^1$H NMR spectrum of complex Ir4 in $d_6$-DMSO.
Fig. S9 $^{19}$F NMR spectrum of complex $\text{Ir1}$ in $d_6$-DMSO.
Fig. S10 $^{19}$F NMR spectrum of complex Ir2 in $d_6$-DMSO.
Fig. S11 $^{19}$F NMR spectrum of complex Ir3 in $d_6$-DMSO.
Fig. S12 $^{19}$F NMR spectrum of complex Ir4 in $d_6$-DMSO.
Fig. S13 Stability of the phosphorescence intensity of Ir1-Ir4 in PBS under irradiation at 405 nm of 21.2 mW/cm².
Fig. S14 Real-time uptake monitoring of the complexes (10 μM) in HeLa cells at incubation times of 0-60 min. The complexes were excited at 405 nm. The phosphorescence was collected at 550 ± 20 nm. Scale: 20 μm.
Fig. S15 Confocal images of HeLa cells co-labeled with the complexes (10 μM, 1 h) and the commercial lysosomal imaging agent LTR (50 nM, 0.5 h). The complexes were excited at 405 nm. LTR was excited at 543 nm. The phosphorescence/fluorescence was collected at 550 ± 20 nm and 620 ± 20 nm for the complexes and LTR, respectively. BF: bright field. The 5th column was the Pearson’s correlation coefficient. Scale bar: 20 μm.
Fig. S16 Confocal images of HeLa cells co-labeled with the complexes (10 μM, 1 h) and the commercial ER imaging agent ERTR (1 μM, 0.5 h). The complexes were excited at 405 nm. ERTR was excited at 543 nm. The phosphorescence/fluorescence was collected at 550 ± 20 nm and 620 ± 20 nm for the complexes and ERTR, respectively. BF: bright field. The 5th column was the Pearson correlation coefficient. Scale bar: 20 μm.
**Fig. S17** Distribution analysis of Ir1-Ir4 in HeLa cells by ICP-MS.
Fig. S18 The log $P$ of Ir1-Ir4 (10 μM).
Confocal images of living HeLa cells incubated with 10 μM Ir1-Ir4 ($\lambda_{ex} = 405$ nm, $\lambda_{em} = 550 \pm 20$ nm) under different conditions. (a) The cells were incubated with 10 μM Ir1-Ir4 at 37 °C for 1 h. (b) The cells were incubated with 10 μM Ir1-Ir4 at 4 °C for 1 h. (c) The cells were pretreated with 50 mM 2-deoxy-D-glucose and 5 μM oligomycin in PBS for 1 h at 37 °C and then incubated with 10 μM Ir1-Ir4 at 37 °C for 1 h. (d and e) The cells were pretreated with endocytic inhibitors NH₄Cl (50 mM), and chloroquine (50 μM) respectively, and then incubated with 10 μM Ir1-Ir4 at 37 °C for 1 h. Scale bar: 20 μm.
**Fig. S20** Confocal images of **Ir1-Ir4** (10 µM, $\lambda_{ex} = 405$ nm, $\lambda_{em} = 550 \pm 20$ nm) and MTR (50 nM, $\lambda_{ex} = 543$ nm, $\lambda_{em} = 620 \pm 20$ nm) before and after photobleaching in HeLa cells. Scale bar: 20 µm.
**Fig. S21** Real-time imaging of HeLa cells stained with **Ir2** (10 μM) for 1 h at 37 °C, followed by treatment with 30 μM CCCP, with increasing scan time. Phosphorescence images of **Ir2** (upper panels), Brightfield images (lower panels). The complex was excited at 405 nm. The phosphorescence was collected at 550 ± 20 nm. Scale: 20 μm.
Fig. S22 Real-time imaging of HeLa cells stained with Ir3 (10 μM) for 1 h at 37 °C, followed by treatment with 30 μM CCCP, with increasing scan time. Phosphorescence images of Ir3 (upper panels), Brightfield images (lower panels). The complex was excited at 405 nm. The phosphorescence was collected at 550 ± 20 nm. Scale: 20 μm.
Fig. S23 Real-time imaging of HeLa cells stained with **Ir4** (10 μM) for 1 h at 37 °C, followed by treatment with 30 μM CCCP, with increasing scan time. Phosphorescence images of **Ir4** (upper panel), Brightfield images (lower panels). The complex was excited at 405 nm. The phosphorescence was collected at 550 ± 20 nm. Scale: 20 μm.
Table S1 Photophysical data for the complexes at 298 K.

<table>
<thead>
<tr>
<th>Complexes</th>
<th>$\lambda_{ab}$</th>
<th>$\varepsilon$</th>
<th>$\lambda_{em}$</th>
<th>$\varphi$</th>
<th>$t$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ir1</td>
<td>457</td>
<td>4.39</td>
<td>549</td>
<td>14.3</td>
<td>731</td>
</tr>
<tr>
<td>Ir2</td>
<td>463</td>
<td>7.41</td>
<td>551</td>
<td>12.7</td>
<td>655</td>
</tr>
<tr>
<td>Ir3</td>
<td>464</td>
<td>6.40</td>
<td>562</td>
<td>9.23</td>
<td>511</td>
</tr>
<tr>
<td>Ir4</td>
<td>468</td>
<td>7.70</td>
<td>569</td>
<td>5.56</td>
<td>400</td>
</tr>
</tbody>
</table>

$^a$ $\lambda_{ab}$ maximum values of the absorption spectra (nm). $^b$ Extinction coefficient in $(1 \times 10^3 \text{ M}^{-1} \text{ cm}^{-1})$. $^c$ $\lambda_{em}$ maximum values of the emission spectra (nm). $^d$ Phosphorescent quantum yield (%). $^e$ Phosphorescent life time (ns).