

# Supporting Information

*for*

## **Cyclometalated Iridium(III) Complex Nanoparticles for Mitochondria-Targeted Photodynamic Therapy**

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## Materials and Methods

### 1. Steady-state emission spectra

The steady-state emission spectra of Ir(tiq)<sub>2</sub>ppy were collected on a Horiba Jobin Yvon FluoroMax-4P fluorescence spectrometer. Ir(tiq)<sub>2</sub>ppy toluene solution was put in the customized deaerating device, and three freeze-pump-thaw cycles were conducted for strict deoxygenation. Phosphorescence quantum yield of Ir(tiq)<sub>2</sub>ppy in toluene was tested using Ir(piq)<sub>3</sub> toluene solution ( $\Phi = 0.60$ ) as the reference, under the excitation wavelength of 480 nm. To avoid reabsorption of the emitted radiation, the absorbance of each sample at the excitation wavelength was kept below 0.1. The quantum yield was calculated by the following formula:

$$\Phi_x = \Phi_{ST} \left( \frac{A_{ST}}{A_x} \right) \left( \frac{I_x}{I_{ST}} \right) \left( \frac{n_x}{n_{ST}} \right)^2 \quad (1)$$

$\Phi$  represents phosphorescence quantum yield,  $A$  represents the absorbance at the excitation wavelength,  $I$  represents the corrected integral area of the emission spectrum,  $n$  represents the refractive index of the solvent, X represents Ir(tiq)<sub>2</sub>ppy, ST represents the reference Ir(piq)<sub>3</sub>. The emission spectra at 77 K were detected in 2-methyltetrahydrofuran (MeTHF).

### 2. Phosphorescence lifetime testing

The phosphorescence decay curve was also collected on a Horiba Jobin Yvon FluoroMax-4P fluorescence spectrometer. Ir(tiq)<sub>2</sub>ppy was dissolved in toluene and put in the customized deaerating device. Three times freeze-pump-thaw was conducted for strict deoxidization. The phosphorescence lifetime was tested by the time-dependent single photon counting technology. The excitation light source was NanoLED at 389 nm, and the attenuation curve was analyzed using Horiba Jobin Yvon DAS6 software.

### 3. Quantum yield of singlet oxygen

Singlet oxygen steady emission spectrum was detected using the spectrophotometer of Fluorolog-3-iHR320, which was equipped with liquid nitrogen under the working conditions of InGaAs detector. Tetraphenylporphyrin (TPP) was used as reference ( $\Phi_{\Delta} = 0.70$ ), the quantum yield of singlet oxygen was calculated by the following formula:

$$\Phi_{\Delta} = \Phi_{\Delta}^{\text{std}} \frac{I}{I_{\text{std}}} \frac{1 - 10^{-A_{\text{std}}}}{1 - 10^{-A}} \quad (2)$$

$\Phi_{\Delta}^{\text{std}}$  (0.70) is the singlet oxygen quantum yield of TPP in air saturated toluene,  $I$  and  $I_{\text{std}}$  represent the singlet oxygen emission intensity of Ir(tiq)<sub>2</sub>ppy and TPP, respectively,  $A$  and  $A_{\text{std}}$  represent the absorbance of Ir(tiq)<sub>2</sub>ppy and TPP at the excitation wavelength.

#### 4. Size measurement and zeta potential using Dynamic Light Scattering (DLS)

The prepared Ir(tiq)<sub>2</sub>ppy NPs (11.41 µg/mL) was diluted to a concentration of 1.6 µg/mL with ultrapure water, then the diameter was measured. After this, the zeta potential was detected on a Malvern Zetasizer Nano ZS90.

#### 5. TEM characterizations

10 µL of the prepared Ir(tiq)<sub>2</sub>ppy NPs (11.41 µg/mL) solution was added onto a copper grid. After the evaporation of water, the images and corresponding electron diffraction patterns were observed by TEM.

#### 6. ROS detection

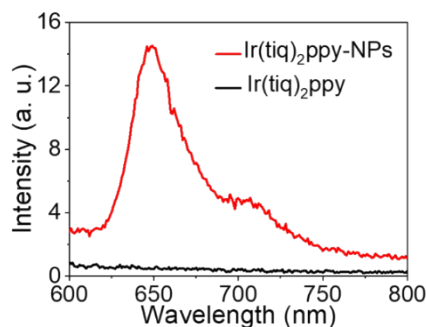
50 µL of 10 µg/mL Ir(tiq)<sub>2</sub>ppy NPs, 10 µg/mL Ir(tiq)<sub>2</sub>ppy and ultra-pure water were added in 96-well plate. After 50 µL of 2',7'-dichlorodihydrofluorescein diacetate (DCFH) solution was added, the fluorescence at 528 nm was measured immediately. Then, under irradiation of white light (5 mW/cm<sup>2</sup>), the fluorescence emission of 2',7'-dichlorofluorescein (DCF) at 528 nm (excited at 485 nm) was measured repeatedly with one-minute intervals. All experiments were conducted in triplicate and presented as mean ± SD.

MCF-7 cells were seeded in 96-well plate at the density of  $4 \times 10^4$  cells/mL and incubated overnight. 1.6 µg/mL of Ir(tiq)<sub>2</sub>ppy NPs and Ir(tiq)<sub>2</sub>ppy were added and incubated at 37°C for 24 h. After incubated with Hoechst 33342 (10 µg/mL) for 30 min and 3 times of PBS washing, DCFH-DA (10 µg/mL) was added for another 20 min. The cells of light groups were irradiated under white light (5 mW/cm<sup>2</sup>) for 10 min after another 3 times of PBS washing, while that of dark groups were kept in dark for 10 min after washing steps. The fluorescence of Hoechst 33342 (461 nm, excited at 350 nm)

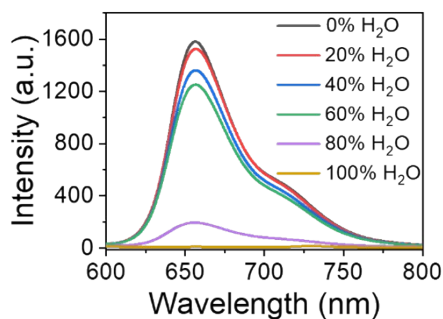
and DCF (525 nm, excited at 480 nm)) were detected by the microplate reader immediately. The ROS capacity was defined as the following equation, the results were shown in Fig. S4.

$$ROS\ capacity = \frac{F(DCF)}{F(Hoechst\ 33342)} \quad (3)$$

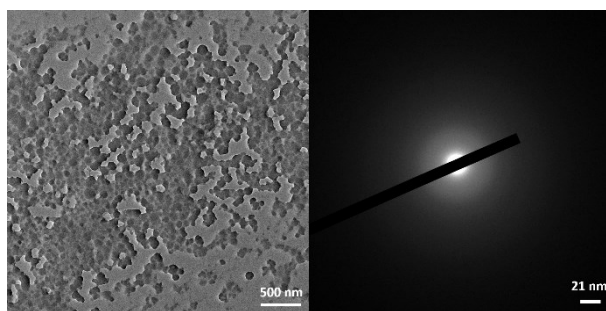
## 7. Figures:



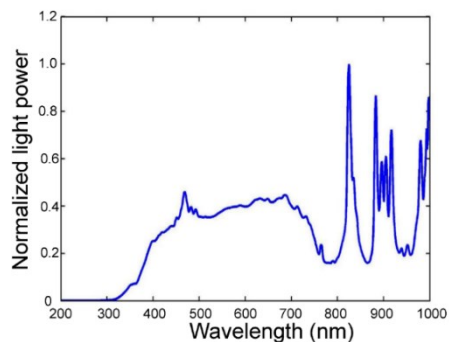
**Figure S1.** Emission spectra of Ir(tiq)<sub>2</sub>ppy NPs and Ir(tiq)<sub>2</sub>ppy in aqueous solution at room temperature without deoxygenation. [Ir(tiq)<sub>2</sub>ppy NPs] = [Ir(tiq)<sub>2</sub>ppy] = 1.6 μg/mL. Ir(tiq)<sub>2</sub>ppy was first dissolved in DMSO, and then diluted by water.



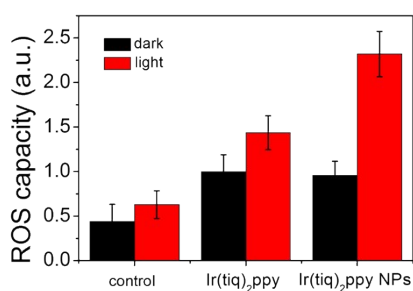
**Figure S2.** Fluorescence spectra of Ir(tiq)<sub>2</sub>ppy in mixed solutions of THF and deionized water at various ratios.



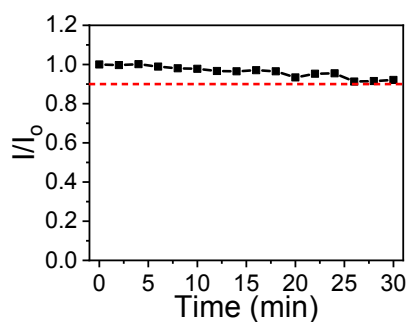
**Figure S3.** TEM image of Ir(tiq)<sub>2</sub>ppy NPs (aggregated during the evaporation of water on copper mesh) and its corresponding electron diffraction pattern.



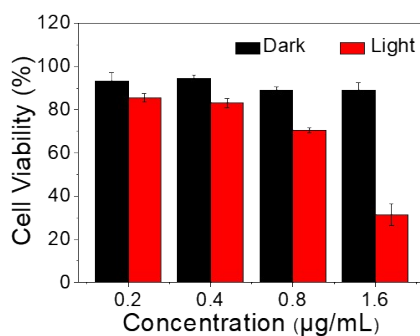
**Figure S4.** The spectrum of the white light used in this work.



**Figure S5.** ROS capacity of MCF-7 cells in the absence and presence of light after treated with Ir(tiq)<sub>2</sub>ppy and Ir(tiq)<sub>2</sub>ppy NPs at the concentration of 1.6  $\mu\text{g/mL}$  for 24 h.



**Figure S6.** Photostability of Ir(tiq)<sub>2</sub>ppy NPs under white light irradiation. Light intensity: 5  $\text{mW/cm}^2$ .



**Figure S7.** Cell viability of MCF-7 cells after incubation with various concentrations of Ir(tiq)<sub>2</sub>ppy for 24 h, irradiation for 30 min at 5  $\text{mW/cm}^2$  by white light followed by another 24 h of incubation.