Supporting Information

Multifunctional zeolitic imidazolate framework-8 for real-time monitoring ATP fluctuation in mitochondria during photodynamic therapy

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Experimental section

Materials

Adenosine triphosphate (ATP) disodium and 2-phenylbenzothiazole (bt) were purchased from Sigma-Aldrich (USA). RhB was purchased from Xi'an Wolsen Biotechnology Co., Ltd. (China). $Zn(NO_3)_2 \cdot 6H_2O$ was purchased by Sinophurm Chemical Reagent Co., Ltd. (China). 2-Methylimidazole (MIm) was purchased by Aladdin Industrial Corporation (China). $IrCl_3 \cdot 3H_2O$ was purchased from Shaanxi Kaida Chemical Engineering Co., Ltd (China). 5-bromo-1,10-phenanthroline (phen-Br) was purchased from Bide Pharmatech Ltd. (China). 2-deoxy-D-glucose, docetaxel, and cisplatin were provided by Shanghai Macklin Biochemical Technology Co., Ltd (China). MitoTracker Green FM and LysoTracker Green DND-26 were purchased from Yeasen Biotech Co., Ltd. (China). Cell Counting Kit-8 (CCK-8) was purchased from 7Sea Pharmatech Co., Ltd. (China). Phosphate buffered saline (10 mM PBS, pH 7.4) was purchased from HyClone Company (USA). All chemical reagents were analytical grade, and Millipore Milli-Q water (18.2 MΩ cm) was used.

Apparatus

Field emission scanning electron microscope (FE-SEM, SU8220, HITACHI, Japan), transmission electron microscope (TEM, JEM-2100 JEOL, and HT7700 HITACHI, Japan) with an accelerating voltage of 200 kV, powder X-ray diffractometer (PXRD, D8 Advance, Bruker, Germany) with CuKα radiation (λ = 1.54056 Å), fluorescence spectrophotometer (Fluorolog-3, Horiba JY, Japan), zeta-potential analyzer (Zetasizer Nano ZS, Malvern, UK), MALDI-TOF mass spectrometer (Autoflex III, Bruker, Germany) equipped with a SmartBeam Nd: YAG laser emitting at 355 nm, ^1H nuclear magnetic resonance spectrometer (NMR, 400 MHz, Bruker, Germany), confocal laser

scanning microscopy images system (CLSM, FV1200-IX81, Olympus, Japan) were used in this work for characterization and measurements.

Synthesis of PS-Ir

Typically, $[(bt)_2Ir(phen\text{-}Br)]PF_6$ (PS-Ir) was synthesized using two steps. Firstly, the chloride-bridged dimer, $[(bt)_2Ir(\mu\text{-}Cl)]_2$ was synthesized according to reported procedures.[S1] Secondly, 0.13 g of $[(bt)_2Ir(\mu\text{-}Cl)]_2$ (0.1 mmol) and 63.16 mg of phen-Br (0.228 mmol) were dissolved in 20 mL of CH_2Cl_2. After it was degassed, the reaction mixture was stirred for 12 h at 40 °C under nitrogen. Subsequently, the solution was cooled to room temperature, and then a 10-fold excess of KPF_6 was added. The resulting mixture was stirred for further 2 h. After that, it was filtered to remove insoluble inorganic salts. Then, the solvent was removed under reduced pressure and the residue was purified by column chromatography on silica gel (eluent: 19/1 CH_2Cl_2/CH_3OH) to recover the pure product. A yellowish-brown solid was obtained with 64% of yield. ^1H NMR (600 MHz, DMSO-d_6) δ 9.00 (dd, J = 8.5, 1.0 Hz, 1H), 8.92 – 8.85 (m, 2H), 8.51 – 8.46 (m, 1H), 8.43 (dd, J = 5.1, 1.1 Hz, 1H), 8.24 (dd, J = 8.5, 5.1 Hz, 1H), 8.20 – 8.11 (m, 3H), 8.05 (d, J = 7.7 Hz, 2H), 7.31 (t, J = 7.7 Hz, 2H), 7.17 (td, J = 7.2, 3.3 Hz, 2H), 6.96 (dt, J = 15.9, 4.2 Hz, 4H), 6.37 (dd, J = 10.2, 7.8 Hz, 2H), 5.74 (dd, J = 8.5, 2.0 Hz, 2H). ESI-HRMS (m/z): $[M]^+$, calculated for $C_{38}H_{23}BrIrN_4S_2$, 871.0177; found, 871.0150.

Cells culture and cytotoxicity assay

HeLa cells were seeded in high-glucose cell culture medium (Dulbecco's modified Eagle's medium, DMEM, Hyclone, USA) supplemented with 10% (v/v) fetal bovine serum (FBS, Gibco, China) and 1% antibiotics (penicillin/streptomycin, Gibco, China)

at 37 °C with 5% CO_2. The HeLa cells were grown to logarithmic phase and trypsinized with 1.0 mL of 0.25% trypsin. 10 μL of the suspension of trypsinized cells was dropped onto a cell counting chamber slide. The density of HeLa cells was detected using an automated cell counter (Beijing Dongsheng Innovation Biotechnology Co., Ltd. China).

HeLa cells were seeded in 96-well plates with a density of 1×10^5 cells/mL in fresh cell culture medium. After incubation 24 h, HeLa cells were cultured in cell culture medium containing different concentrations of RhB@ZIF-8 or PS-Ir for 1 h or 0.5 h for dark-toxicity, respectively. A microplate reader (EMax Plus Microplate Reader, MOLECULAR DEVICES, USA) was used to study the cytotoxicity of RhB@ZIF-8 or PS-Ir at 450 nm according to the manufacturer's instruction (Beijing Solarbio Science & Technology Co., Ltd. China).

Results

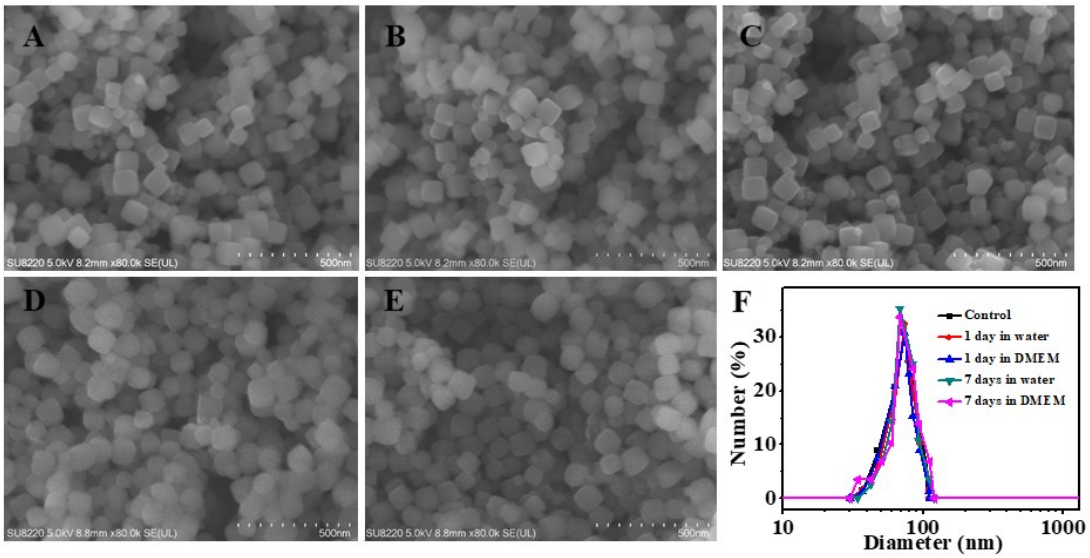

Fig. S1. SEM images (A-E) and hydrodynamic diameter (F) of RhB@ZIF-8 after dispersion in aqueous solution (B, D) and cell culture medium (C, E) for one and seven days, respectively.

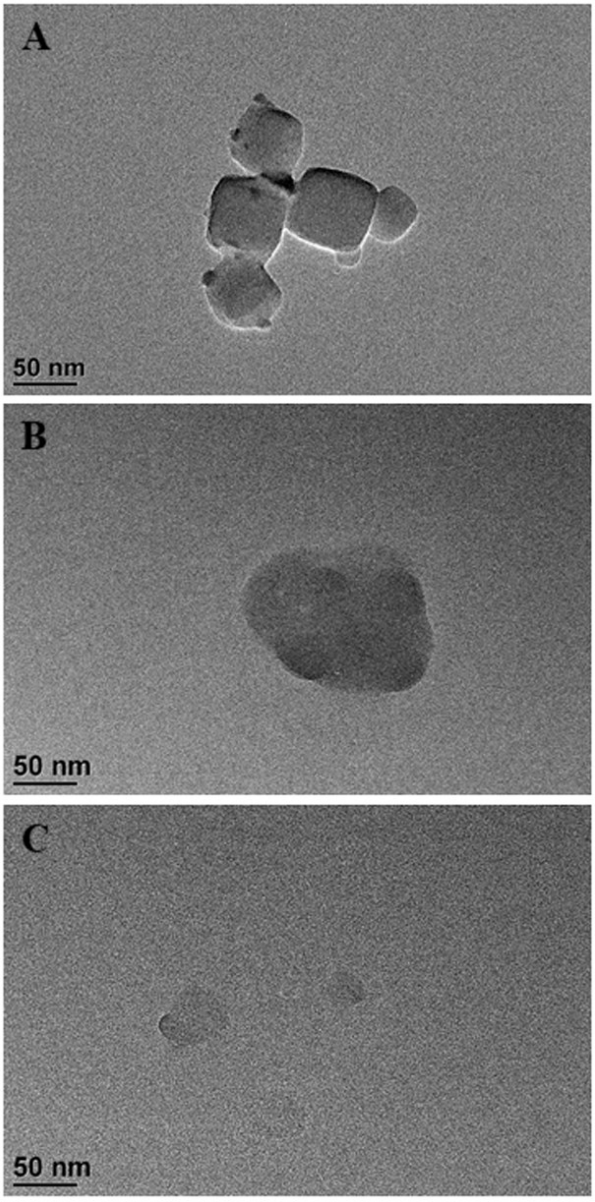

Fig. S2. TEM images of RhB@ZIF-8 before (A) and after (B, C) ATP treatment. RhB@ZIF-8 (0.1 $mg \cdot mL^{-1}$) was incubated with 0.6 mM (B) and 6 mM (C) ATP for 3 min, respectively.

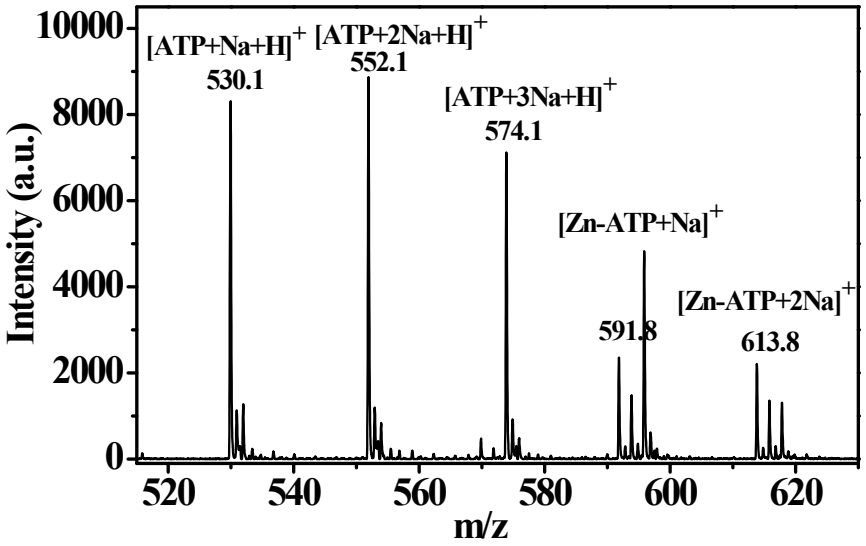

Fig. S3. Positive ion MALDI-TOF mass spectrum of reaction products of ATP and ZIF-8.

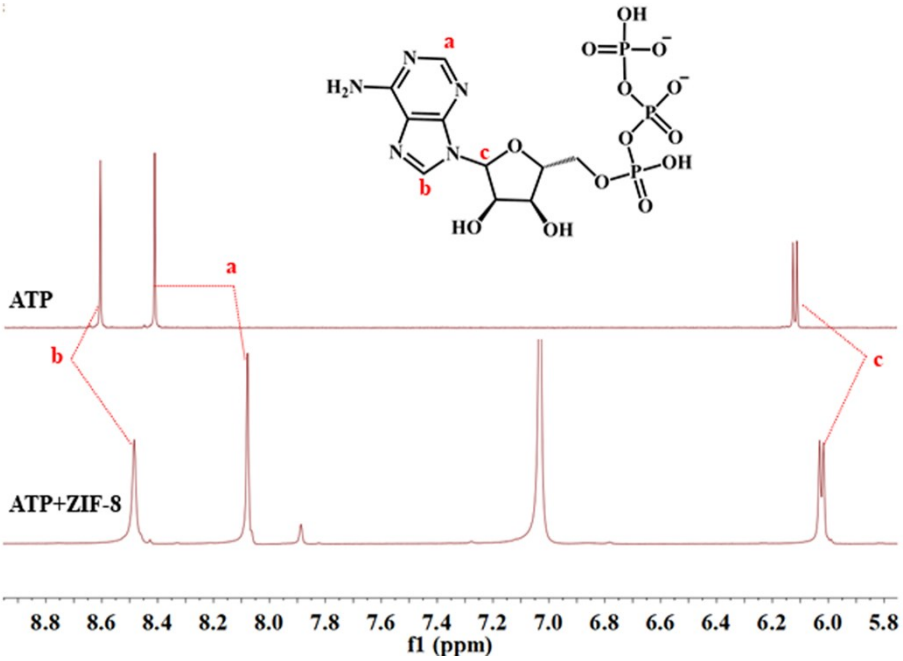

Fig. S4. Partial ^{1}H-NMR spectra of ATP and ATP+ZIF-8 in D_2O.

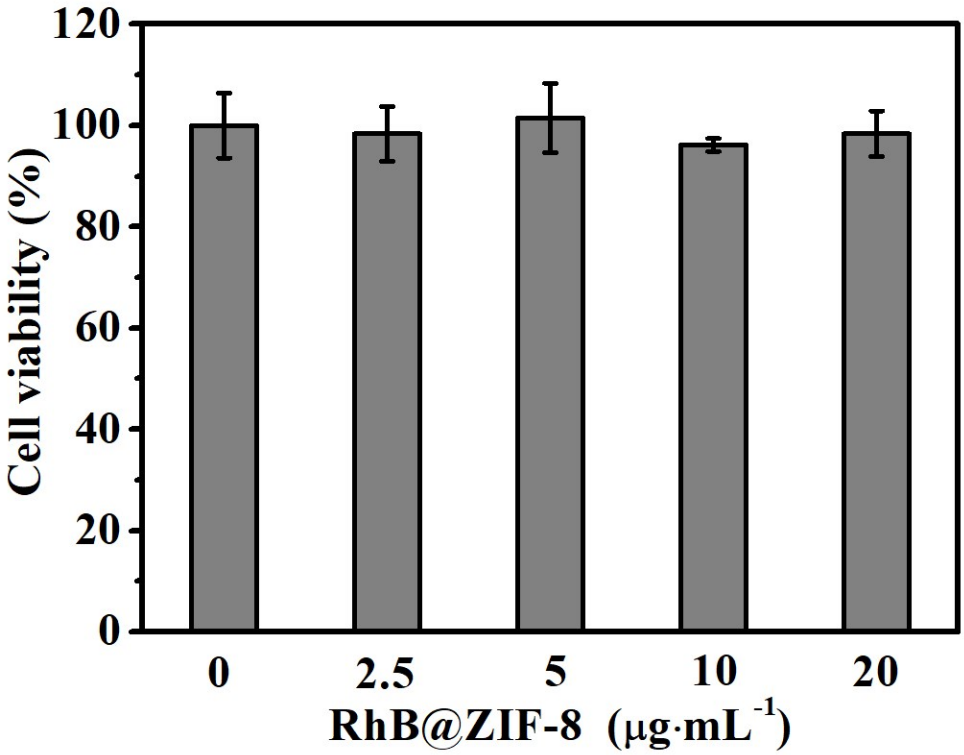

Fig. S5. Cell viability after treatment of HeLa cells with RhB@ZIF-8. HeLa cells were incubated with RhB@ZIF-8 dispersion solution at different concentrations for 24 h.

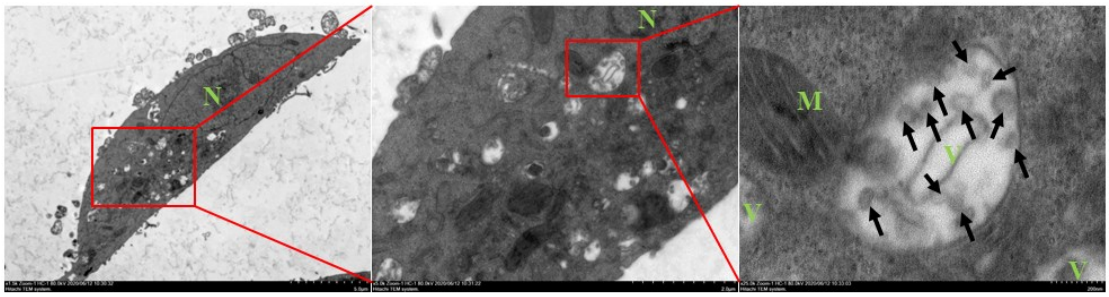

Fig. S6. Bio-TEM images of HeLa cells after treatment with 20 μg·mL^{-1} RhB@ZIF-8 for 0.5 h. Black arrows show intact RhB@ZIF-8. N represents nucleus, M represents mitochondria, and V represents vesicles.

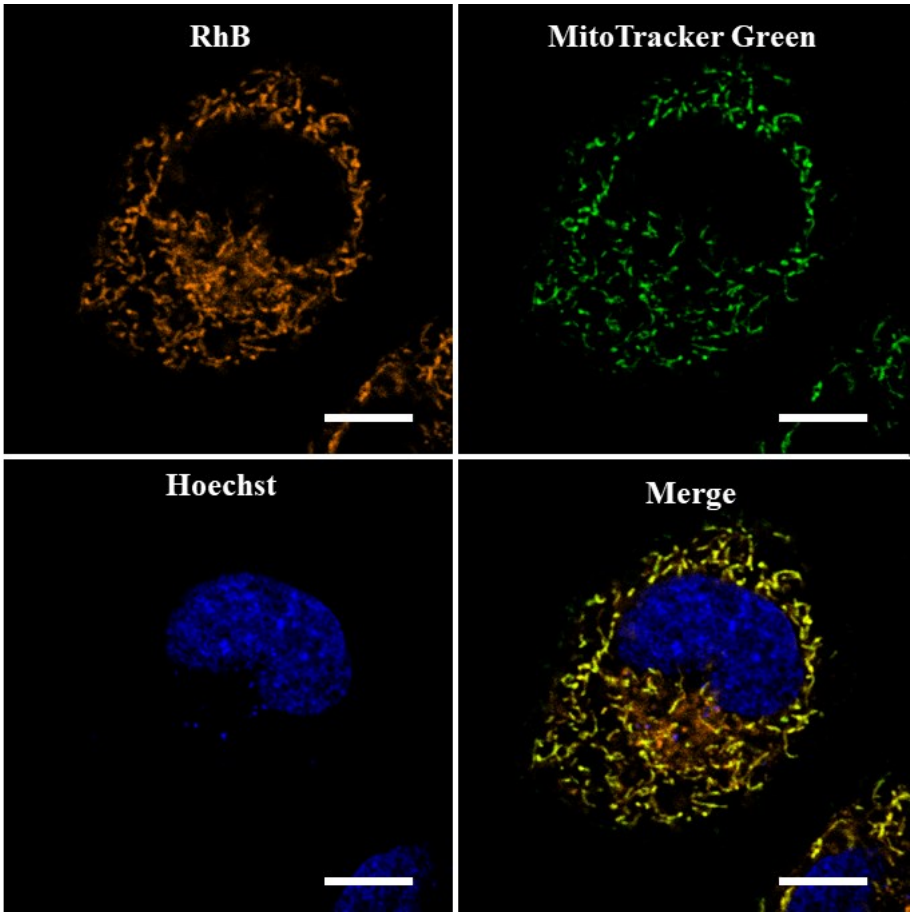

Fig. S7. CLSM images of HeLa cells incubated with RhB for 1 h, and co-stained with MitoTracker Green and Hoechst for 0.5 h in 1.0 mL culture medium without phenol red. MitoTracker Green FM fluorescence image (500-540 nm) obtained upon the excitation at 488 nm. Hoechst fluorescence image (420-460 nm) obtained upon the excitation at 405 nm. RhB fluorescence image (570-670 nm) obtained upon the excitation at 559 nm. Scale bar: 10 μm.

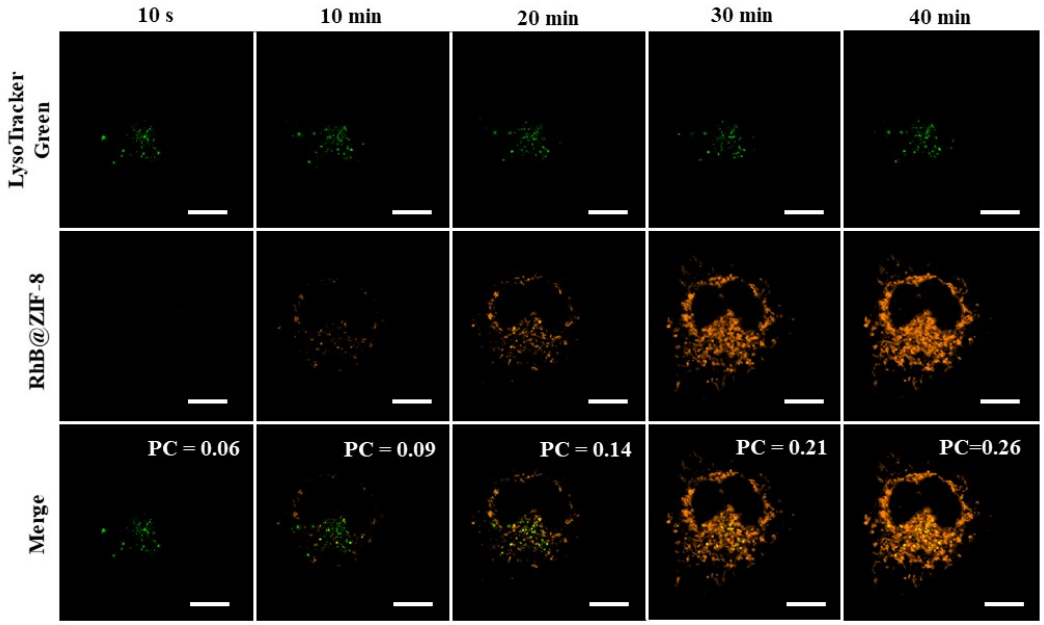

Fig. S8. CLSM images of HeLa cells co-stained with RhB@ZIF-8 and LysoTracker Green. LysoTracker Green fluorescence image (500-540 nm) obtained upon the excitation at 488 nm. RhB fluorescence image (570-670 nm) obtained upon the excitation at 559 nm. Scale bar: 10 μm.

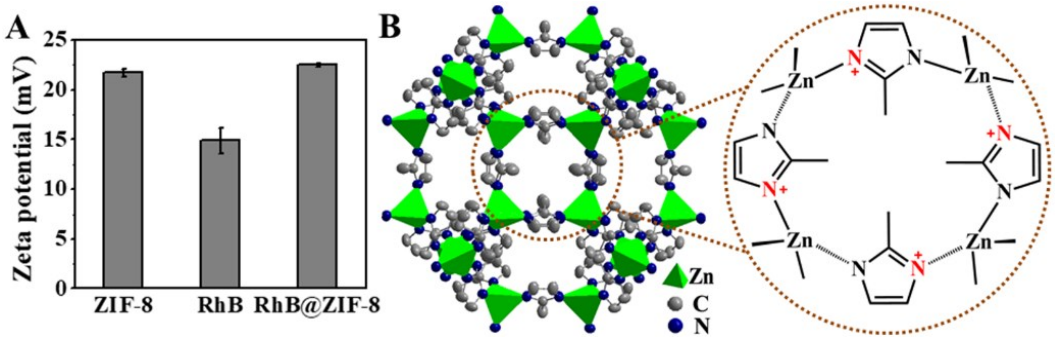

Fig. S9. (A) Zeta potential of free ZIF-8, RhB, and RhB@ZIF-8 in H_2O. (B) A unit framework and local molecular structure of ZIF-8.

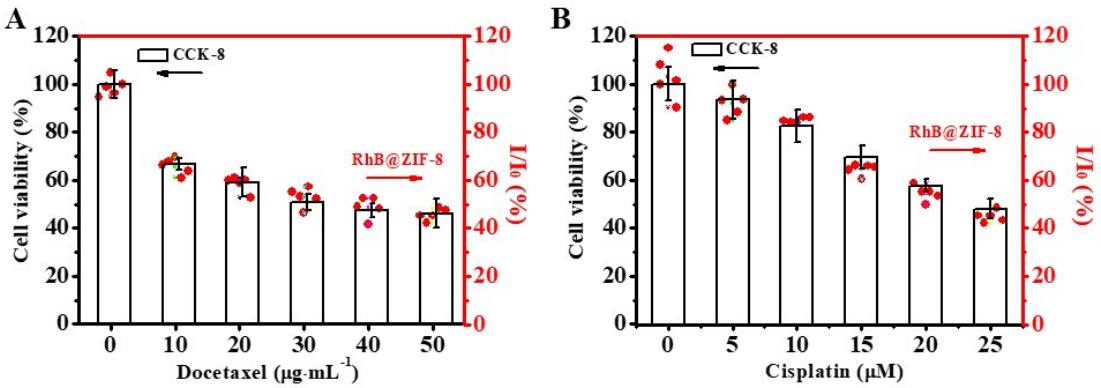

Fig. S10. Cell viability and the change of cellular fluorescence intensity of HeLa cells treated with docetaxel (A) and cisplatin (B) for 24 h.

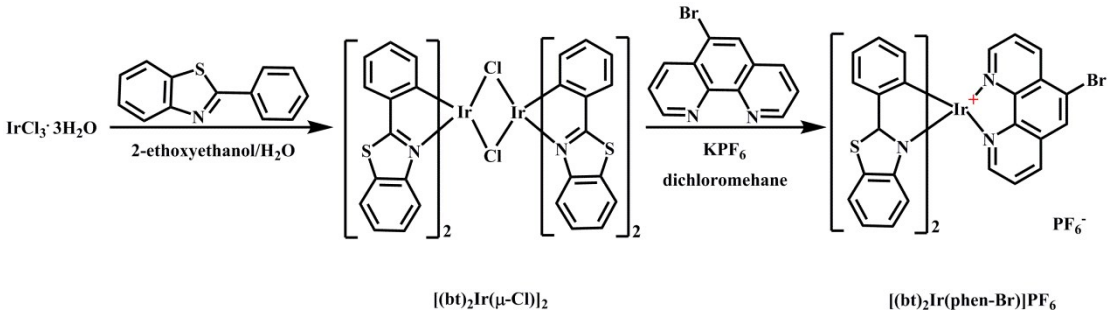

Fig. S11. Synthetic routes of PS-Ir.

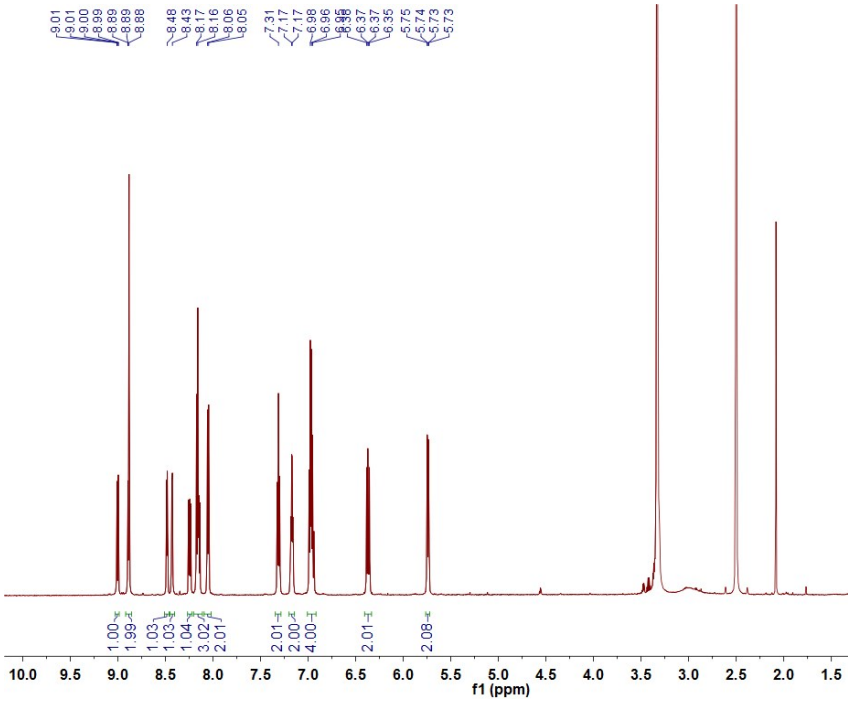

Fig. S12. ^{1}H NMR spectrum of $[(bt)_2Ir(phen\text{-}Br)]PF_6$ in DMSO-d_6.

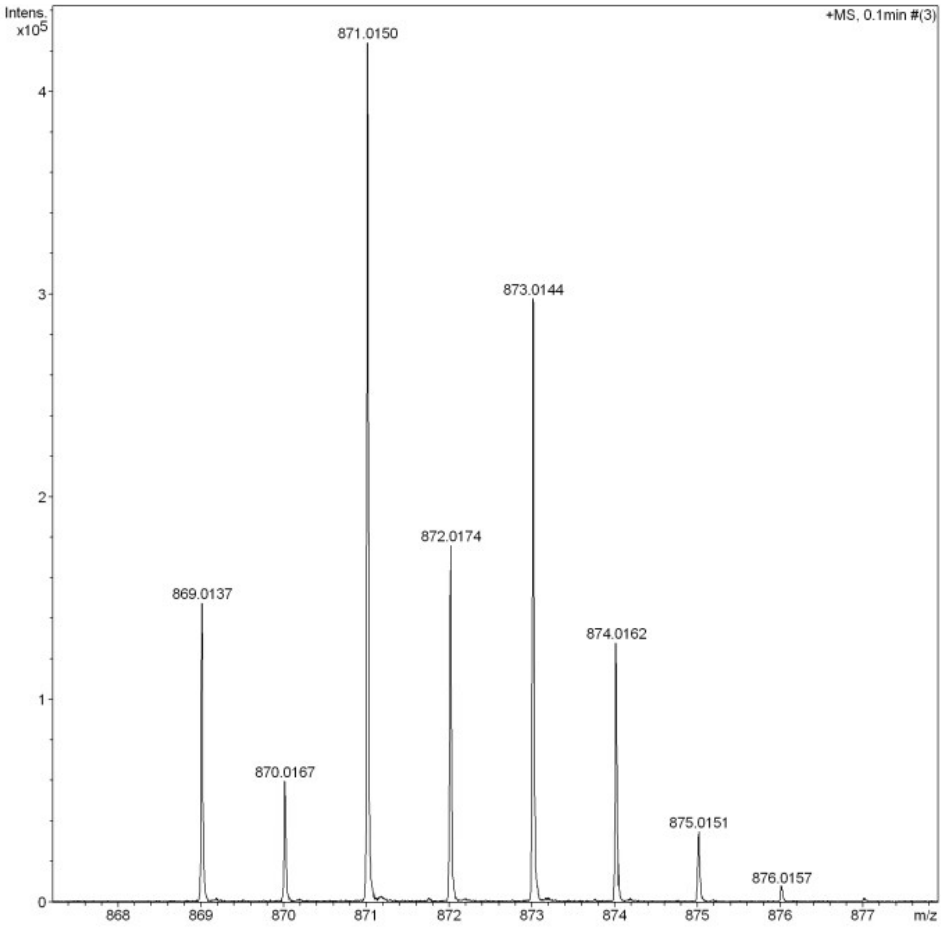

Fig. S13. ESI-MS spectrum of $[(bt)_2Ir(phen\text{-}Br)]^+$.

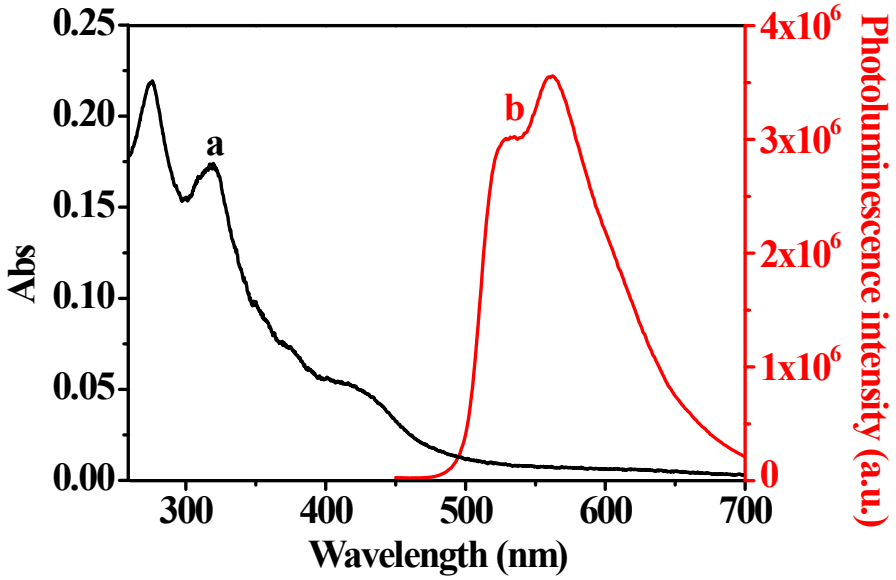

Fig. S14. UV-Vis absorption spectrum (a) and photoluminescence spectrum (b) of PS-Ir (6 μM) in 25 mM PBS (pH 7.4), λ_{ex} = 320 nm.

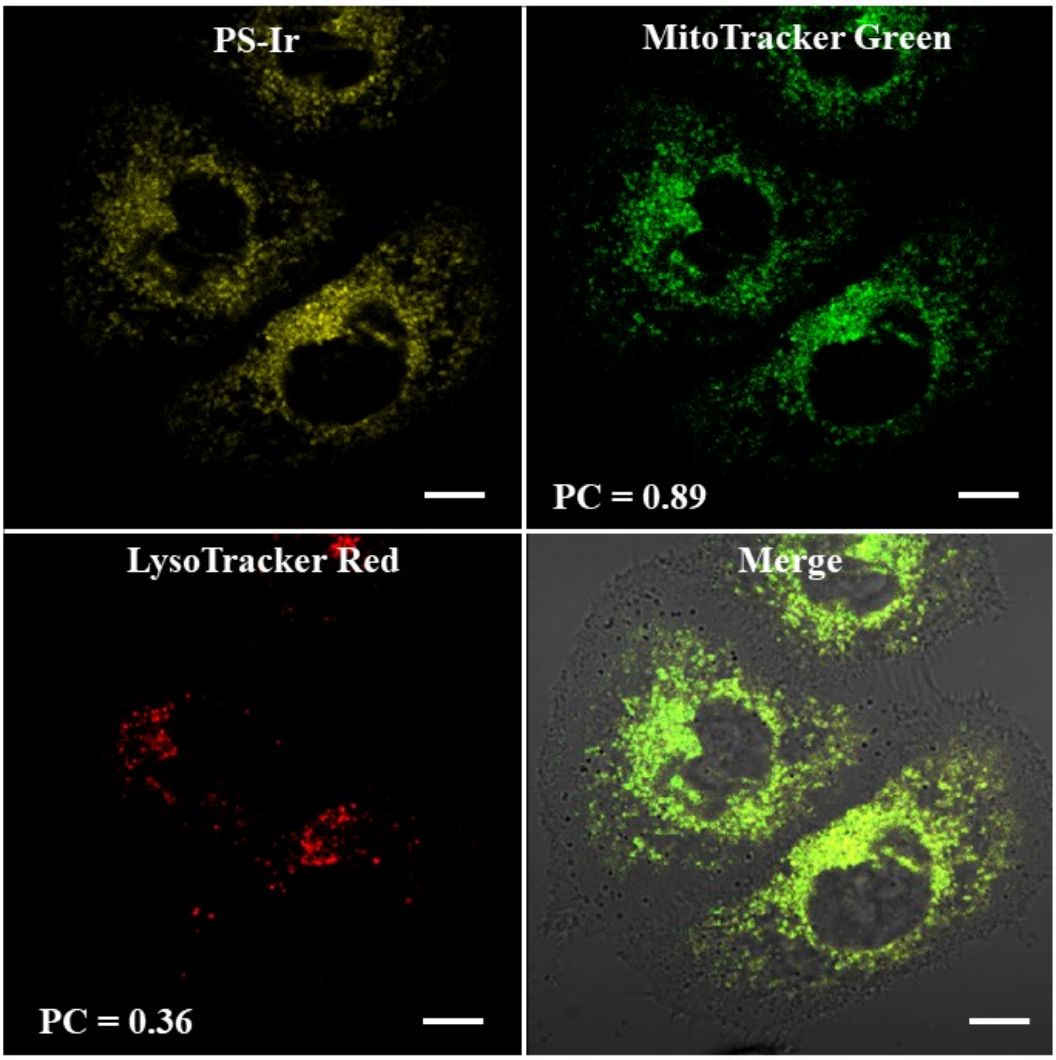

Fig. S15. CLSM images of HeLa cells treated with PS-Ir (6 μM) for 0.5 h, and co-stained with MitoTracker Green for 0.5 h and LysoTracker Red for 1 h in 1.0 mL culture medium without phenol red. Scale bar: 10 μm.

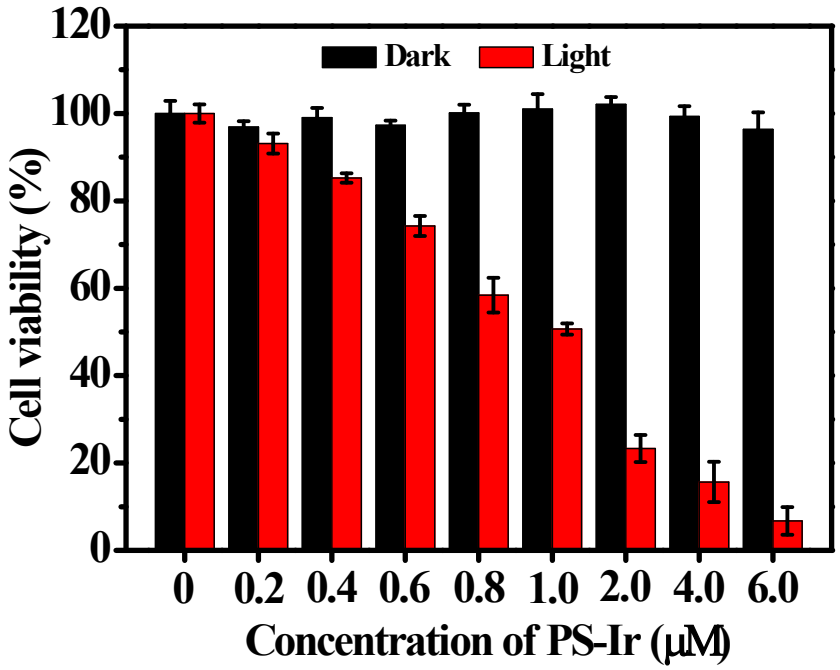

Fig. S16. Cell viability of different concentrations of PS-Ir against HeLa cells under dark or light (20 mW·cm^{-2}, 2 min).

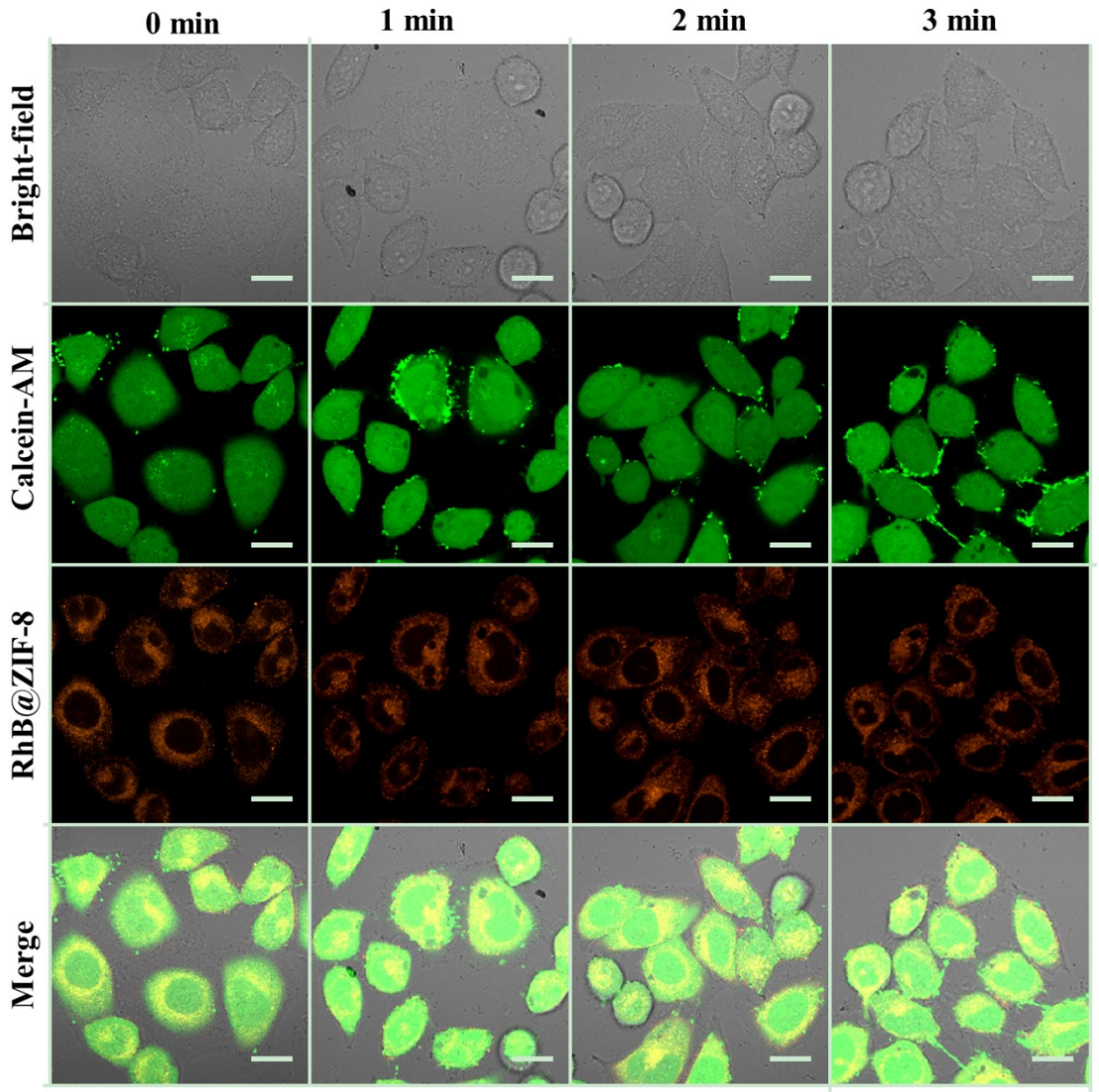

Fig. S17. CLSM images of HeLa cells treated 6 μM PS-Ir for 0.5 h, and then without light irradiation for different time, and finally treated with Calcein-AM and RhB@ZIF-8 (20 μg·mL^{-1}) for 0.5 h, respectively. Scale bar: 20 μm.

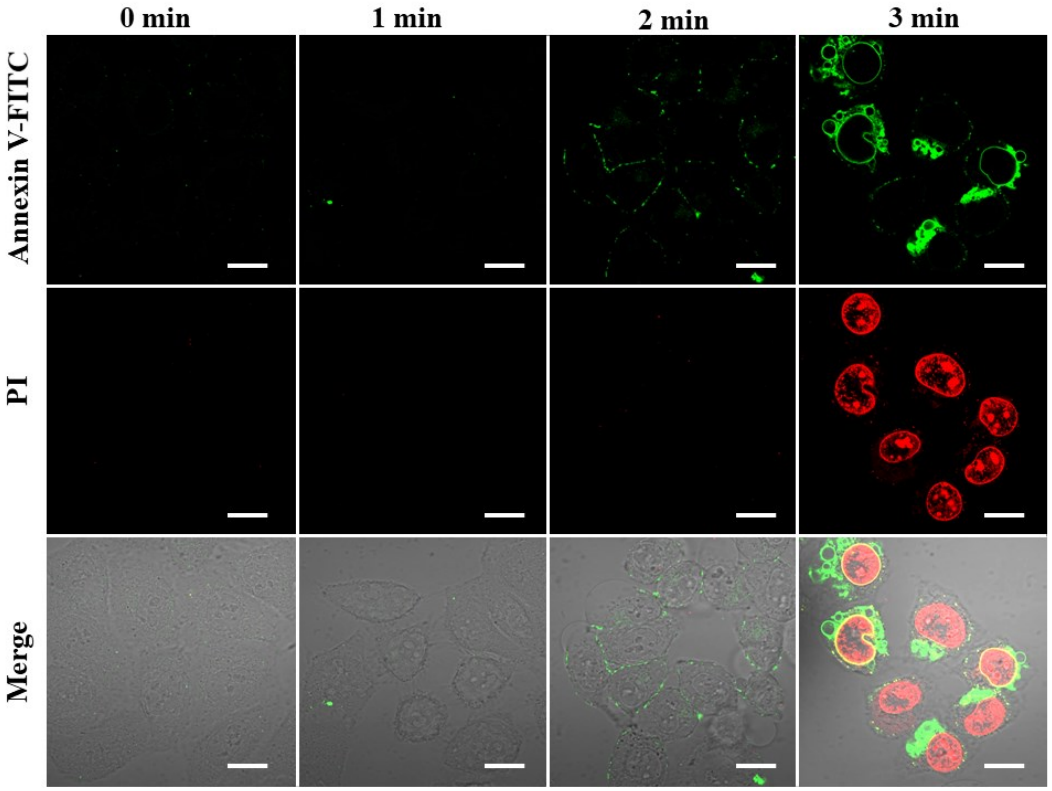

Fig. S18. CLSM images of HeLa cells treated with PS-Ir (6 μM) under white light irradiation (20 mW·cm^{-2}) for different time, and then treated with PI and Annexin V-FITC for 0.5 h. Scale bar: 20 μm.

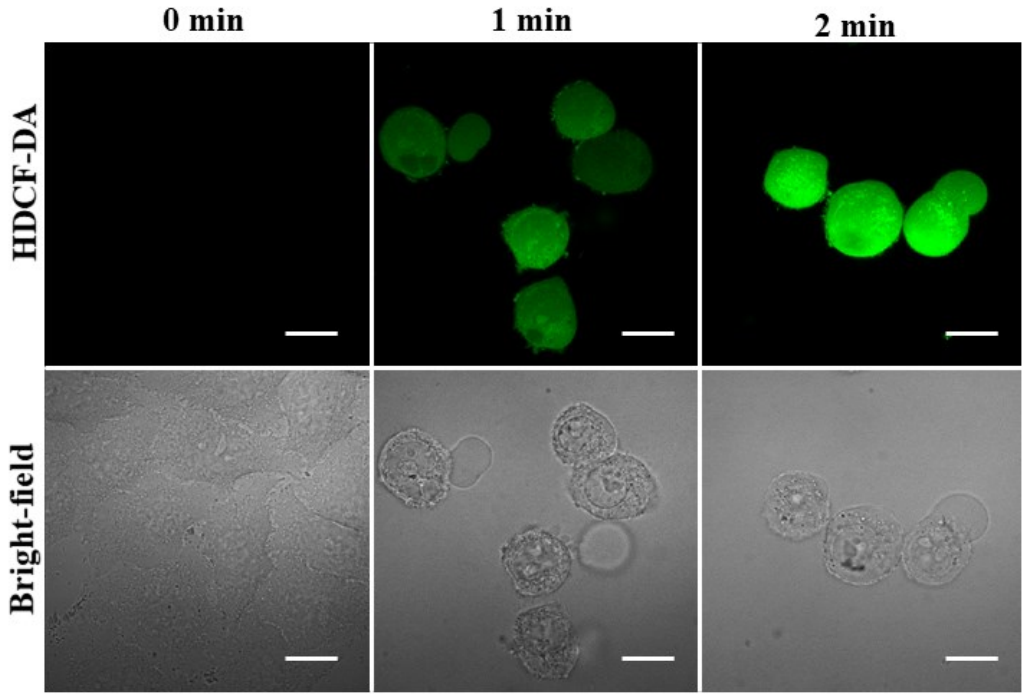

Fig. S19. CLSM images of HeLa cells after incubation with PS-Ir (6 μM) and HDCF-DA with white light irradiation (20 mW·cm^{-2}) for different time. Scale bar: 20 μm.

Reference

[S1] H. Huang, S. Banerjee, K. Qiu, P. Zhang, O. Blacque, T. Malcomson, M. J. Paterson, G. J. Clarkson, M. Staniforth, V. G. Stavros, G. Gasser, H. Chao and P. J. Sadler, *Nat. Chem.* 2019, **11**, 1041-1048.