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Supplementary Information

Quantitative Tracking of Endoplasmic Reticulum Viscosity during Ferroptosis by an Iridium Complex via TPPLM

Liang Hao[#], Yan-Mei Zhong[#], Cai-Ping Tan* and Zong-Wan Mao*

MOE Key Laboratory of Bioinorganic and Synthetic Chemistry, School of Chemistry, Sun Yat-Sen University, Guangzhou 510275, China.

[#]These authors contributes equally to this work.

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Methods

Materials

Iridium trichloride (J&K Chemical, China), 4-(2-Pyridinyl)benzaldehyde (ppy-CHO, J&K Chemical, China), Potassium carbonate (J&K Chemical, China), dimethyl sulfoxide (DMSO, Sigma Aldrich, USA), Cys (Sigma Aldrich, USA), Ala (Sigma Aldrich, USA), Glucose (Sigma Aldrich, USA), GSH (Sigma Aldrich, USA), HSA (Sigma Aldrich, USA),glycerol (J&K Chemical, China), trypsin (Hyclone Laboratoreis Inc, USA), Roswell Park Memorial Institute Medium (RPMI1640 Medium, Hyclone Laboratoreis Inc, USA), phosphate buffered saline (PBS, Sigma Aldrich, USA), fetal bovine serum (FBS, Hyclone Laboratoreis Inc), 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-H-tetrazolium bromide (MTT, J&K Chemical, China), LysoTracker® Deep Red (LTDR, Life Technologies, USA), ER-Tracker Red (ERTR, Beyotime, China), Mito-Tracker Red (MTR, Beyotime, China), Lyso-Tracker Deep Red (LTDR, Beyotime, China) were used as received. Other materials and chemicals were purchased from the commercial sources. All the tested compounds were dissolved in DMSO as mother liquor before diluted into the experimental concentration with 1% (v/v) DMSO in the solvents.

Instrumentation

^{1}H NMR spectra were recorded on a Bruker Avance 400 spectrometer (Germany). Shifts were referenced relative to the internal solvent signals. ESI-MS were recorded on a Thermo Finnigan LCQ DECA XP spectrometer (USA). The quoted m/z values represented the major peaks in the isotopic distribution. Microanalysis (C, H, and N) was carried out using an Elemental Vario EL CHNS analyzer (Germany). HPLC spectra were carried out with a Hewlett Packard High Performance Liquid Chromatograph (USA). UV/Vis spectra were recorded on a Varian Cary 300 spectrophotometer (USA). Emission measurements were conducted on an FLS 920 combined fluorescence lifetime and steady state spectrometer (Japan). Confocal and TPFLIM images were

recorded on a Carl Zeiss LSM 810 laser scanning confocal microscope (Germany). The cofocal microscope was combined with a Becker & Hickl (BH) time-correlated single photon counting (TCSPC) system. The PLIM data were analyzed using the SPCImage software available on www.becker-hickl.com (Becker & Hickl GmbH, the bh TCSPC Handbook sixth Edition). Tecan Infinite M200 Pro microplate reader (Switzerland) was used in MTT assay. FluorChem M (Protein Simple, USA) was used for chemiluminescence detection in western blot.

Supplementary experimental section

Synthetic protocols and characterizations

$[Ir_2(ppy\text{-}CHO)_4Cl_2]$: The precursor was synthesized by literature methods.[1] Briefly, $IrCl_3 \bullet 3H_2O$ (1.00 g, 2.84 mmol) and ppy-CHO (1.145 g, 6.25 mmol) were refluxed in 2-ethoxyethanol (100 mL) for 18 h. After cooling to room temperature and filtration, the residue was washed with methanol and ether. $[Ir_2(ppy\text{-}CHO)_4Cl_2]$ were obtained as orange solids.

Ir-ER: **Ir-ER** was synthesized by reacting $[Ir_2(ppy\text{-}CHO)_4Cl_2]$ (0.200 g, 0.186 mmol) with acetylacetone (0.410 g, 0.410 mmol) in 2-ethoxyethanol under reflux for 8 h. The solvent was removed by vacuum evaporation to obtain a yellow solid. The crude product was then purified with silica gel (CH_2Cl_2:CH_3OH = 9:1). ^1H NMR (400 MHz, $CDCl_3$ δ 8.43 (d, J = 5.7 Hz, 2H), 7.79 (d, J = 8.0 Hz, 2H), 7.68 (t, J = 7.8 Hz, 2H), 7.47 (d, J = 8.0 Hz, 2H), 7.12 – 7.05 (m, 2H), 6.77 (d, J = 7.9 Hz, 2H), 6.14 (s, 2H), 1.72 (s, 6H). Purity by HPLC = 100%.

Photophysical properties

The UV-Vis spectra, emission spectraand lifetimes of **Ir-ER** in PBS, CH_3CN and CH_2Cl_2 at 298K were obtained on a Varian Cary 300 spectrophotometer (USA) and an FLS 920 combined fluorescence lifetime and steady spectrometer (Japan). The emission spectrum of **Ir-ER** under two-photon excitation was measured on a

Femtosecond-Nanosecond Transient Absorption Spectrometer. The resulting data were processed with Origin Pro v8.0. All media contain minimum DMSO (1% v/v) for better solvency.

Viscosity-responsive emission properties

The emission properties of **Ir-ER** in mixed solvents containing methanol and glycerol representing different viscosity at 298K were obtained on an FLS 920 combined fluorescence lifetime and steady spectrometer (Japan). The resulting data were processed with Origin Pro v8.0. All media contain minimum DMSO (1% v/v) for better solvency.

Reponse to solvents

The emission lifetimes of **Ir-ER** in different solvents at 298 K were obtained on an FLS 920 combined fluorescence lifetime and steady spectrometer (Japan). The resulting data were processed with Origin Pro v8.0. All media contain minimum DMSO (1% v/v) for better solvency.

Response to ions and biomolecules

The fluorescent lifetime of **Ir-ER** in aqueous solution with varies ions and biomolecules (c >1 M) at 298 K were obtained on a FLS 920 combined fluorescence lifetime and steady spectrometer (Japan) after an incubation for a week. The resulting data were processed with Origin Pro v8.0. All media contain minimum DMSO (1% v/v) for better solvency.

Mechanism of viscosity-responsive emission

The phosphorescence lifetime of **Ir-ER** in CH_3OH and Gly (percentage of Gly: 70%, v/v) at different temperatures (278 K, 283 K, 288 K, 293 K, 298 K, 303 K, 308 K, 313 K, 318 K, 323 K) were obtained on an FLS 920 combined fluorescence lifetime

and steady spectrometer (Japan). The resulting data were processed with Origin Pro v8.0. All media contain minimum DMSO (1% v/v) for better solvency.

Cytotoxicity

The cytotoxicity of **Ir-ER** towards MCF-7 cell lines was determined by MTT assay.

For cytotoxicity in the dark, the compound were dissolved in DMSO and diluted into gradient concentration with a final DMSO proportion of 1% (v/v). Cells cultured in 96-well plates were grown to confluence before incubated with **Ir-ER** for 44 h. 20 μL of MTT solution (5 mg/mL) was then added to each well. The plates were incubated for an additional 4 h before the media was carefully removed, and DMSO was added (150 μL per well). The plate was shaken for 3 min. The absorbance at 595 nm was measured using a microplate reader (Infinite M200 Pro, Tecan, Switzerland).

For phototoxicity, cells cultured in 96-well plates were grown to confluence before incubated with **Ir-ER** for 20 h. The media containing **Ir-ER** was removed and fresh media without the complexes was added. Then the cells were irradiated with a 405 nm light array (20 mW cm^{-2}) for 15 min (18 J cm^{-2}) and further incubated for 24 h. 20 μL of MTT solution (5 mg/mL) was then added to each well. The plates were incubated for an additional 4 h before the media was carefully removed, and DMSO was added (150 μL per well). The plate was shaken for 3 min. The absorbance at 595 nm was measured using a microplate reader (Infinite M200 Pro, Tecan, Switzerland).

Cellular localization studies

MCF-7 cells were seeded in 35 mm culture dishes (Corning) and incubated for 24 h. The growth media was replaced by PBS solution of Ir-ER (1 μM) containing DMSO (1%, v/v) and further incubated for 1 h. The trackers were then added into the media at the indicated concentrations and incubated for 30 min. The media was then removed and the cells were washed with PBS before visualized by confocal microscopy. λ_{ex} = 405 nm (**Ir-ER**); 561 nm (ER-Tracker Red/Mito-Tracker Red); 633 nm (Lyso-Tracker Deep Red); λ_{em} = 550 ± 20 nm (**Ir-ER**); 610 ± 20 nm (ER-Tracker Red/Mito-Tracker); 660 ± 20 nm (Lyso-Tracker Deep Red).

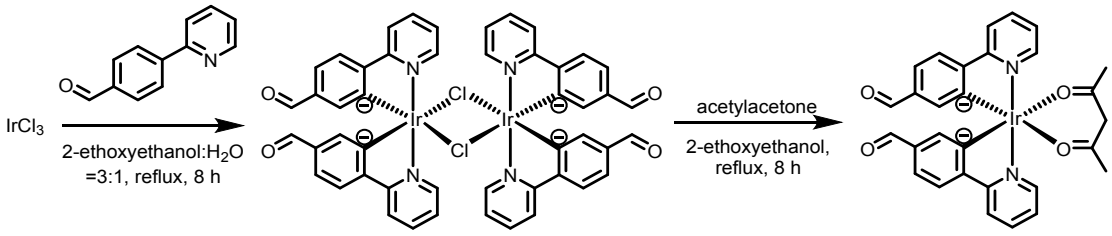
Cellular lipid peroxide detection

MCF-7 cells were seeded in 35 mm culture dishes (Corning) and incubated for 24 h. The cells were then incubated with erastin (10 μM) for 6 h. The media was replaced by PBS solution with 10 μM Image-iT® Lipid Peroxidation Sensor. After 30 min, The cells were washed with PBS for 3 times before visualized by confocal microscopy. λ_{ex} = 488; λ_{em} = 510 ± 20 nm (oxidation state); λ_{ex} = 561; λ_{em} = 591 ± 20 nm (reduction state).

Real-time tracking of ER viscosity via TPPLIM

MCF-7 cells were seeded in 35 mm culture dishes (Corning) and incubated for 24 h. The growth media was replaced with fresh culture medium with **Ir-ER** (10 μM), erastin (10 μM) and DMSO (1%, v/v) before further incubated for 6 h. Cells were then visualized by TPFLIM. The lifetime value is given by Becker & Hickl SPCImage. λ_{ex} = 810 nm; λ_{em} = 570 ± 20 nm.

Supporting Scheme, Figures and Tables



Scheme S1. Synthetic protocols of **Ir-ER**.

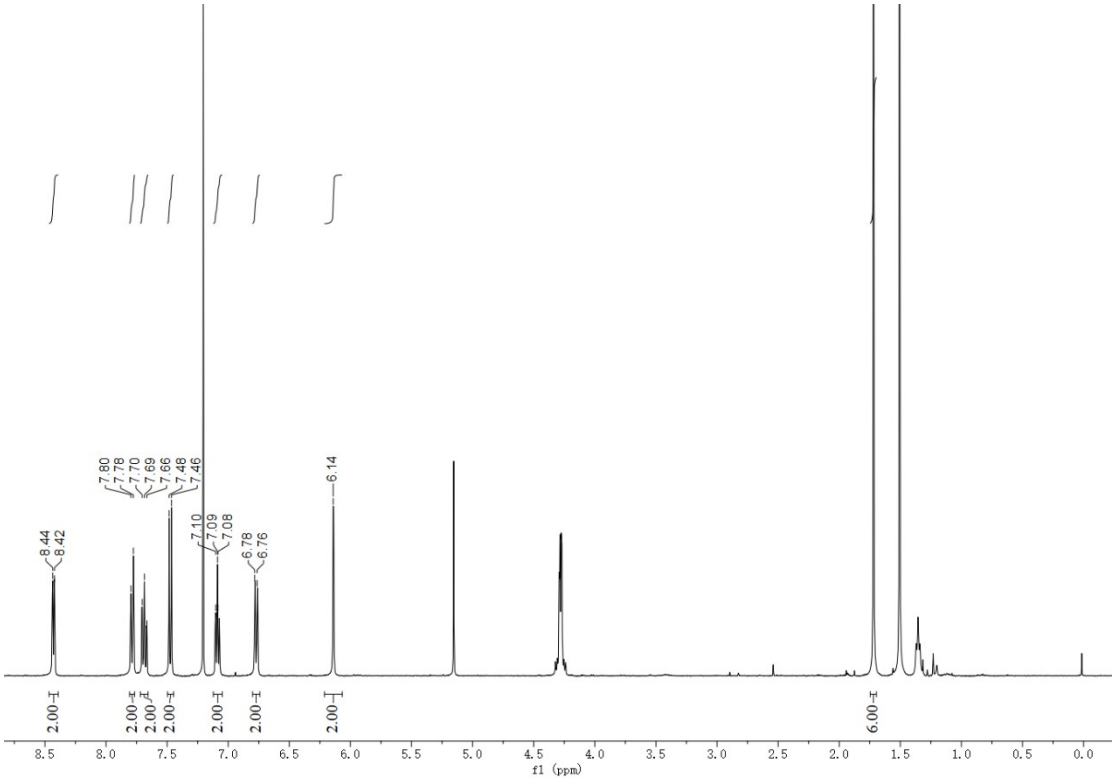


Figure S1 ^{1}H NMR spectrum of **Ir-ER** in CD_3Cl.

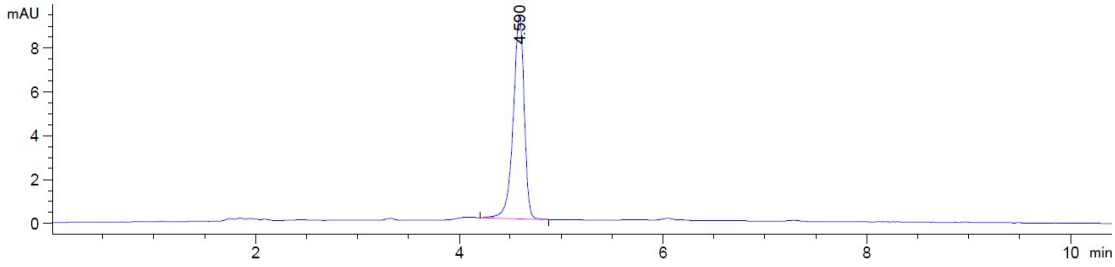


Figure S2 HPLC spectrum of **Ir-ER**.

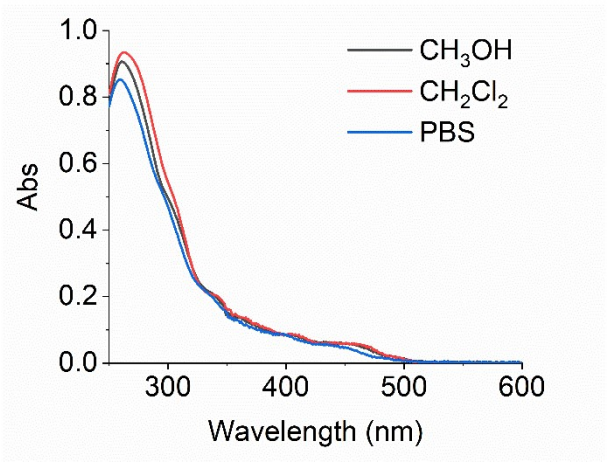


Figure S3 UV-Vis spectra of **Ir-ER** in CH_3OH, CH_2Cl_2 and PBS.

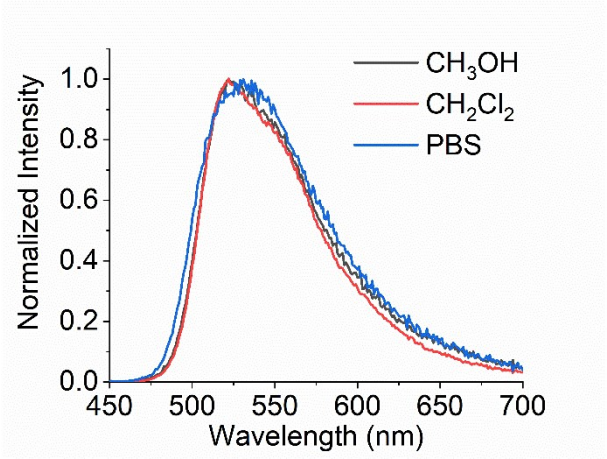


Figure S4 Emission spectra of **Ir-ER** in CH_3OH, CH_2Cl_2 and PBS. λ_{ex} = 405 nm.

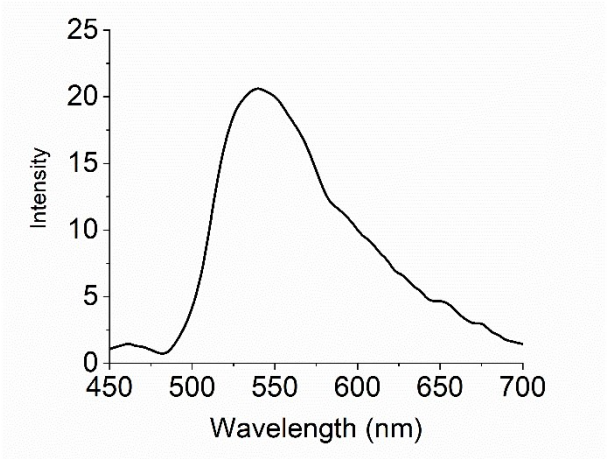


Figure S5 Emission spectrum f **Ir-ER** in DMSO under two-photon excitation. λ_{ex} = 810 nm.

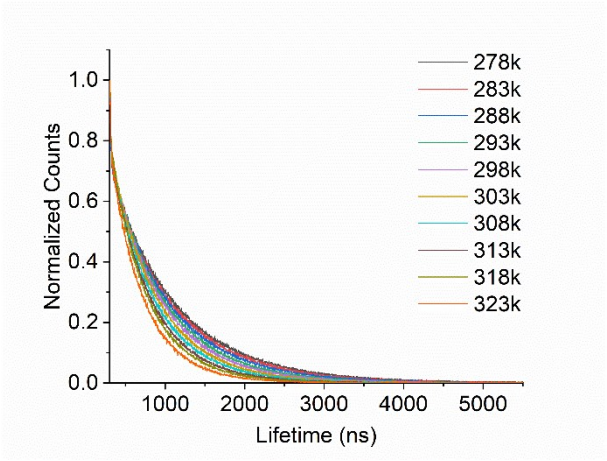


Figure S6 Lifetime spectra of **Ir-ER** (20 μM) at different temperature. λ_{ex} = 405 nm.

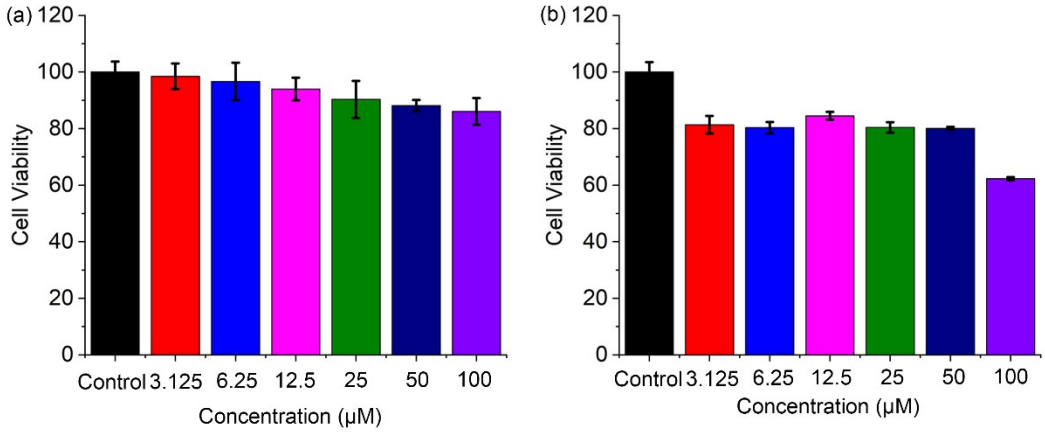


Figure S7 Cytotoxicity of **Ir-ER** against MCF-7 cells in dark (a) and under light (b) conditions in 48 h. λ_{ex} = 405 nm.

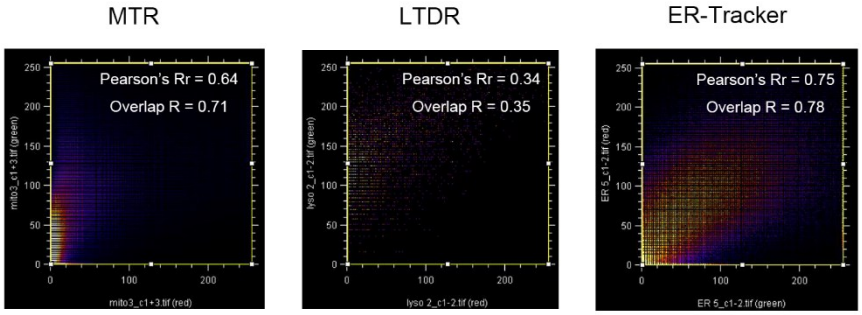


Figure S8 Colocalization coefficients of **Ir-ER** with the organelle trackers. λ_{ex} = 405 nm (**Ir-ER**); 561 nm (ER-Tracker/MTR); 633 nm (LTDR); λ_{em} = 550 ± 20 nm (**Ir-ER**); 610 ± 20 nm (ER-Tracker/MTR); 660 ± 20 nm (LTDR).

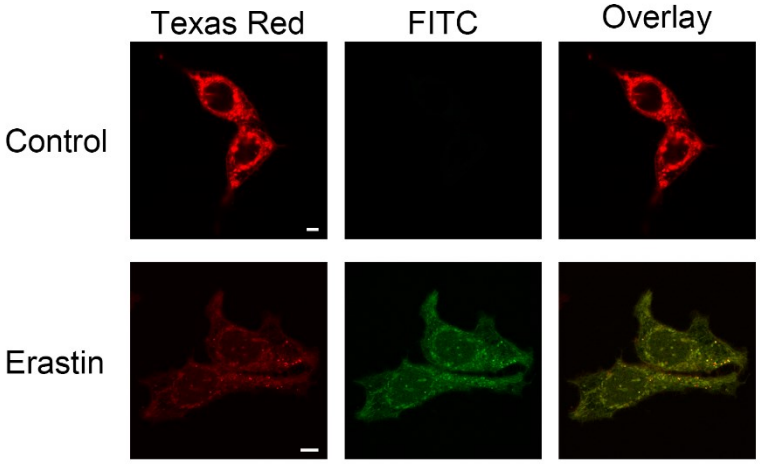


Figure S9 Detection of lipid peroxide in MCF-7 cells. Cells were treated with erastin (10 μM) for 6 h. λ_{ex} = 488; λ_{em} = 510 ± 20 nm (oxidation state); λ_{ex} = 561; λ_{em} = 591 ± 20 nm (reduction state). Scale bar : 5 μm.

References

1. C. Li., M. Yu., Y. Sun., Y. Wu., C. Huang. and F. Li., *J. Am. Chem. Soc.*, 2011, **133**: 11231-11239.