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Supporting Information

Cationic Iridium(III) Complexes for Phosphorescence Staining in the Cytoplasm of Living Cells

Mengxiao Yu,[a] Qiang Zhao,[a] Linxi Shi,[b] Fuyou Li,[*, a] Zhiguo Zhou,[a] Hong Yang,[a] Tao Yi,[a] Chunhui Huang[a]

[a] *Department of Chemistry & Laboratory of Advanced Materials, Fudan University, Shanghai, 200433, P. R. China;*

[b] *Department of Microbiology and Immunolog, Kunming Medical College, Kunming, 650031, P. R. China.*

To whom correspondence should be addressed. E-mail: fyli@fudan.edu.cn.

Materials.

Commercially available chemical reagents were used without further purification. 2-Ethoxyethanol, bipyridine (bpy) was obtained from Acros. $IrCl_3{\cdot}3H_2O$ was industrial products and used without further purification. The ligand 2-(2-quniolinyl)quinoxaline (quqo) was synthesized according to our previous report.[1]

General Experiments.

^{1}H NMR spectra were recorded with Varian spectrometer at 400 MHz. Mass spectra were obtained on a SHIMADZU matrix-assisted laser desorption/ionization time-of-flight mass spectrometer (MALDI-TOF-MASS). Elemental analyses were carried out with VarioEL III O-Element Analyzer system. UV-visible spectra were recorded on Shimadzu UV-2550 spectrometer. Steady-state emission experiments at room temperature were measured on an Edinburgh instrument Xe-900 spectrometer. Lifetime studies were performed with an Edinburgh FL 900 photo-counting system with a hydrogen-filled as the excitation source. Luminescence quantum yields of these complexes in solution were measured using an aerated aqueous solution of $[Ru(bpy)_3]Cl_2$ (ϕ = 0.028)[2] as the standard solution.. Solutions of complexes in CH_2Cl_2 were degassed by three freeze-pump-thaw cycles, while those in DMSO/phosphate buffer solution (PBS) (pH 7, 1:49, v/v) were determined without specific treatment.

Synthesis of Iridium(III) Complexes.

The two iridium(III) complexes $[Ir(dfpy)_2(bpy)]^+PF_6^-$ and $[Ir(dfpy)_2(quqo)]^+PF_6^-$ were prepared by the same procedure[1]. Herein, only the synthesis of $[Ir(dfpy)_2(bpy)]^+PF_6^-$ (**1**) was described in detail.

$[Ir(dfpy)_2(bpy)]^+PF_6^-$ (1). The cyclometalated iridium(III) chlorobridged dimer $[Ir(dfpy)_2Cl]_2$ were prepared according to the literature method.[3] The solution of $[Ir(dfpy)_2Cl]_2$ (0.10 g, 0.079 mmol) and bpy (0.024 g, 0.158 mmol) in CH_2Cl_2/MeOH (30 mL, 2:1, v/v) was heated to reflux. After 4 hours, the red solution was cooled to room temperature and then added 10-fold excess of potassium hexafluorophosphate. The suspension was stirred for 2 h and then was filtered to remove insoluble inorganic salts. The solution was evaporated to dryness under reduced pressure. The crude product was applied to a silica gel column and eluted with CH_2Cl_2/acetone (15:1, v/v) to afford red solid in 59% yield. ^{1}H NMR: (400 MHz, d^6-DMSO), δ(ppm): 8.82 (d, 2H), 8.23-8.27 (m, 4H), 7.95-8.00 (t, 2H), 7.86 (d, 2H), 7.64-7.68 (t, 2H), 7.60 (d, 2H), 7.16-7.19 (t, 2H), 6.76-6.82 (t, 2H), 5.56 (d, 2H). Anal. Calcd for $IrC_{32}H_{20}N_4F_{10}P$: C, 43.99; H, 2.31; N, 6.41. Found: C, 43.73; H, 2.62; N, 6.81. MS(MALDI-TOF): m/e 729.2 ($M-PF_6$).

$[Ir(dfpy)_2(quqo)]^+PF_6^-$ (2). Yield 55%. ^{1}H NMR: (400 MHz, $CDCl_3$), δ(ppm): 10.07 (s, 1H), 8.91 (d, 1H), 8.76 (d, 1H) , 8.18-8.28 (m, 3H), 7.98 (d, 1H), 7.72-7.84 (m, 7H), 7.57-7.62 (t, 1H), 7.36-7.40 (t, 1H), 7.28-7.32 (t, 1H), 7.02-7.06 (m, 2H), 6.55-6.62 (m, 2H), 6.66-6.72 (m, 2H). Anal. Calcd for $IrC_{39}H_{23}N_5F_{10}P$: C, 48.05; H, 2.38; N, 7.18. Found: C, 48.33; H, 2.57; N, 7.52. MS(MALDI-TOF): m/e 830.1 ($M-PF_6$).

Cell Culture.

The HeLa cell line was provided by the Institute of Biochemistry and Cell Biology, SIBS, CAS (China). The HeLa cells were grown in MEM (Modified Eagle's Medium) supplemented with 10 % FBS (Fetal Bovine Serum) at 37 ^{o}C and 5 % CO_2. Cells (5×10^8/ L) were plated on 14 mm glass coverslips and allowed to adhere for 24 hours. The MCF-7 and HCT-8 cell lines were provided by Institute of Basic Medical Sciences Chinese Academy of Medical Sciences. The MCF-7 and HCT-8 cells were grown in RPMI 1640 supplemented with 10 % FBS (Fetal Bovine Serum) at 37 ^{o}C and 5 % CO_2.

Luminescence Imaging.

Before the experiments, cells were washed with PBS buffer and then incubated solely with 20 µM **1** or 2 in DMSO/PBS (pH 7, 1:49, v/v) for 10 min at 25 °C. Cell imaging was then carried out after washing cells with PBS.

Confocal luminescence imaging, including xy-scan, spectrum-scan and Z-scan luminescence imaging, was performed with an OLYMPUS IX81 laser scanning microscopy and a 60X oil-immersion objective lens. Excitation of the HeLa cells incubated with **1** or **2** at 405 nm was carried out with a semiconductor laser. Emission was collected at 520 ± 20 or 620 ± 20 nm for the HeLa cells incubated with **1** or **2**, respectively. Quantization by line plots and luminescent decay were analyzed using software package provided by OLYMPUS instrument.

Cytotoxicity Assay.

The in vitro cytotoxicity was measured using the 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt (MTS) assay[4] in human colorectal adenocarcinoma cell line HCT-8 and an breast cancer cell line MCF-7. Briefly, Cells growing in log phase were seeded into 96-well cell-culture plate at 1×10^4/well. The cells were incubated for 24 h at 37 °C under 5% CO_2. The complexe **1** or **2** (100 uL/well) at concentrations of 5,

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20 and 100 μM was added to the wells of the treatment group, and 100 uL/well DMSO diluted in RPMI 1640 at final concentration of 0.2% to the negative control group, respectively. The cells were incubated for 24 h at 37 °C under 5% CO_2. The combined MTS/PMS solution was added to each well of the 96-well assay plate, and incubated for an additional 2 h. An enzyme-linked immunosorbent assay (ELISA) reader (Bio-Rad, Model 550) was used to measure the OD490 (Absorbance value) of each well referenced at 630 nm. The following formula was used to calculate the viability of cell growth:

Viability (%) = (mean of Absorbance value of treatment group/mean Absorbance value of control) ·100.

The results are expressed as an average over five nominally identical measurements. Statistical significance was tested using one-way analysis of variance followed by the Student-Newman-Keuls test on SPSS 11.0 software. Statistical significance was set at $P < 0.05$.

Table S1. Absorption and photoluminescent properties of **1** and **2** in deaerated CH_2Cl_2 and aerated buffer solutions at 298 K.

Complex	Medium	λ_{abs}(log ε) [nm]	λ_{PL} [nm]	τ (μs)	Φ_{em}
1	CH_2Cl_2	236 (4.82), 244 (4.78), 281 (4.83), 360 (3.58)	517	0.90	0.25
	Buffer[a]	255 (4.52), 295 (4.26), 360 (3.62)	530	0.35	0.10
2	CH_2Cl_2	270 (4.70), 307 (4.29), 367 (4.21), 380 (4.25)	632	1.10	0.13
	Buffer[a]	275 (4.49), 302 (4.27), 388 (4.25)	634		~0.035

[a] DMSO/phosphate buffer solution (PBS) (pH 7, 1:49, v/v).

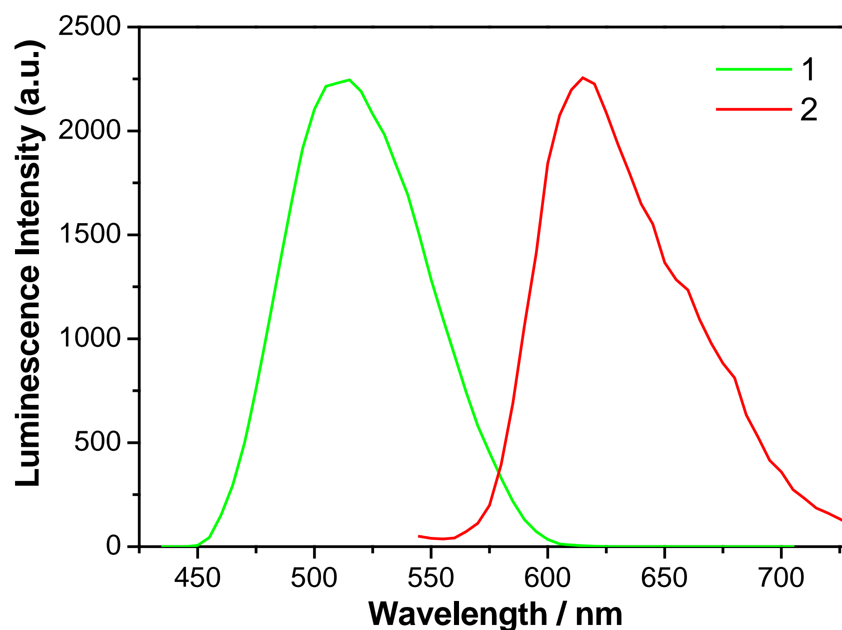

Figure S1. Photoluminescence spectra obtained from the living HeLa cells incubated with 20 μM **1** or **2** for 10 min at 25 °C (λ_{ex} = 405 nm).

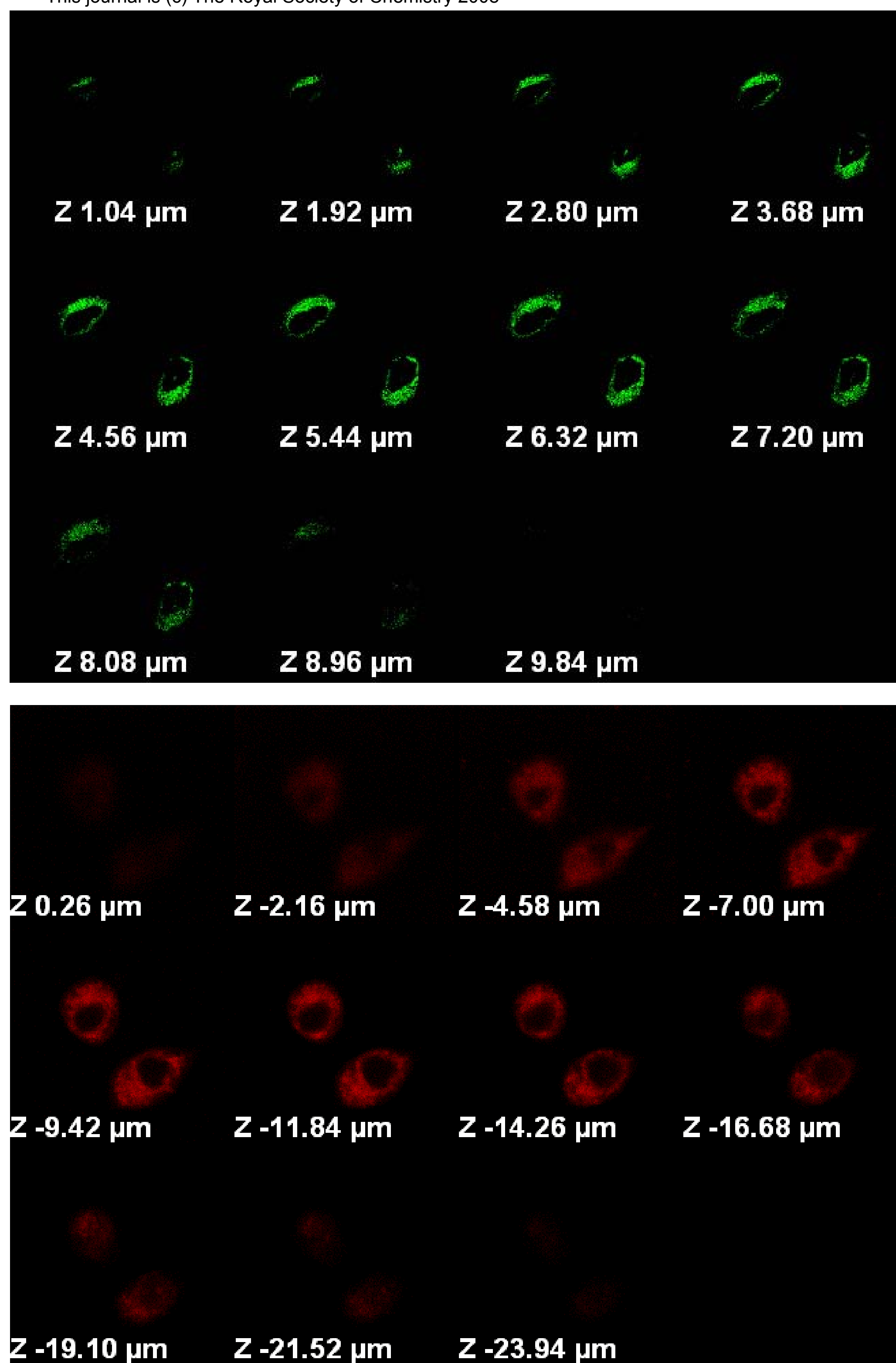

Figure S2. Z-scan images of the living HeLa cells incubated with 20 µM **1** (top) or **2** (bottom) in DMSO/PBS (pH 7, 1:49, v/v) for 10 min at 25 °C (λ_{ex} = 405 nm).

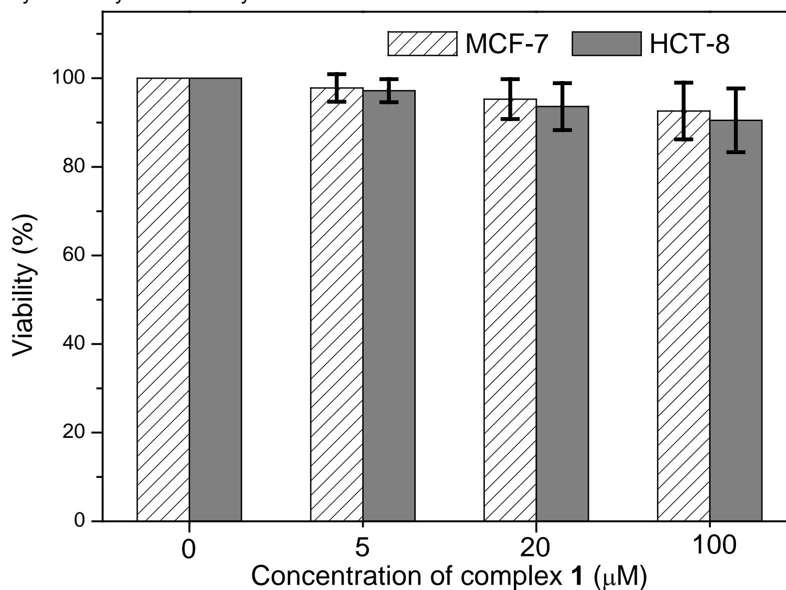

Figure S3. Cell viability values (%) estimated by MTS proliferation test versus concentrations of the iridium(III) complexes **1** after 24 h incubation at 37 ^{o}C. Two cell lines, MCF-7 and HCT-8, were cultured in the presence of 5−100 μM **1** at 37 °C for 24 h.

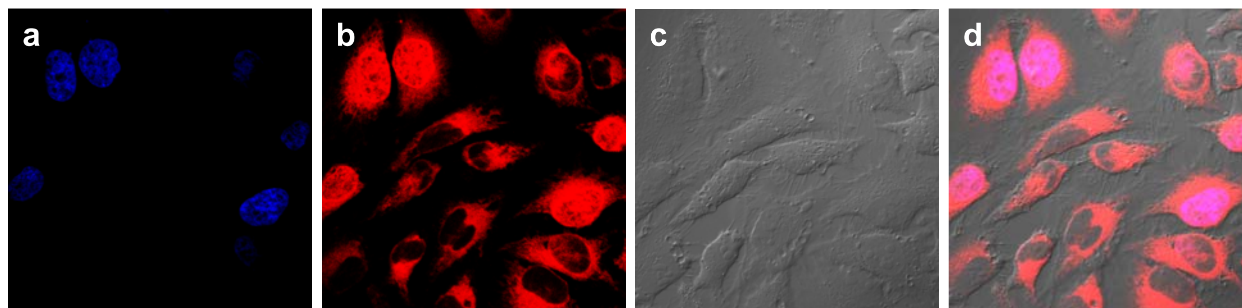

Figure S4. Confocal luminescence (*a* and *b*) and brightfield (*c*) images of the fixed HeLa cells stained with 20 μM **2** and 0.5 μg/mL DAPI (λ_{ex} = 405 nm). The signals of DAPI and **2** were collected from the blue channel (channel-1: 460 ± 20 nm) and red channel (channel-2: 620 ± 20 nm), respectively. Overlay of panels (*a*), (*b*) and (*c*) is shown in panel (*d*). Herein, to avoid the interference of DAPI in obtaining signal from **2** in the cytoplasm, low concentration (0.5 μg/mL) of DAPI was used to stain the nuclei of HeLa cells.

Movie: Comparison of **2** and DAPI for resistance to photobleaching.

References

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