

Ratiometric Phosphorescence Imaging of Hg(II) in Living Cells Based on a Neutral Iridium(III) Complex

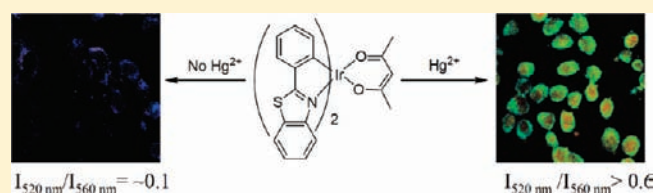
Yongquan Wu,[†] Hao Jing,[†] Zesheng Dong,[†] Qiang Zhao,^{*,†} Huazhou Wu,[†] and Fuyou Li^{*,†}

[†]Department of Chemistry, Fudan University, Shanghai, 200433, P. R. China

^{*}Key Laboratory for Organic Electronics & Information Displays (KLOEID) and Institute of Advanced Materials (IAM), Nanjing University of Posts and Telecommunications, Nanjing 210046, P. R. China

S Supporting Information

ABSTRACT: In this work, a neutral iridium(III) complex [Ir(bt)₂(acac)] (Hbt = 2-phenylbenzothiazole; Hacac = acetylacetonate) has been realized as a Hg(II)-selective sensor through UV–vis absorption, phosphorescence emission, and electrochemical measurements and was further developed as a phosphorescent agent for monitoring intracellular Hg(II). Upon addition of Hg(II) to a solution of [Ir(bt)₂(acac)], a noticeable spectral blue shift in both absorption and phosphorescent emission bands was measured. ¹H NMR spectroscopic titration experiments indicated that coordination of Hg(II) to the complex induces fast decomposition of [Ir(bt)₂(acac)] to form a new complex, which is responsible for the significant variations in optical and electrochemical signals. Importantly, cell imaging experiments have shown that [Ir(bt)₂(acac)] is membrane permeable and can be used to monitor the changes in Hg(II) levels within cells in a ratiometric phosphorescence mode.



INTRODUCTION

Cyclometalized iridium(III) complexes that possess a d⁶-electron configuration show strong spin–orbit coupling, efficient intersystem crossing from the singlet excited state to the triplet manifold, as well as an enhancement of the T₁–S₀ transition, thereby displaying efficient phosphorescent emission at room temperature.^{1,2} Due to their rich excited states, these iridium(III) complexes show unique photophysical properties, such as significant Stokes shifts for easy separation of excitation and emission and relatively long lifetimes compared to purely organic luminophores for eliminating fluorescent backgrounds by time-gated technology.³ Therefore, in recent years, phosphorescent iridium(III) complexes have been applied in the fields of chemosensors,^{4–6} biolabeling, and bioimaging.^{7–9} For example, Lo's group and our group independently reported approximately 50 phosphorescent iridium(III) complexes suitable for cellular imaging; some of these are capable of staining compartments within living cells.^{8–10} The effects of charge and lipophilicity on the cellular uptake of the iridium complexes were investigated in detail. Since positively charged compounds can generally traverse cell membranes more easily, this prior research focused exclusively on cationic iridium complexes. In addition, most of the reported chemosensors based on iridium(III) complexes can detect analytes in solution but not in biological samples.

Combinations of the recognition properties of chemosensors and the monitoring capability of bioimaging based on phosphorescent iridium(III) complexes have rarely been reported. To date, there has been only one example of such of phosphorescent cationic iridium(III) complexes to monitor cysteine/homocysteine in

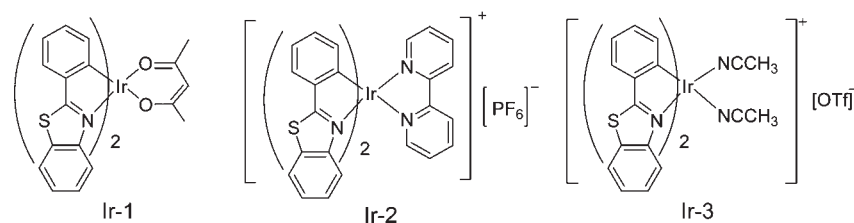
living cells reported by us.¹⁰ We are interested in developing the potential application of neutral phosphorescent iridium(III) complexes for cell imaging and intracellular detection. In the work described herein, we synthesized a neutral phosphorescent iridium(III) complex [Ir(bt)₂(acac)] (**Ir-1**, Hbt = 2-phenylbenzothiazole, Hacac = acetylacetonate, Scheme 1) containing sulfur atoms and found that it can enter the living cells and monitor intracellular Hg(II). The photophysical and electrochemical properties of **Ir-1** have been investigated in the absence and presence of Hg(II) ions, and the complex showed an obvious blue shift in its absorption and emission spectra upon addition of Hg(II). Furthermore, internalization of **Ir-1** into the living cells was investigated, and monitoring intracellular Hg(II) has been achieved with ratiometric luminescence bioimaging. For comparison, two other cationic iridium(III) complex salts, [Ir(bt)(bpy)]PF₆ (**Ir-2**, bpy = bipyridine) and [Ir(bt)₂(CH₃CN)₂]OTf (**Ir-3**, OTf = trifluoromethanesulfonate) (Scheme 1), have also been studied to compare their sensing ability.

EXPERIMENTAL SECTION

Materials. 2-Phenylbenzothiazole (bt), bipyridine (bpy), silver trifluoromethanesulfonate (AgOTf), tetrabutylammonium hexafluorophosphate (Bu₄NPF₆), KPF₆, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), and acetylacetonate (Hacac) were purchased from Acros. IrCl₃·3H₂O (Ir content > 54.51%) was an industrial product and used without further purification. Hg(ClO₄)₂·3H₂O,

Received: October 14, 2010

Published: July 20, 2011

Scheme 1. Chemical Structures of $[\text{Ir}(\text{bt})_2(\text{acac})]$ (**Ir-1**), $[\text{Ir}(\text{bt})_2(\text{bpy})]\text{PF}_6$ (**Ir-2**), and $[\text{Ir}(\text{bt})_2(\text{CH}_3\text{CN})_2]\text{OTf}$ (**Ir-3**)

$\text{Pb}(\text{NO}_3)_2$, $\text{Mg}(\text{NO}_3)_2$, $\text{Zn}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$, $\text{Ni}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$, KNO_3 , NaNO_3 , $\text{Co}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$, $\text{Cd}(\text{NO}_3)_2$, $\text{Cr}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$, $\text{Cu}(\text{NO}_3)_2 \cdot 3\text{H}_2\text{O}$, $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, CuSO_4 , and other reagents were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China).

General Experiments. ^1H NMR spectra were recorded with a Varian spectrometer at 400 MHz. Electrospray ionization mass spectra (ESI-MS) were measured on a Micromass LCTTM system. The UV-vis spectra were recorded on a Shimadzu UV-2550 spectrometer. Steady-state emission experiments at room temperature were measured on an Edinburgh Instruments spectrometer. The luminescence quantum yields of iridium complexes in air-equilibrated solution were measured with reference to quinine sulfate as a standard (yield = 0.53, in 0.1 M H_2SO_4 , $\lambda_{\text{ex}} = 365$ nm). Electrochemical measurements were performed with an Eco Chemie Autolab. All measurements were carried out in a one-compartment cell under N_2 gas, equipped with a glassy-carbon working electrode, a platinum wire counter electrode, and a Ag/Ag^+ reference electrode. The supported electrolyte was a 0.10 mol L^{-1} CH_3CN solution of tetrabutylammonium hexafluorophosphate (Bu_4NPF_6). The ferrocene/ferrocenium couple was added and used as the internal standard. The scan rate was 100 $\text{mV} \cdot \text{s}^{-1}$.

Synthesis of $[\text{Ir}(\text{bt})_2(\text{acac})]$ (Ir-1**).** Complex **Ir-1** was synthesized according to previous literature.¹¹ A mixture of 2-ethoxyethanol and water (3:1, v/v) was added to a flask containing $\text{IrCl}_3 \cdot 3\text{H}_2\text{O}$ (1 mmol) and phenylbenzothiazole (2.2 mmol). The mixture was heated under reflux for 24 h. After cooling to room temperature, the red solid precipitate was filtered to give crude cyclometalated iridium(III) chloro-bridged dimer. To the mixture of crude chloro-bridged dimer $[\text{Ir}_2(\text{bt})_4\text{Cl}_2]$ (0.2 mmol) and K_2CO_3 (1.4 mmol) were added 2-ethoxyethanol and Hacac (0.5 mmol), and then the slurry was refluxed for 12 h. After cooling to room temperature, a brown-red precipitate was collected by filtration and chromatographed using $\text{CH}_2\text{Cl}_2/\text{petroleum ether}$ (3:2, v/v) to give **Ir-1**, red solid. Yield: 45%; mp 269–270 °C. ^1H NMR (CDCl_3 , 400 MHz): δ (ppm) 8.09 (d, $J = 5.6$ Hz, 2H), 7.92 (d, $J = 8.0$ Hz, 2H), 7.68 (t, $J = 7.6$ Hz, 2H), 7.45 (d, $J = 7.4$ Hz, 2H), 7.24 (t, $J = 5.8$ Hz, 2H), 6.81 (t, $J = 7.0$ Hz, 2H), 6.68 (t, $J = 7.0$ Hz, 2H), 6.31 (d, $J = 8.0$ Hz, 2H), 5.13 (s, 1H), 1.78 (s, 6H). Anal. Calcd for $\text{C}_{31}\text{H}_{23}\text{IrN}_2\text{O}_2\text{S}_2$: C, 52.30; H, 3.26; N, 3.94. Found: C, 52.08; H, 3.30; N, 3.47. ESI-MS: m/z 712.1 (M^+).

Synthesis of $[\text{Ir}(\text{bt})_2(\text{bpy})]\text{PF}_6$ (Ir-2**).** A mixture of cyclometalated iridium(III) chloro-bridged dimer $[\text{Ir}_2(\text{bt})_4\text{Cl}_2]$ (120 mg, 0.1 mmol) and bipyridine (37 mg, 0.24 mmol) in 30 mL of $\text{CH}_2\text{Cl}_2/\text{MeOH}$ (1:1 v/v) was heated at reflux under nitrogen for 6 h. The solution was then cooled to room temperature, and KPF_6 (0.24 mmol) was added to the solution. Then, the mixture was evaporated under reduced pressure. Lastly, chromatography on silica gel with $\text{CH}_2\text{Cl}_2/\text{acetone}$ (4:1, v/v, $R_f = 0.37$) as the eluent gave 0.10 g (yield 56%) of **Ir-2** as a yellow solid. ^1H NMR (400 MHz, CDCl_3): δ (ppm) 8.57 (d, $J = 8.4$ Hz, 2H), 8.16 (t, $J = 7.6$ Hz, 2H), 8.07 (d, $J = 5.2$ Hz, 2H), 7.85 (d, $J = 8.0$ Hz, 2H), 7.80 (d, $J = 7.2$ Hz, 2H), 7.50 (t, $J = 6.4$ Hz, 2H), 7.33 (t, $J = 8.0$ Hz, 2H), 7.07 (m, 4H), 6.85 (t, $J = 7.2$ Hz, 2H), 6.38 (d, $J = 7.6$ Hz, 2H), 6.10 (d, $J = 8.4$ Hz, 2H). Anal. Calcd for $\text{C}_{36}\text{H}_{24}\text{IrF}_6\text{N}_4\text{P}_2\text{S}_2$: C, 47.31; H, 2.65; N, 6.13. Found: C, 47.55; H, 3.01; N, 5.80. ESI-MS: m/z 769.1 ($[\text{M} - \text{PF}_6]^+$).

Synthesis of $[\text{Ir}(\text{bt})_2(\text{CH}_3\text{CN})_2]\text{OTf}$ (Ir-3**).** $[\text{Ir}_2(\text{bt})_4\text{Cl}_2]$ (120 mg, 0.1 mmol) and AgOTf (62 mg, 0.24 mmol) were placed in a flask. Acetonitrile (15 mL) and dichloromethane (15 mL) were added, and the slurry was stirred at room temperature for 6 h. The solution was filtered, and the precipitate was washed three times with CH_3CN (0.5 mL \times 3). The filtrate and washings were combined and reduced by evaporation to a volume of 1 mL. The solution was cooled to -10 °C, and ether (15 mL) was slowly added while stirring. The yellow precipitate which formed was collected by filtration, washed with ether, and dried under vacuum for 24 h to yield the product. The product is a yellow solid (0.12 g, 70% yield). ^1H NMR (CDCl_3 , δ (ppm): 8.45 (d, $J = 8.4$ Hz, 2H), 8.01 (d, $J = 8.0$ Hz, 2H), 7.78 (t, $J = 5.8$ Hz, 2H), 7.62 (t, $J = 8.0$ Hz, 4H), 6.93 (t, $J = 8.0$ Hz, 2H), 6.71–6.75 (m, 2H), 6.15 (d, $J = 7.6$ Hz, 2H), 2.45 (s, 6H). Anal. Calcd for $\text{C}_{31}\text{H}_{22}\text{IrF}_3\text{N}_4\text{O}_3\text{S}_3$: C, 44.12; H, 2.63; N, 6.64. Found: C, 43.75; H, 2.73; N, 6.35. ESI-MS: m/z 695.1 ($[\text{M} - \text{OTf}]^+$).

X-ray Structure Determinations. A single crystal of complex $[\text{Ir}(\text{bt})_2(\text{CH}_3\text{CN})_2]\text{OTf}$ (**Ir-3**) was mounted on a glass fiber and transferred to a Bruker SMART CCD area detector. Crystallographic measurement was carried out using a Bruker SMART CCD diffractometer, σ scans, and graphite-monochromated $\text{Mo K}\alpha$ radiation ($\lambda = 0.71073$ Å) at room temperature. The structure was solved by direct methods and refined by full-matrix least-squares on F^2 using the program SHELXS-97.¹² All non-hydrogen atoms were refined anisotropically. Hydrogen atoms were calculated in ideal geometries. For the full-matrix least-squares refinements [$I > 2\sigma(I)$], the unweighted and weighted agreement factors of $R1 = \Sigma(F_o - F_c)/\Sigma F_o$ and $wR2 = [\Sigma w(F_o^2 - F_c^2)^2/\Sigma w F_o^4]^{1/2}$ were used. The CCDC reference number for $[\text{Ir}(\text{bt})_2(\text{CH}_3\text{CN})_2]\text{OTf}$ is 764139.

Mercury(II) Titration and Selectivity Experiments. A stock solution of $\text{Hg}(\text{II})$ ($\text{Hg}(\text{ClO}_4)_2 \cdot 3\text{H}_2\text{O}$) was prepared in deionized water, and other stock solutions of the metal ions Ag^+ , Cd^{2+} , Co^{2+} , Cr^{3+} , Cu^+ , Fe^{2+} , Mg^{2+} , Ni^{2+} , Pb^{2+} , Zn^{2+} , K^+ , and Na^+ (1 mM) were also prepared in deionized water. A stock solution of **Ir-1** (1 mM) was prepared in CH_3CN and then diluted to 20 μM with CH_3CN for titration and selectivity experiments. Titration experiments were performed by placing 2 mL of a solution of **Ir-1** (20 μM) in a quartz cuvette of 1 cm optical path length and then adding the $\text{Hg}(\text{II})$ stock solution incrementally through a micropipet. Test samples for selective optical response experiments were prepared by adding appropriate amounts of metal ion stock solutions to 2 mL of a solution of **Ir-1** (20 μM). All test solutions were stirred for 5 min and then tested by UV-vis absorption and fluorescence spectrometers. For fluorescence measurements, excitation was provided at 365 nm and emission was collected from 450 to 750 nm.

Cell Culture. The KB and HeLa cell lines were provided by the Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences. The KB and HeLa cells were grown in RPMI 1640 (Roswell Park Memorial Institute's Medium) supplemented with 10% FBS (Fetal Bovine Serum) at 37 °C and 5% CO_2 . Cells were plated on 18 mm glass coverslips and allowed to adhere for 24 h.

Confocal Luminescence Imaging. A stock solution of **Ir-1** (1 mM) was prepared in dimethyl sulfoxide (DMSO) and then diluted

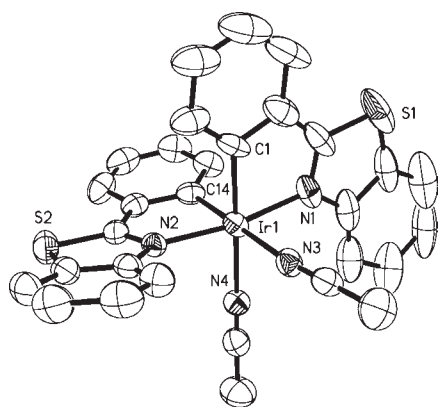


Figure 1. ORTEP diagram of Ir-3. Hydrogen atoms and OTf anion were omitted for clarity.

to 10 or 2 μM with phosphate-buffered saline (PBS) for imaging experiments. Confocal luminescence imaging of cells was performed with an OLYMPUS FV1000 IX8 confocal fluorescence microscope equipped with a $60\times$ oil-immersion objective lens, excitation at 405 nm was carried out with a semiconductor laser, and emission was collected at 515 ± 15 and 560 ± 10 nm.

Theoretical Calculations. Geometric and energy optimizations were performed with the Gaussian 03 program based on the density functional theory (DFT) method.¹³ The Becke's three-parameter hybrid functional with the Lee–Yang–Parr correlation functional (B3LYP) was employed for all calculations. The LANL2DZ basis set was used to treat the iridium atom, whereas the 6-31G* basis set was used to treat all other atoms.

RESULTS AND DISCUSSION

Crystal Structure of Ir-3. The single crystal of Ir-3 was obtained from the mixed solution of CH_2Cl_2 and ether. Figure 1 shows the ORTEP view of Ir-3. The crystallographic refinement parameters of Ir-3 are summarized in Table 1, and the selected bond distances (Angstroms) and angles (degrees) are listed in Table S1 (see Supporting Information).

As shown in Figure 1, the iridium(III) center in Ir-3 adopts a distorted octahedral coordination geometry with cis-metallated carbons atoms and trans nitrogen atoms, similar to what has been revealed by previous structural studies on mononuclear species containing the C[^]N cyclometalated ligands.¹⁴ Moreover, the Ir–C (~ 2.04 Å) and Ir–N (C[^]N) (~ 2.06 Å) distances are similar to those reported for other iridium(III) complexes.¹⁴ However, the Ir–N (CH₃CN) distances (2.142(7) and 2.161(7) Å) are longer than the corresponding Ir–N (bt) distances (2.061(5) and 2.064(4) Å) because of their trans influence. In addition, the relatively wide bite angle (87.39°) of N₃–Ir–N₄ may be attributed to the small size of CH₃CN.

Photophysical Properties. The electronic absorption and emission spectra of Ir-1, Ir-2, and Ir-3 in CH_2Cl_2 and CH_3CN solutions at room temperature are shown in Figure 2, and the corresponding data are listed in Table 2. With reference to previous photophysical studies on related iridium(III) systems,¹⁵ all complexes have intense absorption bands in the ultraviolet region ca. 260–340 nm, with an extinction coefficient ϵ of $\sim 10^5$ $\text{dm}^3 \text{mol}^{-1} \text{cm}^{-1}$, which are assigned to spin-allowed singlet intraligand (¹LC) transitions ($\pi \rightarrow \pi^*$) (bt and bpy). The weak bands at 350–500 nm with ϵ of $\sim 10^3$ $\text{dm}^3 \text{mol}^{-1} \text{cm}^{-1}$ have

Table 1. Crystallographic Data for Ir-3

Ir-3 [Ir(bt) ₂ (CH ₃ CN) ₂] ₂ OTf	
empirical formula	C ₃₁ H ₂₂ F ₃ IrN ₄ O ₃ S ₃
cryst syst	monoclinic
space group	P2(1)/c
crystal size (mm)	0.18 × 0.16 × 0.15
a, Å	13.054(3)
b, Å	13.683(3)
c, Å	17.743(4)
α, deg	90
β, deg	91.47(3)
γ, deg	90
V, Å ³	3168.0(11)
Z	4
calcd density, g/cm ³	1.769
μ, mm ⁻¹	4.469
F(000)	1648
final R indices [<i>I</i> > 2σ(<i>I</i>)]	0.0364
wR2 [<i>I</i> > 2σ(<i>I</i>)]	0.0810
R1 (all data)	0.0668
wR2 (all data)	0.0930
GOF on F ²	0.981

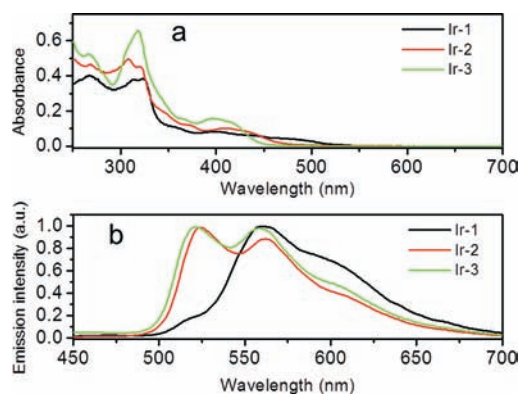


Figure 2. Absorption (a) and emission spectra (b) of Ir-1, Ir-2, and Ir-3 in CH_3CN solution with a concentration of 10 μM , $\lambda_{\text{ex}} = 365$ nm.

been assigned to the mixed singlet and triplet metal-to-ligand charge-transfer (MLCT) transition ($d\pi(\text{Ir}) \rightarrow \pi^*(\text{bpy})$) and intraligand (LC_{bt}) transitions.

In CH_3CN solution, Ir-1 displays a structured emission band with a maximum at a wavelength of 563 nm and a shoulder at 603 nm. With reference to quinine sulfate as a standard in an air-equilibrated solution, the quantum efficiencies (Φ_{em}) of Ir-1 and Ir-2 in CH_3CN solution were measured to be 0.016 and 0.031, respectively. For the cationic iridium(III) complex Ir-2 containing a diimine ligand, the emission commonly comes from a mixed excited state containing ³MLCT ($d\pi(\text{Ir}) \rightarrow \pi^*_{\text{bpy}}$) and ³LLCT ($\pi_{\text{bt}} \rightarrow \pi^*_{\text{bpy}}$) transitions. However, for the neutral complex Ir-1 containing O[^]O ligand with a higher triplet energy level, the emission comes from a mixed excited state containing ³LC ($\pi_{\text{bt}} \rightarrow \pi^*_{\text{bt}}$) and ³MLCT ($d\pi(\text{Ir}) \rightarrow \pi^*_{\text{bt}}$) transitions.¹⁶

Optical Response of Ir-1 to Hg(II). Recently, fluorescent chemosensors for mercury ions have attracted increasing attention, because mercury is a dangerous and widespread global

Table 2. Photophysical Data of Complexes Ir-1, Ir-2, and Ir-3

complexes	solvent	$\lambda_{\text{abs}}/\text{nm}$ ($\epsilon/\text{dm}^3 \text{ mol}^{-1} \text{ cm}^{-1}$)	$\lambda_{\text{PL,max}}(\text{nm})$	Φ_{em}
Ir-1	CH ₂ Cl ₂	266 (34 673), 324 (30 199), 435 (5754)	557, 590sh	0.016
	CH ₃ CN	269 (33 831), 323 (30 597), 438 (5577)	563, 603sh	
Ir-2	CH ₂ Cl ₂	274 (33 884), 310 (28 840), 412 (6309)	525, 561sh	0.031
	CH ₃ CN	270 (31 735), 309 (28 953), 409 (6882)	524, 560sh	
Ir-3	CH ₂ Cl ₂	268 (15 135), 322 (19 054), 400 (5011)	516, 548sh	0.008
	CH ₃ CN	266 (14 876), 318 (19 354), 396 (4851)	520, 560sh	

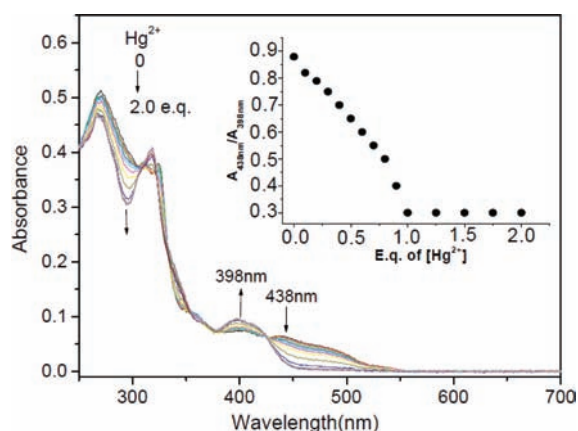


Figure 3. Changes in the UV–vis absorption spectra of Ir-1 (20 μM) in CH₃CN solution with various amounts of Hg(II) ions (0–40 μM). (Inset) Titration curve of the absorbance ratio at 438 nm to 398 nm for Ir-1 upon addition of Hg(II) ions.

pollutant and causes serious environmental and health problems.¹⁷ The responses of Ir-1, Ir-2, and Ir-3 to Hg(II) were investigated through titration in UV–vis absorption, phosphorescence emission, and electrochemical titration measurements. No significant changes in the absorption and phosphorescence emissions were observed for Ir-2 or Ir-3 (Figures S1–S4 in the Supporting Information). However, Ir-1 proved to be able to specifically sense Hg(II), as manifested in an evident optical change.

a. UV–vis Absorption Spectroscopy. Figure 3 shows the absorption changes of Ir-1 as a function of Hg(II) concentration in CH₃CN solution. Upon addition of Hg(II) to a solution of Ir-1, the absorption band at 438 nm progressively disappears while a new band appears at 398 nm. The color of the solution changes from yellow to green (Figure 4), indicating that Ir-1 can serve as a sensitive indicator for Hg(II). The stoichiometry of Ir-1 is given by the variation of $A_{438 \text{ nm}}/A_{398 \text{ nm}}$ with respect to the number of equivalents of Hg(II) added. Effectively, $A_{438 \text{ nm}}/A_{398 \text{ nm}}$ decreases continuously until addition of 1 equiv of Hg(II). Further addition of Hg(II) induces only very minor changes in $A_{438 \text{ nm}}/A_{398 \text{ nm}}$ (Figure 3, inset), indicating that Ir-1 forms a 1:1 complex with Hg(II). The binding constant (K) calculated from absorption titration data is $9.3 \times 10^4 \text{ M}^{-1}$. More importantly, the binding process is fairly fast and we could observe a distinct color change within 2 min. Therefore, Ir-1 could be potentially used for real-time tracking of Hg(II) in organisms.

b. Luminescent Properties of Ir-1 at Different pH Values. The pH dependence of the luminescent emission of Ir-1 was investigated. As shown in Figure 5, Ir-1 exhibits the same emission color (yellow) and intensity at various pH values. Similar phenomena

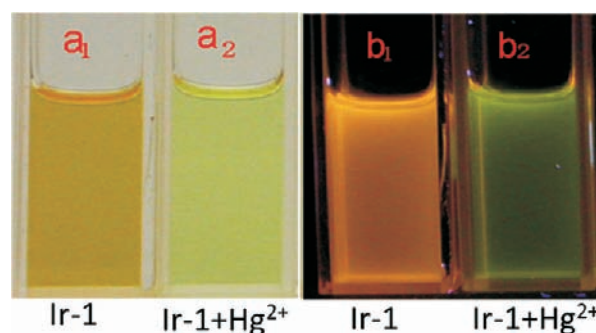


Figure 4. Solution (a) and luminescence (b) color photographs of Ir-1 (40 μM in CH₃CN solution) before (a₁ and b₁) and after (a₂ and b₂) addition of 1 equiv of Hg(II).

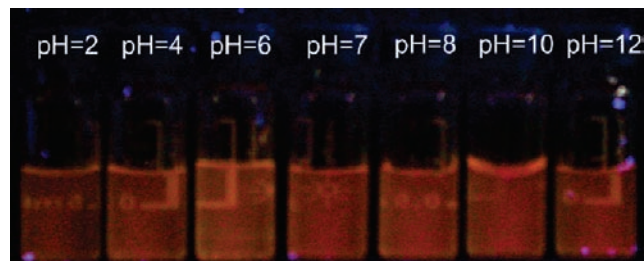


Figure 5. Luminescent photographs of Ir-1 at different pH values.

were also observed for Ir-2 and Ir-3 (Figure S5 in the Supporting Information). Furthermore, as shown in Figure S6 in the Supporting Information, the emission integral counts of Ir-1 (10 μM) at different pH values could be calculated to be 7.05×10^6 , 6.96×10^6 , 6.78×10^6 , 6.75×10^6 , 7.17×10^6 , 7.01×10^6 , and 7.24×10^6 , corresponding to the pH range from 2 to 12. The average value of emission integral counts at different pH values is 7.05×10^6 , and the error of emission integral counts is 0.14×10^6 . These facts revealed that Ir-1 is stable within the pH range from 2 to 12, and its response ability toward Hg(II) is almost invariable. Therefore, complex Ir-1 can be used to detect Hg(II) without pH interference.

c. Luminescent Spectroscopy. It is well known that luminescent emission spectroscopy is more sensitive toward small changes that affect the electronic properties of molecular receptors. Hence, the response of Ir-1 to Hg(II) was further investigated by a photoluminescence technique. Upon addition of 1 equiv of Hg(II), the emission band of Ir-1 exhibited a blue shift of $\sim 40 \text{ nm}$ (Figure 6), corresponding to an evident change in emission color from yellow to green (Figure 4). Hence, Ir-1 shows efficient colorimetric sensing of Hg(II). As determined by

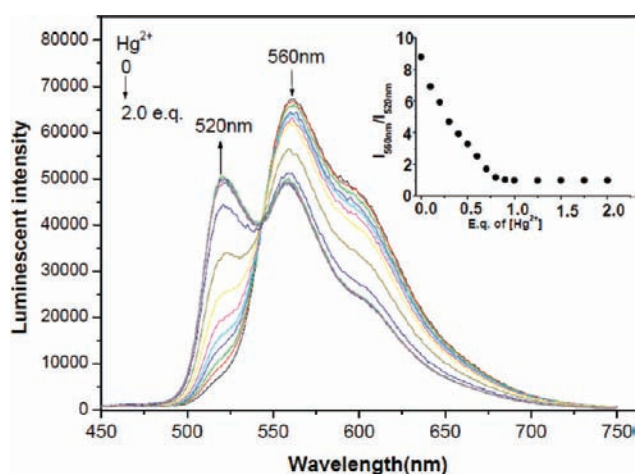


Figure 6. Changes in the luminescence spectra of Ir-1 (20 μM) in air-equilibrated CH_3CN solution with various amounts of $\text{Hg}(\text{II})$ (0–40 μM). (Inset) Titration curve of Ir-1 with $\text{Hg}(\text{II})$.

variations in the luminescence response of Ir-1 in the presence of various amounts of $\text{Hg}(\text{II})$, the stoichiometry of the complex formed between Ir-1 and $\text{Hg}(\text{II})$ is 1:1, which is consistent with the result obtained from the UV–vis absorption spectra. Moreover, in a control experiment, the response of ligand 2-phenylbenzothiazole to $\text{Hg}(\text{II})$ was also investigated by absorption and emission spectroscopies. It can be seen from the Figure S7 (see Supporting Information) that no significant changes in absorption and phosphorescence emission were observed for ligand Hbt in CH_3CN solution before and after addition of 2 equiv of $\text{Hg}(\text{II})$, which are different from complex Ir-1. These observations indicate that Ir-1 could serve as a “naked eye” indicator for $\text{Hg}(\text{II})$ ions.

Electrochemical Studies. Furthermore, the electrochemical response of Ir-1 to $\text{Hg}(\text{II})$ was studied by cyclic voltammetry (CV). In the absence of $\text{Hg}(\text{II})$, Ir-1 shows a quasi-reversible oxidation wave at 0.60 V, which can be assigned to the metal-centered $\text{Ir}^{\text{III}}/\text{Ir}^{\text{IV}}$ and cyclometalated ligand oxidation processes.¹⁸ A significant modification in the cyclic voltammetry was observed upon addition of increasing amounts of $\text{Hg}(\text{II})$ to a solution of Ir-1 (Figure 7). The intensity of the oxidation wave at 0.60 V decreased and a new reversible oxidation wave at 1.03 V appeared with the progressive addition of $\text{Hg}(\text{II})$. However, the reduction potential of Ir-1 was hardly changed with various amounts of $\text{Hg}(\text{II})$ ions (Figure S8 in the Supporting Information). Moreover, a control experiment about the effect of $\text{Hg}(\text{II})$ on the electrochemical signal of ligand Hbt was conducted. As shown in Figure S9 in the Supporting Information, ligand Hbt has a quasi-reversible oxidation wave at 1.67 V. Some changes in the cyclic voltammetry were observed upon addition of excess amounts of $\text{Hg}(\text{II})$ to a solution of Hbt, that is, the oxidation wave was shifted positively. Hence, the new reversible oxidation wave at 1.03 V with addition of $\text{Hg}(\text{II})$ should be assigned to the metal-centered $\text{Ir}^{\text{III}}/\text{Ir}^{\text{IV}}$ and cyclometalated ligand oxidation processes of new complex $[\text{Ir}(\text{bt})_2(\text{CH}_3\text{CN})_2]^+$ rather than oxidation of $\text{Hg}(\text{II})$ –Hbt complex.

Selective Optical Response of Ir-1 to Various Metal Ions.

For an excellent chemosensor, high selectivity is a matter of necessity. The selectivity of Ir-1 for $\text{Hg}(\text{II})$ over other cations was evaluated by examination of the emission spectra. In light of the obvious blue shift of the emission spectra for Ir-1 upon addition of $\text{Hg}(\text{II})$, we investigated the selective response of Ir-1

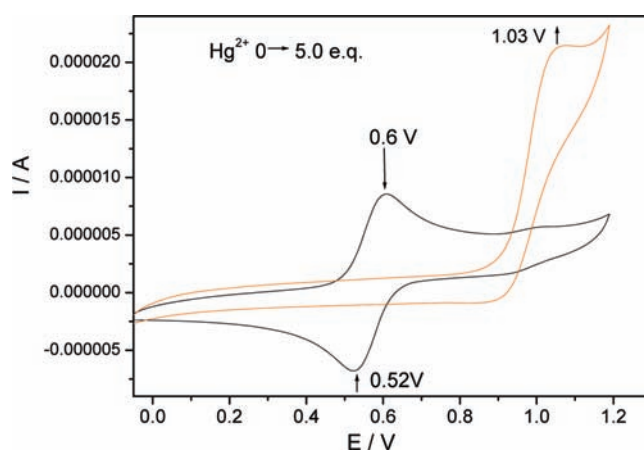


Figure 7. Cyclic voltammograms of Ir-1 (50 μM) in CH_3CN solution in the absence and presence of 5 equiv of $\text{Hg}(\text{II})$ ions.

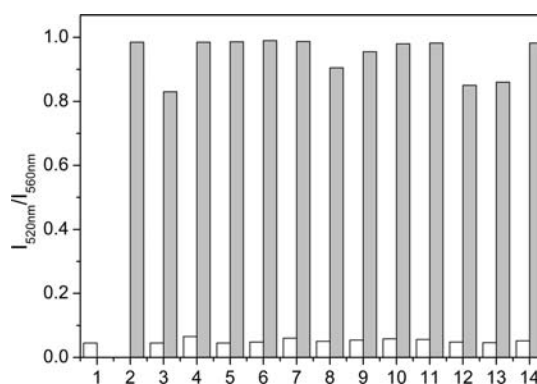


Figure 8. Luminescent response of Ir-1 (20 μM) in the presence of various metal cations (1 equiv) in CH_3CN solutions. Bars represent the ratio ($I_{520\text{ nm}}/I_{560\text{ nm}}$) of emission intensity at 520 and 560 nm. White bars represent addition of 1-fold various metal cations to a 20 μM solution of Ir-1. Solid bars represent the $I_{520\text{ nm}}/I_{560\text{ nm}}$ ratio after addition of $\text{Hg}(\text{II})$ (20 μM): (1) without metal cation; (2) Hg^{2+} ; (3) Ag^+ ; (4) Cd^{2+} ; (5) Co^{2+} ; (6) Cr^{3+} ; (7) Cu^+ ; (8) Fe^{2+} ; (9) Mg^{2+} ; (10) Ni^{2+} ; (11) Pb^{2+} ; (12) Zn^{2+} ; (13) K^+ ; (14) Na^+ .

to various metal ions by using the ratio of the phosphorescent emission intensity at 560 and 520 nm ($I_{560\text{ nm}}/I_{520\text{ nm}}$) as the output signal. To verify a specific response to $\text{Hg}(\text{II})$, variations of the luminescence spectra of Ir-1 caused by other related heavy, transition, and main group metal ions were recorded. As shown in Figure 8 and Figure S10 (in the Supporting Information), only addition of $\text{Hg}(\text{II})$ resulted in a prominent luminescence change, whereas addition of 1 equiv of other competitive cations (such as Ag^+ , Cd^{2+} , Co^{2+} , Cr^{3+} , Cu^+ , Fe^{2+} , Mg^{2+} , Ni^{2+} , Pb^{2+} , Zn^{2+} , K^+ , and Na^+) caused only slight luminescent changes. Moreover, a competition experiment was done to further explore the utility of Ir-1 as an ion-selective probe for $\text{Hg}(\text{II})$. The coexistent metal cations had a negligible interfering effect on the luminescent blue shift of Ir-1 upon addition of $\text{Hg}(\text{II})$ (Figure S11 in the Supporting Information). All results indicate that the selectivity of Ir-1 for $\text{Hg}(\text{II})$ over other cations is remarkably high.

Furthermore, the selectivity of Ir-1 for $\text{Hg}(\text{II})$ over other cations was also evaluated by examination of the absorption spectra (Figure S12 in the Supporting Information). Herein, the ratio of the absorbance intensity at 438 and 398 nm ($A_{438\text{ nm}}/A_{398\text{ nm}}$)

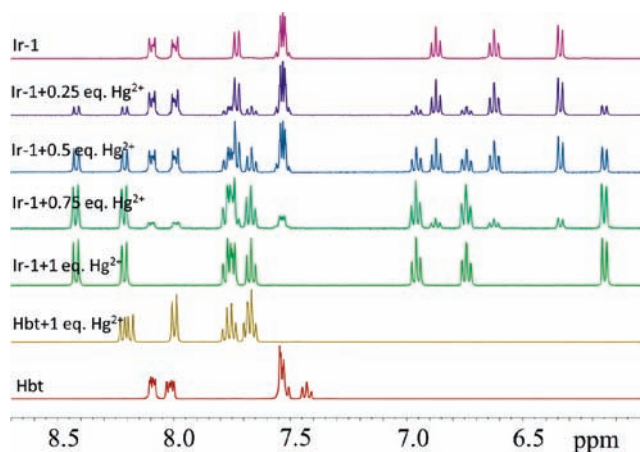


Figure 9. Partial ^1H NMR spectral changes of Ir-1 in CD_3CN in the presence of $\text{Hg}(\text{II})$ (0–1 equiv).

was used as the output signal. Due to the low affinity with Ir-1, other metal ions (such as Ag^+ , Cd^{2+} , Co^{2+} , Cr^{3+} , Cu^+ , Fe^{2+} , Mg^{2+} , Ni^{2+} , Pb^{2+} , Zn^{2+} , K^+ , and Na^+) showed hardly any change in $A_{438\text{nm}}/A_{398\text{nm}}$. However, addition of $\text{Hg}(\text{II})$ resulted in a prominent absorption change. These facts indicate that Ir-1 could be used as a highly selective and ratiometric sensor for $\text{Hg}(\text{II})$.

Mechanism of $\text{Hg}(\text{II})$ Sensing by Ir-1. In terms of Pearson's hard–soft acid–base theory, 19 $\text{Hg}(\text{II})$ is a soft ion (soft acid) and can interact preferentially with sulfur (a soft base). Palomares and co-workers reported that the interaction of $\text{Hg}(\text{II})$ with the sulfur atoms in ruthenium complexes induces a color change. 20 We can deduce that the variations in the optical-electrochemical properties of Ir-1 are due to coordination of $\text{Hg}(\text{II})$ by the lone-pair electrons on the sulfur atom of the cyclometalated ligand 2-phenylbenzothiazole (bt). Herein, to seek further detailed information on the binding mechanism, ^1H NMR spectroscopic titration experiments of complex Ir-1 with different equivalents of $\text{Hg}(\text{II})$ ion were conducted. Upon addition of $\text{Hg}(\text{II})$ ion, a variation in the ^1H NMR spectra of Ir-1, especially in the cyclometalated ligand bt moiety (see Figure 9), was observed. However, no ^1H NMR characteristic peaks at $\delta = 8.09, 8.07, 7.53,$ and 7.43 ppm that correspond to free Hbt appeared upon addition of various amounts of $\text{Hg}(\text{II})$, indicating that the ligand bt still bound with iridium(III). Interestingly, the characteristic peaks of free Hacac at 2.01 ppm appeared for Ir-1 in the presence of 1.0 equiv of $\text{Hg}(\text{II})$ (Figure S13 in the Supporting Information), suggesting that ligand acac was removed from complex Ir-1. Hence, we can deduce that rupture of Ir–O coordinated bonds occurs and free acac ligand is released from Ir-1 after addition of $\text{Hg}(\text{II})$. Furthermore, the ^1H NMR peaks of $[\text{Ir}(\text{bt})_2(\text{CH}_3\text{CN})_2]^+$ can be found in the spectrum of the mixture of Ir-1 with $\text{Hg}(\text{II})$ (Figure S14 in the Supporting Information). Therefore, we tentatively summarize the sensing mechanism of Ir-1 with $\text{Hg}(\text{II})$ as follows (see Scheme 2): the sulfur atoms (soft base) on the bt ligand bind with $\text{Hg}(\text{II})$ (soft acid) according to Pearson's soft–hard acid–base theory, which induces fast decomposition of Ir-1 with departure of acac from the complex to form a new complex $[\text{Ir}(\text{bt})_2(\text{CH}_3\text{CN})_2]^+$. Such a process results in the evident changes of absorption, emission, and electrochemical properties.

The sensing responses of complexes Ir-2 and Ir-3 to $\text{Hg}(\text{II})$ were very weak, which is different from those of Ir-1. In order to clarify such difference, the charge distributions of these complexes

Scheme 2. Illustration of the Sensing Mechanism

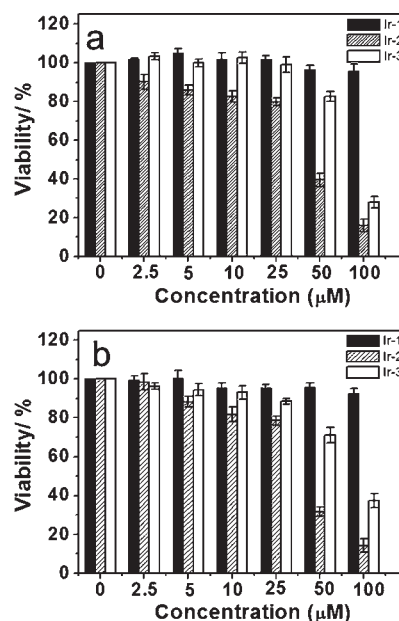
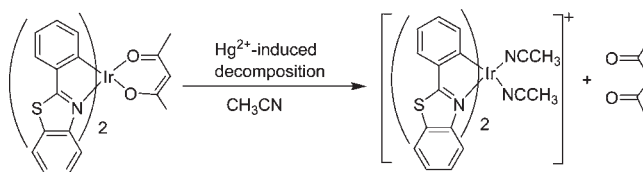


Figure 10. Cell viability values (%) estimated by MTT proliferation test versus incubation concentrations of Ir-1, Ir-2, and Ir-3. KB (a) and HeLa (b) cells were cultured in the presence of 5–100 μM complexes at 37 $^\circ\text{C}$ for 24 h.

were investigated through DFT calculations. Surface charge analysis based on DFT calculations indicated that the charge on the sulfur atom of Ir-1 (the electron cloud density 0.247) is more negative than those of Ir-2 (the electron cloud density 0.310) and Ir-3 (the electron cloud density 0.292). Thus, Ir-1 combines with $\text{Hg}(\text{II})$ to form a coordinated complex more easily, whereas Ir-2 and Ir-3 exhibit weaker coordination ability toward $\text{Hg}(\text{II})$. In addition, Ir-2 and Ir-3 are cationic, making coordination with $\text{Hg}(\text{II})$ more difficult.

Cytotoxicity of Ir-1. We selected two cell lines (KB and HeLa cells) for investigation of the toxicity about three Ir complexes, and the toxicity data are shown in Figure 10. Upon incubation with Ir-1 (10 μM) for 24 h, only less than 5% of the KB and HeLa cells died. When the concentration of Ir-1 was increased to 25 and 100 μM , the cell viability still remains above 90%. For the other two complexes Ir-2 and Ir-3, their cytotoxicity is higher than that of Ir-1, especially at high concentration (>50 μM). However, at the concentration (10 μM) of the cell imaging experiment, the cell viability of these two complexes for both KB and HeLa remains above 80%, even with an incubation time of 24 h. These three complexes show relatively low cytotoxicity in the cell imaging experiment (concentration of 10 μM and incubation time of 15 min). In particular, complex Ir-1 exhibits the lowest cytotoxicity both at low and at high concentration and will not kill the cell being probed.

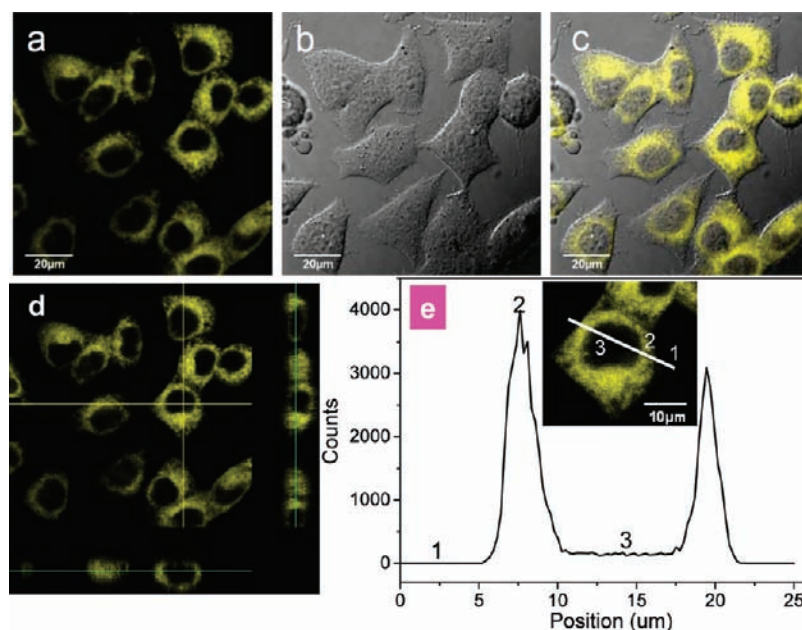


Figure 11. Confocal luminescence (a), bright-field (b), and overlay (c) images of living KB cells incubated with Ir-1 ($10 \mu\text{M}$) in PBS for 15 min at 37°C ($\lambda_{\text{ex}} = 405 \text{ nm}$). (d) Overlay Z-scan confocal image of the living KB cells incubated with Ir-1 ($10 \mu\text{M}$). (e) Luminescence intensity profile and luminescence image (across the line) of KB cells.

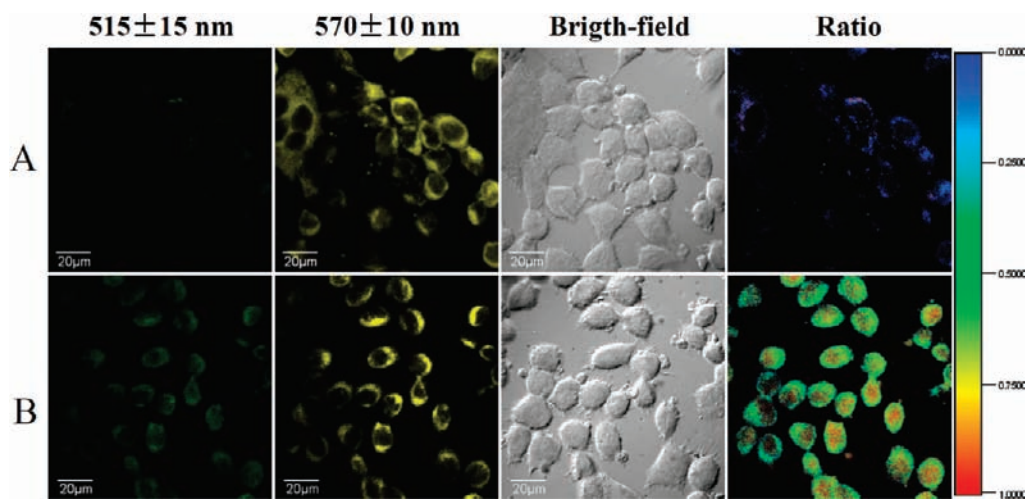


Figure 12. Ratiometric phosphorescence images of Ir-1-incubated KB cells treated without or with Hg(II). (A) KB cells incubated with Ir-1 for 15 min. (B) KB cells incubated with Ir-1 ($10 \mu\text{M}$) for 15 min and then further treated with $100 \mu\text{M}$ Hg(II) for 1 h. Emission was collected by the green channel ($515 \pm 15 \text{ nm}$) and yellow channel ($570 \pm 10 \text{ nm}$) ($\lambda_{\text{ex}} = 405 \text{ nm}$). Ratio of emission intensity at 515 ± 15 to $570 \pm 10 \text{ nm}$ is also shown.

Luminescence Bioimaging of Hg(II) in Living Cells Using Ir-1. In contrast to conventional luminescence-enhanced detection, ratiometric measurement allows simultaneous recording of two emission intensities at different wavelengths in the presence and absence of analytes and thus provides built-in correction for environmental effects.²¹ As described above, addition of Hg(II) into an Ir-1 solution induced a blue shift of approximately 40 nm in the emission spectrum. Therefore, Ir-1 has the potential to serve as a luminescent probe in ratiometric bioimaging. The applicability of Ir-1 in the ratiometric monitoring of intracellular Hg(II) was investigated by confocal luminescence microscopy. For this, optical windows at 515 ± 15 (green channel) and $570 \pm 10 \text{ nm}$ (yellow channel) were chosen as the two signal outputs.

For adequate interaction between Ir-1 and intracellular Hg(II), the reaction was allowed to proceed at 37°C for 1 h. As shown in Figure 11, intense emission was observed within the KB cells incubated with $10 \mu\text{M}$ Ir-1 in PBS for 15 min at 37°C . Overlay of confocal luminescence and bright-field images demonstrated that the luminescence was evident in the cytoplasm over the nucleus and membrane (Figure 11c), which was further confirmed by Z-scan confocal microscopy (Figure 11d and movie in the Supporting Information) and quantization analysis of luminescence intensity. As shown in Figure 11e, further quantization by line plots (inset) revealed that the signal ratio (I_2/I_3) between the cytoplasm (region 2) and nucleus (region 3) reached 16, implying weak nuclear uptake of Ir-1. The bioimaging results

indicated that the neutral complex **Ir-1** had good cell membrane permeability and entered the living cells easily. This is the first case of a neutral iridium(III) complex as a luminescent probe for cell imaging application, although approximately 50 cationic iridium(III) complexes have been used as bioimaging probes.^{7–10}

Concerning the ratiometric bioimaging, KB cells incubated with 10 μM **Ir-1** for 15 min showed an emission ratio $I_{515\text{nm}}/I_{570\text{nm}}$ of ~ 0.1 between the green luminescence channel (515 ± 15 nm) and the yellow luminescence channel (570 ± 10 nm), as shown in Figure 12A. The **Ir-1**-treated cells were then exposed to 100 μM Hg(II) for 1 h at 37 $^{\circ}\text{C}$. The phosphorescence intensity of the green channel increased, while that of the yellow channel decreased slightly, and the ratio of luminescence intensities at 515 ± 15 and 570 ± 10 nm was increased to >0.6 (Figure 12B). Furthermore, **Ir-1** can sense the change of Hg(II) in living cell incubated with 10 μM Hg(II) (Figure S15 in Supporting Information). KB cells incubated with 2 μM **Ir-1** for 30 min showed an emission ratio $I_{515\text{nm}}/I_{570\text{nm}}$ of <0.2 . The **Ir-1**-incubated cells were then exposed to 10 μM Hg(II) for 8 h at 37 $^{\circ}\text{C}$. The phosphorescence intensity of the green luminescence channel increased, and the emission ratio $I_{515\text{nm}}/I_{570\text{nm}}$ was increased to ≥ 0.6 . These data suggest that **Ir-1** could still be used for monitoring changes in a low concentration of Hg(II).

CONCLUSIONS

In summary, we demonstrated the internalization of a neutral iridium(III) complex **Ir-1** into living cells and further selective detection of Hg(II) in cell samples by ratiometric measurement. Compared to two cationic complexes **Ir-2** and **Ir-3**, **Ir-1** specifically responds to Hg(II) with a noticeable change in phosphorescent emission color from yellow to green. The interaction between Hg(II) and the sulfur atom of the C^N cyclometalated ligand is responsible for the significant variations in the optical and electrochemical signals. Importantly, cell imaging experiments have demonstrated that **Ir-1** is membrane permeable and can readily reveal changes in intracellular Hg(II) concentration in a ratiometric way. To our knowledge, this is the first report of a neutral iridium complex entering living cells and realizing ratiometric bioimaging of Hg(II). The result described herein may well be of interest to others working in the areas of phosphorescent heavy metal complexes, bioimaging, and luminescent molecular probes.

ASSOCIATED CONTENT

S Supporting Information. Selected bond distances and angles for **Ir-3**, changes in the absorption and luminescence spectra of **Ir-2** in CH_3CN solution with various amounts of Hg(II), changes in the luminescence spectra of **Ir-3** (5 μM) in air-equilibrated CH_3CN solution with various amounts of Hg(II) ions (0–250 μM), luminescent photographs of **Ir-2** and **Ir-3** at different pH values, luminescent spectra and luminescent integral counts of **Ir-1** (10 μM) at different pH values, absorption and emission spectra of ligand 2-phenylbenzothiazole (Hbt) in CH_3CN solution without and with 2 equiv of Hg(II) ions, changes in the reduction potential of **Ir-1** with various amounts of Hg(II), cyclic voltammograms of Hbt (0.5 mM) in CH_3CN solution in the absence and presence of various amounts of Hg(II) ions (0–2.5 mM), response of luminescent spectra of **Ir-1** (5 μM) in CH_3CN solution to different competition ions, absorption response of **Ir-1** in the presence of various metal

cations, partial ^1H NMR spectral changes of **Ir-1** in CD_3CN in the presence of mercury(II) (0–1 equiv), partial ^1H NMR spectra of **Ir-1** with mercury(II) and **Ir-3** in CD_3CN , ratiometric phosphorescence images of **Ir-1**-incubated KB cells treated without or with Hg(II) (10 μM), and cell imaging for $[\text{Ir}(\text{bt})_2(\text{bpy})]\text{PF}_6$. This material is available free of charge via the Internet at <http://pubs.acs.org>.

AUTHOR INFORMATION

Corresponding Author

*Phone: 86-21-55664185. Fax: 86-21-55664621. E-mail: fyli@fudan.edu.cn (F.Y.L.), iamqzhao@njupt.edu.cn (Q.Z.).

ACKNOWLEDGMENT

The authors thank the National Natural Science Funds for Distinguished Young Scholars (20825101), National Natural Science Foundation of China (50803028 and 20804019), Shanghai Sci. Tech. Comm. (1052 nm03400), Shanghai Leading Academic Discipline Project (B108), IRT0911, and Scientific and Technological Activities for Returned Personnel in Nanjing City (NJ209001) for financial support.

REFERENCES

- (1) (a) Chi, Y.; Chou, P. T. *Chem. Soc. Rev.* **2010**, *39*, 638. (b) Lo, K. K. W.; Louie, M. W.; Zhang, K. Y. *Coord. Chem. Rev.* **2010**, *254*, 2603. (c) Chen, Z. Q.; Bian, Z. Q.; Huang, C. H. *Adv. Mater.* **2010**, *22*, 1534. (d) Zhao, Q.; Liu, S. J.; Huang, W. *Macromol. Rapid Commun.* **2010**, *31*, 794. (e) Wong, W. Y.; Ho, C. L. *Coord. Chem. Rev.* **2009**, *253*, 1709. (f) You, Y.; Park, S. Y. *Dalton Trans.* **2009**, *8*, 1267. (g) Ulbricht, C.; Beyer, B.; Friebe, C.; Winter, A.; Schubert, U. S. *Adv. Mater.* **2009**, *21*, 4418. (h) Thompson, M. E. *MRS Bull.* **2007**, *32*, 694. (i) Liu, S. J.; Zhao, Q.; Fan, Q. L.; Huang, W. *Eur. J. Inorg. Chem.* **2008**, 2177. (j) Liu, S. J.; Zhao, Q.; Huang, W. *Adv. Polym. Sci.* **2008**, *212*, 125.
- (2) (a) Wu, C.; Chen, H. F.; Wong, K. T.; Thompson, M. E. *J. Am. Chem. Soc.* **2010**, *132*, 3133. (b) Mauro, M.; Schuermann, K. C.; Prétôt, R.; Hafner, A.; Mercandelli, P.; Sironi, A.; De Cola, L. *Angew. Chem., Int. Ed.* **2010**, *49*, 1222. (c) Chiu, Y. C.; Hung, J. Y.; Chi, Y.; Chen, C. C.; Chang, C. H.; Wu, C. C.; Cheng, Y. M.; Yu, Y. C.; Lee, G. H.; Chou, P. T. *Adv. Mater.* **2009**, *21*, 2221. (d) Tan, W. J.; Zhang, Q.; Zhang, J. J.; Tian, H. *Org. Lett.* **2009**, *11*, 161. (e) He, L.; Qiao, J.; Duan, L.; Dong, G. F.; Zhang, D. Q.; Wang, L. D.; Qiu, Y. *Adv. Funct. Mater.* **2009**, *19*, 1. (f) Lee, S. J.; Park, K. M.; Yang, K.; Kang, Y. *Inorg. Chem.* **2009**, *48*, 1030.
- (3) (a) Botchway, S. W.; Charnley, M.; Haycock, J. W.; Parker, A. W.; Rochester, D. L.; Weinstein, J. A.; Gareth Williams, J. A. *Proc. Natl. Acad. Sci. U.S.A.* **2008**, *105*, 16071. (b) Murphy, L.; Congreve, A.; Palsson, L. O.; Gareth Williams, J. A. *Chem. Commun.* **2010**, *46*, 8743.
- (4) Zhao, Q.; Li, F. Y.; Huang, C. H. *Chem. Soc. Rev.* **2010**, *39*, 3007.
- (5) (a) Ho, M. L.; Hwang, F. M.; Chen, P. N.; Hu, Y. H.; Cheng, Y. M.; Chen, K. S.; Lee, G. H.; Chi, Y.; Chou, P. T. *Org. Biomol. Chem.* **2006**, *4*, 98. (b) Ho, M. L.; Cheng, Y. M.; Wu, L. C.; Chou, P. T.; Lee, G. H.; Hsu, F. C.; Chi, Y. *Polyhedron* **2007**, *26*, 4886. (c) Schmittel, M.; Lin, H. *Inorg. Chem.* **2007**, *46*, 9139. (d) You, Y.; Park, S. Y. *Adv. Mater.* **2008**, *20*, 3820.
- (6) (a) Zhao, Q.; Cao, T. Y.; Li, F. Y.; Li, X. H.; Jing, H.; Yi, T.; Huang, C. H. *Organometallics* **2007**, *26*, 2077. (b) Zhao, Q.; Liu, S. J.; Shi, M.; Li, F. Y.; Jing, H.; Yi, T.; Huang, C. H. *Organometallics* **2007**, *26*, 5922. (c) Chen, H. L.; Zhao, Q.; Wu, Y. B.; Li, F. Y.; Yang, H.; Yi, T.; Huang, C. H. *Inorg. Chem.* **2007**, *46*, 11075. (d) Zhao, Q.; Li, F. Y.; Liu, S. J.; Yu, M. X.; Liu, Z. Q.; Yi, T.; Huang, C. H. *Inorg. Chem.* **2008**, *47*, 9256. (e) Zhao, Q.; Liu, S. J.; Li, F. Y.; Yi, T.; Huang, C. H. *Dalton Trans.* **2008**, *29*, 3836. (f) Xu, W. J.; Liu, S. J.; Zhao, X. Y.; Sun, S.; Cheng, S.; Ma, T. C.; Sun, H. B.; Zhao, Q.; Huang, W. *Chem.—Eur. J.* **2010**, *16*, 7125. (g) Shi, H. F.; Liu, S. J.; Sun, H. B.; Xu, W. J.; An, Z. F.; Chen, J.;

- Sun, S.; Lu, X. M.; Zhao, Q.; Huang, W. *Chem.—Eur. J.* **2010**, *16*, 12158.
- (h) Xu, W. J.; Liu, S. J.; Sun, H. B.; Zhao, X. Y.; Zhao, Q.; Sun, S.; Cheng, S.; Ma, T. C.; Zhou, L. X.; Huang, W. *J. Mater. Chem.* **2011**, *21*, 7572. (j) Liu, Y.; Li, M. Y.; Zhao, Q.; Wu, H. Z.; Huang, K. W.; Li, F. Y. *Inorg. Chem.* **2011**, ASAP. doi/10.1021/ic102481x
- (7) (a) Lo, K. K. W.; Tsang, K. H. K.; Sze, K. S.; Chung, C. K.; Lee, T. K. M.; Zhang, K. Y.; Hui, W. K.; Li, C. K.; Lau, J. S. Y.; Ng, D. C. M.; Zhu, N. *Coord. Chem. Rev.* **2007**, *251*, 2292. (b) Moreira, V. F.; Greenwood, F. L. T.; Coogan, M. P. *Chem. Commun.* **2010**, *46*, 186. (c) Zhao, Q.; Huang, C. H.; Li, F. Y. *Chem. Soc. Rev.* **2011**, *40*, 2508.
- (8) (a) Lo, K. K. W.; Lee, P. K.; Lau, J. S. Y. *Organometallics* **2008**, *27*, 2998. (b) Lau, J. S. Y.; Lee, P. K.; Tsang, K. H. K.; Ng, C. H. C.; Lam, Y. W.; Cheng, S. H.; Lo, K. K. W. *Inorg. Chem.* **2009**, *48*, 708. (c) Zhang, K. Y.; Lo, K. K. W. *Inorg. Chem.* **2009**, *48*, 6011. (d) Zhang, K. Y.; Li, S. P. Y.; Zhu, N. Y.; Or, I. W. S.; Cheung, M. S. H.; Lam, Y. W.; Lo, K. K. W. *Inorg. Chem.* **2010**, *49*, 2530. (e) Leung, S. K.; Kwok, K. Y.; Zhang, K. Y.; Lo, K. K. W. *Inorg. Chem.* **2010**, *49*, 4984. (f) Zhang, K. Y.; Liu, H. W.; Fong, T. T. H.; Chen, X. G.; Lo, K. K. W. *Inorg. Chem.* **2010**, *49*, 5432. (g) Liu, H. W.; Zhang, K. Y.; Law, W. H. T.; Lo, K. K. W. *Organometallics* **2010**, *29*, 3474.
- (9) (a) Yu, M. X.; Zhao, Q.; Shi, L. X.; Li, F. Y.; Zhou, Z. G.; Yang, H.; Yi, T.; Huang, C. H. *Chem. Commun.* **2008**, 2115. (b) Zhao, Q.; Yu, M. X.; Shi, L. X.; Liu, S. J.; Li, C. Y.; Shi, M.; Zhou, Z. G.; Huang, C. H.; Li, F. Y. *Organometallics* **2010**, *29*, 1085. (c) Jiang, W. L.; Gao, Y.; Sun, Y.; Ding, F.; Xu, Y.; Bian, Z. Q.; Li, F. Y.; Bian, J.; Huang, C. H. *Inorg. Chem.* **2010**, *49*, 3252. (d) Wu, H. Z.; Yang, T. S.; Zhao, Q.; Zhou, J.; Li, C. Y.; Li, F. Y. *Dalton Trans.* **2011**, *40*, 1969.
- (10) Xiong, L. Q.; Zhao, Q.; Chen, H. L.; Wu, Y. B.; Dong, Z. S.; Zhou, Z. G.; Li, F. Y. *Inorg. Chem.* **2010**, *49*, 6402.
- (11) Lamansky, S.; Djurovich, P.; Murphy, D.; Abdel-Razzaq, F.; Lee, H. E.; Adachi, C.; Burrows, P. E.; Forrest, S. R.; Thompson, M. E. *J. Am. Chem. Soc.* **2001**, *123*, 4304. (b) Lamansky, S.; Djurovich, P.; Murphy, D.; Abdel-Razzaq, F.; Kwong, R.; Tsyba, I.; Bortz, M.; Mui, B.; Bau, R.; Thompson, M. E. *Inorg. Chem.* **2001**, *40*, 1704.
- (12) Sheldrick, G. M. *SHELXTL-Plus V5.1 software Reference Manual*; Bruker AXS Inc.: Madison, WI, 1997.
- (13) Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Montgomery, J. A., Jr.; Vreven, T.; Kudin, K. N.; Burant, J. C.; Millam, J. M.; Iyengar, S. S.; Tomasi, J.; Barone, V.; Mennucci, B.; Cossi, M.; Scalmani, G.; Rega, N.; Petersson, G. A.; Nakatsuji, H.; Hada, M.; Ehara, M.; Toyota, M.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Klene, M.; Li, X.; Knox, J. E.; Hratchian, H. P.; Cross, J. B.; Adamo, C.; Jaramillo, J.; Gomperts, R.; Stratmann, R. E.; Yazyev, O.; Austin, A. J.; Cammi, R.; Pomelli, C.; Ochterski, J. W.; Ayala, P. Y.; Morokuma, K.; Voth, G. A.; Salvador, P.; Dannenberg, J. J.; Zakrzewski, V. G.; Dapprich, S.; Daniels, A. D.; Strain, M. C.; Farkas, O.; Malick, D. K.; Rabuck, A. D.; Raghavachari, K.; Foresman, J. B.; Ortiz, J. V.; Cui, Q.; Baboul, A. G.; Clifford, S.; Cioslowski, J.; Stefanov, B. B.; Liu, G.; Liashenko, A.; Piskorz, P.; Komaromi, I.; Martin, R. L.; Fox, D. J.; Keith, T.; Al-Laham, M. A.; Peng, C. Y.; Nanayakkara, A.; Challacombe, M.; Gill, P. M. W.; Johnson, B.; Chen, W.; Wong, M. W.; Gonzalez, C.; Pople, J. A. *GAUSSIAN 03*, Revision C.02; Gaussian, Inc.: Wallingford, CT, 2004.
- (14) Neve, F.; Deda, M. L.; Crispini, A.; Bellusci, A.; Puntoriero, F.; Campagna, S. *Organometallics* **2004**, *23*, 5856.
- (15) (a) Sprouse, S.; King, K. A.; Spellane, P. J.; Watts, R. J. *J. Am. Chem. Soc.* **1984**, *106*, 6647. (b) Didier, P.; Ortmans, I.; Kirsch-De Mesmaeker, A.; Watts, R. J. *Inorg. Chem.* **1993**, *32*, 5239. (c) Ortmans, I.; Didier, P.; Kirsch-De Mesmaeker, A. *Inorg. Chem.* **1995**, *34*, 3695. (d) Dixon, I. M.; Collin, J.-P.; Sauvage, J. P.; Flamigni, L.; Encinas, S.; Barigelletti, F. *Chem. Soc. Rev.* **2000**, *29*, 385. (e) Auffrant, A.; Barbieri, A.; Barigelletti, F.; Lacour, J.; Mobian, P.; Collin, J. P.; Sauvage, J. P.; Ventura, B. *Inorg. Chem.* **2007**, *46*, 6911.
- (16) Zhao, Q.; Liu, S. J.; Shi, M.; Wang, C. M.; Yu, M. X.; Li, L.; Li, F. Y.; Yi, T.; Huang, C. H. *Inorg. Chem.* **2006**, *45*, 6152.
- (17) (a) U.S. EPA, *Regulatory Impact Analysis of the Clean Air Mercury Rule*; EPA-452/R-05-003, 2005. (b) Wu, D. Y.; Huang, W.; Liu, Z. H.; Duan, C. Y.; He, C.; Wu, S.; Wang, D. H. *Inorg. Chem.* **2008**, *47*, 7190. (c) He, G. J.; Zhao, Y. G.; He, C.; Liu, Y.; Duan, C. Y. *Inorg. Chem.* **2008**, *47*, 5169. (d) Zhang, X. L.; Xiao, Y.; Qian, X. H. *Angew. Chem., Int. Ed.* **2008**, *47*, 8025. (e) Yang, H.; Zhou, Z. G.; Huang, K. W.; Yu, M. X.; Li, F. Y.; Yi, T.; Huang, C. H. *Org. Lett.* **2007**, *9*, 4729. (f) Liu, S. J.; Fang, C.; Zhao, Q.; Fan, Q. L.; Huang, W. *Macromol. Rapid Commun.* **2008**, *29*, 1212.
- (18) (a) van Diemen, J. H.; Hage, R.; Haasnoot, J. G.; Lempers, H. E. B.; Reedijk, J.; Vos, J. G.; De Cola, L.; Barigelletti, F.; Balzani, V. *Inorg. Chem.* **1992**, *31*, 3518. (b) McDaniel, N. D.; Coughlin, F. J.; Tinker, L. L.; Bernhard, S. *J. Am. Chem. Soc.* **2008**, *130*, 210. (c) Lo, K. K.-W.; Li, C.-K.; Lau, J. S.-Y. *Organometallics* **2005**, *24*, 4594. (d) Lo, K. K. W.; Lau, J. S. Y. *Inorg. Chem.* **2007**, *46*, 700.
- (19) Pearson, R. G. *J. Am. Chem. Soc.* **1963**, *85*, 3533.
- (20) Coronado, E.; Galan-Mascaros, J. R.; Marti-Gastaldo, C.; Palomares, E. J.; Durrant, R. R.; Vilar, R.; Gratzel, M.; Nazeeruddin, Md. K. *J. Am. Chem. Soc.* **2005**, *127*, 12351.
- (21) (a) Maruyama, S.; Kikuchi, K.; Hirano, T.; Urano, Y.; Nagano, T. *J. Am. Chem. Soc.* **2002**, *124*, 1065. (b) Taki, M.; Wolford, J. L.; O'Halloran, T. V. *J. Am. Chem. Soc.* **2004**, *126*, 712. (c) Jares-Erijman, E. A.; Jovin, T. M. *Nat. Biotechnol.* **2003**, *21*, 1387. (d) Sapsford, K. E.; Berti, L.; Medintz, I. L. *Angew. Chem., Int. Ed.* **2006**, *45*, 4562. (e) Liu, B.; Tian, H. *Chem. Commun.* **2005**, 3156. (e) Zhou, Z. G.; Yu, M. X.; Yang, H.; Huang, K. W.; Li, F. Y.; Yi, T.; Huang, C. H. *Chem. Commun.* **2008**, 3387.