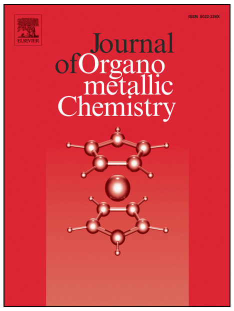


Synthesis, characterization and photophysical properties of a new Cu^{2+} selective phosphorescent sensor

Tianzhi Yu, Qianguang Yang, Zeyang Zhu, Yuling Zhao, Xiaoxiao Liu, Chengjin Wei, Hui Zhang

PII: S0022-328X(17)30619-8

DOI: 10.1016/j.jorganchem.2017.10.032

Reference: JOM 20152

To appear in: *Journal of Organometallic Chemistry*

Received Date: 11 September 2017

Revised Date: 20 October 2017

Accepted Date: 22 October 2017

Please cite this article as: T. Yu, Q. Yang, Z. Zhu, Y. Zhao, X. Liu, C. Wei, H. Zhang, Synthesis, characterization and photophysical properties of a new Cu^{2+} selective phosphorescent sensor, *Journal of Organometallic Chemistry* (2017), doi: 10.1016/j.jorganchem.2017.10.032.

Synthesis, characterization and photophysical properties of a new Cu^{2+} selective phosphorescent sensor

Tianzhi Yu[1,*], Qianguang Yang[1], Zeyang Zhu[1], Yuling Zhao[2], Xiaoxiao Liu[3], Chengjin Wei[1], Hui Zhang[1]

[1]Key Laboratory of Opto-Electronic Technology and Intelligent Control (Ministry of Education), Lanzhou Jiaotong University, Lanzhou 730070, China

[2]School of Chemical and Biological Engineering, Lanzhou Jiaotong University, Lanzhou 730070, China

[3]School of Mechatronic Engineering, Lanzhou Jiaotong University, Lanzhou 730070, China

Abstract: A new Cu(II) selective phosphorescent sensor based on a cationic iridium(III) complex was synthesized and characterized. Compared with other commonly coexisting metal ions, the Ir(III) complex displayed significant phosphorescence quenching for detecting Cu^{2+} with a 1:1 stoichiometry in MeOH/H_2O (1:1, v/v) solution. Upon addition of Cu^{2+}, the rapid quenching of emission intensity provided a sensitive method for detection of Cu^{2+}. The corresponding detection limit was determined to be 7.6×10^{-8} M and the association constant between the Ir(III) complex and Cu^{2+} was calculated to be 0.31×10^{5} M^{-1}. Luminescence imaging experiments demonstrated that this Ir(III) complex was cell permeable and suitable for monitoring intracellular Cu^{2+} in living Hela cells by confocal microscopy.

Keywords: Cationic iridium(III) complex; Phosphorescence quenching; Phosphorescent

---

[*] Corresponding Author. Tel. +86-931-4956935; Fax: +86-931-4938756; e-mail: yutzh@mail.lzjtu.cn (T.Z. Yu).

chemosensor; Cu^{2+} detection; Bioimaging

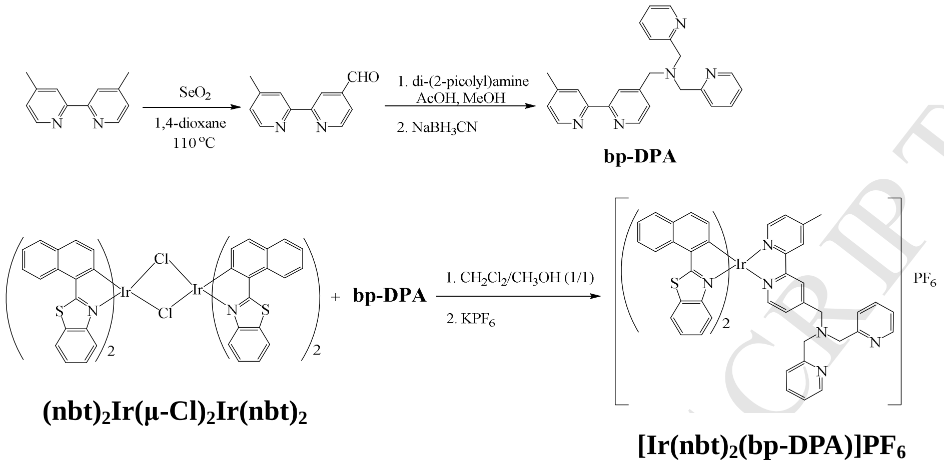
1. Introduction

Recently, phosphorescent heavy-metal complexes have attracted tremendous attentions due to their efficient luminescent properties and potential applications in organic light-emitting diodes (OLEDs) [1-6], light-emitting electrochemical cells [7-10], chemosensors [11-14] and biological labeling [15,16]. Compared with the fluorescent chemosensors, the phosphorescent heavy-metal complexes have attracted more attentions and increasingly used as colorimetric or luminescent probes for biomolecules and metal ions because their relatively long phosphorescence emission is unaffected by short-lived background noise such as fluorescence and scattered light [17-20]. Among phosphorescent heavy-metal complexes, the phosphorescent Ir(III) complexes show some advantageous photophysical properties such as short excited-state lifetime, high quantum efficiency, color diversity and high photostability, and some Ir(III) complexes have been used as luminescent probes.

As trace element and nutrient in living organisms, the copper(Ⅱ) is essential and plays a significant role in a basic physiological process [13]. However, the copper(Ⅱ) is also one of significant metal pollutants and toxic to living cells [21]. Aberrant levels of copper(Ⅱ) ions can result in neurodegenerative diseases such as Alzheimer's disease, prion disease, Menkes disease, Parkinson’s disease and Wilson disease [22-24]. As a result, to seek a rapid sensitive sensor to detect and quantify copper(Ⅱ) ion in environment and in living systems is very importance for biologic research and medical diagnosis. A number of fluorescent chemosensors for the highly sensitive and selective detection of Cu (II) ion have been reported in the literatures [25-31]. Recently, the phosphorescent Ir(III)-based chemosensors have been successfully applied for the detection of Cu^{2+} ions [13,14,19,32-35]. Moreover, the phosphorescent Ir(III) complexes as luminescent probes present important advantages as their simplicity, easy visualization and high sensitivity. The incorporation of a Lewis base as a cation receptor on the ancillary ligands of the luminescent Ir(III) complexes is a very used strategy for the recognition of cations. The coordination interaction of the Cu^{2+} with an ion recognition group on the Ir(III) complex causes variations in the luminescence intensity that may be used to detect Cu^{2+}. For example, You et al. [19] synthesized the Ir(III) complex having two cyclometalating 2-phenylpyridine (ppy) ligands and one 2-(2′-benzo[*b*]thienyl)pyridine (btp) tethered a di(2-picolyl)amine (DPA) copper ion receptor by a methylene linker, which was used as a Cu^{2+}-sensitive probe. This Ir(III) complex has a 1:2 stoichiometry (complex : Cu^{2+}) that was demonstrated by the binding titration data, and the limit of detection for Cu^{2+} was measured to be range of 5.8 – 8.1 μM.

The di(2-picolyl)amine moiety (DPA) always exhibits high affinity and specificity for Zn^{2+} and Cu^{2+} ions over the other metal ions, which has been frequently used as turn-on sensors for Zn^{2+} and Cu^{2+} ions. In this paper we used 2-(1-naphthyl)benzothiazole (**nbt**) as cyclometalated ligand and 2,2′-dipyridine tethered DPA moiety by a methylene linker (**bp-DPA**) as the neutral auxiliary ligand to prepare a new cationic Ir(III) complex, **$[Ir(nbt)_2(bp\text{-}DPA)]PF_6$**. The photophysical properties and ion-recognition behaviors of the cationic Ir(III) complex with some heavy-metal ions are investigated. This cationic Ir(III) complex exhibited good sensitivity and selective on–off type response toward Cu^{2+} over other metal ions including Zn^{2+} and Hg^{2+}. Furthermore, the confocal luminescence imaging was performed to validate the ability of the Ir(III) complex to monitor intracellular Cu^{2+} ions.

The synthetic routes of the neutral auxiliary ligand **bp-DPA** and the cationic Ir(III) complex were shown in Scheme 1.



Scheme 1. Synthetic routes of **bp-DPA** and **[Ir(nbt)$_2$(bp-DPA)]PF$_6$**.

2. Experimental

2.1. Materials and instruments

2-Aminothiophenol, 1-naphthaldehyde and 4,4′-dimethyl-2,2′-bipyridine were bought from Alfa Aesar. 2,2'-Dipicolylamine was obtained from Energy Chemical (China). $IrCl_3 \cdot nH_2O$ (iridium content > 60.0%) was bought from Shanxi Kaida Chemical Co. Ltd. (China) and used without further purification. All the other chemicals were analytical grade reagent. All of the organic solvents used in this study were dried over appropriate drying agents and freshly distilled prior in the related reaction.

4-Methyl-2,2′-bipyridine-4′-carboxaldehyde was synthesized as the procedure described in literature [36]. The cyclometalated Ir(III) μ-chlorobridged dimer of **(nbt)$_2$Ir(μ-Cl)$_2$Ir(nbt)$_2$** was prepared as previously described [37].

^{1}H NMR spectra were recorded on Agilent Technologies DDZ 600 MHz. Mass spectrum was obtained from a Thermo Scientific Orbitrap Elite mass spectrometer. Thermogravimetric analysis

(TGA) was performed on a Perkin-Elmer Pyris system. UV-vis absorption and photoluminescent spectra were recorded on a Shimadzu UV-2550 spectrometer and on a Perkin-Elmer LS-55 spectrometer, respectively. All pH measurements were made with apH-10C digital pH meter.

2.2. Synthesis and characterization of bp-DPA and [Ir(nbt)$_2$(bp-DPA)]PF$_6$

4-Methyl-4′-(di(2-picolyl)aminomethyl)-2,2′-bipyridine (bp-DPA): Di(2-picolyl)amine (0.729 g, 3.66 mmol) and 4-Methyl-2,2′-bipyridine-4′-carboxaldehyde (0.725 g, 3.66 mmol) were dissolved in fresh MeOH (70 mL). A catalytic amount (two drops) of glacial acetic acid was added to the solution, which was refluxed for 0.5 h. The reaction mixture was cooled by using an ice bath, and then $NaBH_3CN$ (0.460 g, 7.32 mmol) was slowly delivered to the solution. The ice bath was removed and the reaction mixture was stirred overnight at room temperature. Basic work-up using saturated aqueous sodium carbonate solution and subsequent extraction using CH_2Cl_2 was performed. The organic layer was dried over anhydrous $MgSO_4$ and concentrated in vacuum. The crude was purified by chromatography on silica gel using acetone/petroleum ether (1:10, v/v) to obtain a sticky brown oily-liquid. Yield (1.185 g, 84.9%). ^{1}H NMR (600 MHz, $CDCl_3$, δ, ppm): 8.50 (d, J = 7.2 Hz, 1H), 8.40 (t, J = 9.6 Hz, 3H), 8.29 (s, 1H), 8.09 (s, 1H), 7.53 (t, J = 9.0 Hz, 2H), 7.47 (d, J = 8.8 Hz, 2H), 7.35 (d, J = 7.2 Hz, 1H), 7.02-6.97 (dd, J = 9.6, 8.4 Hz, 3H), 3.74 (s, 4H), 3.69 (s, 2H), 2.26 (s, 3H).

[Ir(nbt)$_2$(bp-DPA)]PF$_6$: The chloro-bridged dimer complex **(nbt)$_2$Ir(μ-Cl)$_2$Ir(nbt)$_2$** (0.799 g, 0.534 mmol) and **bp-DPA** (0.5111 g, 1.340 mmol) were refluxed in 60 mL of dichloromethane/methanol (1:1, v/v) under nitrogen atmosphere at 75 ℃ for 4 h with keeping in dark place. After cooling, an excess of KPF_6 (15 equiv) was slowly added to the reaction mixture and the solution was stirred for additional 12 h. After completion of the reaction, the solids in the solution were removed by filtration. The residue was purified through chromatography on silica gel using CH_2Cl_2/MeOH (9:1, v/v) as the eluent to obtain a red powder (0.98 g, 74%). ^{1}H NMR (600 MHz, $CDCl_3$, δ, ppm): δ 8.66 (dd, J = 9.0, 8.4 Hz, 3H), 8.44 (t, J = 6.8 Hz, 3H), 7.95 (dd, J = 8.4, 7.8 Hz, 2H), 7.87 (d, J = 6.0 Hz, 1H), 7.82 (d, J = 6.0 Hz, 1H), 7.76-7.68 (m, 4H), 7.57 (t, J = 7.8 Hz, 2H), 7.51-7.44 (m, 5H), 7.41 (t, J = 7.2 Hz, 1H), 7.35 (t, J = 7.8 Hz, 1H), 7.25 (t, J = 7.2 Hz, 2H), 7.20 (dd, J = 8.4, 6.0 Hz, 2H), 7.03 (t, J = 6.6 Hz, 2H), 6.98 (t, J = 7.8 Hz, 1H), 6.56 (dd, J = 8.4, 8.4 Hz, 2H), 6.37 (d, J = 8.4 Hz, 1H), 6.15 (d, J = 8.4 Hz, 1H), 4.03 (s, 2H), 3.85 (s, 4H), 2.63 (s, 3H). HR EI-MS: Calcd. for $[C_{58}H_{43}IrN_7S_2]^+$, 1094.27; Found, 1094.2637.

2.3. Spectroscopic experiments

Spectrophotometric titrations of the Ir(III) complex were performed in air-equilibrated CH_3OH solution. The Ir(III) complex stock solution (1.0×10^{-3} M) was prepared in CH_3OH. The fresh metal stock solutions (typically 1.0×10^{-2} M) were prepared in Milli-Q water using the corresponding chloride salts (Na^+, K^+, Ca^{2+}, Mg^{2+}, Mn^{2+}, Cu^{2+}, Zn^{2+}, Cd^{2+}, Ni^{2+}, Co^{2+}, Pb^{2+}, Al^{3+} and Fe^{3+}), nitrate salt (Ag^+) and perchlorate salt (Hg^{2+}). For emission titration experiment, 10μL of methanol solution of the Ir(III) complex (1.0×10^{-3} M) was taken in a quartz optical cell of 1.0 cm optical path length in 2000 μL and then stock solutions of metal ions were added gradually to it by using micropipette. Spectral data were recorded at 1 min after the addition of the metal ions for both titrations.

2.4. Cell cytotoxicity

The cell cytotoxicity of **$[Ir(nbt)_2(bp\text{-}DPA)]PF_6$** was determined by MTT (3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide) assays. Hela cells were placed in microassay culture plates (5×10^4 cells per milliliter) and grown for 24 h at 37 °C in a

5% CO_2 incubator. Then **[Ir(nbt)$_2$(bp-DPA)]PF$_6$** was added to the wells to achieve final concentrations of 1.25, 2.5, 5, 10 and 20 μM. The plates were incubated at 37 °C in a 5% CO_2 incubator for another 24 h. Upon completion of the incubation, stock MTT dye solution (20 μL, 1 mg/mL) was added to each well. After 4 h, dimethyl sulfoxide (150 μL) was added to solubilize the MTT formazan.

2.5. Cell culture and confocal luminescence imaging

Vitro experiments were performed by using Hela cells. Hela cells were cultured in RPMI-1640 medium supplemented with 10% FBS (fetal bovine serum), 100 units per mL penicillin, and 100 μg/mL streptomycin at 37 °C in a humid atmosphere containing 5% CO_2. Cells (5×10^8 L^{-1}) were plated on 18 mm glass coverslips and allowed to adhere for 24 h. Then the cells were treated with **[Ir(nbt)$_2$(bp-DPA)]PF$_6$** (20 μM) and incubated for 30 min. Subsequently, the cells were treated with 100 μM $CuCl_2 \cdot 2H_2O$. The cells were incubated for 30 min and washed with PBS three times to remove free compound and ions before analysis. Hela cells cells only incubated with 20 μM **[Ir(nbt)$_2$(bp-DPA)]PF$_6$** for 30 min acted as a control. Confocal luminescence images of Hela cells cells were carried out on an Olympus FV1000 laser scanning confocal microscope and a 100x oil-immersion objective lens. Emission was collected at 550–630 nm for the Hela cells.

3. Results and discussion

3.1. Synthesis and characterization

The synthesis of the cationic Ir(III) complexes from Scheme 1 began first with preparing the ancillary ligand **bp-DPA**. The ligand **bp-DPA** was successfully obtained through two step reactions. First, 4,4′-dimethyl-2,2′-bipyridine was selectively oxidized to be 4-Methyl-2,2′-bipyridine-4′-carboxaldehyde by SeO_2 catalyst. Subsequently, this intermediate was subjected to reductive amination with DPA. The ligand **bp-DPA** was purified by column chromatography to give the pure product. The Ir(III) complex **[Ir(nbt)$_2$(bp-DPA)]PF$_6$** was synthesized by reacting the Ir(III) chloro-bridged dimer with the ancillary ligand **bp-DPA**, and followed by a counter ion-exchange reaction from Cl^- to PF_6^-. The Ir(III) complex was structurally characterized by ^{1}H NMR and the high resolution electro spray ionization mass spectra (HR ESI-MS).

3.2. Photophysical and thermal properties of [Ir(nbt)$_2$(bp-DPA)]PF$_6$

The UV-vis absorption and photoluminescence (PL) spectra of **[Ir(nbt)$_2$(bp-DPA)]PF$_6$** in MeOH solution is shown in Fig. 1. As shown in Fig.1, the intense absorption bands before 300 nm in the absorption spectrum are assigned to the spin-allowed $^1(\pi$–$\pi^*)$ ligand-centered transition of the cyclometalated primary ligand and the ancillary ligand in the complex. The following relatively weak absorption band at 345 nm could be attributed to both spin-allowed metal-to-ligand charge-transfer (^{1}MLCT) and ligand-to-ligand charge-transfer (^{1}LLCT) transitions. Moreover, the weaker absorption band that found at 468 nm is ascribed to the spin-forbidden ^{3}MLCT and ^{3}LLCT [38-40].

Under the excitation of 468 nm, the complex **[Ir(nbt)$_2$(bp-DPA)]PF$_6$** exhibits red emission with a maximum peak at 594 nm and a shoulder peak at 642 nm in MeOH solution at room temperature. The CIE coordinates of **[Ir(nbt)$_2$(bp-DPA)]PF$_6$** is (0.610, 0.388). In addition, the photoluminescence quantum yield of **[Ir(nbt)$_2$(bp-DPA)]PF$_6$** at room temperature was measured to be 5.05% in MeOH solution (ca. 10^{-6} mol/L) through an absolute method using the Edinburgh

Instruments spectrometer (FLS920) integrating sphere excited at 468 nm with a Xe lamp. The photoluminescence decay lifetime of **[(nbt)$_2$Ir(bp-DPA)](PF$_6$)** in MeOH solution was measured to be 2.21 μs with a time-correlated single photon counting spectrometer using an Edinburgh Instruments spectrometer (FLS920) with a microsecond flash lamp as the excitation source (repetition rate 90 Hz) at room temperature. The observed emission lifetime is in the microsecond time scale, which indicates the phosphorescent nature of the emission. The result indicates that the Ir(III) complex possesses a relatively long lifetime to differentiate short-lived background fluorescence and enhances the signal-to-noise ratio of detection through the use of time-resolved fluorescence analysis.

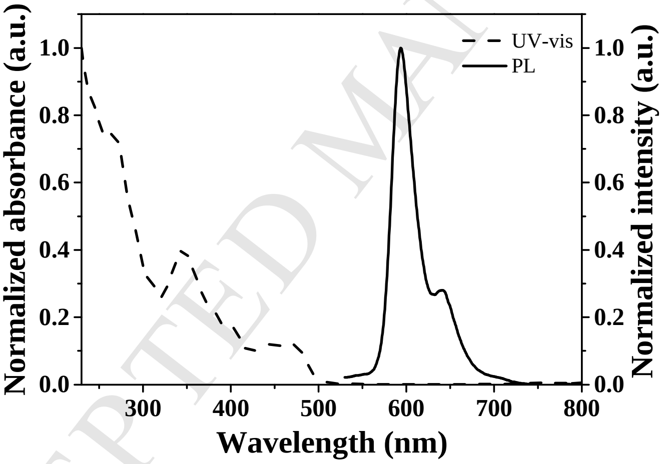


Fig. 1. The UV-vis absorption and photoluminescence (PL) spectra of **[Ir(nbt)$_2$(bp-DPA)]PF$_6$** in MeOH solution.

The thermal behavior of the cationic Ir(III) complex was investigated using thermogravimetric analysis (TGA) under a nitrogen atmosphere. The result of its TGA measurement is shown in Fig. 2. The complex **[(nbt)$_2$Ir(bp-DPA)](PF$_6$)** exhibited good thermal stability up to 256 °C. With increasing temperature, **[(nbt)2Ir(bp-DPA)](PF6)** undergoes two large-stage decomposition at 259 and 382 °C accompanied by two sharp weight losses in its TGA curve. It indicates that the complex meets with the thermal stability requirement of chemical sensor application.

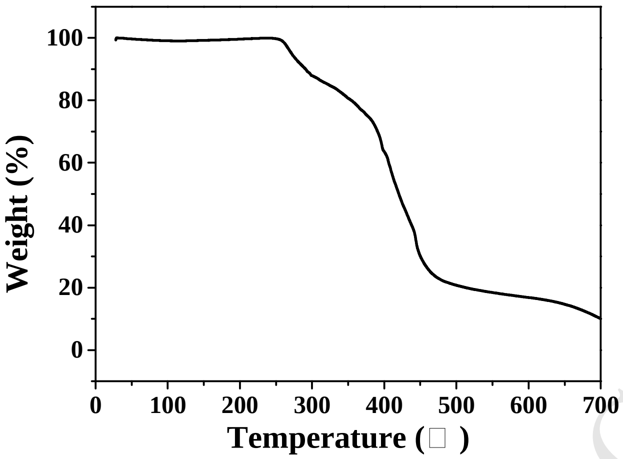


Fig. 2. Thermogravimetric analysis (TGA) of **[Ir(nbt)$_2$(bp-DPA)]PF$_6$** in nitrogen atmosphere (heating rate 10 ℃/min).

3.2. Selectivity and cation-competitive experiments

The selectivity of the Ir(III) complex (10 μM) for metal ions investigated by monitoring its photoluminescence changes upon addition of various metal ions (1 equiv) in MeOH solution is shown in Fig. 3a. The result indicated that the photoluminescence of the Ir(III) complex was quenched significantly upon addition of Cu^{2+} ion and quenched slightly upon addition Ca^{2+} or Ni^{2+} ion, whereas very weak variations in the emission spectra of the Ir(III) complex were observed upon addition of other metal ions, such as Zn^{2+}, Na^{+}, Mg^{2+}, Cd^{2+}, Fe^{3+}, Mn^{2+}, K^{+}, Co^{2+}, Hg^{2+}, Al^{3+}, Pb^{2+} and Ag^{+}. Therefore, the Ir(III) complex displayed a turn-off phosphorescence response and high selectivity in sensing Cu^{2+} ion.

To further investigate the interference of other metal ions on the detection of Cu^{2+} ion, the cation-competitive experiments were also performed by adding Cu^{2+} ion to solutions of the Ir(III) complex in the presence of other metal ions (Fig. 3b). As shown in Fig. 3b, whether in the absence or in the presence of the other metal ions, obvious spectral changes were observed for **[Ir(nbt)$_2$(bp-DPA)]PF$_6$** only upon addition of Cu^{2+} ion. The results suggests that

[Ir(nbt)$_2$(bp-DPA)]PF$_6$ possesses good selectivity for Cu^{2+} ion.

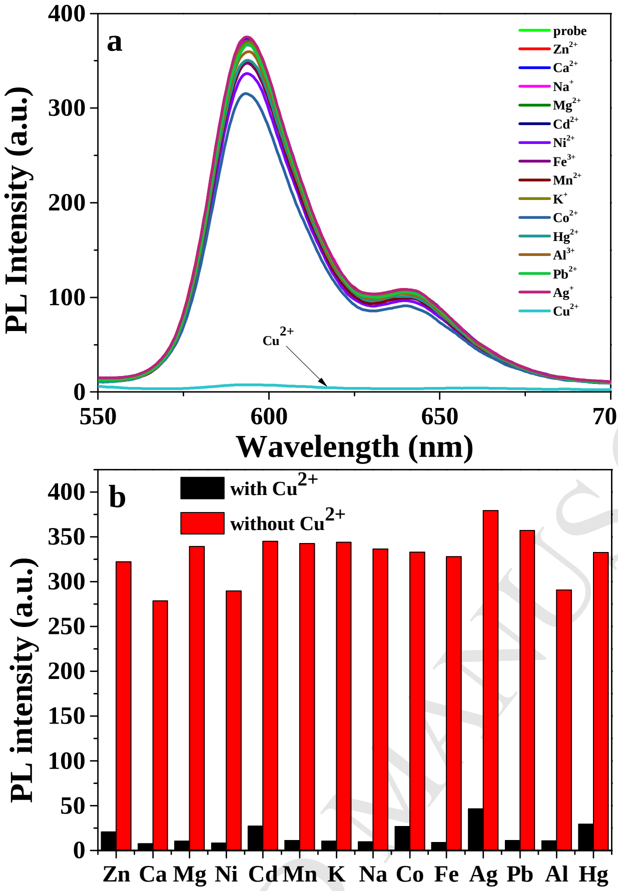


Fig. 3. (a) Photoluminescence spectra of **[Ir(nbt)$_2$(bp-DPA)]PF$_6$** (10 μM) upon addition of various metal ions (1 equiv) in MeOH solution; (b) Column diagrams of the PL intensity of **[Ir(nbt)$_2$(bp-DPA)]PF$_6$** + M^{n+}. Red bars represent the addition of 10 equiv of various metal ions to the blank solution and black bars represent the subsequent addition of Cu^{2+} (1 equiv) to the aforementioned solutions. (λ_{ex} = 468 nm, λ_{ex} = 594 nm)

3.3. Cu^{2+} titration analysis

To further evaluate the sensing properties of the Ir(III) complex towards Cu^{2+} ion, the emission titration experiments of **[Ir(nbt)$_2$(bp-DPA)]PF$_6$** with Cu^{2+} ion in varying concentrations were carried out by monitoring the emission intensity changes of **[Ir(nbt)$_2$(bp-DPA)]PF$_6$**. As shown in Fig. 4a, the lone Ir(III) complex exhibited strong phosphorescence emission with a maximum peak at 594 nm and a shoulder peak at 642 nm upon excitation at 468 nm, and the PL intensity of the Ir(III) complex gradually decreased with continuous addition of Cu^{2+} (0–2.0 eq.) to the solution. The emission intensity of the Ir(III) complex drops by 97.4% as Cu^{2+} ion concentration achieves 1.0 equivalents, which indicates that there is a strong association interaction between Cu^{2+} and the Ir(III) complex.

The Job's plot experiment was performed by monitoring the PL intensity change of **[Ir(nbt)$_2$(bp-DPA)]PF$_6$** with the variation of the concentration of both **[Ir(nbt)$_2$(bp-DPA)]PF$_6$** and Cu^{2+} ion (Fig. 4b), indicating a 1:1 stoichiometry between the Ir(III) complex and Cu^{2+} ions.

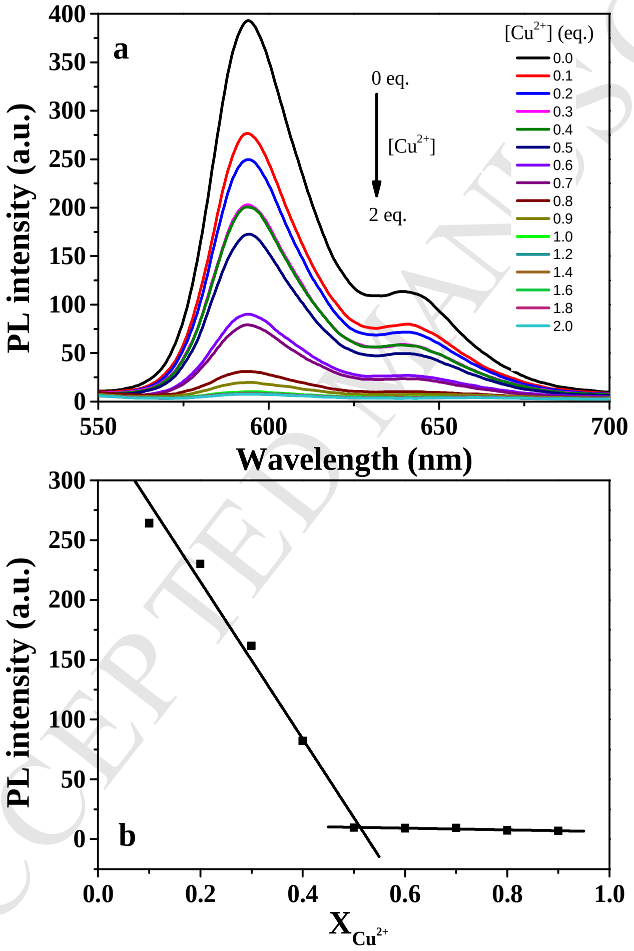


Fig. 4. (a) Photoluminescence spectra of **[Ir(nbt)$_2$(bp-DPA)]PF$_6$** (10 μM) upon titration with Cu^{2+} (0–2.0 eq.) in MeOH solution; (b) Job's plot for the complex formation between **[Ir(nbt)$_2$(bp-DPA)]PF$_6$** and Cu^{2+} ion in MeOH solution. (λ_{ex} = 468 nm, λ_{ex} = 594 nm, $X_{Cu^{2+}}$ = $[Cu^{2+}]/[$**[Ir(nbt)$_2$(bp-DPA)]PF$_6$**$]+[Cu^{2+}]$)

From the titration experiments, plotting emission intensity versus Cu^{2+} concentration (0–1.0 equiv.) afforded a good linear relationship (R = 0.9897) (Fig. 5a). The detection limit of

[Ir(nbt)$_2$(bp-DPA)]PF$_6$ was calculated from the equation DL = 3S/k, where S is the standard deviation of blank measurement, k is the slope between intensity versus sample concentration [33,41,42]. The limit of detection was calculated to be 7.6 × 10^{-8} M. The association constant was determined by the Benesi-Hildebrand equation and calculated to be 0.31 × 10^5 M^{-1} (Fig. 5b). Compared with the similar Ir(III) complex [19], our synthesized Ir(III) complex exhibited lower detection limit for Cu^{2+} ion.

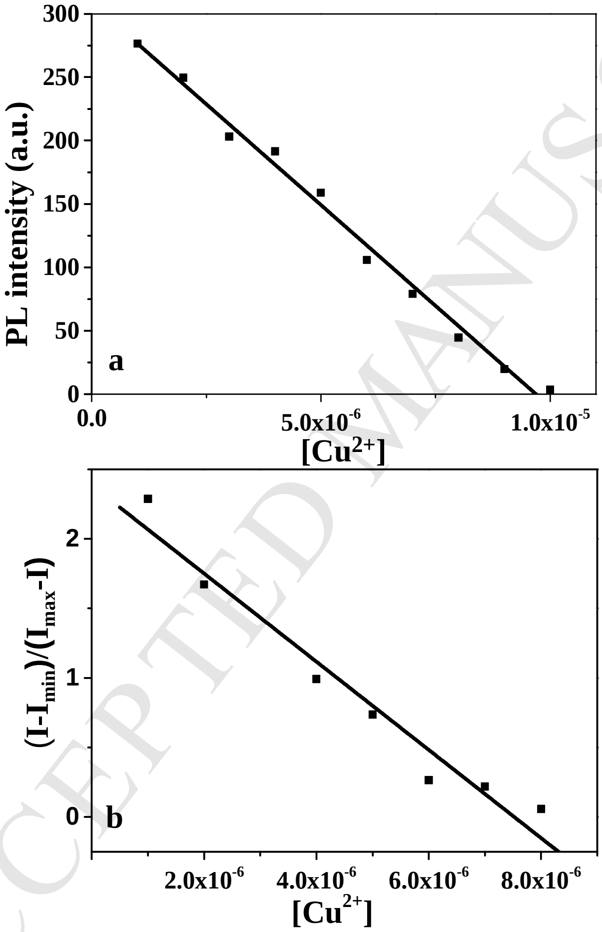


Fig. 5. (a) The linear response of emission intensity at 594 nm of **[Ir(nbt)$_2$(bp-DPA)]PF$_6$** (10 μM) to the Cu^{2+} concentrations changes (0–1.0 equiv.) in MeOH solution; (b) Benesi-Hildebrand plot of **[Ir(nbt)$_2$(bp-DPA)]PF$_6$** at different Cu^{2+} concentrations in MeOH solution. (λ_{ex} = 468 nm)

Reversibility of the probe is an important consideration in practical application. The reversibility of interaction between **[Ir(nbt)$_2$(bp-DPA)]PF$_6$** and Cu^{2+} ion was performed by addition of an aqueous of EDTA disodium (1 eq.) to the mixture of **[Ir(nbt)$_2$(bp-DPA)]PF$_6$** (10 μM) and Cu^{2+} ion (10 μM) in MeOH/H_2O (1:1, v/v) solution (Fig. 6). As shown in Fig. 6, after adding of Na_2EDTA (1 eq.) to the mixture of the Ir(III) complex and Cu^{2+} ion, the emission intensity at 594 nm was almost completely recovered. The result indicated that the interaction between **[Ir(nbt)$_2$(bp-DPA)]PF$_6$** and Cu^{2+} ion is chemically reversible.

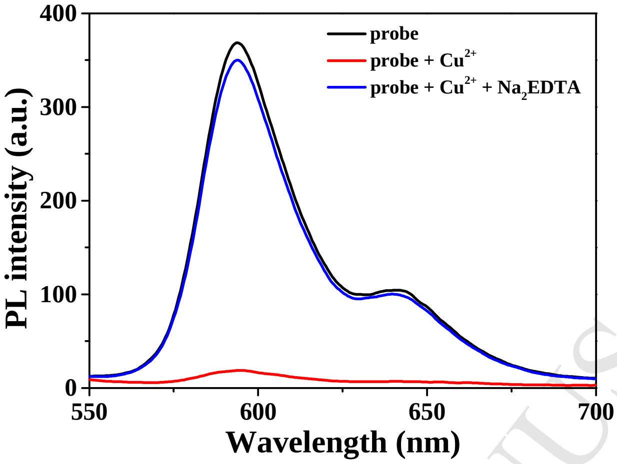


Fig. 6. The emission spectra of **[Ir(nbt)$_2$(bp-DPA)]PF$_6$** (10 μM), **[Ir(nbt)$_2$(bp-DPA)]PF$_6$** (10 μM) + Cu^{2+} (10 μM) and **[Ir(nbt)$_2$(bp-DPA)]PF$_6$** (10 μM) + Cu^{2+} (10 μM) + Na_2EDTA (10 μM) in MeOH/H_2O (1:1, v/v) solution. (λ_{ex} = 468 nm, λ_{ex} = 594 nm)

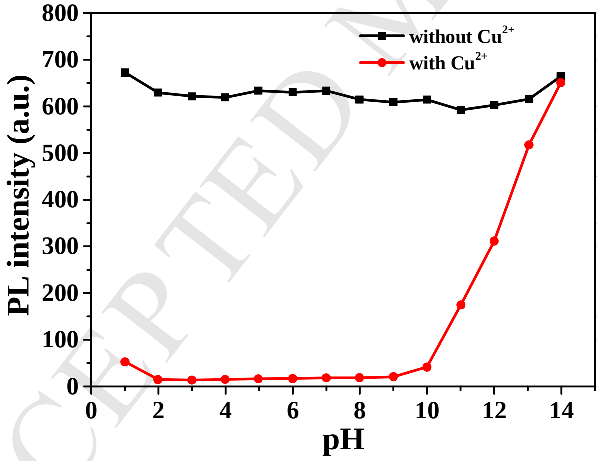


Fig. 7. The emission intensity of **[Ir(nbt)$_2$(bp-DPA)]PF$_6$** (10 μM) with and without Cu^{2+} (10 μM) as a function of pH in MeOH/H_2O (1:1, v/v) solution. (λ_{ex} = 468 nm, λ_{ex} = 594 nm)

The effect of pH on the photoluminescence response of **[Ir(nbt)$_2$(bp-DPA)]PF$_6$** to Cu^{2+} ions was also investigated in MeOH/H_2O (1:1, v/v) solution (Fig. 7). As shown in Fig. 7, the PL intensity of **[Ir(nbt)$_2$(bp-DPA)]PF$_6$** solutions in the presence and absence of Cu^{2+} were both no dramatic change over a pH range of 2–10. However, pH＞10，with the coordinated Cu^{2+} ions gradually dissociating from the Ir(III) complex–Cu^{2+} system, the emission of free the Ir(III) complex recovered gradually.

From the above results, the possible mechanisms of detection and the structures of **[Ir(nbt)$_2$(bp-DPA)]PF$_6$**, both with and without the addition of Cu^{2+}, are shown in Fig. 8. In the absence of Cu^{2+}, the Ir(III) complex exhibits strong red emission with a maximum peak at 594 nm when excited at 468 nm. After coordinated with Cu^{2+}, the paramagnetic effect from spin-orbit coupling of the Cu^{2+} induces the emission of the Ir(III) complex quenching. Upon the addition of Na$_2$EDTA into the **[Ir(nbt)$_2$(bp-DPA)]PF$_6$**–Cu^{2+} system, the emission of the Ir(III) complex was restored due to dissociating from the Ir(III) complex–Cu^{2+} system by EDTA, which indicates the regeneration of the free **[Ir(nbt)$_2$(bp-DPA)]PF$_6$**.

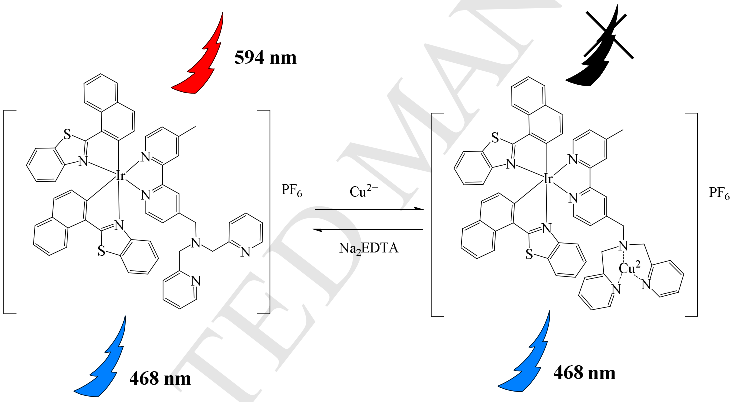


Fig. 8. The possible sensing mechanism of **[Ir(nbt)$_2$(bp-DPA)]PF$_6$** for Cu^{2+}.

3.4. Cell cytotoxicity and confocal luminescence imaging

The cytotoxicity of **[Ir(nbt)$_2$(bp-DPA)]PF$_6$** was investigated in Hela cells using an MTT assay (Fig. 9). As shown in Fig. 9, the Ir(III) complex had some cytotoxicity to Hela cells. When the concentration of **[Ir(nbt)$_2$(bp-DPA)]PF$_6$** was greater than 5 μM, the viability of the cells kept changeless and the cell viability remained above 40%. Herein, we selected 20 μM **[Ir(nbt)$_2$(bp-DPA)]PF$_6$** to analyze its recognition ability for Cu^{2+} ions in living Hela cells by confocal luminescence imaging (Fig. 10). As shown in Fig. 10a, the Hela cells treated with 20 μM

[Ir(nbt)$_2$(bp-DPA)]PF$_6$ alone for 30 min at 37 ^{o}C exhibited bright red emission. However, when Cu^{2+} (100 µM) was introduced into the cells via incubation under the same condition, remarkable intracellular luminescence quenching was observed (Fig. 10d). Bright-field measurements after the treatment with **[Ir(nbt)$_2$(bp-DPA)]PF$_6$** confirmed that the cells were viable throughout the imaging experiments (Figs. 10b,e). Overlays of confocal luminescence images also demonstrated that the luminescence was evident (Figs. 10c,f). Thus, the results indicated that the Ir(III) complex was cell membrane-permeable and had the ability to detect Cu^{2+} in living cells.

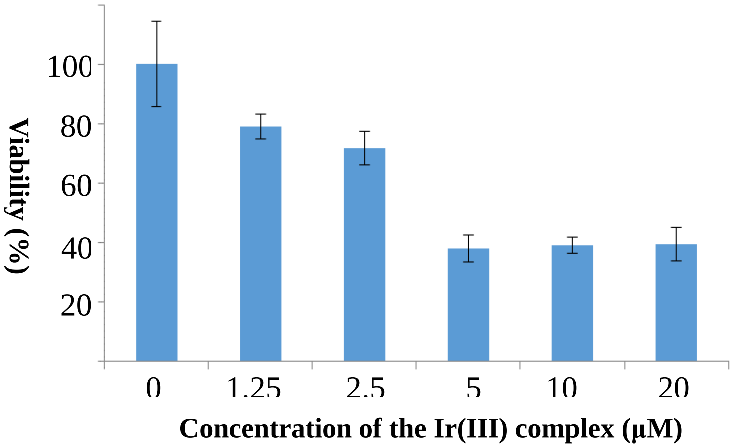


Fig. 9. Hela cell viability values (%) estimated by MTT proliferation test vs. incubation concentrations of **[Ir(nbt)$_2$(bp-DPA)]PF$_6$**. Cells were cultured in the presence of 0–20 µM **[Ir(nbt)$_2$(bp-DPA)]PF$_6$** at 37 ^{o}C for 24 h.

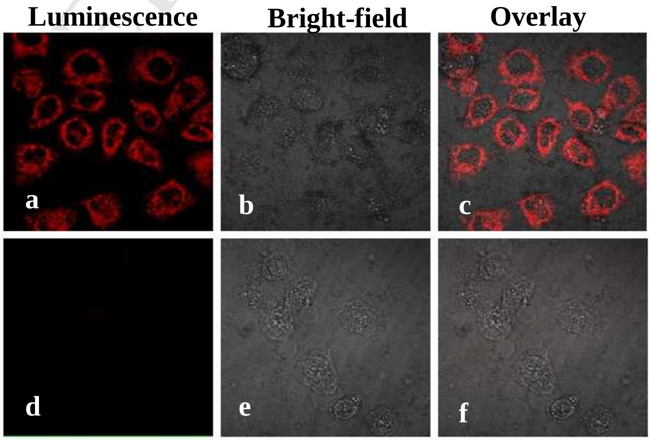


Fig. 10. Confocal luminescence (a and d), bright field (b and e) and overly images (c and f) of Hela cells. Hela cells were incubated with **[Ir(nbt)$_2$(bp-DPA)]PF$_6$** (20 µM) for 30 min at 37 ^{o}C (a–c), and subsequently treated with Cu^{2+} (100 µM) for another 30 min at 37 ^{o}C (d–f).

4. Conclusion

To summarize, we have presented a new cationic Ir(III) complex as phosphorescent sensor for quantitating Cu^{2+} ion. The Ir(III) complex exhibited a rapid turn-off phosphorescent response toward Cu^{2+} ion and displayed excellent reversibility and selectivity for Cu^{2+} ion in MeOH/H_2O (1:1) solution with a broad pH range of 2–10. The detection limit and the association constant were determined to be 7.6×10^{-8} M and 0.31×10^{5} M^{-1} for Cu^{2+}, respectively. Although the Ir(III) complex had some cytotoxicity to Hela cells, it exhibited well cell-permeable and good imaging characteristics for the detection of Cu^{2+} ion in living cells.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (Grant 51563014).

References

[1] (a) W.-Y. Wong, C.-L. Ho, Heavy metal organometallic electrophosphors derived from multi-component chromophores, Coord. Chem. Rev., 2009, 253, 1709–1758; (b) X.L. Yang, G.J. Zhou, W.-Y. Wong, Functionalization of phosphorescent emitters and their host materials by main-group elements for phosphorescent organic light-emitting devices, Chem. Soc. Rev., 2015, 44, 8484–8575; (c) W.-Y. Wong, C.-L. Ho, Functional metallophosphors for effective charge carrier injection/transport: new robust OLED materials with emerging applications, J. Mater. Chem., 2009, 19, 4457–4482; (d) G.J. Zhou, W.-Y. Wong, S. Suo, Recent progress and current challenges in phosphorescent white organic light-emitting diodes (WOLEDs), J.

Photochem. Photobiol. C: Photochem. Rev., 2010, 11, 133–156; (e) G.J. Zhou, W.-Y. Wong, X.L. Yang, New design tactics in OLEDs using functionalized 2-phenylpyridine-type cyclometalates of iridium(III) and platinum(II), Chem. Asian J., 2011, 6, 1706–1727; (f) L. Ying, C.-L. Ho, H.B. Wu, Y. Cao, W.-Y. Wong, White polymer light-emitting devices for solid-state lighting: Materials, devices, and recent progress, Adv. Mater., 2014, 26, 2459–2473; (g) X.L. Yang, G.J. Zhou, W.-Y. Wong, Recent design tactics for high performance white polymer light-emitting diodes, J. Mater. Chem. C, 2014, 2, 1760–1778; (h) C.-L. Ho, H. Li, W.-Y. Wong, Red to near-infrared organometallic phosphorescent dyes for OLED applications, J. Organomet. Chem., 2014, 751, 261–285; (i) X.B. Xu, X.L. Yang, J. Zhao, G.J. Zhou, W.-Y. Wong, Recent advances in solution-processable dendrimers for highly efficient phosphorescent organic light-emitting diodes (PHOLEDs), Asian J. Org. Chem., 2015, 4, 394–429; (j) C.-L. Ho, W.-Y. Wong, Small-molecular blue phosphorescent dyes for organic light-emitting devices, New J. Chem., 2013, 37, 1665–1683; (k) C.-L. Ho, W.-Y. Wong, Charge and energy transfers in functional metallophosphors and metallopolyynes, Coord. Chem. Rev., 2013, 257, 1614–1649.

[2] Y. Chi, P.-T. Chou, Transition-metal phosphors with cyclometalating ligands: fundamentals and applications, Chem. Soc. Rev., 2010, 39, 638–655.

[3] Y. You, S.Y. Park, Phosphorescent iridium(iii) complexes: toward high phosphorescence quantum efficiency through ligand control, Dalton Trans., 2009, 1267–1282.

[4] P.-T. Chou, Y. Chi, Phosphorescent Dyes for Organic Light-Emitting Diodes, Chem.-Eur. J., 2007, 13, 380–395.

[5] Z.Q. Chen, Z.Q. Bian, C.H. Huang, Functional Ir^{III} complexes and their applications, Adv.

Mater., 2010, 22, 1534–1539.

[6] L.X. Xiao, Z.J. Chen, B. Qu, J.X. Luo, S. Kong, Q.H. Gong, J. Kido, Recent Progresses on Materials for Electrophosphorescent Organic Light-Emitting Devices, Adv. Mater., 2011, 23, 926–952.

[7] R.D. Costa, E. Ortí, H.J. Bolink, S. Graber, C.E. Housecroft, E.C. Constable, Efficient and long-living light-emitting electrochemical cells, Adv. Funct. Mater., 2010, 20, 1511–1520.

[8] C. Rothe, C.-J. Chiang, V. Jankus, K. Abdullah, X. Zeng, R. Jitchati, A.S. Batsanov, M.R. Bryce, A.P. Monkman, Ionic iridium(III) complexes with bulky side groups for use in light emitting cells: Reduction of concentration quenching, Adv. Funct. Mater., 2009, 19, 2038–2044.

[9] X.S. Zeng, M. Tavasli, I.F. Perepichka, A.S. Batsanov, M.R. Bryce, C.-J. Chiang, C. Rothe, A.P. Monkman, Cationic bis-cyclometallated iridium(III) phenanthroline complexes with pendant fluorenyl substituents: Synthesis, redox, photophysical properties and light-emitting cells, Chem.-Eur. J., 2008, 14, 933–943.

[10] H.C. Su, H.F. Chen, C.C. Wu, K.T. Wong, Decreased turn-on times of single-component light-emitting electrochemical cells by tethering an ionic iridium complex with imidazolium moieties, Chem.-Asian J., 2008, 3, 1922–1928.

[11] (a) M.X. Yu, Q. Zhao, L.X. Shi, F.Y. Li, Z.G. Zhou, H. Yang, T. Yi, C.H. Huang, Cationic iridium(III) complexes for phosphorescence staining in the cytoplasm of living cells, Chem. Commun., 2008, 2115–2117; (b) Y. Ma, H. Xu, Y. Zeng, C.-L. Ho, C.-H. Chui, Q. Zhao, W. Huang, W.-Y. Wong, A charged iridophosphor for time-resolved luminescent CO_2 gas identification, J. Mater. Chem. C, 2015, 3, 66–72; (c) X.L. Yang, Z. Huang, C.-L. Ho, G.J.

Zhou, D.R. Whang, C.L. Yao, X.B. Xu, S.Y. Park, C.-H. Chui, W.-Y. Wong, Dynamic dual stage phosphorescence chromatic change in a diborylated iridium phosphor for fluoride ion sensing with concentration discriminating capability, RSC Adv., 2013, 3, 6553–6563.

[12] D.S.-H. Chan, W.-C. Fu, M. Wang, L.-J. Liu, C.-H. Leung, D.-L. Ma, A highly selective and non-reaction based chemosensor for the detection of Hg^{2+} ions using a luminescent iridium(III) complex, PLOS ONE, 2013, 8, e60114.

[13] D.-L. Ma, H.-Z. He, D.S.-H. Chan, C.-Y. Wong, C.-H. Leung, A colorimetric and luminescent dual-modal assay for Cu(II) ion detection using an iridium(III) complex, PLOS ONE, 2014, 9, e99930.

[14] M. Wang, K.-H. Leung, S. Lin, D.S.-H. Chan, D.W.J. Kwong, C.-H. Leung, D.-L. Ma, A colorimetric chemosensor for Cu^{2+} ion detection based on an iridium(III) complex, Sci. Rep., 2014, 4, 6794.

[15] W.H.-T. Law, L.C.-C. Lee, M.-W. Louie, H.-W. Liu, T.W.-H. Ang, K.K.-W. Lo, Phosphorescent cellular probes and uptake indicators derived from cyclometalated iridium(III) bipyridine complexes appended with a glucose or galactose entity, Inorg. Chem., 2013, 52, 13029–13041.

[16] K.K.-W. Lo, W.-K. Hui, C.-K. Chung, K.H.-K. Tsang, D.C.-M. Ng, N. Zhu, K.-K. Cheung, Biological labelling reagents and probes derived from luminescent transition metal polypyridine complexes, Coord. Chem. Rev., 2005, 249, 1434–1450.

[17] L.Q. Xiong, Q. Zhao, H.L. Chen, Y.B. Wu, Z.S. Dong, Z.G Zhou, F.Y. Li, Phosphorescence imaging of homocysteine and cysteine in living cells based on a cationic iridium(III) complex, Inorg. Chem., 2010, 49, 6402–6408.

[18] Q. Zhao, C.H. Huang, F.Y. Li, Phosphorescent heavy-metal complexes for bioimaging, Chem. Soc. Rev., 2011, 40, 2508–2524.

[19] Y.M. You, Y. Han, Y.-M. Lee, S.Y. Park, W. Nam, S.J. Lippard, Phosphorescent sensor for robust quantification of copper(II) ion, J. Am. Chem. Soc., 2011, 133, 11488–11491.

[20] Y. Liu, M.Y. Li, Q. Zhao, H.Z. Wu, K.W. Huang, F.Y. Li, Phosphorescent iridium(III) complex with an N^O ligand as a Hg^{2+}-selective chemodosimeter and logic gate, Inorg. Chem., 2011, 50, 5969–5977.

[21] R. Kramer, Fluorescent chemosensors for Cu^{2+} ions: fast, selective, and highly sensitive, Angew. Chem. Int. Ed., 1998, 37, 772–773.

[22] G.L. Millhauser, Copper binding in the prion protein, Acc. Chem. Res., 2004, 37, 79–85.

[23] R. Seth, S. Yang, S. Choi, M. Sabean, E.A. Roberts, In vitro assessment of copper-induced toxicity in the human hepatoma line, Hep G2, Toxicol. in Vitro, 2004, 18, 501–509.

[24] K.J. Barnham, C.L. Masters, A.I. Bush, Neurodegenerative diseases and oxidative stress, Nat. Rev. Drug. Discov., 2004, 3, 205–214.

[25] V. Chandrasekhar, S. Das, R. Yadav, S. Hossain, R. Parihar, G. Subramaniam, P. Sen, Novel chemosensor for the visual detection of copper(II) in aqueous solution at the ppm level, Inorg. Chem. 2012, 51, 8664–8666.

[26] Y.R. Sadaphal, S.S. Gholap, A highly selective colorimetric chemosensor for copper(II) based on N-phenyl-N′-(pyridin-2-yl)thiourea(HPyPT), Sens. Actuators B: Chem., 2017, 253, 173–179.

[27] S.S. Zhang, Q.F. Niu, L.X. Lan, T.D. Li, Novel oligothiophene-phenylamine based Schiff base as a fluorescent chemosensor for the dual-channel detection of Hg^{2+} and Cu^{2+} with high

sensitivity and selectivity, Sens. Actuators B: Chem., 2017, 240, 793–800.

[28] G. Li, F.R. Tao, H. Wang, Y.C. Li, L.P. Wang, A novel reversible colorimetric chemosensor for rapid naked-eye detection of Cu2+ in pure aqueous solution, Sens. Actuators B: Chem., 2015 211, 325–331.

[29] S. Zhang, T. Yu, M.T. Sun, H. Yu, Z.P. Zhang, S.H. Wang, H. Jiang, Highly sensitive and selective fluorescence detection of copper (II) ion based on multi-ligand metal chelation, Talanta, 2014, 126, 185–190.

[30] A.N. Uglov, A. Bessmertnykh-Lemeune, R. Guilard, A.D. Averin, I.P. Beletskaya, Optical methods for the detection of heavy metal ions, Russ. Chem. Rev., 2014, 83, 196–224.

[31] R. Krämer, Fluorescent chemosensors for Cu^{2+} ions: Fast, selective, and highly sensitive, Angew. Chem. Int. Ed., 1998, 37, 772–773.

[32] H.-B. Kim, Y. Li, M.H. Hyun, Phosphorescent chemosensor based on iridium(III) complex for the selective detection of Cu(II) ion in aqueous acetonitrile, Bull. Korean Chem. Soc., 2013, 34, 653–656.

[33] W. Shen, L.Q. Yan, W.W. Tian, X. Cui, Z.J. Qi, Y.M. Sun, A novel aggregation induced emission active cyclometalated Ir(III) complex as a luminescent probe for detection of copper(II) ion in aqueous solution, J. Lumin., 2016, 177, 299–305.

[34] H. Yang, Y.C. Zhu, L.T. Li, Z.G. Zhou, S.P. Yang, A phosphorescent chemosensor for Cu^{2+} based on cationic iridium(III) complexes, Inorg. Chem. Commun., 2012, 16, 1–3.

[35] N. Zhao, Y.H. Wu, J. Luo, L.X. Shia, Z.N. Chen, Aggregation-induced phosphorescence of iridium(III) complexes with 2,2′-bipyridine-acylhydrazone and their highly selective recognition to Cu^{2+}, Analyst, 2013, 138, 894–900.

[36] A.A. Farah, W.J. Pietro, Telechelic poly(ε-caprolactones) with tethered mixed ligand ruthenium(II) chromophores, Can. J. Chem., 2004, 82, 595–607.

[37] T.Z. Yu, Y.J. Bao, Y.L. Zhao, H. Zhang, Z.X. Xu and X.M. Wu, Synthesis, photo- and electro-luminescence of red-emitting Ir(III)complexes with 2-(1-naphthyl) benzothiazole and carrier transporting group-functionalized picolinate ligands, J. Organomet. Chem., 2016, 825–826, 33–40.

[38] S.F. Huang, H.Z. Sun, G.G. Shan, F.S. Li, Q.Y. Zeng, K.Y. Zhao, Z.M. Su, Rational design and synthesis of cationic Ir(III) complexes with triazolate cyclometalated and ancillary ligands for multi-color tuning, Dyes and Pigments, 2017, 139, 524–532.

[39] W.Y. Wong, G.J. Zhou, X.M. Yu, H.S. Kwok, B.Z. Tang, Amorphous diphenylaminofluorene-functionalized iridium complexes for high-efficiency electrophosphorescent light-emitting diodes, Adv. Funct. Mater., 2006, 16, 838–846.

[40] S. Bettington, M. Tavasli, M.R. Bryce, A. Beeby, H. Al-Attar, A.P. Monkman, Tris-cyclometalated iridium (III) complexes of carbazole(fluorenyl)pyridine ligands: Synthesis, redox and photophysical properties, and electrophosphorescent light-emitting diodes, Chem. Eur. J., 2007, 13, 1423–1431.

[41] C. Yi, W.W. Tian, B. Song, Y.P. Zheng, Z.J. Qi, Q. Qi, Y.M. Sun, A new turn-off fluorescent chemosensor for iron(III) based on new diphenylfluorenes with phosphonic acid, J. Lumin., 2013, 141, 15–22.

[42] Y.M. Liu, J.Y. Zhang, J.X. Ru, X. Yao, Y. Yang, X.H. Li, X.L. Tang, G.L. Zhang, W.S. Liu, A naked-eye visible and turn-on fluorescence probe for Fe^{3+} and its bioimaging application in living cells, Sens. Actuators B: Chem., 2016, 237, 501–508.

Highlights:

1. A cationic Ir(III) complex was synthesized for detection of Cu^{2+} in vitro and cells.

2. The detection limit for Cu^{2+} was measured to be 10^{-8} M.

3. The complex has well cell-permeable and imaging characteristics for Cu^{2+} in cells.