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Correspondence and requests for materials should be addressed to D.W.J.K. (dkwong@hkbu.edu.hk); C.-H.L. (duncanleung@umac.mo) or D.-L.M. (edmondma@hkbu.edu.hk)

* These authors contribute equally to this work.

A colorimetric chemosensor for Cu^{2+} ion detection based on an iridium(III) complex

Modi Wang[1]*, Ka-Ho Leung[1]*, Sheng Lin[1], Daniel Shiu-Hin Chan[1], Daniel W. J. Kwong[1], Chung-Hang Leung[2] & Dik-Lung Ma[1,3]

[1]Department of Chemistry, Hong Kong Baptist University, Kowloon Tong, Hong Kong, China, [2]State Key Laboratory of Quality Research in Chinese Medicine, Institute of Chinese Medical Sciences, University of Macau, Macau, China, [3]Partner State Key Laboratory of Environmental and Biological Analysis.

We report herein the synthesis and application of a series of novel cyclometalated iridium(III) complexes 1−3 bearing a rhodamine-linked N^N ligand for the detection of Cu^{2+} ions. Under the optimised conditions, the complexes exhibited high sensitivity and selectivity for Cu^{2+} ions over a panel of other metal ions, and showed consistent performance in a pH value range of 6 to 8. Furthermore, the potential application of this system for the monitoring of Cu^{2+} ions in tap water or natural river water samples was demonstrated.

The Cu^{2+} ion plays an important role in a number of biological processes, including iron absorption, haemopoiesis, various enzyme-catalyzed and redox reactions[1]. Abnormal levels of copper ions can lead to vomiting, lethargy, increased blood pressure and respiratory rates, acute haemolytic anaemia, liver damage, neurotoxicity, and neurodegenerative disease[2,3]. Furthermore, copper ions can disrupt natural ecosystems due to their adverse effects on microorganisms[4]. Therefore, the development of selective and reliable detection methods for Cu^{2+} ions is of significant interest to the scientific and environmental communities.

A number of analytical methods have been developed for the accurate determination of Cu^{2+} ions, including atomic absorption/emission spectroscopy (AAS/AES)[5], inductively-coupled plasma mass spectrometry (ICP-MS)[6], electrochemical methods[7] and X-ray fluorescence spectroscopy (XRF). However, these techniques involve time-consuming sample pre-treatment methods and/or the use of sophisticated instrumentation, thus precluded their application for in-field detection of Cu^{2+} ions. This has stimulated the development of a variety of small molecule probes for the rapid and sensitive detection of Cu^{2+} ions[4,8–22] (Table 1).

Transition metal complexes have found increasing use as colorimetric or luminescent probes for biomolecules and metal ions[23–35]. In particular, octahedral metal complexes possess a unique geometry around which functional co-ligands can be arranged in a defined fashion. For example, Li, Huang and co-workers have previously developed a dual colorimetric and luminescent iridium(III)-based sensor for Hg^{2+} ions by the use of sulfur-containing cyclometalated ligands[24]. In the context of Cu^{2+} ion detection, several groups have reported DPA-based iridium(III) complexes for the luminescent sensing of Cu^{2+} ions[25,36]. However, to our knowledge, no colorimetric iridium(III)-based Cu^{2+} ion probe has yet been described in the literature. We report herein the synthesis and application of a series of novel cyclometalated iridium(III) complexes **1**–**3** bearing a rhodamine-linked N^N ligand for the highly sensitive and selective detection of Cu^{2+} ions (Figure 1).

The synthetic pathway for the synthesis of complexes **1**−**3** is depicted in Scheme S1. In our design strategy, the interaction of Cu^{2+} ions with the rhodamine-linked N^N ligand causes the rhodamine moiety to undergo a conformational change from a spirolactam form into a ring-opened amide form[9]. This structural transition results in a change to the photophysical properties of these complexes, allowing them to function as optical chemosensors for Cu^{2+} ions.

Results

Cu^{2+} ion detection. To investigate the Cu^{2+} ion detection properties of complexes **1**–**3**, we studied the UV-Vis absorption behaviour of these complexes in response to Cu^{2+} ions. Remarkably, a new absorption peak at 555 nm was observed in the spectra of complexes **1**–**3** (5 μM) upon the addition of Cu^{2+} ions (Figure S1), and the color of the solution turned pink. This behavior was attributed to the interaction of Cu^{2+} ions with the rhodamine-linked

Table 1 | Comparison of the advantages, disadvantages and LOD of our newly developed probe with other methods

Method	Detection limit (μM)	Advantages	Disadvantages	Recovery in real sample(%)/ $[Cu^{2+}]_{spiked}$ (μM)	Reference
Fluorescent probe of rhodamine B derivative	0.64	Selective towards Cu(II) ions	Needs organic solvent for detection	95−106/3 and 30	8
Colorimetric rhodamine-based chemosensor	1	Selective towards Cu(II) ions	Low sensitivity	N/A	22
Chromogenic and fluorescent rhodamine-based chemosensor	41	Selective towards Cu(II) ions	Low sensitivity	104−106/163	17
Ratiometric fluorescence chemosensors	0.025	Selective towards Cu(II) ions	Needs organic solvent for detection	N/A	9
Fluorescent chemosensor with two 5-nitro-salicylaldehyde	0.6	Selective towards Cu(II) ions	Long synthetic protocol	N/A	40
Fluorescent rhodamine-based chemosensors	0.02	Selective towards Cu(II) ions/ intracellular imaging	Long synthetic protocol	N/A	41
Iridium(III) complex	0.0045	Selective towards Cu(II) ions, modular synthesis and low detection limit	Needs organic solvent for detection	80−110/3 60−130/0.05	This study

N^N ligand, causing the rhodamine moiety to undergo a conformational change from a spirolactam form into a ring-opened amide form (Figure 1), giving rise to a pink color that is consistent with previous work[9]. Moreover, the UV-Vis spectra showed that the complexes were stable in the absence or presence of Cu^{2+} ions for at least 24 h (Figure S2).

To optimize the performance of the sensor, the choice of organic solvent, aqueous buffer and overall solvent composition were investigated. The absorption increase of complexes **1–3** in response to Cu^{2+} ions was highest with acetonitrile (ACN) compared to dimethyl sulfoxide (DMSO), tetrahydrofuran (THF) or *N*,*N*-dimethylformamide (DMF) (Figure S3). Additionally, complexes **1–3** displayed similar responses to Cu^{2+} ions in buffer systems containing 90, 70 or 50% of acetonitrile (Figure S4). However, the absorption of the complexes was seen to decrease by 30-50% when 30% of acetonitrile was used (Figure S4). Hence, 50% of acetonitrile was considered optimal for further study. We also found that the use of 2-amino-2-hydroxymethyl-propane-1,3-diol (Tris), 2-(*N*-morpholino)ethanesulfonic acid (MES) and 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffer gave similar performances, whereas the use of phosphate buffer resulted in a 25% decrease in absorption intensity of the complexes in response to Cu^{2+} ions (Figure S5). Moreover, the optimal concentration of Tris buffer was determined to be 10 mM (Figure S6). After optimization of the assay conditions, we performed an absorption titration experiment to investigate the absorption response of complexes **1**−**3** to Cu^{2+} ions. Encouragingly, the absorption of complexes **1**−**3** was enhanced as the concentration of Cu^{2+} ions increased (Figure 2a, c, e). Saturation of the absorbance value was reached at 35 μM of Cu^{2+} ions for all three complexes. Complexes **1** and **3** exhibited a linear range of detection for Cu^{2+} ions from 10 nM to 8 μM ($R^2 = 0.99$), while complex **2** displayed a linear range of detection for Cu^{2+} ions from 10 nM to 6 μM (Figure 2b, d, f). The detection limit of complexes **1**−**3** for Cu^{2+} ions was estimated to be 4.5, 5.2 and 4.9 nM using the 3σ criterion, indicating that these complexes were highly sensitive for Cu^{2+} ions. Additionally, the color change of the solution from colorless to pink occurred within 10 s upon the addition of Cu^{2+} ions, suggesting that complexes **1**−**3** could potentially serve as simple and rapid 'naked-eye' indicators for Cu^{2+} ions (Figure 3a).

Selectivity analysis. The selectivity of complexes **1**−**3** for Cu^{2+} ions was examined by testing the response of the system to various other metal ions, including Zn^{2+}, Cd^{2+}, Mg^{2+}, Hg^{2+}, Ca^{2+}, Pb^{2+}, Ni^{2+}, Co^{2+}, Fe^{3+}, K^+, Na^+ and Ag^+. In the absence of metal ions, the UV-Vis absorption spectra of complexes **1**−**3** displayed no significant absorption in the 400 to 600 nm region (Figure 4). The addition of 10 μM of Cu^{2+} ions to complexes **1–3** generated a new absorption band at 555 nm, with a 50-fold increase in absorbance

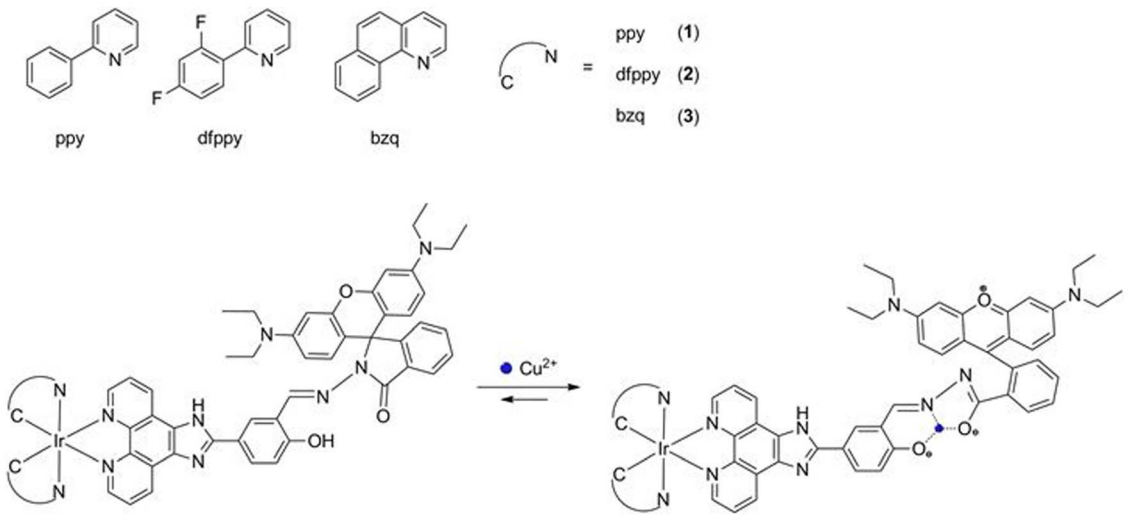

Figure 1 | Chemical structures of cyclometallated iridium(III) complexes 1–3.

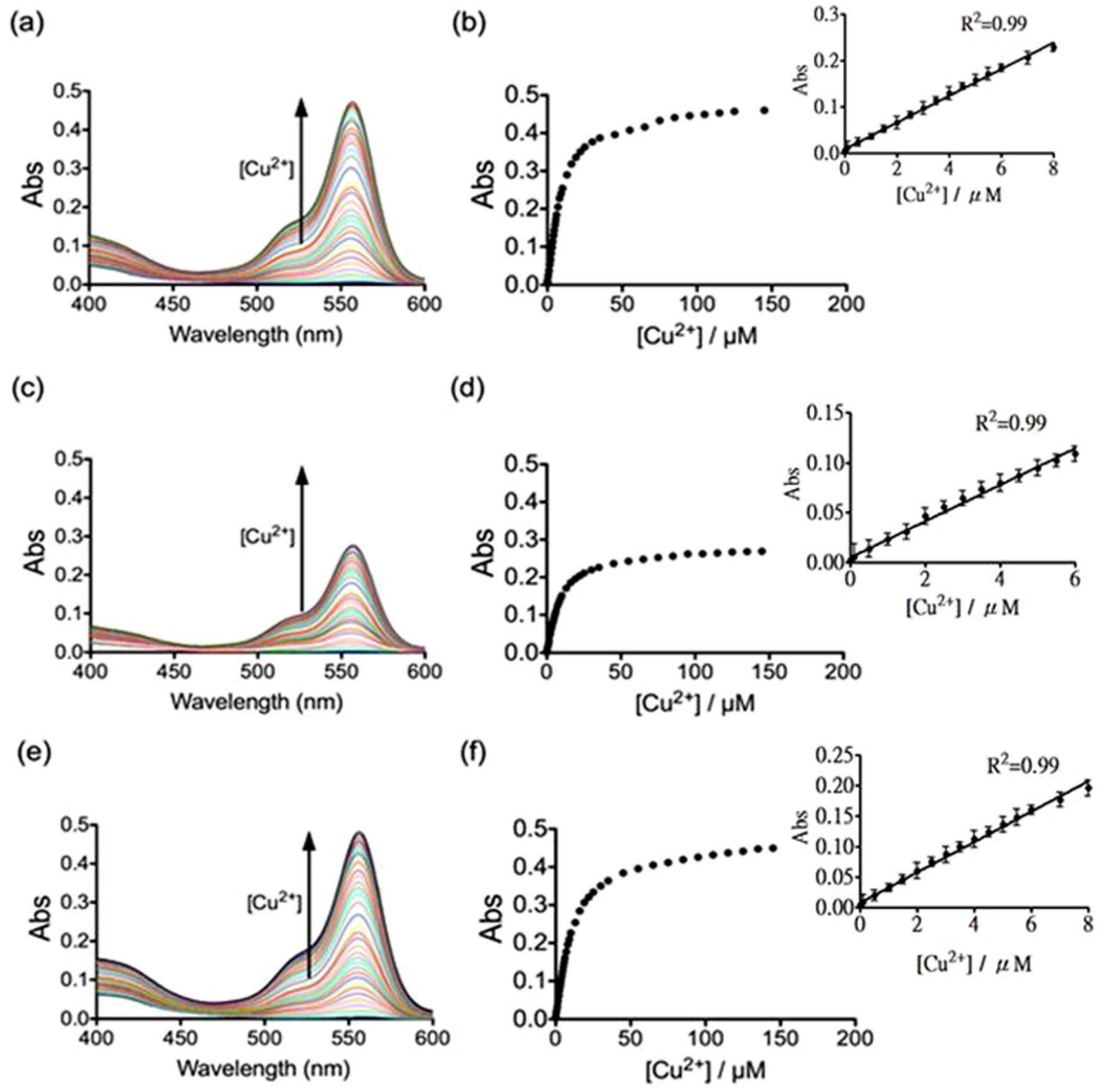

Figure 2 | Absorption spectra of 5 μM of complexes (a) **1**, (c) **2** and (e) **3** in the presence of increasing concentrations of Cu^{2+} ion (1 : 1 ACN-Tris, pH 7.0). Absorbance of complexes (b) **1**, (d) **2** and (f) **3** at 555 nm vs. [Cu^{2+}]. Inset: linear plot of the change in absorbance of the system vs. [Cu^{2+}].

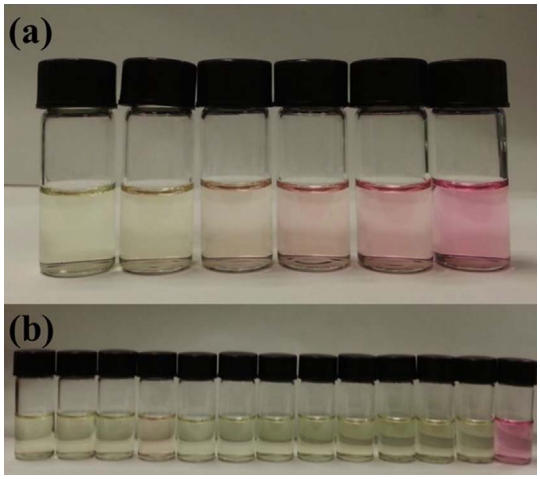

Figure 3 | **Photograph images of complex 1 (5 μM) in the presence of (a) 0, 1, 3, 5, 10 and 20 μM of Cu^{2+} (left to right) and (b) 25 μM of Zn^{2+}, Cd^{2+}, Mg^{2+}, Ag^{+}, Hg^{2+}, Ca^{2+}, Pb^{2+}, Ni^{2+}, Co^{2+}, Fe^{3+}, K^{+} and Na^{+} or 10 μM of Cu^{2+} (left to right).**

signal. By comparison, only minor effects on the absorbance signals of complex **1**–**3** were observed upon the addition of 25 μM of the other metal ions. Importantly, the difference in absorbance intensity of complex **1** in the presence of Cu^{2+} ions compared to the other metal ions could be distinguished by the naked eye (Figure 3b). These results demonstrate the high selectivity of complexes **1**−**3** for Cu^{2+} ions over other metal ions, which originates presumably from the specific interaction of Cu^{2+} ions with the rhodamine-linked N^N ligand. The rhodamine moiety is known to be highly selective for Cu^{2+} ions over other common metal ions[9]. A competition experiment was also carried out by adding 25 μM of other metal ions to solutions of complexes **1**–**3** containing 10 μM of Cu^{2+} ions. The absorbance intensity of complexes **1**–**3** was not significantly abrogated by the presence of the other metal ions (Figure 5), suggesting that the system could potentially be used to detect Cu^{2+} ions in a sample matrix containing interfering metal ions.

Job's plot analysis. The absorbance spectra were analysed using a Job's plot to determine the binding stoichiometry of the iridium(III) complexes **1**−**3** with Cu^{2+} ions. The maximum absorbance of the **1**–Cu^{2+}, **2**–Cu^{2+} and **3**–Cu^{2+} complexes were all achieved at a mole fraction of approximately 50% of Cu^{2+} ions (Figure 6), suggesting

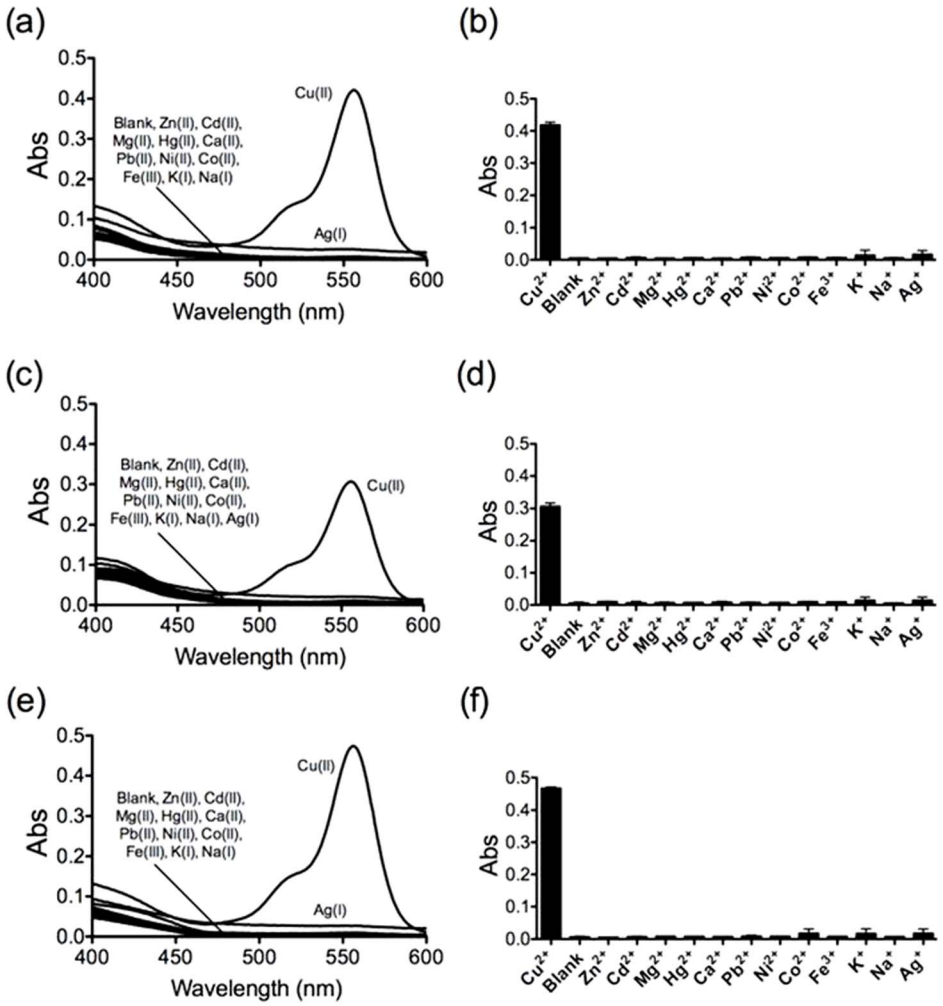

Figure 4 | UV-Vis spectra of 5 μM of complexes **1** (a), **2** (c) and **3** (e) in the presence of 10 μM of Cu^{2+} or 25 μM of various metal ions (1 : 1 ACN-Tris, pH 7.0). Absorbance of 5 μM of complexes **1** (b), **2** (d) and **3** (f) at 555 nm in the presence of 10 μM of Cu^{2+} and 25 μM of various metal ions.

that a 1 : 1 ratio is the likely binding stoichiometry of complexes **1**−**3** with Cu^{2+} ions.

Influence of pH. We next investigated the effect of pH on the response of complexes **1**−**3** to Cu^{2+} ions. The results showed that the performances of the complexes were quite consistent over a pH range of 6 to 8 (Figure S7). This result is consistent with a previous study by Yin and co-workers, who showed that a rhodamine-based sensor could function normally under this pH range[22]. Hence, complexes **1**–**3** are suitable for use as Cu^{2+} chemosensors in the typical pH range of biological systems.

Cu^{2+} ion detection in a tap water sample. In order to investigate the applicability of the system for real sample analysis, we investigated the ability of complexes **1**–**3** to detect Cu^{2+} ions in three water samples using the spike-and-recovery method. Two of the samples were tap water taken from laboratory water pipe located at Hong Kong Baptist University, Hong Kong, while the third sample was a natural water sample taken from Shing Mun River, Hong Kong. The Cu^{2+} ion content in the water sample was first determined by atomic absorption spectroscopy. Then, 0.05 and 2 μM of Cu^{2+} ions was spiked into the sample. Using iridium(III) complexes **1**–**3** as Cu^{2+} ion probes, the recovery factor was determined to be *ca.* 80−110% for Cu^{2+} ion concentrations in the middle region of the linear range of detection and *ca.* 60−130% for Cu^{2+} ion concentrations close to the detection limit (Table 2)[37]. This result demonstrates the potential application of the system in monitoring Cu^{2+} ion content in real water samples. Although the detection limit of complexes **1**–**3** for Cu^{2+} ions in the buffer/organic solvent mixture system is much lower than those reported in other studies, the accuracy of the complexes in real samples seems worse. This may be attributed to the influence of interfering matrix substances in the real water samples, which is a significant factor especially when the spiked Cu^{2+} concentration in this study is as low as 50 nM, compared to 3, 30 or 160 μM that was used in other studies (Table 1).

Discussion

In summary, we have developed a series of novel iridium(III) complexes **1**−**3** as colorimetric probes for the sensitive detection of Cu^{2+} ions, and have demonstrated the potential application of the system for the monitoring of Cu^{2+} ions in real life water samples. Under the optimised conditions, the complexes exhibited high selectivity for Cu^{2+} ions over a panel of other metal ions, and showed consistent performance over a pH range of 6 to 8. Our method is simple, rapid, cost-effective, and could find potential application as a 'naked-eye' indicator for Cu^{2+} ions in water samples. Due to the modular nature of metal complex synthesis, we envisage that this iridium-based chemosensor for Cu^{2+} ions could be further readily fine-tuned without the need for labor-intensive synthetic protocols. We anticipate that this report of a colorimetric iridium(III)-based chemosensor for Cu^{2+} ions could prompt the development of more sensitive and cost-effective methods for Cu^{2+} ion detection from the scientific community.

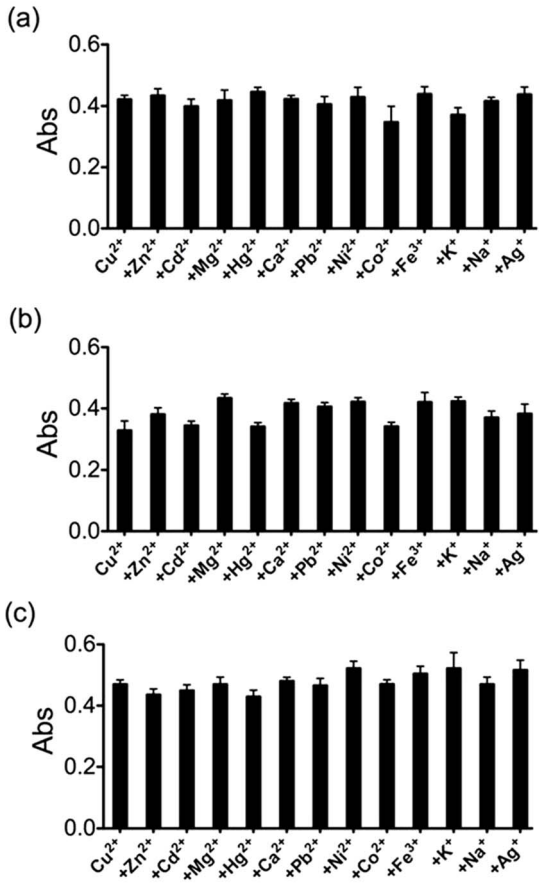

Figure 5 | Absorbance of 5 μM of complexes (a) 1, (b) 2 and (c) 3 at 555 nm in the presence of both 10 μM of Cu^{2+} and 25 μM of various metal ions (1 : 1 ACN-Tris, pH 7.0).

Methods

Chemicals and materials. Reagents were purchased from Sigma Aldrich (St. Louis, MO) and were used as received. Iridium chloride hydrate ($IrCl_3.xH_2O$) was purchased from Precious Metals Online (Australia).

General experimental. Mass spectrometric measurements were performed at the Mass Spectroscopy Unit at the Department of Chemistry, Hong Kong Baptist University, Hong Kong (China). Melting points were determined using a Gallenkamp melting apparatus and are uncorrected. Deuterated solvents for NMR purposes were obtained from Armar and used as received. ^1H and ^{13}C NMR were recorded on a Bruker Avance 400 spectrometer operating at 400 MHz (^1H) and 100 MHz (^{13}C). ^1H and ^{13}C chemical shifts were referenced internally to solvent shift (CD_3CN: ^1H, δ 1.94, ^{13}C δ 118.7; d_6-DMSO: ^1H δ 2.50, ^{13}C δ 39.5). Chemical shifts (δ) are quoted in ppm, the downfield direction being defined as positive. Uncertainties in chemical shifts are typically ±0.01 ppm for ^1H and ±0.05 for ^{13}C. Coupling constants are typically ±0.1 Hz for ^1H-^1H and ±0.5 Hz for ^1H-^{13}C couplings. The following abbreviations are used for convenience in reporting the multiplicity of NMR resonances: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad. All NMR data was acquired and processed using standard Bruker software (Topspin).

Cu^{2+} ion detection. 10 mM of complex stock solution was prepared by dissolving complexes **1**−**3** in acetonitrile. The complexes were then added into 1 : 1 (v/v) acetonitrile: Tris buffer to a final concentration of 5 μM. Different concentrations of Cu^{2+} ions (as $Cu(NO_3)_2$) were then added to 1 mL of 1 : 1 (v/v) acetonitrile: Tris buffer (10 mM, pH 7.0) containing complexes **1**–**3** (5 μM) in a cuvette. UV-Vis absorption spectra were recorded on a Cary UV-300 spectrophotometer (double beam).

Water sample analysis. Two tap water samples were taken from a laboratory water pipe located in Hong Kong Baptist University, Hong Kong, and a river water sample was taken from Shing Mun River, Hong Kong. Standard solution of Cu^{2+} ions was spiked into the real water sample up to a final concentration of 2 μM of Cu^{2+} ions. 0.5 mL of the resulting solution was mixed with 0.5 mL of acetonitrile in a cuvette, and then complexes **1**−**3** were added at a final concentration of 5 μM. UV-Vis absorption spectra were recorded on a Cary UV-300 spectrophotometer (double beam).

Synthesis of compounds 5 and 6. These compounds were synthesised according to a reported literature procedure[38]. Following a Steck-Day reaction procedure, 500 mg of 1,10-phenanthroline-5,6-dione (2.4 mmol) and 350 mg of 4-hydroxybenzaldehyde (2.8 mmol, 1.1 eq.) were dissolved in 10 mL of acetic acid. The mixture was heated at 80°C for 30 min. 3.70 g of dry ammonium acetate (48 mmol, 20 eq.) was added and the reaction was stirred at 100°C for 3 h. The reaction was allowed to cool to room temperature and the product was filtered through a fritted funnel and washed with copious amounts of acetone and ether to yield 510 mg (68%) of compound **5**. Following a modified Duff reaction, 500 mg (1.6 mmol) of **5** and 896 mg of hexamethylene tetraamine (6.4 mmol, 4 eq.) were dissolved in 10 mL of trifluoroacetic acid and refluxed for 3 days. The mixture was allowed to cool to room temperature and 50 mL of 4 M HCl solution was added. The resulting mixture was stirred for 2 h, during which time the solution became cloudy. The solid was collected by filtration, washed with acetone and ether, and dried in vacuum to yield 452 mg (56%) of pure compound **6**.

Synthesis of compound 8. This compound was synthesised according to a reported literature procedure[9]. Rhodamine B (2.5 mmol) was dissolved in 30 mL of ethanol, and 3.0 mL of hydrazine hydrate (85%, excess) was added dropwise with vigorous stirring at room temperature. The mixture was heated to reflux in air for 2 h with stirring. The solution changed from dark purple to light orange, and then became clear. The mixture was cooled and the solvent was removed under reduced pressure. 1 M HCl (50 mL) was added to the solid in the flask to generate a clear red solution. The solution was then basified by the slow addition of 1 M NaOH until the pH of the solution reached 9–10. The resulting precipitate was filtered and washed three times with 15 mL of water. Drying under an IR light afforded 0.83 g (75%) of **8** as a pink solid.

Synthesis of compound 9. This compound was synthesised according to a reported literature procedure[9]. Rhodamine hydrazide (**8**, 1 mmol) was dissolved in 30 mL of absolute ethanol. An excess amount of compound **6** (4 mmol) was added and the mixture was refluxed in air for 6 h. The solution was cooled, concentrated to 10 mL and allowed to stand at room temperature overnight. The precipitate which appeared the next day was filtered and washed three times with 10 mL of cold ethanol. Drying under reduced pressure afforded 0.58 g (74%) of **9** as a pink solid. Yield: 65%. ^1H NMR (400 MHz, $CDCl_3$) δ 13.15 (s, 1H), 11.32 (s, 1H), 9.00 (s, 1H), 8.93 (d, J = 7.7 Hz, 2H), 8.84 (d, J = 3.2 Hz, 1H), 8.62 (d, J = 7.8 Hz, 1H), 7.89 (t, J = 7.7 Hz, 2H), 7.82 (s, 1H), 7.59–7.47 (m, 2H), 7.43 (t, J = 7.3 Hz, 1H), 7.31 (dd, J = 7.8, 4.2 Hz, 1H), 7.15 (d, J = 7.6 Hz, 1H), 6.69 (d, J = 8.6 Hz, 1H), 6.52 (d, J = 8.9 Hz, 2H), 6.46 (d, J = 2.4 Hz, 2H), 6.24 (dd, J = 9.0, 2.4 Hz, 2H), 3.25 (d, J = 7.4 Hz, 8H), 1.09 (t, J = 7.0 Hz, 12H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.49, 159.52, 153.49, 152.31, 151.00, 149.10, 147.59, 143.53, 136.00, 133.74, 130.44, 129.96, 129.17, 128.65, 126.53, 124.10, 123.38, 122.74, 121.49, 119.66, 118.64, 117.43, 108.13, 104.95, 98.06, 66.65, 58.15, 44.30, 30.97, 18.36; HRMS: Calcd. for $C_{48}H_{42}N_8O_3$: 778.3380, Found: 779.4721.

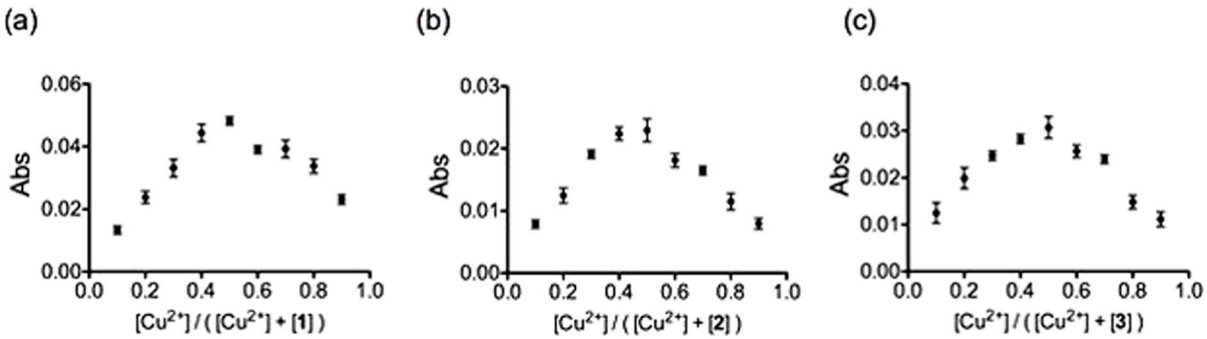

Figure 6 | Job's plot of complexes (a) 1, (b) 2 and (c) 3 with Cu^{2+} ions (1 : 1 ACN-Tris, pH 7.0). Total concentration of 5 μM, λ_{abs} = 555 nm.

Table 2 | Determination of Cu^{2+} ion content in water samples

Complex	Sample	$[Cu^{2+}]$/μM	Spiked $[Cu^{2+}]$/μM	Found $[Cu^{2+}]$/μM	Recovery (%)
1	Tap water 1	0.89	2	2.65 ± 0.08	88
1	Tap water 1	0.89	0.05	0.93 ± 0.06	80
1	Tap water 2	0.67	2	2.45 ± 0.09	89
1	Tap water 2	0.67	0.05	0.735 ± 0.06	130
1	Shin Mun River	0	2	1.565 ± 0.105	78.25
1	Shin Mun River	0	0.05	0.059 ± 0.01	118
2	Tap water 1	0.89	2	2.73 ± 0.3	92
2	Tap water 1	0.89	0.05	0.92 ± 0.06	60
2	Tap water 2	0.67	2	2.77 ± 0.12	105
2	Tap water 2	0.67	0.05	0.71 ± 0.06	80
2	Shin Mun River	0	2	1.695 ± 0.035	84.75
2	Shin Mun River	0	0.05	0.067 ± 0.003	134
3	Tap water 1	0.89	2	3.085 ± 0.035	109.75
3	Tap water 1	0.89	0.05	0.925 0.055	70
3	Tap water 2	0.67	2	2.365 ± 0.215	84.75
3	Tap water 2	0.67	0.05	0.735 ± 0.005	130
3	Shin Mun River	0	2	1.665 ± 0.115	83.25
3	Shin Mun River	0	0.05	0.065 ± 0.005	130

Synthesis of complexes 1–3. The precursor complexes $[Ir_2(ppy)_4Cl_2]$, $[Ir_2(dfppy)_4Cl_2]$ and $[Ir_2(bzq)_4Cl_2]$, and the metal complexes **1–3** were prepared according to modified literature methods[39].

*Representative procedure for complex **1***: A suspension of $[Ir_2(ppy)_4Cl_2]$ (0.2 mmol) and compound **6** (0.44 mmol) in 20 mL dimethylformamide was refluxed overnight under a nitrogen atmosphere. The resulting solution was allowed to cool to room temperature. An aqueous solution of ammonium hexafluorophosphate (excess) was added to the mixture and the resulting precipitate was then filtered. The crude product was purified by silica gel chromatography with a dichloromethane-methanol solvent system (30 : 1 v/v) as eluent. The product was obtained as a brown-orange solid.

Complex **1**. Yield: 55%. ^{1}H NMR (400 MHz, Acetone-d_6) δ 9.46 (s, 1H), 9.06 (d, J = 4.0 Hz, 2H), 8.34 (d, J = 4.0 Hz, 2H), 8.27 (d, J = 8.0 Hz, 2H), 8.12 (t, J = 8.0 Hz, 2H), 7.96–7.89 (m, 7H), 7.70–7.74 (m, 3H), 7.67 (t, J = 8.0 Hz, 1H), 7.24 (d, J = 8.0 Hz, 1H), 7.10 (t, J = 8.0 Hz, 2H), 7.02–6.96 (m, 5H), 6.59–6.42 (m, 8H), 3.43–3.37 (m, 8H), 1.17 (t, J = 8.0 Hz, 12H); ^{13}C NMR (100 MHz, Acetone-d_6) δ 168.7, 165.0, 161.1, 160.8, 154.5, 153.3, 152.6, 151.9, 151.3, 150.3, 150.1, 149.7, 145.1, 139.5, 134.9, 133.0, 132.7, 131.3, 130.5, 130.4, 130.2, 129.9, 129.0, 128.9, 127.5, 127.4, 125.8, 125.1, 124.4, 124.1, 123.4, 121.8, 120.8, 119.8, 118.5, 109.3, 106.1, 98.6, 67.3, 44.8, 12.7 HRMS: Calcd. for $C_{70}H_{58}IrN_{10}O_3$ $[M–PF_6]^+$:1279.4917 Found: 1278.7978.

Complex **2**. Yield: 57%. ^{1}H NMR (400 MHz, Acetone-d_6) δ 9.49 (s, 1H), 9.20 (d, J = 8.0 Hz, 2H), 8.48 (d, J = 4.0 Hz, 4H), 8.14–7.96 (m, 7H), 7.81 (s, J = 4.0 Hz, 2H), 7.73 (t, J = 8.0 Hz, 2H), 7.67 (t, J = 8.0 Hz, 2H), 7.22 (s, J = 8.0 Hz, 1H), 7.08 (t, J = 8.0 Hz, 2H), 6.85 (t, J = 10.0 Hz, 2H), 6.60–6.52 (m, 4H), 6.45–6.42 (m, 2H), 5.92 (d, J = 8.0 Hz, 2H), 3.42 (d, J = 8.0 Hz, 8H), 1.18–1.13 (m, 12H); ^{13}C NMR (100 MHz, Acetone-d_6) δ 164.2, 163.9, 163.8, 162.7, 162.6, 160.0, 154.6, 153.5, 152.8, 150.0, 149.2, 144.2, 139.7, 134.1, 132.7, 129.7, 129.2, 128.9, 128.0, 127.9, 126.9, 124.2, 124.1, 123.7, 123.5, 123.4, 123.1, 121.1, 117.8, 117.5, 114.0, 113.8, 108.4, 99.1, 98.8, 98.5, 97.7, 66.4, 45.8, 44.0, 11.9; HRMS: Calcd. for $C_{70}H_{54}F_4IrN_{10}O_3$ $[M–PF_6]^+$: 1351.4536, Found: 1350.8763.

Complex **3**. Yield: 58%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 14.23 (s, 1H), 10.87 (s, 1H), 9.08 (s, 2H), 8.95 (s, 1H), 8.54 (d, J = 7.7 Hz, 2H), 8.35 (s, 1H), 8.12 (s, 2H), 8.08–7.83 (m, 10H), 7.62 (dd, J = 19.6, 7.6 Hz, 4H), 7.47 (s, 2H), 7.23 (t, J = 7.0 Hz, 2H), 7.09 (dd, J = 26.3, 8.0 Hz, 2H), 6.60–6.24 (m, 8H), 3.32 (s, 8H), 1.07 (t, J = 6.4 Hz, 12H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 163.8, 162.3, 158.8, 156.3, 152.6, 152.3, 151.5, 148.9, 148.6, 147.1, 144.4, 140.3, 137.5, 136.6, 134.2, 133.7, 131.8, 129.7, 129.4, 128.9, 128.5, 127.9, 127.6, 126.7, 124.2, 123.8, 123.2, 122.7, 120.9, 120.3, 119.9, 117.1, 108.2, 108.1, 104.5, 97.4, 65.2, 54.9, 45.4, 43.6, 35.7, 30.7, 12.4; HRMS: Calcd. for $C_{74}H_{58}IrN_{10}O_3$ $[M–PF_6]^+$: 1327.5345 Found: 1326.9231.

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Author contributions

M.W., K.H.L., S.L. and D.S.H.C. carried out all the experiments, performed the data analysis and wrote the manuscript. D.L.M. and C.H.L. designed the experiments and analyzed the results. D.W.J.K. analyzed the results.

Additional information

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