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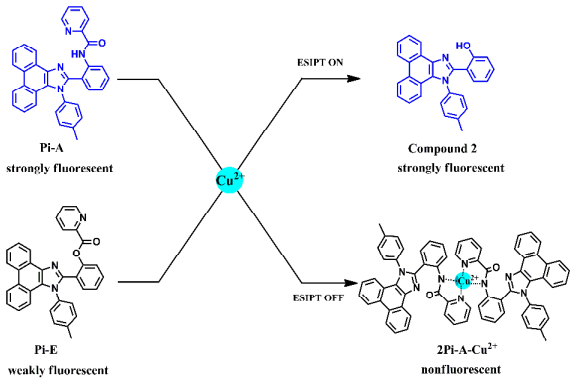
Graphical Abstract



Highlights

- Two novel ESIPT-based fluorescent probes for Cu^{2+} detection were developed.
- Altering linking atom in **Pi-A** reversed its *fluorescence turn-off* to *turn-on* feature.
- Both probes exhibited high selectivity and sensitivity in Cu^{2+} detection.
- These probes can be used for fluorescence imaging of Cu^{2+} in living cell.

Journal Name

COMMUNICATION

Proton donor modulating ESIPT-based fluorescent probes for highly sensitive and selective detection of Cu^{2+}

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Two novel ESIPT-based fluorescent probes, N-(2-(1-(p-tolyl)-1H-phenenanthro[9,10-d]imidazol-2-yl)phenyl) picolinamide (**Pi-A**) and 2-(1-(p-tolyl)-1H-phenanthro[9,10-d]imidazol-2-yl)phenylpicolinate (**Pi-E**), were designed and synthesized. The favorable features of the proposed probes included excellent water solubility, wide pH range, high selectivity and sensitivity to Cu^{2+}. Interestingly, the difference in the linker of the probe molecules led to radical change in the fluorescent response of the probes to Cu^{2+}. **Pi-A** showed fluorescence *turn-off* towards Cu^{2+} with a detection limit of 1.6×10^{-9} M and **Pi-E** showed fluorescence *turn-on* towards Cu^{2+} with a detection limit of 1.8×10^{-8} M in the same condition. The Cu^{2+} recognition mechanisms of the probes were investigated by Job's plot analyses, NMR and ESI-MS spectroscopy experiments. Moreover, our experiments also showed that the probes are of excellent cell permeability and are capable of imaging Cu2+ within living cells.

Introduction

Copper (II) is one of necessary microelement in human body and play crucial roles in many physiological processes.[1] It is also known to be a catalytic cofactor for many metalloenzymes and proteins in organisms.[2] However, the alteration of intracellular Cu^{2+} homeostasis is implicated with various diseases including prion disorders, parkinson's, and other ailments.[3, 4] Therefore, the development of efficient and convenient methods for sensing Cu^{2+} in biological samples is highly valuable to understand its physiological functions and pathological events. Several traditional analytical methods[5-7] like inductively coupled plasma mass spectrometry (ICP–MS), inductively coupled plasma atomic emission spectrometry (ICP–AES) and atomic absorption spectrometry (AAS) have been reported to detect Cu^{2+}. However, these methods involve complicated sample preparation and require expensive and sophisticated instrumentation. The fluorescent probes have been considered as ideal tools that can monitor and image trace amounts of metal ions in live cells or tissues due to its high sensitivity, operational simplicity, and re al-time and nondestructive imaging properties.[8, 9]

Up to now, various fluorescent probes for Cu^{2+} have been designed and synthesized by employing different mechanisms such as coordination to receptors,[10-14] Cu^{2+}–mediated hydrolysis of amides and esters,[15-19] oxidation of phenothiazine, phenol and dihydrorosamine.[20-23] However, many of them still have drawbacks. For instance, some were only utilized in solutions with large amounts of organic solvents or a narrow pH range,[16, 17, 24-26] which would limit their further applications in biological systems. Some probe involved complicated synthetic procedures.[10, 11] In particular, some suffered from incomplete selectivity[20, 27-29] over the competing metal ions such as Fe^{3+}, Co^{2+}, Ni^{2+} and Hg^{2+} due to the similar ionic radius and chemical properties. Thus, it is still necessary to devise new simple-structured fluorescent probes for Cu^{2+} detection *in vitro* and *in vivo*.

Recently, excited-state intramolecular proton transfer (ESIPT) dyes have attracted much attention in probe design due to their intriguing photophysical properties, such as the ultrafast ESIPT reaction and the large Stokes shift.[30, 31] Among various ESIPT dyes, 2-(2-hydroxyphenyl)-9,10-phenanthro imidazoles (HPI) are a new class of π-expanded imidazole derivatives, and have been studied for their synthesis and optical spectroscopic properties.[32-35] As the fluorophore, HPI seem to be especially suitable for constructing fluorescent probes for detection of analytes due to their ease of synthetic modification and fluorescence modulation by functionalization of the hydroxyl groups, and large Stokes shift.[36, 37] Moreover, their analogues bearing an acidic NH group as proton donor of the intramolecular H-bond system show higher quantum yield and more active ESIPT process compared with HPI molecules.[33, 38, 39] Given the similarities and differences in the ESIPT mechanism between O–H and N–H H-bonding systems, it is possible to obtain more ESIPT-fluorescent probes by adjustment of the proton donor group (typically an OH or NH_2 group). Herein, we synthesized a pair of Cu^{2+} fluorescent probes by protection of the amine group and the hydroxyl in compound **1** and **2** with a 2-pyridinecarbonyl group,

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respectively (Scheme 1). The linker between the signal group and recognition union of Cu^{2+} ions was explored. Interestingly, using -**NH** group as the linker, the probe **Pi**-**A** exhibited a fluorescent "*turn–off*" behavior toward Cu^{2+}. While **O** atom was used as the linker, the probe **Pi-E** showed a fluorescent "*turn–on*" behavior to Cu^{2+} (Scheme 2). The result showed that both probes have many advantages including good water solubility, wide pH range, high sensitivity and selectivity to Cu^{2+}. They were successfully used in imaging of Cu^{2+} in living cells.

Experimental section

Reagents and Apparatus

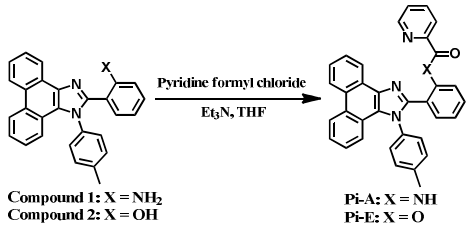
Salicylaldehyde, picolinic acid, *o*-nitrobbenzaldehyde and phenanthrenequinone were purchased from TCI (Shanghai) Development Co., Ltd. Dimethyl sulfoxide (chromatographic grade) was gained from Merck Chemicals Co., Ltd. Other analytes were obtained from Aladdin Chemistry Co., Ltd. Silica gel plates (60F-254) and silica gels (mesh 300–400) were used for thin layer chromatography analyses and column chromatography, respectively. UV–vis absorption spectra were collected on a UV2450 spectrophotometer (Shimadzu Co., Japan). Fluorescence spectra were recorded on a Hitachi F–4500 spectrophotometer (Hitachi Ltd, Japan). ^{1}H NMR and ^{13}C NMR spectra were obtained with tetramethyl silane (TMS) as the internal standard on a Bruker AVB–500 spectrometer. Mass analyses were performed using an API 4000 QTRAP LC/MS/MS system with ESI Ion Source (AB SCIEX Co., U. S. A.). All pH measurements were carried out on a Model pHS–3C pH meter (Shanghai, China). The probe Pi-A and Pi-E were characterized by ^{1}H NMR, ^{13}C NMR and ESI-MS (Figs. S1–S6).

Synthesis of probe Pi-A

Compound 1 and compound 2 were synthesized according to the reported methods.[40] Pi-A as a "*turn–off*" probe for Cu^{2+} was synthesized. The synthesis route of the probe was shown in Scheme 1. Picolinic acid (1.23 g, 10.00 mmol) was dissolved in $SOCl_2$ (20 ml), and the reaction mixture was refluxed for 5 h under N_2 atmosphere. After removing the excess $SOCl_2$, the obtained oily liquid was dissolved in CH_2Cl_2 (10 mL) and cooled in the ice-water bath. Then, the corresponding solution was slowly added into CH_2Cl_2 solution (30 mL) containing compound **1** (0.50 g, 1.25 mmol) and triethylamine (2.54 g, 25.08 mmol). After stirring for 8 h, the reaction mixture was washed with water (100 mL). Subsequently, the organic layer was separated, dried with anhydrous Na_2SO_4, filtered, and concentrated. The crude product was purified by silica gel column chromatography (15:1, petroleum ether: ethyl acetate, v/v) to give probe **Pi-A** as a yellow solid (0.39 g, 61% yield). ^{1}H NMR (500 MHz, $CDCl_3$): δ 13.50 (s, 1H), 9.22 (d, J = 7.5 Hz, 1H), 8.84 (d, J = 8.3 Hz, 1H), 8.79 (d, J = 8.4 Hz, 1H), 8.75 (d, J = 8.4 Hz, 1H), 8.55 (d, J = 4.3 Hz, 1H), 8.29 (d, J = 7.8 Hz, 1H), 7.91–7.79 (m, 2H), 7.72 (t, J = 7.1 Hz, 1H), 7.52 (ddd, J = 8.3, 6.0, 2.1 Hz, 1H), 7.41–7.34 (m, 6H), 7.29 (dd, J = 10.2, 4.7 Hz, 2H), 7.08 (dd, J = 7.8, 1.1 Hz, 1H), 6.86 (t, J = 7.5 Hz, 1H), 2.53 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$): δ 163.28, 150.80, 148.44, 147.94, 139.94, 137.99, 137.17, 136.82, 135.76, 130.73, 129.71, 129.68, 129.43, 128.83, 128.52, 127.72, 127.14, 126.34, 126.08, 125.75, 125.04,124.07, 123.23, 123.12, 122.60, 122.50, 121.67, 121.18, 118.91, 21.42. MS (EI) m/z: 504.40 (M^+).

Synthesis of probe Pi-E

Pi-E was synthesized following the route showed in Scheme 1. Picolinic acid (1.23 g, 10.00 mmol) was dissolved in $SOCl_2$ (20 ml). The reaction mixture was refluxed for 5 h under N_2 atmosphere and then the volatiles were evaporated under reduced pressure. The residue dissolved in CH_2Cl_2 (10 mL), and the resultant solution was added dropwise to the solution of compound **2** (0.50 g, 1.25 mmol) and triethylamine (2.54 g, 25.08 mmol) in CH_2Cl_2 (20 mL). The mixture was stirred at room temperature for 10 h. Then, the solvent was removed by rotary evaporation. The residue was purified by silica gel column chromatography (5:1, petroleum ether: ethyl acetate, v/v) to afford probe **Pi-E** as a brown solid (0.47 g, 75% yield). ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.70 (t, *J* = 7.1 Hz, 1H), 8.61 (d, *J* = 7.7 Hz, 1H), 8.48 (dd, *J* = 9.2, 6.0 Hz, 2H), 8.34 (d, *J* = 2.0 Hz, 1H), 7.61–7.55 (m, 2H), 7.54–7.45 (m, 3H), 7.44–7.36 (m, 2H), 7.33 (dd, *J* = 5.3, 2.6 Hz, 3H), 7.30–7.24 (m, 3H), 7.22 (dd, *J* = 8.9, 7.6 Hz, 1H), 7.12 (t, *J* = 7.5 Hz, 1H), 2.50 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$): δ 163.51, 150.14, 149.95, 147.46, 146.80, 139.64, 137.13, 136.97, 135.22, 131.64, 130.46, 130.34, 129,15, 128.71, 128.11, 127.48, 127.12, 127.01, 126.90, 126.22, 126.03, 125.45, 125.31, 124.91, 123.92, 123.61, 123.10, 123.03, 122.94, 122.43, 121.19, 21.45. MS (EI) m/z: 505.36 (M^+).



Scheme 1 Synthesis route of **Pi-A** and **Pi-E**.

Spectroscopic measurements

Stock solutions of metal ions (10.0 mM) including $CuCl_2{\cdot}2H_2O$, $Pb(NO_3)_2$, NaCl, $CaCl_2{\cdot}2H_2O$, $NiCl_2{\cdot}6H_2O$, $MnCl_2{\cdot}4H_2O$, $AgNO_3$, CuCl, $FeCl_2{\cdot}4H_2O$, $CdCl_2{\cdot}2.5H_2O$, $MgCl_2{\cdot}6H_2O$, $ZnCl_2$, $CoCl_2{\cdot}6H_2O$, $BaCl_2{\cdot}2H_2O$, $Fe(NO_3)_3{\cdot}9H_2O$, and $HgCl_2$ were prepared in deionised water. Stock solutions of **Pi-A** or **Pi-E** (1.0 mM) were prepared in DMSO. These stock solutions were further diluted to required concentration for measurement. NaAc-HAc and Tris-HCl buffer solutions (10.0 mM) were prepared for pH 4.0-6.0 and 7.0-10.0, respectively. Test solutions were prepared as follows: 40 μL of **Pi-A** or **Pi-E** solution (0.5 mM) and proper amounts of Cu^{2+} solution were added into a test tube, and the final volume was adjusted to 2 mL with Tris–HCl buffer solution. After the solutions were incubated at room

temperature for 30 min, absorption and fluorescence spectra were measured.

Cell culture and imaging

HeLa cells were grown in culture media (Dulbecco's modified Eagle's medium containing 10% fetal bovine serum, 1% penicillin and 1% streptomycin) at 37 °C in a humidified incubator with 5% CO_2. Before the experiments, the cells were seeded in 96–well plates and allowed to adhere for 24 h. For fluorescence imaging, the cells were treated within 10 μM probes (**Pi-A** or **Pi-E**) in culture media for 30 min at 37 °C. After washing with PBS buffer three times to remove the remaining probes, the cells were further incubated with different concentration of $CuCl_2$ (0, 10 μM) for 30 min at 37 °C. Subsequently, the fluorescence images were collected with an inverted fluorescent microscope (Nikon, Eclipse Ti-S).

Results and discussion

Optical response of Pi-A and Pi-E to Cu^{2+}

In the UV–vis absorption spectra, **Pi-A** and **Pi-E** exhibited a maximum absorption peak at 272 nm and 258 nm, respectively, which are attributed to the conjugated π-electron systems[21, 41] (Fig. S7). The fluorescence responses of **Pi-A** and **Pi-E** toward Cu^{2+} were investigated under the identical conditions. As shown in Fig. 1, **Pi-A** and **Pi-E** present strong and Very weak fluorescence respectively. The addition of Cu^{2+} caused remarkable changes in the fluorescence spectra of the probes. Dramatically, **Pi-A** exhibited a 158-fold fluorescence *turn-off* response toward Cu^{2+}, whereas **Pi-E** displayed a 12-fold fluorescence *turn-on* response to Cu^{2+}. In addition, fluorescence spectra of **Pi-E**–Cu^{2+} were compared with that of standard compound **2** in DMSO with different water content. Although the enol or keto form emission of compound **2** was effected by water content in solutions, the spectra of these two compounds were nearly identical (Fig. S11).

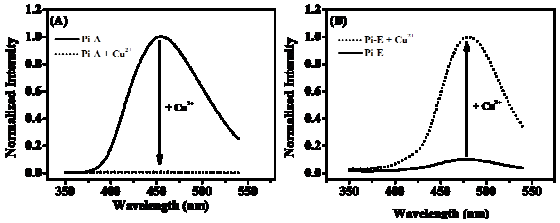
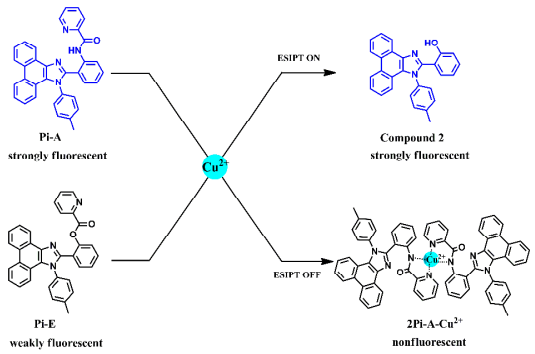


Fig. 1 Fluorescence spectra of 10 μM **Pi-A** (A) or **Pi-E** (B) in the absence and presence of 10 μM Cu^{2+}. Buffer: Tris-HCl (10 mM, pH 7.4), 2% (v/v) DMSO/water. λ_{ex} = 296 nm.

The different fluorescence responses of **Pi-A** and **Pi-E** toward Cu^{2+} were possibly due to the difference in linker which played an indispensable role in Cu^{2+} sensing. The plausible mechanism for the fluorescent changes of probes and the interaction of probes with Cu^{2+} were shown in Scheme 2. The excited state intramolecular proton transfer (ESIPT) process occurred in **Pi-A** molecule[42-46]. The H atom of the amide was transferred to the N atom of the phenanthroimidazole group, which gave a strong fluorescence. Upon addition of Cu^{2+}, the deprotonation took place in the amide group, so the ESIPT process was inhibited which led to a fluorescence quenching of **Pi-A**. On the contrary, the ESIPT process was blocked in **Pi-E** molecule due to protection of the hydroxyl group by a 2-pyridinecarboxylate group, thus the free **Pi-E** showed relatively weak fluorescence. However, a significant fluorescence enhancement of **Pi-E** could be shown after addition of Cu^{2+}. The fluorescence enhancement should be ascribed to the cleavage of ester bond mediated by Cu^{2+}, which restored the ESIPT process and recovered the tautomer emission.



Scheme 2 Proposed detection mechanism of **Pi-A** and **Pi-E** with Cu^{2+}.

The sensing mechanism of **Pi-A** toward Cu^{2+} was investigated by ^1H NMR titration experiments at room temperature. As shown in Fig. 2, the amide NH signal appeared at 13.5 ppm in free **Pi-A**, which disappeared gradually upon the addition of Cu^{2+}. Meanwhile, the proton signals in the pyridine ring displayed a slight downfield shift, and the peaks broaden relatively because of the turbidity of the solution.[47] The NMR studies suggested that the Cu^{2+} chelates with N atoms of the amide and pyridine moiety, and forms a copper complex. Furthermore, the 2:1 stoichiometry of the complexation was confirmed by the Job's plot (Fig. S8). The interaction mechanism of **Pi-E** with Cu^{2+} was confirmed by ESI-Mass in which a peak (m/z 400.50) corresponding to the authentic compound **2** was obtained (Figs. S9 and S10). According to Job's plot (Fig. S8), the stoichiometry in the reaction of **Pi-E** with Cu^{2+} was 1:1. Thus, all these results confirmed the correct of the submitted mechanism.

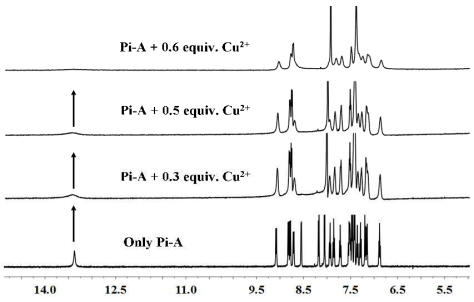


Fig. 2 Partial ^{1}H NMR spectra of **Pi-A** (10 mM) upon titration with various equivalents of Cu^{2+}.

Effects of solvents on emission performance

It is well known that the ESIPT process is affected by external parameters (e.g. solvent). Therefore, the emission spectra of the proposed ESIPT dyes (probe **Pi-A** and compound **2**) in different solvents were investigated. As shown in Fig. S12A, **Pi-A** exhibited an enol emission with small Stokes shift (about 78 nm) in dimethyl sulfoxide (DMSO). In dichloromethane (DCM), two emission peaks appeared at 352 nm and 416 nm, which were assigned to the enol and keto form emission. However, in methanol no obvious emission was found. In case of compound **2,** enol emission prevailed in DMSO and only keto emission appeared in DCM, whereas, dual emissions from the enol and keto forms are observed in protic methanol (Fig. S12B), which were consistent with results reported in the literature.[40] Interestingly, the emission spectra of both **Pi-A** and compound **2** in DMSO solution showed a large bathochromic shift and enol emission disappearance with increasing water proportions (Figs. S12C and S12D). This may be due to the aggregation of **Pi-A** and compound **2**.[37] Based on the above observations, a DMSO/Tris–HCl (v/v = 1:49) solution was selected as the working buffer in the following experiments.

Effects of pH and time

In order to achieve high sensitivity of probes for the detection of Cu^{2+}, the optimization of pH and reaction time is essential. At first, the effect of pH on fluorescence properties of two probes and their response to Cu^{2+} were investigated. As shown in Fig. S13, the pH value had no obvious effect on the fluorescence intensity of **Pi-A** between pH 4.0 and 10.0 both in the absence and presence of Cu^{2+}, suggesting that the fluorescence response of **Pi-A** toward Cu^{2+} was actually pH-independent. On the other hand, **Pi-E** itself showed very weak emission intensity in the pH range of 4.0 to 8.0. When pH >8.0, the emission intensity of **Pi-E** was gradually increased, which might be attribute to the partial hydrolysis of the carboxylic ester. Similar to **Pi-A**, **Pi-E** showed a good response to Cu^{2+} in the pH range from 6.0 to 9.0. These suggest that both probes are able to work effectively at the range of physiological pH to more alkaline condition (6.0–9.0). Time-dependent variations in the fluorescence spectra of **Pi-A** and **Pi-E** were monitored in the presence of 1 equiv. of Cu^{2+}. It was found that the interaction of these probes with Cu^{2+} was completed in less than 30 min (Fig. S14). On the basis of those observations, a physiological pH (pH 7.4) and an assay time of 30 min were selected for the following experiments.

Performance of Cu^{2+} assays

Under the optimal conditions, the fluorescence responses of **Pi-A** and **Pi-E** to various concentrations of Cu^{2+} were researched. As shown in Fig. 3A, **Pi-A** itself showed a very strong emission. However, the fluorescence of **Pi-A** (10 μM) was dramatically decreased upon the addition of Cu^{2+} and finally sustained to a stable value with the molar ration of Cu^{2+}/**Pi-A** up to 1:2. A good linear relationship between the fluorescence intensity and the Cu^{2+} concentration ranging from 0 to 3.5 μM was obtained (Fig. 3B). In addition, the limit of detection (LOD) of Cu^{2+} was calculated to be 1.6×10^{-9} M, indicating that **Pi-A** is highly sensitive to low concentration of Cu^{2+}.

Compared to **Pi-A**, **Pi-E** showed obvious fluorescence increasement along with the titration of Cu^{2+}, and a maximal fluorescence enhancement was obtained when the concentration of Cu^{2+} reached 1.0 equiv (Fig. 3C). The plot of the emission intensity as a function of the Cu^{2+} concentration shows a good linearity within the concentration range of 0–10.0 μM (Fig. 3D), and the LOD of **Pi-E** toward Cu^{2+} was estimated to be 1.8×10^{-8} M. Above results demonstrated that both probes are able to detect Cu^{2+} quantitatively by the fluorescence method.

The selectivity is another crucial parameter to assess the performance of a new fluorescent probe. Therefore, the selectivity experiments of **Pi-A** and **Pi-E** were extended to a variety of environmentally and physiologically active metal ions (Cu^{2+}, Pb^{2+}, Na^+, Ca^{2+}, Mg^{2+}, Fe^{2+}, Mn^{2+}, Ag^+, Cu^+, Cd^{2+}, Zn^{2+}, Ni^{2+}, Fe^{3+}, Co^{2+}, Ba^{2+} and Hg^{2+}). As illustrated in Fig. 4, the addition of Cu^{2+} to the solution of **Pi-A** led to an almost complete fluorescence quenching. In contrast, the addition of Cu^{2+} produced a significant increase in the fluorescence intensity of **Pi-E**. However, the addition of the other metal ions

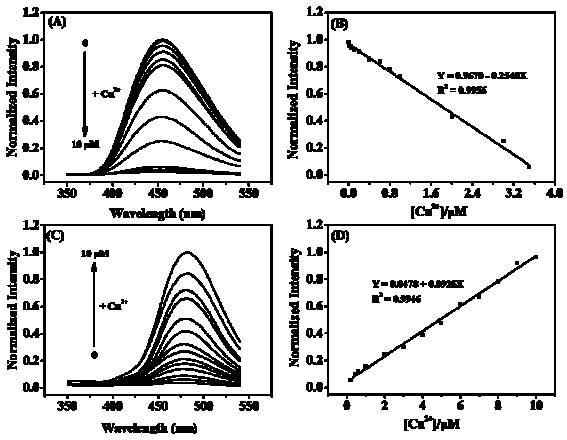


Fig. 3 Fluorescence spectra of 10 μM **Pi-A** (A) or **Pi-E** (C) upon addition of varied concentrations of Cu^{2+}. A plot of fluorescence intensity changes of 10 μM **Pi-A** (B) or **Pi-E** (D) vs. Cu^{2+} concentration. Conditions: for **Pi-A**, $\lambda_{ex}/\lambda_{em}$ = 296/455 nm; for **Pi-E**, $\lambda_{ex}/\lambda_{em}$ = 296/481 nm. Buffer: Tris-HCl (10 mM, pH 7.4), 2% (v/v) DMSO/water.

did not cause any apparent variations in the fluorescence spectra of both probes. All these results indicated that **Pi-A** and **Pi-E** possess high selectivity toward Cu^{2+} and could meet the selective requirements for practical application.

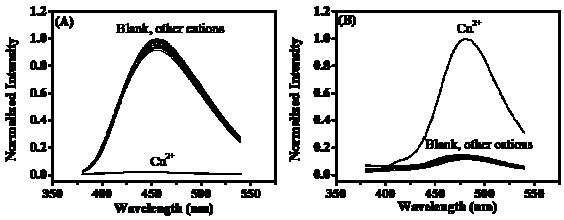


Fig. 4 Fluorescence spectra of 10 μM **Pi-A** (A) or **Pi-E** (B) upon addition of various metal ions. Cu^{2+} was added at a concentration of 10 μM, and other cations (Pb^{2+}, Na^{+}, Ca^{2+}, Mg^{2+}, Fe^{2+}, Mn^{2+}, Ag^{+}, Cu^{+}, Cd^{2+}, Zn^{2+}, Ni^{2+}, Fe^{3+}, Co^{2+}, Ba^{2+} and Hg^{2+}) were added at a concentration of 200 μM.

Imaging of intracellular Cu^{2+}

In order to evaluate the ability of the probes for biological applications, and clarify whether it is sensitive enough to for Cu^{2+} detecting in living cells, we performed an assay to detect Cu^{2+} in living cells using **Pi-A** and **Pi-E**. HeLa cells were pretreated with 10 μM **Pi-A** or **Pi-E** for 30 min, and then incubated with either buffer or buffer containing 10 μM Cu^{2+} for another 30 min and imaged by inverted fluorescence microscopy. As shown in Fig. 5, after addition of Cu^{2+} to the cells pretreated with **Pi-A**, the fluorescence emission from the cells was quenched. But for the cells pretreated with **Pi-E**, upon adding Cu^{2+}, a remarkable blue emission was observed. The auto-fluorescence from the cells treated with PBS was negligible compared to the blue fluorescence from the cells pretreated with probes (Fig. S15). These results established that both probes can permeate through cell membranes and sense Cu^{2+} in the living cells. Due to its *off-on* response feature, **Pi-E** is more suitable for bioimaging applications.

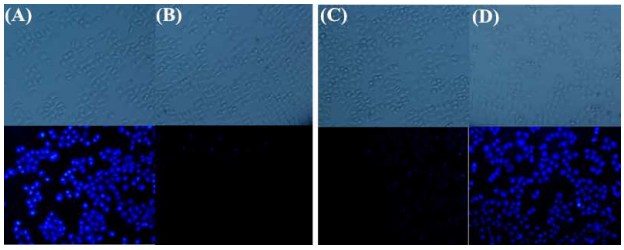


Fig. 5 Fluorescence microscope images of living HeLa cells. Cells incubated with 10 μM **Pi-A** (A) or **Pi-E** (C) for 30 min at 37 °C. HeLa cells pretreated with 10 μM **Pi-A** (B) or **Pi-E** (D) for 30 min at 37 °C and then further incubated with 10 μM Cu^{2+} for another 30 min. Top: bright field image, Bottom: fluorescence image.

Conclusions

We have synthesized a pair of ESIPT-based fluorescent probes (**Pi-A** and **Pi-E**) for *in vitro* and *in vivo* detection of Cu^{2+} by modulating the proton donor in the ESIPT system. Differences in the linker of the probes molecules resulted in completely different sensing behaviour. **Pi-A** and **Pi-E** exhibited high selectivity and sensitivity to Cu^{2+} detection with fluorescence *turn-off* and *turn-on* feature under the same conditions, respectively. The experimental results showed that **Pi-A** and Cu^{2+} form an 2 : 1 stoichiometric ratio complex, while **Pi-E** undergo Cu^{2+}-promoted hydrolysis to generate the corresponding product with a stoichiometric ratio of 1 : 1. Both probes have the following attractive properties: convenience in preparation, good water solubility and tolerating a wide pH range. Cell experiments showed that **Pi-E** is more encouraging for imaging Cu^{2+} in live cells due to its fluorescence enhancement and good cell–membrane permeability. By utilizing the strategy of the modulation of the proton donor in the ESIPT system, more ESIPT–based probes for other analytes might be developed with similar structure but different performance.

Acknowledgements

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