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Grapgical abstract

A highly selective turn-off fluorescent probe for Cu(Ⅱ) based on a dansyl derivative and its application in living cell imaging

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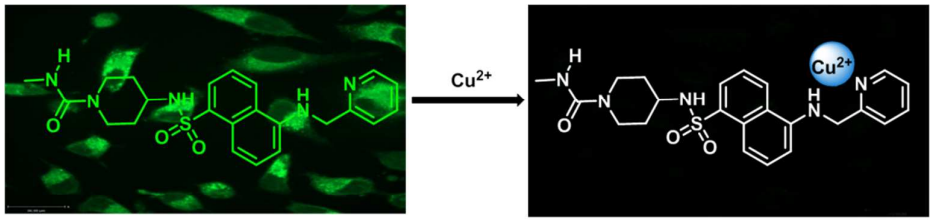

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ARTICLE TYPE

A highly selective turn-off fluorescent probe for Cu(□) based on a dansyl derivative and its application in living cell imaging

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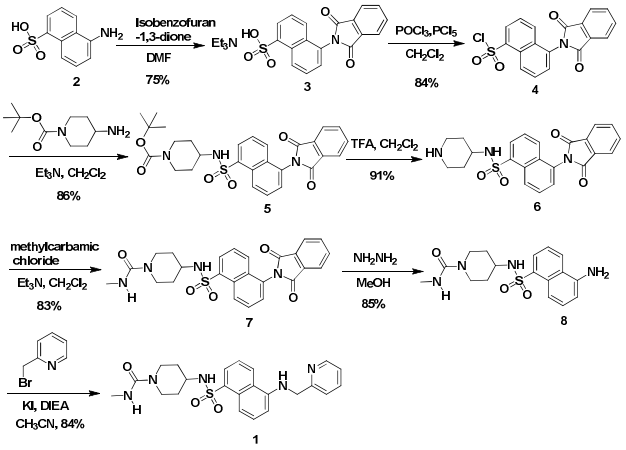
A novel dansyl derivative (1) was designed and synthesized with high yield. It is highly selective to Cu^{2+} over many other competing metal ions, such as Ca^{2+}, Co^{2+}, Cr^{3+}, Cu^{+}, Fe^{2+}, Ga^{3+}, Hg^{2+}, Mg^{2+}, Na^{+}, Ni^{2+} and Pb^{2+}. Zn^{2+} and Fe^{3+} only slightly changed the fluorescence of probe 1. The linear relationship between fluorescence intensity and Cu^{2+} concentration indicates that 1 can be used for quantification. The binding ratio of probe 1 and Cu^{2+} was found to be 1:1 according to the Job's plot experiments. Probe 1 can be used in a broad pH value window ranging from 5 to 11. The limit of detection (LOD) based on $3\times \delta_{blank}/k$ was calculated with a value as low as 1.6×10^{-6} M for Cu^{2+}. Additionally, the association constant of probe 1-Cu^{2+} complexes were found to be 5.08×10^{4} M^{-1}. Moreover, fluorescence microscopy experiments showed that 1 can be used as a fluorescent probe for evaluating the presence of exogenous Cu^{2+} in living cells.

Introduction

Among the various transition metal ions, copper is an essential metal elements not only for human beings and plants but also for extensive microorganism, which playing a crucial role in various fundamental physiological functions including enzyme functions and transcriptional events.[1] However, copper deficiency may lead to hematological manifestations and affect the corresponding enzyme activity and normal metabolism of organisms, whereas excess level of Cu^{2+} can exhibit toxicity and cause gastrointestinal disturbance and neurodegenerative diseases, including Wilson's and Alzheimer's disease.[2] In addition, copper ions are considered as an important environmental pollutant because of its widespread use by man-made sources, such as the paper industry, agriculture and fossil fuel combustion.[3] Thus, it is of great significance to detect copper ion in living cells and environmental setting.[4-6] In the past few years, traditional analytical assays, ranging from atomic absorption spectrometry to inductively coupled plasma mass spectrometry, are used to detect metal ions. However, those strategies are always limited by several dissatisfied aspects, such as high costs, complicate operation and unable to on-site analyze. Therefore, more convenient and economic approaches for copper ions detection are urgently desirable.[7] Recently, the design and development of detection tools based on optical signal with high sensitivity and excellent selectivity for metal ions as a hot research topic is booming in the field of chemo-sensing. Synthetic fluorescent ions probes provides gratifying advantages over other strategies for the selective recognition of chemically and biologically important ions, with the advantages of high sensitivity, simplicity, instantaneous response and low cost.[8-10] Up to now, several studies have aimed at obtaining fluorescent probes for Cu^{2+} detection.[11, 12] Although a few probes shown a fluorescence enhancement with the binding of Cu^{2+} ions, their selectivity and pH-independent ability need to be improved for a more suitable and broad for practical application.[13] Thus, the research related to this area are of great challenge and interest. In this study, we developed a novel fluorescence copper probe, and investigated its fluorescence properties and bio-imaging application. Dansyl derivant was chosen as the fluorophore because of its outstanding intramolecular charge transfer (ICT) structure and desirable spectroscopic properties, such as large stokes shift, absorbance in near UV-region and as the smallest available flurophore.[14] Pyridine group was further introduced into dansyl molecular skeleton, since it can be utilized as a chelator for Cu^{2+} binding owing to nitrogen atoms bearing lone pair electrons show certain coordination ability with Cu^{2+}. Additionally, the piperidine carboxamide substituent is conjugated to the sulfamine side and the folding spatial conformation of piperidine carboxamide is expected to synergy recognize Cu^{2+} with pyridine group. While probe **1** interacts with copper ions, the fluorescence signal is envisioned to turn-off. The fluorescent quenching can be attributed to the decrease of the electron-donating ability of the aniline nitrogen atoms and the occurrence of the intramolecular charge transfer process. Furthermore, this probe shows a fast response and high selectivity towards Cu^{2+} over other tested metal ions and anions. The results show that this probe can be used to detect Cu^{2+} in micromole level and in a broad range of pH. It was finally successfully applied to image exogenous copper ions in living HeLa cells, demonstrating its practical applications.

Results and discussion

The detailed synthetic route probe **1** was depicted in Scheme 1. It was synthesized with 29.2% overall yield by a seven-steps synthetic procedure from readily available starting material, 5-



Scheme 1 Synthesis of probe **1**

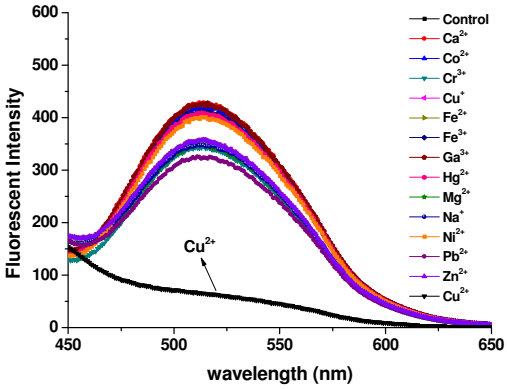


Fig .1 The fluorescence response of probe **1** (16 μM) to various metal ions (400 μM) in CH_3CN/HEPES buffer (8/2, v/v, pH =7.0) excited at 348 nm.

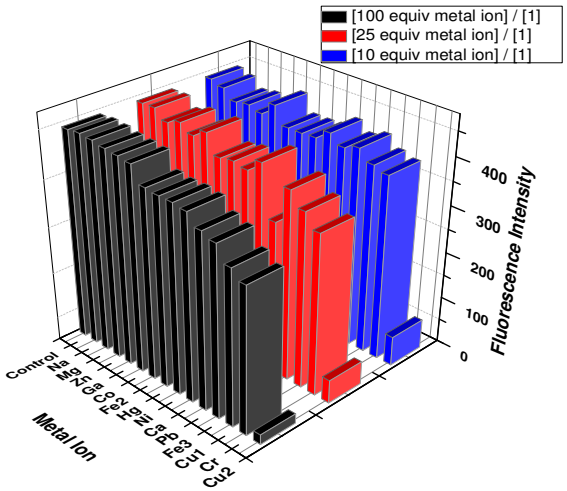


Fig .2 Fluorescence responses of **1** (16 μM) in the presence of 10, 25, 100 equiv of various metal ions, respectively (CH_3CN/HEPES buffer = 8/2, v/v, pH=7.0, $\lambda_{ex}/\lambda_{em}$= 348 nm /515 nm).

aminonaphthalene-1-sulfonic acid. These chemical structures were fully characterized and confirmed by ^1H NMR, ^{13}C NMR, MS (ESI) and/or HRMS spectra (See Supporting Information).

In order to investigate the ion recognition capability of probe **1** in an optimal aqueous solution, the HEPES buffer was used to keep probe solution in physiological pH window. The spectroscopic properties of probe **1** were evaluated in different concentrations of HEPES buffer solution. This novel probe displayed strong fluorescence intensity under neutral condition (Fig. S1, ESI), which indicated that **1** is pH-insensitive in a physiological environment and would work well in physiological conditions. Additionally, the optimal concentration of HEPES for probe solution is 20 mmol/L as its fluorescence intensity is higher than that in other concentrations. Thus, the 20 mmol/L was selected as experimental media concentration in further spectroscopic measurement.

UV-vis analyses were firstly performed in order to investigate the response to metal ions. As seen in Fig. S2 (ESI), the absorption maximum wavelength of probe **1** at 348 nm vanished drastically upon addition of Cu^{2+}. However, no significant changes were observed towards other tested metal ions. The absorption titration of probe 1 with Cu^{2+} was also investigated in Fig. S3. Without Cu^{2+}, probe 1 shows a distinctive absorption band at 325nm to 425nm with a maximum absorption at 348 nm. Cu^{2+} titration leads to a decrease in this absorption, along with an increase in 300 nm absorption bond. To further verify the selectivity towards Cu^{2+}, probe **1** was titrated over a wide range of metal ions. The wavelength of maximum emission peak of the probe in CH_3CN/H_2O (8/2, v/v) HEPES buffer solution (pH 7.0) was 515 nm when the fluorimetric detector was used with the excitation wavelength at 348 nm. The fluorescence intensity was slightly change by addition of 25 equiv of Ca^{2+}, Co^{2+}, Cr^{3+}, Cu^{+}, Fe^{2+}, Ga^{3+}, Hg^{2+}, Mg^{2+}, Na^{+}, Ni^{2+}, Pb^{2+}, Zn^{2+} and Fe^{3+} (Fig. 1). However, a drastic fluorescence quenching could easily be observed upon the addition of 25 equiv of Cu^{2+}, which indicated that **1** could selectively recognize Cu^{2+} in aqueous condition. For the reproducible recognition ability in a lower or higher concentration of various metal ions, an extensive selectivity experiment in three different concentration of various metal ions was carried out as shown in Fig. 2, the fluorescence response of **1** upon the addition of 10, 25 and 100 equiv of a variety of metal ions demonstrated that **1** has a high selectivity for Cu^{2+} over other tested cations and can even well discriminate Cu^{2+} from Cu^{+}. Beside, the spectroscopic response of **1** in the presence of various common anions was also studied in Fig. S4 (ESI), including NO_3^- ,S^{2-}, Cl^-, CH_3COO^-, Br^-, CO_3^{2-}, SO_4^{2-} and HCO_3^-. It is concluded that none of these anions induced any significant changes in the fluorescent spectrum of **1**, which illustrated that probe **1** has excellent selectivity for Cu^{2+} from all tested cations and anions.

The experiment of Cu^{2+} titration was presented in Fig. 3 and Fig. S3. Cu^{2+} addition gives rise to a decrease in the maximum emission peak, along with an increase in 400 nm bond, with isosbestic points at 460 nm. Upon addition of Cu^{2+}, it displays a fierce decrease and finally saturates at about 3 equiv of Cu^{2+}. From the photograph which was recorded under UV light, the emission color of the solution continuously changes from green to dark in Fig. 3a, which can be easily observed by the naked eye. There was a good linear correlation (R^2= 0.994, in the inset of Fig.

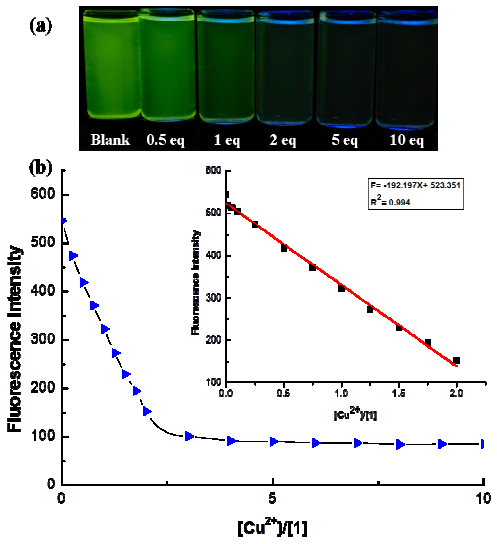


Fig. 3 (a) Fluorescence changes of **1** (16 μM) in the presence of an increasing Cu^{2+} in CH_3CN/HEPES buffer (8/2, v/v, pH = 7.0); (b) the saturation curve between the relative fluorescence intensity and the equiv of Cu^{2+}. Insert: the linear responses of **1** (16 μM) as a function of Cu^{2+} concentration (CH_3CN/HEPES buffer = 8/2, v/v, pH=7.0, $\lambda_{ex}/\lambda_{em}$= 348 nm /515 nm).

3b) between the fluorescence intensity and the concentration of Cu^{2+} in the range from $1.6*10^{-6}$ M to $3.2*10^{-5}$ M with 16 μM of probe **1**. The linear regression equation was F= −192.197X + 523.351, where X denotes the equiv of Cu^{2+}. The association constant between **1** and Cu^{2+} was estimated as $5.08x10^4$ M^{-1} from the titration experiment (Fig. S5, ESI). The detection limit of this probe was calculated to be $1.6x10^{-6}$ M (based on S/N=3), which is much lower than the copper content in blood (11.8 - 23.6 μM). In short, probe **1** can be a sensitive fluorescent sensor for the quantitative detection of Cu^{2+} in micromole level.

To determine the binding stoichiometry of probe **1**-Cu^{2+} complexes, Job's plot [15] experiments were performed. A series of solution containing certain Cu^{2+} and probe **1** were prepared, in which their total concentration are constants. Fluorescence intensity of those mixtures of Cu^{2+} and probe **1** in varying molar ratios (1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2 and 9:1) were measured. Fig. S6 exhibited that a maximum fluorescence intensity appears at 0.5 fraction, indicated that the formation of a 1:1 complex between **1** and Cu^{2+} (Fig. 4a). To gain a clearer understanding of the structure of **1**-Cu^{2+} and the binding site, ^{1}H-NMR data of the complex was investigated. Cu^{2+} is a paramagnetic ion and enables to affect the proton signals that are close to the Cu^{2+} binding site. The ^{1}H-NMR spectra of **1** recorded with increasing equiv of Cu^{2+} show that the proton (H_e) signals gradually shifted upfield (Fig. 4b). Thus, the specific recognition site for Cu(II) is proposed between the site of the aniline and pyridyl nitrogen atoms. These results indicated that a plausible mode of 1/Cu^{2+} as proposed in Fig. 4a. To validate the pH applicability of **1**, the effect of pH on the fluorescence intensity of probe **1** in the presence and absence of Cu^{2+} were also investigated. As presented in Fig. 5, Figure S7 and S8 (ESI), the fluorescence intensity of probe **1** alone was independent on the pH ranging from 5 to 13, which indicated

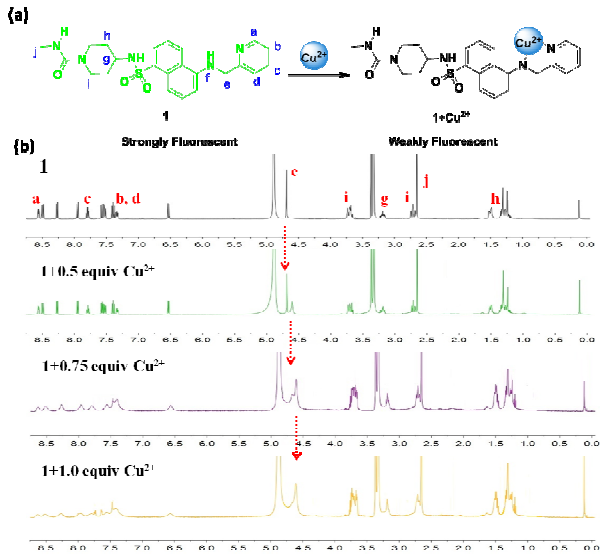


Fig. 4 (a) Proposed binding mode between probe **1**/Cu^{2+}; (b) ^{1}H-NMR 400 MHz spectra of **1** (10.0 mM) upon titration with 0.5 equiv, 0.75 equiv and 1.0 equiv of Cu^{2+}.

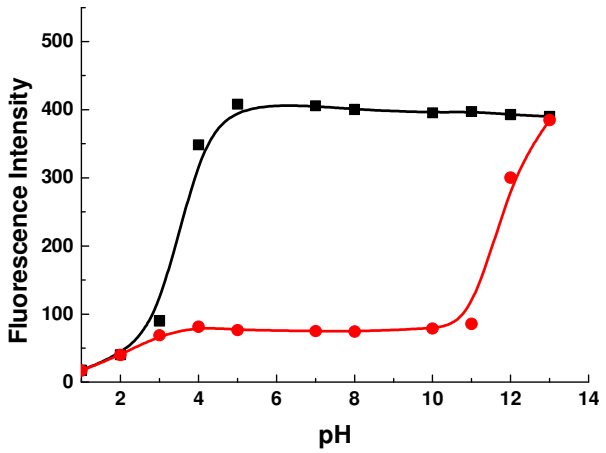


Fig. 5 Emission of **1** alone (black curve) and **1**-Cu^{2+} complex (Red curve) in different pH ($\lambda_{ex}/\lambda_{em}$= 348 nm /515 nm).

both the chemical structure and fluorescence property of probe **1** are relatively stable over a wide pH range from 5 to 13. However, upon the addition of Cu^{2+}, the fluorescence intensity of **1** drastically decreased and these phenomena keeps stable from pH 4 to 11. However, when pH is lower than 4, the fluorescence quenching of probe **1** was most likely caused by the mechanism of photo induced electron transfer (PET) from the fluorophore to protonated aniline under the strong acid environment. This observation might be resulted from the strongly electron withdrawing character of the protonated aniline, which may serve as acceptor in its protonated form. Thus, a strong acid environment gave rise to an enhancement in the PET process upon protonation of the aniline. Additionally, when the pH above 11, the fluorescence intensity of **1**-Cu^{2+} complex increased gradually which can be attributed to the competition of hydroxyl ion (OH^-) as well as the formation of $Cu(OH)_2$. The results confirmed that **1** is able to detect Cu^{2+} in a wide pH window from 5 to 11, which is the overlap pH range of both the solely probe **1**

and the mixture of probe **1**-Cu^{2+}. The above study indicated that probe **1** is able to use in the physiological pH window and even some complicated environment.

To exclude potential interference from other metal ions on Cu^{2+} binding with probe **1**, competition experiments were conducted with other 13 kinds of metal ions in the presence of Cu^{2+}. As presented in Fig. 6, fluorescence quenching of **1** resulted from the mixture of Cu^{2+} and each of various metal ions was similar to that caused by Cu^{2+} alone, indicating that all of those metal ions did not significantly affect the emission intensity of proble **1** and probe **1**-Cu^{2+} complex. Therefore, it confirmed that **1** is a highly Cu^{2+}-specific probe with an 'ON-OFF' optical function. The fluorescent quantum yield[16] of **1** in the absence and the presence of Cu^{2+} is 8.2% and 0.42%, respectively (Table S1, ESI).

Besides high selectivity and pH-independent, a fast response is another necessity for a fluorescent probe to monitor Cu^{2+}. To explore the response time of probe **1**, the kinetics of fluorescence intensity upon the addition of different concentrations of Cu^{2+} was recorded. As depicted in Fig. 7, a clearly decrease of fluorescence intensity was observed within 1 minute and the response time of probe **1** to Cu^{2+} is concentration-dependent. Therefore, it is a favorable property of **1** for Cu^{2+} detection in comparison of other reports copper ion probes [17a] and traditional analytical assay aforementioned. This feature of fast response also suggested that this probe enables to detect Cu^{2+} in real time.

In order to get insight into the reversibility of the binding between probe **1** and Cu^{2+}, N,N,N',N'-9-tetra(2-picolyl)-ethylenediamine (TPEN), a well-known metal ion chelator,[17b] was used in reversibility studies. The fluorescence intensity restored to the status of free probe **1** by adding 4 equiv of TPEN into the probe **1**-Cu^{2+} complex (Fig. 8a), which means the response of probe **1** to Cu^{2+} can be reversed and probe **1** have a possibility of recycle or reutilization after reversible treatment. Furthermore, the time course kinetics fluorescent experiments showed that fluorescence intensity of **1**, **1**-Cu^{2+} and TPEN/**1**-Cu^{2+} remained almost unaltered over a period of 3 min (Fig. 8b). It was concluded that **1**, **1**-Cu^{2+} and the complex of TPEN/**1**-Cu^{2+} have good photo-stability.

With the understanding of the fluorescence turn-off response, and based on its favorable fluorescence properties. We explored the possibility of using **1** for the recognition of exogenous Cu^{2+} in

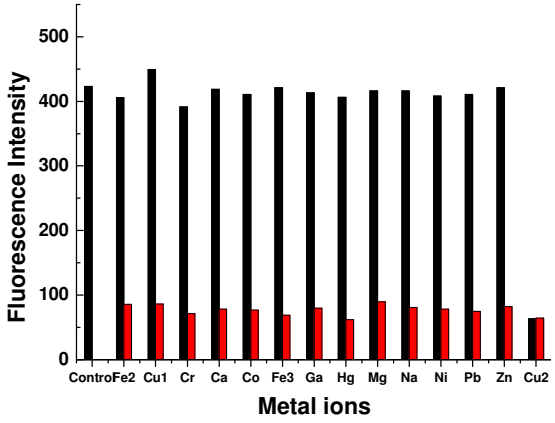


Fig. 6 Fluorescence responses of probe **1** (16 μM) to the addition of Cu^{2+} (320 μM) or 160 μM of other metal ions (black bars) and to the mixture of other metal ions (160 μM) with 320 μM of Cu^{2+} (red bars) in CH_3CN/HEPES buffer solution (8/2, v/v, pH=7.0, $\lambda_{ex}/\lambda_{em}$= 348 nm /515 nm).

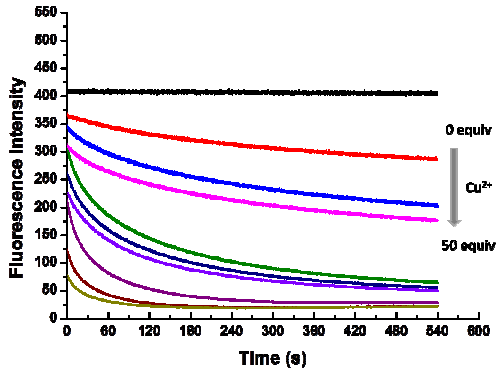


Fig. 7 Reaction-time profiles of **1** (16 μM) in the absence (Black) or presence of Cu^{2+} [1, 3, 4, 5, 8, 9, 10, 25, 50 equiv]. Fluorescence intensities at 515 nm were continuously monitored at time intervals in CH_3CN/HEPES buffer solution (8/2, v/v, pH=7.0, $\lambda_{ex}/\lambda_{em}$= 348 nm /515 nm).

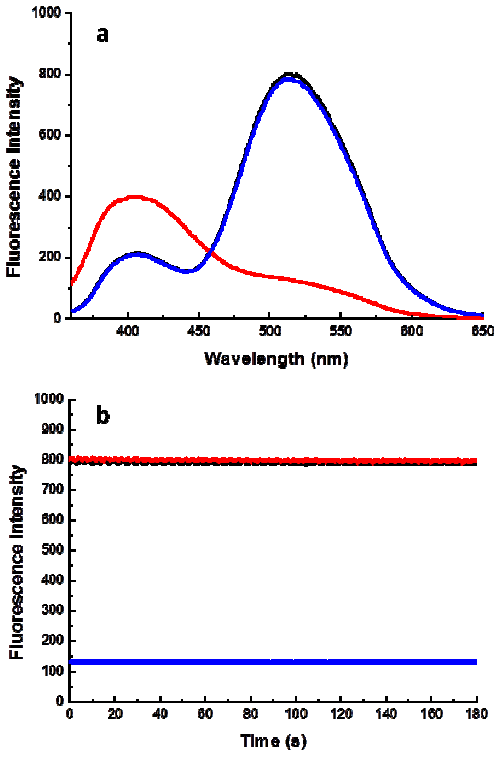


Fig. 8 (a) Reversibility of Cu^{2+} binding to **1** upon addition of TPEN. Black line: only probe **1**; Red line: **1**- Cu^{2+} (2 equiv); Blue line: **1**- Cu^{2+} (2 equiv) plus TPEN (4 equiv) in CH_3CN/HEPES buffer (8/2, v/v, pH=7.0) solution on excitation at 348 nm; (b) Time course kinetics fluorescent experiments. Bed line: only probe **1**; Blue line: **1**- Cu^{2+} (2 equiv); Black line: **1**- Cu^{2+} (2 equiv) plus TPEN (4 equiv) in CH_3CN/HEPES buffer (8/2, v/v, pH=7.0, $\lambda_{ex}/\lambda_{em}$= 348 nm /515 nm).

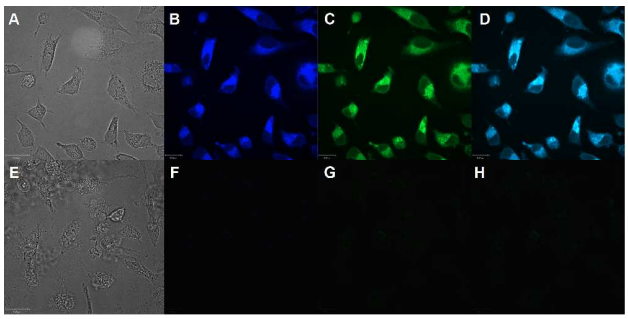


Fig. 9 Confocal fluorescence images in HeLa cells. (A) Bright field image of HeLa cells; (B) Fluorescence image of HeLa cells incubated with **1** (16 μM) by blue channel; (C) the green channel image of cells shown in panel (B); (D) Merged images of B and C; (E) Bright field image of HeLa cells; (F) Fluorescence image of HeLa cells incubated with **1** (16 μM) then addition of 50 μM $CuCl_2$ by blue channel; (G) the green channel image of cells shown in panel (F); (H) Merged images of F and G.

living cells. For *in vitro* cell imaging, since considering CH_3CN is not good for cell, 16 μM of probe **1** is prepared only in HEPES buffer solution, instead of CH_3CN/HEPES buffer solution. HeLa cells were chosen and their fluorescence images were recorded before and after the incubation of Cu^{2+}. The cells were supplemented with **1** alone for 15 min at 37ºC and washed with PBS to remove the remaining probe. A strong and bright fluorescence images could be observed by confocal fluorescence microscopy (Fig. 9B, 9C and 9D), indicating that probe **1** have excellent cell-membrane permeability. In contrast, HeLa cells displayed significant fluorescence quenching upon addition of $CuCl_2$ for 20 min at 37ºC, no obvious fluorescence was found in Fig. 9F, 9G and 9H, which suggested that probe **1** is able to detect Cu^{2+} uptake in living cells. Moreover, it is important to point out that after a long time of incubation with probe **1** for 24 h, most HeLa cells remained at good condition, which demonstrated the low cytotoxicity of **1**.

Conclusions

In summary, a novel fluorescence probe **1** was developed for detection of Cu^{2+} in both aqueous solutions and living cells. It shows excellent selectivity and high sensitivity for Cu^{2+} over other common metal ions and anions based on intramolecular charge transfer mechanism. **1** can be used to quantitatively detect micromole level of Cu^{2+} with a fast response and in a broad pH window range from 5 to 11. We also successfully demonstrated the utility of **1** for the determination of exogenous Cu^{2+} in living HeLa cells.

Experimental section

Materials and instrumentations

All chemicals used in this paper were obtained from commercial suppliers and used without further purification. Solvents used were purified by standard methods prior to use. NMR spectra were taken on a Bruker AMX400 spectrometer with chemical shifts reported as ppm (TMS as an internal standard). High Resolution Mass (HRMS) spectra were obtained using Waters Q-Tof Permies Mass Spectrometer. UV-vis absorption spectra were obtained on a Shimadzu UV-2450 spectrophotometer with a quartz cuvette (path length = 1 cm). Fluorescence measurements were taken on a Shimadzu RF-5301PC fluorescence spectrometer. The fluorescence image of intracellular Cu^{2+} was observed under a Perkin Elmer Ultra ViEW VOX confocal imaging system. The pH measurements were made with a Sartorius basic pH-meter PB-10. TLC analysis was conducted on silica gel plates and column chromatography was performed over silica gel (mesh 200-300), both of which were purchased from the Qingdao Ocean chemicals.

Preparation of the test solution

A stock solution of 20 μM **1** was prepared in acetonitrile. A stock solution of 0.004 M Cu^{2+} was also prepared by dissolving $Cu(NO_3)_2{\cdot}3H_2O$ in 20 mmol/L HEPES buffer which was prepared doubly distilled water. The working solution of **1** (16 μM) was prepared by mixing 2 mL stock solution of Cu^{2+} with 8 mL stock solution of **1** (20 μM). For metal ions selectivity study, various metal ions solutions of CuCl, $CoCl_2$, $CrCl_3$, $FeCl_2$, $GaCl_3{\cdot}6H_2O$, $HgCl_2$, $MgCl_2$, NaCl, $NiCl_2{\cdot}6H_2O$, $Pb(NO_3)_2$, $ZnCl_2$ and $Fe(NO_3)_3{\cdot}9H_2O$ were used. And the anions study was performed in different anions of sodium salt. For all measurements of fluorescence spectra, excitation was at 348 nm with excitation and emission slit widths at 5 nm and 3 nm, respectively. Fluorescence titration experiments were conducted using 16 μM of **1** in HEPES buffer/CH_3CN solution (2/8, v/v) with varying concentration of Cu^{2+}.

Fluorescence quantum yield

The fluorescence quantum yields were determined using quinine as a standard with a known Φ value of 0.546 in 0.1 M H_2SO_4. The sample and the standard were excited at the same wavelength (348 nm), maintaining nearly equal absorbance (0.05). The quantum yield was calculated according to the following:

$\Phi_s/\Phi_f = (A_s/A_f) \times (Abs_s/Abs_f) \times (\eta s^2/\eta f^2)$,

Where Φ_s and Φ_f are the fluorescence quantum yields of the standard and the samples (**1** and **1**+Cu^{2+}), respectively; A_s and A_f are the emission areas of the standard and the samples, respectively; Abs_s and Abs_f are the absorbance of the standard and the samples at the wavelength of excitation; η_s and η_f are the refractive indices of the standard and the reference, respectively.

Imaging in Hela cells

HeLa cells were cultured in RPMI 1640 (Gibco) medium supplement with 10% new born serum (NBS, Gibco), 100 units/ml penicillin (Gibco) and 100ug/ml streptomycin (Gibco) at 37 ºC in a humidified atmosphere of 5.0% CO2 incubator. For cell imaging, cells (2 x 105) were plated in 2 mL of complete cell growth medium on a 35 mm diameter glass-bottomed dish and incubated for 24hours.

After cell adhesion, culture media was removed and washed with PBS three times, and then 800 μl PBS was added into each dish. All the cell lines were incubated with **1** (16 μM) for 10 min at room temperature. The fluorescence images were obtained by blue and green channel, excitation wavelength of laser was 405 nm and 488 nm, respectively. Upon addition of 50 μM Cu^{2+} into the cell line, the fluorescence images of intracellular Cu^{2+} were also obtained by blue and green channel, excitation wavelength of laser was 405 nm and 488 nm, respectively.

General procedures for the synthesis of probe 1

Synthesis of **3**. Triethylamine (2 mL) was added dropwise to a solution of 5-aminonaphthalene -1-sulfonic acid (5576 mg, 25 mmol) **(2)** and isobenzofuran-1, 3-dione (4440 mg, 30 mmol) in DMF (20 mL) and the mixture was stirred and refluxed at 170 °C for 48h. The solvent was evaporated and the residue was taken up in chloroform, dried by $MgSO_4$ and evaporated. The product was obtained following purification by silica gel column chromatography as a colorless solid **3** (8516 mg, yield 75%). ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 9.14 (1H, d), 8.25 (1H, dd), 7.99 (2H, d), 7.83 (2H, d), 7.66 (2H, t), 7.46 (2H, t), 3.08 (6H, m), 1.29 (9H, t); ^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm): 167.7, 141.8, 134.5, 131.9, 131.1, 130.3, 128.9, 128.3, 127.1, 126.2, 126.0, 125.5, 125.1, 123.9, 46.2; HRMS (ESI) m/z: Calcd, $[M+Na]^+$ 477.1460; Found, $[M+Na]^+$ 477.1447.

Synthesis of **4**. To a solution of 5-(1, 3-dioxoisoindolin-2-yl)naphthalene-1-sulfonic acid **3** (6810 mg, 15 mmol) in phosphorus oxychloride (18400 mg, 120 mmol) was added portionwise phosphorus pentachloride (8500 mg, 40 mmol). The reaction mixture was stirred for 12 h under nitrogen at room temperature. The reaction was quenched by pouring into ice water and stirring, neutralized with solid sodium bicarbonate. Chloroform (250 ml) was employed to extract the cold aqueous solution. The combined organic layers were dried by $MgSO_4$, concentrated and left under high vacuum to provide a pale yellow product **4** (4452 mg, yield 84%) without further purify.

Synthesis of **5**. To a CH_2Cl_2 (20 mL) solution of 5-(1,3-dioxoisoindolin-2-yl)naphthalene-1-sulfonyl chloride **4** (5565 mg, 15 mmol) were added Et_3N (2 mL) and tert-butyl 4-aminopiperidine-1-carboxylate (3309 mg, 16.5 mmol). The reaction mixture was stirred at 25°C for 18 h. Concentration and purification by column chromatography provided **5** (6903 mg, 86%) as a colorless solid. ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 8.80 (1H, d), 8.33 (1H, d), 8.02 (2H, d), 7.80 (3H, t), 7.73 (1H, t), 7.55 (2H, t), 5.11(1H, d), 3.87 (2H, s), 3.30(1H, m), 2.73(2H, t), 1.73(2H, t), 1.41(9H, s), 1.30(2H, t); ^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm): 167.7, 154.6, 136.7, 134.9, 132.0, 131.6, 130.0, 129.5, 129.3, 128.8, 128.2, 128.0, 126.4, 125.5, 124.2, 79.9, 51.3, 33.0, 28.5; HRMS (ESI) m/z: Calcd, $[M+H]^+$ 536.1856; Found, $[M+H]^+$ 536.1846.

Synthesis of **6**. To a solution of compound **5** (5352 mg, 10 mmol) in CH_2Cl_2 (15 mL) trifluoroacetic acid (15 mL) was added dropwise at 0°C over 5 minutes. The reaction mixture was stirred for 12 hours at room temperature. The reaction mixture was then evaporated three times with dichloromethane. A yellow residue was obtained after evaporation. The residue was purified by silica gel column chromatography to yield **6** as a white solid (3860 mg, 91%). ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 8.81 (1H, d), 8.34 (1H, d), 8.03 (2H, t), 7.87 (3H, m), 7.75 (1H, t), 7.55(2H, dd), 3.49 (1H, m), 2.93 (2H, d), 2.52 (2H, m), 1.71 (2H, m), 1.30(2H, m); MS (m/z): Calcd, $[M+H]^+$ 436.1; found, $[M+H]^+$ 436.01.

Synthesis of **7**. To a solution of 5-(1, 3-dioxoisoindolin-2-yl)-N-(piperidin-4-yl)naphthalene-1-sulfonamide **6** (4351 mg, 10 mmol) in CH_2Cl_2 (15 mL) were added Et_3N (2 mL) and methylcarbamic chloride (1116 mg, 12 mmol). The reaction mixture was stirred at 25°C for 8 h, at which point the solvent was removed in vacuum. The crude residue was directly purified via column chromatography to afford **7** as a white solid (4083 mg, 83%). ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 8.79 (1H, d), 8.34 (1H, d), 8.02 (2H, m), 7.87(3H, m), 7.76 (1H, m), 7.56 (2H, m), 4.89 (1H, d), 4.40(1H, m), 3.71 (2H, d), 3.31 (1H, m), 2.76 (2H, m), 2.74 (3H, d), 1.73 (2H, m), 1.33 (2H, m); HRMS (ESI) m/z: Calcd, $[M+H]^+$ 493.1546; Found, $[M+H]^+$ 493.1532.

Synthesis of **8**. To a solution of **7** (2460 mg, 5 mmol) in MeOH (20 mL) was added hydrazine (6250 mg, 125 mmol). The resultant solution was stirred at 70°C for 24 h, at which point the precipitate was removed via filtration and washed with MeOH (30 mL). The filtrate was collected and the solvent was removed in vacuum to provide a crude product. The crude product was directly purified via column chromatography to afford **8** as a yellow solid (1539 mg, 85%). ^{1}H-NMR (400 MHz, CD_3OD) δ (ppm): 8.31 (1H, d), 8.20 (1H, d), 7.97 (1H, d), 7.44 (2H, m), 6.90 (1H, d), 3.68 (2H, d), 3.15 (1H, m), 2.64 (2H, t), 2.62 (3H, s), 1.485 (2H, m), 1.22 (2H, m); ^{13}C-NMR (100 MHz, CD_3OD) δ (ppm): 160.4, 146.2, 137.2, 130.1, 129.7, 128.9, 128.0, 125.6, 123.2, 115.0, 111.2, 51.8, 43.5, 33.5, 27.5; HRMS (ESI) m/z: Calcd, $[M+H]^+$ 363.1492; Found, $[M+H]^+$ 363.1491.

Synthesis of **1**. To a stirred solution of primary amine **8** (181 mg, 0.5 mmol) in CH_3CN (5 ml), 2-(bromomethyl)pyridine (128 mg, 0.75 mmol) was added dropwise. KI (8 mg, 0.05mmol) was added. The mixture was refluxed at 90°C for 6 h. The precipitate was filtered off and the solution was evaporated to dryness. The resulting precipitate was purified via column chromatography to afford **1** as a greenish solid (190 mg, 84%). ^{1}H-NMR (400 MHz, CD_3OD) δ (ppm): 8.52 (1H, d), 8.46 (1H, d), 8.23 (1H, d), 7.93 (1H, d), 7.73 (1H, t), 7.50 (2H, dd), 7.35 (1H, t), 7.29 (1H, dd), 6.49 (1H, d), 4.65 (2H, s), 3.68 (2H, d), 3.16 (1H, m), 2.69 (2H, m), 2.63 (3H, s), 1.47 (2H, m), 1.23 (2H, m); ^{13}C-NMR (100 MHz, CD_3OD) δ (ppm): 160.4, 149.4, 145.6, 139.1, 137.4, 130.6, 130.2, 129.8, 128.1, 125.7, 123.8, 123.5, 122.9, 114.3, 106.4, 66.6, 51.8, 43.5, 33.5, 27.6, 20.2; HRMS (ESI) m/z: Calcd, $[M+H]^+$ 454.1835; Found, $[M+H]^+$ 454.1913.

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† Data include UV-vis spectra, fluorescence quantum yield, association constant and figures of ^{1}H NMR, ^{13}C NMR spectra, ESI-MS and HRMS of comounds 3-8 and probe 1 are available. See DOI: 10.1039/b000000x/

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