Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy 264 (2022) 120313

Contents lists available at ScienceDirect

Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy

journal homepage: www.elsevier.com/locate/saa

Irreversible coumarin based fluorescent probe for selective detection of Cu^{2+} in living cells

Nadeem Ahmed [a], Wajeeha Zareen [a], Di Zhang [b,*], Xiaopeng Yang [a], Yong Ye [a,*]

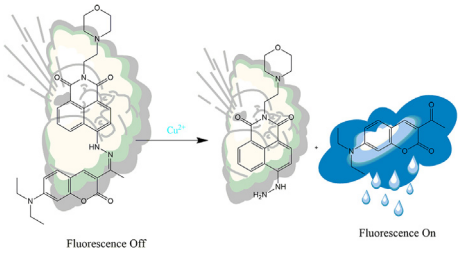
[a] Green Catalysis Center, and College of Chemistry, Zhengzhou University, Zhengzhou 450001, China
[b] Institute of Agricultural Quality Standards and Testing Technology, Henan Academy of Agricultural Sciences, Zhengzhou 450002, China

HIGHLIGHTS

- A selective Coumarin fluorophore based probe was developed.
- Newly synthesized probe shows strong fluorescence induced by Copper promoted catalytic hydrolysis of hydrazone.
- Imaging Cu^{2+} in KYSE30 cells was attained with NC-Cu.

GRAPHICAL ABSTRACT

Probe NC-Cu displayed high selectivity, fast response (20 min), ultrasensitivity (0.65μM) and fluorescence imaging of Cu^{2+} was attained in KYSE30 cells.



ARTICLE INFO

Article history:
Received 30 June 2021
Received in revised form 19 August 2021
Accepted 22 August 2021
Available online 26 August 2021

Keywords:
Fluorescent probe
Copper ions
KYSE30 cells
Catalytic hydrolysis
Fluorescence imaging

ABSTRACT

Copper ion (Cu^{2+}) is an essential part of the living organisms. Cu^{2+} ions play a vital role in many biotic processes. An abnormal amount of Cu^{2+} ions may result in serious diseases. Herein, a novel "fluorescent ON" probe NC-Cu to trace minute levels of Cu^{2+} ions in presence of various biological active species has been developed. Lysosomal cells targeting group (Morpholine) was added to the probe. The spectral properties of probe NC-Cu were recorded in HEPES buffer (0.01 M, pH = 7.4, comprising 50% CH_3CN, λ_{ex} = 430-nm, slit: 5 nm). The synthesized probe NC-Cu work based on copper promoted catalytic hydrolysis of hydrazone and shows remarkable fluorescence enhancement. The reaction of the probe with Cu^{2+} ions was completed within 20 min. An excellent linear relationship (R^2 = 0.9952) was found and the limit of detection (LOD, according to the 3σ/slope) for Cu^{2+} ions was calculated to be 5.8 μM. Furthermore, NC-Cu was effectively functional in the living cells (KYSE30 cells) to trace Cu^{2+} ions.

1. Introduction

Copper is a transition metal that has four oxidation states Copper[0], Copper[1], Copper[11], and Copper[111] [1]. Mostly copper in water is present in divalent (Cu^{2+}) form. Copper is considered the third plentiful trace element in the human after zinc and iron [2]. It is a vital trace element and is present in low deliberation in the different parts of the body [3]. The key amount of Cu^{2+} ion is present in the liver. The average concentration of blood Cu^{2+} ion is found to be 15.7–23.6 μM [4]. Limits of Cu^{2+} ions in drinking water were set as 1.3 ppm by the U.S. environmental protection agency (EPA) [5]. Cu^{2+} ions play a fundamental role in physiological processes in living organisms [6]. As Cu^{2+} ions have electrophilic nature it can be used as a cofactor of many enzymes such as copper amine oxidase [7], dopamine b-hydroxylase [8], Cu\Zn superoxide dismutase [9], tyrosinase [10], ceruloplasmin and cytochrome *c* oxidase [11,12]. These cofactors are responsible for many functions in the body such as handling of dietary amines, pigmentation, antioxidant

* Corresponding authors.
E-mail address: yeyong03@tsinghua.org.cn (Y. Ye).

https://doi.org/10.1016/j.saa.2021.120313
1386-1425/

defense, neurotransmitter synthesis, and metabolism. On the other-hand deficiency and excess concentration of Cu^{2+} ions leads to many serious human genetic and metabolic disorders such as Indian childhood cirrhosis (ICC), Indian prion disease, Huntington's disease, Alzheimer's disease, Parkinson's disease, Menkes disease, Wilson disease, mitochondrial dysfunction to damage cells, obesity, and diabetes [6,13–19]. Therefore, the development of effective and suitable methods for sensing Cu^{2+} ions in biological samples is highly appreciated to understand its physiological functions and uncontrolled events.

The conventional analytical methods for the detection of Cu^{2+} ions are mostly dependent upon instrumental techniques such as atomic absorption spectroscopy (AAS), voltammetry, inductively coupled plasma atomic emission (ICP-AES), and piezoelectric crystals. These methods have some drawbacks such as required classy instrumentation, time-consuming, and expensive [20–22]. Many fluorescent probes have been reported for sensing copper ions [23–29]. Most of them form complex with Cu^{2+} ions and as a result, the fluorescence of the probe was quenched. Some probes showed low water solubility and less selectivity. Recently, fluorescent methods are getting the attention of the researchers. These methods are inexpensive, selective, and sensitive to trace copper ions. Previously many synthesized probes work as 'Turn Off' mode because of the paramagnetic nature of copper [30–32]. Now a day, some 'Turn On' probes are synthesized for Cu^{2+} ions detection [2,33–35].

Recently, our group has reported some fluorescent probes for selective detection of Cu^{2+}ions [36–38]. Herein, we have reported a novel 'fluorescence On' probe NC-Cu for selective and sensitive detection of Cu^{2+} ions among various biological active species because of the hydrolysis of hydrazone derivatives by Cu^{2+} ions (see Scheme 1). Carbonyl compounds can be protected by forming hydrazone and these carbonyl compounds can be regenerated by Cu^{2+} ions promoted hydrolysis of hydrazone [39]. To develop probe NC-Cu we have used coumarin as a carbonyl source. Due to its high water solubility, 1, 8-naphthalic anhydride derivative was used as a source of the amino group. Also, we introduced a lysosomal targeting group in our new design probe NC-Cu. Newly synthesized probe NC-Cu work well in HEPES buffer solution having 50% CH_3-CN. Photo-physical properties of probe NC-Cu have been studied in detail by some spectroscopic techniques such as UV–vis, fluorescence, and mass spectra. Cell imaging was taken by fluorescence confocal microscopy.

2. Experimental

2.1. Materials

All chemicals and reagents including 1,8-Naphthalic anhydride derivative, 2-morpholinoethan-1-amine, Hydrazine monohydrate (80%), 7-(diethylamino) benzaldehyde, ethyl-3-oxobutanoate, piperidine, acetic acid, ethanol, dichloromethane, and methanol were used as such as provided by the seller. According to standard procedures, solvents were purified for chemical synthesis and analysis. Double purified water was used during the experimentation. Probe standard solution ($1*10^{-3}$ M) was made in DMSO. The solutions of ions and amino acid (10 mM) were made from conforming salts counting: NaCl, KCl, $MgCl_2$, $CuCl_2$, $CaCl_2$, $ZnCl_2$, Na_2SO_4, $PbCl_2$, $CdCl_2$, $AgNO_3$, $BaCl_2$, $NiCl_2$, NaHS, $HgCl_2$, $NaNO_3$, $NaNO_2$, HSO_3^-, SO_3^{2-}, NaOCl, Na_2HPO_4, Cysteine and Homo-cysteine.

2.2. Apparatus

Nuclear magnetic resonance (NMR) spectra were noted on a Bruker DTX-400 spectrometer in d-chloroform with tetramethylsilane (TMS) as inner standard. UV–vis spectra were plaid on a Lambda 35 UV–visible spectrometer, PerkinElmer. Fluorescence saturation was done on a HITACHI F-4600 fluorescence spectrometer, and the excitation and emission wavelength cuts were set at 5 nm, excitation voltage was 700 V. By means of methanol as mobile phase mass spectra were recorded on a Q-Tof HR-MS spectrometer (Waters Micro mass). Fluorescence imaging by using KYSE30 cells was studied under a LEICA TCS SP8 laser scanning confocal microscope.

2.3. Cell culture and imaging

KYSE30 cells were cultivated in Dulbecco's Modified Eagle Medium (DMEM) added with 10% fetal bovine serum, at 37° C, in 5% CO_2 incubator. For imaging, probe **NC-Cu** (10 μM) was added to KYSE30 cells and incubated at 37 °C for 30 min in 5% CO_2 incubator. PBS buffer (pH 7.4) solution was used to wash (three times) and again treated with Cu^{2+} ions solution, and incubate them in 5% CO_2 incubator for 1 h. Cells were washed three times again with PBS buffer (pH 7.4). Then the cells were imaged using Leica TCS SP8 confocal microscope.

2.4. Synthesis

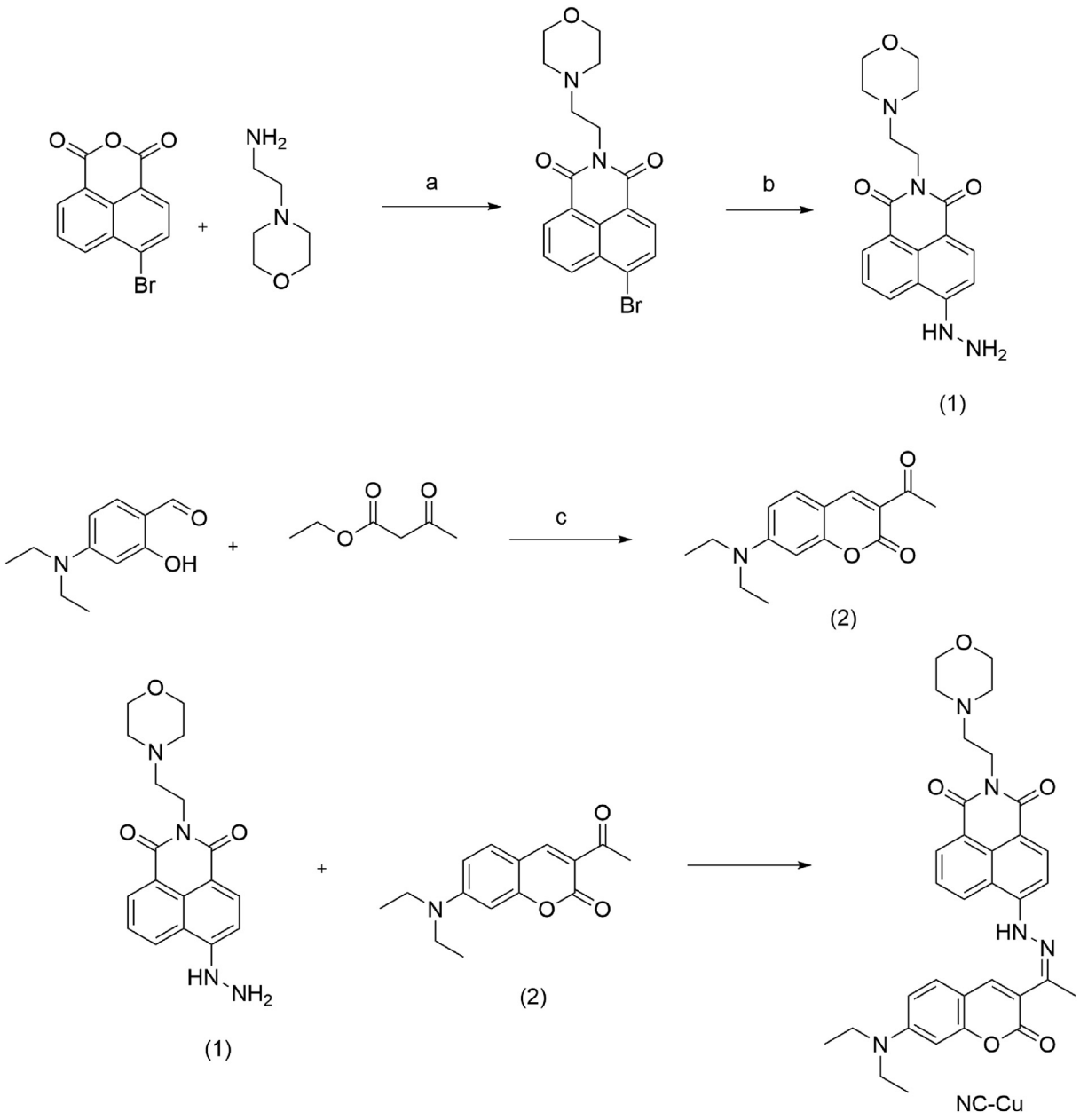
Starting material (Naphthalic anhydride derivative **1** and Coumarin **2)** were synthesised by previous reported method [40,41].

Synthesis of probe NC-Cu: probe NC-Cu was synthesized by dissolving reactant 1 (0.131 g, 0.38 mmol) and 2 (0.10 g, 0.38 mmol) in absolute ethanol, catalytic amount of glacial acetic acid (3 drops) was added and reflux for 12 h, reaction progress was checked by thin layer chromatography (TLC). After consumption of reactant as confirmed by TLC the solvent was removed and crude product was subjected to column chromatography for purification (eluant: CH_2Cl_2/MeOH = 50 /1, v/v) to get probe NC-Cu as a red solid (112 mg) ^{1}H NMR (400 MHz, $CDCl_3$, TMS) δ (ppm): 1.26 (t, *J* = 7.4 Hz, 6H, CH_2CH_3), 2.53 (s, *J* = 13.76 Hz, 3H, CH_3), 2.71 (s, *J* = 5.73 Hz, 4H, NCH_2CH_2N), 3.47 (q, *J* = 6.30 Hz, 4H, CH_2CH_3), 3.73 (s, *J* = 8.13 Hz, 4H, OCH_2), 4.39 (q, *J* = 3.43 Hz, 2H, NCH_2), 6.50 (s, *J* = 3.25 Hz, 1H, PhH), 6.65 (s, *J* = 3.25 Hz, 1H, PhH), 7.29 (s, *J* = 1.72 Hz, 1H, PhH), 7.41 (s, *J* = 2.60 Hz, 1H, PhH), 7.71 (s, *J* = 2.20 Hz, 1H, PhH), 7.81 (s, *J* = 2.53 Hz, 1H, PhH), 8.10 (s, *J* = 4.86 Hz, 1H, PhH), 8.19 (s, *J* = 2.15 Hz, 1H, PhH), 8.55 (s, *J* = 5.18 Hz, 2H, PhH), 8.61 (s, *J* = 2.55 Hz, 1H, NH = N); ^{13}C NMR ($CDCl_3$, 100 MHz): *δ* = 164.17, 163.47, 160.56, 156.68, 151.42, 150.22, 147.48, 142.36, 134.59, 130.61, 130.10, 125.28, 122.25, 119.75, 118.80, 111.90, 109.86, 108.51, 108.34, 96.53, 66.64, 60.21, 59.72, 53.69, 44.63, 16.51, 14.67 ppm; HR-MS: *m*/*z* calcd for $[C_{33}H_{35}N_5O_5][H^+]$ = 582.2711, Found: 582.2709.

3. Results and discussion

3.1. Plan and synthesis of NC-Cu

7-diethylaminocoumarin was used as a fluorophore due to its good photostability, high emission effect, and large stoke shift [42–45]. To increase water solubility hydrazine was attached with 1,8-Naphthalic anhydride derivative [46–48], and lysosomes targeting group was added to newly synthesized probe NC-Cu. Probe work in HEPES buffer solution having 50 % CH_3CN. Cu^{2+} ions promote hydrolysis of hydrazone. We have planned and prepared a new probe NC-Cu in a very easy method with a good yield. The new probe NC-Cu was confirmed by ^{1}H NMR, ^{13}C NMR, and HR-MS [Fig. S1-S3].



Scheme 1. Synthetic route for title compound. Reagents and conditions: a) 1,8-Naphthalic anhydride derivative, 2-morpholinoethan-1-amine, Ethanol, 12 h reflux, b) Hydrazine monohydrate (80%), EtOH, reflux 24 h c) 7-(diethyl amino) benzaldehyde, ethyl-3-oxobutanoate, piperidine, CH_3COOH, EtOH, reflux 12 h. d) 1 and 2, acetic acid, ethanol, reflux 12 h.

3.2. Spectral reaction of probe NC-Cu toward Cu^{2+} ions

The Cu^{2+} ions tracing ability of newly synthesized probe NC-Cu was confirmed by some spectroscopic techniques. The selectivity of probe NC-Cu was studied by ultra-violet (UV) and fluorescence spectroscopy. Firstly, UV selectively was performed. We patterned experiments in HEPES buffer (0.01 M, pH = 7.4, containing 50% CH_3CN). The probe itself had an intense absorption band centered at 492 nm. When the solution of probe NC-Cu was treated with Cu^{2+}, Zn^{2+}, HS^-, Cd^{2+}, Pb^{2+}, Ba^{2+}, Mg^{2+}, Hg^{2+}, Ni^{2+}, Ag^+, Na^+, K^+, NO_2^-, NO_3^-, SO_3^{2-}, HSO_3^-, ClO^-, HPO_4^-, Cys and Hcy. The only changes were observed with Cu^{2+} ions. The absorption band centered at 492 nm was disappeared and two new absorption bands centered at 444 nm and 497 nm appeared (as shown in Fig. 1) after the addition of Cu^{2+} ions. There was no change upon the addition of other ions and amino acids to the solution **(Fig. S4)**. UV selectivity result of probe NC-Cu shows a high selectivity toward Cu^{2+} ions which demonstrates that probe **NC-Cu** could used for Cu^{2+} ions detecting by the absorption spectra method.

Next, we achieved fluorescence selectivity of probe NC-Cu. The probe had almost no fluorescence in absence of Cu^{2+} ions due to C=N isomerization (protection of carbonyl group), however, a strong emission peak centered at 495 nm emerged as Cu^{2+} ions solution was added to the probe solution (Fig. 2). Cu^{2+} ions promote hydrolysis of hydrazone and free the highly fluorescent coumarin derivative. The quantum yields of probe NC-Cu is 0.007. After its reaction with Cu^{2+}, the quantum yield was calculated as 0.23. There was no change in fluorescent intensity when other ions such as Zn^{2+}, HS^-, Cd^{2+}, Pb^{2+}, Ba^{2+}, Mg^{2+}, Hg^{2+}, Ni^{2+}, Ag^+, Na^+, K^+, NO_2^-, NO_3^-, SO_3^{2-}, HSO_3^-, ClO^-, HPO_4^-, and amino acid (Cys and Hcy) were added to probe solution **(Fig. S5)**. This result indicates that probe NC-Cu has high discrimination toward Cu^{2+} ions.

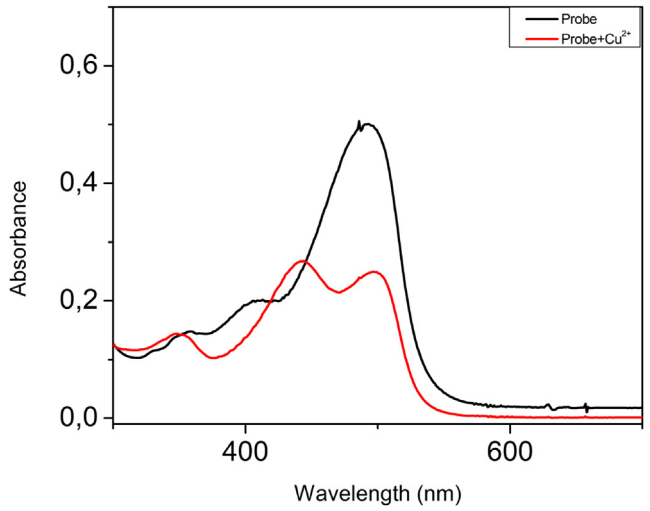


Fig. 1. The UV–vis spectra of probe NC-Cu (10 μM) with Cu^{2+} ions (10 equiv.) in HEPES buffer (0.01 M, pH = 7.4, containing 50% CH_3CN).

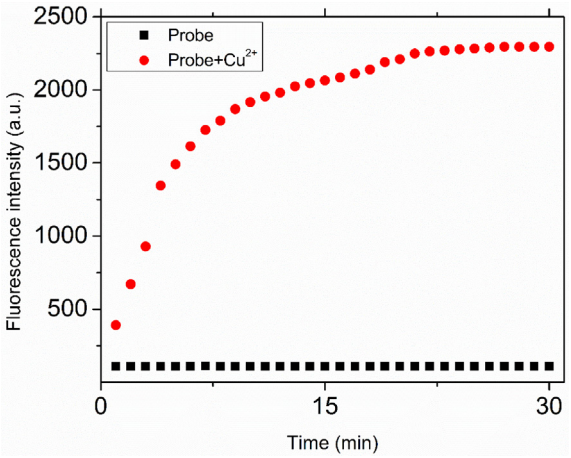


Fig. 4. The emission spectra of probe NC-Cu (10 μM) at 430 nm when treated with Cu^{2+} ions (10 equiv.) with time in HEPES buffer (0.01 M, pH = 7.4, containing 50% CH_3CN, λ_{ex} = 430 nm, slit: 5 nm).

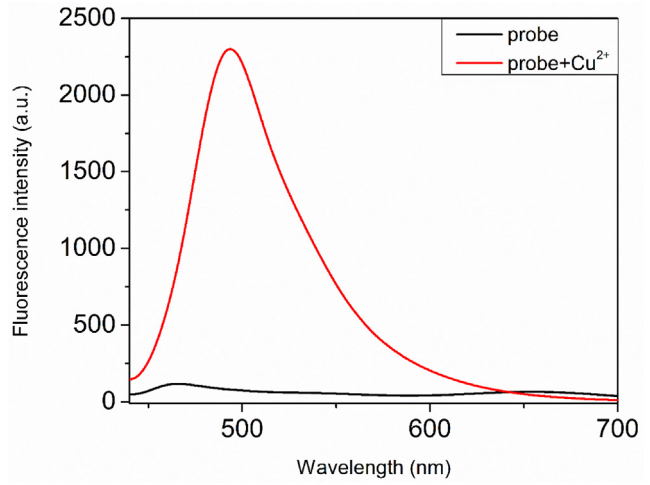


Fig. 2. The emission spectra of probe NC-Cu (10 μM) with Cu^{2+} (10 equiv.) in HEPES buffer (0.01 M, pH = 7.4, containing 50% CH_3CN, λ_{ex} = 430 nm, cuts: 5 nm).

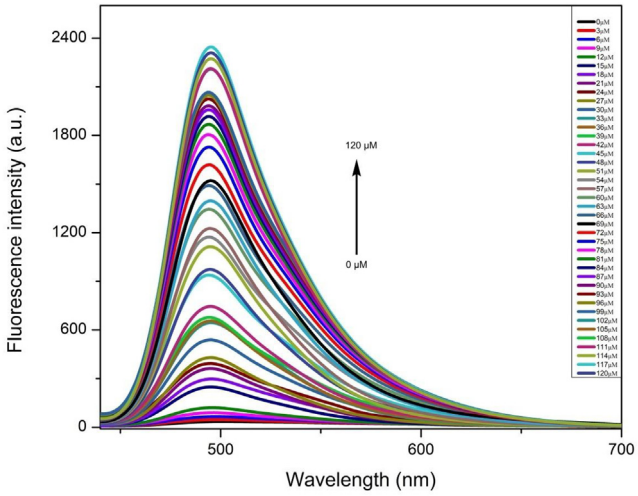


Fig. 5. Emission spectra of probe NC-Cu (10 μM) in the existence of Cu^{2+} ions (0–120 μM) in HEPES buffer (0.01 M, pH = 7.4, containing 50% CH_3CN, λ_{ex} = 430 nm, slit: 5 nm).

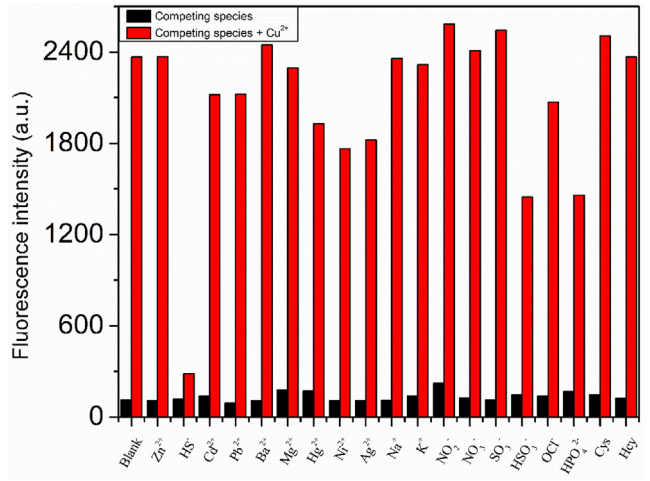


Fig. 3. The emission spectra of NC-Cu (10 μM) at 495 nm changes upon accumulation of ions such as Zn^{2+}, HS^-, Cd^{2+}, Pb^{2+}, Ba^{2+}, Mg^{2+}, Hg^{2+}, Ni^{2+}, Ag^+, Na^+, K^+, NO_2^-, NO_3^-, SO_3^{2-}, HSO_3^-, ClO^-, HPO_4^-, Cys and Hcy (10 equiv.) in the occurrence of Cu^{2+} ions (10 equiv.) in HEPES buffer (0.01 M, pH = 7.4, containing 50% CH_3CN, λ_{ex} = 430 nm, slit: 5 nm).

Next, we performed a competitive study to sightsee the functionality of probe NC-Cu as a fluorescence sensor to trace Cu^{2+} ions in the occurrence of interfering species. When probe NC-Cu was treated with Cu^{2+} ions in presence of ions such as Zn^{2+}, HS^-, Cd^{2+}, Pb^{2+}, Ba^{2+}, Mg^{2+}, Hg^{2+}, Ni^{2+}, Ag^+, Na^+, K^+, NO_2^-, NO_3^-, SO_3^{2-}, HSO_3^-, ClO^-, HPO_4^-, and amino acid (Cys and Hcy), only a slight change was found with concomitant species, except for H_2S that can attack the double bond of hydrazone and prohibit the copper promoted hydrolysis (Fig. 3**).** Competitive study with other metal ions and amino acids show that fluorescence intensity of probe NC-Cu was not changed by other ions and amino acids except H_2S. Probe NC-Cu shows a fast response to Cu^{2+} ions and complete hydrolysis of probe NC-Cu with Cu^{2+} ions was materialized within 20 min (Fig. 4 **and Fig. S6)**. These results show that the probe NC-Cu can be used for the detection of Cu^{2+} ions and cell imaging.

3.3. Fluorescent titration of probe NC-Cu with Cu^{2+} ions

Next, fluorescence titration spectra of probe NC-Cu with Cu^{2+} ions were recorded. The probe itself had almost no emission, but

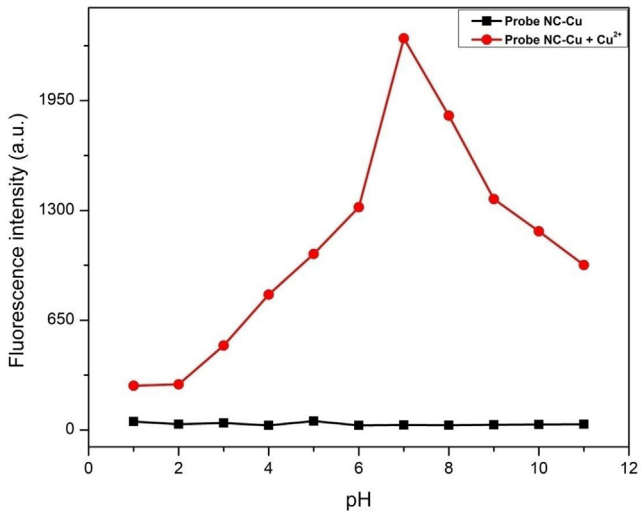


Fig. 6. The emission spectra of probe NC-Cu (10 μM) with pH (1–13) in the absence and presence of Cu^{2+} ions (10 equiv.).

the emission intensity of the probe was slowly increased upon treatment with a low concentration of Cu^{2+} ions solution. The inclusion of Cu^{2+} ion was sustained till a stage touched when there were no more enhancements in fluorescence intensity (Fig. 5). With the addition of Cu^{2+}, probe **NC-Cu** exhibited a significant and progressive emission enhancement at 495 nm. This enhancement in fluorescence intensity was linearly associated with the addition of Cu^{2+} solution in the range of 0–120 μM (R^2 = 0.9952, **Fig. S7**). The limit of detection (LOD, conferring to the 3σ/slope) for Cu^{2+} ions was determined as low as 5.8 μM. This showed a fairly high sensitivity for Cu^{2+} ions.

3.4. The outcome of pH on the fluorescence

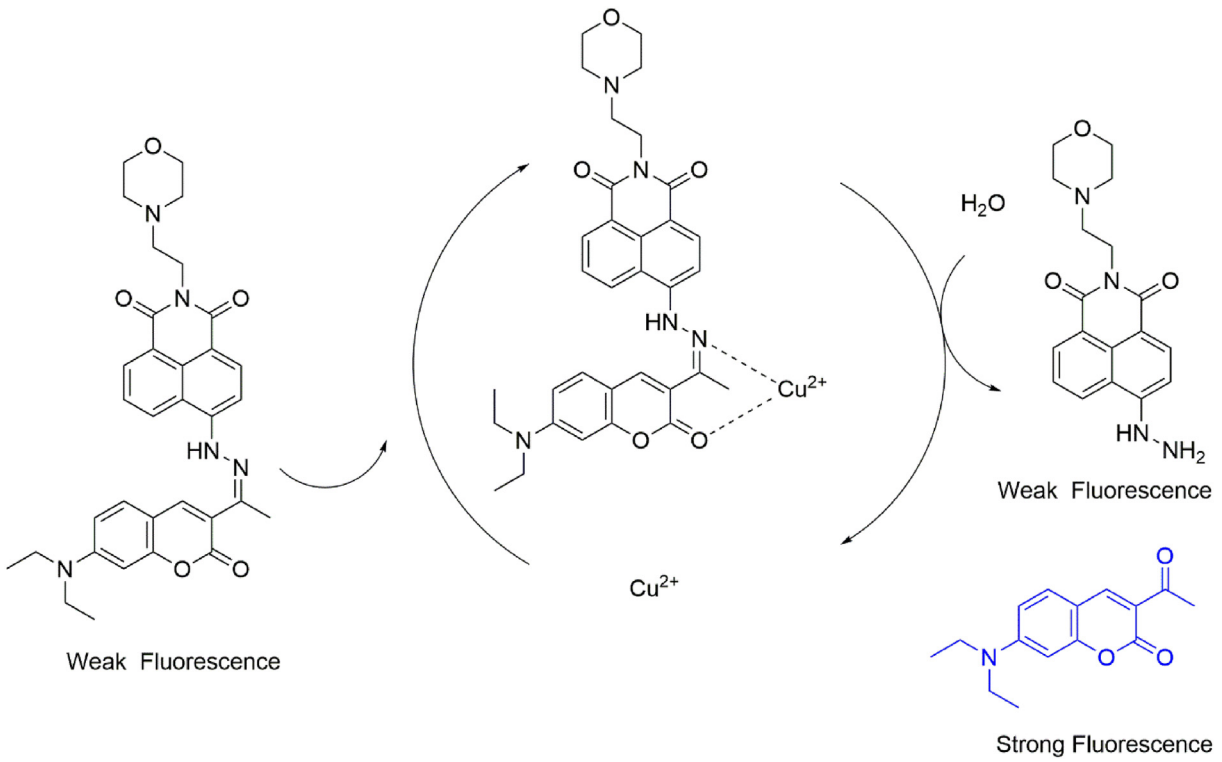
The consequence of pH on the probe respond to Cu^{2+} ions was studied. The pH solutions 1 to 13 were prepared. The experiment was performed with the fixed concentration of probe NC-Cu and Cu^{2+} ions (10.0 μM). The result shows that probe NC-Cu in the free-state has low fluorescence intensity. On the other hand, the fluorescence intensity of the probe in presence of Cu^{2+} ions is enhanced at pH 7 (Fig. 6). However, the fluorescence intensity at lower than 7 or higher than 7 pH is decreased because of protonation of the nitrogen atom at lower pH and deprotonation at higher pH. The pH result shows that the probe NC-Cu can work at the physiological pH range.

3.5. Sensing mechanism

According to the described literature [49], subsequently, an anticipated reaction mechanism was labeled in Scheme 2. The probe **NC-Cu** was hydrolyzed to **1** and **2.** As a result, ICT process was enhanced and strong emission showed. The product of probe **-NC-Cu** with Cu^{2+} ions was confirmed by the HRMS spectrum. A peak at *m*/*z* 768.88 consistent with $[C_{33}H_{41}Cl_2CuN_5O_8]^+$ was present in the MS spectrum (Fig. S8). The intense peak at *m*/*z* 260.14 was related to the coumarin derivative $[C_{15}H_{18}NO_3]^+$ (Fig. S9). The sensing mechanism was also proved by ^{1}H NMR spectra. After the addition of Cu^{2+} ions to the probe solution, the mixture was subjected to column chromatography and coumarin 1 was obtained. The ^{1}H NMR spectra confirmed it (Fig. S10). These results demonstrated that Cu^{2+} could promote hydrolysis of hydrazone.

3.6. Cell cytotoxicity and imaging

To examine whether probe **NC-Cu** can be useful to screen intracellular Cu^{2+} ions levels in living cells, bioimaging investigation



Scheme 2. Proposed reaction mechanism of probe NC-Cu in presence of Cu^{2+}.

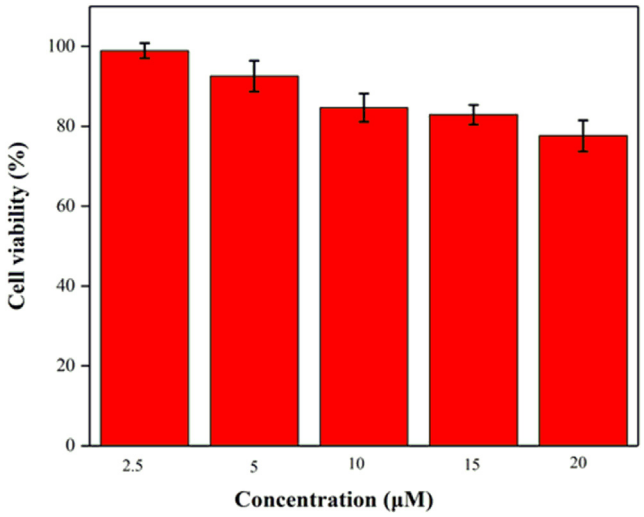


Fig. 7. Toxicity assay of probe NC-Cu at different concentration in KYSE30 cells.

was achieved in KYSE30 cells by confocal fluorescence microscopy. Earlier to these tests, we confirm the toxicity of the probe NC-Cu in living cells. MIT assay was done to confirm the toxicity of probe in cells. As is clarified in Fig. 7, cell feasibility was greater than 80% even when the concentration of probe NC-Cu was 20 μM. This represented the low toxicity of probe **NC-Cu** in live KYSE30 cells.

Due to the low cytotoxicity, high selectivity and sensitivity of probe NC-Cu, we further exposed the probe to image Cu^{2+} in the living cells. The cells (KYSE30) were nurtured with probe NC-Cu (10 μM) for 30 min at 37 °C in 5% CO_2. As shown in Fig. 8**b**, the image of probe showed no emission in the blue region in absence of Cu^{2+} ions. When the cells (KYSE30) were incubated with Cu^{2+} ions (10 equiv.), a blue emission was detected from the confocal microscopic images (Fig. 8**e**). This indicated that Cu^{2+} ions could easily promote hydrolysis of probe NC-Cu. The outcome proposes that probe NC-Cu is an effective indicator for monitoring the level of Cu^{2+} ions in the living cells. This probe is promising as an effective lysosomal-targetable fluorescent probe for exploring Cu^{2+} ions in biological samples.

4. Conclusions

Here, we have planned and prepared a new probe NC-Cu (coumarin based hydrazone) by a simple and easy method in a single step. The newly synthesized hydrazone derivative showed high selectivity and sensitivity toward Cu^{2+} ions in the presence of various coexisting species and amino acids. Hydrazone derivative is a very effective fluorescent sensor and exhibits strong fluorescence intensity in the presence of paramagnetic Cu^{2+}. This fluorescence enhancement is because of the catalytic hydrolysis of hydrazone by Cu^{2+} ions. The probe is promising as an effective lysosomal-targetable fluorescent probe for exploring Cu^{2+} ions in biological samples. The synthesis and application of more precise fluorescent probes for imaging in living cells, tissues and animals is our present attention and target in future.

5. Credit author statement

Mr. Nadeem Ahmed does the experiments and prepares the manuscript.

Miss. Wajeeha Zareen helps to write the manuscript.

Dr. Di Zhang revises the manuscript.

Mr. Xiaopeng Yang helps to draw some schematics and analysis the data.

Prof. Yong Ye helps to design experiments and to revise the manuscript thoroughly.

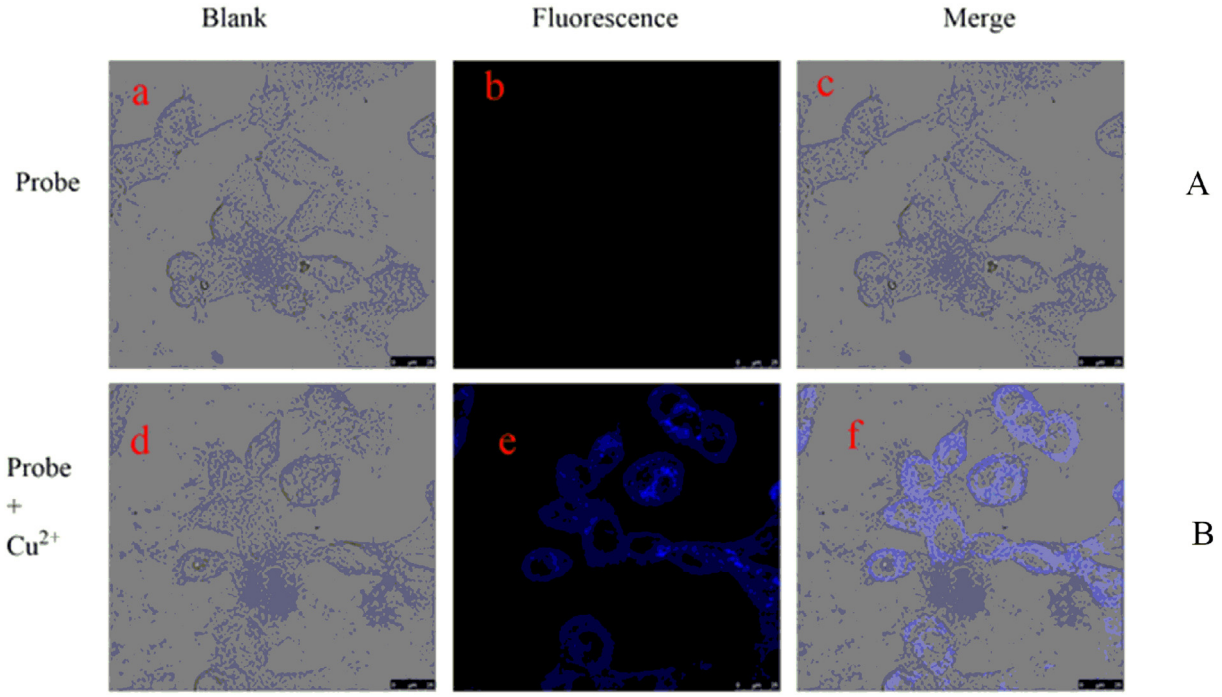


Fig. 8. Confocal fluorescence images of Cu^{2+} ions with probe NC-Cu in KYSE30 cells. (a,b) KYSE30 cells pretreated with NC-Cu (10 μM) for 30 min. (d,e) KYSE30 cells were pretreated with probe NC-Cu (10 μM) for 30 min and then incubated with Cu^{2+} ions (10 equiv.) for 1 h. (c) and (f) are fused images of a with b, d with e.(excitation: 440 nm, emission: 495 nm).

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

This work was financially supported by the Natural Science Foundation of China (21907025) and Scientific and Technological Innovation Project of Henan Academy of Agricultural Sciences (2020CX05).

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.saa.2021.120313.

References

[1] A.R. G. Georgopoulos, M.J. Yonone-Lioy, R.E. Opiekun, P.J. Lioy, P, Environmental copper: its dynamics and human exposure issues, J. Toxicol. Environ. Health Part B: Crit. Rev. 4 (2001) 341–394.
[2] B. Gu, L. Huang, Z. Xu, Z. Tan, H.u. Meng, Z. Yang, Y. Chen, C. Peng, W. Xiao, D. Yu, H. Li, A reaction-based, colorimetric and near-infrared fluorescent probe for Cu^{2+} and its applications, Sens. Actuat. B 273 (2018) 118–125.
[3] C.-I. Shih, Y.-C. Chou, H.-Y. Chen, K.-H. Chen, I.-H. Wang, Y.-C. Yeh, Colorimetric and fluorometric paper-based assay for Cu2+ detection based on green synthesis of 2-aminoterephthalic acid-derived pigments, ACS Appl. Bio Mater. 3 (4) (2020) 2516–2521.
[4] A. Helal, M.H. Or Rashid, C.-H. Choi, H.-S. Kim, Chromogenic and fluorogenic sensing of Cu2+ based on coumarin, Tetrahedron 67 (15) (2011) 2794–2802.
[5] J. Wang, Q. Niu, T. Wei, T. Li, T. Hu, J. Chen, X. Qin, Q. Yang, L. Yang, Novel phenothiazine-based fast-responsive colori/fluorimetric sensor for highly sensitive, selective and reversible detection of Cu2+ in real water samples and its application as an efficient solid-state sensor, Microchem. J. 157 (2020) 104990, https://doi.org/10.1016/j.microc.2020.104990.
[6] Y. Wang, J. Zhou, L. Zhao, B. Xu, A dual-responsive and highly sensitive fluorescent probe for Cu2+ and pH based on a dansyl derivative, Dyes Pigm. 180 (2020) 108513.
[7] Z. Zhou, N. Li, A. Tong, A new coumarin-based fluorescence turn-on chemodosimeter for Cu2+ in water, Anal. Chim. Acta 702 (2011) 81–86.
[8] N. Herranz, N. Dave, A. Millanes-Romero, L. Morey, V.M. Díaz, V. Lórenz-Fonfría, R. Gutierrez-Gallego, C. Jerónimo, L. Di Croce, A.G. de Herreros, RETRACTED: lysyl oxidase-like 2 deaminates lysine 4 in histone H3, in, Elsevier, 2012.
[9] A.R. Reddi, V.C. Culotta, SOD1 integrates signals from oxygen and glucose to repress respiration, Cell 152 (2013) 224–235.
[10] C. Olivares, F. Solano, New insights into the active site structure and catalytic mechanism of tyrosinase and its related proteins, Pigment Cell Melanoma Res. 22 (2009) 750–760.
[11] V.B. Borisov, S.A. Siletsky, Features of organization and mechanism of catalysis of two families of terminal oxidases: heme-copper and bd-type, Biochem. (Moscow) 84 (11) (2019) 1390–1402.
[12] N.E. Hellman, J.D. Gitlin, Ceruloplasmin metabolism and function, Annu. Rev. Nutr. 22 (2002) 439–458.
[13] J.S. Ganesan, S. Gandhi, K. Radhakrishnan, A. Balasubramaniem, M. Sepperumal, S. Ayyanar, Execution of julolidine based derivative as bifunctional chemosensor for Zn2+ and Cu^{2+} ions: applications in bio-imaging and molecular logic gate, Spectrochim. Acta Part A Mol. Biomol. Spectrosc. 219 (2019) 33–43.
[14] Y. Hu, A. Chen, Z. Kong, D. Sun, A reversible colorimetric and fluorescence "turn-off" chemosensor for detection of Cu^{2+} and its application in living cell imaging, Molecules 24 (2019) 4283.
[15] Q. Jiang, Z. Wang, M. Li, J. Song, Y. Yang, X. Xu, H. Xu, S. Wang, A nopinone based multi-functional probe for colorimetric detection of Cu^{2+} and ratiometric detection of Ag+, Photochem. Photobiol. Sci. 19 (2020) 49–55.
[16] M. Johncilla, K.A. Mitchell, Pathology of the liver in copper overload, in: Seminars in liver disease, © Thieme Medical Publishers, 2011, pp. 239-244.
[17] A. Maji, S. Lohar, S. Pal, P. Chattopadhyay, A new rhodamine based 'turn-on'$$ \hbox Cu}^{2+ $$ Cu 2+ ion selective chemosensor in aqueous system applicable in bioimaging, J. Chem. Sci. 129 (2017) 1423–1430.
[18] J.-X. Wang, Z.-Y. Xing, Z.-N. Tian, D.-Q. Wu, Y.-Y. Xiang, J.-L. Li, A dual-functional probe for sensing pH change and ratiometric detection of Cu^{2+}, Spectrochim. Acta Part A Mol. Biomol. Spectrosc. 235 (2020) 118318, https://doi.org/10.1016/j.saa.2020.118318.
[19] J. Xiong, Z. Li, J. Tan, S. Ji, J. Sun, X. Li, Y. Huo, Two new quinoline-based regenerable fluorescent probes with AIE characteristics for selective recognition of Cu^{2+} in aqueous solution and test strips, Analyst 143 (2018) 4870–4886.
[20] Y. An, P. Wang, Z. Yue, A sequential and reversibility fluorescent pentapeptide probe for Cu (II) ions and hydrogen sulfide detections and its application in two different living cells imaging, Spectrochim. Acta Part A Mol. Biomol. Spectrosc. 216 (2019) 319–327.
[21] T. Shu, Z. Yang, Z. Cen, X. Deng, Y. Deng, C. Dong, Y. Yu, A novel ratiometric fluorescent probe based on a BODIPY derivative for Cu^{2+} detection in aqueous solution, Anal. Methods 10 (2018) 5755–5762.
[22] J. Yin, Q. Bing, L. Wang, G. Wang, Ultrasensitive and highly selective detection of Cu^{2+} ions based on a new carbazole-schiff, Spectrochim. Acta Part A Mol. Biomol. Spectrosc. 189 (2018) 495–501.
[23] M. Aarjane, S. Slassi, A. Amine, Novel highly selective and sensitive fluorescent sensor for copper detection based on N-acylhydrazone acridone derivative, J. Mol. Struct. 1199 (2020) 126990.
[24] Z. Li, Y. Xu, H. Xu, M. Cui, T. Liu, X. Ren, J. Sun, D. Deng, Y. Gu, P. Wang, A dicyanomethylene-4H-pyran-based fluorescence probe with high selectivity and sensitivity for detecting copper (II) and its bioimaging in living cells and tissue, Spectrochim. Acta Part A Mol. Biomol. Spectrosc. 244 (2021) 118819, https://doi.org/10.1016/j.saa.2020.118819.
[25] X. Qian, W. Zhu, H. Yu, Y. Xu, W. Liu, H.-Y. Wang, Y.i. Liu, Mitochondria-targeted near-infrared fluorescent probe for monitoring of a copper (I) in living cells and in vivo, Dyes Pigm. 194 (2021) 109561, https://doi.org/10.1016/j.dyepig.2021.109561.
[26] Y. Song, J. Tao, Y.i. Wang, Z. Cai, X. Fang, S. Wang, H. Xu, A novel dual-responsive fluorescent probe for the detection of copper (II) and nickel (II) based on BODIPY derivatives, Inorg. Chim. Acta 516 (2021) 120099, https://doi.org/10.1016/j.ica.2020.120099.
[27] X.-Q. Yi, Y.-F. He, Y.-S. Cao, W.-X. Shen, Y.-Y. Lv, Porphyrinic probe for fluorescence "turn-on" monitoring of Cu+ in aqueous buffer and mitochondria, ACS Sensors 4 (4) (2019) 856–864.
[28] X. Zeng, S. Gao, C. Jiang, X. Jin, M. Ma, Z. Liu, J. Chen, A colorimetric and long-wavelength "turn-on" fluorescent probe for copper ions detection with high selectivity and sensitivity, Chem. Select 6 (25) (2021) 6619–6624.
[29] Y.u. Zhang, L. Li, J. Wang, L. Jia, R. Yang, X. Guo, A 4, 5-quinolimide-based fluorescent sensor for sequential detection of Cu2+ and cysteine in water and living cells with application in a memorized device, Spectrochim. Acta Part A Mol. Biomol. Spectrosc. 230 (2020) 118030, https://doi.org/10.1016/j.saa.2020.118030.
[30] Z.-E. Chen, X.-F. Zang, M. Yang, H. Zhang, A simple indolo [2, 3-a] carbazole based colorimetric chemosensor for simultaneous detection of Cu^{2+} and Fe^{3+} ions, Spectrochim. Acta Part A Mol. Biomol. Spectrosc. 234 (2020) 118236, https://doi.org/10.1016/j.saa.2020.118236.
[31] B. Zhang, H. Liu, F. Wu, G. Hao, Y. Chen, C. Tan, Y. Tan, Y. Jiang, A dual-response quinoline-based fluorescent sensor for the detection of Copper (II) and Iron (III) ions in aqueous medium, Sens. Actuat. B 243 (2017) 765–774.
[32] C. Zhang, B. Gao, Q. Zhang, G. Zhang, S. Shuang, C. Dong, A simple Schiff base fluorescence probe for highly sensitive and selective detection of Hg^{2+} and Cu^{2+}, Talanta 154 (2016) 278–283.
[33] Q. Qiu, B. Yu, K. Huang, D. Qin, A fluoran-based Cu^{2+}-selective fluorescent probe and its application in cell imaging, J. Fluorescence 30 (2020) 859–866.
[34] H. Zhang, L. Feng, Y. Jiang, Y.-T. Wong, Y. He, G. Zheng, J. He, Y. Tan, H. Sun, D. Ho, A reaction-based near-infrared fluorescent sensor for Cu^{2+} detection in aqueous buffer and its application in living cells and tissues imaging, Biosens. Bioelectron. 94 (2017) 24–29.
[35] D. Zhu, Y. Luo, L. Shuai, W. Xie, X. Yan, Z. Duan, W. Cai, A hemicyanine-based selective and sensitive colorimetric and fluorescent turn-on probe for Cu^{2+}, Tetrahedron Lett. 57 (2016) 5326–5329.
[36] N. Ahmed, W. Zareen, D. Zhang, X. Yang, Y. Ye, Coumarin-based reversible fluorescent probe for selective detection of Cu^{2+} in living cells, J. Fluorescence 30 (2020) 1171–1179.
[37] J. Tang, S. Ma, D. Zhang, Y. Liu, Y. Zhao, Y. Ye, Highly sensitive and fast responsive ratiometric fluorescent probe for Cu2+ based on a naphthalimide-rhodamine dyad and its application in living cell imaging, Sens. Actuat. B 236 (2016) 109–115.
[38] B. Zhang, F. Qin, H. Niu, Y. Liu, D. Zhang, Y. Ye, A highly sensitive and fast responsive naphthalimide-based fluorescent probe for Cu^{2+} and its application, New J. Chem. 41 (2017) 14683–14688.
[39] E. Corey, S. Knapp, α-Functionalization of ketones via N, N-dimethylhydrazones, Tetrahedron Lett. 17 (1976) 4687–4690.
[40] Y. Tang, Y. Zhao, W. Lin, Preparation of robust fluorescent probes for tracking endogenous formaldehyde in living cells and mouse tissue slices, Nat. Protoc. 15 (2020) 3499–3526.
[41] Q. Zhou, K. Li, Y.-H. Liu, L.-L. Li, K.-K. Yu, H. Zhang, X.-Q. Yu, Fluorescent Wittig reagent as a novel ratiometric probe for the quantification of 5-formyluracil and its application in cell imaging, Chem. Commun. 54 (2018) 13722–13725.
[42] X. Dai, Q.-H. Wu, P.-C. Wang, J. Tian, Y. Xu, S.-Q. Wang, J.-Y. Miao, B.-X. Zhao, A simple and effective coumarin-based fluorescent probe for cysteine, Biosens. Bioelectron. 59 (2014) 35–39.
[43] J. Li, C.-F. Zhang, S.-H. Yang, W.-C. Yang, G.-F. Yang, A coumarin-based fluorescent probe for selective and sensitive detection of thiophenols and its application, Anal. Chem. 86 (2014) 3037–3042.
[44] X. Meng, S. Li, W. Ma, J. Wang, Z. Hu, D. Cao, Highly sensitive and selective chemosensor for Cu2+ and H2PO4− based on coumarin fluorophore, Dyes Pigm. 154 (2018) 194–198.
[45] P. Qu, X. Ma, W. Chen, D. Zhu, H. Bai, X. Wei, S. Chen, M. Xu, A coumarin-based fluorescent probe for ratiometric detection of hydrazine and its application in

living cells, Spectrochim. Acta Part A Mol. Biomol. Spectrosc. 210 (2019) 381–386.
[46] F. Dai, F. Jin, Y. Long, X.-L. Jin, B.o. Zhou, A 1, 8-naphthalimide-based turn-on fluorescent probe for imaging mitochondrial hydrogen peroxide in living cells, Free Radical Res. 52 (11-12) (2018) 1288–1295.
[47] X. Li, J. Hou, C. Peng, L.i. Chen, W. Liu, Y. Liu, A 1, 8-naphthalimide-based fluorescent probe for selective and sensitive detection of peroxynitrite and its applications in living cell imaging, RSC Adv. 7 (54) (2017) 34287–34292.
[48] J. Shi, Y. Wang, X. Tang, W. Liu, H. Jiang, W. Dou, W. Liu, A colorimetric and fluorescent probe for thiols based on 1, 8-naphthalimide and its application for bioimaging, Dyes Pigm. 100 (2014) 255–260.
[49] M.H. Kim, H.H. Jang, S. Yi, S.-K. Chang, M.S. Han, Coumarin-derivative-based off–on catalytic chemodosimeter for Cu^{2+} ions, Chem. Commun. (32) (2009) 4838, https://doi.org/10.1039/b908638b.