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ARTICLE

Simple quinoline-based "turn-on" fluorescent sensor for imaging copper (II) in living cells

Chen Zhou, Ning Xiao, and Yapeng Li

Abstract: In this work, a novel fluorescent sensor based on a quinoline derivative was designed and synthesized for detecting copper ions in a near-aqueous media. According to its extra-nuclear structure, copper (II) normally exhibits effectiveness for quenching the singlet excited state of organic chromophores through a fast electron transfer mechanism. However, this molecule displayed a strong fluorescence "turn-on" phenomenon upon addition of Cu^{2+}, which was rarely reported in previous research. In addition, density functional theory calculations were adopted to investigate the molecular orbitals as well as the spatial structure. Furthermore, this sensor was applied into living SGC-7901 cells to extend its application in biological systems.

Key words: fluorescent sensor, copper, quinoline derivative, DFT, cell-imaging.

Résumé : Dans la présente étude, un nouveau capteur fluorescent à base d'un dérivé de la quinoléine a été élaboré et synthétisé dans le but de détecter les ions cuivre dans un milieu quasi aqueux. En raison de sa structure extranucléaire, le cuivre (II) s'avère normalement efficace vis-à-vis de l'extinction de l'état excité singulet de chromophores organiques par l'intermédiaire d'un mécanisme de transfert électronique rapide. Cependant, cette molécule a présenté un phénomène important « d'allumage » de la fluorescence après addition de Cu^{2+}, ce qui a été rarement décrit dans la littérature jusqu'à présent. Par ailleurs, les calculs de densité de la fonctionnelle de la densité (TFD) ont été adoptés pour étudier les orbitales moléculaires et la structure spatiale. En outre, ce capteur a été utilisé dans des cellules vivantes SGC-7901 en vue d'étendre son champ d'application aux systèmes biologiques.[Traduit par la Rédaction]

Mots-clés : capteur fluorescent, cuivre, dérivé de la quinoléine, TFD, imagerie cellulaire.

Introduction

In recent years there has been a growing desire to construct optical chemosensors for fast and accurate monitoring of hazardous heavy metals. Among different kinds of chemosensors, a fluorescence-based one is a very advantageous motif for practical applications on account of its sensitivity, selectivity, and rapid response.[1] A common fluorescent sensor would involve the covalent linking of a "receptor" domain with a fluorescent fragment. The two components are intramolecularly connected together, such that the binding of the target analyte causes significant changes to the photophysical properties of the fluorescent fragment, achieving the purpose of identification.[2]

Copper, the third most abundant transition metal ion in the human body, plays a vital role in many fundamental physiological processes.[3,4] Many cytosolic, mitochondrial, and vesicular oxygen-processing enzymes require copper as a redox cofactor, but uncontrolled reactions of copper ions with oxygen result in the formation of reactive oxygen species (ROS), which trigger oxidative damage to proteins, nucleic acids, and lipids. A variety of serious neurodegenerative diseases such as Alzheimers, Parkinson's, Prion, Menkes, and Wilson's diseases are also closely related to the disorder of copper metabolism.[5] As a transition metal ion known for its efficient fluorescent quenching character, most of the previous reported cation sensors generally display fluorescent quenching upon binding with Cu^{2+} under an electron or energy transfer mechanism. In terms of sensitivity concerns, sensors exhibiting fluorescence enhancement as a result of metal-ion binding are favored over those showing fluorescence quenching.[6–8] The fluorescent enhanced phenomenon also contributes to potential value in practical analytical application.[9] Due to the extensive use of copper and the growing awareness of environmental protection in modern society, the development of new Cu^{2+} selective "turn-on" fluorescent sensor is in great demand.

In the present study, 2-(quinoline-8-yloxy) acetohydrazide (HQ), a novel simple-structured fluorescent sensor, was successfully synthesized. The fluoroionophoric properties of HQ were studied in combination of density functional theory (DFT) and applied in living SGC-7901cells. This work had elucidated that HQ is a highly selective and sensitive turn-on fluorescence sensor for Cu^{2+} in near-aqueous media. It would provide useful guidance for the selective recognition of Cu^{2+} ions based on PET (photo-induced electron transfer) mechanism.

Results and discussion

Compound HQ (m.p. 140–142 °C) was easily synthesized with isolated yield of 69% through the reaction of 8-hydroxyquinoline, ethyl bromoacetate, and hydrazine (Scheme 1). The structure of HQ (m.p. 140–142 °C) was confirmed by ^{1}H NMR (Supplementary data, Fig. S1), ^{13}C NMR (Supplementary data, Fig. S2), and LC-Mass (Supplementary data, Fig. S3). The binding mode between HQ and Cu^{2+} was investigated by fluorescence spectra, Job's plot, Benesi–Hildebrand expression, ^{1}H NMR titration, and DFT calculations. Cell imaging experiments were carried out to prove the biocompatibility of HQ. The sensitivity and selectivity of HQ were demonstrated by means of titration experiments.

Cu^{2+}-titration and spectral responses

To gain insight into the signaling properties of HQ toward Cu^{2+}, titration experiments were conducted in a mixed solvent

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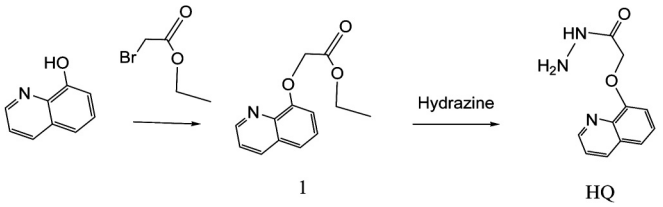
Scheme 1. Synthesis of HQ.



1 HQ

(0.1 mol/L Tris-HCl buffer solution at pH = 7.2, [$v(C_2H_5OH)$/v(buffer) = 1:9]). As illustrated in Fig. 1, HQ displayed an absorption maximum at 319 nm in the absorption spectrum; with the addition of Cu^{2+}, the absorption band at 319 nm increased until the first stoichiometry. Moreover, according to the Benesi–Hildebrand (Fig. 2) expression,[10–12] a linear response of HQ as a function of Cu^{2+} concentration at 319 nm was observed in the range 5 μmol/L: –10 μmol/L. The association constant (K_a) calculated from UV–vis titration of HQ with Cu^{2+} was 5.716×10^4 mol^{-1} (R = 0.99407). In the fluorescence emission, HQ (0.1 (mmol/L)/L) alone exhibited negligible fluorescence (excited at 319 nm, Φ_{HQ} = 0.019), whereas evident enhanced fluorescence intensity was displayed at 384 nm (excited at 319 nm, Φ_{Cu} = 0.21, Fig. 3) after adding Cu^{2+}. Furthermore, as depicted in Scheme 2, the turn-on process could be observed under the irradiation of a UV lamp. In our design of HQ, there was no separate binding site and fluorophore, so we believed that the whole molecule was involved in the emission process. Hence we hypothesized that the obvious fluorescence enhancement was due to the prevention of nonradiative relaxation pathways of N-lone electron pairs by Cu^{2+} binding, and the 1:1 binding mode between HQ and Cu^{2+} was determined by the Job's plot evaluated from the fluorescence spectra of HQ and Cu^{2+} with a total concentration of 0.2 mmol/L (Fig. 4); this supported our hypothesis for a 1:1 binding stoichiometry between them. Remarkably, Fig. 5 illustrated that the fluorescence enhancement of HQ increased linearly with the addition of Cu^{2+}, with a coefficient R = 0.99853, so we might consider that HQ had a potential application for the quantitative determination of Cu^{2+}. In addition, the detecting limit was calculated to be 4.23×10^{-6} mol/L from this linear relation (based on DL = KSb_1/S).

The fluorescence quantum yields of HQ and HQ-Cu^{2+} were determined according to the equation: $Y_u = Y_S \times \frac{F_U}{F_S} \times \frac{A_S}{A_U}$, where F_U and F_S denoted the integral fluorescence intensity of determinand and standard substance, A_U and A_S denoted the relevant maximum absorbance of determinand and standard substance, respectively (quinine sulphate was used as reference quantum yield standard, λ_{ex} = 410 nm, quantum yield = 0.54 in 0.1 mol/L H_2SO_4). The quantum yields for HQ and HQ-Cu^{2+} calculated from the equation in aqueous ethanol were 0.019 and 0.21, respectively.

To obtain further details of the coordination, we carried out ^1H NMR titration experiments in DMSO-d_6. As shown in Fig. 6, upon addition the Cu^{2+} dissolved in D_2O, the chemical shift of protons in HQ changed, especially in the acceptor moiety, with a dramatic change between δ 4.51 and δ 4.39 in the spectrum demonstrating the existence of proton H_a (amino proton) during Cu^{2+} binding. The proton H_c and H_i displayed a shift from δ 4.76 to δ 4.86 and δ 8.91 to δ 9.05 with Δδ up to 0.1 and 0.14, respectively. Simultaneously, other aromatic protons showed a weak shift. These data further confirmed that both amino moiety and fluorophore are involved in the coordination with Cu^{2+} as envisaged.

Response of HQ to various metal ions and metal-ion competition experiments

An important feature of HQ is high selectivity toward Cu^{2+} in comparison with other competitive metal ions, the changes of the fluorometric behavior of HQ (1.0×10^{-4} mol/L) caused by Cu^{2+} and

Fig. 1. UV–vis absorption response of HQ (0.1 (mmol/L)/L) upon addition of different concentrations of Cu^{2+} (0.005 (mmol/L)/L) in Tris-HCl solution [$v(C_2H_5OH)$/$v(H_2O)$ = 1:9, pH = 7.2].

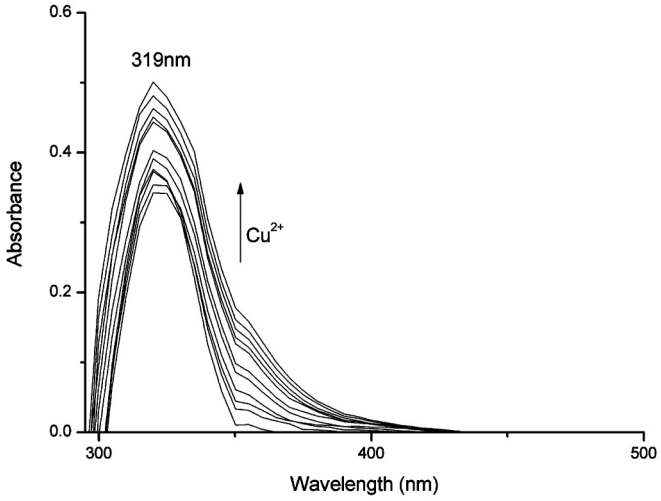


Fig. 2. Benesi Hilderbrand plot of HQ with Cu^{2+}.

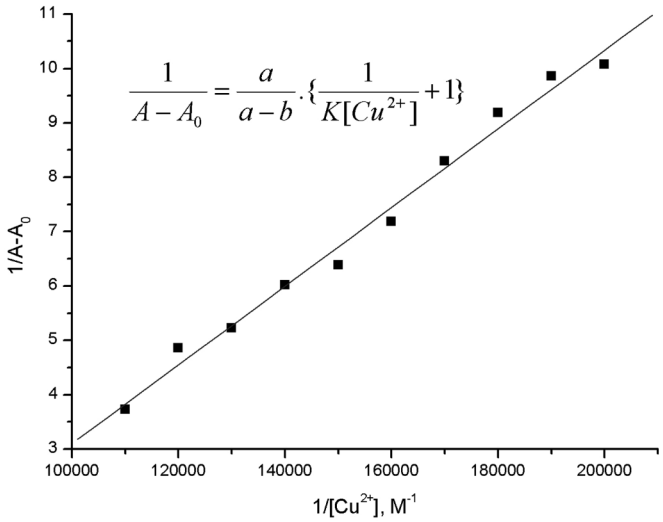
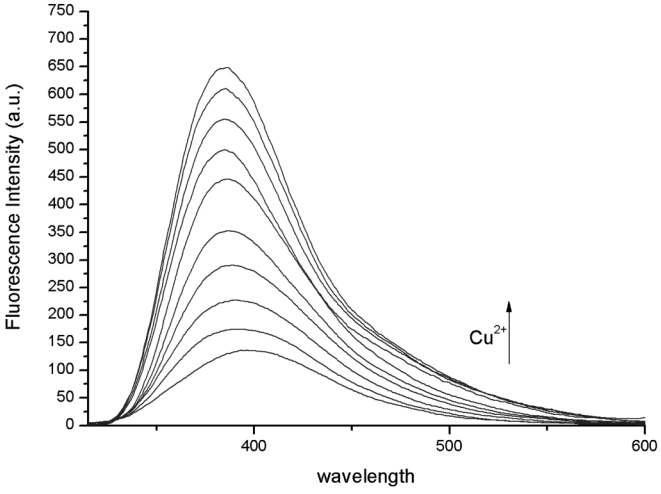


Fig. 3. Fluorescence spectra of HQ (0.1 (mmol/L)/L) upon addition of different concentrations of Cu^{2+} (0.01 (mmol/L)/L) in Tris-HCl solution [$v(C_2H_5OH)$/$v(H_2O)$ = 1:9, pH = 7.2] (λ_{ex} = 319 nm).



Scheme 2. Solutions of HQ without (left) and with (right) Cu^{2+} illuminated with a UV lamp and the binding mode of HQ-Cu^{2+} system.

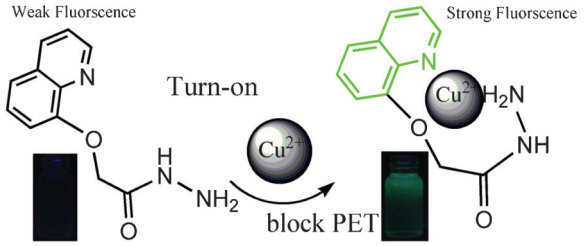


Fig. 4. Job's plot for determining the stoichiometry of HQ and Cu^{2+} in Tris-HCl solution [$v(C_2H_5OH)/v(H_2O)$ = 1:9, pH = 7.2] [Cu^{2+}] + [HQ], the total concentration of HQ and Cu^{2+} was 0.2 (mnol/L)/L (λ_{ex} = 319 nm).

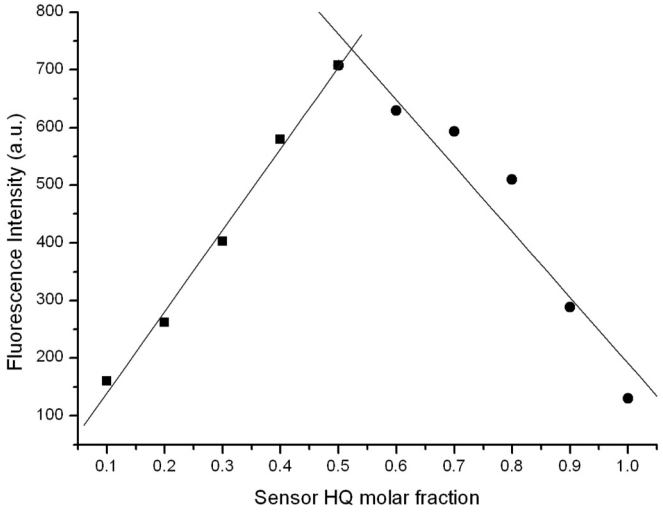
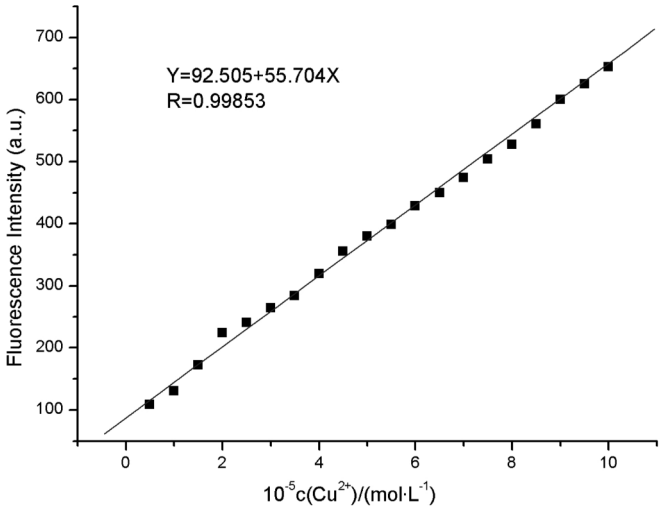


Fig. 5. Normalized response of the fluorescence signal to changing Cu^{2+} concentrations.



miscellaneous competing species (1.0×10^{-4} mol/L) in C_2H_5OH–Tris buffer solution (1: 9, volume ratio, pH = 7.2) were investigated and the results are shown in Fig. 7. The selectivity experiment is illustrated as black bars, only Cu^{2+} caused a strong flourescence enhancement at 384 nm in emission spectrum among common metal ions (excitated at 319 nm). Meanwhile the introduction of other metal ions such as Na^+, K^+, Ca^{2+}, Mg^{2+}, Ba^{2+}, Co^{2+}, Ni^{2+}, Fe^{3+}, Mn^{2+}, Hg^{2+}, Pb^{2+}, Cd^{2+}, Zn^{2+}, Al^{3+} did not cause any significant changes. The competition experiments were also performed for further study of the interference from other cations in the determination of Cu^{2+}, and they were conducted with addition of 1.5 equiv. Cu^{2+} in HQ solution to induce fluorescence enhancement before mixing 10 equiv. interferential metal ions, as shown in red bars. The mixtures of HQ with most mentioned of the above metal ions still exhibited strong fluorescence, except that Fe^{3+} induced a certain degree of quenching. In general, the fluorometric analysis has proven that the flourescence change of HQ at 384 nm was specific to Cu^{2+}.

The reversibility of the chemosensor is a significant for practical applications. HQ was alternately exposed to the Cu^{2+} and EDTA aqueous solution, and the corresponding fluorescence emission was measured, as illustrated in Fig. 8. Unfortunately, the experiment showed that the emission of HQ could not be restored. The irreversibility might be attributed to the stronger complexation capability of HQ to Cu^{2+}.

DFT calculations

To understand the recognition ability of HQ towards Cu^{2+}, the HOMO (highest occupied molecular orbitals) and LUMO (lowest unoccupied molecular orbitals) distributions of HQ were determined by DFT calculations with B_3LYP, using the Gaussian 09 package.[13–19] The 6-31G basis set was used for the H, C, N, and O atoms; for the Cu atom, the LANL2DZ effective core potential was employed.[20,21] As illustrated in Fig. 9, the DFT calculations showed the oscillator strength (*f*), molecular orbital energy levels in the ground (Fig. 9A), and excited states (Fig. 9B) of HQ and HQ-Cu^{2+}, respectively. An obvious structural difference between HQ and HQ-Cu^{2+} is shown in Fig. 10. For HQ, π electrons on both the HOMO and LUMO in the ground (Fig. 9A) and excited states (Fig. 9B) were mainly located on the quinoline group in a non-coplanar geometry. However, when the nitrogen atom lone pair interacts with Cu^{2+}, the energy levels of both HOMO and LUMO in the ground and excited states were lower than those of free HQ, while the π electrons on the HOMO and LUMO of the HQ-Cu^{2+} were located on the whole framework. Furthermore, the lone-pair-carrying N atom exhibited a nearly planar geometry with the quinoline group in HQ-Cu^{2+} (Fig. 10), which prevented the same out-of-phase π-interaction conjugation in HQ (prevented the nonradiative relaxation pathways of N-lone electron pairs by binding Cu^{2+} and blocked photoinduced electron transfer (PET) between 8-hydroxyquinoline and hydrazide moiety). The molecular orbital energy levels lay well below the frontier orbitals of the fluorophore, and it could not serve as an electron donor for the purposes of quenching. It was possible to offer a qualitative rationale for the experimentally observed fluorescence turn-on phenomenon. Meanwhile, it was comparable to the experimental results that the maximum absorption wavelength in simulated absorption spectra of HQ–Cu^{2+} was 327 nm. As for the simulated emission wavelength at 384 nm, it was also in good agreement with the experimental results.

Detection of Cu^{2+} in living cells

To explore the potential biological application of this sensor, we researched the capability of HQ to track Cu^{2+} in live SGC-7901 (human gastric cancer cell) cell lines. The images were obtained upon irradiation at 365 nm with a band path from 300 to 380 nm under identical exposure conditions. The living cells were first incubated with 1 mmol/L HQ in DMF for 30 min at 37 °C in 5% CO_2 atmosphere, then washed with phosphate buffered saline (PBS, pH = 7.4) 3 times, which was sufficient for the intracellular accumulation of HQ, judged from weak self-fluorescence inside the living cells (Fig. 11*a*). However, as shown in Fig. 11*b*, bright intracellular fluorescence was observed in the cells supplemented with HQ followed by adding 2 mmol/L Cu^{2+} under the same loading

Fig. 6. Partial ^{1}H NMR (300 MHz) spectral changes of HQ (10 μmol/L) in DMSO-d_6 upon addition of Cu^{2+}.

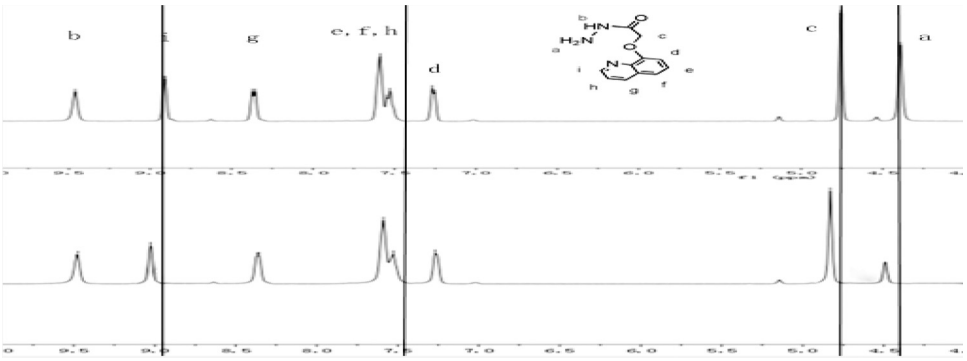


Fig. 7. Fluorescence intensities of HQ (0.1 (mmol/L)/L) in the presence of various metal ions (black bar) and competition experiment (red bar) in Tris-HCl solution [$v(C_2H_5OH)/v(H_2O)$ = 1:9, pH = 7.2] (λ_{ex} = 319 nm, λ_{em} = 384 nm).

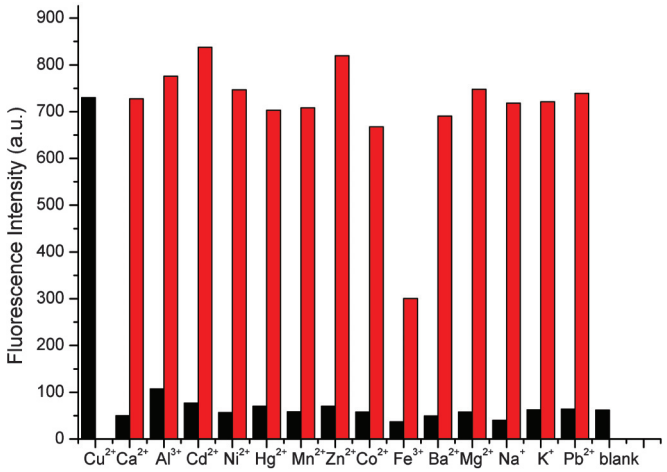
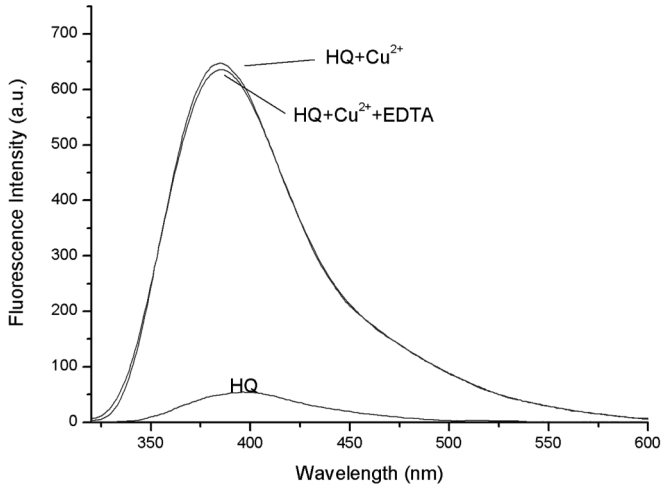


Fig. 8. Curves of fluorescence intensity change for HQ after treated by aqueous solutions of Cu^{2+} and Cu^{2+}+EDTA.



conditions. The bright-field transmission image of SGC-7901 cells incubated with HQ (1 mmol/L) were shown in (Fig. 11*c*). Fig. 11 displays the fluorescence images for the SGC-7901 stained with the sensor before and after being treated with Cu^{2+}, revealing that HQ could be a valuable molecular sensor for studying biological processes involving Cu^{2+} within living cells.

Conclusion

We have developed a simple turn-on fluorescent chemosensor for Cu^{2+} by the conjugation of quinoline, ethyl bromoacetate, and hydrazine. The titration experimental results demonstrated that HQ had excellent selectivity and sensitivity towards Cu^{2+} over other metal ions in near-aqueous media, and the specific recognition of HQ towards Cu^{2+} was supported by the DFT calculations. Moreover, the cell-permeable experiment indicated that this sensor could indeed visualize the change of intracellular Cu^{2+} in living cells. Thus, it is expected that HQ could serve as a valuable turn on fluorescent sensor in biomedical and environmental detections.

Experimental section

Materials and instruments

All the materials for synthesis were purchased from commercial suppliers and used without further purification. Solvents for spectra detection was HPLC reagent without fluorescent impurity. ^{1}H and ^{13}C NMR spectra were taken on a Varian mercury-400 spectrometer with TMS as an internal standard and DMSO as solvent. Melting point was determined using an XT-4 digital melting point apparatus. LC-MS spectra was analysed on an Agilent 1290-micro TOF QII. Fluorescence spectra measurements were performed on a Hitachi F-4500 spectrofluorimeter. The pH measurements were made with Metteler-Toledo Instruments DELTE 320 pH. Cell experiment were applied on an inverted fluorescence microscope (Olympus IX-70) connected to a digital camera (Olympus, c-5050).

UV–vis and fluorometric analysis

In titration and selectivity experiments, the stock solution of HQ was prepared in ethanol. Stock solutions of various cations were prepared in Tris-HCl buffer solution at pH 7.2, the test samples were prepared by placing appropriate amounts of ions stock into matching concentration solution of HQ [$v(C_2H_5OH)/v(H_2O)$ = 1:9, pH = 7.2]. For fluorescence measurements, excitation was provided at 319 nm, and emission was collected from 320 to 600 nm; both the excitation and emission slit widths were 5 nm and 5 nm, respectively.

Preparation of HQ

A mixture of (2.0 g, 14 mmol) 8-hydroxyquinoline and (3.8 g, 28 mmol) K_2CO_3 was added to 50 mL acetonitrile with stirring for 30 min, then (2.5 g, 15 mmol) ethyl bromoacetate was added. The mixture was kept stirred for 8 h at room temperature and then extracted it with CH_2Cl_2 and H_2O 3 times, and the organic layer was dried on $MgSO_4$ for 12 h before distilling the solvent. The crude product compound 1 was purified by column chromatography [silica gel, v(EtOAc)/v(petroleum ether) = 1:2] and obtained as red oil with a yield of 80%.[22]

Compound 1 (0.50 g, 2.2 mmol) in 5 mL MeOH was added dropwise into 1 mL hydrazine and stirred for 2 h, then filtered the

Fig. 9. Frontier molecular orbitals at ground (A) and excited (B) states of HQ and HQ-Cu^{2+}.

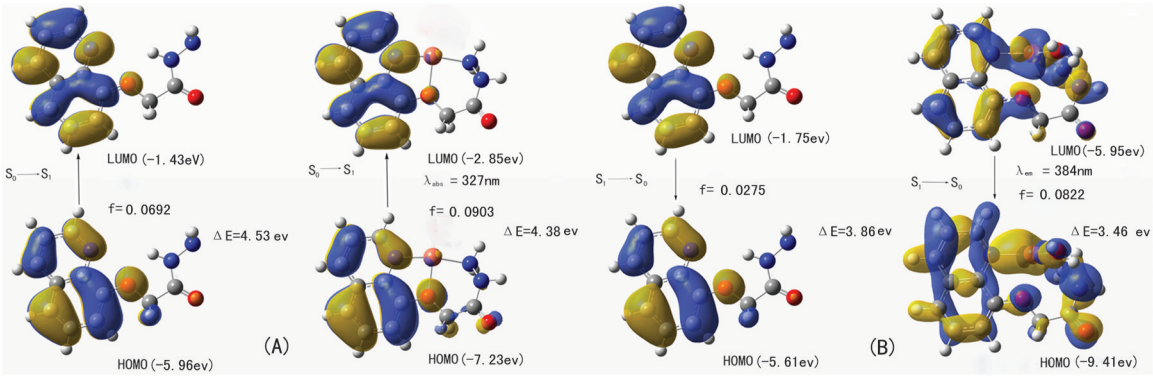


Fig. 10. Calculated energy-minimized structure of free HQ and HQ-Cu^{2+}.

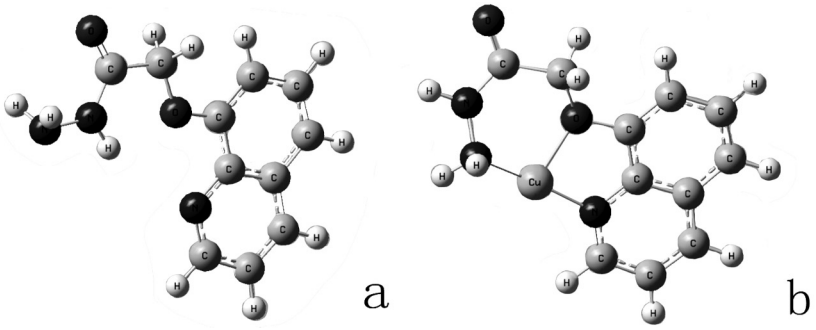
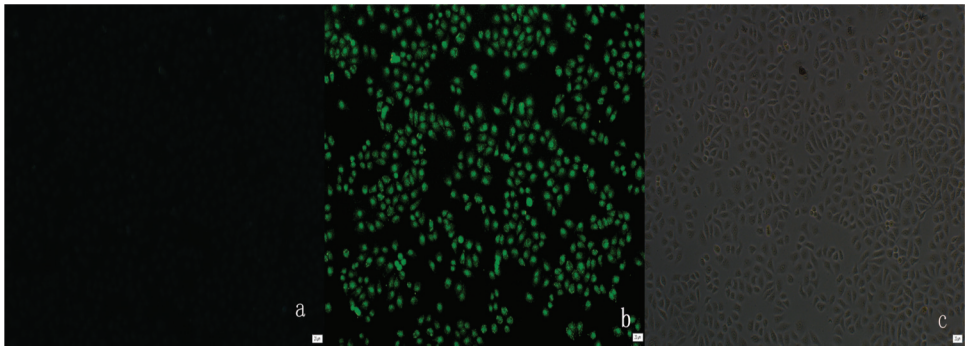


Fig. 11. Fluorescence microscope imaging of SGC-7901 cells stained with 1 mmol/L HQ before (*a*) and after treating with 2 mmol/L of Cu^{2+} (*b*). Bright-field transmission image of SGC-7901 cells incubated with HQ (1 mmol/L) are shown in (*c*).



generated precipitates and washed them with H_2O and CH_2Cl_2 3 times, respectively. The residue was purified through column chromatography [silica gel, $v(C_2H_5OH)/v(CH_2Cl_2)$ = 2:1]. The product HQ was collected as white solid with a yield of 69% (Scheme 1). ^{1}H NMR (300 MHz DMSO, 25 °C, TMS): δ 4.39 (s, 2H), 4.76 (s, 2H), 7.26 (d, J = 6.0 Hz, 1H), 7.53 (d, J = 3.0 Hz, 2H), 7.59 (s, 1H), 8.36 (d, J = 3.9 Hz, 1H), 8.91 (d, J = 1.6 Hz, 1H), 9.46 (s, 1H). ^{13}C NMR (75 MHz DMSO, 25 °C, TMS): δ 68.77, 112.19, 121.42, 122.48, 127.23, 129.6, 136.51, 140.33, 149.88, 154.42, 167.32. LC-M. calcd. $[(M+1)]^+$ m/z for: $C_{11}H_{11}N_3O_2$ = 218.09, found $[(M+1)]^+$ m/z = 218.1

Supplementary data

Supplementary data are available with the article through the journal Web site at http://nrcresearchpress.com/doi/suppl/10.1139/cjc-2014-0011.

References

(1) Bell, T. W.; Hext, N. M. *Chem. Soc. Rev.* **2004**, *33*, 589. doi:10.1039/b207182g.
(2) Wang, W.; Wen, Q.; Zhang, Y.; Fei, X. L.; Li, Y. X.; Yang, Q. B.; Xu, X. Y. *Dalton. Trans.* **2013**, *42*, 1827. doi:10.1039/c2dt32279j.
(3) Kaur, N.; Singh, P.; Kaur, S. *Tetrahedron* **2007**, *3*, 11724.
(4) Hansen, L. M.; Smith, D. J.; Reneker, D. H. *J. Appl. Polym. Sci.* **2005**, *95*, 427. doi:10.1002/app.21117.
(5) Wang, W.; Yang, Q. B.; Sun, L.; Wang, H. G.; Zhang, C. Q.; Fei, X. L.; Sun, M. D.; Li, Y. X. *J. Hazard. Mater.* **2011**, *194*, 185. doi:10.1016/j.jhazmat.2011.07.083.
(6) Li, J.; Uversky, V. N.; Fink, A. L. *Biochemistry.* **2001**, *40*, 11604. doi:10.1021/bi010616g.
(7) Karr, J. W.; Szalai, V. A. *J. Am. Chem. Soc.* **2007**, *129*, 3796. doi:10.1021/ja068952d.
(8) Jackson, R. K.; Yu, S.; Yao, X. D.; Burdette, S. C. *Dalton. Trans.* **2010**, *39*, 4155. doi:10.1039/c000248h.
(9) Loving, G.; Imperiali, B. *Bioconjugate. Chem.* **2009**, *20*, 2133. doi:10.1021/bc900319z.
(10) Benesi, H. A.; Hildebrand, J. H. *J. Am. Chem. Soc.* **1949**, *71*, 2703. doi:10.1021/ja01176a030.
(11) Barra, M.; Bohne, C.; Scaiano, J. C. *J. Am. Chem. Soc.* **1990**, *112*, 8075. doi:10.1021/ja00178a034.
(12) Zhu, M.; Yuan, M. J.; Liu, X. F.; Xu, J. L.; Lv, J.; Huang, C. S.; Liu, H. B.; Li, Y. L.; Wang, S.; Zhu, D. B. *Org. Lett.* **2008**, *10*, 1481. doi:10.1021/ol800197t.
(13) Treutler, O.; Ahlrichs, R. *J. Chem. Phys.* **1995**, *102*, 346. doi:10.1063/1.469408.

(14) Becke, A. D. *J. Chem. Phys.* **1993**, *98*, 1372. doi:10.1063/1.464304.
(15) Lee, C.; Yang, W.; Parr, R. G. *Phys. Rev. B* **1988**, *37*, 785. doi:10.1103/PhysRevB.37.785.
(16) Zhu, S. S.; Lin, W. Y.; Yuan, L. *Dyes Pigm.* **2013**, *99*, 465. doi:10.1016/j.dyepig.2013.05.010.
(17) Ma, L. J.; Cao, W. G.; Liu, J. L.; Deng, D. Y.; Wu, Y. Q.; Yan, Y. H.; Yang, L. T. *Sens. Actuators, B* **2012**, *169*, 243. doi:10.1016/j.snb.2012.04.076.
(18) Li, G. K.; Xu, Z. X.; Chen, C. F.; Huang, Z. T. *Chem. Commun.* **2008**, 1774. doi:10.1039/b800258d.
(19) Kim, C. H.; Park, J.; Seo, J.; Park, S. J.; Joo, T. J. *J. Phys. Chem. A* **2010**, *114*, 5618. doi:10.1021/jp909438p.
(20) Santra, M.; Moon, H.; Park, M. H.; Lee, T. W.; Kim, Y.; Ahn, K. H. *Chem. Eur. J.* **2012**, *18*, 9886. doi:10.1002/chem.201200726.
(21) Li, H.; Niu, L.; Xu, X.; Zhang, S.; Gao, F. *J. Fluoresc.* **2011**, *21*, 1721. doi:10.1007/s10895-011-0867-6.
(22) Chen, F. J.; Hou, F. P.; Huang, L.; Cheng, J.; Liu, H. Y.; Xi, P. X.; Bai, D. C.; Zeng, Z. Z. *Dyes Pigm.* **2013**, *98*, 146. doi:10.1016/j.dyepig.2013.01.026.