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BODIPY Fluorescent Chemosensor for Cu²⁺ Detection and Its Applications in Living Cells: Fast Response and High Sensitivity

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Abstract Copper is an essential trace element for the proper functioning of organ and metabolic process in humans. However, both its excess and deficiency in the body can result in adverse health effects. A BODIPY containing 2,2′-bipyridyl group was synthesized and used as a fluorescent chemodosimeter for selective Cu2+ detection in mild condition. This BODIPY shows fast response (~1 min) and high sensitivity for Cu2+ in aqueous solution due to the photoinduced electron transfer from the excited state of fluorophore to the bipyridyl unit complexed to Cu2+. The fluorescence quenching mechanism revealed by MALDI-TOF Mass spectra showed one Cu2+ could coordinate with two BODIPY molecules, and this coordination is reversible. This simple BODIPY dyes also could be used for sensing the Cu2+ in living cell. This work contributes to extend the potential applications of BODIPY to the biological and environmental areas.

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Introduction

Cu²⁺ plays many important roles in biological processes such as gene expression, structural enhancement of proteins, and metalloenzymatic reactions [1]. However, exposure to a high level of copper even for a short period of time can cause gastrointestinal disturbance, while long-term exposure can cause liver or kidney damage [2]. Therefore, it is highly desirable to design molecular receptors for selective and quantitative determination of Cu²⁺. Up to date, a variety of fluorometric or colorimetric Cu²⁺ receptors have been proposed [3–7]. Among which, many fluorescent chemosensors for Cu²⁺-selective detection were reported and some of them were successfully applied in biology [4, 8–16]. However, some of them show poor selectivity to Cu²⁺ over other cations such as Fe³⁺ and Zn²⁺ and lack sufficient sensitivity and require specific reaction conditions such as acidic [17] or basic media [18]. The chemosensors based on fluorescence enhancement upon binding with Cu²⁺ usually were unrecoverable and irreversible [16, 19–22]. For example, Shiraishi et al. reported that a spiropyran derivative captured Cu²⁺ to produce 1:2 Cu²⁺-amine complex intermediately. This is converted to the 1:2 Cu⁺-imine complex via oxidative dehydrogenation of amine moieties, along with the reduction of Cu²⁺ [23]. Tang's group developed a fluorescent chemodosimeter based on 1,8-naphthyridine which exhibited high selectivity to Cu²⁺. When 1-(7-acetamino-1,8-naphthyridyl)-2- (6-diacetaminopyridyl) ethene was mixed with CuCl₂, Cu²⁺ was bound to the ligand [24]. Whereas, Lee's group reported a novel coumarin-based fluorogenic probe developed as a fluorescent chemosensor with high selectivity and suitable affinity in biological systems toward Cu²⁺. And return of intracellular Cu²⁺ to the resting level could be achieved by addition of EDTA [25]. Great achievement in the field of metal ion-chemosernors has been obtained, however, there is still a demand for new indicators

with improved properties, especially fluorescent probes with high efficiency in the spectral visible region [26–29].

BODIPYs (4,4-difluoro-4-bora-3a,4a-diaza-s-indacene) as fluorescent indicators for real-time sensing and fluorescence imaging are indispensable tools in life and materials science [27, 30]. Fluorescence sensing and imaging of analytes are critically dependent on the availability of the appropriate probes. In many BODIPY-based probes, appending the chelator at the *meso*-position of the BODIPY fluorophore decouples the two subunits because of the almost perpendicular arrangement of the fluorophore and the chelator. The ratiometric BODIPY-linked azacrown ether sensor with high selectivity for potassium over other alkali ions in MeCN is the first example of a probe synthesized using aromatic substitution of 3,5-dichloro BODIPY [31]. Distyryl BODIPY derivative was also reported as a fluorescent probe for the detection of Cr^{3+} [32]. BODIPY derivative with a dipicolylaminylethylamine group was reported forming a 1:1 complex with Zn^{2+} [33]. Addition of anions (F^-, HPO_4^{2-}, OAc^-) to this 1:1 Zn^{2+}-BODIPY complex resulted in large changes in the absorption and emission spectra. The fluorescence switch is often controlled by adjusting the process of photoinduced electron transfer (PET). For example, many pH indicators contain a p-(N,N-dialkyl) aniline subunit as a pH sensitive group showing nonfluorescent in the neutral form which is attributed to reductive PET from the meso p-N,N-dimethylamino-phenyl group to BODIPY. However, protonation prevents PET so that the protonated form displays strong emission [34, 35]. BODIPY appended calix[4]arene diethyl ester shows Ca^{2+} selectivity over other metal ions [36]. Upon Ca^{2+} binding, fluorescence quenching occurs, which is attributed to oxidative PET from the BODIPY donor to the electron-deficient Ca^{2+}- bound carbonyl groups of the esters. Herein, we proposed a new Cu^{2+}-selective colorimetric chemosensor, a *meso*-(4′-methyl-[2,2′-bipyridin]- 4-yl)-BODIPY (MBDP) dye, which facilitates fluorometric detection of Cu^{2+} via coordination of bipyridyl moiety (Scheme 1). Strong fluorescence of MBDP was intermediately quenched

by addition of Cu^{2+} due to PET from the excited state of fluorophore to the bipyridyl unit. Interestingly, EDTA or cyanoborohydride ($NaBH_3CN$) could immediately resume the strong fluorescence. We also study application of this fluorescent indicator to monitor Cu^{2+} in living cells.

Results and Discussion

MBDP was synthesized by using 5-formyl-5′-methyl-2,2′-bipyridine as the bridging unit for pyrrole units with high quantum yield (86 % in methanol). And its structure was confirmed by ^1H NMR spectra and Mass spectra, and all the protons and the intensity of corresponding peaks were shown in the ^1H NMR spectra (Fig. S1). The single crystal was grown in methanol solution. Seen from its molecular structure (Fig. 1), two N atoms of bipyridyl group set at the trans-place to reduce internuclear repulsion. However, the bond C18-C19 could swing and turn to adapt to metal ions (Scheme 1).

Absorption and fluorescence spectra of MBDP were recorded in methanol at room temperature. As shown in Fig. 2a, the λ_{max} of absorption and emission are 501 and 518 nm, respectively. A comparative study of the effects of added metal cations was performed. Fe^{3+}, Zn^{2+} and Cu^{2+} showed on the fluorescence quenching. However, Cu^{2+} cation is the most effective fluorescence quencher in all tested metal cations (Fig. 2b). When 10 equiv of various metal ions (Li^+, Na^+, Mn^{2+}, Mg^{2+}, K^+, La^{3+}, Sn^{2+}, Cr^{3+}, Al^{3+}, Cd^{2+}, Bi^{3+}, Fe^{2+}, Fe^{3+}, Zn^{2+} and Cu^{2+}) in aqueous solution were added to a 20 μM methanol solution of MBDP, it was found that only Cu^{2+} produced an instant color change from green to colorless (Fig. 3a). Almost no change in color was observed with the addition of the other metal ions. The changes in the absorption spectra of MBDP as a function of addition of Cu^{2+} are shown in Fig. 3a. Intensity of absorption at 501 nm was gradually decreased with bathochromic shift after adding of Cu^{2+} ions. This red-shift was ascribed to the addition of water [37, 38]. However, water didn't cause turbidity, which indicated that MBDP or copper complex accommodated the mixed solvent. Absorption intensity was decreased linearly with addition of Cu^{2+}. Until mole ratio of MBDP/Cu^{2+} was 2:1, the intensity had been kept at a numerical value. Compared to other probes, such as quinone derivatives which are relevant to redox reaction, MBDP relies on coordination [39, 40].

To further study the fluorescence-sensing behavior of MBDP, a quantitative investigation of the binding affinity of MBDP with Cu^{2+} was studied by fluorescence titration (Fig. 3b). When the receptor MBDP was titrated with Cu^{2+}, the fluorescence intensity decreased until fluorescence was completely quenched. With the addition of Cu^{2+}, the fluorescence intensity at λ_{max}=518 nm was of a linear decrease, then invariant at zero (Fig. 3b and c). Fluorescence quenching process usually includes dynamic and static quenching.
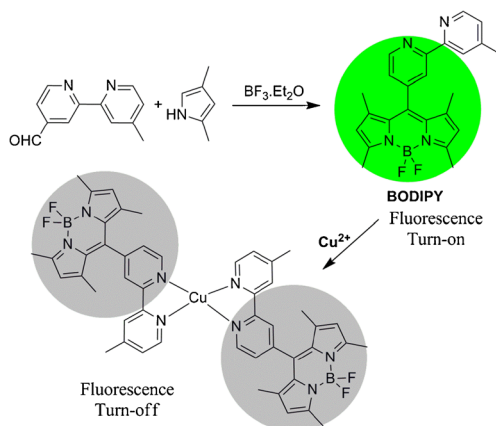
Scheme 1 Synthesis of MBDP and its fluorescence sensing for Cu^{2+}

Fig. 1 Molecular structure of MBDP
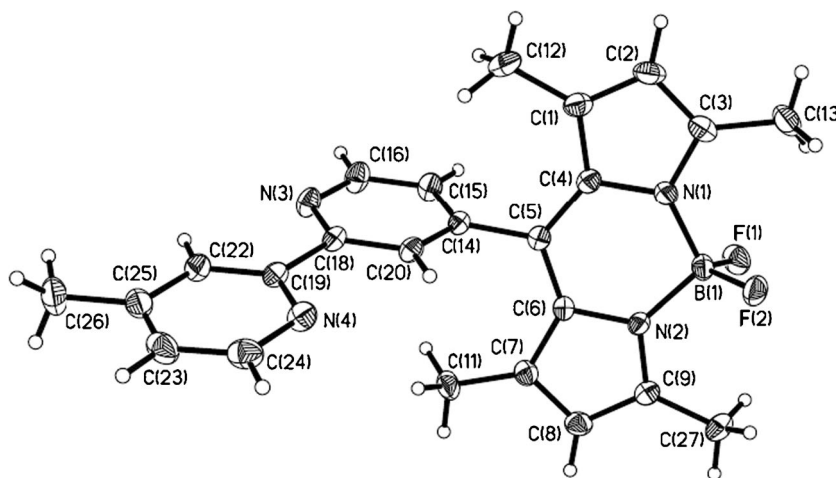
Dynamic quenching process accords to Eq. 1, and static quenching process according to Eq. 2.

$$I_0/I = 1 + K_{SV}[M] \qquad (1)$$

$$\log(I_0/I)/I = \log K_S + n \log[M] \qquad (2)$$

(K_{SV} is the constant of dynamic quenching. K_S is the constant of static quenching. [M] is the metal ion molar concentration.) In our experiments, the experimental results kept to neither the linear Eq. 1 nor Eq. 2, but in line with the $\log(I_0/I) = A+B$ (Fig. S2), indicating quenching process should be joint action of dynamic and static quenching. As shown in Fig. 3c, the quenched fluorescence intensity vs [Cu^{2+}] plot can be curve-fitted into $\frac{I_0-I}{I_0} = 0.11[Cu^{2+}] + 0.10$, where I_0 and I are the luminescent intensity before and after metal ion incorporation, respectively; [Cu^{2+}] is the metal ion molar concentration; and 0.11 is the slope, in a sense, which is on behalf of the fluorescence quenching rate.

Interestingly, when an excess amount of strong chelating agent EDTA or strong reducing agent sodium cyanoborohydride ($NaBH_3CN$) were added to the colorless fluorescent quenched solution by Cu^{2+}, the fluorescence and color immediately resumed but then quenched again by addition of an excess of Cu^{2+} (Fig. S3), implying the reversible coordination binding of Cu^{2+} to MBDP. Furthermore, the matrix assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) analysis of a colorless solution for the capture of MBDP with Cu^{2+} shows a peak at m/z 895.3 (calcd, 895.3), which is assigned to the formation of the 2MBDP-imine-Cu^{2+} (2BC) complex (Fig. 4). PET is often the cause of fluorescence quenching, when the PET process is followed by a nonluminescent process returning to the ground state [41]. The intense green fluorescence of MBDP is quenched by oxidative PET from the excited-state fluorophore to the bipyridyl moiety coordinated to Cu^{2+}. Cu^{2+} formed the most Stable 1:2 complex with MBDP. In addition, other peaks at m/z 981.1 and 914.3 are assigned to 2BC+Na^++NO_3^- and 2BC+F^-. This suggests possible interaction of fluoride with a sodium cation which may be true even in the reaction of $NaBH_3CN$ while reacting with the copper complex. And the peak at m/z 729.7 is assigned to MBDP-Cu^{2+}-Two methyl pyridine possibly due to bond
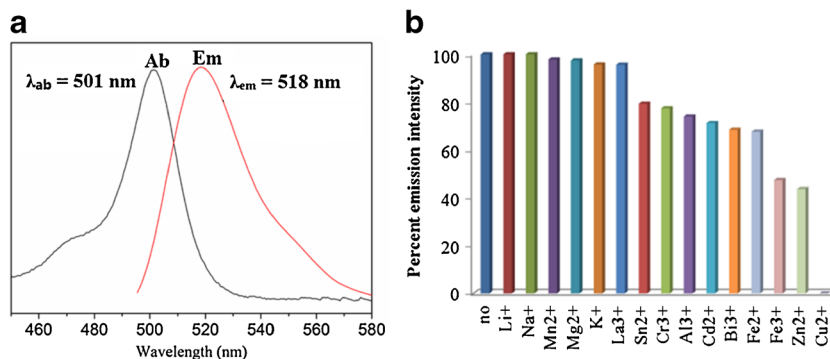
Fig. 2 **a** Absorption and emission (λ_{ex}=480 nm) of MBDP in methanol at 2×10^{-5} mol L^{-1}; **b** Fluorescence emission intensity of MBDP (2×10^{-5} mol L^{-1}) in methanol, which contains 10 equiv of various cations, as a percentage of the emission intensity of the free MBDP. Free MBDP emission intensity was recorded at 518 nm; excitation was at 480 nm

$$\frac{I_0 - I}{I_0} = 0.11[Cu^{2+}] + 0.10$$
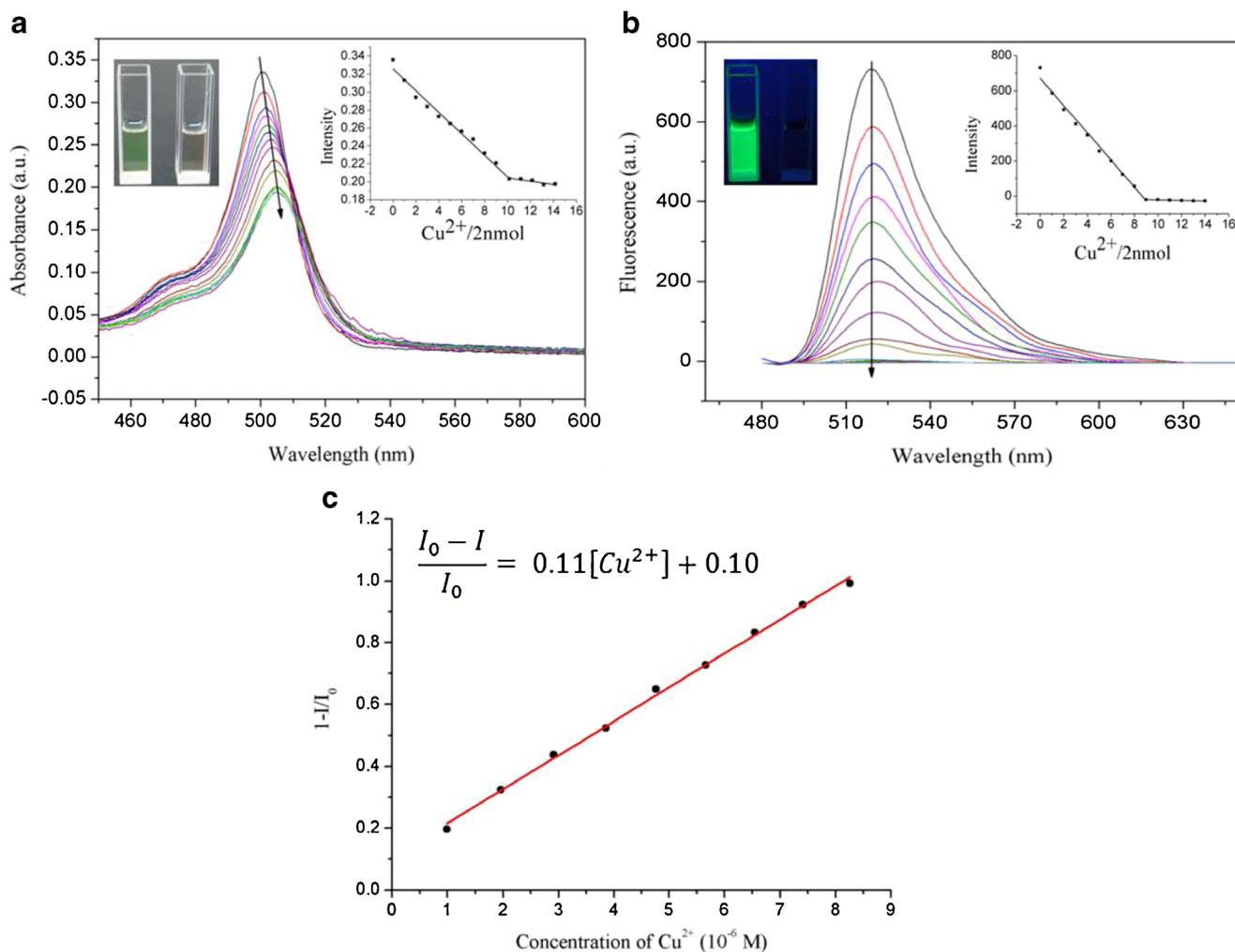
Fig. 3 Fluorescence of MBDP in methanol (2×10^{-5} mol L^{-1}, 2.0 ml) was quenched by successive addition of Cu^{2+} aqueous soultion (10^{-4} mol L^{-1}, 20 µl). **a** Absorbance. Insert: linear relation between N_{Cu2+} and intensity at λ_{max}, and photographs of free MBDP (*left*) and MBDP-Cu^{2+} (*right*) under natural light; **b** Fluorescence change according to the addition of Cu^{2+}. Insert: linear relation between N_{Cu}^{2+} and intensity at 518 nm, and photographs of free MBDP (*left*) and MBDP-Cu^{2+} (*right*) under a 365 nm UV lamp; **c** The curve-fitted equation of MBDP quenched by $CuCl_2$ aqueous solution

breaking of C5-C14 (Fig. 1a). The stoichiometry of the interaction is 1:2 for the MBDP and Cu^{2+}. The fluorescence quantum yields of receptor MBDP in the absence and presence of Cu^{2+} were 0.86 and <0.01 (compared with quinine), respectively. Moreover, Cu^{2+} salts with different counteranions such as $Cu(CH_3COO)_2$, $CuCl_2$, $Cu(NO_3)_2$ $CuSO_4$ show similar spectral change (Fig. S4). To further check the practical applicability of receptor MBDP as Cu^{2+}-selective fluorescent sensor, we carried out competition experiments. When MBDP was treated with 1 equiv of Cu^{2+} in the presence of other metal ions of the same concentration, the fluorescence decrease caused by Cu^{2+} was retained with Li^+, Na^+, Mn^{2+}, Mg^{2+}, K^+, La^{3+}, Sn^{2+}, Cr^{3+}, Al^{3+}, Cd^{2+}, Bi^{3+}, Fe^{2+}, Fe^{3+}, Zn^{2+} and Cu^{2+} (Fig. S5).

The application in living cells for Cu^{2+} sensing was studied using HepG2 (liver cancer cells of human) cell. First, stock solutions of MBDP were prepared in H_2O/DMSO solution (9/1, v/v). To determine the cell permeability of MBDP,

HepG2 cells were incubated with MBDP (50 µM) for 15 min at 37 °C, and washed with PBS to remove the remaining MBDP. The results are shown in Fig. 5a. It is obvious that the MBDP could readily penetrate cell membranes and preferred to accumulate in the cytoplasm. As we all know, excessive intake of Cu^{2+} will cause physical discomfort [2]. As shown in Fig. 5b, (Fig. 5a and d). The biosensing process was monitored through time driver. As shown in Fig. 5d-I, HepG2 cells incubated with MBDP initially display a fast and strong fluorescence quenching. Interestingly, addition of EDTA resumes the fluorescence in one minute (Fig. 5c).

Conclusions

Copper is an essential trace element for the proper functioning of organ and metabolic process in humans. However, both its
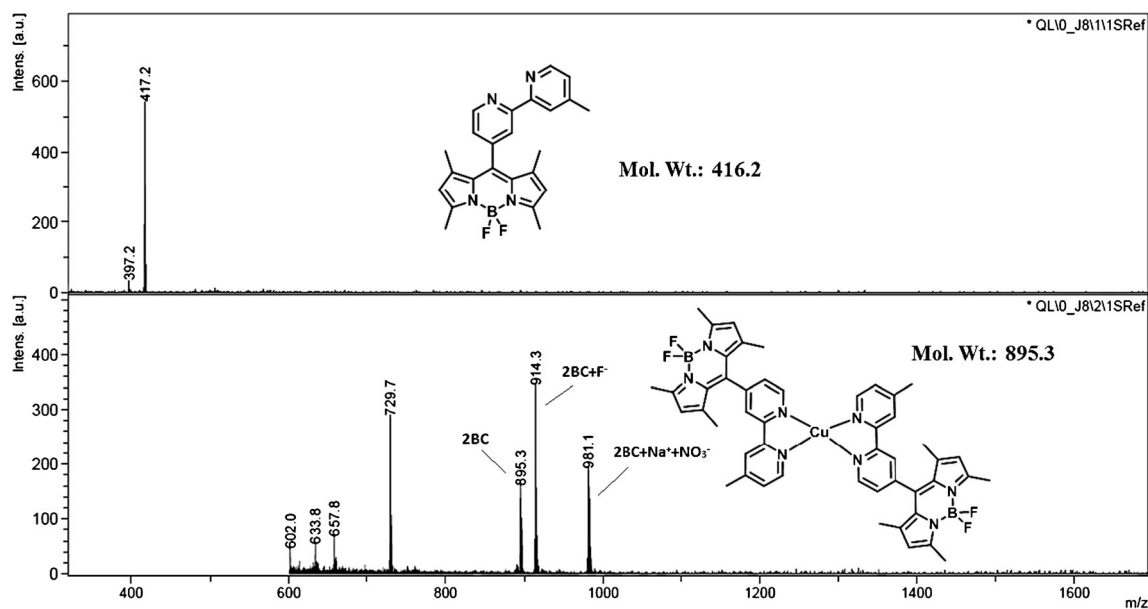
Fig. 4 MALDI-TOF MS of MBDP and capture of MBDP with Cu²⁺

excess and deficiency in the body can result in adverse health effects. A MBDP containing 2,2′-bipyridyl group was synthesized and used as a fluorescent chemodosimeter for selective Cu²⁺ detection in mild condition. This MBDP shows fast response (~1 min) and high sensitivity for Cu²⁺ in aqueous solution due to the photoinduced electron transfer from the excited state of fluorophore to the bipyridyl unit complexed to Cu²⁺. The fluorescence quenching mechanism revealed by MALDI-TOF Mass spectra showed one Cu²⁺ could coordinate with two MBDP molecules, and this coordination is
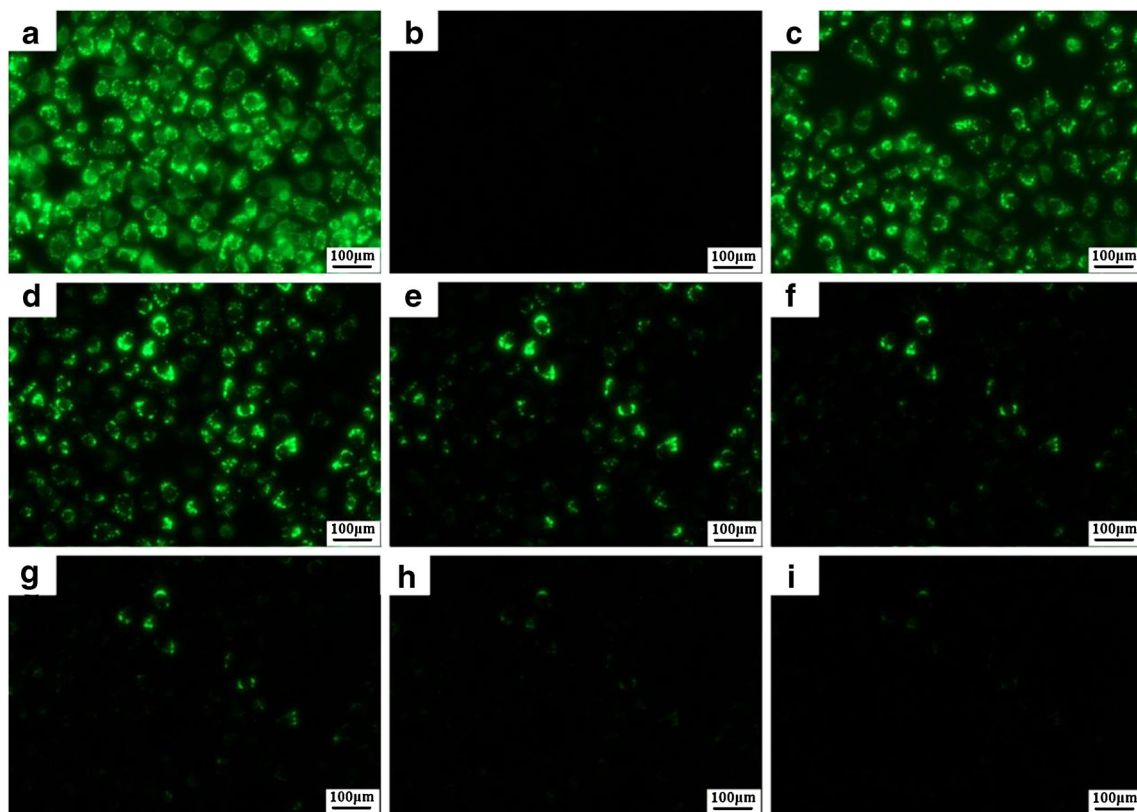
Fig. 5 Fluorescence images in HepG2 cells (Nikon Eclipse Ti, 20× objective lens). **a** Incubated with MBDP (50 μM) for 15 min; **b** Further incubated with addition of CuCl₂ (20 equiv) for 14 min; **c** Return of fluorescence by addition of EDTA (500 μM); **d–i** Time driver about fluorescence quenching by incubation with CuCl₂ (20 equiv): **d** for 2 min; **e** for 4 min; **f** for 6 min; **g** for 8 min; **h** for 10 min; **i** for 12 min

reversible. This simple MBDP dyes also could be used for sensing the Cu^{2+} in living cell. This work contributes to extend the potential applications of MBDP to the biological and environmental areas.

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