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ORIGINAL PAPER

A dual-channel chemosensor based on rhodamine and BODIPY conjugated dyad for ratiometric detection of Hg^{2+} and fluorescence on–off recognition of Cu^{2+} in aqueous solution and living cells

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Abstract

A novel probe **RB** for discriminative detection of Hg^{2+} and Cu^{2+} constructed by rhodamine and BODIPY was designed and synthesized. Probe **RB** consists of three parts: BODIPY as the energy donor, rhodamine as the energy receptor, and thiosemicarbazide as the reacting site. Probe **RB** selectively binds with Hg^{2+} and Cu^{2+} ions in in EtOH/H_2O (V/V = 1:1) among tested metal ions. Probe RB exhibited a fluorescence red shift from 513 to 593 nm for Hg^{2+} and a fluorescence quenching for Cu^{2+}. The mechanism study showed that the fluorescence changes of probe **RB** after the addition of Hg^{2+} was due to oxadiazole formation when the thiosemicarbazide moiety was liberated by Hg^{2+}-facilitated ring opening of the spirocyclic group, while Cu^{2+} not only opened the spirolactam ring, but also bonded to the opening product through 1:2 mode. It is worth noting that all these types of probes could only detect Hg^{2+} or Cu^{2+} through metal ions-facilitated ring opening of the spirocyclic group mechanism. To the best of our knowledge, probe **RB** was firstly reported to detect Hg^{2+} and Cu^{2+} discriminatively. Moreover, probe **RB** demonstrated excellent sensitivity toward Hg^{2+} (LOD 8.36 nM) and Cu^{2+} (LOD 0.16 μM), fast responsive time (within 30 s) and wide pH range (6.0–10.0 for Hg^{2+}, 6.0–11 for Cu^{2+}). This probe was further successfully applied to Hela cells and could easily discriminate Hg^{2+} and Cu^{2+} through double-channel imaging.

Keywords Rhodamine · BODIPY · Hg^{2+} and Cu^{2+} discriminative detection · Cell imaging

Introduction

Heavy metal pollutants have attracted much attention because of their harm to environments and human health. Therefore, selective detection of heavy metal pollutants, especially using fluorescent probes, is currently under in-depth research (Lian et al.2020; Nagarajan et al. 2021; Duong and Kim 2010). Among them, Cu^{2+} and Hg^{2+} ions as two common metal pollutants have been fully researched due to their wide application and high toxicity. Cu^{2+} is closely related to the hematopoietic function of human body. However, when a large number of Cu^{2+} ions are retained in the human body, it is easy to cause a burden to various organs in the body, especially the liver and gallbladder. When problems occur in these two organs, the maintenance of the metabolism in the human body will appear disorder (Hosseini et al. 2014; Zhao et al.2018). While its wide application in infrastructure constructions, power generation, as well as in the production of electronic products and equipment, industrial machinery and transport vehicles, the level of Cu^{2+} pollution in the environment is constantly increasing. Hence, it is of great significance to develop fluorescent probe for Cu^{2+} detection.

Unlike Cu^{2+}, Hg^{2+} is considered to be one of the most toxic heavy metal ions to human health because it can coordinate with many negatively charged groups in enzymes or proteins, such as sulfhydryl groups, which affect many metabolic pathways in cells, such as energy generation, protein and nucleic acid synthesis, thus further affecting cell function and growth of cells (Tetsuro et al. 2005). Moreover, Hg^{2+} ions could easily pass through membranes and induce

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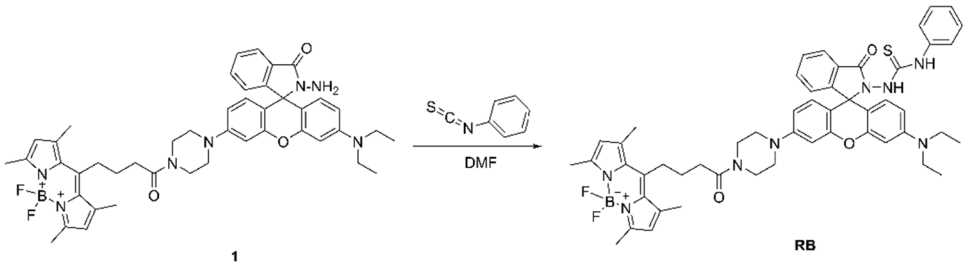
Scheme 1 Synthesis route of probe **RB**



massive damage to central nervous system and organs, such as brain and kidney. Therefore, it is important to develop probes which could detect trace amounts of Hg^{2+} ions in living cells.

Currently, most reported fluorescent probes could only detect Cu^{2+} or Hg^{2+} (Zhu et al. 2021; Slassi et al. 2021; Du et al. 2021; Zhang et al. 2021; Yang et al. 2013, 2021; Culzoni et al. 2012; Chen et al. 2020, 2019; Li et al. 2020; Petdum et al. 2020; Liu et al. 2020a, b; Hu et al. 2020; Lin et al.2020), the fluorescent probes which could discriminately detect two metal ions through different spectra changes were rarely developed, especially in living cells (Huang et al.2013, 2019; Divya and Thennarasu 2020; Shi et al. 2019). Some of reported dual-signal fluorescence sensors have shortcomings in practical applications, such as the indistinguishability of Hg^{2+} and Cu^{2+} ions, long response time, low sensitivity and interference from other metal ions. Therefore, it is still needed to develop dual-signal fluorescence sensors which could discriminately detect Hg^{2+} and Cu^{2+} ions with large spectral differences.

Rhodamine and BODIPY dyes are widely used in the construction of fluorescent probes due to their excellent spectral properties, such as long absorption and emission wavelength, high fluorescence quantum yield, high extinction coefficient and excellent photostability (Zhang et al. 2020, 2016; Zhang and Wong 2020; Beija et al. 2009; Nguyen et al. 2021; Kaur and Singh 2019; Kowada et al. 2015). Most reported rhodamine probes for Hg^{2+} detection undergo a transformation from the spirocyclic type to the open-loop amide type, whose fluorescence properties are completely different. The spirocyclic (closed loop) form is basically non-fluorescent, while the open-loop form produces strong fluorescence emission. Based on this mechanism, some chemical sensors for Hg^{2+} and Cu^{2+} detection have been developed (Zhang and Zhang 2014; Saleem and Lee 2014; Chen et al. 2020). Among them, a series of ratiometric fluorescent probes by fluorescence resonance energy transfer (FRET) mechanism have been reported. Previously, our group has synthesized a FRET probe based on BODIPY- rhodamine system (Compound **1**, Scheme 1), which shows high selectivity toward Hg^{2+} [Wen et al. 2021]. While all these types of probes could only detect Hg^{2+} or Cu^{2+} (Table S1). Recently, our group found a novel FRET probe system with thiosemicarbazide moiety (probe **RB**, Scheme 1) could discriminatively detect Hg^{2+} and Cu^{2+} with excellent sensitivity. Most importantly, probe **RB** was successfully applied to Hela cells and could easily discriminate Hg^{2+} and Cu^{2+} through double-channel imaging. To the best of our knowledge, this type of probe was firstly reported to discriminatively detect Hg^{2+} and Cu^{2+} through different mechanisms.

Experimental section

Materials and apparatus

All the materials used for the synthesis of probe **RB** and analytical experiments were purchased from Sigma-Aldrich without further purification. EtOH in HPLC grade purity and redistilled water were used in all analytical experiments. PerkinElmer Lambda 25 and HITACHI F-4600 Fluorescence spectrophotometer were used for absorption and fluorescence spectra measuring. Bruker Advance 400 MHz spectrometer was used for ^{1}H-NMR and ^{13}C-NMR recording. HRMS data were obtained from an SCIEX TripleTOF 5600 + high resolution spectrometer (American). Leica TCS SP8 Confocal Laser Scanning Microscope was used to obtain the fluorescence images of living cells.

Synthesis of probe RB

Compound **1** (0.3 g, 0.4 mmol) and phenyl isothiocyanate (0.2 mL, 1.3 mmol) were dissolved in dry DMF (5 mL), the mixture was stirred overnight at room temperature. After completion of the reaction, the solvent was removed by a rotary evaporator under reduced pressure to give a red residue, which was purified directly by gel column chromatography (CH_2Cl_2/MeOH = 50/1) to give a red solid (0.33 g, 0.36 mmol) in 90% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.04 (d, J = 7.9 Hz, 1H), 7.69–7.60 (m, 2H), 7.52 (s, 1H), 7.18 (t, J = 7.5 Hz, 2H), 7.08 (dd, J = 19.0, 7.4 Hz, 3H), 6.90 (s, 1H), 6.68 (s, 1H), 6.53–6.46 (m, 4H), 6.33–6.31 (m, 1H), 6.06 (s, 2H), 3.79–3.75 (m, 2H), 3.62–3.58 (m, 2H), 3.44–3.26 (m, 4H), 3.21–3.20 (m, 4H), 3.13–3.00 (m, 2H), 2.51 (s, 6H), 2.46 (s, 6H), 2.15–1.91 (m, 2H), 1.66 (s,

3H), 1.16 (t, $J = 7.0$ Hz, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.28, 167.02, 154.10, 153.99, 125.12, 145.52, 140.46, 137.58, 134.46, 131.61, 129.36, 129.08, 128.34, 127.73, 126.12, 124.88, 124.70, 124.05, 121.81, 112.30, 103.23, 66.83, 48.27, 48.11, 45.19, 44.46, 41.25, 32.68, 27.57, 26.99, 16.51, 14.49, 12.51. HRMS (m/z): calculated for $C_{52}H_{56}BF_2N_8O_3S$ $[M+H]^+$, 921.4257; found, 921.4333.

Absorption and fluorescence spectra measurement procedure

The stock solution of probe **RB** (1 mM) was prepared with EtOH in HPLC grade purity and stored in refrigerator at 4 °C. All the tested metal ions (Ag^+, Ba^{2+}, Ca^{2+}, Cd^{2+}, Co^{2+}, Cu^{2+}, Fe^{3+}, K^+, Hg^{2+}, Mg^{2+}, Mn^{2+}, Na^+, Ni^+ and Zn^{2+}, 10 mM) were prepared in ultrapure water. The UV–vis and fluorescence spectra were recorded in the mixture solution EtOH/H_2O (V/V = 1:1) at room temperature.

Cell imaging

Hela cells were cultured in Dulbecco's modified Eagle medium (DMEM), which contains 10% Fetal Bovine Serum (FBS) in two days. Then Hela cells were firstly treated with probe **RB** (5 μM**,** PBS containing 1% DMSO), which were incubated over 30 min at 37 °C. Then the Hela cells were washed with phosphate buffer saline (PBS) (1 mL × 3). The treated cells were further incubated with Cu^{2+} or Hg^{2+} (5 μM**,** PBS containing 1% DMSO) over 30 min. After removing the culture solvent, the cells were washed with PBS for three times. The fluorescence images of living cells were captured in PBS with Leica TCS SP8 Confocal Laser Scanning Microscope, upon excitation at 488 nm. Emissions were collected with green channel (513 ± 15 nm) and red channel (593 ± 15 nm).

Results and discussion

Probe RB synthesis

The procedures of probe **RB** synthesis are depicted in Scheme 1. Probe **RB** was obtained by the reaction of phenyl isothiocyanate with compound **1** in 90% yield. Probe **RB** was fully characterized by ^{1}H NMR, ^{13}C NMR and HRMS data, which were offered in in the Experimental Section. The spectra of ^{1}H NMR, ^{13}C NMR and HRMS were provided in the supporting information.

Studies of spectral response of probe RB to different metal ions

With probe **RB** in hand, we firstly investigated its sensing ability toward several metal ions (Ag^+, Ba^{2+}, Ca^{2+}, Cd^{2+}, Co^{2+}, Cu^{2+}, Fe^{3+}, K^+, Hg^{2+}, Mg^{2+}, Mn^{2+}, Na^+, Ni^+ and Zn^{2+}) in the mixture solution EtOH/H_2O (V/V = 1:1) by absorption and fluorescence spectra. As shown in Fig. 1a, probe **RB** displays an absorption band around 499 nm, which is the characteristic absorption band of BODIPY. Addition of Cu^{2+} or Ag^+ led to partial decrease in absorption band around 499 nm, accompanied by the appearance of a new band around 564 nm, which was attributed to the typical absorption spectrum of rhodamine group. While with the addition of Hg^{2+}, the absorption spectrum of open-loop rhodamine appeared immediately. The above phenomenon indicated that probe **RB** interacted with Cu^{2+}/Hg^{2+}/Ag^+, further enhancing the conjugation degree of **RB**-Cu^{2+}/Hg^{2+}/Ag^+ system, resulting in the red shift of **RB** absorption spectrum. In addition, probe **RB** is slight pink in the mixed solution of EtOH/H_2O (V/V = 1:1). When Hg^{2+} was added, its color changed from pink to purple, while the addition of Cu^{2+} or Ag^+ induced color

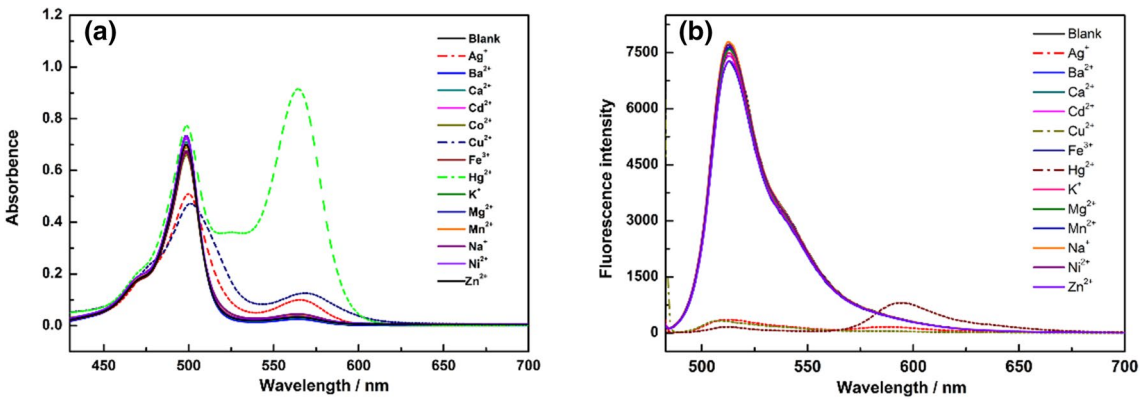


Fig. 1 **a** Absorption spectra of probe **RB** (10 μM) with different metal ions (100 μM) in the mixture solution EtOH/H_2O (V/V = 1:1). **b** fluorescence spectra of probe **RB** (10 μM) with different metal ions (100 μM) in the mixture solution EtOH/H_2O (V/V = 1:1)

changed from pink to lavender, indicating that probe **RB** can detect Hg^{2+} by naked eye (Fig. 2a). Moreover, the fluorescence spectra of probe **RB** in the presence of different metal ions were also measured. As shown in Fig. 1b, probe **RB** exhibited an emission band around 513 nm upon excitation at 480 nm. With addition of Cu^{2+}, its fluorescence was almost quenched completely; while addition of Hg^{2+} induced the disappearance of emission band at 513 nm, along with the appearance of a new emission band centered at 593 nm. As shown in Fig. 2b, emission color changed from green to blank or red were easily observed by addition of Cu^{2+} or Hg^{2+} through naked eye under the illumination with a 365 nm UV-lamp. In addition, Ag^{+} ions had a slight effect, because it could also induce the fluorescence quenching of BODIPY, with the appearance of a new weak emission band centered at 593 nm. However, it has much longer equilibrium time (10 min, Fig. S1) than that of Hg^{2+} ions (30 s).

Fluorescence titrations of probe **RB** with different amounts of Cu^{2+} or Hg^{2+} were carried out in the mixture solution EtOH/H_2O (V/V = 1:1). As depicted in Fig. 3a, probe **RB** showed a fluorescence emission band centered at 513 nm, which was decreased gradually and remained unchanged after addition of 8 Mμ Cu^{2+}. Plot of fluorescence intensity changes at 513 nm as a function of concentrations of Cu^{2+} (0–2.5 Mμ) exhibited an excellent linearity ($R^2 = 0.99277$). While upon progressive addition of Hg^{2+} (0–20 Mμ), fluorescence intensity at 513 nm decreased, along with a new fluorescence emission band around 593 nm appeared and increased gradually (Fig. 3b). In addition, the fluorescence titration curve showed that the ratio of fluorescence intensity of different emission band ($I_{593\ nm}/I_{513\ nm}$) increased dramatically with the increasing concentrations of Hg^{2+} and reached a plateau with addition of 20 μM Hg^{2+}. Plot of $I_{593\ nm}/I_{513\ nm}$ changes of probe **RB** as a function of concentrations of Hg^{2+} (5–15 μM) exhibited an excellent

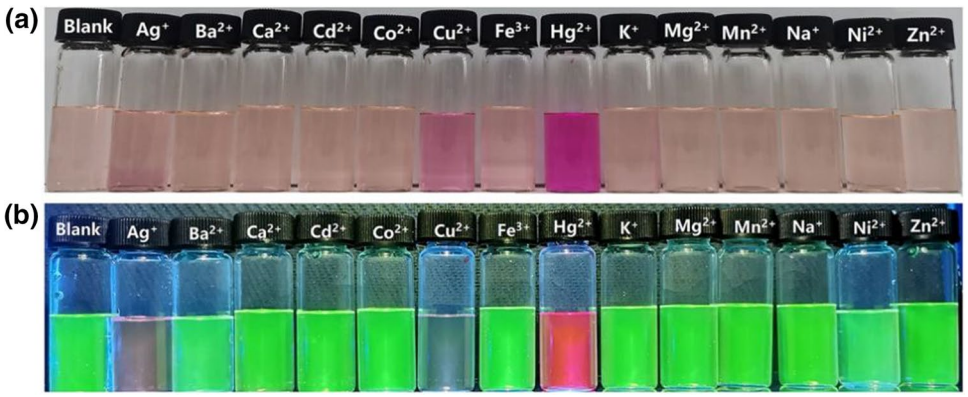
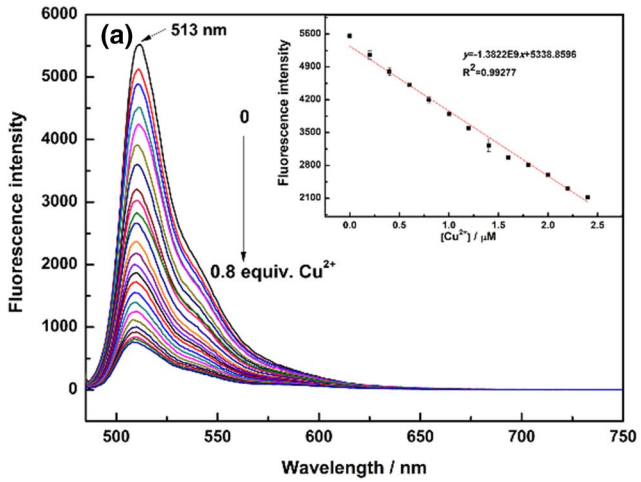


Fig. 2 **a** The color changes of probe **RB** (10 μM) with different metal ions (100 μM) in the mixture solution EtOH/H_2O (V/V = 1:1) under day light. **b** The color changes of probe **RB** (10 μM) with different metal ions (100 μM) in the mixture solution EtOH/H_2O (V/V = 1:1) under 365 nm UV-lamp



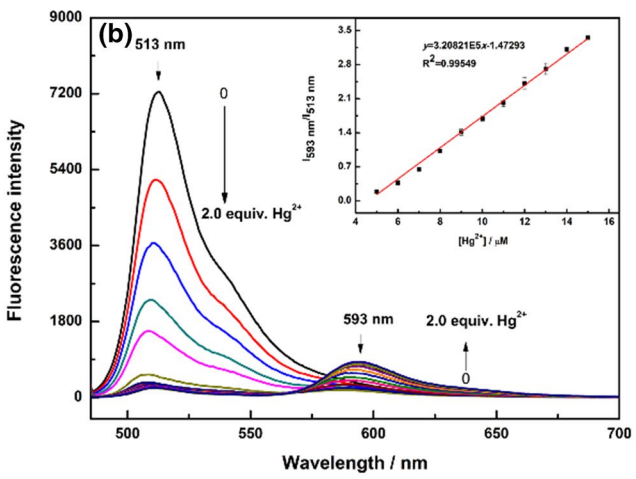


Fig. 3 **a** The fluorescence spectra changes of probe **RB** (10 μM) with increasing concentration of Cu^{2+} (0–8 μM) in EtOH/H_2O (V/V = 1:1). Insert: plot of $I_{513\ nm}$ changes of probe **RB** (10 μM) as a function of Cu^{2+} concentration (0–2.5 μM). **b** The fluorescence spectra changes of probe **RB** (10 μM) with increasing concentration of Hg^{2+} (0–20 μM) in EtOH/H_2O (V/V = 1:1). Insert: plot of $I_{593\ nm}/I_{513\ nm}$ changes of probe **RB** (10 μM) as a function of Hg^{2+} concentration (5–15 μM)

linearity ($R^2 = 0.99549$). The detection limits of probe **RB** to Cu^{2+} and Hg^{2+} were calculated to be 0.16 μM (0.01 ppm) and 8.36 nM (1.68 ppb) (SI), respectively, which were lower than the maximum permissible amount of Cu^{2+} (1.3 ppm) and Hg^{2+} (2.0 ppb) in drinking water that proposed by USA Environmental Protection Agency (EPA).

Moreover, in order to further confirm the sensing ability of probe **RB** to Cu^{2+} and Hg^{2+} in complicated environments, competition experiments of probe **RB** for Cu^{2+} and Hg^{2+} detection were measured through adding the above-mentioned tested metal ions, respectively. As shown in Fig. 4a, the fluorescence intensity of probe **RB** with tested metal ions (Ba^{2+}, Ca^{2+}, Cd^{2+}, Co^{2+}, Fe^{3+}, K^+, Mg^{2+}, Mn^{2+}, Na^+, Ni^+ and Zn^{2+}) remained basically unchanged, while Cu^{2+} addition resulted a ca. 96% quenching of fluorescence at 513 nm. In addition, Ag^+ could lead to a circa (ca.) 91% quenching of fluorescence at 513 nm, while the fluorescence intensity was further decreased to ca. 96%, following addition of Cu^{2+}. Moreover, $I_{593\ nm}/I_{513\ nm}$ was significantly increased with addition of Hg^{2+} in presence of other tested ions (Ag^+, Ba^{2+}, Ca^{2+}, Cd^{2+}, Co^{2+}, Fe^{3+}, K^+, Mg^{2+}, Mn^{2+}, Na^+, Ni^+ and Zn^{2+}), indicating excellent anti-interference ability of probe **RB** for the detection of Cu^{2+} and Hg^{2+}. In addition, we also investigated whether Cu^{2+} and Hg^{2+} interfered with each other (Figs. S2, S3). The results showed that when Cu^{2+} was firstly added into the solution of probe **RB** in EtOH/H_2O (V/V = 1:1), it induced a ca. 90% quenching of fluorescence at 513 nm, and the fluorescence intensity at 513 nm was further decreased, along with the appearance of a new emission band centered at 593 nm after another addition of Hg^{2+}. While when Hg^{2+} was firstly added into the solution of probe **RB** in EtOH/H_2O (V/V = 1:1), it led to the appearance of a new emission band centered at 593 nm, and then another addition of Cu^{2+}had no effect on the new emission band. The above results indicated that the probe **RB** for detecting Hg^{2+} was not disturbed by other measured ions, while for detecting Cu^{2+} was interfered by Hg^{2+}.

Sensing mechanism study

According to the literature (Yang et al. 2005; Ko et al. 2006; Wang et al. 2015; Li et al. 2019; Zhu et al. 2020; Ji et al. 2017), rhodamine fluorophore containing thiosemicarbazide group usually shows no fluorescence, and it could be easily transformed into 1,3,4-oxadiazole by Hg^{2+}-promoted cyclization reaction. Therefore, we assume that probe **RB** with thiosemicarbazide group shows green fluorescence of BODIPY upon excitation at 480 nm, which is due to no FRET process. When Hg^{2+} facilitated the cyclization reaction of thiosemicarbazide to form oxadiazole, the FRET was switched on, accompanied by fluorescence emission of rhodamine appearance upon excitation at 480 nm. To verify this hypothesis, we carried out ^{1}H NMR titration and HRMS experiments. The ^{1}H NMR titration experiment was carried out in $(CD_3)_2SO$, due to its high solubility for probe **RB.** Moreover, the response of probe **RB** to Hg^{2+} in $(CD_3)_2SO$ was the same as that in EtOH/H_2O (V/V = 1:1). As shown in Fig. 5, the peaks at 8.86 (s, 1H) and 9.40 (s, 1H) are the protons of amine 1 and amine 2, addition of Hg^{2+} resulted the disappearance of proton 1 and downfield shift to 10.63 of proton 2. Moreover, the protons of rhodamine group showed a downfield shift and the protons of the pyrrole group on BODIPY kept the same at 6.23 (s, 2H) upon addition of Hg^{2+}. These results clearly showed that thiosemicarbazide group could be transformed into oxadiazole by Hg^{2+} addition. In addition, the HRMS spectrum of probe **RB** with Hg^{2+} was recorded. The peak at m/z 887.4457 corresponded to the calculated m/z at 887.4375 for rhodamine containing

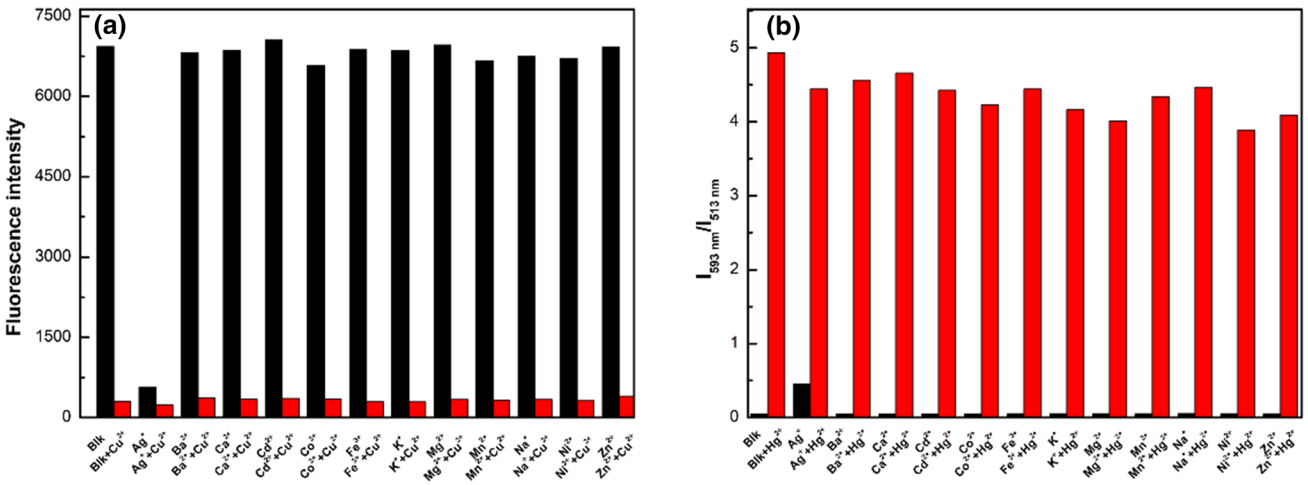


Fig. 4 **a** $I_{513\ nm}$ of probe **RB** (10 μM) toward Cu^{2+} in presence of other metal ions (100 μM) in EtOH/H_2O (V/V = 1:1). **b** $I_{593\ nm}/I_{513\ nm}$ of probe **RB** (10 μM) toward Hg^{2+} in presence of different metal ions (100 μM) in EtOH/H_2O (V/V = 1:1)

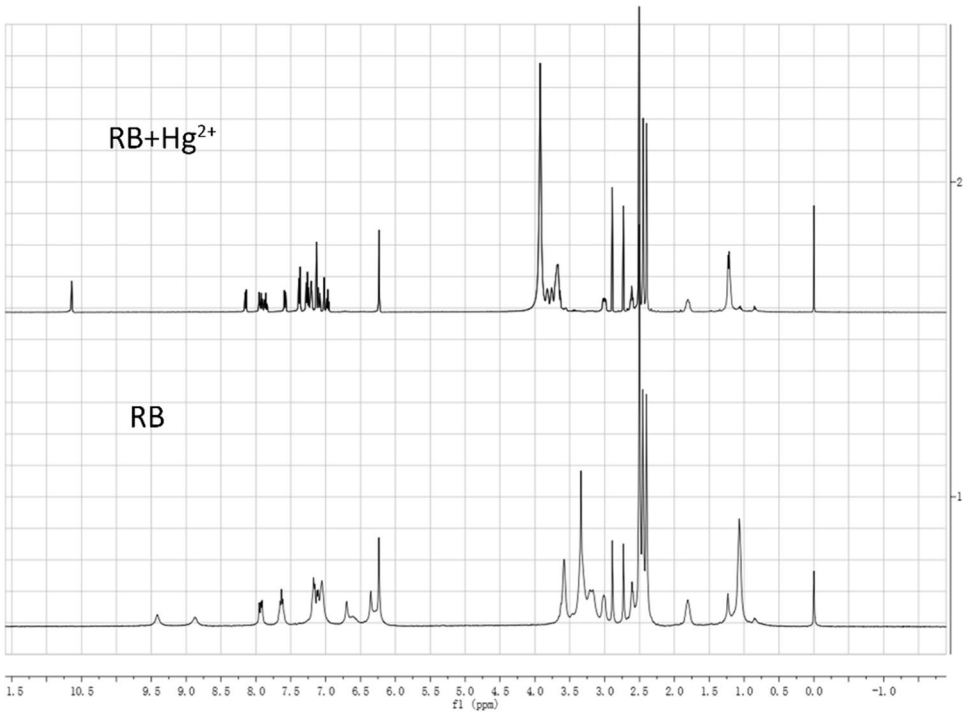
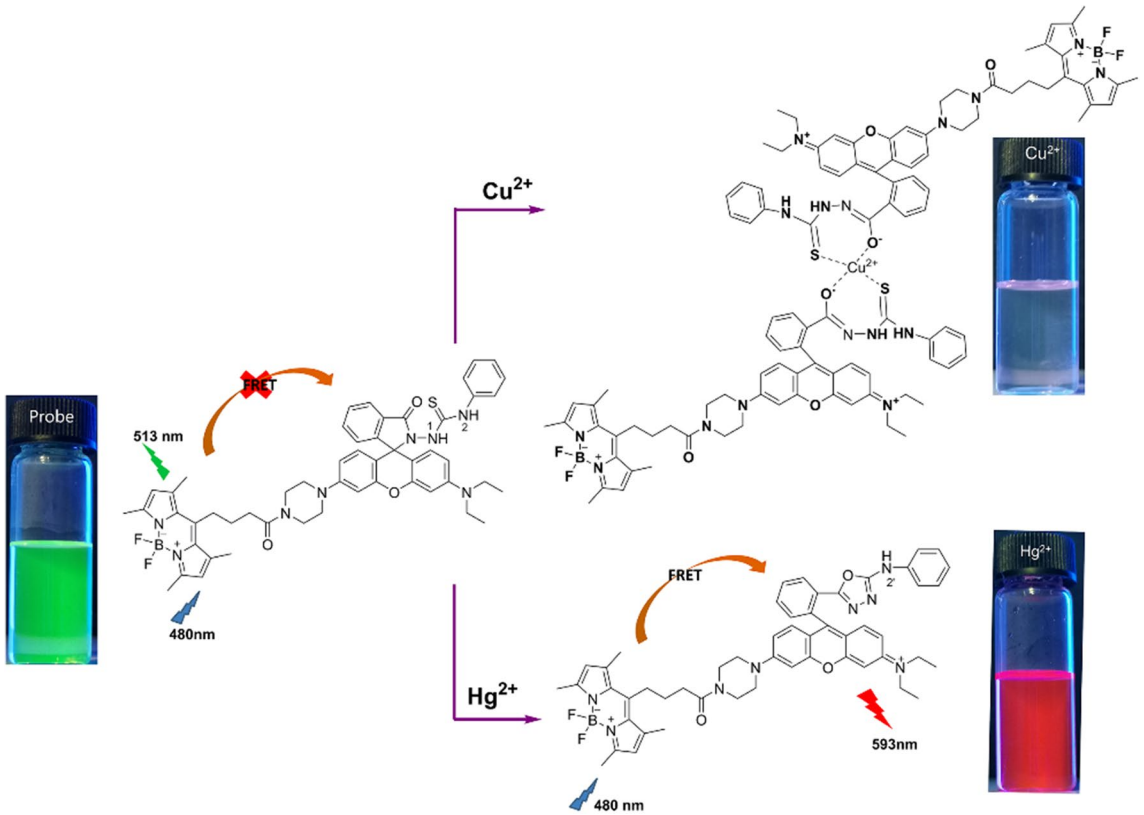


Fig.5 The ^{1}H NMR of probe **RB** and probe **RB** with Hg^{2+} in $(CD_3)_2SO$ solvent



Scheme 2 The proposed mechanism of probe **RB** for Cu^{2+} and Hg^{2+} detection

oxadiazole group (Fig. S4). ^{1}H NMR and HRMS data supported the mechanism which is depicted in Scheme 2. Moreover, The FRET energy transfer efficiency (E) was calculated as 97.8% ($E = 1 - I_{DA}/I_D$) through testing the fluorescence intensity of BODIPY and probe **RB** (Fig. S5).

Most reported rhodamine containing thiosemicarbazide group showed no response toward Cu^{2+}, in order to understand the sensing mechanism of probe **RB** to Cu^{2+}, we investigated reversibility, job plot, HRMS experiments. As shown in Fig. 6a, Cu^{2+} addition induced a ca. 96% quenching of fluorescence at 513 nm, and the fluorescence intensity was almost recovered after addition of EDTA, indicating that the coordination of probe **RB** with Cu^{2+} is chemically reversible. While the fluorescence changes induced by Hg^{2+} could not recover through addition of EDTA, due to the formation of oxadiazole (Fig. 6b). Job plot based on fluorescence change was applied to study the binding stoichiometry of probe **RB** and Cu^{2+}. The fitting result is depicted in Fig S6 and 2:1 stoichiometry was calculated for probe **RB** bonding with Cu^{2+}. In addition, the HRMS spectrum of probe RB with Cu^{2+} showed peaks at 1926.46, which was corresponding to the calculated m/z for 2(**RB**) + Cu^{2+} + Na^+ (Fig. S7). All above results indicated that the response of probe **RB** to Cu^{2+} was due to the coordination of probe **RB** with Cu^{2+} through 2:1 binding mode (Scheme 2).

Kinetics study

Time dependent fluorescence change of probe **RB** in the presence of different concentrations of Cu^{2+} or Hg^{2+} were studied. As shown in Fig. 7a, the fluorescence intensity at 513 nm decreased sharply with the increasing concentrations of Cu^{2+}. It was noted that $I_{513\,nm}$ significantly decreased after 30 s and reached equilibrium within 1 min, which

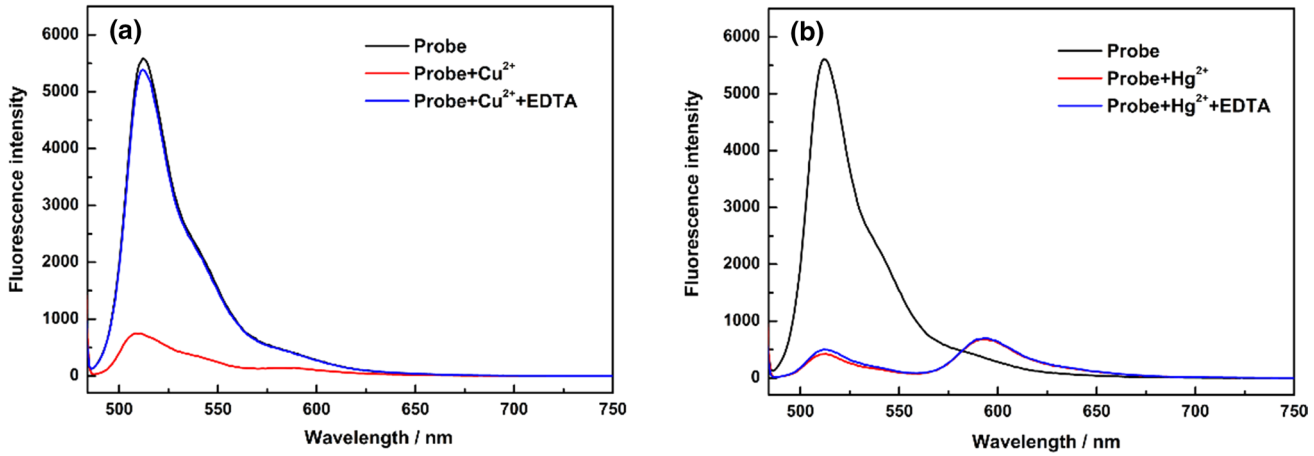


Fig. 6 **a** The fluorescence spectra changes of probe **RB** after the addition of Cu^{2+} and EDTA; **b** The fluorescence spectra changes of probe **RB** after the addition of Hg^{2+} and EDTA

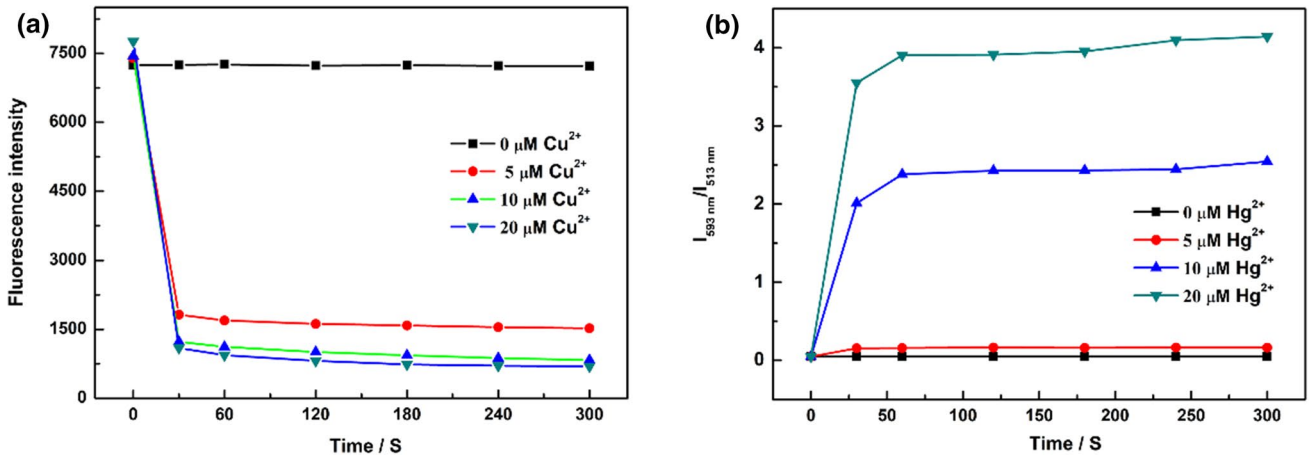


Fig. 7 Time dependent I_{513nm} or $I_{593\ nm}/I_{513\ nm}$ of probe **RB** (10 μM) with different concentrations of Cu^{2+} or Hg^{2+} (0–20 μM) in EtOH/H_2O (V/V = 1:1)

was much faster than most reported probes (5–30 min, Table S1). Moreover, Hg^{2+} addition also induced the value of $I_{593\ nm}/I_{513\ nm}$ augmented with the increasing concentration of Hg^{2+}, and reached equilibrium within 1 min (Fig. 7b). Because of its fast Kinetics, probe **RB** has a broad application prospect in real-time detection of Cu^{2+} and Hg^{2+}.

pH influence

To evaluate the practical applicability of probe **RB**, the suitable operating pH ranges for Cu^{2+} and Hg^{2+} detection were measured through fluorescence spectra, respectively. As shown in Fig. 8, the fluorescence intensity at 513 nm or $I_{593\ nm}/I_{513\ nm}$ of probe **RB** was basically stable in the pH range of 4–12. In the presence of Cu^{2+}, the fluorescence intensity at 513 nm was decreased 45–75% of its initial level in the range of pH = 4–5. Interestingly, the fluorescence intensity decreased significantly (~95% of the initial level) at pH 6 to 12, especially at pH = 6–11 (Fig. 8a). The result showed that probe **RB** was adequate for Cu^{2+} detection in biological surroundings. On the other hand, upon addition of Hg^{2+}, $I_{593\ nm}/I_{513\ nm}$ of probe **RB** showed no obvious changes at pH below 5 or above 11, which indicated that Hg^{2+} could not promote the ring-closed reaction of thiosemicarbazides under strong acidic condition or basic condition. In the range of pH 6–10, the presence of Hg^{2+} significantly improved $I_{593\ nm}/I_{513\ nm}$ of probe **RB** (Fig. 8b), indicating that probe **RB** is suitable for Hg^{2+} detection under physiological conditions.

Living cellular imaging

In order to further study the practical applicability of the probe RB in biological systems, fluorescence microscopy was used to conduct cell imaging experiments using Leica TCS SP8 Confocal Laser Scanning Microscope. Green channel (513 nm ± 15 nm) and red channel (593 nm ± 15 nm) were used to capture fluorescence emission images. As shown in Fig. 9, obvious green fluorescence in the cells was observed, after incubating living Hela cells with probe **RB** (5 μM) for 30 min at room temperature, indicating the cell permeability of probe **RB**. Moreover, in red channel no fluorescence can be observed, which demonstrated that probe **RB** is very steady and there is no interference for Hg^{2+} detection in Hela cells. Incubated the Hela cells stained with the probe **RB** with Cu^{2+} and Hg^{2+} (5 μM) in PBS for 30 min, respectively, and then washed with PBS three times. As shown in Fig. 9d, e, the green fluorescence intensity in the green channel was partially quenched (Fig. 9d), and the red color increased significantly, which indicated Hg^{2+} could promote the cyclization reaction of thiosemicarbazide group to form oxadiazole in living cells. Compared to Hg^{2+}, Cu^{2+} only induced the green fluorescence intensity partially quenched, which was in accordance with the fluorescence spectrum changes in EtOH/H_2O (V/V = 1:1) solution. All above results showed that the probe **RB** could be used as a fluorescent probe to discriminate Hg^{2+} and Cu^{2+} through double-channel imaging in living cells.

Conclusion

In summary, we have synthesized a dual-function probe **RB** containing thiosemicarbazide group, which showed high sensitivity and selectivity toward Cu^{2+} and Hg^{2+}. This probe could detect Cu^{2+} and Hg^{2+} through significant fluorescence on–off and ratiometric fluorescence changes in EtOH/H_2O (V/V = 1:1) solution and living cells. The detection limits of probe **RB** to Cu^{2+} and Hg^{2+} were calculated to be 0.01 ppm and 1.68 ppb, respectively,

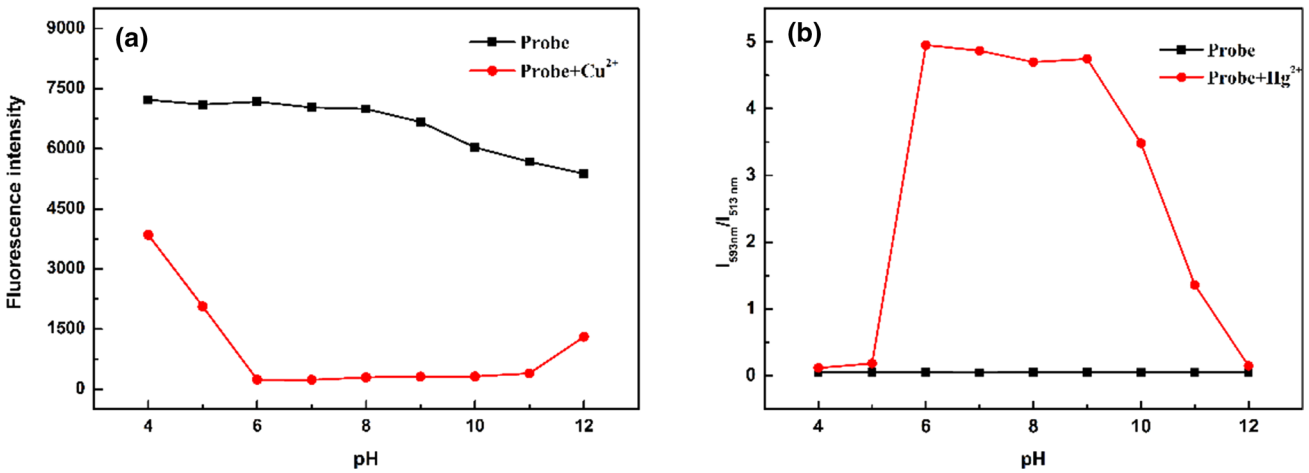


Fig. 8 $I_{513\ nm}$ and $I_{593\ nm}/I_{513\ nm}$ of probe **RB** (10 μM) without and with Cu^{2+} or Hg^{2+} (100 μM) as a function of pH

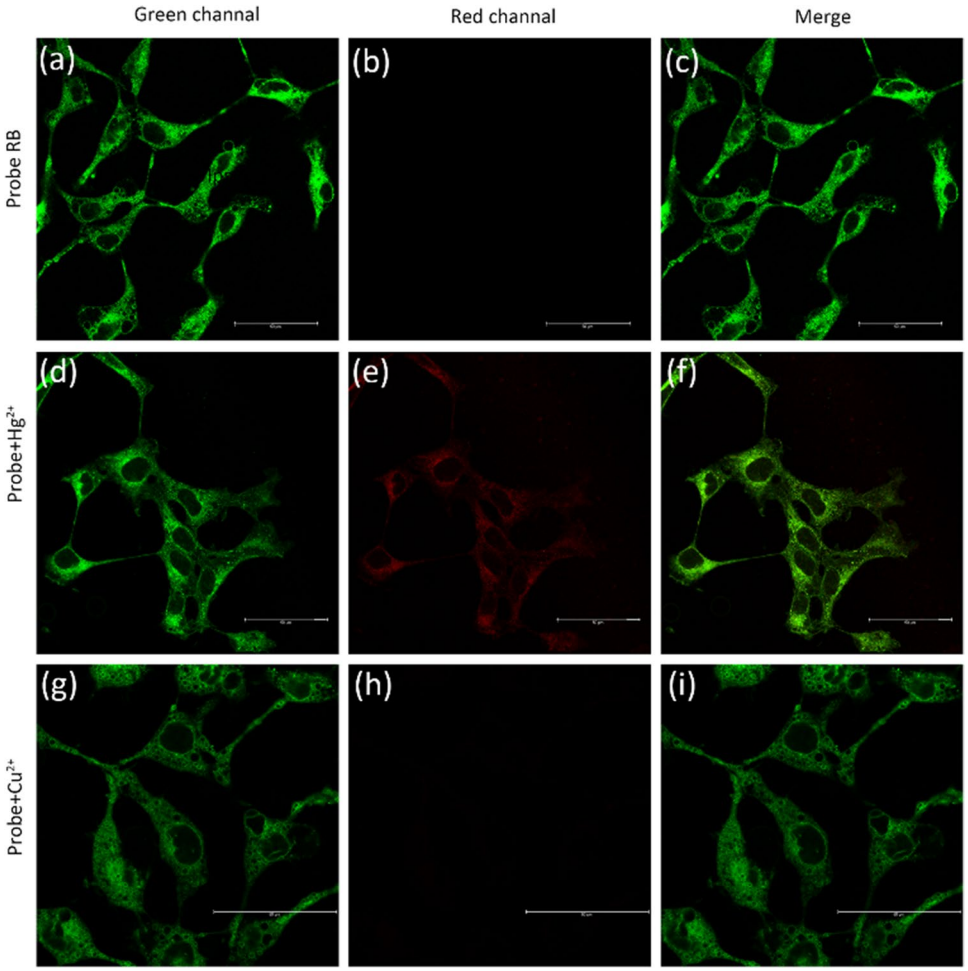


Fig. 9 The confocal fluorescence images of Hela cells incubated with probe **BR** (5 μM) over 30 min **a** green channel, **b** red channel, **c** overlay image of **a**, **b**; the fluorescence images of Hela cells stained with the probe **RB** incubated with Hg^{2+} (5 μM) over 30 min **d** green channel, **e** red channel, **f** overlay image of **d**, **e**; the fluorescence images of Hela cells stained with the probe **RB** incubated with Cu^{2+} (5 μM) over 30 min **g** green channel, **h** red channel, **i** overlay image of **g**, **h**

which was lower than the maximum permissible amount of Cu^{2+} (1.3 ppm) and Hg^{2+} (2.0 ppb) in drinking water proposed by USA Environmental Protection Agency (EPA). Probe **RB** containing thiosemicarbazide group was firstly reported to detect Cu^{2+} through 2:1 binding mechanism. Most importantly, probe **RB** has been successfully applied for discriminating Hg^{2+} and Cu^{2+} through double-channel imaging in living cells.

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Declarations

Conflict of 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.

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