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A terbium-based time-resolved luminescent probe for sulfide ions mediated by copper in aqueous solution

Yanmeng Xiao [a,b], Yanqin Yang [a,b], Guiyan Zhao [a,b], Xinxiu Fang [a,b], Yongxia Zhao [a,b], Pengran Guo [c], Wei Yang [a,*], Jingwei Xu [a,*]

[a] State Key Laboratory of Electroanalytical Chemistry, Changchun Institute of Applied Chemistry, Changchun 130022, China
[b] University of Chinese Academy of Sciences, Beijing 100039, China
[c] Guangdong Provincial Public Laboratory of Analysis and Testing Technology, Guangdong Institute of Analysis, Guangzhou 510070, China

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ABSTRACT

A novel Tb(III) complex-based time-resolved luminescent probe, BBPNA-(DO3A-Tb^{3+})$_2$ (**L**), was designed and synthesized for the recognition of Cu^{2+} and sulfide ions in aqueous solutions. The luminescent probe **L** was efficiently quenched by Cu^{2+} and displayed "on–off" type luminescence change towards Cu^{2+}. Once combined with Cu^{2+} to form the non-luminescent **L**–**Cu** complex, the **L**–**Cu** complex could serve as an "off–on" probe for sulfide ions due to the high affinity of sulfide ions towards Cu^{2+}. In the presence of S^{2-}, the **L**–**Cu** complex reacted with S^{2-} to cause the release of the Cu^{2+} and turn on the luminescence of **L** again. The rebuilding of luminescence was highly selective to S^{2-} over other common anions, which allowed **L** function as an "on–off–on" probe for Cu^{2+} and S^{2-} alternately. Especially, the luminescence lifetime of the probe was about 1.91 ms, much longer than most organic fluorophores. Due to the long luminescence lifetime of the probe **L**, the probe could be used for time-resolved luminescence measurements to eliminate the interferences from autofluorescence and scattering lights and significantly to improve the signal-to-noise for detection.

Besides iron and zinc ions, copper ion (Cu^{2+}) is the third most abundant essential transition metal ion in the human body. It is involved in various vital physiological processes [1,2]. On the other hand, taking in excess amount of copper may lead to liver damage and kidney damage, gastrointestinal disturbance, and a series of neurological problems [3,4]. In addition, copper ion is also a significant environment pollutant which is widely spread in soil and water. In view of the critical role of copper ion in the biological systems and environment, development of rapid and sensitive probes for the detection of Cu^{2+} in biological and environment systems becomes very important. Due to the paramagnetic nature of Cu^{2+}, the binding of Cu^{2+} could induce intrinsic fluorescence quenching and lead to a turn-off signal [5,6]. Interestingly, the resultant non-fluorescence ensemble Cu^{2+} complex could be explored as an anion selective probe.

As a toxic traditional pollutant, sulfide anion is not only generated from industrial processes but also from biological metabolism. To our knowledge, high concentration of sulfide ions can damage mucous membranes of living organisms and cause various biological issues in living organisms. The protonated HS^- or H_2S forms are even more toxic and caustic than the sulfide ion itself. However, recent studies have recognized H_2S as the third gaseous transmitter, in addition to nitric oxide (NO) and carbon monoxide (CO) [7,8]. It is produced endogenously in mammalian systems by enzymes, including pyridoxal-5′-phosphate(PLP)-dependent enzymes and pyridoxal-5′-phosphate-independent enzymes [9,10]. The concentration of H_2S in blood is about 10–100 μM [11]. H_2S is involved in a number of physiological and pathological processes, including inhibition of insulin signaling [12], relaxation of vascular smooth muscles [13], regulation of inflammation and O_2 sensing [14,15]. Otherwise, its deregulation has been correlated with the symptoms of Alzheimer's disease [16], Down syndrome [17], diabetes [18], chronic kidney disease [19], and liver cirrhosis [20]. Accordingly, the development of sensitive and selective techniques for its detection and measurement is very important.

Other than the traditional destructive methods including colorimetric [21], electrochemical [22,23], and gas chromatography assays [24], fluorescence has received great attention recently due to its high selectivity and sensitivity, cell permeability, and offering real-time monitoring in vivo. Several fluorescent probes for H_2S have been reported since 2009, mainly through three mechanisms [25] (Table S1): H_2S-mediated reduction of azides to amines, trapping of H_2S via nucleophilic addition, and copper sulfide precipitation. The former two types of "reactive" probes based on irreversible sulfide-specific chemical reactions are sensitive but limited by relatively long reaction time, poor selectivity over reactive oxygen species (ROS) [26], and relatively strict reaction conditions. The latter takes advantage of the competition of Cu^{2+} between the probe and S^{2-}, which reacts with Cu^{2+} to form a low-solubility CuS

* Corresponding authors.
E-mail addresses: yangwei@ciac.ac.cn (W. Yang), jwxu@ciac.ac.cn (J. Xu).

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($K_{sp} = 1.27 \times 10^{-36}$) [27]. Utilizing this strategy, several fluorescence-based copper complex sensors have been designed.

Compared to organic fluorescence dyes, luminescent lanthanide complex-based probes possess unique luminescent properties including long luminescence lifetime, large Stokes shift, nice photo stability, and sharp emission profile [28,29]. Considering these advantages, luminescent lanthanide probes have been demonstrated as a powerful tool to eliminate background noise for the time-resolved luminescence detection of various biological and bioactive molecules in biological environments [30–37]. Now, we report a time-resolved probe for the detection of S^{2-}. So far as we know, this kind of probe should be the first reported.

In this study, we designed and synthesized a binuclear Tb^{3+} complex according to the previous method [38], in which two DO3A rings (1,4,7,10-tetraazacyclododecane 1,4,7-triacetic acid) [39] for Tb^{3+} chelating were connected with a 2,6-bis(pyrazol-1-yl) pyridine scaffold that functioned as a receptor for Cu^{2+} coordination. The luminescence probe **L** was synthesized by the nucleophilic substitution reaction of compound 1 with compound 2 by three steps (Scheme 1), the detailed synthesis steps were described in the support information (Scheme S1). Once chelating with Cu^{2+} to form the **L**–**Cu** complex, the luminescence of **L** was quenched by the paramagnetic Cu^{2+} center [40]. In the presence of S^{2-}, Cu^{2+} was taken off from the non-luminescence **L**–**Cu** complex to form CuS precipitation, resulting in luminescence recovery of **L**. Thus, complex **L** exhibited "on–off–on" luminescence response along with the addition of Cu^{2+} and S^{2-} alternately.

The UV–Vis spectrum of **L** was selectively affected by Cu^{2+} among various metal ions (Na^+, K^+, Ca^{2+}, Mg^{2+}, Cu^{2+}, Zn^{2+}, Al^{3+}, Mn^{2+}, Fe^{3+}, Fe^{2+}, Cd^{2+}, Ni^{2+}) (Fig. S7). Upon addition of Cu^{2+}, the absorbance at 247 and 295 nm decreased gradually and three new peaks at 272, 280, and 340 nm appeared which indicated the interaction between Cu^{2+} and **L**.

To further confirm the selectivity of **L** towards Cu^{2+} over other metal ions, the luminescence emission spectra were carried out. Both the steady-state and time-resolved luminescence emission spectra of **L** displayed the Tb^{3+} emission pattern with a main emission peak at 545 nm when excited at 295 nm, showing a large Stokes shift of approximately 250 nm. Besides, the steady-state spectrum also included fluorescence around 367 nm derived from the connector. With the addition of different 1 equiv. metal ions (Fig. 1), the luminescence intensities were not significantly altered, except with 1 equiv. Cu^{2+}, which effectively quenched the luminescence (Fig. 1). According to the luminescence turn-off behavior shown in Fig. 1, the probe **L** could recognize Cu^{2+} ions among the test metal ions.

Job's plot analysis (Fig. S8) and titration studies were carried out to investigate the binding property of probe **L** towards Cu^{2+}, which indicated that **L** responded to Cu^{2+} in 1:1 stoichiometry (Fig. 2a, inset). The association constant of Cu^{2+} was obtained as 3.37×10^4 M^{-1} from the titration data (Fig. S9). Moreover, the detection limit of the **L** for Cu^{2+} was estimated to be 1.7×10^{-5} M (Fig. S10).

To evaluate the selectivity of the **L**–**Cu** complex towards S^{2-}, the non-luminescence **L**–**Cu** complex was incubated with a series of physiological and environmental important anions, including F^-, Cl^-, Br^-, I^-, SCN^-, NO^{3-}, CO_3^{2-}, $P_2O_7^{4-}$, PO_4^{3-}, HCO_3^-, and $C_2O_4^{2-}$ and sulfates, such as SO_4^{2-}, SO_3^{2-}, $S_2O_3^{2-}$, $S_2O_8^{2-}$, and sulfoacetic acid disodium salt (SAS), which resulted in negligible luminescence regeneration. Only S^{2-} restored the luminescence completely (Fig. S11). In addition, some competitive biothiols such as L-cysteine (L-Cys), reduced glutathione (GSH) and dithiothreitol (DTT) were also tested to evaluate the selectivity and the result revealed that these competitive biothiols did not induce luminescence regeneration either. Interestingly, $C_2O_4^{2-}$ scarcely influenced the signal at 545 nm, but increased the luminescence intensity at 367 nm (Fig. S11a). In addition, competition experiments were performed in the presence of 10 equivalents of interfering anions, as

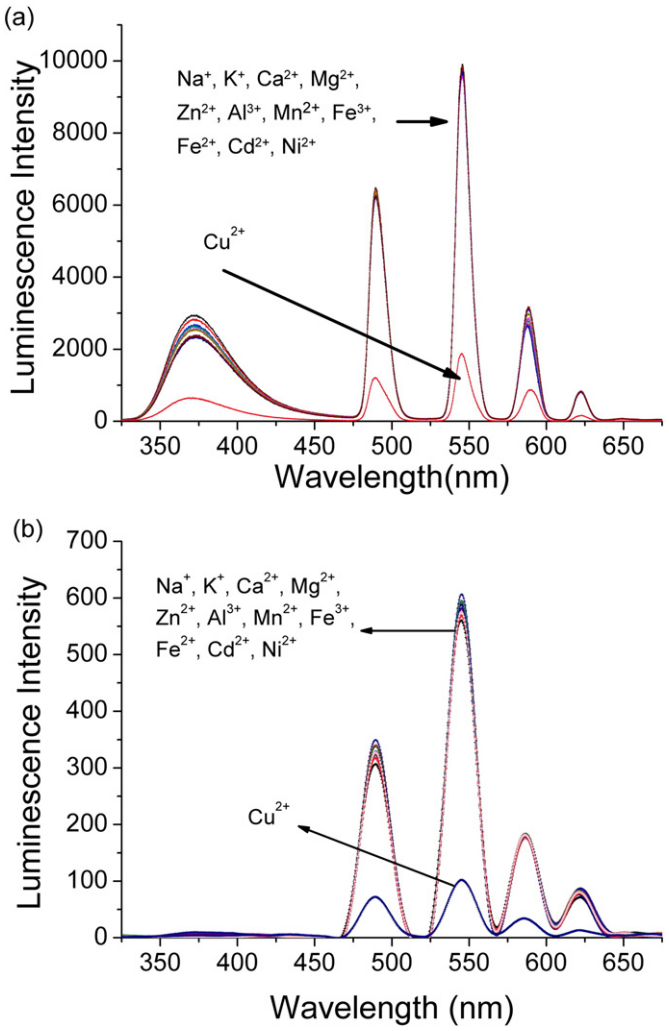
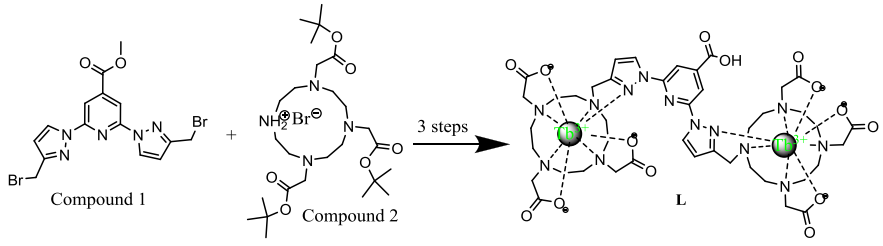


Fig. 1. (a) Steady-state and (b) time-resolved (delay time, 20 μs; gate time, 2.0 ms) luminescence emission spectra of **L** (10 μM, 295 nm) with the addition of various ions (10 equiv. for Na^+, K^+, Ca^{2+}, Mg^{2+}, Zn^{2+}, Al^{3+}, Mn^{2+}, Fe^{3+}, Fe^{2+}, Cd^{2+}, and Ni^{2+}; 1 equiv. for Cu^{2+}) in HEPES-buffered (pH 7.4, 100 mM) aqueous solutions.



Scheme 1. Synthesis of **L**.