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Short Communication

# Simultaneous determination of Hg(II) and Zn(II) using a GFP inspired chromophore

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ABSTRACT

A dual-function chemosensor for $Hg^{2+}$ and $Zn^{2+}$ ions, inspired by the green fluorescent protein (GFP) chromophore, was designed and synthesized, which could specifically recognize $Zn^{2+}$ through an "OFF–ON" fluorescence mechanism due to the restriction of the free rotation of the aryl–alkene bond, whilst also producing a selective visible colorimetric response from yellow to pink with $Hg^{2+}$ ions. The response upon exposure to $Zn^{2+}$ and $Hg^{2+}$ is instantaneous, and the detect limits of $Zn^{2+}$ and $Hg^{2+}$ are $2.18\times10^{-8}$ M and $4.91\times10^{-7}$ M respectively.



## 1. Introduction

Mercury is highly toxic even at low levels [1–3] and can be easily absorbed and accumulated from the environment by human beings, resulting in brain damage and other chronic diseases. Therefore, the design of sensitive sensors and devices for $Hg^{2+}$ detection has attracted considerable attention [4–8]. Whilst $Zn^{2+}$ ions are the second most abundant transition heavy metal in the human body and play an important role in metabolism [9]. However, $Zn^{2+}$ is also a metal pollutant of the environment in water or soil, especially at high concentrations[10]. Making the sensitive and selective determination of $Zn^{2+}$ is of great interest [11–14], resulting in much effort being devoted to the selective detection of $Zn^{2+}$ ions [15,16].

In order to prevent heavy metal pollution, significant effort has been made to develop methods for the detection of these polluting metal ions [17–21]. In particular optical chemosensors, which are simple, quick and sensitive, have attracted much attention for the detection of heavy metal ions [22–24]. However, although fluorescent and colorimetric chemosensors are sensitive and effective, very few examples of chemosensors have been designed to detect two or more kinds of metal ions simultaneously [25].

The green fluorescent protein (GFP) has been widely applied in molecular biology, cell biology, biotechnology, and molecular genetics, since it was isolated from jellyfish (Aequorea victoria) [26,27]. The GFP chromophore, p-hydroxybenzyl ideneimidazolinone (*p*-HBDI, Fig. 1), is formed via autocatalytic cyclization and dehydration of a Ser–Tyr–Gly tripeptide motif followed by air-oxidization [28]. However, this chromophore does not fluoresce due to the free rotation of aryl–alkene bond [29–31]. Burgess et al. have used a $BF_2$ unit to restrict the free rotation of synthetic GFP chromophores [32]. Although the GFP has been known for more than 20 years, GFP or its derivatives have seldom been used to construct sensors to detect metal ions. Tolbert et al. have reported a GFP analog, in which the fluorescence is turned on by complexation with $Zn^{2+}$ and $Cd^{2+}$ [33]. In our previous work, we synthesized a GFP analog, which behaved as an excellent fluorescent chemosensor for $Zn^{2+}$ detection [28].

In this work, we designed and synthesized a novel chemosensor 1 based on the GFP chromophore for the simultaneous detection of $Zn^{2+}$ and $Hg^{2+}$ ions. The sensing capacities of compound 1 for $Zn^{2+}$ and $Hg^{2+}$ were investigated using UV/vis and fluorescence spectroscopic methods. On addition of $Zn^{2+}$ a fluorescence turn ON response was observed, whilst on addition of $Hg^{2+}$ ions a visible colorimetric change was observed. We believe that one possible practical application of this chemosensor is to detect residual $Zn^{2+}$ and $Hg^{2+}$ found after the preparation of zinc amalgam. The proposed scheme for the detection of $Zn^{2+}$ and $Hg^{2+}$ is shown in Fig. S1.

## 2. Experimental

### 2.1. Materials

All the chemicals were of analytic grade and used as received. All solutions were prepared with Nanopure water (18 MΩ $cm^{-1}$)

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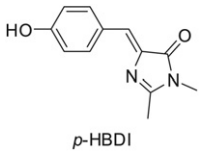


**Fig. 1.** Strcucture of *p*-HBDI.

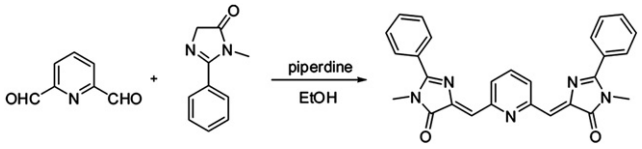


**Fig. 2.** Synthesis of chemosensor 1.

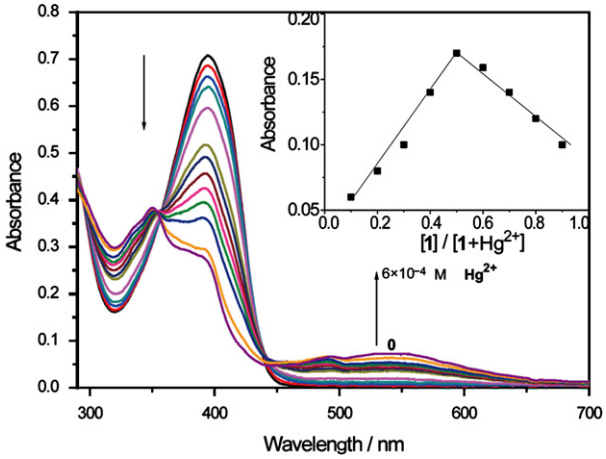


**Fig. 3.** Absorption of **1** (20 μM) $CH_3CN$/HEPES (95/5, v/v, HEPES, 50 mM, pH=7.4) upon complexation with increasing concentration of $Hg^{2+}$. Inset: Job's plot, absorption measured at 550 nm.

from a Millipore system and distilled $CH_3CN$. All the metal ion solutions ($Zn^{2+}$, $Ca^{2+}$, $Mg^{2+}$, $Na^{+}$, $Co^{2+}$, $Ni^{2+}$, $Ag^{+}$, $Cd^{2+}$, $Pb^{2+}$, $Cu^{2+}$ and $Hg^{2+}$) were prepared by adding 5 volumes of aqueous solution to 95 volumes of $CH_3CN$. $^1H$ NMR and $^{13}C$ NMR were acquired in $CDCl_3$ on a BRUKER AVANCE 500 spectrometer using TMS as an internal standard. HRMS were obtained on a HP 5989 mass spectrometer. Infrared spectra were recorded on a Bruker tensor 27 spectrometer. Melting points were determined with a melting point apparatus without correction. Fluorescence spectra were determined on a Varian Cary Eclipse fluorescence spectrometer. UV/vis spectra were measured on a Varian Cary 500 spectrophotometer.

### 2.2. Synthesis of 1-methyl-4-((6-((1-methyl-2-oxo-5-phenyl-1H-pyrrol-3(2H)-ylidene)methyl)pyridine-2-yl)methylene)-2-phenyl-1H-imidazol-5(4H)-one (compound **1**)

A mixture 147 mg of pyridine-2,6-dicarbaldehyde (0.6 mmol) and 200 mg 1-methyl-2-phenyl-1H-imidazol-5(4H)-one (1.25 mmol) in 6 mL absolute ethanol, and piperidine (1–2 drops) were stirred at reflux for 4 h. The reaction mixture was cooled, and the precipitated solid was filtered, washed with ethanol to obtain a yellow solid product **1**, Fig. 2 119 mg, 54% yield. $^1H$ NMR, ($CDCl_3$, $\delta$ ppm, 500 MHz): $\delta$ 8.87 (d, $J$=7.9 Hz, 1H), 7.85 (m, 3H), 7.74–7.51(m, 3H), 7.43 (s, 1H), 3.36 (s, 3H). $^{13}C$ NMR (500 MHz, $\delta$ ppm, $CDCl_3$): $\delta$ 171.45, 164.20, 153.77, 141.35, 136.56, 131.93, 128.90, 128.37, 127.70, 29.15. MS (ESI) *m/z* calcd. for (M+) $C_{27}H_{21}N_5O_2$ 447.1695; found 447.1697.

### 2.3. Preparation of 1-doped sol–gel [34]

TEOS sol–gel was prepared by mixing 20 mL of TEOS with 2 mL ethanol and 0.2 mL of 0.01 M HCl. The sol–gel was stirred vigorously for 1 h and doped with 2 mL compound **1** ($10\times10^{-6}$ M in $CH_3CN$); the sol was stirred overnight before use.

## 3. Results and discussions

The UV/vis spectroscopic properties of compound **1** ($2\times10^{-5}$ M) were investigated in aqueous buffer ($CH_3CN$/HEPES, 95/5, v/v; HEPES, 50 mM, pH 7.4). As shown in Fig. 3, a solution of compound **1** which is of yellow color shows a maximum absorption centered at 400 nm ($\varepsilon=3.54\times10^5$ L M$^{-1}$ cm$^{-1}$), which is similar to that of natural GFP. Upon addition of $Hg^{2+}$, a new broad absorption band around 550 nm ($\varepsilon=3.6\times10^3$ L M$^{-1}$ cm$^{-1}$) develops which results in a color change from yellow to pink. Similar results were reported from some previous works. To investigate the effects of other metal ions on the UV/vis spectroscopic properties of compound **1**, several other metal ions were investigated (Fig. S2). As expected, addition of the other metals to solutions of compound **1** resulted in no or little absorption change at 550 nm. This is likely due to the $d^{10}$ configuration of $Hg^{2+}$ with tetrahedral coordination geometry, and the large ionic radius is likely to take advantage of the specific binding to the ligand having two closed imidazole rings [35,36]. The pink color occurs only in the presence of $Hg^{2+}$ with or without competing metal ions (from yellow to pink, Fig. S3B). As a competing ion, the interference of $Zn^{2+}$ in the detection of $Hg^{2+}$ was investigated. $Hg^{2+}$ was added to a solution of compound **1** containing 12 equivalents of $Zn^{2+}$; the absorbance of the mixed solution was measured (Fig. S4), indicating that $Zn^{2+}$ does not interfere with the detection of $Hg^{2+}$ at 550 nm. These results demonstrate that compound **1** can be used as a visible colorimetric chemosensor for $Hg^{2+}$ determination.

The fluorescence spectra of compound **1** and its titration with $Zn^{2+}$ were also measured in aqueous buffer ($CH_3CN$/HEPES, 95/5, v/v; HEPES, 50 mM, pH 7.4). From Fig. 4 it can be seen that $Zn^{2+}$ binding leads to a fluorescence enhancement of the chromophore centered at 474 nm. The fluorescence increase is caused by restriction of the free rotation of the aryl–alkene bond between pyridine and imidazolone unit on $Zn^{2+}$ binding. Titration of compound **1** with $Zn^{2+}$ results in a decrease of the fluorescence emission peak at 450 nm and increase of the fluorescence emission peak at 474 nm. The interference of other metal ions was investigated under the same conditions. As shown in Fig. 4A, the addition of $Zn^{2+}$ resulted in strong fluorescence emission at 474 nm, while $Ca^{2+}$, $Mg^{2+}$, $Na^{+}$, $Co^{2+}$, $Ni^{2+}$, $Ag^{+}$, $Cd^{2+}$ and $Pb^{2+}$ caused no or little fluorescence changes and $Hg^{2+}$ and $Cu^{2+}$ totally quench the fluorescence. The quenching of emission band is attributed to the spin–orbit coupling effects in case of $Hg^{2+}$ [37] ions and the paramagnetic nature of $Cu^{2+}$ [38].

To further explore the selectivity of compound **1** for $Zn^{2+}$, we measured the fluorescence intensity of compound **1** in the presence of $Zn^{2+}$ and other metal ions. As depicted in Fig. 5, the addition of **1** equivalent of $Zn^{2+}$ to a solution of compound **1** (10 μM) containing 12 equivalents of various metal ions can enhance or recover the fluorescence emission intensity except for $Cu^{2+}$ and $Hg^{2+}$. To solve this problem, thiourea was used as a masking agent for $Hg^{2+}$ (thiourea can also mask $Cu^{2+}$) [39]. As shown in Fig. S5 after masking by thiourea, on addition of $Zn^{2+}$ to a solution of **1** containing $Hg^{2+}$ the fluorescence intensity is significantly enhanced. Fig. S6 shows the visible fluorescence changes. Our observations demonstrate that compound **1** can be used as a fluorescent chemosensor for $Zn^{2+}$ determination.

Benesi–Hildebrand analysis [40] of the absorption data and the Job's plot (inset of Fig. 3) indicate a 1:1 stoichiometry for the **1**–$Hg^{2+}$ complex, Kass = $2.25 \times 10^4\ M^{-1}$. The emission data and Job's plot (inset of Fig. 4) also clearly indicate a 1:1 stoichiometry for the **1**–$Zn^{2+}$ complex, and the association equilibrium constant (Kass) is $2.079 \times 10^3\ M^{-1}$. Since the Kass of $Hg^{2+}$ is larger than that of $Zn^{2+}$, $Zn^{2+}$ cannot compete with $Hg^{2+}$ which is consistent with our observations. The detection limits for $Zn^{2+}$ and $Hg^{2+}$ can be estimated from the titration results to be $2.18 \times 10^{-8}$ M and $4.91 \times 10^{-7}$ M respectively (Figs. S7 and S8). The complexes of $Zn^{2+}$ and $Hg^{2+}$ were also further analyzed by MALDI mass spectroscopy (Fig. S12). It should be noted that the peak at *m/e* 511.0987 of **1**/$Zn^{2+}$ and the peak at m/e 644.1262 of 1/$Hg^{2+}$ could be attributed to the **1**–$Zn^{2+}$ and **1**–$Hg^{2+}$ complexes respectively.

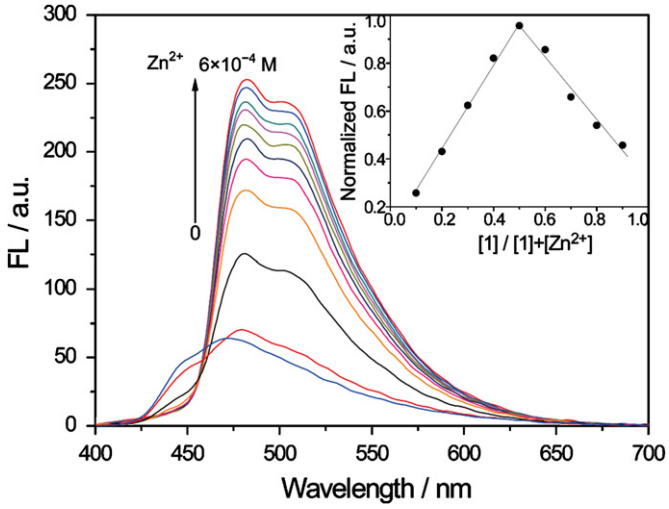


**Fig. 4.** Fluorescence spectrum of **1** (10 μM) in $CH_3CN$/HEPES (95/5, v/v, HEPES, 50 mM, pH 7.4) upon titrating different molar ratios of $Zn^{2+}$ with excitation at 400 nm. Inset: Job's plot; the total concentration of **1** and $Zn^{2+}$ ion is 5.0 mM.

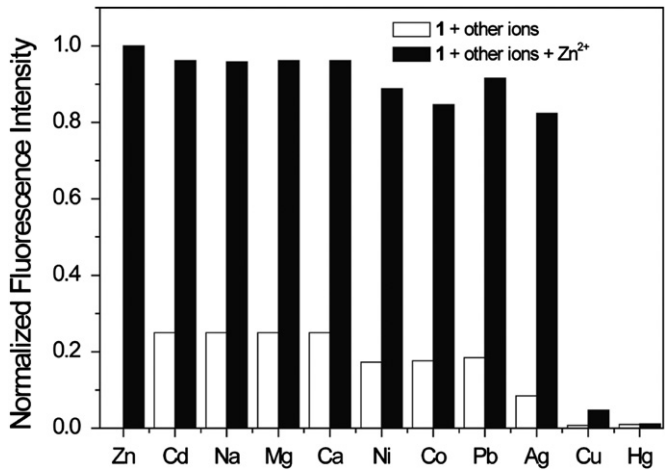


**Fig. 5.** Fluorescence response of **1** (10 μM) in $CH_3CN$/HEPES (95/5, v/v, HEPES, 50 mM, pH 7.4) solution of various metal ions ($Ca^{2+}$, $Mg^{2+}$, $Na^+$, $Co^{2+}$, $Ni^{2+}$, $Ag^+$, $Cd^{2+}$, $Pb^{2+}$, $Cu^{2+}$ and $Hg^{2+}$). The white bars represent the solution mixed with 1 and 12 equivalents of various ions; the black bars represent the solution mixed with 1 and 12 equivalents of ions and 12 equivalents of $Zn^{2+}$.

Next we investigated the properties of the sensor in water. However, given that compound **1** is quite insoluble in water, we encapsulated the dye in a sol–gel to help measure $Zn^{2+}$ and $Hg^{2+}$ in water. Compound **1** can be entrapped in a sol–gel matrix while still being accessible from solution [41]. Fig. 6 shows the responses of a **1**-doped sol–gel towards different metal ions. The results obtained are consistent with Fig. 4, where only the addition of $Zn^{2+}$ (1 μM, $H_2O$) caused a fluorescence enhancement (Fig. 6A), while only $Hg^{2+}$ (1 μM, $H_2O$) caused a color change (yellow to pink, Fig. 6B). Furthermore, the **1**-doped sol–gel was applied to the determination of $Hg^{2+}$ and $Zn^{2+}$ in river water (Qingchun River, Shanghai). The water sample was percolated before using. The results obtained are shown in Table 1. These results indicate that compound **1** can be effectively used as a chemosensor for detecting $Zn^{2+}$ and $Hg^{2+}$ in aqueous solution.

Fig. S9 shows the pH dependent fluorescence changes obtained for **1**. The plot of the integrated emission intensity reveals that at lower pH values ($<6$) the fluorescence of **1** is quenched, which is in agreement with our previous work [28]. The presence of water has significant effect on the selectivity of **1**, so the percentage of water on the fluorescence intensity of **1** was investigated. Fig. S10 shows that the chromophore has maximum emission in 5% water solution.

## 4. Conclusion

In conclusion, we have designed and synthesized a new chemosensor **1** inspired by GFP which can behave as a $Zn^{2+}$ specific fluorescence turn-on chemosensor that exhibits good selectivity. Furthermore, **1** can also be used as a visible colorimetric chemosensor for $Hg^{2+}$ determination. We believe that this

**Table 1**
Recovery test for $Hg^{2+}$ and $Zn^{2+}$ from spiked samples in river water.

| Metal ions | Added ($10^{-6}$ M) | Found ($10^{-6}$ M) | Recovery (%) |
|---|---|---|---|
| $Hg^{2+}$ | 1.00 | 0.971 | 97.1 |
| $Zn^{2+}$ | 1.00 | 0.936 | 93.6 |

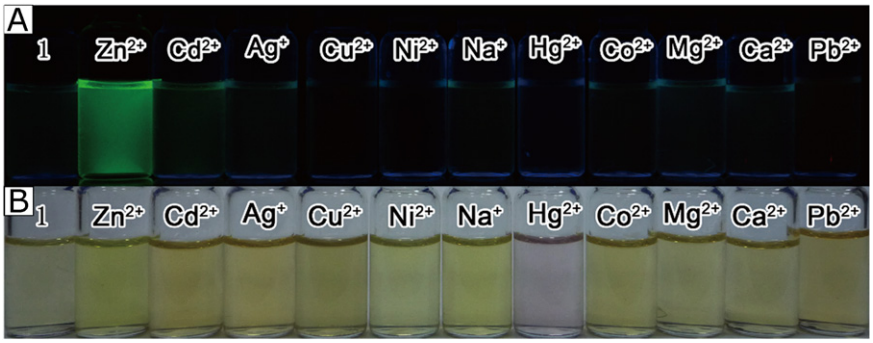


**Fig. 6.** (A) Visible fluorescence emission responses of **1**-doped sol–gel with 1 equivalent $Zn^{2+}$ and various metal ions ($Ca^{2+}$, $Mg^{2+}$, $Na^+$, $Co^{2+}$, $Ni^{2+}$, $Ag^+$, $Cd^{2+}$, $Pb^{2+}$, $Cu^{2+}$ and $Hg^{2+}$) (B) Color changes of **1**-doped sol–gel in the presence of $Hg^{2+}$ and 1 equivalent other metal ions ($Zn^{2+}$, $Ca^{2+}$, $Mg^{2+}$, $Na^+$, $Co^{2+}$, $Ni^{2+}$, $Ag^+$, $Cd^{2+}$, $Pb^{2+}$ and $Cu^{2+}$).

system will be useful to simultaneously detect $Zn^{2+}$ and $Hg^{2+}$ ions in practical application.

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## Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.talanta.2012.07.097.

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