Received: 20 August 2018 | Revised: 5 November 2018 | Accepted: 3 December 2018

DOI: 10.1002/bio.3641

RESEARCH ARTICLE

WILEY LUMINESCENCE
The Journal of Biological and Chemical Luminescence

Multifunctional peptide-based fluorescent chemosensor for detection of Hg^{2+}, Cu^{2+} and S^{2-} ions

Xuliang Pang[1] | Lei Wang[1] | Lei Gao[2] | Huiyun Feng[1] | Jinming Kong[3] | Lianzhi Li[1]

[1] School of Chemistry and Chemical Engineering, Liaocheng University, Liaocheng, China

[2] Zhong Yuan Academy of Biological Medicine, Liaocheng People's Hospital, Liaocheng, China

[3] School of Environmental and Biological Engineering, Nanjing University of Science and Technology, 200 Xiaolingwei, Nanjing, China

Correspondence
Lianzhi Li, School of Chemistry and Chemical Engineering, Liaocheng University; Address: No. 1, Hunan Road, Liaocheng; 252059, P. R. China.
Email: lilianzhi1963@163.com

Funding information
The National Natural Science Foundation of China, Grant/Award Number: (21142003, 20471025); The Natural Science Foundation of Shandong Province of China, Grant/Award Number: ZR2016HB73; The Scientific Research Foundation of Liaocheng University, China, Grant/Award Number: 318011513; Natural Science Foundation of Shandong Province of China, Grant/Award Number: ZR2016HB73; National Natural Science Foundation of China, Grant/Award Number: 21142003, 20471025

Abstract

A novel multifunctional fluorescent peptide sensor based on pentapeptide dansyl-Gly-His-Gly-Gly-Trp-COOH (D-P5) was designed and synthesized efficiently using Fmoc solid-phase peptide synthesis (SPPS). This fluorescent peptide sensor shows selective and sensitive responses to Hg^{2+} and Cu^{2+} among 17 metal ions and six anions studied in *N*-2-hydroxyethylpiperazine-N-2-ethane sulfonic acid (HEPES) buffer solution. The peptide probe differentiates Hg^{2+} and Cu^{2+} ions by a 'turn-on' response to Hg^{2+} and a 'turn-off' response to Cu^{2+}. Upon addition of Hg^{2+} or Cu^{2+} ions, the sensor displayed an apparent color change that was visible under an ultraviolet lamp to the naked eye. The limits of detection (LOD) of DP-5 were 25.0 nM for Hg^{2+} and 85.0 nM for Cu^{2+}; the detection limits for Cu^{2+} were much lower than the drinking water maximum contaminant levels set out by the United States Environmental Protection Agency (USEPA). It is noteworthy that both D-P5-Hg and D-P5-Cu systems were also used to detect S^{2-} successfully based on the formation of ternary complexes. The LODs of D-P5-Hg and D-P5-Cu systems for S^{2-} were 217.0 nM and 380.0 nM, respectively. Furthermore, the binding stoichiometry, binding affinity and pH sensitivity of the probe for Hg^{2+} and Cu^{2+} were investigated. This study gives new possibilities for using a short fluorescent peptide sensor for multifunctional detection, especially for anions.

KEYWORDS

cupric ion, fluorescent sensor, mercury ion, peptide-based sensor, sulfide ion

1 | INTRODUCTION

Living organisms require trace amounts of some metallic elements such as copper, iron, zinc and manganese for nutrition, however excessive levels of these metals can be detrimental to humans and biological organisms.[1] Other heavy metals, such as arsenic, mercury, lead and cadmium, are toxic. Their accumulation can be harmful to organisms and have a negative effect on the environment.[2] Heavy and transition metals (HTM) are introduced into the environment through industrial products and processes.[3] Among these HTM ions, Hg^{2+} has been regarded as the most toxic and hazardous.[4,5] Environmental pollution from mercury has received considerable attention because the accumulation of low concentrations of mercury in organisms through the food chain has caused various potential effects including motion disorders, and serious cognitive prenatal brain damage.[3–5] As one of the most abundant trace metal elements in the human body, Cu^{2+} plays a crucial role in metabolic homeostasis,

Abbreviations used: D-P5, Dansyl-Gly-His-Gly-Gly-Trp-COOH; DCM, Dichloromethane; DIEA, *N*,*N*-diisopropylethylamine; DMF, *N*,*N*-dimethylformamide; EDT, Ethanedithiol; EPA, Environmental Protection Agency; ESI-MS, Electrospray ionization mass spectrometry; FRET, fluorescence resonance energy transfer; HBTU, 2-(1H-Benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate; HEPES, *N*-2-hydroxyethylpiperazine-*N*-2-ethane sulfonic acid;; His, Histidine; HPLC, High performance liquid chromatography; HTM, Heavy and transition metals; LOD, Limit of detection; SPPS, Solid-phase peptide synthesis; TFA, Trifluoroacetic acid; TIS, Tri-isopropylsilane; Trp, Tryptophan; UV, Ultraviolet

protein regulation, and the development of living organisms.[6–9] Therefore, selective and sensitive detection of Hg^{2+} and Cu^{2+} is needed due to fundamental application in environmental and biomedical sciences.[10–17]

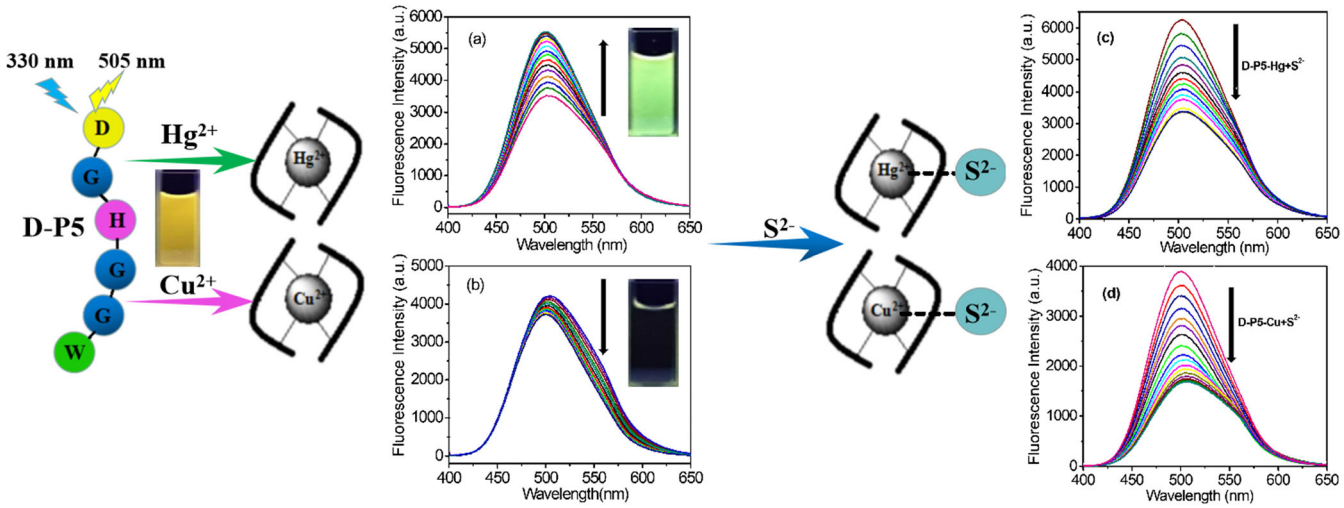
Fluorescent chemical probes are a useful tool for detecting low concentrations of HTM ions, which are significant in living organisms and the environment.[18,19] Fluorescent chemosensors can be used as portable and less costly alternatives to traditional atomic spectrometric techniques.[20–22] Recently, peptide-based fluorescent chemosensors for detecting HTM ions have been investigated because of their increased advantages.[23] Peptide-based sensors can be effectively prepared with high yields using Fmoc solid-phase peptide synthesis (SPPS). The chelation ability of a peptide for HTM ions is attributed to its amino acid composition and its sequence, determining which kind of metal ion can be monitored, as well as the selectivity and sensitivity of chemosensors for HTM ions.[24,25] Therefore, the selectivity and sensitivity of peptide-based sensors for metal ions can be optimized by changing their amino acid composition and sequence. Furthermore, specially designed peptide sensors have strong metal-binding affinities and are environmentally compatible, water soluble and biocompatible.[25–28] Peptide-based sensors containing tryptophan (Trp) have been reported for detecting HTM ions such as Cu(II), Ag(I), Hg(II) and Ni(II), plus those containing histidine (His) for detection of As(III), Zn(II), Cu(II), Ag(I), and Ni(II).[20,29,30] In general, the development of peptide-based chemosensors for detection and monitoring of heavy metal ions, with high sensitivity and quick response is a great challenge.[31,32] S^{2-}, a toxic traditional pollutant, is an important anion in environmental and biological systems. The design of effective probes for monitoring S^{2-} is significant because abnormal levels of S^{2-} have been associated with increased risk of many diseases[33], however peptide-based sensors for S^{2-} detection are rarely reported. Recently, Tang and colleagues described a novel peptide-based fluorescence sensor for detection of Cu(II) and S^{2-}.[34–36] These chemosensors display an 'on–off–on' fluorescence response change on the addition of Cu^{2+} and $S^{2-}$to aqueous solution, however these sensors were often affected by Hg(II) when selectively detecting Cu(II).[35,36] In this study, we synthesized a new multifunctional chemosensor based on a pentapeptide, dansyl-Gly-His-Gly-Gly-Trp-COOH (D-P5), to monitor Hg(II) and Cu(II) and especially S^{2-}. The fluorescent peptide probe could detect Cu(II) via a turn-off response and Hg(II) via turn-on response among various metal ions and anions. More importantly, we successfully detected sulfide using the D-P5-Hg and D-P5-Cu systems. The binding stoichiometry, binding affinity, limit of detection (LOD) and pH influence of the fluorescence peptide chemosensor for these ions were also studied. Scheme 1 displays possible fluorescence detection mode of D-P4 with Cu^{2+}, Hg^{2+} and S^{2-}.

2 | EXPERIMENTAL

2.1 | Materials and instruments

Fmoc-protected amino acids (Fmoc-Gly-OH, Fmoc-L-Trp (Boc)-OH, and Fmoc-L-His (Trt)-OH) and Wang resin (0.71 mol/g) were purchased from C S Bio. Co., USA. Dansyl chloride and trifluoroacetic acid (TFA) were obtained from Shanghai Macklin Biochemical Co., Ltd. *N*,*N*-Diisopropylethylamine (DIEA), triisopropylsilane (TIS) and 2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU) were obtained from Shanghai GL Biochem Ltd. All other chemicals used were of analytical reagent grade unless otherwise noted. Stock solutions of the various metal salts were prepared with 50 mM *N*-2-hydroxyethylpiperazine-*N*-2-ethane sulfonic acid (HEPES) buffer (pH 7.2), and were used throughout the study.

Instruments were: CS 136 Peptide Synthesizer (CS Bio Co., USA); Hitachi F-7000 spectrofluorometer (Hitachi Inc., Japan); Lambda 750 spectrophotometer (Perkin Elmer, USA); API 4500 QTRAP mass spectrometer (Applied Biosystems/MDS SCIEX, USA); high performance liquid chromatography (HPLC) system (model 426 HPLC pump, UVIS 201 detector, Alltech, USA).



SCHEME 1 Proposed fluorescence detection mode of D-P5 for Cu^{2+}, Hg^{2+} and S^{2-}

2.2 | Synthesis of D-P5

Dansyl-Gly-His-Gly-Gly-Trp-COOH was synthesized using Fmoc chemistry by solid-phase peptide synthesis (SPPS) technology. Fmoc-protected Fmoc-L-Trp-OH (1.2 mM) was added to Wang resin (0.4 mM). The Fmoc group was then deprotected, followed by assembly of Fmoc-Gly-OH (1.2 mM), Fmoc-Gly-OH (1.2 mmol), Fmoc–L-His–OH (1.2 mmol) and Fmoc-Gly-OH (1.2 mmol), successively. Dansyl chloride (1.2 mmol) in *N*,*N*-dimethylformamide (DMF) was added to the resin-bound pentapeptide. Then, the resin was dried using pumps after being washed with DMF (15 ml), dichloromethane (DCM) (15 ml) and methanol (15 ml), four times respectively. The peptide was obtained by cleavage from the Wang resin and treated with a mixture of 10 ml trifluoroacetic acid (TFA):thioanisole:phenol:H_2O:ethanedithiol (EDT) (82.5:5:5:5:2.5, v/v/v/v/v) in a dark room at room temperature for 3.5 h. The crude peptide was precipitated in ice-cold ether and then centrifuged at 8000 rpm for 8 min at 4°C, subjected to multiple washing and centrifugation, and finally freeze dried. The obtained crude peptide was analyzed and purified using a HPLC using C18 column, to give a yield of 76% DP-5. Here, 0.1% TFA and 80% CH_3CN were used as mobile phase A, mobile phase B was 0.1% TFA. ESI mass spectrometry was operated in positive ion mode with the following operating parameters: electrospray voltage set to 5500 V; curtain gas, 20 psi; GS 1, 45 psi; GS 2, 50 psi. Temperature: 500°C. Nitrogen was used as the neulizer and desolvation gas.

2.3 | General fluorescence and UV–vis measurements

A stock solution of 2.15 mM D-P5 was prepared in distilled water and stored at 4°C in a dark room. This stock solution was used for all fluorescence and UV spectral measurements after appropriate dilution with 50 mM HEPES buffer containing 100 mM $NaClO_4$ at pH 7.2. UV absorption spectra were recorded using a Perkin Elmer Lambda 750 spectrophotometer in the wavelength range 400–200 nm with a 1 cm path length quartz cuvette. Fluorescence emission spectra were measured using a 1 cm path length quartz cuvette at an excitation wavelength of 330 nm. Slit widths of 5 nm were used for both excitation and emission. Fluorescence spectra for this peptide probe system were measured in the absence or presence of 17 metal ions (Cr^{3+}, Mn^{2+}, Fe^{3+}, Co^{2+}, Zn^{2+}, Cd^{2+}, Hg^{2+}, Pb^{2+}, Al^{3+}, Ca^{2+}, Mg^{2+}, K^+ and Na^+ as chloride anions; Fe^{2+} as a sulfate anion; Ni^{2+}, Cu^{2+} as acetate anions and Ag^+ as a nitrate anion) and six anions ($NaNO_3$, Na_2SO_4, Na_3PO_4, $NaClO_4$, NaAc and NaCl). The peptide sensor concentration was determined using an absorption spectrum at 330 nm for fluorophore dansyl chloride.

2.4 | Fluorescence response of D-P5-hg and D-P5-cu to S^{2-}

Fluorescence emission spectra of D-P5-Hg and D-P5-Cu in the presence of 15 anions (Ac^-, F^-, Cl^-, ClO_4^-, Br^-, I^-, PO_4^{3-}, CO_3^{2-}, NO_2^-, NO_3^-, SCN^-, SO_4^{2-}, SO_3^{2-}, $S_2O_3^{2-}$ and S^{2-}) were measured in 50 mM HEPES buffer solution at pH 7.2 with excitation at 330 nm. Slit widths of 5 nm were used for both excitation and emission. UV–vis absorption spectra for D-P5-Hg and D-P5-Cu with the addition of S^{2-} were recorded in 50 mM HEPES buffer solution at pH 7.2.

3 | RESULTS AND DISCUSSION

3.1 | Solid-phase synthesis of D-P5

Peptide chemosensor (dansyl-Gly-His-Gly-Gly-Trp-COOH) was efficiently prepared by solid-phase synthesis with a yield of 76% using Fmoc chemistry. After cleavage of the product from the Wang resin, D-P5 was purified from the crude product by semi-preparative HPLC using a C18 column. Successful synthesis was confirmed by electrospray ionization mass spectrometry (ESI-MS). The ESI mass of DP-5 was calculated: 746.26 [M + H^+]. Observed: 746.8 [M + H^+]. The fluorescent peptide DP-5 exhibited good solubility in 100% aqueous solution.

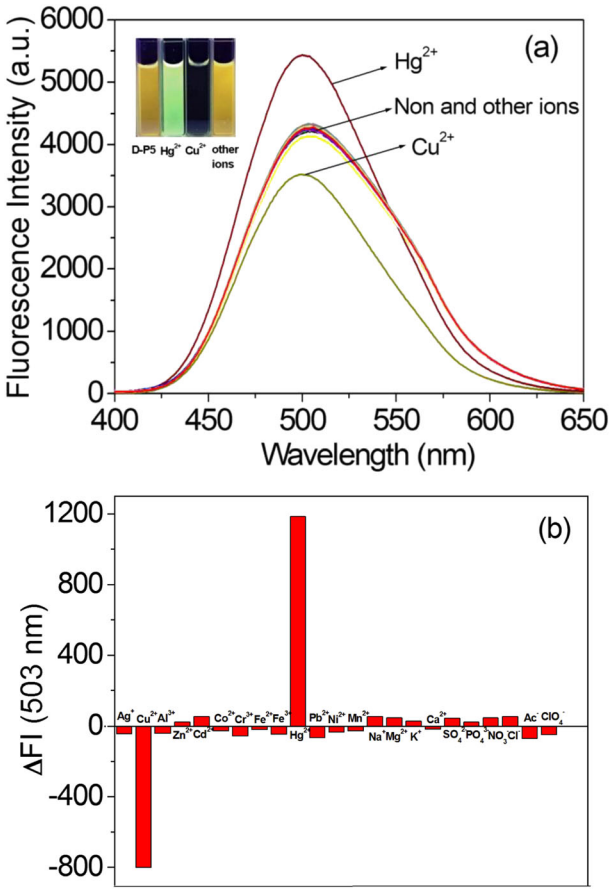


FIGURE 1 Fluorescence emission spectra of DP-5 in the presence of various metal ions excited at 330 nm (a) and the fluorescence response of DP-5 to metal ions and anions (b) in 50 mM, pH 7.2 HEPES buffer solution. The molar ratio of metal/D-P5 is 1:2. Inset in (a) is the color changes of the systems excited by a 365 nm UV lamp

3.2 | Fluorescence and UV–vis spectra

The fluorescent peptide probe D-P5 exhibited good solubility in aqueous solution. The fluorescence responses of this sensor to various metal ions and anions were measured in 50 mM HEPES buffer solution at pH 7.2. Figure 1 indicates the fluorescence emission spectra of D-P5 in the presence of different metal ions (Cr^{3+}, Mn^{2+}, Fe^{3+}, Co^{2+}, Zn^{2+}, Cd^{2+}, Hg^{2+}, Pb^{2+}, Al^{3+}, Ca^{2+}, Mg^{2+}, K^{+} and Na^{+} as chloride anions, Fe^{2+} as a sulfate anion, Ni^{2+}, Cu^{2+} as acetate anions and Ag^{+} as a nitrate anion) and anions ($NaNO_3$, $NaSO_4$, Na_3PO_4, $NaClO_4$, NaAc and NaCl) by excitation with 330 nm wavelength light. D-P5 showed a maximum fluorescence emission peak at 505 nm, and only Hg^{2+} and Cu^{2+} ions had strong responses to this sensor among these ions. This fluorescent peptide probe can differentiate Cu^{2+} and Hg^{2+} by different response types, performing a turn-on response to Hg^{2+} and a turn-off response to Cu^{2+}. Tang and colleagues reported two novel peptide fluorescent chemosensors that exhibited fluorescence quenching responses for Cu^{2+} and Hg^{2+}, and that distinguished these two heavy metal ions by the same response type.[35,36]

Figure 1(a, inset) shows the visible emission color changes of the systems excited by a 365 nm UV lamp. The D-P5 solution itself was yellow in color and the addition of Hg^{2+} into the D-P5 solution changed the fluorescence color from yellow to green. D-P5 solution containing Cu^{2+} was colorless, but the D-P5 solution containing the mixture of other 15 metal ions retained the yellow color. This phenomenon of color change indicated which metal ions, either Hg^{2+} or Cu^{2+} ions, were contained in the peptide solution.

The peptide contained two fluorophores (Trp and dansyl). An excitation wavelength of 330 nm was used to monitor dansyl fluorophore emission; the 290 nm wavelength was used to monitor both Trp and Dansyl fluorophore emissions. The fluorescence emission spectra and fluorescence responses of the peptide probe to different concentrations of Cu^{2+} and Hg^{2+} in 50 mM HEPES buffer solution at pH 7.2 are presented in Figures 2 and 3. As shown in Figure 2, when excited at 330 nm wavelength, the fluorescence intensity of D-P5 at 503 nm continuously increased with addition of Hg^{2+}, but Cu^{2+} addition caused the fluorescence intensity of D-P5 at 503 nm to decrease with a small blue shift. This reduction of fluorescence intensity may result from electron transfer between the excited dansyl group and the complexed Cu^{2+} ion.[37] As shown in Figure 3, when excited at 290 nm wavelength, with the addition of increasing Hg^{2+} concentrations, fluorescence intensity decreased at 360 nm and increased at 500 nm. Fluorescence emission at 360 nm is a characteristic of Trp residues, whereas fluorescence emission at 500 nm is attributed to dansyl chromophores.[18] When Hg^{2+} was added to the D-P5 solution, amino acid residues in D-P5 (including imidazole group of histidine and indole group of tryptophan) can interact with Hg^{2+}, therefore the peptide may fold and the distance between the dansyl fluorophore (acceptor) and Trp residue (donor) would be shortened, resulting in a decrease in emission intensity for Trp (360 nm) and an increase in emission intensity for the dansyl group (500 nm) due to the fluorescence resonance energy transfer (FRET) effect.[7] This change suggests that DP-5 has a selective and sensitive response to Hg^{2+} and Cu^{2+} using different types of response; this may be due to the formation of 2:1 coordination compounds of this sensor with Hg^{2+} and Cu^{2+}, respectively (Figure 2c,d).

UV–vis absorption spectra from the D-P5 solution in the presence of different metal ions were recorded (Figure S1). The results showed that only Hg^{2+} and Cu^{2+} among 17 metal ions could induce noticeable spectral changes in D-P5, in accordance with the results of the above fluorescence spectra. Furthermore, we measured absorption titration

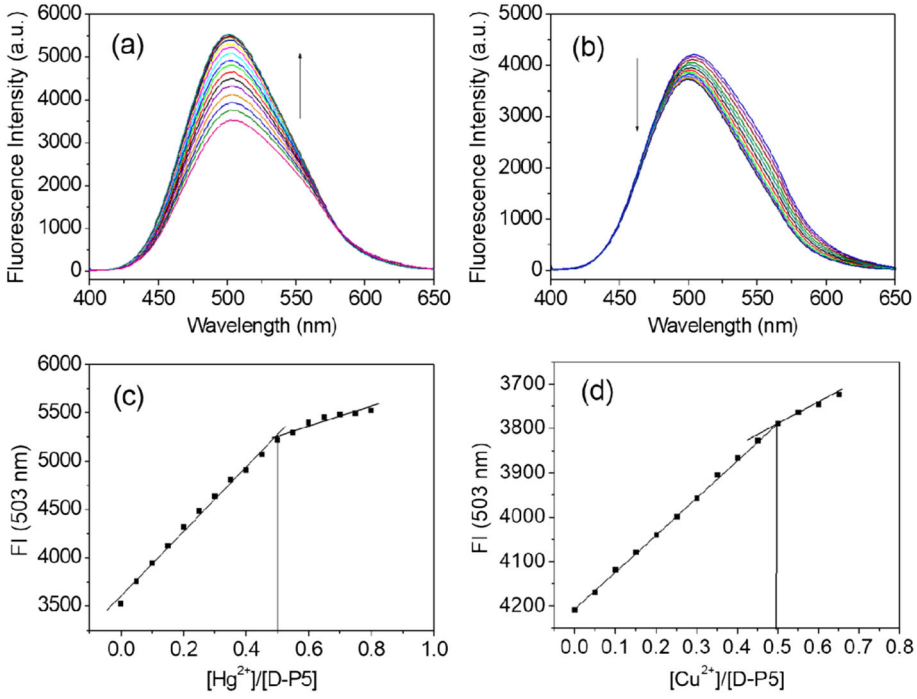


FIGURE 2 Fluorescence emission spectra of D-P5 (100.0 μM) in the presence of increasing concentrations of Hg^{2+} (a) and Cu^{2+} (b) excited at 330 nm in 50 mM HEPES buffer at pH 7.2. Plots of fluorescence intensity versus concentration ratio of $[Hg^{2+}]$/D-P5 (c) and $[Hg^{2+}]$/D-P5 (d)

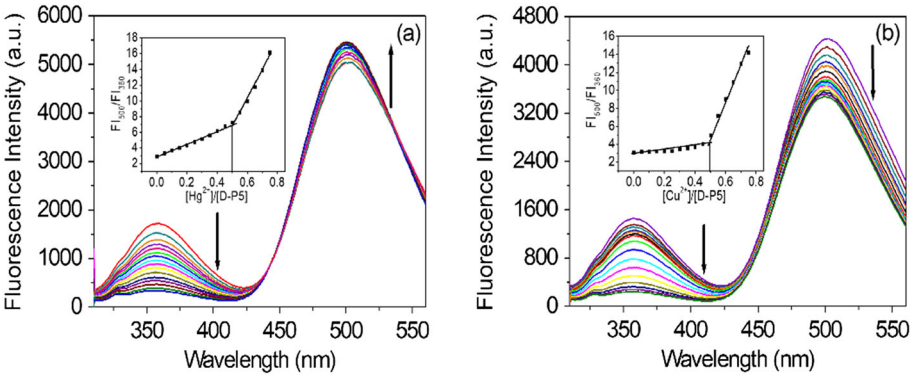


FIGURE 3 Fluorescence emission spectra of D-P5 (100.0 μM) with the addition of increasing concentrations of Hg^{2+} (a) and Cu^{2+} (b) in 50 mM HEPES buffer at pH 7.2 (λ_{ex} = 290 nm). Insets are plots of fluorescence ratio of $\lambda_{500}/\lambda_{360}$ versus concentration ratio of metal ion/D-P5

spectra by addition of increasing amounts of Hg^{2+} and Cu^{2+} ions to the D-P5 solution (Figure S2). With the addition of increasing concentrations of Hg^{2+}, the intensities of the absorption bands at 220 nm and 245 nm wavelength constantly decreased and remained almost unchanged after more than 0.5 equiv of added Hg^{2+}, in addition the absorption band at 330 nm increased slightly. By contrast, on the addition of increasing concentrations of Cu^{2+}, the absorbance peaks at 220 nm and 245 nm consistently increased, and absorption intensity at 330 nm also increased slightly. All results indicated that the different binding mechanisms for the D-P5 sensor with metal ions depended on the properties of the metal ion themselves.

3.3 | Binding stoichiometry and binding constant

The binding stoichiometry of this fluorescent peptide probe to Hg^{2+} and Cu^{2+} was investigated. For the fluorescence titration curve, a 50 μM concentration of Hg^{2+} and Cu^{2+} was needed for the saturation

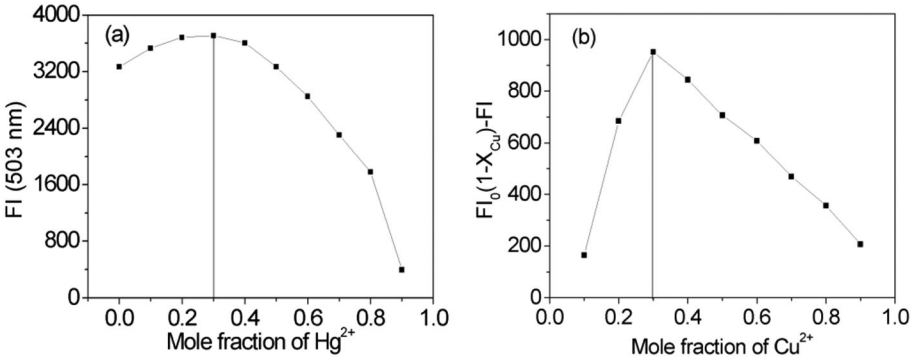


FIGURE 4 Job's plots for D-P5 with Hg^{2+} (a) and Cu^{2+} (b) in 50 mM HEPES buffer solution at pH 7.2

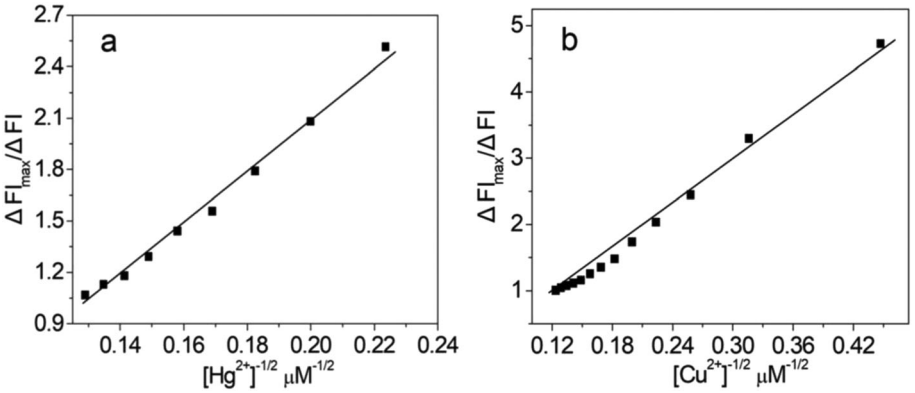


FIGURE 5 Benesi–Hildebrand plot for the determination of binding constants of D-P5 (100.0 μM) with Hg^{2+} ion (a) and Cu^{2+} ion (b)

of fluorescence intensity of the peptide-based probe (100 μM), showing that the binding ratio of this sensor with Hg^{2+} and Cu^{2+} was 2:1, respectively. According to reported literature, a Job's plot was also used to evaluate the stoichiometry of the systems.[38] A Job's plot was established using the mole fraction of metal ions and the relative fluorescence intensity (Figure 4). These result exhibited a maximum at 0.3 mole fraction for Hg^{2+} and Cu^{2+} in HEPES buffer solution at the concentration of 100.0 μM for the sensor (Figure 4). This result also suggested that D-P5 formed a 2:1 complex with Hg^{2+} and Cu^{2+} in HEPES buffer solution. The binding constants for D-P5 with Hg^{2+} and Cu^{2+} ions were determined using the Benesi–Hildebrand equation: $\Delta FI_{max}/\Delta FI = 1 + ([M]^{-n}/K)$. Here, ΔFI_{max} is $FI_{max} - FI_0$, ΔFI is $F_x - F_0$, and $n = 0.5$; F_0 is the fluorescence intensity of D-P5 and F_x is the fluorescence intensity of D-P5 at different concentrations of Hg^{2+} and Cu^{2+}; [M] is the concentration of Hg^{2+} and Cu^{2+} and K is the binding constant.[39,40] As shown in Figure 5(a,b), the binding constants of D-P5 with Hg^{2+} and Cu^{2+} were estimated and the K values were found to be 6.54×10^4 $M^{-1/2}$ and 1.23×10^5 $M^{-1/2}$, respectively.

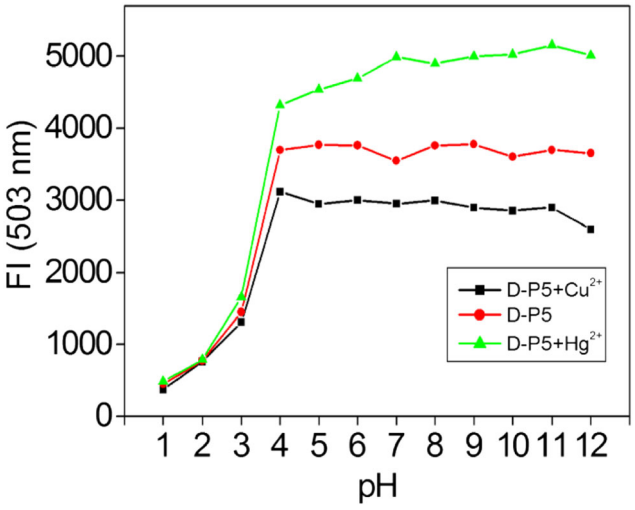


FIGURE 6 Fluorescence intensities at 330 nm of D-P5 in the absence or presence of Hg^{2+} (0.5 equiv.) and Cu^{2+} (0.5 equiv.) at different pH

3.4 | Effects of pH on detection

Fluorescence emission spectra of the D-P5, D-P5-Hg^{2+} and D-P5-Cu^{2+} systems were measured at different pH solution with excitation at 330 nm. The pH value of the sample solution was adjusted by appropriate additions of $HClO_4$ or NaOH solution. The influence of pH on the fluorescence detection of D-P5 to $Hg^{2+}$and Cu^{2+} was measured, as shown in Figure 6. Results indicated that D-P5, D-P5-Hg and D-P5-Cu showed a weak fluorescence intensity in acidic solution (pH < 4) that could be attributed to charge transfer between the dimethylamino group and napthyl moiety and was prevented by the protonated dimethylamino group (pK_a ~4) of fluorophore dansyl chloride.[41,42] However, a weakly alkaline environment had less influence on these fluorescence peaks. At pH 9–12, the negative charge for the indole group of Trp and imidazole ring of His residues in D-P5 increased and could enhance the formation of the D-P5-Hg and D-P5-Cu complexes, therefore resulting in changes of emission intensity.

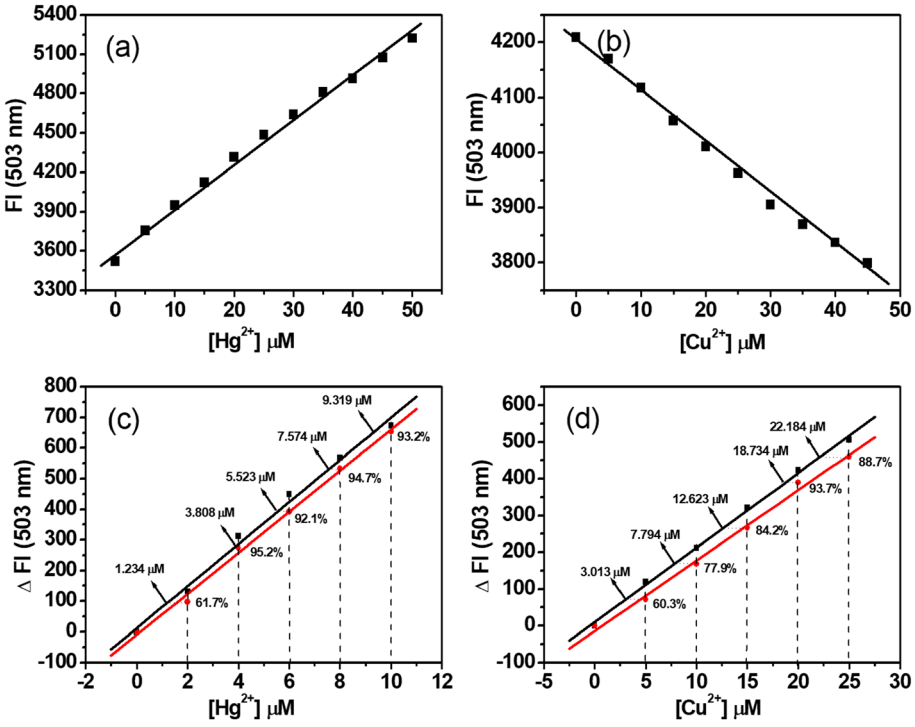


FIGURE 7 Linear fluorescence emission intensity as a function of the concentrations of Hg^{2+} ion (a) and Cu^{2+} ion (b), and the recovery of the sensor for different concentrations of Hg^{2+} ion (c) and Cu^{2+} ion (d)

Overall results indicated that D-P5 was a useful analytical tool for monitoring $Hg^{2+}$and Cu^{2+} at neutral and weak basic pH.

3.5 | Detection limit of D-P5 for Hg^{2+} and Cu^{2+} and application

The limits of detection (LODs) were calculated according to the fluorescence titration experiments. To ascertain the S/N ratio, the fluorescence emission intensity of D-P5 in the absence of Hg^{2+} and Cu^{2+} ions was measured 10 times and the standard deviation of the blank measurement was obtained. Four independent duplicate measurements for fluorescence intensity were evaluated in the presence of Hg^{2+} and Cu^{2+} ions and mean intensity was plotted as a function of Hg^{2+} and Cu^{2+} concentration for determining the slope. Then, LODs for Hg^{2+} and Cu^{2+} ions were obtained using the equation: LOD = 3σ/m, where σ is the standard deviation of the emission intensity of peptide probe itself, and m is the slope of the curve of the plot.[27] LODs for the peptide sensor for Hg^{2+} and Cu^{2+} were calculated using the linear relationships of fluorescence emission intensity at 503 nm with the addition of increasing concentrations of Hg^{2+} and Cu^{2+}. D-P5 showed an instant response to Hg^{2+} and Cu^{2+} in 50 mM HEPES buffer solution. Figure 7(a,b) showed a good linear relationship between fluorescence intensity and Hg^{2+} and Cu^{2+} concentrations in the range 0–50 μM. According to the equation LOD = 3σ/m, the detection limits for DP-5 were 25.0 nM for Hg^{2+} and 85.0 nM for Cu^{2+}. These results indicated that the detection limit for Cu^{2+} was lower than the drinking water maximum contaminant levels of EPA. The detection limit of this peptide-based sensor was compared with that of other optical chemosensors (Table 1). Simple synthesis, sensitive response, water solubility and biocompatibility were good properties for this sensor.

To test practical application of this peptide sensor, we designed a real sample test. Standard detection curves for $Hg^{2+}$and Cu^{2+} were obtained by standard addition of Hg^{2+} and Cu^{2+} to D-P5 sensor solution, as shown in Figure 7(c,d) (shown as a black line). In the same way, Hg^{2+} and Cu^{2+} ions as the same concentrations as the standard addition method were added to river water solution containing the D-P5 sensor to obtain a measurement curve, as shown in Figure 7(c,d) (shown as a red line). A standard curve was used to calculate the concentration of Hg^{2+} and Cu^{2+} ions in the samples. By comparing the calculated ion concentration with the actual concentration, the accuracy and practicability of the detection method could be judged. From Figure 7(c,d), this peptide sensor presented a good recovery of 94.0% for Hg^{2+} in the range 4–10 μM, and 86.1% for Cu^{2+} in the range 10–25 μM for monitoring Hg^{2+} and Cu^{2+} ions in river water. Therefore, the results demonstrated that this D-P5 fluorescent peptide sensor could be used as an effective means for detecting Hg^{2+} and Cu^{2+} ions in water samples and presented potential applications.

In addition, environmental applications are still faced with challenges and detailed studies of chemosensor performance in environmental samples are required. Therefore, we next proceeded to test the sensor on three water samples. We chose samples from three different sources: sewage water, river water and tap water. All samples were tested after filtering. The results are summarized in Table 2.

TABLE 1 Various spectroscopic sensing systems for determination of Hg^{2+} (a), Cu^{2+} (b) and S^{2-} (c)

Method	System	LOD	Reference
(a)			
Fluorescence	Perylene-bisimide sensor	2.2 μM	[43]
Fluorescence	Rhodamine derivative	120 nM	[44]
Fluorescence	Cyclam derivative	7.9 μM	[45]
Fluorescence	Naphthol AS-based sensor	500 nM	[46]
Absorbance	Schiff base	390 nM	[47]
Absorbance	DNA-functionalized gold nanoparticles	60 nM	[48]
Fluorescence	D-GHGGW-COOH	25 nM	This study
(b)			
Fluorescence	Peptide-based sensor	78 nM	[34]
Fluorescence	Carbazole-azine-based sensor	35 nM	[49]
Fluorescence	Pyrene derivative	7.8 nM	[50]
Fluorescence	Rhodamine B-containing diarylethene	28.6 nM	[51]
Fluorescence	Cyclam derivative	260 nM	[45]
Absorbance	*N*-Butylbenzene-1,2-diamine	3.97 μM	[52]
Absorbance	Naphthalimide-based Schiff base	10 μM	[53]
Fluorescence	D-GHGGW-COOH	85 nM	This study
(c)			
Fluorescence	Porphyrin-based sensor	53 nM	[54]
Fluorescence	Naphthalimide derivative	2.46 μM	[55]
Fluorescence	QLBA–Cu^{2+} complex	460 nM	[56]
Fluorescence	FITC-P5-Cu	31 nM	[35]
Fluorescence	L-Cu system	75 nM	[36]
Fluorescence	D-P5-Cu	380 nM	This study
Fluorescence	D-P5-Hg	217 nM	This study

For the three samples, similar results and good recovery rates were obtained. These results implied that this chemosensor possesses analytical capability for detection of Cu^{2+} and Hg^{2+} in real water samples.

3.6 | Fluorescence response of DP-5-Hg and DP-5-Cu to S^{2-}

It is very interesting and noteworthy that the D-P5-Hg and D-P5-Cu systems can be used to detect the S^{2-} anion in HEPES buffer solution.

TABLE 2 Determination of Cu^{2+} and Hg^{2+} from three water sample

Water sample	Cu^{2+}			Hg^{2+}		
	Detection	Added	Found	Detection	Added	Found
Sewage water	0.34 μM	5.0 μM	5.4 μM	0 μM	5.0 μM	5.1 μM
River water	0.16 μM	5.0 μM	5.1 μM	0 μM	5.0 μM	4.8 μM
Tap water	0.01 μM	5.0 μM	4.9 μM	0 μM	5.0 μM	5.0 μM

The fluorescence emission spectrum of the D-P5 sensor was not affected by S^{2-} itself (Figure S3), however when S^{2-} was added to the DP-5-Hg/DP-5-Cu solution, the fluorescence intensities for both DP-5-Hg and DP-5-Cu systems were strongly quenched (Figure 8). As shown in Figure 8(a), the fluorescence intensity of D-P5 + Hg^{2+} was quenched significantly after adding S^{2-}, even lower than originally. This result may be explained as S^{2-} competes strongly with Hg^{2+} for the bound amino acid residues of D-P5 to form the Danyl-Hg-S system. This process restored the unfolded D-P5 structure and destroyed the FRET effect between dansyl fluorophore (acceptor) and Trp residue (donor), inducing fluorescence quenching in this system. That the deduced fluorescence intensity was even lower than that of the original may be due to electron transfer between the excited dansyl group and the complexed Hg^{2+}–S^{2-} system.[37] Several common anions such as Ac^-, F^-, Cl^-, ClO_4^-, Br^-, I^-, PO_4^{3-}, CO_3^{2-}, NO_2^-, NO_3^- and, especially, other sulfur-containing ions including SCN^-, SO_4^{2-}, SO_3^{2-} and $S_2O_3^{2-}$ did not affect the spectra of DP-5-Hg and DP-5-Cu systems, inferring that the two systems have selective responses for S^{2-}. Addition of S^{2-} resulted in decrease in fluorescence emission intensity rather than the regeneration of fluorescence intensity reported by Tang and colleagues for the peptide–Cu system sensor for detection of S^{2-}.[34–36] Differences in these two fluorescent quenching behaviors could be

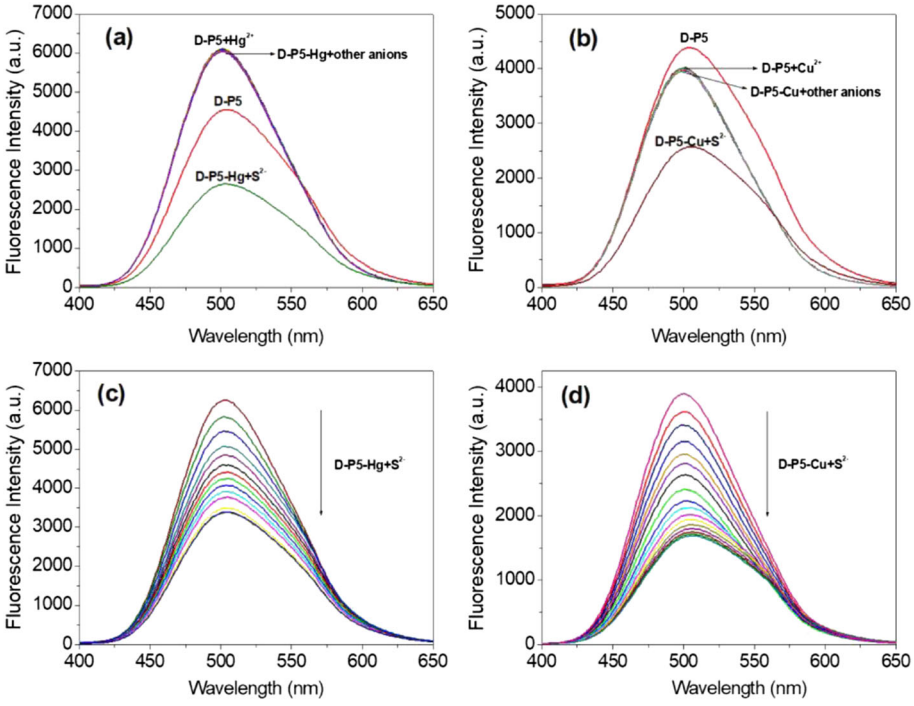


FIGURE 8 Fluorescence emission spectra of D-P5–Hg (a) and D-P5–Cu (b) in the presence of various anions and fluorescence titration of D-P5-Hg (c) and D-P5-Cu (d) following the addition of different concentrations of S^{2-}(D-P5-Hg: 0–200 μM, D-P5-Cu: 0–400 μM) in 50 mM HEPES buffer solution at pH 7.2. Excitation wavelength: 330 nm. D-P5 = 100.0 μM, [Hg^{2+}] = [Cu^{2+}] = 50.0 μM

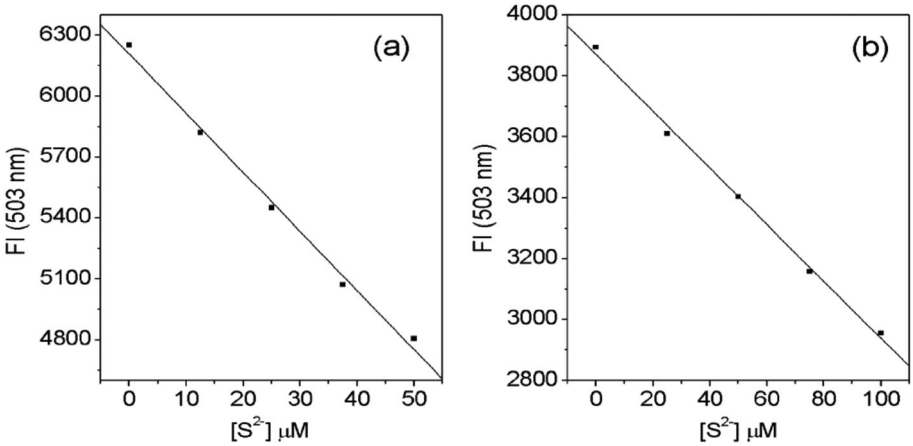


FIGURE 9 Fluorescence intensity at 330 nm of D-P5-Hg (a) and D-P5-Cu (b) in 50 mM HEPES buffer as a function of S^{2-} concentration

attributed to S^{2-} induction of Cu^{2+} release from the peptide–Cu complex and resulted in formation of CuS for the latter; S^{2-} did not induce Cu^{2+} release from the peptide–Cu complex and resulted in the formation of a ternary complex for the former.

It can be seen that fluorescence intensity gradually decreased when increasing concentrations of sulfide ion were added to DP-5-Hg (Figure 8c) and DP-5-Cu (Figure 8d) solutions. For the DP-5-Cu system, the fluorescence emission wavelength was faintly red shift from 500 to 507 nm. This indicated that the sulfide ion interacted with both DP-5-Hg and DP-5-Cu systems, resulting in the formation of new ternary complexes. The fluorescence intensity changes presented a good linear relationship to S^{2-}at low concentrations for these two systems. Based on this analysis method, the LODs of S^{2-} were 217.0 nM for DP-5-Hg and 380.0 nM for DP-5-Cu, respectively (Figure 9). Compared with the previous reported literature (Table 2), D-P5-Hg and D-P5-Cu system also showed good S^{2-} detection performance.

4 | CONCLUSIONS

In summary, we synthesized a novel multifunctional peptidyl chemosensor (Dansyl-Gly-His-Gly-Gly-Trp-COOH) for Hg^{2+} and Cu^{2+} using SPPS. The sensor showed highly selective and sensitive fluorescence responses to Hg^{2+} by a turn-on response and to Cu^{2+} by a turn-off response without interference from the tested metal ions in HEPES buffer solution, which presented a low detection limit for both Hg^{2+} and Cu^{2+}. In addition, it exhibited good practical application for river water samples without performing tedious sample pretreatments. Moreover, we successfully detected sulfide ions using the D-P5-Hg and D-P5-Cu systems. Therefore, these results suggested that a fluorescent peptidyl chemosensor could perform multifunctional monitoring by different mechanisms in environmental systems. We hope that this study promotes the further development of multifunctional probes based on peptides by optimizing modifiable amino acid sequences for many practical applications in environmental and biological systems.

ACKNOWLEDGEMENTS

This work was supported by The Scientific Research Foundation of Liaocheng University, China (318011513), The National Natural Science Foundation of China (21142003, 20471025), and The Natural Science Foundation of Shandong Province of China (ZR2016HB73).

SUPPLEMENTARY MATERIAL

Absorption spectra of D-P5 (20 μM) in the presence of different metal ions (1 equiv.) are shown in Figure S1. Absorption spectra of D-P5 (20 μM) with increasing concentrations of Hg^{2+} (0–0.7 equiv.) (a) and Cu^{2+} (0–0.7 equiv.) (b) in 50 mM HEPES buffer at pH 7.2 are shown in Figure S2. Fluorescence spectra of D-P5 (100 μM) in the absence or presence of S^{2-} (2 equiv.) in 50 mM HEPES buffer at pH 7.2 are shown in Figure S3.

ORCID

Lianzhi Li https://orcid.org/0000-0002-8447-3803

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SUPPORTING INFORMATION

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How to cite this article: Pang X, Wang L, Gao L, Feng H, Kong J, Li L. Multifunctional peptide-based fluorescent chemosensor for detection of Hg^{2+}, Cu^{2+} and S^{2-} ions. *Luminescence*. 2019;34:585–594. https://doi.org/10.1002/bio.3641