RSC Advances

ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/advances

Journal Name

ARTICLE

Single fluorescent probe for reversible detecting copper ions and cysteine in pure water system†

Received 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

www.rsc.org/

Yong Liu, Fangfang Meng and Weiying Lin*

Selective detection of copper ion and cysteine was very challenging, especially in pure water system. In this work, we have engineered a novel fluorescent probe **PI**, which remarkably can reversible detect copper ions (Cu^{2+}) and cysteine(Cys) in pure water system. In addition, the time dependent fluorescence intensity changes of the probe **PI** revealed that the probe showed higher fluorescence intensity within 2 s in the presence of Cu^{2+} and Cys than in the presence of Cu^{2+}. Furthermore, we demonstrated that this probe could effectively detect Cu^{2+} and Cys in living cells. The present study provided a unique strategy which a single fluorescent probe detected multiple targets.

Introduction

Cysteine (Cys) plays a key role in various biological processes including maintaining the homeostasis of biological thiols, protein synthesis, metabolism, post-translational modifications, and detoxification of xenobiotics.[1-3] Abnormal levels of Cys are associated with a variety of diseases such as hair depigmentation, edema, lethargy, liver damage, loss of muscle and fat, skin lesions, and asthma.[4, 5] Similarly, as the third most abundant essential trace element after iron and zinc in the human body, copper ion (Cu^{2+}) in abnormal levels are toxic and can cause oxidative stress and neurological disorders, including Alzheimer's, Parkinson's, Menkes', and Wilson's diseases.[6] Thus, reliable detection of Cu^{2+} and Cys is of great importance and has caused the attention of academic circles.

In the past few years, although a wide variety of techniques have been developed to detect Cu^{2+} and Cys, fluorescence sensing has become the gold standard, due to its high sensitivity, high selectivity, and useful applications in the environment, chemistry, biology, and medicine.[7-11] Thus, the design and synthesis of fluorescent $Cu^{2+}$and Cys probes has received intense attention. First, the fluorescence probes based on reaction-based recognition sites as an important detection means to apply to the detection of Cys (Scheme S1†). Some Cys recognition sites including aldehyde,[12-14] active site,[15] electron-deficient alkynones,[16] disulfide group,[17-19] arenesulfonate ester,[20] acrylate group,[21] selenium-nitrogen bond,[22] rare earth metals,[23] thioester [24] and α, β-unsaturated ketones[25] have been developed. In addition, fluorescent probes for Cu^{2+} have been extensively explored owing to the biological significance of these metal ions.[26-28] However, no precedents exist for reversible detecting Cu^{2+} and Cys in pure water system based on single fluorescent probe.

Thus, in this work, as shown in Fig. 1, we get ready to develop a novel probe **PI**, which was the hybrid of traditional phenanthraquinone and imidazole dye. We envisioned that the free probe **PI** showed strong fluorescence in water. It is known that heavy and transition metal ions have a strong fluorescence quenching character. Thereby, in the presence of Cu^{2+}, the complexation of Cu^{2+} to the compound **PI** will provide the **PI-Cu(II)** ensemble with the overall conjugation breaking, which will lead to fluorescence quenching. We considered that if **PI** can be efficiently quenched by a metal species due to complexation, this may provide a basis for development of fluorescence turn-on sensors for Cys by the displacement method.

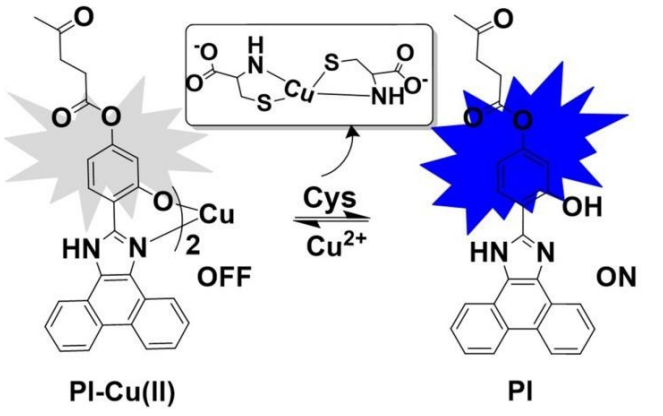


Fig. 1 New strategy for detecting Cys and Cu^{2+} in pure water.

*Institute of Fluorescent Probes for Biological Imaging, School of Chemistry and Chemical Engineering, School of Biological Science and Technology, University of Jinan, Jinan, Shandong 250022, P. R. China. E-mail: weiyinglin2013@163.com

†Electronic Supplementary Information (ESI) available: Experimental procedure, and characterization data See DOI: 10.1039/x0xx00000x.

Experimental

Measurements equipments and materials

Unless otherwise stated, all reagents and experimental materials were purchased from Leon Technology Co. Ltd. and used without further purification. The reagent was analysis pure and used in organic experiment, all the solvents used in optical experiment were chemical pure. TLC analysis was performed on silica gel plates and column chromatography was conducted over silica gel (mesh 200–300), both of which were obtained from the Qingdao Ocean Chemicals. All the reagents used in biological experiments were purchased from the US Molecular Probe Inc. NMR spectra were recorded on an AVANCE III 400 MHz Digital NMR Spectrometer spectrometer, using TMS as an internal standard. Mass spectra were performed using an LCQ Advantage ion trap mass spectrometer from Thermo Finnigan or Agilent 1100 HPLC/MSD spectrometer. The UV-visible absorption spectra were recorded on a Shimadzu UV-2700 spectrophotometer using a quartz cuvette having 1 cm path length. Photoluminescent spectra were recorded at room temperature with a HITACHI F4600 fluorescence spectrophotometer. All image experiments use Nikon ANDOR microscope.

Measurement of the fluorescence quantum yields

In this work, the fluorescence quantum yields (Φ) of the probe **PI** were calculated by means of equation (1): [29-31]

$$\Phi_s = \Phi_r \left(\frac{A_r(\lambda_r)}{A_s(\lambda_s)} \right) \left(\frac{n_s^2}{n_r^2} \right) \frac{F_s}{F_r} \quad (1)$$

Where the subscripts *s* and *r* represent the sample and reference materials, respectively. Φ stands for the quantum yield, *F* expresses the integrated emission intensity, *A* stands for the absorbance, and *n* is the refractive index. In this paper, Fluorescence quantum yields for **PI** was determined by using fluorescein (Φ = 0.95 in water) as fluorescence standard.

Preparation of the test solutions

The stock solution of the probe **PI** was prepared at 1 mM in DMSO. The solutions of various testing species were prepared from KAc, KCl, $CaCl_2$, NaCl, Na_2CO_3, NaF, H_2O_2, $NaHCO_3$, $KHPO_4$, $NaHSO_3$, KI, N_2H_4, $NH_3 \bullet H_2O$, $NaNO_2$, $NaNO_3$, K_3PO_4, $Na_2S_2O_3$, Na_2SO_3, MgCl, $ZnCl_2$, cysteine (Cys), glutathione (GSH), $FeCl_2$, $FeCl_3$, $CoCl_2$ and $CuSO_4 \bullet 5H_2O$ in the twice-distilled water. The test solution of the probe **PI** (10 μM) in 5 mL PBS buffer (pH 7.4). Unless otherwise noted, for titration and selectivity experiments, the excitation wavelength was 365 nm, excitation and emission slit widths were 5 nm and 5 nm.

Cell culture and image

SiHa cells were culture in the medium overnight on a 35 mm petri dish with a cover slip or a plasma-treated 25 mm round cover slip mounted to the bottom of a 35 mm petri dish with an observation window of microscope. In the experiment of using the dye **PI** incubate cell. The SiHa cells were stained with 5 μM **PI** for 20 min.

Control experiment: First, for the experimental group, the culture medium of the cells was changed to a fresh media with 5 μM probe, and then incubated for 20 min. And then the medium was removed and washed three times with PBS to remove the excess probe. Second, we carried out the negative control experiment, in which the culture medium of the cells was changed to a fresh medium with NEM (5 mM) and incubated for 1 h. Then, the medium was removed and washed three times with PBS to remove the excess NEM. After that, 1mL of the medium containing 5 μM probe was added and then incubated for 20 min. Finally, the cells imaging was done by Nikon ANDOR microscope. **PI** was excited at 340-380 nm and the fluorescence was collected at 435-485 nm.

Cell viability evaluated by MTT

In experiments of cell activity, using cell proliferation Kit I with the absorbance of 492 nm being detected using a Perkine Elmer Victor plate reader to detect cell activity. First, counted by cell counter, six thousand cells were seeded per well in a 96-well plate. Second, after overnight culture in a 96-well plate, various concentrations of **PI** ensemble were added into per well of the 96-well plate. After 2h treatment, 20 μL 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyltetrazolium bromide (MTT) solution (5 mg/mL in phosphate buffer solution) was added into the each well of 96-well plate. After 12h incubation in a 96-well plate in 5% CO_2 at 37 °C. 200 μL DMSO was added to dissolve the purple crystals. And further incubate 20 min in 5% CO2 at 37 °C, using a plate reader to read the optical density readings at 492 nm.

Results and discussion

Optical properties

With **PI** in hand, we set out to investigate the solvation effects of the probe in different solvents. Its maximum linear absorption and emission wavelengths in various polarity solvents were in the range of 300–370 nm and 400-550 nm (Fig. 2A and 2B). Its fluorescence intensities were high in organic solvents and very low in aqueous solution (Fig. 2B). The relevant photophysical properties of compound **PI** were summarized in Table 1. From Table 1, one can also found that its fluorescent quantum yields (Φ) were high in organic solvents and low in aqueous solutions.

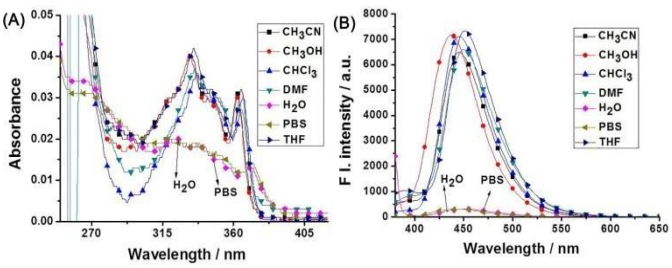


Fig. 2 (A) Absorption spectra and (B) fluorescence responses of **PI** in various solvents. CH_3CN (■), CH_3OH (●), $CHCl_3$ (▲), DMF (▼), H_2O (◆), PBS (◀), THF (▶). [PI]: 10 μM.

Table. 1 The photophysical properties of **PI** in various solvents.

Probe	Solvents	λ^a/nm	λ^b/nm	Stokes shift/nm	Φ^c/%
PI	PBS	370	442	72	11
	H_2O	370	442	72	10
	DMF	365	450	85	96
	MeOH	365	436	71	97
	CH_3CN	365	444	79	83
	THF	365	450	85	87
	CH_3Cl	365	445	80	90

λ^a and λ^b are maximum absorption and maximum fluorescent emission peak, repectively; $^c\Phi$ is fluorescence quantum yield (Error limit: 8%) determined by using fluorescein (Φ = 0.95) in aqueous NaOH (pH 11) as the standard.

Additionally, its Φ values in methanol (Φ=97%) and DMF (Φ=96%) were obviously higher than those in other organic solvents. Although fluorescence intensity was relatively weak in PBS buffer solution, Φ value reached 11%. The results demonstrated that the dye **PI** possessed good water soluble, and could be used as excellent platform for constructing organic fluorescent probe.

Fluorescence response to Cu^{2+} ions

Next, we investigated whether the probe can detect Cu^{2+} ions in aqueous buffer. The fluorescence titration experiment was then carried out (Fig. 3A and inset). When the probe was excited at 365 nm, the fluorescence intensity evidently decreased at 445 nm with the addition of Cu^{2+}. Different from the fluorescence spectra, whole absorption spectra of compound PI was gradually increasing with the increasing Cu^{2+} ions in pH 7.4 PBS at around 365 nm (Fig. S1†).

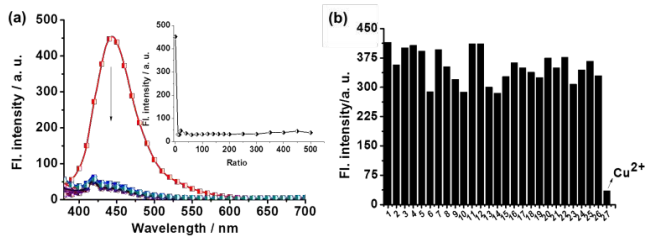


Fig. 3 (A) Fluorescence spectra of compound PI (10 μM) with the increasing concentrations of Cu^{2+} ions (0-500 equiv) in pH 7.4 PBS; (B) Fluorescence intensity (I_{443}) of sensor PI (10 μM) in the presence of various analytes in pH 7.4 PBS. 1, blank; 2, Ac^-; 3, K^+; 4, Ca^{2+}; 5, Na^+; 6, CO_3^{2-}; 7, F^-; 8, H_2O_2; 9, HCO_3^-; 10, HPO_4^-; 11, HSO_3^-; 12, I^-; 13, N_2H_4; 14, NH_3; 15, NO_2^-; 16, NO_3^-; 17, PO_4^{3-}; 18, $S_2O_3^{2-}$; 19, SO_3^{2-}; 20, Mg^{2+}; 21, Zn^{2+}; 22, Cys; 23, GSH; 24, Fe^{2+}; 25, Fe^{3+}; 26, Co^{2+}; 27, Cu^{2+}.

To examine the selectivity, PI was treated with various biologically relevant analytes. As shown in Fig. 3B, addition of Cu^{2+} to the probe PI induced fluorescence quenching at 445 nm. However, no marked changes in the emission were noted upon addition of the representative species such as Ac^-, K^+, Ca^{2+}, Na^+, CO_3^{2-}, F^-, H_2O_2, HCO_3^-, HPO_4^-, HSO_3^-, I^-, N_2H_4, NH_3, NO_2^-, NO_3^-, PO_4^{3-}, $S_2O_3^{2-}$, SO_3^{2-}, Mg^{2+}, Zn^{2+}, and Cys, indicating that the probe had a high selectivity for Cu^{2+}. In addition, the probe showed higher fluorescence intensity than in the presence of Cu^{2+} in different pH buffer solution (Fig. S2†). This was also supported by the observation that different species pH condition and had negligible interference with the fluorescence response. We proposed that Cu^{2+} could coordinate with **PI** and formed the stable species **PI-Cu(II)**.

Fluorescence response to Cys

As we all know, the sulfur atoms of amino acid had very strong coordination ability to metal. We speculated that Cys may move the Cu^{2+} away to free the phenol moiety. The **PI-Cu(II)** ensemble was promising as a turn-on fluorescent sensor for Cys. To further demonstrate the above inference, the dye **PI** was incubated with Cu^{2+} ions, and the resulting ensemble was titrated with Cys in PBS buffer solution (pH 7.4). As shown in Fig. 4A, the free **PI-Cu(II)** was essentially non-fluorescent. However, addition of Cys elicited a dramatic change in the fluorescence spectra. A significant fluorescence turn-on response was observed at 445 nm (extending into 550 nm). And the fluorescence intensity evidently increased and leveled off with the addition of Cys (Fig. 4B). The above results indicated that Cys added to the **PI-Cu(II)** ensemble resulted in the release of the free dye **PI**. Consistent with this observation, treatment of Cys caused a significant fluorescence turn-on response at 445 nm, and up to a 6-fold fluorescence enhancement was observed.

In the selective experiments, the three analytes (GSH, Hcy, sulfur anions) were the biological components which the most likely to interfere with detecting Cys. However, the **PI-Cu(II)** ensemble exhibited highly selective for Cys by the analysis of selective experiments (Fig. 4C), and no marked changes in the emission were noted upon addition of the other species (Fig. 5). Thus, these data demonstrated that the **PI-Cu(II)** ensemble had a high selectivity for Cys over other biological species tested.

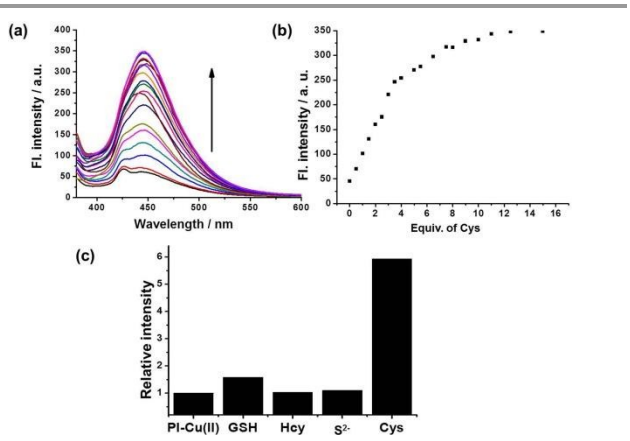


Fig. 4 (A) Fluorescence changes of the **PI-Cu(II)** ensemble with the increasing of Cys (0-16 equiv.) in pH 7.4 PBS buffer solution; (B) Change curve of the fluorescence intensity; (C) Fluorescence intensity (I_{443}) of sensor **PI-Cu(II)** in the presence of various analytes in pH 7.4 PBS. From left to right: **PI-Cu(II)**, GSH, Hcy, S^{2-}, Cys.

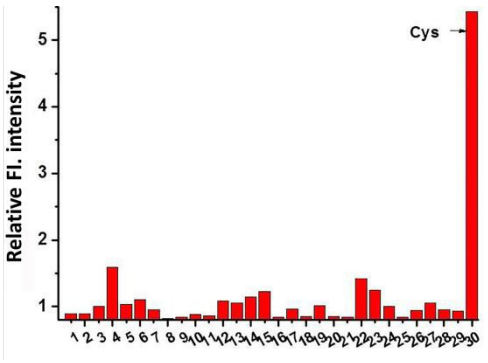


Fig. 5 Fluorescence intensity (I_{443}) of sensor **PI-Cu(II)** in the presence of various analytes in pH 7.4 PBS buffer solution. 1, blank; 2, Ala; 3, Gln; 4, Gly; 5, GSH; 6, Hcy; 7, Ile; 8, Phe; 9, Trp; 10, Tyr; 11, Val; 12, Nac; 13, Ser; 14, Thr; 15, His; 16, AC^-; 17, Br^-; 18, Ca^{2+}; 19, Cl^-; 20, CO_3^{2-}; 21, F^-; 22, H_2O_2; 23, HCO^{3-}; 24, HPO^{4-}; 25, HSO^{3-}; 26, I^-; 27, N_2H_4; 28, NH_3; 29, S^{2-}; 30, Cys.

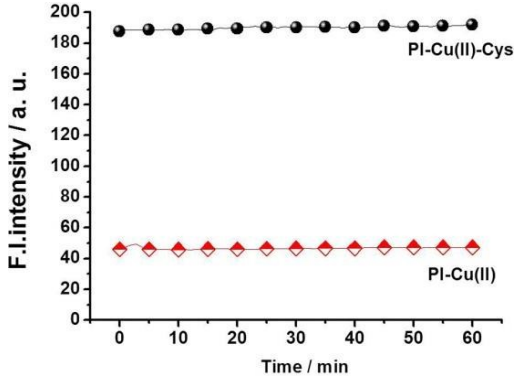


Fig. 6 Changes curve of respond time of the probe **PI-Cu(II)** (PI/Cu(II)=1/250, v:v) and **PI-Cu(II)**-Cys. [**PI**]: 10 μM.

Moreover, the time dependent fluorescence intensity changes of the probe **PI** revealed that the probe showed higher fluorescence intensity within 2 s in the presence of Cu^{2+} and Cys than in the presence of Cu^{2+} (Fig. 6), making it applicable for reversible detecting Cu^{2+} and Cys in pure water system.

We continue to study spectral changes of the **PI-Cu(II)** ensemble in the presence of sulfur anions. As shown in Fig. S3A†, under certain conditions, the **PI-Cu(II)** ensemble showed higher fluorescence intensity in the presence of Cys than sulfide anions. In fluorescence spectroscopic titration experiments, the fluorescence intensity changes of the probe **PI-Cu(II)** ensemble was shown in Fig. S3B†, and fluorescence intensity had no change along with addition of sulfide anions. The results demonstrated that the **PI-Cu(II)** ensemble ruled out the interference of sulfur anions.

Mechanism

To gain insight into the sensing mechanism, we continue to study the sensing process by ^{1}H NMR and HRMS. As shown in Fig. S4†, the addition of 1-2 equiv Cu^{2+} ions to dye **PI** in *d6*-DMSO/D_2O (4:1, v/v) rendered the ^{1}H NMR spectrum vanish due to the complexation of paramagnetic Cu^{2+} to the dye. However, further addition of 3-4 equiv of Cys to the ensemble **PI-Cu(II)** resulted in the spectrum of **PI** recovered. In addition, after treatment of dye **PI** with Cu^{2+} in *d6*-DMSO/D_2O (4:1, v/v), an intense peak at m/z 911.2 corresponding to (2**PI-Cu(II)** + H)$^+$ was present in the HRMS spectrum (Fig. S5†). The results demonstrated that Cu^{2+} could form four- coordinated complexes with dye **PI**. However, the further addition of Cys to the above system will led to the formation of a new peak at 298.2 corresponding to metal complex the 2Cys-Cu(II), and appeared peak of **PI** at 425.1 (Fig. S6†). The results proved that the probe **PI** could reversible detect Cu^{2+} ions and Cys.

Fluorescent imaging and eliminate thiols

The cytotoxicity of the dye PI ensemble was evaluated using commercially compound MTT assay (Fig. S7†).[32, 33] The result indicated that the probe generally present low toxicity for imaging living cell. We then proceeded to examine the ability that the probe monitored Cys in living cells. For proof-of concept, **PI** (5 μM) was initially incubated with SiHa cells for 20 min, and then were treated with different concentration Cu^{2+}. The imaging results showed that the cells incubated with the probe **PI** displayed strong fluorescence in blue channel (Fig. 7B). However, the fluorescence signals of **PI** decreased 72% with increasing of Cu^{2+} (Fig. 7B-G), indicating that Cu^{2+} can coordinate with **PI** and form the stable species in cell.

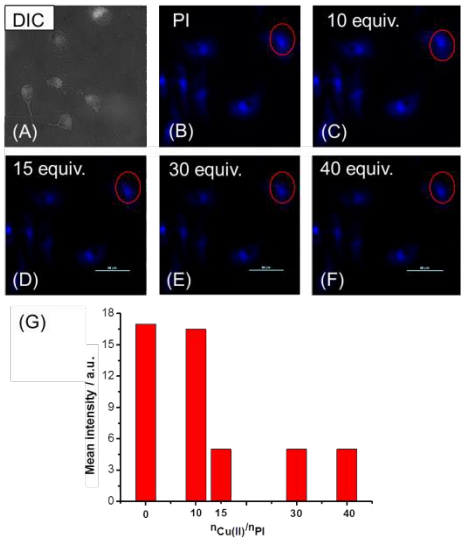


Fig. 7 (A) Differential interference contrast (DIC) images; SiHa cells fluorescence microscope images of the dye **PI** (B), cells treated with Cu^{2+} (C) (10 equiv.), (D) (15 equiv.), (E) (30 equiv.), (F) (40 equiv.), incubated with hydrazine (20 equiv.). (G) Fluorescent changes of within the red circle about (B)-(F). Excitation wavelength: 340-380 nm; Detection wavelengths: 435-485 nm. Scale bar: 50 μm.

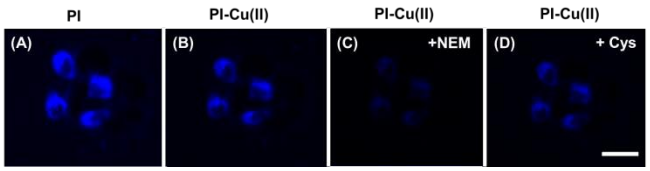


Fig. 8 (A) SiHa cells images of incubated with the probe **PI** (10 μM) for 30 min; (B) Cells Images after adding Cu^{2+} (10 equiv.) to (A) for 1 h; (C) Cells Images after adding NEM (5 mM) to (B) for 1 h; (D) Cells Images after adding Cys (35 equiv.) to (C) for 1h. Excitation wavelength: 340-380 nm; Detection wavelength: 435-485 nm. Scale bar: 50 μm.

As shown in Fig. 8, we first proved that the dye **PI** was capable of emitting strong fluorescence in living cells (Fig. 8A). Second, when the probe **PI-Cu(II)** entered into the cell, and intracellular fluorescence could not be quenched due to existence of endogenous Cys (Fig. 8B). Third, when SiHa cells were pretreated with thiol-blocking reagent N-ethylmaleimide (NEM), 34-36 no fluorescence could be recorded (Fig. 8C). Finally, the cells pre-treated with Cys exhibited enhanced fluorescence in the blue channel (Fig. 8D). The results indicated that the probe **PI-Cu(II)** was capable of imaging endogenous Cys.

Conclusions

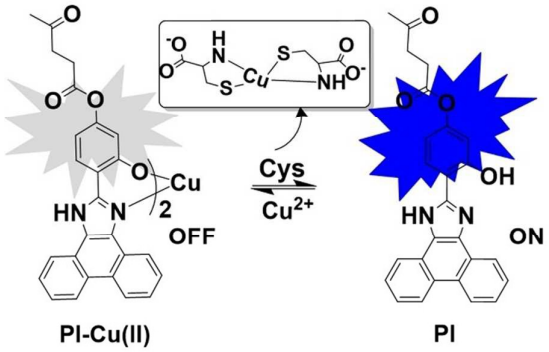
In this work, we have constructed a unique fluorescent probe PI, which remarkably can reversible detect Cu^{2+} and Cys in pure water system. Moreover, we further proved that this novel probe could image Cu^{2+} and Cys in living cells. Compared with previously reported Cu^{2+} and Cys probes, it provided a unique strategy which a single fluorescent probe detected multiple targets. This strategy might open up new opportunities for developing unique molecular tools to reversibly detect target molecules in pure water system.

Acknowledgements

For financial support, this work was financially supported by the NSFC (51503077, 21172063, 21472067), Taishan Scholar Foundation (TS 201511041), NSFSP (ZR2015PE001) and the start-up fund of University of Jinan and doctor start up fund (160082102) of University of Jinan. In addition, we thank Shandong University 2011JC006, Open Project of State Key Laboratory for Supramolecular Structure and Materials (SKLSSM201207), and the NSFC (51303097).

Notes and references

1. M. –Y. Jia, L. –Y. Niu, Y. Zhang, Q. –Z. Yang, C. –H. Tung, Y. –F. Guan and L. Feng, *ACS Appl. Mater. Interfaces*., 2015, **7**, 5907.
2. Z. Yang, N. Zhao, Y. Sun, F. Miao, Y. Liu, X. Liu, Y. Zhang, W. Ai, G. Song, X. Shen, X. Yu, J. Sun and W. –Y. Wong, *Chem. Comm*., 2012, **48**, 3442.
3. V. V. Mummidivarapu, S. D. S. Yarramala, K. K. Kondaveeti and C. P. Rao, *J. Org. Chem*., 2014, **79**, 10477.
4. S. R. Lentz and W. G. Haynes, *Clin. J. Med*., 2004, **71**, 729.
5. S. Seshadri, A. Beiser, J. Selhub, P. F. Jacques, I. H. Rosenberg, R. B. D'Agostino and P. W. F. Wilson, *New Engl. J. Med*., 2002, **346**, 476.
6. D. G. J. Barceloux and D. Barceloux, *J. Clin. Toxicol*., 1999, **37**, 217.
7. J. Liu, Y. –Q. Sun, Y. Huo, H. Zhang, L. Wang, P. Zhang, D. Song, Y. Shi and W. Guo, *J. Am. Chem. Soc*., 2014, **136**, 574.
8. T. Jiang, D. Guo, Q. Wang, X. Wua, Z. Li, Z. Zheng, B. Yin, L. Xia, J. Tang, W. Luo, N. Xia and Y. Jiang, *Anal. Chim. Acta*., 2015, **876**, 77.
9. X. -L. Ni, X. Zeng, C. Redshaw and T. Yamato, *J. Org. Chem*., 2011 **76**, 5696.
10. D. -P. Li, Z. -Y. Wang, X. -J. Cao, J. Cui, X. Wang, H. -Z. Cui, J. -Y. Miao and B. -X. Zhao, 2016, DOI: 10.1039/c5cc09092j .
11. M. Yu, X. Wu, B. Lin, J. Han, L. Yang and S. Han, *Anal. Chem*., 2015, **87**, 6688.
12. S. J. Lim, O. Escobedo, M. Lowry, X. Y. Xu and R. Strongin, *Chem. Commun*., 2010, **46**, 5707.
13. H. L. Li, J. L. Fan, J. Y. Wang, M. Z. Tian, J. J. Du, S. G. Sun, P. P. Sun and X. J. Peng, *Chem. Commun*., 2009, **39**, 5904.
14. W. Wang, O. Rusin, X. Xu, K. Kim, J. O. Escobedo, S. O. Fakayode, K. A. Fletcher, M. Lowry, C. M. Schowalter, C. M. Lawrence, F. R. Fronczek, I. M. Warner and R. M. Strongin, *J. Am. Chem. Soc*., 2005, **127**, 15949.
15. M. Zhang, M. Yu, F. Li, M. Zhu, M. Li, Y. Gao, L. Li, Z. Liu, J. Zhang, D. Zhang, T. Yi and C. Huang, *J. Am. Chem. Soc*., 2007, **129**, 10322.
16. H. –Y. Shiu, H. –C. Chong, Y. –C. Leung, M. –K. Wong and C. –M. Che, *Chem. Eur. J*., 2010, **16**, 3308.
17. J. H. Lee, C. S. Lim, Y. S. Tian, J. H. Hee and B. R. Cho, *J. Am. Chem. Soc*., 2010, **132**, 1216.
18. B. Zhu, X. Zhang, Y. Li, P. Wang, H. Zhang and X. Zhuang, *Chem. Commun*., 2010, **46**, 5710.
19. X. Cao, W. Lin and Q. Yu, *J. Org. Chem*., 2011, **76**, 7423.
20. X. Li, S. J. Qian, Q. J. He, B. Yang, J. Li and Y. Z. Hu, *Org. Biomol. Chem*., 2010, **8**, 3627.
21. Y. Liu, D. Yu, S. Ding, Q. Xiao, J. Guo and G. Feng, *ACS Appl. Mater. Interfaces.*, 2014, **6**, 17543.
22. B. Tang, Y. L. Xing, P. Li, N. Zhang, F. B. Yu and G. W. Yang, *J. Am. Chem. Soc*., 2007, **129**, 11666.
23. H. Chen, X. Li, R. Bai, Y. Wu and Y. Fan, *J. Organometallics*., 2013, **32**, 6226.
24. H. Lv, X. –F. Yang, Y. Zhong, Y. Guo, Z. Li and H. Li, *Anal. Chem*., 2014, **86**, 1800.
25. H. S. Jung, K. C. Ko, G. –H. Kim, A. –R. Lee, Y. –C. Na, C. Kang, J. Y. Lee and J. S. Kim, *Org. Lett*., 2011,**13**, 1498.
26. C. Yua, L. Chen, J. Zhang, J. Li, P. Liu, W.Wang and B. Yan, *Talanta*, 2011, **85**, 1627.
27. C. Yu, J. Zhang, J. Li, P. Liu, P. Wei and L. Chen, *Microchim. Acta*., 2011, **174**, 247.
28. C. Yu, J. Zhang, R. Wang and L. Chen, *Org. Biomol. Chem.*, 2010, **8**, 5277.
29. D. Magde, G. E. Rojas and P. Seybold, *Photochem. Photobiol*., 1999, **70**, 737.
30. D. Oushiki, H. Kojima, T. Terai, M. Arita, K. Hanaoka, Y. Urano and T. Nagano, *J. Am. Chem. Soc*., 2010, **132**, 2795.
31. J. Fan, X. Liu, M. Hu, H. Zhu, F. Song and X. Peng, *Anal. Chim. Acta*., 2012, **735**, 107.
32. Y. –W. Duan, X. –F. Yang, Y. Zhong, Y. Guo, Z. Li and H. Li, *Anal. Chim. Acta*., 2015, **859**, 59.
33. L. Li, Z. Li, W. Shi, X. Li and H. Ma, *Anal. Chem*., 2014, **86**, 6115.
34. P. Lee, A. Gorrell, H. J. Fromm and R. F. Colman, *Biochem*., 1999, **38**, 5754.
35. Y. Zhang, Y. Li and X. –P. Yan, *Anal. Chem*., 2009, **81**, 5001.
36. M. Tian, F. Guo, Y. Sun, W. Zhang, F. Miao, Y. Liu, G. Song, C. –L. Ho, X. Yu, J. –Z. Sun and W. –Y. Wong, *Org. Biomol. Chem*., 2013, **11**, 7721.



In this work, we have engineered a novel fluorescent probe PI, which remarkably can reversible detect copper ion and cysteine in pure water system for the first time.