Highly sensitive and selective turn-on fluorescent chemosensor for Pb^{2+} and Hg^{2+} based on a rhodamine-phenylurea conjugate[†]

Zhi-Qiang Hu,*^a Cun-sheng Lin,^a Xiao-Ming Wang,^a Lei Ding,^a Cun-Li Cui,^a Shu-Feng Liu*^a and Hai Yan Lu*^b

Received 25th January 2010, Accepted 23rd March 2010 First published as an Advance Article on the web 16th April 2010 DOI: 10.1039/c001587c

A novel easily available turn-on fluorescent chemosensor RPU based on a rhodamine–phenylurea conjugate was synthesized, which showed highly selective and sensitive recognition toward Pb^{2+} in CH₃CN and Hg²⁺ in aqueous media over a wide range of tested metal ions with remarkably enhanced fluorescent intensities and also clear color changes from colorless to pink.

The detection of heavy metal ions is an important issue because contamination with heavy metal ions may have severe effects on human health and the environment.¹ Lead(II) and mercury ions are two of the most toxic heavy metal ions. The accumulation of lead in the body can result in neurological, reproductive, cardiovascular and developmental disorders² while mercury can cause a wide variety of diseases such as prenatal brain damage, serious cognitive and motion disorders.³ Currently, the common techniques for detection of lead(II) ion and mercury ion, such as atomic absorption spectrometry and inductively coupled plasma mass spectrometry, are often expensive, complex, and unsuitable for on-site analyses. Therefore, the development of new analytical methods for the sensitive and selective determination of Pb^{2+} and Hg^{2+} , is highly desirable. Because of its operational simplicity, low cost, real time monitoring and high selectivity, fluorescent detection has become the promising strategy used for Pb²⁺ and Hg²⁺ detection.⁴

Recently, considerable efforts have been undertaken to develop fluorescent probes for ead(II) ion,⁵ and mercury ions.⁶ However, many reported fluorescent chemosensors generally undergo fluorescence quenching upon binding with these two metal ions, which is not as sensitive as a fluorescence enhancement response. In addition, the fluorescence enhancement in most cases is small and usually suffers from a high background. In particular, most of the reported fluorescent chemosensors can selectively sense only one of them. The only example reported by Liu *et al.* that can detect these two metal ions are involved in the use of DNA,⁷ which usually lack the simplicity that a small-molecule fluorescence probe can offer. For practical

applications, the development of a small molecule probe that can sense both $Pb^{2\,+}$ and $Hg^{2\,+}$ simultaneously is more desirable.

The rhodamine framework is an ideal mode to construct fluorescent chemosensors due to its excellent photophysical properties such as long absorption and emission wavelength, large absorption coefficient and high fluorescence quantum yield.8 Several rhodamine-based probes for mercury ion have been recently developed.9 In contrast, only few rhodamine based probes for lead(II) has been reported.¹⁰ Most of the reported rhodamine fluorescent probes are based on rhodamine hydrazide derivatives or rhodamine amine conjugates.¹¹ Herein, we report a new turn-on fluorescent chemosensor based on rhodamine-phenylurea conjugate (RPU), which can sensitively and selectively detect Pb²⁺ in CH₃CN, and Hg²⁺ in aqueous media and display remarkably enhanced fluorescence intensities and clear color changes upon recognition. To the best of our knowledge, this is the first example of a fluorescent sensor based on a small molecule that allows the detection of both Pb^{2+} and Hg^{2+} ions.

Synthesis of **RPU** is shown in Scheme 1. Reaction of rhodamine B base 1 with POCl₃ and then NH₃ afforded 2, which further reacted with phenyl isocyanate to give **RPU** in 47% yield. Similarly, rhodamine–phenylthiourea conjugate (**RPTU**) was also synthesized by the reaction of 2 and phenyl isothiocyanate. The structures of **RPU** and **RPTU** were confirmed by their ¹H NMR, ¹³C NMR, MS spectra and EA (Fig. S1–S4, ESI†).

All solutions of **RPU** in CH₃CN and CH₃CN–H₂O are colorless. The absorption spectra of **RPU** (10 μ M) in CH₃CN exhibited only very weak bands over 500 nm. Addition of 50 equiv. Pb²⁺ into CH₃CN solution immediately resulted in a significant enhancement of absorbance at about 560 nm and a shoulder peak appeared at 525 nm (Fig. S5, ESI†). Under the identical condition, only a very mild increase of absorbance at 564 nm was observed with the same amount of Hg²⁺ while no



Scheme 1 Synthesis of RPU and RPTU.

^a College of Chemistry and Molecular Engineering, Qingdao University of Science and Technology, Qingdao 266042, China. E-mail: huzhiqiang@iccas.ac.cn; Fax: + 86-0532-84023405

^b College of Chemistry and Chemical Engineering, Graduate

University of Chinese Academy of Sciences, Beijing 100049, China † Electronic supplementary information (ESI) available: Apparatus and reagents; synthesis and structural characterization of **RPU** and **RPTU**, absorption spectral responses of **RPU** for Pb²⁺ and Hg²⁺, Job plot of complex, reversibility analysis of **RPU** and Hg²⁺, competitive experiments, fluorescence response of **RPTU** for Hg²⁺. See DOI: 10.1039/c001587c



Fig. 1 Fluorescence intensity of **RPU** (1 μ M) in (a) CH₃CN upon the addition of 100 equiv. metal ions and (b) CH₃CN–H₂O (3/7, v/v) upon the addition of 130 equiv. metal ions (Pb²⁺, Hg²⁺, Mg²⁺, Ce³⁺, Cd²⁺, Na⁺, Cu²⁺, K⁺, Ag⁺, Co²⁺, Zn²⁺, Ni²⁺, Ba²⁺, Ca²⁺). $\lambda_{ex} = 530$ nm.

obvious response could be observed upon the addition of Mg^{2+} , Ce^{3+} , Cd^{2+} , Na^+ , Zn^{2+} , K^+ , Cu^{2+} , Ag^+ , Co^{2+} , Ni^{2+} , Ca^{2+} , Ba^{2+} into CH_3CN solution. Fig. 1a shows the fluorescence spectra ($\lambda_{ex} = 530 \text{ nm}$) of **RPU** (1 μ M) measured in CH₃CN with given metal cations (100 equiv.). RPU shows a very weak fluorescence in the absence of metal ions. Its fluorescence quantum yield is about 0.003 with rhodamine B in methanol as standard. However, the addition of Pb^{2+} resulted in a remarkably enhanced fluorescence at 579 nm (about 1000-fold), and the fluorescence quantum yield is 0.32. The color of the solution also changed from colorless to pink-red. This strongly suggested that RPU can serve as a "naked eve" probe for Pb^{2+} . Under the same conditions, additions of Hg²⁺ only induced about 40-fold fluorescence enhancement at 575 nm but no color change of the solution. Addition of other metals led to no obvious fluorescent enhancements and color changes. Similar results could be obtained upon changing the solvent from CH₃CN to CH₃COCH₃.

The response of **RPU** to Pb^{2+} is significantly affected by the content of water in solution. No obvious absorption and emission response could be observed in CH₃CN-H₂O (3/7, v/v). This suggests that water molecule could compete with RPU for coordination to Pb^{2+} and result in degradation of the emission response.¹² However, addition of Hg²⁺ into CH₃CN-H₂O (3/7, v/v) solution of **RPU** resulted in a significant enhancement of absorbance (Fig. S6, ESI⁺) and fluorescence at 584 nm (Fig. 1b) with the change of color from colorless to pink. Additions of other metal ions to CH₃CN-H₂O (3/7, v/v) solution of RPU caused no obvious response. Herein, it should be pointed out that the same phenomenon could be also observed in solutions with other volume ratios of water and CH₃CN (1:4 to 4:1) (Fig. S7, ESI[†]). Moreover, addition of Hg(ClO₄)₂ made the fluorescence of RPU increase as strongly as that of Hg(NO₃)₂ and Hg(AcO)₂ (Fig. S8, ESI[†]). Furthermore, addition of KI to a solution containing RPU and Hg²⁺ led to immediate disappearance of both pink color and fluorescence (Fig. S9, ESI[†]). This indicates that RPU reversibly coordinates with Hg^{2+} . These results show that **RPU** can selectively and sensitively sense Pb^{2+} and Hg^{2+} in the different media.

Fluorescence titrations of **RPU** with Pb^{2+} in CH₃CN and Hg^{2+} in CH₃CN–H₂O (3:7, v/v) were then performed. As shown in Fig. 2, an increase of fluorescence intensity could be observed with increasing Pb^{2+} and Hg^{2+} concentration. Binding analysis using the method of continuous variations (Job plot)

established a 2:1 stoichiometry for the **RPU**–Pb²⁺ complex and a 1:1 stoichiometry for the **RPU**–Hg²⁺ complex (Fig. S10 and S11, ESI[†]). The different binding stoichiometry between **RPU**–Pb²⁺ and **RPU**–Hg²⁺ was also confirmed by the Benesi– Hidebrand method (Fig. S12 and S13, ESI[†]). The association constants were further determined to be 7.4 × 10⁷ M⁻¹ for the **RPU**–Pb²⁺ complex and 3.08 × 10⁷ M⁻² for the **RPU**–Hg²⁺ complex. The inset pictures (Fig. 2) show relative intensities (*I*/*I*₀) *vs.* the concentrations of Pb²⁺ and Hg²⁺ in the low concentration region up to 7 × 10⁻⁹ and 3.5 × 10⁻⁸ M, respectively. These revealed the detection limits of **RPU** for Pb²⁺ and Hg²⁺ around 7 × 10⁻⁹ and 3.5 × 10⁻⁸ M, respectively.

The possible interferences by other metal ions were assessed through competitive experiments. The fluorescence changes of **RPU** in CH₃CN were measured by the treatment of 10 equiv. Pb²⁺ ions in the presence of 200 equiv. other interfering metal ions including Zn²⁺, Mg²⁺, Ca²⁺, Cd²⁺, Cu²⁺, Ba²⁺, K⁺, Co²⁺, Ce³⁺, Na⁺, Ag⁺, Ni²⁺, Hg²⁺. The tested background metal ions showed small or no interference with the detection of Pb²⁺ ion. Similarly, the competitive experiments also confirmed that background metal ions showed small or no interference with the detection of Hg²⁺ ion in CH₃CN–H₂O (3/7, v/v) (Fig. S14, ESI[†]).

The binding mechanism was preliminarily investigated through ¹H NMR. In the spectrum of **RPU**, the resonance



Fig. 2 (a) Fluorescence titration spectra of **RPU** (1 μ M) with Pb²⁺ (0–100 μ M) in CH₃CN; Inset: the fluorescence at 579 nm of **RPU** (0.1 μ M) as a function of the Pb²⁺ concentration (0–7 × 10⁻⁹ M); (b) Fluorescence titration spectra of **RPU** (1 μ M) with Hg²⁺ (0–130 μ M) in CH₃CN–H₂O (3/7, v/v); Inset: the fluorescence at 575 nm of **RPU** (0.1 μ M) as a function of the Hg²⁺ concentration (0–3.5 × 10⁻⁸ M). $\lambda_{ex} = 530$ nm.

for NH appears at 10.92 ppm. Addition of Hg²⁺ into the solution led to an apparent upfield shift to 10.63 ppm. From this observation it can be deduced that NH does not participate in the complexation of **RPU** and Hg²⁺. The downshift peaks near 6.3 ppm, which were assigned to the proton signals of xanthene, proved the delocalization of xanthene. With the addition of Pb²⁺ instead of Hg²⁺, similar results in ¹H NMR of RPU could be also obtained (Fig. S15, ESI⁺). To further explore the binding mechanism, RPTU was synthesized as a substitute of RPU and tested for fluorescence changes upon the addition of Hg^{2+} and Pb^{2+} ions. Unlike **RPU**, **RPTU** exhibited high selectivity and sensitivity for Hg²⁺ in CH₃CN (Fig. S16-S19, ESI[†]). However, in the CH₃CN-H₂O system, additions of the tested metal ions including Hg^{2+} or Pb^{2+} ions, created no obvious fluorescence enhancements and color changes. This confirmed that the C=O group in RPU played a crucial role in the complexation with Hg^{2+} or Pb^{2+} . From these data, it could be deduced that the possible coordination atoms for Pb²⁺ and Hg²⁺ were two O atoms. The exact binding modes between **RPU** and Hg^{2+} or Pb^{2+} are being investigated.

In conclusion, we have reported a novel easily available turn-on fluorescent chemosensor based on a rhodamine– phenylurea conjugate. It displayed an excellent selectivity and a high sensitivity toward the detection of Pb^{2+} in CH_3CN and Hg^{2+} in aqueous media over a wide range of tested metal ions with remarkably enhanced fluorescent intensities and also clear color changes from colorless to pink. The background metal ions showed small or no interference with the detection of Pb^{2+} or Hg^{2+} ion. Moreover, compared with the reported probes for Pb^{2+} or Hg^{2+} , this is the first chemosensor based on a small molecule that can detect both Pb^{2+} and Hg^{2+} at 1 nM and 10 nM level, respectively. We believe that the **RPU** can be used for many practical applications in chemical, environmental and biological systems.

The authors are grateful for the financial support from the National Natural Science Foundation of China No. 20602022 and Natural Science Foundation of Shandong province No. Y2007B39.

Notes and references

- 1 L. M. Campbell, D. G. Dixon and R. E. Hecky, J. Toxicol. Environ. Health, Part B, 2003, 6, 325.
- 2 J. S. Liu-Fu, Lead Poisoning, A Century of Discovery and Rediscovery, in *Human Lead Exposure*, ed. H. L. Needleman, Lewis Publishing, Boca Raton, FL, 1992.

- 3 (a) N. Basu, A. Scheuhammer, N. Grochowina, K. Klenavic, F. Evans, M. Obrien and M. Chan, *Environ. Sci. Technol.*, 2005, **39**, 3585; (b) P. Grandjean, P. hihe, R. F. White and F. Debes, *Environ. Res.*, 1998, **77**, 165.
- 4 (a) A. W. Czarnik, Fluorescent Chemosensors for Ion and Molecule Recognition, in *Supramolecular Chemistry, Fluorescence, and Sensing*, ch. 1, 1993, pp. 1–9; (b) A. P. de Silva, H. Q. N. Gunaratne, T. Gunnlaugsson, A. J. M. Huxley, A. C. P. McCoy, J. T. Rademacher and T. E. Rice, *Chem. Rev.*, 1997, 97, 1515.
- 5 (a) L. Marbella, B. Serli-Mitasev and P. Basu, Angew. Chem., Int. Ed., 2009, 48, 3996; (b) H. Y. Lee, D. R. Bae, J. C. Park, H. Song, W. S. Han and J. H. Jung, Angew. Chem., Int. Ed., 2009, 48, 1239; (c) F. Zapata, A. Caballero, A. Espinosa, A. Tárraga and P. Molina, Org. Lett., 2008, 10, 41; (d) K.-C. Chang, I.-H. Su, A. Senthilvelan and W.-S. Chung, Org. Lett., 2007, 9, 3363; (e) L.-J. Ma, Y.-F. Liu and Y. Wu, Chem. Commun., 2006, 2702; (f) K. Kavallieratos, J. M. Rosenberg, W.-Z. Chen and T. Ren, J. Am. Chem. Soc., 2005, 127, 6514; (g) K. Kavallieratos, J. M. Rosenberg and J. C. Bryan, Inorg. Chem., 2005, 44, 2573.
- 6 Some recent examples of Hg^{2+} -probe see: (a) E. M. Nolan and S. Lippard, Chem. Rev., 2008, 108, 3443; (b) M. G. Choi, Y. H. Kim, J. E. Namgoong and S.-K. Chang, Chem. Commun., 2009, 3560; (c) M. H. Lee, S. W. Lee, S. H. Kim, C. Kang and J. S. Kim, Org. Lett., 2009, 11, 2101; (d) W. Jiang and W. Wang, Chem. Commun., 2009, 3913; (e) M. Suresh, S. Mishra, S. K. Mishra, M. E. Suresh, A. K. Mandal, A. Shrivastav and A. Das, Org. Lett., 2009, 11, 2740; (f) M. Zhu, M. Yuan, X. Liu, X. Xu, J. Lv, C. Huang, H. Liu, Y. Li, S. Wang and D. Zhu, Org. Lett., 2008, 10, 1481; (g) M. G. Choi, D. H. Ryu, H. L. Jeon, S. Cha, J. Cho, H. H. Joo, K. S. Hong, C. Lee, S. Ahn and S. Chang, Org. Lett., 2008, 10, 3717; (h) X. Xue, F. Wang and X. J. Liu, J. Am. Chem. Soc., 2008, 130, 3244; (i) R. R. Avirah, K. Jyothish and D. Ramaiah, Org. Lett., 2007, 9, 121; (j) K. C. Song, J. S. Kim, S. M. Park, K.-C. Chung, S. Ahn and S. Chang, Org. Lett., 2006, 8, 3413; (k) Q.-Y. Chen and C.-F. Chen, Tetrahedron Lett., 2005, 46, 165
- 7 The only reported example of a DNA-based sensor for lead(II) and mercury(II) ions: C.-W. Liu, C.-C. Huang and H.-T. Chang, *Anal. Chem.*, 2009, **81**, 2383.
- 8 For a review, see: H. N. Kim, M. H. Lee, H. J. Kim, J. S. Kim and J. Yoon, *Chem. Soc. Rev.*, 2008, **37**, 1465.
- 9 (a) W. Shi and H. Ma, Chem. Commun., 2008, 1856; (b) X. Zhang, Y. Xiao and X. Qian, Angew. Chem., Int. Ed., 2008, 47, 8025; (c) J. Huang, Y. Xu and X. Qian, J. Org. Chem., 2009, 74, 2167; (d) Y. Shiraishi, S. Sumiya, Y. Kohno and T. Hirai, J. Org. Chem., 2008, 73, 8571; (e) W. Huang, C. Song, C. He, G. Lv, X. Hu, X. Zhu and C. Duan, Inorg. Chem., 2009, 48, 5061.
- 10 J. Y. Kwon, Y. J. Jang, Y. J. Lee, K. M. Kim, M. S. Seo, W. Nam and J. Yoo, J. Am. Chem. Soc., 2005, **127**, 10107.
- 11 M. Beija, C. A. M. Afonso and J. M. G. Martinho, *Chem. Soc. Rev.*, 2009, **38**, 2410.
- 12 D. Ray and P. K. Bharadwaj, Inorg. Chem., 2008, 47, 2252.