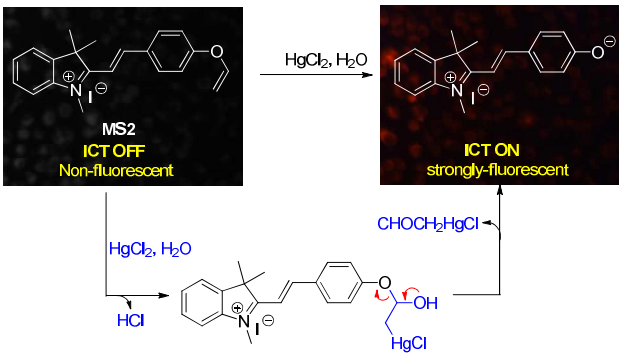
RSC Advances

ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/advances



A novel hemicyanine-based fluorescence turn-on chemodosimeter for Hg^{2+} by mercury triggered hydrolysis of vinylether group has been reported.

Cite this: DOI: 10.1039/c0xx00000x

www.rsc.org/xxxxxx

ARTICLE TYPE

A Highly Sensitive Hemicyanine-Based Fluorescent Chemodosimeter for Mercury Ions in Aqueous Solution and Living Cells

Yu Chen, Chengyu Yang, Zhenni Yu, Bo Chen, Yifeng Han*

Received (in XXX, XXX) Xth XXXXXXXXX 20XX, Accepted Xth XXXXXXXXX 20XX

DOI: 10.1039/b000000x

A novel hemicyanine-based fluorescence turn-on chemodosimeter for Hg^{2+} by mercury triggered hydrolysis of vinylether group has been reported. The probe has the unique advantages of easy-preparation, good water solubility, excellent selectivity, high sensitivity, and fast response time (~ 60 sec) towards Hg^{2+} in aqueous solution. Furthermore, the probe is demonstrated to be qualified to detect Hg^{2+} in living cells.

Mercury is one of the most ubiquitous and poisonous heavy metals.[1] Mercury ions are not biodegradable, and hence can concentrate through the food chain in the tissues of fish and marine mammals. Excess mercury accumulation may induce strong damage to the central nervous system, various cognitive and motor disorders, and Minamata disease.[2] Therefore, the determination of mercury in biological and environmental samples is crucial both to the monitoring of environmental pollution and to the diagnosis of clinical disorders. Whereas standard techniques, such as atomic absorption-emission spectrometry,[3] inductively coupled plasma mass spectrometry,[4] and anodic stripping voltammetry,[5] often require expensive and sophisticated instrumentation, and/or sample preparation, and are therefore not suitable for real-time and in situ analysis.

Optical sensors involving fluoroionophores are becoming popular because of their ease of application in solution as well as their high sensitivity to and selectivity for trace analytes with spatial and temporal resolution.[6] In the past several years, considerable efforts have been made to develop fluorescent chemosensors for Hg^{2+} based on the coordination of Hg^{2+} to heteroatom-based ligands, Hg^{2+} catalyzed desulfurization, and Hg^{2+} promoted hydrolysis of the vinyl ether group and β-alkynyl ether group.[7] However, most of them still have limitations such as interference from other coexisting metal ions, poor water-solubility, and long response time.[8] Therefore, for practical applications, it is still strongly desirable to develop fluorescent sensors with good water solubility, high sensitivity, and quick response for real-time detection of Hg^{2+}.

Merocyanine dye is a well-known chromophore exhibiting strong intramolecular charge transfer (ICT) due to "push-pull" substituent pairs which results in a large spectral shift. To date, a number of probes have been reported based on the hemicyanine moiety for different analytes through the

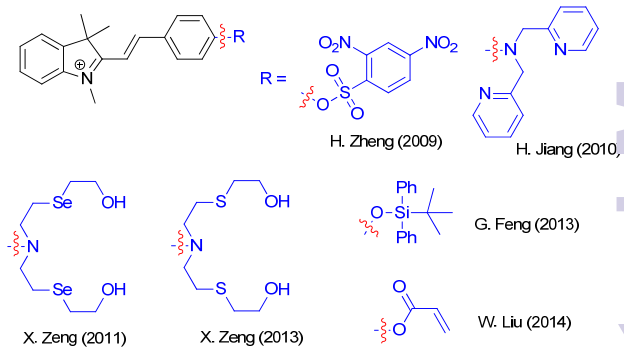
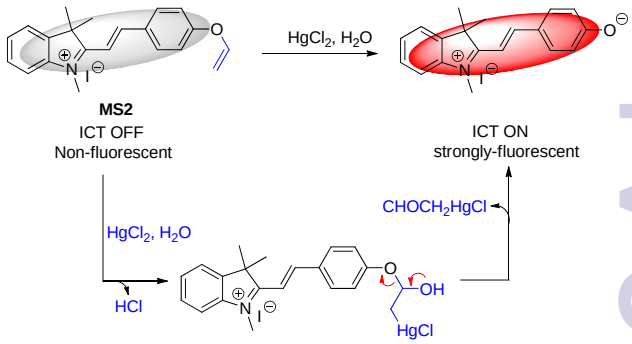


Fig. 1 Hemicyanine-based probes.

modulation of ICT efficiency (Fig. 1).[9] Inspired by these works, we report here a novel and simple hemicyanine-based fluorescence turn-on chemodosimeters **MS2**, a vinylether derivative of hemicyanine, for the detection of Hg^{2+}. The design of probe **MS2** is based on the mercury ion-promoted hydrolysis reaction of vinyl ethers. It is well known that the oxymercuration of vinyl ether will generate the corresponding hemiacetal intermediate, which undergoes fragmentation to release free hydroxyl group.[7j,7n,10] We envisioned that the protection of the hydroxyl group of hemicyanine with a vinyl group would block the ICT process. However, the deprotection of the electron-withdrawing vinyl enol ether group of **MS2** by Hg^{2+} promoted hydrolysis reaction would releases a hydroxy donor which will increase the "push-pull" character of the hemicyanine dye and recover its ICT property (Scheme 1).

As shown in Scheme S1 (ESI†), **MS2** can be readily



Scheme 1 Mercury triggered hydrolysis of **MS2**.

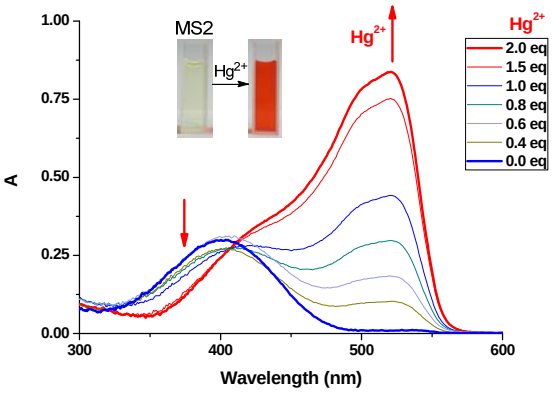


Fig. 2 Absorption spectra of **MS2** (20.0 μM) in PBS buffer solution (10 mM, pH = 7.4, containing 1% CH_3CN) in the presence of different concentrations of Hg^{2+} (0-2.0 equiv.).

prepared in three convenient steps under facile conditions with high yield starting with commercially available 4-hydroxybenzaldehyde. The product (**MS2**) was well characterized by ^1H, ^{13}C NMR, and HR-MS (ESI†).

We firstly assessed the UV-vis spectroscopic properties of **MS2** in PBS buffer solution (10 mM, pH = 7.4, containing 1% CH_3CN). **MS2** (20.0 μM) displayed a moderate UV-vis absorption around 400 nm. Upon incremental addition of Hg^{2+} (0-2.0 equiv.), the peak at 400 nm slightly decreased, and a new band at 520 nm, which is characteristic of hemicyanine fluorophore,[9f] appeared instantly with a clear isosbestic point at 412 nm (Fig. 2). Furthermore, a good linear relationship was observed between the changes in the absorbance at 520 nm with Hg^{2+} in the range of 0-30.0 μM (Fig. S1, ESI†).

The emission spectra of **MS2** and its fluorescence titration with Hg^{2+} were recorded in PBS buffer (10 mM, pH = 7.4, containing 1% CH_3CN). As expected, **MS2** alone is almost non-fluorescent (λ_{ex} = 510 nm, Φ = 0.004, Table S1, ESI†). However, upon progressive addition of Hg^{2+}, the emission band at 551 nm rapidly increased (Fig. 3), which was attributed to the cleavage of vinyl enol group by mercury ion promoted hydrolysis reaction and the formation of free ICT

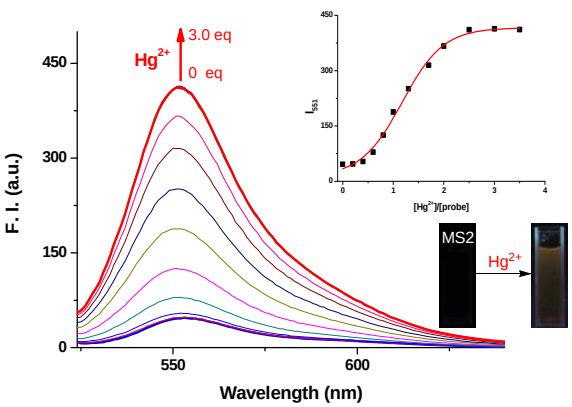


Fig. 3 Fluorescence spectra of **MS2** (10.0 μM) in PBS buffer solution (pH = 7.4, containing 1% CH_3CN) in the presence of different concentrations of Hg^{2+} (0-30.0 μM) (λ_{ex} = 510 nm). Inset: fluorescence intensity changes as a function of Hg^{2+} concentration.

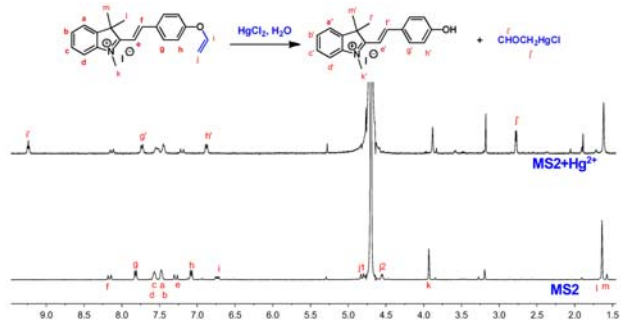


Fig. 4 ^1H NMR spectra of **MS2** in the absence and presence of Hg^{2+} (1.0 equiv. in D_2O).

active hemicyanine fluorophore (Scheme 1). Moreover, the fluorescence titration curve revealed that the fluorescence intensity at 551 nm increased linearly with increasing concentration of Hg^{2+} (R^2 = 0.99) (Fig. 3 and S2, ESI†) and further smoothly increased until a maximum was reached up to 25.0 μM Hg^{2+} (λ_{ex} = 510 nm, Φ = 0.018, Table S1, ESI†).

Efforts were then made to check the detecting mechanism as envisioned that the Hg^{2+} induced hydrolytic cleavage of vinyl enol ether group of **MS2** to the free hemicyanine. To this end, ^1H NMR titration experiment was conducted. As shown in Fig. 4, the three olefinic protons at δ 6.74 (dd, J = 13.5, 5.6 Hz, 1H), 4.81 (d, J = 13.5 Hz, 1H), and 4.55 (d, J = 5.6 Hz, 1H) attributed to the vinyl enol ether group of **MS2**, disappeared after the addition of Hg^{2+}. Meanwhile, chemical shifts of three protons at δ 9.24 (t, J = 5.2 Hz, 1H), and 2.78 (d, J = 5.2 Hz, 2H) attributed to the (2-oxoethyl) mercury chloride were discovered, indicating that the hydrolytic cleavage of vinyl enol ether group of **MS2** occurred in the presence of Hg^{2+} (Scheme 1). Furthermore, the aromatic protons at δ 7.08 (d, J = 8.4 Hz, 2H) attributed to H-h were dramatically shifted upfield after the addition of Hg^{2+} due to the deprotection process strengthens the electron-donating ability from oxygen atom. Those results are in agreement with the optical responses. We also carried out the HPLC-MS measurements for the **MS2**-Hg^{2+} solution (Fig. S10, ESI†). All those results agree well with the proposed Hg^{2+} induce

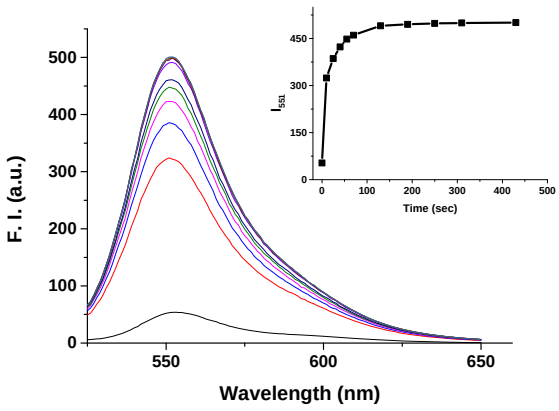


Fig. 5 Time-dependent fluorescence intensity changes of **MS2** (10.0 μM) upon addition of Hg^{2+} (3.0 equiv.) in PBS buffer solution (pH = 7.4, containing 1% CH_3CN) (λ_{ex} = 510 nm). Inset: fluorescence intensity changes as a function of response time.

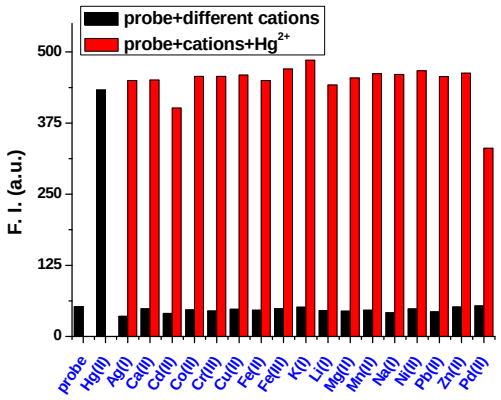


Fig. 6 Fluorescence responses of **MS2** to various metal ions (including Hg^{2+}, Ag^{+}, Ca^{2+}, Cd^{2+}, Co^{2+}, Cr^{3+}, Cu^{2+}, Fe^{2+}, Fe^{3+}, K^{+}, Li^{+}, Mg^{2+}, Mn^{2+}, Na^{+}, Ni^{2+}, Pb^{2+}, Zn^{2+}, and Pd^{2+}). Black bars represent the addition of 3.0 equiv. of the appropriate metal ion to a 10.0 μM solution of **MS2** (in PBS buffer solution, pH = 7.4, containing 1% CH_3CN). Red bars represent the addition of 3.0 equiv. of Hg^{2+} to the solutions containing **MS2** (10.0 μM) and the appropriated metals (3.0 equiv.) (λ_{ex} = 510 nm).

deprotection of **MS2**.

Subsequently, the time-dependence of **MS2** fluorescence was evaluated in the presence of Hg^{2+} in PBS buffer (10 mM, pH = 7.4, containing 1% CH_3CN). The results showed that the fluorescence of tested solutions remarkably increased to their maximum value within 1 minute (Fig. 5), which indicated that **MS2** features the fastest response chemodosimeters to date for Hg^{2+}.

Further, the fluorescence titration of **MS2** with various metal ions was conducted to examine the selectivity (Fig. 6, and S3, ESI†). Much to our delight, the examined alkali, alkaline-earth metal ions, transition metal ions, and even Pd^{2+} ions showed nominal changes to the fluorescence spectra of **MS2**. It should be mentioned that **MS2** still responded to Hg^{2+} sensitively even in the presence of other relevant competing ions (Fig. 6, and S4, ESI†). Therefore, these results suggest that **MS2** displays high selectivity toward Hg^{2+} in aqueous solution.

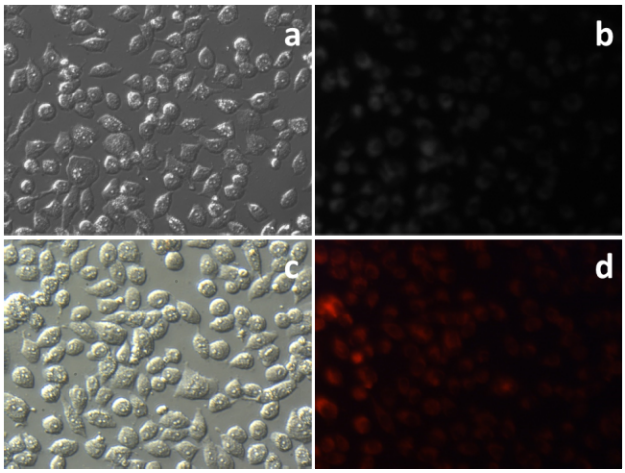


Fig. 7 Fluorescence image of HeLa cells incubated with **MS2** (5.0 μM) for 0.5 h, and then washed quickly with PBS for imaging (b). The cells were then treated with $HgCl_2$ (10.0 μM) for 0.5 h which resulted in a dramatic increase in intracellular red fluorescence (d). (a), (c) Bright-field images of live cells in (b) and (d).

pH impacts on the fluorescence of **MS2** and the **MS2**-Hg^{2+} system were also investigated. As depicted in Fig. S11, ESI†, **MS2** alone was inert to pH in the range of 5.5-9.8. But in the presence of Hg^{2+}, the fluorescence response of **MS2** decreased as pH of test solutions decreased, which was attributed to the formation of enol-form of free hemicyanine fluorophore by mercury-induced hydrolytic cleavage of probe (Scheme S2, ESI†).[9f] Moreover, it reacted more difficultly with Hg^{2+} when increased the pH of test solutions due to the reaction rate of mercury ion-promoted hydrolysis of vinyl enol ether becomes slow at high pH value.[7j,10b] However, satisfactory Hg^{2+}-sensing abilities were exhibited in the range of pH from 7.0 to 9.0, indicating that **MS2** could be used in living cells without interference from pH effects.

Due to the favorable properties of **MS2** in vitro, the potential utility of **MS2** in living cells was studied. HeLa cells were incubated with 5.0 μM of **MS2** for 30 min at 37 ^{o}C and exhibited only weak fluorescence (Fig. 7b). The cells were then treated with $HgCl_2$ (10.0 μM) for 30 min at 37 ^{o}C, which resulted in a dramatic increase of intracellular red fluorescence (Fig. 7d). These indicated that **MS2** was cell membrane permeable and capable of image Hg^{2+} in living cells.

In conclusion, we have rationally developed a novel and simple hemicyanine-based sensitive fluorescence turn-on chemodosimeter for Hg^{2+} *via* mercury triggered hydrolysis reaction. The probe has the unique advantages of easy-preparation, good water solubility, excellent selectivity, high sensitivity, and fast response towards Hg^{2+} in aqueous solution. Furthermore, fluorescence imaging of Hg^{2+} in living cells indicates that this probe is favorable for biological applications.

This work was supported by the National Natural Science Foundation of China (20902082), the Graduate Innovative Research Program of Zhejiang Sci-Tech University (YCX14002), and the Program for Innovative Research Team of Zhejiang Sci-Tech University (13060052-Y).

Notes and references

Department of Chemistry, The Key Laboratory of Advanced Textile Materials and Manufacturing Technology, Zhejiang Sci-Tech University, Hangzhou, 310018, China.
E-mail: zstuchem@gmail.com; Tel: +86-571-86843550

† Electronic Supplementary Information (ESI) available: Experimental details, characterization of the compounds, and additional spectroscopic data. See DOI: 10.1039/b000000x/

1 (*a*) L. Magos, *Met. Ions Biol. Syst*., 1997, **34**, 321-370; (*b*) M. F. Wolfe, S. Schwarzbach and R. A. Sulaiman, *Environ. Toxicol. Chem*., 1998, **17**, 146-160; (*c*) P. B. Tchounwou, W. K. Ayensu, N. Ninashvili and D. Sutton, *Environ. Toxicol*., 2003, **18**, 149-175; (*d*) P. Grandjean, P. Weihe, R. F. White and F. Debes, *Environ. Res*., 1998, **77**, 165-172.

2 (*a*) C. R. Baum, *Curr. Opin. Pediatr*., 1999, **11**, 265-268; (*b*) E. K. Silbergeld, I. A. Silva and J. F. Nyland, *Toxicol. Appl. Pharmacol*., 2005, **207**, S282-S292; (*c*) R. K. Zalups and S. Ahmad, *J. Am. Soc*.

Nephrol., 2004, **15**, 2023-2031; (*d*) Z. Zhang, X. Guo, X. Qian, Z. Lu and F. Liu, *Kidney Int*., 2004, **66**, 2279-2282; (*e*) J. Huang, X. Ma, B. Liu, L. Cai, Q. Li, Y. Zhang, K. Jiang and S. Yin, *J. Lumin*., 2013, **141**, 130-140.

3 (*a*) E. Kopysc, K. Pyrzynska, S. Garbos, E. Bulska, *Anal. Sci.*, 2000, **16**, 1309-1312; (*b*) A. Bernaus, X. Gaona, J. M. Esbrí, P. Higueras, G. Falkenberg, M. Valiente, *Environ. Sci. Technol*., 2006, **40**, 4090-4095.

4 (*a*) D. Karunasagar, J. Arunachalam, S. Gangadharan, *J. Anal. Atom. Spectrom*., 1998, **13**, 679-682; (*b*) B. Fong, W. Mei, T. S. Siu, J. Lee, K. Sai, S. Tam, *J. Anal. Toxicol*., 2007, **31**, 281-287.

5 (*a*) O. Abollino, A. Giacomino, M. Malandrino, G. Piscionieri and E. Mentasti, *Electroanalysis*, 2008, **20**, 75-83; (*b*) L. H. Marcolino-Junior, B. C. Janegitz, B. C. Lourencao, and O. Fatibello-Filho, *Anal. Lett*., 2007, **40**, 3119-3128.

6 (*a*) M. Y. Berezin and S. Achilefu, *Chem. Rev*., 2010, **110**, 2641-2684; (*b*) K. P. Carter, A. M. Young and A. E. Palmer, *Chem. Rev*., 2014, **114**, 4564-4601; (*c*) E. M. Nolan and S. J. Lippard, *Chem. Rev*., 2008, **108**, 3443-3480; (*d*) X. Chen, T. Pradhan, F. Wang, J. S. Kim and J. Yoon, *Chem. Rev*., 2012, **112**, 1910-1956; (*e*) V. S. Lin, W. Chen, M. Xian and C. J. Chang, *Chem. Soc. Rev*., 2015, doi: 10.1039/C4CS00298A; (*f*) H. Zhu, J. Fan, B. Wang and X. Peng, *Chem. Soc. Rev*., 2015, doi: 10.1039/C4CS00285G; (*g*) R. T. K. Kwok, C. W. T. Leung, J. W. Y. Lam and B. Z. Tang, *Chem. Soc. Rev*., 2015, doi: 10.1039/C4CS00325J; (*h*) J. Yin, Y. Hu and J. Yoon, *Chem. Soc. Rev*., 2015, doi: 10.1039/C4CS00275J; (*i*) M. Kaur and D. H. Choi, *Chem. Soc. Rev*., 2015, **44**, 58-77; (*j*) Z. Guo, S. Park, J. Yoon and I. Shin, *Chem. Soc. Rev*., 2014, **43**, 16-29; (*k*) L. Yuan, W. Lin, K. Zheng, L. He and W. Huang, *Chem. Soc. Rev*., 2013, **42**, 622-661; (*l*) J. Fan, M. Hu, P. Zhan and X. Peng, *Chem. Soc. Rev*., 2013, **42**, 29-43; (*m*) J. Du, M. Hu, J. Fan and X. Peng, *Chem. Soc. Rev*., 2012, **41**, 4511-4535; (*n*) K. Wu, Y. Gao, Z. Yu, F. Yu, J. Jiang, J. Guo and Y. Han, *Anal. Methods*, 2014, **6**, 3560-3563; (*o*) X. Li, Y. Gong, K. Wu, S. H. Liang, J. Cao, B. Yang, Y. Hu and Y. Han, *RSC Adv*., 2014, **4**, 36106-36109; (*p*) X. Li, C. Yang, K. Wu, Y. Hu, Y. Han and S. H. Liang, *Theranostics*, 2014, **4**, 1233-1238; (*q*) J. Liu, K. Wu, S. Li, T. Song, Y. Han and X. Li, *Dalton Trans*., 2013, **42**, 3854-3859; (*r*) J. Liu, K. Wu, X. Li, Y. Han and M. Xia, *RSC Adv*., 2013, **3**, 8924-8928; (*s*) X. Li, S. Zhang, J. Cao, N. Xie, T. Liu, B. Yang, Q. He and Y. Hu, *Chem. Commun*., 2013, **49**, 8656-8658.

7 (*a*) W. Xuan, C. Chen, Y. Cao, W. He, W. Jiang, K. Liu and W. Wang, *Chem. Commun*., 2012, **48**, 7292-7294; (*b*) M. Vedamalai and S. P. Wu, *Org. Biomol. Chem*., 2012, **10**, 5410-5416; (*c*) J. Liu, Y. Q. Sun, P. Wang, J. Zhang and W. Guo, *Analyst*, 2013, **138**, 2654-2660; (*d*) F. Song, S. Watanabe, P. E. Floreancig, and K. Koide, *J. Am. Chem. Soc*., 2008, **130**, 16460-16461; (*e*) H. Jiang, J. Jiang, J. Cheng, W. Dou, X. Tang, L. Yang, W. Liu and D. Bai, *New J.Chem*., 2014, **38**, 109-114; (*f*) L. Chen, L. Yang, H. Li, Y. Gao, D. Deng, Y. Wu and L. Ma, *Inorg. Chem*., 2011, **50**, 10028-10032; (*g*) M. Saleem, R. Abdullah, A. Ali, B. J. Park, E. H. Choi, I. S. Hong and K. H. Lee, *Anal. Methods*, 2014, **6**, 3588-3597; (*h*) Q. Li, M. Peng, H. Li, C. Zhong, L. Zhang, X. Cheng, X. Peng, Q. Wang, J. Qin and Z. Li, *Org. Lett*., 2012, **14**, 2094-2097; (*i*) J. Ding, H. Li, C. Wang, J. Yang, Y. Xie, Q. Peng, Q. Li and Z. Li, *ACS Appl. Mater. Interfaces*, 2015, **7**, 11369-11376; (*j*) M. Santra, B. Roy and K. H. Ahn, *Org. Lett*., 2011, **13**, 3422-3425; (*k*) S. Ando and K. Koide, *J. Am. Chem. Soc*., 2011, **133**, 2556-2566; (*l*) J. Jiang, W. Liu, J. Cheng, L. Yang, H. Jiang, D. Bai and W. Liu, *Chem. Commun*., 2012, **48**, 8371-8373; (*m*) S. Zhang, J. Geng, W. Yang and X. Zhang, *RSC Adv*., 2014, **4**, 12596-12600; (*n*) B. Gu, L. Huang, N. Mi, P. Yin, Y. Zhang, X. Tu, X. Luo, S. Luo and S. Yao, *Analyst*, 2015, **140**, 2778-2784; (*o*) G. Chen, Z. Guo, G. Zeng and L. Tang, *Analyst*, 2015, **140**, 5400-5443; (*p*) P. Mahato, S. Saha, P. Das, H. Agarwalla and A. Das, *RSC Adv*., 2014, **4**, 36140-36174.

8 (*a*) M. Tian and H. Ihmels, *Chem. Commun*., 2009, 3175-3177; (*b*) N. Kumari, N. Dey, S. Jha and S. Bhattachary, *ACS Appl. Mater. Interfaces*, 2013, **5**, 2438-2445; (*c*) S. Madhu, R. Kalaiyarasi, S. K. Basu, S. Jadhav and M. Ravikanth, *J. Mater. Chem. C*, 2014, **2**, 2534-2544; (*d*) M. Tian, L. Liu, Y. Li, R. Hu, T. Liu, H. Liu, S. Wang and Y. Li, *Chem. Commun*., 2014, **50**, 2055-2057; (*e*) Y. Han, C. Yang, K. Wu, Y. Chen, B. Zhou and M. Xia, *RSC Adv*., 2015, **5**, 16723-16726.

9 (*a*) S.-P. Wang, W.-J. Deng, D. Sun, M. Yan, H. Zheng, and J.-G. Xu, *Org. Biomol. Chem*., 2009, **7**, 4017-4020; (*b*) H.-H. Wang, L. Xue, Z.-J. Fang, G.-P. Li, and H. Jiang, *New J. Chem*., 2010, **34**, 1239-1242; (*c*) Y. Li, S. He, Y. Lu, and X. Zeng, *Org. Biomol. Chem*., 2011, **9**, 2606-2609; (*d*) Y. Li, F. Wei, Y. Lu, S. He, L. Zhao, X. Zeng, *Dyes Pigments*, 2013, **96**, 424-429; (*e*) S. Yang, Y. Liu, and G. Feng, *RSC Adv*., 2013, **3**, 20171-20178; (*f*) Q. Han, Z. Shi, X. Tang, L. Yang, Z. Mou, J. Li, J. Shi, C. Chen, W. Liu, H. Yang, and W. Liu, *Org. Biomol. Chem*., 2014, **12**, 5023-5030.

10 (*a*) M. Santra, D. Ryu, A. Chatterjee, S.-K. Ko, I. Shin and K. H. Ahn, *Chem. Commun*., 2009, 2115-2117; (*b*) Y. S. Cho, K. H. Ahn, *Tetrahedron Lett*., 2010, **51**, 3852-3854.