ORGANIC LETTERS

2013
Vol. 15, No. 3
492–495

A Fluorescent Ratiometric Chemodosimeter for Cu^{2+} Based on TBET and Its Application in Living Cells

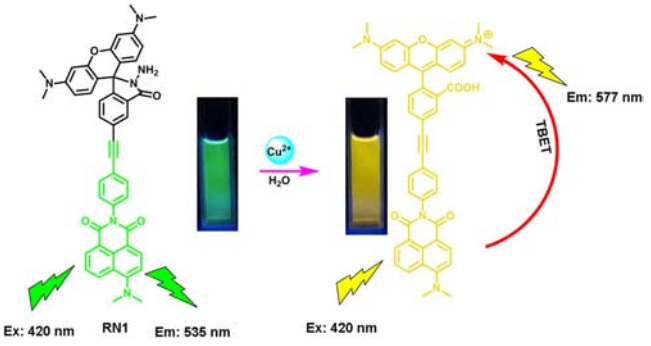
Jiangli Fan,* Peng Zhan, Mingming Hu, Wen Sun, Jizhou Tang, Jingyun Wang, Shiguo Sun, Fengling Song, and Xiaojun Peng*

State Key Laboratory of Fine Chemicals, Dalian University of Technology, No. 2 Linggong Road, High-tech District, Dalian 116024, China

fanjl@dlut.edu.cn; pengxj@dlut.edu.cn

Received November 30, 2012

ABSTRACT



Based on a through bond energy transfer (TBET) between Rhodamine and a naphthalimide fluorophore, a fluorescent ratiometric chemodosimeter RN1 was designed and prepared for single selective detection of Cu^{2+} in aqueous solution and in living cells, as Cu^{2+} acts as not only a selective recognizing guest but also a hydrolytic promoter.

Copper, the third most abundant transition metal in the human body, is vital for both environmental and biological systems. Nevertheless, at high concentrations, copper becomes a toxic and hazardous element to organisms such as many bacteria.[1] Owing to its toxicity to bacteria, elevated concentrations of copper hamper the self-purification capability of the sea or rivers and destroys the biological reprocessing systems in water. Furthermore, alterations in the cellular homeostasis of copper ions can cause neurodegenerative diseases, such as Menkes and Wilson's diseases,[2] familial amyotrophic lateral sclerosis, prion disease, and Alzheimer's disease,[3] probably by its involvement in the production of reactive oxygen species. Therefore, considerable efforts have been devoted to develop Cu^{2+}-selective fluorescent probes due to the nondestructive, quick, and sensitive advantages of emission signals.[4]

Most reported Cu^{2+} fluorescent probes were based on fluorescence intensity.[5] Although turn-on probes are more sensitive due to the lack of background signal, a major limitation is that variations in the sample environment (pH, polarity, temperature, and so forth) might influence the fluorescence intensity measurements. Besides an internal charge transfer (ICT) mechanism using a single fluorophore to obtain ratiometric changes, the exploration of

(1) Barranguet, C.; van den Ende, F. P.; Rutgers, M.; Breure, A. M.; Greijdanus, M.; Sinke, J. J.; Admiraal, W. *Environ. Toxicol. Chem.* **2003**, *22*, 1340.

(2) Waggoner, D. J.; Bartnikas, T. B.; Gitlin, J. D. *Neurobiol. Dis.* **1999**, *6*, 221.

(3) Løvstad, R. A. *Biomaterials* **2004**, *17*, 111.

(4) Krämer, R. *Angew. Chem., Int. Ed.* **1998**, *37*, 772.

(5) (a) Boens, N.; Leen, V.; Dehaen, W. *Chem. Soc. Rev.* **2012**, *41*, 1130. (b) Ruan, Y.-B.; Li, C.; Tang, J.; Xie, J. *Chem. Commun.* **2010**, *46*, 9220. (c) Swamy, K. M. K.; Ko, S.-K.; Kwon, S. K.; Lee, H. N.; Mao, C.; Kim, J.-M.; Lee, K.-H.; Kim, J.; Shin, I.; Yoon, J. *Chem. Commun.* **2008**, *45*, 5915. (d) Wang, D.; Shiraishi, Y.; Hirai, T. *Chem. Commun.* **2011**, *47*, 2673. (e) Yin, S.; Leen, V.; Snick, S. V.; Boens, N.; Dehaen, W. *Chem. Commun.* **2010**, *46*, 6329. (f) Yu, M.; Shi, M.; Chen, Z.; Li, F.; Li, X.; Gao, Y.; Xu, J.; Yang, H.; Zhou, Z.; Yi, T.; Huang, C. *Chem.—Eur. J.* **2008**, *14*, 6892. (g) Que, E. L.; Domaille, D. W.; Chang, C. J. *Chem. Rev.* **2008**, *108*, 1517. (h) Huang, L.; Wang, X.; Xie, G.; Xi, P.; Li, Z.; Xu, M.; Wu, Y.; Bai, D.; Zeng, Z. *Dalton Trans.* **2010**, *39*, 7894. (i) Dujols, V.; Ford, F.; Czarnik, A. W. *J. Am. Chem. Soc.* **1997**, *119*, 7386.

10.1021/ol3032889
Published on Web 01/22/2013

multifluorophores with energy donor−acceptor architectures can achieve large pseudo-Stokes shifts, meanwhile affording simultaneous recorded ratio signals of two emission intensities at different wavelengths, which could provide a built-in correction for the environmental effects. Förster Resonance Energy Transfer (FRET) is generally the most adopted methodology for addressing this issue. Förster Resonance Energy Transfer (FRET) is generally the most adopted methodology for addressing this issue. Normally, dyes based on FRET processes are usually linked by a nonconjugated spacer, and the energy transfer occurs through space. For FRET to be effective, a substantial spectral overlap for the donor emission and acceptor absorption bands is required, which sometimes restrict the choice and the design of these kinds of probe molecules. The dyes based on TBET (through-bond energy transfer) are the ones which have a donor connected to an acceptor via electronically conjugated linkers which prevent the donor and acceptor fragments from becoming planar, and the energy transfer occurs through a bond. For a TBET system there is no such prerequisite, thus it exhibits fast energy transfer rates, large pseudo-Stokes shifts, and flexibility in fluorophores.[6] Actually, to the best of our knowledge, there are a few fluorescent probes for Hg^{2+} reported on TBET (naphthalimide appended rhodamine)[7] and just only one for Cu^{2+} with two different approaches which has not been applied in living cells.[8] Thus, it is important to develop ratiometric fluorescent probes for Cu^{2+} with favorable chemical and spectroscopic properties suitable for the imaging of Cu^{2+} in living cells.

As the fluorescence of the naphthalimide moiety was often quenched probably due to the efficient photoinduced electron transfer (PET) from the amide of rhodamine to the naphthalimide fluorophore, the probes could not exhibit any ratiometric fluorescence for metal ion detection. To solve this problem, herein, we report a ratiometric fluorescent chemodosimeter **RN1** for Cu^{2+} based on TBET, in which (4-*N*,*N*-dimethylamino)-1,8-naphthalide (energy donor) and rhodamine (energy acceptor) were linked by a rigid and conjugated spacer 4-ethynylaniline at the naphthalic anhydride position. A hydrazide functional group was selected as the potential reaction site for Cu^{2+}.[9]

Fortunately, this connection efficiently prevented the fluorescence quenching of naphthalimide. In the absence of Cu^{2+}, the excited energy of the naphthalimide donor could not be transferred to the rhodamine acceptor, as the rhodamine acceptor is in the closed form. Thus, only the emission of the dye naphthalimide is observed. A Cu^{2+}-induced process could change the emission maximum of the system from 535 nm (the characteristic peak of naphthalimide) to 577 nm (the characteristic peak of rhodamine). This wavelength shift allows highly selective ratiometric detection of Cu^{2+} both in acetonitrile/water solution and in living cells.

To explore the mechanism, an **RN1**-Cu^{2+} complex was detected by TOF mass spectrum analysis (Figure S14). A peak at m/z 725.2745 corresponding to intermediate **6** was observed after the addition of Cu^{2+} to **RN1** aqueous solution, which suggested that Cu^{2+} induces the hydrolysis and opening of the spirolactam ring of the rhodamine moiety.

RN1 was synthesized on the basis of the route shown in Scheme 1. Compounds **4** and **3** were successfully linked together by a Sonogashira reaction between terminal alkynyl and bromine in the presence of $PdCl_2(PPh_3)_2$, PPh_3, and CuI as the catalyst to afford **6** in 75% yield. Finally, **RN1** was obtained by refluxing **6** and hydrazine hydrate for 6 h in ethanol with a yield of 74%. All the new intermediates and **RN1** were well characterized by ^1H NMR, ^{13}C NMR, and TOF-MS (Figures S9−S24).

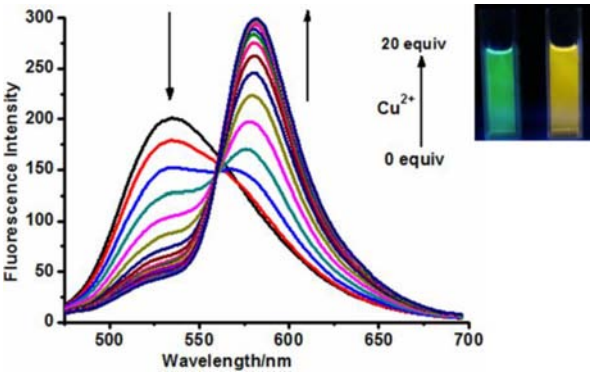
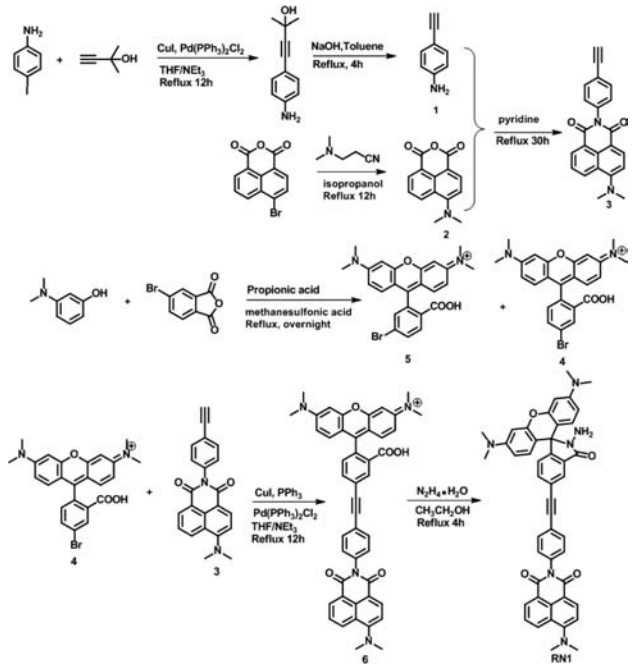


Figure 1. Fluorescence ratio of **RN1** (5 μM) in response to the presence of Cu^{2+} (0−20 equiv) in CH_3CN/H_2O (20:1, v/v) buffered with Tris-HCl (pH 7.4, 10 mM). λ_{ex} = 420 nm. Inset showing the fluorescence before and after the addition of Cu^{2+}.

The absorption and emission properties (Figure S1) of **RN1** (5 μM) were investigated in CH_3CN/Tris-HCl buffer (v/v = 20:1, pH 7.4). In the absence of Cu^{2+}, **RN1** showed one absorption band at 431 nm due to the naphthalimide moiety. On addition of Cu^{2+} (0−20 equiv), a new absorption band appeared at 546 nm corresponding to the rhodamine acceptor. Such a large red shift (115 nm) in absorption behavior changed the color of the solution from yellow-green to pink, allowing colorimetric detection of Cu^{2+} by the naked eye. Accordingly, as shown in Figure 1, upon excitation at 420 nm, the free **RN1** displays a single emission band centered at 535 nm, attributed to the emission of the naphthalimide moiety. There is no TBET in the free **RN1**, as the rhodamine acceptor is in a ring-closed form. Addition of Cu^{2+} significantly decreases the fluorescence intensity at around 535 nm, and simultaneously a

(6) Fan, J.; Hu, M.; Zhan, P.; Peng, X. *Chem. Soc. Rev.* **2013**, *42*, 29.
(7) (a) Kumar, M.; Kumar, N.; Bhalla, V.; Singh, H.; Sharma, P. R.; Kaur, T. *Org. Lett.* **2011**, *13*, 1422. (b) Mahato, P.; Saha, S.; Suresh, E.; Di Liddo, R.; Parnigotto, P. P.; Conconi, M. T.; Kesharwani, M. K.; Ganguly, B.; Das, A. *Inorg. Chem.* **2012**, *51*, 1769. (c) Luxami, V.; Verma, M.; Rani, R.; Paul, K.; Kumar, S. *Org. Biomol. Chem.* **2012**, *10*, 8076.
(8) Zhang, J. F.; Zhou, Y.; Yoon, J.; Kim, Y.; Kim, S. J.; Kim, J. S. *Org. Lett.* **2010**, *12*, 3852.
(9) (a) Dujols, V.; Ford, F.; Czarnik, A. W. *J. Am. Chem. Soc.* **1997**, *119*, 7386. (b) Yuan, L.; Lin, W.; Chen, B.; Xie, Y. *Org. Lett.* **2012**, *14*, 432.

Scheme 1. Design and Synthesis of TBET-Based Ratiometric Fluorescent Cu^{2+} Chemodosimeter **RN1**



new red-shifted emission band at around 577 nm gradually increases. These changes in the fluorescence spectrum stopped when the amount of added Cu^{2+} reached 20 equiv of the probe. At this amount the ratio of the emission intensities at 577 and 535 nm (F577/F535) became as high as 10 times that in the absence of Cu^{2+}. The optimized spatial structure of compound **6** (by GaussView 5.0, Figure S7) shows that there is a 5° torsion angle between the naphthalimide donor and rhodamine acceptor, which further ensures the TBET process between the two fluorophores. Therefore, although the donor emission has overlap with the acceptor absorption in the system, the energy transfer should be mainly based on TBET. Actually, TBET is sometimes accompanied by FRET.[10] The energy transfer efficiency was calculated as 81.3%.[11] The detection limit (3σ/slope)[12] was as low as 3.88×10^{-7} M (Figure S4) for Cu^{2+} which is sufficiently low for the detection of the submillimolar concentration range of Cu^{2+} found in many chemical systems.

Other metal ions such as Na^+, Cd^{2+}, Ni^{2+}, Pd^{2+}, Zn^{2+}, K^+, Hg^{2+}, Co^{2+}, Fe^{3+}, Mn^{2+}, Cr^{3+}, Mg^{2+}, and Ag^+ (Figure 2) as well as anions such as S^{2-}, ClO, I, Cl, HPO_4, Ac, Br, ClO_4, CO_3^{2-}, NO_3, and H_2PO_4 (Figure 3) found in environmental and biological settings exhibited almost no change in emission behavior. They also did not interfere with the fluorescence ratiometric detection of Cu^{2+} by the fluorescence spectrum of **RN1** in CH_3CN/H_2O (20:1, v/v) buffered with Tris-HCl (pH 7.4, 10 mM).

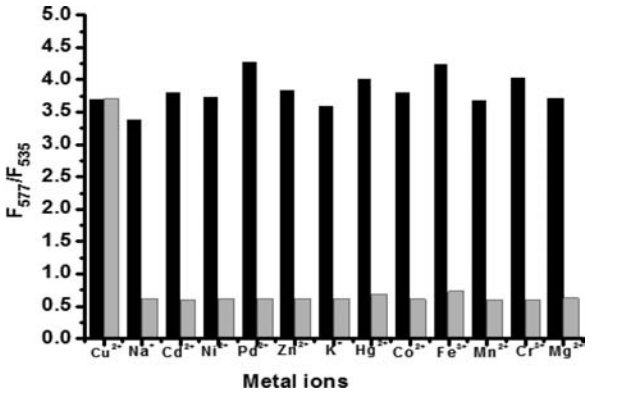


Figure 2. Fluorescence ratio (F_{577}/F_{535}) of **RN1** (5 μM) in the presence of various analytes (50 μM) (Cu^{2+}, Na^+, Cd^{2+}, Ni^{2+}, Pd^{2+}, Zn^{2+}, K^+, Hg^{2+}, Co^{2+}, Fe^{3+}, Mn^{2+}, Cr^{3+}, Mg^{2+}, Ag^+) in CH_3CN/H_2O (20:1, v/v) buffered with Tris-HCl (pH 7.4, 10 mM); λ_{ex} = 420 nm. Red bars represent the addition of 10 equiv of the appropriate metal ion to a 5 μM solution of **RN1**. Black bars represent the subsequent addition of 50 μM Cu^{2+} to the solution.

To apply **RN1** in more complicated systems, such as in organisms and the environment, the fluorescence responses of **RN1** in the absence and presence of Cu^{2+} in different pH values were evaluated. The pK_a of **RN1** alone is 1.2 from the sigmoidal curve. The fluorescence responses (F_{577}/F_{535}) of **RN1-Cu^{2+}** were not changed for pH values above 5.5, which means that **RN1** can work in near-neutral and weak acidic media. The time course of the respective fluorescence intensity at 535 and 577 nm (Figure 4) in the presence of Cu^{2+} indicates that the reaction essentially

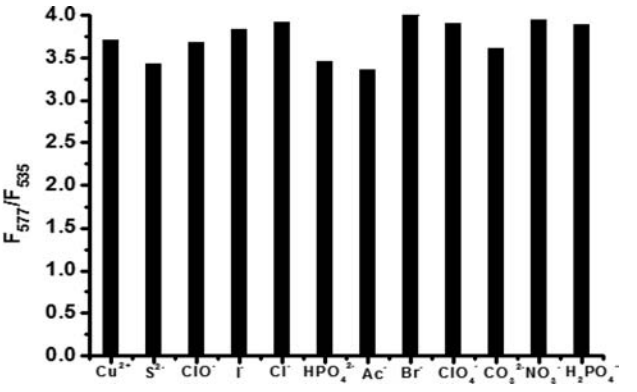


Figure 3. Fluorescence ratio (F_{577}/F_{535}) of **RN1** (5 μM) with addition of 5 μM Cu^{2+} subsequent addition of various appropriate anions in CH_3CN/H_2O (20:1, v/v) buffered with Tris-HCl (pH 7.4, 10 mM). λ_{ex} = 420 nm.

(10) (a) Wan, C. W.; Burghart, A.; Chen, J.; Bergstrom, F.; Johansson, L. B. A.; Wolford, M. F.; Kim, T. G.; Topp, M. R.; Hochstrasser, R. M.; Burgess, K. *Chem.—Eur. J.* **2003**, *9*, 4430. (b) Speiser, S. *Chem. Rev.* **1996**, *96*, 1953.

(11) Bhalla, V.; Roopa; Kumar, M.; Sharma, P. R.; Kaur, T. *Inorg. Chem.* **2012**, *51*, 2150.

(12) Zhu, B.; Gao, C.; Zhao, Y.; Liu, C.; Li, Y.; Wei, Q.; Ma, Z.; Du, B.; Zhang, X. *Chem. Commun.* **2011**, *47*, 8656.

reached completion within minutes so that it could be used as a fluorescent probe for the fast detection of Cu^{2+}.

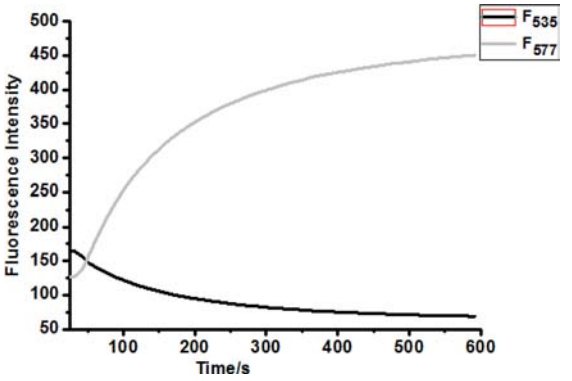


Figure 4. Time-dependent fluorescence ratio (F_{577}/F_{535}) of **RN1** (5 μM) with Cu^{2+} (10 equiv) in CH_3CN/H_2O (20/1, v/v) buffered with Tris-HCl (pH 7.4, 10 mM). λ_{ex} = 420 nm.

Finally, due to the good chemical and spectroscopic properties of the probe, the ratiometric **RN1** was applied for ratiometric fluorescence imaging in living cells. When MCF-7 cells were incubated with only **RN1** (5 μM) for 30 min at 37 °C, the cells showed intense fluorescence in the green channel (Figure 5b) and weak fluorescence in the red channel (Figure 5c). However, treatment of Cu^{2+} (50 μM) with **RN1**-loaded cells elicited a partial fluorescence decrease in the green channel (Figure 5f) and strong fluorescence in the red channel (Figure 5g). Cytotoxicity testing in MCF-7 cells (Figure S8) shows that **RN1** is almost nontoxic to living cells in our cell imaging conditions. Thus, all the results demomstrated that **RN1** was cell membrane permeable and could be used to image Cu^{2+} in living cells.

In conclusion, by changing the connection between the naphthalimide (energy donor) and rhodamine (energy acceptor), a novel fluorescent ratiometric chemodosimeter for Cu^{2+} based on TBET was synthesized and could be used to image Cu^{2+} in living cells which will help in the understanding of biological processes of Cu^{2+} at the molecular level. Unlike standard FRET-based probes, TBET is apparently not constrained by the overlap requirement and enables greater freedom in the selection of fluorophores, thus enabling the development of more wonderful probes based on TBET to satisfy different applications.

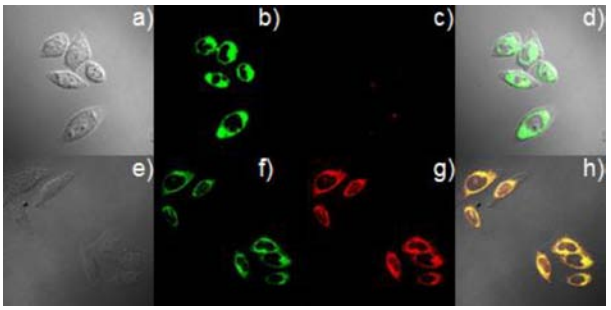


Figure 5. Images of MCF-7 cells treated with the ratiometric **RN1**: (a) bright field image of MCF-7 cells incubated with **RN1** (5 μM); (b) fluorescence image (a) from green channel; (c) fluorescence image (a) from red channel; (d) overlay image of (b) and (c); (e) bright field image of MCF-7 cells incubated with **RN1** (5 μM) for 30 min, and then further incubation with Cu^{2+} (50 μM) for 30 min at 37 °C; (f) fluorescence image (e) from green channel; (g) fluorescence image (e) from red channel; (h) overlay image of (f) and (g). λ_{ex} = 405 nm.

Acknowledgment. This work was financially supported by NSF of China (21136002, 20923006, 21222605, 21272030, and 21076032), National Basic Research Program of China (2009CB724706 and 2013CB733702), and National High Technology Research and Development Program of China (863 Program, 2011AA02A105).

Supporting Information Available. Experimental procedures, characterization data of compounds, and additional spectroscopic data. This material is available free of charge via the Internet at http://pubs.acs.org.

The authors declare no competing financial interest.