# An NBD fluorophore-based sensitive and selective fluorescent probe for zinc ion†

**Wei Jiang,[a] Qingquan Fu,[a] Hongyou Fan[b] and Wei Wang*[a]**

*Received (in Austin, TX, USA) 13th August 2007, Accepted 24th October 2007*
*First published as an Advance Article on the web 9th November 2007*
**DOI: 10.1039/b712377a**

**A novel NBD-derived fluorescent probe for $Zn^{2+}$ is described; the probe features ready availability, good water solubility, high sensitivity and selectivity, and ability to quantify the concentration of $Zn^{2+}$.**

Zinc ion ($Zn^{2+}$), the second most abundant transition metal in the human body, plays myriad roles in numerous cellular functions such as the regulation of gene expression, apoptosis, co-factors in metalloenzyme catalysis, and neurotransmission in biological systems.[1] Deregulation of $Zn^{2+}$ is implicated in several diseases including Alzheimer's disease,[2] prostate cancer,[3] and diabetes.[4] Accordingly, the development of $Zn^{2+}$-specific molecular probes has been of considerable interest in the areas of chemical and biological sciences.

An ideal $Zn^{2+}$ chemical probe with potential for biological applications should possess: 1) good water solubility, 2) the capability to quantitatively determine $Zn^{2+}$ concentration, 3) long excitation wavelength to avoid cell damage, 4) high stability, 5) high selectivity and sensitivity toward $Zn^{2+}$, and 6) easy preparation. The development of fluorescent chemosensors for probing $Zn^{2+}$ has been an active topic as a result of operational simplicity and high sensitivity. However, the search for readily accessible fluorescent $Zn^{2+}$ probes with good water solubility and high specificity is still a challenging task. It is a particular challenge to develop a chemosensor which makes it possible to determine the concentration of $Zn^{2+}$ and to discriminate $Zn^{2+}$ from $Cd^{2+}$ owing to their closely related properties. Fluorescent probes for sensing $Zn^{2+}$, based on various fluorophores such as quinoline,[5] bipyridyl,[6] dansyl,[7] ferrocene,[8] fluorescein,[9] anthracene,[10] benzofuran and benzoxazole,[11] naphthalimide[12] and cyanine[13] have been reported. The bipyridyl,[6] dansyl,[7] ferrocene,[8] anthracene[10] and naphthalimide[12] based probes have poor water solubility. Generally a mixture of organic solvent and water is used, thus limiting their biological applications. The widely used quinoline[5] and fluorescein[9] derived chemosensors provide good solubility; however, their selectivity for $Zn^{2+}$ and $Cd^{2+}$ is not clear. Moreover, the syntheses are not trivial. However, to the best of our knowledge, a 7-nitrobenz-2-oxa-1,3-diazole (NBD) derived fluorescent $Zn^{2+}$ chemical probe has not been described, despite the fact that it has been widely used in molecular imaging in biological systems.[14] Moreover, some of these reported fluorescent probes suffer from problems such as poor water solubility, inadequate selectivity, insufficient sensitivity and the lack of ability to quantitatively measure $Zn^{2+}$. In this communication, we wish to report a newly designed NBD based fluorescent probe for $Zn^{2+}$ based on the photoinduced electron transfer (PET) mechanism. The probe displays high selectivity and sensitivity toward $Zn^{2+}$ ion and good water solubility, demonstrating its potential for biological imaging. More importantly, the chemosensor allows easy quantification of $Zn^{2+}$ concentration.

The PET mechanism has been widely exploited for molecular sensing.[15] In the design of PET fluorescent sensors for $Zn^{2+}$, the critical issue is that $Zn^{2+}$ binding to the sensor should generate a detectable signal so that the binding event can be monitored conveniently. We envisioned that chelation of $Zn^{2+}$ with compounds **1** or **2** would cause the fluorescence intensity to increase as a result of blocking PET of the nitrogen atoms. Accordingly, the newly designed NBD fluorescent probes for $Zn^{2+}$ are composed of two essential components: an NBD moiety as a reporter and *N*,*N*,*N*′,*N*′-tetrakis(2-pyridylmethyl)ethylenediamine (TPEN) or *N*,*N*-bis(pyridin-2-ylmethyl)amine (BPA) as chelators for $Zn^{2+}$ (Fig. 1). TPEN and BPA are the chelators of choice since they display high specificity for binding to $Zn^{2+}$ over other metal cations, and favorable kinetic and thermodynamic properties which result in quick formation of a stable $Zn^{2+}$ complex.[9c–f,13] Furthermore, they are readily incorporated into a fluorophore. The two moieties are directly tethered together through a robust C–N bond without a linker (Fig. 1).

Sensors **1** and **2** were synthesized in a straightforward manner (see ESI†). The investigation of the fluorescence properties of probes **1** and **2** reveals that free **1** exhibits very weak fluorescence (Fig. 2), while **2** displays a strong signal (Fig. S1 in ESI†). Upon addition of $Zn^{2+}$, the fluorescence intensity of probe **1** is enhanced significantly in a concentration dependent manner (Fig. 2). Moreover, when $\geqslant$1.0 equiv. of $Zn^{2+}$ is added, the maximum

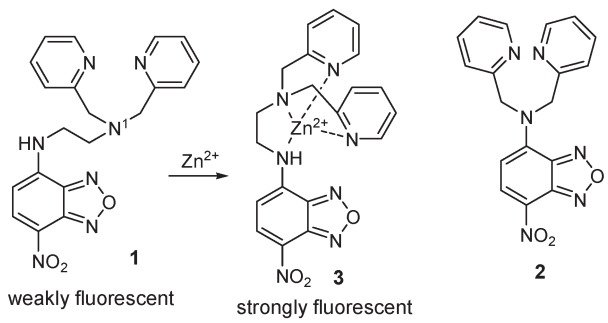


**Fig. 1** Design of NBD-derived fluorescent probes for $Zn^{2+}$.

[a]*Department of Chemistry & Chemical Biology, University of New Mexico, Albuquerque, NM 87131, USA. E-mail: wwang@unm.edu; Fax: (+1) 505 277 2609; Tel: (+1) 505 277 0756*
[b]*Ceramic Processing and Inorganic Materials Department, 01815, Sandia National Laboratories, Albuquerque, NM 87185, USA*
† Electronic supplementary information (ESI) available: Experimental and NMR data. See DOI: 10.1039/b712377a

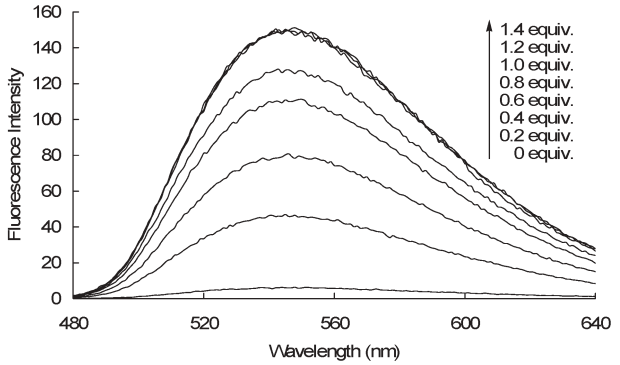


**Fig. 2** Emission spectra ($\lambda_{ex}$ = 470 nm) of probe **1** ($10^{-5}$ M) after addition of a range of amounts of $Zn^{2+}$ (0–1.4 × $10^{-5}$ M) at room temperature in PBS buffer (pH 7.3).

fluorescence intensity is reached. The intensity is increased by more than 25-fold. This indicates the probe is highly sensitive to $Zn^{2+}$ with a $K_d$ of 4.6 μM (see ESI†). Furthermore, as expected, the sensor **1** forms a 1 : 1 complex with $Zn^{2+}$ (see ESI†).

More significantly, a linear relationship between the fluorescence intensity of probe **1** and the concentration of $Zn^{2+}$ is observed (Fig. 3). Therefore the sensor could be used for the quantitative determination of the concentration of $Zn^{2+}$. In contrast, no fluorescence alternation for sensor **2** is observed (Fig. S1 in ESI†). This indicates that the "$N^{1}$" in probe **1** is critical in the PET (Fig. 1).

The above studies prompt us to select chemical probe **1** for further evaluation aimed at determining its selectivity. The fluorescence titration of **1** with various metal ions exhibits high selectivity to $Zn^{2+}$ (Fig. 4). Metal ions which possess a broad spectrum of biological activities and functions in living cells, such as $Na^+$, $K^+$, $Mg^{2+}$, $Ca^{2+}$, $Mn^{2+}$, $Fe^{2+}$, and $Fe^{3+}$, do not give rise to any responses under the same conditions. Most heavy transition metal ions, including $Cd^{2+}$, $Ni^{2+}$, and $Co^{2+}$, also show no interference. $Hg^{2+}$ induces very limited fluorescence enhancement, while $Cu^{2+}$ quenches fluorescence.

In conclusion, a novel NBD-derived water-soluble fluorescent chemical probe has been designed and synthesized, and it displays high selectivity and sensitivity for $Zn^{2+}$ in a neutral buffer aqueous solution. In the presence of $Zn^{2+}$, significant fluorescence

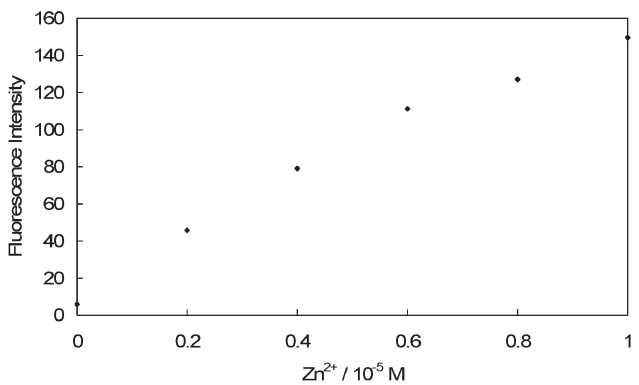


**Fig. 3** Plot of the concentration of $Zn^{2+}$ *vs.* $\Delta I/I_0$, where $\Delta I = I - I_0$, I: the fluorescence intensity of probe **1** ($10^{-5}$ M) with addition of $Zn^{2+}$ and $I_0$: the fluorescence intensity of probe **1** without $Zn^{2+}$ at $\lambda_{em}$: 550 nm.

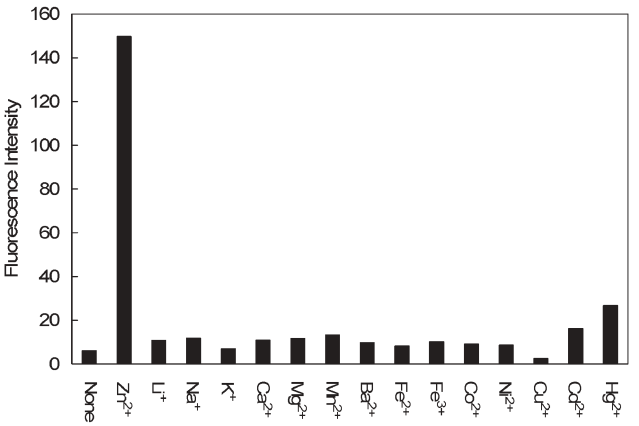


**Fig. 4** The selectivity of probe **1** toward $Zn^{2+}$ and other metal ions. In these experiments, the fluorescence measurement was taken at $\lambda_{ex}$ = 470 nm from $10^{-5}$ M of probe **1** in a PBS buffer (pH 7.3) at room temperature and in the absence and presence of 1.0 equiv. of a metal ion. The fluorescence intensity at $\lambda_{em}$ = 550 nm is used for plotting *versus* an analyte.

enhancement is achieved. Since the concentration of $Zn^{2+}$ in a biological system, for example, in synaptic vesicles, is reported to be in the micro- to millimolar range,[16] the probe **1**, which displays a sensitivity in the micro-range, can be used for the imaging of $Zn^{2+}$. Moreover, the magnitude of the fluorescence intensity increase corresponds nearly linearly to the concentration of $Zn^{2+}$, indicating that the sensor could be used for the quantitative measurement of $Zn^{2+}$ concentrations.

Financial support for this work provided by the Department of Chemistry & Chemical Biology and the Research Allocation Committee, the University of New Mexico, NIH-INBRE (P20 RR016480), and the Sandia University Research Program (SURP) is gratefully acknowledged.

## Notes and references

1 (*a*) B. L. Vallee and K. H. Falchuk, *Physiol. Rev.*, 1993, **73**, 79; (*b*) J. J. R. F. de Silva and R. J. P. Williams, in *The Biological Chemistry of Elements: The Inorganic Chemistry of Life*, Oxford University Press, New York, 2001; (*c*) R. J. P. Williams and J. J. R. F. de Silva, *Coord. Chem. Rev.*, 2000, **200–202**, 247.
2 A. I. Bush, *Trends Neurosci.*, 2003, **26**, 207.
3 M. B. Sorensen, M. Stoltenberg, S. Juhl, G. Danscher and E. Ernst, *Prostate*, 1997, **31**, 125.
4 A. B. Chausmer, *J. Am. Coll. Nutr.*, 1998, **17**, 109.
5 (*a*) E. Kimura and S. Aoki, *BioMetals*, 2001, **14**, 191; (*b*) C. J. Fahrni and T. V. O'Halloran, *J. Am. Chem. Soc.*, 1999, **121**, 11448; (*c*) M. S. Nasir, C. J. Fahrni, D. A. Suhy, K. J. Kolodsick, C. P. Singer and T. V. O'Halloran, *JBIC, J. Biol. Inorg. Chem.*, 1999, **4**, 775; (*d*) M. D. Shults, D. A. Pearce and B. Imperiali, *J. Am. Chem. Soc.*, 2003, **125**, 10591; (*e*) M. Royzen, A. Durandin, V. G. Young, Jr., N. E. Geacintov and J. W. Canary, *J. Am. Chem. Soc.*, 2006, **128**, 3854; (*f*) Y. Liu, N. Zhang, Y. Chen and L.-H. Wang, *Org. Lett.*, 2007, **9**, 315.
6 (*a*) A. Ajayaghosh, P. Carol and S. Sreejith, *J. Am. Chem. Soc.*, 2005, **127**, 14962; (*b*) X. Peng, Y. Xu, S. Sun, Y. Wiu and J. Fan, *Org. Biomol. Chem.*, 2007, **5**, 226.
7 (*a*) T. Koike, T. Watanabe, S. Aoki, E. Kimura and M. Shiro, *J. Am. Chem. Soc.*, 1996, **118**, 12696; (*b*) R. B. Thompson, B. P. Maliwal, V. L. Feliccia, C. A. Fierke and K. McCall, *Anal. Chem.*, 1998, **70**, 4717; (*c*) L. Prodi, F. Bolletta, M. Montalti and N. Zaccheroni, *Eur. J. Inorg. Chem.*, 1999, 455.
8 F. Zapata, A. Caballero, A. Espinosa, A. Tarraga and P. Molina, *Org. Lett.*, 2007, **9**, 2385.

 

9 (*a*) H. A. Godwin and J. M. Berg, *J. Am. Chem. Soc.*, 1996, **118**, 6514; (*b*) D. Elbaum, S. K. Nair, M. W. Patchan, R. B. Thompson and D. W. Christianson, *J. Am. Chem. Soc.*, 1996, **118**, 8381; (*c*) S. C. Burdette, G. K. Walkup, B. Spingler, R. Y. Tsien and S. J. Lippard, *J. Am. Chem. Soc.*, 2001, **123**, 7831; (*d*) T. Hirano, K. Kikuchi, Y. Urano and T. Nagano, *J. Am. Chem. Soc.*, 2002, **124**, 6555; (*e*) S. C. Burdette, C. J. Frederickson, W. Bu and S. J. Lippard, *J. Am. Chem. Soc.*, 2003, **125**, 1778; (*f*) C. C. Woodroofe and S. J. Lippard, *J. Am. Chem. Soc.*, 2003, **125**, 11458.
10 (*a*) E. U. Akkaya, M. E. Huston and A. W. Czarnik, *J. Am. Chem. Soc.*, 1990, **112**, 3590; (*b*) G. Hennrich, H. Sonnenschein and U. Resch-Genger, *J. Am. Chem. Soc.*, 1999, **121**, 5073.
11 (*a*) S. Maruyama, K. Kikuchi, T. Hirano, Y. Urano and T. Nagano, *J. Am. Chem. Soc.*, 2002, **124**, 10650; (*b*) M. M. Henary and C. J. Fahrni, *J. Phys. Chem. A*, 2002, **106**, 5210; (*c*) A. Ohshima, A. Mototake and T. Arai, *Tetrahedron Lett.*, 2004, **45**, 9377; (*d*) M. Taki, J. L. Wolford and T. V. O'Halloran, *J. Am. Chem. Soc.*, 2004, **126**, 712.
12 Z. Xu, X. Qian, J. Cui and R. Zhang, *Tetrahedron*, 2006, **62**, 10117.
13 K. Kiyose, H. Kojima, Y. Urano and T. Nagano, *J. Am. Chem. Soc.*, 2006, **128**, 6548.
14 *Handbook of Fluorescent Probes and Research Products*, ed. R. P. Haugland, Molecular Probes, Inc., Eugene, OR, 2002.
15 (*a*) A. P. de Silva, H. Q. N. Gunaratne, T. Gunnlaugsson, A. J. M. Uhuxley, C. P. McCoy, J. T. Rademacher and T. E. Rice, *Chem. Rev.*, 1997, **97**, 1515; (*b*) F. W. Scheller, F. Schubert and J. Fedrowitz, *Frontiers in Biosensorics I. Fundamental Aspects*, Birkhauser Verlag, Berlin, 1997; (*c*) F. W. Scheller, F. Schubert and J. Fedrowitz, *Frontiers in Biosensorics II. Practical Applications*, Birkhauser Verlag, Berlin, 1997; (*d*) W. Wang, S. Gao and B. Wang, *Curr. Org. Chem.*, 2002, **6**, 1285.
16 Y. Li, C. J. Hough, C. J. Frederickson and J. M. Sarvey, *J. Neurosci.*, 2001, **21**, 8015.