

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}

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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 naphthalimide¹² and cyanine¹³ have been reported. The bipyridyl,⁶ dansyl,⁷ ferrocene,⁸ anthracene¹⁰ and naphthalimide¹² based probes have poor water solubility. Generally a mixture of organic solvent and water is used, thus limiting their biological applications. The widely used quinoline⁵ and fluorescein⁹ 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 ≥ 1.0 equiv. of Zn^{2+} is added, the maximum

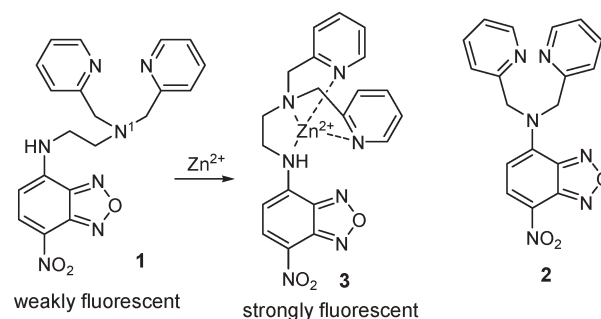


Fig. 1 Design of NBD-derived fluorescent probes for Zn^{2+} .

^aDepartment 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

^bCeramic Processing and Inorganic Materials Department, 01815, Sandia National Laboratories, Albuquerque, NM 87185, USA

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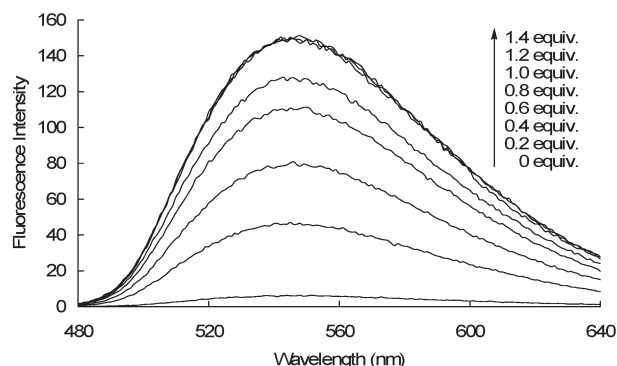


Fig. 2 Emission spectra ($\lambda_{\text{ex}} = 470$ nm) of probe **1** (10^{-5} M) after addition of a range of amounts of Zn^{2+} (0 – 1.4×10^{-5} M) at room temperature in PBS buffer (pH 7.3).

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 $4.6 \mu\text{M}$ (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” 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

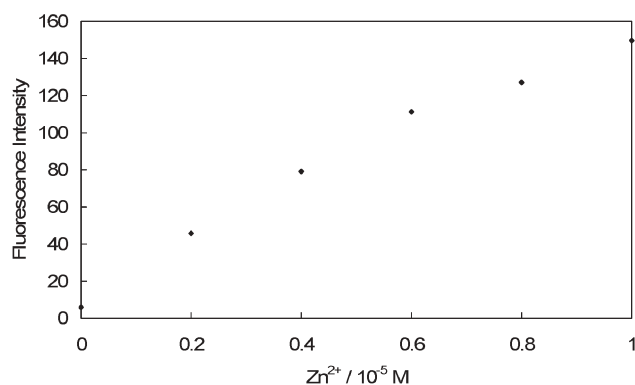


Fig. 3 Plot of the concentration of Zn^{2+} vs. $\Delta I/I_0$, where $\Delta I = I - I_0$, I : the fluorescence intensity of probe **1** (10^{-5} M) with addition of Zn^{2+} and I_0 : the fluorescence intensity of probe **1** without Zn^{2+} at $\lambda_{\text{em}} = 550$ nm.

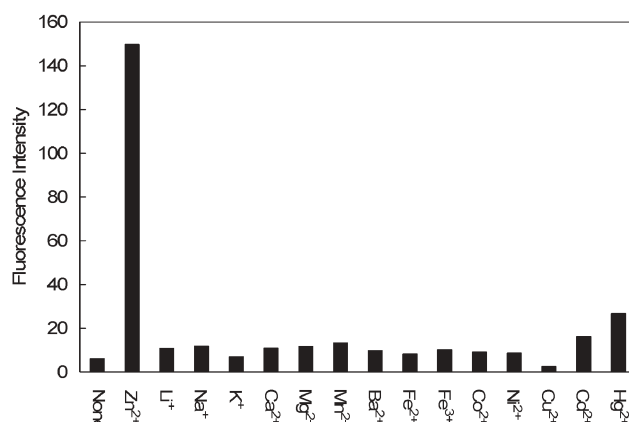


Fig. 4 The selectivity of probe **1** toward Zn^{2+} and other metal ions. In these experiments, the fluorescence measurement was taken at $\lambda_{\text{ex}} = 470$ nm from 10^{-5} M of probe **1** in a PBS buffer (pH 7.3) at room temperature and in the absence and presence of 1.0 equiv. of a metal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

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