# Highly sensitive and selective near-infrared fluorescent probe for zinc and its application to macrophage cells†

**Bo Tang,*[a] Hui Huang,[a] Kehua Xu,[a] Lili Tong,[a] Guiwen Yang,[b] Xia Liu[a] and Liguo An[b]**

*Received (in Cambridge, UK) 15th May 2006, Accepted 21st June 2006*
*First published as an Advance Article on the web 26th July 2006*
**DOI: 10.1039/b606809j**

**A new highly sensitive and selective near-infrared fluorescent probe for zinc ion, based on photoinduced electron transfer (PET) mechanism, has been designed, synthesized, and applied to macrophage cells.**

Metal ions though scarcely present in the human body, play essential roles in many biological processes. Among them zinc is the second most abundant transition metal in humans, fulfilling a magnitude of biological roles. It can influence DNA synthesis,[1] apoptosis,[2,3] gene expression,[4,5] neurotransmission,[6–9] signal transduction.[10] It has the ability to modulate a variety of ion channels.[11] Zinc ion is also an essential component of many enzymes and transcription factors, such as carbonic anhydrase, zinc finger proteins, *etc.*, in which it plays structural or catalytic roles.[12] Failure to maintain zinc homeostasis in the body, organ or cell, may lead to a broad range of developmental defects and malfunctions. Although, the structure chemistry of zinc is well understood, many questions related to zinc homeostasis and action remain unanswered. Thus, it is appealing to make zinc "visible" in tissues, even in living cells.

The fluorescence imaging method has revolutionized the process of quantifying trace zinc ion within biological samples owing to its sensitivity, selectivity and ease of use. Many fluorescent probes for zinc imaging have been developed recently, some of which are well-suited for biological applications.[13,14]

However, they still have several limitations when applied to detect zinc in biological samples. First, most of them need to be excited by UV light, which can cause damage to living cells. Second, their fluorescence lies in the visible region, which cannot penetrate deep enough into human tissue. Compared to visible light, near-infrared (NIR) region at around 650–900 nm is less absorbed by biomolecules and exhibits the least autofluorescence background. Several NIR fluorescence probes have been developed and used in imaging in biological systems,[15,16] but none of them are available for metal ions. Very recently, Nagano *et al.* reported a new NIR fluorescence probe for zinc and received a good result.[17] But there is no report on a NIR fluorescence probe for imaging zinc in biological samples.

[a]*College of Chemistry, Chemical Engineering and Materials Science, Shandong Normal University, Jinan 250014, China. E-mail: tangb@sdnu.edu.cn; Fax: +86-531-86180017; Tel: +86-531-86180010*
[b]*College of Life Science, Shandong Normal University, Jinan 250014, China. E-mail: yanggw@sdnu.edu.cn; Fax: (+86) 531-86180107; Tel: (+86) 531-861880143*

† Electronic supplementary information (ESI) available: detailed experimental procedures, synthesis and characterization data for DPA-Cy, spectral property and pH profile of DPA-Cy, time course of DPA-Cy complexed with zinc. Job plots of DPA-Cy.. See DOI: 10.1039/b606809j

We report here the design, synthesis, and application of a novel NIR fluorescent probe, which is sensitive and selective for imaging zinc.

The most common class of fluorescent probe for metal ions is based on photoinduced electron transfer (PET) quenching mechanisms (shown in Fig. 1).[18–20] PET probes consist of two moieties: an ion selective receptor and a fluorophore. For designing a proper probe for zinc imaging, we chose tricarbocyanine with two propyl groups which can penetrate cellular membranes without any modification as the NIR fluorophore, and 2,2′-dipicolylamine (DPA) to chelate zinc. Inhibition of PET by coordination of $d^{10}$ transition metals or protons to amines is a commonly observed mechanism for fluorescent enhancement.[21] The $[Zn(DPA)]^{2+}$ complex has an apparent $k_d$ of 70 nM and almost no measurable affinity for $Ca^{2+}$ or $Mg^{2+}$.[22] Furthermore, owing to its structural similarity to the membrane-permeable heavy metal chelator *N*,*N*,*N*′,*N*′-tetra (2- picolyl)ethylenediamine (TPEN), the DPA ligand is expected to be membrane permeable.[23,24] Besides, our probe can be easily synthesized through a one-step reaction of tricarbocyanine with DPA under mild conditions with a large throughput (about 50%).[25] So we are sure that the probe can be applied in practice easily and rapidly.

The vinyl chlorine on the cyclohexane bridgehead of tricarbocyanine is reactive and can be replaced by DPA, a very strong nucleophile (Scheme 1).

We then examined the spectral properties of our probe. DPA-Cy shows absorption at 606 nm and emission at 800 nm in acetonitrile with a large Stokes shift of 194 nm (shown in Fig. 1). The shift is much larger than common tricarbocyanine, which can

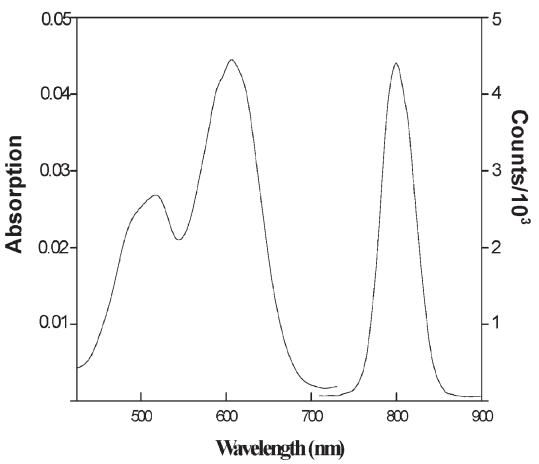
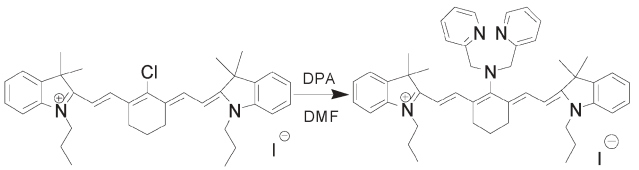



**Fig. 1** Absorption spectra and fluorescence spectra of DPA-Cy in acetonitrile.




**Scheme 1**

avoid self-quenching and measurement errors caused by excitation light and scattered light.

In buffered aqueous acetonitrile solution (HEPES buffer, 100 mM pH 7.4, 100 mM KCl), DPA-Cy showed a 20 nm blue-shift in emission to 780 nm, while absorption red-shifted to 730 nm. DPA-Cy reacted immediately with zinc and the fluorescence intensity increased in a zinc concentration dependent way (Fig. 2).

There is no evident change in the absorption and emission wavelength, but the fluorescence quantum yields of the DPA-Cy–Zn ($\alpha$) were 20-fold higher than that in absence of $Zn^{2+}$ (Table 1 in the Supporting Information†). We then evaluate the effect of pH on the fluorescence of DPA-Cy (the Supporting Information, Fig. S1†). The fluorescence is high and independent of pH from 6.4 to 7.5. That is to say, the probe works well under physiological conditions. The apparent dissociation constants, $k_d$, was determined to be 63 nM (the Supporting Information, Fig. S2†). Job plot analysis revealed that the inflection point was at 0.5, indicating that DPA-Cy forms a 1 : 1 species with zinc in solution (shown in Fig. 3).

Other cations, which exist at high concentration in living cells, $Na^+$, $K^+$, $Ca^{2+}$, and $Mg^{2+}$, do not enhance the fluorescence even at high concentration (1 mM) (Fig. 4). This is due to the low complexation of alkaline metals or alkaline earth metals with the chelator of DPA-Cy. Among first-row transition metal cations $Fe^{2+}$, $Fe^{3+}$, $Co^{2+}$, $Ni^{2+}$ and $Hg^{2+}$ (1 μM) induced a slight enhancement of the fluorescence intensity, and $Cu^{2+}$ quenched the fluorescence.

DPA-Cy probably forms complexes with $Cu^{2+}$, but the fluorescence is weakened because of an electron or energy transfer between metal cation and fluorophore, which is known as the fluorescence quenching mechanism.[21] However, $Cu^{2+}$ will have little influence on *in vivo* detection because of its low concentration.

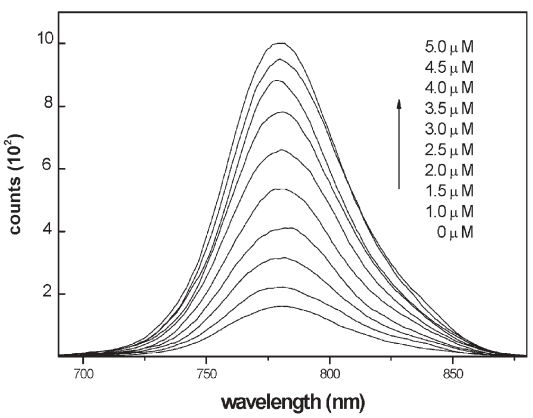



**Fig. 2** Emission spectra (excitation at 731 nm) of 5 μM DPA-Cy in the presence of various concentrations of $Zn^{2+}$ ranging from 0 to 5 μM. These spectra were measured in HEPES buffer, (100 mM, pH 7.4, 100 mM KCl).

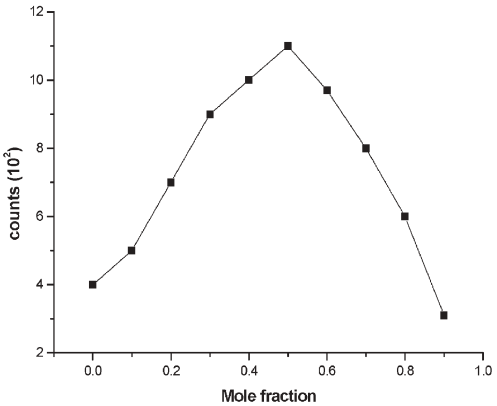



**Fig. 3** Job plot showing the 1 : 1 binding of DPA-Cy to $Zn^{2+}$.

Finally, we applied DPA-Cy to macrophage cells to examine whether it worked in biological systems. Cultured macrophages (RAW 264.7) were incubated with phosphate-buffered saline (PBS) containing the probe (5 μM) at 37 °C for 0.5 h. The cells were well stained because DPA-Cy is lipophilic. The fluorescence was increased immediately by the addition of zinc and 2-mercaptopyridine *N*-oxide, a zinc-selective ionophore. On treatment of the cells with an excess of the cell-permeable, high affinity zinc chelator, *N*,*N*,*N*′,*N*′-tetra (2-picolyl)ethylenediamine (TPEN), the fluorescence decreased to baseline (Fig. 5).

In conclusion, we have described the synthesis, properties and cellular applications of DPA-Cy, the first NIR fluorescence probe for the imaging of zinc. DPA-Cy is based on a PET mechanism and gives a 20-fold turn-on response for detecting zinc. The probe has a large Stokes shift and is highly sensitive and selective to zinc. Besides, DPA-Cy is cell-permeable and can respond to zinc quickly, demonstrating that DPA-Cy is an excellent NIR fluorescence probe for zinc imaging. Efforts to utilize the probe for biological imaging application are in progress.

This work was supported by Program for New Century Excellent Talents in University (NCET-04-0651), the National Natural Science Foundation of China (Nos. 20335030 and 20575036).

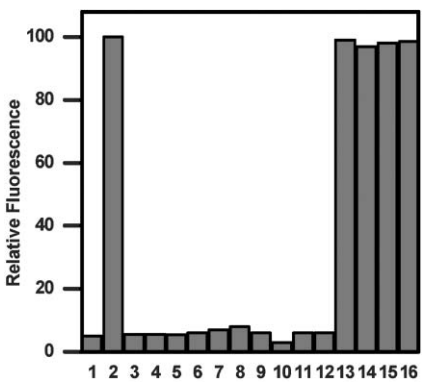



**Fig. 4** The relative fluorescence intensity of DPA-Cy (1 μM) in the presence of various cations of interest. 1) none, 2) 1 μM $Zn^{2+}$, 3) 1 mM $K^+$, 4) 1 mM $Na^+$, 5) 1 mM $Ca^{2+}$, 6) 1 mM $Mg^{2+}$, 7) 1 μM $Ni^{2+}$, 8) 1 μM $Hg^{2+}$, 9) 1 μM $Co^{2+}$, 10) 1 μM $Cu^{2+}$, 11) 1 μM $Fe^{2+}$ ,12) 1 μM $Fe^{3+}$, 13) 1 μM $Zn^{2+}$ + 1 mM $K^+$, 14) 1 mM $Zn^{2+}$, + 1 mM $Na^+$, 15) 1 μM $Zn^{2+}$ + 1 mM $Ca^{2+}$, 16) 1 μM $Zn^{2+}$ + 1 mM $Mg^{2+}$. These data were obtained at pH 7.4 (100 mM HEPES buffer, 100 mM KCl).

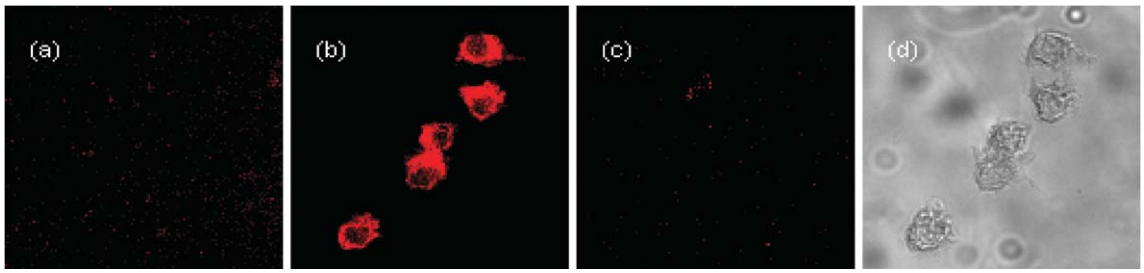



**Fig. 5** Confocal fluorescence images of live macrophage cells. (a) Cells incubated with 5 μM DPA-Cy for 30 min at 37 °C. (b) Cells supplemented with 100 μM $Zn^{2+}$ to the DPA-Cy-treated macrophage cells. A 10 : 2 $Zn^{2+}$/pyrithione ratio was employed and the cells were incubated with $Zn^{2+}$ for 30 min at 37 °C. (c) Treatment with 100 μM TPEN, a high-affinity membrane-permeable heavy-metal chelator, for 5 min at 37 °C. (d) Bright field image of live macrophage cells shown in panel (b), confirming their viability.

## Notes and references


1 J. J. Fraústo da Silva and R. J. P. Williams, *The Biological Chemistry of the Elements*, Clarendon Press: Oxford, 1991, p. 302.
2 P. D. Zalewski, I. J. Forbes, G. Mazdai and C. Giannakis, *Biochem. Inter.*, 1991, **24**, 1093–1101.
3 S. J. Martin, G. Mazdai, J. J. Strain, T. G. Cotter and B. M. Hannigan, *Clin. Exp. Immunol.*, 1991, **83**, 338–344.
4 D. E. Epner and H. R. Herschman, *J. Cell Physiol.*, 1991, **148**, 68–74.
5 R. J. Cousins and L. M. Lee-Ambrose, *J. Nutr.*, 1992, **122**, 56–64.
6 X. M. Xie and T. G. Smart, *Nature*, 1991, **349**, 521–524.
7 C. J. Frederickson, *Int. Rev. Neurobiol.*, 1989, **31**, 145–238.
8 L. M. T. Canzoniero, S. L. Sensi and D. W. Choi, *Neurobiol. Dis.*, 1997, **4**, 275–279.
9 D. W. Choi and J. Y. Koh, *Annu. Rev. Neurosci.*, 1998, **21**, 347–375.
10 F. Grummt, C. Wienmann-Dorsch, J. Schneider-Schaulies and A. Lux, *Exp. Cell Res.*, 1986, **163**, 191–200.
11 N. L. Harrison and S. Gibbons, *J. Neuropharmacology*, 1994, **33**, 935–952.
12 B. L. Vallee and K. H. Falchuk, *Physiol. Rev.*, 1993, **73**, 79–118.
13 J. J. R. Fraústo da Silva and R. J. P. Williams, *The Biological Chemistry of the Elements: The Inorganic Chemistry of Life*, 2rd ed.; Oxford University Press: Oxford, 2001.
14 S. Aoki, S. Kaido, H. Fujioka and E. Kimura, *Inorg. Chem.*, 2003, **42**, 1023–1030.
15 K. Komatsu, K. Kikuchi, H. Kojima, Y. Urano and T. Nagano, *J. Am. Chem. Soc.*, 2005, **127**, 10197–10204.
16 E. Sasaki, H. Kojima, H. Nishimatsu, Y. Urano and K. Kikuchi, *J. Am. Chem. Soc.*, 2005, **127**, 3684–3685.
17 K. Kiyose, H. Kojima, Y. Urano and T. Nagano, *J. Am. Chem. Soc.*, 2006, DOI: 10.1021/ja060399c.
18 K. H. Xu, B. Tang, H. Huang, G. W. Yang, Z. Z. Chen, P. Li and L. G. An, *Chem. Commun.*, 2005, 5974–5976.
19 Sungho. Yoon, A. E. Albers, A. P. Wong and C. J. Chang, *J. Am. Chem. Soc.*, 2005, **127**, 16030–16031.
20 L. Zeng, E. W. Miller, A. Pralle, E. Y. Isacoff and C. J. Chang, *J. Am. Chem. Soc.*, 2006, **128**, 10–11.
21 A. P. de Silva, H. Q. N. Gunaratne, T. Gunnlaugsson, A. J. Huxley, C. P. McCoy, J. T. Rademacher and T. E. Rice, *Chem. Rev.*, 1997, **97**, 1515–1566.
22 G. Anderegg, E. Hubmann, N. G. Podder and F. Wenk, *Helv. Chim. Acta*, 1977, **60**, 123–140.
23 P. Arslan, F. Di Virgilio, M. Meltrame, R. Y. Tsien and T. Pozzan, *J. Biol. Chem.*, 1985, **260**, 2719–2727.
24 L. Fabbrizzi, M. Licchelli, P. Pallavicini and A. Taglietti, *Analyst*, 1996, **121**, 1763–1768.
25 X. Peng, F. Song, E. Lu, Y. Wang, W. Zhou, J. Fan and Y. Gao, *J. Am. Chem. Soc.*, 2005, **127**, 4170–4171.