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Target-triggered deprotonation of 6-hydroxyindole-based BODIPY: specially switch on NIR fluorescence upon selectively binding to Zn^{2+}†

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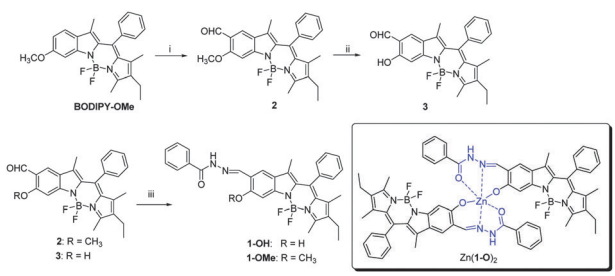
Based on 6-hydroxyindole BODIPY with a Schiff-base structure, NIR fluorescence with impressively high selectivity is triggered by deprotonation of the phenol group upon binding with Zn^{2+} due to the chelation-enhanced fluorescence effect, thus realizing a promising application in bioimaging of Zn^{2+}.

Zinc is a vital component in biological systems since it plays vital roles in catalytic functions of proteins, gene expression, apoptosis, neurotransmission and so forth.[1] As an active research field, rapid progress has been made in fluorescent probes for Zn^{2+}.[2–4] Near Infra-red (NIR) fluorescent sensors are highly desirable due to the induction of minimum background fluorescence and less photodamage as well as deep penetration into tissue.[5,6] The commonly employed approach to construct NIR Zn^{2+} probes is to install responsive receptor units into chromophores with extended π-conjugation moieties so as to shift the absorption or emission to long wavelengths. Alternatively, sensors based on the chelation-enhanced fluorescence (CHEF) effect are relatively convenient with incorporation of proper ligands into the chromophore. Phenol-based Schiff bases (imines) are known as good π-conjugated ligands for metal ions.[7] In general, dyes containing acyclic C=N bonds are poorly fluorescent due to the isomerization of C=N bonds in excited states, but dyes with cyclic C=N bonds are highly fluorescent.[8] Both the imine nitrogen and phenol oxygen can serve as good ligand donors for metal ions, giving rise to structural rigidity *via* coordination with metal ions. Thus, the isomerization of the C=N bond can be inhibited, resulting in the specific CHEF effect. More importantly, the phenol group is deprotonated in the coordination process, which can be seen in a characteristic emission of phenolate.[9] Accordingly, construction of phenol-based Schiff bases on the basis of the CHEF effect would be expected to provide opportunities to the further development of NIR probes.

Herein, we report a novel BODIPY derivative (**1-OH**) with salicylaldehyde benzoyl hydrazone as a specific tridentate chelating unit to develop a turn-on NIR fluorescence probe for Zn^{2+} (Scheme 1). Upon titration with Zn^{2+}, probe **1-OH** undergoes a significant increase in NIR fluorescence. The enhanced fluorescence might be attributed to the Zn^{2+} binding with the Schiff-base ligand, whereupon it would deprotonate the phenol group and rigidify the C=N bond. Interestingly, the resulting complex of **Zn(1-O)$_2$** (Scheme 1) exhibits a long emission wavelength at 680 nm, thus making the determining wavelength fall in the desirable NIR region. Furthermore, the selectivity of **1-OH** towards Zn^{2+} is impressively high, especially with little interference from Cd^{2+}. As demonstrated, **1-OH** can be successfully applied for sensing Zn^{2+} in aqueous media and living cells, showing high promise in clarifying the roles of Zn^{2+} in biological processes.

As shown in Scheme 1, **1-OH** and the reference compound **1-OMe** were synthesized straightforwardly. Formylation in the 5-position *via* a standard Vilsmeier–Haack reaction generated aldehyde **2** with 68% isolated yield. BODIPY based salicylaldehyde **3** was then obtained by removal of the methoxy protecting group. Both **2** and **3** were treated with benzohydrazide in methanol to afford **1-OMe** and **1-OH**, respectively. Their chemical structures were fully characterized by ^{1}H NMR, ^{13}C NMR and HRMS (ESI†).

To demonstrate the potential of **1-OH** as a turn-on NIR fluorescent probe, we firstly evaluated the binding property of **1-OH** toward Zn^{2+} in CH_3CN. Upon titration of Zn^{2+} with **1-OH**, distinct spectral changes were observed (Fig. 1). The intense absorption band at 548 nm was found to decrease while a new band at 617 nm increased with a well-defined isosbestic point at 567 nm, consistent with the presence of two species, free **1-OH** and the resulting complex between Zn^{2+}



Scheme 1 Synthesis of **1-OH** and reference compound **1-OMe**: (i) DMF, $POCl_3$, CH_2Cl_2, r. t., 68% yield; (ii) BBr_3, CH_2Cl_2, −78 °C, 40% yield; (iii) benzohydrazide, EtOH, reflux.

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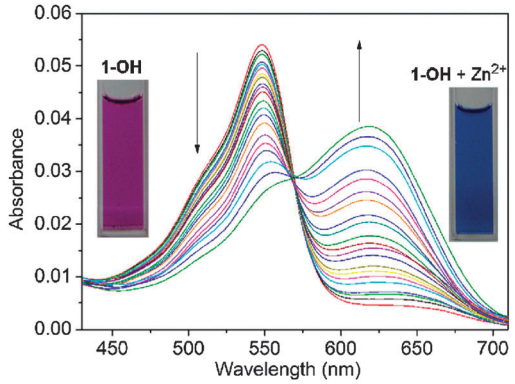

Fig. 1 Absorption spectra of **1-OH** (5.0 μM) in the presence of different concentrations of Zn^{2+} (0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.2, 1.4, 1.6, 1.8, 2.0, 2.2, 2.4, 2.6, 2.8 and 3.0 equiv.) in CH_3CN. Inset: the color change of **1-OH** in the absence and presence of Zn^{2+}.

and **1-OH**. Also, the solution color changed from pink to blue upon addition of Zn^{2+}.

Next, we investigated the Zn^{2+}-binding ability of **1-OH** by fluorescence titration (Fig. 2). When excited at the isosbestic wavelength (567 nm) or the newly formed band (617 nm), the fluorescence signal at 680 nm increased significantly upon addition of Zn^{2+}. The fluorescence quantum yield (Φ_f) increased from 0.006 for free **1-OH** to 0.19 in the presence of excess Zn^{2+} upon excitation at 617 nm, showing a 32-fold enhancement in Φ_f. This is indicative of a sufficient fluorescence response of **1-OH** to Zn^{2+} to serve as a NIR turn-on probe for Zn^{2+}. Moreover, the Job plot indicated that the coordination between Zn^{2+} and **1-OH** has a 1 : 2 binding stoichiometry (Fig. 2b), exhibiting a binding constant of 3.5×10^6 M^{-2}, from the theoretical nonlinear fit of experimental data to a 1 : 2 binding model. In contrast, control experiments with reference compound **1-OMe** under the same conditions showed no change in the spectra of absorption and fluorescence upon addition of Zn^{2+} (Fig. S1, ESI†), hinting that the *ortho*-hydroxy group in **1-OH** plays a vital role in the coordination of Zn^{2+}. Obviously, such a large enhancement in NIR fluorescence could be attributed to the inhibition of C=N isomerization and the deprotonation of the phenolic group conjugated to the BODIPY core upon stable chelation of **1-OH** with Zn^{2+} (Scheme 1), which is a typical CHEF effect.

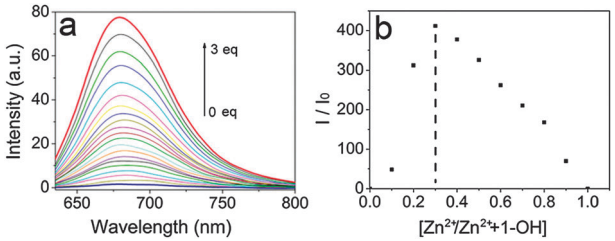

Fig. 2 (a) Fluorescence spectra of **1-OH** (5.0 μM) in CH_3CN upon different concentrations of Zn^{2+} (0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.2, 1.4, 1.6, 1.8, 2.0, 2.2, 2.4, 2.6, 2.8 and 3.0 equiv.) upon excitation at 617 nm. (b) Job plot of **1-OH** obtained from fluorescence titration in CH_3CN. The total concentration of **1-OH** and $Zn(ClO_4)_2$ is 10.0 μM.

Based on the fluorescence titration experiments, the detection limit was evaluated to be 9.7×10^{-7} M.

The coordination properties of **1-OH** with Zn^{2+} were also examined by means of ^1H NMR spectra (Fig. S2, ESI†). Upon addition of Zn^{2+}, the phenolic proton signal at 11.13 ppm disappeared, and the aromatic protons on the phenol ring underwent a significant downfield shift, suggesting that the coordination occurred between the deprotonated phenolic group and Zn^{2+}. Moreover, the downfield shift from 11.88 to 12.69 ppm, corresponding to the amide signal, is indicative of the coordination of Schiff-base ligand to Zn^{2+}. The possible 1 : 2 binding model of Zn^{2+} and **1-OH** (Scheme 1) was further confirmed by the ESI mass spectrum of Zn^{2+} (0.5 equiv.) + **1-OH** (Fig. S3, ESI†). A peak found at 1163.5160 was consistent with the calculated data for $[\mathbf{Zn(1\text{-}O)_2} + H]^+$ (1163.3916).

The Zn^{2+}-binding ability of **1-OH** was also investigated in aqueous solution (0.05 M Tris-HCl, 50% CH_3CN, pH = 7.5). **1-OH** showed almost no fluorescence with a fluorescent quantum yield of 0.003 upon excitation at 617 nm, suggesting that **1-OH** is a promising probe with very low background fluorescence. The large fluorescence enhancement was observed upon addition of Zn^{2+} to the solution of **1-OH**. More interestingly, **1-OH** can only sense Zn^{2+} in aqueous solution with high selectivity (Fig. 3). For instance, other cations such as Ca^{2+}, Mg^{2+}, Na^+, K^+, Li^+, Fe^{2+}, Hg^{2+}, Mn^{2+}, Cu^{2+}, Co^{2+} and Ni^{2+} gave no observable change. Even Cd^{2+}, which interferes with Zn^{2+} detection in most reported Zn^{2+} sensors, has little effect on the fluorescence of **1-OH**.

We finally sought to apply **1-OH** for the bioimaging of Zn^{2+} in living Breast Cancer MCF-7 cells (Fig. 4). Upon incubation with 5.0 μM of **1-OH** for 30 min at 37 °C, the cells displayed a faintly intracellular fluorescence image. When further treated with Zn^{2+} in the culture medium for 30 min, and washed with phosphate buffered saline to remove extracellular Zn^{2+}, the bright red fluorescence image was then observed in these

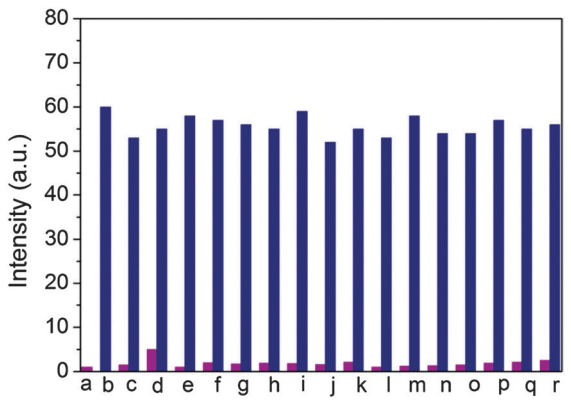

Fig. 3 Fluorescence response of 5.0 μM **1-OH** to various metal ions in aqueous solution (CH_3CN/Tris-HCl = 1 : 1 v/v, pH = 7.5) upon excitation at 617 nm: (a) free **1-OH**, (b) Zn^{2+}, (c) Cu^{2+}, (d) Pb^{2+}, (e) Ni^{2+}, (f) Hg^{2+}, (g) Cd^{2+}, (h) Mg^{2+}, (i) Li^+, (j) Na^+, (k) Co^{2+}, (l) Fe^{2+}, (m) K^+, (n) Ba^{2+}, (o) Mn^{2+}, (p) Ca^{2+}, (q) Ag^+, (r) Cr^{2+}. Note: pink bars for selectivity experiments with **1-OH** and 4.0 equiv. of metal ions; blue bars for interfering experiments which were conducted in the presence of 100 equiv. of Ca^{2+}, Mg^{2+}, Na^+, K^+, and 4 equiv. of Fe^{2+}, Hg^{2+}, Mn^{2+}, Cu^{2+}, Co^{2+}, Ni^{2+}, Cd^{2+}, with the subsequent addition of 1 equiv. of Zn^{2+}.

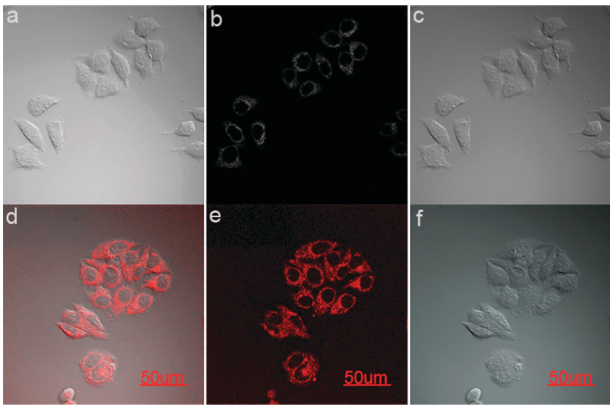

Fig. 4 Fluorescence confocal images of Breast Cancer MCF-7 cells: top, (a–c) cells incubated with 5.0 μM **1-OH** for 30 min; bottom, (d–f) cells incubated with 5.0 μM **1-OH** for 30 min, then treated with Zn^{2+} for another 30 min and washed with PBS. Overlap field (a and d), fluorescence image (b and e), bright field (c and f). The samples were excited with 638 nm, under observation between 662–737 nm.

cells. The overlap of fluorescence and bright-field images revealed that the fluorescence signals were localized in the perinuclear area of the cytosol, indicative of a subcellular distribution and good cell membrane permeability of **1-OH**. These results suggest that **1-OH** can be explored for monitoring Zn^{2+} within living cells.

In summary, we reported the synthesis and photophysical evaluation of a NIR turn-on fluorescent probe **1-OH** for Zn^{2+} with high selectivity. The coordination of **1-OH** with Zn^{2+} induces distinct emission enhancement in the NIR region (680 nm), which is attributable to the Zn^{2+} binding with the Schiff-base ligand on the basis of the CHEF effect, resulting in the deprotonation of the phenol group and causing the C=N bond to rigidify. No detection disturbance of Zn^{2+} by other cations is successfully realized, especially with little interference from Cd^{2+}. With the particularly attractive emission in the NIR region, **1-OH** can be explored for the bioimaging of Zn^{2+} with several advantages such as cell-permeability, and desirable NIR turn-on emission, beneficial for deep light penetration and weak autofluorescence of biological tissues. The CHEF strategy is expected to further help construct turn-on NIR fluorescent probes for metal ions.

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Notes and references

1 (*a*) J. M. Berg and Y. Shi, *Science*, 1996, **271**, 1081–1085; (*b*) A. C. Burdette and S. J. Lippard, *Proc. Natl. Acad. Sci. U. S. A.*, 2003, **100**, 3605–3610; (*c*) B. L. Vallee and K. H. Falchuk, *Psychol. Rep.*, 1993, **73**, 79–118; (*d*) Z. C. Xu, J. Y. Yoon and D. R. Spring, *Chem. Soc. Rev.*, 2010, **39**, 1996–2006.

2 Recent reviews for zinc sensors: (*a*) E. L. Que, D. W. Domaille and C. J. Chang, *Chem. Rev.*, 2008, **108**, 1517–1549; (*b*) E. M. Nolan and S. J. Lippard, *Acc. Chem. Res.*, 2009, **42**, 193–203; (*c*) P. J. Jiang and Z. J. Guo, *Coord. Chem. Rev.*, 2004, **248**, 205–229; (*d*) P. Carol, S. Sreejith and A. Ajayaghosh, *Chem.–Asian J.*, 2007, **2**, 338–348; (*e*) N. C. Lim, H. C. Freake and C. Brückner, *Chem.–Eur. J.*, 2005, **11**, 38–49; (*f*) B. Tang, H. Huang, K. H. Xu, L. L. Tong, G. W. Yang, X. Liu and L. G. An, *Chem. Commun.*, 2006, 3609–3611.

3 (*a*) X. Zhang, D. Hayes, S. J. Smith, S. Friedle and S. J. Lippard, *J. Am. Chem. Soc.*, 2008, **130**, 15788–15789; (*b*) X. J. Peng, Y. Q. Xu, S. G. Sun, Y. K. Wu and J. L. Fan, *Org. Biomol. Chem.*, 2007, **5**, 226–228; (*c*) Q. Zhao, F. Y. Li and C. H. Huang, *Chem. Soc. Rev.*, 2010, **39**, 3007–3030; (*d*) A. Ajayaghosh, P. Carol and S. Sreejith, *J. Am. Chem. Soc.*, 2005, **127**, 14962–14963.

4 (*a*) F. Qian, C. Zhang, Y. Zhang, W. He, X. Gao, P. Hu and Z. Guo, *J. Am. Chem. Soc.*, 2009, **131**, 1460–1468; (*b*) Z. Xu, K. H. Baek, H. N. Kim, J. Cui, X. Qian, D. R. Spring, I. Shin and J. Yoon, *J. Am. Chem. Soc.*, 2010, **132**, 601–610; (*c*) K. Hanaoka, Y. Muramatsu, Y. Urano, T. Terai and T. Nagano, *Chem.–Eur. J.*, 2010, **16**, 568–572; (*d*) Z. Li, M. Yu, L. Zhang, M. Yu, J. Liu, L. Wei and H. Zhang, *Chem. Commun.*, 2010, **46**, 7169–7171; (*e*) C. Zhao, Y. Zhang, P. Feng and J. Cao, *Dalton Trans.*, 2012, **41**, 831–838; (*f*) K. Komatsu, Y. Urano, H. Kojima and T. Nagano, *J. Am. Chem. Soc.*, 2007, **129**, 13447–13454.

5 (*a*) Z. Q. Guo, W. H. Zhu, M. M. Zhu, X. M. Wu and H. Tian, *Chem.–Eur. J.*, 2010, **16**, 14424–14432; (*b*) E. Sasaki, H. Kojima, H. Nishimatsu, Y. Urano, K. Kikuchi, Y. Hirata and T. Nagano, *J. Am. Chem. Soc.*, 2005, **127**, 3684–3685; (*c*) B. Tang, F. Yu, P. Li, L. Tong, X. Duan, T. Xie and X. Wang, *J. Am. Chem. Soc.*, 2009, **131**, 3016–3023; (*d*) L. Yuan, W. Y. Lin, Y. T. Yang and H. Chen, *J. Am. Chem. Soc.*, 2012, **134**, 1200–1211; (*e*) Q. Zhao, C. H. Huang and F. Y. Li, *Chem. Soc. Rev.*, 2011, **40**, 2508–2524; (*f*) P. Li, X. Duan, Z. Z. Chen, Y. Liu, T. Xie, L. Fang, X. R. Li, M. Lin and B. Tang, *Chem. Commun.*, 2011, **47**, 7755–7757; (*g*) K. T. Arun and D. Ramaiah, *J. Phys. Chem. A*, 2005, **109**, 5571–5578; (*h*) J. Y. Shao, H. Y. Sun, H. M. Guo, S. M. Ji, J. Z. Zhao, W. T. Wu, X. L. Yuan, C. L. Zhang and T. D. James, *Chem. Sci.*, 2012, **3**, 1049–1061.

6 (*a*) K. Kiyose, H. Kojima, Y. Urano and T. Nagano, *J. Am. Chem. Soc.*, 2006, **128**, 6548–6549; (*b*) C. H. Hung, G. F. Chang, A. Kumar, G. F. Lin, L. Y. Luo, W. M. Ching and E. W. G. Diau, *Chem. Commun.*, 2008, 978–980; (*c*) S. Atilgan, T. Ozdemir and E. U. Akkaya, *Org. Lett.*, 2008, **10**, 4065–4067; (*d*) X. Y. Lu, W. H. Zhu, Y. S. Xie, X. Li, Y. Gao, F. Y. Li and H. Tian, *Chem.–Eur. J.*, 2010, **16**, 8355–8364; (*e*) X. W. Cao, W. Y. Lin and W. Wan, *Chem. Commun.*, 2012, **48**, 6247–6249.

7 (*a*) L. Salmon, P. Thuéry, E. Rivière and M. Ephritikhine, *Inorg. Chem.*, 2006, **45**, 83–93; (*b*) D. M. Epstein, S. Choudhary, M. R. Churchill, K. M. Keil, A. V. Eliseev and J. R. Morrow, *Inorg. Chem.*, 2001, **40**, 1591–1596; (*c*) L. N. Wang, W. W. Qin, X. L. Tang, W. Dou and W. S. Liu, *J. Phys. Chem. A*, 2011, **115**, 1609–1616; (*d*) S. Dutta, P. Biswas, U. Flörke and K. Nag, *Inorg. Chem.*, 2010, **49**, 7382–7400; (*e*) C. Ma, A. Lo, A. Abdolmaleki and M. J. MacLachlan, *Org. Lett.*, 2004, **6**, 3841–3844; (*f*) J. Wang, C. He, P. Y. Wu, J. Wang and C. Y. Duan, *J. Am. Chem. Soc.*, 2011, **133**, 12402–12405; (*g*) S. Panda, P. B. Pati and S. S. Zade, *Chem. Commun.*, 2011, **47**, 4174–4176.

8 (*a*) J. Wu, W. Liu, X. Zhuang, F. Wang, P. Wang, S. Tao, X. Zhang, S. Wu and S. T. Lee, *Org. Lett.*, 2007, **9**, 33–36; (*b*) J. S. Wu, J. H. Zhou, P. F. Wang, X. H. Zhang and S. K. Wu, *Org. Lett.*, 2005, **7**, 2133–2136; (*c*) M. A. Hortalá, L. Fabbrizzi, N. Marcotte, F. Stomeo and A. Taglietti, *J. Am. Chem. Soc.*, 2003, **125**, 20–21.

9 (*a*) C. Zhao, P. Feng, J. Cao, Y. Zhang, X. Wang, Y. Yang, Y. Zhang and J. Zhang, *Org. Biomol. Chem.*, 2012, **10**, 267–272; (*b*) C. Zhao, Y. Zhou, Q. Lin, L. Zhu, P. Feng, Y. Zhang and J. Cao, *J. Phys. Chem. B*, 2011, **115**, 642–647; (*c*) C. Zhao, P. Feng, J. Cao, X. Wang, Y. Yang, Y. Zhang, J. Zhang and Y. Zhang, *Org. Biomol. Chem.*, 2012, **10**, 3104–3109; (*d*) J. Cao, C. Zhao, P. Feng, Y. Zhang and W. Zhu, *RSC Adv.*, 2012, **2**, 418–420; (*e*) H. M. Guo, Y. Y. Jing, X. L. Yuan, S. M. Ji, J. Z. Zhao, X. H. Li and Y. Y. Kan, *Org. Biomol. Chem.*, 2011, **9**, 3844–3853; (*f*) X. J. Peng, J. J. Du, J. L. Fan, J. Y. Wang, Y. K. Wu, J. Z. Zhao, S. G. Sun and X. Tao, *J. Am. Chem. Soc.*, 2007, **129**, 1500–1501; (*g*) M. Royzen, A. Durandin, V. G. Young, N. E. Geacintov and J. W. Canary, *J. Am. Chem. Soc.*, 2006, **128**, 3854–3855.