A visible light excitable fluorescent sensor for triphosphate/pyrophosphate based on a diZn^{2+} complex bearing an intramolecular charge transfer fluorophore†

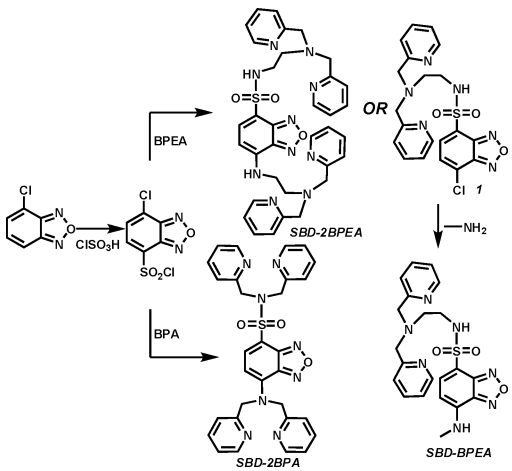
Guangyu Su, Zhipeng Liu, Zhijun Xie, Fang Qian, Weijiang He* and Zijian Guo*

Received 21st May 2009, Accepted 17th August 2009
First published as an Advance Article on the web 24th August 2009
DOI: 10.1039/b914802g

Triphosphate or pyrophosphate can be recognised by a diZn^{2+} complex of bis(BPEA)-appended intramolecular charge transfer fluorophore 4-amino-7-aminosulfonyl-2,1,3-benzoxadiazole, displaying a 5–6 fold fluorescent enhancement at 576 nm.

Phosphates play an essential role in the human body and other living systems. Among them, pyrophosphate (PPi) and adenosine triphosphate (ATP) are involved in energy transduction, metabolic processes, extracellular signal mediations, DNA polymerisation and cyclic adenosine monophosphate synthesis, *etc*.[1,2] Selective sensing and discrimination of different phosphate anions are essential for clarifying their roles in these processes and has been extensively studied.[3–10] The fluorescent sensing strategy *via* metal coordination-altered emission is found to be more effective than that of hydrogen bonding-altered emission, since metal coordination leads to a higher phosphate affinity in aqueous solution.[3,11] Besides the high phosphate affinity, Zn^{2+} does not exhibit any emission quenching effects to fluorophore due to its $3d^{10}4s^0$ configuration. Therefore, Zn^{2+} fluorescent complexes with a vacant coordination site have been adopted as fluorescent probes for different phosphates. In fact, sensors employing bis(BPA-Zn^{2+})-appended fluorophores such as (naphthalen-2-yl)phenol, xanthone and anthracenes display selective fluorescent response to phosphates due to the synergic Zn^{2+} coordination to the fluorophore-spaced bis(BPA-Zn^{2+}) motif (BPA, bis(pyridin-2-ylmethyl)amine).[12–14] However, their UV excited sensors often suffer from the UV irradiation-induced cell damage and autofluorescence,[10,15] which precludes their application in living systems. Sensors derived from intramolecular charge transfer (ICT) fluorophores may overcome these problems since they normally display visible excitability and large Stokes shift. Their ICT effect from its electron-donating group (D) to electron-withdrawing group (A) are responsible for these properties.[16–18]

In this work, a visible light excited sensor, **SBD-2BPEA**, was designed for phosphate sensing *via* Zn^{2+}-mediated binding. In this compound, the distal D and A of ICT fluorophore 4-amino-7-aminosulfonyl-2,1,3-benzoxidiazole (ASBD) has been modified as Zn^{2+} ionophore BPEA (N^1,N^1-bis(pyridin-2-ylmethyl)ethane-1,2-diamine) and sulfonated BPEA, respectively. The analogue, **SBD-2BPA**, which was devised in the normal bis(BPA-Zn^{2+})-appending strategy was also investigated for its phosphate sensing behaviour (Scheme 1). Both **SBD-2BPEA** and **SBD-2BPA** were prepared from 4-chloro-7-chlorosulfonyl-2,1,3-benzoxadiazole reacting with BPEA or BPA.[16] The phosphate sensing behaviours of their diZn^{2+} complexes have been determined by fluorescence spectroscopy. In addition, **SBD-BPEA**, the analogue of **SBD-2BPEA** with methylamine replacing 4-BPEA, was also prepared for comparison.



Scheme 1 Synthesis of **SBD-2BPEA**, **SBD-BPEA** and **SBD-2BPA**.

SBD-2BPEA exhibits stable fluorescence in aqueous solution from pH 7–10 (3% DMSO, v/v; λ_{ex} 468 nm, λ_{em} 591 nm; Fig. S1†), while **SBD-BPEA** exhibits stable fluorescence from pH 2–10 (λ_{ex} 451 nm, λ_{em} 591 nm). Both compounds undergo distinct emission decrease at pH >10.0, which can be ascribed to the reduced ICT effect caused by the deprotonation of sulfonamide. The stable emission of **SBD-BPEA** at even lower pH implies there should be no photo-induced electron transfer (PET) effect from 7-sulfonated BPEA, which may be due to the intramolecular hydrogen bonding between sulfonamide and the *tert*-amine of 7-sulfonated BPEA. However, **SBD-2BPEA** displays an enhanced emission at pH ≤6. Their different fluorescent pH-dependence at lower pH suggests that only 4-BPEA in **SBD-2BPEA** has a PET effect on the fluorophore, and that the enhanced emission of **SBD-2BPEA** at low pH originates from the PET elimination induced by the *tert*-amine protonation in 4-BPEA.

The phosphate sensing behaviour of the diZn^{2+} complex of **SBD-2BPEA** was investigated in buffer solution containing 10 μM **SBD-2BPEA** and 20 μM Zn^{2+} (2Zn^{2+}–**SBD-2BPEA** system,

State Key Laboratory of Coordination Chemistry, School of Chemistry and Chemical Engineering, Nanjing University, Nanjing 210093, P. R. China. E-mail: heweij69@nju.edu.cn, zguo@nju.edu.cn; Fax: +86 25-83314502; Tel: +86 25-83597066; +86 25-83594549

† Electronic supplementary information (ESI) available: Experimental details; fluorescent, emission and ESI-MS spectra; the proposed binding mode of 2Zn^{2+}–**SBD-2BPEA** and PPi. See DOI: 10.1039/b914802g

10 mM HEPES, 1% DMSO, pH 7.2). The instant fluorescence enhancement was observed upon triphosphate (TP) addition. The emission increases almost linearly with the $[TP]_{total}$ and the emission enhancement is no longer observed when the $[TP]_{total}$: [$2Zn^{2+}$–**SBD-2BPEA**] ratio is higher than 1 : 1. The emission enhancement factor (EEF) induced by one equivalent TP is around six. The results suggest that the TP binding ratio of the $2Zn^{2+}$–**SBD-2BPEA** system is around 1 : 1. Moreover, the emission of **SBD-2BPEA** is blue shifted from 591 to 576 nm in this process (Fig. 1(a)). A similar fluorescent response was also observed for the PPi anion with an EEF of ~5 accompanied by a blue shift of 10 nm (Fig. S2†). However, the addition of monophosphate anions $HPO_4^{2-}/H_2PO_4^-$ and other common anions such as $CH_3CO_2^-$, NO_3^-, Cl^-, CO_3^{2-}, SO_4^{2-}, ClO_4^- and F^- does not induce any distinct emission change (Fig. 1(b)). Interestingly, TP or PPi do not induce any evident emission enhancement of the $1Zn^{2+}$–**SBD-2BPEA** system (HEPES buffer of 10 μM **SBD-2BPEA** and 10 μM Zn^{2+}), which suggests that the presence of two equivalent Zn^{2+} is essential for the fluorescent discrimination of TP/PPi from monophosphate anions. On the other hand, **SBD-BPEA** displays a silent response to TP and PPi in both the $2Zn^{2+}$–**SBD-BPEA** (HEPES buffer with 10 μM **SBD-BPEA** and 20 μM Zn^{2+}) and the $1Zn^{2+}$–**SBD-BPEA** system (HEPES buffer of 10 μM **SBD-BPEA** and 10 μM Zn^{2+}). All these results demonstrate that the additional 4-BPEA in **SBD-2BPEA** is essential for the TP/PPi sensing ability of the $2Zn^{2+}$–**SBD-2BPEA** system.

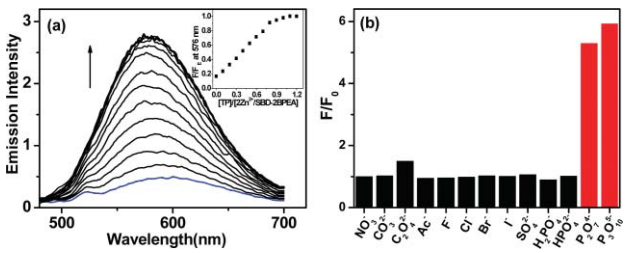


Fig. 1 (a) The emission spectra of the $2Zn^{2+}$–**SBD-2BPEA** (10 μM) system in HEPES buffer when titrated by $Na_5P_3O_{10}$ solution. The $[P_3O_{10}^{5-}]$ for each spectrum is 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 and 13 μM, respectively (from bottom to top). λ_{ex}, 468 nm. Inset: the titration profile according to the emission at 576 nm. F_0 is the emission intensity of free **SBD-2BPEA** at 576 nm. (b) The different emission enhancement factors of the $2Zn^{2+}$–**SBD-2BPEA** system in HEPES buffer (10 mM, pH 7.2, 1% DMSO) induced by different anions. The EEFs were obtained according to the emission at 576 nm when irradiated at 468 nm.

Under similar conditions, neither the $2Zn^{2+}$–**SBD-2BPA** system (10 μM **SBD-2BPA** and 20 μM Zn^{2+}) nor the $1Zn^{2+}$–**SBD-2BPA** system (10 μM **SBD-2BPA** and 10 μM Zn^{2+}) displays any fluorescent response to TP or PPi. There have been successful examples for the design of phosphate sensors by modifying fluorophores such as xanthone and anthracenes with an appended bis(BPA-Zn^{2+}) moiety,[12–14] however, current data show that such a strategy does not work for ASBD fluorophore modification.

The different phosphate sensing behaviour of **SBD-2BPA** and **SBD-2BPEA** should be correlated to their different Zn^{2+} binding behaviours. In fact, **SBD-2BPA** displays no evident emission change upon Zn^{2+} titration, suggesting that **SBD-2BPA** has a very poor Zn^{2+} binding ability as supported from its UV Zn^{2+} titration data. The poor Zn^{2+} binding ability is due to the electron withdrawing effect of 7-sulfonamide on 4-BPA, which in turn invalidates the strategy of appending the bis(BPA-Zn^{2+}) moiety to the fluorophore for phosphate sensing. A similar case has also been reported for its analogue, **NBD-BPA**, in which the electron-withdrawing 7-NO_2 decreases the electron donating ability of 4-amine and disables 4-BPA from Zn^{2+} binding.[18,19] The fluorescent Zn^{2+} titration of **SBD-BPEA** exhibits a linear emission decrement with the increase of $[Zn^{2+}]_{total}$, and the emission becomes steady when the $[Zn^{2+}]_{total}$–[**SBD-BPEA**] ratio reaches higher than 1 : 1 (Fig. S3†). The result suggests a 1 : 1 Zn^{2+}-binding stoichiometry for **SBD-BPEA**, and the Zn^{2+}-triggered sulfonamide deprotonation should reduce the ICT effect and result in the emission decrease similar to the decreased emission at pH >10.0.

The fluorescent Zn^{2+} titration of **SBD-2BPEA** also demonstrates a linear emission decrease with an increase of $[Zn^{2+}]_{total}$ when the $[Zn^{2+}]_{total}$–[**SBD-2BPEA**] ratio is lower than 0.5 (Fig. 2). Afterwards, the emission decrease rate becomes smaller at higher $[Zn^{2+}]_{total}$ and reaches a steady state when the $[Zn^{2+}]_{total}$–[**SBD-2BPEA**] ratio is ~1.4. Only minor emission enhancement can be observed when the $[Zn^{2+}]_{total}$–[**SBD-2BPEA**] ratio increases from 1.4 to 4. Comparison between the fluorescent Zn^{2+} titration data of **SBD-2BPEA** and **SBD-BPEA** implies that Zn^{2+} is firstly bound to the 7-sulfonated BPEA in **SBD-2BPEA** at lower $[Zn^{2+}]_{total}$, and that deprotonation of sulfonamide results in the emission decrease. Since 4-BPEA displays the sole PET effect in **SBD-2BPEA**, the second Zn^{2+} to 4-BPEA forming the diZn^{2+} complex at higher $[Zn^{2+}]_{total}$ should lead to the reduced emission decrease rate or even emission enhancement. In fact, all of these have been observed when the $[Zn^{2+}]_{total}$–[**SBD-2BPEA**] ratio reaches higher than 0.5. The stability of the diZn^{2+} complex of **SBD-2BPEA** should be low, therefore only minor emission enhancement was observed even when four equivalents of Zn^{2+} was added. In addition, the ESI-MS data show only the peak for the mono-Zn^{2+} complex (727.4, $[M - H + Zn]^+$, Fig. S4†), suggesting that the stability of the diZn^{2+} complex is poor in the conditions used for MS determination.

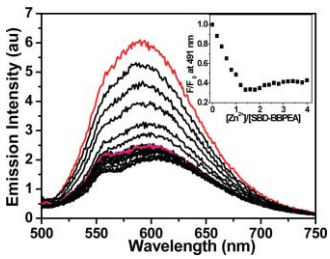


Fig. 2 The fluorescent spectra of 10 μM **SBD-2BPEA** in aqueous HEPES buffer (10 mM, pH 7.2) containing 3% DMSO (v/v) obtained when titrated by adding aliquots of 5 μL $Zn(NO_3)_2$ solution (1.2 mM). λ_{ex}, 468 nm. The top line is for free **SBD-2BPEA**, the bottom line is for the presence of 1.4 equivalent Zn^{2+}. The inset displays the titration profile obtained according to the emission intensity at 591 nm.

The TP-triggered emission enhancement of the $2Zn^{2+}$–**SBD-2BPEA** system should be induced by the synergic TP coordination to both Zn^{2+} centres with the two spaced BPEA motifs to form the diZn^{2+} complex (Fig. 3), since the $1Zn^{2+}$–**SBD-2BPEA** system does not show any response. The mono-Zn^{2+} complex of **SBD-2BPEA** alone displays lower emission than the diZn^{2+} complex due to the deprotonation of sulfonamide and the

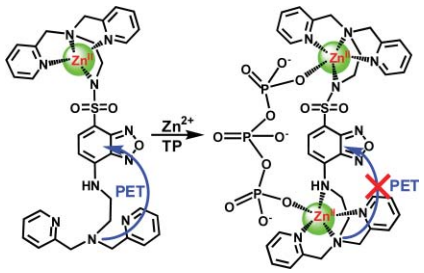


Fig. 3 The proposed sensing mechanism and binding mode of $2Zn^{2+}$–**SBD-2BPEA** system to triphosphate anion.

PET effect of 4-BPEA. The affinity of **SBD-2BPEA** to the second Zn^{2+} could be largely enhanced by the synergic coordination effect and the negative charges of TP. As a result, the PET effect of 4-BPEA is effectively blocked and emission enhancement is achieved.

In order to verify the coordination mode of the ternary complex of **SBD-2BPEA**, solid samples were prepared by adding TP to the methanol solution of **SBD-2BPEA** and $Zn(NO_3)_2$. The ESI-MS spectrum (negative mode, in DMSO–methanol) of the sample exhibits an intensive signal at m/z 1047.4 (Fig. S5†). The signal can be assigned to $[2Zn^{2+}$ + **SBD-2BPEA** + $P_3O_{10}^{5-}]^-$, which confirms the binding ratio of 2 : 1 : 1 ([Zn^{2+}]–[**SBD-2BPEA**]–[$P_3O_{10}^{5-}$]) for the diZn^{2+} complex. The intramolecular synergic coordination of TP to dual Zn^{2+} centers favours the stabilisation of the diZn^{2+} complex even in the conditions for MS determination. Further evidence for such a binding mode comes from the ^{31}P NMR spectral data. Two signals of free TP at −5.62 (distal P) and −20.47 (middle P) ppm are shifted to −7.58 and −19.35 ppm, respectively, when mixed with one equivalent **SBD-2BPEA** and two equivalents $Zn(NO_3)_2$ (Fig. 4).

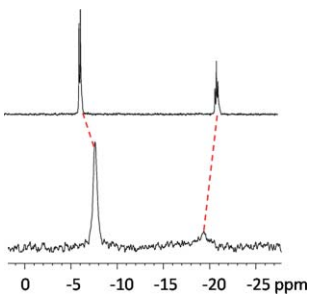


Fig. 4 ^{31}P NMR spectra of TP (upper) and TP in the presence of one equivalent **SBD-2BPEA** and two equivalents Zn^{2+} in CD_3OD–D_2O (lower).

Due to the flexibility of the BPEA motif, PPi is able to coordinate to the two spaced (BPEA-Zn^{2+}) motif in a similar synergic manner (Fig. S6†). However, HPO_4^{2-} and $H_2PO_4^-$ fail to do so and no emission change is observed, although their Zn^{2+} affinity is comparable to that of TP and PPi. Their smaller size means that one such anion cannot coordinate with the spaced bis(BPEA-Zn^{2+}) motif simultaneously. The minor emission enhancement induced by the bulk $C_2O_4^{2-}$ (EEF, ~1.5) also supports this proposal. According to the fluorescence titration, the apparent binding constants of diZn^{2+}–**SBD-2BPEA** complex to TP and PPi were estimated to be 4.1×10^5 and 2.8×10^5 M^{-1} (Fig. S7†). The constants are comparable to those reported by Hamachi *et al.*, which displays the confirmed synergic coordination of phosphates to the unsaturated coordinated bisZn^{2+}.[14a]

In conclusion, a novel visible light excited fluorescent TP/PPi sensor was successfully constructed by appending the bis(BPEA-Zn^{2+}) motif to the ICT fluorophore ASBD. The $2Zn^{2+}$–**SBD-2BPEA** system displays a selective PPi/TP-amplified fluorescence upon excitation at 468 nm. However, the analogous $2Zn^{2+}$–**SBD-2BPA** system based on the reported bis(BPA-Zn^{2+}) appending strategy fails to exhibit any fluorescent response. This work provides a new promising approach for the development of visible light excited sensors for phosphates by coupling two Zn^{2+} binding motifs with an ICT fluorophore. Moreover, the resulting sensors can be fine tuned so that different phosphate anions can be distinguished.

We thank the financial support from the National Natural Science Foundation of China (No. 20871066, 30370351, 90713001, 20721002, 20571043) and the Natural Science Foundation of Jiangsu Province (BK2008015).

Notes and references

1 (*a*) W. N. Limpcombe and N. Sträter, *Chem. Rev.*, 1996, **96**, 2375; (*b*) P. Nyrén, *Anal. Biochem.*, 1987, **167**, 235–238; (*c*) T. Tabary and L. Ju, *J. Immunol. Methods*, 1992, **156**, 55–60; (*d*) D. J. McCarty, *Arthritis Rheum.*, 1976, **19**, 275–285; (*e*) A. Caswell, D. F. Guilland-Cumming, P. R. Hearn, M. K. McGuire and R. G. Russell, *Ann. Rheum. Dis.*, 1983, **42**(Suppl. 1), 27–37.
2 (*a*) A. V. Gourine, E. Llaudet, N. Dale and K. M. Spyer, *Nature*, 2005, **436**, 108–111; (*b*) S. A. Johnson and T. Hunter, *Nat. Methods*, 2005, **2**, 17–25.
3 R. Martínez-Máñez and F. Sancenón, *Chem. Rev.*, 2003, **103**, 4419–4476.
4 S. E. García-Garrido, C. Caltagirone, M. E. Light and P. A. Gale, *Chem. Commun.*, 2007, 1450–1452.
5 F. Pina, M. A. Bernardo and E. García-España, *Eur. J. Inorg. Chem.*, 2000, 2143–2157.
6 N. Marcotte and A. Taglietti, *Supramol. Chem.*, 2003, **15**, 617–625.
7 S. Sasaki, D. Citterio, S. Ozawa and K. Suzuki, *J. Chem. Soc., Perkin Trans. 2*, 2001, 2309–2313.
8 H. K. Cho, D. H. Lee and J.-I. Hong, *Chem. Commun.*, 2005, 1690–1692.
9 Z. Chen, X. Wang, J. Chen, X. Yang, Y. Li and Z. Guo, *New J. Chem.*, 2007, **31**, 357–362.
10 C. Lakshmi, R. G. Hanshaw and B. D. Smith, *Tetrahedron*, 2004, **60**, 11307–11315.
11 (*a*) E. J. O'Neil and B. D. Smith, *Coord. Chem. Rev.*, 2006, **250**, 3068–3080; (*b*) P. D. Beer and P. A. Gale, *Angew. Chem., Int. Ed.*, 2001, **40**, 486–516.
12 D. H. Lee, S. Y. Kim and J.-I. Hong, *Angew. Chem., Int. Ed.*, 2004, **43**, 4777–4780.
13 D. J. Oh and K. H. Ahn, *Org. Lett.*, 2008, **10**, 3539–3542.
14 (*a*) A. Ojida, Y. Mito-oka, K. Sada and I. Hamachi, *J. Am. Chem. Soc.*, 2004, **126**, 2454–2463; (*b*) A. Ojida, H. Nonaka, Y. Miyahara, S. Tamaru, K. Sada and I. Hamachi, *Angew. Chem., Int. Ed.*, 2006, **45**, 5518–5521; (*c*) A. Ojida, I. Takashima, T. Kohira, H. Nonaka and I. Hamachi, I, *J. Am. Chem. Soc.*, 2008, **130**, 12095–12101.
15 H. Jiang, E. J. O'Neil, K. M. DiVittorio and B. D. Smith, *Org. Lett.*, 2005, **7**, 3013–3016.
16 S. Uchiyama, T. Santa, T. Fukushima, H. Homma and K. Imai, *J. Chem. Soc., Perkin Trans. 2*, 1998, 2165–2173.
17 Z. R. Grabowski, K. Rotkiewicz and W. Rettig, *Chem. Rev.*, 2003, **103**, 3899–4031.
18 F. Qian, C. Zhang, Y. Zhang, W. He, X. Gao, P. Hu and Z. Guo, *J. Am. Chem. Soc.*, 2009, **131**, 1460–1468.
19 S. Banthia and A. Samanta, *New J. Chem.*, 2005, **29**, 1007–1010.

