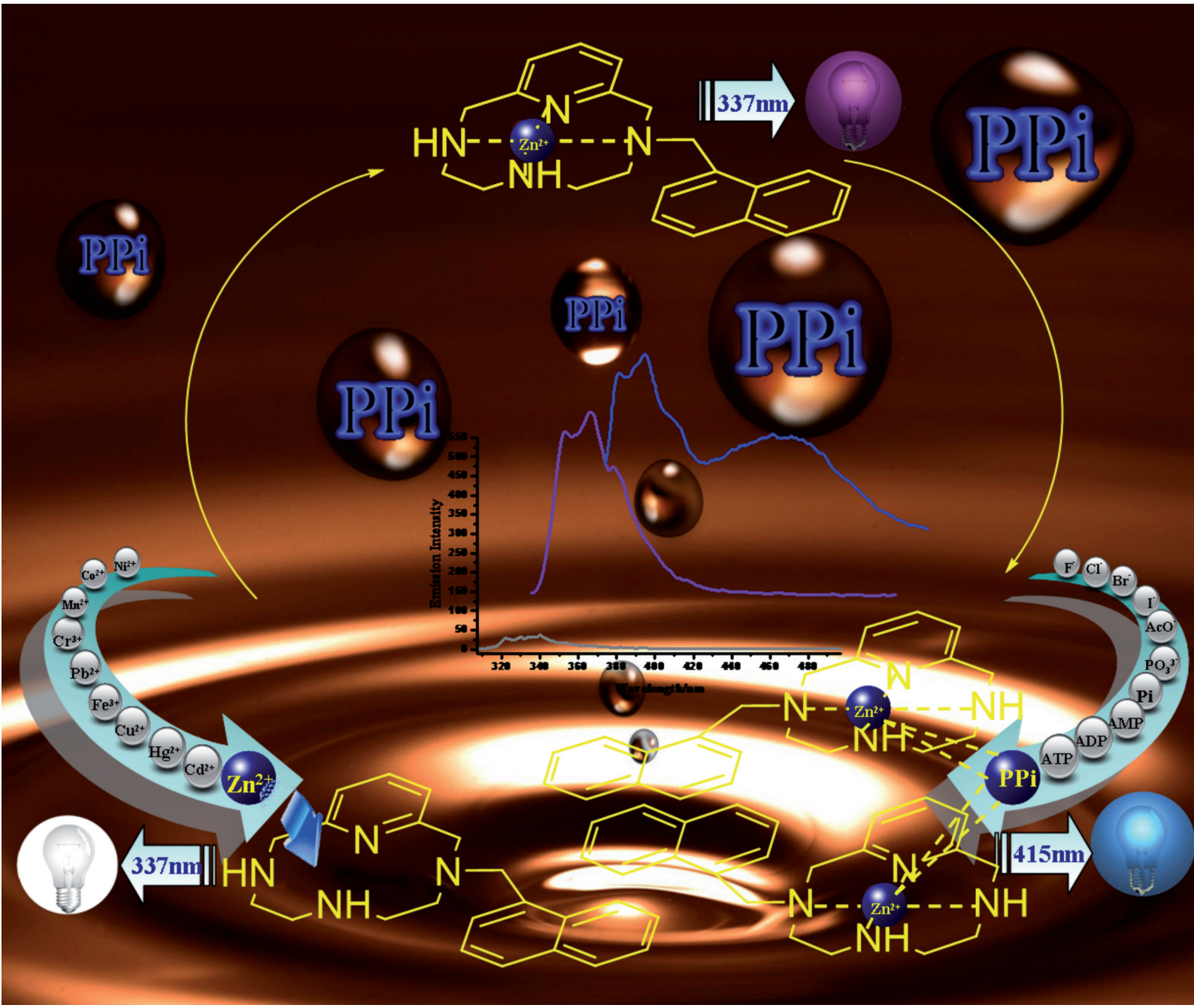
Celebrating 40 years

Dalton Transactions

An international journal of inorganic chemistry

www.rsc.org/dalton

Volume 40 | Number 9 | 7 March 2011 | Pages 1825–2076



ISSN 1477-9226

COVER ARTICLE

Wang *et al.*

A Zn^{2+}-specific turn-on fluorescent probe for ratiometric sensing of pyrophosphate in both water and blood serum

International Year of CHEMISTRY 2011

1477-9226(2011)40:9;1-F

Dalton Transactions

Dynamic Article Links

Cite this: *Dalton Trans.*, 2011, **40**, 1984

www.rsc.org/dalton

PAPER

A Zn^{2+}-specific turn-on fluorescent probe for ratiometric sensing of pyrophosphate in both water and blood serum†

Jinghan Wen,[a] Zhirong Geng,[a] Yuxin Yin,[a] Zhong Zhang[b] and Zhilin Wang*[a]

Received 19th September 2010, Accepted 12th November 2010
DOI: 10.1039/c0dt01262a

A novel fluorescent sensor composed of a naphthalene functionalized tetraazamacrocycle ligand 3,6,9,15-tetraazabicyclo[9.3.1]pentadeca-1(15),11,13-triene-3-methyl naphthalene (**1**) and Zn^{2+} has been designed and prepared, which can be utilized for selective and ratiometric sensing of pyrophosphate (PPi) over other phosphate-containing anions in aqueous solution at physiological pH. Notably, the water soluble **1** itself also exhibits a selective enhanced fluorescent response to Zn^{2+}, and the complex **1**-Zn^{2+} thus formed eventually fulfils the synergic Zn^{2+} coordination-altered strategy with PPi. Furthermore, the ratiometric sensing of **1**-Zn^{2+} towards PPi performed well even in blood serum milieu. Finally, the sensor **1**-Zn^{2+} was successfully employed to monitor a real-time assay of inorganic pyrophosphatase (PPase) by means of ratiometric fluorescent measurements for the first time.

Introduction

The development of highly selective and sensitive fluorescent sensors has received considerable attention in modern analytical chemistry[1,2] owing to their utility in clarifying and analyzing the roles of ions and biomolecules in living systems. However, compared to the comprehensively investigated water soluble metal sensors,[2a–c] fluorescent sensors for biologically relevant anions have been considered a challenging issue, especially in aqueous solution, due to the strong hydration effects[3,4] and their various complexation geometries.[3,5]

As mentioned above, the rational design of water soluble fluorescent sensors for anions is still in need of a thorough study. Among the commonly accessed anion sensing targets,[2d–h] pyrophosphate (PPi) is of particular importance for its essential role in several bioenergetic and metabolic process.[6] Besides, the relative lack of PPi will result in medial calcification[7] (also known as Mönckeberg's arteriosclerosis). On the other hand, calcium pyrophosphate dihydrate deposition disease (CPPD) has been verified to be associated with an excess of PPi.[8] Thus, over the last ten years,[2g] it has been of great interest to utilize fluorescent sensors to detect and discriminate PPi over other anions, especially phosphate (Pi) or ATP. However, most of these sensors respond to PPi merely by changing the fluorescent intensity, which may be disturbed by the environment. Contrarily, ratiometric fluorescent sensors will be independent of environmental disturbance by calculating the ratio of the fluorescent intensities at two different wavelengths.[9]

Thereby motivated, we herein describe a naphthalene appended tetraazamacrocycle (**1**)-Zn^{2+} complex, which is formed in water and shows high selectivity and sensitivity for ratiometric recognition of PPi above 0.2 μM at physiological pH. The ligand **1** itself is also water soluble and can selectively bind to Zn^{2+} at least down to 0.4 μM with an enhanced fluorescent response, which eventually assists to form the novel host for PPi. Notably, the binding of PPi can be probed and confirmed by a characteristic naphthalene fluorescent excimer emission, which is attributed to the assembly of **1**-Zn^{2+}. Although there are examples[10] showing that PPi directed the hosts to be self-assembled and which could be quantitatively analyzed by means of fluorescent excimer formation measurements, our sensor outweighs others in the satisfactory water solubility and the emergence of the unique excimer emission peak that is not induced by any other anions. Besides, their utility in blood serum has never been explored, and the complex biological environment may interfere with their performance. The blood serum concentrations of PPi are only micromolar, whereas ATP is present in serum in nanomolar quantities and Pi concentrations are millimolar. Moreover, Zn^{2+}-based sensors may be less efficient in serum because the concentrations of Mg^{2+} and Ca^{2+} are significant, which both bind to phosphate-containing anions tightly and thus lead to the decomplexation of the probes. Bearing the above considerations in mind, the specific ratiometric sensing of **1**-Zn^{2+} towards PPi is evaluated in the blood serum milieu, and it has been demonstrated that the ratiometric calibration can still function. Finally, we apply **1**-Zn^{2+}

[a]*State Key Laboratory of Coordination Chemistry, Coordination Chemistry Institute, School of Chemistry and Chemical Engineering, Nanjing University, Nanjing 210093, P. R. China. E-mail: wangzl@nju.edu.cn*
[b]*School of Chemistry and Chemical Engineering, Guangxi Normal University, Guilin, 541004, P. R. China.*

† Electronic supplementary information (ESI) available: Experimental details, characterization of **1** (^{1}H NMR, ^{13}C NMR and ESI-MS), binding behaviors of **1** and **1**-Zn^{2+} (fluorescent titrations, UV titrations, Job's plots, ESI-MS and ^{31}P NMR), fluorescent titrations of **1**-Zn^{2+} in blood serum. See DOI: 10.1039/c0dt01262a

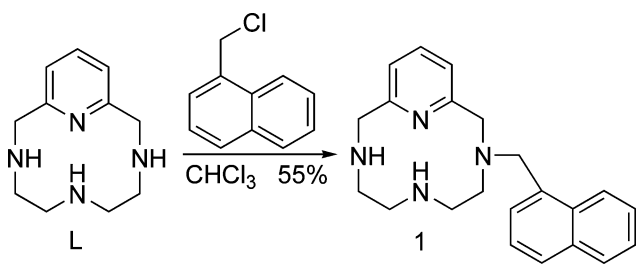
to a nondestructive, real-time assay to monitor the hydrolysis of PPi by inorganic pyrophosphatase (PPase) utilizing ratiometric fluorescent measurements for the first time.

Results and discussion

The aim of our work is to develop a fluorescent sensor which shows a sensitive and selective response to PPi in neutral aqueous solution. First, we have to select an anion host that is able to recognize anions in aqueous solution. It has been known that the fluorescent sensing strategy *via* hydrogen bonding-altered emission is found to be less effective than that of metal coordination-altered one, which leads to a higher phosphate affinity in aqueous solution.[2d–f,5d] Therefore, we prefer metal complexes to free ligand in order to achieve PPi sensing. We hope that the chosen ligand will probably show a selective fluorescent response to Zn^{2+}, which exhibits both high phosphate affinity and emission enhancing effects as a fluorophore due to its $3d^{10}4s^0$ configuration. Moreover, the Zn^{2+}-cyclen (1,4,7,10-tetraazacyclododecane) complex has been known as a good host molecule for anions in neutral aqueous solution.[11] Thus, we decided to employ an analogous compound**L** (3,6,9,15-tetrazabicyclo [9.3.1]pentadeca-1(15),11,13-triene) as the frame to which a naphthalene fluorophore is appended. Its Zn^{2+} fluorescent complex with vacant coordination sites is thus suitable for adoption as a proper fluorescent sensor for PPi. In short, we expect the selective fluorescent response to be triggered due to the synergic Zn^{2+}-ligand coordination to PPi as we have proposed.

Preparation of compounds

Ligand **1** was obtained as illustrated in Scheme 1. The synthesis method of the pyridine containing tetraazamacrocycle ligand 3,6,9,15-tetrazabicyclo[9.3.1]pentadeca-1(15),11,13-triene(**L**) was previously known (see the Experimental section in the supporting information†). Mono *N*-alkylation of **L** at the 3-position with a naphthalene fluorophore was realized in one step by strictly controlling the ratio of the reagents, which made the synthetic procedure effort-saving. It is worth noting that methylnaphthalene group is anchored to the nitrogen in position 3 rather than in position 6, which has been justified by the NMR data.



Scheme 1 Synthesis of the naphthalene appended tetraazamacrocycle ligand (**1**).

Fluorescence of 1 and its pH dependence

Firstly, fluorescence pH-dependence determination of **1** (Fig. 1) was carried out in 0.15 M NaCl aqueous solution to exclude proton disturbance in the detection of metal ions. Ligand **1** dissolves in water readily and fluoresces with λ_{max} at 337 nm at low pH range,

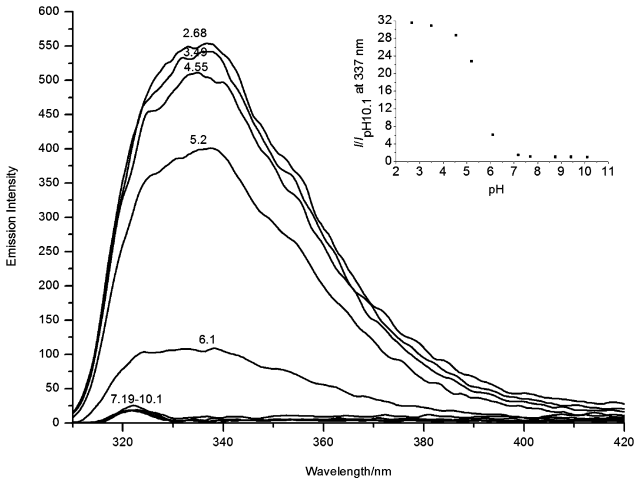


Fig. 1 Emission spectra of 20 μM **1** in water containing 0.15 M NaCl at different pH. λ_{ex} = 280 nm. Inset: The fluorescent pH titration profile of **1** according to $I/I_{pH10.1}$ at 337 nm.

which is assigned to the naphthalene emission.[12] This phenomenon can be ascribed to the reduced photo-induced electron transfer (PET) effect between the tertiary nitrogen atom of the macrocycle moiety and the naphthalene fragment. By increasing the pH, the fluorescent intensity of **1** undergoes a sharp decrease (inset of Fig. 1) and maintains a constant minimal value when pH > 7.2 in aqueous solution. Therefore, all of the detections of metal ions were operated in neutral HEPES buffer (10 mM, pH 7.4).

Zn^{2+} fluorescent response and binding behavior of 1

Various metal ions (5 equiv. each) were added to HEPES buffer (10 mM, pH 7.4) containing 20 μM **1** respectively to verify the extent to which Zn^{2+} will fit the proposed design concept. As expected, **1** exhibits a very weak fluorescence (Fig. 2), and a significant increase of fluorescence is observed for **1** upon addition of Zn^{2+} compared to that of only **1** in the solution. Although a small enhancement in fluorescent intensity is also induced upon the addition of Cd^{2+}, its effect is much less pronounced than that

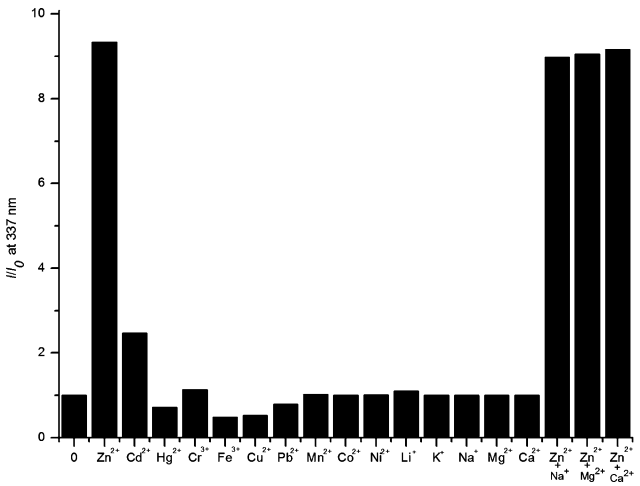


Fig. 2 Histogram of I/I_0 at 337 nm induced by 5 equiv. of different transition-metal cations and 1000 equiv. of Na^+, Ca^{2+}, and Mg^{2+} in HEPES buffer (10 mM, pH 7.4), the concentration of **1** is 20 μM. λ_{ex} = 280 nm.

of Zn^{2+} and it will bring little interference due to its scarcity in living systems. Besides, competitive experiments were carried out by subsequently adding 1000 equiv. of the biologically abundant metal ions (Na^+, Mg^{2+}, Ca^{2+}) to buffer which already contained **1** and Zn^{2+}, and the Zn^{2+}-specific amplified fluorescence of **1** was still unaffected.

The fluorescent response of **1** towards Zn^{2+} (Fig. 3) was preserved in the HEPES buffer aqueous solution (10 mM, pH 7.4). Its fluorescent intensity increases linearly with the concentration of Zn^{2+} (Fig. S4† in the Supporting Information, (0.04–4.0)×10^{-5} M, linearly dependent coefficient: $R^2 = 0.9975$). An enhancement factor of ~19 is observed when the mole ratio (**1**/Zn^{2+}) attains 1 : 1, and higher $[Zn^{2+}]_{total}$ does not result in any further evident fluorescent changes, suggesting a 1 : 1 stoichiometry for the **1**-Zn^{2+} complex (inset of Fig. 3). Besides, Zn^{2+} could be detected at least down to 0.4 μM from the fluorescent titration experiments. The fluorescent Job's plot carried out between **1** and Zn^{2+} also unequivocally determines that the binding stoichiometry is 1 : 1 (Fig. S5†). Moreover, the association constant (K_{ass}) of Zn^{2+} with **1** is determined to be about 4.4×10^6 M^{-1} by nonlinear fitting to the fluorescent titration curve[13] (Fig. S6†). In short, the capture of Zn^{2+} by the receptor **1** can decrease the electron-donating ability of the tertiary nitrogen atom, which results in a decrease in PET efficiency.

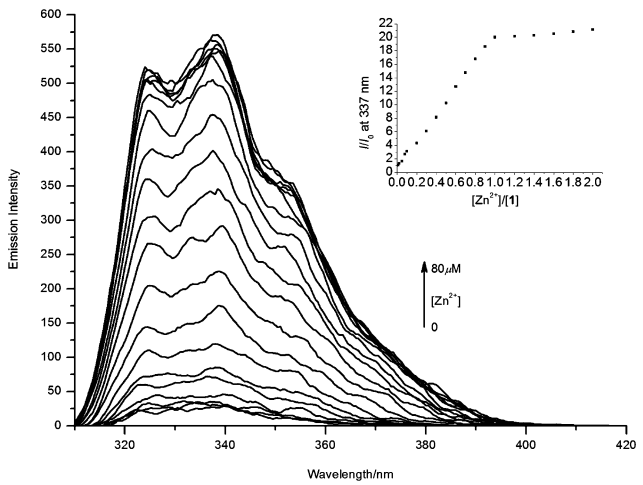


Fig. 3 Emission spectra of 40 μM **1** in HEPES buffer (10 mM, pH 7.4) titrated by $ZnCl_2$ (1 mM) solution (0–80 μM, from bottom to top). λ_{ex} = 280 nm. Inset: The fluorescent titration profile according to I/I_0 at 337 nm.

The Zn^{2+} binding behaviour of **1** was further investigated by UV-vis titration and electrospray ionization mass spectrometry (ESI–MS). The UV-vis spectra (Fig. 4) demonstrate that free **1** has two main absorption bands centered at around 223 nm and 275 nm, which can both be ascribed to π–π* transitions in the naphthalene ring. When Zn^{2+} was added, the two bands both experienced an intensity increase, also denoting the binding of Zn^{2+} to the nitrogen atoms. Moreover, the absorption of **1** increases linearly with the concentration of Zn^{2+} (Fig. S7† , (0.2–2.0)×10^{-5} M, $R^2 = 0.9768$) until the molar ratio (**1**/Zn^{2+}) attains 1 : 1, and thereafter it almost remains unchanged (inset of Fig. 4). Furthermore, although the single crystal structure of **1**-Zn^{2+} has not been obtained yet, its formation is verified by ESI–MS.

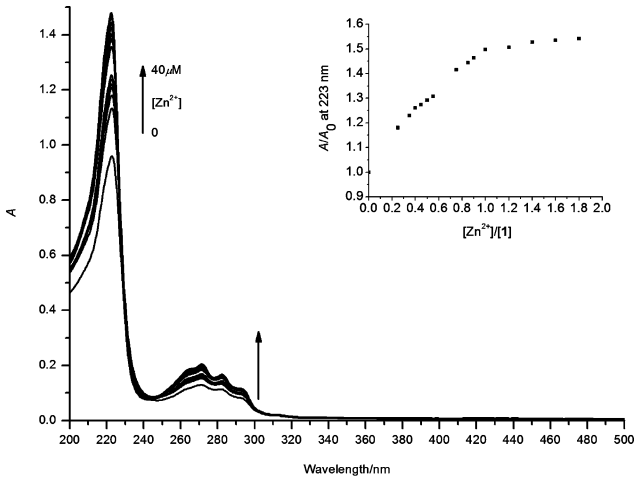


Fig. 4 UV spectra of 20 μM **1** in HEPES buffer (10 mM, pH 7.4) titrated by $ZnCl_2$ (1 mM) solution (0–40 μM). Inset: The UV titration profile according to A/A_0 at 223 nm.

Intensive peaks at m/z 445 (major) and 469 (minor) correspond to $[\mathbf{1}+Zn+Cl]^+$ and $[\mathbf{1}+Zn+H_2O+CH_3CN]^+$, respectively, which are consistent with the calculated isotope results (Fig. S8†).

In addition, we studied the effect of pH on the fluorescent emission of both **1** and **1**-Zn^{2+} in 0.15 M NaCl aqueous solution to clarify the pH range in which the fluorescence of **1**-Zn^{2+} will attain the maximum compared to that of **1**. As shown in Fig. 5, the curve of **1**-Zn^{2+} exhibits a bell shape. It can be explained that the protonation of nitrogen atoms of **1** inhibits the binding of Zn^{2+} to **1** at low pH range. The plateau from 4.0 to 6.0 of the titration curve in the presence of Zn^{2+} can be ascribed to complex **1**-Zn^{2+}, which prevents the PET from occurring. At higher pH (>6.0), the fluorescent intensity decreases quickly, accompanied by the precipitation of Zn^{2+} hydroxide. Compared to the fluorescent spectra of **1**, a remarkable enhancement of fluorescence is observed in the presence of Zn^{2+} (1 equiv.) over almost the whole pH range with a maximum at around 7.5. Thus, this result also suggests that **1**-Zn^{2+} meets our design principle.

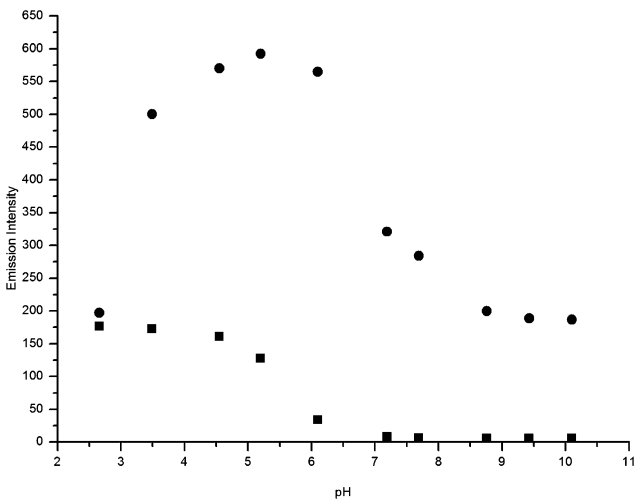


Fig. 5 Effect of pH on the fluorescent intensity at 337 nm of 20 μM **1** in the absence (■) and presence of 20 μM Zn^{2+} (●) in HEPES buffer (10 mM, pH 7.4). λ_{ex} = 280 nm.

Fluorescent response and binding pattern of 1-Zn^{2+} with PPi

The fluorescent changes of **1**-Zn^{2+} upon addition of anions (10 equiv. each) were also examined in neutral HEPES buffer (10 mM, pH 7.4) (Fig. 6). Anions such as F^-, Cl^-, Br^-, I^-, AcO^-,PO_3^{3-}, Pi, ATP, ADP and AMP merely show the structured naphthalene monomer emission centered at 337 nm. Most anions induce negligible fluorescent variations. Nucleoside triphosphates like ATP, ADP, and AMP apparently quench the monomer emission compared to the fluorescent spectrum of **1**-Zn^{2+}, which may be attributed to the enhanced PET process due to the introduction of the electron-rich adenine moieties.[14] Only with PPi does a structureless band with an emission maximum at 415 nm appear, and there is quenching of the monomer emission. Moreover, the new peak at 415 nm can be attributed to excimer formation because no significant changes are observed in UV absorption (Fig. S9†) even by adding a large amount of PPi, which can exclude a charge-transfer mechanism. Furthermore, a more extensive photophysical study was executed to examine the type of the excimer formed. A shift in wavelength ($\Delta\lambda$ = 5 nm) of the excitation spectra is observed (Fig. S10†) when monitored at the monomer (λ_{em} = 337 nm) emission and the excimer (λ_{em} = 415 nm) emission respectively, which identifies the excimer as a preformed dimer rather than a dynamic one.[15]

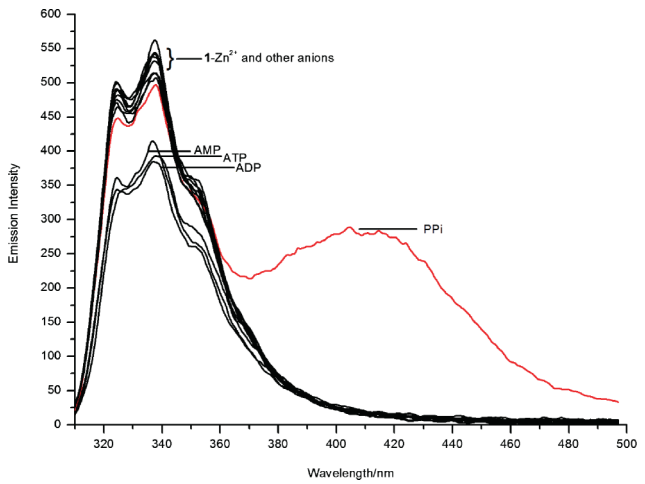


Fig. 6 Emission spectra of 20 μM **1**-Zn^{2+} in the presence of various anions (10 equiv.) in HEPES buffer (10 mM, pH 7.4). λ_{ex} = 280 nm.

Fig. 7 explains the dependence of fluorescent spectra of **1**- Zn^{2+} in neutral HEPES buffer (10 mM, pH 7.4) on the PPi concentration. Increasing the PPi concentration up to about 0.6 equiv. relative to the host concentration results in an approximately 20-fold enhancement in the intensity of the excimer emission, while the monomer emission quenches a little. However, the reverse fluorescent change is observed upon further addition of PPi. As shown in the inset of Fig. 7, the dependence of the emission intensity ratio at 415 nm to that at 337 nm (I_{415}/I_{337}) also exhibits the same trend. The minimum amount of PPi that could be determined is above 0.2 μM from the fluorescent titrations, which is basically equivalent to the lower limit of PPi concentration measured through other chemosensors.[10,11k,16–18]

The binding pattern of **1**-Zn^{2+} with PPi was further investigated by Job's plot, ESI–MS and ^{31}P NMR. As suggested by Job's plot

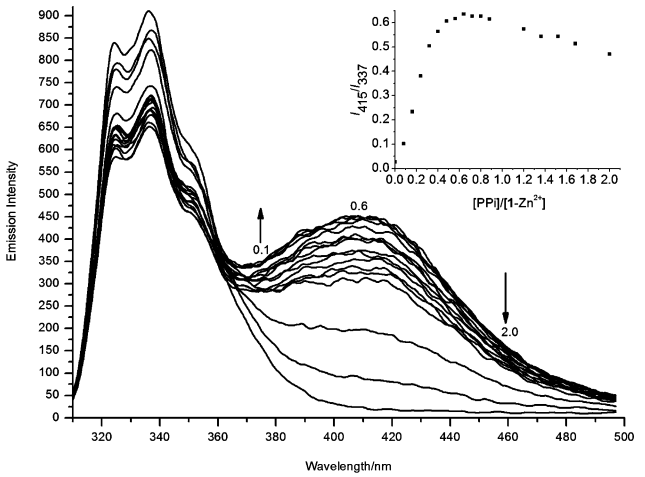
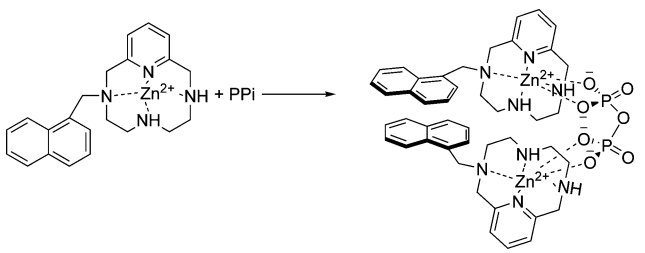


Fig. 7 Emission spectra of 20 μM **1**-Zn^{2+} upon addition of PPi (0.1–2.0 equiv.) in HEPES buffer (10 mM, pH 7.4). Inset: Dependence of I_{415}/I_{337} on the concentration of PPi. λ_{ex} = 280 nm.

(Fig. S11†), **1**-Zn^{2+} binds PPi in a 2 : 1 stoichiometry. Taking into consideration that Zn^{2+} was well known to bind PPi,[10b,10c,11k,16] it is probable that **1**-Zn^{2+} forms a 2 : 1 complex with PPi, which is shown in Scheme 2. The interaction of **1**-Zn^{2+} with PPi in a 2 : 1 stoichiometry brings the two naphthalene subunits belonging to each **1**-Zn^{2+} complex in a face-to-face position favouring the formation of an intramolecular excimer by π–π interaction, which has been signalled and confirmed by the fluorescent titration. Besides, from the results of fluorescent titration experiments and Job's plot, K_{ass} between **1**-Zn^{2+} and PPi is calculated as 2.1×10^8 M^{-2} by nonlinear fitting to the fluorescent titration curve[13] (Fig. S12†), which is comparable with the values of other reported sensors and PPi *via* metal coordination-altered strategy.[10b,10c,16,17]



Scheme 2 Proposed binding mechanism of **1**-Zn^{2+} with PPi.

Moreover, ESI–MS data also justify this hypothesized excimer formation motif. As shown in Fig. S13† in the supporting information, two main sets of peaks are observed in the range of m/z = 990–1030 when the sodium salt of PPi (0.5 equiv.) was added. And these two signals at m/z 999 (major) and 1021 (minor) correspond to $[(\mathbf{1}\cdot Zn)_2+PPi+H]^+$ and $[(\mathbf{1}\cdot Zn)_2+PPi+Na]^+$, respectively, which are confirmed by the isotope calculations. Further evidence for such a binding mode was obtained by the ^{31}P NMR spectral data (Fig. S14†). The signal for the two equivalent phosphorus atoms of PPi clearly shifts downfield (–5.5 ppm) with broadening upon the addition of 2 equiv. of **1** and $ZnCl_2$, which signifies the binding to the zinc atom of **1**-Zn^{2+}.

Then, to further test the role of **1**-Zn^{2+} as a potential PPi sensor in living systems, competitive binding experiments were conducted in the presence of other phosphate-containing anions (Pi, PO_3^{3-},

AMP, ADP, ATP) at 10 equiv., respectively, with the subsequent addition of 0.5 equiv. of PPi. Before PPi was added, as shown in Fig. 8, no significant variation in the emission intensity ratio (I_{415}/I_{337}) is found. The addition of PPi greatly enhances this ratio, which is not evidently affected by the presence of other ions except in the system containing ATP. Even if the ratiometric calibration (I_{415}/I_{337}) does not work well with 10 equiv. of ATP, it is the unique excimer emission that guarantees **1**-Zn^{2+} to be effectively and accurately selective for PPi over ATP.

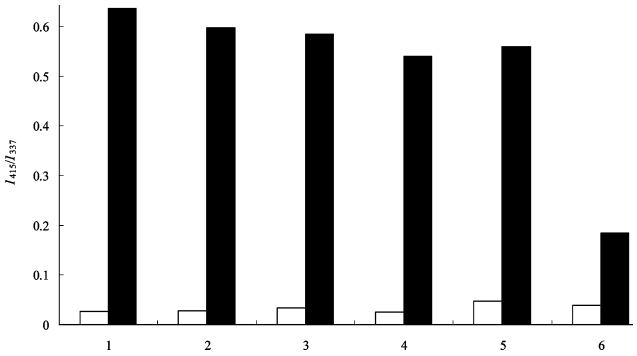


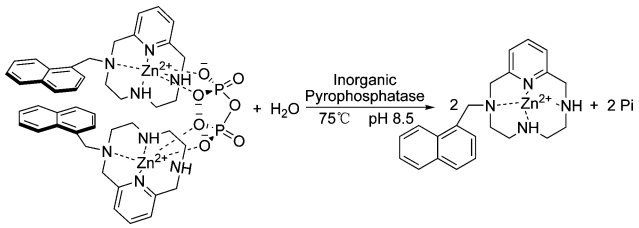
Fig. 8 Fluorescent responses of **1**-Zn^{2+} to various anions in HEPES buffer (10 mM, pH 7.4). λ_{ex} = 280 nm. White bars represent the addition of 10 equiv. of competitive anions to a solution of 20 μM **1**-Zn^{2+}. Black bars represent the subsequent addition of 0.5 equiv of PPi to the solution. (1) No anion, (2) Pi, (3) PO_3^{3-}, (4) AMP, (5) ADP, (6) ATP.

Application of 1-Zn^{2+} in blood serum and inorganic PPase assay

So far the **1**-Zn^{2+} sensing system has been shown to meet our fundamental design expectation. Nevertheless, further investigations should be carried out to demonstrate its bioanalytical potential as a ratiometric fluorescent sensor in a more sophisticated environment.

First of all, biological applicability of **1**-Zn^{2+} has been addressed by carrying out fluorescent titrations in fetal bovine serum.[19] The serum we prepared contained numerous salts, glucose, hormones,[20] and large proteins were removed prior to analysis owing to the possible competitive coordination to **1**-Zn^{2+}.[21] By varying the concentrations of the serum, no apparent changes were observed (Fig. S15†) for PPi-specific ratiometric fluorescent sensing of **1**-Zn^{2+} compared to that of its performance in water discussed above. Therefore, it has been proven that **1**-Zn^{2+} is an effective ratiometric sensor towards PPi in both water and simulated physiological media.

Moreover, sensor **1**-Zn^{2+} can be applied to set up a ratiometric fluorescence assay for monitoring PPi-relevant enzyme activity. Inorganic PPase is a ubiquitous Mg^{2+} dependent[22] hydrolase that catalyzes the hydrolysis of PPi. Such enzymes have been known to be essential for cell growth,[23] which was presumed to control the concentration balance between PPi and Pi. The ion selectivity for PPi over Pi allows for a real-time monitor of PPi hydrolysis. During the hydrolysis, a decrease of the emission intensity ratio (I_{415}/I_{337}) is expected to occur when PPi is converted into Pi at constant pH (Scheme 3). It has been proven above that the formation of **1**-Zn^{2+} is not disturbed by Mg^{2+} even at 1000 equiv. To examine the potential of the sensor for this purpose, different units of inorganic PPase preserved in Tris-HCl (20 mM, pH 8.0) were added to a



Scheme 3 Mechanism of inorganic PPase assay monitored by **1**-Zn^{2+}.

Tricine buffer (50 mM, pH 8.5, 1 mM $MgCl_2$) containing 25 μM PPi and 40 μM **1**-Zn^{2+} at the temperature of 75 °C. The ratiometric fluorescent calibration I_{415}/I_{337} was monitored as a function of time (Fig. 9). Once all of PPi ions in the assay were hydrolyzed (or the concentration of PPi became very low), the emission ratio of the solution remained constant (390 s for 0.4 units; 150 s for 0.8 units; 30 s for 1.6 units). In the presence of each specific amount of inorganic PPase, the recorded emission ratio gradually decreases as the time increases during the hydrolysis of PPi, which matches the predicted trend. Moreover, it is also observed that the decrease in the emission ratio is accelerated with increasing concentrations of the enzyme. Although other chemosensors have been reported to be applied to the fluorescence monitoring of inorganic PPase assay,[16] 1-Zn^{2+} is the first example to be utilized to set up a ratiometric fluorescence assay of PPi hydrolysis, which is feasible in biological systems by providing built-in correction for environmental effects.

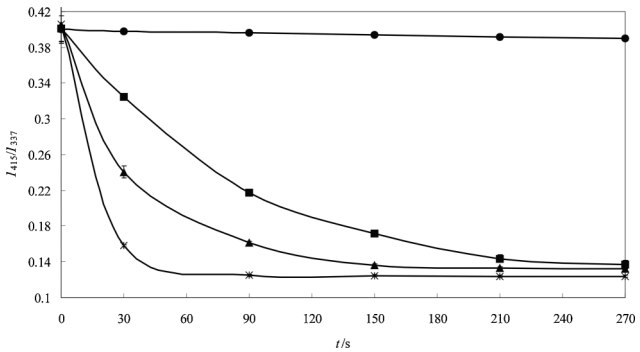


Fig. 9 Fluorescence real-time monitoring of PPi (25 μM) hydrolysis catalyzed by inorganic PPase using 40 μM **1**-Zn^{2+}. Concentrations of inorganic PPase: 0 units (●); 0.4 units (■); 0.8 units (▲); 1.6 units (*).

Conclusion

In summary, we have designed and obtained a novel ratiometric fluorescent sensor, **1**-Zn^{2+}, for PPi, which performs well in both aqueous solution and blood serum milieux at physiological pH. The water soluble ligand **1** employing a naphthalene fluorophore exhibits selective amplified response to at least 0.4 μM Zn^{2+}. It is observed that the sensor **1**-Zn^{2+} responds to PPi selectively owing to the synergic coordination of the central metal Zn^{2+} to the anion. In other words, it has been verified that PPi can act as a template of this complexation-induced self-assembly of **1**-Zn^{2+}. Thus the formed characteristic excimer emission peak can be employed to significantly discern above 0.2 μM PPi over other anions including Pi and ATP that may potentially bring interference. Finally, these intriguing results permitted us

to utilize **1**-Zn^{2+} to monitor PPi hydrolysis by means of real-time ratiometric fluorescent measurements, which will be promising for its capability of avoiding environmental disturbance.

Acknowledgements

This work is supported by the National Natural Science Foundation of China (20721002, 21027013, 21075064 and 90813020) and the National Basic Research Program of China (2007CB925102).

Notes and references

1 (*a*) A. W. Czarnik, *Acc. Chem. Res.*, 1994, **27**, 302–308; (*b*) A. P. de Silva, H. Q. N. Gunaratne, T. Gunnlaugsson, A. J. M. Huxley, C. P. McCoy, J. T. Rademacher and T. E. Rice, *Chem. Rev.*, 1997, **97**, 1515–1566; (*c*) M. H. Keefe, K. D. Benkstein and J. T. Hupp, *Coord. Chem. Rev.*, 2000, **205**, 201–228; (*d*) K. Rurack and U. Resch-Genger, *Chem. Soc. Rev.*, 2002, **31**, 116–127; (*e*) J. F. Callan, A. P. de Silva and D. C. Magri, *Tetrahedron*, 2005, **61**, 8551–8588; (*f*) C. Lodeiro and F. Pina, *Coord. Chem. Rev.*, 2009, **253**, 1353–1383; (*g*) Z. Xu, X. Chen, H. N. Kim and J. Yoon, *Chem. Soc. Rev.*, 2010, **39**, 127–137; (*h*) X. Chen, Y. Zhou, X. Peng and J. Yoon, *Chem. Soc. Rev.*, 2010, **39**, 2120–2135; (*i*) C. Lodeiro, J. L. Capelo, J. C. Mejuto, E. Oliveira, H. M. Santos, B. Pedras and C. Nuñez, *Chem. Soc. Rev.*, 2010, **39**, 2948–2976.

2 (*a*) B. Valeur and I. Leray, *Coord. Chem. Rev.*, 2000, **205**, 3–40; (*b*) L. Prodi, F. Bolletta, M. Montalti and N. Zaccheroni, *Coord. Chem. Rev.*, 2000, **205**, 59–83; (*c*) C. Bargossi, M. C. Fiorini, M. Montalti, L. Prodi and N. Zaccheroni, *Coord. Chem. Rev.*, 2000, **208**, 17–32; (*d*) R. Martínez-Máñez and F. Sancenón, *Chem. Rev.*, 2003, **103**, 4419–4476; (*e*) P. D. Beer and S. R. Bayly, *Top. Curr. Chem.*, 2005, **255**, 125–162; (*f*) E. J. O'Neil and B. D. Smith, *Coord. Chem. Rev.*, 2006, **250**, 3068–3080; (*g*) S. K. Kim, D. H. Lee, J.-I. Hong and J. Yoon, *Acc. Chem. Res.*, 2009, **42**, 23–31; (*h*) C. Caltagirone and P. A. Gale, *Chem. Soc. Rev.*, 2009, **38**, 520–563.

3 S. Kubik, C. Reyheller and S. Stüwe, *J. Inclusion Phenom. Macrocyclic Chem.*, 2005, **52**, 137–187.

4 (*a*) Y. Marcus, *Ion Properties*, Marcel Dekker, New York, 1997; (*b*) K. D. Collins, G. W. Neilson and J. E. Enderby, *Biophys. Chem.*, 2007, **128**, 95–104.

5 (*a*) A. Bianchi and K. Bowman-James, *Supramolecular Chemistry of Anions* ed. E. García-España, Wiley-VCH, New York, 1997; (*b*) F. P. Schmidtchen and M. Berger, *Chem. Rev.*, 1997, **97**, 1609–1646; (*c*) M. M. G. Antonisse and D. N. Reinhoudt, *Chem. Commun.*, 1998, 443–448; (*d*) P. D. Beer and P. A. Gale, *Angew. Chem., Int. Ed.*, 2001, **40**, 486–516; (*e*) K. Bowman-James, *Acc. Chem. Res.*, 2005, **38**, 671–678; (*f*) V. Amendola, M. Bonizzoni, D. Esteban-Gómez, L. Fabbrizzi, M. Licchelli, F. Sancenón and A. Taglietti, *Coord. Chem. Rev.*, 2006, **250**, 1451–1470; (*g*) P. S. Gale, S. E. García-Garrido and J. Garric, *Chem. Soc. Rev.*, 2008, **37**, 151–190.

6 (*a*) P. Nyren, *Anal. Biochem.*, 1987, **167**, 235–238; (*b*) C. P. Mathews and K. E. van Hold, *Biochemistry*, The Benjamin/Cummings Publishing Company, Inc., Redwood City, CA, 1990; (*c*) W. N. Lipscomb and N. Sträter, *Chem. Rev.*, 1996, **96**, 2375–2433.

7 (*a*) *Biomaterials Science* ed. B. Ratner, A. S. Hoffman, F. J. Schoen and J. E. Lemons, Academic Press, London, 1996; (*b*) J. L. Meyer, *Arch. Biochem. Biophys.*, 1984, **231**, 1–8; (*c*) K. A. Lomashvili, S. Cobbs, R. A. Hennigar, K. I. Hardcastle and W. C. O'Neill, *J. Am. Soc. Nephrol.*, 2004, **15**, 1392–1401.

8 (*a*) M. Doherty, C. Becher, M. Regan, A. Jones and J. Ledingham, *Ann. Rheum. Dis.*, 1996, **66**, 432–436; (*b*) A. E. Timms, Y. Zhang, R. G. Russell and M. A. Brown, *Rheumatology*, 2002, **41**, 725–729.

9 (*a*) G. Grynkiewicz, M. Poenie and R. Y. Tsien, *J. Biol. Chem.*, 1985, **260**, 3440–3450; (*b*) S. Deo and H. A. Godwin, *J. Am. Chem. Soc.*, 2000, **122**, 174–175; (*c*) J. V. Mello and N. S. Finney, *Angew. Chem., Int. Ed.*, 2001, **40**, 1536–1538; (*d*) M. S. Tremblay, M. Halim and D. Sames, *J. Am. Chem. Soc.*, 2007, **129**, 7570–7577; (*e*) Z. Xu, N. J Singh, J. Lim, J. Pan, H. N. Kim, S. Park, K. S. Kim and J. Yoon, *J. Am. Chem. Soc.*, 2009, **131**, 15528–15533.

10 (*a*) S. Nishizawa, Y. Kato and N. Teramae, *J. Am. Chem. Soc.*, 1999, **121**, 9463–9464; (*b*) H. K. Cho, D. H. Lee and J.-I. Hong, *Chem. Commun.*, 2005, 1690–1692; (*c*) H. N. Lee, Z. Xu, S. K. Kim, K. M. K. Swamy, Y. Kim, S.-J. Kim and J. Yoon, *J. Am. Chem. Soc.*, 2007, **129**, 3828–3829.

11 (*a*) T. Koike and E. Kimura, *J. Am. Chem. Soc.*, 1991, **113**, 8935–8941; (*b*) E. Kimura, I. Nakamura, T. Koike, M. Shionoya, Y. Kodama, T. Ikeda and M. Shiro, *J. Am. Chem. Soc.*, 1994, **116**, 4764–4771; (*c*) T. Koike, S. Kajitani, I Nakamura, E. Kimura and M. Shiro, *J. Am. Chem. Soc.*, 1995, **117**, 1210–1219; (*d*) E. Kimura, Y. Kodama, T. Koike and M. Shiro, *J. Am. Chem. Soc.*, 1995, **117**, 8304–8311; (*e*) E. Kimura, S. Aoki, T. Koike and M. Shiro, *J. Am. Chem. Soc.*, 1997, **119**, 3068–3076; (*f*) E. Kimura, H. Kitamura, T. Koike and M. Shiro, *J. Am. Chem. Soc.*, 1997, **119**, 10909–10919; (*g*) S. Aoki, Y. Honda and E. Kimura, *J. Am. Chem. Soc.*, 1998, **120**, 10018–10026; (*h*) S. Aoki and E. Kimura, *J. Am. Chem. Soc.*, 2000, **122**, 4542–4548; (*i*) S. Aoki, M. Zulkefeli, M. Shiro, M. Kohsako, K. Takeda and E. Kimura, *J. Am. Chem. Soc.*, 2005, **127**, 9129–9139; (*j*) G. Gasser, M. J Belousoff, A. M. Bond, Z. Kosowski and L. Spiccia, *Inorg. Chem.*, 2007, **46**, 1665–1674; (*k*) D. A. Jose, S. Stadlbauer and B. Konig, *Chem.–Eur. J.*, 2009, **15**, 7404–7412.

12 H. Du, R. A. Fuh, J. Li, L. A. Corkan and J. S. Lindsey, *Photochem. Photobiol.*, 1998, **68**, 141–142.

13 B. Valeur, *Molecular Fluorescence: Principles and Applications*, Wiley-VCH, Weinheim, 2002.

14 C. Bazzicalupi, A. Bencini, S. Biagini, E. Faggi, S. Meini, C. Giorgi, A. Spepi and B. Valtancoli, *J. Org. Chem.*, 2009, **74**, 7349–7363.

15 K. Schillén, D. F. Anghel, M. da G. Miguel and B. Lindman, *Langmuir*, 2000, **16**, 10528–10539.

16 (*a*) D. H. Lee, J. H. Im, S. U. Son, Y. K. Chung and J.-I. Hong, *J. Am. Chem. Soc.*, 2003, **125**, 7752–7753; (*b*) D. H. Lee, S. Y. Kim and J.-I. Hong, *Angew. Chem., Int. Ed.*, 2004, **43**, 4777–4780; (*c*) M. J McDonough, A. J. Reynolds, W. Y. G. Lee and K. A. Jolliffe, *Chem. Commun.*, 2006, 2971–2973; (*d*) N. Shao, H. Wang, X. D. Gao, R. H. Yang and W. H. Chan, *Anal. Chem.*, 2010, **82**, 4628–4636.

17 (*a*) S. Mizukami, T. Nagano, Y. Urano, A. Odani and K. Kikuchi, *J. Am. Chem. Soc.*, 2002, **124**, 3920–3925; (*b*) L. Fabbrizzi, N. Marcotte, F. Stomeo and A. Taglietti, *Angew. Chem., Int. Ed.*, 2002, **41**, 3811–3814; (*c*) X. M. Huang, Z. Q. Guo, W. H. Zhu, Y. S. Xie and H. Tian, *Chem. Commun.*, 2008, 5143–5145; (*d*) M. J. Kim, K. M. K. Swamy, K. M. Lee, A. R. Jagdale, Y. Kim, S.-J. Kim, K. H. Yoo and J. Yoon, *Chem. Commun.*, 2009, 7215–7217.

18 (*a*) D. H. Vance and A. W. Czarnik, *J. Am. Chem. Soc.*, 1994, **116**, 9397–9398; (*b*) E. Climent, Rosa. Casasús, M. D. Marcos, R. Martínez-Máñez, F. Sancenón and J. Soto, *Chem. Commun.*, 2008, 6531–6533; (*c*) I.-B. Kim, M. H. Han, R. L. Phillips, B. Samanta, V. M. Rotello, Z. J. Zhang and U. H. F. Bunz, *Chem.–Eur. J.*, 2009, **15**, 449–456; (*d*) D. Curiel, A. Espinosa, M. Más-Montoya, G. Sánchez, A. Tárraga and P. Molina, *Chem. Commun.*, 2009, 7539–7541.

19 A. J. Alpert and A. K. Shukla, *ABRF 2003: Translating biology using proteomics and functional genomics*, Poster n°P111-W, Denver, 2003.

20 *Geigy Scientific Tables* Ed. C. Lentner, Ciba-Geigy, Basel, 8th edn, 1984, Vol. 3, p. 65.

21 S. Rochat, Z. Grote and K. Severin, *Org. Biomol. Chem.*, 2009, **7**, 1147–1153.

22 (*a*) J. Josse, *J. Biol. Chem.*, 1966, **241**, 1948–1957; (*b*) S. A. Kurilova, T. I. Nazarova and S. M. Avaeva, *Bioorg. Khim.*, 1983, **9**, 1032–1039.

23 (*a*) J. Chen, A. Brevet, M. Fromant, F. Lévêque, J.-M. Schmitter, S. Blanquet and P. Plateau, *J. Bacteriol.*, 1990, **172**, 5686–5689; (*b*) M. Lundin, H. Baltscheffsky and H. Ronne, *J. Biol. Chem.*, 1991, **266**, 12168–12172.