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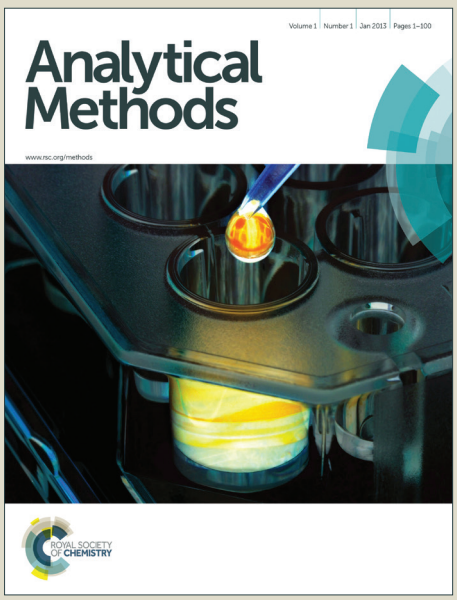

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ARTICLE TYPE

Fluorescence turn-on detection of pyrophosphate based on aggregation-induced emission property of 5-chlorosalicylaldehyde azine

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5-Chlorosalicylaldehyde azine (**1**) has been reported exhibiting typical aggregation-induced emission (AIE) feature in our previous work. In this work, we further discovered that AIE fluorescence of **1** could be selectively quenched by Cu^{2+}, hence **1** and Cu^{2+} complex was applied as a turn-on fluorescence probe for pyrophosphate (PPi) detection in aqueous solution. ^{1}H-NMR data of the isolated products revealed that addition of PPi underwent displacement of **1** in **1**-Cu^{2+} complex, which led to the releasing of free **1** to aqueous solution, resulting in recovered AIE fluorescence. With fluorescence enhancement detection by **1**-Cu^{2+} complex, the linear range and detection limit for PPi were obtained as 0-15 μmol/L (R^2 = 0.997) and 0.064 μmol/L respectively with satisfied selectivity over other anions. The method was also used for PPi detection in serum sample.

1. Introduction

Pyrophosphate (PPi) is known to be one of the most important biologically related anions. It is involved in the elongations of DNA and RNA chain, and it even occurs in protein synthesis and signal transduction.[1] Beyond its physiological functions in biological systems, the relation between its abnormal concentration level and several diseases, such as arteriosclerosis[2] and osteoarthritis,[3] was revealed. Thus, development of highly sensitive and selective detection method for PPi in biological samples is of great interest in recent years due to its indispensible roles.

As to detection of PPi, fluorescent method is considered to be a powerful tool because of its sensitivity, visualization and simplicity. To date, cooperative interaction[4-16] and competing displacement methods[17-21] have been the most ubiquitously utilized two methods to construct the fluorescent probes for PPi, in which organic dyes with conjugated structures are frequently selected as the fluorescent units.[22] However, such organic dyes typically undergo aggregation-caused quenching (ACQ) at high probe concentration level or in poor solvent such as aqueous solution, which is hard to construct a perfect fluorescent assay for biological samples. Promisingly, a new class of fluorescence probes, aggregation-induced emission (AIE) molecules are receiving increasingly attention because of their featured AIE property. The molecules are highly emissive in "aggregate" state and non-emissive or weakly emissive in their corresponding "solution" state.[22, 23] Thus their unique emitting property endows them great potential candidates in the applications of fluorescence sensors,[24-28] bioprobes,[29-39] as well as organic laser emitting diodes (OLED)[24, 40] *etc*.

In our previous work, salicylaldehyde azine (SAA) derivatives was reported possessing AIE characteristics due to the restriction of N-N single bond rotation in aggregate state.[41] SAA derivatives could be facilely synthesized through condensation reaction between hydrazine and corresponding salicylaldehydes. These molecules had been designed based on the following considerations: 1) Schiff base bearing *o*-hydroxyl group on the benzene ring was introduced to form intramolecular hydrogen bond, which could undergo a process known as excited state intramolecular proton transfer (ESIPT);[42] 2) N-N bridge with adjacent intramolecular hydrogen bond enabled fluorophores to rotate around the single bond symmetrically. As a result, the unique structure endows SAA derivatives the ability of rotating freely under "solution" condition, which provided a nonradiative releasing way for the excited state, consequently, weak emission was observed due to the block of intrinsic ESIPT process. However, ESIPT process would be maintained while SAA derivatives were under their "aggregate" condition, thus, strong fluorescence was induced by aggregation.

The unique structure not only assured SAA derivatives of aggregation-induced emission characteristics, it also provided potential chelating sites for metal ions. According to our previous studies, Schiff base bearing *o*-hydroxyl group on benzene ring was shown a better ability of binding Cu^{2+} over other metal ions.[43, 44] Herein, we found that 5-chlorosalicylaldehyde azine (**1**), showing typical aggregation-induced emission at long wavelength (570 nm) with a large stokes shift (~182 nm), could coordinate with Cu^{2+} as well, resulting in considerable fluorescence quenching of **1**.

Copper complexes are considered to be good candidates for constructing fluorescent probes towards PPi according to either cooperative interaction or competing displacement mode.[10-12, 17, 19, 21] In this study, **1** and Cu^{2+} complex was firstly formed in 20% DMSO-water solution with non-fluorescence emission around 570 nm. When PPi was added to the solution, strong fluorescence

peaked at 570 nm was observed. This phenomenon indicated the feasibility of **1**-Cu as a turn-on fluorescent probe for detecting PPi. ^{1}H-NMR data of the isolated products proved that the turn-on response of PPi underwent a competing displacement mechanism, leading to releasing of free **1** into the solution and resulting in recovered AIE fluorescence. The method exhibited good selectivity over other anionic species and detection limit for PPi was obtained as low as 0.064 μmol/L.

2. Experimental section

2.1 Materials

1 was synthesized according to the procedure described in our previous work.[41] All the regents were of analytical grade and used as received unless mentioned in the following parts. Water is twice distilled. Metal ion stock solutions were prepared from their corresponding nitrate salts in water, and anion stock solutions were prepared from their corresponding sodium salts in water, respectively.

2.2 UV-Vis and fluorescence spectra.

Absorption spectra were recorded on a V-550 (JASCO, Japan) UV-Vis spectrometer. Fluorescence spectra were performed on a FP-6500 (JASCO, Japan) spectrofluorimeter under the excitation wavelength of 388 nm. All spectra were measured in quartz cuvettes at 25 ^{o}C (length path 1 cm), excitation and emission slits were set as 3 nm, sensitivity was chosen as medium.

Procedures of preparing samples for UV-Vis and fluorescence spectra measurement: 8 μL 5 mM stock solution of **1** was added into 400 μL DMSO, followed by addition of corresponding amount of Cu^{2+}, and mixture was kept stirring for 2 min, then 1600 μL buffer was added at last and kept stirring until it became homogeneous, then it was left alone for 20 min before the fluorescence spectrum was measured.

Procedures of preparing samples for PPi detection: in 400 μL DMSO solution, stock solutions of 1 (final concentration: 20 μmol/L) and Cu^{2+} (final concentration: 18.5 μmol/L) were added in sequence with vigorously stirring. After the mixture was stirred for 2 min, 1600 μL buffer was added and kept stirring for another 2 min. After that, corresponding amount of PPi was added and thoroughly mixed, then the mixture was left alone and fluorescence spectrum was taken 30 min later.

2.3 Dynamic light scattering (DLS)

DLS data were measured using a ZETA3000HS (Malvern Instruments, United Kingdom) particle size and zeta potential analyzer with a He-Ne laser of 633 nm as the light source.

2.4 Confocal laser scanning microscopy

Confocal laser scanning images of aggregates in thin liquid membrane (an aliquot of 4 μL sample was dropped on the surface of a glass slide treated with H_2SO_4 and H_2O_2 previously, then covered with a cover glass and sealed at last) were obtained with the assistance of an FV1000 confocal system (Olympus, Japan) equipped with a 60 × objective immersed in oil. To avoid the interference of background, photon within the range from 545 to 645 nm, which locates in the emission range of **1**, was collected upon excitation at 405 nm.

2.5 Transmission electron microscopy (TEM)

Transmission electron microscopy (TEM) was performed using a JEOL JEM-1011 instrument.

2.6 ^{1}H-NMR spectra

^{1}H-NMR spectra were recorded using a JNM-ECA300 (JOEL, Japan) spectrometer operated at 300 MHz. DMSO-d_6 containing 0.03% (*v*/*v*) tetramethylsilane (TMS) as the internal standard was selected to dissolve the sample.

Preparation of the isolated products: 400 mL 20% DMSO aqueous solution containing 20 μmol/L **1** and, 18.5 μmol/L Cu^{2+} was prepared first, then PPi with final concentration of 200 μmol/L was added and stirred vigorously. After the mixture was stood for 30 min, it was filtrated with microfiltration membrane (0.22 μm) under vacuum followed by washing with 50% EtOH (EtOH/H_2O, 1/1, *v*/*v*) three times, and then it was dried under infrared lamp for 5 h before NMR characterization.

3. Results and discussions

3.1Aggregation-induced emission characteristics of 1 in DMSO/H_2O

In our previous work,[41] AIE characteristics of **1** had been demonstrated in ethanol/water mixed solvent. Given the good stability required for further analytical experiments, DMSO containing aqueous solution was selected. Hence whether AIE characteristic of **1** could be maintained in such a system was investigated first. As shown in Fig. 1, 20 μmol/L of **1** showed strong fluorescence in the DMSO volume fraction range of 10~50% which was assigned as AIE fluorescence of **1** in its aggregated state, while in high DMSO volume fractions as 70~90%, weak fluorescence was observed due to the free rotation of N-N single bond in **1**'s solution state. DLS characterization of the particles formed in 20% DMSO–water solution revealed that a mean particle size of 533.5 nm was obtained (Table S1) and the fluorescence intensity remained nearly unchanged throughout the experiment.

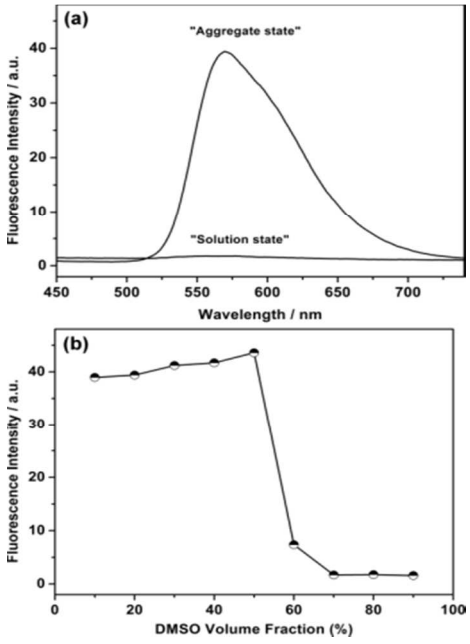

Fig.1 AIE characteristics of **1** in DMSO/H_2O solution: a. Fluorescence spectra of **1** (20 μmol/L) in "aggregate state" (DMSO/water, 2/8, *v*/*v*) and

"solution state" (DMSO/water, 8/2, *v*/*v*); b. Effect of DMSO volume fraction on the fluorescence intensity (peaks in fluorescence spectra) of **1** (20 μmol/L) in DMSO/water containing 10 mmol/L Tris/HCl at pH 7.00. Excitation was performed at 388 nm.

3.2 Effect of metal ions on the AIE characteristics of 1

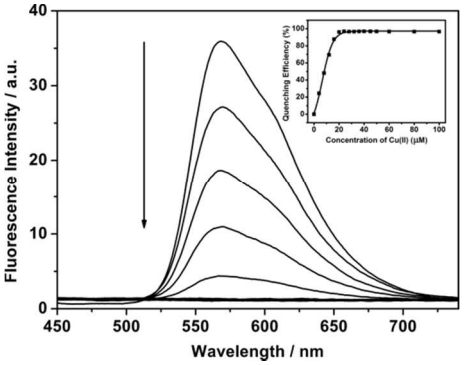

Fig. 2 Fluorescence spectra of 20 μmol/L **1** in the presence of different amounts of Cu^{2+} in 20% DMSO aqueous solution (10 mmol/L Tris-HCl, pH = 7.00). Inset: quenching efficiency* of 20 μmol/L **1** in the presence of different amounts of Cu^{2+}. *Quenching efficiency = $(F_0\text{-}F)/F_0$, where F_0 and F indicate the fluorescence intensity of **1** and after addition of Cu^{2+}, respectively. Excitation was performed at 388 nm.

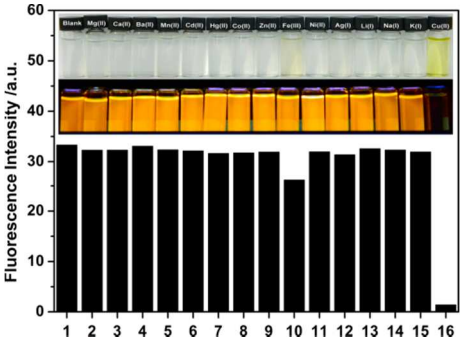

Fig. 3 Fluorescence intensity of 20 μM **1** in 20% DMSO aqueous solution (10 mmol/L Tris-HCl, pH = 7.00) in the absence and presence of different cations. 1: blank, 2: Cd^{2+} (2 mmol/L), 3: Mg^{2+} (2 mmol/L), 4: Mn^{2+} (2 mmol/L), 5: Ba^{2+} (2 mmol/L), 6: Ca^{2+} (2 mmol/L), 7: Hg^{2+} (200 μmol/L), 8: Co^{2+} (200 μmol/L), 9: Zn^{2+} (2 mmol/L), 10: Fe^{3+} (200 μmol/L), 11: Ni^{2+} (2 mmol/L), 12: Ag^{+} (2 mmol/L), 13: Li^{+} (2 mmol/L), 14: Na^{+} (2 mmol/L), 15: K^{+} (2 mmol/L), 16: Cu^{2+}(200 μmol/L). Inset: photos of **1** in the absence and presence of different cations, the upper and lower row were taken under the natural light and illumination of 365nm, respectively.

Schiff base moiety is well known for its chelating abilities with metal ions via the imino N and phenol O atoms.[43-45] For understanding the coordination behaviour of **1**, absorption spectra upon increasing amount of Cu^{2+} were recorded under its "solution" state in 20% DMSO aqueous solution. As shown in Fig. S1, the absorption band at 367 nm decreased after addition of Cu^{2+}, while a newly appeared absorption band centred at 452 nm increased accordingly with an isobestic point emerging at 395 nm. These observations demonstrated the existence of coordination between **1** and Cu^{2+}. When 1 equivalent of Cu^{2+} was added in 20% DMSO aqueous solution, as shown in Fig. 2, the fluorescence was quenched and the inset implies a nearly 1:1 binding between **1** and Cu^{2+}. Furthermore, the ESI-MS spectrum supplied a more direct evidence for the 1:1 binding ratio in the presence of 1 equiv. Cu^{2+}, clear peaks at m/z = 371.1 ($[M + Cu - H]^+$, cald. 370.9) and 450.0 ($[M + Cu + DMSO - H]^+$, cald. 450.0) could be clearly observed (Fig. S2).

Effect of other metal ions on fluorescence spectra of **1** was then studied. As shown in Fig. 3 and Fig. S3, most metal ions showed no interference on the fluorescence intensity of **1**, indicating coordination was not occurred between these cations and aggregates of **1**, except for Fe^{3+}, which are paramagnetic metal ions leading to slightly fluorescence quenching. High selectivity of **1** for Cu^{2+} over other metal ions was because Cu^{2+} has a particularly high thermodynamic affinity with typical N, O-chelate ligands than other metal ions [44].

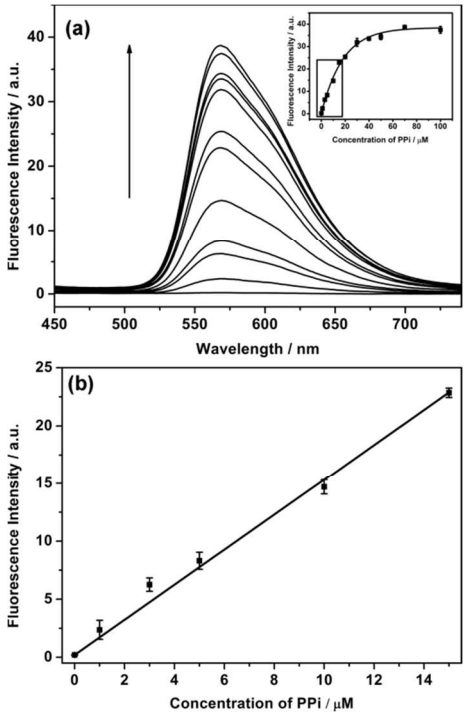

Fig. 4 Fluorescence response of **1**-Cu toward PPi in 20% DMSO aqueous solution: a. Fluorescence spectra of **1**-Cu (20 μmol/L **1** and 18.5 μmol/L Cu^{2+}) upon addition of the increasing amount of PPi (0, 1, 3, 5, 10, 15, 20, 30, 40 50, 70, 100 μmol/L with the arrow direction); b. linear range for PPi detection with **1**-Cu. Other conditions are the same as in Fig. 1.

3.3 Turn-on detection of PPi

Considering pyrophosphate (PPi) could coordinate with Cu^{2+}, addition of PPi to **1**-Cu complex may result in competing displacement of **1** by PPi in **1**-Cu complex. Thus, free **1** in poor solvent aqueous solution could emit AIE fluorescence. Herein, **1**-Cu complex was supposed to be a potential candidate for fluorescence turn-on detection of PPi. In order to obtain a satisfied detection limit (Fig. S4), slightly less amount of Cu^{2+} (18.5 μmol/L) than that of **1** (20 μmol/L) was chosen to form the **1**-Cu complex. As shown in Fig. 4, fluorescence intensity increased upon increasing the amount of PPi with a linear response range of 0~15 μmol/L ($y = 1.51x + 0.20$, $R^2 = 0.997$), and detection limit was determined to be 0.064 μmol/L (n = 10).

To find out the selectivity of this method, fluorescence spectra of **1**-Cu complex were taken in the presence of excess amount of different anions. As shown in Fig. 5, anions of Cl^-, Br^-, I^-, NO_3^-, ClO_4^-, AcO^-, CNS^-, S^{2-}, CO_3^{2-}, PO_4^{3-} do not shown any interference on the detection of PPi. For nucleoside polyphosphates ATP, ADP and AMP, AMP caused no

fluorescence enhancement, but ADP and ATP produced one forth and nearly the same fluorescence response compared with that of PPi. Negative charges at neutral pH for these polyphosphate, namely AMP, ADP, ATP and PPi, were supposed to be responsible for their coordinating ability for Cu^{2+}.[3] Although three negative charges appeared in PO_4^{3-}, but no obvious fluorescence enhancement was observed, we deduced it might be caused by the fact that PO_4^{3-} transformed to $H_2PO_4^-$ and HPO_4^{2-} as the mainly existing species in buffers at neutral pH.

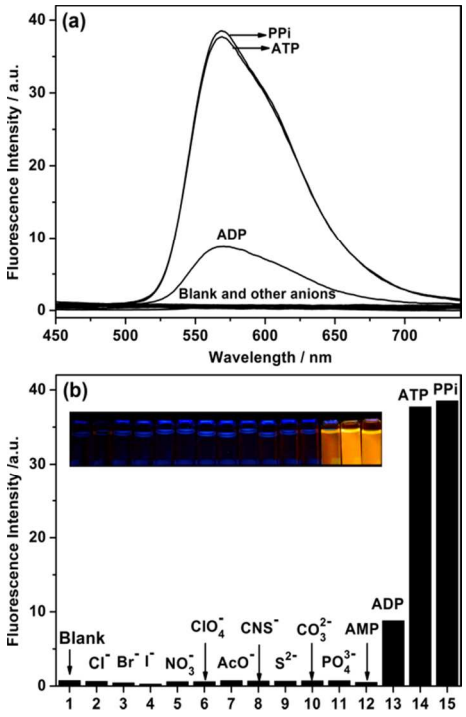

Fig. 5 Fluorescence response of **1**-Cu for different anions in 20% DMSO aqueous solution (20 μmol/L **1** and 18.5 μmol/L Cu^{2+}, 10 mmol/L Tris-HCl, pH=7.00): (a) Fluorescence spectra; (b) fluorescence intensity at 570 nm. Concentrations of anions were kept at 200 μmol/L.

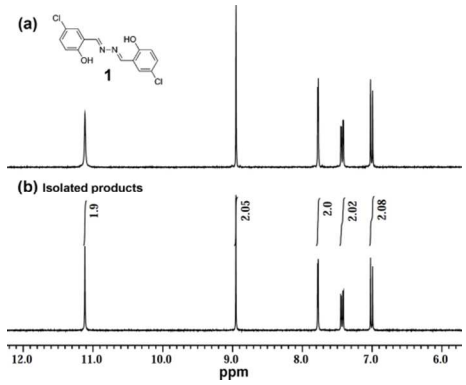

Fig. 6 ^{1}H-NMR data (300 MHz) of 1 (a) and isolated products (b).

3.4 Mechanism of turn-on response towards PPi

Mechanism of the turn-on fluorescence response towards PPi was demonstrated with the assistance of ^{1}H-NMR characterization, confocal laser scanning images and dynamic light scattering data. Aggregates was first isolated from the mixture containing 1-Cu complex and excessive PPi, as shown in Fig. 6, NMR profile of the isolated products was same as that of 1, which revealed that free 1 was released after PPi was added. It should be ascribed to the relative stronger binding ability of PPi with Cu^{2+}. Once PPi was added into solution containing 1-Cu complex, Cu^{2+} preferred to coordinate with PPi, thus 1 was displaced by PPi. Confocal laser scanning system, TEM analysis and particle size analyzer were further employed to observe the fluorescent aggregates and determine their mean diameter in the aqueous mixture, respectively. From Fig. 7 and Fig. S5, it could be seen that fluorescent aggregates of **1** formed in 20% DMSO-water, and their mean diameter is 533.5 nm according to DLS data. For the mixture containing 20 μmol/L **1** and 18.5 μmol/L Cu^{2+}, much fewer fluorescent aggregates could be seen, and their mean particle size decreased to 279.4 nm accordingly (Table S1), which might be due to better solubility of **1**-Cu complex in 20% DMSO-water. Addition of PPi leads to generation of fluorescent aggregates again, and their mean size was determined to be 506.0 nm, which is close to that of **1** in 20% DMSO-water. Therefore, these findings revealed that the turn-on response of **1**-Cu complex towards PPi underwent a competing displacement mechanism, resulting in release of free **1** to reform aggregates and AIE fluorescence recovered.

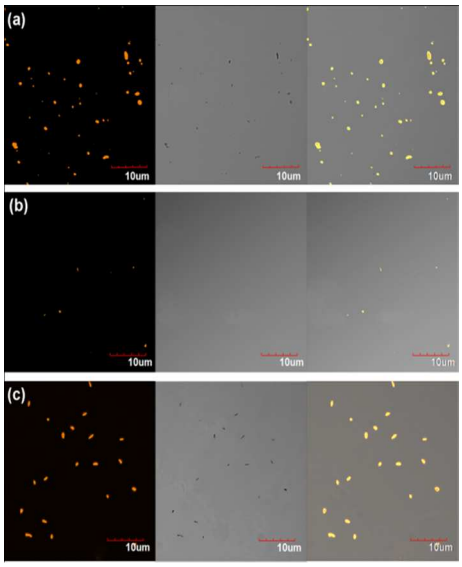

Fig. 7 Confocal laser scanning images: (a) 20 μmol/L **1** in 20% DMSO aqueous solution; (b) 20 μmol/L **1** and 18.5 μmol/L Cu^{2+} in 20% DMSO aqueous solution; (c) 200 μmol/L PPi was added into mixtures of b. For each group pictures, left: confocal laser scanning images; middle: bright-field transmission images; right: merged.

3.5 Application in real sample analysis

The proposed method was also applied to the analysis of PPi in a fetal bovine serum sample. The serum contained numerous salts, glucose, hormones, and proteins (albumin, transferrin and immunoglobulins)[46], to avoid the possible interference caused by these biomolecules, we removed large proteins by a simple precipitation procedure according to the reported[47] and used a 50-fold diluted reconstituted serum for sample treatment. Recovery experiments showed this method had good recoveries (Table 1). The results suggested that the proposed method could be used for PPi detection in serum sample.

Table 1 The results of real sample measurements[a]

Added Cys (μM)	Measured (μM)	Recovery (%)	RSD (n=3, %)
4.0	3.9	97.5	3.6
8.0	8.3	103.8	4.5

[a] Conditions: 1-Cu (20 μmol/L 1 and 18.5 μmol/L Cu^{2+}) in 20% DMSO/H_2O aqueous solution (10 mmol/L Tris-HCl, pH = 7.00), $\lambda_{ex}/\lambda_{em}$ = 388/570 nm.

4. Conclusions

In conclusion, a turn-on detection of pyrophosphate based on aggregation-induced emission property of 5-chlorosalicylaldehyde azine **1** has been established. AIE fluorescence of **1** could be selectively quenched by Cu^{2+} forming **1**-Cu complex. The complex showed turn-on fluorescence response selectively towards PPi with a linear range of 0~15 μmol/L and limit of detection as 0.064 μmol/L, respectively. And mechanism of turn-on response of **1**-Cu complex towards PPi was confirmed as it underwent a competing displacement of **1** in **1**-Cu complex by PPi, as a result, free **1** was released to poor solvent and AIE fluorescence recovered. In serum sample, the method also showed good performance for PPi detection.

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Notes and references

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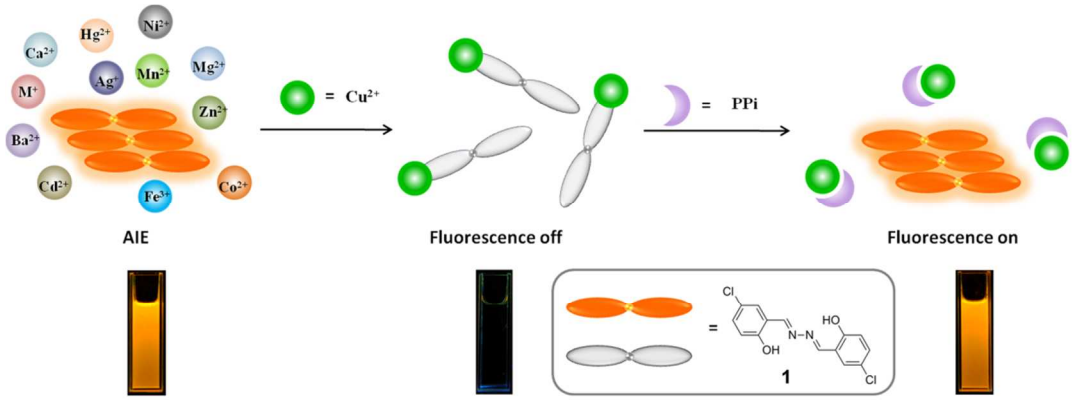
† Electronic Supplementary Information (ESI) available: [details of any supplementary information available should be included here]. See DOI: 10.1039/b000000x/

1. T. M. Devlin, *Textbook of Biochemistry with Clinical Correlations* fifth edition edn., Wiley-Liss, New York, 2002.
2. F. Rutsch, S. Vaingankar, K. Johnson, I. Goldfine, B. Maddux, P. Schauerte, H. Kalhoff, K. Sano, W. A. Boisvert, A. Superti-Furga and R. Terkeltaub, *Am. J. Pathol.*, 2001, **158**, 543-554.
3. Michael Doherty, Carolyn Belcher, Marian Regan, Adrian Jones and J. Ledingham, *Ann. Rheum. Dis.*, 1996, **55**, 432-436.
4. D. H. Lee, S. Y. Kim and J. I. Hong, *Angew. Chem. Int. Edit.*, 2004, **43**, 4777-4780.
5. H. K. Cho, D. H. Lee and J. I. Hong, *Chem. Commun.*, 2005, 1690-1692.
6. Y. J. Jang, E. J. Jun, Y. J. Lee, Y. S. Kim, J. S. Kim and J. Yoon, *J. Org. Chem.*, 2005, **70**, 9603-9606.
7. H. N. Lee, Z. C. Xu, S. K. Kim, K. M. K. Swamy, Y. Kim, S. J. Kim and J. Yoon, *J. Am. Chem. Soc.*, 2007, **129**, 3828-3829.
8. J. H. Lee, J. Park, M. S. Lah, A. Chin and J. I. Hong, *Org. Lett.*, 2007, **9**, 3729-3731.
9. K. M. K. Swamy, S. K. Kwon, H. N. Lee, S. M. Shantha Kumar, J. S. Kim and J. Yoon, *Tetrahedron Lett.*, 2007, **48**, 8683-8686.
10. X. Huang, Z. Guo, W. Zhu, Y. Xie and H. Tian, *Chem. Commun.*, 2008, 5143-5145.
11. Z. Guo, W. Zhu and H. Tian, *Macromolecules*, 2009, **43**, 739-744.
12. 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.
13. X. H. Huang, Y. Lu, Y. B. He and Z. H. Chen, *Eur. J. Org. Chem.*, 2010, 1921-1927.
14. N. Shao, H. Wang, X. D. Gao, R. H. Yang and W. H. Chan, *Anal. Chem.*, 2010, **82**, 4628-4636.
15. W. H. Chen, Y. Xing and Y. Pang, *Org. Lett.*, 2011, **13**, 1362-1365.
16. B. Roy, A. S. Rao and K. H. Ahn, *Org. Biomol. Chem.*, 2011, **9**, 7774-7779.
17. L. Fabbrizzi, N. Marcotte, F. Stomeo and A. Taglietti, *Angew. Chem. Int. Edit.*, 2002, **41**, 3811-3814.
18. D. H. Lee, S. Y. Kim and J. I. Hong, *Tetrahedron Lett.*, 2007, **48**, 4477-4480.
19. X. Zhao, Y. Liu and K. S. Schanze, *Chem. Commun.*, 2007, 2914-2916.
20. L. J. Tang, M. H. Liu, F. F. Li and R. Nandhakumar, *J. Fluoresc.*, 2011, **21**, 701-705.
21. X. J. Zhao and C. Z. Huang, *Biosens. Bioelectron.*, 2011, **30**, 282-286.
22. S. K. Kim, D. H. Lee, J. I. Hong and J. Yoon, *Acc. Chem. Res.*, 2009, **42**, 23-31.
23. Y. Hong, J. W. Y. Lam and B. Z. Tang, *Chem. Soc. Rev.*, 2011, **40**, 5361-5388.
24. L. Liu, G. Zhang, J. Xiang, D. Zhang and D. Zhu, *Org. Lett.*, 2008, **10**, 4581-4584.
25. J.-P. Xu, Z.-G. Song, Y. Fang, J. Mei, L. Jia, A. J. Qin, J. Z. Sun, J. Ji and B. Z. Tang, *Analyst*, 2010, **135**, 3002-3007.
26. S. J. Toal, K. A. Jones, D. Magde and W. C. Trogler, *J. Am. Chem. Soc.*, 2005, **127**, 11661-11665.
27. S. J. Toal, D. Magde and W. C. Trogler, *Chem. Commun.*, 2005, 5465-5467.
28. L. Peng, M. Wang, G. Zhang, D. Zhang and D. Zhu, *Org. Lett.*, 2009, **11**, 1943-1946.
29. X.-t. Chen, Y. Xiang, N. Li, P.-S. Song and A.-j. Tong, *Analyst*, 2010, **135**, 1098-1105.
30. W. Z. Yuan, P. Lu, S. Chen, J. W. Y. Lam, Z. Wang, Y. Liu, H. S. Kwok, Y. Ma and B. Z. Tang, *Adv. Mater.*, 2010, **22**, 2159-2163.
31. W. Xue, G. Zhang, D. Zhang and D. Zhu, *Org. Lett.*, 2010, **12**, 2274-2277.
32. M. Zhao, M. Wang, H. Liu, D. Liu, G. Zhang, D. Zhang and D. Zhu, *Langmuir*, 2008, **25**, 676-678.
33. M. Wang, D. Zhang, G. Zhang and D. Zhu, *Chem. Commun.*, 2008, 4469-4471.
34. M. Wang, D. Zhang, G. Zhang, Y. Tang, S. Wang and D. Zhu, *Anal. Chem.*, 2008, **80**, 6443-6448.
35. Y. Liu, Y. Yu, J. Lam, Y. Hong, M. Faisal, W. Yuan and B. Tang, *Chem. Eur. J.*, 2010, **16**, 8433-8438.
36. M. Faisal, Y. Hong, J. Liu, Y. Yu, J. Lam, A. Qin, P. Lu and B. Tang, *Chem. Eur. J.*, 2010, **16**, 4266-4272.
37. Y. Hong, M. Häuβler, J. Lam, Z. Li, K. Sin, Y. Dong, H. Tong, J. Liu, A. Qin, R. Renneberg and B. Tang, *Chem. Eur. J.*, 2008, **14**, 6428-6437.
38. H. Tong, Y. Hong, Y. Dong, M. Häussler, Z. Li, J. W. Y. Lam, Y. Dong, H. H. Y. Sung, I. D. Williams and B. Z. Tang, *J. Phys. Chem. B*, 2007, **111**, 11817-11823.
39. H. Tong, Y. Hong, Y. Dong, Hau, J. W. Y. Lam, Z. Li, Z. Guo, Z. Guo and B. Z. Tang, *Chem. Commun.*, 2006, 3705-3707.
40. Z. Ning, Z. Chen, Q. Zhang, Y. Yan, S. Qian, Y. Cao and H. Tian, *Adv. Funct. Mater.*, 2007, **17**, 3799-3807.
41. W. Tang, Y. Xiang and A. Tong, *J. Org. Chem.*, 2009, **74**, 2163-2166.
42. S. Scheiner and V. M. Kolb, *Proc. Natl. Acad. Sci.*, 1980, **77**, 5602-5605.
43. Y. Xiang, A. Tong, P. Jin and Y. Ju, *Org. Lett.*, 2006, **8**, 2863-2866.
44. X. Chen, A. Yamaguchi, M. Namekawa, T. Kamijo, N. Teramae and A. Tong, *Anal. Chim. Acta*, 2011, **696**, 94-100.
45. V. Dujols, F. Ford, A.W. Czarnik, *J. Am. Chem. Soc.*, 1997, 119, 7386-7387.
46. *Geigy Scientific Tables (Ed.: C. Lentner,)*, Ciba-Geigy, Basel, 8th edn, 1984, Vol. 3, p. 65.
47. S. Rochat, Z. Grote and K. Severin, *Org. Biomol. Chem.*, 2009, 7, 1147–1153

Graphical abstract



A turn-on detection of pyrophosphate based on aggregation-induced emission property of 5-chlorosalicylaldehyde azine has been established