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Dual signal (color change and fluorescence ON–OFF) ensemble system based on bis(Dpa-Cu^{II}) complex for detection of PPi in water

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ABSTRACT

We have developed simple dual signal (color change and fluorescence ON–OFF) ensemble systems based on a bis(Dpa-Cu^{II}) complex **1** for the detection of PPi in water. Dual signal takes place because of weak binding and fluorescence quenching effect of coordinatively unsaturated Cu^{II} complex for indicators and replacement of the indicators by more strongly binding PPi. As a consequence, these ensembles show a high selectivity and sensitivity for PPi over various anions including phosphate and its derivatives (AMP, ADP, and ATP) in water.

In these days, a growing number of research groups are becoming interested in the development of sensors for biologically important anions using color and fluorescence responses.[1] In particular, pyrophosphate ($P_2O_7^{4-}$, PPi) is known to be involved in several biochemical reactions, such as the hydrolysis of adenosine triphosphate (ATP), DNA polymerization, and other metabolic processes.[2,3] Hence, the selective detection of pyrophosphate has been a matter of investigation by several groups.[4,5a]

Among the known design principles for anion sensing, the displacement (ensemble) approach is simple and convenient because a signaling unit (indicator) is bound to a binding site (receptor) by noncovalent interactions.[1a,c] There are several advantages of this approach because of the noncovalent binding between receptor and indicator: (1) No synthetic effort is required to chemically link an indicator and a receptor; (2) there is considerable freedom in the choice of an appropriate indicator; and (3) signal transduction is induced by the simple addition of an analyte to the receptor-indicator mixture.[5]

Recently, a few fluorescent ensembles that can recognize PPi were reported.[5d–f] These ensembles, which used a bis(Dpa-Zn^{II}) (Dpa = dipicolylamine) complex, displayed quenching phenomena due to either binding effect with receptor and indicator or energy transfer between quencher and fluorophore. However, there were a few disadvantages in these systems. First, several synthetic steps were required to link the quencher with receptor and to synthesize an indicator unit, which was time consuming and tedious.[5d] Second, because quenching should be accompanied by binding, the screening process required to choose the right indicator was restricted.[5e,f] Also, the above ensembles showed only fluorescence ON–OFF.[5d–f]

Fabbrizzi et al. reported a fluorescent PPi ensemble based on a Cu(II) complex,[5a] where the Cu(II) ion was used to completely quench the fluorescence emission of a bound indicator and a coordinately unsaturated Cu(II) complex showed the capability to bind anionic substrates. However, Fabbrizzi's system showed only fluorescence ON–OFF, in comparison with our dual signaling effect, upon PPi binding. Further, no selectivity was reported for PPi over strong biological competitors such as AMP, ADP, and ATP.

Using these characteristics of the Cu(II) ion, we developed a dual signal (color change and fluorescence ON–OFF) ensemble system based on a bis(Dpa-Cu^{II}) complex (**1**) for detection of PPi. In order to realize a dual signal, the well-known indicators, pyrocatechol violet (PV) as a chromophore capable of instantly detecting by naked eyes and fluorescein disodium salts as a fluorophore, were used. These ensembles showed a high selectivity and sensitivity for PPi over various anions including phosphate and its derivatives (AMP, ADP, and ATP) in water.

The synthesis and characterization of complex **1** had been reported previously.[6] α,α′-Dibromo-*m*-xylene was reacted with Dpa, KI and Cs_2CO_3 in acetonitrile to give rise to 1,3-bis[(bis(2-pyridylmethyl)amino)methyl]benzene (**2**). Finally, complex **1** was prepared by complexing **2** with $Cu(ClO_4)_2$ in DMSO.[7]

First, the UV–vis absorption changes of PV (1.98×10^{-5} M) for complex **1** (4.41×10^{-4} M) were observed in a 10 mM HEPES buffer (pH 7.4) at 25 °C (Fig. 1). The UV–vis absorption curves of PV gradually decreased at 442 nm and increased at 664 nm upon the addition of complex **1**, showing a bathochromic shift. When 1.2 equiv of complex **1** was added into PV solution, the UV–vis absorption changes were completely saturated. Also, the color of PV solution turned from pale yellow to sky blue upon the addition of complex **1**. The association constant (K_a) between PV (abs = 664 nm) and complex **1** was estimated to be 1.01×10^6 M^{-1}.[8]

The UV–vis absorption spectra of ensemble **1** ([**1**] = 2.1×10^{-5} M, ensemble **1** = complex **1** + PV) for various anions (2 equiv, sodium

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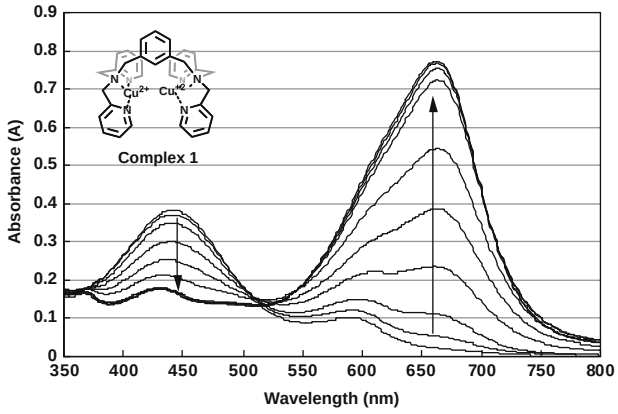


Figure 1. Changes of UV–vis absorption of pyrocatechol violet (PV, 1.98×10^{-5} M) upon the addition of complex **1** (4.41×10^{-4} M) in a 10 mM HEPES buffer (pH 7.4) at 25 °C: Complex **1** (μL) = 0, 10, 20, 40, 60, 80, 100, 130, 160, 200.

salts) were investigated in a 10 mM HEPES buffer (pH 7.4) at 25 °C (Fig. 2). When PPi was gradually added to the solution of ensemble **1**, the UV–vis absorption curves increased at 441 nm and decreased at 663 nm (Hypsochromic shift). The UV–vis absorption spectrum (at 663 nm) was completely saturated at 2 equiv of PPi (a 20-fold decrease). According to displacement titration, the association constant (K_a) between complex **1** and PPi was estimated to be 3.93×10^7 M^{-1}.[9] Also, the color of ensemble **1** returned from sky blue to pale yellow upon the addition of PPi (Fig. 3). However, each addition of 2 equiv of ATP or ADP showed only a 1.9-fold or 1.3-fold decrease in UV–vis absorbance at 663 nm, respectively. Upon the addition of 2 equiv of ATP, ensemble **1** turned from sky blue to pale green, but the solution of ensemble **1** after the addition of ADP (2 equiv) showed no color changes (Fig. 3). Other anions, such as F^-, Cl^-, Br^-, I^-, NO_3^-, AcO^-, HPO_4^{2-}, and AMP, did not give rise to any UV–vis spectral changes, nor, accordingly, induced any color changes (Fig. 3).

In the second place, the fluorescence emission changes (λ_{ex} = 495 nm) of fluorescein (1.25×10^{-6} M, disodium salts) for complex **1** (4.41×10^{-4} M) were examined in a 10 mM HEPES buffer (pH 7.4) at 25 °C (Fig. 4). According to the quenching effect of a Cu(II) ion, the fluorescence emission intensity of fluorescein decreased at 514 nm upon the addition of complex **1**. When 52.9 equiv of complex **1** was added to fluorescein solution, about 80% of the fluorescence emission intensity was quenched. The

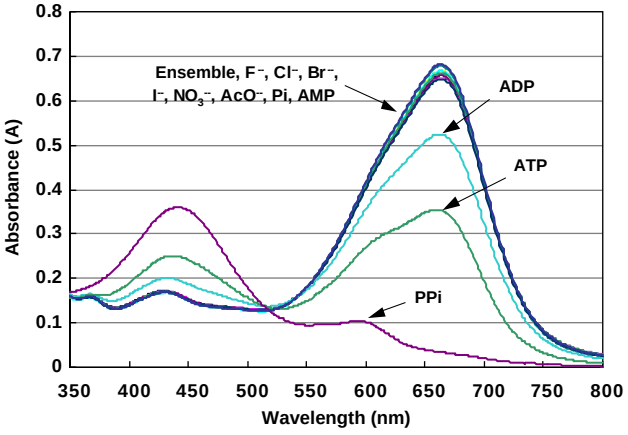


Figure 2. Changes of UV–vis absorption for ensemble **1** ([**1**] = 2.1×10^{-5} M) upon the addition of various anions (2 equiv, sodium salts) in a 10 mM HEPES buffer (pH 7.4) at 25 °C (Ensemble **1** = complex **1** + PV).

Figure 3. Color changes of ensemble **1** ([**1**] = 2.1×10^{-5} M) in a 10 mM HEPES buffer (pH 7.4) at 25 °C after the addition of various anions (2 equiv, sodium salts). From left to right: Ensemble **1**, PPi, ATP, ADP, AMP, Pi, AcO^-, NO_3^-, I^-, Br^-, Cl^-, F^-.

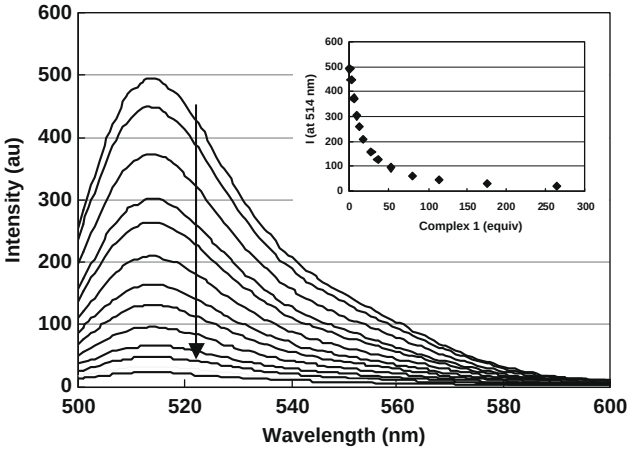


Figure 4. The fluorescence emission changes (λ_{ex} = 495 nm) of fluorescein (1.25×10^{-6} M, disodium salts) upon the addition of complex **1** (4.41×10^{-4} M) in a 10 mM HEPES buffer (pH 7.4) at 25 °C: Complex **1** (μL) = 0, 10, 30, 50, 70, 100, 150, 200, 300, 450, 650, 1000, 1500.

association constant (K_a) between **1** and fluorescein was estimated to be 5.83×10^4 M^{-1}.[8]

The fluorescence emission spectra of ensemble **2** ([**1**] = 7.35×10^{-5} M, ensemble **2** = **1** + fluorescein disodium salts) for various anions (1.2 equiv, sodium salts) were examined in a 10 mM HEPES buffer (pH 7.4) at 25 °C (Fig. 5). The fluorescence emission at 514 nm was completely saturated at 1.2 equiv of PPi, which corresponded to a 5.7-fold increase in the emission intensity (Fig. 5). Each addition of 1.2 equiv of ATP or ADP caused a 3.6-fold or 3.2-fold increase in fluorescence emission intensities at 514 nm,

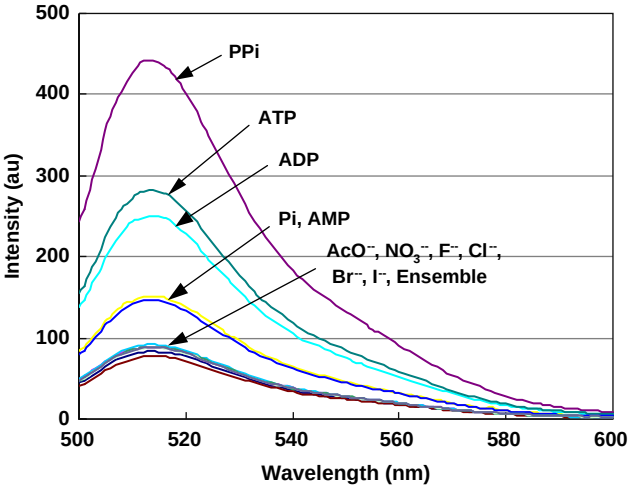
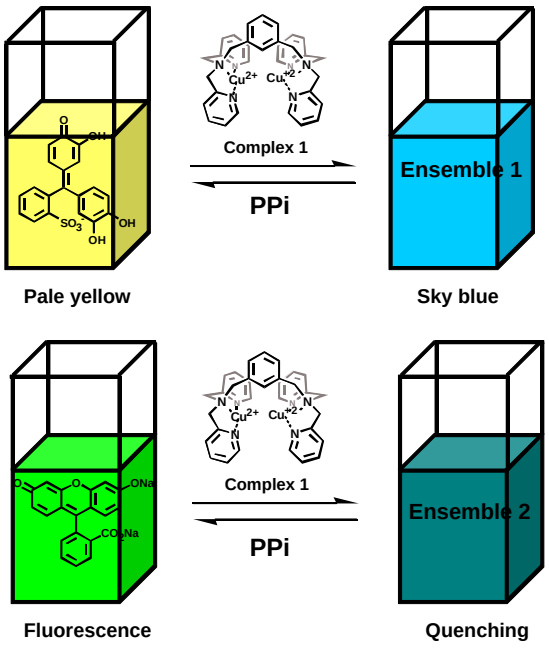


Figure 5. Changes of fluorescence emission for ensemble **2** ([**1**] = 7.35×10^{-5} M) upon the addition of various anions (1.2 equiv, sodium salts) in a 10 mM HEPES buffer (pH 7.4) at 25 °C (Ensemble **2** = complex **1** + Fluorescein disodium salts).



Scheme 1. A schematic representation of dual signal PPi ensemble systems.

respectively. When 1.2 equiv of AMP or Pi was added into solution of ensemble **2**, the fluorescence emission intensities increased two-fold or 1.9-fold, respectively. Other anions such as F^-, Cl^-, Br^-, I^-, NO_3^-, and AcO^- barely showed any changes in the fluorescence emission intensities.

The schematic representation for the dual signal ensemble system is illustrated in Scheme 1. Ensemble **1**, composed of complex **1** and PV, turned from yellow to blue upon the addition of PPi to the ensemble, which was caused by the displacement of weakly bound PV by PPi. Similarly, ensemble **2**, consisting of complex **1** and fluorescein, revived the original fluorescence of fluorescein upon the addition of the more strongly binding PPi to ensemble **2**.

In conclusion, using quenching effect of a Cu(II) ion, we developed simple dual signal (color change and fluorescence ON–OFF) ensemble system for detection of PPi, based on a bis(Dpa-Cu^{II}) complex **1**. There are several advantages to this system: (1) an easy synthesis of the receptor molecules; (2) the use of commercially available indicators; and (3) dual signaling effect (color and fluorescence changes).

Acknowledgments

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Supplementary data

Synthesis and HRMS data of complex **1**, color change of PV for addition of complex **1**, fluorescence emission change of ensemble **2** after addition of PPi, and the determination of association constant by displacement titration are available. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2009.02.036.

References and notes

1. (a) Martínez-Máñez, R.; Sancenón, F. *Chem. Rev.* **2003**, *103*, 4419–4476; (b) Gale, P. A. *Coord. Chem. Rev.* **2003**, *240*, 1–226; (c) Wiskur, S. L.; A-Haddou, H.; Lavigne, J. J.; Anslyn, E. V. *Acc. Chem. Res.* **2001**, *34*, 963–972; (d) Yoon, J.; Kim, S. K.; Singh, N. J.; Kim, K. S. *Chem. Soc. Rev.* **2006**, *35*, 355–360.
2. (a) Limpcombe, W. N.; Sträter, N. *Chem. Rev.* **1996**, *96*, 2375–2434; (b) Nyrén, P. *Anal. Biochem.* **1987**, *167*, 235–238; (c) Tabary, T.; Ju, L. *J. Immunol. Methods* **1992**, *156*, 55–60.
3. (a) McCarty, D. J. *Arthritis Rheum.* **1976**, *19*, 275–285; (b) Doherty, M. *Ann. Rheum. Dis.* **1983**, *42*, 38–44.
4. (a) Nishizawa, S.; Kato, Y.; Teramae, N. *J. Am. Chem. Soc.* **1999**, *121*, 9463–9464; (b) Lee, D. H.; Im, J. H.; Son, S. U.; Chung, Y. K.; Hong, J.-I. *J. Am. Chem. Soc.* **2003**, *125*, 7752–7753; (c) Lee, D. H.; Kim, S. Y.; Hong, J.-I. *Angew. Chem., Int. Ed.* **2004**, *43*, 4777–4780; (d) Lee, H. N.; Xu, Z.; Kim, S. K.; Swamy, K. M. K.; Kim, Y.; Kim, S.-J.; Yoon, J. *J. Am. Chem. Soc.* **2007**, *129*, 3828–3829.
5. (a) Fabbrizzi, L.; Marcotte, N.; Stomeo, F.; Taglietti, A. *Angew. Chem., Int. Ed.* **2002**, *41*, 3811–3814; (b) Han, M. S.; Kim, D. H. *Angew. Chem., Int. Ed.* **2002**, *41*, 3809–3811; (c) Folmer-Andersen, J. F.; Lynch, V. M.; Anslyn, E. V. *J. Am. Chem. Soc.* **2005**, *127*, 7986–7987; (d) Lee, D. H.; Kim, S. Y.; Hong, J.-I. *Tetrahedron Lett.* **2007**, *48*, 4477–4480; (e) McDonough, M. J.; Reynolds, A. J.; Lee, W. Y. G.; Jolliffe, K. A. *Chem. Commun.* **2006**, 2971–2973; (f) Hanshaw, R. G.; Hilkert, S. M.; Jiang, H.; Smith, B. D. *Tetrahedron Lett.* **2004**, *45*, 8721–8724.
6. Yoshiro, M. *Bull. Chem. Soc. Jpn.* **2002**, *75*, 1383–1384.
7. *Synthesis of complex* **1**. To a solution of α,α′-dibromo-*m*-xylene (200 mg, 0.76 mmol) in MeCN were added 2.1 equiv of Cs_2CO_3 (520 mg), di-2-(picolyl) amine (318 mg) and 1.5 equiv of KI (189 mg). The reaction mixture was stirred at 60 °C overnight, and then all the volatile components were evaporated. The residue was partitioned between CH_2Cl_2 and brine (×2). The combined organic phase was washed with water, and then dried in anhydrous Na_2SO_4. Flash chromatographic purification (only CH_2Cl_2–CH_2Cl_2:MeOH = 20:1) afforded **2** (52% yield). Finally, complex **1** was prepared by adding aqueous solution of $Cu(ClO_4)_2$ (2 equiv) to **2** (1 mg) in DMSO (1 ml). Characterization for complex **1** was reported previously.[6] HRMS (FAB): *m*/*e* calcd for $C_{32}H_{32}Cl_3N_6O_{12}$ $[M]^+$ 922.9736, found 922.9734.
8. Conners, K. A.. *Binding Constants*. In *The Measurement of Molecular Complex Stability*;; John Wiley and Sons: New York, 1987.
9. Zhong, Z.; Anslyn, E. V. *J. Am. Chem. Soc.* **2002**, *124*, 9014–9015.