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A simple method for improving the optical properties of a dimetallic coordination fluorescent chemosensor for adenosine triphosphate

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ABSTRACT

The selectivity and sensitivity of $[Zn_2(9,10\text{-bis}[(2,2\text{-dipicolylamino})\text{methyl}]\text{anthracene})]^{4+}$ (**1**) were enhanced by eliminating its intrinsic fluorescence with the introduction of pyrocatechol violet. An ensemble ($[Zn_2(9,10\text{-bis}[(2,2\text{-dipicolylamino})\text{methyl}]\text{anthracene})(\text{pyrocatechol violet})]^{2+}$) can easily detect less than 1 μM of ATP and can discriminate between ATP and various other anions including adenosine diphosphate (ADP).

Fluorescent chemosensors have multiple advantages over other types of chemosensors including high sensitivity, low cost, ease of application, and versatility.[1] Moreover, for the efficient detection of analytes, the photophysical properties of a fluorescent chemosensor can be modulated by a variety of methods such as the introduction of proton-, energy-, and electron-transfer processes, the presence of heavy-atom effects, changes in electronic density, and destabilization of non-emissive $n\pi^*$-excited states.[2] However, in spite of the existence of many approaches for modulating fluorescent chemosensors, many suffer from background fluorescence signals in the absence of a target analyte.[2]

Numerous sensors for anions, including ATP, have been devised based upon metal–ligand interactions for the recognition of analytes.[3] A metal–ligand interaction is useful in the formulation of an anion sensor because the metal–anion bonding interaction is very strong in water and the metal ion can exhibit geometrical preferences which impart selective binding tendencies toward anions of a given shape.[3d,4] Numerous metal complexes, such as cyclone–Zn^{2+}, dipicolylamine–Zn^{2+}, azamacrocyle–Cu^{2+}, for example, have been used in the design of anion sensors.[5] Among these metal complexes, dipicolylamine–Zn^{2+} complexes are popular for molecular recognition because they do not quench the fluorescence of an attached dye, and they have up to two vacant coordination sites for an anionic guest. Also, these metal complexes are easily introduced into an organic scaffold.[6] Recently, numerous fluorescent sensors with two dipicolylamine–Zn^{2+} moieties as anion recognition sites have been developed.[7] These sensors are effective fluorescent sensors of phosphate derivatives such as ATP, pyrophosphate, and peptides with phosphotyrosine residues, but they also have some background fluorescence in the absence of an analyte which is undesirable for analytical purposes.

In this Letter, we report a simple method to enhance the sensitivity and selectivity of a dimetallic coordination fluorescent chemosensor for ATP by eliminating the background fluorescence of the sensor using a competitive assay approach.[8] Previously reported fluorescent chemo-ensembles consist of fluorogenic indicator and receptor moieties with a quencher such as a paramagnetic metal ion or chromogenic azo-compound.[9] However, to the best of our knowledge, a fluorogenic chemo-ensemble constructed with a fluorogenic sensor and its quencher have not been reported. This reverse-type fluorogenic chemo-ensemble can have advantages over a fluorogenic sensor because sensitivity can be increased by eliminating the background fluorescence of the receptor, and selectivity can be enhanced by choosing a quencher with the proper binding affinity for the receptor. To validate this concept, we adapted the sensor (**1**) developed by Hamachi that efficiently detects phosphate residues in aqueous solutions and is easily synthesized, but also has some background fluorescence.[10] In order to eliminate background fluorescence in the fluorogenic sensor (**1**), we chose pyrocatechol violet as a quencher. Pyrocatechol violet is known to coordinate with the two metal ions in a binuclear metal complex.[11] Furthermore, we expected that pyrocatechol violet would be a good fluorescence quencher, similar to a dabsyl group,[12] and that the displacement of the receptor-bound pyrocatechol violet by ATP would be communicated fluorogenically. The competition approach to analyte recognition used by this modified sensor is schematically illustrated in Scheme 1.

A fluorescence titration of pyrocatechol violet was conducted using a 5 μM solution of **1** in aqueous buffer at pH 7.2. Fluorescence emission spectra of **1** in the presence of varied concentrations of pyrocatechol violet are shown in Figure 1. The addition of pyrocatechol violet induced a reduction in fluorescence, as shown in Figure 1 inset, that was nearly proportional to the pyrocatechol violet concentration and was saturated with approximately 2 equiv of pyrocatechol violet. This indicates that

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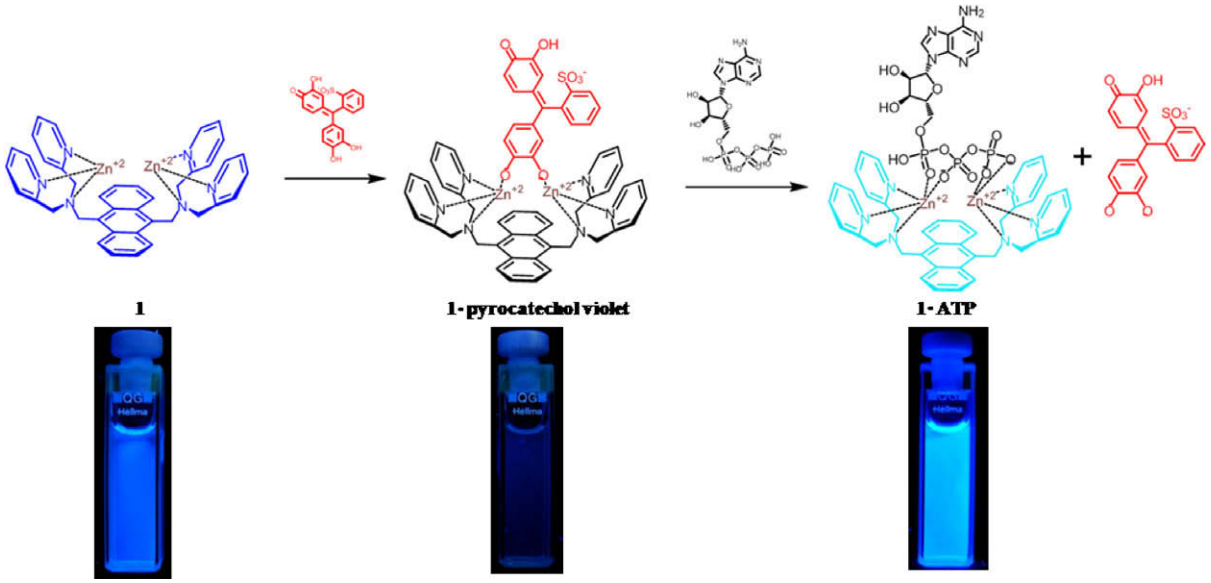

Scheme 1. A schematic representation of the dimetallic coordination fluorescent chemosensor recognition of ATP.

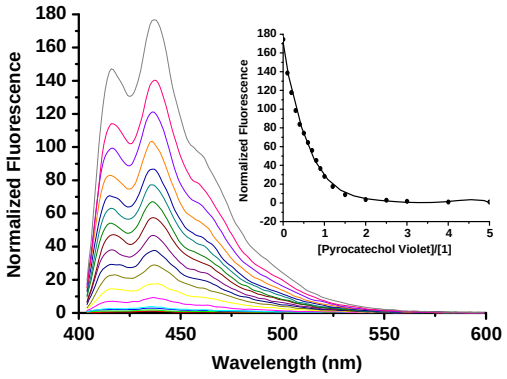

Figure 1. Fluorescence spectra obtained by the addition of pyrocatechol violet solution to a pH 7.2 aqueous buffer (Trizma, 0.05 M) containing **1** (5 μM).

pyrocatechol violet is strongly bound to **1** and is an efficient quencher. To investigate the binding mode of the pyrocatechol violet to **1**, a Job plot was carried out.[13] As shown in Figure 2, despite 2:1 complex being included since the Job plot is not a perfect symmetry, the result of the plot suggests major binding species as a 1:1 complex as depicted in Scheme 1. The association constant of 2.89×10^6 for the binding of pyrocatechol violet to **1** was obtained by non-linear regression analysis of the titration data according to the 1:1 binding model.[13]

The sensor ensemble was prepared by simply mixing **1** and pyrocatechol violet in an aqueous solution of 0.05 M Trizma buffer at pH 7.2 in a 1:2 molar ratio since the fluorescent quenching of **1** was saturated with 2 equiv of pyrocatechol violet. Fluorescence changes of the ensemble in the presence of various amounts of ATP were examined. As expected, the addition of ATP to an aqueous solution of the ensemble resulted in a large enhancement of fluorescence. The chemosensor ensemble displayed a saturation point upon the addition of 16 equiv of ATP (Fig. 3), while less than 1 μM of ATP could be easily detected using our system. Moreover, in the presence of 0.5 μM of ATP, fluorescence enhancement with **1** was only 6% over the initial state (see supporting information) but, in our new system, it was >250% due to elimination of the fluorescence of the initial state by the fluorescence quencher pyrocatechol violet. In addition, the detection range of the chemosensing ensemble (80 μM) was eight times greater than that of **1** (10 μM). In an effort to understand this broadening of the detection range, we

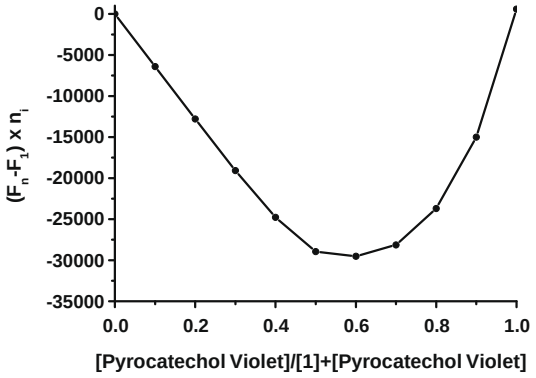

Figure 2. The Job plot for the binding of pyrocatechol violet with the receptor (**1**) in a pH 7.2 (Trizma 0.05 M) buffer solution. Aqueous solutions of **1** (5 μM) and pyrocatechol violet (5 μM) were mixed in varying ratios and the change in fluorescence at 436 nm was measured.

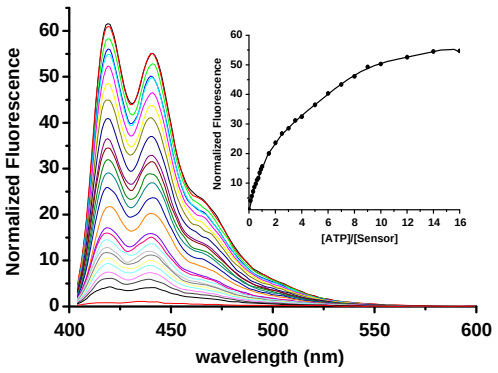

Figure 3. Fluorescence emission spectra obtained by the addition of ATP (0–80 μM) to pH 7.2 buffer solution containing the sensor ensemble (5 μM). Inset: plot of normalized fluorescence intensities of the sensing ensemble at 426 nm versus ATP concentration.

determined the association constant for the binding of ATP to **1** and compared it with that of the receptor–pyrocatechol violet. The binding of ATP to **1** was determined to be 1.36×10^6 by non-linear regression analysis of a titration data (see Supplementary data). Thus, the binding affinity of pyrocatechol violet to the receptor was 2.1-fold greater than with ATP. This result may be used to rationalize the increase in detection range.

Another important property of the chemosensing ensemble is its high selectivity toward ATP over other anions and moderate selectivity over ADP. To evaluate the ATP-selective property of the ensemble, changes in the fluorescence properties of the ensemble caused by other anions were measured. Fluorescence spectra of solutions of the ensemble (5 μM) recorded in the presence of 1 equiv of anions including AMP and ADP are displayed in Figure 4. The ensemble gave a negative response to other anions including AMP but gave a positive response to ADP. Although the ensemble gave a positive response to ADP because of its high binding affinity for the receptor (see Supplementary data), it could discriminate ATP over ADP. This is remarkable since the fluorescence of **1** in the presence of ADP or ATP is almost the same (see Supplementary data). This implies that the selectivity of **1** for anions can be modulated by the pyrocatechol violet.

In conclusion, the sensing properties of a dimetallic coordination fluorescent chemosensor for ATP were improved by eliminating the intrinsic background fluorescence of the sensor by simply incorporating pyrocatechol violet as a quencher. Although the sensing ensemble cannot be applicable to detect ATP in vivo, it can easily detect less than 0.5 μM of ATP and has selectivity for ATP over other anions including ADP in vivo. Moreover, because there is no background fluorescence in our system, in the presence of an analyte the fluorescence was dramatically enhanced from the initial state. This approach might be applicable to the improvement of other fluorogenic sensing systems.

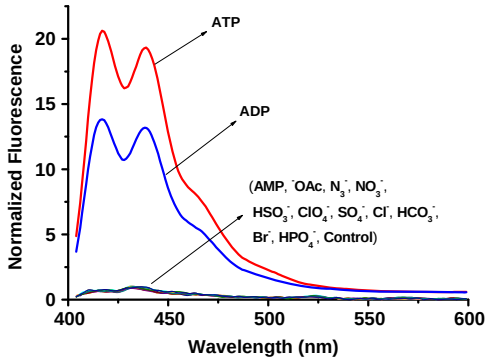

Figure 4. Fluorescence spectra of the ensemble solution (5 μM) in the presence of various anions (5 μM).

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2009.09.016.

References and notes

1. (a) Czarnik, A. W.. *Acc. Chem. Res.* **1994**, *27*, 302–308; (b) Schmidtchen, F. P.; Berger, M. *Chem. Rev.* **1997**, *97*, 1609–1646.
2. de Silva, A. P.; Nimal Gunaratne, H. Q.; Gunnlaugsson, T.; Huxley, A. J. M.; McCoy, C. P.; Rademacher, J. T.; Rice, T. E. *Chem. Rev.* **1997**, *97*, 1515–1566.
3. (a) Rogers, C. W.; Wolf, M. O. *Coordin. Chem. Rev.* **2002**, *233–234*, 341–350; (b) Martínez-Máñez, R.; Sancenón, F. *Chem. Rev.* **2003**, *103*, 4419–4476; (c) Amendola, V.; Fabbrizzi, L.; Mangano, C.; Pallavicini, P.; Poggi, A.; Taglietti, A. *Coordin. Chem. Rev.* **2001**, *219–221*, 821–837; (d) Fabbrizzi, L.; Licchelli, M.; Rabaioli, G.; Taglietti, A. *Coordin. Chem. Rev.* **2000**, *205*, 85–108.
4. Beer, P. D. *Acc. Chem. Res.* **1998**, *31*, 71–80.
5. O'Neil, E. J.; Smith, B. D. *Coordin. Chem. Rev.* **2006**, *250*, 3068–3080.
6. (a) Ojida, A.; Mito-oka, Y.; Sada, K.; Hamachi, I. *J. Am. Chem. Soc.* **2004**, *126*, 2454–2463; (b) Ojida, A.; Sakamoto, T.; Inoue, M.; Fujishima, S.; Lippens, G.; Hamachi, I. *J. Am. Chem. Soc.* **2009**, *131*, 6543–6548; (c) Ojida, A.; Takashima, I.; Kohira, T.; Nonaka, H.; Hamachi, I. *J. Am. Chem. Soc.* **2008**, *130*, 12095–12101; (d) Ojida, A.; Honda, K.; Shinmi, D.; Kiyonaka, S.; Mori, Y.; Hamachi, I. *J. Am. Chem. Soc.* **2006**, *128*, 10452–10459; (e) Ojida, A.; Inoue, M.; Mito-oka, Y.; Tsutsumi, H.; Sada, K.; Hamachi, I. *J. Am. Chem. Soc.* **2006**, *128*, 2052–2058.
7. (a) Kim, S. K.; Lee, D. H.; Hong, J.-I.; Yoon, J. *Acc. Chem. Res.* **2009**, *42*, 23–31; (b) Kohira, T.; Honda, K.; Ojida, A.; Hamachi, I. *ChemBioChem* **2008**, *9*, 698–701; (c) Lee, D. H.; Kim, S. Y.; Hong, J. I. *Angew. Chem., Int. Ed.* **2004**, *43*, 4777–4780; (d) Kwon, T. H.; Kim, H. J.; Hong, J.-I. *Chem. Eur. J.* **2008**, *14*, 9613–9619; (e) Rhee, H.-W.; Lee, C.-R.; Cho, S.-H.; Song, M.-R.; Cashel, M.; Choy, H. E.; Seok, Y.-J.; Hong, J.-I. *J. Am. Chem. Soc.* **2008**, *130*, 784–785; (f) Chen, X.; Jou, M. J.; Yoon, J. *Org. Lett.* **2009**, *11*, 2181–2184; (g) Ojida, A.; Nonaka, H.; Miyahara, Y.; Tamaru, S.; Sada, K.; Hamachi, I. *Angew. Chem., Int. Ed.* **2006**, *45*, 5518–5521; (h) Ojida, A.; Miyahara, Y.; Wongkongkatep, J.; Tamaru, S.; Sada, K.; Hamachi, I. *Chem. Asian J.* **2006**, *1*, 555–563; (i) 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.
8. Wiskur, S. L.; Ait-Haddou, H.; Lavigne, J. J.; Anslyn, E. V. *Acc. Chem. Res.* **2001**, *34*, 963–972.
9. (a) Fabbrizzi, L.; Marcotte, N.; Stomeo, F.; Taglietti, A. *Angew. Chem., Int. Ed.* **2002**, *41*, 3811–3814; (b) Kim, S. Y.; Hong, J.-I. *Tetrahedron Lett.* **2009**, *50*, 1951–1953; (c) Lee, D. H.; Kim, S. Y.; Hong, J.-I. *Tetrahedron Lett.* **2007**, *48*, 4477–4480; (d) Oh, D. J.; Han, M. S.; Ahn, K. H. *Supramol. Chem.* **2007**, *19*, 315–320.
10. Ojida, A.; Mito-oka, Y.; Inoue, M.; Hamachi, I. *J. Am. Chem. Soc.* **2002**, *124*, 6256–6258.
11. Han, M. S.; Kim, D. H. *Angew. Chem., Int. Ed.* **2002**, *41*, 3809–3811.
12. Kadirvel, M.; Gbaj, A.; Mansell, D.; Miles, S. M.; Arsic, B.; Bichenkova, E. V.; Freeman, S. *Tetrahedron* **2008**, *64*, 5598–5603.
13. Conners, K. A. *Binding constants, The Measurement of Molecular Complex Stability*; John Wiley & Sons: New York, 1987.