

Fluorescent Sensors

A Fluorescent Pyrophosphate Sensor with High Selectivity over ATP in Water**

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Recently, considerable attention has been focused on the design of receptors and sensors that have the ability to bind and sense biologically important anions selectively through electrochemical and optical responses.^[1] Anions such as pyrophosphate ($P_2O_7^{4-}$, PPI) and adenosine triphosphate (ATP) play an important role in energy transduction in organisms and control metabolic processes by participation in enzymatic reactions.^[2,3] ATP hydrolysis with the concomitant release of PPI is central to many biochemical reactions, such as DNA polymerization and the synthesis of cyclic adenosine monophosphate (AMP) catalyzed by DNA polymerase and adenylate cyclase, respectively.^[2] Therefore, the detection and

discrimination of these anions has been the main focus of the efforts of several research groups. Although traditional methods of anion sensing such as the use of ion-selective electrodes continue to hold their ground, there is an increasing incentive to find alternative means of analysis, including those based on the use of selective chemosensors.^[1b,4] Among the various chemosensors, fluorescent chemosensors present many advantages, as they benefit from high sensitivity, low cost, easy detection, and versatility.^[1,5] Unfortunately, few, if any, fluorescent anion sensors exist that display both high PPI/ATP selectivities and function in aqueous solution over a wide range of pH values.^[6,7] Herein, we present a new fluorescent PPI sensor based on a naphthalene-dpa system (dpa = bis(2-pyridylmethyl)amine), which exhibits high sensitivity and selectivity over a wide range of pH values for PPI ions relative to other anions, including ATP and ADP (which are structurally similar to PPI in aqueous solution).

A fluorescent reporting naphthalene group was easily introduced into the sensor by a Suzuki coupling reaction. Compound **1** was obtained in 35% overall yield from acetone.^[8] Zn^{II} complexation with the dpa moieties generates an anion-binding site through the formation of a well-known phenoxo-bridged binuclear metal complex (Scheme 1):^[9] Sensor **1**:2Zn, a binuclear Zn^{II} complex of compound **1**, was easily formed by the addition of a methanolic solution of **1** to an aqueous solution of $Zn(NO_3)_2$ (2 equiv).

First, the effect of anions (sodium salts) on the absorption spectrum of sensor **1**:2Zn (30 μ M) was examined in an aqueous solution of HEPES buffer (0.01M, pH 7.4) (HEPES = 2-[4-(2-hydroxyethyl)-1-piperazinyl]ethanesulfonic acid) at 25 °C (Figure 1). In the absence of an anion guest, the absorption spectrum of sensor **1**:2Zn is characterized by an intense band centered at 305 nm. Upon the addition of PPI in increasing amounts, the peak at 305 nm decreases and a new peak appears at 316 nm. The degree of absorption change is not affected even by the addition of more than 1 equiv of PPI (Figure 1a). Unlike the large 11-nm red shift observed with PPI, relatively small red shifts are seen with ATP (5 nm) and ADP (2 nm) upon complexation. Furthermore, sensor **1**:2Zn does not exhibit any clear spectral change upon addition of AMP, HPO_4^{2-} ; other monovalent anions such as $CH_3CO_2^-$, F^- , HCO_3^- , and Cl^- do not affect the spectrum, even when present in excess (up to 100 equiv). The degree of red shift was determined to be $PPI \gg ATP > ADP > AMP \approx HPO_4^{2-}$. These results suggest that sensor **1**:2Zn has a higher selectivity for PPI over other anions.

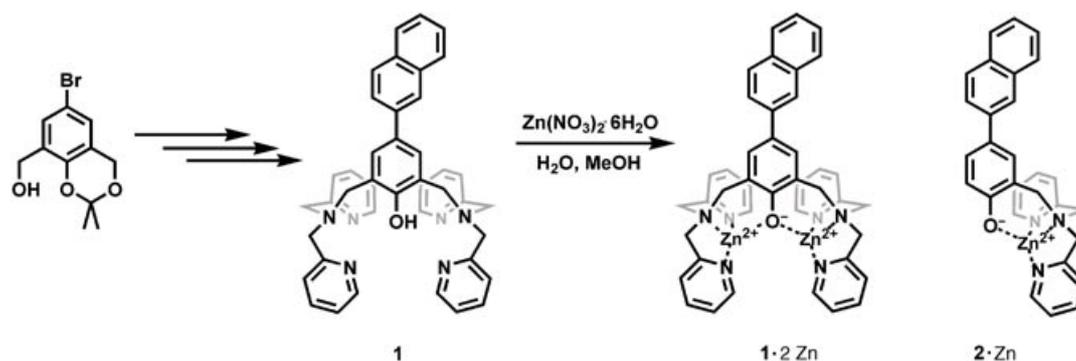
The optical sensing of anions was more drastically demonstrated by virtue of the fluorescent response of **1**:2Zn to complex formation. The effect of anions (sodium salts) on the fluorescent emission spectrum of sensor **1**:2Zn (6 μ M) was investigated in an aqueous solution of HEPES buffer (0.01M, pH 7.4) at 25 °C (Figure 2). When PPI was added to an aqueous solution of **1**:2Zn, the fluorescent emission spectrum shifted in a dose-dependent manner toward longer wavelengths. As shown in Figure 2a, the λ_{max} shifted from 436 nm to 456 nm. An increase in the PPI concentration up to 1 equiv resulted in a 9.5-fold fluorescence enhancement. However, the addition of more than 4 equiv of ATP shows only twofold

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Supporting information for this article is available on the WWW under <http://www.angewandte.org> or from the author.



Scheme 1. Synthetic strategy for sensors 1·2Zn and 2·Zn.

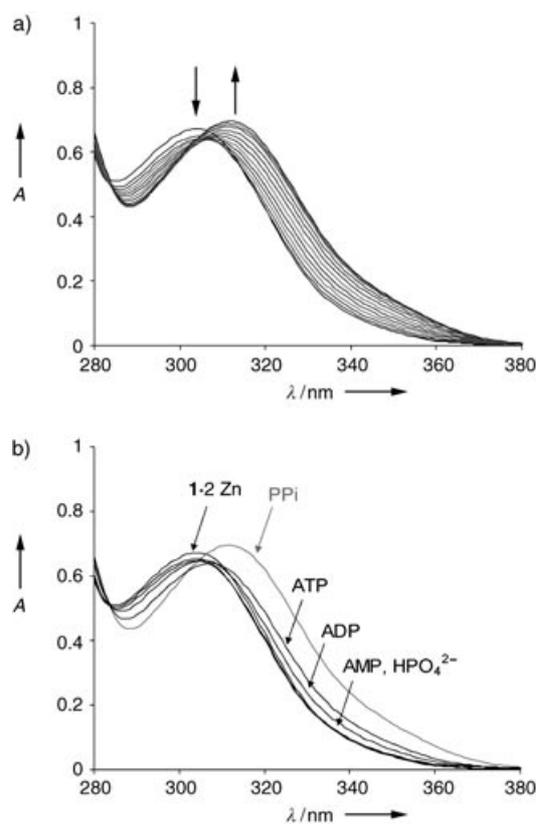


Figure 1. a) Change in absorbance for sensor 1·2Zn (30 μM) upon addition of PPI (sodium salt): [PPI]=0, 3, 6, 9, 12, 15, 18, 21, 24, 27, 30, 32, 34, 36, 38 μM . The spectra were measured in an aqueous solvent of HEPES buffer (10 mM, pH 7.4) at 25 °C. b) UV/Vis spectra of sensor 1·2Zn (30 μM) in HEPES buffer (0.01 M, pH 7.4) at 25 °C in the presence of various anions (30 μM).

enhancement accompanied by a 12-nm red shift. In the case of ADP, sensor 1·2Zn shows only a subtle emission change (1.5-fold increase) upon addition of a 50-fold excess of ADP. The addition of AMP and HPO_4^{2-} does not lead to an emission enhancement, even after the addition of 100 equivalents of each anion (Figure 2b).

The Job plot for the binding between 1·2Zn and anions (PPI and ATP) shows a 1:1 stoichiometry (inset of Figure 2a). The apparent association constant (K_a) was determined to be

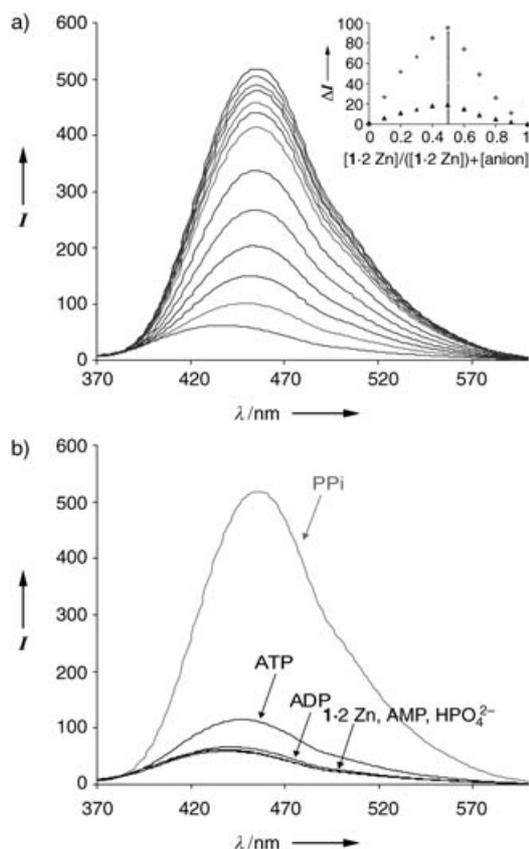


Figure 2. a) Change in fluorescence emission for sensor 1·2Zn (6 μM) upon addition of PPI (sodium salt): [PPI]=0, 0.6, 1.2, 1.8, 2.4, 3.0, 3.6, 4.2, 4.8, 5.4, 6.0, 6.6, 7.2 μM . The spectra were measured in an aqueous solvent of HEPES buffer (0.01 M, pH 7.4) at 25 °C. (Inset: Job plot of 1·2Zn and anions: \blacklozenge PPI, \blacktriangle ATP.) b) Fluorescence emission spectra of sensor 1·2Zn (6 μM) in HEPES buffer (10 mM, pH 7.4) at 25 °C in the presence of various anions (8 μM). I = Intensity (arbitrary units).

$(2.9 \pm 0.7) \times 10^8 \text{ M}^{-1}$ for PPI–1·2Zn by a standard algorithm for competitive binding in the presence of excess HPO_4^{2-} in an aqueous solvent of HEPES buffer (0.01 M, pH 7.4) at 25 °C.^[8,10] By the same method, K_a for ATP–1·2Zn was found to be $(7.2 \pm 1.0) \times 10^6 \text{ M}^{-1}$, which is 40-fold lower than for PPI–1·2Zn. This observation means that 1·2Zn can even detect PPI at nanomolar concentrations in water.

The same selectivity trend found in the absorbance spectra (Figure 1b) is observed in the fluorescence spectra (Figure 2b). However the signal difference is greatly enhanced because of the high sensitivity of fluorescence spectroscopy in favor of PPI. This selectivity enables PPI detection by fluorescence, even in the presence of a large excess of ATP (Figure 3). Figure 3 shows that 1:2Zn can

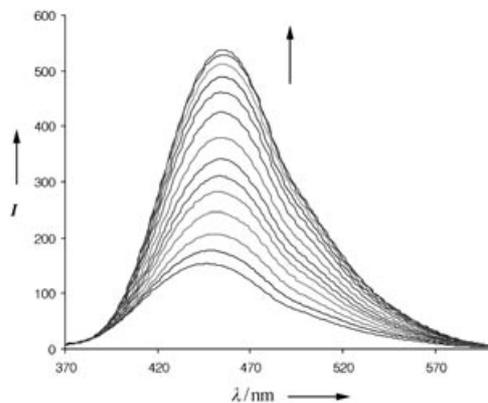
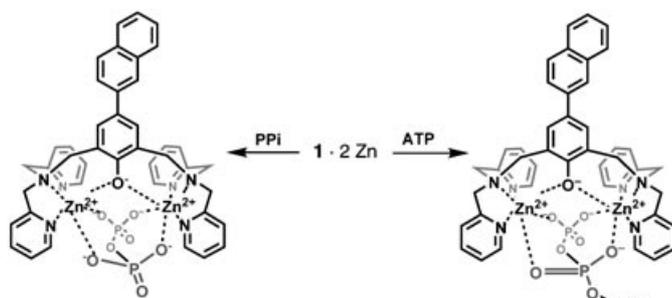


Figure 3. a) Change in fluorescence emission for sensor 1:2Zn ($6 \mu\text{M}$) in the presence of ATP ($300 \mu\text{M}$) upon addition of PPI (sodium salt): $[\text{PPI}] = 0, 1.2, 2.4, 3.6, 4.8, 6.0, 8.0, 11, 17, 24, 34, 45, 65, 85 \mu\text{M}$. The spectra were measured in a pure aqueous solution of HEPES buffer (0.01 M , $\text{pH } 7.4$) at 25°C . I = Intensity (arbitrary units).

detect less than 1 equivalent of PPI even in the presence of a 50- to 250-fold excess of ATP (based on the amount of PPI detected). In other words, 1:2Zn can selectively detect PPI in an aqueous solution with remarkable selectivity over ATP with a detection limit at micromolar concentrations. This is significant in view of the fact that there are many biochemical reactions in which PPI is released in the presence of ATP. An efficient PPI sensor for bioanalytical applications requires that PPI be detected in small amounts in the presence of a large excess of ATP,^[8] thus making our sensor suitable for bioanalytical applications.

The binding mode for PPI-1:2Zn is illustrated in Scheme 2, which is based on our previous work involving a structurally similar sensor and its X-ray crystal structure.^[11] The proposed complex shows that the two sets of oxygen anions on each P atom of PPI bind to the binuclear zinc



Scheme 2. Proposed mechanism for the complexation of sensor 1:2Zn with PPI and ATP.

complex by bridging the two metal ions to give rise to two hexacoordinated Zn^{II} ions in 1:2Zn.^[11] PPI induces a pronounced red shift of λ_{max} of 1:2Zn because the weakening of the bond between the phenolate oxygen atom and Zn^{II} induces a more-negative charge characteristic on the phenolate oxygen atom and thus the bathochromic shift of λ_{max} of 1:2Zn occurs. Simultaneously, an increased charge characteristic on the phenolate oxygen atom induces a fluorescence enhancement.

The selectivity for PPI over ATP can be understood on the basis of the structure of the guest and the charge density of four O-P oxygen atoms of the guest involved in the complexation. The total anionic charge density of the four O-P oxygen atoms involved in the complexation of ATP with 1:2Zn is relatively smaller than that of the four O-P oxygen atoms of PPI (Scheme 2). Therefore, the binding affinity of ATP is drastically reduced, and the degree of fluorescence change becomes smaller relative to PPI binding.

A control sensor, mononuclear 2:Zn (Scheme 1), does not show emission λ_{max} shift and fluorescence enhancement upon addition of PPI. This result means that the cooperative action of two Zn^{II} -dpa units is required for the selective sensing of PPI. Finally, to check for the working pH range for sensing, the effect of the pH value of the medium on the PPI sensing was investigated. Fortunately, fluorescence emission changes shown in Figure 2b were also observed over a wide range of pH values (6.5–10.1) with a similar tendency. This result shows that even if the external pH value is disturbed, sensor 1:2Zn can still detect PPI.^[8]

In summary, we have developed a naphthalene-based fluorescent sensor that selectively detects PPI with high affinity in aqueous solution over a wide pH range. This system shows remarkable selectivity for PPI over other anions, including strong competitors such as HPO_4^{2-} and ATP. This system can be applied for biochemical and analytical enzyme assays involving ATP and PPI.

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