

A fluorescent pyrophosphate sensor *via* excimer formation in water†

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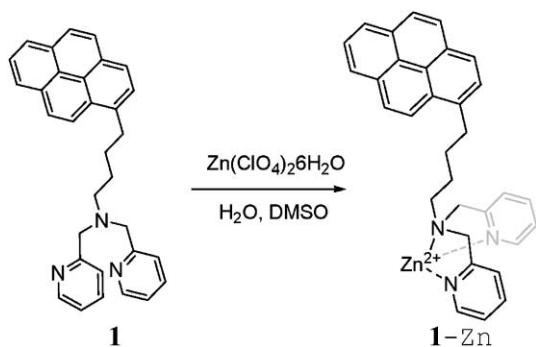
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A new fluorescent sensor based on a pyrene/ Zn^{II} -dpa (dpa = bis(2-pyridylmethyl)amine) conjugate displays excimer emission selective for pyrophosphate over other anions.

Biological anions such as phosphate, pyrophosphate ($\text{P}_2\text{O}_7^{4-}$, PPI), AMP and ATP are involved in important biological processes. For example, anions such as PPI and ATP are involved in energy transduction in organisms, and control metabolic processes *via* participation in various enzymatic reactions.^{1,2} Several important biochemical reactions such as DNA polymerization, synthesis of cyclic AMP (second messenger), and formation of the activated intermediates (aminoacyl-tRNA) in protein synthesis are catalysed by DNA polymerase, adenylate cyclase and aminoacyl-tRNA synthetase, respectively, where ATP hydrolysis with the concomitant release of PPI is at the heart of biochemical pathways.¹ Therefore, several research groups have focused on the detection and discrimination of these biological anions. Until now, very few sensors were reported that displayed high selectivity and reliability, and which functioned in aqueous media.³

Herein, we report a new PPI sensor (1-Zn) based on a pyrene-dpa (dpa = bis(2-pyridylmethyl)amine) conjugate, which shows PPI selective excimer fluorescence *via* excimer formation through a PPI-induced self-assembly of the two PPI sensors.⁴



Synthesis of compound **1** is described in the ESI†. The sensor (1-Zn), Zn^{II} complex of compound **1**, was easily formed by the addition of a DMSO solution of **1** to an aqueous solution of $\text{Zn}(\text{ClO}_4)_2 \cdot 6\text{H}_2\text{O}$ (1 equiv.). Bis(Zn^{II} -dpa)-based artificial receptors are known to show strong binding affinity toward phosphate derivatives in aqueous solution.⁵ Therefore, we expect that the two

Zn complexes at an appropriate distance would cooperatively act to generate an anion binding site. Bringing the two Zn complexes in the presence of phosphate derivatives allows for the two pyrenyl moieties to form an intermolecular pyrenyl complex. Formation of the self-assembled pyrenyl complex would result in a remarkable change in the ratio of excimer emission intensity to monomer emission due to the sandwich-type pyrene dimer of 1-Zn. In addition, the ratiometric sensing for anions is possible by exploiting monomer and excimer emissions.⁶ The effect of anion binding on the absorption spectrum of 1-Zn (0.02 mM) was examined in an aqueous solution of 10 mM HEPES buffer (pH = 7.4) at 25 °C (HEPES = 2-[4-(2-hydroxyethyl)-1-piperazinyl]ethanesulfonic acid).⁷ In the absence of anions, the absorption spectrum of 1-Zn is characterized by five well-shaped and fine peaks between 260 and 350 nm, similar to the absorption spectrum of pyrene. Sensor 1-Zn did not show any obvious spectral change upon addition of HPO_4^{2-} , as well as AMP except for a decrease in intensity. However, addition of PPI or ATP caused a red-shifted spectral change (Fig. 1).

The effect of anions on the fluorescence emission spectrum of sensor 1-Zn (0.02 mM) was also examined in an aqueous solution of HEPES buffer (10 mM, pH 7.4) at 25 °C (Fig. 2). In the absence of anions, the fluorescence spectrum of 1-Zn was characterized by a structured emission band at 360–450 nm which was assigned to the pyrene monomer emission. Upon the addition of PPI to an aqueous solution of 1-Zn, a structureless band with an emission maximum at 475 nm appeared, and there was quenching of the

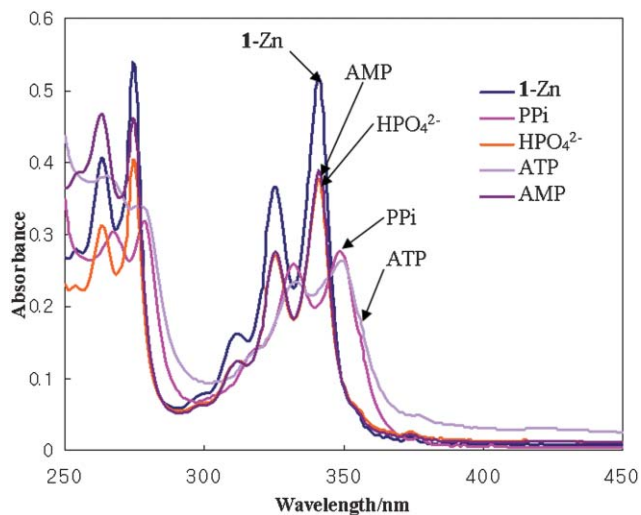


Fig. 1 Absorption spectra of 1-Zn (0.02 mM) at room temperature in the presence of various anions (0.5 equiv.). The spectra were measured in aqueous solvent of 10 mM HEPES buffer (pH = 7.4).

† Electronic supplementary information (ESI) available: Synthesis and spectral data of **1** and 1-Zn, fluorescence spectra of 1-Zn and various anions, fluorescence spectra of 1-Zn in the presence of 10 equiv. of ATP upon addition of PPI, Job plot and photographs of light emission of 1-Zn. See <http://www.rsc.org/suppdata/cc/b4/b417845a/> *jihong@plaza.snu.ac.kr

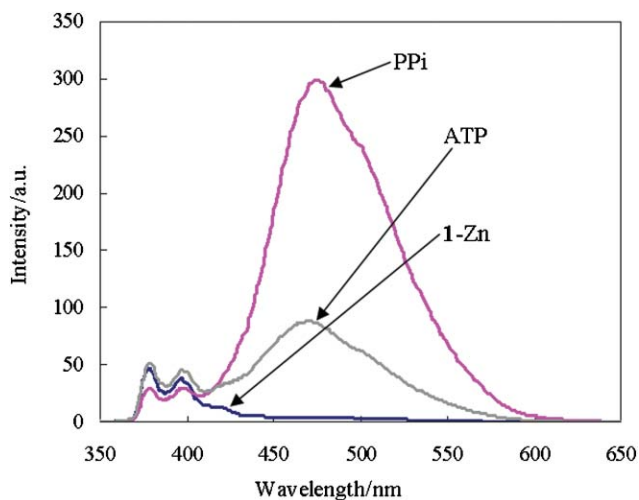


Fig. 2 Fluorescence spectra of **1-Zn** (0.02 mM) in aqueous solvent of 10 mM HEPES buffer (pH = 7.4) in the presence of anions (0.4 equiv). Excitation wavelength 343 nm.

monomer emission. A similar but much less effective response was observed upon addition of ATP. However, **1-Zn** did not show any obvious spectral change upon addition of HPO_4^{2-} , AMP as well as other monovalent anions such as CH_3CO_2^- , NO_3^- , Cl^- and F^- .⁸ These results suggest that sensor **1-Zn** has a higher selectivity for PPI over other anions ($\text{PPI} > \text{ATP} \gg \text{HPO}_4^{2-} > \text{AMP}$, CH_3CO_2^- , NO_3^- , Cl^- , F^-).

The fluorescence dependence of **1-Zn** in an aqueous solution of HEPES buffer (10 mM, pH 7.4) on the PPI concentration is shown in Fig. 3. Increasing the PPI concentration up to approximately 0.3 equiv., relative to the sensor concentration, results in a drastic increase in the intensity of the long-wavelength emission, while the monomer fluorescence intensity decreases a little. However, further addition of PPI decreases the intensity of the long-wavelength emission. Similarly, the ratio of excimer emission intensity at 476 nm to monomer emission intensity at 397 nm depends on the equiv. of added anions; the emission intensity ratio (I_{476}/I_{397}) increases until 0.3 equiv. of PPI or ATP is added, and then decreases after further addition of PPI or ATP (see Fig. 3). In particular, a larger ratio of I_{476}/I_{397} appears when PPI is added, compared with the result of adding ATP. This result also suggests that sensor **1-Zn** has higher selectivity for PPI over ATP as well as HPO_4^{2-} in aqueous media.

Job's plot for the binding between **1-Zn** and PPI suggests a 2 : 1 stoichiometry (see ESI†). Quantum yields of **1-Zn** and **1-Zn**·PPI complex in water were determined as compared to the reference, pyrene in cyclohexane ($\Phi = 0.32$).⁹ Quantum yields of **1-Zn**, monomer of **1-Zn**·PPI complex and excimer of **1-Zn**·PPI complex in water are 0.14, 0.099 and 0.58, respectively. Addition of PPI to the solution of **1-Zn** resulted in brighter light emission upon UV exposure compared to emission of **1-Zn** itself (see ESI†).

While the excimer peak is hardly visible in the presence of 10 equiv. of ATP, adding increasing amounts of PPI (0–2.3 equiv.) clearly shows the formation of excimer peaks (see ESI†). This indicates that our system can selectively detect PPI in the presence of excess ATP by excimer formation. The lowest limit of the binding constant between **1-Zn** and PPI was estimated to be 10^5 .

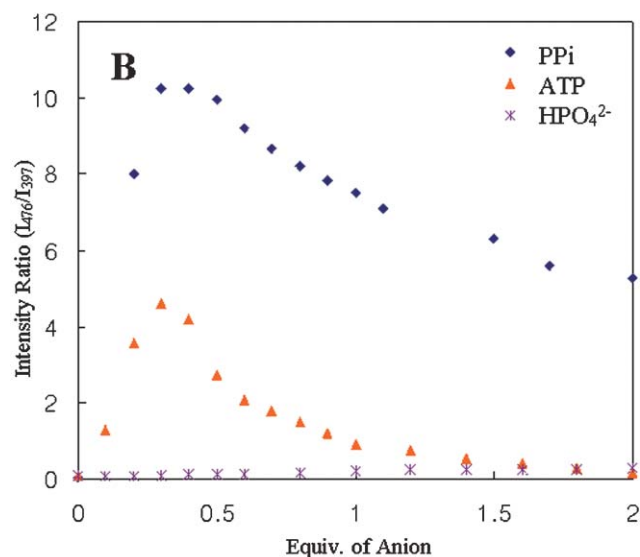
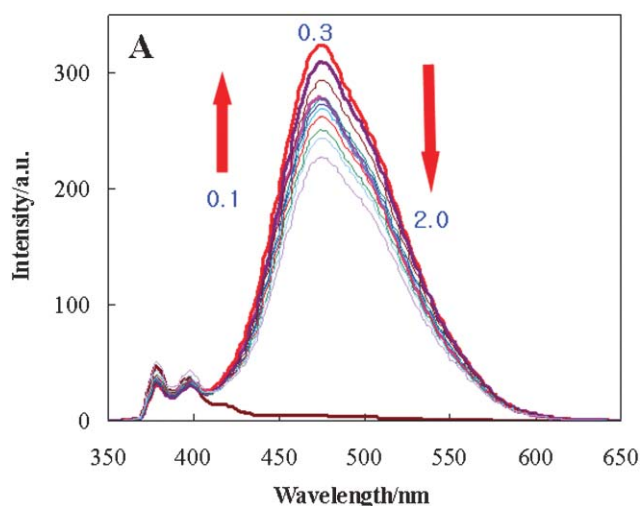


Fig. 3 (A) Fluorescence spectra of **1-Zn** upon addition of PPI (0.1 ~ 2.0 equiv). [**1-Zn**] = 0.02 mM in 10 mM HEPES buffer (pH = 7.4). Excitation wavelength = 343 nm. (B) Dependence of I_{476}/I_{397} on the concentration of PPI, ATP and HPO_4^{2-} .

As suggested by Job's plot, it is likely that **1-Zn** forms a 2 : 1 complex with PPI, as shown in Fig. 4, considering that the bis(Zn^{II} -dpa) unit is known to bind phosphates.⁵ The energy-minimized structure shows that a π - π stacked pyrene dimer is formed upon PPI complexation.

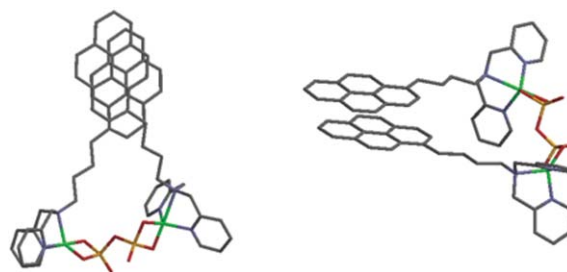


Fig. 4 Energy-minimized structure of the complex between **1-Zn** and PPI. Spartan '02 program (Wavefunction Inc.) was used.

The selectivity for PPI over ATP can be explained on the basis of the structure of the anion guest and the charge density of four O–P oxygen atoms of the guest involved in the complexation.^{3a} The total anionic charge density of the four O–P oxygen atoms involved in the complexation of ATP with two of 1-Zn is relatively smaller than that of the four O–P oxygen atoms of PPI. The binding affinity of ATP would presumably decrease, and therefore the degree of increase of excimer emission upon ATP binding would become smaller relative to that upon PPI binding.

In summary, we have developed a pyrene/Zn^{II}-dpa conjugate-based fluorescent sensor, which shows selective detection for PPI in an aqueous solution. This system shows excimer emission through self-assembly induced more selectively by PPI over other anions, including its strong competitor, ATP. Therefore, this system enables selective detection of PPI amongst other anions by complexation-induced excimer formation.

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- In the absorption and fluorescence experiments, all anions were used in the form of sodium salts.
- In the presence of HPO₄²⁻, the fluorescence spectrum of 1-Zn shows a very small increase of excimer emission intensity at 475 nm, which is negligible compared to those of PPI and ATP. For more detailed fluorescence spectra of 1-Zn and other anions, see ESI†.
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