

High-Affinity Pyrophosphate Receptor by a Synergistic Effect between Metal Coordination and Hydrogen Bonding in Water

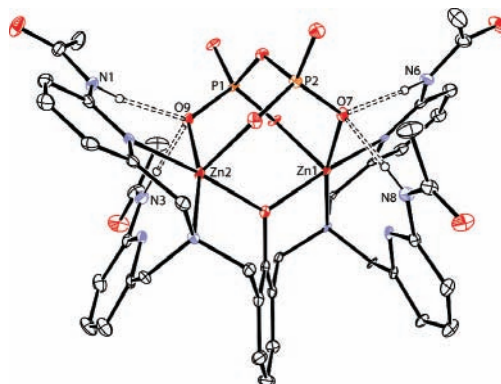
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ABSTRACT



We have developed the tightest binding PPI receptor reported to date by a combination of metal coordination and hydrogen bonding interaction in water.

Pyrophosphate (PPI) is involved in several important enzymatic reactions such as the adenylate cyclase catalyzed synthesis of cyclic AMP from ATP, the attachment of a given amino acid to a particular tRNA catalyzed by aminoacyl-tRNA synthetase in protein synthesis, and DNA replication catalyzed by DNA polymerase.¹ In addition, patients with calcium pyrophosphate dihydrate (CPPD) crystals and chondrocalcinosis have been shown to have high synovial fluid

PPI levels.² Therefore, selective and sensitive detection of PPI is a prerequisite for monitoring the above enzyme reactions and diagnostics of diseases related to the release of certain levels of PPI. We and others have reported on optical sensors for PPI.^{3,4} Even though PPI sensors previously reported show a high affinity and selectivity in aqueous solvent,³ in order to develop more effective PPI sensors that can operate in biological systems, the binding affinity of PPI sensors needs to be further improved because biological PPI ions exist at very low concentrations. Previous PPI sensors

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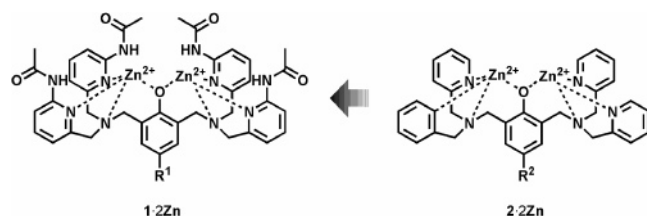
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developed in our group relied solely on a metal coordination effect.^{3e–g} The introduction of additional hydrogen-bonding sites to **2**·2Zn is expected to further increase its binding affinity to PPI through a synergy effect from metal coordination and hydrogen bonding.⁵ Herein, we report a new PPI receptor (**1**·2Zn) that exhibits better affinity while maintaining the same selectivity for PPI in aqueous solvent, compared to **2**·2Zn.

Scheme 1. Introduction of Acetamido Groups To Improve Binding Affinity to PPI (R¹ = H, R² = 2-naphthyl)



1 was prepared by treatment of bis(6-acetamido-2-pyridylmethyl)amine (Bapa) with 2,6-bis(bromomethyl)phenyl acetate in the presence of Cs₂CO₃ and KI followed by hydrolysis. **1**·2Zn was readily obtained by dissolving **1** and zinc nitrate in methanol. Pyrocatechol violet (PV) is known to coordinate the two metal ions in a phenoxo-bridged dinuclear metal complex.^{6,7} Furthermore, PV at a neutral pH turns from yellow to blue when it binds to a phenoxo-bridged dinuclear metal complex.⁸ Therefore, displacement of the weakly bound PV to **1**·2Zn by PPI will restore the yellow color of PV, which can be used for examining the characteristics of **1**·2Zn as a PPI sensor.^{6c,9}

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The characteristics of the sensing ensemble, **1**·2Zn·PV, were examined in an aqueous HEPES buffer solution (0.01 M, pH 7.4) (HEPES = 2-[4-(2-hydroxyethyl)-1-piperazinyl]-ethanesulfonic acid) at 25 °C (Figures 1 and 2). In the

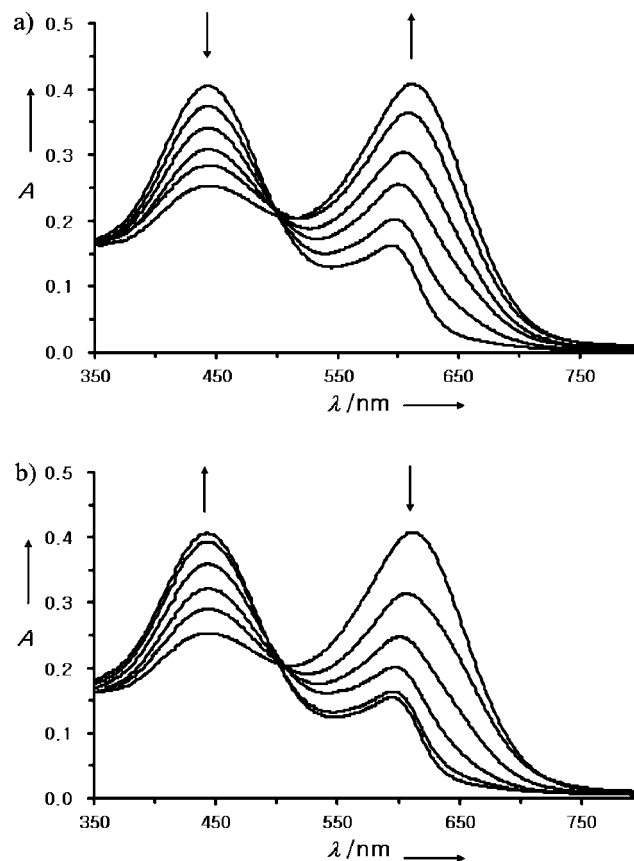


Figure 1. (a) UV/vis spectra obtained by addition of **1**·2Zn solution (final concentrations: 0, 4, 8, 12, 16, and 20 μM) to an aqueous buffer solution (10 mM HEPES, pH 7.4) containing PV (20 μM); (b) UV/vis spectra obtained by addition of PPI solution (final concentrations: 0, 4, 8, 16, 30, 60 μM) to an aqueous solution of 10 mM HEPES buffer (pH 7.4) containing **1**·2Zn·PV (20 μM).

absence of **1**·2Zn, the absorption spectrum of PV is characterized by an intense band centered at 444 nm. Upon addition of **1**·2Zn in increasing amounts, the peak at 444 nm decreased and the peak at 624 nm increased (Figure 1a). As expected, PPI addition to the aqueous solution of **1**·2Zn·PV caused changes in the UV/vis absorption spectra; the peak at λ_{max} = 624 nm decreased while the peak at 444 nm increased (Figure 1b).

The effect of anions (sodium salts) on the absorption spectrum of **1**·2Zn·PV was examined in an aqueous solution of HEPES buffer (0.01 M, pH 7.4) at 25 °C (Figure 2). **1**·2Zn·PV exhibited a color change from blue to yellow upon adding PPI ions while other anions including PO₄^{3−} and

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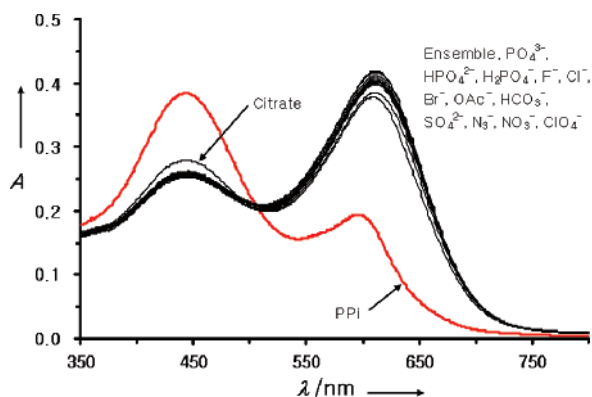


Figure 2. UV/vis spectra of **1**·2Zn·PV (20 μ M, pH 7.4 in 10 mM HEPES buffer) in the presence of various anions (20 μ M).

citrate ions showed no color changes.¹⁰ These results suggest that **1**·2Zn has a high selectivity for PPI over other anions. Job's plots for the binding between **1**·2Zn and PV, and between **1**·2Zn and PPI show a 1:1 stoichiometry, respectively.¹⁰

The association constant for binding of PV to **1**·2Zn was determined to be $1.4(\pm 0.1) \times 10^5 \text{ M}^{-1}$ by UV/vis titration.¹⁰ The association constant between **1**·2Zn and PPI was estimated to be $5.39(\pm 0.03) \times 10^{10} \text{ M}^{-1}$ in an aqueous solvent of 10 mM HEPES buffer (pH 7.4) at 25 °C by using an algorithm for competitive binding between **1**·2Zn and **2**·2Zn in which **2**·2Zn binds PPI and then is displaced by **1**·2Zn. It shows the direct comparison of **1**·2Zn with **2**·2Zn and the possibility of assay at the level of subnanomolar concentrations.

The four amide NH's in **1**·2Zn are expected to stabilize the bound PPI and increase binding affinity more than 100 times. K_d is about 20 pM. It is generally thought that a few hydrogen bonds in water cannot create any significant effects, because competing water molecules can easily hydrogen bond with host and guest, and therefore, host–guest binding is weakened.¹¹ For this reason, it is remarkable that the introduction of a few hydrogen-bonding sites causes an increase in binding affinity of more than 2 orders of magnitude in water. It turns out that this molecule represents the tightest binding PPI receptor reported to date.

The binding mode for PPI·**1**·2Zn is illustrated in Figure 3, which was unambiguously elucidated by an X-ray analysis.¹² As expected, the X-ray structure of the complex reveals that the two phosphoryl oxygen anions (O7 and O9, Figure 3) bridging the two Zn(II) ions are hydrogen bonded to the four amino groups of **1**. The four hydrogen bond distances (N–O) range between 2.73 and 2.82 Å and the hydrogen bond angles range between 163° and 170°, which

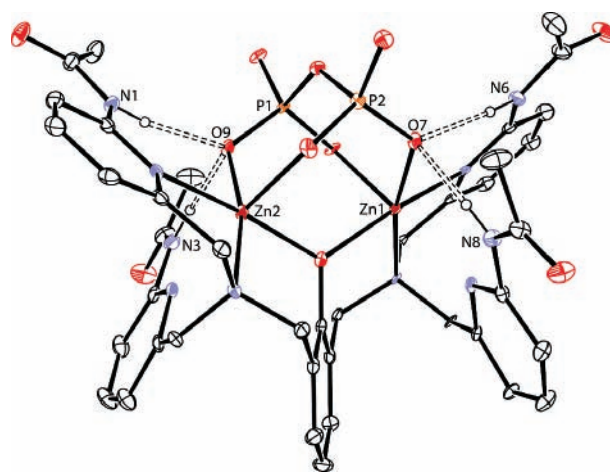


Figure 3. X-ray crystal structure of PPI·**1**·2Zn. All the hydrogen atoms except amide NH's are omitted for clarity.

are well within the range of normal hydrogen bond distances and angles. And it also confirms that the metal coordination interactions in both PPI·**1**·2Zn and PPI·**2**·2Zn have almost the same geometry.^{3c}

In summary, we have developed the tightest binding PPI receptor (**1**·2Zn) reported to date and a new sensing ensemble (**1**·2Zn·PV) that exhibits a selective coloration for PPI and high affinity in an aqueous solution. The improved binding affinity is accomplished by introducing four amide hydrogen bond donors that are rigidly pre-organized to interact with PPI coordinated to the two Zn(II) ions. This study shows that a combination of metal coordination and hydrogen bonding interactions can provide substantial synergistic effects contributing to molecular recognition in water. We have also devised a new method of binding assay that could be used to examine the binding event of new biological receptors.

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Supporting Information Available: Experimental procedure and selected spectral data for compounds **1** and **1**·2Zn, binding constant determination data, and X-ray crystallography data. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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