The development of receptors and sensors for biologically important anions is emerging as a research area of great importance.\textsuperscript{1} Pyrophosphate anion (PPi), in particular, participates in several biodiversity and metabolic processes.\textsuperscript{2-3} Therefore, the detection of PPi has been the main focus of several research groups. While PPi analysis such as ion chromatography remains important, there is mounting incentive to find alternative means of analysis, including those based on the use of selective chemosensors.\textsuperscript{1b,4} Particularly useful would be systems that can recognize PPi in an aqueous solution and signal its presence via an optical signal. Until now, very few examples of optical sensors for PPi in aqueous solution have been reported.\textsuperscript{5a,b} In this communication, we present a new chromogenic PPi sensor based on an azophenol-Dpa (bis[2-pyridylmethyl]amine) system, which shows a high sensitivity and selectivity for PPi over other anions in aqueous solvent of a wide pH range.\textsuperscript{7}

A color-inducing p-nitrophenylazo group can be easily introduced on the para position of the phenolic moiety of 2,6-bis[2-pyridylmethyl]amino)methyl]phenol (H-bpp).\textsuperscript{8} Zn\textsuperscript{2+} complexation with bis[2-pyridylmethyl]amine (Dpa) motley generates an anion-binding site through the formation of a well-known phenoxo-bridged dinuclear metal complex (Scheme 1).\textsuperscript{9} Compound 1 was obtained in an overall yield of 38% from 2,6-dimethylphenol.\textsuperscript{10a} Sensor 1-2Zn, the dinuclear Zn\textsuperscript{2+} complex of compound 1, is easily formed by the addition of a methanolic solution of 1 to an aqueous solution of 2 equiv of Zn(NO\textsubscript{3})\textsubscript{2}.

First, the effect of anions (sodium salts) on the absorption spectrum of 1-2Zn (30 \textmu M) was examined in an aqueous solution of 10 mM HEPES buffer (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 417 nm.

Sensor 1-2Zn does not show any obvious spectral change upon addition of H\textsubscript{2}PO\textsubscript{4}\textsuperscript{−} as well as other monovalent anions such as CH\textsubscript{3}CO\textsubscript{2}−, F\textsuperscript{−}, HCO\textsubscript{3}−, Cl\textsuperscript{−} even up to an excess of 100 equiv. Moreover, no detectable spectral change is observed upon addition of dibasic anions HPO\textsubscript{4}\textsuperscript{2−} and citrate. However, the addition of P\textsubscript{2}O\textsubscript{7}\textsuperscript{4−} (PPi) causes bathochromic shifts from 417 nm (\lambda\textsubscript{max} = 465 nm). It is remarkable that the degree of absorption changes is no longer affected by the addition of more than 1 equiv of PPi. As expected from the UV--vis absorption data, color change occurs by the addition of PPi to the solution of 1-2Zn from yellow to red. Job’s plot for the binding between 1-2Zn and PPi shows a 1:1 stoichiometry (inset of Figure 1a).\textsuperscript{11} Even in the presence of 10 equiv of HPO\textsubscript{4}\textsuperscript{2−}, sensor 1-2Zn shows a similar detection ability for PPi. It is surprising that the apparent association constant (K\textsubscript{a}) was determined as (6.6 ± 1.2) × 10\textsuperscript{8} M\textsuperscript{−1} for PPi–1-2Zn by a standard algorithm for competitive binding in the presence of excess HPO\textsubscript{4}\textsuperscript{2−} in a pure aqueous solvent of 10 mM HEPES buffer (pH 7.4) at 25 °C.\textsuperscript{11} These results suggest that sensor 1-2Zn has high selectivity for PPi over other anions.

Similar results are obtained in an aqueous solvent of 100 mM HEPES buffer (pH 7.4) at 25 °C. The addition of PPi also makes a color change from yellow (\lambda\textsubscript{max} = 417 nm) to red (\lambda\textsubscript{max} = 463 nm). In 100 mM HEPES buffer, sensor 1-2Zn shows the reduced affinity for PPi (K\textsubscript{a} = (8.3 ± 1.8) × 10\textsuperscript{4} M\textsuperscript{−1}), compared with 10 mM HEPES buffer system.

The novel binding mode for PPi–1-2Zn is illustrated in Figure 2, which was unambiguously elucidated by an X-ray analysis. The X-ray structure of the complex reveals that the two sets of oxygen anions on each P of PPi bind to the dinuclear zinc complex by bridging the two metal ions to give rise to the two hexacoordinated Zn\textsuperscript{2+} ions in 1-2Zn.\textsuperscript{12a} The binding mode for HPO\textsubscript{4}\textsuperscript{2−}–1-2Zn should be the same as that of HPO\textsubscript{4}\textsuperscript{2−}–H-bpp.\textsuperscript{9c,13} Despite this, HPO\textsubscript{4}\textsuperscript{2−} does not make a large UV--vis absorption change upon complexation with 1-2Zn. Instead, only PPi induces the selective red-shift of \lambda\textsubscript{max} of 1-2Zn because weakening the bond between p-nitrophenylazo phenolate oxygen and Zn\textsuperscript{2+} induces more negative charge character on the phenolate oxygen and thus the bathochromic shift of \lambda\textsubscript{max} of 1-2Zn occurs. As revealed by previous works,\textsuperscript{9c,13} HPO\textsubscript{4}\textsuperscript{2−} does not coordinate in tetradentate fashion as PPi does. This explains why HPO\textsubscript{4}\textsuperscript{2−} does not alter \lambda\textsubscript{max} of 1-2Zn. Stronger coordination of PPi to dinuclear zinc complex enables sensor 1-2Zn to show color changes and higher selectivity over HPO\textsubscript{4}\textsuperscript{2−} (Figure 3). Hexacoordination of Zn\textsuperscript{2+} ions is clearly reflected in the extremely high K\textsubscript{a} of PPi–1-2Zn in water (K\textsubscript{a} = 6.6 × 10\textsuperscript{8} M\textsuperscript{−1}). It is worthwhile noting that PPi binds 1-2Zn over 10\textsuperscript{2}-fold more tightly than HPO\textsubscript{4}\textsuperscript{2−} does.\textsuperscript{9e}

A control sensor, mononuclear 2Zn does not show \lambda\textsubscript{max} and color changes upon the addition of PPi. This result means that the cooperative action of two Zn\textsuperscript{2+}–Dpa is needed for the selective sensing of PPi.\textsuperscript{14} Finally, to check the working pH, the effect of the pH value of the medium on the PPi sensing was checked. UV--vis absorption changes shown in Figure 1b occur in the wide pH range of 6.5–8.3 with a similar tendency.\textsuperscript{10b} This result shows that even if the external pH is disturbed, sensor 1-2Zn can still detect PPi.

In summary, we have developed a new azophenol-based colorimetric sensor, which shows a selective coloration for PPi with...
high affinity in aqueous solution in a wide pH range. This system shows good selectivity for PPI even in the presence of a strong competitor such as HPO$_4^{2-}$. In addition, the novel binding mode for PPI–1·2Zn was unambiguously confirmed by an X-ray analysis. For biochemical and analytical applications, work is directed toward the development of fluorescent sensors capable of detecting PPI at lower concentrations.

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Supporting Information Available: Experimental procedure and selected spectral data for compounds 1, 2, 1·2Zn, and 2·Zn. UV–vis absorption data (PDF). X-ray crystallography data (CIF). This material is available free of charge via the Internet at http://pubs.acs.org.