

Chemosensors for Pyrophosphate

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CON SPECTUS

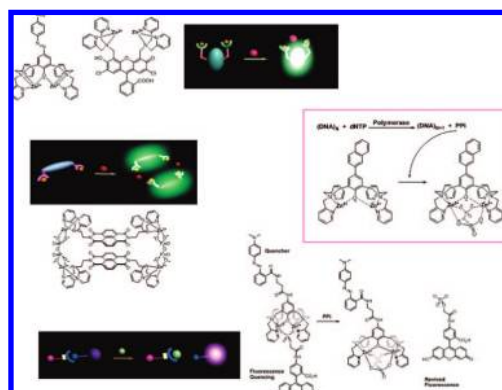
The selective detection of the anion pyrophosphate (PPI) is a major research focus. PPI is a biologically important target because it is the product of ATP hydrolysis under cellular conditions, and because it is involved in DNA replication catalyzed by DNA polymerase, its detection is being investigated as a real-time DNA sequencing method. In addition, within the past decade, the ability to detect PPI has become important in cancer research.

In general, the sensing of anions in aqueous solution requires a strong affinity for anions in water as well as the ability to convert anion recognition into a fluorescent or colorimetric signal. Among the variety of methods for detecting PPI, fluorescent chemosensors and colorimetric sensors for PPI have attracted considerable attention during the past 10 years. Compared with the recognition of metal ions, it is much more challenging to selectively recognize anions in an aqueous system due to the strong hydration effects of anions. Consequently, the design of PPI sensors requires the following: an understanding of the molecular recognition between PPI and the binding sites, the desired solubility in aqueous solutions, the communicating and signaling mechanism, and most importantly, selectivity for PPI over other anions such as AMP and ADP, and particularly phosphate and ATP.

This Account classifies chemosensors for PPI according to topological and structural characteristics. Types of chemosensors investigated and reported in this study include those that contain metal ion complexes, metal complexes combined with excimers, those that function with a displacement approach, and those based on hydrogen-bonding interaction.

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Thus far, the utilization of a metal ion complex as a binding site for PPI has been the most successful strategy. The strong binding affinity between metal ions and PPI allows the detection of PPI in a 100% aqueous solution. We have demonstrated that carefully designed receptors can distinguish between PPI and ATP based on their different total anionic charge densities. We have also demonstrated that a PPI metal ion complex sensor has a bioanalytical application. This sensor can be used in a simple and quick, one-step, homogeneous phase detection method in order to confirm DNA amplification after polymerase chain reaction (PCR).



Introduction

A fluorescent or colorimetric chemosensor is defined as a compound of abiotic origin that complexes to an analyte with concomitant fluorescent or colorimetric signal transduction.

Generally, there are three different approaches to designing chemosensors (Figure 1). The most popular approach involves covalently introducing

binding sites and signaling subunits to the chemosensors. On the other hand, a coordination complex (called a displacement approach) can be used, where the introduction of anions revives the noncoordinated spectroscopic behavior of the indicator. In both approaches, the change in fluorescence or color is reversible in principle. However, a chemodosimeter approach involves the use of

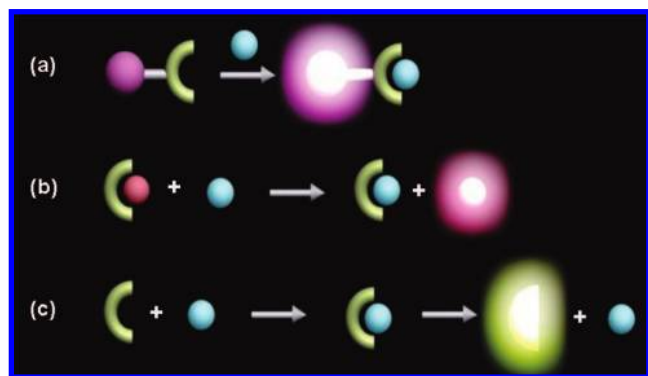


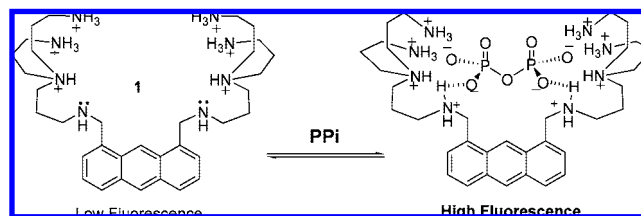
FIGURE 1. Three different approaches for chemosensors: (a) chemosensor bearing a signaling subunit as well as a binding site; (b) displacement approach; (c) chemodosimeter.

specific chemical reactions upon binding with anions, which is usually irreversible.

Anions play a fundamental role in a wide range of chemical and biological processes, and considerable effort has been devoted to the development of abiotic receptors for anionic species.¹ Anion-selective fluorescent sensors^{2,3} and colorimetric sensors² have attracted increasing attention. In particular, pyrophosphate (PPI) is a biologically important target because it is the product of ATP hydrolysis under cellular conditions,⁴ and the detection of pyrophosphate is being investigated as a real-time DNA sequencing method.⁵ Recently, the detection of PPI has become an important issue in cancer research.⁶ Patients with calcium pyrophosphate dehydrate (CPPD) crystals and chondrocalcinosis have also been shown to have a high synovial fluid PPI level.⁷ In this regard, the detection and discrimination of PPI has been the main focus of several studies over the last 10 years with particular attention being paid to fluorescent chemosensors for PPI.

In general, sensing anions in aqueous solution requires a strong affinity for anions in water as well as the ability to convert anion recognition into a fluorescent or colorimetric signal. Compared with the recognition of metal ions, it is much more challenging to selectively recognize anions in an aqueous system due to the strong hydration effects of anions. Consequently, the design of PPI sensors also requires an understanding of the molecular recognition between PPI and the binding sites, the desired solubility in an aqueous solution, the communicating and signaling mechanism, and most importantly, the selectivity for PPI over other anions, particularly phosphate (Pi) or ATP. For applications in biological systems, PPI sensing normally requires selectivity in the presence of Pi, AMP, ADP, and ATP. Thus far, the utilization of a metal ion complex as a binding site for PPI has been found to be the most successful strategy because the strong binding affinity

SCHEME 1. Fluorescent Chemosensor **1** with PPI



between metal ions and PPI allows the detection of PPI in 100% aqueous solutions.

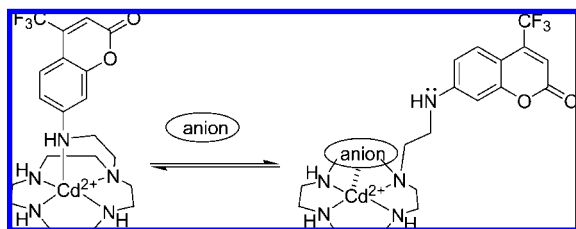
Receptors based on hydrogen-bonding interactions usually have several disadvantages. For example, sensing PPI in aqueous solution based on hydrogen-bonding interactions is quite challenging and fluoride, acetate, or biscalboxylate anions compete strongly with strong hydrogen bonding in this system.

In this regard, metal ion complexes are ideal binding sites for PPI recognition in aqueous solutions. As shown in the text, the discrimination of PPI from Pi is somewhat less challenging than the selective binding of PPI in the presence of ATP. However, it was demonstrated that carefully designed receptors can distinguish between PPI and ATP based on their different total anionic charge densities. This chemosensor was also used as a simple and rapid alternative for assessing the outcome of PCR. This Account classifies the chemosensors for PPI according to their topological and structural classification, which includes chemosensors containing metal ion complexes, the utilization of metal complexes as well as excimers, displacement approaches, and receptors based on hydrogen-bonding interaction.

In 1994, Czarnik et al. reported their pioneering work in which an anthracene derivative bearing polyamine groups (**1**, Scheme 1) was used as a PPI sensor in a 100% aqueous solution.⁸ The chemosensor **1** binds pyrophosphate with fluorescence enhancement, and 1:1 complexation occurred with $K_d = 2.9 \mu\text{M}$ at pH 7 (0.05 M HEPES). This chemosensor shows a 2200-fold increase in pyrophosphate/phosphate discrimination. A real-time assay of pyrophosphate hydrolysis catalyzed by inorganic pyrophosphatase was also reported using this fluorescent chemosensor.

Chemosensors Using Metal Ion Complexes

Kikuchi et al. used the Cd^{2+} –cyclen–coumarin system as a fluorescent chemosensor (**2**) for PPI in an aqueous solution.⁹ As shown in Scheme 2, Cd^{2+} in complex **2**· Cd^{2+} is coordinated by the four nitrogen atoms of cyclen and an aromatic amino group. The aromatic amino group of coumarin is displaced when various anions are added to a buffer solution

SCHEME 2. Design Concept of Sensing Anions with **2**·Cd²⁺

(0.1 M HEPES, pH 7.4) containing complex **2**·Cd²⁺, which causes a change in the excitation spectrum. Among the various anions examined, this system showed tight binding with citrate ($K_d = 9.0 \times 10^{-5}$ M) and PPI ($K_d = 7.5 \times 10^{-5}$ M).

Among the metal ion complex based sensors, Zn²⁺ complexes with a bis(2-pyridylmethyl)amine (DPA) unit have attracted considerable attention. Recently, Hong et al. reported an azophenol-based colorimetric sensor **3**·2Zn²⁺ containing two Zn²⁺–DPA units (**3**), which can selectively recognize PPI ($K_a = 6.6 \times 10^8$ M⁻¹) among the various anions in water (Figure 2).¹⁰ This particular sensor can bind PPI approximately 1000 times more tightly than Pi and can be used over a wide range of pH (6.5–8.3). The X-ray structure of this complex showed that 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 two hexacoordinated Zn²⁺ ions. As shown in Figure 2b, complex **3**·2Zn²⁺ shows a selective color change only with PPI. This bathochromic shift can be attributed to a weakening of the bond between *p*-nitrophenylazo phenolate oxygen and Zn²⁺ upon the addition of PPI. These results suggest that the azophenol-based chemosensor containing two Zn²⁺–DPA units can be a promising candidate for sensing PPI in aqueous systems. In addition, compared with Kikuchi's receptor, citrate did not cause any significant changes.

Hong et al. extended this DPA–2Zn²⁺–phenoxide system to a fluorescent chemosensor for the detection of PPI. A naphthalene derivative **4**·2Zn²⁺ exhibited a selective fluorescence change upon the addition of PPI, as shown in Figure 3.¹¹ The λ_{max} shifted from 436 to 456 nm, with 9.5-fold fluorescence enhancement upon the addition of 1 equiv of PPI at pH 7.4. The association constant was calculated to be 2.9×10^8 M⁻¹, which means that complex **4**·2Zn²⁺ can detect PPI in water at nanomolar concentrations. It turns out that **3**·2Zn can detect less than 1 equiv of PPI even in the presence of a 50- to 250-fold excess of ATP (based on the amount of PPI detected). This is the first example of a complex that can discriminate PPI from ATP in aqueous solution. The selectivity for PPI over ATP can be understood as follows: The total anionic charge density of the four O–P oxygen atoms involved in the complexation of ATP with complex **4**·2Zn²⁺

is smaller than that of the four O–P oxygen atoms of PPI. This reduces the binding affinity of ATP significantly.

Based on our previous two examples, DPA–2Zn²⁺–phenoxide has been demonstrated to be a good system for the selective recognition of PPI and is probably the best system reported thus far. In order to improve the binding affinity and selectivity toward PPI, Hong et al. examined the synergistic effect of metal coordination and hydrogen bonding (Figure 4).¹² Compound **5**·2Zn²⁺ was synthesized as a highly selective colorimetric sensor for PPI in water. Improved binding affinity was achieved by introducing four amide hydrogen bond donors that are rigidly preorganized to interact with the PPI coordinated to the two Zn²⁺ ions. The association constant and K_d for PPI was estimated to be 5.39×10^{10} M⁻¹ and approximately 20 pM, respectively. This system is the strongest binding PPI receptor in water. Figure 4 shows the X-ray crystal structure of complex **5**·2Zn²⁺–PPI, in which additional H-bonds by the amide groups are clearly shown. This approach clearly shows that careful design of the receptor for anions based on molecular recognition chemistry certainly has its merits for applications to sensors.

Yoon et al. recently attached a DPA–2Zn²⁺ system to a fluorescein moiety. A complex **6**·2Zn²⁺ showed unique changes in the fluorescence emission and color only with PPI when HSO₄⁻, CH₃COO⁻, I⁻, Br⁻, Cl⁻, F⁻, Pi, and PPI were also added to the 100% aqueous solution (Figure 5).¹³ Upon the addition of PPI, the emission maximum of complex **6**·2Zn²⁺ shifted gradually from 523 to 534 nm, and chelation enhanced fluorescence (CHEF) effects (~150%) were observed (Figure 5). The UV absorption spectra of complex **6**·2Zn²⁺ also showed a similar bathochromic shift (~13 nm) after adding pyrophosphate. From fluorescence titrations, the association constant of complex **6**·2Zn²⁺ was observed to be 9.8×10^4 M⁻¹. However, when excess Pi was added to the **6**·2Zn²⁺ complex, there was almost no change in both the λ_{max} and fluorescence intensity. Complex **6**·2Zn²⁺ certainly has important advantages over the pyrophosphate-selective fluorescent chemosensors reported thus far. Both the emission and excitation wavelengths are suitable for biological applications. In addition, all the fluorescence changes can be monitored in a 100% aqueous solution at pH 7.4. Most importantly, **6**·2Zn²⁺ is a ratiometric fluorescent sensor.

Yoon et al. reported a new acridine–DPA–Zn²⁺ complex (**7**·2Zn²⁺) that exhibits different signal responses to pyrophosphate and phosphate in water.¹⁴ Figure 6 shows the crystal structure of the cation of compound **7**. Among the various anions examined, complex **7**·2Zn²⁺ had a selective CHEF effect with Pi and a selective CHEQ (chelation-enhanced fluo-

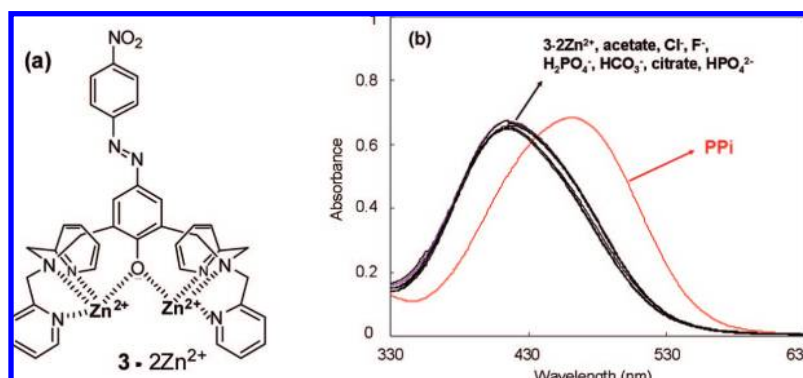


FIGURE 2. (a) Structure of $3 \cdot 2Zn^{2+}$ and (b) the color changes of $3 \cdot 2Zn^{2+}$ ($30 \mu M$) in 10 mM HEPES buffer (pH 7.4) upon the addition of various anions ($30 \mu M$).

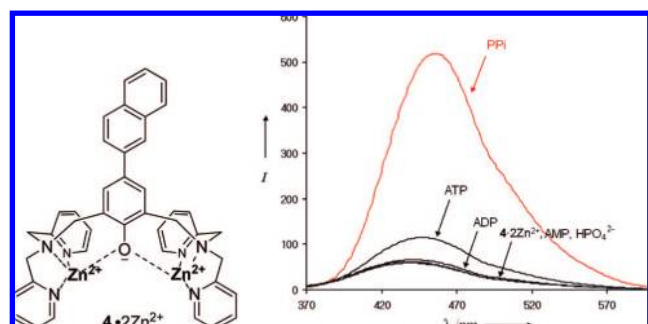


FIGURE 3. Structure of $4 \cdot 2Zn^{2+}$ and fluorescence changes of $4 \cdot 2Zn^{2+}$ ($6 \mu M$) upon the addition of various anions ($8 \mu M$) in HEPES buffer (10 mM, pH 7.4).

rescence quenching) effect with PPI in a 100% aqueous solution (Figure 6). From fluorescent titrations, the association constants for PPI and Pi were calculated to be 4.85×10^7 and $9.36 \times 10^4 M^{-1}$, respectively. The large CHEF effect with Pi was attributed to the additional hydrogen bonding between the nitrogen on the acridine and the hydrogen of Pi.

Sensing PPI Utilizing Metal Complexes and Excimers

The most interesting feature of pyrene derivatives is their capacity to form excimers and ability to impart dual monomer–excimer fluorescence. From previous studies, it was found that excimer formation may be induced upon the binding of the DPA– Zn^{2+} system with PPI because PPI needs to bind two Zn^{2+} sites as shown in the X-ray crystal structures. Hong, et al. reported a DPA– Zn^{2+} –pyrene based fluorescent chemosensor $8 \cdot Zn^{2+}$, which exhibited a strong and selective excimer peak with PPI (Figure 7).¹⁵ The Job plot for the binding between chemosensor $8 \cdot Zn^{2+}$ and PPI suggested a 2:1 stoichiometry, as expected. While the excimer peak was barely visible in the presence of 10 equiv of ATP, adding increasing amounts of PPI (0–2.3 equiv) clearly showed the

formation of excimer peaks. Hence, this system can selectively detect PPI in the presence of excess ATP through excimer formation.

Yoon et al. recently extended this concept to naphthaldimide system $9 \cdot 2Zn^{2+}$ as a highly selective fluorescent chemosensor for PPI, which can function in a 100% aqueous solution (Figure 8).¹⁶ This sensor shows a unique excimer peak at 490 nm only in the presence of PPI. Four zinc sites as well as a π – π interaction induced the unique 2 + 2 type excimer in the presence of PPI, as shown in Figure 8. This 2 + 2 type excimer formation is supported by the ESI data and unique excimer fluorescence peak. Furthermore, the detection of PPI is selective over ATP or Pi. The association constant for PPI was reported to be $4.1 \times 10^5 M^{-1}$.

Displacement Approach

There are some metal ion complex systems that lack an available fluorophore, and fluorescence competition assays are used to detect PPI in water. The Fabbrizzi group used an azacrown– Cu^{2+} complex ($10 \cdot 2Cu^{2+}$) and fluorescent dye as the so-called “chemosensing ensemble (CE)” approach (Figure 9).¹⁷ In this metal-containing CE system, efficient quenching is provided by Cu^{2+} ions, which is suppressed when the indicator is released into the solution with full revival of the fluorescence. From the competition assay, the $\log K_s$ values for PPI and Pi using coumarin 343 (**11**) at pH 7 were 7.2 and 4.4, respectively.

The Zn^{2+} –DPA moiety was also used by Jolliffe et al., in which two Zn^{2+} –DPA units were introduced onto a cyclic peptide, as shown in Figure 10.¹⁸ Ensemble $12 \cdot 2Zn^{2+}$ detected PPI ($\log K_{assoc} = 8.0$) in water with a selectivity 2 orders of magnitude greater than ADP and ATP. Coumarin **13** was used as an indicator for the binding study.

Hong et al. recently used a new system bearing a quencher–receptor conjugate (Q–R) **14** and a fluorophore–

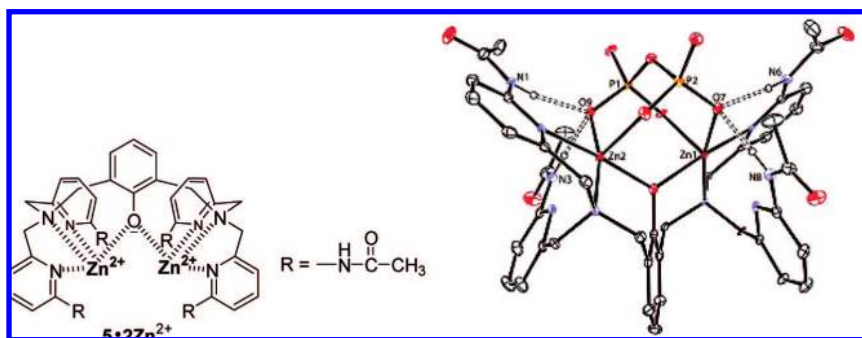


FIGURE 4. The structure of compound $5 \cdot 2\text{Zn}^{2+}$ and the crystal structure of $5 \cdot 2\text{Zn}^{2+}$ -PPI.

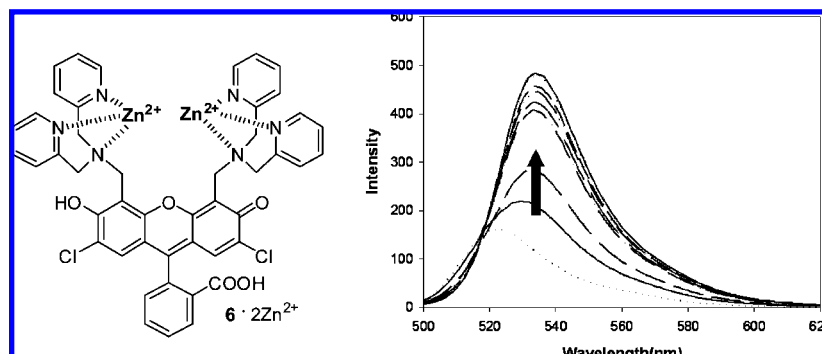


FIGURE 5. Structure of $6 \cdot 2\text{Zn}^{2+}$ complex and fluorescent titrations of compound $6 \cdot 2\text{Zn}^{2+}$ complex ($1 \mu\text{M}$) with PPI at pH 7.4 (20 mM HEPES; excitation at 517 nm).

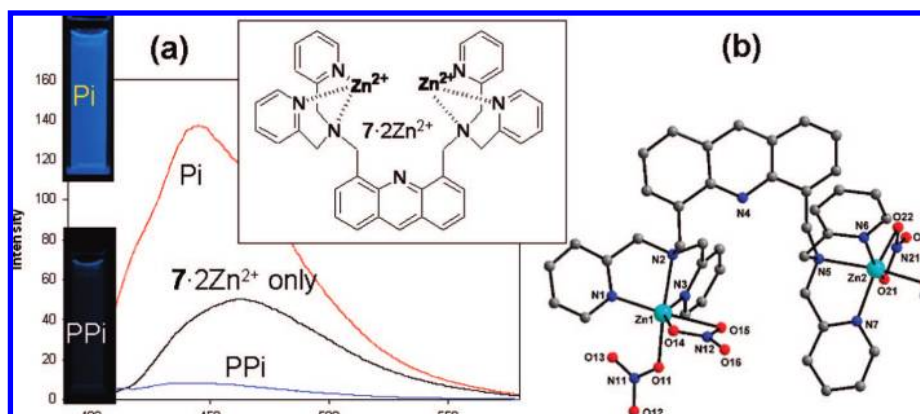


FIGURE 6. (a) Fluorescent changes of compound $7 \cdot 2\text{Zn}^{2+}$ ($3 \mu\text{M}$) upon the addition of Pi and PPI at pH 7.4 (10 mM HEPES) and (b) the crystal structure of the cation of $7 \cdot 2\text{Zn}^{2+}$.

substrate conjugate (F-S) **15** as the on-off switching fluorescent sensor for PPI,¹⁹ as shown in Scheme 3. An increase in the PPI concentration of up to 3 equiv. relative to the Q-R concentration resulted in a 45-fold increase in fluorescence. The selectivity trend of Q-R **14** was $\text{PPI} > \text{ATP} > \text{ADP} > \text{AMP} \sim \text{Pi} > \text{CH}_3\text{CO}_2^- \sim \text{F}^-$.

Receptors Based on Hydrogen-Bonding Interaction

Receptors based on the hydrogen-bonding interaction certainly have drawbacks compared with the metal complexes. Recognition in aqueous solution is quite rare in hydrogen-

bonding systems. Furthermore, fluoride, acetate, or biscalboxylate ions are serious competitors in most cases. However, from the molecular recognition point of view, important results have been reported by many groups.

For example, Teramae et al. reported a pyrene-functionalized guanidinium receptor as a fluorescent chemosensor for PPI in methanol.²⁰ This particular system uses a complexation-induced self-assembly approach for the detection of PPI. An intramolecular excimer peak was observed in the presence of PPI, which was attributed to the PPI complex. Sessler et al. reported fluorescent calixpyrrole receptors bearing thiourea groups that show high affinity toward PPI and fluoride anions

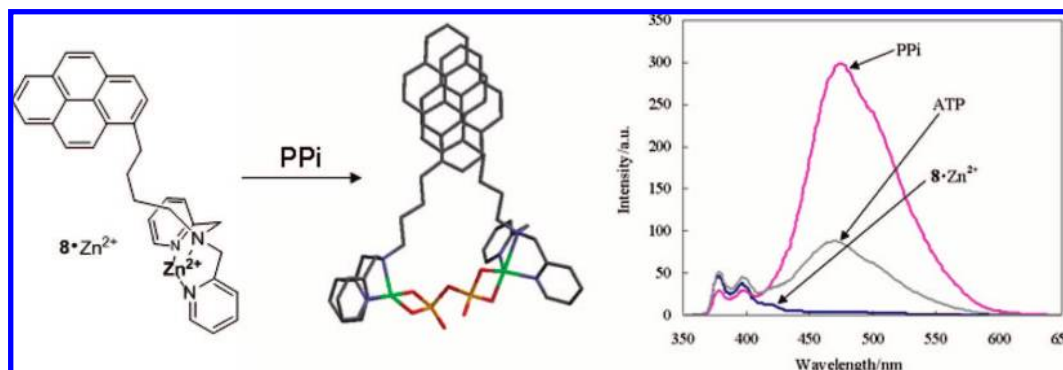


FIGURE 7. Energy-minimized structure of $8 \cdot \text{Zn}^{2+} - \text{PPI}$ (Spartan '02 program, Wavefunction Inc.) and fluorescent changes of $8 \cdot \text{Zn}^{2+}$ (0.02 mM) upon the addition of PPI and ATP (0.4 equiv) in HEPES buffer (10 mM, pH 7.4).

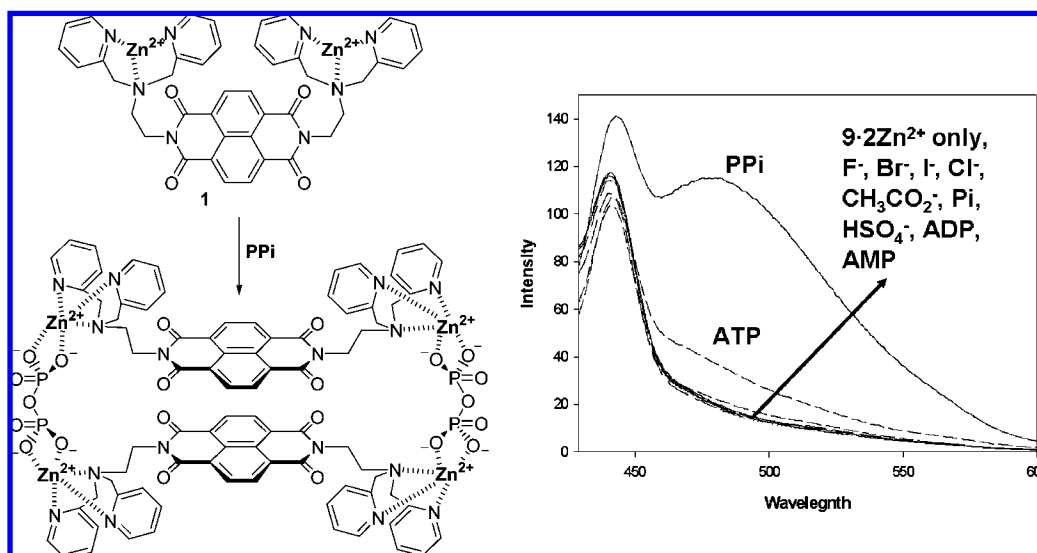


FIGURE 8. Proposed binding mechanism of chemosensors $9 \cdot 2\text{Zn}^{2+}$ with PPI and fluorescent changes of $9 \cdot 2\text{Zn}^{2+}$ (6 μM) upon the addition of various anions (10 equiv) in HEPES buffer (10 mM, pH 7.4).

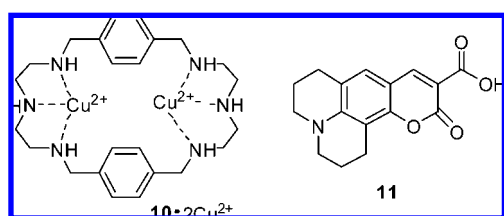


FIGURE 9. The structure of $10 \cdot 2\text{Cu}^{2+}$ and fluorescent indicator 11.

in acetonitrile.²¹ In this case, PPI can make multiple hydrogen bonds with both calixpyrrole and the thiourea moiety. Dipyrrolyl quinoxalines have been actively examined by the Anzenbacher group as chromogenic sensors or fluorescent sensors for PPI.²² These results have been applied to multi-well assays using polyurethane-embedded sensors bearing dipyrrolyl quinoxaline moieties, which allowed the colorimetric screening of aqueous anion solutions. These chromogenic conductive polymers exhibited reversible anion-specific changes both in color and in conductivity after increasing the concentration of anions such as PPI and fluoride ions.^{22a}

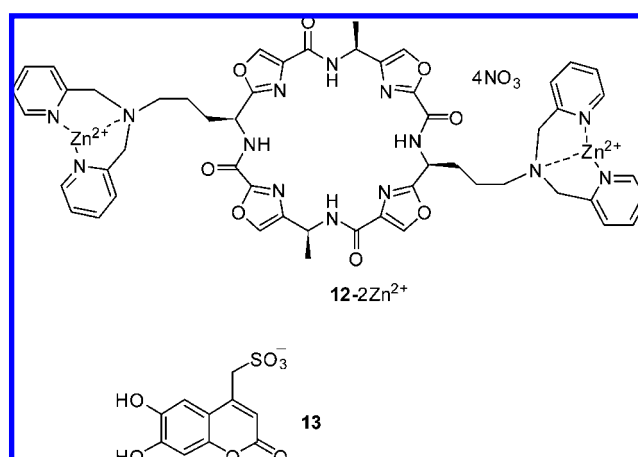
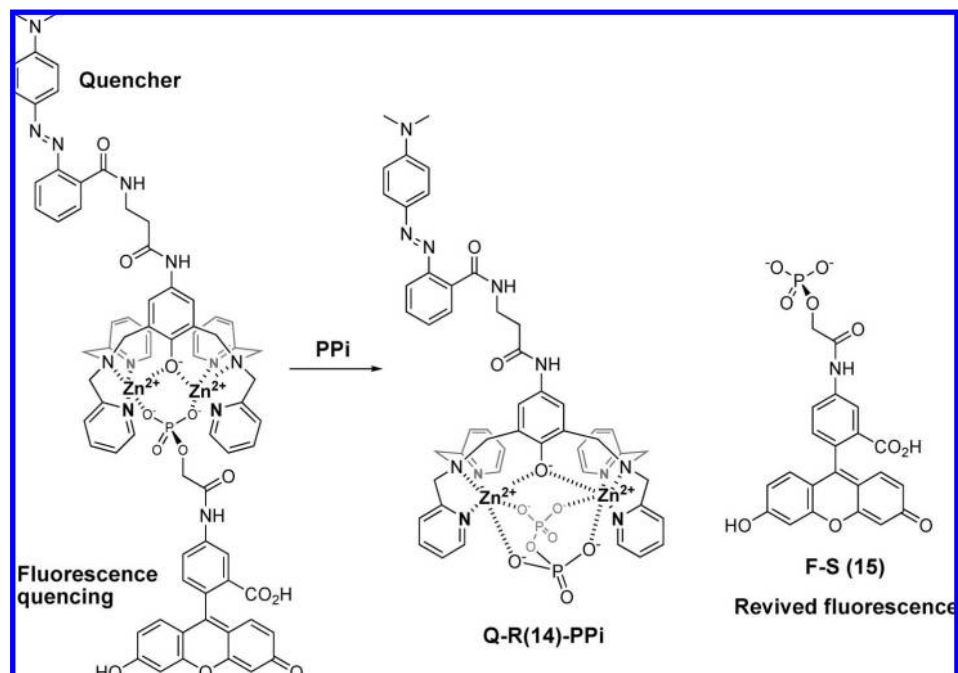


FIGURE 10. The structure of $12 \cdot 2\text{Zn}^{2+}$ and coumarin indicator 13.

In addition to the well-known type of hydrogen bonding for the anion binding, such as amide, pyrrole, and urea, various types of receptors containing imidazolium moieties have been used as anion chemosensors.^{1b,23} These hosts can produce strong and unique $(\text{C}-\text{H})^+ - \text{X}^-$ hydrogen bonding between

SCHEME 3. Chemical Structures of a Quencher–Receptor Conjugate (Q–R) **14** and a Fluorophore–Substrate Conjugate (F–S) **15** Used in an Ensemble Approach



the imidazolium moieties and various anions.²³ Yoon and Kim et al. recently reported four different fluorescent imidazolium systems (**16**–**19**) as fluorescent chemosensors for PPI (Figure 11).²⁴ Among the various anions, such as HSO_4^- , CH_3CO_2^- , I^- , Br^- , Cl^- , F^- , H_2PO_4^- , and PPI, compounds **16**–**19** showed the highest binding affinity with PPI in acetonitrile. The fluorescent quenching effect upon the addition of PPI was explained by a fluorescent photoinduced electron transfer (PET) mechanism. The association constants of compounds **16**–**19** with PPI were calculated to be 5.43×10^6 ,

$\sim 1.01 \times 10^8$, 3.58×10^6 , and $6.76 \times 10^6 \text{ M}^{-1}$, respectively. Among the series of hosts examined, dimer host **17** showed the largest binding constant with PPI, which suggests that a preorganized rigid binding pocket might play an important role in the binding with PPI.

Application

Hong et al. recently reported an example of the bioanalytical application of a PPI sensor, in which a simple and quick, one-step, homogeneous phase detection method was used to confirm DNA amplification after polymerase chain reactions (PCR).²⁵ The basis of this new method is to detect the PPI released from the dNTPs, which occurs stoichiometrically when DNA is synthesized by the action of DNA polymerase (Figure 12). As an example, complex $\mathbf{4} \cdot 2\text{Zn}^{2+}$ was used for this purpose, because $\mathbf{4} \cdot 2\text{Zn}^{2+}$ can detect a small amount of PPI in the presence of a large excess of ATP.¹¹ Figure 12 shows the results of gel electrophoresis on the finished PCR mixture and fluorescence intensity at 464 nm of sensor $\mathbf{4} \cdot 2\text{Zn}^{2+}$ after adding the finished PCR mixture. The change in signal observed in each experiment in lanes B, C, D, and E was almost linear (Figure 13). This confirms the hypothesis that the extent of the fluorescence changes in sensor $\mathbf{4} \cdot 2\text{Zn}^{2+}$ is proportional to not only the amount of PPI generated from PCR but also the DNA amplified. This method might be a simple and rapid alternative for assessing the outcome of PCR.

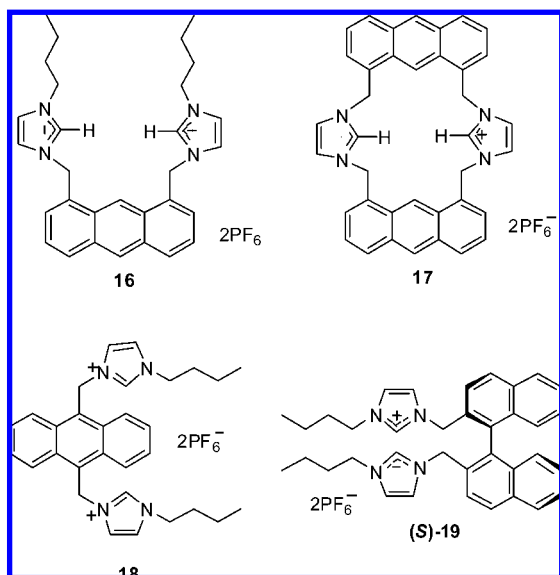


FIGURE 11. Structures of fluorescent imidazolium receptors **16**–**19**.

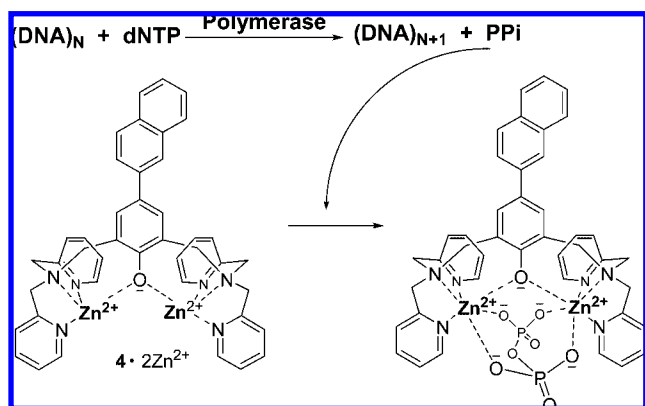


FIGURE 12. Detection method for DNA amplification in PCR.

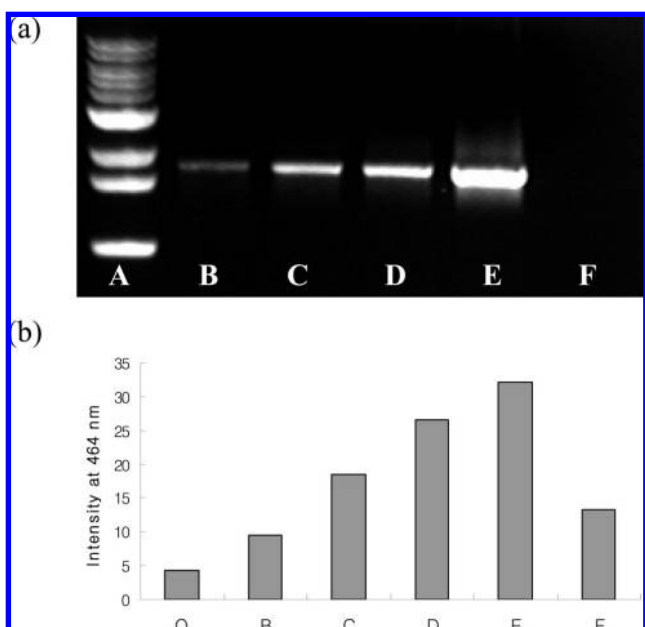


FIGURE 13. (a) Gel electrophoresis of finished PCR mixtures and (b) fluorescence intensity of sensor $4 \cdot 2\text{Zn}^{2+}$ ($5 \mu\text{M}$) at 464 nm upon addition of the finished PCR product mixture in 10 mM HEPES buffer (pH 7.4) at 25 °C: (A) standard bp ladder, (O) 0 μL , (B) 1 μL , (C) 3 μL , (D) 5 μL , and (E) 9 μL of finished PCR product mixture performed with template DNA and (F) 9 μL of finished PCR product mixture performed without template DNA.

Conclusions

This Account concentrated on our work and some representative studies from other groups on the chemosensing of PPI. There are many reports on sensing PPI using organic artificial sensors, which require an understanding of molecular recognition as well as the mechanisms for the fluorescent changes and colorimetric changes. A careful design of the binding site as well as the communicating system will provide better systems for sensing PPI in the future.

We also presented the application of this approach as a simple and rapid alternative for assessing the outcome of PCR.

It is expected that these artificial sensors will be used to detect PPI in living cells or organs for a variety of purposes.

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BIOGRAPHICAL INFORMATION

Sook Kyung Kim was born in Busan in 1977. In 2000 and 2003, she received her B.S. and Master from Silla University. Currently she is working with Professor Juyoung Yoon at Ewha Womans University for her Ph.D. degree. For her thesis, she is working on investigating various fluorescent chemosensors for ions.

Dong Hoon Lee was born in Seoul, Korea (1975). He received his B.S. degree (1998) and Ph.D. (2004) from Seoul National University. He is currently a senior researcher at LG. He is now involved in the development of HTL materials for efficient OLEDs.

Jong-In Hong was born in Daegu, Korea (1959). He received his B.S. (1982) and M.S. (1984) degrees in Chemistry from Seoul National University and Ph.D. (1991) in Organic Chemistry from Columbia University. He completed postdoctoral research at MIT (1991–1992) and assumed a faculty position in the Department of Chemistry of Seoul National University where he is now a Professor. His research involves the fields of molecular recognition and supramolecular chemistry. A major focus of his study is on the development of optical and electrochemical sensors, self-assembled superstructures based on metal–ligand and hydrogen-bonding interactions, and organic materials for organic light-emitting diodes, organic thin film transistors, and organic solar cells.

Juyoung Yoon was born in Busan in 1964. He was graduated with a B.S. from Seoul National University (1987) and received his Ph.D. (1994) from The Ohio State University. After completing postdoctoral research at the University of California, Los Angeles (1994–1996), and at The Scripps Research Institute (1996–1998), he joined the faculty at Silla University in 1998. In 2002, he moved to Ewha Womans University, where he is currently an Associate Professor of Division of Nano Sciences and the Department of Chemistry. His research interests include investigations of fluorescent chemosensors, molecular recognition, organo-EL materials, and organic synthesis of biologically active compounds.

FOOTNOTES

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