DOI: 10.1002/asia.201100149

# Nanoparticle-based Indicator-Displacement Assay for Pyrophosphate

**Dong Ju Oh, Kyung Mi Kim, and Kyo Han Ahn*[a]**

*Dedicated to Professor Eun Lee on the occasion of his retirement and 65th birthday*

**Abstract:** Silica nanoparticles functionalized with a $Zn^{II}$-dipicolylamine derivative are used as the receptor component of a sensing ensemble for an indicator-displacement assay. The nano-ensemble system, constructed with pyrocatechol violet as an indicator, selectively senses pyrophosphate over other anions including hydrogen phosphate, thus showing a visible blue-to-yellow color change and the corresponding absorption spectral changes in a buffer solution of pH 7. A distinct recognition behavior—the nano-ensemble does not sense hydrogen phosphate that was sensed by the traditional ensemble with the same molecular receptor—is explained by an incomplete-displacement model in the case of the integrated receptor system. The present work demonstrates that the nanoparticle-based competition assay is as effective as the traditional approach with molecular receptors. Furthermore, such an integrated receptor system can provide distinct recognition behavior from its molecular receptor.

**Keywords:** indicator-displacement assay · nanoparticles · receptors · supramolecular chemistry · zinc

## Introduction

A typical chemical sensing system is composed of two key components, recognition and signaling parts (or receptor and indicator). Usually, the recognition site is covalently linked to the indicator in such a way that the supramolecular interactions between an analyte and the recognition site can generate signals, usually as absorption and emission changes. This binding site/signaling subunit approach has been traditionally used in the development of various chemosensors. A different approach is to use an ensemble of a receptor and an indicator, which is called the indicator-displacement (or competition) assay.[1] In this ensemble approach, the receptor is not linked to the indicator by covalent bonds, which discriminates it from the traditional approach. In the indicator-displacement assay, an indicator molecule bound to the receptor through supramolecular interactions, such as hydrogen bonding and metal-coordination bonding, is released when the receptor–indicator ensemble is exposed to an analyte that has stronger affinity to the receptor rather than to the indicator. As the indicator is displaced, a change in the color or fluorescence of the ensemble system is observed, thereby enabling detection of the analyte. Such a sensing ensemble can be created simply by mixing a molecular receptor with a suitable indicator; here, the task of covalent linking between the receptor and indicator parts is not required. In the traditional approach, covalent attachment of an indicator to a receptor structure without affecting the recognition and the signaling events is sometimes challenging.

The indicator-displacement assay is now widely used as a standard sensing method[1] since the Anslyn group demonstrated its usefulness in the detection of various oxoanions such as citrate, tartrate, phosphate, inositol triphosphate ($IP_3$), heparin (a sulfate-containing biomolecule), and 2,3-bisphosphoglycerate.[2]

Although ample examples of indicator-displacement assays for various analytes are known, almost all the assays are based on molecular receptors.[1] Martínez-Máñez and coworkers reported a heterogeneous competition assay for citrate, using mesoporous silica solids functionalized with guanidinium groups.[3] The solid ensemble was suspended in water, and the displaced indicator was analyzed spectrophotometrically after isolation. This is a rare example of competition assays in which material-based receptor systems are used as the ensemble component. Nanoscale cavities of the mesoporous materials are presumed to impart a good selec-

[a] D. J. Oh, K. M. Kim, Prof. K. H. Ahn
Department of Chemistry and Center for Electro-Photo Behaviors in Advanced Molecular Systems
Pohang University of Science and Technology (POSTECH)
San 31 Hyoja-dong, Pohang 790-784 (Republic of Korea)
Fax: (+82) 54-279-5877
E-mail: ahn@postech.ac.kr

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/asia.201100149.

tivity toward citrate over several carboxylates examined. Our experiences from integrated receptor systems, including this study,[4] suggest that congested "micro-environments" generated by the assembled receptors on a silica surface also seem to influence the guest selectivity.

We have been studying how "integrated" supramolecular systems, recognition systems in which many receptor units, either the same or different types, are integrated onto nanomaterials.[4,5] With such integrated supramolecular systems, we may realize novel recognition features, such as multiple interactions, signal enhancement, or analyte specificity, which are commonly realized in biological systems but are difficult to achieve with non-integrated systems. Also, a chemical ensemble sensing system based on an integrated receptor system would extend the usefulness of the indicator-displacement assay in solution to that on sensory materials. To this end, we have investigated an indicator-displacement assay by exploiting receptor-functionalized silica nanoparticles as the molecular receptor component.

## Results and Discussions

To realize the "nano-ensemble" system, we have chosen silica nanoparticles as the integration platform, as they are readily available and show no optical interference. The functionalization of a silica surface is also well established.

We have chosen $Zn^{II}$-dipicolylamine (DPA) as the recognition motif. The DPA ligand coordinates strongly to the $Zn^{2+}$ cation to form the Zn(DPA) complex (Figure 1), which provides one or two vacant coordination sites for an anionic guest such as phosphate anion. Accordingly, bis- or tris-Zn(DPA) derivatives have been widely used for the recognition of biologically important phosphate derivatives such as phosphate, pyrophosphate (PPi), $IP_3$, and phytate (a hexaphosphate compound). Particularly, bis-Zn(DPA) complexes, such as **1** and **2** (Figure 1), recognize PPi, an important biomarker, with a good selectivity over phosphate and mono-, di-, and tri-phosphate nucleotides. Hence, they have been widely used for the development of chemosensors for PPi.[6] Kim and co-workers first reported an indicator-displacement assay for $HPO_4^{2-}$ based on bis-Zn(DPA) **1** (R = Me).[7] Since then, other types of indicator-displacement assays for phosphate and its derivatives have been developed.[2d,8] Recently, we also developed indicator-displacement assays for phytate and $IP_3$ using tripodal receptors composed of the Zn(DPA) binding motif.[9]

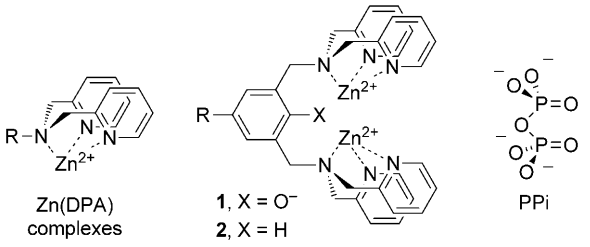


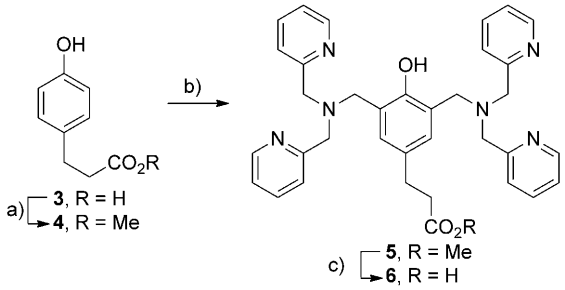

Figure 1. Structures of Zn(DPA), bis-Zn(DPA), and PPi.

**Abstract in Korean:**

표면을 인산 기를 인지하는 $Zn^{II}$-디피콜일아민 착물로 기능화한 실리카 나노입자를 합성하고, 지시약(파이로카테콜 바이올렛)-나노입자 수용체로 구성되는 나노-앙상블 계를 이용하여 피로인산을 선택적으로 감지할 수 있음을 입증하였다. 경쟁 치환에 의해서 나노-앙상블 용액이 청색에서 노란색으로의 변화와 그에 따르는 자외선/가시광선 흡수띠의 변화를 나타내었다. 기존의 지시약-분자 수용체로 구성되는 경쟁분석법은 인산에 대해서 색 변화를 보이나 나노-앙상블 계는 오직 피로인산에 대해서만 색변화를 보이는데, 그 이유는 나노수용체가 제공하는 집적된 환경에서 인산 이온이 지시약을 완전히 치환하지 못한 결합 구조로서 설명될 수 있다. 본 연구는 나노입자 수용체를 앙상블 감지 계에 적용한 최초의 예이며, 또한 집적된 수용체 계를 통해서 분자 수용체와는 차별적인 기질 선택성을 구현할 수 있음을 보여준다.

### Synthesis

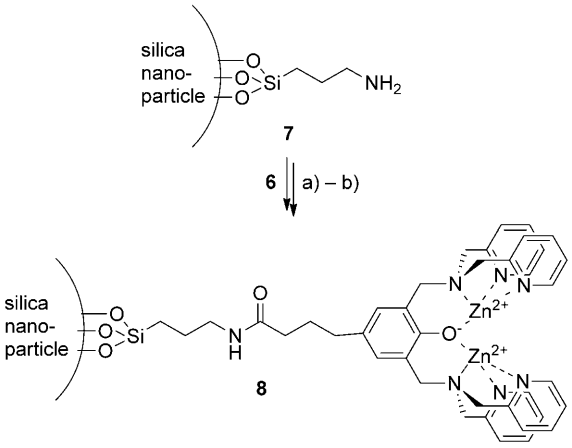
To prepare the desired Zn(DPA)-functionalized silica nanoparticles, we have designed bis(DPA)-acid **6**; its synthesis is straightforward as depicted in Scheme 1. Thus, 3-(4-hydroxyphenyl)propanoic acid methyl ester (**4**), which was obtained from the commercially available acid **3**, was treated with DPA under Mannich reaction conditions to afford bis(DPA)-ester **5** as pale brown oil in 64% yield after careful purification by silica-gel column chromatography. Hydrolysis of the methyl ester provided bis(DPA) acid **6** in 85% yield.




Scheme 1. Synthesis of bis(DPA) **6**: a) $SOCl_2$, MeOH, −40→25 °C, 12 h, 95%; b) paraformaldehyde, bis(2-pyridylmethyl)amine (DPA), 1 M HCl (cat.), EtOH/$H_2O$ (1:3), reflux, 36 h, 64%; c) NaOH, $H_2O$/MeOH (1:3), 25 °C, 1 day, 85%.

Silica nanoparticles (~50 nm) with terminal aminopropyl groups (**7**) were used as a nanotemplate; they were coupled with bis(DPA)-acid **6** under the standard EDC-HOBt coupling protocol (EDC = 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide; HOBt = 1-hydroxybenzotriazole). The resulting nanoparticles were treated with an excess amount of $Zn(ClO_4)_2$ dissolved in water to give the corresponding functional nanoparticle (NP **8**), which was purified by dialysis in deionized water to remove excess $Zn(ClO_4)_2$, unreacted **6**, and other reagents (Scheme 2). On the basis of the amount of the nanoparticle **7** used in the coupling reaction, a stock solution of the functional nanoparticles was prepared




Scheme 2. Preparation of bis-Zn(DPA)-functionalized NP **8**: a) silica NP **7**, bis(DPA)-acid **6**, EDC, HOBt, $H_2O$/DMF, 25 °C, 3 days; b) excess $Zn(ClO_4)_2 \cdot H_2O$.

(0.1 mg NPs $mL^{-1}$) and used for sensing purposes after dilution.

The functionalized nanoparticle (NP **8**) was characterized by IR and TEM. The amide bond of the organic moiety on the surface of silica nanoparticles was identified by the band at 1627 $cm^{-1}$. Also, the hydroxy stretching band around 3500 $cm^{-1}$ increased upon the coordination of a $Zn^{II}$ ion to the bis(DPA) moiety, which is likely owing to an increased hydrophilicity of the metal complex (Figure 2a). The functional nanoparticles show little change in the particle size from that of the starting aminopropyl-terminated **7** (particle diameter ~50 nm) (Figure 2b).

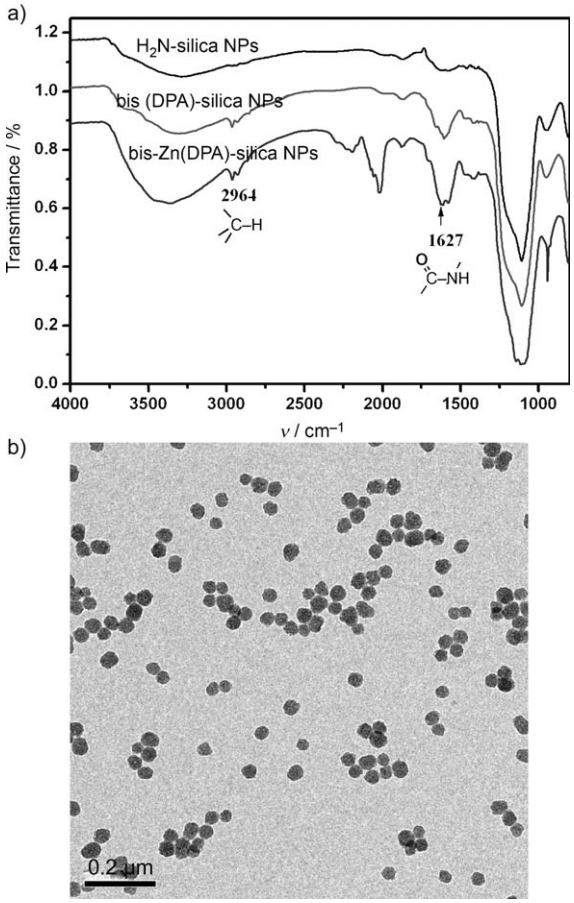



Figure 2. a) IR spectra of silica nanoparticle 7 and its bis(DPA)- and bis-Zn(DPA)-functionalized NPs. b) TEM image of the bis-Zn(DPA)-functionalized NPs (particle diameter ~50 nm).

### Competition Assay

The nanoparticle ensemble system was prepared by simply mixing NP **8** (0.02 mg $mL^{-1}$) with pyrocatechol violet (PV) (50 μM) in an aqueous HEPES buffer at pH 7.0 (HEPES = 2-[4-(2-hydroxyethyl)-1-piperazinyl]ethanesulfonic acid). Figure 3 shows the UV/Vis spectra obtained when the indicator solution was titrated with NP **8**. A color change from yellow ($\lambda_{max}$ = 444 nm) to blue ($\lambda_{max}$ = 625 nm) was observed upon addition of the nanoparticles, which can be ascribed to the binding of PV to the $Zn^{II}$ complexes on the nanoparticle surface.[7a]

The UV/Vis spectral changes upon addition of the nanoparticle solution to the PV solution were dependent on the pH of the solution. The absorption peak at $\lambda_{max}$ = 625 nm increased slightly at pH 6.0; the result indicates that the coordination of PV to the Zn(DPA) sites requires deprotonation of the catechol hydroxy groups, which is unfavorable at acidic pH. As expected, the indicator complexation seems to be favorable at pH 7 or higher, as the absorption peak showed essentially the same increase (see the Supporting Information). At higher pH than 7, however, PV can exist in its diphenolate form, the absorption peak of which overlaps

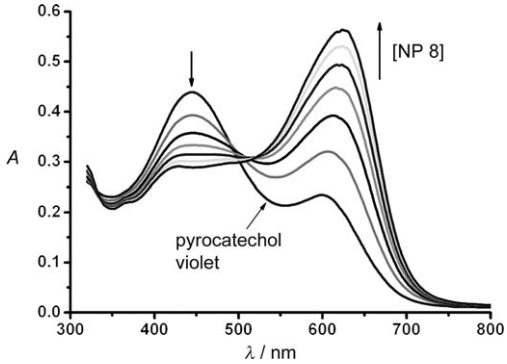



Figure 3. UV/Vis spectral changes of a solution of PV (50 μM) in HEPES buffer (pH 7.0, 10 mM) upon addition of NP **8** (final concentrations: 0.004, 0.008, 0.012, 0.016, 0.020, 0.024, 0.028 mg $mL^{-1}$ water).

the peak at $\lambda_{max}$ = 625 nm. Therefore, pH 7 is chosen as an optimum value for our sensing purposes.

On the basis of the above experiments, an optimum nano-ensemble solution is composed of PV (50 μM) and NP **8** (0.02 mg $mL^{-1}$) in a pH 7 buffer solution. As expected, the absorption peak at $\lambda_{max}$ = 625 nm decreased while the peak at $\lambda_{max}$ = 444 nm increased upon addition of PPi to the nano-

CHEMISTRY
AN ASIAN JOURNAL

ensemble solution at pH 7. These absorption changes indicate that the PV molecules bound to the nanoparticles are released upon addition of PPi, which is a much stronger binder to the bis-Zn(DPA) complex ($K_{ass}=2–6\times10^{8}\,M^{-1}$ in water) (Figure 4).[6d]

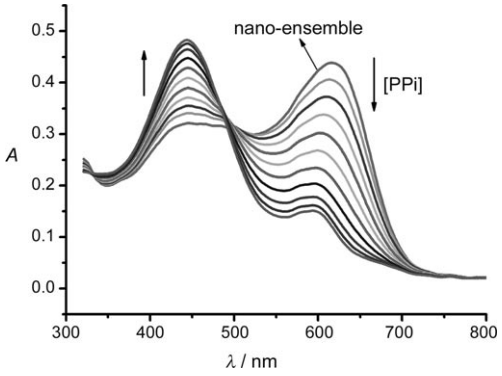



Figure 4. UV/Vis spectral changes of the nano-ensemble (50 μM PV+0.02 mg mL$^{-1}$ of NP **8**) upon addition of PPi (final concentrations: 0, 20, 40, 60, 80, 100, 120, 140, 160, 180, 200 μM) in water buffered at pH 7.0 (HEPES buffer, 10 mM).

The replacement is highly selective to PPi; other anions ($AcO^-$, citrate, $CO_3^{2-}$, $Cl^-$, $Br^-$, $N_3^-$, $ClO_4^-$,$SO_4^{2-}$, and $NO_3^-$) show little optical changes (Figure 5). The selectivity

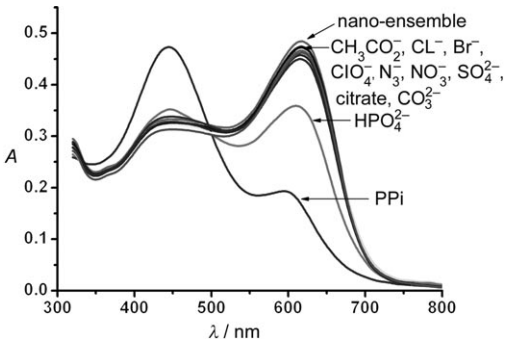



Figure 5. UV/Vis spectra of the nano-ensemble system (50 μM PV+0.02 mg mL$^{-1}$ of NP **8**) in a buffer solution at pH 7.0 (HEPES buffer, 10 mM) in the presence of various anions (150 μM; $AcO^-$, $Cl^-$, $Br^-$, $ClO_4^-$, $N_3^-$, $NO_3^-$, $SO_4^{2-}$, citrate, $CO_3^{2-}$, $HPO_4^{2-}$, and PPi).

for PPi over $HCO_3^-$, $Cl^-$, $AcO^-$, and $SO_4^{2-}$ is also an important feature because these anions play important roles in the body. Only in the case of $HPO_4^{2-}$, the absorption peak at $\lambda_{max}=625$ nm shows a 40% decrease compared to that caused by PPi. Although addition of $HPO_4^{2-}$ caused an absorbance change at the blue wavelength region, interestingly there was a small increase in the absorbance at $\lambda_{max}=$ 444 nm.

Nanosensing-ensembles that can be used to detect analytes with the naked eye, without resorting to any spectrometric instrument, are of interest because of their operational simplicity. The present nano-ensemble system shows an apparent color change from blue to yellow only when PPi was added to an aqueous buffered solution of the nano-ensemble (HEPES pH 7.0); other anions including $HPO_4^{2-}$ failed to cause the color change (Figure 6). It may thus be concluded that the nano-ensemble system may serve as a specific tool for PPi, which can be effectively detected by the naked eye in a neutral aqueous solution.

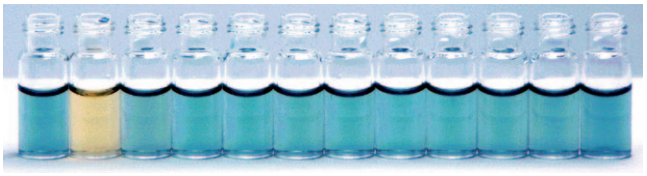


Figure 6. Photos that show the color of the nano-ensemble solution (50 μM PV+0.02 mg mL$^{-1}$ of NP **8**) in the absence and presence of anions (140 μM) in a buffer solution at pH 7.0 (HEPES, 10 mM): from left to right; no anion, PPi, $HPO_4^{2-}$, citrate, $ClO_4^-$, $CH_3CO_2^-$, $NO_3^-$, $SO_4^{2-}$, $N_3^-$, $CO_3^{2-}$, $Cl^-$, $Br^-$.

In the above, we have noted that $HPO_4^{2-}$ causes unusual absorption changes when it interacts with the nano-ensemble; the absorption peak of bound PV at $\lambda_{max}=625$ nm decreases as expected in the presence of $HPO_4^{2-}$, whereas the peak of "free" PV at $\lambda_{max}=444$ nm increases only a little, thus suggesting an incomplete restoration. As a consequence, the nano-ensemble does not show a blue-to-yellow color change toward $HPO_4^{2-}$ (Figure 6). The conventional sensing ensemble composed of a 1:2:1 molar ratio of bis-(DPA) **1** (R = Me), zinc perchlorate, and pyrocatechol violet shows a color change in the case of $HPO_4^{2-}$.[7] The contrasting behavior of the integrated receptor system from the traditional molecular receptor system toward $HPO_4^{2-}$ may be ascribed to a complex binding mode between $HPO_4^{2-}$ and the nano-ensemble. In this case, the PV molecules coordinated to the Zn(DPA) site are resistant to the displacement by $HPO_4^{2-}$, owing to the "congested" microenvironment, or they are not completely displaced from it but are still in a bound state, possibly as in its mono-catecholate form (Figure 7). The normal decrease in the absorption peak at $\lambda_{max}=625$ nm indicates that the PV is no longer in its dicatecholate form. Also, the abnormal increase in the absorption peak $\lambda_{max}=444$ nm (a little increase) suggests that most of PV molecules are not in the unbound state, but somewhere in the intermediate state. Probably, this incomplete displacement state, which does not occur in the non-integrated receptor system, seems to persist in a congested micro-environment of the integrated receptor system. But in the case of PPi, the incomplete displacement state is not conceivable because it is a much stronger competitor than $HPO_4^{2-}$ or PV toward the metal binding site (association constant values of PPi, $HPO_4^{2-}$, and PV toward the bis-Zn(DPA) complex are $2–6\times10^{8}$, $11\times10^{4}$, and $5\times10^{4}\,M^{-1}$, respectively).[6d,7] The binding mode between PPi and the bis-Zn-(DPA) moiety has been established by Hong and co-workers.[6f] It should be noted that the molecular interactions be-

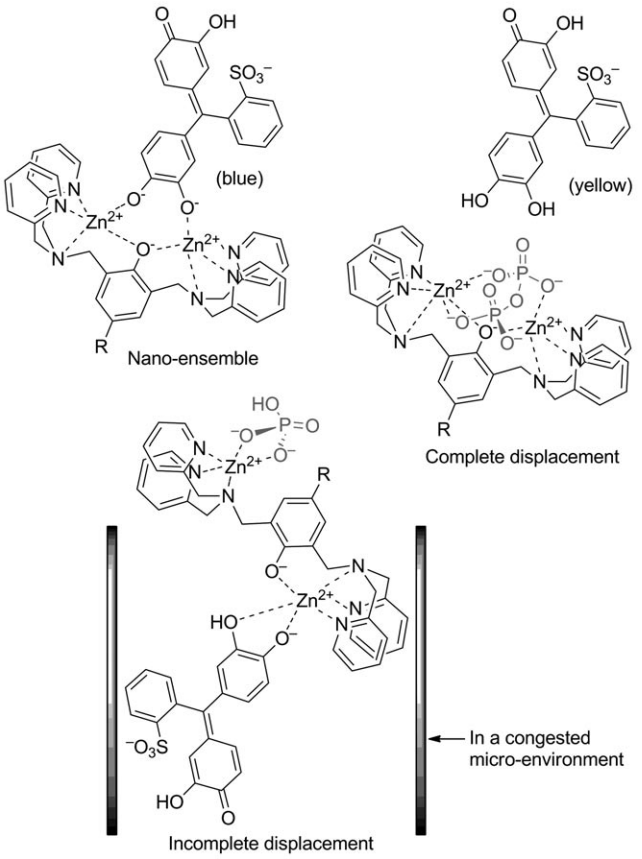



Figure 7. Complete and incomplete displacement complexes in a congested micro-environment conceivable in the cases of PPi and $HPO_4^{2-}$, respectively.

tween the indicator, the analyte, and the $Zn^{II}$ centers can be discrete in part but are complex in total; for example, intermolecular interactions with neighboring $Zn^{II}$ sites are also conceivable, which will lead to the formation of ternary or higher complexes.

The different selectivity behavior observed by our nano-ensemble system compared with the conventional sensing ensemble provides an additional example of notable features obtainable with integrated recognition systems. We demonstrated such an example recently in the case of a liposome-based integrated sensing system.[4]

The present nano-ensemble system might allow quantitative assay of PPi in an aqueous solution at physiological pH. The calibration curve gives a good correlation with respect to the concentration of PPi down to a low μM level (Figure 8).

## Conclusions

We have demonstrated that the traditional indicator-displacement assay can be extended to a nanoparticle-based assay. Specifically, we have introduced silica nanoparticles functionalized with a pyrophosphate-binding bis-

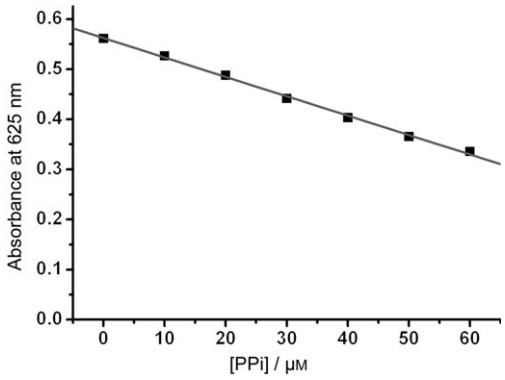



Figure 8. A plot of absorbance (at 625 nm) of the nano-ensemble system (50 μM PV+0.028 mg mL$^{-1}$ of NP **8**) against [PPi] in a buffer solution at pH 7.0 (HEPES).

$Zn^{II}$(dipicolylamine) moiety as the receptor component of the sensing ensemble. Together with pyrocatechol violet as the indicator component, this nano-ensemble sensing system senses pyrophosphate with an excellent selectivity over hydrogen phosphate and other mono- and divalent anions, showing a blue-to-yellow color change along with the corresponding spectral changes. A distinct recognition behavior—the nano-ensemble does not sense hydrogen phosphate that was sensed by the traditional ensemble with the same molecular receptor—is explained by an incomplete-displacement model in the case of the integrated receptor system. The present work demonstrates that the nanoparticle-based competition assay is as effective as the traditional approach with molecular receptors. Furthermore, such an integrated receptor system can provide a distinct recognition behavior from the corresponding molecular receptor.

## Experimental Section

*Methyl 3-(4-hydroxyphenyl)propanoate (**4**)*

Thionyl chloride (0.5 mL, 6.6 mmol) was added dropwise with stirring to a pre-cooled solution of 3-(4-hydroxyphenyl)propanoic acid (**3**) (1.0 g, 6.0 mmol) in MeOH (40 mL) at −40 °C with an acetonitrile–dry ice bath; the reaction mixture was allowed to stand at room temperature for 12 h. Solvent was evaporated under reduced pressure and the residue was purified by column chromatography on silica gel (EtOAc/*n*-hexane = 1:1, by volume) to give the ester as a yellow oil (1.0 g, 95 %). $^1$H NMR ($CDCl_3$, 300 MHz): $\delta$ = 2.58 (t, 2H), 2.87 (t, 2H), 3.64 (s, 3H), 6.75 (d, 2H), 7.00 (d, 2H), 7.20 ppm (s, 1H); $^{13}$C NMR ($CDCl_3$, 75 MHz): $\delta$ = 30.06, 36.08, 51.87, 115.47, 129.31, 131.94, 154.49, 174.56 ppm.

*Methyl 3-(3,5-bis{bis[(pyridin-2-yl)methyl]aminomethyl}-4-hydroxyphenyl)propanoate (**5**)*

Phenol **4** (1.0 g, 5.6 mmol) and HCl (1 M, 1.4 mL) were added to a suspension of paraformaldehyde (0.55 g, 18.3 mmol) and di(2-picolyl)amine (DPA) (2.76 g, 13.9 mmol) in ethanol (15 mL) and water (45 mL), and the resulting mixture was refluxed for 36 h. The reaction mixture was cooled to room temperature, neutralized with $Na_2CO_3$, and then extracted into chloroform. The organic layer was dried over magnesium sulfate and concentrated to give a brown oil. The crude product was purified by column chromatography on silica gel ($CHCl_3$/MeOH = 20:1) to give bis(DPA) **5** as a pale brown oil (2.1 g, 64 %): $^1$H NMR ($CDCl_3$, 300 MHz): $\delta$ = 2.56 (t, 2H), 2.84 (t, 2H), 3.62 (s, 3H), 3.78 (s, 4H), 3.93 (s, 8H), 7.03

CHEMISTRY
AN ASIAN JOURNAL

(s, 2H), 7.10–7.15 (m, 4H), 7.50 (d, 4H), 7.58–7.70 (m, 4H), 8.53 (d, 4H), 10.95 ppm (s, 1H); $^{13}$CNMR ($CDCl_3$, 75 MHz): $\delta$=30.38, 36.27, 51.68, 54.91, 59.95, 122.12, 123.09, 124.20, 129.02, 130.28, 136.69, 149.02, 154.45, 159.39, 173.75 ppm; MS (FAB+) $[M+H]^+$: 603.2; HRMS (FAB+) $[M+H]^+$ calcd for $C_{36}H_{39}N_6O_3$: 603.3005; found: 603.3088.

*3-(3,5-Bis[bis[(pyridin-2-yl)methyl]aminomethyl]-4-hydroxyphenyl)propanoic acid (**6**)*

A solution of bis(DPA)-methyl ester **5** (350 mg, 0.58 mmol) in MeOH/water (3:1, 5 mL), was treated with NaOH (23 mg, 0.58 mmol), and the mixture was stirred for 24 h at room temperature. Most of the methanol was evaporated under reduced pressure, and the aqueous solution was washed with $CH_2Cl_2$ (3×20 mL). The aqueous solution was neutralized with 1 N HCl and extracted with $CH_2Cl_2$ (3×30 mL). The combined organic layer was dried over anhydrous $Na_2SO_4$ and concentrated in vacuo to give bis(DPA)-acid **6** (290 mg, 85%), which was used for the nanoparticle functionalization. $^1$HNMR ($CDCl_3$, 300 MHz): $\delta$=2.57 (t, 2H), 2.91 (t, 2H), 3.79 (s, 4H), 3.85 (s, 8H), 7.08 (s, 2H), 7.12–7.16 (m, 4H), 7.50 (d, 4H), 7.59–7.65 (m, 4H), 8.53 ppm (d, 4H); $^{13}$CNMR ($CDCl_3$, 75 MHz)): $\delta$=31.48, 37.83, 55.07, 59.57, 122.39, 123.52, 123.81, 129.95, 130.51, 137.06, 148.83, 154.54, 159.08, 176.25 ppm; MS (FAB+) $[M+H]^+$: 589.2; HRMS (FAB+) $[M+H]^+$ calcd for $C_{35}H_{37}N_6O_3$: 589.2849; found: 589.2930.

*Preparation of bis-Zn(DPA)-silica nanoparticle **8***

A solution of bis(DPA)-acid **6** (185 mg, 0.315 mmol), aminopropyl-terminated silica nanoparticles (2.5 mg; available as 25 mg/1 mL, 50 nm; www.kisker-biotech.com), EDC (60 mg, 0.315 mmol), and HOBt (43 mg, 0.315 mmol) in water/DMF (2 mL, 1:1) was stirred at room temperature for 3 days. After centrifugation twice (12000 rpm, 30 min, 20 °C), the nanoparticles were subjected two times to dialysis (membrane pore 3500 MW, 2000 mL water×2) to remove any remaining reagents. As dialysis proceeded, nanoparticles became solid and subsided onto the bottom of the membrane. Then, bis(DPA)-functionalized nanoparticles were dissolved in water/MeOH (1:1) and treated slowly with an aqueous solution (10 mL) of $ZnClO_4{\cdot}6H_2O$ (235 mg, 0.63 mmol), and the resulting solution was stirred at room temperature for 24 h. The reaction mixture was subjected to centrifugation (5000 rpm, three times) and then dialysis to afford bis-Zn(DPA)-functionalized silica nanoparticle **8**. For sensing purposes, a solution of nanoparticles (0.02 mg mL$^{-1}$) was prepared from a stock solution of the nanoparticles (0.1 mg mL$^{-1}$, water). The functionalized nanoparticle was characterized by IR and TEM.

*UV/Vis Titration of the Nanoparticle Ensemble System with PPi*

The nanoparticle ensemble system was prepared by mixing bis-Zn(DPA)-silica nanoparticle **8** and pyrocatechol violet in HEPES buffer (pH 7). A nano-ensemble solution (1.0 mL) containing the functionalized nanoparticle (0.02 mg mL$^{-1}$) and pyrocatechol violet (50 μM) in HEPES buffer (pH 7, 10 mM) was titrated with pyrophosphate aliquots (in the final concentration range of 0–200 μM) and the corresponding absorption changes were followed by UV/Vis spectrometry.

## Acknowledgements

This work was supported by grants from the Center for Electro-Photo Behaviors in Advanced Molecular Systems (R11-2008-052-01001).

[1] For reviews, see: a) B. T. Nguyen, E. V. Anslyn, *Coord. Chem. Rev.* **2006**, *250*, 3118; b) E. V. Anslyn, *J. Org. Chem.* **2007**, *72*, 687; c) N. Marcotte, A. Taglietti, *Supramol. Chem.* **2003**, *15*, 617.

[2] a) A. Metzger, E. V. Anslyn, *Angew. Chem.* **1998**, *110*, 682; *Angew. Chem. Int. Ed.* **1998**, *37*, 649; b) K. Niikura, A. Metzger, E. V. Ansyln, *J. Am. Chem. Soc.* **1998**, *120*, 8533; c) J. J. Lavigne, E. V. Anslyn, *Angew. Chem.* **1999**, *111*, 3903; *Angew. Chem. Int. Ed.* **1999**, *38*, 3666; d) S. L. Tobey, B. D. Jones, E. V. Anslyn, *J. Am. Chem. Soc.* **2003**, *125*, 4026; e) Z. Zhong, E. V. Anslyn, *J. Am. Chem. Soc.* **2002**, *124*, 9014; f) Z. Zhong, E. V. Anslyn, *Angew. Chem.* **2003**, *115*, 3113; *Angew. Chem. Int. Ed.* **2003**, *42*, 3005.

[3] M. Comes, G. Rodríguez-López, M. D. Marcos, R. Martínez-Máñez, F. Sancenón, J. Soto, L. A. Villaescusa, P. Amorós, D. Beltrán, *Angew. Chem.* **2005**, *117*, 2978; *Angew. Chem. Int. Ed.* **2005**, *44*, 2918.

[4] K. M. Kim, D. J. Oh, K. H. Ahn, *Chem. Asian J.* **2011**, *6*, 122.

[5] A. Chatterjee, D. J. Oh, K. M. Kim, K.-S. Youk, K. H. Ahn, *Chem. Asian J.* **2008**, *3*, 1962.

[6] For recent reviews, see: a) S. K. Kim, D. H. Lee, J.-I. Hong, J. Yoon, *Acc. Chem. Res.* **2009**, *42*, 23; b) T. Sakamoto, A. Ojida, I. Hamachi, *Chem. Commun.* **2009**, 141. For selected examples, see: c) D. H. Vance, A. W. Czarnik, *J. Am. Chem. Soc.* **1994**, *116*, 9397; d) D. H. Lee, J. H. Im, S. U. Son, Y. K. Chung, J.-I. Hong, *J. Am. Chem. Soc.* **2003**, *125*, 7752; e) D. H. Lee, S. Y. Kim, J.-I. Hong, *Angew. Chem.* **2004**, *116*, 4881; *Angew. Chem. Int. Ed.* **2004**, *43*, 4777; f) J. H. Lee, J. Park, M. S. Lah, J. Chin, J.-I. Hong, *Org. Lett.* **2007**, *9*, 3729; g) H. N. Lee, Z. Xu, S. K. Kim, K. M. K. Swamy, Y. Kim, S.-J. Kim, J. Yoon, *J. Am. Chem. Soc.* **2007**, *129*, 3828.

[7] M. S. Han, D. H. Kim, *Angew. Chem.* **2002**, *114*, 3963; *Angew. Chem. Int. Ed.* **2002**, *41*, 3809.

[8] a) L. Fabbrizzi, N. Marcotte, F. Stomeo, A. Taglietti, *Angew. Chem.* **2002**, *114*, 3965; *Angew. Chem. Int. Ed.* **2002**, *41*, 3811; b) R. G. Hanshaw, S. M. Hilkert, H. Jiang, B. D. Smith, *Tetrahedron Lett.* **2004**, *45*, 8721; c) M. J. McDonough, A. J. Reynolds, W. Y. G. Lee, K. A. Jolliffe, *Chem. Commun.* **2006**, 2971; d) M. S. Saeed, D. R. Powell, M. A. Hossain, *Tetrahedron Lett.* **2010**, *51*, 4904;.

[9] a) D. J. Oh, M. S. Han, K. H. Ahn, *Supramol. Chem.* **2007**, *19*, 315; b) D. J. Oh, K. H. Ahn, *Org. Lett.* **2008**, *10*, 3539.

Received: February 14, 2011
Published online: May 19, 2011