Supporting Information

A highly sensitive gold nanoparticle-based colorimetric probe for pyrophosphate using a competition assay approach

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General methods.

All reagents used in this work were purchased from Sigma-Aldrich Chemical Co. and were used without further purification. All anion solutions were prepared from the respective sodium anions and deionized water. Buffer solution was prepared using deionized water to a final pH 7.0 (HEPES 10 mM, NaCl 10 mM). The ¹H and ¹³C-NMR spectra were recorded on a Varian Gemini 2000 (300MHz) and VNS (600MHz). UV-Vis spectrometric studies were carried out on an S-3100 purchased from SCINCO. TEM images of 11mercaptoundecylphosphoric acid functionalized gold nanoparticles (Phos-AuNPs) were captured on JEM-2010 purchased from JEOL (operating at 200 kV). Sample for TEM measurements were prepared by placing 30 μ L of the Phos-AuNPs solution on a carbon-coated copper grid and then drying at room temperature.

Synthesis of 1,3-bis[bis(2-pyridylmethyl)aminomethyl]benzene (BBPAB)¹

m-Xylylenedibromide (1.32 g, 5.00 mmol), di-2-pyridylmethylamine (2.00 g, 9,63 mmol), and *N*,*N*-diisopropylethylamine (1.32 g, 10.06 mmol) were dissolved in CHCl₃ (50 mL). After 4 h of stirring at room temperature, the resulting solution was washed once with water (25 mL) and dried over Na₂SO₄. The product was purified by silica gel chromatography with CHCl₃/MeOH as the eluent. The yield of the product was 89% (2.22 g). ¹H-NMR (300 MHz, CDCl₃) δ 3.70 (s, 4H), 3.82 (s, 8H), 7.09-7.14 (m, 4H), 7.28-7.62 (m, 12H), 8.50-8.52 (m, 4H); ¹³C-NMR (150 MHz, CDCl₃) δ 58.51, 60.02, 121.87, 122.68, 127.54, 128.23, 129.15, 136.32, 139.03, 148.90, 159.78.



Figure S1. ¹H-NMR spectrum of BBPAB



Figure S2. ¹³C-NMR spectrum of BBPAB

Preparation of [Zn₂(1,3-*bis*[*bis*(2-pyridylmethyl)aminomethyl]benzene)]⁴⁺

 $([Zn_2(BBPAB)]^{4+})$ $[Zn_2(BBPAB)]^{4+}$ was obtained by simply mixing BBPAB, zinc perchlorate in an 1 : 2 molar ratio in the aqueous solution of HEPES buffer pH 7.0.

Preparation of Phos-AuNPs

All glassware was washed with freshly prepared aqua regia (3:1 = HCl:HNO₃) followed by extensive rinsing with doubly distilled H₂O. Citric acid-stabilized Au particles with a diameter of 13 nm were prepared by adding 50 mL of a citrate solution (38.8 mM) to 500 mL of boiling 1.0 mM HAuCl₄ with vigorous stirring. After the appearance of a deep red color, boiling and stirring were continued for 15 min. The solution was then allowed to cool to room temperature with continued stirring. One percent TWEEN 20 solution (1.5 mL) was added to 30 mL of the citric-acid-stabilized AuNPs (11.41 nM), and 12 mL of 11-mercaptoundecyl phosphoric acid (10 mM) in THF was added to the solution 5 times in 36 hours at equivalent intervals. The solution was adjusted to 1 mM HEPES buffer (pH 7.0) using 30 mM HEPES buffer and incubated for 12 hours. After incubation, unreacted 11-mercaptoundecyl phosphoric acid and TWEEN 20 were eliminated by centrifuging twice. The Phos-AuNPs were stored in distilled water. The final concentration of the Phos-AuNPs was 6 nM.

Determination of the stability of Phos-AuNPs in the presence of [Zn₂(BBPAB)]⁴⁺

The solution of Phos-AuNPs (6 nM) was mixed 0.5 mL of 10 mM HEPES buffer (pH 7.0, 10 mM NaCl) containing various concentrations of $[Zn_2(BBPAB)]^{4+}$ (0, 1.0, 1.6, 2.0, 2.2, 2.4, 3.0, 4.0 and 5.0 μ M). The mixtures were analyzed using UV-Vis spectroscopy at the extinction wavelength of 580 nm to determine the stability of the Phos-AuNPs. The mixtures were continuously stirred at room temperature with a magnetic stir bar to ensure homogeneity.

Colorimetric assay for PPi using ensemble system

The solution of Phos-AuNPs (6 nM) was mixed 0.5 mL of 10 mM HEPES buffer (pH 7.0, 10 mM NaCl) containing $[Zn_2(BBPAB)]^{4+}$ (3.0 μ M). After 60 sec, the assay was initiated by adding various concentration of PPi to the assay mixture, and kinetics were monitored using UV/Vis spectroscopy at the extinction wavelength of 580 nm. The solution was continuously stirred at room temperature with a magnetic stir bar to ensure homogeneity.

Figure S3. Plot of assay solution absorbance intensities at 580 nm versus PPi concentration (60 sec).

Colorimetric assay for PPi using a mixture of [Zn₂(BBPAB)]⁴⁺ and Phos-AuNPs

0.5 mL of $[Zn_2(BBPAB)]^{4+}$ (3 µM) was pretreated for 5 minutes with various concentrations of PPi ions in a buffer solution (10 mM HEPES, 10 mM NaCl) and then added to 0.5 mL of Phos-AuNPs (6.0 nM) in the same buffer solution. Absorbance spectra of the Phos-AuNP (3.0 nM) solutions were recorded 1 minute after the addition of $[Zn_2(BBPAB)]^{4+}$ (1.5 µM) that had been pretreated with various concentrations of PPi ions.

Determination of the detection limit of Phos-AuNPs for PPi²

Figure S4. The detection limit of Phos-AuNPs for PPi Intercept = 5.4385Slope = 0.79546 $R^2 = 0.99834$

 $(A_0-A) = 0.79546 * (Log[P_2O_7^{4-}]) + 5.4385$

LOD = 146 nM

Colorimetric assay for ATP/ADP/AMP using a mixture of $\left[Zn_2(BBPAB)\right]^{4_+}$ and PhosAuNPs

Figure S5. Plot of assay solution absorbance intensities at 580 nm versus ATP/ADP/AMP concentration (60 sec).

Determination of the PPi selectivity over various anions

0.5 mL of $[Zn_2(BBPAB)]^{4+}$ (3.0 μ M) was pretreated for 5 minutes with various anions in a buffer solution (10 mM HEPES, 10 mM NaCl) and then added to 0.5 mL Phos-AuNP (6.0 nM) in the same buffer solution. Absorbance spectra of the Phos-AuNP (3.0 nM) solution were recorded 1 minute after the addition of $[Zn_2(BBPAB)]^{4+}$ (1.5 μ M) that had been pretreated with various anions (0.5 μ M).

Figure S6. The color of the solution in the presence of $[Zn_2(BBPAB)]^{4+}$ that has been pretreated with anions (0.5 µM), from left to right: control, $P_2O_7^{4-}$, HPO_4^{2-} , F^- , CI^- , Br^- , Γ , CN^- , HSO_4^- , SO_4^{2-} , NO_3^- , N_3^- , AcO^- , HCO_3^- , CIO_4^- .

Determination of the interference in the presence of other anions

0.5 mL of $[Zn_2(BBPAB)]^{4+}$ (3.0 μ M) was pretreated for 5 minutes with PPi in the presence of various anions in a buffer solution (10 mM HEPES, 10 mM NaCl) and then added to 0.5 mL of Phos-AuNPs (6.0 nM) in the same buffer solution. Absorbance spectra of the Phos-AuNPs (3.0 nM) solution were recorded 1 minute after the addition of $[Zn_2(BBPAB)]^{4+}$ (1.5 μ M) that had been pretreated with PPi (1.0 μ M) in the presence of various anions (1.0 μ M).

Figure S7. UV/Vis spectra obtained by the addition of $[Zn_2(BBPAB)]^{4+}$ (1.5 µM) pretreated with PPi (1.0 µM) and other anions (1.0 µM) to a pH 7.0 buffer solution (10 mM HEPES, 10 mM NaCl) containing Phos-AuNPs (3.0 nM).

Determination of the pH dependence

0.5 mL of $[Zn_2(BBPAB)]^{4+}$ (3.0 μ M) was pretreated for 5 minutes with PPi (1.0 μ M) in various buffer solution and then added to 0.5 mL of Phos-AuNPs (6.0 nM) in the same buffer solution. Absorbance spectra of the Phos-AuNPs (3.0 nM) solution were recorded 1 minute after the addition of $[Zn_2(BBPAB)]^{4+}$ (1.5 μ M) that had been pretreated with PPi (1.0 μ M). The composition of the buffer solution are as follow: pH 4.0 (10 mM acetate buffer, 10 mM NaCl), pH 5.0 (10 mM acetate buffer, 10 mM NaCl), pH 6.0 (10 mM acetate buffer, 10 mM NaCl), pH 7.0 (10 mM HEPES buffer, 10 mM NaCl), pH 8.0 (10 mM tris buffer, 10 mM NaCl), pH 9.0 (10 mM tris buffer, 10 mM NaCl).

Figure S8. Absorbance intensities of Phos-AuNPs (3 nM) at 580 nm in the presence and absence of PPi (1.0 µM) at various pH.

Figure S9. UV/Vis spectra obtained by the addition of $[Zn_2(BBPAB)]^{4+}$ that has been pretreated with PPi (1.0 µM) in pH 7.0 buffer solution (10 mM HEPES, 10 mM NaCl) containing Phos-AuNPs (3 nM), bare AuNPs (3 nM).

The stability of Phos-AuNPs in the presence of CN⁻ or I⁻

Figure S10. UV/Vis spectra obtained by the addition of cyanide, iodide $(1.0 \ \mu\text{M})$ to a pH 7.0 buffer solution (10mM HEPES, 10 mM NaCl) containing Phos-AuNPs (3.0 nM).

Determination of the interference in the presence of other cations

0.5 mL of $[Zn_2(BBPAB)]^{4+}$ (3.0 µM) was pretreated for 5 minutes with PPi in the presence of various cations in a buffer solution (10 mM HEPES, 10 mM NaCl) and then added to 0.5 mL of Phos-AuNPs (6.0 nM) in the same buffer solution. Absorbance spectra of the Phos-AuNPs (3.0 nM) solution were recorded 1 minute after the addition of $[Zn_2(BBPAB)]^{4+}$ (1.5 µM) that had been pretreated with PPi (1.0 µM) in the presence of various cations (1.0 µM).

Figure S11. UV/Vis spectra obtained by the addition of $[Zn_2(BBPAB)]^{4+}$ (1.5 µM) pretreated with PPi (1.0 µM) and other cations (1.0 µM) to a pH 7.0 buffer solution (10 mM HEPES, 10 mM NaCl) containing Phos-AuNPs (3.0 nM).

References

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