A New Fluorescent Chemosensor for Highly Selective and Sensitive Detection of Inorganic Phosphate (Pi) in Aqueous Solution and Living Cells

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Scheme S1. Synthesis of the fluorescence chemosensor FP.











Fig. S3. ESI-mass spectra of FP



Fig. S4. Time-dependent fluorescence changes of **FP** and **FP**-Fe³⁺ in HEPES aqueous buffer (THF: $H_2O = 3.7$, 20 mM, pH = 7.4). Excitation at 430 nm.



Fig. S5. ESI-mass spectra of FP-Fe³⁺



Fig. S6. ESI-mass spectra of FP-Fe³⁺ in the presence of Pi



Fig. S7. Job's plot of **FP** toward Fe³⁺ in HEPES aqueous buffer (THF: $H_2O = 3:7, 20$ mM, pH = 7.4).



Fig. S8. Benesi-Hildebrand plot (emission at 515 nm) of **FP** (10 μ M) based on 1:1 binding stoichiometry with Fe³⁺ in HEPES aqueous buffer (THF: H₂O = 3:7, 20 mM, pH = 7.4). Excitation at 430 nm.



Fig. S9. The fluorescence intensity changes of **FP** (1 μ M) versus low concentration Fe³⁺ (0–20 μ M) at 515 nm in HEPES aqueous buffer (THF: H₂O = 3:7, 20 mM, pH = 7.4). Excitation was performed at 430 nm.



Fig. S10. Absorption spectra of FP (10 μ M) in HEPES aqueous buffer (THF: H₂O = 3:7, 20 mM, pH = 7.4) upon addition of various metal ions (3×10⁻⁴ M).



Fig. S11. The time courses fluorescence intensities at 515 nm of **FP** (10 μ M) towards 3×10⁻⁴ M Fe³⁺ (a) and **FP**-Fe³⁺ (10 μ M) in the presence of 3×10⁻⁴ M Pi (b) in HEPES aqueous buffer (THF: H₂O = 3:7, 20 mM, pH = 7.4). Excitation was performed at 430 nm.



Fig. S12. Fluorescent emission intensity changes of FP (10 μ M) at 515 nm in HEPES aqueous buffer (THF: H₂O = 3:7, 20 mM, pH = 7.4) upon the alternate addition of Fe³⁺–Pi with several concentrations ratio (0 : 0, 10 : 0, 10 : 20, 20 : 10, 20 : 40, 40 : 20, respectively). Excitation at 430 nm.



Fig. S13. Confocal bright-field (top), and fluorescence (bottom) imaging of Fe³⁺ and Pi in living U-343 MGa cells. (A) Cells stained with **FP** (1 μ M) at 37 °C in a 5% CO₂ incubator for 30 min, (B) and treated with Fe³⁺ (10 μ M) for 20 min, (C) then the cells incubated with Pi (30 μ M) for another 20 min. Scale bar = 40 μ m.



Fig. S14. Confocal bright-field (top), and fluorescence (bottom) imaging of Pi in living U-343 MGa cells. (A) Cells stained with **FP**-Fe³⁺ (1 μ M) at 37 °C in a 5% CO₂ incubator for 30 min, (B) U-343 MGa cells treated with 100 μ M Pi and then incubated with **FP**-Fe³⁺ (1 μ M) for 30 min. Scale bar = 40 μ m.