A Ratiometric Fluorescent Sensor for Phosphates: Zn^{2+}-enhanced ICT and Ligand Competition

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Electronic Supplementary Information

Materials and Methods

Deionized water (distilled) were used throughout the experiment. All the materials for synthesis were purchased from commercial suppliers and used without further purification. All anions used were Cl^-, Br^-, I^-, HSO_4^- and $CH_3CO_2^-$ in the form of their tetrabutylammonium salts, except for $Na_4P_2O_7$ and Na_2HPO_4. HEPES buffer solutions (pH = 7.4) were prepared using 0.02 M HEPES, 0.02 M $NaNO_3$ and proper amount of NaOH under adjustment by a pH meter. Melting points were measured on a X-6 melting point apparatus and listed without correlation. IR spectra were recorded on a Nicolet-20DXM spectrometer at room temperature. ^1H NMR spectrum was recorded on a Varian INOVA 400 MHz spectrometer, using TMS as internal standard. The electro-spray ionization mass spectrometry (ESI-MS) was performed on a HP 1100LC/MSD with acetronitrile as solvent. The emission measurements was performed at room temperature in air saturated solvent of spectroscopic grade. The pH of the solution was carried out on a digital pH meter. The emission spectra were recorded by a PTI-C-700 fluorometer.

Procedures of anions sensing by **1**-Zn

A 1.0×10^{-3} M stock solution of **1**-Zn was prepared in absolute CH_3CN. To 5 mL glass tubes with 5.0 mL solvent (1:9 (v/v) buffer/CH_3CN) containing different amount of anions, 0.5 mL of the stock solution was added to each tube by a pippet to obtain solutions of 100 µM **1**-Zn. Then the tubes were shaken for 2 min and followed by standing at room temperature for 5 min. 3.0 mL of the solutions in the tubes was later transferred out for the fluorescence measurement. If the concentration of **1**-Zn needs to be 10 µM, then a 1.0×10^{-4} M stock solution in CH_3CN of **1**-Zn was prepared following a similar way.

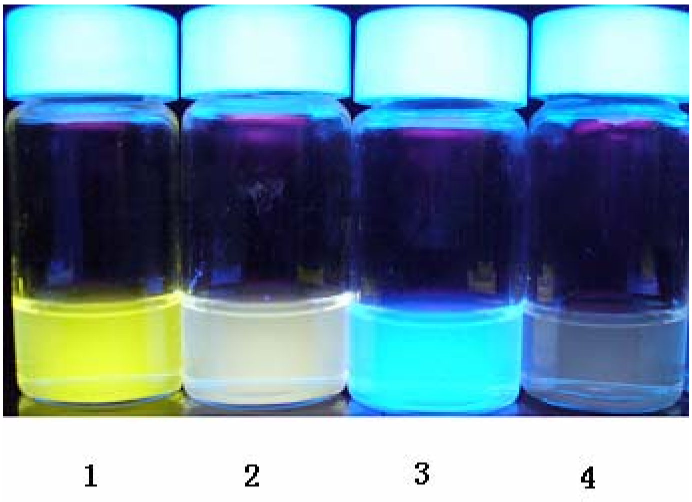

Fig. S1 The fluorescent change of **1** (50 mM) and **1**-Zn (50 mM) upon addition of different equivalent PPi in CH_3CN-HEPES (9:1,v/v). (excitation at 365 nm). (1: **1**-Zn; 2: **1**-Zn + 0.5 eq PPi; 3: **1**-Zn + 1 eq PPi; 4: **1**).

Synthesis and Characteristics of Compounds 1 and 1-Zn.

1: Compound **1** was synthesized according to the modification of published procedures.[S1] 2-Acetylpyridine (0.43 mL, 3.86 mmol) was slowly added in dropwise under nitrogen flowing at room temperature to a suspension of NaOH (0.238 g, 5.95 mmol) in anhydrous EtOH (20 mL), and the mixture was stirred at room temperature for 2 h. A solution of 1-pyrenylaldehyde (0.40 g, 1.75 mmol) in anhydrous EtOH (6 mL) was thus added. After stirring overnight, 95% aq. EtOH (30 mL) and solid $AcONH_4$ (4.7 g, 61.25 mmol) were added and the mixture refluxed for 4 h. The solvent was removed in vacuum and 10% aq. HCl (10 mL) was added, affording a red powder, which was filtered, dissolved in CH_2Cl_2 (50 mL) and washed with 10% aq NaOH, until pH was alkaline. The organic phase was dried over Na_2SO_4 and evaporated. The brown thick residue thus obtained was stirred with CH_3OH (10 mL), affording a yellow powder, which was purified by column chromatography on silica gel (CH_2Cl_2), leading to a bright yellow solid (44%). mp 212.2-213.5 °C. ν_{max}(film)/cm^{-1} 3242, 3047, 1646, 1344, 1320, 1284, 848, 840, 791, 761, 737, 722. ^1H NMR(δ ppm, $CDCl_3$): 7.34 (dd, 2H, *J* = 5.6 Hz, *J* = 6.0 Hz), 7.90 (dd, 2H, *J* = 7.6 Hz, *J* = 7.6 Hz), 7.99-8.25 (m, 9H), 8.69 (d, *J* = 4.0 Hz), 8.74 (d, *J* = 8.0 Hz, 2H), 8.78 (s, 2H). ESI-MS Positive: $[M+H]^+$ (m/z 434.1).

1-Zn: A solution of **1** (1.0 mmol) in CH_2Cl_2 (8 mL) was added to a solution of $ZnCl_2 \times 2H_2O$ (0.136 g, 1.0 mmol) in dry ethanol (25 ml). The reaction mixture was refluxed for 3 h, then cooled to room temperature. The crude yellowed precipitate was filtered and washed with ethanol and dietheylether. The product was recrystallized from warm nitrobenzene, giving yellow powder (85%). mp > 300 °C. ν_{max}(film)/cm^{-1} 1594, 1519, 1470, 1410, 1384, 1344, 886, 852, 840, 790, 759, 731, 716, 702. ^1H NMR(δ ppm, $CDCl_3$): 7.52 (dd, 2H, *J* = 8.0 Hz, *J* = 6.4 Hz), 7.87 (dd, 2H, *J* = 6.4 Hz, *J* = 7.2 Hz), 8.01 (d, *J* = 8.4 Hz), 8.14-8.26 (m, 9H), 8.31 (d, *J* = 6.4 Hz, 2H), 8.45 (s, 2H). HRMS (EI) calcd for $C_{31}H_{19}ClN_3Zn$ [M+] 532.0247, found 531.9500.

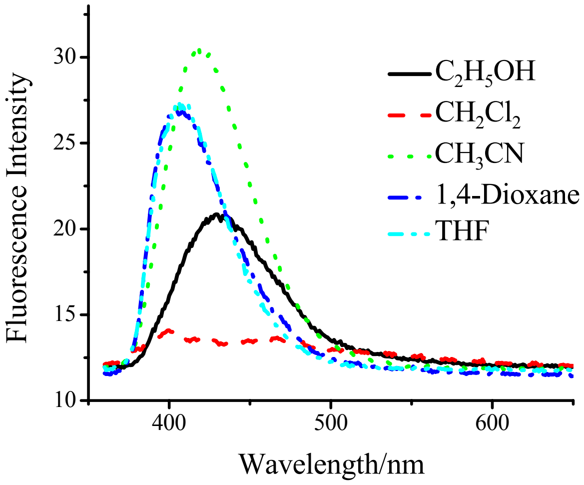

Fig. S2 The fluorescence spectra of ligand **1** (1 μM) in different solvents.

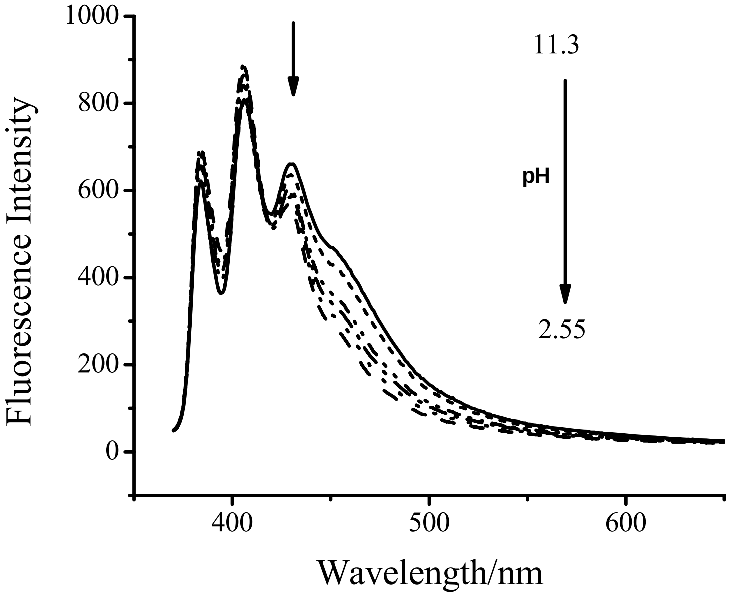

Fig. S3 Effect of pH on the fluorescence spectra of ligand **1** (1 μM) in CH_3CN-H_2O (9:1, v/v). (excitation at 346 nm)

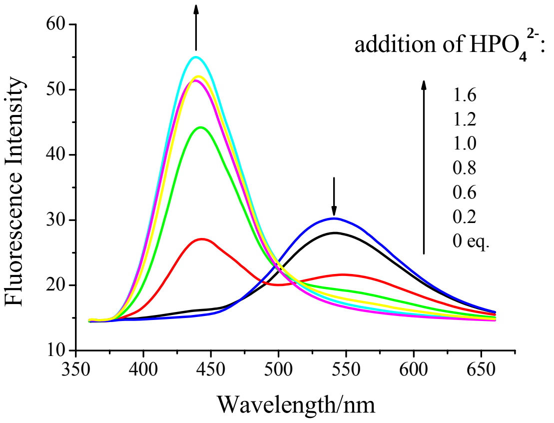

Fig. S4 Change of fluorescence emission for sensor **1**-Zn (100 μM) upon addition of 0-1.6 eq. of HPO$_4^{2-}$. The spectra were measured excitation at 346 nm in CH$_3$CN-HEPES (9:1, v/v; pH=7.4).

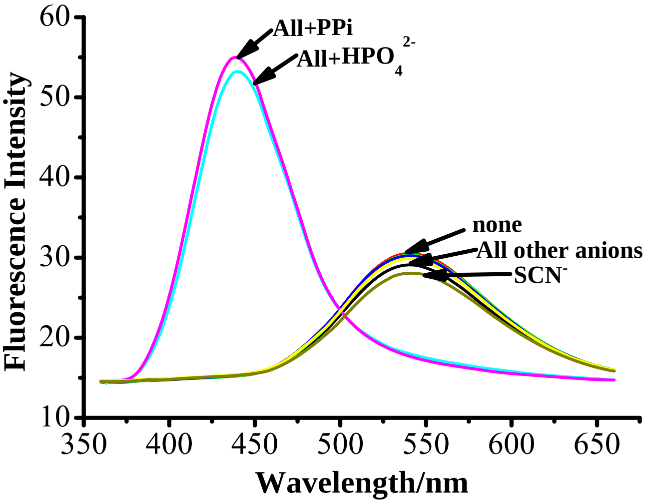

Fig. S5 Fluorescence emission for sensor **1**-Zn (100 μM) upon addition of 50 equiv. of HPO$_4^{2-}$, PPi, Cl$^-$, Br$^-$, I$^-$, CH$_3$CO$_2^-$, HSO$_4^-$ and SCN$^-$. The spectra were measured upon excitation 346 nm in CH$_3$CN-HEPES (9:1, v/v. pH=7.4).

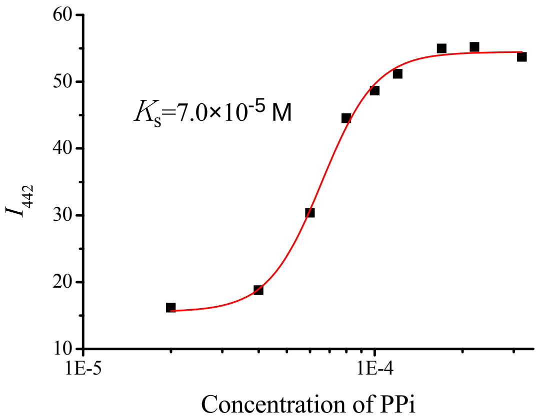

Fig. S6 Fit plot of the fluorescence intensity of I_{442} with log concentration of anion for sensor **1**-Zn (100 μM) upon addition of PPi. The spectra were measured excitation at 346 nm in CH_3CN-HEPES (9:1, v/v; pH=7.4).

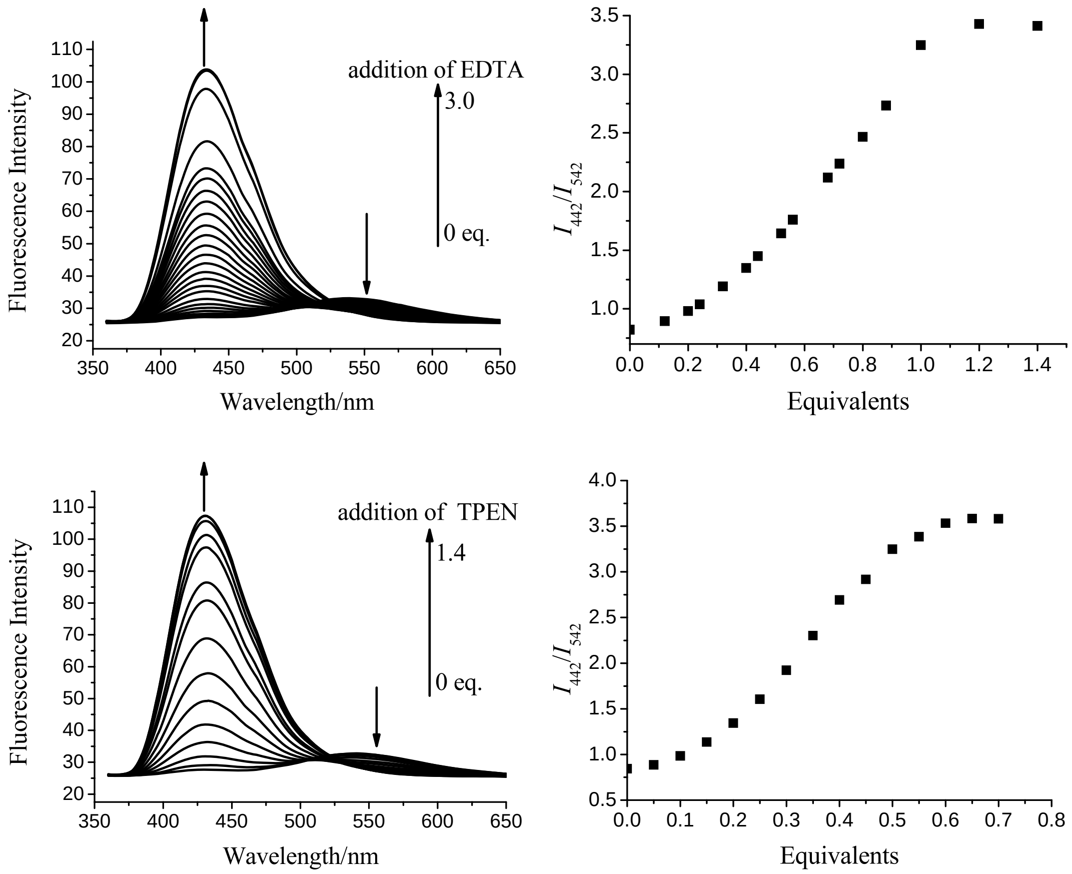

Fig S7 Change of fluorescence emission for sensor **1-Zn** (10 μM) upon addition of 0-3 eq. of EDTA (upper) and 0-1.4 eq. of TPEN (*N*,*N*,*N*′,*N*′-Tetrakis(2-pyridylmethyl)ethylenediamine) (bottom). The spectra were measured excitation at 346 nm in CH_3CN-HEPES (9:1, v/v; pH=7.4).

Reference:

S1. A. Gulyania, R. G. Srinivasa, G. U. Kulkarnib and S. Bhattacharyaa, *J. Mol. Struct*., 2002, **616**, 103.

Graphic abstract