Electronic Supplementary Information

Azobenzene-based System for Fluorimetric Sensing of H$_2$PO$_4^-$ (Pi) that Works as a Molecular Keypad Lock

Jing Wang and Chang-Sik Ha*

Department of Polymer Science and Engineering, Pusan National University, Busan 609-735, Korea
E-mail: csha@pusan.ac.kr

Experimental

1. Materials and Instrumentations

Unless otherwise noted, materials were obtained from commercial suppliers and were used without further purification. The Fourier-Transform Infrared (FTIR) spectra were recorded at room temperature on a JASCO FTIR 460 Plus operated at a resolution 4cm$^{-1}$. The $^1$H nuclear magnetic resonance (NMR) spectra were recorded on a Varian Gemimi-2000 (300 MHz) spectrometer. Electrospray Ionization (ESI) Mass-Spectrometry was performed using an agilent 1100 LC/MSD SL instrument. All spectroscopic measurements were performed in a mixture of H$_2$O-MeOH (1/1, v/v, 10 mM HEPES (4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid) buffer solution, pH 7.5). The UV–vis spectra were measured using a Hitachi U-2010 spectrometer at 25 °C.
The fluorescence emission spectra were measured using a Hitachi F-4500 spectrometer at 25 °C. Excitation slit size was set at 5 nm or 10 nm and emission slit size was set at 10.0 nm. Scan speed was set at 240 nm/min.

2. Synthesis of complex DHAB-Zn(II)

An aqueous solution of ZnCl$_2$ (0.043 g, 0.32 mmol) was added dropwise to 2, 2’ – dihydroxyazobenzene (DHAB) (0.069 g, 0.32 mmol) in 25 mL MeOH, and the mixture was stirred for 12 h at room temperature. Then the precipitate was filtered and washed with cold water to give complex DHAB-Zn(II) (0.081g, 91 %) as a light red powder. MS (ESI) [M+Zn(II)-H+] 278.2; $^1$H NMR (300 MHz, CH$_3$OH/H$_2$O, TMS): 6.81–7.94 (m, 16H); IR: (KBr, disc): $\nu$ = 3481.3, 3055.2, 1612.1, 1571.2, 1472.2, 1350.1, 1317.5, 1261.9, 944.5, 872.4, 743.9 cm$^{-1}$.

3. Calculation of the association constant ($K_{ass}$) between DHAB and Zn(II)

The relationship among absorbance of the mixture (A), the initially prepared DHAB concentration ($C_0$), and the concentration of zinc ions ($C_g$) can be expressed by the following equation:\textsuperscript{1}

$$\frac{C_0}{A - \epsilon_M C_0} = \left[ \frac{1}{K_a(\epsilon_D - 2\epsilon_M)} \right] \frac{1}{C_0 C_g} + \frac{4}{\epsilon_D - 2\epsilon_M}$$
Where $\varepsilon_M$ and $\varepsilon_D$ are molar extinction coefficients of the DHAB in the absence and presence of zinc ions respectively, at a wavelength of 499 nm. The association constant $K_{ass}$ can thus be calculated to be $1.94 \times 10^9$ M$^{-2}$, extracted from intercept/[4 × slope].


Fig. S1 Fluorescence emission spectra of DHAB ($2 \times 10^{-5}$ M, PH 7.5) in a mixture of H$_2$O-MeOH (1:1, v/v, 10 mM HEPES) upon progressive addition of Zn(II), $\lambda_{ex}=436$ nm. Excitation and emission slit width were both set at 5.0 nm.
Fig. S2. (Left) fluorescence emission spectra of DHAB/Zn(II) ($3 \times 10^{-5}$ M/$3 \times 10^{-5}$ M, PH 7.5) in a mixture of H$_2$O-MeOH (1:1, v/v, 10 mM HEPES) upon addition of various anions (100 equiv of DHAB); $\lambda_{ex}$=436 nm; (right) change of fluorescence intensity of DHAB/Zn(II) ($3 \times 10^{-5}$ M / $3 \times 10^{-5}$ M, PH 7.5) at 610 nm. I and I$_0$ are the fluorescence intensity at 610 nm in the presence and absence of various anions, respectively. Excitation and emission slit width were both set at 5.0 nm.

Fig. S3 Fluorescence emission spectra of DHAB/Zn(II) ($3 \times 10^{-5}$ M/$3 \times 10^{-5}$ M, PH 7.5) in a mixture of H$_2$O-MeOH (1:1, v/v, 10 mM HEPES) upon progressive addition of CN$^-$. $\lambda_{ex}$=436 nm. Excitation and emission slit width were both set at 10.0 nm.
Fig. S4 Fluorescence emission spectra of DHAB/Zn(II) (3 × 10^{-5} M/ 3 × 10^{-5} M, PH 7.5) in a mixture of H_{2}O-MeOH (1:1, v/v, 10 mM HEPES) upon progressive addition of H_{2}PO_{4}^{-}. \lambda_{ex}=436 \text{ nm}. Excitation and emission slit width were both set at 10.0 \text{ nm}.

Fig. S5 The plot with \(I_{0}/(I - I_{0})\) versus \([G]^{-2}\). \(I\) and \(I_{0}\) are the fluorescence intensity of DHAB-Zn(II) at 610 nm in the presence and absence Pi anions, respectively. \([G]\) is the concentration of Pi anions.
Fig. S6 Fluorescence emission spectra of DHAB-Zn(II) (1 × 10^{-5} M, PH 7.5) in a mixture of H2O-MeOH (1:1, v/v, 10 mM HEPES) upon progressive addition of CN\(^{-}\). \(\lambda_{ex}=436\) nm. Excitation and emission slit width were set at 5nm and 10.0 nm, respectively.

Fig. S7 Fluorescence spectra of DHAB and complex DHAB-Zn(II) with different inputs in a mixture of H2O-MeOH (1:1, v/v, 10 mM HEPES, PH 7.5). The letters P, U, S, A, N represent DHAB (1 × 10^{-5} M), Zn(II) (1 × 10^{-5} M), complex DHAB-Zn(II) (1 × 10^{-5} M), CN\(^{-}\) (3 × 10^{-5} M) and H2PO4\(^{-}\) (1.5 × 10^{-4} M), respectively. Excitation and emission slit width were both set at 5.0 nm.
Fig. S8 (Left) The effect of Zn(II) and CN\textsuperscript{−} on the fluorescence spectra of DHAB. The letters P, U and A represent DHAB (1 × 10\textsuperscript{-5} M), Zn(II) (1 × 10\textsuperscript{-5} M) and CN\textsuperscript{−} (3 × 10\textsuperscript{-5} M), respectively. PUA represents sequence 1: Zn(II) (IN1) and CN\textsuperscript{−} anions (IN2); PAU represents sequence 2: Zn(II) (IN2) and CN\textsuperscript{−} anions (IN1); (right) The effect of CN\textsuperscript{−} and H\textsubscript{2}PO\textsubscript{4}\textsuperscript{−} on the fluorescence spectra of complex DHAB-Zn(II). The letters S, A and N represent complex DHAB-Zn(II) (1 × 10\textsuperscript{-5} M), CN\textsuperscript{−} (3 × 10\textsuperscript{-5} M) and H\textsubscript{2}PO\textsubscript{4}\textsuperscript{−} (1.5 × 10\textsuperscript{-4} M), respectively; SAN represents sequence 1: CN\textsuperscript{−} (IN1) and Pi anions (IN2); SNA represents sequence 2: CN\textsuperscript{−} (IN2) and Pi anions (IN1). Note that the output is defined as '0' or '1' depending on whether the fluorescence intensity here is lower or higher than 40, respectively.