A novel NIR fluorescent turn-on sensor for the detection of pyrophosphate anion in complete water system

Weihong Zhu,* Xiaomei Huang, Zhiqian Guo, Xumeng Wu, Huihui Yu and He Tian

Shanghai Key Laboratory of Functional Materials Chemistry, Key Laboratory for Advanced Materials and Institute of Fine Chemicals, East China University of Science & Technology, Shanghai 200237, P. R. China. Fax: (+86) 21-6425-2758. E-mail: whzhu@ecust.edu.cn.

Contents:

1. Experimental

1.1 General

1.2 Synthesis of (E)-diethyl 2,2'-(4-(2-(4-(dicyanomethylene)-4H-chromen-2-yl)vinyl)phenyl)azanediyl)diacetate (DCEA)

1.3 Synthesis of (E)-2,2'-(4-(2-(4-(dicyanomethylene)-4H-chromen-2-yl)vinyl)phenyl)azanediyl)diacetic acid (DCAA)

2. Absorption spectra and emission spectra of DCAA upon titration of Cu$^{2+}$ (Fig. S1 - Fig. S3)

3. Emission spectra of DCAA-Cu$^{2+}$ upon titration of amino acids such as Glycine and Histidine (Fig. S4)

4. Emission spectra of DCAA-Cu$^{2+}$ upon titration of PPi (Fig. S5 - Fig. S7)

5. $^1$H NMR, $^{13}$C NMR and HRMS characterization of DCEA (Fig. S8 - Fig. S10)

6. $^1$H NMR, $^{13}$C NMR and HRMS characterization of DCAA (Fig. S11 - Fig. S13)
1. Experimental

1.1 General

All solvents were of analytical grade. Diethyl 2,2'-(4-formylphenyl)azanediyl)diacetate (2) was synthesized from diethyl 2,2'-(phenylazanediyl)diacetate (1) via Vilsmeier formylation in 60% yield (X. Meng, M. Z. Zhu, L. Liu and Q. X. Guo. *Tetrahedron Lett.*, 2006, **47**, 1559). The intermediate of 2-(2-methyl-4H-chromen-4-ylidene)malononitrile was prepared by the established literature procedure (G. G. Badcock, F. M. Dean, A. Robertson and W. B. Whalley, *J. Chem. Soc.*, 1950, 903). $^1$H NMR and $^{13}$C NMR in CDCl$_3$ or DMSO-$d_6$ were measured on a Bruker AV-400 spectrometer with tetramethylsilane (TMS) as internal standard. High Resolution Mass spectra (HRMS) were measured on a Waters LCT Premier XE spectrometer. UV-vis spectra were obtained with a Varian Cary 500 spectrophotometer (1 cm quartz cell) at 25 °C. Fluorescent spectra were recorded on a Varian Cary Eclipse fluorescence spectrophotometer (1 cm quartz cell) at 25 °C.

**Cell culture:** A human nasopharyngeal epidermal carcinoma cell line (KB cell) was provided by the Institute of Biochemistry and Cell Biology, SIBS, CAS (China). Cells were grown at 37°C and with 5% CO$_2$ in Roswell Park Memorial Institute medium (RPMI) 1640 supplemented with 10% fetal bovine serum (FBS). Cells ($5 \times 10^8$ L$^{-1}$) were plated on 18 mm glass coverslips and allowed to adhere for 24 h. The cells were washed three times with PBS buffer, and the medium was replaced with PBS buffer before imaging.

**Microscopy and imaging methods:** Confocal luminescence imaging: Confocal
luminescence imaging of cells was performed with a modified Olympus FV1000 laser-scanning microscope. A 60 × oil-immersion objective lens was used. Excitation was carried out with a semiconductor laser at $\lambda = 405$ nm, and emission was collected in the range $\lambda = 630 - 730$ nm, including the maximum emission wavelength of DCAA. KB cells were incubated with a PBS solution of DCAA-Cu$^{2+}$ (10 $\mu$M) for dye loading for 0.5 h at 37°C, then the cells were incubated with a PBS solution of PPi (30 $\mu$M) for 0.5 h at 37°C. The stained cells were washed three times with PBS buffer. Then the treated cells were imaged by fluorescence microscopy.

1.2 Synthesis of ($E$)-diethyl 2,2'-(4-(2-(4-(dicyanomethylene)-4H-chromen-2-yl)vinyl)phenyl)azanediyl)diacetate (DCEA)

2-(2-methyl-4H-chromen-4-ylidene)malononitrile (0.62 g, 3.0 mmol) and diethyl 2,2'-(4-formylphenyl)azanediyl)diacetate (1.09 g, 3.7 mmol) were dissolved in toluene (40 ml) with piperidine (0.5 ml) and acetic acid (0.5 ml) under argon protection at room temperature. Then the mixture was refluxed and stirred for 8 h. The solvent was evaporated in vacuo, and the crude solid was purified by column chromatography on silica gel eluting with petroleum ether/ethyl acetate (5/1, v/v) to afford a red solid in 48% yield. $^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 8.92 (d, $J = 8.4$ Hz, 1H, phenyl-H), 7.71 (t, $J = 6.8$ Hz, 1H, phenyl-H), 7.56 (d, $J = 15.6$ Hz, 1H, alkene-H), 7.54 (d, $J = 3.2$ Hz, 1H, phenyl-H), 7.45 (m, 3H, phenyl-H), 6.81 (s, 1H, pyran-H), 6.65 (d, $J = 8.8$ Hz, 2H, phenyl-H), 6.62 (d, $J = 15.6$ Hz, 1H, alkene-H), 4.25 (q, 4H, $-CH_2CH_3$), 4.19 (s, 4H, $-NCH_2$), 1.30 (t, $J = 6.8$ Hz, 6H, $-CH_2CH_3$). $^{13}$C NMR (100 MHz, CDCl$_3$): $\delta$ 170.09, 158.48, 152.88, 152.37, 149.83, 139.18, 134.36, 129.88,
125.78, 125.74, 124.73, 118.51, 117.94, 117.24, 116.17, 114.55, 112.60, 105.78, 61.50, 61.07, 53.37, 14.25. HRMS (ESI): calcd for C$_{28}$H$_{24}$N$_{3}$O$_{5}$ [M - H]$^-$ 482.1716, found 482.1715.

1.3 Synthesis of (E)-2,2'-(4-(2-(4-(dicyanomethylene)-4H-chromen-2-yl)vinyl)phenyl)azanediyl)diacetic acid (DCAA)

(E)-diethyl 2,2'-(4-(2-(4-(dicyanomethylene)-4H-chromen-2-yl)vinyl)phenyl)azanediyl)diacetate (0.15 g, 0.31 mmol) was dissolved in a 1:1 THF/CH$_3$OH mixture (30 ml), and treated with 10 equiv. of lithium hydroxide (0.13 g, 3.1 mol). The mixture was reacted at room temperature for 5 min, at which time TLC analysis showed complete consumption of starting material. The reaction was evaporated to dryness, then the crude product was purified by silica gel column chromatography (CH$_2$Cl$_2$/MeOH = 15:1 v/v) to give DCAA as a red solid (0.13 g, 48% yield). $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$ 8.72 (d, $J = 8.0$ Hz, 1H, phenyl-H), 7.89 (t, $J = 7.6$ Hz, 1H, phenyl-H), 7.80 (d, $J = 8.3$ Hz, 1H, phenyl-H), 7.69 (d, $J = 16.0$ Hz, 1H, alkene-H), 7.59 (m, 3H, phenyl-H), 7.20 (d, $J = 16.0$ Hz, 1H, alkene-H), 6.91 (s, 1H, pyran-H), 6.52 (d, $J = 8.1$ Hz, 2H, phenyl-H), 4.05 (s, 4H, -NCH$_2$-). $^{13}$C NMR (100 MHz, DMSO-$d_6$): $\delta$ 173.40, 159.35, 152.60, 152.01, 139.89, 135.02, 130.18, 125.86, 124.47, 118.93, 117.13, 116.29, 113.78, 111.44, 105.10, 79.27, 78.94, 78.61. HRMS (ESI): calcd for C$_{24}$H$_{16}$N$_{3}$O$_{5}$ [M – 2Li + H]$^-$ 426.1090, found 426.1089.
2 Absorption spectra and emission spectra of DCAA upon titration of Cu$^{2+}$
**Fig. S1** Absorption spectra (A) and emission spectra (B) of DCAA (10 μM) with excitation wavelength at 500 nm in MOPS (10 mM, pH = 7.0) buffer solution of upon titration of Cu$^{2+}$ (0, 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4, 1.5, 1.6, 1.8, 2.0, 3.0, 5.0 equiv.). Inset: the job plots with both absorption and fluorescence titrations exhibited a maximum at about 0.33 mol fractions, indicating that DCAA forms a 2:1 complex with Cu$^{2+}$. (C) The possible sensing mechanism of DCAA-Cu and PPi, and the high resolution mass spectra (HRMS) of DCAA-Cu. Note, the mass peak for DCAA-Cu$^{2+}$ at m/z 915.1472 corresponding to [C₄₈H₃₂N₆O₁₀Cu]⁻ (= [2DCAA + Cu$^{2+}$ + 2H]⁻ calculated as 915.1476) gives strong evidence that DCAA forms a 2:1 complex with Cu$^{2+}$. 
**Fig. S2** Fluorescence spectra of DCAA in the absence and presence of Cu$^{2+}$ and the addition of EDTA.
Fig. S3 A) Fluorescence changes during the titration of DCAA (3 μM) with Cu$^{2+}$ (0-10 equiv.) in MOPS (10 mM, pH = 7.0) buffer solution; B) A plot of $(I - I_{\text{min}}) / (I_{\text{max}} - I_{\text{min}})$ vs Log([Cu$^{2+}$]), the calculated detection limit of DCAA is $1.26 \times 10^{-6}$ M.
3  Emission spectra of DCAA-Cu$^{2+}$ upon titration of amino acids such as
Glycine and Histidine

![Graph A](image1)

![Graph B](image2)

Fig. S4 Emission change of DCAA-Cu$^{2+}$ (10 µM) in an aqueous solution buffered
with MOPS (10 mM, pH = 7.0) upon titration with 50.0 equiv. of Histidine and
Glycine.
4 Emission spectra of DCAA-Cu$^{2+}$ upon titration of PPi

![Emission spectra of DCAA-Cu$^{2+}$ upon titration of PPi](image)

**Fig. S5** Emission change of DCAA-Cu$^{2+}$ (10 µM) in an aqueous solution buffered with MOPS (10 mM, pH = 7.0) upon titration with 15 equiv. of PPi.
Fig. S6  A) Fluorescence changes during the titration of DCAA-Cu$^{2+}$ (3 μM) with PPI (0-20 equiv.) in MOPS (10 mM, pH = 7.0) buffer solution; B) A plot of $(I - I_{\text{min}}) / (I_{\text{max}} - I_{\text{min}})$ vs Log([PPI]), the calculated detection limit of DCAA-Cu$^{2+}$ is 2.02 × 10$^{-6}$ M.
**Fig. S7** Emission change of DCAA-Cu\(^{2+}\) (10 µM) in an aqueous solution buffered with MOPS (10 mM, pH = 7.0) upon titration with 15 equiv. of PPI and 150 equiv. of Cu\(^{2+}\). Note: The chemosensor detection of PPI is reversible. When PPI was added gradually to the solution of DCAA-Cu\(^{2+}\), the fluorescence intensity was obviously enhanced and stabilized upon addition of 15 equiv. of PPI. Reversibly, when adding exceeded Cu\(^{2+}\) to the solution above, the fluorescence intensity was quenched gradually and it’s quenched completely in 150 equiv. of Cu\(^{2+}\).
5 $^1$H NMR, $^{13}$C NMR and HRMS characterization of DCEA

![Fig. S8 $^1$H NMR spectra of DCEA in CDCl$_3$](image)

**Fig. S8** $^1$H NMR spectra of **DCEA** in CDCl$_3$

![Fig. S9 $^{13}$C NMR spectra of DCEA in CDCl$_3$ (100 MHz)](image)

**Fig. S9** $^{13}$C NMR spectra of **DCEA** in CDCl$_3$ (100 MHz)
Fig. S10 High resolution mass spectra (HRMS) of DCEA

6 $^1$H NMR, $^{13}$C NMR and HRMS characterization of DCAA

Fig. S11 $^1$H NMR spectra of DCAA in DMSO-$d_6$
**Fig. S12** $^{13}$C NMR spectra of DCAA in DMSO-$d_6$ (100 MHz)

**Fig. S13** High resolution mass spectra (HRMS) of DCAA