

A colorimetric and fluorescent turn-on sensor for pyrophosphate anion based on a dicyanomethylene-4*H*-chromene framework†

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A new red fluorescent sensor DCCP-Cu²⁺ based on dicyanomethylene-4*H*-chromene shows turn-on fluorescence with high selectivity for pyrophosphate over other anions.

The design and synthesis of highly sensitive and selective chemosensors capable of biologically binding important anions continues to be an active area in supramolecular chemistry.¹ Particularly, pyrophosphate anion (PPi) plays an important role in bioenergetic and metabolic processes such as cellular signal transduction and protein synthesis.² Therefore, the development of fluorescent sensors for PPi has become a focus of considerable research in biological markers due to their advantages such as high sensitivity, low background noise, and wide dynamic ranges.³ Up to now, some examples of selective recognition sensors for PPi^{4,5} have been reported, but only a few examples can display turn-on in emission spectra.^{4*b,d,5b*} Furthermore, emission spectra in the red or near infra-red (NIR) region are beneficial to their application as biological anion sensors in biological samples with much lower background signal and smaller scattering being obtained along with little damage to living cells.^{6*b*}

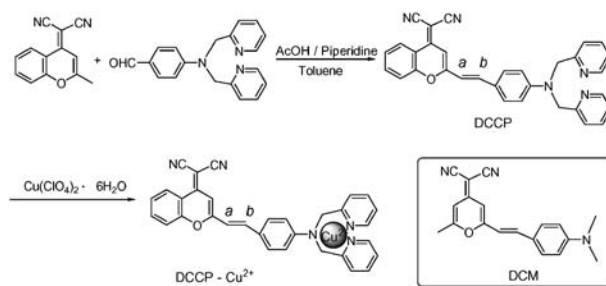
With this in mind, herein we present a novel colorimetric and fluorescent turn-on PPi sensor DCCP-Cu²⁺ (Scheme 1) possessing both dicyanomethylene-4*H*-chromene and bis(2-pyridylmethyl) amine (DPA) moieties. Notably, we attached a benzene unit to a dicyanopyran moiety, and extended the conjugation system with an emission band centered at about 650 nm, thus successfully making the determining wavelength fall in the desired NIR region.⁶ To the best of our knowledge, this is the first red turn-on fluorescence sensor for PPi based on an intramolecular charge-transfer (ICT) mechanism.

DCCP is derived from a well known laser dye, DCM (4-dicyanomethylene-2-methyl-6-[4-(dimethylamino)styryl]-4*H*-pyran, Scheme 1).⁷ The synthesis of sensor DCCP is straightforward as depicted in Scheme 1; it was obtained in 48% yield from the treatment of (2-methylchromen-4-ylidene)malononitrile and 4-[bis(pyridin-2-ylmethyl)amino]benzaldehyde in the presence of acetic acid, piperidine and toluene with concomitant removal of water. The chemical structures were

characterized by ¹H NMR, ¹³C NMR, and HRMS as shown in the Electronic Supplementary Information (ESI).† In ¹H NMR of DCCP, the characteristic coupling constant ($J = 15.6$ Hz) of protons (*a*) and (*b*) is indicative of the predominant *trans*-isomer (Scheme 1).

The intramolecular charge transfer (ICT) mechanism has been widely exploited for ion sensing and molecular switching,^{3*a,b,e*} even for molecular logic gates.⁸ As a matter of fact, DCCP is also D-π-A type with a broad absorption band resulting from an ultra-fast process of ICT. As shown in Fig. S1B,† free DCCP shows a characteristic emission band at around 650 nm with high efficiency ($\Phi_{\text{DCCP}} = 0.40$)⁹ resulting from the ICT process. Notably, incorporating a benzene unit into a dicyanopyran moiety can result in a remarkable red shift of about 55 nm in the emission spectra with respect to DCM ($\lambda_{\text{em}} = 595$ nm). In DCCP, the DPA moiety acts as an electron-donating substituent whose donor character strongly depends on cation coordination. If the electron-donating character of the DPA moiety is reduced, hypsochromic shifts in both absorption and fluorescence spectra of DCCP are expected, resulting from a decrease in ICT efficiency.¹⁰

In order to further investigate DCCP, the fluorescence behavior of DCCP toward various metal ions was studied in a mixture of ethanol-water (60 : 40, v/v) with a buffer solution of 3-(*N*-morpholino)propanesulfonic acid (MOPS, 10 mM, pH = 7.0) on the basis of the effect of water on fluorescence (Fig. S2).† Metal ions such as Na⁺, Zn²⁺, Ag⁺, Fe³⁺, Fe²⁺, Ni²⁺, Cd²⁺, Hg²⁺, and Pb²⁺ also show subtle disturbance (Fig. 1), but only the addition of Cu²⁺ to DCCP causes a significant decrease of fluorescence intensity acting as an efficient fluorescence quencher. Generally, DPA has been extensively used as a Zn²⁺ ligand for its high affinity and excellent selectivity.¹¹ Unexpectedly, there is a slight change in the emission spectra of DCCP with the titration of Zn²⁺.



Scheme 1 Synthesis of DCCP-Cu²⁺ and chemical structure of DCM.

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† Electronic supplementary information (ESI) available: Synthesis and full characterization of DCCP, Fig. S1–S14 and Table S1. See DOI: 10.1039/b809983a

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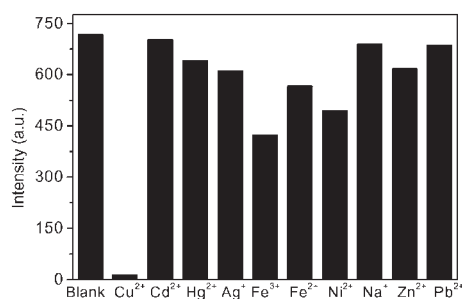


Fig. 1 Fluorescent intensity change of DCCP (10 μM) in a mixture of ethanol–water (60 : 40, v/v) with a buffer solution of MOPS (10 mM, pH = 7.0) in the presence of various metal ions (50 μM , λ_{ex} = 505 nm).

In the system of DCCP, we found that Cu^{2+} showed stronger binding affinity, almost two orders of magnitude higher than Zn^{2+} and Ni^{2+} , which might be the result of the slight change in the emission spectra of DCCP with the titration of Zn^{2+} (Fig. S3–S5, Table S1).[†] As illustrated in Fig. S1A,[†] the presence of an isosbestic point at 447 nm clearly reveals the presence of an equilibrium process corresponding to free DCCP and copper complex DCCP-Cu^{2+} . The maximum absorption peak also exhibits an obvious hypsochromic shift from 505 to 413 nm upon titration of Cu^{2+} with DCCP (Fig. S1A),[†] accompanied by a color change from light pink to pale yellow which can be seen by the naked eye. It indicates that the capture of Cu^{2+} by the receptor DPA can result in a decrease of the electron-donating ability of the amino groups, thus resulting in a decrease in ICT efficiency.^{11e} Moreover, both the UV/Vis titration curve and the linear decrease of fluorescence intensity within the equivalent range of Cu^{2+} ion indicate that DCCP forms a 1 : 1 complex with Cu^{2+} , whose association constant (K_{ass}) is determined to be about $1.1(2) \times 10^6 \text{ M}^{-1}$ from the UV/Vis titration curve (Fig. S3).^{†12}

The complex DCCP-Cu^{2+} is easily obtained at room temperature by the addition of an aqueous solution of $\text{Cu}(\text{ClO}_4)_2 \cdot 6\text{H}_2\text{O}$ to a methanolic solution of DCCP. Unfortunately, the $^1\text{H-NMR}$ spectrum of DCCP-Cu^{2+} does not provide useful information due to the paramagnetic character of Cu^{2+} . An intensive peak at m/z 556.14 provides strong evidence for DCCP-Cu^{2+} in the electron-spray ionization (ESI) HPLC-MS measurements. Moreover, the peaks at 1097 and 620 cm^{-1} in the infra-red (IR) spectra corresponding to the characteristic vibration of ClO_4^- also confirm the formation of complex DCCP-Cu^{2+} (Fig. S6).[†]

Previously, many sensors have relied on a metal coordination effect to detect anions.¹³ Since the d^9 electronic configuration of Cu^{2+} displays strong binding tendencies towards anion substrates, it can recognize anions effectively *via* ensuring high stabilization effects on the ligand field.^{4b} In addition, the titration of Cu^{2+} with DCCP can almost completely quench fluorescence to the baseline. Bearing in mind all the above considerations, it is reasonable to predict that DCCP-Cu^{2+} might be designed as a turn-on fluorescent sensor for specific anions.

The selectivity and sensitivity of DCCP-Cu^{2+} have been examined with various anions (Fig. S7),[†] including F^- , Cl^- , Br^- , I^- , H_2PO_4^- , HCO_3^- , HSO_4^- , CH_3COO^- , NO_3^- ,

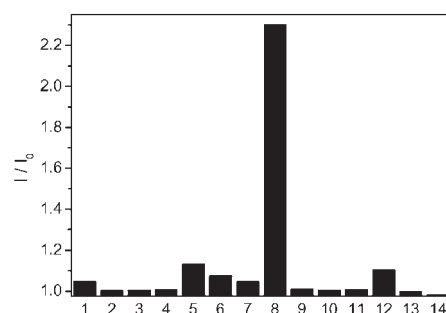


Fig. 2 Fluorescent response of DCCP-Cu^{2+} (10 μM) to various anions at 30 μM concentration in a mixture of ethanol–water (60 : 40, v/v) with a buffer solution of MOPS (10 mM, pH = 7.0): 1, F^- ; 2, Cl^- ; 3, Br^- ; 4, I^- ; 5, H_2PO_4^- ; 6, HPO_4^{2-} ; 7, PO_4^{3-} ; 8, $\text{P}_2\text{O}_7^{4-}$; 9, CO_3^{2-} ; 10, HCO_3^- ; 11, SO_4^{2-} ; 12, HSO_4^- ; 13, CH_3COO^- ; 14, NO_3^- .

HPO_4^{2-} , SO_4^{2-} , CO_3^{2-} , PO_4^{3-} (Pi), and $\text{P}_2\text{O}_7^{4-}$ (PPI). As illustrated in Fig. 2, unexpectedly, the addition of 3 equiv. of PPI to 10 μM DCCP-Cu^{2+} causes a distinctive increase of fluorescent intensity using the isosbestic point at 447 nm as excitation wavelength, while the disturbance of fluorescence intensity for other anions can be neglected. Obviously, DCCP-Cu^{2+} shows high selectivity and sensitivity towards PPI compared with other investigated anions including phosphate anion.

To get further insight into the binding of PPI and DCCP-Cu^{2+} , the absorption spectra of DCCP-Cu^{2+} upon titration with PPI were recorded; they exhibit an obvious increase at the peak of 505 nm with an isosbestic point at

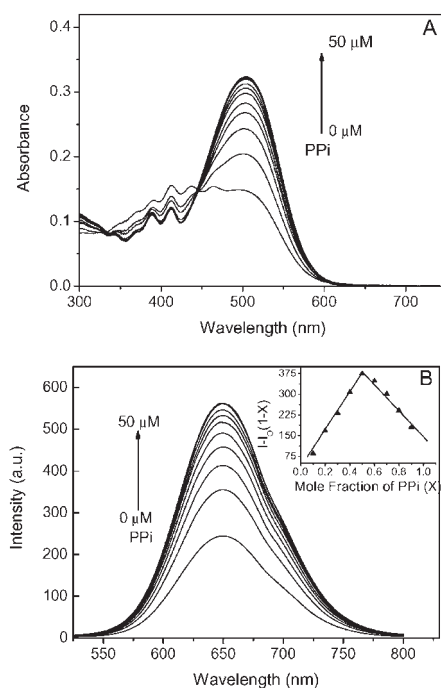


Fig. 3 Spectra of DCCP-Cu^{2+} (10 μM) upon titration with PPI from 0 to 50 μM in a mixture of ethanol–water (60 : 40, v/v) with a buffer solution of MOPS (10 mM, pH = 7.0): (A) absorption change; (B) emission change (λ_{ex} = 447 nm). Inset: Job's plot of DCCP-Cu^{2+} (the total concentration of DCCP-Cu^{2+} and PPI is 50.0 μM).

447 nm (Fig. 3A). The associated color change from pale yellow to light pink can be easily distinguished by the naked eye in Fig. S8.† At the same time, the fluorescence intensity at 650 nm is obviously enhanced (Fig. 3B). The enhancement of fluorescence intensity within the equivalent range of Cu^{2+} ion testifies that DCCP– Cu^{2+} forms a 1 : 1 complex with PPI, whose association constant (K_{ass}) is determined to be about $4.6 \times 10^5 \text{ M}^{-1}$ from the fluorimetric titration curve. Moreover, in ESI HPLC-MS (Fig. S9),† a high abundance peak at m/z 556.29 corresponding to DCCP– Cu^{2+} was clearly observed even when adding excess PPI (100 equiv.) to DCCP– Cu^{2+} , which indirectly indicates that PPI does not form a strong enough complex to remove Cu^{2+} from DCCP– Cu^{2+} . Therefore, the significant fluorescence enhancement might be resulting from the electrostatic interaction between PPI and DCCP– Cu^{2+} , in which two oxygen atoms of PPI coordinate with the center of Cu^{2+} (Fig. S10)† to reduce the magnitude of the electron withdrawal *via* partial neutralization of the charge on the Cu^{2+} ion,^{4b} thus increasing the electron-donating character of the DPA moiety and finally resulting in an increased efficiency of ICT.

In summary, a red turn-on fluorescent sensor DCCP– Cu^{2+} based on a dicyanomethylene-4*H*-chromene framework is presented which has high selectivity and sensitivity towards PPI. It has several advantages: the determining wavelength falling in the desired red or NIR region beneficial to good transmission and low autofluorescence in biological samples, fluorescent type turn-on to get the maximum signal-to-noise ratio, and determination with an internal standard using the isosbestic point at 447 nm as excitation wavelength to avoid troublesome calibration. Accordingly, DCCP– Cu^{2+} is a potential candidate for sensing biologically important anions in aqueous environments under physiological pH.

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