A Conjugated Polymer Fluorescent Sensor for Continuous Identification of Copper (II) and Pyrophosphate in Blood Serum and Synovial Fluid

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Abstract

A novel “on-off-on” super-sensitive conjugated polymer fluorescence sensor (PPE-DPA) was developed and realized the continuous recognition of Cu$^{2+}$ and pyrophosphate (PPI). The fluorescence intensity was decreased linearly with the change of Cu$^{2+}$ from 0.05 to 5.0 μmol L$^{-1}$ and the limit of detection was 24 nmol L$^{-1}$. And the fluorescence intensity was linearly enhanced with the increase of PPI from 0.5 to 12.0 μmol L$^{-1}$ and the limit of detection was 230 nmol L$^{-1}$. In addition, this method was applied to detect PPI in the blood serum and synovial fluid of patients with arthritis and satisfactory results were obtained. Thus, PPE-DPA not only provides an effective tool for detecting Cu$^{2+}$ and PPI in samples, but also gives a potential way to diagnose arthritis.

Keywords: Conjugated polymer, fluorescence sensor, Cu$^{2+}$, pyrophosphate, synovial fluid, blood serum
Introduction

Cu$^{2+}$ and pyrophosphate (PPi) are widely found in organisms and they play very important roles in life science, medicine and chemical processes. Cu$^{2+}$, as the third most abundant transition metal ion after Fe$^{3+}$ and Zn$^{2+}$ in the human body, is involved in many critical physiological processes. Besides, many diseases could be caused by the disorder of Cu$^{2+}$ in human body. Similarly, as one of the most important biological anions, PPi is produced by hydrolysis of adenosine triphosphate (ATP) catalyzed by hydrolases, and participates in the replication of DNA, processing of genetic information and other bioenergetic and metabolic processes. But some diseases would be caused by the abnormal concentrations of PPi, such as calcium pyrophosphate dehydrate crystals, chondrocalcinosis, vascular calcification, kidney stones, arthritis, hypophosphatasis, medial arteriosclerosis and so on. And the levels of intracellular PPi had become an important indicator in cancer and real time of DNA sequences research. In addition, it is reported that the levels of PPi in synovial fluid is higher for patients with arthritis than normal, but in serum is the same. So that, PPi could be a potential biomarker for the treatment and clinical diagnosis of arthritis. In a word, selective identification and detection of Cu$^{2+}$ and PPi are important for life science and disease diagnosis.

In recent years, fluorescence probes for separate identification of Cu$^{2+}$ and PPi had been widely reported, there are also some sensors for identifying PPi with DPA-M$^{n+}$ as a chelate group, but for continuous identification of Cu$^{2+}$ and PPi were still rarely developed. Obviously, for continuous identification sensors, the workload of the synthesis and the cost can be reduced, the operation is simpler and the efficiency is higher. Among all fluorescence probes, conjugated polymer (CP) fluorescence probes have unique $\pi$-$\pi^*$ conjugated molecular wire structure, which makes local perturbation can be amplified and utilized in the whole polymer molecular chain or even in the whole polymer system, namely, superquenching phenomenon.
And, this property determines that CP have the ability to detect ultra-low content of substances to be measured, and show stronger sensitivity than small molecular fluorescence sensors.\textsuperscript{31,32}

Our research was focus on how to use the advantage of CP to develop a fluorescence sensor for Cu$^{2+}$ and PPI.

In our previous research works, carboxylate sodium or guanidinium side groups was modified to the side chain of PPE\textsuperscript{32, 33} and these conjugated polymers are not responsive to copper ion. In this paper, a new “on-off-on” relay identification CP fluorescence sensor, poly(2,5-bis(4-(bis(pyridin-2-ylmethyl)amino)butoxyl)-1,4-Phenylethylenylene-1,4-poly(phenylene ethynylene)) (PPE-DPA), was synthesized successfully according to DPA has a certain complexation effect on Cu$^{2+}$. Based on the fluorescence “on-off-on” phenomenon, the continuous recognition of Cu$^{2+}$ and PPI were realized. Through optimization experiments and titration experiments, it was resulted that detection of Cu$^{2+}$ and PPI had good selectivity, high sensitivity, good linear range and low detection limit. Most importantly, the PPE-DPA has been successfully applied to detect PPI in blood serum and synovial fluid of patients with arthritis and the results were consistent with the previous literatures\textsuperscript{16,34,35}. Therefore, PPE-DPA provides a new feasible method for the quantitative determination of PPI in synovial fluid and blood serum, thus achieving the diagnosis of arthritis.

**Experimental**

*Reagents and chemicals*

1, 4-Diethynylbenzene (96%), (PPh$_3$)$_4$Pd (99%) were purchased from J&K SCIENTIFIC LTD (Beijing, China). NaCl, HCl, NaOH, K$_2$CO$_3$, Na$_2$S$_2$O$_3$, KH$_2$PO$_4$, Na$_2$HPO$_4$·12H$_2$O and NaH$_2$PO$_4$·2H$_2$O, H$_3$PO$_4$, CH$_3$COOH, sulfuric acid (98%) and H$_3$BO$_3$ were purchased from Beijing Chemical Works (Beijing, China). 1,4-dimethoxybenzene, iodine, potassium iodate,
boron tribromide, 1,4-dibromobutane, CuI and DPA were purchased from Energy chemical technology co., LTD (Shanghai, China). Triethylamine and diisopropylamine were purchased from Tianjin Fu Yu Chemical Co., Ltd. (Tianjin, China). Dichloromethane, methanol, ethanol, tetrahydrofuran, N,N-Dimethylformamide, acetonitrile, acetone, ether, petroleum ether and acetic ether purchased from Tianjin Tiantai Fine Chemical Co., Ltd. (Tianjin, China). The solutions of metal ions and anions were prepared from their salts which were purchased from Beijing Chemical Reagent Corporation (Beijing, China). Moreover, the counter-anion of metal ion salts is generally Cl\(^{-}\), SO\(_4^{2-}\) or NO\(_3^{-}\), and the counter-cation of anion salts is generally Na\(^{+}\) or K\(^{+}\). Anhydrous MgSO\(_4\) and Na\(_2\)SO\(_4\) were purchased from Tianjin Dong Li Big Chemical Reagent Factory (Tianjin, China). Heps was purchased from Promega Corporation. Tris was purchased from Sinopharm Chemical Reagent Co., Ltd. Thin layer chromatography (TLC) was carried out using silica gel 60 F254, and column chromatography was conducted over silica gel (300-400 mesh), both of which were obtained from the Qingdao Ocean Chemicals (Qingdao, China). Twice-distilled water was used throughout all experiments. All chemicals were analytical reagent and were used without further treatment. For the practicality experiments, blood serum 1, blood serum 2, synovial fluid 1 and synovial fluid 2 were obtained from in the second affiliated hospital of Jilin University in Changchun, China.

**Apparatus**

The nuclear magnetic resonance spectroscopy (NMR) of compounds were identified by \(^1\)H NMR and \(^{13}\)C NMR (Varian Mercury YH-400 NMR spectrometer), using tetramethylsilane (TMS) as an internal standard. ESI Mass spectra were obtained using a Q-Trap 2000 (Applied Biosystems Corporation, American) without using the liquid phase part. MOLDI-TOF Mass spectra were obtained using a Autoflex speed TOF by Brucker. The average molecular weight and the molecular weight distribution were determined by GPC (Agilent 1260). All fluorescence
measurements were carried out in a 1 cm path length quartz cuvette with a Hitachi F-2700 spectrometer (Shimadzu Corporation, Japan). Fluorescence quantum yields was carried out with a FLS920 steady state and transient state fluorescence spectrometer (Edinburgh Instrument). All pH measurements were tested with a Sartorius PB-10 digital pH meter. All the optical measurements were carried out at room temperature (298 K) under ambient conditions.

Synthesis of PPE-DPA

PPE-DPA was synthesized by introducing di-(2-picolyl) amine (DPA) into the side chains of poly(phenylene) ethynylene (PPE), and the specific synthetic route consisting of 5 steps are shown in Scheme 1. The first step was to synthesize 1,4-diodo-2,5-dimethoxybenzene (M1) from 1,4-dimethoxybenzene with iodine and potassium iodate; then the second step was to remove methyl group from methoxy group of M1 to get 1,4-diodo-2,5-dihydroxy- benzene (M2) with boron tribromide. Thirdly, the hydroxyl of M2 was halogenated with 1,4-dibromobutane to give 1,4-bis(4-bromobutoxy)-2,5-diiodobenz-ene (M3). The fourth step was to turn M3 into monomer of PPE-DPA (M4) through ammonolysis reaction with DPA. The last step was Sonogashira-coupling reaction, namely, the PPE-DPA was obtained by polymerization of M4 and 1,4-diethynylbenzene (specific operations are seen in SI). As the GPC characterization showed, PPE-DPA featured the average molecular weight of 7230 (PDI=1.120, about 9 repeat units). All syntheses were carried out under argon atmosphere.

Experimental Method

A stock solution of PPE-DPA in DMSO was prepared with a concentration of 0.5 mmol L⁻¹ and the concentration was calculated with respect to its monomer unit. The standard solutions of some metal ions, anions and PPi (1.0 mmol L⁻¹) mentioned above were prepared with distilled water. When the stock solution of PPE-DPA was diluted to 5.0 μmol L⁻¹, a
fluorescence emission peak was found at 420 nm upon excitation at 310 nm. Then, 5.0 μmol L⁻¹ metal ion solution was added and fluorescence emission intensity was measured after the mixture reacted for 20 min. Next, to the same solution above, was added 12.0 μmol L⁻¹ solution of PPi, which also needed to react 20 min, and then the fluorescence emission signal was recorded.

Preparation of Practical Sample Solutions

At first, the synovial fluid of arthritis patients and blood serum were purified by centrifugation to remove proteins which may affect the detection of PPi. After centrifugation, the synovial fluid and blood serum were diluted 6 times with Hepes buffer. Then the resulting solutions were referred to as the practical sample solutions.

Results and Discussion

Spectral properties of PPE-DPA

The excitation and emission spectra of PPE-DPA measured was shown in Fig.1 (a) and (b). The strong fluorescence emission peak at 420 nm was obtained when 310 nm was selected as the experimental excitation wavelength. In addition, it could be found that the strong fluorescence emission peak of PPE-DPA (5.0 μmol L⁻¹) can be quenched to about 18% of the initial strength due to Cu²⁺ (5.0 μmol L⁻¹) existed in Fig. 1 (c). And it could be seen from Fig. 1 (d) that the weak fluorescence emission peak was increased to 70% of the original intensity by adding PPi solution (12.0 μmol L⁻¹). Meanwhile, the fluorescence quantum yield of PPE-DPA (5.0 μmol L⁻¹) was also measured. The absolutely fluorescence quantum yield is 2.48%. Based on this fluorescent “on-off-on” phenomenon, a novel fluorescent CP sensor with DPA for label-free detection of Cu²⁺ and its complex with Cu²⁺ for detecting PPi were studied.
The specific sensing mechanism was shown in Fig. 2. PPE acted as fluorophore in this sensor and then its side chain was modified by DPA which has the advantages of fast metal ion coordination rate, good biocompatibility, high selectivity and so on. The action of DPA with Cu$^{2+}$ led to the photo-induced electron transfer (PET) process from the fluorophore to the Cu$^{2+}$, showing the fluorescence quenching.\textsuperscript{36,37} While, the oxygen atoms on the P-O in PPI have strong coordination with Cu$^{2+}$.\textsuperscript{28} The addition of PPi to the above system would be competed with the PPE-DPA system in the binding of Cu$^{2+}$, and then led to a partial recovery in the fluorescence intensity of PPE-DPA-Cu$^{2+}$ by blocking the PET pathway. Therefore, this label-free fluorescence CP sensor can be used to detect continuously Cu$^{2+}$ and PPI.

\textit{Optimization of testing conditions}

In order to get a better as well as a more stable fluorescence phenomenon, conditions were explored, including solvent, pH, buffer solution and time. Firstly, for conjugated polymers, because of their own stability may not be particularly good, there are many substances may lead to their own aggregation so that their fluorescence self-quenching and we need to find a stable condition for conjugated polymer sensors to play the greatest role. Therefore, the effect of organic solvents on the sensing performance of PPE-DPA was studied, including CH$_3$OH, CH$_3$CH$_2$OH, MeCN, THF, DMF and DMSO. Besides, the effect of organic solvent content was also determined and the content ranged from 10% to 90%. As shown in Fig. S7 and S8, it was found that PPE-DPA in the mixture of MeCN and Hepes buffer (v/v=1/1) has the better fluorescence response to Cu$^{2+}$. Secondly, we discussed that the effect of pH value from 3.0 to 12.0 on detection of Cu$^{2+}$. As shown in Fig. S9, the degree of fluorescence quenching increased with the increase of pH from 3.0 to 8.0, which can be ascribed that the tertiary amine part of PPE-DPA could become quaternary ammonium salts at low pH levels so that they could not react with Cu$^{2+}$. When pH=8.0, the fluorescence quenching reached the maximum extent. From
pH=8.0 to pH=12.0, the degree of fluorescence quenching gradually decreased because Cu$^{2+}$ could be hydrolyzed under alkaline conditions. So, pH=8.0 was chosen for the determination process. Meanwhile, the effect of several buffer systems (pH=8.0) were compared (in Fig. S10). And finally, Hepes buffer solution (pH=8.0) was chosen for PPE-DPA to detect Cu$^{2+}$. Thirdly, the effect of reaction time of PPE-DPA with Cu$^{2+}$ was studied. It was shown in Fig. S11 that the degree of fluorescence quenching was gradually increased and the fluorescence was almost unchanged after 20 minutes. To sum up, the MeCN/Hepes buffer solution (v/v=1/1; 1.0 mmol L$^{-1}$, pH=8.0) of PPE-DPA was fully reacted for 20 minutes after adding Cu$^{2+}$ before the fluorescence experiments were carried.

**Metal ion sensing properties of PPE-DPA**

The selective experiments of PPE-DPA to various metal ions were measured, and the concentrations of PPE-DPA and metal ions were all 5.0 µmol L$^{-1}$. As shown in Fig. 3 (black bar), the relative fluorescence intensities of PPE-DPA were no obvious change in the presence of Pb$^{2+}$, Al$^{3+}$, Co$^{2+}$, Cr$^{6+}$, Zn$^{2+}$, Ba$^{2+}$, Mg$^{2+}$, Pt$^{2+}$, Hg$^{2+}$, Ca$^{2+}$, Ni$^{2+}$, Mn$^{2+}$, Fe$^{3+}$, Na$^{+}$, Be$^{2+}$, Cd$^{2+}$, Cs$^{+}$, As$^{3+}$, Ag$^{+}$, Mo$^{2+}$, V$^{5+}$, Sr$^{2+}$, Li$^{+}$, K$^{+}$ and Rb$^{+}$, but only Cu$^{2+}$ could produce an obvious fluorescence quenching phenomenon. These results showed that PPE-DPA has a good selectivity to Cu$^{2+}$.

In order to further investigate the high selectivity of PPE-DPA to Cu$^{2+}$, the interference experiments of other metal ions were carried out. The tested results were illustrated in Fig. 3 (red bar) and it showed that these competing metal ions have no significant interference to the detection of Cu$^{2+}$. In a word, PPE-DPA showed a high selectivity and a good anti-interference ability to the detection of Cu$^{2+}$.

**Spectral titration experiment of PPE-DPA for Cu$^{2+}$ determination**

In order to obtain a better insight into the response performance of PPE-DPA to Cu$^{2+}$, the
spectral titration experiment was developed under the optimal conditions. In Fig. 4, it was recorded the fluorescent change of PPE-DPA (5.0 μmol L\(^{-1}\)) with adding Cu\(^{2+}\) (0, 0.05, 0.1, 0.5, 1.0, 2.0, 3.0, 4.0, 5.0 and 6.0 μmol L\(^{-1}\)). The results showed that the degree of fluorescence quenching was increased with the increase of Cu\(^{2+}\) concentration. **But, when the coordination reaction of DPA and copper ion get in equilibrium and the fluorescent emission intensity of PPE-DPA cannot be changed with the increase of copper ion concentration.** And, there was a good linear relationship from 0.05 to 5.0 μmol L\(^{-1}\) and the square of correlation coefficient was 0.996. The limit of detection (LOD) was 24 nmol L\(^{-1}\) (based on S/N=3). Compared with conventional methods\(^{37-39}\) in Table 1, PPE-DPA for Cu\(^{2+}\) offered lower LOD and better linear range. Therefore, it can be seen from the above that PPE-DPA has high sensitivity to Cu\(^{2+}\).

**Anion sensing properties of PPE-DPA-Cu\(^{2+}\) system**

PPi can partially recover the fluorescence of PPE-DPA quenched by Cu\(^{2+}\). According to this phenomenon, the complexes of PPE-DPA with Cu\(^{2+}\) would be also used to detect PPi in this work. At first, selective experiments of PPE-DPA-Cu\(^{2+}\) system to some common anions were considered, including PPi, CO\(_3^{2-}\), F\(^-\), Br\(^-\), I\(^-\), Ac\(^-\), SO\(_4^{2-}\), CI\(^-\), NO\(_3^-\), SCN\(^-\), H\(_2\)PO\(_4^-\), HPO\(_4^{2-}\) and PO\(_4^{3-}\) (12.0 μmol L\(^{-1}\)). The result was shown in Fig. 5 (black bar) that only PPi could recover the fluorescence of PPE-DPA.

Besides, interference experiments were also carried out. The fluorescence intensity was detected after adding PPi to the mixture of PPE-DPA-Cu\(^{2+}\) and another anion. From Fig. 5 (red bar), it indicated that other anions have no obvious interference to detection of PPi. In other words, the PPE-DPA-Cu\(^{2+}\) system had a good selectivity and anti-interference ability to the detection of PPi.

**Spectral titration experiment of PPE-DPA-Cu\(^{2+}\) system for PPi determination**
In this part, we discussed the PPi titration experiment. The different concentration of PPi (0, 0.5, 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, 10.0, 11.0, 12.0 μmol L⁻¹) was added to the PPE-DPA-Cu²⁺ system and the fluorescence was measured and recorded in Fig. 6. It was shown that the fluorescence gradually recovered with the increasing of PPi concentration and there was a good linear in the range from 0.5 to 12.0 μmol L⁻¹ with square of correlation coefficient of 0.997 (R²=0.997). The limit of detection (LOD) was 230 nmol L⁻¹ (based on S/N=3). Compared with other previous sensors in Table 1, the PPE-DPA-Cu²⁺ system still has obviously improved in terms of linear detection and lower detection limit. Therefore, as a high sensitive and selective fluorescent sensor, PPE-DPA-Cu²⁺ could be used for the detection of PPi in real samples.

Analysis of PPi in blood serum and synovial fluid

In order to test the reliability of this method, we applied the PPE-DPA-Cu²⁺ system to detect PPi in the prepared synovial fluid and blood serum. The initial contents of PPi in blood serum and synovial fluid of arthritis patients using PPE-DPA-Cu²⁺ as fluorescent sensor were listed in Table 2, which was consistent with the values reported in literatures. And the recovery experiments for varying added amounts of PPi were carried out. The results are summarized in Table 2. The recovery of PPi was range from 97.1% to 104.0% and RSD was less than 1.67%. Besides, we compared PPE-DPA-Cu²⁺ with conventional PPi analytical methods for the same sample in Table 3. These results showed that the PPE-DPA-Cu²⁺ system provided a useful method for the detection of PPi in synovial fluid and blood serum of patients with arthritis.

Conclusions
In conclusion, a novel “on-off-on” conjugated polymer fluorescence sensor PPE-DPA was synthesized, which showed good selectivity and high sensitivity for continuous detection of Cu$^{2+}$ and PPI. Fluorescence intensity of PPE-DPA decreased linearly with the change of Cu$^{2+}$ concentration from 0.05 to 5.0 μmol L$^{-1}$ and the limit of detection (LOD) was 24 nmol L$^{-1}$ (based on S/N=3). Similarly, the fluorescence intensity of PPE-DPA recovered linearly with the addition of PPI from 0.5 to 12.0 μmol L$^{-1}$ and the limit of detection (LOD) was 230 nmol L$^{-1}$ (based on S/N=3). Besides, the PPE-DPA-Cu$^{2+}$ system has also been successfully applied to detect PPI in blood serum and synovial fluid of arthritic patients. The results showed that the fluorescence sensor can be used to qualitatively and quantitatively detect PPI for early diagnosis of arthritic patients. Therefore, this work not only developed a high selective and sensitive fluorescent sensor based on conjugated polymer for Cu$^{2+}$ and PPI, but also offered a new feasible method for the clinical diagnosis of arthritis.

Acknowledgements

This work was supported by the State Major Project for Science and Technology Development, China (No. 2013YQ47078102-3), the Science-Technology Development Project of Jilin Province of China (No. 20150204060GX), the Natural Science Foundation of Jilin Province (No. 20160101314JC).

Supporting Information

The Supporting Information is the synthesis and characterization data of intermediate and the final product and supplementary spectral data (PDF). This material is available free of charge on the Web at http://www.jsac.or.jp/analsci/.
References


42. P. Yao, Z. Liu, J. Ge, Y Chen, Q. Cao, Dalton Trans, 2015, 44, 7470.


44. X. Zhao, K. Schanze, Chem. Commun., 2010, 46, 6075.
### Table 1  Comparison of different methods for continuous detection of Mn⁺ and PPi

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<th>Fluorescent sensor type</th>
<th>Mn⁺</th>
<th>PPi</th>
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<tr>
<td></td>
<td>Range of linearity/μmol L⁻¹</td>
<td>LOD/ nmol L⁻¹</td>
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<tr>
<td>Flavonoid derivative</td>
<td>0-10</td>
<td>100</td>
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<tr>
<td>Chromene derivative</td>
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<tr>
<td>NTPA-Fe₃O₄@SiO₂</td>
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<tr>
<td>Conjugated polymers</td>
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### Table 2  Results of PPi detection in serum and synovial fluid

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<th>Sample</th>
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<th>PPi found/μmol L⁻¹</th>
<th>Recovery/%</th>
<th>RSD/%</th>
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<td>0.0</td>
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<td>—</td>
<td>—</td>
<td>6.1 ± 1.4</td>
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<td>Blood serum 2</td>
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</tr>
<tr>
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<td>98.8</td>
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Table 3 Comparison of conventional analytical methods for PPI in blood serum and synovial fluid

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<td>enzymatic analytical method</td>
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Figure Captions

Scheme 1. Synthetic route of PPE-DPA.

Fig. 1 (a) The fluorescence excitation spectrum of PPE-DPA; (b) the fluorescence emission spectrum and image of PPE-DPA; (c) the fluorescence emission spectrum and image of PPE-DPA-Cu$^{2+}$; (d) the fluorescence emission spectrum and image of PPE-DPA-Cu$^{2+}$-PPi. Entrance slit, 5 nm; exit slit, 5 nm. These images were taken under a UV-lamp with a light wavelength of 365 nm.

Concentration of PPE-DPA and Cu$^{2+}$, 5.0 μmol L$^{-1}$; Concentration of PPi, 12.0 μmol L$^{-1}$.

Fig. 2 Sensing mechanism of PPE-DPA.

Fig. 3 The relative fluorescence intensities of PPE-DPA upon the addition of different metal ions (black bar); and the addition of Cu$^{2+}$ in MeCN-Hepes buffer solution with another cations (red bar). $I_0$: Fluorescence intensity of PPE-DPA; $I$: Fluorescence intensity of PPE-DPA in the presence of different metal ions.

Concentration of PPE-DPA, 5.0 μmol L$^{-1}$; Concentration of metals, 5.0 μmol L$^{-1}$.

Fig. 4 Spectral titration curve of PPE-DPA for Cu$^{2+}$ determination.

Concentration of PPE-DPA, 5.0 μmol L$^{-1}$; Concentration of Cu$^{2+}$, 0, 0.05, 0.1, 0.5, 1.0, 2.0, 3.0, 4.0, 5.0 and 6.0 μmol L$^{-1}$.

Fig. 5 The relative fluorescence intensities of PPE-DPA-Cu$^{2+}$ system adding different anions (black bar); and adding PPi separately in MeCN-Hepes buffer solution with different interfering anions (red bar). $I_0$: The fluorescence intensity of PPE-DPA-Cu$^{2+}$ system. $I$: The fluorescence
intensity of PPE-DPA-Cu$^{2+}$ system in the presence of different anions.

Concentration of PPE-DPA and Cu$^{2+}$, 5.0 μmol L$^{-1}$; Concentration of anions, 12.0 μmol L$^{-1}$.

Fig. 6  Spectral titration curve of PPE-DPA-Cu$^{2+}$ system for PPI determination.

Concentration of PPE-DPA and Cu$^{2+}$, 5.0 μmol L$^{-1}$; Concentration of anions, 0, 0.5, 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, 10.0, 11.0, 12.0 μmol L$^{-1}$. 
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Fig. 4  Spectral titration curve of PPE-DPA for Cu$^{2+}$ determination.

Concentration of PPE-DPA, 5.0 μmol L$^{-1}$; Concentration of Cu$^{2+}$, 0, 0.05, 0.1, 0.5, 1.0, 2.0, 3.0, 4.0, 5.0 and 6.0 μmol L$^{-1}$. 

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Fig. 5  The relative fluorescence intensities of PPE-DPA-Cu$^{2+}$ system adding different anions (black bar); and adding PPi separately in MeCN-Hepes buffer solution with different interfering anions (red bar). I$_0$: The fluorescence intensity of PPE-DPA-Cu$^{2+}$ system. I: The fluorescence intensity of PPE-DPA-Cu$^{2+}$ system in the presence of different anions.

Concentration of PPE-DPA and Cu$^{2+}$, 5.0 μmol L$^{-1}$; Concentration of anions, 12.0 μmol L$^{-1}$.

Fig. 6  Spectral titration curve of PPE-DPA-Cu$^{2+}$ system for PPi determination.

Concentration of PPE-DPA and Cu$^{2+}$, 5.0 μmol L$^{-1}$; Concentration of anions, 0, 0.5, 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, 10.0, 11.0, 12.0 μmol L$^{-1}$.