

Polythiophene based fluorescent probe for copper ions with high sensitivity

Chaoxia Guo,¹ Shouxiang Jiang,¹ Wenxue Zhu,² Xiuxia Yang,² Meishan Pei,¹ Guangyou Zhang¹

¹School of Chemistry and Chemical Engineering, University of Jinan, Jinan 250022, China

²Yidu Central Hospital of Weifang, Weifang 262500, China

Correspondence to: G. Zhang (E-mail: chm_zhanggy@ujn.edu.cn)

ABSTRACT: Two new conjugated polymers poly{3-([4-((2-hydrazino-2-oxoethyl)(methyl)amino)cyclohexylidene)methyl]thiophene}(P1) and poly{3-([4-((3-hydrazino-3-oxopropyl)(methyl)amino)cyclohexylidene)methyl]thiophene}(P2) were synthesized, and their optical properties were investigated. P1 exhibited excellent selectivity toward Cu²⁺ ions in 50% water solution, the fluorescence color of P1 changed distinctly from greenyellow to colorless in the presence of Cu²⁺ under UV-light, while introduction of other metal ions could not induce such significant variation. Moreover, highly sensitive detection of Cu²⁺ ions was demonstrated in 90% water solution. Its high metal-chelating capability allowed Cu²⁺ recognition with a detection limit of 3.2×10^{-10} M. These results indicated that this kind of nonionic polymer containing multidentate ligand could be used as a highly selective and sensitive chemosensor for Cu²⁺ detection. The proposed binding mode of P1 with Cu²⁺ was supported by DFT calculation using Gaussian 03. Unlike P1, P2 showed no obvious fluorescent change in the presence of various metal ions due to its space steric hindrance resulted from N/O distribution on the side chain of P2. © 2015 Wiley Periodicals, Inc. *J. Appl. Polym. Sci.* 2015, 132, 42440.

KEYWORDS: applications; conducting polymers; optical properties; sensors and actuators; synthesis and processing

Received 30 December 2014; accepted 28 April 2015

DOI: 10.1002/app.42440

INTRODUCTION

Although copper (Cu²⁺) plays crucial roles in many fundamental physiological processes in organisms, excessive Cu²⁺ can be toxic and cause aberrant oxidative and nitrosative stress events that accompany diseases such as cancer, cardiovascular disorders, and neurodegenerative diseases.^{1–6} Now it has become one of the major components of the environmental pollutants.^{7,8} Therefore, research and development of effective, rapid, and simple method for monitoring low concentrations of Cu²⁺ in aqueous media is still imperative.

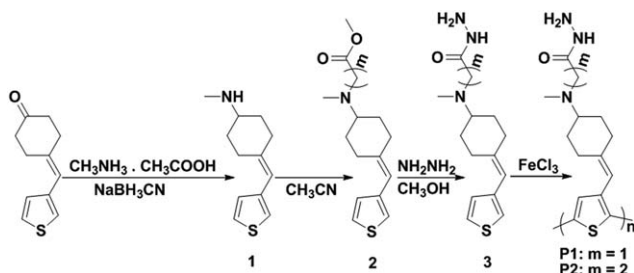
Conjugated polymers (CPs) have attracted considerable attention due to their signal amplification effects and potential applications in sensing of metal ions and biologically relevant targets. CPs are very interesting and prospective tools for designing optical sensors. One of the advantages with this type of sensors is the collective system response given by the series of chromophores building the polymer chain.^{9,10} A variety of schemes has been formulated in developing ideal optical platforms based on CPs.^{11–14} Among all the tools available for this purpose, fluorescent chemosensors are particularly desired due to their distinct advantages in terms of sensitivity, selectivity,

response time, and in situ observation.^{15–17} In recent years, there has been interest in exploiting polythiophene based polymers as chemical and biological sensors due to their strong fluorescence quenching.^{18–22} Nonetheless, simple and easily synthesized polythiophene-based probes for direct detection of trace metal ions, for example Cu²⁺, remain rare and there are still challenges in this area. This encouraged us to develop new polythiophene derivatives with favorable characters for metal ion sensing.

At the heart of any chemosensor or biosensor is its recognition unit (receptor). It is constructed for providing selective target binding from a mixture of different and sometimes closely related compounds. The acylhydrazine functionalized thiophene chromophore design was adopted based on our previous experience on the π -conjugated polymer photosensitizers.²³ In the present work, we prepared two polythiophene derivatives P1 and P2 (Scheme 1), of which P1 was successfully applied as a fluorescent probe for sensing Cu²⁺ in Tris-HCl solutions. The sensory system provides some advantages: (1) Incorporation of multidentate ligand in the side chain of polythiophene was used for efficient complexation of Cu²⁺. (2) Facile and simple synthesis. Classical FeCl₃-catalyzed oxidative polymerization was

Additional Supporting Information may be found in the online version of this article.

© 2015 Wiley Periodicals, Inc.



Scheme 1. The synthetic routes of P1 and P2, m represents the number of methylene units.

chosen for synthesizing the conjugated polymer. (3) Obvious fluorescence quenching, this sensory system performed a rapid “turn off” response with the color change from greenyellow to colorless.

EXPERIMENTAL

Materials

All metal salts such as $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$, $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$, $\text{BaCl}_2 \cdot 3\text{H}_2\text{O}$, $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$, AgNO_3 , $\text{CdCl}_2 \cdot 2.5\text{H}_2\text{O}$, PbCl_2 , $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$, $\text{SrCl}_3 \cdot 6\text{H}_2\text{O}$, $\text{CrCl}_3 \cdot 6\text{H}_2\text{O}$, ZnCl_2 , HgCl_2 , $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$, and LiCl were analytical grade and used without further purification. All other organic reagents were purchased and used as received.

Measurements

UV-vis spectra were recorded on a Shimadzu 3100 spectrometer. Fluorescence measurements were carried out using an Edinburgh Instruments-FLS920 fluorescence spectrometer. ^1H NMR was recorded on a Bruker AV III 400 MHz NMR spectrometer. Infrared spectra were recorded on a Bruker Vertex 70 FT-IR spectrometer using KBr pellets. Gel permeation chromatography (GPC) measurements were performed with a Waters 2410 refractive-index detector at 35°C and THF was used as the eluent at a flow rate of 1.0 mL min^{-1} . GPC data were calibrated with polystyrene standards. Mass spectra were obtained using a Perkin Elmer Clarus 500 mass spectrometer.

Sample Preparation

All tests described in this paper were carried out at room temperature (25°C) with distilled water. In the experiments involving titration with various metal ions, the polymers were dissolved in THF/Tris-HCl (1/9, v/v) or THF/Tris-HCl (1/1, v/v) to afford the test solutions ($1 \times 10^{-5} \text{ M}$). Stock solutions ($1 \times 10^{-5} \text{ M}$) of the metal salts in water were prepared. The sum volume of each ion solution introduced to the test solution was no more than $20 \mu\text{L}$.

Computational Details

Density functional theory (DFT) structural optimizations were performed with the Gaussian 03 program. In all cases, the structures were optimized using the B3LYP functional and the mixed basis set 6-31+G(d).²⁴

Synthesis of N-Methyl-4-(thiophen-3-ylmethylidene)cyclohexanamine (1)

A mixture of 3-[(4-oxocyclohexylidene)methyl]thiophene (0.74 g, 3.84 mmol) in 10 mL methylene chloride and 5 mL acetic acid was stirred at room temperature. Then a solution of

methylamine acetate (0.70 g, 7.69 mmol) in methylene chloride (10 mL) was added drop-wise over 30 min and the reaction mixture was stirred for 1 h. After adding sodium cyanoborohydride (0.25 g, 3.98 mmol), the reaction mixture was kept in an ice bath for 1 h and subsequently for 3 h at room temperature. The final reaction mixture was diluted with water and extracted with CHCl_3 ($3 \times 50 \text{ mL}$), the organic layer was washed with water ($3 \times 50 \text{ mL}$) and dried over anhydrous Na_2SO_4 . After filtration and evaporation, the crude product was isolated by preparative thin layer chromatography (TLC) on silica gel to yield compound 1 (0.47 g, 59.8%) as a yellowish oil. FTIR (KBr, cm^{-1}): 1441 (CH=C), 1240 (C–N). ^1H NMR (CDCl_3 , 400 MHz, ppm): $\delta = 7.23\text{--}7.25$ (d, 1H, CH, $J = 8 \text{ Hz}$), 7.02 (s, 1H, CH), 6.99–7.01 (d, 1H, CH=CH, $J = 8 \text{ Hz}$), 6.16 (s, 1H, CH=C), 2.44 (s, 3H, CH_3), 1.94–2.06 (m, 4H, CH_2), 1.69 (s, 1H, NH), 1.13–1.32 (m, 4H, CH_2).

Synthesis of Methyl{methyl[4-(thiophen-3-ylmethylidene)cyclohexyl]amino}acetate (2a)

A solution of compound 1 (0.45 g, 2.17 mmol), NaHCO_3 (2.37 g, 28.2 mmol), and methyl chloroacetate (0.26 g, 2.38 mmol) in 15 mL acetonitrile was stirred for 12 h at room temperature. The solvent was removed, and saturated sodium carbonate solution (25 mL) was added. The mixture was extracted with CHCl_3 ($3 \times 20 \text{ mL}$). The organic phase was washed with saturated sodium carbonate solution several times and dried over Na_2SO_4 , the crude product was purified by TLC on silica gel (ethyl acetate : n-hexyl acetate = 4 : 1) to give the product (0.24 g, 40%) as a faint yellow oil. FTIR (KBr, cm^{-1}): 1740 (C=O), 1433 (CH=C), 1196 (C–N), 1049 (C–O–C). ^1H NMR (CDCl_3 , 400 MHz, ppm): $\delta = 7.23\text{--}7.25$ (d, 1H, CH, $J = 8 \text{ Hz}$), 7.02 (s, 1H, CH), 6.98–7.00 (d, 1H, CH=CH, $J = 8 \text{ Hz}$), 6.14 (s, 1H, CH=C), 3.71 (s, 3H, O– CH_3), 3.31 (s, 2H, N– CH_2), 2.68–2.74 (m, 1H, CH), 2.39 (s, 3H, N– CH_3), 1.91–1.99 (m, 4H, CH_2), 1.24–1.41 (m, 4H, CH_2).

Synthesis of Methyl 3-{methyl[4-(thiophen-3-ylmethylidene)cyclohexyl]amino}propanoate (2b)

A solution of compound 1 (0.15 g, 0.72 mmol), NaHCO_3 (0.59 g, 9.39 mmol) and methyl acrylate (0.10 g, 1.16 mmol) in 15 mL acetonitrile was stirred for 12 h at room temperature. The solvent was removed, and saturated sodium carbonate solution (25 mL) was added. The mixture was extracted with CHCl_3 ($3 \times 20 \text{ mL}$). The organic phase was washed with saturated sodium carbonate solution several times and dried over Na_2SO_4 , the crude product was purified by TLC on silica gel (ethyl acetate : methanol = 1 : 2) to give the product (0.17 g, 80.6%) as a faint yellow oil. FTIR (KBr, cm^{-1}): 1735 (C=O), 1436 (CH=C), 1210 (C–N), 1043 (C–O–C). ^1H NMR (CDCl_3 , 400 MHz, ppm): $\delta = 7.26\text{--}7.28$ (d, 1H, CH, $J = 8 \text{ Hz}$), 7.05 (s, 1H, CH), 7.02–7.04 (d, 1H, CH=CH, $J = 8 \text{ Hz}$), 6.17 (s, 1H, CH=C), 3.70 (s, 3H, O– CH_3), 3.80–2.82 (t, 2H, N– CH_2), 2.48–2.52 (t, 2H, C– CH_2), 2.62–2.63 (m, 1H, CH), 2.22 (s, 3H, N– CH_3), 1.86–1.96 (m, 4H, CH_2), 1.26–1.51 (m, 4H, CH_2).

Synthesis of 3-[(4-[(2-Hydrazino-2-oxoethyl)(methyl)amino]cyclohexylidene)methyl]thiophene (3a)

Compound 2a (0.25 g, 0.90 mmol) was dissolved in 10 mL ethanol. Then 80% hydrazine hydrate (1.35 g, 22 mmol) was

added. The resulting mixture was stirred at room temperature for 12 h. After remove of the solvent, the mixture was extracted with CH_2Cl_2 (3×20 mL). The organic phase was washed with brine for several times and dried over Na_2SO_4 . The crude product was purified by TLC on silica gel (ethyl acetate : methanol : triethylamine = 15 : 1 : 5), the final product **3a** (0.23 g, 92%) was collected as a faint yellow oil. Ms (ESI): $m/z = 279.14[\text{M}]^+$. FTIR (KBr, cm^{-1}): 1670 (C=O), 1492 (CH=C), 1245 (C-N). ^1H NMR (CDCl_3 , 400 MHz, ppm): $\delta = 8.38$ (s, 1H, NH), 7.24–7.26 (d, 1H, CH, $J = 8$ Hz), 7.02 (s, 1H, CH), 6.98–7.00 (d, 1H, CH, $J = 8$ Hz), 6.16 (s, 1H, CH=C), 3.17 (s, 2H, CH_2), 2.60–2.64 (m, 1H, CH), 2.38–2.41 (d, 2H, NH_2 , $J = 12$ Hz), 2.30 (s, 3H, N- CH_3), 1.85–1.96 (m, 4H, CH_2), 1.24–1.43 (m, 4H, CH_2).

Synthesis of 3-({4-[(3-Hydrazino-3-oxopropyl)(methyl)amino]cyclohexylidene}methyl)thiophene (3b)

Compound **2b** (0.10 g, 0.34 mmol) was dissolved in 10 mL ethanol. Then 80% hydrazine hydrate (0.65 g, 13 mmol) was added. The resulting mixture was stirred at room temperature for 12 h. After removing the solvent, the mixture was extracted with CH_2Cl_2 (3×20 mL). The organic phase was washed with brine for several times and dried over Na_2SO_4 . The crude product was purified by TLC on silica gel (ethyl acetate : methanol : triethylamine = 4 : 1 : 1), the final product (0.09 g, 90.3%) was collected as a yellow oil. Ms (ESI): $m/z = 293.16[\text{M}]^+$. FTIR (KBr, cm^{-1}): 1658 (C=O), 1458 (CH=C), 1244 (C-N). ^1H NMR (CDCl_3 , 400 MHz, ppm): $\delta = 9.50$ (s, 1H, NH), 7.24–7.26 (d, 1H, CH, $J = 8$ Hz), 7.02 (s, 1H, CH), 6.99–7.00 (d, 1H, CH, $J = 4$ Hz), 6.15 (s, 1H, CH=C), 2.64–2.70 (t, 2H, N- CH_2), 2.42–2.48 (m, 1H, CH), 2.37–2.40 (t, 2H, C- CH_2), 2.23 (s, 3H, N- CH_3), 2.01–2.03 (d, 2H, NH_2 , $J = 8$ Hz), 1.84–1.96 (m, 4H, CH_2), 1.31–1.49 (m, 4H, CH_2).

Synthesis of Poly{3-({4-[(2-hydrazino-2-oxoethyl)(methyl)amino]cyclohexylidene}methyl)thiophene} (P1)

Anhydrous ferric chloride (0.45 g, 1.7 mmol) was suspended in 20 mL dry chloroform and stirred for 30 min under nitrogen and then compound **3a** (0.12 g, 0.43 mmol) was added dropwise. The mixture was stirred at 0°C for 24 h. Treatment of the reaction mixture with methanol (10 mL) resulted in the precipitation of the polymer which was carefully de-doped by repeatedly treating a chloroform solution of the polymer with 25% ammonium hydroxide at ambient temperature. The crude product was obtained as a red solid after evaporation of the chloroform. The solid polymer was then purified by Soxhlet extraction with n-hexane and THF to remove impurities and oligomers. Finally **P1** (0.04 g, 33.3%) was obtained as a red solid. FTIR (KBr, cm^{-1}): 1671 (C=O), 1447 (CH=C), 1220 (C-N). ^1H NMR (CDCl_3 , 400 MHz, ppm): $\delta = 8.38$ (s, 1H, NH), $\delta = 7.68$ (s, 1H, CH), 6.19 (s, 1H, CH). GPC: $M_n = 3183$ g/mol, PDI = 1.23.

Synthesis of Poly{3-({4-[(3-hydrazino-3-oxopropyl)(methyl)amino]cyclohexylidene}methyl)thiophene} (P2)

P2 was synthesized in a similar way with **P1**, and a claret-colored solid was obtained with a yield of 41.1%. FTIR (KBr, cm^{-1}): 1661 (C=O), 1441 (CH=C), 1232 (C-N). ^1H NMR (CDCl_3 , 400 MHz, ppm): $\delta = 9.50$ (s, 1H, NH), $\delta = 7.51$ (s,

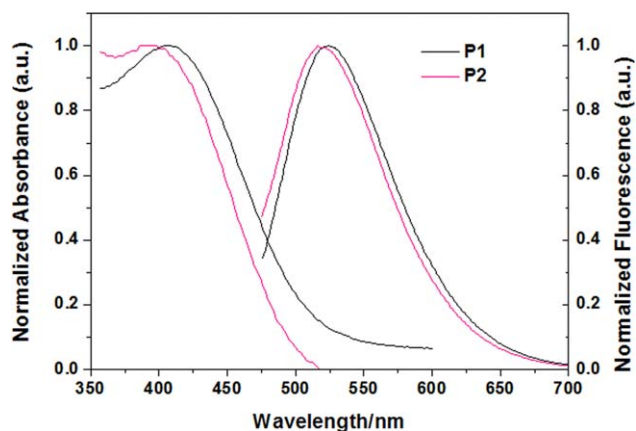


Figure 1. Normalized absorption and emission spectra of **P1** and **P2** (1×10^{-5} M). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

1H, CH), 6.16 (s, 1H, CH=C). GPC: $M_n = 4940$ g/mol, PDI = 1.21.

RESULTS AND DISCUSSION

Synthesis and Characterization of the Monomers and Polymers

The synthesis of **P1** and **P2** is described in Scheme 1. The starting material was prepared according to the reported methods,²⁵ then the target products were obtained by several steps of reductive amination, esterification, hydrazinolysis, and FeCl_3 oxidative polymerization. All monomers have been validated with ^1H NMR, FTIR, and MS characterization, and the resulting polymers were characterized by ^1H NMR, FTIR, and GPC (Supporting Information Figures S1–S14). The molecular weight and polydispersity index (PDI) of **P1** and **P2** measured by GPC were $M_n = 3183$ g/mol, PDI = 1.23 and $M_n = 4940$ g/mol, PDI = 1.21, respectively. The GPC results of this two polymers show the moderate molecular weight.

Photophysical Properties

P1 and **P2** displayed maximum absorption peaks at 408 nm and 398 nm in their UV-vis spectra and maximum emission peaks at 524 nm and 516 nm respectively in THF-tris buffer solution (Figure 1). The similar absorption and emission spectra observed for **P1** and **P2** was due to their same conjugated backbone. UV-vis spectra of **P1** showed a larger maximum absorption wavelength relative to **P2**, indicating an increase in effective conjugation and increased planarity. In addition, the conjugated polymers readily dissolve in common solvents, such as DMSO, CH_3OH , THF, and CHCl_3 , which can be attributed to the polar and flexible substituents on thiophene units as side chain of the polymers.

The Selective Fluorescence Recognition of **P1** on Cu^{2+}

In our previous report,²³ we incorporated acylhydrazine unit into the conjugated polymer, and found that the hydrochloride of the polymer (**PM1-HCl** and **PM2-HCl**) showed strong interaction with Hg^{2+} and Cu^{2+} ions. For **P1** and **P2** in this work, the methylamine was attached to the hexacyclic ring, and non-ionic polymers were obtained. These structural differences had a

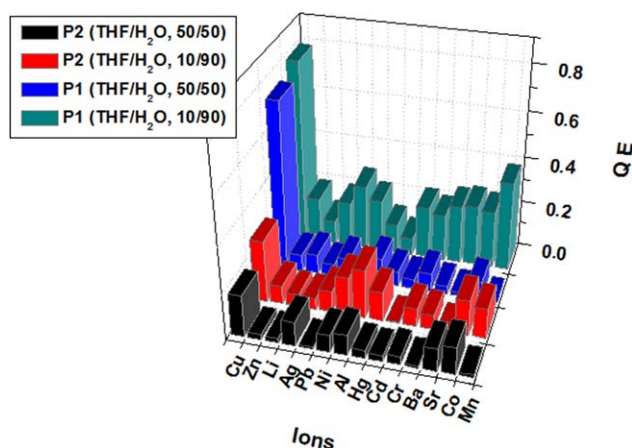


Figure 2. The fluorescence quenching efficiency ($1 - I/I_0$) of **P1** and **P2** (1×10^{-5} M) with various metal ions in 5 mM Tris-HCl buffer solutions (THF/Tris, v/v, pH 7.4). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

prodigious influence on their fluorescence properties relative to **PM1·HCl** and **PM2·HCl**.

The selectivity of a probe should be taken into consideration when designing a metal ion sensor. Investigation of the sensing properties of the polymers was carried out in Tris-HCl (5 mM, pH = 7.4) buffer solution by a fluorescence method. Preliminary observations (Supporting Information Figures S10 and S11) indicated that in both aqueous solutions containing different ratio of water the fluorescence “on-off” effect by coordination with copper ions could be conducted in neutral and alkaline conditions. To avoid the formation of insoluble hydroxides in alkaline systems, Cu^{2+} sensing experiments were carried out in neutral condition which conformed to biological conditions. Both **P1** and **P2** were treated with Mn^{2+} , Co^{2+} , Sr^{3+} , Ba^{2+} , Cr^{3+} , Cd^{2+} , Hg^{2+} , Al^{3+} , Ni^{2+} , Pb^{2+} , Ag^+ , Li^+ , Zn^{2+} , and Cu^{2+} as the chloride or nitrate salts to compare the different binding ability of **P1** and **P2** toward metal ions in THF/Tris-HCl buffer containing 50% and 90% water. As can be seen from Figure 2, **P1** exhibited pronounced selective quenching behavior toward Cu^{2+} ions relative to the other metal ions in 50% water solution. The quenching efficiency ($1 - I/I_0$) expressed by the ratio of the fluorescence intensity of polymers in the presence (I) and absence (I_0) of 1 equivalent of metal ions was 72% for Cu^{2+} ions. Other metal ions did not present such significant effects and the ratio was less than 10% even for the most interfering metal ions. In addition, the fluorescence variations of **P1** from greenyellow to colorless can also be readily

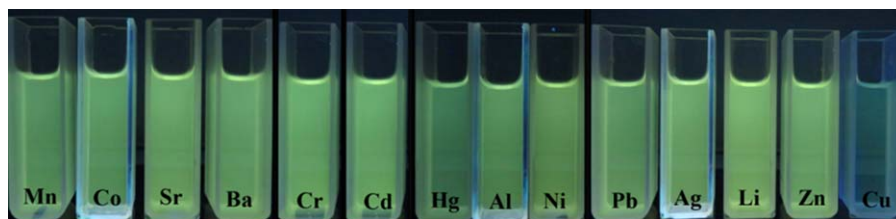


Figure 3. Changes in the color of **P1** (1×10^{-5} M) in THF/Tris buffer (50/50, v/v, pH 7.4, Tris-HCl 5mM) induced by the addition of various metal ions (1×10^{-5} M). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

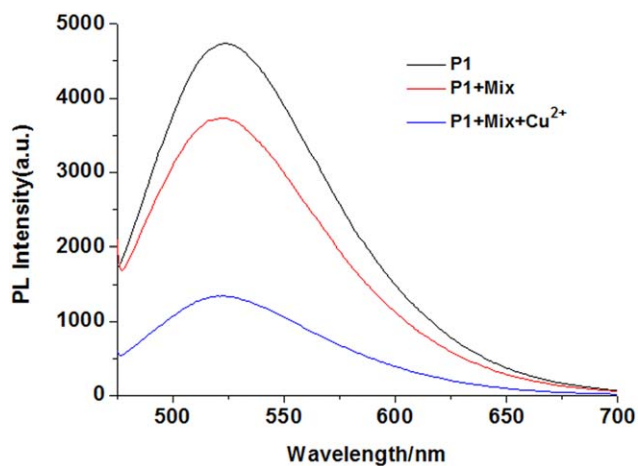


Figure 4. Fluorescence spectra of **P1** (1×10^{-5} M) with addition of equimolar metal ions. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

observed by the naked eye (Figure 3). While in 90% water solution, the quenching efficiency reached 79% for Cu^{2+} ions, other metal ions also induced non-ignorable influences on fluorescence intensity of **P1**. Apparently, **P1** showed a higher selectivity toward Cu^{2+} in 50% water solution, which could be attributed to higher affinity of **P1** with Cu^{2+} over other ions in a more polar system. For **P2**, it can be observed that none of the metal ions exerts virtually any effect on the fluorescence intensity in any of the two systems containing different proportion of water. The fluorescence quenching efficiency is below 20% even for Cu^{2+} . Thus, **P2** had a very low ability to complex metal ions. This suggested that nitrogen or oxygen atoms on the side chain of **P2** failed to form stable coordinate bonds with metal ions due to their distribution.

Competition experiments were also carried out by monitoring the change in fluorescence intensity at 524 nm upon addition of 1 equivalent Cu^{2+} ion to a mixed solution of **P1** and different metal ions (1 equivalent) in 50% water solution (Figure 4). Although the addition of mixed metal ions to the **P1** solution caused a fluorescence quenching of the sensor, the fluorescence intensity of the sensor was largely reduced upon further addition of Cu^{2+} ions into the above mixture. These results indicated that the recognition of Cu^{2+} by **P1** was not significantly influenced by other coexisting metals.

The Sensitive Fluorescence Recognition of **P1** on Cu^{2+}

To evaluate the suitability of **P1** as an active fluorescence sensor for the turn-off detection of Cu^{2+} in aqueous solution, the

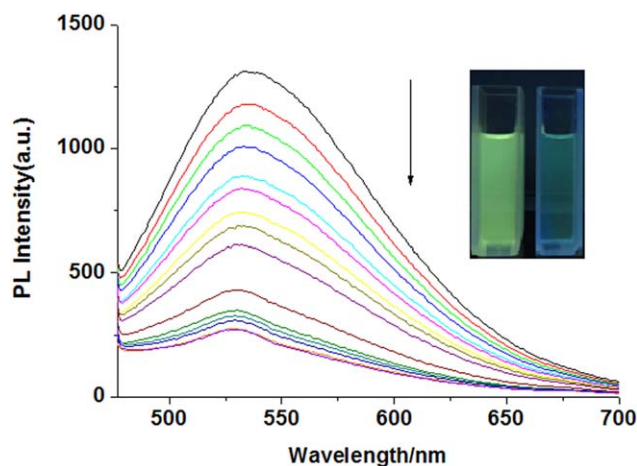


Figure 5. Fluorescence spectra of **P1** (10 μM) with addition of various concentrations of CuCl_2 (0.0004–50 μM) in THF/Tris-HCl buffer (10/90, v/v, pH 7.4, Tris-HCl 5mM) with an excitation at 450 nm. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

emission properties of polymer **P1** and its turn-off behaviors were investigated in 90% water solution. The conjugated polymer displayed greenyellow fluorescence centered at 527 nm with the excitation at 450 nm. It was evident from Figure 5 that obvious fluorescence quenching of **P1** could be observed in the presence of 4×10^{-10} M Cu^{2+} , indicating an upper detection limit. The fluorescence intensity of **P1** solution kept decreasing with increasing amount of Cu^{2+} ions. When the addition amount reached 5×10^{-6} M, which was only half amount of **P1**, the emission was almost completely quenched and was accompanied with an obvious color change of the solution from greenyellow to colorless under UV light (Figure 5, inset). The fluorescence intensity remained unchanged upon addition of excess amount of Cu^{2+} ions. The quenching effect could be attributed to the coordination of the amino groups on the side chain of **P1** with Cu^{2+} , resulting in the chelation-induced fluorescent turn off effect. The fluorescent wavelength of the metal-chelated polymer appears a slight hypochromatic shift, which may be caused by the deconjugation effect on the polymer backbone after coordination of specific copper ions.²⁶

To further quantify the turn-off efficiency of **P1**, the fluorescence intensities of the polymer with Cu^{2+} ion at various concentrations were fitted into the Stern-Volmer equation: $I_0/I = 1 + K_{sv}[\text{Cu}^{2+}]$, where I_0 and I are the initial and final emis-

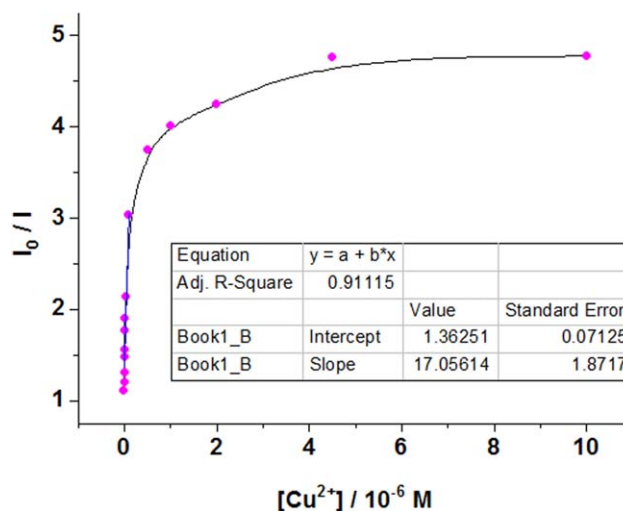


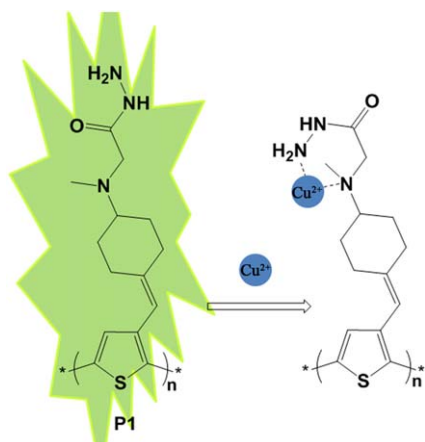
Figure 6. The Stern-Volmer plot of **P1** in THF/Tris-HCl buffer (10/90, v/v, pH 7.4, Tris-HCl 5mM) upon addition of increasing amounts of Cu^{2+} . [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

sion intensity of the chromophores and K_{sv} is the Stern-Volmer quenching constant.²⁷ The quenching efficiency increases with increasing tendency of the polymer to associate with the quencher in solution. This association can occur either through the formation of a non-luminescent complex between the polymer and the quencher (static quenching) or due to collisions between the photo-luminescent macromolecule and the quencher (dynamic quenching). As depicted in Figure 6, the Stern-Volmer plot for **P1** displayed a linear relationship in a Cu^{2+} concentration range of 0– 0.1×10^{-6} M, the corresponding K_{sv} was determined to be $1.7 \times 10^7 \text{ M}^{-1}$ and the detection limit for Cu^{2+} of 3.2×10^{-10} M was obtained based on $3\delta/s$, where δ is the standard deviation of blank measurements, and s is the slope between fluorescence intensity versus Cu^{2+} concentration. The high sensitivity is consistent with the signal amplification ability of conjugated polymers due to facile energy migration along the polymer backbone.²⁸ Simultaneously, a curvilinear relationship of the plot within the concentration range of 0.1 – 10×10^{-6} M demonstrated the coexistence of static and dynamic fluorescence quenching of **P1** toward the analytes.

P1 was also titrated with Cu^{2+} ions in 50% water solution (Supporting Information Figure S12). Unlike the fluorescent characteristics of it in 90% water solution, the emission attenuated gradually after the addition of 2×10^{-9} M Cu^{2+} , and it

Table I. Comparison of Proposed Cu^{2+} -Selective Sensor with Reported Sensors

Fluorophore	Solvent	Limit of detection (M)	K_{sv}	Selectivity	pH
Poly(HEMA-co-DCPDP) ²⁹	Water/ethanol buffer	ND	$1.42 \times 10^5 \text{ M}^{-1}$	High	7
1,3,5-Triphenylbenzene ³⁰	Phosphate buffer	1.9×10^{-10}	$1.50 \times 10^7 \text{ M}^{-1}$	High	8
Polyfluoreneethynylene ³¹	Methanol	ND	$7.5 \times 10^6 \text{ M}^{-1}$	High	ND
Polyfluorene copolymers ³²	Aqueous solution	2×10^{-8}	$1.44 \times 10^7 \text{ M}^{-1}$	Moderate	ND
Polythiophene derivative	Water/THF buffer	3.2×10^{-10}	$1.70 \times 10^7 \text{ M}^{-1}$	High	7.4



Scheme 2. Proposed binding mode of **P1** toward Cu^{2+} ions. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

could not be quenched by introduction of access amount of Cu^{2+} . From Supporting Information Figure S13, the correlation between I_0/I and $[\text{Cu}^{2+}]$ indicates that multiple equilibria existed in the copper binding process.

Previous reports on fluorescent detection of Cu^{2+} ions using CPs were listed by Table I for comparison. The newly developed polymer **P1** exhibits relatively good performance in terms of the sensitivity, detection limits, and selectivity. Moreover, the sensing material has several advantages, such as facile preparation, low cost, and label free detection.

Proposed Binding Mode

Based on the above discussion, a possible binding mode between **P1** and Cu^{2+} was proposed in Scheme 2. For **P1**, lone-pair nitrogen atoms of primary and tertiary amine could participate in binding with Cu^{2+} ions, where electron transfer from the π^* orbit at the excited state of **P1** to the 3d orbital of Cu^{2+} ions, thereby causing the subsequent Cu^{2+} -mediated interpolymer π -stacking aggregation, resulting in the rapid quenching of fluorescence.^{19,33,34} Moreover, the main chain structures of polythiophene are easily changed in aqueous solution with stimuli. The complexing disrupts the coplanarity of polymer chain, and thus lead to a hypochromatic shift of maximum emission to some extent. In more polar solution, solvent effect may benefit thiophene aggregates, which act synergistically in the fluorescence quenching of **P1**. Hence **P1** exhibited high sensitivity toward Cu^{2+} in 90% water solution. On the other hand, this kind of high affinity lowered its selective target binding from different metal ions. So, **P1** presented excellent selectivity in less polar condition, that is, in 50% water solution where the metal ion coordination is very weak except for Cu^{2+} . For **P2**, the nitrogen atoms could not join in the Cu^{2+} binding simultaneously due to space steric hindrance resulted by their relatively distant location on the side chain. Therefore, less prominent fluorescence quenching of **P2** by metal ions could be observed.

The proposed binding mode was supported by quantum computation using Gaussian 03 program. The main coordinated bond lengths at the probable binding sites are summarized in

Supporting Information Table S1. The coordinated bond lengths of $\text{Cu-N}(21)$ and $\text{Cu-N}(38)$ are slightly shorter than the reported Cu-N bond length, suggesting a strong interaction between the amino groups and Cu^{2+} . By contrast, the $\text{Cu-O}(31)$ and $\text{Cu-N}(32)$ bond lengths exceed the range of the bond force, inferring that the carbonyl group and secondary amine moieties have almost no interaction with Cu^{2+} . Thus, only primary and tertiary amine of **P1** could act as binding sites for Cu^{2+} ions to form **P1**- Cu^{2+} complex.

CONCLUSIONS

In conclusion, we have synthesized and characterized two new conjugated polymers based on 3-(cyclohexylidene)methylthiophene. Thereinto, **P1** presented excellent selectivity toward Cu^{2+} ions in 50% water solution, the fluorescence color of **P1** disappeared only after addition of Cu^{2+} , which can be easily detected by naked eyes. Highly sensitive detection of Cu^{2+} ions was demonstrated in 90% water solution. This sensor allowed Cu^{2+} recognition in a range of nanomolar concentrations with a detection limit of 3.2×10^{-10} M. These results indicated that this kind of nonionic polymer could be used as a highly selective and sensitive chemo-sensor for Cu^{2+} detection. Unlike **P1**, **P2** showed no significant fluorescent changes in the presence of various metal ions. Therefore, subtle structure adjustment of a conjugated polymer can make a big difference on its optical properties, which is pivotal guidance to our later work on designing some other type of sensors with desired attributes.

ACKNOWLEDGMENTS

The authors thank Sanmenxia Aoke Chemical Industry Co., w0920 for financial support.

AUTHOR CONTRIBUTIONS

Chaoxia Guo: She did most of the work on polymer synthesis and completed paper writing. Shouxiang Jiang: He took part in the synthesis of the polymers. Wenxue Zhu: She helped to study practical applications of the synthesized polymers. Xiuxia Yang: She analyzed the experimental data. Meishan Pei: He is an expert in organic synthesis and participated in the design of the polymers. Guangyou Zhang: He provided guidance for the design and synthesis of the polymers.

REFERENCES

- Sarkar, B.; Siegel, H.; Siegel, A.; In *Metal Ions in Biological Systems*. Marcel Dekker: New York, **1981**; Vol. 12, p 233.
- Que, E. L.; Domaille, D. W.; Chang, C. J. *Chem. Rev.* **2008**, *108*, 1517.
- Stern, B. R. *J. Toxicol. Environ. Health* **2010**, *73*, 114.
- Finkel, T.; Serrano, M.; Blasco, M. A. *Nature* **2007**, *448*, 767.
- Kang, Y. J. *Pharmacol. Ther.* **2011**, *129*, 321.
- Gaggelli, E.; Kozlowski, H.; Valensin, D.; Valensin, G. *Chem. Rev.* **2006**, *106*, 1995.

7. Merian, E. *Metals and Their Compounds in the Environments*; Wiley-VCH: Weinheim, **1991**; p 893.
8. Zietz, B. P.; Dassel de Vergara, J.; Dunkelberg, H. *Germany. Environ. Res.* **2003**, *92*, 129.
9. Chen, L.; McBranch, D. W.; Wang, H. -L.; Helgeson, R.; Wudl, F.; Whitten, D. G. *Proc. Nat. Acad. Sci.* **1999**, *96*, 12287.
10. Heeger, P. S.; Heeger, A. *Proc. Nat. Acad. Sci.* **1999**, *96*, 12219.
11. He, F.; Tang, Y. L.; Wang, S.; Li, Y. L.; Zhu, D. B. *J. Am. Chem. Soc.* **2005**, *127*, 12343.
12. Lange, U.; Roznyatovskaya, N. V.; Mirsky, V. M. *Anal. Chim. Acta.* **2008**, *614*, 1.
13. Song, X.; Wang, H. L.; Shi, J.; Park, J. W.; Swanson, B. I. *Chem. Mater.* **2002**, *14*, 2342.
14. Liu, B.; Yu, W. L.; Pei, J.; Liu, S. Y.; Lai, Y. -H.; Huang, W. *Macromolecules* **2001**, *34*, 7932.
15. Demchenko, A. P. *Introduction to Fluorescence Sensing*; Springer: New York, **2009**.
16. Haupt, K.; Mosbach, K. *Chem. Rev.* **2000**, *100*, 2495.
17. Callan, J. F.; De Silva, A. P.; Magri, D. C. *Tetrahedron* **2005**, *61*, 8551.
18. Li, C.; Numata, M.; Takeuchi, M.; Shinkai, S. *Angew. Chem. Int. Ed.* **2005**, *44*, 6371.
19. Tang, Y. L.; He, F.; Yu, M.; Feng, F.; An, L.; Sun, H.; Wang, S.; Li, Y.; Zhu, D. *Macromol. Rapid Commun.* **2006**, *27*, 389.
20. Costa, S. P. G.; Oliveira, E.; Lodeiro, C.; Raposo, M. M. M. *Tetrahedron Lett.* **2008**, *49*, 5258.
21. Ho, H. A.; Leclerc, M. *J. Am. Chem. Soc.* **2003**, *125*, 4412.
22. Kim, Y. G.; Samuelson, L. A.; Kumar, J.; Tripathy, S. K. *J. Macromol. Sci.* **2002**, *9*, 1127.
23. Wang, X. Y.; Zhao, J. J.; Guo, C. X.; Pei, M. S.; Zhang, G. Y. *Sens. Actuators B* **2014**, *193*, 157.
24. Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Montgomery, J. A.; Jr., Vreven, T.; Kudin, K. N.; Burant, J. C.; Millam, J. M.; Iyengar, S. S.; Tomasi, J.; Barone, V.; Mennucci, B.; Cossi, M.; Scalmani, G.; Rega, N.; Petersson, G. A.; Nakatsuji, H.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Klene, M.; Li, X.; Knox, J. E.; Hratchian, H. P.; Cross, J. B.; Adamo, C.; Jaramillo, J.; Gomperts, R.; Stratmann, R. E.; Yazyev, O.; Austin, A. J.; Cammi, R.; Pomelli, C.; Ochterski, J. W.; Ayala, P. Y.; Morokuma, K.; Voth, G. A.; Salvador, P.; Dannenberg, J. J.; Zakrzewski, V. G.; Dapprich, S.; Daniels, A. D.; Strain, M. C.; Farkas, O.; Malick, D. K.; Rabuck, A. D.; Raghavachari, K.; Foresman, J. B.; Ortiz, J. V.; Cui, Q.; Baboul, A. G.; Clifford, S.; Cioslowski, J.; Stefanov, B. B.; Liu, G.; Liashenko, A.; Piskorz, P.; Komaromi, I.; Martin, R. L.; Fox, D. J.; Keith, T.; Al-Laham, M. A.; Peng, C. Y.; Nanayakkara, A.; Challacombe, M.; Gill, P. M. W.; Johnson, B.; Chen, W.; Wong, M. W.; Gonzalez, C.; Pople, J. A. GAUSSIAN 03, Revision D.02, Gaussian: Pittsburg, PA, **2006**.
25. Guo, C. X.; Yang, X. F.; Yang, X. X.; Zhu, W. X.; Pei, M. S.; Zhang, G. Y. *Sens. Actuators B* **2014**, *205*, 345.
26. McQuade, D. T.; Pullen, A. E.; Swager, T. M. *Chem. Rev.* **2000**, *100*, 2537.
27. Lakowicz, J. R. *Principles of Fluorescence Spectroscopy*. Plenum Press: New York, **1983**.
28. Swager, T. M. *Acc. Chem. Res.* **1998**, *31*, 201.
29. Guo, Z. Q.; Zhu, W. H.; Tian, H. *Macromolecules* **2010**, *43*, 739.
30. Sirilaksanapong, S.; Sukwattanasinitt, M.; Rashatasakhon, P. *Chem. Commun.* **2012**, *48*, 293.
31. Zeng, D.; Cheng, J. G.; Ren, S. J.; Sun, J. S.; Zhong, H. L.; Xu, E. J.; Du, J. P.; Fang, Q. *React. Funct. Polym.* **2008**, *68*, 1715.
32. Xing, C. F.; Shi, Z. Q.; Yu, M. H.; Wang, S. *Polymer* **2008**, *49*, 2698.
33. McQuade, D. T.; Pullen, A. E.; Swager, T. M. *Chem. Rev.* **2000**, *100*, 2537.
34. Xing, C. F.; Yuan, H. B.; Xu, S. C.; An, H. L.; Niu, R. M.; Zhan, Y.; Zhan, Y. *ACS Appl. Mater. Interfaces* **2014**, *6*, 9601.