

Thiourea Based Conjugated Polymer Fluorescent Chemosensor for Cu^+ and Its Use for the Detection of Hydrogen Peroxide and Glucose^{*}

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Abstract A novel conjugated polymer, poly(**1**), containing thiourea moieties in main chain is synthesized *via* Suzuki coupling reaction. The addition of cuprous ion quenches the fluorescence of poly(**1**), whereas the fluorescence changes slightly upon addition of other metal ions, exhibiting the fluorescent almost turn-off sensing ability towards Cu^+ . When hydrogen peroxide was added to the solution containing poly(**1**) and Cu^+ , Cu^+ was oxidized into Cu^{2+} , resulting in the fluorescence recovery. The H_2O_2 released from glucose oxidation by glucose oxidase (GOD) also recovered the fluorescence of poly(**1**)/ Cu^+ solution. The results indicated that the poly(**1**)/ Cu^+ solution could serve as a sensing platform for hydrogen peroxide and glucose.

Keywords: Conjugated polymer; Thiourea; Cuprous ion; Hydrogen peroxide; Glucose.

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INTRODUCTION

Conjugated polymer has attracted significant attention due to its wide application including light-emitting diodes, plastic lasers and solar cells^[1–6]. Among them, one of the present interests in the field of conjugated polymer focused on the application of chemo-/biosensor with high sensitivity. A number of conjugated polymers bearing diverse structures and functionalities have been prepared and developed for detection of metal ions^[7–10], explosive^[11, 12], saccharide^[13, 14], and protein^[15–20]. For example, poly(phenyleneethynylene) bearing functional side groups such as 15-crown-5-ether^[8], carboxylate^[21–23], amino^[24], and sulfonate group^[25, 26] interacted with metal ion as turn-off fluorescent sensor. Moreover, conjugated polymer/metal ion system can also be served as turn-on fluorescent assay. Jin reported a platform for sensitive assay of hydrogen peroxide and glucose based on the conjugated polymer/ Fe^{2+} system^[27]. We prepared a turn-on fluorescent assay containing poly(phenyleneethynylene) bearing thiohydantoin group and Cu^+ to detect hydrogen peroxide and glucose^[28]. Schanze developed a fluorescent turn-on platform bearing carboxylate-substituted poly(phenyleneethynylene) (PPECO2) and Cu^{2+} to detect phosphatase and alkaline phosphatase^[29]. We also synthesized poly(phenyleneethynylene) containing thiourea moieties and developed a fluorescent turn-on assay containing

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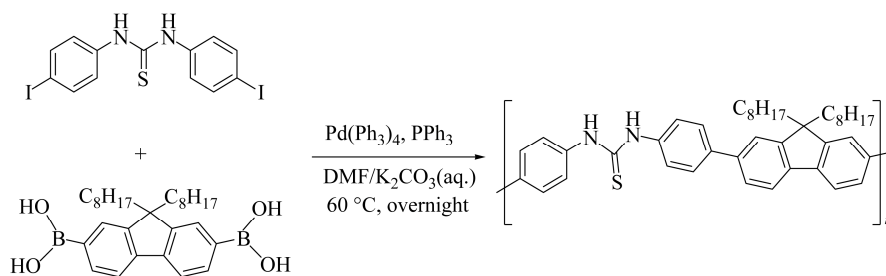
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corresponding conjugated polymer and silver ion to detect ADP which depended on the recovery of Ag^+ quenched fluorescence of polymer by ADP^[30].

Among most of chemosensors based on the conjugated polymer, the functional group was attached in the side chain. The reports that functional moieties were attached in main chain were rare. To date, several of functional backbone polymers have been reported for metal ions such as Zn^{2+} , Cd^{2+} and Ag^+ ^[31, 32]. Poly(phenyleneethynylene) containing diazobenzene as functional group in the main chain was reported. In the presence of hydrazine, the diazo unit was reduced to phenylhydrazine and the conjugation of the main chain was changed, resulting in a decoloring and fluorescence turn-on effect^[33]. Therefore, we can image that if the functional moieties are attached in the main chain of a conjugated polymer, the conjugation of the main chain changes in presence of analyte, leading to the colorimetric and/or fluorescent response.

Hydrogen peroxide (H_2O_2) is a precursor molecule of other reactive oxygen species (ROS) which widely causes oxidative damage on proteins and nucleic acids and is closely correlated to various human diseases such as cancer and Alzheimer's disease^[34, 35]. In addition, hydrogen peroxide can be generated as a byproduct from various cellular aerobic oxidation reactions such as glucose oxidation by glucose oxidase (GOx). Consequently, the development of novel H_2O_2 sensors is crucial for clinical diagnostics and biological analysis.

Herein, we synthesized 1,3-bis(4-iodophenyl)-thiourea and polymerized it with (9,9-dihexyl-9,9a-dihydro-4aH-fluorene-2,7-diyl)diboronic acid to obtain poly(**1**) (scheme 1). The thiourea group was embedded in the main chain of poly(**1**). We investigated the interactions between poly(**1**) and metal ions. It was very interesting to find that Cu^{2+} and Cu^+ exhibit different effects on the fluorescence of poly(**1**). Cu^+ quenched the fluorescence of poly(**1**), while Cu^{2+} at the same concentration resulted in slightly change. Now, we report a fluorescence platform containing poly(**1**) and Cu^+ to detect hydrogen peroxide and glucose.



Scheme 1 Synthesis of poly(**1**)

EXPERIMENTAL

Reagents

DMF, as polymerization solvent, was distilled over CaH_2 . 4-Iodoaniline, 4-iodophenyl isothiocyanate and (9,9-dioctyl-9H-fluorene-2,7-diyl)bisboronic acid were obtained from TCI. All other reagents were commercially obtained and used as received.

Apparatus

^1H - (400 MHz) and ^{13}C - (100 MHz) NMR spectra were recorded on a Bruker Avance/DMX 400 MHz NMR spectrometer with $\text{DMSO}-d_6$ as solvent and tetramethylsilane as an internal reference. IR spectra were measured using a Shimadzu FTIR-8100 spectrophotometer. Absorbance detections were carried out on Shimadzu UV-2450 spectrophotometer. Fluorescence spectra were obtained using a Hitachi F-4500 spectrofluorometer with a xenon lamp and 1.0 cm quartz cells. Melting points (mp) were measured on a digital melting point apparatus (WRS-1B). Elemental analysis were performed on an Eager 300 elemental microanalyzer. The number- and weight-average molecular weights (M_n and M_w , respectively) of the polymer were determined using gel permeation chromatography (GPC) on a Jasco Gulliver system (PU-980, CO-965, RI-930, and UV-1570) equipped with polystyrene gel columns (Shodex columns K804, K805, and J806) and calibrated by polystyrene

standards at 30 °C using THF as the eluent. Fluorescence emission spectra were obtained at room temperature on an FLS-920 Edinburgh Fluorescence Spectrophotometer, with a Xenon lamp and 1.0 cm quartz cells.

Synthesis of **1**

First, 4-iodoaniline (2.2 g, 10 mmol) was added into a 100 mL flask in the present of 30 mL THF. Then 4-iodophenyl isothiocyanate (2.61 g, 10 mmol) in 10 mL of THF was dropwise added into the flask. The reaction mixture was stirred at room temperature overnight. The mixture was concentrated by a rotary evaporator and the residue was extracted with 200 mL of CH₂Cl₂. The organic phase was washed with 1 mol/L HCl, saturated aq. NaHCO₃ and brine. The organic layer was dried over anhydrous MgSO₄ and evaporated to dryness. The crude mixture was concentrated under reduced pressure and the product was purified *via* flash column chromatography (EtOAc:hexane = 1:4 *V/V*). White solid was obtained in 83% yield (3.04 g).

Mp 199.2–199.4 °C; ¹H-NMR (400 MHz, DMSO-d₆, δ): 7.33 (d, *J* = 8.2 Hz; 4H, Ar), 7.68 (d, *J* = 8.1 Hz; 4H, Ar), 9.92 (s, 2H, NH); ¹³C-NMR (100 MHz, DMSO-d₆, δ): 88.72 (Ar), 125.71 (Ar), 137.10 (Ar), 139.22 (Ar), 179.34 (C=S); IR (KBr, cm⁻¹): 3199(s), 3157(s), 3008(s), 1637(w), 1583(s), 1525(s), 1002(s), 819(s); Anal. Calcd for C₁₃H₁₀I₂N₂O: C 32.52, H 2.10, N 5.83; found: C 32.98, H 2.01, N 5.14.

Polymerization

A solution of **1** (480 mg, 1.00 mmol) and (9,9-dioctyl-9H-fluorene-2,7-diyl)bisboronic acid (478 mg, 1.00 mmol), Pd(PPh₃)₄ (57 mg, 50 μmol) and PPh₃ (26.2 mg, 100 μmol) in DMF (3.00 mL) and saturated aq. K₂CO₃ (2 mL) was stirred at 60 °C overnight. The resulting mixture was poured into MeOH/4 mol/L HCl [4/1 (*V/V*), 300 mL] to precipitate the polymer. It was separated by filtration using a membrane filter (ADVANTEC H100A047A) and dried under reduced pressure.

GPC (THF, polystyrene standard): *M*_w = 34300, *M*_n = 14200, PDI = 2.41; ¹H-NMR (400 MHz, DMSO-d₆, δ): 0.65 (d, *J* = 107.4 Hz; 6H, CH₃), 0.91–1.52 (m, 24H, CH₂), 1.94 (dd, *J* = 52.9, 79.6 Hz; 4H, CH₂), 7.16–8.02 (m, 14H, Ar), 9.15 (s, 2H, NH); IR (KBr, cm⁻¹): 3440(s), 2925(s), 2852(s), 1656(s), 1434(s), 1116(s), 1004(s), 829(s), 717(s).

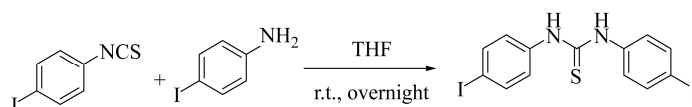
Typical Experimental Procedure

A typical experimental procedure is described as follows: Stock solution of poly(**1**) (1.8 mmol/L) in dimethyl sulfoxide (DMSO) and CuCl (1.8 mmol/L) in DMSO were prepared in flasks equipped with stopcocks, respectively. Aliquots of the poly(**1**) solution (0.1 mL), DMSO (5 mL), the CuCl solution (1 mL) and H₂O (1 mL) were transferred to a vial, and then the resulting mixture was diluted to 10 mL with DMSO to give the sample solution, of which the polymer repeat unit concentration ([PRU]) and [CuCl]/[PRU] were adjusted to be 18 μmol/L and 10, respectively. The concentration of poly(**1**), which was calculated on the basis of the monomeric unit, was 18 μmol/L for all the measurements except it was otherwise pointed out. The fluorescence intensity was measured with the excitation wavelength 330 nm except as otherwise noted, and the excitation and emission slits were set to 1 and 1 nm, respectively.

RESULTS AND DISCUSSION

Synthesis

Thiourea monomer **1** was prepared by reaction of 4-iodoaniline with 4-iodophenyl isothiocyanate (Scheme 2). The product was isolated in high yields (83%) after purification by silica gel chromatography. The monomer was obtained as white powder and characterized by spectroscopic methods and satisfactory analysis data corresponding to its molecular structure (Figs. S1–S3).

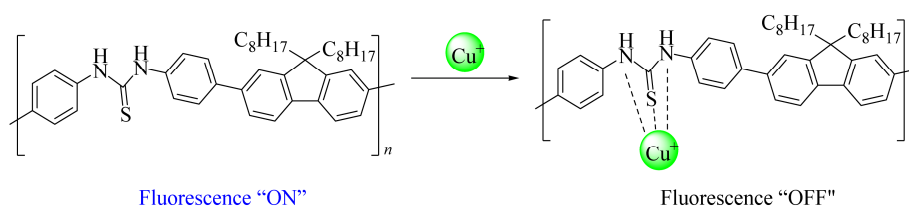


Scheme 2 Synthesis of **1**

The Suzuki coupling reaction of **1** with (9,9-dioctyl-9H-fluorene-2,7-diyl)bisboronic acid was carried out in DMF at 60 °C overnight (Scheme 1). The corresponding polymer poly(**1**) with M_n 's about 14200 and PDI about 2.41 was obtained in 57% yield. The structure of poly(**1**) was characterized spectroscopically (Figs. S4 and S5).

Interaction between Poly(**1**) and Metal Ions

To examine the interaction between poly(**1**) and metal ions, the emission spectra of poly(**1**) were measured in the presence of various metal ions. As shown in Fig 1(a), 10 equivalents of Cu^+ almost completely quenched the fluorescence, while other metal ions (Mn^{2+} , Zn^{2+} , Na^+ , Ca^{2+} , Cd^{2+} , Pb^{2+} , Mg^{2+} , K^+ , Cu^{2+} , Hg^{2+} , Ni^{2+} and Ag^+) changed the fluorescence slightly. Therefore, the result demonstrates that poly(**1**) displays fluorescent almost turn-off sensing ability towards cuprous ion. Cuprous ion and poly(**1**) may form chelate *via* coordinate bond resulting in fluorescence quenching. The mechanism is depicted in Scheme 3.



Scheme 3 Proposed complexation mechanism of poly(**1**) with Cu^+

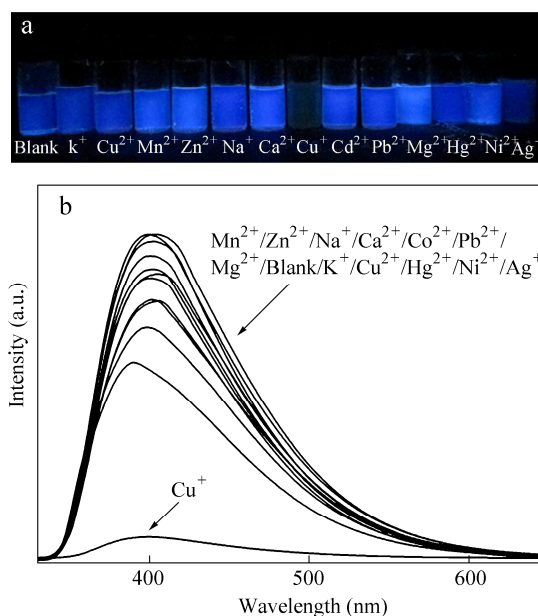


Fig. 1 (a) Visual fluorescence colour variations and (b) emission spectra of poly(**1**) in DMSO (containing 10% H_2O) ($[\text{PRU}] = 18 \mu\text{mol/L}$) upon the addition of various metal ions ($[\text{metal ion}]/[\text{PRU}] = 10$, Excitation at 330 nm.) The visual fluorescence was obtained with excitation at 365 nm using a hand-held UV lamp

Figure 1(b) shows the emission spectra of poly(**1**) in the presence of various metal ions. The addition of 10 equivalents of Mn^{2+} , Zn^{2+} , Na^+ , Ca^{2+} , Cd^{2+} , Pb^{2+} and Mg^{2+} increased the fluorescence intensity by 8% to 25%, while the fluorescence intensity decreased by 1% to 15% with the addition of K^+ , Cu^{2+} , Hg^{2+} , Ni^{2+} and Ag^+ . Nevertheless, the addition of cuprous ion quenched 90% of the original polymer fluorescence. It seems that Cu^{2+} and Cu^+ show opposite effects on the fluorescence of poly(**1**).

The interaction of poly(**1**) with Cu^+ was investigated in more detail. Figure S6 shows the absorption spectrum of poly(**1**) with various concentrations of Cu^+ . The absorption of polymer features a broad maximum

around 320 nm. The addition of Cu^+ to poly(**1**) enhanced the intensity. As shown in Fig. 2(a), with the increase of Cu^+ , the fluorescence intensity of poly(**1**) decreased continuously. When 10 equivalents of cuprous ion was added into the solution, 90% of the original polymer fluorescence was quenched. In low concentration region, *i.e.*, from 0 to 7.14×10^{-5} mol/L, a linear correlation was observed. The association constant (K_a) was determined to be 9.81×10^4 L/mol (Fig. 2b). The detection limit was 4.13×10^{-6} mol/L which was calculated using fluorescence data according to the IUPAC definition. To test the performance of poly(**1**) towards Cu^+ , we synthesized 1,3-bis-[4-(9,9-dimethyl-9H-fluoren-2-yl)-phenyl]-thiourea (probe **1**) according to Fig. S7. The probe **1** was obtained with analysis data corresponding to its molecular structure (Figs. S8 and S9). As the amount of Cu^+ increased, the fluorescence intensity of probe **1** decreased continuously. However, 1000 equivalents of Cu^+ added into the solution quenched 87% of original polymer fluorescence and the association constant (K_a) was determined to be 1.55×10^3 L/mol (Fig. S10), which reconfirm the sensitivity of poly(**1**) towards Cu^+ .

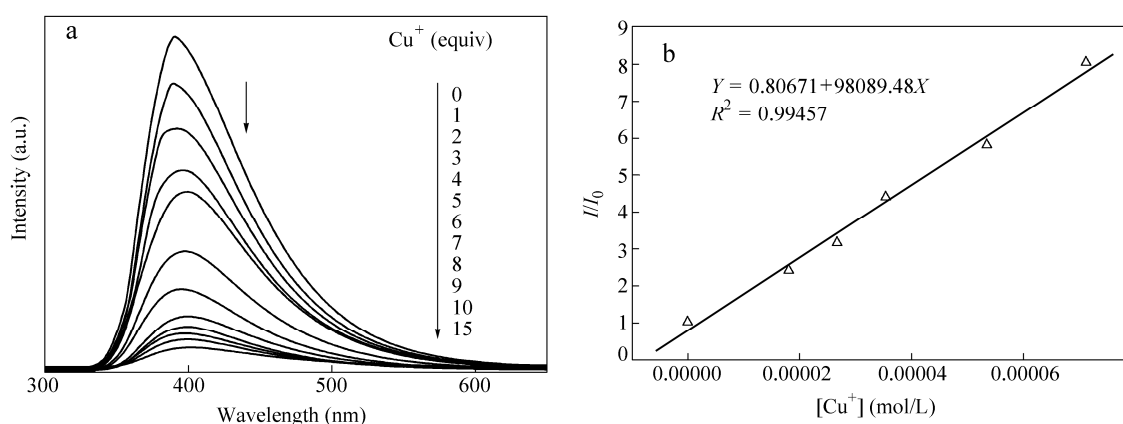
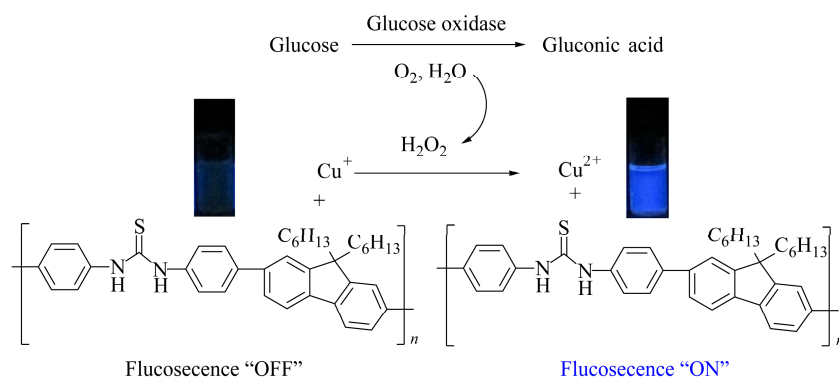


Fig. 2 (a) Emission spectra of a solution of poly(**1**) ([PRU] = 18 μmol/L) in DMSO (containing 10% H₂O) titration with Cu^+ ; (b) Fluorescence intensity enhancement (I/I_0) at 395 nm with titration $[\text{Cu}^+]/[\text{PRU}] = 0$ to 4 (Excitation at 330 nm.)

According to the hard and soft acids and bases (HSAB) theory^[36–40], soft bases prefer to bind soft acids and the interaction between soft bases and soft acids is stronger than that between soft bases and borderline acids. The sulfur atom has an easily polarizable lone pair of electron and is considered as a ‘soft base’. Cuprous ion is a typical soft acid and copper ion is considered as a borderline acid. In presence of Cu^+ , the Cu^+ -thiourea chelating complex was formed (Fig. S11), which changed conjugation of main chain and consequently quenched the fluorescence.

Application to Detect Hydrogen Peroxide and Glucose

From what has been mentioned above, there were opposite effects of Cu^+ and Cu^{2+} on poly(**1**). Cu^+ can be oxidized into Cu^{2+} in presence of hydrogen peroxide, which means the fluorescence of the poly(**1**)/ Cu^+ solution might be recovered upon the addition of hydrogen peroxide (Scheme 4). We first examined the influence of incubation time upon addition of hydrogen peroxide into the poly(**1**)/ Cu^+ solution ([PRU] = 18 μmol/L, $[\text{Cu}^+]/[\text{PRU}] = 10$, $[\text{H}_2\text{O}_2]/[\text{PRU}] = 5$, $[\text{H}^+]/[\text{PRU}] = 5$). Figure S12 shows the alteration of the fluorescence intensity at 395 nm with the incubation time. The fluorescence intensity at 395 nm increased with increasing the incubation time from 0 min to 10 min and then tended to be a constant value after 10 min. Therefore, the incubation time was controlled to be 10 min.



Scheme 4 Schematic illustration of the hydrogen peroxide and glucose assays

For further investigation, we measured the emission spectra of poly(**1**)/ Cu^+ solution upon addition of various amounts of H_2O_2 . As shown in Fig. 3(a), with the increase of H_2O_2 , more and more Cu^+ were oxidized into Cu^{2+} , leading to the further recovery of the fluorescence. Upon addition of 6 equivalents of H_2O_2 , 90% of the original polymer fluorescence was recovered. In low concentration region, *i.e.*, from 0 to 7.14×10^{-5} mol/L, a linear correlation was observed. The association constant (K_a) was determined to be 8.78×10^4 L/mol (Fig. 3b). The detection limit was 4.64×10^{-6} mol/L which was calculated using fluorescence data according to the IUPAC definition. Thus, it demonstrates that H_2O_2 can be recognized *via* the fluorescence recovery of the poly(**1**)/ Cu^+ solution.

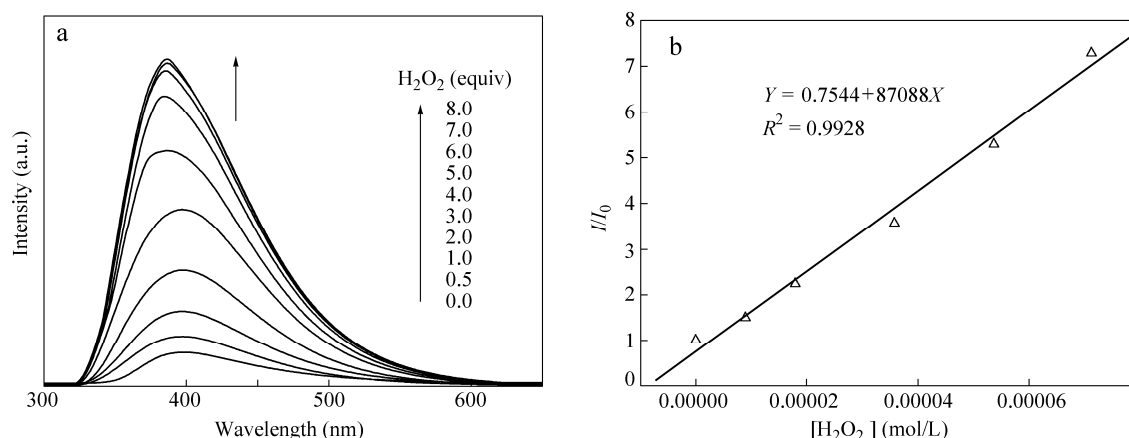


Fig. 3 (a) Emission spectra of poly(**1**)/ Cu^+ solution ($[\text{PRU}] = 18 \mu\text{mol/L}$, $[\text{Cu}^+]/[\text{PRU}] = 10$, $[\text{H}^+]/[\text{PRU}] = 5$) in DMSO (containing 10% H_2O) with H_2O_2 titration; (b) Fluorescence intensity enhancement (I/I_0) at 395 nm with titration $[\text{H}_2\text{O}_2]/[\text{PRU}] = 0$ to 4 (Excitation at 330 nm).

In order to demonstrate the potential of poly(**1**)/ Cu^+ assay to detect bioanalyte such as glucose, we used poly(**1**)/ Cu^+ solution to monitor glucose oxidation by GOD. The poly(**1**)/ Cu^+ solution ($[\text{PRU}] = 18 \mu\text{mol/L}$), Cu^+ ($180 \mu\text{mol/L}$), GOD (100U), H^+ ($90 \mu\text{mol/L}$) and glucose ($90 \mu\text{mol/L}$) was prepared, and the changes in the fluorescence emission *via* incubation time were recorded (Fig. S13).

When the GOD was added into glucose solution, the fluorescence intensity at 395 nm increased dramatically by H_2O_2 produced from glucose oxidation. In addition, the fluorescence intensity at 395 nm tended to be a constant value after 30 min.

As shown in Scheme 3, glucose will be specifically oxidized into gluconic acid in the catalysis of glucose oxidase (GOD), releasing an equivalent hydrogen peroxide. The produced H_2O_2 can oxidize Cu^+ into Cu^{2+} ,

leading to the recovery of the fluorescence. Using a mixed-solution containing poly(**1**) ([PRU] = 18 $\mu\text{mol/L}$, [GOD] = 100U, $[\text{Cu}^+]/[\text{PRU}] = 10$, $[\text{H}^+]/[\text{PRU}] = 5$), a fluorescence experiment with glucose was carried out. As predicted, the addition of glucose recovered the fluorescence (Fig. 4a). When 9 equivalents of glucose were added, 72% of the original polymer fluorescence was recovered. In low concentration region, *i.e.*, from 0 to 8.93×10^{-6} mol/L, a linear correlation was observed. The association constant (K_a) was determined to be 1.07×10^5 L/mol (Fig. 4b). The detection limit was 3.80×10^{-6} mol/L. Consequently, the poly(**1**)/ Cu^+ solution can also act as a glucose probe.

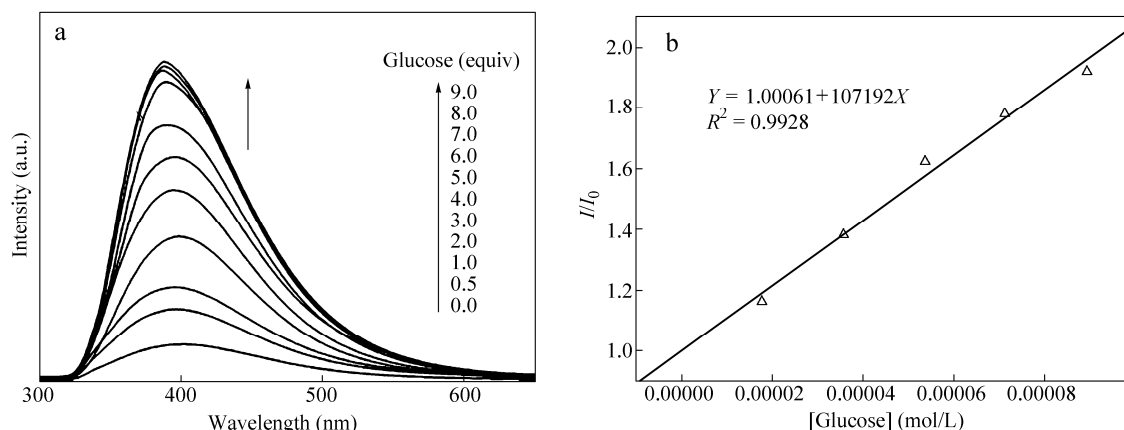


Fig. 4 (a) Emission spectra of a poly(**1**)/ Cu^+ solution ([PRU] = 18 $\mu\text{mol/L}$, $[\text{Cu}^+]/[\text{PRU}] = 10$, $[\text{H}^+]/[\text{PRU}] = 5$, [GOD = 100U]) in DMSO (containing 10% H_2O) with glucose titration; (b) Fluorescence intensity enhancement (I/I_0) at 395 nm with titration [Glucose]/[PRU] = 0 to 0.5 (Excitation at 330 nm.)

CONCLUSIONS

In conclusion, we have reported a novel fluorescence chemosensor containing thiourea group in main chain. Poly(**1**) exhibited sensing ability towards cuprous ion. The phenomenon of Cu^+ and Cu^{2+} showing opposite effects on poly(**1**) allowed poly(**1**)/ Cu^+ solution serving as a sensing platform for oxidant. In the presence of hydrogen peroxide and glucose (containing GOD), the fluorescence of the poly(**1**)/ Cu^+ solution could be recovered. Therefore, poly(**1**)/ Cu^+ solution could act as a sensing platform for hydrogen peroxide and glucose.

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