Macromolecules
ARTICLE

Macromolecules **2010**, *43*, 739–744 **739**
DOI: 10.1021/ma902466g

Hydrophilic Copolymer Bearing Dicyanomethylene-4*H*-pyran Moiety As Fluorescent Film Sensor for Cu^{2+} and Pyrophosphate Anion

Zhiqian Guo, Weihong Zhu,* and He Tian*

Key Laboratory for Advanced Materials and Institute of Fine Chemicals, East China University of Science & Technology, Shanghai 200237, P. R. China

Received November 9, 2009; Revised Manuscript Received November 29, 2009

ABSTRACT: The design of fluorescent sensors for pyrophosphate anion ($P_2O_7^{4-}$, PPi) has become a focus of considerable research in biological markers with realizing distinct advantages such as high sensitivity, easy detection, and wide dynamic ranges. A new strategy of incorporating the ion-sensitive fluorescent unit to form hydrophilic copolymer poly(HEMA-*co*-DCPDP) has been developed as a film sensor for Cu^{2+} and PPi. In general, the responsive characteristics of polymer-based fluorescent film for bioprocess are dependent on several factors, such as the permeability of ions into polymer matrix, the aggregation of polymer chains, and the strength of binding interaction between ions and polymeric sensors. To overcome these limitations, 2-hydroxyethyl methacrylate (HEMA) as a neutral hydrophilic chain segment is chosen as the monomer for its high hydrophilicity to improve ion permeability into the polymer matrix. Using the ensemble method, the related metal complex, poly(HEMA-*co*-DCPDP)-Cu^{2+}, shows turn-on fluorescence and high sensitivity for PPi in both solution and thin film over other anions such as adenosine triphosphate (ATP) and phosphate (Pi). The low-cost hydyophilic copolymer film of poly(HEMA-*co*-DCPDP)-Cu^{2+} exhibits high sensitivity and rapid response to PPi with turn-on orange-red fluorescence, showing an ideal hydrophilic film strategy for low-cost anion chemosensors or chips to the online monitor and high-throughput bioprocessing in continuous system.

Introduction

The development of highly sensitive and selective chemosensors for biologically important anions continues to be an active area in supramolecular chemistry, especially sensing ions in aqueous solution and solid-state chemo-/biosensors.[1] Anions such as pyrophosphate ($P_2O_7^{4-}$, PPi) and adenosine triphosphate (ATP) play a fundamental role in a wide range of chemical and biological processes.[2] In particular, PPi as the product of ATP hydrolysis under cellular condition is an important issue in cancer research.[3] As a consequence, the design of fluorescent sensors for PPi has become a focus of considerable research in biological markers with realizing distinct advantages such as high sensitivity, easy detection, and wide dynamic ranges.[4] Even though several PPi sensors based on small molecules displayed turn-on fluorescence mode in an aqueous media have been reported,[5] the design of fluorescent PPi sensors for application in the continuous online system[6] is still a challenge because of the limitations of small molecules in fabrication devices. Recently, as novel important functional materials, polymeric sensors that can be tailored to meet specific needs via rational design approaches have been paid particular attention because of exploiting chemical and biological species by various energy transduction principles, such as radiant, electrical, mechanical, and thermal process.[7] Until now, only very few polymer sensors that display the off−on fluorescence mode for PPi in solution were reported.[8] However, no case has been reported in utilizing polymers as the fluorescent sensor film to real-time monitor of PPi.

In contrast with molecular sensors, film sensors based on polymers exhibit prominent advantages, such as easy fabrication in device, a wide choice of incorporating specific units into functional polymer, low cost, and so on.[9] Hydrophilic polymeric films as pH sensors have been widely studied for monitoring high-throughput bioprocessing in continuous system.[6] Accordingly, incorporating ion-sensitive fluorophore receptors to form hydrophilic polymers for wide-range analytes might pave a way to develop a novel real-time monitoring system for bioprocess. Recently, we have reported a new metal complex sensor DCCP-Cu^{2+} that shows turn-on fluorescence for PPi in the near-infrared region, which is derived from a typical laser dye of DCM (4-dicyanomethylene-2-methyl-6-[4-(dimethylamino)-styryl]-4*H*-pyran).[5b] Keeping the above strategies in mind, we design a hydrophilic fluorescent copolymer sensor poly(HEMA-*co*-DCPDP), which possesses 2-hydroxyethyl methacrylate (HEMA) as hydrophilic unit and 2-{2-[4-(bis(pyridin-2-ylmethyl) amino)styryl]-6-[4-(piperazin-1-yl)styryl]-4*H*-pyran-4-ylidenmalononitrile (DCPDP) as the ion-sensitive fluorophore unit (Scheme 1). Intriguingly, not only does poly(HEMA-*co*-DCPDP) exhibit sensitivity to Cu^{2+} but also the relative metal complex, poly(HEMA-*co*-DCPDP)-Cu^{2+}, shows turn-on fluorescence with high sensitivity for PPi both in solution and in thin film over other anions such as ATP and phosphate (Pi). To the best of our knowledge, this is the first hydyophilic polymer-based film sensor with turn-on fluorescence, high selectivity, and rapid response to PPi anion, showing an ideal hydrophilic film strategy for low-cost chemosensors or chips to the online monitor and high-throughput bioprocessing.

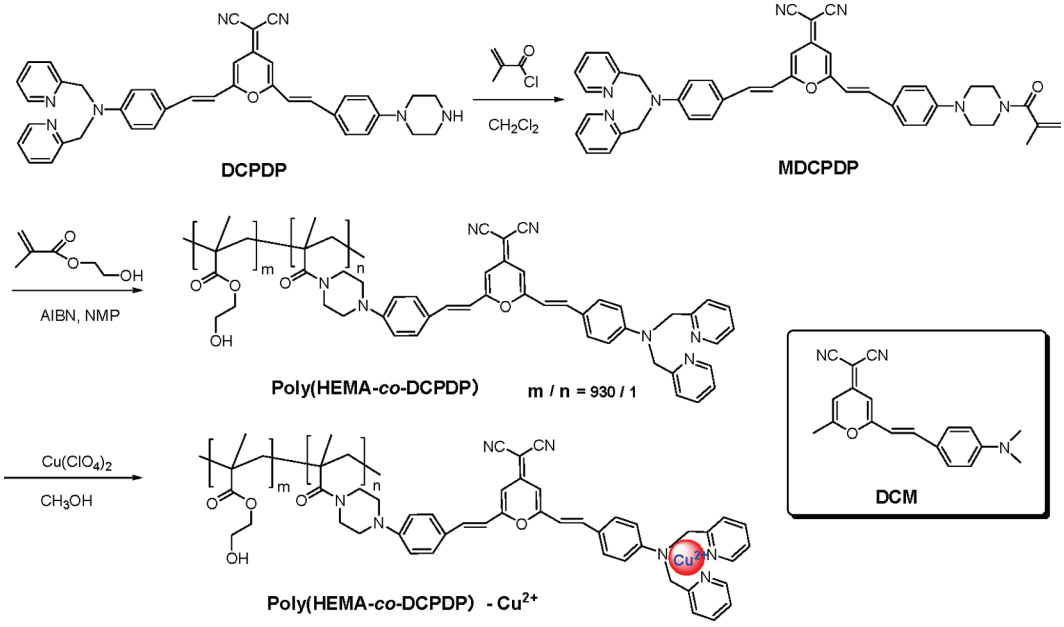
Results and Discussion

Design and Synthesis. As is well known, the response characteristics of polymer-based fluorescent film for bioprocess are dependent on several factors, such as the permeability of ions into polymer matrix, the aggregation of polymer chains, and the strength of binding interaction between ions and polymeric sensors.[10a] To overcome these

*Corresponding author. Fax: (+86) 21-6425-2758. E-mail: whzhu@ecust.edu.cn (W.Z.); tianhe@ecust.edu.cn (H.T.).

Published on Web 12/09/2009

Scheme 1. Synthesis of Poly(HEMA-*co*-DCPDP)-Cu^{2+}



problems, 2-hydroxyethyl methacrylate as a neutral hydrophilic chain segment is chosen as monomer for its high hydrophilicity to improve the permeability of ions into polymer matrix. Poly(2-hydroxyethyl methacrylate) (PHEMA) has been extensively studied and used in biomedical application, such as a major component in contact lenses, drug delivery, and biocompatible hydrogels.[10] Again, because of 2-hydroxyethyl groups, PHEMA exhibits a high hydrophilicity but is insoluble in water by polymerization.[10d]

The fluorescent unit of DCPDP is straightforwardly synthesized (Scheme 1).[11a] DCPDP was functionalized with methacryloyl chloride to gain the corresponding monomer MDCPDP (in Scheme 1). In ^{1}H NMR of DCPDP and MDCPDP, the characteristic coupling constant (J = 16.0 Hz) of alkene protons is indicative of the predominant trans isomer. Finally, poly(HEMA-*co*-DCPDP) was prepared by free-radical copolymerization of MDCPDP and 2-hydroxyethyl methacrylate (HEMA) in anhydrous solution of *N*-methylpyrrolidin-2-one (NMP) with 2,2-azobis(isobutyronitrile) (AIBN) as an initiator. The resulting molar ratio of DCPDP and HEMA units in the copolymer composition was 1:930, derived from ^{1}H NMR spectra or standard Job's plot of MDCPDP absorption spectra in ethanol solution (ξ = 15 581 $M^{-1}\cdot cm^{-1}$ at 460 nm). The experimental details and characterization data are given in the Supporting Information (Figures S1 and S2). Apparently, the molar ratio of DCPDP units in the copolymer is much less than that of HEMA units so as not to affect distinctly the copolymer hydrophilicity and its good film-forming property. Furthermore, the treatment of poly(HEMA-*co*-DCPDP) with $Cu(ClO_4)_2$ in methanol affords the desired metal complex poly(HEMA-*co*-DCPDP)-Cu^{2+} as the anion sensor candidate.

Fluorescence Properties and Ion Recognition. As a derivative of DCM (4-dicyanomethylene-2-methyl-6-[4-(dimethylamino)styryl]-4*H*-pyran), the monomer of MDCPDP is also a typical donor-π-acceptor (D-π-A) structure with a broad absorption band resulting from an ultrafast process of intramolecular charge transfer (ICT).[12] Actually, we have exploited the ICT mechanism of DCM derivatives for ion sensing and molecular switching, even for molecular logic gates.[5b,11] In MDCPDP, the DPA moiety acts as an electron-donating substituent whose donor character strongly depends on cation coordination. If the electron-donating character of DPA moiety is reduced, then the significant decrease in fluorescence intensity MDCPDP is resulted from a decrease in ICT efficiency.[12,13]

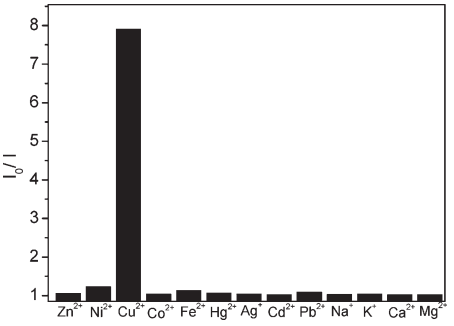


Figure 1. Fluorescence response of poly(HMAM-*co*-MDCPDP) (1.2 g/L) in a mixture solution of water−ethanol (2:5 v/v) at pH 7 in the presence of various metal ions (50 μM, λ_{ex} = 460 nm, λ_{em} = 605 nm). Note: I_0/I represent the ratio of the fluorescence intensity change before and after the addition of various metal ions with poly(HEMA-*co*-DCPDP).

Although bis(2-pyridylmethyl)amine (DPA) has been extensively used as a Zn^{2+} ligand owing to its high affinity and excellent selectivity,[14] the DPA units of previous reported DCM derivatives[5b,11a] display high selectivity and sensitivity to Cu^{2+}. Therefore, poly(HEMA-*co*-DCPDP) should be a candidate for the Cu^{2+} ion sensor in that the DCPDP monomer contains the DPA moiety. The selectivity and sensitivity of poly(NIPMAM-*co*-MDCPDP) were investigated with various metal ions (Figure 1). Other metal ions such as Na^+, Ag^+, Fe^{3+}, Fe^{2+}, Ni^{2+}, Cd^{2+}, Hg^{2+}, Pb^{2+}, and Zn^{2+} show subtle disturbance, but only the addition of Cu^{2+} ions to poly(HEMA-*co*-DCPDP) causes a significant decrease in fluorescence intensity acting as an efficient fluorescence quencher. It implies that the capture of the Cu^{2+} ion by the receptor DPA exhibits stronger binding affinity and decreases the electron-donating ability of the amino groups,[15] thus resulting in a decrease in the ICT efficiency.[11,14b] Moreover, the slight hypochromic shift in the absorption spectra of poly(HEMA-*co*-DCPDP) with the addition Cu^{2+} further confirms the disturbance of the ICT process (Figure S3 in the Supporting Information). As previously reported, fluorescent sensors based on the D-π-A type are usually disturbed by

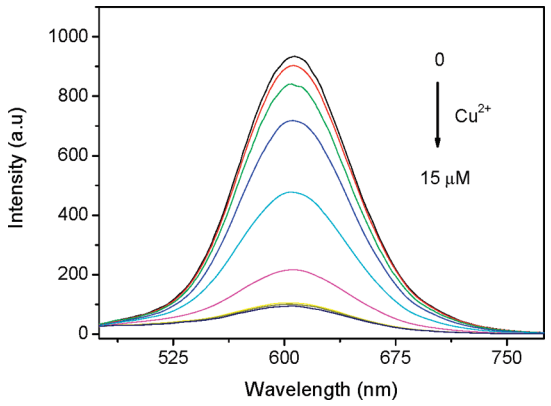


Figure 2. Emission spectra of poly(HEMA-*co*-DCPDP) (1.2 g/L) upon titration with Cu^{2+} ions from 0 to 15 μM in a mixture of water−ethanol (2:5 v/v) solution at pH 7 (λ_{ex} = 460 nm).

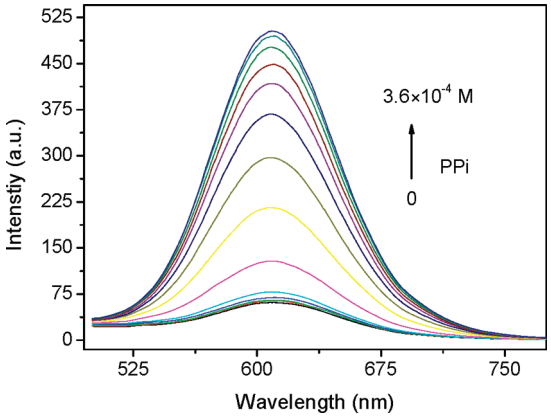


Figure 3. Emission spectra of poly(HEMA-*co*-DCPDP)-Cu^{2+} (0.8 g/L) upon titration with PPi from 0 to 3.6 × 10^{-4} M in a mixture of water−ethanol (2:5 v/v) with a buffer solution of MOPS (10 mM, pH 7.0, λ_{ex} = 460 nm).

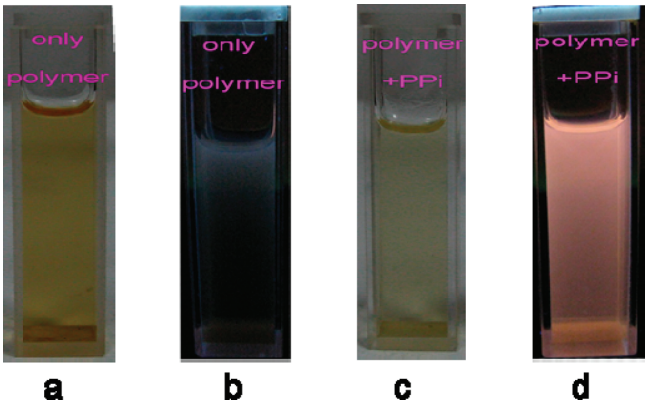


Figure 4. Fluorescence visual response of poly(HEMA-*co*-DCPDP)-Cu^{2+} in (a,b) the absence and (c,d) the presence of PPi under (a,c) a visual light and (b,d) a UV light 365 nm in a mixture of water−ethanol (2:5 v/v) with a buffer solution of MOPS (10 mM, pH 7.0).

protons in the detection of metal ions, and MDCPDP only captures the Cu^{2+} ion in neutral and acidic but not under a basic condition.[11a] Therefore, pH 7 is chosen to guarantee MDCPDP effective coordination with Cu^{2+} in buffer solution. Moreover, the Stern−Volmer constant (K_{sv})[16] is determined to be ~1.42 × 10^5 M^{-1} by fluorometric titration curves with the addition of the Cu^{2+} in a mixture solution of water−ethanol (2:5 v/v) at pH 7 (Figure 2).

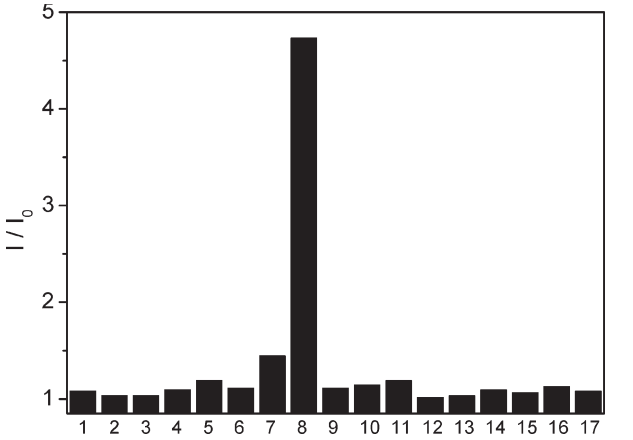


Figure 5. Fluorescence response of poly(HEMA-*co*-DCPDP)-Cu^{2+} (0.8 g/L) to various anions at 1.8 × 10^{-4} M concentration in a mixture of water−ethanol (2:5 v/v) with a buffer solution of MOPS (10 mM, pH 7.0): 1, F^-; 2, Cl^-; 3, Br^-; 4, I^-; 5, $H_2PO_4^-$; 6, HPO_4^{2-}; 7, PO_4^{3-}; 8, $P_2O_7^{4-}$; 9, ADP; 10, AMP; 11, ATP; 12, CO_3^{2-}; 13, HCO_3^-; 14, SO_4^{2-}; 15, HSO_4^-; 16, CH_3COO^-; 17, NO_3^- (λ_{ex} = 460 nm, λ_{em} = 605 nm). Note: I/I_0 represents the ratio of the fluorescence intensity change after and before the addition of various anions with poly(HEMA-*co*-DCPDP)-Cu^{2+}.

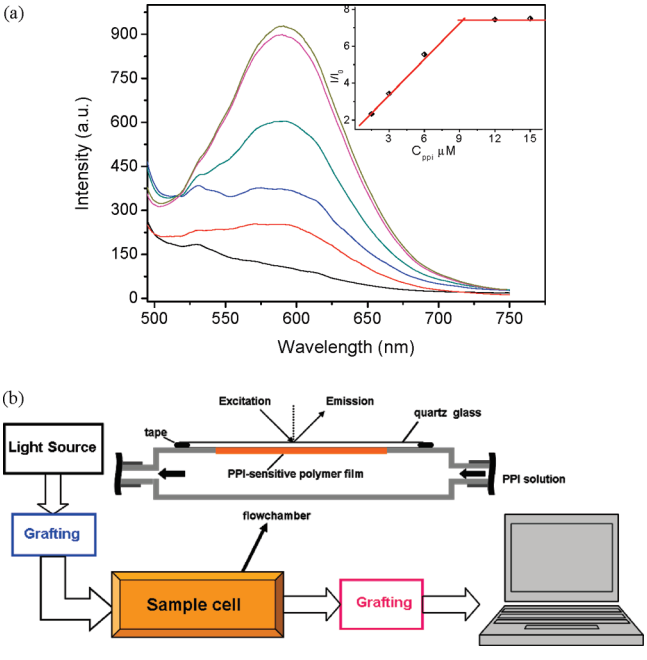


Figure 6. (a) Fluorescence response of poly(HEMA-*co*-DCPDP)-Cu^{2+} film (1.0 g/m^2) immersed in aqueous solution of PPi for ~5 min: 1.5, 3.0, 6.0, 12.0, and 15.0 μM. Inset: Job plot of polymer film at λ_{em} = 590 nm. Note: I/I_0 represents the ratio of the fluorescence intensity change after and before the addition of PPi with poly(HEMA-*co*-DCPDP)-Cu^{2+}. (b) Schematic drawing of the experimental setup using polymer film sensor on a quartz slide in combination with a spectrofluorometer.

Metal-ion complexes are ideal binding sites for anion recognitions such as PPi in aqueous solutions.[2,17] In general, the design of PPi sensors for bioprocess requires the following principles: (a) understanding the recognition mechanism between PPi and the binding sites, (b) high hydrophilicity, and (c) high selectivity for PPi over other anions such as AMP and ADP, and, in particular, phosphate and ATP.[2] The unsaturated coordinative Cu^{2+} complex exhibits strong binding tendencies toward anion substrates due to the d^9 electronic configuration of the copper ion.[5j] Taking into

consideration the fact that the addition of Cu^{2+} to poly(HEMA-*co*-DCPDP) almost completely quenches fluorescence to baseline, naturally, it is reasonable to predict that the ensemble of metal complex poly(HEMA-*co*-DCPDP)-Cu^{2+} might be a candidate as a turn-on fluorescent sensor for specific anions. As expected in Figure 3, the addition of PPi to poly(HEMA-*co*-DCPDP)-Cu^{2+} causes a fluorescent enhancement factor (I/I_0) of ~4.8. This obvious change in fluorescence can be visualized under UV lamp in Figure 4. The selectivity and sensitivity of poly(HEMA-*co*-DCPDP)-Cu^{2+} is conducted to examine various anions including F^-, Cl^-, Br^-, I^-, $H_2PO_4^-$, HCO_3^-, HSO_4^-, CH_3COO^-, NO_3^-, HPO_4^{2-}, SO_4^{2-}, CO_3^{2-}, AMP, ADP, ATP, PO_4^{3-} (Pi), and $P_2O_7^{4-}$ (PPi) (Figure 5). Interestingly, other investigated anions give nearly no disturbance to the selective sensing of PPi, except for only a little influence from PO_4^{3-}. The association constant (K_{ass}) poly(HEMA-*co*-DCPDP)-Cu^{2+} with PPi is estimated to be ~3.1×10^4 M^{-1} by the fluorometric titrations curve (Figure 3). The significant fluorescence enhancement might be attributed to the electrostatic interaction between PPi and poly(HEMA-*co*-DCPDP)-Cu^{2+}, in which two oxygen atoms of PPi coordinate with the center of Cu^{2+} to reduce the magnitude of the electron withdrawal via partial neutralization of charge on the Cu^{2+} ion,[5j,11a] thus increasing the electron-donating character of DPA moiety and finally resulting in an increased efficiency of ICT (Scheme 2). Moreover, the possible coordination manner in which two oxygen atoms of PPi coordinate with the Cu^{2+} complex is consistent with the reported crystal structures.[18]

Film Properties. As our initial strategy in the incorporation of HEMA units as hydrophilicity, poly(HEMA-*co*-DCPDP) indeed displays high hydrophilicity due to the side chain of 2-hydroxyethyl groups.[10] Exactly, the obtained copolymer poly(HEMA-*co*-DCPDP) is well soluble in methanol and ethanol but seldom dissolves in water. For the good film-forming property of PHEMA, poly(HEMA-*co*-DCPDP) can be easily spin-coated to form a high quality film. Therefore, the copolymer films of poly(HEMA-*co*-DCPDP) and poly(HEMA-*co*-DCPDP)-Cu^{2+} were fabricated on the quartz slides for further study. As mentioned above, the responsive film characteristics of polymer-based fluorescent sensors are dependent on the permeability of ions into polymer matrix and the strength of binding interaction between ions and polymer sensors.[9a] Expectably, the film of poly(HEMA-*co*-DCPDP) shows high sensitivity to Cu^{2+} with a fast response (Figure S4 in the Supporting Information). For further understanding the film characteristic of poly(HEMA-*co*-DCPDP)-Cu^{2+}, we investigated the influence of film thickness by adjusting the amount of materials employed on per square meter. The fluorescence responses based on two films (1.0, 0.8 g/m^2) are investigated with various concentration of PPi (Figures 6 and 7). It shows that the fluorescence intensity change (I/I_0) of the polymer film (1.0 g/m^2) reaches the equilibrium for ~5 min (Figure 7). Notably, the fluorescence response of the film to reach equilibrium is much faster compared with the reported examples (up to 20 min),[9a] which can be attributed to the high permeability of its side chains.[10a] Exactly, to visualize the distinct fluorescence changes of the polymer film, the patterned fluorescence image of word "ECUST" is formatted by using a writing brush with PPi on a quartz plate under UV lamp at 365 nm (Figure 8). The changes in fluorescence intensity for the polymer film (1.0 g/m^2) with titration of PPi are shown in Figure 6. Obviously, similar to the polymer in solution, the film of poly(HEMA-*co*-DCPDP)-Cu^{2+} also displays sensitive to PPi and shows turn-on fluorescence of ~590 nm and a slight red shift (Figure S5 in the Supporting Information). The job curves of polymer films with linear response are capable of meeting further application in practice (Figure 6). Moreover, as our initial aim for practically fluorescent film sensors to monitor PPi, the film stability of polymer in flowing water is also examined. For determining the repeatability, the film is

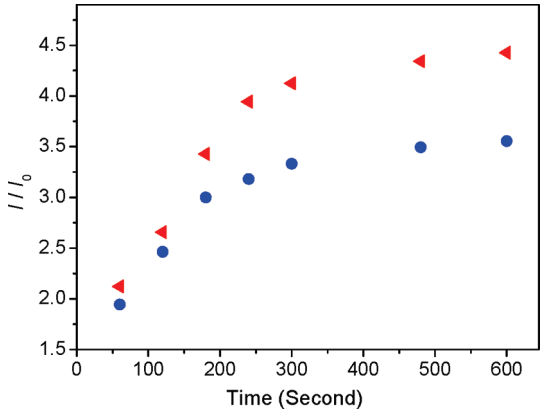
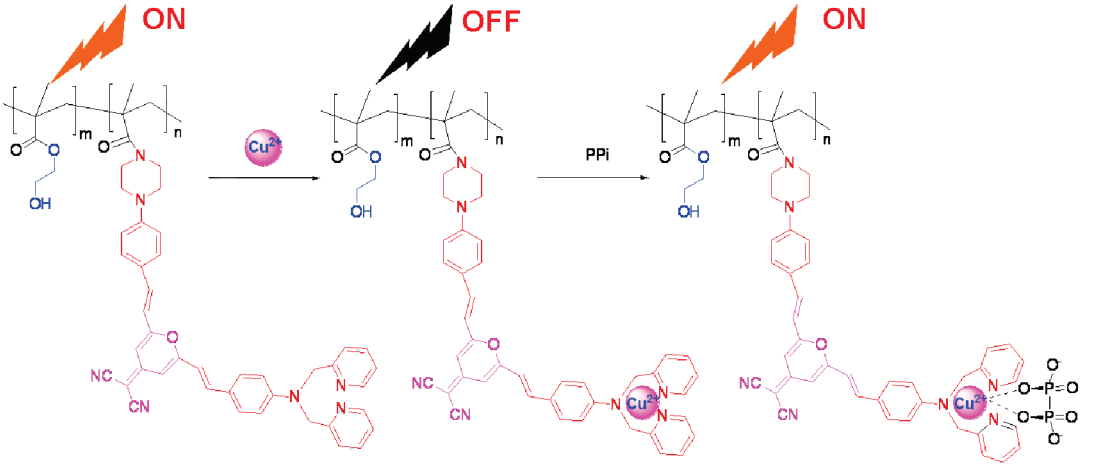


Figure 7. Fluorescence intensity changes (I/I_0) as a function of time after poly(HEMA-*co*-DCPDP)-Cu^{2+} film (●, 1.0 g/m^2; ▲, 0.8 g/m^2) immersing in 6 μM PPi solution. Note: I/I_0 represents the ratio of the fluorescence intensity change after and before the addition of PPi with poly(HEMA-*co*-DCPDP)-Cu^{2+}.

Scheme 2. Schematic Representation of Cu^{2+} and PPi Sensors Based on the Fluorescence "on−off" and "off−on" of Poly(HEMA-*co*-DCPDP) and Poly(HEMA-*co*-DCPDP)-Cu^{2+}, Respectively



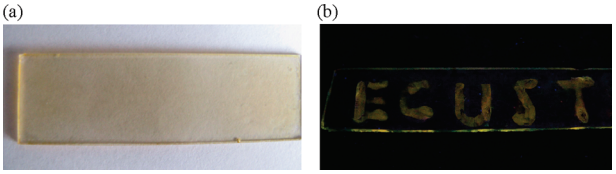


Figure 8. Image patterns obtained with a poly(HEMA-*co*-DCPDP)-Cu^{2+} film (1.0 g/m^2) on a quartz plate (a) in the visible light and (b) the formation of patterned fluorescence image of word "ECUST" with PPi solution under UV lamp at 365 nm.

tested every 3 h in water. The result shows that the fluorescence properties of polymer films are rarely changed in flowing water. Consequently, the polymer films are hydrophilic with high stability, fully meeting the requirements of a continuous bioprocessing or chip, such as monitoring alkaline phosphatase (ALP, EC 3.1.3.1) activity in clinical practice in real time under physiological conditions as PPi hydrolyzed.[8a]

Conclusions

A novel copolymer of poly(HEMA-*co*-DCPDP) possessing DCPDP derived from dicyanomethylene-4*H*-pyran chromophore as ion-sensing units has been synthesized by incorporating HEMA as the hydrophilic function unit. Not only does poly(HEMA-*co*-DCPDP) exhibit turn-off fluorescence to targeting Cu^{2+} ions but also poly(HEMA-*co*-DCPDP)-Cu^{2+} shows selectivity and sensitivity off−on fluorescence mode with PPi over other anions such as Pi, ATP, ADP, and AMP. Furthermore, the thin film of poly(HEMA-*co*-DCPDP)-Cu^{2+} can be fabricated on quartz slides with high sensitivity to PPi as a convenient fluorescent sensor. The utilization of a metal-ion complex as a binding site for probe anions (so-called ensemble method) is a novel strategy for developing novel turn-on fluorescent chemosensors. A general design platform that includes the incorporation of special function monomer with metal-ion complex to form a hydrophilic copolymer may pave the way to develop novel anion sensors for high-throughput bioprocess in continuous system.

Experimental Section

All solvents were of analytical grade. NMP was dried over and distilled under vacuum. The intermediates of 2-{2-[4-(bis(pyridine-2-ylmethyl)amino)styryl]-6-[4-(piperazin-1-yl)styryl]-4*H*-pyran-4-ylidene}malononitrile were prepared by the established literature procedure.[11a] ^{1}H NMR and ^{13}C NMR in $CDCl_3$ or DMSO-d_6 were measured on a Bruker AV-400 or AV-500 spectrometer with tetramethylsilane (TMS) as internal standard. Mass spectra were measured on a Micromass LCT. UV−vis spectra were obtained using a Varian Cary 500 spectrophotometer (1 cm quartz cell). Fluorescent spectra were recorded on a Varian Cary Eclipse fluorescence spectrophotometer (1 cm quartz cell). The weight-average molecular weights (M_n) and polydispersity (M_w/M_n) of the polymer were measured by GPC (Waters 1515/Wyatt Technology) at 30 °C using *N*,*N*-dimethylformamide (DMF) as the eluent and standard polystyrene as the reference.

2-{2-[4-(4-Methacryloylpiperazin-1-yl)styryl]-6-[4-(bis(pyridin-2-ylmethyl)amino)styryl]-4*H*-pyran-4-ylidene}malononitrile (MDCPDP). DCPDP (125 mg, 0.19 mmol) and methacryloyl chloride (60 mg, 0.6 mmol) were both dissolved in CH_2Cl_2 (20 mL) with triethylamine (0.5 mL) under argon protection at room temperature for 24 h. Then, the solvent was evaporated in vacuo. The crude solid was purified by column chromatography on silica gel eluting with EtOAc and CH_2Cl_2 (1:1) to afford a purple solid in 67% yield. ^{1}H NMR (500 MHz, $CDCl_3$, δ): 1.99 (s, 3H, $-CH_3$), 3.31 (t, 4H, J = 5.0 Hz, piperazine-H), 3.77 (t, 4H, J = 5.0 Hz, piperazine-H), 4.91 (s, 4H, $-NCH_2-$), 5.09 (s, 1H, $-C=CH_2$), 5.26 (s, 1H, $-C=CH_2$), 6.50 (d, 1H, J = 16.0 Hz, alkene-H), 6.57 (d, 1H, J = 2.0 Hz, pyran-H), 6.58 (d, 1H, J = 16.0 Hz, alkene-H), 6.59 (d, 1H, J = 2.0 Hz, pyran-H), 6.77 (d, 2H, J = 8.8 Hz, phenyl-H), 6.92 (d, 2H, J = 8.8 Hz, phenyl-H), 7.22 (m, 2H, pyridine-H), 7.25 (d, 2H, J = 8.0 Hz, pyridine-H), 7.40 (d, 1 H, J = 16.0 Hz, alkene-H), 7.41 (d, 2H, J = 8.6 Hz, phenyl-H), 7.42 (d, 1H, J = 16.0 Hz, alkene-H), 7.48 (d, 2H, J = 8.8 Hz, phenyl-H), 7.66 (t × d, 2H, J = 7.7 Hz, 1.6 Hz, pyridine-H), 8.63 (d, 2H, J = 4.4 Hz, pyridine-H). ^{13}C NMR (100 MHz, $CDCl_3$, δ): 20.50, 48.22, 48.73, 57.22, 57.50, 105.67, 106.12, 112.82, 113.82, 115.41, 115.57, 115.87, 116.00, 120.82, 122.39, 123.72, 125.97, 129.35, 129.73, 136.99, 137.36, 138.01, 140.08, 149.90, 150.20, 152.03, 156.00, 157.79, 158.69, 159.18, 171.26. HRMS: $C_{44}H_{39}N_7O_2$ (ESI positive ion mode for [M + H]): Calcd, 698.3271; Found, 698.3243; (ESI positive ion mode for [M + Na]): Calcd, 720.3034; Found, 720.3063 (100%).

Poly(HEMA$_m$-*co*-MDCPDP$_n$) (m/n = 930/1). The mixture of 2-hydroxyethyl methacrylamide (840 mg, 6.45 mmol), MDCPDP (20 mg, 0.029 mmol), and the initiator of α,α′-azoisobutyronitrile AIBN (8 mg, 0.05 mmol) was dissolved in anhydrous NMP (5 mL). Then, the mixture was shaken for 5 min at ambient temperature and degassed by being subjected to the freeze−thaw cycle three times. After being heated for 2 days at 70−80 °C, the resultant mixture was poured into ether, precipitated with ether four times, and then filtrated. The resulting copolymer was dried under vacuum to give an orange-yellow solid (0.45 g). ^{1}H NMR (400 MHz, DMSO-d_6, δ): 0.78−1.18 (m, $-CH(CH_3)_2$, $-CH_3$), 1.78−1.99 (br, $-CCH_2$), 2.75 (br, piperazine-H), 3.17 (br, piperazine-H), 3.58 (br, 2H, $-CH-CH_2-OH$), 3.90 (br, $-CH_2-CH_2-OH$), 4.80 (br, $-CH_2-CH_2-OH$), 5.01 (br, $-N-CH_2$), 7.10−7.75 (br, weak aromatic and pyridine H), 8.60 (br, 2H, pyridine-H). GPC (DMF): M_n = 42 195, M_w = 96 862, M_p = 126 117, M_w/M_n = 2.30.

poly(HEMA-*co*-DCPDP)-Cu^{2+}. To a solution of poly(HEMA-*co*-DCPDP) (0.2 g) in CH_3OH (15 mL) was added dropwise $Cu(ClO_4)_2 \cdot 6H_2O$ (0.3 g, 0.76 mmol) in MeOH (5 mL), and the mixture was stirred for 24 h at room temperature. Then, the solvent was evaporated under vacuum, and the obtained solid was washed with cold water four times to give poly(HEMA-*co*-DCPDP)-Cu^{2+} (0.20 g) as a yellow-brown solid.

Preparation of Polymer Films. The operation process of film investigation was as follows: preparing the concentrated polymer solution in methanol (25 mg/mL) and filtering the solution with filter membranes to remove tiny fibers for guaranteeing the film smoothness. The quartz plates were cleaned by distilled water and methanol sequentially in ultrasonic bath before use. The polymer solution was dropped on the quartz plate, and the polymer drops were evenly extended on the whole quartz plates by the use of surface extension. Finally, the films were put in a vacuum box under room temperature for 24 h to remove residual solvents.

Acknowledgment. This work was financially supported by NSFC/China, National Basic Research 973 Project (2006CB806200), Project for New Century Excellent Talents in University (NCET-06-0418), Shanghai Shuguang Project (07SG34), and Specialized Research Fund for the Doctoral Program of Higher Education SRFDP 200802510011.

Supporting Information Available: Structure characterization of poly(HEMA-*co*-DCPDP) and poly(HEMA-*co*-DCPDP)-Cu^{2+}, the absorption and fluorescence spectra with Cu^{2+} ions, and PPi. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) For recent reviews for anion receptors: (a) Gale, P. A.; Garcia-Garrido, S. E.; Garric, J. *Chem. Soc. Rev.* **2008**, *37*, 151–190. (b) Yoon, J.; Kim, S. K.; Singh, N. J.; Kim, K. S. *Chem. Soc. Rev.* **2006**, *35*, 355–360. (c) Gale, P. A. *Acc. Chem. Res.* **2006**, *39*, 465–475.

(d) Beer, P. D.; Gale, P. A. *Angew. Chem., Int. Ed.* **2001**, *40*, 486–516. (e) Fabbrizzi, L.; Licchelli, M.; Rabaioli, G.; Taglietti, A. *Coord. Chem. Rev.* **2000**, *205*, 85–108. (f) Schmidtchen, F. P.; Berger, M. *Chem. Rev.* **1997**, *97*, 1609–1646. (g) Ojida, A.; Miyahara, Y.; Wongkongkatep, J.; Tamaru, S.; Sada, K.; Hamachi, I. *Chem. Asian J.* **2006**, *1*, 555–563.

(2) Kim, S. K.; Lee, D. H.; Hong, J. I.; Yoon, J. *Acc. Chem. Res.* **2009**, *42*, 23–31.

(3) (a) Lipscomb, W. N.; Strater, N. *Chem. Rev.* **1996**, *96*, 2375–2433. (b) Ronaghi, M.; Karamohamed, S.; Pettersson, B.; Uhlen, M.; Nyren, P. *Anal. Biochem.* **1996**, *242*, 84–89.

(4) (a) Callan, J. F.; de Silva, A. P.; Magri, D. C. *Tetrahedron* **2005**, *61*, 8551–8588. (b) Martinez-Manez, R.; Sancenon, F. *Chem. Rev.* **2003**, *103*, 4419–4476. (c) Suksai, C.; Tuntulani, T. *Chem. Soc. Rev.* **2003**, *32*, 192–202. (d) de Silva, A. P.; Gunaratne, H. Q. N.; Gunnlaugsson, T.; Huxley, A. J. M.; McCoy, C. P.; Rademacher, J. T.; Rice, T. E. *Chem. Rev.* **1997**, *97*, 1515–1566.

(5) (a) Kim, M. J.; Swamy, K. M. K.; Lee, K. M.; Jagdale, A. R.; Kim, Y.; Kim, S. J.; Yoo, K. H.; Yoon, J. *Chem. Commun.* **2009**, 7215–7217. (b) Huang, X. M; Guo, Z. Q; Zhu, W. H; Xie, Y. S; Tian, H. *Chem. Commun.* **2008**, 5143–5145. (c) Shao, N.; Jin, J.; Wang, G.; Zhang, Y.; Yang, R.; Yuan, J. *Chem. Commun.* **2008**, 1127–1129. (d) Lee, H. N.; Xu, Z.; Kim, S. K.; Swamy, K. M. K.; Kim, Y.; Kim, S. J.; Yoon, J. *J. Am. Chem. Soc.* **2007**, *129*, 3828–3829. (e) Lee, H. N.; Swamy, K. M. K.; Kim, S. K.; Kwon, J. Y.; Kim, Y.; Kim, S. J.; Yoon, Y. J.; Yoon, J. *Org. Lett.* **2007**, *9*, 243–246. (f) Lee, D. H.; Kim, S. Y.; Hong, J. I. *Angew. Chem., Int. Ed.* **2004**, *43*, 4777–4780. (g) Aldakov, D.; Anzenbacher, P., Jr. *J. Am. Chem. Soc.* **2004**, *126*, 4752–4753. (h) Lee, D. H.; Im, J. H.; Son, S. UK.; Chung, Y. K.; Hong, J. I. *J. Am. Chem. Soc.* **2003**, *125*, 7752–7753. (i) Mizukami, S.; Nagano, T.; Urano, Y.; Odani, A.; Kikuchi, K. *J. Am. Chem. Soc.* **2002**, *124*, 3920–3925. (j) Fabbrizzi, L.; Marcotte, N.; Stomeo, F.; Taglietti, A. *Angew. Chem., Int. Ed.* **2002**, *41*, 3811–3814.

(6) (a) Badugu, R.; Kostov, Y.; Rao, G.; Tolosa, L. *Biotechnol. Prog.* **2008**, *24*, 1393–1401. (b) Kermis, H. R.; Kostov, Y.; Harms, P.; Rao, G. *Biotechnol. Prog.* **2002**, *18*, 1047–1053.

(7) (a) Bosch, P.; Catalina, F.; Corrales, T.; Peinado, C. *Chem.—Eur. J.* **2005**, *11*, 4314–4325. (b) Potyrailo, R. A. *Angew. Chem., Int. Ed.* **2006**, *45*, 702–723. (c) Kumar, A.; Srivastava, A.; Galaev, I. Y.; Mattiasson, B. *Prog. Polym. Sci.* **2007**, *32*, 1205–1237. (d) Thomas, S. W.; Joly, G. D.; Swager, T. M. *Chem. Rev.* **2007**, *107*, 1339–1386.

(8) (a) Liu, Y.; Schanze, K. S. *Anal. Chem.* **2008**, *80*, 8605–8612. (b) Liu, Y.; Schanze, K. S. *Anal. Chem.* **2009**, *81*, 231–239. (c) Zhao, X.; Liu, Y.; Schanze, K. S. *Chem. Commun.* **2007**, 2914–2916.

(9) (a) Liu, Y.; Mills, R. C.; Boncella, J. M.; Schanze, K. S. *Langmuir* **2001**, *17*, 7452–7455. (b) Adhikari, B.; Majumdar, S. *Prog. Polym. Sci.* **2004**, *29*, 699–766. (c) Wolfbeis, O. S. *J. Mater. Chem.* **2005**, *15*, 2657–2669. (d) Joly, G. D.; Geiger, L.; Kooi, S. E.; Swager, T. M. *Macromolecules* **2006**, *39*, 7175–7177. (e) An, L.; Liu, L.; Wang, S. *Chem. Asian J.* **2009**, *4*, 1196–1206.

(10) (a) Montheard, J. P.; Chatzopoulos, M.; Chappard, D. *Polym. Rev.* **1992**, *32*, 1–34. (b) Bayramoglu, G.; Arica, M. Y. *Macromol. Symp.* **2003**, *203*, 213–218. (c) Filmon, R.; Grizon, F.; Baslé, M. F.; Chappard, D. *Biomaterials* **2002**, *23*, 3053–3059. (d) Shen, L. J.; Zhu, W. H.; Meng, X. L.; Guo, Z. Q.; Tian, H. *Sci. China., Ser. B: Chem.* **2009**, *52*, 821–826. (e) Tian, H.; Gan, J. A.; Chen, K. C.; He, J.; Song, Q. L.; Hou, X. Y. *J. Mater. Chem.* **2002**, *12*, 1262–1267.

(11) (a) Guo, Z. Q.; Zhu, W. H.; Xiong, Y. Y.; Tian, H. *Macromolecules* **2009**, *42*, 1448–1453. (b) Guo, Z. Q.; Zhu, W. H.; Shen, L. J.; Tian, H. *Angew. Chem., Int. Ed.* **2007**, *46*, 5549–5553. (c) Guo, Z. Q.; Zhao, P.; Zhu, W. H.; Xie, Y. S.; Tian, H. *J. Phys. Chem. C.* **2008**, *112*, 7047–7053.

(12) (a) Bourson, J.; Valeur, B. *J. Phys. Chem.* **1989**, *93*, 3871–3876. (b) Valeur, B.; Leray, I. *Coord. Chem. Rev.* **2000**, *205*, 3–40. (c) de Silva, A. P.; McClenaghan, N. D. *Chem.—Eur. J.* **2002**, *8*, 4935–4945.

(13) Zhang, X.; Li, Z. C.; Li, K. B.; Lin, S.; Du, F. S.; Li, F. M. *Prog. Polym. Sci.* **2006**, *31*, 893–948.

(14) For very recent examples of fluorescent probes for zinc ion: (a) Nolan, E. M.; Lippard, S. J. *Acc. Chem. Res.* **2009**, *42*, 193–203. (b) Carol, P.; Sreejith, S.; Ajayaghosh, A. *Chem. Asian J.* **2007**, *2*, 338–348. (c) Jiang, W.; Fu, Q.; Fan, H.; Wang, W. *Chem. Commun.* **2008**, 259–261. (d) Qian, F.; Zhang, C.; Zhang, Y.; He, W.; Gao, X.; Hu, P.; Guo, Z. *J. Am. Chem. Soc.* **2009**, *131*, 1460–1468.

(15) (a) Ballesteros, E.; Moreno, D.; Gomez, T.; Rodri guez, T.; Rojo, J.; Garcia-Valverde, M.; Torroba, T. *Org. Lett.* **2009**, *11*, 1269–1272. (b) Jung, H. S.; Kwon, P. S.; Lee, J. W.; Kim, J. I.; Hong, C. S.; Kim, J. W.; Yan, S.; Lee, J. Y.; Lee, J. H.; Joo, T.; Kim, J. S. *J. Am. Chem. Soc.* **2009**, *131*, 2008–2012.

(16) The constant K_{sv} can be calculated from Stern−Volmer equation: $I_0/I = 1 + K_q\tau_0 c_q = 1 + K_{sv}c_q$.

(17) For very recent metalion complexes examples for molecular probes: (a) Sakamoto, T.; Ojida, A.; Hamachi, I. *Chem. Commun.* **2009**, 141–152. (b) Chung, S. Y.; Nam, S. W.; Lim, J.; Park, S.; Yoon, J. *Chem. Commun.* **2009**, 2866–2868. (c) Chen, X.; Jou, M. J.; Yoon, J. *Org. Lett.* **2009**, *11*, 2181–2184. (d) An, L.; Liu, L.; Wang, S. *Biomacromolecules* **2009**, *10*, 454–457. (e) Zeng, Q.; Cai, P.; Li, Z.; Qin, J.; Tang, B. Z. *Chem. Commun.* **2008**, 1094–1096. (f) Li, Z.; Lou, X.; Yu, H.; Li, Z.; Qin, J. *Macromolecules* **2008**, *41*, 7433–7439.

(18) Erragh, F.; Boukhari, A.; Abraham, F.; Elouadi, B. *J. Solid State Chem.* **1995**, *120*, 23–31.