Fluorescent Conjugated Polymer $PPESO_3$: A Novel Synthetic Route and the Application for Sensing Protease Activities

Tao Zhang,[†,‡] Hongliang Fan,[†] Jianguang Zhou,[†] Guoliang Liu,[†] Guodong Feng,[†] and Qinhan Jin*[,†,‡]

College of Chemistry, Jilin University, Changchun 130021, P.R. China, and College of Instrument Science & Electrical Engineering, Jilin University, Changchun 130021, P.R. China

Received July 10, 2006; Revised Manuscript Received August 29, 2006

ABSTRACT: A facile synthetic route for preparing fluorescent conjugated polymer $PPESO_3$ has been proposed. Compared with a commonly used synthetic procedure, not only is the newly developed procedure simplified but also the dealkylation step with BBr_3 is avoided. Therefore, the preparing difficulty and danger are reduced, which is obviously beneficial to the preparation and application of $PPESO_3$ and other PPEs with similar structure. Also, the nonspecific bindings between $PPESO_3$ and proteins were investigated. Encouraged by the results, a rather simple but effective sensor system to assay protease activity was developed. This sensor took advantage of the nonspecific interactions of the ionic conjugated polymers with proteins and the enzyme-catalyzed hydrolysis of proteins. It was known that bovine serum albumin (BSA) could enhance the fluorescence of $PPESO_3$ with an apparent blue shift of the peak emission wavelength. Such phenomena have been successfully utilized to monitor trypsin and pepsin activities in this study for the first time.

Introduction

Fluorescent conjugated polymers (FCPs), e.g. PPEs[1] and PPVs, are π-conjugated polymers exhibiting many interesting and useful properties, such as strong absorption, strong fluorescence, and good conductivity.[2] Because of the "molecular effect",[3] highly delocalized excitons or electrons can migrate freely and rapidly along the polymer backbone and serve several fluorophores. Thus, a single quencher molecule can cause a superquenching of the entire polymer chain,[4] which will greatly enhance the ability to sense analytes at very low concentration. Therefore, in recent years, chemo- and biosensors based on the superquenching of conjugated polymers are gaining increasing attention in many fields such as the assays of metal ions,[5−12] organic compounds,[2−4,13−17] and biomolecules.[18−23] It is worthy to note that the development of new conjugated polymers and the designs of highly selective sensing systems will remain a hot area in the future.

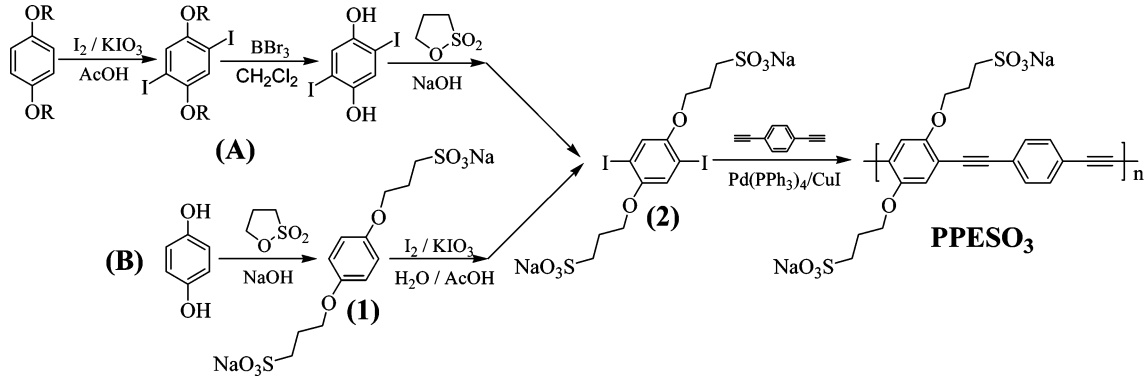
Our group has been also working on the preparation and application of conjugated polymers with great interest. To our knowledge, for many materials of this type, the inherent water insolubility resulted from the long, rigid polymer backbone and its organic characteristics often makes their applications much difficult in many fields, especially in the field of biomolecular analysis. Comparatively, $PPESO_3$ (also other ionic conjugated polymers known as conjugated polyelectrolytes) featuring charged side groups and subsequently good water solubility has made it more superior for its two obvious advantages: (1) more suitable for biosensory systems in aqueous solution; (2) further enhanced sensitivity to oppositely charged quenchers. Consequently, during the past decade, quantitative biosensors based on $PPESO_3$ and its analogues have been successfully developed, ranging from the detection of sugar,[24] anthrax lethal factor,[25] protease,[26] kinase, and phosphatase[27] to the monitoring of protease activity.[28] However, up to now, almost all PPE-based conjugated polyelectrolytes were prepared according to a procedure shown in Scheme 1A.[2,11,16,17,19,26,27] As for that scheme, the preparation of 1,4-diiodohydroquinone is a necessary step in which the dealkylation of 1,4-dialkyloxy-2,5-diiodobenzene by BBr_3 is required. It is well-known that BBr_3 can violently hydrolyze even in the air; therefore, it is very hard to control and store it. Besides, in this reaction some rigorous conditions such as anhydrous and low temperature (−78 °C) environment are needed, and a poisonous gas is released. All the above-mentioned will increase the preparing difficulty and danger. And surely, the relatively low activity of hydroxyls on 1,4-diiodohydroquinone is of some disadvantage for the installation of specific side groups. In this paper, a new synthetic route for preparing $PPESO_3$ has been developed independently. Compared with the procedure reported by Tan et al.,[2,16] this new route is much simpler and more effective. Not only the procedure is simplified, but most importantly the preparing difficulty, complexity, and danger were greatly reduced by avoiding the use of BBr_3, which will be beneficial to the preparation and application of $PPESO_3$ and other PPEs with similar structures.

Proteases play a key role in cell biology and are very important for disease diagnostics and pharmacy. Therefore, it will be of great practical significance to develop a rapid and sensitive assay for protease activity. Though biosensors based on conjugated polymers can lead to high sensitivity, the nonspecific bindings with proteins often make the problem complicated. For example, Bazan et al. have demonstrated the fluorescence enhancement of PPV-based polyelectrolytes caused by avidin,[29] which is somewhat in conflict with the previous results obtained by Chen.[18] And as for nonspecific bindings of conjugated polyelectrolytes with proteins, several groups have also published their results. Heeger et al.[30] reported the interactions of a sulfonated PPV with cytochrome *c*, myoglobin, or lysozyme, giving the results that the fluorescence of their polyelectrolyte could be strongly quenched by cytochrome *c* but to a less extent by lysozyme and by myoglobin. Likewise, in Bunz's report,[31] lysozyme, histone, myoglobin, and hemoglobin could also quench carboxylated PPE effectively, but BSA

[†] College of Chemistry.
[‡] College of Instrument Science & Electrical Engineering.

10.1021/ma061541x CCC: $33.50
Published on Web 10/18/2006

Scheme 1. Synthetic Routes for $PPESO_3$



enhanced the fluorescence due to its surfactant effect. Unfortunately, such phenomena were seldom positively utilized with the exception that Kim et al. improved the sensitivity in detecting Hg^{2+} with PPE−proteins complex,[32] and certainly, these undesired processes will cause interferences in protease assays. However, if considering conversely, fluorescence changes caused by proteins also provide some possibilities for fabricating protease sensors based on the enzyme-catalyzed reactions, and also its kinetics can be easily studied. Herein, the nonspecific interactions between $PPESO_3$ and proteins have been investigated, and the potential for constructing sensitive sensors based on such interactions to assay protease activity has been demonstrated. Namely, the results that bovine serum albumin (BSA) can greatly enhance the fluorescence of $PPESO_3$ have been successfully utilized to monitor trypsin and pepsin activities.

Results and Discussion

Synthesis of $PPESO_3$. A synthetic route developed by us for preparing $PPESO_3$ is shown in Scheme 1B. Starting from hydroquinone, compared with the route (A) proposed by Tan et al.,[2,16] this new route requires only three steps: (1) Under basic conditions, sulfonated receptors were installed via 1,3-propane sultone, followed by (2) the electrophilic substitution of iodine in a mixture of glacial acetic acid and water to form compound **2**, and (3) $PPESO_3$ was prepared by palladium-catalyzed cross-coupled polymerization with 1,4-diethynylbenzene. By using hydroquinone, the high activity of hydroxyls made the installation of sulfonated side groups much easier as compared with the use of 1,4-diiodohydroquinone. It was found that by stirring at room temperature for 4 h the reaction was completed, and the yield was over 90%. As for compound **2**, it seems that sulfonates will somehow block electrophilic substitutions of iodine. In fact, the relatively long distance has weakened the attraction to electrons by sulfonates; therefore, the effect of alkyloxy still dominates, which favors the formation of para-substituted product. In the literature, the solvent used for preparing 1,4-dialkyloxy-2,5-diiodobenzene is often glacial acetic acid.[33] Considering the solubility of 1,4-di(propyloxysulfonate)benzene and the product of interest, water was introduced into this system in our procedure. Our results show that when the ratio of glacial acetic acid/water is 4:3, the yield is optimal (70%). Figure 1 shows the ^1H NMR spectra of the intermediate products 1,4-di(propyloxysulfonate)benzene and 1,4-diiodo-2,5-di(propyloxysulfonate)benzene. And $PPESO_3$ was finally prepared in a mixture of water, DMF, and diisopropylamide. To avoid any deactivation of the catalyst, this reaction was carried out in a dark room, and a constant pressure funnel was used to help deoxygen by insulating substrates from solvent at the initial stage. The final product was collected by centrifugation as light yellow powder and was characterized by NMR and FTIR.

Nonspecific Interactions between $PPESO_3$ and Proteins. In Bunz's article,[31] fluorescence enhancement of carboxylated PPE by BSA was reported, but they did not notice any change in emission wavelength. However, as can be seen in Figure 2A obtained by our study, fluorescent changes of our polymer upon addition of BSA are very significant. With the increasing of BSA concentration, not only the fluorescence quantum yield increases greatly but also an apparent blue shift of maximum emission wavelength (from 530 to 457 nm) occurs. When the BSA concentration is up to 20 μg/mL, the spectrum shape was closely analogous to that of unaggregated $PPESO_3$ in methanol. It is well-known that the optical properties of conjugated polyelctrolytes are highly sensitive to surfactants.[34,35] Our previous work[36] has also demonstrated that nonionic surfactant poly(vinylpyrrolidone) (PVP) can cause fluorescent changes of $PPESO_3$, and they are almost identical to those by BSA. Therefore, it is believed that these phenomena result mainly from the surfactant effect of BSA. When BSA was replaced

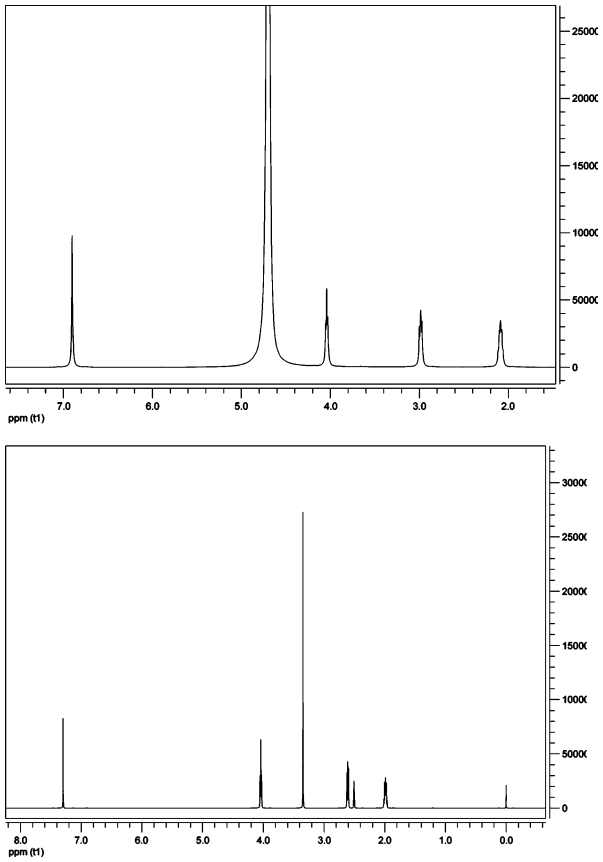


Figure 1. ^1H NMR spectra of 1,4-di(propyloxysulfonate)benzene (top) and 1,4-diiodo-2,5-di(propyloxysulfonate)benzene (bottom).

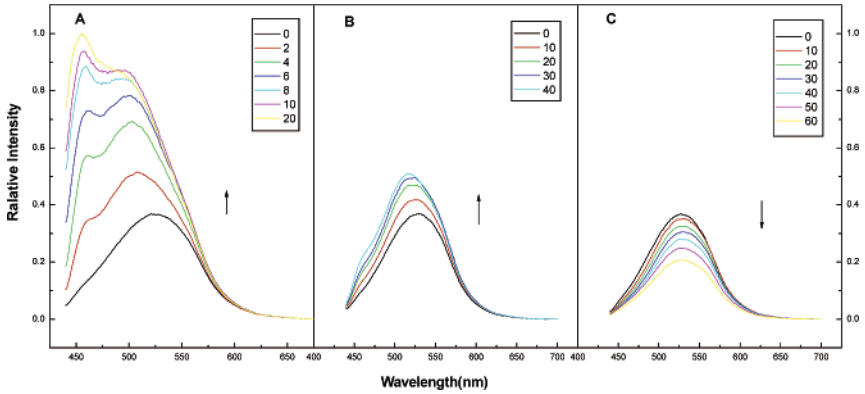


Figure 2. Normalized fluorescence changes of $PPESO_3$ upon addition of proteins: (A) BSA, (B) trypsin, and (C) pepsin.

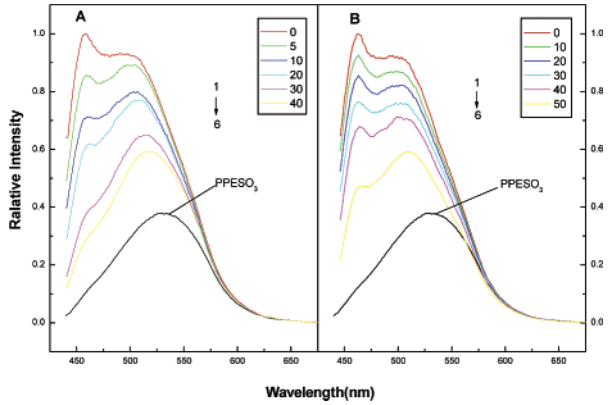


Figure 3. Normalized fluorescence spectra of $PPESO_3$−BSA complex as a function of trypsin (A) and pepsin (B) concentrations. BSA concentration is 10 μg/mL.

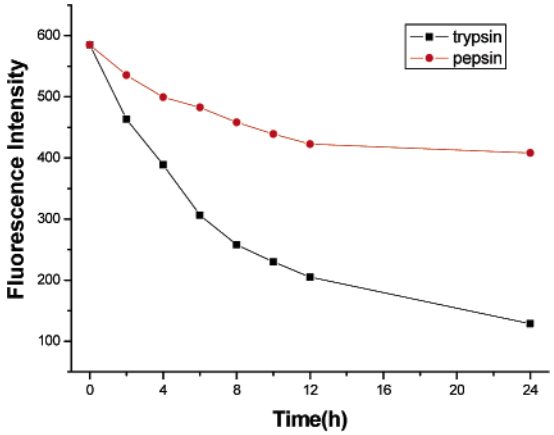


Figure 4. Fluorescence intensity changes of $PPESO_3$−BSA complex at 457 nm against time after addition of trypsin (10 μg/mL) or pepsin (10 μg/mL). The incubating temperature is 30 °C.

with trypsin or pepsin, different results were obtained though they are both negatively charged proteins under neutral conditions. As shown in Figure 2B,C, trypsin at lower concentration could increase the polymer fluorescence, while pepsin led to a "quenching", but both to a less extent. In our opinion, the electrostatic repulsion between the polymer and trypsin may be responsible for the fluorescence enhancement by trypsin, in which the *J*-aggregation of $PPESO_3$ was weakened. However, it was noted that when the trypsin concentration is up to 60 μg/mL or higher, the fluorescence intensity begins to decrease (data not shown). Though the proper interpretation for fluorescence "quenching" is still to be worked out, we suspect that the polymer is probably heavily coated by these enzymes.

Assay for Protease Activity. Unlike reported in previous literature where the nonspecific bindings of conjugated polyelectrolytes with proteins were called "a cautionary tale",[31] in our study, BSA-induced fluorescence enhancement and blue shift of $PPESO_3$ were successfully utilized to monitor the activity of protease. Our initial expectation was that the fluorescence could be restored once BSA was fully hydrolyzed. It is well-known that trypsin can catalyze the hydrolysis of the peptide bonds by the C side of lysine or arginine in proteins. Figure 3A illustrates the fluorescence spectral changes when adding trypsin to a mixture of $PPESO_3$ and BSA (10 μg/mL). As can be seen, the fluorescence intensity decreases gradually and the spectral band shows a red shift apparently with the increasing of trypsin concentration, until the spectral shape was almost identical to that of a complex of $PPESO_3$ and trypsin at a concentration level of 40 μg/mL. As known from the above that trypsin increases the emission of $PPESO_3$, therefore, the efficient fluorescence quenching and gradual red shift can only be attributed to the hydrolysis of BSA by trypsin. Encouraged by these results, pepsin was tested by us as well. And it was found that the fluorescence band of the $PPESO_3$−BSA complex underwent the same changes upon adding pepsin (see Figure 3B) with those upon adding trypsin. Such phenomena have implied to us that the fluorescence of the $PPESO_3$−BSA complex is highly sensitive to trypsin and pepsin; therefore, on the basis of this process one can easily monitor the enzyme-catalyzed reaction, and of course, the protease activity can be estimated as well. To further prove the applicability, some supplemental investigations have also been carried out. As shown in Figure 4, when trypsin concentration was fixed, the emission of $PPESO_3$−BSA was strongly "quenched" against time, which revealed directly the high activity of trypsin. On the other hand, for pepsin only a small reduction in fluorescence intensity against time is observed, and there is nearly no more change occurs after 12 h. The reason for this result is mainly because that pepsin (whose proper pH is about 2.0) will lose the activity more easily under the neutral conditions. And in our results, the gradual decrease of slope shown in Figure 4 represents the loss of protease activity. Overall, the highly sensitive fluorescence changes of the $PPESO_3$−BSA complex caused by protease have provided a general method to monitor protease activity, and of course, the kinetics of enzyme-catalyzed reaction can be easily studied as well.

Conclusions

In this paper, a facile three-step synthetic route for preparing fluorescent conjugated polymer $PPESO_3$ has been proposed. As for installing sulfonated side groups on hydroquinone, it is much easier when compared to commonly used 1,4-diiodohy-

droquinone due to the high activity of the hydroxyls. To prepare compound **2**, the solubility of the substrates is crucial; therefore, the proper solvent chosen by us has made a key role for the success. Finally, $PPESO_3$ was prepared in a dark environment and was collected by centrifugation. The method proposed here is rather simple and effective and certainly will facilitate the preparation and application of $PPESO_3$ as well as other PPEs with similar structure.

Also, the nonspecific interactions between $PPESO_3$ and proteins were investigated. The results indicate that BSA can greatly increase the fluorescence of $PPESO_3$ due to its surfactant effect, and trypsin can only result in an enhancement to a less extent in which the aggregation of $PPESO_3$ was weakened by the electrostatic repulsions. On the contrary, pepsin "quenched" the polymer fluorescence weakly. The property that $PPESO_3$ is highly sensitive to BSA has encouraged us to study on the enzyme-catalyzed hydrolysis of BSA. And our subsequent researches have demonstrated that, upon adding trypsin or pepsin with bioactivity to the $PPESO_3$−BSA complex, the fluorescence can be fully restored when BSA was decomposed into smaller fragments, which has proved to a certain extent the potential for constructing highly sensitive biosensors for protease activity based on the nonspecific bindings of ionic conjugated polymers with proteins. And in our opinion, it may be used as a general method for more proteases, not just limited to either the polymer or the enzymes we have studied.

Experimental Section

General Information. 1,4-Diethynylbenzene (96%) and $(PPh_3)_4$-Pd were purchased from Aldrich Chemical Co. and Hangzhou Kaida Metal Catalyst & Compounds Co. Ltd. (Hangzhou, China), respectively. Hydroquinone, 1,3-propane sultone, cuprous iodide, and all other chemicals are of analytical purity. BSA, trypsin, and pepsin were provided by Key Laboratory for Molecular Enzymology and Engineering, the Ministry of Education (Changchun, China). NMR and FTIR data were obtained using a Bruker Advance-500 spectrometer and Perkin-Elmer SPECTRUM ONE FTIR(CIS), respectively. All fluorescence experiments were performed on a Shimadzu RF-5301 spectrometer at 30 °C. And without special mention, the concentrations of $PPESO_3$ and BSA are 10^{-6} M and 10 μg/mL, respectively, and the unit used for protease is μg/mL.

Synthesis and Characterization of $PPESO_3$. Conjugated polymer $PPESO_3$ was prepared according to a procedure shown in Scheme 1B.

1,4-Di(propyloxysulfonate)benzene (1). Under an argon atmosphere, 8.8 g (80 mmol) of *p*-hydroquinone was rapidly dissolved in 100 mL of NaOH solution (10%) in an Erlenmeyer flask. A solution that contained 24.4 g (200 mmol) of 1,3-propane sultone and 160 mL of dioxane was added to the above-mentioned solution at once. The resulting mixture was then stirred at room temperature for 4 h, and a quantitative precipitate formed. After cooled in an ice−water bath, the reaction mixture was vacuum filtered. The obtained solid was washed with cold acetone and collected as white powder of 30.2 g. The powder was characterized with ^{1}H NMR (D_2O, δ_{ppm}): 2.09 (t, 4H), 2.99 (t, 4H), 4.04 (t, 4H), 6.90 (s, 4H). FTIR (υ_{max}, cm^{-1}, KBr): 3071, 3049, 2975, 2906, 2871, 1511, 1476, 1455, 1402, 1344, 1230, 1046, 950, 931, 863, 829, 767, 604, 563, 531.

1,4-Diiodo-2,5-di(propyloxysulfonate)benzene (2). 3.86 g (15.2 mmol) of I_2 and 1.55 g (7.24 mmol) of KIO_3 were dissolved in a mixture of 40 mL of glacial acetic acid, 3 mL of H_2SO_4, and 30 mL of water, and then 5.77 g (14.5 mmol) of compound **1** was added to the above-mentioned solution. The resulting mixture was refluxed at about 60 °C for 12 h. After cooled in an ice−water bath, the reaction mixture was filtered and then washed with a large amount of cold ethanol. Without further purification, the product was collected as fine white powder with high purity. The powder was characterized with ^{1}H NMR (DMSO-d_6, δ_{ppm}): 1.99 (t, 4H), 2.61 (t, 4H), 4.04 (t, 4H), 7.30 (s, 2H). FTIR (υ_{max}, cm^{-1}, KBr): 2975, 2956, 2940, 2923, 1625, 1489, 1464, 1438, 1391, 1353, 1262, 1214, 1179, 1156, 1057, 1031, 937, 850, 812, 795, 739, 625, 553.

$PPESO_3$. This polymer was prepared by a procedure similar to that proposed by Tan et al.[16] A mixture of 20 mL of water, 30 mL of DMF, and 10 mL of diisopropylamine were placed in an isobarically funnel which was connected to a Schlenk flask. And then 1.008 g (1.55 mmol) of monomer **1**, 0.189 g (1.50 mmol) of 1,4-diethynylbenzene, 52.0 mg (45.0 μmol) of $Pd(PPh_3)_4$, and 10.0 mg (45 μmol) of CuI were added to the flask rapidly. The whole system was deoxygenated by a gentle flow of argon for 30 min, and the solvent mixture was dropped into the reaction flask slowly. The final mixture was then stirred at 55 °C for 20 h. After cooled to room temperature, the resulted solution was slowly added to 1 L of a methanol/acetone/ether mixture (10:40:50). The polymer precipitated was filtered, then redissolved in 200 mL of water/methanol (70:30), and treated with 0.1 g of sodium sulfide (Na_2S), and then the solution was filtered with quantitative filter paper, followed by a 10−20 μm fritted glass filter. The polymer was reprecipitated by addition to a large volume of methanol/acetone/ether (10:40:50). The upper liquid was carefully removed and the resulting mixture was centrifugated to collect $PPESO_3$ as a light yellow powder. The powder was characterized with ^{1}H NMR (DMSO-d_6, δ_{ppm}): 2.09 (t, 4H), 2.70 (t, 4H), 4.17 (t, 4H), 7.21 (s, 2H), 7.61 (4H). FTIR (υ_{max}, cm^{-1}, KBr): 2943, 2878, 1626, 1515, 1467, 1413, 1281, 1191, 1049, 1003, 878, 835, 735, 666, 614, 532.

Acknowledgment. This research is supported by Key Laboratory for Molecular Enzymology and Engineering of the Ministry of Education (Changchun, China). We thank Dr. Ying Mu and Shaowu Lv for kindly providing proteins and helpful discussions.

References and Notes

(1) Bunz, U. H. F. *Chem. Rev.* **2000**, *100*, 1605−1644.
(2) Tan, C.; Atas, E.; Müller, J. G.; Pinto, M. R.; Kleiman, V. D.; Schanze, K. S. *J. Am. Chem. Soc.* **2004**, *126*, 13685−13694.
(3) Zhou, Q.; Swager, T. M. *J. Am. Chem. Soc.* **1995**, *117*, 12593−12602.
(4) Dalvi-Malhotra, J.; Chen, L. *J. Phys. Chem. B* **2005**, *109*, 3873−3878.
(5) Wang, B.; Wasielewski, M. R. *J. Am. Chem. Soc.* **1997**, *119*, 12−21.
(6) Kimura, M.; Horai, T.; Hanabusa, K.; Shirai, H. *Adv. Mater.* **1998**, *10*, 459−462.
(7) Kim, J.; McQuade, D. T.; McHugh, S. K.; Swager, T. M. *Angew. Chem., Int. Ed.* **2000**, *39*, 3868−3872.
(8) Bangcuyo, C. G.; Rampey-Vaughn, M. E.; Quan, L. T.; Angel, S. M.; Smith, M. D.; Bunz, U. H. F. *Macromolecules* **2002**, *35*, 1563−1568.
(9) Fan, L. J.; Zhang, Y.; Jones, W. E., Jr. *Macromolecules* **2005**, *38*, 2844−2849.
(10) Kim, I.-B.; Erdogan, B.; Wilson, J. N.; Bunz, U. H. F. *Chem.−Eur. J.* **2004**, *10*, 6247−6254.
(11) Kim, I.-B.; Dunkhorst, A.; Gilbert, J.; Bunz, U. H. F. *Macromolecules* **2005**, *38*, 4560−4562.
(12) Tang, Y.; He, F.; Yu, M.; Feng, F.; An, L.; Sun, H.; Wang, S.; Li, Y.; Zhu, D. *Macromol. Rapid Commun.* **2006**, *27*, 389−392.
(13) Zhou, Q.; Swager, T. M. *J. Am. Chem. Soc.* **1995**, *117*, 7017−7018.
(14) Yang, J.-S.; Swager, T. M. *J. Am. Chem. Soc.* **1998**, *120*, 5321−5322.
(15) Yang, J.-S.; Swager, T. M. *J. Am. Chem. Soc.* **1998**, *120*, 11864−11873.
(16) Tan, C.; Pinto, M. R.; Schanze, K. S. *Chem. Commun.* **2002**, 446−447.
(17) Haskins-Glusac, K.; Pinto, M. R.; Tan, C.; Schanze, K. S. *J. Am. Chem. Soc.* **2004**, *126*, 14964−14971.
(18) Chen, L.; McBranch, D. W.; Wang, H.-L.; Helgeson, R.; Wudl, F.; Whitten, D. G. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 12287−12292.
(19) Wosnick, J. H.; Mello, C. M.; Swager, T. M. *J. Am. Chem. Soc.* **2005**, *127*, 3400−3405.
(20) Gaylord, B. S.; Massie, M. R.; Feinstein, S. C.; Bazan, G. C. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 34−39.
(21) Liu, B.; Bazan, G. C. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 589−593.
(22) Hong, J. W.; Hemme, W. L.; Keller, G. E.; Rinke, M. T. *Adv. Mater.* **2006**, *18*, 878−882.

(23) Wang, D.; Gong, X.; Heeger, P. S.; Rininsland, F.; Bazan, G. C.; Heeger, A. J. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 49−53.
(24) DiCesare, N.; Pinto, M. R.; Schanze, K. S.; Lakowicz, J. R. *Langmuir* **2002**, *18*, 7785−7787.
(25) Kushon, S. A.; Ley, K. D.; Bradford, K.; Jones, R. M.; McBranch, D.; Whitten, D. *Langmuir* **2002**, *18*, 7245−7249.
(26) Kumaraswamy, S.; Bergstedt, T.; Shi, X.; Rininsland, F.; Kusion, S.; Xia, W.; Ley, K.; Achyuthan, K.; McBranch, D.; Whitten, D. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 7511−7515.
(27) Rininsland, F.; Xia, W.; Wittenburg, S.; Shi, X.; Stankewicz, C.; Achyuthan, K.; McBranch, D.; Whitten, D. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 15925−15300.
(28) Pinto, M. R.; Schanze, K. S. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 7505−7510.
(29) Dwight, S. J.; Gaylord, B. S.; Hong, J. W.; Bazan, G. C. *J. Am. Chem. Soc.* **2004**, *126*, 16850−16859.
(30) Fan, C.; Plaxco, K. W.; Heeger, A. J. *J. Am. Chem. Soc.* **2002**, *124*, 5642−5643.
(31) Kim, I.-B.; Dunkhorst, A.; Bunz, U. H. F. *Langmuir* **2005**, *21*, 7985−7989.
(32) Kim, I.-B.; Bunz, U. H. F. *J. Am. Chem. Soc.* **2006**, *128*, 2818−2819.
(33) Wariishi, K.; Morishima, S. I.; Inagaki, Y. *Org. Process Res. Dev.* **2003**, *7*, 98−100.
(34) Lavigne, J. J.; Broughton, D. L.; Wilson, J. N.; Erdogan, B.; Bunz, U. H. F. *Macromolecules* **2003**, *36*, 7409−7412.
(35) Chen, L. H.; Xu, S.; McBranch, D.; Whitten, D. *J. Am. Chem. Soc.* **2000**, *122*, 9302−9303.
(36) Zhang, T.; Fan, H. L.; Zhou, J. G.; Liu, G. L.; Jin, Q. H. *Chem. J. Chin. Univ.* **2006**, *27*, 853−855.

MA061541X