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Study on the interaction between nitroxide free radical and conjugated polyelectrolytes by fluorimetry

Wenchao Dou and Xingguang Su*

ABSTRACT: In this work, we studied the fluorescence quenching of the anionic conjugated polyelectrolyte PPE-SO₃ by the paramagnetic species 2,2,6,6-tetramethylpiperidine-*N*-oxide free radical (TEMPO) in aqueous solution. At low quencher concentration the Stern–Volmer constant is 94 mol/L; as the quencher concentration increases the Stern–Volmer plots become superlinear. Ascorbic acid is used to reduce TEMPO to the corresponding hydroxylamine and the PPE-SO₃ fluorescence is fully recovered. Under a large excess of ascorbic acid over TEMPO, the rise of fluorescence followed pseudo-first-order kinetics. The second-order rate constant calculated from this time course is 0.7 mol/L/s. Copyright © 2008 John Wiley & Sons, Ltd.

Keywords: conjugated polyelectrolytes; nitroxide free radical; fluorescence quenching

Introduction

Conjugated polyelectrolytes (CPEs) are π -conjugated polymers that feature ionic solubilizing side groups (1). These materials show many of the interesting and useful properties exhibited by π -conjugated polymers, such as high fluorescence quantum yields, unique solution behaviour, the ability to interact electrostatically with other charged species, and an extraordinarily high sensitivity to fluorescence quenchers (2). This latter property has been exploited in sensing experiments, in which a variety of organic and inorganic analytes and biomolecules can be detected at picomolar concentration levels (3–6).

Nitroxides are persistent free radicals frequently used as radical scavengers or as quenchers of excited states (7). Blough and co-workers have shown that these covalently-linked nitroxide–fluorophore adducts can be employed as very sensitive optical sensors of radical/redox reactions (8–11). Scaiano and co-workers have also developed a number of prefluorescent probes allowing radical and/or antioxidant detection in homogeneous and heterogeneous systems (12–14).

However, there is no report of studies on the interaction between nitroxides radicals and conjugated polyelectrolytes until now and the mechanism is also not clear. In an effort to construct highly sensitive optical probes of radical/redox species, we sought to exploit both the radical trapping and fluorescence quenching properties of the nitroxides by constructing a system that contains nitroxides and conjugated polyelectrolytes.

In this work, we studied the fluorescence quenching of the sulphonate-substituted poly(phenylene ethynylene) PPE-SO₃ by the paramagnetic species 2,2,6,6-tetramethylpiperidine-*N*-oxide free radical (TEMPO) in aqueous solution (structures in Fig. 1). PPE-SO₃ is a water- and alcohol-soluble polymer that exhibits a strong blue or green fluorescence (15, 16). The possible mechanism of fluorescence quenching was studied. Additionally, chemical reduction of the TEMPO to a diamagnetic hydroxylamine can lead a fluorescence recovery of PPE-SO₃.



Figure 1. Structure of PPE-SO₃.

Experimental

Chemicals

PPE-SO₃ was synthesized according to a previously described method (16), and the structure (Fig. 1) was confirmed by ¹H-NMR spectroscopy [(DMSO-*d*₆, δ_{ppm}): 2.10(4H) 2.72(4H) 4.17(4H) 7.21(2H) 7.62(4H)]. 2,2,6,6-Tetramethylpiperidine-*N*-oxide free radical (TEMPO) (98%) was purchased from Changzhou Jiana Chemical Co., Ltd (Changzhou, China). Ascorbic acid (99%) was purchased from Tianjin Fuchen Chemical Reagents Factory (Tianjin China). 1,4-Diethynylbenzene (96%) and (PPh₃)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. The water used in all experiments had a resistivity of 18 M Ω /cm.

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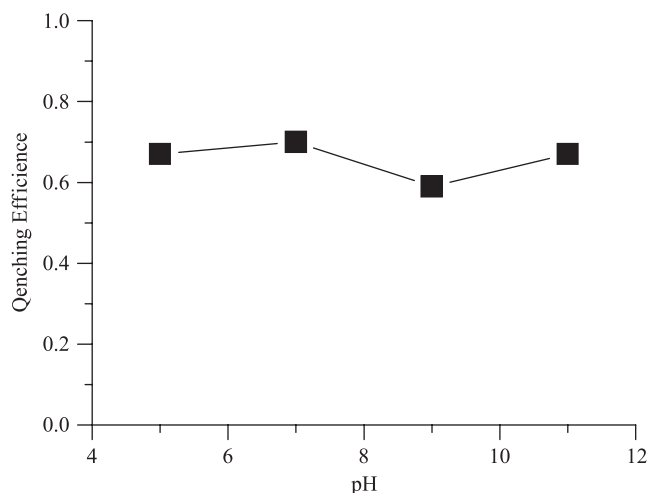


Figure 2. The effect of pH on the interaction between PPE-SO₃ and TEMPO. The concentrations of PPE-SO₃ and TEMPO were 5 μmol/L and 0.02 mol/L, respectively.

Apparatus

Fluorescence experiments were performed on a Shimadzu RF-5301 PC spectrofluorophotometer. UV-vis absorption spectra were obtained using a Varian GBC Cintra 10e UV-visible spectrometer. In both experiments, a 1 cm path-length quartz cuvette was used to measure the absorption or fluorescence spectrum. NMR spectra were recorded using a Bruker Advance-500 spectrometer, operating at 500 MHz for ¹H-NMR. The fluorescence lifetimes of the samples were measured with a Lecroy Wave Runner 6100 Digital Oscilloscope (1 GHz) using a 325 nm laser wavelength (pulse width = 4 ns) from YAG:Nd as the excitation source. All optical measurements were carried out at room temperature under ambient conditions.

Results and Discussion

Effect of TEMPO on the FL spectra of PPE-SO₃

First of all, we examined the effect of pH on the interaction of PPE-SO₃ and TEMPO. The quenching efficiency ($1 - I/I_0$) of PPE-SO₃ by TEMPO was obtained in 2 mmol/L phosphate buffer solution at pH 5–11; the results are shown in Fig. 2. It can be seen that the quenching efficiency is not strongly dependent on pH. The quenching efficiency is highest (67%) at pH 7. Since the quenching efficiency in water is 70%, our experiments were all carried in pure water solution.

The fluorescence quenching of the PPE-SO₃ was studied by systematically monitoring the steady-state fluorescence intensity changes with different concentrations of the paramagnetic compound TEMPO; the results are shown in Fig. 3, from which it can be seen that the addition of TEMPO to PPE-SO₃ solution results in a large decrease in the fluorescence intensity of PPE-SO₃, which is not accompanied by a new red-shifted emission peak. This result suggests that the fluorescence decrease is not the result of conformational changes induced by paramagnetic compound, but solely through electron-exchange quenching.

There are two general types of quenching: (a) static quenching through the formation of a complex; and (b) dynamic quenching due to random collisions between the photoluminescence emitter and the acceptor (17). Both processes can be quantitatively described by the Stern–Volmer equation:

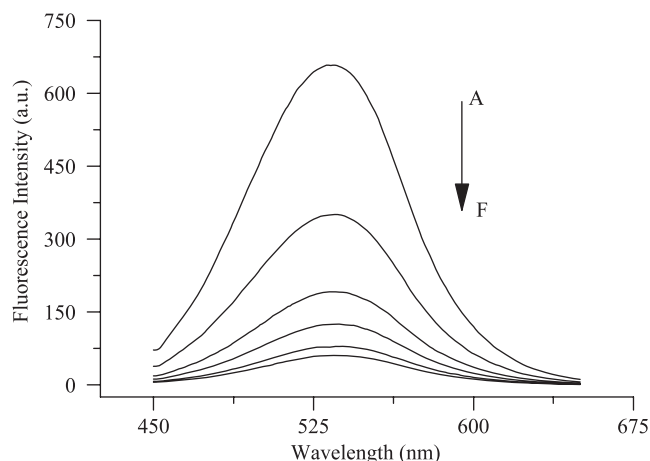


Figure 3. The effect of TEMPO on the fluorescence spectra of PPE-SO₃ aqueous solution (5 μmol/L). The TEMPO concentrations A–F were 0, 0.01, 0.02, 0.03, 0.04 and 0.05 mol/L, respectively. Excitation wavelength was 441 nm.

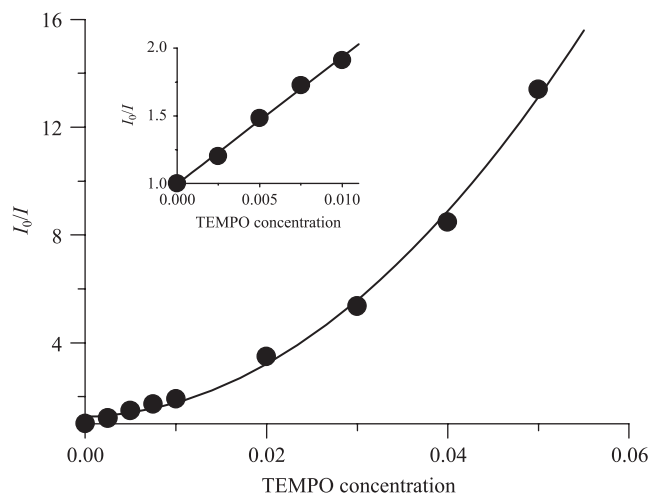


Figure 4. The relationship between fluorescence intensity of PPE-SO₃ aqueous solution (5 μmol/L) and TEMPO concentration. (Inset) The relationship between fluorescence intensity of PPE-SO₃ aqueous solution and TEMPO concentration in the low concentration range.

$$I_0/I = 1 + K[Q] \quad (1)$$

where I_0 is the fluorescence intensity of fluorophore in absence of quencher; I is the fluorescence intensity of fluorophore when the quencher is present at concentration $[Q]$, and K is the Stern–Volmer constant. The Stern–Volmer constant provides a quantitative measure of the quenching. Figure 4 shows the relation between I_0/I and TEMPO concentration in this work. From the inset of Fig. 4, it can be seen that I_0/I increases linearly with TEMPO concentration in a low TEMPO concentrations range. By fitting the data with the linear equation, the Stern–Volmer quenching constant in low TEMPO concentrations range is found to be 94 mol/L.

The quenching of anionic conjugated polyelectrolyte PPE-SO₃ by cationic quencher has been investigated (1, 15, 18, 19). The Stern–Volmer constants (K_{sv}) for MV²⁺ quenching of PPE-SO₃ were in the range 10⁶–10⁷ mol/L (15). The amplified quenching has been attributed to: (a) the formation of a strong association complex between the polymer and the quencher ion, with electrostatic interaction; and (b) the ability of the singlet exciton to rapidly diffuse to the quencher ‘trap’ site (1). However, in our

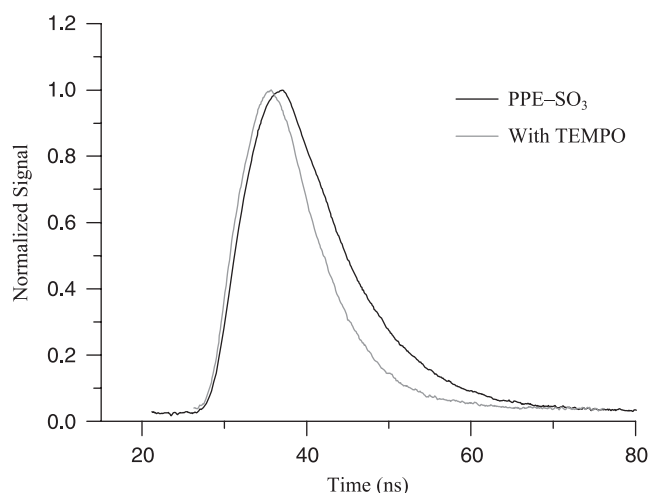


Figure 5. Normalized fluorescence decays of PPE-SO₃ (5 μmol/L) in aqueous solution with and without TEMPO (0.02 mol/L). Fluorescence emission spectra were measured with excitation wavelength at 325 nm and detection at 535 nm.

study, the Stern–Volmer constant in low TEMPO concentrations range is only found to be 94 mol/L; the smaller *K*_{sv} is because the neutral quencher TEMPO and the anionic polymer cannot form an association complex. Therefore, the *K*_{sv} obtained at low TEMPO concentrations may result from dynamic quenching. This proposal is also confirmed by a fluorescence decay experiment. Figure 5 shows fluorescence decays detected at 535 nm with 325 nm excitations for solutions of PPE-SO₃ with and without TEMPO. It can be seen that, with the addition of TEMPO to the polymer, the polymer's fluorescence decay time decreased for 7.8 ns to 5.7 ns. The fluorescence decay measurement confirmed that the fluorescence quenching of PPE-SO₃ by TEMPO is involved in the dynamic mechanism.

However, with the TEMPO concentration increases, the Stern–Volmer plots curved upward. The deviation from linearity implies the onset of an additional quenching mechanism. We used the combination of 'sphere-of-action quenching' and dynamic quenching to explain the positive deviation from the linear curve at the high concentration of TEMPO.

The sphere-of-action quenching has been used to study the upward curve of the Stern–Volmer plots in the MBL-PPV and neutral quencher systems (20). While there is large gyration radius of the conjugated polymer in aqueous solution, at high quencher concentration the quencher molecules may be within the electron-exchange range of conjugated polymer chain (20). Previous reports have confirmed that PPE-SO₃ exists in a strongly aggregated state in water, with extensive interchain π -stacking (1). Considering the large size of the conjugated polymer in aqueous solution, in a high quencher concentration regime, it may be that there are quencher molecules within the electron-exchange distance from one of the conjugated macromolecules. So it can be deduced that the superlinearity in the high TEMPO concentration range (>0.01 mol/L) may result from a combination of sphere-of-action quenching and dynamic quenching:

$$I_0/I = (1 + K_{sv}[Q]) \exp(V[Q]) \quad (2)$$

Equation 2 describes the combined quenching in terms of the Stern–Volmer constant, *K*_{sv}, and the sphere-of-action volume, *V*. On the basis of equation (2) and the lifetime from the decay

curves shown in Fig. 5, the sphere volume and *K*_{sv} are calculated to be 41 and 18 mol/L, respectively. As shown in equation 2, at high quencher concentration, the Stern–Volmer plots for the quenchers become superlinear, implying a combination of sphere-of-action quenching and dynamic quenching (20).

Effect of ascorbic acid on the FL spectra of the PPE-SO₃–TEMPO system

The paramagnetic nitroxides are well known to be efficient quenchers of excited singlet states of aromatic hydrocarbons, presumably through an intermolecular electron-exchange interaction between the ground-state nitroxide and excited-state compound within a collision complex. Preferential reaction of the nitroxide with ascorbic acid leads to the formation of a diamagnetic product, thereby eliminating the intermolecular quenching pathway and resulting in an increased fluorescence yield that reflects radical/redox scavenging (8).

In this work, we used ascorbic acid to reduce TEMPO to the corresponding hydroxylamine and studied the fluorescence recovery of PPE-SO₃. The results are shown in Fig. 6, which shows that the initial fluorescence of PPE-SO₃ is quenched 70% by the addition of TEMPO. After addition of ascorbic acid to the reaction system, a recovery in the PPE-SO₃ fluorescence intensity can be observed, owing to the reduction of nitroxide, which lead a loss of paramagnetic of nitroxide (see Fig. 7). A subtle blue

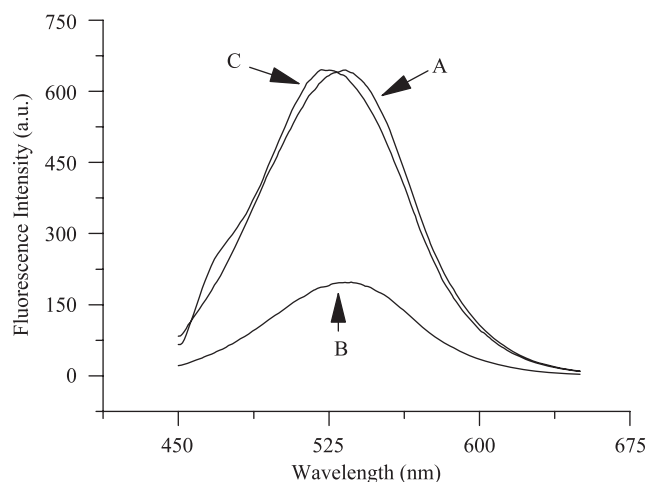


Figure 6. The effect of ascorbic acid on the fluorescence spectra of the PPE-SO₃–TEMPO system. (A) Fluorescence spectra of PPE-SO₃ in pure water; (B) fluorescence spectra of the PPE-SO₃ (5 μmol/L)–TEMPO (0.02 mol/L) system; (C) the fluorescence spectra of PPE-SO₃ (5 μmol/L)–TEMPO (0.02 mol/L) system after the addition of ascorbic acid (0.02 mol/L) for 6 min. Excitation wavelength was 441 nm.

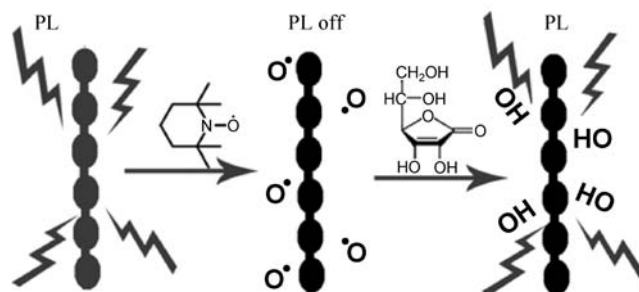


Figure 7. Schematic representation of fluorescence quenching and recovery.

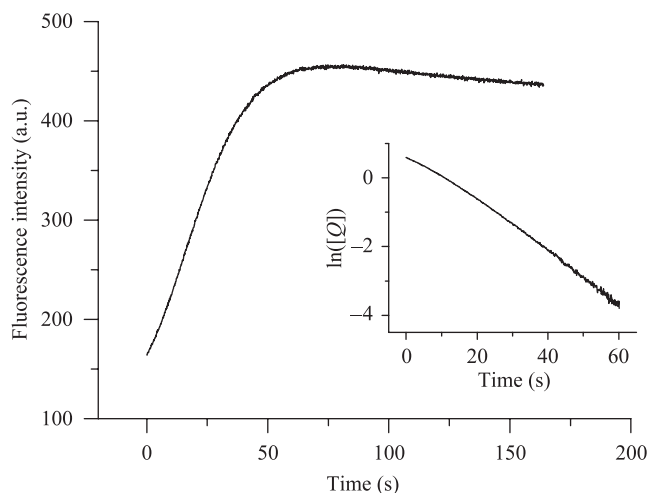


Figure 8. The relationship between the fluorescence intensities and incubation time. Reaction system contains 0.2 mol/L ascorbic acid, 0.02 mol/L solution of TEMPO and 5 μ mol/L PPE-SO₃. (Inset) Relationship between the logarithm of TEMPO concentration and the incubation time. Excitation wavelength was 441 nm.

shift in the PPE-SO₃ fluorescence spectra can also be observed after the addition of ascorbic acid; it may be caused by the non-specific interactions between the polymer and L-diketogulonic acid (the oxidation product of ascorbic acid), which can break the polymer aggregates (21).

The reaction of nitroxide radical with ascorbic acid was found to be first order with respect to both ascorbic acid and the nitroxide radicals (22). Under a large excess of ascorbate over TEMPO, the reaction follows pseudo-first-order kinetics. In this work, the kinetic study was carried out by monitoring the fluorescence intensity of PPE-SO₃ with the incubation time after the addition of excess ascorbic acid; the result is shown in Fig. 8. It can be seen that the fluorescence intensity of PPE-SO₃ increased with the incubation time. The fluorescence intensities can be converted to substrate concentration as a function of time by using Eqn. 3, which is derived from the Stern–Volmer equation (18):

$$[Q_t] = [Q_0] [(I_\infty/I_t - 1)/(I_\infty/I_Q - 1)] \quad (3)$$

where $[Q_t]$ is the quencher concentration at time t , $[Q_0]$ is the initial quencher concentration, I_∞ is the final fluorescence intensity after the addition of ascorbic acid, I_Q is the fluorescence intensity after addition of quencher and before the addition of ascorbic acid, and I_t is the fluorescence intensity at time t after ascorbic acid addition.

Figure 8 inset illustrates the relationship of $\ln([Q])$ and the incubation time in this work. From Fig. 8 it can be seen that the logarithmic plot of the TEMPO concentration has a good linear relationships with time. Thus, the reaction is first order with respect to the TEMPO, which concurs with a previous report (22). The second-order rate constant calculated from the time courses, 0.7 mol/L/s, is not in very good agreement with the value (2 mol/L/s) obtained by using the same substrate (23). This may be caused by the reason that the reaction rate is proportional to the pH value of the solution (22).

Conclusion

In conclusion, this study investigated the interactions between TEMPO and PPE-SO₃ in aqueous solution. The results showed that

the fluorescence emission of PPE-SO₃ can be efficiently quenched by the paramagnetic compound TEMPO, and the PPE-SO₃ fluorescence can be substantially recovered by the addition of ascorbic acid. The possible mechanism of FL quenching was studied. This study also demonstrates that by using the PPE-SO₃–TEMPO–ascorbic acid system, it is possible to study the dynamic processes of radical/redox scavenging, and this system has the potential as a sensor for the detection of ascorbic acid. One potential limitation of this approach is that the quenching efficiency of conjugated polyelectrolyte by TEMPO is not very high. In our laboratory, the development of some other analogous nitroxides as quenchers for improving the Stern–Volmer constant is under progress.

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References

1. Tan CY, Atas E, Muller JG, Pinto MR, Kleiman VD, Schanze KS. Amplified quenching of a conjugated polyelectrolyte by cyanine dyes. *J. Am. Chem. Soc.* 2004; **126**: 13685–13694.
2. Pinto MR, Kristal BM, Schanze KS. A water-soluble poly(phenylene ethynylene) with pendant phosphonate groups. Synthesis, photophysics, and layer-by-layer self-assembled films. *Langmuir* 2003; **19**: 6523–6533.
3. Chen LH, McBranch DW, Wang HL, Helgeson R, Wudl F, Whitten DG. Highly sensitive biological and chemical sensors based on reversible fluorescence quenching in a conjugated polymer. *Proc. Natl Acad. Sci. USA* 1999; **96**: 12287–12292.
4. Harrison BS, Ramey MB, Reynolds JR, Schanze KS. Amplified fluorescence quenching in a poly(p-phenylene)-based cationic polyelectrolyte. *J. Am. Chem. Soc.* 2000; **122**: 8561–8562.
5. Kushon SA, Ley KD, Bradford K, Jones RM, McBranch D, Whitten D. Detection of DNA hybridization via fluorescent polymer super-quenching. *Langmuir* 2002; **18**: 7245–7249.
6. Gaylord BS, Heeger AJ, Bazan GC. DNA detection using water-soluble conjugated polymers and peptide nucleic acid probes. *Proc. Natl Acad. Sci. USA* 2002; **99**: 10954–10957.
7. Ballesteros OG, Marette L, Sastre R, Scaiano JC. Kinetics of cap separation in nitroxide-regulated 'living' free radical polymerization: application of a novel methodology involving a prefluorescent nitroxide switch. *Macromolecules* 2001; **34**: 6184–6187.
8. Blough NV, Simpson DJ. Chemically mediated fluorescence yield switching in nitroxide–fluorophore adducts: optical sensors of radical/redox reactions. *J. Am. Chem. Soc.* 1988; **110**: 1915–1917.
9. Green SA, Simpson DJ, Zhou G, Ho PS, Blough NV. *J. Am. Chem. Soc.* Intramolecular quenching of excited singlet states by stable nitroxyl radicals. 1990; **112**: 7337–7346.
10. Herbelin SE, Blough NV. Intramolecular quenching of excited singlet states in a series of fluorescamine-derivatized nitroxides. *J. Phys. Chem. B* 1998; **102**: 8170–8176.
11. Pou S, Huang Y, Bhan A, Bhadti VS, Hosmane RS, Wu SY, Cao G, Rosen GM. A fluorophore-containing nitroxide as a probe to detect superoxide and hydroxyl radical generated by stimulated neutrophils. *Anal. Biochem* 1993; **212**: 85–90.
12. Aspee A, Marette L, Scaiano JC. Monitoring photodecomposition of dibenzyl ketone within NaY zeolite with a pre-fluorescent nitroxide compound. *Photochem. Photobiol. Sci.* 2003; **2**: 1125–1129.
13. Ballesteros OG, Marette L, Sastre R, Scaiano JC. Kinetics of cap separation in nitroxide-regulated 'living' free radical polymerization: application of a novel methodology involving a prefluorescent nitroxide switch. *Macromolecules* 2001; **34**: 6184–6187.
14. Ricci A, Chretien MN, Scaiano JC. Base-catalyzed ship-in-a-bottle synthesis of a prefluorescent, zeolite-incorporated sensor for monitoring radical processes in zeolites. *Chem. Mater.* 2004; **16**: 2669–2674.
15. Tan CY, Pinto MR, Schanze KS. Photophysics, aggregation and amplified quenching of a water-soluble poly(phenylene ethynylene). *Chem. Commun.* 2002; 446–447.

16. Zhang T, Fan HL, Zhou JG, Liu GL, Feng GD, Jin QH. Fluorescent conjugated polymer PPESO3 a novel synthetic route and the application for sensing protease activities. *Macromolecules* 2006; **39**: 7839–7843.
17. Lakowicz JR. Quenching of fluorescence. In *Principles of Fluorescence Spectroscopy*, 2nd edn. Kluwer Academic/Plenum: New York, 1999; 237–265.
18. Pinto MR, Schanze KS. Fluorescent conjugated polymer PPESO3 A novel synthetic route and the application for sensing protease activities. *Proc. Natl Acad. Sci. USA* 2004; **101**: 7505–7510.
19. DiCesare N, Pinto MR, Schanze KS, Lakowicz JR. Saccharide detection based on the amplified fluorescence quenching of a water-soluble poly(phenylene ethynylene) by a boronic acid functionalized benzyl viologen derivative. *Langmuir* 2002; **18**: 7785–7787.
20. Wang DL, Wang J, Moses D, Bazan GC, Heeger AJ. Photoluminescence quenching of conjugated macromolecules by bipyridinium derivatives in aqueous media: charge dependence. *Langmuir* 2001; **17**: 1262–1266.
21. Lavigne JJ, Broughton DL, Wilson JN, Erdogan B, Bunz UHF. 'Surfactochromic' conjugated polymers: surfactant effects on sugar-substituted PPEs. *Macromolecules* 2003; **36**: 7409–7412.
22. Okazaki M, Kuwata K. A stopped-flow ESR study on the reactivity of some nitroxide radicals with ascorbic acid in the presence of β -cyclodextrin. *J. Phys. Chem.* 1985; **89**: 4437–4440.
23. Couet WR, Brasch RC, Sosnousky G, Lukszo J, Prakash I, Gnewuch CT, Tozer TN. Influence of chemical structure of nitroxyl spin labels on their reduction by ascorbic acid. *Tetrahedron* 1985; **41**: 1165–1172.