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Synthesis and Characterization of a Water-Soluble Carboxylated Polyfluorene and Its Fluorescence Quenching by Cationic Quenchers and Proteins

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Abstract: We have developed a new intermediate monomer, 2,7-[bis(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-9,9-bis(3-(*tert*-butyl propanoate))]fluorene, that allows the easy synthesis of water-soluble carboxylated polyfluorenes. As an example, poly[9,9′-bis(3′′-propanoate)fluoren-2,7-yl] sodium salt was synthesized by the Suzuki coupling reaction, and the properties of the polymer were studied in aqueous solutions of different pH. Fluorescence quenching of the polymer by different cationic quenchers (MV^{2+}, MV^{4+}, and NO_2MV^{2+}; MV = methyl viologen) was studied, and the quenching constants were found to be dependent on the charge and electron affinity of the quencher molecule and the pH of the medium. The largest quenching constant was observed to be $1.39\times10^{8}\,M^{-1}$ for NO_2MV^{2+} at pH 7. The change in polymer fluorescence upon interaction with different proteins was also studied. Strong fluorescence quenching of the polymer was observed in the presence of cytochrome c, whereas weak quenching was observed in the presence of myoglobin and bovine serum albumin. Lysozyme quenched the polymer emission at low protein concentrations, and the quenching became saturated at high protein concentrations. Under similar experimental conditions, the polymer showed improved quenching efficiencies toward cationic quenchers and a more selective response to proteins relative to other carboxylated conjugated polymers.

Keywords: biosensors · fluorescence quenching · polyfluorenes · polymers · proteins

Introduction

Conjugated polyelectrolytes (CPEs) are π-conjugated polymers with ionic side chains that are soluble in water and other polar solvents.[1] The fluorescence of CPEs can be efficiently quenched by oppositely charged quenchers.[1c] Superquenching or amplified quenching was first demonstrated through a single methyl viologen (MV^{2+}) molecule, which can quench the emission of several sulfonated poly(phenylenevinylene) (PPV) chains containing hundreds of repeat units.[2] This phenomenon has been widely applied in chemo/biosensors for the detection of various analytes, such as metal ions, carbohydrates, proteins, and DNA.[2–4]

The optical properties and sensor applications of carboxylated conjugated polymers, such as carboxylated poly(*p*-phenyleneethynylene)s (PPEs), have been widely investigated by Swager and co-workers as well as other groups.[5] The quenching behavior of these polymers were studied by using different quenchers, such as metal ions, MV^{2+}, and proteins; the polymers showed values of the Stern–Volmer (SV) constant (K_{sv}) in the range 10^4–10^6.[5] In a recent publication, the fluorescence change of carboxylated PPEs in the presence of lysozyme, histone, myoglobin, hemoglobin, and bovine serum albumin was studied.[5b] It was found that lysozyme, histone, myoglobin, and hemoglobin quenched the polymer fluorescence, whereas BSA enhanced it. This random behavior of polymer fluorescence changes in the presence of different proteins is due to the nonspecific binding between proteins and the polymer as well as the different aggregation behavior of conjugated polyelectrolytes in solution.

For chemo/biosensors that operate by superquenching mechanisms, the sensor sensitivity is directly related to the superquenching efficiency of the polymer/quencher pairs. In general, a higher K_{sv} value requires a lower quencher concentration to achieve a given level of fluorescence quench-

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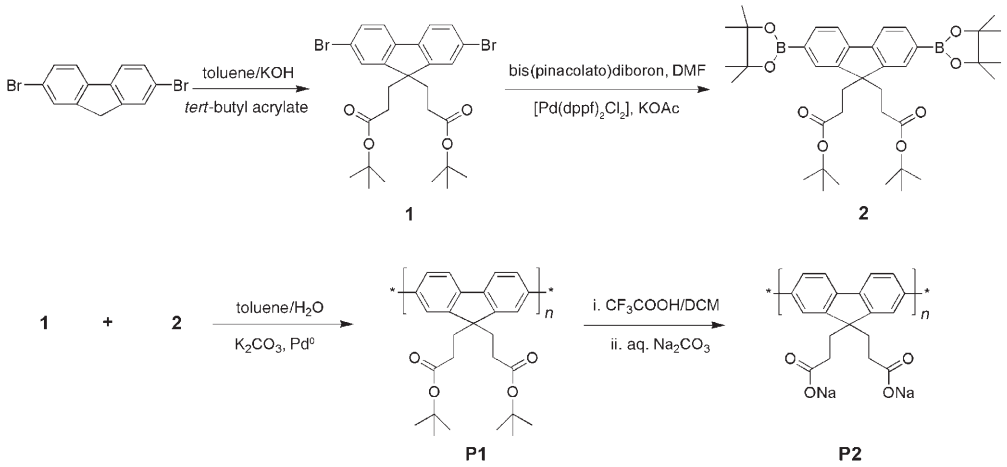
ing, which leads to high detection sensitivity. It is thus important to develop systems that provide as high a K_{sv} value as possible. With this aim, much effort has been made to study how polymer concentration, polymer aggregation, quencher charge, and quencher hydrophobicity affect K_{sv}.[2,3] On the other hand, very little effort has been devoted to the development of new highly fluorescent polyelectrolytes that allow for greater contrast between quenched and unquenched polymer.

Water-soluble polyfluorenes (PFs) and their derivatives have emerged as a new class of materials for polymer light-emitting devices and chemo/biosensors because of their high fluorescence quantum yields and good water solubility.[6,7] Cationic PFs, in particular, have proven useful for sequence-specific DNA detection.[4] However, anionic PFs are not as well-reported,[7] and there are almost no existing examples of water-soluble carboxylate-functionalized PFs.[8] As opposed to carboxylated PPEs, which are synthesized under mild Sonogashira cross-coupling conditions,[5] carboxylated PFs are more difficult to synthesize. It is generally believed that the basic reagents used for traditional Suzuki coupling reactions could hydrolyze the carboxylic ester groups during the polymerization. Recently, a base-free Suzuki polymerization method was developed to synthesize an amphiphilic alcohol-soluble carboxylated PF copolymer.[8] Herein, we report a simple and efficient method of synthesizing water-soluble carboxylated PFs by using a newly developed intermediate, 2,7-[bis(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-9,9-bis(3-(*tert*-butyl propanoate))]fluorene. The availability of water-soluble carboxylated PFs allows us to examine the effect of pH on the optical properties of the polymer and how these properties affect its quenching behavior by different cationic quenchers. Relative to carboxylated PPEs, the new polymer showed a higher fluorescence quantum yield in water, a larger K_{sv} value toward different quenchers, and a better selective response to different proteins.

Results and Discussion

Scheme 1 shows the synthetic strategy for the anionic carboxylated PFs. Direct alkylation of 2,7-dibromofluorene with *tert*-butylacrylate in a mixture of toluene/aqueous KOH gave monomer **1** in 51% yield. Conversion of **1** into diboronate ester **2** was achieved under Miyaura reaction conditions in the presence of bis(pinacolato)diborane, [Pd(dppf)$_2$Cl$_2$], and KOAc with dry DMF as the solvent.[9] Traditional methods, such as lithiation of the bromo-substituted fluorene **1**,[6d] followed by rapid addition of 2-isopropoxy-4,4,5,5-tetramethyl-1,3,2-dioxaborolane did not yield the desired product due to the side reaction between *n*BuLi and the ester groups. The availability of alkylcarboxylate-containing aryl boronates simplifies the synthesis of the precursor polymers for anionic water-soluble PFs that rely on Suzuki cross-coupling protocols. A library of carboxylated water-soluble PFs could be synthesized immediately through coupling of **2** with various co-monomers. Compound **2** was obtained in 93% yield after purification by silica-gel column chromatography. The structures of **1** and **2** were confirmed with NMR spectroscopy, MS, and elemental analysis.

The coupling of 1 equivalent of **1** with **2** in a mixture of toluene and 2 M K_2CO_3 under Suzuki conditions for 24 h yielded poly[9,9′-bis(*tert*-butyl-3″-propanoate)fluoren-2,7-yl] (**P1**) in 71% yield. The ^{1}H NMR spectrum clearly shows the peak for the C(CH$_3$)$_3$ group at 1.31 ppm, which indicates the existence of the carboxylic ester groups. **P1** is readily soluble in chloroform, dichloromethane, and THF, but is insoluble in water. Hydrolysis of the ester groups was done in two steps. First, **P1** was dissolved in dichloromethane and treated with CF_3COOH at room temperature for 5 h. After solvent removal, the residue was treated with Na_2CO_3 (0.005 M) for 4 h. The polymer was purified by dialysis (MW cutoff: 12000–14000 Da) against deionized (DI) water for 3 days to remove the salt and low-molecular-weight fractions. Poly[9,9′-bis(3″-propanoate)fluoren-2,7-yl] sodium salt (**P2**) was obtained in 62% yield after freeze-drying. For **P2**, there was



Scheme 1. The synthetic route to monomers **1** and **2** and polymers **P1** and **P2**. DCM = dichloromethane, DMF = *N*,*N*-dimethylformamide, dppf = 1,1′-bis(diphenylphosphanyl)ferrocene.

no residual peak observed at around 1.31 ppm in the ^{1}H NMR spectrum, which indicates the complete conversion of $COOC(CH_3)_3$ into COONa. **P2** is readily soluble in water ($>5\,mg\,mL^{-1}$) and other polar solvents such as methanol and DMF.

The absorption and photoluminescence spectra of **P2** were recorded in DI water at pH 2→7 at a concentration of 1.2×10^{-6} M. In general, **P2** has a strong absorption band with a peak at around 390 nm, which can be attributed to the π–π* transition of the polymer backbone.[10] The absorption maximum did not change clearly with pH; however, the absorbance increased with increasing pH. The absorption spectra of **P2** at pH 4, 5, and 7 are shown in Figure S1 of the Supporting Information. At pH 7 and a **P2** concentration of 1.2×10^{-6} M, the absorbance was over two times higher than that at pH 4, which indicates the presence of fewer interchain interactions (i.e., hydrogen bonding) at pH 7. **P2** emits blue fluorescence with a main peak at around 434 nm and a shoulder at about 457 nm (Figure 1). The absolute fluorescence intensities for solutions of **P2** also change with pH. Within the tested pH range, the highest fluorescence intensity for **P2** was observed at pH 7, and the lowest was observed at pH 2. The photoluminescence quantum yields (PLQYs) at different pH values were measured with quinine sulfate as a reference ($\Phi_F=55\%$ in 0.1 N H_2SO_4),[11] and the results are shown in Figure 1. At pH 7, the PLQY of **P2** was about 25%, which is higher than that of most carboxylated PPE derivatives (3–10% in aqueous solution).[5] When PLQY was plotted versus pH, the PLQY values for solutions of **P2** at pH 5→7 were found to be nearly the same. However, a sharp transition in PLQY was observed for solutions of **P2** at pH 4→5. At pH 4, the PLQY was around 4%. There was almost no detectable fluorescence from the polymer solution at pH 2. This pH-dependent optical behavior is believed to be associated with protonation and deprotonation of the side chains. Due to the similarity between heptane diacid and the side chains of **P2**, we estimated the pK_a values of **P2** to be 4–6 (pK_a values for heptane diacid are p$K_{a1}=4.48$ and p$K_{a2}=5.42$).[12] At pH<4, the carboxylate groups are protonated, and **P2** is in the acid form. Protonation leads to decreased solubility of **P2** in DI water. Furthermore, a low pH value favors hydrogen bonding between the protonated polymers, which leads to a more aggregated polymer state. The broad energy distribution due to the hydrogen-bonded states also increases the probability of a nonradiative decay process to lower the solution fluorescence. This is in agreement with the observation that at low pH (pH<4), the PLQYs are very low. When the pH is higher than the pK_a values of the polymer, such as at pH 7, deprotonation is a more favorable process. Under these circumstances, most polymer chains are negatively charged. The repulsion between negatively charged side chains would cause **P2** to be in a less aggregated state. Therefore, the solution has a high PLQY. For solutions of **P2** of pH 4–6, it is most likely that there is a coexistence of protonated and partially deprotonated end groups, and the PLQYs are in the range 4–25%.

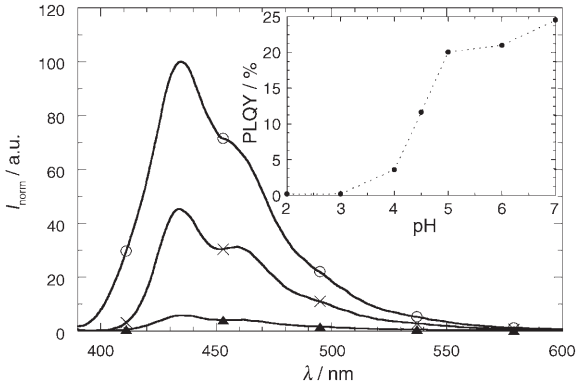
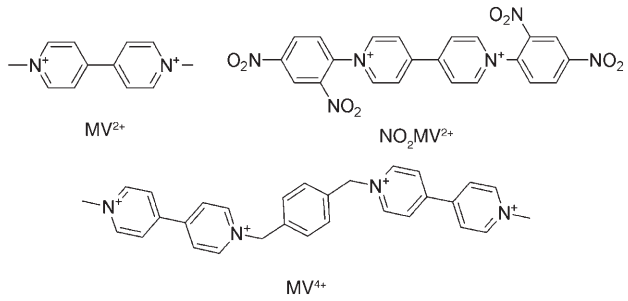


Figure 1. Photoluminescence spectra of **P2** at pH 4 (▲), 5 (×), and 7 (○). Inset: Plot of PLQY of **P2** as a function of pH.

The polymer fluorescence quenching was studied with MV^{2+} (*N*,*N*′-dimethyl-4,4′-bipyridinium diiodide), NO_2MV^{2+} (*N*,*N*′-bis(2,4-dinitro-1-benzyl)-4,4′-bipyridinium dichloride), and MV^{4+} (α,α′-bis(1′-methyl-4,4′bipyridinium)-*p*-xylene tetrachloride) as quenchers. The structures of the quenchers are shown in Scheme 2. These quenchers were chosen as they are good electron acceptors and have different net charges and/or LUMO (lowest unoccupied molecular orbital) energy levels that could affect electron transfer. Cyclic voltammetry of the quenchers and **P1** was performed in DMF and dichloromethane, respectively, with $Ag/AgNO_3$ as the reference electrode.[13] The onset potentials were measured to be −0.69, −0.34, −0.67, and −1.64 V for MV^{2+}, NO_2MV^{2+}, MV^{4+}, and **P1**, respectively. The corresponding LUMO levels were calculated to be −3.49, −4.14, −3.81, and −2.84 eV (Table 1).[13] The LUMO level of **P2** was estimated to be similar to that of **P1**, as the side chains have little effect on the oxidation and reduction of the polymer backbone.[4c] The differences in LUMO energy levels between the quenchers and **P2** were 0.95, 1.30, and 0.97 eV for MV^{2+}, NO_2MV^{2+}, and MV^{4+}, respectively.



Scheme 2. Chemical structures of the cationic quenchers.

The quenching efficiency for **P2** and the different quenchers can be quantified by measuring K_{sv}, which can be obtained from the following equation: $I_0/I=1+K_{sv}[\text{quencher}]$, in which I_0 and I are the fluorescence intensities of **P2** in the absence and presence of the quenchers, respectively.[2,3] To

Table 1. K_{sv} values of **P2** with three quenchers at different pH and their LUMO energy levels.

Chromophores	K_{sv} [$\times 10^6$ M^{-1}]			E_{red}/LUMO[a]
	pH 4	pH 5	pH 7	[V/eV]
P2	–	–	–	−1.64/−2.84
MV^{2+}	0.48	1.24	31.7	−0.69/−3.79
NO_2MV^{2+}	6.31	11.6	139	−0.34/−4.14
MV^{4+}	0.89	2.04	78.5	−0.67/−3.81

[a] Cyclic voltammograms were recorded for [quencher] = 1 mM in DMF and [**P1**] = 1 mM in dichloromethane with $Ag/AgNO_3$ as the reference electrode and glassy carbon as the working electrode at a scan rate of 50 mV s^{-1} with 0.1 M tetrabutylammonium hexafluorophosphate as the electrolyte. E_{red} was determined from the onset of reduction, and the LUMO energy level (eV) was calculated by using the formula $E_{LUMO} = -e(E_{red}+4.48)$.

evaluate how charge density and polymer aggregation affect the quenching process, we studied the SV plots at pH 4, 5, and 7. This corresponds to **P2** in the neutral, partially deprotonated, and mostly deprotonated state, respectively. The K_{sv} values were calculated from the linear part at low quencher concentrations, at which the quenching process occurs by static quenching through interactions of ground states. However, at higher quencher concentrations, the quenching process occurs through diffusion-controlled dynamic quenching.[3d,14] Figure 2a shows the SV plots of **P2** (1.2×10^{-6} M in repeat units) with three quenchers at pH 4. For MV^{2+} and MV^{4+}, weak fluorescence quenching was observed, and the K_{sv} values were very small (4.8×10^5 M^{-1} for MV^{2+}, 8.9×10^5 M^{-1} for MV^{4+}; Table 1). The largest K_{sv} value of 6.31×10^6 M^{-1} was calculated for NO_2MV^{2+}. This value is over 10 times higher than that for MV^{2+}. At pH 4, **P2** is nearly in the neutral state, and there are almost no electrostatic interactions between **P2** and the quenchers. Close contact between **P2** and the quenchers is thus mainly due to the hydrophobic interactions. For NO_2MV^{2+}, owing to the larger driving force for electron transfer (0.95 eV for MV^{2+}, 1.30 eV for NO_2MV^{2+}) and the greater van der Waals forces between the electron-poor quencher (NO_2MV^{2+} relative to MV^{2+}) and the electron-rich polymer, a larger K_{sv} value was observed.

The SV plots of **P2** with different quenchers at pH 5 are shown in Figure 2b, and the K_{sv} values are summarized in Table 1. The K_{sv} values for NO_2MV^{2+} and MV^{4+} increased from 6.31×10^6 and 8.9×10^5 M^{-1} to 1.16×10^7 and 2.04×10^6 M^{-1}, respectively. A similar increase in K_{sv} value was observed for MV^{2+}. Relative to the solution at pH 4, the number of deprotonated carboxylate groups should be higher in solution at pH 5. Both electrostatic and hydrophobic interactions between **P2** and the quenchers are possible. The increased K_{sv} values at pH 5 could be mainly due to the increased electrostatic interactions between **P2** and the quenchers.

Figure 2c shows the SV plots for **P2** at pH 7 upon addition of MV^{2+}, NO_2MV^{2+}, and MV^{4+}. As seen in Figure 2c and Table 1, the K_{sv} values were significantly improved relative to those obtained at pH 4 and 5. The response of **P2** to the quenchers became very sensitive even at very low

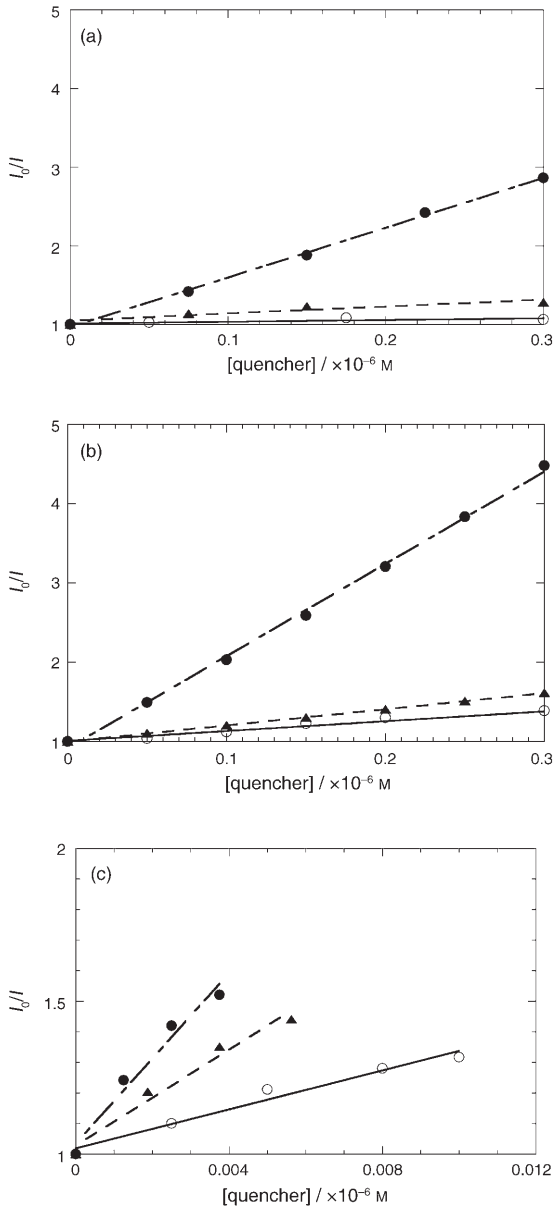


Figure 2. SV curves of **P2** with different quenchers at pH 4 (a), 5 (b), and 7 (c). ○ = MV^{2+}, ● = NO_2MV^{2+}, ▲ = MV^{4+}.

quencher concentrations. A K_{sv} value of 7.85×10^7 M^{-1} was determined for MV^{4+}, which is about twice that for MV^{2+} ($K_{sv} = 3.17\times10^7$ M^{-1}). This indicates that MV^{4+} can quench the **P2** emission about two times more efficiently than MV^{2+}. The higher K_{sv} value for MV^{4+} is believed to be due to the greater ability of the multivalent quencher to induce aggregation of the polymer.[3a] Aggregation allows more rapid interchain diffusion of the excition, and hence a more efficient quenching of the polymer fluorescence. A K_{sv} value of 1.39×10^8 M^{-1} was obtained for NO_2MV^{2+}, which is about four times higher than that for MV^{2+}. As NO_2MV^{2+} and MV^{2+} have the same number of positive charges per quencher, it is unlikely that anything related to charge pairing could have such a tremendous effect on electron transfer. The significant difference in K_{sv} is thus more likely to be

due to the difference in the LUMO energy levels between NO_2MV^{2+}/**P2** and MV^{2+}/**P2**. The large difference in energy levels for NO_2MV^{2+}/**P2** provides more efficient electron transfer than the other two quenchers.[3a,b] The large difference in K_{sv} values between MV^{2+} and NO_2MV^{2+} relative to that between MV^{2+} and MV^{4+} indicates that the electron affinity of the quencher plays a more important role in the amplified quenching than the net charge of the quenchers. For MV^{2+} and MV^{4+}, the K_{sv} values at pH 7 were almost 100 times larger than those at pH 4, and over 30 times larger than those at pH 5. This is due to the increased amount of deprotonated carboxylate groups at pH 7, which favors stronger electrostatic interactions and, thus, more efficient electron transfer. Importantly, at pH 7, the K_{sv} values for **P2** are one to two orders of magnitude greater than those reported for carboxylated PPEs (i.e., $6\times10^5\,M^{-1}$ with MV^{2+} and $3.6\times10^6\,M^{-1}$ with MV^{4+}).[15]

The fast response of **P2** to different quenchers suggests the potential application of **P2** in biodetection, for example, to detect certain proteins by superquenching. We thus tested the fluorescence quenching of **P2** by four different proteins in phosphate-buffered saline (PBS) buffer at pH 7.2. The four proteins are bovine serum albumin (BSA; pI = 4.8–4.9), myoglobin (Myo; pI = 7.0–7.2), lysozyme (Lys; pI = 11) and cytochrome c (Cyt c; pI = 10.2–10.7).[5b] As protein charge is dependent on solution pH,[5c] at pH 7.2, BSA is negatively charged, Lys and Cyt c are both positively charged, and Myo is neutral. Furthermore, Myo and Cyt c are metalloproteins, which favors energy/electron transfer.[5b]

The fluorescence spectral changes of **P2** upon interaction with each protein are shown in the Supporting Information, and the corresponding SV curves are shown in Figure 3. The fluorescence response of **P2** to Cyt c is the most significant of the four proteins, whereby a K_{sv} value of around $7.0\times10^7\,M^{-1}$ was shown. At pH 7.2, Cyt c can easily form complexes with anionic **P2** through electrostatic interaction, which allows energy and/or charge transfer from **P2** to Cyt c. This is due to the metalloporphyrin functionality in Cyt c, which is capable of quenching the excited state of **P2**.[16] Furthermore, the protein-induced aggregation of **P2** should also affect its fluorescence quenching. To clarify the contribution of these two factors, we performed experiments with lysozyme and myoglobin. Lysozyme is highly charged at pH 7.2, but it does not contain any electron-transfer center.[17] The polymer fluorescence was also quenched by lysozyme. However, the fluorescence quenching saturated at around 35 % of its original fluorescence, whereas under similar conditions, the polymer fluorescence was totally quenched by Cyt c. These results indicate that the polymer fluorescence is sensitive to low concentrations of polycations, in which the charge pairing can induce polymer aggregation that decreases fluorescence. On the other hand, myoglobin is a heme protein with low electron-transfer reactivity in vivo and in vitro.[17] At pH 7.2, the net charge of myoglobin is close to zero, which does not favor electrostatic interactions with the polymer. Under similar experimental conditions, the fluorescence quenching of **P2** by myoglobin was much less obvious than for Cyt c. A K_{sv} value of around $5.0\times10^6\,M^{-1}$ was observed for myoglobin, which is over an order of magnitude lower than that for Cyt c. BSA as a model protein for many bioassays was also used to study its effect on polymer fluorescence. Owing to its negative charge in nature, the presence of BSA in polymer solution only slightly affected the polymer emission. As opposed to previous reports in which the surfactant nature of BSA was observed to enhance greatly the fluorescence of both carboxylated and sulfonated PPEs,[18] the only slight interference of BSA on the optical properties of the polymer indicates that the synthesized polymer is less aggregated in water and has an improved selectivity in response to different proteins.

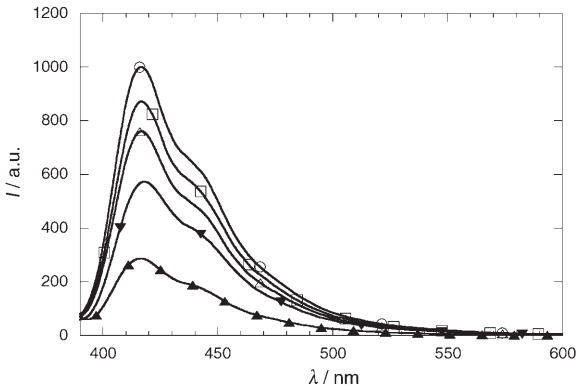


Figure 3. SV curves of **P2** with different proteins in PBS buffer (pH 7.2). ○ = No protein, □ = BSA, △ = myoglobin, ▼ = lysozyme, ▲ = Cyt c.

Conclusions

In summary, we have developed a new strategy for synthesizing a key intermediate for anionic polyfluorenes. A water-soluble carboxylated polyfluorene **P2** has been synthesized as an example. **P2** has strong blue fluorescence with a PLQY of about 25 % in water at pH 7. Both the absorption and emission of **P2** could be fine-tuned at pH 2–7. At pH 4, the polymer was in an aggregated state with a very low charge density. Low K_{sv} values were observed for all quenchers. At pH 5, the polymer had a greater amount of charged side groups, which led to higher K_{sv} values for all quenchers than those at pH 4. At pH 7, a K_{sv} value of $1.39\times10^8\,M^{-1}$ was observed for **P2** with NO_2MV^{2+} as the quencher, which is over two orders of magnitude higher than those reported for carboxylated PPEs. The response of **P2** to different proteins showed high fluorescence quenching toward cytochrome c. Little change in fluorescence in the presence of BSA and myoglobin indicated that **P2** is highly soluble in buffer and is less aggregated than carboxylated and sulfonated PPEs. The high quantum yield and large K_{sv} value of **P2** toward cationic quenchers and proteins make anionic polyfluorenes a good candidate for chemo/biosensor applications that take advantage of the amplified fluorescence quenching.

Experimental Section

Materials

2,7-Dibromofluorene, *tert*-butyl acrylate, and bis(pinacolato)diboron were purchased from Aldrich. Bovine serum albumin, myoglobin, lysozyme and cytochrome c were also purchased from Aldrich and used as received. Other reagents were purchased from Aldrich and used as received unless otherwise specified.

Instrumentation

^{1}H and ^{13}C NMR spectra were recorded on a Burker 300-MHz spectrometer with a probe at 300 MHz for ^{1}H and 75 MHz for ^{13}C. Elemental analysis was performed on a Perkin–Elmer 2400 CHN/CHNS elemental analysis instrument. UV/Vis spectra were collected with a Shimadzu UV-2401 recording spectrophotometer. Fluorescence was measured by using a Perkin–Elmer LS-55 instrument equipped with a xenon lamp excitation source and a Hamamatsu (Japan) 928 photomultiplier tube, with 90° angle detection for solution samples. Fluorescence quantum yields were measured with quinine sulfate in 0.1 N H_2SO_4 as reference. Cyclic voltammetry was carried out on a computer-controlled CHI660A electrochemical workstation with a glassy carbon electrode as the working electrode, a platinum electrode as the counter electrode, and $Ag/AgNO_3$ as the reference electrode. The scan rate was 50 mV s^{-1}. A solution of tetrabutylammonium hexafluorophosphate (0.1 M) in DMF and dichloromethane were used as the electrolyte for the quenchers and the polymer, respectively. The pH of the solution was measured by using a pH meter (Sartorius PB-10) with a glass/reference electrode calibrated with buffer solutions of pH 4, 7, and 10.

Fluorescence-Quenching Experiments

Quenching experiments were performed by successive addition of quenchers to a solution of **P2** (1.2×10^{-6} M) in water at different pH values at room temperature. Quenching experiments with proteins were performed by addition of proteins to a solution of **P2** (1.2×10^{-6} M) in PBS buffer (pH 7.2) at room temperature. Fluorescence spectra were recorded for each solution at different quencher/protein concentrations, and I_0/I was used for the K_{sv} plots.

Syntheses

1: Aqueous KOH (50 wt%, 5 mL) was added dropwise to a solution of 2,7-dibromofluorene (3.3 g, 10.2 mmol) and tetrabutylammonium bromide (250 mg, 0.78 mmol) in toluene (25 mL). The solution was stirred for 20 min at room temperature. *tert*-Butyl acrylate (5.25 g, 41 mmol) was added dropwise, and the mixture was stirred at room temperature for 5 h. The mixture was diluted with dichloromethane (15 mL) and washed with water (2×10 mL). The organic layer was dried over anhydrous $MgSO_4$. After the solvent was removed, the residue was purified by column chromatography (dichloromethane/hexane = 1:1) to give 2,7-dibromo-9,9-bis(3-(*tert*-butyl propanoate))fluorene (**1**; 3.0 g, 51%) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.53 (d, J = 8.37 Hz, 2H), 7.48 (d, J = 6.27 Hz, 4H), 2.30 (t, J = 8.18 Hz, 4H), 1.46 (t, J = 8.36 Hz, 4H), 1.33 ppm (s, 18H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 172.4, 150.1, 139.2, 131.2, 126.6, 122.2, 121.6, 80.6, 54.2, 34.5, 30.0, 28.2 ppm; MS (EI): m/z calcd for $C_{27}H_{32}Br_2O_4$: 580.35 $[M]^+$; found: 580.02; elemental analysis: calcd (%) for $C_{27}H_{32}Br_2O_4$: C 55.88, H 5.56; found: C 55.51, H 5.53.

2: A mixture of **1** (2.13 g, 3.7 mmol), KOAc (1.7 g, 17 mmol), and bis(pinacolato)diboron (2.4 g, 9.5 mmol) in DMF (15 mL) was placed in a 100-mL flask. After the mixture was stirred for 10 min, $[Pd(dppf)_2Cl_2]$ (80 mg) was added quickly. The mixture was stirred overnight at 90 °C. After cooling to room temperature, the mixture was poured into water and extracted with dichloromethane (2×15 mL). The organic layer was washed with water (2×10 mL) and then dried over anhydrous $MgSO_4$. After the solvent was removed, the residue was purified by column chromatography (ethyl acetate/hexane = 1:4) to give 2,7-bis(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-9,9-bis(3-(*tert*-butyl propanoate))fluorene (**2**; 2.3 g, 93%) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.80 (t, J = 8.54 Hz, 4H), 7.71 (d, J = 7.68 Hz, 2H), 2.38 (t, J = 8.37 Hz, 4H), 1.44 (t, J = 8.37 Hz, 4H), 1.37 (s, 24H), 1.29 ppm (s, 18H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 173.0, 148.0, 144.0, 134.5, 129.1, 119.8, 84.0, 80.1, 53.6, 34.6, 30.1, 28.2, 25.1 ppm; MS (EI): m/z calcd for $C_{39}H_{56}B_2O_8$: 674.48 $[M]^+$; found: 674.37; elemental analysis: calcd (%) for $C_{39}H_{56}Br_2O_8$: C 69.45, H 8.37; found: C 69.18, H 8.38.

P1: Monomers **1** (291 mg, 0.5 mmol) and **2** (340 mg, 0.5 mmol) were added into a 50-mL two-necked flask. K_2CO_3 (560 mg), $[Pd(PPh_3)_4]$ (10 mg), toluene (10 mL), and water (3 mL) were then added. After degassing, the mixture was heated at 90 °C with vigorous stirring for 24 h. After the mixture was cooled to room temperature, it was poured into methanol. The precipitated solid was collected by filtration to yield poly-[9,9′-bis(*tert*-butyl-3″-propanoate)fluoren-2,7-yl] (**P1**; 300 mg, 71%) as a greenish solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.87–7.73 (br m, 6H), 7.73 (s, 4H), 2.54 (br s, 4H), 1.64 (br s, 4H), 1.31 ppm (s, 18H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 172.8, 149.3, 140.2, 127.2, 121.6, 120.4, 80.1, 54.0, 34.8, 30.2, 29.7, 28.0 ppm.

P2: **P1** (30 mg) was dissolved in dichloromethane (25 mL) in a 50-mL flask. After addition of trifluoroacetic acid (2 mL), the mixture was stirred overnight at room temperature. After the solvent was removed, the yellowish-green residue was treated with aqueous Na_2CO_3 (0.005 M, 30 mL) at room temperature for 4 h. The polymer was purified by dialysis against DI water for 3 days. The solution was freeze-dried to give poly[9,9′-bis(3″-propanoate)fluoren-2,7-yl] sodium salt (**P2**; 23 mg, 62%) as a gray solid. ^{1}H NMR (300 MHz, CD_3OD): δ = 7.94–7.82 (m, 6H), 2.61 (br s, 4H), 1.65 ppm (br s, 4H).

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