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# Synthesis and properties of a novel water-soluble anionic polyfluorenes for highly sensitive biosensors

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**Abstract**

A new anionic water-soluble polyfluorene—poly(9,9-bis(4′-sulfonatobutyl)fluorene-*co-alt*-1,4-phenylene) sodium salt (PFS) was synthesized by Suzuki-coupling reaction. The anionic polyfluorene shows bright blue photoluminescence and it was found the photoluminescence could be highly quenched by a bioactive dye (Lucifer yellow cadaverine biotin-X dipotassium salt). PFS photoluminescence quenching only occurs effectively upon addition of avidin. The photoluminescence quenching process is sensitive to even subnanomolar amount of avidin ($10^{-10}$ M). The avidin facilitated photoluminescence quenching opens up opportunities of new ultra-high efficiency and rapid response biosensors based on the polyfluorene electrolyte.


*Keywords:* Polyelectrolyte; Water-soluble; Biosensor

## 1. Introduction

More and more attention was paid to water-soluble conjugated polymers, because the unique ionic groups of them may offer many new application opportunities [1]. For example, water-soluble conjugated polymers could be used as active layers in light emitting diodes (LEDs) through a layer-by-layer self-assembly approach and the environment-friendly solvent (such as water) can be used in device fabrication [2]. The most interesting and promising property of the water-soluble polymers was their use in chemical [3] and biological [4] sensors. Water-soluble conjugated polymers are attractive sensor materials for the reasons that their electrical, optical, electrochemical and optoelectronic properties can be greatly modified by very minor perturbations of the environmental stimuli and there exists amplification actions by a collective system response in macromolecules [5]. This amplification property offer a key advantage compared with the small molecules as the sensitive materials in sensors and extensive research works have been made recently to develop the use of the water-soluble conjugated polymers as the sensing materials in chemo and biosensors. Chen et al. [6] first reported sensitive, rapid-response biosensors for detection aromatics and proteins through a sensitive reversible fluorescence quenching actions between the conjugated polymers (fluorophore, PPV) and the small molecule (methyl viologin, $MV^{2+}$) quenchers. Biosensors detect protein (antibody) or DNA were demonstrated using other conjugate polyelectrolytes [7] and polyelectrolyte complexes [8,9]. But in most cases, the sensing action requires both photoluminescence quenching and unquenching (photoluminescence recovery).

Herein, we report the synthesis of a new anionic water-soluble polyfluorene—poly(9,9-bis(4′-sulfonatobutyl)fluorene-*co-alt*-1,4-phenylene) sodium salt (PFS) and the use as the highly sensitive material in a novel biosensor system. PFS was synthesized by Suzuki-coupling reaction and structures were characterized using $^{1}$H NMR and $^{13}$C NMR. The photoluminescence from PFS in water solution was tested and was found to be quenched by a bioactive dye—Lucifer yellow cadaverine biotin-X, dipotassium salt (LYCBDP), which is a good energy acceptor for PFS. The quenching was found only occur with the presence of avidin with very high

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sensitivity. This avidin-induced photoluminescence quenching enables a novel, simple, rapid but highly sensitive biodetection technique.

Fig. 2 shows the mechanism of our biosensor. The initial solution only contains the anionic polyfluorene—PFS (donor) and the biotin bonded dye—LYCBDP, which all are negatively charged. Thereby, it is difficult for them to move closely enough for fluorescence resonance energy transfer (FRET). When avidin was added into the solution, it will form a complex with PFS due to the attractive Coulomb interactions, because avidin is positively charged in neutral water. At the same time, avidin has a very high affinity ($K_d \sim 10^{-15}$ M) [10] with biotin groups of LYCBDP. Thereby, it will cause the formation of PFS, avidin and LYCBDP complex, in results of which the average distance between PFS and LYCBDP becomes close enough to allow FRET and induce the highly quenching of PFS. The quantity of the added avidin can be highly determined by the change of PFS' emission. Indeed, this biosensor has a very high sensitivity on detecting the special protein—avidin, which can detect avidin with the concentration of $10^{-10}$ M. The quenching was found only occur in the presence of avidin. This avidin-induced photoluminescence quenching enables a novel, simple, rapid but highly sensitive biodetection technique.

## 2. Experimental section

### 2.1. General details

$^1$H and $^{13}$C NMR spectra were collected on a Bruker DRX 400 spectrometer in Deuterated chloroform solution operating, respectively, at 400 MHz (for $^1$H) and 100 MHz (for $^{13}$C), with tetramethylsilane as reference. Number-average ($M_n$) and weight-average ($M_w$) molecular weights were determined by multi-angle laser light scattering combined with size exclusion chromatography (MALLS-SEC), which was performed with a multi-angle photometer (DAWN-DSP, Wyatt Technology Co.) at 633 nm at 25 °C with DMSO eluent at a flow rate of 1.0 ml/min. Elemental analyses were performed on Vario EL Elemental Analysis Instrument (Elementar Co.). UV–vis absorption spectra were recorded on a HP 8453 UV–vis spectrophotometer. The fluorescence spectra were recorded on a Jobin–Yvon spectrometer with 90° detection for solution samples. Emission spectra were measured in situ as described in previous literature [7b,c]. Deionized water obtained by Milli-Q system (Millipore) was used. All the experiments for absorption and fluorescence spectra were collected at ambient temperature. Lucifer yellow cadaverine biotin-X, dipotassium salt (LYCBDP) was purchased from Molecular Probes, Inc. (Eugene, Oregon). The concentrations of LYCBDP in aqueous solutions are estimated based on their extinction coefficient constant of $11 \times 10^3$ at 428 nm. Avidin and bovine serum albumin (BSA) were purchased from Sigma. Other materials used in this study were purchased from Aldrich and used directly as received.

### 2.2. 2,7-Dibromo-9,9-bis(4′-sulfonatobutyl)fluorene disodium (*1*)

To a stirred mixture of 2,7-dibromofluorene (4 g, 12 mmol) and 60 ml of dimethylsulfoxide (DMSO) under nitrogen were added tetrabutylammoium bromide (80 mg) and 8 ml of a 50 wt% aqueous solution of sodium hydroxide. 20 ml DMSO solution of 1,4-butane sultone (4 g, 29 mmol) was added dropwise to the mixture. The reaction mixture was stirred at room temperature for 3 h and then the reaction mixture was precipitated into 500 ml of acetone. The resulting product was collected by filtration, washed with ethanol, and recrystallized twice from acetone/$H_2O$ to yield (**1**) 4.3 g (58.6%) after drying in vacuum at 100 °C for 24 h.

$^1$H NMR (400 MHz, $D_2O$). $\delta$ (ppm): 7.55 (s, 2H), 7.51–7.50 (d, 2H), 7.33–7.31 (d, 2H), 2.50–2.46 (m, 4H), 1.86–1.82 (m, 4H), 1.40–1.32 (m, 4H), 0.48–0.45 (m, 4H).

$^{13}$C NMR (100 MHz, $D_2O$). $\delta$ (ppm): 152.24, 138.91, 130.39, 126.55, 121.54, 121.30, 55.26, 50.85, 38.62, 24.33, 22.49. Element Anal. Calcd for $C_{21}H_{22}Br_2O_6S_2\,Na_2 \cdot 2H_2O$: C, 37.28; H, 3.85; S, 9.47. Found: C, 37.70; H, 3.53; S, 9.32.

### 2.3. Poly(9,9-bis(4′-sulfonatobutyl)fluorene-co-alt-1,4-phenylene) sodium salt (PFS)

To the mixture of 2,7-dibromo-9,9-bis(4′-sulfonatobutyl) fluorene disodium (**1**) (1.224 g, 1.81 mmol), 1,4-phenylene-bisboronic acid (0.3 g, 1.81 mmol) and $Pd(OAc)_2$ (12 mg) was added a degassed mixture of 80 ml aqueous buffer (pH 10.0) and 30 ml DMF. The mixture was vigorously stirred at 85–90 °C for 48 h under argon atmosphere. After the mixture was cooled down to room temperature, it was poured into 1 l of acetone. The resulting precipitate was separated and re-d issolved in deionized water and dialyzed using a membrane with a 10,000–12,000 cutoff for 3 days. The final product was obtained after drying in vacuum at 110 °C for 24 h, yielding 0.46 g (46%) after dialysis. $^1$H NMR (400 MHz, DMSO-$d_6$). $\delta$ (ppm): 7.88–7.37 (m, 10H), 2.25–2.18 (m, 4H), 2.07–2.03 (m, 4H), 1.38 (m, 4H), 0.61 (m, 4H). $^{13}$C NMR (100 MHz, DMSO-$d_6$). $\delta$ (ppm): 151.70, 139.73, 139.27, 129.51, 127.84, 127.24, 126.25, 120.93, 55.28, 51.48, 31.06, 25.34, 23.53.

Element Anal. Calcd for $C_{27}H_{26}O_6S_2Na_2 \cdot 2H_2O$: C, 54.73; H, 5.07; S, 10.81. Found: C, 55.17; H, 5.93; S, 10.36.

## 3. Results and discussion

PFS is obtained by the sequence of reactions shown in Fig. 1. The monomer 2,7-dibromo-9,9-bis(4′-sulfonatobutyl)-fluorene disodium was prepared from 2,7-dibromofluorene by reaction with 1,4-butane sultone in DMSO at room temperature in the presence of an excess of 50% NaOH aqueous solution. Then the polymerization with the 1,4-phenylenebisboronic acid was carried out in a mixture of 30% DMF and 70% 0.2 M aqueous sodium carbonate solution containing 1 mol% $Pd(OAc)_2$ under rigorous stirring at 85–90 °C for 2 days. The final reaction mixture was precipitated from acetone. The resulting precipitate was redissolved in deionized water and

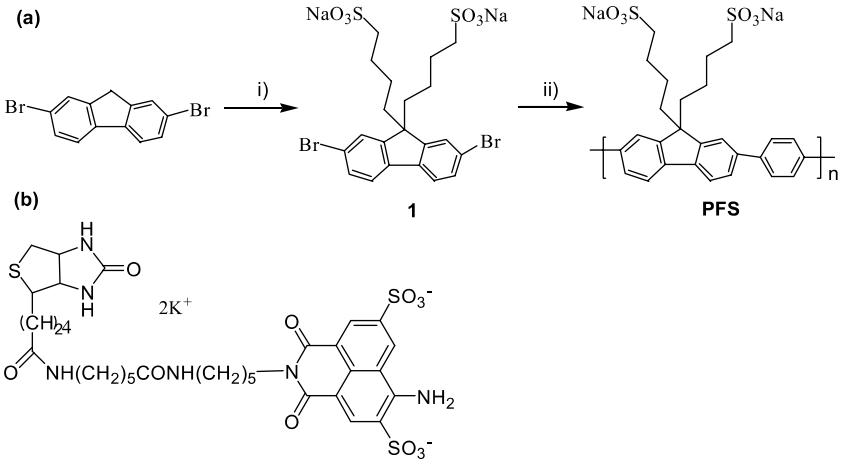


Fig. 1. (a) Synthesis of water-soluble PFS; (i) NaOH/$H_2O$/DMSO/1,4-butane sultone; (ii) 1,4-phenylenebisboronic acid/$Pd(OAC)_2$/DMF/$H_2O$/$Na_2CO_3$. (b) The chemical structure of Lucifer yellow cadaverine biotin-X, dipotassium salt (LYCBDP).

further purified by dialysis using a membrane with a 10,000–12,000 cutoff for 3 days to get the final product with a yield of ca. 46%. The structure of the polymer was confirmed by NMR spectroscopy and elemental analysis. The polymer has good solubility (more than 5 mg PFS in 1 ml) in water because of the sulfonate groups on side chains. Multi-angle laser light scattering combined with size exclusion chromatography (MALLS-SEC) analysis shows the average molecular weight of $M_n = 103{,}000$ ($\pm 2\%$) and $M_w = 214{,}000$ ($\pm 1.6\%$), respectively. The photoluminescence efficiency of PFS in water ($10^{-8}$ M) was found to be $90 \pm 5\%$ with 9,10-diphenylanthracene in ethanol as the standard [11]. The excellent water solubility, high molecular weight, and high PL efficiency allow PFS to be a very attractive material for biosensor applications.

The Fig. 2 shows the mechanism of our biosensor. This sensor is based on the highly selective quenching of the sensor material and no unquenching process is needed, indicating a greatly simplification for rapid detection. The testing agent we used is a bioactive dye—Lucifer yellow cadaverine biotin-X, dipotassium salt (LYCBDP) (Fig. 1(b)), which is a good energy acceptor for PFS. Fig. 3 shows the absorbance and fluorescence spectra of PFS and LYCBDP in water. The fluorescence spectrum of PFS has a large overlap with absorption spectra of LYCBDP, and LYCBDP are expected to quench via the FRET mechanism.

The initial solution only contains the anionic polyfluorene—PFS (donor) and the biotin bonded dye—LYCBDP, which all are negatively charged. Thus it is difficult for them to move closely enough for FRET. When avidin was added to the solution containing PFS and LYCBDP, immediate quenching of photoluminescence from PFS occurred. Fig. 4 shows the PL spectra of the solution containing $8.1 \times 10^{-8}$ M PFS and $2.5 \times 10^{-7}$ M LYCBDP before adding avidin (line a) and the PL spectra after adding $5.6 \times 10^{-9}$ M avidin to the solution (line b) under 370 nm excitation. The initial solution exhibits a strong blue fluorescence at around 420 nm specific to PFS (Line a, Fig. 4). After adding $5.6 \times 10^{-9}$ M avidin to the solution, the overall PL emission is highly quenched, whereas the emission shoulder in the long wavelength due to LYCBDP slightly increased, indicating that the polymer fluorescence quenching mechanism is singlet–singlet energy transfer from PFS to LYCBDP.

Fig. 5(a) shows change of PL emission under 370 nm excitation, when avidin of concentration from $3.8 \times 10^{-10}$ to $7.1 \times 10^{-9}$ M was added into the solution containing $8.1 \times 10^{-8}$ M PFS and $2.5 \times 10^{-7}$ M LYCBDP. PL intensity peaked at around 420 nm responsible for PFS decreased quickly with the increasing content of the avidin. It is expected that positively charged avidin added into negatively charged PFS solution could form a complex with PFS due to the attractive coulomb interactions. At the mean time, avidin has a very high

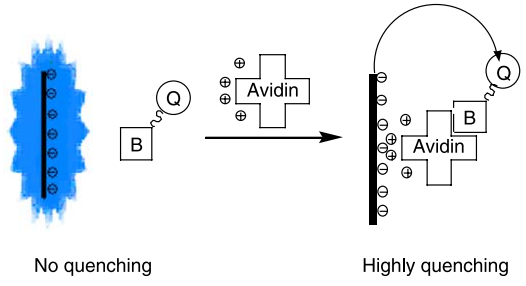


Fig. 2. Schematic of the mechanism of the biosensor.

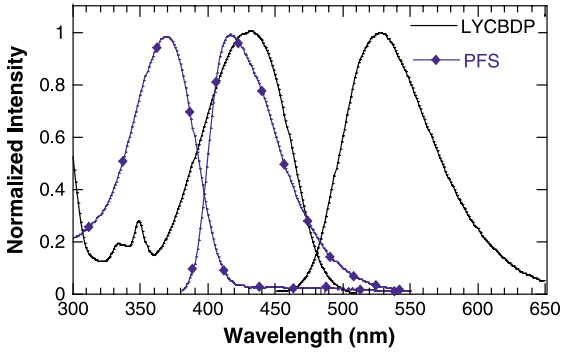


Fig. 3. Absorbance and PL emission spectra of PFS ($10^{-5}$ M) and LYCBDP ($10^{-5}$ M) in water.

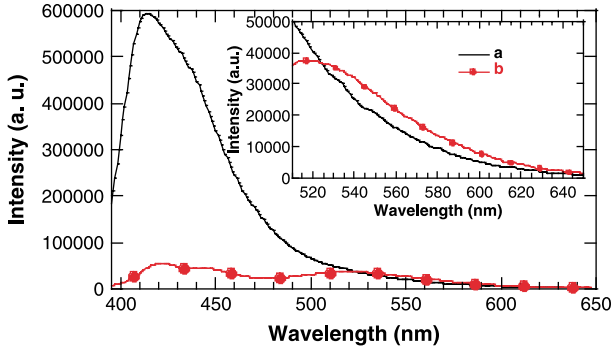


Fig. 4. Fluorescence spectra. (a) $8.1\times10^{-8}$ M PFS and $2.5\times10^{-7}$ M LYCBDP (b) $8.1\times10^{-8}$ M PFS and $2.5\times10^{-7}$ M LYCBDP and $5.6\times10^{-9}$ M avidin. $\lambda_{ex}=370$ nm.

affinity ($K_d\sim10^{-15}$ M) [10] with biotin groups of LYCBDP. Thereby, it will cause the formation of PFS, avidin and LYCBDP complex, which will bring a decrease in the average PFS-LYCBDP distance, allowing for FRET and inducing the highly quenching of PFS. Fig. 5(b) shows the PL spectra of Fig. 5(a) normalized to 420 nm peak. It was found that the ratio of LYCBDP's emission to PFS's emission increases with the continuous addition of avidin while the overall intensity of the PFS emission decreased significantly with increasing avidin content (Fig. 5(a)). This again indicates that quenching mechanism is avidin-mediated singlet–singlet energy transfer from PFS to LYCBDP.

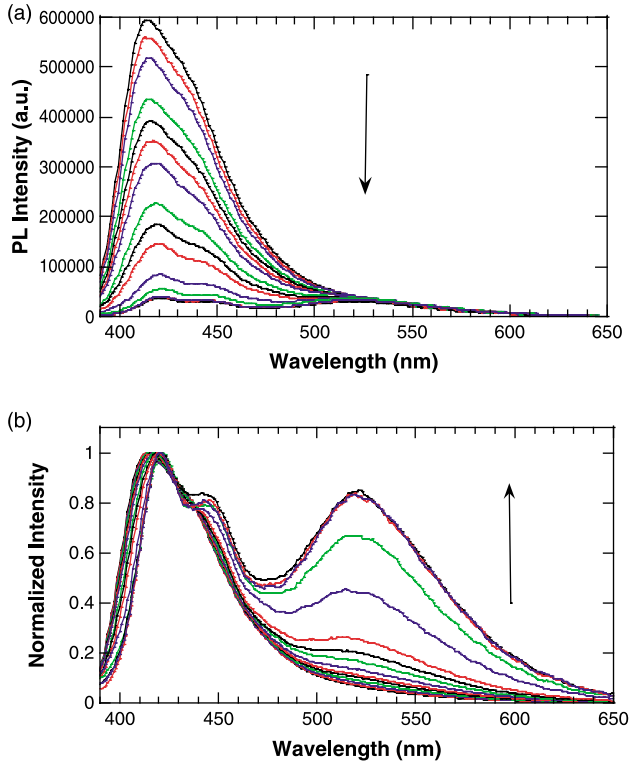


Fig. 5. (a) Fluorescence quenching. To the solution containing $8.1\times10^{-8}$ M PFS and $2.5\times10^{-7}$ M LYCBDP, was added avidin with the concentration from $3.8\times10^{-10}$ to $7.1\times10^{-9}$ M. (b) Normalized fluorescence spectra. $\lambda_{exe}=370$ nm.

Normally the quenching of the PFS's fluorescence follows a conventional 'Stern–Volmer' relationship:

$$\frac{PL_0}{PL} = 1 + K_{sv}[\text{Quencher}] \quad (1)$$

Where $PL_0$ and PL are the quantum efficiencies (or intensities) of fluorescence in the absence and presence of the quencher, and the [Quencher] is the quencher concentration, respectively. The constant $K_{sv}$ provides a direct measure of the quenching sensitivity as well as the sensor's sensitivity [6]. In Fig. 6, we show Stern–Volmer plot of $PL_0$/PL versus [avidin] for data set of Fig. 5. As shown in Fig. 6(a) the $PL_0$/PL increased with the increased content of added avidin. A linear region is observed at low avidin concentrations, followed by an up-sloping nonlinear region. This behavior is very similar as those observed by other groups in different water-soluble conjugated polymer and quencher systems, which can be caused by many reasons, such as the mixed static and dynamic quenching and polymer aggregation [7]. From the linear region of Fig. 6(a) a Stern–Volmer constant $K$ was calculated as $3.2\times10^{8}$ $M^{-1}$, which indicates a great sensitivity of this type of biosensor (Fig. 6(b)). The quantity of added avidin can be determined by the change of PFS' emission in linear region. Indeed, this biosensor has a very high sensitivity on detecting avidin, as low as detectable concentration of $10^{-10}$ M. In addition, this fluorescence quenching occurred instantaneously when avidin was added to the solution, which enables a rapid response biosensor.

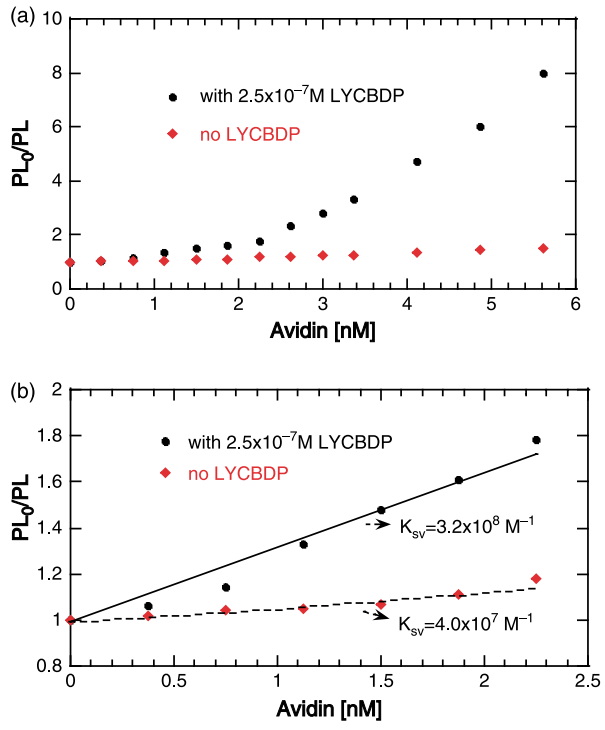


Fig. 6. (a) Stern–Volmer plot obtained by addition of avidin to the solution containing $8.1\times10^{-8}$ M PFS with or without LYCBDP ($2.5\times10^{-7}$ M). The concentration of avidin is from $3.8\times10^{-10}$ to $5.6\times10^{-9}$ M. (b) Zoom-out of the low concentration region of avidin. $\lambda_{ex}=370$ nm.

It should be noticed that avidin is positively charged and would potentially affect the fluorescence quenching of PFS. Avidin has Coulombic interaction with anionic PFS, which could possibly cause the aggregation of the polymers so that induces the nonspecific quenching [9]. In order to investigate the effect of the quenching caused by the aggregation of PFS on the protein surface, Fig. 6(a) also shows the Stern–Volmer plot of $PL_0/PL$ versus [avidin] when avidin of concentration from $3.8\times10^{-10}$ to $7.1\times10^{-9}$ M was added into the solution containing $8.1\times10^{-8}$ M PFS without presence of LYCBDP. It can be found that the quenching caused by the aggregation of PFS on the avidin surface is negligible compared with the quenching in presence of LYCBDP. Stern–Volmer constant (Fig. 6(b)) responsible for the aggregation quenching ($K_{sv}$ is $10^7$) is much lower than that by LYCBDP/avidin complex ($K_{sv}$ is $10^8$).

Fig. 7 shows that when $6.1\times10^{-9}$ M avidin was added to $7.9\times10^{-8}$ M PFS solution, PFS's fluorescence has a significant loss (~37%) and the PL spectra has an obvious red shift (about 10 nm) due to the formation of a complex between them. This complex solution from PFS and avidin can be used as the original materials to detect the LYCBDP. When LYCBDP ($2.4\times10^{-9}$ to $3.5\times10^{-8}$ M) was added to the solution, PFS's emission was further quenched by LYCBDP upon the binding between biotin and avidin. The emission does not change any more when the concentration of LYCBDP is more than $2.5\times10^{-8}$ M, and PFS's emission was almost completely quenched by LYCBDP. Therefore, by using different compositions of the original sensory solution, one can get highly sensitive sensors to detect either avidin or LYCBDP with sensitivity at subnanomolar level. The quenching of PFS happens instantaneously with the addition of avidin or LYCBDP, enabling real time detection.

As stated previously, the sensor's sensitivity was based on the two factors: one is the attractive Coulomb interactions between PFS and avidin, and the other is the high affinity between avidin and LYCBDP. Only when both two factors work, the PFS and LYCBDP will be bounded closely enough for efficient FRET. According to the Debye–Hückel theory,

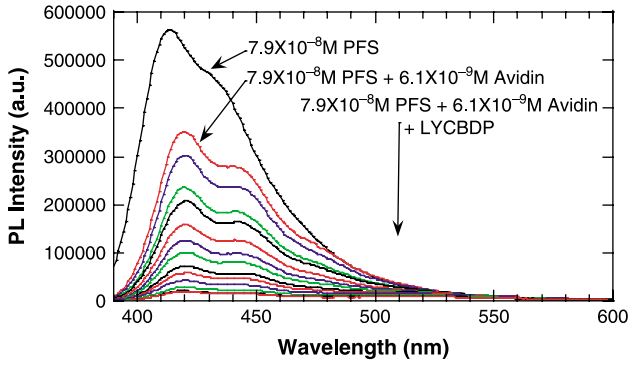


Fig. 7. Fluorescence quenching. To the solution containing $7.9\times10^{-8}$ M PFS and $6.1\times10^{-9}$ M avidin, was added LYCBDP with the concentration from $2.4\times10^{-9}$ to $3.1\times10^{-8}$ M. $\lambda_{ex}=370$ nm.

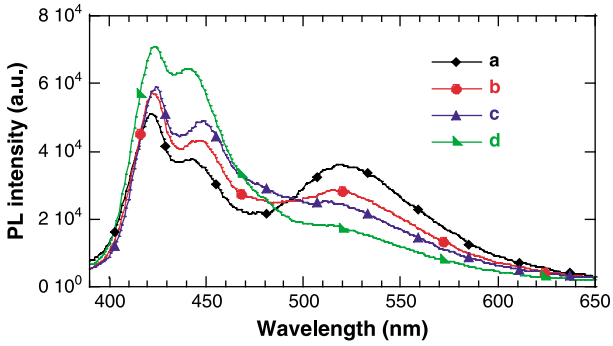


Fig. 8. Influence of [NaCl] on the energy transfer between PFS ($8.1\times10^{-8}$ M) /avidin ($6.1\times10^{-9}$ M) and LYCBDP ($2.5\times10^{-7}$ M). The concentration of NaCl was 0, 0.256, 0.771 and 1.67 M for a, b, c and d, respectively.

with increasing the salt concentration in the solution, the attraction between PFS and avidin decreases due to columbic screening and results in reduction of the energy transfer between LYCBDP and PFS. As shown in Fig. 8, with the increase of the NaCl concentration, the emission of LYCBDP decreased quickly, while the emission of PFS increased, which confirm the importance of electrostatic interactions in determining the success of the biosensor. With the increased concentration of NaCl, the PFS/avidin complexes begin to dissociate because of the electrostatic screening action of the added ions. Thus, the LYCBDP cannot be bounded closely to PFS, and the energy transfer between them will decrease greatly, because the Förster energy transfer largely depends on the distance between the donor and acceptor. However, as can be seen from Fig. 8 the influence of salts only occurs in high concentration (e.g. >25 mM) and has been found to have only a negligible effect on the energy transfer in low salt concentration region.

A useful biosensor requires not only the high sensitivity but also the good specificity. The specific recognition between biotin and avidin and the attractive Coulomb interaction between the fluorescent polymer chains and the quenchers are two crucial factors for the novel conjugated polyelectrolyte based biosensor. To demonstrate this point, control experiments were carried out. It is expected that no efficient quenching will occur when Lucifer yellow cadaverine dipotassium salt (LY) (with no biotin linkage), streptavidin, bovine serum albumin (BSA) or lysozyme (instead of avidin) were used in the system. Fig. 9 compares PL quenching ability ($PL/PL_0$) for different control experiments. When $5.6\times10^{-9}$ M avidin was added to the solution containing $8.1\times10^{-8}$ M PFS and $2.6\times10^{-8}$ M LYCBDP (Fig. 9(O)), avidin–biotin complex formation brings LYCBDP to the close vicinity of PFS chains and blue emission from PFS was greatly quenched by 95% (Fig. 9(A)).Because there is no biotin group on lucifer yellow cadaverine dipotassium salt (LY), there is no avidin–biotin complex formation and consequently no effective quenching. In Fig. 9(B), it was shown that when $5.7\times10^{-9}$ M avidin was added to the solution containing $8.1\times10^{-8}$ M PFS and $2.6\times10^{-8}$ M

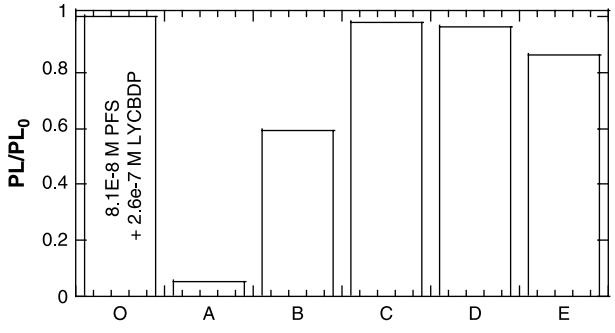


Fig. 9. The specificity of the biosensor. (A) $8.1\times10^{-8}$ M PFS + $2.6\times10^{-8}$ M LYCBDP + $5.6\times10^{-9}$ M avidin. (B) $8.1\times10^{-8}$ M PFS + $2.6\times10^{-8}$ M LY + $5.7\times10^{-9}$ M avidin. (C) $8.1\times10^{-8}$ M PFS + $2.6\times10^{-8}$ M LYCBDP + $5.7\times10^{-9}$ M BSA. (D) $8.1\times10^{-8}$ M PFS + $2.6\times10^{-8}$ M LYCBDP + $5.6\times10^{-9}$ M streptavidin. (E) $8.1\times10^{-8}$ M PFS + $2.6\times10^{-8}$ M LYCBDP + $5.6\times10^{-9}$ M Lysozyme. $\lambda_{ex}=370$ nm.

LY, the photoluminescence from PFS can be quenched but much less effectively (41%). This quenching is mainly due to the attractive coulomb interaction between PFS chains and avidin (Fig. 7) [9]. Bovine serum albumin (BSA) is a negatively charged protein that does not have specific interaction with biotin. As shown in Fig. 9(C), there is almost no photoluminescence quenching due to the facts that BSA does not form complex with biotin groups on LYCBDP and there is no coulomb attraction to bring LYCBDP close to PFS. Streptavidin, similar to avidin, is a biotin-binding protein with a high affinity virtually unmatched in nature ($K_d\sim10^{-15}$ M). Streptavidin has an isoelectric point near to neutrality (5–6, compared to 10 for avidin) and does not have attractive interaction with negatively charged PFS, which results in much less effective photoluminescence quenching of PFS (less than 5%, Fig. 8(D)). Lysozyme is also a positively charged protein. When $5.6\times10^{-9}$ M lysozyme was added to the solution containing $8.1\times10^{-8}$ M PFS and $2.6\times10^{-8}$ M LYCBDP (Fig. 8(E)), there is only a little of quenching (<15%) observed, which is mainly caused by the aggregation of PFS in presence of lysozyme. All these facts indicate the high sensitivity and high specificity/selectivity of the biosensor of this study.

These results provide strong evidence that the highly efficient quenching of PFS occurs not only because of the complex formation between the PFS and avidin due to coulomb interaction but also due to the specific biotin–avidin interactions. The biosensor scheme (as shown in Fig. 2) has the advantages of high sensitivity, rapid-response and excellent selectivity and could be also utilized in the fabrication of biosensors for DNA detection. For example, one can use cationic polyfluorene electrolytes [2d,7e] as sensory materials, which form complexes with negatively charged DNA or oligonucleatide, to detect complementary DNA or PNA that linked with a neutral or positive charged quencher. The quenching can only be observed when the hybridization of DNA/PNA and the DNA with complementary sequence occurs, leading specific biodetection.

## 4. Conclusion

In summary, we synthesized a new conjugated polyelectrolyte—poly(9,9-bis(4′-sufonatobutyl)fluorene-*co-alt*-1,4-phenylene) sodium salt (PFS) and exploited the usage in a novel sensor system. Different from the sensors published so far in scientific literatures, this sensor is based on the highly selective quenching of the sensor material and no unquenching process is needed, indicating a greatly simplification for rapid detection. It was found that the photoluminescence from PFS can be quenched by a biotin-labelled dye—lucifer yellow cadaverine biotin-X dipotassium salt (LYCBDP) and the quenching only occurred in the presence of avidin. Thus, PFS can be used as biosensor for the detection, with the detection sensitivity as low as subnanomolar ($1\times10^{-10}$ M). Control experiments using LY, streptavidin, BSA and lysozyme were performed and the results support strongly the proposed biosensing mechanism. The scheme could be utilized in the fabrication of biosensors for DNA detection and enables a novel, simple, rapid and highly sensitive biodetection technique.

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