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Fluorescent sensor based on a novel conjugated polyfluorene derivative

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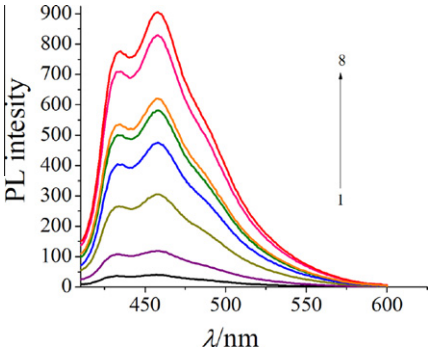
HIGHLIGHTS

- A novel water-soluble polyfluorene derivative (P-2) was synthesized.
- P-2 is more suitable for biosensory systems in aqueous solution.
- Strong fluorescence enhanced sensitivity to oppositely charged quenchers BSA.
- Simplicity of operation, a novel luminescence probe.

GRAPHICAL ABSTRACT

A novel water-soluble polyfluorene derivative (P-2) was synthesized. P-2 showed good fluorescence performance which is beneficial to the application of P-2 for fluorescence sensor, we established a novel fluorescence probe-P-2 as a probe to determine BSA. The method has high sensitivity and simplicity of operation.



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ABSTRACT

A novel water-soluble polyfluorene derivative, poly[(9,9-bis(3′-((*N*,*N*-dimethylamino)*N*-ethylammonium) propyl)-2,7-fluorene)-alt-2,7-(9,9-*p*-divinylbenzene)]dibromide (P-2) was synthesized by the palladium-catalyzed Suzuki coupling reaction and it's quaternized ammonium polyelectrolyte derivatives was obtained through a postpolymerization treatment on the terminal amino groups. The electrochemical and optical properties of the copolymers was fully investigated. The results showed that the new polyfluorene derivative had high electronic conductivity and strong fluorescence, therefore it had good potential to be used in chemical and biological sensors, as shown in optical sensing of bovine albumin (BSA) in this study.

Introduction

There is an increasing demand for chemical and biological sensors in many fields. High sensitivity and easy operation are the two main issues for sensor development. Recently it has attracted much attention to use conjugated polymers (CPs) as optical transducers in highly sensitive biosensors. In comparison to typical fluorescent dyes, CPs contain a large number of repeated absorbing units, and the transfer of excitation energy along the whole backbone of the CPs to an acceptor results in the amplification of fluorescence signals by a collective optical response.

Fluorescence techniques are based on fluorescence resonance energy transfer (FRET) in which an excited-stated donor

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chromophore can transfer energy to a proximal acceptor chromophore (typically <10 nm) through a long-range nonradiative dipole–dipole coupling. It has been extensively and intensively studied as a powerful analytical technique to interrogate changes in molecular conformation, association and the assembly or disassembly of biomolecular machinery [1–5] and therefore provide potential can fulfill requirements of chemical and biosensors sensing. However, implementation of fluorescent probes in functional devices maintaining the sensitivity is still very challenging. Thus, extensive efforts are currently being done in the search of improved materials for fluorescent sensing [6].

Conjugated polymers (CPs) contain a large number of absorbing units, and the transfer of excitation energy along the whole backbone of the CP to the chromophore reporter results in the amplification of fluorescence signals [7–14]. Fluorescent CPs have been employed in different sensing applications, and particularly as sensitive probes for the detection of biomolecules [15–18]. Typically, CPs chemosensors interacted electrostatically with other charged species, and displayed an extraordinarily high sensitivity to fluorescence quenchers [19]. However, most reported CPs exhibited rather poor solubility in aqueous media, probably due to their low charge densities which compete with the aromatic π–π stacking of the hydrophobic backbones. Herein we have synthesized a novel water-soluble fluorescent CP poly[(9,9-bis(3′-((*N*,*N*-dimethylamino)*N*-ethylammonium)propyl)-2,7-fluorene)-alt-2,7-(9,9-*p*-divinylbenzene)]dibromide (P-2). P-2 showed good structure flexile. Because of the flexibility of the molecular chain, P-2 has a good processability. In addition, P-2 (also other conjugated polyelectrolytes) features charged side groups and subsequently good water solubility, which together rendered its two obvious advantages: (1) more suitable for biosensory systems in aqueous solution; (2) further enhanced sensitivity to oppositely charged quenchers. Moreover its strong fluorescence will be beneficial to the application of P-2.

Protein is not only the final executant of life functions but also the key to understand the physiology, pathology and pharmacology in a biological system and is also very important in disease diagnostics. In terms of the determination of proteins, reported methods include polarographic [20], FT-IR spectroscopy [21], quartz crystal microgravimetry sensor [22], fluorimetric determination [23] and flow injection chemiluminescence (FI-CL) [24]. Though some of the methods could be very accurate, they need expensive equipments and complicated sample pretreatment. Some methods have limited practical use due to low selectivity. In this study, P-2 has been successfully applied to the determination of BSA, indicating its potential application in clinical studies.

Experimental procedures

Materials

All manipulations involving air-sensitive reagents were performed under an atmosphere of dry nitrogen. All reagents, unless otherwise specified, were obtained from Alfa Aesar, Sinopharm Chemical Reagent Co. Ltd, and Tian Jin Da Mao Chemical Reagent Factory (Tian Jin, China) and used as received. All the solvents used were further purified before use.

Instrument

Nuclear magnetic resonance (NMR) spectra were recorded on a INOVA 300 MHz spectrometer with tetramethylsilane as the internal standard. The elemental analysis was performed on a PERKIN ELMER 2400 II elemental analyzer. UV–vis absorption and photoluminescence (PL) emission spectra were measured using a Shimadzu TU-1901 spectrophotometer and a Shimadzu RF-5301PC spectrophotometer, respectively. Electrical conductivity was determined by dual display potentiostat. The lifetime of P-2 was obtained with FLS920 combined fluorescence lifetime and steady state spectrometer. Thermal gravimetric analysis (TGA) was performed with Simultaneous Thermal Analysis-STA 409EP under N_2 atmosphere. All optical measurements were performed at room temperature unless otherwise stated.

Synthesis

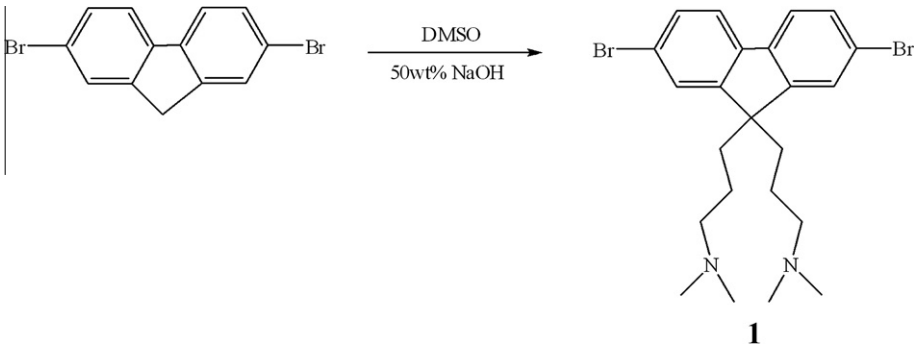
Monomer synthesis

Monomers preparation of 2,7-dibromo-9,9-bis(3′-(N,N-dimethylamino)propyl)-fluorene (1) (Scheme 1). To a stirred mixture of 2,7-dibromofluorene (4 g, 12 mmol) and 60 mL of dimethyl sulfoxide (DMSO) under nitrogen were added tetrabutyl ammonium bromide (80 mg) and 8 mL of a 50 wt.% aqueous solution of sodium hydroxide. 20 mL DMSO solution of 3-dimethylaminopropylchloride hydrochloride (5 g, 32 mmol) was added dropwise to the mixture. The reaction mixture was stirred at room temperature for 6 h and then diluted with 50 mL of water, to dissolve all salts. The product was extracted with ether (3 × 100 mL) and the combined organic layer was washed with 10% NaOH (aq) (2 × 100 mL), water (3 × 100 mL) and brine (1 × 100 mL). The solution was dried over $MgSO_4$, filtered, and stripped of solvent by vacuum evaporation to yield a crude solid. The crude solid was recrystallized from MeOH/H_2O to afford 1 [25]. (2.93 g, 48.2% yield) as white crystals. IR (cm^{-1}): 653 (C–Br), 1104 (C–N), 1607 (fluorene ring C=C), 2820 (methyl C–H), 3100 (fluorene ring C–H). ^1H NMR (300 MHz, DMSO-d^6): ä 7.82–7.80 (d, 2H, fluorene ring), 7.69 (s, 2H, fluorene ring), 7.56–7.53 (d, 2H, fluorene ring), 2.04–2.00 (t, 4H, $-CH_2N$), 1.92–1.88 (m, 16H, $-NCH_3$, $-CH_2-$), 0.60–0.52 (m, 4H, $-CH_2-$). Elemental Anal. Calcd. for $C_{23}H_{30}Br_2N_2$: C, 55.89; H, 6.12; N, 5.67. Found: C, 55.76; H, 6.12; N, 5.60.

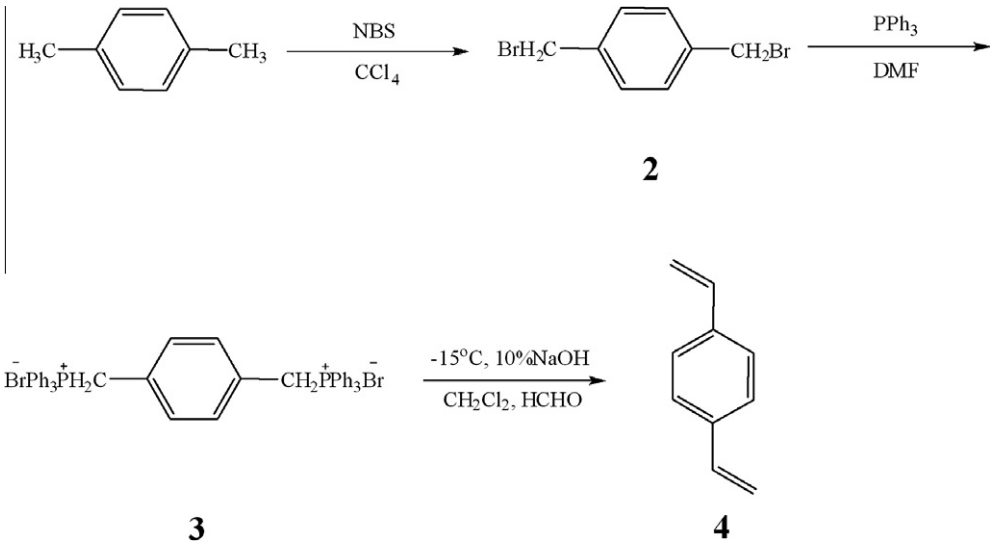
Monomers preparation of p-divinylbenzene (4) (Scheme 2). To the solution of 2.0 mL (21.82 mmol) 1,4-dimethylbenzene in CCl_4 (60 mL), 5.8 g (26 mmol) of NBS and 0.10 g (1.03 mmol) of benzoyl mixture peroxide were added, then stirred at 80 °C for 6 h. A crude product was obtained after heat filter and washed with absolute ethyl alcohol. Compound 1 was obtained as a white acicular crystalline solid (4.94 g, 85.8% yield) from the crude product by recrystallization with absolute ethyl alcohol [26].

A mixture of 2 (4.94 g, 18.5 mmol) and triphenylphosphine (9.0 g, 34.32 mmol) in 60 mL DMF was refluxed at 160 °C for 10 h in a nitrogen atmosphere. The precipitate was filtered and washed with ether [27], and then transferred into a 100 mL three-necked flask for compound 4 synthesis.

A mixture of CH_2Cl_2 (30 mL) and 40% HCHO aqueous solution (12 mL) was added into the previous flask. The solution was cooled down to −15 °C and stirred vigorously. Ten percent of aqueous NaOH (20 mL) was added dropwise over 1 h with constant stirring under N_2. The mixture was stirred at room temperature overnight, and then 100 mL water was added to the solution. The solution was extracted three times with CH_2Cl_2 (20 mL). The combined organic layers were washed with saturated brine twice and dried over anhydrous $MgSO_4$. The solvent was removed to dryness under reduced pressure [28]. The pure product was obtained as white acicular crystalline solid in 58.9% yield (1.42 g) by the recrystallization of 50% ethanol twice. IR (cm^{-1}): 833 (benzene 1,4 substituted), 1607 (aromatic ring C=C), 1620 (alkene C=C), 3009 (alkene C–H) ^1H NMR (300 MHz, $CDCl_3$): d 5.43 (d, 2H, J = 10.9 Hz), 5.92 (d, 2H, J = 17.6 Hz), 6.80 (dd, 2H, J = 17.6, 10.9 Hz), 7.59 (d, 4H, J = 8.3 Hz), 8.12 (d, 4H, J = 8.3 Hz); Elemental Anal. Calcd. for C_8H_{10}: C, 90.57; H, 9.43. Found: C, 90.42; H, 9.52.



Scheme 1. Reaction pathway to 2,7-dibromo-9,9-bis(3′-(*N*,*N*-dimethylamino)propyl)-fluorene (1).



Scheme 2. Reaction pathway to *p*-divinylbenzene (4).

Preparation of poly[(9,9-bis(3′-(N,N-dimethylamino)propyl)-2,7-fluorene)-alt-2,7-(9,9-p-divinylbenzene)] (P-1) (Scheme 3). Compound 1 (2.93 g, 5.78 mmol), Compound 4 (0.29 g, 2.23 mmol), triphenyl phosphine (PPh_3) (0.3 g) and palladium acetate ($Pd(OAc)_2$), (25 mg) were dissolved in a mixture of 60 mL of DMF and 80 mL of triethylamine. The mixture was refluxed with vigorous stirring at 90 °C for 36 h under nitrogen. After the mixture was cooled to room temperature, it was poured into 200 mL methanol. The precipitated material was recovered by filtration through a funnel [25]. The resulting solid material was washed for 24 h using acetone to remove oligomers and catalyst residues (0.46 g, 46.3% yield). IR (cm^{-1}): 1660 (C═C), 1326 (C–N), 968 (alkene C–H), 3025 (phenyl ring C–H); ^1H NMR (δ, ppm): 7.67–7.64 (6H, fluorene ring), 7.48–7.43 (4H, phenyl ring), 7.27 (2H alkene), 2.21 (6H, methyl). Elemental Anal. Calcd. for $C_{28}H_{29}N_2$: C, 85.53; H, 8.24; N, 6.23. Found: C, 83.85; H, 8.42; N, 6.14.

Preparation of [(9,9-bis(3′-((N,N-dimethylamino)N-ethylammonium) propyl)-2,7-fluorene)-alt-2,7-(9,9-p-divinylbenzene)]dibromide (P-2) (Scheme 4). Solution of 2.92 g P-1 in 40 mL of *N*,*N*-dimethylformamide (DMF) with 10 mL of bromoethane was stirred for 5 days at 50 °C temperature. The resulting precipitate was collected on a frit at reduced pressure and dried in an air stream and then in vacuo at 50 °C for 5 days [28].

IR (cm^{-1}): 1326 (C–N), 3085 (C–H phenyl ring), 1086 (C–H alkene), 1668 (C═C). ^1H NMR (δ, ppm): 7.78–7.64 (6H, fluorene ring), 7.44–7.38 (4H, phenyl ring), 7.27 (2H alkene), 2.27 (6H, N–CH_3), 1.25 (6H, –N–C–CH_3). Elemental Anal. Calcd. for P-2: C, 85.16; H, 9.32; N, 5.52. Found: C, 83.55; H, 9.53; N, 5.43.

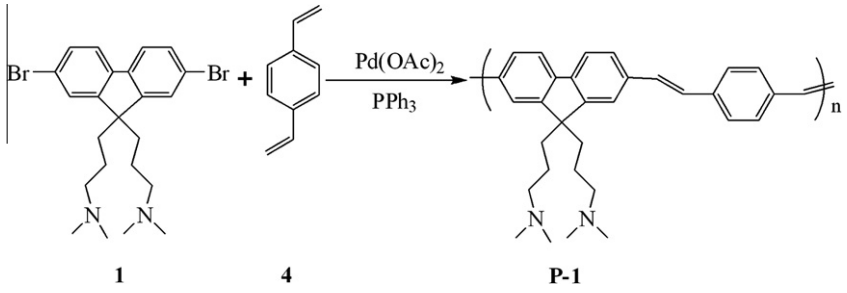
Results and discussion

Thermal stability

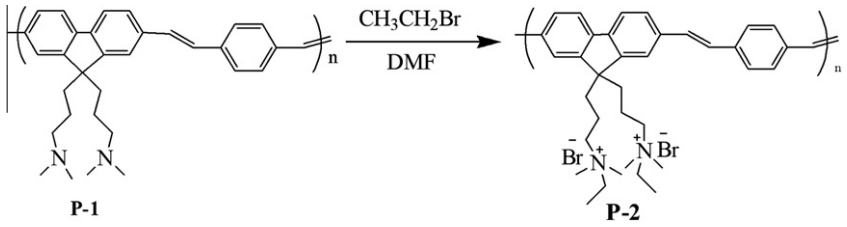
The thermal stability of the polymers was studied by thermogravimetric analysis (TGA) under an inert (argon) atmosphere. The thermograms (Fig. 1) showed that the polymer P-2 exhibit good thermal stability with a weight loss of 3.6% at 180 °C. This 3.6% weight loss is likely due to residual moisture or molecular impurities encapsulated within the polymers from the reaction mixture. P-2 had a degradation onset at 238 °C with the initial weight loss derived from associated water. P-2 showed a weight loss of 71.1% as the temperature reached 508 °C. The enhanced water solubility may have compromised the polymer's thermal stability. In either case, the relatively high thermostability renders the polymer good candidates for fabricating light-emitting diodes and fluorescence-based sensors using spin casting or polyelectrolyte self-assembly methods.

Electrical properties of polymer P-2

To investigate the electrical conductivity of P-2, we used the four-point probe method to measure the current and voltage of P-2 fixed on the surface of glass by spin coating method. The



Scheme 3. Reaction pathway to P-1.



Scheme 4. Reaction pathway to P-2.

results are shown in Fig. 2. According to the theory formula below we obtained the electrical conductivity of P-2: $\sigma = 1/\rho = 1/(5.4448 \times 10^{-4})$ S cm^{-1} = 1836.6 S cm^{-1}. From the viewpoint of conductivity, P-2 has the potential to be used as materials for electronic conductivity.

$$\rho = [4.5324\chi_j(U/I)] \times \left[F_4\left(\frac{a}{d}, \frac{d}{s}\right)\right] \Big/ F_3(\chi_j/s)$$

where U is voltage, I is current, a (40 mm) is the long edge of the film, b (10 mm) is the short edge; s is probespacing, χ_j is made 10^{-4} mm which is less than 0.5 times of the probespacing, so F_3 is negligible; F_4 (4, 10) is 1.0700.

The lifetime of polymer P-2

Fluorescence lifetime is an important parameter for luminescent materials. As shown in Fig. 3, when 388 nm was selected as the excitation wavelength, a fluorescent attenuation curve 1 was obtained at 457 nm. Fluorescence life of P-2 is 1.0356 ns and fitting degree is up to 1.108 when fluorescence attenuation curve were fitted by single exponential 2. These results indicated that P-2 was a polymer of short fluorescence lifetime.

Optical properties of polymer P-2

The synthesized P-2 was generally brown solids and the polymerization solution was orange-brown. P-2 showed intrinsic water-solubility. The optical properties of the polymer P-2 was shown in Fig. 4. The emission spectra varied remarkably with the content of P-2 in water, indicating a good dispersion of P-2 in this concentration range.

Sensor based on fluorescence energy transfer (FET)

Sensor BSA based on fluorescence energy transfer (FET) are described. The resulting fluorescence spectra showed that fluorescence intensity was reduced by addition of BSA. The nonspecific bindings between P-2 and proteins were investigated. Subsequently a FET-based sensor BSA for a specific BSA was designed. FET property was investigated before and after forming a complex with the BSA as analyte. The resulting fluorescence spectra clearly changed by addition of the analyte BSA. This is probably due to nonspecific bindings and quenching by addition of the analyte BSA. The FET behavior strongly depended on sequence of the analyte BSA as well as analyte concentration.

In the emission spectrum, a characteristic fluorescence peak of P-2 was observed at 433 and 458 nm; however, it was quenched

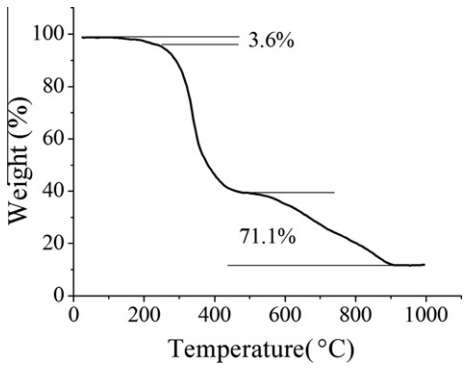


Fig. 1. Analysis of the thermal decomposition of P-2.

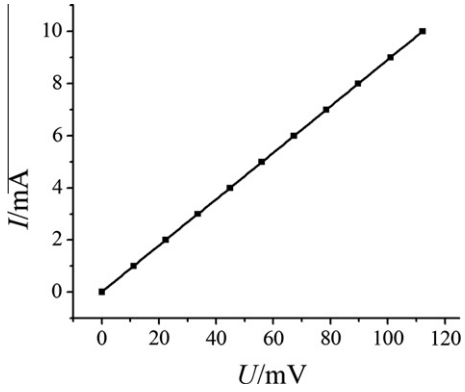


Fig. 2. The conductivity of P-2.

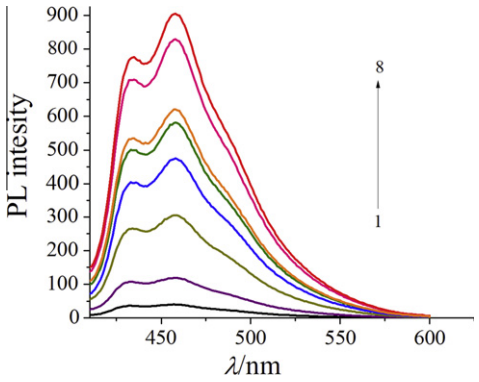


Fig. 3. The fluorescence lifetime of P-2: (1) fluorescent attenuation curve; (2) fitting curve.

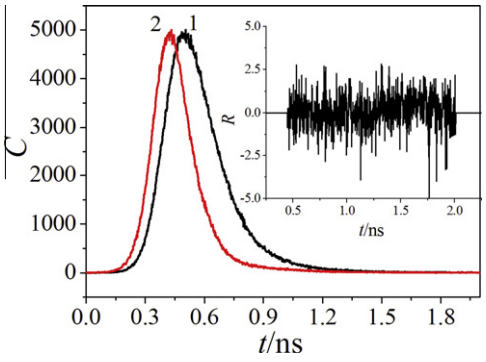


Fig. 4. Photoluminescence spectra of P-2, the concentration of 1–8 were, respectively 4.0×10^{-6}, 1.0×10^{-5}, 2.0×10^{-5}, 2.5×10^{-5}, 3.0×10^{-5}, 3.5×10^{-5}, 4.0×10^{-5}, 5.0×10^{-5} g mL^{-1}.

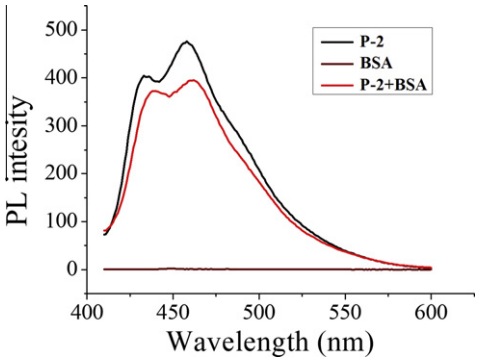


Fig. 5. The fluorescence intensity of P-2, BSA and P-2 + BSA, respectively.

when BSA was added. Therefore, a wavelength of 458 nm was selected as the emission wavelength for the determination of BSA. The changes of emission spectra of the P-2 system in the absence and presence of BSA were shown in Fig. 5. The emission peak at 458 nm corresponded to the concentration of the BSA. It indicated that the interaction between the P-2 and BSA was generated in the P-2–BSA system, which permitted the use of P-2 for determination of BSA. This observation suggested its potential application in clinical studies.

Influence factors on the system

Effect of pH and the concentration of P-2 and BSA: As BSA is amphoteric, the fluorescence intensity of the P-2 system with BSA is strongly dependent upon the pH value. The results indicated

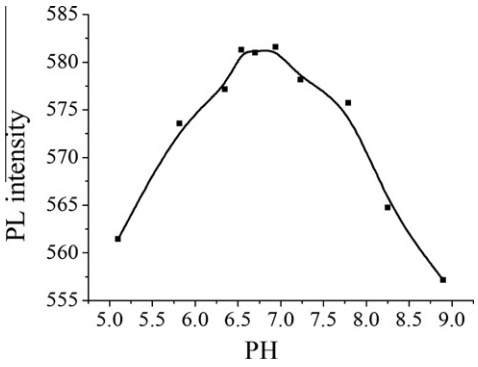


Fig. 6. Effect of pH to the P-2–BSA system.

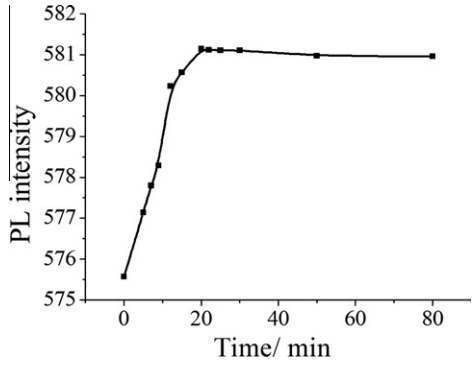


Fig. 7. Stability time of the P-2–BSA system (C_{BSA}, 1.0×10^{-5} g mL^{-1}; P-2, 5.0×10^{-6} g L^{-1}; pH, 6.84).

that the maximum fluorescence intensity of the system was stable in the pH range of 6.54–6.91 (Fig. 6). Therefore, we selected pH 6.84 for further investigation. The effect of the buffer solutions, Na_2HPO_4–sodium citrate, citric acid–sodium citrate, NaAc–HAc, KH_2PO_4–NaOH and Na_2HPO_4–KH_2PO_4 on the fluorescence intensity was examined and 0.2 mol L^{-1} NaAc–HAc buffer offered the highest sensitivity. Based on the result of the effect of the P-2 complex concentration and time on the fluorescence intensity of the P-2–BSA system, the optimal conditions were selected as follows: the P-2 complex, 5.0×10^{-6} mol L^{-1}; NaAc–Hac, 0.2 mol L^{-1}.

Fluorescence stability

As shown in Fig 7, the fluorescence intensity of the P-2–BSA system reached its maximum in 20 min after all of the reagents had been added and remained stable for at least 2 h at room temperature (Fig. 7).

Determination of BSA

Calibration curve and detection limit. Under optimal conditions the quenched fluorescence intensity of the system (ΔI) showed an excellent linear relationship with the concentration of BSA, The linear equation was $\Delta I = 37.07 + 75.14C_{BSA}$ (10^{-5} g mL^{-1}), with a correlation coefficient of 0.973. The detection limit (3σ) was 2.0×10^{-8} g mL^{-1} (Fig. 8). The quenched relative fluorescence has good reproducibility with standard deviation usually less than 5%. Possible reasons may explain this excellent performance of P-2–BSA FRET sensor. The quenching of fluorescence is highly dependent on the energy transfer between P-2 and BSA, which is critical for P-2–BSA FRET sensor. The high fluorescence quenching efficiency leads to the low background of signals and results in the high sensitivity.

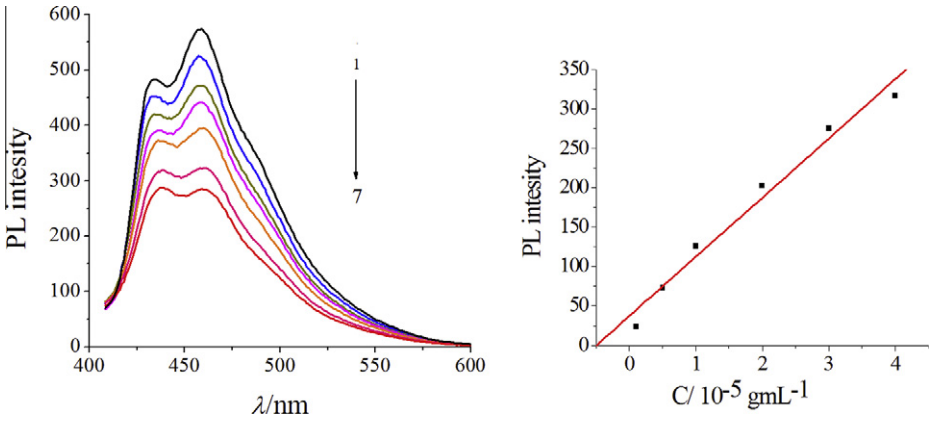


Fig. 8. Calibration line towards BSA.

To assess the applicability of the proposed method in samples, the effect of the potential interferents, such as metal ions, amino acids, sugars, pyrimidines, purines and surfactants, was examined. The tolerance levels of various interferents were summarized in Table 1. It was shown that most species had little effect on the fluorescence intensity, except for Fe^{3+}, Al^{3+}, L-methionine and L-cysteine, which generated a significant effect at concentrations over 2.0×10^{-5} mol L^{-1}. These three substances all interfered the interaction of P-2 and BSA.

Conclusions

A novel water-soluble polyfluorene derivative P-2 was synthesized by the palladium-catalyzed Suzuki coupling reaction and it's quaternized ammonium polyelectrolyte derivatives was obtained through a postpolymerization treatment on the terminal amino groups. The electrochemical and optical properties of the resulting copolymers suggested that P-2 has excellent electro-optical properties. Due to its fluorescence property, P-2 can also be applied in biological sensors. In our study P-2 was successfully applied to the determination of BSA due to fluorescence sensing, because of the "molecular wire effect", which causes a polymer to be quenched by a considerably lower analyte concentration. This effect is highly important and significant in clinical inspection, hence indicated the potential clinical application of P-2.

Table 1
Effects of coexistence interferents (C_{BSA}, 1.0×10^{-5} g mL^{-1}; P-2, 5.0×10^{-6} g L^{-1}; pH, 6.84).

Substance	Concentration (mol L^{-1})	Change of luminescence intensity (%)
Co^{2+}	2.0×10^{-4}	6.25
Cu^{2+}	2.0×10^{-4}	7.5
Fe^{3+}	2.0×10^{-5}	10
Zn^{2+}	2.0×10^{-4}	6.3
Al^{3+}	2.0×10^{-5}	9.6
L-Histidine	1.6×10^{-3}	7.9
L-Leucine	3.2×10^{-3}	10.7
L-Tryptophan	1.6×10^{-3}	8.07
L-Methionine	2.0×10^{-5}	8.5
Lysine	4.0×10^{-4}	4.8
L(+)-Arginine	6.0×10^{-3}	3.49
L-Phenylalanine	1.6×10^{-3}	13
L-Cysteine	2.0×10^{-5}	12.4
Vitamin C	1.6×10^{-3}	4.9
Vitamin B_{12}	1.6×10^{-3}	5.6

Acknowledgments

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