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Copolythiophene-Derived Colorimetric and Fluorometric Sensor for Lysophosphatidic Acid Based on Multipoint Interactions

Minhuan Lan,[§,†] Weimin Liu,*[,†] Ying Wang,[†] Jiechao Ge,[†] Jiasheng Wu,[†] Hongyan Zhang,[†] Jianhong Chen,[§,†] Wenjun Zhang,[‡] and Pengfei Wang*[,†]

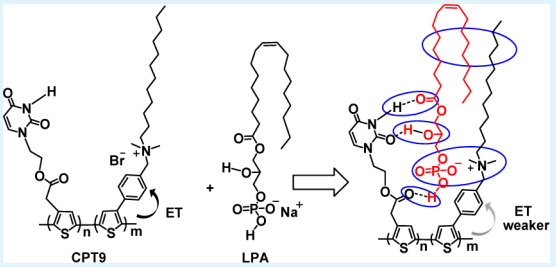
[†]Key Laboratory of Photochemical Conversion and Optoelectronic Materials, Technical Institute of Physics and Chemistry, Chinese Academy of Sciences, Beijing, 100190, People's Republic of China

[‡]Center of Super-Diamond and Advanced Films (COSDAF) and Department of Physics and Materials Science, City University of Hong Kong, Hong Kong SAR (P.R. China)

[§]Graduate School of the Chinese Academy of Sciences, Beijing, 100049, China

Supporting Information

ABSTRACT: 3-Phenylthiophene-based water-soluble copolythiophenes (**CPT9**) were designed for colorimetric and fluorometric detection of lysophosphatidic acid (LPA) based on electrostatic interaction, hydrophobic interaction, and hydrogen bonding. Other negatively charged species gave nearly no interference, and the detection limit reached to 0.6 μM, which is below the requisite detection limits for LPA in human plasma samples. The appealing performance of **CPT9** was demonstrated to originate from the multipoint interaction-induced conformational change of conjugated backbone and weakened electron transfer effect. To our best knowledge, this is the first polythiophene based optical sensor which displays emission peak red-shift followed with fluorescence enhancement.



KEYWORDS: *conjugated polythiophene, biosensors, lysophosphatidic acid, ratiometric fluorescent, colorimetric, electron transfer*

1. INTRODUCTION

Lysophosphatidic acid (LPA), as a membrane component and metabolic intermediate, plays a key role in biochemical, physiological, and pathological processes, such as stimulating the proliferation of cancer cells, smoothing muscle contraction, neurotransmitter release, and promoting aggregation of platelets.[1−4] The LPA physiological concentrations in plasma are approximately 0.1−6.3 μM. Elevated levels of plasma LPA are associated with gynecologic cancers, especially ovarian cancer.[5] Therefore, plasma LPA levels may represent a potential biomarker for the early detection of malignant cancer.[6,7] To date, although significant efforts have been put into the development of high sensitivity and selectivity, low cost methods to quantify LPA, including chromatography, capillary electrophoresis, and immunochemical methods,[8−12] the requirement of expensive instrumentation as well as time-consuming and labor-intensive procedures hampered their practical application. Fluorescence probes have attracted considerable attention because of their convenient and real-time analysis; however, limited successful examples for LPA detection have been developed.[13−15] Given the increasing interest in early cancer diagnosis, developing a simple and efficient analytical method to rapidly quantify LPA is desirable.

A power optical sensing platform based on water-soluble conjugated polythiophene offers a unique combination of high sensitivity and visual detection. This platform shows good sensitivity because of the amplification by a collective system response, thereby offering a great superiority over the small molecule-based sensors.[16−20] In recent years, different kinds of polythiophene derivates were used to sense important biological species (such as nucleic acids, proteins, and heparin),[21−24] and small molecules.[25−27] However, given the simplex electrostatic interaction, they often display low selectivity to analytes[28,29] and show single signal response to binding events. As a result, sensing may be affected by some uncertain factors, such as instrumental efficiency, environment condition, and molecular concentration.[30,31]

Along with our continuing efforts in the exploration of fluorescent chemosensors for the selective detection of significant biological species based on multipoint interactions,[15,32−37] herein we report a conjugated copolythiophene sensor for the detection of LPA with good sensitivity and selectivity based on electrostatic interaction, hydrophobic interaction, and hydrogen bonding. In our previous studies, we discovered that 3-phenylthiophene can regulate the conformation of the polythiophene chain and result in signal amplification followed by a significant red shift in emission wavelength after binding the target.[38,39] To our knowledge, this study was the first to observe that polythiophene, as an optical sensing platform, displays a red shift in the emission peak,

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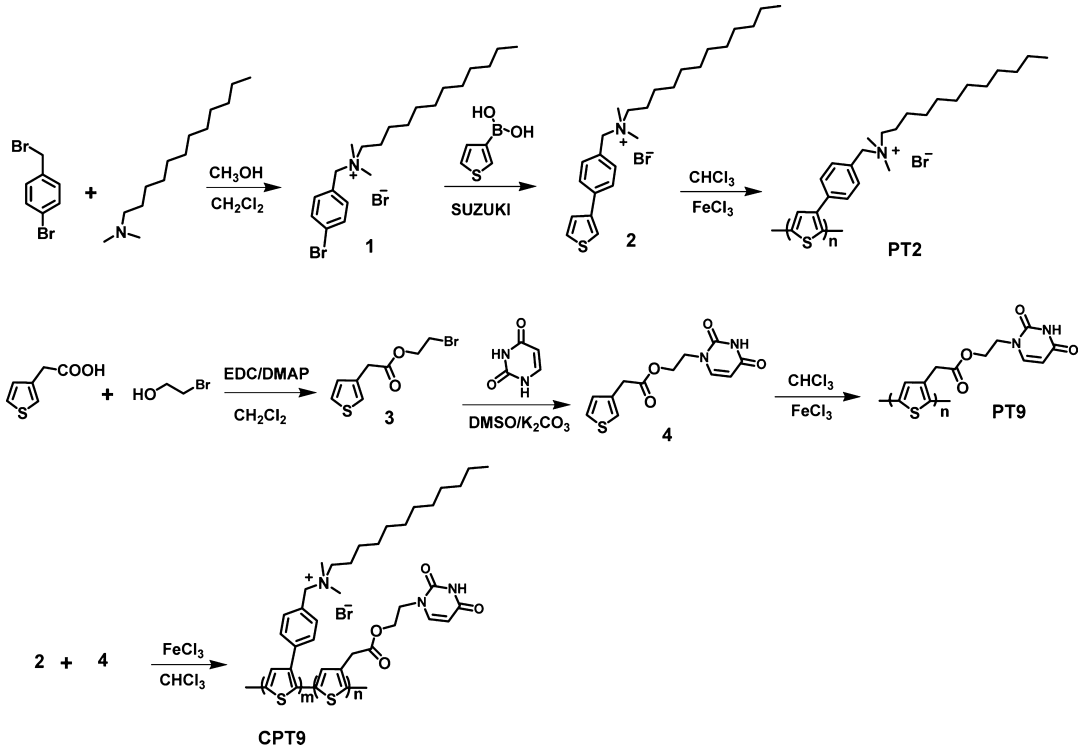
Scheme 1. Synthetic Routes of CPT9, PT2, and PT9



followed by fluorescence enhancement. These results prompted us to investigate a new design strategy utilizing the properties of 3-phenylthiophene by introducing a suitable recognition group to the backbone.

2. EXPERIMENTAL SECTION

2.1. Materials and Measurements. 4-Bromobenzyl bromide, *N*,*N*-dimethyldodecylamine, thiophene-3-boronic acid, tetrakis-(triphenylphosphine)palladium(0), 3-thiopheneacetic acid, 2-bromorthanol 4-(dimethylamino)pyridine, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride, iron(III) chloride, anhydrous, and uracil were purchased form Alfa Aesar. ctDNA, RNA, and heparin were purchased from Sigma. 1-Oleyoyl lysophosphatidic acid (sodium salt) (LPA) was purchased from Cayman Company. Other reagents were purchased from Beijing Chemical Regent Co. All reagents and chemicals were analytical reagent (AR) grade and used without further purification unless otherwise noted. CH_2Cl_2 and $CHCl_3$ were distilled from CaH_2 under nitrogen.

All UV−vis and fluorescence spectra in this work were recorded in Hitachi U3010 and Hitachi F-4600 fluorescence spectrometers. The water was purified by Millipore filtration system. ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were collected on a Bruker Advance-400 spectrometer with tetramethylsilane as an internal standard. Electron impact (EI) mass spectroscopy was carried out on a Waters GCT Premier mass spectrometer. Matrix-assisted laser desorption ionization-time-of-flight (MALDI-TOF) mass spectra were obtained on a Bruker Microflex mass spectrometer, and electrospray ionization (ESI) mass spectra on a Shimadzu LC-MS 2010 instrument. The gel-permeation chromatography was performed using polystyrene as the standard, and THF was employed as the eluent. Zeta potentials were recorded on Zetasize 3000 HS (Malvern, UK).

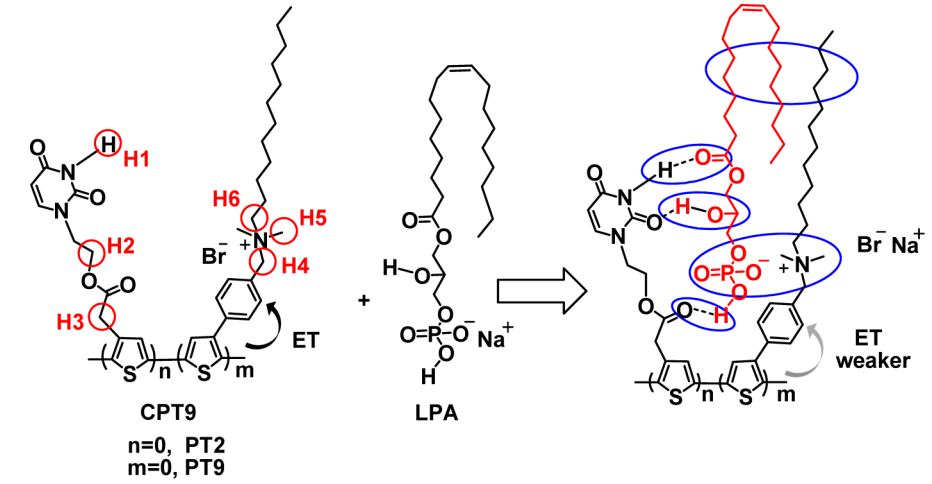
2.2. Syntheses of Sensor Probe and Model Polymers. The synthetic routes of **CPT9**, **PT2**, and **PT9** were outlined in Scheme 1, and the details were described below. **PT2** was synthetic in our preview report.[38]

*2.2.1. 2-Bromoethyl 3-Thiopheneacetate (Compound **3**).* 2-Bromorthanol (0.4 g, 3.2 mmol) was added slowly with syringe to a mixture of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (0.5 g, 2.6 mmol), 4-(dimethylamino)pyridine (70 mg, 0.6 mmol), and 3-thiopheneacetic acid (0.284 g 2 mmol) in 40 mL of dry CH_2Cl_2 under nitrogen. The mixture was stirred at room temperature for 12 h to complete the reaction. The solution was washed with H_2O (3 × 20 mL), and the organic layer was dried with anhydrous $MgSO_4$. After removal of the solvent under reduced pressure, the residue was purified by column chromatography (eluent: petroleum ether/ethyl acetate = 5:1) to give compound **3** (0.34 g, yield 68%) as a colorless liquid. ^{1}H NMR (400 MHz, $CDCl_3$, TMS, ppm): 3.50−3.53 (t, J = 12 Hz, 2H), 3.71 (s, 2H), 4.41−4.44 (t, J = 12 Hz, 2H), 7.05−7.07 (d, J = 5 Hz, 1H), 7.18 (s, 1H), 7.29−7.31 (m, 1H). ^{13}C NMR (100 MHz, $CDCl_3$, TMS, ppm): 28.6, 35.8, 64.3, 123.2, 126.0, 128.6, 133.2, 170.7. EI mass spectrum m/z: calculated 249.95 (100%), 247.95 (97.8%); found 249.95, 247.95.

*2.2.2. (3-Uracil-ethyl)-3-thiopheneacetate (Compound **4**).* A solution of 0.25 g (1 mmol) of compound **3**, 0.2 g (1.8 mmol) of uracil, and 0.5 g (3.6 mmol) of K_2CO_3 in 8 mL of DMSO was reacted at 70 °C for 10 h and then cooled to room temperature. The resulting solution was poured into 30 mL of H_2O. The residue was extracted with CH_2Cl_2 (3 × 20 mL), and the resulting organic layer was collected and dried with anhydrous $MgSO_4$. After removal of the solvent under reduced pressure, the residue was purified by column chromatography (eluent: CH_2Cl_2/CH_3OH = 30:1) to give compound **4** (0.12 g, yield 42%) as a white powder. ^{1}H NMR (400 MHz, $CDCl_3$, TMS, ppm): 3.67 (s, 2H), 3.91−3.94 (t, J = 9.8 Hz, 2H), 4.34−4.36 (t, J = 9.8 Hz, 2H), 5.53−5.55 (d, J = 8 Hz, 1H), 6.75−6.77 (d, J = 8 Hz, 1H), 7.00−7.01 (d, J = 5 Hz, 1H), 7.14 (s, 1H), 7.31−7.33 (m, 1H), 8.79 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$, TMS, ppm): 35.9, 47.8, 62.4, 102.2, 123.4, 126.3, 128.5, 133.1, 144.7, 150.9, 163.8, 170.6. EI mass spectrum m/z: calculated 280.05; found 280.05.

2.2.3. General Syntheses of Polythiophenes. All polymers in this paper were prepared via an oxidative polymerization under nitrogen in the presence of iron(III) chloride, anhydrous. General method for preparation of polythiophenes was carried out as follows: 4 equiv of $FeCl_3$ was dissolved in 30 mL of dry $CHCl_3$ under nitrogen, and then 1 equiv of corresponding monomers dissolved in 20 mL of $CHCl_3$ was added dropwise. The reaction mixture was stirred at room temperature for 2 days. The resulting precipitate was collected, washed with methanol, and finally dried under vacuum to give the desired polymers

Scheme 2. Schematic of the Proposed Interaction Mechanism of CPT9 with LPA Utilizing the Electrostatic Interaction, Hydrophobic Interaction, and Multiple Hydrogen Bonds



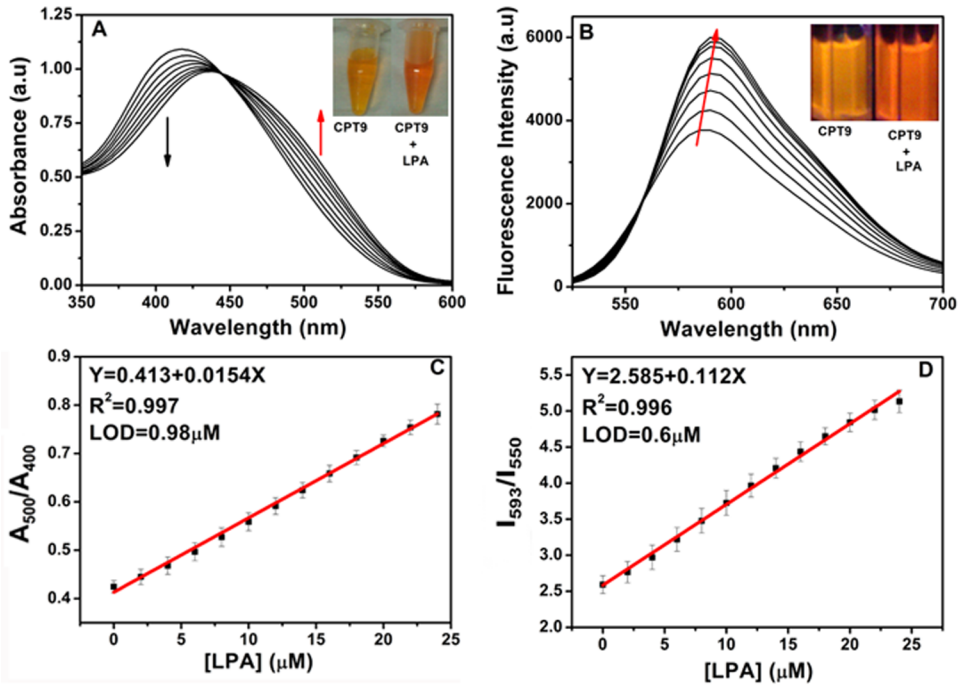


Figure 1. (A) Absorbance and (B) fluorescence titration spectra of **CPT9** (100 μM) in 10 mM of HEPES buffer solution (1% CH_3CN, pH = 7.4) upon gradual addition of LPA from 0 to 25 μM (λ_{ex} = 500 nm). The inset shows the solution color change and the fluorescent color change of the resulting solution. (C) Absorbance intensity ratio (A_{500}/A_{400}) and (D) fluorescence intensity ratio (I_{593}/I_{550}) of **CPT9** vs the concentrations of LPA (0–25 μM), respectively.

as a solid. For the copolymerization, 5 equiv compound **2** and 1 equiv compound **4** were used to give the corresponding copolythiophene (**CPT9**). The polymerization of compound **2** and compound **4** gives the corresponding homopolythiophenes **PT2** and **PT9**, respectively.

CPT9 *(Yield: 35%).* Gel-permeation chromatography analysis (GPC): Mn = 1.28 ×10^5, polydispersity index (PDI) = 1.39. ^{1}H NMR (400 Mz, DMSO, TMS, ppm) 0.78 (br), 1.17 (br), 1.75 (br), 3.04 (br), 3.88 (br), 4.22 (br), 4.73 (br), 5.73 (br), 6.72 (br), 6.97 (br), 7.47–7.68 (br), 9.36 (br), 11.26 (br).

PT2 *(Yield: 50.0%).* GPC: Mn = 6.655 × 10^4 (PDI = 1.161). ^{1}H NMR (400 Mz, CD_3CN–D_2O (v/v = 1/1), TMS, ppm) 0.69 (br), 1.06 (s,br), 1.60 (s, br), 2.85 (s, br), 2.99 (s, br), 4.46 (s, br), 6.81 (s, br), 7.35–7.37 (dbr), 7.49–7.50 (dbr).

PT9 *(Yield: 60%).* GPC: Mn = 7.12 × 10^4 (PDI = 2.03). ^{1}H NMR (400 Mz, DMSO, TMS, ppm) 3.60–3.66 (br), 3.91 (br), 4.26 (br), 5.41 (br), 7.30–7.46 (br), 11.23 (br).

3. RESULTS AND DISCUSSION

3.1. Design of Copolythiophenes for LPA Detection.

The molecular structure of LPA coexists with hydrogen bond donor (−OH) and hydrogen bond acceptor (C═O) (Scheme 2). The phosphate in the molecule head results in high negative charged under physiological conditions. Moreover, the long hydrophobic chain make the overall molecule highly amphiphilic. Given the detailed structural information, we designed a water-soluble conjugated copolythiophene (**CPT9**) for LPA recognition.

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Table 1. Characteristic Chemical Shifts of CPT9 in the Absence and Presence of LPA

	H1	H2	H3	H4	H5	H6
CPT9	11.26	3.88	4.22	4.73	3.04	1.75
CPT9 + LPA	11.24	4.25	4.68	5.30	3.42	1.96

Scheme 2 shows that the phenyl substituent in **CPT9**, as a rigid group, is attached to the 3-position of thiophene to tune the conformation of copolymer chain, resulting in signal amplification.[40−42] The ammonium in **CPT9** improves its water solubility and provides positive center to bind with the negatively charged LPA via electrostatic interaction. Moreover, it is a strong electron-withdrawing group that can induce the electron transport from the polythiophene backbone to the 3-aryl unit. The uracil moiety is introduced via its strong affinity to LPA through the three hydrogen bonds. The two corresponding homopolymers (**PT2** and **PT9**) were also synthesized as model polymers to illustrate the importance of copolymerization applied in our design for highly selective and sensitive determination of LPA.

3.2. LPA Sensing with CPT9. The interaction between **CPT9** (100 μM, calculated on monomers basis) and LPA was studied in HEPES buffer (10 mM, pH 7.4, 1% CH_3CN) at room temperature by absorption and emission spectroscopy. The UV−vis spectrum of **CPT9** in the HEPES solution displays a characteristic absorption band of polythiophene at 400 nm (Figure 1A). The addition of LPA casused the absorption peak to gradually decrease at 400 nm, whereas a new absorption band at around 500 nm emerged with an isosbestic point at 442 nm. Figure 1B shows their corresponding changes in the fluorescence spectra. The conjugated backbone emission peak at 585 nm was gradually increased with red-shift to 593 nm upon LPA addition. The absorbance intensity ratio (A_{500}/A_{400}), and the fluorescence intensity ratio (I_{593}/I_{550}) show a good linear relationship with LPA amount ranging from 0−25 μM (Figure 1C and D). The detection limit, defined as three times the standard deviation of background, reached 0.6 μM, which is below the requisite detection limits for LPA in human plasma samples.[5]

Such significant changes in the absorption and fluorescence spectra can be well explained by the LPA-induced combined conformational change of conjugated backbone and weakened electron transfer effect.[43] The strong evidence for the electrostatic interaction and multiple hydrogen bonds between **CPT9** and LPA comes from the zeta-potential analysis and ^{1}H NMR titrations. For example, the zeta-potential of CPT9 was +37.8 mV resulting from the mass of positive charges on its surface. Upon the addition of 25 μM LPA, the zeta-potential of the solution was reduced to +29.9 mV, indicating that the negatively charged LPA interacted with **CPT9** through the electrostatic interaction. Table 1 summarizes the ^{1}H NMR titrations results. The chemical shift of proton **H3** increased from 4.22 to 4.68 upon LPA addition. Meanwhile, the chemical shifts of proton **H4** also increased from 4.73 to 5.30. These results suggest that carbonyl groups and ammonium groups of **CPT9** participate in the interaction with LPA.

3.3. Interaction between Two Homopolythiophenes and LPA. Two corresponding homopolythiophenes (**PT2** and **PT9**) were prepared as model polymers for comparison to isolate the contribution of the electrostatic, hydrophobic and multiply hydrogen bonds interactions. The LPA addition results in smaller red shifts both in the absorbance and fluorescence spectra of **PT2** (Figure 2A and B). In contrast with **PT2**, a slight blue shift occurs combining the intensity decrease in the absorption spectroscopy of **PT9** upon LPA addition (Figure 2C). Meanwhile, the fluorescence intensity at 560 nm was monotonously decreased in the presence of 25 μM of LPA (Figure 2D). These results strongly support the suggestion that the electronic interaction, multiple hydrogen bonds, and hydrophobic interaction have essential roles in molecular recognition. Each functional substituent in **CPT9** is indispensable for highly sensitive LPA detection.

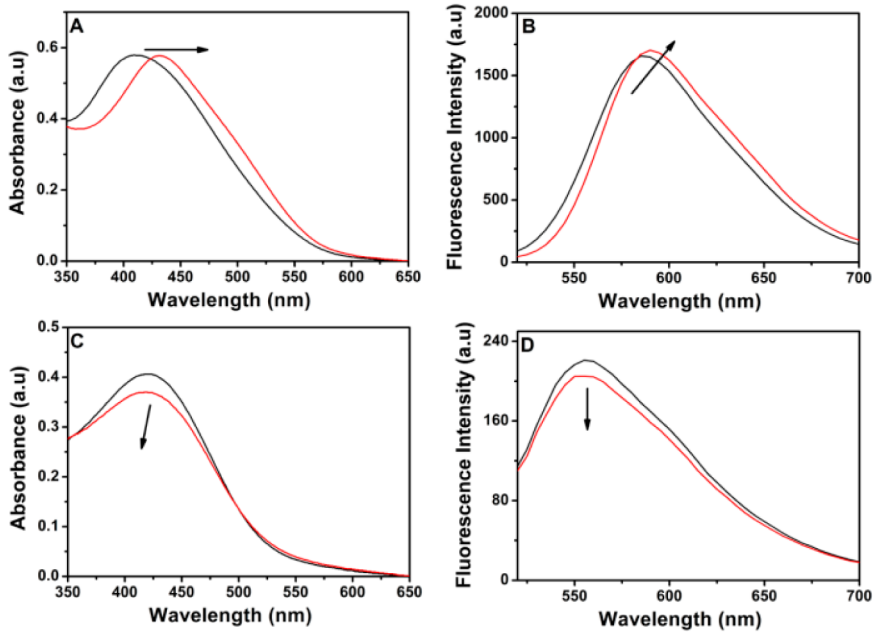

Figure 2. Absorbance and fluorescence spectra of **PT2** (A and B) and **PT9** (C and D) (100 μM) in 10 mM of HEPES buffer solution (1% CH_3CN, pH = 7.4) in the absence and presence of 25 μM of LPA, respectively. λ_{ex}= 500 nm.

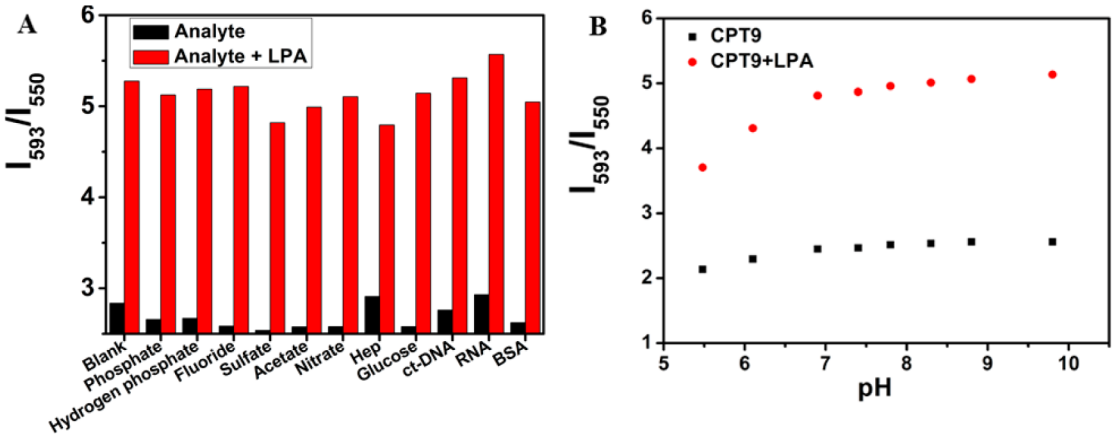

Figure 3. (A) Selectivity of **CPT9** (100 μM) to LPA (30 μM) under 10 mM of HEPES buffer solution (1% CH_3CN, pH = 7.4) in the presence of some coexisting biological species. ctDNA, RNA, and BSA were 5 mg/L, and other anions or biological molecules were 30 μM. (B) pH effect on the fluorescence intensity ratio (I_{593}/I_{550}) of free **CPT9** (100 μM) (black squares) and **CPT9**/LPA (20 μM) mixtures (red dots) at room temperature.

3.3. Selectivity Toward LPA and pH-Dependent Response. Changes in the fluorescence intensity ratio (I_{593}/I_{550}) of **CPT9** caused by other biologically important species were also tested in the buffer solution to evaluate the selectivity of reported sensor for LPA. The lack of hydrophobic interaction, other anions including phosphate, hydrogen phosphate, fluoride, sulfate, acetate, and nitrate did not significantly increase the fluorescence intensity, as shown in Figure 3A. Moreover, negligible variations in the fluorescence intensity ratio (I_{593}/I_{550}) were observed upon the addition of other biological species including glucose, ct-DNA, RNA, and BSA. In contrast, LPA binds with **CPT9** through the electrostatic, hydrophobic, and multiple hydrogen bonds interaction, thereby resulting in a distinct change in fluorescence intensity at 593 nm. The pH effects on the sensing process were also investigated. The experimental results indicated that **CPT9** can be used to detect LPA in a wide range of pH values from 6 to 10 (Figure 3B). But in the acidic condition (pH < 7), the negative phosphate unit in the LPA may be partially protonated which decreases the electrostatic interaction of **CPT9** and LPA.

CONCLUSIONS

In summary, we developed, for the first time, a highly sensitive and selective fluorometric sensor for LPA in aqueous solution using a copolythiophene (**CPT9**). The appealing performance of the sensor was demonstrated to originate from the electrostatic, hydrophobic, and multihydrogen bond cooperative interactions, synergetic with signal amplification via the conformational change of the conjugated polymer main chain. In addition, the present probe shows fluorescence enhancement and emission peak red shift upon binding with LPA, which is different with previous polythiophene-based optical sensors. We believe that the newly proposed design strategy based on the copolymerization method can also be extended to design other sensors for highly sensitive and selective sensing of various biomoleculars.

ASSOCIATED CONTENT

Supporting Information

Characterization of new compounds and the spectra of ^1H NMR titrations. This material is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Author

*Tel.: +86-10-82543435. Fax: +86-10-82543435. E-mail: wangpf@mail.ipc.ac.cn (P.W.) or wmliu@mail.ipc.ac.cn (W.L.).

Notes

The authors declare no competing financial interest.

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