# Water-Soluble Conjugated Polymers for Amplification of One- and Two-photon Properties of Photosensitizers

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ABSTRACT: Two new water-soluble polyfluorene-based conjugated polymers have been designed and synthesized to act as two-photon light harvesting complexes to amplify the two-photon property of photosensitizers and organic dyes through fluorescence resonance energy transfer (FRET). The two-photon absorption cross sections of the conjugated polymers at 800 nm have been significantly improved by inserting ethynylene and vinylene groups as the bridges, which extend the conjugation length and electron delocalization. By using these conjugated polymers as two-photon light harvesting complexes, the two-photon excitation emission of the photosensitizer, Rose Bengal, was found to be significantly enhanced (by up to 85 fold) through FRET. These studies are expected to provide insight on designing systems with further improved performance for potential applications in two-photon photodynamic therapy, sensing, and imaging.
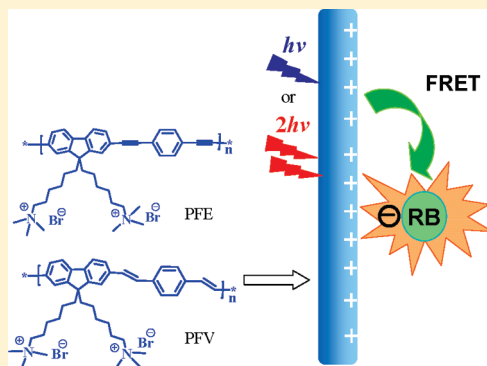
INTRODUCTION

Chromophores can simultaneously absorb two photons to be promoted onto the excited state in the presence of intense laser irradiation, which is known as two-photon absorption (TPA). The transition probability from the ground state to the excited state is proportional to the square of the incident beam intensity, which allows three-dimensional (3D) spatial resolution for two-photon induced chemical or physical processes in materials. Two-photon absorption materials have many potential and promising applications such as two-photon photodynamic therapy (PDT),[1,2] fluorescence microscopy,[3,4] 3-D microfabrication and optical data storage.[5] These potential applications have strongly stimulated the research activity on materials with large two-photon absorption cross sections (δ) in aqueous media.[1−6]

Photodynamic therapy (PDT) has been known as a noninvasive treatment method and has been used for treatment of several types of cancer.[7−9] Two-photon photodynamic therapy (TP−PDT) and imaging have advantages over their one-photon counterparts by offering the advantages of (a) the ability to treat deeper into diseased tissues; (b) the ability to work with a smaller, more confined treatment area; and most importantly, (c) TP-PDT renders selective activity in *z*-direction, which significantly reduces side effects. Furthermore, the photosensitizers for TP-PDT are not limited to those absorbing at longer wavelengths, thus the choice of photosensitizers is more versatile and flexible. However, conventional photosensitizing drugs in clinical use usually have small two-photon absorption cross sections and their efficiencies are limited.[10−12] To make TP-PDT more generally applicable, design of new photosensitizers or chemical modification of existing ones to produce efficient two-photon sensitization is required, which is however complex, expensive and

needs long periods.[13−16] An alternative method is to use materials with large TPA cross sections as two-photon light harvesting complexes in combination with existing photosensitizing drugs. In this approach, materials with large TPA cross sections act as energy donor to transfer the harvested energy to the photosensitizers (acting as acceptor). The photosensitizer molecules are thus indirectly excited through two-photon excitation fluorescence resonance energy transfer (TPE-FRET). The efficiency of the existing drug molecules could be significantly enhanced through energy transfer processes. On the basis of this concept, different TPE-FRET schemes have been developed,[17−22] in which the TPA donors and photosensitizers are assembled together to achieve enhanced two-photon photosensitizing activity. These approaches include chemically assembling TPA donors into dendrimer arms with a photosensitizer moiety as the central core,[20] assembly or covalently linking photosensitizer molecules and materials with larger TPA cross sections such as semiconducting nanoparticles,[18] small molecules[17,19] and conjugated polymers.[21,22] In these assemblies, materials with large TPA cross sections act as two-photon light harvesting complexes to enhance two-photon excitation efficiency of the photosensitizers.

Water-soluble conjugated polymers have been known to display excellent light-harvesting property and can result in fluorescence amplification of organic dyes in aqueous media through FRET.[23−26] This property has been widely used for developing novel biosensors with enhanced sensitivity.[23−26] In addition, conjugated polymers also have large TPA cross sections

compared to their small molecule counterparts.[1,27,28] Water-soluble conjugated polymers have been utilized to enhance two-photon property of photosensitizers and organic dyes in aqueous media through FRET.[21,22] The two-photon excitation fluorescence of the photosensitizer molecules and dyes has been reported to be enhanced by a factor of over 30 times based on commercially available conjugated polymers.[21,22] There are still a lot of room for further improvement in enhancement factor if conjugated polymers with optimized structures and large TPA cross sections are used, which will be particularly useful for clinical applications.

Many different strategies have been used to improve TPA cross section of molecules, particularly at 800 nm, the central wavelength of most readily available femtosecond laser source used in many applications. Several factors, including the properties of π-conjugated segment, electron donating/withdrawing strength of donor and/or acceptor substitutes, molecular symmetry, and the molecular dimension have been known to play important roles in the TPA cross section of organic molecules.[1] A few recent studies showed that TPA cross sections of organic molecules can be improved by orders of magnitude through increasing the conjugation length and coplanarity.[29−32] It has also been reported that the solvent also played an important role in the TPA cross section of conjugated polymers.[28,33,34] The TPA cross sections of conjugated polymers in aqueous media were reported to be much smaller than those in organic solvents, which will restrict their enhancement capability in biological applications. There is strong demand to develop new water-soluble conjugated polymers with improved TPA cross sections.

In this work, we have designed and synthesized two new cationic conjugated polymers (CCPs) with improved TPA cross sections at 800 nm, which were subsequently utilized as two-photon light harvesting complex to enhance two-photon excitation emission of photosensitizers. These two polymers, poly[9,9′-bis(6″- (N,N,N-trimethylammonium)hexyl)fluorene-2,7-ylene-ethylene-co-alt-1,4-phenylene] (PFE) and poly[9,9′-bis(6″-(N,N,N-trimethylammonium)hexyl)fluorene-2,7-ylene-vinylene-co-alt-1,4-phenylene] (PFV), have modified backbone structures from poly [9,9′-bis(6″-(N,N,N-trimethylammonium)hexyl)fluorene-co-alt-1,4-phenylene dibromide] (PFP), a cationic conjugated polymer that was previously utilized as two-photon light harvesting complex.[21,22] By inserting ethynylene and vinylene units as the bridges, which changed conjugation delocalization and rigidity of the polymer backbones, the TPA cross section of the two new CCPs at 800 nm have been significantly improved compared to PFP. The TPA cross sections of PFE (with the ethynylene bridge) and PFV (with the vinylene bridge) at 800 nm are 8 times and 36 times of that of PFP. The improved TPA cross sections of CCPs at 800 nm make them useful in acting as two-photon light harvesting complexes to significantly enhance two-photon excitation emission of photosensitizer, Rose Bengal (RB), via FRET. The two-photon emission of RB under excitation at 800 nm was found to be enhanced by up to about 20 times when PFE was used and more than 85 times when PFV was used, respectively. Our results will provide useful information for the further application of CCPs in the field of two-photon PDT, sensing and imaging.

■ EXPERIMENTAL SECTION

Materials and Measurements. Solvents and chemicals were purchased from Sigma-Aldrich and used without further purification

unless otherwise noted. THF was dried and distilled under sodium. Polymers PFP and PFV were synthesized using a procedure as described in the literature and shown in Scheme 1.[35,36] 2,7-Diiodo-9,9-di(6′-bromohexyl)fluorene (**1**),[37] 2,7-Dibromo-9,9-di(6′-bromohexyl)fluorene (**4**),[35] and 1,4-bis(diethylphosphinatyl methyl)phenylene (**6**)[38] were prepared according to the reported procedures.

^{1}H NMR and ^{13}C NMR spectra were recorded on 300 MHz AC Bruker spectrometers. Mass spectra were measured by using an AEI-M850-MS spectrometer. FT-IR spectra were taken on a Varian 3100 FT-IR spectrometer in a KBr pellet form. GPC analysis was carried out on a Waters Styragel system using polystyrene as the calibration standard and tetrahydrofuran (THF) as eluent. UV−vis absorption and fluorescence spectra were measured by using a Shimadzu UV-2450 spectrophotometer and a Perkin-Elmer LS50 spectrophotometer, respectively. The emission quantum yields (QE) of the polymers were measured by using quinine sulfate (0.1 M H_2SO_4) and Rhodamine 6G in methanol as standards.

The two-photon excitation fluorescence measurement was performed by using Spectra Physics femtosecond Ti:sapphire oscillator (Tsunami) as the excitation source. The output laser pulses have a center wavelength at 800 nm with pulse duration of 40 fs and a repetition rate of 76 MHz. The laser beam was focused onto the samples contained in a cuvette with path length of 1 cm. The emission from the sample was collected at a 90° angle by a pair of lenses and an optical fiber that was connected to a monochromator (Acton, Spectra Pro 2300i) coupled with CCD (Princeton Instruments, Pixis 100B) system. A short pass filter with cutoff wavelength at 700 nm was paced before the spectrometer to minimize the scattering from the pump beam. All the measurements were performed in phosphate buffer (25 mM, pH = 7.5) solutions at room temperature. The aqueous solutions of the cationic polymers were prepared by first dissolving polymers in DMSO (1 mM) and then diluting to the desired concentration in phosphate buffer (25 mM, pH = 7.5) solutions.

Preparation of 2,7-Bis[(trimethylsilyl)ethynyl]-9,9-di(6′-bromohexyl)fluorene (2). A solution of 2,7-diiodo-9,9-di(6′-bromohexyl)fluorene (1.48 g, 2 mmol), copper iodine (80 mg, 0.42 mmol) and triethylamine (2 mL, 14 mmol) in 20 mL of THF were stirred under N_2 for 30 min. Trimethylsilyl acetylene (490 mg, 5 mmol) and Pd(PPh$_3$)$_2$Cl$_2$ (50 mg, 0.072 mmol) were then added and the mixture were stirred for 24 h at room temperature. After removing the solvent in vacuo and purification procedures by passing through a silica gel column using DCM/hexane = 1:10 as the eluent, a yellow solid product (800 mg, 59%) was obtained. The NMR and mass spectra results are as follows. (a) ^{1}H NMR (300 MHz, CDCl$_3$): δ 7.59 (d, 2H), 7.46 (d, 2H), 7.41 (s, 2H), 3.28 (t, 4H), 1.97−1.91 (m, 4H), 1.70−1.60 (m, 4H), 1.16−1.08 (m, 8H), 0.53 (m, 4H), 0.29 (s, 18H). (b) ^{13}C NMR (300 MHz, CDCl$_3$): 150.5, 140.8, 131.4, 126.1, 121.9, 119.9, 105.9, 94.5, 55.1, 40.2, 33.9, 32.7, 29.0, 27.8, 23.4, 0.04. (c) MS (EI): m/z = 685 (M+).

Preparation of 2,7-Diethylnyl-9,9-di(6′-bromohexyl)fluorene (3). Monomer **2** (680 mg, 1 mmol) and potassium carbonate (1.38 g, 10 mmol) were mixed into 50 mL of methanol and the solution was stirred overnight at room temperature. Then the solvent was stripped off under a reduced pressure. The residue was dissolved into 100 mL of chloroform. The organic solution was washed successively with 1.0 M aqueous HCl and water, and then dried over anhydrous MgSO$_4$. A yellow oil product (460 mg, 95%) was obtained after the solvent was removed. The NMR and mass spectra results are as follows. (a) ^{1}H NMR (300 MHz, CDCl$_3$): δ 7.62 (d, 2H), 7.49 (d, 2H), 7.45 (s, 2H), 3.28 (t, 4H), 3.16 (s, 2H), 1.98−1.92 (m, 4H), 1.68−1.61 (m, 4H), 1.16−1.08 (m, 8H), 0.62−0.54 (m, 4H). (b) ^{13}C NMR (300 MHz, CDCl$_3$): 150.8, 141.0, 131.4, 126.5, 120.9, 120.0, 72.7, 58.4, 55.1, 40.2, 33.9, 32.7, 29.0, 27.8, 23.6. (c) MS (EI): m/z = 540 (M+).

Preparation of Poly[9,9′-bis(6″-(bromohexyl)fluorene-2,7-yleneethylene-co-alt-1,4- phenylene] (PFE-Br). 1,4-Dibromophenylene (128 mg, 0.5 mmol), monomer **3** (270 mg, 0.5 mmol),

Scheme 1. Synthesis Routes of Monomers and Conjugated Polymers
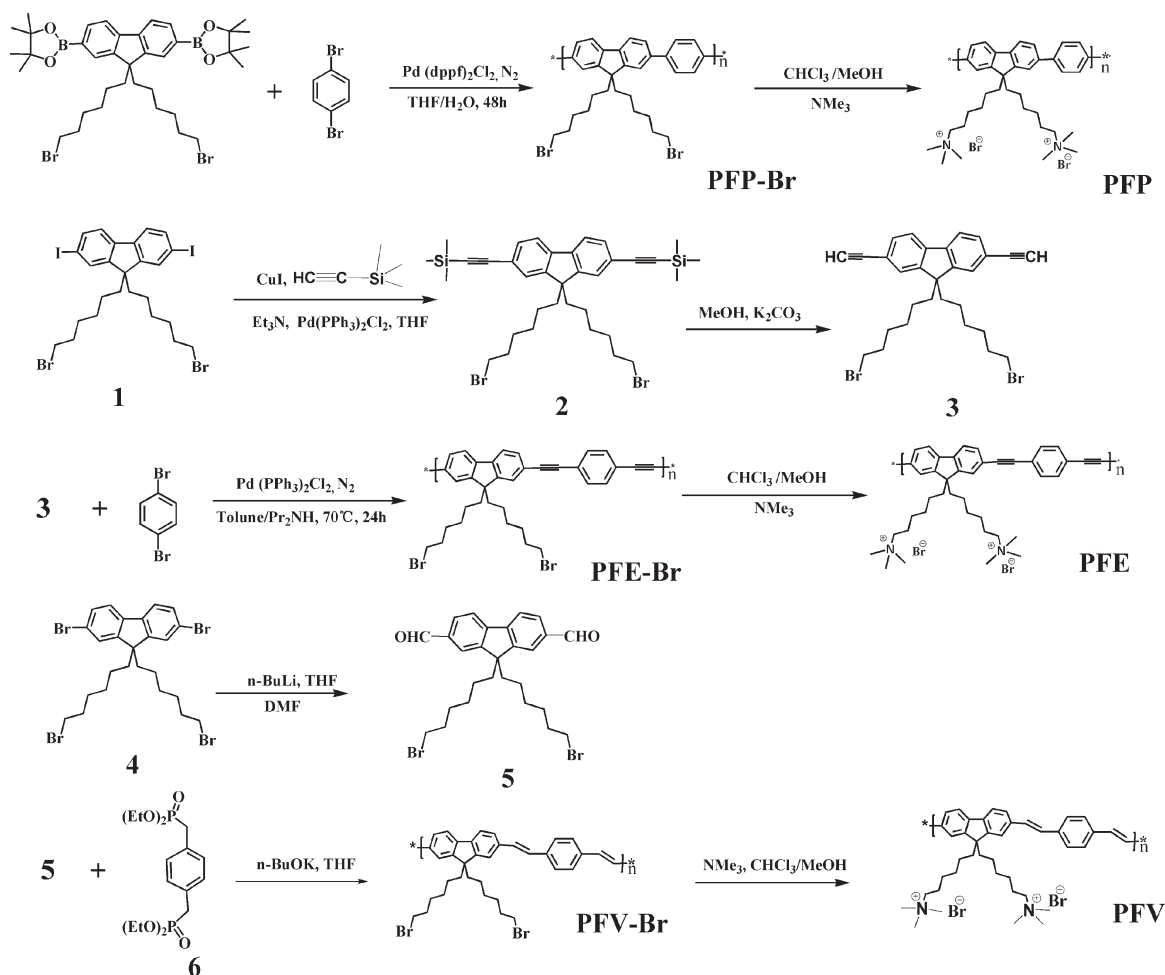
Pd(PPh$_3$)$_2$Cl$_2$ (50 mg, 0.072 mmol), and cuprous iodide (19.2 mg, 0.1 mmol) were combined in degassed toluene (10 mL) and diisopropylaine (4 mL). The reaction mixture was stirred at 70 °C for 24 h. After cooling to the room temperature, the solvent was removed under vacuum. The residue was dissolved into 50 mL of chloroform and washed with water. The organic layer was dried over with MgSO$_4$, and then precipitated in methanol. The precipitate was filtered off and reprecipitated twice in methanol to afford the brown solid product (180 mg, 58%). NMR spectra FTIR and GPC results are as follows. (a) ^{1}H NMR (300 MHz, CDCl$_3$): δ 7.72 (br, 2H), 7.58−7.49 (br, 8H), 3.26 (br, 4H), 2.08 (br, 4H), 1.63−1.55 (br, 4H), 1.15−1.07 (br, 8H), 0.65 (br, 4H). (b) FT-IR (KBr, cm^{-1}): 2931−2858 (v_{C-H}, v_{Ar-H}), 2201 ($v_{C≡C}$), 1653−1606 ($v_{Ar,C=C}$), 1465−1436 ($δ_{C-H}$), 1267−1233 (v_{C-C} of fluorene), 1161−1123 (weak, $v_{R,C-C}$), 1070−1008 (v_{C-C} of fluorene), 894 ($v_{≡C-C}$), 825 ($δ_{C-H}$ of p-Ar), 750 ($δ_{Ar,C-H}$), 726 ($δ(CH_2)_n$, n ≥ 4), 690−646 (v_{C-H} of fluorene), 560−526 (v_{C-Br}). (c) GPC: M_n = 13181, M_w = 31926, PDI = 2.42.

Preparation of the Ionic Polymers. PFP-Br, PFE-Br, and PFV-Br were dissolved into 20 mL of chloroform. Then, 5 mL of trimethylamine solution in ethanol (30%) were then added. The mixture was stirred for 48 h at room temperature. The solvent were then evaporated at vacuum and ionic polymers were obtained after dried at vacuum. (a) ^{1}H NMR (300 MHz, DMSO-d) for PFP: δ 8.09−7.58 (m, 10H), 3.17 (br, 18H), 2.98 (br, 4H), 2.14 (br, 4H), 1.47 (br, 4H), 1.08 (br, 8H), 0.54 (br, 4H). (b) ^{1}H NMR (300 MHz, DMSO-d) for PFE: δ 8.01−7.55 (m, 10H), 3.17 (br, 18H), 2.99 (br, 4H), 2.07 (br, 4H), 1.47 (br, 4H),

1.07 (br, 8H), 0.54 (br, 4H). (c) ^{1}H NMR (300 MHz, DMSO-d$_6$) for PFV: δ 8.09−7.27 (m, 14H), 3.18 (br, 18H), 2.95 (br, 4H), 2.09 (br, 4H), 1.45 (br, 4H), 1.26−1.05 (br, 8H), 0.54 (br, 4H).

■ RESULTS AND DISCUSSION

Synthesis and Physical Properties of Polymers. Scheme 1 shows the synthesis routes of the conjugated polymers used in this study. Poly[9,9′-bis(6″-(N,N,N-trimethylammonium)hexyl)-fluorene-co-alt-1,4-phenylene dibromide] (PFP) was synthesized using a previously reported method[35] via the Suzuki reaction. Monomer **3** was prepared from 2,7-diiodo-9,9-di(6′-bromohexyl)-fluorene and trimethylsilyl acetylene by a palladium(II)-catalyzed cross-coupling reaction, followed by cleavage of the trimethylsilyl protecting group under basic condition. The neutral polymer, poly[9,9′-bis(6″-(bromohexyl)fluorene-2,7-yleneethylene-co-alt-1,4-phenylene] (PFE-Br), was prepared by palladium-catalyzed crossing-coupling condensation between monomer 3 and 1,4-dibromophenyl in the mixture of toluene/diisopropylaine (2.5:1) solution containing CuI under N$_2$ at 70 °C for 24 h. Monomer **5** was prepared from 2,7-dibromo-9,9-di(6′-bromohexyl)-fluorene with a yield of 55%. The bromides at the 2,7- position are activated by adding 2 equiv of t-BuLi in dry THF at −78 °C and excess N,N-dimethylformamide (DMF) and stirring the reaction mixture overnight at room temperature.
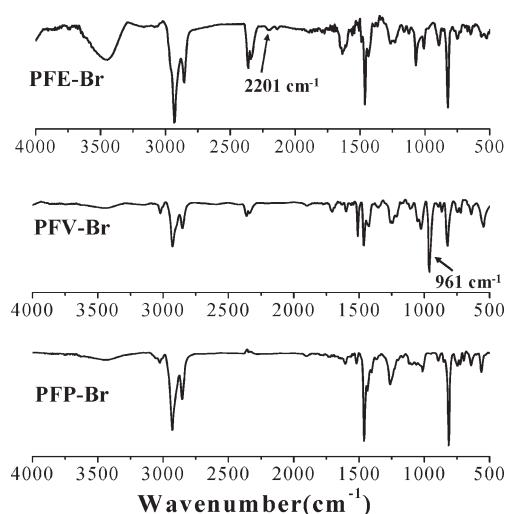
Figure 1. FT-IR spectra of polymers PFE-Br, PFV-Br, and PFP-Br.

Table 1. Polydispersity Properties of Polymers

polymers	M_w (g/mol)	M_n (g/mol)	PDI
PFP-Br	17 249	6876	2.51
PFE-Br	31 926	13 180	2.42
PFV-Br	31 095	17 141	1.81

Figure 2. (a) UV—visible absorption (solid lines) and emission spectra (dash lines) of PFP, PFE, and PFV; (b) TPA cross sections of polymers with different excitation wavelength.

The poly[9,9′-bis(6″-(bromohexyl)fluorene-2,7-ylenevinylene-*co-alt*-1,4-phenylene] (PFV-Br) was prepared via the Wittig—Horner condensation reaction between the dialdehyde mono-mer **5** and the 1,4-bis(diethylphosphinatylmethyl)phenylene in dry THF by slowly adding 1 equiv of *t*-BuOK. The prepared neutral polymers PFE-Br and PFV-Br were then dissolved into chloroform and treated with 30% trimethylamine in ethanol solution at room temperature for 48 h to obtain water-soluble cationic polymers PFE and PFV, respectively.

The structures and purity of polymers were confirmed by using NMR spectroscopy and FT-IR spectra. As shown in Figure 1, the FT-IR spectrum of PFE-Br gives a weak character-istic absorption peak of $-C{\equiv}C-$ at 2201 cm^{-1}, which demon-strate the symmetrical structure and high molecule weight of the polymer PFE-Br. The absorption peak at 961 cm^{-1} of PFV-Br is due to the C=C bridge, which confirms the *trans-* configuration of the vinylene double bonds of PFV-Br. The disappearance of the peak around 1650 cm^{-1} is due to the reduced C—C rotation in aromatic ring upon polymerization, which indicates a relatively high molecular weight of PFV-Br. The detail assignments of all peaks are summarized in the Experimental Section.

The weight-averaged molecular weight (M_w) and number-averaged molecular weight (M_n) with the polydispersity index PDI (PDI = M_w/M_n) of neutral polymers PFP-Br, PFE-Br, and PFV-Br were determined by the gel permeation chromatography (GPC) analysis using THF as the eluent. The data are summar-ized in Table 1. On the basis of the molecular weight, the numbers of repeat units for three polymers are 12 for PFP-Br, 21 for PFE-Br, and 28 for PFV-Br, respectively.

Absorption and Fluorescence Properties of the Polymers. The absorption and fluorescence spectra of the polymers PFP, PFE, and PFV in the aqueous media are shown in Figure 2a, and some optical properties are summarized in Table 2. The
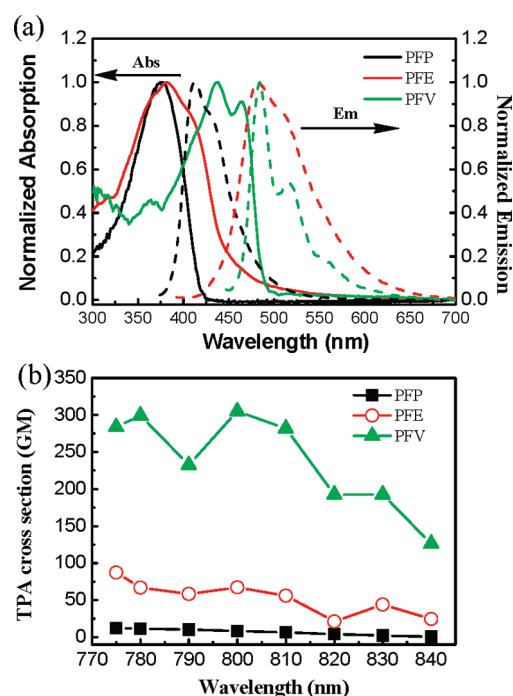
absorption spectrum of PFP exhibits a maximum at 375 nm and the fluorescence spectrum peaks at 418 nm with a shoulder around 430 nm, similar to other polyfluorene derivatives. Both absorption and fluorescence spectra of PFE and PFV are red-shifted relative to those of PFP, which can be understood as a result of increasing conjugation length and extended delocaliza-tion along the polymer backbone chain upon inserting the ethynylene and vinylene units. The absorption spectrum of PFV is further red-shifted by 55 nm relative to that of PFE. These results suggest that the double bond allows better electron delocalization than the triple bond, similar to the previous studies on polyethylene and polyvinylene compounds.[39] However, it is noted that PFE and PFV exhibited similar emission spectra despite the large difference in their absorption maximum. This discrepancy can be ascribed due to the low solubility of PFE in aqueous solution. The solubility of PFP, PFE and PFV in aqueous solution were measured to be 2.8, 0.4, and 2.2 mg/mL, respectively. Because of the low solubility, PFE aggregates severely in the aqueous solution. The observed emission mainly originates from the emission of the aggregated form, which is red-shifted to ∼483 nm from its nonaggregated emission at ∼425 nm (see Supporting Information). The fluorescence quantum yields (QY, φ) of three polymers in water were measured by using quinine sulfate or rhodamine 6G as the standard. The QY value of PFP, PFE, and PFV are 9.8%, 9.8%, and 3.0% respectively, which is significantly lower than the quantum yields of the neutral precursors in organic solvents. The quantum yields of PFP-Br, PFE-Br, and PFV-Br in THF which were measured to be 48%, 60%, and 48% respectively. The low quantum yields of cationic polymers in aqueous solution are likely due to a self-quenching effect induced by the π−π stacking aggregation. The extinction coefficients (ε) of the polymers also increase upon inserting the vinylene and ethynylene bridges

Table 2. Photophysical Properties of PFP, PFE, PFV, and RB in Aqueous Solution
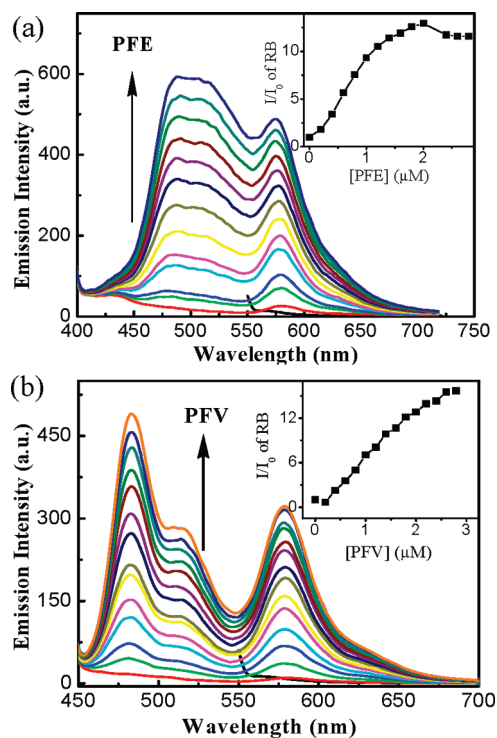
polymers	$\lambda_{max,abs}$ (nm)	$\lambda_{max,em.}$ (nm)	ε ($M^{-1} \cdot cm^{-1}$)	φ (%)	δ (GM) (800 nm)
PFP	375	418	3.65×10^4	9.8	8.4
PFE	382	483	3.73×10^4	9.8	67.3
PFV	437, 464	484	4.17×10^4	3.0	305
RB	550	568	1.95×10^4	0.84	21.0
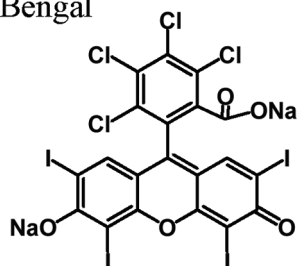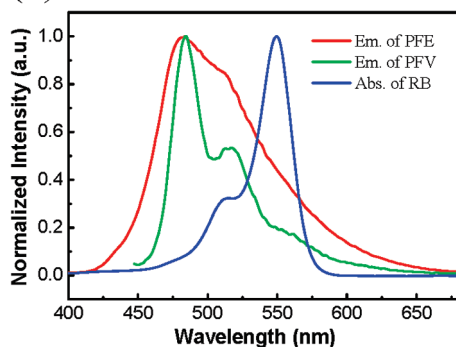
Figure 3. (a) Chemical structure of Rose Bengal; (b) absorption spectrum of RB and emission spectra of PFE and PFV.

Figure 4. (a) Emission spectra of RB/PFE complex upon gradual addition of PFE into PBS buffered RB solution: $[PFE] = 0-2.8 \times 10^{-6}$ M in repeat units, $[RB] = 2.5 \times 10^{-8}$ M), $\lambda_{EX} = 385$ nm; b) Emission spectra of RB/PFV complex upon gradual addition of PFV into PBS buffered RB solution (25 mM, pH = 7.5): $[PFV] = 0-2.8 \times 10^{-6}$ M in repeat units, $[RB] = 2.5 \times 10^{-8}$ M, $\lambda_{EX} = 435$ nm. The insets plot enhancement factor of the RB emission versus the polymer concentrations.

(Table 2), which further confirms that PFV and PFE have larger effective conjugation lengths than PFP.[40]

The TPA cross sections of three water-soluble conjugated polymers in water have also been measured by using rhodamine 6G in methanol and fluorescein (pH =11) as the standard. Although the TPA cross sections are strongly dependent on the excitation wavelength (Figure 2b), here we mainly focus our studies on the TPA cross sections of polymers at 800 nm due to its practical importance. The applications of two-photon excitation fluorescence microscopy and two-photon PDT usually use a Ti:sapphire oscillator, which has a central wavelength at 800 nm. As summarized in Table 2, the TPA cross section of PFV at 800 nm is 305 GM per repeat unit, which is 4.5 times that of PFE (67 GM) and 36 times that of PFP (8.4 GM). If we consider the entire polymer chain, the TPA cross sections for PFP, PFE and PFV will be 100 GM, 1410 GM, and 8540 GM per polymer chain, respectively.

Two factors are responsible for much larger TPA cross sections (per repeat unit) of PFE and PFV at 800 nm compared to PFP. First, due to improved conjugation length and electron delocalization by inserting ethynylene and vinylene units, both one-photon and two-photon absorption peaks shifts to red. PFP has a one-photon absorption peak at 375 nm. Its TPA peak was known to be ~650 nm,[41] far away from 800 nm. The red-shift in

absorption spectra will help to increase TPA cross sections at 800 nm. Second, the improvement in conjugation length and electron delocalization will drastically increase the TPA responses of the conjugated polymers. It has been previously established that there is a strong correlation between electron delocalization and two-photon absorptivity.[1] The extent of conjugation and coplanarity of the polymer chain have been known particularly important for improving TPA cross section of the materials. Larger conjugation and better coplanarity can lead to states with a larger extent of charge separation thus a larger TPA cross section. The TPA cross section of three polymers at different excitation wavelengths available from our laser system are summarized in Figure 2b.

One-Photon Excitation Fluorescence Resonance Energy Transfer. Rose Bengal (RB, Figure 3a) is an important photosensitizer that has been widely used in photodynamic therapy due to their good water solubility and high efficiency in generating molecular singlet oxygen.[11] However, RB has a relatively small TPA cross section (21 GM based on our measurements) and its application in two-photon PDT is limited. The absorption spectrum of RB overlaps well with the emission spectra of both PFE and PFV, as shown in Figure 3b. PFE and PFV can thus be used as light harvesting complexes to improve the one-photon and two-photon excitation efficiency of RB via FRET.

The one-photon emission spectra of polymers/RB systems were measured to examine the FRET efficiency and one-photon optical amplification effects of the systems. The measurements were carried out in the PBS buffered (25 mM, pH = 7.4)
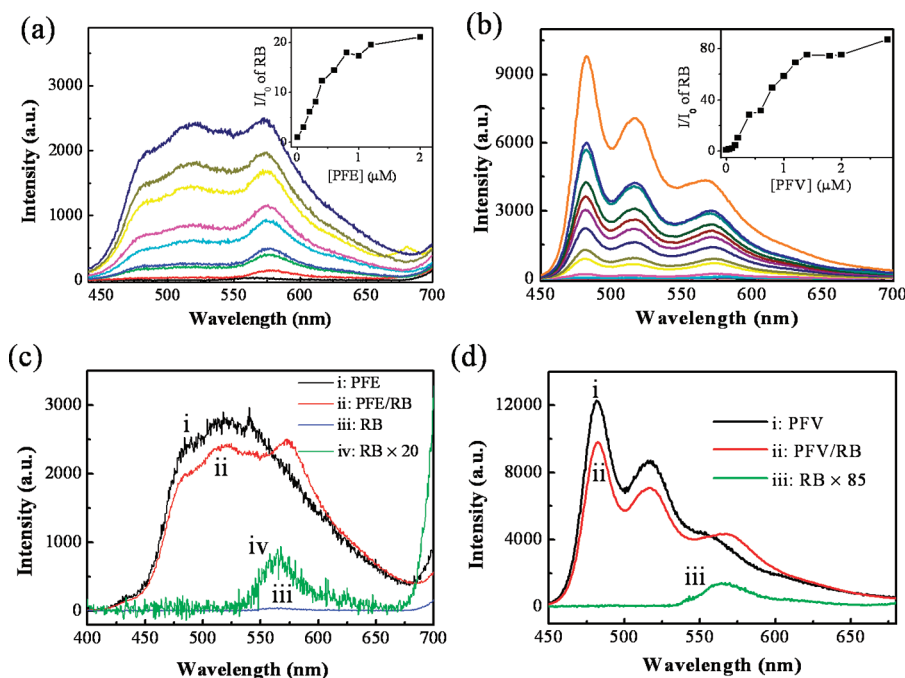
Figure 5. (a) Two-photon excitation emission spectra of PFE/RB complex upon gradual addition of PFE into the RB solution in PBS buffer solution: $[PFE] = 0-2.0 \times 10^{-6}$ M in repeat units. (b) Two-photon excitation emission spectra of PFV/RB complex upon gradual addition of PFV into the RB solution in PBS solutuion: $[PFV] = 0-2.8 \times 10^{-6}$ M in repeat units; Insets are the plot of enhancement factor of RB emission versus polymer concentration. (c) Two-photon excitation emission spectra of PFE (i), PFE/RB complex (ii), RB (iii), and RB amplified 20 times (iv), $[PFE] = 1.2 \times 10^{-6}$ M in repeat units. (d) Two-photon excitation emission spectra of PFV (i) and RB (iii) and PFV/RB complex (ii), $[PFV] = 2.8 \times 10^{-6}$ M in repeat units. $[RB] = 2.5 \times 10^{-8}$ M in all samples. All two-photon experiments were performed under excitation at 800 nm.

solutions with a fixed RB concentration (2.5×10^{-8} M) and increasing concentration of polymers. The anionic RB can form a complex with cationic polymers via electrostatic interactions to bring the energy donor and acceptor into close proximity to ensure efficient energy transfer. As shown in Figure 4, the emission intensity of RB gradually increases upon addition of cationic polymers, indicating efficient FRET from the polymers to RB. Under excitation at the maximum absorption of polymers (385 nm for PFE and 435 nm for PFV), the emission intensity of RB at 580 nm (I_1) was found to be significantly enhanced compared to that when RB was directly excited at its absorption maximum at 540 nm (I_0). The RB emission was found to be enhanced by up to 13-fold upon addition of PFE, and about 15-fold upon addition of PFV. The slightly different enhancement factor by PFE and PFV are consistent with slightly larger absorption coefficient (ε) of PFV compared to that of PFE. These results confirmed efficient FRET from the cationic conjugated polymers to the photosensitizer, RB. The one-photon excitation capability of RB can be significantly enhanced by using these CCPs as light harvesting complexes.

Two-Photon Excitation Fluorescence Resonance Energy Transfer (TPE-FRET). The two-photon excitation fluorescence assays were similarly examined by adding cationic polymers into the RB solution (2.5×10^{-8} M). The samples were excited with femtosecond laser pulses with a central wavelength at 800 nm and pulse duration of 40 fs. As can be seen from Figure 5, the intensities of two-photon excitation emission of RB were significantly enhanced upon gradual addition of the polymers (PFE or PFV) into the RB solution until saturation. The enhancement factor (the ratio of RB emission intensity in the presence of the polymers over that in the absence of the polymers) as a function

of polymer concentration is plotted in Figure 5. The two-photon emission of RB was enhanced by up to about 20 times by addition of PFE, and more than 85 times by addition of PFV, respectively.

The observed large enhancement factors via TPE-FRET is mainly due to larger TPA cross sections of the conjugated polymers at 800 nm compared to that of RB (see data in Table 2), owing to improved conjugation lengths of the conjugated polymers. In addition, conjugated polymers are made of many repeat units or conjugation segments, the excitation energy harvested by the entire polymer chain can migrate along the polymer chain[39] and eventually transfer to bound RB molecules via FRET, which result in optical amplification as in the one-photon excitation case. Both factors are responsible for the observed large enhancement in the two-photon excitation emission of the RB molecules.

Under one-photon excitation, addition of PFE and PFV resulted in similar magnitudes of enhancement in the emission intensities of RB, which is consistent with similar absorption coefficients (ε) of PFV and of PFE. However, addition of PFE and PFV result in quite different magnitudes of enhancement in two−photon excitation emission of RB. The enhancement factor when using PFV as the two-photon light harvesting complex is about 4.5 times that when using PFE as the two-photon light harvesting complex. This different enhancement factors are consistent with the relative TPA cross sections of PFE and PFV at 800 nm (Table 2). The inherent two-photon property of CCPs is the primary factor for their amplification ability. The difference in other factors, such as quantum yield of polymers, polymer chain length and energy transfer efficiency are also partially responsible for the observed different enhancement factors. To further design efficient system for application in

two-photon photodynamic therapy and multiphoton imaging, it is essential to design conjugated polymers with large TPA cross sections at 800 nm as two-photon light harvesting complexes.

CONCLUSION

In summary, two new water-soluble conjugated polymers, PFE and PFV, have been designed and synthesized to act as two-photon light harvesting complexes to amplify the two-photon property of water-soluble photosensitizers and organic dyes through FRET. The TPA cross sections at 800 nm have been significantly improved by inserting the ethynylene and vinylene groups as the bridges, which extend the conjugation length. The TPA cross section (per repeat unit) of PFV (with a vinylene bridge) at 800 nm is 4.5 times than that of PFE (with an ethynylene bridge), and 36 times than that of PFP. The two-photon excitation emission of the photosensitizer RB was found to be significantly enhanced through FRET by using these conjugated polymers as two-photon light harvesting complexes. The two-photon emission of RB was enhanced by up to about 20 times when PFE was used and more than 85 times when PFV was used, respectively. The inherent two-photon property of CCPs is believed to be the primary factor responsible for the observed huge enhancement in the two-photon emission of the photosensitizer. These studies are expected to provide insight on designing systems with further improved performance for potential applications in two-photon PDT, sensing, and imaging.

ASSOCIATED CONTENT

ⓢ **Supporting Information.** Solvent-dependent absorption spectra and solvent and concentration dependent emission spectra of PFE. This material is available free of charge via the Internet at http://pubs.acs.org.

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REFERENCES

(1) He, G. S.; Tan, L. S.; Zheng, Q.; Prasad, P. N. *Chem. Rev.* **2008**, *108*, 1245–1330.

(2) Pawlicki, M.; Collins, H. A.; Denning, R. G.; Anderson, H. L. *Angew. Chem., Int. Ed.* **2009**, *48*, 3244–3266.

(3) Denk, W.; Strickler, J. H.; Webb, W. W. *Science* **1990**, *248*, 73–76.

(4) So, P. T. C.; Dong, C. Y.; Masters, B. R.; Berland, K. M. *Annu. Rev. Biomed. Eng.* **2000**, *2*, 399–429.

(5) Parthenopoulos, D. A.; Rentzepis, P. M. *Science* **1989**, *245*, 843–845.

(6) Dichtel, W. R.; Serin, J. M.; Edder, C.; Frechet, J. M. J.; Matuszewski, M.; Tan, L. S.; Ohulchanskyy, T. Y.; Prasad, P. N. *J. Am. Chem. Soc.* **2004**, *126*, 5380–5381.

(7) Fisher, W. G.; Partridge, W. P.; Dees, C.; Wachter, E. A. *Photochem. Photobiol.* **1997**, *66*, 141–155.

(8) Karotki, A.; Khurana, M.; Lepock, J. R.; Wilson, B. C. *Photochem. Photobiol.* **2006**, *82*, 443–452.

(9) Khurana, M.; Collins, H. A.; Karotki, A.; Anderson, H. L.; Cramb, D. T.; Wilson, B. C. *Photochem. Photobiol.* **2007**, *83*, 1441–1448.

(10) Kruk, M.; Karotki, A.; Drobizhev, M.; Kuzmitsky, V.; Gael, V.; Rebane, A. *J. Lumin.* **2003**, *105*, 45–55.

(11) Wilkinson, F.; Helman, W. P.; Ross, A. B. *J. Phys. Chem. Ref. Data* **1993**, *22*, 113–262.

(12) Xu, C.; Shear, J. B.; Albota, M.; Webb, W. W. *Biophys. J.* **1997**, *72*, MP379–MP379.

(13) Drobizhev, M.; Karotki, A.; Kruk, M.; Mamardashvili, N. Z.; Rebane, A. *Chem. Phys. Lett.* **2002**, *361*, 504–512.

(14) Drobizhev, M.; Makarov, N. S.; Rebane, A.; de la Torre, G.; Torres, T. *J. Phys. Chem. C* **2008**, *112*, 848–859.

(15) Kuimova, M. K.; Collins, H. A.; Balaz, M.; Dahlstedt, E.; Levitt, J. A.; Sergent, N.; Suhling, K.; Drobizhev, M.; Makarov, N. S.; Rebane, A.; Anderson, H. L.; Phillips, D. *Org. Biomol. Chem.* **2009**, *7*, 889–896.

(16) Suo, Z. Y.; Drobizhev, M.; Spangler, C. W.; Christensson, N.; Rebane, A. *Org. Lett.* **2005**, *7*, 4807–4810.

(17) Chen, C. Y.; Tian, Y. Q.; Cheng, Y. J.; Young, A. C.; Ka, J. W.; Jen, A. K. Y. *J. Am. Chem. Soc.* **2007**, *129*, 7220–7221.

(18) Clapp, A. R.; Pons, T.; Medintz, I. L.; Delehanty, J. B.; Melinger, J. S.; Tiefenbrunn, T.; Dawson, P. E.; Fisher, B. R.; O'Rourke, B.; Mattoussi, H. *Adv. Mater.* **2007**, *19*, 1921–1926.

(19) Kim, S.; Ohulchanskyy, T. Y.; Pudavar, H. E.; Pandey, R. K.; Prasad, P. N. *J. Am. Chem. Soc.* **2007**, *129*, 2669–2675.

(20) Oar, M. A.; Serin, J. A.; Dichtel, W. R.; Frechet, J. M. J. *Chem. Mater.* **2005**, *17*, 2267–2275.

(21) Tian, N.; Xu, Q. H. *Adv. Mater.* **2007**, *19*, 1988–1991.

(22) Wu, C. L.; Xu, Q. H. *Macromol. Rapid Commun.* **2009**, *30*, 504–508.

(23) Feng, F. D.; He, F.; An, L. L.; Wang, S.; Li, Y. H.; Zhu, D. B. *Adv. Mater.* **2008**, *20*, 2959–2964.

(24) Ren, X. S.; Xu, Q. H. *Langmuir* **2009**, *25*, 43–47.

(25) Thomas, S. W.; Joly, G. D.; Swager, T. M. *Chem. Rev.* **2007**, *107*, 1339–1386.

(26) Wang, S.; Gaylord, B. S.; Bazan, G. C. *J. Am. Chem. Soc.* **2004**, *126*, 5446–5451.

(27) Schroeder, R.; Ullrich, B.; Graupner, W.; Scherf, U. *J. Phys.-Condes. Matter* **2001**, *13*, L313–L320.

(28) Woo, H. Y.; Korystov, D.; Mikhailovsky, A.; Nguyen, T. Q.; Bazan, G. C. *J. Am. Chem. Soc.* **2005**, *127*, 13794–13795.

(29) Albota, M.; Beljonne, D.; Bredas, J. L.; Ehrlich, J. E.; Fu, J. Y.; Heikal, A. A.; Hess, S. E.; Kogej, T.; Levin, M. D.; Marder, S. R.; McCord-Maughon, D.; Perry, J. W.; Rockel, H.; Rumi, M.; Subramaniam, C.; Webb, W. W.; Wu, X. L.; Xu, C. *Science* **1998**, *281*, 1653–1656.

(30) Lee, S.; Thomas, K. R. J.; Thayumanavan, S.; Bardeen, C. J. *J. Phys. Chem. A* **2005**, *109*, 9767–9774.

(31) Marder, S. R.; Torruellas, W. E.; BlanchardDesce, M.; Ricci, V.; Stegeman, G. I.; Gilmour, S.; Bredas, J. L.; Li, J.; Bublitz, G. U.; Boxer, S. G. *Science* **1997**, *276*, 1233–1236.

(32) Norman, P.; Luo, Y.; Agren, H. *J. Chem. Phys.* **1999**, *111*, 7758–7765.

(33) Baur, J. W.; Alexander, M. D.; Banach, M.; Denny, L. R.; Reinhardt, B. A.; Vaia, R. A.; Fleitz, P. A.; Kirkpatrick, S. M. *Chem. Mater.* **1999**, *11*, 2899–2906.

(34) Woo, H. Y.; Bazan, G. C. *Mol. Cryst. Liq. Cryst.* **2008**, *485*, 928–933.

(35) Stork, M.; Gaylord, B. S.; Heeger, A. J.; Bazan, G. C. *Adv. Mater.* **2002**, *14*, 361–366.

(36) Tang, F.; He, F.; Cheng, H. C.; Li, L. D. *Langmuir* **2010**, *26*, 11774–11778.

(37) Sun, H.; Feng, F.; Yu, M. H.; Wang, S. *Macromol. Rapid Commun.* **2007**, *28*, 1905–1911.

(38) Lee, J. K.; Schrock, R. R.; Baigent, D. R.; Friend, R. H. *Macromolecules* **1995**, *28*, 1966–1971.

(39) Liu, B.; Yu, W. L.; Pei, J.; Liu, S. Y.; Lai, Y. H.; Huang, W. *Macromolecules* **2001**, *34*, 7932–7940.

(40) Bredas, J. L.; Cornil, J.; Heeger, A. J. *Adv. Mater.* **1996**, *8*, 447–452.

(41) Najechalski, P.; Morel, Y.; Stephan, O.; Baldeck, P. L. *Chem. Phys. Lett.* **2001**, *343*, 44–48.