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Interpolyelectrolyte Complexes of Conjugated Copolymers and DNA: Platforms for Multicolor Biosensors

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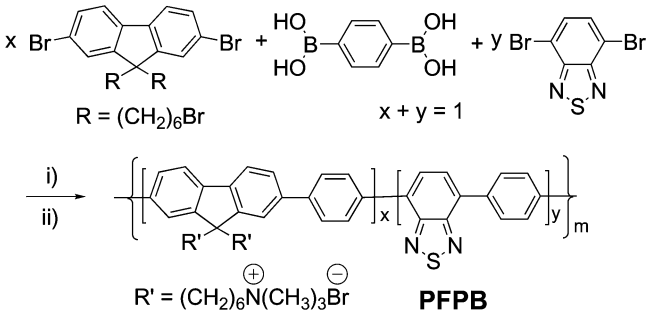
DNA and RNA detection methods are of considerable scientific and technological importance.[1−3] Water-soluble conjugated polymers are being increasingly studied for this purpose because their molecular structure allows for collective response and, therefore, optical amplification of fluorescent signals.[4,5] The large number of optically active units along the polymer chain increases the probability of light absorption, relative to small-molecule counterparts.[5] Facile energy transfer makes it possible to deliver excitations to fluorophores, which signal the presence of a target DNA sequence.[6,7]

Recent studies indicate that energy transfer *between* segments in a conjugated polymer may be substantially more important than along the backbone.[8,9] Molecular interactions that decrease the elongation of the backbone or that bring segments closer together could therefore be used to increase sensitivity. It occurred to us that a small number of optically active units within a polymer sequence could be activated by structural changes that compressed or aggregated the polymer chains. In this communication, we disclose a cationic conjugated polymer structure that incorporates these design guidelines. Furthermore, electrostatic complexation with negatively charged DNA can be used to change the emission of the polymer by reducing the average intersegment distance. When combined with a fluorophore (C*)-labeled peptide nucleic acid strand (PNA-C*), the polymer can be used to design a three-color DNA detection assay.

To obtain two emission colors from a single polymer chain, we introduced 5% of the 2,1,3-benzothiadiazole (BT) chromophore into a cationic poly(fluorene-*co*-phenylene) polymer chain (Scheme 1). The synthetic approach involves a Suzuki copolymerization of *p*-phenylenebisboronic acid with a 95:5 mixture of 2,7-dibromo-9,9-bis(6′-bromohexyl)fluorene and 4,7-dibromo-2,1,3-benzothiadiazole.[10] Elemental analysis of the resulting polymer is consistent with a chemical composition similar to the monomer feed. Since the GPC-determined molecular weight (M_n) is ∼11000 amu, one can estimate that there is, on average, one BT molecule per polymer chain. In a second step, quarternization of the pendant groups by addition of NMe_3 provides the polycationic water soluble **PFPB** (see Scheme 1).

In deionized water, at concentrations below 1×10^{-6} M (in repeat units, RUs), **PFPB** emits predominantly in the 400−500 nm region, with a fluorescence quantum yield (Φ) of 22%. Indeed, both the absorption ($\lambda_{max} = 380$ nm) and the emission are nearly identical to that of poly(9,9-bis(6′-*N*,*N*,*N*,-trimethylammonium)-hexyl)fluorene-*co*-*alt*-1,4-phenylene) dibromide (**PFP**), which lacks BT sites (see Figure 1, A and B).[11] Under dilute conditions, the emission of **PFPB** is dominated by the more abundant oligo(fluorene-*co*-phenylene) segments. When [**PFPB**] $> 1 \times 10^{-6}$ M, one observes the emergence of green emission (500−650 nm), characteristic of the BT sites. As [**PFPB**] increases, the green emission grows at the expense of the blue emission. These data

Scheme 1[a]



[a] i) 2 M K_2CO_3, THF, $Pd(PPh_3)_4$; ii) $N(CH_3)_3$, THF/H_2O.

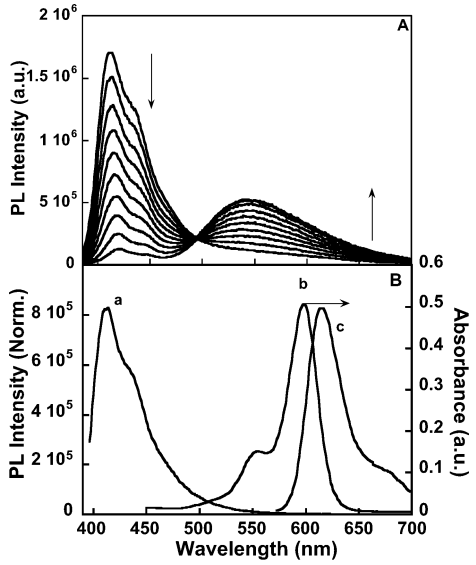


Figure 1. (A) Fluorescence spectra of **PFPB** in water as a function of [ss-DNA] ([RU] $= 5 \times 10^{-7}$ M, [ss-DNA] = 0 M to 2.7×10^{-8} M in 3.0×10^{-9} M increments, $\lambda_{exc} = 380$ nm). (B) (a) Normalized photoluminescence spectra of **PFP**, (b) absorption and (c) emission spectra of ss-DNA-TR in water.

indicate aggregation in the more concentrated regime, which leads to a reduction of the distance between polymer segments and enhances energy transfer to units containing lower-energy BT chromophores.[12,13]

On the basis of well-known interpolymer interactions between oppositely charged polyelectrolytes,[14−17] we anticipated that complexation of **PFPB** with ss-DNA (5′-ATC TTG ACT ATG TGG GTG CT) in water[18] would lead to contraction and aggregation of polymer chains, a concomitant reduction of intersegment distances, and more efficient energy transfer to the BT sites. Figure 1A shows the emission from **PFPB**/ss-DNA solutions upon addition of ss-DNA ([RU] $= 5 \times 10^{-7}$ M, ss-DNA = (5′-ATC TTG ACT ATG

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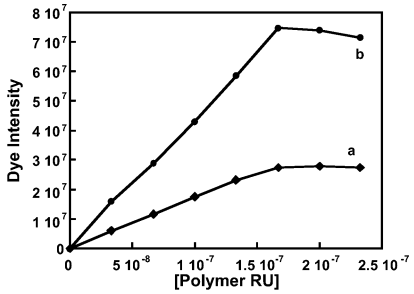

Figure 2. (a) TR emission intensity for **PFP**/ss-DNA-TR and (b) **PFPB**/ss-DNA-TR in water ($\lambda_{exc} = 380$ nm, [ss-DNA-TR] $= 2.0 \times 10^{-8}$ M, TR intensity is corrected to reflect the difference in optical density for the two polymers).

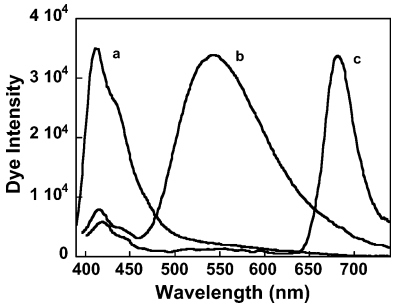

Figure 3. Normalized fluorescence in water of (a) **PFPB**/PNA-Cy5, (b) **PFPB**/DNAn+PNA-Cy5, and (c) **PFPB**/DNAc/PNA-Cy5 ([PNA-Cy5] $= 2.0 \times 10^{-8}$ M, [RU] $= 1.6 \times 10^{-7}$ M, ($\lambda_{exc} = 380$ nm).

TGG GTG CT), [ss-DNA] varies from 0 M to 2.7×10^{-8} M). The isosbestic point at 492 nm highlights the transition from blue to green emission with increasing [ssDNA].

Figure 1B shows the emission spectra of **PFP**, and the absorption and emission of ss-DNA-TR (TR = Texas Red dye and ssDNA-TR = 5′-TR-ATC TTG ACT ATG TGG GTG CT). Note that the spectral overlap between the absorption of TR and the green emission band of **PFPB**/ss-DNA is substantially larger than that with the **PFP** emission. Therefore, we anticipated a larger value for the overlap integral in the Förster equation and more efficient fluorescence resonance energy transfer (FRET) with **PFPB**.[19] Indeed, as shown in Figure 2, the TR emission intensity as a function of polymer concentration, is greater when **PFPB** is excited, relative to **PFB** (the value of Φ for TR is the same in the two solutions). The spectra in Figure 2 were measured by excitation at 380 nm, which selectively creates polymer-based excited states.

On the basis of the mechanistic information above, we postulated that **PFPB** could be used in a three-color DNA assay by using a PNA-C* strand.[6] PNA serves to provide a base sequence that searches complementary ssDNA. However, because PNA is neutral, it is possible to use water without buffer or other ions that are required to screen the negatively charged phosphate backbone during duplex formation.[20] Since PNA-TR is not available commercially, we used PNA-Cy5 (5′-Cy5-CAGTCCAGTGATACG) as the PNA-probe instead. The absorption and emission of Cy5 ($\lambda_{abs} = 648$ nm, $\lambda_{em} = 681$ nm) are similar to those of TR (Supporting Information). Hybridization of PNA-Cy5 with a complementary ss-DNA (ss-DNAc = 5′-CGTATCACTGGACTG) endows the ss-DNAc/PNA-Cy5 duplex with multiple negative charges. Complexation of ss-DNAc/PNA-Cy5 by electrostatic forces to the positively charged **PFPB** allows for energy transfer from the polymer to Cy5 and should lead to red emission. In the case of a noncomplementary ss-DNA (ss-DNAn = 5′-ACTGACGATA-GACTG), electrostatic complexation occurs only between **PFPB** and the ss-DNAn, which should give rise to emission from the BT units.

Figure 3 shows the different emission colors that are observed in this detection scheme. In water (pH = 7.0), a solution of **PFPB** ([RU] $= 1.6 \times 10^{-7}$ M) and PNA-Cy5 emits blue. For the noncomplementary situation ss-DNAn+PNA-Cy5 (annealing protocols are done independently), green emission is predominant. Under similar conditions, when ss-DNAc/PNA-Cy5 is used, only red emission from the Cy5 units takes place. These data indicate that FRET from **PFPB** to the Cy5 signaling chromophore is essentially complete.

In summary, we report design guidelines for water-soluble conjugated polymer structures that change emission color as a result of conformational and aggregation changes. Complexation with oppositely charge polyelectrolytes (such as DNA) brings together polymer segments and encourages energy migration to low-energy emissive sites (BT in the case of **PFPB**). With the aid of PNA-C* probe strands, one obtains three different colors, depending on the solution content: (1) blue, in the absence of DNA, (2) green, when noncomplementary ssDNA is present, (3) and red, when the complementary ssDNA is found. Fine-tuning of these electrostatic and optical events could lead to multicolor biosensor schemes that take advantage of the fluorescence amplification characteristic of conjugated polymers.

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Supporting Information Available: Details for the synthesis of **PFPB** and FRET experiments (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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