Communication

Shape-Adaptable Water-Soluble Conjugated Polymers

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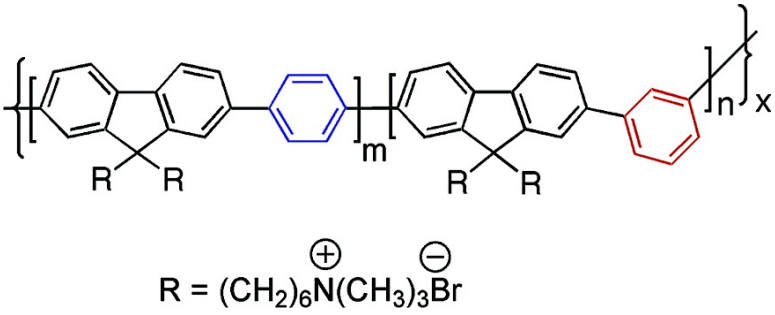

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Shape-Adaptable Water-Soluble Conjugated Polymers

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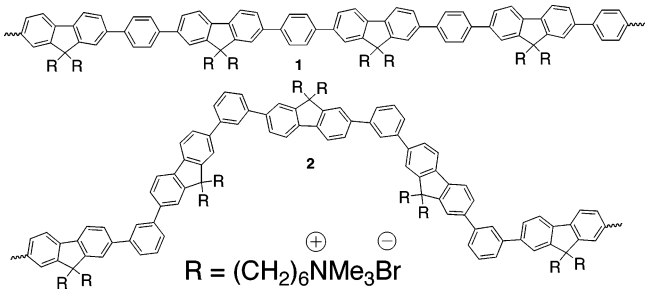
There is substantial and growing interest in using conjugated polymers (CPs) as the responsive basis for chemical and biological detection schemes. It has been pointed out, and experimentally demonstrated, that CPs provide the advantage of collective response, relative to noninteracting small molecules.[1,2] This collective response influences optoelectronic properties, such as Förster resonance energy transfer (FRET), electrical conductivity and fluorescence efficiency; properties which can be used to report, or "transduce", target analyte presence.[2]

Water solubility, a prerequisite for interrogating biological substrates, is usually achieved by charged groups on the CP backbone.[3] These charges permit the coordination of electrostatic forces with oppositely charged analyte targets,[4,5] so that the collective optical response of CPs can be used to amplify fluorescence-based transduction.[6]

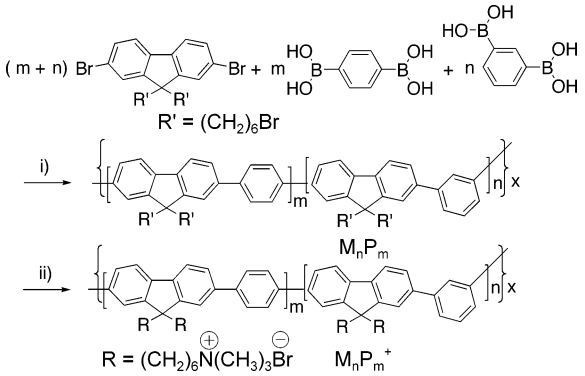
Cationic CPs (CCPs), in particular, have proven useful for strand-specific DNA detection,[7−9] a topic of intense current interest.[10−12] CCPs reported in the literature have linear backbone structures and thus a "rigid-rod" aspect ratio.[3] Polymer **1** is typical of this class of materials.[6] Such a structure cannot adapt to the range of secondary structures presented by biological macromolecules. A backbone such as that in polymer **2**, with a 120° orientation between the fluorenyl units, would have more conformational freedom and improved registry with analyte shape. With better spatial interactions one can expect improved contacts between optically active groups and therefore more efficient FRET from the CCP to dyes on diagnostic probe structures.



In this communication we report on a synthetic method for producing CCPs with a range of backbone regiochemistries. We show that, despite structural differences which affect the average conjugation length, there is facile energy transfer among polymer segments which results in similar emission properties and FRET function. Furthermore, the nonlinear CCPs are more efficient excitation donors.

The introduction of nonlinear "kinks" along a linear polymer structure is accomplished by varying the ratio of *p*- and *m*-phenylene bisboronic acids in a Suzuki copolymerization with 2,7-dibromo-9,9-bis(6′-bromohexyl)fluorene (Scheme 1).[13] Purification by precipitation yields the resulting M_nP_m polymers, where the subscripts in M_n and P_m correspond to the percentage meta units and para units in the polymer structure. NMR spectroscopy of the polymers is consistent with a stereochemical composition equal to that in the monomer feed. A random incorporation of meta and para linkages is most likely. Addition of NMe_3 to the neutral polymers gives the cationic species ($M_nP_m^+$). GPC analysis shows molecular weights in the 15 000 to 20 000 amu range.

Scheme 1[a]



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

Table 1. Optical Properties of the Polymers

$M_nP_m^+$	$\lambda_{max,abs}$	$\lambda_{max,em}$	ϵ[a]	Φ_{buffer}[b]
$M_{100}P_0^+$	335	369	37	0.51
$M_{90}P_{10}^+$	337	403	32	0.57
$M_{75}P_{25}^+$	347	410	30	0.50
$M_{50}P_{50}^+$	361	421	32	0.44
$M_{25}P_{75}^+$	376	417	42	0.42
$M_0P_{100}^+$	384	417	46	0.42

[a] unit: 10^3 L cm^{-1} mol^{-1}. [b] 50 mmol phosphate buffer, quinine bisulfite as the standard.

UV−vis and fluorescence spectra for a range of compositions are summarized in Table 1. There is a progressive blue shift in absorption with increasing meta content, consistent with the more effective electronic delocalization across para linkages. The ϵ values are lowest for polymers with intermediate compositions because the random distribution of conjugated segments results in broader absorption bands.

Figure 1 shows the fluorescence spectra in water as a function of polymer composition. Increasing the para content past the 50:50 ratio does not perturb the emission maxima. Fast energy transfer, either by intra- or interchain mechanisms, localizes excitations on the longest conjugation segments within the lifetime of the excited state.[14] Table 1 shows that there is little variation in the fluorescence quantum yields (Φ in Table 1).

Equation 1 describes how the FRET rate changes as a function of the donor−acceptor distance (r), the orientation factor (κ), and the overlap integral (J).

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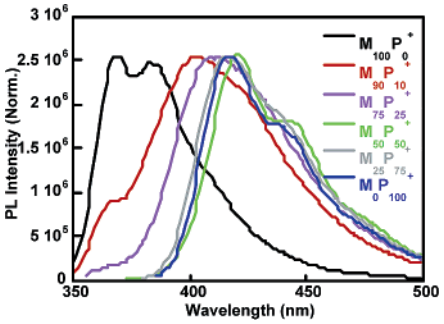

Figure 1. Normalized photoluminescence spectra of $M_nP_m^+$ in water. The excitation wavelength is the absorption maximum for each polymer.

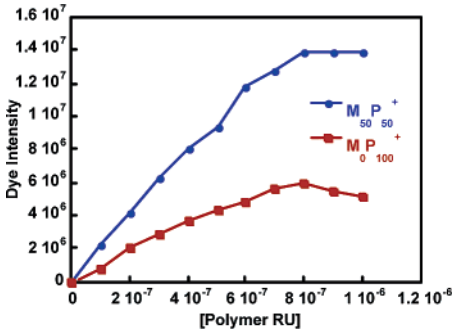

Figure 2. C* emission intensity for $M_{50}P_{50}^+$/ds-DNA−C* (blue) and $M_0P_{100}^+$/ds-DNA−C* (red) in 50 mmol phosphate buffer (pH = 8.0) at a [ds-DNA−C*] = 2.0×10^{-8} M.

$$k_{t(r)} \propto \frac{1}{r^6} \cdot k^2 \cdot J(\lambda) \qquad (1)$$

$$J(\lambda) = \int_0^\infty F_D(\lambda)\varepsilon_A(\lambda)\lambda^4 d\lambda$$

Since $M_{50}P_{50}^+$ and $M_0P_{100}^+$ have similar emission frequencies, the value of J using a common acceptor dye should be nearly identical between the two polymers. We also note that the fluorescence lifetimes of the two polymers are similar (400 ± 50 ps). Therefore, differences in FRET efficiencies to a common acceptor chromophore will extract information relevant to the average polymer/acceptor chromophore distance and the orientation of transition moments.

To examine the effect of polymer structure on the interactions with a biological substrate, we examined FRET from $M_{50}P_{50}^+$ or $M_0P_{100}^+$ to a double-stranded DNA containing fluorescein (C*) at the 5′ position (dsDNA−C*). Figure 2 shows the C* emission intensity as a function of polymer concentration, upon excitation of $M_{50}P_{50}^+$ or $M_0P_{100}^+$ at 363 nm. This wavelength was chosen because there is no significant C* absorption and the two polymers have similar ϵ values. Excitation thus leads to a similar number of polymer-based excited states. We also confirmed that the value of Φ for C* is the same in the two sets of solutions. The data in Figure 2 show more efficient FRET from $M_{50}P_{50}^+$, consistent with a shorter distance to dsDNA−C*, or with more variable orientation of the transition moments (improved κ).

A second set of experiments involved FRET from the CCPs to ds-DNA with intercalated ethidium bromide (EB).[15,16] EB emission occurs only from FRET to the intercalated moieties, upon excitation of the CCPs. Figure 3 shows more efficient transfer in the series $M_0P_{100}^+ \rightarrow M_{25}P_{75}^+ \rightarrow M_{50}P_{50}^+ \rightarrow M_{75}P_{25}^+$ ($M_{100}P_0^+$ was not tested because its emission spectrum does not overlap significantly with

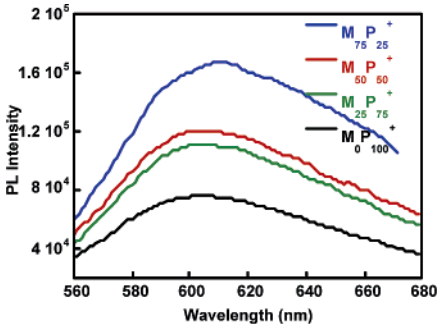

Figure 3. Comparison of the intensity of EB emission for polymer/ds-DNA/EB in 50 mmol phosphate buffer (pH = 7.4) with [ds-DNA] = 1.0×10^{-8} M, [Polymer RU] = 2.0×10^{-7} M, [EB] = 1.1×10^{-6} M. Emission intensity was normalized relative to the ϵ value at the excitation wavelength.

the absorption spectra of EB ($\lambda_{max,abs}$ = 530 nm)). A clear improvement in FRET therefore takes place with increased meta contents in the polymer.

In summary, a novel synthetic entry into CCPs with increased conformational freedom is now available (Scheme 1). These materials conform more tightly to the secondary structure of ds-DNA, resulting in improved FRET efficiencies. These findings should enable the improvement of fluorescent DNA assays that take advantage of the collective response of conjugated polymers. Similar results are expected for analogous sensors based on specific protein−DNA or protein−RNA interactions.[17]

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Note Added After ASAP Posting. The minus signs were missing from the exponents in the captions for Figures 2 and 3 in the version posted ASAP on October 10, 2003; the corrected version was posted on October 15, 2003.

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

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