A novel cationic conjugated polymer for homogeneous fluorescence-based DNA detection†

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A novel water-soluble cationic conjugated polymer, poly({2,5-bis[3-(*N,N*-diethylamino)-1-oxapropyl]-*para*-phenylenevinylene}-*alt*-*para*-phenylenevinylene) dibromide, was synthesized and used to develop a simple label-free DNA detection essay.

Conjugated polyelectrolytes (CPEs) are versatile new materials that combine features of conventional conjugated polymers and polyelectrolytes, resulting in unique electrical and optical properties that have made them ideally suited for applications in electrooptic devices,[1] biosensors and drug delivery.[2–4] Modification of CPEs with anionic or cationic functional groups yields materials that possess the properties of conjugated polymers but are also water soluble which is essential for interfacing with biological substrates such as proteins and DNA. These water-soluble conjugated polymers are attractive sensor materials because their electrical, optical and optoelectric properties can be greatly modified by small perturbations of the local electrochemical environment.[5] The enormous potential of this approach was first demonstrated by Chen *et al.* who developed a sensitive 'real-time' biosensor for detection of aromatics and proteins based on poly(2-methoxy-(5-propyloxy)sulfonate phenylenevinylene).[6]

Cationic conjugated polymers (CCPs), in particular, have proven to be very useful for DNA sequence detection based on electrostatic interaction with the negatively charged phosphate backbone.[4,7–9] Previously reported CCPs for DNA detection were either polythiophene derivatives[4,10,11] or poly(fluorene-*co*-phenylene) derivatives.[7,12–14]

Poly(*para*-phenylenevinylene) (PPV) is a well-known luminescent conjugated polymer. Recently, considerable research has been focused on anionic PPV derivatives as substrates for chemo- or bio-sensors.[6,15–18] In these sensors, amplified fluorescence quenching is achieved by electron or energy transfer due to a strong association between polymers and oppositely charged quenchers resulting in efficient energy migration and exciton delocalization across the polymer.[6,16] However, the anionic charge of these PPV derivatives makes detection of equally charged anionic biomolecules, such as DNA, problematic. Here, we report the synthesis of a novel cationic water-soluble PPV, poly({2,5-bis[3-(*N,N*-diethylamino)-1-oxapropyl]-*para*-phenylenevinylene}-*alt*-*para*-phenylenevinylene dibromide ($PPVNEt_2Br_2$) that overcomes this limitation as demonstrated by a novel DNA assay and is an addition to the currently limited number of available CCPs.

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Fig. 1 shows the sequence of reactions to obtain poly({2,5-bis[3-(*N,N*-diethylamino)-1-oxapropyl]-*para*-phenylenevinylene}-*alt*-*para*-phenylenevinylene) ($PPVNEt_2$). 2,5-Bis(3-[*N,N*-diethylamino]-1-oxapropyl)-1,4-diiodobenzene was synthesized according to ref. 19. $PPVNEt_2$ was obtained by a Heck coupling reaction, using a catalytic system containing palladium acetate, tributylamine and tri-*ortho*-tolylphosphine under a nitrogen atmosphere (see ESI† for details). The weight-average molecular weight of $PPVNEt_2$, measured by gel permeation chromatography (GPC) in THF, is 9 kDa with a polydispersity of 2.8.

The cationic, water soluble polymer was prepared from neutral $PPVNEt_2$ by quaternization with bromoethane, as shown in Fig. 1. Fig. 2 presents UV-vis absorption and photoluminescence spectra of $PPVNEt_2$ (in chloroform) and $PPVNEt_2Br_2$ (in water). Clearly, a shift in absorption maxima occurred between the neutral and charged polymers. $PPVNEt_2$ exhibits maximal absorption at 454 nm while the absorption peak of $PPVNEt_2Br_2$ is blue shifted by 42 nm to 412 nm. This shift may be due to a solvatochromic effect, also observed in substituted polythiophenes[20] and poly(diacetylene).[21] The photoluminescence spectra of $PPVNEt_2$ and $PPVNEt_2Br_2$ were recorded in chloroform and water, respectively, while excited at the wavelength of maximal absorption. The emission peak wavelengths were 517 and 520 nm for $PPVNEt_2$ and $PPVNEt_2Br_2$, respectively.

Homogeneous DNA fluorescence analysis has great importance due to its high sensitivity and simple operation. In general, labeling of two nucleic acids or dual modification of the same altered strand are necessary in such methods to achieve sequence specificity.[22] In this work, the synthesized cationic polymer was used to detect specific hybridization with minimal DNA

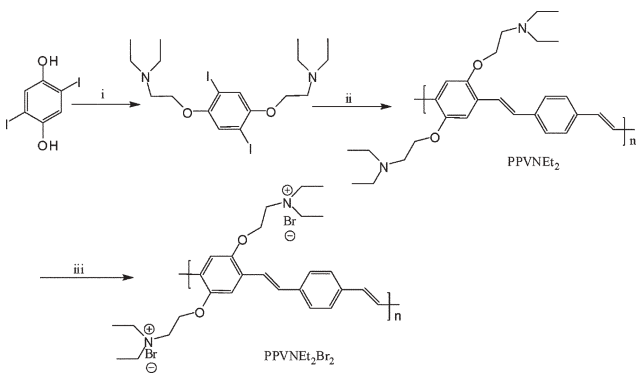

Fig. 1 Synthesis of $PPVNEt_2^+$. i, 2-Chlorotriethylamine hydrochloride, K_2CO_3, acetone, 71%; ii, *para*-divinylbenzene, palladium acetate, tri-*ortho*-tolylphosphine, tri-*n*-butylamine, DMF, 65%; iii, bromoethane, THF, 90%.

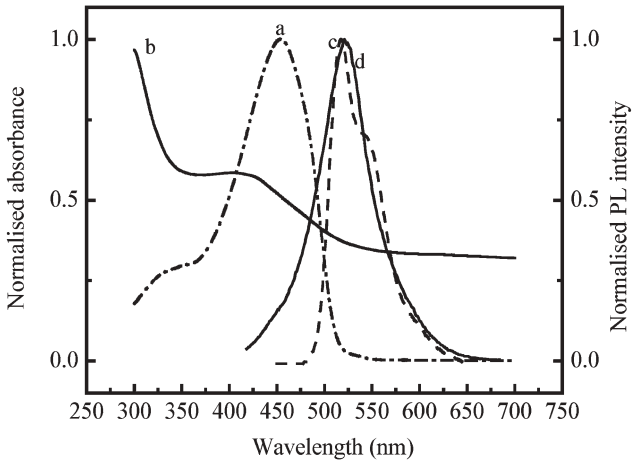

Fig. 2 UV-vis absorption and normalised photoluminescence spectra. $PPVNEt_2$ absorption (a) and emission (c) in chloroform. $PPVNEt_2Br_2$ absorption (b) and emission (d) in water.

modification. The chromophore dye Cy3 was chosen in order to facilitate Förster resonance energy transfer (FRET) from the polymer (donor) to the dye (acceptor).[23] The absorption and emission spectra of Cy3 labeled oligonucleotides (ODNs) in 100 mM NaCl and 10 mM sodium citrate (SSC buffer, pH 7.9) are shown in Fig. 3. The absorption spectrum of Cy3-ODN has significant overlap with the emission of $PPVNEt_2Br_2$ (Fig. 2d), an important condition for FRET.[23] Fig. 3 also shows the emission spectrum of a solution containing $PPVNEt_2Br_2$ and Cy3-ODN when exited at 400 nm exhibiting strong emission from Cy3 (with negligible direct excitation of Cy3 at 400 nm, see inset, Fig. 3), which illustrates efficient FRET from the polymer to Cy3. There is a ~5 nm red shift in the Cy3 emission in the presence of the polymer, presumably due to an increase in polarity in the vicinity of Cy3 by the interaction with the cationic polymer.[8]

To test how FRET was affected by the presence of sample ODNs hybridization was carried out in SSC buffer (pH 7.9) at room temperature for half an hour in the presence of $PPVNEt_2Br_2$ and Cy3-ODN. Fig. 4 shows FRET emission spectra of $PPVNEt_2Br_2$/Cy3-ODN after hybridization with different concentrations of complementary ODN. It can be seen from Fig. 4 that the FRET ratios progressively decreased with increasing concentration of complementary ODN. One likely reason is that a polymer chain, which is in conformation of a flexible random coil, strongly interacts with single stranded ODNs through the electrostatic and hydrophobic interactions that bring them in intimate contact. On the formation of the stiffer ODN duplex structure the distance between polymer and dye increases resulting in a decrease of FRET efficiency. Liu *et al.* illustrated that hydrophobic interactions also play an important role for efficient FRET besides electrostatic interactions.[24] After hybridization, the hydrophobic bases in the double-stranded ODN are packed inside the helix, which minimizes external hydrophobic interactions and thus contributes to a decrease of the FRET ratio.[24]

After incubation with non-complementary ODN, FRET ratios also decrease (Fig. 5), but to a much smaller extent than in the presence of corresponding concentrations of complementary ODN, as shown in the inset of Fig. 4 and Fig. 5. This non-specific interaction probably occurs because added negatively charged non-complementary ODNs compete with ODN-Cy3 in the ODN-Cy3/$PPVNEt_2^+$ duplex causing a decrease of the FRET ratio.

In summary, we synthesized a novel cationic water-soluble conjugated polymer, $PPVNEt_2Br_2$. This polymer has been used for homogeneous DNA hybridization detection in real time. The results illustrate that it is possible to detect specific DNA fragments in solution by using a singly labelled ODN probe strand. Further optimization of the structure and property of CCPs may lead to simple and practical analysis platforms for DNA hybridization.

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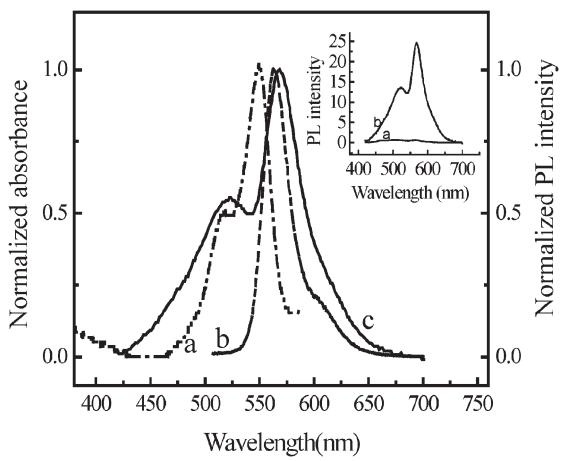

Fig. 3 Normalized absorption (a) and emission (b) spectra of ODN-Cy3 (5′-Cy3-TCGGCATCAATACTCATC-3′) in SSC buffer upon excitation at 488 nm. Normalized emission spectrum (c) of $PPVNEt_2^+$/ODN-Cy^3 (0.6 μM)) duplex in SSC buffer upon excitation at 400 nm. Inset: Relative spectral emission intensity of ODN-Cy^3 in SSC buffer upon excitation at 400 nm: (a) in the absence of $PPVNEt_2Br_2$; (b) in the presence of $PPVNEt_2Br_2$.

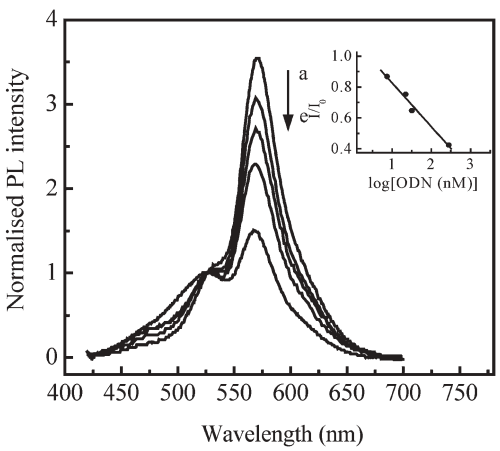

Fig. 4 Emission spectra of $PPVNEt_2^+$ (0.037 mg ml^{-1})/ODN-Cy3 (1.2 μM) duplex after hybridization with different concentrations of complementary ODN target (5′-GATGAGTATTGATGCCGA–3′): (a) 0 nM; (b) 7.41 nM; (c) 22.2 nM; (d) 32.1 nM; (e) 279.1 nM. The spectra were normalised to polymer emission. Inset: Normalised peak intensity decrease in the presence of different concentrations of complementary ODN (I_0 is the peak emission at 0 nM ODN).

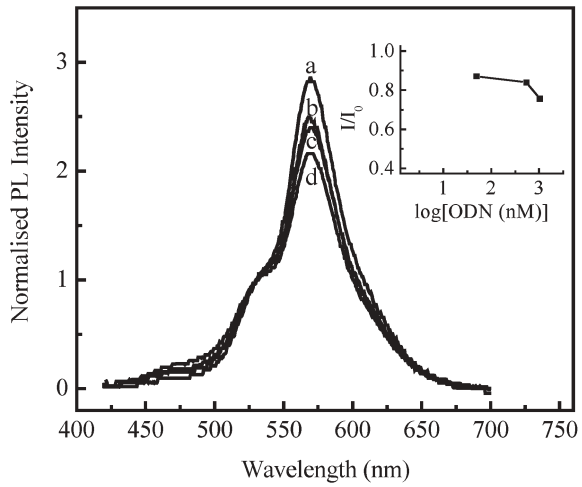

Fig. 5 Emission spectra of $PPVNEt_2^+$ (0.37 mg ml^{-1})/ODN-Cy3 (1.2 μM) duplex after addition of different concentrations of non-complementary ODN (5′-TCGGCATCAACACTCATC-3): (a) 0 nM; (b) 49.4 nM; (c) 543.4 nM; (d) 1.04 μM. The spectra were normalised to polymer emission. Inset: Normalised peak intensity decrease in the presence of different concentrations of non-complementary ODN (I_0 is the peak emission at 0 nM ODN).

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