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Biotinylated poly(*p*-phenylene ethynylene): unexpected energy transfer results in the detection of biological analytes†

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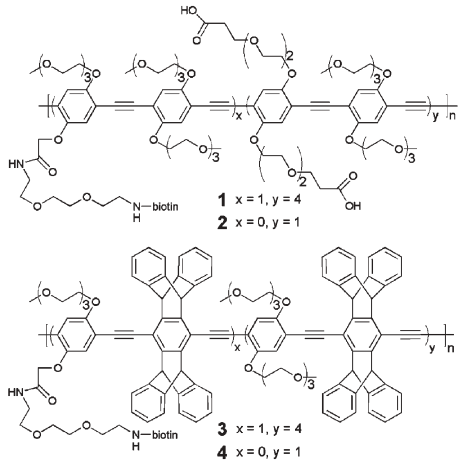
Decreased spectral overlap between a donor biotinylated poly(*p*-phenylene ethynylene) and a chromophore-labeled streptavidin acceptor leads to better observed fluorescence resonance energy transfer.

In recent years, the fluorescence properties of conjugated polymers (CPs) have been actively investigated in the design of highly sensitive chemical and biological sensors, the majority of which have been based upon the amplification of fluorescence quenching.[1] In contrast to turn-off sensors, a turn-on sensor using fluorescence resonance energy transfer (FRET) with CPs as light-harvesting donors[2] has the advantage of being more sensitive and selective. Although FRET is a tool widely used in biology to study biomolecular structure and dynamics,[3,4] its use with CPs as a method of transduction for sensing biological molecules is not common.[5,6] Here, we report a model biosensor based on the multivalent interactions between biotinylated poly(*p*-phenylene ethynylene) and fluorophore-labeled streptavidin.

Streptavidin is a tetrameric protein that binds up to four molecules of *d*-biotin with the dissociation constant estimated to be 4×10^{-14} M.[7] Because of this high affinity, the streptavidin–biotin recognition system has been applied to model biosensor design in conjunction with conjugated polymers in affinitychromic[8,9] and agglutination assays.[10] Here a water-soluble biotinylated poly(*p*-phenylene ethynylene) (PPE) (**1**) and its non-biotinylated relative (**2**) were synthesized for solution energy transfer (ET) experiments *via* a Sonagashira–Hagihara cross-coupling reaction.[11,12] Analogously, an organic solvent-soluble biotinylated PPE (**3**) and its non-biotinylated variation (**4**) useful for solid phase thin-film experiments were also synthesized.[13]



Polymer **1** was constructed from two diiodobenzene monomers at loading ratios of 1 : 4 (biotinylated to non-biotinylated monomers) that were polymerized by a cross-coupling reaction with a diacetylene monomer. The mono-substituted biotinylated monomer used in the synthesis of **1** was designed to provide binding accessibility for streptavidin while minimizing the divalent binding of one streptavidin onto the same repeat unit, if it were symmetrically functionalized with biotin.[14] Polymers **3** and **4** were designed with a pentiptycene in the backbone to promote greater thin-film quantum yield.[1b]

As an initial assay, biotinylated polymers **1** and control polymer **2** were incubated with fluorescein-labeled streptavidin (3.5 dyes/protein) at room temperature, in 50 mM Tris buffer at pH 7.5 for five minutes. Fluorescein was selected as its absorbance maximum at 490 nm overlaps well with the emission maximum of polymer **1** at 486 nm (excitation at 440 nm). This would favor FRET by the Förster mechanism between the polymer donor and dye acceptor upon binding of labeled streptavidin to biotin. When 0.030 nmol of labeled streptavidin was added to 2.16 nmol of **1**, an increase in the fluorescein's emission was observed. The overlapping fluorescence spectra were deconvoluted to separate fluorescein's emission from that of the polymer. Although the degree of enhancement in the fluorescence emission was low, these results indicate that biological recognition is necessary for ET from the polymer to the dye-labeled streptavidin.

In order to better visualize ET between the polymer donor and dye acceptor, a more red-shifted rhodamine B-labeled streptavidin (RhB-strept) was used in the solution phase ET assays with **1**. To our surprise, higher ET was observed even though RhB had a diminished spectral overlap with **1** (emission maximum **1**: 486 nm, absorption maximum RhB-strept: 574 nm, 4.6 dyes/protein). At this point we decided to screen **1** with Texas red X™-labeled streptavidin (T-red-strept) (absorption maximum 591 nm, 2.9 dyes/protein). Remarkable ET was observed. For both dyes the emission due to ET was amplified compared to direct excitation of the dyes at their absorbance maximum (Fig. 1). This is consistent with the light-harvesting properties of conjugated polymers. Control experiments with **2** showed no ET upon addition of both dye-labeled streptavidin derivatives. A control experiment with the addition of a biotin pre-saturated solution of T-red-strept to biotinylated **1** was also carried out. Again no decrease in fluorescence of the polymer and no ET to the dye were observed.

The quantum yields of the streptavidin-bound dyes vary upon binding to polymer **1**, presumably due to an aggregation or an environmental change in their vicinity. This effect is observed by directly exciting the dyes at their maximum absorbance (where the polymer does not absorb) using the same polymer concentration as in Fig. 1. In the presence of **1**, RhB-strept's quantum yield was diminished by 38% while that of T-red decreased by 63%. Nevertheless, greater emission intensity was observed for T-red-strept (Fig. 1) despite the greater decrease in its quantum yield as compared to RhB-strept. The strong emission response from T-red-strept is therefore not due to a simple improvement in its quantum efficiency.

To study the nature of the interactions between dye-labeled streptavidin and **1** we determined the Stern–Volmer quenching constants from fluorescence emission and lifetime measurements in 50 mM Tris buffer at pH 7.4. Upon addition of the streptavidin-free fluorescent dyes (fluorescein, RhB and sulfo-rhodamine 101 (Texas red™ parent dye)) to **1**, the apparent K_{SV} values were determined to be 26 300, 91 800 and 97 900 M^{-1} respectively. The

† Electronic supplementary information (ESI) available: experimental details, synthesis of the polymers and control experiments. See http://www.rsc.org/suppdata/cc/b4/b408478k/

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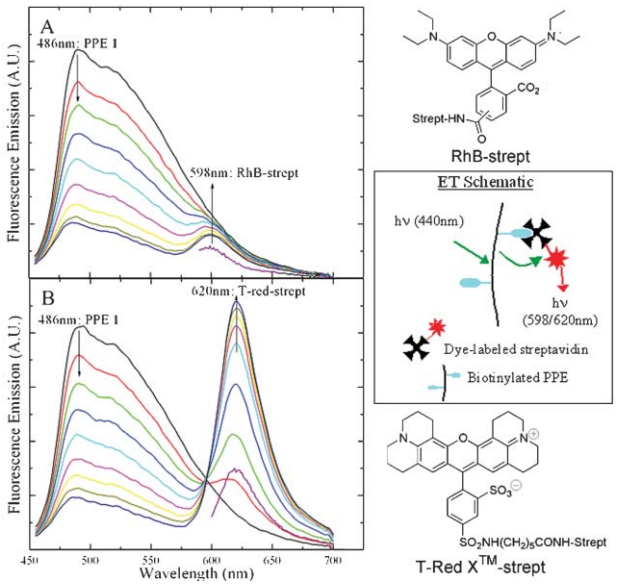

Fig. 1 Addition of 0.017 nmol aliquots of (A) RhB-strept, (B) T-red-strept to 1.51 nmol of **1**. ET observed in both cases with amplified emission of dyes due to the light-harvesting conjugated polymers. Direct excitation of the dyes at 575 and 585 nm correspond to 0.100 nmol of streptavidin.

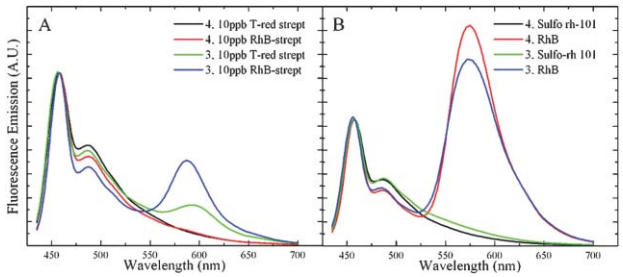

Fig. 2 Thin-films of (A) **3** and **4** treated with dye-labeled streptavidin and 1% Triton X-100: ET upon binding of streptavidin to **3**. (B) **3** and **4** treated with free dyes and 1% Triton X-100. RhB exhibits better association to both polymers. Sulfo-rhodamine 101 had little affinity. Spectra scaled to 454 nm.

bi-molecular quenching constant k_q ranged from 1.25×10^{14} to 3.4×10^{14} $M^{-1}s^{-1}$ for the three dyes, which greatly exceeded the diffusion constant and is indicative of static quenching. The dyes therefore had an inherent affinity for the conjugated polymer backbone. A more planar conformation and greater hydrophobic character for Texas red™ compared to RhB and fluorescein may permit better stacking and orbital interaction with the CP backbone, allowing for greater ET. In the case of dye-labeled streptavidin, the biological recognition first brought the dyes into closer proximity with the polymer. Conformational and hydrophobic characteristics of the dyes then tailored the extent of orbital mixing with the polymer; the flatter Texas Red™ interacted most intimately with the planar conjugated polymer backbone. This may contribute to the better ET even at decreased spectral overlap between the CP donor and dye acceptor.

Thin film experiments have demonstrated to have superior sensitivity[1b] and these were conducted with **3** and **4**. Incubation with the dye-labeled streptavidin was performed in the presence of Triton X-100, a non-ionic detergent, to diminish non-specific binding. It was observed that RhB-strept exhibited better ET than T-red-strept (Fig. 2A). However a small shoulder due to non-specific binding was nonetheless observed in the case of polymer **4** incubated with RhB-strept. This finding suggests that the smaller RhB dye is able to interact more intimately with the sterically restrictive structure of polymers **3** and **4**, leading to greater ET. To verify the affinity of the dyes with the conjugated polymers, incubation of polymers **3** and **4** was carried out with the streptavidin-free dyes (Fig. 2B). Indeed, the free RhB dye associated with both **3** and **4**, while free sulfo-rhodamine 101 (Texas red™) associated with neither (Fig. 2B). ET is therefore significantly dependent on factors that influence the degree of interaction between the polymer and dye.

In summary, we have designed a sensitive turn-on model biosensor based on ET between biotinylated polymer and dye-labeled streptavidin. An increased energy transfer was observed with decreased spectral overlap and may be due to the degree of orbital interaction between the dye and conjugated polymer.

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