Biocompatible post-polymerization functionalization of a water soluble poly(*p*-phenylene ethynylene)†

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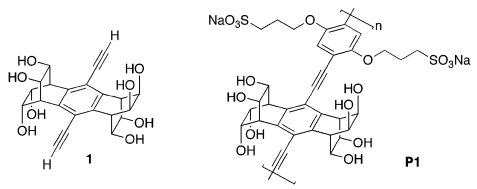
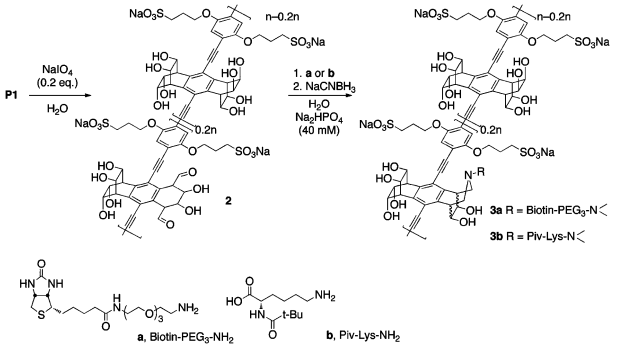
A biocompatible post-polymerization functionalization reaction takes advantage of a polymer's structural motif for the controllable attachment of biotin as a model biosensor that responds to streptavidin.

Strategies for the post-polymerization functionalization (PPF) of polymers are advantageous in that they allow for tuning of a polymer's properties without synthetically retreating to the monomer stage. Further, PPF permits the incorporation of functional groups that may be incompatible with polymerization conditions. Several strategies have been reported for conjugated polymers. A number of designs involve substitution reactions with pendant halogen,[1] alcohol,[2] or carboxylic acid moieties,[3] and application of high yielding click chemistries[4] like the 1,3-dipolar cycloaddition of alkynes and azides[5] or thiol-conjugate addition[6] have also been reported. Two potential drawbacks are characteristic of the above strategies: (i) an appropriately functionalized monomer specific for the intended PPF must be incorporated into the polymerization—often in protected form and (ii) it can be difficult to control the extent of functionalization.



We recently reported the synthesis of a rigid hydrophilic monomer (**1**) that—when incorporated into poly(*p*-phenylene ethynylenes) (PPEs)[7] (**P1**)—leads to increased spectral purity by preventing hydrophobically induced aggregate emission.[8] We envisioned that the three dimensional array of vicinal hydroxyl groups might be further elaborated through periodate oxidation and reductive amination (**P1** → **2** → **3**, Scheme 1).[9] Similar processes have been widely applied for bioconjugation through periodate oxidation of carbohydrate residues, making this process compatible with existing bioconjugation schemes. Herein we report a biocompatible post-polymerization biotinylation of **P1**, where (i) the need for a PPF specific monomer is negated by activation of an existing structural motif (already present in the polymer for purposes outlined above) and (ii) the extent of functionalization can be controlled by the equivalents of the $NaIO_4$ reagent. Further, the improved spectral purity imparted by the presence of **1** in **3a** is not lost. In turn, this results in a more sensitive signal amplified



Scheme 1 Periodate oxidative activation and reductive amination.

biosensor[10] response to fluorophore-labeled streptavidin, a tetrameric protein with high biotin affinity (4×10^{-14} M)[11] that has been applied to a variety of conjugated polymer affinitychromic[3,12] and agglutination[2b] biosensor designs.

Treatment of **P1** with 0.2 equivalents of $NaIO_4$ in water generated 1,6-dialdehyde moieties at random positions along the backbone (**2**, Scheme 1).[13] Subsequent incubation with an excess of amine-containing compound (**a** or **b**) in aqueous alkaline solution generated the putative Schiff base, which was reduced *in situ* to the tertiary amine **3** with $NaCNBH_3$.

The azepane linkage in **3** is proposed based on two model studies. Firstly, the broad nature of the ^{1}H NMR signals of **3a** overlapped with the weaker biotin signals making determination of the extent of functionalization difficult. Thus, **3b**—exhibiting a strong, unobstructed pivalamide signal—was prepared under identical conditions for **3a**. Integration analysis revealed a 18–20% incorporation of **b** (Fig. S3, ESI†).[14] Therefore, while 0.2 equivalents of $NaIO_4$ oxidant should generate 0.4 aldehyde equivalents, there appears to only be 0.2 equivalents of the incorporated amine.

Secondly, acetonide protected **4**—a synthetic intermediate in the synthesis of **1**[8]—was treated with periodate anion and produced the tetraaldehyde **5** (Scheme 2a). Addition of an excess of butyl amine and $NaCNBH_3$ to **5** in methanol gave **6** as the major product. Such products have been observed for bridging 1,6-dialdehydes[15] and likely form *via* a 7-*exo*-trig reductive cyclization to install one amine for every dialdehyde present (Scheme 2b). Thus, we propose the PPF in Scheme 1 proceeds in an analogous manner, allowing for the extent of functionalization to be controlled by the molar equivalents of $NaIO_4$.

The effect of the described PPF method on the photophysical properties of the polymer can be seen in Fig. 1a and b. The absorbance and fluorescence maxima of **3a** show excellent overlap with the parent polymer **P1** in both water and PBS solution, indicating that the oxidation and reductive

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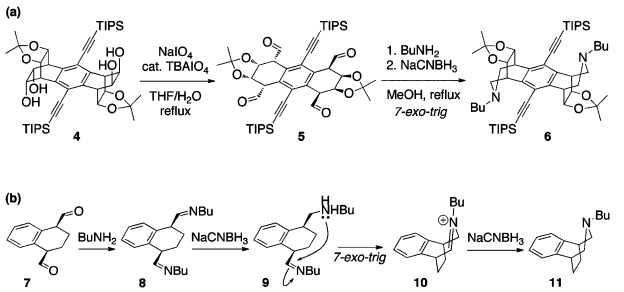

Scheme 2 (a) Model reductive amination product and (b) proposed mechanism.

amination reactions leave the conjugated polymer backbone intact. The origin of the reduced quantum yield of **3a** is unclear. The possibility of excited state photo-electron transfer from the newly installed amine lone pairs to the polymer was examined by varying the pH but no effect was found (pH = 1–12, Fig. S5, ESI†). The reduced quantum yield may be attributed to replacing diol moieties with the relatively insoluble biotin, leading to a more aggregated state of the polymer and diminished quantum yield. In any event, the effect of incorporating **1** in **3a** is still present as no lower energy excimer emission is observed and spectral purity is maintained.

The response of **3a** in the presence of streptavidin is represented schematically in Fig. 1c, where Texas Red X™-labeled streptavidin (TRXS) is able to aggregate the biotinylated polymers (**3a**). Amplification is achieved through the funneling of polymer excitons to the lower energy Texas Red X™ dyes through intra- and interchain energy migration within the supramolecular aggregate. The results of serial additions of TRXS to **3a** at room temperature in PBS solution are shown in Fig. 1d. As anticipated, a decrease in the **3a** emission and a corresponding increase in dye emission was observed. The amplifying effect of the polymer sensor can be seen through direct excitation of the dye (Fig. 1e, red). Finally, incubation of TRXS with **P1** showed no response (Fig. 1e, black).

To better understand the nature of the interaction between TRXS and **3a**, we determined the Stern–Volmer quenching constant for the polymer emission (460 nm) in Fig. 1d. The Stern–Volmer plot showed positive curvature (Fig. S6, ESI†), which is likely due to additional energy migration pathways within the polymer assembly[16] produced by the strong biotin–streptavidin association. Further, no detectable excited state lifetime change was observed with increasing TRXS concentration, indicating that static quenching is the dominant mechanism of energy transfer.

Compared with previous systems,[10] a 100 fold greater K_{SV} of 2×10^7 mol^{-1} was found. This higher sensitivity is likely due to enhanced energy transfer through avoidance of lower energy excimers. These states—negated by the presence of **1**[8]—are localized and perhaps too low in energy to undergo transfer to the dye.

In summary, a biocompatible PPF strategy has been developed, which takes advantage of existing monomer functionality and design. Further, the extent of functionalization can be controlled through the equivalents of $NaIO_4$. Finally, a highly sensitive ($K_{SV} = 2 \times 10^7$ mol^{-1}) turn-on model biosensor

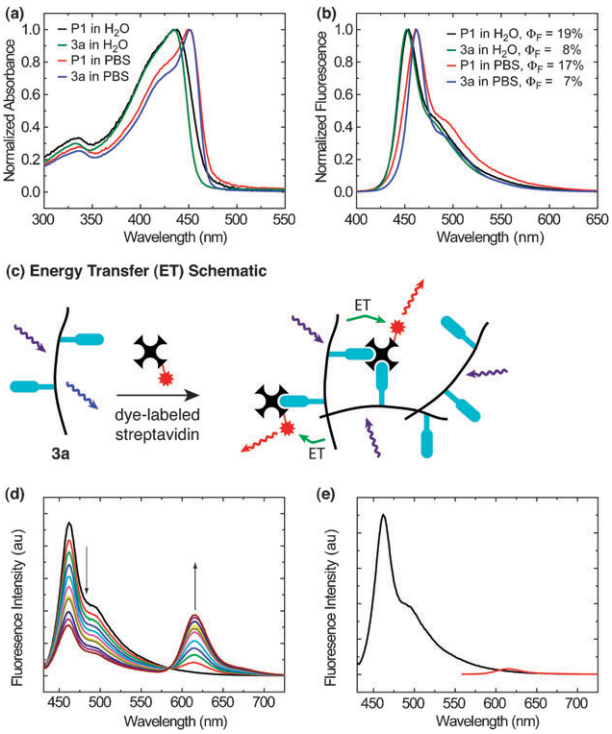

Fig. 1 (a) Absorbance spectra of **P1** and **3a** in water and PBS solution. (b) Fluorescence spectra of **P1** and **3a** in water and PBS solution. (c) Energy transfer schematic showing how intra- and interchain exciton migration and energy transfer to TRXS can lead to amplification. (d) Addition of 9.15 pmol aliquots of Texas red X™-labeled streptavidin to 3.46 nmol (based on repeat unit of **3a**). (e) Excitation of **P1** in the presence of 100 pmol TRXS (black) and direct excitation of 100 pmol of TRXS (red).

based on ET between **3a** and TRXS was demonstrated where the presence of **1** led to dramatically increased sensitivity.

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