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

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# 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 retreatting 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, alcohol, or carboxylic acid moieties, and application of high yielding click chemistries like the 1,3-dipolar cycloaddition of alkynes and azides or thiol-conjugate addition 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 polymer synthesis—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 ethynylene) (PPEs) (P1)—leads to increased spectral purity by preventing hydrophobically induced aggregate emission. 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). 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, and (ii) the extent of functionalization can be controlled by the equivalents of the NaIO4 reagent. Further, the improved spectral purity imparted by the presence of 1 in 3a is not lost. In turn, this demonstrates an improved signal amplified biosensor response to fluorophore-labeled streptavidin, a tetrameric protein with high biotin affinity (4 x 10^{14} M) that has been applied to a variety of conjugated polymer affinity chromic and agglutination biosensor designs.

Treatment of P1 with 0.2 equivalents of NaIO4 in water generated 1,6-dialdehyde moieties at random positions along the backbone (2, Scheme 1). 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 NaCNBH3 (vide infra, Scheme 2).

The azepane linkage in 3 is proposed based on two model studies. Firstly, the broad nature of the 1H 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). Therefore, while 0.2 equivalents of NaIO4 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—was treated with periodate anion and...
produced the tetraaldehyde 5 (Scheme 2a). Addition of an excess of butyl amine and NaCNBH₃ as the major product. Such products have been observed for bridging 1,6-dialdehydes 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 NaIO₄.

The effect of the described PPF method on the photophysical properties of the polymer can be seen in Fig. 1a and 1b. 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 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 to streptavidin in the presence of 3a 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 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, a 100 fold greater Kₜₐₜₐ of 2x10⁷ 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—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 NaIO₄. Finally, a highly sensitive (Kₜₐₜₐ = 2x10⁷) turn-on model biosensor based on ET between 3a and TRXS was demonstrated where the presence of 1 lead to dramatically increased sensitivity.

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We are herein submitting a manuscript entitled “Biocompatible Post-Polymerization Functionalization of a Water Soluble Poly(p-Phenylene Ethynylene)” for consideration as a publication in *Chemical Communications*. The approach given takes advantage of existing biocompatible reactivity for the functionalization of polymers in a predictable manner. Further, the modified polymers were evaluated within the context of a biotin–streptavidin model biosensor.
Supporting Information for

Controllable Biocompatible Post-Polymerization Functionalization of Poly(\(p\)-Phenylene Ethynylene)s and Highly Sensitive Detection of Streptavidin

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**Synthetic Procedures**

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Materials: Silica gel (40 µm) was purchased from SiliCycle. All solvents used for photophysical experiments were spectral grade. Pd(PPh₃)₄ was purchased from Strem Chemicals, Inc. All other reagent grade materials were purchased from Aldrich, TCI America, and Alfa Aesar, and used without further purification.

Experimental:
NMR Spectroscopy: ¹H and ¹³C NMR spectra for all compounds were acquired in CDCl₃, D₂O and DMF-d₇ on a Bruker Avance Spectrometer operating at 400 and 100 MHz, respectively. The chemical shift data are reported in units of δ (ppm) relative to residual solvent.

Gel Permeation Chromatography (GPC): Polymer molecular weights were determined using a triple detection method for calibration with poly(acrylic acid) standards on a Viscotek TDA 305-040 instrument equipped with two Viscotek A-MBHMW-3078 columns and analyzed with light scattering and refractive index detectors. Samples were dissolved in 5% NH₄OH.

Absorption and Emission Spectroscopy: Fluorescence spectra were measured on a SPEX Fluorolog-τ3 fluorometer (model FL-321, 450 W Xenon lamp) using right-angle detection. Ultraviolet-visible absorption spectra were measured with an Agilent 8453 diode array spectrophotometer and corrected for background signal with a solvent filled cuvette. Fluorescence quantum yields of #### in both water and 1X PBS were determined relative to perylene and are corrected for solvent refractive index and absorption differences at the excitation wavelength.

Lifetime measurements: Time resolved fluorescence measurements were performed by exciting the samples with 160 femtosecond pulses at 390 nm from the double output of a Coherent RegA Ti:Sapphire amplifier. The resulting fluorescence was spectrally and temporally resolved with a Hamamatsu C4780 Streak Camera system.
Synthetic Procedures

**Biotin functionalization, synthesis of 3a:** Polymer 1 (11.8 mg, 14.6 µmol based on repeat unit) was dissolved in 4 mL of H₂O and NaIO₄ (2.92 µmol in 0.2 mL) was added dropwise under vigorous stirring. After 30 min, a (Biotin-PEG₃-NH₂, 3 mg, 7 µmol in 1 mL of 0.2M Na₂HPO₄) was added and the reaction was stirred for 20 min. A solution of NaCNBH₃ (15 mg, 239 µmol in 1 mL of 40 mM Na₂HPO₄) was added and the reaction stirred for 3 hours. The reaction was dialyzed against water with 5 changes of water and lyophilized to yield 3a. GPC gave Mₙ = 38,474, PDI = 3.4. ¹H NMR (600 MHz, D₂O): δ 7.44 (s, 2H), 4.64 (broad, 4H), 4.37 (broad, 4H), 4.05 (broad, 4H), 3.89 (broad, 4H), 3.80-3.30 (biotin, PEG), 3.18 (broad, 4H) 2.35 (broad, 4H), 1.30-0.90 (biotin).

**Piv-Lysine functionalization, synthesis of 3b:** Prepared using identical conditions as above for 3a except that b (Piv-Lys-NH₂) was used in place of a. GPC gave Mₙ = 49,073, PDI = 4.9. ¹H NMR (600 MHz, D₂O): δ 7.42 (s, 2H), 4.61 (broad, 4H), 4.35 (broad, 4H), 4.03 (broad, 4H), 3.88 (broad, 4H), 3.16 (broad, 4H), 2.33 (broad, 4H), 1.05 (broad, tBu, 1.6–1.8*).

*Based on three experiments and corresponds to 18–20%.

**Synthesis of tetraaldehyde 5:** A solution NaIO₄ (0.200 g, 0.935 mmol) in 10 mL of water was added to a solution of 4 (0.200 g, 0.248 mmol) in 10 mL of THF. Solid TBAIO₄ (54 mg, 0.124 mmol) was added directly and the solution was refluxed for 30 min. After cooling, the reaction was partitioned between EtOAc and brine and the organic phase collected. The aqueous layer was washed with fresh EtOAc and the combined organic layers were dried over Na₂SO₄ and concentrated in vacuo. The residue was eluted through a silica gel plug using EtOAc to give 5 (95%). ¹H NMR (400 MHz, CDCl₃): δ 9.65 (d, J=2, 4H), 4.98 (nfo, actual ddd, J=6.6, 2.8, 2, 4H), 4.89 (dd, J=6.6, 2.8, 4H), 1.48 (s, 6H), 1.46 (s, 6H), 1.12 (s, 42H). ¹³C NMR (125 MHz, CDCl₃) δ 197.6, 134.1, 125.9, 110.7, 105.7, 101.2, 73.9, 54.5, 25.9, 24.2, 18.8, 11.4. HRMS (EI) calcd. for C₄₆H₆₆O₈Si₂[M+H] 803.4369, found 803.4344.

**Synthesis of amine 6:** To a solution of 5 (0.150 g, 0.186 mmol) in 10 mL of MeOH was added butyl amine (82 mg, 1.12 mmol). After stirring for 10 min at room temperature, NaCNBH₃ (0.250 g, 3.98 mmol) was added and the mixture was refluxed for 3 hours. Once cool, 1 mL of sat. NaHCO₃ was added and the solvent was removed in vacuo. The residue was_partitioned between DCM and sat. NaHCO₃. The organic layer was dried over Na₂SO₄ and evaporated to dryness. Silica gel chromatography (EtOAc:Hex, 8:2) provided 6 (65%) as a white solid. ¹H NMR (400 MHz, CDCl₃): δ 4.33 (dd, J=4.0, 2.4, 4H), 4.00 (m, 4H), 2.84 (d, J=12, 4H), 2.58 (dd, J=11.8, 7.5, 4H), 2.29 (br t, 4H), 1.64 (s, 6H), 1.40 (s, 6H), 1.28 (m, 4H), 1.12 (br s, 42H), 1.06 (m, 4H), 0.74 (t, J=7.4, 6H). ¹³C NMR (125 MHz, CDCl₃) δ 139.5, 119.8, 110.8, 103.2, 98.3, 76.5, 57.4, 48.7, 41.5, 28.7, 25.9, 24.7, 20.7, 19.0, 14.1, 11.6. HRMS (EI) calcd. for C₅₄H₈₈N₂O₂₄Si₂ [M+H] 885.6355, found 885.6357.
NMR Spectra

**Figure S1:** $^1$H and $^{13}$C NMR spectra of compound 5
Figure S2: $^1$H and $^{13}$C NMR spectra of compound 6
Figure S3: $^1$H spectrum of compound 3b

Figure S4: $^1$H spectrum of compound 3a
UV-vis and Fluorescence data

**Table S1:** Summary of photophysical data of 3a

|          | Abs $\lambda_{max}$ (nm) | Em $\lambda_{max}$ (nm) | log $\varepsilon$ | $\Phi_F$ |
|----------|---------------------------|--------------------------|--------------------|-----------|
| 3a, water| 436                       | 451                      | 4.52               | 8%        |
| 3a, 1X PBS| 450                       | 461                      | 4.58               | 7%        |

**General protocol for energy transfer assays in PBS:**

50 µL of a stock polymer solution (0.056 mg/mL in PBS) was diluted with PBS to a total volume of 3 mL in a fluorescence cuvette. To this was added aliquots of Texas Red-X™ labeled streptavidin (0.5 µL of a 1 mg/mL solution) and fluorescence emission was taken at each addition. Excitation wavelength was 426 nm.

![Graph showing quantum yield vs pH](image)

**Figure S5:** Effect of quantum yield of 3a at different pH. Measurements performed in PBS where pH was adjusted with HCl or NaOH.

![Graph showing Stern–Volmer quenching analysis](image)

**Figure S6:** Stern–Volmer quenching analysis of Figure 1d in main text.