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Biocompatible Post-Polymerization Functionalization of a Water Soluble Poly(*p*-Phenylene Ethynylene)

Brett VanVeller and Timothy M. Swager*

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
10 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
15 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
20 click chemistries[4] like the 1,3-dipolar cycloaddtion 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
25 polymer synthesis—often in protected form and (ii) it can be difficult to control the extent of functionalization.
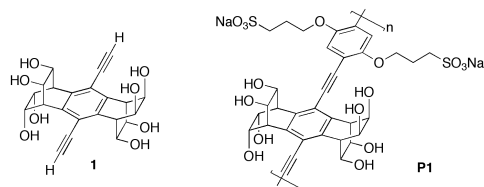
We recently reported the synthesis of a rigid hydrophilic monomer (**1**) that—when incorporated into poly(*p*-phenylene
30 ethynylenes) (PPEs)[7] (**P1**)—leads to increased spectral purity by preventing hydrophobically induced aggregate emission.[8] We envisioned that the three dimensional array of vicinal hydroxyl groups might be further elaborated through periodate oxidation and reductive amination (**P1**→**2**→**3**, Scheme 1).[9]
35 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
40 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 NaIO$_4$ reagent. Further, the improved spectral purity imparted by the presence of **1** in **3a** is not lost. In turn, this
45 demonstrates an improved signal amplified biosensor[10] response to fluorophore-labeled streptavidin, a tetrameric

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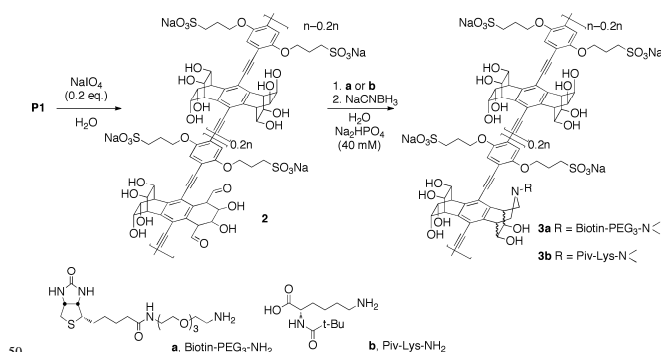

50 **Scheme 1** Periodate oxidative activation and reductive amination

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
55 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$_3$ (*vide infra*, Scheme 2).

The azepane linkage in **3** is proposed based on two model
60 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**.
65 Integration analysis revealed a 18–20% incorporation of **b** (Fig. S3, ESI). 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
70 in the synthesis of **1**[8]—was treated with periodate anion and
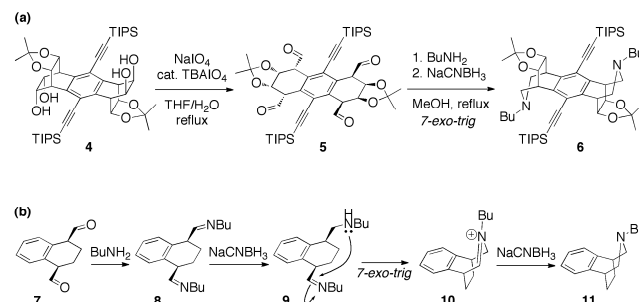
Scheme 2 a) Model reductive amination product, **b)** proposed mechanism
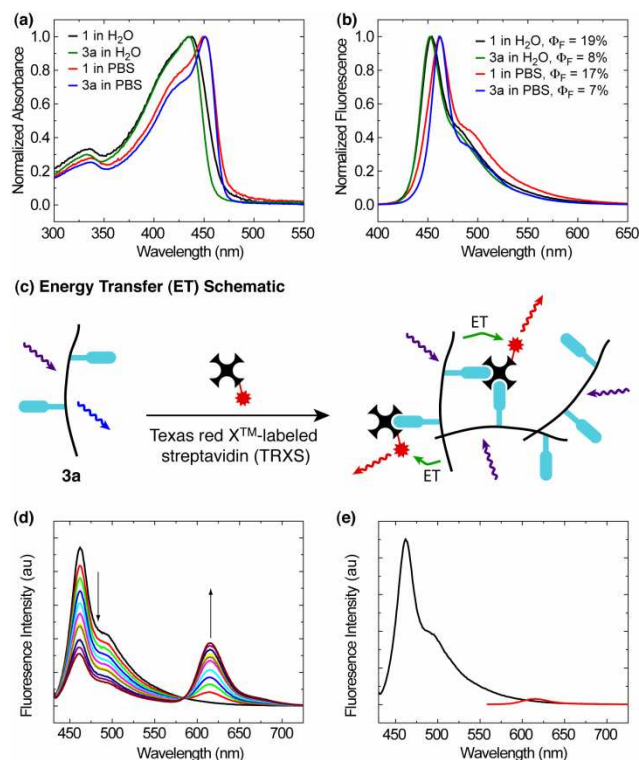
Fig. 1 (a) Absorbance spectra of **1** and **3a** in water and PBS solution. (b) Fluorescence spectra of **1** and **3a** in water and PBS solution. (c) Energy transfer schematic showing how intra- and interchain exciton migration and energy transfer to TRXS can lead to amplification. (d) Addition of 9.15 pmol aliquots of Texas red X™-labeled streptavidin to 3.46 nmol (based on repeat unit of **3a**). (e) Excitation of **1** in the presence of 100 pmol TRXS (black) and direct excitation of 100 pmol of TRXS (red).

produced the tetraaldehyde **5** (Scheme 2a). Addition of an excess of butyl amine and NaCNBH$_3$ to **4** in methanol gave **5** 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 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[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 $2x10^7$ 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$_{SV}$ = $2x10^7$) turn-on model biosensor based on ET between **3a** and TRXS was demonstrated where the presence of **1** lead to dramatically increased sensitivity.

Notes and references
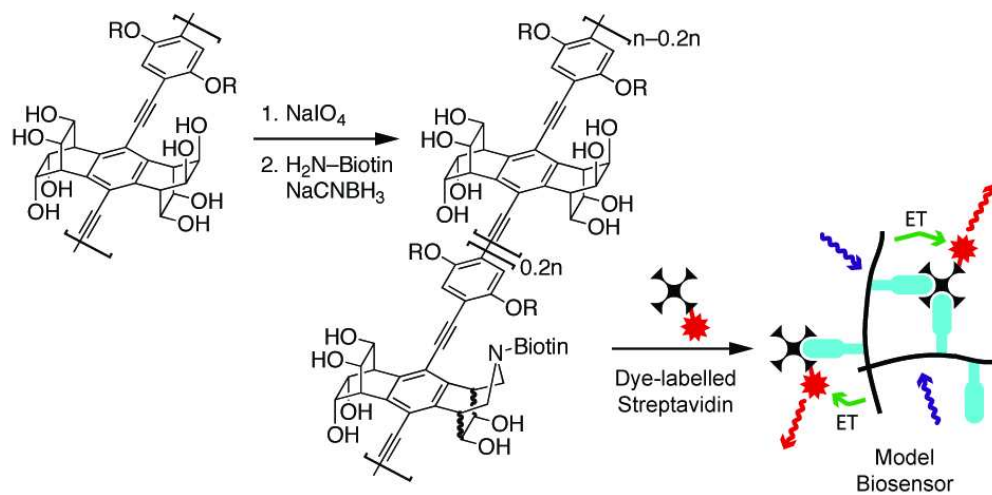
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† Electronic Supplementary Information (ESI) available: Experimental details. See DOI: 10.1039/b000000x/

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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 predicatble manner. Further, the modified polymers were ealuated within the context of a biotin–streptavidin model biosensor.

80x41mm (300 x 300 DPI)

S1

Supporting Information for

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

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S2

Materials: Silica gel (40 μm) was purchased from SiliCycle. All solvents used for photophysical experiments were spectral grade. Pd(PPh$_3$)$_4$ 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: ^{1}H and ^{13}C NMR spectra for all compounds were acquired in CDCl$_3$, D$_2$O and DMF-d$_7$ 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% NH$_4$OH.

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 H_2O and $NaIO_4$ (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 Na_2HPO_4) was added and the reaction was stirred for 20 min. A solution of $NaCNBH_3$ (15 mg, 239 µmol in 1 mL of 40 mM Na_2HPO_4) 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 M_n = 38,474, PDI = 3.4. ^{1}H NMR (600 MHz, D_2O): δ7.44 (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 M_n = 49,073, PDI = 4.9. ^{1}H NMR (600 MHz, D_2O): δ7.42 (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 $NaIO_4$ (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 $TBAIO_4$ (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 Na_2SO_4 and concentrated *in vacuo.* The residue was eluted through a silica gel plug using EtOAc to give **5** (95%). ^{1}H NMR (400 MHz, $CDCl_3$): 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). ^{13}C NMR (125 MHz, $CDCl_3$) 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 $C_{46}H_{66}O_8Si_2$ [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, $NaCNBH_3$ (0.250 g, 3.98 mmol) was added and the mixture was refluxed for 3 hours. Once cool, 1 mL of sat. $NaHCO_3$ was added and the solvent was removed *in vacuo*. The residue was partitioned between DCM and sat. $NaHCO_3$. The organic layer was dried over Na_2SO_4 and evaporated to dryness. Silica gel chromatography (EtOAc:Hex, 8:2) provided **6** (65%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$): 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). ^{13}C NMR (125 MHz, $CDCl_3$) 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 $C_{54}H_{88}N_2O_4Si_2$ [M+H] 885.6355, found 885.6357.

S4
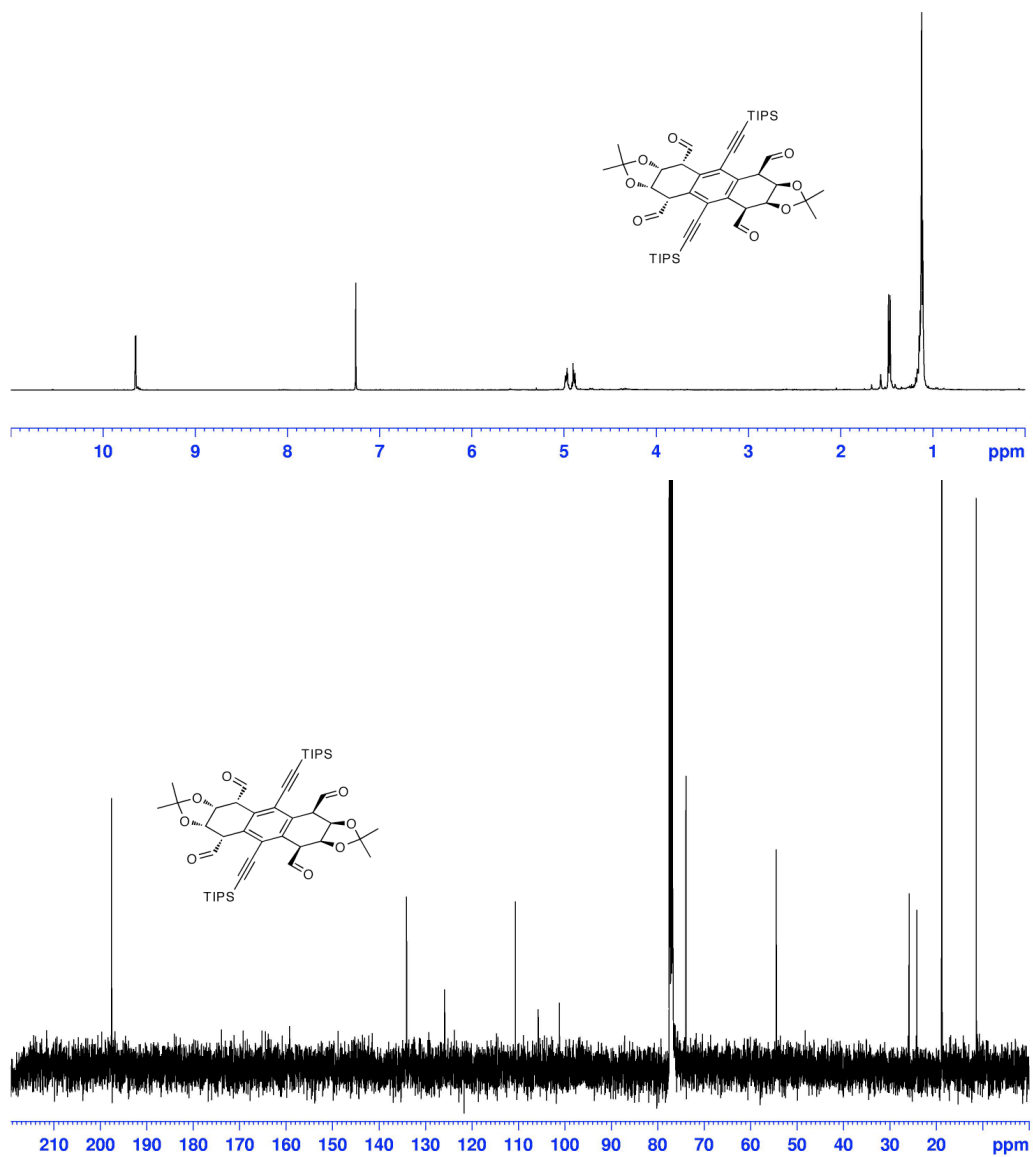
NMR Spectra

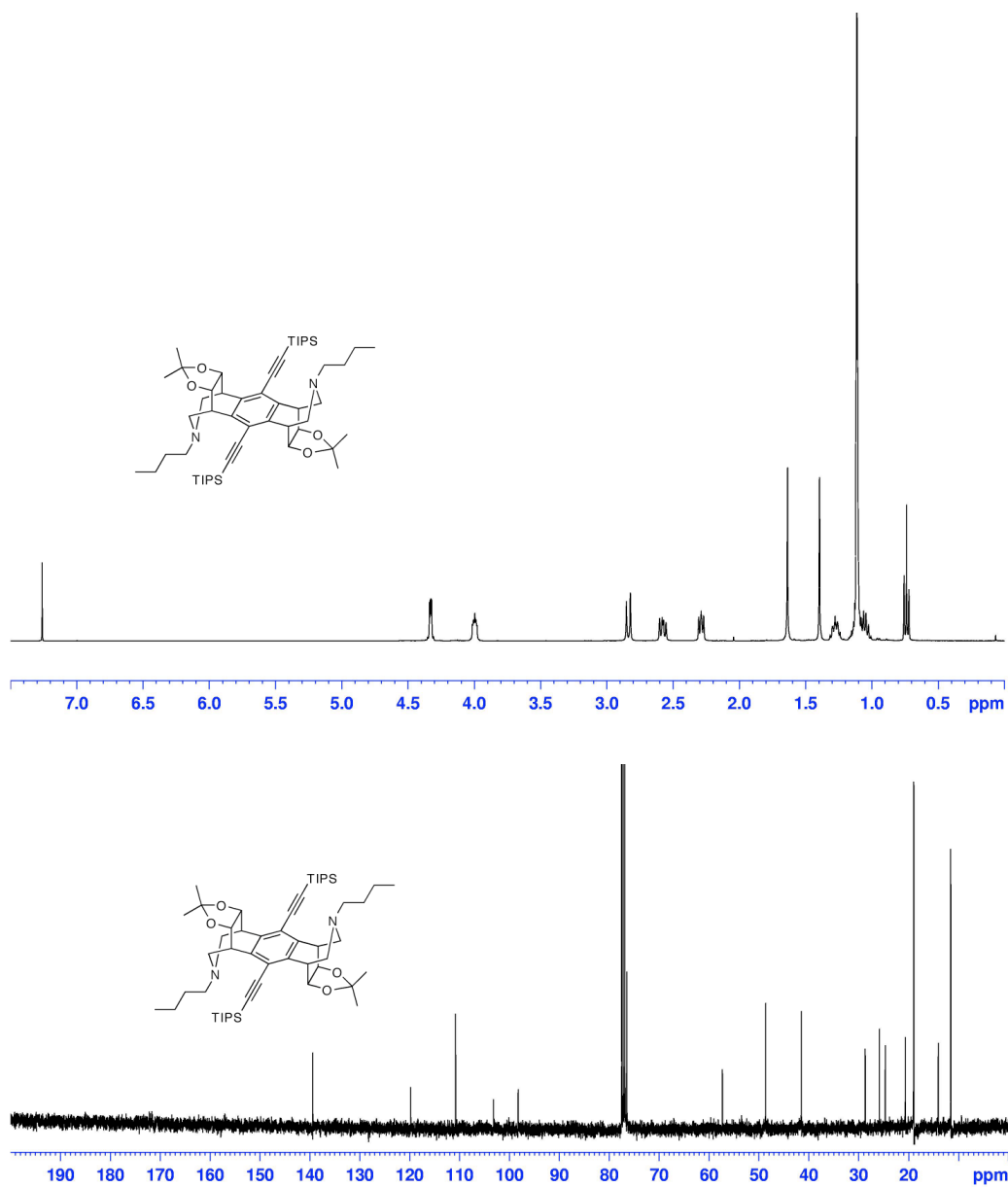**Figure S1:** ^{1}H and ^{13}C NMR spectra of compound **5**
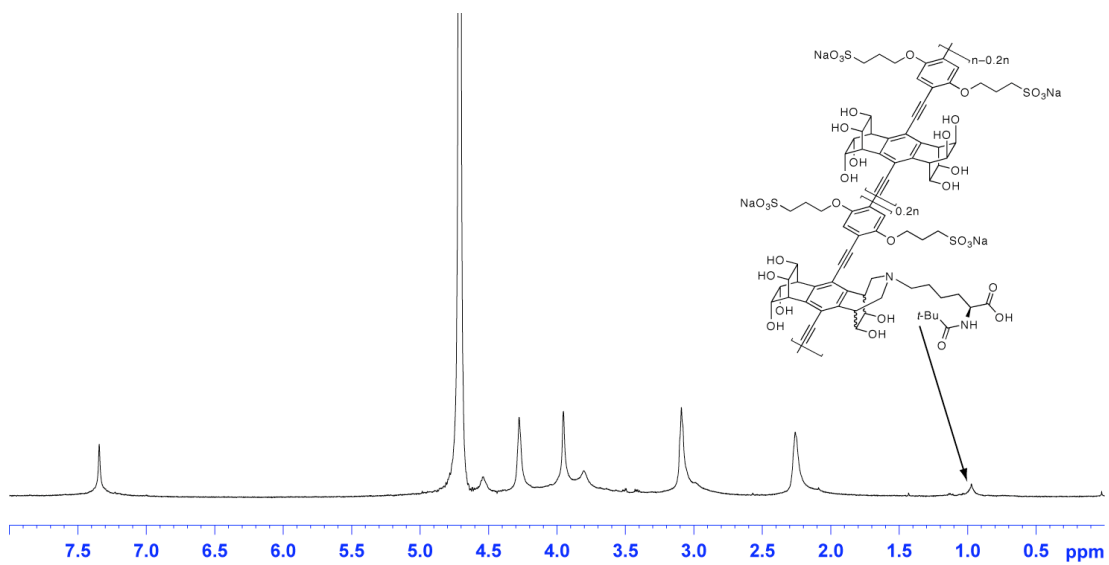
S5

Figure S2: ^{1}H and ^{13}C NMR spectra of compound **6**

Figure S3: [1]H spectrum of compound **3b**

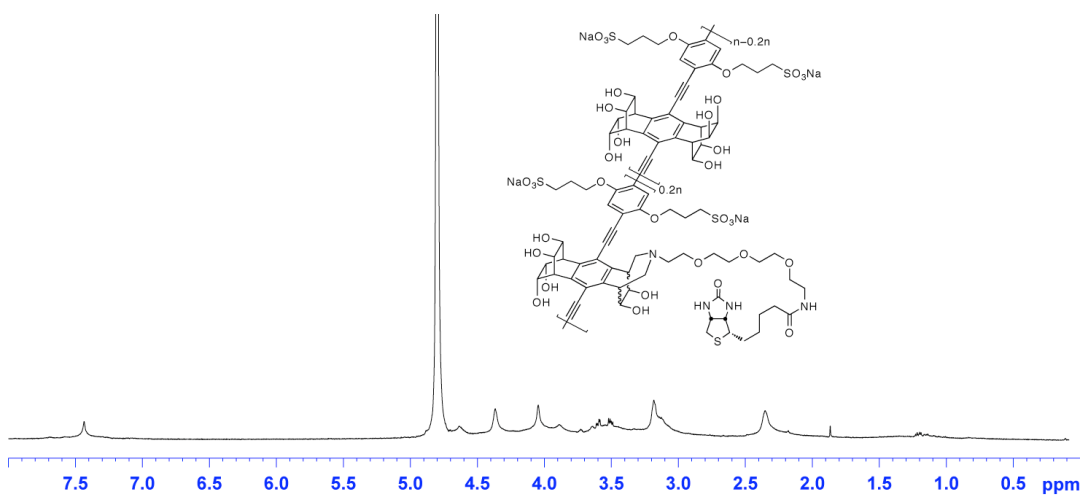**Figure S4:** [1]H spectrum of compound **3a**

UV-vis and Fluorescence data

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

	Abs λ_{max} (nm)	Em λ_{max} (nm)	log ε	Φ_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.
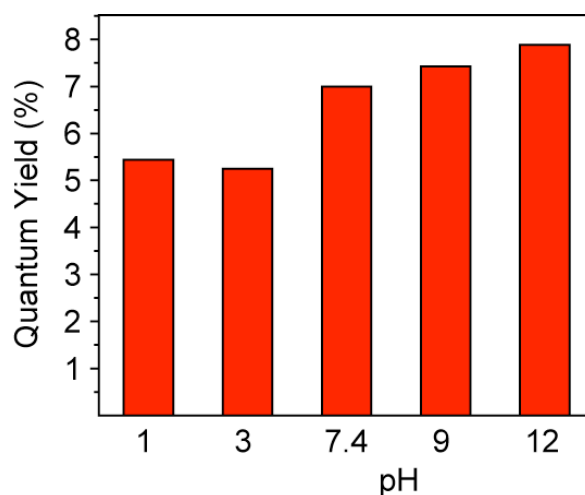
Figure S5: Effect of quantum yield of **3a** at different pH. Measurements performed in PBS where pH was adjusted with HCl or NaOH.
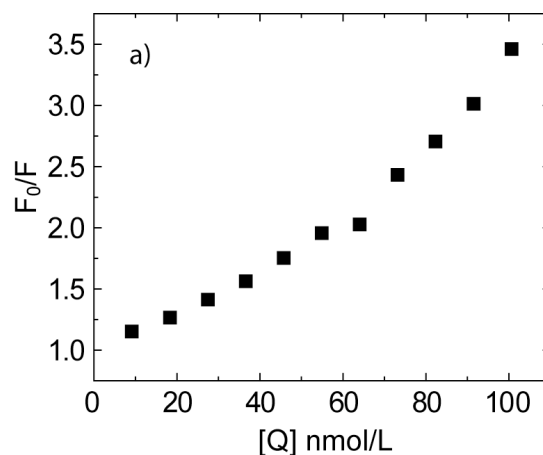
Figure S6: Stern–Volmer quenching analysis of Figure 1d in main text.