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Electronic Supplementary Information

for

Biotinylated poly(*p*-phenylene ethynylene): using energy transfer for the detection of biological analytes

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Experimental details, synthesis of the polymers and control experiments.

(9 pages)

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Experimental Section

General. ^{1}H and ^{13}C NMR spectra for monomers and polymers were recorded on a (Varian 300 MHz) or on a Varian VXR-500 (500 MHz) instrument. The chemical shift data for each signal are given in units of δ (ppm) relative to tetramethylsilane (TMS) where δ (TMS) = 0, and referenced to the solvent residual. High-resolution mass spectra were obtained on a Finnigan MAT 8200 system using sector double focus and an electron impact source with an ionizing voltage of 70V, and with a Bruker DALTONICS APEX II, 3 Tesla, FT-ICR-MS with ESI source or EI/CI source. UV-visible absorption spectra were measured with a Cary 50 UV/visible spectrometer. Fluorescence spectra were measured with a SPEX Fluorolog-2 fluorometer (model FL112, 450W xenon lamp). The spectra in solution were obtained at room temperature using a quartz cuvette with a 1cm path length. Polymer thin film spectra were recorded by front-face (22.5°) detection. Fluorescence quantum yields of polymers in Tris buffer (100mM, pH 7.4) were determined relative to solutions of coumarin 6 (Φ_F = 0.78 in ethanol) as a reference. Te quantum yields for solid state thin films were obtained relative to 0.01 mol% 9, 10-dipheynylanthracene in PMMA ((Φ_F = 0.83) as a reference. The molecular weights of polymers were determined by using three PLgel 5μm 10^5, 10^4, 10^3 (300 x 7.5 mm I.D) columns in series and a diode detector at 254nm at a flow rate of 1.0ml/min in THF or in DMF. The molecular weights were reported relative to polystyrene or poly(ethylene oxide) standards purchased from Agilent Inc. Polymer thin films on a cover glass (18 x 18 mm, pretreated with 1,1,1,3,3,3-hexamethyldisilazane) were spin cast on an EC101DT photoresist spinner (Headway Research Inc.) using a spin rate of 3000 rpm from a chloroform solution. Melting point (m.p.) determination was performed using a Laboratory Devices MEL-TEMP instrument (open capillaries used) and was uncorrected.

Materials.

All solvents were spectral grade unless otherwise noted. Morpholine and biotin were purchased from Alfa Aesar and used as received. Fluorescein conjugated streptavidin, rhodamine-conjugated streptavidin, Texas Red-X conjugated streptavidin and sulforhodamine 101 were purchased from Molecular Probes Inc. and used as received. All other chemicals were purchased from Aldrich Chemical In. and used as received. All air and water sensitive synthetic manipulations were performed under a nitrogen atmosphere using standard schlenk techniques.

Synthesis.

Monomer **5** was prepared according to Scheme S-1

Scheme S-1

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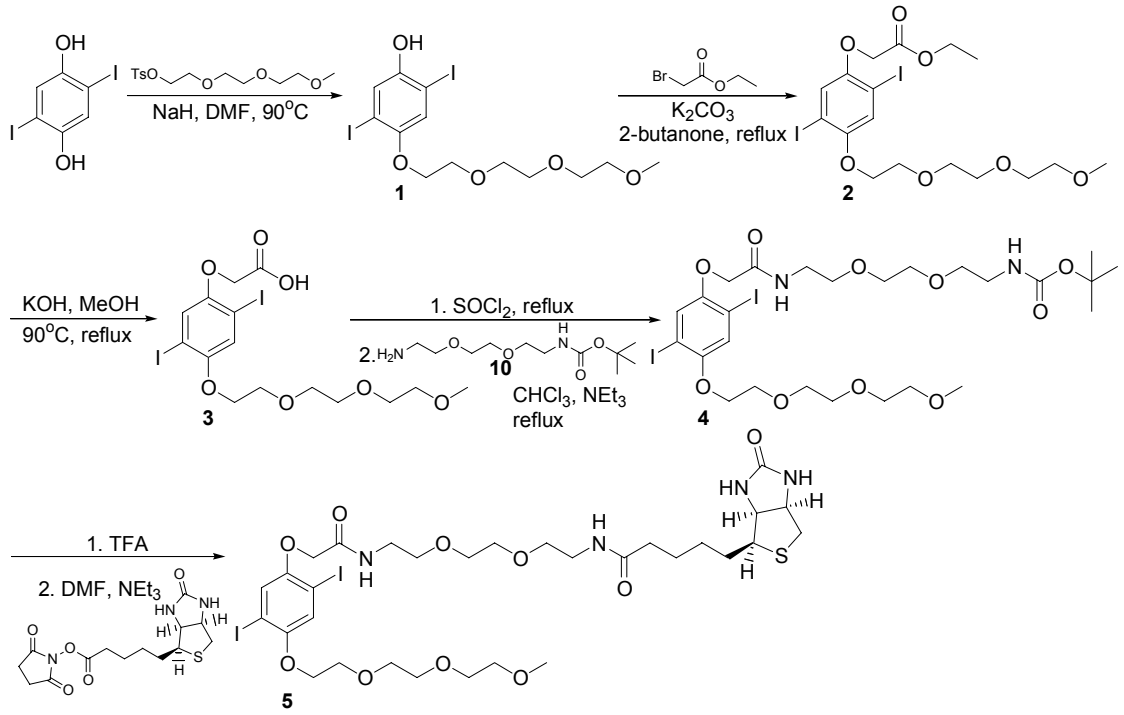


(1): To a 250ml round bottom flask equipped with a reflux condenser containing 2,5-diiodo-1,4-dihydroxybenzene (10.00g, 27.6mmol) was added 125 ml anhydrous N, N'-dimethyl formamide (DMF) under nitrogen. The solution was cooled to 0°C, and nitrogen was bubbled through the solution for 15 minutes. NaH as a 60% dispersion in mineral oil (1.326g, 33.2mmol) was added and the resulting suspension was stirred for 20 min at 0oC. Triethylene glycol monomethyl ether *p*-toluenesulfonate (9,94g, 31.2mmol) was then transferred to the solution via syringe. The reaction was heated at 65°C for 14h under nitrogen. A light clear brown solution was obtained. DMF was removed under reduced pressure and the resulting brown oil was extracted with ethyl acetate (500 ml total) against 200ml H_2O. The organic layer was washed with 50ml brine and the solvent was removed under reduced pressure. The product was purified by column chromatography with 6:4 hexane/ethyl acetate to afford a colorless oil which solidified to a white solid upon standing (3.98g, 28%). m.p. 81-83°C. ^{1}H NMR (300 MHz, $CDCl_3$): 7.38 (1H, s), 7.09 (1H, s), 5.27 (1H, s), 4.08 (2H, t, J=4.5Hz), 3.88 (2H, t, J=4.5Hz), 3.79 (2H, t, J=4.5Hz), 3.69 (2H, t, J=4.5Hz), 3.67 (2H, t, J=4.5Hz), 3.38 (3H, s); ^{13}C NMR (125 MHz, $CDCl_3$): 152.6, 150.5, 125.0, 121.9, 87.8, 84.4, 72.1, 71.3, 71.0, 70.8, 70.5, 69.8, 59.3; HR-MS (EI) calcd. For $C_{13}H_{18}I_2O_5$ (M+): 507.9238, found: 507.9239.

(2): In a 250ml round bottom flask were combined 1 (2.00 g, 3.94mmol), K_2CO_3 (1.632g, 11.81mmol), ethyl bromoacetate (0.567ml, 5.12mmol) and 100ml acetone. The flask was fitted with a reflux condenser and the reaction mixture was refluxed for 12h. A pale yellow suspension resulted. This was cooled, filtered and the solvent was removed under reduced pressure. The residue was purified by column chromatography with 6:4 hexane/ethyl acetate and the product was isolated as a colorless oil which solidified upon standing to a white solid (2.02g, 86%). m.p. 44-45°C. ^{1}H NMR (300 MHz, $CDCl_3$): 7.26 (1H, s), 7.17 (1H, s), 4.61 (2H, s), 4.30 (2H, q, J=4.2), 4.13 (2H, t, J=3Hz), 3.88 (2H, t,

J=3Hz), 3.80 (2H, t, J=3Hz), 3.70 (2H, t, J=3Hz), 3.68 (2H, t, J=3Hz), 3.57 (2H, t, J=3Hz), 3.39 (3H, s), 1.32 (3H, t, J=4.2Hz); ^{13}C NMR (125 MHz, $CDCl_3$): 168.4, 153.9, 152.4, 123.9, 123.6, 86.7, 86.4, 72.2, 71.4, 71.0, 70.8, 70.4, 69.8, 67.7, 61.7, 59.3, 14.5. HR-MS (EI) calcd. For $C_{17}H_{24}I_2O_7$ (M+): 593.9606, found: 593.9625.

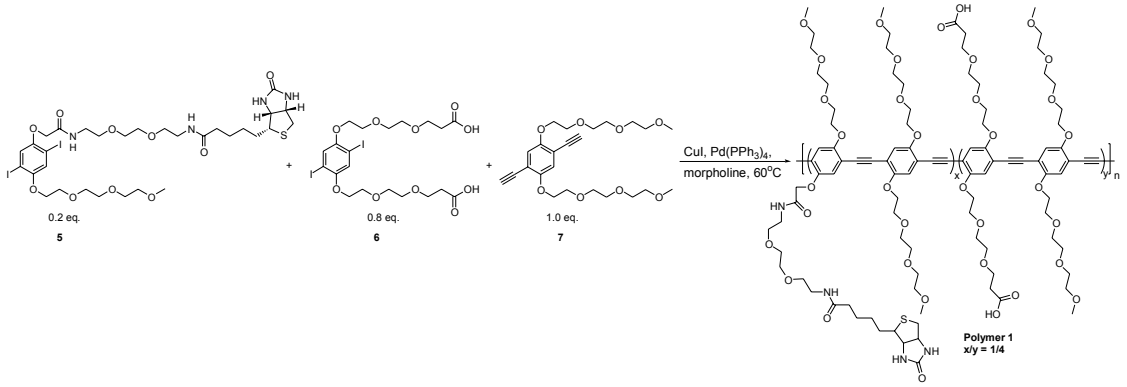
(3): In a 250 ml round bottom flask were combined **2** (2.00g, 3.36mmol) and KOH (0.944g, 16.8mmol) in 70ml methanol. A reflux condenser was fitted and the reaction was heated to reflux for 14h. The solvent was removed under reduced pressure. 45ml 10% $HCl_{(aq)}$ was added. The product precipitated and was isolated by centrifugation followed by lyophilization. A white solid was obtained (1.79g, 94%). m.p. 74-76^{o}C. ^{1}H NMR (300 MHz, DMSO): 7.38 (1H, s), 7.24 (1H, s), 4.72 (2H, s), 4.10 (2H, t, J=4.5Hz), 3.73(2H, t, J=4.5Hz), 3.62 (2H, t, J=4.5Hz), 3.53 (2H, t, J=4.5Hz), 3.52 (2H, t, J=4.5Hz), 3.42 (2H, t, J=4.5Hz), 3.23 (3H, s); ^{13}C NMR (125 MHz, DMSO): 169.8, 152.7, 151.7, 123.0, 122.2, 86.7, 86.5, 71.3, 70.2, 69.9, 69.7, 69.6, 69.0, 66.1, 58.0; HR-MS (ESI) calcd. For $C_{15}H_{20}I_2O_7$ (M+Na): 588.9191, found: 588.9182.

(4): In a 50ml round bottom flask equipped with a reflux condenser containing **3** (0.500g, 0.883mmol) was added 5ml $SOCl_2$. This was refluxed for 10h. The thionyl chloride was then removed under reduced pressure to afford the acid chloride as a pale yellow oil (0.521g, 0.883mmol). To this was then added 20ml CH_2Cl_2. Anhydrous NEt_3 was then added (0.185ml, 1.32mmol) and the mixture was stirred for 5min. **10**[1] (0.329g, 1.32mmol) was added as a solution in 10ml CH_2Cl_2. The reaction mixture was refluxed for 12h. The solvent was removed under reduced pressure. The residue was dissolved in 100ml $CHCl_3$ and washed with 30ml H_2O. The organic layer was washed with 15ml brine, dried over $MgSO_4$. The organic solvent was removed under reduced pressure to afford a colorless oil which solidified upon standing to a white solid (0.560g, 80%). m.p. 81-83^{o}C. ^{1}H NMR (300 MHz, $CDCl_3$): 7.28 (1H, br), 7.25 (1H, s), 7.17 (1H, s), 4.90 (1H, br), 4.13 (2H, t, J=4.5Hz), 3.90 (2H, t, J=4.5Hz), 3.80 (2H, t, J=4.5Hz), 3.81-3.52 (16H, m), 3.39 (3H, s), 3.32 (2H, t, J=5.1Hz), 1.45 (9H, s); ^{13}C NMR (125 MHz, $CDCl_3$): 206.1, 167.4, 156.2, 154.1, 151.4, 123.3, 86.8, 86.3, 79.5, 72.2, 71.4, 71.0, 70.8, 70.6, 70.6, 70.5, 70.0, 69.8, 69.1, 59.3, 40.5, 39.1, 28.7; HR-MS (ESI) calcd. For $C_{26}H_{42}I_2N_2O_{10}$ (M+H): 797.1002, found: 797.1022.

(5): A 50ml round bottom flask containing **4** (0.487g, 0.611mmol) was loaded with 2ml TFA. The clear yellow solution was stirred for 30min. The TFA was removed, 2ml H_2O was added and was also removed under reduced pressure. The deprotected product was dried under high vacuum. To this was added 5ml anhydrous DMF, NEt_3 (0.450ml, 3.22mmol). This was stirred for 15min, then N-hydroxysuccinimido biotin[2] (0.212g, 0.624mmol) was added. The pale yellow solution quickly became a thick white slurry and was stirred at room temperature for 40h. The solvent was removed under reduced pressure at 40^{o}C and the reaction mixture was washed with 25ml H_2O. The product was isolated by centrifugation and lyophilized to afford a white powder (0.525g, 94%). m.p. 175-176^{o}C. ^{1}H NMR (500 MHz, $CDCl_3$): 7.85 (2H, m), 7.39 (1H, s), 7.31 (1H, s), 6.43 (1H, s), 6.36 (1H, s), 4.52 (2H, s), 4.30 (1H, m), 4.11 (1H, m), 3.74 (2H, t, J=5.0Hz), 3.62 (2H, t, J=5.0Hz), 3.54-3.30 (16H, m), 3.22 (3H, s), 3.18 (2H, m), 3.08 (2H, m), 2.80 (1H, dd, J=12.5, 5.0Hz), 2.58 (J=12.5Hz), 2.06 (2H, t, J=7.5Hz), 1.62-1.57 (1H, m), 1.52-1.43

(3H, m), 1.32-1.26 (2H, m); ^{13}C NMR (125 MHz, $CDCl_3$): 172.1, 167.2, 162.7, 153.0, 151.7, 123.3, 122.7, 86.9, 86.8, 71.3, 70.2, 69.9, 69.7,69.2, 69.0, 68.8, 61.3, 61.0, 59.2, 58.1, 55.5, 38.44, 30.37, 35.1, 28.2, 28.1, 25.3; HR-MS (ESI) calcd. For $C_{31}H_{48}I_2N_4O_{10}S$ (M+H): 923.1253, found: 923.1210.

Scheme S-2



Polymer 1: A 25ml schlenk flask was charged with **5** (0.0205g, 0.022mmol), **6** (0.0606g, 0.089mmol, synthesis to be reported in a forthcoming publication) and **7**[3] (0.050g, 0.111mmol), $Pd(PPh_3)_4$ (6.41mg, 0.0056mmol) and CuI (1.06mg, 0.0056mmol) under N_2. To this was added 1.5ml freshly degassed morpholine under N_2. The reaction vessel was sealed and heated at 60^{o}C for 48h. 3ml H_2O was added and the reaction mixture was dialyzed (cellulose membrane, MWCO 10000) against 1L deionized water for 2 days (6 water changes). The polymer was then lyophilized to afford an orange polymer (97mg, 95%). Mn= 130,000, PDI=1.48 for DMF soluble fraction. ^{1}H NMR (500 MHz, DMF): 7.29 (20H, br), 6.39 (1H, s), 6.32 (1H, s), 4.78 (2H, s), 4.33 (38H, br), 3.94 (24H, br), 3.78-3.46 (160H, broad multiplet), 3.28 (33H, br), 1.60 (8H, br).

Scheme S-3

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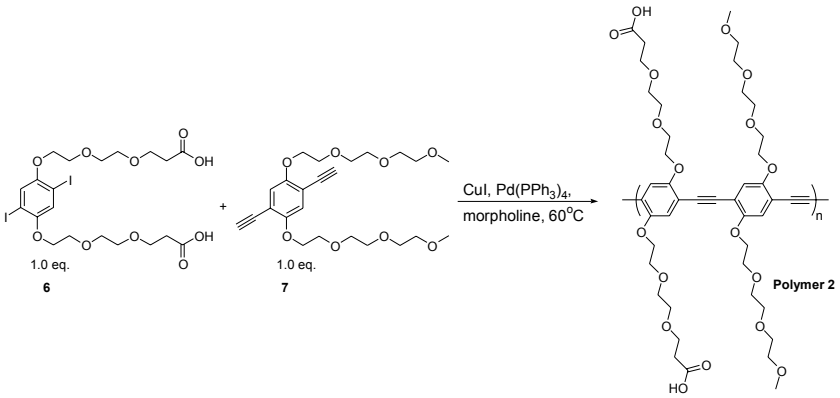
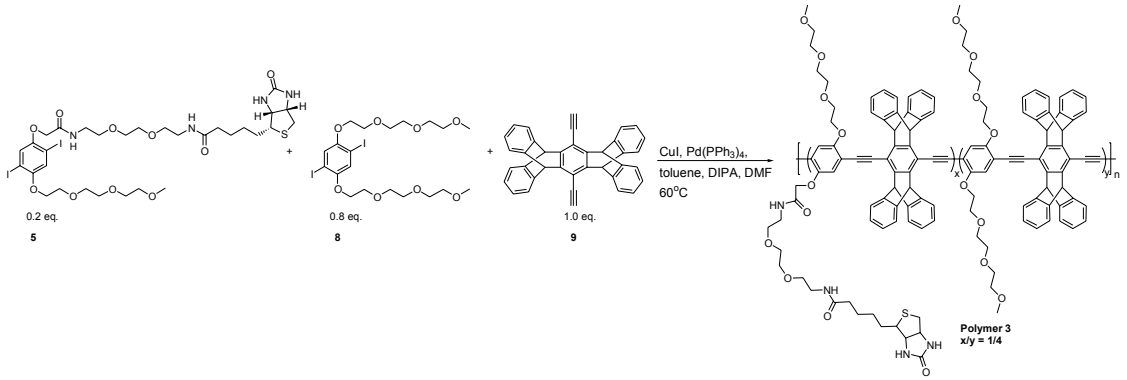


Polymer 2: A 25 ml schlenk flask was charged with **6** (0.0454g, 0.066mmol) and **7** (0.030g, 0.066mmol), $Pd(PPh_3)_4$ (3.85mg, 0.00333mmol) and CuI (0.634g, 0.00333mmol) under N_2. To this was added 1.0ml freshly degassed morpholine under N_2. the reaction vessel was then sealed and heated at 60°C for 48h. 3ml H_2O was added and the mixture was dialyzed against 1L deionized water for 2 days (6 water changes). It was then lyophilized to afford an orange polymer (56mg, 96%). Mn=128,000, PDI=1.53 for DMF soluble fraction. ^{1}H NMR (500 MHz, DMF): 7.30 (4H, s), 4.34 (8H, br), 3.95 (8H, br), 3.79-3.46 (32H, br), 3.29 (6H, s)

Scheme S-4



Polymer 3: A 25ml schlenk flask was charged with **5** (0.00796g, 0.00819mmol) and **8** (0.0214g, 0.0328mmol), **9**[4] (0.020g, 0.418mmol), $Pd(PPh_3)_4$ (2.367mg, 0.00205mmol) and CuI (0.390mg, 0.00205mmol) under N_2. 1.5ml of a freshly degassed, mixture of 4:1 toluene/diisopropylamine and 0.5ml freshly degassed DMF were added via syringe. The reaction vessel was sealed and heated at 60°C for 5 days. The polymer was isolated by precipitation into methanol followed by centrifugation. A yellow powder was obtained (32mg, 83%). Mn=7700, PDI=2.04 for THF soluble fraction. ^{1}H NMR (500MHz, $CDCl_3$): 7.66-7.47 (60H, broad multiplet), 7.05 (40H, br), 6.42 (1H, s), 6.39 (1H, s), 6.10 (20H, br), 5.30 (2H, br), 4.68 (20H, br), 4.26 (22H, br), 3.83 (20H, br), 3.65 (20H, br), 3.55 (20H, br), 3.44 (20H, br), 3.31 (19H, br), 2.78 (2H), 1.40-1.25 (6H, broad multiplet).

Scheme S-5

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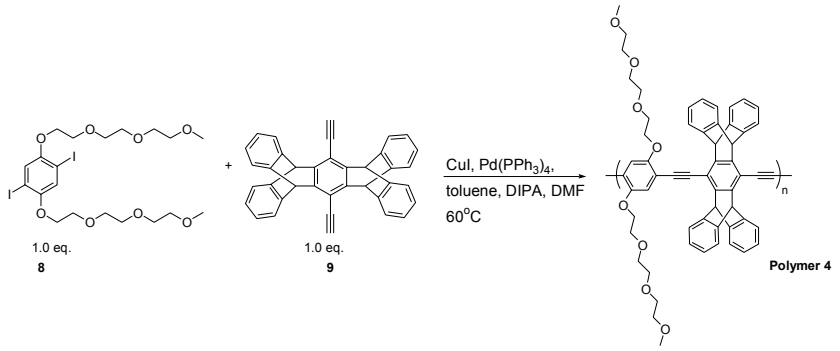

Polymer 4: A 25ml schlenk flask was charged with **8** (0.020g, 0.0306mmol) and **9** (0.0149g, 0.0312mmol), $Pd(PPh_3)_4$ (1.766mg, 0.00153mmol) and CuI (0.291mg, 0.00153mmol) under N_2. 1.5ml of a freshly degassed mixture of 4:1 toluene/diisopropylamine was added via syringe. The reaction vessel was sealed and heated at 60°C for 5 days. The polymer was isolated by precipitation into ethyl acetate followed by centrifugation. A yellow powder was obtained (21.3mg, 80%). Mn=14000, PDI=2.02 for THF soluble fraction. ^{1}H NMR (500 MHz, $CDCl_3$): 7.53 (10H, broad multiplet), 7.05 (8H, br), 6.20 (4H, br), 4.68 (4H, br), 4.26 (4H, br), 3.82 (4H, br), 3.65 (4H, br), 3.55 (4H, br), 3.44 (4H, br), 3.31 (6H, br)

General protocol for energy transfer assays in solution phase
7.5µl of a stock polymer solution (1mg/ml in Tris buffer, 40mM at pH7.4) was diluted with the same Tris buffer to a total volume of 3ml in a fluorescence cuvette. To this was added aliquots of dye-labeled streptavidin (1µl of a 1mg/ml solution) and fluorescence emission was taken at each addition. Excitation wavelength at 440nm was chosen, and emission spectrum was taken from 455-700nm.

General protocol for energy transfer assays in solid phase
Microscope coverslips were pretreated in 1,1,1,3,3,3-hexamethyldisilazane.[5]
Polymer solutions at 1mg/ml in chloroform were spin-cast onto microscope coverslips at a spin rate of 3000rpm for 1 minute. The coverslips were put under vacuum for 2h, then were incubated in a solution of dye labeled streptavidin or dye for 1h. The coverslips were then washed with deionized water, blotted dry and dried under vacuum for a minimum of 5h. Excitation wavelength at 400nm was chosen, and emission spectrum was taken from 415-700nm.

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Control experiments

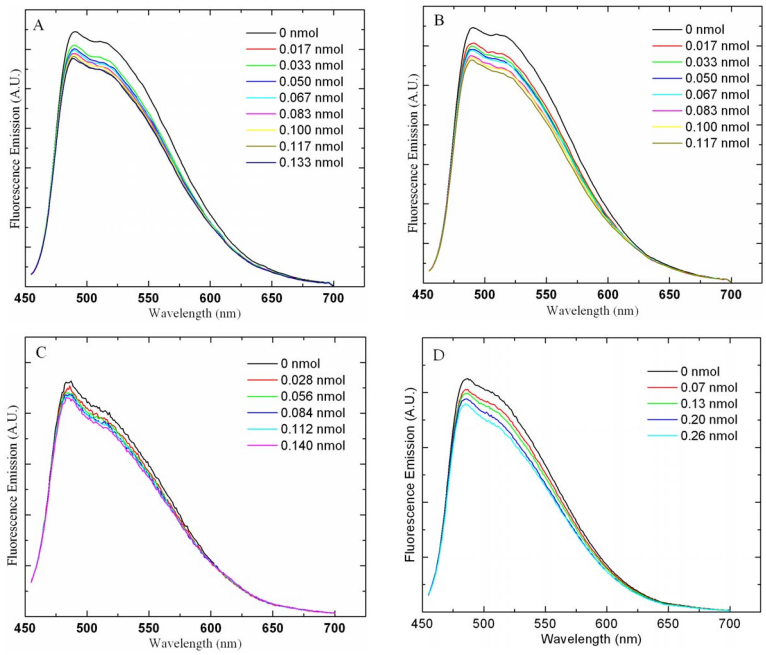

Figure 1. Emission spectra of polymer **2** when incubated with A) rhodamine Red-labeled streptavidin; B) Texas Red-X labeled streptavidin; C) polymer **1** and biotin-saturated Texas Red-X labeled streptavidin; D) polymer **1** and streptavidin.

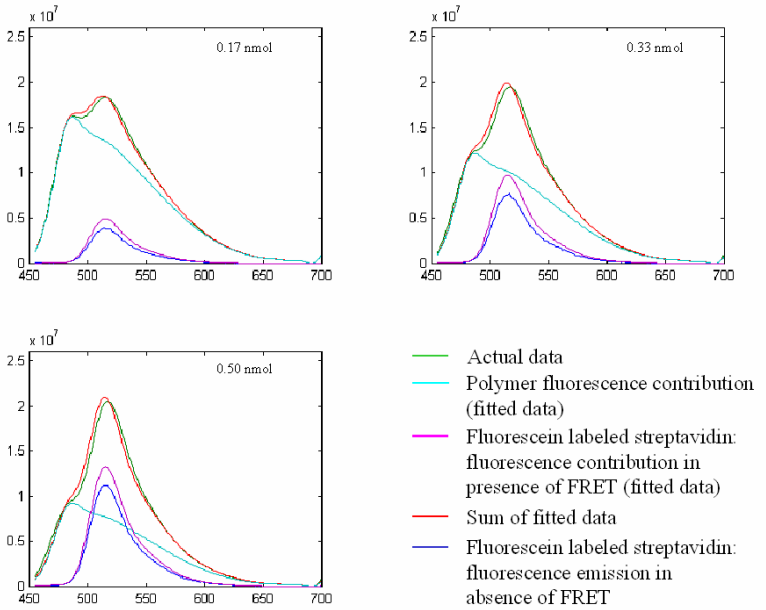

Figure 2. Polymer **1** with addition of fluorescein-labeled streptavidin. Deconvolution of fluorescence emission spectra. Difference between the magenta and blue curves corresponds to emission contribution due to energy transfer from **1** to fluorescein.

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