Supporting Information for:

Selective Sensing of Metalloproteins from Non-selective Binding using a Fluorogenic Amphiphilic Polymer

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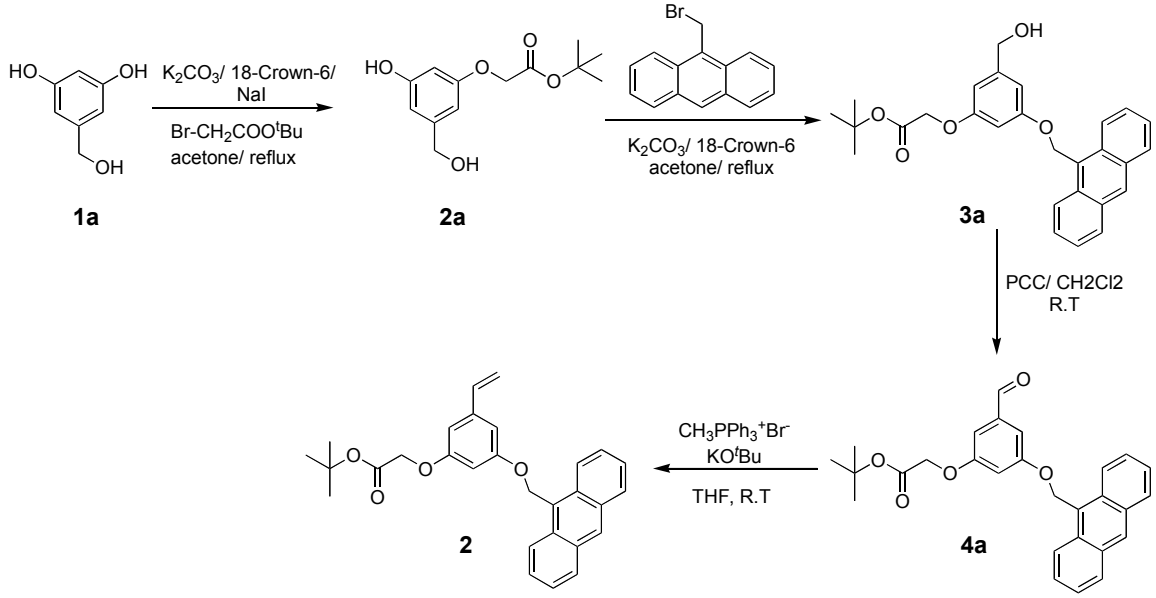
Experimental:

General methods: All reagents except cytochrome c peroxidase (CcP) were commercially available and used as received unless stated otherwise. CcP was obtained from Professor Michael Knapp's laboratory at the University of Massachusetts Amherst. ^{1}H-NMR spectra were recorded on a 400 MHz NMR spectrometer using residual proton resonance of the solvents as internal standard. Chemical shifts are reported in parts per million (ppm). ^{13}C-NMR spectra were proton decoupled and recorded on a 100 MHz NMR spectrometer using the carbon signal of the deuterated solvent as the internal standard. Mass spectra were obtained at the Molecular Weight Characterization facility at University of Massachusetts. The molecular weights of the polymers were determined by size exclusion chromatography on a single injector mode GPC, using THF as eluent and toluene as the internal reference; polystyrene standards were used for calibration and output was received and analyzed using a RI detector. The monomer **1** was synthesized as per the reported procedure[1] and the monomer **2** was synthesized starting from 3,5-dihydroxybenzyl alcohol. 9-bromomethyl anthracene[2] also was prepared using reported procedure. All the compounds were characterized using ^{1}H, ^{13}C NMR.

Scheme I- Synthesis of anthracene containing monomer 2



Synthesis of monomer **2** was achieved from 3,5-dihydroxybenzyl alcohol as shown in above scheme. Compounds **2a** was prepared by the reaction of 3,5-dihydroxybenzyl alcohol (**1a**) with 1 equiv of *tert*-butyl bromoacetate under alkylation conditions. The yield of this reaction was rather low, because of the disubstituted byproduct obtained in the reaction along with the product as a statistical mixture. The mono substituted phenol **2a** was then alkylated with 9-bromomethyl anthracene under alkylation condition to get compound **3a**. The alcohol **3a** upon oxidation by PCC afforded the corresponding aldehydes **4a**. Treatment of aldehyde **4a** with methyl triphenylphosphonium bromide and potassium *tert*-butoxide in THF afforded the monomer **2**. The block copolymer **3** was synthesized by using NMP polymerization conditions. The polymer **3** was then hydrolyzed using potassium hydroxide as the base to afford carboxylic acid polymers **4**.

Synthesis of Compound 2a

3,5-Dihydroxybenzyl alcohol (**1a)** (25 g, 178 mmol, 1 equiv) was dissolved in acetone (300 mL). To this solution were added K_2CO_3 (49.3 g, 356 mmol, 2 equiv), NaI (32.0 g, 214 mmol, 1.2 equiv) and 18-Crown-6 (4.7 g, 17.8 mmol, 0.1 equiv) and stirred for 10 min. To this mixture, *tert*-butyl bromoacetate (26.4 mL, 178 mmol, 1 equiv) was added dropwise and the mixture was refluxed for 30 h. TLC was monitored to check the reaction completion. Upon reaction completion the reaction mixture was then cooled to room temperature and the solvent was evaporated to dryness. To this residue, water and ethyl acetate were added and stirred for 30 min. The organic layer was separated and aqueous layer was extracted with ethyl acetate. The combined organic layer was washed with water and brine solution. The organic layer was evaporated to dryness and purified by silica gel column chromatography (45-50% ethyl acetate in hexanes) to afford 19.1 g (42% yield) of **2a**. The major by-product of this reaction is the compound, where *tert*-butyl acetate groups are added to both the phenolic groups of compound **1a**.

^{1}H NMR (400MHz, CD_3COCD_3) δ 8.34 (s,1H), 6.48 (s, 1H), 6.41 (s, 1H), 6.27 (t, J = 2.0 Hz, 1H), 4.52 (m, 4H), 4.22 (s, 1H), 1.45 (s, 9H).; ^{13}C NMR (100 MHz, CD_3COCD_3) δ 168.7, 160.3, 159.2, 145.9, 107.3, 104.5, 101.3, 82.0, 81.8, 66.1, 64.5, 64.4, 28.2.

Synthesis of Compound 3a

Compound **2a** (5.63 g, 22.1 mmol, 1.2 equiv) was dissolved in acetone (100 mL). To this solution were added, K_2CO_3 (9.2 g, 66.4 mmol, 3 equiv), 18-Crown-6 (0.59 g, 2.21 mmol, 0.1 equiv) and 9-bromomethyl anthracene (5.0 g, 18.4 mmol, 1 equiv). The reaction mixture was refluxed for 24 h. TLC was monitored to check the complete consumption of 9-bromomethyl anthracene. The reaction mixture was then cooled to room temperature and solvent was evaporated to dryness. The residue was partitioned between water and ethyl acetate. The organic layer was separated, and the aqueous layer was extracted again with ethyl acetate. The combined organic layer was dried over Na_2SO_4 and evaporated to dryness. The crude product was purified by silica gel column chromatography (25-30% ethyl acetate in hexane) to afford 7.54 g (92% yield) of **3a**.

^{1}H NMR (400MHz, $CDCl_3$) δ 8.49 (s, 1H), 8.23 (d, J = 8.4 Hz, 2H), 8.02 (d, J = 7.6 Hz, 2H), 7.54-7.46 (m, 4H), 7.32 (s, 1H), 6.81 (s, 1H), 6.63 (t, J = 2.0 Hz, 1H), 6.61 (s, 1H), 5.89 (s, 2H), 4.66 (s, 2H), 4.51 (s, 2H), 2.05 (s, 1H), 1.49 (s, 9H); ^{13}C NMR (100

MHz, $CDCl_3$) δ 167.9, 160.5, 159.2, 143.7, 131.4, 130.9, 129.1, 129.0, 126.5, 125.0, 123.8, 106.1, 105.5, 100.9, 82.4, 65.5, 65.1, 62.7, 28.0.

Synthesis of Compound 4a

To a stirred solution of compound **3a** (7.2 g, 16.2 mmol, 1 equiv) in dry dichloromethane (250 mL) under argon atmosphere was added pyridinium chlorochromate (4.6 g, 21.0 mmol, 1.3 equiv). It was stirred at room temperature for 5 h. The reaction mixture was filtered over celite and the filtrate was concentrated and purified by silica gel column chromatography (10-15% ethyl acetate in hexanes) to afford 6.1 g (85% yield) of **4a**.

^{1}H NMR (400MHz, $CDCl_3$) δ 9.93 (s, 1H), 8.51 (s, 1H), 8.21 (d, J = 8.8 Hz, 2H), 8.03 (d, J = 8.0 Hz, 2H), 7.56-7.46 (m, 4H), 7.32 (s, 1H), 7.07 (s, 1H), 6.94 (t, J = 2.4 Hz, 1H), 5.92 (s, 2H), 4.56 (s, 2H), 1.49 (s, 9H); ^{13}C NMR (100 MHz , $CDCl_3$) δ 191.6, 167.4, 160.8, 159.6, 138.5, 131.3, 130.9, 129.2, 129.1, 126.7, 125.9, 125.1, 123.7, 108.8, 108.3, 107.8, 82.7, 65.6, 63.1, 28.0.

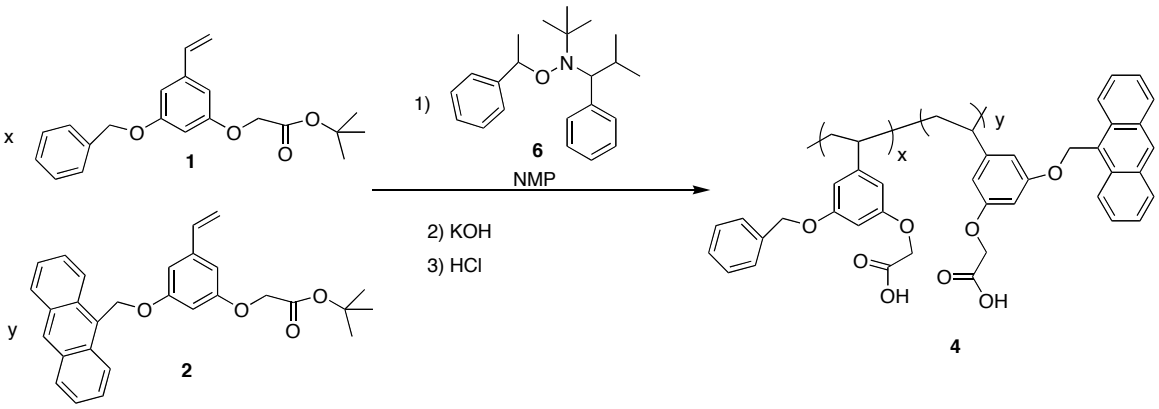
Synthesis of monomer 2

Methyl triphenylphosphonium bromide (CH_3PPh_3Br) (7.0 g, 19.7 mmol, 1.5 equiv) was taken in dry THF (150 mL) and KO^{t}Bu (2.2 g, 19.7 mmol, 1.5 equiv) was added to this under argon atmosphere. This mixture was stirred for 20 min and a solution of compound **4a** (5.8 g, 13.1 mmol, 1 equiv) in 30 mL of dry THF was added drop wise through syringe. The reaction mixture was further stirred at room temperature for 4 h. Then it was quenched with water and extracted using ethyl acetate and the crude mixture was purified by silica gel column chromatography (15-20 % ethyl acetate in hexanes) to afford 4.2 g (72% yield) of **2**.

^{1}H NMR (400MHz, $CDCl_3$) δ 8.50 (s, 1H), 8.27 (d, J = 8.4 Hz, 2H), 8.03 (d, J = 8.4 Hz, 2H), 7.56-7.46 (m, 4H), 6.88 (s, 1H), 6.72-6.69 (m, 3H), 5.90 (s, 2H), 5.80 (d, J = 17.6 Hz, 1H), 5.30 (t, J = 10.4 Hz, 1H), 4.56 (s, 2H), 1.52 (s, 9H); ^{13}C NMR (100 MHz , $CDCl_3$) δ 167.8, 160.4, 159.2, 139.8, 136.6, 131.3, 130.9, 129.1, 129.0, 126.5, 125.0, 123.8, 114.6, 106.0, 105.2, 101.3, 82.3, 65.6, 62.6, 28.0.

Synthesis of copolymer 3 and 4

To the dry single neck round-bottomed flask NMP alkoxyamine initiator **6** (0.0035 g, 0.0108 mmol), compound **2** (0.03 g, 0.0682 mmol) and **1** (0.5 g, 1.47 mmol) were added. Then the reaction flask was degassed by freeze-pump-thaw cycles. The reaction flask was kept at room temperature for 15 minutes and transferred to the preheated oil bath at 130 ˚C. Stirred at this temperature for 24 h. later this reaction was quenched by dissolving it in THF (2 mL) and precipitated in methanol. The obtained solid was again dissolved in THF (2 mL) and precipitated in methanol. The precipitate was filtered and dried under vacuum. Yield 0.42 g (75 %), SEC (polystyrene/THF) M_n 12 KDa, PDI 1.34; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.40 (bs, 0.045H), 8.20 (bs, 0.045H), 7.80 (bs, 0.18H), 7.259 (bs, 5H), 6.11-5.81 (m, 3H), 4.67 (bs, 2H), 4.19 (bs, 2H), 2.1-1.5 (m, 3H), 1.37 (s, 9H). The percentage incorporation of monomer **2** was 4%.

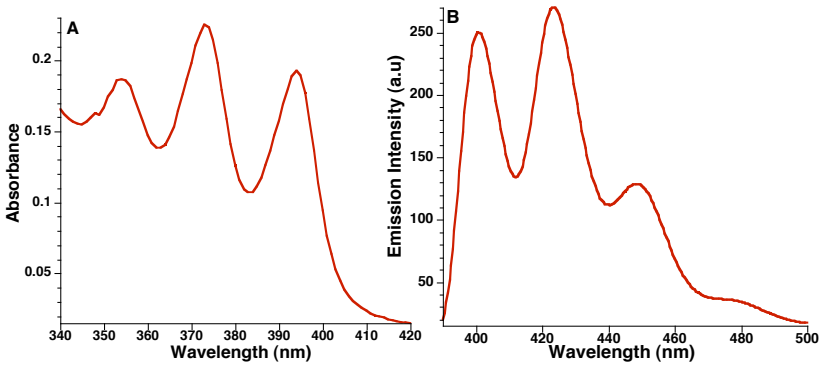


The compound **3** (0.4 g) was dissolved in THF, methanol and water mixture (7: 2: 1), to that potassium hydroxide (10 equiv.) was added. The reaction mixture was refluxed for 16 h followed by removal of solvent using rotary evaporator. The resultant mixture was dissolved in water and refluxed for 12h. Finally, polymer was precipitated by treating it with 3 M HCL. The precipitate was filtered and washed with water and dried under vacuum. Yield 0.3 g (81%). ^{1}H NMR (CD_3SOCD_3) 8.20-7.20 (m, 5.5H), 6.11-5.8 (m, 3H), 4.69 (bs, 2H), 4.32 (bs, 2H), 1.24-2.25 (m, 3H).

Spectroscopic Measurements:

The UV-Vis absorption spectra were recorded on a varian (model EL 01125047) spectrophotometer using quartz cells. Fluorescence spectra were recorded on a Jasco (FP-6500) fluorimeter. The spectra were recoded using a 200 μL quartz cuvette. Excitation and emission bandwidth was kept at 3 nm. For Stern-Volmer quenching studies, optical value at the peak wavelength was set between 0.1 to 0.2 to avoid self-absorption effects. Polymer **4** excited at 373 nm, and the emission was recorded from 390 to 500 nm. For proflavin hydrochloride, the emission was recorded from 390 to 650 nm. For fluorescence studies, 50 μM Polymer **4** was incubated with various concentrations of analytes (10 – 50 μM of protein) in 5 mM sodium phosphate buffer pH (7.4) and the measurements were taken after 30 minutes. Stern-Volmer studies for some proteins (elastase and β-glucosidase) were carried out at much lower concentrations (2 – 10 μM) due to solubility problems.

Absorption and Emission Spectra of Polymer 4:



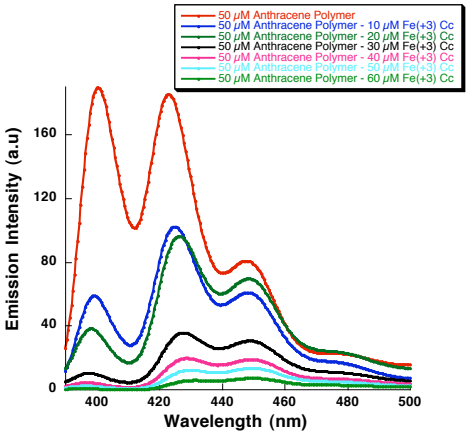
Concentration of polymer **4** = 100 µM

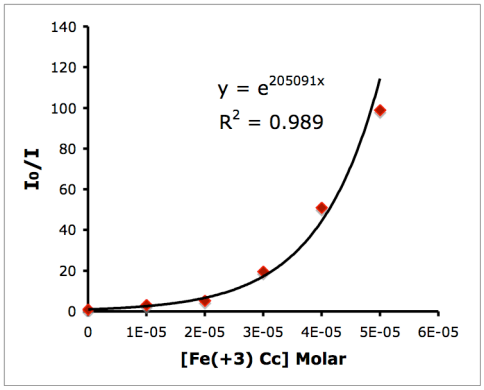
***Stern-Volmer Quenching Studies**:*

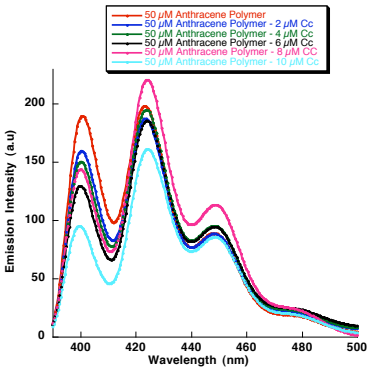
Metalloproteins:

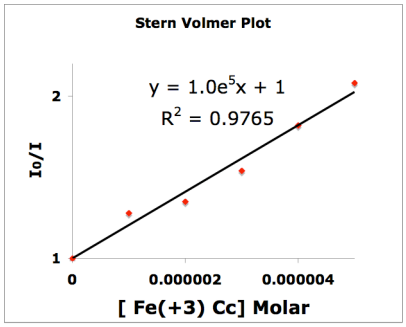
For Cytochrome c, we have carried out Stern-Volmer quenching studies at two differerent concentrations. At lower concentration of Cc (2-10 µM), I_0/I increases linearly with the quencher concentrations. However, at higher concentration of Cc (10 - 60 µM) superlinear behavior was observed due to sphere of action mechanism.[3]

Cytochrome c:

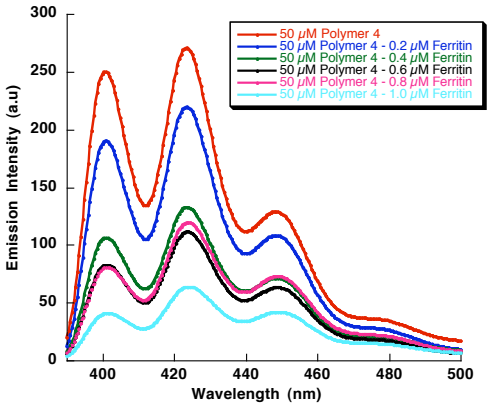


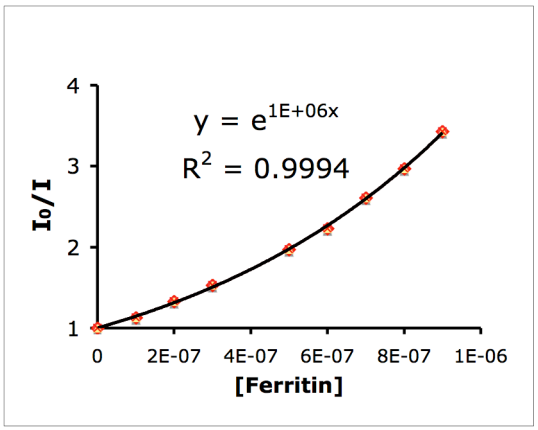




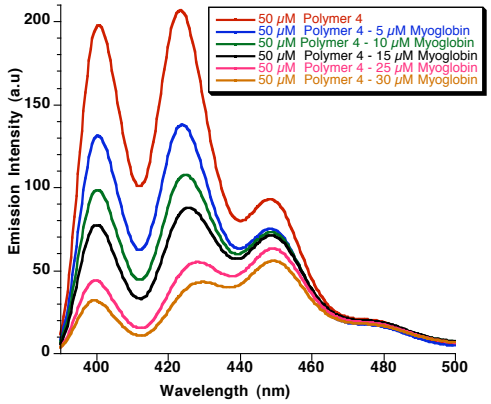


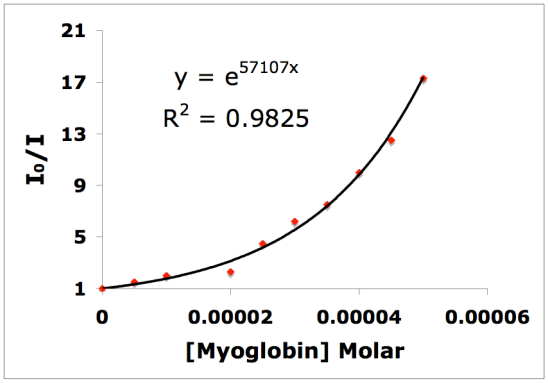
Ferritin:



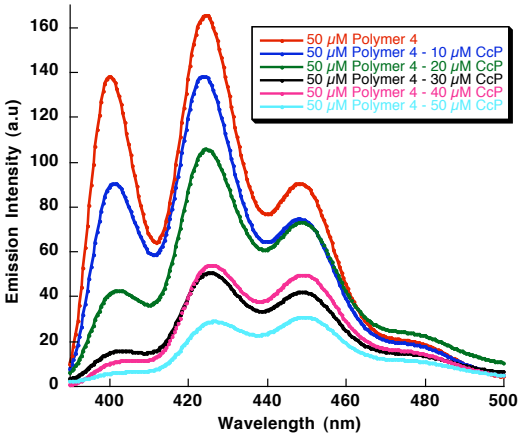


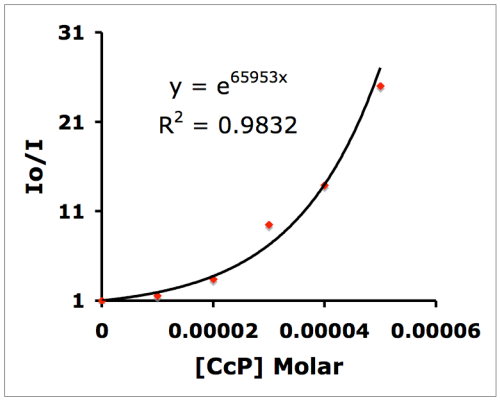
Myoglobin:



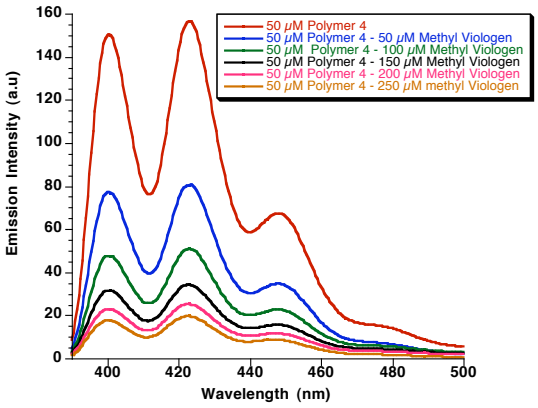


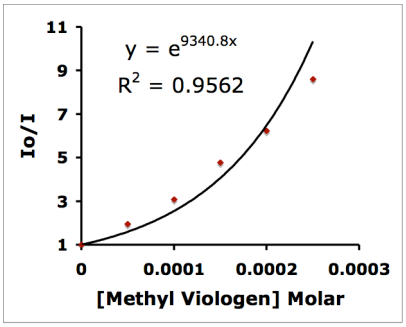
Cytochrome c Peroxidase (CcP):



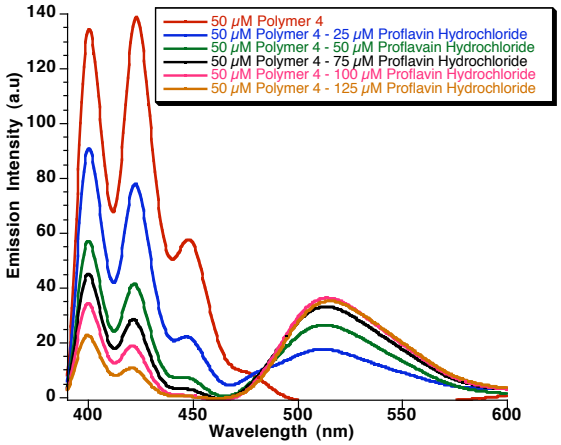


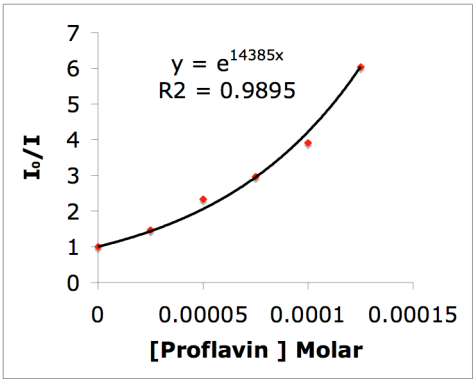
Electron Acceptor - Methyl Viologen :





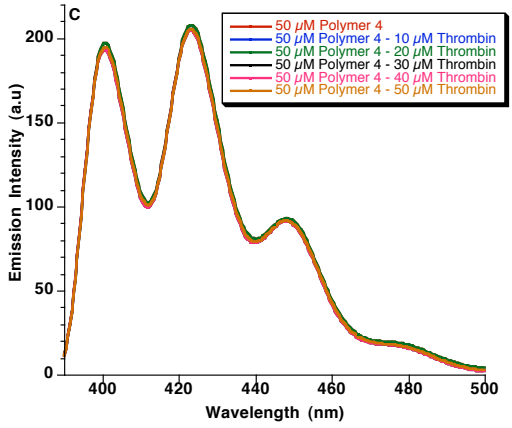
Energy Acceptor - Proflavin hydrochloride



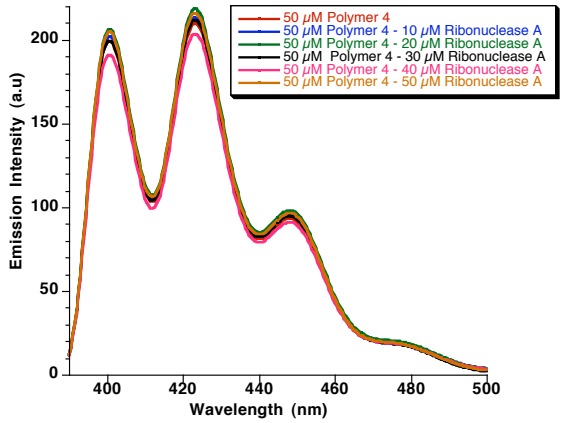


Non-Metalloproteins

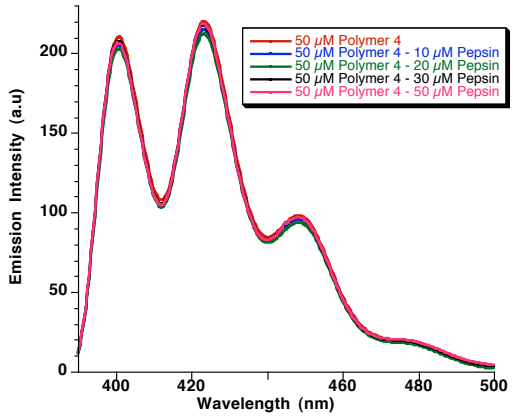
Thrombin:



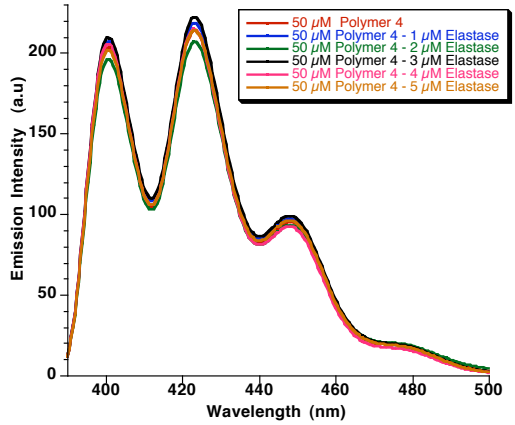
Ribonuclease A:



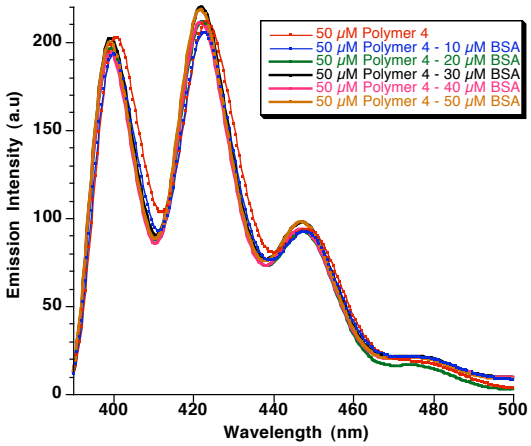
Pepsin:



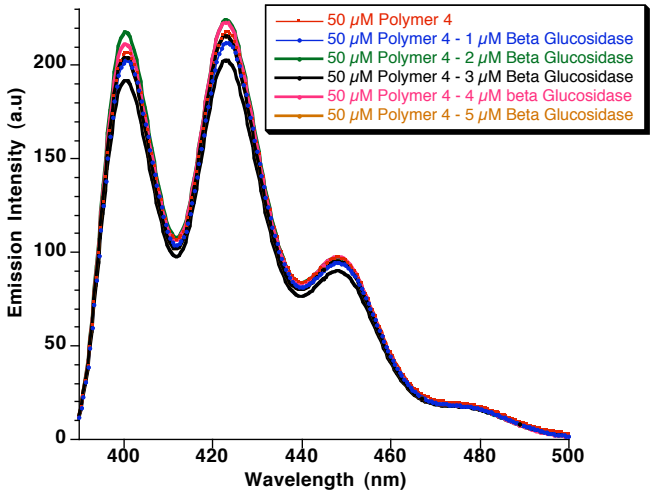
Elastase:



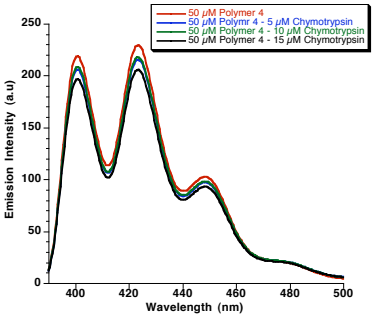
Bovine Serum Albumin (BSA):



β-Glucosidase:



α-Chymotrypsin:



References:

1. Basu, S.; Vutukuri, D. R.; Thayumanavan, S. *J. Am. Chem. Soc*. **2005**, *127*, 16794-16795.
2. Stack, D. E.; Hill, A. L.; Diffendaffer, C. B.; Burns, N. M. *Organic Letters*. **2002**, *4*, 4487-4490.
3. Wang, J.; Wang, D.; Miller, E. K.; Moses, D.; Bazan, G. C.; Heeger, A. J. *Macromolecules* **2000**, *33*, 5153-5158.