A biosensing model system: Selective interaction of biotinylated PPEs with streptavidin-coated polystyrene microspheres

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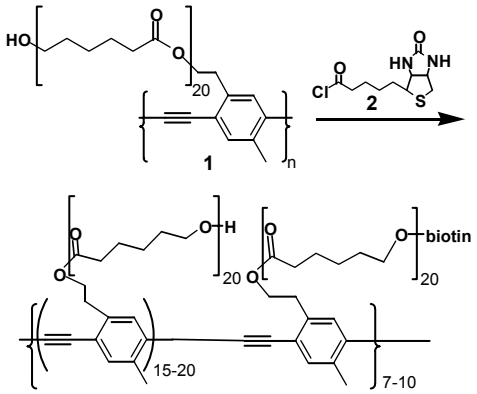
Supplemental Material

Biotinylation of **1** to form **3**: An oven-dried Schlenk flask with stirbar was cooled under N_2 gas and charged with biotin (122 mg, 0.500 mmol). Excess (~2 mL) thionyl chloride was added and the



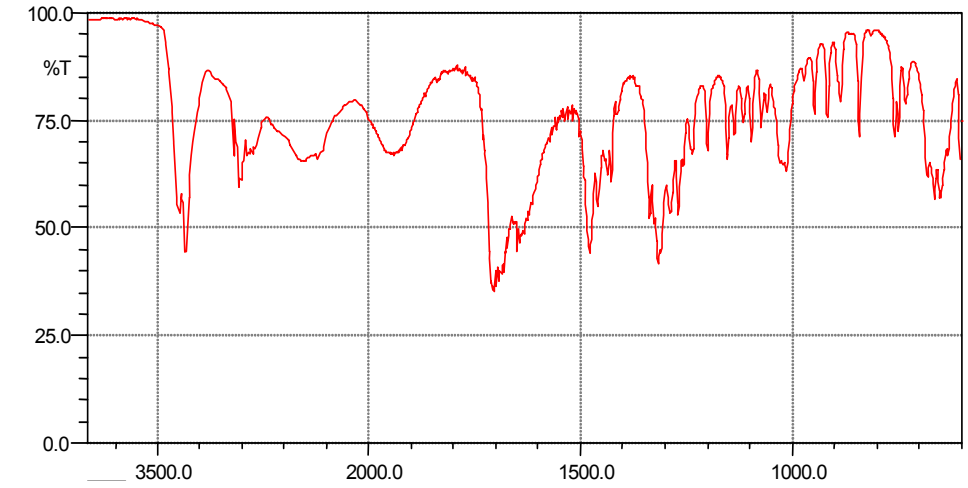
3

reaction was capped with a septum and placed into an ice bath. The reaction was occasionally vented with a needle as it reached ambient temperature over a 2h period. Excess thionyl chloride was removed by vacuum distillation. The product **2** was used without purification. Polymer **1** (120 mg, 0.0500 mmol) was dissolved in a freshly distilled mixture of THF/triethylamine (5:1) (~2 mL). This solution was pipetted into the reaction vessel containing **2**. The reaction mixture was placed again into an ice bath and allowed to reach ambient temperature over the course of 4 h. The polymer **3** was precipitated into excess methanol, collected over a fritted funnel, re-dissolved in THF and precipitated again into water (110 mg collected).

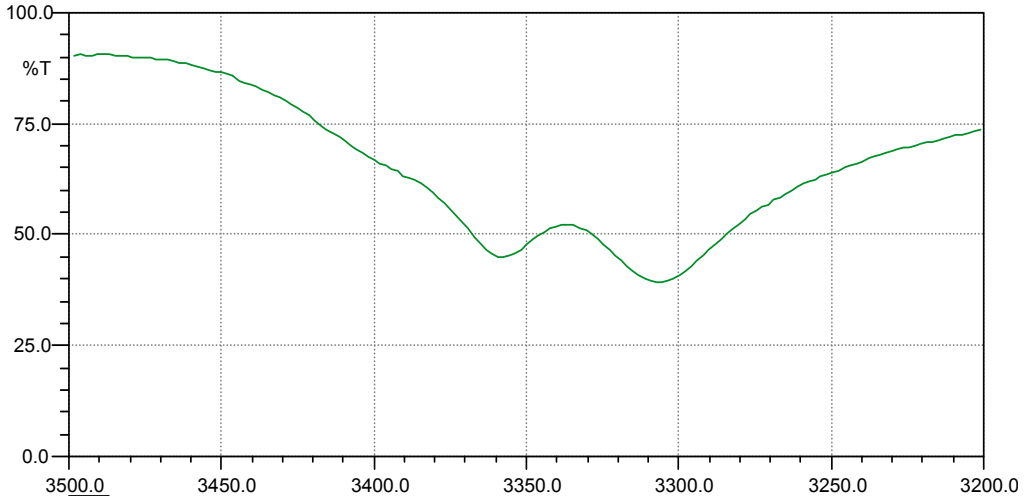
Characterization of 1, 3 and biotin by IR:

Spectra were obtained on a Shimadzu 8400 FTIR with a Pike Technologies Diffuse Reflectance attachment and processed with Shimadzu Hyper-IR v 1.57 software including the Kubelka-Munk fuction to help resolve the peaks. All samples were scanned 3000 times. The characteristic bands observed at 3307 and 3358 cm^{-1} were visible in the expansion in the region of 3500 cm^{-1} to 3200 cm^{-1} for polymer **3**.

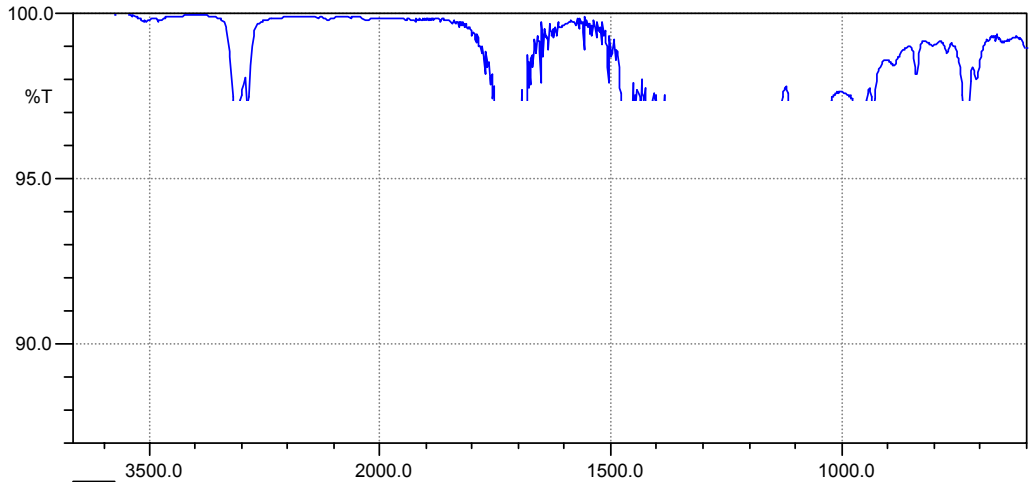
Biotin:



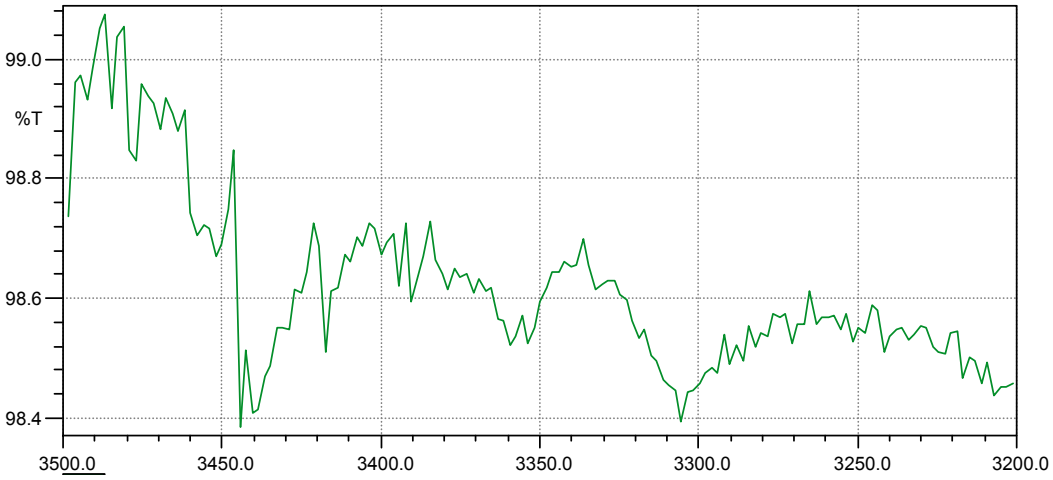
Biotin (expansion from 3500 cm^{-1} to 3200 cm^{-1})



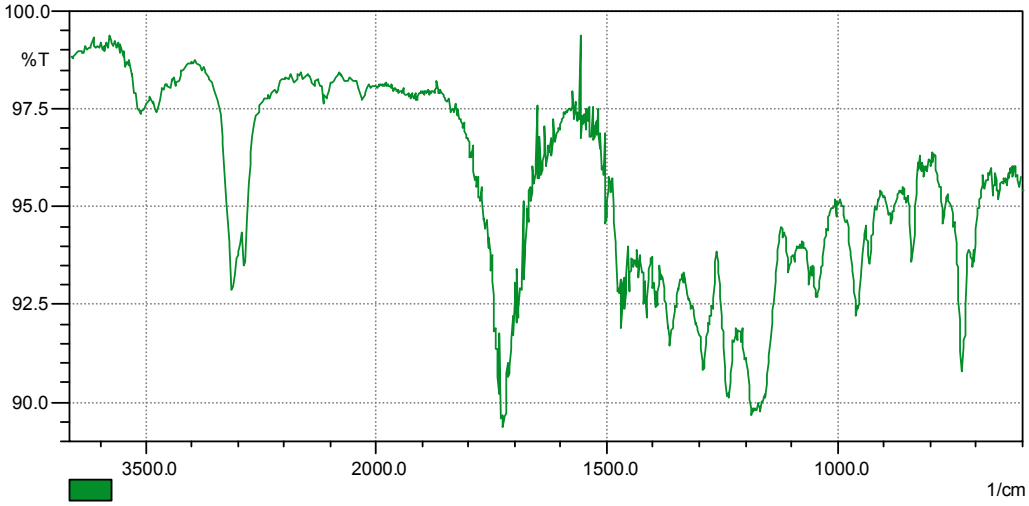
Polymer **1**:



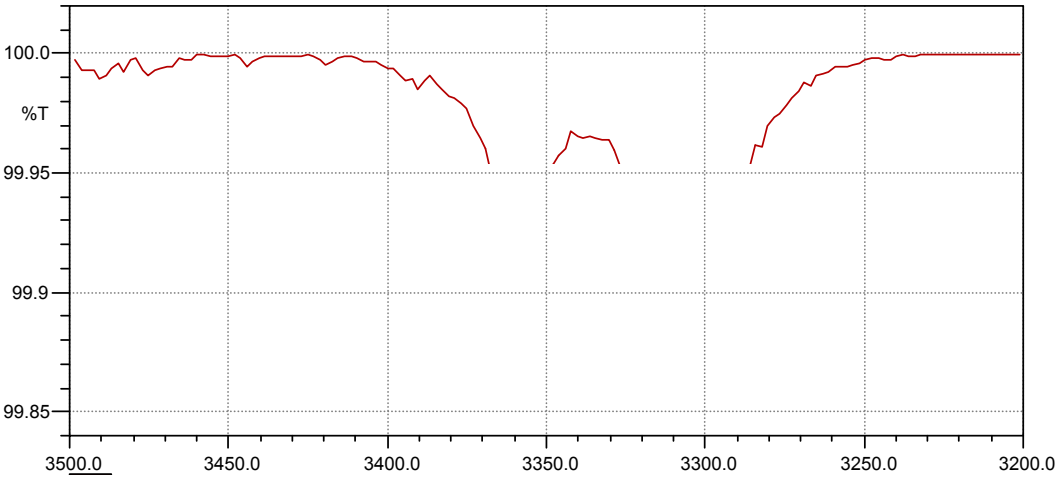
Polymer **1** (expansion from 3500 cm^{-1} to 3200 cm^{-1})



Polymer **3**:



Polymer **3** (expansion from 3500 cm^{-1} to 3200 cm^{-1}):



Preparation of the polymer solutions for exposure to microspheres:

Polymer **3** (20.0 mg, 5.95 x 10^{-5} mmol) was taken up in a small amount (~1 mL) of THF. Sodium dioctyl sulfosuccinate (100 mg, 0.225 mmol) was dissolved in 100 mL of water. The THF solution was added dropwise to the vigorously stirred surfactant solution. This mixture was then heated at ~50^{o} C for 12 hours, then diluted to a total volume of 1.0 L (Stock A); 10 mL of stock A was lyophilized, then redissolved in 20 mL of 0.1 M sodium phosphate buffer. 1 mL (containing 10 μg of polymer) of this buffered polymer solution was placed into an Eppendorf tube containing 0.5 mg of streptavidin-coated polystyrene microspheres. The Eppendorf tube was capped and placed onto a mechanical wrist shaker for 12h. The agglutinated composite was found to be immobilized on the side of the capsule as seen in Figure 1.

SEM Images:

Samples of **1** and **3** exposed to streptavidin-coated beads were prepared for SEM by placing them onto an aluminum sample plate which was covered with a conducting graphitic tape. The samples were placed in a vacuum sputterer and coated with 0.40 nm of gold. Images were obtained digitally on a Hitachi 2500 Delta.

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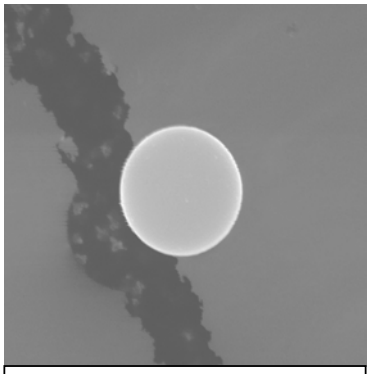
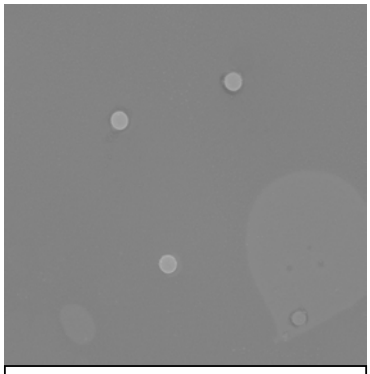
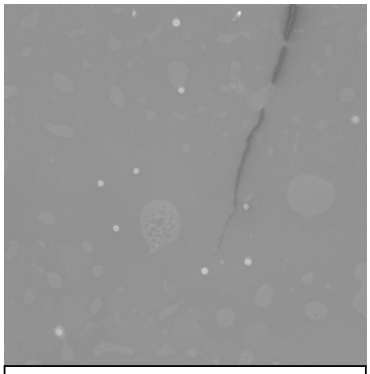
Control Sample: Polymer **1** + streptavidin-coated polystyrene microspheres



←18 μm →

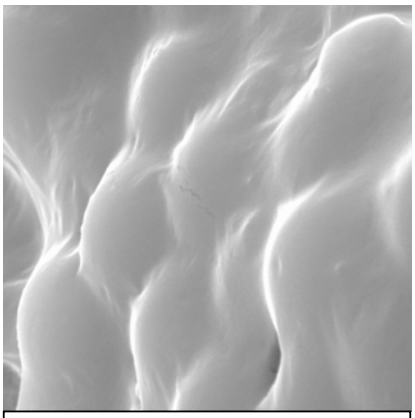


←130 μm →

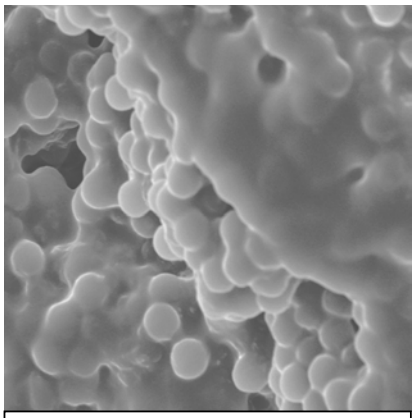


←410 μm →

Polymer **3** combined with streptavidin-coated polystyrene spheres

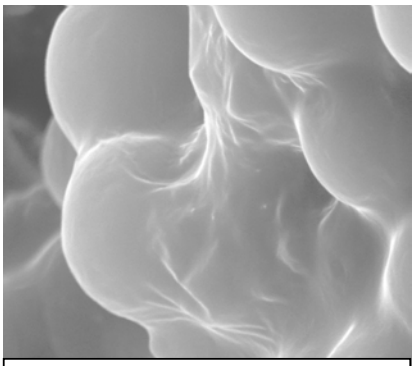


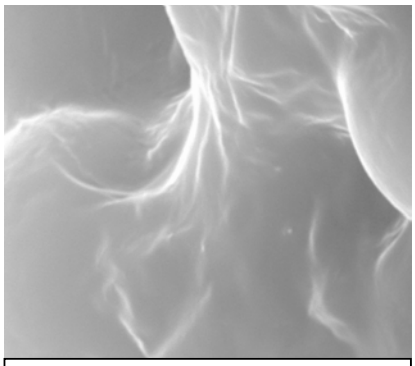
←18 μm →



←75 μm →

Polymer **3** combined with streptavidin-coated polystyrene spheres





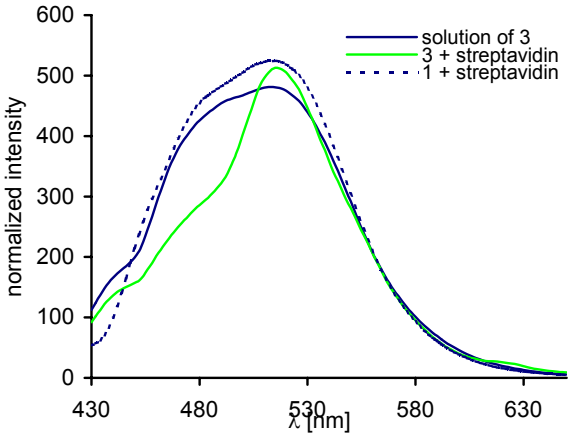
Stoichiometric Calculations:

Calculations used to estimate the approximate percent functionalization of the biotinylated polymer **3** utilizing streptavidin-coated microspheres:

Bangs Laboratories states that 1 mg of streptavidin coated polystyrene microspheres (Product Code: CP01N/5622) can bind 0.098 μg of biotin. Thus, the 0.5 mg of beads utilized in this experiment must bind 0.049 μg of biotin. Assuming perfect binding (1:1 streptavidin:biotin) this would mean that 10 μg of polymer (2400 g/mol-repeat) would be ~5% functionalized.

A titration experiment was then performed with free streptavidin to confirm this approximation. A buffered polymer solution of 15 mg/L (Stock B) was prepared for this experiment—the dilutions per flask are listed in the table below. 1 μg of streptavidin was added to each flask. Aldrich streptavidin can bind 14 pg (0.057 pmol) of biotin per μg. Streptavidin *may* bind up to four biotin molecules, but aggregation can occur if only 2 biotin molecules from separate polymer molecules are bound. To further complicate the matter, *inter*polymer biotin binding must compete with *intra*polymer biotin binding. Thus, the table below shows a range of binding modes (2,3,4). As the spectroscopic changes began with the third dilution (flask 3), the minimum percent functionalization can be found here assuming a binding mode of only 2 biotin molecules per streptavidin. From these data we can conclude that the polymer **3** is 12% functionalized with biotin.

Flask	1	2	3	4	5
mL stock B	1.0	0.20	0.04	0.008	0.00016
μg polymer	15	3	0.6	0.12	0.024
pmol of polymer	6.25	1.25	0.250	0.050	0.010
4:1 biotin:streptavidin	-	-	0.057 / 0.250 = 23%	-	-
3:1 biotin:streptavidin	-	-	0.043 / 0.250 = 17%	-	-
2:1 biotin:streptavidin	-	-	0.029 / 0.250 = 12%	-	-



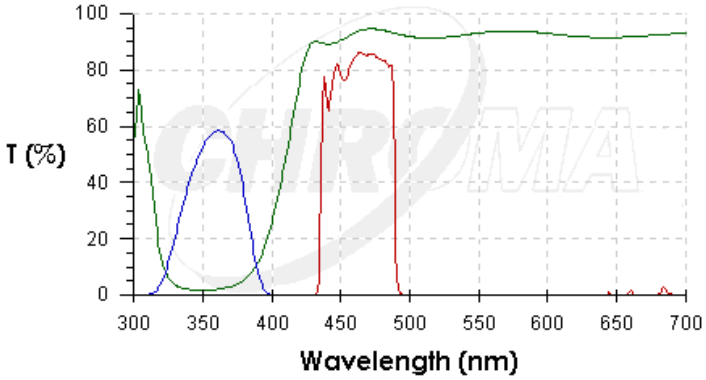
Emission spectra of **3**, **1** with streptavidin and **3** with streptavidin (suspension)

Fluorescence Microscopy

The following filter sets and spectra were purchased/obtained from Chroma and www.chroma.com.

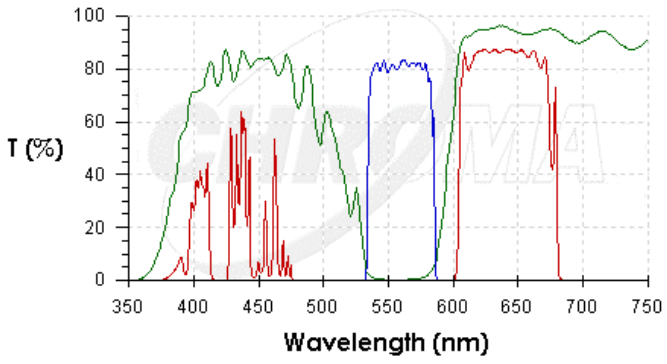
Filter	***Product Code***	***Spectra Link***
DAPI	31000	http://www.chroma.com/data/spectra.cfm?setnum=31000
Texas Red	41004	http://www.chroma.com/data/spectra.cfm?setnum=41004

Spectra: *DAPI*



Blue = exciter, green = dichroic, red = emitter

Texas Red



Blue = exciter, green = dichroic, red = emitter