# Probing Biological Recognition Using Conjugated Polymers at the Air−Water Interface

**Juan Zheng and Timothy M. Swager***

*Department of Chemistry, 77 Massachusetts Avenue, Cambridge, Massachusetts 02139*

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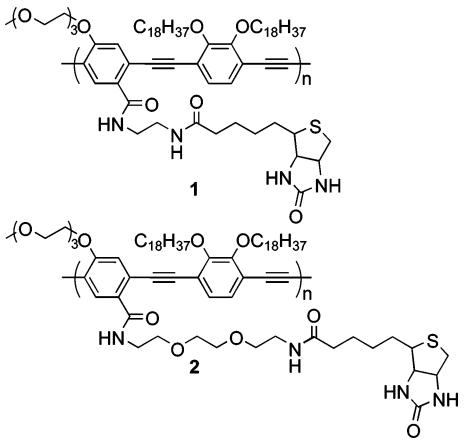
Conjugated polymer chemosensors such as poly(*p*-phenylene ethynylene)s (PPEs) function with superior sensitivity (when compared to their monomeric counterparts) in the detection of trace amounts of analytes by amplified fluorescence quenching.[1] Although the operative molecular wire effect is advantageous to these "turn-off" sensing schemes, the excellent light-harvesting capability of PPEs can also be harnessed for energy transfer in detection methods.[2] Previous studies investigated the detection of biological analytes using energy transfer in solution phase, which led to surprising insights into its mechanism.[3] However, in biological systems the recognition often occurs at amphiphilic interfaces. Previous biosensor models using such amphiphilic surfaces have been devised using phospholipids,[4] amphiphilic diacetylene monomers, and supported poly(diacetylene) polymers.[5] Here, we present a biotinylated fluorescent amphiphilic polymer as a mimic for cellular interfaces. This was studied at the air−water interface using a Langmuir trough, which allows for controlled orientation of the hydrophilic and hydrophobic side chains, thus providing a controllable model for understanding the basic mechanisms for recognition processes in amphiphilic cellular environments.

Streptavidin is a tetrameric protein that binds up to four molecules of *d*-biotin with the dissociation constant estimated to be $4 \times 10^{-14}$ M.[6] Because of this high affinity, the streptavidin−biotin recognition system has been applied to model systems in conjunction with conjugated polymers in affinitychromic,[7] energy transfer,[3] and agglutination assays.[8] Here, amphiphilic biotinylated PPEs **1** and **2** were synthesized via a Sonagashira−Hagihara cross-coupling reaction.[9,10] The polymers differed only in their linker length with respect to the biotin moieties.




When deposited on the air−water interface without any applied pressure, both polymers assume alternating face-on and edge-on conformations for the hydrophilic biotinylated monomers and the hydrophobic octadecyloxy-substituted monomers, respectively. Upon application of mechanical pressure using the Langmuir trough barriers, this alternating conformation—referred to as zipper phase—rotates to assume an aggregated edge-on conformation.[11]

This conformation change may be monitored using a variety of methods. In particular, the pressure−area isotherms show a distinct slope change corresponding to the change in polymer conformation. The polymers show aggregation behavior when compressed to the edge-on phase as evidenced by the UV−vis absorption and fluorescence emission spectra (Figure 1).[11] Red-shifted aggregation peaks at 441 and 447 nm arise for polymers **1** and **2**, respectively, at surface pressures between 10 and 15 mN/m. Concurrently, fluorescence is dramatically quenched at the transition between zipper and edge-on phases and continues to quench when further compressed. This is consistent with increased interchain $\pi$-stacking interactions.[11]

To test the ability of the polymers to interact with avidin and streptavidin, 0.25 mg of avidin in 0.5 mL of Tris buffer was added to the Tris buffer subphase (60 mL) of the annealed polymer monolayer and incubated for 1 h. The pressure−area isotherms for **1** and **2** following incubation show a less marked slope change upon phase transition. Less aggregation results from avidin binding as evidenced by the fluorescence and UV−vis spectra (see Supporting Information). Compared to the native polymer, the fluorescence was less quenched at 15 mN/m and higher surface pressures. Relative quantum yields for the polymers at 35 mN/m (monitored at 500 nm) were calculated for both polymers **1** and **2**. Following incubation with avidin, the quantum yields were 1.58 and 1.69 times the original quantum yields of the native polymers **1** and **2**. Control experiments using biotin presaturated avidin to preclude biological recognition were carried out. These relative quantum yields remained constant, at 1.01 and 1.05 times the original values for **1** and **2**, respectively.

To verify that the biotin groups at the polymer monolayer still retained their ability to bind to proteins, energy transfer assays were carried out with 0.040 mg of Texas Red-X-labeled streptavidin injected into the subphase (60 mL) following film annealing. After incubation of the labeled streptavidin with the monolayer, a minimum amount of energy transfer was observed with polymer **1**, while polymer **2** displayed significantly higher energy transfer under similar conditions (Figure 2a,b). This dramatic difference may be related to the linker length of the biotin to the polymer, which tailors the biotin availability by changing the hydrophilic characteristics of the side chain. The longer linker provided better access of the biotin to the binding pocket of streptavidin, which is located at the center of each $\beta$-barrel. For the shorter linker, biotin cannot bind as efficiently to the streptavidin located in the aqueous subphase, resulting in a weaker energy transfer signal. In addition, energy transfer was more efficient when the polymer was in the zipper phase (0−15 mN/m). As there were less interchain interactions in the zipper phase, the polymer was less likely to undergo quenching through nonradiative processes, and the chances of energy transfer may be increased. In other words, at the edge-on conformation (15−35 mN/m), quenching through polymer aggregation competed with energy transfer, and it was thus less efficient.

* Corresponding author. E-mail: tswager@mit.edu.

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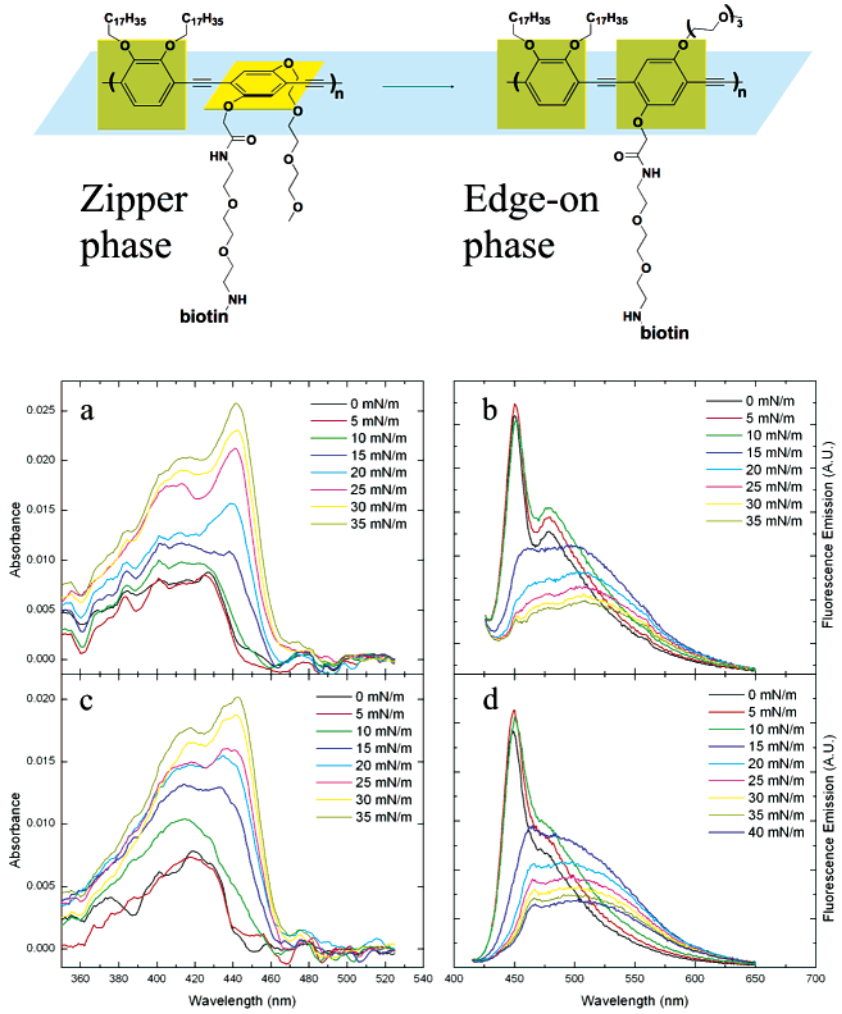


**Figure 1.** (a) In situ UV−vis absorption spectra of monolayer of **1** at the air−water interface. (b) Fluorescence emission of polymer **1**. (c) In-situ UV−vis absorption of monolayer of **2** at the air−water interface. (d) Fluorescence emission of polymer **2**.

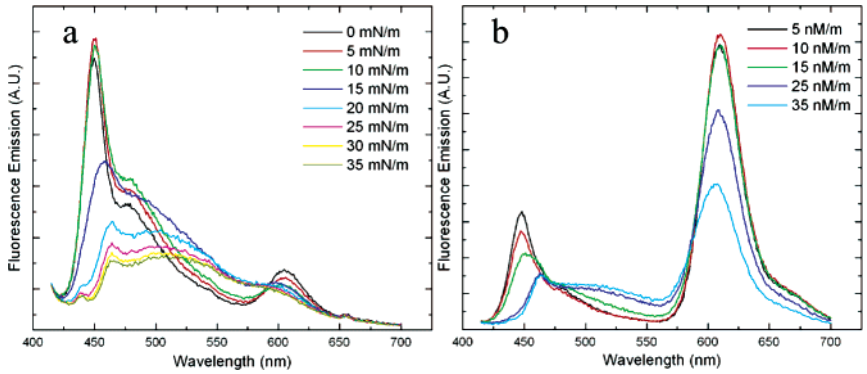


**Figure 2.** (a) Fluorescence emission of Texas Red X-labeled streptavidin incubated with **1**. (b) Fluorescence emission of Texas Red X-labeled streptavidin incubated with **2**.

Excitation spectra were obtained in order to ascertain that the emission of Texas Red dye was indeed due to energy transfer. The spectra corresponded largely to the polymer's unaggregated absorption profile. Furthermore, direct excitation at 400 nm (where the polymer was irradiated) of only Texas Red X-labeled streptavidin in the Langmuir trough failed to produce any fluorescence. The observed fluorescence was therefore due to energy transfer from the polymer to the dye.

Control experiments using free Texas Red dye (not conjugated to any protein) were also conducted. While some energy transfer occurred due to nonspecific binding for both polymers, they do not explain the greater amount of energy transfer observed for the protein experiments, thus further suggesting the importance of biological recognition. Control experiments using biotin presaturated Texas Red-X-labeled streptavidin added to the polymer monolayers demonstrated that, once again, minimal energy transfer was observed between the polymer and the dye, as biological recognition was now precluded.

In summary, we have studied the effect of linker length on biological recognition at the air−water interface using energy transfer between a fluorescent conjugated polymer and a dye-labeled protein. A longer linker provided greater binding of the protein as evidenced by the dramatically increased energy transfer. This may be due to an increased hydrophilicity of the

side chain, which allowed the biotin to better access the streptavidin located in the aqueous subphase. Subtle changes in the polymer structure can thus have important consequences for analyte recognition.

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**Supporting Information Available:** Experimental details, synthesis of the polymers, and control experiments. This material is available free of charge via the Internet at http://pubs.acs.org.

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