Electronic Supplementary Information

for

Probing biological recognition using conjugated polymers at the air-water interface

Juan Zheng and Timothy M. Swager*

Experimental details, synthesis of the polymers and control experiments.

(8 pages)

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

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. 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. Melting point (m.p.) determination was performed using a Laboratory Devices MEL-TEMP instrument (open capillaries used) and was uncorrected.

Monolayer studies were performed on a NIMA 201 M Langmuir-Blodgett trough with a quartz window. The *in situ* UV-visible absorption spectra were measured with a Cary 50 UV/visible spectrometer with fiber optics. Fluorescence spectra were measured with a SPEX Fluorolog-2 fluorometer (model FL112, 450W xenon lamp) equipped with a bifurcated fiber optic cable oriented at about 60^{o} relative to the flat surface of the subphase.

Materials.

All solvents were spectral grade unless otherwise noted. Morpholine and biotin were purchased from Alfa Aesar and used as received. Texas Red-X conjugated streptavidin and avidin 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.

(**3**): To **1** (0.367 g, 0.618 mmol) was added 5 ml CH_2Cl_2. Anhydrous NEt_3 was then added (0.129 ml, 0.927 mmol) and the mixture was stirred for 5min. **2**[51] (0.129 g, 0.804 mmol) was added as a solution in 5 ml CH_2Cl_2. The reaction mixture was refluxed for 12h. The solvent was removed under reduced pressure. The residue was dissolved in 50 ml $CHCl_3$ and washed with 15ml H_2O. The organic layer was washed with 15ml brine, dried over $MgSO_4$. The resulting yellow oil was eluted with 7:3 ethyl acetate/hexane through a plug of silica. Fractions containing the product were combined. The organic solvent was removed under reduced pressure to afford a pale yellow solid (0.228g, 52%).. M.p.: 53-54^oC.^1H NMR (500 MHz, $CDCl_3$): 7.24 (1H, s), 7.19 (1H, br), 7.16 (1H, s), 4.95 (1H, br), 4.45(2H, s), 4.13 (2H, t, J=4.5Hz), 3.90 (2H, t, J=4.5Hz), 3.80 (2H, t, J=4.5Hz), 3.70-3.66 (4H, m), 3.56 (2H, t, J=4.5Hz), 3.54 (4H, m), 3.38 (3H, s), 3.33 (2H, m), 1.43 (9H, s); ^{13}C NMR (125 MHz, $CDCl_3$): 167.9, 156.3, 154.1, 151.2, 123.4, 123.2, 86.8, 86.2, 79.8, 72.1, 71.3, 70.9, 70.8, 70.4, 69.7, 69.1, 59.3, 40.6, 39.3, 28.6; HR-MS (ESI) calcd. For $C_{22}H_{34}I_2N_2O_8$ (M+H): 708.32, found: 709.0.

(**4**): A 50ml round bottom flask containing **3** (0.228 g, 0.322 mmol) was loaded with 5ml 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 3ml anhydrous DMF, NEt_3 (0.049 ml, 0.483 mmol). This was stirred for 15min, then N-hydroxysuccinimido biotin[52] (0.111g, 0.325 mmol) was added. The pale yellow solution quickly became a thick white slurry and was stirred at room temperature for 12h. The solvent was removed under reduced pressure at 40^oC and the reaction mixture was washed with 10 ml H_2O. The product was isolated by centrifugation and lyophilized to afford a white powder (0.244 g, 91%). M.p.: 203-205^oC. ^1H NMR (500 MHz, DMSO): 7.88 (2H, m), 7.39 (1H, s), 7.31 (1H, s), 6.43 (1H, s), 6.36 (1H, s), 4.49 (2H, s), 4.28 (1H, m), 4.11 (2H, m), 3.74 (2H, t, J=5.0Hz), 3.62 (2H, t, J=5.0Hz), 3.53 (4H, m), 3.42 (2H, t, J=5.0Hz), 3.23 (3H, s), 3.19 (2H, t, J=5.0Hz)), 3.14 (2H, t, J=5.0Hz), 3.08 (2H, m), 2.80 (1H, dd, J=12.5, 5.0Hz), 2.58 (1H, 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, DMSO): 172.3, 167.3, 162.6, 153.0, 151.9, 123.6, 122.7, 87.1, 86.7, 71.3,

70.1, 69.8, 69.7, 69.6, 69.1, 68.9, 61.0, 59.2, 58.0, 55.4, 38.3, 38.1, 35.2, 28.2, 28.0, 25.2; HR-MS (ESI) calcd. For $C_{27}H_{40}I_2N_4O_8S$ (M+H): 834.50, found: 835.0.

Polymer **1**: A 25ml schlenk flask was charged with **4** (20.00 mg, 0.0240 mmol), **5** (15.892 mg, 0.0240 mmol), $Pd(PPh_3)_4$ (1.39mg, 0.00120 mmol) and CuI (0.206 mg, 0.00120 mmol) under N_2. To this was added 1.0 ml freshly degassed morpholine under N_2. The reaction vessel was sealed and heated at 60^{o}C for 48h. The polymer solution was then precipitated into ~125ml methanol and collected by centrifugation, followed by drying on high vaccum. Yield: 25mg, 86%. Mn = 15000, PDI = 1.78 for THF soluble fraction. ^{1}H NMR (500 MHz, $CDCl_3$, ppm): broad peaks at 7.1, 4.6, 4.4, 4.2, 3.9, 3.8, 3.6, 3.5, 3.4, 3.1, 2.9, 2.8, 2.0, 1.8, 1.6, 0.9

Polymer **2**: A 25ml schlenk flask was charged with **7** (20.00 mg, 0.0217 mmol), **5** (14.375mg, 0.0217 mmol), $Pd(PPh_3)_4$ (1.25mg, 0.00108 mmol) and CuI (0.206 mg, 0.00108 mmol) under N_2. To this was added 1.0 ml freshly degassed morpholine under N_2. The reaction vessel was sealed and heated at 60^{o}C for 48h. The polymer solution was then precipitated into ~125ml methanol and collected by centrifugation, followed by drying on high vaccum. Yield: 25mg, 89%. Mn = 9600, PDI – 1.41 for THF soluble fraction. ^{1}H NMR (500 MHz, $CDCl_3$): broad peaks at 7.1, 4.6, 4.3, 3.9, 3.8, 3.6, 3.5, 3.4, 3.1, 2.9, 2.7, 2.2, 1.8, 1.3, 0.9.

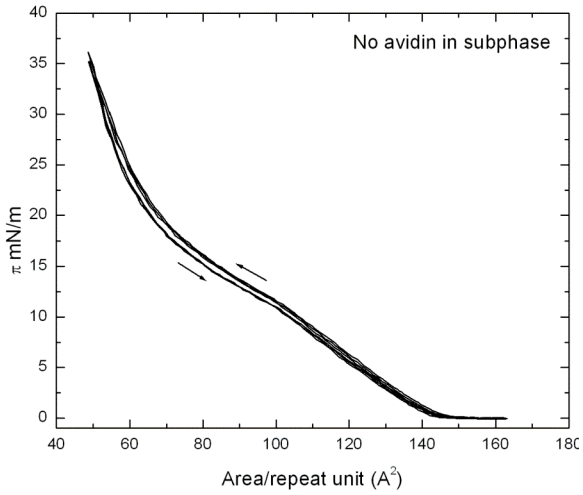

Figure 1. Pressure-area isotherms for polymer **1** with no avidin added to the subphase, after three annealing cycles. Change from zipper to edge-on at around 15 mN/m.

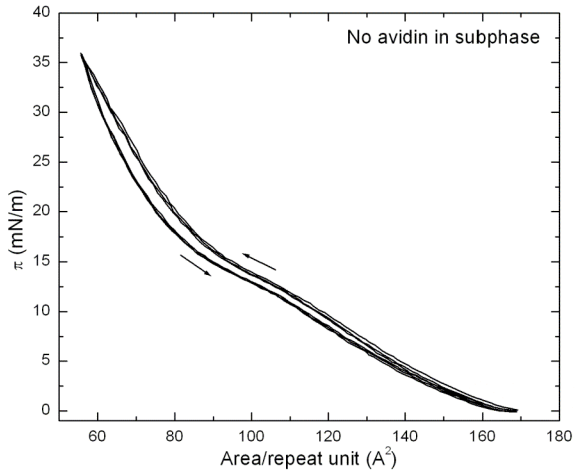

Figure 2. Pressure-area isotherm of polymer **2** with no avidin added to the subphase, after three annealing cycles. Change from zipper to edge-on phase at around 15 mN/m.

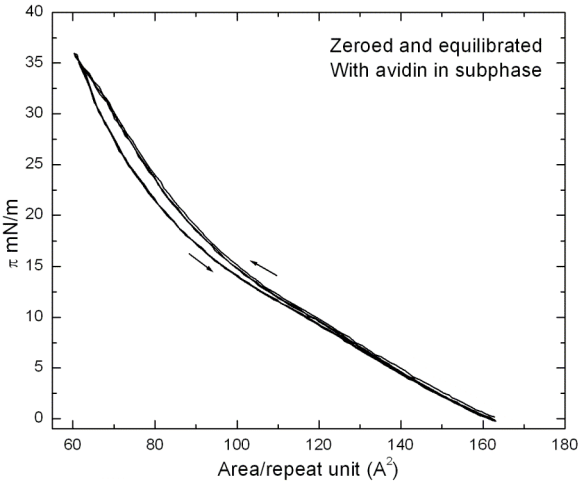

Figure 3. Pressure-area isotherm of polymer **1** when avidin was added to subphase, reproducible isotherms after initial annealing cycles.

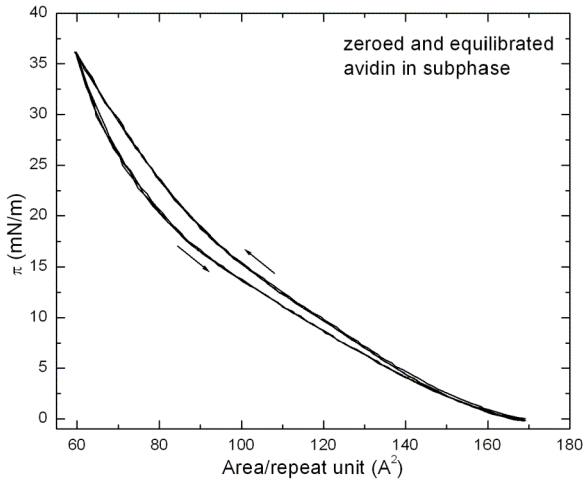

Figure 4. Pressure-area isotherms of polymer **2**, after initial annealing cycles. Hysteresis on expansion from edge-on to zipper phase.

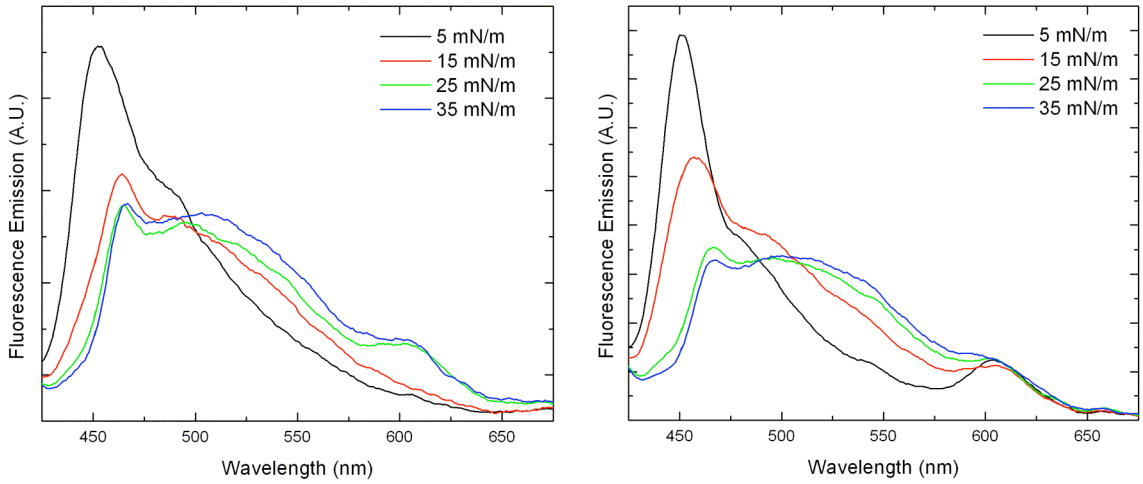

Figure 5. Incubation with sulforhodamine 101 (Texas Red parent dye). Left: polymer **1**. Right: polymer **2**. Difference between the polymers was not as great as when Texas Red X-labeled streptavidin was used.

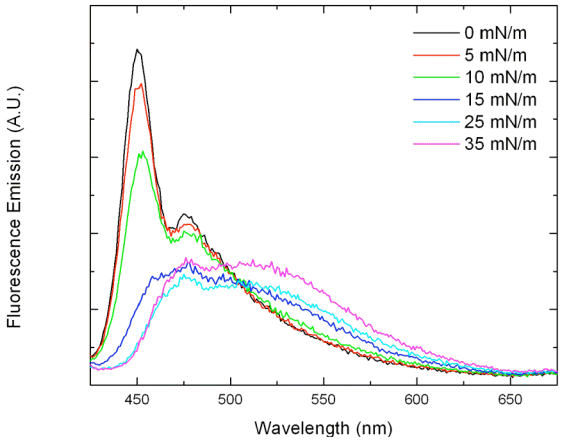

Figure 6. Fluorescence emission of polymer **1** with biotin pre-saturated dye-labeled streptavidin, no energy transfer observed due to lack of biological recognition.

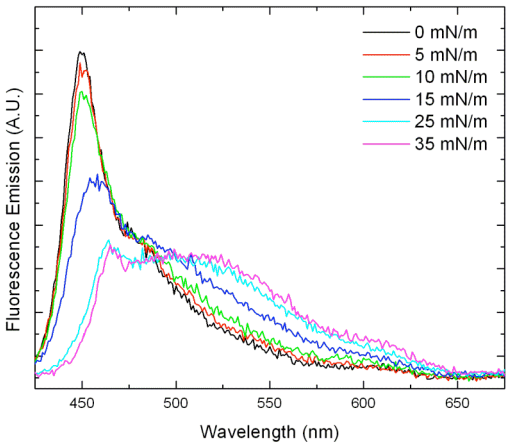

Figure 7. Fluorescence emission of polymer **2** with biotin pre-saturated dye-labeled streptavidin, virtually no energy transfer observed due to lack of biological recognition.

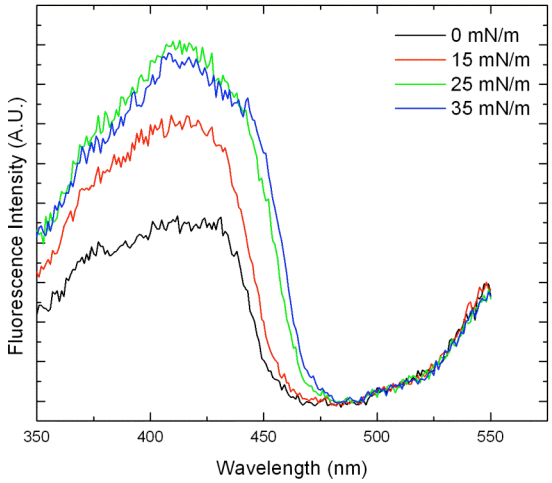

Figure 8. Excitation spectra of Texas Red X-labeled streptavidin, when incubated with polymer **2**.