

Electronic Supporting Information

Polyelectrolyte Mediated Intra and Intermolecular Crosslinking in Microgel-Based Etalons for Sensing Protein Concentration in Solution

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Experimental Details

Materials

N-Isopropylacrylamide was purchased from TCI (Portland, Oregon) and purified by recrystallization from hexanes (ACS reagent grade, EMD, Gibbstown, NJ) prior to use. *N,N'*-methylenebisacrylamide (BIS) (99%), acrylic acid (AAc) (99%), and ammonium persulfate (APS) (98.5%), and Poly(allylamine hydrochloride) (PAH, MW=58000) were obtained from Sigma–Aldrich (Oakville, ON) and were used as received. NHS-Biotin ((+)-Biotin *N*-hydroxysuccinimide ester) was purchased from Sigma-Aldrich (St. Louis, MO, USA). Sodium chloride and sodium hydroxide were obtained from Fisher (Ottawa, ON). All deionized (DI) water was filtered to have a resistivity of 18.2 MΩ•cm and was obtained from a Milli-Q Plus system from Millipore (Billerica, MA). Chromium (Cr) and Gold (Au) were deposited using a model THEUPG thermal evaporation system from Torr International Inc. (New Windsor, NY). The annealing of Cr/Au layer was done in a Thermolyne muffle furnace from Thermo Fisher Scientific (Ottawa, Ontario). Anhydrous ethanol was obtained from Commercial Alcohols (Brampton, Ontario). Sodium Hydroxide (NaOH, 99.8%) and hydrochloric acid were purchased from Caledon Chemicals (Georgetown, Ontario) and were used as received. Fisher's finest prewashed glass coverslips were 25×25 mm and obtained from Fisher Scientific (Ottawa, Ontario). Cr (99.999%) was obtained from ESPI (Ashland, OR), while Au (99.99%) was obtained from MRCS Canada (Edmonton, AB). EDC (1-Ethyl-3-[3-dimethylaminopropyl] carbodiimide hydrochloride), Pierce® Streptavidin Magnetic Beads were purchased from Thermo Scientific (Rockford, USA).

Procedures

Poly (*N*-isopropylacrylamide-co-acrylic acid) Microgel (pNIPAm-co-AAc) Synthesis

Microgels composed of poly(*N*-isopropylacrylamide-co-acrylic acid) (pNIPAm-co-AAc) were synthesized via temperature-ramp, surfactant free, free radical precipitation polymerization as described previously.¹⁻⁴ The reaction mixture was comprised of 85% *N*-isopropylacrylamide (NIPAm) and 10% acrylic acid (AAc) with a 5% *N,N'*-methylenebisacrylamide (BIS) crosslinker. The monomer, NIPAm (17.0 mmol), and BIS (1.0 mmol) were dissolved in deionized water (100 mL) with stirring in a beaker. The mixture was filtered through a 0.2 μm filter affixed to a 20 mL syringe into a 200 mL 3-neck round-bottom flask. The beaker was rinsed with 25 mL of deionized water and then filtered into the NIPAm/BIS solution. The flask was then equipped with a temperature probe connected to a temperature control system, a condenser, N₂ gas inlet (a needle), and a stir bar. The solution was purged with N₂ gas for about 1.5 h, with stirring set to a rate of 450 rpm, while the temperature was allowed to reach 45 °C. AAc (2.0 mmol) was then added to the heated mixture with a micropipette in one aliquot. A 0.078 M aqueous solution of APS (5 mL) was delivered to the reaction flask with a transfer pipet to initiate the reaction. Immediately following initiation, a temperature ramp of 45 to 65 °C was applied to the solution at a rate of 30 °C/h. The reaction was allowed to proceed overnight at 65 °C. After polymerization, the reaction mixture was allowed to cool down to room temperature and filtered through glass wool to remove any large aggregates. The coagulum was rinsed with deionized water and filtered. Aliquots of these microgels (12 mL) were centrifuged at a speed of ~8500 relative centrifugal force (rcf) at 23 °C for about 40 minutes to produce a pellet at the bottom of the centrifuge tube. The supernatant was removed from the pellet of microgels, which was then resuspended to the same volume (12 mL) of deionized water. Centrifugation and resuspension was repeated five more times to remove any unreacted reagents, linear polymers, and oligomers present with the microgel. After repeated centrifugation pure, concentrated and very viscous microgel pellet was formed and kept in the centrifuge tube for further use.

Synthesis of Poly(allylamine hydrochloride)-Biotin (PAH-Biotin)

PAH-Biotin was prepared following a procedure mentioned elsewhere.⁵ PAH (40 mg mL⁻¹) solution in water was adjusted to pH 8.1 with 1.0 M sodium hydroxide solution. An aliquot of 50 μL NHS-Biotin (1.51 mg in 50μL DMSO) was added per milliliter of PAH. The mixtures were gently stirred and incubated for 3 hours at room temperature and then dialyzed for two weeks against water in a dialysis tube with a cut off MW 10,000 to remove unreacted NHS-Biotin. The DI water of the dialysis chamber was changed every day. The ratio of PAH's amines

to biotin was calculated as 100:1 and will be represented as PAH-Biotin_{100:1}. PAH-Biotin with amines to biotin ratio 1000:1 was also synthesized according to the same procedure described above and will be designated as PAH-Biotin_{1000:1}.

Etalon Fabrication:

The details of the paint-on technique used to fabricate microgel etalons for this study have been reported elsewhere.¹ In short, 25 × 25 mm pre-cleaned glass coverslips were rinsed with DI water and ethanol and dried with N₂ gas, and thin layer of 2 nm of Cr followed by 15 nm of Au were thermally evaporated onto them at a rate of ~0.2 Å s⁻¹ and ~0.1 Å s⁻¹, respectively, using a thermal evaporation system by Torr International Inc. model THEUPG (New Windsor, NY). The Cr act as adhesion layer to hold Au layer on glass and the Au coated substrates were annealed at 250 °C for 3 h to form a conformal layer of Au onto glass cover slip and then cooled to room temperature by removing the slides from the oven and kept inside covered petri dishes for future use. An aliquot of about 12 mL of previously purified microgel solution was centrifuged for 30 min at 23 °C at ~8500 relative centrifugal force (rcf) to pack the microgels into a pellet. The microgel pellet contained in a centrifuge tube was vortexed to loosen and was placed into a hot-plate at 30 °C. A previously coated Cr/ Au substrate was rinsed with ethanol, dried with N₂, and then placed onto hot plate (Corning, NY) set to 30 °C. A 40 μL aliquot of the concentrated microgels was put onto the substrate and then spread toward each edge using the side of a micropipette tip. The film was rotated 90°, and the microgel solution was spread again. The spreading and rotation continued until the microgels covered the entire substrate and became viscous. The microgels were allowed to dry completely on the substrate for 2 h with the hot plate temperature set to 35 °C. After 2 hours, the dry film was rinsed with deionized water to remove any excess microgels not bound directly to the Au. Next, the film was placed into a deionized water bath and allowed to incubate overnight on a hot plate set to ~30 °C. Following this step, the substrate was again rinsed with DI water to further remove any microgels not bound directly to the Au substrate surface. Then, the film was dried with N₂ gas and placed into the thermal evaporator, and an additional 2 nm Cr followed by 5 nm Au film were deposited onto the microgels as an overlayer. After the overlayer, this sandwich arrangement of microgel by two thin Au layers makes our optical device, etalon. The device was soaked in DI water overnight on a hot plate at 30 °C and rinsed with DI water and dried with N₂, and subsequently used for experiments.

Reflectance Spectroscopy:

Reflectance measurements were conducted in a specially designed sample holder using a USB2000+ spectrophotometer, a HL-2000- FHS tungsten light source, and a R400-7-VIS-NIR optical fiber reflectance probe all from Ocean Optics (Dunedin, FL). The spectra were recorded using Ocean Optics Spectra Suite Spectroscopy Software over a wavelength range of 350-1025 nm. Measurements were performed in the sample holder, which allows for careful sample positioning, sample stability, solvent injection, and fine temperature control. The light source was always set up on the center of the etalon.

Experimental Setup for Biosensing:

PNIPAm-co-AAc etalons were sealed at the sides using nail polish to resist any PAH penetration through the side of the etalon. After sealing, they were dried in air overnight followed by soaking in DI water overnight. Before each experiment, the etalons were placed in a specially designed aluminum sample chamber and soaked with 30 mL pH 7.2 solution (2 mM ionic strength). The chamber was connected with a digital heater to control the temperature of liquid in the chamber. The chamber was covered with a replaceable metal cover with a sample inlet where a pH electrode can be set and the center of the cover was fitted with the HL-2000-FHS tungsten light source fiber optically coupled to the reflectance probe. The temperature (25 °C) and the pH of the solution were monitored continuously. The light source for the reflectance spectra was adjusted with height and intensity to get best reflectance spectra. The spectrum was recorded in real time and waited until the spectra ceased shifting before additional PAH or PAH-Biotin was added to it. Each experiment was repeated at least three times.

Reaction of PAH-biotin with magnetic streptavidin beads:

A specific amount of PAH-biotin solution was transferred to a microcentrifuge tube, and a given amount of streptavidin modified magnetic particles were added. The reaction mixture was stirred gently for 2 hours at room temperature. After the reaction, an external magnet was placed on the tube and held for 2 minutes. The magnet visibly pulled the streptavidin modified magnetic particles out of the solution presumably with bound PAH-Biotin. The solution containing "free" PAH-biotin left in the microcentrifuge tube was subsequently added to the chamber with the etalon. As a control, a specific amount of PAH-biotin (both PAH-biotin_{100:1} and PAH-biotin_{1000:1}) was reacted with excess amount of streptavidin modified magnetic particles similar to above reaction. An external magnet was used to remove all of the magnetic

particles, both bound to PAH-biotin and unbound. The supernatant solution was subsequently added to the chamber with the etalon. No significant shift was recorded confirming there is no excess unreacted PAH-biotin (Fig S1a and S1b). This also confirms that the "reaction" conditions are sufficient to allow all of the magnetic particles to bind to the PAH-biotin. It was also found that magnetic streptavidin beads bound with PAH-biotin cannot penetrate through Au overlayer and cause the microgels to collapse. Finally, it was determined that the storage buffer of magnetic streptavidin beads does not influence the etalon's optical properties. This was accomplished by removing the magnetic beads (via magnet exposure) from a volume of the magnetic bead solution. The resultant solution was subsequently added to the etalon, which showed no spectral response to its addition (Fig. S1c).

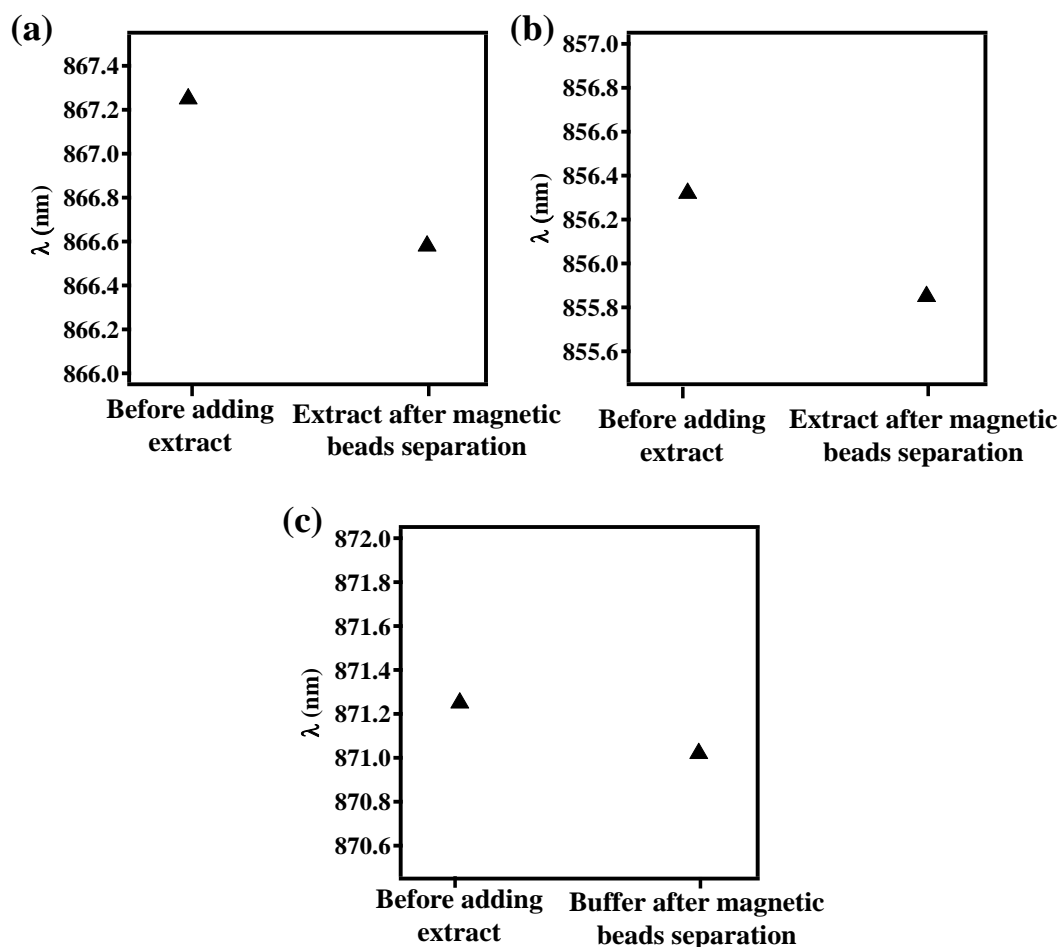


Fig. S1: a) Addition of solution left in the reaction vessel to the etalon after adding enough streptavidin-modified magnetic particles to bind all of the PAH-biotin_{100:1} in solution and magnetic isolation; b) Addition of solution left in the reaction vessel to the etalon after adding enough streptavidin-modified magnetic particles to bind all of the PAH-biotin_{1000:1} in solution and magnetic isolation; c) Addition of the solution left in the reaction vessel to the etalon after removing the streptavidin-modified magnetic particles from its native buffer solution -- isolation of the magnetic particles from its storage buffer followed by addition of the buffer to the etalon.

References:

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