Stimuli-Responsive Microgel-Based Surface Plasmon Resonance Transducer for Glucose Detection Using a Competitive Assay with Concanavalin A

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**EXPERIMENTAL SECTION**

**Materials:** Unless stated otherwise, all reagents and chemicals were purchased from commercial sources and used without further purification. N-isopropylacrylamide (NIPAm) was purchased from TCI (Portland, Oregon) and purified by recrystallization from hexanes (ACS reagent grade, EMD, Gibbstown, NJ) before use. Glycosyloxyethyl methacrylate solution (5% w/v in ethanol, Sigma Aldrich) was concentrated by removing the ethanol with a rotovap (IKA RV8, Wilmington, NC), resulting in a pale green viscous fluid. N, N'-methylenbisacrylamide (BIS) (99%), ammonium persulfate (>98%), Trizma base (>99%), α-D-glucose, D-(+)-galactose, sucrose, Concanavalin A (ConA), erythrina crystagalli (Ery), Bovine serum albumin (BSA) was purchased from Sigma Aldrich. Calcium chloride (anhydrous) (CaCl$_2$) was purchased from Fisher Chemical (Fair Lawn, New Jersey). Manganese chloride tetrahydrate (MnCl$_2$•4H$_2$O) was purchased from Matheson Coleman & Bell (Norwood, Ohio). Hydrochloric acid (HCl) was purchased from Caledon (Georgetown, Ontario). Anhydrous ethanol (Brampton, Ontario) was processed by adding 3 Å molecular sieve to remove trace amounts of water. All deionized water (DI H$_2$O) was obtained from Milli-Q Plus system from Millipore (Billerica, MA) with a resistivity of 18 MΩ•cm. Glass cover slips were purchased from Fisher Scientific (Ottawa, Ontario). Chromium (99.999%) was purchased from ESPI (Ashland, OR) and Gold (99.99%) was purchased from MRCS Canada (Edmonton, AB, Canada).

**Microgel Synthesis:** Microgel particles were synthesized via surfactant free, free radical precipitation polymerization. The monomer mixture, with a total concentration of 112.2 mM, was composed of 78% (mol %) N-isopropylacrylamide (NIPAM), 21% glycosyloxyethyl methacrylate (GEMA), and 1% N,N'-methylenbisacrylamide (BIS) crosslinker. Briefly, NIPAm (0.88 mmol) and BIS (0.11 mmol) were dissolved in 10 mL DI H$_2$O and filtered through a 0.2 µm filter into a 50 mL, 3-neck round bottom flask. Additionally, the reaction vessel was equipped with a reflux condenser and temperature probe. The mixtures were degassed with dry N$_2$ and heated to 65 °C for ~1 h. Next, GEMA (0.23 mmol) was dissolved in 500 µL anhydrous ethanol, added to the solution in an aliquot and allowed to stabilize for 5 min before adding APS (0.078M, 500 µL) solution. The mixtures were allowed to react at 65 °C for 4 h under a N$_2$ environment. The pale yellow clear solution became turbid within the first 15 min after initiating the reaction. The dispersions were allowed to cool down to room temperature and filtered through glass wood to remove all the large aggregates. The microgel solution was cleaned by repeated centrifugation at ~10,000 rpm for 30 min (× 6). The resultant suspension was concentrated into a pale yellow pellet.

**P(NIPAm-co-GEMA) Microgel-Modified SPR Sensor Surface:** The glass cover slips were washed copiously with DI H$_2$O, 95% ethanol, more DI H$_2$O and dried with flowing N$_2$ gas. The cleaned glass surface was coated with 2 nm Cr and then with 50 nm Au using a Torr International Inc. (New Windsor, NY) thermal evaporation system under a pressure of 10$^{-6}$ torr. The microgel film was generated using a previously described “paint-on” technique. Briefly, an aliquot of 40 µL p(NIPAm-co-GEMA) microgel solution was deposited on an Au coated substrate and spread toward each edge using the side of a micropipette tip. The film was rotated 90° to spread the
microgel solution to fully covered slides. The painting procedure was processed on a hot plate at 30 °C. With the temperature was increased to 35 °C, the microgel solution on the Au substrate was allowed to dry for 2 h. The excess microgels were removed by washing the surface with a large amount of DI H2O and further soaking in DI H2O for overnight.

**Tris-HCl Buffer Preparation**: A 0.1 M pH 7.5 Tris-HCl buffer solution was prepared by dissolving 12.144 g Trizma base in 990 mL DI H2O in a 2 L beaker. After adjusting the pH of solution to 7.5 by adding a concentrated HCl, 0.1979 g MnCl2•4H2O and 0.1110 g CaCl2 were added to the mixture; the final volume of the solution was adjusted to 1 L in a volumetric flask. Different concentrations of ConA were prepared with the 0.1M pH 7.5 Tris-HCl buffer. The BSA, Ery, and BSA/ConA mixtures were prepared using this buffer.

**Microgel and Microgel Film Characterization**: The chemical composition of the microgels was confirmed by nuclear magnetic resonance (NMR) spectroscopy (Agilent/Varian Inova two-channel 400 MHz). The transmission electron microscope (TEM) images of the microgel particles were acquired using a JEOL, JEM 2100 (JEOL USA, Inc., MA, USA) with an accelerating voltage of 200 kV, and the images were analyzed further by Image-J software. The specimens were prepared by adding 10 μL of highly diluted microgel solutions onto a carbon coated copper grid and air-dried overnight. The hydrodynamic diameter of p(NIPAm-co-GEMA) microgel particle was measured by dynamic light scattering (DLS) (Brookhaven Instruments ZetaPlus zeta potential analyzer, Holtsville, NY) as a function of temperature from 25 °C to 60 °C in 5 °C increments. All the measurements were taken in DI water with an average of ten 30 s acquisitions and an average of three measurements per sample at each temperature. The surface morphology of the microgel modified SPR sensor chip (before and after soaking in ConA solution) was characterized by atomic force microscopy (AFM) (Digital Instrument, Dimension 3100) in air. The images were acquired in a 10×10-μm area using a scan rate of 0.5 Hz and 512 scan points and lines in the tapping mode. The p(NIPAm-co-GEMA) microgels coated SPR sensor surface was soaked in 1 mg/mL ConA solution for 45 min, rinsed with pH 7.5 Tris-buffer and dried with N2 gas before imaging. The glucose responsiveness of p(NIPAm-co-GEMA)-ConA film was monitored by measuring the film thickness change in liquid by an AFM (Asylum Research MFP 3D AFM, Santa Barbara, CA) and the reflectance changes by a surface plasmon resonance (SPR) spectrometer. The SPR spectrometer is a custom-built instrument equipped with a 632.8 nm HeNe laser (1.5 mW, R-32734, Newport) and a photodiode detector (918D-SL-OD3, Newport) with a scanning range of 25.000° to 90.000° and a resolution of 0.001°. The 50 nm Au sensor chip was coupled to the back of a BK7 hemicylindrical prism with a refractive index of 1.51. The motion of the rotation stages were controlled by a XPS C-8 controller (Newport) with a self-developed LabVIEW program. In addition, the position of rotation stage and the readout of photodiode detector were recorded synchronically with an in-house developed LabVIEW program. The SPR instrument was calibrated using a series of sucrose solutions with different concentrations. The calibration curve of the resonance angle (RA) as a function of refractive index unit (RIU) is shown in Figure S4.

**Resonance Angle Shift with ConA Solution Addition**. The calibration curve of RA vs. RIU (Figure S4) can be expressed as Equation (1),
\[ RA = 124.058RIU - 89.500 \]  

(S1)

The RA shift (\( \Delta RA \)) can be derived from Equation (1) as

\[ \Delta RA = RA_1 - RA_2 = (124.058RIU_1 - 89.500) - (124.058RIU_2 - 89.500) \]  

(S2)

Thus, \( \Delta RA \) can be expressed as Equation (3),

\[ \Delta RA = 124.058\Delta RIU \]  

(S3)

When the solvent was changed from buffer to 1 mg/mL ConA in buffer solution, the \( \Delta RIU \) can be calculated based on the dn/dc value of ConA, which is \( \sim 0.185 \text{ mL/g} \). Thus, the RIU changes can be expressed as Equation (4),

\[ \Delta RIU = \text{dn} = 0.185 \text{mL/g} \times \text{dc} \]  

(4)

which dc is 0.001 g/mL (1 mg/mL) as the ConA concentration changes. By using \( \Delta RIU = 1.85 \times 10^{-4} \) in equation (3), we obtained \( \Delta RA = 0.023^\circ \)

Figure S1. The \(^1\text{H NMR spectra of p(NIPAm-co-GEMA), GEMA and pNIPAm.} \)
Figure S2. AFM images of a pNIPAm microgel-based film (a,b) before, and (c,d) after soaking in 1 mg/mL ConA solution. Images a) and c) are height profile; b) and d) are the phase profile.

Figure S3. The resonance angle of p(NIPAm-co-GEMA) film in different concentrations of ConA. The data were derived from Figure 5a. The resonance angle at each ConA concentration was determined from SPR spectra by picking the point with the lowest reflected light intensity. Each point represents the average of three different measurements and the error bars represent their standard deviation. The line in the graph is used for guiding the eye.
Figure S4. Calibration curve of our custom-built SPR. Different concentrations of sucrose solutions were prepared and their refractive index at different concentration was cited from the reference. The resonance angle at each concentration was determined and plotted as a function of refractive index. Each point represents the average of three different measurements, and the error bar represent their standard deviation. The red line is the best fit with a slope of 124.058 degree/RIU, an intercept of -89.500 degree and a $R^2$ of 0.997.

Table S1. The calculated effective refractive index at each resonance angle based on the calibration curve in Figure S4.

| Concentration of ConA (mg/mL) | Resonance Angle | Refractive Index |
|-------------------------------|----------------|-----------------|
| 0                             | 78.246         | 1.35216         |
| 0.001                         | 78.228         | 1.35201         |
| 0.01                          | 78.284         | 1.35246         |
| 0.1                           | 79.067         | 1.35878         |
| 0.5                           | 79.291         | 1.36058         |
| 1                             | 79.328         | 1.36088         |
Figure S5. A real-time SPR profile of p(NIPAm-co-GEMA) film (black line) and pNIPAm film (red line) after exposure to 1 mg/mL ConA in a pH 7.5 Tris-HCl buffer. The first arrow in each line represents the point where 1 mg/mL ConA was introduced, and the second arrow in each line represents the point where pH 7.5 Tris-HCl buffer was added.

Figure S6. A real-time SPR profile of p(NIPAm-co-GEMA) film in different solutions in the order: buffer/ConA/buffer/galactose (200 mg/dL)/buffer. The arrows in the plot represent the point at which a different solution was switched in the fluidic cell.

Reference

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