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Supporting Information

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Label-Free Biosensing with Hydrogel Microlenses

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Materials

All reagents were acquired from Sigma-Aldrich unless otherwise specified. *N*-Isopropylacrylamide (NIPAm) was prepared by re-crystallization using hexanes (J.T. Baker) prior to use. *N*,*N*'-Methylene(bisacrylamide) (BIS) and ammonium persulfate (APS) were used without further purification. Acrylic acid (AAc) was distilled under reduced pressure. 3-Aminopropyltrimethoxysilane (APTMS) was used for the functionalization of cover glasses. The glass substrates used were 24 x 50 mm Fisher Finest brand cover glass obtained from Fisher Scientific. Absolute (200 proof) and 95% ethanol was used for various purposes in this investigation. 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) and biotin hydrazide were purchased from Pierce. Dimethyl sulfoxide (DMSO) was obtained from J.T. Baker. Polyclonal anti-avidin (raised in rabbit) and polyclonal anti-biotin (raised in goat) were purchased from Sigma-Aldrich. Labled anti-goat IgG (Alexa Fluor 594, raised in rabbit) was obtained from Molecular Probes. Water was distilled and then deionized (DI) to a resistance of at least 18 MΩ (Barnstead Thermolyne E-Pure system) and then filtered through a 0.2 μm filter to remove particulate matter. 3M transparency film for laser printers and a Hewlett Packard LaserJet 4000N printer was used for pattern printing.

Methods

1. Microgel Synthesis.

Microgels with a molar composition of 89.4% NIPAm, 0.5% BIS, 10% AAc, and 0.1% 4-acrylamido-fluorescein were synthesized *via* aqueous free-radical precipitation polymerization, using 300 mM total monomer concentration. In a three-neck, 200 mL round-bottom flask, 100 mL of a filtered aqueous solution of NIPAm and BIS was added and the mixture was heated to ~70 °C under a N_2 atmosphere while stirring with a magnetic stir bar. After 1 hr, AAc and 4-acrylamidofluorescein solutions were added to the flask to bring the total monomer concentration

up to 300 mM. Polymerization was immediately initiated by injecting 1 mL of a hot (~ 70 °C) APS solution (6.13 mM). The reaction was kept at 70 °C for 4 hours under a N_2 environment. The particles were purified by dialysis against water for ~2 weeks with the water being changed twice per day, using 10 000 MW cut-off dialysis tubing (VWR).

2. Microgel Functionalization.

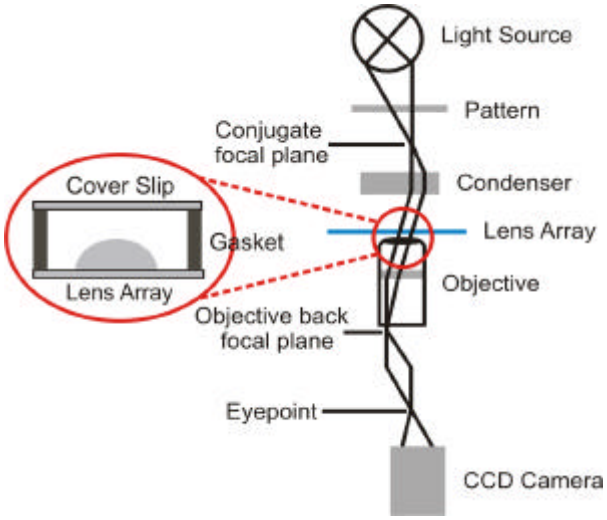
The anionic microgel was functionalized with biotin and 4-aminobenzophenone by 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) and dicyclohexylcarbodiimide (DCC) coupling reactions respectively. The functionalization was planned by assuming the consumption of 50% of the carboxyl groups on the particles by biotin and other 50% by 4-aminobenzophenone (ABP). Since the reaction efficiency of the coupling is <100 %, some portion of AAc groups are expected to remain available for binding to the cationic glass substrate. First, the biotinylation of 10-fold 2-[*N*-morpholino]ehtanesulfonic acid (MES) (pH 4.7) diluted anionic microgel (1 mL) was done by adding biotin hydrazide (3.8 mg dissolved in 0.5 mL of dimethyl sulfoxide (DMSO), 50 % of the total amount of acrylic acid in the microgel solution) to the dilute microgel solution. EDC (15 mg) was added to the microgel and biotin solution to activate the coupling reaction. The solution was stirred overnight at 4 °C and the unreacted biotin hydrazide was removed by several cycles of centrifugation followed by resuspension in phosphate buffered saline (PBS) (pH 7.5). The biotinylated acrylic acid microgel particles were then modified by ABP by the DCC coupling reaction. The PBS (pH 7.5) solution of biotinylated acrylic acid microgel particle (1 mL) was centrifuged and redispersed in DMSO several times to replace the buffer medium by DMSO and finally redispersed in 700 μL of DMSO. Further, 150 μL of 0.01 M ABP in DMSO was added to the microgel solution followed by the addition of 150 μL of 0.01 M DCC in DMSO, making the total volume of reaction solution 1 mL. The reaction solution was stirred overnight at room temperature in dark. The side product N,N'-dicyclohexylurea was removed by adding 0.5 mL de-ionized water to the reaction solution and filtering out the solid. The filtrate was subjected to several centrifugation cycles (centrifugation and resuspension) to clean the particles and replace the solvent by PBS buffer (pH 7.5).

3. Bioresponsive Hydrogel Microlens Substrate Preparation.

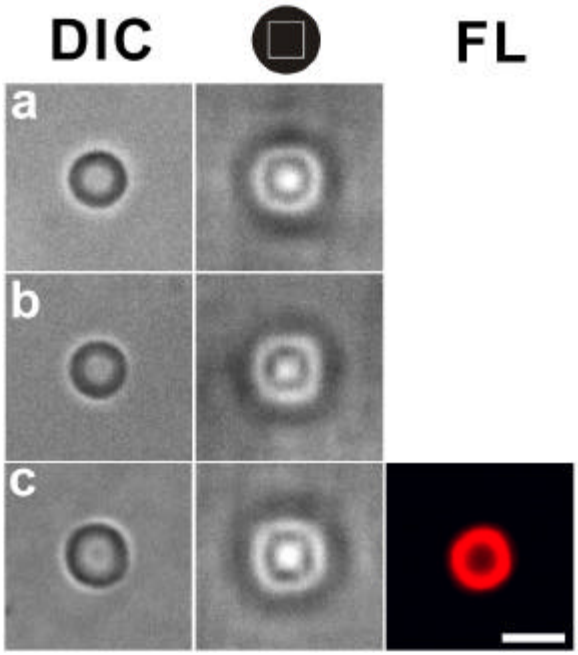
Glass cover slips were cleaned in an Ar plasma (Harrick Scientific) for 30 min to remove organic residuals from the glass surface. The glass substrates were immersed in an ethanolic (absolute ethanol) 1% APTMS solution for ~2 hrs, after which they were removed from the APTMS solution and rinsed several times with 95% ethanol. These substrates were stored in 95% ethanol for no longer than 5 days prior to use. The substrates were rinsed with DI water and dried by a stream of N_2 gas prior to assembly. The substrate was then exposed to an aqueous 10% (v/v dilution of initial concentration following synthesis) biotin-ABP functionalized microgel solution buffered by 10 mM PBS buffer pH 7.5. After 30 min, the substrate was rinsed with DI water, and dried with nitrogen gas to leave behind microgels that are strongly attached to the substrate by Coulombic interactions. A microlens array/silicone gasket/coverslip sandwich assembly was prepared and buffered solution of polyclonal anti-biotin was introduced into the void space. After 3 hrs of incubation, the substrates were rinsed and the medium was replaced with PBS buffer pH 7.5. Photoligation of the microgel-tethered ABP to the antigen-bound antibody was accomplished via UV irradiation using a 100W longwave UV lamp for 30 min while cooling the coverslip on an ice bath. For microscopic investigations of microlens response to competitive protein binding, 150 μL of various biocytin, anti-avidin, and anti-goat IgG solutions buffered in 10 mM PBS were introduced into the void space of the assembly.

4. Microscopy.

The bioresponsivity of hydrogel microlenses were monitored using brightfield and fluorescence optical microscopies. Brightfield transmission and differential interference contrast (DIC) optical microscopies were used to study the changes in the optical properties of the hydrogel microlens attached to the substrate, while epi-fluorescence microscopy was used to visualize the binding of fluorescently labeled IgG to the microlenses. An Olympus IX70 inverted microscope equipped with a high numerical aperture, oil immersion 100X objective (NA=1.30) was used for all microscopies reported here. Images were captured using either a black/white or a color CCD camera (PixelFly, Cooke Corporation).



SI Figure 1. Inverted light microscopy setup used for aqueous phase imaging experiments. The lenses at the imaging plane move the objective back focal plane to the eyepoint, thus bringing the pattern near the source into focus.



SI Figure 2. Effects of nonspecific adsorption on the optical properties of bioresponsive microlenses prepared with 1 μM anti-biotin: (left column) DIC image of the hydrogel microlens and (right column) projected square pattern images through the hydrogel microlens in: (a) 10 mM PBS buffer pH 7.5, (b) 5.5 μM anti-avidin and, (c) 5.5 μM rabbit anti-goat IgG conjugated with Alexa Fluor 594. The fluorescence microscopy image of hydrogel microlens (FL) is also shown in the extra panel. Note that 150 μL of each solution was used for this experiment. The scale bar is 2 μm.