

Supplementary Information

Direct Observation of Ligand-Induced Receptor Dimerization with a Bioresponsive Hydrogel

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Materials

All reagents were purchased from Sigma-Aldrich unless otherwise specified. *N*-Isopropylacrylamide (NIPAm) was re-crystallized using hexanes (J.T. Baker) prior to use. Acrylic acid (AAc) was distilled under reduced pressure. *N,N'*-Methylene(bisacrylamide) (BIS) and ammonium persulfate (APS) were used as received. 3-Aminopropyltrimethoxysilane (APTMS) was used for the functionalization of cover glasses. The gold seal cover glasses (24×40, No 1.5, Electron Microscopy Sciences) were used as a substrate for hydrogel assembly. Absolute (200 proof) ethanol was used for various purposes in this study. 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) and coupling buffer (50 mM 2-[*N*-morpholino]ethanesulfonic acid pH 5.2, 0.05% ProClin 300) were purchased from Bangs Labs. Phosphate buffered saline (PBS) (pH 7.4) and Tris-HCl (pH 7.5) were purchased from Invitrogen. Active-site blocked human α -thrombin (HCT-BFPRCK) was purchased from Haematologic Technologies Inc. Monoclonal Anti-His antibody was purchased from GE Healthcare, and mAb 6D1 for GPIIb α was a gift from Dr. B. Coller (Rockefeller U., New York, NY).

Materials and Methods

Hydrogel Synthesis. Hydrogel particles 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 of total monomer concentration. In a 200 mL

round-bottom flask with three-necks, 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) and remained for 4 hours at 70 °C under a N_2 environment. The hydrogels 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).

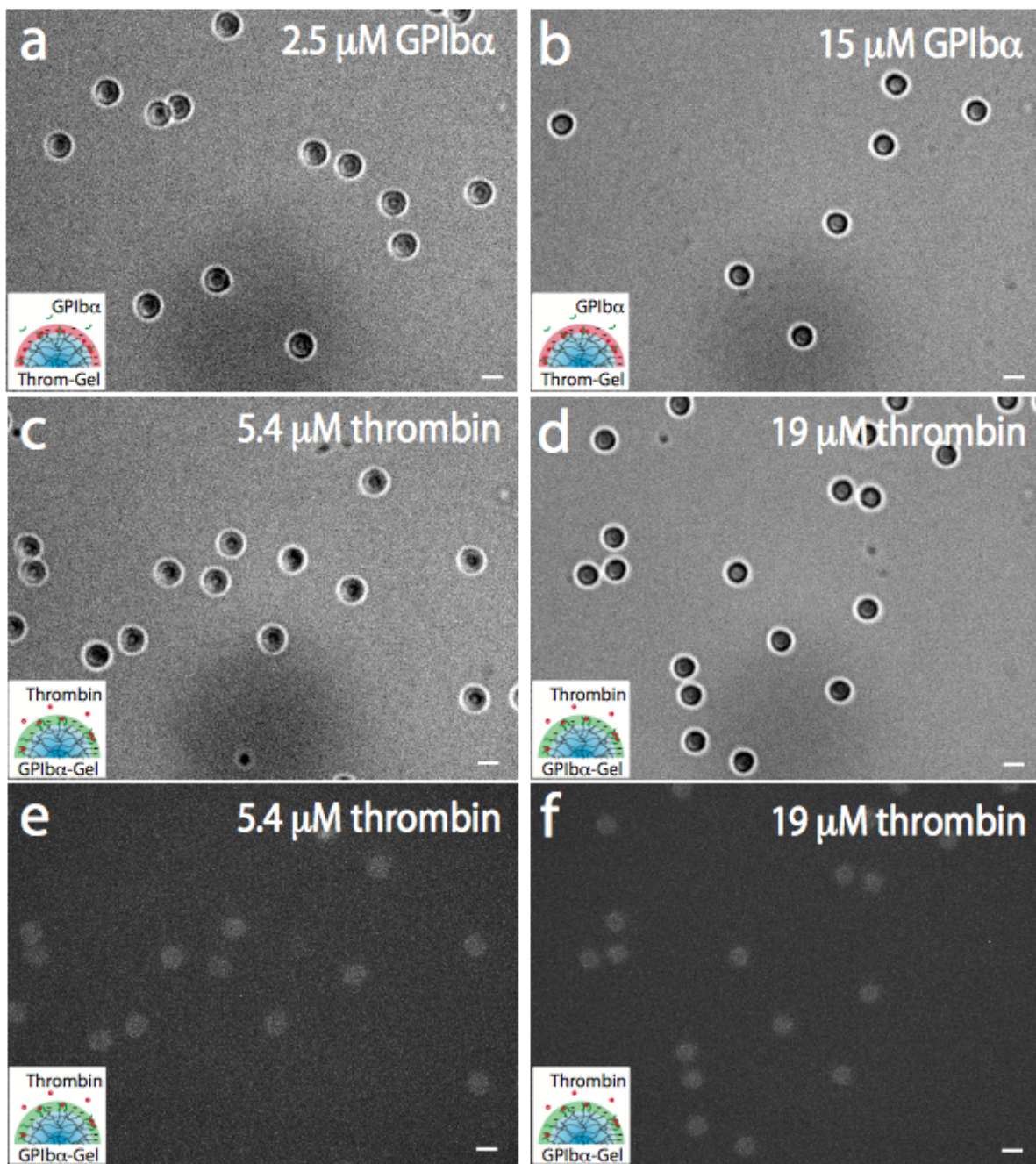
Protein Expression. The cDNA of human platelet GPIIb α (His¹ to Arg²⁹⁰) and vWF A1 domain (Asp¹²⁶¹ to Pro¹⁴⁶⁶) were cloned into Xho I and Mlu I sites of ExpressTag-6 (ET6) vector, a modified version of the pIRES2-GFP vector (Clontech, Inc.), which encodes a Kozak sequence, a N-terminal secretion signal sequence, and a C-terminal His₆ tag. HEK293T cells were transiently transfected using calcium phosphate or Lipofectamine. Culture supernatants were harvested 3 days after transfection and proteins were purified using Ni-NTA affinity chromatography followed by size-exclusion chromatography in 20 mM Tris, pH 7.5, 150 mM NaCl, 0.1 mM BME, and 0.2 mM EDTA.

Bioresponsive Microgel Preparation. The anionic hydrogel was functionalized with the human GPIIb α or α -thrombin by 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) coupling reactions. 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 anionic hydrogel (100 μ L) was centrifuged at 15,871g, and the pellet was resuspended in 200 μ L of the coupling buffer (MES pH 5.2). EDC (40 mg in 200 μ L) was added, followed by 20 μ g of 0.2 mg/mL GPIIb α or 40 μ g of 3.3 mg/mL α -thrombin. After shaking for 1 hour at room temperature, the hydrogels were washed 5 times using centrifugation followed by resuspension in phosphate buffered saline (PBS) (pH 7.4).

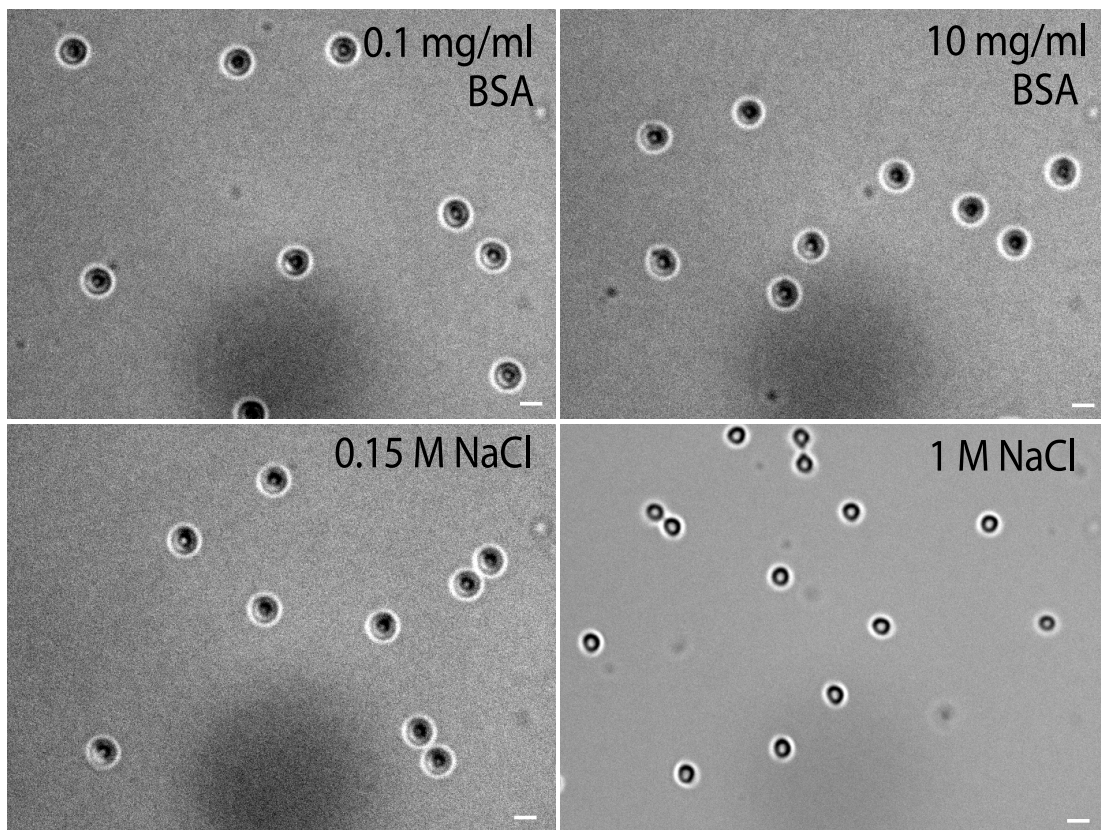
Microgel Substrate Preparation for the Dimerization Assay. Glass cover slips were cleaned in plasma cleaner for 20 min to remove organic residuals from the glass surface. The glass substrates were immersed in an ethanolic (absolute ethanol) 1% APTMS solution for ~ 2 hours, after which they were removed from the APTMS solution and rinsed several times with ethanol (200 proof). These substrates were stored in ethanol for no longer than 3 days prior to use. The substrates were rinsed with DI water and dried by a stream of Ar gas prior to assembly.

The substrate was then assembled with 50-well silicone gasket, and the each well was exposed to 4 μL of an aqueous 5% (v/v dilution of initial concentration following synthesis) bio-microgel solution buffered by PBS buffer (pH 7.4). After 30 min, the substrate was rinsed with PBS buffer, and gently dried with Ar gas. A bionanogel array/gasket assembly was prepared and 6 μL buffered solution of the GPIIb α , α -thrombin, BSA, and NaCl was introduced into the void space, followed by covering the assembly with a cover glass. After 1 hour of incubation, microscopic investigations of bionanogel response to ligand-induced dimerization were conducted.

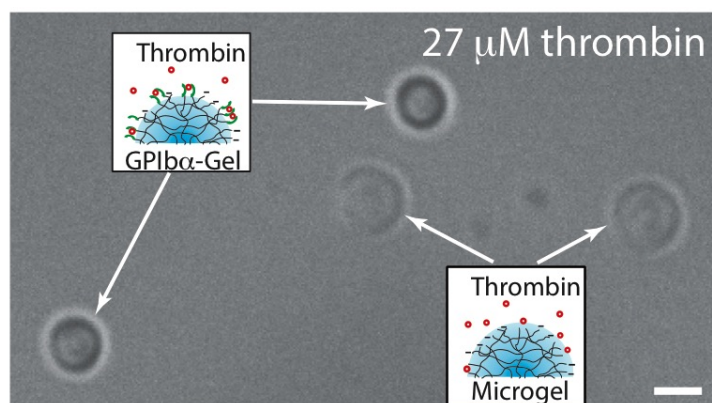
Microscopy. Brightfield transmission, differential interference contrast (DIC), and epifluorescence optical microscopies were used to study the changes in the optical properties of the microgel particle attached to the substrate. A Carl Zeiss Axiovert 200M inverted microscope equipped with a high numerical aperture, oil immersion 100X objective (NA=1.30) was used for all microscopies reported here. Images were captured using CCD camera (AxioCamMR, Zeiss).



SI Figure 1. Wider view image of thrombin- (a,b) or GPIb α - (c-f) functionalized microgel particles in response to the indicated concentration of GPIb α or thrombin, respectively. Fluorescence microscopy images of the microgels of c and d are shown in e and f, respectively. Note that 6 μl of each 1X PBS buffered solution was used for the experiment. The scale bars are 2 μm .



SI Figure 2. Wider view image of GPIb α -functionalized microgel particles in response to the indicated concentrations of BSA in 1X PBS buffer (pH 7.4, top row) or NaCl in 50 mM Tris-HCl buffer (pH 7.5, bottom row). Note that 6 μ l of each solution was used for the experiment. The scale bars are 2 μ m.



SI Figure 3. Wider view image of GPIIb/IIIa-functionalized and non-functionalized microgel particles in response to the indicated concentrations of thrombin in 1X PBS buffer. Note that 6 μl of each solution was used for the experiment. The scale bars are 2 μm .