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Glucose-Responsive Polymer Brushes for Micro-cantilever Sensing

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Experimental Section

Materials: NIPAAM (99%) monomer, Sodium Acrylate (97%) monomer, copper (I) bromide (CuBr, 99.9%), methanol (MeOH, 99.9%), N-Hydroxysuccinimide (NHS, 98%), 3-Aminophenylboronic acid monohydrate (98%), and α-D-Glucose (96%), 4-Mercaptophenylboronic acid were obtained from Sigma-Aldrich (Milwaukee, WI). Milli-Q (Millipore, Billerica, MA), water (18MΩ/cm) and methanol were used as polymerization solvents. N,N,N,N,N-Pentamethyldiethylenetriamine (PMDETA) was used as received from Acros Organics (Hampton, NH). 1-Ethyl-3-[3-dimethylaminopropyl]carbodiimide Hydrochloride (EDC) was used as received from Thermo Scientific (Rockford, IL). The thiol initiator ($BrC(CH_3)_2COO(CH_2)_{11}SH$) was synthesized as reported. [1, 2] To immobilize the initiators for surface-initiated polymerization, gold substrates with an average grain diameter of 45 nm were prepared by thermal evaporation under a vacuum (4×10^{-7} Torr). For this purpose an adhesion layer of chromium (50Å) followed by a layer of gold (450 Å) was evaporated onto silicon wafers. Before deposition, silicon wafers were cleaned in a mixture of H_2O_2/H_2SO_4 (1:3, v/v) at 80 °C ("piranha solution") for 10 min and washed thoroughly with Milli-Q-grade water. (Caution: Piranha solution reacts violently with organic matter!)

Micro-contact printing for immobilizing initiator on gold surface: The PDMS stamp was inked in a solution of thiol initiator in ethanol (2mM) for 1 min, dried with nitrogen, and brought into contact with the gold substrate for 30s with constant applied loads to ensure good contact throughout the entire printing time. Inked substrates were then dried in nitrogen for ATRP.

PBA-SAM, and Polymer brushes on cantilever chips: All experiments were conducted using either the Cantichip® (Cantion A/S, Denmark) piezoresistive lever array or the Octosensis® (Micromotive GmbH, Germany) lever array. The micro-cantilever substrates were coated with a 5 nm chromium adhesion layer followed by a 45 nm gold layer using an electron beam evaporator (CHA Industries, Fremont, CA). In all cases, prior to SAM formation, the microcantilevers were carefully cleaned for 10 min in acetone and for 10 min in absolute ethanol. They were first rinsed with water and then with ethanol. The thiols of mercaptophenylboronic, and thiol initiator were selectively transferred onto the micro-cantilever arrays with a micropipette manipulation system (Signatone, Gilory, CA) while monitoring with an optical microscope. Coated micro-cantilevers were then dried in nitrogen for ATRP and subsequent chemical modifications.

SI-ATRP for growing polymer brushes: The polymerization solution was prepared by adding a solution of NIPAAM monomer and NaAc monomer to an organometallic catalyst according to our previous report with some modification. [3] The organometallic catalyst was formed in a nitrogen atmosphere by adding CuBr (1.8 mg, 0.013 mmol) and PMDETA (14 µL, 0.064 mmol) in a 1:5 molar ratio to 1.0 mL of MeOH as solvent. The mixture was then sonicated for 1-2 min to facilitate the formation of the CuBr/PMDETA complex. Next, 1.55 g (17.5 mmol) of NIPAAM monomer and 0.18 g NaAc (1.9 mmol, 10 mol% of NaAc against NIPAAM) dissolved in 11 mL of water was filtered into the catalyst-complex solution through a 0.45 µm Millipore Millex filter. The polymerization solution was then transferred into flasks containing the sample substrates with immobilized patterned initiator. The flasks were sealed with rubber septa and kept at room temperature under nitrogen. After the desired reaction time, substrates were removed from the polymerization solution, exhaustively rinsed with DI water to remove all traces of the polymerization solution, and subsequently dried in a stream of nitrogen.

PBA immobilization: The protonation of PNIPAAM-co-PNaAc to PNIPAAM-co-PAA followed a published procedure, and involved immersing the sample into acid solution (pH=2.0) with subsequent rinsing with DI water. [4] Conversion of carboxylic acid groups in PNIPAAM-co-PAA into active esters (PNIPAAM-co-PAA-NHS) was achieved by placing polymer brush coated substrates in a solution containing EDC and NHS in water for 20min and rinsing with ethanol. [5] Two ratios and concentrations of EDC/NHS have been investigated in an attempt to maximize brush swelling response.

RUN 1: EDC, 8 mg/mL, NHS, 5 mg/mL, PBA: 16 mg/mL

RUN 2: EDC, 100mg/mL, NHS, 62.5mg/mL, PBA: 16mg/mL

To covalently bind APBA, the PNIPAAM-co-PAA-NHS coated substrate was immersed overnight in a solution of PBA in PBS buffer (5mM). Subsequently, the sample was rinsed with PBS buffer and Milli-Q grade water.

Characterization: The polymer brush substrates were rinsed with Milli-Q water, dried under a stream of nitrogen, and mounted on steel sample disks prior to AFM measurements. The AFM topographic images were collected in contact mode using V-shaped silicon nitride cantilevers (Nanoprobe, Veeco, spring constant 0.12 N/m; tip radius 20-60 nm) using a Multi Mode atomic force microscope (Digital Instruments, Santa Barbara, CA). Topographic imaging was performed in air and in glucose solution using a fluid cell. The AFM topographic images were collected under low applied normal forces (~1nN) to minimize compression and lateral damage of the polymer brushes.

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