Electronic Supplementary Material (ESI) for Chemical Communications

Supporting Information

Binding Behavior of Protein on Spatially Controlled Poly[Oligo(Ethylene Glycol) Methacrylate] Brushes Grafted from Mixed Self-Assembled Monolayers on Gold

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Materials. Absolute ethanol (99.9+%, Merck), absolute methanol (MeOH, 99.9+%, Merck), anhydrous *N,N'*-dimethylformamide (DMF, 99.8+%, Aldrich), toluene (J. T. Baker), copper(I) bromide (Cu(I)Br, 99.999%, Aldrich), 1-undecanethiol (98%, Aldrich), 2,2'-dipyridyl (bpy, 99+%, Aldrich), *N,N'*-disuccinimidyl carbonate (DSC, Aldrich), 4-(dimethylamino)pyridine (DMAP, Fluka), 2-(2-aminoethoxy)ethanol (EG_2-NH_2, 98%, Aldrich), (+)-biotinyl-3,6,9-trioxaundecanediamine (Biotin-NH_2, Pierce), streptavidin (Pierce) and phosphate buffered saline (PBS, Sigma) were used as received. Oligo(ethylene glycol) methacrylate (OEGMA, Mn: ~360, Aldrich) was passed through a column consisting of the activated, basic aluminum oxide to remove inhibitors. $[BrC(CH_3)_2COO(CH_2)_{11}S]_2$ were prepared according to the literature.[1]

Preparation of the Mixed SAMs on gold surfaces. Gold substrates were prepared by thermal evaporation of 5 nm titanium and 100 nm of gold onto silicon wafers. The mixed SAMs of (**1**) and (**2**) were prepared by immersing a gold substrate (2 × 2 cm^2) in 2 mM ethanolic solution, including the different mole fractions of (**2**) for 12h at room temperature. After the formation of the mixed SAMs presenting a polymerization initiator, the surface was thoroughly washed with ethanol several times and then dried in a stream of argon.

Formation of biotin/*p*OEGMA Film on gold surfaces. The surface-initiated, atomic transfer radical polymerization (SI-ATRP) from the initiator anchored on the gold surface was carried out with different reaction times at room temperature under argon atmosphere. Cu(I)Br (143 ng, 1.0 mmol) and 2,2'-bipyridyl (312 mg, 2.0 mmol) were added into a schlenk tube, containing the water/methanol (2 mL/8 mL). OEGMA (0.44 mL, 1.0 mmol) was then added, and the resulting dark red solution was bubbled with argon for 10 min. The polymerization was quenched by exposing the reaction mixture to air and pouring water into

the tube. The termination step caused the reaction solution to turn blue indication the oxidation of Cu(I) to Cu(II). The pOEGMA-coated substrate was thoroughly rinsed with pure water and methanol to remove any remained monomers and physisorbed polymers, and then dried in a stream of argon.

The *p*OEGMA film was activated in dry DMF solution of *N*,*N'*-disuccinimidyl carbonate (DSC, 0.1 M) and 4-(dimethylamino)pyridine (DMAP, 10 mM) for overnight at room temperature. The sample was thoroughly rinsed with DMF and CH_2Cl_2, and then dried with a stream of argon. The DSC-activated *p*OEGMA films were soaked in an ethanolic solution of Biotin-NH_2 (1 mg/mL) for 2 h at room temperature. After the reaction, the substrates were washed with ethanol, and dried in a stream of argon. Subsequently, to deactivate the nonreactive NHS carbonate ester groups, the substrates were immersed in an ethanolic solution of EG_2-NH_2 (0.1 mg/mL) for 1 h at room temperature. After treating with the blocking agent, the substrates were rinsed with ethanol and dried in a stream of argon.

Immobilization of streptavidin on biotin/*p*OEGMA Film. The concentration of streptavidin was set to be 0.1 mg/mL in PBS solution (pH 7.4). After the biotin/*p*OEGMA immersed into the protein solution for 1 h at ambient temperature, the resulting substrate was carefully washed with distilled water and then dried in a stream of argon.

Characterizations. The thicknesses of the films were measured with Gaertner L116s ellipsometer (Gaertner Scientific Corporation, IL), equipped with a He-Ne laser (632.8 nm) at a 70o angle of incidence. A refractive index of 1.46 was used for all the samples. More than five different points on each sample were measured, and the average values were recorded.

Polarized infrared external reflectance spectroscopy (PIERS) spectra were recorded with a nitrogen-purged by Thermo Nicolet Nexus FT-IR spectrophotometer equipped with the SAGA (Smart Apertured Grazing Angle) accessory. The spectra were taken by adding approximately 4000 scans for background and 1000-2000 scans for the samples (a resolution of 4 cm^{-1}, gain of 4, mirror velocity of 3.1647 cm/sec, beam splitter of KBr). AFM experiments were performed with the Nanoscope multimode with a NanoScope III controller (Digital Instruments Veeco Metrology Group, Santa Barbara, CA, US). with tapping-mode. All experimental data were collected by using scanner E and tapping mode AFM tips.

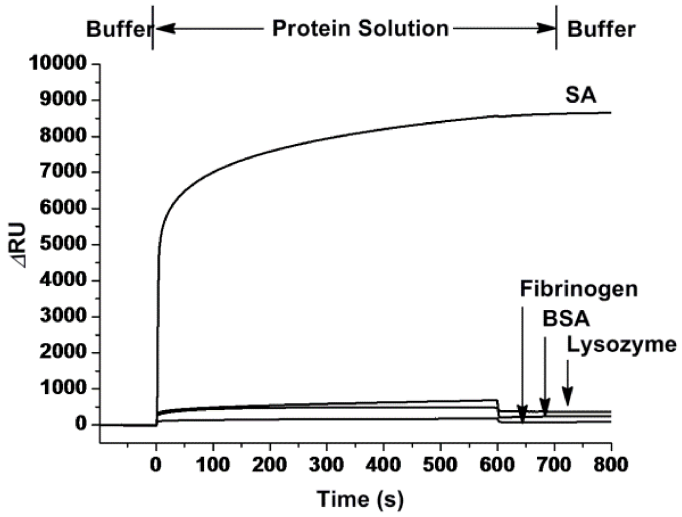

Figure S1. SPR data for specific binding of streptavidin (SA) and non-specific binding of model proteins on biotinylated-*p*OEGMA films (thickness:~6 nm) grown from **7** (the mole fraction of **2**: 0.8), where model proteins such as BSA, fibrinogen, and lysozyme are used. This results show that the sparse surface coverage not only reduced the non-specific bindings from the model proteins, but also provided the selective immobilization of streptavidin.

(1) R. R. Shah, D. Merreceyes, M. Husemann, I. Rees, N. L. Abbott, C. J. Hawker, J. L. Hedrick, *Macromolecules* 2000, **33**, 597.