Electronic Supplementary Information

Photoreactive Immobilization of 11-(2,4-dinitro-5-fluorobenzene)-undecenamide on a Hydrogenated Silicon (100) Surface for Protein Immobilizations.

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Comparative fluorescence analyses of protein adsorption on undecenoic acid (UA), NHS-UA, and stearic acid (SA) prepared on silicon (100) surface

Comparative fluorescence image analyses of the photo-immobilized DFUA, UA, NHS-UA, and SA monolayers on a silicon (100) surface were carried out in Figure S1; the captured proteins are FITC labeled BSA (0.1 mg/ml). On the UA immobilization, a large fluorescent signal as intense as in a DFUA layer was observed as shown in Figure S1(a), which is mainly attributed to a nonspecific (or electrostatic) attraction between carboxyl-terminated surface and proteins. This interaction was manifested in a phosphate buffered saline (phosphate 10 mM, NaCl 50 mM, pH 7.4, PBS) and substantially alleviated by using a buffer with higher ionic strength (PBS plus 1M NaCl) as shown in Figure S1(b).

On the other hand, for the immobilization of SA, a ten times as concentrated solution (100 mM) as a DFUA one (10 mM) was spin-coated on a hydrogenated silicon surface at 1000 rpm and a mask pattern was laid on in contact with the silicon substrate. The sandwiched silicon wafer was exposed to UV light (365 nm) for 10 min. Due to the absence of an alkene group, SA molecules were photo-immobilized at its carboxyl group, and the corresponding fluorescence image of SA substrate reflects a nonspecific adsorption of the FITC-BSA on methyl-terminated SA layer on the silicon surface. In a SA concentration less than 20 mM, no noticeable fluorescence was observed in this protocol, suggesting that only a negligible amount of UA is tethered through the carbonyl immobilization during the present surface photoreaction. This picture was further fortified with the hydrophobic feature of the SA surface and with the hydrophilic characteristic of the UA surface (data not shown).

Moreover, another control experiment using NHS-UA was also performed. In this immobilization, the protein layer prepared on an NHS-UA layer was relatively more robust than that on a simple UA layer, and a comparable fluorescence intensity with the image of a DFUA layer was observable as in Figure S1(a) (data not shown); the proteins on this surface appeared not to be affected severely with the use of a buffer solution of high ionic strength. One more advantage of this ligand might be avoiding carboxyl-immobilization as mentioned above. In spite of these advantages, the ligand was quite unstable in a prolonged exposure to an aqueous buffer solution because the hydrolysis of NHS ester is inevitable in the aqueous solution. As shown in Figure S1(d), a negligible fluorescence intensity was observed on the NHS-UA surface after soaking the patterned wafer in a concentrated buffer solution (PBS plus 1M NaCl) for 2 h. On the other hand, the protein capture on a DFUA layer was relatively invariant with soaking it in the concentrated buffer solution.

The present DFUA layer has a unique advantage over other alternatives such as UA or NHS-UA in that it is more reactive to thiol group than amine groups at a specific pH range (pH 6.0 ~ 6.5). Therefore, a glutathione immobilization was performed as a special nucleophile to the dinitorfluorophenyl (DNF) group in a PBS buffer solution, and GST-EGFP proteins (0.1 mg/ml) was allowed for the non-covalent association with the tethered glutathione molecules. As shown in Figure S2(a), GST-EGFP proteins was successfully immobilized on the glutathione-DFUA layer. However, EGFP proteins without the tag, by contrast, showed a substantially lower intensity; the residual fluorescence is attributed in part to a nonspecific protein adsorption or to a covalent association via an unreacted region of DFUA in the previous reaction step.

EXPEIMENTALS

Chemicals

Silicon (100) wafer was purchased from Sino-American Silicon Products Inc.. Undecenoic acid (99.9%), *N*-hydroxysuccinimide (NHS, 97%), dicyclohexylcarbo-diimide (DCC, 99%) and dinitro-fluoro-bezene (DNB, 99.9%) was purchased from Aldrich and used as received. Bovine serum albumin (BSA), FITC-labeled BSA, and Cy5-labeled streptavidin were purchased from Sigma and used as received. A recombinant EGFP was expressed using conventional biotechnology. The affinity tags were attached on the N-terminal of the protein and the detailed procedures for manufacturing recombinant proteins are described in the previous study. All the solvent otherwise specified were reagent grade, and triply distilled water of resitivity greater than 18.0 MΩ·cm was used in making aqueous solutions.

Syntheses of DFUA and NHS-UA

2,4-dinitro-5-fluoroaniline (2 g, 10.4 mmol) and sodium hydride (0.48 g, 20.8 mmol) were dissolved in 100 ml of distilled THF, and stirred for 30 min at 0 ^{o}C. Then, 10-undecenoyl chloride (2.8 ml, 10.4 mmol) dissolved in distilled THF was gradually added to the solution prepared above, and the reaction vessel was allowed for 5 h at ambient temperature. The resultant product was washed with dichloromethane and water three times, dehydrated with magnesium sulfate, and finally dried over in vacuuo. The final product (1.538 g, 40%) was separated with column chromatography (n-hexane : ethyl acetate = 30 : 1).

^{1}H NMR (400 MHz, $CDCl_3$): 1.32~1.38 (m, 12 H), 1.75 (t, 3H, *J*=19Hz), 2.03 (t, 3H, *J*=17 Hz), 2.56 (t, 3H, *J*=18 Hz), 4.91~5.00 (m, 2H), 5.75~5.84 (m, 1H), 9.00 (d, 1H, *J*=17 Hz), 9.11 (d, 1H, *J*=9 Hz), 10.74 (s, 1H)

For a synthesis of NHS-UA, undecenoic acid in 10 ml of dichloromethane (2 g, 10 mmol) was added into 5ml of dichloromethane solution containing DCC and NHS (3g and 2 g, respectively). After 5 h reaction at room temperature, the resultant product was concentrated in vacuuo, and was separated with column chromatography (n-hexane : ethyl acetate = 10 : 1).

Preparation of a hydrogenated silicon surface

Approximately 20ml of toluene solution containing 10 mM DFUA was placed in a vial and continuously degassed with dry nitrogen at least for 30 min. A piece of silicon (~1x1 cm^2) was submerged into a 10% HF solution to remove a native oxide layer, then washed with deoxygenated toluene and copious ethanol. Typically, the followed micro-contact printing or photolithographic works were carried out less than 2 min after the preparation of the hydrogenated silicon surface.

Microcontact printing for fluorescence and AFM measurements

A silicon wafer featured with 4 μm photoresistant polymer lines (SU8, Dow corning) and 3 μm spacing were used as a master for an elastomeric PDMS stamp. Into the master on a Petri dish was poured viscous PDMS oligomers. After 4 hrs for the oligomer to polymerize at 70 ^oC, the PDMS stamp was peeled off from the master. The PDMS stamp was oxidized with O_2 plasma for 1 min, just before micro-contact printing. A PDMS stamp inked by spin-coating the stamp with 10 mM DFUA in ethanol was

mildly pressed for 10 min onto a silicon surface (100); the silicon surface was in advance hydrogenated by soaking in 10% HF solution for 30 min at ambient temperature. Then, the wafer with the PDMS stamp was quickly brought to the exposure of 365 nm (25mW/cm^2) UV light from a mask aligner (MIDAS, Daejeon, Korea) to tether a DFUA monolayer typically for 10 min. After removing the stamp, the resultant pattern on gold film was rinsed with ethanol and deionized water, and was blown with N_2 gas for further interactions.

Photolithographic immobilization of DFUA, UA, and SA

On a silicon chip spin-coated with 10 mM DFUA, UA, NHS-UA, and SA in ethanol, respectively, a coverslip and a pattern mask made of stainless steel with spherical holes (d ~ 1mm) was sequentially placed. Then, the sandwiched silicon wafer was exposed to UV light for 10 min as employed in the micro-contact printing. After removing the pattern mask, the resultant pattern on the silicon wafer was thoroughly rinsed with copious toluene, ethanol, and deionized water, and was blown with nitrogen gas. The silicon wafer was allowed for protein adsorption typically in a concentration of 0.1 mg/ml, and further investigated with a fluorescence analyzer.

Fluorescence Image Analyses

For the fluorescence measurement, the silicon surface modified with monolayers prepared by schemes mentioned above was soaked in a PBS solution containing dye-labeled proteins or EGFP (0.1 mg/ml) for 1 h at 8℃. After immobilization, the silicon surface was incubated for 1 hr in 80% humidity to confirm a specific protein interaction. Then, the silicon surface was rinsed five times with PBST, and followed by rinsing with

deionized water. For the alleviation of ionic nonspecific interactions, high ionic strength buffer solution containing 1N NaCl was used in the monolayer of UA and NHS-UA. The fluorescent spots were analyzed by using a specialized analyzer, GenPix 4200 (Axon, USA).

AFM measurements

AFM images of line-patterned DFUA on a silicon surface were acquired via a Bioscope II apparatus (Veeco Inc.). Commercialized silicon tips were used for the tapping mode (20 nN/m, resonant frequency: ~320 kHz). Most of the images were obtained at a scan rate of ~2 Hz and were flattened by the software provided by the manufacturer.

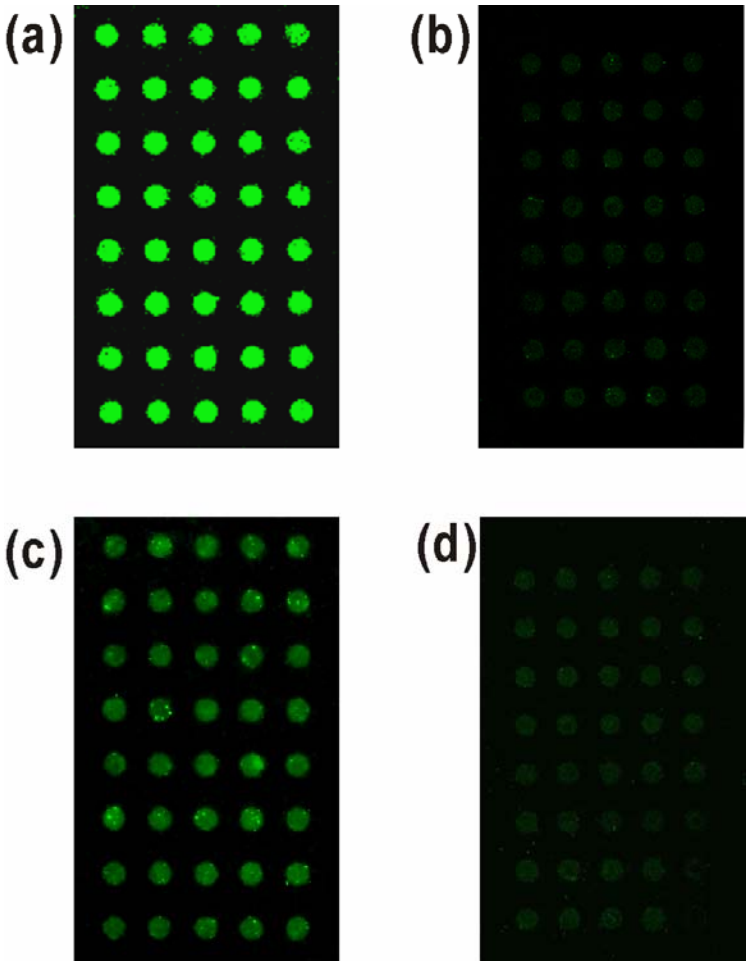

Figure S1. Fluorescence images of captured FITC-labeled BSA in a phosphate buffered saline (PBS, pH 7.4) (a) on a photolithographically patterned UA surface, (b) on the same UA surface but under a buffer condition with a high ionic strength (PBS plus NaCl 1M), (c) on a SA surface prepared by spin-coating it in the concentration of 100 mM, and (d) on an NHS-UA surface pre-soaked with a buffer solution for 2 h before treating it with the protein solution (PBS plus NaCl 1M), respectively.

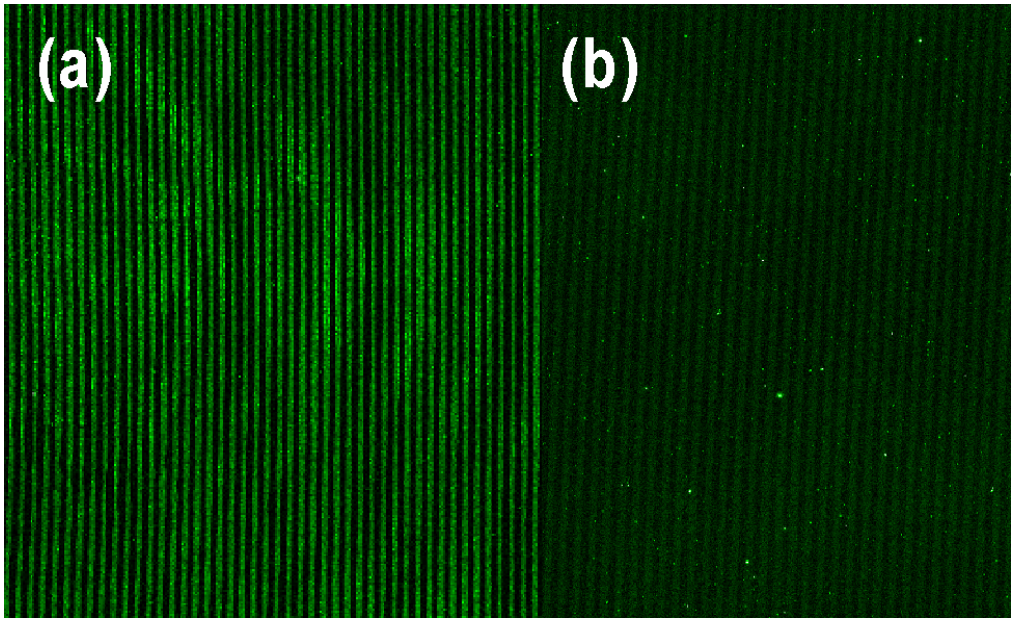

Figure S2. (a) Fluorescence image of GST-EGFP specifically captured on a patterned glutathione-DFUA layer, and (b) image of a simple EGFP immobilized nonspecifically on the same surface.