Supplementary Information for

Microstamping on an Activated Polymer Surface: Patterning Biotin and Streptavidin onto Common Polymeric Biomaterials

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1. Materials. PE, PS, PMMA (GoodFellow Inc., Berwyn, PA) and PET films (Dupont Inc., Wilmington, DE) were cleaned by rinsing with absolute ethanol for 2 h. EZ-Link™ biotin-PEO-LC-amine ((+)-biotinyl-3,6,9-trioxaundecanediamine) (termed biotin-amine hereafter) was obtained from Pierce (Rockford, IL). All other chemical reagents were obtained from Aldrich (Milwaukee, WI). Unlabeled streptavidin was purchased from Molecular Probes (Eugene, OR). ^{15}N-labeled streptavidin was synthesized in house by expression of a plasmid-borne synthetic gene of streptavidin in an *E. coli* host, which were grown in ^{15}N-labeled culture media, as described in section 3 below.

2. Surface Chemical Modification of Polymers. All surface derivatization reactions of polymer films were carried out without stirring.

Oxidation of PE (PE-OH). A cleaned PE film (1×3 cm) was introduced into a vial containing 20 ml of 0.5 M chromic oxide in a solution of acetic acid and acetic anhydride for 4 h. The PE sample was removed from the solution, and then rinsed with deionized water. The oxidation with chromic acid is relatively non-specific, and in addition to the introduction of hydroxyl groups, some fraction of the introduced oxygen-containing moieties are probably carboxylic acid.[1]

Hydroxylation of PS (PS-OH). The cleaned PS films were immersed into 18.5% (v/v) formaldehyde/1 M acetic acid for 4 h. at room temperature, rinsed with deionized water, and then dried under nitrogen.

Carboxylation of PE-OH and PS-OH (PE-COOH and PS-COOH). After drying, PE-OH and PS-OH films were reacted with 1 M bromoacetic acid/2 M NaOH overnight, to convert hydroxyl groups to carboxylic acid groups in the surface region.[2]

Carboxylation of PMMA (PMMA-COOH). A cleaned PMMA film sample was introduced into a vial containing 20 ml of 2 M NaOH that had been equilibrated to 40 °C in a constant-temperature bath for 2 h. After the reaction, the film sample was removed and rinsed with 0.1 M HCl, rinsed with distilled water and then dried under nitrogen.[3]

Carboxylation of PET (PET-COOH). A cleaned PET film sample was introduced to a vial containing 20 ml of 1 M NaOH for 2 h. After the hydrolysis, the film sample was removed and rinsed with 0.1 M HCl, rinsed with distilled water and then dried under nitrogen.[4]

Activation of Polymer Surfaces. The derivatized polymer films were dried in a vacuum oven at 50 °C for at least 2 h, and were then activated by immersion in an ethanol solution of 1-ethyl-3-(dimethylamino)propylcarbodiimide (EDAC, 0.1 M) and pentafluorophenol (PFP, 0.2 M) for 15 min. The residual pentafluorophenyl esters were inactivated by reaction with 10 mM 2-aminoethoxyethanol (AEE) in 100 mM sodium bicarbonate buffer, pH 8.3 for 20 min. The samples were then rinsed with 200-proof anhydrous ethanol, dried under a stream of nitrogen, and used immediately thereafter.

3. Synthesis, Expression and Purification of ^{15}N-labeled Recombinant Streptavidin Gene Synthesis of Core Streptavidin. Our gene design for streptavidin is similar to those described previously.[5] Briefly, a synthetic gene for core streptavidin (encoding residues 13-139 of wild-type streptavidin)[6] was designed by reverse transcription of preferred *E. coli* codons, and then modified to introduce restriction endonuclease recognition sites for cloning and cassette mutagenesis. EcoRI and HindIII recognition sites were added to the 5' and 3' ends respectively of the gene, to enable ligation of the synthetic gene into pUC19 plasmid (NEB Inc.). An Nde I site was also included at the 5' end, downstream of the EcoRI recognition site to allow subsequent insertion of the streptavidin gene into the pET25b expression vector (Novagen Inc.) and also provided the initiating Methionine codon.

Standard molecular biology protocols were used for the synthesis of the core streptavidin gene.[7] Chemically synthesized oligonucleotides (Integrated DNA Technologies) corresponding to the designed sequence were annealed in solution, ligated into linearized, dephosphorylated, pUC19 (NEB Inc.), and the ligation product was then transformed into XL1 Blue *E. coli* cells. Ligation of the core streptavidin gene into pUC19 was confirmed by DNA sequencing using dye

terminator chemistry (ABI 373 fluorescent sequencer, PE Biosystems). The gene was then subcloned into the expression vector pET25b (Novagen Inc.) to yield the expression vector pSTREP, and transformed into the BL21(DE3) strain of *E. coli* (Novagen Inc.).

Expression and ^{15}N-Labeling of Recombinant Streptavidin. BL21(DE3) (Novagen Inc.) cells transformed with pSTREP were cultured overnight in 50 ml Circlegrow liquid media (Bio 101 Inc.) supplemented with ampicillin (100 μg/ml), at 37 °C. The cells were centrifuged, resuspended in 5 ml of fresh Circlegrow medium containing 100 μg/ml ampicillin, and 3 ml of the resuspended culture were used to inoculate 1 liter Circlegrow medium supplemented with 100 μg/ml ampicillin in shaker flasks. The culture was incubated with shaking at 37 °C until the absorbance at 600 nm reached 0.8, at which time the cells were removed, pelleted at 3000 g, resuspended in M9 minimal media containing $^{15}NH_4Cl$ as the sole nitrogen source and isopropyl β-D-thiogalactopyranoside (IPTG) was added (1 mM) to induce protein expression. Non-isotopically labeled streptavidin was produced without switching the media from Circlegrow media to minimal media prior to induction, and the 1 L culture was allowed to reach an OD of 0.8, at which protein expression was induced by adding IPTG to a final concentration of 1 mM. Cells were cultured for a further 5 h (3 h for non-isotopically labeled protein expression), after which they were harvested by centrifuging at 3000 g for 15 min. The cell pellets were stored at -70 °C till further use.

Purification of ^{15}N-Labeled Streptavidin. The frozen cell pellet was resuspended and thawed in 50 ml lysis buffer (50 mM Tris, 200 mM NaCl, 5 mM EDTA, 8% (w/v) sucrose, 1 mM phenyl methyl sulfonyl chloride, 2% (v/v) Triton X-100, pH 8.0). The cells were lysed by repeated rounds of sonication (Sonic Dismembrator 550, Fisher Scientific). Because streptavidin is

sequestered in insoluble inclusion bodies in *E. coli* upon overexpression,[5] the insoluble fraction of the cell lysate, containing the inclusion bodies, were washed in Triton X-100 and then dissolved overnight in in 50 mL of 6 M guanidine.HCl. The solubilized protein was then dialyzed overnight against 20 L of 50 mM Tris, 8 mM EDTA, 100 mM NaCl, pH 7.9. The dialysis buffer was exchanged twice over 24 h. Insoluble protein was removed by centrifugation, and the refolded protein was then concentrated by ultrafiltration, and purified by iminobiotin affinity chromatography.[8] The yield of ^{15}N-labeled streptavidin was ~2 mg/L of culture.

4. Surface Characterization

Fluorescence Microscopy. Fluorescence images of Alexa488-labeled streptavidin patterns were acquired on a confocal microscope (Zeiss LSM 510) at 10X or 20X magnification. The gain of the fluorescence detector was adjusted to avoid saturation of the most intense signals originating from the patterned areas.

X-ray photoelectron spectroscopy. XPS analysis was performed on a SSX-100 spectrometer (Surface Science Inc., Mountain View, CA), equipped with a monochromatized AlK_α X-ray source, a hemispherical electron analyzer, and a low-energy electron flood gun for charge compensation of insulators. Samples were typically introduced into a preparation chamber, maintained at a pressure of 10^{-4} torr, and then transferred into the analysis chamber, typically maintained at 10^{-8} torr. The samples were typically analyzed at 35° take-off angle; the take-off angle is defined as the angle between the sample plane and the entrance axis of the hemispherical

analyzer. The typical X-ray spot size was 1000 μm. Survey scan spectra were acquired from 0-1000 eV for elemental composition.

Time-of-Flight Secondary Ion Mass Spectrometry. TOF-SIMS spectra and ion images were obtained on a TOF-SIMS instrument (TOF-SIMS IV, ION-TOF, Münster, Germany). A 25-keV monoisotopic $^{69}Ga^+$ primary ion beam generated by a Ga^+ gun were used. "Bunched mode" was used to achieve highest mass resolution ($m/\Delta m \approx 10{,}000$) in the mass spectra. The typical target current of the primary Ga^+ beam in the bunched mode for TOF-SIMS was 3 pA with a pre-bunched pulse width of 20 ns. The raster area of the Ga^+ ion gun was 384 × 384 μm^2 and the raster resolution was 128 × 128 pixels, in order to attempt to match the pixel size with the Ga^+ ion beam spot size (i.e. the Ga^+ ion beam spot size was approximately 384÷128 ≈ 3 μm), thereby optimizing both spatial resolution and data rates for these gun conditions. All primary Ga^+ ion fluences were below the damage threshold of 1×10^{13} ions cm^{-2} for static SIMS.

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