Supporting Information

Single Molecule Protein Patterning Using Hole Mask Colloidal Lithography

William Lum[1], Dinesh Gautam[2], Jixin Chen[2], Laura B. Sagle[1]*

EXPERIMENTAL METHODS

Sample Preparation. First (S1A), round #1.5 25 mm borosilicate coverslips (Fisherbrand) are piranha cleaned. Individual coverslips are spin-coated with 350kDa 4% w/v polymethylmethacrylate (Sigma Aldrich) in 99% anisole (Acros Organic) at 3000 rpm for 30 seconds with a 5 second ramp (S1B). The coverslips are soft baked at 180 °C for five minutes to remove the anisole and fix the polymer to the surface. The PMMA covered coverslip is exposed to a 5 second oxygen plasma etch. Positively charged 0.2%-0.005% 200-350 kDa poly(diallyldimethylammonium chloride) solution (PDDA) (Sigma Aldrich) is drop coated for 30 seconds and rinsed with DI water (S1C). Carboxylated polystyrene (PS) nanospheres (Corpuscular) of varying sizes are diluted between 0.05 % to 0.00005% v/v for concentrated and dilute samples and drop coated onto the substrate for 2 minutes and rinsed with DI water (S1D). The negatively charged PS spheres electrostatically repulse one another to limit aggregation and bind the positive PDDA surface. Adjustment of PDAA to lower concentrations are needed for lower concentration of PS spheres in order to balance the charges between the PS and PDDA. 50 angstroms of gold is electron beam physical vapor deposited over the entire surface of the substrate (S1E). Household invisible tape is applied to the surface and stripped away to remove the raised PS nanospheres (S1F). The substrate surface is still covered in a planar gold surface but is dotted with holes exposing PDDA where the polystyrene spheres once were. An additional 2 minute and 30 second oxygen plasma etch removes the exposed PDDA and PMMA. The area that has gold are protected from the oxygen plasma while the exposed holes become wells extending to the glass (S1G).

Protein Patterning. Patterning protein into the HCL substrate and begins with depositing 11-aminoundecyltriethoxysilane (AUTES) (Gelest) through either vapor deposition (<64nm) or solution (>64nm) (S1I). Once the exposed glass is functionalized with AUTES, the polymers and gold are removed by sonicating in acetone and rinsed with ethanol and Milli Q water (S2J). The substrate is dried with nitrogen then biotin-Poly(ethylene glycol)(1K)-carboxylic acid (Nanocs) is

reacted with the amino group of AUTES through carbodiimide chemistry (S1K). 10 μM biotin-PEG(1K)-COOH in pH 5.5 4-morpholinoethanesulfonic acid (MES) buffer is dissolved with 10 fold excess N-Hydroxysuccinimide (NHS) and 1-ethyl-3-(-3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) (ThermoFisher). The Biotin-PEG(1K)-COOH : NHS : EDC solution is drop coated to the substrate for 1 hour. After which, the substrate is rinsed with MilliQ water and dried with nitrogen. Finally, 1-10 nM of streptavidin (Sigma Aldrich) in pH 7 2-[4-(2-hydroxyethyl)piperazin-1-yl] ethanesulfonic acid (HEPES) buffer (Scheme 1L). The substrate is rinsed and soaked in buffer until characterization.

AFM Characterization. All AFM characterization was with done with dried samples on a Veeco Dimension AFM with MikroMasch NSC15 aluminum backside coated probes at the University of Cincinnati Advanced Materials Characterization Center. AFM images and particle analysis was done with Nanoscope Analysis (Bruker). The bin width of both height histograms is 1 nm. The bin width of 15 nm diameter histogram is 3 nm. The bin width of the 210 nm diameter histogram is 10 nm.

Fluorescence Microscopy Characterization. All fluorescence measurements were carried out using a home-built Nikon Ti-U inverted microscope under total internal reflection fluorescence (TIRF) mode connected with four solid-state lasers (Dragon Lasers, China), two beam expanders along with a Nikon 100× oil-immersed TIRF objective (CFI Apo 100×, NA 1.49, WD 0.12 mm), and an EMCCD camera (Andor iXon Ultra 897). Each laser source works together

with a TIRF fluorescent filter cube. In this study, we used red filter model TRF49913 (Chroma, USA) for the 640 nm laser.

Flow cells for fluorescence measurement were prepared from combining the patterned sample glass coverslips with PDMS blocks that have been connected with tubings and syringe pump. The size and shape of the flow cell are defined by a channel (hole) cut in a double-sided tape that sticks the sample and the PDMS. The size of the channel is approximately 2 cm × 2 mm × 100 μm (length × width × height). The PDMS blocks were prepared by mixing Sylgard 184 silicone elastomer base (Dow Corning, USA) with the curing agent in 10:1 by mass for 15 minutes. Then, vacuum desiccated to remove bubbles on the surface transferred to the Petri dish and cured overnight at room temperature. Cured PDMS blocks were cut into smaller sizes as the coverslips. Syringe tips were cut and punched through the PDMS block and connected the other end with a PTFE tubing.

These flow cells were used to flow ≈ 0.1 mL of 1 nM streptavidin in the 1× PBS buffer solution (pH = 7.4) at a rate of 0.02 mL/min. Incubated for 15 minutes followed by flowing with 1 mL buffer solution at 0.2 mL/min to wash away any excess of streptavidin that did not bind to the target. Then ≈ 0.1 mL of 1 nM Cy5 labeled biotin was flowed through the channel at 0.02 mL/min. Incubated for 15 minutes followed by flowing 1 mL of buffer at 0.2 mL/min to wash away unbound biotin-Cy5. Then we recorded a video of Cy5 biotin binding to streptavidin under TIRF illumination with the 640 nm laser at a camera exposure time 50 ms each frame and an EM gain of 300×. The length of the video was 500 frames or 25 s.

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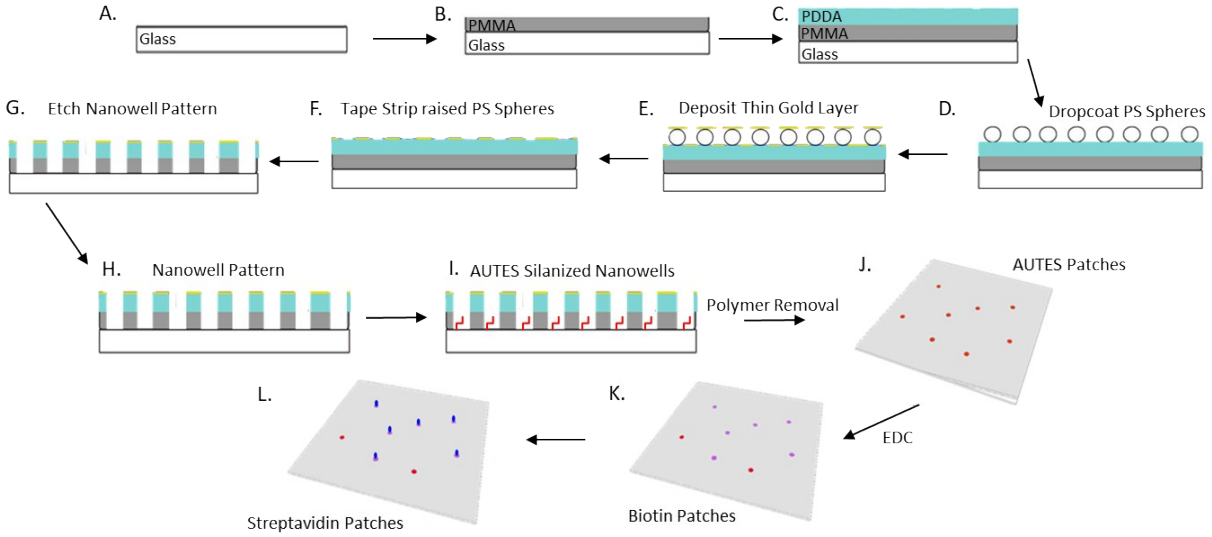

Figure S1. Schematic of the hole mask colloidal lithography technique to make nano-wells and pattern streptavidin on the glass. Cleaned glass is spin-coated with PMMA and PDDA (A-C). Polystyrene nanospheres are deposited on the sacrificial PDDA/PMAA polymer layer (D). Followed by a 5 nm gold is deposition over the entire surface (E). The nanospheres are taped stripped away (F). The exposed polymer is etched to form nano-wells where the bottom is exposed glass (G). To pattern the hole-mask substrate, the glass is salinized with an amine functional group (I). Finally, the AUTES is EDC coupled to biotin and then bound with streptavidin. (K, L)

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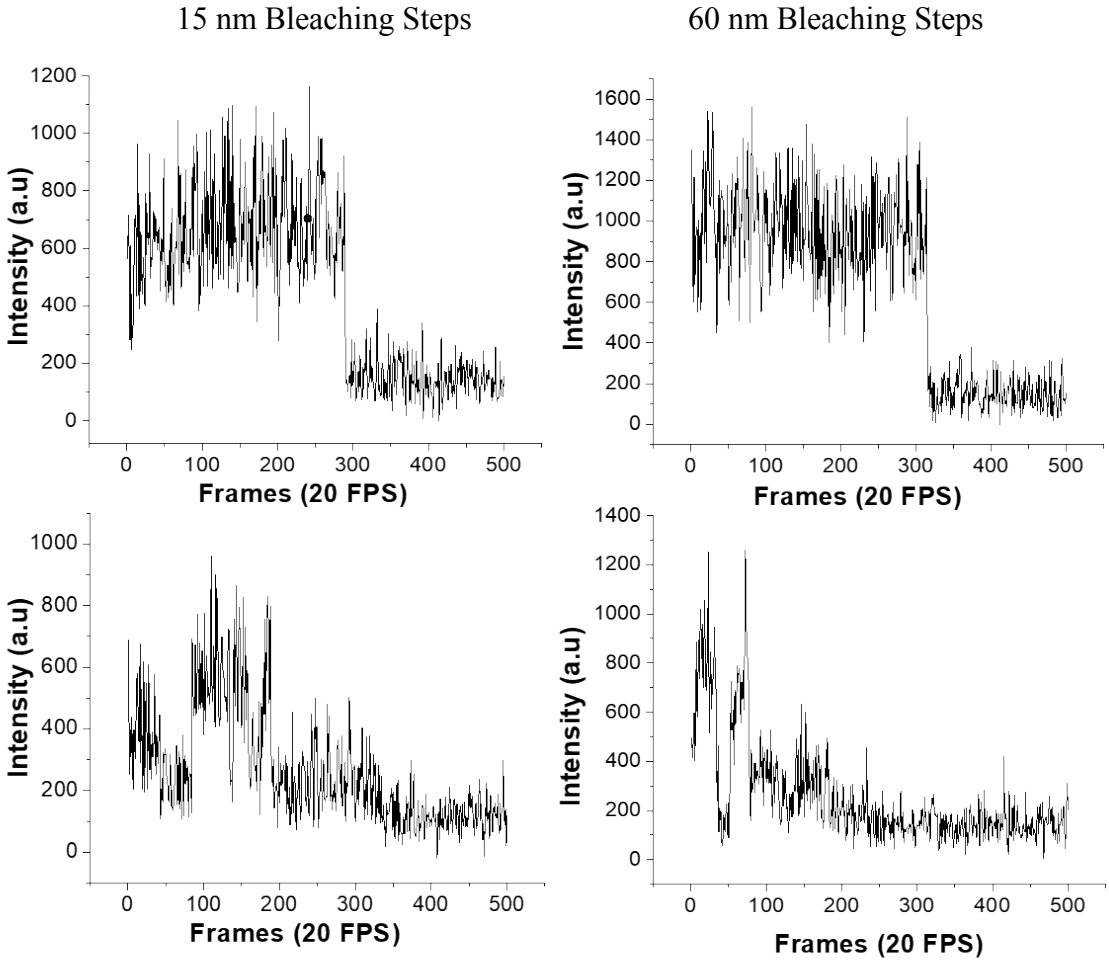

Figure S2. Example bleaching step data. Top are examples of single steps, while the bottom are examples of multiple steps. Bottom left is 2 steps, while the bottom right is 3 steps.

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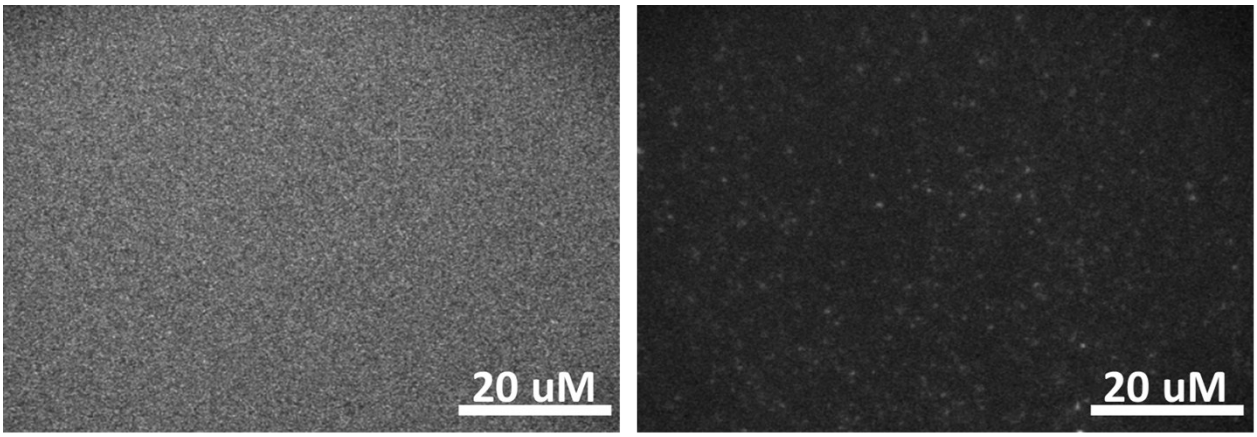

Figure S3. Fluorescence image of the 15 nm sample pre (left) and post (right) adding biotin-cy5 fluorescent dye.

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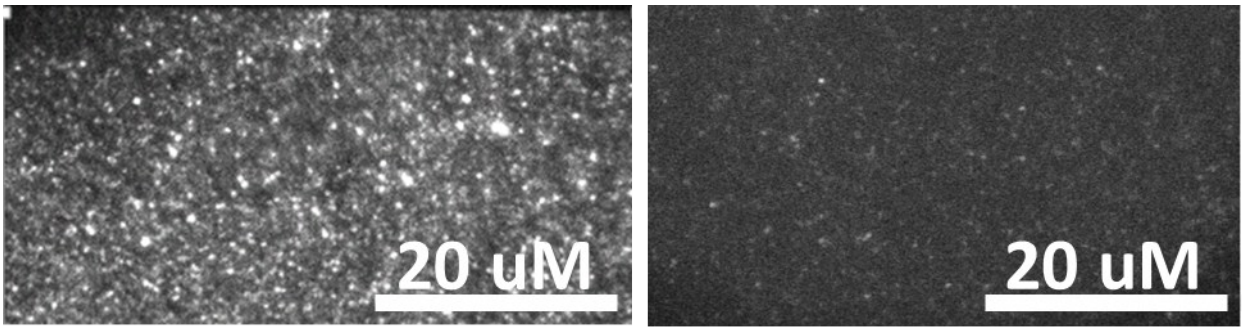

Figure S4. Fluorescence images illustrating the density of fluorescence spots can be tuned by the concentration of the PS spheres. The left image is of a sample made with 0.001% PS spheres, while the right is made with 0.00002% PS spheres. Concentration of AUTES, biotin, streptavidin, and biotin-cy5 remain constant. Decreasing the amount of PS spheres reduces the density of the hole-mask template, that directly reduces fluorescence spots.

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Figure S5. Negative controls demonstrate that each iterative step of amino silane, biotin, and streptavidin, is necessary for biotin-Cy5 binding. Without biotin and streptavidin, biotin-cy5 does not bind. Omitting biotin results in no biotin-cy5 binding. Lastly, omitting amino silane and streptavidin results in no biotin-cy5 binding.