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Supporting Information

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Facile Preparation of Complex Protein Architectures on Surfaces with sub-100 nm Resolution

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MATERIALS AND METHODS

Preparation of Nanotemplates

High-resolution nanotemplates were produced using electron-beam lithography. Silicon wafers (4") were spin-coated with poly(methyl methacrylate) (PMMA):anisole at a ratio of 2:1 for 40 s at 3000 RPM followed by a postbaking step at 180 °C for 90 s. The resulting thickness of the PMMA layer was ~100 nm. The PMMA resist was exposed in an e-LiNE electron-beam lithography system (voltage: 20 kV, aperture: 10 μm, beam current: 29 pA) (Raith GmbH, Dortmund, Germany), developed in a solution of MIBK:isopropanol at a 1:3 ratio for 30 s, immersed in isopropanol for 1 min, and blown dry under a stream of N_2. The PMMA pattern was transferred into the silicon substrate using a low-etch-rate reactive ion etcher in a balanced process that used SF_6 as a precursor for the etching and C_4F_8 for passivation of the sidewalls (Alcatel Vacuum Technology France, Annecy, France) that lasted for 25 s. The resulting Si structures were ~60 nm deep. The PMMA resist was removed by immersion in acetone for ~1 min. Generally, dicing was not completed prior to or after patterning to prevent particle contamination of the wafer. If dicing had to be completed prior to or after patterning, a sacrificial layer of PMMA was spin-coated on the wafer, dicing was completed, and then the PMMA was removed.

Nanotemplate design

Several types of geometries were used during these experiments, Table S1. Patterns of lines, meshes of lines overlapping to form right angles, linelets, and squares gave results with different resolution limits and ease of use. Patterns with large spaces between features (squares, linelets) were difficult to locate using atomic force microscopy (AFM). The highest-resolution features were obtained with patterns of meshes. High-resolution patterns of squares were difficult to create.

Table S1. Assessment of the quality of various geometries, sizes, and spacings of patterns of proteins provided as well as suggestions for visualization.

Geometries	Width (nm)	Spacing (μm)	Quality of Pattern	Visible with Immunofluorescence	Ease of location with AFM
Mesh	40	0.8	Low	40 nm meshes could be seen, but not distinguished; 60 nm and larger meshes were distinguishable	Easily located
Mesh	60	1.2	Medium		
Mesh	100	2	High		
Mesh	200	4	High		
Linelets	250 nm × 3 μm	1, 2, 4, 8, 16, 32, 64	High	Easily located	Large spacing between linelets made them difficult to locate with AFM
Line	100	1	High	Easily located	easily located
Line	250	2.5	High	Easily located	easily located
Square	50	Spacing between features in clusters = 1× width, spacing between clusters = 2 to 8.5	No data available	500 and 250 nm features were always visible, a slight signal from 100 nm was sometimes visible	With large spaces between clusters, squares were very difficult to locate
Square	100		No data available		
Square	250		Medium		
Square	500		High		

Preparation of planar elastomers

PDMS planar elastomers were polymerized using Sylgard 184 prepolymers (Dow Corning, Midland, MI) at a ratio of 10:1 (base polymer:curing agent). An automatic mixer/dispenser (DOPAG Micro-Mix E, Cham, Switzerland) was used to uniformly mix the prepolymers before dispensing them into planar Petri dishes (Falcon 1001 and 1013, Becton Dickinson, NJ). PDMS was then cured at 60 ºC for at least 24 h. The planar elastomers had a final thickness of ~2 mm. Complete mixing of the PDMS is crucial to ensure homogeneous curing of the elastomer. If incomplete curing occurs locally, this may result in PDMS residues left on the nanotemplate, which might be difficult to remove.

Protein inking of planar elastomers

The polymerized PDMS was cut while still in contact with the Petri dish into ~5 × 5 mm^2 pieces of elastomer. The side of the elastomer that was not in contact with the Petri dish was marked by making a shallow cut with a scalpel. The elastomers were cleaned by sonication in a 1:3 solution of isopropanol:deionized water for 5–10 min, rinsed using deionized water produced using a Millipore Simplicity System (Millipore Corporation, Billerica, MA), rinsed using ethanol, and blown dry under a stream of N_2. The side of the elastomer that was in contact with the Petri dish was inked with ~100 μL of antibody solution for 30 min at room temperature. Using the pipette tip, the antibody solution was spread over the entire elastomer surface without contacting the surface. TRITC-labeled goat anti-rabbit IgG (T6778, Sigma, St. Louis, MO) was used at a concentration of 0.5 mg mL^{-1} in phosphate buffered saline (PBS) (A7906, Sigma). AlexaFluor 647-labeled goat anti-rabbit IgG (A21244, Invitrogen, Carlsbad, CA) was used at a concentration of 0.1 mg mL^{-1} in PBS. Elastomers inked with antibodies were rinsed using PBS and deionized water, and blown dry under a stream of N_2 for approximately 30 s.

Subtraction and Printing of proteins

Microscope glass slides (Menzel GmbH, Braunschweig, Germany) and silicon wafer pieces (Siltronic AG, Munich, Germany) were used as substrates for printing. Prior to printing, substrates were cleaned by sonication in a 1:3 solution of isopropanol:deionized water for 5-10 min, rinsed in deionized water, rinsed in ethanol, and blown dry under a stream of Ni. Silicon substrates and nanotemplates were treated in an oxygen plasma at 200 W for 60 s (Technics Plasma 100-E, Florence, KY). Proteins on homogenously inked elastomers were removed in selected areas by bringing the elastomers into contact with the nanotemplate for 15 s. The elastomers were brought into contact and released from the nanotemplate by hand without bending the elastomers to prevent the introduction of distortions in the resulting protein patterns. The protein patterns were transferred from the elastomers to the final substrates using a 30-s-long printing step. Intimate contact between the elastomer and the nanotemplate/substrate occurred after placing the elastomer on the nanotemplate/substrate by hand and applying a slight pressure with tweezers. The displacement of air by a propagating contact line between

the elastomer and the contacted surface was easily seen by eye and confirmed uniform contact between the surfaces. Nanotemplates were cleaned of organic material by repeating the treatment with oxygen plasma before reusing. When completing patterns involving multiple subtraction or printing steps, a mark on the back of the elastomer created by making a shallow cut with a scalpel was used as an alignment marker to assure that successive steps were being completed in the same area.

Visualization

Fluorescence micrographs were acquired using a Nikon Eclipse 90i (Nikon Corporation, Tokyo, Japan) fitted with a Nikon Digital Sight DS-1QM/H-cooled CCD camera (Nikon Corporation). Black and white images captured from individual channels were color-coded and combined into one image for analysis using NIS-Elements 2.30 (Nikon Corporation). Scanning electron microscopy images were obtained using a LEO 1550 (LEO Electron Microscopy, Inc., Throrwood, NY) to verify the quality and dimensions of the nanotemplate. AFM images were obtained using a Nanoscope Dimension 3000 (Digital Instruments, Santa Barbara, CA) operated in tapping mode using standard silicon cantilevers (174–191 kHz, Nanosensors, Neuchâtel, Switzerland). AFM images were planarized, displayed, and analyzed using WSxM (Nanotec Electronica, Madrid, Spain).

The size of the total area containing patterns on the nanotemplate was important for (i) locating the pattern of proteins on the final substrate using immunofluorescence microscopy or AFM, (ii) locating the patterns on the nanotemplate using scanning electron microscopy, and (iii) ensuring correct location of the elastomer on the nanotemplate and substrate during contact steps. A pattern size of 1000 μm × 1000 μm was found to be optimal. Rectangular features with dimensions of 80 μm × 30 μm were placed at the corners of the area containing patterns on the nanotemplate to help locate the pattern.