Supporting Information

Ultrahigh Resolution, Serial Fabrication of Three Dimensionally-Patterned Protein Nanotructures by Liquid-Mediated Non-contact Scanning Probe Lithography

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Experimental Section

Materials: Bovine serum albumin (BSA, $M_w \sim 6.8 \times 10^4$ Da, pI ~ 4.7), human plasma fibrinogen ($M_w \sim 3.4 \times 10^5$ Da, pI ~ 4.8) and lysozyme ($M_w \sim 1.4 \times 10^4$ Da, pI ~ 11.1) were purchased from Calbiochem and used without further purification. 11-(2-(2-(2-methoxyethoxy)ethoxy)ethoxy)undec-1-ene ($C_{11}EO_3Me$) was synthesized following a procedure reported before. Mesitylene was purchased from Aladdin and redistilled from sodium under vacuum before used. Sodium chloride (NaCl), potassium chloride (KCl), sodium phosphate dibasic (Na_2HPO_4), and potassium phosphate (KH_2PO_4) brought from Sinopharm Chemical Reagent Co., Ltd were all AR grade and were used as received. Phosphate buffered saline (PBS, 0.14 M, pH 7.4) solution was prepared using NaCl, KCl, Na_2HPO_4 and KH_2PO_4 and filtered by 0.2 um hydrophilic milipore filter prior to AFM experiment. The water used was purified by filtration through a Millipore gradient system after distillation, giving a resistivity of 18.2 MΩ·cm. Au coated silicon wafers which the thickness of Au is 50 nm and RMS is 0.5 nm were immersed into Piranha solution (H_2SO_4/30% H_2O_2, v:v=7:3) at 70 °C for 5 min, rinsed with plenty of Millipore-Q water and blown dried by a stream of high-purity nitrogen gas, then immediately fixed onto the bottom of AFM liquid cell for subsequent experiment. 1-Dodecanethiol was purchased from Sigma-aldrich and used without further purification. 1-Dodecanethiol modified Au

substrates were prepared by immersing the substrates into a 5 mM 1-Dodecanethiol ethanol solution at room temperature for 12 h. The substrates were rinsed sequentially with ethanol and water, and then dried under a stream of nitrogen. Other reagents were AR grade and used as received.

Liquid-mediated Non-contact Scanning Probe Lithography (L-NCSPL): The atomic force microscopy (Park Systems, XE-100) used in our experiments has two scanners, XY-piezo scanner and Z-piezo scanner. When imaging sample, XY-piezo scanner moves sample in the xy two-dimensional plane and Z-piezo scanner moves the cantilever in z direction. In non-contact AFM, the cantilever oscillates just above the sample surface. And the set point value in non-contact AFM correlates to the amplitude of cantilever vibration. As the set point value decreases, the amplitude of the cantilever vibration and tip-to-sample distance decrease. When non-contact AFM operated in top-to-bottom slow scan direction, XY-piezo scanner linearly scans the Au surface in x direction, after the line scanning is completed, the XY-piezo scanner moves the sample surface in y direction (from bottom to top) by one pixel point, and then linearly scans the sample surface in x direction again. When the non-contact AFM is performed in left-to-right or right-to-left slow scan direction, the XY-piezo scanner linearly scans the Au surface in y direction. After the line scanning is completed, the XY-piezo scanner moves the sample surface in y direction (from left to right or right to left) by one pixel point, and then linearly scans the sample surface in x direction again. The movement of sample cycles in this way until the scan is completed.

Micro- and nanopatterning of protein was performed by using an AFM equipped with an open liquid cell. In a typical experiment, an Au substrate was placed at the bottom of the liquid cell, followed by filling with a certain amount of BSA solution. A cantilever (NCHR, NanoSensors, spring constant 42 N/m) was mounted onto the liquid probe hand on the Z-piezo scanner and then fully immersed into protein solution. The AFM tip was brought close to the Au substrate and oscillated. Patterned structures of BSA were fabricated at different set point value and scan line density by non-contact AFM in top-to-bottom slow scan direction, scan rate was 2 Hz. As for comparison, patterns of protein were fabricated at different scan rates increasing from 1 to 9 Hz. In addition, non-contact AFM performed in left-to-right or right-to-left slow scan direction was used to fabricated patterned structures of BSA on Au substrate. The set point was 20 nm, the scan pixel density was 256 × 256 and the scan rate was 2 Hz. The as-made patterned structures of protein were imaged by non-contact AFM in bottom-to-top slow scan direction, scan rate was 1 Hz to ensure the optimal resolution of image during scanning.

Characterization of patterned structures of BSA in air: After the fabrication of the patterned structures of BSA on Au substrates by L-NCSPL in BSA solution, the substrates were removed from the solution, and then rinsed sequentially with PBS buffer and water, and dried under a stream of nitrogen. The substrates were imaged with non-contact AFM in air with a NCHR cantilever. The scan rate was 1 Hz and the image resolution was 256 by 256.

Preparation and Characterization of $C_{11}EO_3Me$ Modified Cantilever: $C_{11}EO_3Me$ was used for modifying the cantilever tip by hydrosilylation reaction. Briefly, NCHR silicon

AFM cantilever was cleaned with Piranha solution at 80 °C for 10 min to remove organic contaminants and followed by etching silica layer on silicon cantilever with 2% HF aqueous solution. Then, the etched cantilever was immersed in a 0.1 M solution of $C_{11}EO_3Me$ in freshly distilled mesitylene under argon and refluxed for 2 h. Meanwhile, one piece of etched silicon wafer was immersed in solution and tracked hydrosilylation reaction. After reaction, cantilever and silicon wafer were then thoroughly washed with petroleum ether, ethanol, and dichloromethane sequentially and blown dried by a stream of high-purity nitrogen gas.

In order to confirm successful hydrosilylation reaction of cantilever, X-ray photoelectron spectroscopy (XPS) measurements of traced silicon wafer was conducted on an X-ray photoelectron spectrometer (ESCA, Axis Ultra DLD, Kratos) with a monochromatic Al K X-ray source (1486.6 eV) as radiation source under 10^{-9} mbar (Figure S5). High resolution spectra were collected by using the monochromatic Mg X-ray source (1253.6 eV). All spectra were calibrated with the C_{1s} peak at 284.6 eV. Three different points was detected on the surface to reduce the error.

SEM Measurements: The topographies of bare AFM tip and $C_{11}EO_3Me$ modified AFM tip before and after fabricating BSA patterns were observed by a field emission scanning electron microscope (ZEISS MERLIN) operated at an accelerating voltage of 5 kV. In this case, to avoid the possible contamination of putter-coated with gold for cantilever surface, all cantilevers were only fixed onto conductive adhesive to minimize sample charging.

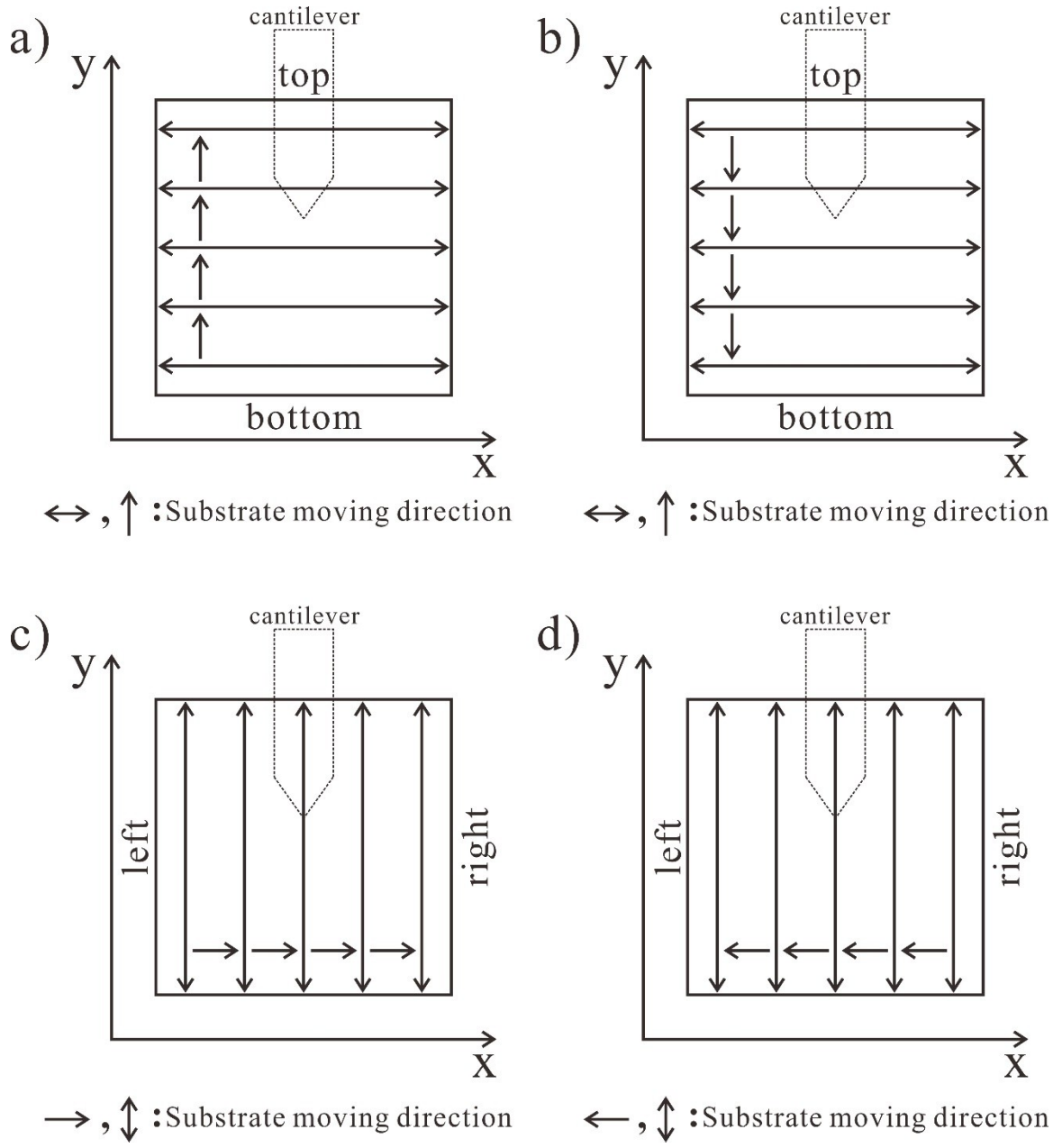

Figure S1. Illustration of a) top-to-bottom, b) bottom-to-top, c) left-to-right and d) right-to-left slow scan direction. The dotted graph is a top view of the rectangular cantilever.

Figure S1 illustrates the slow scan direction. The slow scan direction is regarded as the direction that AFM tip scans the substrate. For example, when AFM is performed in top-to-bottom slow scan direction, XY-piezo scanner moves the substrate back and forth fast in x direction, and subsequently moves it from bottom to top in y direction at the

distance of one pixel (Figure S1a). Then the movement of substrate cycles in this way until the scan is completed. According to the relative movement between the substrate and the AFM tip, the AFM tip will scan the substrate in top-to-bottom slow scan direction.

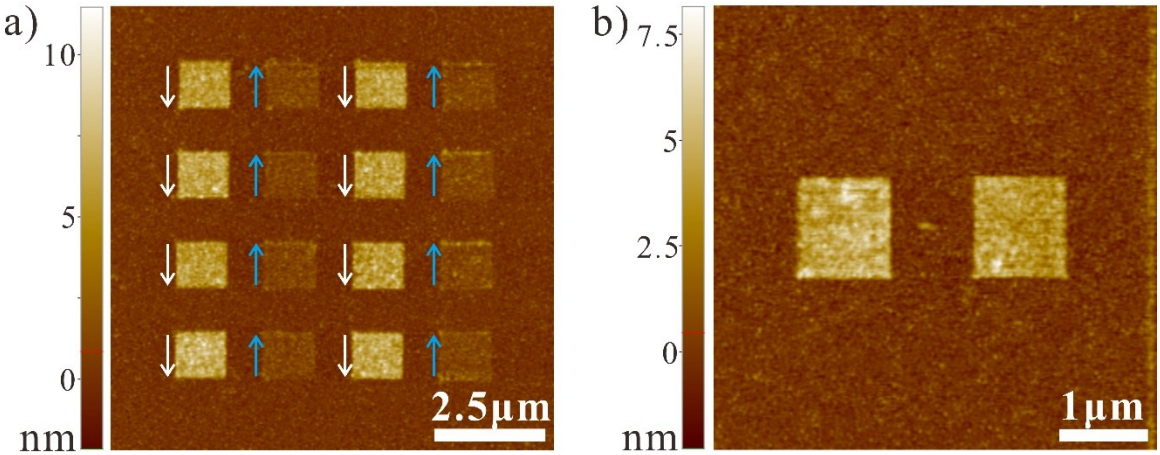

Figure S2. a) AFM topographic view of an array of 1 µm × 1 µm square-shaped microstructures of BSA fabricated via L-NCSPL by switching slow scan direction alternately. The arrows indicate the slow scan directions. b) AFM topographic view of two square-shaped microstructures of BSA fabricated by non-contact scanning in left-to-right (left) and right-to-left (right) slow scan direction, respectively.

Figure S2 shows the impact of slow scan direction on the patterning of BSA. A series of 1 µm × 1 µm squares of BSA structures on Au substrate were scanned with non-contact AFM with set point of 20 nm in top-to-bottom and bottom-to-top slow scan direction alternately under a BSA solution. As shown in Figure S2a, a larger amount of BSA are deposited onto the scanned areas when scanned by non-contact AFM in top-to-bottom slow scan direction. As shown in Figure S2b, non-contact AFM were used to scan

Au substrate in left-to-right and right-to-left slow scan direction respectively. A large amount of BSA were deposited onto Au substrate in both directions.

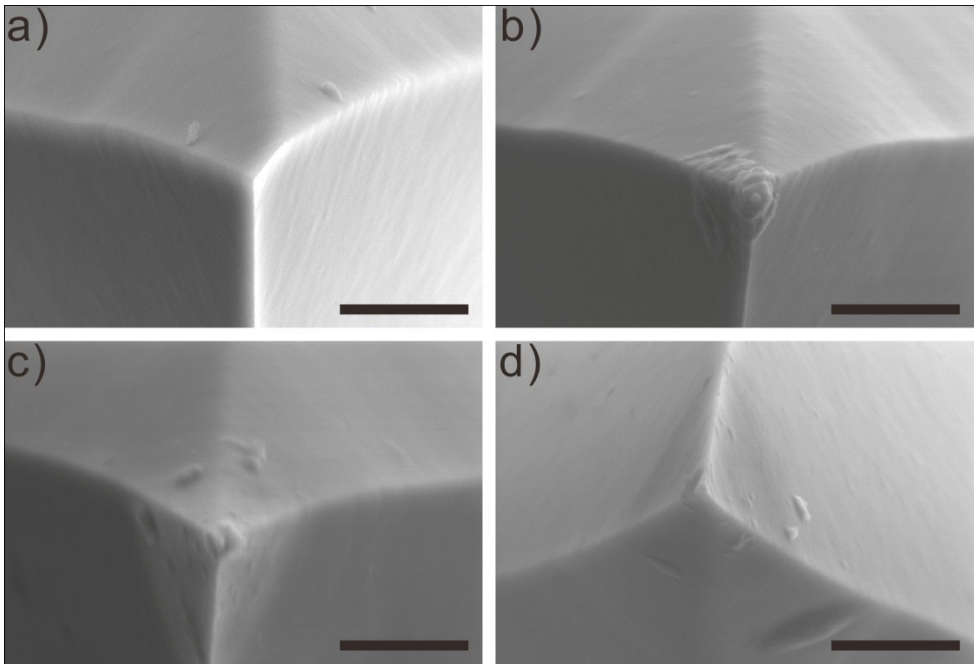

Figure S3. SEM images of a) new AFM tip, b) new AFM tip after patterning BSA on Au substrate by L-NCSPL, c) AFM tip modified with $C_{11}EO_3Me$, and d) $C_{11}EO_3Me$ modified tip after patterning BSA on Au substrate by L-NCSPL. Scale bar is 1μm.

Figure S3 shows the SEM images of AFM tip before and after the fabrication of the patterned structures of BSA. After the fabrication of patterned structures of BSA, the AFM tip was imaged with SEM. Compared with a new tip (Figure S3a), little amount of BSA adsorbs on the AFM tip after the fabrication process.

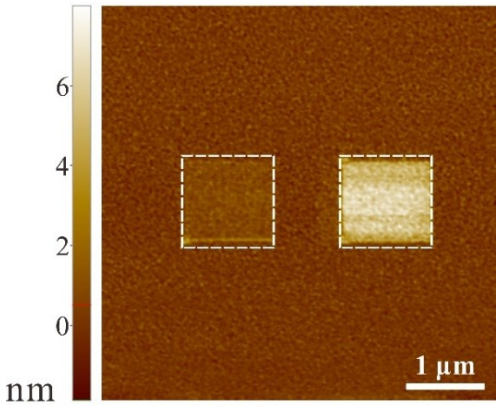

Figure S4. AFM topographic view of patterned structure of BSA fabricated by L-NCSPL with the modified tip to scan left and right dotted area in bottom-to-top (left square) and top-to-bottom (right square) slow scan direction, respectively.

Figure S4 shows patterned structures of BSA is successfully fabricated with this modified tip. After the fabrication process, there is no BSA molecules adsorb on the modified tip due to the protein resistant property of C11EO3Me (Figure S3d). This may indicate that BSA molecules in solution are trapped by AFM tip first and then pushed onto the scanned area.

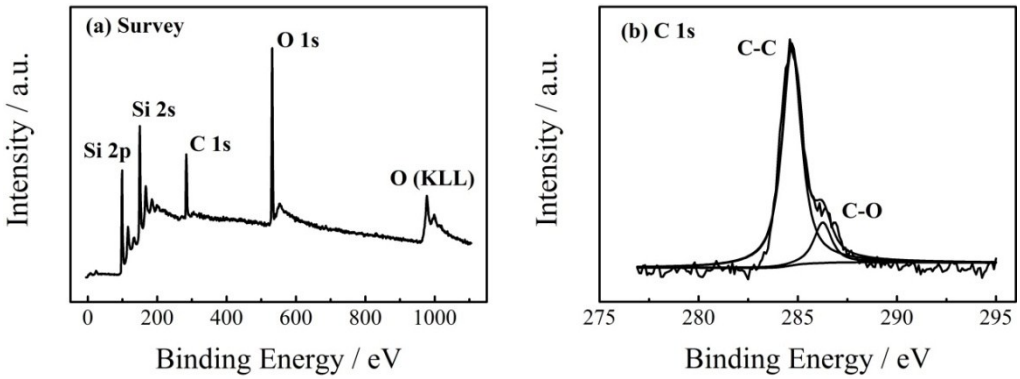

Figure S5. (a) XPS survey spectrum of $C_{11}EO_3Me$ modified silicon wafer. (b) High resolution spectrum of C 1s in (a).

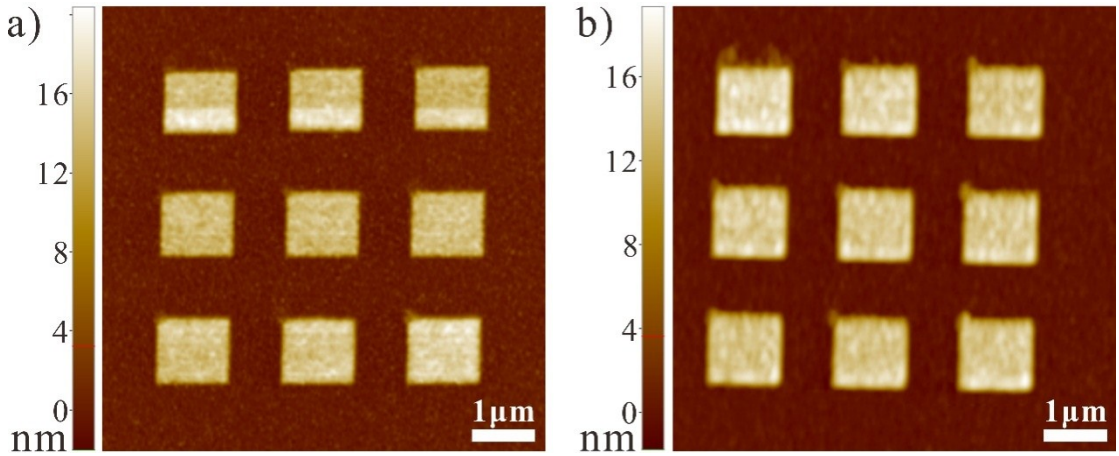

Figure S6. a) AFM topographic view of patterned structure of BSA fabricated by L-NCSPL with set point of 20 nm. This image was obtained in PBS buffer immediately after the fabrication. b) Patterned structure of BSA was imaged with non-contact AFM in air. Before imaging, the substrate was rinsed sequentially with PBS buffer and water, and then dried under a stream of nitrogen.

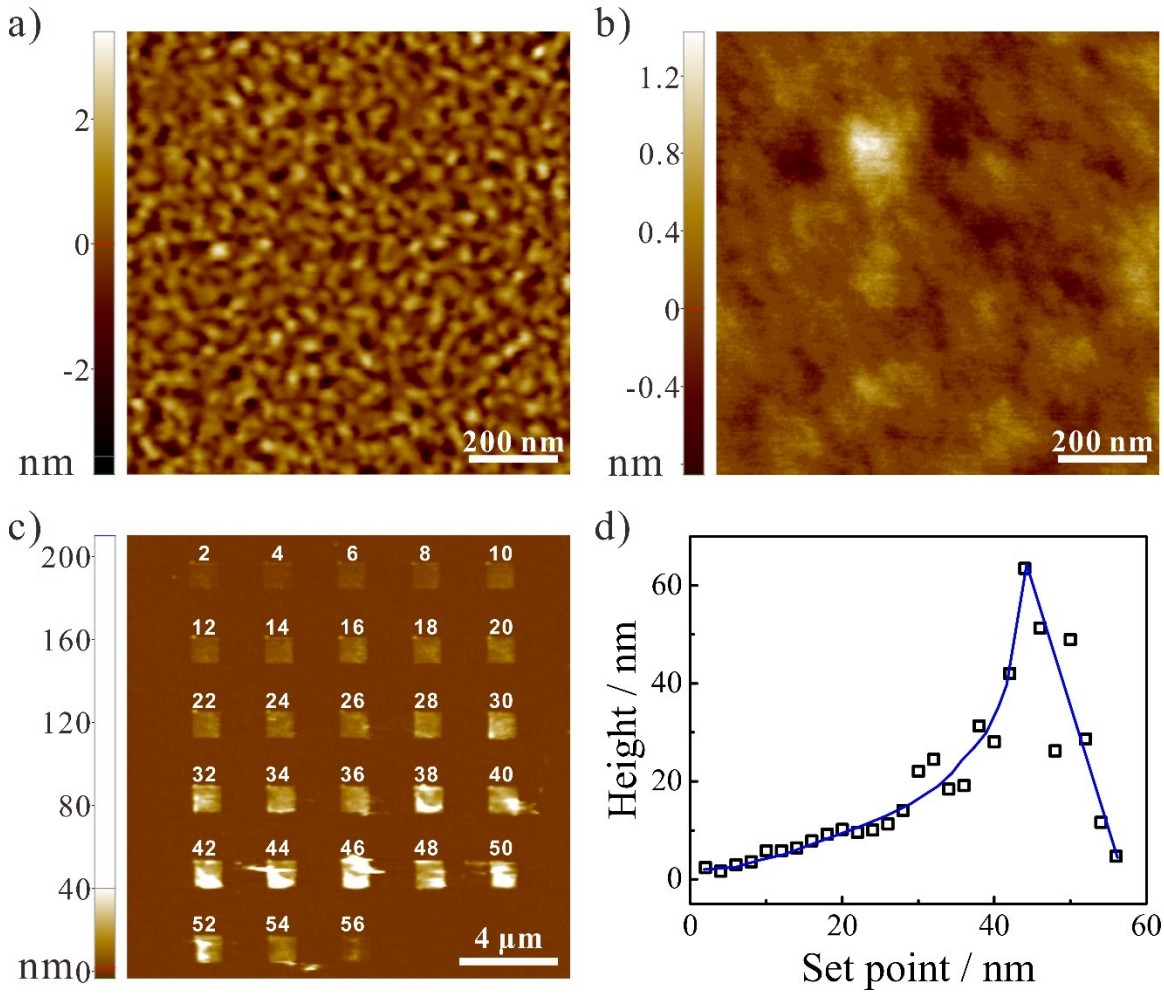

Figure S7. a) AFM topographic view of Au substrate obtained by non-contact AFM under PBS buffer. b) AFM topographic view of adsorption of lysozyme on Au substrate. c) AFM topographic view of patterned structure of fibrinogen fabricated by using the non-contact AFM to scan a series of 1 µm × 1 µm squares on Au substrate in fibrinogen solution with set point from 2 nm to 56 nm in top-to-bottom slow scan direction. d) Plot of the Height of square-shaped microstructures of fibrinogen versus the set point.

Figure S7 shows that L-NCSPL was used to fabricate the patterned structures of lysozyme and human plasma fibrinogen. When Au substrate was immersed under a

lysozyme solution (10 mg·mL^{-1} in PBS buffer), we found that lysozyme quickly adsorbs on the substrate and forms a densely packed layer (Figure S7b). Therefore, L-NCSPL is not suitable for patterning lysozyme on Au substrate. L-NCSPL was also used to fabricate patterned structures of human plasma fibrinogen on Au substrate. Non-contact AFM was used to scan a series of 1 μm × 1 μm squares with set point from 2 to 56 nm in top-to-bottom slow scan direction. As shown in Figure S7c, the patterned structure is successfully fabricated. The height of square-shaped structures increase as the set point increases from 2 to 44 nm, and then decrease with the set point larger than 44 nm (Figure S7d).

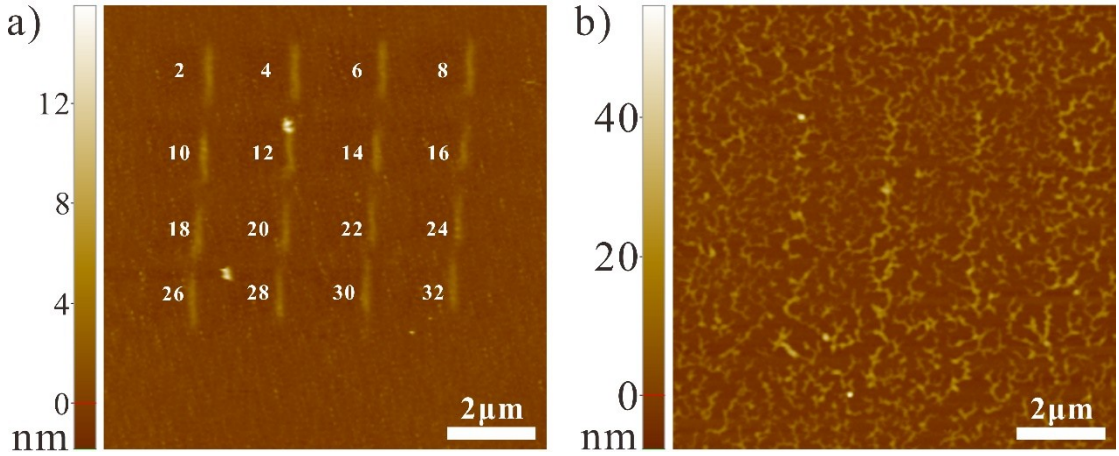

Figure S8. An AFM tip operated in the non-contact AFM was used to scan a series of 1 μm × 1 μm squares on1-Dodecanethiol modified Au substrate in BSA solution with set point from 2 nm to 32 nm in top-to-bottom slow scan direction. After the fabrication process, this area was immediately imaged with non-contact AFM in solution, as shown in a). The substrate was rinsed with sequentially with PBS buffer and water, and then dried under a stream of nitrogen. After that, the same area was imaged with non-contact AFM in air, as shown in b).

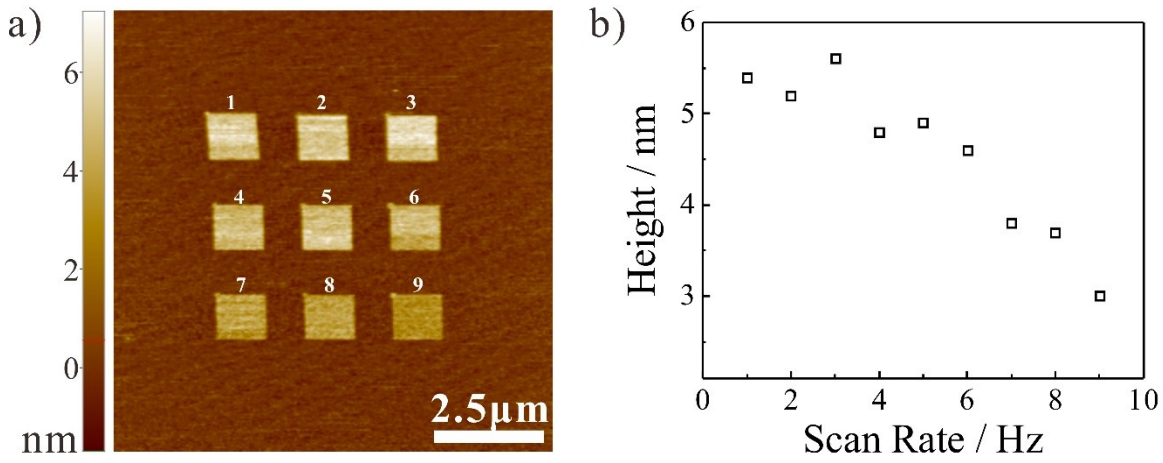

Figure S9. a) AFM topographic view of an array of 1 µm × 1 µm square-shaped microstructures of BSA fabricated by L-NCSPL with increasing the scan rate from 1 to 9 Hz. b) Change of height for BSA patterned structures as function of scan rate.