Protein printing with an atomic force sensing nanofountainpen

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We demonstrate the direct printing of proteins on a surface using a cantilevered nanopipette as the probe of a scanned probe microscope. Protein features as small as ~200 nm were directly delivered through the ~100 nm aperture of the nanopipette by simply contacting the probe with any surface. This allows for the direct connection of this methodology to standard separation techniques so that multiple proteins can be printed through one nanopipette at different locations in ambient conditions. [DOI: 10.1063/1.1594844]

The deposition and confinement of molecules in nanometric domains is a problem of considerable current interest. It is of particular importance when the molecules are of a biological nature, such as deoxyribonucleic acid (DNA) or proteins. The age of genomics and proteomics has triggered the development of the ''biochip'', an array of dots, each consisting of a small volume of molecules or dots consist of fragments of DNA, in a protein chip, the spots consist of various proteins. The biochip allows researchers to study the interaction of a very large number of molecules at once, on a single platform. This is a crucial requirement for processing the vast amount of information involved with the fields of genomics and proteomics. Reading of the chips is typically done using fluorescent probes.

Protein printing is a problem that has been investigated. The work of MacBeath and Schreiber,[1] showed that protein microarrays spotted using a conventional arrayer GMS 417 (Affymetrix, Santa Clara, CA) could be produced for high throughput screening with spot diameters of between 150–200 microns. This study highlighted the problem of the size of the arrays that would result from conventional techniques of protein printing (dot dimensions are of ~200 μm).

In an attempt to produce smaller features consisting of proteins, several articles have been published, using scanning probe microscopy (SPM) techniques to create dots of proteins on surfaces. They were based on earlier methods to deliver molecules to substrates. One of them, Dip-pen lithography (DPN) has been pioneered by Piner *et al.*,[2] and consists of dipping an atomic force microscope (AFM) probe in an ''ink'', and delivering molecules from the AFM tip to a solid substrate of interest via capillary transport. The other, ''fountain pen nanochemistry'',[3] is based on the development of cantilevered nanopipettes as AFM sensors,[4] and uses these nanopipettes to flow molecules to the substrate. In this work,[3] it was shown that such a nanopipette AFM sensor could act to write defined patterns with AFM control. This was demonstrated through the deposition of a chemical etchant, to chemically alter a metal film.

Recently,[5,6] DPN was used to print proteins on gold surfaces as has been previously demonstrated with much smaller molecules. In the first example,[5] the proteins were chemically modified with thiol groups in order to make a covalent linkage with the gold surfaces. In the second example,[6] the protein was not directly written but a small molecule was deposited first to which the proteins had an affinity and thus could absorb to these regions. A third example,[7] of biomolecule printing did not use standard AFM control but relied on working in a solution environment and used the ionic current between two electrodes, one in a straight pipette and the other in the solution, to allow for a feedback signal based on the ionic current. In this experiment, the solution contained in the pipette included solubilized biotinylated DNA that was ejected by the electrochemical current onto a streptavidin-coated glass surface.

We here show that protein solutions can readily flow through cantilevered nanopipettes using the methods of Ref. 3 using a scanned probe microscopy (NSOM)/SPM-100 Confocal System with cantilevered nanopipettes (Nanonics

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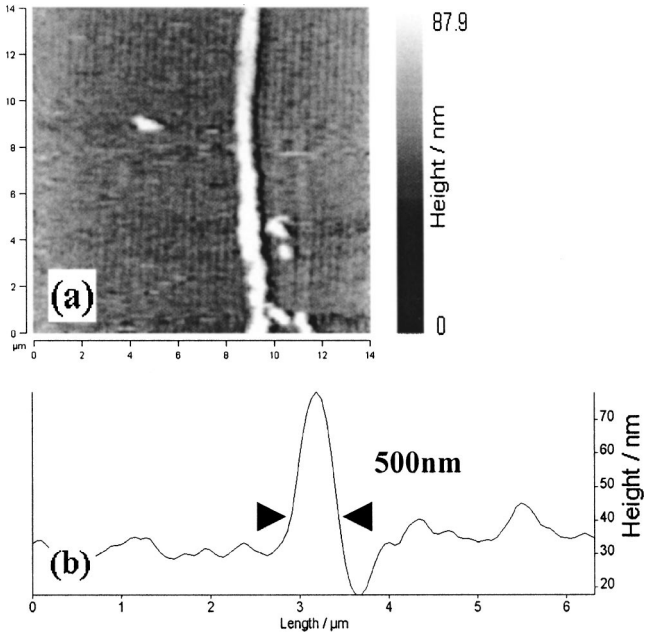

FIG. 1. (a) AFM topography image shows protein G line printed on Super-Aldehyde surface. (Scan area of 14×14 μm^2, line scan rate of 2 Hz, and AFM tip with resonance frequency of 125 kHz). (b) Line profile of the protein G line. The linewidth is ~500 nm and height of ~40 nm.

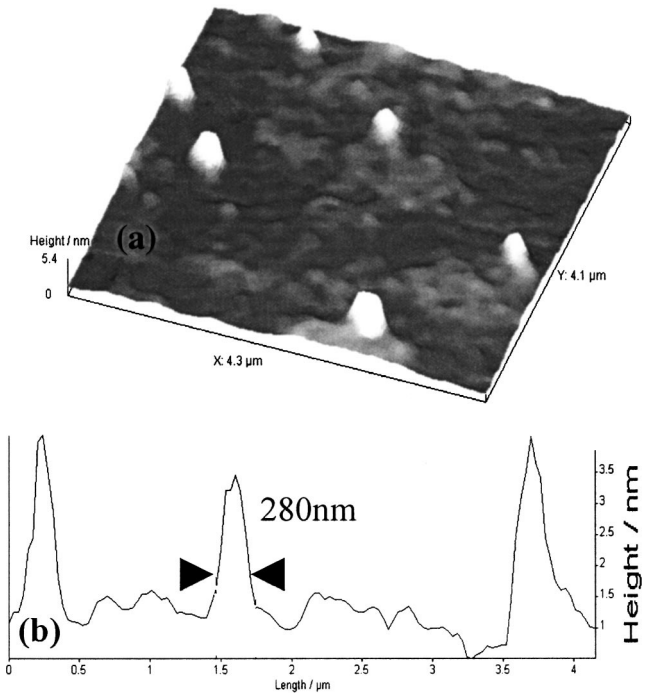

FIG. 2. (a) AFM image with 4.3×4.3 μm^2 scan area of GFP dots printed on a BSA substrate. (b) Line profile of the printed protein dots shows <300 nm width and ~2 nm height. Based on the measurement of the height it is possible that these are monolayer dots of GFP.

Imaging Ltd., Jerusalem) to print proteins in ambient environments under standard normal force AFM control without the need for external electric fields and liquid environments. The writing can also take place with little limitation on the surface character. Thus, in this study either a surface without the need for covalent linkage chemistry or the surface previously used by MacBeath and Schreiber[1] were employed. We have printed structures which are 1000 times smaller than those printed with the conventional methods of MacBeath and Schreiber.[1]

The advantage of our methodology over the techniques of DPN[5,6] or the ionic current feedback method[7] is that our technique can be readily connected to standard separation methodologies such as high performance liquid chromatography (HPLC) and can be used in ambient conditions without the need for liquid environments. The connection to HPLC instrumentation would allow the writing of dots of many different proteins with one cantilevered nanopipette connected to an HPLC system even in an air environment on standard substrates that have been used previously for protein printing. Furthermore, it is important to emphasize that the technique does not require multiple dipping of the tip and specific treatment of the substrate for protein printing.

As an initial experiment, our goal was to see whether the same conditions used in the report by MacBeath and Schreiber,[1] i.e., printing of ''protein G'', a yeast protein, onto aldehyde-coated glass slides[8] could be used at the scale of our experiments which was 1000 times smaller than this previous work. Furthermore, this investigation defined the chemistry of the system in which an aldehyde group on the surface reacts preferentially with the primary amino group of the N terminus on the protein to form a Schiff base linkage. A line of protein G printed on such a surface and imaged in intermittent contact mode is presented in Fig. 1. The linewidth is ~500 nm and the thickness is ~40 nm. The solution used for protein G was phosphate buffered saline solution 100 μM with 10% glycerol to keep the proteins hydrated.

The solutions for both proteins used for the protein printing in this letter were loaded from the large end of the pipette with a syringe, and capillary action rapidly sucked the solution to the pipette tip. The temperature for both systems was room temperature and the writing speed was approximately a line scan rate of 2 Hz. The humidity was not controlled although there is some evidence that such control of humidity could improve the writing in terms of dimensionality and uniformity.

Protein patterns formed on such surfaces were strongly linked and were not removed by washing the slides, and could be imaged repeatedly by using intermittent contact mode AFM without any disruption of the pattern.

Green fluorescent protein (GFP) is a fluorescent protein isolated from the Pacific jellyfish, *Aequoria victoria* which has an absorption at 490 nm and an emission at 509 nm. This allowed characterization of the printed line by both atomic force and near-field optical imaging. Unlike the case of protein G, in the case of GFP, a simple association with bovine serum albumin (BSA) is used for the printing. To demonstrate the ability to print a line even without any covalent linkage, we show a sequence of GFP dots on a glass slide coated with BSA. The dots have a diameter of ~250 nm, as shown in the cross section in Fig. 2. The GFP was also in PBS solution at a concentration 1.5 μg per μl.

We also show a line of GFP in Fig. 3, and its cross section in Fig. 3(c), showing its width to be ~450 nm. We stress the fact that no chemical bond is formed between the printed GFP and the BSA substrate (as may be the case for protein G on aldehyde substrates). However, the adsorption is strong enough to allow multiple scans of the area without

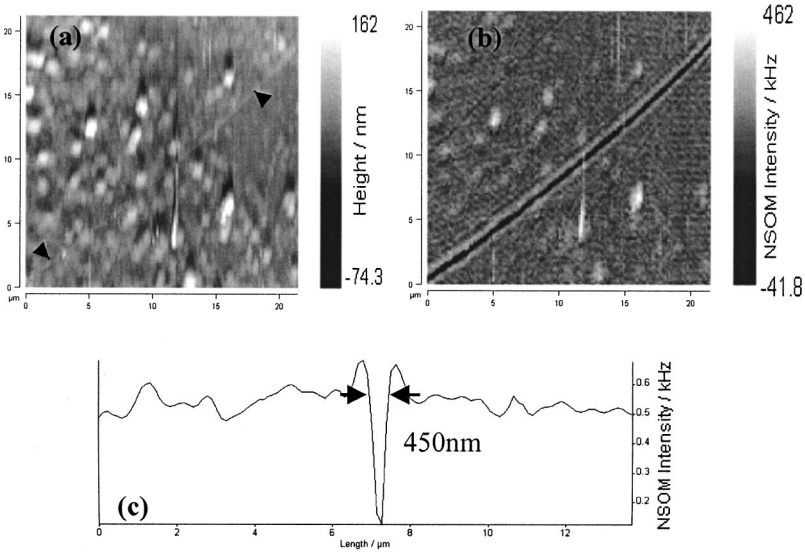

FIG. 3. (a) AFM topography image ($24.8\times21.6\ \mu m^2$ scan area in 1 Hz line scan rate) shows a GFP protein line printed on a BSA substrate. The line was imaged by using a cantilevered NSOM probe in noncontact mode with a resonance frequency of 125 kHz. (b) NSOM image of the protein line in (a) shows the absorption of the GFP protein with laser emission wavelength of 488 nm. (c) Cross section of the line in (b) shows an absorption line width of 450 nm.

alteration of the features. The BSA substrate is more corrugated than the aldehyde slides. In spite of this, the printed features are clearly visible when imaged with AFM.

We further characterized the printed GFP patterns using NSOM, which can be readily implemented on the very same NSOM/SPM-100 Confocal platform. Using a cantilevered NSOM probe, with a 100 nm aperture, we measured the 488 nm light from an Ar-ion laser, reflected from the sample. We show in Fig. 3(b) the result of such an experiment. The topography image [Fig. 3(a)] shows a dim line with a noisy background from the BSA substrate due to the less than flat BSA background in this case. The reflected light image, acquired simultaneously, is presented in Fig. 3(b). The GFP line appears as a black line across the image, due to the strong absorption at 488 nm of the protein. Also, most of the noisy background seen in the topography image does not appear here. The only feature that absorbs light is the GFP line and the background is brighter because the image, which was obtained using reflection mode, has more scattered light when there is less absorption and even more scattered light in regions which have a larger topography.

In summary, we present a very simple method for directly printing nanometeric patterns of proteins. It is shown that cantilevered nanopipette fountain pens can be used, without the need for flow control or environmental control mechanisms, to print proteins. Moreover, under conditions that have been previously used in protein printing protocols that use conventional spot arrayers, this method yields spots that are almost 1000 times smaller ($\sim$200 μm compared with $\sim$200 nm).

The printing process and the adsorption of the protein on the substrate does not seem to induce significant changes in the printed protein, as indicated by the fact that GFP does not lose its property to absorb light at 488 nm. In addition, we show that future nanobiochips can be read with NSOM, to overcome the inherent diffraction limit problem, posed by the small separation between the spots.

[1] G. MacBeath and S. Schreiber, Science **289**, 1760 (2000); P. Arenkov, Anal. Biochem. **278**, 123 (2000).
[2] R. D. Piner, J. Zhu, F. Xu, S. Hong, and C. A. Mirkin, Science **283**, 661 (1999).
[3] A. Lewis, Y. Kheifetz, E. Shambrodt, A. Radko, and E. Khatchatryan, Appl. Phys. Lett. **75**, 2689 (1999).
[4] S. Shalom, K. Lieberman, A. Lewis, and S. R. Cohen, Rev. Sci. Instr. **63**, 4061 (1992).
[5] D. L. Wilson, R. Martin, S. Hong, M. Cronin-Golomb, C. A. Mirkin, and D. L. Kaplan, Proc. Natl. Acad. Sci. U.S.A. **98**, 13660 (2001).
[6] K. Lee, S. Park, C. A. Mirkin, J. Smith, and M. Mrksich, Science **295**, 1702 (2002).
[7] A. Bruckbauer, L. Ying, A. Rothery, D. Zhou, A. Shevchuk, C. Abell, Y. Korchev, and D. Klenerman, J. Am. Chem. Soc. **124**, 8810 (2002).
[8] Aldehyde slides were purchased from TeleChem International (Cupertino, CA) under the tradename SuperAldehyde Substrates.