Nanostamp Optimization for Single-Molecule DNA/Protein Array Studies

Mónica M. López Martínez
Chemical Engineering, University of Puerto Rico-Mayaguez Campus

REU Program: 2017 Cornell NanoScale Science & Technology Facility
Research Experience for Undergraduates (CNF REU) Program
CNF REU Principal Investigator: Dr. Michelle D. Wang, Laboratory of Atomic and Solid State Physics, Howard Hughes Medical Institute, Cornell University
CNF REU Mentors: Ryan Badman, Laboratory of Atomic and Solid State Physics, Cornell University; Dr. James E. Baker, Laboratory of Atomic and Solid State Physics, Cornell University
Primary Source of CNF REU Funding: National Science Foundation via the National Nanotechnology Coordinated Infrastructure (NNCI) Grant No. ECCS-1542081
Primary CNF Tools Used: JEOL 6300 e-beam lithography, Oxford Cobra etcher, AFM, SEM
Contact: monica.lopez8@upr.edu, mdw17@cornell.edu, rpb226@cornell.edu, jeb94@cornell.edu
Website: http://www.cnf.cornell.edu/cnf_2017reu.html

Abstract:

Over the past two decades, biophysical single molecule DNA, RNA and motor protein studies have demonstrated the important role that the structural and mechanical properties of single molecules play in gene replication and expression. Specifically, genetic processes can be significantly affected when DNA experiences torque or protein interaction forces, which happens often *in vivo*. Single molecule DNA studies typically "tether" DNA between a protein anchor on a surface, and a microbead in solution, that can be twisted or pulled with optical or magnetic tweezers. Nanostamping of protein spot anchor arrays, compared to blanket coating a substrate with protein for DNA anchoring, aids in single molecule studies by precisely controlling the DNA anchor position. This improves resolution and throughput of the technique. Thus, we are exploring a nanostamping method called the "Ink Subtract Print" method by optimizing the dimensions of electron beam-patterned nanostamps that can selectively pattern arrays of 100-300 nm wide circles of protein on a glass surface.

Summary:

Introduction. The deoxyribonucleic acid molecule (DNA) has a double helix structure with unique mechanical properties that subsequently influence the way that other proteins interact with DNA to achieve complex biological functions of transcription and replication.

Our lab is interested in studying the interactions between DNA and motor proteins that are present in DNA processing activities. Although DNA can be macroscopically long, it is only 2 nm wide and cannot be visualized with light microscopy. DNA is therefore tethered between a microsphere handle and microscope slide for observation and manipulation.

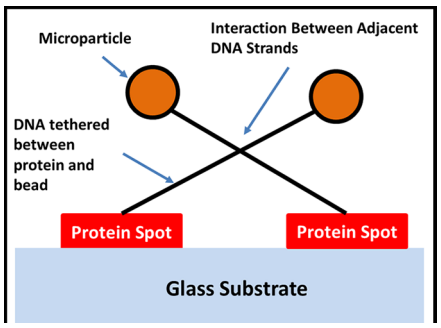

Figure 1: Schematic of protein-DNA-bead tethers.

To obtain organized observations of these microscopic molecules and investigate interactions between a protein and multiple DNA strands, we precisely placed the position of the separate DNA molecules on a glass slide so that the distance between strands was well-defined. Specifically, we patterned a protein surface array using the Ink-Subtract-Print (ISP) nanostamping method [1], so the DNA could be anchored on the surface at one end of the DNA molecule with high spatial precision. (Figure 1).

Ink-Subtract-Print Method (ISP). This protein patterning procedure consists of inking a PDMS square with protein to cover the whole surface, and then stamping the square on the patterned silicon template to remove the protein everywhere on the PDMS except in the array of small circles [1]. This subtractive step is physically possible because plasma cleaning the silicon template gives hydrophilic properties to the wafer. Since the protein on the PDMS is also hydrophilic, it will be attracted to the top surface of the subtraction template wafer.

When the PDMS is pulled away, the protein will be attached to the wafer in the non-patterned areas. In contrast, the PDMS will retain protein where the silicon wafer had holes. The patterned protein spots on the

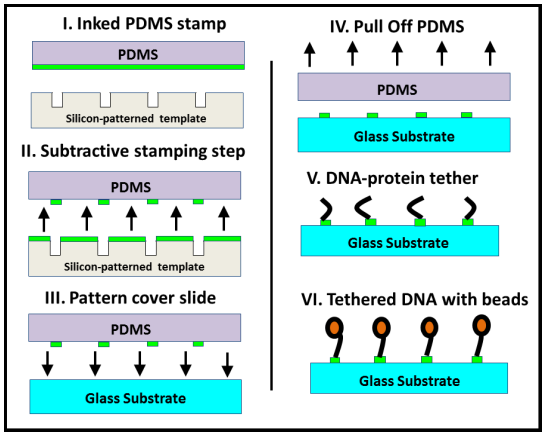

Figure 2. Ink-Subtract-Print stamping method.

PDMS after subtraction are, then, stamped or "printed" on a plasma-cleaned glass surface which will be the area used to perform the DNA-binding experiments. (Figure 2)

Nanofabrication of Patterned Silicon Template Method. Our main goal thus was to create patterned silicon templates containing periodic hexagonal arrays of 100-300 nm circular holes spaced at a 3 µm pitch that could be used as a subtraction template (Figure 3a) to produce patterned protein spots on PDMS (Figure 3b).

To acquire the target pattern needed to perform the ISP stamping procedure on our silicon wafer, the first step was to expose a silicon wafer coated with ZEP520A resist using the JEOL JBX-6300FS 100kV electron-beam (e-beam) lithography system. Once the wafer was exposed and developed, the Oxford Cobra inductively coupled plasma (ICP) etcher was used to etch the silicon wafer according to the e-beam pattern. To remove the ZEP520A and obtain our final cleaned, patterned nanostamp template, we used a bath of 1165 organic stripper for approximately four hours. Finally, we obtained four 1 cm × 1 cm stamp areas patterned on each wafer.

Results:

After four hours of DNA incubation time (13.7 kbp DNA, 25 pM), we observed an average of 10 tethers in each microscope field of view, which contained ~ 1,500, 150 nm wide protein spots. To increase the number of tethers, the incubation time was increased to 17 hours and yielded ~ 50-100 DNA-protein tethers per field of view. By increasing anchor protein spots to 300 nm in diameter, we were able to obtain more tethers in a shorter amount of time compared to 150 nm wide protein spots. An analysis of these results shows that since the area of each protein anchor spot is small compared to anchor spots described in previously published reports [2], it will take more time for the DNA to bind to these small features (Figure 4).

There are several ways to improve the number of DNA tethers in the future work: (1) Increase DNA concentration (2) tether DNA to magnetic beads and place a magnet under the glass so the DNA is more likely to dwell near the cover slide surface and thus more likely to bind to the protein anchor or (3) test larger stamp circles.

References:

[1] Coyer, Sean R., Andrés J. García, and Emmanuel Delamarche. "Facile Preparation of Complex Protein Architectures with Sub-100-nm Resolution on Surfaces." Angewandte Chemie International Edition 46.36 (2007): 6837-6840.

[2] De Vlaminck, Iwijn, et al. "Highly parallel magnetic tweezers by targeted DNA tethering." Nano letters 11.12 (2011): 5489-5493.

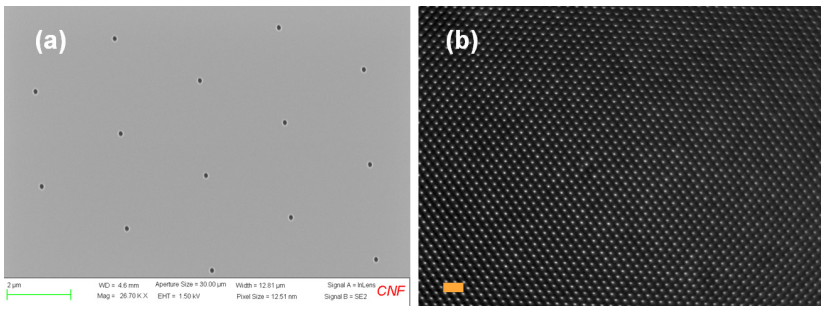

Figure 3: (a) A scanning electron microscope image of the silicon template with a hexagonal array of holes for subtractive stamping, scale bar is 2 µm. (b) A fluorescent microscope image of the final protein array on glass, scale bar is 6 µm.

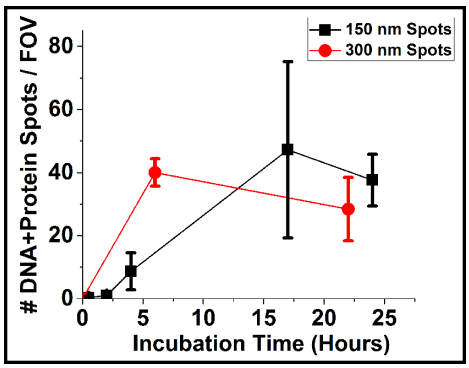

Figure 4: The number of DNA tethers bound to protein anchors per field of view (FOV) under a fluorescent microscope, per hour of incubation, using (1) 150 nm wide protein anchors and (2) 300 nm wide protein anchors.