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Recent advances in molecular lithography

Michael Norton[a], Mashiur Rahman[a], B. Scott Day[a], Chad Huffman[a], Huan Cao[a], David Neff[a], Heather Butts[a], and Aaron Gin.S[b].
[a] Department of Chemistry, Marshall University, Huntington, WV 25755 USA;
[b] Center for Integrated Nanotechnologies and Sandia National Laboratories, Albuquerque, NM 87185 USA

ABSTRACT

The precise placement of molecular and nanoparticle species at predetermined locations on a substrate surface remains a current challenge. Some barriers are particularly relevant to soft matter such as biomolecules. The advent of DNA Origami, invented by Rothemund, provides partial solutions to some challenges while raising new challenges. In this paper, two particular levels of molecular placement will be discussed, associating large DNA based molecular nanostructures with traditional lithographic nanostructures and the association of molecular scale species with particular locations within large Origami structures. Typical plasmid based DNA Origami nanostructures are approximately 100 nm in diameter. This size scale closely matches that of gold nanoscale structures which are readily produced using e-beam and other lithographic techniques. The strategy for associating large DNA based nanostructures with these lithographic structures employs the placement of thiol terminated DNA molecules within the molecular assembly, positioned to allow tethering of the biomolecular nanostructure to the substrate through gold-thiol bonds. Although a number of soft chemistry mechanisms can be employed to associate DNA molecules with with substrates, the use of the origami constructs as substrates suggests that single stranded DNA provides the optimum attachment strategy. A solid state asymmetric PCR process for ssDNA fabrication is therefore described and demonstrated. Structures generated with the three tiered attachment strategy described here are amenable to characterization and assembly verification using AFM and NSOM. While a complete convergence of top down and bottom up approaches cannot be claimed, it is clear that the practice and methods of molecular lithography are rapidly advancing.

Keywords: Sequential self-assembly, origami, molecular lithography, nanoarchitectures, nanolithography, nanosensors, sensors, solid state asymmetric PCR.

1. INTRODUCTION

As nanoscale devices for the detection, recognition and signaling of single molecules are developed, methods will have to be developed for individual characterization and quality control of these devices. The development of testbeds which can isolate and assist in the interrogation of single devices or arrays of these devices currently presents a challenge. There are at least two requirements driving the development of such testbeds. One is the simple desire to obtain, in addition to individual performance characteristics for the devices, yield data for the devices. A more complex driver for the fabrication of arrays is the case in which current technology does not provide sufficient sensitivity to detect the signal from a single device, therefore requiring, at this time, the fabrication of large scale device arrays from which ensemble responses can be detected. It is reasonable to assume that the nature of the array substrate will strongly influence the range of chemical and physical methods which can be used for both the interrogation and for the fabrication of the devices. Particularly one can anticipate that future nanostructure fabrication may resemble silicon fabrication in a number of ways. Although silicon technology is often described as a process of sculpting, or removing materials, the sum product is actually additive, bit by bit structures are built up on top of a base structure which is solid. At this time, soft matter based organic molecular nanotechnology has been for the most part liquid based. It is the thrust of this paper to describe the generation of array substrates compatible with the next generation of soft nanostructures. In this introduction, an overview of the tremendous steps already made in DNA based molecular lithography will be presented, followed with a description of the assemblies motivating the current efforts in this laboratory.

It is important to recognize that the current level of development of nanostructures based on DNA derives from the significant foundational contributions to the understanding of design and fabrication of DNA based structures provided by a set of pioneering researchers. These contributions include the generation of arrays from small block structures

(Winfree and Seeman) (1), the generation of networks (Mao (2)), the integration of larger blocks into both extended systems (Kiehl (3) and into finite systems (Dwyer (4)) and the production of even larger constructs via a method termed origami by Rothemund (5). This short, incomplete list of foundational publications is meant to provide an understanding of the progressive evolution of the field.

If a consensus definition of lithography is that it is "a writing process", then molecular lithography will for the purposes of this paper address both the placement of molecules on surfaces in designed patterns, and the writing of molecules on other, substrate, molecules. Braun (6,7) has presented a pathway to the production of active devices, nanotube transistors, using a novel, RecA protein based additive lithography. Briefly, in solution, a RecA covered single stranded DNA (ssDNA) molecule is deposited, in a sequence specific manner, on a lengthy double stranded DNA template due to the sequence recognition properties of RecA-ssDNA complexes. Anti-RecA antibodies are then attached, after which multiple biotinylated secondary antibodies bind to the complex, providing docking sites. Nanotubes are prepared for attachment through nonspecific adhesion of a bridging molecule, streptavidin, each of which can bind up to 4 molecules of biotin with high affinity. When contacted in solution, the carbon nanotubes are bound to the "substrate" DNA molecule through a bridge of nonspecifically bound streptavidin/biotin/antibody2/antibody1/RecA-ssDNA complex. After the entire nanotube/protein/DNA complex is deposited on a silicon substrate, the DNA substrate strand not covered with the RecA complex is metallized using selective silver, then gold deposition. Upon establishing electrical contact to the metalized leads, transistor function was then demonstrated using the silicon substrate as a gate electrode. One characteristic of this entire, marvelous assembly process is that all steps are additive, ie each layer is "written" on prior layers, and there are no etching steps.

It would be advantageous to perform the majority of the steps involved in this assembly on a tethered DNA substrate. This approach would enable not only the production of multiple identical device copies simultaneously, but also the potential for cross reactions could be eliminated due to spatial isolation of the species involved in the reactions. This is particularly important in the case of use of the biotin/streptavidin (biotin/SA) interaction. This interaction is one of the strongest non-covalent interactions known and it is therefore a central tool for fabrication. The multivalency of the streptavidin enables it to cross link, or bridge more than two biotin tethered species. In many cases one might seek to use the same biotin/SA linkage for two completely different steps. In substrate immobilized conditions, the ability to rinse away materials between reactions enables such sequential assembly using the same components. "Tool reuse" is an important concept in additive molecular lithography and is only possible if cleansing/purification/isolation steps can be employed. Attention in our laboratory has been directed toward immobilization of DNA substrate strands to enable sequential assembly.

In order to produce an array of devices, it would be necessary to first generate an orderly array of assembly sites. A schematic diagram of one potential model assembly site is produced in Figure 1. Not shown is the lithographically produced metal attachment site necessary to bind it to a substrate (e.g. silicon, silicon oxide, gold, etc). The disk in Figure 1 represents an example of an adapter structure, a macromolecular assembly sufficiently large to bridge the gap between the size of readily available microfabricated noble metal attachment sites and yet capable of binding a single (DNA) substrate molecule. The molecular assemblies which appear to readily span the necessary size range are the molecular origami constructs of Rothemund (5). The large disk construct would require self-assembly of attachment sites capable of binding to microfabricated structures on one side (e.g. gold thiol bindings) and the other side of the disk would provide a mechanism for forming a strong bond with a single DNA molecule. In a sense, three levels of platforms must be produced in order to generate a localized double stranded DNA substrate, a metal foundation, a scale spanning molecular adapter and the double stranded DNA molecule itself.

The remainder of this paper addresses these three crucial areas at the micro-nano interface. Production of gold based attachment sites utilizing soft lithography and e-beam lithography, evaluation of the relative suitability of these attachment sites for origami based bridges across size scales and the relatively large scale production of the single stranded DNA macromolecules required in DNA based molecular lithography.

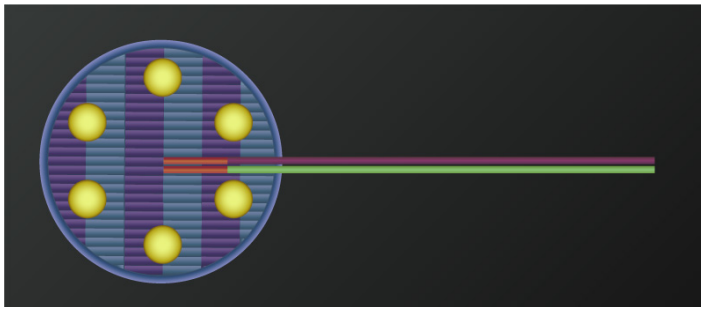

Fig. 1. Schematic of molecular adapter structure. Large disk represents an origami construct, attached to array sites via smaller disks which represent potential surface binding moieties (e.g. thiols). The long horizontal cylinders represent DNA molecular substrates for additional lithography.

2. METHODOLOGY

2.1 Attachment site fabrication via soft lithography

Following methods modified from reference 8, a one dimensional grating style polydimethlysiloxane (PDMS) stamp with a pitch of 1.66 microns was used to generate a two dimensional array of bare gold spots. The overall procedure, including a silver development step, is diagramed in Figure 2 below. The PDMS stamp was rinsed with ethanol twice before use. The gold on glass substrates were purchased from EMF (Evaporated Metal Films, Inc.) and the gold surfaces were cleaned with piranha solution for one hour pattern application. (Caution: Piranha solution is highly reactive and should be prepared and used with appropriate safety equipment and disposed of soon after use.) The PDMS stamp was inked with hexadecanethiol (~50 microliters of 1 mM hexadecanethiol in EtOH per square centimeter of stamp is allowed to coat the surface, then the surface is dried with a stream of nitrogen) and used immediately to stamp the gold surface in two orthogonal directions.

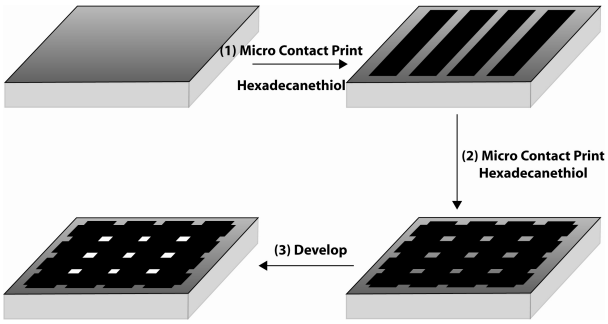

Fig. 2. Schematic of the two step process for soft lithography prepation of patterned surface, which is followed by a silver development step to disclose the pattern.

The exposed gold surface was disclosed using a silver enhancement kit (# 15718 from Ted Pella, Inc.).

2.2 Attachment site fabrication using e-beam lithography

Standard e-beam methods were employed to generate 10X10 arrays of gold dots with a pitch of 5 micrometers in the x (horizontal) and a 6 micrometer pitch in the y (vertical) direction on the silicon/native silicon oxide surface. Briefly, a JEOL JBX-5FE electron beam lithography system was used to expose 250nm thick 495K C4 polymethlymethacrylate

(PMMA) on silicon substrates. Various electron doses were used to slightly vary the size of the patterned dots. Development was performed in 1:3 MIBK (Methylisobutylketone) : IPA (Isopropyl Alcohol) for 60 seconds. Using an electron beam evaporation system, a 20Å Ti layer was deposited followed by a 20Å Au layer. The metal was lifted off by soaking in acetone. Inspection using SEM revealed dot diameters around 100nm and profilometry confirmed the ~40Å metal layer thickness on a witness sample. Samples were then separated into chips using a dicing saw. Chips were rinsed vigorously with acetone before characterization via AFM surface imaging.

2.3 Production of DNA Origami Shapes

The assembly of the three holed disk origami construct was performed using the protocols developed by Rothemund (5)

2.4 AFM Characterization/Imaging

The AFM characterizations were performed using a Nano-R AFM (Pacific Nanotechnology, Inc.) operated in the close contact (tapping) mode. P-MAN-SICC-0 AFM cantilevers were used with a nominal tip radius of ~ 10 nm. The raw data were processed using Nano Rule Version 2.17 software (Pacific Nanotechnology, Inc.)

2.5 Asymmetric Synthesis of Single Stranded DNA

2.5.1 Target sequence

The 582 bp sequence targeted for amplification is part of a 3167 bp larger strand described in more detail in reference 9. Briefly, the larger sequence, a 1 micron long strand of DNA, is comprised of several subsections. The 582 bp long subsequence functions as a linker to attachment sites and as a standoff to isolate other parts of the long strand from the metallic attachment sites. The 582 base long single stranded construct is intended to serve both as a method to increase the mechanical rigidity of this standoff domain of the larger construct and to block interaction of bases in this region with other single stranded sections of the construct. The sequence for the 582 base target is:

(ATTGGTTATCCGGCTTTAGCCGTTTTAGGGAATATTTAGTTTTCTTATCTGGCTCTATCCCAACTCACAACA
AGGTCAAACCTTGTTCTCAGGTGATAATTTCTTGCACCTGAGGTTGCAGTTTCCCGCTTTTTGGCAGATAGT
CCCGCTACCGGGTGATGCACTTGGTAGTGGGATTAGTTCAAAAAACCCCAGCTCCACGGCATTTCGTGATT
TAGCCTTGGGATTTCCCTCGGGGGCTAAATCTCGAACTGCCCCTTTCGGCCGCTTGCACCGCTCTTTCCTTC
CCTTCTTTCGCTTTCCTCGCCCGCGATCCCGCGACCGTTCACATCGCCAGTGCGACGCGCATTGGTGGTGTG
GGCGGCGCGAATTACGCGGCGATGTCCCGCGCAGGTAAGCGGTAAGTCCGACGCGTTGACAACCCTTCCC
GCTAGCCACGCCCGGAGAAGCGATAATGCGGTCGACCGCTTTCCCCCTACACGACGTTCCGCTAATTCAAC
CCATTGCGGTCCCAAAAGGGTCAGTGCTGCAACATTTTGCTGCCGGTCACTTAACATTATGCTGAGTGATA
TCCCGCTTAAG).

2.5.2 Generation of double stranded singly biotinylated 582 bp template strand

PCR (the polymerase chain reaction) was performed using 25µL of 2X SYBR Green PCR Master Mix from Applied Biosystems (ABI), 3µL of a 10 micromolar solution of each of the primers (forward primer IT193MN (sequence: 5'biotin-TAACCAATAGGCCGAAATCGGC AAA) and reverse primer IT197MN (sequence: GAATTCGCCCTATAGTGAGTCGTAT), 0.75µL of 140 ng/µL PGEM plasmid as template, and 18.25µL of autoclaved distilled H_2O to bring the reaction volume to 50µL total. Thermal cycling conditions were: 2 min at 95° (initial denaturation), and 20 cycles of the following: 20 sec at 95°C, 20 sec at 60°C, and 45 sec at 72°C on an ABI 7300 Real-Time PCR system. After cycling, PCR product was removed from remaining primers using a QIAquick PCR Purification kit (Quiagen). The product concentration was estimated from the absorbance value measured at a wavelength of 260 nm. The yield determined for the PCR reaction was approximately 20 pmol.

2.5.3 Attachment of DNA to magnetic beads

0.2 mg of Dynal Dynabeads M-280 (bead diameter is 2.8µm) streptavidin coated beads from Invitrogen were washed and bound with 20 pmol of biotinylated 582 bp DNA following the manufacturer's protocol. The incubating step was extended to 45 minutes to maximize binding. The DNA carrying beads were magnetically separated from the solution and the supernatant was pipetted away.

2.5.4 Asymmetric PCR on magnetic beads

The DNA-beads were resuspended in 156 microliters of PCR solution and divided into three tubes. Each tube then contained 37.75µL autoclaved distilled H_2O, 5µL 10X PCR Buffer (Mg^{2+} free), 3µL 25 mM MgCl2, 3µL dNTP mixture (2.5 mM of each), 0.25µL Taq DNA Polymerase (all of the above reagents from the PCR Amplification Kit, TaKaRa Bio), 3µL IT197 primer, and the biotinylated 582 bp DNA template on magnetic beads. Each of the three tubes were subjected to the same reaction conditions, only varying in the number of PCR amplification cycles (three, six, or nine cycles). These conditions were initial denaturation of 95°C for 1 min, then cycling through 20 sec at 95°C, 20 sec at 60°C, and 45 sec at 72°C on an ABI 7300 Real-Time PCR system. 2 microliters of the products taken from each tube were separated by electrophoresis on 1.2% agarose Flashgels (Cambrex) and photodocumented using a MiniBIS Pro gel imaging system (DNR Bio-Imaging Systems). A FlashGel ladder (Cambrex) was used as a calibration standard, with bands at 100,200,300,500,800,1250,1500,2000,4000 bp.

3. RESULTS AND DISCUSSION

3.1 Attachment site fabrication via soft lithography

The surface roughness of the 100 nm thick gold film on these glass substrates is sufficient to obscure the thiol patterns created using the soft lithography method. The patterns are readily disclosed using a silver development kit. Such kits are designed for use in microscopy for developing traces of metallic silver or gold and can be expected not only to highlight the areas intentionally left uncovered, but to also amplify any metal surface exposed by defects in the thiol masking layer. An atomic force micrograph of a typical product resulting from silver development of an attachment site array fabricated using soft lithography is shown in figure 3**.** The attachment or anchoring sites appear to be on the order of 200 nm in diameter. The shapes of these features may partially reflect the shape of the probe tip due to convolution. It should be noted that the silver decorations are only for visualization purposes. It should also be noted that these structures are extremely fragile, and the variation in height observed may be due to particle dislodgement during processing.

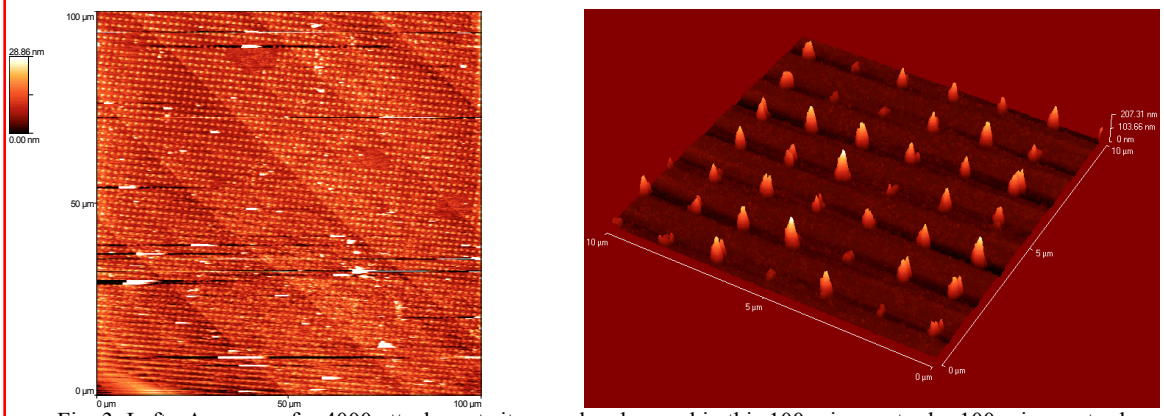

Fig. 3. Left: An array of ~ 4000 attachment sites can be observed in this 100 micrometer by 100 micrometer low resolution AFM image (z scale 28 nm). Right: Higher resolution AFM image of array disclosed using silver development kit (z scale 207 nm).

There are several advantages to this fabrication technique relevant to sensors and particularly relevant to molecular sensors. Because the thiol resist mask produced via the soft lithography technique is very thin (~2.3 nm (ref 10) the geometry of the attachment site itself(recessed by 2.3 nm) will not necessarily interfere in imaging processes because the site is only recessed by 2.3 nm. This advantage could not be realized in this particular set of samples because these samples are relatively rough. However thinner, smoother thin films can be prepared. A second advantage of the soft lithography method is the small number of steps involved in fabrication of an array of uncovered gold spots. This naturally leads to a smaller number of processing defects. A third advantage is the compatibility of this preparation method with surface plasmon detection techniques, which require the use of thin precious metal films. One of the greatest advantages of the method is the highly parallel nature of the process. Large surface area substrates can be covered with nearly identical sites. This advantage is most relevant to the characterization of large numbers of nanosensors.

3.2 Attachment site fabrication via e-beam lithography

An example AFM image of attachment sites produced using e-beam lithography followed by evaporation and liftoff processes is provided in Figure 4.

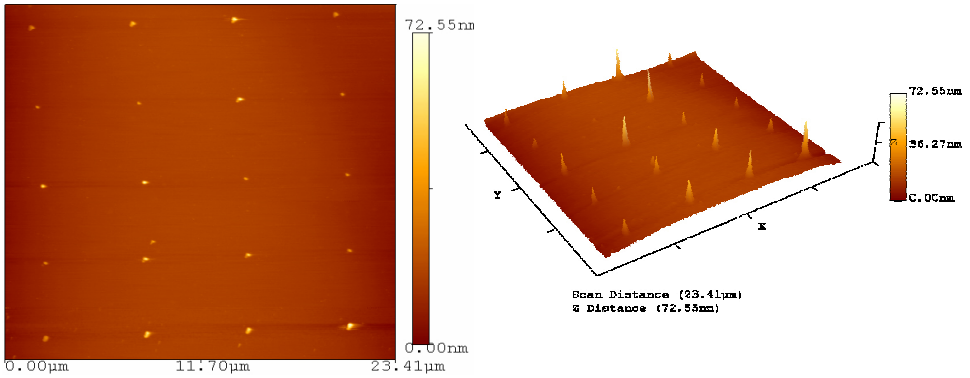

Fig. 4. Left: Top view AFM image of representative section of 10X10 dot array (23 micrometer by 23 micrometer image, z scale 73 nm. Right: Side view of same spot array.

When contrasted to the soft lithography approach employed above, the e-beam method has many advantages. The most important advantages include ready control of pattern dimensions (including departure from regular arrays), control of spot dimensions and control of attachment site height. However, some variation in apparent height of the sites is can be seen in the side view image of Figure 4. Although this could be due to handling, tip or contamination artifacts, the attachment site height does present another variable requiring control during fabrication. The importance of control of this parameter is dependent on whether molecular lithography is to be performed on the top of the attachment sites or between attachment sites. An extreme advantage of the e-beam method is that the silicon surface roughness is entirely decoupled from the metal attachment site dimensional control process. Silicon processing can readily achieve near atomic level smoothness and a very low level of roughness can be preserved though the lithographic process. In regions between these dots, RMS roughness on the order of 0.4 nm has been measured using AFM. This level of surface quality is necessary if fine structure within DNA architectures are to be observed routinely using scanning probe techniques. The minimum achievable size of a gold attachment site which will display significant stability on a silicon surface in long term contact with salt/buffer solution remains to be determined. However the optimum size would be 2 nm in height or less, equivalent to the diameter of double stranded DNA, since this would provide minimal interference in scanning probe characterization of the attached species. The lower limit for readily producible TiOx adhesion layer/gold

top layer structures is unlikely to fall into this size range due to limits in film continuity. It is possible that attachment sites composed of very thin discontinuous films will be sufficient for some applications. This may be particularly true in cases where the molecules to be attached are unusually large, as are the origami species discussed below.

3.3 Origami Structures

The finite, two dimensional, DNA based nanostructures generated by Rothemund (5) represent an extreme example of macromolecular engineering. AFM images of example structures, containing three pores, are shown in Figure 5.

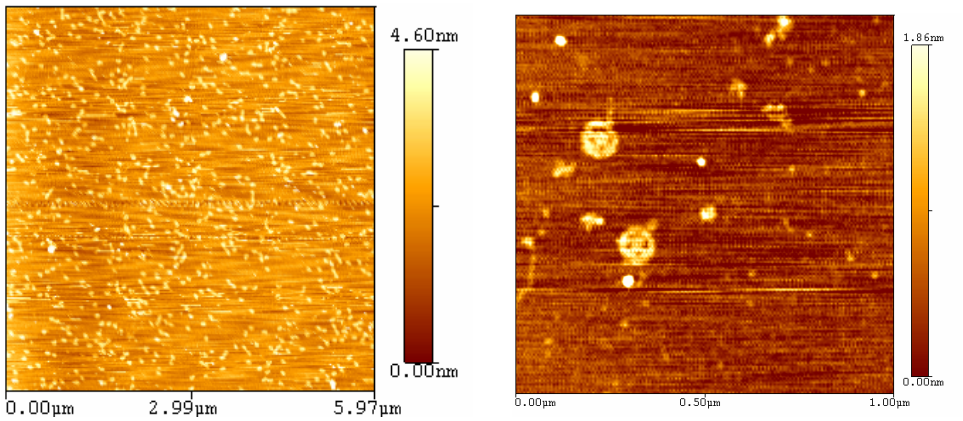

Fig. 5. Left: AFM image of 100's of origami disks on a mica surface (5.97 micrometer X 5.97 micrometer view, z scale 4.6 nm) Right: High magnification AFM micrograph of disks (1 micrometer X 1 micrometer view, z scale 1.87 nm).

These porous disks, with a diameter to ~100 nm, have a footprint of 31,000 square nanometers. Several characteristic features common to nanostructures prepared via solution phase synthesis are immediately apparent. Apparent in the left image is the fact that large numbers of structures are readily produced from a homogeneous solution self assembly process. Considerable surface debris is apparent in the right image. Dry AFM imaging requires a compromise between rinsing the sample enough to remove most of the debris (100X as many DNA fragments are included in the reaction mix than necessary for self assembly, the remainder of these strands must be removed before imaging) while not rinsing so vigorously that the disks themselves are removed. Although these structures would appear to be ideal substrates for the surface assembly of significant numbers of nanoscale components, there is no control over origami placement on the substrate surface, no control over angle of orientation or even control of top face up vs bottom face up. Although the metal attachment site approach discussed above for nanostructure immobilization does address the first two issues, control of the rotational angle is expected to be less readily achievable.

If the origami structure is to be used as a substrate for further lithography, requiring verification via imaging, a lithographic technique capable of providing a near atomically flat surface may be required. The apparent thickness of these origami ultrathin films is on the order of 1 nm. It has not been possible for us to determine the surface roughness of the top of the gold pillars generated via ebeam lithography. It may be assumed, however, that thin metal films sufficiently smooth to enable topographical imaging can be produced and that this smoothness can be maintained through the soft lithography process. In contrast, if the origami structure will simply serve as an adapter and attachment point, the flexibility of patterning afforded by e-beam lithography may be advantageous.

3.4 Attachment strand fabrication via solid phase asymmetric PCR

Origami structures are composed entirely of DNA. The component strands are all single stranded DNA. ssDNA is not readily produced. Chemical synthesis is generally limited to molecules 100 bp and less in length. Pure samples of single

stranded molecules longer than this have until recently required the employment of the methods of biotechnology, growing the molecules in living cells, then performing complex separation processes in order to isolate them. The technique of solid phase asymmetric PCR amplification of DNA has recently been employed to generate ssDNA for diagnostic purposes (11). Here we describe larger scale experiments for the purpose of generating quantities sufficient for use in nanofabrication. Particularly, the 582 base sequence used as an example target strand in this discussion will be used for securing larger sequences of DNA to origami structures. Similar sequences could be woven into the fabric of origami structures to provide the strong binding sites required to bridge between microfabricated structures and single DNA molecules.

Figure 6 presents the results of gel electrophoresis experiments which indicate significant production of ssDNA using the solid phase method. The 582 base target is represented by the bright band in the center of lane 3. In lanes 4, 5 and 6 the increasing intensity of the similarly central band indicates an increase in product concentration. The disproportionate increase in intensity of a band above the target band indicates the growth of a side product, ascribed to concatamers. Under the conditions employed here, it would appear that sufficient product for experimentation could be obtained with as few as three thermal cycles.

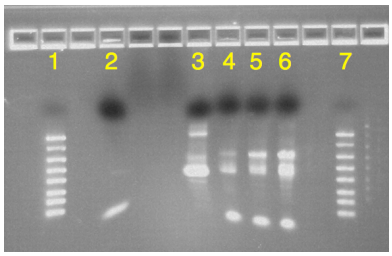

Figure 6 Image of gel used for electrophoresis of DNA products. Lanes 1&7, ladder with bands at 100,200,300,500,800,1250,1500,2000,4000 bp). Lane 2 contains solution from the beads with no thermal cycling, Lane 3 contains the target (582 construct) and the 3167 bp template, Lanes 4, 5 and 6 contain the products from 3, 6 and 9 thermal cycles, respectively.

4. CONCLUSION

The selection of substrate and metal binding site fabrication method must be performed with recognition of the impact these choices will have on the ease with which devices can be characterized and on the functionality of these devices. The e-beam and soft lithography approaches provide extremely different surface topographies for attachment of nanostructures. Two other competitive methods for the fabrication of large regular arrays of metallic binding sites have not been addressed here, interference lithography (12) and microsphere lithography(13,14). At this time it would appear that both of these methods would lead to the preparation of metallic binding sites with morphologies most closely resembling those resulting from e-beam lithography. E-beam, interference and microsphere lithograpy can be expected to find broadest use in addressing tethering requirements, where the binding site is for simple attachment, rather than acting as a platform to support multiple levels of lithography, in cases where high resolution imaging is required for process development.

Although many applications may be addressable through the use of origami, hybrid structures will likely be necessary to overcome the rotational disorder seemingly intrinsic to these systems. Orientation of origami structures is not necessary in order for them to act as multiscale adapters, connecting 2 nm diameter DNA strands to 100 nm diameter precious metal attachment points.

One of the remaining barriers to molecular lithography, the production of single stranded DNA in quantities sufficient for use in the large scale array experiments described in this paper, has now been addressed through the solid state asymmetric PCR process.

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REFERENCES

1. E Winfree, F Liu, LA Wenzler, NC Seeman , " Design and self-assembly of two-dimensional DNA crystals ", *Nature*, 394, 539–544 (1998).
2. Yu He, Yi Chen, Haipeng Liu, Alexander E. Ribbe, and Chengde Mao, "Self-Assembly of Hexagonal DNA Two-Dimensional (2D) Arrays", *J. Am. Chem. Soc*., 127(35), 12202-12203 (2005)
3. T. LaBean, H. Yan, J. Kopatsch, F. Liu, E. Winfree, J.H. Reif and N.C. Seeman, "The construction, analysis, ligation and self-assembly of DNA triple crossover complexes", *J. Am. Chem. Soc.*, 122, 1848-1860 (2000).
4. S. H. Park, C. Pistol, S. J. Ahn, J. H. Reif, A. R. Lebeck, C. Dwyer, T. H. LaBean, "Finite-size, Fully-Addressable DNA Tile Lattices Formed by Hierarchical Assembly Procedures", *Angewandte Chemie*, 45, 735-739 (2006).
5. Paul W. K. Rothemund, "Folding DNA to create nanoscale shapes and patterns", *Nature*, 440, 297- 302 (2006).
6. Kinneret Keren, Michael Krueger, Rachel Gilad, Gdalyahu Ben-Yoseph, Uri Sivan and Erez Braun, "Sequence-Specific Molecular Lithography on Single DNA Molecules", *Science*, 297, 72- 75 (2002).
7. Kinneret Keren, Rotem S. Berman, Evgeny Buchstab, Uri Sivan and Erez Braun, "DNA-Templated Carbon Nanotube Field-Effect Transistor", *Science*, 302, 1380 – 1382 (2003).
8. Y. Xia and G. M. Whitesides, "Soft Lithography", *Angew. Chem.. Int. Ed. Engl*., 37, 550-575 (1998).
9. Michael Norton, David Neff, B. Scott Day, Zachary Grambos, Mikala Shremshock, Heather Butts and Huan Cao, "Single Molecule Substrates for Lithography", *Sixth IEEE Conference on Nanotechnology, 2006, IEEE-NANO 2006*, 2, 531-533 (2006).
10. Gregory S. McCarty, "Monitoring the addition of molecular species to electrodes utilizing inherent electronic properties", *J. App. Phys*., 99, 64701-64707 (2006).
11. Takaaki Kojima, Yoshiaki Takei, Miharu Ohtsuka, Yasuaki Kawarasaki, Tsuneo Yamane, and Hideo Nakano, "PCR amplification from single DNA molecules on magnetic beads in emulsion: application for high-throughput screening of transcription factor targets", *Nucleic Acids Res*., 33(17), e-150-e159 (2005).
12. S. R. J. Brueck, "Optical and Interferometric Lithography - Nanotechnology Enablers", *Proc. IEEE*, 93, 1704-1721 (2005).
13. X. Zhang, A. V. Whitney, J. Zhao, E. M. Hicks, and R. P. Van Duyne, "Advances in Contemporary Nanosphere Lithographic Techniques", *J. Nanoscience and Nanotechnology*, 6, 1920-1934 (2006).
14. X. Zhang, E. M. Hicks, J. Zhao, G. C. Schatz, and R. P. Van Duyne, "Electrochemical Tuning of Silver Nanoparticles Fabricated by Nanosphere Lithography", *Nano Lett.* 5, 1503-1507(2005).