Nanometer scale rafts built from DNA tiles

Koshala Sarveswaran, Paul Huber, and Marya Lieberman*
Department of Chemistry and Biochemistry
University of Notre Dame
Notre Dame, IN 46556, USA
mlieberm@nd.edu

Chris Russo and Craig Lent
Department of Electrical Engineering
University of Notre Dame
Notre Dame, IN 46556, USA

Abstract **Hierarchical self-assembly was used to construct meso-scale DNA objects as eventual templates for molecular electronic circuitry. DNA tiles were assembled from single-stranded precursors. The tiles measure 4 nm x 12 nm x 2 nm. They can be programmed to self-assemble into larger objects, such as a 4-tile raft 37 nm long. The composition and structure of the DNA rafts were characterized in solution by biochemical assays. Rafts were imaged on mica substrates under isopropanol by non-contact mode AFM, or alternately, were deposited on silicon wafers and imaged by non-contact mode AFM in air.**

Keywords: DNA computing, molecular electronics, silicon oxide, AFM, self-assembly, hierarchical self-assembly.

INTRODUCTION

Most architectures for molecular electronics are based on a simple functional unit, e.g. a field programmable gate or crossbar intersection, which is repeated over a large area. Ideally, this simple functional unit would be a single molecule or small group of molecules; in reality, the need for power gain is likely to require several types of molecules to form, eg. Goto pairs [1]. However, the scale of molecules (a few nm at most) is incommensurate with the scale of lithography (200 nm for optical lithography, 10 nm at best for X-ray or e-beam lithography). There is a general need for ways to marshal large numbers of molecular electronics components in ordered arrays and to integrate them with lithographic I/O structures. We are particularly interested in techniques which are capable of forming molecular QCA circuits [2] with the requisite sub-nm precision. The common question is how to integrate bottom-up methods (chemical synthesis and self-assembly) with top-down methods (lithography). This paper describes an approach to this patterning problem using Winfree/Seeman DNA tiles.

DNA is a natural choice for a programmable structural element. The base-pairing interaction between DNA strands is strong and selective, so the sequence of the oligonucleotides controls binding and structure. Not only can sequences of nucleic acids be designed to hybridize in a predictable fashion, long strands (>100 bp) can be synthesized and characterized using the methods of biotechnology. Although DNA normally forms linear polymers, not branched structures, DNA can exhibit structures with increased valence [3] including replication forks (valence 3) and Holliday junctions (valence 4).

The need for stable crossover sites was recognized by Seeman *et al.*, who worked out sequences which can form stable junctions with valences of 3 or 4 [4]. By placing two DNA duplexes next to one another and including two crossover sites, it is possible to arrive at a rigid DNA tile with a well-defined shape [5]. Each tile contains up to four short sections of single-stranded DNA that extend from the corners of the tile. These four "sticky ends" can be designed to match the ends of other DNA tiles (Fig. 1). Winfree and Seeman demonstrated the self-assembly of two-dimensional periodic lattices consisting of hundreds of thousands of double crossover tiles [6]. These lattices self-assembled in solution and were sturdy enough to be deposited onto mica substrates for AFM imaging.

Figure 1. Double crossover DNA tile

Unlike Seeman and Winfree, whose 2-D tiling patterns create orderly arrangements of the DNA tiles, our objective is to create a maximum amount of controlled heterogeneity. This paper describes the self-assembly of DNA rafts that are 8 nm by 37 nm in size. Each part of the raft has a different sequence, so in principle, molecular recognition could be used to differentiate locations on the raft. In this way, the DNA raft could act as a circuit board for molecular components. The rafts can be attached to silicon wafers by using a very thin poly(lysine) adhesion layer. There is thus the potential for integration of DNA rafts as "holders" for molecular components at the end of a normal CMOS process stream.

RESULTS AND DISCUSSION

A. Sequence Design

The schematic structure of a 4-tile raft is shown at the top of Fig. 2. Each tile is made up of four single strands of DNA; the sequences used are shown at the bottom of Fig. 2.

0-7803-7976-4/03/$17.00 ©2003 IEEE

Designing the tiles for these rafts requires two stages: first, geometric design and second, sequence design. The geometric design involves choosing the spacing of crossover points, length of the duplex region, and length of the sticky ends. The design we are using yields tiles that are approximately 4 nm wide, 12.5 nm long, and 2 nm in height. The four-tile raft is a diamond shape 37 nm long by 8 nm wide.

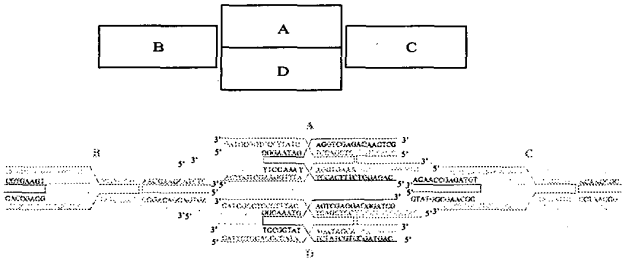

Figure 2. Top: schematic structure for 4-tile raft. Bottom: sequences of the A, B, C, and D tiles.

Proper design of the base pair sequences of these single strands requires that they provide the desired crossovers and sticky ends and that they hold no significant complementarity to any of the other DNA strands in the raft, thus avoiding formation of unwanted structures. The sixteen strands required for tiles A, B, C, and D of the 4-tile raft were designed based on the previous methods of [6]. Several heuristic guidelines were followed. Since AT base pairs form two H-bonds vs. the three H-bonds in a GC base pair, local ratios of AT and GC pairs were kept constant over the tile. All sequences were checked to prevent misalignment, hairpin formation, and alternate conformations.

The sequences of the single-stranded regions are designed so that tile A matches only with one of the single-stranded regions on tile B, while tile D matches the other single-stranded segment on tile B. Thus, tile B holds tiles A and D in position. Tile C likewise binds to the single-stranded regions on the opposite side of tiles A and D. The other ends of tiles B and C lack single stranded segments to prevent formation of larger aggregates.

B. Assembly of tiles and rafts

The tiles shown in Fig. 2 each self-assemble from four single-stranded DNA oligonucleotides. An equimolar solution of the single strand components was heated to 90... C to melt out base-paired structures, then slowly cooled to 4... C. Two experiments verified the oligonucleotide composition of each tile. First, a slow-migrating band of DNA which, based on its molecular mass, corresponds to a tile can be isolated from mixtures of the four constituent single strands by non-denaturing gel electrophoresis (Fig 3). The slow-migrating band was isolated, dissolved in buffer and re-run on a denaturing gel, which revealed the presence of each of the four single strands.

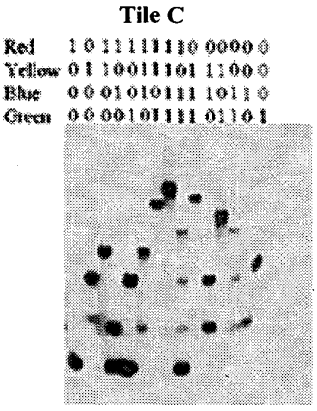

Figure 3. Non-denaturing gel show formation of tile C (lowest mobility band). Presence of different strands is indicated at top of each lane.

Second, by annealing different ratios of the single strands and running the products out on a non-denaturing gel, we can show that the tile only forms in good yield when all four strands are present in a 1:1:1:1 ratio. The yield of properly assembled tile ranges from 70% to 95% for the tiles we have investigated.

The self-assembly of the individual tiles to form a four-tile raft was demonstrated using biochemical assays.

The key points to be established are a) that the tiles form a self-limiting structure rather than an infinite array or uncharacterizable aggregate, b) that each raft includes one of each of its component tiles, c) that each tile is hybridized to its proper partner or partners, and d) that the physical structure of the raft is what we expect.

The 16 DNA strands needed for the 4-tile raft were mixed in stoichiometric ratio and annealed from 50...C to 20...C over the period of 36 h. The formation of soluble products argues that non-specific aggregation does not occur.

The structure of the four-tile raft was probed using enzymatic ligation of the nicks in the raft DNA with T4 DNA ligase. For example, in Fig. 4a, the three yellow strands of DNA from tiles A (21bp), B (48bp), and D (21bp) were joined to form strands of length 90 or 69 bp. By carrying out the ligation reaction and then denaturing the raft, we confirm that ligation had occurred only between the expected strands of the raft.

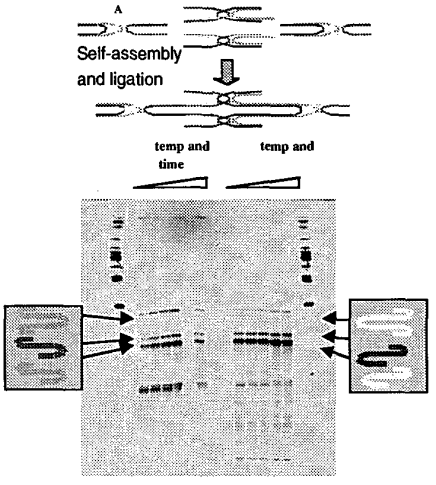

Figure 4. a) Four tile raft and its ligation sites. b) Denaturing gel electrophoresis after ligation of the four-tile raft. Identities of single stranded fragments are indicated.

Fig. 4b shows the result of one such ligation experiment. Two main conclusions may be drawn: first, that tiles A, B, C, and D are hybridized in the proper fashion in the raft, and second, that access to the four ligation sites is not uniform. The "outside" ligation sites which produce the 90 bp yellow and green strands shown in Fig. 4 are accessible to T4 ligase, but the "inside" ligation sites which would produce the analogous long red and blue strands are blocked. The inaccessible sites lie on the inside of the raft, tucked between two tiles, while the red and blue sites lie on the outside surface of the raft (top or bottom), so this result is consistent with the proposed raft structure.

C Surface deposition and AFM imaging

Mica surfaces adsorb DNA strongly and can be used as substrates for imaging DNA tiles and rafts by atomic force microscopy (AFM). A drop of the DNA raft in micromolar concentration in a buffer that contains divalent cations is placed on the mica for a few minutes, then rinsed. Control experiments with the buffer solution show smooth (rms roughness .8-1.2) surfaces. Silicon wafers must first be treated to form a smooth native oxide, and then primed with a poly(lysine) adhesion layer. This layer increases the rms roughness of the native silicon oxide surface from 0.7 to about 2.5 . DNA rafts can then be deposited from solution.

AFM images (Fig. 5) are obtained in tapping mode. The 4-tile rafts show a height of 1.9-2.4 , which compares well to the expected 2 nm thickness of Z-form DNA. Lateral dimensions are harder to interpret since the AFM tip shape is convoluted with the true dimensions of the object being analyzed. In Fig. 5a, the large white spot has dimensions of 27 nm x 56 nm x 2.1 nm. If the tip radius is about 20 , these lateral dimensions match up well with the predicted 8 nm x 37 nm dimensions of the rafts.

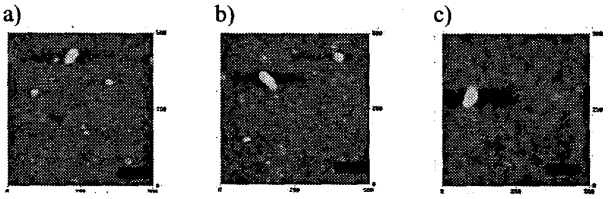

Figure 5. Non-contact AFM images of 4-tile raft a) Mica substrate, isopropanol. b) Silicon oxide/poly(lysine) substrate, air. Scale bar = 100 nm.

EXPERIMENTAL

A. DNA sequences

The 16 different strands used in this experiment were obtained from Integrated DNA Technologies, Inc, Coralville, IA. For visualization of gel experiments, oligos (1 μg) were labeled at their 5 -end with T4 polynucleotide kinase (Promega) and 85 μCi (7000 Ci/mmol) [γ-^{32}P]-ATP in 10 μl of kinase buffer (70mM Tris-HCl (pH 7.6), 10 mM $MgCl_2$ and 5mM DTT) for 30 minutes at 37°C. The labeled strands were mixed with an excess of unlabelled strands. Concentration of DNA solutions was measured by their absorbance at 260 nm. The strands of each tile were mixed in stoichiometric ratio and dissolved to 0.5-1.5 μM in TAE/Mg^{2+} buffer (40 mM Tris-HCl (pH 8.0), 2 mM EDTA, 20 mM acetic acid, 12.5 mM Mg^{2+}). The solutions were annealed using a linear gradient from 90°C to 4°C over 12 hours in a thermocycler. To produce the rafts, all 16 strands were mixed from the start and annealed from 50ßC to 20ßC over 36 hr.

B. Non-denaturing Polyacrylamide Gels.

Non-denaturing gel electrophoresis was carried out using 8-12% (19:1 acrylamide:bisacrylamide) gels containing 12.5 mM Mg^{2+}. The running buffer was TAE/Mg^{2+} and the loading buffer also contained 0.02% bromophenol blue and Xylene cyanol and 50% glycerol. Electrophoresis was run at 7 V/cm at 4°C for 12-24 hr.

C. Denaturing gels

Denaturing gel electrophoresis was carried out using gels containing 8.3 M urea and 10% (19:1 acrylamide: bisacrylamide). The running buffer was TBE (50mM Tris-HCl (pH 8.0), 50mM Boric acid and 1mM EDTA). Samples were heated at 90°C for 5 minutes to denature them, then were kept on ice before loading to prevent rehybridization.

D. AFM Imaging

Atomic force microscopy was used to image the rafts on mica and silicon surfaces. In a typical experiment, 15μl of a roughly one micromolar solution of annealed (but not ligated) rafts was placed on a freshly cleaved mica surface and left for 2 minutes to dry. Then 10 drops of doubly distilled water were placed on the mica as a rinse solution, and the liquids were shaken off the mica. The surface was then dried with nitrogen.

For silicon substrates, silicon [100] (MEMC Electronic Materials, Inc, Malaysia) wafers were cleaned in Pirhana solution (1:3 30% H_2O_2/conc. H_2SO_4, CAUTION: Pirhana solution is a strong oxidant and can cause explosions when mixed with organic solvents) at 90°C for 30 minutes, rinsed with distilled water, and dried with a stream of N_2. The cleaned wafers were etched in a solution of 10% HF until they became completely hydrophobic, indicating removal of the surface oxide. RCA cleaning procedures [7] were carried out next; the silicon wafers were first cleaned in a NH_4OH : H_2O_2 : H_2O (1 : 1 : 5) solution at 70°C for 10 minutes, rinsed with water for 5 minutes, then immersed in HCl : H_2O_2 : H_2O (1 : 1 : 5) solution at 70°C for 10 minutes and rinsed again with water and finally dried with nitrogen. The wafers were soaked in a 0.01% w/v poly-L-lysine solution for 10 minutes and dried before deposition of DNA. 15μl of DNA solution was deposited on the surface and dried for 2 minutes, then the surface was rinsed with water and dried with nitrogen.

The atomic force microscope images shown were obtained by scanning the surface in air, using a Nanoscope IIIa multimode instrument (Digital instruments, CA) in tapping mode. When imaging biological samples with AFM at high resolution it is important to minimize the tip tapping force. Silicon cantilevers were chosen (Nanosensors) with a force constant of 30-50 N/m. The tip radius curvature of these tips ranged from 20-50 nm, as determined by apparent widths of

calibration samples (5 nm gold nanoparticles and plasmid DNA.)

AFM scans were taken at 256x256 pixel resolution and produced topographic images of the samples. Typical scanning parameters used: tapping frequency 300kHz, RMS amplitude before engage 1-1.2 V, integral and proportional gains 0.1-0.4 and 0.4-0.8 respectively, setpoint 0.8-1.2 V, scanning speed 1-2 Hz/line.

ACKNOWLEGEMENTS

Aimee Robinson and Stacy Wagner both contributed to this project as REU students.

REFERENCES

[1] a) J. M. Tour, W. L. Van Zandt, C. P. Husband, S. M. Husband, L. S. Wilson, P. D. Franzon, and D. P. Nackashi, "Nanocell logic gates for molecular computing," IEEE T. Nano. 2002, vol. 1, pp. 100-109. b) K. L. Wang, "Issues of nanoelectronics: A possible roadmap," J. Nanosci. Nanotech. 2002, vol. 2, pp 235-266.

[2] M. Lieberman, S. Chellamma, B. Varughese, Y. Wang, C. S. Lent, G. H. Bernstein, G. L. Snider, and F. C. Peiris, "Quantum-dot Cellular Automata at a Molecular Scale," in Molecular Electronics II, Ari Aviram, Mark Ratner, and Vladimiro Mujica, Eds. Annals of the New York Academy of Sciences, 2002, vol. 960, pp. 225-239.

[3] Lee, J.; Voziyanor, Y.; Pathania. S.; Jeyaram, M.; " Structural alterations and conformational dynamics in Holliday junctions induced by binding of a site-specific recombinase " Molecular Cell, 1998, vol. 1, pp. 483-493; Lilly, D. J. M.; Quart. Rev. Biophys.,2000, vol. 33, pp. 109-159.

[4] Seeman, N. C. " Nucleic acid junctions and lattices," J. Theor. Bio, 1982, vol. 99, pp. 237-247; Seeman, N. C. " De novo Design of Sequences for Nucleic-Acid Structural-Engineering," J. Biomol. Struct. Desyn. 1990, vol. 8, pp. 573-581.

[5] Fu, T-J.; Seeman, N. C. " DNA Double-Crossover Molecules," Biochemistry, 1993, vol. 32, pp. 3211-3220.

[6] Winfree, E.; Liu, F.; Wenzler, L. A.; Seeman, N. C. " Design and self-assembly of two-dimensional DNA crystals," Nature, 1998, vol. 394, pp. 539-544.

[7] Kern, W.; Puotinen, D. A. *RCA Rev.* 1970, vol. 31, p. 187