The Design and Fabrication of a Fully Addressable 8-tile DNA Lattice

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Abstract— We have designed and experimentally demonstrated the self-assembly of an addressable DNA lattice (i.e., a unique tile for each position in the lattice) using a two-step tile annealing procedure. Our method can be applied to a variety of systems including algorithmic tiling systems to expand the basic tile set.

1. Introduction

Since the introduction of DNA self-assembly as a technique for nanoscale patterning and synthesis [1-6], advances in computer-aided design and material synthesis have begun to pave the way for the application of DNA nanostructures to the integration of nanoelectronic components into new kinds of computer architectures. Self-assembly is beginning to emerge as an alternative to conventional photolithography for patterning large numbers of nanoelectronic devices at nanoscale resolutions. Much of the work in this area is driven by the limitations of conventional very-large-scale-integrated (VLSI) circuit fabrication techniques as spatial resolutions and device pitch approach nanometers and device performance suffers because of the fundamental difference in material properties at the nanoscale [7]. Further, new models for computation have shown that fundamental paradigm shifts can be enabled by controlled self-assembly [8-10]

An important challenge that remains is to design a sufficiently large set of DNA tiles to assemble the kinds of aperiodic structures that appear in logic circuitry. Efficient use of tiles (e.g., by algorithmic assembly) will no doubt lead to near-term reductions in the tile set size but more complex designs will require larger sets to improve assembly time and/or yield which motivates the design of new tiles [11]. Many aperiodic patterns (and some periodic patterns) appear at various levels in the placement of wires and devices in modern circuitry and the tile set must accommodate this complexity. One difficulty in creating new tiles is to find nucleotide sequences that minimize the strength of unintentional interactions with the other tiles in the set while maximizing the strength of intentional interactions. Another important challenge is to understand the sensitivity of the tile shape (beyond the intentional geometric shape) to specific sequence choices.

The workhorse in the tile creation process is an optimization algorithm that is aware of both the intentional geometric constraints that form the tile and all possible

unintentional interactions. Clearly, this space is vast and computer-aided design is necessary to make progress. There are many varieties of computer tools available for DNA sequence and tile design [12-16]. However, many of these tools are single-processor implementations or heuristic methods that are unable to find sufficient numbers of sequences for large tile sets and can not guarantee the optimality of each solution.

To overcome these challenges we have developed a parallel algorithm to design "4x4" tiles (see [17]) for use in assembling large aperiodic DNA lattices. Our method is similar to that used by Yin, et al. in [16] but designed around the 4x4 tile and implemented as a parallel algorithm as described in section 2. We have experimentally verified our method by designing, synthesizing, and assembling an 8-tile system and characterized it with an atomic force microscope (AFM) as described in section 3.

2. Methods

DNA sequence design— The tile system we have adopted is based on the 4x4 tile system developed by Yan et al. Figure 1 illustrates the three major components of a tile in this system: a core strand, four shell strands, and four arm strands.

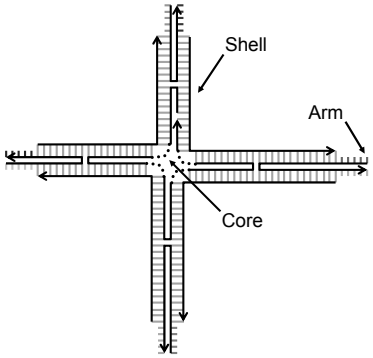

Figure 1: Schematic of a 4x4 tile. The arrows indicate the 5'-3' direction for each DNA strand

The core and arm strands interact with the shell strands to form each distal extension of the cruciform. When this system is tiled each core is separated by four full turns (42 bp) from the adjacent cores. The inner part of the core (dotted lines in figure 1) is occupied by four regions of unpaired poly-T_4.

We have redesigned each of the 5-bp arm overhangs (two per arm) of the original 4x4 tiles to satisfy the constraint that each tile has only one position in an 8-tile lattice. That is, we conserved sequences between all tiles in the core and shells. Only the arm strand overhangs change. We will describe how this is possible with hierarchical assembly.

The new overhangs are appended to the conserved portions of each arm strand and compared against the other strands in the tile. This verification ensures that new overhangs do not disturb the known-good structure of the original tile. We evaluated all the 5-bp overhangs and ranked them in descending order of stability with existing sequences in the tile. The topmost sequences were then appended to each conserved arm strand to make eight new tiles.

We used a nearest-neighbor mismatch algorithm based on the open source MELTING4 tool to evaluate our sequence designs [18]. We modified the original code to run on a cluster of 300 1-GHz x86 Linux workstations and analyzed all possible alignment configurations of each new candidate arm strand. This analysis

included self-binding and 3 – 6 consecutive nucleotide region mismatching and took about 75 CPU-months to complete.

An important part of our modifications to the original MELTING4 code was to handle internal and terminal sequence mismatches. Internal mismatching data missing from the MELTING4 source were incorporated from Peyret et al. [19] and terminal mismatches were resolved by using a "test fixture". That is, each alignment configuration was padded with a 3-bp region of matching sequences on each end to simulate the environment of a complete tile. The test fixture slightly increases the stability of a configuration because of the matching base pairs on the ends but this is true for all configurations. Therefore, a systematic bias in stability is introduced to all configurations which make relative comparisons more reliable than absolute T_m comparisons.

Hierarchical Self-Assembly— Each tile must be annealed in isolation from the other tiles because all tiles share the same core-shell nucleotide sequences. Otherwise, each tile's arm strands can bind to a core-shell proto-tile that has already bound another tile's arm strands which will create random tiles. This will destroy the full addressability of the system.

We annealed each tile (1 through 8) in individual microcentrifuge tubes. Tiles were prepared by the methods described in [17] and only briefly outlined here. The constituent DNA strands for each tile were synthesized by a commercial vendor (IDT-DNA, Inc.) and resuspended in 1X Tris-acetic-EDTA (TAE) buffer at 15 μM. The 9 strands per tile were mixed and diluted to a final concentration of 1 μM. Each tile was annealed over 48 hours from 95 °C to ambient and then from ambient to 4 °C. DNA lattice was made by mixing pre-annealed tiles at room temperature for 4 hours and then incubating at 4 °C overnight.

AFM Imaging— Samples were prepared by depositing 5 μL of DNA lattice onto a freshly cleaved mica surface for 5 minutes. Then 35 μL of 1X TAE was added and the entire sample placed under a Digital Instruments Nanoscope AFM fluid cell for tapping-mode imaging.

3. Results

Each new tile design was verified by using our modified MELTING4 tool to find the maximally stable configuration between all pairs of strands in the tile. Figure 2 is the "cross-plot" of maximal T_m (approximates stability) for all pairs of strands in the tile (tile-1 in this case).

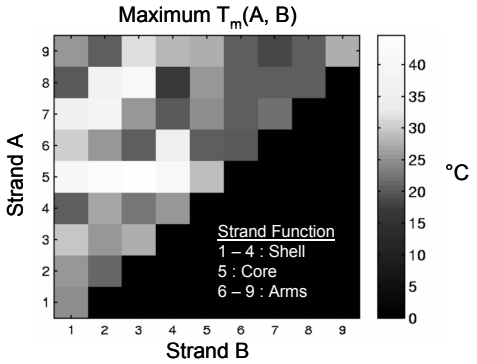

Figure 2: Cross-plot of the maximum interaction strength over all configurations of strands in the tile

The plot illustrates how the geometric binding constraints of the original tile system from figure 1 create a characteristic signature. Unintentional sequence

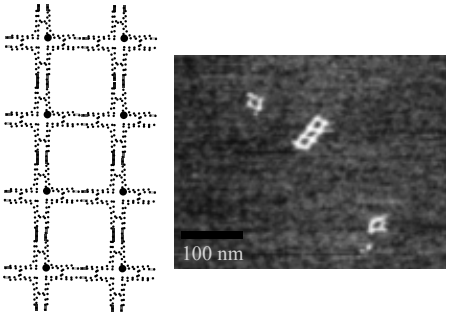

Figure 3: Schematic (left) and AFM image (right) of an 8-tile assembly (a 4x2 lattice)

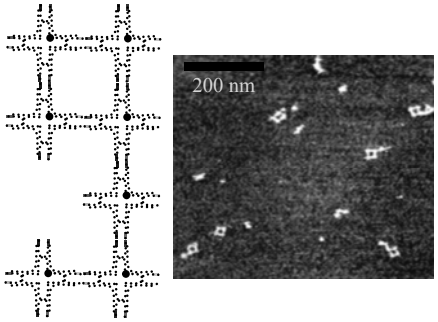

Figure 4: Schematic (left) and AFM image (right) of a partial 4x2 lattice

matches that might disrupt the tile's formation will change the cross-plot signature and indicate a design error based on the thermodynamic behavior of the strands.

We examined the tiles by pre-annealing each tile and then mixing groups of tiles at room temperature (see section 2). Figures 3 & 4 show the groups of tiles we mixed and the AFM images of the lattice that formed.

The lattice fragments dynamically interact with the mica surface and this complicates the interpretation of the AFM images since portions of the lattice can diffuse or be knocked away from the surface during a scan. Strong tip-sample interactions are probably also responsible for making the lattice appear bent or warped since the lattice persistence length (> 50 nm) is comparable to the size of an individual lattice (60 x 20 nm).

4. Conclusions

The cluster based nearest-neighbor mismatch algorithm can be used to design and verify new sets of cruciform or "4x4" DNA tiles. We have shown that this design process is robust by experimentally verifying the self-assembly of an 8-tile lattice system. Algorithmic approaches to generating fully addressable lattice may benefit from using larger basis sets to either improve the speed or yield of the assembly. Our method can be adapted to analyze the interaction of DNA in many types of tile systems and can enable the exploration of large and complex tile systems.

Acknowledgements

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