Molecular Assembly and Computation: From Theory to Experimental Demonstrations

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Abstract. While the topic of Molecular Computation would have appeared even a half dozen years ago to be purely conjectural, it now is an emerging subfield of computer science with the development of its theoretical basis and a number of moderate to large-scale experimental demonstrations. This paper focuses on a subarea of Molecular Computation known as *DNA self-assembly*. Self-assembly is the spontaneous self-ordering of substructures into superstructures driven by the selective affinity of the substructures. DNA provides a molecular scale material for effecting this programmable self-assembly, using the selective affinity of pairs of DNA strands to form DNA nanostructures. DNA self-assembly is the most advanced and versatile system known for programmable construction of patterned systems on the molecular scale. The methodology of DNA self-assembly begins with the synthesis of single-strand DNA molecules that self-assemble into macromolecular building blocks called DNA tiles. These tiles have sticky ends that match the sticky ends of other DNA tiles, facilitating further assembly into large structures known as DNA tiling lattices. In principal you can make the DNA tiling assemblies form any computable two- or three-dimensional pattern, however complex, with the appropriate choice of the tiles component DNA. This paper overviews the evolution of DNA self-assembly techniques from pure theory to experimental practice. We describe how some theoretical developments have made a major impact on the design of self-assembly experiments, as well as a number of theoretical challenges remaining in the area of DNA self-assembly. We descuss algorithms and software for the design, simulation and optimization of DNA tiling assemblies. We also describe the first experimental demonstrations of DNA self-assemblies that execute molecular computations and the assembly of patterned objects at the molecular scale. Recent experimental results indicate that this technique is scalable. Molecular imaging devices such as atomic force microscopes and transmission electron microscopes allow visualization of self-assembled two-dimensional DNA tiling lattices composed of hundreds of thousands of tiles. These assemblies can be used as scaffolding on which to position molecular electronics and robotics components with precision and specificity. The programmability lets this scaffolding have the patterning required for fabricating complex devices made of these components.

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1 Introduction

There is a long history of theoretical ideas in computer science that have led to major practical advances in experimental and applied computer science: for example, formal language and automata theory led to practical programming language design and parsing techniques, and number theoretic algorithms led to the development of public key cryptography forming the basis of many of the cryptographic protocols currently used on the internet.

This paper describes the development of the theory of self-assembly starting with a theory of tiling in the 1960s (that first established that tilings can form any computable 2D pattern), and its on-going evolution, including theoretical works on its parallel complexity as well as sophisticated stochastic and kinetic theories of self-assembly. We also describe our experimental demonstrations of DNA nanostructure self-assembly to execute computation and to form 2D lattices with regular patterns. See [Reif, et al 01] for a more detailed survey of current experimental work in self-assembled DNA nanostructures. Also, see [Reif, 98 and 02] for comprehensive surveys of the larger field of DNA computation (also known as biomolecular computation).

Motivation: The Need to Form Complex Patterned Objects at the Molecular Scale. As a motivating application of self-assembled nanostructures, we first briefly describe a major challenge to be faced by the field of computer science in the immediate future, namely the scale limitations of known fabrication techniques for microelectronics and MEMS(microelectrocal mechanical systems). We describe how bottom-up techniques based on self-assembly may be used to address and overcome this challenge.

Top-Down Techniques. The microelectronics industry currently uses optical lithography for constructing microelectronic and MEMS devices on silicon chips. Because of wavelength resolution limits, it is unlikely that optical lithography will scale much below a few nanometers. It has been projected that in approximately 15 years, this top-down method for patterning microelectronics will reach its ultimate limits and rate of progress in miniaturization of microelectronics will either halt or be very much reduced. It should be noticed that the use of lithography in microelectronics and MEMS is but one example of how our current engineering technology is based entirely on top-down manufacturing methods – whereas engineering progress at the molecular scale will require replacement of the current engineering technology by bottom-up self-assembly methods. Other top-down approaches to assembling nanoscale objects (such as microelectronics or MEMS) use ebeam lithography or a macroscale instrument, such as a scanning probe microscope, that can move and probe at molecular-size scales. Major obstacles to using such instruments to construct complex devices such as microelectronics at the molecular scale include the sequential nature of these technologies and their controllability and scalability. Although certain ebeam lithography systems and scanning probe microscopes can make use of a small amount of parallelism (e.g., a number of simultaneous probes), those numbers are dwarfed by the vast number of molecules that need to be manipulated.

To overcome this key problem of patterning structures below a few nanometers and into the molecular scale, we need new approaches. Known patterning methods used for manufacture at the microscale can be categorized as either top-down or bottom-up.

Bottom-Up methods for Self-assembly at the Molecular Scale. All known bottom-up approaches for patterned assembly rely on self-assembly. Self-assembly is the spontaneous self-ordering of substructures into superstructures driven by the selective affinity of the substructures. Self-assembly processes are well studied in biological systems such as the cell, in chemistry, and protein engineering. How can we program them to design new structures or to execute computations at the molecular scale?

(i) Cellular Self-Assembly. One can get inspiration from biological cells which operate on the nanoscale and use bottom-up methods for assembly. Cells perform a multiplicity of self-assembly tasks, including the self-assembly of cell walls (via lipids), of microtubules, etc. Many of these biological self-assembly processes utilize the specificity of ligand affinities to direct the self-assembly. However it is not at all easy to reprogram these biological cells for specialized assemblies at the nanoscale since the machinery available in biological cells is exceptionally difficult to predict and control, and we are only now beginning to understand the complexity of its control systems.

(ii) Chemical Self-Assembly. Self-assembly methods are well known in chemistry and have long been used for the self-assembly of lipid or polymer layers, but they generally result in structures that have limited complexity and are not readily programmable.

(iii) Protein Self-Assembly. Protein engineering is a bottom-up approach that offers great promise for molecular devices, but we do not yet fully understand the folding rules for protein assembly. The main difficulty here is that when you attempt to engineer proteins, you have at this time only a very limited degree of predictability in the resulting protein conformations.

(iv) DNA self-assembly. Finally, DNA self-assembly is a bottom-up approach which entails the spontaneous self-assembly of DNA strands into nanostructures driven by selective hybridization among DNA strands. As we shall see, in contrast to the other methods for self-assembly just mentioned, DNA self-assembly is readily programmable and has already been experimentally demonstrated.

Goals and Organization of this Paper. The goal of this paper is describe techniques for self-assembly of DNA tiling arrays and applications of this technology, including DNA computation. *Section 2* overviews the emerging theory of self-assembly starting from the theory of Domino Tiling Problems developed in the 1960s to some kinetic and stochastic theoretical models of tiling self-assembly processes. Turing-universality, NP completeness, and program-size complexity results self-assembly processes are cited. Also, we describe the parallel depth complexity of self-assembled tilings and in particular, linear self-assemblies which have been used in practice. *Section 3* describes the experimental demonstration of self-assembled DNA tiling lattices. This section introduces various classes of DNA nanostructures known as DNA tiles, and describe some the software devel-

oped for the design of the DNA strands composing these tiles. We also describe some 2D DNA tiling lattice assemblies and their visualization by atomic force and electron microscopes. *Section 4* describes our experimental demonstration of DNA tiling computations, which used linear DNA tiling assemblies. *Section 5* concludes the paper.

2 The Theory of Self-Assembly

This Section overviews the emerging theory of self-assembly. We note a number of techniques that have been used in the experimental demonstrations of DNA tiling assemblies, as described in the following Section 3.

Domino Tiling Problems. The theoretical basis for self-assembly has its roots in *Domino Tiling Problems*(also known as Wang tilings) as defined by Wang [Wang61] (Also see the comprehensive text [Grunbaum, et al, 87]). The input is a finite set of unit size square tiles, each of whose sides are labeled with symbols over a finite alphabet. Additional restrictions may include the initial placement of a subset of these tiles, and the dimensions of the region where tiles must be placed. Assuming an arbitrarily large supply of each tile, the problem is to place the tiles, without rotation (a criterion that cannot apply to physical tiles), to completely fill the given region so that each pair of abutting tiles have identical symbols on their contacting sides. (See Figure 1 for 'Smart Bricks' tiling assembly generalized to polygons.)

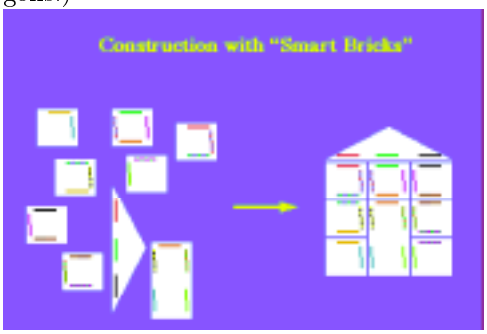

Fig. 1. A tiling assembly using 'Smart Bricks' with affinity between colored pads.

Theoretical Models of Tiling Self-assembly Processes. Domino tiling problems do not presume or require a specific process for tiling. Winfree [Winfree95] is responsible for invention of a key idea: self-assembly processes can be used for computation via the construction of DNA tiling lattices. (These will be further discussed in Section 3.) The sides of the tiles are assumed to have some methodology for selective affinity, which we call *pads*. Pads function as programmable binding domains, which hold together the tiles. Each pair of pads have specified binding strengths The self-assembly process is initiated by a singleton tile (the *seed tile*) and proceeds by tiles binding together at their pads to form aggregates known as *tiling assemblies*. The preferential matching of tile pads facilitates the further assembly into tiling assemblies.

Using the kinetic modeling techniques of physical chemistry, [Winfree98] developed a kinetic model for the self-assembly of DNA tiles. Following the classical

literature of models for crystal assembly processes, [Winfree95] considers assembly processes where the tiling assembly is only augmented by singleton tiles (known in crystallography as *monomers*) which bind to the assembly at their tile pads. The likelihood of a particular tile binding at (or disassociating from) a particular site of the assembly is assumed to be a fixed probability dependent on that tile's concentration, the respective pad's binding affinity, and a temperature parameter. Winfree [W98] developed software for discrete time simulation of the tiling assembly processes, using approximate probabilities for the insertion or removal individual tiles from the assembly. These simulations gave an approximation to the kinetics of self-assembly chemistry and provided some validation of the feasibility of tiling self-assembly processes. Using this software as a basis, Yuan [Guangwei00] at Duke developed improved (sped up by use of an improved method for computing on/of likelihood suggested by Winfree) simulation software with a Java interface (http://www.cs.duke.edu/∼yuangw/project/test.html) for a number of example tilings, such as string tilings for integer addition and XOR computations. In spite of an extensive literature on the kinetics of the assembly of regular crystalline lattices, the fundamental thermodynamic and kinetic aspects of self-assembly of tiling assemblies are still not yet well understood. For example, the affect of distinct tile concentrations and different relative numbers of tiles is not yet known; for this there is the possible application of Le Chatelier's principle.

[Adleman, et al 00] developed stochastic differential equation models for self-assembly of tiling assemblies and determined equilibrium probability distributions convergence rates for some 1-dimensional self-assemblies. His model allowed for binding between subassemblies and assumed a fixed probability for tile binding events independent of the size of tile assemblies. Since the movement of tile assemblies may depend on their size (and thus mass), this model might in the future be refined to make the probability for tile binding events dependent of the size of tile assemblies.

Meso-Scale Physical Simulation of Tile Assemblies. Pad binding mechanisms for the preferential matching of tile sides can be provided by various methods: (i) *molecular affinity*, using for example hydrogen bonding of complementary DNA or RNA bases, (ii) *magnetic attraction*, e.g., pads with magnetic orientations constructed by curing the polymer/ferrite composites in the presence of strong magnet fields, and also pads with patterned strips of magnetic orientations, (iii) *capillary force*, using hydrophobic/hydrophilic (capillary) effects at surface boundaries that generate lateral forces, (iv) *shape complementarity* (or conformational affinity), using the shape of the tile sides to hold them together. There is a variety of distinct materials for tiles, at a variety of scales:

(a) Molecular-Scale Tiling Assemblies have tiles of size up to a few hundred Angstroms. Specifically, DNA tiles will be the focus of our discussions in the following sections.

(a) Meso-Scale Tiling Assemblies have tiles of size a few millimeters up to a few centimeters. Whitesides at Harvard University has developed and tested multiple technologies [Zhao, et al, 98] [Xia et al, 98a,98b], [Bowden,et al 98], [Harder,et

al 00] for meso-scale self-assembly, using capillary forces, shape complementarity, and magnetic forces (see http://www-chem.harvard.edu/GeorgeWhitesides.html). [Rothemund, 2000] also gave some meso-scale tiling assemblies using polymer tiles on fluid boundaries with pads that use hydrophobic/hydrophilic forces. A materials science group at the U. of Wisconsin also tested meso-scale self-assembly using magnetic tiles (http://mrsec.wisc.edu/edetc/selfassembly). These meso-scale tiling assemblies were demonstrated by a number of methods, including placement of tiles on a liquid surface interface (e.g., at the interface between two liquids of distinct density or on the surface of an air/liquid interface). These meso-scale tiling experiments have used mechanical agitation with shakers to provide a temperature setting for the assembly kinetics (that is, a temperature setting is made by fixing the rate and intensity of shaker agitation). These meso-scale tilings also have potential to illustrate fundamental thermodynamic and kinetic aspects of self-assembly chemistry.

Optimization of Tiling Assembly Processes. There are various techniques that may promote assembly processes in practice. One important technique is the tuning of the parameters (tile concentration, temperature, etc.) governing the kinetics of the process. [Adleman,et al 02] considers the problem of determining tile concentrations for given assemblies and conjectures this problem is $\sharp P$ complete. Nevertheless, the above tiling simulation software may be useful in the determination of heuristic estimates for parameters values such as tile concentration.

Various other techniques may improve convergence rates to the intended assembly. A blockage of tiling assembly process can occur if an incorrect tile binds in a unintended location of the assembly. While such a tile may be dislodged by the kinetics of subsequent time steps, it still may slow down the convergence rate of the tiling assembly process to the intended final assembly. To reduce the possibility of blockages of tiling assembly processes, [Reif97] proposed the use of distinct tile pads for distinct time steps during the assembly. [Reif97] also described the use of self-assembled tiling *nano-frames* to constrain the region of the tiling assemblies.

Turing-universal and NP Complete Self-assemblies. Domino tiling problems over an infinite domain with only a constant number of tiles were first proved by [Berger66] to be undecidable. This and subsequent proofs [Berger66, Robinson71, Wang75] rely on constructions where tiling patterns simulate single-tape Turing Machines or cellular arrays[Winfree95]. They require only a constant number of distinct tiles. These undecidability proof techniques allow [Winfree95] to show (he used the first and last layers of the assembly for input and output of computations, respectively) that computation by self-assembly is Turing-universal and so tiling self-assemblies can theoretically provide arbitrarily complex assemblies even with a constant number of distinct tile types. [Winfree,96] also demonstrated various families of assemblies which can be viewed as computing languages from families(e.g., regular, CFL, etc.) of the Chomsky hierarchy.

[LewisPapa81, Moore00] proved the NP-completeness of Domino tiling problems over polynomial-size regions. Subsequently, [Winfree96], [Jonoska97, Jonoska98]

and [Lagoudakis and LaBean,99] proposed the use of self-assembly processes (in the context of DNA tiling and nanostructures) to solve NP complete combinatorial search problems such as SAT and graph coloring. However, the practical scale of these tiling methods to solve NP complete problems is limited to only moderate size problems at best.

Program-size Complexity of Tiling Self-assemblies. The programming of tiling assemblies is determined simply by the set of tiles, their pads, and the choice of the initial seed tile. A basic issue is the number of distinct tile types required to produce a specified tile assembly. The *program size complexity* of a specified tiling is the number of distinct tiles (with replacement) to produce it. [Rothemund and Winfree, 00b] show that the assembly of an $n \times n$ size square can be done using $\theta(\log n / \log \log n)$ distinct tiles. They also show that largest square uniquely produced by a tiling of a given number of distinct tiles grows faster than any computable function. [Adleman,et al 02] recently gave program size complexity bounds for tree shaped assemblies.

Massively Parallel Computation by Tiling. In computation by self-assembly, parallelism reveals itself in many ways. Each superstructure may contain information representing a different calculation (*global parallelism*). Due to the extremely small size of DNA strands, as many as 10^{18} DNA tiling assemblies may be made simultaneously in a small test tube. Growth on each individual superstructure may also occur at many locations simultaneously via *local parallelism*. The *depth* of a tiling superstructure is the maximum number of self-assembly reactions experienced by any substructure (the depth of the graph of reaction events), and the *size* of a superstructure is the number of tiles it contains. Likewise for the number of layers. For example, a superstructure consisting of an array of $n \times m$ tiles, where $n > m$ has depth m. Tiling systems with low depth, small size, and few layers are considered desirable, motivating the search for efficient computations performed by such systems. [Reif97] was the first to consider the parallel depth complexity of tiling assemblies and gave DNA self-assemblies of linear size and logarithmic depth for a number of fundamental problems (e.g., prefix computation, permutation, integer addition and subtraction, finite state automata simulation, and string fingerprinting) that form the basis for the design of many parallel algorithms. Furthermore, [Reif97] showed these elementary operations can be combined to perform more complex computations, such as bitonic sorting and general circuit evaluation with polylog depth assemblies.

Linear Self-Assemblies. Tiling systems that produce only superstructures with k layers, for some constant k, are said to use *linear self-assembly*. [Reif97] gave some simple linear tiling self-assemblies for integer addition as well as related operations (e.g., prefix XOR summing of n Boolean bits). These were the basis of the DNA tiling experiments of [Mao,00] that demonstrated the first example of DNA computation using DNA assembly, as described in Section 3. These linear tilings were refined by [Winfree, Eng, and Rozenberg,00] to a class of String Tilings that have been the basis for further ongoing DNA tiling experiments of [LaBean, et al 00] of integer addition described in Section 3.

3 The Practice: Self-Assembly of DNA Tiling Lattices

DNA Hybridization. Single-strand DNA is a polymer that consists of a sequence of four types of bases grouped into two disjoint pairsknown as Watson-Crick complementary pairsthat can bind together through hydrogen bonding in an operation known as hybridization. DNA enjoys a unique advantage for a nanostructure construction material because two single strands of DNA can be designed and constructed by the experimental scientist to be selectively sticky and bind together to form doubly stranded DNA. (see Figure 2.) Hybridization is much more likely to occur if the DNA base sequences are complementarythat is, if the component bases are Watson-Crick pairsand the temperature and salinity are set appropriately. The resulting doubly stranded DNA is relatively rigid and forms the well-known double-helix geometry. If the sticky single-strand segments that hybridize abut doubly stranded segments of DNA, you can use an enzymic reaction known as ligation to concatenate these segments.

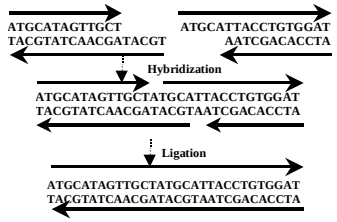

Fig. 2. Hybridization of sticky single-strand DNA segments.

DNA Nanostructures. [Seeman, 82] first pioneered DNA structure nanofabrication in the 1980s by assembling a multitude of DNA nanostructures (such as rings, cubes, and octahedrons) using DNA branched junctions and remains a leader in this area [Seeman, 98, Seeman et al 94, 98, 99]. However, these early DNA nanostructures werent very rigid. To increase the rigidity of DNA nanostructures, Seeman made use of a DNA nanostructure known as a DNA crossover [Seeman, 82 and Seeman et al 89] (also known as a *branched Holiday junction*) which consists of two doubly stranded DNA, each having a single strand that crosses over to the other. Pairs of crossovers, known as double crossovers, provide a significant increase in rigidity of a DNA nanostructure. Also, certain crossovers (known as antiparallel crossovers) cause a reversal in the direction of strand propagation following the exchange of the strand to a new helix.

DNA Tiles. These are quite rigid and stable DNA nanostructures that are formed from multiple DNA antiparallel crossovers. DNA tiles typically have a roughly rectangular geometry. These tiles come in multiple varieties that differ from one another in the geometry of strand exchange and the topology of the strand paths through the tile. The first DNA tiles developed [Winfree, et al 86,98] were known as double-crossover (DX) tiles and composed of two DNA double helices with two crossovers. LaBean, Reif, and Seeman [LaBean, et al 00] have developed some novel DNA tiles known as triple-crossover(TX) tiles that are composed of three DNA double helices with four crossovers. These TX tiles have properties that can facilitate one and two dimensional tiling assemblies and computations. (See Figure 3.)

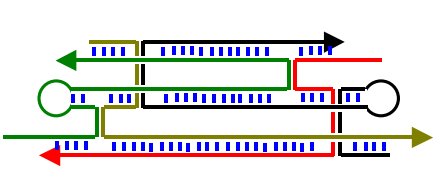

Figure 3a *A TX Tile*

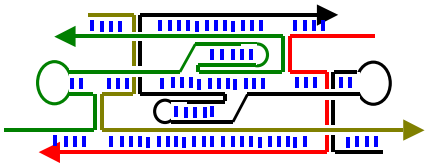

Figure 3b *A TX tile with two extra stem-loops which project into (black) and out of (green) the plane of the page.*

Fig. 3. (a) A triple-crossover tile and (b) a triple-crossover tile that has two extra stem-loops that project into (black) and out of (green) the plane of the page.

Each DNA tile is designed to match the ends of certain other DNA tiles, a process that facilitates the assembly into tiling lattices. In particular, DNA tiles are designed to contain several short sections of unpaired, single-strand DNA (ssDNA) extending from the ends of selected helices (often called 'sticky ends') that function as programmable binding domains, which are the *tile pads*. Both double- and triple-crossover tiles are useful for doing tiling assemblies. The DX tiles provide up to four pads for encoding associations with neighboring tiles, whereas the TX tiles provide up to six pads that are designed to function as binding domains with other DNA tiles. Use of pads with complementary base sequences provides control the neighbor relations of tiles in the final assembly. In particular, the tile pads hybridize to the pads of other chosen DNA tiles. Individual tiles interact by binding with other specific tiles through hybridization of their pads to self-assemble into desired superstructures. (See Figure 4.)

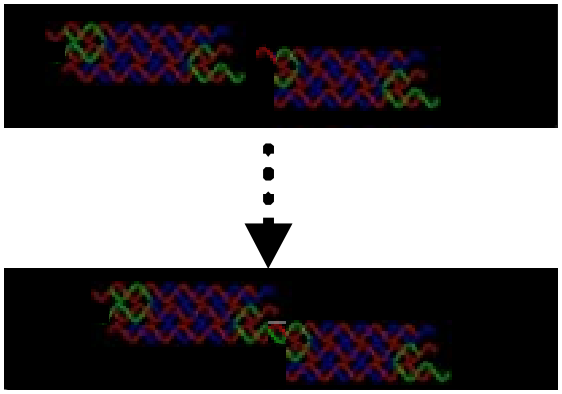

Fig. 4. The binding of DNA tile pad pairs. The two tiles interact by hybridization at their adjacent pads to form a two-tile assembly.

Software for the optimized design of DNA tiles was first developed in Mathlab by Winfree. This software used a greedy search method to optimize the choice of DNA strands comprising the DNA tiles. The software was improved at Duke Univ. by Bo [Bo01] to allow for a more sophisticated optimization heuristic (providing improved sequence specificity of the DNA words used for tile pads,

minimizing the likelihood of incorrect hybridizations from non-matching pads), to include more realistic models of DNA hybridization, and to provide a Java interface. (see Figure 5.)

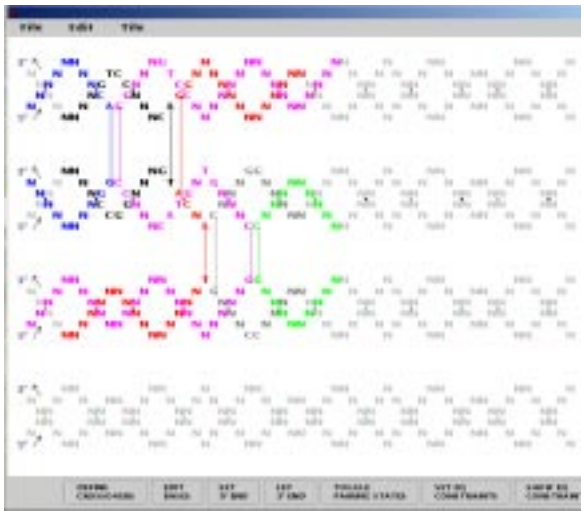

Fig. 5. An illustration of our Java software for design of a TX tile.

DNA Tiling Lattices. These are superstructures built up from smaller component structures(DNA tiles). Individual DNA tiles interact by annealing with other specific tiles via their ssDNA pads to self-assemble into desired superstructures. These lattices can be either: **(a)** *non-computational,* containing a fairly small number of distinct tile types in a repetitive, periodic pattern; or **(b)** *computational,* containing a larger number of tile types with more complicated association rules which perform a computation during lattice assembly. The direct assembly of DNA lattices from component ssDNA has been demonstrated for non-computational DNA lattices described below.

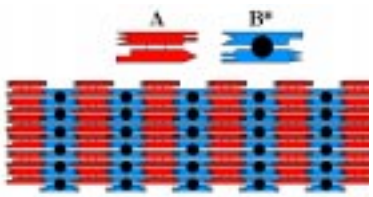

Fig. 6. AB* Array. *Lattice formed from two varieties of DNA tiles, including one (B*) containing an extra loop of DNA projecting out of the lattice plane, faciliting atomic force microscope imaging of the lattice.*

Winfree and Seeman [Winfree, et al 98 and Mao, 99] demonstrated the self-assembly of two-dimensional periodic lattices consisting of at hundreds of thousands of double-crossover tiles, which is strong evidence of this approachs scalability. In addition, LaBean, Reif, and Seeman [LaBean, et al 00] have constructed DNA TX molecules which produced tiling lattices of even larger numbers of tiles. Both classes of self-assembled DNA lattices were observed through atomic force microscopy (AFM), a mechanical scanning process that provides images of

molecular structures on a two-dimensional plate, as well as by use of transmission electron microscopy (TEM). Distinguishing surface features can be designed into individual tiles by slightly modifying the DNA strands composing the tiles. These modified DNA strands form short loops that protrude above the tile. (See Figure 6.)

To enhance definition, we have also affixed metallic balls to these DNA loops using known methods for affixing gold balls to DNA. Surface features - such as two-dimensional banding patterns - have been programmed into these DNA lattices by using DNA tiles that assemble into regular repetitive patterns.

These topographical features were observed on the DNA tiling lattices with atomic force and transmission electron microscopy imaging devices [Winfree et al., 98; Liu et al., 99; Mao, et al 99]. (See Figure 7.) These tiling assemblies

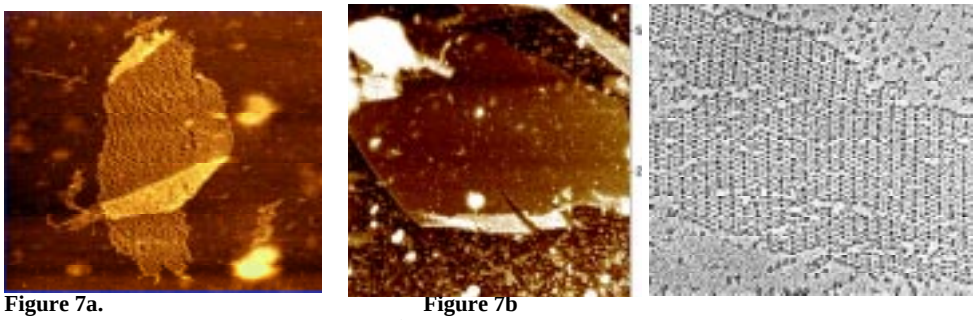

Figure 7a.
Figures 7a and 7b *give AFM images of DNA lattices with TX tiles 3-4 microns on a side.*

Figure 7b

Figure7c. *TEM image of platinum rotary shadowed TX lattice.*

Fig. 7. DNA tiling lattices.

had no fixed limit on their size. Recall that [Reif97] introduced the concept of a *nano-frame*, which is a self-assembled nanostructure that constrains the subsequent timing assembly (e.g., to a fixed size rectangle). A tiling assembly might be designed to be *self-delimitating* (growing to only a fixed size) by the choice of tile pads that essentially 'count' to their intended boundaries in the dimensions to be delimitated.

Directed Nucleation Assembly Techniques. We have recently developed another method for assembly of complex patterns, where an input DNA strand is synthesized that encodes the required pattern, and then specified tiles assemble around blocks of this input DNA strand, forming the required 1D or 2D pattern of tiles. This method makes the use of artificially synthesized DNA strands that specify the pattern and around which 2D DNA tiles assemble into the specified pattern; in this method, the permanent features of the 2D pattern are generated uniquely for each case. (See Figure 8.)

Application to Layout of Molecular-Scale Circuit Components. Molecular-scale circuits have the potential of replacing the traditional microelectronics with densities up to millions of times current circuit densities. There have been a

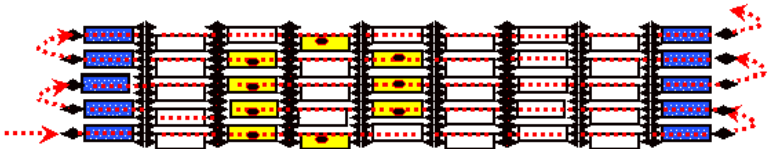

Fig. 8. In red is an input pattern strand of DNA. It encodes a 2D pattern in modified row major order (each odd row traversing alternately left to right, and each even row traversing right to left). Specific DNA tiles self-assemble around each segment of this input pattern strand of DNA. Then the tiles self-assemble into a 2D tiling lattice with a pattern determined by the pattern strand. A small instance of this method has been successfully executed where up to 10 TX tiles assembled around a preformed scaffold DNA strand.

number of recent efforts to design molecular circuit components ([Petty et al 95] [Aviram,Ratner98]). Tour at Rice Univ. in collaboration with Reed at Yale have designed and demonstrated [Chen et al 99] organic molecules that act as conducting wires [Reed et al.97],[Zhou99] and also rectifying diodes (showing negative differential resistance (NDR), and as well as [CRR+,99], [RCR+,00], and have the potential to provide dynamic random access memory (DRAM) cells. These generally use $\sim 1,000$ molecules per device, but they have also addressed single molecules and recorded current through single molecules [BAC+96], [RZM+97]. These molecular electronic components make conformational changes when they do electrical switching. One key open problem in molecular electronics is to develop molecular electronic components that exhibit restoration of a signal to binary values; one possible approach may be to make use of multi-component assemblies that exhibit cooperative thresholding. A key problem is to develop methods for assembling these molecular electronic components into a molecular scale circuit. Progress in the molecular circuit assembly problem could have revolutionary impact on the electronic industry, since it is one of the key problems delaying the development of molecular-scale circuits. As explained in the Introduction, the usual approach of laying out circuits by top-down techniques (e.g., lithography) is not practical at the molecular scale; instead bottom-up approaches (e.g., self-assembly) need to be used. Hence this may be a key area of application of DNA tiling assemblies. There are a number of possible methods for the selective attachment of the molecular electronic components to particular tiles of the DNA tiling array, using annealing: (i) using DNA as a selective assembly glue for linking chemistry between and molecular electronics [Tour and Bunz, 00], and (ii) the use of gold beads with attached DNA strands that can hybridize at selected locations of a self-assembled DNA arrays, so the molecular electronics components may self-assemble between the gold breads. Also, DNA lattices may be useful as a foundation upon which to grow nano-scale gold wires. This might be done by depositions of gold from colloid onto nano-spheres immobilized on DNA tiling lattices. Molecular probe devices may be used to test the electrical properties of the resulting molecular circuit attached to the DNA tiling array. *Computational* lattices (as opposed to regular, non-computational lattices), may also be employed to provide for the layout of highly complex circuits, e.g., the layout of the electronic components of an arithmetic unit. (For a discussion of possible schemes for encorporating molecular motors into tiling assemblies, see [Reif, et al 00].)

4 Computation by DNA Self-Assembly.

Programming Self-Assembly of DNA Tilings. Programming DNA self-assembly of tilings amounts to the design of the pads of the DNA tiles (recall these are sticky ends of ssDNA that function as programmable binding domains, and that individual tiles interact by annealing with other specific tiles via their ssDNA pads to self-assemble into desired superstructures). The use of pads with complementary base sequences allows the neighbor relations of tiles in the final assembly to be intimately controlled; thus the only large-scale superstructures formed during assembly are those that encode valid mappings of input to output. The self-assembly approach for computation only uses four laboratory steps: (i) mixing the input oligonucleotides to form the DNA tiles, (ii) allowing the tiles to self-assemble into superstructures, (iii) ligating strands that have been co-localized, and (iv) then performing a single separation to identify the correct output.

The Speed of Computing via DNA Tiling Assemblies (compared with silicon-based computing.) The speed of DNA tiling assemblies is limited by the annealing time, which can be many minutes, and can be 10^{10} slower than a conventional computer. A DNA computation via self-assembly must take into account the fact that the time to execute an assembly can range from a few minutes up to hours. Therefore, a reasonable assessment of the power of DNA computation must take into account both the speed of operation as well as the degree of massive parallelism. Nevertheless, the massive parallelism (both within assemblies and also via the parallel construction of distinct assemblies) possibly ranging up to 10^{18} provides a potential that may be advantageous for classes of computational problems that can be parallelized.

String-Tiles: A Mechanism for Small-Depth Tiling. An approach for small-depth computations is to compress several tile layers into single tiles, so that the simplest form of linear self-assembly suffices. Linear self-assembly schemes for integer addition were first described by [Reif97]; in this scheme each tile performed essentially the operation of a single carry-bit logic step. This linear self-assembly approach works particularly well when the topology and routing of the strands in the DNA tiles is carefully considered, leading to the notion of string tiles. The concept of string tile assemblies derives from Eng's observation that allowing neighboring tiles in an assembly to associate by two sticky ends on each side, he could increase the computational complexity of languages generated by linear self-assembly. [Winfree99a] showed that by allowing contiguous strings of DNA to trace through individual tiles and the entire assembly multiple times, surprisingly sophisticated calculations can be performed with 1-layer linear assemblies of string tiles. The TAE tiles recently developed by [LaBean, et al 99] are particularly useful as string tiles.

Input/Output to Tiling Assemblies Using Scaffold and Reporter Strands. Recall that the TX tiles are constructed of three double-helices linked by strand exchange. The TX tiles have an interesting property, namely that certain distinguished single stranded DNA (to be called scaffold and reporter strands, respectively) wind through all the tiles of a tiling assembly. This property provides a more sophisticated method for input and output of DNA computations in string

tiling assemblies. In particular, there are two types of ; the TAE tile contains an Even (and the TAO tiles contains an Odd) number of helical half-turns between crossover points. Even spacing of crossovers of the TAE tile allows reporter strands to stretch straight through each helix from one side of the tile to the other. These reporter segments are used for building up a long strand which records inputs and outputs for the entire assembly computations.

(a) Input via Scaffold Strands: We take as input the scaffold strands and which encode the data input to the assembly computation. They are long DNA strands capable of serving as nucleation points for assembly. Preformed, multimeric scaffold strands are added to the hybridization/annealing mixture in place of the monomeric oligo corresponding to the tile's reporter segment. The remaining portion of the component ssDNA comprising the tiles are also added. In the resulting annealing process, tiles assemble around the scaffold strand, automatically forming a chain of connected tiles which can subsequently be used as the input layer in a computational assembly.

(b) Output via Reporter Strands: After ligation of the tiling assembly (this joins together each tiles segments of the reporter strands), the reporter strand provides an encoding of the output of the tiling assembly computation (and typically also the inputs). Note this input/output can occur in parallel for multiple distinct tiling assemblies. Finally, the tiling assembly is disassembled by denaturing (e.g., via heating) and the resulting ssDNA Reporter Strands provide the result (these may be used as scaffold strands for later cycles of assembly computation, or the readout may be by PCR, restriction cutting, sequencing, or DNA expression chips).

One Dimensional DNA Tiling Computations for Parallel Arithmetic. We now outline (See Figure 9.) procedures for using the string tiles described above that self-assemble into linear tiling assemblies to perform massively parallel arithmetic. [LaBean, et al 99] describes string tile systems that compute binary number addition (where the binary numbers are encoded by strands of DNA) by using two distinct sets of sticky-ends between adjacent tiles in the assembly to effectively communicate the values of the carry-bits. (They can also be used for computation of bit-wise XOR of Boolean vectors encoded by strands of DNA.) The assemblies result in the appending of these strands to the addition sums. For computations on specific inputs, these procedures make use of the scaffold strands mentioned above. The inputs are self-assembled strands of DNA composed of sequences DNA words encoding the pairs of binary numbers to be summed. Otherwise, the input tiles can be (using known techniques uses for the assembly of combinatorial libraries of DNA strands) randomly assembled and thereby generate a molecular look-up table in which each reporter strand encodes the random inputs and resultant outputs of a single calculation. After denaturing the assemblies back to individual strands, one may sample the resulting reporter strands to verify the outputs are correctly computed. A sufficient number of DNA tile molecules provide full coverage of all possible n-bit input strings. Such look-up tables may be useful as input for further computations as they represent a unique library of sequences with a complex structural theme.

An experimental demonstration of an XOR tiling computation based on TAO tiles is reported in [Mao, LaBean, Reif, and Seeman, 00].

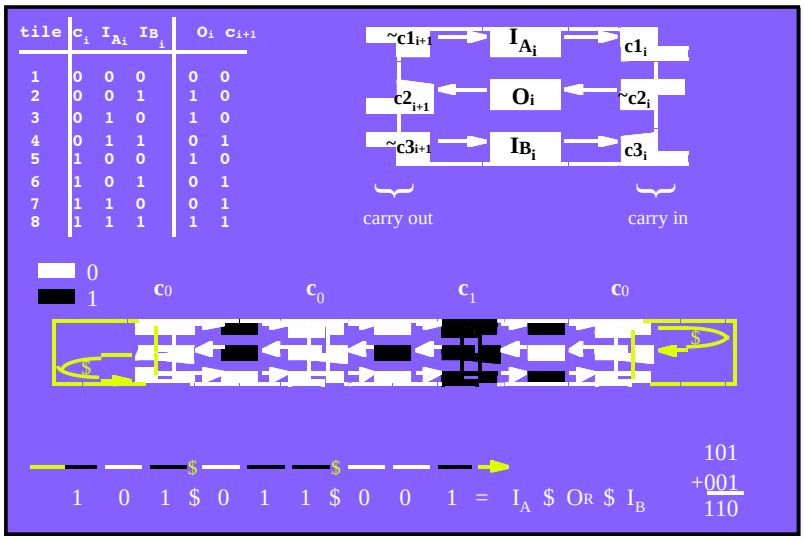

Fig. 9. String Tile Addition with TAE Building Blocks. *Upper left shows the truth table for addition; one tile type will be required for each row in the table. Upper right shows a schematic of a tile including the sequence positions for encoding values for input bits (IAi and IBi), the output bit (Oi), and the carry bit values on the tile's sticky-ends. The center schematic shows a five tile complex carrying out the addition of two 3-bit numbers. Arrows indicate the trace of the reporter strand as it winds through the entire assembly three times. The left and right extreme tiles act to reroute the reporter strand back through the lattice. The two corner tiles have been successfully built and shown to properly associate with one another.*

Two Dimensional DNA Tiling Computations. In the immediate future, it may be possible to extend the one dimensional DNA tiling assembly methods to two dimensional tilings, and to demonstrate these methods experimentally. One interesting goal is integer multiplication. The most direct and relatively straightforward way is to multiply via repeated additions and bit shifts, applying known VLSI systolic array architecture designs for integer multiplication. This would require a two dimensional $n \times n$ tiling assembly, with some increased complexity over the linear assembly for integer addition. Two dimensional computational tilings may also be used to do logical processing. [Lagoudakis and LaBean,99] proposed a 2D DNA self-assembly for Boolean variable satisfiability, which uses parallel construction of multiple self-assembling 2D DNA lattices to solve the problem. Such methods for solving combinatorial search problems do not scale well with the input size (the number of parallel tiling assemblies grows exponentially with the number of Boolean variables of the formula). However, similar constructions may be used for evaluating Boolean queries and circuits in massively parallel fashion, for multiple input settings of the input Boolean variable, and in this context it may be appropriate to consider the Boolean formulas a to be of fixed size.

Three Dimensional DNA Tiling Computations. There is a number of possible methods for executing computations experimentally on 3D DNA lattices, providing computations with (implicit) data movement in three dimensions. Matrix inner produc might be executed by a three dimensional computational tiling by applying known VLSI systolic array architecture designs for matrix inner product. Another possible three dimensional computational tiling is that of the time-evolution (time is the third dimension of the tiling) of a 2D cellular automata (e.g., 2D cellular automata simulation of fluid flow).

5 Conclusion

The self-assembly of DNA tilings is a promising emerging method for molecular scale constructions and computation. We have overviewed the theory of DNA tiling self-assemblies and noted a number of open problems. We have discussed the potential advantages of self-assembly techniques for DNA computation; particularly the decreased number of laboratory steps required. We also discussed the potential broader technological impacts of DNA tiling lattices and identified some technological impacts of non-computational DNA assemblies: including their use as substrates for surface chemistry and particularly molecular electronics, robotics. Many of these applications are dependent on the further development of the appropriate attachment chemistry between DNA and the molecules attached to the arrays.

Error Control in DNA Tiling Assemblies. A chief challenge in DNA tiling self-assemblies is the control of assembly errors. As stated above, two dimensional self-assembled *non-computational* tilings have been demonstrated (and imaged via atom force microscopy) that involve up to a hundred thousand tiles. Certain of these appear to suffer from relatively low defect rates, perhaps in the order of less than a fraction of a percentage or less. But even such low error rates should be reduced. The factors influencing these defect rates are not yet well understood and there are no known estimates on the error-rates for self-assembled *computation* tilings, since such tilings have been achieved only very recently and have only been done on a very small scale(error rates appear to be less than 5% [Mao et al 00]). There is reason (see the construction of a potential assembly blockage described in [Reif, 98]) to believe that in computational tilings, defect errors may be more prevalent; and moreover, they can have catastrophic effects on the computation. Experiments need to be done to determine the error rates of the various types of self-assembly reactions, both computational and non-computational. There are a number of possible methods to decrease errors in DNA tilings: *(a) Annealing Temperature Optimization* is a well known technique used in hybridization and also crystallization experiments. It can be used decrease in defect rates at the expense in increased overall annealing time duration. In the context of DNA tiling lattices, the parameters for the temperature variation that minimize defects have not yet been determined. *(b) Error Control by Redundancy.* There are a number of ways to introduce redundancy into a computational tiling assembly. One simple method that can be developed for linear tiling assemblies, is to replace each tile with a stack of three tiles executing the same function, and then add additional tiles that essentially 'vote' on the pad associations associated with these redundant tiles. This results

in a tiling of increased complexity but still linear size. This error resistant design can easily be applied to the integer addition linear tiling described above, and similar redundancy methods may be applied to higher dimension tilings. *(c) Error Control by step-wise assembly:* [Reif, 98] suggested the use of serial self-assembly to decrease errors in self-assembly. It is as yet uncertain which of these methods will turn out to be effective and it is likely that a combination of at least a few of the methods will prove most effective.

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