Challenges and Applications for Self-Assembled DNA Nanostructures *

John H. Reif ** , Thomas H. LaBean and Nadrian C. Seeman

Duke University   New York University

Abstract. *DNA self-assembly* is a methodology for the construction of molecular scale structures. In this method, artificially synthesized single stranded DNA self-assemble into DNA crossover molecules (tiles). These DNA tiles have sticky ends that preferentially match the sticky ends of certain other DNA tiles, facilitating the further assembly into tiling lattices. We discuss key theoretical and practical challenges of DNA self-assembly, as well as numerous potential applications.

The self-assembly of large 2D lattices consisting of up to thousands of tiles have been recently demonstrated, and 3D DNA lattices may soon be feasible to construct. We describe various novel DNA tiles with properties that facilitate self-assembly and their visualization by imaging devices such as atomic force microscope. We discuss bounds on the speed and error rates of the various types of self-assembly reactions, as well as methods that may minimize errors in self-assembly. We briefly discuss the ongoing development of attachment chemistry from DNA lattices to various types of molecules, and consider application of DNA lattices (assuming the development of such appropriate attachment chemistry from DNA lattices to these objects) as a substrate for:

(a) layout of molecular electronic circuit components,

(b) surface chemistry, for example ultra compact annealing arrays,

(c) molecular robotics; for manipulation of molecules using molecular motor devices.

DNA self-assembly can, using only a small number of component tiles, provide arbitrarily complex assemblies. It can be used to execute computation, using tiles that specify individual steps of the computation. In this emerging new methodology for computation:

-input is provided by sets of single stranded DNA that serve as nucleation sites for assemblies, and

* A postscript version of this paper is at URL http://www.cs.duke.edu/~reif/paper/SELFASSEMBLE/selfassemble.ps .

** Contact address: Department of Computer Science, Duke University, Box 90129, Durham, NC 27708-0129. E-mail: reif@cs.duke.edu. John Reif is supported by Grants NSF/DARPA CCR-9725021, CCR-96-33567, NSF IRI- 9619647 and EIA-0086015, ARO contract DAAH-04-96-1-0448, and ONR contract N00014-99-1-0406. Nadrian C. Seeman is supported by Grants GM-29554 from the National Insititute of General Medical Sciences, ONR contract N00014-98-1-0093, DARPA/NSF grant NSF-CCR-97-2502, AFSOR contract F30602-98-C-0148 NSF grants CTS-9986512 and EIA-0086015.

-output can be made by the ligation of reporter strands of DNA that run though the resulting assembly, and then released by denaturing.
DNA self-assembly can be used to execute massively parallel computations at the molecular scale, with concurrent assemblies that may execute computations independently. Due to the very compact form of DNA molecules, the degree of parallelism (due to distinct tiling assemblies) may be up to 10^{15} to possibly 10^{18}. We describe various DNA tiling assemblies that execute various basic computational tasks, such as sequences of arithmetic and logical computations executed in massively parallel fashion. We also consider extensions of these computational methods to 3D DNA tiling lattices and to assemblies that hold state.

1 Introduction to Tiling Self-Assemblies

1.1 Self-Assembly. This is a process involving the spontaneous self-ordering of substructures into superstructures.

Biological Self-Assembly. We take inspiration from the cell, which performs 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. We will focus instead on self-assemblies whose components are artificially constructed tiles.

1.2 Domino Tiling Problems. These were defined by Wang [Wang61] (Also see the 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 (the pads). 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 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.)

Domino tiling problems over an infinite domain with only a constant number of tiles was first proved by [Berger66] to be undecidable; proofs rely on constructions wherein tiling patterns simulate single-tape Turing Machines (see also [Berger66, Robinson71, Wang75]). Other results include reductions of NP-complete problems to finite-size tiling problems [LewisPapa81, Moore00]

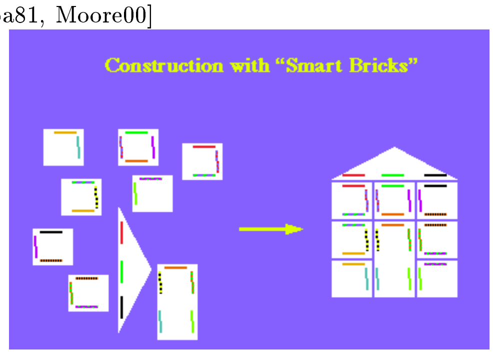

Fig. 1. A tiling assembly using 'Smart Bricks' with affinity between colored pads.

1.3 Self-Assembly of Tiling Lattices. Domino tiling problems do not presume or require a specific process for tiling. However, we will presume the use of the self-assembly processes for construction of tiling lattices. In this self-assembly process, the preferential matching of tile sides facilitates the further assembly into tiling lattices.

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.

Since domino tiling problems are undecidable (see Section 3), tiling self-assemblies can theoretically provide arbitrarily complex assemblies even with a constant number of distinct tile types. As a very simple example, it possible to construct tiling assemblies with self-delimiting boundaries (e.g., rectangular boundaries of a give width w and length h), by use of a set of wh distinct tiles, with w distinct pads types on the bottom and top of a set of square tiles and a set of h pad types on the other sides of these tiles, and this can also be done with a constant number of distinct tiles. (See [Rothemund and Winfree, 2000b] for improved program-size complexity of self-assembled squares.)

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 are a variety of distinct materials for tiles, at a variety of scales:
(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).
(b) 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.

1.4 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. We give in Section 2 a description of self-assembly techniques for DNA tilings and discuss applications in Section 3. Section 4 describes DNA tiling computations and their applications. Section 5 discusses the kinetics of self-assemblies and error control and Section 6 concludes the paper.

2 DNA Self-Assembly of DNA Tilings

2.1 DNA as a Construction Material. Nano-fabrication of structures in DNA was pioneered by the Seeman laboratory ([Seeman82, 94b, 96a]), who built a multitude of DNA nano-structures using DNA branched junctions [Seeman89, Wang91a, Du92]. These previous systems were flexible, so control over synthesis and proof of synthesis were both limited to the topological level, rather than the geometrical level (in contrast to the tiles described below). These DNA nano-structures included: DNA knots [Seeman93], Borromean rings [Mao97], a cube [Chen91], and a truncated octahedron [Zhang94] (reviewed in e.g. [Seeman98] [Seeman94b] and [Seeman96a]).

2.2 DNA Tiles Constructed from DX and TX Complexes. The building blocks in the tiling constructions to be discussed are branched DNA complexes, which we call *DNA tiles*, consisting of several individual DNA oligonucleotides that associate with well-defined secondary and tertiary structure (see [Winfree, et al 98] and below description of DX and TX tiles). These associate with well-defined secondary and tertiary geometric structure (which is much more predictable and less flexible than DNA nano-structures using DNA branched junctions). These complexes come in a number of varieties that differ from one another in the geometry of strand exchange and the topology of the strand paths through the tile. The branched DNA complexes used for tiling assemblies include the double-crossover (DX) and triple-crossover (TX) complexes, The DX and TX complexes consists of two (three, respectively) double-helices fused by exchange (crossover) of oligonucleotide strands at a number of separate crossover points. Anti-parallel crossovers cause a reversal in direction of strand propagation through the tile following exchange of strand to a new helix. For example, DAO and DAE are double-crossover DX tiles with two anti-parallel crossovers[1]. (See Figure 2.) .

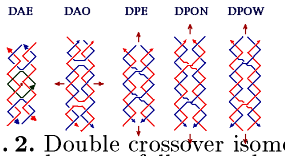

Fig. 2. Double crossover isomers.

DX complexes have been used successfully as substrates for enzymatic reactions including cleavage and ligation [Liu, Sha and Seeman,99]. The TX (see [LaBean,99]) TAO and TAE tiles, are similar except that they have three double-helices interlocked by exchange of oligonucleotide strands at four separate crossover points, two between the first pair of helices, two between the second (See the TAO in Figure 3 and the TAE in Figure 4. Both DX and TX motifs are useful for tiling assemblies; the DX (TX) complexes provide up to four (six, respectively) ssDNA pads [Liu, et al 99a] for encoding associations with neighboring tiles.

2.3 DNA Tiling Lattices. Tile assemblies, or simply tilings, can be defined as superstructures or lattices built up from smaller, possibly repetitive, component structures. Individual 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.

---

[1] The structure of the TAE resembles the TAO in that it is constructed of three double-helices linked by strand exchange, however, it contains an Even (rather than Odd) number of helical half-turns between crossover points. Even spacing of crossovers allows reporter strands (shown in black) to stretch straight through each helix from one side of the tile to the other. These three horizontal reporter segments are used for building up a long strand which records inputs and outputs for the entire assembly computations. A 3D confirmation of the TAE tile has been rendered by Brendon Murphy at Duke University; see http://www.duke.edu/∼bkm2/taehtml/present.html .

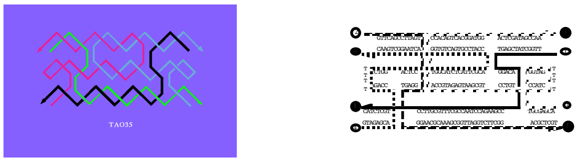

Fig. 3. The TAO tile and a Strand and Sequence Trace through the TAO Tile.

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*.

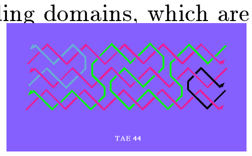

Fig. 4. The TAE Tile.

These DNA self-assembly procedures generally will be described as occurring in two distinct stages:

(i) annealing of ssDNA into tiles; and

(ii) assembly of tiles into superstructures.

However, direct assembly of DNA lattices from component ssDNA is also possible, and has in fact already been demonstrated for non-computational DNA lattices described below.

2.4 Two Dimensional DNA Tiling Assemblies. Recently Winfree and Seeman have demonstrated the use DX tiles to construct 2D periodic lattices consisting of upto a hundred thousand DX units [Winfree, et al 98] as observed by atomic force microscopy[2] The surface features are readily programmed [Winfree et al., 98; Liu et al., 99; Mao, et al 99]. (See Figure 5.) In addition, [LaBean et al, 99] constructed produced tiling arrays (see Figure 6) composed of DNA triple crossover molecules (TX); these appear to assemble at least as readily as DX tiles. These tiling assemblies had no fixed limit on their size. [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). Alternatively, 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.

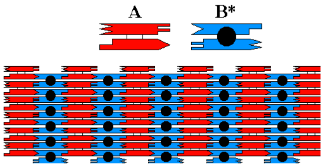

Fig. 5. AB* Array. *Lattice formed from two varieties of DX, one containing an extra loop of DNA projecting out of the lattice plane, faciliting atomic force microscope imaging of the lattice.*

2.5 Three Dimensional DNA Tiling Assemblies. There are a number of possible methods for constructing 3D periodic (non-computational) tilings. For example, stable tiling arrays with well-defined helices that come out of the plane (e.g., the TX tiling array constructed in [LaBean et al, 99]) may lead to ways to provide 3D tiling assemblies.

[2] An atomic force microscope [AFM] is a mechanical scanning device that provides images of molecular structures laying on a flat 2D plate.

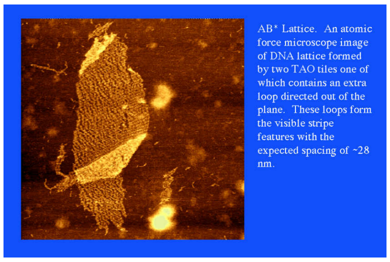

Fig. 6. A non-computational DNA tiling formed by sets of two types of TAO tiles.

3 Applications of Non-Computational DNA Tiling Arrays

We now identify some further technological impacts of non-computational DNA assemblies; particularly their use as substrates for surface chemistry and molecular electronics, robotics. Many of these applications are dependant on the development of the appropriate attachment chemistry between DNA and the molecules attached to the arrays.

3.1 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 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 (see Figure 7) 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 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.

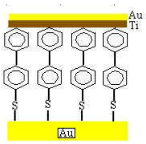

Fig. 7. The Tour-Reed molecular electronic diode.

The Molecular Circuit Assembly Problem: This 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.

Top-down techniques versus bottom-up approaches. The usual approach of laying out circuits by top-down techniques (e.g., lithography) may not be practical at the molecular scale; instead bottom-up approaches (e.g., self-assembly) may 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) linking chemistry between DNA and molecular electronics. Tour and Bunz recently prepared DNA-linked systems where the DNA could serve as a selective assembly glue for device configurations [WST+00].

(ii) The use of gold beads. In this approach, DNA strands attached to the gold beads can hybridize at selected locations of the arrays, and 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. (See Figures 8 and 9.)

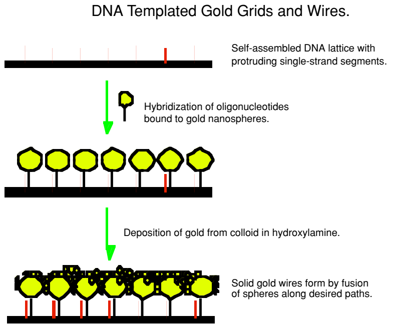

Fig. 8. Diffusion of gold on beads to form molecular-scale gold wires.

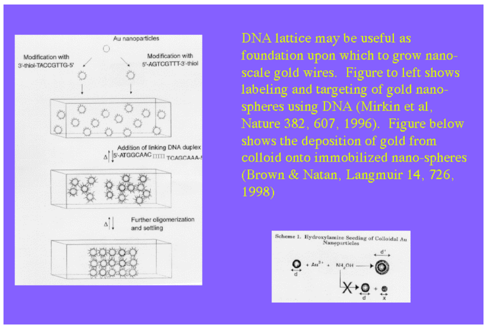

Fig. 9. A scheme for molecular-scale gold wires.

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.

(Other related approaches for positioning of molecular electronic molecules without lithography include that of [Bumm, et al., 1999, 2000], which describes the use of directed self-assembly of molecular terrace structures in organic monolayers.)

3.2 Application to Surface Chemistry and Impact on Biotechnology. One intriguing application for DNA lattices is their use as an attachment substrate for an array of DNA strands, using hybridization with single stranded DNA on individual tiles. This has a number of applications that impact DNA computations (e.g., see Brockman, et al 98][Smith,98]), as well as more general biotechnology:

(a) It may provide a dramatic miniaturization of the DNA chip technology (a technology that we have noted above might be used for I/O in DNA computations, among other applications), to molecular scale aspect widths.

(b) It may provide a dramatic miniaturization of DNA computation methods using surface chemistry [Corn, et al 99], again to molecular scale aspect widths.

Two dimensional DNA tiling lattices may in the future be self-assembled with each of the tiles modified to have dangling *hybridization*strands, which will be single stranded, as used by [Liu et al., 1999]. These hybridization strands will be thus assembled in a very regular, dense fashion, and may have sequences determined by the computational tiling. The differential hybridization of these hybridization strands may be demonstrated with fluorescence tagged complementary DNA.

(3.3) Tiling Assemblies with Molecular Motors. Several types of molecules are known to couple chemical energy to the generation mechanical force, thereby functioning as molecular motors. Possible schemes for molecular motors include:

(a) Re-Engineering Biological Molecular Motors. Cells make use of a variety of such motor-like devices in processes such as mitosis. The best characterized of these fall into three categories.

(i) *ATP synthase and ADP* acts as a rotary motor, coupling proton flux through a membrane with the phosphorylation of ADP to ATP. The F1 component of ATP synthase can also be run in reverse, coupling hydrolysis of one ATP molecule to 120 B0 of rotation about the motor axis.

(ii) *Myosin* acts as a molecular running machine, skipping many steps along an actin filament with each molecule of ATP consumed. All of these motors are modular and can be re-engineered to accomplish linear or rotational motion of essentially any type of molecular component.

(iii) *Kinesin* acts as a molecular walking machine, translocating itself (and any attached components) in step-wise fashion along a microtubule. Each step along the microtubule consumes one ATP molecule.

Construction of these biological molecular motors and their linking chemistry to DNA arrays. These motors are composed of proteins with well known transcription sequences. There are also well known proteins (binding proteins) that provide linking chemistry to DNA. Hence it seems feasible that these molecular motors and attached linking elements may be synthesized from sequences obtained by concatenation of these transcription sequences. [Bachand, et al., 1999 and 2000], describes a biomolecular motor constructed of expressed ADP protein [Montemagno,et al, 1998 and 1999] with an attached [Soong, et al, 1999] silicon arm.

(b) DNA Motors. The Seeman laboratory made a DNA construction of a mechanical device capable of controlled movement [Mao, et al 99a, Seeman, et al 99]. This device consists of two DX molecules connected by a DNA double helix that contains a segment of DNA that can be converted to the left-handed Z-DNA structure. In B-promoting conditions, the two unconnected helices of the device are on the same side of the connecting helix, but they are on opposite sides in Z-promoting conditions. This results in an apparent rotary motion of about a half-revolution, leading to atomic displacements ranging from 2 to 6 nm, depending on the location of the atom relative to the axis of the stationary helix. This motion has been demonstrated by fluorescent resonance energy transfer (FRET). It is important to point out that the device based on the B-Z transition is only a prototype that was used to learn how to characterize motion in DNA systems. It lacks programmability, except to the limited extent that one can orient the two DX molecules at a variety of relative torsion angles in the B-state. Thus, all of the molecules must be in either the B-state or in the Z-state, assuming one has robust chemical control.

These molecular motors might be combined with the 2-D arrays, to achieve an array of devices (This has not been possible to do with the DX system, since it is most convenient there for the pivoting part of the system to point normal to the array. However, the TX system does not have this difficulty.). As an example, an array with attached kinesin may provide for the movement of objects across the surface of a two dimensional tiling array, similar to a conveyer belt, and this may be the basis of a transport system (*a molecular conveyer belt*) for molecular objects.

Programmable Sequence-Specific Control of DNA Mechanical Motion. However, such an array of molecular motors would be more useful if they can be selectively controled. Such a system would lead to the ability to manipulate specific molecules and more generally, to do chemistry at chemically identical but spatially distinct sites. Because it couples a series of distinct structural states with programmability, such a system offers the potential of direct route to molecular robotics. The Seeman laboratory is developing a related system based on the paranemic crossover (PX) system, which leads to sequence-specific nanomechanical motion. It may be switched readily between two discrete states, PX or JX, in which the helices at one end of the molecule reverse positions in the transition between states. An array of these molecular devices would contain individually programmed PX/JX molecules, whose conformational state would be amenable to specific reversal (or not, depending on the program) from cycle to cycle. A DNA array with programmability of this sort may offer a mechanism to do DNA computation of arrays whose elements (the tiles) hold state, as discussed in the next section.

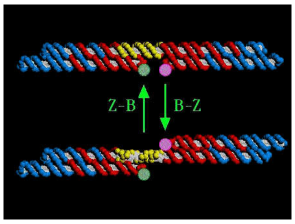

Fig. 10. A prototype DNA nanomechanical device.

4 Computation by Self-Assembled Tilings

4.1 DNA Computation. In his seminal paper on molecular computation [Adleman94], Adleman demonstrated the use of recombinant DNA techniques for solving a small combinatorial search problem. This work spawned considerable further work in DNA computation (See survey of [Reif, 1998]). However, one difficulty with such methods for DNA computation is the number of laboratory procedures, each time consuming and error-prone, grows with the size of the problem.

4.2 Computation by DNA Self-Assembly.

We now focus on another approach: computation by self-assembly. In this case Self-assembly is the spontaneous self-ordering of substructures into superstructures driven by annealing of Watson-Crick base-pairing DNA sequences. Computation by self-assembly entails the building up of superstructures from starting units such that the assembly process itself performs the actual computation. Adleman made use of a simple form of computation by self-assembly in his original experiment [Adleman94]: instead of blindly generating all possible sequences of vertices; instead, the oligonucleotide sequences and the logic of Watson-Crick complementarity guide the self-assembly processes so that only valid paths are generated. [Winfree95] generalized this approach to two-dimensional (2D) self-assembly processes and showed that computation by self-assembly is Turing-universal (also see prior Turing-universal results for tiling [Buchi62, Wang63, 75, Berger66, Robinson71] discussed below).

[Winfree96,Eng97] proposed self-assembly of linear, hairpin, and branched DNA molecules to generate regular, bilinear, and context-free languages, respectively. [Winfree96], Jonoska et al [Jonoska97, Jonoska98], and [Lagoudakis and LaBean,99] all proposed the use of self-assembled DNA nanostructures to solve NP complete combinatorial search problems (but the scale is limited to only moderate size problems at best).

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. Consequently, the difficulty mentioned previously (with respect to DNA computation) has been addressed: rather than implementing a DNA computing algorithm using a sequence of multiple laboratory procedures, our approach essentially uses only four:

(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.

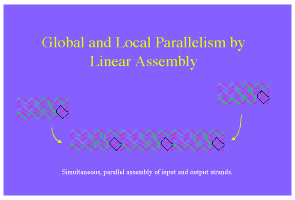

Fig. 11. Global and Local Assembly Parallelism. *Local tile association rules dictate that only valid computational lattices are able to form regardless of temporal ordering of binding events.*

4.3 Massively Parallel Computation by Tiling

The massive parallelism inherent in DNA-based computers has, since its inception, driven thinking in the field. In computation by self-assembly, parallelism reveals itself in many ways. Each superstructure may contain information representing a different calculation (*global parallelism*). Growth on each individual superstructure may occur at many locations simultaneously *local parallelism*. (See Figure 4.)

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. A superstructure consisting of an array of $n \times m$ tiles, where $n > m$, is said to have m layers. Again, although it needs further study, tiling systems with low depth, small size, and few layers are considered desirable, motivating the search for efficient computations performed by such systems. Tiling systems that produce only superstructures with k layers, for some constant k, are said to use *linear self-assembly.* As an example, the two tiling systems for addition discussed in [LaBean, et al 99] for $n-$bit sums produce linear superstructures that are arrays of size $3 \times n$ and $1 \times n$, but known tiling systems for multiplication produce $n \times n$ for n-bit products [Winfree99a], and hence are not linear.

[Reif97] described DNA self-assembly methods of linear size and small depth to solve a number of fundamental problems (e.g., prefix computation, permutation, certain integer arithmetic operations, finite state automata simulation, and string fingerprinting) that form the basis for the design of many parallel algorithms. Furthermore, these elementary operations can be combined to perform more complex computations, such as bitonic sorting and general circuit evaluation in $O(\log n)$ experimental steps.

4.4 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^{11} 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 from 10^{15} to 10^{18} provides a potential that may be advantageous for classes of computational problems that can be parallelized.

4.5 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.

4.6 Input/Output to Tiling Assemblies Using Scaffold and Reporter Strands

Input and output are critical to the practical use of DNA-based computing[3]Winfree [Winfree95] used the first and last layers of the assembly for input and output, respectively. The TAO and TAE tiles have an interesting property, namely that certain

[3] There are a number of known methods for synthesis of DNA from sequence information provided by conventional media, (e.g., by PCR, restriction cutting, or sequencing).but these provide limited I/O rates. While it is not the purpose of this work to develop improved biotechnology techniques for DNA sequence synthesis and sequencing, we note this is a key goal for an entire sector of the biotechnology industry, and therefore it is likely that the capabilities will significantly improve in the future. One of the most promising is the possible use of large arrays of (perhaps up to a $1,000$) DNA chips. Each DNA chip can have up to $100,000$ individually addressed locations, each capable of light-directed chemical synthesis of DNA strands. Similar technology, using fluorescently tagged DNA hybridized to DNA chip annealing arrays, can be used for output of DNA information to conventional media. This could potentially provide up to approximately a factor of $100,000,000$ parallelism in I/O. See [Reif, et al, 2000] for a discussion of these techniques and for methods for error-correction methods for DNA chip synthesis).

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:

(a) Input via Scaffold Strands: We take as input the scaffold strands and which encode the data input to the assembly computation. (See Figure 4.) 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).

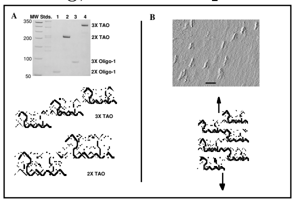

Fig. 12. Use of scaffold strands in assemblies.

4.7 One Dimensional DNA Tiling Computations or Parallel Arithmetic.

We now outline (See Figure 13.) 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 will be 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 will 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 this tiling is described in [Mao, LaBean, Reif, and Seeman, 2000].

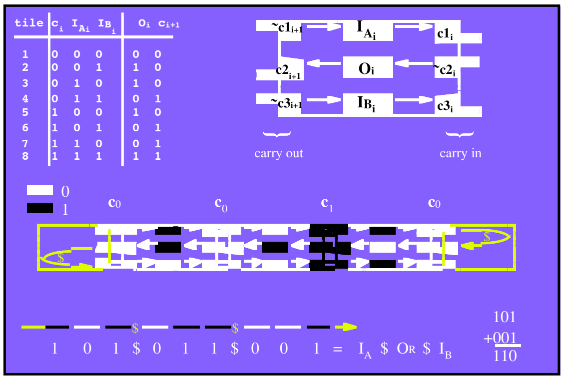

Fig. 13. String Tile Addition with TAE Building Blocks. *Upper left shows the truth table for addition; one tile type will be required for each row in the table. Upper right shows a schematic of a tile including the sequence positions for encoding values for input bits (IAi and IBi), the output bit (Oi), 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.*

4.8 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.

4.9 Three Dimensional DNA Tiling Computations.

There are 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 two dimensional cellular automata (e.g., a two dimensional cellular automata simulation of fluid flow).

4.10 Recycling of the Component ssDNA

In principle, after a cycle of tiling assembly and disassembly, the component ssDNA comprising these tiles can simply be separated (for example, by magnetic bead separation), and reused for further cycles of assembly computations. For non-computational tiling assemblies, heat cycling has exactly this affect, and a few such cycles does not appear to result in noticeable degradation of the component ssDNA. Such re-cycling methods might be used for computational tiling assemblies, though there seems no apparent reason why there would be more degradation in this case of computational assemblies. (If ssDNA degradation does occur over many cycles, one may need to consider methods for repair, such as enzymatic repair of component ssDNA.) Another possibly serious issue is that of contaminating the 'result' ssDNA holding the results of successive computations; for example these result ssDNA strands may be contaminated by partial products due to incomplete ligation of the tiling assembly. In principle, these errors might be corrected by additional steps that provide purification of the result ssDNA (e.g., separate out the ssDNA of the correct mass or having specific primers at the start and end of the calculation to amplify the correct answer by PCR) using conventional recombinant DNA techniques. Varied DNA backbones [e.g., Neilsen, 1995] may prove another approach to increasing the covalent stability

4.11 Arrays of Finite State Machines. A DNA array of motors, as described in the previous section, may offer a mechanism to do DNA computation of arrays whose elements (the tiles) hold state. That is, the DNA assemblies may be able to simulate a parallel computing model known as cellular automata, which consist of arrays of finite state automata, each of which holds state. The transitions of these automata and communication of values to their neighbors might be done by conformal (geometry) changes, again using this programmability. There are numerous examples of 1 D (2 D, respectively) cellular automata that can do computations for which tiling assemblies would have required a further dimension (for example, integer multiplication in one dimension instead of two).

5 The Kinetics and Error Control in Self-Assembled Tiling Assemblies

5.1 Kinetics of Self-Assembled Tiling Assemblies

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 not yet well understood. For example, it is not yet known the affect of distinct tile concentrations and different relative numbers of tiles, and the possible application of Le Chatelier's principle.

Winfree [W98] developed computer simulation of tiling self-assemblies. This software makes a discrete time simulation of the tiling assembly processes, using approximate probabilities for the insertion or removal individual tiles from the assembly. These simulations provide an approximation to the kinetics of self-assembly chemistry. Using this software as a basis, Guangwei Yuan at Duke developed improved 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. This simulation software was recently speed up by use of an improved method for computing on/of likelihood, as suggested by Winfree.

The meso-scale tiling experiments described in Section 1 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.

5.2 Error Control in Self-Assembled Tiling Assemblies

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. 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. It is as yet uncertain which methods will turn out to be effective and it is likely that a combination of at least a few of the following methods will prove most effective.

(a) Error Control by Annealing Temperature Variation. This is a well known technique used in hybridization and also crystallization experiments. It is likely that this will provide some 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 Improved Sequence Specificity of DNA Annealing. The most obvious methodology here is to improve the choice of the DNA words used for tile pads (that is, to decrease the likelihood of incorrect hybridizations from non-matching pads).DNA word design software for DNA tiles was developed by Winfree. This software has recently been improved by Horatiu Voicu at Duke University (see http://www.cs.duke.edu/∼hvoicu/app.html) to include more realistic models of DNA hybridization, and was also speed up by use of a technique of Rajasakaran and Reif [RR95] known as nested annealing. Another possible approach is to use the observation and experimental evidence of [Herschlag 91] that stressed DNA molecules can have much higher hybridization fidelity (sequence specificity) than a relaxed molecule. This would entail redesigning DNA tiles, so their pads are strained single stranded loop segments with higher sequence specificity, or by the use of stressed DNA motifs known as "PX dumbbells" [Shen, Z. Ph.D. Thesis, NYU, 1999].

(c) 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.

(d) Error Control by Free Versus Step-wise Assembly. Self-assembly may be restricted so that certain assembly reactions can proceed only after others have been completed (*serial (or step-wise) self-assembly*). Alternatively, self-assembly reactions may be limited by no such restrictions *free self-assembly*). It is expected, but unproven, that free self-assembly is faster than serial self-assembly. [Reif, 98] suggested the use of serial self-assembly to decrease errors in self-assembly. There is not yet any experimental data on the error rates of self-assembly reactions and error control/repair of 'self-assembly' versus 'serial-assembly'. To decrease the human effort in serial assembly, assembly steps might be executed automatically with the use of a robotic machines (E.g., the Nanogen machine, which employs a chip that contains DNA sequences above electrodes. Tile components hybridized to these DNA sequences can be released in sequence by making the electrode sufficiently negative.).

(e) Use of DNA Lattices as a Reactive Substrate for Error Repair. DX complexes and lattices have been used successfully as substrates for enzymatic reactions including restriction cleavage and ligation of exposed hairpins attached to the tiles [Liu99a]. One approach is the use of DNA lattices to execute a broader class of reactions. For example, if restriction enzymes, topoisomerases or site-specific recombinases can be shown to operate on exposed portions of the DNA lattices, then it may be possible to modify the topology and geometry of the DNA lattice. This may aid in the DNA tiling computations described above, for example by providing mechanisms for error repair in DNA tiling computations. (Note: as mentioned above, this approach may also be of use for recycling of the component ssDNA for the next computation cycles.)

6 Conclusion

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 identify some further possible applications. The chief difficulties are that of error control and predicable kinetics, as described in the previous section. Nevertheless, the self-assembly of DNA tilings seems a very promising emerging method for molecular scale constructions and computation.

References:

1. Adleman, L., Molecular Computation of Solution to Combinatorial Problems, Science, 266, 1021, (1994).
2. Aviram and M. Ratner (Eds), Molecular Electronics: Science and Technology, Annals of the New York Academy of Sciences, New York, Vol. 852 (1998).
3. Bachand, G.D., and Montemagno, C.D. Constructing organic/inorgancis NEMS devices powered by bio-molecular motors. Biomedical Microdevices, in press, (2000).
4. Bachand, G. D., and Montemagno, C. D. Constructing biomolecular motor-powered, hybrid NEMS devices. Technical Proceedings of the International Symposium on Microelectronics and Micro Electro Mechanical Systems (MICRO/MEMS). Queensland, Australia, (1999).
5. Berger, R. The Undecidability of the Domino Problem, Memoirs of the American Mathematical Society, 66 (1966).
6. S. Blawas, T. F. Oliver, M. C. Pirrung, and W. M. Reichert, Step-and-repeat Photopatterning of Protein Features Using Caged-biotin-BSA: Characterization and Resolution, Langmuir, 14, 4243 (1998).
7. Bowden, N.; Brittain, S.; Evans, A. G., Hutchinson, J. W. and Whitesides, G. M. Spontaneous formation of ordered structures in thin films of metals supported on an elastomeric polymer, Nature 1998,393, 146-149.
8. Buchi, J.R Turing Machines and the Entscheidungsproblem, Mathematiche Annalen, 148, 201-213, (1962).
9. Brockman, J.M.; Frutos, A.G. and Corn, R.M. A Multi-Step Chemical Modification Procedure to Create DNA Arrays on Gold Surfaces for the Study of Protein-DNA Interactions with Surface Plasmon Resonance Imaging, J. Am. Chem. Soc., 121 8044-8051 (1999).
10. Bumm, L. A. and P. S. Weiss, Positioning Atoms and Molecules without Lithography: Self-Assembly and Directed Assembly, invited manuscript in preparation for Superlattices and Nanostructures, (2000). http://stm1.chem.psu.edu/~psw/papers/ DirectAsmblyJACS99.pdf

11. Bumm, L. A., J. J. Arnold, L. F. Charles, T. D. Dunbar, D. L. Allara, and P. S. Weiss, Directed Self-Assembly to Create Molecular Terraces with Molecularly Sharp Boundaries in Organic Monolayers, Journal of the American Chemical Society 121, 8017 (1999). (ABSTRACT)
12. J. Chen, M. A. Reed, A. M. Rawlett, and J. M. Tour, Observation of a Large On-Off Ratio and Negative Differential Resistance in an Molecular Electronic Device, Science, Vol 286, 19, Nov. 1999, p 1550–1552.
13. Clelland, C.T., Risca, V., and C. Bancroft. Genomic Steganography: Amplifiable Microdots. Under review by Nature, 1999.
14. E. Coven, N. Jonoska: DNA Hybridization, Shifts of Finite type and Tiling of the Integers, (to appear in: Words, Sequences, Languages: where computer science and linguistics meet, published by Kluwer, edited by Carlos Martin-Vide).
15. Du, S.M., S. Zhang and N.C. Seeman, DNA Junctions, Antijunctions and Mesojunctions, Biochem., 31, 10955- 7pt963, (1992).
16. Eng, T., Linear DNA self-assembly with hairpins generates the equivalent of linear context-free grammars, 3rd DIMACS Meeting on DNA Based Computers, Univ. of Penn., (June, 1997).
17. Faulhammer, D., Cukras, A.R., Lipton, R.J. and Landweber, L.F., Molecular computation: RNA solutions to chess problems, Proc. Nat. Acad. Sci. (USA) 97, 1385-1389 (2000).
18. Fu, T.-J., and N.C. Seeman, DNA Double Crossover Structures, Biochemistry, 32, 3211-3220, (1993).
19. Garey, M. R., and D. S. Johnson Computers and Intractability: A Guide to the Theory of NP-Completeness, W.H Freeman and Company, page 257, (1979).
20. Gehani, A and J.H. Reif, Microflow Bio-Molecular Computation, 4th DIMACS Workshop on DNA Based Computers, University of Pennsylvania, June, 1998. DNA Based Computers, IV, DIMACS Series in Discrete Mathematics and Theoretical Computer Science, (ed. H. Rubin), American Mathematical Society, 1999. Appeared also in Biosystems, Journal of Biological and Informational Processing Sciences, Vol. 52, Nos. 1-3, (ed. By L. Kari, H. Rubin, and D. H. Wood), pp 197-216, (1999). www.cs.duke.edu/∼reif/paper/geha/microflow.ps
21. Gehani, A., T. H. LaBean, and J.H. Reif, DNA-based Cryptography, 5th DIMACS Workshop on DNA Based Computers, MIT, June, 1999. DNA Based Computers, V, DIMACS Series in Discrete Mathematics and Theoretical CS (ed. E. Winfree), American Mathematical Society, 2000. www.cs.duke.edu/∼reif/paper/DNAcrypt/crypt.ps
22. Gillmor, S.D., Liu, Q., Thiel, A.J., Condon, A.E., Corn, R.M., Smith, L.M. and Lagally, M.G. Hydrophobic/Hydrophilic Patterned Surfaces to Create DNA Arrays, to be submitted (to Langmuir), 1999.
23. Grunbaum, S., Branko, and G.C. Shepard, Tilings and Patterns, H Freeman and Company, Chapter 11, (1987).
24. Harada, K. and Orgel, L.E., Unexpected substrate specificity of T4 DNA ligase revealed by in vitro selection, Nucleic Acids Res 21, 2287-2291 (1993).
25. Harder, P.; Grunze, M.; Dahint, R.; Whitesides, G. M. and Laibinis, P. E., Molecular Conformation in Oligo(ethylene glycol)-Terminated Self-Assembled Monolayers on Gold and Silver Surfaces Determines Their Ability To Resist Protein Adsorption, J. Phys. Chem. B 1998,102, 426-436.
26. D. Herschlag (1991), Implications of ribozyme kinetics for targeting the cleavage of specific RNA molecules in vivo, Proc. Nat. Acad. Sci. (USA), 88, pp. 6921-6925.
27. Jhaveri, S., Kirby, R., Conrad, R., Magion, E.J., Glick, G., Ellington, A.D. (1999) Signaling aptamers. Accepted to JACS.

28. Jonoska, N., S. Karl, M. Saito: Creating 3-Dimensional Graph Structures With DNA in DNA based computer III (Editors: H. Rubin, D. Wood) DIMACS series in Discrete Math. and Theoretical Comp. Sci. vol 48 (1999) 123-136.
29. Jonoska, N., S. Karl, M. Saito: Three dimensional DNA structures in computing (to appear in BioSystems).
30. Jonoska, N., 3D DNA patterns and Computing (to appear) collection of papers on Patterns in Biology edited by M. Gromov and A. Carbone (to be published by World Scientific).
31. Jonoska, N., S. Karl: Ligation Experiments in Computing with DNA, Proceedings of 1997 IEEE International Conference on Evolutionary Computation (ICEC'97), April 13-16, (1997) 261-265.
32. Jonoska, N., S. Karl: A molecular computation of the road coloring problem in DNA based computer II (Editors: L. Landwaber, E. Baum) DIMACS series in Discrete Math. and Theoretical Comp. Sci. vol 44 (1999) 87 - 96,
33. Jonoska, N., S. Karl, M. Saito: Graph structuires in DNA computing in Computing with Bio-Molecules, theory and experiments, (editor Gh. Paun) Springer-Verlag (1998), 93-110.
34. Jonoska, N., Sofic Shifts with Synchronizing Presentations, Theoretical Computer Science vol. 158 1-2 (1996) 81-115.
35. Jonoska, N., Constants in factorial and prolongable languages, Pure Math. Appl. vol 7 1-2 (1996) 99-110.
36. LaBean, T. H., E. Winfree, J. H. Reif, Experimental Progress in Computation by Self-Assembly of DNA Tilings, 5th International Meeting on DNA Based Computers(DNA5), MIT, Cambridge, MA, (June, 1999). To appear in DIMACS Series in Discrete Mathematics and Theoretical Computer Science, ed. E. Winfree, to appear American Mathematical Society, 2000. http://www.cs.duke.edu/~thl/tilings/labean.ps
37. LaBean, T. H., Yan, H., Kopatsch, J., Liu, F., Winfree, E., Reif, J.H. and Seeman, N.C., The construction, analysis, ligation and self-assembly of DNA triple crossover complexes, J. Am. Chem. Soc. 122, 1848-1860 (2000).
www.cs.duke.edu/~reif/paper/DNAtiling/tilings/JACS.pdf
38. Lagoudakis, M. G., T. H. LaBean, 2D DNA Self-Assembly for Satisfiability, 5th International Meeting on DNA Based Computers(DNA5), MIT, Cambridge, MA, (June, 1999). DIMACS Series in Discrete Mathematics and Theoretical Computer Science, vol.44, American Mathematical Society, ed. E. Winfree, (1999).
39. Lewis, H.R., and C.H. Papadimitriou, Elements of the Theory of Computation, Prentice-Hall, pages 296-300 and 345-348 (1981).
40. Li, X.J., X.P. Yang, J. Qi, and N.C. Seeman, Antiparallel DNA Double Crossover Molecules as Components for Nanoconstruction, J. Am. Chem. Soc., 118, 6131-6140, (1996).
41. Liu, F., H. Wang and N.C. Seeman, Short Extensions to Sticky Ends for DNA Nanotechnology and DNA-Based Computation, Nanobiology 4, 257-262 (1999).
42. Liu, F., R. Sha and N.C. Seeman, Modifying the Surface Features of Two-Dimensional DNA Crystals, J. Am. Chem. Soc. 121, 917-922 (1999).
43. Liu, F., M.F. Bruist and N.C. Seeman, Parallel Helical Domains in DNA Branched Junctions Containing 5', 5' and 3', 3' Linkages, Biochemistry 38, 2832-2841 (1999).
44. Liu, Q., Wang, L., Frutos, A.G., Condon, A.E., Corn, R.M. and Smith, L.M., DNA computing on surfaces, Nature 403, 175-179 (2000).
45. Mao, W. Sun, Z. Shen and N.C. Seeman, A DNA Nanomechanical Device Based on the B-Z Transition, Nature 397, 144-146 (1999).

46. Mao, C., W. Sun, and N.C. Seeman, Construction of Borromean Rings from DNA, Nature, 386(6621), 137-138, (March,1997).
47. Mao, W. Sun and N.C. Seeman, Designed Two-Dimensional DNA Holliday Junction Arrays Visualized by Atomic Force Microscopy, J. Am. Chem. Soc. 121, 5437-5443 (1999).
48. Mao, C., T.H. LaBean, J. H. Reif, and N.C. Seeman, An Algorithmic Self-Assembly, Nature, Sept 28, (2000).
www.cs.duke.edu/~reif/paper/SELFASSEMBLE/AlgorithmicAssembly.pdf
49. Montemagno, C. D., and Bachand, G. D. Constructing nanomechanical devices powered by biomolecular motors. Nanotechnology 10: 225-331 (1999).
50. Montemagno, C. D., Bachand, G. D., Stelick, S. J., and Bachand, M. Constructing biological motor powered nanomechanical devices. Sixth Foresight Conference on Molecular Nanotechnology, (1998).
51. Moore, C. and J. M. Robson, Hard Tiling Problems with Simple Tiles, to appear, 2000.
52. Neilsen, P.E., Peptide Nucleic Acids, Ann. Rev. Biophys. and Biophys. Chem. 1995 14, 167-183.
53. Petty, M. C., M. R. Bryce and D. Bloor (Eds.), An Introduction to Molecular Electronics, Oxford University Press, New York (1995).
54. Reed, M. A., C. Zhou, C. J. Muller, T. P. Burgin and J. M. Tour, Conductance of a molecular junction, Science, Vol. 278, pages 252254, October 10, 1997.
55. Reed, M. A. and J. M. Tour Computing with Molecules, Scientific American, June 2000. http://www.scientificamerican.com/2000/0600issue/0600reed.html
56. Reif, J.H., Local Parallel Biomolecular Computation, Third Annual DIMACS Workshop on DNA Based Computers, University of Pennsylvania, June 23-26, 1997. Published in DNA Based Computers, III, DIMACS Series in Discrete Mathematics and Theoretical Computer Science, Vol 48 (ed. H. Rubin), American Mathematical Society, 1999, p 217-254. Postscript versions of this paper and its figures are at
http://www.cs.duke.edu/~reif/paper/Assembly.ps and Assembly.fig.ps
57. Reif, J.H., Parallel Molecular Computation: Models and Simulations. Proceedings: 7th Annual ACM Symposium on Parallel Algorithms and Architectures (SPAA'95) Santa Barbara, CA, July 1995, pp. 213-223. Published in Algorithmica, special issue on Computational Biology,25:142-176, 1999. www.cs.duke.edu/~reif/paper/Molecular.ps
58. Reif, J.H., Synthesis of Parallel Algorithms. 22 chapters, 1011 pages. Published by Morgan Kaufmann, Spring, 1993.
59. Reif, J.H., Paradigms for Biomolecular Computation, First International Conference on Unconventional Models of Computation, Auckland, New Zealand, January 1998. Published in Unconventional Models of Computation, edited by C.S. Calude, J. Casti, and M.J. Dinneen, Springer Publishers, 1998, pp 72-93.
http://www.cs.duke.edu/~reif/paper/paradigm.ps
60. Reif, J.H., and T. H. LaBean, Computationally Inspired Biotechnologies: Improved DNA Synthesis and Associative Search Using Error-Correcting Codes and Vector-Quantization, Sixth International Meeting on DNA Based Computers (DNA6), DIMACS Series in Discrete Mathematics and Theoretical Computer Science, Leiden, The Netherlands, (June, 2000) ed. A. Condon. To be published by the American Mathematical Society, 2000. www.cs.duke.edu/~reif/paper/Error-Restore/Error-Restore.ps
61. Reif, J.H. and Zheng Sun, Nano-Robotics Motion Planning and Its Applications in Nanotechnology and Biomolecular Computing, NSF Design & Manufacturing Grantees Conference, Jan 5-8, 1999. http://www.cs.duke.edu/~reif/paper/NanoRobotics.html

62. Robinson, R.M. Undecidablility and Nonperiodicity for Tilings of the Plane, Inventiones Mathematicae, 12, 177-209, (1971).
63. Rothemund, P.W.K., Using lateral capillary forces to compute by self-assembly, Proc. Nat. Acad. Sci. (USA) 97, 984-989 (2000a).
64. Rothemund, P.W.K., and E. Winfree, The Program-Size Complexity of Self-Assembled Squares, Symposium on Theory of Computing(STOC 2000), Portland, OR, (May, 2000b).
65. Roweis, S. E. Winfree. On the reduction of errors in DNA computation. Journal of Computational Biology, 6(1): 65–75, 1999.
66. Roweis, S. E. Winfree, R. Burgoyne, N. V. Chelyapov, M. F. Goodman, P. W. K. Rothemund, L. M. Adleman. A Sticker-Based Model for DNA Computation. Journal of Computational Biology, 5(4): 615–629, 1998
67. Seeman, N.C., Nucleic Acid Nanostructures and Topology. Angewandte Chemie. 110, 3408-3428 (1998); Angewandte Chemie International Edition 37, 3220-3238 (1998).
68. Seeman, N.C., DNA Engineering and its Application to Nanotechnology, Trends in Biotech. 17, 437-443 (1999).
69. Seeman, N.C. Nucleic Acid Junctions and Lattices, J. Theor. Biol., 99, 237-247, (1982).
70. Seeman, N. C., J. Chen, S.M. Du, John E. Mueller, Yuwen Zhang, Tsu-Ju Fu, Yinli Wang, Hui Wang,Siwei Zhang, Synthetic DNA knots and catenanes, New Jour. of Chemistry, 17, 739-755, (1993).
71. Seeman, N. C., J.-H. Chen, N.R. Kallenbach, Gel electrophoretic analysis of DNA branched junctions, Electrophoresis, 10, 345-354, (1989).
72. Seeman, N. C., F. Liu, C. Mao, X. Yang, L.A. Wenzler, R. Sha, W. Sun, Z. Shen, X. Li, J. Qi, Y. Zhang, T. Fu, J.-H. Chen, and E. Winfree, Two Dimensions and Two States in DNA Nanotechnology, Journal of Biomolecular Structure and Dynamics, ISSN 0739-1102, Conversion 11, Issue 1, June, 1999.
73. Seeman, N. C., H. Wang, X. Yang, F. Liu, C. Mao, W. Sun, L.A. Wenzler, Z. Shen, R. Sha, H. Yan, M.H. Wong, P. Sa-Ardyen, B. Lui, H. Qiu, X. Li, J. Qi, S.M. Du, Y. Zhang, J.E. Mueller, T.-J. Fu, Y. Wang, amd J. Chen, New Motifs in DNA nanotechnology, Nanotechnology 9, p 257-273 (1998).
74. Seeman, N. C., H. Wang, B. Liu, J. Qi, X. Li, X. Yang, F. Liu, W. Sun, Z. Shen, R. Sha, C. Mao, Y. Wang, S. Zhang, T.-J. Fu, S. Du, J. E. Mueller, Y. Zhang, and J. Chen, The Perils of Polynucleotides: The Experimental Gap Between the Design and Assembly of Unusual DNA Structures, The 2nd Annual Workshop on DNA Based Computers, American Mathematical Society, June 1996.
75. Seeman, N. C., Y. Zhang, and J. Chen, DNA nanoconstructions, J. Vac. Sci. Technol., 12:4, 1895-1905, (1994).
76. Seeman, N. C., Y. Zhang, S. Du, H. Wang, J.E. Mueller, and J. Chen, The control of DNA structure and topology: An overview, Mat. Res. Soc. Symp. Proc., 356, 57-66, (1994). H. Wang, Proving Theorems by Pattern Recognition, Bell System Technical Journal, 40, 1-141, (1961).
77. Sha, R., F. Liu, M.F. Bruist and N.C. Seeman, Parallel Helical Domains in DNA Branched Junctions Containing 5', 5' and 3', 3' Linkages, Biochemistry 38, 2832-2841 (1999).
78. Smith, L. M. , R. M. Corn, A. E. Condon, M. G. Lagally, A. G. Frutos, Q. Liu, and A. J. Thiel, A Surface-Based Approach to DNA Computation. J. Computational Biology, 5(2), 255-266. 1998.
79. Soong, R. K., Stelick, S. J., Bachand, G. D., and Montemagno, C. D. Evaluating adhesion strength of biological molecules to nanofabricated substrates. Technical Pro-

ceedings of the Second Conference on Modeling and Simulation of Microsystems 99 Conference; Puerto Rico (1999).
80. Sun, W., C. Mao, F. Liu and N.C. Seeman, Sequence Dependence of Branch Migratory Minima. J. Mol. Biol. 282, 59-70 (1998).
81. Wang, H., In Proc. Symp. Math. Theory of Automata, 23-26 (Polytechnic Press, New York, 1963).
82. Wang, Y., J.E. Mueller, B. Kemper, and N.C. Seeman, Assembly and characterization of five-arm and six-arm branched junctions, Biochem. 30, 5667-5674 (1991)
83. Winfree, E. Simulations of Computing by Self-Assembly. In Proceedings of the Fourth Annual Meeting on DNA Based Computers, held at the University of Pennsylvania, June 16-19, 1998.
84. Winfree, E., X. Yang, N.C. Seeman, Universal Computation via Self-assembly of DNA: Some Theory and Experiments, 2nd Annual DIMACS Meeting on DNA Based Computers, Princeton, June, 1996.
85. Winfree, E., T. Eng, G. Rozenberg String tile models for DNA computing by self-assembly, unpublished notes, September 1998.
86. Winfree, E., Furong Liu, Lisa A. Wenzler, Nadrian C. Seeman (1998) Design and Self-Assembly of Two Dimensional DNA Crystals. Nature 394: 539–544, 1998.
87. Winfree, E., Xiaoping Yang, Nadrian C. Seeman. Universal Computation via Self-assembly of DNA: Some Theory and Experiments. In DNA Based Computers II: DIMACS Workshop, June 10-12, 1996 (Volume 44 in DIMACS). Laura F. Landweber and Eric B. Baum, editors. American Mathematical Society, 1998, pp. 191–213.
88. Winfree, E., On the Computational Power of DNA Annealing and Ligation. In DNA Based Computers: Proceedings of a DIMACS Workshop, April 4, 1995, Princeton University (Volume 27 in DIMACS). Richard J. Lipton and Eric B. Baum, editors. American Mathematical Society, 1996, pp. 187–198.
89. Winfree, E., F. Liu, L. A. Wenzler, and N.C. Seeman, Design and Self-Assembly of Two-Dimensional DNA Crystals, Nature 394, 539-544 (1998).
90. Winfree, E., 5th International Meeting on DNA Based Computers(DNA5), MIT, Cambridge, MA, (June, 1999). To appear in DIMACS Series in Discrete Mathematics and Theoretical Computer Science, ed. E. Winfree, (1999).
91. Xia, Y. and Whitesides, G. M., Angew. Soft Lithography, Chem. Int. Ed. Engl. 1998,37, 550-575.
92. Xia, Y. and Whitesides, G. M., Soft Lithography, Annu. Rev. Mater. Sci. 1998,28, 153-184. Yan, L.; Zhao, X.-M. and Whitesides, G. M. Patterning a Preformed, Reactive SAM Using Microcontact Printing, J. Am. Chem. Soc. 1998,120, 6179-6180.
93. Yang, X., L.A. Wenzler, J. Qi, X. Li and N.C. Seeman, Ligation of DNA Triangles Containing Double Crossover Molecules, J. Am. Chem. Soc. 120, 9779-9786 (1998).
94. Zhao, X.; Votruba, P.G.; Strother, T.C.; Ellison, M.D.; Smith, L.M. and Hamers, R.J. Formation of Organic Films on Si(111) Surfaces via Photochemical Reaction (in preparation), 1999.
95. Zhou, C. , et al., Appl. Phys. Lett. 71, 611 (1997).
96. Zhou, C. , Atomic and Molecular Wires, Ph.D. thesis, Yale University (1999).