Overview of New Structures for DNA-Based Nanofabrication and Computation

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Summary

This paper presents an overview of recent experimental progress by the Duke DNA NanoTech Group in our efforts to utilize novel DNA nanostructures for computational self-assembly as well as for templates in the fabrication of functional nano-patterned materials. We have prototyped a new DNA tile type known as the 4x4 (a cross-like structure composed of four four-arm junctions) upon which we have deposited metal to form highly conductive nanowires and also are adapting multi-tile 4x4 sets for a variety of computational applications. We have recently described a DNA barcode lattice composed of DX tiles assembled on a long scaffold strand; the system propagates 1-dimensional (1D) information encoded on the scaffold strand into a specific and reprogrammable barcode pattern which is visible in 2D by atomic force microscopy. We have succeeded in demonstrating the first highly parallel computation via DNA tile self-assembly by using a single-layer superstructure made of DX tiles which computes the entire lookup table of pairwise XOR calculations up to a modest size input string length. We have prototyped a 2-state DNA lattice assembly containing actuator components and demonstrated its ability to be controllably switched between the two states. We are currently working on a molecular robotics experiment aimed at demonstrating unidirectional motion of a small DNA fragment along a track constructed of DNA. We have demonstrated a diverse set of novel structures and applications which extend the inherent information carrying capacity of DNA in a variety of novel directions.

Background and Context

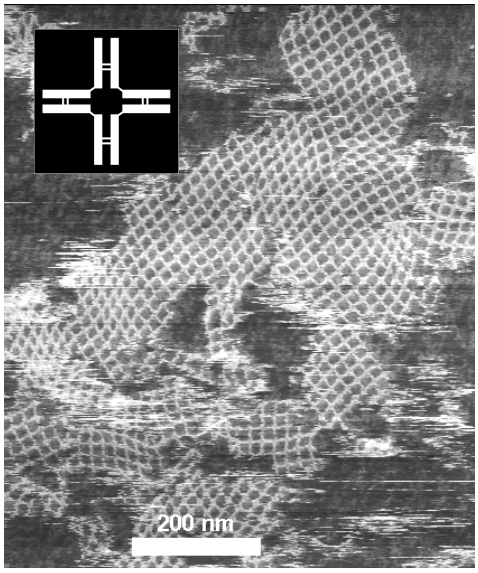
The control of information flow on the molecular level in artificial systems holds future promise for molecular computation applications as well as for nanotechnology investigations where we desire the intricate control of matter on the scale of a few nanometers to a few tens of nanometers. DNA-based nanotechnology, seeks to engineer synthetic DNA polymers to encode information necessary for realization of desired structures or processes on the molecular level[1-3]. Properly designed sets of DNA oligonucleotides are able to self-assemble into complex, highly organized structures. The major advantage of DNA as a self-assembling structural material over other known chemical systems is that DNA's well-understood Watson-Crick base-pairing rules act as a programming language with which to input organizational instructions. In particular, we understand how B-form DNA, the structure adopted by double-stranded DNA (dsDNA) under standard solution conditions, forms during the

annealing of complementary strands. The specifically programmable molecular recognition ability of DNA, as well as the large vocabulary of available sequences, make it ideal for controlling the assembly of nanostructures. Annealing of complementary strands is achieved by simply heating dissolved oligonucleotide strands above their dissociation temperature then cooling them very slowly thus allowing strands to find and bind their sequence complements. Unpaired, single-strand regions (ssDNA) extending from the ends of double-strand domains can be used to specifically bind other dsDNA displaying a complementary ssDNA sticky-end. Regions of ssDNA therefore, can act as address codes capable of orienting attached substructures to neighboring points in 3-space. If DNA is appended to nanoscale objects and chemical moieties, it can act as "smart glue" for organizing such objects and moieties in space. An obvious advantage of DNA self-assembly for fabrication is the simultaneous creation of at least millions or billions of copies of a desired structure via the massive parallelism inherent in molecular processes.

Self-assembling nanostructures composed of DNA molecules offer great potential for bottom-up nanofabrication of materials and objects with smaller features than ever previously possible[1-3]. Recently DNA has been demonstrated to be a useful material for constructing periodically patterned structures[4-6], nanomechanical devices[7-10], and molecular computing systems[11-15]. DNA has also been employed, using appropriate attachment chemistries, to direct the assembly of other functional molecules[16-21]. Potential applications of DNA self-assembly and scaffolding include nanoelectronics, biosensors, and programmable/autonomous molecular machines. Subsequent sections of this paper highlight our recent progress in DNA nanotechnology; in the final section, some potential future directions are mentioned.

4x4 Tiles, Lattices and Nanowires

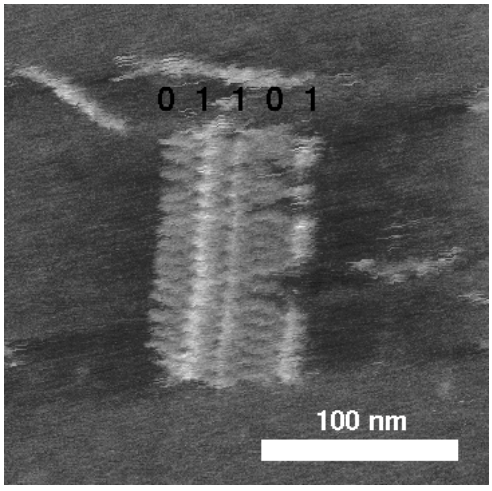
We have successfully completed the design, construction, and characterization of a novel DNA tile (4x4) and its self-assembled lattice forms. The novelty of this structure includes a square aspect ratio with helix stacking and sticky-end connections in four directions (north, south, east, and west) within the lattice plane. Self-assembly of 4x4 tiles results in two distinct lattice morphologies: long (~5 μm on average) uniform nanoribbons (~60 nm wide) and flat two-dimensional nanogrids which both display periodic square cavities. Control of the relative proportions of the two lattice morphologies has been achieved with only slight reprogramming of the tile spacing and sticky-end associations. The 4x4 nanoribbon provides an excellent scaffold for production of uniform width nanowires via glutaraldehyde-treated DNA metallization using both silver and gold. We have, for the first time, produced highly conductive nanowires on self-assembled DNA tiling structures.



The figure above shows an atomic force microscope (AFM) image of lattice formed from 4x4 tiles. The inset (upper left) gives a schematic of a single 4x4 tile where the white rectangles each represent double helical DNA segments, each one of which is linked to a neighboring dsDNA domain via a crossover point at which 2 strands are exchanged between the double helices (shown as thin, parallel lines). The arms of the 4x4 tile are joined to one another in the central region using linkers of four thymidine residues (not shown). Detailed description of the molecules and their structural properties are presented elsewhere[22].

Aperiodic Barcode Lattice

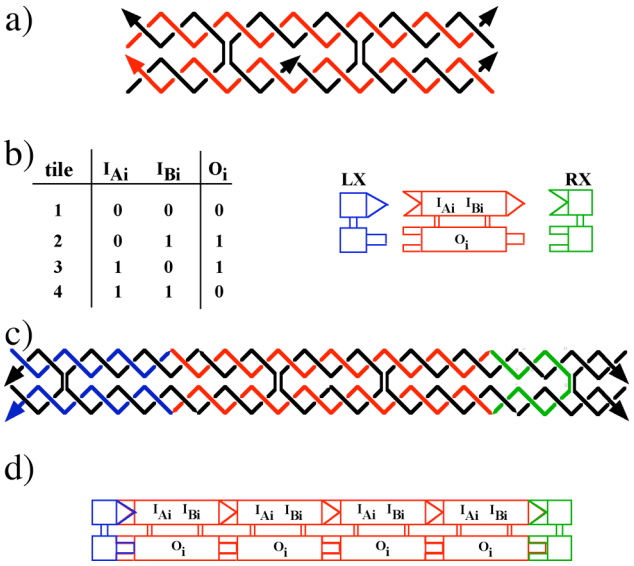
We have recently reported the construction of a novel aperiodic patterned DNA lattice (Barcode Lattice) by directed-nucleation self-assembly of DNA tiles around a scaffold DNA strand[23]. The input DNA scaffold strand, constructed by ligation of shorter synthetic oligonucleotides, provides layers of the DNA lattice with barcode patterning information represented by the presence or absence of DNA hairpin loops protruding out of the lattice plane. The scaffold strands provide nucleation points for the assembly of double-crossover (DX) tiles into linear layers which then specifically stack to form the barcode lattice.



The figure above shows an AFM image of a section of patterned DNA lattice containing barcode information of 01101.; the 1 and 0 bit values are clearly visible as lighter and darker stripes, respectively. We have also demonstrated the reprogramming of the system to another patterning; an inverted barcode pattern of 10010 was achieved by modifying the scaffold strands and one of the strands composing each tile. A ribbon lattice, consisting of repetitions of the barcode pattern with expected periodicity, was also constructed by the addition of sticky-ends. The patterning of both classes of lattices was clearly observable via atomic force microscopy. These results represent a first step toward implementation of a visual readout system capable of converting information encoded on a 1-dimensional DNA strand into a 2-dimensional form readable by advanced microscopic techniques. A functioning visual output method would not only increase the readout speed of DNA-based computers, but may also find use in other sequence identification techniques such as mutation or allele mapping. The aperiodic barcode lattice described is a step toward future applications which exploit self-assembling DNA nanostructures for fabrication of specifically patterned materials (e.g. nanoelectronic components) since addressable arrays with control of feature patterning in two or three dimensions will be required.

DX XOR Algorithmic Assembly

Computation by self-assembly of DNA is an efficient method of executing parallel DNA computing. We have reported a multi-bit parallel computation of pair-wise XOR using DNA 'string tile' self-assembly. XOR is the logical Boolean operation whose output is 0 if its two input bits agree and 1 if the inputs disagree. A set of DNA double-crossover (DX) tiles encoding the truth table for XOR was constructed. The assembly followed the "string tile" design model in which information carrying DNA fragments were brought together within each tile and organized via tile-to-tile associations such that only structures representing valid computations were formed. Parallel tile self-assembly and ligation led to reporter strands representing a molecular look-up table containing all possible pair-wise XOR calculations up to a certain size input string. Computation was read-out by sequencing the cloned reporter strands. This is the first experimental demonstration of a parallel computation by DNA tile self-assembly in which large numbers of distinct inputs were simultaneously processed. Further details of the computation are presented elsewhere[24].



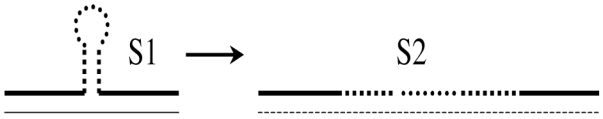
The figure above illustrates the molecular structure of the DX tiles used in this construction (Panel-a). It contains five strands that self-assemble through Watson-Crick base pairing to produce two double helices which are connected to each other at two points where their strands cross over between them. There are two continuous strands (red) going in opposite directions through the DX tile and are used to encode input and output information. Panel-b gives the truth table for XOR and a representation of the computational tile types. The two helical domains are drawn as rectangles, flanked by sticky ends shown as geometrical shapes. The two input values of each tile are in the upper rectangle and the output value is in the lower rectangle. The corner tiles are labeled as LX and RX. One tile type is required for each of the four rows in the truth table, plus one tile type is needed for each of the two ends of the assembly. In parallel computation of pair-wise XOR, multiple DX tiles will self-assemble into multi-tile linear superstructures via sticky-end associations. Panel-c shows an example of the reporter strand structure with one computational tile and two corner tiles. Panel-d shows an example of a multi-tile assembly capable of calculating 4-bit XOR. I_{Ai} and I_{Bi} indicate sequences encoding binary values for the two input bits of the operation. O_i encodes the value of the output bit. The linear assembly shown in Panel-d is composed of six tiles, starting on the left with an LX tile, followed by four computational tiles and ending with an RX tile. A reporter strand starts on its 5' end with a blue LX strand, then it runs through all computational tiles and records the input bits, then it is rerouted back through the assembly by the green RX strand and passes back through the entire assembly to pick up the output bit values.

2-State Lattice

Controlled mechanical movement in molecular-scale devices is one of the key goals of nanotechnology. DNA is an excellent candidate for construction of such devices due to the specificity of base pairing and its robust physicochemical properties. A variety of DNA-based molecular machines displaying rotational and open/close movements have recently been demonstrated [reviewed in ref. 25]. Reversible shifting of equilibrium between two conformational states is triggered by changes in experimental conditions or by the addition of a " DNA fuel strand" that provides the driving force for such changes. Incorporation of DNA devices into arrays could lead to complex

structural states suitable for nanorobotic applications, if each individual device can be addressed separately. This has numerous potential applications. 1) The size and shape of the lattice can be programmed through the control of sequence-dependent devices, leading to controlled nanofabrication of molecular nanoelectronic wires with on and off states. For example, tunneling effects of quantum-dot cellular automata could be actuated by controlling the distance between adjacent cells. 2) Molecules or nanoparticles can be selectively manipulated, e.g. sorted and transported, using molecular motor devices arranged on DNA tiling arrays, which may lead to programmed chemical synthesis. 3) It may offer a mechanism to do DNA computation on arrays whose tile elements are capable of holding separate states which can be used for recording or transmitting information.

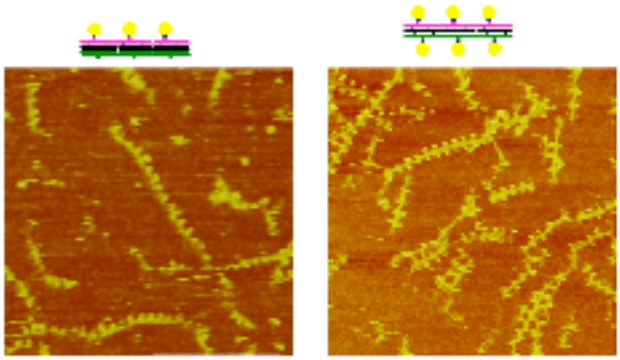
We have reported the construction of a robust sequence dependent DNA device, which we call a "nano-actuator" and the incorporation of such devices into a 2D parallelogram DNA lattice.



The figure above illustrates the design and operation of a single nano-actuator device. The two states of the device are shown as S1 and S2. S1 represents the shortened state containing a bulged three-arm DNA branch junction. S2 represents the elongated state and contains two perfectly complementary strands of DNA. Bulged 3-arm DNA branch-junctions have been well characterized and extensively used in DNA nanoconstruction and as topographic markers in self-assembly of 2D DNA lattices. Thus, DNA devices based on bulged three-arm junctions are an excellent candidate to serve as actuators for DNA lattices. The parallelogram lattice contained one such device on each wall of each unit cell. Large alterations in lattice dimension were observed due to the additive changes from each unit cell. Complete details of the 2-state lattice system are reported elsewhere[26].

TX Tile Array for Organizing Nanospheres

We have reported a recent experiment using a linear array of DNA triple crossover tiles (TX) to controllably template the self-assembly of two forms (single-layer or double-layer) of streptavidin linear arrays through biotin-streptavidin interaction[28]. The TX tile consisted of seven oligonucleotides hybridized to form three double-stranded helices lying in a plane and linked by strand exchange at four immobile crossover points. The tiles were designed to contain two stem-loops protruding, one each out of the upper and the lower helices, in the plane of the tile. To template the assembly of streptavidin molecules, the hairpin loops were modified to incorporate two biotin groups per loop, indicated by the small blue dots. Formation of single-layer or double-layer streptavidin linear arrays was controlled using two different templates which are illustrated in the figure below.



The figure above shows AFM images (500 x 500 nm) of the single (left) and double (right) layer TX tile assemblies displaying the protein streptavidin in linear arrays. Schematics of the tile assemblies are given just above each AFM image. In the first template (left panel), only one stem loop in each TX molecule was modified with biotin groups. In the second template (right panel), both stem loops were modified to incorporate biotins. The binding of streptavidin molecules (represented as yellow dots in the schematics) to the two different templates resulted in single-layer or double-layer streptavidin linear arrays, as shown. In a further experiment, streptavidin molecules were labeled with gold nanoparticles and again organized into linear arrays on the DNA templates (data not shown here). See reference 28 for complete details.

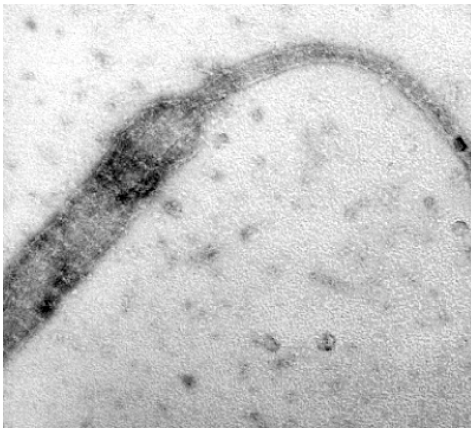
Autonomous DNA Robotics

A DNA nano-device that achieves autonomous unidirectional movement holds great potential in nano-fabrication and DNA computation. DNA actuators demonstrating cycles of motions have been built by several research groups[7-10], but they are not autonomous in the sense that they are mediated by external environmental changes such as the changes of the ionic conditions or the addition of fuel DNA strands. We have designed autonomous DNA robots[27] and are currently working on experimental prototypes. One of the proposed schemes is a "walking" DNA nano-device that moves randomly along a track composed of DNA. This walking DNA device is powered by the ATP consumption of a ligase. We have extended the construction of this walking DNA device and designed a unidirectional autonomous DNA robot. The device is similarly powered by ATP consumption of a ligase, and the unidirectionality of its movement is ensured by using two carefully selected restriction enzymes. We have implemented this design and will be publishing the details of its experimental execution shortly (P. Yin *et al.*, *manuscript submitted*).

TX Nanotubes and Nanowires

We have recently presented results on the construction and characterization of DNA nanotubes, a new self-assembling superstructure composed of DNA tiles[29,30]. Triple-crossover (TX) tiles modified with thiol-containing dsDNA stems projected out of the tile plane were utilized as the basic building block. TX nanotubes display a constant diameter of approximately 25 nm and have been observed with lengths up to 20 microns. The TX nanotubes are interesting to compare with the 4x4 nanotubes (nanoribbons prior to flattening on a substrate) because the design alterations which enable morphologic switching of the superstructures are quite different. In the 4x4 case

neighbor relations in the lattice were changed so that neighboring tiles alternated orientation and canceled out tile curvature thus avoiding accumulation of curvature in the lattice. In the TX nanotube case, the addition of a new interaction between tiles (the formation of disulfide bridges between thiol groups on neighboring tiles) appears to be the cause of lattice curvature and tube formation.



The figure above shows a negative stained transmission electron microscopy (TEM) image of a section of TX DNA nanotube with the left side apparently unwrapped while the right side remains in a tight tube-like structure. Lighter colored bands are visible and identified as protruding stem-loops on the B tiles of the 2-tile AB set. The bands have spacing of approximately 28 nm, in perfect agreement with the design. Other high resolution images of the constructs from TEM and AFM as well as preliminary data on successful metallization of the nanotubes have been published[29,30]. DNA nanotubes represent a potential breakthrough in the self-assembly of nanometer scale circuits for electronics layout since they can be targeted to connect at specific locations on larger-scale structures and can subsequently be metallized to form nanometer scale wires. The dimensions of these nanotubes are also perfectly suited for applications involving interconnection of molecular scale devices with macroscale components fabricated by conventional photolithographic methods.

Discussion

DNA continues to be a leading choice as programmable building material for micron-scale objects with nanometer-scale feature precision. Periodic structures remain the easiest to prototype in high yield, however recent advances in programmable patterning promise much more complex aperiodic DNA arrays soon. Fabrication of nanoelectronic circuits by bottom-up parallel self-assembly is a current goal. Programmable molecular systems useful for monitoring or controlling biochemical systems have a wide variety of potential applications in diagnostic and therapeutic medicine. Future DNA-based computing and control systems may represent the perfect media for interfacing on the molecular level with complex biological systems from the control of expression of an individual gene in an individual cell up to intervention of complex disease states in whole organisms.

References

1. Seeman, N.C. DNA in a material world. *Nature* **421**, 427-431 (2003).

2. LaBean, T.H. Introduction to self-assembling DNA nanostructures for computation and nanofabrication. in *Computational Biology and Genome Informatics*, eds. Wang, J.T.L., Wu, C.H. & Wang, P.P. (World Scientific Publishing, River Edge, NJ, 2003).
3. J. H. Reif, DNA lattices: a programmable method for molecular scale patterning and computation, *Computer and Scientific Engineering Magazine*, special issue on Bio-Computation, IEEE Computer Society, pp 32-41 (2002).
4. Winfree, E., Liu, F., Wenzler, L. A. & Seeman, N.C. Design and self-assembly of two-dimensional DNA crystals. *Nature* **394**, 539-544 (1998).
5. LaBean, T. H. *et al*. Construction, analysis, ligation, and self-assembly of DNA triple crossover complexes. *J. Am. Chem. Soc.* **122**, 1848-1860 (2000).
6. Mao, C., Sun, W. & Seeman, N.C. Designed two-dimensional DNA Holliday junction arrays visualized by atomic force microscopy*, J. Am. Chem. Soc.* **121**, 5437-5442 (1999).
7. Mao, C., Sun, W., Shen, Z. & Seeman, N.C. A DNA nanomechanical device based on the B-Z transition, *Nature* **397**, 144-146 (1999).
8. Yurke, B, Turberfield, A.J., Mills, A.P., Jr, Simmel, F.C., & Jennifer L. Neumann, A DNA-fueled molecular machine made of DNA. *Nature* **406**, 605-608 (2000).
9. Yan, H., Zhang, X., Shen, Z. & Seeman, N.C. A robust DNA mechanical device controlled by hybridization topology. *Nature* **415**,62-65, (2002).
10. Li, J.J, & Tan, W. A single DNA molecule nanomotor. *Nano Lett.* **2,** 315-318 (2002).11. Adleman, L.M. Molecular computation of solutions to combinatorial problems. *Science* **266**, 1021-1024 (1994).
12. Liu, Q. *et al.* DNA Computing on surfaces. *Nature* **403**, 175-179 (2000).
13. Mao, C., LaBean, T. H., Reif, J.H., & Seeman, N.C. Logical computation using algorithmic self-assembly of DNA triple crossover molecules. *Nature* **407**, 493-496 (2000).
14. Benenson, Y. *et al.* Programmable and autonomous computing machine made of biomolecules, *Nature* **414**, 430 – 434 (2001).
15. Ravinderjit B.S., Chelyapov, N., Johnson, C., Rothemund, P.W. K. & Adleman, L. Solution of a 20-variable 3-SAT problem on a DNA computer. *Science* **296**, 499-502 (2002).
16. Mirkin, C. A. Programming the assembly of two- and three-dimensional architectures with DNA and nanoscale inorganic building blocks. *Inorg. Chem, **39***, 2258 (2000).
17. Alivisatos, A.P. *et al.* Organization of 'nanocrystal molecules' using DNA molecules. Nature, **382**, 609-611 (1996).
18. Braun, E., Eichen, Y., Sivan, U., Ben-Yoseph, G. DNA-templated assembly and electrode attachment of a conducting silver wire. *Nature* **391**, 775 (1998).
19. Keren, K., *et al.* Sequence-specific molecular lithography on single DNA molecules. *Science*, **297**, 72 (2002).
20. Niemeyer, C. M., Burger, W., Peplies, J. Covalvent DNA-streptavidin conjugates as building blocks for novel biometallic nanostructures. *Angew. Chem.-Int. Edit.* **37**, 2265-2268 (1998).
21. Xiao, S. et al. Self-assembly of nanoparticle arrays by DNA scaffolding. *J. Nanoparticle Research* **4**, 313 (2002).

22. H. Yan, S.H. Park, G. Finkelstein, J.H. Reif, and T.H. LaBean (2003) DNA-Templated Self-Assembly of Protein Arrays and Highly Conductive Nanowires. *Science* **301**, 1882-1884.

23. Hao Yan, Thomas H. LaBean, Liping Feng, and John H. Reif, (2003) Directed Nucleation Assembly of Barcode Patterned DNA Lattices, *Proc. Natl. Acad. Sci. USA*, **100**, 8103-8108.

24. Yan, H., Feng, L., LaBean, T.H., and Reif, J.H. (2003) Parallel Molecular Computation of Pair-wise XOR Using DNA "String Tile" Self-Assembly, *J. Am. Chem. Soc.*, **125**, 14246-14247.

25. C. Niemeyer and M. Adler, *Angew. Chem.* 2002, **114**, 3933-3937; *Angew. Chem.-Int. Edit.* **41**, 3779-3783.

26. L. Feng, S.H. Park, J.H. Reif, and H. Yan, (2003) A Two State DNA Lattice Switched by DNA nanoactuator, *Angew. Chem.-Int. Edit.* **42**, 4342-4346.

27. John H. Reif (2003) The Design of Autonomous DNA Nanomechanical Devices, Walking and Rolling DNA. *DNA Based Computers (DNA8)*, Sapporo, Japan, June 10-13, 2002, (Edited by M. Hagiya and A. Ohuchi), *Lecture Notes in Computer Science*, **2568**, Springer-Verlag, New York, pp 22-37.

28. H. Li, S- H. Park, J. H. Reif, T. H. LaBean, and H. Yan, (2004) DNA Templated Self-Assembly of Protein and Nanoparticle Linear Arrays, *J. Am. Chem. Soc.* **126**, 418-419.

29. D. Liu, J.H. Reif, and T.H. LaBean, (2003) DNA Nanotubes, Construction and Characterization of Filaments Composed of TX-tile Lattice. *DNA Based Computers (DNA8)*, Sapporo, Japan, June 10-13, 2002, (Edited by M. Hagiya and A. Ohuchi), *Lecture Notes in Computer Science*, **2568**, Springer-Verlag, New York, pp 10-21.

30. D. Liu, S- H. Park, J. H. Reif, and T.H. LaBean (2004) DNA nanotubes self-assembled from TX tiles as templates for conductive nanowires. *Proc. Nat. Acad. Sci., USA* **101**, 717-722.