Self-assembled DNA Structures for Nanoconstruction

Hao Yan, Peng Yin, Sung Ha Park, Hanying Li, Liping Feng, Xiaoju Guan, Dage Liu, John H. Reif, Thomas H. LaBean

Department of Computer Science, Duke University, USA

Abstract. In recent years, a number of research groups have begun developing nanofabrication methods based on DNA self-assembly. Here we review our recent experimental progress to utilize novel DNA nanostructures for self-assembly as well as for templates in the fabrication of functional nano-patterned materials. We have prototyped a new DNA nanostructure known as a cross structure. This nanostructure has a 4-fold symmetry which promotes its self-assembly into tetragonal 2D lattices. We have utilized the tetragonal 2D lattices as templates for highly conductive metallic nanowires and periodic 2D protein nano-arrays. We have constructed and characterized a DNA nanotube, a new self-assembling superstructure composed of DNA tiles. We have also demonstrated an aperiodic DNA lattice composed of DNA tiles assembled around a long scaffold strand; the system translates information encoded in the scaffold strand into a specific and reprogrammable barcode pattern. We have achieved metallic nanoparticle linear arrays templated on self-assembled 1D DNA arrays. We have designed and demonstrated a 2-state DNA lattice, which displays expand/contract motion switched by DNA nanoactuators. We have also achieved an autonomous DNA motor executing unidirectional motion along a linear DNA track.

INTRODUCTION

DNA is an extraordinarily versatile material for designing nano-architectural motifs, due in large part to its programmable G-C and A-T base pairing into well-defined secondary structures. These encoded structures are complemented by a sophisticated array of tools developed for DNA biotechnology: DNA can be manipulated using commercially available enzymes for site-selective DNA cleavage, ligation, labeling, transcription, replication, kination, and methylation. DNA nanotechnology is further empowered by well-established methods for purification and structural characterization and by solid-phase synthesis, so that any designer DNA strand can be constructed. The above advantages of DNA as a nanoconstruction material explain the rapid and exciting progress in DNA based nanotechnology in recent years [1-3], especially in self-assembled nanostructures [4-6], nanorobotics [7-16], and nanocomputation [17-24]. In this paper, we review our recent experimental progress in constructing novel self-assembled DNA structures for nanofabrication and nanorobotics, and we also discuss their applications in nanotechnology, with an emphasis on DNA-based nannoelectronics.

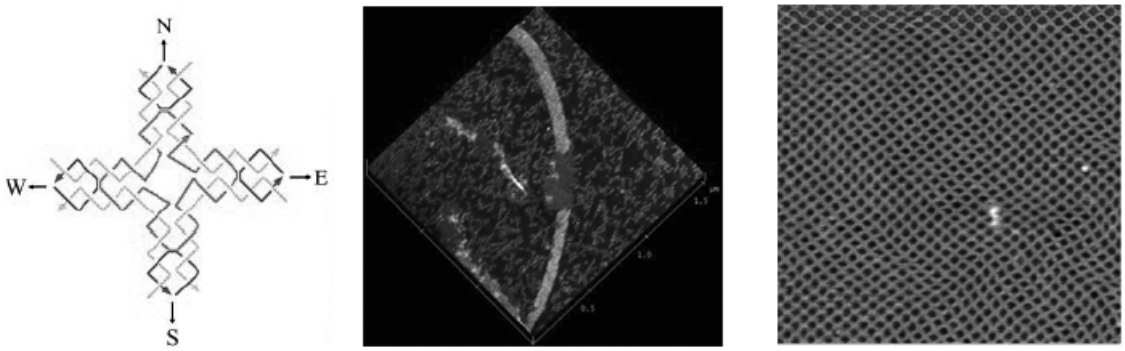

FIGURE 1 (a) Strand structure of the cross DNA nanostructure (4x4 DNA tile). **(b)** Atomic Force Microscope (AFM) image of a nanoribbon self-assembled from the cross DNA nanostructure tiles. **(c)** AFM image of a nanoribbon composed of 4x4 tiles. Scale: 1.5 um x 1.5 um **(e)** AFM image of a 2D nanogrid self-assembled from the cross structure tiles. Scale: 600 nm x 600 nm.

SELF-ASSEMBLED DNA LATTICES

DNA Nanogrids and Nanoribbons

Self-assembling nanostructures composed of DNA molecules offer great potential for bottom-up nanofabrication of materials and objects with nanometer scale features. Potential applications of DNA self-assembly and scaffolding include nanoelectronics, biosensors, and programmable/autonomous molecular machines. We have recently achieved the design, characterization and self-assembly of a novel DNA nanostructure (referred to as DNA cross structures or 4x4 tiles) [25]. This planar tile consists of four four-armed branch junctions pointing at four directions (north, east, south, and west in the tile plane). Special features of this tile structure include a square aspect ratio, which may help regularize lattice growth by balancing helix stacking and sticky-end connections in all four directions within the lattice plane. Figure 1a gives a schematic drawing of the strand structure of the 4x4 tile. Note that a central strand weaves through all the four four-armed junctions. Though each arm is of flexible Holliday junction structure, when combined with junctions on neighboring tiles they are able to form reasonably rigid nanostructures.

With slight modification of the tile spacing and its sticky-end association, we are able to program the self-assembly of the above 4x4 tiles into two distinct lattice morphologies: a uniform width nanoribbon and a ‘waffle-like 2D planar nanogrid structure. A nanoribbon is formed when the identical face of every constituent tile points toward the same direction in the lattice; the planar grid, in contrast, is formed when the identical face of each adjacent tile points up and down alternatively. Figures 1b and c give AFM images of the 1D nanoribbon and 2D nanogrid.

The DNA nanogrid and nanoribbon can serve as scaffolds to organize other molecular components. In particular, we have achieved a) periodic streptavidin nanoarrays templated on 2D nanogrids via affinity binding to biotin labeled DNA strands, and b) highly conductive sliver and gold nanowires via electroless deposition. Details can be found in ref. 25.

DNA Lattice Nanotubes from TX Tiles

We have recently reported results on the construction and characterization of DNA nanotubes, a new self-assembling superstructure composed of DNA tiles. 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.

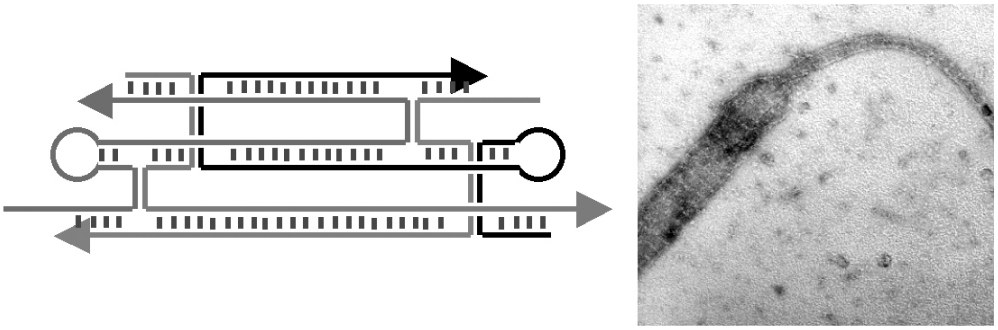

FIGURE 2. ***Left panel:*** Schematic drawing of a TAO DNA tile. ***Right panel:*** TEM image of a DNA nanotube constructed from two different tile types.

Figure 2 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 good 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 [26]. 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.

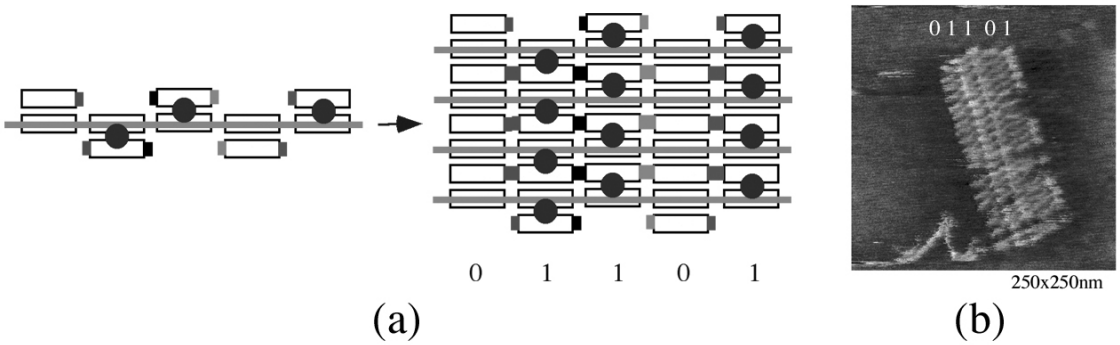

FIGURE 3 (a) Simplified schematic drawing of the self-assembly of the DNA barcode lattice 01101. The gray thick line represents a scaffold strand weaving continuously through five separate DNA tile structures. Each black dot represents a DNA stem loop coming out from its corresponding DNA tile. Multiple layers of such structure will associate with each other via sticky end pairings and form a 2D grid displaying the barcode information, which can be detected by AFM. **(b)** AFM image of a lattice displaying the barcode information of 01101. 1 and 0 bit values are clearly visible as lighter and darker stripes.

Directed Nucleation of Barcode DNA Lattices

We recently reported the construction of an aperiodic patterned DNA lattice (barcode lattice) formed by a self-assembly process via the directed nucleation of DNA double crossover (DX) tiles [28] around a scaffold DNA strand [27]. Figure 3a shows a schematic drawing of the self-assembly of a barcode lattice representing bit values of 01101. A scaffold strand (shown as gray thick line in Figure 2a) encodes information 01101, and serves as the nucleation point for the assembly of DX tiles, with each bit represented by a DX tile. To aid in visual read-out of the encoded information 01101, each bit 1 is displayed as two stem loops perpendicular to the tile plane, with one protruding upward and the other downward; each bit 0, in contrast, is represented as the absence of such stem loops. Multiple layers of such structure will associate with each other via sticky ends pairing and form a 2D grid displaying the barcode information, which can be easily detected by AFM. Figure 3b shows one such image.

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 DX 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 1D DNA strand into a 2D 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. Details of the barcode lattice construction and analysis can be found in ref. 27.

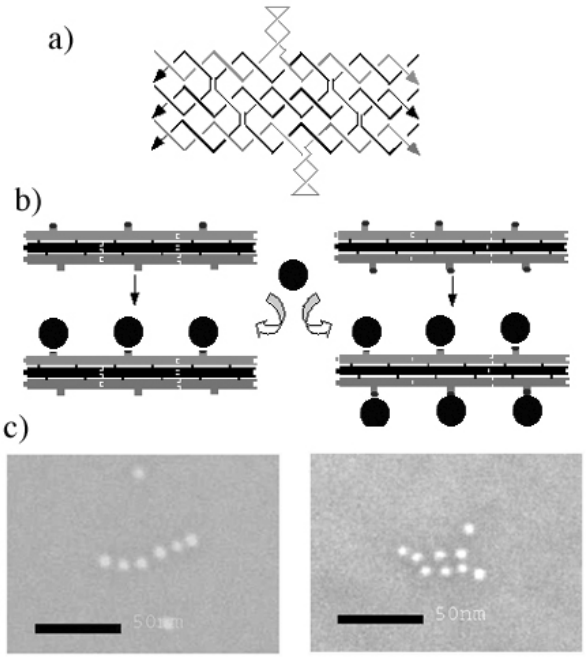

FIGURE 4 (a) Strand structures of triple crossover DNA tile with two stem loops protruding out the tile on two sides. **(b)** schematic drawings of the TX DNA-templated self-assembly of single-layer and double-layer streptavidin-gold linear arrays. Left: streptavidin-gold binds to one side of the 1D TX arraysingle layer through streptavidin-biotin interatction to form single layer gold nanoparticle arrays; Right: streptavidin-gold binds to both side of the 1D TX arraysingle layer through streptavidin-biotin interatction to form double layer gold nanoparticle arrays. Streptavidin-gold is represented by black ball. **(c)** scanning electron microscopy (SEM) images of single-layer (left panel) and double-layer (right panel) streptavidin-gold arrays. Scale bars: 50 nm.

DNA Templated Linear Nano-Particle Arrays

Self-assembled DNA arrays provide an excellent template for spatially positioning other molecules with increased relative precision and programmability. One potential application of DNA nanotechnology is the use of self-assembled DNA lattices to scaffold assembly of nanoelectronic components, especially metallic nanoparticles. We have recently demonstrated the use of a linear triple crossover (TX) DNA array for the assembly of streptavidin conjugated 5 nm gold nanoparticles, where the gold can be precisely positioned periodically on the self-assembled DNA array [29]. Two forms (single-layer and double-layer) of streptavidin-coated gold nanoparticle linear arrays were achieved on DNA template. In this system, each TX tile (Figure 4a) is designed to contain two stem loops protruding on two opposite sides of the TX molecule in the tile plane. To obtain a single-layer streptavidin-gold array, only the stem loop on one side of each TX tile is modified with biotin; to obtain double-layer of strepatividin-gold array, stem loops on both sides of each TX tile are modified with biotin. Streptavidin coated gold nanoparticles (shown as dark black balls in Figure 4b) specifically bind to the biotin-modified stem loops, and thus self-organize into single- or double-layer arrays respectively. Figure 4c shows scanning electron microscopy (SEM) images demonstrating the TX array templated self-assembly of single layer and

double layer streptavidin-gold particles. The distance between each pair of adjacent gold particles in the single layer and double layer arrays is ~17 nm, matching the designed parameter.

The use of DNA nanostructure to organize nanoparticles into programmable arrays could provide a powerful tool to assemble architectures for nanoelectronic device construction and electrical measurements. It may also aid in building logical molecular electronic devices such as quantum cellular automata. In addition, an array of uniformly gapped metallic particles might serve to interconnect other nanoelectronic components.

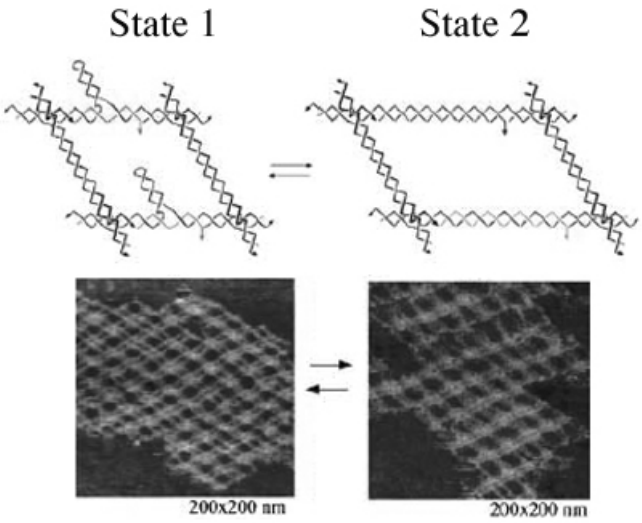

FIGURE 5 ***Upper panel:*** Schematic drawing of the two states of the lattice unit structure. ***Lower panel:*** Representative AFM images of the rhombus lattices displaying different morphologies, which can be reversibly switched by the DNA nanoactuator.

DNA NANOMECHANICAL DEVICES

A Two-state DNA Lattice Switched by DNA Nanoactuator

Controlled mechanical movement in molecular-scale devices is one of the key goals of nanotechnology. DNA is an excellent candidate for the 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. 30]. 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.

We have recently 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 [31]. The nanoactuator can exist in two states. State 1

is the shortened state with a bulged three-arm DNA branch junction; state 2 is the elongated state with 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, a DNA device based on bulged three-arm junction is an excellent candidate to serve as actuator for DNA lattices. The parallelogram lattice contained one such device two opposite edges of each unit cell (Figure 5). Large alterations in lattice dimension were observed due to the additive changes from each unit cell. Lower panel of Figure 5 shows the AFM images demonstrating the inter-conversion of the two states of the rhombus lattice actuated by the actuator devices. The sizes of the cavities in the rhombus lattice were switched from ~ 14 nm x 14 nm (the left image) to ~ 14 nm x 20 nm (the right image). Reverse process from extended lattice to contracted lattice was also observed. Details of the 2-state lattice system are reported in ref. 31.

The above switchable DNA lattices promise numerous potential applications in nanofabrication, nanocomputation, and nanoelectronics. One particularly attractive application could be the controlled nanofabrication of molecular nanoelectronic wires with ‘on’ and ‘off’ states, since the size and the shape of the underlying DNA lattice templates can be programmably controlled using DNA sequence dependent nanoactuator devices.

An Autonomous DNA Walker Moving Along a Track

Most molecular machines executing cellular functions in human body are autonomous and in many cases unidirectional, which makes the construction of such autonomous unidirectional devices in artificial systems promising and attractive. We have recently reported the design and experimental construction of an autonomous unidirectional DNA walker along a DNA track [32]. This walker device has the following features: 1) The device is free of any external environmental mediations, and hence is autonomous. It is powered by the hydrolysis of ATP consumed by T4 ligase. 2) The motion of the device is unidirectional. 3) The device executes motion along a DNA track and renders a DNA fragment moving unidirectionally from one end of the track to the other end.

The structural design and the operation of the device are shown in Figures 6. The walker device is composed of two parts: the ‘track’ and the ‘walker’. The track consists of three evenly spaced DNA double helical ‘anchorages’ (A, B, and C), each tethered to another DNA double helical segment ‘backbone’ via a flexible single strand DNA ‘hinge’; the walker is a 6-nucleotide DNA initially residing on top of anchorage A (labeled * and represented as bold fragments in Figure 6).

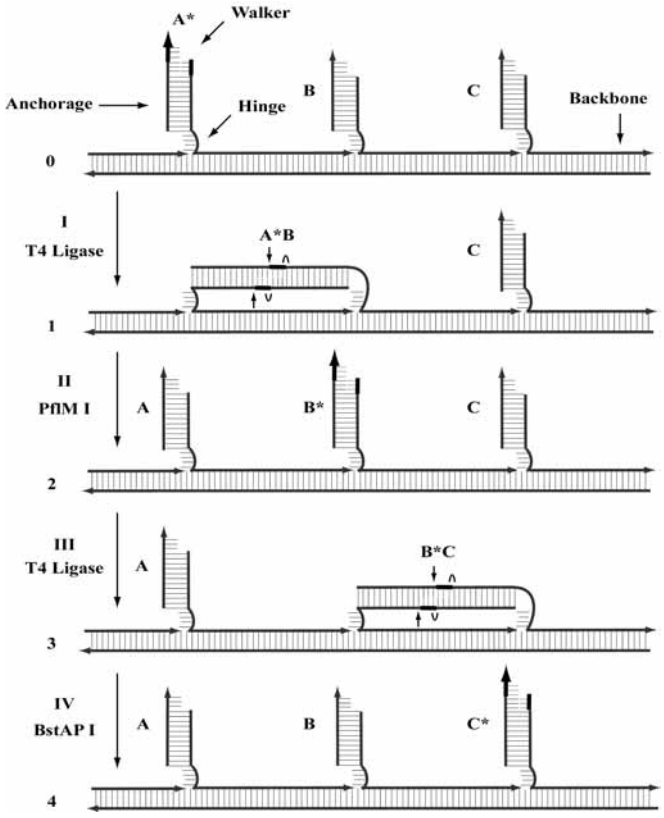

FIGURE 6 The structural design and operation of the autonomous unidirectional device. The bold fragments are the walker fragments. The arrows indicate the PflM I and BstAP I restriction sites. The curves indicate the ligation sites.

The walker moves sequentially along the track from anchorage A to B, then to C in an autonomous, unidirectional fashion. The motion is facilitated by the alternate actions of ligase and restriction endonucleases, and powered by the hydrolysis of ATP. Panel 0 of Figure 6 shows the walker (indicated by *) residing at anchorage A before the motion starts. At this point, the anchorage/walker complex A* and the anchorage B have complementary sticky ends, which will hybridize with each other. The nicks in the hybridization complex A*B are subsequently sealed by T4 ligase, which covalently joins the two anchorages (process I in Figure 6). This is an irreversible step driven by the energy released from the hydrolysis of ATP. Next, a restriction endonuclease PflM I recognizes the newly generated recognition site in A*B and cuts the walker to anchorage B* (process II). Now the newly generated sticky end of B* is complementary to that of C, and the two will hybridize with each other. Again, T4 ligase seals the nicks (process III), producing a new recognition site for another endonuclease BstAP I, which will cut the walker to anchorage C, completing the autonomous unidirectional movement of the walker (process IV). The above autonomous, unidirectional operation of the walker device was verified via the careful tracking of the radioactively labeled walker using gel electrophoresis. For details, see ref. 32.

In principle, we can extend the motion of the walker well beyond the 3-anchorage system demonstrated above. By encoding information into the walker and the

anchorages, we have accomplished a theoretical design of an autonomous nano-mechanical device capable of universal computation and hence universal translational motion [33]. It is also conceivable to embed the walking device into well defined DNA lattices [4-6, 27] and thus obtain ('intelligent') robotics lattices. Nanorobotics systems of this kind would have many applications in nano-computing, nano-fabrication, and nano-electronics.

CONCLUSION

In this review, DNA as a designer molecule for constructing self-assembled DNA lattices and nanomechanical devices was discussed. The experimental demonstrations described here further attest to DNA's role as a leading material for nanoconstruction. The fascinating potential applications of self-assembled DNA structures for nanoelectronic, nanorobotics, and nanocomputation are awaiting us to explore.

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