JACS
JOURNAL OF THE AMERICAN CHEMICAL SOCIETY

COMMUNICATION

pubs.acs.org/JACS

Design and Construction of Double-Decker Tile as a Route to Three-Dimensional Periodic Assembly of DNA

Urmi Majumder,[§] Abhijit Rangnekar,[§,∥] Kurt V. Gothelf,[∥] John H. Reif,[*,§] and Thomas H. LaBean[*,§,†,‡]

Departments of [§]Computer Science, [†]Chemistry, and [‡]Biomedical Engineering, Duke University, Durham, North Carolina 27708, United States

[∥]Centre for DNA Nanotechnology, Department of Chemistry and iNANO, Aarhus University, DK-8000 Århus, Denmark

Supporting Information

ABSTRACT: DNA is a useful material for nanoscale construction. Due to highly specific Watson−Crick base pairing, the DNA sequences can be designed to form small tiles or origami. Adjacent helices in such nanostructures are connected via Holliday junction-like crossovers. DNA tiles can have sticky ends which can then be programmed to form large one-dimensional and two-dimensional periodic lattices. Recently, a three-dimensional DNA lattice has also been constructed. Here we report the design and construction of a novel DNA cross tile, called the double-decker tile. Its arms are symmetric and have four double helices each. Using its sticky ends, large two-dimensional square lattices have been constructed which are on the order of tens of micrometers. Furthermore, it is proposed that the sticky ends of the double-decker tile can be programmed to form a three-dimensional periodic lattice with large cavities that could be used as a scaffold for precise positioning of molecules in space.

Over the past decade, the use of DNA for the construction of designed nanostructures and the programmed assembly of particles and biomolecules has attracted much attention.[1] Due to the predictable Watson−Crick base pairing, stability, high persistence length of the DNA double helix, and ease of chemical synthesis of short oligonucleotides (up to ∼200 bases long), DNA has become a popular material for self-assembly-based fabrication in bionanotechnology.

One of the fundamental goals of DNA nanotechnology has been to use branched DNA junctions (crossovers) to construct ordered arrays and assemble DNA into three-dimensional crystalline lattices for use as scaffolds for organization of biological macromolecules, nanodevices, and nanoelectronic components.[2] Over a period of time, many DNA tiles containing crossovers have been synthesized and then used to create large ordered arrays in one- and two-dimensions.[3]

The DNA 4×4 cross-tile was one of the first tile designs (along with the parallelogram tiles[3h]) that provided helix-stacking in both directions in the lattice plane, thus providing more regular lattice growth and 2D lattices with square aspect ratios.[4] Moreover, the 4×4 cross-tile architecture has been experimentally demonstrated to be capable of producing very large lattices with edge dimensions on the millimeter length scale.[5] There have also been several reports in which three-dimensional DNA nanostructures have been synthesized.[6] However, these structures typically exist in solution as individual entities, incapable of assembly into larger structural arrays in three-dimensions.

Only recently, a well-ordered macromolecular three-dimensional crystalline lattice using the DNA tensegrity triangle has been demonstrated.[7] The tensegrity triangle is a rigid DNA motif with three-fold rotational symmetry.[3d] The resulting three-dimensional lattice has periodic rhombohedral cavities of approximately 103 nm^3 in size. The relatively small size of the cavity puts a limitation on the usefulness of the lattice for scaffolding other nanostructures. Moreover, the tightly packed structure would also deny access for the guest molecules to reach the inner strata of the lattice.

Here we describe the design and synthesis of a cross-tile, called the double-decker tile, that has been assembled into periodic two-dimensional lattice and which is also capable of self-assembling into three-dimensional lattice with much larger cavity size.

The double-decker tile comprises of two 4×4 cross-tiles, lying one on top the other and linked by two crossovers in each arm arranged perpendicular to the plane of the tile (Figure 1A,B). Thus, each arm of the double-decker tile consists of four helices. The distance between the horizontal crossovers and vertical crossovers within the tile arms was set to 18 nucleotides, a value which is one full turn of DNA double helix greater than the minimum possible value of approximately 8 nucleotides. In order to reduce possible strain between the layers, one of the strands in the helix connecting the two crossover points was nicked.

The double-decker tile was designed so that all of its four arms are symmetric. Consequently, the sequence composition of each arm is exactly the same. This provides a reduction in the number of different DNA strands required and also a simplification of the sequence design. Moreover, it also cancels possible geometric distortions and asymmetric, sequence-specific curvature in the tile. It has been shown previously that by adopting sequence symmetry in the 4×4 cross-tile, it was possible to obtain much larger lattices compared to asymmetric tiles.[5]

Nucleotide sequences were designed using Uniquimer, which is a software tool for generating *de novo* DNA sequences for DNA self-assembly.[8] The algorithm for this software is based on a previously published program, SEQUIN.[9] Both of the sequence design tools assign DNA sequences on the basis of sequence symmetry minimization. However, in Uniquimer, the sequence optimization is automated based on heuristics and has been

Received: December 3, 2010
Published: February 28, 2011

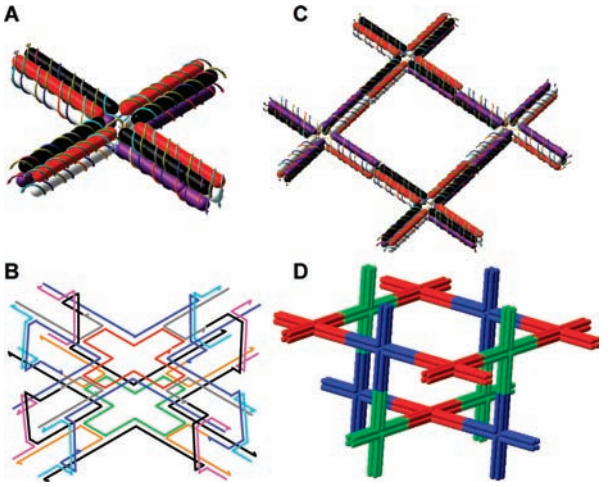

Figure 1. (A) Schematic representation of the double-decker tile and (B) schematic drawing of the strand trace through the tile. (C) Self-assembly of the double-decker tile into two-dimensional lattice using corrugation. (D) Suggested design for the extension of double-decker tile into three dimesions to form a 3D lattice.

observed to sometimes over use particular subsequences. Consequently, a Perl program was developed to find subsequence repeats in a given set of sequences of user defined length, and this information was then used to eliminate excessive repeats before assigning the final DNA sequences.

Sticky ends were then designed so that the adjacent tile centers would have integral numbers of half-turns (15 in this case) of double helix between them. Consequently, the length of the sticky ends was set to 4 bases. Based on the complementarity of sticky end sequences, the double-decker tile can be programmed to self-assemble into either two-dimensional lattices or three-dimensional lattices (Figure 1C,D). It is well established that the original design[4a] of the 4×4 tile introduces some inherent curvature within the tiles that can be eliminated by using a corrugation strategy (flipping adjacent tiles) within the 2D lattice.[4b] The same strategy was employed here, and the sticky ends of the double-decker tile were designed in order to accomplish the flipping of the adjacent tiles with respect to one another (Figure 1C).

The lattice was formed by heating a stoichiometric mixture of participating strands to 90 °C and slowly cooling it to room temperature in a styrofoam box over 16 h, followed by overnight incubation at 4 °C. The corrugated design successfully assembled into enormous lattices of tens of micrometers in size (Figure 2A−C). Height analysis of the AFM images showed that the lattice was approximately 3.4 nm high (Figure S2), which corresponds to the height of two DNA double helices on mica. Also, analysis of the AFM images of center-to-center distance between two adjacent tiles corroborates the theoretically predicted value of approximately 30 nm (which corresponds to 15 half-turns of DNA double helix plus the width of two double helices) (Figure S2).

One interesting observation from the AFM images is that the lattices typically display fairly straight edges and quite sharp corners. This may be attributable to the high degree of cooperativity between the double-decker tiles due to the presence of four pairs of sticky ends within each arm-to-arm association. In previous 4×4 cross-tile lattices with only two sticky-end associations per tile binding interaction, somewhat more ragged or jagged lattice edges have been observed.[4]

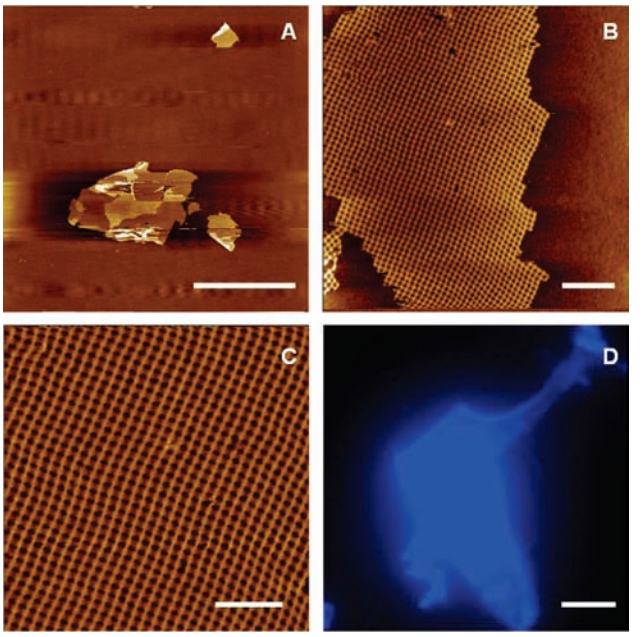

Figure 2. (A−C) Atomic force microscopy images of the double-decker 2D lattice with corrugation. The scale bars are (A) 10 μm, (B) 300 nm, and (C) 200 nm. (D) Fluorescence microscopy image of the same sample. The scale bar is 20 μm. The lattices are tens of micrometers in size.

One drawback with AFM imaging is that the imaging area can be rather small compared to that for the 2D lattices. To circumvent this problem and determine the size of the bigger lattices, fluorescence microscopy was performed. Figure 2D shows a fluorescence microscopy image of the 2D lattice stained with 4′,6-diamidino-2-phenylindole (DAPI). It shows one of the largest lattices observed, with dimensions on the order of tens of micrometers. A wider field image with multiple lattice pieces is given in Figure S3. It should be noted that the DAPI staining process can destroy large lattice, and therefore the pieces observed here should be taken as low estimates for the possible size of assembled lattice.

The sticky ends of the double-decker tile can be programmed to rotate neighboring tiles by 90° and thus to form 3D lattices as well (Figure 1D). The resulting 3D lattice would display cavities of substantial size, with periodicity of approximately 60 nm. This should enable it to act as a host material and allow easy access to guest macromolecules and nanostructures, such as proteins or nanoparticles.

This study presents a new DNA tile design with two 4×4 cross-tiles one on top of another, connected via Holliday junction-like crossovers. The tile was designed to have symmetric arms and the sticky ends were programmed to create corrugated associations between neighboring tiles. This led to large two-dimensional lattices which extended to tens of micrometers in size. One of the advantages of this tile is that the sticky ends can be designed such that a three-dimensional periodic lattice can be formed with large cavity size. Such a lattice could be used for precise positioning of other molecules in 3D space.

■ ASSOCIATED CONTENT

Supporting Information. Detailed experimental procedures, DNA sequences, and analysis of AFM images. This material is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Author
thomas.labean@duke.edu

ACKNOWLEDGMENT

This work was supported by grants DMR-0706397, OISE-0624012, CCF-0650083, CCF-05-23555, and CCF-04-32038 from the U.S. National Science Foundation and by the Danish National Research Foundation (support to A.R. and K.V.G.). We thank Prof. Jie Liu for providing access to a DI Veeco Nanoscope IIIa AFM.

REFERENCES

(1) (a) Seeman, N. C. *Nature* **2003**, *421*, 427–431. (b) Li, H.; Carter, J. D.; LaBean, T. H. *Mater. Today* **2009**, *12*, 24–32. (c) Amin, R.; Kim, S.; Park, S. H.; LaBean, T. H. *NANO* **2009**, *4*, 119–139.

(2) Seeman, N. C. *J. Theor. Biol.* **1982**, *99*, 237–247.

(3) (a) Winfree, E.; Liu, F.; Wenzler, L. A.; Seeman, N. C. *Nature* **1998**, *394*, 539–544. (b) LaBean, T. H.; Yan, H.; Kopatsch, J.; Liu, F.; Winfree, E.; Reif, J. H.; Seeman, N. C. *J. Am. Chem. Soc.* **2000**, *122*, 1848–1860. (c) Malo, J.; Mitchell, J. C.; Venien-Bryan, C.; Harris, J. R.; Wille, H.; Sherratt, D. J.; Turberfield, A. J. *Angew. Chem., Int. Ed.* **2005**, *44*, 3057–3061. (d) Liu, D.; Wang, M.; Deng, Z.; Walulu, R.; Mao, C. *J. Am. Chem. Soc.* **2004**, *126*, 2324–2325. (e) Mathieu, F.; Liao, S.; Kopatsch, J.; Wang, T.; Mao, C.; Seeman, N. C. *Nano Lett.* **2005**, *5*, 661–665. (f) He, Y.; Chen, Y.; Liu, H.; Ribbe, A. E.; Mao, C. *J. Am. Chem. Soc.* **2005**, *127*, 12202–12203. (g) Reishus, D.; Shaw, B.; Brun, Y.; Chelyapov, N.; Adleman, L. *J. Am. Chem. Soc.* **2005**, *127*, 17590–17591. (h) Mao, C.; Sun, W.; Seeman, N. C. *J. Am. Chem. Soc.* **1999**, *121*, 5437–5443.

(4) (a) Yan, H.; Park, S. H.; Finkelstein, G.; Reif, J. H.; LaBean, T. H. *Science* **2003**, *301*, 1882–1884. (b) Park, S.H.; Yin, P.; Liu, Y.; Reif, J. H.; LaBean, T. H.; Yan, H. *Nano Lett.* **2005**, *5*, 729–733.

(5) He, Y.; Tian, Y.; Chen, Y.; Deng, Z.; Ribbe, A. E.; Mao, C. *Angew. Chem., Int. Ed.* **2005**, *44*, 6694–6696.

(6) (a) Goodman, R. P.; Schaap, I. A. T.; Tardin, C. F.; Erben, C. M.; Berry, R. M.; Schmidt, C. F.; Turberfield, A. J. *Science* **2005**, *310*, 1661–1665. (b) He, Y.; Ye, T.; Su, M.; Zhang, C.; Ribbe, A. E.; Jiang, W.; Mao, C. *Nature* **2008**, *452*, 198–201. (c) Douglas, S. M.; Dietz, H.; Liedl, T.; Högberg, B.; Graf, F.; Shih, W. M. *Nature* **2009**, *459*, 414–418. (d) Andersen, E. S.; Dong, M.; Nielsen, M. M.; Jahn, K.; Subramani, R.; Mamdouh, W.; Golas, M. M.; Sander, B.; Stark, H.; Oliveira, C. L. P.; Pedersen, J. S.; Birkedal, V.; Besenbacher, F.; Gothelf, K. V.; Kjems, J. *Nature* **2009**, *459*, 73–76.

(7) Zheng, J.; Birktoft, J. J.; Chen, Y.; Wang, T.; Sha, R.; Constantinou, P. E.; Ginell, S. L.; Mao, C.; Seeman, N. C. *Nature* **2009**, *461*, 74–77.

(8) Wei, B.; Wang, Z.; Mi, Y. *J. Comput. Theor. Nanosci.* **2007**, *4*, 133–141.

(9) Seeman, N. C. *J. Biomol. Struct. Dyn.* **1990**, *8*, 573–581.