Communication

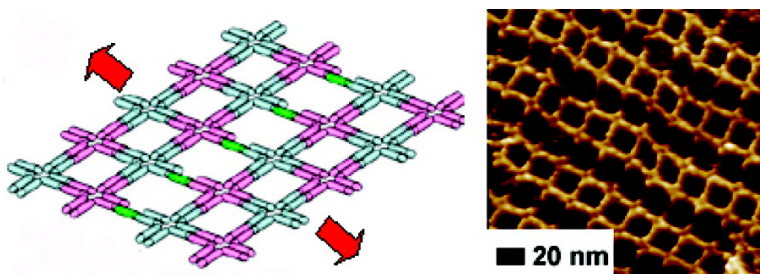# Stepwise Self-Assembly of DNA Tile Lattices Using dsDNA Bridges

Sung Ha Park, Gleb Finkelstein, and Thomas H. LaBean

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Stepwise Self-Assembly of DNA Tile Lattices Using dsDNA Bridges

Sung Ha Park,‡ Gleb Finkelstein, and Thomas H. LaBean*

Departments of Physics, Chemistry, and Computer Science, Duke University, Durham, North Carolina 27708

Received October 23, 2007; E-mail: thomas.labean@duke.edu

With few exceptions,[1] the simple helical motif of double-strand DNA (dsDNA) has typically been judged to be uninteresting in DNA-based nanotechnology applications. Here, we present constructions consisting of heterogeneous DNA motifs using dsDNA in conjunction with complex, cross-tile building blocks.[2−4] We demonstrate control of length and directionality of superstructures by incorporation of dsDNA bridges in stepwise assembly processes. This strategy is analogous to on-the-fly "reprogramming" of the sticky-ends displayed on DNA tiles.

For over 20 years, DNA has been recognized as a useful construction material for nanotechnology because of its readily programmable molecular recognition and predictable local geometry.[5] Many artificial, self-assembled DNA nanostructures have been reported using various geometric structures and functionalities, including one- and two-dimensional periodically patterned structures,[2−4,6] three-dimensional polyhedra,[7] DNA computers,[8] and mechanical devices.[9] Although dsDNA has been used as a scaffold for constructing metallized nanowires,[10] it has not previously been considered a useful motif for complicated self-assemblies.

Here we demonstrate dsDNA nanobridges in joining preformed lattice pieces in controlled ways. Two distinct self-assembled DNA superstructures are implemented and observed by atomic force microscopy (AFM): (i) finite-size lattice formed from 2×2 nanoarrays (NAs)[3] plus dsDNA bridges and (ii) extended lattices formed from nanotracks[4] (NTs) plus dsDNA bridges.

Schematic diagrams of the self-assembled cross-tiles are shown in Figure 1a and 1b. Arrows indicate simplified strands running from 5′ to 3′. A-tiles (shown in blue) and B-tiles (in red) each consist of three kinds of strands: a central loop-strand, four shell-strands, and four arm-strands. Arm-strands each carry 5-base sticky-ends at both 5′- and 3′-ends. Detailed nucleotide sequences and Watson−Crick complementary sticky-ends are given in the Supporting Information (Figures S1 and Table S1). The 1×2 and 2×2 NAs were assembled by placing equimolar mixtures of desired strands in 1xTAE/Mg buffer and cooling slowly from 95 to 20 °C. We assembled two forms of 2×2 NAs: without arms (a cartoon and an AFM image in Figure 1d) and with noncomplementary sticky-ends on the outer arm-strands (Figure 1e). We observed that 1×2 and 2×2 NAs without outer arms were sufficiently structurally robust to visualize by tapping-mode AFM under buffer (Figure 1c and 1d, respectively). Among the structures visualized in AFM images, we estimate that roughly 80% of 1×2 NAs, about 90% of 2×2 NAs without arm-strands, and approximately 75% of 2×2 NAs with arm-strands have assembled correctly.

Next, we tested the sequential build-up of hierarchical superstructures via stable substructures, thus allowing the reuse of DNA base sequences. A two-step method was used: first, a high-temperature anneal (as above) and, second, a low-temperature anneal, by cooling slowly from 42 to 20 °C over ca. 4 h (see Supporting Information for details). Low-temperature annealing
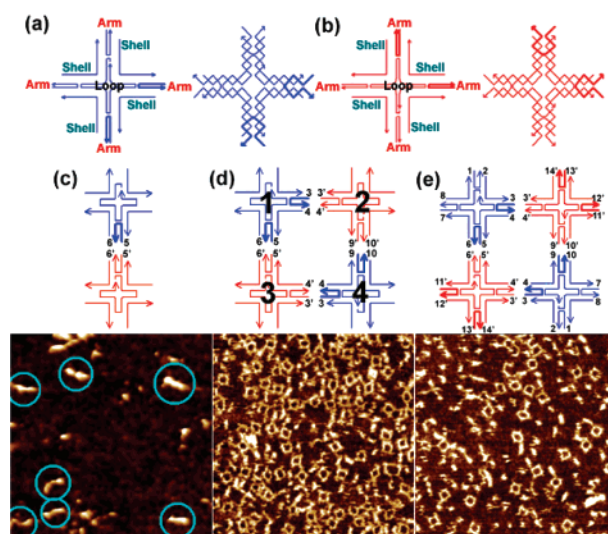
‡ Current address: Department of Computer Science, Caltech, Pasadena, CA 91125.

Figure 1. Schematic drawings and AFM images of tiles and NAs (without dsDNA bridges): (a) A-tile, (b) B-tile, (c) 1×2 NA, (d and e) 2×2 NA without and with outer sticky-end arm strands, respectively. Scans are (c) 250×250 nm and (d and e) 500×500 nm.
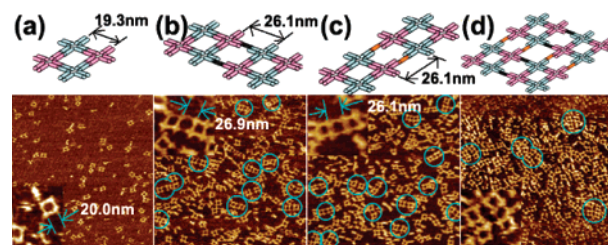

Figure 2. Schematic cartoons and AFM images of four-step self-organization of finite-size superstructures composed of 2×2 NAs and dsDNA bridges: (a) 2×2 NA, (b) 2×2 NAs with horizontal bridges, (c) with vertical bridges, and (d) with bridges in both directions (scan sizes, 800×800 nm and insets, 100×100 nm). Circled in cyan are the properly assembled superstructures. Production yields are discussed in the text.

minimizes dissociation of preformed-lattices ($T_m \approx 43−45$ °C) and provides enough thermal energy to facilitate further hybridization. We assembled 2×2 NAs by first annealing two versions of 1×2 NA ($1 + 2$ and $3 + 4$, shown in Figure 1d) in separate test tubes, followed by mixing and low-temperature annealing. This two-step assembly provides fully addressable 2×2 NAs with four unique pixel sites. NAs were modified with biotinylated loop-strands and mixed with streptavidin (SA), thus decorating the arrays with programmed patterns of SA. The decoration of NAs with protein was visualized by AFM (data not shown); protein patterns on similar NAs have been described previously.[3]

We then demonstrated construction of DNA superstructures composed of 2×2 NAs and dsDNA bridges (2 full-turns, ~6.8 nm) (Figure 2). Although the tiles typically interact via two sticky-ends per arm, here we connect neighboring tiles with individual
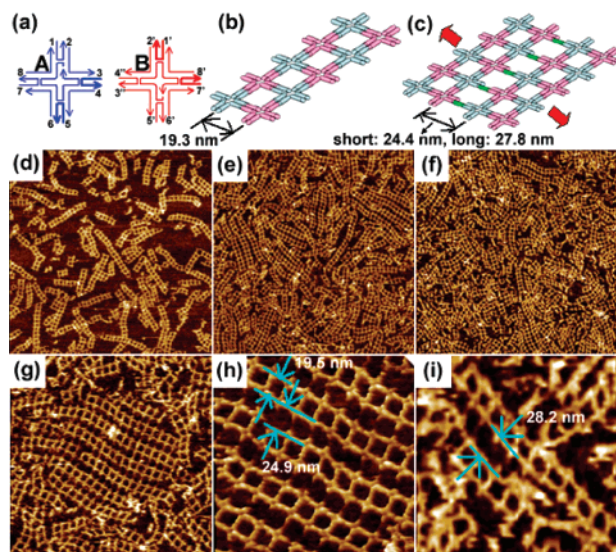
Figure 3. (a) Drawings of A and B cross-tiles, (b) cartoon of a nanotrack (NT), (c) NT with dsDNA bridges. Red arrows indicate growth-directions due to the possibility of assembling additional dsDNA bridges. AFM images are given (1 × 1 μm scans) for (d) NT without bridges, (e) NT with short-bridges, and (f) NT with long-bridges; (g and h) high-resolution AFM images of NTs with short-bridges (500 × 500 nm and 200 × 200 nm scans); (i) AFM image of NTs with long-bridges (200 × 200 nm scan). Observed dimensions are in good agreement with designed structures.

dsDNA bridges. A diagram of the four-step assembly process is given in the Supporting Information (Figure S3). Figure 2 panels a through d show cartoons and AFM images of 2 × 2 NAs (after second-step annealing), 2 × 2 NAs with horizontal bridges (after third), with vertical (after third), and with both directions (after final-step), respectively. Horizontal and vertical are relative directions and are defined in the Figure 2d schematic drawing. All experimental distance measurements are in good agreement with designed superstructures with less than 5% deviation. Structures circled in cyan in Figure 2b through 2d are the properly assembled superstructures. We estimate final production yields of 2 × 2 NAs at roughly 80%, 2 × 2 NAs with either horizontal or vertical bridges at 30%, and 2 × 2 NAs with both at 20%, respectively. Here noticeably low yields of final products of 2 × 2 NAs with either horizontal or vertical, and 2 × 2 NAs with both bridges may come from error accumulation through each annealing step. To realize the full potential of stepwise assembly procedures, we will need to maximize the yield of final product, probably by increasing production yields of intermediate products. This could be achieved by including separation steps, for example, native gel electrophoresis, to remove unassociated and/or poorly associated strands.

We also demonstrated size control of self-assembled DNA NTs utilized by two different lengths of dsDNA bridges, the short (16 bases, 1.5 full-turns, ~5.1 nm) and the long (26 bases, 2.5 full-turns, ~8.5 nm) bridges. By keeping the core structure of the A and B cross-tiles and intentionally introducing noncomplementarity into the sticky-ends on one side of the A and B tiles (sticky-ends 3, 4, 3″, and 4″ in Figure 3a) self-assembly of 1D DNA NT can be obtained. A cartoon of NT is shown in Figure 3b and AFM image in Figure 3d. A cartoon of nanotracks with multipath bridges are shown in Figure 3c; these can be achieved by a two-step

annealing procedure. Figure 3e through 3i shows AFM images of NTs with dsDNA bridges with various lengths. Figure 3g shows a crystal of seven NT units aligned by linking bridges. The average measured distances between NTs with short/long bridges are ~24.5/~28.0 nm, closely matching the designed distances, 24.4/27.8 nm.

In summary, we have rationally designed, constructed, and examined a set of novel DNA superstructures consisting of two different DNA motifs; cross-tiles and dsDNA domains using sequential assembly. The use of synthetic dsDNA motifs as nanobridges for connecting complex DNA tile lattices is equivalent to reprogramming the neighbor relations between tiles while minimizing cost and maximizing efficiency. Duplex DNA molecules can serve as nanobridges that control size and directionality of superstructures assembled from complex DNA substructures. Since dsDNA has a persistence length of ~50 nm (roughly 150 base pairs), future studies are needed to determine whether longer DNA bridges are also appropriate for stepwise assembly projects.

Supporting Information Available: DNA base sequences, complete experimental methods, additional AFM images, and image analysis. This material is available free of charge via the Internet at http://pubs.acs.org.

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