

Programmed-Assembly System Using DNA Jigsaw Pieces

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Abstract: A novel method for assembling multiple DNA origami structures has been developed by using designed 2D DNA origami rectangles, so-called “DNA jigsaw pieces” that have sequence-programmed connectors. Shape and sequence complementarity were introduced to the concavity and convex connectors in the DNA rectangles for selective connection with the help of

nonselective π -stacking interactions between the side edges of the DNA jigsaw piece structures. Single DNA jigsaw piece units were assembled into

unidirectional nanostructures with the correct alignment and uniform orientation. Three and five different DNA jigsaw pieces were assembled into pre-designed and ordered nanostructures in a programmed fashion. Finally, three-, four-, and five-letter words have been displayed by using this programmed DNA jigsaw piece system.

Keywords: DNA structures • nanostructures • nanotechnology • self-assembly • supramolecular chemistry

Introduction

Programmed self-assembly of heterogeneous molecules is one of the major issues in supramolecular chemistry and nanotechnology.^[1,2] DNA is the most promising molecule for the creation of various self-assembled components and scaffolds for the selective placement of functional molecules and nanomaterials.^[2–5] The DNA origami method developed for the preparation of fully addressable 2D structures allows the design of complicated patterns and the construction of 3D architectures.^[5–10] In nanotechnology, mesoscale assemblies with a size of approximately 100 nm can be used to prepare functional components for practical devices. Although programmed DNA and RNA assemblies that use

small DNA and RNA units have been achieved,^[11,12] a strategy for the construction of defined mesoscale DNA assemblies has not been developed. Since DNA origami structures have been employed for the selective positioning of functional molecules and nanoparticles,^[5–10] novel methods should be explored for further development of the programmed arrangement of multiple prefunctionalized DNA origami structures. To achieve this aim, two processes are required: the first is the assembly of the monomeric components from the template DNA and staple strands, and the second is the arrangement of the multiple monomers in a programmed manner.

In this study, we designed and prepared 2D DNA origami rectangles, DNA jigsaw pieces containing sequence-programmed connection sites, a convex connector, and a corresponding concavity, to allow assembly of adjacent DNA rectangles through these adhesive connections (Figure 1). The three principal rules for our programmed assembly of DNA origami rectangles are: 1) specific Watson–Crick base pairing at the concavity and convex connector in part through staple strands; 2) shape fitting of the side edges for selective connection and exclusion of undesired pairings of DNA rectangle units; and 3) π -stacking interactions of the side edges,^[6] which enables the formation of stable connection of the side edges. Using these designed DNA jigsaw pieces, we examined the programmed arrangements of the multiple mesoscale DNA assemblies. For the controlled self-assembly of these DNA jigsaw piece monomers, we divided the self-assembly processes into two steps by employing different

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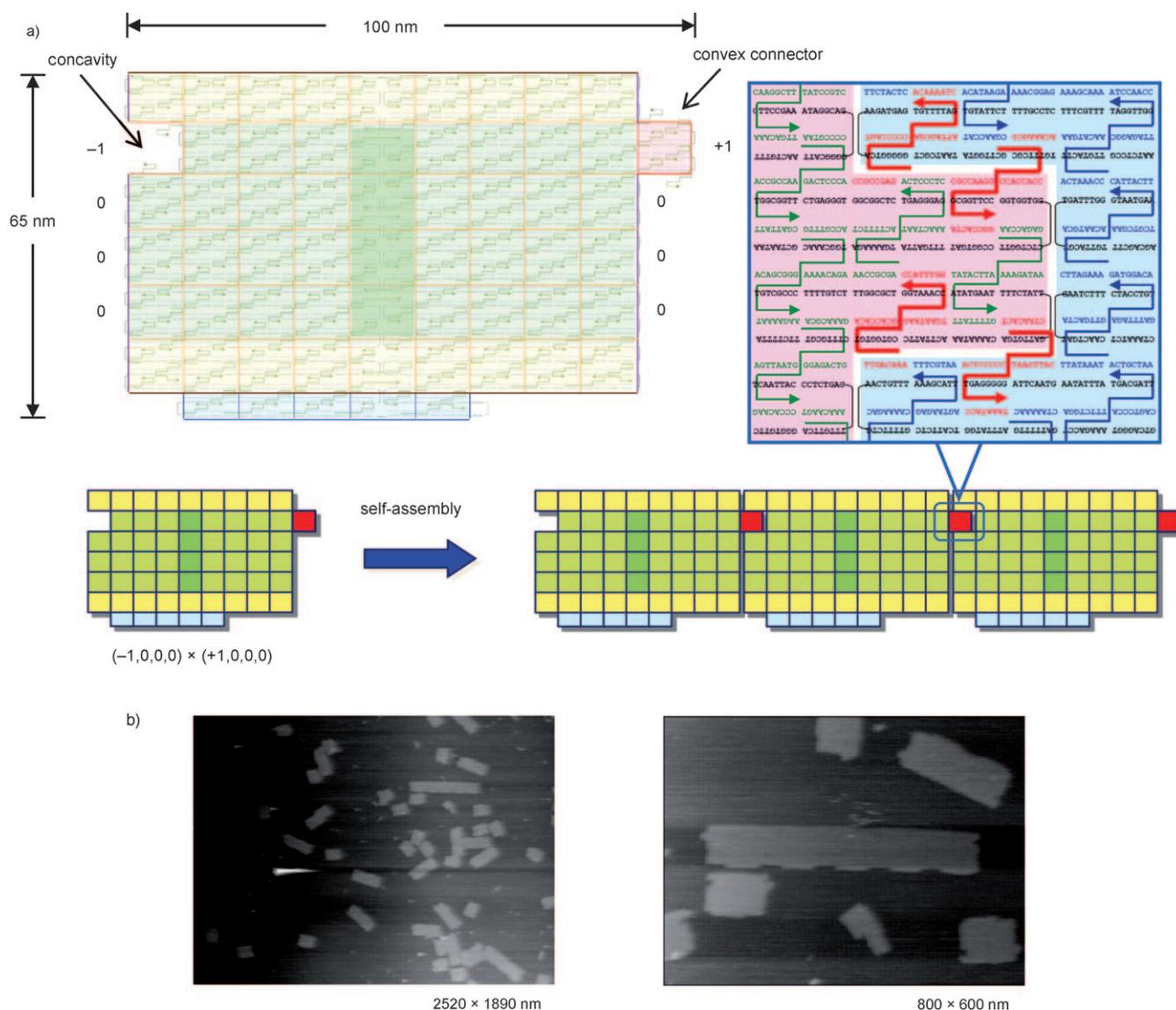


Figure 1. "DNA jigsaw piece" structure and self-assembly of the DNA piece and the assembled structures. a) Top left: Structure of the designed DNA jigsaw piece with a concavity and convex connector. Simple drawing of the jigsaw piece represented as a matrix of blocks. One block contains four 32-mer double strands. Bottom: Scheme for the self-assembly of the DNA jigsaw piece monomer. Top right: The detailed connection around the connector and the concavity between neighboring DNA jigsaw piece monomers. Black and colored DNA sequences denote the M13 single strand and the staple strands, respectively. Green and blue arrows (staple strands) represent the left and right side edges of the DNA jigsaw piece monomers, respectively. Red arrows represent the hybridization strands (connective staple strands) bridging both DNA jigsaw pieces. b) AFM images after self-assembly of the DNA pieces. The right image is an expanded image of the left; image sizes are presented below the images.

annealing procedures: one was the formation of the individual DNA jigsaw piece monomers by fast annealing, and the other was the arrangement of the assembled DNA jigsaw piece monomers into the programmed unidirectional DNA assemblies by slow annealing.

Results and Discussion

Design and preparation of the DNA jigsaw piece and assembly of the monomers: For self-assembly with single DNA jigsaw pieces, the convex connector and the counter-

part concavity were situated on opposite edges of the DNA origami rectangle and in the same rows. The design of a jigsaw piece may be specified by the code $(a,b,c,d) \times (e,f,g,h)$, in which the two brackets describe the structure of the two opposite edges. The symbols specify the shape of each edge, divided into four-helix blocks. Each four-helix block can be flush (0), a convex connector (+1) or a concavity (-1). For example, the left and right edges of the DNA jigsaw piece shown in Figure 1 are expressed as $(-1,0,0,0) \times (+1,0,0,0)$. An additional asymmetric domain (light blue) was also introduced at the bottom edge of the

rectangle structure for identification of the orientation of the DNA jigsaw piece.

As reported in the initial study by Rothmund in 2006, the DNA origami rectangle assembled in the direction of the side edges through π -stacking interactions, and even when the extra tetrathymidine (T_4) loops were introduced at both side edges, the connection still worked.^[6] We utilized the π -stacking interactions between the side edges of neighboring DNA origami rectangles as the driving force for the 1D assembly. In this study, we introduced two additional factors that allowed the precise connection of the side edges: one was the fit of the side edges by using a concavity and convex connector, and the other was the hybridization of the connection strands introduced between the concavity and the convex connector. In the DNA jigsaw piece design, we introduced an extra T_4 -loop to the staple strands located on both side edges, to prevent any undesired connections during self-assembly as described in the original DNA rectangle design.^[6] However, we did not introduce extra loops around the concavity and the convex connector to promote specific connections.

The detailed design of a DNA jigsaw piece is shown in Figure 1a, in which four connective hybridization strands colored in red were introduced as parts of staple strands to connect the convex connector and the concavity during the adhesion of the neighboring DNA jigsaw pieces. Formation of the DNA assemblies was carried out by using M13mp18 single-stranded DNA and complementary DNA strands (staple strands) in a solution containing Tris buffer (pH 7.6), EDTA (ethylenediaminetetraacetic acid), and Mg^{2+} . M13mp18 DNA and staple strands (five equivalents) were annealed from 85 to 15 °C by decreasing the temperature at a rate of 1.0 °C min⁻¹. The detailed design of the DNA jigsaw pieces and the sequences of the staple strands are included in the Supporting Information.

AFM measurements clearly demonstrated that the DNA jigsaw piece formed the designed structure, and further elongated assemblies, including a five-successive DNA jigsaw piece assembly, were observed after annealing (Figure 1b). The result shows that the introduction of the concavity and convex connector with the connective hybridization strands allows correct alignment and uniform orientation between the neighboring DNA jigsaw pieces without significantly perturbing the π -stacking interaction between the side edges.

Importantly, when the connection strands in the connector and the concavity were removed, such multiple assembly was not observed (Figure S1 in the Supporting Information). The result indicates that the selective base pairing between the connector and the concavity predominantly controlled the alignment and orientation of the arrangement of the DNA jigsaw piece assembly.

Self-assembly of single DNA jigsaw pieces: To expand the variety of DNA jigsaw pieces, we introduced pairs of concavity and convex connectors at different positions in the same row of the side edges to prepare four different DNA

jigsaw pieces, DNA jigsaw pieces A, B, C, and D as shown in Figure 2. For identification of the individual DNA jigsaw pieces, two dumbbell hairpin DNA strands^[6] shown as pink blocks were introduced as markers that were adjacent to the concavity and convex connector. In this experiment, we segregated the formation of the individual DNA jigsaw pieces and the arrangement of the DNA jigsaw pieces into unidirectional assemblies by using different annealing procedures.

First, the formation of the DNA jigsaw pieces was carried out from a mixture of M13mp18 DNA and staple strands, which was annealed from 85 to 25 °C by decreasing the temperature at a rate of 2.0 °C min⁻¹; this process, which we term “fast annealing”, reduces multimer formation. The monomers were then purified by gel filtration to remove the excess staple strands. The samples were subjected to AFM measurements in the same buffer solution. As shown in Figure 2a and 2b, DNA jigsaw piece monomer, dimer, and trimer structures were observed after fast annealing, and the monomers formed the predesigned shapes. The concavity and convex connector and the corresponding hairpin markers were observed clearly and were identified in the predesigned places. The size of the DNA jigsaw pieces A, B, C, and D was approximately 100 × 70 nm, which is consistent with the original design.

Next, DNA jigsaw pieces were further assembled by slow annealing from 50 to 15 °C at a rate of -0.05 °C min⁻¹, which we term “slow annealing”. After self-assembly of the single pieces, elongated assemblies of the pieces were obtained from all four monomers (Figure 2a and 2b). In the case of the A piece, more than 10 pieces of the multiple DNA monomers were connected to form a structure > 1 μ m in length, which showed 1D and aligned assembly with uniform orientation (Figure 2c and 2d). For extension to the various monomers, we performed self-assembly of the B, C, and D pieces. As shown in Figure 2c and 2d, similar unidirectional assemblies were formed from the DNA jigsaw pieces B, C, and D with a uniform orientation of the individual monomers.

To examine the specific joining of the DNA jigsaw pieces, we mixed the four different DNA monomers A–D and investigated the self-assembly from the mixed monomers. Under slow annealing, the same DNA jigsaw pieces self-assembled selectively, and connections between different DNA monomers were not observed (Figure S2 in the Supporting Information). These results confirm that the hybridization, shape, and π -stacking between the same monomers effectively work for the appropriate pairing and connection of the DNA jigsaw pieces during self-assembly.

Programmed self-assembly of different DNA jigsaw pieces:

This strategy was further expanded to more complicated self-assembly processes by using different DNA jigsaw pieces. To construct the predesigned assemblies, we prepared DNA jigsaw pieces containing a concavity and convex connector in different rows of the side edges of the DNA jigsaw piece structures. To prepare the trimer-assembly E-F-G from the three DNA jigsaw pieces E, F, and G, the DNA

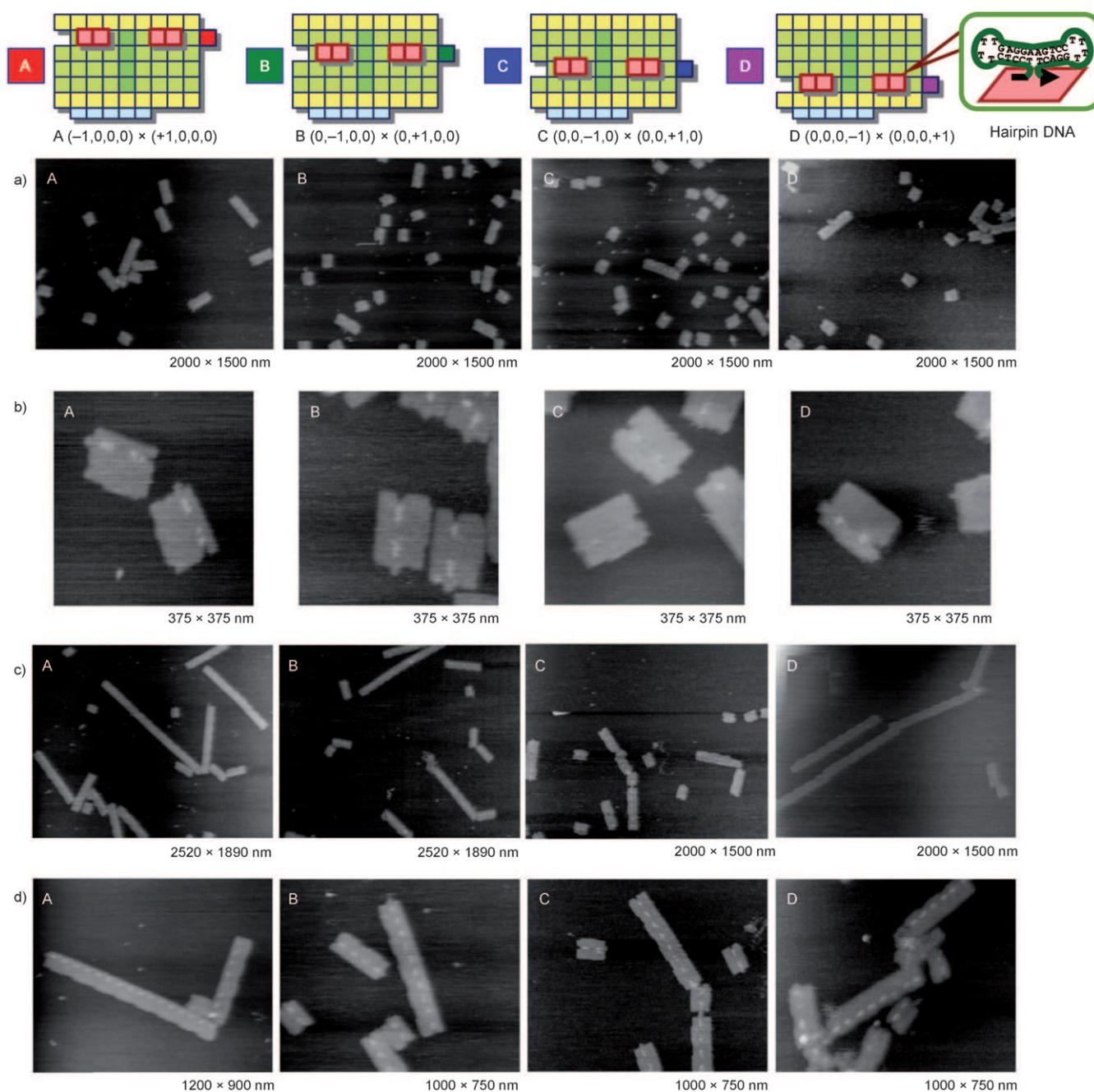


Figure 2. DNA jigsaw piece monomers and self-assembled structures. Simple drawings of the monomers A–D. The positions of the concavity and convex connector on the side edges of the piece are represented as (a,b,c,d) and (e,f,g,h), respectively. Pink blocks on the DNA jigsaw piece monomers represent hairpin DNA markers for AFM imaging. a) AFM images of the DNA jigsaw piece monomers A, B, C, and D after fast annealing from 85 to 25 °C at a rate of $-2.0^{\circ}\text{Cmin}^{-1}$. b) Expanded images of DNA jigsaw piece monomers A–D. c) Self-assembly of the DNA jigsaw piece monomers A–D after slow annealing from 50 to 15 °C at a rate of $-0.05^{\circ}\text{Cmin}^{-1}$. d) Expanded image of the assemblies from the pieces A–D after slow annealing.

strands in the connectors of the E and F pieces were designed as complementary to those in the concavity of the F and G pieces, respectively (Figure 3a). By changing the row, self-assembly from the single piece monomers should be prevented because of the specific hybridization and the incomplete overlap of the shape and π -stacking between the same DNA jigsaw pieces. These DNA jigsaw pieces formed monomers after fast annealing (Figure 3a). When these

pieces were individually assembled by slow annealing, most remained as monomers (Figure S3 in the Supporting Information), in contrast to what was observed for the assembly of the DNA jigsaw pieces A–D (Figure 2). Next, the pieces E, F, and G were mixed and assembled by slow annealing from 50 to 15 °C. Trimers were observed that were assembled from the three DNA jigsaw pieces E, F, and G, in that order, which were identified by the presence of specific hair-

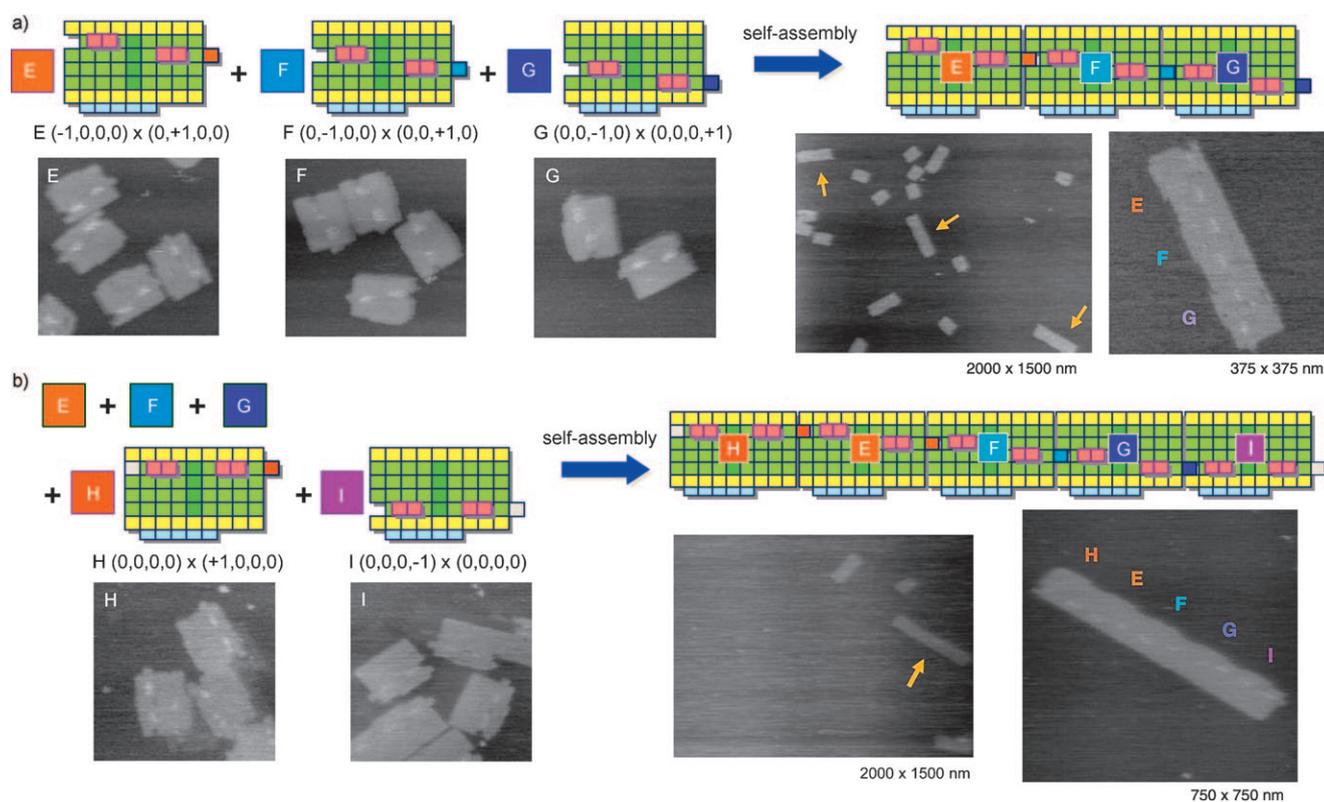


Figure 3. Self-assembly of three and five different DNA jigsaw piece monomers. a) Simple drawings of the DNA jigsaw pieces E, F, and G, in which the concavity and convex connectors in the DNA jigsaw piece are in different rows, and the AFM images of the monomers. Scheme for the assembly of the three DNA jigsaw piece monomers E, F, and G. AFM image of the trimer-assemblies (yellow arrow) from the three pieces E, F, and G (left) and the expanded image of the trimer-assembly E-F-G (right). b) Simple drawings of the DNA jigsaw pieces H and I, in which hybridization (connection) strands in the concavity of piece A and the convex connector of piece D are deleted, and the AFM images of the monomers. Scheme for the assembly of the five pieces, E, F, G, H, and I. AFM images of pentamer-assembly (yellow arrow) from five pieces (left) and the expanded image of the pentamer-assembly H-E-F-G-I (right). Image sizes for monomers are 375 × 375 nm, and other sizes are presented below the images.

pin markers (Figure 3a). In addition to the trimer, monomers and dimers were also observed. The ratio of monomers/dimers/trimers was 16:36:48 with respect to monomer conversion. These results clearly show that the connections designed on the DNA jigsaw pieces promoted the correct assembly of the designed nanostructures.

We next examined the programmed self-assembly of five different monomers. We prepared H and I pieces that did not have hybridization strands in the concavity and convex connector, respectively (Figure 3b and S4 in the Supporting Information). The five pieces E, F, G, H, and I were used for self-assembly. DNA jigsaw pieces E, F, and G formed the center of the three units and pieces H and I acted as terminators of the assembly. Five successive assemblies of the five DNA jigsaw pieces were observed after slow annealing from 50 to 15 °C at a rate of $-0.05\text{ }^{\circ}\text{C min}^{-1}$. We also observed monomers and other multimers. The yield of pentamer formation was 19% with respect to monomer conversion. The pentamers were formed from the five DNA jigsaw pieces in the order H, E, F, G, and I; the assembled DNA jigsaw pieces were identified by the presence of specific hairpin markers (Figure 3b). These results clearly show that the designed connection on the DNA jigsaw pieces allowed

construction of the predesigned unidirectional assemblies from different DNA jigsaw pieces in a programmed manner.

The yields of the assemblies from the different DNA jigsaw pieces in the present work are still not high enough for practical use as carriers for self-assembly-based fabrication. Higher concentrations of DNA jigsaw pieces tended to increase the yields of the target assembly; for example, the five-DNA jigsaw piece assembly yielded 24% as opposed to the previous 10% yield by increasing the concentration of monomers from 2 to 8 nM. In addition, the yield of the slower annealing step for assembling the five monomers was improved from 19 to 24% by changing the temperature-decreasing slope from 0.05 to 0.01 $^{\circ}\text{C min}^{-1}$.

Programmed assembly system for displaying words by using DNA jigsaw pieces carrying alphabet letters:

Finally, this programmed DNA jigsaw piece system was used as a word display. We built the words from the alphabet letters formed by introduction of dumbbell hairpin DNAs onto the individual DNA jigsaw pieces. We noticed from previous work that the introduction of multiple hairpins onto the surface of a DNA jigsaw piece led to the preferential attachment of the hairpin-incorporated side of the DNA jigsaw piece onto the

mica surface. This means that hairpins should be introduced to the backside of the DNA jigsaw pieces presented in Figure 1a. Therefore, we incorporated the hairpin letters into the backside, and the order of the DNA jigsaw pieces was arranged in reverse to display the words (Figure 4). For the three-letter word, we tried to obtain “DNA” by using pieces I, G, and F as representative of D, N, and A, respectively. For the four-letter word “NANO”, we used pieces G, F, E, and I as representative of N, A, N, and O, respectively. The five-letter word “KYOTO” was made from pieces I, G, F, E, and H, as representative of K, Y, O, T, and O, respectively. DNA jigsaw pieces with alphabet letters were prepared by fast annealing, and the corresponding letters were observed on the individual DNA jigsaw pieces (Figure 4a). After self-assembly of these DNA jigsaw pieces by slow annealing, we clearly observed the programmed words in the AFM images. The yields for the three-, four-, and five-letter words were 40, 16, and 9% for monomer conversion, respectively,

which are lower than observations in previous studies for the assembly of monomers containing eight hairpin markers. Introduction of several hairpins may induce curvature of the face of the DNA jigsaw piece, thus, distorting the flatness, which may decrease the adhesive forces necessary for the well-fitted connection required for elongated assembly.

Conclusion

We have demonstrated a novel method for the preparation of predesigned unidirectional DNA assemblies from single and different DNA jigsaw pieces by using the programmed-assembly system. The designed DNA jigsaw piece monomers assembled preferentially through the defined connection in a “key-and-lock” fashion after slow annealing. The three factors mentioned earlier worked effectively to promote programmed assembly; in particular, the DNA se-

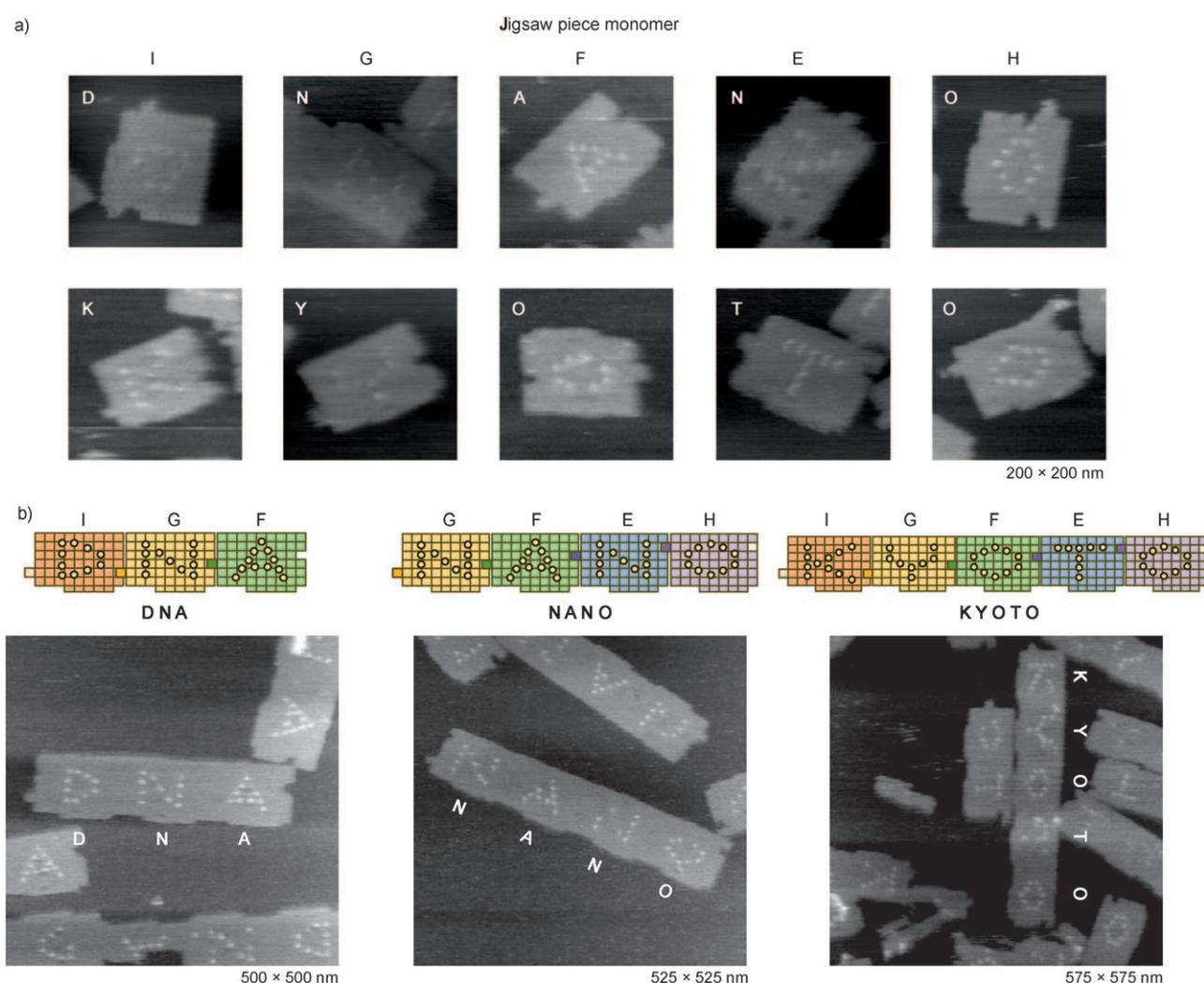


Figure 4. Programmed self-assembly of DNA jigsaw pieces carrying alphabet letters for displaying words. a) The alphabet letters are displayed on the individual DNA jigsaw pieces, and the names of the jigsaw piece monomers (I, G, F, E, and H) are indicated above. b) Unidirectional DNA jigsaw piece assemblies for displaying words. Three letters “DNA” from DNA jigsaw piece units I, G, and F. Four letters “NANO” from DNA jigsaw piece units I, G, F, and E. Five letters “KYOTO” from DNA jigsaw piece units I, G, F, E, and H. Image sizes are presented below the images.

quences of the connection strands controlled the selective assembly of the different DNA jigsaw pieces. The shape of the side edges worked not only for selective connections, but also for the exclusion of incorrect connections during self-assembly. In addition, nonselective π -stacking interactions between the designed side edges promoted the precise connection to maximize the overlap of the side edges for stable adhesion. Our method can be expanded to create self-assembled modules carrying various molecules and nanomaterials, which can be used for programmed self-assembly-based nanofabrication.

Experimental Section

Preparation of the samples: The sample solution (50 μ L) containing M13mp18 single-stranded DNA (New England Biolabs, 0.01 μ M), staple DNA strands (5 equiv, 0.05 μ M), Tris-HCl (pH 7.6, 20 mM), EDTA (1 mM), and MgCl₂ (10 mM) was annealed from 85 to 25 °C at a rate of -2.0 °C min⁻¹, for the preparation of monomers (fast annealing) by using a thermal cycler. The samples were purified twice by using a gel-filtration column (Sephacryl S-300, GE Healthcare). The purified monomers were annealed from 50 to 15 °C at a rate of -0.05 or -0.01 °C min⁻¹, for the assembly of the monomers (slow annealing).

AFM imaging: AFM images were obtained by using an AFM system (Nano Live Vision, RIBM, Tsukuba, Japan) with a silicon nitride cantilever (Olympus BL-AC10EGS). Samples (2 μ L) were adsorbed onto a freshly cleaved mica plate for 5 min at RT and then washed three times with the same buffer solution. Scanning was performed in the same buffer solution using a tapping mode.

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