Subtractive assembly of DNA nanoarchitectures driven by fuel strand displacement†

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Herein we demonstrate that flexible DNA architectures with larger cavities can be efficiently constructed by first assembling a relatively more rigid DNA tile architecture and subsequently subtracting a center tile through fuel strand displacement; such structures are otherwise difficult to obtain if the center tile is missing in the beginning, proving a new strategy for DNA self-assembly.

In the past decade, structural DNA nanotechnology has proven to be successful in creating nanostructures through self-assembly with increased complexity and accuracy at nanometre scale.[1–4] Currently existing strategies of DNA tile based self-assembly include: (1) tile–tile association through sticky ends, that each composite DNA tile possesses unique sticky ends to pair with the sticky ends on its neighboring tiles; when they are mixed together, the sticky ends pair with each other leading the tiles to self-assemble into a larger tile-array structure;[5–9] (2) scaffolded DNA origami[10] or nucleated self-assembly,[11] which relies on using long single stranded DNA, *e.g.* a viral genome, as the scaffold to nucleate a large number of short helper strands (>200) into a predetermined pattern, where the sequences of the helper strands depend on the folding path and the pattern generated; each of these DNA origami tiles with proper sticky ends can be further assembled into a super-array;[10] (3) algorithmic self-assembly, in which DNA tiles carrying computational information are assembled together cooperatively to generate complex patterns. Through these methods, both simple periodic and complex aperiodic DNA arrays have been assembled.[12,13] All these strategies are additive in that the composite DNA tiles or strands are added all together in one step or in a step-wise hierarchical manner.

It has been suggested and demonstrated that DNA nanostructures with different patterns can be used as masks for molecular lithography applications to generate nanometre scale features from a variety of materials.[14] For example, it has been proposed to use DNA nanostructures as templates for generating nanoscale integrated electronic circuits.[6] In these applications, precise control of the size and shape of complex patterning is in high demand.

In the efforts to create fixed sized DNA nanoarrays with a minimal number of unique DNA tiles, we used a symmetric design that took advantage of the intrinsic symmetry of the DNA tile, but broke the symmetry of individual tiles by attaching different sticky ends on each arm, so that the tiles at the corner, side and center positions were differentiated.[15] Finite sized and symmetrically shaped DNA tile arrays were created using a minimal number of tiles defined by the size and symmetry of the array. In that study, we found that a small fraction of arrays failed to form the designed shape because of a missing central tile, which was possibly due to a small error in the stoichiometry. On the other hand, missing 1–2 corner tiles or side tiles did not affect the assembly process thus did not change the overall shape of the final tile array. These observations inspired us to find a reliable alternative way to create a frame structure without the central tile.

Here we demonstrate a new strategy, termed "subtractive DNA self-assembly", that can be used to effectively create flexible DNA nanoarchitectures with relatively large cavities that are otherwise difficult to achieve. Fig. 1 shows one such example where a cross-shaped DNA tile[6,15] (Fig. 1a) was used as a building block to prove this strategy. We used the symmetric array assembly approach we developed before to create a fixed size multi-tile array system for this purpose. Fig. 1b shows the three unique tiles used (A, B, C), each carrying unique sticky ends numbered, *e.g.* 1 pairs with 1′ *etc*. One of the sticky ends (numbered 3′) on the B tile has an elongated toe-hold region, which will be later used for the strand displacement in the subtractive assembly. When all three tiles are mixed in a 4 : 4 : 1 molar ratio of A : B : C, they self-assemble into a symmetric 9-tile array (Fig. 1c). Fig. 1d shows the atomic force microscope (AFM) images of such arrays, with ~70% yield of the complete 9-tile structures. Upon the addition of a pair of fuel strands that are fully complementary to the sticky-end 3′ and the toe-hold regions, the C tile will be released out of the 9-tile structure, leading to an 8-tile square frame with a large central cavity (about 40 × 40 nm). AFM images shown in Fig. 1f clearly demonstrate the success of this subtractive assembly, with ~64% yield of the correctly formed 8-tile closed frame structure. Note there are also ~22% triangular shaped structures in Fig. 1f, which presumably result from incomplete tile arrays that are missing a corner tile before subtraction. There are also ~13% other structures that are open or without a well-defined shape.

To prove that our subtractive assembly strategy is needed to effectively obtain the desired 8-tile frame structure, we did a control experiment by directly attempting to generate the structure in Fig. 1e using A and B tiles only, leaving the C tiles out in the beginning. The structures created are shown in Fig. 1h and schematically in Fig. 1g, where incomplete

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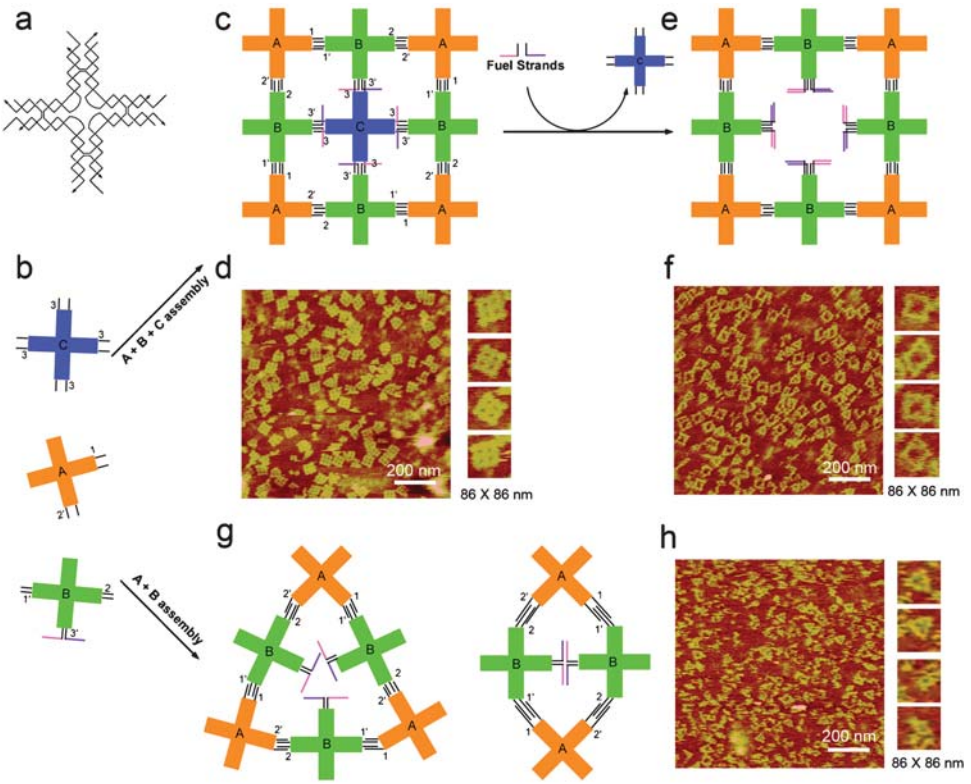

Fig. 1 (a) A 4-arm cross-shaped DNA tile in which each arm carries a pair of uniquely sequenced sticky ends. (b) The three tiles A, B, and C used here. Their sticky ends (numbered such that 1 pairs with 1′, *etc.*) are designed so that when they are mixed in a 4 : 4 : 1 molar ratio, they will self-assemble into 3 × 3 square shaped arrays as shown in (c), and the AFM images in (d). The pair of sticky ends labeled as 3′ on the B tile are elongated to include single-stranded segments that will act as toe-holds in the following strand displacement. (e) After the first step of assembly, upon addition of the fuel strands that are fully complementary to the sticky ends 3′ and the toe-hold segments, the central C tile is released, leaving a square frame with a large central cavity, AFM images shown in (f). The yield of the desired structure is ~64%. (g) When the central C tile is missing in the assembly process to begin with, the A and B tiles will form a variety of smaller structures, such as 6-tile and 4-tile assemblies, due to the flexibility of the cross-shaped tiles. (h) AFM images reveal that no 8-tile square shaped frame structure is found in this assembly.

structures dominate, possibly due to the flexibility of the cross-shaped building blocks. This experiment demonstrates that the central tile is essential in the self-assembly process of the symmetrical 9-tile array, which may act as a starting point (seed tile) for the side tiles and subsequently the corner tiles to attach on. Missing a corner tile is common in the symmetric assembly, but missing the central tile totally prevents the desired structure to form. Therefore the subtractive assembly through strand displacement is a valuable strategy to create the frame structure with a large central cavity.

To further confirm that the subtractive assembly strategy can be effective for a different tile array system, we used a relatively more rigid tile, an 8-helix tile system[16] as a second proof. Fig. 2a illustrates the 8-helix tile building block used in this design. Fig. 2b shows that 13 different 8-helix tiles labeled A to L are used to assemble a 25 tile array (Fig. 2c) with a 2-fold symmetry, where the A tile is in the central position. In this design, the sticky ends on the C and E tiles that pair with those on the A tiles are modified, so that they each contain an 8-base protruding toe-hold segment. When their corresponding fuel strands are added into the solution, the A tile is released, leaving a central cavity in the tile array. AFM images in Fig. 2e and f show the tile array structures before and after fuel strand displacement, with 56% and 44% yield of the desired structure (counting the tile arrays with fewer than 2 tiles missing over all discernible discrete structures), respectively, demonstrating the success of the subtractive assembly. On the other hand, if only 12 tiles are mixed without the central A tile, the structures obtained are all irregularly shaped, with no complete 24-tile structure observed, indicating that the A tile is required as a scaffold element in the correct assembly of the desired architecture, while missing some of the corner tiles does not affect the assembly of the other tiles into a structure only missing that tile. This further confirms that our subtractive assembly strategy is crucial in making a central cavity in the tile array, because it is otherwise hard to achieve without this initial additive and then subtractive approach.

In summary, we have demonstrated that subtractive assembly can be a new strategy for structural DNA nanotechnology. It is foreseeable that such large frame structures with central cavities may be used as masks in molecular lithography. In the future, it is desirable to use strand displacement to create addressable cavities which would be hard to achieve using conventional tile based assembly. However, it is worthy to point out that one can also create holes using the origami approach by following a specific folding path, *e.g.* the smiley face pattern generated by Rothemund,[10] although it is necessary to redesign a relatively large numbers of strands to create

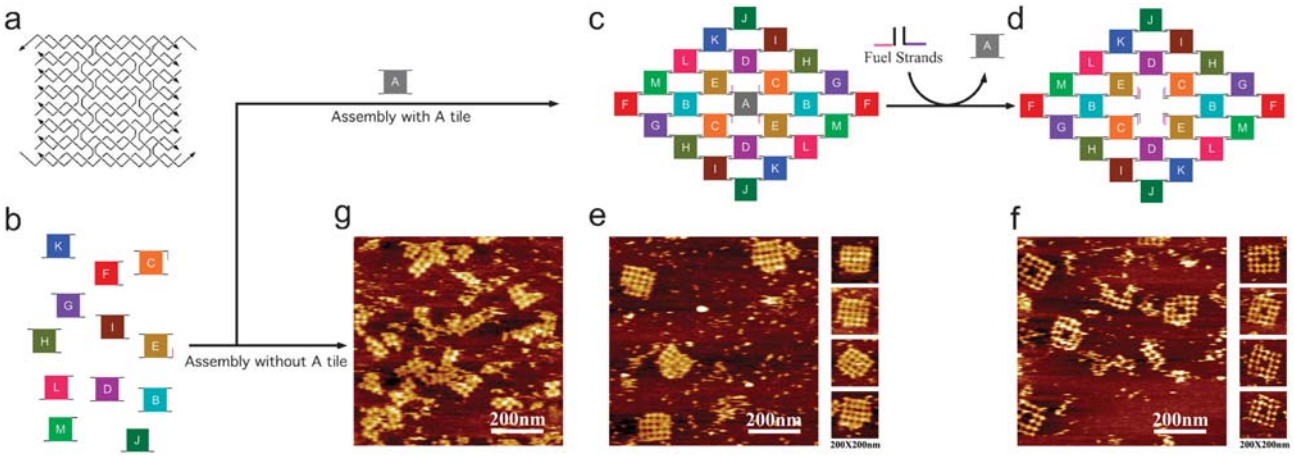

Fig. 2 (a) An 8-helix tile with sticky ends in the 4 corners. (b) The combination of 13 tiles with the sticky ends designed to self-assemble into a 5 × 5 array with 2-fold symmetry as shown in (c). The A tile is in the center. The sticky ends of the C and E tiles that pair with those of the A tiles are each extended with an 8-base segment that acts as a toe-hold for the following fuel strand displacement. (d) Addition of the fuel strands causes release of the central A tile, leaving a cavity in the tile array. The AFM images shown in (e) and (f) are of assembly with the A tile before and after adding fuel strands. (g) AFM image of the assembly with 12 tiles without the central A tile.

a different folding pattern. Our approach complements this and indeed may also be applied to displace helper strands in a preformed DNA origami structure to create holes.

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