Chinese Science Bulletin 2006 Vol. 51 No. 24 2973—2976

DOI: 10.1007/s11434-006-2223-9

Analogic China map constructed by DNA

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Received September 30, 2006; accepted November 19, 2006

Abstract In this research, a nanoscale DNA structure of analogic China map is created. The nanostructure of roughly 150 nm in diameter with a spatial resolution of 6 nm is purely constructed by folding DNA. The picture observed by atomic force microscopy (AFM) is almost identical with the designed shape. The DNA origami technology invented by Rothemund in 2006 is employed in the construction of this shape, which has proved the capability of constructing almost any complicated shape enabled by DNA origami, and provides new bottom-up method for constructing nanostructures.

Keywords: DNA, origami, self-assembly, nanostructure, China map, AFM.

Compared to conventional top-down routines of shaping, bottom-up self-assembly for constructing shapes (especially nanoscale shapes) is undoubtedly an important method. The accumulated knowledge and technology on DNA and the coding capacity of DNA itself have made DNA self-assembly the most promising self-assembly technology.

New methods on constructing nanoscale shapes with DNA self-assembly emerge frequently. In 1989, Seeman[1] first proposed a DNA branched junction as the basic self-assembly unit. After that, Yan *et al.*[2] have improved these four-arm junctions which self-assemble into DNA nanogrids, as have been clearly observed by AFM. In 1998, Winfree *et al.*[3] constructed a DNA double crossover molecule, called DX motif. Each DX motif contains four sticky ends, and they self-assembled into two-dimensional DNA crystals. DX motif can be designed to make DNA triangles[4] and nanotubes[5]. In 2000, LaBean *et al.*[6] constructed a DNA triple crossover molecule, called TX motif. Each TX motif contained six sticky ends, and allowed for better two-dimensional DNA arrays. The common idea of DNA self-assembly is to patch the big shape by assembling small basic units through Watson-Click complementary. This shared property has induced their common limitation; that is, it will be very difficult, if possible, to construct non-periodical complicated shapes.

In 2006, Rothemund[7] first proposed a completely new DNA self-assembly method, that is, DNA origami, which could successfully construct a variety of relatively complicated nanoscale shapes and patterns. This technology is undoubtedly a breakthrough in the field of DNA self-assembly. With DNA origami, Rothemund[7] has constructed six desired shapes of ~100 nm in diameter, including square, rectangle, star, disk with three holes, etc., and several patterns including the map of western hemisphere. The method of DNA origami is folding a long single-stranded DNA molecule by choosing a number of short complementary oligonucleotides to hold it in place. The structures can be programmed to desired shapes. Meanwhile, each oligonucleotide can also serve as a pixel to form designed patterns on the surfaces of those structures.

In this research, DNA origami technology is used to construct an analogic China map shape by folding a 7-kb single-stranded DNA by over 200 complementary oligonucleotides, resulting in a double-stranded DNA structure of ~150 nm in diameter. Imagine the long single-stranded DNA as a soft rope. Fill DNA into the raster in horizontal direction with specified offset to simulate the given shape, and short oligonucleotides perform like a number of staples, binding the long single-stranded DNA in appropriate sites. This structure is observed by AFM, and a nanoscale China map shape is successfully built. This research used DNA origami to construct an asymmetrical complicated two-dimensional shape, and therefore provided a further proof of the complicated nanoscale shape constructing capacity enabled by DNA origami technology.

1 Material and method

1.1 Design of DNA sequence

To fold DNA into China map shape with DNA origami technology, the sequence design strategy comprises: Choose a long single-stranded DNA (called

scaffold strand), and choose appropriate binding site and design complementary short oligonucleotides (called staple strands) to hold the scaffold strand into a desired shape.

The design is performed in two steps:

(i) Design the scaffold according to the outline of China map. Treat the scaffold strand as a long line, filling the raster to form the China map shape by folding the strand in horizontal direction. The two parts of northwest and northeast of China map has make it a concave shape, so this shape cannot be achieved only by folding from north to south. As shown in Fig. 1, separate the main part of China map into east part and west part by the longitude on Hainan Island. Also separate the convex part of northeast and northwest into east and west parts. The folding starts with the south end of Hainan Island, goes from south to north to finish west part first, and after reaching the most north point of northwest part, goes from north to south. Finish the folding of northeast part in the same way, and continue folding on east coast, which will end, again, with Hainan Island. Taiwan Island is the most difficult part, as it is not conjoint with the mainland and not in the central longitude. This issue is roughly solved by linking lines between the island and the mainland. The linking lines are part of the scaffold but not counted into the horizontal folding. Islands in South China Sea are not included in this analogic shape.

The length of each folding line corresponds with the number of bases on the scaffold strand. One turn of DNA double-helix is around 10.67 bases. Each offset has to be an integer of half turns (e.g. $10.67\times0.5\times3\approx16$ bases). One turn is around 3.6 nm in length and 2 nm in width. The gap between horizontal helices depends on the spacing of crossovers, which is set at 1.5 turn. Therefore, the inter-helix gap is ~1 nm. This data shall be considered into the length-width proportions when we are designing the scaffold. Meanwhile, the constraint of ~7000-nt in full length shall also be satisfied. The total number of designed bases is supposed to be close to, but not exceeds, the full length.

Therefore, the designed scaffold shown in Fig. 1 can be represented as the following data set.

Each entry is the number of bases for each line on the scaffold, which is proportional to the offset. This set of data, which is obtained by measuring the China map, is also the input for next step programming: (16 16 6 6 32 32 112 112 107 107 112 112 208 208 230 230 256 256 267 267 272 112 112 112 80 80 48 48 32 48 16 16 16 48 48 64 64 256 80 80 64 64 32 32 48 48 32 32 16 32 16 48 48 80 80 64 64 64 64 32 32 16 112 64 64 80 80 96 96 112 112 112 112 96 96 128 16 16 128 48 48 6 6 16 16). Each integer has to be the multiple times of 16, or with residue 6 or 11 when divided by 16. Under that presupposition, the integer shall be adjusted to be as proportional to offset as possible to achieve a tight simulation of the shape. The specification of residue 6 and 11 will minimize the strain. This issue is discussed in the Supplementary Note S1 of Ruthemund paper[7].

(ii) Generate the sequence of staple strands by program. In order to make the folded rope-like DNA to be fixed to the shape, it is necessary to staple between horizontal lines and vertical gaps, which is implemented by Watson-Crick complementary. We can generate the sequence of staple strands according to the known scaffold sequence and A-T C-G pair matching principals. Staple strands could be classified into eight types of a—h, as shown in Fig. 2 (a). The four types of a—d are used for horizontal fixing, as shown in Fig. 2 (b); their combination can form different types of horizontal fixings. Some staple strands of certain type lack several bases, but it is still considered as this type. For instance, at the upper border of the shape, some staple strands of type a or b will lack 8-nt in 3′; at the left or

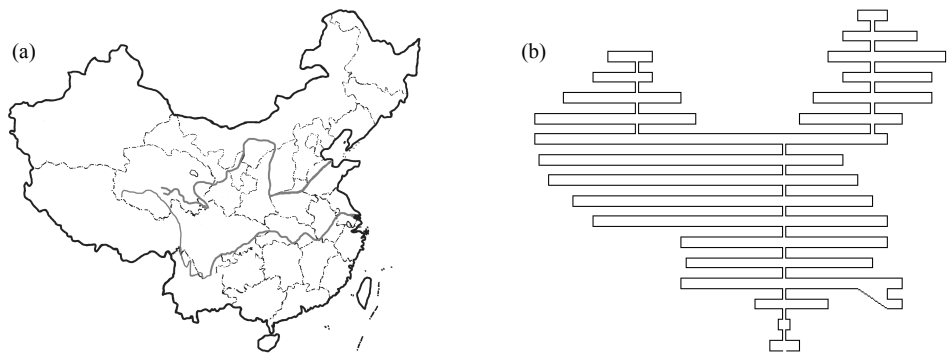

Fig. 1. The map of the main part of China (a) and the corresponding designed scaffold (b).