Stepwise Assembly of DNA Tile on Surfaces

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Abstract. A method of solid-phase self-assembly for building DNA nanostructure in a microfluidic device is proposed in this paper. In this method, pre-assembled DNA lattice is anchored on solid surface, which gives nuclei for further growth of the lattice. Flushing out the solution around the nuclei by flow and replace it by different solution enables us stepwise self-assembly in a single chamber at the constant temperature. The results of experiment to verify feasibility of the proposed method will be shown.

Keywords: DNA tile, self-assembly, stepwise assembly, microfluidic device.

1 Introduction

The production technology of the nanometer order is divided roughly into two categories: top-down, and bottom-up. The top-down production technology, typified by semiconductor processing technology, achieves the resolution of less than a hundred nanometer based on lithography technology. This technology is applied in the MEMS/NEMS, enabling the fabrication of microsensors, micromotors and other micromachines. However, top-down processing technology has an essential drawback; the size of manufacturing facilities greatly increases as the processing method evolves to be more sophisticated. On the other hand, the bottom-up technology realizes nanoscale objects made of atoms, molecules and nanoparticles, not by using external apparatus but by designing interaction among them. This kind of technique, where those components coalesce into complicated nanostructures by self-organization, is now drawing increased attention. Especially, active researches are done on the self-assembly of biomolecules such as DNA and proteins, and on the self-organization using polymer. The bottom-up technology does not require a huge plant for production while making minute processing possible; however there still remain numerous problems with practical applications such as low reliability during the assembly process.

The DNA nanotechnology was initiated in 1982 by Seeman when he proposed self-assembled nanostructures made of DNA molecules [1]. The key in this technology is immobilization of Holliday junction (crossover) to make well-defined DNA structures. Winfree and Seeman utilized one of such structures called DX (double

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crossover) tile to realize a patterned lattice made of these tiles [2]. This method allows us to construct not only simple pattern such as periodic stripes or barcodes, but also the complex algorithmic pattern such as Sierpinski's fractal [3]. However, it is very difficult to obtain perfect DNA lattices in one-pot reaction. The growth process of DNA lattice is strongly influenced by the concentration of monomer tiles as well as the temperature of the solution [4]. As the reaction progresses, decreased concentration of monomer tiles in the tube is inevitable which results in erroneous assembly.

Reif proposed another method of DNA tile self-assembly called the hierarchical assembly procedure [5]. In this method, instead of mixing all kinds of DNA in a single step, several equimolar DNA solutions are combined at an appropriate temperature for the self-assembly of one specific subcomponent. Each subcomponent is independently assembled, and then mixed with solution of another subcomponent at higher temperature to build higher order components and so on. In this manner, they built fully addressable DNA lattice made of larger DNA tiles called 4×4 tile [6]. Advantage of this method is that it requires less kinds of orthogonal sequence of DNA than that of one-pot self-assembly.

We have proposed a method of DNA tile assembly by using microfluidic device [7,8]. We expect that the microfluidic device is advantageous to obtain large high-quality DNA lattice, because it provides a reaction chamber in which the concentration of each DNA component can be kept optimal concentration for the crystals' growth. For this purpose, we have designed and fabricated a special microfluidic device utilizing capillary pump and open reaction chamber that enables real-time, direct AFM observation [7]. We also confirm the flow in the microchannel enhance the hybridization efficiency between immobilized DNA on the wall and DNA molecule in the solution [8].

In this paper, we propose a stepwise self-assembly on surfaces for building DNA nanostructure in a microfluidic device. In our method, pre-assembled DNA lattices are anchored on the microfluidic channel, which serves as nuclei for further growth of the DNA lattices. Flushing out the solution around the nuclei and switching solutions by flow enables us to realize stepwise self-assembly whose can produce the results similar to those of the hierarchical assembly procedure, even in a single reaction chamber. This method also enables us to build a layered structure of DNA lattice at a constant temperature. In the following sections, we show the detail of the concept of the stepwise self-assembly on surfaces and the results of experiment to verify feasibility of the proposed method.

2 Concept of Stepwise Self-assembly on Surfaces

Schematic of the stepwise self-assembly on surfaces is given in Fig.1. This method is suitable for building layered DNA tile lattices with reduced number of orthogonal sticky ends [7]. Note that our method is not limited to this but also effective in building nano-objects with structured hierarchy.

For ease of understanding, a simple example is used hereafter. Three kinds of solution are prepared,

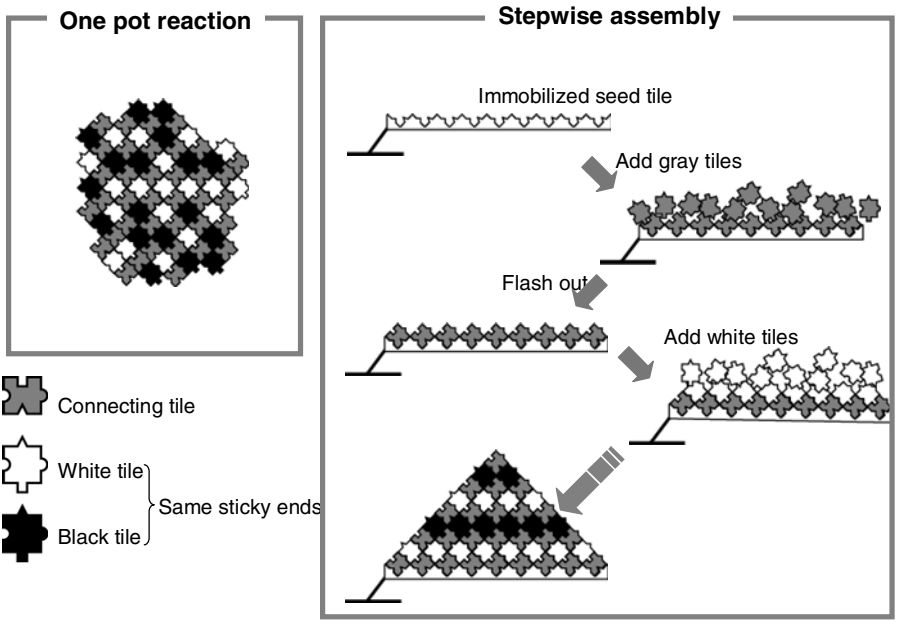

Fig. 1. Stepwise assembly on surfaces

each of which containing a single type of DNA tiles. They are shown as black, gray, and white tiles in the Figure. The incision of four edges represents the sequence of sticky end. Each tile can only be connected to the particular tiles with complementary sticky ends. Here, we assume that the white tiles and black tiles have the identically shaped sticky ends to reduce the number of different sticky ends. Moreover, we designed so that both the white and the black tiles are allowed to attach themselves only to gray tiles, limiting the possibility to the combination of either gray-and-white or gray-and-black.

One-pot reaction, where all three types of the tiles are simultaneously thrown into a tube, results in randomly patterned lattice (Fig.1 left). On the other hand, the solid-phase self-assembly enables us to build well-defined pattern in stepwise fashion. First, pre-assembled seed lattices (initial nuclei) are anchored on a surface by hybridization between the immobilized DNA and the seed lattice. Next, a solution containing only gray tiles is applied. After the reaction, any unconnected DNA tile is washed off with the buffer flow. Then, another solution containing either black or white tiles is applied so the new tiles can hybridize with the previous layer of gray tiles. Further application of the solution containing gray tiles will build a new foundation for yet another layer of black or white tiles. By repeating this process, arbitrary layered pattern can be made from only three kinds of tiles. Also note that the whole process can be done under the same temperature, thus we do not have to change the length of sticky ends for each stage of assembly.

3 Pre-assembly of Nuclei and Their Anchoring on a Gold Surface

In order to initialize lattice growth in the solid-phase, we need crystal nuclei (seed lattices). They must have well-defined lattice structure for the further growth. For this purpose, we prepared a DNA tile lattice made of two columns of DNA tiles (called 2-column DNA lattice, hereafter) (Fig.2.A).

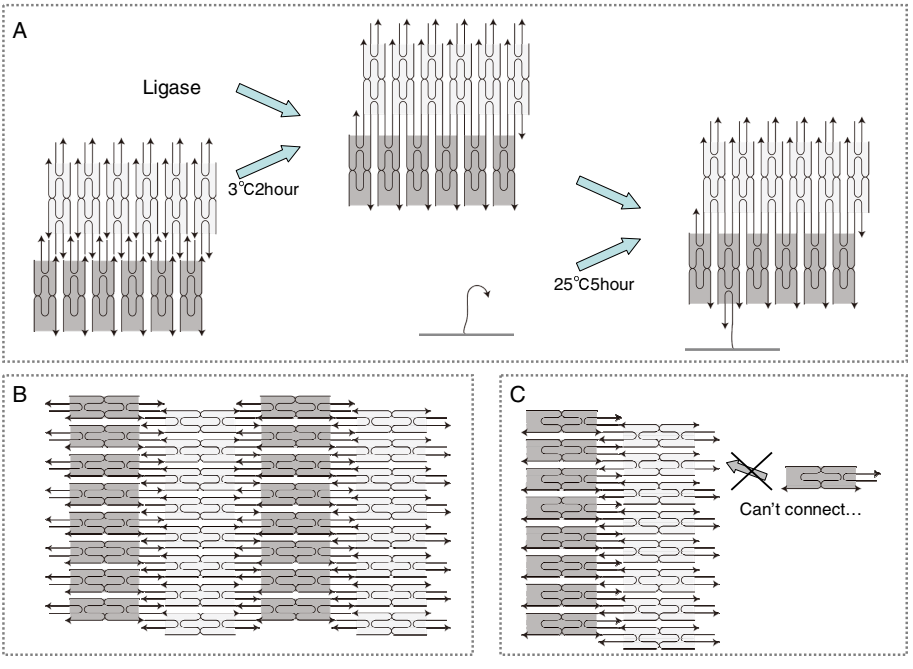

Fig. 2. Pre-assembly of 2-column lattice

3.1 2-Column DNA Lattice

A simple DNA tile set, consisting of two DX tiles is used to make a 2-column DNA lattice. Original tile set generates a large periodic lattice with alternating row of two kinds of tiles, however the shape of the lattice cannot be defined (Fig.2.B). We modified the original tile set to obtain a 2-columned lattice (Fig. 2.C). Here, the strands that comprise lower sticky ends for the gray tile is removed from the original set. We used the same sequence as in the literature [2] for all the strands. The solution must be kept at low temperature (3ºC), because this lattice is formed by only one matching sticky ends. Also we have to stabilize the structure by ligation. Then the solution containing the 2-columin lattices is applied on the gold surface, and anchored by an immobilized ssDNA (Fig.2.A).

The pre-assembly of the 2-column DNA lattice was evaluated by gel electrophoresis. The experimental protocols are as follows: 1) A DNA solution for the 2-column lattice is heated up to 95ºC (ssDNA 1 μM, 1×TAE, $MgCl_2$ 12.5 mM). 2) It is slowly cooled down to room temperature in water bath (Styrofoam container filled with hot water). It

takes overnight. 3) Ligase (T4 DNA Liagase; Takara) is applied to the solution and kept at 10 ℃ for 2 hours. The resultant solution was evaluated by gel electrophoresis (15%PAGE, 250V, 45 min, room temperature) (Table.1 and Fig.3). Lane 1 is a reference experiment where a full DNA lattice is formed. Most ingredients of the solution remained on the top of gel because of their size. The 2-colum lattice is in Lane 3. The long smear region between full lattice and a band of monomer tile indicates it

Table 1. Experimental condition

	Sample	Ligase
Lane 1	Full DNA lattice	O
Lane 2	Full DNA lattice	X
Lane 3	2-column DNA lattice	O
Lane 4	2-column DNA lattice	X
Lane 5	White tile	O
Lane 6	White tile	X

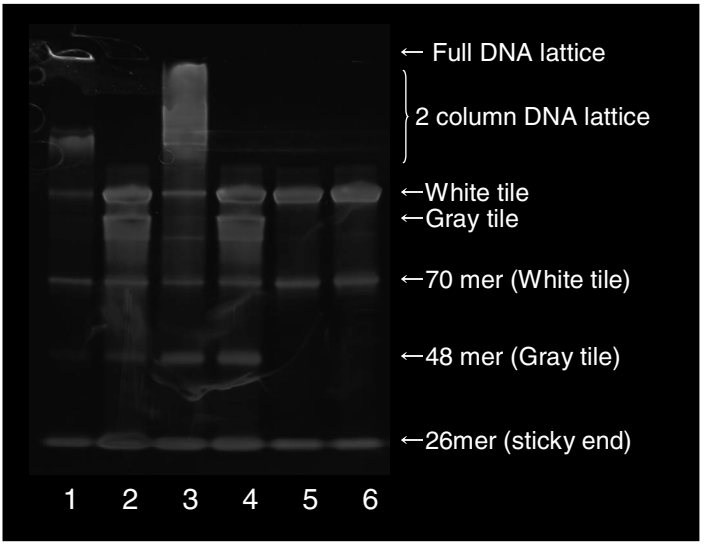

Fig. 3. Gel electrophoresis

forms aggregated structure of various sizes. Ligation process was omitted for lanes 2 and 4. Here, we did not observe any larger structure than a monomer tile. It implies that the 2-column lattices are broken during the electrophoresis.

3.2 Anchoring of 2-Column Lattice

The pre-assembled 2-column lattice is anchored on a solid surface by the following protocols: 1) A 36-base ssDNA (5'-TCA CTC TAC CGC ACC AGA ATG GAG ATT

TTT TTT TTT-SH-3') is put onto gold surface patterned on a glass substrate (72mm x 50 mm, Matsunami). This strand was immobilized by Au-SH bonding (DNA: 50 μM, $MgCl_2$: 200 mM). 2) The surface was rinsed with buffer (1×TAE, $MgCl_2$ 12.5 mM). 3) Solution of 2-column lattice (1μM of each ssDNA, 1×TAE, $MgCl_2$ 12.5 mM) was applied on the surface. The lattice is anchored by hybridization with the immobilized ssDNA (4 hours). One of the sticky end for upper (light gray) tile was modified with FITC for evaluation by fluorescence. 4) The surface was rinsed again with buffer.
Anchoring of the 2-colimn lattice is confirmed by fluorescence intensity (Table.2, Fig.4, 5). As control experiments, another ssDNA with FITC that do not match with the immobilized strand were also tested. The correct combination of the immobilized

Table 2. Experimental condition

	Immobilization	Sample
No.1	O	2-column lattice
No.2	X	2-column lattice
No.3	O	Mismatched ssDNA
No.4	X	Mismatched ssDNA
No.5	O	None

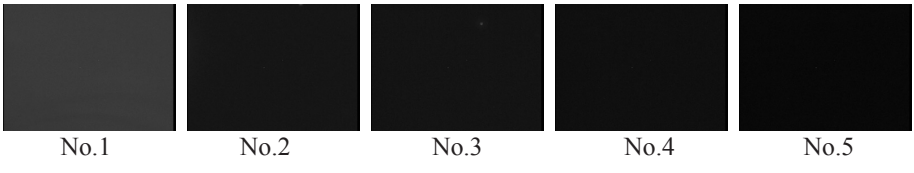

Fig. 4. Image of fluorescence microscope (excitation wavelength: 488nm, Emission wavelength: 530nm)

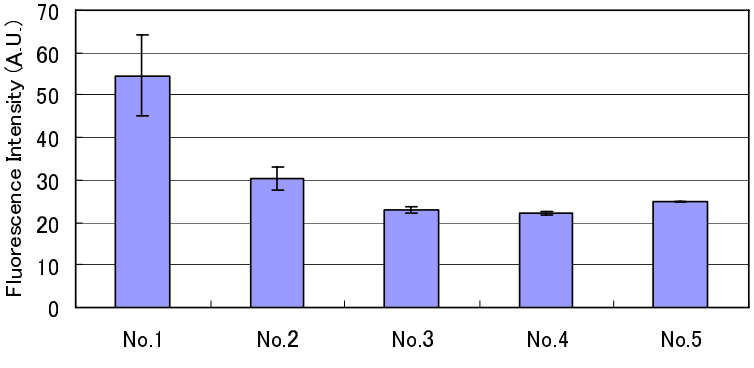

Fig. 5. Fluorescent intensity of each sample

strand and the 2-column lattice showed the highest intensity. Intensity of other cases were at the same level of background (No.5).

4 Stepwise Self-assembly of DNA Tile on Surfaces

We examined the stepwise self-assembly initiated by the anchored pre-assembled 2-column DNA lattice. In order to demonstrate the method, two kinds of DNA tiles (gray and white tile in Fig.6) are prepared. Only the white tile was modified by FITC, and also, only the gray tiles can associate with the 2-column lattice. The lattice can grow only when applying solutions in the order of "gray, wash, white, wash, gray, wash …." Fig.6.A illustrates first two steps of such "correct" sequence for the growth. By contrast, Fig.6.B illustrates "wrong" sequence (white, wash, gray, wash, …). In this case, white tiles cannot associate with the 2-column lattice, thus no further growth occurs. In other words, the growth of DNA lattice must be dependent on the order of solution applied to the nuclei.

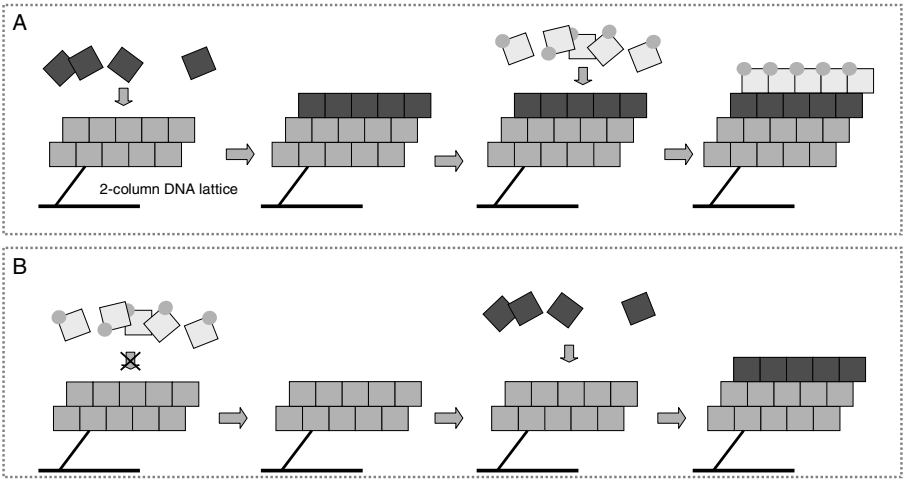

Fig. 6. Experimental scheme of stepwise assembly

This was examined by the following protocols: 1) A 2-column DNA is anchored by the same process described in section 3.2. 2) 2 hours later, its surface was washed by buffer and was dried quickly by N_2 blower. 3) A solution of monomer DNA tiles was applied on the surface and left for two hours to allow the association of the tiles. 4) Then the surface was washed and dried again. This process was repeated according to the specific order of application.

Four kinds of different sequence were compared (Table.3). Fig.7 and 8 show the results of fluorescence intensity measurement. Only No.1 has high intensity, which implies that DNA lattice grows from 2-column DNA lattice only when the monomer solution is applied in the correct order.

Table 3. Experimental condition

	1_{st} Step	2_{nd} Step	3_{rd} Step
No.1	2-column DNA lattice	Gray tile	White tile
No.2	2-column DNA lattice	White tile	Gray tile
No.3	2-column DNA lattice	Gray tile	None
No.4	2-column DNA lattice	White tile	None

Fig. 7. Image of fluorescence microscope

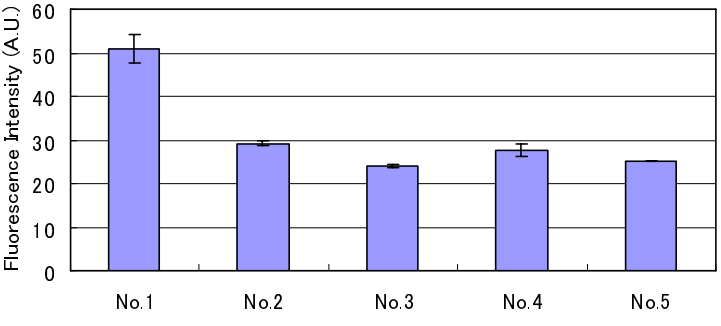

Fig. 8. Fluorescent intensity

5 Conclusion

In this paper, a method of stepwise self-assembly of DNA tile on surfaces is proposed. First, a 2-column DNA lattice as a seed structure is made and anchored on a surface by hybridization. The formation of the 2-colum lattice and its anchoring on the surface is confirmed by fluorescence microscope. Next, the stepwise self-assembly around the anchored seed lattice is evaluated by applying solutions in different order. We verified that only the correct sequence allows the lattice to grow. Although our experiment was simple, it was designed to demonstrate the feasibility and potential of fabricating complicated DNA nanostructure by the solid-phase self-assembly.

All the experiments shown here were done on a glass plate. We have to verify that the same process is valid in the microfluidic device. According to our preliminary experiment, there are hardly any problems except for the absorption of fluorescent particle in PDMS matrix of the microfluidic device. We think this is solved by some

straightforward approach. For the future work, we have to observe the surface with AFM to directly examine the product. Improving yield for multi-step assembly is also an important issue.

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