

Heat-resistant DNA tile arrays constructed by template-directed photoligation through 5-carboxyvinyl-2'-deoxyuridine

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ABSTRACT

Template-directed DNA photoligation has been applied to a method to construct heat-resistant two-dimensional (2D) DNA arrays that can work as scaffolds in bottom-up assembly of functional biomolecules and nano-electronic components. DNA double-crossover AB-staggered (DXAB) tiles were covalently connected by enzyme-free template-directed photoligation, which enables a specific ligation reaction in an extremely tight space and under buffer conditions where no enzymes work efficiently. DNA nanostructures created by self-assembly of the DXAB tiles before and after photoligation have been visualized by high-resolution, tapping mode atomic force microscopy in buffer. The improvement of the heat tolerance of 2D DNA arrays was confirmed by heating and visualizing the DNA nanostructures. The heat-resistant DNA arrays may expand the potential of DNA as functional materials in biotechnology and nanotechnology.

INTRODUCTION

Template-directed DNA photoligation (1–4) has recently attracted considerable interest because of its potential application as a tool for molecular biology research such as the specific detection of DNA or RNA sequences. Advantages of photoligation over enzymatic ligation are that the former is very efficient, enzyme-free, independent of the buffer condition and effective in ligation in an extremely tight space where no enzymes can gain access. Due to these advantages, the template-directed photoligation can be variously applied not only to

molecular biology research but also to DNA nanotechnology.

DNA tiles used in DNA nanotechnology are molecular tiles made of DNA. They have 'sticky ends' on their edges. The sticky ends preferentially match the edges of other DNA tiles according to the Watson–Crick base-pairing rule. In this manner, the arrays of DNA tiles keep growing throughout the crystallization process. One of the popular DNA tiles is a double-crossover (DX) tile that consists of two side-by-side double-stranded helices linked at two crossover junctions. The DX tiles have the ability to create two-dimensional (2D) periodic DNA arrays by self-assembly (5,6).

DNA self-assembly through specific base-pairing provides a method of DNA nanotechnology to construct nanostructures, as well as a method of DNA computation to solve complex problems. DNA has thus been recognized as a useful building material in the field of nanotechnology (7–9). Recently, great progress has been made in DNA self-assembly such as periodic DNA arrays with nanometer-scale precision (6,10–14), patterned structures (15–18), polyhedrons (19–21), nano-mechanical devices (22–28) and computing systems (29–31). Applications of template-directed DNA photoligation to these self-assembled DNA nanostructures will considerably expand the potential of DNA nanotechnology, because the DNA structures are given new properties such as heat tolerance, rigidity and branched-strand structure by photoligation. Heat-resistant DNA structures can be used as components for constructing more complex structures/systems that are required for practical application in electronic engineering, material science, medicine and biology. In particular, the heat-resistant 2D DNA array has great potential for use as a scaffold in the construction of nano-devices made through stepwise binding of various molecules or nano-electronic components, because the stepwise binding requires repeated

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temperature rising and falling. Here, we describe heat-resistant 2D DNA arrays self-assembled from double-crossover AB-staggered (DXAB) tiles that were covalently connected by template-directed photoligation.

MATERIALS AND METHODS

Design of DXAB tile

The DXAB tile (Figure 1a) used in the construction of 2D DNA arrays by self-assembly consists of two parts, namely A and B, derived from the well-known DX tiles (5). Part A and part B are held together by strand-ab, and their junction point has a certain degree of flexibility due to the nick. The complementary sticky-end pairs labeled as n and n' ($n = 1, 2, 3$) can bind to each other, and the DXAB tiles can form the 2D arrays (Figure 1b). The sequences of sticky ends were designed to carry out the photoligation reactions. Both 5'-ends of one double-helix of part A have UV-sensitive 5-carboxyvinyl-2'-deoxyuridine (^{CV}U) (2), which can bind to the thymine or cytosine base at the neighboring 3'-end by 366-nm UV-ray exposure (Figure 1c).

DNA sequences, synthesis and purification

The DNA strand sequences of the DXAB tile were based on the DAE tiles by Winfree *et al.* (6), and modified in order to carry out photoligation. The strands were also designed to have no stable self-folded structures that prevent planned hybridization. The stability of self-folded structures of the strands were checked by mfold (32,33) and HyFol (<http://www.nanobiophys-sakura.net/>

HyFol/index.html). The DNA strand sequences of the DXAB tile are shown in Figure 1d. All DNA strands in this study were synthesized and purified by HPLC or PAGE commercially (SIGMA Genosys Inc, Operon Biotechnologies, Inc., and Nihon Gene Research Laboratories Inc.). Strands containing ^{CV}U s were synthesized using the cyanoethylphosphoramidite of ^{CV}U that was synthesized according to the previously reported method (2).

Annealing of DNA strands of DNA tiles

Sets of strands for constructing the DXAB tiles were stoichiometrically mixed and dissolved to $0.8\mu\text{M}$ in $1\times\text{TAE}/\text{Mg}^{2+}$ buffer (40 mM Tris-acetate, 1 mM EDTA, 12.5 mM Mg acetate, pH 8.3). The solutions were annealed from 95°C to 50°C for 2 h 40 min, and annealed further from 50°C to 24°C for 19 h in a Peltier Thermal Cycler PTC-200 (MJ Research Inc.). During the first annealing process, DNA single strands were assembled into DXAB tiles, and during the second annealing process, the tiles were assembled into 2D DNA arrays through complementary base sequences in their sticky ends.

Photoligation reactions

For photoligation reactions, an aliquot of the annealed sample was dispensed to another tube and exposed to 366-nm UV-rays on ice for 5 min using UV-LED illuminator ZUV-C10 (OMRON Inc.).

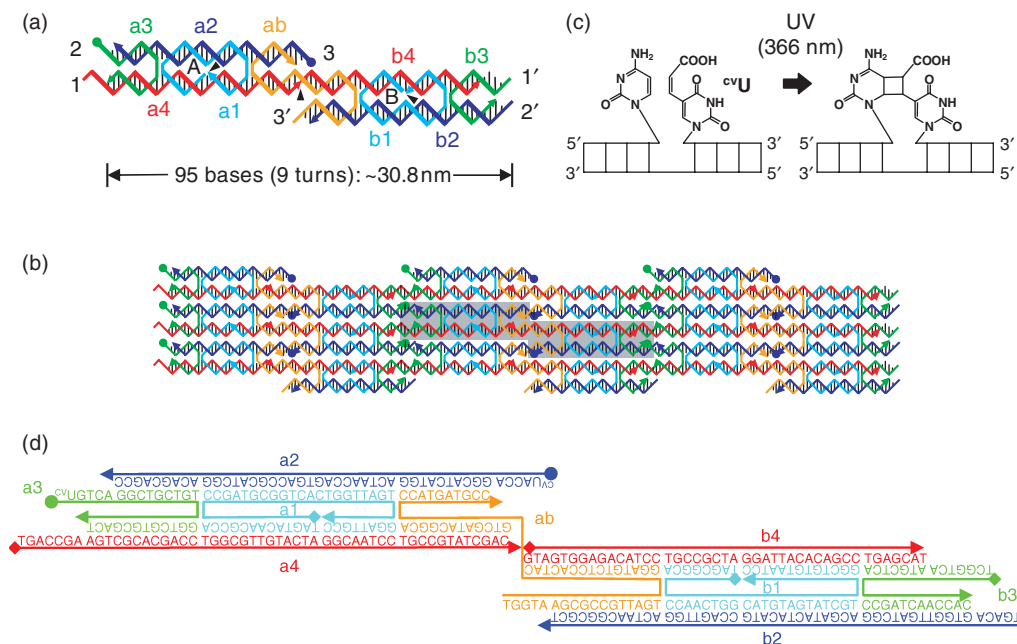


Figure 1. Design of a DXAB tile and arrangement into a 2D DNA array. (a) Strand structure of the DXAB tile for construction of heat-resistant 2D DNA arrays. Complementary sticky-end pairs are labeled as n and n' ($n = 1, 2, 3$). Arrowheads at the ends of strands indicate the 3'-terminals. Black triangles indicate nicks in the strands. The solid circles at the 5'-ends represent ^{CV}U bases. (b) The lattice topology of a 2D DNA array produced by the DXAB tiles. The strand color is the same as that used in (a). The reverse side of the tile is designated by a gray rectangle. (c) Template-directed DNA photoligation with ^{CV}U . (d) The sequences of the DXAB tile. The solid rhomboids at the 5'-ends represent phosphorylation.

Non-denaturing gel electrophoresis

For the analysis of the sizes of DNA nanostructures by gel electrophoresis, samples were prepared with an appropriate amount of 6×loading buffer [36% (v/v) glycerol, 30mM EDTA and 0.05% (w/v) each of bromophenol blue and xylene cyanol] and loaded onto gels prepared with 5% polyacrylamide (29:1, polyacrylamide:bisacrylamide) and running buffer (1×TBE). The gels were run at 20mA on a BIO CRAFT BE-130 gel electrophoresis apparatus (Ever Seiko, Co., Japan) placed in a refrigerator controlled at 4°C. The temperature in a gel, which was affected by the balance between Joule heating caused by an electrophoresis current and heat dissipation into the surroundings, gave a condition that the hydrogen bonds between the sticky ends of unligated DXAB tiles were broken while the DNA tiles in themselves did not melt into their component strands. After electrophoresis, the gels were stained with ethidium bromide dye (Continental Lab Products Inc.) and imaged on an Imaging System FLA-5100 (FUJIFILM Inc.).

AFM imaging

For atomic force microscopy (AFM) imaging in buffer, a 4μl sample drop was spotted on freshly cleaved mica (Nihon Shoji Co., Ltd) and left to adsorb onto the surface for 3 min. Then 22μl of 1×TAE/Mg²⁺ buffer was placed onto the mica and another 22μl of the buffer was pipetted onto the mica and another 22μl of the buffer was pipetted onto the mica. Atomic force micrographs were obtained by the tapping mode in buffer on a NanoScope IIIa (Digital Instruments) equipped with a multimode head with OMCL-TR400PSA-1 tips (Olympus Inc.).

Heat treatment

For the heat treatment in buffer solutions, the sample solutions of the self-assembled 2D DNA arrays before and after UV exposure were incubated at a specified temperature for 5 min on a Peltier Thermal Cycler PTC-200 (MJ Research Inc.) and cooled at room temperature for 3 min.

For the heat treatment on the mica surface, the self-assembled 2D DNA arrays before and after UV exposure were heated in the adsorbed state on the mica surfaces under 30μl buffer droplets on Thermo Block ND-M01 (NISSIN Inc.) at 100% relative humidity to prevent drying.

RESULTS

Construction of photoligated DNA arrays

Photoligation of 2D DNA arrays was first applied to conventional DAE arrays. The sticky ends of two types of DAE tiles, named A and B, were modified for photoligation. Each 5'-end of four sticky ends of the A tile has ^CVU, and each 3'-end of four sticky ends of the B tile has thymine or cytosine base (see Supplementary Data). These tiles were successfully self-assembled and formed into 2D DNA arrays. After UV exposure,

however, the DNA arrays changed their shapes and no periodic structures were observed by AFM imaging. One of the reasons for the failure of the DAE tiles to form photoligated 2D DNA arrays may be excess conformational strains around photoligation sites. The DXAB tile was thus designed to effectively relieve the conformational strains by decreasing the number of photoligation sites. In the DXAB tile, the A and B DAE tiles were held together through a DNA strand to reduce the number of photoligation sites.

The self-assembly of the DXAB tiles were examined by gel electrophoresis. Figure 2 shows a 5% polyacrylamide non-denaturing gel electrophoresis for three samples: non-UV-exposed self-assembled DXAB arrays with phosphorylation at the 5'-ends of strand-a1, a4, b1, b3 and b4 (Lane L1), non-UV-exposed self-assembled DXAB arrays without phosphorylation (Lane L2) and UV-exposed self-assembled DXAB arrays with phosphorylation (Lane L3). The gel was run under a condition that the hydrogen bonds between the sticky ends of unligated DXAB tiles were broken while the DNA tiles in themselves did not melt into their component strands. The temperature in a gel meeting this condition was achieved by adjusting the balance between Joule heating caused by an electrophoresis current and heat dissipation

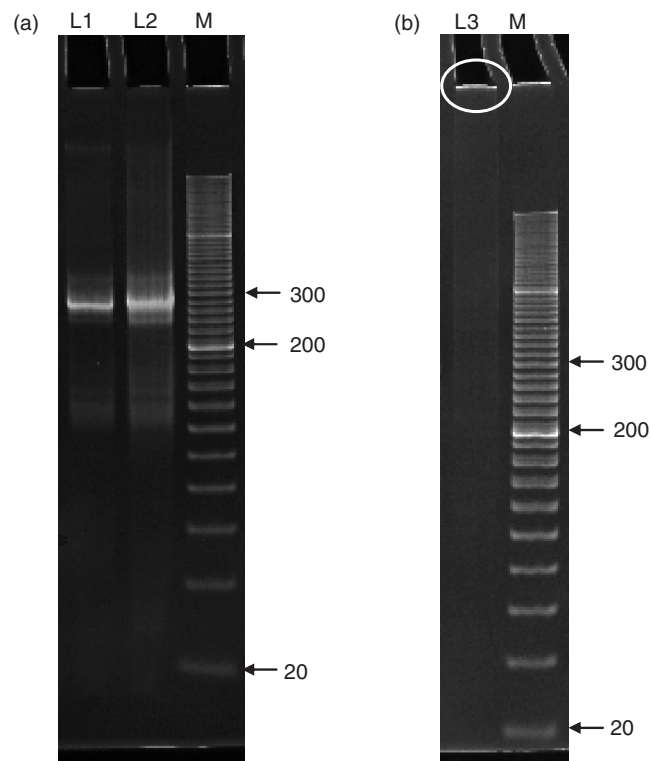


Figure 2. Polyacrylamide non-denaturing gel (5%) electrophoresis of DXAB tiles and arrays. Equal amounts (0.8μM) of strands that constitute the DXAB tile were mixed and annealed to make DNA arrays. The gels were stained with ethidium bromide. The gel was run at 20mA for 90 min (a) and 130 min (b). Lane M: 20 bp DNA ladder; Lane L1: non-UV-exposed self-assembled DXAB tiles with phosphorylation; Lane L2: non-UV-exposed self-assembled DXAB tiles without phosphorylation; Lane L3: self-assembled DXAB tiles with phosphorylation after UV-ray exposure for 5 min.

into the surroundings at a lower temperature. In Lanes L1 and L2, the DXAB arrays were falling apart into individual tile, so that the single bands corresponding to the unligated DXAB tile appeared. The mobility of the bands was equal to that of the sticky-ends-free DXAB tile. In Lane L3, no band corresponding to the unligated DXAB tile appeared. No band corresponding to the dimer or trimer of the unligated DXAB tile appeared either. Instead, there was a band that did not migrate into the gel and accumulated in the well. This band may be the photoligated DNA arrays with the enlargement in molecular weight by UV exposure. Figure 2a also indicates the comparison of the DXAB tile with and without phosphorylation of the 5'-ends of strand-a1, a4, b1, b3 and b4. The single prominent band corresponding to the unligated DXAB tile with phosphorylation (Lane L1) was slightly sharper than the one without phosphorylation (Lane L2).

The self-assembled DXAB tile arrays before and after photoligation reactions were visualized by atomic force microscopy. The samples corresponding to those analyzed in Lanes L1 and L3 in Figure 2 were deposited for adsorption on atomically flat mica surfaces and then imaged in $1 \times \text{TAE}/\text{Mg}^{2+}$ buffer. The DXAB arrays before photoligation had the periodic structure grown to a micro-scale (Figure 3a). The average distance of the longitudinal AB period (the long axis period) was about 30 nm, which is in good agreement with the designed parameters. The average distance of the short axis period measured from section profiles was about 6 nm. The difference in elevation of the periodic corrugation was about 0.8 nm, which is shorter than the diameter of a hydrated DNA duplex (2 nm). This is because the tip cannot touch the mica surface through the narrow interspaces between DNA duplexes. No significant conformation changes occurred after UV-ray exposure in buffer solutions (Figure 3c). The photoligated arrays show almost the same periodic structure with the long and short axis periods of about 30 and 6 nm, respectively. The self-assembled DXAB tiles using strand-a1, a4, b1, b3 and b4 without phosphorylation were also examined by atomic force microscopy. The sample corresponding to the one that was analyzed in Lane 2 in Figure 2 had no periodic array structure.

Heat-resistance of photoligated DNA array

The effect of photoligation on the heat tolerance of 2D DNA arrays was examined by heating the sample solutions of the DXAB arrays. Figure 3b is an AFM image of a non-UV-exposed sample after heating at 40°C for 5 min in buffer solutions. The array has been broken completely, and the section profile showed an aperiodic form. Figure 3d–f are AFM images of UV-exposed samples after heating at 40°C, 45°C, and 50°C, respectively, for 5 min in buffer solutions. In Figure 3d, the arrays are shown as bright areas in the large scan image, while in Figure 3e they are shown as dark areas. Heating the DNA arrays in buffer solutions may cause stacking of the arrays, so that they were observed as brighter (thicker) areas in AFM images.

However, the UV-exposed arrays were not broken after heating at 40°C and 45°C, and well-ordered periodic structure images were obtained. The long and short axis periods were about 30 and 6 nm, respectively, indicating that almost the same periodic structure as that of the arrays in Figure 3c was formed. After heating at 50°C the arrays were broken completely. The periodic arrays were not observed, and the section profile showed disordered structures (Figure 3f).

The effect of photoligation on the heat tolerance of 2D DNA arrays was also examined by heating the DXAB arrays in adsorbed states on mica surfaces under buffer droplets (Figure 4). This heat treatment will be closer to a heating method for practical use to bind or remove other molecules or particles. The heat treatment on the surface can also prevent the further stacking of the arrays due to adsorption to the mica surfaces. Figure 4a–c shows AFM images of non-UV-exposed samples after heating on the mica surface under buffer droplets at 40, 50, and 65°C for 5 min and cooling at room temperature for 3 min. After heating at 40°C, the non-UV-exposed arrays barely maintained their forms (Figure 4a). They began to fall apart to about ~100 nm scale pieces after heating at 50°C. The section profiles in Figure 4b show corrugations without clear periodicity. After heating at 65°C, the arrays were broken up completely (Figure 4c). The most noticeable results we obtained are shown in Figure 4d–f, indicating AFM images of UV-exposed samples after heating on mica surface under buffer droplets at 60, 65, and 70°C for 5 min and cooling at room temperature for 3 min. After heating at 60°C, the arrays were not broken; instead, the lattice appears to have been rearranged in a more orderly fashion than the one before heat treatment (Figure 4d). After heating at 65°C, the arrays maintained their forms, but they were not more ordered structures than the one in Figure 4d. After heating at 70°C, the arrays were broken up completely (Figure 4f). We confirmed that it is absolutely safe to heat arrays at 60°C for 5 min. Therefore, the heat tolerance is practically sufficient for binding or removing other molecules or particles.

We objectively judged the complete breaking of the arrays by confirming the occurrence of the similar pattern of torn pieces and the aperiodic section profiles of broken arrays at several spots on the mica surfaces. In the case of the non-UV-exposed samples after heating at 40°C in the buffer solutions, we confirmed almost the same patterns as those shown in Figure 3b at eight or more areas of $1 \mu\text{m} \times 1 \mu\text{m}$. The same tendency was confirmed in the UV-exposed samples after heating at 50°C in buffer solutions (Figure 3f), non-UV-exposed samples after heating at 50°C and 65°C on the mica surface (Figure 4b and c), and UV-exposed samples after heating at 70°C on the mica surface (Figure 4f).

DISCUSSION

The DAE tiles modified for photoligation were formed into 2D DNA arrays. After UV exposure, however, the arrays changed their shapes. This is probably because

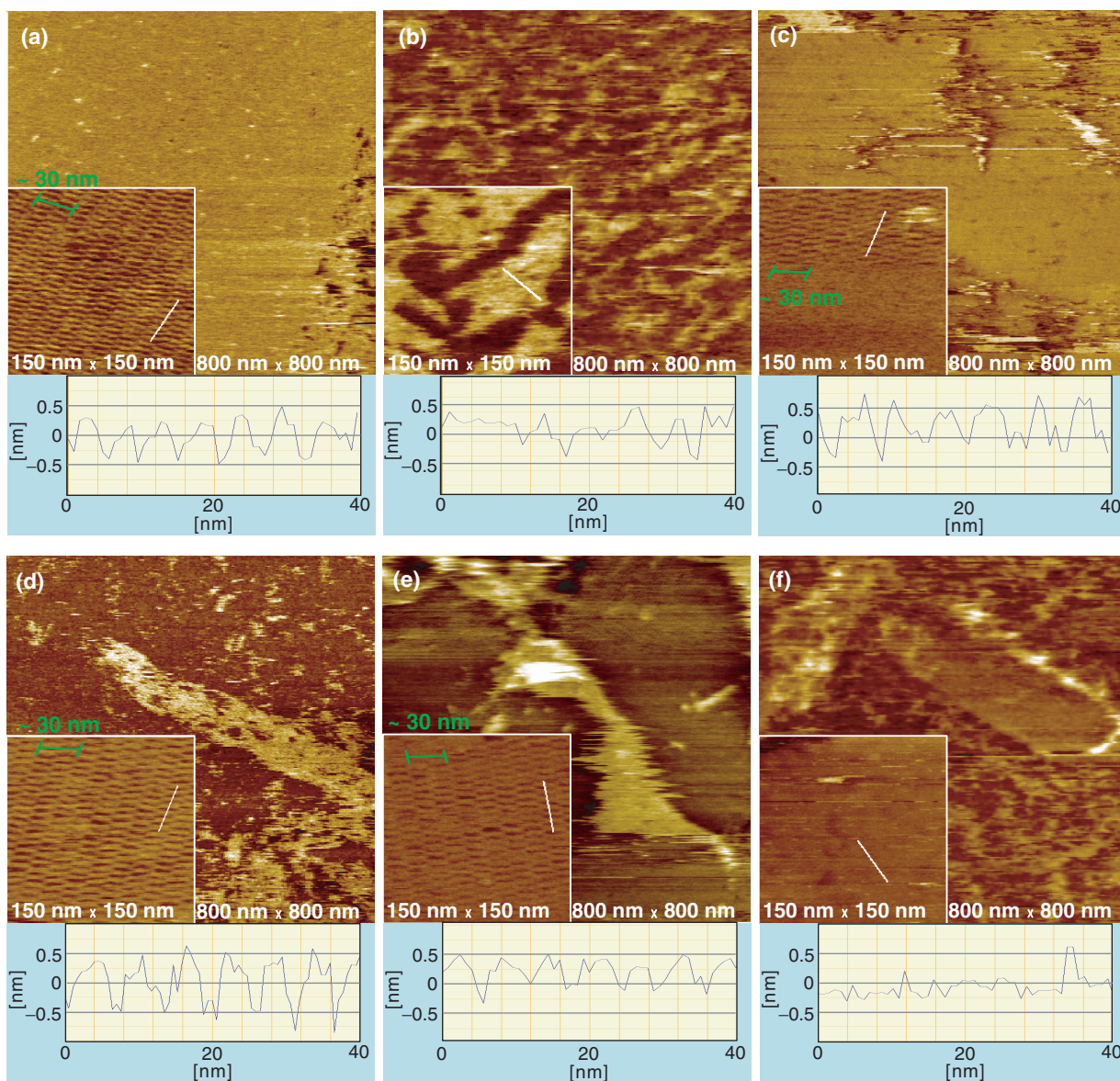


Figure 3. AFM images and section profiles of self-assembled 2D DNA arrays of DXAB tiles with or without heat treatment in buffer solutions. The scan sizes of images are 800 nm × 800 nm for large images and 150 nm × 150 nm for inset images. The section profiles correspond to the white lines in the inset images. (a) Non-UV-exposed DNA arrays without heat treatment. (b) Non-UV-exposed DNA arrays after heating at 40°C in buffer solutions. (c) UV-exposed DNA arrays without heat treatment. UV-exposed DNA arrays after heating at 40°C (d), 45°C (e) and 50°C (f) in buffer solutions.

the arrays cannot relieve the conformational strains caused by photoligation. To apply photoligation to various types of DNA tiles, we may have to design DNA strand sequences that can relieve the conformational strains for each case by lowering the density of photoligation sites as well as by nicking appropriate sites. Further study is also required to determine the relation between photoligation and conformational changes in DNA arrays for widespread use.

In the present study, it remains unclear why the DXAB tiles using strand-a1, a4, b1, b3 and b4 without phosphorylation formed no 2D DNA arrays, while the DXAB tiles with phosphorylation formed 2D DNA arrays. The presence of the 5'-phosphorylation increased

the melting temperature of duplexes (34). Therefore, phosphorylation may enhance the formation of DXAB tiles with crucial structures to their self-assembly at an array-forming temperature. Phosphorylation may also cause slight conformational changes that make DXAB tiles flat enough to self-assemble into DNA arrays as large as a micro-meter scale. The fact that the single band corresponding to the unligated DXAB tile with phosphorylation was slightly sharper than the one without phosphorylation (Figure 2) may indicate a slight conformational difference between the tiles with and without phosphorylation.

Template-directed photoligation has already been shown to be useful for a tool for molecular biology

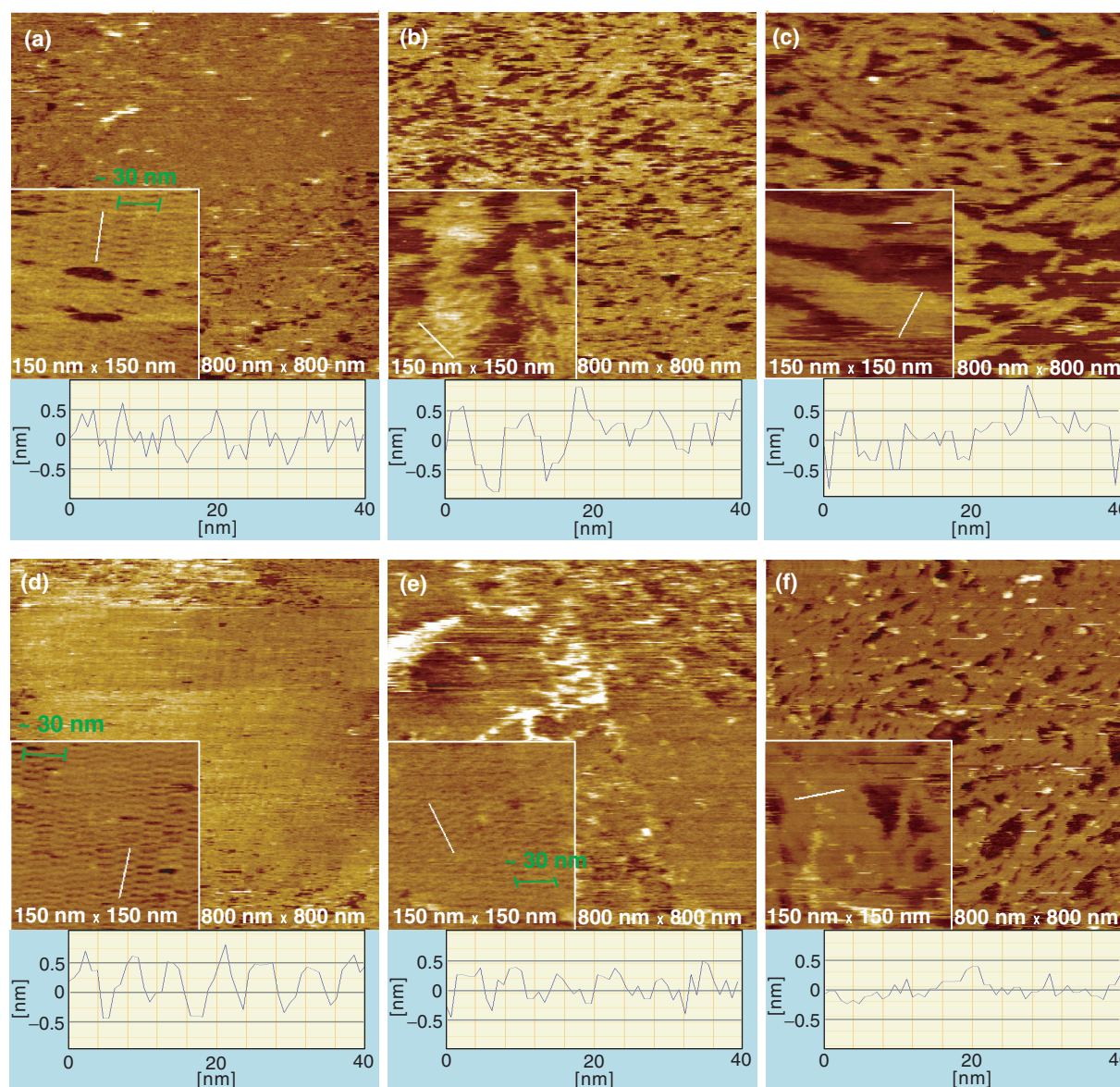


Figure 4. AFM images and section profiles of self-assembled 2D DNA arrays after heat treatment on the mica surfaces under buffer droplets. The scan sizes of images are $800\text{ nm} \times 800\text{ nm}$ for large images and $150\text{ nm} \times 150\text{ nm}$ for inset images. The section profiles correspond to the white lines in the inset images. Non-UV-exposed DNA arrays after heating at 40°C (a), 50°C (b) and 65°C (c) on the mica surfaces. UV-exposed DNA arrays after heating at 60°C (d), 65°C (e) and 70°C (f) on the mica surfaces.

research (1–4). The present study demonstrates that the template-directed photoligation has also a huge potential for use in DNA nanotechnology. A combination of photoligation and programmable self-assembly can realize addressable heat-resistant 2D DNA arrays, which would be available as rewritable memories or scaffolding with the ability to address DNA data-strands, nano-electronic components and other molecules on a nanometer scale through repeating thermal changes. 3D complex structures can also be constructed by stepwise assembly of heat-resistant DNA components. Branched DNA strands created by photoligation will facilitate the construction of substantially small DNA tiles and more complex 3D DNA nanostructures that would be

successfully used in the fields of biotechnology and nanotechnology.

CONCLUSION

We have demonstrated the availability of photoligation reactions on DNA nanostructures. The self-assembly of DXAB tiles including CVUs generates 2D periodic arrays, and after photoligation the arrays maintained their forms without breakup or remarkable conformation changes. The heat resistance of the arrays was improved by photoligation. A heat treatment assay on the mica surface showed that heat resistance improved at least 20°C .

SUPPLEMENTARY DATA

Supplementary Data are available at NAR Online.

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Conflict of interest statement. None declared.

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