Modifying the Surface Features of Two-Dimensional DNA Crystals

Furong Liu, Ruojie Sha, and Nadrian C. Seeman*

Contribution from the Department of Chemistry, New York University, New York, New York 10003

Received August 7, 1998

Abstract: DNA double-crossover (DX) molecules are rigid DNA motifs that contain two double helices linked at two different points. It is possible to form hydrogen-bonded two-dimensional crystals from DX molecules and to observe those arrays by atomic force microscopy (AFM) [Winfree, E.; Liu, F.; Wenzler, L. A.; Seeman, N. C. *Nature* **1998**, *394*, 539−544]. The sticky ends that hold the arrays together can be varied, so as to include diverse periodic arrangements of molecules in the crystal. The inclusion of extra DNA hairpins designed to protrude from the plane of the crystal provides a topographic label that is detected readily in AFM images: By using these labels, it is possible to produce stripes at predicted spacings on the surface of the crystal. The experiments presented here demonstrate that it is possible to modify these patterns, by both enzymatic and nonenzymatic procedures. We show that a hairpin containing a restriction site can be removed quantitatively from the array. We also demonstrate that a sticky end protruding from the array can be ligated to a hairpin containing its complement. In addition, it is possible to anneal a hairpin to the crystalline array by hydrogen bonding, both in solution and after deposition on a mica surface. The ability to modify these arrays increases the diversity of patterns that can be produced from an initial set of DX components. Thus, a single array can be modified in a large number of ways that can alter its physical or chemical features.

DNA offers a particularly convenient molecular medium for the construction of objects, devices, and arrays. The combination of sticky ended ligation[1] with stable branched DNA species[2] has permitted us to construct DNA molecules whose edges have the connectivity of a cube[3] and of a truncated octahedron.[4] Similarly, branched DNA molecules have provided the basis for the deliberate syntheses of knots[5,6] and Borromean rings.[7] These constructions were achieved by utilizing DNA molecules whose components were known to be flexible when their concatenation is catalyzed by T4 DNA ligase.[8,9] This problem led us to seek stiffer branched units; we have found that antiparallel DNA double-crossover (DX) molecules[10] (Figure 1) can behave as rigid constituents of DNA constructions that entail ligation.[11] We have used this fact to construct a nano-mechanical device predicated on the B→Z transition of DNA.[12]

We have reported recently the formation of two-dimensional (2D) DNA crystals constructed from DX components.[13] The DX molecules function as individual tiles that cover a plane,
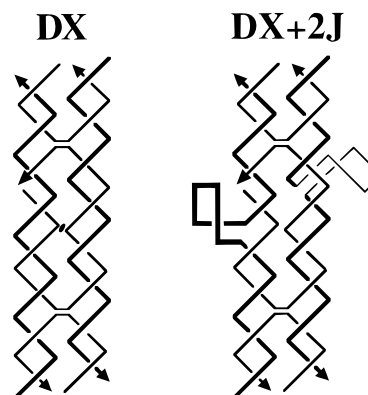

DX　　　**DX+2J**

Figure 1. Molecules used in this work. The molecule on the left, labeled DX, is a conventional antiparallel DNA DX molecule with an even number of half-turns (four) between the crossover points. This molecule consists of five strands, two continuous antiparallel helical strands, drawn with a thick line, and three strands drawn with thinner lines: two strands on either end forming the crossovers and a cyclic strand in the middle that also participates in the crossovers. It is very difficult to close this cyclic strand, so it is drawn containing a nick. Except for this nick, the system is 2-fold symmetric; this feature is indicated by the small elliptical object at the center of the molecule. The arrowheads indicate the 3′ ends of the strands. The molecule on the right, labeled DX+2J, is derived from the DX molecule. It differs from the DX molecule by having two extra hairpin loops included in the helical strands at the sites of bulged three-arm junctions. These two loops are drawn to protrude out of the plane of the DX helix axes: the one on the right, drawn with a very thin line, goes into the page, and the one on the left, drawn with a very thick line, comes out of the page. These hairpins act as topographic markers that are visible in the AFM. These molecules are drawn to contain four turns of DNA, but the repeat distance of the molecules used here is 4.5 turns of DNA in each repeat.

when connected by sticky-ended hydrogen bonding without ligation. The DX molecular dimensions are approximately 4 nm wide (two helical diameters) × 16 nm long, with a thickness

* Address correspondence to this author.

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of 2 nm. By altering the variety and nature of the sticky ends, one can change the features of the ordered array to produce specific periodic nanofabricated patterns on the mesoscopic scale; these can be visualized by atomic force microscopy (AFM). AFM features derive from DNA hairpins that protrude in a direction perpendicular to the crystalline layer. These hairpins consist of about one turn of DNA that is connected to the DX helices through bulged three-arm DNA branched junctions[14] to form one or more DX + junction (DX+J) motifs.[11,13] The DX+2J motif used contains two bulged junctions, one oriented into the plane and the other oriented out of the plane. An example of a DX+2J molecule is shown schematically in Figure 1. The DX+J motif exhibits rigidity similar to that of the DX motif when tested in the same assay.[11]

The ability to produce a variety of patterns in 2D crystals opens up a large number of possibilities for nanofabrication. We have shown previously that it is possible to tile the plane with two different molecules, one a conventional DX molecule and the other a DX molecule containing two bulged junctions (DX+2J), where one hairpin points out of the layer on each side. This arrangement results in rows of protrusions that appear in the AFM as stripes separated by ∼33 nm, about two molecular lengths. In a second arrangement, three conventional DX molecules have been combined with a DX+2J molecule to produce stripes separated by ∼65 nm, about four molecular lengths.[13] In this scheme, every new pattern requires different starting components, which can be expensive. The work presented here addresses this problem.

One can regard ordered DX assemblies as a basic substrate on which chemical operations can produce both useful modifications and varied patterns. For example, the addition of protruding hairpins to specific sites on a crystal or their removal could be used to alter the properties, the pattern, or the information content of the substrate. Here, we demonstrate that it is possible to add features to a 2D DNA crystal by ligating or by hydrogen bonding hairpins to it; likewise, we demonstrate that it is possible to remove hairpins by the use of restriction enzymes. Thus, from a small set of starting DX crystal components, it is possible to produce a diversity of ordered DNA arrays, each displaying different surface features.

Materials and Methods

Synthesis and Purification of DNA. All DNA molecules in this study have been synthesized on an Applied Biosystems 380B automatic DNA synthesizer, removed from the support, and deprotected, using routine phosphoramidite procedures.[15] DNA strands have been purified by electrophoresis; bands are cut out of 12−20% denaturing gels and eluted in a solution containing 500 mM ammonium acetate, 10 mM magnesium acetate, and 1 mM EDTA.

Formation of Hydrogen-Bonded Arrays. Complexes are formed by mixing a stoichiometric quantity of each strand, as estimated by OD_{260}. Exact stoichiometry is determined, if necessary, by titrating pairs of strands designed to hydrogen bond together and visualizing them by nondenaturing gel electrophoresis; absence of monomer is taken to indicate the endpoint. All 20 strands are mixed either in 10 mM HEPES (pH 7.8), 6 mM MgCl₂, and 1 mM EDTA (for restriction) or 20 mM Tris (pH 7.6) and 10 mM MgCl₂ (for restriction or ligation). The final concentration of DNA is 0.4 μM, and the final volume is 50 μL. The tube containing the DNA solution is put in about 2 L of boiled water and placed in a Styrofoam box for at least 40 h to facilitate hybridization.

Enzymatic Reactions. A. Phosphorylation. An individual strand of DNA (100 pmol) is dissolved in 10 μL of a solution containing 50

mM Tris·HCl (pH 7.6), 10 mM MgCl₂, 10 mM 2-mercaptoethanol, and 1 mM ATP and incubated with 3 units of polynucleotide kinase (Amersham) for 120 min at 37 °C. The reaction is stopped by heating the solution to 90 °C for 10 min, followed by gel purification.

B. Restriction of Arrays. Five units of *Pvu* II (New England Biolabs) is added to a 20 μL aliquot of a solution containing arrays, which is then incubated for 1.5 h at 37 °C. The array is then imaged directly.

C. Ligation to Arrays. The two hairpin strands are added to a 12.5 μL aliquot of the annealed lattice. The molar ratio of sticky ends on the lattice to each hairpin is 1:2, the final ligation volume is 25 μL, and the final DNA concentration of array components is 0.2 μM. The ligation solution contains 20 mM Tris (pH 7.6), 10 mM MgCl₂, 0.4 mM ATP, and 1 mM DTT. This ligation solution is incubated in about 2 L of water (37 °C) and cooled slowly to about 10 °C. At that point, 5 units of T4 DNA ligase (Amersham) are added to the solution and incubated overnight at 16 °C. After ligation, the product is dialyzed against 2 L of a solution containing 10 mM HEPES (pH 7.8), 6 mM MgCl₂, and 1 mM EDTA in a microdialysis system whose reservoir is circulated by a pump.

Hydrogen Bonding of Hairpins. Hairpins are incubated with the array in the same conditions used for ligation, but no dialysis is performed.

AFM Imaging. A 3−5 μL aliquot of a solution containing arrays is deposited on a freshly cleaved mica surface for 1.5 min. It is then washed with double-distilled water and dried with compressed air. Samples were imaged under 2-propanol in a fluid cell on a Nanoscope II and commercial 100 or 200 μm oxide-sharpened silicon nitride oriented twin tips (Digital Instruments).

Results

Molecules Used in Enzymatic Modification of 2D DNA Crystals. The DX and DX+2J molecules used in the enzymatic modification experiments are shown in Figure 2. The sequences of the molecules have been designed using the program SEQUIN[16] to minimize their sequence symmetry. The molecules labeled **A**, **C**, and **D*** in Figure 2 have been used in both of the enzymatic DNA array modification studies reported here. Molecules **A** and **C** are conventional DX molecules, a DNA motif that has been characterized extensively by gel electrophoresis and chemical probes.[10,11,17−19] The molecule labeled **D*** is a DX+2J motif, similar to one used in our previous study.[13] Bulged three-arm branched junctions are used to produce the protruding hairpins, rather than conventional branched junctions. Bulged junctions are used because the presence of the bulge supplies the leeway necessary for the DX helical domain to maintain its stacking without distortion.[20]

The molecule labeled **Bˆ** is a DX+2J molecule that has been used in the experiments involving modification of the 2D array by restriction. Each of its two bulged junction hairpins contains 12 nucleotide pairs and a loop consisting of dT₄. In addition, each contains a **5′-CAGCTG-3′** sequence; this is the recognition site for cleavage by *Pvu II* restriction endonuclease. This enzyme has been chosen deliberately because the products of its digestion are blunt-ended fragments; blunt-ended fragments do not require special treatment (e.g., ref 21) to be removed after cleavage. The site has been located within the bulged hairpin

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A
```
ACTATGCTAGTCC TGTATGTCATGCCGTTGTGCC TGAGCACCAGTCG
    GATCAGG ACATACAGTACGGCAACACGG ACTCGTGGTCAGCCGTCAG
CACAGCGGTAGCGT CCGATACGATGCAGTACGTGT CCGTAGTTGCTGG
    CCATCGCA GGCTATGCTACGTCATGCACA GGCATCAACGACCGCAGTC
```

B^
```
        T  T  ATGCCAGCTG TA T
        T  T  TACGGTCGAC AT  T

GCAGTCGCACGACC TGGCGT TGTACTACGCAATCC TGCCGTATCGACG
    CGTGCTGG ACCGCA ACATGATGCGTTAGG ACGGCATAGCTGCCTACCG
CGTCAGGCTGCTGT CCGATGCGGT CACTGGTTAGT CCATGATGCACG
    CGACGACA GGCTACGCCA GTGACCAATCA GGTACTACGTGCCATCGC

        T CTCAGCTG CCTA T  T
        T GAGTCGAC GGAT T  T
```

B°
```
    T  T GAGCC      GACACCACGAG T
    T  T CTCGGCTGTGG      TGCTC  T

GCAGTCGCACGACC TGGCGT TGTACTACGCAATCC TGCCGTATCGACG
    CGTGCTGG ACCGCA ACATGATGCGTTAGG ACGGCATAGCTGCCTACCG
CGTCAGGCTGCTGT CCGATGCGGT CACTGGTTAGT CCATGATGCACG
    CGACGACA GGCTACGCCA GTGACCAATCA GGTACTACGTGCCATCGC

    T GCAAC                  GCTTCGCTGAC T  T
    T CGTTGCGAAGC                 GACTG  T  T
```

C
```
GATGGCGACATCC TGCCGCTATGATTACACAGCC TGAGCATTGACAC
    CTGTAGG ACGGCGATACTAATGTGTCGG ACTCGTAACTGTGACTTGA
GTAGCGCCGTTAGT CCAACTGGCATGTAGTATCGT CCGATTCAACCAG
    GGCAATCA GGTTGACCGTACATCATAGCA GGCTAAGTTGGTCGTTGCT
```

D*
```
    T  T AGCATGTTGCCAGC   T
    T  T TCGTACAACGGTCG    T

TGAACTCGGCAGCC TGTAAC GGTTGGTCGCTATCC TGATGACACTACG
    GCCGTCGG ACATTG CCAACCAGCGATAGG ACTACTGTGATGCTGATAC
CAACGAGCAATCGT CCGATGCGGT ACATACGCAGT CCAGTTGTATCG
    CGTTAGCA GGCTACGCCA TGTATGCGTCA GGTCAACATAGCGTGTCG

        T GTGACGTGCTGACA T  T
        T CACTGCACGACTGT  T  T
```

Figure 2. Sequences of the molecules used in this work. The five molecules used in these studies are illustrated in a form that unwinds the helices but permits easy labeling of their DNA sequences. The 3′ end of each strand is indicated by an arrowhead. Molecules **A** and **C** are DX molecules, and **B^**, **B°**, and **D*** are DX+2J molecules. The restriction study uses molecules **A**, **B^**, **C**, and **D***, and the ligation study uses molecules **A**, **B°**, **C**, and **D***. Molecule **B^** contains a recognition site for the restriction endonuclease *Pvu II*; this site is indicated in lighter text, and the points of scission are indicted by filled triangles. Molecule **B°** is included in the array as just the central molecule containing sticky ends. The hairpins, containing sticky ends complementary to **B°**, are also shown; these hairpin molecules are ligated to **B°** in the ligation experiment; other than their hairpins, **B^** and **B°** are identical. Note that the sticky ends are complementary in alphabetical order but that 2.5 turns separate crossover points between molecules. Consequently, the molecules bridge between each other at an interface and thereby tile the plane, rather than forming a simple linear structure.

so that only five nucleotide pairs (and the bulge) remain after scission. It is important to realize that **D*** does not contain the *Pvu II* recognition site.

The molecule labeled **B°** is a DX+2J molecule that has been used in experiments involving ligation of hairpins to the array. Both the molecule **B°** and the two hairpins to be ligated to it are shown in Figure 2. Its size and sticky ends within the plane of the array are the same as those of **B^**. The nonplanar part of **B°** is similar to the product of digesting **B^** with *Pvu II* because its bulged arm contains five nucleotide pairs. However, it also contains two asymmetric (non-self-complementary) sticky ends, **5′-GACACC-3′** and **5′-CGAAGC-3′**, that are used for the

ligation of hairpin loops. Following ligation, the intact product contains 16 nucleotide pairs, in addition to the dT$_4$ hairpin loop. The molecules used for hydrogen-bonded addition of hydrogen bonding are similar to **B°** and its hairpins, except that the sticky ends are twice as long (see below).

Modification of DNA Arrays by Restriction. Figure 3 illustrates the modifications performed on the arrays in a schematic fashion. The four components of the array to be restricted, molecules **A**, **B^**, **C**, and **D***, are shown in an enlarged representation at the top of Figure 3a. Each molecule is drawn as a pair of rectangles connected by two lines. The molecules are shaded uniquely, to clarify the way in which they form rows
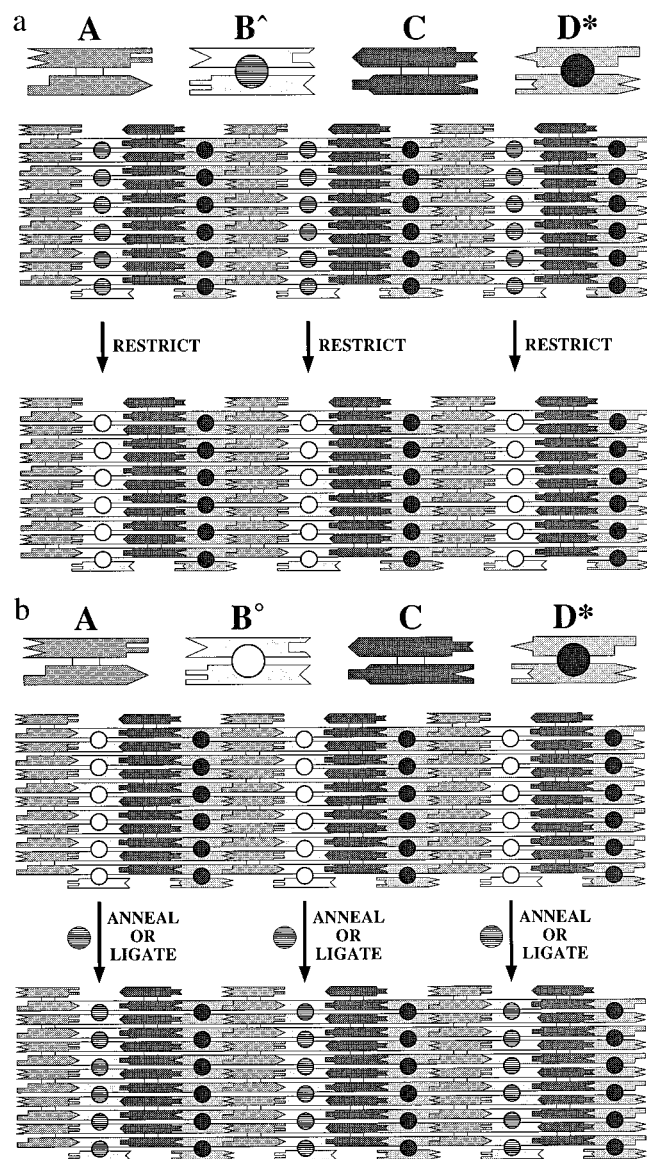
Figure 3. Experiments performed here. (a) Restriction of a crystalline array. The four components of the array are shown schematically at the top of the drawing. The four tiles are labeled, and each is shaded differently. The sticky ends are shown as complementary geometric shapes. **A** and **C** are DX molecules, and **B^** and **D*** are DX+2J molecules. Their protruding hairpins are represented by filled circles. The different circle fillings indicate that the hairpins differ between the two DX+2J molecules. Beneath the components, the array is drawn with the same components reduced in size. The topographic features of the DX+2J molecules appear as stripes (vertical rows of filled circles) in the AFM, whose resolution is sufficient to resolve stripes but insufficient to resolve individual hairpins packed together with 4 nm spacings. The bottom part of the diagram illustrates the effect of removing the hairpin of **B^** by restriction: The prominent stripe is replaced with a much less intense feature. (b) Ligation or hydrogen-bonded annealing to a crystalline array. The same conventions apply to this panel as to panel a. The difference here is that **B°** replaces **B^**. This component contains short arms ending in sticky ends that do not produce an intense feature in the AFM. The drawing illustrates that the addition of hairpins to this array produces a pattern similar to the starting pattern of panel a. Annealing and ligation produce the same result, although the sticky ends used for annealing are longer.

within the assembled crystal. The sticky ends of each molecule are represented as geometrical shapes at the left and right ends of the rectangles. Complementarity is indicated by complementary shapes on the ends of adjacent molecules; for example,
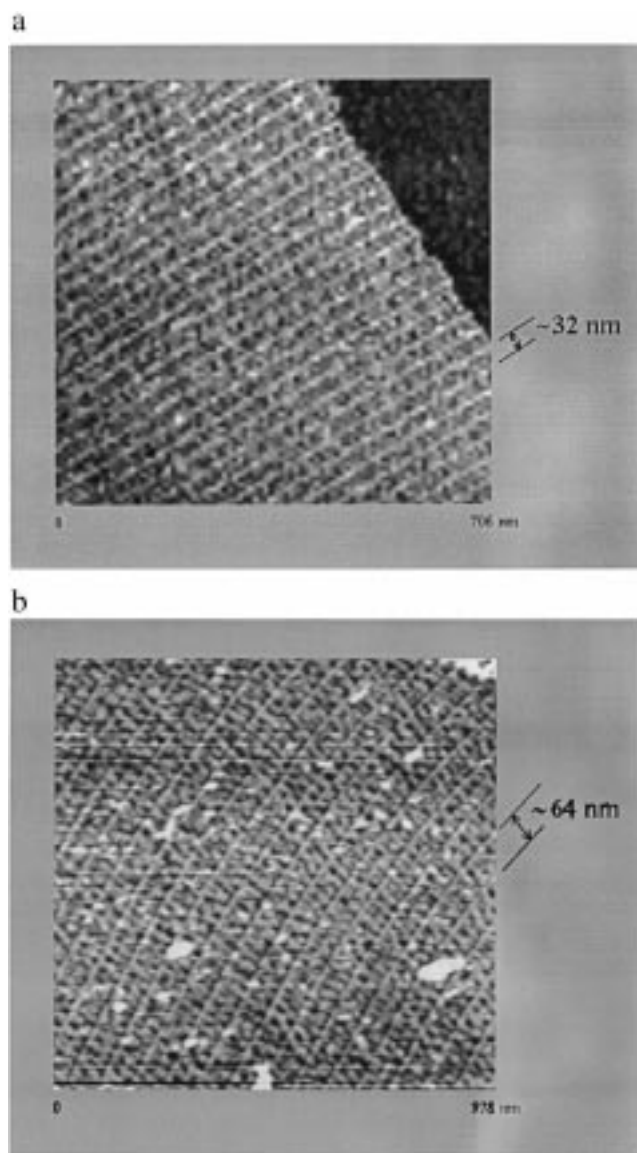
Figure 4. AFM images illustrating the restriction of a 2D crystal. (a) The array before restriction. Prominent stripes are visible in this image. These result from the DX+2J motifs of the **B^** and **D*** components of the array. Individual hairpins are not resolved in the narrow direction of the molecules (ca. 4 nm). The spacing between the stripes is shown to be about 32 nm, the expected distance for nine turns of DNA. (b) The array after restriction. The spacing of the prominent stripes is seen to double to about 64 nm. This is consistent with removal of the **B^** hairpins, while retaining the **D*** hairpins. Weaker stripes are visible halfway between the prominent stripes, perhaps resulting from the residual arms on **B^** following restriction. There is a certain amount of debris visible, but the array appears undamaged.

the lower right side of **A** consists of a male V-shape, and the upper left side of **B^** is a female V-shape. Both **B^** and **D*** are DX+2J molecules, and this fact is denoted by shaded circles at the centers of the molecules. The differences between the hairpins of **B^** and **D*** are indicated by differences in the shading of these circles. The aim of the experiment is to change the pattern by digestion of the array by *Pvu II*, so that the hairpin of **B^** is removed, leaving a remnant of just five nucleotide pairs; this remnant is represented by an unfilled circle in the lower half of Figure 3a.

Our primary means of characterization is AFM observation of 2D crystalline arrays. Figure 4a is an AFM image of the array built from **A**, **B^**, **C**, and **D***. Stripes with a spacing of

roughly 32 nm are the most prominent features of this array. This is the spacing expected for an unmodified array and is similar to the spacing observed previously for an array containing only two components, a conventional DX molecule, **A**, and a DX+2J molecule, **B***.[13] Figure 4b is an AFM image of an **A−B^−C−D*** array following digestion by *Pvu II*. In this case, the most prominent feature of the array is a series of stripes separated by about 64 nm. The array is similar to one observed previously that contained four components, **A**, **B**, **C**, and **D***, where **A**, **B**, and **C** are conventional DX molecules and **D*** is a DX+2J molecule. In addition to the major 64 nm stripes, a far less prominent striped feature is visible halfway between these stripes. This stripe is likely to represent the residual bulged arm containing five nucleotide pairs (see below). We cannot exclude the possibility that some intact hairpins remain. This image also contains a certain amount of high (white) debris resulting from the contact of the array with the restriction enzyme extract.

We have checked by AFM the stability of the **A−B^−C−D*** arrays under the 37 °C restriction conditions; this control experiment has been performed to be sure that intact arrays, and not their components, are the substrates of the restriction enzyme. We have incubated the arrays for 1.5 h at 37 °C and then placed them in the AFM at room temperature. The arrays appear to be stable under these conditions; they are not known to assemble on the support, so we are not observing a rapid formation. The proportion of material in arrays after incubation also appears to be normal (data not shown).

Modification of DNA Arrays by Ligation. Figure 3b illustrates the ligation of sticky-ended DNA hairpins to the arrays, the complementary experiment to restriction. Three of the components, **A**, **C**, and **D***, are exactly the same as in the previous experiment, illustrated in Figure 3a. The difference here is that **B^** has been replaced by **B°**, a different DX+2J molecule. Rather than a pair of hairpins in its bulged arms, it contains a pair of short double helices (five nucleotide pairs) terminating in six-nucleotide sticky ends. The experiment here is to ligate hairpins to those sticky ends; only the sticky ends associated with ligation contain phosphate groups. As illustrated in Figure 3b, the ligation is expected to produce an array resembling the starting material in Figure 3a, although the hairpins are slightly longer.

Figure 5a shows an AFM image of the starting crystalline array for this experiment. The image is characterized by alternating rows of prominent stripes and secondary stripes. Figure 5a resembles Figure 4b closely, suggesting that the prominent stripes result from the **D*** molecules and that the secondary stripes are the short arms on the **B°** molecules. From this observation it is possible to infer that the less prominent stripes of Figure 4b are indeed the residual hairpins left after restriction of the **A−B^−C−D*** array. Figure 5a indicates that the separation of the prominent stripes is about 64 nm. Figure 5b is an AFM image of the product of the ligation reaction. It is clear that the differences between the stripes have disappeared. Both images of the product contain stripes of equally strong prominence, separated by about 32 nm; these product images are consistent with successful ligation of the hairpins to the array.

Hydrogen Bonding of Hairpins to DNA Arrays. We find that it is also possible to attach hairpins noncovalently, annealed by means of hydrogen bonding. The six-nucleotide sticky ends of the **B°** molecules are not long enough to stabilize the noncovalent attachment of hairpins to the arrays. Nevertheless,
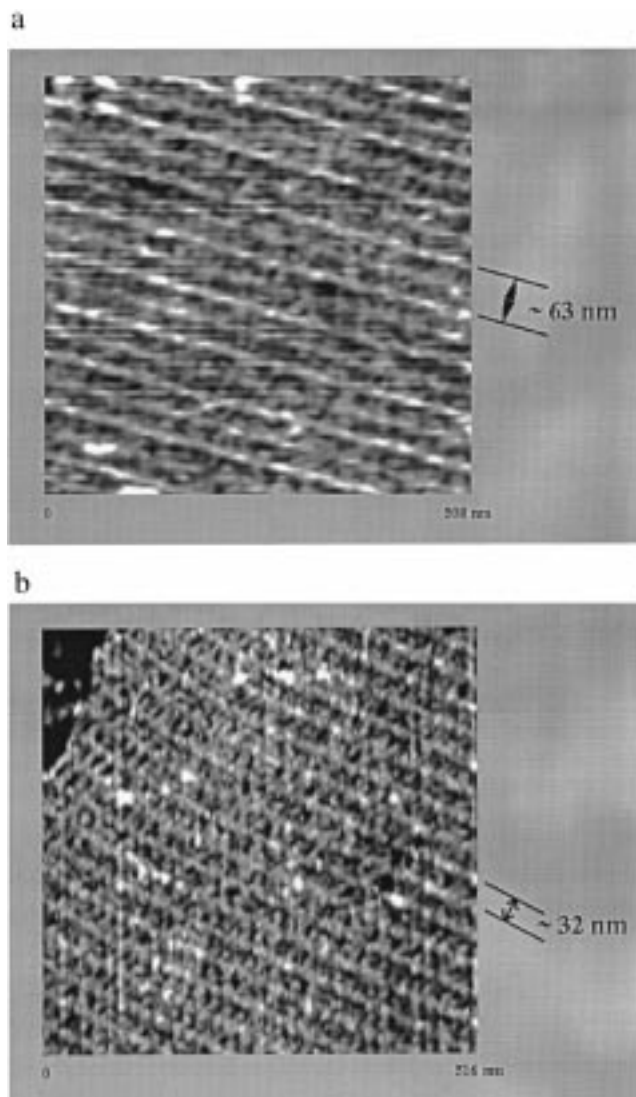
Figure 5. AFM images illustrating the modification of a two-dimensional crystal by ligation. (a) A crystal before ligation. Prominent stripes are apparent in this large array. Less prominent stripes are visible halfway between these stripes, similar to the image seen in Figure 4b. The spacing of the prominent stripes is about 63 nm, the expected distance for 18 turns of DNA. (b) A crystal after ligation. This image shows a series of uniformly spaced stripes of equal prominence, in contrast to panel a, where the stripes alternated in intensity. The spacing of the stripe seen clearly in this view is about 32 nm, the expected distance if the ligation has been successful in adding the hairpins to **B°**.

this means of attachment can be used, if one lengthens the sticky ends to 12 nucleotides (**5′-CGATTCCGAAGC-3′** and **5′-GCTCCAGACACC-3′** on the DX+2J molecules and their complements on the hairpins), so that the hairpins ultimately contain 22 nucleotide pairs. A variation on the **D*** hairpin containing 22 nucleotide pairs is also used. Figure 6a is an AFM image of the array before annealing the additional hairpins; it is very similar to Figure 5a, showing a 64 nm spacing of prominent stripes, alternating with weaker stripes halfway between them. Figure 6b shows an array after the hairpins have annealed, where the 32 nm spacing is prominent. This arrangement is similar to that seen in the ligation experiment shown in Figure 5b. The extent of modification is also similar to that of the ligation experiment. In addition, we are able to anneal the hairpins to the exposed surface of the array when it is attached to the surface (data not shown).
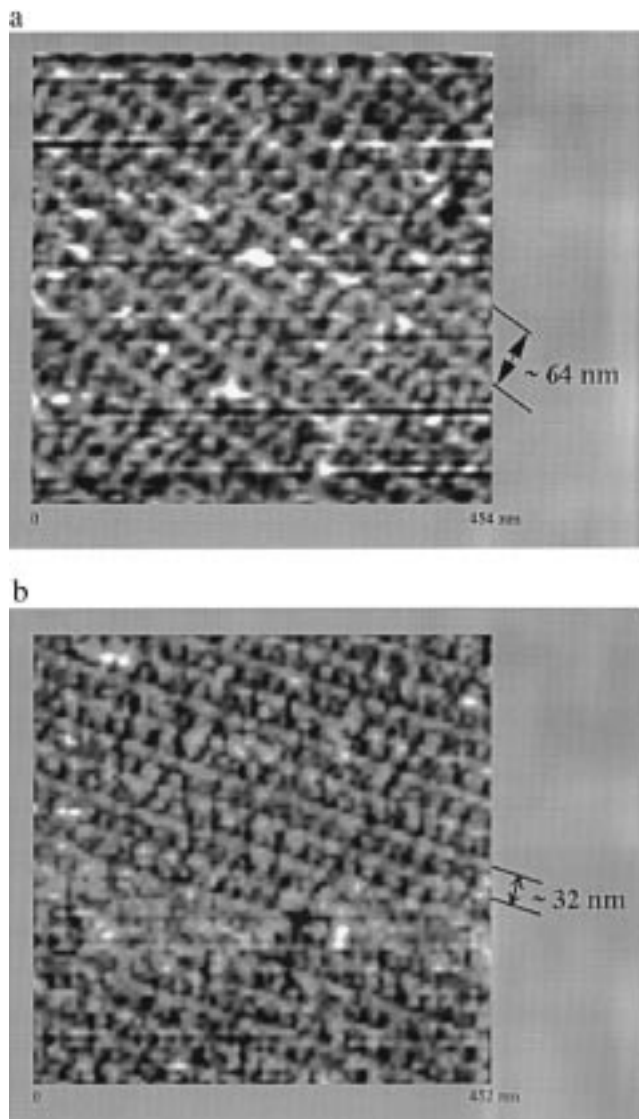
Figure 6. AFM images illustrating the modification of a two-dimensional crystal by hydrogen bonding. (a) A crystal before hydrogen bonding. Similar to Figures 5a and 4b, an array containing prominent stripes with a separation of ∼64 nm is seen; weaker stripes alternate with the prominent ones, halfway between them. (b) A crystal after hydrogen bonding. Both stripes are equally prominent, and they are separated by ∼32 nm.

Discussion

Modification of the Arrays. We have described a pair of complementary modifications to hydrogen-bonded arrays of DNA tiles that alter the covalent structures of their components. We have shown that it is possible both to remove and to add hairpins that produce topographic features on the mesoscopic scale. The extent of restriction appears to be virtually complete, and only a few positions of ligation failure are detectable. We can also add hairpins to the 2D DNA crystal by noncovalent attachment. It is important to point out that we have performed the enzymatic operations on arrays in solution; the enzymology is less successful if the arrays to be modified have already been deposited on the mica support. Nevertheless, we have been successful in attaching hairpins to long sticky ends by hydrogen bonding them after the array has been deposited.

Potential Applications of Array Modification. Our previous report of 2D tilings of the plane demonstrated that, in principle, it is possible to construct virtually any periodic surface pattern,

with a sufficient diversity of components.[13] The work presented here suggests that the same components can lead to altered properties or multiple patterns, thereby lessening the expense of synthesis and increasing the flexibility of the design. Altered properties could be obtained by adding or removing hairpins that contain fluorescent labels or sites for protein decoration.[22]

As an example of increased pattern diversity, the one-dimensional striped system described here could be expanded from two stripes to a larger number, each with a different restriction site, or containing a different sticky end. Each of these sites could be modified individually by restriction or by ligation. For simplicity, let us say there are four different rows that can contain a hairpin or not, in a system that could be called **A−B*−C−D#−E−F^−G−H•**, where each of the nonalphabetic symbols represents a different hairpin or hairpin attachment site. Therefore, 2^4 (= 16) different patterns could be produced from eight starting components in about 128 running nanometers, using any of the systems described here. Similarly, 20 components with 10 variable features could produce 2^{10} (= 1024) patterns. Clearly, some means of phasing the array would be necessary: For example, DX+2J molecules on the normally blank components might be used; a grouping such as **A*B*C*DE*F*** would contain the right features to establish both the origin and orientation of the starting point. Alternatively, a protein that bound to a particular hairpin[22] could also delimit the border. Without such phasing, the number of distinct patterns in the four-stripe system would decrease from 16 to 6. In general, the number of patterns phased by a border will be 2^p for **p**-hairpin patterns that are not otherwise distinct to the AFM, but unphased patterns are markedly fewer; the number of unphased patterns is the same as the number of different necklaces that be made with **p** beads of two colors.[23]

It is evident how to extend this system to two dimensions, but the aspect ratio of DX molecules, ca. 4 × 1, would lead to some extra effort. A **p × q** repeat unit assembled with 16 nm square tiles could be made with 4**pq** tiles, assuming a feature every 32 nm. However, 16**pq** tiles would be necessary if 16 × 4 nm DX molecules were used in both directions.

It is clear that global treatment involving restriction or ligation will lead to a periodic array containing the same repetitive pattern. If one wished to store information in the array, some means would have to be found to modify the array locally after deposition on a support. It is possible to manipulate molecules individually by means of scanning probe microscopic techniques (e.g., ref 24). If one could restrict, ligate, bind, or dissociate locally, this system could be converted into a means of writing information very densely. The information could then be read by a scanning probe instrument, such as the AFM used in this work.

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