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Two dimensional PNA/DNA arrays: estimating the helicity of unusual nucleic acid polymers†

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Received (in Columbia, MO, USA) 22nd January 2004, Accepted 19th May 2004
First published as an Advance Article on the web 24th June 2004

We extend the generality of nucleic acid-based structural nanotechnology by incorporating non-natural nucleic acids into a DNA double crossover (DX) molecule; visualizing two-dimensional arrays of these DX molecules by Atomic Force Microscopy (AFM) enables us to measure the helical repeat of any heteroduplex sequence capable of forming the outer arms of a DX.

Structural DNA nanotechnology uses reciprocal exchange between DNA double helices or hairpins to produce branched DNA motifs, like Holliday junctions, or related structures, such as crossover (DX), triple crossover (TX), paranemic crossover (PX) and DNA parallelogram motifs. These motifs are combined using sticky-ended cohesion to produce specific structures. The strength of sticky-ended cohesion is that it produces predictable adhesion combined with known structure. DNA stick-polyhedra, deliberately designed knots, Borromean rings, nanomechanical devices and a variety of 2D periodic arrays have been constructed.[1]

Peptide Nucleic Acid[2] (PNA) is an uncharged, achiral synthetic oligomer with a polyamide backbone. The backbone displays adenine, thymine, guanine and cytosine with a through-bond repeat distance similar to DNA. PNA has been hybridized successfully into Watson–Crick duplexes containing DNA. Relative to DNA–DNA duplexes a hybrid PNA–DNA duplex is less dependent[3] on ionic strength for stability and more resistant[4] to modification or cleavage by enzymes. Its uncharged nature makes it an excellent candidate[5] for scaffolding nanoelectronics.

The use of PNA would enable the functional advantages described above for hybrid duplexes to apply to the motifs used in DNA nanotechnology. These motifs appear to be amenable to the incorporation of PNA if the structural and chemical differences of PNA can be taken into account in the design and assembly of new constructs. In this regard, the key structural difference noted between PNA and DNA is that the helical repeat of PNA–DNA duplexes is substantially different[6] from DNA–DNA duplexes.

We decided to test the incorporation of PNA into crossover-based molecules by incorporating it into a DNA double crossover[7,8] (DX) molecule. Stable DX molecules consist of two double helical DNA domains; the domains are linked by two crossover points separated by an integral number of DNA half turns. They are roughly 2 nm thick and 4 nm wide, with a length that depends on the number of nucleotide pairs in their helical domains. DX molecules can be elaborated further by appending a hairpin between the two crossovers; the hairpin in this 'DX + J' molecule[8] is perpendicular to the plane of the molecule. When equipped with sticky ends, DX and DX + J molecules can tile the plane, so they are referred to as 'tiles'.[9]

The "**AB***" system[9] consists of two tiles: a DX tile (**A**) and a DX + J tile (**B***), each of which is equipped with sticky ends. When they assemble correctly, two-dimensional arrays are formed. These arrays display the hairpins as 'stripes', with a spacing of ~32 nm when visualized by AFM. We utilized a modified **AB*** system (Fig. 1) where the **A** tile was redesigned to allow the incorporation of relatively short segments of PNA. The **B*** tile was designed to allow systematic variation of the overall twist of the helices, to which lattice formation is likely to be sensitive.

Two versions of the **A** tile were assembled, **Ad**, which is entirely DNA and **Ap** where the two short crossover strands consist of PNA. Eight versions of the **B*** tile were assembled, **B0**–**B7**; the numeral indicates the number of nucleotide pairs added to compensate for the expected twist difference when **Ap** is incorporated into the arrays. Nondenaturing gel electrophoresis† demonstrated the clean formation of all these tiles with mobilities corresponding to the appropriate molecular weights.

We used AFM to monitor the formation of arrays that included all combinations of the tiles (Fig. 2). The **Ad** tile forms arrays cleanly with tiles **B0**, **B1**, and to some extent with **B2**. As the number of added bases increases, the self-assembly fails and random aggregates are obtained. The corresponding arrays combining the **Ap** tile with **B0**, **B1**, **B2** or **B3** are also ill-formed. However, combining the **Ap** tile with **B4**, **B5** and (to some extent) **B6** leads to clean, well-patterned arrays; these arrays display stripes with the expected ~34 nm periodicity (**B5** is ~2 nm longer than **B0**). These data demonstrate the incorporation of an unnatural oligomer into a nucleic acid nanostructure capable of forming ordered 2D arrays.

The helical repeat of the DNA/PNA segment of **Ap** can be calculated assuming that the average helical repeat for the rest of the system remains at 10.5 base pairs per helical turn (bp/t) and that

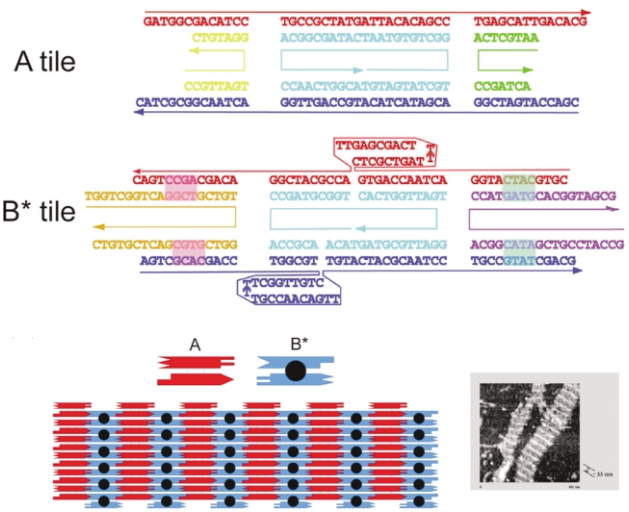

Fig. 1 The DX tiles used in this study. The upper tile is the '**A**' tile: in **Ad** the green strand and the yellow strand consist of DNA whereas in **Ap** they consist of PNA. The lower tile is the **B0** tile – base pairs are added alternately to the left and right pairs of outer arms to form tiles **B1**–**B7** as shown by the green and pink highlights: *e.g.* to form **B1** from **B0** a base pair is added to both pink regions; to form **B2** from **B1** a base pair is added to both green regions. The lower part of the figure shows the tiles forming a two dimensional array. Here, complementary sticky ends are shown as complementary geometrical shapes. An AFM image of the standard striped pattern with a periodicity of 32 nm is visible to the right.

† Electronic supplementary information (ESI) available: sequence data, experimental protocols for assembly of the tiles and arrays and gel electrophoresis data demonstrating formation of the tiles. See http://www.rsc.org/suppdata/cc/b4/b401103a/

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DOI: 10.1039/b401103a

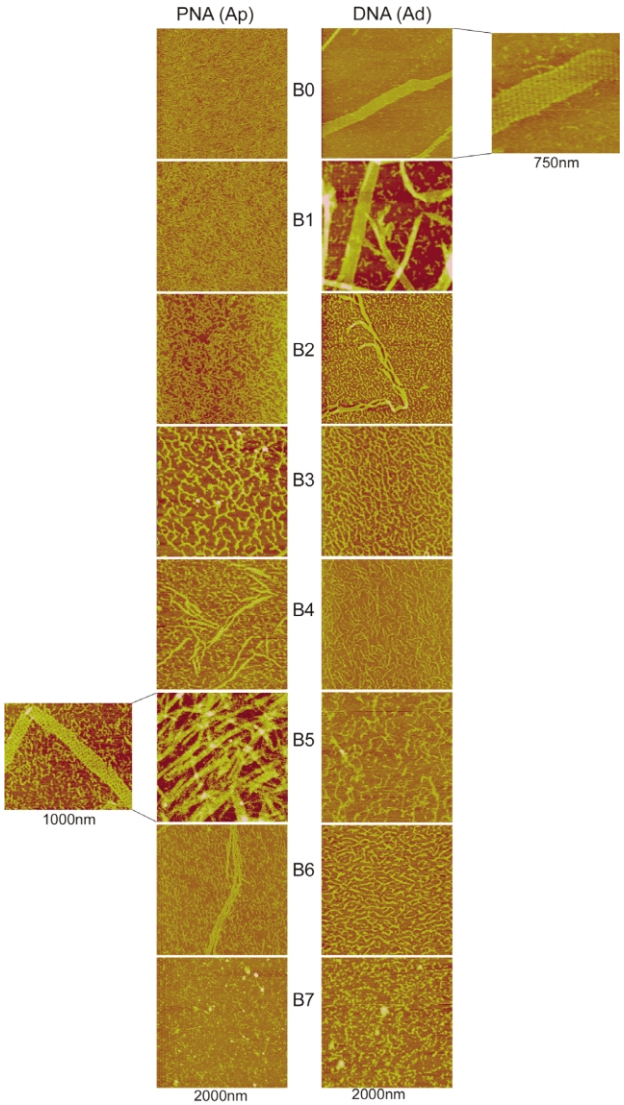

Fig. 2 AFM images of arrays formed from the tiles **Ap** and **Ad** with tiles **B0**–**B7**. The images in the two columns are of a 2000 nm field and the maximum height is 6.0 nm. **Ap**/**B5** and **Ad**/**B0** have zoomed images on the left and right, respectively, showing the periodic features of the arrays.

the helices between crossovers on neighboring tiles contain 2.5 turns – which must occur to form well-structured arrays.

The **Ap**/**B5** system forms arrays most like those containing only DNA; this combination of tiles corresponds to a helical repeat of 15.6 bp/t with a maximum error of ± 1.4 bp/t measured over the 30 DNA–PNA base pairs present per tile. This figure differs somewhat from the value[6] of 13.0 bp/t obtained by NMR using an octamer heteroduplex molecule. These differences could be due to a variety of factors including sequence effects, confinement in the double crossover motif, and interaction with the mica substrate upon which the sample is deposited for AFM analysis. However, from other studies of two-dimensional arrays under identical conditions[10–12] it has been found that well-formed arrays can be obtained if the design is based on helical repeat values identical to those found in solution by other[13–15] techniques. The mica surface is not expected to play a role in the assembly of these arrays, since they are formed in solution before deposition onto the mica.

We have demonstrated that it is possible to replace DNA strands with PNA strands in the formation of 2D nucleic acid arrays. We have also shown that this system can serve as an analytical tool: The **AB*** system serves as a prototype for analyzing the helical repeat of a polynucleotide if it can form the outer arms of a DX molecule. The amounts of material required are low (< 5 pm per analysis) and the results can be obtained readily from the visual display of the AFM. These hetero-polymeric arrays are also likely to allow investigators to utilize the rich functional possibilities of unnatural oligomeric variants of DNA molecules in structural nucleic acid nanotechnology.

This research has been supported by grants GM-29554 from the NIGMS, N00014-98-1-0093 from ONR, grants CTS-9986512, EIA-0086015, DMR-01138790, and CTS-0103002 from NSF, and F30602-01-2-0561 from DARPA/AFSOR.

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