Two Dimensional DNA Arrays

- A key goal of DNA nanotechnology is the construction of periodic arrays in 2 and 3 dimensions.

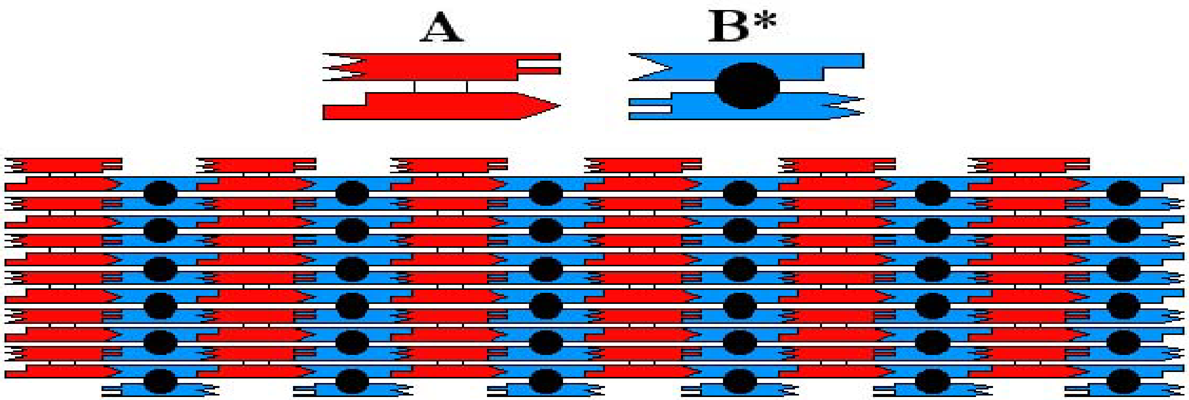
- 2-D arrays

  (1) antiparallel double crossover molecules,

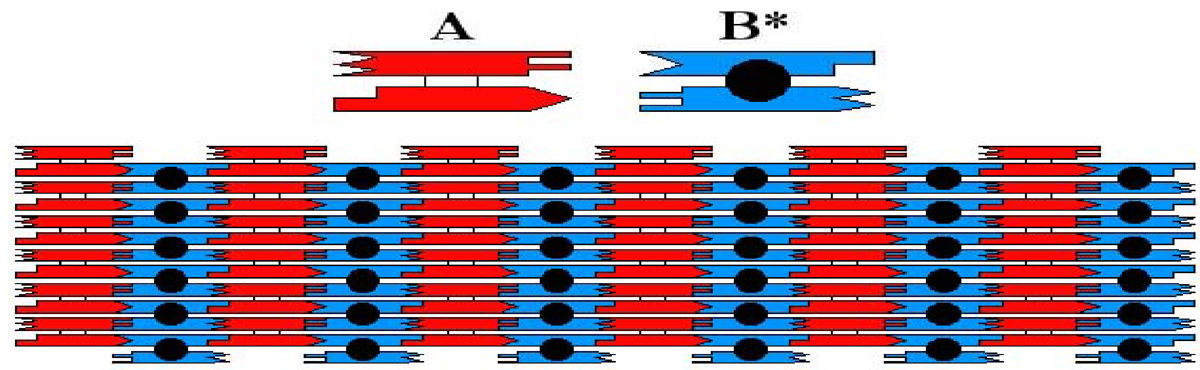
  (2) triple crossover molecules,

  (3) Holliday junction parallelograms

Double Crossover DNA Arrays

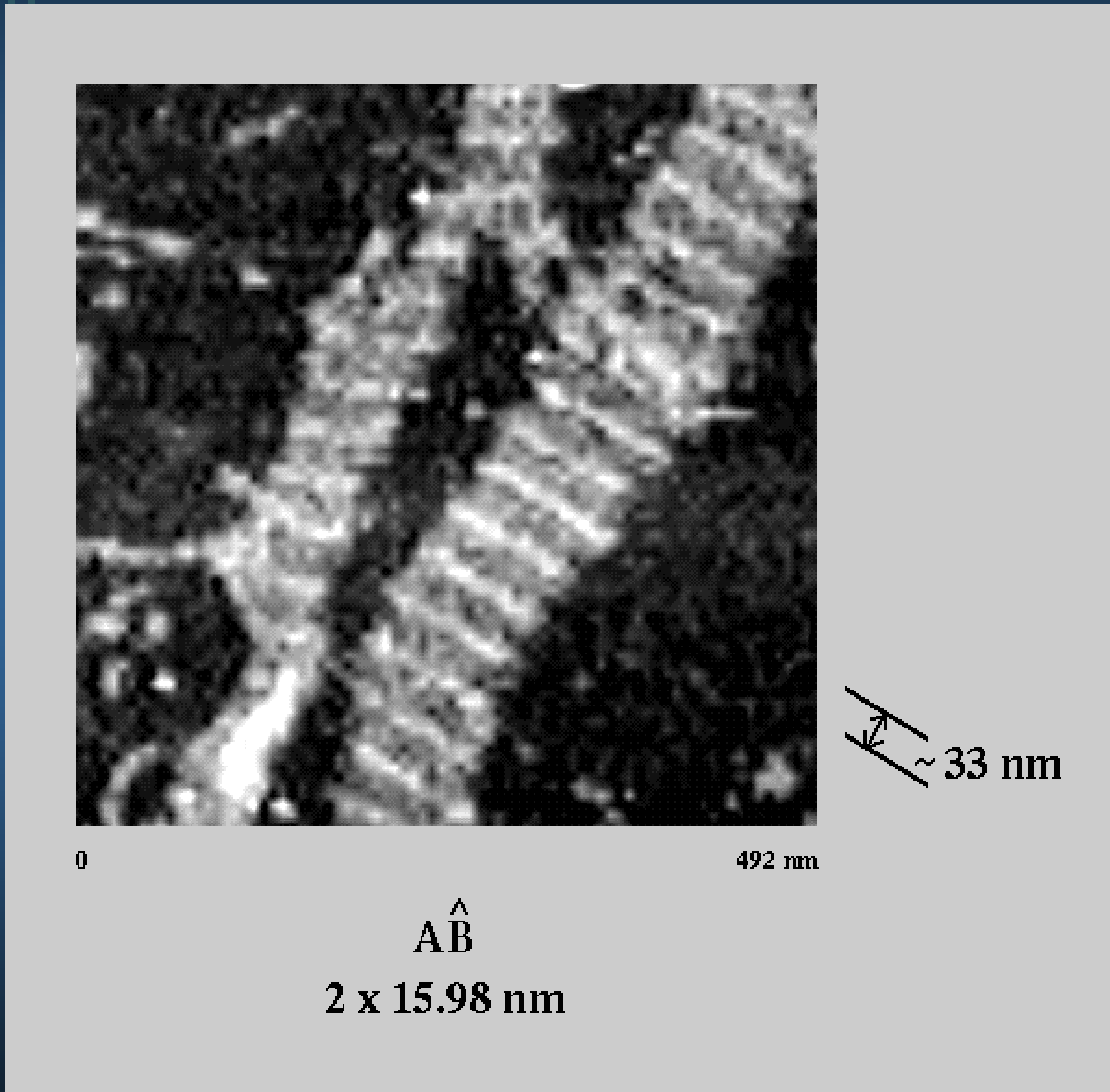


- using two different double crossover molecules (A and B*)
- When these two tiles are mixed in solution, they form hydrogen bonded 2-D arrays.



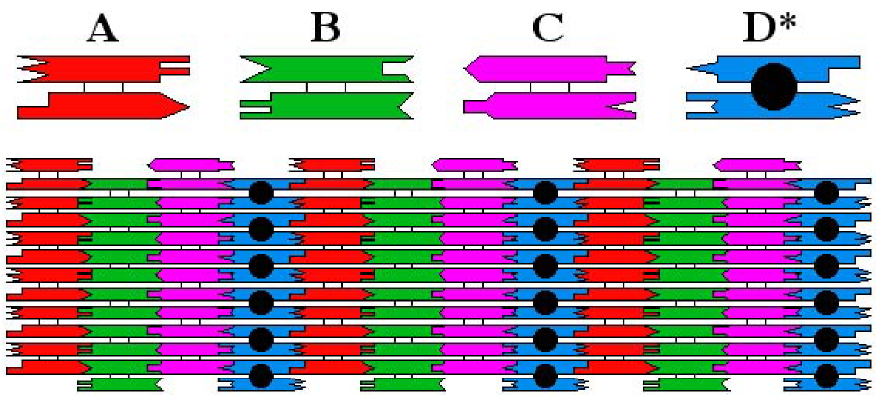
- A, B*: 4 nm wide, 16 nm long and 2 nm thick
- The A and B* have complementarity between them.
- * in B
  - --- DNA hairpins that project out of the plane of the helices
  - --- act as topographic markers in AFM

AFM Image



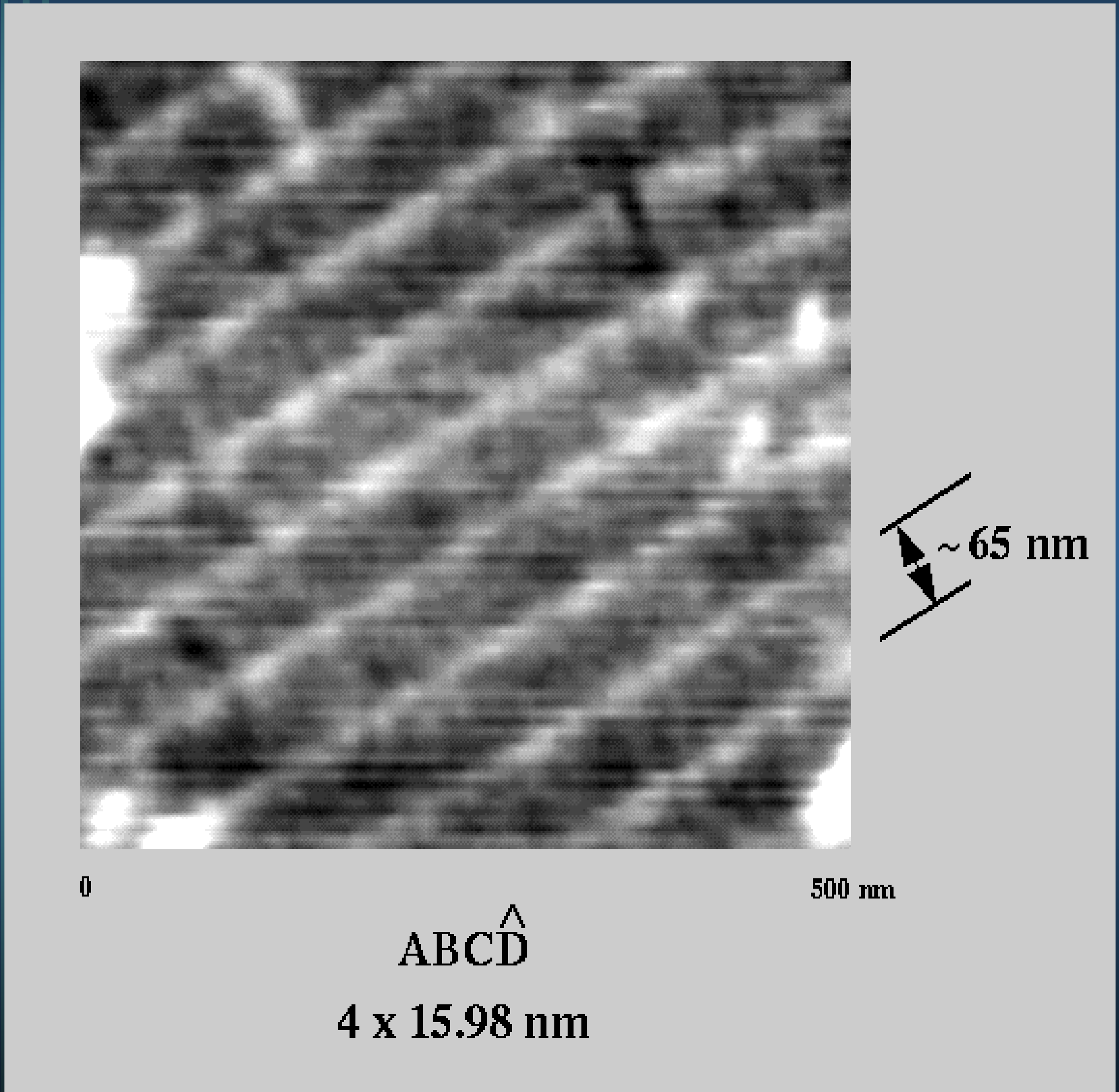
- several µm long, and hundreds nm wide
- The separation of the stripes is about 32 nm as expected.

Versatility of Double Crossover DNA Arrays



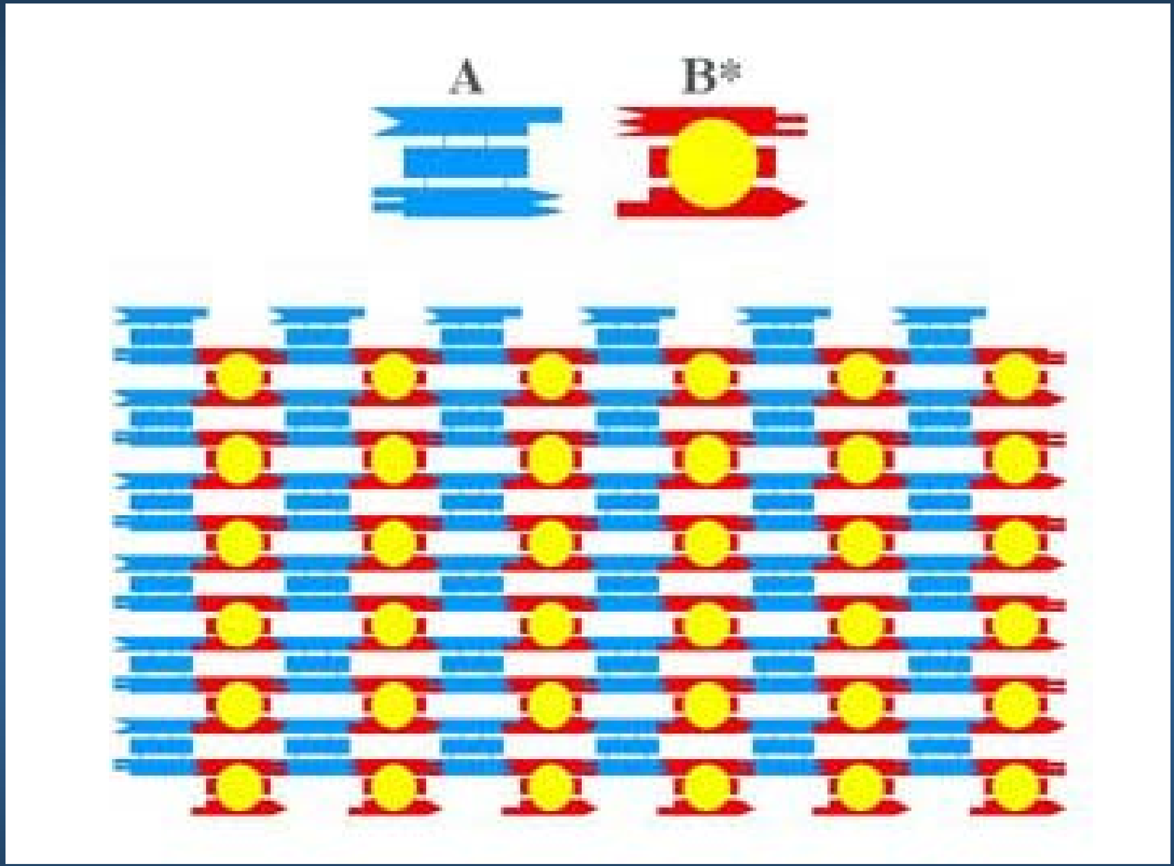
- Four different double crossover molecules (A, B, C, and D*) D* contains the protruding hairpins.

AFM Image



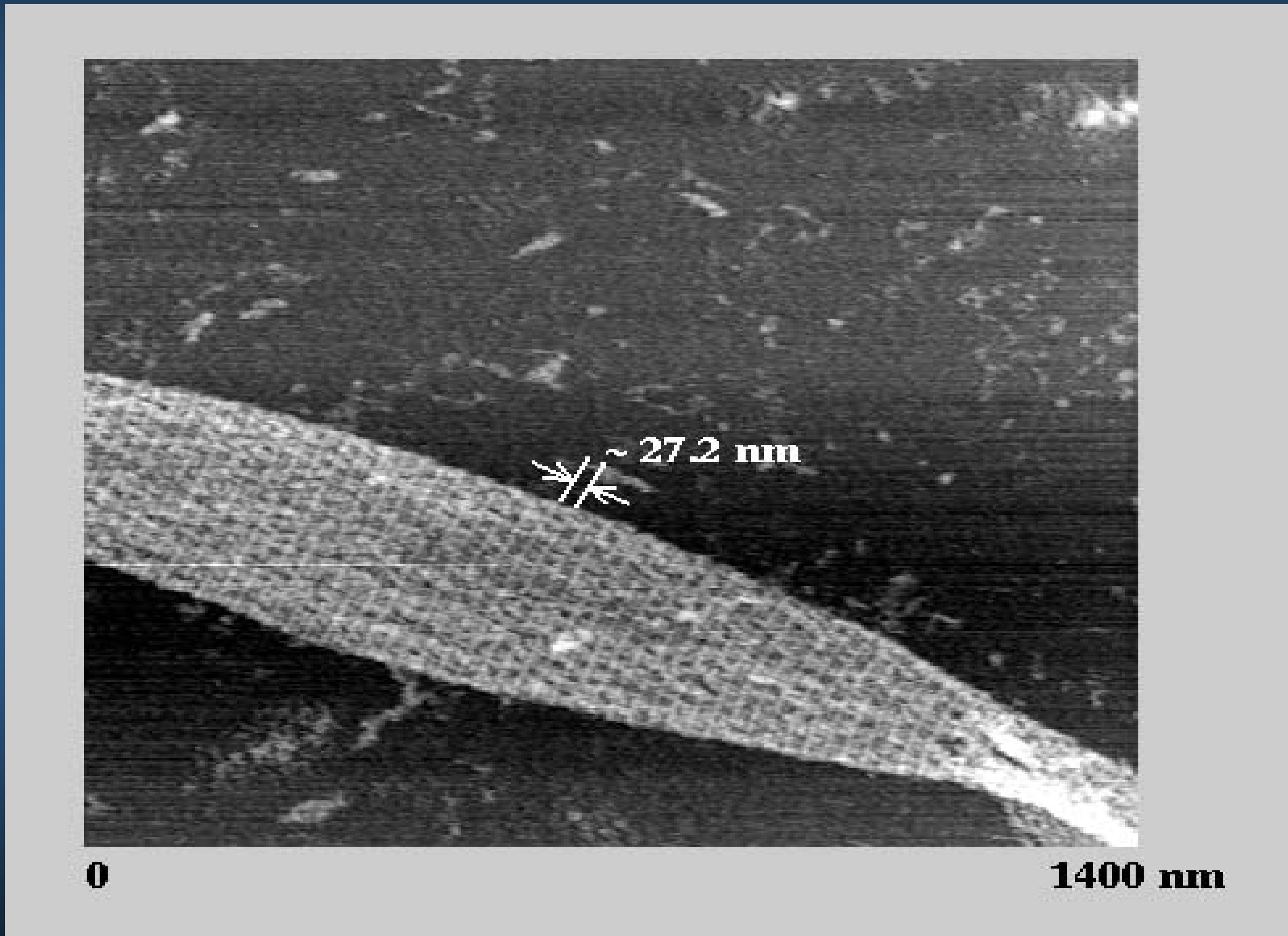
- The separation of the stripes is about 64 nanometers, rather than 32 nanometers.

Triple Crossover DNA Arrays



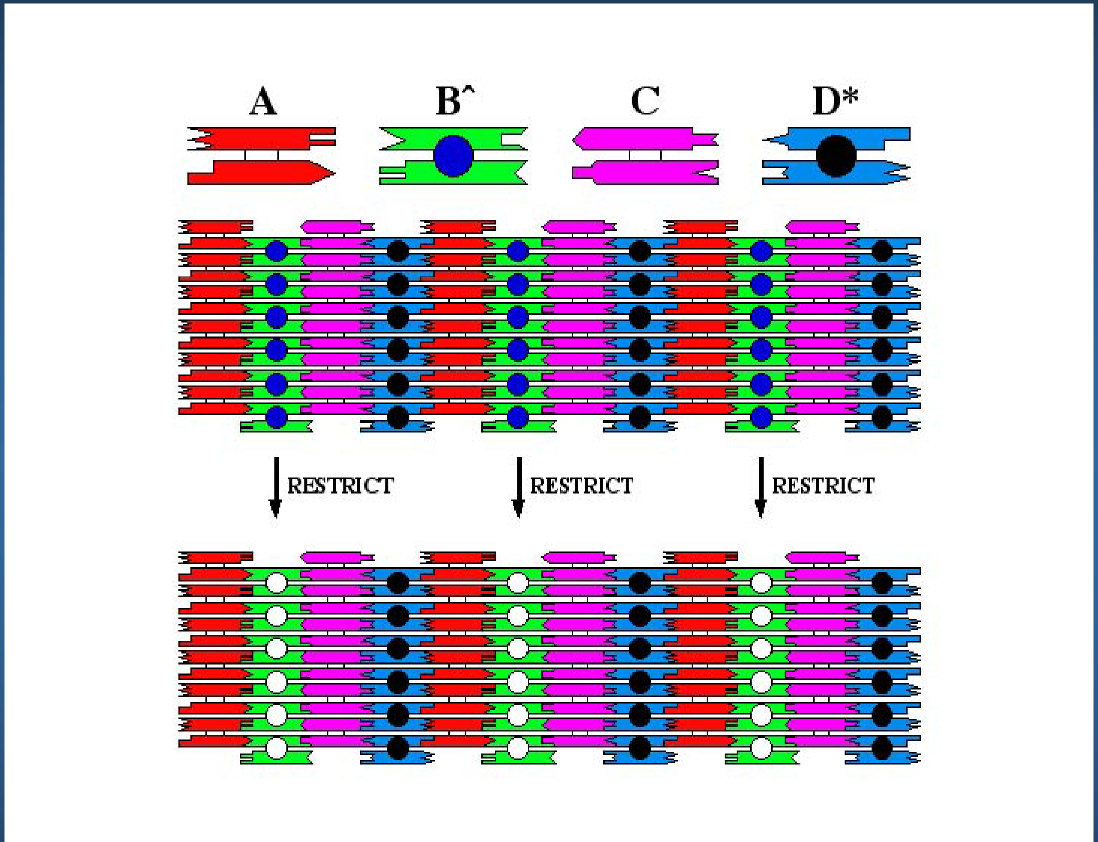
- using two different triple crossover molecules (A and B*)

AFM Image



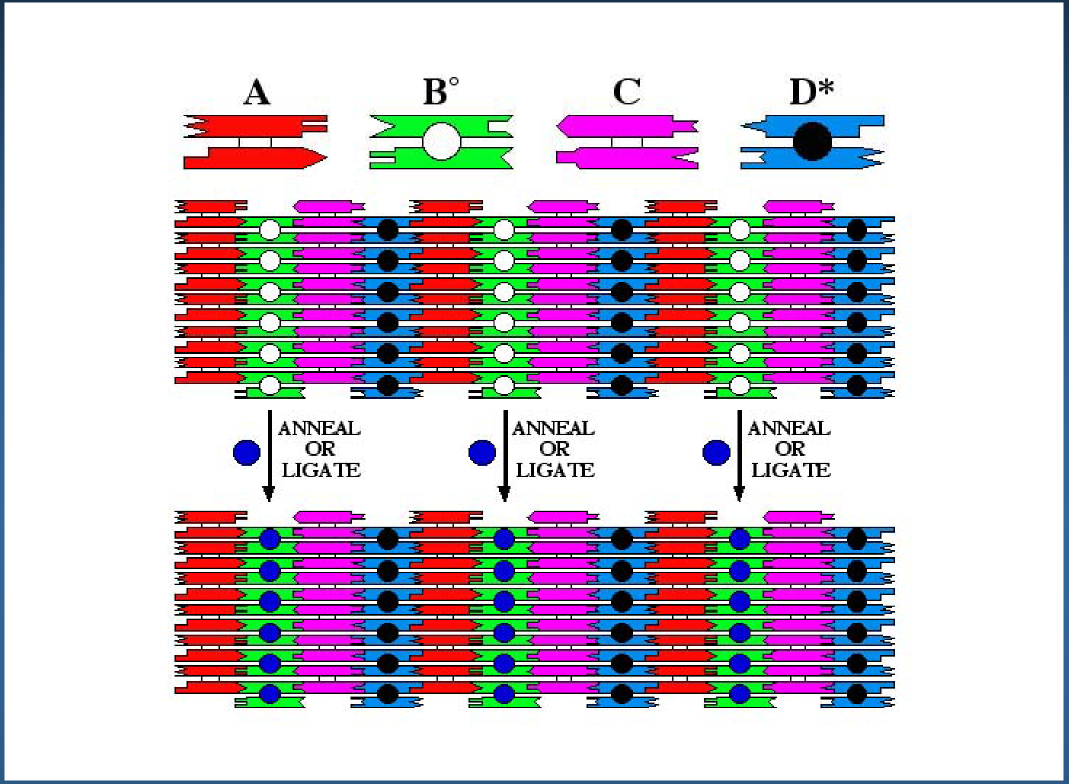
Two Dimensional DNA Array Modification

(1) Modification by restricting hairpins



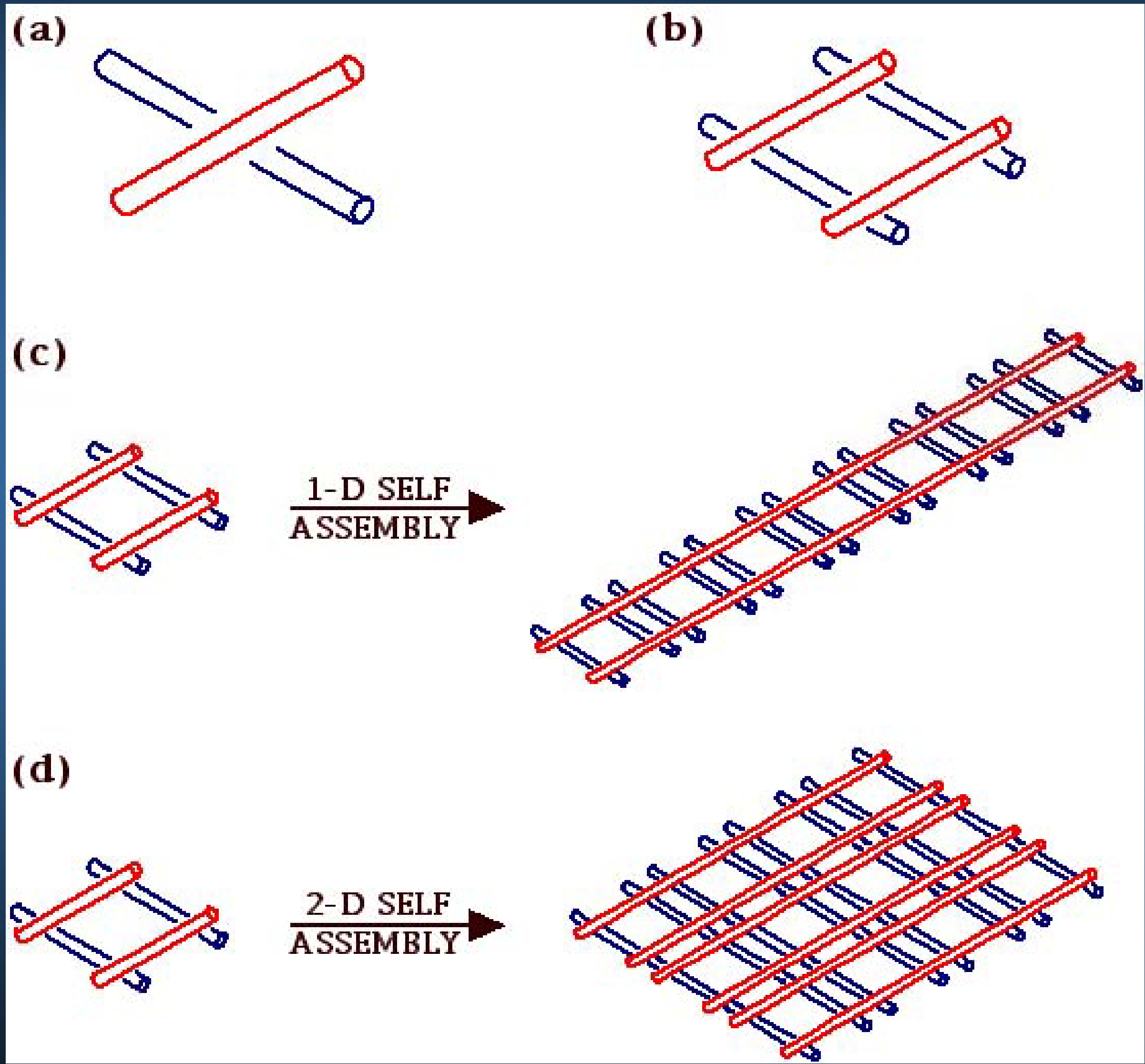
- Hairpin B^ contains a restriction site missing on D*.
- The 32 nm pattern on the top is converted to a 64 nm pattern after restriction.

(2) Modification by annealing or ligation

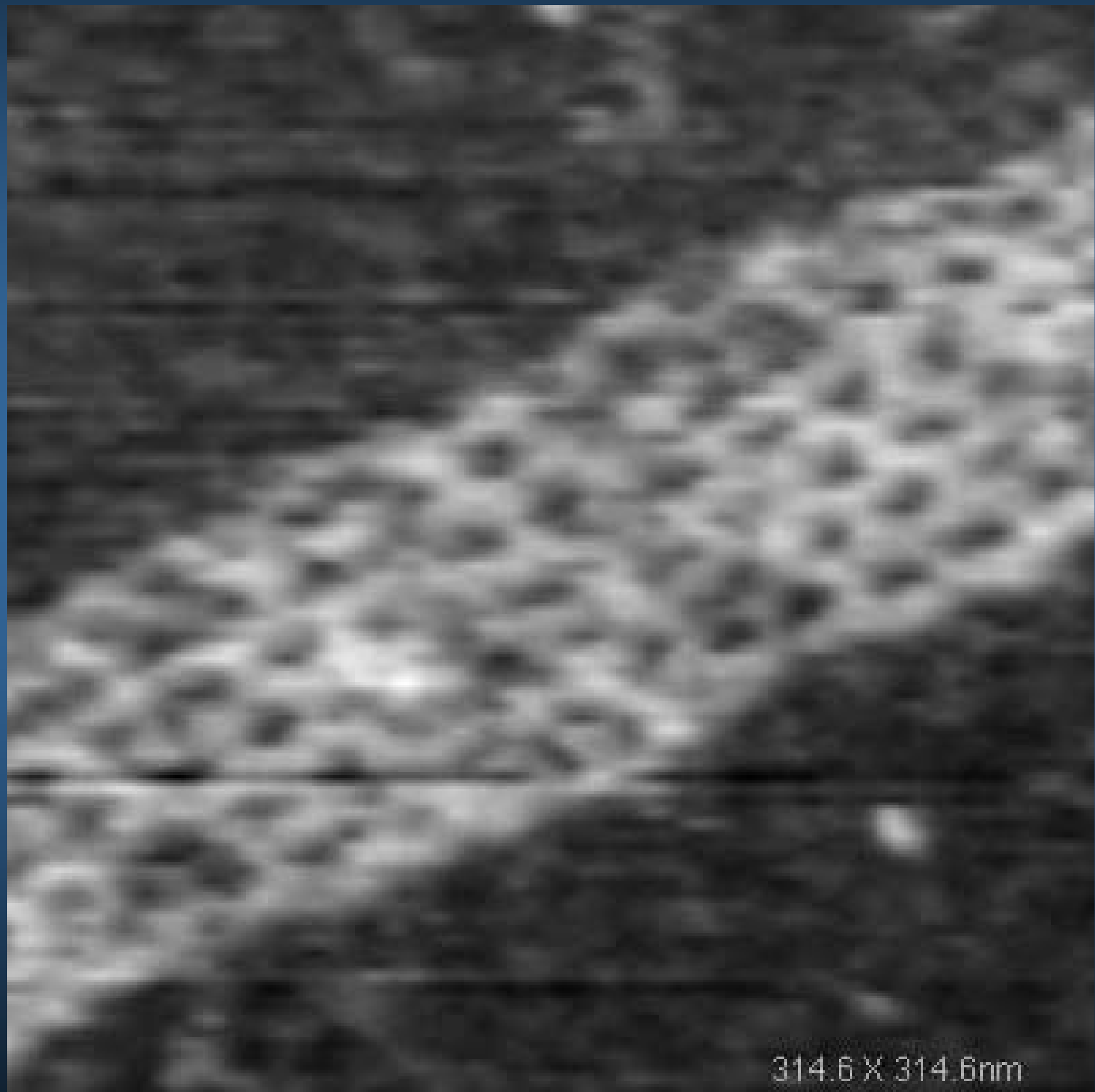


- B° contains a sticky end, that can pair with a sticky end on a hairpin in solution.
- The upper array produces a 64 nm pattern, but when a hairpin is ligated to it, or even just hydrogen bonded to it, a 32 nm pattern results.

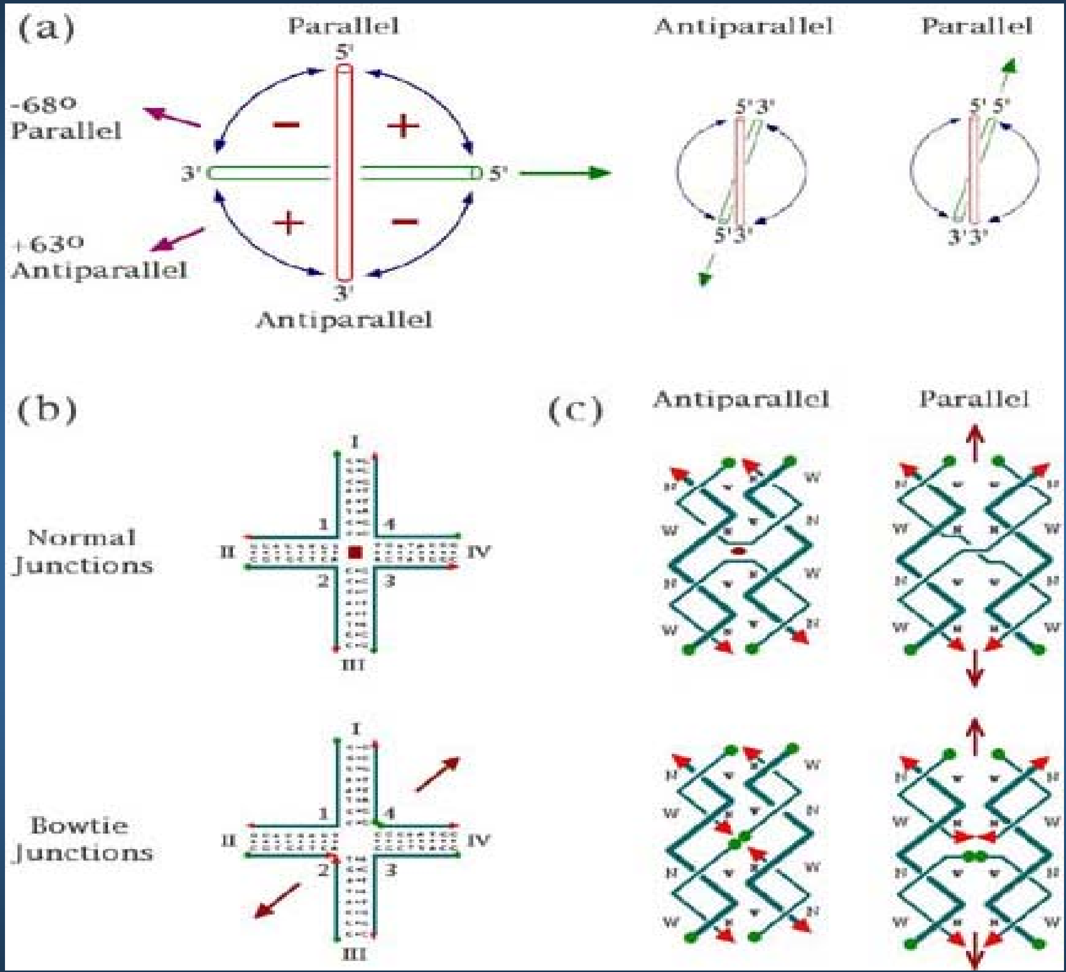
Holliday Junction Parallelogram DNA Arrays



AFM Image

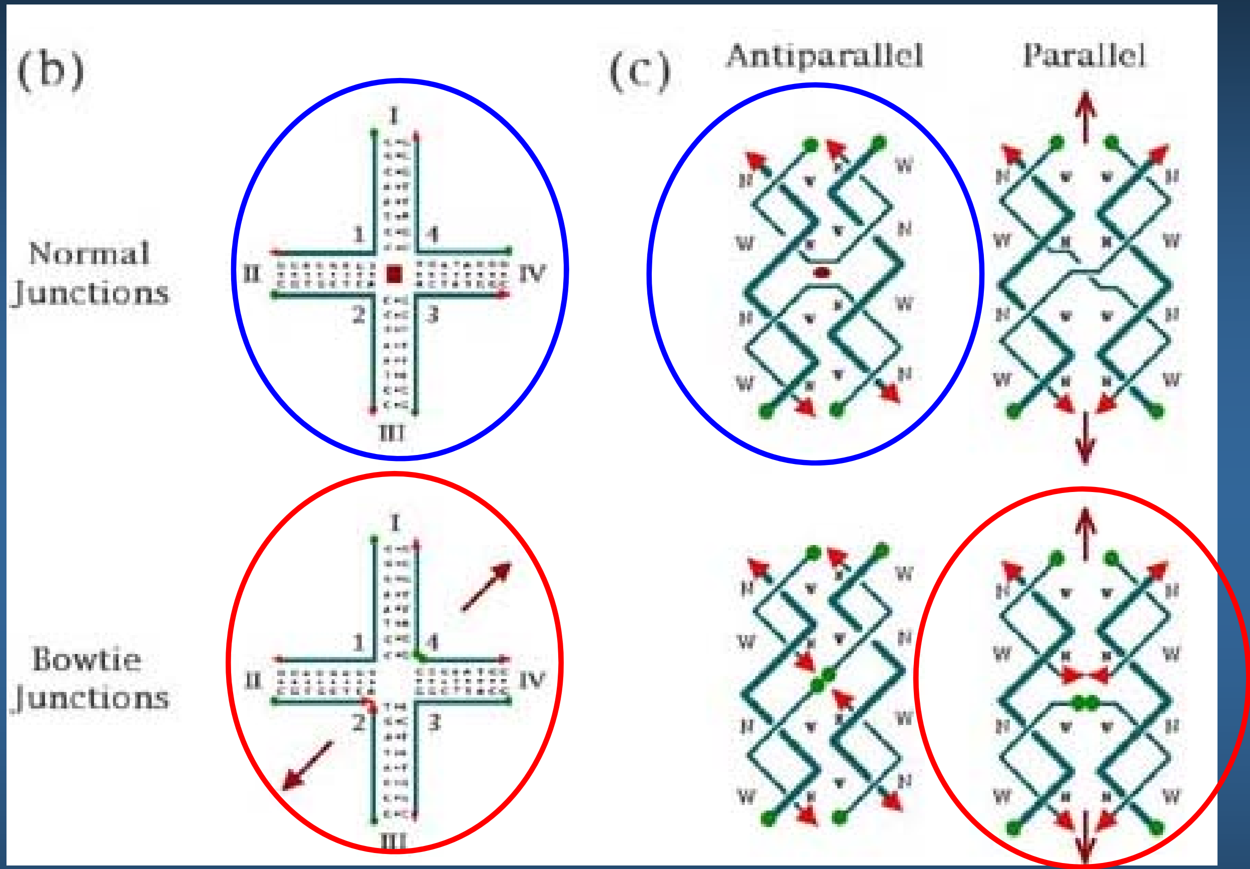


Bowtie Junctions



Note that the Bowtie junction has 5', 5' and 3', 3' linkages in two of its strands.

Bowtie Junctions



In Mg(+2)- containing solutions, the normal junction looks like the antiparallel junction on the upper left, and the Bowtie junction looks like the parallel junction on the lower right. The key feature is that both prefer a chain-direction-reversing structure for the crossover strands.

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- How do we know that the Bowtie junction is parallel, rather than antiparallel?
- To establish which is the correct structure, we made the four parallelograms below, labeled I, II, III and IV.
- If parallel, the parallelograms will look like the blue ones on the second line.
- If antiparallel, the parallelograms will look like the red ones on the third line.

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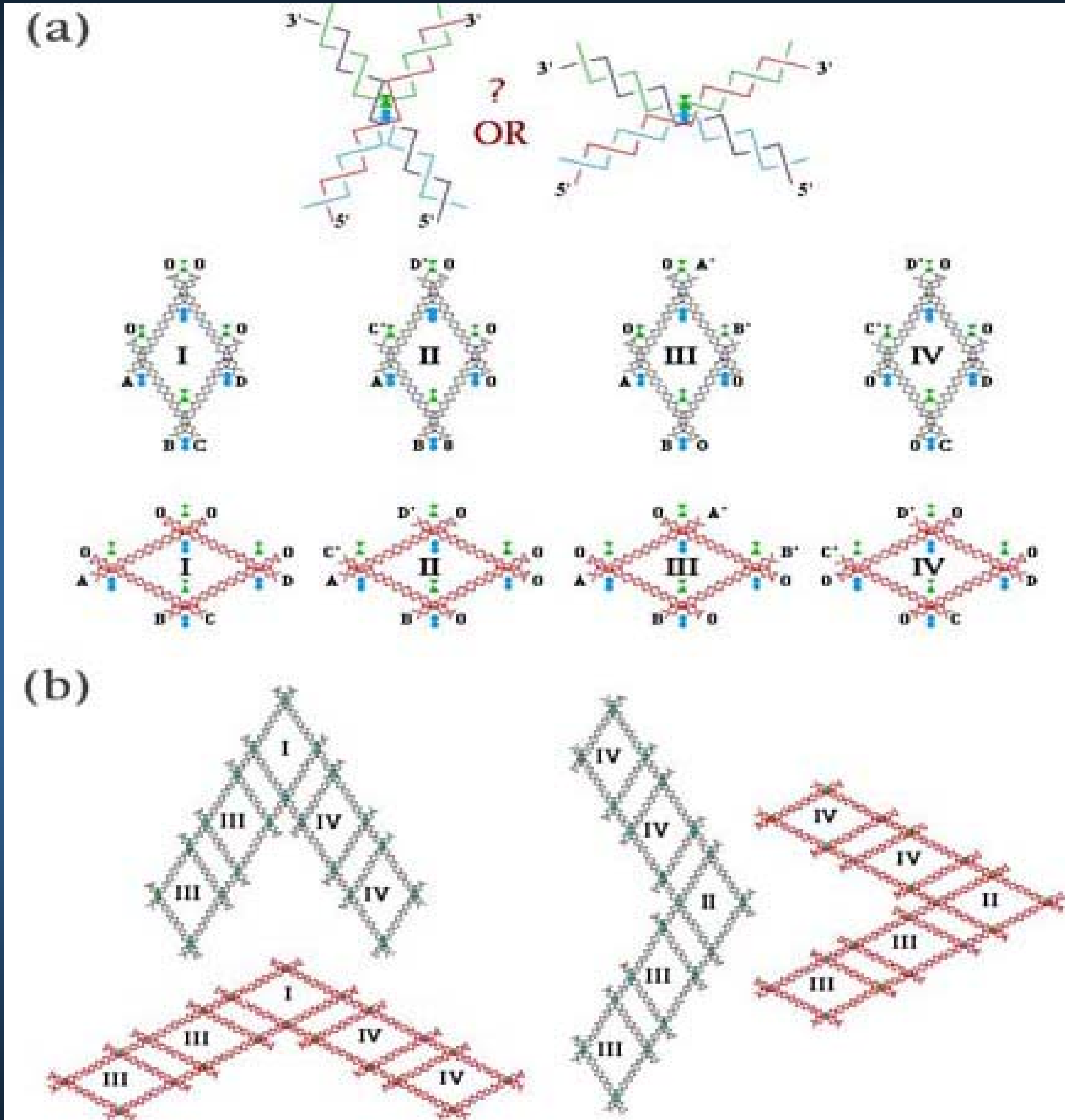
(a)
3'
3'
?
OR
5'
5'
I
II
III
IV
(b)
I
III
IV
III
IV
IV
IV
II
III
III
IV
IV
II
III
III
I
III
IV
III
IV

....

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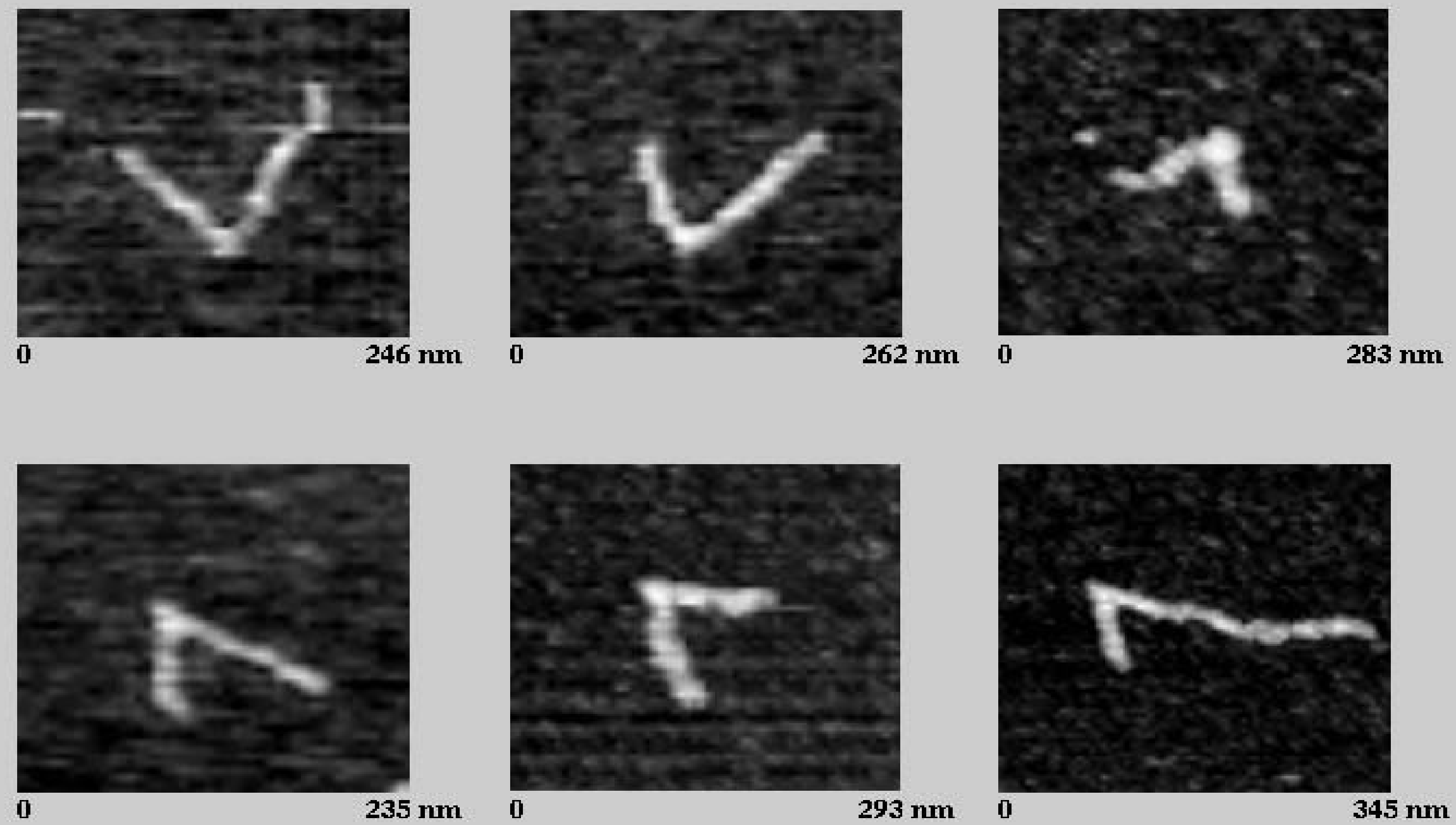
- **V-shaped array made from I, III, and IV**
  - It will have an acute angle if parallel.
  - It will have an obtuse angle if antiparallel.

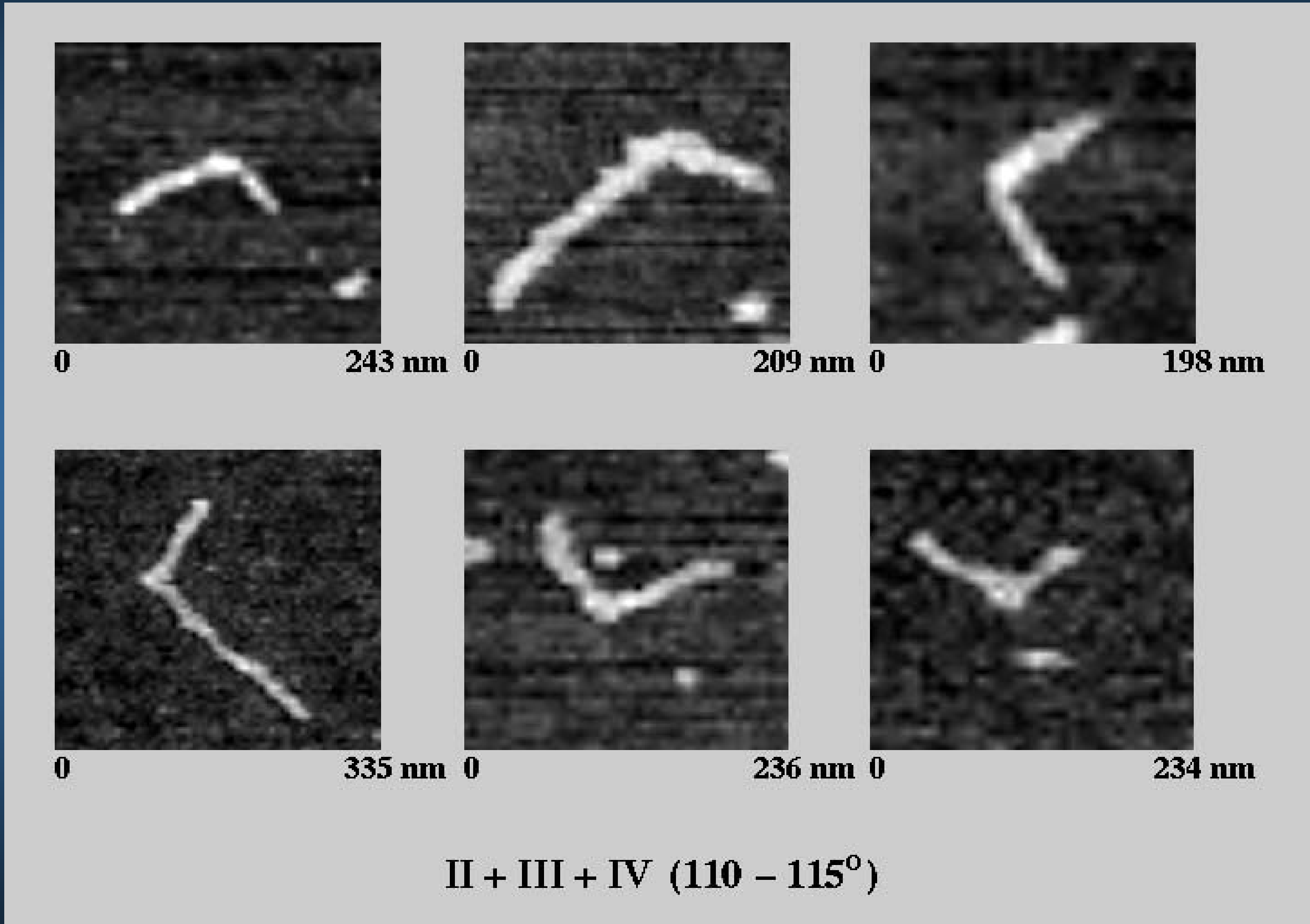
- **V-shaped array made from II, III and IV**
  - It will have an obtuse angle if parallel.
  - It will have an acute angle if antiparallel.

I, III, IV Array



I + III + IV (65 – 69°)

II, III, IV Array



From this experiment it is clear that Bowtie junctions are parallel.