The "LEGO" properties of DNA.

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DNA has long been recognized and studied for its effective code carrying properties, but recent studies suggest that the "molecule of life" might be ready for an entirely new kind of fame. Novel experiments demonstrate that the same properties that make DNA the ideal molecule for storing the genetic code of living beings, also cause it to be an ideal starting point for building nanoscale-sized structures, devices, and perhaps even robots. In this essay, I will provide an overview of recent accomplishments in DNA nanotechnology performed by Nadrian C. Seeman's chemistry group [1, 2].

The Advantages of DNA Nanotechnology

There are many aspects to DNA that make it a powerful construction material. Probably the most important one is the ability of DNA to carry code. Because of this, we can incorporate the blueprints for an entire structure into the DNA itself, so that it can act as both the raw material and the building instructions simultaneously. However, simply "coding" the molecules is not sufficient for building structures: we must also have a means of assembling them. DNA strands have the fortuitous property that they like to bind to other DNA strands that have a complementary sequence. It is easy to see that by creating many short DNA strands with specially chosen sequences and combining them together in the appropriate environment, we can have them self-assemble into complicated structures of our own design. As the DNA fragments bind to each other according to the programmed plan, they form progressively larger structures which consist of, on one hand, a stable non-reactive part composed of hydrogen-bonded DNA segments pairs, and, on the other hand, "sticky" ends which have yet to react with a matching "sticky" sequence on another DNA strand (or structure). At every step of

the building process, we can treat the structure to DNA-ligase enzymes that covalently bind the broken desoxyribose chains together. This whole process requires very little external manipulation and it is the DNA *itself* that carries out its own instructions. This chronological assembly is the basic process by which DNA nanostructures are built.

The many advantages (mentioned above) of using DNA as a building molecule would not be nearly as important if it were not for the abundance of DNA-manipulation tools that are available to us. Sizeable research and technological investments in the field of genomic research have provided us with powerful tools for synthesizing arbitrary DNA strands that encode sequences of our choosing. We thus have the freedom of completely specifying our entire system with relative ease and speed. Furthermore, we also have access to the entire gamut of biological enzymes that act on DNA and permit us to carry forth diverse complex operations, such as binding, denaturing, splicing, twisting, etc.

The Basic Building Motifs

Before making the claim that DNA is universal building material, we must demonstrate the existence of elementary building blocks, as well as of operations on these, that would lead to the assembly of a larger structure. Of prime importance to any wireframe structure are connecting rods. These can be made from two DNA strands bound together into a helix. A second very important element of any extended structure are junctions. If we can create branching molecules with sticky arms at each end, this alone will yield a large number of possible structures. Immobile junctions of arbitrary coordination number can easily be formed by judiciously binding halves of DNA strands to each other into star-shaped formations, as illustrated in figure 1. These junctions can then be used to connect DNA rods into intricate "wireframe models". Seeman *et al.* have demonstrated the feasibility of such a scheme by successfully synthesizing DNA molecules that have the connectivity of a cube and a truncated octahedron, respectively (see figure 2).

There are limitations to these techniques, however. The angles between the arms of a simple DNA junction are not fixed, but instead, they have a lot of angular freedom over which we have very little control. This means, for example that DNA "cubes" would not necessarily always be upright in a solution environment. In order to confer rigidity to DNA structures, we must either resort to using triangulation (which removes the floppy degrees of

freedom through constraints), or else we must design new junctions that are rigid under bending. Such a junction has been discovered[3], that uses DNA double-crossover molecules, which are essentially linear fibers made from four DNA strands intertwined in a regular motif (see figure 3 for an illustration of multi-crossover DNA molecules). Together with control over branching and linking, rigid junctions and arms confer us control over geometry.

Complementary to our ability to control the branching is our degree of control over the topology of our molecules. The topology of any string-like object can be fixed by completely specifying the polarities of its nodes. A node is a crossing of two strands and can be positive ("over") or negative ("under"). Positive and negative nodes are mirror images of each other and one cannot change the polarity of a node without cutting a strand. It turns out that nodes of either polarities can be made from a half-turn of two different species of DNA. DNA is usually found in a right-handed conformation (B-DNA), but it is possible to form a left-handed DNA helix (Z-DNA) given an appropriate sequence (e.g.: the repeated CCGG motif) and a favorable environment (e.g: Mg^{2+} ion concentration). The authors of the reviewed reports claim to be able to selectively generate closed loops which have the topology of a circle, trefoil knots with either all positive or all negative nodes, and a figure-8 knot, by starting from the *same* DNA strand and simply adjusting the concentration of Mg^{2+} before performing the ligation of the strand's ends. The prerequisite for such a feat is that the strand's sequence be specially chosen so that it is subdivided into regions which will each undergo the B-DNA to Z-DNA transition at different ionic concentrations. This powerful method can be used to construct arbitrary knots and chains of DNA molecules and has been successfully used to create a triplet of Boromean rings (see figure 4 for an explanation), which are topologically non-trivial

There are additional feats which have been accomplished with DNA. These include kinks, in which a DNA double-strand takes on a "V"-shape. Another exciting new element is the discovery of a DNA "motor"[4] which takes advantage of the B-DNA to Z-DNA transition to flip between two structurally different states.

Stiffness and Arrays

Most of the current work on DNA structures is in trying to design DNA crystals (i.e.: periodic lattices). The ability to create rigid scaffolds is of paramount importance if DNA is ever to be used in nanotechnology. Addi-

tionally, one promising application of DNA lattices of immediate importance is the facilitated crystallization of proteins. Current methods for the crystallization of water-soluble proteins involve trying to form a unit cell by compounding the proteins with stabilizing molecules, a problem which involves a lot of guesswork and no guarantee of success. However, if one is given a mesoscopic three-dimensional lattice, the proteins could then be "tied" to the lattice, such that the proteins would be in a periodic array which could subsequently be analyzed by standard diffraction techniques. DNA crystals would then provide a powerful method for *easily* determining the structures of a much wider range of proteins than is currently possible.

So far, only two-dimensional lattices have been constructed. The current building strategy is to use double-crossover molecules since they are known to be rigid and are suitable for high symmetry applications such as a lattice. One way of building a lattice would be to stack many DNA helices on top of one another and then have them be intertwined (in double-crossover fashion) with the helix above as well as with the helix below it in a periodic fashion. The way to achieve this is to create two species of short double-crossover molecules (or "DNA brick") that will self-assemble in a staggered array, as depicted in figure 5. Figure 6 shows an experimental realization of such a lattice as seen by an atomic force microscope.

Researchers are now trying to go beyond the closely knit weaving patterns seen so far, and there have been attempts to generate lattice structures that incorporate empty space. Current designs use triangles made out of three DNA crossover segments[5] as the basic building block (as opposed to the "DNA bricks"). Three-dimensional lattices, of course, are the next step, and there are already efforts under way to create coordinated junctions which will form rigid 3D structures.

Conclusion

We have summarized the recent developments in DNA nanotechnology. Our control over the shape of DNA structures seems almost limitless. We have already demonstrated our ability to control the linking, branching, crossing, geometry, dynamics (to a certain extent) and topology of DNA structures, and these feats provide us with a large range of operations from which we can base future engineering exploits. The promise of DNA technology lies not primarily in its ability to generate miniature structures and robots, but rather in its amazing simplicity and availability (linear nucleotide sequences,

no need for "factories", etc.). These advantages, derived from nature, are perhaps DNA greatest strength in competing with and/or complementing silicon technologies. After all, to paraphrase the authors, isn't biochemistry already just "nanotechnology that works"?

References

[1] N. C. Seeman, C. Mao *et al.*, *Nanotechnology* **9**, 257-273 (1998)

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[3] S. Zhang, T.-J. Fu *et al.*, *Biochemistry* **32**, 8062-8067, (1994)

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[5] X. Yang, L.A. Wenzler *et al.*, *J. Am. Chem Soc.* **120**, 9779-9786, (1998)

All figures are taken from the Seeman Lab website at `http://seemanlab4.chem.nyu.edu`

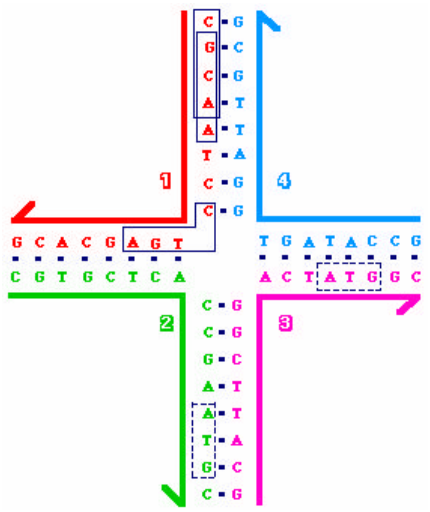

Figure 1: A four-way junction made by combining four DNA strands with specially chosen sequences.

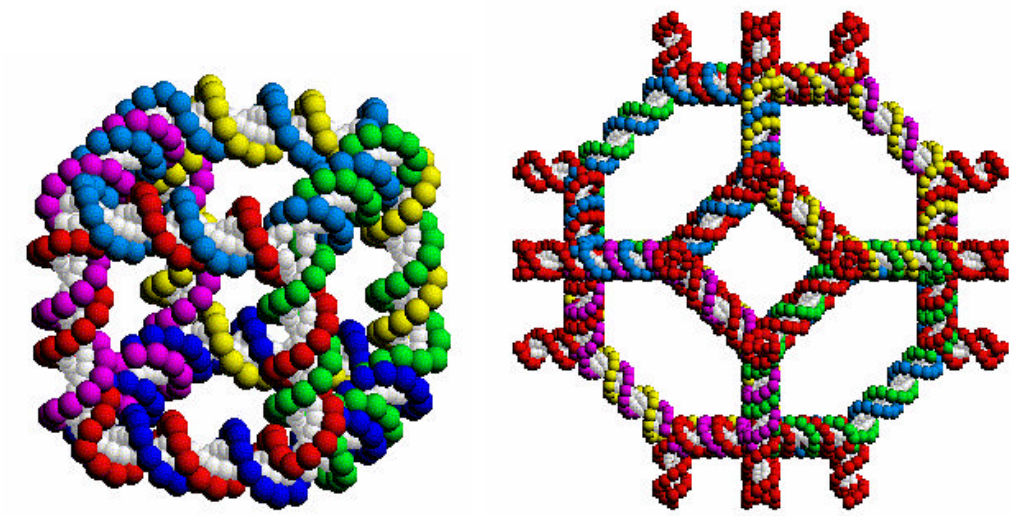

Figure 2: A cube and a truncated octahedron made entirely of DNA. The different colors represent the different strands. There is one strand associated with each face, and two bound strands (i.e.: one double-helix) for each joint.

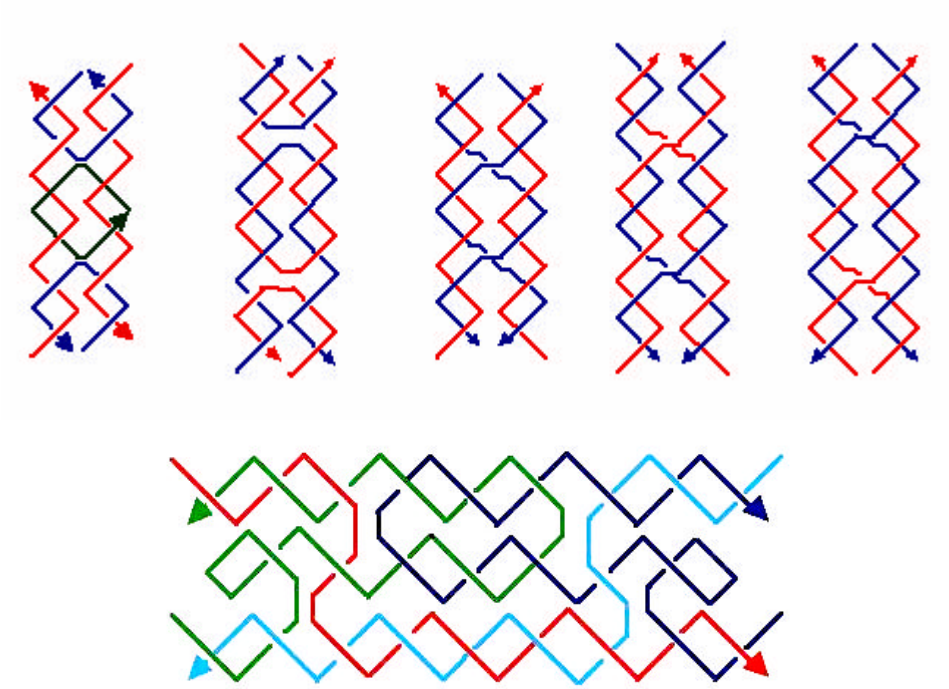

Figure 3: Illustration of multi-crossover DNA molecules: the top row illustrates the variants of the double-crossover DNA configurations, while the bottom row is an example of a triple-crossover molecule.

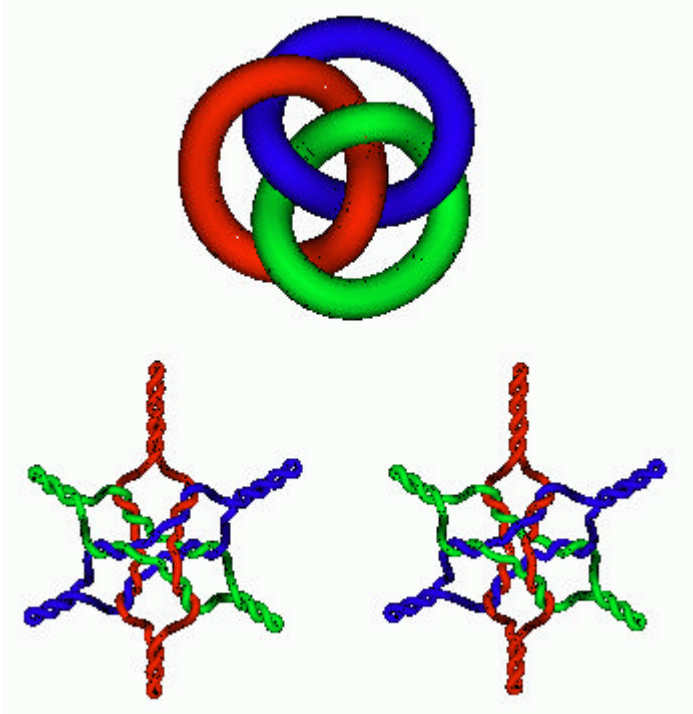

Figure 4: Boromean Rings: these structures will become completely unknotted if any of their rings are severed. The reason for building such a structure is that its synthesis can be tested by cutting only one loop and then detecting that the structure has been destroyed. The top structure is a set of three Boromean rings, while the bottom structure (in stereographic view: focus your eyes properly to see it in 3D) shows the geometry of the DNA structures that were actually synthesised. The complicated design was necessary to allow for the subsequent verification of the synthesis.

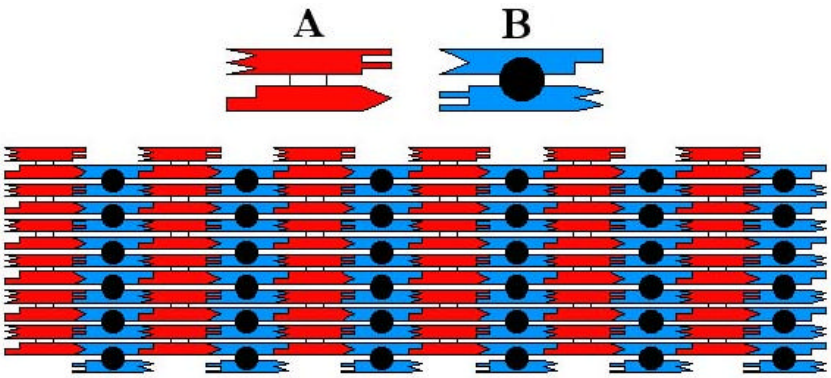

Figure 5: A microscopic cartoon of a double-crossover DNA lattice. Short DNA crossover blocks are ligated in a staggered manner such that a given DNA helix (corresponding to a linear string of half-blocks in the illustration) is intertwined with both its top and bottom neighbors.

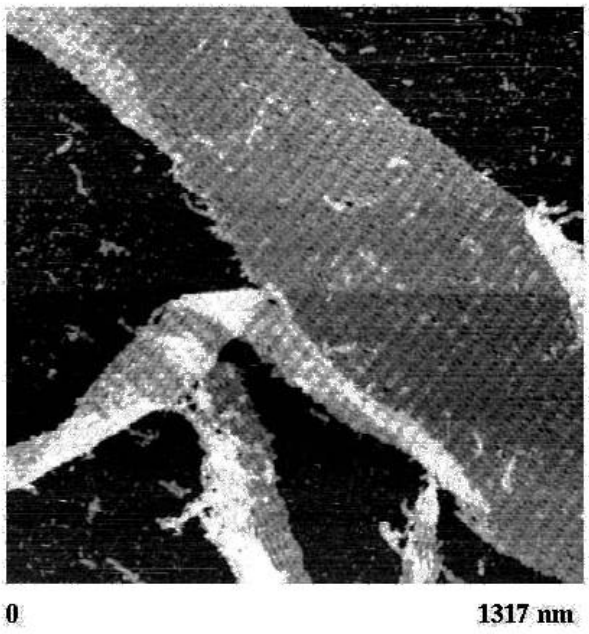

Figure 6: An AFM picture of a DNA lattice made from connected double-crossover DNA molecules.