DNA AS CLAY

Roald Hoffmann

Chemists are builders by nature, master riggers of the atomic stuff. Single molecules of moderate complexity under their belt, they now long to move on to the construction of more elaborate structures. The pull of the architectonic metaphor is strong (and based in childhood play), so people have naturally thought of building-block, ball-and-stick, Tinkertoy, and assembly-line construction at the molecular level. Chemists have ingeniously designed small modular units of varying rigidity that can be assembled or assemble themselves, or that they wish would assemble, into larger, ordered structures of substantial complexity.

One of the most surprising (and thought-provoking) feats of molecular engineering in this burgeoning area is the construction by Nadrian Seeman and his coworkers at New York University of giant (relative to most molecules) little (on the scale of macroscopic matter) stick figures, polyhedra and knots out of DNA. Models of two of these creations are shown in Figures 1 and 2.

Out of DNA? The idea seems wild—DNA is not your typical synthetic master sculptor's clay. The notion also seems transgressive of natural order—to build geometric objects of no intrinsic value from genetic material. Let me face the second concern first, even before I show you the principles of this beautiful sculpture.

The nucleic-acid "system" that operates in terrestrial life is optimized (through evolution) chemistry incarnate. Why not use it? Not to make genetic manipulations of human DNA, which quite justifiably provokes ethical questions. But to allow human beings to sculpt something new, perhaps beautiful, perhaps useful, certainly unnatural. As beautiful and unnatural as a Schubert song or the American Constitution.

The essence of that DNA system is the sugar-phosphate-base polymer that serves as the backbone of each DNA strand and the nucleotide bases that form complementary pairs—guanine (G) with cytosine (C), and adenine (A) with thymine (T)—with another DNA strand, giving rise to the corollary double-helical structure. Through some ingenious solid-state support chemistry, drawn by the incentive of a market for diagnostics, automated DNA synthesizers have developed to the stage that even a novice at chemistry can assemble moderate amounts of a 100-base-long nucleic-acid strand in a day.

Figure 1. Cubes are among the fantastic structures Seeman and his colleagues made of DNA.

It has also become pretty easy to link two helices together. The idea is shown in Figure 3. A double strand is assembled from synthesized complementary single strands, except that one of the strands is designed to have a few extra bases (here four) at one terminus. This generates a "sticky end." Another double helix with a complementary single-stranded extension may be bound, and the polymer backbone is "annealed" by an enzyme called DNA ligase.

Figure 2. Truncated octahedron is also built of DNA.

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Figure 3. Pieces of DNA can be joined by complementary base-pairing of sticky ends, followed by annealing the backbones with a DNA ligase.

This generates an easy way of extending synthetic DNA linearly. Suppose you want to build a polyhedron. A line is fine, it makes the edges. But it is the vertices—three-, four- or five-connected—that define the polyhedron. It is possible by clever design of nucleic-acid sequences (Seeman is a specialist in this) to build such junctions. One is shown in Figure 4. An essential feature of the design of this vertex is that the sequence of bases in each strand minimizes the chance that some other structure, such as the two-strand pairing, will form (and it also eliminates a kind of coordinated slipping of the four strands).

The junction is a branch point, but it is not rigid. The double-helical arms (here about one turn of a double helix long) can bend out of the plane they are drawn in; this allows their linkage into a polyhedron.

Now we can see the ingenious way in which Seeman and Junghuei Chen built a cube of DNA (Figure 5). First they constructed two rings (Figure 5, *top*) destined to become the left (*L*) and right (*R*) faces of the cube. By combining with the appropriate strands, they ligated these faces into a three-square belt of *L*, *F* (front face) and *R*. This intermediate structure was eventually ligated into a cube. A group of ligations at the end seal up the structure.

This cube-like molecule, shown in detail at the bottom of Figure 5, is built up from 10 synthetic DNA strands—two strands that contain 80 nucleotide bases and eight strands that are a little longer or shorter (to provide sticky ends) than 40 nucleotides. Each edge contains 20 nucleotide pairs, two turns of a double helix. An edge is then around 68 Angströms long. The cube-like object is more than just a polyhedron; if one focuses on the DNA strands, it is a complex catenane, a system of six intertwined rings not covalently bonded to each other.

The truncated octahedron in Figure 2 is built by

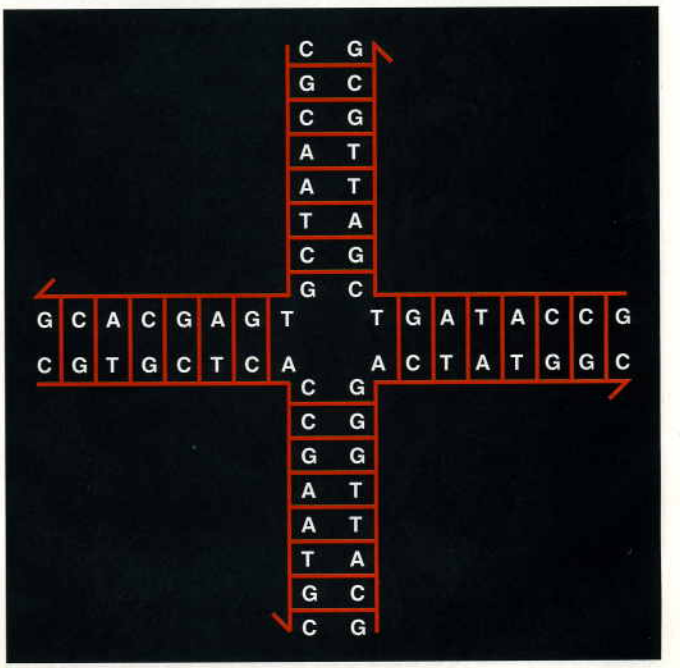

Figure 4. Junctions, like the one shown, are essential for making three-dimensional structures.

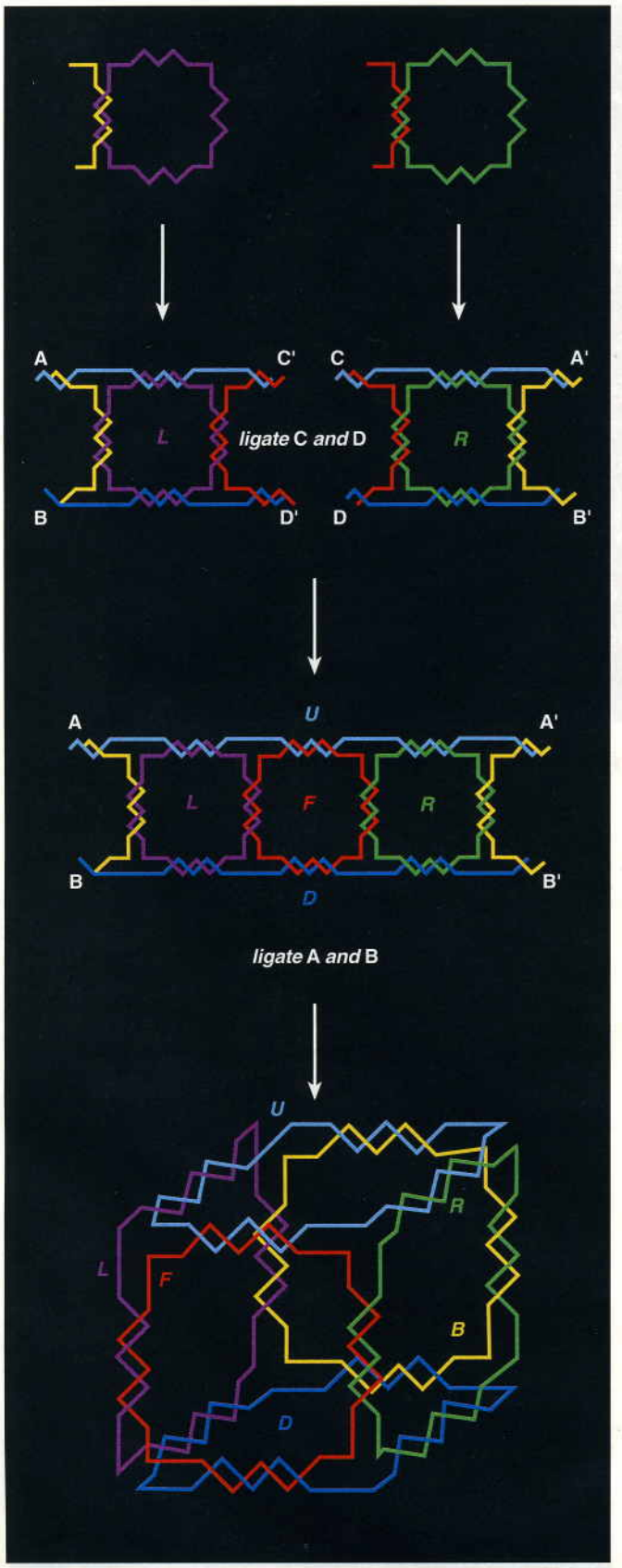

Figure 5. Stepwise assembly of a DNA cube is shown. Left (*L*), right (*R*), front (*F*), back (*B*), upper (*U*), and lower (*D*) faces of the cube are indicated. Sticky ends A, B, C and D are used to join the individual DNA strands in such a way that the desired three-dimensional structure is ensured.

Yuwen Zhang and Seeman to have the same edge length as the cube, 20 nucleotides. The molecule then contains 1,440 nucleotides in its framework. It also has some extra arms for potential three-dimensional linking; its total estimated molecular weight approaches 800,000. That's big.

In the building and analysis of these incredible objects still another biomolecular tool was used, a restriction enzyme. Call it a clever cleaver—an enzyme that is known to break a nucleotide double helix at or near a specific base sequence. You can buy a good number of these. In the synthetic mode, the cleaver can be used to create new sticky ends from a built-in loop containing the sequence specific to the restriction enzyme—the principle is shown in Figure 6. The synthesis of the truncated octahedron (but not the cube) used this strategy.

The restriction enzyme also allows analysis of the tiny amount produced of these shapes. Each edge of the polyhedron, consisting of two DNA strands, can be constructed with a specific restriction site. Then that given edge, and no other, can be cleaved, leaving behind an object with a specific intertwined complexity. Gel electrophoresis, the workhorse analytical technique of molecular biology, is then used to identify the fragments. By a sequence of such cuts and some good detective work the topology of the composite assembly may be determined.

Note that I said "topology" and not "shape." The junctions and the intervening DNA are not as rigid as the models shown here make them out to be. Only a few nanograms of the final product is made, insufficient for structure determination by magnetic resonance or crystallographic techniques. We don't yet know the real shape of these beasts. The cube may turn out to be a parallelepiped, partially collapsed or distorted in some other way. But its cubical connectivity is beyond doubt.

One interesting point about these shapes is that although their supramolecular topology is symmetrical—cube or truncated octahedron-like, edges of equal "length"—in microscopic detail the component building elements are distinct. Each edge has a different, designed sequence. We are playing on the molecular level here with the notion of similarity—building like objects from unlike building blocks and unlike objects from like building blocks.

Why did Seeman and his coworkers make these stick figures? In part to test the design principles; putting a complex structure together reveals instantly to the real and molecular carpenter the shortcomings of the modular units or the assembly principle. There are potential uses in drug delivery, as templates or scaffolding for crystallization, and in general for nanoconstruction. I think the DNA shapelets were also made for the sheer fun of it.

Organic synthesis used to be familiar, paradigmatic ground—pure reagents were used, subjected to well-defined conditions, perhaps with a catalyst thrown in. The practice was and is ingenious, and very clean. With luck and skill, enough mate-

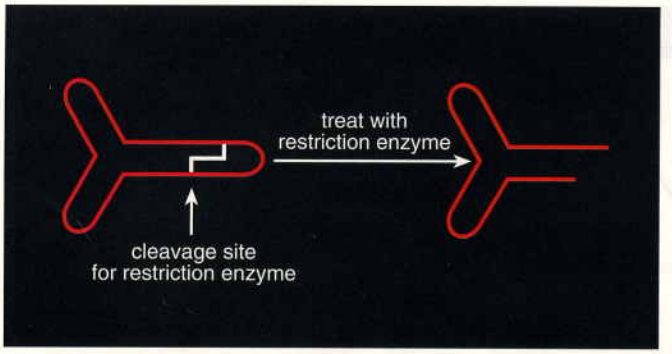

Figure 6. Creation of new sticky ends from a DNA loop requires a restriction enzyme that recognizes the specific sequence to be cut.

rial could be made for structure determination by physical methods. But things have sure changed, just in a few years. Witness:

The syntheses here, using ligating and restriction enzymes, never making enough material for the usual structure determination.

The use of genetic engineering methods—binding, synthesizing the gene, cloning, expressing. Bacteria or rabbits then do the chemistry for you.

The use of "libraries"—vast, random families of molecules produced by designed chance, then selected for a task.

I suspect that there is a little resistance in the minds of many of my organic chemist friends to these newfangled ways of making molecules, and to the ways of determining that they have been made. I would ask them to think of the organic chemists of the '40s faced with the then new physical methods of structure determination. Or if a ligating enzyme or gene expression bothers them, to reflect on what it means to use a metal or an oxide catalyst in an "ordinary" synthesis, not knowing in molecular detail how that catalyst works.

The making of molecules matters; whether a method is "sporting" or not is defined by fashion and tradition. These DNA sculptures are marvelous achievements of organic synthesis.

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