DNA Nanotechnology

credit: Chelsea Gordon, HSR

By Amrita Goyal

"We wish to suggest a structure for the salt of deoxyribose nucleic acid (DNA). This structure has novel features which are of considerable biological interest… It has not escaped our notice that the specific [complementary base] pairing we have postulated immediately suggests a possible copying mechanism for the genetic material" (1).

With these famous words, James Watson and Francis Crick ushered in a new era in biology—the age of DNA. Just as the implications of DNA's complementary base pairing and helical structure for a possible mechanism of replication did not escape them, the possible applications of DNA to materials science, based on the same elements of DNA's structure, have not escaped the notice of present-day chemists. DNA is the next frontier of nanotechnology and will become the basis of the construction of many three-dimensional nanostructures.

DNA is the ideal macromolecule to utilize in the quest to develop self-assembling nanostructures. Molecular self-assembly—the synthetic strategy of choice for scientists developing new nanotechnologies—is the spontaneous, non-covalent association of molecules to form stable and well-defined aggregates, and is a consequence of the molecules' intrinsic chemical properties (2).

DNA is perhaps one of the best-studied polymers in all of science, and is an ideal material for generating nanostructures that form by self-assembly. DNA's anti-parallel strands are held together by hydrogen bonds between complementary bases—adenine always pairs with thymine, guanine with cytosine. The larger helical structure of the DNA molecule has been studied extensively since its discovery by Watson and Crick. As a result, the geometry of double-stranded DNA and the energetics of DNA folding in solution are highly predictable (3). Specific DNA sequences of hundreds of base pairs can be easily synthesized in the laboratory. DNA is also extremely easy to replicate, either by the polymerase chain reaction (PCR) or by bacterial plasmid methods.

Molecular engineers are thus in possession of two of the three requirements for constructing nanostructures—a well understood material with which to build, and methods by which to produce and manipulate the material. The final ingredient necessary is a versatile design strategy (3). Over the last ten years, DNA nanostructure technology has gone through three distinct phases. Dr. Nadrian Seeman of New York University was the first scientist to construct a three-dimensional DNA nanostructure—a cube (4). Moreover, he devised the most highly developed method of DNA nanostructure synthesis, known as the "tile model," which is the most suitable for the production of two-dimensional arrays and nanostructures (3).

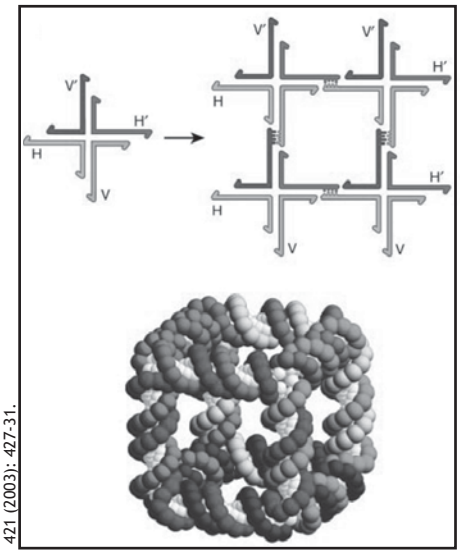
DNA Branches Out

Nadrian Seeman's tile model of synthesis is based on the construction of two-dimensional geometric building blocks out of DNA, which are then able to self-assemble into two- and three-dimensional arrays and nanostructures. This method of construction is made possible by exploiting two naturally

occurring DNA structures: sticky ends and branched DNA (5).

Sticky ends are short, single-stranded regions of DNA that extend from the end of a double-stranded DNA molecule. Sticky ends are able to act like Velcro flaps by virtue of DNA's inherent ability to engage in complementary base pairing; they can come together to form normal, double helical DNA of predictable geometry. However, although they are very useful, sticky ends are not sufficient to enable scientists to construct highly interesting shapes. Sticky ends are invaluable in self-assembly, and allow the construction of circular DNAs and extended linear sequences, but are limited in terms of expanding the possible geometries of DNA nanostructures (5).

Although DNA is unbranched and linear the vast majority of the time, during the crossing-over step of meiosis, a process responsible for generating sex cells, DNA takes on a branched conformation at short-lived sites known as Holliday junctions. Naturally occurring Holliday junctions consist of four strands of double helical DNA bound together at a branch point (Figure 1). Synthetic DNA complexes based on Holliday junctions have branch points flanked by three to eight arms of DNA (5). These artificial complexes provide nanomaterials engineers with the flexibility to construct shapes that range from cubes to truncated octahedrons to Borromean rings (6).



credit: Adapted from Seeman, N. "DNA in a material world." *Nature* 421 (2003): 427-31.

Figure 1. On the top left is a diagram of a Holliday junction. The portions marked H, H', V, and V' are complementary sticky ends, and they adhere to one another as shown in the top right figure. These two dimensional crystals can then be ligated to form three dimensional cubes. (5)

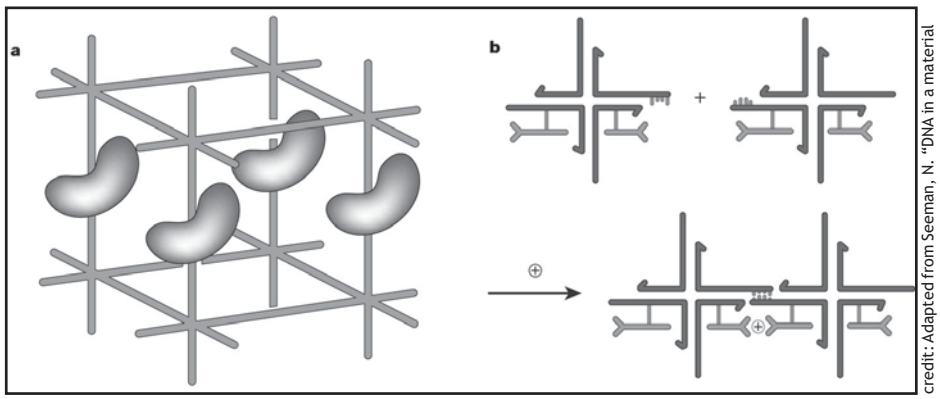


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Figure 2. An illustration of the possible applications of DNA scaffolds. (a) Proteins are attached to a scaffold for the purpose of X-ray crystallography. (b) The deposition of metal ions on DNA scaffolds at a key step in the synthesis of nanoelectronic materials (5).

Armed with these tools, the synthesis of a nanostructure such as a cube is relatively simple. In his experiments, Seeman created four-branched DNA junctions, and from each double helical branch extended a sticky end. Large numbers of these junctions were then able to assemble into two-dimensional crystals that consisted of interconnected rings of DNA. These crystals could then be ligated to form a three-dimensional cube that was several nanometers across (Figure 1) (5).

Thus, the prescription for two-dimensional DNA nanostructure synthesis according to Seeman's tile method is a straightforward one—synthesize branched DNA with programmed sticky ends, and allow it to assemble into a closed object or crystalline array. Such crystalline scaffolds could be used as platforms to hold other molecules for the purpose of X-ray crystallography, or to create complexes of proteins. Three-dimensional lattices could be used as scaffolds for assembling other materials. For example, metal atoms could be incorporated into the structure to make a nanoelectric assembly, which could function as a circuit. DNA binding enzymes could even be used to direct or restrict metal deposition onto the structures, which would facilitate very precise synthesis (Figure 2) (5).

However, as useful as the tile method is in synthesizing nanocrystals, it has distinct limitations. Because of the topology of the requisite branched structure, it is not possible to reproduce the DNA using regular DNA polymerase, the enzyme that synthesizes DNA strands in nature. In 2004, Dr. William Shih of Harvard University achieved the mammoth task of successfully synthesizing a nanostructure from a single strand of DNA that was replicable by PCR (7).

DNA Gets a Bit Cagey

Shih's triumph of molecular engineering came in the shape of an octahedron. This octahedron was constructed from a single, 1700 base pair-long strand of DNA, the folding of which was driven by a denaturation-renaturation reaction, in conjunction with the addition of five 40 nucleotide-long DNA strands that served to guide its folding process (7).

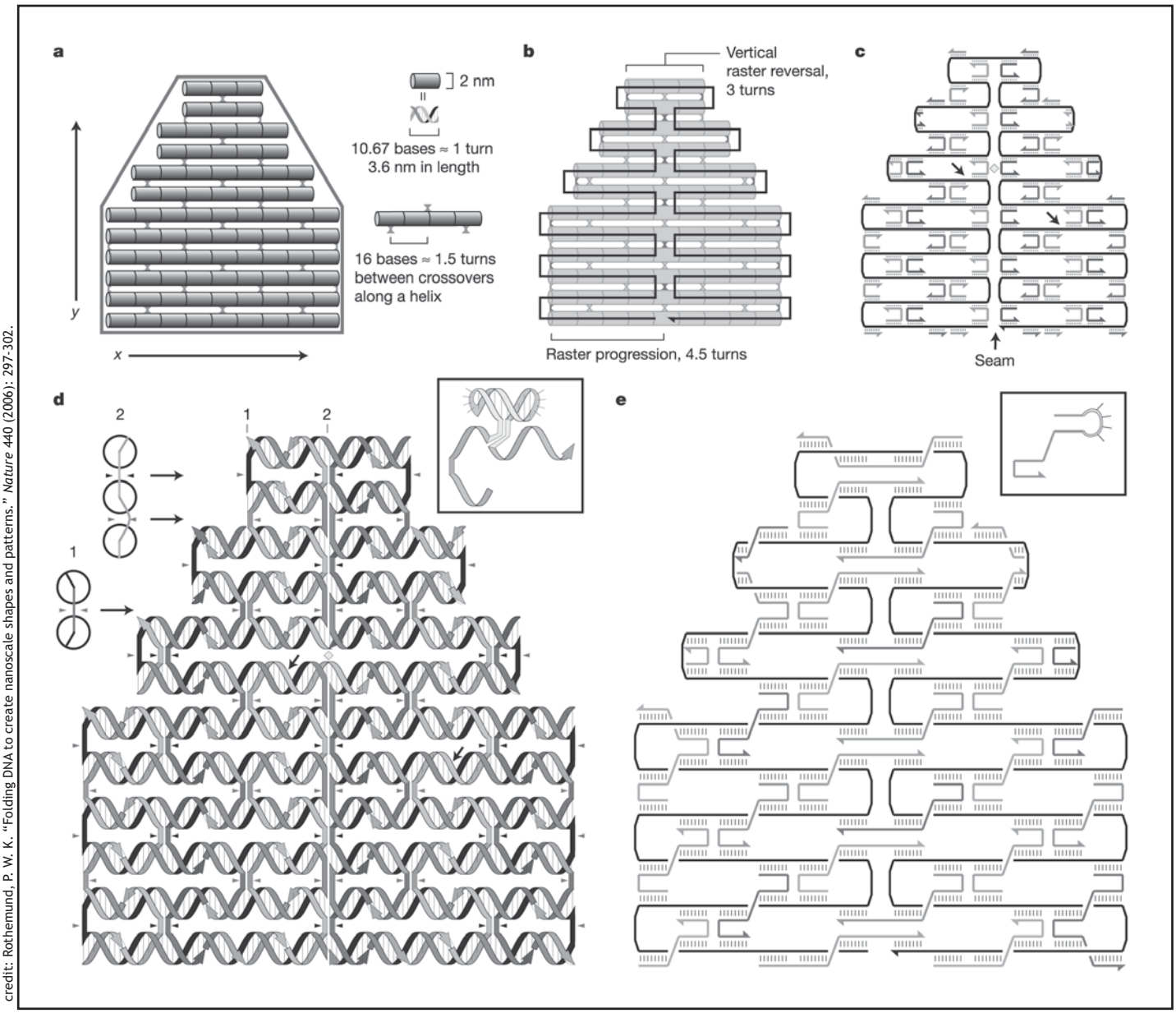
There are several things that make Shih's strategy for synthesizing the octahedron quite notable. First, it is possible to replicate the 1700 base pair DNA strand using lab techniques as simple as PCR. Second, no covalent bonds were either made or broken during the assembly of the octahedron. Most importantly, however, Shih's method introduced the concept of using short DNA strands to direct the folding of a longer strand. However, this process

represents a significant challenge in that the components must be mixed in their exact stoichiometric ratios for efficient assembly to occur (7).

The octahedral geometry of Shih's nanocrystal is extremely important. Each of the twelve edges of the octahedron is made of double stranded helical DNA. These helical struts frame the eight equilateral, triangular faces. Many of the other shapes that have been synthesized, including the cube and truncated octahedron, are subject to deformation by even moderate amounts of force. The octahedron, on the other hand, very strongly resists deformation because of the triangular shape of its faces (7). Numerous structures in nature also take advantage of the strength of this geometry, including the protein coat of several viruses, which is icosahedral in shape.

Each octahedron has a diameter of approximately 20 nanometers, which is equivalent to the size of a small virus. The cavities of these octahedrons are sized to accommodate a sphere with a diameter of about 14 nanometers, which would make the octahedrons useful for such applications as the transport of proteins and other macromolecules, and possibly even for drug delivery. Complexes of these nanostructures could be used to position other materials at specific locations in the structure; it would even be possible to encode binding sites for the attachment of sequence-specific binding proteins along the three dimensional framework (7). Most importantly for synthesis of rigid supernanostructures, a tetrahedral nanocrystal has been synthesized as well (8). Together with its octahedral counterpart, such a structure could eventually be used as a building block in the synthesis of larger structures held together with linkers (specially designed strands of single stranded DNA) (9).

The DNA cages might also be integrated with biological macromolecules



credit: Rothemund, P. W. K. "Folding DNA to create nanoscale shapes and patterns." *Nature* 440 (2006): 297-302.

Figure 3. An illustration of the DNA origami method (10).

and used as carriers for other molecules, including proteins and drugs. Additional chemical capabilities could be added to the structures by conjugating them to aptamers, which are folded, single-stranded lengths of DNA that bind specifically to small molecules, or to ribozymes, or RNA molecules with catalytic properties (5).

DNA Origami

Stars, smiley faces, rectangles, triangles, and squares—this seemingly random assortment of shapes represents a handful of the two-dimensional nanostructures that Dr. Paul Rothemund of the California Institute of Technology has been able to synthesize out of DNA using a self-assembly technique he has called "DNA origami" (10).

Rothemund's new synthetic strategy shatters many of the rules governing nanostructure assembly that have been established in the field of DNA nanotechnology. His method uses a few hundred 16 to 32 nucleotide-long single-stranded DNA molecules to "staple" a very long single strand into any desired two-dimensional shape. Individual staples can be made into nanometer-scale pixels that create surface patterns on a given 100 nanometer shape or can combine shapes into larger structures. This process is capable of producing nanostructures that are larger and more complex than any of those generated by its predecessors (10).

Rothemund's method is a surprisingly straightforward one and is largely computer-based. First, a shape is chosen, and on the computer, a model of it is filled from top to bottom with an even number of parallel double helices. Then, the single long scaffold strand of DNA is folded back along the double helices, and periodic crossovers are introduced to provide rigidity. Staple strands with a length of 16 to 32 nucleotides are added to hold the scaffold together in the desired shape by means of complementary binding (Figure 3). Finally, the structure is optimized on the computer to minimize any destabilizing

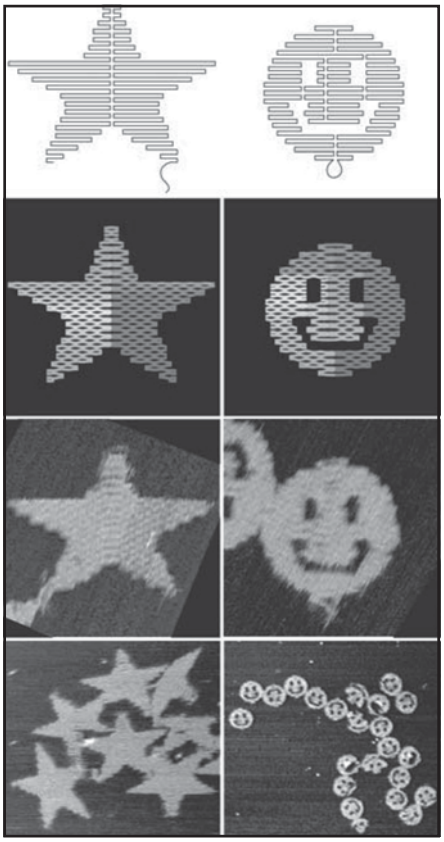


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Figure 4. Pictures of some of the nanostructures synthesized by Rothemund (10).

strains (10).

The variety of shapes that can be synthesized using this method is limited only by the imagination. The synthesis process is also remarkably efficient. For any given shape, over 70% of the nanostructures synthesized come out "well formed"(10). Moreover, the staples that hold the scaffold together provide a means of generating patterns of pixels, as they can be labeled with fluorophores, or small molecules that give off light when irradiated with a particular frequency. Each oligonucleotide can serve as a six-nanometer pixel and be used to pattern complex designs on the nanostructures. Examples of patterns created by Rothemund include a picture of the DNA double helix accompanied by the letters "DNA," snowflakes, and a map of the world (Figure 4) (10).

Although these nanostructures are all two-dimensional, the extension of this system from two to three dimensions should not be difficult given the established precedents. The design and synthesis of functional materials in a "bottom-up" fashion at the nanoscale level has become significantly more feasible with the development of DNA origami (10). One of the goals of nanostructure synthesis through patterned DNA origami is the development of a nanobreadboard, or a very small circuit to which proteins, aptamers, fluorophores, nanotubes, nanowires, gold nanoparticles or any number of other molecules could be added. These might ultimately be used to model complex protein assemblies and molecular electronic circuits (10).

DNA nanotechnology has applications as disparate as X-ray crystallography, protein array assembly, self-assembling nanomachinery, nanoelectronics, and nanocircuitry. The structure of DNA was initially resolved through a combination of chemistry, physics, and the insight of many brilliant scientists. Now, scientists are using their knowledge of the structure and characteristics of DNA, in combination with their intuition about the potential of this biological polymer, to revolutionize materials science, chemistry, biology, and computing. H

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