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Author
Powers, Alexander

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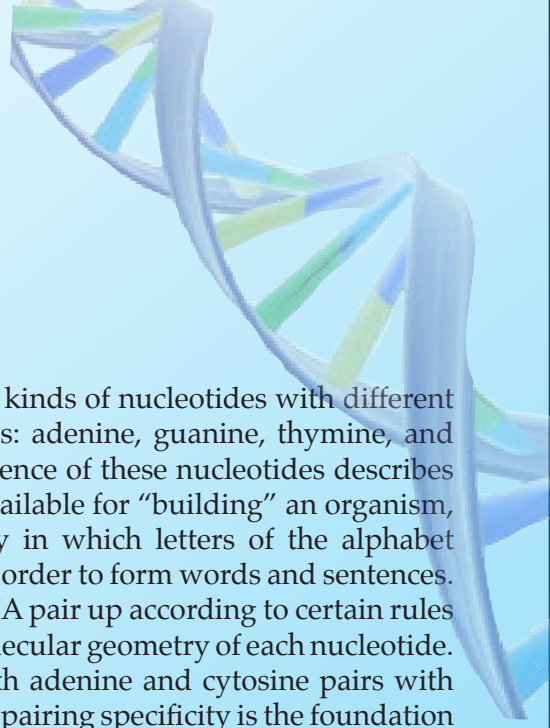
DNA : Building Blocks of Nanotechnology

Alexander Powers



> "A friend of mine suggests a very interesting possibility for relatively small machines. He says that, although it is a very wild idea, it would be interesting in surgery if you could swallow the surgeon. You put the mechanical surgeon inside the blood vessel and it goes into the heart and 'looks' around...it find out which valve is faulty and takes a little knife and slices it."

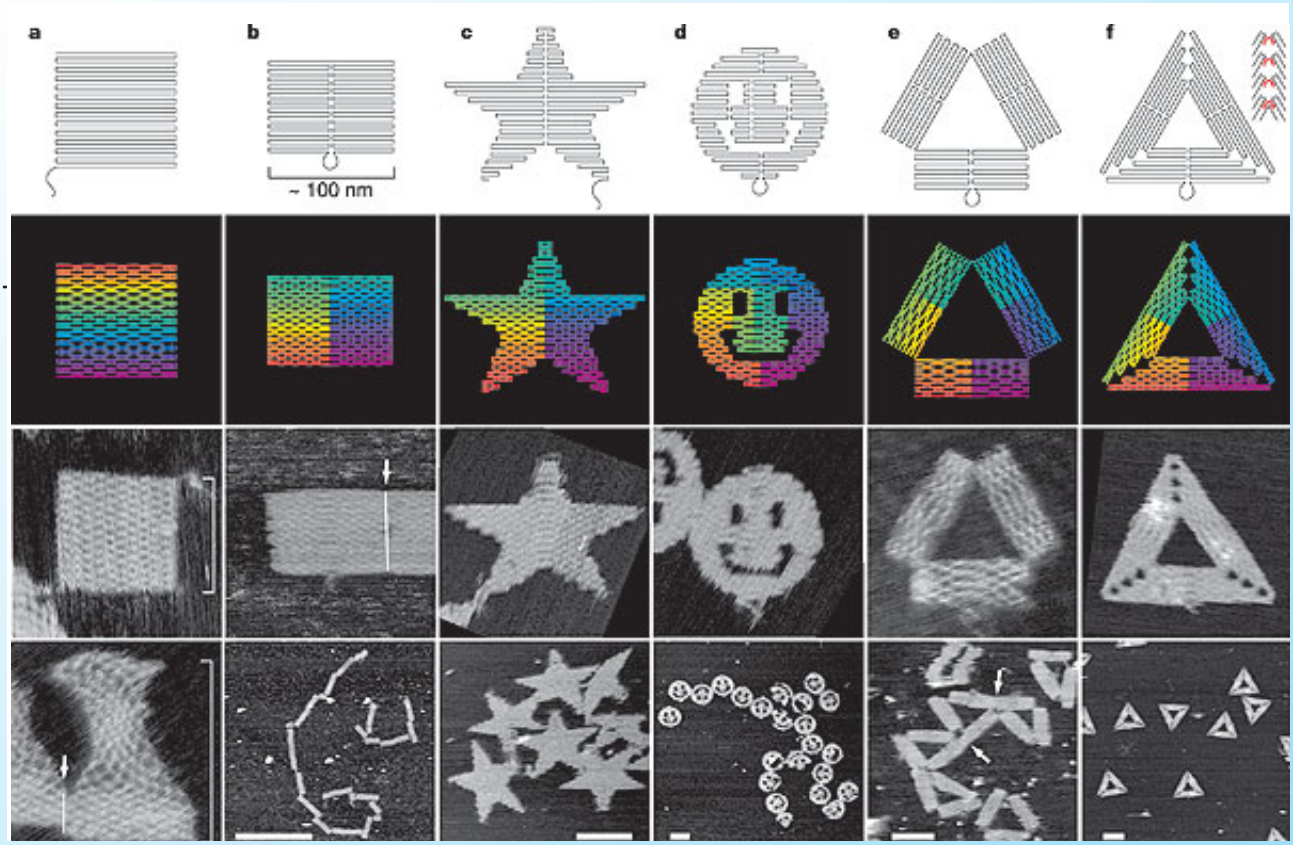
Richard Feynman offered this prophetic vision at his famous 1959 Caltech lecture "There's Plenty of Room at the Bottom" – a seminal event in the history of nanotechnology. In developing nanoscale machines, Feynman suggested scientists take a hint from biology. After all, proteins zip around cells on elaborate transport systems while DNA molecules encode vast quantities of information on a molecular scale. Feynman asked innovators to, "consider the possibility that we too can make an object that maneuvers at that level"(Feynman, 1960). Little did he know that biology would be the key to making his vision a tangible reality nearly 50 years later. The burgeoning field of DNA nanotechnology -- using nucleic acids as a building material in an nonbiological context -- has recently yielded some incredible breakthroughs ranging from programmable drug delivery capsules to enzyme "spiders" and chemical logic gates. DNA nanotechnology has the potential to finally realize the nano-surgeon.

Deoxyribonucleic acid (DNA) makes for an extremely effective building material at the nanoscale; afterall, nucleic acids are life's information storage molecule of choice. Nearly everyone learns about DNA by the time they graduate middle school - and with good reason. Just as computers derive vast amounts of information from a simple code of 1's and 0's stored electronically, DNA encodes the vast complexity of life in simple chemicals. Deoxyribonucleic acids are composed of long strands of repeating subunits known as nucleotides. DNA uses four different kinds of nucleotides with different chemical structures: adenine, guanine, thymine, and cytosine. The sequence of these nucleotides describes the information available for "building" an organism, similar to the way in which letters of the alphabet appear in a certain order to form words and sentences. Two strands of DNA pair up according to certain rules dictated by the molecular geometry of each nucleotide. Thymine pairs with adenine and cytosine pairs with guanine. This base pairing specificity is the foundation of designing DNA nanostructures. The key to building small is encoding the assembly information into the molecules themselves rather than using external forces to arrange them. Early successes often relied on these outside forces like atomic force microscopy or scanning tunneling microscopy to build structures molecule by molecule - approaches which cannot be easily scaled up to create large, complex structures (Shankland, 2009). The main advantage of DNA as a building material is that it can spontaneously self assemble, the sequence of nucleotides can be precisely controlled and the 3D structure is well understand (in contrast to the complexity of proteins).

DNA nanostructures fall into one of two categories: structural and dynamic. Static structures are fixed arrangements of DNA in specific shapes. A variety of strategies exist to do this, one of the most successful of which is DNA origami. Dynamic structures are formed similarly but are designed to move using techniques like strand displacement - this is essential for any sort of computational or mechanical functionality.

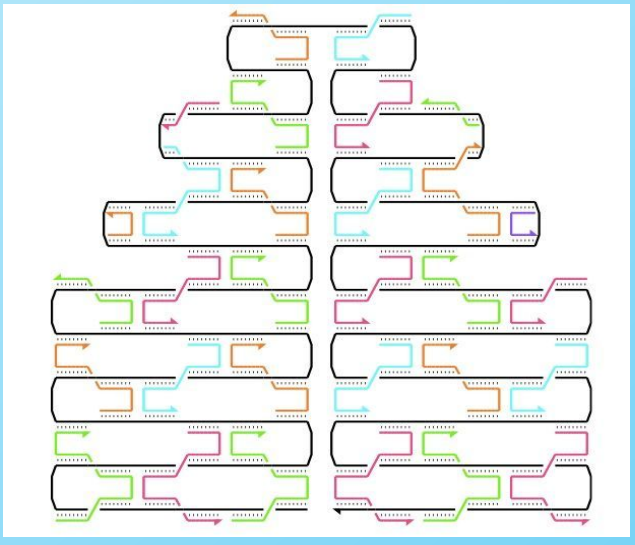
"Deoxyribonucleic acid (DNA) make for an extremely effective building material at the nanoscale"

In a 2006 Nature article, Paul Rothemund coined the fanciful term "DNA Origami" to describe his successful manipulation of DNA strands into a variety of shapes. He synthesized six different shapes including squares, triangles, and five-pointed stars consisted of flat lattices of DNA. His revolutionary technique utilized a single long "scaffold" strand of



DNA Origami can used used to fold long strands of DNA into a variety of shapes as seen in these computer models and electron microscope images (Rothemund 2006).

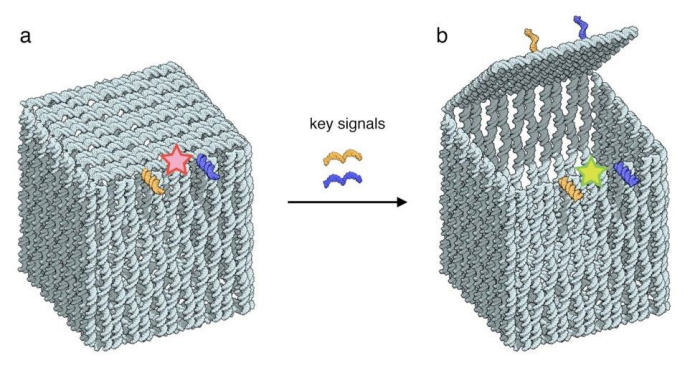
genomic DNA from a virus (7,000 nucleotides long), which was coiled, twisted, and stacked by small custom "staple" strands. The long single strand won't bind to itself, so a computer algorithm designs short strands complementary to certain regions - these are designed so as to maximize the connectivity and tightly hold everything together. Perhaps the most surprising part of the method is its simplicity – staple strands are mixed with the long strand and heated for 2 hours at 95C. The process is entirely spontaneous - strands join together to maximize complementary binding thus forming the correct structure. With the steadily falling cost of synthesizing custom DNA strands, this method is relatively inexpensive. Synthetic DNA strands have been available by mail order for the past 20 years - now most cost less than 10 cents per base (Carlson, 2009).



Staple strands bind to complementary sites on the long scaffold strand winding it back and forth into an energetically favored configuration (Rothemund 2006).

" squares, triangles, and five-pointed stars consisted of flat lattices of DNA"

Rothemund's second achievement was developing a method to pattern the 2-D shapes. He designed staple strands that would stick up from the flat lattice, increasing the height of the nanostructure at desired locations. The staple strand, normally in plane with the flat DNA lattice, contains "hairpins" - short regions that don't bind to the scaffold. The hairpin structures were clearly visible by atomic force microscopy. A world map as well as the word "DNA" were successfully created and visualized. These letters



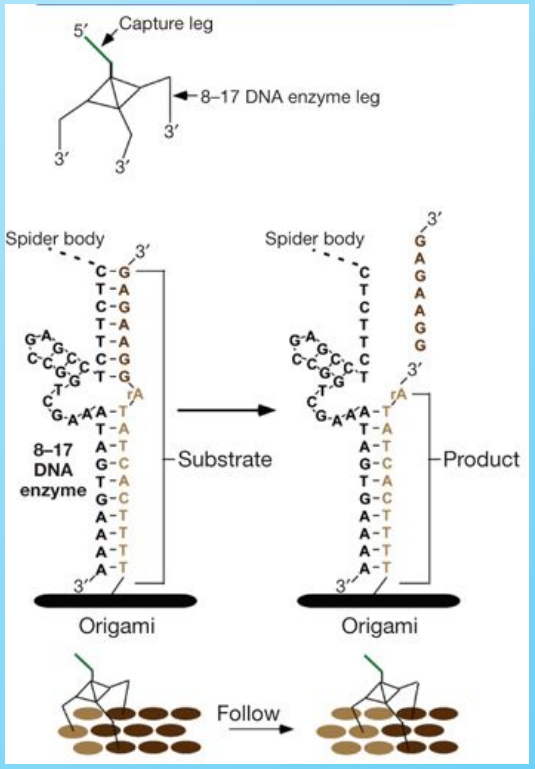
DNA boxes can be unlocked by key strands and have potential for drug delivery (Andersen 2009).

are about 30nm high - 6000 times smaller than the width of a human hair.

Rothemund's method has been extended to the construction of dynamic structures, for example, a hollow cube with a controllable lid composed of six sheets (Andersen, 2009). This technology has its main application in targeted drug delivery that concentrates therapeutics in some regions of the body relative to others and can do so in response to desired stimuli. The entire box is composed solely of a single long strand of DNA from the M13 bacteriophage and hundreds of staple strands. The lid is functionalized with a lock key system consisting of 2 strands of DNA - a mere 2.5nm wide. The lid is initially closed by these two nearly complementary strands, one attached to the lid and the other to the cube side wall. This system takes advantage of a method called toehold strand displacement to allow the box to open in response to the presence of a unique "key" oligonucleotide that displaces one of the lock strands. This key strand attaches to the toehold region initially and, having a better match than one of the complementary strands, replaces the other lock strand. The lid is now free to open. The lab detected the opening of the box by incorporating two fluorescent dyes into the faces of the box; when close together, fluorescence is increased through a process known as FRET - fluorescence resonance energy transfer. Therefore, when the box opens and the dyes are further apart, FRET decreases and is detectable via spectroscopy. Further experiments utilized two locks each with distinct keys. In order for the box to open, both keys had to be present. Further experiments demonstrated that the box could respond to complex combinations of strands and even cellular messenger RNAs. This opens the possibility for smart systems that could respond to

The above methodologies - DNA origami as well as dynamic strand displacement strategies, provide the foundation for more complex functional devices. Nanoscale machinery will require tiny moving parts to interact with and manipulate their environment. Moving machinery synthesized at the nanoscale is a daunting challenge; relatively simple molecules must move in desired paths. Scientists again looked to biology for inspiration. While cells might appear to be stationary and static, they are in fact buzzing with tiny protein machines. Motors, like the enzyme ATP synthetase or proteins that power flagellum, spin at up to 6,000 to 17,000 rpm (Rice, 1999). Other motor proteins include "walkers" like kinesin which transport payloads along cellular highways of microtubule filaments. Kinesin travels in a controlled, specific direction because the they only attach in one orientation dictated by the microtubules structure (Rice, 1999). Motor proteins like kinesin have inspired scientists to artificially create walkers using DNA nanotechnology (Lund, 2010). One of the biggest obstacles to an artificial walker is its simple molecular structure which prevents it from containing "programmed" instructions. Thus it must take its cues for movement from its environment, in this case,

"nanoscale robot- in this case termed "DNA spiders" which can walk across a flat sheet of DNA"



DNA walkers composed of enzymes can move along 2D sheets of DNA origami along paths containing substrate strands sticking up orthogonally (Lund 2010).

patterning of short strands sticking up from a 2D DNA origami sheet. Published in Nature in 2010, the paper describes nanoscale robot - in this case termed "DNA spiders" which can walk across a flat sheet of DNA in a complex pre-determined path. This is very comparable to creating a robot which moves forward and turns based on preprogrammed instructions - except about 109 times smaller.

The walker is actually not composed of DNA but rather proteins and enzymes that act on DNA. These include streptavidin (a protein often used as a connector between different proteins of interest) as the body of the spider and 3 deoxyribozyme "legs" each connected to the streptavidin. Using the DNA origami patterning techniques developed by Rothemund, surfaces are designed that layout paths for the spiders to follow. These are made up of characteristic strands that stick up perpendicular to the 2D surface. The legs of the spider bind to the short strands, cleaving them into two upon contact with the enzymatic leg. Each leg moves independently from one site to an accessible substrate site. Thus, the body of a spider at the interface between cleaved strands and fresh substrate (uncleaved strands) will move towards the substrate region. This amounts to the spider moving directionally along a track as the substrates are cleaved. In comparison to protein walkers, these are more predictable, programmable, and can interact with designed landscapes.

In conclusion, DNA has great potential beyond its biological role. Its capacity for information storage and self assembly makes DNA is a powerful tool for nanotechnology.

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