

44 DNA NANOROBOTS

Life's genetic blueprints can fold into macroscopic machines.

BY HAI-JUN SU AND CARLOS E. CASTRO

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The Rise of the **DNA** Nanorobots

When designed properly, DNA folds into tiny devices that move like macroscopic machines.



BY **HAI-JUN SU AND CARLOS E. CASTRO**

The patient's fever has reached 104, he's drenched with sweat, and he can barely talk, let alone move. The doctor orders a blood test. Soon the diagnosis is clear: malaria. But throughout the region, malaria parasites have acquired resistance to the drugs meant to kill them. The doctors turn to the last-ditch solution. They send in the nanorobots.

Throughout the patient's body, the tiny robots home in on red blood cells, binding only to those that are infected, ignoring the healthy blood cells nearby. Then, one by one, they punch holes in the membranes of the infected cells, injecting a powerful drug that dispatch the parasites within. Later the body breaks down the nanorobots themselves, which are made entirely of DNA, into harmless byproducts, and the body safely excretes them.

Such a vision may seem fantastical, but it isn't so far-fetched. Working together, our research groups have pioneered methods for designing and building nanometer-scale mechanical devices whose motions can be precisely controlled. Our work opens a door to nanometer-scale robots (nanorobots) that can sense, respond to, and manipulate their local environment.

Our devices, which we developed with support from the U.S. National Science Foundation, also accomplish a task that macroscopic machines cannot. Their molecular components self-assemble into a working device with no help at all, much as the molecular complexes inside living cells do.

We have just begun to tap the possibilities of these self-assembling DNA devices, but in the future DNA nanorobots may be able to control chemical reactions, sense how fast fluid is flowing, measure forces exerted by a specific molecule, or change their shape to perform different tasks. They may even be able to manipulate various molecules and synthetic nanoparticles to fabricate even more complex nanoscale devices, much as industrial robots construct circuit boards or automobiles.

To build DNA nanorobots, we combined our expertise. One of us (Su) had previously conducted research on kinematic theories for macroscopic mechanisms and robots. The other (Castro) had delved into the field of bioengineering known as

DNA origami, in which DNA—the same molecule that carries genetic information—is folded into useful structures.

Both of us arrived at Ohio State at roughly the same time, and early on we decided to collaborate. By then bioengineers had used the DNA origami technique to fold DNA into 2-D and 3-D structures such as a smiley face, a five-pointed star, a tetrahedron, and a nanometer-sized pore. But most of these devices were incapable of motion, and ones that moved did so in a simple or poorly controlled fashion. Our central goal in teaming up was to apply the principles of rigid-body kinematics to design DNA origami structures that could carry out familiar mechanical motions.

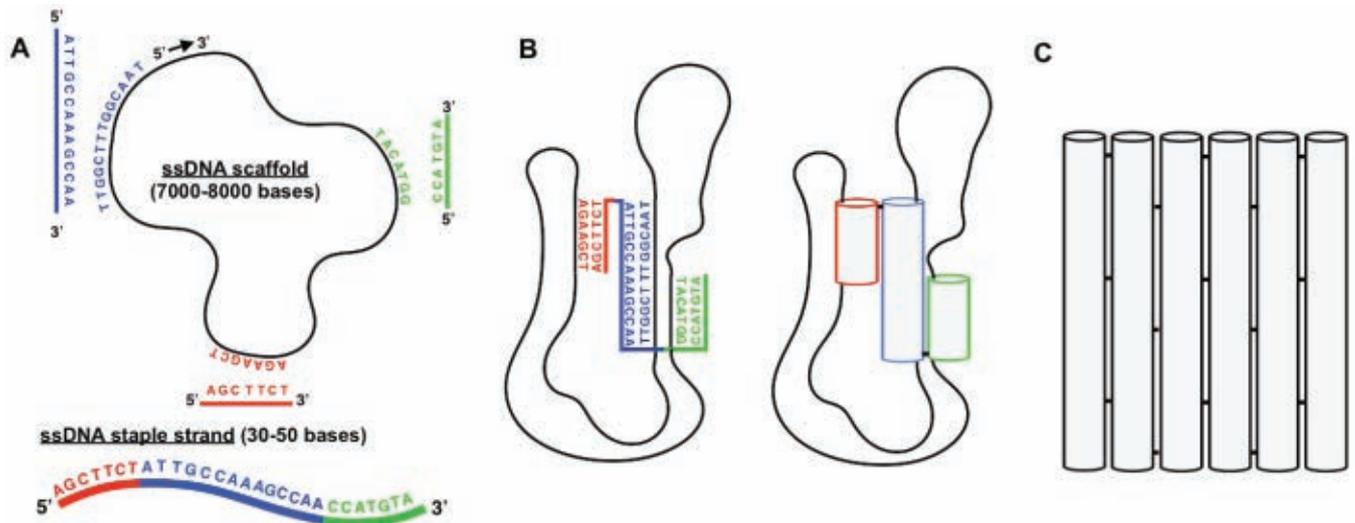
Folding DNA

Inside cells, DNA exists mostly as a twisting double-stranded ladder—the famous double helix discovered by American biologist James Watson and English physicist Francis Crick. But in the early 1980s, Nadrian (Ned) Seeman, a nanotechnologist and crystallographer at New York University, realized that DNA could also be induced to fold into more complex shapes. To do so, Seeman took advantage of two biochemical properties that allow DNA to encode genetic information.

First, each strand of DNA's double helix consists of a string of chemical building blocks called nucleotides. The four nucleotides that make up DNA contain components called bases that are often denoted as letters. (Specific sequences of these "letters" – adenine (A), thymidine (T), cytosine (C), and guanine (G) spell out genes, thereby encoding the myriad functions of living tissues.)

Second, when one strand of DNA encounters a second strand that matches it, the two zip together tightly to form a double helix. When the second strand does not match, according to rules

Building kinematic links with DNA origami



Short, custom-made DNA strands "staple" parts of a large DNA loop to each other (left and center), causing it to fold onto itself repeatedly to create a rigid link.

laid out by Watson and Crick, the two strands instead go their separate ways. The rules are simple: A's on one strand must pair with T's on the second strand, and C's must pair with G's. This allows scientists to design strands to either float freely or stick to each other and zip together.

Seeman had experimented with complex DNA structures such as a 4-arm junction that resembles the letter "X." In a moment of insight, he realized that giving DNA the correct sequence of nucleotides could program it to fold—on its own—into a specific nanoscale structure.

Seeman's insight and his follow-up work on the rational design and construction of DNA structures launched the field of DNA nanotechnology. Then, in 2006, the field underwent a quantum leap. That year Paul Rothemund of California Institute of Technology developed a new approach called scaffolded DNA origami. We have used this approach to build our mechanisms and machines.

Scaffolded DNA origami begins with a long loop of single-stranded DNA from a well-studied and harmless virus. This loop, called the scaffold, contains between 7200 and 8100 nucleotides.

As the DNA scaffold molecule wriggles and writhes in solution, it adopts many different con-

figurations. Some of these place distant scaffold sections in close proximity. When designed with the correct nucleotide sequence, these scaffold sections bind to each other, causing the scaffold to fold in on itself to a degree.

To fashion more intricate folds, however, we also need short snippets of single-stranded DNA called staple strands. We design these strands, which are 30-50 nucleotides long, with a nucleotide sequence that enables part of the strand to bind to one scaffold section and part of the strand to bind to another. This holds the two sections close, much as a metal staple holds together opposite ends of a folded paper.

Simply adding a staple strand drives the DNA scaffold, which is a large loop of DNA, to fold into a particular shape, and it holds it there. Additional staples drive subsequent folding steps, ultimately fixing the DNA into the desired 2-D or 3-D structure. The strategic folding employed gives DNA origami its name.

Researchers have built a large and growing variety of complex DNA origami structures, including nanotubes; nanopores; and templates for proteins, nanoparticles, small molecules, and carbon nanotubes. But so far few of them can move in controlled ways.

DNA on the Move

To design the motion of a robotic arm or other macroscopic mechanism, engineers use the principles of kinematics. We used the same principles to design DNA origami mechanisms.

Kinematic theory assumes infinitely rigid links and infinitely flexible joints. In practical terms, this means that the links must be far stiffer than the joints. Double-stranded DNA exists as a double helix, a structure that's about 20 times stiffer than a strand of single-stranded DNA. For that reason, we use double-stranded DNA for the rigid links and single-stranded DNA for the flexible joints. We do this by inducing the scaffold to fold into double stranded structures in places and leaving it single stranded in others. We also make the links even more rigid—a thousand times stiffer than single-stranded DNA—by combining DNA double helices into bundles.

If we design the scaffold and the staples with the correct base sequence, we can control which parts of the final DNA origami structure will be double-stranded and which will be single-stranded. In this way, we determine the location of the links and the joints.

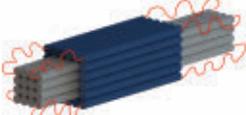
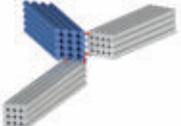
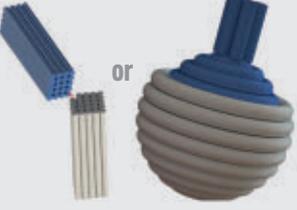
Recently we have developed

ways to build DNA origami mechanisms with a variety of complex shapes. We do this by controlling the length, cross-sectional dimensions, and shape of a link, and sometimes by designing links with corners, branch junctions, or curves.

We can also design joints and control their flexibility and degrees of freedom. Typically we use a very short stretch (2-4 nucleotides) of single-stranded DNA to make the joint flexible, and we arrange these flexible regions to constrain their movement. For example, aligning flexible connections forms an axis of rotation, much as aligning two separate hinges on the edge of a door keep the door vertical as it opens. Another example is a slider joint where a pipe-like tube slides back and forth along a solid cylinder.

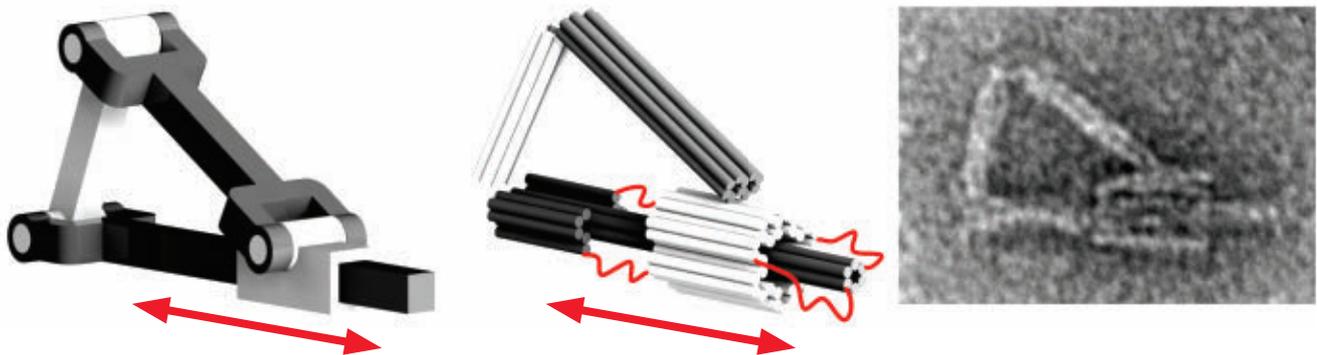
Over the past decade, bioengineers have trans-

From links to joints

Joint Type	Example DNA Origami Design	Macroscopic Machine Design
Revolute		
Prismatic		
Cylindrical		
Universal		
Spherical	 or 	

Using a method called scaffolded DNA origami, the authors programmed DNA to fold into five common types of joints, from a revolute (hinge) joint to a spherical (ball-and-socket) joint. Each joint consists of rigid links (blue and gray) with a characteristic bundle of DNA double helices, connected by very short, flexible joints consisting of single-stranded DNA (red).

DNA Origami Mechanisms



A crank-slider mechanism (left) converts the energy of a rotating wheel into a linear back-and-forth motion, or vice versa. The authors built a crank-slider mechanism from DNA (center) by combining DNA origami links and joints. They used electron microscopy (right) to visualize the tiny device and confirm that it actually moved.

formed the design of DNA scaffold origami from art to engineering. Now we can generate 2-D blueprints for DNA origami structures using an open-source computer-aided design program, caDNAno, then run those blueprints through simulation software called CANDO (Computer-Aided eNginneering for DNA Origami) to predict a 3-D folded structure.

We can also pull blueprints from catalogues of past DNA origami designs and modify them as needed, just as engineers do with CAD drawings. We can assemble components of a mechanism virtually as well. CANDO also lets us check for errors, and ensure we can fabricate the resulting molecule at high yields. All this reduces the need to perform experimental design iterations, which can be expensive and time-consuming.

To implement DNA origami mechanisms as nanorobots, we need to control their motion in real time. To this end, we recently adapted an actuation method that earlier DNA origami researchers have used to close DNA tweezers and perform other simple motions.

We built into our DNA origami mechanisms short sections of single-stranded DNA that each protrudes from the device like a sewn-on tag inside a coat. We distribute these tags throughout the structure. Then we add short snippets of single-stranded DNA we call “closing strands,” each of which binds tightly to two of these tags.

Binding two tags simultaneously pulls them together and drives the DNA origami device to move in a particular way. To reverse the motion, we add “opening strands”—single-stranded DNA snippets that lure the closing strands off the tags, releasing the device to reverse its motion.

To validate our design and fabrication strategy, we designed several joints with rotational and linear motion, including a hinge joint, a piston-like slider joint, and a universal joint like that in a car. We also combined these to form mechanisms with multiple degrees of freedom: a crank-slider mechanism that transforms rotational motion to linear motion or vice versa, and a Bennett four-bar linkage that folds up into a compact, closed bundle. Using transmission electron microscopy, we visualized the nanodevices in several conformations. As we’d hoped, their shapes matched the predictions of macroscopic kinematic theory closely, and actuation was reversible.

Toward a Working Nanorobot

Before we can combine our mechanisms into the type of nanorobot we envision, several technical challenges must be addressed. We’ve begun to tackle them, and we hope that other bioengineers and mechanical engineers will join us to advance the field.

New computational tools are needed to man-

age uncertainties in device self-assembly and in single-stranded DNA strand flexibility, both of which can alter a DNA device's kinematics. New computational and statistical tools are also needed to predict the final device's kinematics and dynamics.

Even fully folded DNA origami structures jostle and shift shape in solution, which makes it hard to control their motion. We've begun to address this challenge by building stiffer joints. To do so, we replaced single-stranded DNA, which is extremely flexible, with a few double-stranded DNA helices. These joints still deform by bending, much as a diving board bends and rebounds. We can then integrate these joints into what we call compliant DNA mechanisms. As a proof of concept, we designed a DNA origami compliant hinge joint and used it in a bistable four-bar nanomechanism that could execute the desired motion.

The design process for DNA origami mechanisms is still too cumbersome and error-prone, often requiring costly design iterations. New software that combines the capabilities of caDNano and CANDO would streamline the process, creating a CAD-like program that would allow mechanical engineers untrained in biology to design DNA origami parts and mechanisms.

We also need better methods to validate a design. Transmission electron microscopy and atomic force microscopy help visualize these nanoscale structures, but they generate 2-D images that make it difficult to determine the object's true

More to explore

NUCLEIC ACID JUNCTIONS AND LATTICES

Nadrian C. Seeman in *Journal of Theoretical Biology*, Vol. 99, pages 237-247, 1982.

PROGRAMMABLE MOTION OF DNA ORIGAMI MECHANISMS

Alexander E. Marras, et al. in *Proceedings of the National Academy of Sciences USA*, Vol. 112, No. 3, pages 713-8, January 20, 2015.

MECHANICAL DESIGN OF DNA NANOSTRUCTURES

Carlos E. Castro, et al. in *Nanoscale*, Vol. 7, pages 5913-21, published online Feb. 6, 2015.

DNA ORIGAMI COMPLIANT NANOSTRUCTURES WITH TUNABLE MECHANICAL PROPERTIES

Lifeng Zhou et al. in *ACS Nano*, Vol. 8, No. 1, pages 27-34, published online December 8, 2013.

DIRECT DESIGN OF AN ENERGY LANDSCAPE WITH BISTABLE DNA ORIGAMI MECHANISMS

Lifeng Zhou et al. in *Nano Letters*, Vol. 15, No. 1, pages 1815-1821, published online February 10, 2015.

structure. We've developed a computational approach called projection kinematics that uses these 2-D images to calculate the device's 3-D structure. But a method that determines 3-D kinematic parameters directly would be better.

In addition, actuation currently takes several minutes, which is too slow for many practical purposes. Faster methods of triggering motion may be possible by changing ionic conditions, temperature, light, or a magnetic field. We also need new computational and statistical tools to better predict a DNA origami device's kinematics and dynamics, and a cheap, fast and high-throughput fabrication process.

As we and other researchers clear these hurdles, engineers will have a design tool and manufacturing method to build DNA origami mechanisms and more complex nanodevices and nanorobots. We envision a nanoscale equivalent of a walking robot that can travel from one position to another, a robotic manipulator or Stewart-Gough six-axis platform to precisely position molecules for specific

tasks, and a mechanism similar to the crank-slider for injecting drugs into individual cells. But many other DNA devices are possible, and soon the field will be clear for practical applications of DNA origami mechanisms in sensing, nanomanufacturing, medicine—anywhere a controlled motion is needed at a nanometer scale.

At that point, in a clinic somewhere, a doctor may decide to send in the nanorobots. **ME**

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