

DNA Nanotechnology Grows Up

Once dismissed as molecular parlor tricks, techniques for piecing ultrasmall structures together with DNA are starting to prove their worth in serious research

SNOWBIRD, UTAH—All scientists face rejection when their proposals are dissected and their papers picked apart. Ned Seeman's worst slapdown came after what he considered at the time his greatest success. Seeman, an x-ray crystallographer at New York University (NYU) in New York City, had spent more than a decade working out the details of how to use DNA not as the master genetic control, but as a construction material for making molecular beams, joints, and girders that could be programmed to weld themselves together in molecular triangles, squares, and other simple shapes. Finally, in 1991, Seeman and colleagues at NYU managed to forge a DNA cube, the first three-dimensional (3D) nanoscale object in which the position of each atom was programmed, defined, and known.

Seeman submitted his manuscript to (ahem) *Science*. But it was promptly returned with a comment from a reviewer who, as Seeman recalls, asked, "Where is the biology?" Today, Seeman is regarded as the founder of the burgeoning field of DNA nanotechnology, in which researchers arrange DNA's four building blocks—molecules of adenine (A), guanine (G), cytosine (C), and thymine (T)—so that they assemble themselves into what-

ever structures the scientists want to build. After cubes, researchers in the field went on to build octahedra, rafts, smiley faces, and even a collection of strands that look like a Rock 'Em Sock 'Em Robot. Many critics are still unconvinced. "People always say, 'Yeah, that's cute. So what? What are you going to do with it?'" Seeman says. Or as Hendrik Dietz, a biophysicist at the Technical University of Munich in Germany, says he often hears it, "'It's an amusing but pointless exercise.'"

"Pointless" may seem harsh for an endeavor that represents nanotechnology's closest thing to building materials atom by atom from the ground up. But until now, the critics had a point: The field has searched for relevance.

No longer. DNA nanotechnology has left its childhood behind and entered adolescence. Like a teenager who clings to parts of childhood, some DNA nanotech continues to be playful and impractical. (The molecular robot boxer can't actually throw a punch.) But the field is also growing in strength and power. It's now churning out applications that are helping researchers map the atomic structure of proteins and compute inside cells (see sidebar, p. 1142), and soon may even start tracking and

Build up. DNA folding has constructed ever-more-complex objects, including a simple cube, two-dimensional faces and figures, and a 3D vase.

curing diseases. "We're still playing around," says Andrew Turberfield, a physicist at the University of Oxford in the United Kingdom. "But we've gotten good enough that we can do some interesting things."

Flying fish

Getting to this stage has been a slog. Thirty years ago, Seeman, then a young assistant professor, was struggling to make a mark in his field of protein crystallography, in which researchers interpret the way beams of x-rays bounce off copies of a protein aligned in a crystal to work out the molecule's structure. Many proteins rebuff efforts to force this order. And Seeman was struggling. "I was confronting this fatal progression of no crystals, no crystallography, no crystallographer," he recalls.

Seeman wondered whether DNA might help. DNA is most commonly thought of as a linear chain of A's, T's, G's, and C's. That's true, of course. But thanks to the propensity of individual nucleotide bases of DNA to pair with one another (T's with A's and G's with C's), DNA in cells forms a double helix, in which two strands of complementary nucleotides zip together. Seeman and others knew that under special circumstances, such as when DNA is copied during cell division, double-stranded DNA can unzip and begin binding to other strands, forming DNAs with branch points, not perfectly linear molecules. By tweaking the DNA's base pair sequence, researchers quickly realized that they could make artificial DNA branches with four arms, six arms, or more.

It was these branched DNAs that gave Seeman his eureka moment. In a tale that has risen to legend in the field, Seeman was drinking a beer in a bar in Albany when an

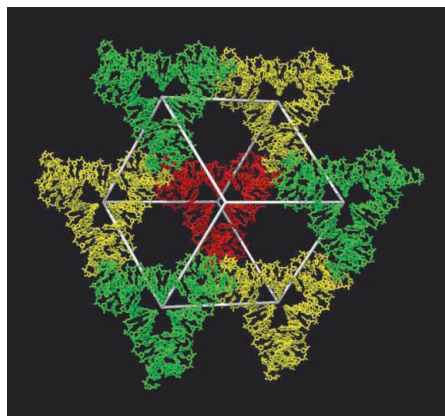
image of the M. C. Escher woodcut *Depth* popped into his head. The woodcut depicts dozens of flying fish soaring in formation, with head and tail, left and right fins, and top and bottom fins all oriented the same way. Seeman realized that artificial DNAs with six arms (front, back, up, down, left, and right) could be tailored to link up into a regular 3D cubic lattice with a large empty space at the center of each cube. And as a crystallographer, Seeman immediately envisioned that such an array could be used to trap copies of a single protein in the voids and get them to line up with the same orientation. In other words, he imagined a tool for determining the structure of virtually any protein at will.

In 1982, Seeman and colleagues laid out their ideas for creating such a lattice and other complex nanostructures in the *Journal of Theoretical Biology*. Actually making the structures was a lot harder. "It took a long time to figure out how to get these experiments to work," Seeman says. Among the challenges were learning how to outfit double-stranded DNAs with single-stranded tails that would link up with a complementary tail on another DNA fragment and how to make normally floppy DNAs rigid enough to form stable structures.

A series of advances cleared these and other hurdles. Over the next few years, Seeman's lab turned out triangles, squares, and other shapes. Then came the 1991 cube, and by 1998 Seeman's team had figured out how to assemble such parts into an extended two-dimensional array. Even then the field remained small, as building new structures required painstaking effort to design and synthesize all the component DNA strands. And synthesizers could churn out DNAs only hundreds of base pairs in length, a constraint that limited the complexity of the final structures.

That changed 4 years ago when Paul Rothemund, a chemist at the California Institute of Technology in Pasadena, and colleagues developed a technique called DNA origami. He and colleagues started with a 7000-base-pair viral genome for which the entire sequence was known.

Next, using a computer, they modeled how they would need to fold this single strand over and back upon itself to create a desired shape. Then they synthesized 250 short "staple" DNA strands designed to bind to sections of the DNA that ended up next to each other when folded, holding the structure in shape. Finally, the researchers added the staples to the viral genome, heated the brew, and cooled it down. Presto, the now iconic image of the DNA smiley face. "Seeing the



Molecular vise? A void in this DNA may hold proteins inside to map their atomic structure.

pictures, my jaw dropped," says William Shih, a DNA nanotech expert at Harvard Medical School (HMS) in Boston. "This was a game changer for the field." Adds Dietz: "With origami, it's gone 'whoosh!' to a completely different scale. Now we can make structures in the megadalton range with 16,000 base pairs where the position of every base pair is at a precisely registered position." And since Rothemund's achievement other groups have added versions of the technique to fold DNA origami in 3D, as well as computer-aided design programs able to automate the task.

Construction boom

These advances haven't quieted all of the critics. At a Foundations of Nanoscience meeting held here in April,* a prominent U.S. chemist who asked not to be named said that he still thought the field lacks widespread utility. But Shih and other proponents argue

that is beginning to change. "Some people see a DNA robot and say, 'You can build a robot,'" Shih says. "Some people see a robot and say, 'You can build anything.'"

Maybe not anything yet. But Seeman, for one, is now aiming to complete his original dream of using DNA nanostructures to help solve protein structures. In 2009, Seeman and a host of collaborators reported in *Nature* that they had stitched together a series of triangles into a rigid crystalline 3D lattice with rhombohedral voids. Then, using x-ray crystallography, they mapped out the structure of their crystal with a resolution of 4 angstroms. When he showed an image of the crystal at the meeting, Seeman exclaimed: "This is the most exciting slide I've ever shown. I know where every atom is."

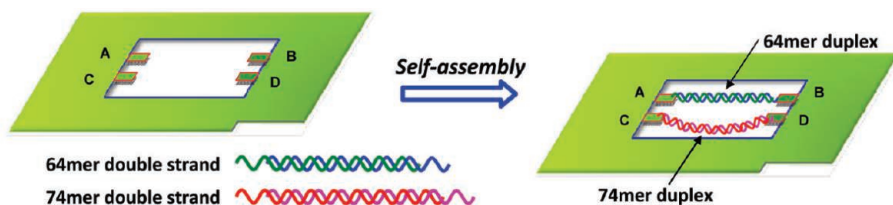
More or less, that is. Getting a picture of a molecule with a 4-angstrom resolution suggests that the arms are still wiggling around too much to determine the structure of copies of a protein held within. So Seeman's team is still working on getting its lattices to diffract at a higher resolution and on anchoring copies of a protein to be imaged inside each cell. If they succeed, they will be able to work out the shape of the protein by carrying out x-ray diffraction on the combination and then subtracting out the scaffolding holding the protein in place.

While Seeman's group tinkers with its protein scaffolds, researchers in other groups have been making progress with alternatives. At the meeting, for example, Shih reported that he and colleagues had for the first time used DNA nanotech tools to map the structure of a previously unsolved protein, using nuclear magnetic resonance (NMR) spectroscopy. The technique works by identifying the magnetic signature of atoms in proteins relative to their neighbors. By knowing each atom's neighbors, researchers can piece together the structure of an overall protein, in a process much like solving a complex jigsaw puzzle. But the technique works only for modest-sized proteins, for which sorting out the interactions between neighbors is manageable.

Tweaks to standard NMR have provided researchers with additional bits of information for helping solve their molecular jigsaws. In 1997, for example, researchers at the National Institute of Diabetes and Digestive and Kidney Diseases spiked a protein-containing NMR solution with a compound that spontaneously forms liquid crystals, mate-

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*8th Annual Conference on Foundations of Nanoscience, 11–15 April, Snowbird, Utah.



Freeze frame. A tiny reaction chamber built from DNA (green) enables chemists to watch how DNA-binding proteins interact with DNA strands of different length.

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rials that flow but have a regular molecular orientation like a crystal. They found that as the protein molecules tumbled around in solution and repeatedly banged into the walls of this soft crystalline material, they wound up spending ever-so-slightly-more time in one particular orientation than in others. The subtle preference biased the NMR results enough for the researchers to spot clues such as the angle between two atoms bonded in a protein. That information made it possible to solve structures for several proteins that reside in cell membranes and are nearly impossible to crystallize. A big drawback to the technique and later variations of it is that many cell-membrane proteins can stay in solution only with the help of detergents, which often tear apart the liquid crystals.

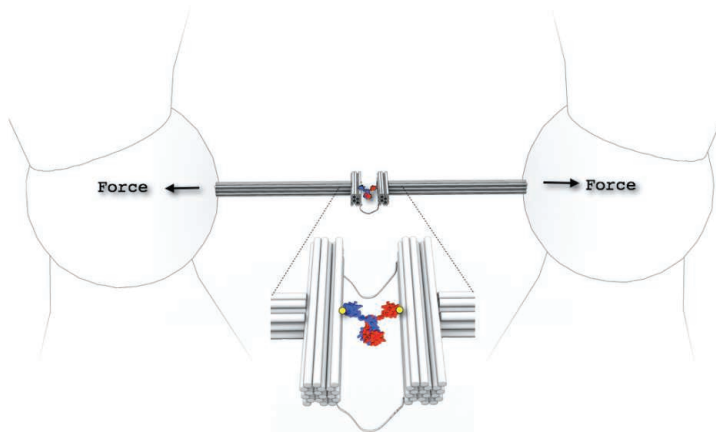
In 2007, Shih and colleagues replaced liquid crystals with origami-based DNA nanotubes that weren't affected by detergents. They showed they could solve the structure of a transmembrane protein domain of a T cell receptor. The structure had previously been determined by other methods, so it wasn't clear whether the technique could be used to solve unknown proteins. At the April meeting, Shih reported that he and his colleagues had used the origami nanotubes to solve the structure of a previously unsolved membrane protein known as UCP2, which helps govern insulin secretion in pancreatic cells.

That's not all. Turberfield's lab at Oxford reported online 10 January in *Nano Letters* that it had used related DNA nanotech tools to improve another protein structure determination technique called cryo-electron microscopy. In this case, Turberfield's team obtained high-resolution images of a hard-to-crystallize protein known as a G protein, the receptor that binds it, and the two paired together.

DNA nanotech's growth isn't limited to mapping proteins. At Kyoto University in Japan, chemical biologist Hiroshi Sugiyama has turned to DNA nanotechnology to help him watch protein catalysts carry out reactions in real time. Sugiyama and colleagues at Kyoto and the Japan Science and Technology Corp. in Tokyo reported online 15 January 2010 in the *Journal of the American Chemical Society* using DNA origami to stitch together what looks like a minuscule picture frame.

They then spanned the frame with two nearly identical double-stranded DNAs tailored to interact with a protein called *M.EcoRI*, which adds methyl groups to specific sites in DNA as a key part of cellular development.

M.EcoRI works by bending its target DNA by as much 59° in order to insert its methyl group. To see this feat in operation, Sugiyama and his colleagues engineered two DNA strands and stretched them across the frame. The first, 64 bases long, was taut; the other, with 74 nucleic acid bases, was floppier. They then watched the protein with a fast rastering atomic force microscope. *M.EcoRI* had a far easier time stuffing its methyl group into the longer, relaxed strand, suggesting that DNA's structure plays a big part in how it is modified. Seeman, for one,



Soft touch. Rigid DNA linkers (gray bars) gently pull apart plastic beads held by laser tweezers (left and right sides) to reveal how proteins stretched between them fold.

says he's impressed by the results: "It's a chemist's dream to watch individual reactions happen."

Biophysicists are also looking to DNA constructions to help them investigate molecules one at a time. At the meeting, Dietz reported that he and his colleagues are using DNA origami to improve a now-standard set of biophysics tools to see what happens to proteins and DNA as they are pulled apart. In the standard approach, researchers use lasers as molecular tweezers to trap plastic beads in a particular location in solution. Then they attach linkers to these beads and anchor a protein or other target molecule between the two linkers. By making slight adjustments to the lasers, researchers can pull the beads apart and bring them back together to see how the changes in tension affect a protein's ability to fold, among other properties.

But the technique has limitations. For starters, current linkers are floppy. This blurs the resolution of techniques used to track the captured protein. What's more, once the protein is pulled apart, the linkers drift away

from one another, so the experiment cannot be run in reverse or repeated. And in many cases, the floppy linkers must be yanked so hard to get the proteins to move that they pull the protein apart.

So Dietz and his colleagues replaced the usual floppy linkers with stiff rods made from DNA origami containing as many as 18 helical tubes each. They haven't tried them on a working protein yet. But initial tests have shown that the rigid DNA bundles are better at transferring force to their targets, making it possible to move the pinned molecules with less than half the applied force. That should make tracking the effect on proteins easier. The rigid linkers should also stay in place once an experiment is run, allowing it to be reversed. "We're excited," Dietz says. "If you can build on the scale of biological macromolecules, it opens up new areas of scientific exploration."

Someday, DNA nanotechnology may also push past basic science to find real-world applications. Shawn Douglas, a postdoc in the genetics technology lab of George Church at HMS, is working on what he calls a DNA origami nanorobot designed to seek out and destroy cancer cells. Douglas's "robot" looks more like a hollow cylinder some 60 nanometers long and 25 nanometers across. He built it from DNA origami and stapled it closed using DNA strands called aptamers, which in this case were designed to bind specifically to molecules specific to cancer cells. He then loaded the cylinders with fluorescent immune-system proteins that bind to cancer cells and induce apoptosis and added them to cancer cells in an in vitro assay. The loaded cylinders bound to their targets, released their cargo, and killed up to 40% of the cells. The work has a way to go before it will be ready for patients. Nevertheless, "it's beautiful data," says Tim Liedl, a condensed matter physicist at Ludwig Maximilian University in Munich, Germany. "It's getting us closer to the vision of something patrolling our own bloodstreams."

Certainly DNA nanotech has not reached that level of maturity. But Shih and others say it's growing up fast. "In the next 5 years, this is where we're going to make a real contribution, getting control over small collections of individual molecules," Shih says. By then the teenager may find itself a young adult.

—ROBERT F. SERVICE