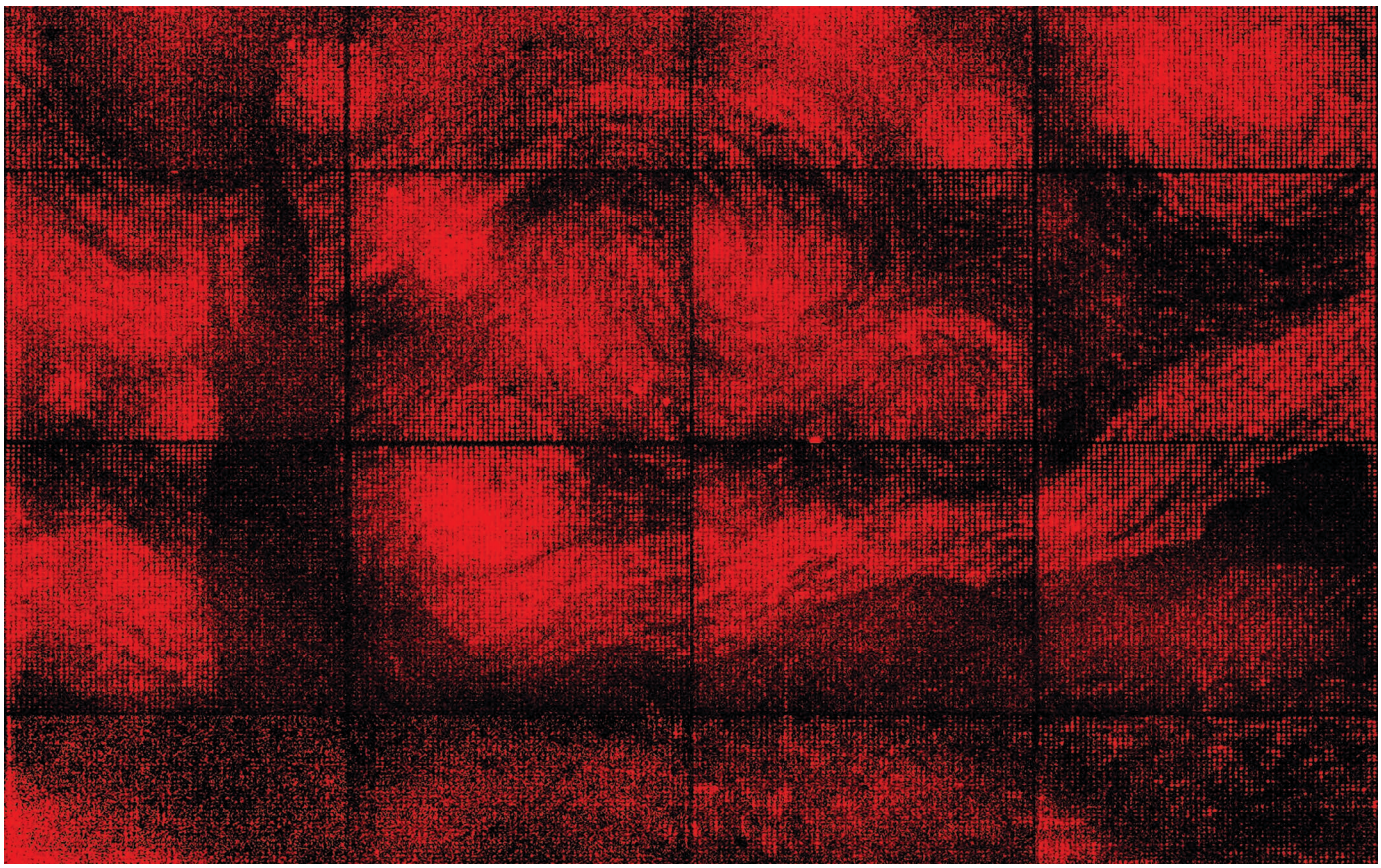
TECHNOLOGY FEATURE

THE ARCHITECTURE OF STRUCTURED DNA

Researchers are exploiting the structural properties of DNA to build nanoscale models for use in medicine and materials science.

ASHWIN GOPINATH/CALTECH



Van Gogh's *The Starry Night* recreated using DNA.

BY XIAOZHI LIM

Vincent van Gogh's *The Starry Night* is a classic of post-Impressionist art. Its whimsical whorls have entranced art lovers since the Dutch artist painted it in 1889. In 2016, Ashwin Gopinath, a bioengineer at the California Institute of Technology in Pasadena, recreated the work. But instead of oils, he drew his copy in DNA.

Drawn on a silicon chip, Gopinath's creation demonstrates the growing power of a once-obscure branch of materials science: DNA nanotechnology. The field emerged in the 1990s when scientists began to dream up nanoscale machines. Today, more than 300 research groups are trying to harness the base-pairing properties of DNA, with the goal of manipulating the molecule as if it were a building material, rather than a carrier of genetic information.

"Once we started to realize that you can use the information in DNA to organize stuff, it started a cascade of activity," says Ned Seeman, a synthetic chemist at New York University who is widely acknowledged to be the founder of DNA nanotechnology.

DNA's dimensions make it ideal for building nanostructures: the double helix is a flexible, configurable rod, 2 nanometres wide, with a twist that repeats every 3.4–3.6 nm. Researchers have exploited the well-characterized structure, and the ease of synthesizing custom DNA, to build ever-more-elaborate designs for applications from drug delivery and diagnostics to nanofabrication. But challenges remain, and nanotechnologists are rethinking the fundamentals of building with DNA.

CONSTRUCTION STRATEGIES

The collection of shapes assembled from DNA ranges from 2D smiley-faced emojis to 3D geometrical objects and blocks of alphabetic characters. But the underlying technology is based on one simple rule: base-pair complementarity. Driven by hydrogen bonds that ▶

pair the bases adenine and thymine, and cytosine and guanine, complementary DNA strands will spontaneously form a double helix. In nature, the two strands are usually fully complementary. If strands are only partially complementary, however, both can accept multiple DNA partners. This concept is the foundation of DNA nantotechnology, says Paul Rothemund, Gopinath's supervisor.

During cell division, DNA forms a four-armed intermediate structure known as a Holliday junction. The structure is unstable and disintegrates quickly into two double helices. In the early 1980s, Seeman managed to stabilize it[1] by pairing each strand's sequence with another at the junction. He went on to produce a junction with six strands, forming the first branched DNA structure in 3D. A series of increasingly complex designs followed: a stick cube in 1991, branched DNA crystals in 1998 and DNA tubes in 2005.

In 2004, William Shih, a biochemist now at the Wyss Institute for Biologically Inspired Engineering at Harvard University in Boston, Massachusetts, took a different approach. He formed a 22-nm-wide octahedron from just a single strand of DNA[2]. The 1,669-base DNA strand was held in shape using five 40-base strands.

Building on this idea, two years later, Rothemund used hundreds of 26- to 32-base segments of DNA that he called staples to guide the folding of a 7-kilobase 'scaffold' strand into a variety of 2D shapes roughly 100 nm in diameter[3]. This was "a landmark achievement", says DNA scientist Peng Yin, also at the Wyss Institute, because it greatly increased the complexity and size of DNA nanostructures.

Rothemund built his structures using the single-stranded DNA of a virus as a scaffold — the DNA required was too long for conventional oligonucleotide synthesis. He worked out how the DNA could be folded and where the 200 or so staples would need to attach to form shapes such as squares, triangles, stars and smiley faces. By mixing the DNA with a 100 times more staples than were needed, heating to 95 °C and cooling to room temperature over 2 hours, the shapes formed spontaneously on the basis of the instructions programmed into their sequences.

DNA 'origami' has come a long way since then. Initially, says Shawn Douglas, a biophysicist at the University of California, San Francisco, it could take an entire month to work out where the folds and staples go for just one design. "It was easy to make mistakes," he says, "and also hard to make modifications." This challenge inspired Douglas to develop software to accelerate origami design (see 'DNA origami'). The first working version of caDNAno was built in 2009, while Douglas was completing his PhD in Shih's group at Harvard. The software cut origami design to one day[4]. "In the next 3 months, we made 30 shapes," says Douglas, including protractors, twisted ribbons and an octahedral globe.

A couple of years later, another team, led by biophysicist Mark Bathe at the Massachusetts Institute of Technology in Cambridge, developed an ancillary tool called CanDo[5] to check the DNA origami blueprint from caDNAno. "It will tell you what it thinks the structure looks like in 3D," says Bathe. Bathe's group has since developed a tool called DAEDALUS that tells users all the sequences, including the scaffold, they need just by entering a desired geometry[6].

Another way to build with DNA is using DNA bricks. In 2012, while a postdoctoral fellow in Shih's lab, Yonggang Ke, a biochemist now at Georgia Institute of Technology and Emory University in Atlanta, developed a technique in which every brick in a DNA nanostructure has a unique sequence of 32 or 42 bases. A quarter of each sequence is complementary to another quarter on a different brick. By connecting and extending the bricks, researchers can assemble a canvas like building a brick wall. "Each brick can bind to two at the top and two at the bottom," Yin explains.

For a flat, 2D canvas, the bricks contain 10.5 bases per quarter, which allows them to connect to each other in a single plane; any 2D pattern can be prepared by simply picking the correct bricks. To add a third dimension, Ke shortened the bricks to eight bases per quarter, which forced them to connect perpendicularly. The researchers produced 102 distinct structures, including hearts, spheres and the Roman alphabet[7]. "In that first paper, we produced more 3D structures than the whole field combined," says Yin.

NANOFABRICATION APPLICATIONS

One use for these novel DNA shapes is to carry materials such as drug molecules, metal nanoparticles and proteins. Positioning these useful materials on the DNA is generally easiest before it is coaxed into a structure. The cargo is typically carried on the staple strands, and because each structure can include some 200 staples, says Rothemund, they offer plenty of opportunities to precisely place the molecular cargo.

DNA molecules are charged, which means that nanostructures can be arranged electrostatically by etching a pattern of negatively charged binding sites on a flat surface using an electron beam. "You can get them exactly where you want, oriented how you want," says Rothemund. This is just what his team demonstrated when it recreated *The Starry Night* from a dense array of photonic crystal cavities — micrometre-sized devices in which light can resonate — that contained meticulously placed DNA nanostructures carrying dyes[8].

"Once we realized you can use the information in DNA to organize stuff, it started a cascade of activity."

Another idea is to cast nanoparticles using DNA nanostructures as the mould. This requires fairly large and stiff DNA nanostructures with internal cavities. In collaboration with Bathe's team, Yin's group built such structures using DNA bricks. The teams then introduced silver nanoparticle seeds into the cavities, and allowed them to develop in the presence of soluble silver, like rock sugar growing in supersaturated solution. The seeds developed to fill the cavities, producing cubic, spherical, triangular and Y-shaped nanoparticles[9].

Chad Mirkin, a chemist at Northwestern University in Evanston, Illinois, is pursuing yet another nano-strategy, which he calls programmable atom equivalents. These nanoparticle cores can range from metals and polymers to proteins. Hundreds of partially double-stranded DNA molecules are attached to the core's surface to form a dense DNA shell. The single-stranded free ends are complementary to the free ends of other 'atom equivalents'. When those structures

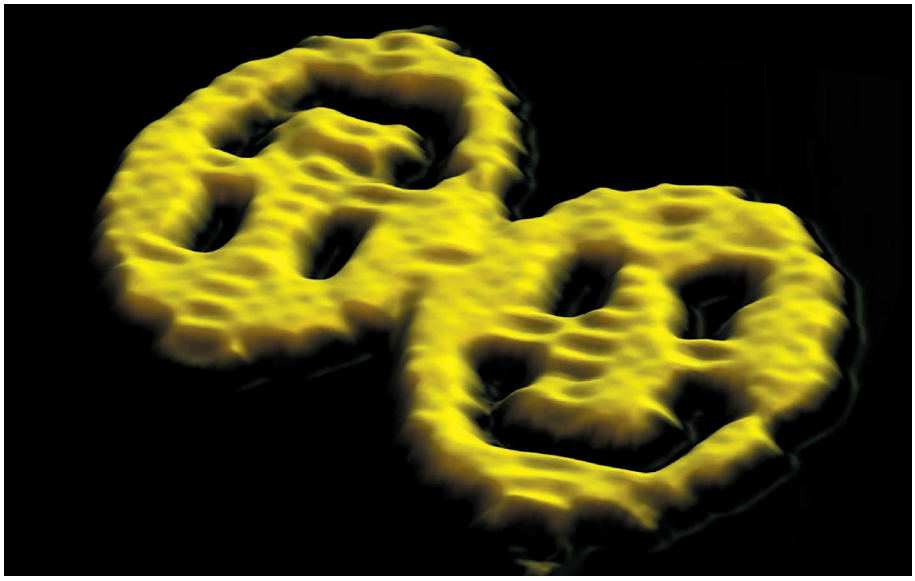
DNA origami

Folding DNA typically begins with choosing a scaffold. Single-stranded sequences of up to 200 bases can be synthesized relatively easily, but beyond that, it is simpler to use viral DNA.

Once a shape has been chosen, the software tools caDNAno or DAEDALUS can be used to help design the structure. caDNAno can map a preselected sequence in the desired shape, identify crossover points and generate the short strands of DNA or 'staples' required to fold it. With DAEDALUS, only the desired geometry is needed; the software generates the scaffold and staple sequences. The blueprints can be checked for accuracy using the tool CanDo, which predicts the 3D structure.

The strands are then mixed together in the right ratios (with an excess of staples), heated and cooled. Well-formed DNA nanostructures are seen on gel electrophoresis as sharp bands that are distinct from the starting material. They can be further characterized using electron microscopy or atomic force microscopy. If no band is present, the DNA either failed to fold or the yield was low. This could be due to a design mistake, especially at crossover points. If the design is correct, the folding conditions may need to be optimized by tuning parameters such as the buffer, temperature and reaction time.

For researchers who don't want to create their own DNA nanomaterials, Tilibit Nanosystems in Garching, Germany, supplies made-to-order structures and prefabricated structures and kits. X.L.



PAUL ROTHEMUND AND NICK PAPADAKIS/CALTECH

Smiley-faced emojis are one of the many shapes assembled from DNA.

are mixed together, they link up and extend into a crystal lattice that positions the desired atoms precisely in space. "This is an incredibly reliable method," says Mirkin.

Remarkably, the crystal's structure and properties can be controlled by varying the sizes and shapes of the nanoparticle cores, as well as the length of the DNA strands — no small achievement, given that crystallization processes are notoriously tricky. "We are trading ill-defined materials chemistry for well-defined and programmable DNA interactions to form high-quality crystals, and we can guide it down a path," says Mirkin, whose research group has churned out more than 40 crystal symmetries, 6 of which have never been observed in nature.

NANO CARGO

One popular adornment to nanostructured DNA is light-emitting materials called fluorophores. GATTAquant DNA Nanotechnologies in Braunschweig, Germany, for instance, makes nanorulers from DNA origami structures and fluorescent molecules to validate super-resolution microscopes. Super-resolution microscopy allows researchers to take images beyond the resolution limit set by the diffraction of light, but "there is no standard to measure the resolution of the system," says Max Scheible, head of research and development at GATTAquant. "DNA nanotechnology really enabled this."

GATTAquant attaches fluorescent molecules at precise distances on an origami structure and mounts them on glass slides. These nanoscale rulers allow researchers to verify the resolution of sub-diffraction-limit microscopes.

The co-founders of Ultivue, a start-up company in Cambridge, Massachusetts, are hoping to use nanostructures to make an impact in cancer research. In cancer tissues, biomarkers such as the proteins BRCA1 and HER2 can herald the onset or progression of disease, and can potentially aid diagnosis, prognosis and treatment. Until now, most biomarkers have been studied in isolation. "What's missing is a fingerprint" of biomarkers as they are seen in cancerous tissue, says Mael Manesse, lead researcher at Ultivue.

At Ultivue's headquarters, Manesse demonstrates the company's technology. Lit on the computer monitor are cells from a thin slice of lung tissue that Manesse has positioned under a microscope. When he switches the microscope's light to red, the cells disappear. In their place is a smattering of bright spots, indicating CD3 — a biomarker for immune cells called T cells. These proteins are marked with Ultivue's DNA-based imaging probe: a short 'docking' strand attached through an antibody, and its complementary 'imaging' strand carrying a fluorescent dye. Each biomarker of interest has its own docking strand; the complementary imaging strands can be added, imaged and removed one at a time. The images are then superimposed to obtain a composite picture of the tissue. This allows almost unlimited numbers of biomarkers to be studied, but the tissue sample remains preserved, says Manesse.

DNA nanostructures can also be used to build sensors, drugs and vaccines for therapeutic or diagnostic applications. For example, researchers have made a synthetic vaccine by anchoring the antigen streptavidin and oligonucleotides with an immune-response-boosting, repeating cytosine–guanine motif on tetrahedral DNA nanostructures[10]. In mouse studies, the vaccine produced higher levels of antibodies against streptavidin than a mixture of just streptavidin and oligonucleotides.

Eventually, Shih hopes to make drug nanofactories: DNA origami nanocapsules that can produce drugs on demand inside the body using building blocks from the cell. "It is very exploratory at this point," he says. In theory, the nanocapsules would hold RNA polymerase — an enzyme that makes RNA — and DNA templates. Once triggered, it would begin manufacturing and releasing its payload, like a virus using cellular materials to replicate itself.

DNA ALTERNATIVES

Although well into its third decade, DNA nanotechnology still faces a number of challenges. One key obstacle, says biophysicist Hendrik Dietz at the Technical University of Munich, Germany, is production yield: researchers have yet to break into gram-scale synthesis. "DNA origami can make a very big difference in health," Dietz says. "But the problem is we can't even make quantities that are big enough to use."

Another hurdle is the limited variety of materials that can be attached to DNA. Researchers are working to expand origami designs to use materials other than DNA. Earlier this year, for example, Dietz reported the preparation of DNA structures that fold using protein staples[11], and Douglas is updating caDNAno to include RNA and protein building blocks.

Perhaps the biggest limitation is the lack of control over the self-assembly process. As structures get larger, the chances of misfolding increase. "We need new strategies to suppress self-assembly errors," says Shih.

One possibility, Rothemund suggests, would be to move away from the standard *in vitro* method of mixing, heating and cooling, and allow cells to build the structures instead. Last year, bioengineer Christopher Voigt at the Massachusetts Institute of Technology engineered the bacterium *Escherichia coli* to produce a simple, branched, four-part junction from single-stranded DNA[12]. But for more-complex origami nanostructures, Rothemund says, a shift to RNA may be necessary. Unlike DNA, single-stranded RNA can hold its shape without staples. Building with RNA is largely uncharted territory, but Rothemund is excited to explore it. "It is like building with wood, but now you can't use nails or notches or glue," he says. "We still need to learn a lot of things." ■

XiaoZhi Lim *is a freelance writer in Natick, Massachusetts.*

1. Seeman, N. C. *J. Theor. Biol.* **99,** 237–247 (1982).
2. Shih, W. M., Quispe, J. D. & Joyce, G. F. *Nature* **427,** 618–621 (2004).
3. Rothemund, P. W. K. *Nature* **440,** 297–302 (2006).
4. Dietz, H., Douglas, S. M. & Shih, W. M. *Science* **325,** 725–730 (2009).
5. Castro, C. E. *et al. Nature Methods* **8,** 221–229 (2011).
6. Veneziano, R. *et al. Science* **352,** 1534 (2016).
7. Ke, Y., Ong, L. L., Shih, W. M. & Yin, P. *Science* **338,** 1177–1183 (2012).
8. Gopinath, A., Miyazono, E., Faraon, A. & Rothemund, P. W. K. *Nature* **535,** 401–405 (2016).
9. Sun, W. *et al. Science* **346,** 1258361 (2014).
10. Liu, X. *et al. Nano Lett.* **12,** 4254–4259 (2012).
11. Praetorius, F. & Dietz, H. *Science* **355,** eaam5488 (2017).
12. Elbaz, J., Yin, P. & Voigt, C. A. *Nature Commun.* **7,** 11179 (2016).

CORRECTION

The Technology Feature 'The architecture of structured DNA' (*Nature* **546,** 687–689; 2017) erroneously credited Paul Rothemund for designing the experiments. In fact, the work was done by his postdoc, Ashwin Gopinath. Furthermore, the examples of shapes created by Shawn Douglas should have been protractors, twisted ribbons and an octahedral globe.