

## Nanotechnology

## Magical materials

By Hubert Filser



Designer molecules: Size-dependent separation of DNA sequences by gel electrophoresis. *Source: Jan Greune*

**Honeycombs, crosses, bridges: Strands of DNA can be programmed to fold into such shapes spontaneously. LMU physicist Tim Liedl exploits this property to build 3D nanostructures for a variety of applications.**

The black box in Professor Tim Liedl's laboratory in the basement of the Physics Institute at LMU Munich looks quite unassuming. But for most people, what's going on inside it sounds like magic. Strands of DNA in aqueous solution are organizing themselves into complex three-dimensional nanostructures. Moreover, the form of the final structures has been precisely determined, in advance, by Liedl and his team. Tim Liedl is an acknowledged master of the art of designing DNA strands that will self-assemble into 3D structures such as honeycombs, crosses or bridges. The secret lies in the propensity of single DNA strands to fold into well-defined shapes determined by the sequences of their basic building blocks.

This method for the synthesis of nanostructures is known as DNA origami. Origami is the Japanese term for the art of creating 3D shapes by folding a sheet of paper according to a predetermined scheme. DNA strands fold up automatically, but the principle is essentially the same. "DNA molecules are actually very simple," says Liedl. Stable and robust, they are made up of a linear sequence of four types of subunit – the nucleotide bases adenine (A), cytosine (C), guanine (G) and thymine (T). What makes DNA special is that these bases interact in

specific ways: A pairs with T, and C with G. DNA strands with "complementary" base sequences therefore automatically "hybridize" to form duplexes, and this property can be utilized for the self-assembly of nanometer (nm)-scale structures.

In the early days, DNA nanotechnology, as it was then known, looked like children at play – serious-minded scientists putting little figures together. In 1991, Ned Seemann, the acknowledged father of the technology, constructed a cube with ~ 10-nm sides out of a few DNA strands that together were just a few hundred bases long. In 2004, biochemist William Shih at Harvard University designed and built an octahedron consisting of 1869 bases. In 2006, computer scientist Paul Rothemund at Caltech created a smiley face, using "scaffold" and "staple" strands, thus establishing the field of DNA origami.

### Extending the geometric possibilities of the DNA-Toolbox

At first, researchers indeed delighted in making playful forms like dolphins, the letters of the alphabet, crosses and other figures (not forgetting an outline map of America), but they were fully aware of the true potential of the new procedure – as a versatile tool for the creation of

components for the growing field of nanotechnology. So they concentrated on extending the geometric possibilities of their toolbox. Junctions allow one to make lattice structures, for instance, and Liedl played a large role in the developments that made 3D objects part of every origami engineer's standard repertoire. Among many other elements, he designed a Y-shaped "joint" that forms a triple junction, making it possible to produce linked hexagons and thus to generate larger lattices. In a further step, hexagons can be "stitched" together to obtain 3D footballs modeled after the fullerenes or "buckyballs" – molecules made up of hexagonal tiles of ordered carbon atoms. Thus the short history of DNA origami reflects the rapid development of the nanosciences.

"To generate structures, we exploit the physical properties of DNA, not its capacity to store genetic information," Liedl says. "Virtually any form can be constructed from mixtures of DNA strands that can interact with each other." With the support of a generous Starting Grant from the European Research Council, Liedl is applying the technique in a wide range of settings. He is building nanostructures that can confine and convert light energy into more useful forms, act as carriers to ferry drugs to their sites

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of action, or be used as optically active nanocomponents in photonic circuits.

Liedl proceeds as would an engineer: He constructs customized giant molecules with dimensions of up to a micrometer, which are nonetheless modifiable on the nanometer scale. He uses the single-stranded 7249-base genome of the bacterial virus M13 (M for Martinsried, where it was first isolated) as his basic scaffold. Up to 8640 bases can be packed into this virus particle, but much longer strands have also been used as scaffolds. The current record is 51,466 bases. Folding of the scaffold strand is directed by several hundred short staple strands, whose sequences are complementary to binding sites that have been preselected to yield the desired shape. As a rule of

thumb, staples must be at least 15 bases long, to ensure that they form stable double helices at room temperature and hybridize only to the correct site in the scaffold. However, the chance that a given 8-base sequence occurs more than once in a molecule the size of M13 is negligible.

### The nanoworld has its own set of rules

Lining up of the bases follows the classical lock-and-key principle, and works like the gears in a cuckoo-clock. Liedl adds the staple strands, together with M13, to a buffered salt solution at 80°C. The solution now contains billions of copies of each strand (with staples present in 10-fold excess), and its temperature is slowly reduced. This parameter determines how long it takes for partners to pair up, and longer strands hybridize at higher temperatures than short ones. Since the researchers know how fast the staple strands take up their predetermined positions in the geometrical shape, they can control the order of binding by regulating the temperature. Unless this step is properly timed, shorter strands may be unable to penetrate into the interior of the structure, because access has already been blocked, and the structure will remain incomplete. Assembly of these systems is often described in terms of attaching Lego bricks to one another, but the nanoworld has its own set of rules. The whole assembly process must be carefully planned. Only if the reaction conditions and all other parameters are just right can the desired structures emerge.

Using the electron microscope in the basement of the building, Liedl can check whether or not the single strands have assembled into the planned structures, such as tetrahedra, cubes, bridges

or tubes – billions of copies of the same object are generated in parallel. It may look easy, but it requires a fund of technical know-how. Liedl learned much of this know-how in William Shih's laboratory at Harvard. In 2007 it was clear to both that the new technology could open up previously unimagined opportunities. For no other method, not even electron-beam lithography, is capable of fabricating nanostructures with such precision.

Different researchers had very specific goals in view. Ned Seemann hoped to manufacture crystalline cages into which proteins could be incorporated for further study. Liedl and his colleagues (recalling physicist Richard Feynman's dictum: 'What I cannot create, I do not understand') wanted to build a cell with all its components. For what makes the strategy exciting is not so much the forms themselves as the possibilities they offer. The objects may look very simple, but virtually all provide sites for diverse binding interactions, and each interaction site is specifically addressable (via hybridization). That is the great advantage of DNA molecules. On a spherical scaffold, one can – in principle – assemble a virus or an artificial cell that is no bigger than the real thing, but is equipped with special functions and properties that one can alter in a targeted fashion and observe what happens.

### The so-called click-chemistry

Meanwhile, open-source programs for the design of novel objects have become available. One such is caDNAno, written by Shawn Douglas of the University of California in San Francisco. One can now delineate the desired structure using computer graphics and order the DNA sequences required for its realization from commercial sources. Liedl



Imaging architectures: Tim Liedl (standing) and his team use transmission electron microscopy (TEM) to view the DNA components they design.

Source: Jan Greune



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demonstrates how this works by sketching a simple 3D shape, an elongated honeycomb structure. In order to generate the correct spatial configuration, the correct distribution of sites of hybridization between two strands is extremely important. The “pitch” of the double helix, which accommodates 10.5 base pairs per 360° turn, must also be taken into account. “In the beginning,” (i.e. less than 10 years ago) “we had to design our sequences at the drawing-board, which was a pretty complicated operation,” says Liedl. Now the computer calculates which binding sites must be used, and prints a list of the sequences required at the end of the procedure. Specialized suppliers synthesize DNA sequences to order and dispatch them in microvials, each containing 1015 molecules of each sequence in a few microliters of fluid. “We order them by mail, and they arrive by post.”

As this infrastructure has grown, so has DNA origami’s range of applications. “Virtually anything can be linked to DNA,” says Liedl – via so-called click-chemistry. Given the appropriate choice of the geometry of the basic forms, docking sites can easily be added at the desired positions. The whole field can be compared to an enormous erector set – with nanoscale components. For example, a DNA strand with fluorescent molecules attached has been used as a kind of nano-

balance to measure the forces exerted by proteins. Two-dimensional checkerboards have been built to which specific molecules can be hooked at precisely defined positions, which allows one to reconstruct biological reaction networks.

### Fascinating optical properties

Liedl’s group is now experimenting with gold particles arranged into helical and ring-shaped patterns via targeted hybridization to helix bundles. These ordered arrays, whose elements are similar in size to the wavelength of light, exhibit fascinating optical properties – such as localized plasmon resonances – and are essential for the fabrication of “metamaterials” that have a negative refractive index. “Using arrays of nanoparticles set in predetermined patterns, we have observed optical effects that are unknown in nature,” says Liedl. One can also make use of the so-called chirality of molecules – the fact that attached groups can bind in different spatial orientations around a central atom, producing variants of the same compound that differ in their spatial structure – to manipulate light. Such “isomers” function as optical switches. Depending on their “handedness”, they interact differently with polarized light. “We have constructed the first origami structure that alters the plane of polarization of light in an orderly fashion,”

says Liedl, and that on a scale and with a degree of precision that classical lithography cannot match.

Medical applications are also in sight. “Nanostructures may well be used for specific purposes within the next few years,” says Liedl, as diagnostic tools for virus detection, as vehicles for targeted delivery of drugs or for the production of novel vaccines, for instance. “Perhaps it will even be possible someday to use DNA scaffolds to facilitate the manufacture of designer vaccines that induce blocking antibodies that can mask viral binding sites in cases of acute infection.” Origami-based structures would be ideal for such an application, as they can reach sizes comparable to those of viruses. “So they should be able to communicate with viruses or the human immune system.” Since Liedl’s research touches on so many different disciplines, including nanophysics, synthetic biology, cell biology and fundamental physics, he often receives inquiries from would-be collaborators in distant fields of research. “I often find myself being pulled in completely unexpected directions,” he says. And perhaps these encounters will lead to strategies that are so mind-boggling as to suggest that specialists in DNA origami are indeed endowed with magical powers.

Prof. Dr. Tim Liedl

Professor of Experimental Physics at LMU. Liedl (b. 1976), studied Physics at LMU, obtaining his PhD in 2007. Following a stint as a postdoctoral fellow in the Dana-Faber Cancer Institute at Harvard Medical School in Boston, he returned to Munich to take up his present position. In 2013 Liedl was awarded a highly endowed Starting Grant by the European Research Council (ERC).

