NANOTECHNOLOGY

The importance of being modular

DNA is the material of choice for making custom-designed, nanoscale shapes and patterns through self-assembly. A new technique revisits old ideas to enable the rapid prototyping of more than 100 such DNA shapes. SEE LETTER P.623

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Carpenters have been turning trees into furniture and dwellings for thousands of years, and so the discipline of woodworking features well-established techniques for joining pieces of wood to achieve a desired form. Nanotechnologists similarly try to use DNA as a material for crafting nanometre-scale shapes, but 'DNA-working' has been in development for a mere three decades. Because our picture of how DNA self-assembles is incomplete, DNA-working techniques are still evolving. The latest development is reported on page 623 of this issue, where Wei *et al.*[1] present a method whose intrinsic modularity enables arbitrary DNA shapes to be constructed with striking speed.

The practice of building nanoscale structures from DNA[2] once required creativity, intimate knowledge of DNA geometry and considerable synthetic effort. For example, in the late 1980s, the design and multistep synthesis of a 7-nanometre cube[3] from ten DNA strands, and its subsequent characterization, took about 2 years (N. C. Seeman, personal communication).

In 2006, DNA origami[4] emerged as a simple method that allows non-experts to rapidly design and synthesize complex DNA structures of approximately 100 nanometres in diameter, with reaction yields that often exceed 90%. In this technique, a single long strand of DNA is folded in one step by approximately 200 short DNA strands called staples, to create whatever shape is desired (Fig. 1a). In less than a week, one can accomplish all the steps required to make a DNA object: the computer-aided design and chemical synthesis of the staples; the formation of the object; and the final characterization of the product by atomic force microscopy (AFM). The original origami method[4] made only two-dimensional shapes, but was quickly extended to enable the construction of three-dimensional architectures[5,6] and curved geometries[7,8].

Because of its modularity, DNA origami provides a general platform for arranging other nanoscale objects — from electronic components to enzymes — as required. For example, DNA has been used to make a 'pegboard' onto which carbon nanotubes were organized into transistors[9]; a 'picture frame' into which individual DNA-repair enzymes were mounted so that their motion could be captured by AFM as they processed a substrate[10]; and a 'clamshell' that was programmed to respond to specific cancer cells *in vitro* by popping open to deliver a potentially therapeutic payload[11]. In each case, the pattern of components on top of the origami can be quickly and inexpensively reconfigured to perform a different task.

The modularity of DNA origami makes it a highly efficient technique for generating patterns, and its efficiency can be quantified. Let us consider how patterns might be added to a DNA origami rectangle. An experimenter can purchase a set of N staples (we'll call these 'white' staples) to fix the long strand into shape, and a second set of N 'black' staples that is identical to the first except that each staple carries a special chemical group. Each white staple, and equivalently the corresponding black staple, specifies a unique position in the final rectangle. By choosing the colour of the staple for each position, a set of just $2N$ strands can be used to create any of 2^N possible black and white patterns. If the chemical group on the black staples can act as a point of attachment for a small piece of a nanowire, for example, then any of 2^N possible patterns of wires can be made.

The most fundamental limitation of DNA origami is that this trick for obtaining an exponential increase in the number of patterns from a linear increase in the number of DNA strands does not generalize to shapes — for each new shape, one must design a new fold for the long strand and purchase another set of staples. Wei and colleagues' technique[1] dispenses with the long strand and so allows different shapes to be generated highly efficiently.

The authors' approach returns to a previously used paradigm for DNA-working, that of DNA tiles[12]. In their system, each tile is a single DNA strand with four different binding domains that specify which four other tiles can bind to it as neighbours. The authors' general scheme specifies a set of N tiles that self-assemble to form a rectangle, within which each tile adopts a particular position. By mixing together appropriate subsets of tiles and allowing them to self-assemble, arbitrary DNA shapes can be prepared (Fig. 1b; $N = 310$).

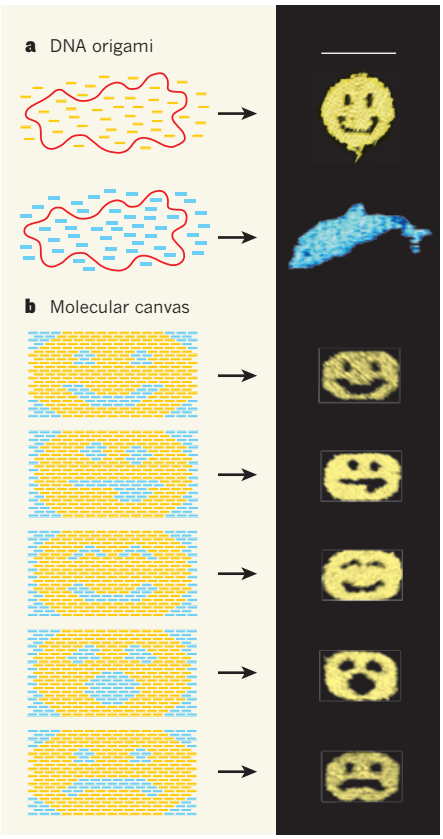

Figure 1 | More emoticons for your money. **a**, In DNA origami, a set of short DNA strands (known as staples) is used to fold a long strand into a shape. For each different shape, a new set of staples must be synthesized, at a cost of roughly US$1,000. Here, a set of 237 staples (yellow; not all are shown) folds a long DNA strand (red) to create a smiley face[4], whereas another completely different set of 204 staples (blue) creates a dolphin[14]. Scale bar, 100 nanometres; colours have been added to the micrographs of the DNA shapes on the right. **b**, In Wei and colleagues' approach[1] for making DNA shapes, a set of 310 different strands acts as a library of tiles that, when mixed together, self-assemble to form a rectangular molecular 'canvas'. To make any other shape, an appropriate subset of these strands is selected and mixed together — the tiles shown in yellow on the left are the subset that self-assembles into the corresponding emoticon at right. An extra set of 1,396 strands (not shown) is also required to seal the shapes' edges and prevent them from aggregating. A fixed set of 1,706 strands, costing roughly $7,000, can therefore make an astronomical $2^{310} = 2\times10^{93}$ potential shapes, such as the emoticons shown.

The DNA strands on the edges of each shape have free binding domains, which can cause the shapes to clump together. To render the edges non-sticky, the authors added edge-protector strands where necessary. Because each of the four domains on *N* different tiles might need to be protected, a set of 4*N* additional strands was required. So, to access any of 2^N potential shapes, the single-stranded tile technique requires just 5*N* different strands. This efficient and modular architecture allowed Wei *et al.* to construct 107 shapes by hand, spending just a few hours on each shape. By using a robot to select and mix strands, the authors reduced the time required to make a shape to one hour. In this way, they constructed 44 shapes in about 44 hours. This advance truly brings DNA nanotechnology into the rapid-prototyping age, and enables DNA shapes to be tailored to every experiment.

Wei and colleagues' technique is the large-scale realization of a concept known as uniquely addressed tiling, which was first formally described[13] 12 years ago. So why is this advance happening only now? One answer is that, according to the predominant thinking about DNA self-assembly, such a technique should not work well — making the concentrations of tile strands perfectly equal is experimentally difficult, and relatively small departures from equality were expected to result in low yields of target structures. This idea followed from the common assumption that many DNA structures would begin self-assembling simultaneously, and then get stuck as partially complete shapes when tiles present at lower concentrations were exhausted. This potential problem was so compelling that DNA origami was invented expressly to avoid it. But the yields of Wei and colleagues' structures are surprisingly high: up to 40% for some shapes.

The success of the method cries out for explanation. The authors suggest that, if the nucleation of self-assembly is rare and the subsequent growth of a DNA shape is fast, then complete structures will form in preference to partial ones. Another possibility is that more-complete structures can gain strands from less-complete ones through a mechanism called Ostwald ripening, in which strands fall off less-stable structures and rejoin more-stable ones. Wei and colleagues' choice of single DNA strands as tiles — rather than the more complex, multistranded tiles used previously[12] — could have a crucial role, because more-complete structures might steal single strands from less-complete structures directly, without any tiles falling off, by strand displacement.

More generally, both the single-stranded-tile method[1] and DNA origami violate several other previous intuitions about what should and should not work. In both cases, careful studies of yields, kinetics and mechanism will be required to circumscribe the conditions under which each method works best and determine whether the single-stranded tile method will supplant DNA origami in practical applications. Wei and colleagues' findings remind us that we are still just apprentice DNA carpenters, and will embolden others to mix hundreds of DNA strands together against prevailing wisdom. The results will probably surprise us. ■

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P.W.K.R. declares competing financial interests.
See go.nature.com/tntyxm for details.

REGENERATIVE MEDICINE

Reprogramming the injured heart

When the heart is injured, the muscle does not regenerate and scars are produced. This process can be attenuated in the hearts of live mice by forcing scar-forming cells to become muscle cells. SEE ARTICLES P.593 & P.599

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Cardiovascular disease remains the leading cause of death worldwide. Because of the heart's limited ability to regenerate, injuries such as myocardial infarction (heart attack) heal by scar formation rather than muscle regeneration. As a result, the heart pumps less efficiently, leading to the burgeoning epidemic of heart failure seen today. Current medical therapies support the heart with its reduced function, but scientists and clinicians are eager to learn how to regenerate damaged heart muscle. On pages 593 and 599 of this issue, Qian *et al.*[1] and Song *et al.*[2] describe how, in an effort to improve cardiac function, they have induced scar-forming cells (fibroblasts) to become muscle cells (cardiomyocytes) in the injured hearts of live mice.

The reprogramming of cells from one fate to another moved from the realm of alchemy to biochemistry after the discovery of MYOD1, a transcription factor that regulates the expression of genes involved in the development of skeletal muscle. When experimentally expressed, MYOD1 can convert many cell types into skeletal muscle *in vitro*[3], as well as cells in the injured hearts of live rats[4]. More recently, it was found[5] that somatic (non-germline) cells from adult mammals could be reprogrammed to become pluripotent stem cells — which can differentiate into any cell type — by expressing 'cocktails' of transcription factors. Researchers have recently used this approach to convert differentiated cells directly into other differentiated cell types such as cardiomyocytes[6–10].

Qian *et al.*[1] and Song *et al.*[2] built on previous work[6] which showed that fibroblasts could be reprogrammed into cardiomyocytes *in vitro* by the introduction of genes coding for three transcription factors that regulate heart development (GATA4, MEF2C and TBX5). Qian *et al.* used only these three genes, whereas Song *et al.* observed better *in vitro* reprogramming efficiency by adding a fourth one, which encodes the transcription factor HAND2. In both studies, the authors induced myocardial infarction in mice by occluding a coronary artery (a blood vessel that supplies blood to heart muscle), and used retroviruses to deliver the transcription-factor genes to the injured heart. These viruses can insert genes into the chromosomes of actively dividing cells, such as scar-forming fibroblasts, but not into those of non-dividing cells such as cardiomyocytes. One month after treatment, reprogrammed cardiomyocyte-like cells comprised 2.4–6.5% of the cardiomyocytes in the region bordering the injured area (the infarct border zone) in the study by Song *et al.* and, remarkably, up to 35%