NEWS & VIEWS

NANOSTRUCTURES

The manifold faces of DNA

Lloyd M. Smith

When it comes to making shapes out of DNA, the material is there, and its properties are understood. What was missing was a convincing, universal design scheme to allow our capabilities to unfold to the full.

As civilization has developed over the past 10,000 years, humankind has learned how to build larger and larger structures; over the past two decades, we have begun to learn how to build smaller and smaller structures. On page 297 of this issue[1], Paul Rothemund presents a material step forward in this second arena: he describes a stunningly simple and versatile approach to the fabrication, by self-assembly, of two-dimensional DNA nanostructures of arbitrary shape.

DNA has emerged over recent years as the molecule of choice for nanodesigners. There are two reasons for this. First, in the 50 years since the discovery of the DNA double helix[2], a detailed understanding of the energetics of its formation has developed[3]. This allows one to predict, with reasonable success, the shapes into which a DNA molecule of a given sequence will fold in solution[4]. Second, the advent of automated chemistry for the rapid synthesis of DNA molecules has made it possible to easily obtain DNA molecules of any desired sequence, of lengths up to 100 nucleotides or so. (A nucleotide is the monomer unit of DNA.)

Longer molecules may also be obtained using biological methods such as the polymerase chain reaction or now-standard molecular cloning approaches. The molecular engineer is thus armed with two of the basic elements needed to build structures of interest: the materials for building and an understanding of their properties. The missing ingredient has been a versatile design strategy.

The best-developed model for DNA design is the 'tile model' developed in Ned Seeman's laboratory[5]. This uses as its basic building-block DNA in two-dimensional, rectangular shapes. These tiles are designed to include crossover points between DNA strands, so imparting stiffness to the structure. Free single-stranded regions ('sticky ends' in the parlance of the molecular biologist) extrude from each corner of the tile and permit tiles to self-assemble into larger, two-dimensional sheets.

This basic concept has been adapted and extended many times, enabling demonstrations of, for instance, the templated self-assembly of protein arrays[6], and the fabrication of the fractal patterns known as Sierpinski triangles[7].

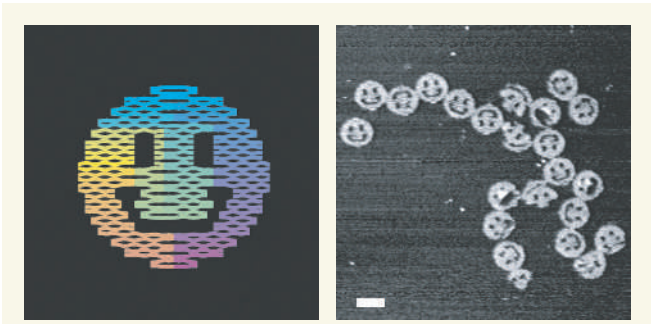

Figure 1 | Give us a smile. **a**, A folding path to make a DNA 'smiley'. An even number of double helices is filled into the desired shape in the order of colours in the spectrum from red (start) to violet (end). **b**, An atomic force microscopy image of the finished product. (From Fig. 2 on page 299; scale bar, 100 nm.)

A second design theme was introduced by William Shih and colleagues[8] in 2004. They showed that a single strand of DNA, 1,669 nucleotides long, could be driven to self-assemble into a nanoscale octahedron by the addition of five short DNA strands complementary to selected regions of the original strand. Again, structural rigidity was obtained by means of crossover points. This work was notable in two respects. First, it permitted assembly of a three-dimensional structure, rather than the two-dimensional sheets that had been the primary focus of most previous work. Second, it introduced the concept of using short DNA strands to direct the folding of a longer DNA strand.

Rothemund[1] builds upon both these models, and expands their generality to permit the fabrication in DNA of any two-dimensional shape. Just as in the approach described by Shih *et al.*[8], he directs the folding of a long single-stranded DNA molecule, in this case the genome of the widely used cloning vector M13 mp18. M13 is a bacteria-destroying virus with a single-stranded DNA genome about 7,000 nucleotides in length; its known sequence and ready availability make it convenient for this application.

The design process[1] has five steps (see Fig. 1 on page 298). First, the desired shape is chosen and is filled from top to bottom by an even number of parallel double helices. Second, a single, long scaffold strand is folded back and forth along the double helices, so introducing periodic crossovers (again for rigidity) between parallel helices. Third, a computer program generates the sequences of many short 'staple strands'. These bind to the DNA scaffold strand — making it double-stranded — and create crossovers between strands. In the two final steps, the design is examined and refined by computer to relieve strain and to strengthen the structure at the nicks and seams produced in the initial design process.

The results that emerge are stunning. Rothemund shows the generality of the approach with six different structures (Fig. 2 on page 299), notably a five-pointed star and a smiley face — myriads of which are a disconcerting sight in an atomic force microscopy image (Fig. 1, above). He further demonstrates the assembly of the individual structures into rather beautiful, higher-order patterns, reminiscent of the designs found in Persian carpets, and shows the absence of any symmetry requirement in the designs by fabricating a map of the world (Fig. 3g on page 300).

Rothemund's basic method is fairly straightforward, and ample experimental and design details are provided in the many pages of supplementary material. He notes that there is a plethora of widely available chemical modifications to DNA strands; this should make it possible to incorporate, for example, dyes or binding elements into these structures at any desired position. The extension of this model from two to three dimensions should not prove too difficult either, given the three-dimensional precedents[8], opening up further possibilities in the design and construction of functional materials at the nanoscale.

Thus equipped not only with DNA building

materials and an understanding of their structural and chemical properties, but also with a versatile general approach to weaving them together[1], we are arriving at a new frontier in our pursuit of ever-smaller structures. The barrier we have to surmount next is to deploy our knowledge to develop structures and devices that are really useful. Happily, in that endeavour we are now perhaps limited more by our imagination than by our ability. ■

Lloyd M. Smith is in the Department of Chemistry, University of Wisconsin, 1101 University Avenue, Madison, Wisconsin 53706, USA.
e-mail: smith@chem.wisc.edu

1. Rothemund, P. W. K. *Nature* **440,** 297–302 (2006).
2. Watson, J. D. & Crick, F. H. *Nature* **171,** 737–738 (1953).
3. Breslauer, K. J., Frank, R., Blocker, H. & Marky, L. A. *Proc. Natl Acad. Sci. USA* **83,** 3746–3750 (1986).
4. Zuker, M. *Nucleic Acids Res.* **31,** 3406–3415 (2003).
5. Seeman, N. C. *Nature* **421,** 427–431 (2003).
6. Yan, H., Park, S. H., Finkelstein, G., Reif, J. H. & LaBean, T. H. *Science* **301,** 1882–1884 (2003).
7. Rothemund, P., Papadakis, N. & Winfree, E. *PLoS Biol.* **2,** e424 (2004).
8. Shih, W., Quispe, J. & Joyce, G. *Nature* **427,** 618–621 (2004).

ALZHEIMER'S DISEASE

A needle from the haystack

Richard Morris and Lennart Mucke

Abnormal protein clumps of many varieties build up in the brains of individuals with Alzheimer's disease. But which types actually cause memory deficits? The behaviour of model mice might help to find out.

A huge array of neurodegenerative diseases seems to be caused by abnormal clusters of certain proteins. Many of these disorders are on the rise and cannot be treated effectively, including the most frequent among them — Alzheimer's disease[1]. To understand and treat these conditions better, much more needs to be learned about the structures and activities of the disease-causing protein assemblies. In this issue, Karen Ashe and her colleagues (Lesne *et al.*, page 352)[2] describe the isolation of a specific protein assembly from mouse brains that may help to explain some of the memory loss seen in Alzheimer's disease. Remarkably, they applied a behavioural screen to select the assemblies that were functionally most relevant.

The key component of Lesne and colleagues' protein assembly is the amyloid-β peptide (Aβ), which is widely believed to have a causal role in Alzheimer's disease. Compelling support for this Aβ hypothesis comes from studies indicating that most, if not all, genetic causes and risk factors for Alzheimer's increase the cerebral accumulation of Aβ (ref. 3), and from a variety of experimental models in which Aβ impairs neural functions[4,5]. However, it is still uncertain how Aβ accumulation may lead to the relentless cognitive decline that characterizes the disease[4,6].

A major source of confusion has been the propensity of Aβ to exist in a variety of complexes that seem to differ not only in their number of peptide building-blocks, but also in their overall conformation and biological activity (Fig. 1). In increasing order of complexity, Aβ can exist as monomers (a single peptide unit each), dimers (pairs), trimers (trios), oligomers (many units), tiny transient structures known as protofibrils, larger stable fibrils, and highly compacted admixtures of fibrils and smaller aggregates (amyloid plaques). To make matters worse, these different assemblies can be bound to a variety of other structures within the complex molecular and cellular environment of the brain. It is therefore not surprising that it has taken a while to determine which assembly is most closely related to cognitive decline.

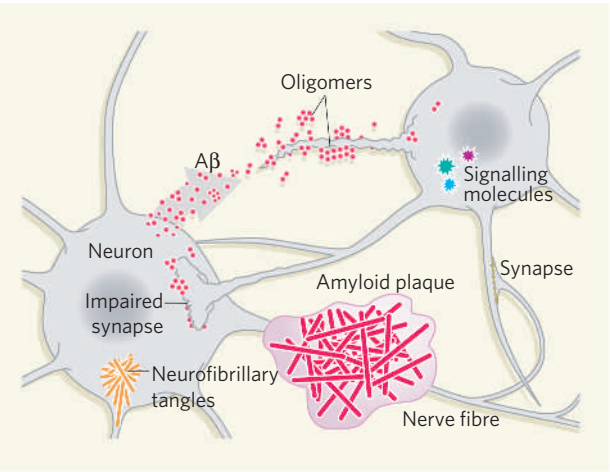

Figure 1 | The role of amyloid-β (Aβ) in Alzheimer's disease. Aβ peptides self-aggregate and form increasingly larger assemblies that accumulate in the brain. The largest of these assemblies (amyloid plaques), a hallmark of Alzheimer's disease, displace and distort neuronal branches. Smaller Aβ assemblies (oligomers) have been much harder to detect in brain tissues but may be even more detrimental than plaques. Lesne *et al.*[2] show that Aβ*56 is among these stealthy moieties, and seems to have a key role in memory decline. Independent of neurofibrillary tangles — another pathological hallmark of the human condition — Aβ oligomers may impair the junctions through which brain cells communicate (the synapses) or alter neurotransmission by other mechanisms. This would deplete signalling molecules that are dependent on synaptic activity and are required for memory and other brain functions.

Building on their own studies and pioneering work of other groups[4,6], Lesne *et al.* went on a biochemical hunt for this Aβ assembly in a mouse model for Alzheimer's disease. Although it is, of course, impossible to recapitulate all aspects of this complex human disease in a mouse, mice genetically engineered to express human amyloid precursor protein (APP) and the APP-derived Aβ peptides in their neurons develop a highly informative array of Alzheimer-like alterations, including cerebral amyloid plaques, distortions of neuronal branches, impairments of synapses (the junctions between neurons) and deficits in learning and memory[5].

Rather than work all the way back from end-state disease to altered physiology, the authors used the onset of early memory deficits in APP mice as an instructive guide in their detection of the Aβ assemblies that are most likely to impair neuronal function. Spatial memory impairments in these animals occurred in discrete stages. APP mice remembered as well as normal control animals when they were young. But at 6 months of age, there was a partial decline in memory to a point that then remained stable for 7–8 months. This was followed by a further memory decline at around 15 months. Lesne and colleagues used this graded pattern of forgetfulness as their route to trapping the molecular culprit.

They combined their behavioural studies with a detailed biochemical analysis of the Aβ assemblies found in the brains of the animals. Previous studies had already shown that plaque load and overall Aβ content are poor predictors of cognitive failure[4,5,7]. A series of tissue fractionation and protein purification steps allowed the researchers to zero in on a specific, functionally relevant Aβ assembly, which they termed Aβ*56. This name arose because the assembly reacted with anti-Aβ antibodies, tandem mass spectrometry showed it contained Aβ sequence, and it had an apparent relative molecular mass of 56,000 in gel electrophoresis. The authors provide evidence that Aβ*56 forms outside cells and that it may be a cluster of 12 Aβ peptides — although the exact origin and atomic structure of this assembly remain to be resolved, and it may be that it has additional constituents other than Aβ.

The early memory deficits in APP mice, starting at 6 months, were negatively correlated with the level of Aβ*56 found in their brains. In more direct support of a causal relationship, an Aβ*56-containing isolate from APP mouse