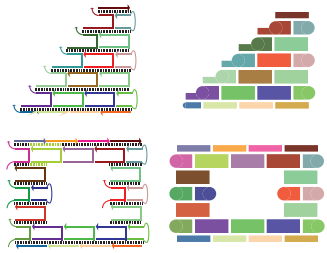
MOLECULAR ENGINEERING

DNA nanoLEGOlogy

Researchers design a simple 'shake & bake' method for the assembly of complex nanostructures from interlocking DNA tiles.



Designer nanoscale shapes self-assemble from short DNA strands. Reprinted from *Nature*.

DNA's high chemical stability and programmable base-paired structure make it not only an ideal carrier of genetic information but also a promising material for engineering sophisticated nanodevices. Peng Yin and colleagues from the Wyss Institute at Harvard recently reported a robust bottom-up approach for the assembly of complex nanostructures in a single melting-annealing cycle from a mix of short synthetic DNAs.

The idea to use DNA as a scaffold in molecular engineering was conceived about 30 years ago when, inspired by M.C. Escher's woodwork 'Depth', crystallographer Ned Seeman reasoned that interlocking branched DNAs could be used as tiles to build carrier lattices for crystallization of target molecules. The concept launched the field of DNA nanotechnology and led to the first reports on the assembly of basic geometric shapes, tubes and lattices from DNA tiles. Nevertheless, complex nanoshapes remained largely out of reach, with experts sharing the intuition that imprecise ratios among the building tiles would lead to jammed assembly.

In 2006, a different concept, DNA origami, emerged as a robust solution for programmed self-assembly of bigger and more complex nanostructures. Rather than using individual tiles, the method relies on folding a large continuous DNA 'scaffold' into a desired shape, guided by multiple short synthetic DNA 'staples'. As the staples do not interact with each other, careful adjustment of input ratios is not required. Versatility in shape design, however, remained

EPIGENETICS

SORTING OUT EPIGENETIC STATES

A nanofluidic device can sort single DNA molecules based on their epigenetic marks.

Epigenetic marks do not act alone. DNA and histone modifications can be grouped into epigenetic 'states' that affect gene expression and cell identity in both normal development and disease. The hunt for these states led the engineering group of Harold Craighead and the epigenetic team of Paul Soloway at Cornell University into a creative collaboration. Their brainchild: a nanofluidic device that can identify and sort single chromatin fragments based on the combination of marks that they carry.

Robust methods to locate various marks in the genome already exist. Chromatin immunoprecipitation detects histone modifications, and bisulfite sequencing identifies DNA methylation. So why the need for a new tool? Epigenetic states are derived by overlaying maps of these marks, but comparing data from different cell populations can obscure how marks are coordinated within each cell. "One thing that you can never resolve from superimposition," notes Soloway, "is whether those epigenetic marks that are identified on a given gene are really present at the very same time, on the same molecule."

Although chromatin immunoprecipitation can be performed in successive rounds or combined with bisulfite sequencing on a single sample, either option requires a lot of starting material (typically more than 1,000 cells per round), whereas nanofluidics can characterize multiple marks directly from a single cell.

Soloway likens nanofluidics to fluorescence-activated cell sorting (FACS), except that nanofluidics uses voltage gradients to propel molecules rather than using pressure to propel cells. Chromatin stained with affinity fluorescent probes is loaded and threads like a noodle through a 250-nm-deep channel. Lasers excite the passing fragments prior to a branch point, and sensitive photodiodes detect the emission of single fluorophores thanks to the tiny interrogation volume of the channel. Signal is processed in real time by on-board circuitry, which can be programmed to score fluorophore combinations and actuate a voltage switch that sends DNA to the positive output channel.

limited because each new shape requires a redesigned scaffold and set of DNA staples.

To overcome this, Yin and colleagues discarded the long scaffold altogether and revisited the original DNA-tile approach in a new form of molecular masonry. "Traditionally, a DNA tile is assembled from several DNA molecules and has a structurally rigid core that displays several different sticky ends," explains Yin. "As the core does not encode information, we got rid of it and concatenated the remaining sticky ends into a single-stranded DNA tile."

The result is a 42-base-long fusion of four sticky ends, each forming a full turn in the DNA helix. Upon annealing, each helical turn hybridizes with a complementary segment in each of four other strands to form a LEGO-like mosaic of interlocked rectangular tiles acting as pixels. The size and shape of the growing pattern can be changed by simply omitting relevant pixels and sealing any exposed edges with polythymidine 'protector' tiles.

Using this rationale, the researchers designed a master pick-and-mix library of approximately 1,700 DNA tiles sufficient to assemble a virtually inexhaustible pool of 100-nanometer shapes. Surprisingly, almost all of the more than 100 tested shapes—including tubes, letters, symbols, Chinese characters and emoticons comparable to those achieved by DNA origami—showed consistent self-assembly with significant yields and without careful adjustment of tile ratios, suggesting sparse and specific nucleation followed by rapid completion of the programmed structures.

"The most important aspect of this new development is to show the huge design space for DNA nanostructures," says Yin. DNA origami assemblies have already proven useful as platforms for the arrangement of other molecules such as fluorophores, proteins or inorganic materials, but the new method has an additional advantage: whereas the long scaffold in origami is typically phage derived, Yin's approach relies entirely on short synthetic strands. This allows incorporation of modified bases, RNA or L-DNA, further increasing potential applications.

Petya V Krasteva

RESEARCH PAPERS

Wei, B. *et al.* Complex shapes self-assembled from single-stranded DNA tiles. *Nature* **485**, 623–626 (2012).

The engineering was tricky, but the groups achieved a sorting rate of more than 500 molecules per minute, with a low (1–2%) incidence of false positives. They improved accuracy by observing output channels as well as the input and tweaking the sorting parameters. Traditional FACS operates at higher speeds, but the tiny nanodevice can be multiplexed to greatly increase throughput.

The researchers demonstrated the ability to sort plasmids, either naked or bearing methyl groups, using a DNA dye and labeled MBD1 protein that binds methylated DNA. They enriched at levels similar to FACS, but missed up to 25% of positive molecules. To lower the false-negative rate, they have now optimized MBD1 binding conditions and are exploring brighter probes.

The technology is still at the proof-of-principle stage, but it has intriguing potential. One attraction of chip-based approaches is their easy integration. Any version of a Lilliputian factory can be dreamed up, conceivably including DNA extraction, probe binding, sorting and library preparation for sequencing, all on the same factory floor. Profiling single cells may also address the provocative question of how epigenetic states are propagated through cell division, or during differentiation or reprogramming. Soloway wants to profile rare and hard-to-culture cells such as mouse primordial germ cells.

The groups are working on directly sorting chromatin, adjusting optics to add a third laser, optimizing probes and attempting unbiased amplification of the mere femtograms of sorted output to allow sequencing. Soloway hopes that commercialization will offer a route for technical improvements like mutliplexing to ramp up throughput.

Ultimately, the device could be used to profile the effect of epigenome-modifying drugs. "This tool," says Soloway, "could provide a very effective means to quite rapidly screen for efficacy of different compounds and query the effective compounds on multiplicities of epigenetic states."

Tal Nawy

RESEARCH PAPERS

Cipriany, B.R. *et al.* Real-time analysis and selection of methylated DNA by fluorescence-activated single molecule sorting in a nanofluidic channel. *Proc. Natl. Acad. Sci. USA* **109**, 8477–8482 (2012).