DNA NANOTECHNOLOGY

Nanoscale cable tacking

Synthetic DNA-labelled polymers can be made to self-assemble on two- and three-dimensional DNA scaffolds in custom routings.

Hendrik Dietz

In the enduring quest to miniaturize electronics, self-assembled DNA-based template nanostructures have been considered as a potential method to create complex three-dimensional electronic circuitry on scales that are not accessible by conventional top-down lithography. DNA is, however, an insulator, and it is therefore necessary to functionalize DNA carrier structures with a conducting component. Writing in *Nature Nanotechnology*, Kurt Gothelf, Mingdong Dong and colleagues[1] have now shown that conducting polymers can be tacked in user-defined paths onto custom DNA-based scaffolds (Fig. 1). The approach provides a compelling route to feature-rich circuitry on the nanoscale, but the ability to bring a polymer into a desired shape could also be of value in other areas, such as light harvesting or light emission.

The researchers — who are based at Aarhus University, Harvard University, Harvard Medical School, the Max Planck Institute of Biochemistry and Ludwig-Maximilians-Universität — used a polymer based on polyphenylene vinylene (PPV). It is a homopolymer that exhibits electroluminescence and is used in organic light diodes, photovoltaics, lasers, and in the displays of devices such as smartphones. Polyphenylene vinylene features a conjugated π-electron system and can be regarded as a one-dimensional semiconductor. I remember fondly a lab class during my undergraduate studies that involved building a simple organic light-emitting diode using PPV as the active medium. I smeared (or, in fact, spin-coated) a PPV film onto a transparent indium tin oxide electrode and placed a metal contact on the other side. The diode functioned to a degree, despite my clumsy handiwork. However, much of the art of making a good (that is, one that has high efficiency) organic light-absorbing or light-emitting cell has to do with organizing or structuring the polymer film in highly controlled ways. Homopolymers are the same from beginning to end and tend to coil up. They are, as a result, generally difficult to organize.

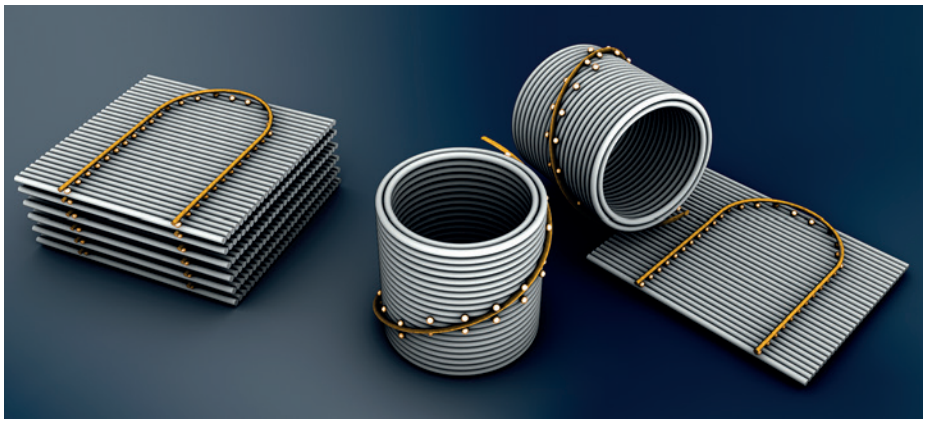

Figure 1 | Routing polymers on DNA scaffolds. Schematic illustration of DNA origami objects on which DNA-labelled PPV molecules can be self-assembled through DNA strand hybridization. The grey cylinders denote the DNA double-helical domains and the gold wires denote the individually routed polymers. Glowing spheres denote the tack positions, as realized by double-helical DNA domains formed by hybridization of complementary DNA strands protruding from the DNA support structures and from the polymers. The stack of rectangles on the left illustrates how complex three-dimensional wiring architectures might be achieved in the future by preparing two-dimensional sheets each with custom cable routings, and performing a layer-by-layer assembly to bring the sheets into contact.

Gothelf and colleagues have developed an attractive strategy for organizing single polymers by using custom DNA origami[2] scaffolds to template the shape individual polymers will adopt. To tack the polymer onto the DNA scaffolds, they synthesized a PPV variant in which single-stranded, 9-base-long DNA tails protrude laterally from the polymer backbone; the synthetic protocol they developed may be generally applicable to polymers that can be prepared with hydroxyl-containing side chains and that are compatible with automated DNA synthesis.

The products of the synthesis were analysed with a range of methods including gel permeation chromatography (GPC), UV–vis and fluorescence spectroscopy, X-ray photoelectron spectroscopy and atomic force microscopy (AFM). The GPC analysis indicated that the size of the polymer molecules ranged from around 340 kDa to around 3,300 kDa. Imaging with AFM revealed polymers with lengths from 20 nm to 200 nm, corresponding to molecules with 30 to 300 repeat units, respectively. In the AFM images, the polymers appeared as flattened ribbons with widths around 10–15 nm and appeared to have persistence lengths of between 100 and 200 nm. The polymers are thus softer than carbon nanotubes, but are stiffer than DNA double-helices, which have a persistence length of 50 nm. The comparably high stiffness is a little surprising when considering the apparently flexible structure of the polymer backbone. Electrostatic repulsion between the like-charged DNA tails along the backbone may stiffen the polymer. In this case the flexibility should depend on the salinity of the solution. An alternative explanation is that the researchers may have occasionally looked at bundles of polymers, rather than individual polymers, where the bundles are stabilized laterally through the DNA 'velcro' tails.

For the programmable tacking of the polymer, Gothelf and colleagues designed multiple two-dimensional DNA origami scaffolds and also a curved

three-dimensional origami scaffold. Each scaffold featured custom patterns of single-stranded DNA tails that were complementary to the 9-base strands on the polymer. The two-dimensional rectangular scaffold, or 'breadboard', was endowed with tails that created tacking points for several one-dimensional paths, including a line, an L-shape, a U-shape, a wave, a staircase, and a circle. Direct imaging with AFM revealed that the DNA-labelled PPV polymer self-assembled successfully onto all of these patterns, but the yield of assembly decreased with increased degree of curvature of the target path, presumably due to the energetic penalty of creating bends on paths with high curvature such as the wave-like or staircase paths. The three-dimensional scaffold had a cylindrical shape, with a height of 100 nm and a diameter of 60 nm. Again, single-stranded DNA tacking points were included at custom positions and were used to template a 360° right-handed helical path for the polymer. To reveal the correct assembly of the polymer into the helical path, the team used a modality of super-resolution fluorescence microscopy called DNA PAINT.

The high-density modification of the PPV polymer variant with DNA labels may affect its physical properties such as its electroluminescence. Although Gothelf and colleagues did not directly study these properties, they do provide data that suggest that the polymer retains some of its interesting physical properties. For example, surface potential measurements with electrostatic force microscopy indicated that the capability of charge transfer at the interface of polymer/DNA is significantly better than for DNA alone. Furthermore, when the researchers attached a single Alexa 647 acceptor dye molecule on one of the origami staple strands that binds to the polymer on the two-dimensional DNA rectangle with the U-like shape, a 20× enhancement of the emission of the acceptor was observed, which may be a consequence of near-field enhancement produced by the conjugated polymer.

For future use in electronics or other applications, several challenges will have to be addressed. Stacking multiple modified DNA origami scaffolds in a layer-by-layer fashion[3], each with its particular polymer routing and potentially including other active components, may be a way to create three-dimensional integrated nanoscale circuits (Fig. 1, left). Important progress has been made in placing DNA objects with user-defined long-range order onto solid-state surfaces[4], which is a necessary step for integrating such components into a device context. But will DNA nanostructures retain their shape when taken out of solution and into a dry electronics environment? Some type of petrification protocol may need to be developed. Furthermore, Gothelf and colleagues note that degradation of the polymer by cleavage into shorter pieces occurred during purification of their synthesis products, and therefore it was not possible to prepare polymers with monodispersity. This finding hints at an increased fragility of the DNA-modified PPV variant, and this will be an important issue to resolve if applications are to emerge. ❐

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BIOSENSORS

Microcantilevers to lift biomolecules

Nanomechanical sensors can now detect femtomolar concentrations of analytes within minutes without the need to passivate the underlying cantilever surface.

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Archimedes, the Greek mathematician, is once believed to have said "Give me a lever long enough and a place to stand, I will lift the earth." Today, this famous quote could be paraphrased as 'Give me a lever small enough, and I will 'weigh' molecules.' Microscale versions of Archimedes levers, called microcantilevers, have been used in several forms to lift or strain molecular-scale structures by invoking nanomechanical signal transduction. Specific binding of analytes to their complementary partners on the cantilever surface induces lateral surface stress, resulting in bending of the free end of the cantilever. The sensitivity of the assay is measured by how tiny a bend one can detect using lasers, whereas the selectivity is governed by specificity of the complementary partner for the analyte. Microcantilevers have been used to study various biological interactions[1–6], for quantifying antibiotics in blood serum[7] and for detecting bacterial resistance to antibiotics[8] with excellent specificity and sensitivity. However, cantilevers are limited by non-specific binding at the bottom surface, which may negate the specific binding that occurs at the top surface. Passivation may resolve these issues but it requires tedious optimization. Writing in *Nature Nanotechnology*, Joseph Ndieyira and colleagues at University College London, Imperial College London and Jomo Kenyatta University of Agriculture and Technology now show that by controlling the concentration of the receptor footprint on the cantilever surface, it is possible to overcome competing stresses from opposing cantilever surfaces[9]. This approach allows direct capture of molecules at femtomolar concentrations within minutes without the need to passivate the underlying cantilever surface.

Human immunodeficiency virus (HIV) mutates very easily resulting in many different strains even within the body of a single infected person. Several methods have been proposed for the detection of HIV but they are typically complicated and not amenable to point-of-care diagnostics. Ndieyira and co-workers used microcantilevers to nanomechanically detect HIV. The cantilevers were functionalized with single-domain antibodies that are specific to HIV. Binding of HIV to the antibodies on the cantilever induces a lateral surface stress and this causes the cantilever to bend. The assay was shown to reliably detect HIV down to about 500 fM, which is