DNA NANOTECHNOLOGY

A cascade of activity

Enzymatic reactions can be coupled together by carefully organizing the enzymes on DNA scaffolds.

Chenxiang Lin and Hao Yan

Nature has evolved a myriad of enzymes to catalyse the chemical reactions that are vital to the metabolism and reproduction of living systems. Different enzymes often work together, sometimes in a cascade fashion. Inspired by the natural world, scientists have been building synthetic molecular scaffolds to copy nature and, ideally, exercise some degree of control over the catalytic activities of enzymes. On page 249 of this issue, Itamar Willner and colleagues from the Hebrew University of Jerusalem take advantage of the organizational power of DNA to activate a cascade of biocatalytic reactions[1].

DNA nanotechnology uses DNA as a generic material to build rigid two- and three-dimensional architectures with molecular binding sites at designated positions that can organize inorganic and biological macromolecules with nanometre precision[2,3]. These self-assembled DNA-based nanostructures allow researchers to study how molecular interactions depend on the distance between the molecules[4]. A number of proteins have been organized on DNA scaffolds in this way, but researchers did not know how the scaffolds affected the biological function of proteins, or if it was possible to build multi-enzyme complexes on these scaffolds.

A pilot step was taken by Christof Niemeyer, then at the University of Bremen, and co-workers in 2002 when they assembled two enzymes — NAD(P)H:FMN oxidoreductase (which reduces flavin mononucleotide (FMN) to FMNH2 by using nicotinamide adenine dinucleotide (NADH) as an electron donor) and luciferase — that catalyse consecutive reaction steps, into a spatially well-defined structure using single-stranded DNA as a guide[5]. They found the overall catalytic activity of the two enzymes to be significantly higher when they were assembled in close proximity on a DNA template, compared with a random distribution on a surface. The work was a proof-of-concept demonstration that an artificial multi-enzyme complex can work on a DNA scaffold.

Willner and co-workers have now attached glucose oxidase and horseradish peroxidase to self-assembled DNA scaffolds — which are shaped like ladders and can be either two or four units wide — and studied cascade reactions catalysed by the two enzymes (Fig. 1a). Each type of enzyme was first covalently linked to a unique anchoring strand of DNA that bound to the scaffold at specific, pre-determined locations. The reduction–oxidation cascade reaction that could not be catalysed in a diffusion-controlled solution of the two enzymes (and the corresponding substrates) was found to be effectively activated when the enzymes were assembled on the DNA scaffold. Furthermore, the bi-enzyme complexes assembled on the 'two-ring' scaffolds showed higher activity than those assembled on 'four-ring' scaffolds (Fig. 1b), owing to the closer proximity of the two enzyme components.

The research team was also able to assemble an enzyme (glucose dehydrogenase) and its cofactor (a non-protein compound required by an enzyme to carry out a reaction, in this case nicotinamide adenine dinucleotide) on the 'two-ring' scaffold. They used a series of anchoring strands with different lengths to immobilize the cofactor, and found that longer anchoring strands bridged the gap between the enzyme and cofactor, allowing them to communicate and generate higher catalytic activity.

These experiments provide an excellent example of programmable DNA-directed assembly of multi-component enzymatic complexes. Moreover, the assembly system is modular and could therefore be adapted for the construction of other functional molecular complexes.

A number of challenges need to be met to achieve more complex and programmable

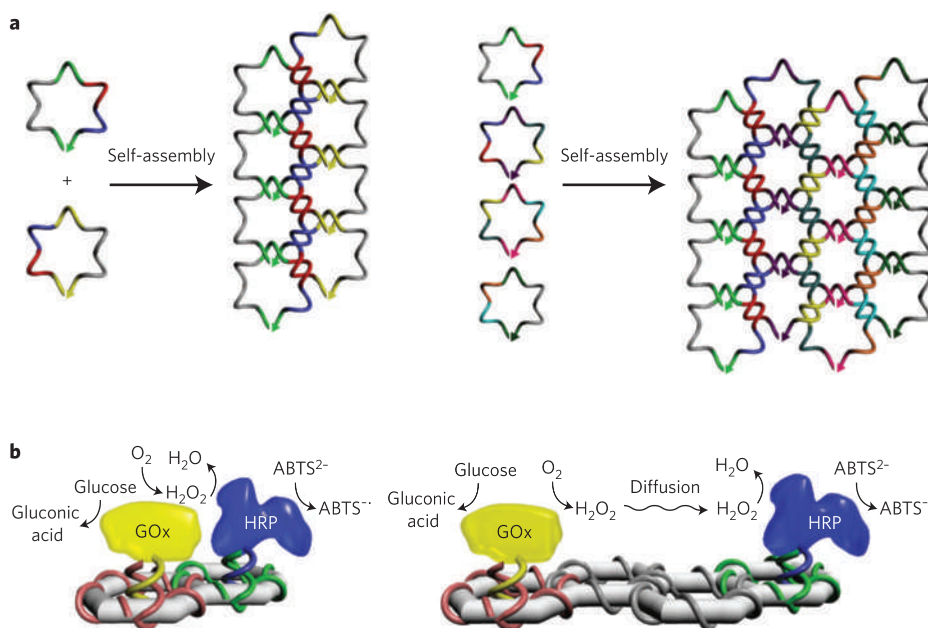

Figure 1 | Biocatalytic cascades activated on programmable DNA scaffolds. **a**, The self-assembly of a ladder-like DNA lattice that can be programmed to be two (left) or four (right) strands of DNA wide. DNA segments with the same colour are complementary to each other, and the 3′ end of each strand is represented by a closed triangle. **b**, Enzyme complexes are assembled as a result of hybridization between the anchoring strands (light green and blue) that are linked to the enzymes and the capturing sites (red and dark green) on the scaffold. Closer enzyme proximity resulted in faster reaction kinetics. For clarity, only one repeating unit of the DNA lattice is shown. GOx, glucose oxidase; HRP, horseradish peroxidase; ABTS, 2′,2′-azino-bis[3-ethylbenzthiazoline-6-sulphonic acid].

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enzyme cascades. First, a one-to-one DNA-protein conjugation technique should be adopted to ensure better control over the spatial orientation of the proteins and minimal loss of their biological function after conjugation and immobilization. The 'click chemistry' conjugation method reported by Distefano's group[6] seems to be a promising candidate for this purpose. Second, although DNA-directed assembly has been intensively investigated in two dimensions, expanding the organizational power of DNA scaffolds to three-dimensional motifs would truly mimic the way protein–protein complexes are assembled in nature. Third, the bienzyme complexes reported so far are all static constructs. It is desirable to incorporate DNA nanomechanical devices[7,8] into the assembly to achieve dynamic complexes with activities that are regulated by biologically relevant signal inputs such as pH change, salt gradient or the addition of a nucleic acid strand. Indeed, it is foreseeable that, by merging the addressability of DNA structure with the versatile functionality of protein libraries, self-assembling, dynamic and functional biomolecular networks are in reach. ❐

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CHEMICAL VAPOUR DEPOSITION

Making graphene on a large scale

Graphene samples with areas of several square centimetres and excellent electrical and optical properties have been fabricated using chemical vapour deposition.

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Graphene — a single layer of carbon atoms bonded into a two-dimensional hexagonal network — has been the subject of intense research since it was first isolated in free-standing form in 2004 (refs 1, 2). Just as related forms of carbon such as fullerenes and nanotubes had previously been found to have many remarkable properties, so too did free-standing graphene. Among the most unexpected and promising of these were its thermodynamic stability, extremely high charge-carrier mobilities, and mechanical stiffness, the last of which distinguishes it from monoatomic metallic films[3].

At first, graphene was produced by the mechanical exfoliation, or peeling of thin layers, of thick graphite[1]. Although this was an easy way to get micron-size flakes suitable for laboratory research, it was difficult to see how it would scale to produce the large areas necessary for some of its most promising potential applications. Subsequently, graphene was grown by epitaxy[4], which requires ultrahigh vacuum conditions and is therefore expensive, and by various chemical methods[3,5,6], which produce graphene with degraded electrical properties.

The fabrication of high-quality, large-area graphene remains one of the hottest topics in nanotechnology and materials science today. Now three independent research groups, headed by Byung Lee Hong of Sungkyunkwan University[7], Jing Kong of MIT[8] and Yong Chen from Purdue University[9], have made a breakthrough by showing that samples of single- and few-layer graphene with areas of square centimetres can be manufactured with a chemical vapour deposition (CVD) technique, and transferred to other substrates.

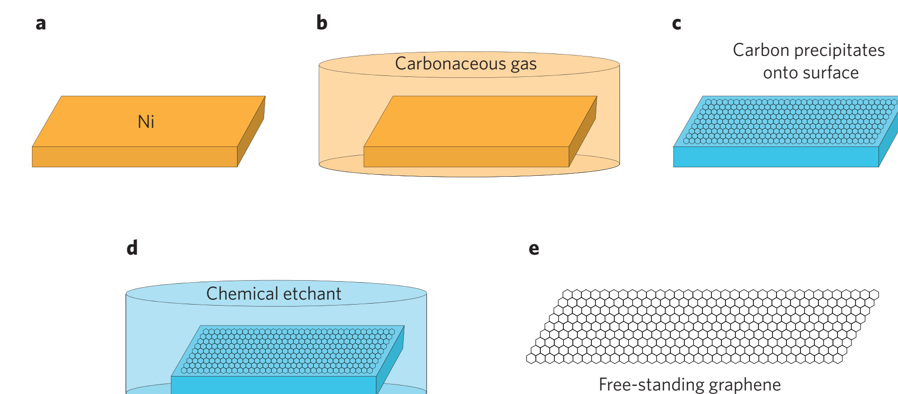

Figure 1 | A chemical vapour deposition route for large-scale graphene production. a, A thin layer of nickel (Ni) is deposited onto a substrate (not shown): the crystallinity of this layer is controlled by its thickness, annealing and the nature of the substrate. **b**, The Ni layer is heated to about 1,000 °C and exposed to a carbonaceous gas environment. Carbon atoms are generated at the Ni surface and diffuse into the metal. **c**, As the nickel is cooled down, the carbon atoms precipitate out of the nickel layer and form graphene on its surface. The graphene samples are expected to form on the (111) faces of Ni crystallites. **d**, The graphene membrane is detached from the Ni layer by gentle chemical etching. **e**, The resulting free-standing graphene layer is transferred onto appropriate substrates (not shown). Colours illustrate the difference in temperature (orange, hot; blue, cold) and chemical composition (orange, gas; blue, etchant).

The three approaches are similar to each other, and also similar to previous work on the deposition of nanometre-thick graphite films on nickel substrates from vapour-phase carbon[10]. The CVD approach to producing graphene (Fig. 1) relies on dissolving carbon into the nickel substrate, and then forcing it to precipitate out by cooling the nickel. The thickness and crystalline ordering of the precipitated carbon is controlled by the cooling rate and by the concentration of carbon dissolved in the nickel. This

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