

Designed DNA molecules: principles and applications of molecular nanotechnology

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Abstract | Long admired for its informational role in the cell, DNA is now emerging as an ideal molecule for molecular nanotechnology. Biologists and biochemists have discovered DNA sequences and structures with new functional properties, which are able to prevent the expression of harmful genes or detect macromolecules at low concentrations. Physical and computational scientists can design rigid DNA structures that serve as scaffolds for the organization of matter at the molecular scale, and can build simple DNA-computing devices, diagnostic machines and DNA motors. The integration of biological and engineering advances offers great potential for therapeutic and diagnostic applications, and for nanoscale electronic engineering.

Rationally designed DNA

DNA sequences that have been designed, using manual or computational methods, to fold into a particular structure or perform a particular function.

In vitro selection (of DNA)

The selection of DNA strands with desired functional properties from an initial set using cycles of selection for functional properties and enrichment of strands with the desired properties.

Molecular evolution (of DNA)

Similar to *in vitro* selection of DNA, except that strands might be mutated between cycles, thereby diversifying the set of DNA strands.

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doi:10.1038/nrg1892
Published online 13 June 2006

The cellular role of DNA is relatively limited, perhaps because of the restrictions imposed by bonding between complementary strands. Outside the cell, however, nanoengineers are uncovering the many hidden talents of DNA. The DNA sequence is able to process information in biochemical assays, its structure is an ideal building material, and its folding pathway allows DNA to move and respond to its environment. Here, I review several innovative applications of designed DNA molecules, some underlying design principles and the prospects for future developments. The applications of new DNA-based technologies range from molecular diagnostics to protein purification and from therapeutics to the assembly of tiny electronic circuits. These uses exploit the properties of DNA at three levels — sequence only, structure (which depends on sequence) and folding pathways (which depend on both sequence and structure) — as the following examples show.

By taking their cue from the genetic code found in nature, nanoengineers are now using DNA sequences *in vitro* to direct the synthesis and evolution of molecules with new functions and reactive properties. When attached to other molecules, detection methods that exploit DNA sequences allow complementary molecules to be identified at extremely low concentrations. DNA sequences can also be used, in a non-biological setting, to control the layout of electronic components in a nanocircuit.

DNA strands can fold into stable structures that have valuable functional and material properties. Sensitive methods for detecting DNA have been extended to

detect proteins and other macromolecular targets of DNA aptamers — that is, nucleic-acid molecules that have high binding specificity for their targets. Lattices and even three-dimensional (3D) objects have been self-assembled from rigid DNA structures. DNA lattices can organize proteins and nanoelectronic components on a surface with unprecedented precision, making it possible to gain a better understanding of the properties and interactions of proteins.

The folding pathway of a DNA molecule to its stable structure allows it to move and perform mechanical functions. The energy released in DNA-folding pathways has been used *in vitro* to drive motors, providing the ability to release, grab or cleave target molecules and even to control the release of drugs, depending on the outcome of certain diagnostic tests.

This review focuses on the recent developments in generating rationally designed DNA molecules and their applications. The past decade has seen huge advances in the development of design principles, particularly by exploiting DNA structure and folding pathways, but also in applications of designed DNA sequences. Together, techniques for the *in vitro* selection or molecular evolution of DNA molecules from random pools have given spectacular yields, including DNA aptamers and enzymes, which can inhibit the expression of harmful genes or disrupt protein function. Excellent reviews of selected DNA molecules can be found elsewhere^{1–3}; this paper includes only examples of designed DNA molecules, or molecules that combine both selected and designed

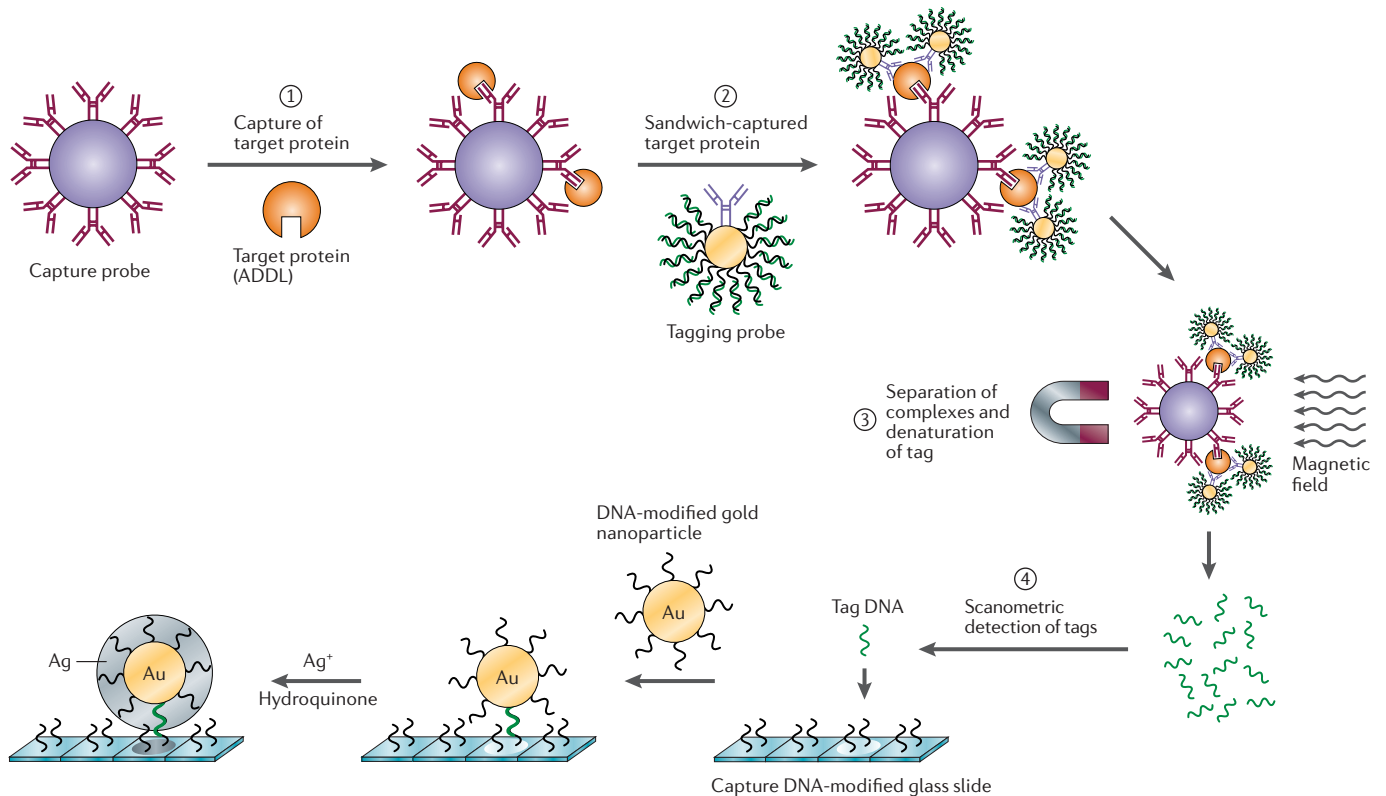


Figure 1 | Exploiting DNA sequences: sensitive molecular detection. Bio-barcode amplification allows detection of molecules, at low attomolar levels, from biological samples, such as sera or spinal fluids^{8,10}. Two types of probe are used to detect a protein target, which, in the illustration, consists of amyloid- β -derived diffusible ligands (ADDLs) in human cerebrospinal fluid. Tagging probes consist of a gold nanoparticle (yellow) to which an antibody for the target (blue) and complements of DNA tags (black) are attached. DNA tags (green) are then hybridized to their complements. Capture probes consist of magnetic beads (blue circles) to which antibodies (pink) are attached. On exposure to the magnetic beads, the target, ADDL, is caught on the capture probes (step 1). It is then 'sandwiched' between capture and tagging probes, by binding to antibodies on both types of probe (step 2). The resulting complexes are separated from solution using a magnetic field and washed (step 3), at which point the tags denature from their complements. The concentration of ADDL in the sample can then be estimated on the basis of the amount of tag that is released. To this end, Georganopolou *et al.*¹⁰ used scanometric DNA detection (step 4). In this method, tag DNA is captured on a glass slide, which has been spotted with DNA strands that are complementary to part of the tag. Gold nanoparticles (Au), which contain DNA strands complementary to the remaining (unhybridized) part of the DNA tag, then sandwich the tag between a slide and nanoparticle. The nanoparticles are coated with silver (Ag), and the light intensity scattered from each spot is measured. Adapted with permission from REF. 8 © (2003) American Association for the Advancement of Science and REF. 10 © (2005) National Academy of Sciences.

features. The examples are organized by the primary property of DNA used: sequence, structure or folding pathway (although the distinctions are sometimes blurred). Overall, I hope that the reader will gain a renewed appreciation for the extraordinary abilities of DNA, and that the range of examples will stimulate ideas for further practical applications.

Innovative uses of DNA sequence

Many uses of DNA exploit its sequence properties, along with the ability of a DNA sequence to hybridize with its complement. Following a short description of DNA-sequence properties and the techniques for manipulating these molecules, I describe several applications, ranging from sensitive molecular detection to DNA computing and the assembly of nanoelectronic components. Finally, I describe some challenges and prospects for this field.

DNA sequences have three major assets: a four-base digital make-up, an ability to bind a complementary sequence with high affinity, and stability in a wide range of environmental conditions. DNA has an incredible information density — roughly 1 bit per nm³ — that can be retrieved from an ssDNA sequence by its ability to recognize and hybridize with its complement. Hybridization is reversible and, in part, thermodynamically driven: when temperature decreases complementary ssDNA in solution tends to hybridize, and when temperature increases dsDNA tends to denature into single strands. The applications that exploit the features of DNA sequences also depend crucially on efficient technologies for the sequencing, synthesis, amplification (by PCR) and detection (by fluorescence spectroscopy and DNA microarray analysis) of DNA strands.

Fluorescence spectroscopy

The analysis of fluorescence spectra that are emitted by fluorophores when they are excited by visible light or ultraviolet rays.

DNA microarray

An array of spots on a planar surface, with copies of an ssDNA immobilized on each spot. The array can be used to detect DNA strands that are complementary to those immobilized to the surface.

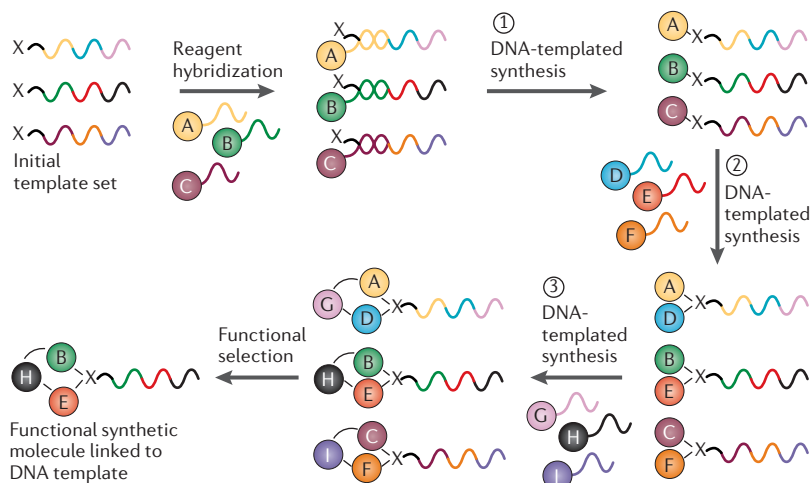


Figure 2 | Exploiting DNA sequences: DNA-templated synthesis of macrocycles. Three macrocycles, each composed of four cyclically-linked units, are synthesized¹⁵. The starting material consists of nine types of reagent (A–I), each of which is linked to a distinct biotinylated DNA tag (indicated by a coloured strand). A set of three DNA templates, each the concatenation of three sequences that are complementary to three distinct tags, is initially synthesized. These templates represent the three macrocycles to be synthesized. Attached to each initial template is a single unit (X; a lysine derivative) that will form part of each macrocycle. The macrocycles are then synthesized in a multi-step process, in which each step increases the length of the synthesized molecule by one. In the first illustrated synthesis step, the tags attached to reagents A, B and C (yellow, green and dark pink, respectively) hybridize to each of the three templates, bringing the X unit into proximity with the A, B and C units (one per template). The units then conjugate with the X unit by amine acylation, to produce synthesized molecules of length 2. The process is repeated (synthesis steps 2 and 3) to produce three synthesized four-unit macrocycles. The biotin present on the tags allows the macrocycles to be purified by capturing them on streptavidin beads and then releasing them. Following synthesis, one macrocycle is selected for function, and its tag reveals its identity. Adapted with permission from REF. 15 © (2001) American Association for the Advancement of Science.

Macrocycle
A cyclic molecule with large molecular weight, typically constructed from subunits. One example is porphyrin, which is a component of heme and the prosthetic group in haemoglobins and myoglobins, and is responsible for oxygen transport in the blood.

DNA code
A mapping from a set of DNA sequences to the set of monomers of other polymers; for example, the genetic code maps the set of three-letter codons to the set of amino acids.

Cytokines
Proteins that aid in the formation and development of blood cells, and are important in immune-system functioning.

DNA tags, which are sequences that are chemically attached to other molecules, represent the most successful and varied application of DNA-sequence properties. DNA tags are used in the highly sensitive detection of disease markers, the parallel synthesis of new compounds and the discovery of new reagents — as such, they have been applied in disease diagnosis and drug development. Two powerful principles unify the applications of DNA tags. First, new DNA codes⁴ can direct the cheap and efficient parallel synthesis of large sets of polymers. In this case, each DNA tag is composed of ordered segments that specify the sequence of polymeric units of one molecule to be synthesized, just as a sequence of natural codons specifies the sequence of amino acids that make up a protein. Second, efficient methods for amplifying, sequencing and detecting DNA can be extended to new types of molecule using their attached DNA tags. Several applications of DNA tags are described next.

Sensitive molecular detection. Molecular-detection methods can help to identify disease markers, such as cytokines in human serum samples, and, in so doing, aid the diagnosis of some cancers and immunodeficiency-related diseases⁵. Detection methods can also identify allergens and pollutants⁶. Highly sensitive detection,

at the attomolar (10^{-18} mole) level or better, is of great practical importance: it allows more efficient screening at blood banks through the pooling of samples, and it facilitates early disease detection when treatment might be more promising and paediatric research when sample sizes are necessarily small⁶.

Early DNA-based detection methods, such as immuno-PCR^{6,7}, involved many cumbersome steps, including PCR. Bio-barcode amplification (BCA), which has been developed by Nam and colleagues⁸, is a new streamlined method that achieves highly sensitive detection at attomolar concentrations without the need for PCR amplification. In BCA, the target protein to be detected is ‘sandwiched’ between two probes: a tagging probe and a capture probe (FIG. 1). Both probes contain antibodies, which bind to the target protein such that the protein is sandwiched between the probes. Two key innovations are that many copies of a DNA tag are attached to the tagging probe, and that tags can be removed for detection purposes using a simple denaturing process. BCA has been successfully applied to the detection of prostate-specific antigen (an indicator of prostate cancer and breast cancer^{8,9}), amyloid- β -derived diffusible ligands (ADDLs; indicators of Alzheimer disease¹⁰) and interleukin 2 (a cytokine protein that is involved in inflammation and immune responses in humans¹¹). Georganopoulou and colleagues¹⁰ quantified differences in ADDL levels in clinically realistic samples of cerebrospinal fluid that were taken from human tissue. Overall, they found a significantly higher (low femtomolar) concentration of ADDLs in diseased patients, compared with samples from control patients who had not been diagnosed with Alzheimer disease.

Fredriksson *et al.*¹² described another ingenious detection scheme that uses a pair of DNA aptamers, rather than antibodies, as the means of binding to the target protein. Unlike the BCA method, this ‘proximal ligation’ method is limited to recognizing protein targets for which DNA aptamers are known, and also requires the use of PCR. However, proximal ligation avoids washing or separation steps, and can detect a cytokine protein at subattomolar concentrations even in biological samples.

DNA-templated synthesis. In the cell, the genetic code supports both translation (whereby protein products encoded by DNA are synthesized) and evolution (whereby cycles of translation, mutation and selection produce better-adapted products in the form of evolved species). The ultimate goal of DNA-templated synthesis (DTS)¹³ is to emulate these processes *in vitro* in a parallel fashion. DTS can create polymers with strong binding affinity to a target of interest, such as a disease marker or toxin (FIG. 2). DTS offers several advantages over traditional methods for developing functional molecules, including parallelism, ease of purification and mutational evolution. Rosenbaum and Liu¹⁴ reported the first DTS of molecules that do not have a ribose backbone, which are called peptide nucleic acids (PNAs); these are valuable substitutes for DNA in environments where the backbone of DNA is prone to degradation, such as in therapeutic uses in living cells. Gartner *et al.*¹⁵ showed

Immuno-PCR

A protein-detection method in which specially designed linker molecules capture a copy of the protein of interest and a DNA tag. The captured tags are then amplified and detected using PCR, followed by either fluorescence spectroscopy or chip-based detection methods.

Enone

An unsaturated chemical compound or functional group consisting of a conjugated system of an alkene and a ketone.

Boolean logic

An algebra for reasoning about logical values ('true' or 'false'), which includes AND, OR and NOT operations, and is also useful for computing with binary ('1' or '0') values.

that DTS can be followed by *in vitro* selection for desired functional properties — in this case, a molecule that inhibits the activity of carbonic anhydrase. Carbonic anhydrase-driven reactions, which convert carbon dioxide and water into hydrogen ions and bicarbonate, have several functions in the body (such as maintaining an acidic environment in the stomach and transporting carbon dioxide by red blood cells) and so inhibitors of such reactions have therapeutic uses.

One limitation of DTS is that synthesis can only occur in environments in which dsDNA will not denature, because binding of complementary strands directs synthesis steps. Halpin and colleagues^{16–18} describe a synthesis method that overcomes this limitation by splitting templates into pools at intermediate stages of synthesis. With the pooling method, synthesis can occur at high temperatures or with organic solvents. They reported both the synthesis of a set of 1 million peptides, each comprised of 5 amino acids, and the selection from this set of a peptide with affinity for the 3-E7 antibody, which inhibits vascular endothelial growth factor.

Parallel reaction discovery. The purpose of parallel reaction-discovery systems is to efficiently test many pairs of reagents simultaneously in a single solution, to identify unknown binding pairs that might have innovative uses^{19,20}. This process has been hindered by the difficulty of bringing each pair of reactants to be tested into close proximity with one another while avoiding crossreactivity among pairs of reagents that are not of interest. Another challenge is to identify those pairs that do react. DNA tags solve both of these problems: the molecules to be paired have complementary regions on their tags so that they come together by hybridization, and sequencing of the tags provides the means to identify reactive pairs. As proof of principle, Kanan *et al.*²⁰ used their method to discover — from 168 combinations of reagents — that an

enone could be produced by an alkyne–alkene coupling reaction in the presence of a palladium catalyst.

Computing with DNA. In the applications described so far, DNA sequence has an informational role. Is computation possible for information stored in DNA? In essence, computation is the process of determining an output from a list of inputs, using elementary instructions, where the available instructions depend on the context of the computation. For example, a schoolchild can determine the sum of two multiple-digit input numbers written on paper, using a sequence of single-digit additions. By contrast, a computer calculates the sum of numbers in binary format, using the principles of Boolean logic. Computation is not, however, limited to paper or silicon media. Indeed, as Adleman²¹ showed (FIG. 3), DNA is an attractive medium because of its high information density. Inputs to the Adleman computation are DNA sequences that represent candidate solutions to a computational problem. The computation repeatedly prunes out incorrect solutions, so that, ultimately, the true solution is selected. This select-and-prune process is analogous to *in vitro* selection, except that sequences are selected on the basis of their information content rather than their functionality. In the largest experiment so far, Braich *et al.*²² reported a search-and-prune computation involving more than 1 million inputs, representing potential solutions to a problem of mathematical logic. Although this approach is not competitive with conventional computers, the work has inspired many creative approaches for DNA computation that exploit structure and folding pathways, which are described in later sections.

Organization of nanoelectronic components and other materials. Single-walled carbon nanotubes (CNTs) have been used to realize tiny electronic transistors, which have potential for assembly into nanocircuits. There are several challenges to building a circuit from CNTs, however, including controlling the layout of the component CNTs and placing the wires to connect the components according to a specific pattern. DNA tags (and also tags made of PNA) offer great promise in meeting these challenges. DNA tags that are attached to CNTs can direct their assembly by binding a tag with its complement²³. DNA strands have been used to spatially position CNTs, making it possible to assemble them into circuits²⁴. In addition, ssDNA can be stretched across a surface to connect two electrodes by binding the ends of the ssDNA with complementary strands that are attached to the electrodes. Silver can then be deposited along the stretched DNA strand, to create a nanowire that connects the electrodes^{25,26}.

Varying the DNA strands that are wrapped around CNTs alters the electrostatic properties of the CNT–DNA hybrid in a way that depends on the diameter of the CNT²⁷. DNA-wrapped CNTs also enhance the sensitivity of nanotube devices in the detection of gases²⁸. Storhoff and Mirkin²⁹, and Niemeyer³⁰ review further uses of DNA for materials synthesis — for example, in the assembly of networks of gold nanoparticles or the organization of enzymes so as to speed up chemical reactions³¹.

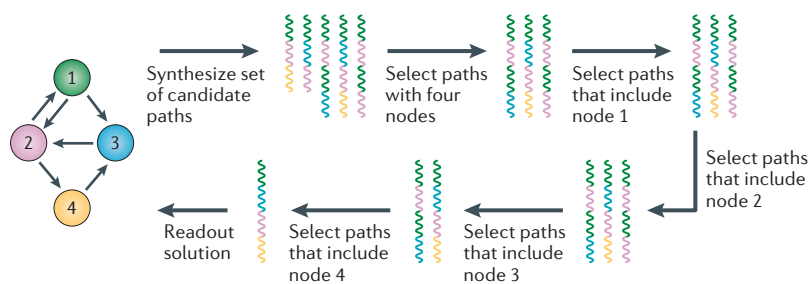


Figure 3 | Exploiting DNA sequences: DNA computation. Adleman²¹ used select-and-prune parallel computation to determine whether there is a path (that is, a sequence of linked nodes) through a network, such that the path visits each node exactly once. In the illustrated example (simplified from the Adleman experiment), the input set contains a sample of five paths in a simple four-node network (shown on the left). Nodes are represented by short DNA strands, and paths are represented as concatenations of the corresponding node strands. The first round, which is accomplished by gel electrophoresis and shown on the right, selects paths that have exactly four nodes, therefore pruning two paths from the input sample. The second, third, fourth and fifth rounds prune out paths that do not visit node 1, 2, 3 or 4, in corresponding order (accomplished using DNA-sequence complementarity and magnetic-bead separation). Only one path remains at the end of the computation, revealing the solution: 1→3→2→4.

Challenges and opportunities. DTS and the involved reactions have excellent potential for use in the discovery of drugs or other new compounds. For practical applications, it will be necessary to broaden the scale and range of these methods. An adaptive approach involving repeated rounds of synthesis, in which templates of already-synthesized molecules that show some useful function could be mutated or recombined, might diversify the synthesis pool¹⁷. Another possibility is to integrate DNA-computing methods, which select molecules on the basis of the information content of their labels, with methods that select or organize molecules on the basis of their function.

Innovative uses of DNA structure

The diverse structures that DNA can have vastly expand its potential uses in nanotechnology and biotechnology applications. Structure is the key feature that allows the specific binding of DNA to proteins. It is now also possible to engineer rigid scaffolds from DNA. The integration of the scaffolding and binding structural properties of DNA yields two-dimensional (2D) ordered arrays of proteins, which might be useful for the detailed study of protein structure. DNA structure even supports computation, making it possible, in principle, to program the layout of arbitrarily complex 2D or 3D patterns from DNA.

DNA structure, which might involve one or more DNA molecules, is largely shaped by the hybridization of complementary intermolecular or intramolecular base pairs. The most stable structure formed by DNA molecules in solution depends on environmental factors, such as temperature, pH level and salt concentration. The double helix is just one possible conformation; structures might have branching helices that stem from junctions or loops (FIG. 4a). The stacking of adjacent base pairs causes short helices to be rigid, unlike ssDNA. Some DNA molecules enjoy further structural complexity, such as G quartets (FIG. 4b), which can fit snugly in crevices of proteins, and therefore can have high binding specificity for important protein targets. One DNA aptamer exploits a G-quartet structure to bind to thrombin and can inhibit thrombin-catalysed fibrin-clot formation *in vitro* in samples of human plasma³².

Substantial progress has also been made recently in the design of branched and rigid shapes from DNA. These new structures support the creation of 2D scaffolds and complex patterns, and even 3D structures. Single molecules can now be arrayed with nanoscale precision on DNA scaffolds. Because many exciting developments in engineered DNA structures have been reported in the past year or so, they are the primary focus of this section.

Molecular detection. Sensitive detection of molecules can take advantage of structure as well as sequence. Li *et al.*³³ and Stavis *et al.*³⁴ considered the case of multiplexed detection, where the goal is to determine which of many DNA fragments, derived from pathogens, are present in solution. Instead of sequence tags, Li *et al.*³³ used branched DNA structures called nanobarcodes, with red or green fluorophores on the branch tips (FIG. 4a). Nanobarcodes are distinguishable, using fluorescence

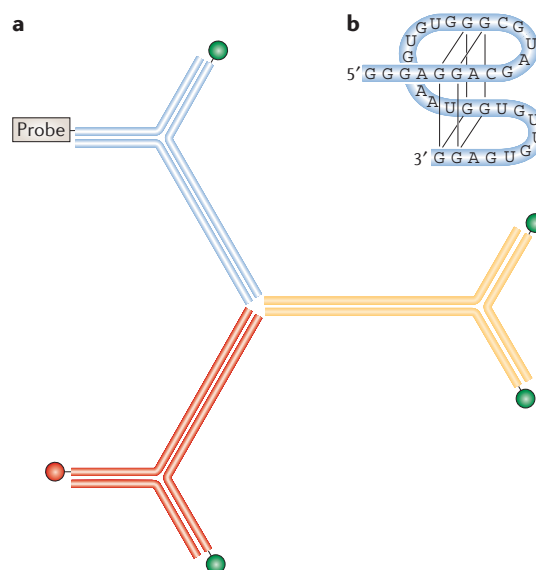


Figure 4 | Exploiting DNA structure: branched structures. **a** | The nanobarcode, which is a branched structure with fluorescent labels at the tips of its branches³³, is constructed from Y-shaped components, each formed from three DNA strands (indicated by colours). Each Y-shaped structure binds to another by complementary sticky ends. Ligation is then used to seal the nicks (at positions where distinct strands meet) in the overall structure. Nanobarcodes are distinguished by the ratio of red and green fluorophores (represented by circles) on their branches, and have been used for multiplexed detection. For each type of protein to be detected, a probe is selected that has high binding specificity for the target. A tagging probe is constructed by attaching nanobarcodes of common type (that is, ratio of red to green fluorophores) to copies of the probe. The tagging probe and an additional large capture probe (not shown) are introduced into a solution; if the target is present in the solution, it is sandwiched between the tagging and capture probes, and detected by fluorescence spectroscopy. **b** | A G-quartet structure in a nucleic-acid aptamer, which binds to thrombin (not shown) in the presence of potassium ions³¹.

spectroscopy, by their ratios of red to green fluorophores. As proof of concept, nanobarcodes were used to detect the presence or absence of DNA fragments from four pathogens (*Bacillus anthracis*, *Francisella tularensis*, Ebola virus and the severe acute respiratory syndrome (SARS) coronavirus) in a prepared solution with a detection limit of 620 attomoles. Stavis *et al.*³⁴ showed that, by flowing nanobarcode tags through microfluidic channels, more rapid detection is possible.

DNA scaffolding. A long-term goal of Seeman and co-workers^{35,36} has been to build lattices and other rigid structures from DNA in a controlled scalable fashion. DNA lattices can form a 2D scaffold on which biological macromolecules can be arrayed. DNA lattices could also bring enzymes into proximity, thereby catalysing numerous reactions on a large scale, and serve as guides for the placement of nanoelectronic components on a surface,

G quartets

Nucleic-acid structures in which four G nucleotides form a stable structure.

Thrombin

A protein that is involved in coagulation and anti-coagulation, and can recognize many macromolecular substrates.

Microfluidic channels

Systems of channels, with diameters that range from micrometres to millimetres, through which fluids can flow. Can be used to separate and detect DNA molecules in the fluids.

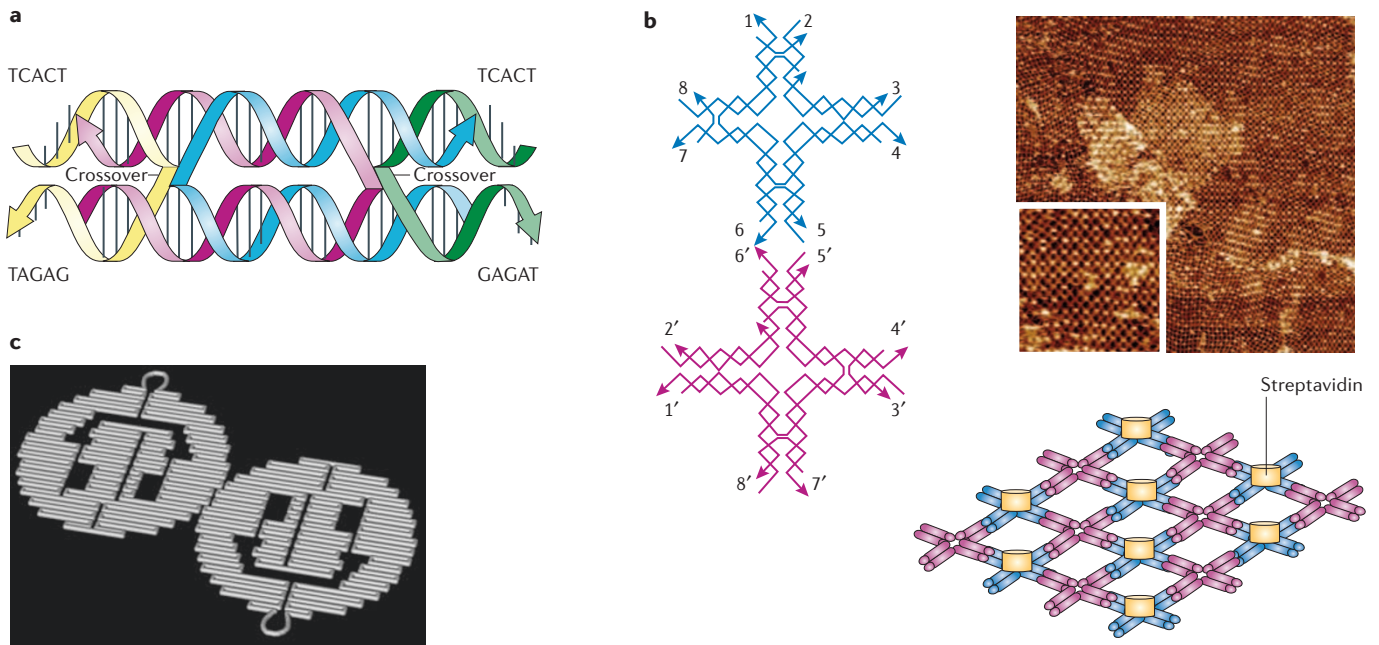


Figure 5 | Exploiting DNA structure: double-crossover structures and self assembly. **a** | A double-crossover (DX) molecule³⁷ composed of two helices with strands that cross at two points, thereby creating a rigid structure. Five-nucleotide single-stranded sequences, called sticky ends, allow DX molecules to bind to each other in a programmable fashion; that is, two DX molecules bind only if they have complementary sticky ends. **b** | Rigid junction molecules with four emanating helices, in which each of the four branches contain a single crossover³⁹. This molecule was used to construct a DNA lattice, with biotinylated components, on which streptavidin was arrayed. The atomic-force microscopy image shows a resulting lattice, arrayed with streptavidin (visible as the light spots; the scale of the image is 1 $\mu\text{m} \times 1 \mu\text{m}$ and the inset is 150 nm \times 150 nm). **c** | Smiley face constructed using the scaffolded origami technique⁴². A long scaffold strand can be laid out in rows, to cover the desired pattern. Rothemund used a 7,249-nucleotide DNA strand from the virus M13mp18 (a strand that is inexpensive to purchase). More than 200 short DNA strands (each about the length of a PCR primer) are used to hold the long scaffold strand in the desired pattern. The diagram shows how the long DNA strand is laid out to create the resulting pattern. Panel **a** reproduced with permission from REF. 48 \copyright (2004) American Chemical Society. Panel **b** adapted with permission from REF. 40 \copyright (2005) American Chemical Society. Panel **c** reproduced with permission from REF. 42 \copyright (2006) Macmillan Publishers Ltd.

thereby forming a nanocircuit. In a 3D DNA lattice, the cavities could serve as cages in which proteins are arrayed, making it possible to uncover new properties of the macromolecules with X-ray crystallography.

Many DNA scaffolds are constructed from rectangular structures, called tiles, which are composed of numerous short DNA strands. Emanating from these tiles are one or more single strands, called sticky ends, which can bind to complementary sticky ends on other tiles. Self-assembly of large structures from small tiles ensues when many tiles bind to each other. Important challenges in tile design are ensuring that the tiles are rigid (a necessary property for scaffolding) and that adjacent assembled tiles are co-planar.

The double-crossover (DX) molecule of Fu and Seeman³⁷ (FIG. 5a) is an early, elegant and widely used design, from which the first 2D DNA lattices were built by Winfree *et al.*³⁸. Other designs and applications have followed^{39–41}, which additionally incorporate strategies to array protein molecules in regular arrangements on the lattice. Such arrays could be used to study protein structure using electron microscopy or to construct spatially well-defined complexes involving several enzymes. Specific examples

are the lattices of Yan *et al.*³⁹ and Park *et al.*⁴⁰, in which streptavidin binds to biotinylated DNA molecules at lattice junctions, thereby precisely controlling the arrangement of the streptavidin molecules, for example, with a distance of approximately 20 nm between adjacent molecules (FIG. 5b). Liu *et al.*⁴¹ incorporated the DNA aptamer for thrombin (FIG. 4b) into a hairpin structure at regular points of a one-dimensional (1D) lattice. This approach should, in principle, allow organization of any molecule for which there is a DNA aptamer.

In a different approach, known as single-stranded origami⁴², a long strand folds in an intricate way, aided by short strands that direct the folding into the desired configuration. Shih *et al.*⁴³ used this approach to construct the first rigid 3D structure, crafting an octahedron from a single long (1,669 nucleotide) strand and five shorter (30–35 nucleotide) strands. The short molecules bind so as to form appropriately placed rigid DX structures, which become edges of the octahedron. Rothemund⁴² illustrated the generality of a different method, known as scaffolded origami, by constructing smiley faces, a world map and many other complex 2D patterns (FIG. 5c). To design a pattern, Rothemund uses a computational tool

Atomic-force microscopy
The analysis of the contours of a surface, which are based on measurements of Van der Waals forces between the surface and a scanning probe that is attached to a tiny cantilever.

X-ray crystallography
The analysis of the diffraction patterns of X-rays of a crystal, used to determine the structure of the molecule.

Double-crossover (DX) molecule
A type of rigid DNA rectangular-shaped molecule that is comprised of four or five DNA strands, with four ssDNA strands emanating from its corners.

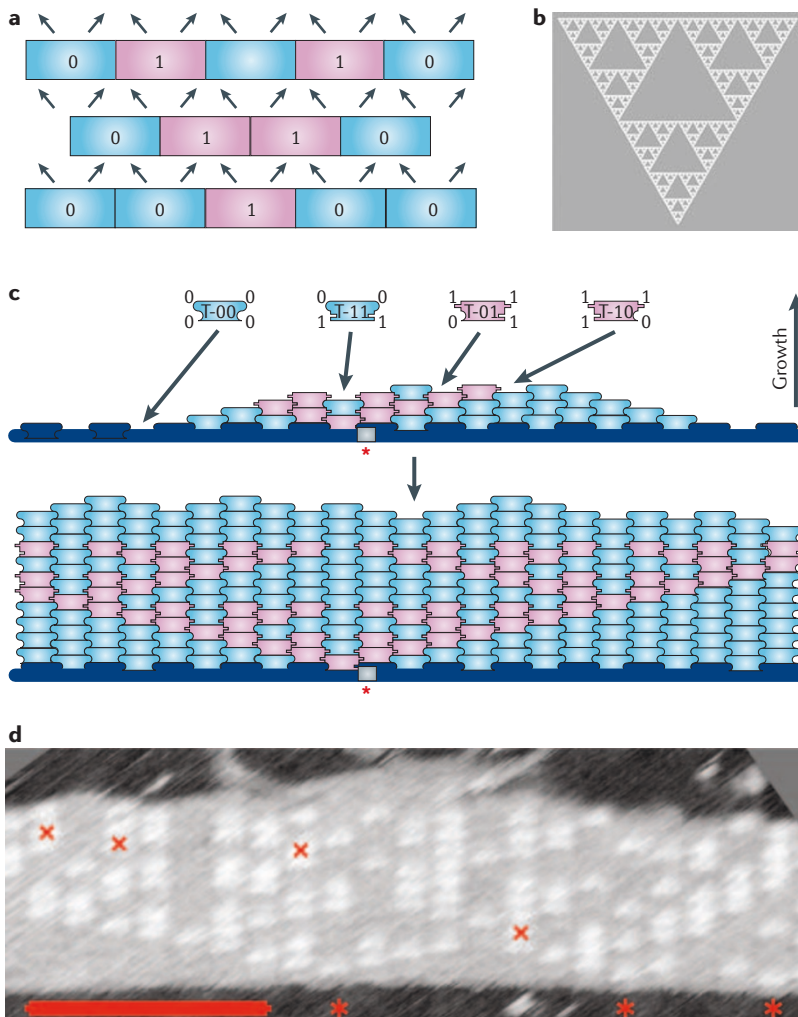


Figure 6 | Construction of a two-dimensional aperiodic lattice. Four types of double-crossover (DX) molecule, distinguished by their sticky ends (a), represent four types of tile from which a Sierpinski triangle can self-assemble (b). Tiles assemble upwards from the blue frame, starting from the position marked with the red asterisk, forming patterned rows (c). Each tile is marked with either 0 or 1 (corresponding to the sticky ends of the DX molecule) on each of its four corners, and is added to the assembly in such a way that the mark on its lower-left corner matches the mark on the adjoining tile immediately to its left in the row below it, and similarly the mark on its lower-right corner matches the mark on the adjoining tile immediately to its right in the row below it. Crucial to the success of the construction is that the two sticky ends on the lower end of a DX molecule (tile) bind cooperatively to the upper sticky ends of the two matching DX molecules (tiles) below it; a tile with just one matching sticky end is considered to be too weak to persist. Analogously, if working on a puzzle, by moving inward from one corner of the frame and ensuring that each inserted puzzle piece properly connects with two pieces already in the puzzle, mistakes can be reduced. The proof proposed by Winfree to show that DNA self-assembly is able to perform general-purpose computation builds on the classical work of Wang⁸², and relies on the cooperative binding of DNA tiles. An atomic-force microscopy image (d; courtesy of Erik Winfree, California Institute of Technology) shows a recognizable Sierpinski triangle fragment, with each error marked by a red cross. Red asterisks again represent starting points of self-assembly. Scale bar represents 100 nm. Reproduced with permission from REF. 54.

juxtaposed on adjacent rows, crossing over from one row to another in a manner resembling DX molecules. The staple strands therefore hold the pattern together rigidly when combined with the scaffold strand in solution.

Goodman *et al.*⁴⁴ gave an elegant design for a rigid 3D DNA tetrahedron that, like the octahedron, has a triangulated frame. Each tetrahedron is formed from five short strands, designed to favour the formation of tetrahedron edges in a hierarchical fashion, thereby providing faster assembly and higher yield than previous methods for the construction of 2D structures. Other new approaches to the construction of rigid DNA structures that can be replicated have been proposed by von Kiedrowski and co-workers^{45–47}.

Nanoelectronic assembly. Self-assembled tile structures made of DNA show promise in the construction of conductive nanowires and 2D arrays of nanocomponents. DNA tiles that are not in fact planar, but have some curvature, self-assemble into nanotubes rather than planar lattices^{39,48}. Liu *et al.*⁴⁹ created a nanowire by metallizing a DNA nanotube with silver. The wire had significantly higher conductivity than wires built from dsDNA.

Many circuit subsystems, such as random-access memory and programmable logic arrays, are arranged in a 2D-array pattern. As a first step towards the construction of nanoelectronic arrays, Le *et al.*⁵⁰ assembled DNA–gold nanocomponents onto a 2D DNA array of DX molecules, with some molecules containing an extruding poly(A) ssDNA strand. Gold nanocomponents covalently linked to poly(T) strands then hybridize to the extruding poly(A) strands, resulting in a 2D array of gold nanoparticles. Such arrays could serve as a nanoscale memory, in which the electronic state of the nanoparticles could be read by scanning probes.

Algorithmic self-assembly. Winfree⁵¹ forged a fundamental link between DNA self-assembly and computation: sticky-ended binding of tiles is, in fact, powerful enough to support general-purpose computation. To understand why this is true, consider the construction of a large jigsaw puzzle after first discarding the picture on the box cover. There is a way to make a coherent whole out of all the pieces. The ‘program’ for doing this is not a traditional sequence of instructions, but rather is implicitly specified by interconnection patterns (which function as sticky ends) between pieces. The DNA lattices and nanotubes described above are examples of periodic structures, the assembly of which is programmed by their sticky ends. Mao *et al.*⁵² and Yan *et al.*⁵³ described the first aperiodic tile assembly in one dimension. A Sierpinski triangle is an elegant example of a 2D aperiodic pattern, which has been assembled using just four tiles, given a suitable starting frame, by Rothmund *et al.*⁵⁴ (FIG. 6).

Challenges and opportunities. Techniques for the scalable assembly of rigid 2D and 3D DNA structures suffer from errors that are due to inherent limitations in the specificity of sticky ends and the control of hybridization kinetics. Error-correction techniques have the potential to rectify mistakes in the self-assembly process^{55,56}. DNA

to plan the layout of a single long DNA strand, called a scaffold strand, which fills in the desired pattern row by row — similar to laying out yarn to create a string picture. Short staple strands are then designed, which can hybridize to segments of the scaffold strand that are

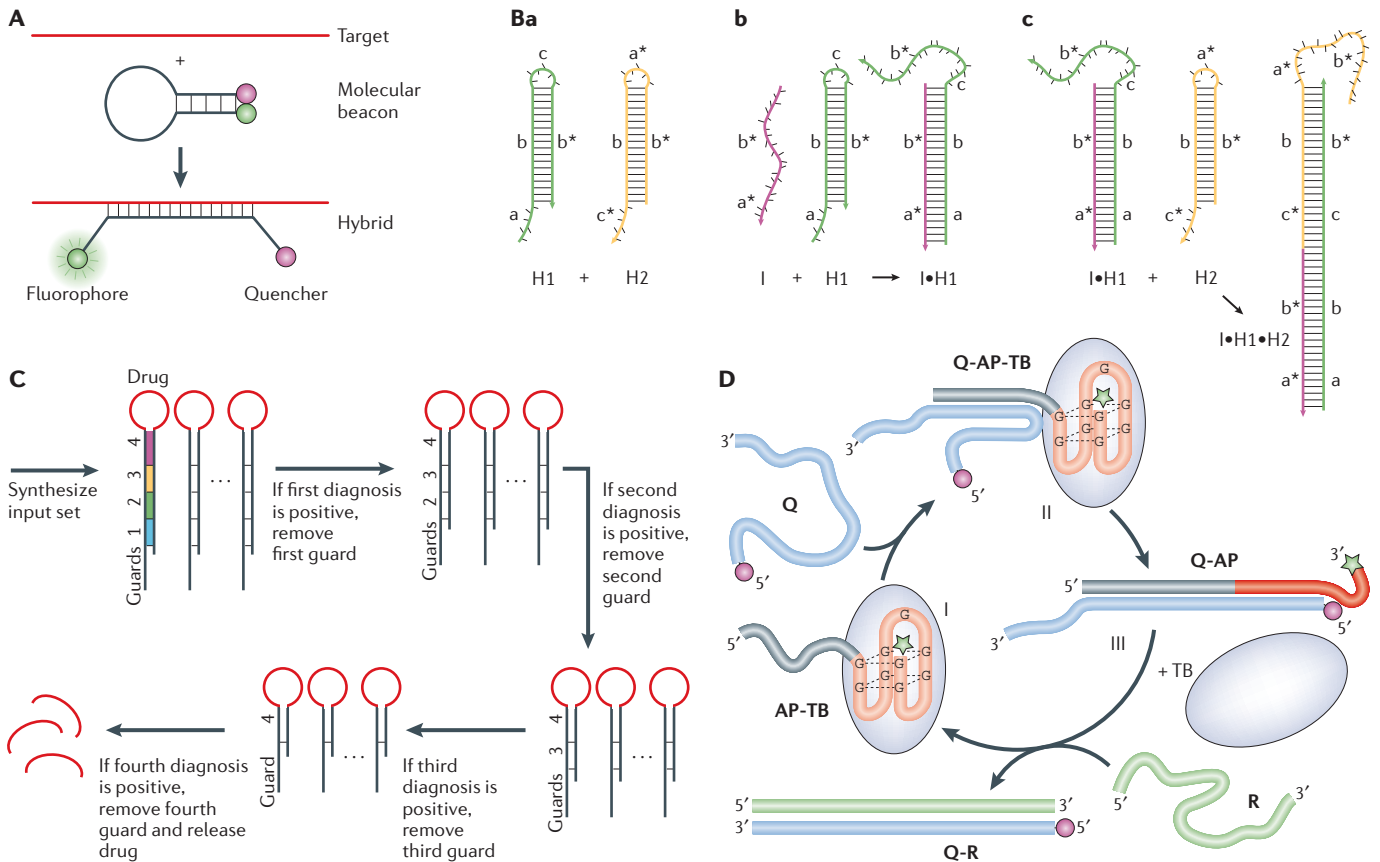


Figure 7 | DNA-folding pathways and their uses. A | A molecular beacon in which a stable hairpin structure, closed by a short helix, switches to a helix with emanating single-stranded ends on introduction of a strand that is complementary to the loop sequence of the hairpin. This change in structure separates the fluorescent label from the quencher, thereby producing a fluorescence signal. **B** | A folding pathway of the hybridization chain reaction⁶⁸, the initial structure of which consists of two types of hairpin, closed by long helices. **Ba** | Initially, two types of sequence fold into hairpin structures. The first type, H1, has a helix formed from complementary sequences (b and b*), a hairpin loop formed by sequence c and an unpaired sequence (a) emanating from the 5' end of the helix. The second type, H2, has a similar helix with reverse polarity, but differs in its hairpin loop, which is the sequence a* complementary to a. **Bb** | On introduction of the target sequence (a*b*), the target binds to the unpaired 'a' region of an H1 hairpin and opens the stem through a branch migration, leaving cb* unpaired. **Bc** | This unpaired region then binds to and opens an H2 hairpin, exposing a new unpaired region (a*b*) on H2. This unpaired a*b* region is similar to the initial target sequence, forming the basis for a chain reaction that produces 'polymers' of alternating H1 and H2 components. **C** | A computation for disease diagnosis and drug delivery⁷¹. The inputs are all identical, consisting of a hairpin structure with a long helix, and a drug sequence encapsulated in the hairpin loop. The computation process is similar to a search-and-prune computation (FIG. 3), except that selection steps are replaced by diagnostic tests for disease indicators (high or low levels of expression of a gene) in the environment. Each positive diagnosis causes shortening of the hairpin helices, so that the drug is released if all diagnoses are positive. Therefore, in this computation, the inputs change at each step, in contrast to the search-and-prune computation of FIG. 3; this highlights the wide range of ways in which DNA-based computations can proceed. **D** | A device that binds and releases thrombin⁷². A DNA sequence contains the aptamer (AP, in red) for thrombin (TB, grey oval), concatenated with an additional ssDNA sequence (grey) and a fluorescence signal (green star). Addition of a blue sequence (a quencher, Q) causes the blue and grey regions to bind, ultimately disrupting the G-quartet structure of the thrombin aptamer and causing thrombin to be released. On addition of the green sequence (a remover, R), the blue and green sequences bind, displacing the black-red aptamer sequence through branch migration, and causing thrombin to be bound again. Further cycles are possible, by cycling addition of blue and green strands. Panel **b** reproduced with permission from REF. 68 © (2004) American Association for the Advancement of Science. Panel **c** reproduced with permission from REF. 72 © (2004) John Wiley & Sons Inc.

self-assembly of electronic components raises new research challenges, and opportunities for the design and modelling of circuit architectures⁵⁷.

Because, at present, there is limited understanding of the rules of formation of DNA tertiary structure, the development of DNA aptamers and enzymes has

employed *in vitro*-selection methods rather than rational design. However, to develop larger DNA molecules with new binding properties, it is likely that a combination of rational design and selection will be effective. Combined approaches have already been used for the design of new RNA molecules⁵⁸.

Innovative uses of DNA-folding pathways

When a DNA molecule is not in its stable structure, perhaps because of a change in environmental conditions, it switches to a form that is stable in the new environment. In doing so, the molecule typically passes through a sequence of intermediate structures, called the folding pathway. The folding pathway of DNA can be put to good use in molecular detection to diagnose and respond to environmental conditions, to release energy (which can be used to drive nanoscale motors) and to execute computations.

FIGURE 7 illustrates several folding-pathway (or structure-switching) scenarios. In a typical case, DNA molecules are initially bound to each other, but have some single-stranded regions. When a new DNA molecule is introduced that can bind with a single-stranded region, it can displace base pairs by a process called branch migration. This displacement can cause the potential energy trapped in loops of the initial structure to be released, thereby providing fuel for DNA devices, such as DNA tweezers⁵⁹, and can also cause the movement of the DNA molecules. Simmel and Dittmer⁶⁰ provide a comprehensive overview of several feats of motion by DNA molecules, including unidirectional walking and movement along a track. Such devices might aid in the transport of tiny molecules in a nanoscale system. Alternatively, changes in buffer conditions, which are achieved by introducing positively charged ions, can cause a DNA helix to come apart and switch from its usual B form (in which the helix twists to the right) to the less standard Z form (in which the helix twists to the left)^{61,62}. The addition of enzymes that cleave DNA also causes a change in the environment and a corresponding change in the stable structure of the collection of DNA molecules, which could be used to detect the presence of the enzymes. The following examples highlight work that directly explores the potential for applications of nanodevices that exploit folding pathways.

Molecular detection. Molecular beacons (FIG. 7A) are used to signal the presence of nucleic-acid pathogens and to monitor amplification by PCR^{63,64}, both in cells and *in vitro*. To detect a target DNA strand, the beacon initially forms a hairpin structure closed by a short helix, in which the loop sequence includes the complement of the target strand. One end of the sequence is linked to a fluorophore, whereas the other end is linked to a quencher. Binding of complement to target causes the hairpin to open, thereby separating the fluorophore and quencher, and causing a fluorescent signal to be emitted. Hamaguchi *et al.*⁶⁵, and Nutiu and Li⁶⁶ showed that features of molecular beacons and DNA aptamers could be integrated to obtain devices that signal on detection of proteins. For example, by incorporating the aptamer into the hairpin of the molecular beacon, the hairpin sequence can bind to the protein target of the aptamer, so that the hairpin opens and a fluorescent signal ensues. Stojanovic *et al.*⁶⁷ showed that, by combining both an aptamer for a reagent and a DNA enzyme in a single strand, it is possible to bring the enzyme and reagent together, thereby catalysing a reaction and detecting the signal.

The signal produced by molecular beacons is, at best, proportional to the amount of target to which the beacons are exposed, as each beacon can bind to, at most, one target. Dirks and Pierce⁶⁸ introduce the hybridization chain reaction (HCR) as a means to amplify the signal of an aptamer (sensor) for a DNA target (FIG. 7B). HCR reactions have the property that the molecular weight of the amplification polymers varies inversely with the concentration of the target molecule.

In a different chemical-sensing scheme, Heller *et al.*⁶⁹ show that dsDNA that is adsorbed onto a single-walled carbon nanotube can switch from the B to the Z form in the presence of cations, which are attracted to the negatively charged DNA backbone. This causes a detectable change in emission energy from the nanotube; moreover, the transition is reversible on removal of the ions. The DNA-coated nanotubes have been used to detect cations in the blood and tissues of living mammalian cells.

Diagnosis and drug release. Benenson *et al.*^{70,71} designed a DNA device that performs several diagnostic tests for high or low concentrations of certain DNA molecules. The device embodies a simple 'if-then' computation: if the results of all tests are positive, then the device releases a drug (FIG. 7C). Their drug is a short antisense DNA that can bind to the mRNA transcribed from harmful genes, such as cancer-causing genes, thereby preventing their expression. The device progresses through several diagnostic states, which indicate the outcomes (positive or negative) of the diagnostic tests performed so far. These states are concretely represented by intermediate structures formed by the DNA components of the device. In the initial structure, the drug sequence is included in the loop of a hairpin closed by a long helix. On each positive diagnosis, the helix becomes shorter (through the action of an enzyme and other participating DNA strands), so that the loop sequence is completely cleaved from its closing helix and is therefore released only if all diagnoses are positive.

Binding and release of a protein target. Dittmer *et al.*⁷² constructed a DNA device that can bind and release thrombin in a controlled fashion (FIG. 7D). The ability to perform such a function is an important step towards the goal of building artificial systems that can emulate the functions performed by molecules in the cell. One of the strands used in their device is the DNA aptamer for thrombin, with additional unpaired bases, called sticky ends, which do not interfere with the formation of the thrombin-binding G-quartet structure of the aptamer. This DNA aptamer is used to bind thrombin, thereby preventing it from performing other functions. To release thrombin, a second DNA strand is introduced, which binds with the sticky end of the aptamer and then displaces thrombin. A third strand can be introduced that binds with the second; this releases the thrombin-binding strand, so that it once again binds with thrombin. By alternating addition of the second and third strands, the device can repeatedly cycle between its binding and releasing functions.

Random-access memory

A means of storing digital information, in which data can be accessed and changed by directly accessing the location where they are stored; this is contrary to serial access memory, such as a tape, where it is necessary to access the data in serial order.

Programmable logic array

An array of logic gates that can be programmed to compute simple logic functions.

DNA tweezers

A device made from DNA, in which the angle subtended by two connected rigid helices can be made large or small by the use of additional DNA 'fuel' strands, thereby emulating the opening and closing of tweezers.

Challenges and opportunities. Using DNA devices *in vivo* presents formidable challenges, such as the need to power the devices with DNA (or other) fuel or to handle waste products. Dittmer *et al.*^{73,74} suggest that the instructions for the synthesis of DNA machine components could be encoded in an artificial gene, and that expression of the gene could then control the workings of the device. They give a simple demonstration *in vitro*. However, the issue of waste-product disposal was not addressed in this work. Other long-term uses of DNA motors could be in the development of systems that are able to undergo synthesis⁷⁵, self-replication or evolution⁷⁶; such systems in the cell rely on mechanisms for controlled transport and release of materials — tasks that DNA can, in principle, perform.

There are undoubtedly many other ways in which DNA-structure switching or folding pathways could be used to keep track of the states of a system; recent work in the use of structure to encode states of a molecular machine suggest some intriguing possibilities^{77,78}.

Conclusions

As shown by the examples above, there has been tremendous recent progress in the design and controlled

use of DNA molecules. The stability and sequence properties of DNA are ideal for information storage and processing in a biological setting, and can facilitate the synthesis and discovery of new molecules. The structural properties of DNA make it effective as a rigid 2D or even 3D scaffold for the assembly of molecules and electronic components. The folding pathway of DNA allows it to work as a mechanical device and adapt to changing environmental conditions.

In turn, the process of designing structures and devices from DNA helps to advance our understanding of the properties of DNA and, more generally, of how complex biological systems work.

In the future, the ability to build devices that integrate several functions of DNA, such as the discovery of new reactions with the synthesis and directed evolution of promising reagents, could further enhance the usefulness of DNA. Additionally, the insertion of genes that encode DNA molecules or devices with diagnostic or therapeutic applications into synthetic gene networks could provide a means for expression of those devices in cells or in other diagnostic environments^{79,80}. DNA might eventually do itself out of business by allowing the synthesis, discovery and control of molecules with more complex functions.

- Breaker, R. R. Natural and engineered nucleic acids as tools to explore biology. *Nature* **432**, 838–845 (2004).
- Emilsson, G. M. & Breaker, R. R. Deoxyribozymes: new activities and new applications. *Cell. Mol. Life Sci.* **59**, 596–607 (2002).
- Joyce, G. F. Directed evolution of nucleic acid enzymes. *Ann. Rev. Biochem.* **73**, 791–836 (2004).
- Brenner, S. & Lerner, R. A. Encoded combinatorial chemistry. *Proc. Natl Acad. Sci. USA* **89**, 5381–5383 (1992).
- Janeway, C. A., Travers, P., Walport, M. & Capra, J. D. *Immunobiology: The Immune System in Health and Disease* 6th edn (Garland Science Publishing, New York, 2005).
- Niemeyer, C. M., Adler, M. & Wacker, R. Immuno-PCR: high sensitivity detection of proteins by nucleic acid amplification. *Trends Biotechnol.* **23**, 208–216 (2005).
- Sano, T., Smith, C. L. & Cantor, C. R. Immuno-PCR: very sensitive antigen detection by means of specific antibody–DNA. *Science* **258**, 120–122 (1992).
- Nam, J.-M., Thaxton, C. S. & Mirkin, C. A. Nanoparticle-based bio-bar codes for the ultrasensitive detection of proteins. *Science* **301**, 1884–1886 (2003).
Introduces the BCA method for the highly sensitive detection of proteins. Subsequent papers extend the use of the method to disease diagnosis.
- Nam, J.-M., Stoeva, S. & Mirkin, C. A. Bio-bar-code-based DNA detection with PCR-like sensitivity. *J. Am. Chem. Soc.* **126**, 5932–5933 (2004).
- Georganopoulou, D. G. *et al.* Nanoparticle-based detection in cerebral spinal fluid of a soluble pathogenic biomarker for Alzheimer's disease. *Proc. Natl Acad. Sci. USA* **102**, 2273–2276 (2005).
- Nam, J.-M., Wise, A. R. & Groves, J. T. Colorimetric bio-barcode amplification assay for cytokines. *Anal. Chem.* **77**, 6985–6988 (2005).
- Fredriksson, S. *et al.* Protein detection using proximity-dependent DNA ligation assays. *Nature Biotechnol.* **20**, 473–477 (2002).
- Gartner, Z. J. & Liu, D. R. The generality of DNA-templated synthesis as a basis for evolving non-natural small molecules. *J. Am. Chem. Soc.* **123**, 6961–6963 (2001).
- Rosenbaum, D. M. & Liu, D. R. Efficient and sequence-specific DNA-templated polymerization of peptide nucleic acid aldehydes. *J. Am. Chem. Soc.* **125**, 13924–13925 (2003).
- Gartner, Z. J. *et al.* DNA-templated organic synthesis and selection of a library of macrocycles. *Science* **305**, 1601–1605 (2004).
Illustrates the use of DTS to create a pool of molecules from which those with new functional properties might be selected.
- Halpin, D. R. & Harbury, P. B. DNA display I. Sequence-encoded routing of DNA populations. *PLoS Biol.* **2**, 1015–1021 (2004).
- Halpin, D. R. & Harbury, P. B. DNA display II. Genetic manipulation of combinatorial chemistry libraries for small-molecule evolution. *PLoS Biol.* **2**, e174 (2004).
Reports a method for the synthesis, selection and evolution of new compounds in parallel from a large pool.
- Halpin, D. R., Lee, J. A., Wrenn, S. J. & Harbury, P. B. DNA display III. Solid-phase organic synthesis on unprotected DNA. *PLoS Biol.* **2**, e175 (2004).
- Calderone, C. T., Puckett, J. W., Gartner, Z. J. & Liu, D. R. Directing otherwise incompatible reactions in a single solution by using DNA-templated organic synthesis. *Angew. Chem. Int. Ed.* **41**, 4104–4108 (2002).
- Kanan, M. W., Rozeman, M. M., Sakurai, K., Snyder, T. M. & Liu, D. R. Reaction discovery enabled by DNA-templated synthesis and *in vitro* selection. *Nature* **431**, 545–549 (2004).
- Adleman, L. Molecular computation of solutions to combinatorial problems. *Science* **266**, 1021–1024 (1994).
Introduces the principle of DNA computation by solving a tiny combinatorial problem in a test tube.
- Braich, R. S., Chelyapov, N., Johnson, C., Rothmund, P. W. K. & Adleman, L. Solution of a 20-variable 3-SAT problem on a DNA computer. *Science* **296**, 499–502 (2002).
- Williams, K. A., Veenhuizen, P. T. M., de la Torre, B. G., Eritja, R. & Dekker, C. Carbon nanotubes with DNA recognition. *Nature* **420**, 19–26 (2002).
- Xin, H. & Woolley, A. T. DNA-templated nanotube localization. *J. Am. Chem. Soc.* **125**, 8710–8711 (2003).
- Braun, E., Eichen, Y., Sivan, U. & Ben-Yoseph, G. DNA-templated assembly and electrode attachment of a conducting silver wire. *Nature* **391**, 775–778 (1998).
- Keren, K., Berman, R. S., Buchstab, E., Sivan, U. & Braun, E. DNA-templated carbon nanotube field-effect transistor. *Science* **302**, 1380–1382 (2003).
- Zheng, M. *et al.* Structure-based carbon nanotube sorting by sequence-dependent DNA assembly. *Science* **302**, 1545–1548 (2003).
- Staii, C. & Johnson, A. T. DNA-decorated carbon nanotubes for chemical sensing. *Nano Lett.* **5**, 1774–1778 (2005).
- Storhoff, J. J. & Mirkin, C. A. Programmed materials synthesis with DNA. *Chem. Rev.* **99**, 1849–1862 (1999).
- Niemeyer, C. M. Nanoparticles, proteins, and nucleic acids: biotechnology meets materials science. *Angew. Chem. Int. Ed.* **40**, 4128–4158 (2001).
- Niemeyer, C. M., Koehler, J. & Wuerdemann, C. DNA-directed assembly of bienzymic complexes from *in vivo* biotinylated NAD(P)H:FMN oxidoreductase and luciferase. *ChemBioChem* **02-03**, 242–245 (2002).
- Bock, L. C., Griffin, L. C., Latham, J. A., Vermaas, E. H. & Toole, J. J. Selection of single-stranded DNA molecules that bind and inhibit human thrombin. *Nature* **355**, 564–566 (1992).
- Li, Y. *et al.* Multiplexed detection of pathogen DNA with DNA-based fluorescence nanobarcodes. *Nature Biotechnol.* **23**, 885–889 (2005).
- Stavis, S. M. *et al.* Detection and identification of nucleic acid engineered fluorescent labels in submicrometre fluidic channels. *Nanotechnology* **16**, S314–S323 (2005).
- Seeman, N. C. DNA nanotechnology: novel DNA constructions. *Annu. Rev. Biophys. Biomol. Struct.* **27**, 225–248 (1998).
- Seeman, N. C. DNA in a material world. *Nature* **421**, 427–431 (2003).
- Fu, T.-J. & Seeman, N. C. DNA double crossover molecules. *Biochemistry* **32**, 3211–3220 (1993).
- Winfree, E., Liu, F., Wenzler, L. A. & Seeman, N. C. Design and self-assembly of two-dimensional DNA crystals. *Nature* **394**, 539–544 (1998).
Illustrates the principle of algorithmic self-assembly computation, through the construction of rigid 2D lattices from rigid DX molecules.
- Yan, H., Park, S.-H., Finkelstein, G., Reif, J. H. & LaBean, T. H. DNA-templated self-assembly of protein arrays and highly conductive nanowires. *Science* **301**, 1882–1884 (2003).
Describes the construction and use of 2D DNA lattices for the organization of proteins, and the construction of DNA nanotubes.
- Park, S.-H. *et al.* Programmable DNA self-assemblies for nanoscale organization of ligands and proteins. *Nano Lett.* **5**, 729–733 (2005).

41. Liu, Y., Lin, C., Li, H. & Yan, H. Aptamer-directed self-assembly of protein arrays on a DNA nanostructure. *Angew. Chem. Int. Ed.* **44**, 4333–4338 (2005).
42. Rothemund, P. Folding DNA to create nanoscale shapes and patterns. *Nature* **440**, 297–302 (2006). **Reports on the scaffolded origami technique for constructing complex 2D patterns.**
43. Shih, W. M., Quispe, J. D. & Joyce, G. F. A 1.7-kilobase single-stranded DNA that folds into a nanoscale octahedron. *Nature* **427**, 618–621 (2004). **Describes the construction of the first 3D rigid shape — an octahedron — from DNA, using short scaffold DNA strands to direct the folding of a long DNA strand.**
44. Goodman, R. P. *et al.* Rapid chiral assembly of rigid DNA building blocks for molecular nanofabrication. *Science* **310**, 1661–1665 (2005).
45. von Kiedrowski, G. *et al.* Toward replicatable, multifunctional, nanoscaffolded machines. a chemical manifesto. *Pure Appl. Chem.* **75**, 609–619 (2003).
46. Scheffler, M., Dorenbeck, A., Jordan, S., Wustefeld, M. & von Kiedrowski, G. Self-assembly of trisiliconucleotidyls: the case for nanoacetylene and nano-cyclobutadiene. *Angew. Chem. Int. Ed.* **38**, 3311–3315 (1999).
47. Eckhardt, L. H. *et al.* DNA nanotechnology: chemical copying of connectivity. *Nature* **420**, 286 (2002).
48. Rothemund, P. W. K. *et al.* Design and characterization of programmable DNA nanotubes. *J. Am. Chem. Soc.* **126**, 16344–16353 (2004).
49. Liu, D., Park, S.-H., Reif, J. H. & LaBean, T. H. DNA nanotubes self-assembled from TX tiles as templates for conductive nanowires. *Proc. Natl Acad. Sci. USA* **101**, 717–722 (2004).
50. Le, J. D. *et al.* DNA-templated self-assembly of metallic nanocomponent arrays on a surface. *Nano Lett.* **4**, 2343–2347 (2004).
51. Winfree, E. On the computational power of DNA annealing and ligation. *DIMACS Ser. Discrete Math. Theoret. Comput. Sci.* **27**, 199–219 (1996).
52. Mao, C., LaBean, T. H., Reif, J. H. & Seeman, N. C. Logical computation using algorithmic self-assembly of DNA triple-crossover molecules. *Nature* **407**, 493–496 (2000).
53. Yan, H., LaBean, T. H., Feng, L. & Reif, J. H. Directed nucleation assembly of DNA tile complexes for barcode-patterned lattices. *Proc. Natl Acad. Sci. USA* **100**, 8103–8108 (2003).
54. Rothemund, P. W. K., Papadakis, N. P. & Winfree, E. Algorithmic self-assembly of DNA Sierpinski triangles. *PLoS Biol.* **2**, e424 (2004).
55. Winfree, E. & Bekbolatov, R. Proofreading tile sets: error correction for algorithmic self-assembly. *Lect. Notes Comput. Sci.* **2943**, 126–144 (2004).
56. Chen, H.-L. & Goel, A. Error free self-assembly using error prone tiles. *Lect. Notes Comput. Sci.* **3384**, 62–75 (2005).
57. Dwyer, C., Lebeck, A. R. & Sorin, D. J. Self-assembled architectures and the temporal aspects of computing. *Computer* **38**, 56–64 (2005).
58. Breaker, R. Engineered allosteric ribozymes as biosensor components. *Curr. Opin. Biotechnol.* **13**, 31–39 (2002).
59. Yurke, B., Turberfield, A. J., Mills, A. P., Simmel, F. C. & Neumann, J. L. A DNA-fuelled molecular machine made of DNA. *Nature* **406**, 605–608 (2000).
60. Simmel, F. C. & Dittmer, W. U. DNA nanodevices. *Small* **1**, 284–299 (2005).
61. Jovin, T. M., Soumpasis, D. M. & McIntosh, L. P. The transition between B-DNA and Z-DNA. *Annu. Rev. Phys. Chem.* **38**, 521–558 (1987).
62. Mao, C. D., Sun, W. Q., Shen, Z. Y. & Seeman, N. C. A nanomechanical device based on the BZ transition of DNA. *Nature* **397**, 144–146 (1999).
63. Tyagi, S. & Kramer, F. R. Molecular beacons: probes that fluoresce upon hybridization. *Nature Biotechnol.* **14**, 303–308 (1996).
64. Anthony, T. & Subramaniam, V. Molecular beacons: nucleic acid hybridization and emerging applications. *J. Biomol. Struct. Dyn.* **19**, 497–504 (2001).
65. Hamaguchi, N., Ellington, A. & Stanton, M. Aptamer beacons for the direct detection of proteins. *Anal. Biochem.* **294**, 126–131 (2001).
66. Nutiu, R. & Li, Y. Structure-switching signaling aptamers. *J. Am. Chem. Soc.* **125**, 4771–4778 (2003).
67. Stojanovic, M. N., de Prada, P. & Landry, D. W. Catalytic molecular beacons. *ChemBiochem* **2**, 411–415 (2001).
68. Dirks, R. M. & Pierce, N. A. Triggered amplification by hybridization chain reaction. *Proc. Natl Acad. Sci. USA* **101**, 15275–15278 (2004).
69. Heller, D. A. *et al.* Optical detection of DNA conformational polymorphism on single-walled carbon nanotubes. *Science* **311**, 508–511 (2006).
70. Benenson, Y. *et al.* Programmable and autonomous computing machine made of biomolecules. *Nature* **414**, 430–434 (2001).
71. Benenson, Y., Gil, B., Ben-Dor, U., Adar, R. & Shapiro, E. An autonomous molecular computer for logical control of gene expression. *Nature* **429**, 423–429 (2004). **Describes the design and uses of a computational DNA device in vitro to control the release of a drug.**
72. Dittmer, W. U., Reuter, A. & Simmel, F. C. A DNA-based machine that can cyclically bind and release thrombin. *Angew. Chem. Int. Ed.* **43**, 3550–3553 (2004). **Presents the design of a mechanical device, built from DNA, which can bind and release thrombin.**
73. Dittmer, W. U. & Simmel, F. C. Transcriptional control of DNA-based nanomachines. *Nano Lett.* **4**, 689–691 (2004).
74. Dittmer, W. U., Kempter, S., Radler, J. O. & Simmel, F. C. Using gene regulation to program DNA-based molecular devices. *Small* **1**, 709–712 (2005).
75. Liao, S. & Seeman, N. C. Translation of DNA signals into polymer assembly instructions. *Science* **306**, 2702–2704 (2004).
76. Yurke, B. & Mills, A. P. Using DNA to power nanostructures. *Gen. Prog. Evol. Mach.* **4**, 111–122 (2003).
77. Uejima, H. & Hagiya, M. Secondary structure design of multi-state DNA machines based on sequential structure transitions. *Lect. Notes Comp. Sci.* **2943**, 74–85 (2004).
78. Stojanovic, M. N. & Stefanovic, D. A deoxyribozyme-based molecular automaton. *Nature Biotechnol.* **21**, 1069–1074 (2003).
79. McDaniel, R. & Weiss, R. Advances in synthetic biology: on the path from prototypes to applications. *Curr. Opin. Biotechnol.* **16**, 476–483 (2005).
80. Weiss, R. Challenges and opportunities in programming living cells. *The Bridge* **33**, 39–46 (2003).
81. Macaya, R. F., Schultze, P., Smith, F. W., Roe, J. A. & Feigon, J. Thrombin-binding DNA aptamer forms a unimolecular quadruplex. *Proc. Natl Acad. Sci. USA* **90**, 3745–3749 (1993).
82. Wang, H. *An Unsolvable Problem on Dominoes. Technical Report BL-30 (II-15)* (Harvard Computation Laboratory, Cambridge, Massachusetts, 1962).

Competing interests statement

The author declares no competing financial interests.

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