Designed DNA molecules: principles and applications of molecular nanotechnology

Anne Condon

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.

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 ability to release, grab or cleave target molecules and even to control the release of drugs, depending on the outcome of certain diagnostic tests.

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; this paper includes only examples of designed DNA molecules, or molecules that combine both selected and designed.
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 nano-electronic 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 nm$^3$ — 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.
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 codes 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 functional 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 diseases. Detection methods can also identify allergens and pollutants. 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 small.

Early DNA-based detection methods, such as immuno-PCR⁶, involved many cumbersome steps, including PCR. Bio-barcode amplification (BCA), which has been developed by Nam and colleagues⁷, 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 cancer⁸,⁹), 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). Georgopanopoulos and colleagues¹² 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.¹³ 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)¹¹ 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 Liu⁴ 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
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 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. 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. 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 Adleman showed, 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. 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 nano electronic 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 complement. DNA strands have been used to spatially position CNTs, making it possible to assemble them into circuits. 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.

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 CNT. DNA-wrapped CNTs also enhance the sensitivity of nanotube devices in the detection of gases. Storhoff and Mirkin 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 reactions.
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 pool. 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 plasma.

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. and Stavis et al. 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. used branched DNA structures called nanobarcodes, with red or green fluorophores on the branch tips (Fig. 4a). Nanobarcodes are distinguishable, using fluorescence 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. 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 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.
Crossover structure of the molecule.

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 5| Exploiting DNA structure: double-crossover structures and self assembly. a | A double-crossover (DX) molecule 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 crossover. 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 µm × 1 µm and the inset is 150 nm × 150 nm). c | Smiley face constructed using the scaffolded origami technique. 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. 42 © (2005) American Chemical Society. Panel b adapted with permission from REF. 40 © (2005) American Chemical Society. Panel c reproduced with permission from REF. 42 © (2006) Macmillan Publishers Ltd.

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.

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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 Seeman (FIG. 5a) is an early, elegant and widely used design, from which the first 2D DNA lattices were built by Winfree et al. Other designs and applications have followed, 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. and Park et al., 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. 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 origami, a long strand folds in an intricate way, aided by short strands that direct the folding into the desired configuration. Shih et al. 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. Rothemund illustrated the generality of a different method, known as scaffolded origami, by constructing smily faces, a world map and many other complex 2D patterns (FIG. 5c). To design a pattern, Rothemund uses a computational tool...
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\(^\text{39,48}\). Liu et al\(^\text{39}\) 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\(^\text{40}\) 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. Winfree\(^\text{31}\) 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\(^\text{34}\) and Yan et al\(^\text{36}\) 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 Rothemund et al\(^\text{34}\) (Fig. 6).
self-assembly of electronic components raises new research challenges, and opportunities for the design and modelling of circuit architectures.

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 molecules.
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 tweezers,

\[ \text{DNA tweezers} \]

and can also cause the movement of the DNA molecules. Simmel and Dittmer\(^\text{59}\) 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)\(^\text{61,62}\). 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\(^\text{63,64}\), 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.\(^\text{65}\), and Nutiu and Li\(^\text{66}\) 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.\(^\text{67}\) 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 Pierce\(^\text{68}\) 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.\(^\text{69}\) 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.\(^\text{70,71}\) 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.\(^\text{72}\) 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.
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.\textsuperscript{3,4} 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 synthesis\textsuperscript{9}, self-replication or evolution\textsuperscript{8}; 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\textsuperscript{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\textsuperscript{79,80}. DNA might eventually do itself out of business by allowing the synthesis, discovery and control of molecules with more complex functions.

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Competing interests statement
The author declares no competing financial interests.

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