DNA nanotechnology

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Abstract | DNA is the molecule that stores and transmits genetic information in biological systems. The field of DNA nanotechnology takes this molecule out of its biological context and uses its information to assemble structural motifs and then to connect them together. This field has had a remarkable impact on nanoscience and nanotechnology, and has been revolutionary in our ability to control molecular self-assembly. In this Review, we summarize the approaches used to assemble DNA nanostructures and examine their emerging applications in areas such as biophysics, diagnostics, nanoparticle and protein assembly, biomolecule structure determination, drug delivery and synthetic biology. The introduction of orthogonal interactions into DNA nanostructures is discussed, and finally, a perspective on the future directions of this field is presented.

The fabrication of nanometre-scale structures uses two different approaches, 'top-down' and 'bottom-up' methods. The top-down approach starts from large structures and reduces their sizes to the required dimensions and patterns by means of an external assembly tool. This is the standard strategy in manufacturing, but it becomes increasingly difficult to reach smaller sizes towards the molecular scale. By contrast, the bottom-up approach uses the internal information of molecules to guide their autonomous self-assembly into nanostructures. Compared with top-down methods, it presents the advantage of large chemical diversity and highly parallel synthesis — even a picomole synthesis makes 10^{12} copies in a single operation[1]. Molecular self-assembly has yielded numerous examples of symmetric or periodic structures with exquisitely organized features. However, the assembly of molecules into complex asymmetric patterns — which may be needed to create useful functionality — is far more challenging.

It is in this forum that DNA-based assembly has truly transformed nanoscience (FIG. 1). DNA has the most predictable and programmable interactions of any natural or synthetic molecule. It possesses remarkable binding specificity and thermodynamic stability and can be created with a nearly infinite choice of sequences that bind reliably to their complementary partners. It is structurally well defined on the nanometre scale and has a persistence length of ~50 nm under conventional conditions. It can be rapidly synthesized and modified using automated methods, and a large variety of DNA-acting enzymes can controllably further tune and modify its structure.

The early years: DNA tile assembly

In 1980, while thinking about six-arm DNA branched junctions, one of the authors, Ned Seeman, realized that these branched junctions could be connected together by the base pairing of single-stranded overhangs, called sticky ends, into a 3D crystalline material. Thus, rather than relying on trial and error to crystallize biological macromolecules, the notion was that crystals could be deliberately assembled using predictable interactions (Watson–Crick base pairing) (FIG. 2a). Ned's dream was to assemble branched junctions into a 3D crystal and to use this crystal as a scaffold for the 3D organization of proteins and other biomacromolecules, thereby solving the macromolecular crystallization problem[2] (FIG. 2b).

However, DNA branched junctions (for example, the four-arm Holliday junction) had two limitations: their geometric flexibility and instability as a result of branch migration due to two-fold symmetry at their branch points. In 1983, Seeman reported that Holliday junctions could be rendered immobile by making arms with unique sequences and hence lacking the detrimental two-fold symmetry[3]. During the next decade, a 3D DNA cube was constructed by the connection of three-arm junctions[4], and double-crossover (DX) molecules comprising two DNA double helices linked together by two strand exchanges, rather than the single exchange involved in the Holliday junction, were reported[5]. A strand exchange is a process whereby a strand starts on one helix and switches to the next. These DX molecules offered the geometric rigidity and stability necessary to start building extended DNA structures with controlled geometry, connectivity and topology[6]. Subsequently, DX molecules were used to assemble 2D DNA crystals by design[7] (FIG. 2c). These DNA building blocks — often called DNA tiles — are made from DX molecules containing sticky ends and can grow autonomously into 2D crystals, which can be characterized by atomic force microscopy (AFM). DNA hairpins placed on these tiles perpendicular to the plane of assembly showed that it

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doi:10.1038/natrevmats.2017.68
Published online 8 Nov 2017

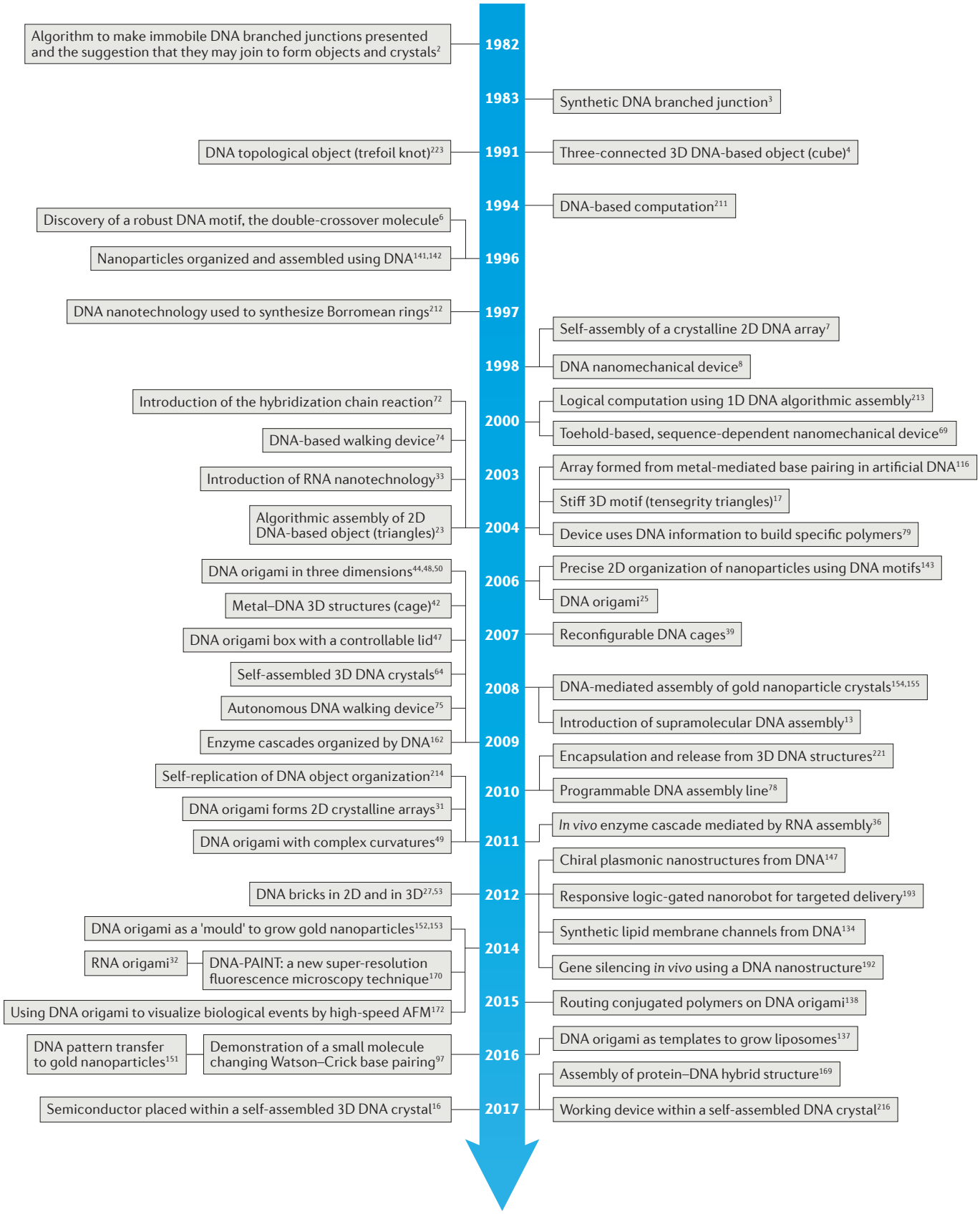


Figure 1 | **A timeline of the field of DNA nanotechnology.** Although not a comprehensive account, discoveries and major developments are listed. AFM, atomic force microscopy; DNA-PAINT, DNA points accumulation for imaging in nanoscale topography.

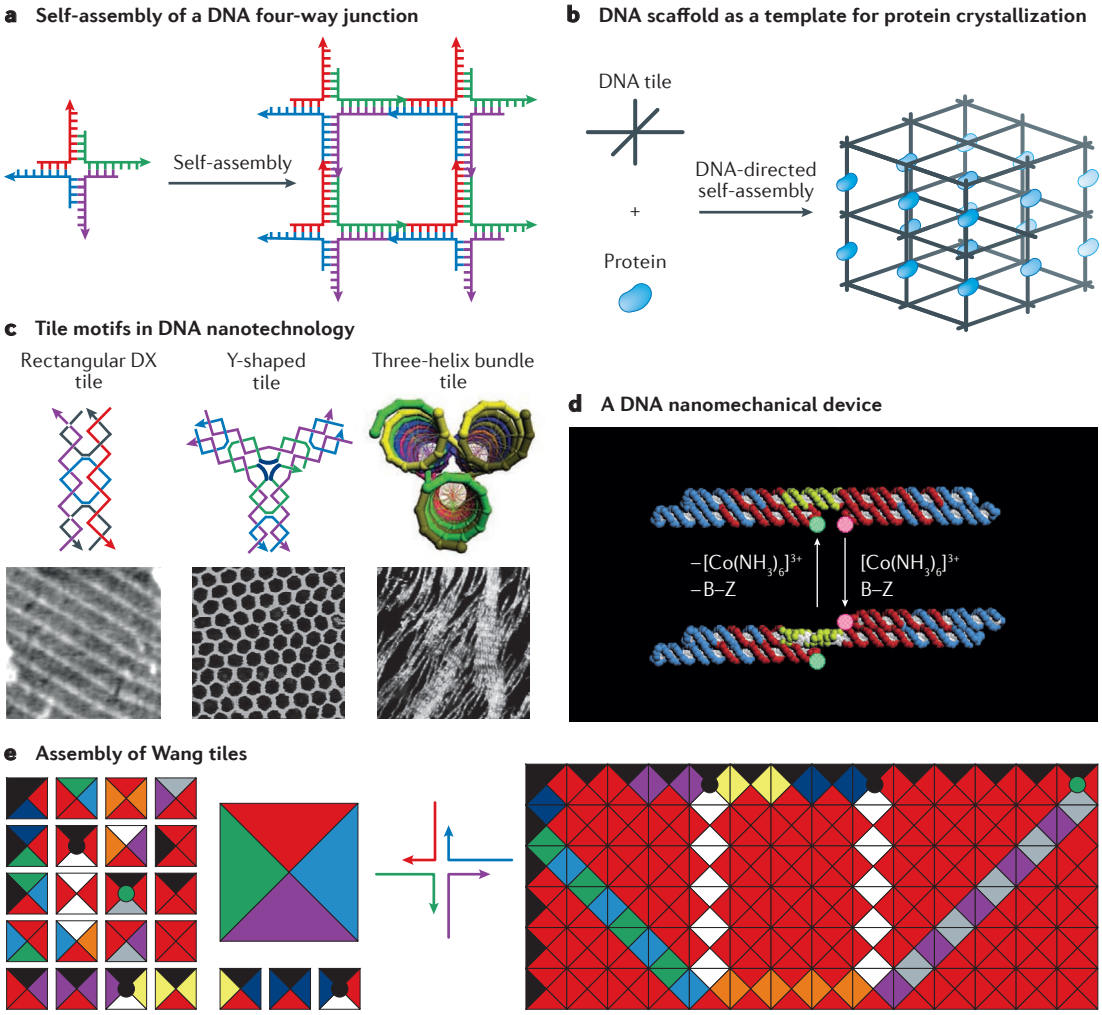


Figure 2 | **The beginning of DNA nanotechnology. a** | A DNA four-way junction with self-complementary, single-stranded ends ('sticky ends') self-assembles into a quadrilateral shape. **b** | A DNA scaffold that aids protein (blue) crystallization. **c** | DNA nanotechnology motifs. The top panels show tile motifs in DNA nanotechnology, and the bottom panels show atomic force microscopy images of their assemblies into lattices. The left panels show an example of a double-crossover (DX) tile[7]; the middle panels show an example of Y-shaped DNA motif self-assembly into hexagonal 2D lattices; and the right panels show an example of three-helix bundles[9]. **d** | Nanomechanical DNA device. When the middle (yellow) DNA section is in the B– or right-handed helical form, the fluorescent donor and acceptor are close together, and fluorescence resonance energy transfer (FRET) occurs. When $[Co(NH_3)_6]^{3+}$ is added, this DNA section undergoes a transition to the Z– or left-handed helix form, separating the two fluorophores and reducing FRET[8]. **e** | Wang tiles are squares with four differently coloured sides that assemble with their neighbours only if the abutting edges are the same colour. In algorithmic DNA assembly, each Wang tile colour can be a specific DNA sequence, leading to the assembly of aperiodic patterns[23]. Panel **c** (left, bottom) is adapted with permission from REF. 7, Macmillan Publishers Limited. Panel **c** (middle, bottom and top) is adapted with permission from REF. 14, American Chemical Society. Panel **c** (top, right) is adapted with permission from REF. 9, Wiley-VCH. Panel **c** (bottom, right) is adapted with permission from REF. 215, American Chemical Society. Panel **d** is adapted with permission from REF. 8, Macmillan Publishers Limited. Part **e** is adapted with permission from REF. 208, Dover Press.

was possible to predesign any periodicity in the placement of these hairpins[7] (FIG. 2c, left). This was the first time that the information in DNA had been used to grow predesigned crystalline arrays.

In 1999, using rigid DX molecules, the first prototype of a DNA nanomechanical device was unveiled[8]. More specifically, the structure comprised two DNA DX motifs separated by a DNA portion that is capable of being reversibly switched between the normal right-handed (B-DNA) double helix and the left-handed (Z-DNA) double helix (FIG. 2d). Two fluorescent reporters were attached to the edges of the DX units. The structure reversibly twisted about the central DNA portion over multiple cycles by the addition and removal of $[Co(NH_3)_6]^{3+}$ (REF. 8).

Between 1980 and 1998, many examples of the rational design of 2D and 3D discrete and extended DNA structures were reported[9]. Moreover, it was shown that DNA structures can be used as dynamic components of molecular machines. It is important to note that this field could not have been possible without

the important discoveries in DNA solid-phase synthesis, which gave ready access to DNA strands of any sequence[10]. Automated DNA synthesis has been transformational in numerous fields, including molecular biology, synthetic biology, DNA-based computation, oligonucleotide therapeutics, molecular diagnostics and sequencing[11,12].

On the basis of this groundwork, the area of structural DNA nanotechnology started taking off. DX tiles were followed by numerous other tile motifs in which DNA strands were connected via strand exchange. These tiles were used to create highly ordered 2D crystalline surfaces of deliberately designed geometries and periodicities[13]. For example, 2D networks were created from other types of crossovers, such as triple-crossover and cross motifs, and different monomer morphologies, such as three-point[14] and six-point[15] star motifs, tensegrity triangles[16,17] and T-junctions[18]. In 'directed nucleation assembly', a DNA single strand was used to template the assembly of DX tiles around itself into well-defined discrete structures rather than simple 2D crystals[19]. More recently, a simple method to construct long single-stranded DNA templates (>1,000 bases) of arbitrary sequences from repetitive motifs was reported[20]. These were used to direct the assembly of DX tiles into linear DNA structures of well-defined length and aperiodic patterning from a minimum number of starting units[21]. The concept of sequence symmetry at positions of DNA tile motifs was introduced to reduce shape distortion and allow the growth of large 2D arrays from a minimum number of strands[22]. Interestingly, algorithmic self-assembly and principles of computer science were used to show that, in principle, aperiodic crystals can be grown from DNA. For example, 'Wang tiles' are squares with four differently coloured sides that can assemble with their neighbours only if the abutting edges are of the same colour[23] (FIG. 2e). In algorithmic DNA tiles, each Wang tile colour is a specific DNA sequence. Using a nucleating strand of defined sequence, these algorithmic tiles can grow into aperiodic lattices. However, errors occur in the growth process, arising from off-template growth, and tile misplacement is among the errors observed. Error-free algorithmic DNA assembly is a goal yet to be attained but will undoubtedly be revisited now that we have more robust tools[24].

DNA origami and brick assembly

In 2006, a publication by Paul Rothemund[25] (then a post-doctoral fellow at the California Institute of Technology) transformed the landscape of DNA nanotechnology. In this article, a method for DNA construction was proposed based on a long, viral 'scaffold' single strand of DNA that folds onto itself into a desired pattern (FIG. 3a). This is achieved by the addition of short 'staple strands' that bring together selected parts of the DNA single strand (FIG. 3a, left). Accordingly, non-periodic 2D structures of arbitrary complexity can be made, such as a map of the Americas, rectangles, smiley faces, stars and other designed patterns[25] (FIG. 3a, right). Although the concept of DNA origami can be attributed solely to Paul Rothemund, previous work, including the construction of an octahedron by folding a continuous DNA strand with the aid of short strands[26] and a DNA 'barcode' system[19], may have provided some inspiration.

In terms of complexity, aperiodicity and ease of self-assembly in general, the DNA origami approach — in which each construct typically contains approximately 200 addressable points in an area of 8,000–10,000 nm^2 — was unprecedented. An appealing feature of DNA origami is that staple strands that interact with the scaffold are usually not purified, thus simplifying the assembly. Now, we can conceive of any arbitrary design, and with the aid of a simple computer interface (freely available) and very robust and efficient techniques, we can create this design in the laboratory. Any researcher, whether a chemist, biologist, physicist or computer scientist, has access to these structures from commercially available strands.

More recently, an approach that gives rise to a level of complexity similar to that of DNA origami but without the need for a long scaffolding strand was reported[27]. Termed 'single-stranded tile' or 'brick' assembly, it involves the computer-aided design of arbitrary structures and their assembly using hundreds of DNA single strands that form interconnected staggered duplexes (FIG. 3b). The building blocks are single strands of DNA containing four modular domains, which are designed to form interconnected staggered duplexes with one another, resulting in DNA lattices. As the sequences are all unique, these motifs can be used as a molecular canvas for which it is possible to make any arbitrary shape by selecting the set of strands that defines the structure.

A distinct limitation of these two approaches is their need for hundreds of DNA strands of different sequences to produce a single nanostructure. However, with the continuous decrease in the cost of oligonucleotide synthesis, this will become less problematic. Indeed, there appears to be a relative of Moore's law in the cost of DNA, whereby the effective cost is halved every 30 months[28,29]. With respect to scalability, an important first step was taken with DNA origami frames with hollow interiors being used as sites for the nucleation and growth of periodic tile arrays[30]. Moreover, cross-shaped origami molecules were shown to form 2D periodic arrays, as a demonstration of Wenyan Liu's rule, that is, DNA arrays in *N* dimensions should be propagated by motifs whose helix axes span those dimensions[31].

Recently, a powerful approach to building structures from single-stranded RNA has been reported[32]. RNA nanostructures were designed to form co-transcriptionally: because transcription is slow on the timescale of RNA assembly, the nascent RNA strand can be folded into a desired shape as soon as it is synthesized using hairpins and RNA-based association motifs. Earlier work[33–35] resulted in rationally designed RNA nanostructures, and the *in vivo* assembly of RNA nanostructures was demonstrated[36] (discussed in detail later). This addresses one of the objectives in synthetic biology: to fold RNA into complex functional shapes (like the ribosome). Synthetic biologists have used nucleic acid sequences to modulate function but not structure in two dimensions or three dimensions.

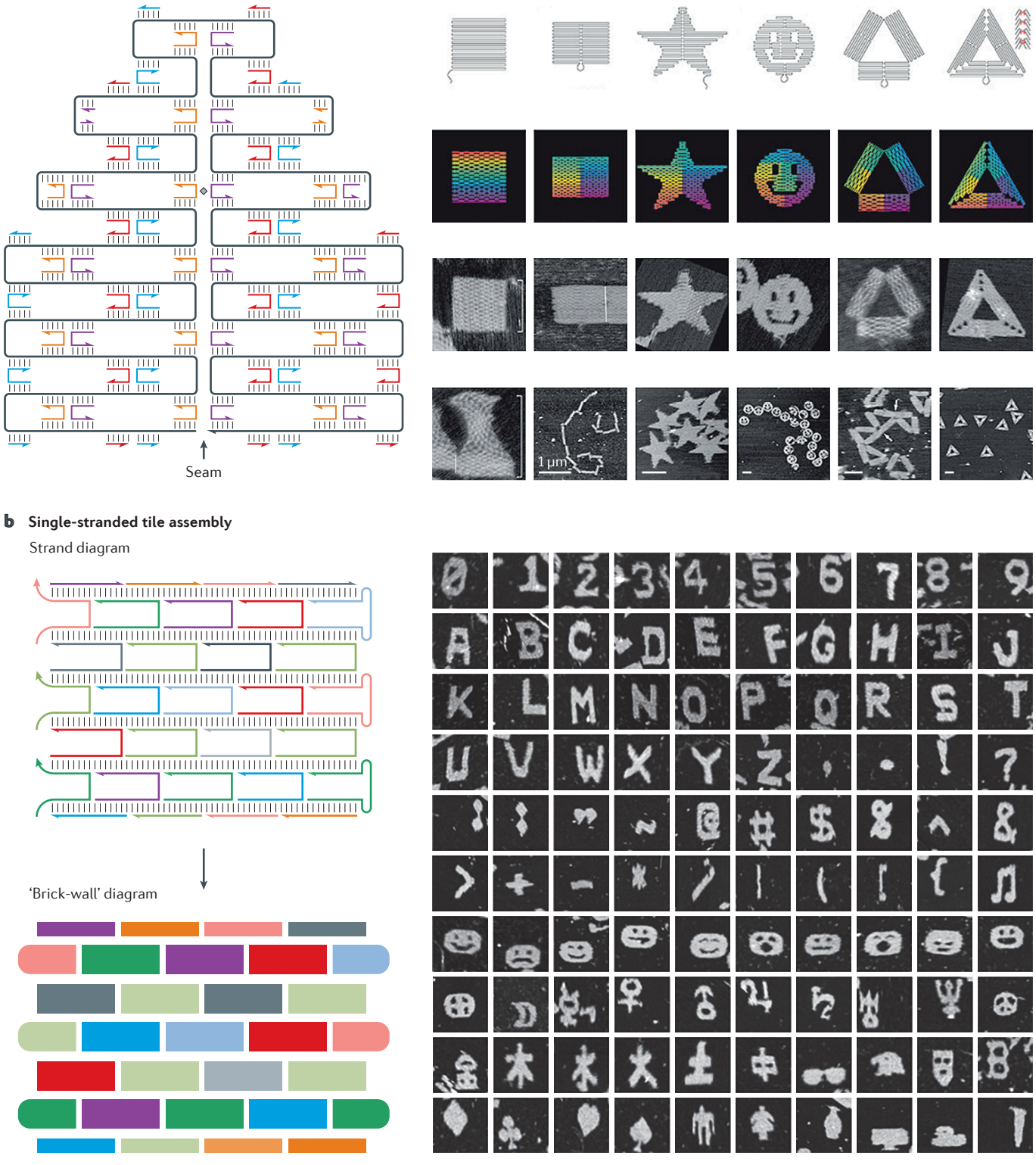


Figure 3 | **DNA origami and single-stranded tile assembly. a** | Scaffolded DNA origami. The left panel shows a long genomic DNA strand folded with the help of small staple strands to give a desired, computationally designed shape. The right panels show atomic force microscopy (AFM) images of DNA origami shapes. Scale bars are 100 nm unless noted otherwise[25]. **b** | Single-stranded tile assembly. The left panel shows single strands of DNA containing four domains that are computationally designed to associate into staggered duplexes, resulting in DNA lattices. The right panel shows AFM images of single-stranded tile assemblies[27]. Panel **a** is adapted with permission from REF. 25, Macmillan Publishers Limited. Panel **b** is adapted with permission from REF. 27, Macmillan Publishers Limited.

Three-dimensional DNA structures

In 1991, the first discrete 3D structure was reported, as described earlier[4] (FIG. 4a, left). These pioneering experiments demonstrated the feasibility of using DNA as a building block for 3D structures. Shortly thereafter, a solid-support technique was used to build a more complex, truncated octahedron[37]. Both structures were formed in low yields because the gel-based structural characterization used at the time could detect strands in closed cycles only. The advent of AFM and transmission electron microscopy to analyse DNA synthesis products enabled unclosed objects to be the targets of construction. Beginning with unclosed DX molecules, the products have been nearly quantitative in yield. An octahedron was constructed by annealing a 1.7-kilobase-long DNA strand with five short staple strands[26] (FIG. 4a, middle). Four different DNA single strands were programmed to form tetrahedra (FIG. 4a, right), the latter two syntheses resulting in significantly better yields[38]. Subsequently, a modular approach to construct a large number of 3D DNA structures, such as triangular, square, pentagonal and hexagonal prisms, was introduced[39] (FIG. 4b). DNA polyhedra, such as tetrahedra, dodecahedra, icosahedra and a buckyball, were assembled using a hierarchical approach that relies on the association of identical symmetrical DNA three-point-star or five-point-star tiles[40,41] (FIG. 4c). Metal–DNA cages were constructed using a face-centred approach[42].

DNA origami has been extended into 3D structures using a number of approaches. A general method to roll DNA origami sheets into a range of discrete 3D structures by connecting parallel DNA duplexes by crossover junctions at specific locations to ensure curvature of one helix with respect to the next was reported[43]. Subsequently, a stacking strategy of multiple layers of DNA helices into honeycomb[44] and square[45] lattices was shown to efficiently create complex and rigid 3D DNA origami. Moreover, twist and curvature can be introduced in multilayer DNA origami by targeted base insertions/deletions to adjust the distance between crossover junctions[46] (FIG. 4d). In a second strategy, the scaffold strand was assembled into eight-connected origami square sheets, which were used as the faces of a cubic box (FIG. 4e). The 'lid' of this 3D structure could be selectively opened or closed with the aid of added DNA strands[47]. In a third approach, a DNA tetrahedron was constructed by wrapping the scaffold strand repeatedly around the entire structure, defining triangular faces separated by 'hinges' of unpaired nucleotides[48]. Later, a strategy to stack concentric double-helical circles to match the contours of a target 3D shape was introduced[49] (FIG. 4f). In addition, a multi-arm DNA origami method was used to prepare 2D DNA scaffolds and then fold them into 3D prism structures through connection strands on the 2D structures[50]. Finally, a method to create wireframe DNA origami 3D structures by converting the structure into a triangulated 3D mesh and using a routing algorithm to trace the contour of this structure with the DNA origami scaffold has been recently reported[51]. This method produces complex 3D structures with mostly double-stranded DNA as edges (FIG. 4g). In a distinct but related approach, complex wireframe 2D and 3D origami structures were constructed, in which each edge is represented by antiparallel DNA crossover tiles and each vertex is represented by a multi-arm tile junction[52]. The single-stranded tile approach was expanded to design an array of 3D motifs from a 'brick' set[53].

DNA nanotubes of increasing complexity and functionality have been designed by a number of approaches, including DNA tile assembly[54–58], DNA origami[59], single-stranded tiles[60] and wireframe nanotubes[61,62].The self-assembly of 3D crystalline arrays from DNA proved to be a more challenging goal than originally anticipated[2]. An unusual motif for a DNA crystalline array with large cavities produced by combination of parallel and antiparallel strands was discovered[63]. The first example of a self-assembled 3D DNA crystal was finally reported in 2009 (REF. 64). The crystal was based on the association of branched, rigid DNA 3D triangular motifs tailed by very short sticky ends[64] (FIG. 4h). The association of these sticky ends in directions that span 3D space generates a crystalline arrangement, with potential applications as templates for protein crystallization and as porous hosts for nanomaterials. More recently, crystals with two molecules per asymmetric unit were built, and the post-assembly stabilization of these crystals with DNA triple helices was shown[65]. New insights into the propensity of rigid motifs to form self-assembled crystals are emerging[66], as are new motifs for 3D DNA crystallization[67,68].

Dynamic DNA structures

A seminal paper in 2000 introduced the concept of isothermal DNA strand displacement, which became essential to the construction of many DNA machines[69]. In this concept, a DNA 'tweezer' (FIG. 5a) is closed by the addition of DNA 'fuel' strands and re-opened with a different DNA strand, with the mechanical motion measured by fluorescence energy transfer between two dyes. The notion is that an unpaired 'toehold' extension to a motif component on the machine can bind to the complete complement of the strand. Upon binding, when the toehold is sufficiently long (usually about eight nucleotides), the complement will branch and migrate to the other end of the strand, thereby removing the strand from the motif because more nucleotide pairs are formed.

Isothermal strand displacement was used to reversibly rotate a series of DNA devices between *cis* and *trans* morphologies, converting a line of parallel DNA trapezoids into alternating 'up' and 'down' trapezoids[70] (FIG. 5b). A DNA actuator that was introduced into a 2D tile lattice enabled switching of the entire lattice between a stretched and a compact conformation[71].

In a different concept — hybridization chain reaction — hairpin DNA monomers assemble only when a trigger DNA strand is added. More specifically, the trigger strand opens the first hairpin, which can then open the second hairpin, which in turn opens the first, and so on (FIG. 5c). Thus, the DNA assembly can be triggered and amplified autonomously[72,73].

Since the above examples were first demonstrated, DNA machines have significantly increased in complexity. A number of DNA 'walkers' have been reported, including a DNA unit with two 'legs' that can walk

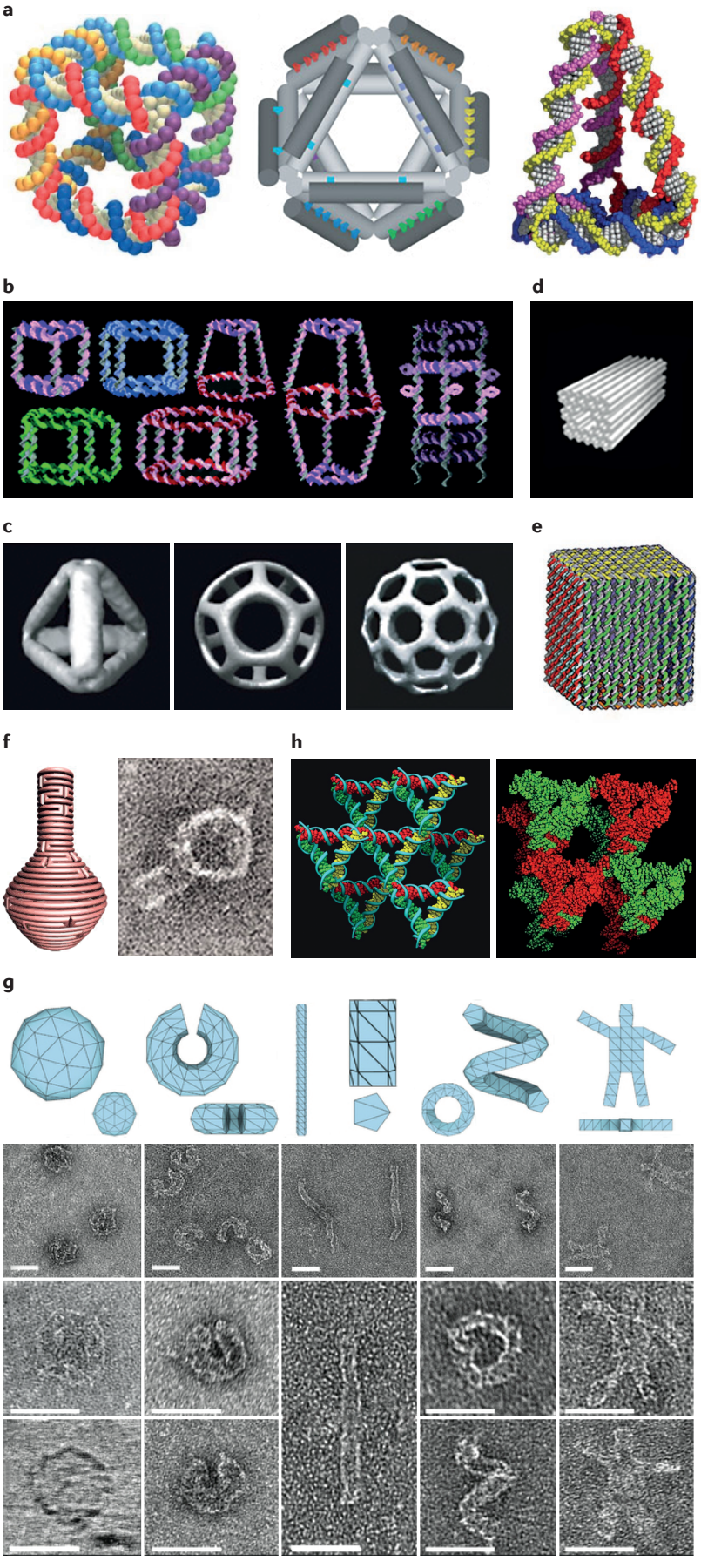
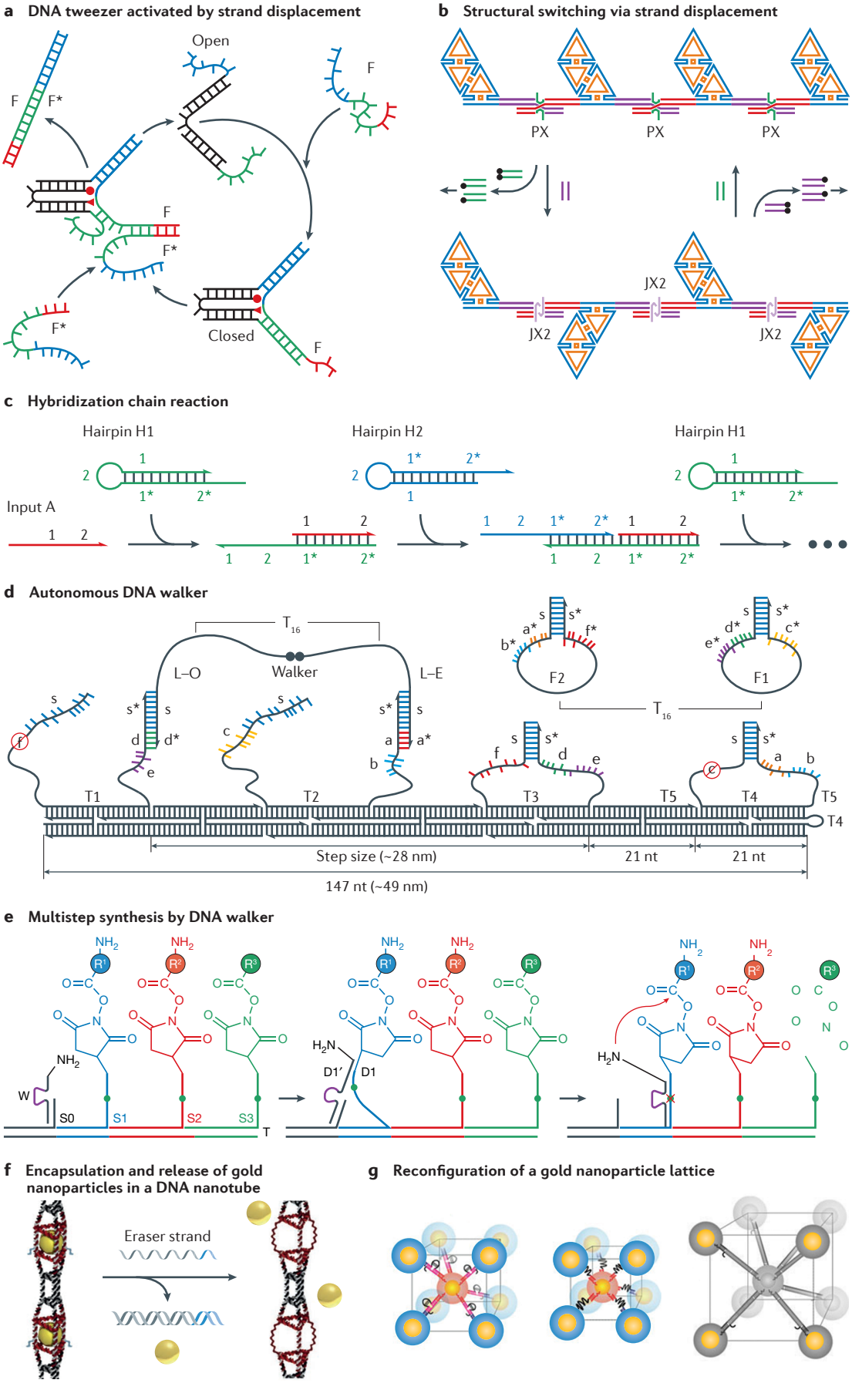


Figure 4 | **Three-dimensional structures from DNA.** **a** | The left panel shows a DNA cube constructed from connected three-arm junctions[4]. The middle panel shows an octahedron constructed from a long DNA strand and five connecting strands[26]. The right panel shows a tetrahedron constructed from four DNA single strands[38]. **b** | A face-centred approach to build prismatic cages[39]. **c** | Cryo-electron microscopy images of polyhedra assembled from DNA crossover tiles[40]. **d** | DNA origami can be rolled into various multilayered 3D structures[216]. **e** | DNA origami sheets connected into a cube[47]. **f** | The left panel shows a DNA 'urn' assembled by stacked concentric circles in an origami design; the right panel shows an electron microscopy image of this structure[49]. **g** | Wireframe DNA origami structures[51]. Scale bars are 50 nm. **h** | Self-assembled DNA crystal from rigid triangles and two-base sticky ends[64]. Panel **a** (left) is adapted with permission from REF. 217, American Chemical Society. Panel **a** (middle) is adapted with permission from REF. 26, Macmillan Publishers Limited. Panel **a** (right) is adapted with permission from REF. 218, AAAS. Panel **b** is adapted with permission from REF. 39, American Chemical Society. Panel **c** is adapted with permission from REF. 40, Macmillan Publishers Limited. Panel **d** is adapted with permission from REF. 44, Macmillan Publishers Limited. Panel **e** is adapted with permission from REF. 47, Macmillan Publishers Limited. Panel **f** is adapted with permission from REF. 49, AAAS. Panel **g** is adapted with permission from REF. 51, Macmillan Publishers Limited. Panel **h** (left) is adapted with permission from REF. 64, Macmillan Publishers Limited. Panel **h** (right) is adapted with permission from REF. 219, American Chemical Society.

directionally and autonomously on a track[74,75] (FIG. 5d). DNA 'gears' have been made to 'roll' against one another[76], and a DNA 'spider' that can walk along a predesigned track on DNA origami has been demonstrated[77]. These strategies were extended to an assembly line that involved a somersaulting walker that can transport and combine cargo (for example, gold particles) along a predesigned track[78]. The same group had earlier programmed a group of devices to translate specific DNA sequences into polymer assembly instructions[79]. A DNA walker was used to perform multistep organic synthesis in a prescribed sequence[80] (FIG. 5e). Further DNA dynamic constructions include cascades[75], conditional assemblies[81] and a 'capture' device[82] that places various patterns on a DNA origami surface. We are now reaching the stage where detailed biophysical measurements can be made on dynamic DNA systems[83].

DNA dynamic motion has also been implemented in three dimensions. Structurally dynamic DNA cages were induced to expand and contract to different sizes with the addition of specific DNA strands[39], and this reconfiguration was also shown for DNA tetrahedra[84]. These mechanisms are relevant for the application of DNA cages in the encapsulation and release of drugs in a biological environment. In a proof-of-concept experiment, a DNA nanotube that encapsulates gold nanoparticles into predesigned plasmonic lines was reported[85]. Upon addition of a DNA 'eraser' strand, the gold nanoparticle guests can be efficiently and rapidly released, which is of relevance for both plasmonic structures and drug delivery



◀ Figure 5 | **Dynamic DNA nanostructures. a** | A DNA tweezer. In the open form (top), the two fluorophores are separated. Strand F brings the blue and green strands together and closes the tweezer, increasing fluorescence resonance energy transfer (FRET). Strand F* removes F by strand displacement, restoring the open form of the tweezer[69]. Asterisks denote complementary DNA sequences. **b** | Strand displacement reversibly converts a DNA nanostructure from a line of parallel trapezoids to an alternating arrangement[70]. PX is a structure that can provide a planar conformation analogous to *trans*,and JX2 provides a planar conformation analogous to *cis*. **c** | Hybridization chain reaction. Hairpin DNA monomers H1 and H2 stay closed until input strand A is introduced. This strand opens H1, revealing a region that opens H2, which in turns opens H1, and so on, until a polymer is formed[72,220]. **d** | A DNA walker with two legs walks autonomously along a double-crossover track[75]. The walker is on loops T1 and T2, and the two black dots denote the walker's 5′,5′ linkage. T_{16} represents flexible polythymidine linkers on the walker and fuel hairpins, F1 and F2. Two T5 regions provide flexibility at the base of the track stem loops. All the binding sites are labelled with lowercase letters, and complementary sequences are represented with an asterisk. The two fuel-grabbing sequences f and c on T1 and T4, respectively, (denoted with a red strikethough) do not function. nt, nucleotides. **e** | A DNA walker that performs multistep organic synthesis[80]. **f** | A DNA nanotube encapsulates gold nanoparticles and releases them by strand displacement[221]. **g** | Reconfiguring a gold nanoparticle lattice[90]. Panel **a** is adapted with permission from REF. 69, Macmillan Publishers Limited. Panel **b** is adapted with permission from REF. 70, Macmillan Publishers Limited. Panel **c** is adapted with permission from REF. 220, Macmillan Publishers Limited. Panel **d** is adapted with permission from REF. 75, AAAS. Panel **e** is adapted with permission from REF. 80, Macmillan Publishers Limited. Panel **f** is adapted with permission from REF. 221, Macmillan Publishers Limited. Panel **g** is adapted with permission from REF. 90, Macmillan Publishers Limited.

(FIG. 5f). Strand displacement was used to interconvert 3D structures of different geometries and to access a structure that cannot be made directly[86]. Dynamic motion has also been induced in DNA origami structures. For example, a DNA origami Möbius strip was dynamically switched to a catenane[87]. Photoswitching of a DNA origami structure was demonstrated using photoresponsive azobenzene DNA[88,89]. Dynamic motion of DNA nanostructures has been used to reconfigure other functional materials, such as a gold nanoparticle 3D lattice[90] (FIG. 5g). Finally, DNA devices have been incorporated into hydrogels, thus transducing DNA recognition and stimuli-responsive behaviour into macroscopic changes, such as directed shape change, shape memory and electrocatalytic behaviour[224].

Supramolecular DNA assembly

The field of DNA nanotechnology has mostly been driven by crystallographers, biochemists, biologists, physicists and computer scientists, with comparatively little contribution from chemists. However, conceptual advances in physical organic, macromolecular and supramolecular chemistry have the potential to revolutionize the field. In the short term, these advances provide tools to create novel nanostructures with heterologous components, and they can stabilize these nanostructures to enable their use in applications in biology and medicine. In the long term, they could provide new DNA assembling paradigms that augment the unmodified DNA alphabet of adenine, thymine, guanine and cytosine, thereby achieving structural complexity[2]. Blending together DNA building blocks and synthetic organic, inorganic and polymeric molecules can also impart functionality to DNA structures, which have minimally exciting chemical properties despite the beauty of their structural diversity and ease of production.

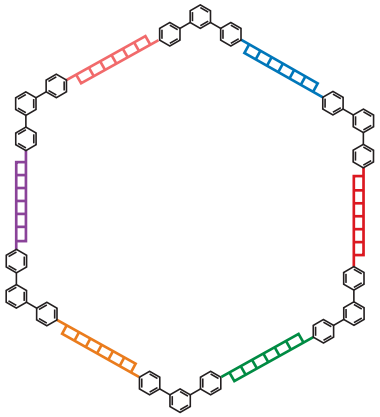
Organic vertices in DNA structures. Rather than using crossover motifs, which are the backbone of DNA nanotechnology, synthetic chemistry offers the possibility to use corner units based on organic molecules or transition metals. This substantially reduces the number of DNA strands needed and introduces structural motifs unavailable to unmodified DNA.

In an early example of this strategy, a central tetrahedral carbon was connected to two identical, self-complementary DNA strands[91]; these structures assembled into cyclic and oligomeric assemblies. To increase the selectivity of the assembly, a rigid organic molecule was inserted in the middle of a DNA strand by automated synthesis. Six of these strands with complementary sequences assembled cleanly into a DNA hexagon (FIG. 6a), which templated the formation of a gold nanoparticle hexagon. To introduce modularity and dynamic character into these assemblies, DNA polygons with rigid organic corner units and single-stranded sides were used as scaffolds with different geometries[92]. These polygons were produced by introducing multiple organic vertices within a strand and then cyclizing this strand. They could be used as reconfigurable templates to organize gold nanoparticles, as well as to create DNA prismatic cages and DNA nanotubes. Interestingly, these small organic corner units were found to profoundly influence the assembly outcome, to stabilize DNA hybridization and to increase its cooperativity[93,94]. They can be efficiently synthesized[95] and will undoubtedly be increasingly used in DNA nanotechnology.

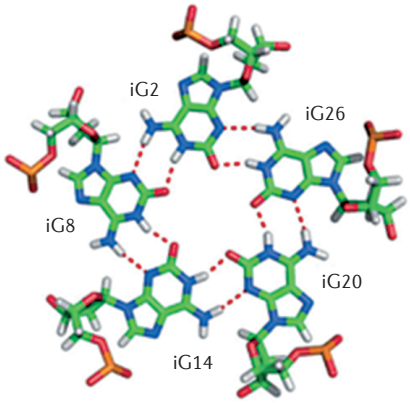
New DNA motifs. Synthetic chemistry enables the modification of the nucleobases in the interior of the DNA double helix. Self-complementary isoguanines have two hydrogen-bonding faces that are oriented at an angle that forms a pentameric assembly. In the presence of caesium ions, poly(isoguanine) results in a higher-order DNA pentaplex rather than the classical duplex[96] (FIG. 6b). More recently, a small molecule with three thymine-like faces (cyanuric acid) was shown to reprogramme the assembly of unmodified poly(adenine) into triple helices with a hexameric rosette internal structure. These triplexes further assemble cooperatively into long fibres (FIG. 6c). The use of externally added small molecules to alter the molecular recognition of DNA is likely to result in a number of new DNA motifs because of its ease and the availability of numerous small molecules with two or three hydrogen-bonding faces[97].

Although the field of artificial DNA bases is too wide to cover, it is worth mentioning that numerous structures and interactions, such as altered hydrogen-bonding motifs[98] or molecules that do not hydrogen-bond but exhibit hydrophobic and π-stacking interactions, have been used to replace DNA Watson–Crick base pairing[99,100]. Several different types of base modification have been replicated by polymerases. In one example, an organism (*Escherichia coli*) was shown to replicate and propagate this expanded genetic alphabet[101,102]. There has also been tremendous progress in creating synthetic oligonucleotide analogues that show greater serum stability and lower immunogenicity than DNA

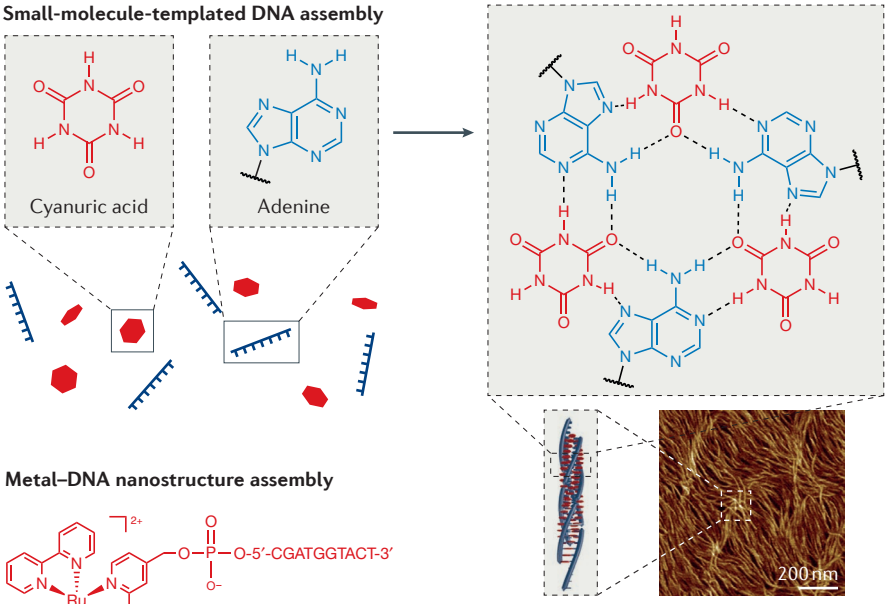
a DNA hexagon with organic corner units



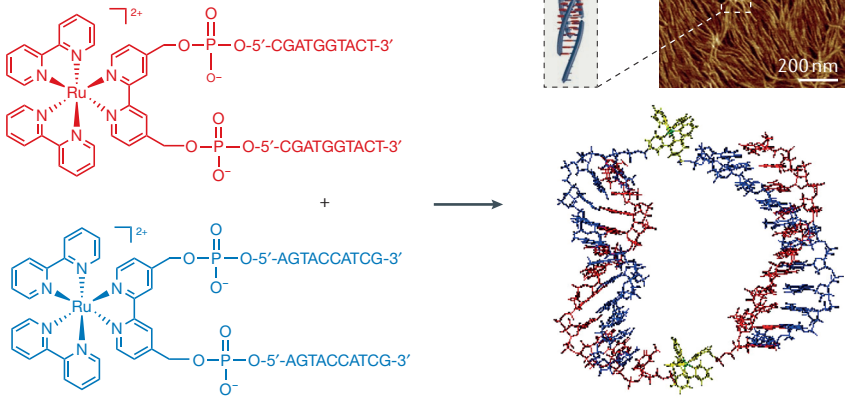
b Pentameric assembly from isoguanine



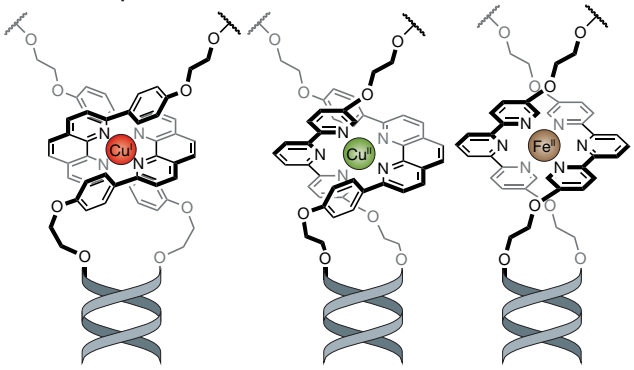
c Small-molecule-templated DNA assembly



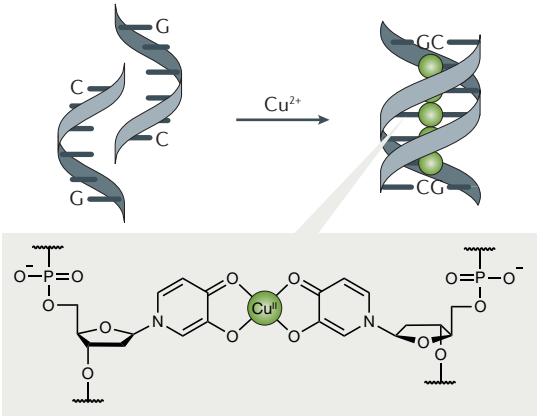
d Metal–DNA nanostructure assembly



e DNA-templated metal coordination



f Metallobase pairs



g Nanotube formation via curvature-inducing porphyrin units

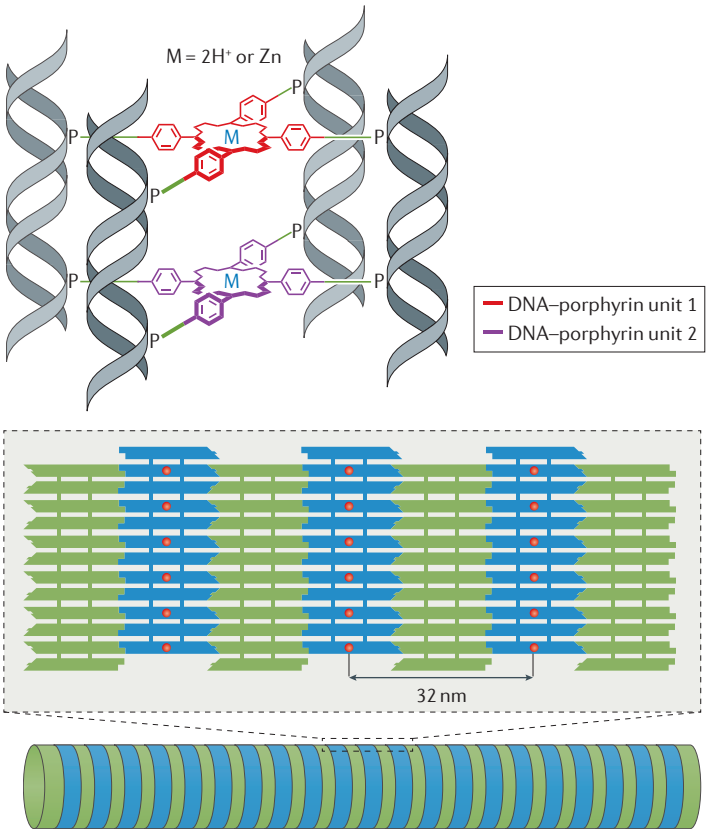


Figure 6 | **Supramolecular DNA assembly. a** | Directed assembly of a DNA hexagon with rigid organic corner units[92]. **b** | Isoguanine nucleobases form a pentameric assembly[96]. **c** | Cyanuric acid chaperones the formation of hexameric rosettes from poly(adenine) strands, which assemble into extended, triple-helical fibres[97]. **d** | Branched DNA strands with transition metal corner units assemble into metal–DNA nanostructures[109]. **e** | DNA double helices template the formation of metal-coordinating environments that are selective for their complementary transition metals[111]. **f** | DNA bases are replaced with ligands and bring together five copper-mediated base pairs[116]. **g** | Porphyrin units join four vertical DNA strands together and introduce curvature into DNA tiles, causing them to form nanotubes[121]. Panel **a** is adapted with permission from REF. 92, Wiley-VCH. Panel **b** is adapted with permission from REF. 96, National Academy of Sciences. Panel **c** is adapted with permission from REF. 97, Macmillan Publishers Limited. Panel **d** is adapted with permission from REF. 222, Elsevier. Panel **e** is adapted with permission from REF. 111, Wiley-VCH. Panel **f** is adapted with permission from REF. 116, AAAS. Panel **g** (top) is adapted with permission from REF. 122, American Chemical Society. Panel **g** (bottom) is adapted with permission from REF. 121, Wiley-VCH.

or RNA. These include peptide nucleic acids[103], locked nucleic acids[104], mirror-image DNA[105], 2′-fluoro[106] and 2′-methoxy derivatives[107]. These will be increasingly used in DNA nanotechnology, particularly for biological applications, but they are currently too expensive to adopt as standard oligonucleotides.

Organizing transition metals with DNA. The introduction of metals into DNA can impart this molecule with important properties, such as increased stability, redox activity and photochemical, catalytic and magnetic properties. In turn, the use of DNA can result in the organization of transition metal complexes into any deliberately designed structure, either periodic or aperiodic. This is currently difficult to achieve using conventional supramolecular chemistry. This programmed organization of transition metals will lead to the application of these molecules as sequence-defined metal–organic frameworks in nanoelectronics, nano-optics, data storage, molecular magnetic behaviour, light harvesting and catalysis.

In 2001, the first metal–DNA branched structure was reported[108]. This structure comprises a luminescent ruthenium bipyridine centre with two DNA strands as appendages. If two complementary complexes are brought together, they assemble into a cyclic metal–DNA nanostructure[108–110] (FIG. 6d). Transition metal corner units resulted in remarkable stabilization of DNA hybridization (for example, the melting temperature jumps from 40 to 80 °C upon introduction of a single metal complex into the DNA duplex), as well as chirality transfer from DNA to the metal and improved DNA charge transport[111,112] (FIG. 6e). These strategies were used to build a metal–nucleic acid 3D cage[42] with site-specific incorporation of transition metals in the vertices of the structure. Chiral metal–DNA four-way junctions[113] were constructed and used to make metal–DNA nanotubes.

The real power of DNA is the ability to organize different transition metals within a nanostructure. For example, the DNA-templated creation of three different ligand environments was demonstrated, whereby each environment was selective for a specific transition metal ion (FIG. 6e). When the 'incorrect' metal ion is added, 'error correction' occurs[111]. DNA hybridization has been used to template the construction of metal–salen complexes and nanostructures[114,115], and these were enzymatically replicated[114]. In an important approach, the DNA bases were replaced with metal-binding ligands, and metal-mediated base pairs were created by DNA hybridization[116–118] (FIG. 6f). Up to five copper-mediated base pairs could be arranged into a short oligonucleotide with the metals coupled ferromagnetically[116]. In this system, the metal centre was found to mediate charge transport within DNA[119]. Subsequently, different metals were incorporated into these artificial base pairs[120]. Finally, metal–DNA structures were used to change the curvature of a DNA nanostructure. In this example, a porphyrin molecule was linked to four DNA strands, and it induced the rolling of DNA tiles into tubular structures[121,122] (FIG. 6g).

Organizing polymers and lipids with DNA. Block copolymer self-assembly is an important area of research that has resulted in predictable morphologies with long-range order, such as spherical micelles, rods, vesicles and bilayers, and with numerous applications in materials science and biomedicine[123]. Object-oriented DNA nanotechnology, however, offers unique programmability and gives rise to assemblies with relatively short-range order. Polymers assemble using a large number of interactions, including but not limited to hydrophobic, electrostatic, π–π stacking and fluorophilic interactions, as opposed to the simple A–T, G–C assembly 'language' of DNA. Combining the two materials can result in long-range organization of DNA into new structures through a number of orthogonal interactions and is an area of great promise.

DNA block copolymers were induced to switch between different long-range morphologies with externally added DNA strands or enzymes[124,125] (FIG. 7a). In addition, a simple and high-yielding synthesis of DNA–polymer conjugates has been reported in which the polymeric block is monodisperse and fully sequence-controlled[126]. By attaching these polymers to 3D DNA prisms, self-assembly via hydrophobic interactions was shown[127,128] (FIG. 7b). When the polymers are on a single prism face, the DNA cages assemble via hydrophobic interactions. Interestingly, the hydrophobic polymer length dictates the aggregation number of these quantized assemblies (FIG. 7b, top left). With a relatively long polymer, monodisperse spherical micelles with a hydrophobic core and DNA prisms on their exterior are seen. The prisms can be 'peeled off' from the micelle by strand displacement, and the cage micelles can also be used as light-harvesting antennas (FIG. 7b, right). By contrast, when polymers are arranged on both sides of the DNA cages, they can undergo an intramolecular 'handshake' to give a micelle structure inside a DNA cage[129] (FIG. 7b, bottom left). In this case, the internal hydrophobic association can encapsulate small molecules and works synergistically with DNA base pairing to increase DNA assembly cooperativity.

Lipid chains and cholesterol units have also been attached to DNA scaffolds to modulate their assembly behaviour. DNA origami sheets could be hydrophobically induced to fold on themselves if their surfaces were modified with cholesterol units[130] (FIG. 7c). Cholesterol-substituted DNA cages can associate, show dynamic

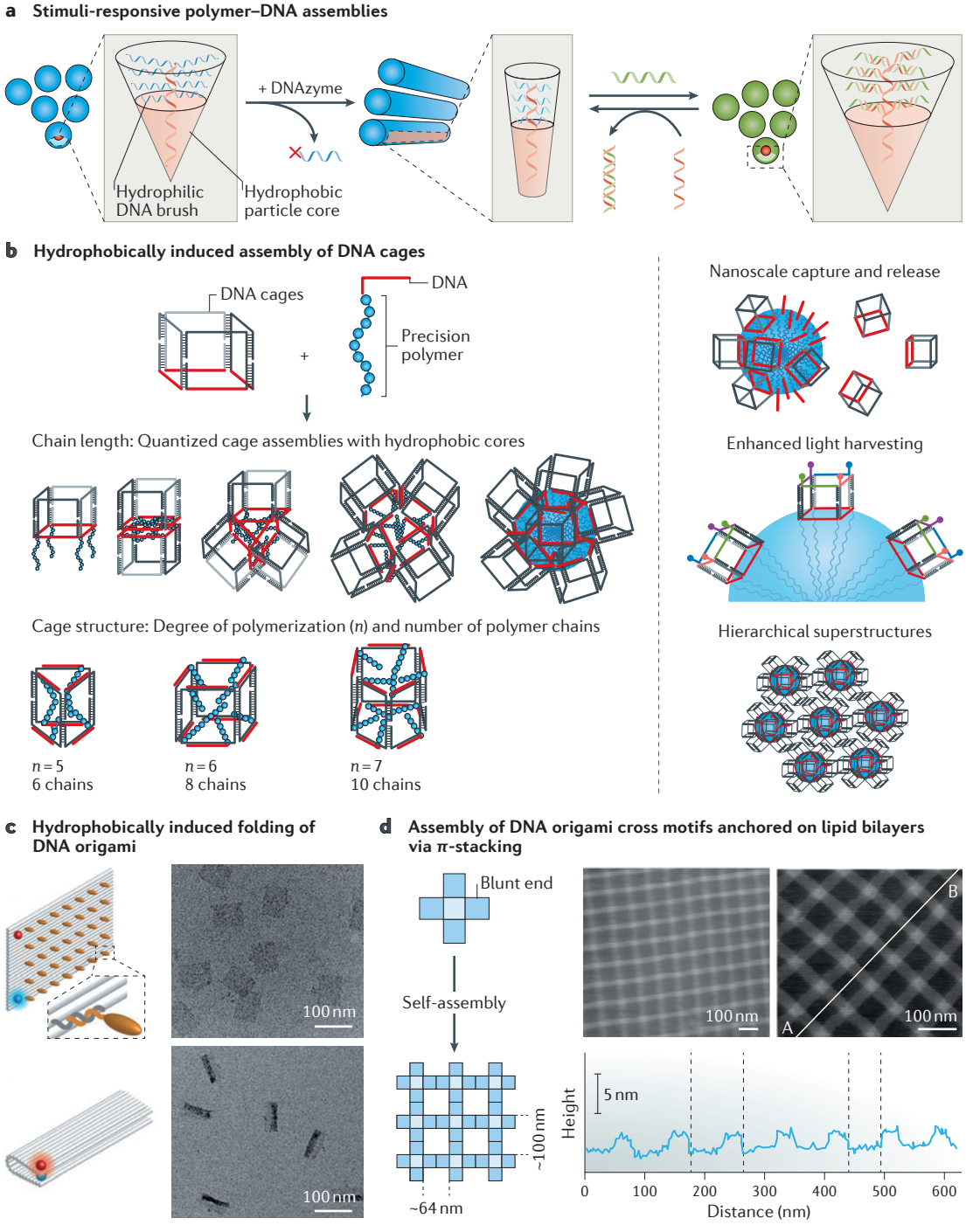


Figure 7 | **Interaction of DNA structures with polymers and lipids. a** | The assembly of DNA–polymer conjugates into spherical micelles. Upon DNA degradation with a DNA enzyme, the morphology becomes rod-like because of the size match between the core and exterior. Upon re-hybridization with DNA strands, the spherical morphology is recovered[124]. **b** | DNA cages are combined with sequence-controlled hydrophobic polymers. When four polymers are on a single face of the cage, hydrophobic interactions bring the DNA cages together into quantized assemblies (cage dimer, trimer, and so on) defined by the length of the hydrophobic polymers (*n* is the number of C_{12} chains in the polymer). When eight polymers are on the cage, they undergo an intramolecular 'handshake' to form a hydrophobic core within the cage[127,128]. **c** | Decorating a DNA origami rectangle with cholesterol units causes it to fold on itself via hydrophobic contact of these units[130]. **d** | DNA origami cross-shaped motifs with blunt ends on each edge self-assemble owing to π-stacking interactions between the blunt ends to form a lattice (left). Atomic force microscopy images of a DNA lattice made from cross-shaped DNA origami (right, top). The profile of the lattice along the A-B line in the AFM image above (right, bottom)[132]. Panel **a** is adapted with permission from REF. 124, Wiley-VCH. Panel **b** is adapted with permission from REF. 127, American Chemical Society. Panel **c** is adapted with permission from REF. 130, Wiley-VCH. Panel **d** is adapted with permission from REF. 132, Macmillan Publishers Limited.

behaviour and then be lifted-off from spherically supported lipid bilayers on silica beads[131]. Origami structures electrostatically anchored on supported lipid bilayers were reported to assemble into highly ordered 2D lattices through blunt-end interactions[132] (FIG. 7d). Additionally, 2D origami structures were photochemically switched from bound to unbound; interestingly, these processes were monitored in real time by high-speed AFM[133]. DNA barrel-shaped nanostructures with hydrophobic anchors have been used as membrane nanopores, with evidence of molecule translocation across the pore[134,135] and of cell toxicity[136].

In addition to the use of lipids to modulate DNA assembly, DNA nanostructures have been used as templates to form size-defined liposomes[137] and to orient conjugated polymer chains[138]. DNA origami rings and cages were decorated with lipids on their interior and were used to encapsulate and 'sculpt' liposome formation, providing a fundamental understanding of how lipid environments grow[137]. In another unique application, DNA nanostructures aligned a conjugated polymer chain along a well-defined path. A polyphenylene vinylene substituted with short DNA strands was hybridized to complementary positions on an origami structure, which oriented this structure into prescribed 'routes' with the potential to organize optical or electronic molecular wires into arbitrary geometries[138]. Thus, lipids and polymers can introduce new assembly motifs into DNA nanotechnology, with protein-like folding as an emergent property. In turn, DNA nanotechnology provides a unique opportunity to arrange polymers or lipids into anisotropic and size-controlled structures.

Applications of DNA nanotechnology

Assembling inorganic nanostructures with DNA. Inorganic nanostructures, such as metals and semiconductor nanoparticles, nanowires and nanorods, have emerged as a promising class of materials for optical, electronic, catalytic and sensing applications. They will undoubtedly have an essential role in future devices, such as solar cells, light-emitting diodes, electronic and photonic circuits and tools for medical diagnostics and therapeutics[139]. Notably, plasmonic materials, such as gold and silver particles, can be used to manipulate the interaction of light with matter precisely[140]. Many of the optical and electronic properties of inorganic nanomaterials arise directly from the geometric arrangement of the particles within an assembly. In addition, applications in optical routing and switching at subwavelength scales will depend on our ability to organize nanoparticle circuits[140]. DNA nanotechnology has played an important part in guiding this geometric arrangement.

In 1996, the first DNA-mediated assembly of gold nanoparticles to give 1D discrete structures was reported[141]. This was achieved by monofunctionalizing a gold nanoparticle with a single DNA strand. At the same time, gold nanoparticles that were polyfunctionalized with DNA were assembled into aggregates with distinct spectral changes[142]. These changes in optical properties upon DNA recognition are the basis for some of the more sensitive detection techniques for DNA (in some cases rivalling amplification techniques by the polymerase chain reaction). After these two seminal contributions, DNA nanotechnology was used to create more complex nanoparticle patterns. Gold nanoparticles of different sizes (5 and 10 nm) were organized using DNA tile assembly into an extended checkerboard pattern[143]. To create discrete 2D patterns, gold nanoparticles were labelled with a single DNA strand, and they were hybridized to their complementary positions on DNA polygon templates (for example, triangles, squares and pentagons). Using strand displacement, these gold nanoparticle assemblies were switched between different geometries, and writing and erasing of different particles was demonstrated[144] (FIG. 8a). In a subsequent study, a 3D gold nanoparticle tetrahedral assembly was created[145], and nanoparticles were regularly organized around a DNA nanotube[146]. Gold nanoparticles were also organized into size-defined linear 'wire' structures by encapsulating them inside DNA nanotubes[85].

DNA origami has been successfully used to precisely pattern gold nanoparticles and nanorods into arbitrary designs. For example, a helical and chiral arrangement of gold nanoparticles was created (FIG. 8b). It was demonstrated that one can predesign a particular circular dichroism spectrum and obtain it experimentally from the nanoparticle plasmon excitation with visible light[147,148]. In another example, two particles were placed on a DNA origami nanotube, which was precisely oriented with respect to incident light. Finely controlled plasmonic coupling between the particles significantly enhanced and focused light into a zeptolitre volume between the two particles, which was able to increase the fluorescence of a dye molecule 117-fold[149] (FIG. 8c). DNA origami was also used to hierarchically assemble a hybrid structure composed of a multichromophoric virus capsid and a gold nanoparticle with controlled spacing between the two functional units[150].

Current methods of nanoparticle assembly rely on generating a complex DNA scaffold and using it to position the particles into the desired functional structure. Recently, DNA nanostructures were used not as permanent scaffolds but as transient, re-usable templates: a programmed pattern of DNA strands contained in the parent template is molecularly 'printed' onto the inorganic material itself[151]. The method produces highly stable particles that are anisotropically labelled with a controlled number, geometry and anisotropic placement of different DNA sequences (FIG. 8d). These nanoparticles can autonomously assemble without the use of DNA scaffolds, and the DNA template nanostructure can be re-used[151]. In two separate studies, DNA origami 3D objects have been used as 'nanocontainers' (REFS 152,153), which template the growth of gold nanoparticles within their interior (FIG. 8e). Using these strategies, particles with arbitrary shapes and monodisperse sizes were obtained, which is an important advance in nanomaterials synthesis.

Ordered 3D plasmonic crystals have been formed by self-assembly of multifunctional gold nanoparticles[154,155], whereby the geometry and flexibility of the spacer DNA modulate the 3D plasmonic structure. In one of these studies, gold nanoparticles were organized into a diamond lattice, which is a difficult structure to

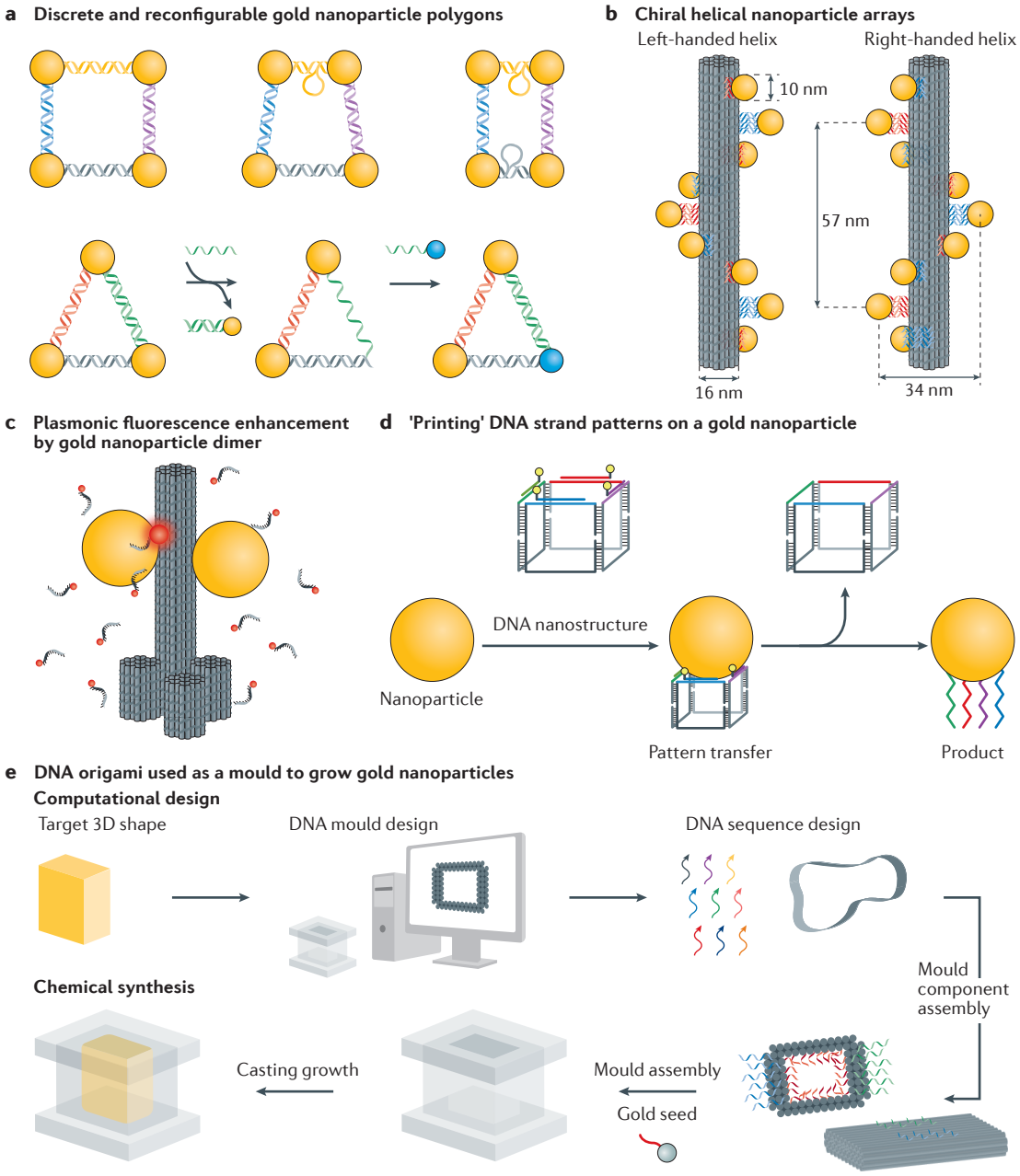


Figure 8 | **Nanoparticle assembly with DNA. a** | DNA polygons with rigid organic vertices template the assembly of gold nanoparticle triangles and squares, which can be reconfigured into trapezoids or rectangles. Strand displacement removes one particle, which can be replaced with a smaller one in the assembly[144]. **b** | A DNA origami nanotube templates the formation of helical gold nanoparticle arrays of opposite chiralities[147]. **c** | Two gold particles positioned on DNA origami can plasmonically focus light into zeptolitre volumes to increase fluorescence[149]. **d** | A DNA cube transfers a square pattern of different DNA strands to a gold nanoparticle. This cube 'printing press' can be re-used, and the particle displays anisotropic binding motifs[151]. **e** | A DNA origami 3D structure is used as a 'mould' to grow gold nanoparticles of precisely programmed shapes[152,153]. Panel **a** is adapted with permission from REF. 144, American Chemical Society. Panel **b** is adapted with permission from REF. 147, Macmillan Publishers Limited. Panel **c** is adapted with permission from REF. 149, AAAS. Panel **d** is adapted with permission from REF. 151, Macmillan Publishers Limited. Panel **e** is adapted with permission from REF. 153, AAAS.

obtain. This strategy relies on encapsulating a DNA nanoparticle within DNA origami tetrahedra and subsequently assembling these host–guest structures with isotropically substituted gold nanoparticles to form the crystalline diamond-like array[156].

Assembling proteins with DNA. Another promising area of application for DNA nanotechnology is the assembly of proteins. In fact, solving protein structures was the original driving force for DNA nanotechnology, and it remains a sought-after goal. Protein–protein

interactions are fundamental in many aspects of biology. Understanding and controlling these phenomena will be essential for moving forward in both fundamental biology and synthetic biology. Small molecules can be used to bring together proteins; however, to control the physical arrangement of multiple proteins and to create complex enzyme cascades or cell signalling events, such as small-molecule organic synthesis, will be prohibitively difficult. DNA nanotechnology is uniquely poised to overcome this problem because of the matched nanometre-scale size of proteins and DNA, the unique programmability of DNA and the compatibility of many nucleic acids with proteins. For example, DNA nanostructures have been used as templates for protein assembly. Nanotubes nucleated the growth of amyloid fibrils, which were then organized on a DNA origami surface[157].

DNA structures have also been used to organize proteins into well-defined patterns. A 2D crystalline network of DNA was created[158], and proteins were attached at regular positions on the lattice. This produced an ordered protein array (FIG. 9a), the structure of which was resolved by cryo-electron microscopy. Moreover, the same group was also able to encapsulate a protein inside a DNA cage[159] (FIG. 9b). DNA structures allowed the assembly of membrane protein dimers and trimers on lipid bilayer nanodiscs, a process that would be low yielding without the DNA template; this is useful to develop an understanding of protein–protein interactions[160]. In addition to cryo-electron microscopy, DNA structures were used to elucidate membrane protein structure by NMR spectroscopy. This was achieved by using a DNA nanotube as a liquid crystalline medium to weakly align them[59].

DNA nanotechnology has started to be used to create enzyme cascades by organizing a number of metabolic proteins on DNA tiles or origami scaffolds. In 1994, the co-assembly of two proteins on a linear DNA scaffold was demonstrated[161]. Then, in a subsequent study, two enzymes were organized on a DNA network into a 'cascade', in which the first enzyme product was the substrate for the second enzyme; enzymatic activity is more efficient the closer together the two enzymes are[162]. Enzyme structures were attached within the internal space of DNA origami structures[163]. In addition, instead of relying on passive diffusion to transport the molecule from one enzyme to the next, a two-enzyme reaction cascade that is mediated by a DNA 'swinging arm' was introduced. A single-stranded DNA carries the substrate and transfers it from one enzyme to the next, thus increasing the enzymatic activity[164]. Importantly, an enzyme cascade was demonstrated *in vivo*. RNA modules were designed to carry binding units for the enzymes (Fe–Fe) hydrogenase and ferredoxin and to spontaneously polymerize into a nanotube upon its formation transcriptionally. The hydrogen output *in vivo* was found to increase when the structures were self-assembled[36] (FIG. 9c). Researchers have also studied the behaviour of motor proteins using DNA nanotechnology. For example, the proteins dynein and kinesin have been organized on a DNA origami structure, and their numbers, distances and locations have been systematically changed. This created a 'molecular tug of war', providing a better understanding of motor proteins and biological motion[165] (FIG. 9d). A photosynthetic mimic was also created by attaching a DNA three-way junction that carries light-harvesting dyes to a photosynthetic reaction centre protein; this extended the absorbance cross section of the reaction centre into a new spectral range[166].

DNA nanotechnology also presents the opportunity to control the spatial organization of ligands that cooperatively bind to proteins. For example, a DNA pentaplex structure was used to spatially organize phosphocholine molecules into a pentavalent array, thus enabling them to cooperatively bind to human C-reactive protein[167] (FIG. 9e). In another example, rigid DNA tiles were able to control the presentation of aptamers for the protein thrombin. It was shown that thrombin prefers to bind two aptamers simultaneously, and the optimal distance between the aptamers was determined[168]. Recently, a novel method for creating protein–DNA hybrid nanostructures was reported[169]. The principle involves folding long double-stranded DNA into desired structures by selectively bringing together DNA double-helix portions using transcription activator-like (TAL) effector protein dimers. Thus, it is protein–DNA interactions that form DNA into the desired structure. This contribution is a departure from traditional Watson–Crick mediated DNA assembly, as well as a potential method for protein organization by fusing the proteins to the TAL effector units.

Biophysical and biomedical applications. DNA nanotechnology has already contributed important biophysical techniques that facilitate single-molecule observations in biology. Here, we discuss these techniques as well as two promising biomedical applications: drug delivery and tissue engineering.

DNA nanotechnology is starting to yield powerful biophysical techniques, such as DNA points accumulation for imaging in nanoscale topography (PAINT), which is a super-resolution fluorescence technique. This method relies on the transient binding and unbinding of oligonucleotide strands to specific positions of an origami scaffold. These strands are labelled with fluorophores, thus resulting in 'blinking' events on the origami scaffold, which is the basis for super-resolution fluorescence imaging. This has led to the simultaneous imaging of microtubules, mitochondria, the Golgi apparatus and peroxisomes inside fixed cells[170]. Fluorophores can be placed on DNA origami nanotubes in different geometric patterns and, combined with DNA-PAINT, can be used as efficient tools for multiplexed detection[171] (FIG. 10a). A more recent development is qPAINT — a procedure for quantifying the number of fluorophores (and thus the biological components linked to them). In a separate approach, a DNA origami structure with a small rectangular inner space has been created to serve as a nanoreactor. This nanoreactor enables the direct observation of single-molecule DNA and DNA–protein dynamic events using high-speed AFM[172,173] (FIG. 10b). Importantly, DNA switches based on the i-motif structure have been used to measure intracellular pH within the endosomal pathway[174].

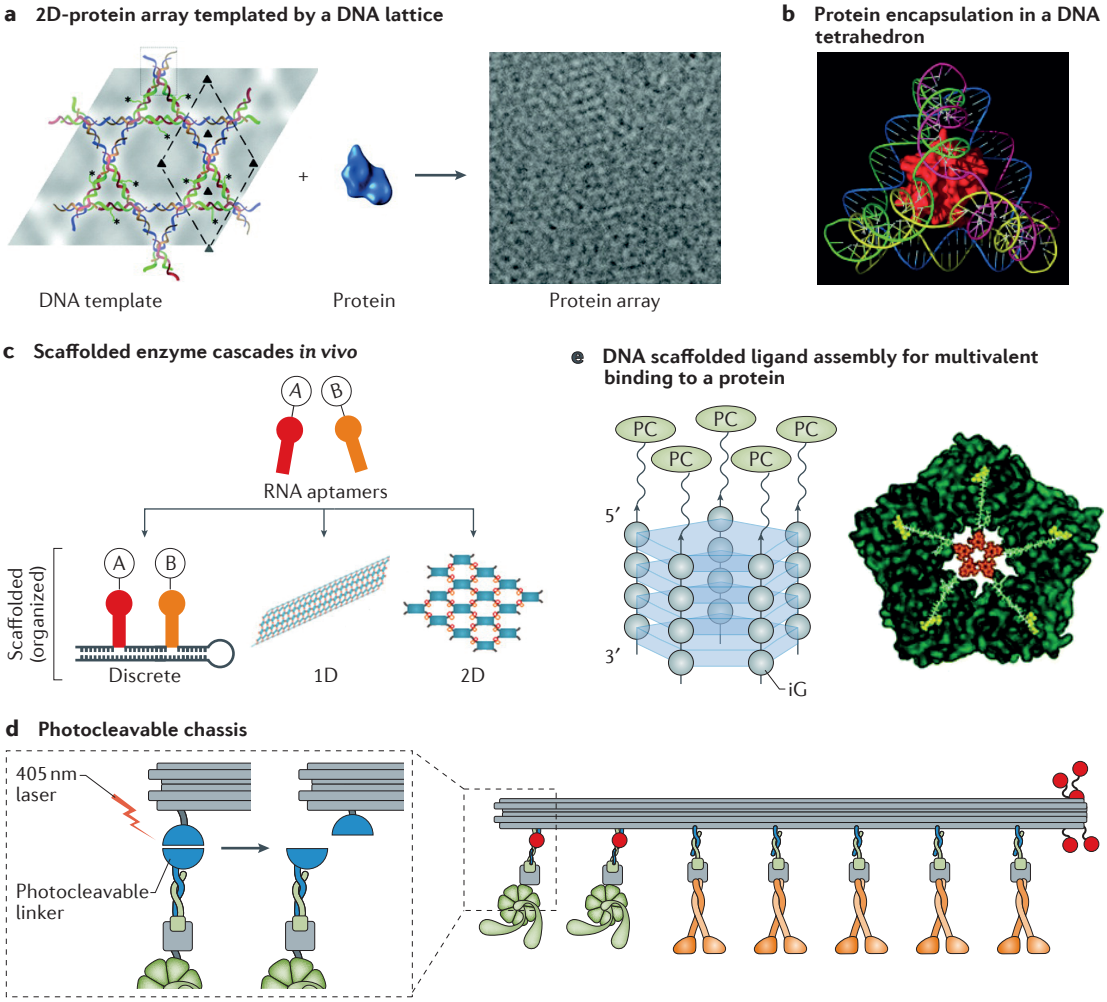


Figure 9 | **Protein assembly with DNA. a** | A DNA lattice with a periodic arrangement of binding sites is used to organize a neuropeptide-binding G protein-coupled membrane receptor into a 2D crystalline array[158]. **b** | A DNA tetrahedron encapsulates the protein cytochrome C[159]. **c** | RNA modules with protein binding sites can autonomously assemble into 1D and 2D assemblies on which proteins A (ferredoxin) and B (hydrogenase) are scaffolded. These structures are generated *in vivo* and are used to control the spatial organization of a hydrogen-producing pathway[36]. **d** | DNA origami is used to attach two motors of opposite polarity, dynein (green) and kinesin (orange), and its motion is slowed or stalled as a result of this 'tug of war'. When dynein is photochemically cleaved (inset), motility is restored[165]. **e** | A self-assembled pentameric DNA structure organizes five protein substrates (phosphocholine, PC) to bind cooperatively with a pentavalent protein (human C-reactive protein, CRP)[167]. Panel **a** is adapted with permission from REF. 158, American Chemical Society. Panel **b** is adapted with permission from REF. 159, Wiley-VCH. Panel **c** is adapted with permission from REF. 36, AAAS. Panel **d** is adapted with permission from REF. 165, AAAS. Panel **e** is adapted with permission from REF. 167, American Chemical Society.

An ideal drug delivery vehicle would be biocompatible, safe, stable *in vivo* and capable of targeting a diseased environment and delivering a high level of drug cargo to this environment[175]. In addition, recent studies have shown that the size and shape of the drug carrier and the placement of ligands on the carrier are strong determinants of its biological fate in organisms[176]. DNA nanostructures are unprecedented in their ability to be programmed to any size, shape and ligand patterning, and are compatible with numerous chemistries for stabilization and biological compatibility. They are also dynamic, enabling us to programme their drug release to respond to specific biological cues. Thus, DNA nanostructures are uniquely positioned to advance the area of drug delivery.

Cellular uptake and gene silencing with DNA nanostructures have been examined. DNA wireframe cages are able to penetrate cells without the aid of transfection agents, at greater efficiency than single-stranded or double-stranded DNA[177,178]. This intriguing finding parallels recent work, in which spherical nucleic acids (gold nanoparticles with a dense shell of thiolated DNA strands) were shown to enter unaided into cells[179]. It raises interesting questions about the mechanisms of cellular entry of DNA nanostructures; answers to these questions will provide us with new methods of delivering oligonucleotide therapeutics. Live-cell imaging techniques were used to show that DNA tetrahedra in which a component strand was end-modified with a cyanine chromophore (Cy3) enter HeLa cells by a

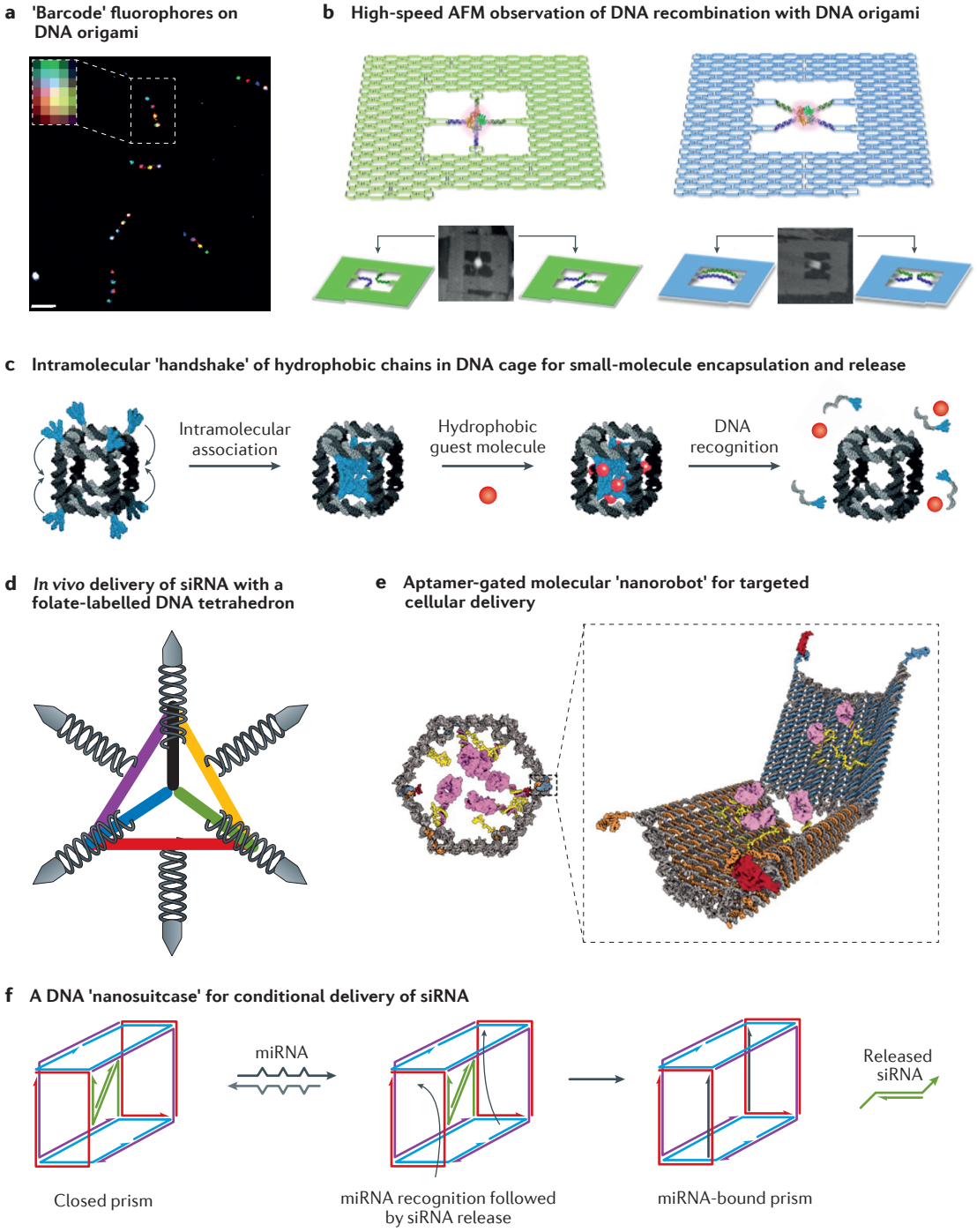


Figure 10 | **Biological applications of DNA nanotechnology. a** | Fluorophores are placed on DNA origami nanotubes in different patterns; combined with super-resolution microscopy, they can be used for the simultaneous detection of multiple analytes[171]. **b** | A DNA origami structure with a small rectangular inner space enables the direct observation of DNA recombination events using high-speed atomic force microscopy (AFM)[172]. **c** | When eight dendritic lipid chains are organized on a DNA cube, they undergo an intramolecular 'handshake' to form scaffolded micelles within the DNA cage; these are capable of encapsulating small molecules and releasing them in the presence of a specific DNA sequence[129]. **d** | A DNA tetrahedron with four small interfering RNA (siRNA) strands terminated with folate groups shows *in vivo* targeting of cancer cells and gene silencing[192]. **e** | A DNA origami cage structure encapsulates antibodies and is closed by two aptamer switches; it opens upon recognition of a protein on leukaemia cells, revealing encapsulated cargo that manipulates cell signalling[193]. **f** | A DNA 'nanosuitcase' selectively delivers its siRNA cargo upon recognition of a specific microRNA (miRNA) sequence[195]. Panel **a** is adapted with permission from REF. 171, Macmillan Publishers Limited. Panel **b** is adapted with permission from REF. 172, American Chemical Society. Panel **c** is adapted with permission from REF. 129, Macmillan Publishers Limited. Panel **d** is adapted with permission from REF. 192, Macmillan Publishers Limited. Panel **e** is adapted with permission from REF. 193, AAAS. Panel **f** is adapted with permission from REF. 195, American Chemical Society.

caveolin-mediated endocytic pathway; most of these localize in lysozomes, pointing to the need for endosomal escape for these molecules[180]. However, the effect of dye-induced cellular uptake, or fluorescence changes of the dye in different cellular environments, will still need to be examined. More recently, octahedral DNA cages were shown to selectively enter cells via recognition by a scavenger receptor associated with cardiovascular disease[181]. Furthermore, DNA cages[182] and micelles[183] were found to be effective for the delivery of antisense oligonucleotides, with a greater ability to silence gene expression than the single-stranded antisense oligonucleotides.

In addition, both wireframe DNA cages[184] and origami structures[185] are remarkably nuclease resistant, with small synthetic modifications greatly increasing this stability[184]. However, DNA origami structures dissociate in low-concentration magnesium environments, and their stability *in vivo* as drug carriers may need to be optimized[186]. Recently, a contribution showed the construction of DNA origami structures that were stable to low salt conditions by increasing the thermal denaturation temperature of their component parts[187], and DNA origami structures were coated with oligolysine polymers to increase their stability and cellular uptake[188].

DNA nanostructures have been used as carriers for intercalating antitumour drugs, such as doxorubicin, and were cytotoxic to doxorubicin-resistant cancer cells[189]. Moreover, the encapsulation capacity and release profiles could be controlled using DNA nanotubes with programmed helical twists[190]. Doxorubicin-loaded DNA cages can also be used for targeted therapy. Labelling these cages with MUC-1 aptamers allowed selective targeting to MUC-1 positive cancer cells[191]. Finally, DNA nanostructure delivery has been demonstrated *in vivo* (FIG. 10c). A DNA tetrahedron with four small interfering RNA (siRNA) strands terminated with folate groups was constructed and showed *in vivo* targeting of cancer cells and gene silencing; this behaviour was dependent on the spatial organization of the RNA strands and was optimal with a minimum of three folate ligands per tetrahedron[192] (FIG. 10d).

Owing to their large pore sizes, DNA cage structures are not normally able to capture small-molecule drugs non-covalently, unless these are direct DNA binders (this class of drugs is normally cytotoxic)[190]. A DNA cage structure has been designed that circumvents this problem[129]. The cage is formed by attaching eight hydrophobic dendritic units on a DNA cube, and because these units prefer to point inside the cube, a cohesive encapsulated hydrophobic environment is created. This structure is capable of carrying many classes of small-molecule drugs, and when specific DNA strands are added, the drugs are selectively released. The hydrophobic environment can be increased in size by changing the geometry of the 3D structure[128].

An exciting promise of DNA nanostructures is their ability to release their therapeutic cargo selectively in response to a specific molecule overexpressed in the disease environment. A DNA origami cage structure that encapsulates antibodies and that is closed by two aptamer switches was reported[193] (FIG. 10e). Upon recognition of an antigen protein expressed on the surface of leukaemia cells, the origami structure opened, revealing its encapsulated structures. These were then able to manipulate the signalling of the cancer cell. A DNA cube was designed such that it opens and unfolds in response to an RNA sequence that is expressed specifically in prostate cancer cells[194], and a DNA 'nanosuitcase' was shown to selectively deliver its siRNA cargo upon recognition of a specific microRNA sequence[195] (FIG. 10f). Recently, a DNA cage was designed so that it binds with low nanomolar affinity to human serum albumin, which is the most abundant protein in the blood[196]. Albumin has been used in the clinic to improve the *in vivo* biodistribution of drugs and localize them to tumours[197]. It was shown to increase the nuclease resistance of the cage while still allowing for gene silencing, and it is expected to improve the biological distribution of DNA nanostructures (although this awaits experimental verification)[196].

In tissue engineering and regenerative medicine, DNA materials offer the possibility of finely positioning growth and differentiation factors into a well-defined structure, thus being able to programme the cellular environment for a predesigned tissue growth outcome. Because tissue growth and regeneration is a highly dynamic and responsive phenomenon, it is an ongoing challenge to find adaptive materials that will reconfigure in a time-dependent manner[198]. Thus, DNA materials are ideally suited for such cue-responsive behaviour.

DNA self-assembled structures can be used as structurally tunable extracellular matrix scaffolds. For example, human cervical cancer cell growth was found to be robust on these scaffolds, and fine-tuning of the rigidity of the DNA scaffold resulted in measurable changes in cell behaviour[199]. More recently, DNA nanotubes functionalized with RGD peptides were shown to enhance the differentiation of neural stem cells into neurons, and this phenomenon is geometry dependent[200]. The use of DNA nanostructures as cellular scaffolds is a nascent field with great promise; however, we will have to wait for future experiments to better judge its applicability.

Conclusions and outlook

Before the inception of DNA nanotechnology, one could never have imagined being able to build such complex structures through bottom-up self-assembly. Although top-down approaches, exemplified by the semiconductor industry, continue to give us more complex and smaller nanopatterns, these are difficult and costly to generate, and importantly, they are limited to specific materials and specialized applications. We have always known that natural systems exceed any top-down method in their assembly complexity, hierarchical features and function. DNA nanotechnology has allowed us to start approaching the complexity of natural structures, machines and devices. It is in this sense that this area has transformed what we can create in the laboratory.

DNA nanotechnology is starting to deliver on its promise for biophysics and plasmonics. Proof-of-concept studies have shown that it will be transformational in numerous other areas. Tools to study biology, protein assemblies for synthetic biology and green

chemistry, targeted and adaptive drug delivery and diagnostic systems, multivalent protein binders as therapeutics, light-harvesting systems and plasmonic devices are all applications within reach. Progress towards most DNA nanotechnology applications, especially *in vivo* work, will require more DNA-minimal fabrication methods (using the fewest number of DNA strands to build a structure). The challenge here is to balance design simplicity with complex function. Chemists will need to be more involved to address issues of stability, scalability, functionalization and drug delivery. Interestingly, this aspect of the field has suffered from the fact that chemists are often intimidated by the learning curve involved with DNA research, whereas biologists, physicists, computer scientists and engineers may be hesitant to take on chemical synthesis approaches with DNA. This intersection of the fields will be essential for the growth of DNA nanotechnology. However, this is currently underpopulated, and young scientists are encouraged to step into this exciting area.

Assembly of inorganic materials with DNA. DNA nanotechnology has demonstrated the assembly of nanoparticles into predesigned arrangements. It offers unparalleled precision and programmability and may be the key to creating superior plasmonic structures, metamaterials, nanoelectronic circuits and light-harvesting materials. In the short term, DNA nanotechnology is starting to reveal the fundamental physics underlying the collective properties of nanomaterials. One day, we expect to design and create negative refractive index materials, surface-enhanced Raman scattering sensors and other plasmonic devices by use of the information-based architectural properties of DNA. However, most of these applications will likely need more robustness than can be accomplished from a DNA material. In this respect, the use of DNA scaffolds as a 'printing press' to transfer binding patterns to inorganic materials is a promising strategy[151]. Biomineralization within or around DNA nanostructures will likely have an important role in the fabrication of electronic or photonic devices. It should also be noted that the persistence length of DNA is 50 nm, but the persistence length of a DNA six-helix bundle is on the micrometre scale[201].

More immediate future applications of DNA-mediated nanoparticle assemblies are biological sensing, imaging and cellular recognition. In these applications, the DNA components that link the nanomaterials can be used for molecular recognition. For example, the plasmonic enhancement in the gaps between gold or silver nanoparticles or collective chiral plasmonic properties[148] can be used to greatly increase the sensitivity of the detection of analytes or allow near-infrared imaging or local heating of specific cellular components in a highly selective manner[202–204]. Much of the foundational work for these applications has already taken place, and the next few years will see research evolving towards these 'smart' nanomaterials and their potential use in the field or the clinic.

Protein assemblies with DNA. The organization of proteins with DNA has already started to contribute to fundamental biological understanding, but this area is less well developed than others. Thus far, the difficult aspect of this research is how to conjugate proteins to DNA in a robust and site-specific manner. Biological conjugation, including *in vivo* conjugation, is an important research area in its own right, and the hope is for DNA nanotechnologists to provide tools towards this goal[205–207]. One solution to this problem is to use aptamers to bind proteins on DNA or RNA nanostructures. An important proof-of-concept contribution here is the *in vivo* transcription of RNA as a self-assembling platform to create enzyme cascades[36]. Now that RNA nanotechnology has taken off on its own, we will likely see important contributions in this direction, such as *in vivo* self-assembling nucleic acid platforms to mediate protein–protein interactions and protein 'green chemistry'. Synthetic biology stands to gain a great deal from self-assembling RNA strategies. Very interesting opportunities exist in the use of DNA–protein assemblies to mediate cellular signalling and cell–cell interactions for biomedical applications. Creating protein-mimicking DNA structures will not only provide a better understanding of protein function but will enable designs that expand the diverse functions exhibited by proteins.

Biophysical and biomedical applications. DNA nanostructures offer unique opportunities for biophysics, drug delivery and regenerative medicine, in particular because of their programmability and their ability to dynamically respond to a complex set of cues. One can imagine that in the future, small DNA and/or RNA devices may be able to function *in vivo*, sense a set of signals and operate selectively on a biochemical pathway. Given the incredible advances in gene sequencing and the promise of precision medicine, these DNA devices may have an important role in patient-specific next-generation nanomedicines. The ability to conjugate numerous photonic and electronic materials on DNA will allow these devices to interact with devices external to the organism (such as a smartphone).

Right now, these applications are just a promise. They face some important hurdles, such as the susceptibility of DNA to nuclease degradation in biological media, its potential immunogenicity, the need for endosomal escape, and the *in vivo* instability of DNA origami. Wireframe structures, by contrast, do not disassemble in biologically relevant media but will also need to be optimized for nuclease resistance and cellular delivery[51,178,182]. DNA origami or single-stranded tiles require the use of a large number of different DNA strands (often >200, in addition to a scaffold strand). Each of these component strands would have to be examined in terms of toxicity and immunogenicity profiles, and the technology is now prohibitively expensive. However, as noted above[208], there is a Moore's Law of DNA synthesis, with a cost-halving time of around 30 months.

Introduction of orthogonal interactions. Proteins fold into diverse 3D structures by using multiple orthogonal interactions, such as hydrogen bonding, hydrophobic interactions, van der Waals interactions, metal coordination and disulfide linkages. For example, a coiled-coil

motif assembles first by hydrogen-bond association into α-helices and then by 'zipping up' hydrophobic side chains at the interface of these helices. DNA nanotechnology has reduced the assembly code to only four letters: A, T, G and C. By adding organic, inorganic and polymeric molecules to DNA nanostructures, we are now able to access increased complexity through interactions that are orthogonal to simple Watson–Crick base pairing. Hopefully, we will be able to understand the interplay between these different interactions, which will allow the generation of self-assembly modules that can be plugged into DNA nanostructures to augment their structural range. In addition, we can take advantage of the diverse functionalities introduced by synthetic molecules and use DNA to organize these functionalities into motifs that allow their use in future devices. This is an area of growth in DNA nanotechnology, and it has tremendous potential. It will require the recruitment of more synthetic, macromolecular and supramolecular chemists to increase the application range and to discover novel components that expand the DNA structural alphabet. Importantly, synthetic methods to create these structures will need to be simplified; considering recent progress in the enzymatic[5,209] and non-enzymatic[210] replication of unnatural DNA, this is expected to be feasible in the future. The potential of chemistry to reduce complexity in DNA nanotechnology methods, to increase scalability, to render DNA structures more robust and more biocompatible, and to increase functionality and applications is tremendous.

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Acknowledgements
This research has been supported by the following grants to H.F.S.: Natural Sciences and Engineering Research Council of Canada (NSERC), Canada Research Chairs Program, the Canadian Institutes of Health Research (CIHR), Fonds de recherche du Québec — Nature et technologies (FRQNT) and Prostate Cancer Canada. The following grants were provided to N.C.S.: EFRI-1332411 and CCF-1526650 from the National Science Foundation (NSF), MURI W911NF-11-1-0024 from the US Army Research Office (ARO), N000141110729 from the US Office of Naval Research (ONR), DE-SC0007991 from the US Department of Energy (DOE) for DNA synthesis, and partial salary support and grant GBMF3849 from the Gordon and Betty Moore Foundation. The authors thank P. Chidchob, A. Lacroix, N. Avakyan, J. Hsu, D. Bousmail, T. Trinh, D. de Rochambeau, H. Fakih, E. Vengut Climent and M. Dore for help proofreading the manuscript.

Author contributions
H.F.S. researched the data for the article. H.F.S. and N.C.S. wrote the article and edited it before submission.

Competing interests statement
The authors declare no competing interests.

How to cite this article
Seeman, N. C. & Sleiman, H. F. DNA nanotechnology. *Nat. Rev. Mater.* **3**, 17068 (2017).