DNA Architectonics: towards the Next Generation of Bio-inspired Materials

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### Introduction

Solid-phase DNA synthesis is certainly one of the most influential developments of the last century. Together with the understanding of DNA structure and function, not only biology and molecular genetics have advanced significantly, but also new emerging fields, such as bionanotechnology, would not have evolved. The detailed understanding of DNA, that is, the specific connectivity of the nucleotides, the Watson–Crick base pairing, and the resulting helical structure of the double-stranded DNA (dsDNA) makes it possible to design DNA sequences that spontaneously form objects through complementary nucleobase recognition. High yielding synthesis and purification is crucial to the success of all approaches involving DNA, both as a template and scaffold. In addition, other fields of research, such as supramolecular chemistry, organic synthesis and inorganic coordination chemistry, add greatly to the expanding field of DNA bionanotechnology through incorporation of nucleoside analogues further adds functionality with addressable groups, which have an influence on the function of the DNA nano-objects. This article highlights the recent achievements in this emerging field and gives an outlook on future perspectives and applications.

### Keywords:
DNA • DNA architectonics • functional oligonucleotides • nanobiotechnology • nucleotides

Although DNA can be regarded as a relatively rigid, one dimensional stick with a persistence length of around 50 nm (about 150 base pairs) certain flexibility can be designed into the structure, for example, through small bulges or single-strand segments; these can be crucial for the successful self-assembly of the architectures. DNA is quite forgiving in terms of modification, and relatively stable duplexes can be obtained even with large amounts of both external and internal modifications. Recent reviews of the literature in the field of synthesis and application of nucleoside analogues demonstrate the breadth of modifications available.

Although many of the basic architectures described below are obtained by using standard DNA, which can easily be purchased from commercial sources, the incorporation of nucleoside analogues with addressable functionalities adds a new level of complexity, and takes the concept a significant step forward. Organic synthesis will certainly continue to play a key role in successfully merging the fields of DNA self-assembly with supramolecular chemistry. In this respect, a steadily increasing number of building blocks for site-specific modification of DNA are also available from commercial sources. These include a range of modifiers for further conjugation, both within the sequence and at the end of the DNA, for example, through amide coupling, sulfide chemistry and click ligation, and modifications that have direct influence on DNA structure, for example, photoswitchable units. This article focuses on the most recent developments in the field of DNA nanotechnology, which increasingly includes modified nucleotides, derived either from post-synthesis modification or through chemical synthesis of the building blocks.

### The Early Days

The evolution of DNA nanotechnology has been reviewed several times in the last few years, including a recently published special themed issue of *Chem. Soc. Rev.* therefore only a limited overview of the early-day systems shall be given. The pioneering work in DNA assembly stems from several laboratories. Among them, Seeman and Turnerfield realised that the self-assembly of designer DNA strands into cubes or octahedra and tetrahedra can be achieved, either in multiple steps or—in the best case—in a single step (Figure 1). Indeed, the concept was laid out already many years ago in a theoretical paper by Seeman. Since then, a wide range of arrays, such as grids and lattices on surfaces, linked frayed wires, nanoscale patterns through folded DNA, tube structures and bipyramids have been assembled successfully. RNA has also been used as a building block in nanotechnology, and is likely to become an additional valuable tool as demonstrated by Jaeger et al. A great advantage is that the length of the edges can be varied relatively easily through elongation of the DNA sequences, though the helicity needs to be taken into the structure, for example, through small bulges or single-strand segments; these can be crucial for the successful self-assembly of the architectures.
into account. Therefore, it sometimes may be better to think of helical turn increments of 3.3 nm (i.e., the helical pitch of B-DNA) rather than of base-pair increments, though this depends very much on the desired structure. The initial syntheses of the DNA objects were rather low yielding and had to be performed under high dilution conditions, with careful adjustment of the annealing process. Current technologies have overcome this problem, mainly through better design of the DNA sequences, and the assemblies can now be obtained in high yields (> 90%). However, all assemblies are based on the precise equimolar association between the complementary strands involved; therefore, elaborate control of the purity and the relative stoichiometry of the strands is vital to ensure a high yield.

DNA–Nanoparticle Conjugates

The early publications on the selective connection of gold nanoparticles (AuNPs) through DNA recognition by Mirkin et al.\textsuperscript{[17]} and Alivisatos and co-workers\textsuperscript{[18]} certainly should be recognised for laying the foundations for a wide range of NP conjugates based on DNA scaffolds. The basic principle includes attachment of thiol end-modified DNA to the AuNPs. Different NPs can be modified with specific DNA sequences, which hybridise either to themselves or to complementary bridging DNA, to give a programmed reversible assembly of NPs (Figure 2a). A vast amount of one-dimen-

Figure 1. Examples of first generation DNA nanostructures. a) DNA cube (reprinted by permission from Macmillan Publishers, Ltd., Nature, copyright 2003).\textsuperscript{[5d]} b) DNA tetrahedron (reproduced by permission from The Royal Society of Chemistry).\textsuperscript{[8b]} c) Scanning force microscopy image showing parallel arrays of frayed wires (adapted with permission from ref. [11], copyright 2002, American Chemical Society). d) Construction of DNA tubes controlled by a four-way-branched DNA connector (reprinted with permission of John Wiley & Sons, copyright 2005, from ref. [13]).

Figure 2. Nanoparticle–DNA conjugates obtained through different approaches. a) Connecting AuNPs by hybridisation of complementary DNA strands (adapted with permission from ref. [17c], copyright 2000, American Chemical Society). b) Induction of helical AuNP arrays by non-covalent DNA binding of peptide–NPs (adapted with permission from ref. [22], copyright 2010, American Chemical Society). c) DNA-hexagonal template for the organisation of AuNPs (adapted with permission of John Wiley & Sons, copyright 2006, from ref. [24]). d) Layer-by-layer hybridisation for photochemically active DNA–NP conjugates (adapted with permission of John Wiley & Sons, copyright 2001, from ref. [26]).
bridisation of DNA-bound NPs\textsuperscript{20} and electrostatic interactions,\textsuperscript{21} or by using noncovalent interactions between DNA and functionalised NPs, for example, peptide–DNA recognition (Figure 2b).	extsuperscript{22} The DNA sequences on the NPs are also available for enzymatic manipulation (restriction and ligation enzymes) as shown by Ivanisevic and colleagues, who made use of specific DNA sequences for reassembling NPs with a DNA scaffold.\textsuperscript{23}

The concept is also adaptable to DNA surfaces (see also below) and NPs have, thus, been arranged on DNA templates, such as hexagons (Figure 2c),\textsuperscript{24} lattices and grids,\textsuperscript{25} but also directly on gold electrodes through attachment of thiol-DNA onto the surface, and subsequent layer-by-layer hybridisation assembly to yield photoactive conjugates (Figure 2d).\textsuperscript{26} DNA-guided assembly of NPs is now being used to design NP crystals. In a seminal work, Gang et al. reported the formation of three-dimensional crystalline assemblies of AuNPs mediated by interactions between complementary DNA molecules attached to the nanoparticle surface.\textsuperscript{27} Their tuneable approach has led to the successful realisation of a body-centred-cubic lattice structure, which is temperature-tuneable and structurally open. The concept is being adopted to create more NP lattices;\textsuperscript{28} Mirkin and co-workers have recently presented six design rules that can be used to deliberately prepare nine distinct colloidal crystal structures, with control over lattice parameters on the 25 to 150 nm length scale.\textsuperscript{29} Their design rules outline a strategy to independently adjust each of the relevant crystallographic parameters, including particle size (5 to 60 nm), periodicity, and interparticle distance.

**DNA Surfaces as Templates**

The two dimensional lattice and grid structures as pioneered by LaBean and colleagues, are a demonstration of the concept of self-assembly of DNA strands with addressable sites for modification (Figure 3a).\textsuperscript{5j,10b} Many extended 2D crystals have now been reported; most recent advances include in situ formation of 2D grids on solid surfaces reported by Mao et al.\textsuperscript{30} The method of sticky-end reuse in a hierarchical fashion by Pistol and Dwyer allows for the assembly of fully programmable, large-molecular-weight DNA complexes from low-cost small precursors (Figure 3b).\textsuperscript{31} The concept should be scalable to larger grids, as demonstrated for an 8×8 DNA waffle grid that gives access to large-scale synthesis of DNA arrays.

![Figure 3. Examples of constructing 2D arrays with the use of DNA tile lattices. a) Schematic drawing of double and triple crossover complexes (DAE, DAO, TAO) and a 4×4 tile (reproduced by permission of The Royal Society of Chemistry).\textsuperscript{5j} b) Formation of (biotinylated) waffle-grids by using the sticky-end approach (reprinted by permission of IOP Publishing Limited).\textsuperscript{31} c) Formation of AuNp arrays with differently sized NPs (reprinted from ref. [32b] with permission, copyright 2005, American Chemical Society). d) Formation of thrombin and PDGF protein arrays on a set of DNA tiles (reprinted from ref. [32c] with permission, copyright 2007, American Chemical Society).](image-url)
The two-dimensional lattice and grid structures can contain specifically addressable sequences to which further functionalities can be attached. Therefore, the surfaces act as platforms, and DNA strands, small molecules, nanoparticles, proteins and antibodies have been anchored in this way (Figure 3c).[32] Yan and colleagues have generalised a highly programmable strategy to self-assemble multiprotein nanoarrays with deterministic positional addressability (Figure 3d).[32a] As proof-of-concept, 2D arrays of thrombin and platelet derived growth factor were made by attaching the corresponding aptamers to rigid DNA tiles; periodic insertion of “blank” tiles act as spacer units. This strategy was used to attach the two proteins to the same tile with nanometre precision.[33] Other biomolecules, such as enhanced green fluorescent protein (EGFP),[34] horseradish peroxidase (HRP) and glucose oxidase (GOx),[35] and virus capsids (Figure 3e)[36] were attached to origami tiles (see also below), usually by using linker-modified staple strands, which interact with the protein, for example, through His-tag–nickel interactions or modification of the biomolecule with a complementary DNA strand.

The seminal work on DNA origami tiles by Rothemund demonstrated that long (usually viral) DNA strands can be stitched together with short staple strands to give well-defined nanosized tiles (Figure 4a). A few hundred staple strands are required to create a DNA origami, and the concept was proven by the successful formation of the famous maps and smiley faces on surfaces.[37] The rectangular origami tiles are now beginning to play a key role in the design of assembly lines, because rectangular tiles can be linked together through dangling DNA at the edges. Key is the design of the origami tile in order to understand important factors involved in the assembly of DNA origami superstructures. Yan and Liu et al. have constructed a new series of rectangular-shaped DNA origami tiles in which parallel DNA helices are arranged in a zigzag pattern when viewed along the DNA helical axis.[37] This design allows to relax an intrinsic global twist found in the original planar, rectangular origami tiles, which can lead to very different folding and assembly patterns as compared to the intended outcome. In this case, the zigzag tiles were designed to promote two-dimensional array formation, but one-dimensional linear arrays and tubular structures were observed instead. Apparently the dimensional aspect ratio of unit tiles and connection design play a key role. The theoretical analysis of double-crossover tiles is equally important for understanding the DNA curvature, which is essential for successful construction of origami tiles.[38]

The 2D origami tiles can further be used as construction tiles to be folded into 3D structures as shown by Endo and Sugiyama et al.[39] Here, Y-shaped, X-shaped, and asterisk-shaped structures formed by self-assembly were stitched together by using staple strands to give hollow prism structures; high-speed AFM (one image per second) showed irreversible disruption of the hollow structures within seconds. DNA origami has also been used to assemble higher order geometrical structures, such as a Möbius strip, which can be reconfigured through strand displacement to create supercoiled rings and catenanes (Figure 4b).[40] It was suggested that this “DNA fold-and-cut strategy, analogous to Japanese kirigami, can be used to create and reconfigure programmable topological structures that are unprecedented in molecular engineering.” A step forward is the development of orthogonal stacking of DNA origami tiles by Woo and Rothemund, who used the geometric arrangements of blunt-end stacking interactions to direct recognition (Figure 4c).[41] They showed that both binary codes and shape complementarity can serve as a basis for such stacking bonds. This work demonstrates how a single attractive interaction, which in this case is not based on the base-pairing of DNA, can be developed to create diverse bonds, and may well guide strategies for molecular recognition in systems beyond DNA nanostructures. Gothelf, LaBean and Rangnekar have added a third dimension by creating four helical bundle tiles,[42] which were found to be extremely rigid and stable. Although these can be assembled to form micrometre-long filaments, the formation of 2D structures failed, and nanorings were observed instead; the reason was unclear. A similar observation was made with an analogous sticky-end approach with a DNA duplex by Stulz et al.[43]

The commonly used concept in origami templating includes addressing the platform through the DNA code itself, in which incorporation of single-stranded segments, for example, elongation of specific staple strands, gives dangling DNA strands; the code of this DNA forms a unique address on the platform, analogous to the addressing of 2D DNA crystals. DNA, which hybridises to this address, can contain the particle of interest to be attached.[44] The concept again has general character: origami templates have DNA binding sites with a unique coding sequence, which can be adsorbed onto surfaces. For example, nanoparticles modified with the complementary DNA sequence can be attached easily to create seed nanoparticles. These seed nanoparticles can be enlarged, and even fused, by electroless deposition of silver. Using this method, LaBean, Finkelstein and colleagues constructed a variety of metallic structures (Figure 4d).[45] Fusion of the origami tiles leads to the formation of rings, pairs of bars, and H shapes. A new aspect is that DNA can be used as template for etching and masking silicon oxide in molecular lithography: Liu et al. have used a triangular DNA origami in vapour-phase etching. This could lead the way to the use of more complex DNA origami structures as templates for bottom-up nanofabrication with about 20 nm resolution.[46] Molecular lithography with DNA nanostructures has, however, been reported earlier by Mao and Deng,[47] and the group has now used a biotemplating strategy for the fabrication of metallic nanoparticle arrays.[48] The templates are self-assembled DNA nanostructures, which dictate nanoparticle synthesis in the gas–solid phase during thermal evaporation.
Figure 4. DNA origami tiles and their use as construction material and platforms. 

a) Principle of forming DNA origami from plasmid DNA (finished design including staples across the “seam” of the DNA tile; reprinted by permission from Macmillan Publishers, Ltd., Nature, copyright 2006).[12] 

b) Formation of a Möbius strip from DNA origami (reprinted by permission from Macmillan Publishers Ltd, Nature Nanotechnol., copyright 2010).[40] 

c) Connection of DNA origami tiles through orthogonal stacking bonds without DNA hybridisation (reprinted by permission from Macmillan Publishers, Ltd., Nature Chemistry, copyright 2011).[41] 

d) Formation of metallic structures by using AuNPs attached to tiles as seeds (reprinted from ref. [45] with permission, copyright 2011, American Chemical Society). 

e) Arrangement of virus capsids with nanoscale precision by using DNA origami (reprinted from ref. [36] with permission, copyright 2010, American Chemical Society).
3D DNA Structures, Empty or Enclosing Cargoes

The great advantage of using a DNA-based stick approach to build up structures is that almost any three-dimensional geometry can be achieved; the limitation is mostly set by the researcher’s imagination. For example, engineered Holliday junctions,[49] DNA scissors,[50] ion-induced switches,[51] DNA tube control by DNA connectors[13] or small-molecule mediated DNA junction induction[52] can be used as building blocks. A recent milestone in the design of three-dimensional structures certainly is the self-assembled crystalline DNA structure by Seeman, Mao and co-workers (Figure 5a).[53] The structure is built up by tensegrity triangles, which are basically rigid 3D triangular units consisting of three connected non-coplanar double helices of DNA. By adding sticky ends, they can bind to each other, to eventually form a symmetric 3D lattice. Surprisingly, branched DNA with exceptionally short sticky ends can assemble into a highly ordered material at temperatures at which genomic DNA is fully denatured, if the branching geometry and linker rigidity favour crystallisation.[54] This system by Richert et al. uses rigid organic linkers to assemble branched DNA, in which...
only two base pairs suffice to form the new material. In conclusion, many factors including increased rigidity, reduced charge, optimal fraction of organic non-DNA matter, and appropriate counter-ions, need to be optimised to achieve hybridisation.

The concept of creating distinct structures seems to take a new turn with the creation of curved tiles, which can be used to build up 2D arrangements of concentric rings and 3D spherical shells, ellipsoidal shells, and a nanoflask. It is possible to engineer complex shapes that twist and curve at the nanometre scale, as shown originally by Dietz, Shih and Douglas. Through programmable self-assembly, strands of DNA are directed to form a custom-shaped bundle of tightly cross-linked double helices, arrayed in parallel to their helical axes. Targeted insertions and deletions of base pairs cause the DNA bundles to develop twist of either handedness or to curve. The degree of curvature could be quantitatively controlled, and a radius of curvature as tight as 6 nm was achieved. The group also combined multiple curved elements to build several different types of intricate nanostructures, such as a wireframe beach ball or square-toothed gears (Figure 5b). The group has extended this method to building custom three-dimensional shapes, which are formed as pleated layers of helices and are constrained to a honeycomb lattice. They have demonstrated the design and assembly of nanostructures approximating six shapes—monolith, square nut, raced bridge, genie bottle, stacked cross, slotted cross—with precisely controlled dimensions ranging from 10 to 100 nm. Building on this concept, Yan and co-workers have designed more 3D objects (Figure 5c). Overall, the design for a 3D object hinges on the careful dissection of the object into a wireframe representation, much like the Earth’s division into latitude circles. The tiles need to be carefully designed manually, including cross-over and staple strands, to obtain the correct curvature. For the successful construction of a DNA nanoflask with a neck diameter of 13.2 nm, maximum flask diameter of 40 nm, and overall height of 70 nm, a total of 35 concentric double-helical DNA rings were used.

Such 3D DNA nanostructures do not just represent a playing-field for the open-minded researcher, but actually start to serve very specific purposes. In view of loading these nanostructures with cargoes, they are promising as delivery systems. A first step towards the use of engineered DNA nanostructures to deliver and control the activity of cargoes within cells was recently made by Turberfield and colleagues, that contains a controllable lid that can be opened by a DNA tetrahedron can enclose an enzyme, and the encapsulated cargo is quantitatively demonstrated by spatially mapping the coelomocytes of C. elegans.

DNA-based tubes can be tailored in their size, simply by using edges of different lengths. Sleiman and co-workers have reported a partitioned DNA tube with smaller and larger compartments, this allows for a specific enclosure of AuNPs in the larger parts, whereas the smaller ones remain empty (Figure 5d). The AuNPs are arranged in linear wires but do not communicate with each other. Peeling of stabilising edge strands selectively opens the compartments to release the NPs. Similarly, metal–nucleic acid cages and the rapid and quantitative method to generate DNA cages of deliberately designed geometry has been described by the same group. Equally convincing are the icosahedral NP capsules designed by Krishnan and colleagues, who constructed the most complex DNA-based plasmonic solid through a unique modular assembly strategy and demonstrated the functional aspect for DNA polyhedra by encapsulating gold nanoparticles from solution. A nanoscale DNA box was designed by Besenbacher, Gothelf, et al., that contains a controllable lid that can be opened by a “key”. The box, crafted by an origami approach, gives controlled access to the interior compartment and opens the way to controllable cargo storage and release (Figure 5f).

It should be noted that chemical modifications at the interior or terminus of DNA can also be used as “chemical glue” to stitch dsDNA together, hydrophobic aromatic molecules, such as porphyrins, stilbene derivatives and perylenes are suitable for this approach. In general, either end or interior modifications on the DNA aggregate due to their hydrophobic nature, and introduce intramolecular interactions between the dsDNA. This can be used to form higher order architectures, such as long rods (end-on stacking) or helical bundles (side-on stacking) from short DNA strands (Figure 5g). In another approach, the combination of DNA with lipids to form amphiphiles is being investigated for programmable nanomaterials. Herrmann and co-workers have demonstrated that virus-like particles, templated by DNA micelles, can easily be assembled in solution, and can be loaded internally with a number of small molecules; such constructs are particularly interesting for drug delivery. The use of copper catalysed alkyne–azide click chemistry is certainly gaining much interest in the formation of novel architectures. The site specific modification of DNA with either alkynes (usually) or azides (rare) adds an addressable moiety, which was explored in the formation of metalated DNA, catenanes, and recently, for a variety of cross-linked oligonucleotides. Three dimensional sys-
tems, such as tetrahedra, were also chemically modified, and led to a functional assembly of chemical groups with tuneable stoichiometry and defined geometry.[77]

Particularly intriguing is the attachment of metal chelators to DNA, which can form specific metal complexes. In this respect, Wengel and co-workers have used a terpyridine moiety attached to the ribose part of “unlocked” nucleic acids (UNA, lacking the C2’–C3’ bond) to interlock two complementary DNA strands, and thus form a very stable duplex.[83] Sleiman showed that incorporating a bipy-unit within the DNA strand leads to a system in which chiral metal–DNA four-arm junctions can be assembled: two DNA strands are linked through metal complexation rather than Watson–Crick base pairing. In principle, an external template strand brings together two ligand-modified DNA strands for metal coordination, which upon metatation form a metal–DNA junction with four different arms. After template removal, a single-stranded four-arm DNA junction is retained. These structures were extended to form metalated nanotubular structures with site-specific metatation. The group of Stulz has inserted a terpyridine moiety into DNA using a rigid acetylene linker; this gives access to site-specific metatation of DNA and potential interstrand linking of dsDNA (Figure 5h).[80] By attaching the terpyridine at selected sites of two short DNA strands with a self-complementary sticky-end, the DNA first assembles into long DNA duplexes, which are then interconnected by zinc or nickel to form bis-terpy complexes between DNA duplexes.[84] AFM has shown the formation of tubular arrays consisting of DNA bundles that are 50–200 nm wide and 2–50 nm high, and several microns long. TEM also showed long-range ordering in the form of fibres. Also, porphyrins have been used as a platform to construct helical bundles of DNA. This concept demonstrates the directed formation of DNA nanoarrays through orthogonal self-assembly by using both base pairing and metal complexation. Post-modification of linker-substituted DNA with a porphyrin has allowed the group of Endo and Majima to attach four DNA single strands onto the porphyrin platform. After formation of the DNA duplex with an unmodified complementary strand to the four-way-branched conjugate, a four double-helix DNA assembled structure is obtained. This system was further incorporated into 2D DNA tiles, which assemble into three-dimensional DNA tubes containing the porphyrin as a control unit in form of a four-way-branched DNA connector.[13]

DNA Switches, Machines and Walkers

Switchable DNA structures have now found their way into the design of nanostructures. Much like folded RNA, DNA can adopt a variety of secondary structures apart from the standard helical duplex. Hairpins, loops, bulges, quadruplexes, B- to Z-DNA change and i-motifs are amongst the most versatile structures suitable for switching. The event can be induced either by changing the environmental (physical) parameters, such as pH, temperature or ionic strength, or by the addition of suitable complementary strands, which are commonly referred to as “fuel” strands. The field of nucleic acid based molecular devices has recently been reviewed by Krishnan and Sinnel.[80] The first report on a DNA nano-mechanical device stems from Mao, Seeman, et al. who used the B- to Z-DNA switching by changing the ionic strength of the buffer solution.[81] A subsequent system by Yurke et al., which is termed “molecular tweezers”, represents the first DNA-fuelled molecular machine made of DNA (Figure 6a).[82] Their “machine” not only uses DNA as a structural material, but also as a “fuel”. The machine is constructed from three strands that hybridise into the form of a pair of tweezers. The tweezers can be opened and closed by the addition of auxiliary strands of fuel DNA, and each cycle produces a duplex of DNA waste product. The concept has now been implemented many times, and a recent example is illustrated by the switching of DNA structures to adjust AuNP distances on a DNA scaffold by Gang et al. (Figure 6b).[83] The switching occurs between a flexible single-strand linkage and either a short stem–loop or a long double strand structure by the addition of the appropriate complementary strand. Hairpin DNA, which is immobilised on a glass surface on one end and conjugated to magnetic NPs on the other end, can be elongated and contracted by using an external magnetic field.[84] In this case, the fuel is not added in form of complementary DNA strands, but by an external, controllable stimulus, with which the DNA responds to the magnetic field rather than perform the motor action itself.

The synthetic modification of DNA with small organic switching units is becoming an attractive concept, and azobenzene derivatives seem to establish themselves as convenient control agents. Azobenzenes can be addressed with different wavelengths, and lead to a switch between a short non-planar Z- and an elongated planar E form. The building block for DNA synthesis is now commercially available and can be incorporated easily into any DNA strand. The advantage of using light over DNA fuels is that the machine can be operated remotely, does not produce waste, and is reversible through a simple input; in reality, the switching is not a hundred per cent efficient, cannot be achieved rapidly (usually in the order of minutes), and overlapping absorbances can make specific addressing difficult. Examples of the use of azobenzene-modified DNA as switching units include a light-driven nanomachine for photoswitching between the resting and active state of a DNAzyme (Figure 6c).[85] Very recently, AuNPs were attached at three vertices of the variational triangular faces of tetrahedron,[86] the contraction of one of the edges of the tetrahedron was initiated by the azobenzene switch, followed by dissociation of the complementary strand to promote a hairpin structure in the edge (Figure 6d). The system was shown to be robust and reversible. Chemical modification of DNA also comes to aid when the DNA structures are to be connected through orthogonal means to the Watson–Crick base pairing to form DNA structures with moving units. An elegant example is the synthesis of DNA catenanes by Heckel and Schmidt.[83] Al-
though the 3D structures described above also represent catenane structures by means of interlinked DNA strands, they are interlocked and cannot move freely. Here, the structures are connected through polyamide–DNA interactions; the polyamides are attached covalently to one DNA ring, which interlocks with another ring through sequence-specific binding. The polyamide can in principle be cleaved to leave the rings to rotate freely, and despite not showing motion, the catenanes are precursors to molecular motors or machines. Similarly, the rotaxanes by Heckel, Famulok, et al. combine the fields of mechanically interlocked molecules and DNA nanotechnology; these constructs inherently contain moveable parts (a macrocycle moving unhindered along an axle). The concept has recently been extended to DNA mimics with light-activated “caged” interaction modules, in which irradiation with near-UV light initiates dimer formation; this adds another level of control for functional nano-architectures. All these structures make use of a modular approach to build up interlocked systems; enzymatic ligation can be used to stitch DNA strands together to prevent disassembly, or new bulky spherical DNA constructs are needed to prevent dethreading. With their movable parts, they could also be suitable components for the construction of molecular motors or machines made from DNA.

In recent years, walking DNA structures have been realised and can be regarded as genuine molecular motors. They show aspects of progressive, repetitive, processive and directionally biased transport of a molecular fragment along a track. A thorough overview can be found within the recent review article by Leigh and von Delius. DNA walkers can be differentiated into non-autonomous and autonomous walkers. For non-autonomous DNA walkers, which were introduced by Sherman and Seeman, the feet are anchored to the track through binding strands, which hybridise to both foot and track. The foot is then freed by addition of the complementary lifting strand to the binding sequence, and leaves the waste duplex behind. The cycle of foot rep-
lease and reattachment at the next forward site is controlled by the operator and causes the device to walk along its track. By designing an autonomous reaction cycle, the requirement for an external operator can be overcome. Here, the toeholds, which are required to initiate a hybridisation reaction, are progressively revealed by the preceding reactions in the cycle; the concept has been explained in detail by Turberfield and co-workers, who designed the first autonomous walker together with Yan, Reif and colleagues.

A step further towards nanoscale machines is achieved by introducing a moving DNA object that can walk along specifically laid-out tracks on DNA origami tiles. The necessary input and fuel for the walking events stem from DNA hybridisation. Both DNA itself (walker) and modified DNA (carrying cargo) have been moved along such DNA tracks. Winfree, Yan and colleagues have shown that DNA spiders can act as molecular robots and autonomously carry out sequences of actions, such as “start”, “follow”, “turn” and “stop” (Figure 7a). Through single molecule microscopy, it was confirmed that the walkers achieve directional movement along a well-defined track. Similarly, Seeman and co-workers have designed a proximity-based programmable assembly line, in which a DNA machine was capable of not only walking along a track, but also loading and donating a cargo. The walker is based on a tensegrity-triangle organisation with three hands and four feet. The hands accept and pick-up a cargo (AuNP), and move it along the track. Recently, Sugiyama, Turberfield and co-workers have demonstrated the controlled motion of a DNA motor along a 100 nm long track on an origami tile, based on the burnt-bridges methodology, which was followed by real-time AFM. The motor was shown to move in a uniform, directional and processive way and does not dissociate from the track; this fulfils the requirement of an effective linear molecular motor. Together with the ability to move cargoes along a branched track (molecular robot, Figure 7b) the systems now become autonomous, and the motion can be programmed by the layout of addresses along the track. Directionality is programmed by the choice of fuel, and the burnt bridges mechanism becomes redundant. This provides a significant step forward in the design of autonomous cargo delivery systems, with directionality input, which is rewritable by an external program encoded in DNA. Autonomous bio-barcode DNA machines can also be used for DNA amplification for sensing applications.

Figure 7. DNA “robots”. a) A molecular robot guided by prescriptive landscapes (walking DNA spider; reprinted by permission from Macmillan Publishers Ltd., Nature Nanotechnol., copyright 2010). b) A programmable and autonomous molecular robot the motion of which is fuelled by DNA hybridization (reprinted from ref. [92] with permission, copyright 2011, American Chemical Society).
Summary and Outlook

The field of DNA architectonics clearly has advanced to a stage at which new materials can be made in a straightforward and predictable manner. DNA mainly serves as a construction material to build up all kinds of geometries. This may be an oversimplified use of DNA, because it is more than just the brick and mortar for the structures. The specific sequence recognition, which is vital for the success of the self-assembly, is programmable. Specific local DNA structures (single strand, double strand, bulges, loops, hairpins, junctions, etc.) can be used to introduce shape, flexibility or rigidity; in addition, DNA can act as fuel to trigger autonomous. It is, however, difficult to predict where exactly they will become increasingly complex and functional.

The next major step forward will be to combine the immense repertoire of chemically synthesised building blocks with the DNA geometries. First successful attempts have been made, but a highly decorated and multifunctional DNA architecture has yet to be synthesised. Certainly, it will not be long before this is realised. We will continue to see more and more geometries being made from DNA, and they will become increasingly complex and functional. Highly sophisticated programmed architectures will emerge, which will find widespread applications in nanotechnology, electronics, drug delivery, diagnostics and sensing, and computing. DNA-based machines are already becoming fully autonomous. It is, however, difficult to predict where exactly the field will be heading as new technologies will continue to emerge: many of the approaches used today could not have been predicted ten years ago as some of the key technologies were not available (e.g., origami tiles). There lie many challenges ahead to be explored, and DNA nanotechnology will certainly play a central role in the realisation of a functional nanoworld.


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