PERSPECTIVE

OBC
www.rsc.org/obc

DNA-programmed assembly of nanostructures

Kurt V. Gothelf*[a] and Thomas H. LaBean*[b]

[a] *Department of Chemistry, Aarhus University, Langelandsgade 140, 8000, Aarhus C, Denmark. E-mail: kvg@chem.au.dk; Tel: (+45) 89423907*

[b] *Department of Computer Science, Duke University, Durham, North Carolina, 27708, USA. E-mail: thomas.labean@duke.edu; Tel: (919)660-1565*

Received 27th July 2005, Accepted 13th September 2005
First published as an Advance Article on the web 6th October 2005

DNA is a unique material for nanotechnology since it is possible to use base sequences to encode instructions for assembly in a predetermined fashion at the nanometre scale. Synthetic oligonucleotides are readily obtained by automated synthesis and numerous techniques have been developed for conjugating DNA with other materials. The exact spatial positioning of materials is crucial for the future development of complex nanodevices and the emerging field of DNA-nanotechnology is now exploring DNA-programmed processes for the assembly of organic compounds, biomolecules, and inorganic materials.

Introduction

Self-assembly is often one of the key approaches discussed when debating future methods for building nanostructures and nanodevices.[1] Our current ability to form nanostructures by self-assembly is, however, very limited compared to the power of lithographic techniques for the formation of solid structures in bulk materials and, in particular, electronic circuits at the nanoscale. The pace of developing and refining lithographic techniques, new scanning probe microscopy and nanoimprinting techniques is impressive and the current bottom-up approaches are far from able to compete. Self-assembly procedures for the production of some non-biological systems are commonly used and have in some cases found commercial applications. These include self-assembled monolayers for the immobilization of compounds and materials on surfaces, Langmuir–Blodgett films, artificial membranes *etc.* However, these structures are typically, polydisperse and only "nanoscaled" in one dimension. Formation of more complex monodisperse and two- or three-dimensional nanostructures consisting of multiple building blocks requires precise control over each interaction when the structure undergoes self-assembly. In this regard supramolecular chemistry is in its infancy and the structures that are accessible *via* this method are mainly limited to a single or a few highly symmetric structures.

The motivation and inspiration to continue exploring self-assembly is, on the other hand, obvious when studying the most advanced nanosystem known: the living cell. This unimaginably complex machinery made of organic molecules and polymers is formed by, and operates by, self-assembly. The precision and efficiency of this process derives from specific molecular interactions between proteins, DNA and RNA in particular, and other compounds including lipids, carbohydrates, and small molecules. The question is: how can we use any part of the cell's self-assembly machinery to assemble artificial nanostructures? A dramatic reduction in complexity is necessary and this should be obtained by focusing on only one or two types of structural element from cells. In this regard DNA (or RNA) is the obvious choice since (i) it is the building block with the highest information content, (ii) it is constructed from only four, quite similar chemical building blocks, (iii) its self-assembly behavior is by far the most predictable compared to other classes of biomolecules, (iv) microgram quantities of oligonucleotides are easily and inexpensively available *via* automated chemical synthesis and (v) a diverse infrastructure developed for biotechnology provides many tools for DNA manipulation, including amplification *via* the polymerase chain reaction (PCR).

Kurt Vesterager Gothelf (1968) performed his PhD research in organic synthesis and asymmetric catalysis at Aarhus University, Denmark, where he obtained his PhD degree in 1995 under the guidance of Professor K. B. G. Torssell and Professor K. A. Jørgensen. Following a period as a postdoc in Professor K. A. Jørgensen's group, he joined Professor M. C. Pirrung's group at Duke University. He returned to Aarhus University in 1999 to initiate research in organic nanochemistry. Since May 2002 he has been an Associate Professor at Aarhus University.

Kurt V. Gothelf

Thomas H. LaBean

Thomas Henry LaBean (1963) earned his PhD at the University of Pennsylvania in 1993. He studied the folding properties of arbitrary sequence proteins expressed by random, synthetic DNA libraries under the guidance of Professor Stuart A. Kauffman and Professor Tauseef R. Butt. He then moved to Duke University and studied protein design with Professors Jane S. and David C. Richardson, and then worked on DNA-based computation with Professor John H. Reif. He now studies self-assembling biomolecular nanostructures as an Associate Research Professor at Duke University.

DOI:10.1039/b510551j

Proteins, antibodies and their small molecule affinity substrates are also efficient and highly specific in self-assembly processes and, if only few types of specific interactions are required, they might be the best choice. The importance of their role in molecular biology, medicine and nanoscience cannot be underestimated. However, when it comes to the individual encoding of multiple building blocks for the assembly into nanostructues they are less suitable due to their complexity, diversity in structure and differences in the nature of their interactions or size. Peptide self-assembly[2] has its own, large technical literature which will not be discussed here.

Since the pioneering work by Ned Seeman[3], non-biological DNA-programmed self-assembly has become a major research area and several recent reviews have described various aspects of the area.[3–15] In this perspective we will focus on the application of DNA and its analogs as a programmable material with which to assemble organic, inorganic, and biomolecular nanostructures, and also for the assembly of complex, nanosized materials and micrometre-scale materials with nanometre-scale features and patterns.

Basic considerations regarding DNA-design

When planning to assemble materials by simple DNA-programmed processes the first things to consider are how to conjugate the materials and DNA (or analogs) and how to design the sequences to obtain the desired assembly.[16] The power of automated oligonucleotide synthesis makes small oligonucleotides easily accessible and, furthermore, many DNA-modifiers are commercially available and can be incorporated during automated DNA-synthesis. These include phosphoramidites with modified nucleoside bases, fluorescent dyes, biolabels such as biotin and a variety of functional chemical groups (FCG).[17] Typically, modifications are incorporated at the 5′-end of the DNA sequence using a modifier with a phosphoramidite group for coupling with the 5′-OH of the DNA sequence and a protected FCG. DNA sequences containing a 5′-terminal 4,4′-dimethoxytrityl (DMTr) ether are often separated from side products by chromatography immediately after synthesis. Therefore, it is advantageous to use modifiers that also contain a DMTr ether. Such modifiers can also be applied to 3′ and internal modification by incorporation at an earlier stage of the automated synthesis. The modifiers typically have FCGs such as amines, thiols or carboxyl groups. It is relatively easy to conjugate DNA with organic molecules that carry functional groups which react with the FCGs.[17] Biomolecules and chemically modified biomolecules can be conjugated with DNA in a similar manner, however, an alternative strategy is to introduce, for example, a biotin modifier in the DNA sequence for subsequent association with streptavidin.[7] Steptavidin has four binding sites with a very strong affinity for biotin, and the remaining three binding sites can be used for binding other biotin-labelled biomolecules or materials.

The inorganic material most frequently used in conjugation with DNA is gold nanoparticles.[5,11] DNA sequences with a thiol modifier can react directly with the naked gold nanoparticle or, by ligand exchange, react with monolayer-covered particles to form thiolate-Au bonds. This method can also be used for the reaction of silver and metal sulfide nanoparticles with thiols. Depending on the stoichiometry this approach typically leads to nanoparticles with more than one oligonucleotide molecule attached, however, purification methods have been described.[18] Alternatively, inorganic materials modified with an organic moiety *e.g.* a maleimide or a *N*-hydroxysuccinimide ester are linked to oligonucleotides by the reaction with a thiol or amine modifier, respectively.

To make DNA assemblies that are stable at room temperature it is required that DNA-sequences are of sufficient length. Typically 10- to 25-mer DNA sequences are used. A 10-mer dsDNA helix has a melting temperature between 25 and 35 °C. Guanine (G) and cytosine (C) interact *via* three hydrogen bonds leading to higher stabilization (*ca.* 6 kcal mol^{-1}) than the two-hydrogen bond interaction between thymine (T) and adenine (A) (*ca.* 4.5 kcal mol^{-1}) (Fig. 1). Therefore the melting point is also dependent on the nucleotide composition. Alternatively, the commercially available DNA analog peptide nucleic acids (PNA)[19] and locked nucleic acids (LNA)[20] can be applied. They have a much higher affinity for duplex formation and fewer bases are required to obtain stable double helix.[19–21] Furthermore they are both stable towards DNases, and by using PNA the negative charge of the DNA phosphate backbone is avoided.

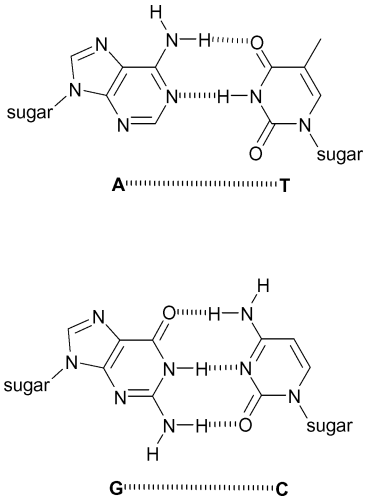


Fig. 1 The two-hydrogen bond interaction between adenine (A) and thymine (T) and the three-hydrogen bond interaction between guanine (G) and cytosine (C).

In the simplest case it is desired to bring two components, each attached to one oligonucleotide sequence, together *via* DNA-programmed assembly (Fig. 2). In principle the same self-complementary (palindromic) sequence can be applied on each component, however, two different complementary sequences are, by far, the most often used to increase control of the hybridization process and to avoid the formation of homodimers if the components are different. If both components are conjugated *via* the 5′ end to complementary sequences they will be separated by the dsDNA formed upon hybridization. In the case where one of the components is conjugated *via* the 3′-end, the two components will be brought in close proximity. What is preferred strongly depends on the size of the appended materials and the details of the particular application. In the template approach the sequences attached to the two components are

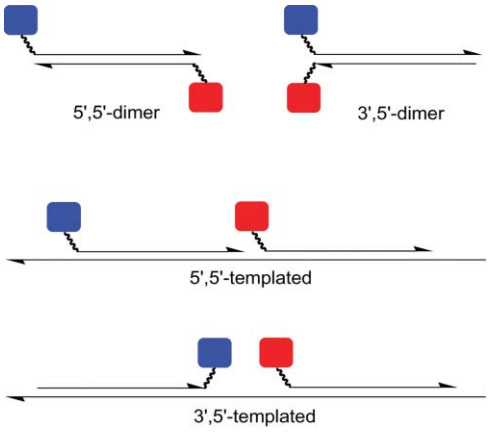


Fig. 2 Strategies for assembly of two components by DNA-programmed assembly. The coloured boxes symbolize an organic, bioorganic or inorganic material and the curved lines indicate linkers between DNA and the material. Arrowheads mark the 3′ ends.

non-complementary, however they are complementary with two successive sequences on a template. Depending on the conjugation point, the components may be aligned head-to-head or head-to-tail (or tail-to-tail). If the alignment of the two components on the template leads to some kind of signalling, this may be used for detection of DNA (RNA) sequences containing the template sequence.[22] More complex structures are easily designed by also attaching materials to the template and/or aligning several DNA-conjugates on one or more templates.

The use of only two complementary sequences or two sequences and a complementary template may also lead to more complex assemblies if multiple copies of the strands are attached to the same component (Fig. 3). This is often the case for materials with multiple attachment points such as gold nanoparticles. If a DNA multiplier *i.e.* a phosphoramidite linked to two or more DMTr ethers is applied in the DNA-synthesis other materials with only one attachment point can also be functionalized with multiple identical sequences.

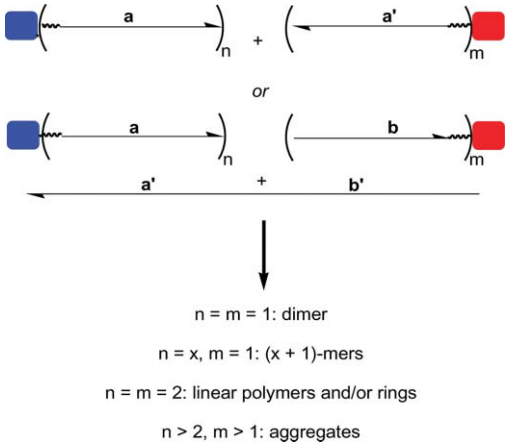


Fig. 3 Application of materials labelled with one or more identical DNA strands for formation of more complex assemblies. The arrowheads mark the 3′ ends.

The conjugation of materials to a modifier in the middle of a DNA-sequence is, in principle, equivalent to the attachment of two different DNA-strands to the same component (Fig. 4A). If the modifier is a nucleoside, *e.g.* having the linker attached *via* the nucleotide base, the copying of the DNA sequence by PCR may be possible. The material may also, in fact, be attached to two different DNA sequences, either by using chemical modifiers or by incorporation during the automated synthesis using phosphoramidite chemistry (Fig. 4A). These types of conjugates allow for the formation of well-defined supramolecular oligomers. The materials can be assembled in a side-by-side fashion or along a double strand depending on the DNA-encoding (Fig. 4B and C). In analogy to the templated approach mentioned above for materials attached with one DNA-sequence, the structures in Fig. 4A can also be aligned on a template, however, in this setup enzymatic ligation of the aligned sequences is in principle possible (Fig. 4D).

Most of the strategies for DNA-programmed assembly introduced above have been applied for the assembly of various materials, and several examples will be given in this perspective. However, much more advanced DNA-designs have been used for the assembly of pure DNA-structures and, in a few cases, also for the assembly of materials. The following section will give examples of such structures.

DNA nanostructures

The last few years have seen a great number of advances in our ability to construct complex nanostructures from nucleic acid building materials.[8] The study of artificial DNA structures for applications in nanotechnology began in the early 80s when Seeman sought to design and construct periodic matter and discrete objects assembled from synthetic DNA oligonucleotides.[23] He noted that simple double-helical DNA could only be used for the construction of linear assemblies and that more complex building blocks would be required for two- and three-dimensional constructs. He also noted that biological systems make use of branched base-pairing complexes such as forks (three-arm junctions) found in replicating DNA and Holliday intermediates (four-arm junctions) found in homologous recombination complexes. These natural branch junction motifs exposed a potential path toward multi-valent structural units. A Holliday junction is formed by four strands of DNA (two identical pairs of complementary strands) where double-helical domains meet at a branch point and exchange base-pairing partner strands. The branch junctions in recombination complexes are free to diffuse up and down the paired homologous dsDNA domains since the partners share sequence identity along their entire lengths. Seeman showed that by specifically designing sequences which were able to exchange strands at a single specified point and by breaking the sequence symmetry which allowed the branch junction to migrate,[24] immobile junctions could be constructed and used in the formation of stable and rigid DNA building blocks. These building blocks (or DNA tiles), especially double-crossover (DX) complexes[25], became the initial building blocks for the construction of periodic assemblies and the formation of the first two-dimensional crystals of DNA tiles.[26] DX tiles

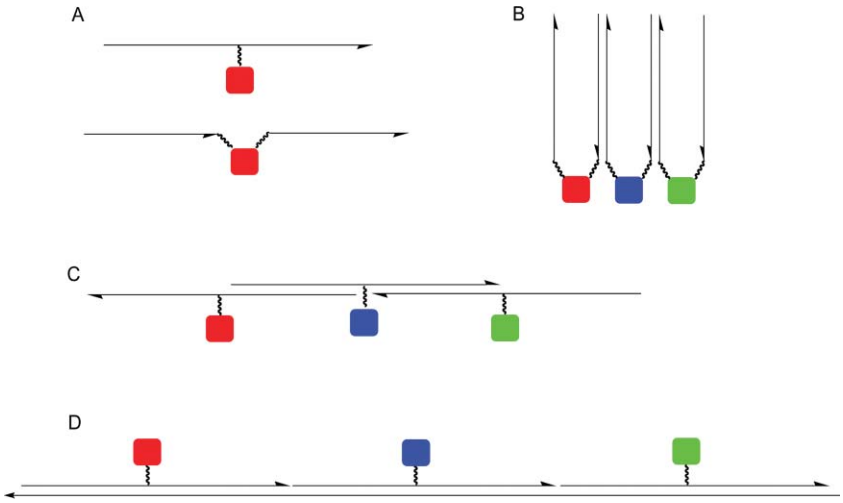


Fig. 4 Assembly of materials incorporated internally in DNA sequences. (A) Materials attached *via* a DNA modifier or *via* two separate sequences. (B) Side-by-side assembly. (C) Assembly along a double strand. (D) Alignment on a template. Arrowheads mark the 3′ ends of strands.

have also been used to produce beautiful algorithmic assemblies displaying fractal design patterns (discussed further, below).[27] These large lattices provide multiple attachment sites both within and between tiles for complex programmed structures and lead to diverse possibilities for scaffolding useful constructs and templating interesting chemistries.

A large number of distinct DNA tile types have now been designed and prototyped; some examples are shown in Fig. 5. The high thermal stability (T_m up to at least 70 °C) of some DNA tiles, the ability to program tile-to-tile association rules *via* ssDNA sticky-ends, and the wide range of available attachment chemistries make these structures extremely useful as molecular scale building blocks for diverse nanofabrication tasks. DNA tiles produced to date have contained double-helical DNA domains as structural members and branch junctions crossovers as connectors. The use of paired crossovers greatly increases the stiffness of the tiles over that of linear dsDNA. Following the success of the DX lattices, triple-crossover (TX) tiles, lattices, and computational nanostructures were demonstrated.[28–30]

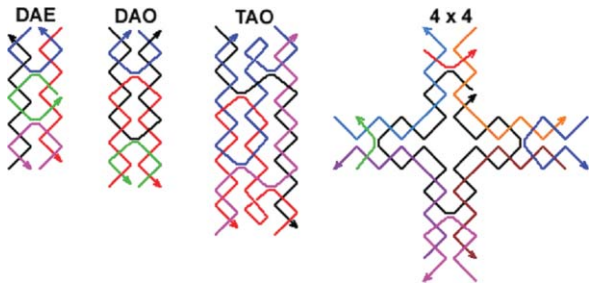


Fig. 5 Schematic drawings of four DNA tiles are shown. Colored lines represent different oligonucleotide strands with arrowheads marking the 3′ ends. DAE and DAO are double crossover complexes (also known as DX), TAO is an example of a triple crossover (or TX) tile, and the 4 × 4 tile is composed of four arms each of which contains a four-arm junction.

Since DX and TX tiles are designed with their helices parallel and coplanar, their lattices tend to grow very well in the direction parallel to the helix axes and fairly poorly in the direction perpendicular to it. Elimination of this problem and the growth of lattices with a square aspect ratio was the primary motivation behind the design of the 4 × 4 cross tile.[31] Long DNA nanotubes (up to 15 μm) and large 2D lattices (many square micrometres) have been assembled from 4 × 4 cross tiles (Fig. 6). Variants of these tiles have been used to pattern proteins and metallic nanoparticles (see below) and provide a versatile toolbox with which to organize nanoscale materials.

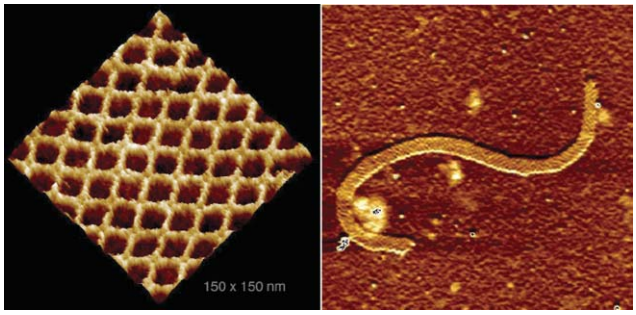


Fig. 6 AFM images of corrugated (planar) and uncorrugated (tube) versions of 4 × 4 cross tile lattices. The right panel is a 1 μm × 1 μm scan. Adapted with permission from ref. 31.

A variety of other tile shapes have been prototyped beyond the rectangular and square tiles shown above. Lattices with rhombus-like units have been made in which the helix crossing angles are closer to the relaxed ~60° angles observed in biological Holliday junctions.[32] At least three different versions of triangular DNA tiles have been prototyped (Fig. 7), one in which the plane is tiled entirely by triangles[33] and two versions which form hexagonal patterns.[34,35] Such triangular lattices have not been shown to grow as large as those from rectangular and square tiles, but they may be useful for assembly applications where slightly more structural flexibility is desired. They have also demonstrated some interesting multilayer structures with symmetrical stacking interactions.[35]

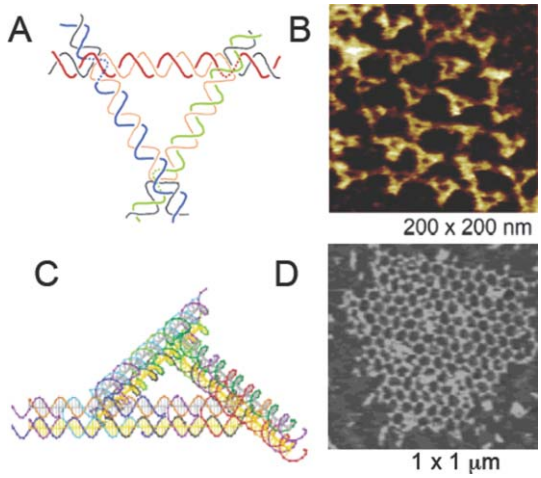


Fig. 7 Schematic drawings of two different triangular tiles (A and C) and AFM images of resulting 2D lattices assembled from trianglular tiles (B and D). Adapted with permission from ref. 33 (A and B) and ref. 34 (C and D).

DNA tiles which hold their helical domains in non-planar arrangements have also been designed, for example a three-helix bundle (Fig. 8).[36] and a six-helix bundle.[37] Non-planar tiles represent one strategy for expanding the tiling into the third dimension, although initial attempts at 3D structures using these tiles have not yet succeeded.

Besides 3D periodic lattices, another long-term goal of DNA self-assembly studies has been the generation of complex patterns on 2D arrays. The most complex pattern yet demonstrated *via* molecular self-assembly is the Sierpinski triangle pattern shown in Fig. 9.[27] These patterns were formed using a small tile set whose sticky-ends represent tile association rules which promote lattice formation according to the specific rules of the encoded algorithm.

Demonstration of this complex algorithmic self-assembly using synthetic DNA tiling shows that any arbitrary structure which can be specified by a set of encoded association rules can be expected to form, albeit at some yield < 100% and with some error rate > 0%. Self-assembling tiles and lattices have also been constructed from RNA with an added feature: the production of finite-sized arrays.[38] Previous DNA tiling systems all resulted in unbounded growth of the lattice and, consequently, polydisperse products following annealing. Demonstration of finite-sized arrays represents another step toward increased control of self-assembled molecular systems.

Three-dimensional building blocks and periodic matter constructed from DNA have been long-term goals of this field. Early attempts to build a cube[39] and a truncated octahedron[40] with dsDNA edges and branch junction vertices met with some success, but the final constructs were produced in very low yields. More recently, a tetrahedral unit with short double helical edges was constructed in much higher yield.[41] Perhaps the most impressive experimental success yet in DNA-based 3D nanostructures produced an octahedron with DX-like edges.[42] This study was noteworthy in that the 1.7 kilobase DNA strand (which folded with the help of five short oligonucleotides) was produced as a single piece by PCR-based assembly, and the octahedron was formed in sufficient yield to permit structural characterization by cryo-electron microscopy.

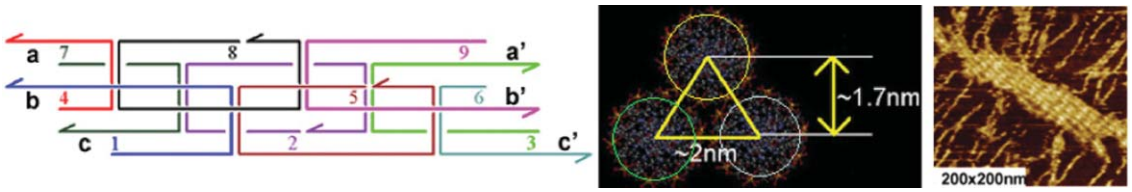


Fig. 8 The three-helix bundle (3HB) DNA tile shown as a schematic trace of the strands through the tile. Different color lines represent different oligonucleotides and arrowheads mark the 3′ ends. Six crossover points (paired vertical lines) connect the three double helices (paired horizontal lines) with two crossovers connecting each of the possible pairs of helices. The middle panel is an end view of the 3HB tile to show the stacking of the helical domains. The right panel is an AFM image of 2D lattice formed from properly programmed 3HB tiles. Adapted with permission from ref. 36.

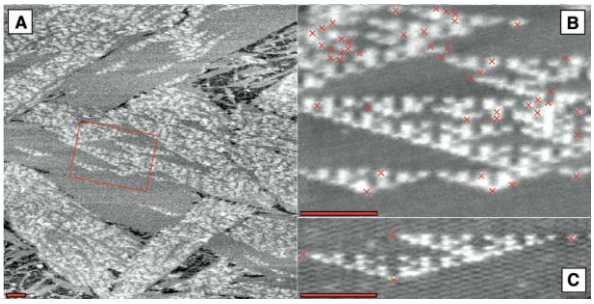


Fig. 9 AFM images of DX tile lattice algorithmically assembled to form fractal Sierpinski triangle patterns. Bright tiles carry an additional stem-loop of DNA projected out of the tile plane which appears taller to the AFM and, therefore, acts as a topographic marker. Panel B is an expanded view of the boxed region in panel A. Panel C is an expanded view from another section of lattice. Tiles which appear to be assembled incorrectly, based on visual inspection of the preceding (input) tiles, are marked by a red X. Scale bars are 100 nm. Adapted with permission from ref. 27.

The 2D lattices and 3D structures assembled from DNA and described here represent interesting objects in their own right, but their real usefulness will come from their application as scaffolds and templates upon which chemistry is performed or with which heteromaterials are organized into functioning nanodevices. We will return to some of these applications later in this perspective.

DNA-programmed assembly of organic building blocks

One wide spread application of DNA-programmed assembly (and disassembly) of organic compounds is the association and dissociation of two organic dyes on each end of a hairpin structure used in molecular beacons and fluorescent resonance energy transfer (FRET).[43] These two designs are widely used for DNA-sequence detection and are good examples of the power of DNA-programmed control over the distance between organic molecules on the nanoscale. For applications such as catalysis, molecular electronics, memory storage, drug delivery, protein mimics, chemical sensors and supramolecular chemistry in general the relative positioning of organic molecules also plays a central role and this area is rapidly developing as demonstrated below.

In pionering work by Shi and Bergstrom the assembly of a rigid and bent organic structure containing two arylethynylaryl moieties which are connected *via* a tetrahedral backbone was reported.[44] At each terminal position the arylethynylaryl moieties are attached to two identical oligonucleotides *via* a 2-hydroxyethyl spacer (Fig. 10). The two oligonucleotides are self-complementary (palindromic) which allows these DNA-conjugates to form cyclic structures in which the organic scaffolds are connected *via* dsDNA. Mixtures of two- to seven-membered rings formed and the size of the rings could, to some extent, be controlled by the annealing conditions.

Linear polymerization of building blocks containing a simple alkyl chain or oligoethyleneglycol chain between two oligonucleotides was reported recently.[45,46] Müllen *et al.* reported the synthesis and self-assembly into linear polymers of two linear perylenediimide–oligonucleotide conjugates with complementary DNA-sequences.[47]

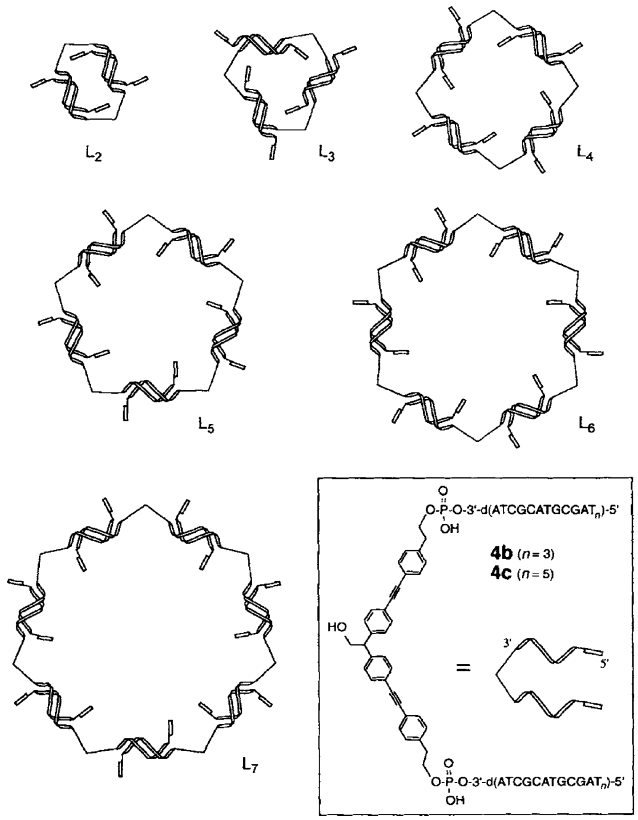
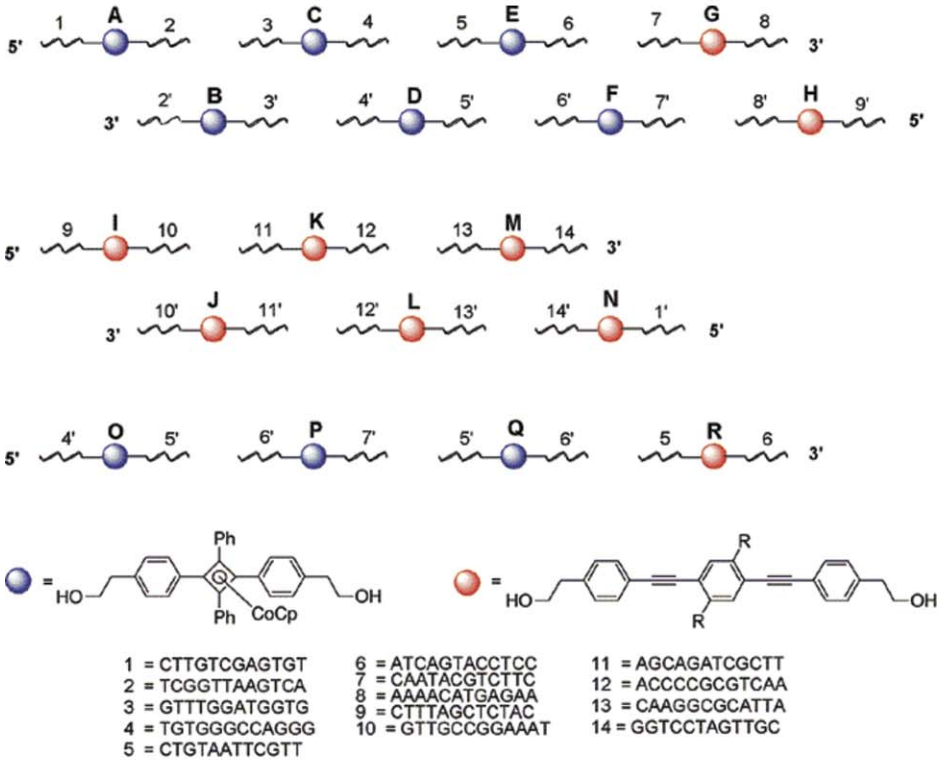


Fig. 10 Self-assembly of two- to seven-membered DNA cycles of organic scaffolds connected *via* self-complementary DNA-sequences. Adapted with permission from ref. 44.

Building blocks with two or more different and individual oligonucleotide sequences are required to form well-defined and monodisperse assemblies. This was the case in work by Bunz *et al.* where a tetraphenylcyclobutadiene(cyclopentadienyl)–cobalt complex and a phenyleneethynylene trimer were incorporated in the middle of 24-mer oligonucleotide sequences using phosphoramidite chemistry (Fig. 11A)).[48,49] The cobalt complex has interesting optical properties and oligo(phenyleneethynylene)s are fluorescent and of interest for organic semiconductor devices. The encoding of the organic modules with two individual 12-mer oligonucleotide sequences allowed them to assemble by DNA hybridization into supramolecular structures with predetermined connectivity. As shown in Fig. 11B, oligomers of up to seven units were formed as determined by native polyacrylamide gel electrophoresis (PAGE) analysis, fluorescence, and the measurement of DNA melting point temperatures.

Sleiman and co-workers have synthesized ruthenium bipyridine complexes with two identical[50] or two different oligonucleotide sequences.[51] The conjugates were formed by a modified

A



B

	Structure	Tm °C	Unannealed ϕ	Annealed ϕ
I	G H	40	0.91	0.90
II	G H I	40	0.96	0.94
III	G H I J	50	0.97	0.96
IV	G H I J K	45	0.84	0.86
V	G H I J K L	40	1.0	1.0
VI	G H I J K L M	55	0.98	0.93
VII	C D		--	--
VIII	C D E	40	--	--
IX	C D E F	35	--	--
X	F G	40	0.60	0.60
XI	E F G		0.41	0.35
XII	E F G H	35	0.25	0.22
XIII	E Q E Q E Q E	47		
XIV	R Q R Q R Q R	47		
XV		--	--	--

Fig. 11 (A) Structures of DNA–organic conjugates. (B). Melting temperature and fluorescence quantum yields for annealed conjugates. Adapted with permission from ref. 48.

phosphoroamidite base strategy. In the latter report both non-complexed and Ru-complexed dioligonucleotide bipyridines were synthesized. In the presence of two of these modules with complementary DNA-sequences they showed a strong tendency to form cyclic dimers, however, higher order structures and polymers were also observed. It is notable that the Ru-complexed dioligonucleotide bipyridines showed a higher tendency to dimerize than the non-complexed analogs.

In a related approach Han *et al.* reported on the synthesis of a terpyridine derivative tethered to a DNA sequence.[52] When two of the conjugates containing DNA sequences of different length were mixed in the presence of Fe(II), a stable bis(terpyridine)iron(II) complex formed (Fig. 12). It was possible to separate heterodimeric modules from homodimeric ones using PAGE. These dimeric metal complexes are two-way branched oligonucleotides. Three of the oligonucleotide modified metal-organic modules were capable of self-assembling into DNA triangles where the bis(terpyridine)Fe(II) complexes are positioned in the vertexes.

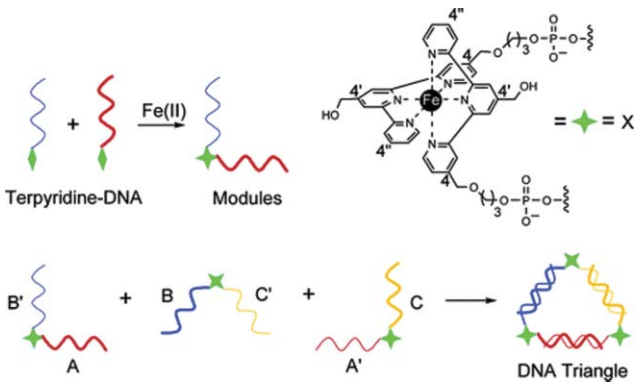


Fig. 12 Assembly terpyridine-DNA conjugates into two-way branched bis(terpyridine)Fe(II) complexes and DNA-programmed assembly of these into DNA triangles. Adapted with permission from ref. 52.

In a series of three papers Majima *et al.* have reported on the side-by-side assembly of two dsDNA strands by the application of multiple cross-linked oligonucleotides.[53–55] Two identical 10-mer oligonucleotides were cross-linked by a disulfide tether attached in the middle of the oligonucleotides. Two 20-mer or 30-mer sequences were assembled side-by-side by annealing with two or three of the short cross-linked oligonucleotides. In a single step, well-defined rigid structures controlling the relative orientation of the two double helix strands were formed.[53]

Branched DNA structures with three or more arms were independently explored by the two research groups and they both used an Ψ-branched phosphoramidite with three protected primary hydroxyl groups.[56–58] Shchepinov *et al.* used a Ψ-branched synthon to form oligonucleotide dendrimers with two, three, six, nine or twenty-seven arms.[57] Hybridization between two of the resulting DNA dendrimers with complementary sequences and also hybridization between DNA dendrimers with complementary strands on a solid support were investigated by thermal melting analysis. Von Kiedrowski *et al.* applied purified and well-characterized three-armed oligonucleotides to investigate the self-assembly of two three-armed structures with complementary sequences.[58] They suggested, based on PAGE analysis, formation of a dimeric structure interconnected *via* three parallel dsDNA helices in an "acetylene-like" fashion and a tetrameric cyclic structure connected in a "cyclobutadiene-like" fashion, along with higher order structures (Fig. 13). The Ψ-structure was also applied to the formation of a tetrahedral structure.[59]

Fig. 13 Structure and assembly of Ψ-branched DNA sequences into dimer and tetramer structures. Adapted with permission from ref 58.

Steward and McLaughlin applied a Ni(II)–cyclam complex as the scaffold for four-armed oligonucleotide conjugates (Fig. 14).[60] The branched oligonucleotide conjugates were synthesized by an advanced phosphoroamidite-based solid-phase method. Initially the first 20-mer oligonucleotide arm was synthesized on the solid support in the reverse 3′ to 5′ direction with reverse nucleoside phosphoramidites. One of the four functional side chains on the Ni-cyclam is attached to the oligonucleotide on the solid support and the synthesis is continued in parallel for the remaining three hydroxyl groups on the Ni–cyclam, but this time in the normal 5′ to 3′ direction. This elegant method enables the synthesis of four arm branched DNA structures where all four arms are 5′ terminated. Using a similar synthetic method, the same group has demonstrated the synthesis of a structure with six oligonucleotide arms by the application of a Ru(II) tris(bipyridyl) complex containing six hydroxyl groups arranged in an octahedral structures as the central building block.[61] The

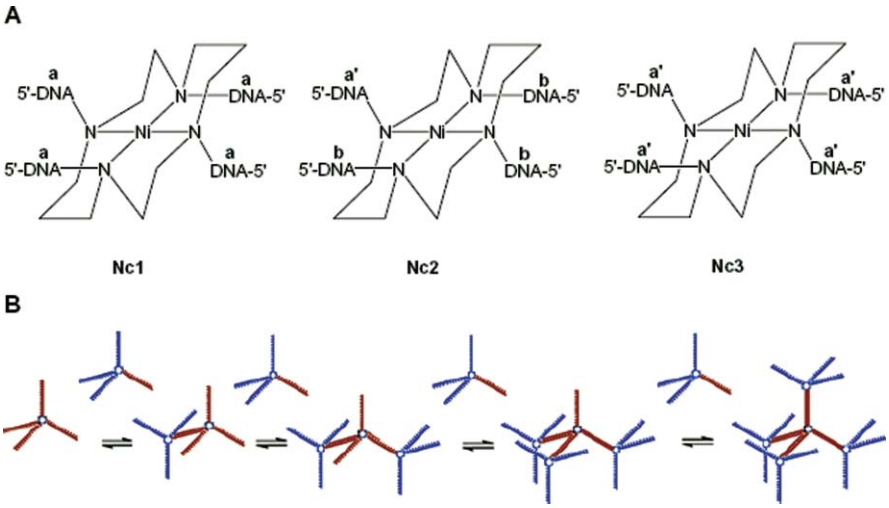


Fig. 14 (A) Structures of the Ni–cyclam DNA conjugates and (B) assembly of four way branched structures. (B) is adapted with permission from ref. 60.

four armed Ni-cyclam structures **Nc1**, **Nc2** and **Nc3** resulting from the former study contain four 20-mer oligonucleotide sequences and are tetrahedral by design (Fig. 14A).[60] The hybridization between **Nc1** and four **Nc2** (which contains one arm complementary to the four arms of **Nc1**) was explored. Only at high (100 mM) Mg^{2+} concentration was it possible to form the pentamer as illustrated in Fig. 14B and it was assumed that charge repulsion between the 12 unpaired arms is reduced by increased counter ion concentration. Attempts to form a 3D DNA crystal were performed by annealing **Nc1** with **Nc3** (all strands complementary to those of **Nc1**). Higher molecular weight bands were observed by PAGE, however, the structure of these assemblies has yet to be determined.

It has been demonstrated above that DNA is excellent for encoding the assembly of organic molecules into well-defined structures. One of the major draw-backs is the lack of control over spatial positioning of the organic groups. This is a particular problem for linear structures and large rings. For some purposes, in which only the average distance between the organic moieties is of importance, as for example for molecular beacons and FRET, this lack of positional control is not crucial, however, for applications where exact distances between the molecules are required, this is a serious limitation. Another aspect is that the organic compounds, in almost all cases described above, are separated by some length of dsDNA helix, hampering direct interaction between the compounds thus limiting the applicability of such assemblies. To avoid these problems, a new strategy is to apply DNA-programming both for assembling the organic structures and for covalently coupling the organic building blocks into new macromolecular structures.[10] Progress in this area is described in the next section.

DNA-programmed covalent coupling of organic building blocks

Recent progress in DNA-programmed synthesis has revealed that a variety of organic reactions can be directed by attached oligonucleotide sequences.[62] The concept of DNA-programmed synthesis is illustrated in Fig. 15A. The functional groups FG1 and FG2 can in principle react with each other without hybridization of the DNA sequences, but at low concentrations (nM to μM) their intermolecular reaction is so slow that practically no conversion occurs. If the DNA sequences attached to the functional groups are complementary, they will hybridize even at very low concentrations and thereby bring the two functional groups into close proximity. Now the local concentrations of the functional groups are significantly increased and the groups can react in a "pseudo-intramolecular" reaction, which will proceed significantly faster than the intermolecular reaction. Two other DNA designs for DNA-directed reactions are based on using either DNA hairpins or a DNA template (Fig. 15B and C).

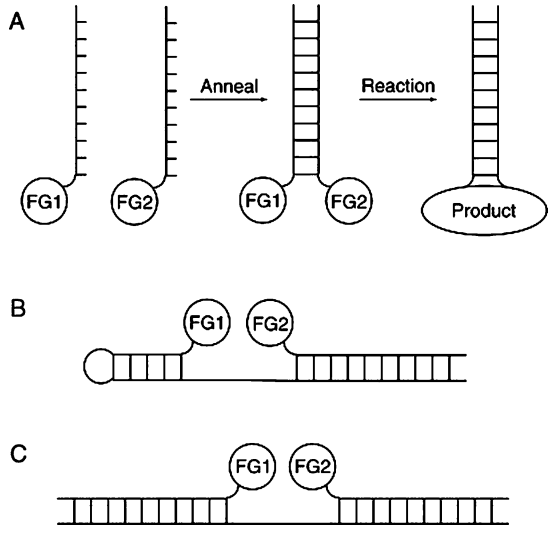


Fig. 15 Principle of DNA-programmed covalent coupling reactions. (A) The functional groups FG1 and FG2 are brought into close proximity by hybridization of their attached complementary DNA sequences leading to reaction and formation of a product. (B) Arrangement of the reactants in a DNA hairpin structure. (C) Arrangement of the reactants on a DNA template. Adapted with permission from ref. 10.

The new field of DNA-programmed chemistry has mainly been developed by Liu *et al.* and they have explored a variety of applications of this concept such as *e.g.* combinatorial chemistry and the discovery of new organic reactions.[62–64] The number of example applications of DNA-programmed chemistry for nano-related sciences is limited.[10] Liu applied a 40-mer DNA-template to align with ten PNA tetramer building blocks for parallel chemical ligation by reductive amination.[65] In a recent report by Chen and Mao it was demonstrated that the pH induced switching between the triplex and duplex DNA structures of a 5′-carboxylate labelled 40-mer DNA template, annealed with two 5′-amino labelled 12-mers, allowed for the selective reaction of the carboxylate with either one of the amino groups.[66]

Assembly and covalent coupling of three different oligonucleotides to a central organic core was reported by von Kiedrowski and co-workers.[67] They used the Ψ-branched three-armed DNA structure mentioned previously (Fig. 13) as the template to align three DNA-conjugated chemical functionalities at the center. The three individual oligonucleotides, having a 5′-hydrazide functionality, were aligned to react with a 1,3,5-triformyl benzene resulting in a trishydrazone attached to three different oligonucleotide sequences. In this method the chemical connectivity information contained in the Ψ-branched DNA sequences is copied by template-directed linking.

In an early report in this field the DNA-programmed synthesis of a organometallic complex was demonstrated. Two salicylaldehyde conjugated oligonucleotides were aligned on a DNA template in the presence of ethylenediamine and Mn(II) or Ni(II) resulting in the formation of metallosalen–DNA complex (Fig. 16).[68]

In the molecular engineering strategy by Gothelf *et al.*, two or three salicylaldehyde groups are contained within the same compound enabling the assembly and covalent coupling of multiple modules[10,69–72] The linear oligonucleotide-functionalized module (LOM) and tripoidal oligonucleotide-functionalized module (TOM) shown in Fig. 17A were synthesized.[69] The backbone of the modules is based on oligo(phenylene ethynylene)s to obtain a rigid, conjugated and potentially conducting structure. The modules contain salicylaldehyde moieties at each terminus. The modules also contain amide spacers at each terminus, which are connected to 15-mer oligonucleotides *via* phosphoramidite chemistry. Oligonucleotides attached at each terminus, are encoded to link up others containing complementary sequences. The salicylaldehyde groups of two modules are brought in close proximity when their complementary DNA sequences are annealed together. DNA-programmed coupling of the modules proceeds *via* manganese–salen formation between two salicylaldehydes groups in the presence of ethylenediamine and Mn(II).[70] The metal–salen forming coupling reaction was deliberately chosen, since the linkages between the individual head groups of the modules will be essentially linear due to the stereochemistry of the manganese–salen complex formed. The metal–salen link constitutes a potential conducting junction with the possibility of varying the central coordinated metal.

Depending on the encoding of LOMs and TOMs with different DNA sequences, assembly and covalent coupling of the modules into a variety of predetermined nanostructures can be formed as depicted for selected structures in Fig. 17B. The products were characterized by denaturing PAGE and dimer and trimer products were characterized by MALDI-TOF MS. It was also found that the melting points of the LOM–LOM, LOM–TOM or TOM–TOM combinations are increased by 15–30 °C after the coupling of the modules.[70]

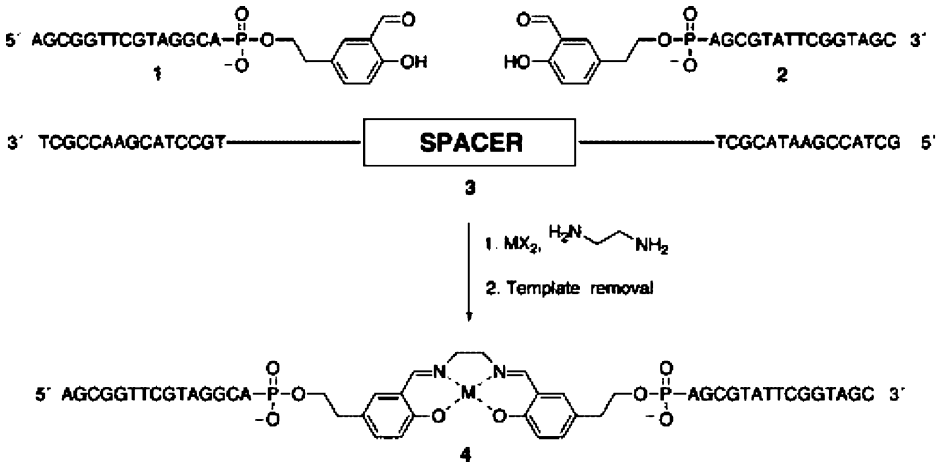


Fig. 16 DNA-templated formation of a metallosalen complex. Adapted with permission from ref. 68.

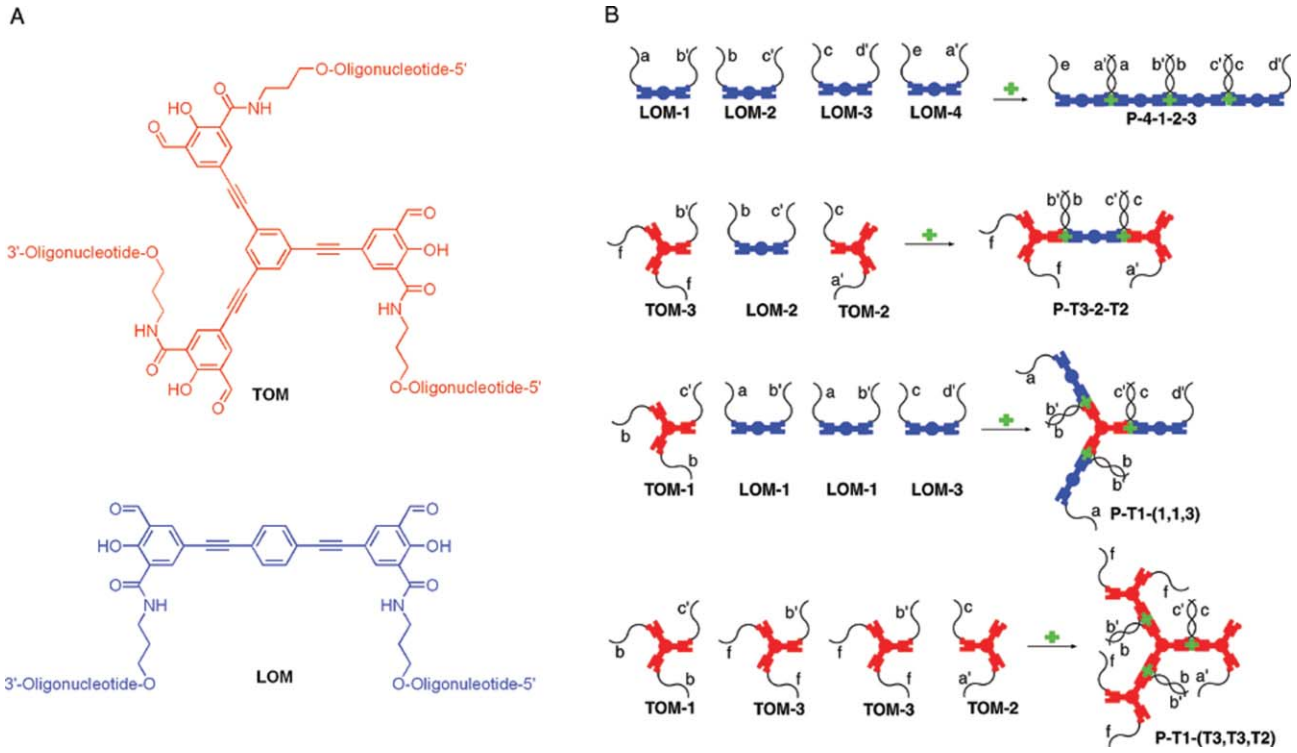


Fig. 17 Modular DNA-programmed assembly of conjugated nanostructures. (A) Structure of LOM and TOM modules. (B) Representative couplings of LOMs and TOMs by formation of multiple manganese–salen complexes between the modules (green colour: ethylenediamine and $Mn(OAc)_2$. (B) is adapted with permission from ref. 10.

The same group later showed that by introducing a disulfide spacer between the organic module and the DNA sequences, it was possible to cleave the DNA sequences off the macromolecular nanostructures.[71,72] It has also been shown that the DNA-programmed coupling could be performed by reductive amination with ethylenediamine and $NaCNBH_3$ resulting in tetrahydrosalen linked structures with significantly increased stability.[72] The principle of using DNA to assemble a macromolecular organic structure from which the DNA sequences are subsequently removed is a new aspect of DNA-nanotechnology. Furthermore, recycling of the liberated thiol modified DNA sequences may be possible.

DNA-programmed assembly of biomolecules

Assembly of other biomolecules on DNA templates and arrays may prove useful for fabrication of biomimetics and other devices with applications such as biochips, immunoassays, biosensors, and a variety of nanopatterned materials. The logical end to the shrinking of microarrays is the self-assembled DNA nanoarray with a library of ligands distributed at addressable locations to bring analyte detection down to the single molecule level. We will return to complex DNA tiling structures momentarily, but first we will look at simpler dsDNA systems.

The conjugation of DNA and streptavidin *via* a covalent linker was reported by Niemeyer *et al.* in 1994, and these conjugates were applied to DNA-programmed assembly on a macroscopic DNA array on a surface and in a nanoscale array made by aligning DNA-tagged proteins to specific positions along a oligonucleotide template (Fig. 18).[7,73,74] The covalent attachment of an oligonucleotide to streptavidin provides a specific recognition domain for a complementary nucleic acid sequence. In addition, the binding capacity for four biotin molecules is utilized as biomolecular adapters for positioning biotinylated components along a nucleic acid backbone (*vide infra*).

Biotin labelled oligonucleotides are commercially available and are routinely used in molecular biology, and their application for nanostructuring is growing in popularity.[7,75] Niemeyer

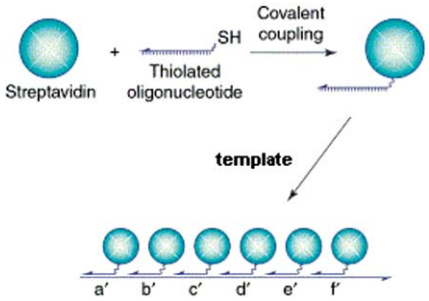


Fig. 18 Conjugation of 5′-thiol oligonucleotide and streptavidin, and alignment of the conjugates on a 170-mer RNA template. Adapted with permission from ref. 7.

applied 169-mer dsDNA sequences labelled with biotin in the 5′-positions for the formation of DNA–streptavidin networks (Fig. 19A).[76–78] Despite the tetravalent binding capacity of streptavidin it serves primarily as a bi- or trivalent linker between the oligonucleotide strands as observed in the AFM pictures of the aggregates shown in Fig. 19B. By thermal denaturation and rapid cooling these aggregates are transformed into DNA–streptavidin nanocircles as imaged by AFM (Fig. 19C). Nanocircles with DNA sequences of sizes varying from 87 to 485-mer sequences were formed and, depending on the length and concentration, up to 77% nanocircles were formed relative to other structures for the larger DNA sequences, whereas 28% nanocircles were formed from the 87-mer sequences.

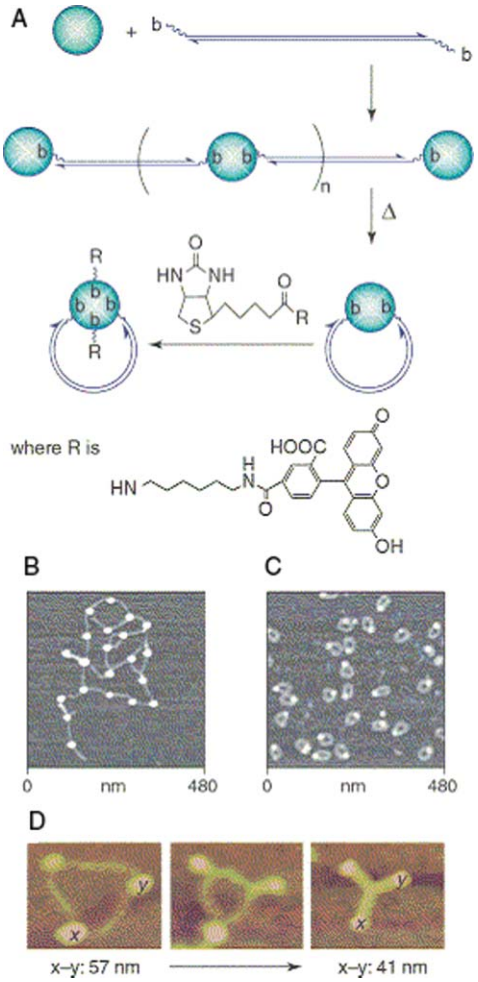


Fig. 19 (A) Self-assembly of DNA–streptavidin conjugates. (B) AFM images of the oligomeric DNA–STV aggregates and (C) of the DNA–streptavidin nanocircles. (D) Ionic-switching of a DNA_3–$(streptavidin)_3$ by increased supercoiling of the interconnecting DNA linkers. Adapted with permission from ref. 7.

The nanocircles were, furthermore, functionalized with hapten groups such as fluorescein and applied in an immuno-PCR assay.[79] During the studies of the aggregates, in this case a DNA_3-$(streptavidin)_3$ structure, supercoiling of the dsDNA-sequences induced by increased Mg^{2+} concentration led to a significant structural change, decreasing the distance between the streptavidins as illustrated in Fig. 19D.

Besides duplex DNA structures, more complex self-assembling DNA tiling structures have been used to organize biomolecules into specific spatial patterns. DNA nanostructures covalently labelled with ligands have been shown to bind protein molecules in programmed patterns, for example, making use of the popular biotin/avidin pair, arrays of evenly spaced streptavidin molecules were assembled on DNA tile lattice.[31] On 4 × 4 cross tile lattice, individual streptavidin molecules are visible as separate peaks in the AFM image (Fig. 20). Single molecule detection could be achieved on DNA nanoarrays displaying a variety of protein binding ligands.

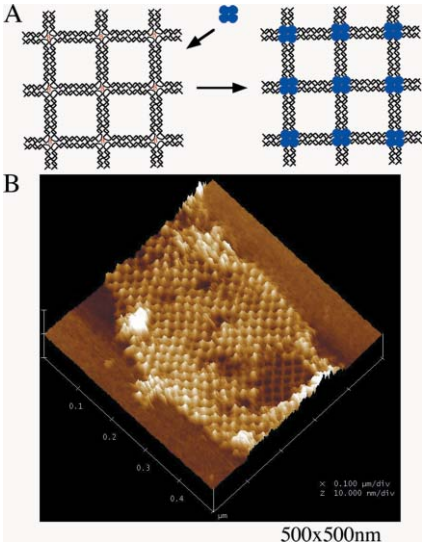


Fig. 20 (A) Schematic drawing of 4 × 4 cross tile lattice carrying a biotinylated central strand and streptavidin molecules (blue) binding to the functionalized sites. (B) AFM image showing individual streptavidin proteins at the vertices of the cross tile array. Adapted with permission from ref. 31.

Further design evolution of the 4 × 4 cross tile system to a two tile type (A and B) tile set allowed for somewhat more complex structures and patterns.[80] In this study, some size control of lattice and partial addressability were demonstrated, but the display patterns were still periodic and symmetric (Fig. 21). In ongoing experiments, finite-sized objects with independent addressing have been used to assemble a range of specifically patterned protein arrays in high yield.[81]

Another exciting future use for biomolecules specifically patterned on self-assembled DNA nanostructures is the specific deposition of inorganic materials *via* crystal nucleation. Natural peptides and proteins have been implicated in the growth of nano-patterned silica by living organisms.[82] Peptides and RNA sequences have been artificially evolved by *in vitro* selection to specifically bind and precipitate or crystallize various semiconductors and metals.[83,84] Patterning these species on 3D DNA lattices could provide a method for bottom-up assembly and controlled deposition resulting in a wide variety of complex inorganic structures for use in nanoelectronics, photonics, and other fields.

DNA-programmed assembly of materials

In analogy to the immobilization of DNA on a variety of solid surfaces, the conjugation of DNA and analogs with metal nanoparticles, semiconductor nanoparticles and polymer

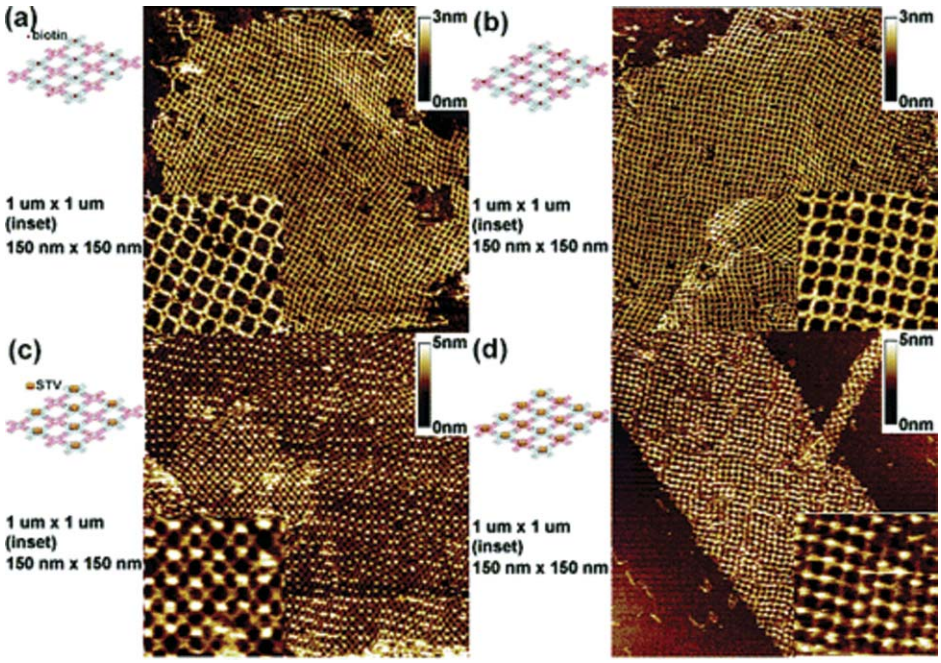


Fig. 21 Atomic force microscopy images of the programmed self-assembly of streptavidin on 1D DNA nanotracks. Panels a and b are AFM images of bare A*B and A*B* nanotrack before streptavidin binding, respectively, where tiles marked with '*' indicate the presence of biotinylated strands. Panels c and d are AFM images of A*B and A*B* nanotrack after binding of streptavidin. All AFM images are 500 nm × 500 nm. Adapted with permission from ref. 80.

particles is becoming increasingly important. Such DNA-conjugated materials have in several studies found application for biosensors and the reader is addressed to recent excellent reviews for a detailed overview.[5,11,85] Here the main focus will be on the application of DNA to assemble materials into well defined nanostructures.[3] Two important pioneering reports on the assembly of gold nanoparticles by hybridization of DNA-nanoparticle conjugates were published back-to-back in *Nature* in 1996.[86,87] Mirkin *et al.* prepared two samples of 13 nm gold nanoparticles functionalized with 3′-thiol-linked DNA sequences and 5′-thiol-linked DNA sequences, respectively.[86] If a solution containing the two DNA-nanoparticles is mixed with a DNA target complementary to both DNA-nanoparticle sequences, hybridization will force the particles to aggregate (see Fig. 3). This change in interparticle distance causes a change in the plasmon absorbance due to plasmon coupling. The resulting color change in the presence of the target is a very efficient and easy method for DNA-detection and has been developed in a series of subsequent reports.[18,88,89] In one extension they reported on the DNA-programmed placement of 8 nm gold particles around larger 31 nm gold particles (Fig. 22).[90] Depending on the ratio between nanoparticles **1** and **2**, the "satellite structures" were observed within extended assemblies or in isolated structures as shown in Fig. 22B.

Whereas the examples mentioned above utilize nanoparticles functionalized with several oligonucleotide strands per nanoparticle, the approach described by Alivisatos, Schultz and co-workers applies gold nanoparticles labelled with only single oligonucleotide molecules.[87] Small 1.4 nm gold nanoparticles containing one maleimide group per cluster were reacted with 5′- or 3′-thiol modified 18-mer oligonucleotides. By annealing two or three of these DNA–nanoparticle conjugates with a DNA template, homodimeric or homotrimeric nanoparticle assemblies were formed as verified by TEM. In more recent studies heterodimeric and heterotrimeric gold nanoparticle assemblies were also obtained by using nanoparticles of different sizes.[18] In Fig. 23 the DNA-programmed arrangement of 5 nm and 10 nm gold nanoparticles into non-periodic assemblies is shown. It is an excellent example of the power of DNA to encode and assemble materials, but it also shows the lack of structurally rigidity of dsDNA, since precise positional arrangement of nanoparticles is not obtained. In a very recent study, homotetrameric DNA–nanoparticle assemblies containing extendable hairpin loops were reported.[91] Related studies using branched DNA-sequences were performed by others.[92]

Niemeyer *et al.* have published several papers on DNA conjugated with biomolecules (*vide supra*) and they have extended this work to the DNA-programmed assembly of gold nanoparticles.[74] Short 5′-thiol modified DNA strands were attached covalently to streptavidin, and gold nanoparticles (1.4 nm) with a single amino substituent were coupled to a biotin moiety. These two components were mixed and four of the biotinylated-gold nanoparticles were linked to the streptavidin–DNA conjugate due to the strong and specific affinity of biotin for the four binding sites of streptavidin. Up to six of these DNA–streptavidin–gold nanoparticle structures with individual DNA sequences were annealed with a complementary 170-mer RNA sequence, resulting in alignment of the six nanoparticle–streptavidin complexes in a line as verified by TEM. In another study they reported the functionalization of gold nanoparticles with up to seven different 3′-thiol and 5′-thiol modified oligonucleotide sequences for the detection of different target sequences using the same oligofunctional DNA gold nanoparticles.[93]

In a recent publication a method for reversible switching of DNA–gold nanoparticle aggregation was developed.[94] Gold nanoparticles (23 nm) bound to two different 12-mer oligonucleotides (a and b) were applied. In the presence of a template containing sequences a′ and b′, the nanoparticles will aggregate upon hybridization as in the concept developed by Mirkin *et al.*[22] Niemeyer *et al.* extended this to a system capable of undergoing DNA-programmed reversible switching between aggregation and dispersion by applying two complementary "fuel" oligonucleotides, F_a and F_d (Fig. 24). The base sequence of F_a is comprised of the template sequences a′ and b′ plus a short 4-mer sequence c′. The hybridization between the F_a template and the nanoparticle DNA-sequences is disrupted by the addition of the fully complementary fuel strand F_d (Fig. 24, stage III) which strips F_a out of the complex, leading to the formation of a waste duplex and redispersion of the nanoparticles. The switching between the aggregated and dispersed nanoparticles was easily detected by UV–visible spectroscopy due to the

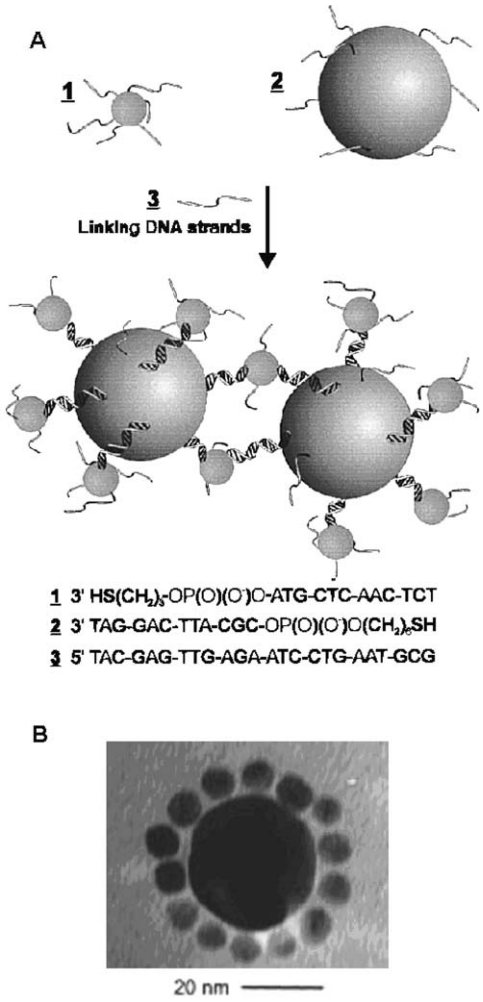


Fig. 22 (A) Formation of DNA–gold nanoparticle aggregates by hybridization of DNA sequences on 8 nm gold nanoparticles and 31 nm gold nanoparticles with a DNA template. (B) TEM image of a nanoparticle satellite structure obtained from the reaction involving a 120:1 ratio of 8 nm and 31 nm nanoparticles. Adapted with permission from ref. 90.

previously mentioned interparticle distance dependent change in the plasmon resonance absorption. It was demonstrated that the switching could be repeated at least seven times, and it is important to note that the waste product (dsDNA of F_a and F_d) was not removed during the seven cycles. It was sufficient to add increasing amounts of F_a and F_d in each cycle.

Combining DNA's ability to organize nanomaterials with the diverse and programmable structures available from self-assembling DNA tile lattice strategies has resulted in several initial steps toward the bottom-up assembly of nanomaterials that may prove useful as electronic components. A TX tile assembly was used to align a modest number of 5 nm gold particles in single and double layer rows.[33] This construction used tiles containing integral biotin-labelled DNA strands and streptavidin bound gold particles. In another study, DX tile arrays were used to pattern 6 nm gold particles into precisely spaced rows covering micrometre scale areas as shown in Fig. 25.[95] This study featured gold nanoparticles labelled with T_{15} oligonucleotides which base-paired with assembled DX lattice displaying single-strand A_{15} sequences hanging off certain tiles. The ability to organize electrically active species such as gold using DNA points the way toward the templating of complex devices and circuits for applications in nanoelectronics.

DNA-programmed assembly of materials other than gold nanoparticles has also been reported. Such materials include, semiconductor nanoparticles,[96,97] nanorods,[98] mesoscale particles,[99,100] and dendrimers[101,102] Most of these examples are

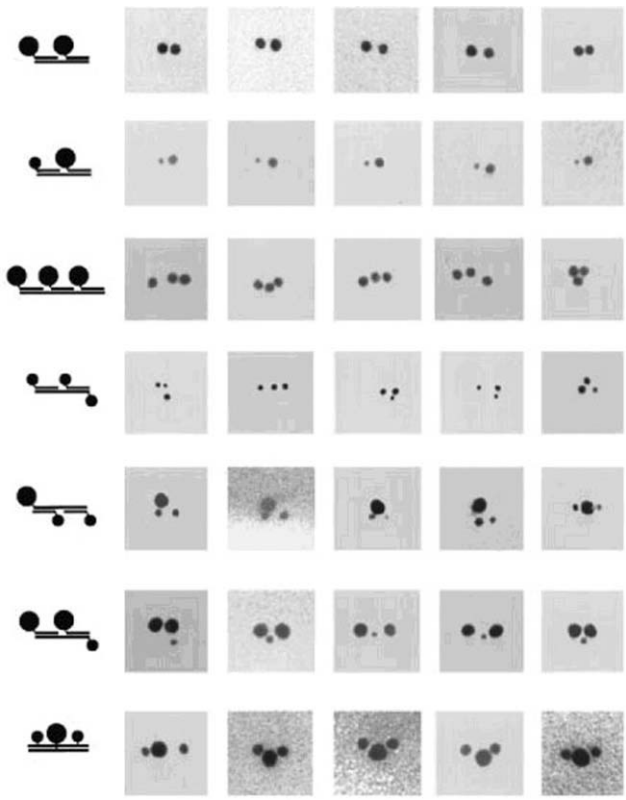


Fig. 23 Schematic illustrations and TEM images for nanocrystal 10 and/or 5 nm gold nanoparticle–DNA structures. Adapted with permission from ref. 18.

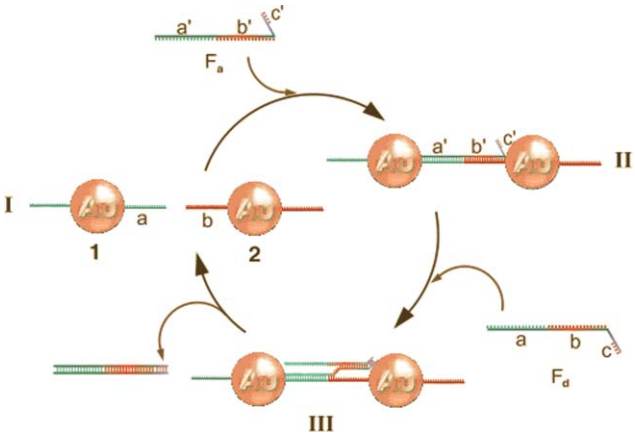


Fig. 24 Reversible aggregation of DNA-modified gold nanoparticles using fueling oligonucleotides F_a and F_d. Adapted with permission from ref. 94.

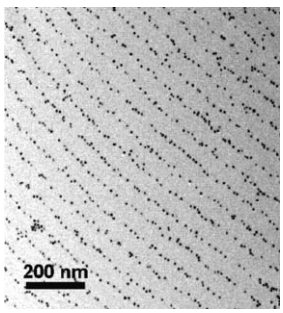


Fig. 25 TEM image of gold nanoparticles organized on a self-assembled DX tile lattice using complementary base-pairing interactions. Adapted with permission from ref. 95.

based on linear assembly of two complementary DNA strands leading to dimers or aggregates. Many examples of nanowires templated on DNA molecules by a variety of electroless deposition protocols (including fabrication of a field effect transistor[103]) have also been reported, but these are beyond the scope of this article.

Carbon nanotubes are one of the most promising materials for nanoscience due to their unique structure and mechanic and electronic properties.[104] In recent years chemical conjugation of organic and bioorganic compounds with carbon nanotubes is a field that has developed rapidly.[105] The ability to control the exact positioning of multiple carbon nanotubes by means of DNA-programmed assembly would be a major achievement in nanoscience. In a few reports, the conjugation of carbon nanotubes with DNA[106,107] and with PNA has been described.[108] In these reports carbon nanotubes were shortened into fragments by oxidation, resulting in carbon nanotube fragments with carboxyl groups in the terminal positions and, to some extent, in their walls. Covalent coupling of 5′-amino DNA-sequences or PNA to carboxyl groups on the nanotubes led to the formation of carbon nanotubes coupled with DNA or PNA sequences. Carbon nanotubes containing 12-mer PNA-sequences were annealed with dsDNA sequences containing 12-mer sticky ends and imaged by AFM.[108] In the work by Dai *et al.*, multi-wall carbon nanotubes (MWNTs) and single-wall carbon nanotubes (SWNT) functionalized with 20-mer DNA sequences were annealed with complementary sequences attached to gold nanoparticles.[107] The resulting aggregates were deposited on mica and imaged by AFM (Fig. 26). The images revealed the occasional interconnection of individual MWNTs by a gold nanoparticle. The surface plot in Fig. 26B shows a gap between the gold nanoparticle and the MWNT corresponding to the 7 nm length of the 20-mer dsDNA connecting the two materials.

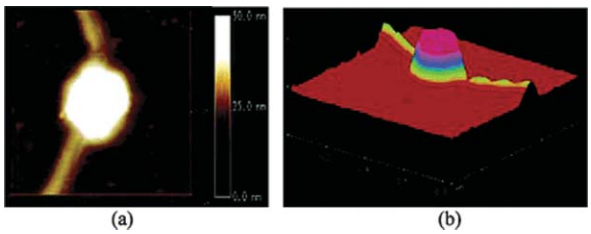


Fig. 26 (A) AFM image of the interconnection of two MWNTs by a gold nanoparticle (scanning area: 0.55 μm × 0.55 μm). (B) 3-D surface plot of (A). Adapted with permission from ref. 107.

In another study, SWNTs were assembled between prepositioned metal electrodes *via* complementary DNA base-pairing by ssDNA on the gold electrodes (thiol-labelled oligos) and the oxidized SWNTs (3′-amino-labelled oligos).[109] Electrical conductivity between the electrode pairs was shown to be highly dependent on the presence of complementary DNA on the electrodes and nanotubes. These initial investigations of carbon nanotube–DNA conjugates hold great promise for future developments in the assembly of nanotube structures with useful electronic and mechanical properties.

Conclusions

In the ultimate development of bottom-up nanofabrication strategies it will be possible to assemble large numbers of easily available building blocks, and depending on the nature and programming of the building blocks they will self-assemble into complex nanostructures with enzyme-like properties, electronic circuits with efficient contacts to larger length scales, memory storage devices, drug delivery robots, multifunctional diagnostic devices for *in vivo* application, or even systems capable of self-reproduction. Only the future will tell how much of this will be realized in practice, but whatever develops, DNA-programmed assembly will undoubtedly play a central role.

We are now learning the basics of how to position materials with DNA. It has been demonstrated that DNA molecules are used to assemble a small number of components (< 10) such as organic molecules, biomolecules and nanoparticles into well defined assemblies or aggregates. Furthermore, complex DNA building blocks have been assembled into highly regular 2D DNA-lattices, which in some cases were used for the periodic incorporation of proteins or nanoparticles. Formation of DNA-wires and 3D constructs has also been described. Most of the reports describe model-studies, but some of the systems have found application, in particular for DNA-sequence detection.[85]

The area of DNA-nanotechnology will undoubtedly continue to evolve and improve our present ability to position materials using DNA. Specific topics we find of particular importance and interest include: (i) the development of new DNA-constructs with enhanced properties for materials assembly, (ii) the design of new DNA structures which can control the formation of nanoparticle devices for application in electronics and photonics, (iii) the assembly of 2D DNA lattices on surfaces with individually addressable connecting points for future development of nanoarrays, (vi) major advances in DNA-programmed assembly of advanced carbon nanotube-based architectures, in particular if the problems regarding the synthesis of monodisperse carbon nanotube fragments of similar size, structure and properties are solved, (v) further development in DNA-programmed assembly *and* covalent coupling to form macromolecular nanostructures with potential application in molecular electronics, catalysis and new macromolecular architectures, (iv) design of other systems for DNA-programmed assembly, in which the organic, bioorganic or inorganic building blocks are connected by other means than DNA-hybridization leading to structures that are stable after removal of the DNA-sequences.

Since the elucidation of its structure, DNA has fascinated mankind, as it reduces the information behind all living organisms to a code based on only four chemical compounds. We are now able to engineer DNA and to conjugate it with other materials. This has opened almost unlimited possibilities for design of structures and for programmed assembly events at the nano-scale.

Acknowledgements

This work has been supported by grants from the National Science Foundation (USA) (EIA-0218376, CCR-0326157) to T. H. LaBean and grants from the Danish Technical Research Council, the Danish National Research Foundation and the Carlsberg Foundation to K. V. Gothelf.

References

1 G. M. Whitesides and B. Gryzbowski, *Science*, 2002, **295**, 2418–2421.
2 *Self-Assembling Peptide Systems in Biology, Medicine and Engineering*, ed. A. Aggeli, N. Boden and S. ZhangKluwer Academic Publishing, Dordrecht, Netherlands, 2000.
3 N. C. Seeman, *Angew. Chem., Int. Ed.*, 1998, **37**, 3220–3238.
4 J. J. Storhoff and C. A. Mirkin, *Chem. Rev.*, 1999, **99**, 1849–1869.
5 C. M. Niemeyer, *Angew. Chem., Int. Ed.*, 2001, **40**, 4128–4158.
6 N. C. Seeman, *Synlett*, 2000, 1536–1548.
7 C. M. Niemeyer, *Trends Biotechnol.*, 2002, **9**, 395–401.
8 N. C. Seeman, *Nature*, 2003, **421**, 427–431.
9 J. Wengel, *Org. Biomol. Chem.*, 2004, **2**, 227–280.
10 K. V. Gothelf and R. S. Brown, *Chem. Eur. J.*, 2005, **11**, 1062–1069.
11 E. Katz and I. Willner, *Angew. Chem., Int. Ed.*, 2004, **43**, 6042–6108.
12 *Nanobiotechnology: Concepts, Applications and Perspectives*, ed. C. M. Niemeyer and C. A. Mirkin, Wiley-VCH, Weinheim 2004.
13 B. Samori and G. Zucheri, *Angew. Chem., Int. Ed.*, 2005, **44**, 1166–1181.
14 N. C. Seeman, *Trends Biochem. Sci.*, 2005, **3**, 119–125.
15 J. Xu, T. H. LaBean, and S. L. Craig, in *Supramolecular Polymers 2*, ed. Alberto Ciferri, CRC Press - Taylor & Francis Group, Boca Raton, FL, 2005, pp. 445-480.

16 P. Pancoska, Z. Moravek and U. M. Moll, *Nucleic Acids Res.*, 2004, **32**, 4630–4645.
17 S. L. Beaucage and R. P Iyer, *Tetrahedron*, 1993, **49**, 1925–1963.
18 C. J. Loweth, W. B. Caldwell, X. Peng, A. P. Alivisatos and P. G. Schultz, *Angew. Chem., Int. Ed.*, 1999, **38**, 1808–1812.
19 P. E. Nielsen, *Acc. Chem. Res.*, 1999, **32**, 624–630.
20 B. Vester and J. Wengel, *Biochemistry*, 2004, **43**, 13233–13241.
21 O.-S. Ng and D. E. Bergstrom, *Nano Lett.*, 2005, **5**, 107–111.
22 R. Elghanian, J. J. Storhoff, R. C. Mucic, R. L. Letsinger and C. A. Mirkin, *Science*, 1997, **277**, 1078–1081.
23 N. C. Seeman, *J. Theor. Biol.*, 1982, **99**, 237–247.
24 N. C. Seeman, *Chem. Intell.*, 1995, **1**, 38–47.
25 X. Li, X. Yang, J. Qi and N. C. Seeman, *J. Am. Chem. Soc.*, 1996, **118**, 6131–6140.
26 E. Winfree, F. Liu, L. A. Wenzler and N. C. Seeman, *Nature*, 1998, **394**, 539–544.
27 P. W. K. Rothemund, N. Papadakis and E. Winfree, *PLoS Biol.*, 2004, **2**, 2041–2053.
28 T. H. LaBean, H. Yan, J. Kopatsch, F. Liu, E. Winfree, J. H. Reif and N. C. Seeman, *J. Am. Chem. Soc.*, 2000, **122**, 1848–1860.
29 C. Mao, T. H. LaBean, J. H. Reif and N. C. Seeman, *Nature*, 2000, **407**, 493–496.
30 H. Li, S.-H. Park, J. H. Reif, T. H. LaBean and H. Yan, *J. Am. Chem. Soc.*, 2004, **126**, 418–419.
31 H. Yan, S. H. Park, G. Finkelstein, J. H. Reif and T. H. LaBean, *Science*, 2003, **301**, 1882–1884.
32 C. Mao, W. Sun and N. C. Seeman, *J. Am. Chem. Soc.*, 1999, **121**, 5437–5443.
33 D. Liu, M. Wang, Z. Deng, R. Walulu and C. Mao, *J. Am. Chem. Soc.*, 2004, **126**, 2324–2325.
34 B. Ding, R. Sha and N. C. Seeman, *J. Am. Chem. Soc.*, 2004, **126**, 10230–10231.
35 Y. Brun, M. Gopalkrishnan, D. Reishus, B. Shaw, N. Chelyapov and L. Adleman, in *Proceedings of the 1st Conference on the Foundations of Nanoscience*, 2004, pp. 2–15.
36 S.-H. Park, R. Barish, H. Li, J. H. Reif, G. Finkelstein, H. Yan and T. H. LaBean, *Nano Lett.*, 2005, **5**, 693–696.
37 F. Mathieu, S. Liao, J. Kopatsch, T. Wang, C. Mao and N. C. Seeman, *Nano Lett.*, 2005, **5**, 661–665.
38 A. Chworos, I. Severcan, A. Y. Koyfman, P. Weinkam, E. Oroudjev, H. G. Hansma and L. Jaeger, *Science*, **306**, 2068–2072.
39 J. Chen and N. C. Seeman, *Nature*, 1991, **350**, 631–633.
40 Y. Zhang and N. C. Seeman, N. C., *J. Am. Chem. Soc.*, 1994, **116**, 1661–1669.
41 R. P. Goodman, R. M. Berry and A. J. Turberfield, *Chem. Commun.*, 2004, 1372–1373.
42 W. M. Shih, J. D. Quispe and G. F. Joyce, *Nature*, 2004, **427**, 618–621.
43 See *e.g.*:C. M. Niemeyer and M. Adler, *Angew. Chem., Int. Ed.*, 2002, **41**, 2779–3783.
44 J. Shi and D. E. Bergstrom, *Angew. Chem., Int. Ed.*, 1997, **36**, 111–113.
45 J. Xu, E. A. Fogleman and S. L. Craig, *Macromolecules*, 2004, **37**, 1863–1870.
46 E. A. Fogleman, W. C. Yount, J. Xu and S. L. Craig, *Angew. Chem., Int. Ed.*, 2002, **41**, 4026–4028.
47 M. A. Abdalla, J. Bayer, J. O. Rädler and K. Müllen, *Angew. Chem., Int. Ed.*, 2004, **43**, 3967–3970.
48 S. M. Waybright, C. P. Singleton, K. Wachter, C. J. Murphy and U. H. Bunz, *J. Am. Chem. Soc.*, 2001, **123**, 1828–1833.
49 S. M. Waybright, C. P. Singleton, J. M. Tour, C. J. Murphy and U. H. F. Bunz, *Organometallics*, 2000, **19**, 368–370.
50 I. Vargas-Baca, D. Mitra, H. J. Zulyniak, J. Banerjee and H. Sleiman, *Angew. Chem., Int. Ed.*, 2001, **40**, 4629–4631.
51 D. Mitra, N. Di Cesare and H. F. Sleiman, *Angew. Chem., Int. Ed.*, 2004, **43**, 5804–5808.
52 J. S. Choi, C. W. Kang, K. Jung, J. W. Yang, Y. G. Kim and H. Y. Han, *J. Am. Chem. Soc.*, 2004, **126**, 8606–8607.
53 M. Endo and T. Majima, *J. Am. Chem. Soc.*, 2003, **125**, 13654–13655.
54 M. Endo, S. Uegaki and T. Majima, *Chem. Commun.*, 2005, 3153–3155.
55 M. Endo and T. Majima, *Chem. Commun.*, 2004, 1308–1309.
56 M. S. Shchepinov, I. A. Udalova, A. J. Bridgman and E. M. Southern, *Nucleic Acids Res.*, 1997, **25**, 4447–4454.
57 M. S. Shchepinov, K. U. Mir, J. K. Elder, M. D. Frank-Kamenetskii and E. M. Southern, *Nucleic Acids Res.*, 1999, **27**, 3035–3041.
58 M. Scheffler, A. Dorenbeck, S. Jordan, M. Wustefeld and G. von Kiedrowski, *Angew. Chem., Int. Ed.*, 1999, **38**, 3311–3315.
59 G. von Kiedrowski, L. H. Eckardt, K. Naumann, W. N. Pankau, M. Reimold and M. Rein, *Pure Appl. Chem.*, 2003, **75**, 609–619.
60 K. M. Steward and L. W. McLaughlin, *J. Am. Chem. Soc.*, 2004, **126**, 2050–2057.
61 K. M. Steward, J. Rojo and L. W. Mclaughlin, *Angew. Chem., Int. Ed.*, 2004, **43**, 5808–5811.
62 X. Li and D. R. Liu, *Angew Chem., Int. Ed.*, 2004, **43**, 4848–4870.
63 Z. J. Gartner, B. N. Tse, R. Grubina, J. B. Doyon, T. M. Snyder and D. R. Liu, *Science*, 2004, **305**, 1601–1605.
64 M. W. Kanan, M. M. Rozenman, K. Sakurai, T. M. Snyder and D. R. Liu, *Nature*, 2004, **431**, 545–549.
65 D. M. Rosenbaum and D. R. Liu, *J. Am. Chem. Soc.*, 2003, **125**, 13924–13925.
66 Y. Chen and C. Mao, *J. Am. Chem. Soc.*, 2004, **126**, 13240–13241.
67 L. H. Eckardt, K. Naumann, W. N. Pankau, M. Rein, M. Schweitzer, N. Windhab and G. von Kiedrowski, *Nature*, 2002, **420**, 286.
68 J. L. Czlapinski and T. L. Sheppard, *J. Am. Chem. Soc.*, 2001, **123**, 8618–8619.
69 M. Nielsen, A. H. Thomsen, E. Cló, F. Kirpekar and K. V. Gothelf, *J. Org. Chem.*, 2004, **69**, 2240–2250.
70 K. V. Gothelf, A. H. Thomsen, M. Nielsen, E. Cló and R. S. Brown, *J. Am. Chem. Soc.*, 2004, **126**, 1044–1046.
71 R. S. Brown, M. Nielsen and K. V. Gothelf, *Chem. Commun.*, 2004, 1464–1465.
72 M. Nielsen, V. Dauksaite, J. Kjems and K. V. Gothelf, *Bioconjugate Chem.*, 2005, **16**, 681–685.
73 C. M. Niemeyer, T. Sano, C. L. Smith and C. R. Cantor, *Nucleic Acids Res.*, 1994, **22**, 5530–5539.
74 C. M. Niemeyer, W. Bürger and J. Peplies, *Angew. Chem., Int. Ed.*, 1998, **37**, 2265.
75 S.-J. Park, A. A. Lazarides, C. A. Mirkin and R. L. Letsinger, *Angew. Chem., Int. Ed.*, 2001, **40**, 2909–2919.
76 C. M. Niemeyer, M. Adler, S. Lenhert, S. Gao, H. Fuchs and L. F. Chi, *ChemBioChem*, 2001, **2**, 260–265.
77 C. M. Niemeyer, M. Adler, B. Pignataro, S. Lenhert, S. Gao, L. F. Chi, H. Fuchs and D. Blohm, *Nucleic Acids Res.*, 1999, **27**, 4553–4561.
78 C. M. Niemeyer, M. Adler, S. Gao and L. Chi, *Angew. Chem., Int. Ed.*, 2000, **39**, 3056–3059.
79 C. M. Niemeyer, R. Wacker and M. Adler, *Angew. Chem., Int. Ed.*, 2001, **40**, 3169–3172.
80 S.-H. Park, P. Yin, Y. Liu, J. H. Reif, T. H. LaBean and H. Yan, *Nano Lett.*, 2005, **5**, 729–733.
81 S.-H. Park, C. Pistol, S.-J. Ahn, J. H. Reif, A. Lebeck, C. Dwyer and T. H. LaBean, *Angew. Chem., Int. Ed.*, 2005, submitted.
82 J. N. Cha, K. Shimizu, Y. Zhou, S. C. Christiansen, B. F. Chmelka, G. D. Stucky and D. E. Morse, *Proc. Natl. Acad. Sci.*, 1999, **96**, 361–365.
83 S. R. Whaley, D. S. English, E. L. Hu, P. F. Barbara and A. M. Belcher, *Nature*, 2000, **405**, 665–668.
84 L. A. Gugliotti, D. L. Feldheim and B. E. Eaton, *Science*, 2004, **304**, 850–852.
85 N. L. Rosi and C. A. Mirkin, *Chem. Rev.*, 2005, **105**, 1547–1562.
86 C. A. Mirkin, R. L. Letsinger, R. C. Mucic and J. J. Storhoff, *Nature*, 1996, **382**, 607–609.
87 A. P. Alivisatos, K. P. Johnsson, X. Peng, T. E. Wilson, T. C. J. Loweth, M. P. Bruchez and P. G. Schultz, *Nature*, 1996, **382**, 609–611.
88 J. J. Storhoff, R. Elghanian, R. C. Mucic, C. A. Mirkin and R. L. Letsinger, *J. Am. Chem. Soc.*, 1998, **120**, 1959–1964.
89 R. A. Reynolds III, C. A. Mirkin and R. L. Letsinger, *J. Am. Chem. Soc.*, 2000, **122**, 3795–3796.
90 R. C. Mucic, J. J. Storhoff, D. A. Mirkin and R. L. Letsinger, *J. Am. Chem. Soc.*, 1998, **120**, 12674–12675.
91 S. A. Claridge, S. L. Goh, J. M. J. Fréchet, S. C. Williams, C. M. Micheel and A. P. Alivisatos, *Chem. Mater.*, 2005, **17**, 1628–1635.
92 M. G. Grimau, D. Iacopino, A. Aviño, B. G. de la Torre, A. Ongaro, D. Fitzmaurice, J. Wessels and R. Eritja, *Helv. Chim. Acta*, 2003, **86**, 2814–2826.
93 C. M. Niemeyer, B. Ceyhan and P. Hazarika, *Angew. Chem., Int. Ed.*, 2003, **42**, 5766.
94 P. Hazarika, B. Ceyhan and C. M. Niemeyer, *Angew. Chem., Int. Ed.*, 2004, **43**, 6469–6471.
95 J. D. Le, Y. Pinto, N. C. Seeman, K. Musier-Forsyth, T. A. Taton and R. A. Kiehl, *Nano Lett.*, 2004, **4**, 2343–2347.
96 G. P. Mitchell, C. A. Mirkin and R. L. Letsinger, *J. Am. Chem. Soc.*, 1999, **121**, 8122–8123.
97 W. J. Parak, D. Gerion, D. Zanchet, A. S. Woerz, T. Pellegrino, C. Micheel, S. C. Williams, M. Seitz, R. E. Bruehl, Z. Bryant, C. Bustamante, C. R. Bertozzi and A. P. Alivistos, *Chem. Mater.*, 2002, **12**, 2113–2119.

98 E. Dujardin, L.-B. Hsin, C. R. C. Wang and S. Mann, *Chem. Commun.*, 2001, 1264–1265.
99 C. M. Soto, A. Srinivasan and B. R. Ratna, *J. Am. Chem. Soc.*, 2002, **124**, 8508–8509.
100 V. T. Milam, A. L. Hiddessen, J. C. Crocker, D. J. Graves and D. A. Hammer, *Langmuir*, 2003, **19**, 10317–10323.
101 Y. Choi, A. Mecke, B. G. Orr, M. M. B. Holl and J. R. Baker Jr., *Nano Lett.*, 2004, **4**, 391–397.
102 C. R. DeMattei, B. Huang and D. A. Tomalia, *Nano Lett.*, 2004, **4**, 771–777.
103 K. Keren, M. Krueger, R. Gilad, G. Ben-Yoseph, U. Sivan and E. Braun, *Science*, 2002, **297**, 72–75.
104 H. Dai, *Acc. Chem. Res.*, 2002, **35**, 1035–1044.
105 S. Banerjee, T. Hemraj-Benny and S. S. Wong, *Adv. Mater.*, 2005, **17**, 17–29.
106 C. Dwyer, M. Guthold, M. Falvo, S. Washburn, R. Superfine and D. Erie, *Nanotechnology*, 2002, **13**, 601–604.
107 S. Li, P. He, J. Dong, Z. Guo and L. Dai, *J. Am. Chem. Soc.*, 2005, **127**, 14–15.
108 K. A. Williams, P. T. M. Veenhuizen, B. G. de la Torre, R. Eritja and C. Dekker, *Nature*, 2002, **420**, 761.
109 M. Hazani, F. Hennrich, M. Kappes, R. Naaman, D. Peled, V. Sidorov and D. Shvarts, *Chem. Phys. Lett.*, 2004, **391**, 389–392.