First blueprint, now bricks: DNA as construction material on the nanoscale

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For the most part DNA was considered Nature's instruction manual for life leading to the popular description 'blueprint of life'. However, DNA is now taking on a new aspect where it is finding use as a construction element for architecture on the nanoscale. This *tutorial review* addresses the importance of building ordered structures with DNA on the nanoscale, the underlying principles and approaches to build such scaffolds, the current limitations and the anticipated trajectory of the area. This is would be of interest to the chemical biology, supramolecular and bioengineering communities in particular.

Introduction

A blueprint is a detailed drawing or map which identifies and directs the construction and development of an object, such as a building. Every cell in an organism's body has the same DNA which is the hereditary material in almost all life forms. DNA is thus a cellular blueprint, containing information that encodes a set of instructions for the cell on how to build and sustain life in the organism. Hence the frequently employed description of DNA as the 'blueprint of life'.[1] Apart from its well-known role as the cellular storehouse of information, DNA is now being used to construct rigid scaffolds in one, two and three dimension on the nanoscale. This field is termed 'Structural DNA Nanotechnology'. It seeks to use the base-complementarity design principle of DNA to create ordered superstructures from a set of DNA sequences that self-assemble into regular, well-defined topologies on the nanoscale.[2] DNA is an attractive building block on this length scale as the double helix has a regular diameter of 2.3 nm and a pitch of 3.4 nm.[3] This inherently nanoscale object has a persistence length of ~150 base pairs, which implies that up to lengths of ~50 nm, the DNA double helix essentially behaves as a rigid rod.[4] Furthermore, the elegant rules of base-pairing allow specific and predictable recognition of distinct sequences facilitating the orientations of the resultant double helices in space. It therefore becomes possible to program a set of linear DNA molecules by encoding as sequence information, instructions to self-assemble into predicted structures on the nanoscale using simple base pairing logic. The end result of such programming in the context of structural DNA nanotechnology is the self-assembly into double helices in specific spatial orientations, whereas the end result of programming DNA in the cellular context is the reading and translation of the DNA code into protein by cellular machinery.

This field is driven primarily by the recent interest in nanoscience and technology, particularly the need for the reproducibly creating ordered structures and devices on the nanoscale. Such nanodevices or structures should have desirable features as (a) a high degree of structural precision (b) high speeds of operation and (c) small size. DNA, being an intrinsically nanoscale object, coupled with its robust mechanical properties and highly predictable and specific recognition,

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can be used to realize devices satisfying all the above conditions. The need to build such structures is driven by the vision that they could offer solutions to problems in science as diverse as biology and information technology. For instance, cells have dimensions of the order of a few microns. But cellular components such as proteins, metabolites, lipids *etc.* are of the order of a few nanometers. The subtle interplay between networks of these nanoscale entities control cellular processes ranging from development to disease in an organism. Abnormalities in a cell that lead to disease are almost always due to the malfunction of one or more components. To counter such malfunctions, we could envisage 'agents' or intelligently designed entities on the same length scales, to target and counteract the glitch in the cellular machinery. Ordered structures built from DNA could thus function as carriers,[5] of drugs that could specifically target abnormalities and use the abnormal cellular signals to trigger cargo release, thereby functioning as search and destroy nanomachines.

Challenges in information science that can be addressed using DNA include computational problems and miniaturization of electronic devices. Computational problems arising from constraints relating to size requirements and operation speeds could potentially be circumvented using DNA. For example, when a simple biochemical reaction involving DNA is performed, the operation is performed simultaneously on trillions of DNA molecules. In information science, this is called 'parallelism' and refers to the ability to share and/or execute a given stimulus by several entities simultaneously, thus reducing the load on a particular entity performing the operation.[6] Even the latest computers are far from realizing the phenomenal parallelism inherent in operations performed in a simple biochemical reaction. Hence, DNA is well-placed to exploit the power of parallelism to address computational problems. In addition, DNA can also be used to make nanoscale assemblies which can perform functions analogous to key components in electronic devices. In the race to make machines smaller faster and more powerful, the fabrication of progressively smaller electronic components are being made at ballooning costs. These components are becoming so small, that it is envisaged that they will soon reach the size of molecules. This has driven the field of molecular electronics, where the function of an electronic component is performed by a large molecule. DNA has already been used to make nanowires which could serve as interconnects in molecular electronics.[7] DNA assemblies have also been used to make logic gates, taking the field closer to constructing nanoscale DNA-based transistors.[8] Thus DNA is a powerful candidate to be the next basic building block in nanoelectronics—a role that nature might not have intended for it.

Creating the basic building blocks

B-DNA and the supramolecular 'glue' of base pairing

The most basic and familiar building block is Watson and Crick base-paired double helical DNA, shown in Fig. 1, which is called B-DNA. B-DNA has a uniform 2.3 nm diameter and behaves as a rigid rod below its persistence length of 50 nm, or 150 base pairs. The construction of frameworks made from rods is only possible if one can make specific contacts between

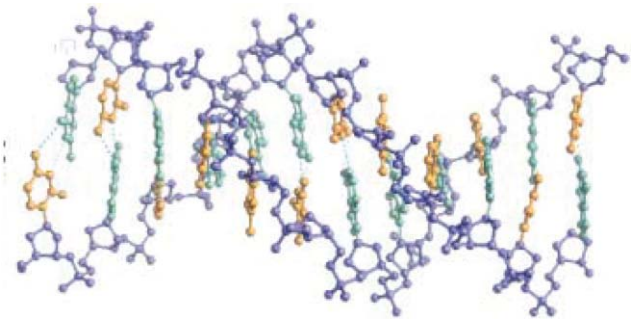

Fig. 1 The structure of B-DNA where the sugar phosphate backbones are in purple, the purine nucleobases are in cyan and the pyrimidine nucleobases in ochre. (Reprinted by permission from R. Wing *et al. Nature* 1980, **287**, 755.[38] Copyright 1980 Nature Publishing Group.)

desired rods at defined locations. With DNA, the exquisite specificity due to base complementarity rules, allows for the formation of a specific contact. Contacts between any two given rods at a desired location may be achieved by introducing at the desired location per B-DNA rod, a single stranded segment or overhang that have complementary sequences. The 'hybridization' or the uniting of the two complementary overhangs *via* Watson–Crick base-pairing brings about a specific contact between the duplex rods at the desired location. Thus a collection of oligonucleotides, encoded with the appropriate sequence information, can function as rigid duplex rods that can be 'welded' or joined at specific points into units of different patterns that can undergo further self-assembly.

Multi-armed junctions

B-DNA is commonly perceived as incapable of much structural variety. Interestingly however, non-linear, multiply branched structures made of B-DNA occur naturally. For example, the replication fork, though only transient, is a 'three-way junction', where three duplex arms radiate from a central point. Three arm junctions are also found in 5S ribosomal RNA[9] and in the terminal repeats of the single-stranded DNA comprising the genome of the adeno-associated virus.[10] Similarly, the naturally occurring Holliday junction[11] is a four-arm junction that occurs transiently during DNA recombination, while the peptidyl transferase centre in 23S r-RNA is a naturally occurring five-arm junction.[12] In fact, several large ribosomal RNAs (r-RNA) contain junctions ranging from three-way to seven-way junctions, most of which include unpaired bases at the branch point. The presence of variously armed junctions in structured RNA molecules implicates their possible role as structure directing agents within these macromolecules. Thus it was only a matter of course before these motifs were used to direct structure in artificially designed nucleic acid assemblies. Fig. 2 shows artificially constructed multi-armed junctions using DNA.

One can construct many-armed junctions from B-DNA, using combinations of Watson–Crick base paired oligonucleotides. Thus to construct an *N*-armed junction, one needs to start with *N* unique oligonucleotides that each have two distinct hybridization sites, one near the 5′ end and one near

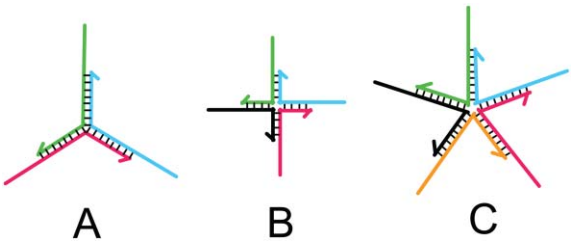

Fig. 2 (A) Three, (B) four and (C) five arm junctions formed from as many DNA sequences.

the 3′ end, for binding to two different oligonucleotides. The 5′ binding site of the m^{th} oligonucleotide must be complementary to the 3′ binding site of the $(m + 1)^{th}$ oligonucleotide, and for $m = N$, the 5′ binding site of the Nth oligonucleotide must be complementary to the 3′ binding site of the first oligonucleotide ($m = 1$). Using this concept, 3-, 4- and 5-arm junctions have been realized (Fig. 2).[13] In order to impart more stability to the overall structure, extra bases that do not pair, are incorporated between both binding sites of a given oligonucleotide, such that these unpaired bases are located at the branch point of the many-armed junction.[13]

DX, TX and PX Junctions

Four-arm junctions or the Holliday junction may also be viewed through the eyes of reciprocal exchange. Consider two independent double helices, of red and black strands respectively as shown in Fig. 3. If say, the 5′ termini of a red and a black strand of the same polarities were to come unstuck, displace each other and 'cross over', into the adjacent helix, this would result in a four-arm junction. We will refer to this as a 'crossover event'. This can also occur with say the 5′ termini of strands with the opposite polarities, but the resultant structure would only be a conformational variant (see Fig. 3). However, when more than one crossover event occurs between pairs of double helices, there are major topological differences between the resultant structures depending on the polarities of the strands that have crossed over or undergone a reciprocal

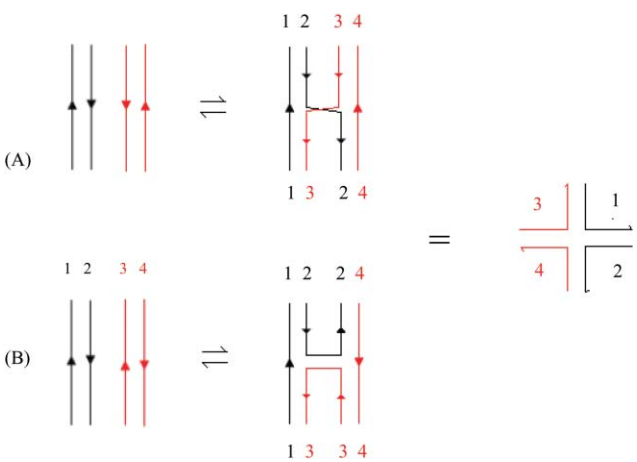

Fig. 3 Schematic showing a single crossover event between two duplexes occurring in configurations that are (A) parallel and (B) antiparallel and the identical four-way junction they form.

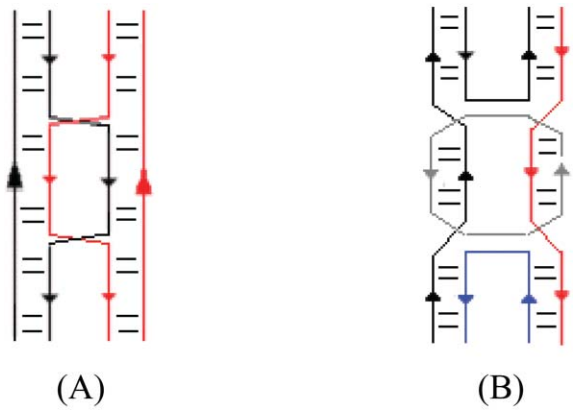

Fig. 4 Double crossover (DX) junctions containing even numbers of helical half turns between crossover points (A) DPE, constructed from four strands and (B) DAE, constructed from five strands.

exchange (Fig. 4). These form a new set of building blocks, called crossover motifs, which are derived from multiple-crossover events with different strand topologies.

Double crossover junctions, or DX junctions,[14] are formed from four or five oligonucleotides sequences that make up two helical domains that are connected by two crossover events. Fig. 5 shows the different topologies possible with double crossover junctions. Crossover junctions are classified as parallel or anti-parallel based on the relative orientation of the two strands of the double helical domains that have not crossed over. Nomenclature in double crossover junctions begin with a D indicating the double crossover events, followed by an A or P depending on whether the junction is anti-parallel or parallel. This is followed by either an E or O indicating an even or odd number of helical half-turns between each crossover point. A fourth letter is required to describe DPO junctions regarding where the extra half helical turn at the crossover point is accommodated. The letters W or N denote whether this extra turn is present at or corresponds to a major groove or minor groove separation respectively. The number of helical half turns depicts the strand orientation (see Fig. 5) and a different number of helical turns can be achieved by repositioning the crossover points. This in turn may be achieved by altering the sequences of segments involved in the formation of the crossover.

DAE junctions can be constructed using five strands, where three of the strands are involved in the crossovers and two are fixed to a given helical domain (Fig. 4). Two of these three sequences are designed such that their 5′-end and 3′end sequences are complementary to the termini of fixed strands. The fifth strand is circular and complementary to the central portion of the fixed strands and is thus centrally located on the DAE junction (Fig. 4).[15] This central strand can also have its 5′ and 3′ ends complementary, projecting out of the DX framework (DAE + J) (see Fig. 5), and can be used to position other building blocks of interest. DAO junctions use typically four sequences,[15] where the two longest strands that cross over have their central portions complementary to each other and two shorter strands base-pair with the spatially close termini.

Parallel DX junctions can be obtained with just four strands. In DPE junctions, two of the four strands are fixed

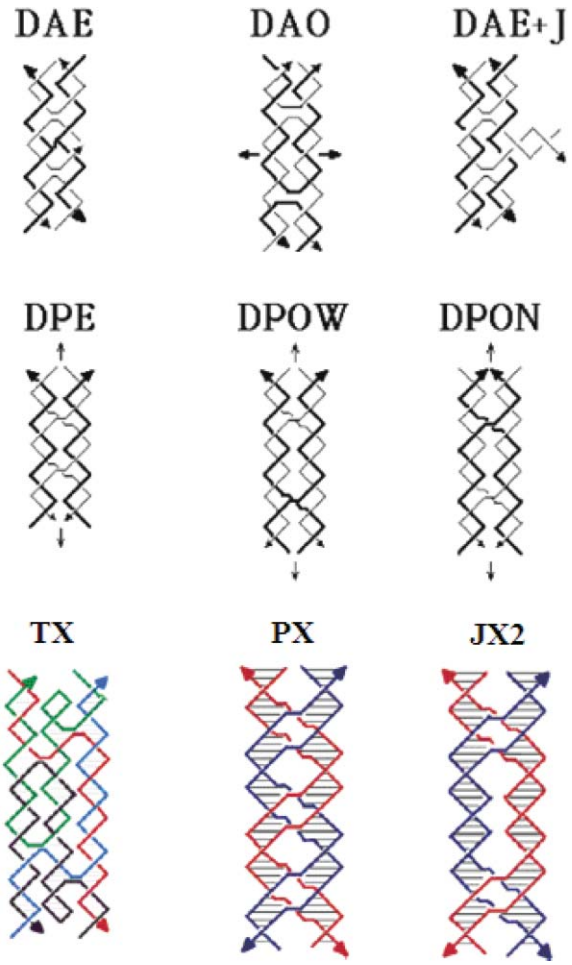

Fig. 5 Schematic representation of the various topologies that may be formed using double-crossover (DX), triple-crossover (TX) and multiple crossover junctions (PX and JX2). (Reprinted with permission from *J. Biomol. Struct. Dyn., Conversation 11*, 2000, **2**.[39] Copyright 2000 Adenine press. Also N. C. Seeman, *Biochemistry*, 2003, **42**, 7259.[2] Copyright 2003 American Chemical Society.)

while the other two cross over. Thus the crossover strands have three regions: two of these are the segments at the 5′ and 3′ that are complementary to the 3′ and 5′ termini of one of the fixed strands and a third region in the middle complementary to the central portion of the other fixed strand. DPO junctions on the other hand have all four strands involved in the crossovers as shown in Fig. 5.

TX[16] tiles or motifs are derived from DX tiles, where there are three distinct helices instead of two, and thus have double the number of crossovers. These tiles can also be constructed using four strands, where each strand is part of each of the three helical domains. Therefore every strand is involved in two crossover events as shown in Fig. 5. Such a structure imparts greater rigidity in comparison to simple duplexes as well as DX tiles. PX motifs result from reciprocal exchange occurring between both strands of both the helices at the same crossover points. PX motifs have multiple crossover points at every helical turn, where strands of the same polarity unite giving rise to parallel crossovers. The PX motif thus bears a superficial resemblance to intertwined duplexes. JX2 motifs are similar to the PX motif, except that reciprocal exchange is omitted at two adjacent points (Fig. 5.)

Non-Watson–Crick base paired building blocks

The ability of some nucleobases to recognize *via* their Hoogsteen sites gives rise to DNA building blocks with structures very different from B-DNA. For example, G-quadruplex DNA is a highly stable four-stranded structure formed from G-rich oligonucleotides in the presence of group IA and IIA cations.[17] The guanines in G-quadruplexes are hydrogen-bonded to each other *via* their Hoogsteen sites into a planar, cyclic, tetrameric arrangement called a G-quartet, or G-tetrad (Fig. 6A).[17] A G-quadruplex consists of typically, 2–4 tetrads stacked upon each other (Fig. 6D). The G-quadruplex has a diameter of 2.3 nm, and this quadruple helical structure is considered parallel when all the strands have the same polarity or antiparallel when only two of the four strands have the same polarity.

Similarly, C-rich oligonucleotides also associate under acidic conditions to form a four-stranded structure called the i-motif due to the capacity of hemiprotonated cytosines to base pair with each other (Fig. 6C). An i-motif consists of two parallel-stranded duplexes that are held together by C-H.C^+ base pairs that are intercalated in an antiparallel orientation (Fig. 6D).[18] Thus diagonal strands in an i-motif have the same strand polarity. The four DNA backbones in an i-motif are not equidistant from each other and, therefore, the strands form two very narrow grooves and two relatively wider grooves.

All the aforementioned building blocks may be endowed with sticky ends at appropriate locations on the building block that facilitate further self-assembly of the unit into higher order structures as outlined below.

One-dimensional superstructures

The processing of information contained in DNA occurs by transcription, in a linear manner, along the length of a sequence of DNA. Information transfer from DNA to protein, occurs unidirectionally, along one dimension. Thus the construction of self assembling DNA motifs in one dimension to a form linear superstructures represents the most rudimentary and yet primary concept in structural DNA nanotechnology.

1D structures using various tiles

A simple DX or TX tile with self-complementary sticky ends, at appropriate concentrations, is enough to grow structures that extend along a line. DX tiles in particular have been extensively used to propagate structures unidirectionally.[19] Each DX tile consists of two parallel double helices and four strands, where two of the four strands first participate in one helix and then exchange to the other helix, thus holding the two helices together. Thus, a given DX tile has four single-stranded sticky ends that may be programmed to bind to complementary ends of other DX tiles, allowing fine control of extension mediated by binding specificity. Superstructures resulting from such association begin to curve upon themselves when they acquire dimensions beyond the persistence length of the associated building block. Thus, different types of tiles

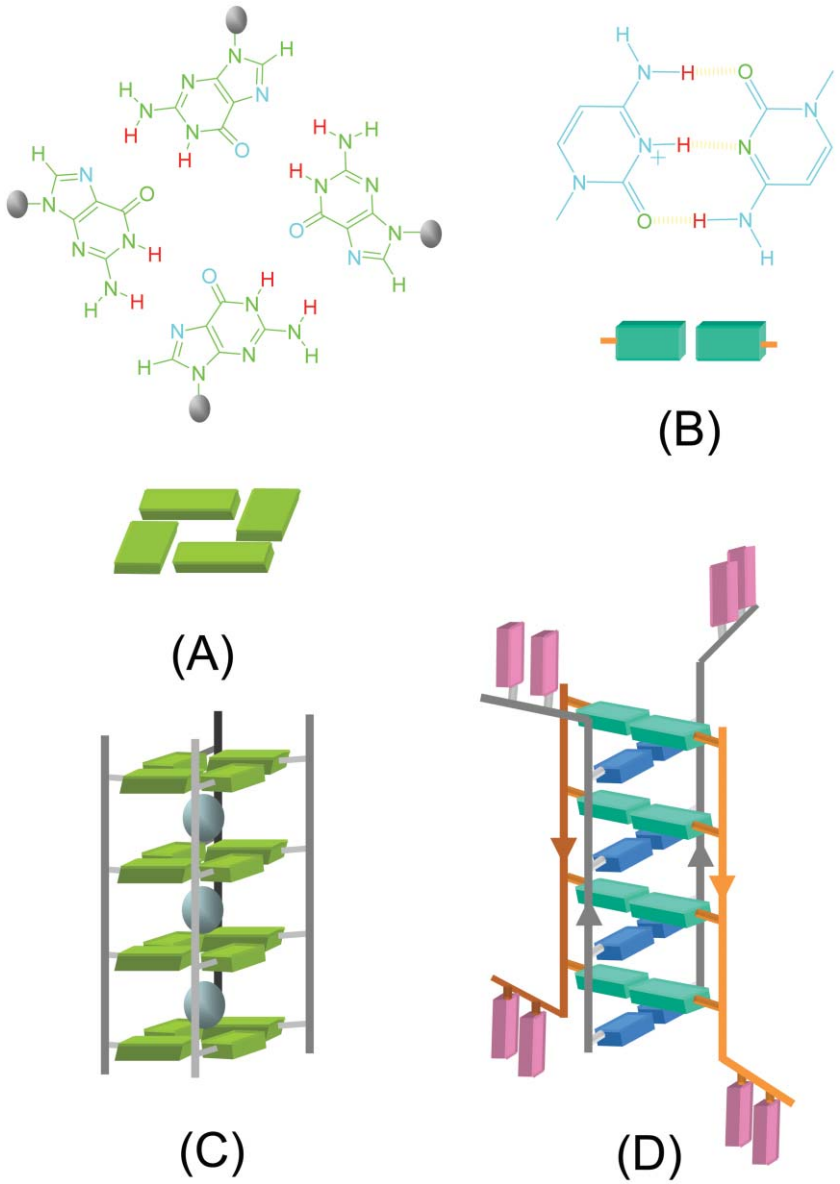

Fig. 6 Unusual base pairing leading to four-stranded building blocks. (A) Hoogsteen base pairing between guanines gives rise to a G-Tetrad. (B) Hemiprotonation of cytosine results in a C–C^+ base pair. (C) A G-quadruplex comprising G-tetrads formed from sequences with tandem G repeats. (D) An I-motif comprising intercalated C–C^+ base pairs formed from sequences with tandem C repeats.

have been used to make extended structures in one dimension. For example, triangular tiles constructed by fusing three four-arm junctions together have been used to make a one-dimensional array of triangles (Fig. 7).[20] Each vertex of the triangle consists of a four-arm junction, and each side of the triangle is a DNA duplex. Thus, each tile would have six free duplexes, two at each vertex. If sticky ends were added to two free duplexes, which are at two different vertices, they self-associate into a linear arrangement with repeating triangular units (Fig. 7a and 7b).

Tubes

Other superstructures that propagate unidirectionally are DNA tubes that have been constructed using DX and TX tiles. This is achieved using two different TX tiles,[21] **A** and **B**, with two looped structures (unpaired TTTT) incorporated at the midpoint of opposing sides of the tiles and two stem-loop structures incorporated at the centre of tile **B** (Fig. 8). Both **A** and **B** bear complementary overhangs, such that **A** associates diagonally with **B**. Additionally tile **B** incorporated thiol modifications in four of its component strands either in the 5′ or the 3′ end. When an equimolar mixture of both tiles was annealed and allowed to oxidize, they self-assembled into tubular structures. Tiles **A** and **B** associated in 2D resulting in the formation of a sheet with alternating **A** and **B** tiles. However, as the sheet attains dimensions beyond the persistence length of the building blocks, it curves and the formation of disulfide bonds between spatially close **B** tiles stabilizes the resultant DNA nanotube (Fig. 8). Tubes have also been constructed by assembling DX tiles and making use of their inherent ability to curve by a judicious choice of the

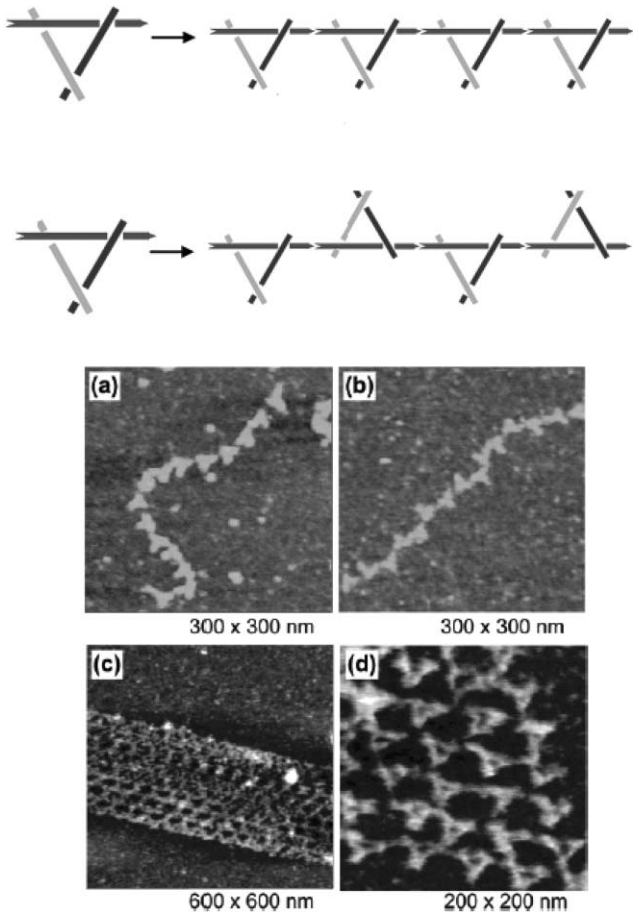

Fig. 7 Triangular building blocks self assemble into (A and B) 1D arrays and (C and D) 2D arrays. The triangular building blocks are formed from the self assembly of four-arm junctions into two different configurations. (Reprinted with permission from D. Liu *et al.*, *J. Am. Chem. Soc.* 2004, **126**, 2324.[26] Copyright 2004 American Chemical Society.)

sticky ends.[22] DNA helix bundles, distinct from protein helix bundles, constructed by an extension of DX and TX tiles, have also been used to construct rigid nanotubes.[23]

Wires

G-rich oligonucleotides associate into four-stranded structures called G-quadruplexes in the presence of monovalent or divalent cations. These can be (i) unimolecular, where a single strand with four G-rich regions folds up, (ii) bimolecular where two strands with two G-rich regions associate or (iii) tetramolecular, formed by the tetramerization of a single G-rich strand.[17] Long polymers of G-quartets can form wire-like structures called G-wires, with a uniform width of 2.3 nm and lengths up to 500 nm.[24] G-wires appear to have higher persistence lengths than B-DNA, and have variable lengths. G-wires have been formed predominantly from bimolecular G-quadruplex forming sequences at high concentrations. One can draw a structural analogy with a tube on the nanoscale where the central canal is filled with tight fitting spheres, corresponding to the cation in the G-quadruplex building block. The length of G-wires is a function of the incubation conditions with particular dependence upon ionic environment, temperature and time. The mode of self association of a bimolecular G-quadruplex-forming sequence into higher order structures or G-wires need not be a single pathway. However, all pathways involve the association of a species **A**, that is folded upon itself, contributing both G-rich segments to the same quadruplex as well as an extended species **B**, that contributes both G-rich segments to two distinct quadruplexes. Various combinations of **A** and **B** or indeed **B** alone, could result in a supramolecular polymerization event leading to G-wire formation as shown in Fig. 9.

2D superstructures

Design principles of 2D array construction

A 2D array can be made from a structural motif that can associate in the x and y coordinates, to give a planar, periodic, 2D crystalline array. 2D arrays made from DNA have been constructed from repeating units that use structural motifs such as DX, TX, PX, DNA parallelograms and four-by-four motifs. The incorporation of sticky ends at appropriate positions on the motif facilitates its self-association in two dimensions, where the motif is the repeating unit in a 2D crystal-like array.

Construction of simple 2D arrays

As a simple example, let us consider a four-way junction, inspired by the naturally occurring Holliday junction. Four such junctions with sticky ends, can self-assemble in a cyclic fashion by donating two sticky ends each, to form a square or

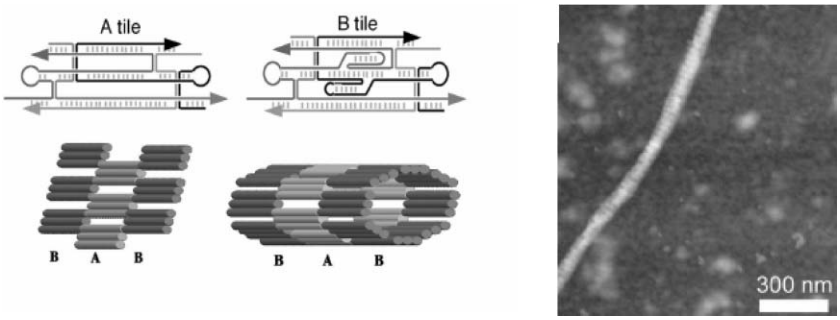

Fig. 8 A combination of triple-crossover (TX) tiles **A** and **B**, self-assemble into a 2D array that curls on its ends, propogating in 1D into DNA nanotubes. (Reprinted from D. Liu *et al.*, *Proc. Natl. Acad. Sci. U. S. A.*, 2004, **101**, 717–722.[21] Copyright 2004 PNAS.)

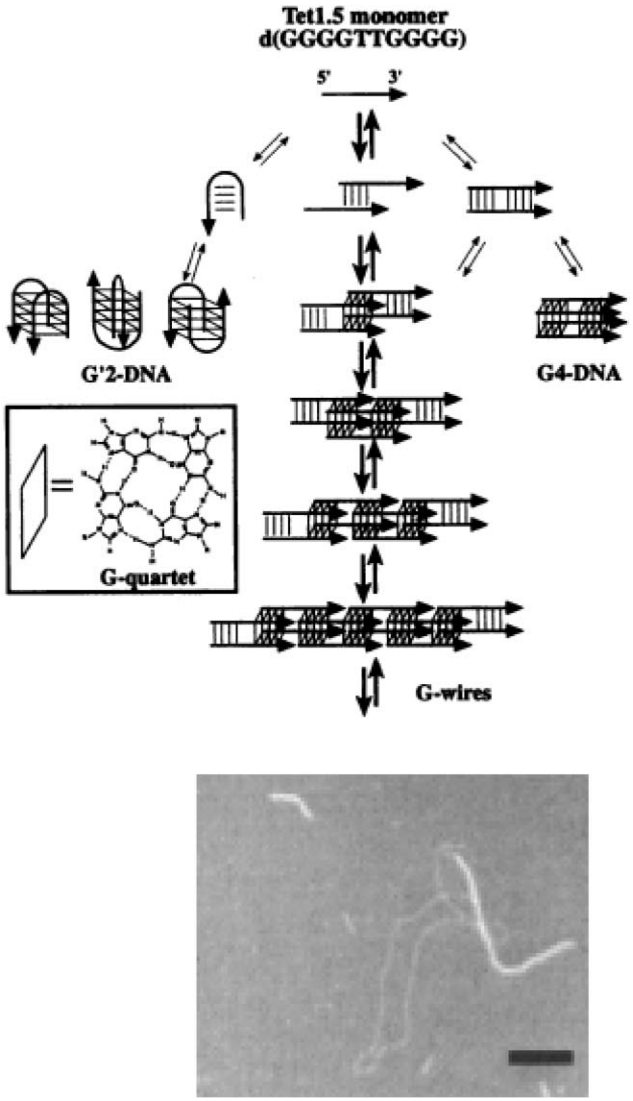

Fig. 9 A G-Quadruplex-forming sequence, propagates quadruplex units in 1D to form a G-wire, *via* interlocking quadruplex units as shown in the schematic. (Reprinted with permission from T. C. Marsh, *et al.*, *Nucleic Acids Res.*, 1995, **23**, 696.[24] Copyright 1995 Oxford University Press.)

quadrilateral 'tile'[25] (Fig. 10). This tile would thus also possess sticky ends at its vertices. Several such square tiles can now self-associate *via* these sticky ends in two dimensions into a net-like structure on the nanoscale, where the cavity size in the net can be tuned by adjusting the lengths of the sequences in Holliday-type junction. Using a similar approach, triangular tiles made from four-arm junctions have been self-assembled into a 2D array of triangles (Fig. 7c & d).[20]

Triangular tiles composed of double crossovers with three sticky ends, have been assembled to produce a pseudo-hexagonal tile[26] with six sticky ends which further associates into a pseudo-hexagonal array (Fig. 11). One of the major factors which control the dimensions of the resulting sheet is the concentration of the component tiles. More recently, double and triple crossovers or DX/TX tiles, have been assembled into 2D arrays that incorporate a design strategy where the sheet ends close into tubes. This concept has also been used to construct helix bundles.[23] Such nanotubes can serve as excellent conductive nanowires.

Controlling array propagation in 2D using molecular logic

2D arrays with different surface topographies have been constructed from combinations of different DX tiles defined by their overhang sequences. By making a prior selection of tiles, the pattern in which self-assembly occurs is dictated by the principles of molecular logic. Thus the output pattern, or surface topography reflects a given molecular computation. This approach has been validated for computational problems such as counting or copying,[27] using logical operations such as OR, AND, XOR, Half Adder, Full Adder logics. For example, counting has been achieved using a half adder, given by recurrent AND and XOR logic as shown in Fig. 12. Numbers 0 to 8 are represented in binary as shown below.

1	0	0	0
0	1	1	1
0	1	1	0
0	1	0	1
0	1	0	0
0	0	1	1
0	0	1	0
0	0	0	1
0	0	0	0

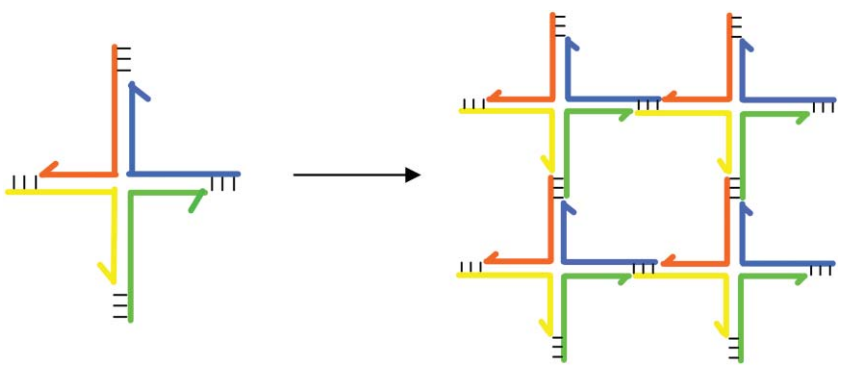

Fig. 10 Self assembly of a four-arm junction with sticky ends into a quadrilateral tile that also has sticky ends, capable of further self-assembly in 2D.

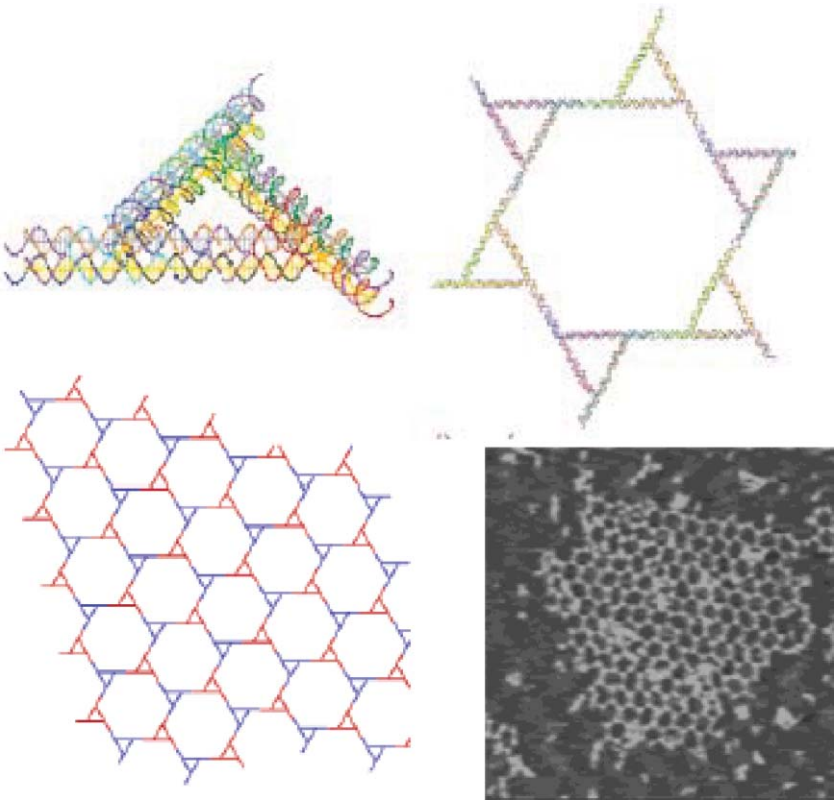

Fig. 11 Self assembly of DX motif-based triangular tiles into a pseudohexagonal 2D array. (Reprinted with permission from B. Ding *et al.*, *J. Am. Chem. Soc.*, 2004, **126**, 10230.[26] Copyright 2004 American Chemical Society.)

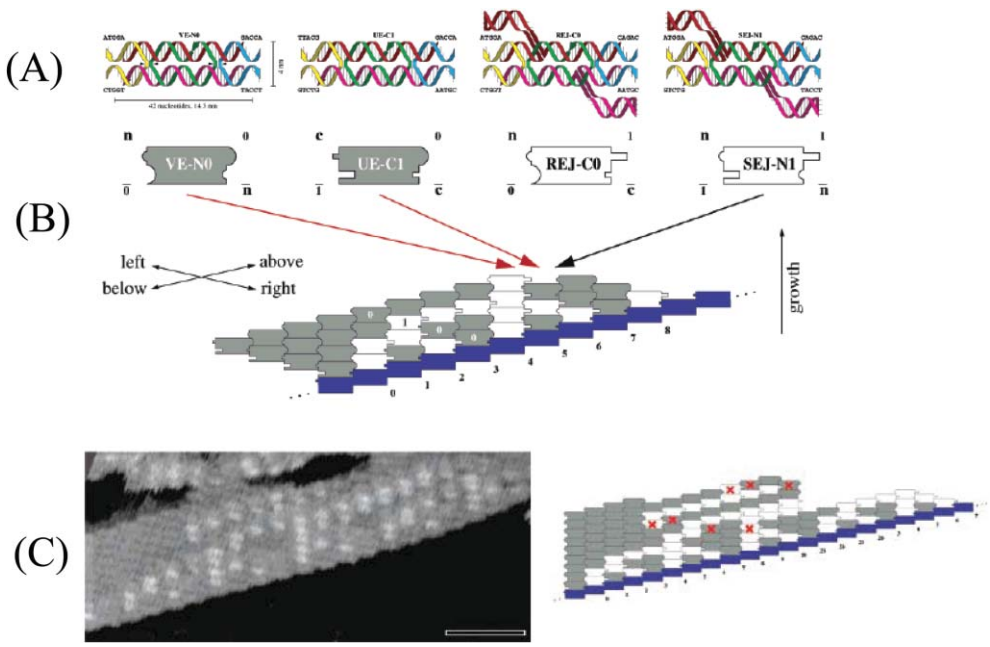

Fig. 12 Using recurrent AND and XOR logic to self-assemble DX-tiles in 2D to achieve counting and copying. (A) The four component sticky-ended DX tiles where the sequences perform the above logic. Two tiles incorporate stem and loop structures to provide a topographic contrast when analyzed by AFM. (B) The four counter rule tiles, VE-N0, UE-C1, REJ-C0, and SEJ-N1, corresponding to the four possible input pairs for ripple-carry adder logic. The two lower domains per tile are the inputs and the upper two act as outputs. Each tile outputs either 0 or 1 to the tile above it and outputs either a carry bit (c) or not (n) to the tile to its left. (C) AFM image of the resultant programmed assembly illustrating counting. Red crosses indicate errors. (Reprinted with permission from R. D. Barish *et al.*, *Nano Lett.*, 2005, **5**, 2586.[27] Copyright 2005 American Chemical Society.)

This sequence from 0–8 can be achieved by implementing the following equations by beginning with a series of 0's,

$$b_{i+1}^{n+1} = b_{i+1}^{n} \text{ XOR } c_i^{n+1}$$

$$c_{i+1}^{n+1} = b_{i+1}^{n} \text{ AND } c_i^{n+1}$$

where b_i^n is the bit in the i^{th} column and the n^{th} row of the counting array and c_i^n is the carry bit provided by that position to its leftward neighbor. Thus the array from top to bottom can be generated using the classical ripple-carry adder.[28] This algorithm for a half adder has been executed successfully using DNA DX tiles (Fig. 12A and B). Four different DX tiles representing the different sets of inputs for the ripple-carry adder were generated, each input represented by a set of single-stranded sequences. Each tile gives an output that is either a 0 or 1, which in turn serves as an input to the tile above. The same tile also gives an output to the left, whether to perform a carry or not. This serves as an input for the incoming tile on the left. Thus, a mixture of the component tiles together with a scaffold which is equivalent to 0's, initiates counting from 0 and proceeds till the occurrence of an error. Experimentally, counting up to values of 24 are achievable with error rates that are less than 10% (Fig. 12C).

Controlled confinement of self-assembly in 2D

Recently, in a paradigm shift in 2D-array formation, self-assembly of such large scale assemblies was restricted in 2D to form defined shapes such as squares, discs and five-point stars. These arrays have been constructed using the concept of raster filling. Several short strands that form regular helices are arranged, or stapled together, in the desired shape with a 7 kb single 'stapling strand' that defines the shape of the scaffold (Fig. 13A).[29] Using this concept, any desired shape within reasonable approximation may be constructed *e.g.*, smileys, stars, triangles *etc.* and this has given rise to the term 'DNA origami' (Fig. 13B).

3D superstructures

DNA has been used to construct regular, albeit discrete structures in three dimensions, namely polyhedra of various kinds. These have been realized using various strategies with appropriately positioned complementary regions engineered into the self-assembling component oligonucleotides. An important design consideration in polygon construction is that the DNA-backbone that defines the polyhedral edges is well within the persistence length. This compels the backbone to bend abruptly at defined angles at pre-designated locations thus creating the vertices of the polygon. The dimensions of these regular polygons can be easily calculated using the dimensions of the constituent DNA segments and simple geometrical principles. The first polygon that was constructed was a cube[30] that was realized by hybridizing two complexes of five oligonucleotides each, in a sequential manner using enzymatic ligation as shown in Fig. 14. This gives six circular, catenated oligonucleotides comprising twelve duplexed regions that go to form the twelve edges of a cube. Cyclic oligonucleotide self assembly has also been used to construct a truncated octahedron.[31] More recently, a one-pot supramolecular synthesis of a tetrahedron[32] was achieved using four

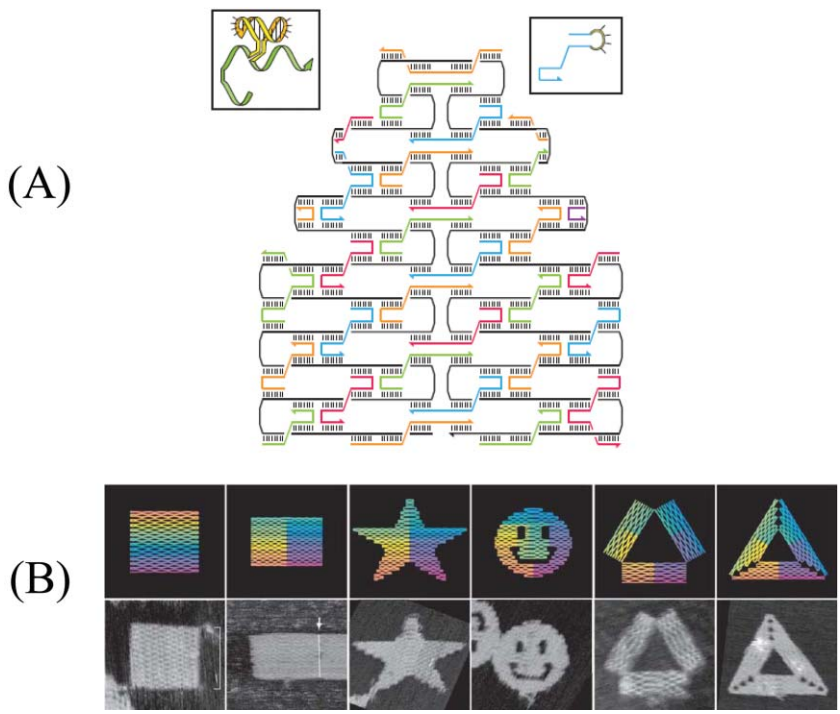

Fig. 13 Confining DNA self-assembly in 2D by using a 'stapler strand' or a raster-filling methodology to create specific shapes on the nanoscale. (A) Design principle: A long oligonucleotide being 'stapled' with several smaller oligonucleotides. Specific surface features are achieved by incorporating stem and loop structures at various stapler sequences. (B) Different shapes obtained by raster filling the long oligo. (Reprinted by permission of Macmillan Publishers Ltd. from P. W. K. Rothemund, *Nature*, 2006, **440**, 297.[29] Copyright 2006 Nature Publishing Group.)

linear oligonucleotides that were allowed to self-assemble and then locked in place *via* enzymatic ligation giving four catenated, circular oligonucleotides in the shape of a tetrahedron. Using a radically different approach, a 1669 bp ssDNA sequence folded into an octahedron upon self-assembly with five 40 bp complementary DNA sequences.[33] The formation and geometry of the vertices is all important to polygon stability. An *N*-connected vertex is generated from an *N*-armed DNA junction, *i.e.*, *N* oligonucleotides integrate into a single complex that radiates *N* duplexes from a single node. Thus the cube and tetrahedron can also potentially be made from 3-arm junctions and an octahedron from 4-arm junctions. Another factor which is important for the construction of such polyhedra is the stability of the vertex. For a specific polygon, the duplex arms at a vertex are required to bend at a specific angle (*e.g.* 90° at each face, for the cube) which could impart immense strain on the flanking DNA sequence. This strain is somewhat mitigated by incorporating unpaired nucleobases between successive arms of the junction in order to aid bending as well as stabilize the *N*-arm junctions.[13] Indeed even bulges containing 4–6 unpaired T bases, have been incorporated into the junctions of DNA-triangles which seems to stabilize the overall structure.[34]

One possible application of ordered 3-D polyhedra, has been postulated to be in aiding macromolecular crystallization. The self-assembly of polyhedra with sticky ends into ordered arrays would result in the presentation of an ordered scaffold to which other molecules, say proteins, could bind thus facilitating X-ray crystallographic analysis of the bound protein. In addition, such regular, closed structures can either be used as carriers by functioning as capsules or as scaffolds. They can be used to enclose entities like terbium ions whose fluorescent properties are greatly enhanced by hydrophobic encapsulation, especially when localized in an aromatic environment.[35]

Conclusions

Structural DNA nanotechnology is still in an embryonic stage where the underlying principles, the design and fabrication of building blocks, are still being explored. Despite the controlled, rational design strategies to assemble DNA into structures that are impossible to attain with other biomolecules, there are still several obstacles that limit the practical applications of such assemblies. Reconfiguration of DNA assemblies require time scales of seconds to milliseconds to achieve a change of state. Thus, there is palpable compromise on speed at the top for room at the bottom. Furthermore,

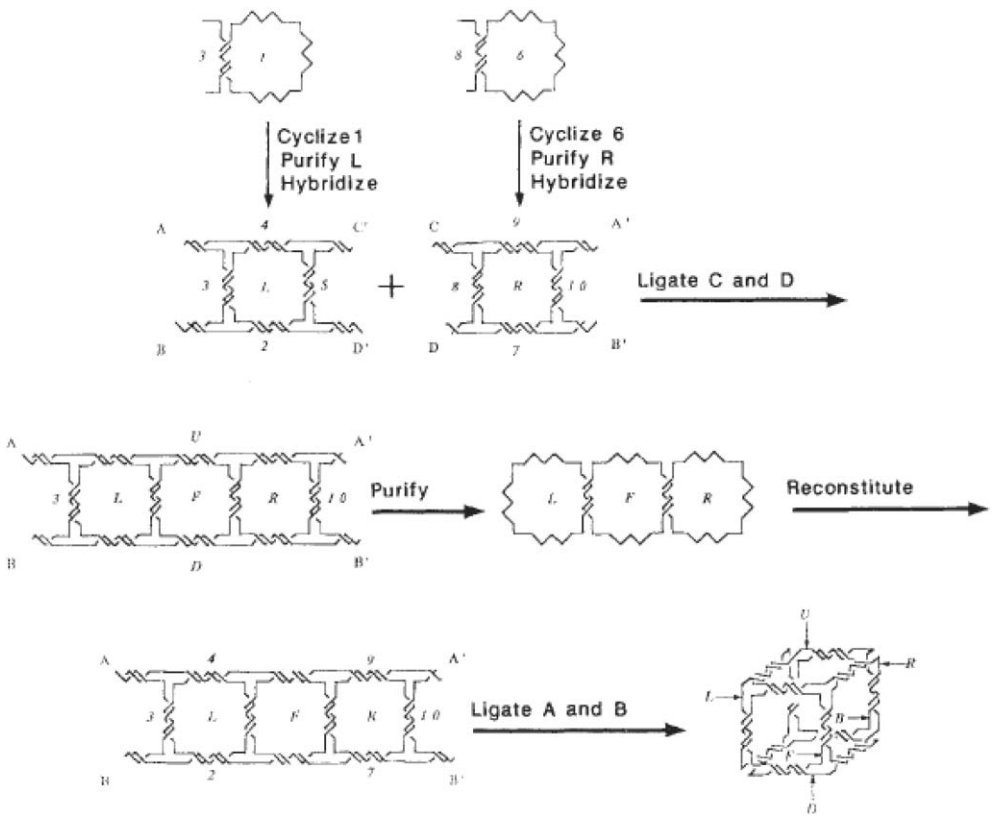

Fig. 14 Synthesis of a cube from ten linear oligonucleotides in six steps. As in classical organic synthesis, different combinations of linear oligonucleotides are subjected to ligation or a circularization reaction, which locks the assembly at each step. In the first step, linear strands 1 and 3 are subjected to ligation, circularizing 1. Addition of strands 2–5 to this assembly followed by ligation circularizes 3 and 5 yielding one face of a cube with four overhangs A, B, C′ and D′. Upon mixing and ligating this with a face containing complementary overhangs A′, B′, C and D synthesized separately in a similar manner, a grid with three faces results. This grid has two overhangs A and B on one side and A′ and B′ on the other, that fold upon itself, resulting in a cube that is then trapped by ligation, giving rise to six, mutually catentated, circular oligonucleotides. (Reprinted by permission of Macmillan Publishers Ltd. from J. Chen, *Nature* 1991, **350**, 631.[30] Copyright 1991 Nature Publishing group.)

errors in self assembly compromises function in nearly all devices. For example, in the case of performing logical operations evidenced by tile self-assembly as discussed in Fig. 12, error rates limited counting capabilities to 24. Although it is possible to design several unique sequences to self assemble, the binding sites may still have moderate affinities for sequences that are not the exact complement. As a result, parts of a given sequence may be capable of hybridizing to multiple sequences, which could disrupt the performance of the logic gate. Thus, the key issues in the area that would need to be addressed if structural DNA nanotechnology is to live up to its potential would be (i) to develop methodologies to achieve error-free assembly of components (ii) to develop design concepts that control the size or confines 2D arrays to defined dimensions and (ii) to push the limits of the dimensions of rigid assemblies and scaffolds beyond what is currently achievable, into micron regimes.

Positively charged metals such as gold, silver or platinum ions can be immobilized on DNA wires *via* electrostatics with the negatively charged, but pre-organized backbone on these assemblies. Such metallated wires can act as nanoscale interconnects in molecular electronics. If the nanoparticles are immobilized such that regimes of plasmon resonance are achieved, then these 1D scaffolds could act as waveguides on the nanoscale. In a world where photonics is superseding electronics, such scaffolds could be extremely valuable. 2D scaffolds could also be made to extend into 3D and form a regular-periodic lattice which could potentially transform crystallographic techniques, as these scaffolds could present the desired protein periodically. There have already been reports of regular arrangements of proteins on 2D arrays.[36] 2D and 3D arrays also have regular cavities that could serve as super-accurate filters on the nanoscale.[37] Thus, despite the current limitations, structural DNA nanotechnology is well-placed to be a vehicle that could revolutionize nanoelectronics, macromolecular crystallography and nanorobotics.

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