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The Challenge of Structural Control on the Nanoscale: Bottom-Up Self-Assembly of Nucleic Acids in 3D

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Abstract

Control of the structure of matter has been a major challenge to humankind for its entire history. The finer the features that that we are able to engineer, the greater the level of control that we have. Here, we summarize progress made in the bottom-up control of structure that is based on the self-assembly of nucleic acids. Nucleic acids are unique among molecular systems in that their intermolecular interactions can be programmed, from the perspectives of both affinity and of structure. Structural DNA nanotechnology has been based on directing the cohesion of branched DNA motifs by the same cohesive interactions used by genetic engineers. As a result, multiply-connected objects, periodic and aperiodic arrays and nanomechanical devices have been produced by these systems. Current experiments are directed at using nucleic acid systems to scaffold the spatial assembly of other species.

Keywords

Branched DNA; unusual DNA motifs; DNA periodic arrays; atomic force microscopy; DNA polyhedra; self-assembly; DNA devices; DNA scaffolding

1. Introduction

The challenge of controlling of the structure of matter is as old as our species. At one time, this quest sought only to produce macroscopic substances with desirable properties, stronger, lighter, more conductive, more beautiful, more durable. However, our civilization has advanced to the point where miniaturization of organized objects and devices has now become another goal, leading to systems that have greater computational or medical utility. We have just about reached the limit where it is useful to think in terms of miniaturization by traditional methods, such as lithography. If we want to conquer the nanoscale, we should consider working from the bottom-up, rather than working exclusively from the top-down.

Of course, chemists have been working for over 150 years building compounds from their constituent atoms. Much has been learned in that interval about how to assemble atoms into molecules with specific spatial arrangements. Nevertheless, the construction of new compounds is extremely tedious, and at some point, 100 atoms, 200 atoms, larger constructs entail assembling those compounds into larger well-defined structural units, such as polymers or crystals. Other than naturally-occurring proteins, polymers are not highly-structured; as for crystals, most compounds can be crystallized, but there is little or no control or predictability associated with the crystals that they form. If one wishes to use the molecules individually for their chemical properties, this poses no problem. However, if the goal is to construct new macroscopic or mesoscopic arrangements of matter, a new and different approach must be taken.

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The unlikely but highly successful system that enables one to do this is the nucleic acids. DNA is well-known as the genetic material of all living organisms [1]. It functions successfully as genetic material because of its chemical properties. These properties include the affinity of complementary sequences, a well-stacked antiparallel double helical backbone that is largely regular regardless of sequence, and a persistence length (a measure of stiffness) around 50 nm under standard conditions. It is logical to examine whether these properties can be exploited outside of biology. Structural DNA nanotechnology aims to use the properties of DNA to produce highly structured and well-ordered materials from DNA. This effort that has been underway since the early 1980's [2].

Indeed, the properties that enable nucleic acids to fulfill their role in nature also enable them to serve as a remarkably diverse and tractable system to build objects, periodic and aperiodic lattices and nanomechanical devices. In this paper, we will cover this new field, known as structural DNA nanotechnology, describing how branched motifs of DNA are designed, how they have been used to produce a variety of objects, how they can be used in the formation of crystalline arrays in 2D and 3D. We will describe how the recognition properties that are exploited by nature in the metabolism of DNA also can be exploited to control their self-assembly and the action of DNA-based nanomechanical devices.

2. DNA as a Construction Material

Figure 1 illustrates the main features of DNA structure. Figure 1a shows an unwound dinucleotide, emphasizing the backbones, which consist of alternating sugar and phosphate units. The backbones are held together by the hydrogen-bonded base pairs. The phosphates are ionized at physiological pH, so the molecule is a polyanion. The well-known double helical structure contains two sugar phosphate backbones, which are antiparallel to each other. This feature is readily apparent in Figure 1a, where the oxygen atoms on the pentagonal sugars on the right side of the structure are pointing down, whereas those on the left side are pointing up. The antiparallel nature of the chain results in a dyad axis normal to the helix axis. The dyad axis not only repeats every unit, but the periodicity of the system leads to other dyad axes half-way in between. Thus, in Figure 1a, we can see a dyad axis squarely in the center of the drawing of the molecule, and two others that are going through the base pairs; only the backbones are related by this symmetry. The diameter of the DNA double helix is about 2 nm, and the repeat in the direction of the helix axis is 3.4 Å; with 10–10.5 nucleotide pairs per turn, this leads to a pitch of roughly 3.5 nm, so DNA is very much a denizen of the nanoscale.

Figure 1b illustrates the Watson-Crick base pairs. These consist of one small pyrimidine and one larger purine each: Thymine (T) pairs with adenine (A) by means of two hydrogen bones, and cytosine (C) pairs with guanine (G) using three hydrogen bonds. Note that the central N-H-->N hydrogen bond in the A-T pair is directed from the pyrimidine (the donor) to the purine (the acceptor); by contrast, that hydrogen bond in the G-C pair is directed from the purine to the pyrimidine. This difference ensures that cross-pairing between the purines and pyrimidines (e.g., A-C or G-T) is not favored. The little chart at the bottom of Figure 1b emphasizes the nature of the Watson-Crick base-pairing interactions: A big unit that donates an N-H-->N hydrogen bond (G) pairs with a small unit that accepts one (C), and a small unit that donates an N-H-->N hydrogen bond (T) pairs with a large unit that accepts one (A). The third hydrogen bond in the G-C pairs leads to additional stability, so that the melting temperature of a particular DNA motif will be roughly a function of its G+C/A+T ratio (see [3] for a more modern treatment of DNA melting). The size complementarity is a consequence of fitting the base pairs into the double helical backbone; in the absence of that constraint, any set of paired interactions can be observed [e.g., 4].

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The early 1970's saw molecular biologists exploit enzymes that modify the DNA molecule to produce new arrangements of DNA sequences. Using restriction enzymes that cleave at specific sites and ligases that fuse nicks in backbones, they were able to cut and splice DNA molecules, in much the same way that a film editor uses his tools to re-order sequences of film, creating a new overall effect [5]. The upshot of this activity was genetic engineering, which has led to the worldwide biotechnology industry. The key feature that concerns us was the use of single stranded overhangs (called 'sticky ends') to specify the order in which molecules were to be assembled. Sticky-ended cohesion between DNA molecules is illustrated in Figure 2a. An important feature of sticky-ended cohesion is that when two sticky ends cohere, they form classical B-DNA [6], the structure established by Watson and Crick and refined by their successors. Thus, if one knows the positions of the atoms of one component of a cohesive pair, one knows the positions of the other component. Hence, sticky-ends provide the most readily programmable and predictable intermolecular interactions known, from the perspectives of both affinity and structure [7].

Of course, naturally-occurring DNA is a linear molecule. From a topological standpoint, the DNA double helix of finite length is just a line segment. The line may be curved, and closed ones can be knotted or catenated; nevertheless, concatenating a series of lines together end-to-end just results in longer lines. Arranging linear DNA molecules in a specific order is of significant value to genetic engineers, because this establishes the presence and regulation of gene products, but linear DNA helical structures are not useful structural components for nanoscale structural engineering. DNA is extended beyond linearity by the notion of making synthetic branched molecules [2].

Branching achieved by changing the covalent nature of DNA (i.e., at the primary structure level) is certainly possible [8], but it has not been exploited yet in DNA nanotechnology. Nevertheless, DNA is found to be branched at the level of secondary structure in biological systems; this means that conventional DNA strands associate to produce junction points: If we think of the strands of DNA as the lanes of a two-lane highway, a junction point would correspond to the lanes going through an intersection. Traffic could flow through four different lanes in a traditional four-way intersection; in countries that drive on the right, northbound traffic following the curb would turn east, westbound traffic would turn north, southbound traffic would turn west and eastbound traffic would turn south. A branched DNA molecule is similar.

Typically, branched DNA is an ephemeral intermediate in DNA metabolism; for example, the four-arm Holliday junction is an intermediate in genetic recombination [9]. Although not made in the same way as in the cell, it is useful to think about the cellular process that produces branched DNA molecule, the process of reciprocal exchange illustrated in Figure 2b. Here, a shaded strand and an unshaded strand are seen to produce two new strands, one shaded-unshaded, and another that is unshaded-shaded. By applying this process once or more, it is possible to produce a series of branched DNA motifs that have been found to be extremely useful in DNA nanotechnology [10]. Some of these are shown in Figure 2c. At the left of this panel, we see that a single exchange between two double helices produces a Holliday junction. A double exchange of the same sort (between strands of opposite polarity) produces the double crossover (DX) structure [11]; adding another helix the same way leads to a triple crossover (TX) molecule [12]. Reciprocal exchange at all possible sites between strands of the same polarity leads to the paranemic crossover (PX) structure [13]; leaving two sites uncrossed produces one of its topoisomers, known as JX_2 [14].

The combination of branched motifs with sticky-ended cohesion is the key to structural DNA nanotechnology. As we have noted, fusing linear pieces of DNA with sticky ends produces just longer lines. However, fusing branched DNA molecules leads to more

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complex topologies. For example, Figure 2d illustrates the combination of four Holliday-junction-like molecules (in a different representation from Figure 2c), tailed with sticky ends, to produce a quadrilateral [2]. Furthermore, the quadrilateral itself is surrounded by sticky ends, so that it could be extended with more units to build a 4-connected lattice in two dimensions. The system is certainly not limited to 2D: The connectors between vertices in such a structure are not ladders, as shown, but double helices, so the orientations of rigid components at their ends is a function of their separation, much like two wing-nuts on a screw. Furthermore, 3-space-spanning motifs have also been reported [15,16], so structural DNA nanotechnology is really a three-dimensional system.

Branched DNA of biological origin usually displays symmetry that allows its branch point to migrate, but it is a simple matter to design [2] and assemble synthetic DNA sequences that are stable because they lack this symmetry. Branched junctions containing three, five or six arms have also been assembled and characterized [17]; in recent work our laboratory has made junctions with eight and twelve arms [X. Wang and NCS, in preparation]. This is important, because the connectivity of the graph produced by cohesion or ligation of junctions is limited by the number of arms that flank its junctions. Space-filling lattices built from the classical Platonic or Archimedean polyhedra have connectivities up to twelve [18]. The ability to generate stable branched DNA molecules has opened new opportunities for the bottom-up construction of nanoscale objects.

It is evident that DNA nanotechnology is a bottom-up approach to nanotechnology. The components are a few nm in size, so the exquisite placement of individual atoms or molecules that characterizes some of the top-down work done with scanning probe microscopes [e.g. 19, 20] is beyond the range of this methodology. However, it is important to recognize that bottom-up approaches are able to take advantage of the vast parallelism of chemical reactions. Thus, in the worst of constructions, a billion or a trillion product molecules typically will be produced. Although beyond the scope of this article, the parallelism of DNA nanotechnology is related to the parallelism exploited in DNA-based computation [21].

3. Advantages of DNA in Construction

The primary reason to use DNA as a bottom-up construction material lies in its outstanding molecular recognition properties, enabling precise structural alignment of diverse DNA molecules that can scaffold various molecular species. DNA and its congeners appear to be unique among biopolymers in this regard. Antibodies, for example, may lead to specific binding, but the detailed geometry of antibody-antigen interactions must be worked out experimentally in every case. It is only with nucleic acids that the detailed 3D geometry is known in advance, because, as noted above, sticky ends form B-DNA when they bind to link two molecules together [6]. It is a very good approximation to assume that all sticky ends, regardless of length have the same overall B-DNA structure when they cohere with their complements.

The convenience of chemical synthesis [22] is another advantage of DNA; “vanilla” DNA (DNA exactly like naturally-occurring DNA) is available from a number of vendors, and DNA synthesizers are readily capable of generating a number of varied molecules based on commercially available phosphoramidites. It is routine to synthesize DNA molecules whose lengths are 120 nucleotides or shorter. A variety of enzymes are commercially available to manipulate DNA and to trouble-shoot errors: DNA ligases catalyze the covalent joining of complexes held together by sticky-ended cohesion; exonucleases (which require an end to digest DNA) are useful in purifying cyclic target molecules from linear failure products [23]; restriction endonucleases are employed both to trouble-shoot syntheses and to create

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cohesive ends from topologically-closed species [24]; topoisomerases, which catalyze strand-passage reactions, can be used to correct folding errors [25].

Several features of DNA help it to perform its genetic functions. One of these is the persistence length, about 500 Å [26], leading to a predictable overall structure for the short (70–100 Å) lengths typically used. In fact, it is possible to design branched species with longer persistence lengths [27]. Thus, the self-assembled structure of DNA constructs and of some of its interactions can be taken for granted, just as one can take for granted the shape of a domino tile. The external code on DNA can be read, even when the double helix is intact [28]; thus, if DNA is used for scaffolding, absolute positions can be addressed within a pre-designed cavity. The ability to pack nanoelectronics or other scaffolded materials very tightly is likely to be aided by the high density of functional groups on DNA; in principle there is a site every 3.4 Å along the helix axis.

Most of the constructions in DNA nanotechnology done so far use the vanilla DNA molecules evolved in nature to be genetic material. However, the information-based advantages of using nucleic acids are not limited to the natural DNA molecule. A vast number of DNA analogs have been produced and analyzed for therapeutic purposes [e.g., 29]. This means that systems prototyped by conventional DNA ultimately may be converted to other backbones and bases, as required by specific applications. For example, it is unlikely that nanoelectronic components will be scaffolded successfully by poly-anionic DNA molecules. However, there are numerous neutral analogs of nucleic acids that may be much better suited to act as scaffolds for this purpose.

4. Targets of DNA Construction

Ideally, the system we have described above should resemble a self-assembling version of molecular Lego: The well-defined combining properties of the various motifs ought to be programmable so that they spontaneously produce a variety of shapes and arrangements. This is a good point for us to think about what we would like to be able to make, if this system behaved in an ideal fashion.

The key motivating goal in this system has been the desire to conquer the macromolecular crystallization experiment. Although an extensive lore now exists, every macromolecule presents different crystallization problems. The problem of crystallizing a macromolecule of unknown structure is that one does not know how to promote the organization of the molecules into a periodic lattice in a rational fashion, because the 3D molecular structure is unknown. Furthermore, in contrast to experiments that are easily debugged, every relevant parameter must be within the correct window for the crystallization to be successful; consequently, the independent factors cannot easily be optimized individually, although incomplete factorial methods have been developed [30].

The approach we suggested is to use DNA to form periodic arrays that organize the macromolecule of interest [2]. The notion is illustrated in Figure 3a. A ‘box’ of nucleic acid is designed to act as the repeating unit of a 3D periodic lattice. A series of boxes are to be held together by sticky ends. The actual motifs to be used are more complex and more structurally robust than the simple schematic box illustrated. These boxes act as the host lattice for a series of macromolecular guests that are positioned and oriented with the boxes, and hence within the lattice. If all are ordered adequately, then the x-ray diffraction experiment can be performed on the entire contents of the crystal. In addition to the ability perhaps to organize molecules that are otherwise resistant to crystallization, this system offers the advantage that guest macromolecules whose structures change when binding to ligands would not destroy the lattice, because only DNA would be involved in maintaining lattice contacts.

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If one can imagine organizing macromolecules, it is also possible to imagine organizing other species that do not readily self-organize. Prominent amongst them would be nanoelectronic components. The idea of using DNA to organize nanoelectronic components is not very new [31], but only recently has it started to gain favor. The concept, illustrated for a simple component in Figure 4b, is that the outstanding architectural properties of DNA be used to act as scaffolding with nanometer precision for species that are intractable to simple bottom-up organization on their own.

A further goal of DNA nanotechnology is the use of DNA's structural and informational properties to build nanomechanical devices. The earliest DNA devices were predicated on structural transitions of the molecule. There are two kinds of DNA-based devices, those based on structural transitions triggered by the addition of small or large molecules to the solution, and those that are sequence dependent, although these can be subdivided further. As we will see below, the latter are more useful, because a variety of them can co-exist in solution at the same time, yet they can be activated individually. This is closer to the notion of acting as a successful basis for nanorobotics, and this is the route that investigators largely are taking at this time.

It is useful to distinguish structural DNA nanotechnology from a less precise form of DNA nanotechnology, termed compositional DNA nanotechnology. Structural DNA nanotechnology uses well-structured components, combined by using both affinity and structure to control geometry, or at least, strand topology; the goal of this approach is structural predictability with a precision (or resolution) of 1 nm or better in the products. By contrast, in compositional DNA nanotechnology, these conditions are not met completely: The components may be flexible or the cohesive interactions by which they are combined are uncharacterized; as a consequence, the composition of the product may be known, but its 3D structure cannot be predicted with high precision.

A detailed description of the work produced by compositional DNA nanotechnology, in which DNA is used largely as ‘smart-glue’, rather than a precise structural component, is beyond the scope of this article. Nevertheless, it is important to recognize that numerous laboratories have made useful and valuable materials by this approach. For example, this approach has been used in diagnostics [32], to organize DNA nanoparticles on small [33] and on large [34] scales, in combination with non-DNA organic components [35,36], and in the production of DNA-protein aggregates [37]. Although they do not provide the high resolution structural features sought in structural DNA nanotechnology, using smart-glue approaches to organize nanoparticles can lead to organized products, but with lower precision, 10's to 100's of nanometers. In a complementary vein, G-wires [38,39] are examples of well-structured nucleic acid systems that lack the sequence diversity central to structural DNA nanotechnology.

5. Sequence Design

Stable branched motifs are derived from synthetic molecules, so there must be a method to assign sequences to them. The method of sequence symmetry minimization [2,40] has proved to be up to the task. The basic idea is that DNA strands will maximize the double helical structures that they form. Although an early approach [41] involved calculating a likelihood of formation from nearest-neighbor equilibrium thermodynamic parameters, no constructs built to date appear to require a calculation this extensive. The method used can be understood readily by reference to Figure 2c. This molecule contains four 16-mers, labeled 1, 2, 3 and 4. We break up each of these single strands into a series of thirteen overlapping tetramers, such as the CGCA or GCAA that have been boxed; we insist that each of these be unique. In addition, we insist that each tetramer that spans a branch point,

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such as the boxed CTGA, not have its linear complement (TCAG) present; this restriction results in these tetramers being unable to form linear double helices. Consequently, competition with the four octamer double helical targets can occur only from trimers, such as the boxed ATG sequences. The reason to go through the exercise of sequence-symmetry minimization is that the molecules that are designed correspond to conformations of DNA that are of higher free energy than the 'ground state', i.e., the simple Watson-Crick double helix. The aim of sequence design is to ensure that the 'excited state' that the system chooses is also the target state sought by the investigator.

The assumption used in all of the sequence design is that molecular geometry and structure formation are sequence-independent. In those cases where it is known that sequences result in structural features, e.g., the bends associated with tracts of several A;s [42], the issue is handled by forbidding those sequence within a molecule. Likewise, runs of G's that often form their own secondary structure [43], are avoided. This approach has largely worked to date. A second issue, of course, is folding. It is difficult to model the folding of and pairing of a large number of DNA strands. Nevertheless, it is often a good idea to examine the associations that one wishes to promulgate in the construction of a given motif. If, for example, it seems that the ends of a strand are likely to pair earlier than the middle of the strand, perhaps because of higher G-C content, then re-design may be indicated, because the middle may not be able to wrap around its complementary strands if its ends are already tied down.

6. Graph-Like Structures from DNA

The earliest interesting DNA constructions involved closed graph structures. Early examples were polyhedral stick figures whose edges consist of double helical DNA, and whose vertices correspond to the branch points of branched junctions. The formation of the edges was implicit in these constructions, just the result of joining junctions together. Figure 5a illustrates a molecule whose edges have the connectivity of a cube or rhombohedron [23]. Although the DNA in the arms of DNA branched junctions is stiff, the angles between the arms are apparently floppy [44,45]. Consequently, individual DNA polyhedra and other graph-like figures are characterized topologically, rather than structurally. The cube in Figure 5a is a hexacatenane, with two turns of DNA per edge. With two turns per edge, each strand corresponds to one of the faces of the cube, and each strand is linked twice to the four strands that flank it. The molecule was constructed in solution, by methods that had little control over the products of the synthesis. Proof of synthesis was obtained by breaking down the final product to sub-catenanes that could be constructed and characterized independently.

Many of the problems of the synthesis of the cube were eliminated by the development of a solid-support-based methodology [24], which allowed the use of the full logical control implicit in sticky-ended construction. As a test case, this approach was used to build a truncated octahedron (Figure 5b), again with two turns per edge [46]. Thus, the DNA representation of this Archimedean polyhedron is a 14-catenane. Six of its faces correspond to squares and eight of its faces correspond to hexagons. The faces corresponding to squares contain an extra arm at each junction (not shown in Figure 5b), so that this 3-connected polyhedron was built with 4-arm junctions. Consequently, it might have been possible to make a 4-connected lattice, such as the zeolite A lattice, but not enough material was produced to use it as starting material for a lattice construction.

Both of these polyhedra correspond to regular graphs. In an experiment related to DNA-based computation, we have also constructed an irregular graph [47]. The construction prototyped a monochromatic version of a solution to a graph 3-colorability problem.

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Whereas the two previous polyhedral constructions used step-wise synthesis to maximize control, the nature of DNA-based computation entails doing as many steps as possible at once. Similarly, the requirements of the calculation involve mixing edges of different properties, so that molecules corresponding to edges were added explicitly to the solution containing the junctions. This single-pot reaction resulted in the construction of the irregular graph illustrated in Figure 6. Unlike the cube and the truncated octahedron, this graph is a single-stranded knot, rather than a catenane. Proof of synthesis in this case requires the breakdown of the final strand to predictable restriction fragments.

7. Two-Dimensional Arrays

Individual objects like polyhedra may demonstrate the versatility and convenience of DNA branched junctions as a system for the construction of difficult targets. Nevertheless, the goals of structural DNA nanotechnology go beyond the construction of individual objects to periodic arrays, and even to aperiodic arrays. The ability to make specifically-patterned 2D and 3D structures is central to the success of this enterprise. The floppiness of individual branched junctions usually makes them unsuitable as components for such constructions. The relationship between cycles and periodicity can lead to cyclization of floppy components. However, DX molecules (Figure 2c) are branched species that are roughly twice as stiff as linear DNA [27,48], and therefore substantially stiffer than simple branched junctions. Consequently, they are well-suited to serve as components for periodic arrangements. TX molecules (Figure 2c) have been used successfully in periodic arrangements without stiffness characterization.

The first examples of two dimensional periodic arrays were DX arrays that contained the capability to produce patterns [49]. Figure 7a illustrates an array produced from a conventional DX molecule and a DX+J molecule, which contains a domain pointing perpendicular to the plane of the helix axes. The dimensions of these 2 nm-thick tiling components are about 4 nm × 16 nm. The extra domains form stripe-like patterns separated by 32 nm that are readily observed in the atomic force microscope. To demonstrate the level of control over the pattern, a second DX array is shown in Figure 7b. Here, three DX tiles are combined with a DX+J tile, to produce a pattern where the stripes are separated by 64 nm. TX molecules can be used to produce similar 2D arrays [12]. It is possible to connect them 1–3 to leave addressable gaps in the lattice that can be filled in by other components, including rotated TX molecules themselves.

Although four-arm junctions are floppy, an effective system results from combining four four of them into a parallelogram [50]. The 4-arm branched junctions assorts its four arms into two double helical domains [51], which are twisted with respect to each other [52]. The twist can be 40–60 from antiparallel [50, 53] or (with 3′,3′ and 5′,5′ linkages in the crossover strands) 70 from parallel [54], so that a variety of parallelograms can be produced. The parallelograms can be connected through sticky-ended cohesion to produce an array containing cavities. It is straightforward to alter the sizes of the cavities, so that the porosity of this system is readily tunable.

Yan, Reif and their colleagues [55] have developed a motif that they call the 4 × 4. it consists of four Holliday junction molecules that flank a central cavity. The molecules fail to stack within the cavity because of loops that proceed from one domain to another. The system is nearly, but not exactly tetragonal. This new motif appears to be robust, and is likely to have a number of applications in DNA nanotechnology.

All of the periodic motifs described so far are basically low-symmetry motifs, corresponding to translations or twofold rotations normal to the plane. Recently, we have developed a trigonal motif that produces a pseudo-hexagonal array [56]. The motivation to make

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hexagonal structures is that they correspond to the 'stick' version of hexagonal close packing within the plane. There have been a number of significant failures to make hexagonal structures. We have adapted the unsuccessful bulged junction triangle motif [25] by doubling its height, so that it is made of DX molecules, rather than double helices. This molecule forms an excellent honeycomb structure. Analysis of the system indicates that the key to the formation of the 2D array is the DX cohesion: Blunting one of the sticky ends leads to failure in honeycomb array generation. This approach to DX cohesion has now been applied to a large number of motifs that were intractable or at least recalcitrant to 2D periodic array formation. An approach from Adleman and his colleagues [57] uses DX cohesion in structures confined to a single plane, and is largely successful, but inspection of their results demonstrates that the stiffness of the DX sides to the triangles is apparently partially responsible for the uniquely stiff nature of the DX triangle motif.

8. DNA Nanomechanical Devices

There are multiple different routes to obtaining nanomechanical devices from DNA-based systems. The first of these is the use of DNA structural transitions. Such transitions are well-known, and they can be used to change the structure of DNA in a reliable fashion. The first robust system was based on the B-Z transition of DNA [58]. Z-DNA has two requirements for formation: An appropriate sequence (called 'proto-Z-DNA') and Z-promoting conditions; in the absence of either, the structure remains in the B-form. The need for a special sequence allows control of the transition in space (i.e., where, and over how much DNA it will occur), and the necessity of Z-promoting conditions provides control over the transition in time (i.e., when it happens). A device that takes advantage of the B-Z transition is illustrated in Figure 8a. It consists of two DX molecules connected by a shaft that can undergo the B-Z transition. In the absence of Z-promoting conditions, the shaft is in the B-conformation, and both domains unconnected to the shaft are on the same side of it. However, addition of the effector, $Co(NH_3)_6^{3+}$, switches it to the Z-conformation. This in turn rotates an unconnected domain of a DX molecule to the other side of the shaft. The transition is demonstrated by a pair of dyes using fluorescent resonance energy transfer (FRET).

The problem with devices based on DNA structural transitions is that they are triggered by a single molecular species, and are not addressable individually. The breakthrough in this area was made by Yurke and his colleagues [59], who developed the second type of device, which is sequence-dependent. This was a molecular tweezers that contracted when a particular strand was added to the solution, and then relaxed to an expanded state when the strand was removed. The removal of the strand was achieved by adding a short, unpaired segment (called a toehold) to its end. Addition of the complete complement to this strand resulted in its binding first to the toehold and then ultimately the complement branch-migrates its way through the structure until the entire strand is in duplex form; this is essentially an irreversible state, because there is more base pairing with the complement than with the device structure, and the duplex may be more stable than the unusual motif. Numerous devices have been built using this approach, including a robust 2-state device [14], a bipedal walker [60], a transcriptionally-controlled device [61] and a device that performs translation [62].

The robust two-state device is based on transitions between the PX and JX_2 molecules shown in Figure 2c; its machine cycle is illustrated in Figure 8b. It shows a molecule in the PX structure (left), with two thick 'set' strands that put it into that state. The addition of the complements to the set strands ('unset' strands) results in their removal, leaving a naked frame (top); the black dots at the ends of the complements represent biotin groups that aid in the removal of the duplex from solution. The addition of two thin set strands puts the system

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in the JX_2 state (right). The feature of the JX_2 structure that is central to its role here is that its bottom is rotated a half-turn from the bottom of the PX structure. Note that the sequence complementary to the set strands can be varied, so that there are a lot of different frames that can be developed, and which can be addressed independently. The bottom part of Figure 8b illustrates that the PX state can be restored in a manner analogous to the formation of the JX_2 state.

The PX-JX_2 device has been used to build a device that performs a translation operation, patterned after the ribosome [62]. Figure 9 illustrates the device and its relationship to the traditional components in cellular translation. The device consists of a diamond-shaped structure made from two edge-sharing DNA triangles, and then two other double diamonds. The units are connected by independently addressable PX-JX_2 devices. The diamonds are tailed in sticky ends, indicated by Arabic numbers. The sticky ends that flank the gaps on the top row are a function of the states of the two devices. For example, in the two PX states shown, the two gaps are flanked by sticky end pairs (1,2) and (4,6). These positions each can accommodate a DX molecule whose ends are complementary to these sticky ends. The DX molecule shown at the bottom of the diagram plays a role analogous to an aminoacyl tRNA: There are a large number of these molecules present simultaneously in solution, and the device binds the correct one specified by the state of its two devices. Thus, the set strands play the role of mRNA, directing the choice of units to be incorporated. The lower domain of the DX molecule plays the role of a tRNA molecule, because it contains the complements to the sticky end pairs. Its top strand corresponds to the amino acid, because it will be incorporated into the product. The only relationship between the coding set strands and the product strands is an arbitrary code, analogous to the genetic code in protein synthesis.

An exciting hybrid of a device and a 2D lattice has been pursued by Yan, Reif and their colleagues [63]. A parallelogram-like lattice has been built that contains a hairpin. A strand can be removed from the base of the hairpin, and a complement to the entire hairpin and base can be added. This expands the lattice dimensions of the parallelogram in one direction from four turns to six turns. Ultimately this approach may be used to change the dimensions of crystalline samples, possibly in 3D.

Another form of device is the activation of a DNA transition by a protein. This is close to phenomena that actually occur within a living cell. This type of device is sequence-specific, but a new protein must be found for each different transition, thereby decreasing the convenience and generality of the approach. This type of device was prototyped recently in a context similar to that of the B-Z device; the proto-Z segment of the device was replaced with the binding site for a DNA-bending protein [64] known as integration host factor (IHF). The binding of IHF to the DNA was monitored by FRET, just as it was in the B-Z device. The interesting feature of this device is that it was used to measure the amount of work that the protein could do upon binding. As schematized in Figure 10, the protein was required to break a number of hydrogen bonded base pairs so as to bind. By titrating the number and type of interactions, it was possible to estimate that the protein is capable of doing 7–8 kcal/mol of work when it binds to DNA.

The devices described above are all clocked devices: They respond to a particular stimulus in a way that is designed. However, there is a great deal of interest in autonomously operating devices. One has been developed recently by Mao and his colleagues [65]. He has employed a DNAzyme, a DNA molecule that binds to a given short sequence of RNA, and then catalyzes its cleavage. When the RNA molecule binds, it brings together a tweezer-like molecule. Following cleavage, however, the two RNA molecules no longer hold the tweezers together, so it expands. In addition, the RNA is so short following cleavage that it

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is no longer bound to the DNA; it dissociates when a new substrate molecule is present, and the cycle repeats.

A review of all of the DNA-based nanomechanical devices is beyond the scope of this article. This is a field that is moving very rapidly. A recent review [66] describes progress in the area through the end of 2004. Developments in DNA-based nanorobotics are occurring so swiftly that any review is likely to be obsolete by the time of its publication.

CONCLUDING REMARKS

Structural DNA nanotechnology is a new architectural system on the nanometer scale that is derived from the central biological molecule, DNA. As recently as a couple years ago, it was possible to describe the highlights of this area in a relatively short review article, [e.g., 67]. Here, we have not described in detail many of the experiments that have contributed to the advances in the field. Prominent among these are Shih *et al.*'s success in making a self-replicable octahedron [68] based on the notion of PX cohesion [69]. Similarly, we have not described the exciting work on algorithmic assembly by Rothemund *et al.*, who have self-assembled a Sierpinski triangle from DX molecules [70]. Extensive work has been done, particularly by Kiehl and his colleagues, on using DNA to scaffold the assembly of metallic nanoparticle species [71,72]. Other work has described metallization as a start on using DNA to organize nanoelectronics [55,73]. A large number of new motifs are being developed for a variety of purposes.

There well may be biological applications to structural DNA nanotechnology, but biology is not the key goal of the effort. Some of the practical aims of the field have been noted earlier, particularly in terms of biological crystallography, nanoelectronics, nanorobotics and new materials. However, the key intellectual goal is to establish the connection between the molecular scale and the macroscopic scale. Being able to design materials by choosing DNA sequences, and then to examine their properties on the macroscopic scale will be a major milestone in our understanding of matter. To this end, there are several efforts currently underway to extend the 2D results noted above to 3D. Crystals of some motifs have been developed that are over a millimeter in length, although they only diffract to about 10 Å resolution at this time. Clearly, a concomitant effort is needed to incorporate nanomechanical devices within such crystals, so that their properties of can be altered in a specific fashion, as a consequence of particular triggers.

In current work we and the other practitioners of structural DNA nanotechnology have used primarily Watson-Crick base pairing. However, many new tertiary interactions for RNA [e.g., 74] and DNA motifs [75] are being discovered. As we learn about the thermodynamics and structural requirements of these interactions, they ultimately will lead to a whole new generation of capabilities for structural nucleic acid nanotechnology. Likewise, as we learn more about the metabolism of DNA, we may discover new motifs that are currently exploited by living systems that can be used in this effort.

What began as the effort of a single laboratory in 1980 has become a field that has recently had the first conference devoted exclusively to its issues. The level of excitement in the field is high, and I hope I have conveyed some of that excitement in this article. Nevertheless, the reader who is stimulated to consider entering the field is urged to learn firsthand from the practitioners of this discipline before embarking on his or her own projects. There are numerous experimental problems and subtleties in the field and the successful and unsuccessful means to solve them are often unpublished. There is little merit in re-inventing the wheel, particularly if it turns out to be a square wheel. Nevertheless, this field, like all fields, will benefit enormously from the fresh insights that new investigators can bring to it. I encourage all who are intrigued to get involved!

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Acknowledgments

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Biography

Nadrian C. Seeman received a BS in biochemistry from the University of Chicago, and his Ph.D. in biological crystallography from the University of Pittsburgh. His postdoctoral training, at Columbia and MIT, emphasized nucleic acid crystallography. He obtained his first independent position at SUNY/Albany, where his frustrations with the macromolecular crystallization experiment led him to the campus pub in the fall of 1980. There, he realized that the similarity between 6-arm DNA branched junctions and the flying fish in the periodic array of Escher's 'Depth' might lead to a rational approach to the organization of matter on the nanometer scale, particularly crystallization. Ever since, he has worked to implement this approach and its spinoffs, such as nanorobotics and the organization of nanoelectronics; since 1988 he has worked at New York University. He has won the Sidhu Award, the Feynman Prize, the Emerging Technologies Award and the Tulip Award in DNA Computing.

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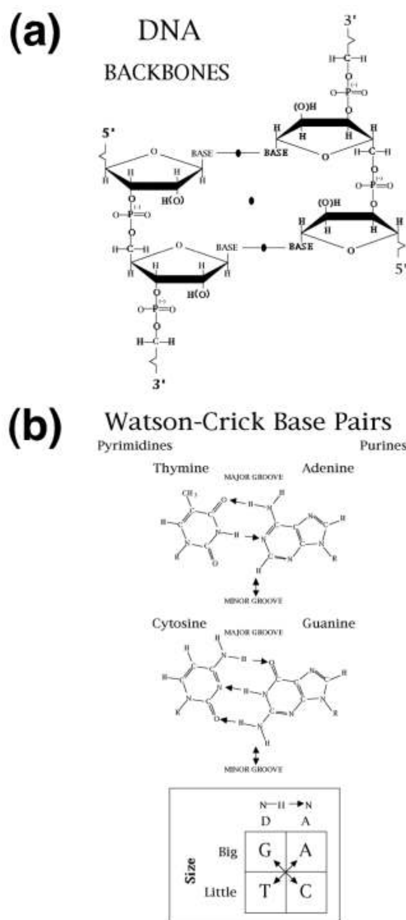

Figure 1. Watson-Crick Base Pairs

The two base pairs, are shown, thymine-adenine (A-T) above cytosine-guanine (C-G). The chemical natures of the bases (pyrimidines and purines) are indicated on the two sides of the drawing. The hydrogen bonds are indicated by arrows from hydrogen atoms to the electronegative recipients of the interactions. The major groove and minor groove regions of the base pairs are indicated. The dyad axis relating the glycosyl (N-R) bonds is indicated by a vertical axis in the minor groove. At the bottom of the drawing is a schematic indicating the two bits of information that determine the base pairing, base size and the direction of the N-H-->N hydrogen bond. The four bases are unique in this regard, with one big donor (G), one big acceptor (A), one little donor (T) and one little acceptor (C). The double headed arrows in the diagram indicate the complementary natures of the Watson-Crick base pairs.

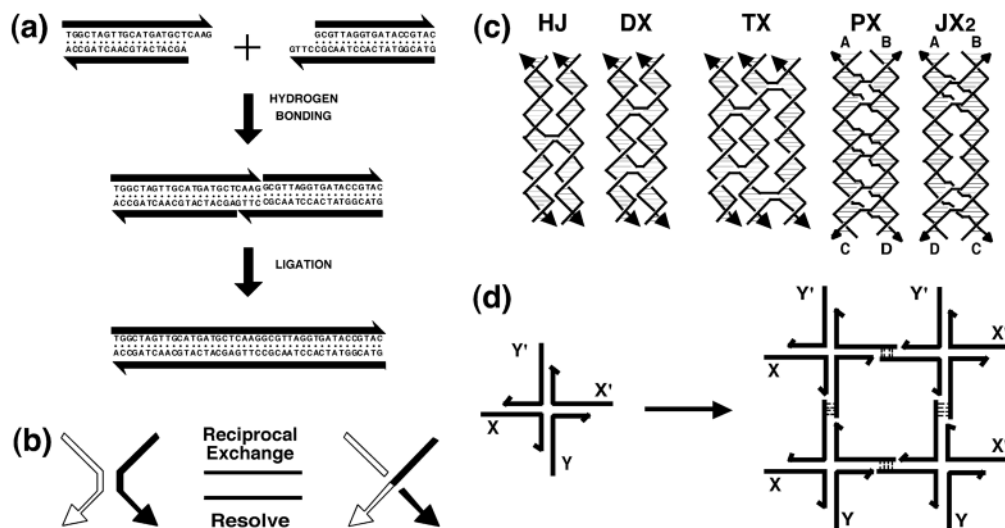

Figure 2. Components of Structural DNA Nanotechnology

(a) Sticky Ended Cohesion. Two linear double helical molecules of DNA are shown at the top of panel *(a)*. The antiparallel backbones are indicated by the black lines terminating in half-arrows. The half-arrows indicate the 5′-->3′ directions of the backbones. The right end of the left molecule and the left end of the right molecule have single-stranded extensions ('sticky ends') that are complementary to each other. The middle portion shows that, under the proper conditions, these bind to each other specifically by hydrogen bonding. The bottom of panel *(a)* shows that they can be ligated to covalence by the proper enzymes and cofactors. *(b) Reciprocal Exchange of DNA Backbones.* Two strands are shown on the left, one filled, and one unfilled. Following reciprocal exchange, one strand is filled-unfilled, and the other strand is unfilled-filled. *(c) Key Motifs in Structural DNA Nanotechnology.* On the left is a Holliday junction (HJ), a 4-arm junction that results from a single reciprocal exchange between double helices. To its right is a double crossover (DX) molecule, resulting from a double exchange. To the right of the DX is a triple crossover (TX) molecule, that results from two successive double reciprocal exchanges. The HJ, the DX and the TX molecules all contain exchanges between strands of opposite polarity. To the right of the TX molecule is a paranemic crossover (PX) molecule, where two double helices exchange strands at every possible point where the helices come into proximity. To the right of the PX molecule is a JX_2 molecule that lacks two of the crossovers of the PX molecule. The exchanges in the PX and JX_2 molecule are between strands of the same polarity. *(d) The Combination of Branched Motifs and Sticky Ends.* At the left is a 4-arm branched junction with sticky ends. On the right four such molecules are combined to produce a quadrilateral. The sticky ends on the outside of the quadrilateral are available so that the structure can be extended to form a 2D lattice.

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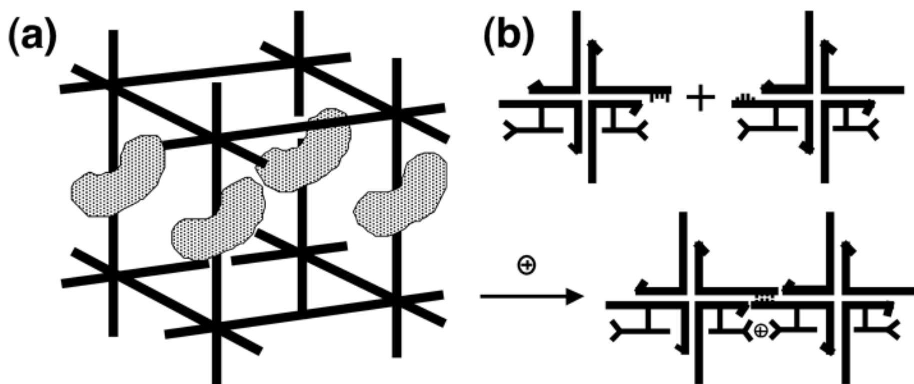

Figure 3. Applications of DNA Periodic Arrays

(a) Biological Macromolecules Organized into a Crystalline Array. A cube-like box motif is shown, with sticky ends protruding from each vertex. Attached to the vertical edges are biological macromolecules that have been aligned to form a crystalline arrangement. The idea is that the boxes are to be organized into a host lattice by sticky ends, thereby arranging the macromolecular guests into a crystalline array, amenable to diffraction analysis. *(b) Nanoelectronic Circuit Components Organized by DNA.* Two DNA branched junctions are shown, with complementary sticky ends. Pendent from the DNA are molecules that can act like molecular wires. The architectural properties of the DNA are seen to organize the wire-like molecules, with the help of a cation, which forms a molecular synapse.

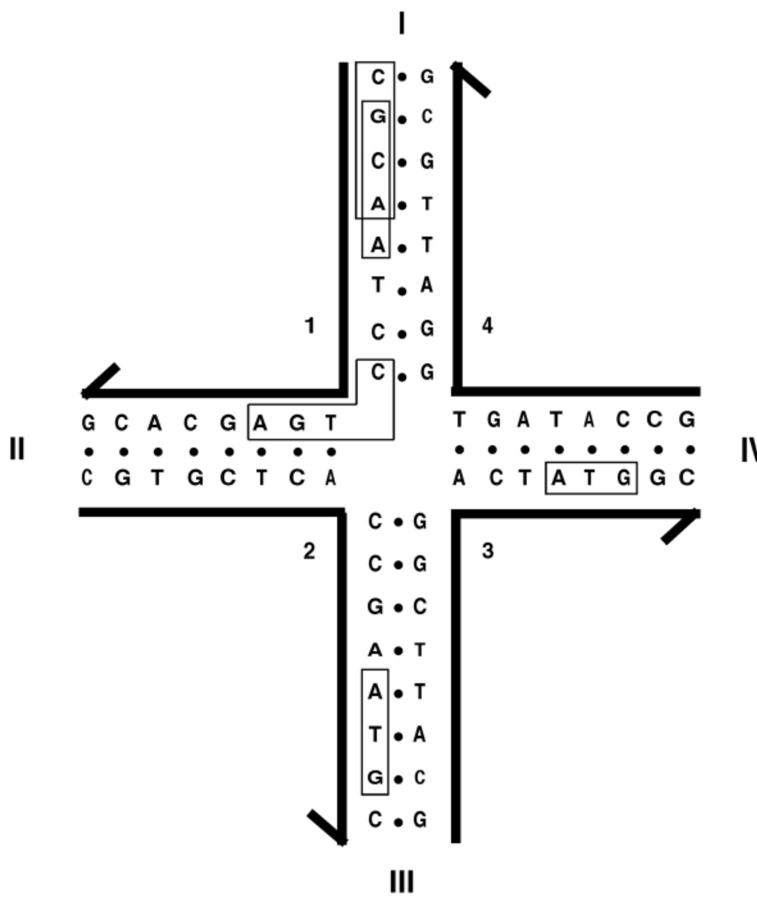

Figure 4. A Stable Branched Junction

There is no dyad symmetry flanking the branch point; tetramers, such as the boxed sequences CGCA and GCAA are unique, and there is no TCAG to complement the CTGA flanking the corner. Thus, competition with the target octamers can come only from the boxed ATG trimers.

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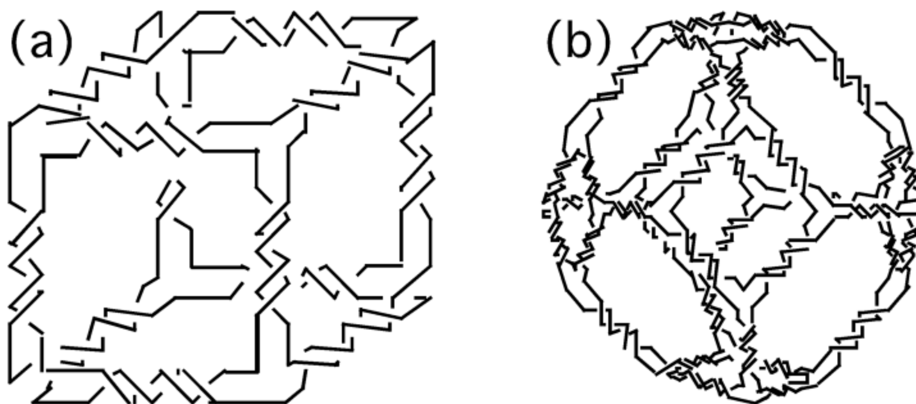

Figure 5. Two Geometrical Constructions Assembled from Branched DNA Molecules

Panel *(a)* shows a DNA molecule whose helix axes are connected like the edges of a cube. The twisting has been confined in the drawing to the central portion of each edge for clarity, but it actually extends to the vertices. Each edge is two turns long, so each face corresponds to a cyclic single strand linked twice to each of its four neighbors, forming a hexacatenane. Panel *(b)* illustrates a molecule with the same design features, but constructed with its helix axes connected like those of a truncated octahedron. It is a 14-catenane, with six strands corresponding to the square faces and eight strands corresponding to the hexagonal faces. Proof of synthesis in both cases consists of digesting the product with various restriction enzymes to produce predicted sub-catenanes that can be produced independently. The topology of each molecule has been demonstrated, but their geometries are unknown.

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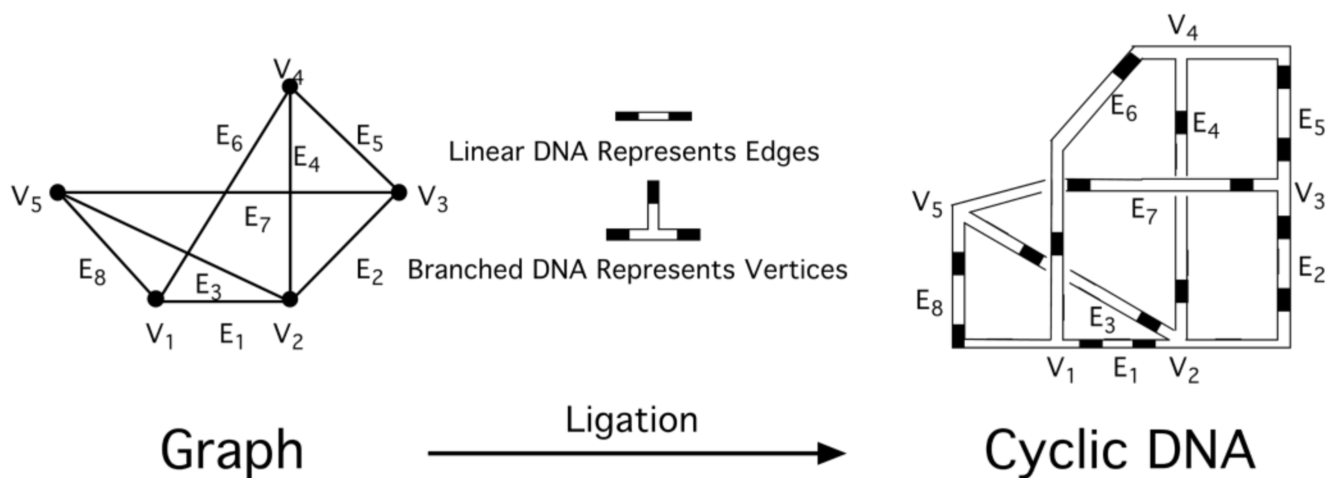

Figure 6. An Irregular Graph Assembled from DNA

The graph is illustrated on the left in a traditional representation. Its vertices and edges are numbered. The edges are made in a DNA construct from double helical DNA and the vertices correspond to the branch points of immobile DNA branched junctions. The dark rectangles at the ends of each object and in each edge of the DNA representation correspond to sticky ends that are used to assemble the cyclic molecule.

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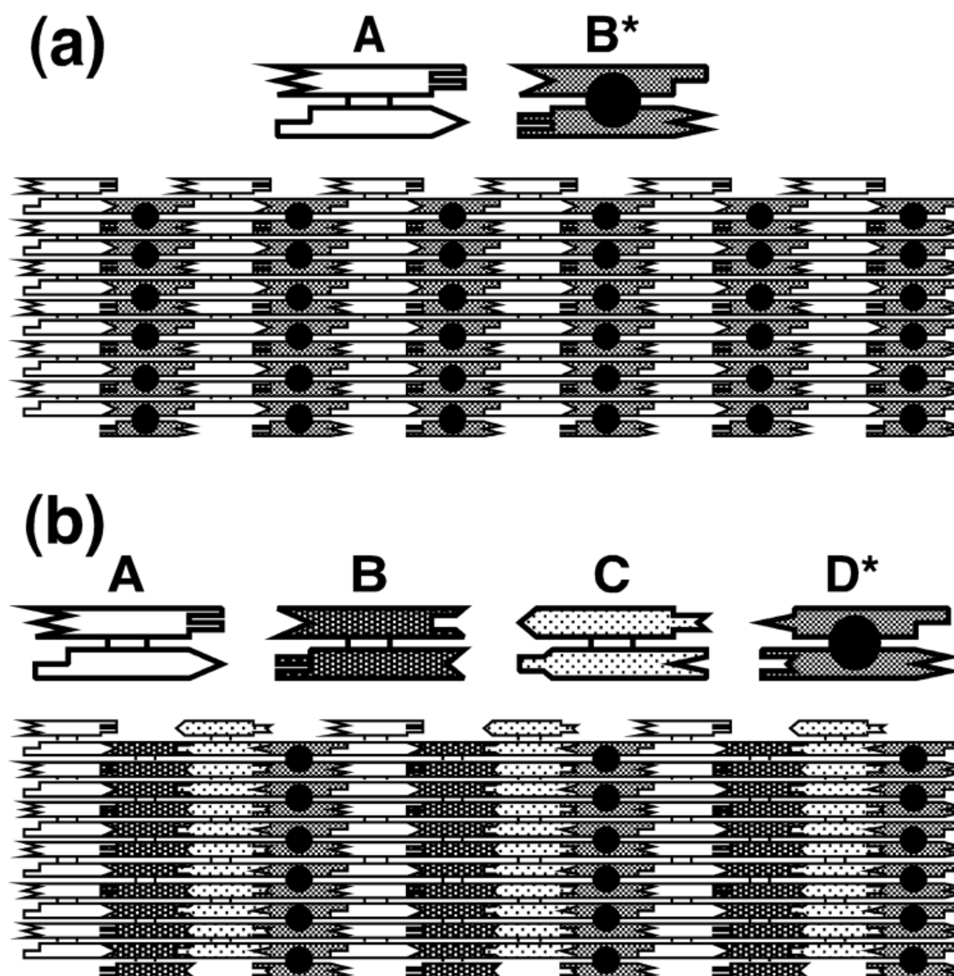

Figure 7. DNA Arrays

(a) Two DX Molecules Tile the Plane. A conventional DX molecule, A, and a DX+J molecule, B*, are seen to tile the plane. The extra domain on B* leads to stripes. The molecules are 4 × 16 nm in this projection, so the stripes are ~32 nm apart. *(b) Four DX Molecules Tile the Plane.* This arrangement is similar to *(a)*, but there is only one DX+J molecule, D*, so the stripes are separated by ~64 nm.

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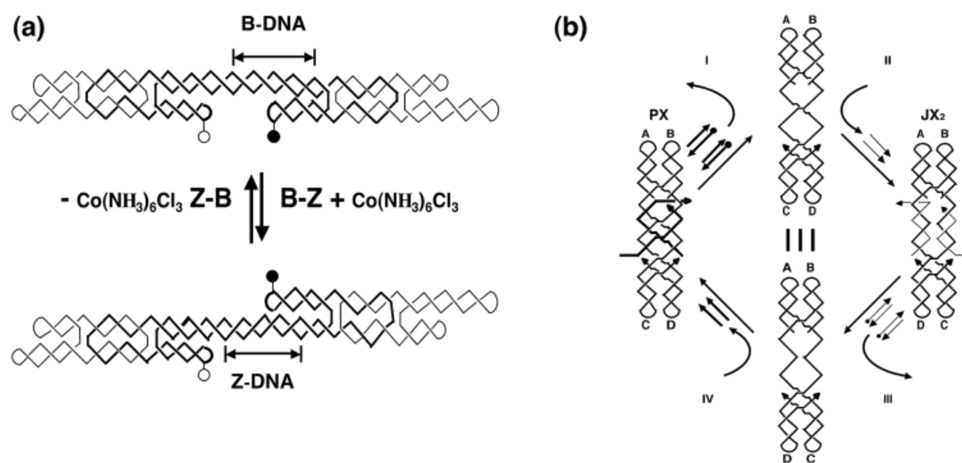

Figure 8. DNA Devices

(a) A DNA Nanomechanical Device Based on the B-Z Transition. The device consists of two DX molecules connected by a shaft containing 20 nucleotide pairs capable of undergoing the B-Z transition. Under B conditions the short domains are on the same side of the shaft, but under Z-conditions (added $Co(NH_3)_6^{3=}$) they are on opposite sides of the shaft. The FRET pair, represented by a filled and unfilled circle, are used to monitor this change.

(b) The Machine Cycle of a PX-JX$_2$ Device. Starting with the PX device on the left, the thick strands are removed by their complements (Process I) to leave an unstructured frame. The addition of the thin strands (process II) converts the frame to the JX_2 structure, in which the top and bottom domains are rotated a half turn relative to their arrangement in the PX conformation. Processes III and IV reverse this process to return to the PX structure.

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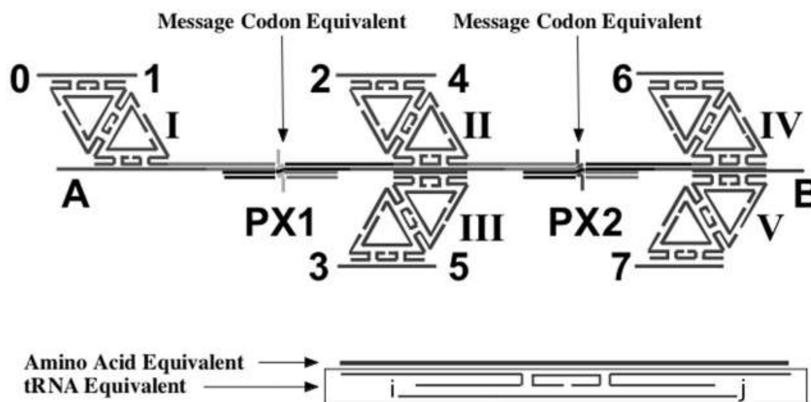

Figure 9. A DNA-Based Translation Device and Its Relationship to Traditional Components
The upper portion of the diagram illustrates the diamond components (indicated by Roman numerals) formed from fused DNA triangles. The sticky ends that flank the gaps in the device are indicated by Arabic numerals. The diamond motifs are connected by two independent PX-JX_2 devices. The control strands that control these devices are indicated as being equivalent to codons on an RNA message. A DX molecule (which will fit via its sticky ends, into one of the gaps between the diamonds) is shown at the bottom. The sticky ends on the DX are indicated by i and j, and they correspond to the anticodon of an aminoacyl tRNA. The top strand of the DX molecule is analogous to the amino acid of traditional protein synthesis.

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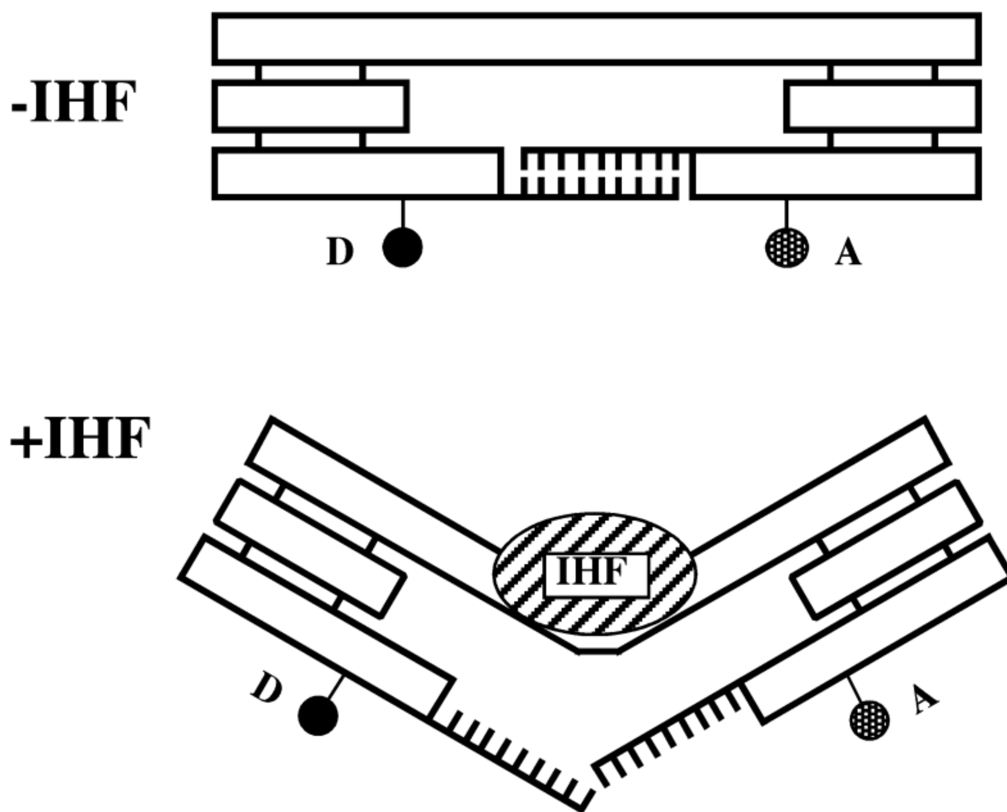

Figure 10. The TX-Based Nanomechanical Measuring Device

Double-helical DNA is shown as rectangular boxes. The triple crossover motifs (TX) are shown as three fused rectangular boxes. The upper domain connects the two TX motifs with the binding site for IHF. IHF is shown as a shaded ellipse, and in the lower panel its binding distorts the connecting shaft. For clarity, the 160° bend is reduced to 45° here. A donor dye (fluorescein) and an acceptor dye (Cy3) are shown as a filled and a stippled circle, respectively. The fluorescence resonance energy transfer reported by this pair will decrease upon IHF binding, as the distance between the dyes increases from DB to DA. The bottom domains are connected by a cohesive tract. IHF must do work to separate the two strands in proportion to the length of the cohesive tract. Increase in the length of the cohesive tract leads to a decreasing ability of IHF to break the base pairs within it.