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Building DNA Nanostructures for Molecular Computation, Templated Assembly, and Biological Applications

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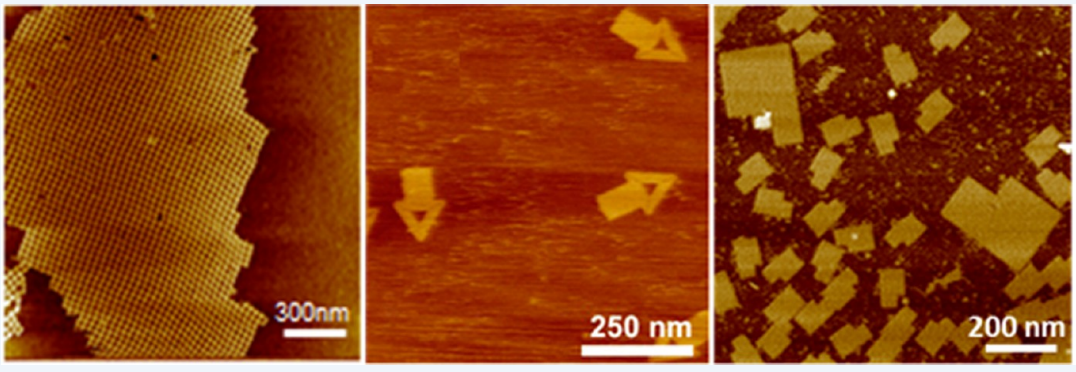

CONSPECTUS: DNA is a critical biomolecule well-known for its roles in biology and genetics. Moreover, its double-helical structure and the Watson−Crick pairing of its bases make DNA structurally predictable. This predictability enables design and synthesis of artificial DNA nanostructures by suitable programming of the base sequences of DNA strands. Since the advent of the field of DNA nanotechnology in 1982, a variety of DNA nanostructures have been designed and used for numerous applications. In this Account, we discuss the progress made by our lab which has contributed toward the overall advancement of the field.

Tile-based DNA nanostructures are an integral part of structural DNA nanotechnology. These structures are formed using several short, chemically synthesized DNA strands by programming their base sequences so that they self-assemble into desired constructs. Design and assembly of several DNA tiles will be discussed in this Account. Tiles include, for example, TX tiles with three parallel, coplanar duplexes, 4 × 4 cross-tiles with four arms, and weave-tiles with weave-like architecture. Another category of tiles we will present involve multiple parallel duplexes that assemble to form closed tubular structures. All of these tile types have been used to form micrometer-scale one- and two-dimensional arrays and lattices. Origami-based structures constitute another category where a long single-stranded DNA scaffold is folded into desired shapes by association with multiple short staple strands. This Account will describe the efforts by our lab in devising new strategies to improve the maximum size of origami structures.

The various DNA nanostructures detailed here have been used in a wide variety of different applications. This Account will discuss the use of DNA tiles for logical computation, encoding information as molecular barcodes, and functionalization for patterning of other nanoscale organic and inorganic materials. Consequently, we have used DNA nanostructures for templating metallic nanowires as well as for programmed assembly of proteins and nanoparticles with controlled spacings. Among other applications, we have used DNA nanotechnology in biosensors that detect target DNA sequences and to affect cell surface receptor clustering for communicating with a cell signaling pathway. We used DNA weave-tiles to control the spacing between thrombin-binding aptamers which resulted in very high antithrombin and anticoagulant activity of the construct. We believe that the tremendous progress in DNA nanotechnology over the past three decades will open even more research avenues in the near future for applications in a wide variety of disciplines including electronics, photonics, biomedical engineering, biosensing, therapeutics, and nucleic-acid-based drug delivery.

INTRODUCTION

Elucidation of the double-helical structure of DNA by James Watson and Francis Crick in 1953 revolutionized the field of modern genetics.[1] Knowledge of DNA's structure subsequently helped researchers ascertain mechanisms behind fundamental cellular functions such as replication, transcription, and translation. The structure showed that the two strands of the double-helix are held together by hydrogen bonding interactions between Watson−Crick base-pairs.[2] These interactions are not only highly specific and predictable but also fairly weak and therefore reversible. Thus, carefully designed nucleotide sequences provide very high levels of programmability as well as structural diversity during the self-assembly of DNA molecules.

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The most commonly occurring form of DNA is called B-DNA, which forms in aqueous solution or in dried fibers in the presence of high humidity.[3] The B-DNA double-helix is periodic and contains 10.5 base-pairs per turn.[4] On average, the rise per base-pair is 3.4 Å and the width of the double-helix is approximately 2.0 nm.[1] A single turn of DNA double-helix can be considered a rigid cylinder of ∼3.6 nm length and ∼2 nm diameter. Apart from Watson−Crick base-pairing and structural properties, DNA has high physiochemical stability and short DNA oligomers can easily be chemically synthesized. These properties make DNA suitable for applications beyond genetics, for example, in nanotechnology.

In 1982, Ned Seeman proposed to use DNA as a building material for construction of nanoscale structures.[5] He hypothesized that nucleic acid sequences could be designed such that the strands would fold into well-defined secondary and tertiary structures. His goal was to assemble DNA into three-dimensional crystalline lattices upon which to scaffold biological macromolecules, nanodevices, or nanoelectronic components.[5] Subsequently, substantial progress has been made in the field of DNA nanotechnology. DNA has been used to construct increasingly complex self-assembled nanostructures in one-, two,- and three-dimensions using various design strategies. Furthermore, these nanostructures have been used for innovative applications in diverse areas of science and technology. In this Account, we will highlight various DNA nanostructures designed and developed in our lab, including DNA tiles, lattices, tubes, and origami-based structures. We will describe the design, assembly, and characterization of these nanostructures. Additionally, we will give an account of the applications of DNA nanotechnology in DNA-based computation, templated assembly of organic and inorganic material, biosensing, and anticoagulation. The progress made by our lab in this field mirrors and complements the overall progress made in the field in the past 32 years. Thus, this Account, while not exhaustive, nonetheless illustrates the development of DNA-based nanotechnology and its potential in future technologies.

DNA TILES AND LATTICES

Seeman's lab designed the double crossover (DX) tile in which the nucleotide sequences of the participating strands were designed such that they formed two parallel DNA duplexes linked by two strand exchange points at which one strand crosses over from each duplex to the other.[6] These strand exchange points, known as "crossovers", are similar to Holliday junctions and have become integral components of structural DNA nanotechnology.

Our lab extended the principle of DNA tiles with parallel duplexes by designing triple crossover (TX) tiles.[7] They consist of four oligonucleotide strands, which assemble to form three coplanar duplexes linked by four crossover points (Figure 1A). The resulting DNA structures were characterized by electrophoresis and thermal melting experiments. Additionally, two-dimensional arrays were constructed by incorporating sticky-ends onto the TX tiles (Figure 1A). Atomic force microscopy (AFM) imaging showed sheetlike structures with dimensions of several micrometers and periodic design suitable for organizing nanomaterials (Figure 1A).

In 2003, we developed a new tile architecture (known as the 4 × 4 or cross-tile) designed to allow growth of lattice with more equal or square aspect ratio.[8] The 4 × 4 cross-tile consists of nine strands and four arms, with a crossover junction in each arm (Figure 1B). Characterization of the annealed structure by electrophoresis and thermal transition analysis confirmed formation of tiles. Sticky-ends were introduced to facilitate formation of two-dimensional lattices (Figure 1B). However, in the original design, adjacent tiles had the same orientation within the lattice, and ribbon-like structures (rather than the expected 2D sheets) were observed under AFM (Figure 2A). These ribbons were due to inherent curvature in the 4 × 4 tiles which led to the formation of tube-like structures that subsequently flattened and formed fixed-width ribbons on the mica substrate. In order to rectify this problem, a second design was developed in which the adjacent tiles faced toward opposite lattice faces (Figure 1B). This corrugated design strategy canceled out the inherent curvature of the tile and produced two-dimensional square lattices (Figure 1B).[8] In addition to the two-dimensional lattices, 4 × 4 tiles were also used to form one-dimensional arrays which were called nanotracks.[9] Subsequent to this work, Mao and co-workers also designed three-, four-, and six-armed tiles with symmetric arms which formed two-dimensional lattices.[10−12]

The corrugated two-dimensional lattices formed from 4 × 4 tiles were periodic and nondeterministic in size. We also developed finite-sized and fully addressable two-dimensional arrays with 4 × 4 tile using hierarchical self-assembly procedures.[13] Two extremes in hierarchical assembly strategies were explored, namely minimization of the number of unique molecular address labels (DNA sticky-end sequences) required for encoding tile associations, versus minimization of the depth (number of sequential steps) of the assembly process. Higher production yields of defect-free assemblies were achieved by procedures that minimized the assembly depth (and maximize diversity of address labels). It was further shown that control over length and directionality of superstructures is possible via stepwise assembly processes by incorporation of dsDNA bridges between 4 × 4 tile arrays.[14]

The 4 × 4 tile was further modified to create a new tile, called the double-decker which was composed of two 4 × 4 tiles, lying one on top of the other and linked by two crossovers in each arm (Figure 1C).[15] Thus, each of the four symmetric arms of the double-decker tile consisted of four duplexes. The double-decker tile was also assembled into two-dimensional square lattices using a corrugation strategy (Figure 1C). The lattices, thus formed, were of micrometer-scale (Figure 1C). Fluorescence microscopy revealed the size of some of the lattices to be at least tens of micrometers on each edge.[15] By suitable programming of the sticky-ends, the double-decker tile has the potential for assembly into three-dimensional crystals[15] as was demonstrated by Seeman and co-workers in a different system involving triangular tiles.[16]

Our lab also developed a novel tile design strategy which lacked rigid crossover motifs, thus making it distinct from all previous tiles. In this strategy, the sequences were designed such that they weaved back and forth, thereby forming a weave-like tile with rigid double-helical domains connected to neighboring domains by single-stranded -TTTT- loops (Figure 1D).[17] The resulting tile was called DNA weave-tile, and it was considerably more flexible than crossover containing tiles. Several weave-tiles were designed with varying numbers of double-helices.[17,18] These tiles were also assembled into two-dimensional lattices via sticky-end associations (Figure 1D). Not surprisingly, owing to the flexibility of individual tiles, the resulting lattices were not as large as those observed with crossover-containing tiles (Figure 1D).

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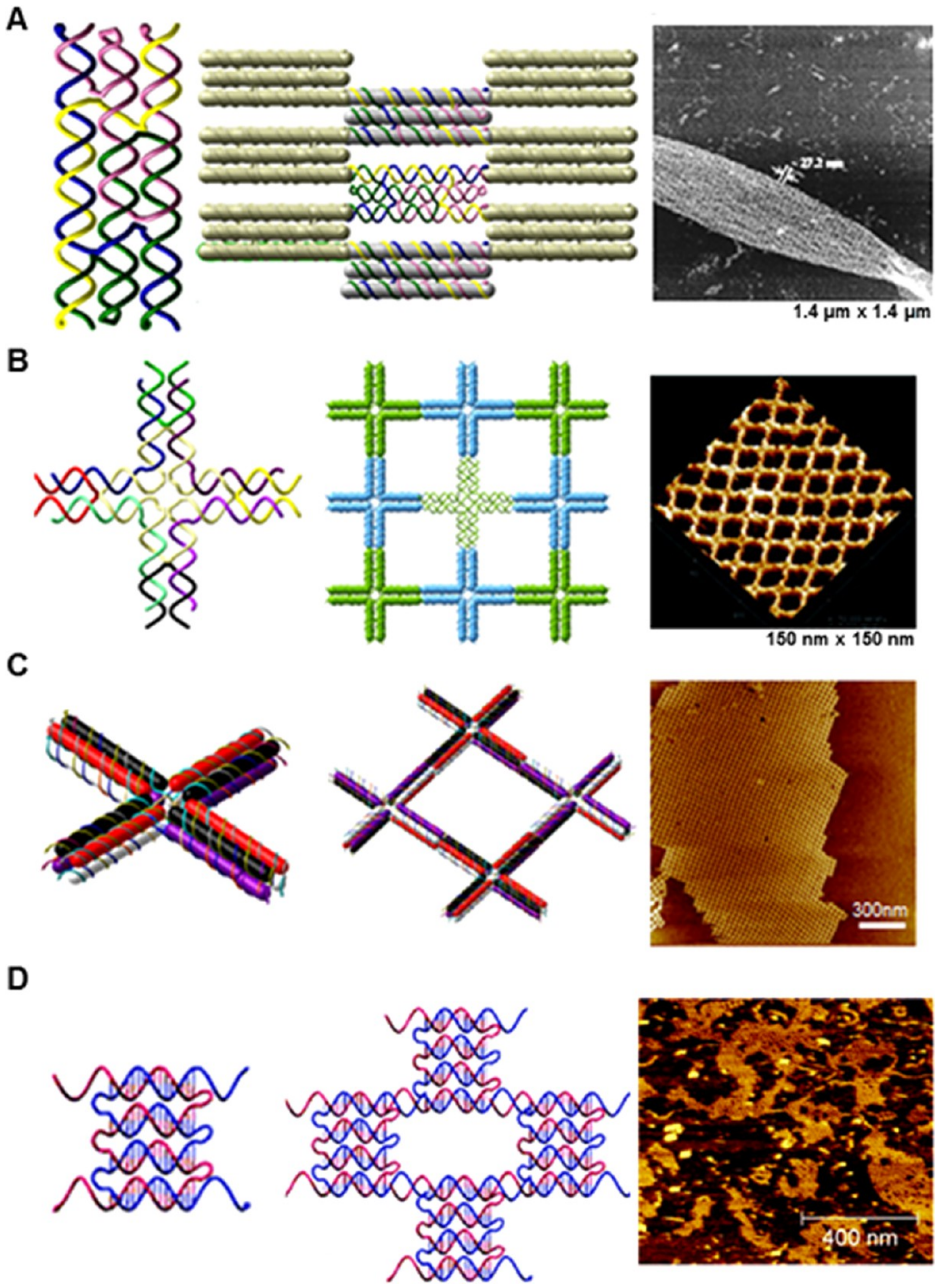

Figure 1. DNA tiles. (A) A triple-crossover (TX) tile (left). Two sets of TX tiles equipped with appropriate sticky-ends will form two-dimensional arrays (center). AFM image of the array (right). Reproduced with permission from ref 7. Copyright 2000 American Chemical Society. (B) A 4 × 4 tile with sticky-ends (left). Two-dimensional 4 × 4 lattice using corrugation strategy (center). AFM image of the 4 × 4 lattice (right). From ref 8. Reprinted with permission from AAAS. (C) A double-decker tile with sticky-ends (left). Two-dimensional double-decker lattice using corrugation strategy (center). AFM image of the double-decker lattice (right). Reproduced with permission from ref 15. Copyright 2011 American Chemical Society. (D) A four-helix weave-tile (left). Two-dimensional array using weave-tile (center). AFM image of the weave-tile array (right). Reproduced with permission from ref 17. Copyright 2010 American Chemical Society.

■ DNA TUBES

As mentioned above, inherent curvature of 4 × 4 tiles resulted in formation of nanotubes when these tiles were equipped with sticky-ends in uncorrugated fashion.[8] These nanotubes displayed a range of diameters and appeared to be quite flexible (Figure 2A). Subsequently, we devised a new strategy to introduce controlled curvature in a two-dimensional array of TAO tiles (a variant of TX tiles), resulting in the formation of fixed-sized nanotubes. In this case, two types of TAO tile (A tile and B tile) were used with a lattice design containing alternating layers of A and B tiles.[19] The B tile further had a protruding double-stranded DNA stem on its central helix that bore a thiol modification. The formation of disulfide linkages between adjacent B tiles caused the otherwise planar lattice to curve and form tubes (Figure 2B). Characterization by transmission electron microscopy (TEM; Figure 2B) and AFM confirmed the formation of nanotubes with uniform width of ∼25 nm and length up to about 20 μm. Subsequently, Rothemund et al. showed the formation of DNA nanotubes using DX tiles in a similar fashion.[20]

These nanotubes also displayed a propensity to collapse upon being deposited onto surfaces prior to imaging.[8,19,20]

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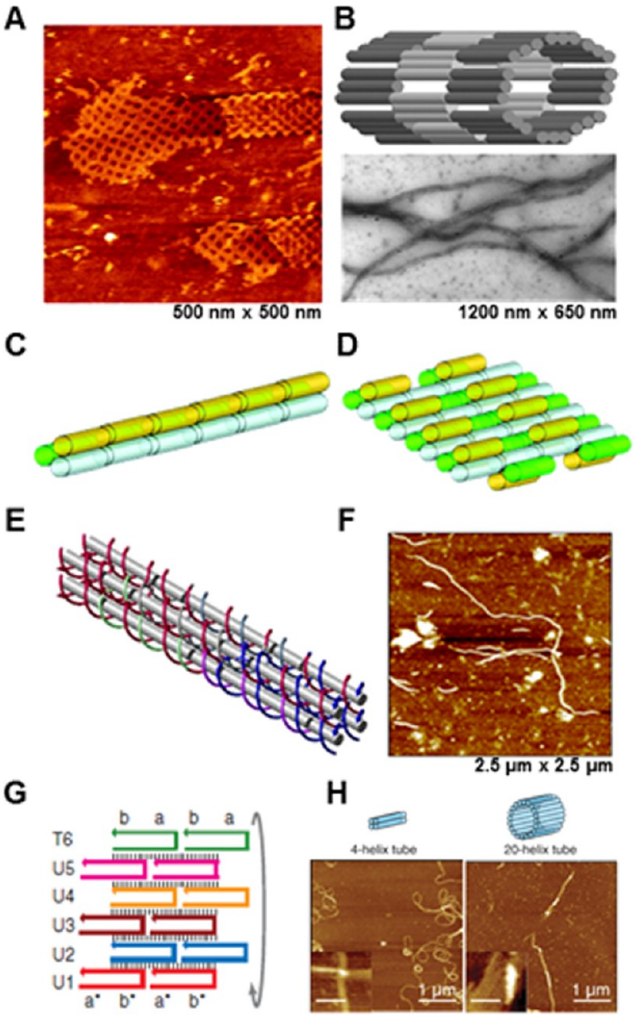

Figure 2. DNA tubes. (A) AFM image of the flattened end of a nanoribbon formed by collapse of 4 × 4 nanotube on mica surface. From ref 8. Reprinted with permission from AAAS. (B) TAO nanotube (top). TEM image of the TAO nanotubes (bottom). Reproduced with permission from ref 19. Copyright 2004 PNAS. (C) One-dimensional array of 3HB. (D) Two-dimensional array of 3HB. (C, D) Reproduced with permission from ref 21. Copyright 2005 American Chemical Society. (E) A four-helix bundle (4HB). (F) AFM image of one-dimensional filaments of 4HB. (E, F) Reproduced by permission of IOP Publishing from ref 22. (G) Single-stranded tile (SST) strategy to form tubes of programmed circumference. (H) AFM image of 4-helix and 20-helix tubes from SST assembly. (G, H) From ref 25. Adapted with permission from AAAS.

Subsequently, we employed a new strategy in which more rigid subunits were used to form tubes. These were three-helix bundles (3HB) and four-helix bundles (4HB).[21,22] Seeman and co-workers also used similar strategies to form six- and eight-helix bundles.[23,24] The 3HB contained three axially parallel helical domains connected by crossover junctions. The helical axes in case of 3HB were not coplanar but instead had one helix lying in the groove formed by the other two, thus resulting in a triangular cross-section.[21] By suitable programming of sticky-ends, one- and two-dimensional arrays were formed using 3HB; these were characterized by AFM (Figure 2C and D).

The 4HB tiles consisted of four axially parallel DNA helices with one helix connected to two adjacent helices via multiple crossovers such that the resulting interior angle was approximately 90° (Figure 2E).[22] Equipping 4HB with suitable sticky-ends resulted in formation of largely unbent linear filaments which were several micrometers in length (Figure 2F).[22] However, when we attempted to form two-dimensional sheet-like arrays of the 4HB, ring-like structures were observed instead.[22] The exact reason behind this ring formation is not yet fully understood. The most likely explanation is that the helices are overwound due to introduction of a twist of 10.67 base-pairs/turn in the design instead of the more natural twist of 10.5 base-pairs/turn.

3HB and 4HB were designed using a multistranded tile strategy where each bundle tile was constituted of multiple strands and where adjacent helices were connected via rigid crossover junctions. In another strategy, known as a single-stranded tile (SST) strategy, a subunit in an array consists of only one DNA strand.[25] These single strands cross over from one helix to the adjacent helix resulting in the formation of single-stranded crossovers sometimes also known as half-crossovers (Figure 2G). This strategy was used to assemble nanoribbons and nanotubes with programmable width and circumference, respectively.[25] The formation of four, five, six, seven, eight, ten and 20 helix tubes was demonstrated using this strategy (Figure 2H).

DNA ORIGAMI: PUSHING THE SIZE LIMIT

The tiles and lattices described above were all made using multiple, short, chemically synthesized oligonucleotide strands. Lattices were formed by suitably appending individual tiles with appropriate sticky-ends. In 1999, however, it was demonstrated by us that multiple TX tiles could be assembled into super-structures without sticky-ends by employing a long single-stranded molecule known as a scaffold strand.[26] Subsequently, in 2003, we further hypothesized that a two-dimensional DNA nanostructure could be formed by folding the scaffold strand with the help of multiple short DNA strands (Figure 3A).[27] Each short strand in this structure would remain individually addressable by virtue of their specific ordering along the large scaffold strand. In 2006, Rothemund greatly improved upon this concept and introduced what is now known as DNA origami.[28] In this strategy, a long single-stranded DNA scaffold (typically biosynthesized) is folded into a desired shape using many short staple strands. Very high structural complexity as well as surprisingly high assembly yields were achieved using the origami-based approach. Moreover, the origami structures contained much greater numbers of addressable pixels (binding sites) than previous nonperiodic, tile-based structures. However, the size of origami structures is limited by the length of the scaffold strand. Rothemund and several researchers used the single-stranded DNA genome of a bacteriophage, M13mp18, which is 7249 bases long. It was, therefore, desirable to explore other designs and sources for the scaffold strand.

An associated hurdle while pushing the size limit of DNA origami is the chemical synthesis of staple strands. In a typical M13mp18-based origami structure, approximately 225 unique staple strands are used. If the size of the origami were to be increased, procurement of staple strands from commercial sources could become cost prohibitive. Consequently, we assisted in the development a novel strategy of concurrent production of entire set of staple strands by a unique, nicking strand-displacement amplification (nSDA) involving reusable surface-bound template strands that were synthesized in situ using a custom piezoelectric inkjet system (Figure 3B).[29] These staple strands were then used to fold both strands of double-stranded M13-bacteriophage genomic DNA in a single-pot

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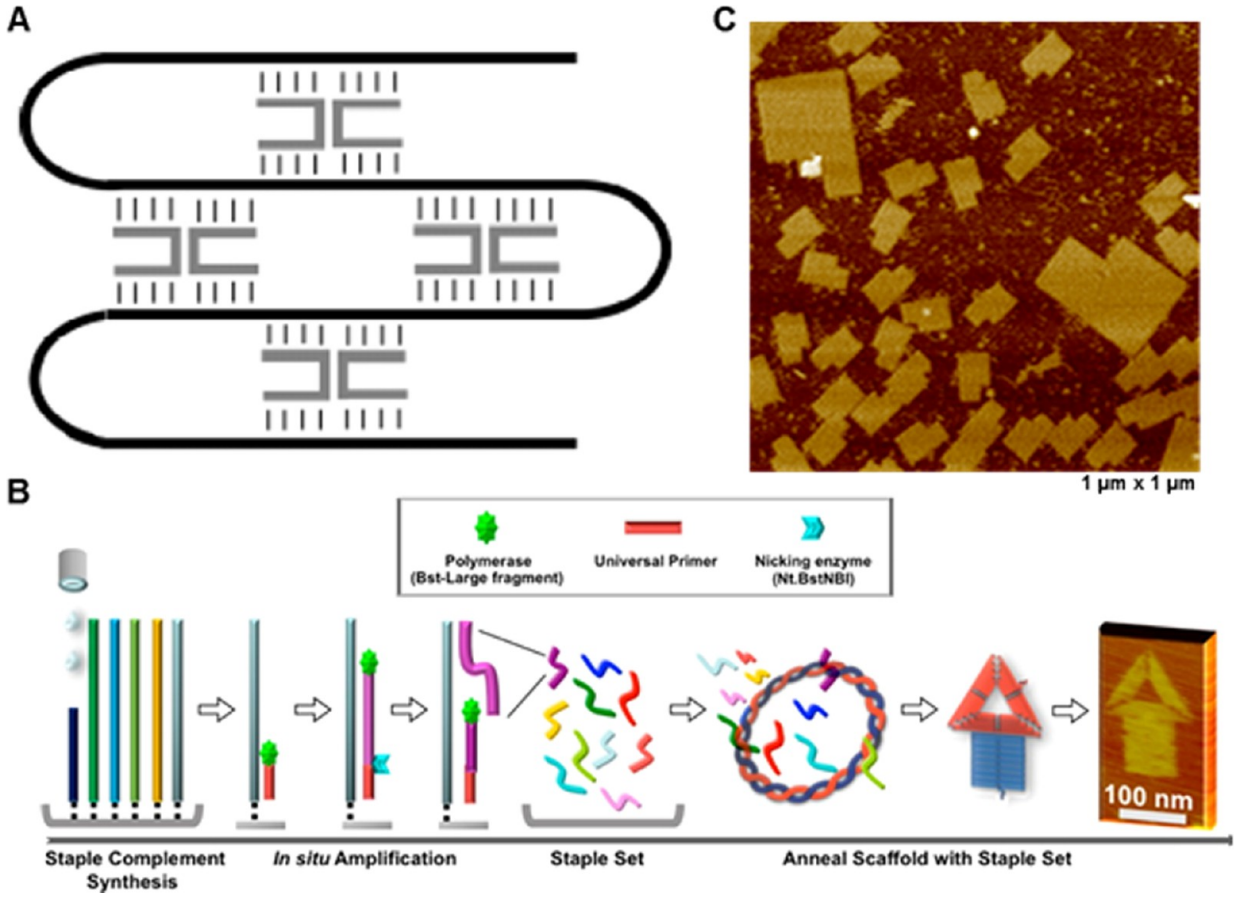

Figure 3. DNA origami. (A) Schematic for folding a long single-stranded scaffold strand with the help of several short strands as proposed in 2003. Reproduced with permission from ref 27. Copyright 2003 World Scientific Publishing. (B) Concurrent production of entire sets of staple strands by strand-displacement amplification involving reusable surface-bound template strands that were synthesized in situ using a custom piezoelectric inkjet system. Staple strands were then used to fold both strands of double-stranded M13 genome into distinct shapes which then formed nanoscale house-like heterodimer. Reproduced with permission from ref 29. Copyright 2013 American Chemical Society. (C) Notched-rectangle shaped large origami constructed using 51 466 base long scaffold from a hybrid phage. Small notched-rectangle origami was synthesized using regular 7249 base long M13mp18 genome. Adapted from ref 31.

reaction into two distinct shapes (a triangle and a rectangle) which then heterodimerized to form a nanoscale houselike structure (Figure 3B). The combination of chip-based staple strand production, double-sized scaffold, and high-yield one-pot assembly substantially increased the useful scale of DNA origami.[29] Earlier, Shih and co-workers had also demonstrated the folding of double-stranded scaffold to form two distinct origami structures.[30]

In order to further push the limit on maximum origami size, we developed a method for producing a large single-stranded DNA scaffold strand by cloning parts of M13 phage, including its origin of replication and ssDNA packaging site, into λ phage.[31] This hybrid phage was propagated as a dsDNA λ phage for several generations and was then switched to M13 growth in order to produce ssDNA genome for purification. The length of the hybrid phage genome was 51 466 bases. This large scaffold strand was folded to form a notched-rectangle origami (Figure 3C) using over 1600 staple strands, each around 32 bases in length. The staple strands were synthesized using the chip-based approach described above.[29] The conventional 7249 base long single-strand scaffold was also folded to form a notched-rectangle origami to provide direct size comparison (Figure 3C).[31] The larger origami provides just over 7 times more surface area than the smaller version. This origami structure, to the best of our knowledge, is the largest origami structure yet produced and in very high assembly yield as well.

■ DNA-BASED COMPUTATION

DNA nanotechnology has a variety of very interesting applications in DNA-based computation. One of the earliest examples of a complex design for an algorithmic assembly was described in 1999 when we presented a tiling for solving Satisfiability Problems using two-dimensional DNA arrays.[32] The proposed design demonstrated a method for assigning a value to each variable and then checking whether the assigned values satisfied all the clauses in the formula. The checking procedure was performed during DNA lattice formation by algorithmic assembly according to rules specified by the tiles' sticky-ends. This procedure is illustrated in Figure 4A, where a successful assembly computation (depicted by the "success" tile shown at the top of the assembly) is performed starting with the bottom layer of input tiles (violet).[32]

This work was followed by experimental demonstration of a different logical computation using algorithmic self-assembly of TX tiles.[33] A one-dimensional algorithmic self-assembly of TX tiles was used to execute four steps of cumulative XOR operations on a string of binary bits. Sticky-ends on the TX tiles contained information to encode the bit values and to direct

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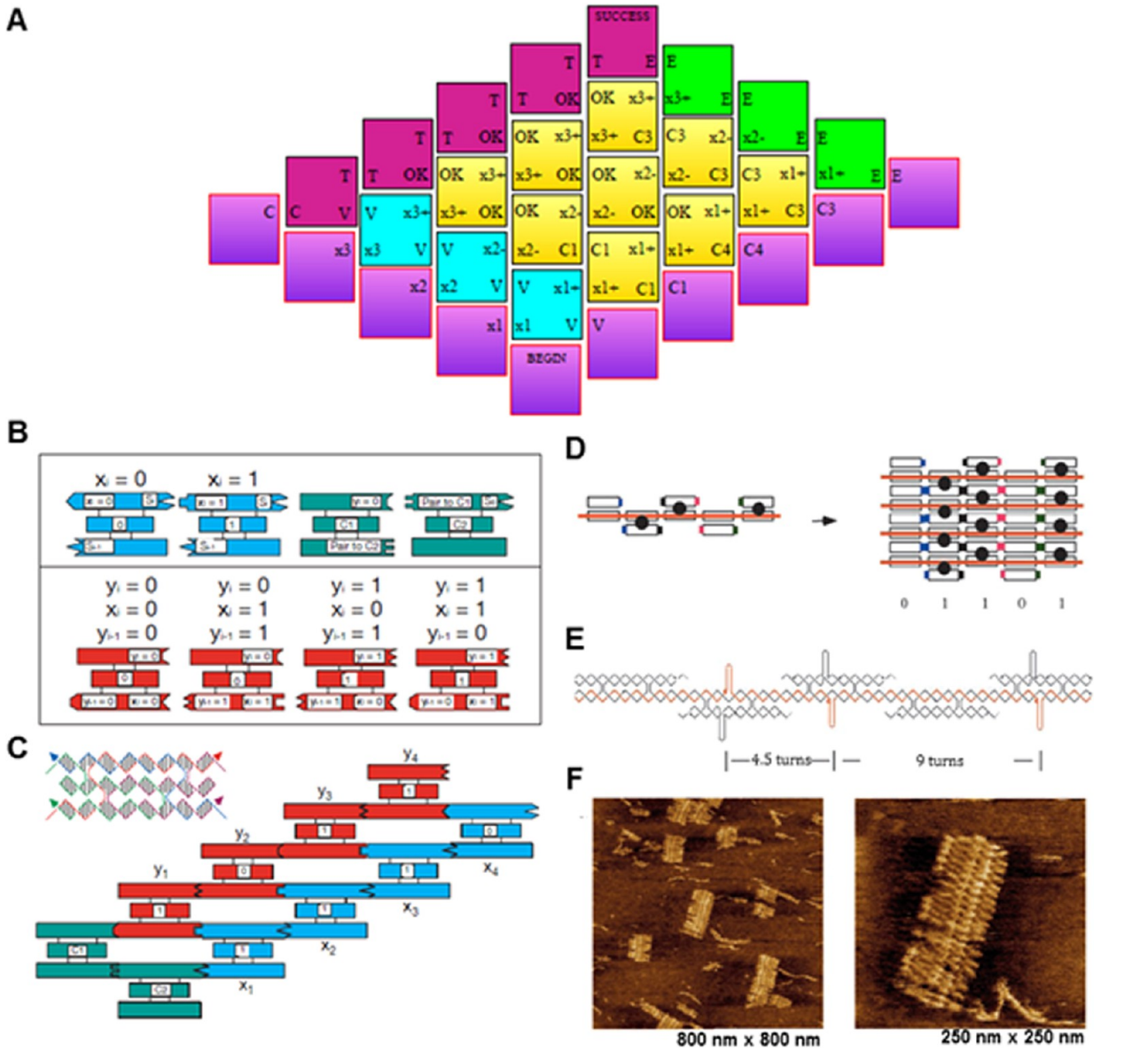

Figure 4. DNA-based computation. (A) A successful assembly computation for solution to a satisfiability problem using input tiles (violet). Reproduced with permission from ref 32. Copyright 1999 American Mathematical Society. (B) Component tiles for cumulative XOR computation. (C) Cumulative XOR operation using a particular set of input *x* tiles. TX tile used for the computation (top-left). (B, C) Reproduced with permission from ref 33. Copyright 2000 Nature Publishing Group. (D) Conversion of one-dimensional information into two-dimensional readout for barcode lattice. (E) Strand trace of the DX tiles for barcode lattice with protruding hairpin loop structures (loops represent 1's in the encoded binary number). (F) AFM images of barcode lattices. (D–F) Reproduced with permission from ref 35. Copyright 2003 PNAS.

lattice assembly. Figure 4B illustrates the component tiles, and an example four-bit cumulative XOR computation is given in Figure 4C for a specific set of inputs. Read-out of this computation was done using gel electrophoresis.[33] In a subsequent study, pairwise-XOR operations were performed on a large number of distinct inputs using DX string tile assembly.[34] Computational output was generated in the form of a reporter strand, which was purified using gel electrophoresis and then sequenced for the readout.

We also reported, prior to the advent of DNA origami, the construction of an aperiodic patterned barcode lattice by directed nucleation of DX tiles around DNA scaffold strands.[35] The input scaffold strand provided each layer of the DX lattice with a bit-string represented by the presence or absence of DNA hairpins protruding out of the lattice plane. Self-assembly of multiple DNA tiles around the scaffold resulted in patterned lattices displaying the desired barcode information (Figure 4D and E) which were characterized by AFM (Figure 4F). These results represented a system capable of converting information encoded on a one-dimensional DNA strand into a two-dimensional visual readout.[35] Another interesting application of DNA-based computation is in molecular cryptography systems based on the one-time pad strategy.[36] This method could be applied for the encryption of either biological DNA or binary data encoded in synthetic DNA.

TEMPLATED ASSEMBLY OF ORGANIC MATERIAL ON DNA NANOSTRUCTURES

Self-assembled DNA nanostructures have also been used as templates for creating programmable and periodic assembly of

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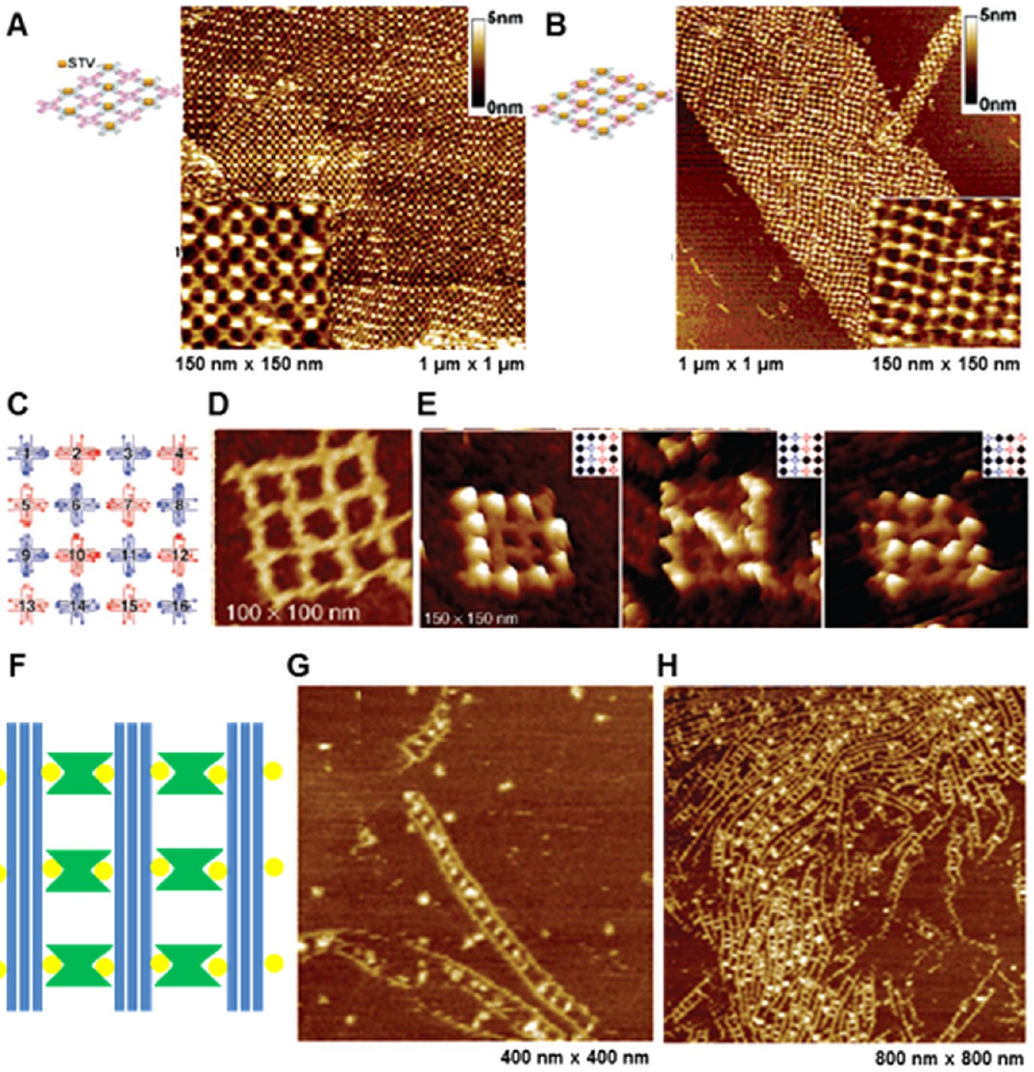

Figure 5. DNA-templated assembly of organic material. (A) AFM image of the 4 × 4 lattice with biotin/streptavidin modifications on alternate tiles. (B) All the tiles in the 4 × 4 lattice are biotin/streptavidin-modified. (A, B) Reproduced with permission from ref 9. Copyright 2005 American Chemical Society. (C) Hierarchical assembly of 4 × 4 tiles into finite-sized and fully-addressable array. (D) AFM image of the same. (E) Selective modification of tiles with biotin gives desired aperiodic pattern with streptavidin molecules. (C–E) Reproduced with permission from ref 13. Copyright 2006 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim. (F) Linear TX arrays functionalized on both edges with aptamers form multiple-ladder-like structure when aptamer-binding antibody is added. (G) Ladderlike structures formed from TX DNA tile rails and dimeric antibody protein rungs. (H) Larger scale DNA–protein lattices showing some multiple parallel organization. (G, H) Reproduced from ref 38 with permission from The Royal Society of Chemistry.

other molecules and nanostructures. Implementing existing strategies of in vitro DNA–protein conjugation on DNA structures provides opportunities for several applications involving controlled protein assemblies. The most commonly used strategy for DNA–protein conjugation is functionalizing DNA with biotin for binding to streptavidin. In one of the earliest demonstrations of this strategy, the 4 × 4 cross-tile was modified with biotin at the tile center.[8] After streptavidin was added and bound, well-formed, two-dimensional periodic streptavidin arrays were observed by AFM. Subsequently, streptavidin was assembled on linear TX tile arrays via biotin along one or both the edges of the array.[37] Streptavidin was also templated onto DNA nanostructures with more precisely controlled spacing.[9] In this case, nanotracks and 4 × 4 lattices were formed using two different tile types, and based on biotin modification of either one or both of the tiles, arrays of proteins with desired spacings were produced on DNA lattice (Figure 5A and B).[9]

The above examples of streptavidin binding to DNA lattices resulted in periodic protein arrays due to the lack of addressability of individual tiles in the lattice. As described earlier, the hierarchical assembly of finite-sized 4 × 4 tile array consisted of 16 distinct 4 × 4 tiles (Figure 5C and D) and each tile was uniquely addressable.[13] This array was, thus, exploited to introduce selective biotin modifications so that aperiodic patterns of streptavidin could be obtained, as shown in Figure 5E.[13]

Apart from biotin–streptavidin conjugation, we have also employed aptamer–antibody interactions to form protein assemblies on DNA nanostructures.[38] One-dimensional TX tile arrays were decorated with aptamer, and single-chain antibodies were selected from a phage display library for binding to the aptamer. When added to the aptamer-decorated TX tile arrays, these antibodies formed ladder-like structures with dimeric antibody rungs and DNA tile rails (Figure 5F–H).[38] We have also covalently conjugated a peptide molecule to an oligonucleotide.[39]

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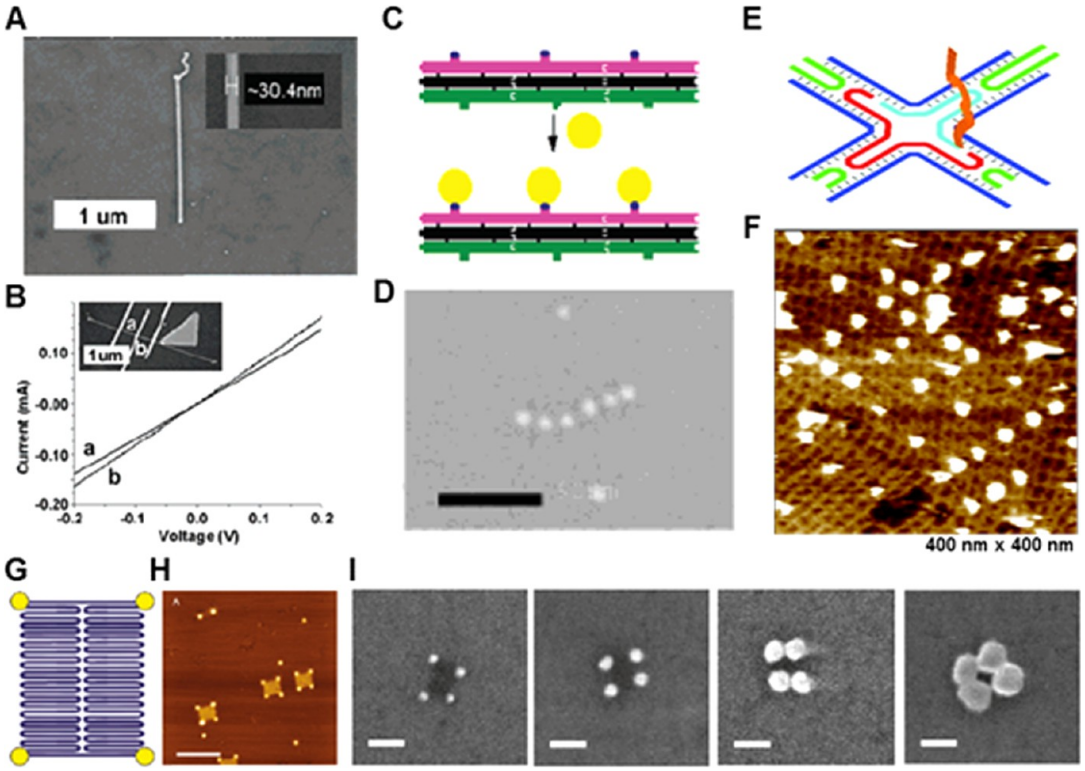

Figure 6. DNA-templated assembly of inorganic material. (A) One-dimensional array of 3HB tiles metallized with silver showing largely uniform thickness. (B) $I-V$ curve of the same over two regions illustrates ohmic behavior. (A, B) Reproduced with permission from ref 21. Copyright 2005 American Chemical Society. (C) Biotin-modified linear TX array templates streptavidin–gold nanoparticle conjugates. (D) SEM image of the same. Scale bar: 50 nm. (C, D) Reproduced with permission from ref 37. Copyright 2004 American Chemical Society. (E) 4 × 4 tile conjugated with gold-binding peptide. (F) AFM image of gold nanoparticles templated on 4x4 lattice using gold-binding peptides. (E, F) Adapted with permission from ref 39. Copyright 2011 American Chemical Society. (G) DNA origami with gold nanoparticles bound at the four corners. (H) AFM image of the same. Scale bar: 250 nm. (I) Controlled growth of silver onto gold nanoparticles bound to the origami. Metallization times (from left to right): 4, 9, 15, and 20 min. All scale bars: 100 nm. (G–I) Reproduced with permission from ref 41. Copyright 2011 American Chemical Society.

The 5′ end of an oligonucleotide from the 4 × 4 tile was labeled with an amino-terminated phosphoramidite, the amine functional group was then used for coupling to the peptide's C-terminus. This peptide–DNA conjugate was then integrated into the 4 × 4 lattice, thereby forming periodic arrays of peptide molecules.

TEMPLATED ASSEMBLY OF INORGANIC MATERIAL ON DNA NANOSTRUCTURES

Of course, DNA nanostructures can also be used for templating inorganic materials. Double-stranded DNA has been used as a template for deposition of various metals. For example, we have used 4 × 4 nanoribbon assemblies for templating highly conductive silver nanowires.[8] The resulting nanowires were characterized by AFM and scanning electron microscopy (SEM). The metallized nanoribbons were uniform in height and width and up to 5 μm long. After patterning metal leads to the wires by electron beam lithography, a current–voltage ($I-V$) measurement was conducted at room temperature. The resulting $I-V$ curve was found to be linear, demonstrating ohmic behavior.[8] One-dimensional array using 3HB was also metallized to construct silver nanowires. These nanowires were approximately 30 nm in width (Figure 6A), and their $I-V$ curve exhibited mostly linear behavior (Figure 6B).[21] Silver metallization of TAO nanotubes also exhibited similar behavior.[19] We subsequently metallized standard dsDNA with silver resulting in ultrathin nanowires with diameters of around 15 nm.[40]

Metal nanoparticles have also been templated on DNA nanostructures in a programmed manner. We have employed several strategies for the templated assembly of nanoparticles. In the first approach, linear TX tile arrays were periodically labeled with biotin.[37] Streptavidin-conjugated 5 nm gold nanoparticles were then precisely positioned on the self-assembled linear array (Figure 6C). Figure 6D shows an SEM image of one such linear array of streptavidin-gold nanoparticles.[37] In the second approach, gold-binding peptide molecules were covalently conjugated with the 4 × 4 tile, as mentioned above (Figure 6E).[39] Subsequent addition of 5 nm gold nanoparticles resulted in specific binding of gold nanoparticles to the peptides at desired locations (Figure 6F).[39]

In the third approach, DNA origami templates were modified to display DNA binding sites with a uniquely coded sequence.[41] Gold nanoparticles of 5 nm were functionalized with the complementary DNA strand and were then bound to the origami using DNA–DNA hybridization (Figure 6G and H).[41] The origami structures were then adsorbed onto silicon dioxide substrates. The seed nanoparticles were later enlarged, and even fused, by electroless deposition of silver and characterized using SEM (Figure 6I).[41] Using this method, several different metallic structures were fabricated.

BIOLOGICAL APPLICATIONS OF DNA NANOSTRUCTURES

We exploited strand-displacement properties of DNA to design a system based on hybridization chain reaction (HCR)[42] for

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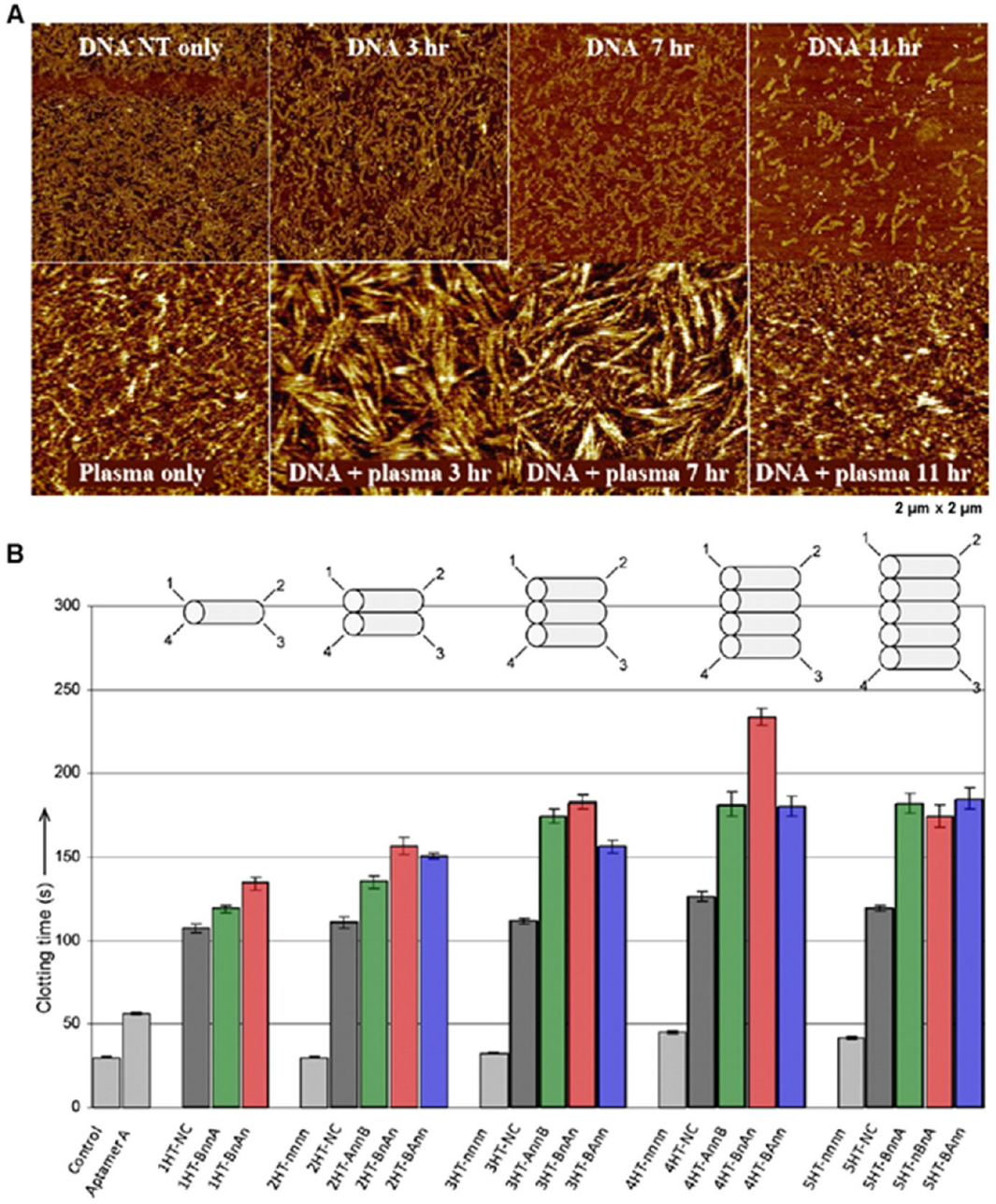

Figure 7. DNA nanostructures in blood plasma. (A) AFM images of DNA nanotrack taken after incubations at 37°C without (top) and with (bottom) plasma. Reproduced from ref 45. (B) Anticoagulant activity, expressed as clotting time of blood plasma, of various weave-tile constructs displaying the two thrombin binding aptamers, A and B, in various configurations. Reprinted from ref 18, Copyright 2012, with permission from Elsevier.

biosensing purposes.[43] A metastable mixture of DNA hairpins was used to detect the target DNA strand. The target sequence bound to and opened the first hairpin which would then bind and open the other hairpins, thus triggering formation of dendrimer assemblies, which were conveniently detected using gel electrophoresis. This system was tested successfully with HIV and *Chlamydia trachomatis* sequences.[43]

DNA nanostructures can potentially play a very important role in the field of biomedical engineering and biotherapeutics. They can act as carrier for targeted drug delivery. Moreover, they can be used for the programmed assembly of multiple drug/ligand molecules for multivalent targets. In one such application, we sensitized a cellular signaling pathway with the help of DNA nanostructures.[44] Signaling by transforming growth factor β (TGFβ) has been shown to be dependent on receptor clustering. By patterning a DNA nanostructure with closely spaced peptides that bind to TGFβ receptor, the sensitivity of cultured cells to TGFβ was markedly increased.[44]

We also proposed and partially constructed a multivalent thrombolytic delivery system to display and deliver human tissue plasminogen activator and plasminogen, two molecules essential in the thrombolytic pathway.[45] Our hypothesis was that the proteins bound to the DNA nanodevice would achieve greater therapeutic efficacy by extending their circulation time, by targeting fibrin clots specifically, and by recruiting plasminogen activators and plasminogen in plasma and forming active fibrinolytic centers on clot surfaces as well as inside the clots.[45]

Considering the possible biomedical applications of these nanostructures, we further studied their stability in plasma.[45] DNA nanotracks were incubated in pooled human plasma at

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37 °C, collected at different time points, and then visualized and analyzed for structural integrity using AFM (Figure 7A). These data revealed two important aspects of the interaction of nanotracks with blood plasma.[45] First, the nanotracks in plasma appeared thicker with less detail visible, probably due to nonspecific protein interactions. Second, the DNA nanostructures were stable at 37 °C without plasma for at least 11 h and in the presence of blood plasma for at least 7 h.[45] This degree of stability is encouraging for future in vivo stability and function test.

We also used thrombin binding aptamers and DNA nanostructures to conduct anticoagulation studies with pooled human plasma.[17,18] In the first study, four copies of a single thrombin binding aptamer with high antithrombin activity (Apt A) were displayed on a weave-tile.[17] Clotting assays showed that Apt A displayed on weave-tile was a much more effective anticoagulant than free Apt A. This was most likely due to the high local concentration of Apt A when displayed on the weave-tile.[17] In the second study, two distinct thrombin binding aptamers, Apt A and a second aptamer which binds to thrombin with much higher affinity (Apt B), were displayed on a weave-tile.[18] The two aptamers target distinct sites on thrombin. By judiciously choosing the size of the weave-tile and positioning the two aptamers in different configurations, we optimized the interaptamer spacing and relative orientation. This increased affinity of the construct to thrombin, resulting in much higher anticoagulant activity.[18] The anticoagulant activity of various constructs, expressed in terms of clotting time of plasma, is illustrated in Figure 7B. Anticoagulant activity was rapidly and stably reversed by using single-stranded DNA strands, complementary to the aptamer, as antidotes, thus enabling significant control over the blood coagulation cascade. Aptamers displayed on the weave-tile were also shown to be more stable in plasma compared to the free aptamers.[18]

■ CONCLUDING REMARKS

The field of DNA nanotechnology has progressed significantly since its inception in 1982. Our lab has made significant contributions by designing novel DNA tiles and lattices as well as by consistently pushing the limits on the maximum size of DNA origami. Moreover, we have used DNA nanostructures for DNA-based computation and for templated assembly of nanowires, nanoparticles and proteins. DNA nanostructures have also been used for various biomedical applications including interfacing with cell surface receptors, thrombolytic delivery, and highly potent anticoagulants. There is, however, still a growing need for new architectural strategies for targeting new functional and application areas such as electronics, photonics, and novel biomedical technologies. Moreover, future DNA nanostructures should be useful for in vivo and sensing applications with key areas including stimulus response, actuation, and drug delivery. We envision DNA-based materials that display *agency* such that they can self-assemble, perform sensing, carry-out computation, and finally respond via inherent actuation.

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