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Knitting complex weaves with DNA origami

William M. Shih and Chenxiang Lin

Department of Cancer Biology, Dana-Farber Cancer Institute, Boston, MA 02115, Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, MA 02115, and Wyss Institute for Biologically Inspired Engineering at Harvard, Cambridge, MA 02138

Summary of recent advances

The past three decades have witnessed steady growth in our ability to harness DNA branched junctions as building blocks for programmable self-assembly of diverse supramolecular architectures. The DNA-origami method, which exploits the availability of long DNA sequences to template sophisticated nanostructures, has played a major role in extending this trend through the past few years. Today, two- and three-dimensional custom-shaped nanostructures comparable in mass to a small virus can be designed, assembled, and characterized with a prototyping cycle on the order of a couple of weeks.

Introduction

Structural DNA nanotechnology [1,2] pursues the construction of devices on the 10–100 nm scale through self-assembly of DNA strands. The greatest advantage of using DNA for nanoconstruction derives from the strong predilection of DNA for adopting Watson-Crick pairing, if it is available, to yield double helices of very regular structure; a base pair typically is 2.0 nm in diameter and 0.34 nm in height with a twist of 34–35 degrees. Each segment along a given strand can be programmed to pair with a different partner, therefore various branched junctions (e.g. Figure 1A) are possible. The antiparallel double-crossover motif [3,4] (Figure 1B), where two double helices are held in parallel by two pairs of antiparallel strand exchanges, has emerged as a particularly useful building block for nanoconstruction. This motif can be conceived as akin to a rigid Lego tile that can be arrayed into two- and three- dimensional lattices of custom size and shape [5] (Figure 1C).

A principal mission for the field is to generate exact structures of ever increasing complexity. Imagining a custom-shaped tapestry composed of a vast number of unique tiles that each occupy a specified physical address is trivial, yet coaxing real double-crossover tiles to coalesce into such large assemblies with appreciable yield is not. Ascension to successive vistas of complexity over the next twenty years will critically depend upon continuous innovation in one key area: suppression or circumvention of DNA–self-assembly errors. The DNA-origami method [6], which employs a long, enzymatically produced single strand as a majority of the assembled mass, has emerged in recent years as an important foundation for decreasing self-assembly errors and thereby realizing more complex nanostructures. This review will focus on progress in the field towards advancing DNA-

Corresponding author: Shih, William (William_Shih@dfci.harvard.edu).

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origami capability; some potential applications will be briefly mentioned (for another perspective, see here [7]).

DNA and the biosynthetic advantage

In our view, the second greatest advantage of DNA for robust self-assembly of complex nanostructures results from the availability of biologically evolved polymerases that efficiently catalyze sequence-complementary monomer addition for faithful replication of long DNA strands. Nanoconstruction with long strands confers the entropic advantage endowed by pre-organization of its component segments at high effective concentration with perfect control over the intended stoichiometry. However, generalized production of highly homogeneous populations of informational molecular polymers that each are thousands to millions of units in length demands extremely accurate and robust elongation, which only is accessible today through the use of enzymes with relatively low error rates (e.g. for DNA polymerases, $1\text{E-}04$ or better). Artificial polymer platforms (e.g. peptide nucleic acids, peptoids) will not be compatible in a practical sense with an extensive scaffolded-assembly strategy until comparably accurate enzymatic or enzyme-like synthesis becomes accessible (e.g. through creation of variant polymerases or ribosomes [8]). In the meantime, such aqueous-compatible polymers can be combined with DNA into hybrid materials that offer the combined functionalities of their constituent parts.

Single-layer DNA origami

In DNA origami [6] (foreshadowed by [9]), one starts with a long single strand that acts as a scaffold, most commonly the 7.3 kilobase genome of the M13 bacteriophage, and mixes it with hundreds of chemically synthesized oligonucleotides, typically 20 to 50 nucleotides in length, that are programmed by Watson-Crick sequence complementarity to act like staples to pinch the scaffold into an antiparallel array of helices after rapid heating followed by slow cooling over the course of an hour (Figure 2A). Neighboring helices are held together by strands that cross over through a bridging phosphate, as in the double-crossover motif. Each helix can be designed with a different length, thus it is as if a designer has two ribosomes worth of molecular silly putty that can be molded by self-assembly into any desired two-dimensional cookie-cutter shape; realized examples from independent groups include a smiley face [6], dolphins [10], and a map of China [11] (Figure 2B). Remarkably, DNA origami efforts to date have not required sequence design, tight control over staple-strand stoichiometry, or purification of staple strands. This robustness is a testament to the entropic advantage of using long strands for scaffolding.

DNA-origami domains can be linked together in various ways. The simplest strategy preserves coaxial stacking of double helices, through either blunt-end or sticky-end cohesion. Origami also can be linked along the axis orthogonal to the helices by shared staple strands that cross over from the helix on the bottom of one monomer to the helix on the top of the next. It is striking, however, that no lattices anywhere near in size to those achieved for simpler multistrand-based tiles (e.g. [12]) have been reported with DNA origami; success may await significant improvements in the folding quality of constituent origami monomers. Domains also can be linked with their respective helical axes at an angle, for example to build a triangle [6] (Figure 2B).

By shifting the register of crossovers along adjacent interhelical interfaces, DNA sheets can be programmed to roll along their helical axes into tube-like structures, such as six-helix bundles [13] and prisms with three, four, or six flat-origami faces [14] (Figure 3). Such domains can be combined to form wireframe polyhedra with open faces, such as an icosahedron built from six-helix struts that is 100 nm in diameter [15]. A box with closed faces roughly 40 nm per dimension can be generated by attaching a square lid to the two

open faces of a square nanotube built from four flat-origami faces. One such box was generated with a top lid attached along a flexible hinge on one wall and two “locks” along the opposite wall in the form of DNA double helices with a “toehold” extension; this extension allowed for opening of the lid by addition of “key” complementary strands [16]. A similar box was generated that can be assembled in two steps, with the second step closing two sets of three walls using a total of nine face-sharing staple strands [17]. Using a quite different approach, a tetrahedron with solid faces has been constructed, with edges that are 54 nm long [18]. In this case, the four triangular faces are not constructed from modular domains of the scaffold. Instead, the scaffold strand passes one helix at a time through each face, such that only two hairpin turns are present in the entire structure.

Multi-layer DNA origami

Sheets of DNA helices can be stacked upon each other to create multi-layer structures, which can be advantageous when greater rigidity or complex three-dimensional cavities and surfaces are desired. The cross section of a multi-layer structure can be designed such that the helices fall either on a honeycomb lattice [15] (Figure 4A) or a square lattice [19], where stability is enforced by strand crossovers between all or most adjacent helices. The honeycomb lattice is more convenient for building objects with a hexagonal cross-section, and enables construction of objects that are more rigid per unit mass, due to the greater cross-sectional area that is afforded. The square lattice is more convenient for building objects with a rectangular cross-section, and enables construction of flatter surfaces, finer grade cavities, and denser objects. Multi-layer origami have the disadvantage that folding requires up to week-long folding times and produces lower yields [15]. As with single-layer origami, helices can be designed with different lengths, and separate domains can be connected together in various orientations. In this way, a variety of honeycomb origami have been demonstrated, including a slotted cross about 30 nm in diameter [15] (Figure 4B).

Further design versatility can be obtained by introducing structures whose helical axes supertwist and bend [20]. For example, increasing the number of base pairs in between crossovers leads to locally underwound helices and a compensatory global right-handed supertwisting of the helical cross-section. Alternatively, if the number of base pairs between crossovers is increased on one face of a nanostructure and decreased on the other face, then the respective supertwisting moments cancel out. However, now the face with deletions is under tension and the face with insertions is under compression. The structure relaxes this stress somewhat by global bending of the axis of the nanostructure towards the face with deletions. These principles were used for multi-layer honeycomb structures to create 60-helix ribbons that supertwist in either direction, 18-helix beams that bend up to 180 degrees with a radius of curvature as tight as 6 nm, and more complex structures such as square-toothed gears (Figure 4B).

Scaling to greater complexity

For creation of increasingly complex DNA nanostructures, the availability of powerful software design tools becomes a rate-limiting factor. An open-source package, caDNAno, greatly aids the design of single-layer and multi-layer origami on either a honeycomb [21] or square lattice [19]. Other software packages are available as well, for example the SARSE program that was used to design a cookie-cutter dolphin and a single-layer box [10]. As our ability to suppress self-assembly improves, there will be a need for increasingly sophisticated CAD programs.

Another important step for increasing complexity will be to transition from M13-based to designed-sequence scaffolds. The cost per base pair of gene synthesis has been dropping steadily [22]. Low-cost enzymatic or biological synthesis of scaffold sequences that each are

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specialized for folding one particular nanostructure will re-introduce sequence design as a key accessible parameter for improving the speed and robustness of DNA-origami folding, which in turn will be required for assembling more complex three-dimensional nanostructures. Recent methods that enable the use of double-stranded DNA as a source of scaffold that can be fed directly into the folding reaction, made possible through combined thermal and chemical denaturation followed by a quick drop to a lower temperature [23], or else methods that enable selective purification of one strand of a PCR product, such as through introduction of a biotin into only one of the primers [24], further enhance our ability to use diverse and longer scaffold sequences beyond what is available from M13. Low-cost gene-synthesis technology also makes more attractive the use of single-stranded DNA origami, where few or no staple strands are used, instead most interactions are programmed to occur via intramolecular base pairing within the long template strand [25-29]. Furthermore, if all the DNA is produced enzymatically or biologically, this raises the long-term prospect of large-scale production of industrial quantities of DNA eventually at a cost similar to other biologically produced commodities such as cotton.

Chemically synthesized DNA is much more heterogeneous than DNA polymerized by enzymes directed by a clonal template, thus the latter provides a possibility for supplying higher quality staple strands to the folding reaction. Here a Type IIS restriction endonuclease might be used to liberate short staple strands from a longer linear template; these clonally-derived, high-quality oligonucleotides can be expected to be advantageous in other applications where high quality is critical, such as DNA computing, where heterogeneities presumably contribute to leakage errors. The long thermal folding ramps currently required for assembling multi-layer DNA origami undoubtedly result in considerable depurination and strand scission defects [30], both in scaffold and staple strands. The use of chemical instead of thermal denaturation may lessen this type of damage [31]. Further optimization of origami architecture and DNA sequence, introduction of folding chaperones, and other strategies such as isothermal active self-assembly to guide the folding pathway or to implement error correction may be useful in reducing the amount of time that the DNA must be subjected to harsh thermal regimes.

Towards applications: DNA origami and templating

Patterning on a rectangle can be enabled by synthesis of two sets of staple strands [6]. For example, a “zero-bit pixel” set specified folding of the scaffold into a rectangle, and a “one-bit pixel” set was identical to the first, except that a hairpin dumbbell was programmed to extrude out of the plane of the rectangle for each of the strands. Then any custom bitmap pattern, for example a map of the Americas, could be created by mixing and matching subsets of strands from the two pools. DNA origami can be used to capture other DNA strands after initial formation [32], with potential application in nanoarray-based sensing [33,34]. DNA origami also have been used as rigid, information-bearing seeds for templating low-error-rate algorithmic assembly of multistrand tiles into cellular automata to build patterns such as binary counters [35] and Sierpinski triangles [36].

DNA origami can be used to template the organization of other matter. DNA origami have been used to template protein guests [37-41] and metallic nanoparticle arrays [42,43], to provide a calibration tool for super-resolution optical microscopy [44]; to self-assemble a cross-junction of carbon nanotubes into a field-effect transistor [45]. Even weak organization can be useful; for example, DNA origami have been used as a weak alignment medium for enabling NMR structure determination of membrane proteins [13].

It is very challenging to maintain long-range coherence by self-assembly, as small errors can accumulate into a large defect over long distances. To scale towards greater lengths, a useful strategy will be to take advantage of top-down strategies to enforce long-range order on a large number of DNA-origami structures. A promising early step has been taken in demonstrating the shape-complementary docking of DNA origami on electronics-compatible surfaces patterned by electron-beam lithography [46]. Here the microfabricated surface can template the microscale position of origami monomers, each of which in turn can template the nanoscale positioning of guest molecules [47]. Origami also have been positioned between nanoelectrodes using dielectrophoretic trapping [48].

Conclusions and future outlook

Scaffolded DNA origami has been recognized as revolutionary because its entropic advantage has enabled researchers to bypass requirements for sequence design, strand purification, and strand stoichiometry control, and thereby rapidly prototype intricate aperiodic nanostructures more than an order of magnitude larger in size than what has previously been possible. However, it may be necessary to introduce sequence design and production of higher-quality staple strands in order to achieve good yields of solid, three-dimensional DNA nanostructures of considerably greater complexity than is practical to achieve today. Fortunately, automated sequence design and low-cost production of clonally derived staple strands appear as achievable objectives for the not-too-distant future. Ultimately, we expect to see development of a large number of strategies for decreasing self-assembly errors, where each method only offers a modest improvement, but the combined effect of many such strategies applied in tandem result enables a large jump in complexity over what can be achieved today.

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Figure 1. Branched DNA molecules as basic building blocks for nanoconstruction. (A) A Holliday junction is formed by four DNA oligonucleotides each pairing with its neighboring two partners. (B) An antiparallel double-crossover molecule with single-stranded overhangs is formed by five DNA oligonucleotides. It can be conceptualized as two parallel DNA double helices brought together through strand exchange at junction points. (C) A two-dimensional array assembled from the molecule shown in (B) through base pairing between the single-stranded overhangs. Note the green-tinted molecules are identical to the red-tinted ones but rotated 180 degrees in the plane of the page.

Figure 2.

Design scheme and examples of planar, single-layer DNA origami. (A) Top: the finished design layout of a planar, single-layer origami. The dark blue strand represents the long DNA scaffold that winds through the entire structure, which is held together by many short DNA oligonucleotides (colored strands) known as staple strands. This diagram was rendered with caDNAno [21]. Bottom: abstract models of the same origami structure with each cylinder representing a DNA double helix. The left diagram is more realistic as it shows the bowing of DNA helices caused by the electrostatic repulsion. (B) Selected examples of planar, single-layer origami. Top row (from left to right): a star [6], a smiley face [6,10], and a dolphin; middle row (from left to right): a long strip with repeated small cavities [37] and a rectangle with a large cavity [38]; bottom row (from left to right): two different triangles and a rectangle [6].

Figure 3.

Objects formed by folding single-layer origami. Front row (from left to right): a spiral [20] and a wireframe triangle [23]. Middle: a six-helical bundle tube. Back row (from left to right): a closed face tetrahedron [18]; a box with the top lid controllable for opening and closing (shown only the closed state) [16]; open end prisms with six, four and three faces [14]; a wire-framed beach ball [20]; a box with two sets of three faces closed by face-sharing staples (detail not shown here) [17]; a wire-framed icosahedron [15]. Note that the depiction of spacing between parallel cylinders used here is meant to reflect qualitatively the relative density of crossovers per helical interface. Each underlying grid square has dimensions 20 nm by 20 nm.

Figure 4.

Design scheme and examples of multi-layer origami. (A) The finished design layout (rendered in caDNAno [21]) of a three-layer origami with a honeycomb-lattice cross section. In total, 18 double helices are held together through crossovers within scaffold DNA (black) and staple strands (orange, grey and blue). (B) Inset figures: upper left: an abstract model of the three-layer origami shown in (A) with each cylinder representing a double helix; lower left: a partially unfolded intermediate, useful for conceptual purposes, of the three-layer origami; upper right: a zoom-out view of a multilayer DNA origami consisting of 64 helices based on the square-lattice design [19]. Main figure: Multi-layer origami with a diverse of shapes. On the sides: 60-helix ribbons with global right-handed or left-handed twist [20]. In the middle, first row in the front: a series of multi-layer DNA origami with different aspect ratios based on the honeycomb lattice design [21]; second row, from left to right: multi-layer, honeycomb DNA origami that resemble the shape of a square nut, a slotted cross, a stacked cross, a genie bottle and a railed bridge [15]; third row: a series of multi-layer DNA origami, based on the square-lattice design, with different aspect ratios [19]; behind the third row: multi-layer, honeycomb DNA origami consisting of 18-helix ribbons bent at angles from 0 to 180° [20]. The bent segments are highlighted in red. Each underlying grid square has dimensions 20 nm by 20 nm.