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Complex DNA Nanostructures from Oligonucleotide Ensembles

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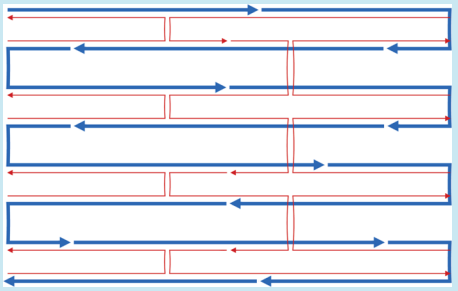
Supporting Information

ABSTRACT: The first synthetic DNA nanostructures were created by self-assembly of a small number of oligonucleotides. Introduction of the DNA origami method provided a new paradigm for designing and creating two- and three-dimensional DNA nanostructures by folding a large single-stranded DNA and 'stapling' it together with a library of oligonucleotides. Despite its power and wide-ranging implementation, the DNA origami technique suffers from some limitations. Foremost among these is the limited number of useful single-stranded scaffolds of biological origin. This report describes a new approach to creating large DNA nanostructures exclusively from synthetic oligonucleotides. The essence of this approach is to replace the single-stranded scaffold in DNA origami with a library of oligonucleotides termed "scaples" (scaffold staples). Scaples eliminate the need for scaffolds of biological origin and create new opportunities for producing larger and more diverse DNA nanostructures as well as simultaneous assembly of distinct structures in a "single-pot" reaction.



KEYWORDS: *DNA nanotechnology, self-assembling nanostructures, DNA origami*

Early studies on the design and construction of DNA nanostructures were inspired by biological archetypes such as the "Holliday Junction"[1−3] and showed that synthetic oligonucleotides could be used to reproduce and greatly elaborate upon these structures. This strategy, assembling synthetic oligonucleotides into larger structures and devices, opened a new and increasingly sophisticated field of DNA nanotechnology capable of producing complex systems such as a DNA-based robot that delivers nanomaterials to predesignated locations[4] and strategies for DNA-based computation.[5−7] A new paradigm for constructing DNA nanostructures was reported in 2006.[8] This approach, termed DNA origami, folds a large single-stranded scaffold and secures it in place with numerous oligonucleotide "staples" to assemble a broad range of two- and three-dimensional structures.[9−15] DNA origami enhances the kinetics of assembly and has been merged with the use of oligonucleotides to create integrated DNA nanosystems with large DNA platforms and smaller moving or functional parts.[16−20]

Despite its well-demonstrated power and breadth of application, the technique of DNA origami suffers from some limitations. Key among these is the need for a large single-stranded scaffold. Scaffolds are of biological origin, the most popular being the bacteriophage M13 genome and derivatives thereof. Double-stranded DNA can also be used but requires either a single-strand preparation step or complex annealing protocol.[21] A second limitation is that, in the absence of a library of single-stranded, sequence-distinct scaffolds, the possibility of simultaneous "single-pot" assembly of multiple DNA nanostructures and nanosystems is significantly compromised.

Reported here is a strategy for design and construction of large DNA nanostructures exclusively from synthetic oligonucleotides (i.e., not requiring a large single-stranded scaffold). The first *design* step borrows from DNA origami by rastering a virtual single-stranded DNA molecule, the "design-scaffold" of any desired sequence to form the shape. Next, a set of staples is computed using open source software (e.g., caDNAno[22]). Finally, in the key step, breakpoints are inserted at strategic locations in the design-scaffold to generate an ensemble of "scaples" (scaffold staples) (Figure 1). Scaples thus generated hybridize to complementary staples to form DNA nanostructures that are comparable to those created by DNA origami. Since the requirement for a single-stranded scaffold has been removed, these structures are no longer sequence or size limited. By eliminating the requirement for a single-stranded scaffold of biological origin, the multiplexed single-pot assembly of DNA nanostructures becomes possible, with significant ramifications for the assembly of complex nanosystems.

To test the idea of using scaples in place of full-length single-stranded scaffold DNA, a triangular structure was designed on the basis of a geometrically distinctive and well described DNA origami structure,[8] shown in Figure 2. After confirming that the structure assembled as expected by the conventional DNA origami protocol, sites along the backbone of the M13 sequence were selected as potential locations for fragmentation and scaple generation. Key considerations were to avoid scaple breakpoints that were close to staple breakpoints (allowing no less than three base pairs of separation), to avoid breakpoints in close proximity to crossovers, and to maintain reasonable scaple

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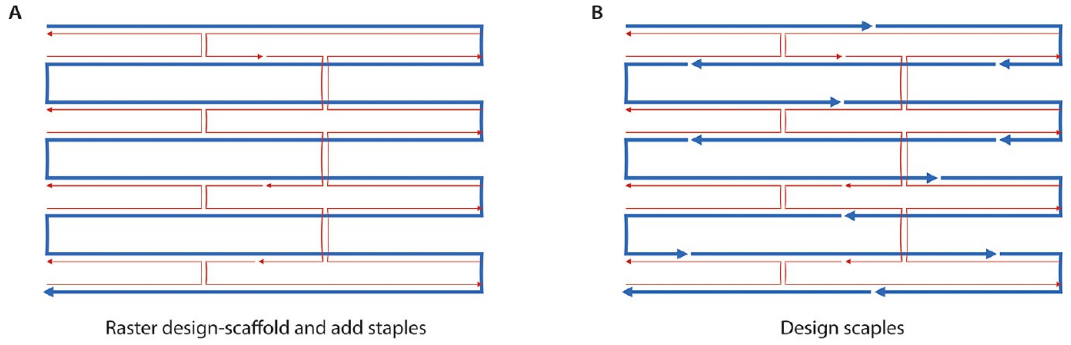

Figure 1. Strategy for the design of scaples-based nanostructures. (A) The first step is to raster a "design-scaffold" through the desired shape. Staples are then introduced using software (e.g., caDNAno[22]) or by hand. (B) In the key step, positions for the insertion of breakpoints on the design-scaffold are determined. The scaples and staples thus generated are synthesized and annealed as described here and in the Supporting Information.

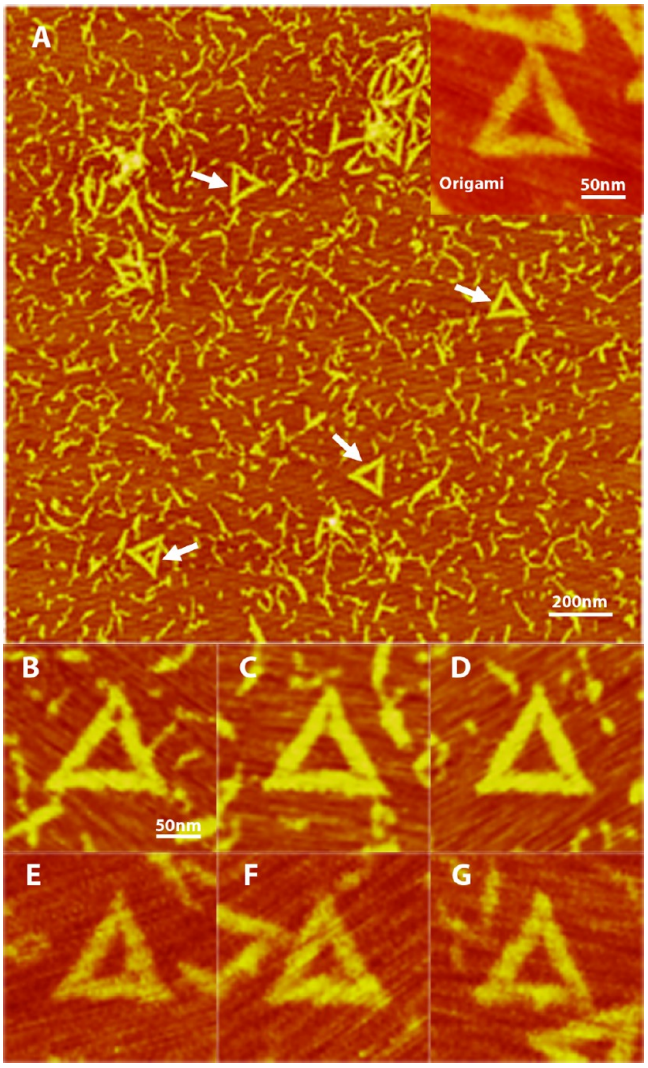

Figure 2. Scaples-based triangle using M13mp18 as the design-scaffold. (A) A representative field of the scaples version of the original DNA origami triangle (shown for comparison in the inset). The 154 scaples created for this triangle were designed using the M13mp18 design-scaffold layout exactly as in the origami triangle.[8] (B–G) Higher magnification AFM images of individual scaples-based triangles.

lengths with respect to limitations of current DNA synthesis methods (see Figure 5). Scaples were, therefore, no longer than about 60 nucleotides and no shorter than about 20 nucleotides (nt), the latter to ensure robust duplex formation and stability.

The staple:scaple ensemble was annealed by a protocol similar to that used for DNA origami (Methods). Figure 2 shows the results of this experiment. A significant number of complete structures was observed in the scaple:staple reaction, with total yield based on electrophoretic mobility of approximately 11% (Figure S2, Supporting Information). These structures were indistinguishable from the same structure created by DNA origami (Figure 2, inset). The number of incomplete structures in the staple:scaple reaction was substantially greater than that observed for the DNA origami reaction. Nonetheless, these data show that large DNA nanostructures can be assembled in the absence of a continuous scaffold backbone and the upper limit in size and complexity need not be constrained by scaffold limitations.

A rigorous test of the scaples approach would be the creation of a structure that was independent of the M13 sequence entirely. To carry out this test, a number of random sequence strings containing 7249 nt (the same number as in the M13mp18 scaffold) was generated. The sequences were tested for internal complementarity and those devoid of excessive predicted secondary structures were further analyzed (Methods). A sequence was chosen from this subset and used to design the same triangular structure shown in Figure 2 (inset). Staples and scaples were computed as in the first experiment. The staples and scaples thus generated were checked once again for undesirable characteristics such as internal base pairing and G-quartet formation and minor adjustments made to minimize the occurrence of these phenomena. Finally, these molecules were synthesized and assembled. The results are shown in Figure 3. Triangular structures were observed that were indistinguishable from those formed with the M13-derived scaples and the DNA origami triangle. Despite the relatively low yield, comparable to that observed with M13 scaples (Figure 2), this experiment shows that it is possible to construct complex DNA nanostructures using sequences that are nonbiological and randomly generated. This capability removes the requirement for large single-stranded scaffolds of biological origin with the concomitant sequence and length limitations.

An important opportunity created by the method described here is the potential to create different DNA nanostructures in a single-pot reaction, something that is very difficult with only one type of scaffold (i.e., M13). To test this possibility, a conventional, geometrically distinct DNA origami structure (a rectangle) was assembled in the same reaction mixture as the

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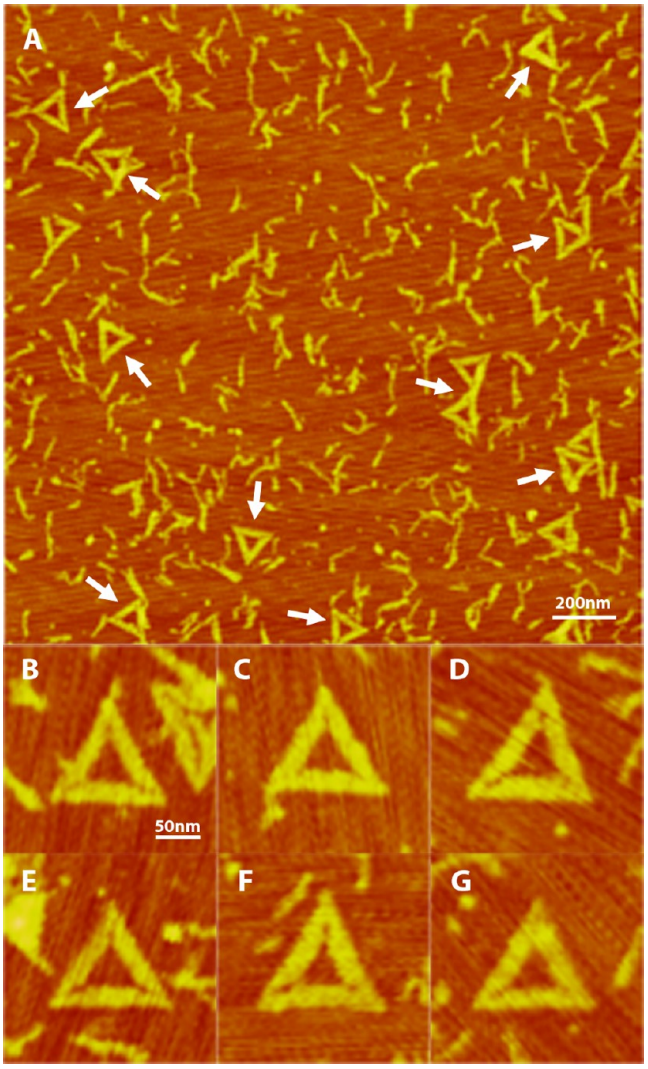

Figure 3. Scaples-based triangles constructed using a random sequence design-scaffold. The nanostructures shown are geometrically identical to those shown in Figure 2 but were created using a nonbiological, random sequence design-scaffold. The sequence was processed to remove internal subsequence similarity, undesired internal complementarity, and sequences formally capable of forming G-quartets. (A) A representative field AFM image of the triangles. (B–G) Individual examples of the same structure.

random sequence scaples-based triangle described above and a scaples-based round cornered square. Figure 4 shows that all three structures formed with yields roughly comparable to those obtained when assembled independently. Thus, with 837 individual oligonucleotides and one large single-stranded scaffold, it is possible to create three defined high molecular weight DNA nanostructures in a single reaction.

This report describes a strategy for the design and construction of large DNA nanostructures that assemble from a library consisting exclusively of small synthetic oligonucleotides. Any biological sequence constraints are lifted, thereby creating new opportunities for expanded and single-pot DNA nanostructure self-assembly. These new opportunities come at a cost. The higher order kinetics of nanostructure assembly by this method enhances the probability of formation of undesirable byproduct and concomitant lower yield of the desired product when compared with DNA origami. Similar limitations in yield have been observed in a related, independently developed strategy for creating DNA nanostructures.[23] In that approach, termed the "single-stranded tile" method, a stencil pattern is used to mask a preconfigured self-assembled DNA "canvas" comprised of a few hundred oligonucleotides. The mask defines the required subset of oligonucleotides to create a large number of different shapes from the same canvas. Although this method requires a large set of "edge protectors" to avoid undesirable aggregation, it allows rapid and automated construction of a vast array of nanoscale DNA shapes. In contrast, the scaples method utilizes a unique set of oligonucleotides for each shape, which incurs greater cost and design effort but in return allows simultaneous assembly of linear and curved, 2D and 3D nanostructures in a single reaction. Moreover, the scaples method uses open source design software (e.g., caDNAno[22]) that is readily available to any laboratory. As this approach is further developed, it should be possible to improve upon scaple and staple designs and optimize reaction conditions and methods to obtain higher yields. It is likely that both the scaples and the single-stranded tile strategies, and possibly a combination of the two, will find applications in DNA nanotechnology, the preferred approach in any particular case being a function of their virtues and liabilities in light of the experimental goal(s). The initial success at building complex nanostructures from libraries consisting exclusively of short synthetic oligonucleotides suggests that further development of this general strategy (executed via scaples or the single-stranded tile method) will lead to significant advances in the use of oligomeric DNA for the construction of large and complex DNA nanostructures and devices.

METHODS

Materials. All staple and scaple strands were purchased from Integrated DNA Technologies (IDT, Coralville, IA) in 96-well plates at 100 uM (25 nmoles) in RNase-free water. M13mp18 ssDNA was ordered from Bayou BioLabs (Metairie, LA) and was used without any further purification.

Assembly of the Nanostructures. The staples and scaples corresponding to each structure are listed below (Tables S1 and S2, Supporting Information). The buffer used to create these structures was the same as the one used in DNA origami, namely, 40 mM Tris, 20 mM acetic acid, 2 mM EDTA (TAE, pH 8.3) and 12.5 mM magnesium acetate (TAEM). Since staples and scaples are of the same size, they were added in 1:1 molar ratio at a final concentration of 40 nM for each oligonucleotide. The desired structures were created by thermal annealing as follows: heat to 95 °C for 3 min, cool from 95 to 40 °C in 4 h, followed by maintenance of temperature at 40 °C for 10 h. After that, the solution was cooled to 24 °C over 1 h (note, no difference was observed if cooled to room temperature at this stage). The solution was stored at 4 °C before imaging by AFM.

AFM Imaging. The structures were imaged by depositing 2 μL of the sample on a freshly cleaved piece of mica and allowing the sample to bind to the mica surface for 1 min. The mica was then rinsed with distilled water (dipping, 10×) and dried using nitrogen gas. Images were collected in Tapping-Mode using a Digital Instruments MultiMode AFM.

Agarose Gel Electrophoresis. Samples were analyzed by electrophoresis through a 1% agarose gel in TAEM at 50 mA. After electrophoresis, the DNA nanostructures were visualized by staining with 1× SYBR Green and illumination with UV light (365 nm).

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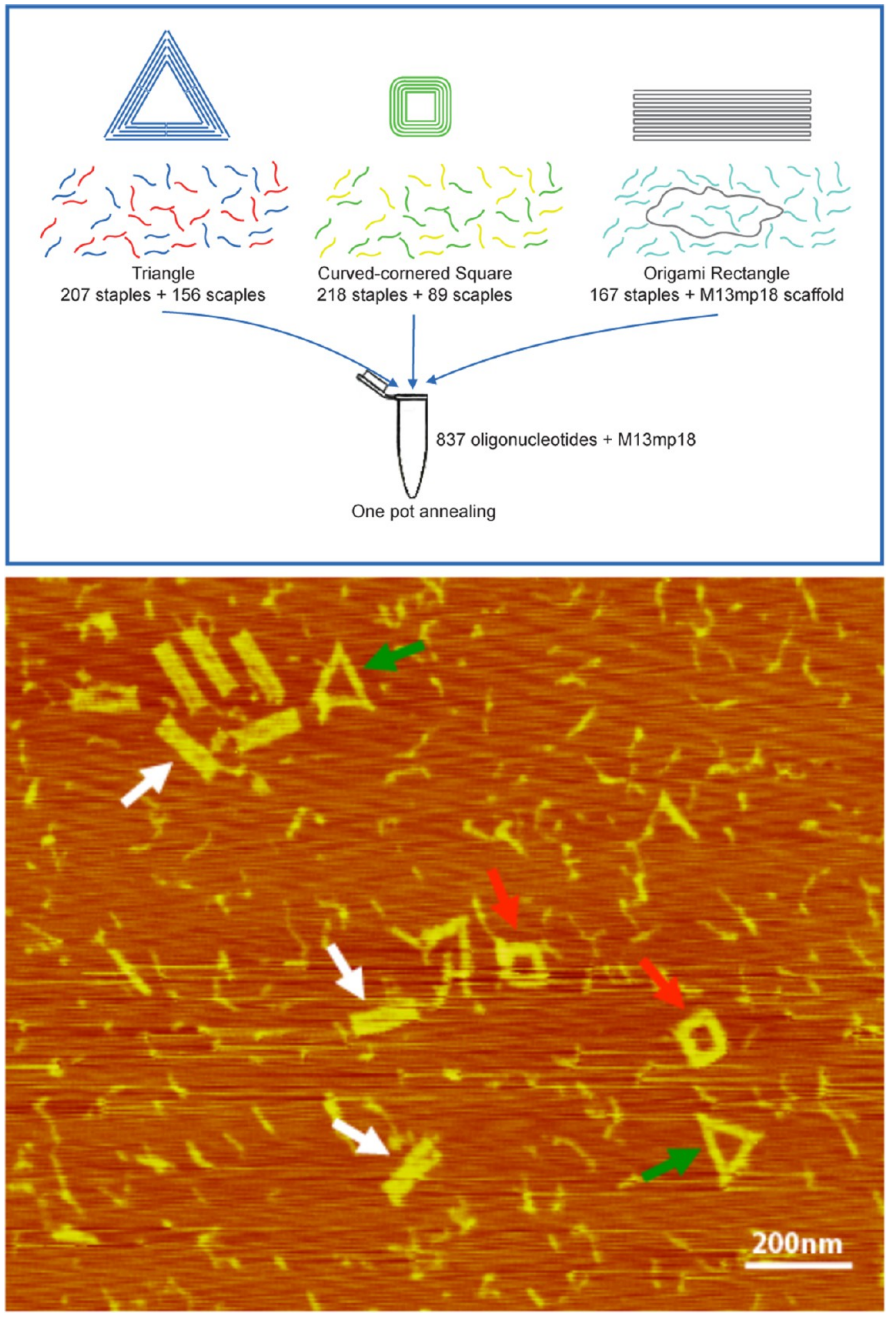

Figure 4. Simultaneous assembly of three DNA nanostructures in a "one-pot" reaction. In the experiment shown here, one DNA origami structure with an M13mp18 scaffold (rectangle, identified by white arrows) and two scaples-based nanostructures, a triangle (green arrows) and a square with curved corners (red arrows), were assembled in a single reaction containing over 800 distinct oligonucleotides. Both the triangle and the round-cornered square were designed with nonbiological random sequences.

Design of Scaples. An open source program, caDNAno,[22] was used to create the design-scaffold and corresponding staples in a manner analogous to that used for DNA origami. A .json file for each side of the triangle was created using the Rothemund triangle as reference and exported as an .svg file. The .svg files were opened in Adobe Illustrator (.ai) and manipulated to construct the complete triangle (Figure 5). Then, starting from one of the innermost helices in the triangle, potential scaple breakpoints were identified on the basis of the following guidelines:

(1) Length of a scaple: Breakpoints were positioned to maintain scaple lengths between 20 and 60 nt. There were instances where the length of the scaples had to be reduced to accommodate other necessary requirements.
(2) Relative position of breakpoints: Scaple breakpoints were introduced such that they were as far as possible from crossovers. With this in mind, scaples were designed to allow hybridization with the maximum number of different staples possible. In cases where a positioning compromise was required, such as in the presence of a staple junction, the breakpoints were offset by 3−5 nt from the midpoint between two crossovers.
(3) Stereochemical considerations: Care was taken to not introduce unwanted degrees of rotation around crossovers. This was accomplished by avoiding the alignment

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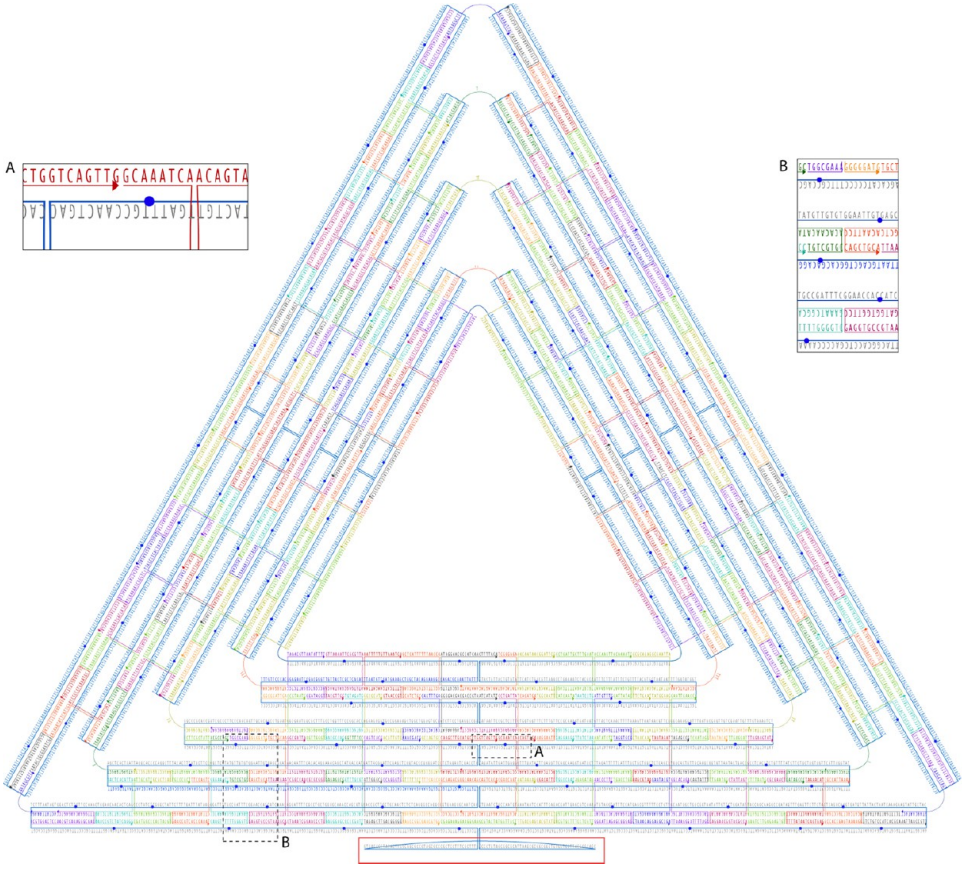

Figure 5. A graphic rendering of triangle structure used in this study.[8] The blue strand represents the design-scaffold and the other colored strands represent the staple sequences. Each side was designed in caDNAno[22] and was exported in .svg format and converted to .ai format in Adobe Illustrator (note: it is possible to make the entire triangle in caDNAno as a single .json file instead of breaking it into three sides, but the resultant design will need the same processing to represent the triangle in a workable format). The sides were consolidated into a single .ai file. The sides were joined by combining the bridge staples on the nonparallel edges of the sides on the corresponding helices together and the addition of 0−4 thymines to introduce flexibility at the vertices. The innermost helices of the three sides were joined by the design-scaffold (later converted to scaples). At this point, breakpoints were introduced in the design-scaffold (blue spots) following the guidelines described earlier. There are 154 scaples in this structure. The loop in the bottom (surrounded by a red box) was omitted from the scaples-based nanostructure, and the scaple sequence on either side of the loop was combined to form one scaple. Two sections (dotted boxes) are enlarged to illustrate specific features. Panel A shows an example of offsetting the breakpoint by 3 nt from the midpoint between two crossovers due to the presence of a staple junction in the center. There are many occurrences of this architecture in the structure. Panel B shows a representative staggered arrangement of breakpoints on adjacent parallel helices of the design-scaffold.

of breakpoints on adjacent parallel helices. This resulted in a staggered arrangement of breakpoints.

(4) Exceptions: In some instances, exceptions to these guidelines were necessary, and in those cases, the overall goal of maintaining structural integrity was the primary driver.

Figure 5A is an example of a case where the breakpoint was offset by 3 nt from the midpoint because of the staple junction in the center. Figure 5B shows the staggered arrangement of breakpoints on different and parallel helices of the design-scaffold. Aligning the breakpoints vertically may make the structure less stable.

We generated 154 scaples for the triangle structure of length between 30 and 60 nt. The 97-nucleotide long loop on one of the sides in the original DNA origami triangle was omitted from the scaples-based nanostructure. This was possible because, unlike M13mp18, scaples are not limited by length. If a loop or any other structure is desirable, it can easily be introduced into the scaple-based structure. It is noteworthy that this set of staples and scaples is just one of many possible sets that could be used to create this nanoshape.

Generation of Random Sequence Design-Scaffolds. To test the creation of nanostructures based on a nonbiological sequence, an algorithm was developed that generated completely random sequences the same length of M13mp18 (7249 nt). One of these sequences was selected, and this sequence was analyzed for internal repeats of length of 15 nt or more. A 97 nt long subsequence (that forms a loop in the origami triangle) was removed, and the resulting sequence was then divided into three segments (2384 nt each) and loaded into caDNAno for the construction of each side of the triangle. Each side was exported as an .svg file and assembled into one triangle as described in Figure 5 (Figure S1, Supporting Information). Scaple sequences were constructed using the consolidated triangle as described above and tested for the presence of undesirable secondary structure features including hairpins and G-quartets.

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■ ASSOCIATED CONTENT

Supporting Information

DNA sequences, additional supporting figures. This material is available free of charge via the Internet at http://pubs.acs.org.

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Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

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