DNA origami: a history and current perspective
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Researchers have been using DNA for the rational design and construction of nanoscale objects for nearly 30 years. Recently, ‘scaffolded DNA origami’ has emerged as one of the most promising assembly techniques in DNA nanotechnology with a broad range of applications. In the past two years alone, DNA origami has been used to assemble water-soluble probe tiles for label-free RNA hybridization, to study single-molecule chemical reactions, to probe distance-dependent multivalent ligand–protein binding effects, and to organize a variety of relevant molecules including proteins, carbon nanotubes, and metal nanoparticles. This review will recount the origin, evolution, and current status of this extremely versatile assembly technique.

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Introduction
In 1982 Nadrian Seeman proposed that immobile junction structures could be generated from DNA and that individual structures could be combined using simple sticky end base pairing to assemble complex, multi-dimensional objects [1]. The suggestion that a nucleic acid polymer, traditionally only considered in the context of molecular genetics, could be used as a construction material for the assembly of nanoscale geometric objects was completely revolutionary. From the creation of the earliest structures to the invention of ‘DNA origami’, DNA nanotechnology has evolved into a diverse, multi-disciplinary field.

Structural development
DNA nanotechnology is founded on the principle that single strands of DNA self assemble into double helices by complementary base pairing. The predictable nature of these intermolecular interactions enables researchers to ‘program’ individual DNA molecules to associate with one another. However, double stranded DNA molecules have a linear topology and will only support one-dimensional assemblies. By precisely designing nucleotide sequence it is possible to produce branched DNA molecules, extending the complexity of potential target structures. Attaching single stranded overhangs, or ‘sticky ends’, to the individual components provides a consistent and convenient method for inter-structure association.

Early attempts to use DNA molecules to construct specific architectures from single branch points were unsuccessful [2]. The flexibility of DNA building blocks with a single junction point did not facilitate the creation of higher order structures. The advent of multiple-crossover motifs overcame this limitation and provided the rigidity necessary for the assembly of larger objects. In particular, the double crossover motif first reported in 1993, remains the central motif in DNA nanotechnology and has been used for the construction of many discrete and periodic assemblies [3].

Following the production of individual DNA objects including a quadrilateral, a cube, and truncated octahedron, researchers used branched DNA molecules (or ‘tiles’) for the synthesis of periodic structures [4–6]. Using a variety of rigid, multiple-crossover motifs such as double and triple crossover molecules, multi-helical planar molecules, and bundled helix molecules as basic building blocks, several types of one and two-dimensional periodic networks were constructed [3,7–11]. The ability to make deliberately patterned structures represented a momentous development and has facilitated the precise positioning of molecules including proteins, aptamers, metal nanoparticles, and quantum dots [10–15]. However, the theoretical boundary of this type of DNA array is infinite and arrays may be non-uniform in size and shape.

For many applications it is desirable to construct finite-sized arrays. Schulman and Winfree achieved the synthesis of fixed width ribbon arrays composed of double crossover molecules by exerting precise control over nucleation and growth processes to influence the characteristics of the final structure [16]. Using a design level approach, Liu et al. reported a novel strategy to produce completely finite-sized DNA arrays by using sets of DNA tiles with unique sticky ends that connect to neighboring tiles [17]. They used the geometric symmetry of the DNA molecules and resulting nanoarrays to reduce the total number of unique tiles needed for the overall assembly and the formation of fixed-sized arrays. Park et al. reported the further development of finite-sized arrays by creating
aperiodic, addressable DNA tile lattices using a novel stepwise, hierarchical assembly technique [18]. Proteins were displayed on the nanoarray surface in the shape of the letters ‘D’, ‘N’, and ‘A’, demonstrating precise control over molecule placement on an underlying DNA scaffold. Pistol and Dwyer used a similar strategy to demonstrate a low-cost hierarchical method to fabricate large molecular weight aperiodic structures [19]. The method employed sticky-end re-use in a hierarchical manner to reduce the cost of fabrication by building larger complexes from smaller precursors. Each of these strategies to construct arrays with fixed dimensions represented a significant achievement; however, the robust and efficient synthesis of addressable nanostructures of fixed size using traditional assembly methods remained a challenge.

Yan et al. employed a unique assembly strategy for the construction of an aperiodic DNA lattice by the direct nucleation of DNA tiles around a scaffold strand [20]. A DNA strand that encoded the overall design information was generated by ligation and additional oligonucleotides were subsequently assembled into double crossover tiles around the scaffold strand. Several discrete barcode lattices and a ribbon lattice composed of repeating units were constructed. Shih et al. reported the construction of an octahedron structure using a similar ‘folding’ strategy [21]. A long single strand of DNA was synthesized, amplified by polymerase and folded into an octahedron structure following the addition of several short oligonucleotides. Folding of the long strand was designed to occur in stages using a simple denaturation-renaturation procedure. The emergence of unique assembly approaches and their success in the formation of discrete DNA objects would foreshadow the appearance of DNA origami.

Assembly approaches

The traditional approach to DNA-based self-assembly involves the systematic design of a number of complementary oligonucleotides that assemble into the intended structures. Component oligonucleotides are combined in equimolar stoichiometry, mixed in buffer solution, heated to a high temperature, and slowly cooled to allow complementary sequences to hybridize with each other. Despite its utility, there are certain disadvantages to this strategy. In order to obtain large structures without flaws, the constituent oligonucleotides must have perfect equimolar stoichiometry, and this requirement can result in a low yield owing to experimental errors. Although the use of hierarchical annealing and additional purification steps can increase the efficiency, this can be a lengthy process. The complexity of the structures that can be created using this strategy is limited to basic geometric shapes and the repetition of basic building blocks makes it difficult to create spatially addressable objects.

One of the most significant advances to DNA nanoconstruction was developed by Paul Rothemund [22]. He reported a fundamentally new approach for the construction of discrete DNA nanostructures using numerous short ‘staple’ strands of DNA to direct the folding of a long ‘scaffold’ strand into a flat array of antiparallel helices (Figure 1). ‘Scaffolded DNA Origami’ has several important advantages as compared to traditional assembly strategies. The folding of a long strand of DNA proceeds without many errors resulting in final structures with fewer defects and significantly higher yield. The method avoids stoichiometric dependence, eliminating the need for purification of the oligonucleotides and reducing the time required for synthesis. It is possible to create more complex shapes and the resulting nanostructures have

Figure 1

Scaffolded DNA origami formation of a two-dimensional rectangular structure. Approximately 200 short staple strands of DNA (red) direct the folding of a long, circular scaffold strand of DNA (blue) into a flat array of antiparallel helices.
fixed dimensions and are fully addressable, allowing the attachment of molecules at prescribed positions. The success of scaffolded DNA origami can be attributed to several factors: as staples are not required to bind to one another but to the scaffold, their relative concentrations are not pertinent. The initial attachment of correct staples partially arranges the long scaffold for subsequent binding of the remaining staples and strand invasion permits correct binding of staples to displace incorrect or truncated staples and eliminate unwanted secondary structure. Since Rothemund’s original report, numerous research groups have used scaffolded DNA origami to construct a variety of architectures and demonstrate several important applications.

**Single-molecule detection**
The first reported DNA origami architectures were two dimensional, including arbitrary shapes such as a rectangle, star, triangle, and a ‘smiley’ face [22]. One of the first applications was reported by Ke et al. where they used DNA origami to construct rectangular nucleic acid probe tiles for label-free RNA hybridization, molecular analogs of macroscopic DNA chips [23]. 20 nucleotide long single stranded probes were extended from the surface of the origami; the probes were complementary to RNA targets and after binding the tiles were adsorbed onto mica with RNA capture easily detected by atomic force microscopy (AFM). As a result of the nanometer-scale precision with which the probes were arranged, the effects of probe placement were also explored and it was determined that the exact position of the probes has a significant effect on the hybridization efficiency of the targets. Although this approach cannot compete with existing microarrays that analyze thousands of targets at once, this success may ultimately permit the detection of low levels of gene expression, possibly down to the single cell level.

One of the most thrilling advances using 2D DNA origami was recently reported by Voigt et al. where they demonstrated that single-molecule chemical reactions can be performed on a DNA origami scaffold [24]. They used a rectangular origami structure as an addressable support to visualize chemical formation and cleavage reactions with readout of chemical reactions achieved via biotin–streptavidin complexes. The formation or cleavage of individual chemical bonds resulted in either attachment or removal of the complexes from the origami surface with the chemical event easily visualized using AFM. For cleavage reactions three types of linkers were incorporated into the biotinylated staple strands: type A (non-cleavable), type B (containing a disulfide moiety that can be cleaved by reduction), and type C (containing an electron-rich 1,2-bis(alkylthio)ethane moiety that can be cleaved by singlet oxygen generated with UV light in the presence of a singlet oxygen photosensitizer) (Figure 2a). Efficient cleavage of linkers B and C was observed using AFM through the selective disappearance of streptavidin from the origami surface. For single-molecule bond formation, DNA origami provided the perfect platform on which to visualize reacting functional groups (Figure 2b). Incoming functional groups were linked to biotin and the incorporation of each group was visualized by the addition of streptavidin. The reaction of three functional groups commonly used for bioconjugation reactions were studied: alkyne, amine, and azide. The reactions were shown to proceed successively on the immobilized DNA origami scaffold with high selectivity. The direct monitoring of chemical reactions on the single-molecule level is a stunning demonstration of the versatility of DNA origami platforms. It may be possible to use this method to study a variety of chemical processes or even for the preparation of macromolecules in a highly selective manner as compared to traditional synthesis.

The ability to study events on the single-molecule level using DNA origami extends beyond chemical reactions. Rinker et al. showed that distance-dependent multivalent binding effects could be systematically investigated by incorporating multiple-affinity ligands into DNA origami [25]. The capture of single protein molecules by bivalent aptamers contained within the origami was directly visualized by AFM (Figure 2c). The spatial separation of the aptamers was varied to determine the ideal inter-ligand distance for efficient binding of the target protein. In addition, DNA structures have shown promise as model systems to study the thermodynamic properties of other multivalent systems. Nangreave et al. demonstrated the use of multihelical DNA tiles as a platform to systematically investigate the thermodynamic characteristics of multivalent interactions [26]. The number and position of the linkers between the tiles was varied and the corresponding change to the stability of the resulting complexes was evaluated. DNA origami offers a unique opportunity to build on this success and study more complex and interactive biomolecular networks. Origami structures are perfectly suited for engineering multivalent systems in which precise control of the spatial placement of all system components is required.

**Material organization**
2D DNA origami has also been used as a template for patterning protein molecules. Kuzyk et al. described two general approaches to template protein assembly using 2D DNA origami [27]. In the first approach they used a rectangular origami tile as a prefabricated template for streptavidin assembly. Several staple strands containing a biotin modification were displayed on the surface and after the structures were annealed, streptavidin was added and the arrays were imaged using AFM. In the second approach they demonstrated the simultaneous assembly of origami and protein (Figure 3a). Biotin modified staples were functionalized with streptavidin and
subsequently annealed directly with the origami. Kuzuya et al. reported an alternative approach to pattern streptavidin where they constructed a punched DNA origami assembly with periodic nanometer-scale wells to selectively capture the protein [28]. Through the attachment of biotins at the edges of each well, single streptavidin molecules were selectively confined and patterned ‘within’ the origami structure (Figure 3b). Researchers are currently developing new bioconjugation strategies that will enable the patterning of other classes of proteins. The ability to precisely pattern and orient protein molecules with nanometer precision may enable researchers to study intricate protein-protein interactions and construct novel biomaterials for applications such as tissue engineering.

Besides proteins, DNA origami is an ideal platform for the organization of other types of molecules. Single-walled carbon nanotubes (SWNT) have attractive electronic properties and have been suggested for use in nanoelectronic devices. Top-down methods such as lithography are traditionally used to produce miniature electronic devices; however it is a challenge to discover a reliable method to organize SWNT. Recently Maune et al. reported a strategy to arrange multi-component populations of SWNT using a rectangular DNA origami template [29]. SWNT were functionalized with DNA linkers complementary to nucleic acid capture strands extended from the surface of the origami template (Figure 3c). Two populations of SWNT were tested and AFM imaging revealed that the nanotubes attached to the templates with good efficiency, specificity, and intended orientation. The nanotubes were aligned into cross-junctions and electric characterization of one junction revealed stable field-effect transistor-like behavior. Although several challenges remain before this strategy can be used for the synthesis of electronic circuits or more complex devices, their work is a demonstration of the organizational power of DNA origami structures.

Assemblies of well-defined metal nanostructures have attracted interest owing to the high local-field
enhancement that is generated when they are excited at their plasmon resonance. To exploit this enhancement it is necessary to fabricate materials with inter-particle spacing less than 10 nanometers. Ding et al. recently reported the use of a triangular DNA origami template to organize a chain of gold nanoparticles [30*]. Six different gold nanoparticles were functionalized with thiolated, single stranded DNA, while the origami triangle displayed the complementary sequences along one edge (Figure 3d). SEM images confirmed the patterning of the gold nanoparticles with the desired inter-particle spacing. UV–visible spectral analysis revealed a plasmonic band shift from 521 to 526 nm indicating there were plasmonic interactions among the assembled gold nanoparticles. Pal et al. reported the use of a triangular DNA origami template to organize silver nanoparticles [31*]. The silver nanoparticles were functionalized with chimeric, phosphorothioated DNA that was complementary to capture strands displayed on the origami surface, with 9 of 24 nucleotides having a phosphorothioate backbone and the remaining 15 containing the traditional phosphodiester linkages (Figure 3c). The nine sulfur atoms in the phosphorothioated domain provided the DNA with high affinity for the surface of the silver nanoparticles and with adequate surface coverage, afforded stability against aggregation in solutions with a high salt concentration. Discrete monomeric, dimeric, and trimeric silver nanoparticle structures and a silver–gold nanoparticle hybrid structure were constructed with precise interparticle spacing. These origami templates could be used to systematically study the photonic and distance-dependent effects of plasmonic coupling of metal nanoparticles with potential applications in sensing and detection, optical waveguides, and resonators.

3D origami

Structurally, DNA origami has undergone significant advancement since its inception. Recently Douglas et al. reported the construction of random three-dimensional (3D) objects using the scaffolding strategy [32*]. They constructed a monolith, square nut, railed bridge, genie bottle, and slotted and stacked cross by generalizing the DNA origami method and folding corrugated layers of helices on a honeycomb lattice (Figure 4a). 3D origami structures can expand the potential applications by permitting greater spatial positioning and scaffolding possibilities, including the ability to encapsulate molecules such as proteins, enzymes, and metallic nanoparticles. Several 3D DNA origami containers have already been reported: Ke et al. constructed a closed tetrahedron molecular container (Figure 4b), Anderson et al. built a dynamic DNA box container with a controllable lid that can be opened in the presence of an externally supplied DNA ‘key’ (Figure 4c), and Kuzuya et al. designed and constructed a 3D box (Figure 4d) [33,34*,35]. In the future, hollow 3D DNA structures could be used to contain proteins for study and crystallization, or as deliv-

The organization of materials by DNA origami structures. (a) Simultaneous assembly of rectangular origami and streptavidin protein. Diagram illustrating the intended design and corresponding zoom in and wide field AFM images of the displayed pattern [27]—adapted with permission from IOP Publishing. (b) Construction of a punched DNA origami tape with periodic wells to capture streptavidin. Schematic diagram of protein capture by single wells followed by formation of a long tape, and AFM image of resulting protein chain [28]—reproduced with permission from Wiley InterScience. (c) Arrangement of two types of single wall carbon nanotubes using a rectangular DNA origami template. Diagrams illustrating the intended tube organization and AFM image showing the underlying DNA origami scaffold with nanotubes arranged in a cross junction [29*]—adapted by permission from Macmillan Publishers Ltd. (d) Organization of six different gold nanoparticles by a triangular DNA origami scaffold. Schematic diagram of the assembly process and corresponding scanning electron microscope (SEM) image of the gold nanoparticles chain with a superimposed triangle showing the position of the DNA template [30*]—adapted with permission from the American Chemical Society. (e) Patterning of three silver nanoparticles by a triangular DNA origami structure. Diagram demonstrating the desired center-to-center particle spacing, transmission electron microscope (TEM) image of the nanoparticles organized on the DNA scaffold after negative staining, and SEM image of the sample without staining [31*]—reproduced with permission from Wiley InterScience. All scalebars have been removed from the original images.
ery vehicles for the transport and release of cargo. DNA origami objects continue to advance with the construction of more compact structures. Ke et al. reported the synthesis of 3D origami cuboids with layers of helices packed on a square lattice [36]. Rather than having a hollow cavity, these structures are ‘filled’ in providing a higher material density that should be more resistant to compression (Figure 4e). Multilayer structures created using this strategy have the potential to precisely orient guest macromolecules by ‘carving’ out a cavity across the layers that is a geometric compliment to the guest molecule. The ability to orient a protein in three dimensions could...
be a revolutionary breakthrough for electron transfer reactions that depend on the precise orientation and accessibility of the immobilized proteins.

Conclusion
To fully realize the potential of DNA origami certain challenges will have to be addressed: the ability to scale up the size of DNA origami structures and exert control over their deposition in solution are among the most crucial. Remarkably, progress is already being made with respect to these challenges. Pound et al. demonstrated a simple, PCR based scaffold preparation method that may enable the construction of larger, arbitrary sized DNA origami structures [37]. Zhao et al. devised a route to scale up origami using DNA tiles as folding staples for the full length m13mp18 scaffold [38]. Improvements to the technique could result in nanostructures reaching the micron size domain of conventional photolithographic techniques. Several research groups have begun the integration of top-down lithography with bottom-up self-assembly to localize DNA origami structures on patterned substrates as demonstrated by recent reports from Kershner et al., Gerdon et al., and Hung et al. [39–41]. Despite the obstacles that remain, DNA origami is poised to serve as a powerful smart material in the future, with seemingly endless possibilities that are limited only by one’s imagination.

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References and recommended reading
Papers of particular interest, published within the annual period of review, have been highlighted as:

• of special interest
** of outstanding interest


This was the first reported construction strategy in which a relatively long DNA strand was used to direct the assembly of smaller DNA components. To this point, most DNA nanostructures were assembled using ‘traditional’ approaches. The most important component of the system was the scaffold strand, constructed by the ligation of shorter synthetic oligonucleotides, around which double crossover tiles were assembled from other short oligonucleotides. Several types of arrays composed of one scaffold strand and many double crossover tiles were constructed with unique ‘barcode’ patterns.


In this work the authors demonstrate a similar ‘folding’ strategy as the previous report by Yan et al. [39]. A DNA octahedron was folded from a mixture of a 1669 nucleotide long scaffold strand and five 40 nucleotide short strands by a denaturation-renaturation procedure. The folding was designed to proceed in two stages with prearrangement of the scaffold and short strands into an intermediate branched tree structure followed by closure of the structure into the octahedron. The scaffolding approach to DNA nanostructure assembly would become a mainstream technique with the formal introduction of ‘scaffolded DNA origami’ in 2006 [41].


This report represents a turning point in DNA nanotechnology. It formally introduced the idea of folding a very long single strand of DNA into a desired shape through the direction of hundreds of short DNA ‘staple’ strands. Analogous to paper origami, the technique has become known as ‘scaffolded DNA origami’. It has become one of the most popular and important approaches for the assembly DNA nanostructures.
23. Ke Y, Lindsay S, Chang Y, Liu Y, Yan H: Self-assembled water-soluble nucleic acid probe tiles for label-free RNA hybridization assays. Science 2008, 319:180-183. This is the first reported use of a DNA origami structure for detection of a biological sample. The authors assembled nanoscopic nucleic acid probe tiles that are molecular analogs of nanometric DNA chips to enable RNA target detection in solution. The ability to detect single-molecule hybridization of the target RNA to probes on the DNA origami chip via atomic force microscopy was enabled by the difference in the elastic properties of single and double stranded DNA (or a DNA-RNA hybrid). This system holds promise for single cell gene expression analysis.

24. Voigt NV, Terrin T, Rotaru A, Jacobsen MF, Ravnsbæk JB, Subramani R, Mamdouh W, Kjems J, Mokhir A, Besenbacher F, Gothelf KV: Single-molecule chemical reactions on DNA origami. Nat Nanotechnol 2010, 5:200-203. This work demonstrates that chemical reactions with single molecules can be performed at prescribed locations on a rectangular DNA origami scaffold and subsequently imaged by atomic force microscopy. The authors carry out single-molecule bond cleavage and bond formation reactions and the high yield and chemoselectivity observed in their experiments demonstrate the feasibility of post-assembly chemical modification of DNA nanostructures and their potential use as locally addressable solid supports.

25. Rinker S, Ke Y, Liu Y, Chhabra R, Yan H: Self-assembled DNA nanostructures for distance-dependent multivalent ligand–protein binding. Nat Nanotechnol 2008, 3:418-422. The authors show that distance-dependent multivalent binding of a protein can be investigated by incorporation of affinity ligands into a DNA origami scaffold. Thrombin aptamers were displayed on the surface of a rectangular origami structure and the capture of single thrombin molecules by the aptamer ‘pincers’ was directly visualized by atomic force microscopy. The results illustrate the potential to use DNA origami scaffolds in the design of complex and interactive biomolecular networks.


28. Kuzuya A, Kimura M, Numajiri K, Koshi N, Ohnishi T, Okada F, Ke Y, Douglas SM, Liu M, Sharma J, Cheng A, Leung A, Liu Y, Shih WM: Self-assembly of DNA into nanoscale three-dimensional shapes. Nature 2010, 469:418-422. This work extends the DNA origami scaffolding technique to three dimensions with the construction of custom shapes formed as pleated layers of helices constrained to a honeycomb lattice. As an initial demonstration six 3D shapes were designed and constructed with precisely controlled dimensions ranging from 10 to 100 nm. In addition, a hierarchical assembly strategy was used to assemble homomultimeric linear tracks and heterotrimeric wireframeicosahedra. 3D DNA origami structures may expand the range of potential applications by providing greater spatial positioning possibilities, including encapsulation or space-filling functionalities.


30. Andersen ES, Dong M, Nielsen MM, Jahn S, Subramani R, Mamdouh W, Golas MM, Sander B, Stark H, Oliveira CLP, Pedersen JS, Birkedal V, Besenbacher F, Gothelf KV, Kjems J: Self-assembly of a nanoscale DNA box with a controllable lid. Nature 2009, 459:73-76. The authors created an addressable DNA origami box that can be opened in the presence of an externally supplied DNA key. The box was constructed with a cavity that is large enough to contain a variety of biomolecules including, for example, a ribosome or poliovirus. The programmability of the box shows that dynamic changes can be induced by external signals in the environment and the application of such a nanorobotic device could result in the controlled release of a payload contained within.


