This review focuses on fundamental advances in DNA origami design and its emerging applications. For convenience, applications are coarsely categorized into nanofabrication, biosensing, and drug delivery. Nonetheless, considering the highly interdisciplinary nature of DNA nanotechnology, many works in the field do not fall within clearly defined boundaries.

INTRODUCTION TO DNA ORIGAMI
Over the past three decades, DNA, the genetic information carrier in most living organisms, has seen an ever-expanding role as a material for the construction of nanoscale objects.1–3 One technique in particular, known as DNA origami, has opened up the ability for researchers to design arbitrarily shaped complex three-dimensional (3D) nanostructures. Origami refers to the art of folding and sculpting a flat sheet of paper into arbitrarily shaped objects. DNA origami is a process of molecular self-folding: a long single-stranded DNA (scaffold), typically M13 phage genomic DNA (~7,000 bp), is folded into prescribed objects by hundreds of short synthetic DNA oligonucleotides, typically 20–60 bp long, which are designed to be complementary to different parts of the scaffold DNA (Figure 1A). The synthetic DNA strands crosslink spatially distant segments of a scaffold together, hence the term “staples.” For a comprehensive elaboration of DNA origami design principles, one can refer to this review article.4 In the first report of DNA origami in 2006 by Paul Rothemund,5 planar, arbitrarily shaped, two-dimensional (2D) objects with length scales around 100 nm were constructed (Figure 1B). Topologically, these 2D DNA origamis contained DNA double helices bundled together within a 2D plane by “crossovers,” where DNA strands crossed between neighboring DNA helices. Simple 3D hollow containers—a tetrahedron6 (Figure 1C) and a cube7 (Figure 1D)—were constructed in 2009 via the folding of 2D planar DNA origami sheets.

The milestone advancement in constructing 3D DNA origami structures was introduced by Shih and coworkers in 2009,8 when they bundled DNA helices into a honeycomb lattice to construct solid 3D objects (Figure 1E). They developed a design platform called caDNAAno,9 which drastically simplifies the design process to enable a general route to the rapid manufacture of sophisticated shapes. The Shih group later demonstrated square10 and hybrid lattice11 design concepts. Subsequent work enabled the introduction of curvature by simply adding or deleting bases between adjacent DNA helical crossovers (Figure 1F).12 In a departure from the traditional rigid lattice model, a study published in 2011 by Yan and coworkers used concentric DNA rings to define the surface feature of 3D objects, inducing curvature through the manipulation of strand crossover positions (Figure 1G).13

The above DNA origami design strategies all pack DNA helixes in parallel to construct objects. In 2013, the Yan group developed a wire-frame design strategy that uses DNA four-arm junctions as vertices within a network of DNA helices to generate DNA gridiron 2D and 3D objects (Figure 1H).14 They subsequently reported another method of creating more complex objects with a variety of multi-arm
Figure 1. Overview of DNA Origami Design Strategies and Structures

(A) DNA origami design strategies for constructing 2D and 3D objects.

(B) DNA origami smiley face. Reprinted by permission from Macmillan Publishers Ltd: Nature (Rothemund), copyright 2006.

(C and D) A hollow tetrahedron (C) and cube (D) formed from the folding of flat DNA origami sheets. (C) Reprinted with permission from Ke et al. Copyright 2009 American Chemical Society. (D) Reprinted by permission from Macmillan Publishers Ltd: Nature (Andersen et al.), copyright 2009.


(F) A curved 6-helix bundle spiral-like object. From Dietz et al. Reprinted with permission from AAAS.

(G) A nanoflask built from concentric rings of DNA helixes. From Han et al. Reprinted with permission from AAAS.

(H) A 3D gridiron structure based on DNA four-arm junctions. From Han et al. Reprinted with permission from AAAS.


(J) A wireframe icosahedron. From Veneziano et al. Reprinted with permission from AAAS.
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15 Controlling the length of the connecting loops can tune the angle between arms (Figure 1). On the basis of this strategy, Bathe and colleagues developed a design algorithm called DAEDALUS to fully automate the design process, which enabled them to create numerous polyhedral objects (Figure 1J). Högberg and colleagues reported an alternative wire-frame strategy that renders designed objects into triangulated meshes. To optimize the scaffold routing, they developed an algorithm (vHelix, based on Autodesk Maya). 3D objects such as the Stanford bunny were fabricated from this method (Figure 1K). The wire-frame method has demonstrated its outstanding design power over creating symmetric and asymmetric objects, which has largely enriched the design toolbox of DNA origami. In addition, these wire-frame objects have greater promise in biomedical applications because they have shown enhanced stability under physiological conditions, largely as a result of their wire-frame nature. Helices of conventional DNA origami are generally densely packed in parallel and thus largely depend on cations (e.g., Mg\(^{2+}\) and Na\(^{+}\)), which physiological fluids lack, to neutralize the negative charge repulsion among helices during and after folding. Wire-frame origami structures typically have only one or two helixes aligned, requiring less shielding and thus facilitating enhanced resistance to cation depletion under physiological conditions.

Expanding the size of DNA origami structures is desired for a variety of applications. One method is to use longer scaffold DNA, as demonstrated by several reports. Nonetheless, this requires the usage of a larger number of staple strands with unique sequences and thus increases the cost. In addition, this method is not suitable for fabricating larger structures on the micron scale. A more feasible strategy is the hierarchical assembly of DNA origami tiles into discrete superstructures or 1D, 2D, and 3D lattice structures through sticky-end cohesion or base-pair stacking. Numerous DNA origami discrete superstructures have been realized, as illustrated in Figures 1L–1P. Micrometer-sized DNA origami lattice structures have also been assembled through polymerization of DNA origami tiles either in aqueous solution (Figures 1Q–1S) or on a solid substrate (Figure 1T).

RNA molecules largely resemble DNA both structurally and chemically but are biologically more versatile. For instance, RNA has some advantages over DNA,
including a greater potential to be cloned and expressed in large quantities, as well as functional capabilities such as scaffolding proteins, detecting ligands, and releasing small interfering RNAs. RNA nanotechnology has grown in parallel along with the development of DNA nanotechnology. Nonetheless, the realization of RNA origami had not been reported until 2013, when the Sugiyama and Mao groups independently demonstrated the folding of a long single-stranded RNA (ssRNA, ~1,000 bp) into prescribed objects by using DNA staples (Figure 2A, left). The ssRNA scaffold was in vitro transcribed from a T7-promoter-containing double-stranded DNA (dsDNA) template by T7 RNA polymerase. Objects were assembled according to a conventional thermal annealing protocol. In 2014, Sugiyama and colleagues made pure RNA origami objects by using RNA staples to fold the RNA scaffold (Figure 2A, right). These RNA staples were also produced by in vitro transcription. In the same year, Andersen and colleagues reported the work of ssRNA origami (Figure 2B), which can self-fold into presdesigned tile structures. Angle-defined kissing loops were incorporated into the tiles to allow their further hierarchical assembly into larger lattice structures, such as hexagonal 2D lattices.
lattices. These ssRNA molecules were produced by in vitro transcription with T7 RNA polymerase. In addition to using conventional thermal annealing, this work demonstrated that these RNA architectures can self-fold and assemble isothermally during the transcriptional process. Although RNA assembly principles are the same as those for DNA, which rely primarily on Watson-Crick base pairing, RNA assembly has its own unique characteristics: (1) RNA-DNA and RNA-RNA duplexes have 11 bp per helical turn, (2) abundant secondary and tertiary structures exist within RNA strands, and (3) RNA is more vulnerable to nuclease degradation. These aforementioned points need to be taken into account in the design and preparation of RNA origami architectures.

In addition to DNA origami, single-stranded DNA (ssDNA) bricks developed by Yin and colleagues are another versatile toolset for constructing well-defined, arbitrarily shaped DNA structures. Both strategies have their own strengths and limitations, and thus people should choose according to their specific applications:

1. DNA origami typically produces higher yields of correct nanostructures. The use of a single scaffold and excess staples minimizes the possibility of missing strands, which can occur in techniques such as DNA bricks, where precise stoichiometry among strands is required.
2. DNA origami objects can have enhanced stability under working conditions because the continuous scaffold DNA routes through and holds the whole object.
3. DNA bricks possess higher modularity than DNA origami. A set of bricks could serve as a canvas to allow for the building of a large number of different objects, whereas a unique set of strands is generally necessary for building a single origami object.
4. DNA bricks offer a better choice for controlling the sequence and functionality of DNA strands. For instance, a 4H × 4T rectangle composed of L-DNA of random sequence has demonstrated high nuclease resistance.

DNA ORIGAMI FOR NANOFABRICATION

The DNA origami technique has been used extensively for the fabrication of nanoscale constructs, including many that are unobtainable (or extremely difficult to obtain) with other approaches. At the macroscopic scale, top-down fabrication of materials is used to create products with specific structures and functions. Unfortunately, this same capability has been difficult to achieve on the nanoscale, given that bottom-up self-assembly methods suffer from limited scope as a result of thermodynamic constraints, and top-down methods are time consuming and costly. DNA origami helps to bridge this fabrication gap, because the ability to self-assemble arbitrary shapes with full addressability allows for the fabrication of nanostructures with well-defined form and the capacity to precisely arrange heteroelements. DNA origami has been used as template structures for the organization of both synthetic nanomaterial components and biomolecules, as molds for nanoparticle synthesis, as masks for nanolithography, and as scaffolds for artificial enzyme cascades, etc. The devices fabricated by these techniques have been utilized for many applications, including nanoplasmonics, nanophotonics, biosensing, and drug delivery. The last two applications are separately reviewed and discussed after the nanofabrication section.

Nanofabrication with Synthetic Nanomaterials

A wide variety of nanomaterials can interface with DNA through a range of conjugation techniques, which have been well summarized in a previous review. By far the
most common material used for nanofabrication with DNA origami is gold, although there have been some reports on the use of silver. Gold nanoparticles (AuNPs) and gold nanorods (AuNRs) have very interesting optical properties that can be modulated through inter-particle interactions. Furthermore, AuNPs can be easily incorporated into DNA nanostructures through the facile conjugation of thiolated-oligonucleotides with the gold surface. There are a vast number of examples in the literature of nanofabrication using AuNPs, but only a few representative studies will be highlighted here. Recent work by Oleg Gang’s group has demonstrated the ability for DNA origami nanostructures to program the fabrication of well-defined 2D networks of AuNPs. The authors used a cross-shaped origami tile with a hole in the center for capturing a single AuNP. They modified the four edges of the tile with unique ssDNA extensions to allow for a wide range of anisotropic and specific connections between different tiles in a lock-and-key mechanism (Figure 3A). The authors used this system to fabricate well-defined AuNP clusters and chains, as well as large 2D arrays. The same group has also used 3D polyhedral DNA origami nanostructures to construct well-defined 3D AuNP crystal structures. Other groups have explored similar AuNP arrays, including the use of hexagonal tiles and “nano-flowers” for constructing linear chains and hexagonal and square lattices. In addition to organizing nanoparticles into well-defined patterns, DNA origami has also been utilized to selectively functionalize the surface of nanoparticles. For instance, Wang and colleagues used a DNA origami clamp to encapsulate a AuNR, creating an addressable surface that facilitated the anchoring of AuNPs at selected positions to form AuNR-AuNP clusters of prescribed configurations. Fan and colleagues transferred a molecular pattern on DNA origami onto AuNPs by removing the origami template while leaving oligonucleotides at selected locations on the surface of AuNPs, which served as docking sites for the attachment of other particles. Prior to this work, a similar strategy was demonstrated by Sleiman and colleagues, who used non-origami DNA polyhedral structures.

The prevailing rationale for using DNA nanostructures to organize AuNPs is the ability to use interactions between the nanoparticles as a means of manipulating light. This was elegantly demonstrated by Liedl and colleagues, who used a 24-helix bundle DNA origami rod to pattern the self-assembly of AuNPs into helices with either a right-handed or left-handed pitch (Figure 3B). These nanostructures exhibited pronounced circular dichroism effects in the visible range of the electromagnetic spectrum, and these effects changed on the basis of the helicity of the organized AuNPs. Subsequently, the same group demonstrated switchable circular dichroism by using a similar DNA origami rod-based architecture, where the orientation of the superstructure in reference to incident light could be controlled. Other groups have similarly used DNA origami to control the plasmonic behavior of AuNRs, including a AuNR capable of walking along a DNA origami track, stacked arrangements of offset AuNRs, chiral AuNP rings, and static and dynamic AuNR dimers.

The fabrication of devices where AuNPs interact with other optically active materials has also been accomplished with DNA origami. In their pioneering work, Tinnefeld and colleagues constructed a zepto-liter electromagnetic “hotspot” by organizing 100 nm AuNP dimers on a 12-helix bundle DNA origami pillar (Figure 3C). When the authors placed a fluorescent dye in the gap between the AuNPs, they demonstrated fluorescence enhancements of up to 117-fold. By using this system, the authors were able to monitor DNA hybridization and Holliday junction conformational dynamics at the single-molecule level. Other groups have conducted similar studies, including distance-dependent quenching and enhancement of fluorescent-dye-AuNP systems on a
Figure 3. DNA Origami for Nanofabrication with Synthetic Nanomaterials

(A) 2D AuNP arrays assembled by DNA origami. Reprinted by permission from Macmillan Publishers Ltd: Nature Chemistry (Liu et al.\textsuperscript{41}), copyright 2016.

(B) Chiral AuNP helixes assembled on DNA origami templates and subsequent interactions with polarized light. Reprinted by permission from Macmillan Publishers Ltd: Nature (Kuzyk et al.\textsuperscript{42}), copyright 2012.

(C) DNA-origami-directed nanoantenna with two AuNPs forming a hotspot that increased the photon count rate nearly 50 times. From Acuna et al.\textsuperscript{43} Reprinted with permission from AAAS.

(D) AuNP and quantum dot assembled on a triangular DNA origami. Reprinted with permission from Samanta et al.\textsuperscript{44} Copyright 2014 American Chemical Society.

(E) DNA-origami-based light-harvesting system (left) and its antenna-effect dependence on the number of donors (right). Reprinted with permission from Hemmig et al.\textsuperscript{45} Copyright 2016 American Chemical Society.

(F) DNA-origami-templated single-walled carbon nanotube (SWNT) cross-junction (inset) and DNA-templated SWNT connection to electrodes. Reprinted by permission from Macmillan Publishers Ltd: Nature Nanotechnology (Maune et al.\textsuperscript{46}), copyright 2010.

(G) Casting of a silver nanoparticle within a DNA origami mold. From Sun et al.\textsuperscript{47} Reprinted with permission from AAAS.

(H) Size-controlled liposomes templated by a DNA origami ring. Reprinted by permission from Macmillan Publishers Ltd: Nature Chemistry (Yang et al.\textsuperscript{48}), copyright 2016.

(I) Hydrofluoric acid (HF)-vapor-mediated negative (top) and positive (bottom) pattern transfer from DNA origami to SiO\textsubscript{2} substrate. Reprinted with permission from Surwade et al.\textsuperscript{49} Copyright 2011 American Chemical Society.

(J) AFM image of triangular DNA origami with three Cy5 dyes (top right) and AFM images with corresponding epifluorescence images of origami structures in a photonic crystal cavity (PCC) (left). Wide-field fluorescence microscopy images show optical emission of dye-modified DNA nanostructures in lithography-patterned PCCs to create an image of van Gogh’s The Starry Night (bottom right). Reprinted by permission from Macmillan Publishers Ltd: Nature (Gopinath et al.\textsuperscript{50}), copyright 2016.

Scale bars, 200 nm (A), 20 nm (B and G), 100 nm (F and I), 5 nm (F, inset), 50 nm (H), 250 nm (J, left), and 50 nm (J, right).
triangular DNA origami tile\(^{63}\) and “nano-antennas” for hotspot-induced enhancement of surface-enhanced Raman spectroscopy.\(^{64}\) AuNP-quantum-dot heteroelement devices have also been constructed for studying the distance-dependent fluorescence quenching between the two elements (Figure 3D).\(^{65}\) Similar work from the Liedl group investigated the fluorescence lifetime of AuNP-quantum-dot pairs on a DNA origami frame.\(^{65}\)

The ability to control the transfer of energy on the nanoscale is another application in which precise fabrication is required. For example, arrangements of small-molecule fluorescent dyes have been fabricated for light-harvesting applications using DNA nanostructure templates. In one study, Tinnefeld, Chin, and colleagues were able to drastically enhance the Förster resonance energy transfer (FRET)-induced emission from an acceptor dye by varying the number and placement of donor dyes.\(^{45}\) When a ring-based structure (mimicking structural motifs found in photosynthetic bacteria) was used, the fluorescence intensity of the acceptor dye increased 2-fold in relation to the emission following direct excitation (Figure 3E). The ability to precisely control the geometry of these energy-transfer pathways by using DNA origami can be used to engineer more efficient light-harvesting systems for applications such as solar cells. The control of electron movement has been demonstrated with carbon nanotube arrangements fabricated with DNA origami. Han and colleagues were able to demonstrate that the construction of cross-junctions between two different DNA-functionalized carbon nanotube populations (semi-conductor and metal) led to the generation of field-effect transistor behavior (Figure 3F).\(^{46}\) Gothelf and colleagues\(^{66}\) recently used a DNA origami template to direct the routing of individual polymers, which can serve as nanoscale wires with polymers that are suitably conductive.

Although DNA origami nanostructures are primarily used in nanofabrication as a scaffold for the attachment of various elements, it has also been used as a scaffold for the synthesis of nanoparticles. In their pioneering work, Yin and colleagues used hollow DNA origami constructs as a mold to facilitate the growth of both silver and gold nanoparticles into shapes defined by the DNA structure.\(^{57}\) They were able to synthesize nanoparticles that differed in both size and shape by enclosing a small AuNP seed inside a structurally rigid DNA origami frame, such that further growth of the nanoparticle was constrained by the DNA nanostructure (Figure 3G). In a similar approach, Lin and colleagues designed DNA origami rings that could template the growth of liposomes.\(^{48}\) With lipid-modified DNA strands incorporated on the DNA frame as nucleation sites, the self-assembly of further lipids into a bilayer structure led to the formation of liposomes with a diameter defined by the DNA origami ring (Figure 3H). This allowed the authors to assemble highly monodisperse liposomes in four different sizes by using a variety of different lipid species. Other studies on the interaction between DNA nanostructures and lipids have been previously reviewed.\(^{67}\)

DNA origami structures have been used as masks for the construction of nanoscale features via lithography, given that the size of the features created in this manner are much smaller than those created by other lithographic techniques. Yin, Liu, and colleagues found that the presence of a silicon dioxide substrate altered the etching rate of the substrate in the presence of hydrofluoric acid vapor (Figure 3I).\(^{49}\) In a low-moisture environment, the DNA nanostructures acted as a protective mask to prevent substrate etching, leading to a positive-tone pattern transfer. However, in a high-moisture environment, substrate etching was accelerated in the presence of DNA, leading to negative-tone pattern transfer. This technique was improved
by Tiron and colleagues, who created patterns with \(<10\) nm resolution,\(^6^8\) and techniques involving DNA metallization have been used to transfer patterns to substrates other than silicon dioxide.\(^6^9\) DNA origami nanostructures have also been used in conjunction with traditionally fabricated lithographic substrates for the creation of hybrid devices with enhanced functionality. Rothemund and colleagues were able to guide the self-assembly of fluorescent-dye-modified DNA origami structures inside lithographic-patterned photonic crystal cavities.\(^5^0\) The use of the DNA nanostructures allowed the authors to precisely control the number and location of dyes within each cavity to create a system in which the intensity of cavity emission could be digitally varied (Figure 3J).

**Nanofabrication with Biomolecules**

In biology, the spatial and temporal arrangement of biomolecules into well-defined complexes leads to enhanced activity or new functionality. The ability to mimic these complexes through nanofabrication with DNA origami is a useful tool for better understanding native multi-protein systems in an effort to construct more efficient enzymatic nanoreactors, conduct single-molecule biophysical studies, or achieve super-resolution optical imaging.

Advances in the ability to functionalize DNA nanostructures with proteins have enabled the synthesis of a wide variety of nanofabricated biomolecule systems; for more information, we direct readers to an excellent review on the subject.\(^7^0\) Although simpler DNA nanostructures had been previously used for the assembly of multi-enzyme systems, Yan and colleagues were the first to use DNA origami to scaffold a more complex, three-protein system.\(^7^1\) Using a 2D rectangular DNA origami tile, the authors were able to investigate the effects of protein distance on a bi-enzymatic process using glucose oxidase and horseradish peroxidase. The authors found that placing a non-enzymatic “bridge” protein between the two enzymes could increase the reaction rate even further (Figure 4A). The authors attributed this effect to the more effective “shuttling” of reaction intermediates between the hydration shells of the two enzymes. Subsequent studies by the same group increased the complexity of the design by utilizing a flexible ssDNA linker modified with cofactor NAD\(^+\) to control the preference for two competing enzymatic reactions.\(^7^2\) 3D DNA origami nanostructures have also been used for modulating and studying enzymatic behavior, such as using a “nanocage” to encapsulate multiple enzymes to improve reaction efficiency.\(^8^0\)

DNA origami nanostructures have also been used for controlling and studying non-enzymatic proteins. Shih, Reck-Peterson, and colleagues used a 12-helix bundle DNA origami tube as a platform for organizing the motor proteins kinesin and dynein.\(^7^3\) Using single-molecule fluorescence techniques to track the DNA nanostructure, the authors were able to study the interplay between motor proteins with opposite-polarity movement (Figure 4B). Hogberg, Teixeira, and colleagues also used a tubular DNA origami structure to pattern proteins, wherein they focused on understanding how the spatial organization of ligands affects receptor-mediated cell responses.\(^7^3\) By controlling the distance between two Ephrin-A2 molecules, the authors were able to show how ligand placement alters receptor EphA2 phosphorylation and subsequent cell invasion (Figure 4C). Shih, Bellot, and colleagues created a trapezoidal “nanoactuator” in which the activity of a split enhanced green fluorescent protein (EGFP) could be controlled allosterically.\(^7^4\) The incorporation of additional DNA oligonucleotides could force the trapezoid into either “closed” or “open” conformations, regulating the distance between the EGFP halves and leading to increased fluorescence only in the closed conformation (Figure 4D).
Figure 4. DNA Origami for Nanofabrication with Biomolecules

(A) Assembly of a GOx-HRP cascade on DNA origami with a protein bridge in between to facilitate H₂O₂ diffusion through a connected hydration layer and increased reaction rates. Reprinted with permission from Fu et al. Copyright 2012 American Chemical Society.

(B) Single-molecule fluorescent measurements of the movement of DNA origami nanostructures attached to opposite-polarity motor proteins. From Derr et al. Reprinted with permission from AAAS.

(C) DNA origami nanostructure modified with Ephrin-A2 molecules (left) and relative phosphorylation activity from structures carrying different ligand organizations (right). Scale bars, 20 nm. Reprinted by permission from Macmillan Publishers Ltd: Nature Methods (Shaw et al.), copyright 2014.


(E) DNA-origami-based single-molecule study of base-stacking forces. From Kilchherr et al. Reprinted with permission from AAAS.

(F) DNA-origami-based single-molecule force clamp for measuring DNA bending induced by TATA-binding protein via changes in FRET intensity. From Nickels et al. Reprinted with permission from AAAS.


(H) Optical imaging of densely packed clusters on a DNA origami substrate with 5 nm resolution by DNA-PAINT. Scale bars, 10 nm. Reprinted by permission from Macmillan Publishers Ltd: Nature Nanotechnology (Dai et al.), copyright 2016.
The nature of DNA origami structures makes them excellent substrates for conducting delicate single-molecule biophysical studies. Dietz and colleagues recently utilized a DNA origami structure to study the forces and lifetimes of DNA base-pair stacking interactions with optical tweezers (Figure 4E). Two DNA origami beams were attached to micrometer-sized beads, which were trapped by optical tweezers. The two beams were tethered together by a 68 bp ssDNA. An array of blunt-end DNA duplexes with varied numbers protruded from each beam. The authors could examine the stacking forces and lifetimes between the arrays of blunt ends on either arm by tracking the force-extension and constant-distance data with optical tweezers. They found that stacking forces depended on the number, orientation, and type of base, in good agreement with previous studies. Another report by Liedl, Tinnefeld, and colleagues employed DNA origami as a nanoscopic force clamp to investigate known biophysical models (e.g., Holliday junction and TATA-binding protein) on a single-molecule basis (Figure 4F). This nanoscopic force clamp is based on measuring the efficiency of FRET at distances correlated to varying entropic forces acting on the system. The authors controlled the entropic force by varying the contour length of the ssDNA spring tethered to the origami substrate. Utilizing this system, they observed that the TATA-binding protein induced Holliday-junction conformer transitions and DNA duplex bending.

Another utilization of DNA origami structures lies in super-resolution microscopy studies based on the DNA-PAINT technology, a variation of point accumulation for imaging in nanoscale topography. DNA-PAINT imaging is carried out with diffusion, dye-labeled short oligonucleotides that interact transiently with the sample. DNA origami could serve as a perfect substrate for studying and improving the DNA-PAINT technology before its broad application in imaging biological samples, where the Yin group has been the pioneer in this exciting field. They have largely demonstrated the capabilities of DNA-PAINT for multiplex super-resolution imaging (Figure 4G), quantitative super-resolution imaging (qPAINT), and super-resolution imaging of biomolecules in densely packed clusters with 5 nm resolution (Figure 4H), all benchmarked on DNA origami substrates.

Challenges and Perspectives
One major constraint on the use of DNA origami for fabrication at the nanoscale is the size limitations—the overall dimensions of these nanostructures are limited by the length of the scaffold strand. Given that ssDNA scaffolds significantly longer than the traditionally used M13 genome are not only technically challenging to acquire but also mechanically fragile, it seems unlikely that the fabrication of large-scale assemblies can be achieved with traditional DNA origami. One potential option is to use DNA origami nanostructures as individual building blocks for the formation of larger superstructures. Another limitation is the relatively static nature of these nanostructures; the ability to fabricate dynamic materials with programmable movement at the macro-level adds a level of utility that cannot easily be translated to the nanoscale. A considerable amount of work has gone into developing dynamic DNA machines, highlighted in a previous review, particularly through the use of DNA-driven strand-displacement reactions. However, further advances in the ability to control the movement of DNA origami structures would open up a range of new utilities for nanofabricated devices. In particular, dynamic mechanisms that occur more quickly, are more complex, or can be achieved without the introduction of new DNA species are needed. Stimuli-responsive alternative nucleic acid structures, such as i-motifs, aptamers, and G-quadruplexes, potentially offer methods for designing “smart” dynamic nanostructures. Work in this field has been highlighted in several excellent reviews.
DNA ORIGAMI FOR BIOSENSING

The characteristics of DNA origami nanostructures, and the associated benefits, offer potential advantages for use in sensing applications. Biosensing is a critically important function in a diverse set of fields ranging from medicine to food science to military defense. Biosensors are used for studying genetic mutations, detecting pathogenic microorganisms, and monitoring air quality, etc. The nanometer addressability of DNA origami allows for the precise placement of various sensing elements in complex configurations, and the bottom-up self-assembly method makes production significantly simpler than comparable top-down approaches. DNA origami nanostructures are especially useful for single-molecule biosensing, which facilitates ultra-low detection limits as well as the ability to directly monitor dynamic processes. The large profile of DNA nanostructures, particularly 2D designs, facilitates immobilization onto surfaces such as mica or glass, and the ability to place sensing elements in asymmetric patterns simplifies target identification. DNA origami sensors are also very promising for multiplexed assays for many of the same reasons listed previously. In a broad sense, DNA origami constructs used for biosensing can be divided into two categories: binding-based devices and nanopores.

Binding-Based Biosensors

DNA origami nanostructures that fall within this category utilize a specific molecular interaction (nucleic acid hybridization, protein-ligand binding, etc.) to alter the shape or profile of the sensor. These conformational changes can be observed via single-molecule techniques such as atomic force microscopy (AFM), transmission electron microscopy (TEM), or optical tweezers for identification of the target. Given the high specificity and well-understood behavior of Watson-Crick base pairing, it is no surprise that DNA origami nanostructures have been used for detecting specific nucleic acid sequences with considerable success. In their pioneering work, Yan and colleagues were the first to demonstrate that DNA origami could be used as a platform for single-molecule mRNA detection. The authors used a rectangular 2D nanostructure with sequences complementary to three different genes (Rag1, Myc, and Actb) extending out from the surface. When the target mRNA species were present, they bound to these “capture” probes and formed a dsDNA V-shaped protrusion, which could be visualized by AFM. By using pre-formed DNA-dumbbell extensions arranged in asymmetric patterns to provide a “barcode,” the authors could discriminate between different nanostructures containing probes for each gene, thus providing multiplexed assaying (Figure 5A). Using this approach, the authors were able to get semiquantitative estimates of target concentration and were able to demonstrate picomolar sensitivity. In a similar work, Fan, He, and colleagues used an asymmetric “Map of China” 2D origami nanostructure, which obviates the need for additional indexing agents for target identification during multiplexed assays. They utilized biotinylated DNA oligomers complementary to the target sequence, which enabled the use of streptavidin-biotin binding to provide a clearer AFM signal. However, this approach also increases cost by using modified oligonucleotides for each target.

Along with detecting specific genes, DNA origami biosensors have also been developed to detect single-nucleotide polymorphisms (SNPs). In subsequent work, Fan, He, and colleagues used their Map of China structure to discriminate between two DNA sequences containing a single-base mismatch. In this instance, the authors utilized a kinetically controlled toe-hold-mediated strand-displacement process in which a perfectly matched target sequence displaced a biotinylated oligomer from...
Figure 5. DNA Origami for Biosensing

(A) Detection of specific mRNA sequences after hybridization to a rectangular DNA origami template. From Ke et al. Reprinted with permission from AAAS.

(B) Detection of SNPs with a kinetically controlled toe-hold-mediated strand-displacement reaction. Reprinted with permission from Subramanian et al. Copyright 2011 American Chemical Society.

(C) Protein detection using DNA origami pliers. Binding of target molecules leads to a conformational change from X-shaped to parallel-shaped structures. Scale bars, 300 nm. Reprinted by permission from Macmillan Publishers Ltd: Nature Communications (Kuzuya et al.), copyright 2011.

(D) Mechanochemical sensing of PDGF with a chain-linked DNA nanostructure. Binding of target molecules leads to increases in structure flexibility and the distance between optical beads. Reprinted with permission from Koirala et al. Copyright 2014 Wiley-VCH.

(E) Funnel-like origami structure with a tail to guide the correct insertion of origami into nanopores and a current-versus-time graph of origami insertion into a solid-state nanopore. Reprinted with permission from Bell et al. Copyright 2012 American Chemical Society.

(F) Origami nanoplate on a nanocapillary with overhanging predator strands (top left) capturing prey strands (bottom left). Current traces show that CGCG prey strands (top right) are retained for a significantly longer time than AT prey strands (bottom right). Reprinted with permission from Hernández-Ainsa et al. Copyright 2013 American Chemical Society.

(G) Origami nanopore and current traces showing the translocation of a single DNA hairpin (HP). From Langecker et al. Reprinted with permission from AAAS.

(H) Large origami nanopore and current traces showing the translocation of dsDNA. Reprinted by permission from Macmillan Publishers Ltd: Nature Communications (Krishnan et al.), copyright 2016.
the nanostructure, leading to a loss of streptavidin binding. When a mismatched target was used, the strand-displacement reaction stalled at the mismatched base, and the biotinylated strand was not removed. Seeman and colleagues extended this work by using a similar kinetically controlled strand-displacement reaction to displace incumbent “signal strands,” leading to a double-stranded to single-stranded conformational change that could be visualized by AFM. The authors patterned the signal strands to form the characters A, C, G, and T within the same nanostructure, such that a specific mutation in the target sequence would lead to displacement of specific signal strands and the subsequent disappearance of the corresponding character (Figure 5B).

3D DNA nanostructures have been used for sensing specific DNA sequences by using a conformational change of the entire structure as the signal output. Komiyama and colleagues used a “plier”-shaped nanostructure that transitioned between three distinct parallel, anti-parallel, and X-shaped conformations upon target binding, whereas Shih, Bellot, and colleagues used a trapezoidal structure in which binding of the target sequence changed the angle between two arms.

Sensors for protein biomarkers have also been fabricated with DNA origami. Although the interface between DNA nanostructures and any arbitrary nucleic acid target is clear, the sensing of proteins is currently limited to a select number of targets, typically proteins that interact directly with DNA (enzymes, binding elements, proteins with aptamer targets, etc.) or with a small number of oligonucleotide modifications (biotin, fluorescein, etc.). Komiyama and colleagues were the first to use a DNA origami nanostructure to detect protein species by using their plier-shaped system. Binding of a target molecule to detection elements on both arms brings the two arms together, leading to a conformational transition between the energetically favorable X shape to the more unfavorable linear-parallel conformation (Figure 5C). This transition was monitored at the single-molecule level by AFM, as well as bulk measurements from FRET-fluorescence microscopy. Although the initial targets used for this study have limited downstream applicability (streptavidin and anti-fluorescein IgG bind to biotin and fluorescein, respectively), it is easy to envision this system adapting to more biomedically relevant species through the addition of new detection elements. The authors also used this system to detect other species, including specific metal ions (sodium, potassium, and silver), ATP, and pH changes.

Subsequent work by other groups extended the use of DNA origami nanostructures for protein biosensing to other target molecules. In 2014, Sugiyama, Mao, and colleagues used a mechanochemical-sensing system in which the presence of a target analyte led to a change in the mechanical properties of a DNA origami nanostructure, which could be detected with optical tweezers. The authors designed a seven-tiled, 2D structure with fixed linkages between tiles formed on one edge and a dsDNA “locking” duplex linkage on the other edge. If the “locking” strands were displaced, the flexibility of the tiled-structure drastically increased, and this change could be observed as a stepwise increase in distance with optical tweezers (Figure 5D). The authors demonstrated that this approach could be used for the sensing of platelet-derived growth factor (PDGF) if the locking strands were formed from a PDGF aptamer—in the presence of PDGF, the locking duplex dissociated in favor of the more energetically stable aptamer-protein interaction. Using this technique, the authors found that concentrations as low as 10 pM could be detected within 10 min. By using two different locking strands within the same tiled structure, they also demonstrated multiplexed assaying.
Other examples of protein-based biosensing using DNA origami nanostructures include the detection of restriction enzymes with the trapezoidal nanoactuator developed by Shih, Bellot, and colleagues\(^7\) and the use of aptamers on 2D DNA origami templates for detecting the malaria biomarker *Plasmodium falciparum* lactate dehydrogenase (PfLDH)\(^9\) and thrombin.\(^9\)

Additionally, DNA origami nanostructures have been used for detecting and studying dynamic processes of biomolecules at the single-molecule level. This work, pioneered by Endo and Sugiyama and discussed in detail in an excellent review, uses high-speed AFM imaging within a structurally rigid DNA origami frame to monitor changes in various substrates, including alternative nucleic acid conformations such as G-quadruplexes and i-motifs, as well as protein activities such as DNA methylation and repair.\(^9\)

**Biosensors Using DNA Origami Nanopores**

Nanopores have been used extensively in biosensing applications. This technology is capable of sensing molecules while foregoing the need for chemical modifications or amplification of the analyte, making it attractive as a versatile method for molecular detection.\(^10\),\(^11\) Application of a voltage across a thin membrane containing a single pore creates an electrophoretic force that causes molecules to translocate through the pore. These molecules displace the conducting ions in the pore, reducing conductivity and causing a measurable drop in current. Although the majority of applications use silicon-based membranes (solid-state nanopores), there is also interest in studying and using biological nanopores.

When solid-state membranes are used, producing nanopores of smaller (<10 nm) diameter requires time-consuming electron-beam lithography techniques, and precisely controlling the placement of binding sites is challenging.\(^10\) Thus, DNA origami has been explored as a tool for offering greater geometrical and chemical control of solid-state nanopores. Recent research has reported the use of DNA origami structures in conjunction with solid-state nanopores for the formation of nanopore-DNA origami hybrids. When subjected to an electrical current, the negatively charged DNA experiences an electrophoretic force toward the positive pole of the nanopore, leading a single origami to settle over the solid-state pore to form a nanopore-DNA origami complex. To demonstrate this concept, Keyser and colleagues used a funnel-shaped origami bearing a long tail (Figure 5E), which they threaded through the pore to allow the funnel to insert in the correct orientation.\(^9\) The DNA origami structures themselves can contain a pore with prescribed dimensions bearing various ligands or other modifications, so any molecules translocating through the nanopore-DNA origami complex could be subject to interactions with the nanostructure pores’ chemical modifications. One example of this has been demonstrated by the Keyser group, who added “predator” strands to the origami pore (Figure 5F).\(^9\) These strands bind the target “prey” strands, increasing their translocation time and creating a more pronounced current drop than non-specific molecules.

Separately from these solid-state nanopores, biological nanopores exist in abundance in all living organisms and are necessary for the intra- and extracellular trafficking of ions and molecules.\(^10\),\(^11\) Their important role in facilitating this transport has made them popular topics of study for understanding how cells move ions and biological material. Researchers have extensively studied nanopore proteins, such as α-hemolysin, and are now interested in designing and fabricating synthetic biological nanopores. Given the need to engineer these nanopores with precisely designed dimensions and characteristics, DNA origami has arisen as the technology...
of choice. With the customizability, versatility, and biological compatibility of DNA, nanopores fabricated from DNA are allowing researchers to better study existing biological pores as well as utilize these pores for various technical applications.

The first biological nanopores fabricated with DNA origami were “nanobarrels,” which contain a channel that can act as the pore. The seminal work in this field was by Dietz, Simmel, and colleagues, who incorporated a cap bearing 26 hydrophobic moieties between the barrel and a protruding stem (Figure 5G). These hydrophobic moieties allowed the structure to thermodynamically favor incorporation into lipid bilayers, effectively spanning the vesicle membrane to form an open pore. This work successfully demonstrated that functioning synthetic biological nanopores could be designed with precise geometrical dimensions.

Although Dietz, Simmel, and colleagues and other groups have demonstrated DNA nanopore integration into lipid bilayers, spontaneous insertion remains highly inconsistent. Subsequent work by the same group addressed this issue by using a new design featuring a larger moiety-bearing platform with a protruding stem of increased pore diameter. This design allows for the attachment of 57 hydrophobic moieties, enabling the biological pore to spontaneously insert into lipid membranes. In addition, these pores demonstrate increased physical and electrically stability and allow for the passage of larger analytes, increasing the potential of origami nanopores for use in versatile sensing applications (Figure 5H).

Challenges and Perspectives
Despite the advances made in biosensing using DNA origami, particularly at the single-molecule level, the technology still has a long way to go before it can live up to its considerable potential. For example, the current use of DNA origami in biosensing applications is limited by the scope of available analytes. The development of new methods for incorporating a wider variety of biomolecules into DNA origami nanostructures, including nitrilotriacetate conjugation for the incorporation of His-tagged proteins and methods for introducing single-stranded oligonucleotides onto specific locations on proteins, is already underway. For binding-based devices, the current techniques used for monitoring analyte-sensor interactions are limited by their relatively low throughput and technically challenging and expensive nature. Although AFM, TEM, and optical tweezers are excellent tools for single-molecule studies in research lab settings, new outputs are needed before DNA origami sensors become practical for real-world applications. Magnetic susceptibility and surface-enhanced Raman scattering are a couple of strategies that could be incorporated into DNA origami nanostructures to provide alternative sensing outputs. For DNA origami nanopores, a major challenge lies in the high ionic permeability of the origami structures. DNA origami nanostructures only a few layers thick leak ions readily, reducing the contrast between background current and a translocation event. To increase detection sensitivity, a method to reduce ion leakage must be developed. Progress in the field of DNA structures as biological nanopores has advanced quickly, such that bilayer integration and transport regulation have been achieved in just a few years. However, there have yet to be any reported studies introducing selectivity to these nanopores to allow for the detection of specific species. Additional modifications to the biological nanopore geometry or chemistry are needed in order for biosensing using this technology to have significant technical applications.

DNA ORIGAMI FOR DRUG DELIVERY
The field of nanomedicine is founded upon the development of nanotechnology, especially the capability of fabricating nanoscale objects to be utilized as delivery
vehicles for a variety of therapeutic cargos, including small molecules, proteins, nucleic acids, etc. DNA nanostructures have some essential characteristics of an effective, customizable delivery system: (1) good biocompatibility, (2) full addressability, and (3) control over size, shape, and surface chemistry. In particular, the addressability and control of nanostructure design are particular strengths that are infeasible with many comparable nanoparticle delivery systems. Although we specifically focus on DNA origami technology, comprehensive reviews of other DNA nanostructures for drug-delivery applications are available elsewhere.106,107

Delivery of Small Molecules
Anthracyclines (e.g., doxorubicin and daunorubicin) are a class of widely used anti-cancer drugs that can intercalate into DNA duplexes and thus inhibit macromolecular biosynthesis. DNA origami nanostructures have served as potent delivery vehicles for doxorubicin or daunorubicin, given that a large number of DNA bases are available for non-covalent loading of these drugs. The first study was reported in 2012 by Ding, Yan, and colleagues (Figure 6A), who utilized tubular and triangular DNA origami structures to deliver doxorubicin or daunorubicin to induce cytotoxicity in doxorubicin-resistant cancer cells.108 The DNA origami carriers led to more cellular accumulation of doxorubicin and cytotoxicity than free doxorubicin. In 2014, the same group used DNA origami structures to deliver doxorubicin for in vivo cancer therapy.117 Relative to controls, doxorubicin-loaded DNA origami showed significantly inhibited tumor growth. Hogberg and coworkers demonstrated that adjusting the global twisting of DNA origami structures can tune the encapsulation efficiency and release rate of doxorubicin (Figure 6B).109 They delivered doxorubicin into three different breast cancer cell lines by using DNA origami nanostructures and found that cytotoxicity was higher and the cellular elimination rate was lower than for free doxorubicin. In another study, Castro and colleagues designed a rod-like DNA origami structure to load and deliver daunorubicin into a drug-resistant leukemia model with significantly enhanced cytotoxicity.118

Delivery of Proteins and Nucleic Acids
Proteins and nucleic acids have long served as functional materials for disease diagnosis and therapy. Unlike the intercalating drugs, proteins and nucleic acids typically need to be loaded through Watson-Crick base pairing between capturing strands on the DNA structures and linking strands conjugated to proteins and nucleic acid cargos. In one study, Church’s group loaded antibodies into a DNA origami barrel nanostructure whose transition between open and closed states was controlled via aptamer-based “locks” (Figure 6C).110 Upon recognition of cell-surface receptor “keys” on the cell membrane, the barrel was opened to release Fab antibody fragments that bind to human CD33 and CDw328 to inhibit growth of leukemia cells. The logic-gated locks with specific recognition of cell membrane markers were designed to enhance targeting specificity. Beyond this in vitro computing, Bachelet and colleagues took DNA origami robot-based computing to in vivo models. They designed a series of DNA-origami-based logic gates that could conduct computational work in a cockroach animal model (Figure 6D). The authors envisioned that their DNA origami robot could be used for simultaneous delivery of multiple therapeutics in a responsive manner, although this was not explicitly demonstrated in the work cited here.111

The loading of functional nucleic acids onto DNA origami structures is relatively straightforward given the predictable Watson-Crick base pairing, such that traditional nucleic acid hybridization can be utilized. Synthetic oligonucleotides containing unmethylated cytosine-phosphate-guanosine (CpG) motifs are a potent activator of innate and acquired immune responses and act primarily through Toll-like receptor 9. The first report of CpG delivery using DNA origami structures was in
Figure 6. DNA Origami for Drug Delivery
(A) Triangular and tubular origami used for the delivery of doxorubicin show increased cytotoxicity. Reprinted with permission from Jiang et al. Copyright 2012 American Chemical Society.
(B) Tubular origami with tunable encapsulation and release properties for delivering doxorubicin. Reprinted with permission from Zhao et al. Copyright 2012 American Chemical Society.
(C) DNA origami hexagonal barrel for the delivery of therapeutic antibodies. Scale bars, 20 nm. From Douglas et al. Reprinted with permission from AAAS.
(E) Tubular origami for the delivery of Cpg oligonucleotides. Reprinted with permission from Schuler et al. Copyright 2011 American Chemical Society.
(F) Triangular and tubular origami for the delivery of AuNRs show higher tumor accumulation and temperature increases under laser irradiation. Reprinted with permission from Jiang et al. Copyright 2015 Wiley-VCH.
(G) Origami grown on AuNP for the delivery of doxorubicin. Reprinted with permission from Yan et al. Copyright 2015 Wiley-VCH.
(H) Rectangular origami encapsulated with viral proteins demonstrates enhanced cell internalization, as shown in the flow cytometry study. Reprinted with permission from Mikkila et al. Copyright 2014 American Chemical Society.
(I) Origami octahedron enveloped by lipids shows enhanced in vivo bioavailability. Reprinted with permission from Perrault and Shih. Copyright 2014 American Chemical Society.
2011 by Liedl and colleagues (Figure 6E). They designed a 30-helix tubular origami structure with 62 CpG molecules decorated on the surface of the structure. CpG-carrying DNA origami in spleen cells stimulated a significantly stronger immune response than equal amounts of CpG delivered with Lipofectamine or without delivery agents. Other functional nucleic acids, such as aptamers and small interfering RNAs, have been extensively used as cargo delivered by non-origami DNA structures. Although, to the best of our knowledge, no study has used DNA origami to deliver these functional nucleic acids, we believe this is an area of active research by several different groups.

**Hybrid Systems with Multiple Functionalities**

Another application of incorporating DNA origami structures with other materials is to achieve multiple functionalities. These materials include but are not limited to inorganic and organic nanomaterials, biomolecules, lipids, etc. Ding and colleagues attached AuNRs onto triangular and tubular DNA origami structures to enable simultaneous two-photon imaging and photothermal therapy after laser irradiation (Figure 6F). In vitro cellular studies demonstrated that the DNA-origami-AuNR complex had significantly higher cell internalization efficiency and cytotoxicity than AuNRs alone. In vivo, a higher accumulation of AuNRs at the tumor site and an increased survival rate of mice bearing xenograft tumors were observed for the complex structure. Fan, Song, and colleagues tethered short DNA oligonucleotides onto AuNPs, which they used as primers to initiate rolling circle amplification to generate a long, repetitive ssDNA protruding from the AuNP cores (Figure 6G). They used this long ssDNA as a scaffold to fabricate DNA origami structures around the AuNP core. They also incorporated cell-penetrating peptides and doxorubicin into this 3D superstructure to enhance cell internalization efficiency and to enable cytotoxicity, respectively. In vitro cellular experiments demonstrated that the as-fabricated 3D superstructures had a high drug-loading capacity and enabled the efficient transport of signal reporters and drugs for cellular imaging and chemotherapy.

**Improving DNA Origami’s Delivery Efficiency and Biostability**

Although DNA origami structures can enter cells alone, their internalization efficiency and biostability need be further improved if they are to survive physiological environments. Inspired by viruses, several groups have found that the encapsulation of DNA origami nanostructures by other materials, such as viral proteins and lipids, can drastically increase cell internalization efficiency and biostability. Kostiainen and colleagues used cowpea chlorotic mottle virus capsid proteins to coat and pack a planar rectangular DNA origami into either a tubular or planar complex via electrostatic interactions (Figure 6H). Compared with DNA origami alone, the encapsulated DNA origami showed a 13-fold increase in internalization in HEK293 cells. Confocal microscopy showed that the protein-encapsulated DNA origami was in close proximity to the cell nucleus, suggesting that this complex could potentially have nuclear uptake. Similarly, Shih and colleagues packaged a DNA origami octahedron into a PEGylated lipid bilayer to increase its in vitro and in vivo stability and bioavailability (Figure 6I). They demonstrated that the envelopment of DNA nanostructures into PEGylated lipids conferred protection against nuclease digestion, decreased immune activation by 2 orders of magnitude, and increased pharmacokinetic bioavailability by a factor of 17 in relation to that of non-encapsulated DNA origami nanostructures.

**Challenges and Perspectives**

Cost is among the most challenging hurdles ahead of DNA origami’s practical applications as a drug-delivery vehicle. Commercially available staple strands of a ~7,000 bp
origami structure at a synthesis scale of about 10 nmol typically cost several hundred dollars. Taking into account additional costs, such as the scaffold DNA, oligonucleotide functionalization, and purification of origami, the actual cost per single DNA origami design would be even higher. Thus, economic production methods of scaffold and staple DNA are urgently needed to lower this cost. In vivo production of DNA strands or even entire origami structures could potentially solve this issue, given that simple DNA tile structures have already been produced in vivo.\textsuperscript{119} In vitro and in vivo stability of DNA origami is another issue that needs to be addressed. Given the highly compact packing of DNA duplexes in these nanostructures, high concentrations of cationic ions (e.g., Mg\textsuperscript{2+} and Na\textsuperscript{+}) are required to neutralize the negative charge of the DNA backbone and thus stabilize DNA origami structures. Typical physiological solutions (e.g., PBS and medium) do not contain high enough concentrations of cationic ions to sufficiently stabilize the DNA origami structures. Many studies have been conducted to test the stability of DNA origami in solutions mimicking physiological conditions. They have found that less compact objects (e.g., wire-frame origami objects) are more stable in cation-depleted solutions.\textsuperscript{16,17,120} In addition, proteins in the medium can nonspecifically coat the DNA origami structures to form a “protein corona” that potentially offers protection against structure dissociation and nuclease digestion.\textsuperscript{121} Further investigations are needed to fully elucidate the stability issue of DNA origami structures. Although DNA origami structures have been experimentally demonstrated to efficiently enter cells, the specific endocytosis pathway remains to be unveiled via systematic mechanistic studies. Another challenging hurdle is that all studies have shown that DNA origami structures are eventually transported to lysosomes for digestion.\textsuperscript{108} To enable efficient cargo release into the cytosol, lysosomal escape of DNA origami vehicles might be necessary. Potential strategies include using targeting ligands to initiate uptake via a non-lysosomal pathway or conjugating functional molecules onto DNA origami to facilitate lysosomal escape. A better understanding of the pharmacokinetics and pharmacodynamics of DNA origami structures in vivo also needs to be studied before any clinical drug-delivery applications. Off-target issues are another challenge that needs to be addressed. The introduction of exogenous DNA has many potential complications: activation of a potent immune response, sequence-specific interference with mRNAs or microRNAs leading to unwanted gene regulation, and long-term integration into the genome. The chemical modification of the staple DNA strands, such as via the introduction of modified phosphoramidites or post-synthetic modification to render them biologically inert, could be one potential solution to these problems.

CONCLUDING REMARKS

In the 10 years since its inception, DNA origami has made remarkable strides toward a wide range of applications. In this review, we provide a relatively comprehensive overview of the advances in DNA origami technology and its broad applications in separate sections on DNA origami design, nanofabrication, biosensing, and drug delivery. In each section, we review and discuss representative works and conclude with challenges and perspectives from our point of view. Despite these many exciting achievements, DNA-origami-based applications are still in their infancy and have a long way to go before they can be used for real-world problems. It is worth noting that the strengths and limitations of DNA origami relatively depend on specific applications. For instance, cost is one of the major concerns for drug delivery, but it might not be as important for building an ultrasensitive biosensor platform that needs only trace amounts of DNA. Therefore, a case-by-case analysis should be conducted when DNA origami is used for certain types of applications. And for some applications that do not need high structural complexity (e.g., intracellular RNA imaging), simple DNA tile structures could be utilized instead of DNA.
origami. Here, we summarize the major challenges that need to be addressed on a general basis before DNA origami can be applied broadly:

1. Design: further simplified and automated design platforms need to be developed, especially for researchers outside the DNA nanotechnology field.
2. Cost: DNA is relatively expensive in comparison with other materials, so straightforward and economic production methods are necessary.
3. Scale up: the size of discrete origami structures is typically constrained within ~100 nm because of the length of M13 scaffold DNA, and thus alternative strategies need to be developed for size expansion.
4. Chemical functionality: DNA is a relatively chemically inert biomolecule, and thus facile methods for adding a wide variety of functionalities needs further development. Current methods for introducing additional reactivity, through the introduction of alternative nucleotides during synthesis or post-synthetic modifications, provide a good starting point but can be cost prohibitive.
5. Stability: DNA is relatively vulnerable to damage caused by harsh fabrication environments or physiological conditions; thus, it requires enhanced stability to survive such environments.
6. Immunogenicity: exogenous DNA has been shown to elicit potent immune responses, and thus methods for minimizing these reactions are needed, primarily for in vivo applications.
7. Defects: DNA origami structures contain assembly defects, which can hinder heteroelement or therapeutic incorporation. Optimizing structural designs (e.g., crossover pattern and staple length) and assembly conditions (e.g., Mg²⁺ concentration and a thermal annealing protocol) can help to minimize structural defects. Straightforward methods should also be developed to allow convenient examination of structural quality.

**AUTHOR CONTRIBUTIONS**

P.W. and T.A.M. investigated the literature and wrote and revised the manuscript. V.P. and P.K.D investigated the literature and wrote sections of the manuscript. Y.K. proposed the review topic, supervised the writing of the manuscript, and reviewed the manuscript.

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