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Review

Discrete DNA Three-dimensional Nanostructures: the Synthesis and Applications*

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Abstract Structural DNA nanotechnology, an emerging technique that utilizes the nucleic acid molecule as generic polymer to programmably assemble well-defined and nano-sized architectures, holds great promise for new material synthesis and constructing functional nanodevices for different purposes. In the past three decades, rapid development of this technique has enabled the syntheses of hundreds and thousands of DNA nanostructures with various morphologies at different scales and dimensions. Among them, discrete three-dimensional (3D) DNA nanostructures not only represent the most advances in new material design, but also can serve as an excellent platform for many important applications. With precise spatial addressability and capability of arbitrary control over size, shape, and function, these nanostructures have drawn particular interests to scientists in different research fields. In this review article, we will briefly summarize the development regarding the synthesis of discrete DNA 3D nanostructures with various size, shape, geometry, and topology, including our previous work and recent progress by other groups. In detail, three methods majorly used to synthesize the DNA 3D objects will be introduced accordingly. Additionally, the principle, design rule, as well as pros and cons of each method will be highlighted. As functions of these discrete 3D nanostructures have drawn great interests to researchers, we will further discuss their cutting-edge applications in different areas, ranging from novel material synthesis, new device fabrication, and biomedical applications, *etc*. Lastly, challenges and outlook of these promising nanostructures will be given based on our point of view.

Keywords DNA self-assembly; DNA 3D nanostructure; Nanocarrier; Biosensor; Nanomedicine

INTRODUCTION

DNA, the well-known genetic information carrier in biology, is also a generic polymer with precise molecular weight that can be employed as building blocks for the construction of nanostructures and nanodevices based on its well-established molecular-recognition and capability of programmable self-assembly. Since the concept proposed by Dr. Nadrian Seeman with the dream that utilizes DNA as structural units to build a crystal-like lattice for protein immobilization and resolving their structures in the early 1980s, DNA nanotechnology, as a single spark, has dramatically kindled a prairie fire in both chemistry and materials science. Similar to many other techniques, the development of DNA nanotechnology also follows the principle of going from simple to complex.

The principle of DNA nanotechnology can be clearly illustrated in Seeman's pioneer work, where stable Holliday junction with four sequence-specific strands was designed and constructed (Fig. 1A)[1–3]. Compared to conventional linear DNA duplex, Holliday junction is a branched structure, which plays key role in DNA

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nanotechnology. At each edge, a short overhang (sticky end) theoretically can be included and enables the branched tiles further assemble into infinite 2D lattices. Based on this concept, many efforts have been put by Seeman and his colleagues to construct more complicated structures. In early 1990s, with more knowledge and skills learned from initial experiments, several complicated 3D polyhedral structures were successfully synthesized. At that moment, however, the design and synthesis of a well-defined DNA nanostructure was a tedious work compared to today's facile DNA self-assembly. For instance, to synthesize a DNA cube or truncated octahedron, multi-step laborious ligations were involved and the overall yield was extremely low[4, 5]. Later on, more robust and versatile DNA tiles, such as double crossover (DX)[6], triple crossover (TX)[7], tensegrity triangle[8], paranemic crossover (PX)[9, 10], multi-arm junction, *etc.*, have been invented for complex nanostructure self-assembly[11–13]. Afterwards, the field of structural DNA nanotechnology has experienced the rapid development and keeps steady growth. With more nanostructures synthesized by DNA nanotechnology, scientists have established multitude of design rules for different strategies, which significantly enhanced the efficiency and complexity of the DNA nanostructure self-assembly. To date, a large variety of one-, two-, and three-dimensional (1D, 2D, and 3D) nanostructures have been engineered using different assembly methods[14–17] and almost any desired DNA nanostructures that one can imagine could be designed and constructed. The rapid development of DNA nanotechnology somehow has significantly renewed our understanding of DNA molecules. In history, the illustrations of DNA structures and their functions let us fully understand the mystery of life on our planet. In contrast, the manipulation and programmable assembly of nucleic acids with DNA nanotechnology allow us to create hundreds and thousands of arbitrary nanostructures with different functions. To the best of our knowledge, it is the first time that a technique grasped by human beings can synthesize so many artificial objects similar to naturally existing components which are created by millions of years' evolution.

Along its trajectory of development, programmable DNA self-assembly moves forward faster and faster, especially in the past decade. Fortunately, we have been intensively engaged in this hot research area and put a lot of efforts on synthesizing a series of highly symmetric and discrete 3D nanocages with rational design in the past years[18–21]. We are particularly interested in complicated 3D structure synthesis not only aiming at building more fancy nanostructures but also trying to explore their functions for a wide range of applications. Compared to 1D and 2D architectures previously synthesized, 3D nanostructures not only expand the structural scope of the DNA-based complex nanomaterials, but also provide a unique platform for interacting with other larger molecular systems in a versatile fashion. Taking advantage of the sequence specificity and spatial addressability of the resulting DNA nanostructures, they have been widely used to direct heteroelements' arrangement, chemical reactions, and biomolecular interactions, *etc*. For instance, DNA 3D structures have been employed as templates to direct the assembly of inorganic nanoparticles and to synthesize photonic metamaterials[22, 23]. Their unique size and geometry are highly feasible to serve as delivery vehicles for diagnostic and therapeutic purposes[24, 25]. In particular, their biocompatible and 3D features at nanometer scale are naturally suitable for building biomimetic systems. As we know, in living organism, proteins play as the most important molecular machines to realize versatile biological functions. The mimicry of artificial molecular machines (*e.g.* proteins) and highly ordered complex structures (*e.g.* organelles) is an attractive but challenging task to scientists. To date, it is still far away to realize the protein mimetics since it is difficult to predict the folding and assembling behaviors of long peptide chains. Alternatively, one can expect that robust DNA nanostructures with well-designed morphologies and functions may resemble the subcellular molecular machines and do similar work. As such, we can envision that the discrete DNA 3D nanostructures with arbitrary shapes and functions may confer significant potentials in bioscience and biomedical studies.

In literature, many review papers have intensively summarized the history, state of the art architectures, the cutting-edge of recent assembling methods, and various applications of DNA nanotechnology[1, 26–28]. To be different, in this review paper, we will particularly focus on the discrete DNA 3D objects, and briefly introduce their development, current stage, and future challenges, as well as discuss their applications. Herein the term "discrete DNA 3D nanostructures" is used to describe the self-assembled DNA architectures with precise 3D geometries and the length of each dimension is larger than the thickness of a single DNA duplex. With such criteria, single-layered 1D and 2D DNA self-assemblies synthesized before are excluded. Meanwhile, DNA

hydrogel also will not be mentioned due to the lack of precise control over size and geometry, although it is an important DNA 3D assembly. In the following sections, we will first concentrate on the recent advances of how to synthesize various discrete DNA 3D nanostructures using different strategies. With special emphasis on the methods developed in recent years, we will demonstrate the powerful capability of DNA nanotechnology to create many fantastic nanostructures in a precisely controlled manner. For each strategy, the design principles will be discussed and their advantages and drawbacks will be illustrated. Further, as the foci of DNA nanotechnology have gradually shifted to finding their potential use in different areas, we would like to highlight recent progress on their applications. Self-assembled DNA nanostructures modified with artificial functional moieties for multi-purposes, including serving as drug carriers, sensing probes, and nano-templates, will be summarized. In the last part, we will give short conclusive remarks of this particular subject, the challenges and perspectives on these promising 3D nanostructures will be rationalized.

SYNTHESIS OF THE DISCRETE DNA 3D NANOSTRUCTURES

Although the synthesis of DNA 3D nanostructures, such as DNA cube, truncated octahedron was considered as the real start of DNA nanotechnology era[4, 5], the well-controlled 3D nanostructure self-assembly remains as a challenging work for a long time at the early stage of this technique. It could be noticed that the first several DNA nanocages with highly symmetric geometry were assembled by deliberate and tedious ligations, which was not an efficient way for the synthesis of more complicated nanostructures. Meanwhile, each strut of these DNA frame cages contains a bare single duplex, resulting in very flexible 3D objects that can be easily deformed. Therefore, facile and one-pot synthesis of 3D nanostructures with satisfied yield is highly motivated. In general, DNA nanotechnology relies on Watson-Crick complementarity of A-T and C-G base-pairing which allows the nucleic acid strands self-assemble into pre-designed and highly ordered structures in a controllable fashion. In rare cases, nonclassical pairing patterns, such as G-rich or C-rich single-stranded nucleic acids self-assemble into G-quadruplexes, i-motifs, triplexes, and parallel-stranded duplexes are also possible to be employed for DNA nanostructure self-assembly[29, 30]. Along with the development of structural DNA nanotechnology, thus far, three different major strategies, including tile-based bottom-up self-assembly, DNA origami, and single stranded tile (SST) self-assembly, have been invented and applied for 3D nanostructure self-assembly. Based on each method or their combinations, a large variety of DNA 3D assemblies have been constructed especially in the past decade. In this section, we will focus on their syntheses and describe how each method works and what nanostructures they can make.

DNA Tile-based 3D Nanostructures

Tile-based method is a typical bottom-up self-assembly that employs a few DNA strands to form structural building blocks (DNA tiles) first. Then multi-copies of the DNA tiles further assemble together *via* sticky-end association, resulting in highly ordered 3D objects. As shown in Fig. 1(A), Holliday junction is a typical DNA tile consisted of four single strands. By changing the numbers of the component strands, similar junction tiles with varied arms can be designed, which have been widely employed as building blocks for larger structure assembly. For instance, Luo's group once utilized Y-shaped tiles to construct dendrimer-like DNA architectures with a controllable manner[31]. The resulting dendrimer-like DNA was relatively stable and almost monodisperse. Their size was easily tuned by designing different number of generation for the dendrimer formation. Furthermore, Liu and coworkers introduced DNA i-motif into the Y-shaped tiles and the size of the resulting dendrimers could be changed in response to varied pH values[32]. However, these simple junction tiles are usually flexible, preventing them from fabricating sophisticated 3D nanostructures. Therefore, more rigid DNA tiles with multiple crossover junctions are frequently designed to enable the controllable DNA nanostructure self-assembly. In general, symmetric design is normally introduced to these building blocks to reduce the use of component DNA strands. Although rigid DNA tiles have made big success in the synthesis of a large variety of brickwork-like periodic 2D lattices[7, 33, 34], how to assemble them into well-defined 3D structure has remained as a challenging work since a long time. Inspired by viral capsid self-assembly (Fig. 1B) and also the curved feature of a 3D object, we have realized that the tiles used for 3D assembly have to bend from its original geometric plane[35]. Based on this consideration, in our previous work, we exploited sequence symmetry to design a series

of DNA point-star motifs with higher flexibility compared to the tiles used for 2D array construction. In our design, each arm of the point-star motif contains a rigid double crossover and the adjacent arms are linked by a single-stranded free loop which provides the tile with appropriate flexibility. By balancing the rigidity and flexibility, DNA tiles are capable of forming a large variety of highly symmetric 3D wireframe polyhedral structures (such as tetrahedron, octahedron, dodecahedron, icosahedron, and Bucky ball, Figs. 1C–1E) under different conditions[18–21]. In our design, curvature and flexibility of the DNA point-star motif are mainly tuned by the length of free hinge loops. When significantly elongating the free loops, the point-star motif can completely bend from its original geometric plane to form a dimer through sticky-end association. As such, tube like DNA nanostructures with defined diameters and lengths can be obtained[36]. Moreover, multitude of DNA tiles can be simultaneously used for the 3D nanostructures. As an example, Mao's group have developed a directed self-assembly strategy to synthesize a range of complex 3D structures (Fig. 2A) by changing flexibility of DNA tiles and using two types of DNA tiles: directing tiles (D-tiles) and assembly tiles (A-tiles) in the system[37]. All of the synthesized DNA polyhedral objects have triangular faces and therefore are non-deformable structures. Recently, Yan and co-workers reported a set of new design rules for engineering more complicated wireframe DNA nanostructures using similar DNA point-star motifs, by which size-limited DNA wireframes with varied symmetries were assembled (Fig. 2B)[38]. Further, Yan's group used four-arm junctions as the building blocks to assemble 3D curvature structures. The 3D architecture was formed by integrating vertices represented by different numbers of multi-arm junctions with controlled angles and DNA crossover tiles by scaffold strands. Through this approach, gridiron-like 3D architectures and spherical hollow containers were achieved by shifting the distance of vertical directions between junctions (Fig. 2C)[39].

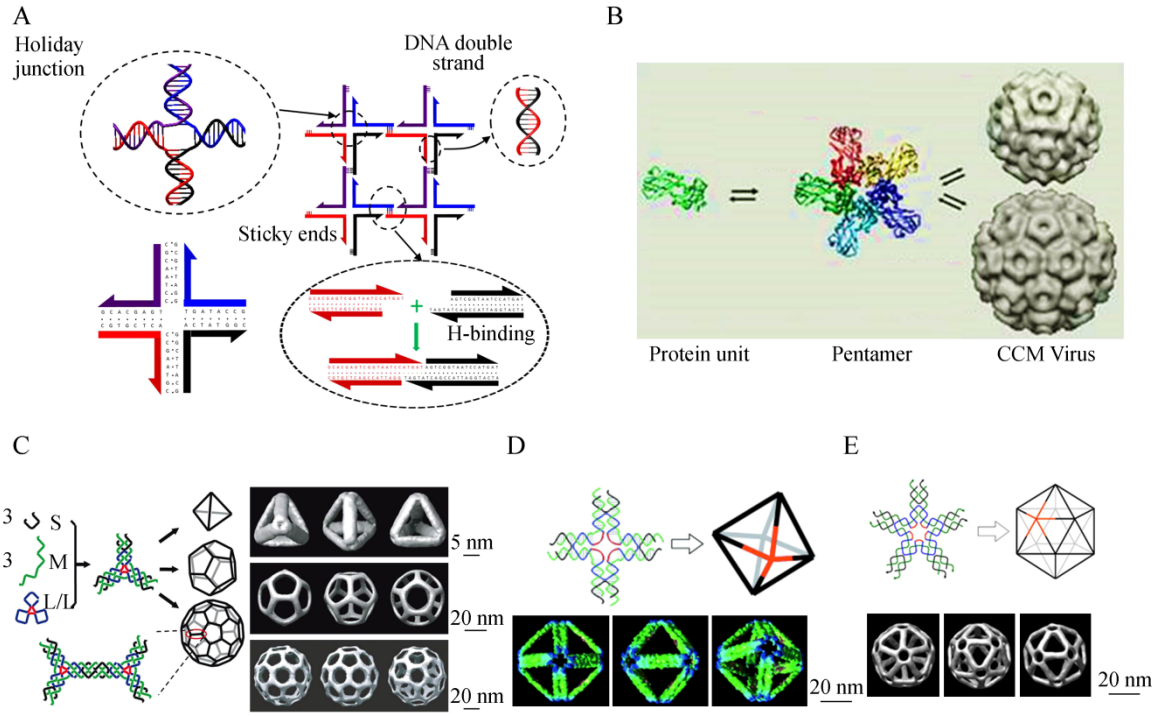

Fig. 1 The principle of DNA nanotechnology and DNA 3D self-assembly based on point-star tiles: (A) The structure of Holliday junction and the principle of DNA self-assembly; (B) A representative process of viral capsid self-assembly (Reproduced by the permission from Ref. [35]; Copyright (1999) Proceedings of the National Academy of Sciences of the United States of America (PNAS)); (C) A tetrahedron, a dodecahedron and a buckyball synthesized from three-point-star DNA tiles and their corresponding cryogenic electron microscopy (cryo-EM) images (The flexibility of the tiles was determined by the length of red segments in the central strands.) (Reprinted with permission from Ref. [18]; Copyright (2008) Nature Publishing Group); (D) An octahedron, synthesized from four-point-star DNA tiles (Reprinted with permission from Ref. [19]; Copyright (2010) John Wiley & Sons Inc); (E) An icosahedron assembled from five-point-star DNA tiles with similar strategy (Reprinted with permission from Ref. [20]; Copyright (2008) PNAS)

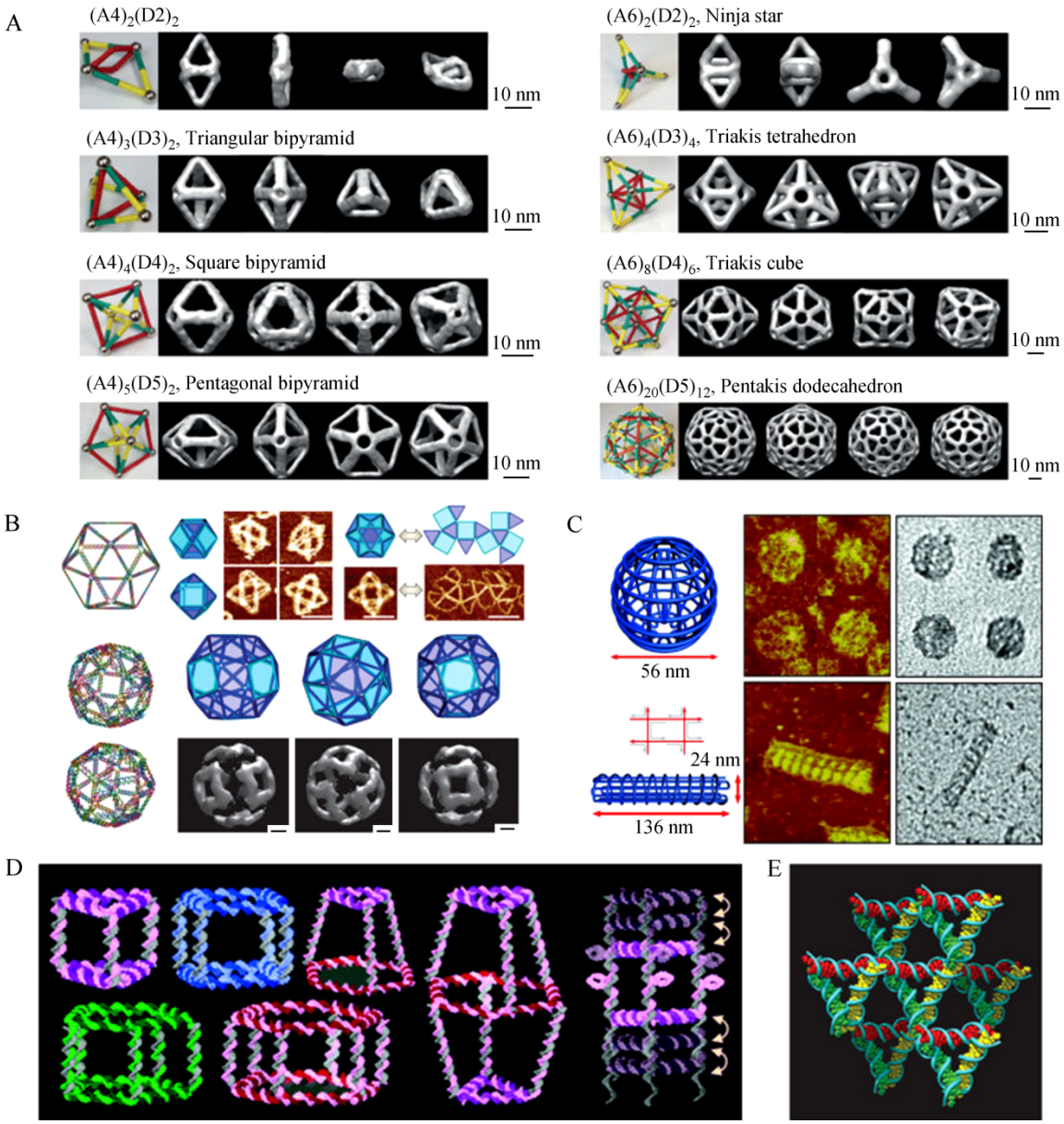

Fig. 2 3D objects assembled by various DNA based-tiles: (A) 3D structural models of the DNA complexes that gained by the combination of two types of DNA tiles and their corresponding cryo-EM images (Reprinted with permission from Ref. [37]; Copyright (2014) John Wiley & Sons Inc); (B) Wireframe Archimedean solid structures including the model of cuboctahedron and corresponding atomic force microscopy (AFM) images, the model of DNA snub cube and its cryo-EM images (scale bar = 10 nm) (Reprinted with permission from Ref. [38]; Copyright (2015) Nature Publishing Group); (C) Spherical structure and tube structure of DNA 3D origamis (Reprinted with permission from Ref. [39]; Copyright (2013) American Association for the Advancement of Science (AAAS)); (D) Triangular, cubic, pentameric, and hexameric prism, heteroprism and biprism assembled with cycle strands that consist of several reporting units (Reprinted with permission from Ref. [40]; Copyright (2007) American Chemical Society); (E) A 3D periodic crystal formed by tensegrity triangles (Reprinted with permission from Ref. [43]; Copyright (2009) Nature Publishing Group)

The key idea of DNA nanotechnology is to design branched structural units for the supramolecular self-assembly. Besides the aforementioned tiles assembled by pure DNA strands with rational designed sequence, some rigid and symmetric organic molecules can be inserted into the DNA strands to serve as branching point and to direct the orientation of DNA self-assembly. Sleiman and her colleagues have extensively investigated the

incorporation of *m*-terphenyl-based organic vertices for modular synthesizing a series of discrete DNA polyhedra with good control over their geometry[40], dimension[41] and flexibility[42]. For instance, they first synthesized cyclic DNA 2D building blocks with organic molecules as vertices and further assembled them into 3D discrete objects in the existence of a number of single strands. Through this facile method, triangular, cubic, pentameric, hexameric prisms and the more complex heteroprism and biprism assemblies were successfully synthesized (Fig. 2D)[40].

When a DNA polyhedral nanostructure is to be synthesized, no matter which DNA tiles listed above are used, it requires all of the tiles bending accordingly to form a closed structure. Therefore, the size of as-synthesized 3D objects is usually small, ranging from several nanometers to several hundred nanometers in diameter. As mentioned above, rigid tiles such as DX, TX motifs originally designed for the self-assembly of 2D lattice are a type of planar structures, where sticky-ends at all branches locate in the same geometric plane. In contrast, if the sticky-ends on a DNA tile are intrinsically distributed in a stereoscopic fashion, they can facilitate the tiles' association in 3D space. As such, macroscopic crystal structures will be generated. Based on this idea, Mao and Seeman invented a triangular tensegrity DNA tile with three-fold rotational symmetry to realize the first self-assembled DNA 3D crystal with the pre-designed lattice parameters[43]. With ten years' continuous efforts, the crystal quality had been improved a lot and the atomic structure was determined by X-ray diffraction with 0.34 nm resolution (Fig. 2E). This achievement is a significant step to realize Seeman's original dream and clearly demonstrates that it is possible to design and construct a well-ordered macromolecular 3D crystalline lattice with precise control at atomic scale.

Overall, the tile-based DNA 3D assembly is a one-pot and hierarchical self-assembling process. Owing to the different strength of DNA hybridization between each component strand, DNA tiles form first in the solution and then further assemble into well-defined 3D objects *via* short sticky-ended cohesions. In general, symmetric design is normally applied to the DNA tile to reduce the total number of DNA strands used in the system. Therefore, only limited numbers of DNA strands are required to synthesize a discrete DNA nanostructure, which significantly simplifies the sample preparation and reduces the cost. Meanwhile, as a large family of DNA tiles has been designed so far, 3D nanostructures can be ready to synthesize by using different tiles at varied assembling conditions. On the other hand, the drawback of tile-based self-assembly is also obvious. Precise stoichiometry of DNA strands is required to obtain right 3D architectures with high yield. Most of the 3D nanostructures assembled by tile-based strategy are framework objects with large cavity inside. Their rigidity becomes a concern in some specific applications, *e.g.* when serving as nano-template to organize large inorganic nanoparticles. Also, the functionalization of these nanostructures may also encounter difficulties since introducing extra DNA strands or molecules into these all base-paired nanostructures may interfere their assembly process and reduce the overall yield.

DNA Origami-based 3D Nanostructures

The second strategy to construct DNA 3D nanostructures is based on a technique called DNA origami. As early as in 2004, Shih and co-workers once employed five 40-nt short helper strands to fold a 1669-nt long DNA scaffold into an octahedron (Fig. 3A), representing a landmark work that laid the foundation of the following DNA origami[44]. Later in 2006, the concept of DNA origami was proposed by Rothemund who systematically investigated its capability by employing a long piece of genomic DNA from the M13 bacteriophage and folding it into a large variety of pre-designed patterns with the assistance of hundreds of staple strands[45]. The invention of DNA origami is regarded as one of the most important breakthroughs in structural DNA nanotechnology. This method offers another general strategy for one-pot synthesis of versatile DNA nanostructures with well-defined shape instead of tile-based self-assembly. To design a DNA origami, the sequences of the staple strands and the number of connectivity of DNA between crossover points need to be carefully considered to achieve the desired shapes. Nowadays, a number of software packages based on computational method have been developed to assist scientists in the design of origami, by which the long single-stranded DNA scaffold is modeled to be folded into arbitrary shape and then the sequences of the short staple strands can be proposed[46, 47]. Compared to

tile-based 3D self-assembly, the nanostructure synthesized by origami strategy is also derived from numerous crossover events between neighboring duplexes, but their complicity and rigidity are much higher than in the former cases due to the large number of crossovers existed in the relatively solid structure. After its invention, DNA origami has become as a powerful tool and has constructed a large number of complex structures. Although DNA origami was initially used to fold the long genomic DNA into arbitrary 2D structures, a leap of building versatile 3D objects was made in 2009 using this method[48]. Based on their structural differences, DNA 3D origami can be divided into multiple categories, including single-layer hollow container structures, closely packed parallel-helix structures, structures consisting of bent or twisted DNA helices, intricate DNA bricks, and wireframe DNA origami. For their synthesis, three different strategies have been employed to construct the DNA 3D origami. The first way is to fold single-layer 2D origami sheets into the 3D single-layer cages[24, 49–52], including a tetrahedron (Fig. 3B)[51], a barrel-shaped nanorobots[24], and a cubic box with a controllable lid (Fig. 3C)[52]. The second strategy is to pack single-layer DNA origami into closely packed 3D solid structures. This approach is a general design strategy for construction of rigid DNA structure. DNA duplexes in a multi-layer origami can be packed into 3D lattices[48, 53–55] such as honeycomb lattices (Fig. 3D), which are not only able to form nanostructures such as monoliths, square nuts, railed bridges, and genie bottles, but can also form hierarchical assemblies of structures such as heterotrimeric wireframe icosahedra[48]. Later Shih *et al.* also created tensegrity 3D origami structures with high strength-to-weight ratios and great resilience[55]. In their design, multiple segments of unpaired scaffold were used for connecting rigid DNA helix-bundle units into 3D shapes (Fig. 3E). The third strategy to create DNA 3D origami is to build the structures by either twisting or bending a multi-layer DNA origami in a controllable manner. This can be achieved by adding and deleting base-pairs at specific sites in a block of honeycomb duplexes to create regions of local strain. The twisting and bending of a regular multi-layer DNA origami structure finally resulted in 3D objects with specific shape. Shih *et al.* used this method to create a group of multi-layer 3D nanostructures with precisely controlled curvatures (Fig. 3F)[56]. Yan and co-workers further developed this strategy and tuned the curvatures of 3D objects to control their topology. For instance, they successfully made a Mobius strip, the first example of topological DNA 3D nanostructure, by precisely tuning the bending and twisting of a DNA 2D origami[57]. Later, they synthesized a series of more interesting single-layer 3D DNA containers with complex curved surfaces of different degrees, including 3D spherical shells, ellipsoidal shells, and a nanoflask (Fig. 3G)[58]. These complex 3D objects need curvatures both in-plane generated by concentric rings and out of the plane realized by adjusting the relative position of crossover points between DNA double helices.

More recently, Högberg and co-workers developed a highly automated origami design method of assembling arbitrary DNA polygonal digital meshes including helix rods, waving stickman, a bottle, and a bunny (Fig. 3H) with a volume inflatable ball. Compared with conventional close-packed helices brick-like shapes, these 3D objects only had one helix per edge as structural elements[59]. The assembly of 3D complex architectures were based on A-trails routeing theory and relaxation simulations, which was difficult to implement using normal origami strategy mentioned above. Moreover, Bathe group developed a top-down strategy to design nearly arbitrary DNA architectures (Fig. 3I) by completing DNA scaffold routing with a spanning tree algorithm instead of operation manually[60]. Through this method, tetrahedra of different edge lengths, an octahedron, two pentagonal bipyramids, a cube, a reinforced cube, an icosahedron, and a cuboctahedron have been folded with scaffolds of custom length and sequence gained by the asymmetric polymerase chain reaction. These new 3D polyhedral DNA structures did not have tightly packed helices like conventional origami structures, but were chemically stable in low-salt conditions in consistence with physiological environments.

The advantages of DNA origami are not only to have provided a tool to construct versatile and rigid 3D nanostructures, but also overcome the stoichiometry issue that exists in the tile-based self-assembly. Normally, excessive staple strands are added for the folding process. As such, the high local concentration of helper strands can frequently hybridize onto the long scaffold strand, which drives the system toward the formation of pre-designed product. Owing to its powerful capability in designing rigid and complicated nanostructures, currently, the number of DNA 3D objects assembled by origami method is much larger than that of tile-based structures.

However, some drawbacks of this method are accompanied. One obstacle is their cost since hundreds of synthetic oligonucleotides are needed for each architecture, and the overall price for each origami set is high, preventing their large scale synthesis. Meanwhile, the types of long scaffold DNAs are limited. Currently, most of the nanostructures are based on genomic DNA of M13 macrophage with a certain length (~7 kilo-bases), resulting in a relatively fixed size for the final product. Moreover, although the software can be used to assist the design of DNA origami, how reliable of the exact structure folded by hundreds of designed staple strands is not very clear.

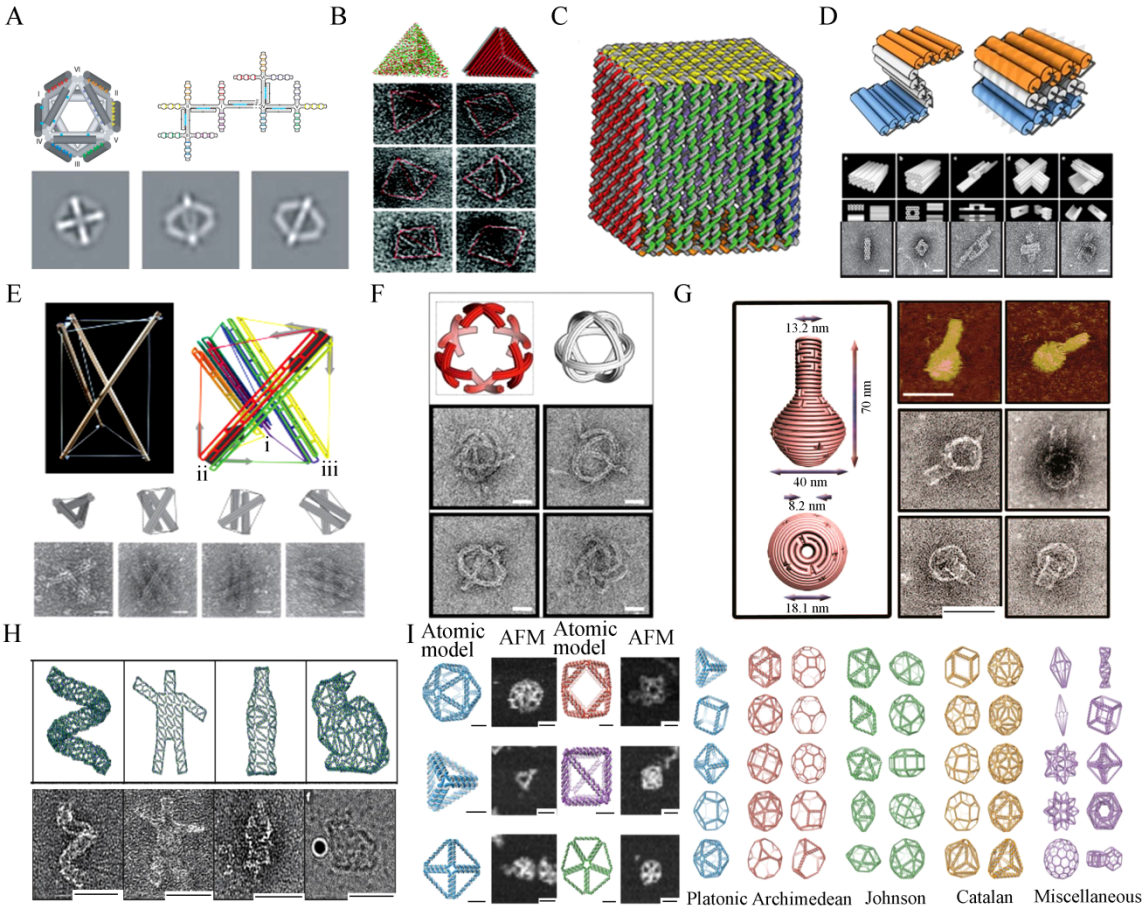

Fig. 3 3D nanostructures assembled by DNA origami: (A) A DNA octahedron composed of a 1.7 k-nt long DNA scaffold and five staple strands and its cryo-EM images (Reprinted with permission from Ref. [44]; Copyright (2004) Nature Publishing Group); (B) A single-layer DNA hollow tetrahedron and its transmission electron microscopy (TEM) images (Reprinted with permission from Ref. [51]; Copyright (2009) American Chemical Society); (C) DNA box with a controllable lid (Reprinted with permission from Ref. [52]; Copyright (2009) Nature Publishing Group); (D) Honeycomb lattices assembled from parallel DNA helices by the staple strands and their corresponding TEM images (scale bar = 20 nm) (Reprinted with permission from Ref. [48]; Copyright (2009) Nature Publishing Group); (E) A tensegrity 3D DNA structure and its TEM images (scale bar = 20 nm) (Reprinted with permission from Ref. [55]; Copyright (2010) Nature Publishing Group); (F) A DNA 3D nanostructure with curvature and its TEM images, curvatures were generated by adding DNA base pairs from the convex faces and deleting base pairs from the concave faces of multi-helix bundle struts (scale bar = 20 nm) (Reprinted with permission from Ref. [56]; Copyright (2009) AAAS); (G) 3D single-layer DNA origami nanoflask with fine curvatures and its TEM images (scale bar = 50 nm) (Reprinted with permission from Ref. [58]; Copyright (2011) AAAS); (H) The design and cryo-EM images of DNA polygonal digital meshes (scale bar = 50 nm) (Reprinted with permission from Ref. [59]; Copyright (2015) Nature Publishing Group); (I) Scaffolded DNA origami nanoparticles and its AFM images (left), 45 diverse scaffolded DNA origami nanoparticles (right) (Scale bars of atomic models and AFM images are 10 and 20 nm, respectively.) (Reprinted with permission from Ref. [60]; Copyright (2016) AAAS)

Single Stranded Tile-based 3D Nanostructures

As described earlier, tile-based self-assembly utilizes multitude of DNA strands to form a rigid motif first, and then assembles them into various nanostructures. To be an extreme case, can a single stranded DNA perform as an individual tile for complicated nanostructure self-assembly? The answer is "yes". Recently, the concept of single-stranded tile (SST) based 3D self-assembly was defined by Yin and co-workers. In this method, the sequence of each single-stranded DNA and their sticky-end associations are deliberately designed. Then they successfully modulated the assembly using only short synthetic strands called DNA bricks to synthesize a large variety of complex discrete 3D objects (Fig. 4A)[61]. Impressed by their obtained structures, it reminds us that many 3D objects previously assembled by multiple individual DNA strands can also be classified as SST self-assembly. For example, Seeman's DNA wireframe polyhedra, including cube[4] and truncated octahedron[5] assembled by ligation (Figs. 4B and 4C), and Turberfield's one-pot synthesized tetrahedrons[62] constructed by four single-stranded strands (Fig. 4D), belong to this category. With increased number of single-stranded DNAs in SST assembly, DNA nanotubes can be constructed through the formation of circular bundle which contains varied number of DNA duplexes. As shown in Fig. 4(E), one of the nanotubes consisting of six DNA duplexes can be synthesized using 14 DNA strands[63]. SST based self-assembly represents another conceptual breakthrough for DNA nanostructure synthesis. It can form prescribed 3D shapes by one-step annealing reactions without considering the purification and stoichiometry of DNA strands. In general, the synthesis yield has an inverse relationship to the total number of DNA strands used in the system. The annoying issue of this method is that the sequence design becomes too complicated when large structures are synthesized.

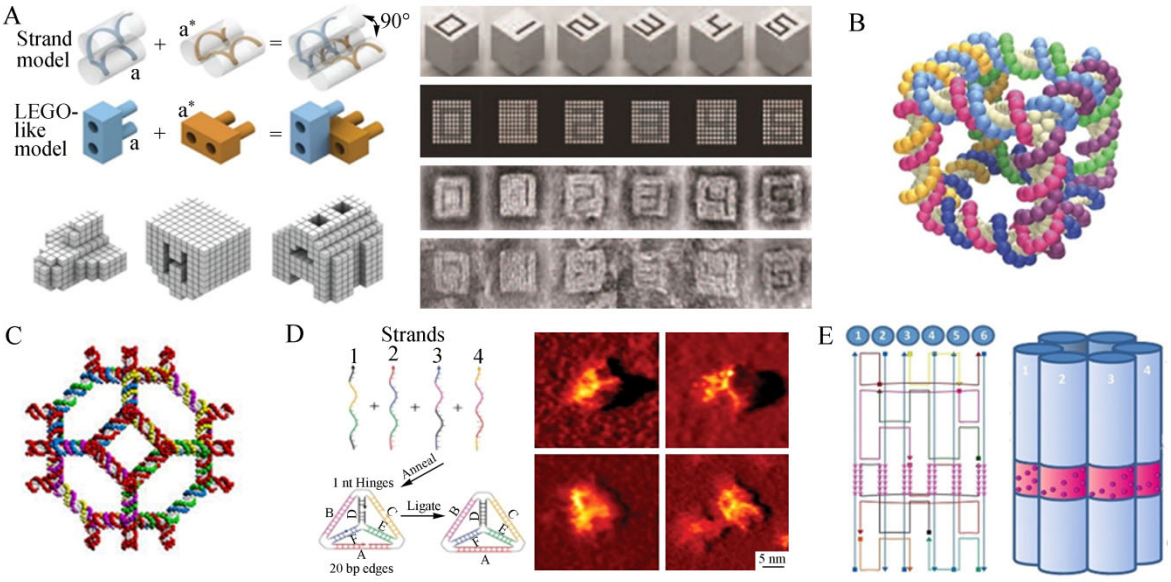

Fig. 4 SST-based 3D nanostructures: (A) Complex 3D discrete structures synthesized from only short strands DNA units and its TEM images (Reprinted with permission from Ref. [61]; Copyright (2012) AAAS); (B) A DNA cube containing twelve equal-length double-helical edges with eight vertices and achieved through multi-step assembly (Reproduced by the permission from Ref. [4]; Copyright (1991) Nature Publishing Group); (C) A DNA truncated octahedron containing six squares and eight hexagons and assembled by combining individual polygons (Reproduced by the permission from Ref. [5]; Copyright (1994) American Chemical Society); (D) A DNA tetrahedron assembled from four DNA single strands and its corresponding AFM images (Reprinted with permission from Ref. [62]; Copyright (2005) AAAS); (E) Schematic representation of a DNA nanopore composed of six interconnected duplexes represented as cylinders (Reprinted with permission from Ref. [63]; Copyright (2013) American Chemical Society)

Self-assembled DNA 3D Complex Nanostructures

In previous parts, we have introduced three different strategies commonly used in DNA 3D nanostructure self-assembly. Nevertheless, the synthesis of 3D objects is not restricted to any one of the methods above.

Consequently, more complex structures can be obtained by combination of different strategies in their synthesis. For example, giant DNA tiles can be synthesized by DNA origami or SST strategy, then these giant tiles can be further assembled into 3D structures following tile-based assembling rule. A representative work was done by Yin *et al.*, who designed a three-point-star motif called "tripod" using origami method. By adjusting the angle between the arms, a series of giant DNA prisms were synthesized, with much bigger size than those obtained by conventional tile assembly (Fig. 5A)[64]. Each tripod has the mass of 60 times more than that of our previous three-point-star tiles. Finally, giant DNA-origami polyhedrons, including tetrahedron, triangular prism, cube, pentagonal prism, and hexagonal prism, were constructed using such 5-MD tripods with different designed inter-arm angles.

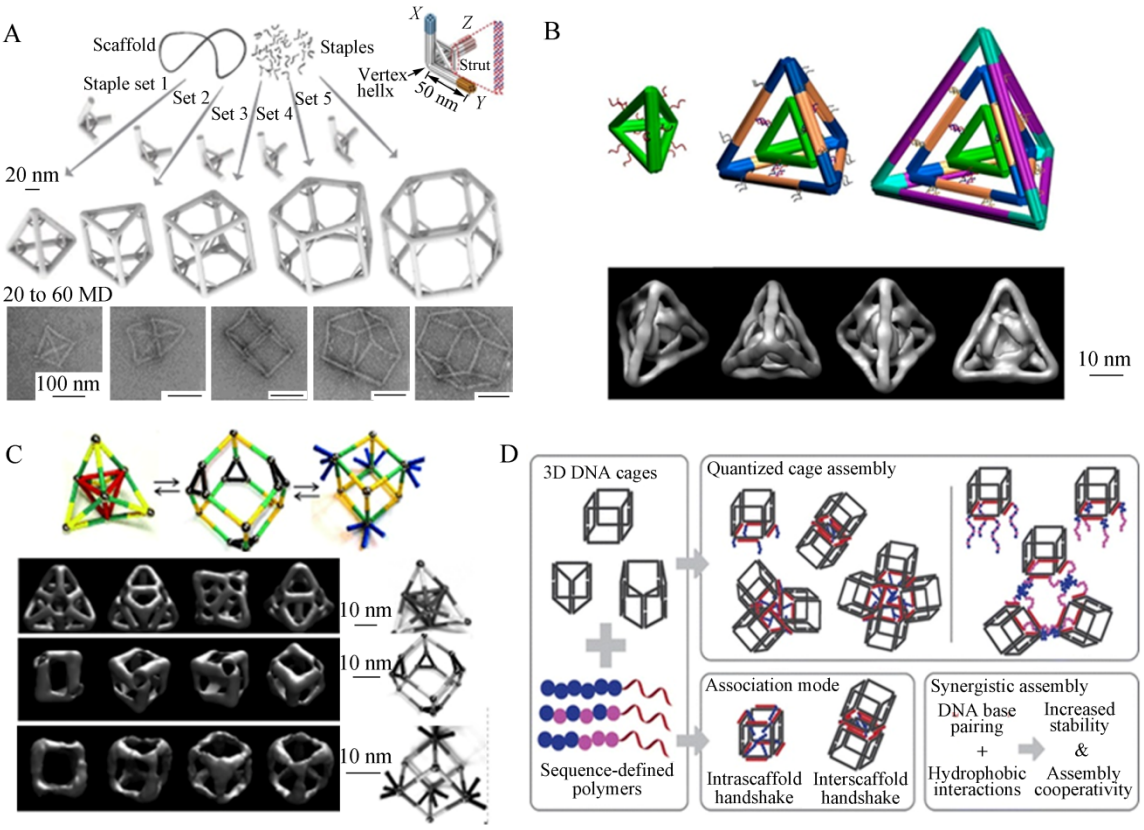

Fig. 5 Self-assembly of DNA 3D complex nanostructures: (A) DNA-origami polyhedral including a tetrahedron, a triangular prism, a cube, a pentagonal prism and a hexagonal prism and the images of TEM (scale bar = 100 nm) (Reprinted with permission from Ref. [64]; Copyright (2014) AAAS); (B) Schematic models and cryo-EM images of "Russian doll"-like TET achieved by layer-by-layer assembly in the presence of ATP (Reprinted with permission from Ref. [65]; Copyright (2015) American Chemical Society); (C) Cryo-EM characterization and the structures of cages gained by change conformation (Reprinted with permission from Ref. [66]; Copyright (2015) John Wiley & Sons Inc); (D) The complex structures by combining DNA nanocages of different geometries with polymers of different length. (Reprinted with permission from Ref. [67]; Copyright (2016) American Chemical Society)

The complexity of 3D nanostructures can be further increased by hierarchically organizing the existing 3D objects together. Recently, Mao's group reported a layer-by-layer assembly method to construct "Russian doll"-like multilayered DNA tetrahedron from inside to outside (Fig. 5B). The layers were similar in structure but different in size. Three different layers of the tetrahedron were linked by single-stranded overhangs extruded from the middle of the struts, finally resulting in an entire "Russian doll"-like complex[65]. Meanwhile, Mao's group also developed another strategy to gain nanocage transformer (Fig. 5C), by which the morphology of 3D nanostructures transformed from one status to another. In this method, the precursor DNA nanocage was

synthesized first and then transformed into a desired structure by adding the complementary strands for isothermal strand displacement[66]. The complex 3D structures synthesized by transformation strategy were usually not achieved directly from DNA tiles. Moreover, in 2016 Sleiman's reported a protein-inspired assembly method to gain quantized cage assemblies and donut-shaped "cage-rings" (Fig. 5D)[67]. The quantized cage assemblies were formed by combining DNA nanocages of different geometries with hydrophobic-hydrophilic copolymers decorated on the same face of the cage. This strategy provided a novel idea for the synthesis of complicated 3D assemblies by using DNA hybrid materials.

APPLICATION OF DNA 3D NANOSTRUCTURES

In the first section, we have witnessed the capability of DNA nanotechnology which performs as powerful tool for the synthesis of 3D nanostructures. Their precise spatial addressability and capability of arbitrary control over size and shape bring them many advantages compared to other methods used for nanomaterials synthesis. More importantly, with excellent programmability of the nucleic acid sequence and the well-established methods for DNA modification, the self-assembled DNA nanostructures can be easily functionalized to generate a large variety of functional systems with molecular level precision, which may be used for a wide range of applications, such as diagnostics, biomedicine, material synthesis, and synthetic biology. To introduce functionalities to a DNA nanostructure, one of the commonly used methods is the site-specific modification on DNA strand, by which various functional moieties, such as small chemicals, biomolecules, and nanoparticles can be conjugated on the nanostructures. For instance, fluorescent dye molecules can be conjugated on DNA for imaging purposes. Targeted molecules, such as biotin, folic acid, and peptide, can also be modified on the DNA strands, enabling the nanostructures with particular functions through biotin-avid in or ligand-receptor interactions. Alternative method to functionalize the DNA nanostructure is by carefully designing the sequences of component DNA strands where functional nucleic acid segments can be introduced and interact with their targets through nucleic acid base-pairing, aptamer-target interaction, or DNA-protein interaction. Moreover, other strategies such as static charge interaction, π-π stacking, and molecular interculation, *etc.*, although less used, are also very useful for DNA nanostructure functionalization.

Indeed, DNA nanotechnology has already become an interdisciplinary research area, with scientists and engineers from chemistry, materials science, physics, computer science, and biology. Based on their excellent controllability and versatility in both structure and function, 3D nanostructures have been intensively used to address many fundamental questions in science and are also amenable to diverse applications. In this section, we will simply review their applications, especially focus on their serving as nanocarriers for cargo encapsulation and delivery, sensing probes for diagnosis, and nano-templates for new material synthesis.

As Nanocarriers for Cargo Delivery

As we mentioned above, the DNA 3D nanostructures with controllable morphology and spatial addressability allow scientists to manipulate specifically DNA modules and precisely present various types of recognition molecules at the nanometer level. Therefore, DNA 3D nanostructures could be easily programmed to form ideal carriers that load versatile payloads through specific interactions. Thus far, different types of cargos, such as small molecules (*e.g.* fluorescent dye, anti-tumor chemotherapeutics)[68–70], biomolecules (*e.g.* Cytosine-phosphate-Guanosine (CpG) sequences, siRNA, aptamers, proteins)[71, 72], *etc.*, have been reported and loaded on various DNA 3D nanostructures for different purposes.

Small molecule delivery

When used for carrying small molecules, the cargo can be either covalently conjugated to DNA strand or absorbed on the duplex by physical interaction. For example, fluorescent dyes are model molecules frequently used for cellular delivery and imaging, which can be easily traced *via* fluorescence microscopes. Sleiman's group has shown that self-assembled DNA nanotubes and cages can act as carriers to deliver cyanine fluorescent dyes into human cancer cells[68]. Moreover, multiple fluorophores can be labeled on a DNA nanostructure where the numbers and positions of these fluorescent cargos can be precisely controlled (Fig. 6A)[69], such as labeling a

DNA origami box for delivery[52]. Besides the direct conjugation, a label-free method was reported by Ding and co-workers, by which fluorescent molecules were bonded with DNA duplex to study the distribution and stability of DNA origami nanostructures in live cells[73]. Furthermore, Krishnan and co-workers reported the encapsulation of fluorescent labeled dextran molecules in a DNA icosahedral nanostructure without using chemical bonds[74]. The principle of this method was that the bound dye molecules were released when the structure was disrupted, resulting in the decrease of the fluorescence intensity. As fluorophore FITC is highly sensitive to pH value, this complex could serve as an *in vivo* pH probe for intracellular detection. Anti-tumor chemotherapeutics are another important type of small molecules that can be delivered by DNA nanostructures. Doxorubicin (Dox), a widely used anti-cancer drug for various cancers' treatment, kills the tumor cells by intercalating the DNA duplex and inhibiting macromolecular biosynthesis. Doxorubicin can be easily loaded on many DNA 3D structures by intercalating the drug molecules into the DNA duplex. Starting from DNA tiles, tetrahedral[75], icosahedral nanostructures[76] and half-icosahedral nanostructure (Fig. 6B)[77], as well as DNA-origami based tube[78] have been demonstrated as effective carriers for Dox delivery to cancer cells. Owing to their unique structural features, DNA nanostructure-based drug carriers exhibit many advantages over conventional drug delivery systems. For instance, no matter which nanostructures were used, it was found that the DNA 3D nanocarriers could promote the cellular internalization of Dox, which significantly enhanced cell-killing activity. Moreover, the release profile of loaded drug could be tuned by changing the design of DNA nanocarriers. As an example, Högberg *et al.* developed two DNA origami nanostructures for delivering Dox to three different breast cancer cell lines[78]. These two nanostructures had different degrees of global twist, hence generating different amounts of relaxation in the DNA double-helix structure to control the release of Dox (Fig. 6C). More importantly, Ahn and co-workers demonstrated that DNA nanostructure-based nanodrug could overcome the multi-drug resistance (MDR)[75]. In their experiment, DNA tetrahedrons were employed for efficient delivery of Dox into Dox-resistant human breast adenocarcinoma cancer cells (MCF-7/ADR) and they found that the drug-bearing DNA nanostructures could significantly inhibit the growth of MDR cells. The effect of reversing MDR was attributed to that the membrane P-glycoprotein was not able to efficiently pump the nano-sized drug carrier out of the cell. Besides loading drug through molecular interculation, recently, Sleiman and co-workers invented a method to introduce hydrophobic and dendritic alkyl residues into the DNA nanostructure (Fig. 6D)[25]. When eight alkyl residues were conjugated at the corner of a DNA cube, they engaged in an intramolecular "handshake" inside the cubic structure, resulting in a monodisperse DNA-amphiphile-based micelle. This novel architecture with well-defined geometry has shown the capability of encapsulating small hydrophobic drug molecules and their release by DNA recognition. All these studies clearly demonstrated that the DNA 3D nanostructures can serve as innovative vehicles for drug delivery. Apparently, DNA 2D origami has been demonstrated to delivery Dox for cancer treatment *in vivo* and shown remarkable therapeutic activity[79]. In a similar way, the 3D drug carriers are also highly expected to possess enhanced passive targeting at the tumor region and long-circulation properties because of their size effect. Thus, the efficacy of loaded drug can be increased and the side effect can be reduced compared to its free counterpart. Nevertheless, there are still some fundamental questions to be addressed in future. For instance, how the morphology (size, shape, and topology) and rigidity affect the cellular uptake remains unclear.

Functional nucleic acid delivery

Besides the small molecules, biomacromolecules which are usually difficult to be delivered can also be loaded into DNA 3D nanostructures. Modern molecular biology has revealed that different types of nucleic acids with specific sequences have different functions in the living organisms. With the homologous constituents, utilizing DNA 3D nanostructure to deliver functional pieces of nucleic acids into cell can be naturally expected. For example, Fan *et al.* employed a DNA tetrahedron as a nanocarrier to deliver multivalent CpG segments (Fig. 7A) into an immunological cell[80]. Compared to single-strand DNA, the multivalent CpG -bearing tetrahedron was more stable in fetal bovine serum and showed higher efficiency to enter macrophage and to stimulate the immune response. Similarly, Liedl and co-workers synthesized hollow 30-helix DNA origami nanotubes bearing up to 62 CpGs (Fig. 7B), which triggered a higher immune response than CpG alone[81]. Another type of functional nucleic acids is small interfering RNAs (siRNAs) which are used to bind complementary mRNA

molecules and to inhibit the protein translation. Lee *et al*. developed a new siRNA delivery system by using tail-bearing DNA tetrahedron (Fig. 7C), which could silence the target genes in tumor cells[82]. Moreover, modification of folate acid on these tetrahedral nanocarries was proved to be effective to improve the efficacy of siRNA delivery and the knocked-down of luciferase expression. Aptamers are artificially selected oligonucleotides that have high affinity to specific targets. Fan *et al.* designed a dynamic DNA tetrahedral nanostructure with an anti-ATP aptamer (Fig. 7D) embedded in one of the edges[83]. This nanostructure could efficiently enter the cell and monitor the intracellular level of ATP based on ATP-induced aptamer conformational change and the altered FRET signals.

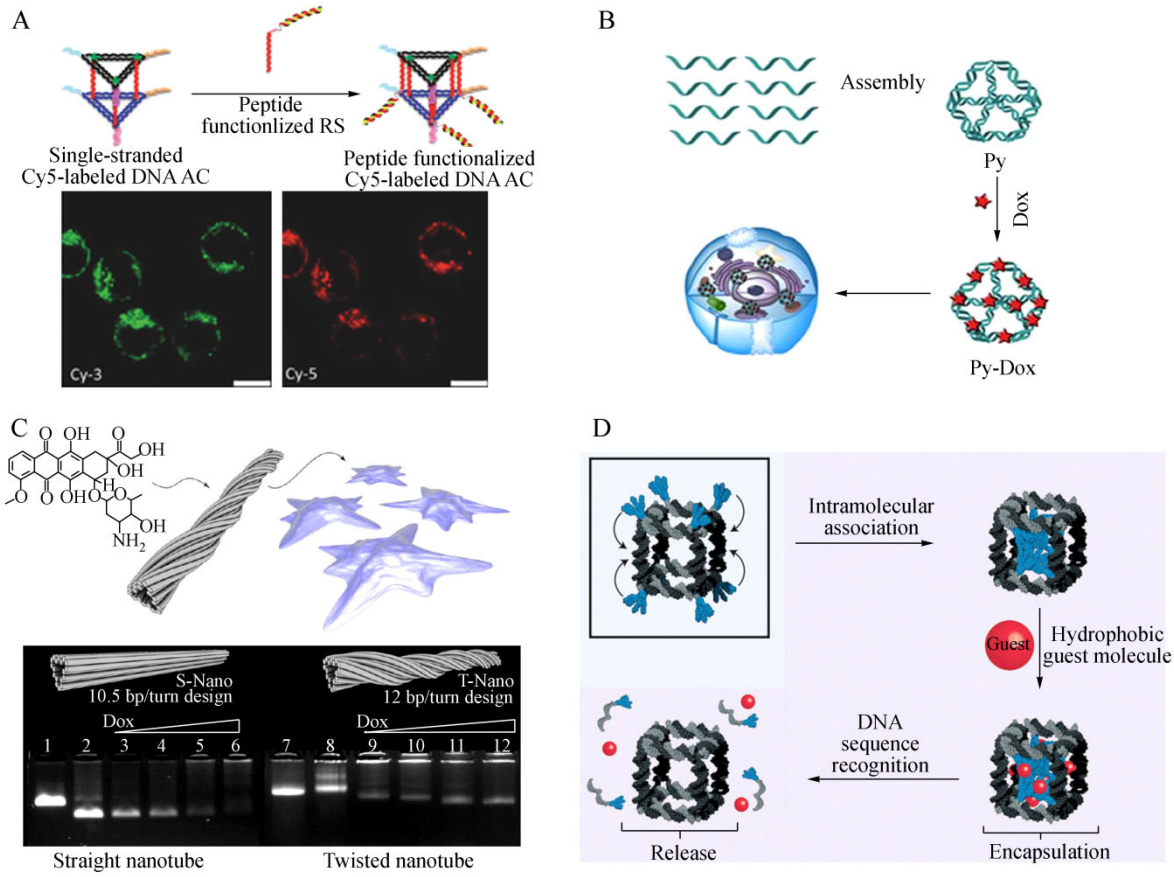

Fig. 6 Delivering small molecules with DNA 3D structures: (A) Self-assembled DNA nanocages containing both Cy3 and Cy5 (a fluorescence dye) and confocal fluorescence images of replated cells (scale bar = 10 μm) (Reprinted with permission from Ref. [69]; Copyright (2014) John Wiley & Sons Inc); (B) Schematic representation for the synthesis, loading and delivery of rigid open-caged pyramidal DNA nanostructure-Dox to the cells and depicting the internalization of Py-Dox hybrid and the release of Dox (Reprinted with permission from Ref. [77]; Copyright (2016) John Wiley & Sons Inc); (C) Two DNA origami nanostructures colocalizing with Dox drugs and 2% Agarose gel electrophoresis (Reprinted with permission from Ref. [78]; Copyright (2012) American Chemical Society); (D) Intramolecular assembly within cubes with eight alkyl chains to form scaffolded micelles, which are capable of encapsulating small molecules and releasing them in the presence of a specific DNA sequence (Reprinted with permission from Ref. [25]; Copyright (2013) Nature Publishing Group)

Protein delivery

Protein is the most important molecular machines in living system. In a cellular environment, there are many different kinds of proteins including antigen, antibody, catabolite activator protein (CAP), *etc.*, which can interact with DNA for various cellular reactions. In our previous studies, we reported the organization of specific proteins, such as streptavidin (STV) and antibody on various highly symmetric DNA polyhedra[84]. Yan *et al.* employed these structures as synthetic vaccines for immunological stimulation (Fig. 8A)[85]. By incorporating

CpG sequence as adjuvant into tetrahedral STV-DNA complex, they found that the assembled tetrahedral antigen-DNA complexes could induce strong and long-lasting antibody response against the antigen as compared to the individual protein antigens alone. In the meantime, DNA nanostructures alone had ignorable immunogenicity, demonstrating the advantages of DNA nanostructure based synthetic vaccines with rational designed properties. As more and more protein-based drugs are used for disease treatment, recently, Church's group loaded therapeutic antibodies on a DNA hexagonal barrel (Fig. 8B), which could be switched to open and close state by an aptamer-triggered lock[24]. In their study, various Fab antibody fragments, which was bound to human CD33 and human CD328 and induced growth arrest in leukemic cells, were covalently attached to 5′-amine-modified linkers and embedded inside the barrel. When the barrel structure was opened, the antibodies were allowed to bind to cell-surface receptors and inhibited the growth of the target cells. Similarly, an increase in T cell activation was induced by the structure loaded with Fab fragments that targeted to human CD3 and flagellin. Moreover, Turberfield and co-workers encapsulated a transcription factor protein (catabolite activator protein, CAP) inside a DNA tetrahedral nanostructure by non-covalent binding (Fig. 8C)[86]. As an important transcription factor, CAP regulates more than 100 genes, which binds to a 22 base-pair DNA recognition site with high affinity in the presence of cyclic adenosine monophosphate (cAMP). In a separate study, Kostiainen *et al.* showed that it was possible to coat a layer of virus capsid proteins onto the surface of DNA origami nanostructure and to significantly enhance the internalization of DNA-protein complex nanostructures[87]. All these results demonstrate that DNA 3D nanostructures can be employed as generic vehicles for effective protein delivery.

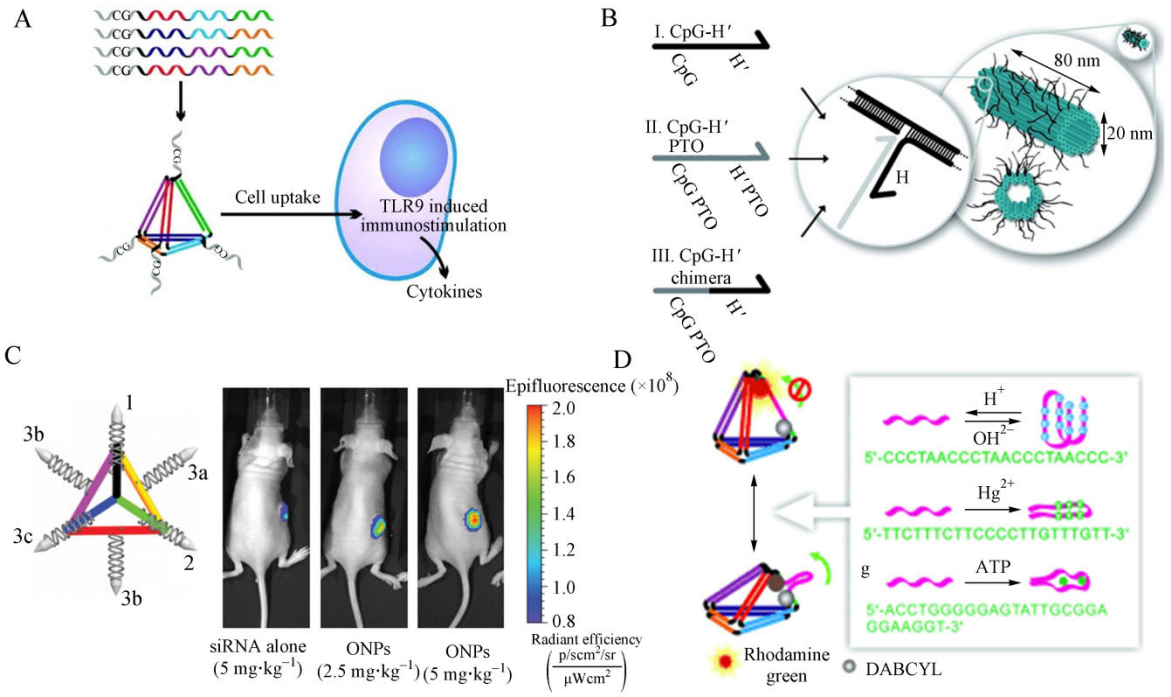

Fig. 7 Delivering nucleic acids with DNA 3D structures: (A) Schematic showing of the assembly of CpG-bearing DNA tetrahedron and its immunostimulatory effect (Reprinted with permission from Ref. [80]; Copyright (2011) American Chemical Society); (B) Design of 30-helix DNA origami tube with different types of CpG-H0s and endocytotic pathway (Reprinted with permission from Ref. [81]; Copyright (2011) American Chemical Society); (C) Schematic of DNA strands for tetrahedron formation (arrow head represents 5′ end of the nucleic acid strand; each color corresponds to one of the six edges of the tetrahedron) and representation showing site-specific hybridization of siRNA to the self-assembled nanoparticles (Reprinted with permission from Ref. [82]; Copyright (2012) Nature Publishing Group); (D) Scheme of DNA tetrahedral containing a nick site to connect aptamer by adapting complementary dynamic sequences in one arm (Green arrow = FRET signal) (Reprinted with permission from Ref. [83]; Copyright (2012) John Wiley & Sons Inc)

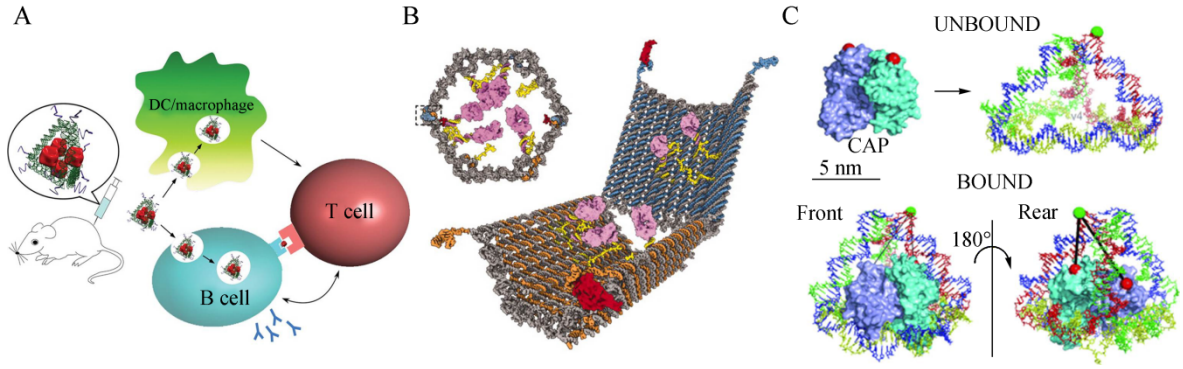

Fig. 8 Delivering proteins with DNA 3D structures: (A) Schematic design of the DNA scaffolded adjuvant-antigen vaccine complex (The model antigen (streptavidin) is shown in red and the tetrahedral DNA scaffold is represented by green helices) (Reprinted with permission from Ref. [85]; Copyright (2014) American Chemical Society); (B) A barrel-shaped nanorobot achieved by combining individual DNA origami sheets (Reprinted with permission from Ref. [24]; Copyright (2012) AAAS); (C) Structural model of CAP and the cage in unbound and bound the two CAP acceptors (Reprinted with permission from Ref. [86]; Copyright (2013) John Wiley & Sons Inc)

As Sensing Probes for Biosensors

DNA-based biosensors utilize short oligonucleotides as probes to recognize the target molecules and generate output signals to determine the quantities of the target. In the past two decades, a huge number of DNA biosensors have been constructed for different target molecule detection. However, improvements are still needed in this field, including enhancement of the sensitivity and decreasing the limit of detection. Compared to conventional DNA sensors with 1D and 2D architectures, DNA 3D nanostructures provide new opportunities to satisfy the requirements of various biosensors owing to their multivalent binding behaviors and precise 3D orientations at nanoscale. In recent years, a variety of sensing strategies based on DNA 3D nanostructure have been developed to detect different biomolecules, including DNAs, miRNAs, pathogen nucleic acids, and disease-related biomarkers. Thus far, DNA tetrahedral nanostructure is the most used platform for biosensor construction due to its simplicity in design and easy modification. In general, 3D DNA nanostructures, anchoring of different biomolecular probes in a specific spatial arrangement, can significantly improve the sensitivity with several orders of magnitude when meeting their target molecules. For example, in 2010, Fan *et al*. reported a DNA tetrahedron-based platform for immobilization of DNA probes on gold electrode surfaces (Fig. 9A) with a pendant single-stranded DNA probe and three thiol groups on four vertexes respectively[88]. This tetrahedral-structured probe (TSP) immobilized on substrates can prevent nonspecific adsorption and improve the recognition ability of single DNA molecules. When detecting a target DNA, it possesses 25 to 100-fold higher selectivity for single-base mismatches than conventional DNA sensors regarding the discrimination factor. Further, the detection events based on TSP sensors could be directly visualized with electrostatic force microscopy[89]. In a particular case, this DNA tetrahedral nanostructure can be employed as both capture probe and reporter in the sensor to achieve ultra-high sensitivity (Fig. 9B)[90]. Meanwhile, miRNA can also be detected by TSP-based DNA sensor. For example, Fan's group designed a DNA sensor, in which signaling modules such as hairpin structure and hybridization chain reaction (HCR) were used for ultrasensitive detection of miRNAs[91, 92]. This sensor can drastically improve the sensitivity, and as low as a few attomolars (1000 copies) of miRNAs can be detected with high single-base discrimination ability (Fig. 9C). Moreover, Zhou *et al.* extended the detection range to living cancer cells[93]. By producing a long helix with multiple biotins for enzyme binding and multiple branched arms for multivalent anchoring on the electrode gold surface, they demonstrated a simple and sensitive electrochemical sensor capable of detecting four different cancer cells.

Besides the hybridization-based recognition, other DNA probe design strategies, such as antigen sensors and aptamer target sensor, also have been developed. For instance, by coupling antibodies to TSP *via* a DNA bridge, a novel electrochemical immunosensor for detection of certain antigens has been realized, which has

superior sensitivity and selectivity compared to conventional sensors without using DNA nanostructures[94, 95]. Notably, the protein-resistant ability of TSP immunosensor can significantly minimize nonspecific adsorption observed in other immunosensors. When aptamer probe is incorporated, the target molecules, detected by DNA 3D nanostructure based biosensors, can be largely expanded. For example, immunological thrombin sensor with an anti-thrombin aptamer on DNA polyhedral vertex exhibits a detection limit of 100 pmol/L proteins in buffer condition. In another case, the aptamer-based assay of cocaine has a remarkably low detection limit of 33 nmol/L (Fig. 9D)[96] by integrating anti-cocaine aptamer in the sensor. In addition, other aptamer-based sensors, including mercury sensors and anti-ATP sensors, have been developed for reliable and quantitative detection as well[97–99].

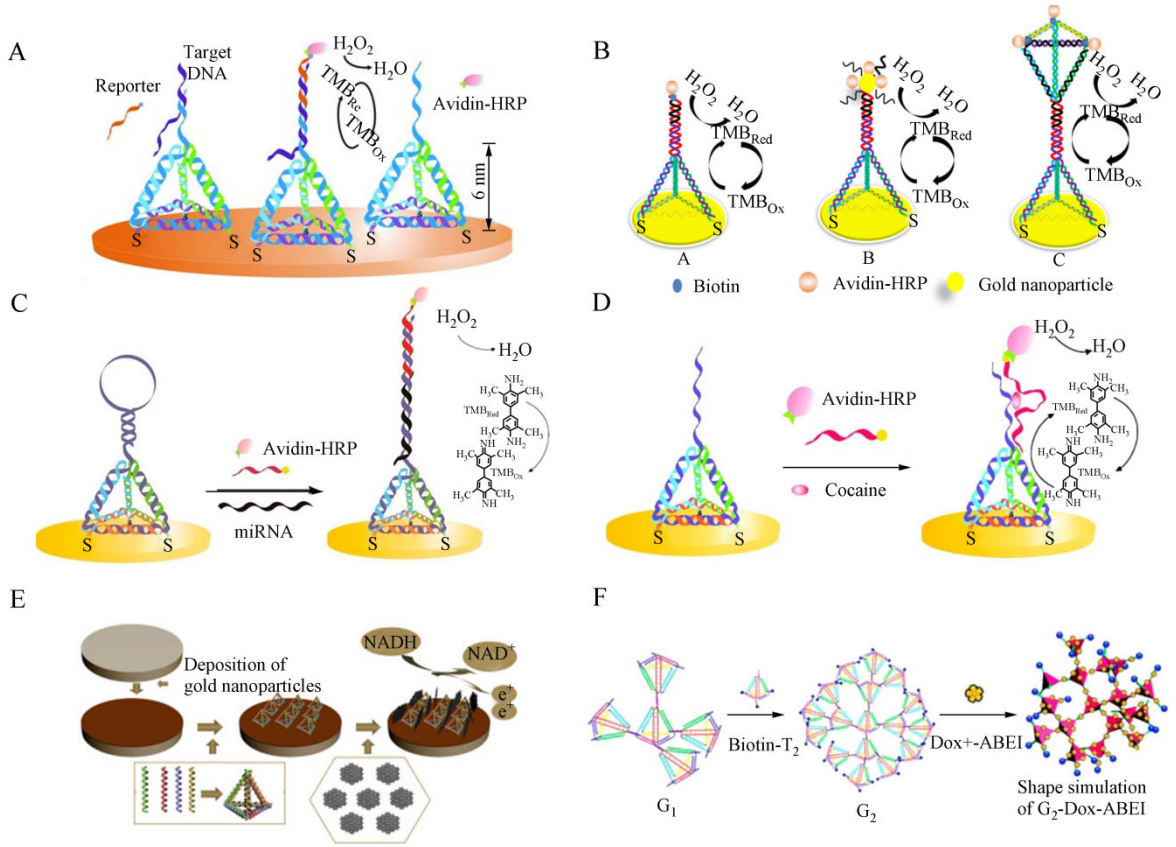

Fig. 9 DNA 3D nanostructure-based biosensors: (A) Electrochemical biosensing strategies based on tetrahedral-structured probes (TSP) for detection of DNA (Reprinted with permission from Ref. [88]; Copyright (2010) John Wiley & Sons Inc); (B) Schematic illustration of RTSPs-based electrochemical DNA sensor, in which A utilizes single-stranded DNA report probes, B utilizes gold nanoparticles report probes, and C utilizes tetrahedral structured report probes (Reprinted with permission from Ref. [90]; Copyright (2015) Elsevier); (C) First generation of E-DNA sensor based on the electron transfer and conformational changes upon target binding for sensitive microRNA detection (Reprinted with permission from Ref. [91]; Copyright (2014) American Chemical Society); (D) Electrochemical biosensing strategies based on TSP for detection of aptamer's target (Reprinted with permission from Ref. [96]; Copyright (2011) American Chemical Society); (E) Construction process for the graphene-DNA tetrahedron-gold nanoparticle modified gold disk electrode for nicotinamide adenine dinucleotide hydride (NADH) detection (Reprinted with permission from Ref. [103]; Copyright (2015) Elsevier); (F) Preparation of the tetrahedron DNA dendrimer (G_2)-Dox-ABEI complex (Reprinted with permission from Ref. [104]; Copyright (2016) American Chemical Society)

Moreover, enzyme can be incorporated into DNA 3D nanostructure to amplify the signals and improve the sensitivity of the biosensor. For instance, by introducing horseradish peroxidase (HRP) on TSPs, DNA sensor was able to detect DNA target as low as 100 femtomole and obtained a large dynamic range[100, 101]. At the

meantime, other type of biomolecules, including various miRNA and Avian Influenza A (H7N9) virus, were detected using this strategy[102]. Furthermore, biosensors can also be constructed with DNA hybrid materials. Recently, Li *et al.* reported an electrochemical biosensor (Fig. 9E) based on DNA tetrahedron/graphene composite film for highly sensitive detection of NADH, a major biomarker related with many diseases such as cancers and bacterial infection[103]. By assembling the DNA tetrahedron/graphene composite film on a gold electrode surface, the detection limit of the NADH sensor was reduced down to 1 fmol/L, with a dynamic range up to 10 pmol/L, which perfectly matched the requirement for NADH detection in clinical diagnostics.

In a more complicated fashion, DNA nanostructures can serve as not only scaffolds for biomolecular probes but also vehicles for drug delivery, which enable the construction of smart nanodevices for theranostic studies. As an example, Yuan and co-workers developed a novel method of high efficiency detection of lipopolysaccharides (LPS), in which a Dox-electrochemiluminescence (ECL) aptasensor was constructed by amplifying ECL signals through formation of tetrahedron DNA dendrimers (Fig. 9F)[104].

As Nano-templates to Synthesize New Materials

Owing to the unique morphology and advantage of precise modification at the specific sites on DNA nanostructure, many guest nano-objects can be arbitrarily decorated at predesigned positions on these 3D scaffolds. Thus far, DNA 3D nanostructures have been widely employed as ideal templates to construct new materials with hallmark properties by organizing multiple nano-objects, such as proteins, lipid molecules, inorganic nanoparticles, *etc*. In our previous work, we have demonstrated that the self-assembled DNA nanostructures could be used to organize proteins in 3D space[84]. By introducing specific interactions, individual protein particles, including streptavidin and antibody, were immobilized on DNA polyhedron scaffolds to form highly ordered DNA-protein chimerical complex structures (Fig. 10A). Such well-defined, multicomponent, protein-containing structures cannot be systematically prepared by other means. In principle, our method can be applied to many other DNA 3D nanostructures and different proteins. Recently, Liu *et al.* developed a new strategy called "Frame Guided Self-assembly" to synthesize DNA-based complex structures (Fig. 10B), in which DNA nanostructures were used to direct assembly of lipophilic molecules in order to synthesize liposome with desired size and shape rather than a normal spherical vesicle[105]. With the same strategy, DNA origami modified with lipophilic moieties have been employed as templates to form liposome-like architectures. For instance, DNA octahedron with an estimated diameter of ~50 nm with cholesteryl on its outer side was used to assemble membrane-enveloped DNA nanostructures (Fig. 10C) that mimick the geometry of a viral protein capsid shell[106]. The authors demonstrated that the precise control over the density of attached lipid conjugates was effective to achieve a high yield of tightly wrapped unilamellar nanostructures. More recently, Lin and co-workers also employed DNA 3D origami as nano-templates to guide the liposome assembly, by which the synthesis of small unilamellar vesicles with programmable size and outstanding monodispersity was realized (Fig. 10D)[107]. It is worthy of noting that the envelopment of DNA nanostructures in a lipid bilayer can confer protection against nuclease digestion for their potential applications.

As scaffolds or templates, DNA 3D nanostructures can guide not only the assembly of lipoic molecules and biomolecules, but also the growth and assembly of inorganic nanoparticles. Nowadays, various DNA 3D nanostructures have been used for template-based synthesis of new materials. In general, DNA nanostructures can play three different roles in synthesizing inorganic nanoparticles with desired morphology and constitution: (1) precisely positioning nanoparticles at designed binding sites (Fig. 11); (2) as a template to confine crystal growth for size and morphology control (Fig. 12A); (3) directing assembly of nanoparticles to form crystal-like superlattices (Figs. 12B and 12C). For instance, Kuzyk *et al.* showed that pairs of chiral helical gold nanoparticles were assembled on DNA origami tubes (Fig. 11A)[22]. Optical properties of the chiral nanoparticle assemblies were rationally designed, resulting in opposite circular dichroism signals. For a hollowed 3D nanostructure, Yan *et al.* demonstrated that the guest nanoparticles were positioned at both inside and outside of the DNA origami cage, resulting in fine control over the particle distribution and interparticle distance (Fig. 11B)[108]. Previously, we reported also a strategy to encapsulate gold nanoparticles in the center of highly symmetric DNA polyhedra to engineer the surface of nanoparticles (Fig. 11C)[109]. Later on, Mao and co-workers used these complex structures as templates for synthesizing molecule-like nanoparticle (NP) clusters

(Fig. 11D)[110]. By introducing different symmetry on nanoparticle surface *via* DNA polyhedron encapsulation, CH_4-like (with a tetrahedral symmetry), SF_6-like (with an octahedral symmetry) and $W(CH_3)_6$-like nanoparticle based clusters have been successfully synthesized.

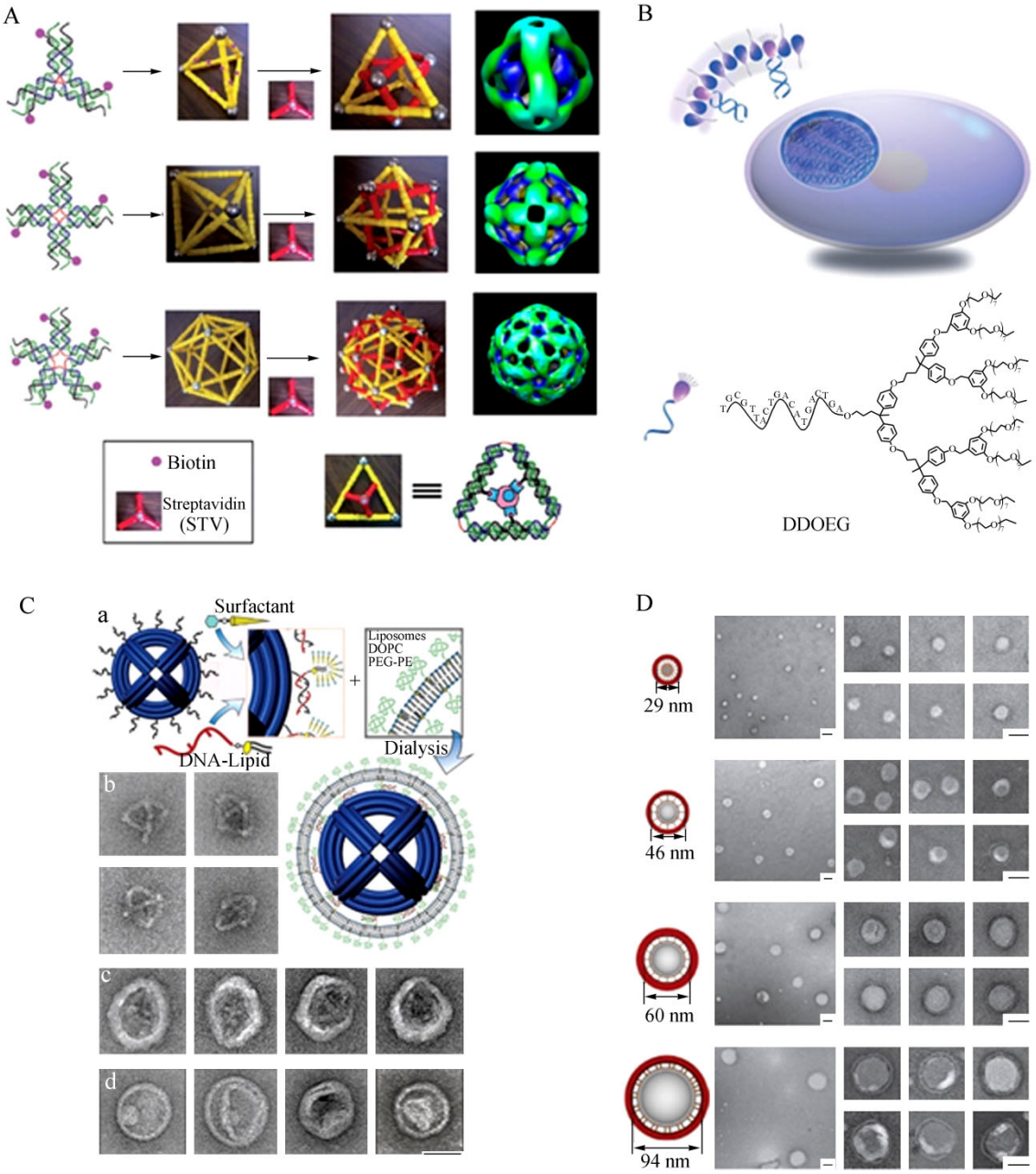

Fig. 10 DNA-guided protein and liposome self-assembly: (A) Schematic representation of the 3D protein organization directed by DNA nanostructures tetrahedron, octahedron, and icosahedron (Reprinted with permission from Ref. [84]; Copyright (2012) John Wiley & Sons Inc); (B) The AuNPs modified with 20 nt and 6 nt single-stranded DNA anchored by DDOEG by hybridization with DNA (Reprinted with permission from Ref. [105]; Copyright (2013) John Wiley & Sons Inc); (C) Schematic of the encapsulation strategy and negative-stain TEM images of non-encapsulated DNA nano-octahedron and encapsulated DNA nano-octahedron (scale bar = 50 nm) (Reprinted with permission from Ref. [106]; Copyright (2014) American Chemical Society); (D) TEM images of liposomes formed inside 29, 46, 60 and 94 nm DNA rings after purification. For each ring size, a cartoon model and corresponding TEM images of DNA-templated liposomes (scale bar = 50 nm) (Reprinted with permission from Ref. [107]; Copyright (2016) Nature Publishing Group)

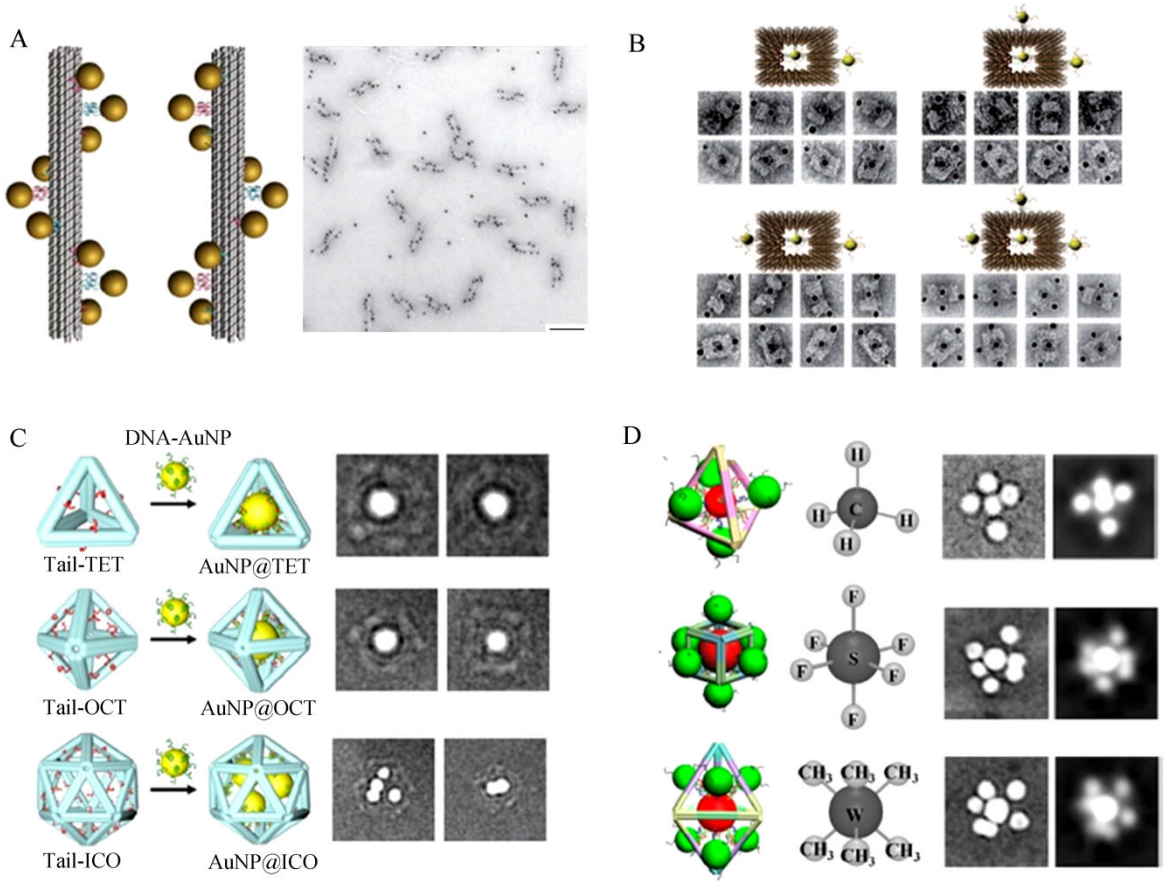

Fig. 11 Position and organize nanoparticles using DNA 3D nanostructures as templates: (A) Gold nanoparticle helices based DNA origami tube and corresponding TEM image (Reprinted with permission from Ref. [22]; Copyright (2012) Nature Publishing Group); (B) Schematic of the encapsulation strategy and negative-stain TEM images of DNA cages with one 5 nm AuNPs inside and various numbers of 5 nm AuNPs outside (Reprinted with permission from Ref. [108]; Copyright (2011) John Wiley & Sons Inc); (C) DNA polyhedra encapsulate AuNPs to form core-shell structures (AuNP@cages) (Reprinted with permission from Ref. [109]; Copyright (2014) American Chemical Society); (D) Nanoparticle clusters CH_4-like, SF_6-like, $W(CH_3)_6$-like structure based self-assembled DNA polyhedral wireframe nanocages and their cryo-EM images (Reprinted with permission from Ref. [110]; Copyright (2015) American Chemical Society)

In 2014, inspired by casting a cubic watermelon through culturing in a cubic shape glassware, Yin and co-workers developed a general strategy for synthesizing inorganic nanoparticles with prescribed shapes, dimensions, and surface modifications using DNA origami based nanocasting method[111]. The close DNA 3D structures with mechanical rigidity acted as the glassware and constrained the seed growth to form nanoparticles with the same shapes as those of DNA origamis (Fig. 12A). Such a method may lead to computational design of many functional materials with unique properties for different applications, such as novel biosensors, optical nanocircuits, electronic nanocomputers, *etc*. Recently, Gang and his colleagues employed DNA origami based polyhedra, such as tetrahedron and octahedron, to guide the self-assembly of nanoparticles in order to synthesize crystal-like superlattices[112]. This approach relied on the association between anisotropic DNA polyhedra with well-defined multi-valent binding topology and spherical DNA modified gold nanoparticles. Based on this method, many desired lattice types of ordered 3D lattices (Fig. 12B) were assembled by connecting the vertices of DNA polyhedra with gold nanoparticles. Some of the superlattice structures (*e.g.* diamond-like lattice) that have never been synthesized before have been also realized (Fig. 12C). With this general DNA frame guided assembly, undoubtedly more crystallographic lattices can be engineered by using other DNA 3D nanostructures, paving the way to study their unique and collective properties aroused by the periodic arrangement of

nanoparticles in 3D space[113].

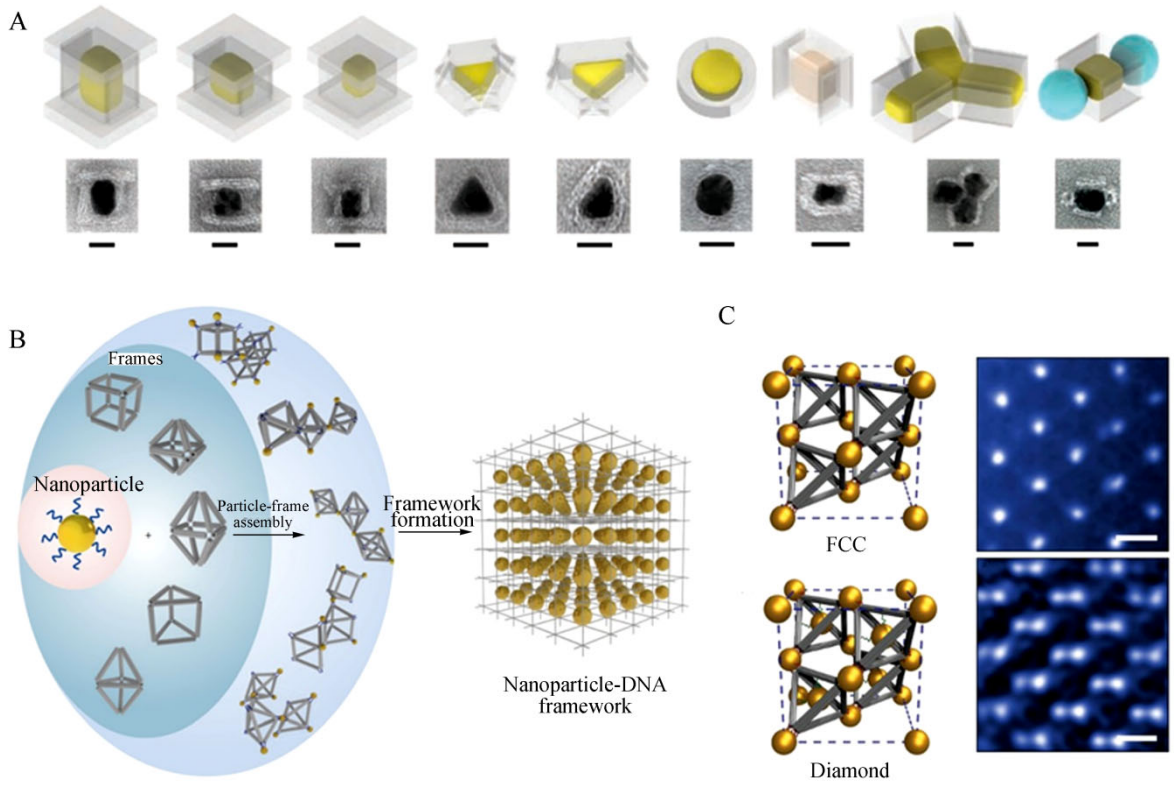

Fig. 12 DNA origami-based nanocasting and the frame-guided assembly of nanoparticle-based superlattices: (A) Casting growth of the metal particle and TEM imaging of products (scale bar = 20 nm) (Reprinted with permission from Ref. [111]; Copyright (2014) AAAS); (B) Lattice assembly through NP-DNA frameworks (NPs (yellow ball) capped with oligonucleotides (blue curves) are mixed with polyhedral DNA frames (from top to bottom): cube, octahedron, elongated square bipyramid, prism, triangular bipyramid.) (Reprinted with permission from Ref. [112]; Copyright (2016) Nature Publishing Group); (C) Unit cell model of the assembled FCC, diamond crystal and cryo-STEM high-magnification image of them (scale bar = 50 nm) (Reprinted with permission from Ref. [113]; Copyright (2016) AAAS)

SUMMARY AND OUTLOOK

As an important category of nano-objects with well-defined structures and functions, discrete DNA 3D nanostructures have been extensively explored and big progress on their synthesis and application have been made. With active efforts by researchers worldwide, many new concepts and assembling strategies have been introduced in DNA nanotechnology, enabling this technique with the capability of building any arbitrary 3D nanostructures with controllable size, shape, geometry and function. The resulting nanomaterials, with precisely tunable and addressable feature, have not been synthesized by any other conventional means. The fantastic 3D nanostructure not only renews our understanding of the magic DNA molecule, but also provides us with an excellent platform to construct mechanical, chemical, physical, and medical devices for different purposes. As more and more researchers from different disciplines are involving in this field, we believe the future of programmable DNA self-assembly will rely on continuous pursuit of integration with other research areas and its enabling applications. For example, as one of the most promising directions, constructing intelligent and refined 3D structures with viable biological functions are highly motivated. When employing as highly sensitive probes or protein-like molecular machines, these structures can assist us to investigate more specific inter- and intracellular interactions in biological system and to better understand their molecular mechanism. Following this direction, the following aspects deserve researchers' attentions, including: (1) How to integrate more

functional moieties into well-defined self-assembled nanostructures? Compared to DNA molecule, RNA, peptide and protein have more significant biological functions in cell. Thus, the synthesis of complex 3D nanostructures bearing these functional molecules is a technical hurdle to overcome. Apparently, although several RNA based 3D architectures have been demonstrated[114–116], their assembling behavior and functionalities have not been fully revealed; (2) The interaction between the discrete DNA 3D structures and cells as well as the related mechanisms are still not clear. How the size and morphology affect the cellular internalization of 3D nanostructures needs to be studied; (3) As an important category of functional carriers for drug delivery, physical stability of the discrete DNA 3D structures have to be examined; besides their cellular metabolism and fate, toxicity, immunogenicity have to be evaluated. Of course, the potentials of 3D nanostructures should not be limited to the scope listed above. Many concepts and applications that have been validated on DNA 1D and 2D assemblies can be naturally expanded to these 3D platforms[117–119]. For instance, DNA nanostructures have been employed as templates for organic synthesis with significantly improved reaction rate and yield[117, 118]. When 3D nanostructures are applied as new templates, inevitably more interesting molecules with unique structures are highly expected to be synthesized, such as precise polymers with 2D and 3D features.

Despite the advances and brilliant future of DNA 3D nanostructures shown in the sections above, it is worthy of noting that DNA nanotechnology is still at a young age and many challenges remain in this hot interdisciplinary research area. One important issue that may hinder its real application is the cost. To synthesize a specific DNA origami, a set of M13 genomic DNA and hundreds of staple DNA strands will cost more than 1000 US dollars, limiting its use in many applications, such as drug delivery which requires a lot of materials for both *in vitro* and *in vivo* study. Secondly, although some software packages have been developed to assist the design of DNA nanostructures, it is reported that only about 30% of the assembled DNA structures are similar to the original design[120]. Therefore, how to effectively design and synthesize the target nanostructure remains as a great challenge in future work. Moreover, to produce a desired shape and useful nanodevices, DNA-containing solution usually undergoes a long thermal annealing process up to 90 °C and 24 h. In some particular cases, such as sensitive protein involved co-assembling, fast and isothermal assembly is highly desired. Lastly, the complexity and functionality of currently available DNA 3D nanostructure are still primitive compared to functional components in the living systems. Studies on integrating multitude of functional moieties, such as proteins, nanoparticles, *etc*., into DNA 3D complex structures are rare. Facing these challenges, scientists in this field need to aggressively pursue new development of DNA nanotechnology.

As a conclusion, discrete 3D nanostructures, synthesized using DNA nanotechnology, have made tremendous achievements in the past decade although challenges remain. We believe it will continue its fast pace of moving forward and there is no doubt that more and more applications based on this promising technology and the resulting DNA 3D objects will appear in a wide range of research areas. With more active efforts put in, it is highly possible that the use of DNA nanostructures will eventually exceed expectations far beyond the scope of this review.

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