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Engineering Artificial Machines from Designable DNA Materials for Biomedical Applications

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Deoxyribonucleic acid (DNA) emerges as building bricks for the fabrication of nanostructure with complete artificial architecture and geometry. The amazing ability of DNA in building two- and three-dimensional structures raises the possibility of developing smart nanomachines with versatile controllability for various applications. Here, we overviewed the recent progresses in engineering DNA machines for specific bioengineering and biomedical applications.

Introduction

Deoxyribonucleic acid (DNA) works as the life information carrying materials through encoding the genetic instructions implemented in the development and functioning of living organisms. DNA is polymerized from four basic nucleobases, which behave under a unique principle, that is, adenine (A) pairs up with thymine (T) and cytosine (C) pairs up with guanine (G) with high specificity, respectively. In the past few decades, material fabrication at sizes from nanometer to millimeter heavily relies on the top-down approaches taking the advantage of designed lithograph tools.[1–3] Whereas, with a strict recognition principle DNA materials exhibit a great potential as building blocks to construct nanostructures in a bottom-up fashion, with significant programmability that engineers and chemists have ever dreamed about.[4,5] Taking the advantage of DNA programmability, objects with specially designed sizes, shapes, and architectures have been successfully constructed from DNA in the nano size range.[6–8] In particular, short single-stranded DNA (ssDNA) strands, termed as DNA tiles, have been rationally designed to self-assemble into higher-order periodic and aperiodic lattice structures.[9,10] Furthermore, structures with arbitrary architectures and dynamic functions (i.e., logically gated nanosize box) can be generated through folding a long nature ssDNA, genome of phage M13 with $\sim$7429 nucleotides in length.[11,12] With the intensive efforts over the past 30 years, significant controllability in the fabrication resolution with up to 4–6 nm has been achieved in the construction of nanostructures from custom-designed DNA.[13,14]

Currently, medicine and therapeutics tend to move into nanoscale, specifically targeting particular biomolecules with key functions in specific diseases,[15,16] for example, integrase, protease, and nonnucleoside reverse transcriptase in curing HIV and specific receptors in cancer therapies. With significant advances in constructing nanostructures with precisely controlled physical features (e.g., size, topology, and architecture), it is reasonable to believe that DNA materials are able to serve as a basic scaffold platform to construct molecular nanorobot with dynamic functions. Therefore, nano-sized DNA machines, nanostructures with precisely designed properties mimicking specific function of biological system, could be promising tools in the emerging medicine and therapeutics. However, it is challenging to create desired dynamic bio-functions on DNA machines and to apply these fantastic DNA robots to biomedical applications. Thirty years have passed, since Dr. Seeman created the first complete artificial nanostructure from DNA strands,[17,18] with the original idea to precisely localize proteins for crystallization, DNA nanostructures with designed functions have been achieved in numerous concept-proved applications for various purposes. Although there exist several good reviews on DNA nanostructures and nanotechnologies,[7,13,14,19,20–24] we highlighted here the recent progresses in engineering artificial machines from fully designed DNA materials and their potential biomedical applications, with special focus on how the dynamic functions were designed and achieved with maximal utilization of the advantages of DNA structures. For ease of understanding, we started with the basic concept of DNA nanostructure, DNA origami and DNA tiles, and then we discussed the applications utilizing

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DNA as scaffold or artificial vehicle for achieving dynamic functions, such as desired drug delivery and designed biosensing.

DNA Structure Technologies

First, the basic concept of DNA nanostructure technology will be briefly overviewed. Fascinatingly, the versatile capabilities of DNA nanostructure technology are all developed from a simple principle. Based on the principle of hybridization between DNA strands encoding complementary sequences, two-dimensional (2D) or three-dimensional (3D) DNA structures with designed architecture or geometry can be built through designing the sequences. Generally, there are two major strategies in building structures from DNA, that is, single-stranded DNA tile (SST) and DNA origami. Either of them has demonstrated profound capability in building nanostructures.

Approaches based on DNA tiles

In the case of DNA tiles, structures are built from rationally designed short ssDNA oligos through specific hybridization. With history tracking back to the very beginning of DNA nanostructure technology started by Seeman,[17,25–27] DNA tiles have been majorly used to assemble both periodic and aperiotic structures, including branch junction structures,[25,26,28] 2D lattices,[27,29,30] ribbon,[31] 3D lattice,[10] tubes,[32,33] and other shapes.[5] Successfully, Seeman's group created a high resolution of around 4 Å in a 3D crystal structure made from tensegrity triangle DNA tile[10] (Fig. 1A, B). This is a landmark to demonstrate that the DNA lattice structure technology provides a super solution for biomolecular crystal application.

Recently, Yin's group at Harvard University achieved one crucial breakthrough. In contrast to conventional SST approaches, in which a structure with repeated architecture unit was assembled from a small number of DNA tiles, they developed a new approach consisting of a large number of uniquely addressable DNA oligos encoding distinct sequences by which basic canvas structure was assembled. Based on a very simple strategy of only incubating selected DNA tiles together, hundreds of different 2D[34] or 3D[35] structures with desired complicate architecture have been built from the basic canvas structure (Fig. 1C, D). This approach significantly extends the capability of DNA tiles in the construction of complicate aperiodic structures.

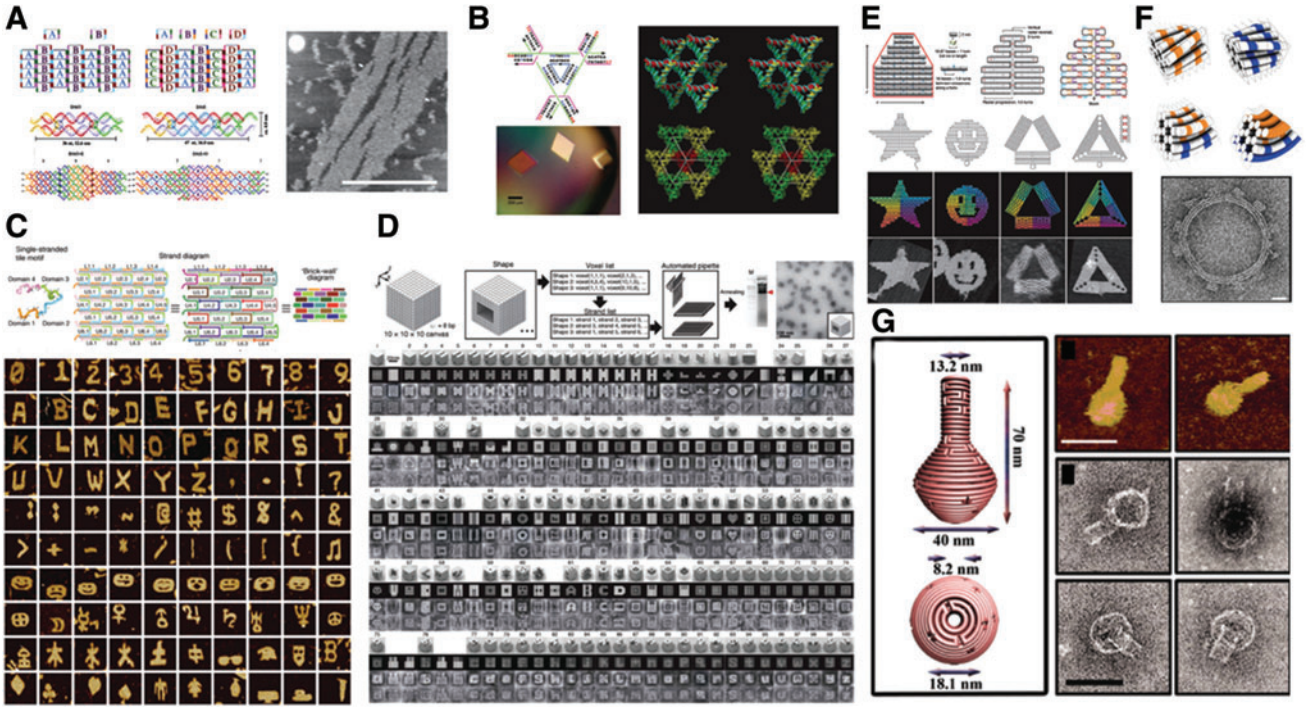

FIG. 1. Deoxyribonucleic acid (DNA) structural technology: Single-stranded DNA tiles (SST) self-assembly and DNA origami. **(A)** Large two-dimensional (2D) lattices assembled from rational designed single-stranded DNA (ssDNA) tiles, schematic for design (*left*), AFM image of assembled lattices (*right*). Scale bar is 300 nm. Reprinted from Winfree *et al.*[27] **(B)** Three-dimensional (3D) crystal assembled from DNA tiles, schematic of tensegrity *triangle* unit (*left top*), and optical image of assembled tensegrity *triangle* crystal (*left bottom*). Stereoscopic image of DNA tensegrity *triangle* (*Right*). Reprinted from Zheng *et al.*[10] **(C)** Two-dimensional complex shapes assembled from SST, assembly schematic (*top*) and AFM images of 107 distinct assembled complex 2D shapes (*bottom*). Reprinted from Wei *et al.*[118] **(D)** Three-dimensional complex shapes assembled from SST, assembly schematic (*top*) and SEM images of 102 distinct assembled complex 3D shapes (*bottom*). Reprinted from Ke *et al.*[119] **(E)** DNA origami designed by Paul W.K. Rothemund, schematic for DNA folding (*top*) and various 2D shapes built from DNA origami (*bottom*). Reprinted from Rothemund.[12] **(F)** Twisted and curved structure built from DNA origami. Schematic for building twisted curved DNA structure (*top*) and 12-tooth gears build from hierarchical assembly of twisted and curved monomers (*bottom*). Reprinted from Dietz *et al.*[36] **(G)** A 3D nanoflask structure with complex designed curvatures. Reprinted from Han *et al.*[37] Color images available online at www.liebertpub.com/teb

Approaches based on DNA origami

Another approach for construction of DNA nanostructures is termed as DNA origami. In this case, one set of short DNA strands termed as staple are used to fold one long scaffold ssDNA isolated as virus genome during a programmed thermo-procedure, like the Japanese art paper folding. In comparison with DNA tiles, DNA origami exhibits several advantages such as high assembly efficiency, versatile ability in constructing higher-order 3D structures with complicated architecture, and high stability in assembled structure. For instance, Rothemund's group at California Institute of Technology successfully demonstrated the first proof of concept for DNA origami in 2006 with folding M13mp18 genome DNA into complete artificial shapes and patterns[12] (Fig. 1E). On the basis of this evolutional invention, Shih's group[36] (Fig. 1F) and Yan's group[37] (Fig. 1G) successively developed extend DNA origami to build 3D structures with designed complex curvatures by simply selecting insertions or deletions of DNA base pairs to induce the hybridized DNA helix bundles forming twist or curve. Thus far, with the intense efforts paid DNA origami has demonstrated its spectacular power in creating arbitrary structures.[20,24,36–39]

Additionally, with precise controllability in the construction of structures at nanoscale, structural DNA technology has also been successfully employed in nanoparticle fabrication and physical applications. For example, Yin's group has developed a DNA technology platform to transfer the shapes of DNA origami into other materials such as graphene[40] and inorganic oxides[41] to fabricate nanomaterials with desired patterns. Also, DNA structures can be used to organize elements including quantum dots,[42–44] colloidal particle,[45] carbon nanotube,[46] nanoparticles,[47–52] and fullerene molecules[53] with high spatial resolution. With the nature of DNA molecules, biocompatibility and specific recognition by other biomolecules and the high stability of DNA nanostructure against degradation,[54] it could be expected that sophisticated DNA nanomachines hold great potential in biomedical applications.

Emerging Applications of DNA Machines in Biomedical Fields

Fully addressable DNA scaffolds for nanoscale organization

Following the basic concept of DNA nanostructure, the progress in utilizing DNA nanostructures as scaffolds for patterning other components will be discussed in this section. The significant advantage in sequence specificity leads to the super-precisely spatial addressability in DNA structures. This has been proven to enable the fabrication of excellent addressable scaffolds for spatial organization of elements including various types of nanoparticles[29,33,39,42,43,45,52,55–59] and proteins.[29,37,60–66] Recently, some structures made from pure DNA strands have been successfully recruited for the organization of bio- or chemical reactions. For instance, single molecules immobilized on a $100 \times 70\,nm^2$ rectangular structure made from DNA origami were successfully visualized by utilizing the high addressability with a nanoscale resolution.[67] By chemically modifying the specific DNA strands on the rectangular structure, streptavidin was precisely immobilized at specific spots and visualized at single molecule level. Based on this platform, single chemical reaction was organized and monitored through the single molecule imaging on the DNA rectangle (Fig. 2A). The precise controllability provided by DNA nanostructures offers an ultrasensitive method with great potential for visualization, organization, and monitoring of single molecular events. Similarly, a versatile DNA nanorod-based fluorescence system has also been developed by taking the advantage of DNA structures in spatial addressability with nanoscale resolution.[35] Particularly, a rod structure with a length of 720 nm was generated by connecting two pieces of small DNA rods made from origami. Three distinct fluorophores (i.e., red, green, and blue) were precisely immobilized at specific positions on the DNA rod surface (Fig. 2B), which introduced the spatial information into the system and generated a nanoscale barcode. It was proven that the spatial information between different fluorophores was unambiguously decoded using epifluorescence and total internal reflection fluorescence microscopy. Based on this nanoscale barcode, 216 distinct fluorescence patterns were generated by simply changing the positions of the three fluorophores on the DNA rod (Fig. 2C). Finally, it was shown that specific receptors on yeast surface were clearly *in situ* imaged using this novel DNA fluorescent barcode (Fig. 2D). This single molecular DNA barcode technology provides a new biological tool to identify multiplex targets in one system, holding great potential in bioengineering and biomedical applications.

Besides organizing molecules as a static scaffold, designed DNA structures have also been successfully recruited as a platform to perform complex biological reactions, in which multiple enzyme factors worked together in a controlled manner. For instance, a mesoscale structure made from ligated branch DNA units has been shown to significantly improve the expression efficiency from linear plasmid DNA tethered on the DNA structures[68] (Fig. 2E). This is a novel platform that organized the free gene DNA into an immobilized 3D form with high local concentration, and converted the *in vitro* protein synthesis reaction from a solution-based system to a spatially controllable system with improved enzyme turnover rates (Fig. 2F). Due to the importance of the technologies of *in vitro* gene expression and protein synthesis in bioengineering applications, it is reasonable to believe that this spatial organization technology could have broader implication in biomedical applications. Directly assembling artificial DNA structure inside the living cell is very crucial for biomedical application. Due to the nature of DNA existing as double-stranded form inside cells, it is almost impossible to apply the technology developed in the *in vitro* application to build DNA structure *in vivo*. However, with the similar molecular structure, RNA provides an alternative for directly building artificial nanostructure inside the living cell. Herein assembled RNA nanostructure will be discussed for highlighting the possibility of applying artificial assembled molecular structure for *in vivo* biomedical application. In one study, the group led by Silver and Lindner has assembled periodic structures from short RNA strands with designed binding motifs inside the living *Escherichia coli* cells,[38] which is the first report of successfully assembled RNA structure *in vivo*. Unlike most DNA structure building processes, the RNA structures were built in a complete isothermal process at 37°C. Interestingly,

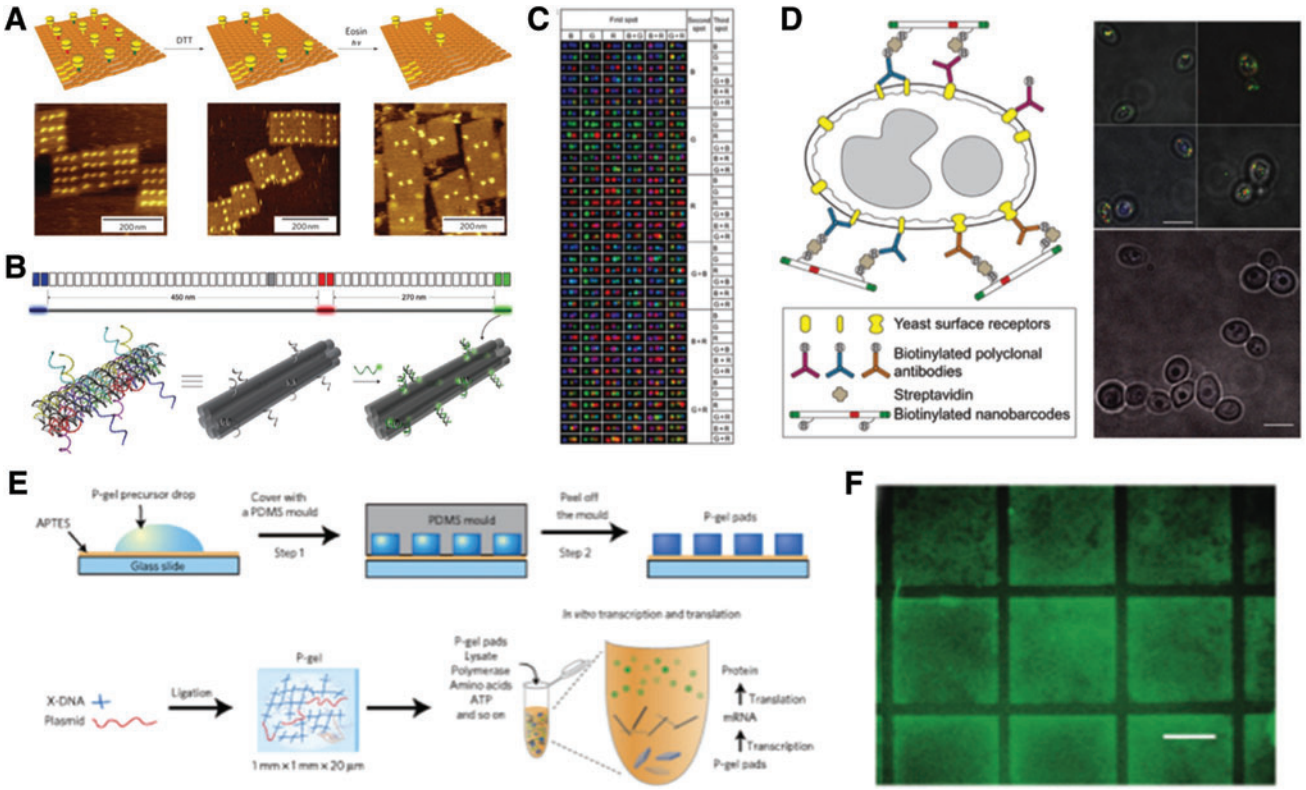

FIG. 2. Organization of molecule and bioreaction on DNA structure. **(A)** Chemical reaction was organized at single molecule level on a *square* of DNA origami. Reprinted from Voigt *et al.*[67] **(B)** Schematic for fluorescent DNA barcode. **(C)** 216 distinct barcodes. **(D)** Visualization of specific receptor on yeast by DNA barcodes. Reprinted from Lin *et al.*[120] **(E)** Schematic for cell-free protein synthesis from assembled plasmid DNA on a DNA hydrogel. **(F)** Fluorescent imaging of the DNA hydrogel, scale bar is 500 µm, reprinted from Park *et al.*[68] Color images available online at www.liebertpub.com/teb

with tethering [FeFe]-hydrogenase and ferredoxin catalyzes in the adjacent position through a specific RNA aptamer–protein interaction on the RNA structure, *in vivo* hydrogen production efficiency was improved significantly compared to cell carrying free enzymes. This is a notable success in applying designed RNA structure into biological applications for desired spatial organization, thus providing bioengineers a new powerful tool for organizing metabolic reactions in living cells. Very recently, another remarkable application was reported by a group at Harvard University. In this application, Qi *et al.* developed ssDNA into versatile programmable molecular glue for the organization of mesoscale hydrogel particles.[69] It was successfully demonstrated that massive ssDNA structures with designed sequences were able to function as specific glue to guide microscale hydrogel particles to interact in a DNA sequence-specific manner. This application paved the way to use DNA structures to organize objects, 4–6 orders of magnitude larger than DNA in size, and set up a new platform for introducing the full addressability of molecular DNA into applications like tissue engineering.

Designed DNA nanomachines for drug delivery

In addition to spatial organization of elements at nanoscale, dynamic functions were developed based on DNA nanostructure technology. In this section, the progress in which the potential of DNA as a controlled artificial vehicle for drug delivery were explored will be discussed. In previous studies, active uptake of DNA structures by cells has been observed although the underlying mechanisms are still not clear.[61,70,71] Moreover, due to the versatile programmability of DNA and the chemical DNA modification and functionalization methods developed in the past few decades, nanostructures made from rationally designed DNA strands could be the most attractive materials for developing efficient drug delivery system, which is able to deliver chemicals in responding to temperature change,[72] special biomolecule recognition,[73,74] magnetic force[75] and photon,[76] that is hard to achieve using other nanoparticles. Moreover, DNA structures have been shown to have high resistance to degradation and low immune reaction.[77,78]

Doxorubicin (DOX), one of the most widely used chemotherapy drugs, has been extensively studied as a model in DNA-based controlled delivery in the last few years. Dabrowiak and coworkers for the first time developed a DNA-capped gold nanoparticle for specific DOX delivery.[78] This is a simple design in which free DNA strands hybridize with its complementary strand tethered on a gold particle and dangling from the center, and DOX stuck on the double-stranded DNA part in a non-sequence-specific manner. It was demonstrated that DOX was released in a thermal dose-dependent manner [Fig. 3A(a)]. Following this, Huang and colleagues designed another DNA structure for DOX delivery, where an icosahedral structure was created from assembled DNA strands and DOX was carried on the double-stranded DNA

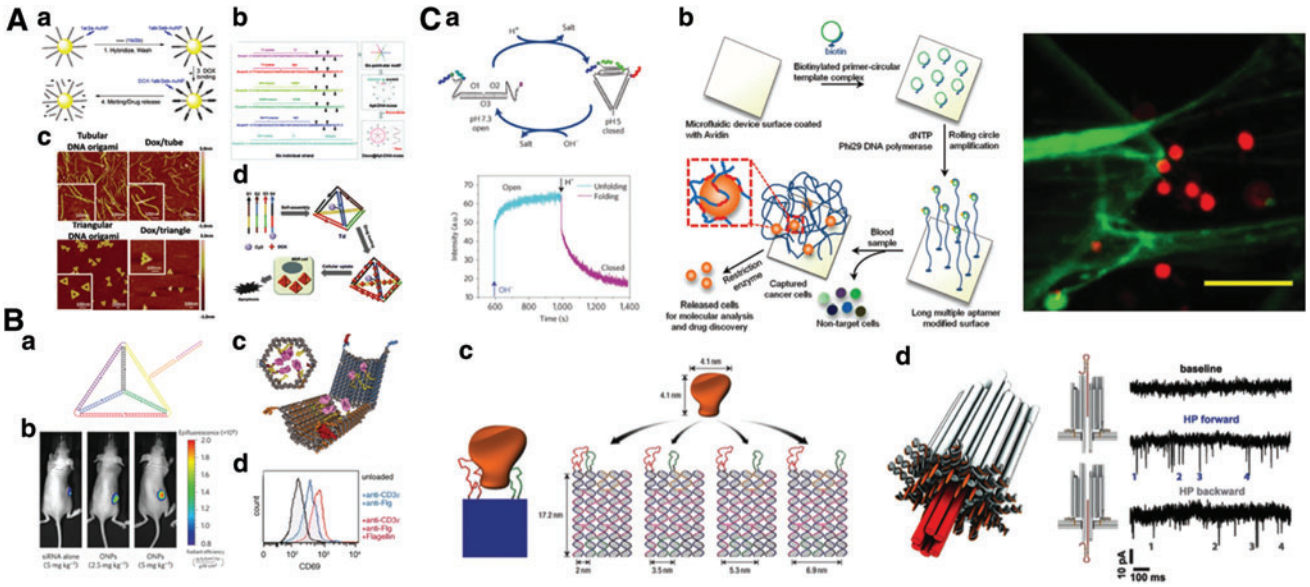

FIG. 3. Design DNA machine for drug delivery and biosensor applications. **(A) (a)** Gold nanoparticle with tethered DNA strand carrying doxorubicin (DOX). Reprinted from Alexander *et al.*[78] **(b)** Assembled DNA icosahedra structure was designed for DOX delivery. The specific cell targeting was guided by a tethered aptamer. Reprinted from Chang *et al.*[79] **(c)** Two-dimensional tube and 3D *triangle* DNA origami for DOX delivery. Reprinted from Jiang *et al.*[80] **(d)** Terahedron assembled from DNA tile delivering DOX overcame drug resistance of cancer cells. Reprinted from Kim *et al.*[81] **(B) (a)** Schematic DNA tetrahedron structure with hybridized siRNA for delivery. **(b)** DNA tetrahedron delivery siRNA to cancer cell in a mouse model. Reprinted from Lee *et al.*[82] **(c)** A DNA box with a logically controlled gate was designed from DNA origami. **(d)** T-cells were activated by DNA machine delivered specific antibodies. Reprinted from Douglas *et al.*[35] **(C) (a)** DNA machine was designed for sensing the pH change inside cells. Reprinted from Modi *et al.*[99] **(b)** A DNA massive material was designed for sensing and capturing specific flowing cells, schematic for the DNA structure (*left*) and captured target cell in the DNA structure (*right*). Reprinted from Zhao *et al.*[100] **(c)** A DNA structure with precisely positioned aptamer for capture single molecule in a distance-dependent manner. Reprinted from Rinker *et al.*[101] **(d)** A nanopore was structured from DNA origami. Schematic (*left*) and sensed the DNA structure as the function of membrane electrical conductivity (*right*). Reprinted from Langecker *et al.*[102] Color images available online at www.liebertpub.com/teb

frame in a sequence-independent manner.[79] The authors demonstrated that conjugated DNA aptamers specifically recognizing MUC1, a crucial marker for a broad range of epithelial cancer cells, could be utilized to guide the DNA icosahedral structure targeting cancer cells. DOX was released via the entire recycling pathway after uptake by cells [Fig. 3A (b)]. Very recently, another research group reported a new system for delivering DOX carried in a specific designed DNA origami.[80] In this study, Yan and colleagues extended the DNA-based DOX delivery by designing a new carrier, a 2D tubular and a 3D triangular DNA origami. Among the DNA particles developed so far, the DNA origami structures can provide the most precise structure control and more double helix DNA structures for DOX loading [Fig. 3A (c)]. Upon administration, it was observed that the whole DNA origami vehicle carrying DOX was efficiently absorbed by MCF-7 cancer cells. Most interestingly, it was found that the DNA origami vehicle even efficiently delivered the DOX into MCF-7 cells, which exhibit high resistance against free DOX in a solution. It is reasonable to conclude that DNA origami vehicle can successfully circumvent the cancer drug resistance system and bring great promise to cancer treatment. Similar outcome was also reported on a tetrahedron structure assembled from designed short DNA strands[81] [Fig. 3A (d)].

Besides the unique binding DOX to double-stranded DNA nonspecifically, engineered DNA structures have been developed for delivery of various types of molecule including proteins and RNA. Anderson and colleagues demonstrated that DNA tetrahedral structure assembled from short DNA tiles successfully delivered siRNA into tumor cells.[82] In this study, a tetrahedral structure, a robust DNA self-assembled nanoparticle, was used as a carrier on which a siRNA of interesting was tethered through hybridization with specific DNA strand [Fig. 3B (a)]. When used in a tumor xenograft mouse model, it was observed that the DNA particles efficiently accumulated to the specific tumor after tail vein injection and released siRNA for silencing the target gene inside the tumor cells [Fig. 3B (b)]. Interestingly, almost no detectable immune response was induced along the efficient delivery in this system. Moreover, Douglas *et al.* at Harvard University reported a DNA device with a very dynamic feature for antibody-based drug delivery.[35] In this study, a DNA origami box was developed for carrying immobilized antibody fragment inside. A logic gate was designed from a DNA aptamer, which changed its conformation in response to specific marker displayed on cell membrane [Fig. 3B (c)]. Upon recognition of the specific ligand, the aptamer-based gate was unlocked to open the box into two parts connected through a designed ssDNA hinge. This was followed by the explosion of antibodies decorated inside of the box to achieve its function. Due to the highly designed dynamic function, this DNA device was designated as ''nanorobot'' by the authors. To prove this concept, the suppression of this DNA nanorobot on the

growth of cells adopted from a patient with large granular lymphocytic leukemia in a dose-dependent manner and induced T-cell activation was demonstrated as well [Fig. 3B (d)]. This finding paved the way to develop DNA carriers with tunable and programmable functions for drug delivery in a precisely controllable manner.

DNA nanostructures as biosensor for diagnostic applications

In this section, progress achieved in developing molecular DNA machine for biosensing, another dynamic function, will be discussed. Ultra sensitivity is another crucial feature that can be offered by DNA nanostructure technology. The advantage of DNA in nanostructure fabrication and controllability with around 2–3 nm in precision holds great promise in developing device with ultra sensitivity, which was hardly achieved in other methods. To date, simple engineered DNA systems have been widely used in the detection and signal sensing applications, such as hybridization for detecting special DNA/RNA molecules and even metal-ion,[83–87] fluorescence resonance transfer (FRET) probes,[88–90] and electrochemical DNA sensors.[91–97] Recently, systems with more complicated design for the detection of specific targets have also been developed. For example, Weissleder and colleagues developed an efficient system for detecting pathogens using novel magneto-DNA probes with high sensitivity.[98] By extending the FRET technology, Krishnan and coworkers developed a DNA nanomachine, named I-switch, to detect the dynamic change of pH value inside the living cells[99] [Fig. 3C (a)]. Moreover, Zhao *et al.* reported a 3D DNA network comprising of repeated adhesive aptamer sequences for capturing flowing cancer cells from blood samples in a designed microfluidic device[100] [Fig. 3C (b)].

With the advances of DNA nanostructure technologies, novel platforms have been developed by taking advantages of precise assembly of DNA nanostructures, thus bringing the DNA machine-based detection to a higher level with more controllability. In 2008, Yan's group designed a system to detect protein binding in a precisely distance-dependent manner.[101] Particularly, two distinct thrombin-binding DNA aptamers, that bind with thrombin from different sides respectively, were immobilized on the border of square DNA tiles and recruited as a sensing unit [Fig. 3C (c)]. Due to the DNA addressability, the positions of two aptamer sensors can be accurately controlled. Interestingly, it was demonstrated that the thrombin protein binding activity was controlled as a function of the distance between two aptamer sensors. Moreover, another remarkable system has been recently reported by Langecker *et al.*[102] In this study, a pure artificial nanopore was created on lipid membrane from a DNA origami structure. Inspired by the structure of a natural ion channel, a 7249 bp M13 ssDNA genome was designed and folded into a channel structure with an interior diameter of 2 nm and length of $\sim$42 nm. Via tethered cholesterol moieties, the DNA structure penetrated through the lipid membrane and formed a channel [Fig. 3C (d)]. Like native ion channel on cell membrane, this synthetic DNA nanochannel exhibited gating behavior in a tailored manner through simply replacing the specific staple DNA with varied lengths. This is an amazing feature in engineering applications for synthetic systems. It is known that, nanopore structures have been proven to be a powerful platform in molecular sensing applications with high sensitivity in the past few years.[21,34,103–107] As a proof of concept, the utilization of synthetic DNA nanopore for sensing applications has been successfully demonstrated, in which short DNA molecules with distinct secondary structures were discriminated as a function of membrane electrical properties when the molecules went through the nanopore. Hence, this is a pronounced engineering achievement, which opens up broad opportunities for biomolecular sensing applications.

Besides the above applications, DNA nanostructure has also been used as platforms for rational design and construction of vaccines.[108,109] In a typical work from Yan and Chang,[108] a synthetic vaccine complex that contains a model antigen and cytosine-phosphate-guanosine adjuvants was developed and showed to induce strong and long-lasting antibody responses against the antigen. Due to the self-assembly and the recognition ability of DNA, devices based on DNA i-motif have attracted increasing interests.[110–112] For example, Fan's group[113] constructed various molecular logic gates by reconfiguring DNA tetrahedra nanostructures with dynamic sequences that are responsive to many factors including protons, metal ions (Hg^{2+}), small molecules (ATP) and complementary DNA strands. Such DNA i-motif-based devices hold great promise for applications in biosensing, diagnostics, and molecular computers that function both *in vitro* and *in vivo*.

Challenges and Future Prospective

Although many exciting achievements have been done in the past few years, most of DNA nanostructure technologies (e.g., DNA self-assembled nanostructures) remain at the stage of proof-of-concept. While excited by significant potentials of DNA nanostructure technology, we have to keep in mind that there still exist huge challenges in applying these technologies to practical biomedical fields.

First, the optimization of the manufacture procedures remains a major technical challenge to engineers, although computer-assisted DNA nanostructure building interfaces (e.g., unpack and caDNAno) have made the design of simple DNA structures convenient even for the beginners. As for now, production efficiency limited the DNA structures only to bench works in laboratories, while there is an urgent need for improved production efficiency and quality for their widespread biomedical applications. In fact, great efforts have been put recently on optimizing these procedures, and it was demonstrated that specific temperature with a narrow range exited for specific origami shape to fold with high yield.[114] Second, DNA origami structure is majorly folded from ssDNA genome of M13 with about 7 kb in length so far. However, this DNA scaffold resource is limited and can hardly meet the requirements for more complicated nanomachine designs in the future. Besides, it is uncertain whether the sequence of M13 genome is suitable for all structures folding. Even though great progresses have been made in the assembly of SSTs recently, this has not been tested for the structure with rigid physical properties and dynamic features. For instance, it is not clear if structure like box with a logic gate controlled lip could be built from short ssDNA tiles assembly. Furthermore, 3D nanostructures with sizes around 100 nm hold great potentials in drug delivery

and tumor targeting, which may requires a scaffold of over 100 kb in size based on a simple calculation. Hence, it is very crucial to develop new technologies to generate ssDNA scaffolds with more capabilities in scaffold sequences and sizes. Moreover, biosafety is also a huge concern for applying DNA nanomachines in medical applications. Although high stability and low immune responses have been reported in previous studies, the stability, toxicity, and immune responses of DNA structures in human body still need comprehensive investigations. It has been indicated that the recognition and absorption of DNA nanostructures into cells is dependent on a specific cell receptor and the size and shape of DNA structures.[15,100] However, the mechanism of DNA structure recognition by mammalian cells and the internalization of DNA structures into cells are still not well understood. Therefore, it is necessary to put more efforts to investigate the behavior of DNA nanostructures *in vivo*.

In addition to technical challenges, the cost is another huge roadblock for popularizing DNA technology. Although the cost for synthetic DNA oligos and even DNA blocks with size around few hundred base pairs has dramatically decreased over the past few decades, there is still no cost-effective way for most laboratories to achieve the complete chemical synthesis of large size DNA (>1000 bp). Furthermore, for example, the total cost for M13-based origami at laboratory scale is still high (around US$700[7]). Hence, manufacture of DNA nanostructures in a large scale for biomedical applications is still a huge challenge. Recently, the development of new technologies for construction of large DNA molecules, such as Gibson assembly[115,116] and DNA microchips[117] for low cost enzymatic DNA synthesis, may bring down the cost of DNA production. Moreover, synthetic biology also holds potential to address such challenges by developing technologies to produce DNA materials in a commercially available way.

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Disclosure Statement

No competing financial interests exist.

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